POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER-IV

BOTDSE T403.1

MICROBIOLOGY (COURSE – II)

Self-LearningMaterial

DIRECTORATEOFOPENANDDISTANCELEARNING
UNIVERSITYOFKALYANIK
ALYANI–
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Director'sMessage Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the three fold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani. Development of printed SLMs for students admitted to the DODL with in a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, NewDelhi, India under Open and Distance Mode DEB Regulations, 2020 had been our endeavour.We are happy to have achieved our goal. Utmost care and precision have been ensured in the development of the SLMs, making the useful to the learners, besides avoid in errors as far as practicable. Further suggestions from the stakeholders in this would be welcome. During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Amalendu Bhunia, Hon'ble Vice Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance. Sincere gratitude is due to the respective chairpersons as well as each and everymember of PGBOS (DODL),University of Kalyani. Heartfelt thanks are also due to the Course Writersfaculty members at the DODL, subject-experts serving at University Post Graduate departments and also to theauthorsandacademicianswhoseacademiccontributionshaveenrichedtheSLMs.We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODLof University of Kalyani. Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode. Self-Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning, University of Kalyani, Kalyani741235,West Bengal and all the copyright reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani. All the Self Learning Materials are self-writing and collected frome-book, journals and websites.

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Director
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University of Kalyani

SYLLABUS

BOTDSE T403.1

MICROBIOLOGY (COURSE – II)

(Full Marks – 100)

Course	Group	Details Contents Structure		Study hour
BOTDSE T403.1	**Microbiology (Course – II)**	Unit 1. Microbial Genetics:	Bacterial genome replication and cell cycle;, prokaryotic transcription and translation.	**1**
		Unit 2. Microbial Genetics:	Plasmid replication	**1**
		Unit 3. Microbial Genetics:	Prokaryotic transcription and translation	**1**
		Unit 4. Microbial Genetics:	Regulation of gene expression in prokaryotes	**1**
		Unit 5. Microbial Genetics:	Genetic recombination in bacteria.	**1**
		Unit 6. Microbial Genetics:	Viral genome replication.	**1**
		Unit 7. Immunology:	Overview of the immune system. Innate immunity and adaptive immunity	**1**
		Unit 8. Immunology:	Major histocompatibility complex (MHC) and their role in antigen presentation,	**1**
		Unit 9. Immunology:	Cytokines	**1**
		Unit 10. Immunology:	Antigen- chemical nature, types; hapten, adjuvant.	**1**
		Unit 11. Immunology:	Monoclonal and polyclonal antibodies.	**1**
		Unit 12. Immunology:	Antigen-antibody reaction.	**1**
		Unit 13. Immunology:	Hypersenitivity and allergy.	**1**
		Unit 14. Immunology:	Vaccines and vaccination.	**1**

	Unit 15. Immunology:	Immunological techniques- ELISA,RIA,	**1**
	Unit 16. Immunology:	Immunofluorescence, Immunoelectrophoresis,	**1**
	Unit 17. Immunology:	Flow cytometry,	**1**
	Unit 18. Immunology:	Fluorescence-Activated Cell Sorting (FACS).	**1**
	Unit 19. Medical Microbiology:	Principle of epidemiology.	**1**
	Unit 20. Medical Microbiology:	Air borne diseases	**1**
	Unit 21. Medical Microbiology:	Water borne diseases,	**1**
	Unit 22. Medical Microbiology:	food borne diseases, arthropod borne diseases,	**1**
	Unit 23. Medical Microbiology:	Sexually transmitted diseases,	**1**
	Unit 24. Medical Microbiology:	Respiratory diseases.	**1**
	Unit 25. Mathematical approach for microbiologists:	Numerical Microbiology Problem solving,	**1**
	Unit 26. Mathematical approach for microbiologists:	Concept of mathematical models,	**1**
	Unit 27. Mathematical approach for microbiologists:	Application of Mathematical models to microbiological processes.	**1**
	Unit 28. Mathematical approach for microbiologists:	Application of Mathematical models to microbiological processes.	**1**

CONTENTS

Microbiology (Course – II)

Theoretical Course Credits: 8

Content Structure:

1. Introduction
2. Objectives
3. **Microbial Genetics:**
   - Bacterial genome replication and cell cycle; Plasmid replication, prokaryotic transcription and translation.
   - Regulation of gene expression in prokaryotes.
   - Genetic recombination in bacteria.
   - Viral genome replication.
4. **Immunology:**
   - Overview of the immune system.
   - Innate immunity and adaptive immunity, major histocompatibility complex (MHC) and their role in antigen presentation, cytokines.
   - Antigen- chemical nature, types; hapten, adjuvant.
   - Monoclonal and polyclonal antibodies.
   - Antigen-antibody reaction.
   - Hypersenitivity and allergy.
   - Vaccines and vaccination.
   - Immunological techniques- ELISA, RIA, Immunofluorescence, Immunoelectrophoresis, Flow cytometry, Fluorescence-Activated Cell Sorting (FACS).
5. **Medical Microbiology:**
   - Principle of epidemiology.
   - Air borne diseases, water borne diseases, food borne diseases, arthropod borne diseases, sexually transmitted diseases, respiratory diseases.
6. **Mathematical approach for microbiologists:**
   - Numerical Microbiology Problem solving,
   - Concept of mathematical models, Application of Mathematical models to microbiological processes.
7. **Suggested reading**
8. **Assignment**

1. Introduction

Microbiology (from Ancient Greek (mīkros) 'small', (bíos) 'life', and (-logía) 'study of') is the scientific study of microorganisms, those being of unicellular (single-celled), multicellular (consisting of complex cells), or acellular (lacking cells). Microbiology encompasses numerous sub-disciplines including virology, bacteriology, protistology, mycology, immunology, and parasitology.

Eukaryotic microorganisms possess membrane-bound organelles and include fungi and protists, whereas prokaryotic organisms—all of which are microorganisms—are conventionally classified as lacking membrane-bound organelles and include Bacteria and Archaea. Microbiologists traditionally relied on culture, staining, and microscopy for the isolation and identification of microorganisms. However, less than 1% of the microorganisms present in common environments can be cultured in isolation using current means. With the emergence of biotechnology, Microbiologists currently rely on molecular biology tools such as DNA sequence-based identification, for example, the 16S rRNA gene sequence used for bacterial identification.

Viruses have been variably classified as organisms, as they have been considered either as very simple microorganisms or very complex molecules. Prions, never considered as microorganisms, have been investigated by virologists; however, as the clinical effects traced to them were originally presumed due to chronic viral infections, virologists took a search—discovering "infectious proteins".

The existence of microorganisms was predicted many centuries before they were first observed, for example by the Jains in India and by Marcus Terentius Varro in ancient Rome. The first recorded microscope observation was of the fruiting bodies of moulds, by Robert Hooke in 1666, but the Jesuit priest Athanasius Kircher was likely the first to see microbes, which he mentioned observing in milk and putrid material in 1658. Antonie van Leeuwenhoek is considered a father of microbiology as he observed and experimented with microscopic organisms in the 1670s, using simple microscopes of his design. Scientific microbiology developed in the 19th century through the work of Louis Pasteur and in medical microbiology Robert Koch.

Here, the discipline is utilised to learn about every part of the organisms in order not only to understand how they exist in their environments but also how they affect their individual surroundings and, in turn, other organisms nearby (human beings, animals, etc.). Microbiology has consistently proved to be one of the most significant fields in biology, making it possible to define how some microorganisms cause diseases, discover treatments for such diseases and even use a few microbes for industrial applications etc.

The majority of the natural elements on the earth contain microorganisms. All living things, including humans, plants, and animals, are intimately connected to the microbes that consistently recycle important nutrients like carbon and nitrogen, break down organic matter, and influence our daily lives.

2. Course Objectives

Microbiology as special paper of M.Sc. Botany course serves to impart advanced training to the students in the field of Microbiology with focus on microbial diversity, bioprospecting and applications of microbes for obtaining various biologically significant metabolites and in bioremediation of polluted environments. Students undergo hands-on training with state-of-the art technologies and are trained so as to develop an aptitude for independent research. The Programme equips students for higher research leading to the Ph.D. Degree in India or in International Universities overseas, or for employment in Research Institutes, in teaching, and in Industry.

Learning Outcomes:

-To provide value-based education, with academic excellence and advanced research and to raise skilled candidates with research caliber in the field of Microbiology

-To inculcate the advanced concepts of Microbiology including taxonomy, physiology ¬ Immunology, biomolecular interactions, etc.

-To impart the scope for the application of concepts learned in the subject.

-To introduce about the recent advances in the field of Microbiology and its importance in research.

3. Microbial Genetics:

- Bacterial genome replication and cell cycle; Plasmid replication, prokaryotic transcription and translation.
- Regulation of gene expression in prokaryotes.
- Genetic recombination in bacteria.
- Viral genome replication.

Bacterial genome replication:

Bacterial genomes are generally smaller and less variant in size among species when compared with genomes of eukaryotes. Bacterial genomes can range in size anywhere from about 130 kbp to over 14 Mbp. A study that included, but was not limited to, 478 bacterial genomes, concluded that as genome size increases, the number of genes increases at a disproportionately slower rate in eukaryotes than in non-eukaryotes. Thus, the proportion of non-coding DNA goes up with genome size more quickly in non-bacteria than in bacteria. This is consistent with the fact that most eukaryotic nuclear DNA is non-gene coding, while the majority of prokaryotic, viral, and organellar genes are coding. Right now, we have genome sequences from 50 different bacterial phyla and 11 different archaeal phyla. Second-generation sequencing has yielded many draft genomes (close to 90% of bacterial genomes in GenBank are currently not complete); third-generation sequencing might eventually yield a complete genome in a few hours. The genome sequences reveal much diversity in bacteria. Analysis of over 2000 *Escherichia coli* genomes reveals an *E. coli* core genome of about 3100 gene families and a total of about 89,000 different gene families. Genome sequences show that parasitic bacteria have 500–1200 genes, free-living bacteria have 1500–7500 genes, and archaea have 1500–2700 genes. A striking discovery by Cole et al. described massive amounts of gene decay when comparing Leprosy bacillus to ancestral bacteria. Studies have since shown that several bacteria have smaller genome sizes than their ancestors did. Over the years, researchers have proposed several theories to explain the general trend of bacterial genome decay and the relatively small size of bacterial genomes. Compelling evidence indicates that the apparent degradation of bacterial genomes is owed to a deletional bias.

Prokaryotic DNA Replication is the process by which a prokaryote duplicates its DNA into another copy that is passed on to daughter cells. Although it is often studied in the model organism *E. coli*, other bacteria show many similarities. Replication is bi-directional and originates at a single origin of replication (OriC). It consists of three steps: Initiation, elongation, and termination.

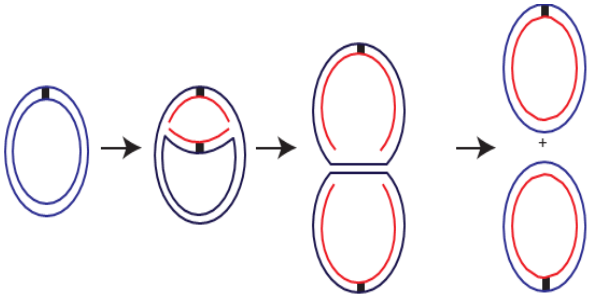
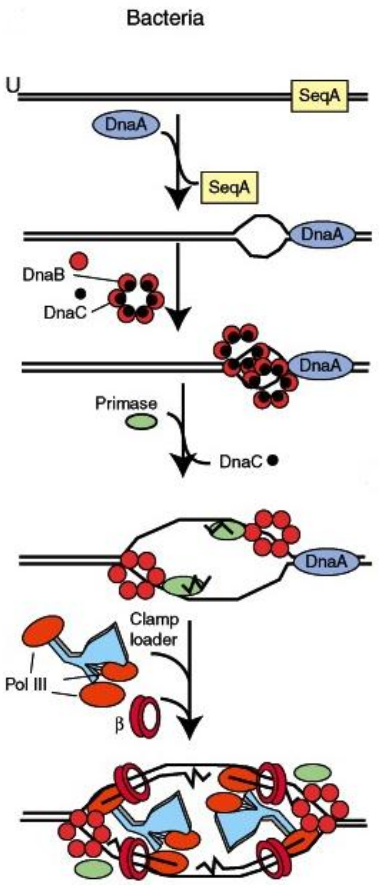


Figure 1. Replication initiation in bacteria. Most bacteria have a circular chromosome with one origin, although there are exceptions to this. Illustrated here is the *E. coli* chromosome that has one origin from which two replication forks proceed in opposite directions.

Initiation

All cells must finish DNA replication before they can proceed for cell division. Media conditions that support fast growth in bacteria also couples with shorter inter-initiation time in them, i.e. the doubling time in fast growing cells is less as compared to the slow growth. In other words, it is possible that in fast growth conditions the grandmother cells starts replicating its DNA for grand daughter cell. For the same reason, the initiation of DNA replication is highly regulated. Bacterial origins regulate orisome assembly, a nuclei-protein complex assembled on the origin responsible for unwinding the origin and loading all the replication machinery. In *E. coli*, the direction for orisome assembly are built into a short stretch of nucleotide sequence called as origin of replication (*oriC*) which contains multiple binding sites for the initiator protein DnaA (a highly homologous protein amongst bacterial kingdom). DnaA has four domains with each domain responsible for a specific task. There are 11 DnaA binding sites/boxes on the *E. coli* origin of replication out of which three boxes R1, R2 and R4 (which have a highly conserved 9 bp consensus sequence 5' - TTATC/ACACA) are high affinity DnaA boxes. They bind to DnaA-ADP and DnaA-ATP with equal affinities and are bound by DnaA throughout most of the cell cycle and forms a scaffold on which rest of the orisome assembles. The rest eight DnaA boxes are low affinity sites that preferentially bind to DnaA-ATP. During initiation, DnaA bound to high affinity DnaA box R4 donates additional DnaA to the adjacent low affinity site and progressively fill all the low affinity DnaA boxes. Filling of the sites changes origin conformation from its native state. It is hypothesized that DNA stretching by DnaA bound to the origin promotes strand separation which allows more DnaA to bind to the unwound region. The DnaC helicase loader then interacts with the DnaA bound to the single-stranded DNA to recruit the DnaB helicase, which will continue to unwind the DNA as the DnaG primase lays down an RNA primer and DNA Polymerase III holoenzyme begins elongation.



Steps in origin activation and replisome assembly in bacteria

Regulation

Chromosome replication in bacteria is regulated at the initiation stage. DnaA-ATP is hydrolyzed into the inactive DnaA-ADP by RIDA (Regulatory Inactivation of DnaA), and converted back to the active DnaA-ATP form by DARS (DnaA Reactivating Sequence, which is itself regulated by Fis and IHF). However, the main source of DnaA-ATP is synthesis of new molecules. Meanwhile, several other proteins interact directly with the *oriC* sequence to regulate initiation, usually by inhibition. In *E. coli* these proteins include DiaA, SeqA, IciA, HU, and ArcA-P, but they vary across other bacterial species. A few other mechanisms in *E. coli* that variously regulate initiation are DDAH (*datA*-Dependent DnaA Hydrolysis, which is also regulated by IHF), inhibition of the *dnaA* gene (by the SeqA protein), and reactivation of DnaA by the lipid membrane.

Elongation

Once priming is complete, DNA polymerase III holoenzyme is loaded into the DNA and replication begins. The catalytic mechanism of DNA polymerase III involves the use of two metal ions in the active site, and a region in the active site that can discriminate between deoxyribonucleotides and ribonucleotides. The metal ions are general divalent cations that help the 3' OH initiate a nucleophilic attack onto the alpha phosphate of the deoxyribonucleotide and orient and stabilize the negatively charged triphosphate on the deoxyribonucleotide. Nucleophilic attack by the 3' OH on the alpha phosphate releases pyrophosphate, which is then subsequently

hydrolyzed (by inorganic phosphatase) into two phosphates. This hydrolysis drives **DNA synthesis to completion.**

Furthermore, DNA polymerase III must be able to distinguish between correctly paired bases and incorrectly paired bases. This is accomplished by distinguishing Watson-Crick base pairs through the use of an active site pocket that is complementary in shape to the structure of correctly paired nucleotides. This pocket has a tyrosine residue that is able to form van der Waals interactions with the correctly paired nucleotide. In addition, dsDNA (double stranded DNA) in the active site has a wider major groove and shallower minor groove that permits the formation of hydrogen bonds with the third nitrogen of purine bases and the second oxygen of pyrimidine bases. Finally, the active site makes extensive hydrogen bonds with the DNA backbone. These interactions result in the DNA polymerase III closing around a correctly paired base. If a base is inserted and incorrectly paired, these interactions could not occur due to disruptions in hydrogen bonding and van der Waals interactions.

DNA is read in the 3' → 5' direction, therefore, nucleotides are synthesized (or attached to the template strand) in the 5' → 3' direction. However, one of the parent strands of DNA is 3' → 5' while the other is 5' → 3'. To solve this, replication occurs in opposite directions. Heading towards the replication fork, the leading strand is synthesized in a continuous fashion, only requiring one primer. On the other hand, the lagging strand, heading away from the replication fork, is synthesized in a series of short fragments known as Okazaki fragments, consequently requiring many primers. The RNA primers of Okazaki fragments are subsequently degraded by RNase H and DNA Polymerase I (exonuclease), and the gaps (or nicks) are filled with deoxyribonucleotides and sealed by the enzyme ligase.

Rate of replication

The rate of DNA replication in a living cell was first measured as the rate of phage T4 DNA elongation in phage-infected E. coli. During the period of exponential DNA increase at 37°C, the rate was 749 nucleotides per second. The mutation rate per base pair per replication during phage T4 DNA synthesis is 1.7 per 10^8.

Termination

Termination of DNA replication in *E. coli* is completed through the use of termination sequences and the Tus protein. These sequences allow the two replication forks to pass through in only one direction, but not the other.

DNA replication initially produces two catenated or linked circular DNA duplexes, each comprising one parental strand and one newly synthesised strand (by nature of semiconservative replication). This catenation can be visualised as two interlinked rings which cannot be separated. Topoisomerase 2 in *E. coli* unlinks or decatenates the two circular DNA duplexes by breaking the phosphodiester

bonds present in two successive nucleotides of either parent DNA or newly formed DNA and thereafter the ligating activity ligates that broken DNA strand and so the two DNA get formed.

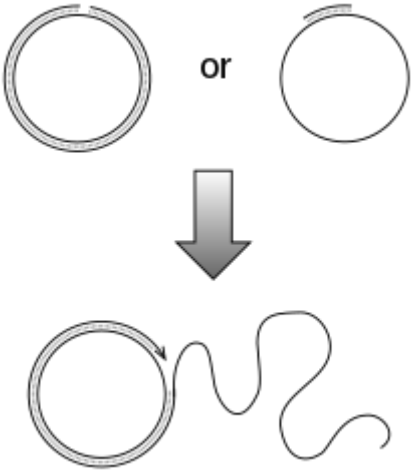
Other Prokaryotic replication models:

The theta type replication has been already mentioned. There are other types of prokaryotic replication such as rolling circle replication and D-loop replication

Rolling Circle Replication

This is seen in bacterial conjugation where the same circulartemplate DNA rotates and around it the new strand develops.

Rolling circle replication



When conjugation is initiated by a signal the **relaxase** enzyme creates a nick in one of the strands of the conjugative plasmid at the *oriT*. Relaxase may work alone or in a complex of over a dozen proteins known collectively as a **relaxosome**. In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF. The nicked strand, or *T-strand*, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated either independent of conjugative action (vegetative replication beginning at the *oriV*) or in concert with conjugation (conjugative replication similar to the rolling circle replication of lambda phage). Conjugative replication may require a second nick before successful transfer can occur. A recent report claims to have inhibited conjugation with chemicals that mimic an intermediate step of this second nicking event.

D-loop replication

D-loop replication is mostly seen in organellar DNA, Where a triple stranded structure called displacement loop is formed.

Cell Cycle, and Cell Growth:

The bacterial cell cycle is traditionally divided into three stages: the period between division (cell 'birth') and the initiation of chromosome replication (known as the B period); the period required for

replication (known as the C period); and the time between the end of replication and completion of division (known as the D period) (FIG. 1). In the enteric organism Escherichia coli and the spore former Bacillus subtilis, DNA replication begins at a single origin (oriC) on a single circular chromosome. Replication proceeds bidirectionally around the circumference of the chromosome, terminating at a region opposite oriC. During replication the chromosome remains in a condensed, highly ordered structure that is known as the nucleoid. Division is initiated near the end of chromosome segregation by the formation of a cytokinetic ring at the nascent division site. The tubulin-like protein FtsZ serves as the foundation for assembly of this ring and is required for recruitment of the division machinery. Nutrient availability and growth rate could potentially affect any of the above steps.

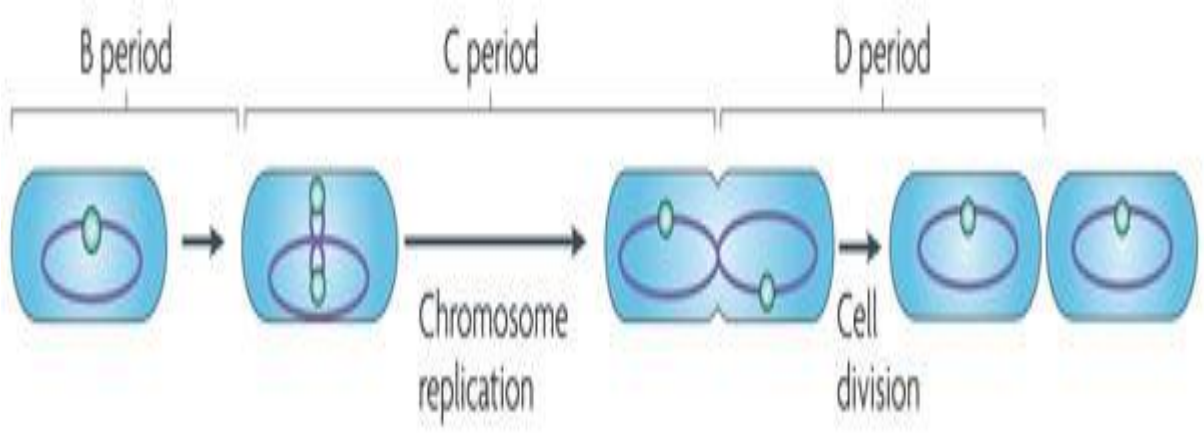
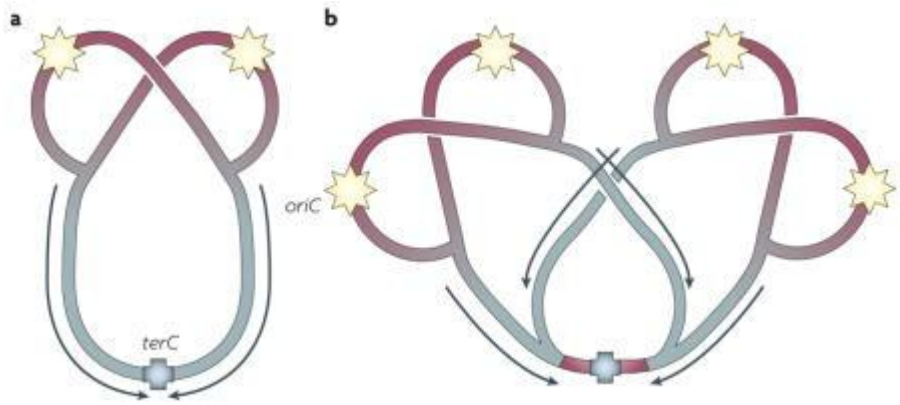


Figure 1 The bacterial cell cycle
The bacterial cell cycle is traditionally divided into three stages: the period between division (birth) and the initiation of chromosome replication (the B period); the period required for chromosome replication (the C period); and the time between the completion of chromosome replication and the completion of cell division (the D period). The bacterial cells (in this case, Escherichia coli) are outlined in black and contain highly schematic chromosomes (purple ovals) with oriC regions shown as green circles.

The bacterial cell cycle under different growth conditions derives largely from early physiological studies of B. subtilis and E. coli. These studies indicated that, at constant temperature, mass doubling time decreases in response to increases in nutrient availability; however, both the C period and the D period remain essentially constant. Consequently, under nutrient-rich conditions, both E. coli and B. subtilis reach growth rates at which the period required for chromosome replication and segregation is greater than the mass doubling time. To resolve this paradox, rapidly growing cells initiate new rounds of chromosome replication before completing the previous round, a situation that results in two, four or even eight rounds of replication proceeding simultaneously. This phenomenon, which was first discovered in B. subtilis and termed ‘multifork replication’ (REF. 6), was formalized and further investigated by Cooper and Helmstetter in their influential 1968 paper5 (BOX 1).

Notably, Cooper and Helmstetter's work illuminated how cells balance largely constant rates of replication fork progression with nutrient-dependent changes in mass doubling time, by initiating replication and dividing more frequently when growing faster.



Cooper and Helmstetter's model

Replication during slow growth

In slow-growing bacterial cells (with a mass doubling time >C + D period), there is a single round of replication per division cycle. This type of growth resembles that of eukaryotes in that there is a gap (the B period), a period in which DNA replication takes place (the C period) and finally a period of chromosome segregation and cell division (the D period). During replication each cell has only two copies of the origin region (*oriC*) and one copy of the terminus (*terC*) (see the figure, part **a**).

Replication during fast growth (multifork replication)

In rapidly growing cells (with a mass doubling time ≤C + D period), each chromosome re-initiates a new round of replication before the first round has terminated, although only one round is initiated per cell division. Multifork replication ensures that at least one round of replication is finished before cytokinesis, to guarantee that each daughter cell receives at least one complete genome. During multifork replication cells can have four or more copies of the region proximal to *oriC* and one copy of the region proximal to *terC* (see the figure, part **b**). This imbalance has implications for gene expression levels as well as for the activity of the initiator protein DnaA.

Although arguably one of the most important insights in the field in the past 40 years, Cooper and Helmstetter's model is limited in that it views the cell cycle as a single process, in which replication initiation is the triggering event that determines the timing of all subsequent steps in replication and cell division. This view does not take into consideration the effects of nutrients and metabolic status on events that occur after replication initiation, nor does it explain how cell cycle events are coordinated with mass doubling to ensure that new rounds of replication are initiated only once per division cycle and cell size homeostasis is maintained. Recent work suggests that, instead of being a

single process, the bacterial cell cycle is a set of coordinated but independent events. This more nuanced view is the model to which we subscribe.

Multifork replication is not a universal feature of the bacterial life cycle: the aquatic bacterium *Caulobacter crescentus* has temporally compartmentalized cell cycle stages, a situation analogous to the eukaryotic cell cycle. For simplicity, however, in this Review we treat *B. subtilis* and *E. coli* as representative Gram-positive and Gram-negative bacteria, respectively. In addition, we use the term 'division cycle' instead of cell cycle to refer to the period of time between the birth of a cell and its own subsequent division.

Cell division

Like replication, division must be coupled to growth to ensure that average cell size is maintained under a given growth condition. Cells that divided before they doubled in mass would, after several generations, become unsustainably small. Conversely, a population of cells that routinely divided a substantial time after they had doubled in mass would ultimately grow into filaments that are no longer viable. In addition to this temporal form of control, a second regulatory network ensures that cell size increases under carbon-rich conditions, allowing cells to maintain a constant DNA to cell mass ratio during multifork replication.

The temporal control of cell division

Precisely how division is coordinated with mass doubling is not clear. One possibility is that a factor analogous to DnaA accumulates in newborn cells, reaching the critical concentration only when cells have doubled in mass. To ensure that division is coordinated with mass doubling, the accumulation of such a factor would need to be dependent on nutrient availability and growth rate. Potential candidates include essential components of the division apparatus. However, none of the cell division genes identified to date, including *ftsZ*, seems to be regulated by growth rate in either *B. subtilis* or *E. coli*.

Alternatively, it has been proposed that replication — a process that is itself dependent on growth rate — serves as a checkpoint for division (FIG. 3a). In support of this idea, inhibiting the initiation of replication in *B. subtilis* through the use of conditional mutants leads to the formation of elongated cells with acentric cytokinetic rings adjacent to centrally positioned nucleoids. These cells experience a notable division delay due to DnaA-dependent repression of FtsL, a crucial component of the division machinery. FtsL is highly unstable and therefore DnaA-dependent transcriptional inhibition quickly leads to a decrease in FtsL levels and a severe division delay.

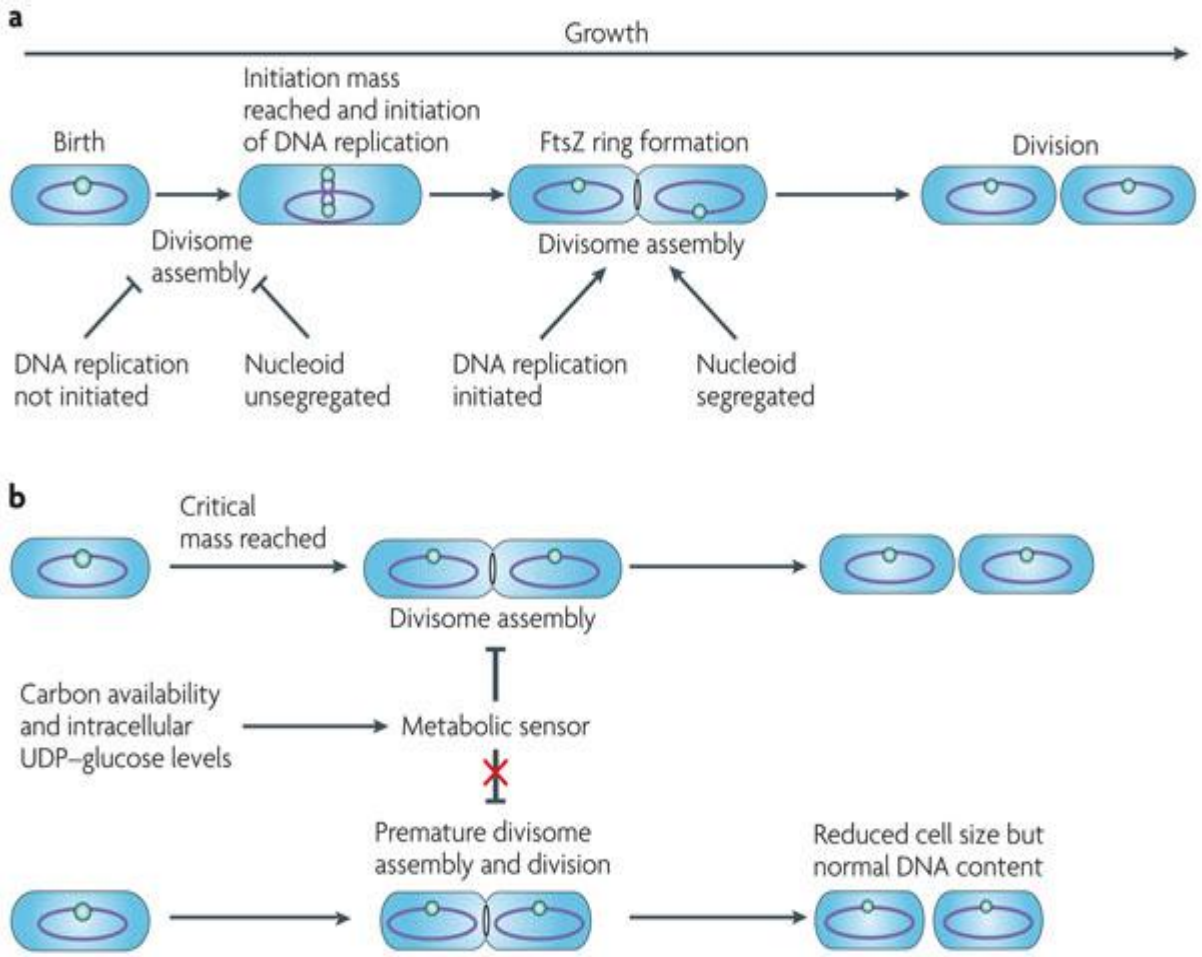


Figure 3 Spatial and temporal regulation of cell division is achieved through multiple layers of control

a| FtsZ assembly is coordinated with the initiation of DNA replication and nucleoid segregation to ensure that daughter cells receive complete copies of the bacterial genome. b| In Bacillus subtilis, the glucolipid biosynthesis pathway serves as a metabolic sensor to transmit information about carbon availability and growth rate, through the intracellular UDP–glucose concentration, to the division apparatus. When this sensor is functional (top), division is coupled to the achievement of a specific size (termed‘critical mass’) as well as to mass doubling time. When this sensor is defective (bottom), division is uncoupled from the achievement of critical mass but remains sensitive to mass doubling time, resulting in reduced cell size.

Coordinating cell size with nutrient availability

Carbon availability is the primary determinant of cell size for rapidly growing bacteria (FIG. 3b). In *B. subtilis* information about carbon availability is transmitted directly to the division apparatus by accumulation of the nucleotide sugar UDP–glucose. UDP–glucose inhibits division through its interaction with the bifunctional diacylglycerol glucosyltransferase, UgtP. Under conditions in which UDP–glucose is high, such as during growth in carbon-rich medium, UgtP inhibits FtsZ assembly and delays maturation of the cytokinetic ring until cells have reached the appropriate length. Under these conditions UgtP is distributed uniformly throughout the cytoplasm and localizes to the cytokinetic ring in an FtsZ-dependent manner, consistent with its role as a division inhibitor.

Conversely, under conditions in which UDP–glucose levels are low, such as during growth in carbon-poor medium, the intracellular concentration of UgtP drops and the remaining protein is sequestered away from mid-cell in small, randomly positioned foci. The exact mechanism underlying this change in UgtP concentration and localization remains to be determined. UgtP inhibits FtsZ assembly *in vitro*, indicating that it interacts directly with FtsZ to inhibit division. The molecular mechanism by which UgtP prevents FtsZ assembly has yet to be determined; possibilities include capping the growing polymers or preventing the formation of stabilizing lateral interactions.

Carbon-dependent increases in cell size ensure that cells have sufficient room to accommodate the extra DNA that is generated by multifork replication. Defects in UDP–glucose biosynthesis reduce cell size by ~30% under carbon-rich conditions (when cells have a mass doubling time of ~25 minutes) and lead to a 3.5-fold increase in the frequency of FtsZ rings that are assembled across unsegregated nucleoids; Noc does not seem to inhibit FtsZ assembly across unsegregated nucleoids in these cells. However, under carbon-poor conditions (when cells have a mass doubling time of ~80 minutes), defects in UDP–glucose biosynthesis have little effect on cell morphology, and FtsZ ring formation takes place across segregated or partially segregated nucleoids. Despite the reduction in cell size, the timing of FtsZ ring formation and division is still precisely coordinated with mass doubling in UDP–glucose-deficient cells. This observation implies that a second, UDP–glucose-independent pathway ensures that FtsZ assembly and division are coupled to cell growth.

E. coli cells also increase in size under carbon-rich conditions, and cells shifted from carbon-poor to carbon-rich medium rapidly increase their growth rate but delay division until they reach the appropriate size for the new conditions. Although UgtP is not conserved in *E. coli*, the accumulation of UDP–glucose does seem to be important for coordinating cell size with carbon availability. Studies indicate that mutations in two genes that are required for UDP–glucose biosynthesis, phosphoglucomutase (*pgm*) and glucose-1-phosphate uridylyltransferase (*galU*), reduce *E. coli* cell size by ~30% without substantially affecting mass doubling time (N.S. Hill and R.B. Weart, unpublished observations) (FIG. 3b). In the absence of a UgtP homologue, it is not clear what the UDP–glucose-sensitive effector is in *E. coli*, but on the basis of the data from *B. subtilis* it is reasonable to speculate that it is a UDP–glucose-binding protein. Systematic analysis of genes encoding such proteins should identify the effector responsible for coordinating nutrient availability with cell size in this Gram-negative organism.

•Plasmid replication:-

Double stranded, few kilo base self-replicating extra DNA fragments is known as “Plasmids” commonly recognized in different gram negative and positive bacterial strains as well as in some fungi including unicellular yeasts. Although plasmids are usually circular but linear plasmids have also been reported.

Though plasmid are not required for bacteria survival but encodes essential genetic determinants that enables bacteria to adapt and resist unfavorable conditions for better survival and to encounter external threats with other microbes occupying the same position in an ecological food chain. Replication mechanisms of plasmid are host specific and effects plasmid copy number. Plasmid replicon consists of one or more origin of replication (ori) and few regulatory elements such as Rep proteins, localized in the 4 kilo base region of the DNA fragment. In addition plasmid also possesses few essential genes that assist in DNA replication. The molecular mechanism of bacterial plasmid replication is similar to the origin of replication of E. coli chromosome.

Plasmid Replication

Bacterial plasmid replication is not dependant on its nuclear genome replication with long intermissions between replication proceedings occurring during the course of cell division. Definite plasmid copy number depends on plasmid type, host organism and the growth conditions. Unintended aberrations from normal copy number are attuned. However dominant and recessive copy mutants to the wild type do exist.

Plasmid replication mechanisms

There are three types of plasmid replication namely rolling circle, Col E1 type and Iteron contain replication

Rolling circle: Rolling circle replication mechanism is specific to bacteriophage family m13 and the fertility F factor which encodes for sex pili formation during recombination by means of conjugation. Fragments smaller than 10 kilo base usually replicate by this replication mechanism as reported in some gram positive bacteria. It allows the transfer of single stranded replication product at a faster rate to the recipient cell through pilus as in case of fertility factor or to the membrane in case of phage.

Mechanism: Rolling circle occurs to a covalently closed circular piece of double-stranded DNA. A nick is produced in one of the strands by enzyme nickases creating a 5’ phosphate and a 3’ hydroxyl. Free 3’ hydroxyl will be used by DNA polymerase to make new DNA pushing the old nicked strand off of the template DNA (**Figure 1**).

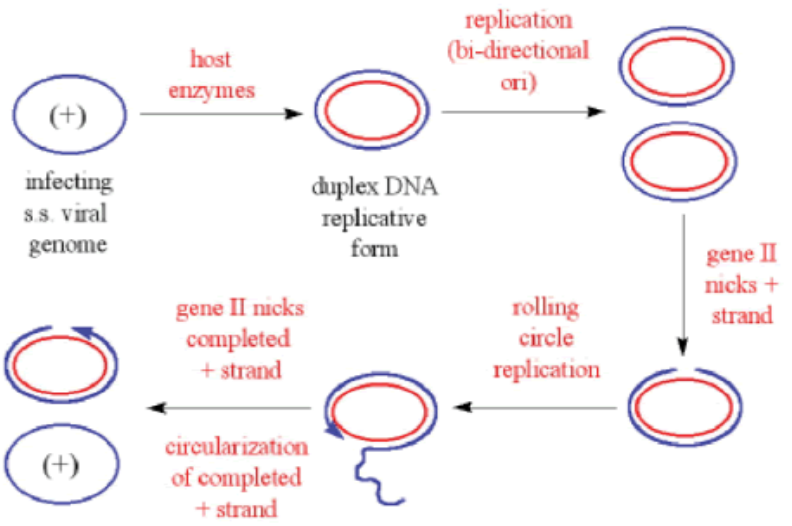


Figure 1: Mechanism of rolling circle replication.

Col E1 type replication: Col E1 replication is a negative regulation mechanism which enables the plasmid to control its own copy numbers by involving RNA type I, RNA type II, Rom protein, and the plasmid itself. Col E1 replication is initiated by means of RNA-RNA interactions and does not rely on replication initiation protein encoded by the plasmid to regulate its copy number.

Mechanism: RNA type II that originates 555 base pairs upstream from the replication origin of Col E1 plasmid is transcribed which marks the start of Col E1 replication. A determined hybrid with the DNA strand is formed by a loop enriched in G nucleotide positioned 290 of RNAII and a C-rich region on the template strand positioned 20 nucleotides upstream from the origin. Several stems and loops are exhibited by the newly formed secondary structure. A DNA/RNA hybrid is recognized by enzyme RNase and dissociates the RNA hybrid to the 3' end of RNAII. The resultant RNA primer is linked to the plasmid with a free 3' hydroxyl group. This RNA enables replication of DNA to begin by providing DNA polymerase a specific site to initiate nucleotides synthesis. Consequently DNA synthesis is commenced with the leading strand is happening (**Figure 2**).

Iteron-containing replicons: This replicon consists of a gene that encodes Rep protein for plasmid replication initiation, set of direct repeat sequences called iteron, adjacent AT-rich region and Dna boxes which is a protein required for bacterial chromosome replication initiation. However length of adjacent AT-rich region and number of iterons and DnaA boxes differs in a replicon.

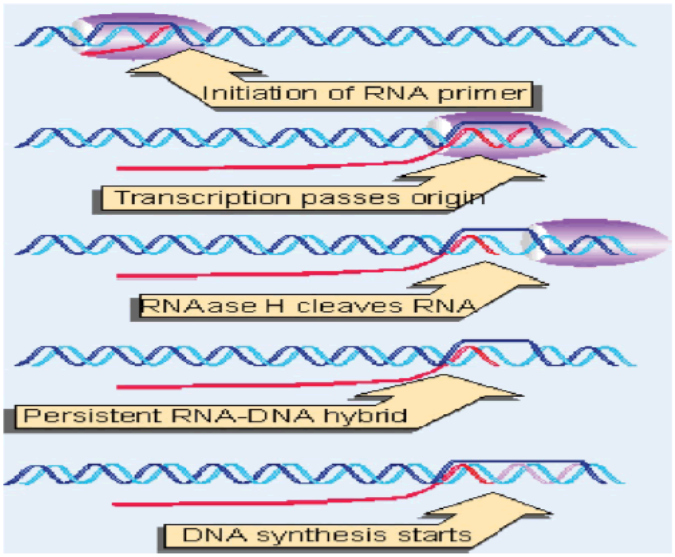


Figure 2: Mechanism of Col E1 type replication.

Mechanism: Iteron contain replication begins with the binding of Rep proteins to the iteron being organized in the same orientation of the DNA helix. And by binding to the DnaA boxes in the replicon the Rep-DnaA-DNA assembly promotes melting of the strand at the nearby AT-rich region to which host replication factors subsequently gain access and promote leading and lagging strand synthesis in a manner analogous to initiation of replication at the chromosomal origin, oriC (**Figure 3**).

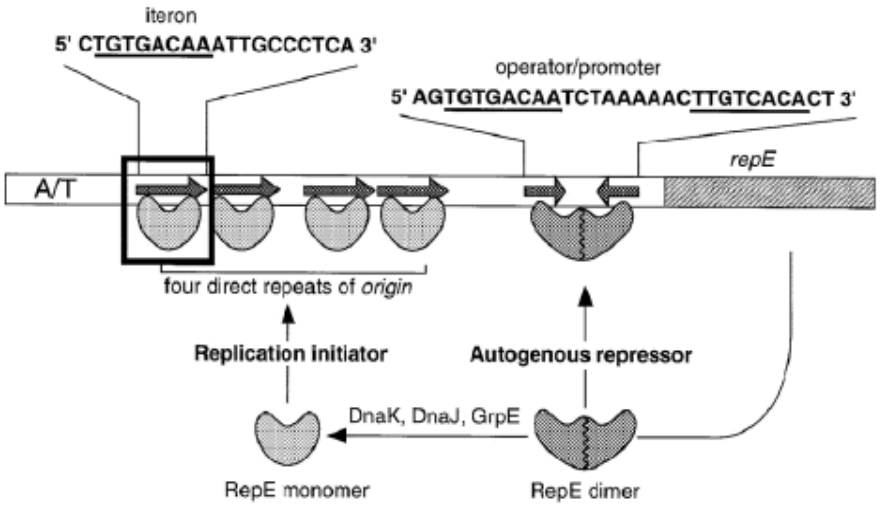


Figure 3: Mechanism of iteron contains replication.

Plasmids copy number is controlled principally at the beginning of replication initiation. The frequency with which initiation of replication of iteron-containing plasmids occurs is modulated in part by sequestration of the origin region in nucleoprotein complexes and intermolecular pairing of complexes on different plasmids, which is referred to as "handcuff".

Thus plasmid replication by means of rolling circle, Col E1 type and Iteron contain replication is an efficient way to control its copy number and compatibility in bacteria and other respective organisms.

•Prokaryotic transcription and translation.

Outline the process of prokaryotic transcription and translation

The prokaryotes, which include bacteria and archaea, are mostly single-celled organisms that, by definition, lack membrane-bound nuclei and other organelles. A bacterial chromosome is a covalently closed circle that, unlike eukaryotic chromosomes, is not organized around histone proteins. The central region of the cell in which prokaryotic DNA resides is called the nucleoid. In addition, prokaryotes often have abundant **plasmids**, which are shorter circular DNA molecules that may only contain one or a few genes. Plasmids can be transferred independently of the bacterial chromosome during cell division and often carry traits such as antibiotic resistance. Because of these unique features, transcription and gene regulation is somewhat different between prokaryotic cells and eukaryotic ones.

LEARNING OBJECTIVES

Understand the basic steps in the transcription of DNA into RNA in prokaryotic cells

Understand the basics of prokaryotic translation and how it differs from eukaryotic translation

Prokaryotic Transcription

Initiation of Transcription in Prokaryotes

Prokaryotes do not have membrane-enclosed nuclei. Therefore, the processes of transcription, translation, and mRNA degradation can all occur simultaneously. The intracellular level of a bacterial protein can quickly be amplified by multiple transcription and translation events occurring concurrently on the same DNA template. Prokaryotic transcription often covers more than one gene and produces polycistronic mRNAs that specify more than one protein.

Our discussion here will exemplify transcription by describing this process in *Escherichia coli*, a well-studied bacterial species. Although some differences exist between transcription in *E. coli* and transcription in archaea, an understanding of *E. coli* transcription can be applied to virtually all bacterial species.

Prokaryotic RNA Polymerase

Prokaryotes use the same RNA polymerase to transcribe all of their genes. In *E. coli*, the polymerase is composed of five polypeptide subunits, two of which are identical. Four of these subunits, denoted α, α, β, and β' comprise the polymerase **core enzyme**. These subunits assemble every time a gene is transcribed, and they disassemble once transcription is complete. Each subunit has a unique role; the two α-subunits are necessary to assemble the polymerase on the DNA; the β-subunit binds to the ribonucleoside triphosphate that will become part of the nascent "recently born" mRNA molecule; and the β' binds the DNA template strand.

The fifth subunit, σ, is involved only in transcription initiation. It confers transcriptional specificity such that the polymerase begins to synthesize mRNA from an appropriate initiation site. Without σ, the core enzyme would transcribe from random sites and would produce mRNA molecules that specified protein gibberish. The polymerase comprised of all five subunits is called the **holoenzyme**.

Prokaryotic Promoters

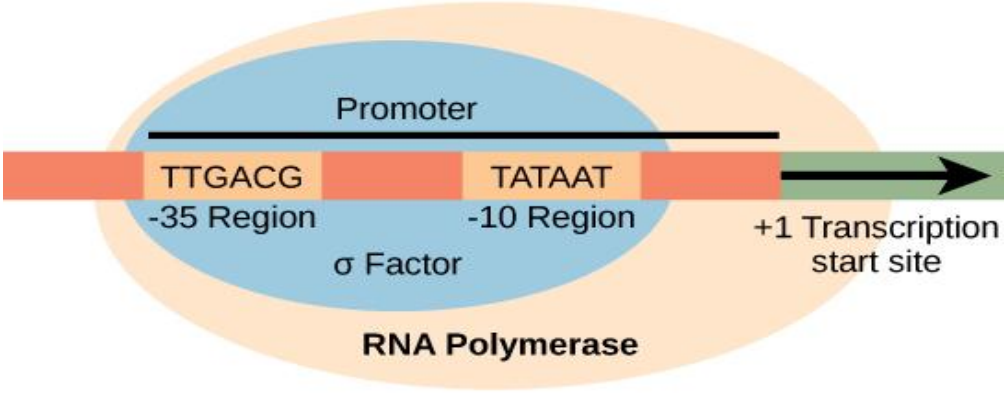


Figure 1. The σ subunit of prokaryotic RNA polymerase recognizes consensus sequences found in the promoter region upstream of the transcription start sight. The σ subunit dissociates from the polymerase after transcription has been initiated.

A **promoter** is a DNA sequence onto which the transcription machinery binds and initiates transcription. In most cases, promoters exist upstream of the genes they regulate. The specific sequence of a promoter is very important because it determines whether the corresponding gene is transcribed all the time, some of the time, or infrequently. Although promoters vary among prokaryotic genomes, a few elements are conserved. At the -10 and -35 regions upstream of the initiation site, there are two promoter **consensus** sequences, or regions that are similar across all promoters and across various bacterial species (Figure 1).

The -10 consensus sequence, called the -10 region, is TATAAT. The -35 sequence, TTGACA, is recognized and bound by σ. Once this interaction is made, the subunits of the core enzyme bind to the site. The A–T-rich -10 region facilitates unwinding of the DNA template, and several phosphodiester bonds are made. The transcription initiation phase ends with the production of abortive transcripts, which are polymers of approximately 10 nucleotides that are made and released.

Elongation and Termination in Prokaryotes

The transcription elongation phase begins with the release of the σ subunit from the polymerase. The dissociation of σ allows the core enzyme to proceed along the DNA template, synthesizing mRNA in the 5′ to 3′ direction at a rate of approximately 40 nucleotides per second. As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it (Figure 2). The base pairing between DNA and RNA is not stable enough to maintain the stability of the mRNA synthesis components. Instead, the RNA polymerase acts as a stable linker between the DNA template and the nascent RNA strands to ensure that elongation is not interrupted prematurely.

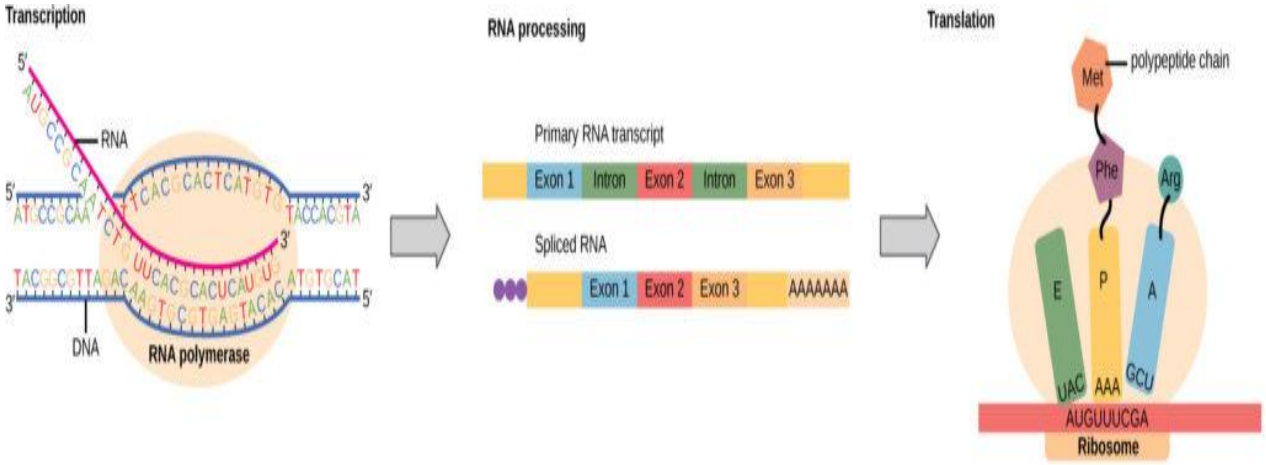


Figure 2. Click for a larger image. During elongation, the prokaryotic RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5′ to 3′ direction, and unwinds and rewinds the DNA as it is read.

Prokaryotic Termination Signals

Once a gene is transcribed, the prokaryotic polymerase needs to be instructed to dissociate from the DNA template and liberate the newly made mRNA. Depending on the gene being transcribed, there are two kinds of termination signals. One is protein-based and the other is RNA-based. **Rho-dependent termination** is controlled by the rho protein, which tracks along behind the polymerase on the growing mRNA chain. Near the end of the gene, the polymerase encounters a run of G nucleotides on the DNA template and it stalls. As a result, the rho protein collides with the polymerase. The interaction with rho releases the mRNA from the transcription bubble.

Rho-independent termination is controlled by specific sequences in the DNA template strand. As the polymerase nears the end of the gene being transcribed, it encounters a region rich in C–G nucleotides. The mRNA folds back on itself, and the complementary C–G nucleotides bind together.

The result is a stable **hairpin** that causes the polymerase to stall as soon as it begins to transcribe a region rich in A–T nucleotides. The complementary U–A region of the mRNA transcript forms only a weak interaction with the template DNA. This, coupled with the stalled polymerase, induces enough instability for the core enzyme to break away and liberate the new mRNA transcript.

Upon termination, the process of transcription is complete. By the time termination occurs, the prokaryotic transcript would already have been used to begin synthesis of numerous copies of the encoded protein because these processes can occur concurrently. The unification of transcription, translation, and even mRNA degradation is possible because all of these processes occur in the same 5′ to 3′ direction, and because there is no membranous compartmentalization in the prokaryotic cell (Figure 3). In contrast, the presence of a nucleus in eukaryotic cells precludes simultaneous transcription and translation.

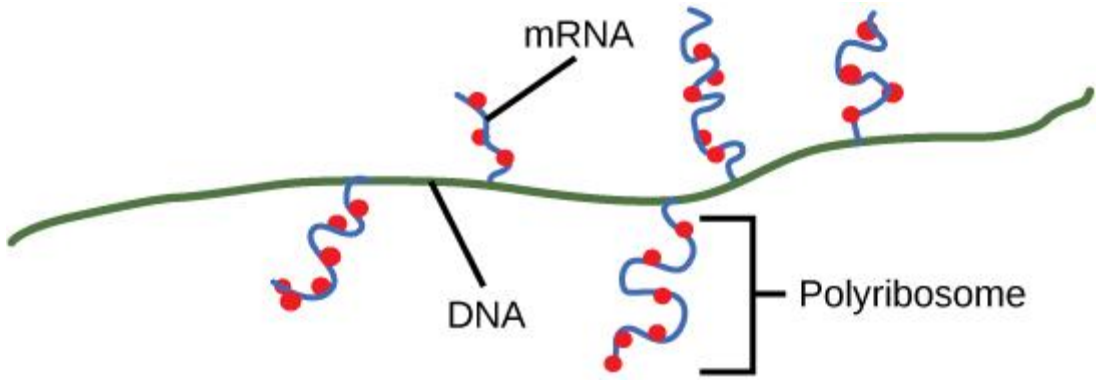


Figure 3. Multiple polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

Prokaryotic Translation

Translation is similar in prokaryotes and eukaryotes. Here we will explore how translation occurs in *E. coli*, a representative prokaryote, and specify any differences between bacterial and eukaryotic translation.

Initiation

The **initiation of protein synthesis** begins with the formation of an initiation complex. In ***E. coli***, this complex involves the small 30S ribosome, the mRNA template, three initiation factors that help the ribosome assemble correctly, guanosine triphosphate (GTP) that acts as an energy source, and a special initiator tRNA carrying ***N*-formyl-methionine** (fMet-tRNAf^{Met}) (Figure 4). The initiator tRNA interacts with the start codon AUG of the mRNA and carries a formylated methionine (fMet). Because of its involvement in initiation, fMet is inserted at the beginning (N terminus) of every polypeptide chain synthesized by *E. coli*.

In *E. coli* mRNA, a leader sequence upstream of the first AUG codon, called the **Shine-Dalgarno sequence** (also known as the ribosomal binding site AGGAGG), interacts through complementary base pairing with the rRNA molecules that compose the ribosome. This interaction anchors the 30S ribosomal subunit at the correct location on the mRNA template. At this point, the 50S ribosomal subunit then binds to the initiation complex, forming an intact ribosome.

In eukaryotes, initiation complex formation is similar, with the following differences:

- The initiator tRNA is a different specialized tRNA carrying methionine, called Met-tRNAi
- Instead of binding to the mRNA at the Shine-Dalgarno sequence, the eukaryotic initiation complex recognizes the 5′ cap of the eukaryotic mRNA, then tracks along the mRNA in the 5′ to 3′ direction until the AUG start codon is recognized. At this point, the 60S subunit binds to the complex of Met-tRNAi, mRNA, and the 40S subunit.

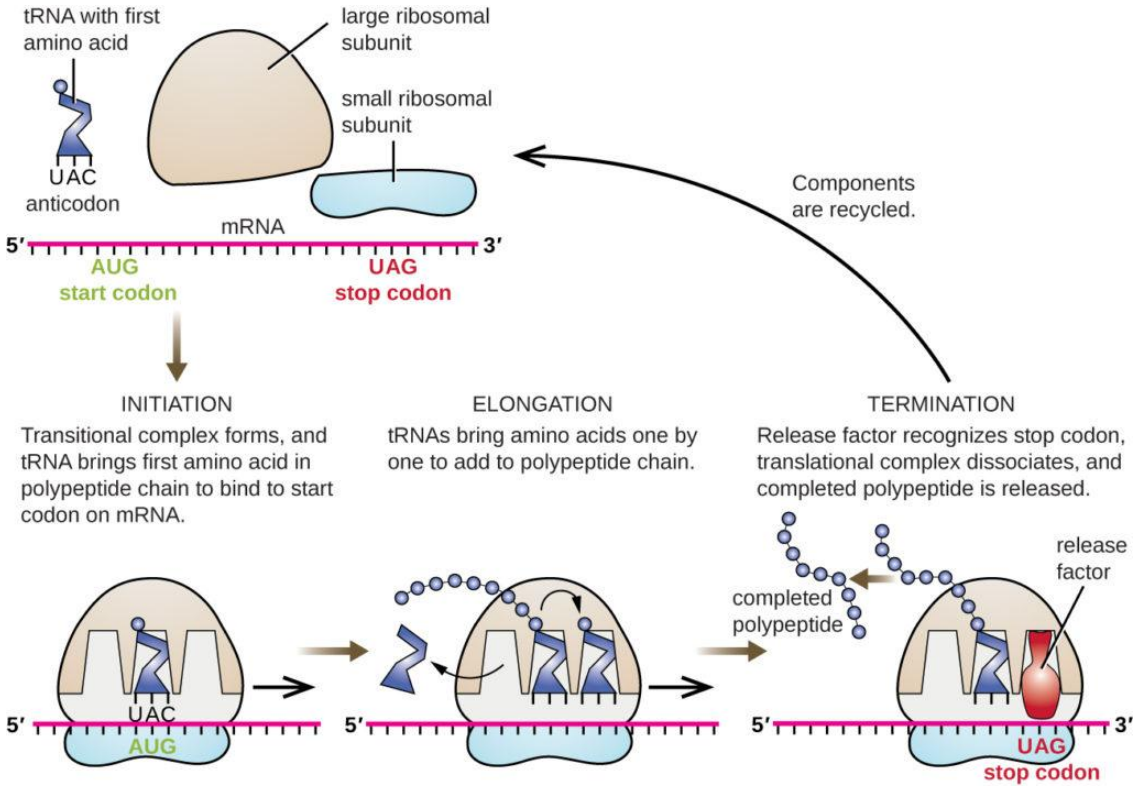


Figure 4. Translation in bacteria begins with the formation of the initiation complex, which includes the small ribosomal subunit, the mRNA, the initiator tRNA carrying N-formyl-methionine, and initiation factors. Then the 50S subunit binds, forming an intact ribosome.

Elongation

In prokaryotes and eukaryotes, the basics of **elongation of translation** are the same. In ***E. coli***, the binding of the 50S ribosomal subunit to produce the intact ribosome forms three functionally important ribosomal sites: The **A (aminoacyl) site** binds incoming charged aminoacyl tRNAs. The **P (peptidyl) site** binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA.

The **E (exit) site** releases dissociated tRNAs so that they can be recharged with free amino acids. There is one notable exception to this assembly line of tRNAs: During initiation complex formation, bacterial fMet−tRNA^{fMet} or eukaryotic Met-tRNAi enters the P site directly without first entering the A site, providing a free A site ready to accept the tRNA corresponding to the first codon after the AUG.

Elongation proceeds with single-codon movements of the ribosome each called a translocation event. During each translocation event, the charged tRNAs enter at the A site, then shift to the P site, and then finally to the E site for removal. Ribosomal movements, or steps, are induced by conformational changes that advance the ribosome by three bases in the 3′ direction. Peptide bonds form between the amino group of the amino acid attached to the A-site tRNA and the carboxyl group of the amino acid attached to the P-site tRNA. The formation of each peptide bond is catalyzed by **peptidyl transferase**, an RNA-based ribozyme that is integrated into the 50S ribosomal subunit. The amino acid bound to the P-site tRNA is also linked to the growing polypeptide chain. As the ribosome steps across the mRNA, the former P-site tRNA enters the E site, detaches from the amino acid, and is expelled. Several of the steps during elongation, including binding of a charged aminoacyl tRNA to the A site and translocation, require energy derived from GTP hydrolysis, which is catalyzed by specific elongation factors. Amazingly, the *E. coli* translation apparatus takes only 0.05 seconds to add each amino acid, meaning that a 200 amino-acid protein can be translated in just 10 seconds.

Termination

The **termination of translation** occurs when a **nonsense codon** (UAA, UAG, or UGA) is encountered for which there is no complementary tRNA. On aligning with the A site, these nonsense codons are recognized by release factors in prokaryotes and eukaryotes that result in the P-site amino acid detaching from its tRNA, releasing the newly made polypeptide. The small and large ribosomal subunits dissociate from the mRNA and from each other; they are recruited almost immediately into another translation init iation complex.

In summary, there are several key features that distinguish prokaryotic gene expression from that seen in eukaryotes. These are illustrated in Figure 5 and listed in Table 1.

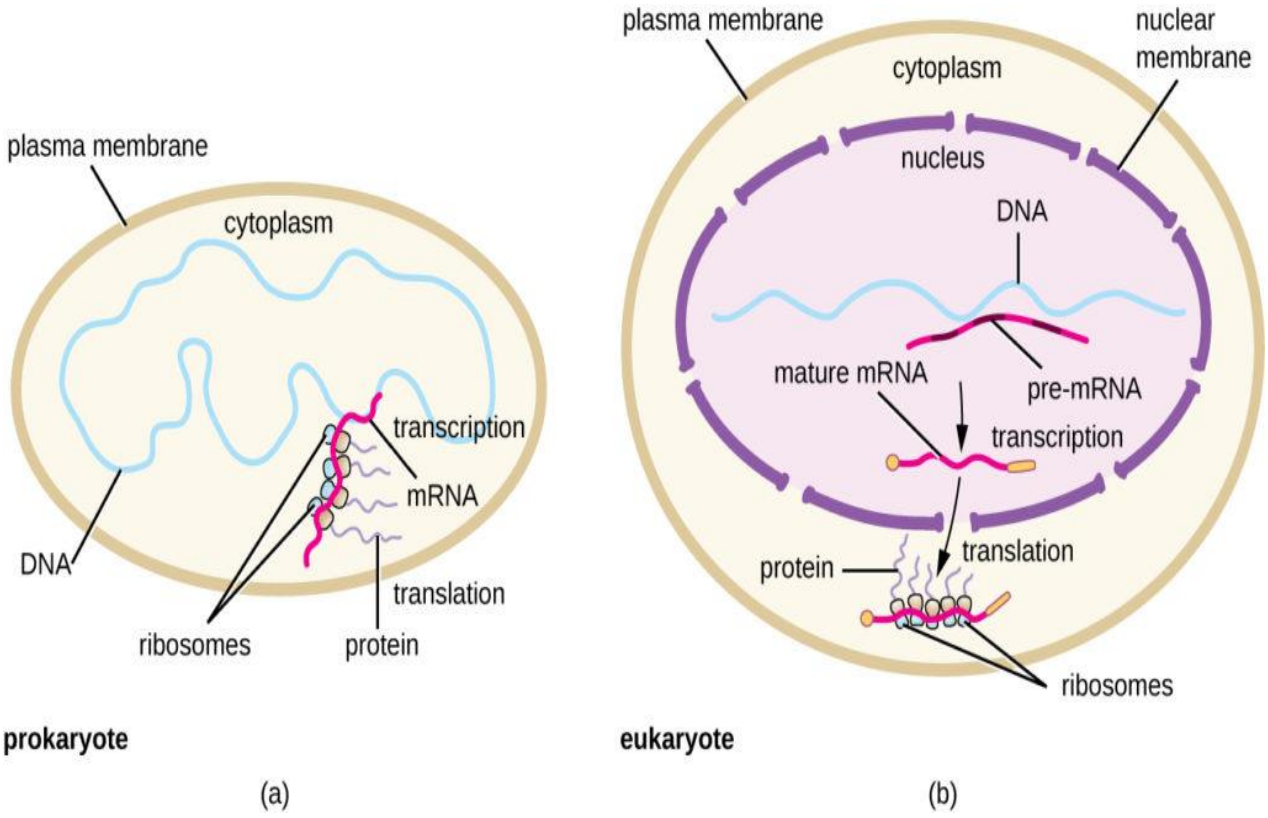


Figure 5. (a) In prokaryotes, the processes of transcription and translation occur simultaneously in the cytoplasm, allowing for a rapid cellular response to an environmental cue. (b) In eukaryotes, transcription is localized to the nucleus and translation is localized to the cytoplasm, separating these processes and necessitating RNA processing for stability.

• Regulation of gene expression in prokaryotes

The DNA of prokaryotes is organized into a circular chromosome that resides in the cell's cytoplasm. Proteins that are needed for a specific function, or that are involved in the same biochemical pathway, are often encoded together in blocks called **operons**. For example, all five of the genes needed to make the amino acid tryptophan in the bacterium *E. coli* are located next to each other in the *trp* operon. The genes in an operon are transcribed into a single mRNA molecule. This allows the genes to be controlled as a unit: either all are expressed, or none is expressed. Each operon needs only one regulatory region, including a **promoter**, where RNA polymerase binds, and an **operator**, where other regulatory proteins bind.

In prokaryotic cells, there are three types of regulatory molecules that can affect the expression of operons. **Activators** are proteins that increase the transcription of a gene. **Repressors** are proteins that suppress transcription of a gene.

Finally, **inducers** are molecules that bind to repressors and inactivate them. Below are two examples of how these molecules regulate different operons.

A) The *trp* Operon: A Repressor Operon

Like all cells, bacteria need amino acids to survive. Tryptophan is one amino acid that the bacterium *E. coli* can either ingest from the environment or synthesize. When E. coli needs to synthesize tryptophan, it must express a set of five proteins that are encoded by five genes. These five genes are located next to each other in the **tryptophan (*trp*) operon** (**Figure 17.3**).

When tryptophan is present in the environment, *E. coli* does not need to synthesize it, and the *trp* operon is switched off. However, when tryptophan availability is low, the *trp* operon is turned on so that the genes are transcribed, the proteins are made, and tryptophan can be synthesized.

A DNA sequence called the operator is located between the promoter and the first *trp* gene. The operator contains the DNA code to which the repressor protein can bind. The repressor protein is regulated by levels of tryptophan in the cell.

When tryptophan is present in the cell, two tryptophan molecules bind to the *trp* repressor. This causes the repressor to change shape and bind to the *trp* operator. Binding of the tryptophan–repressor complex at the operator physically blocks the RNA polymerase from binding, and transcribing the downstream genes. Thus, when the cell has enough tryptophan, it is preventing from making more.

When tryptophan is not present in the cell, the repressor has no tryptophan to bind to it. The repressor is not activated and it does not bind to the operator. Therefore, RNA polymerase can transcribe the operon and make the enzymes to synthesize tryptophan. Thus, when the cell does not have enough tryptophan, it synthesizes it.

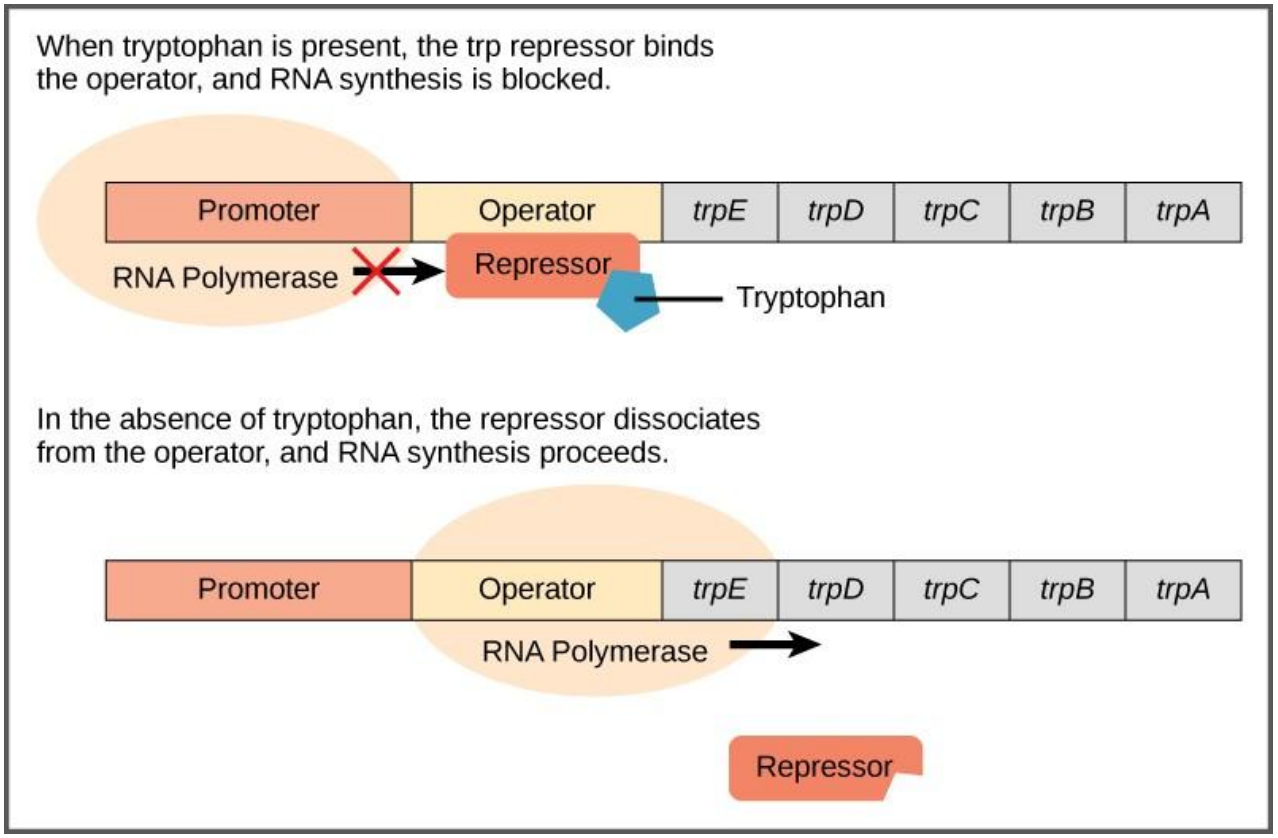


Figure 17.3 *The five genes that are needed to synthesize tryptophan in E. coli are located next to each other in the trp operon.*

B) The *lac* Operon: An Inducer Operon

The *lac* operon in *E. coli* has more complex regulation, involving both a repressor and an activator. *E. coli* uses glucose for food, but is able to use other sugars, such as lactose, when glucose concentrations are low. Three proteins are needed to break down lactose; they are encoded by the three genes of the *lac* operon.

When lactose is not present, the proteins to digest lactose are not needed. Therefore, a repressor binds to the operator and prevents RNA polymerase from transcribing the operon.

When lactose is present, lactose binds to the repressor and removes it from the operator. RNA polymerase is now free to transcribe the genes necessary to digest lactose (**Figure 17.4**).

However, the story is more complex than this. Since *E. coli* prefers to use glucose for food, the *lac* operon is only expressed at low levels even when the repressor is removed. But what happens when ONLY lactose is present? Now the bacterium needs to ramp up production of the lactose-digesting proteins. It does so by using an activator protein called catabolite activator protein (CAP).

When glucose levels drop, cyclic AMP (cAMP) begins to accumulate in the cell. cAMP binds to CAP and the complex binds to the *lac* operon promoter (**Figure 17.5**). This increases the binding ability of RNA polymerase to the promoter and ramps up transcription of the genes.

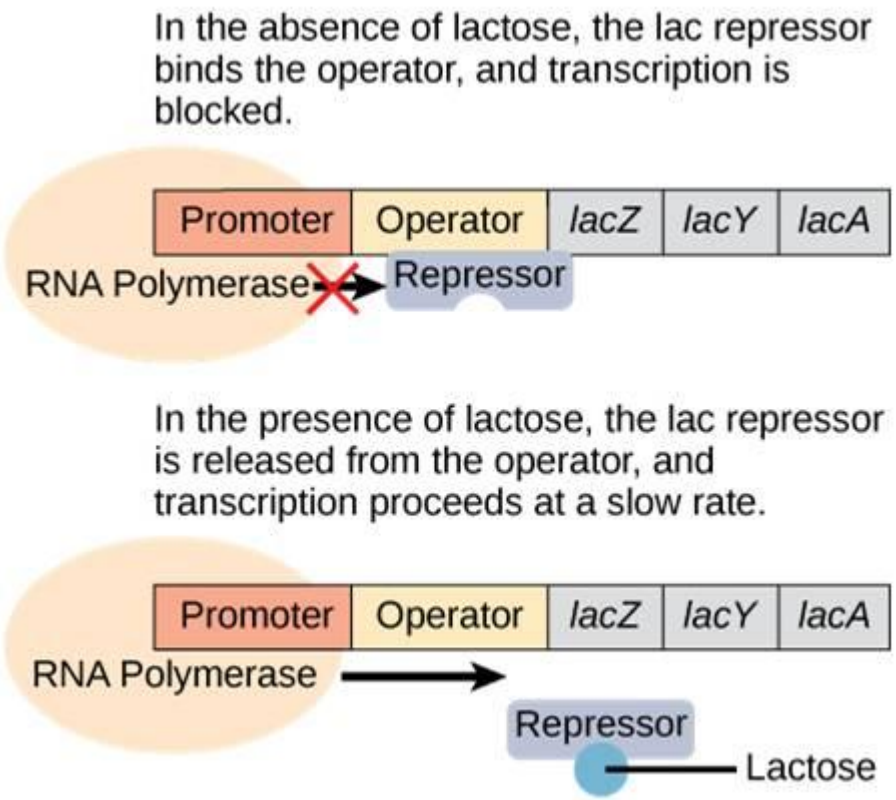


Figure 17.4 *Transcription of the lac operon only occurs when lactose is present. Lactose binds to the repressor and removes it from the operator.*

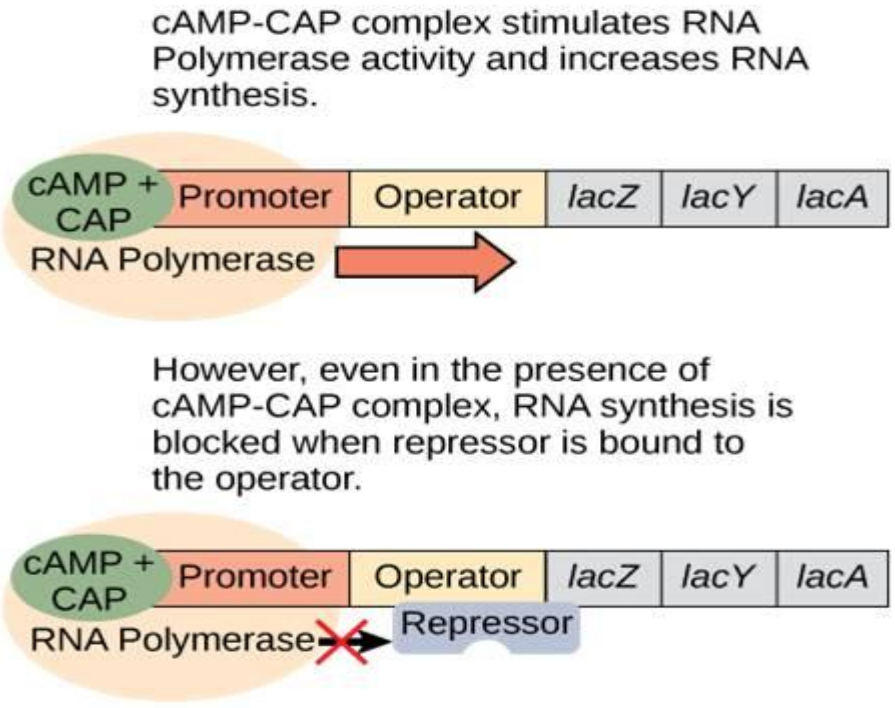


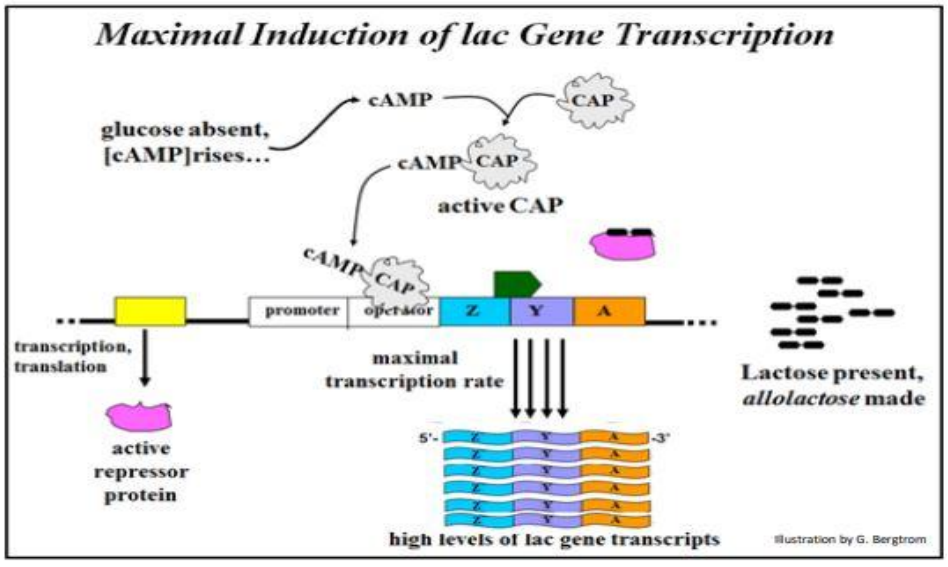
Figure 17.5 *When there is no glucose, the CAP activator increases transcription of the lac operon. However, if no lactose is present, the operon is not activated.*

In summary, for the *lac* operon to be fully activated, two conditions must be met. First, the level of glucose must be very low or non-existent. Second, lactose must be present. Only when glucose is absent and lactose is present will the *lac* operon be transcribed maximally. This makes sense for the cell, because it would be energetically wasteful to create the proteins to process lactose if glucose was plentiful or lactose was not available (**Table 17.2**).

Lactose present?	Repressor bound?	Glucose present?	CAP bound?	Transcription of *lac* operon?
-	Yes	+	No	No
-	Yes	-	Yes	No
+	No	+	No	Some
+	No	-	Yes	Lots

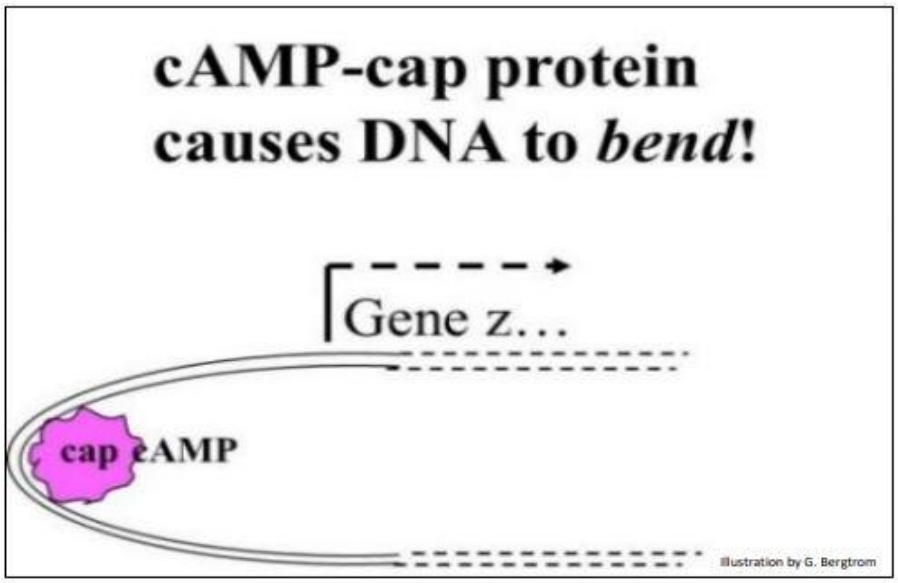
Positive Regulation of the Lac Operon; Induction by Catabolite Activation

The second control mechanism regulating lac operon expression is mediated by CAP (cAMP-bound **catabolite activator protein** or cAMP receptor protein). When glucose is available, cellular levels of cAMP are low in the cells and CAP is in an inactive conformation. On the other hand, if glucose levels are low, cAMP levels rise and bind to the CAP, activating it. If lactose levels are also low, the cAMP-bound CAP will have no effect. If lactose is present and glucose levels are low, then allolactose binds the lac repressor causing it to dissociate from the operator region. Under these conditions, the cAMP-bound CAP can bind to the operator in lieu of the repressor protein. In this case, rather than blocking the RNA polymerase, the activated Camp-bound CAP induces even more efficient lac operon transcription. The result is synthesis of higher levels of lac enzymes that facilitate efficient cellular use of lactose as an alternative to glucose as an energy source. Maximal *activation* of the lac operon in high lactose and low glucose is shown below.



Regulation of the Lac Operon- cAMP-bound CAP is an **inducer** of transcription. It does

this by forcing the DNA in the promoter-operator region to bend. And since bending the double helix loosens H-bonds, it becomes easier for RNA polymerase to find and bind the promoter on the DNA strand to be transcribed…, and for transcription to begin. cAMP-CAPinduced bending of DNA is illustrated below.

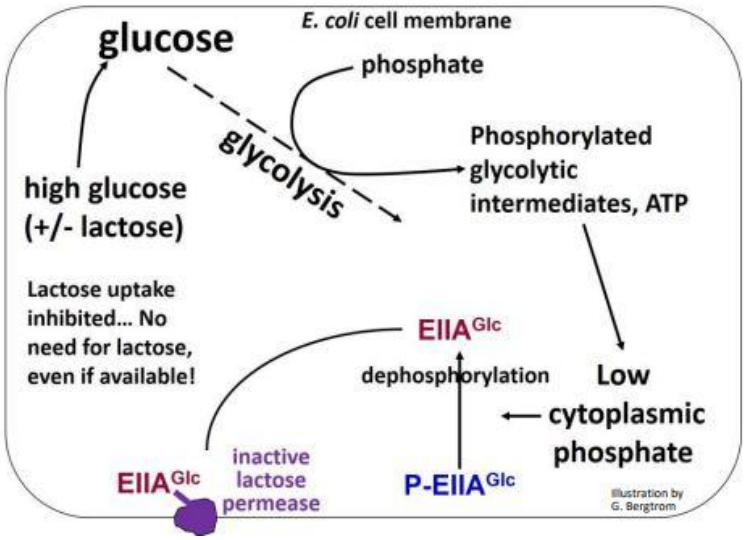


Lac Operon Regulation by Inducer Exclusion and Multiple Operators

In recent years, additional layers of lac operon regulation have been uncovered. In one case, the ability of *lac permease* to transport lactose across the cell membrane is regulated. In another, additional operator sequences have been discovered to interact with a multimeric repressor to control lac gene expression.

a) ***Regulation of Lactose use by Inducer Exclusion***

When glucose levels are high (even in the presence of lactose), phosphate is consumed to phosphorylate glycolytic intermediates, keeping cytoplasmic phosphate levels low. Under these conditions, unphosphorylated EIIAGlc binds to the *lactose permease* enzyme in the cell membrane, preventing it from bringing lactose into the cell. The role of phosphorylated and unphosphorylated $EIIA^{Glc}$ in regulating the lac operon are shown below.



High glucose levels block lactose entry into the cells, effectively preventing allolactose formation and the derepression of the lac operon. Inducer exclusion is thus a logical way for the cells to handle an abundance of glucose, whether or not lactose is present. On the other hand, if glucose levels are low in the growth medium, phosphate concentrations in the cells rise sufficiently for a specific kinase to phosphorylate the EIIAGlc. Phosphorylated EIIAGlc then undergoes an allosteric change and dissociates from the lactose permease, making it active so that more lactose can enter the cell. In other words, the inducer is not "excluded" under these conditions!

The kinase that phosphorylates $EIIA^{Glc}$ is part of a phosphoenolpyruvate (PEP)- *dependent phosphotransferase* system (PTS) cascade. When extracellular glucose levels are low, the cell activates the PTS system in an effort to bring whatever glucose is around into the cell. But the last enzyme in the PTS phosphorylation cascade is the kinase that phosphorylates $EIIA^{Glc}$. Phosphorylated $EIIA^{Glc}$ dissociates from the lactose permease, re-activating it, bringing available lactose into the cell from the medium.

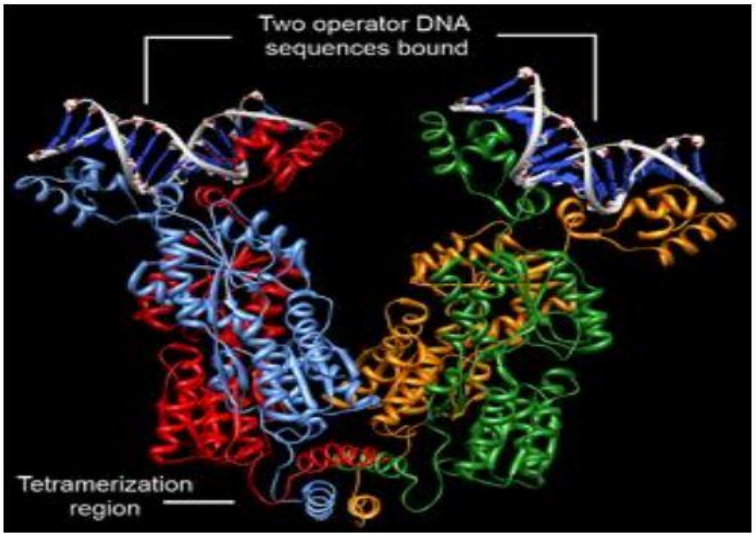
b) Repressor Protein Structure and Additional Operator Sequences

The lac repressor is a tetramer of identical subunits (below).

Each subunit contains a *helix-turn-helix* motif capable of binding to DNA. However, the operator DNA sequence downstream of the promoter in the operon consists of a pair of *inverted repeats* spaced apart in such a way that they can only interact two of the repressor subunits, leaving the function of the other two subunits unknown… that is, until recently!

Two more operator regions were recently characterized in the lac operon. One, called $\mathbf{O_2}$, is within the *lac z gene* itself and the other, called $\mathbf{O_3}$, lies near the end of, but within the *lac I* gene. Apart from their unusual location within actual genes, these operators, which interact with the remaining two repressor subunits, went undetected at first because mutations in the O2 or the O3 region individually do not contribute substantially to the effect of lactose in derepressing the lac operon.

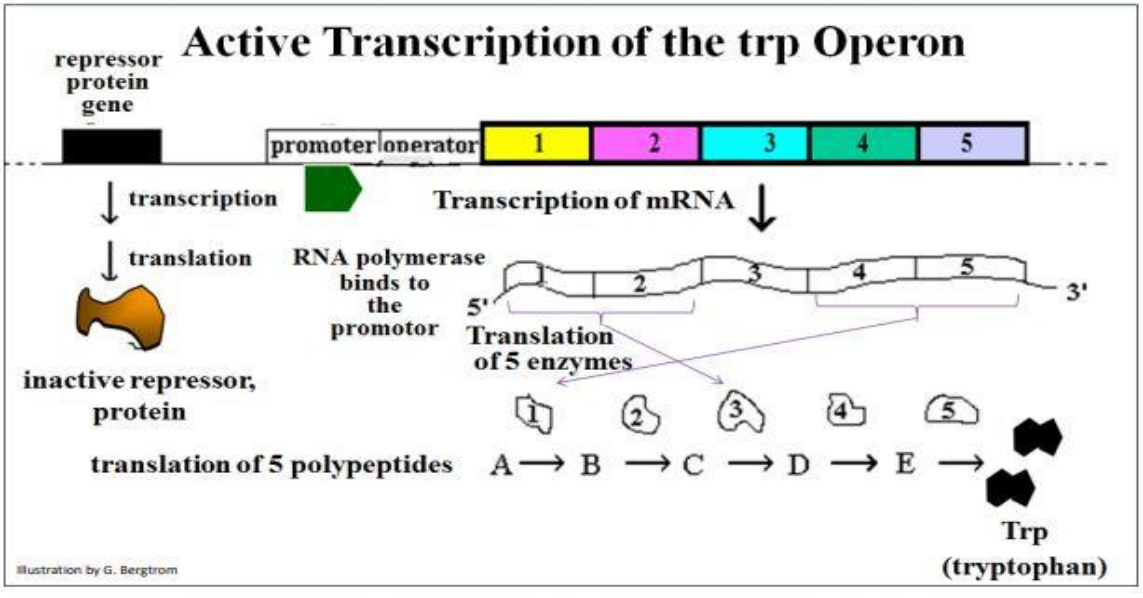
Only mutating both regions at the same time results in a substantial reduction in binding of the repressor to the operon.



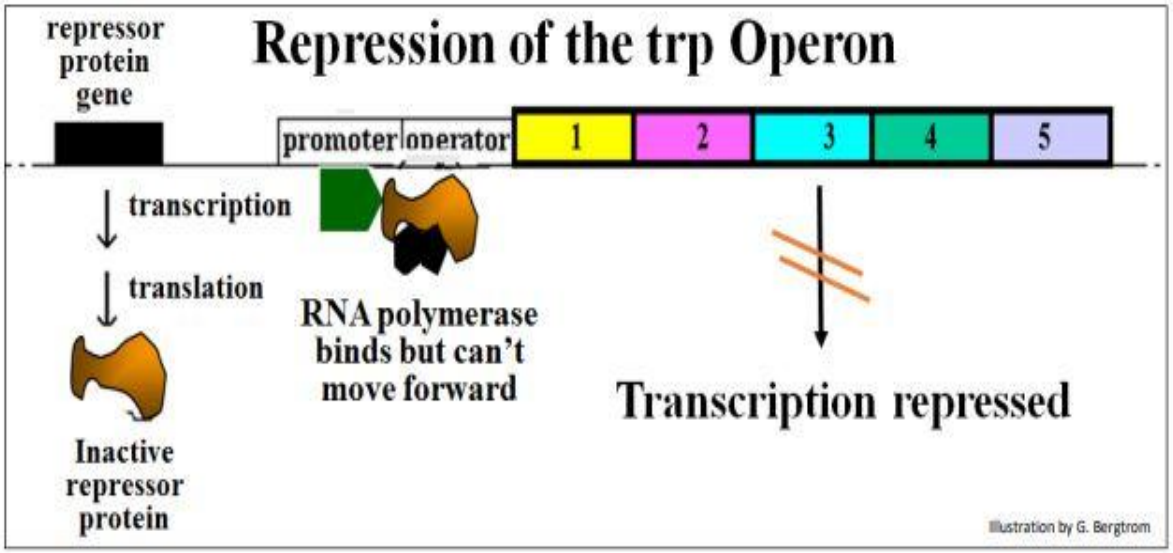
B. Mechanism of Control of the Tryptophan Operon

If ample tryptophan (**trp**) is available, the tryptophan synthesis pathway can be inhibited in two ways. First, recall how feedback inhibition by excess trp can allosterically inhibit the trp synthesis pathway. A rapid response occurs when tryptophan is present in excess, resulting in rapid feedback inhibition by blocking the first of five enzymes in the trp synthesis pathway. The *trp operon* encodes polypeptides that make up two of these enzymes.

Enzyme 1 is a *multimeric* protein, made from polypeptides encoded by the *trp5* and *trp4* genes. The trp1 and trp2 gene products make up **Enzyme 3**. If cellular tryptophan levels drop because the amino acid is rapidly consumed (e.g., due to demands for proteins during rapid growth), E. coli cells will continue to synthesize the amino acid, as illustrated below.



On the other hand, if tryptophan consumption slows down, tryptophan accumulates in the cytoplasm. Excess tryptophan will bind to the trp repressor. The trp-bound repressor then binds to the trp operator, blocking RNA polymerase from transcribing the operon. The repression of the trp operon by trp is shown below.



In this scenario, tryptophan is a **co-repressor**. The function of a co-repressor is to bind to a repressor protein and change its conformation so that it can bind to the operator.

• Genetic recombination in bacteria:

Bacteria are able to respond to selective pressures and adapt to new environments by acquiring new genetic traits as a result of mutation, a modification of gene function within a bacterium, and as a result of horizontal gene transfer, the acquisition of new genes from other bacteria. Mutation occurs relatively slowly. The normal mutation rate in nature is in the range of 10^{-6} to 10^{-9} per nucleotide per bacterial generation, although when bacterial populations are under stress, they can greatly increase their mutation rate. Furthermore, most mutations are harmful to the bacterium. Horizontal gene transfer, on the other hand, enables bacteria to respond and adapt to their environment much more rapidly by acquiring large DNA sequences from another bacterium in a single transfer.

Horizontal gene transfer, also known as lateral gene transfer, is a process in which an organism transfers genetic material to another organism that is not its offspring. The ability of *Bacteria* and *Archaea* to adapt to new environments as a part of bacterial evolution most frequently results from the acquisition of new genes through horizontal gene transfer rather than by the alteration of gene functions through mutations. (It is estimated that as much as 20% of the genome of *Escherichia coli* originated from horizontal gene transfer.)

Horizontal gene transfer is able to cause rather large-scale changes in a bacterial genome. For example, certain bacteria contain multiple virulence genes called pathogenicity islands that are

located on large, unstable regions of the bacterial genome. These pathogenicity islands can be transmitted to other bacteria by horizontal gene transfer. However, if these transferred genes provide no selective advantage to the bacteria that acquire them, they are usually lost by deletion. In this way the size of the bacterium's genome can remain approximately the same size over time.

There are three mechanisms of horizontal gene transfer in bacteria: transformation, transduction, and conjugation. The most common mechanism for horizontal gene transmission among bacteria, especially from a donor bacterial species to different recipient species, is conjugation. Although bacteria can acquire new genes through transformation and transduction, this is usually a more rare transfer among bacteria of the same species or closely related species.

Transformation

Transformation is a form of genetic recombination in which a DNA fragment from a dead, degraded bacterium enters a competent recipient bacterium and is exchanged for a piece of DNA of the recipient. Transformation usually involves only homologous recombination, a recombination of homologous DNA regions having nearly the same nucleotide sequences. Typically this involves similar bacterial strains or strains of the same bacterial species.

A few bacteria, such as *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Hemophilus influenzae*, *Legionella pneomophila*, *Streptococcus pneumoniae*, and *Helicobacter pylori* tend to be naturally competent and transformable. Competent bacteria are able to bind much more DNA than noncompetent bacteria. Some of these genera also undergo autolysis that then provides DNA for homologous recombination. In addition, some competent bacteria kill noncompetent cells to release DNA for transformation.

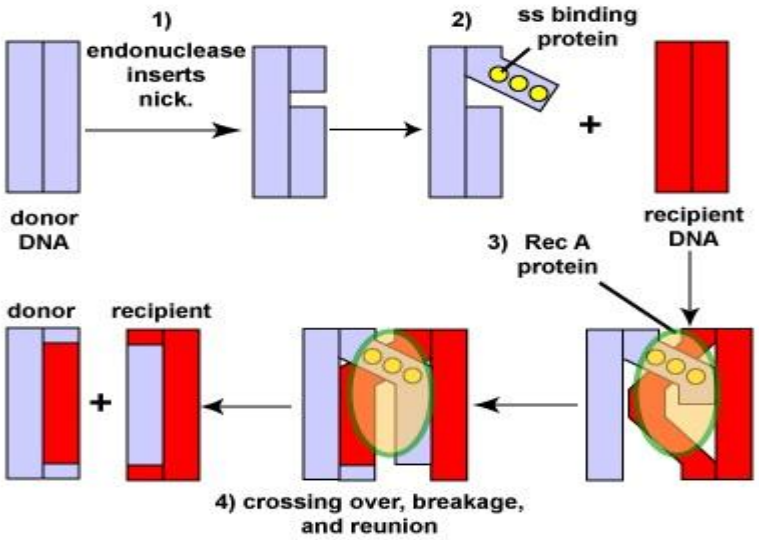
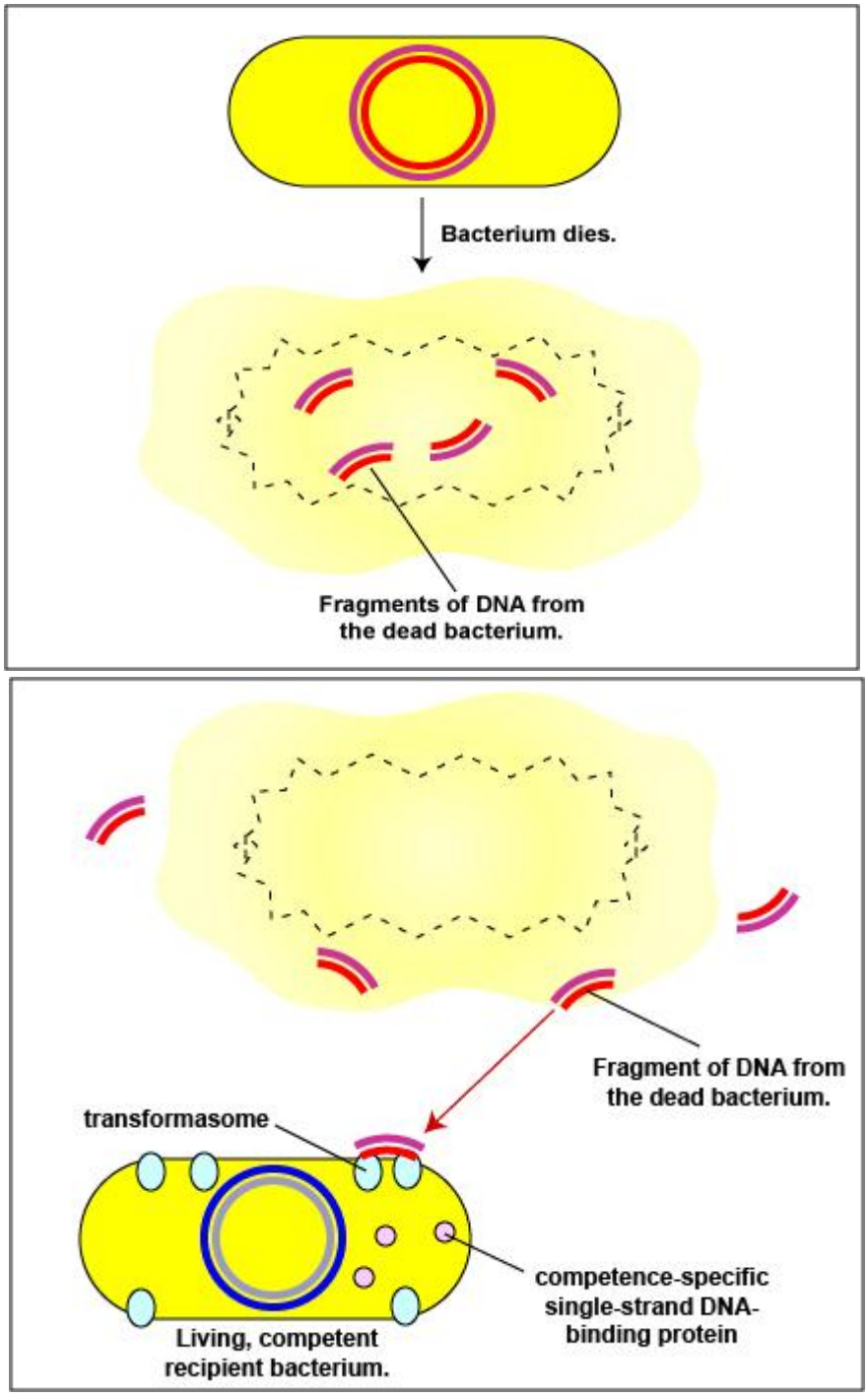


Figure 1. Pairing of Homologous DNA molecules and Exchange of DNA Segments by way of Rec A Protein. 1) A DNA endonuclease inserts a nick in one strand of the donor DNA. 2) The nicked strand is separated from its partner strand by proteins functioning as a helicase. Molecules of single-stranded binding protein (yellow) then bind. 3) Rec A protein then binds to the single-strand fragment and promotes base pairing of the donor DNA with the recipient DNA (crossing over). 4) The linked molecules are separated by resolvases, enzymes that cut and rejoin the cross-linked DNA molecules.

During transformation, DNA fragments (usually about 10 genes long) are released from a dead degraded bacterium and bind to DNA binding proteins on the surface of a competent living recipient bacterium. Depending on the bacterium, either both strands of DNA penetrate the recipient, or a nuclease degrades one strand of the fragment and the remaining DNA strand enters the recipient. This DNA fragment from the donor is then exchanged for a piece of the recipient's DNA by means of RecA proteins and other molecules and involves breakage and reunion of the paired DNA segments as seen in (Figure 1.). Transformation is summarized in Figure 1.



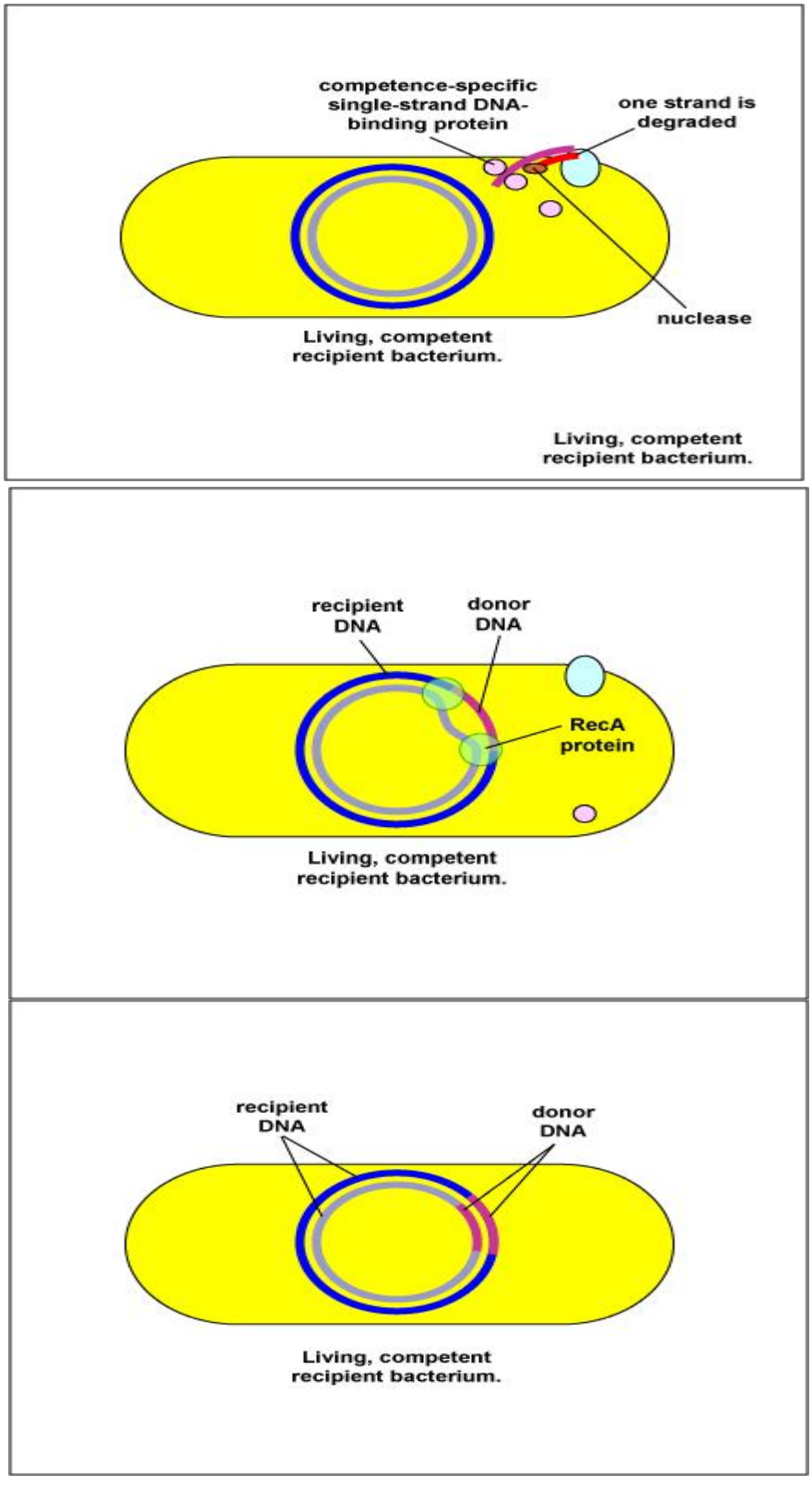


Figure **2*:*** Transformation: Step 1: A donor bacterium dies and is degraded.Step 2: DNA fragments, typically around 10 genes long, from the dead donor bacterium bind to transformasomes on the cell wall of a competent, living recipient bacterium.Step 3: In this example, a nuclease degrades one strand of the donor fragment and the remaining DNA strand enters the recipient. Competence-specific single-stranded DNA-binding proteins bind to the donor DNA strand to prevent it from being degraded in the cytoplasm. Step 4: RecA proteins promotes genetic exchange between a fragment of the donor's DNA and the recipient's DNA. This involves breakage and reunion of paired DNA segments. Step 5: Transformation is complete.

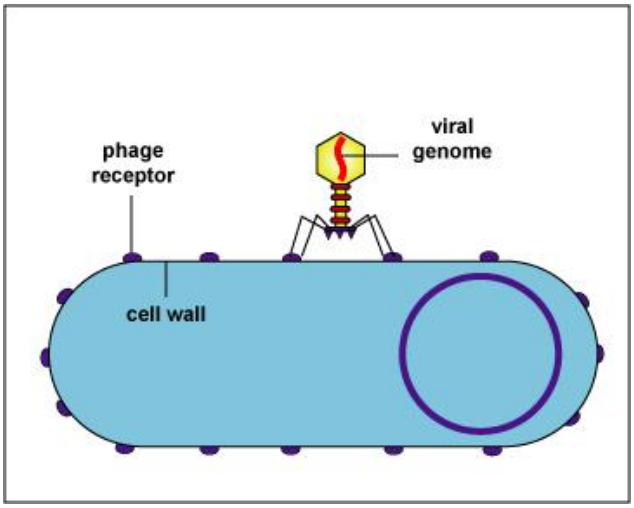
Transduction

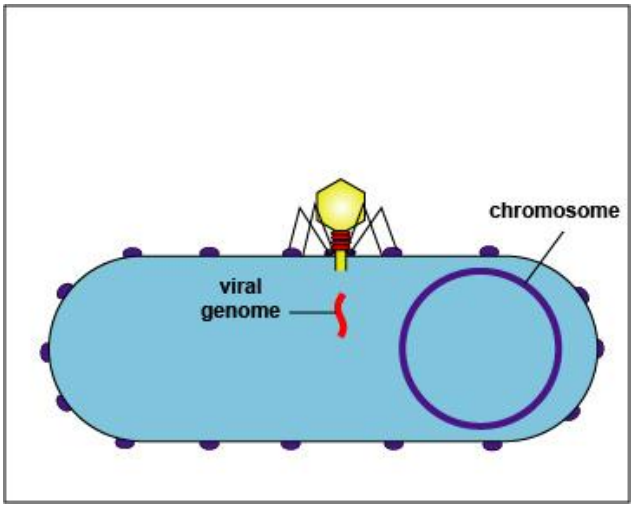
Transduction involves the transfer of a DNA fragment from one bacterium to another by a bacteriophage. There are two forms of transduction: **generalized transduction** and **specialized transduction**.

During the replication of *lytic bacteriophages* and *temperate bacteriophages*, occasionally the phage capsid accidently assembles around a small fragment of bacterial DNA. When this bacteriophage, called a transducing particle, infects another bacterium, it injects the fragment of donor bacterial DNA it is carrying into the recipient where it can subsequently be exchanged for a piece of the recipient's DNA by homologous recombination. Generalized transduction is summarized in Figure 3.

- **Step 1**: A bacteriophage adsorbs to a susceptible bacterium.
- **Step 2**: The bacteriophage genome enters the bacterium. The genome directs the bacterium's metabolic machinery to manufacture bacteriophage components and enzymes. Bacteriophage-coded enzymes will also breakup the bacterial chromosome.
- **Step 3:** Occasionally, a bacteriophage capsid mistakenly assembles around either a fragment of the donor bacterium's chromosome or around a plasmid instead of around a phage genome.
- **Step 4**: The bacteriophages are released as the bacterium is lysed. Note that one bacteriophage is carrying a fragment of the donor bacterium's DNA rather than a bacteriophage genome.
- **Step 5:** The bacteriophage carrying the donor bacterium's DNA adsorbs to a recipient bacterium.
- **Step 6:** The bacteriophage inserts the donor bacterium's DNA it is carrying into the recipient bacterium.
- **Step 7:** Homologous recombination occurs and the donor bacterium's DNA is exchanged for some of the recipient's DNA. (Figure 3.3.1 shows the functions of the RecA proteins involved in homologous recombination.)

Generalized transduction occurs in a variety of bacteria, including *Staphylococcus, Escherichia, Salmonella*, and *Pseudomonas*.


phage
receptor
viral
genome
cell wall


chromosome
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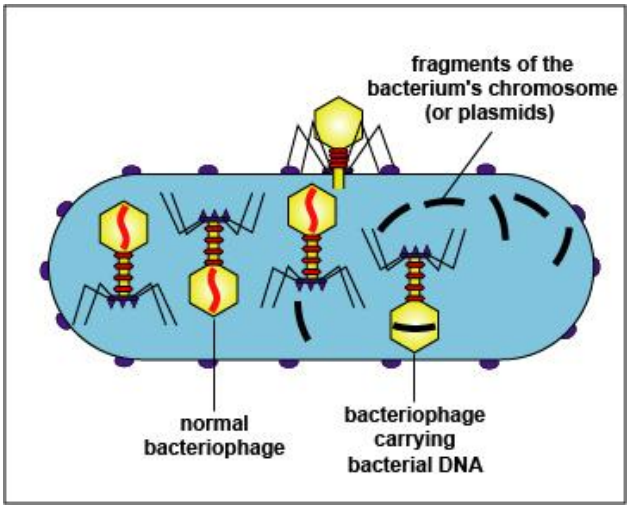
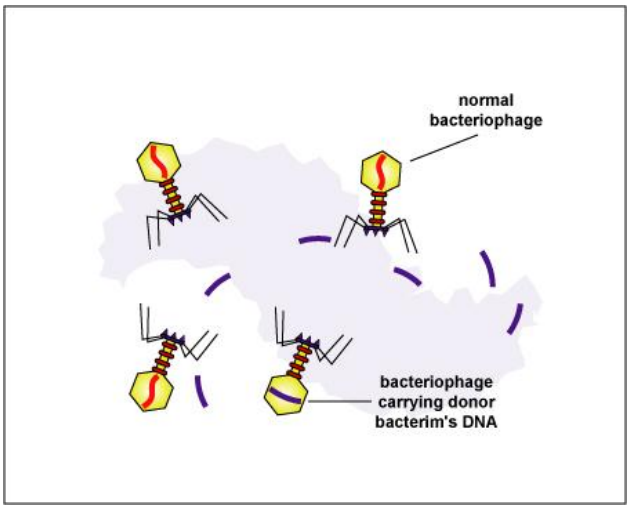

fragments of the
bacterium's chromosome
(or plasmids)
normal
bacteriophage
bacteriophage
carrying
bacterial DNA


normal
bacteriophage
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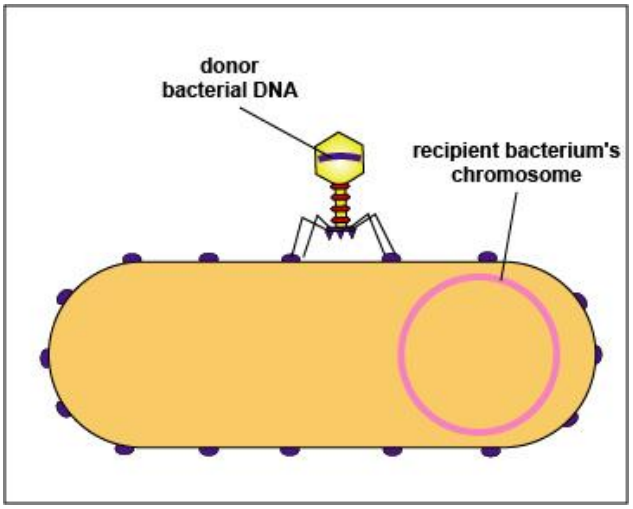
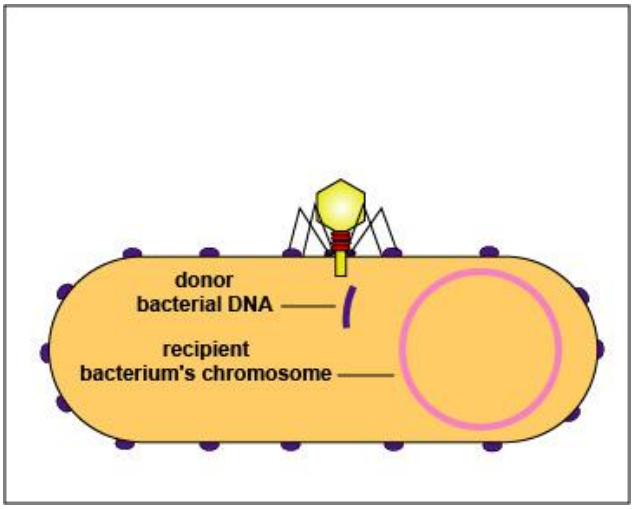
carrying donor
bacterim's DNA


donor
bacterial DNA
recipient bacterium's
chromosome


donor
bacterial DNA
recipient
bacterium's chromosome

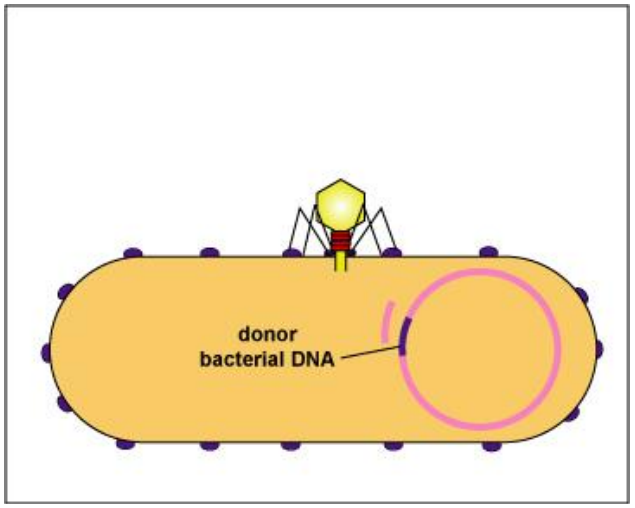


Figure 3.3. Generalized Transduction by Lytic Bacteriophage,Plasmids, such as the penicillinase plasmid of *Staphylococcus aureus*, may also be carried from one bacterium to another by generalized transduction.

Specialized transduction: This may occur occasionally during the lysogenic life cycle of a temperate bacteriophage. During spontaneous induction, a small piece of bacterial DNA may sometimes be exchanged for a piece of the bacteriophage genome, which remains in the bacterial nucleoid. This piece of bacterial DNA replicates as a part of the bacteriophage genome and is put into each phage capsid. The bacteriophages are released, adsorb to recipient bacteria, and inject the donor bacterium DNA/phage DNA complex into the recipient bacterium where it inserts into the bacterial chromosome (Figure 3).

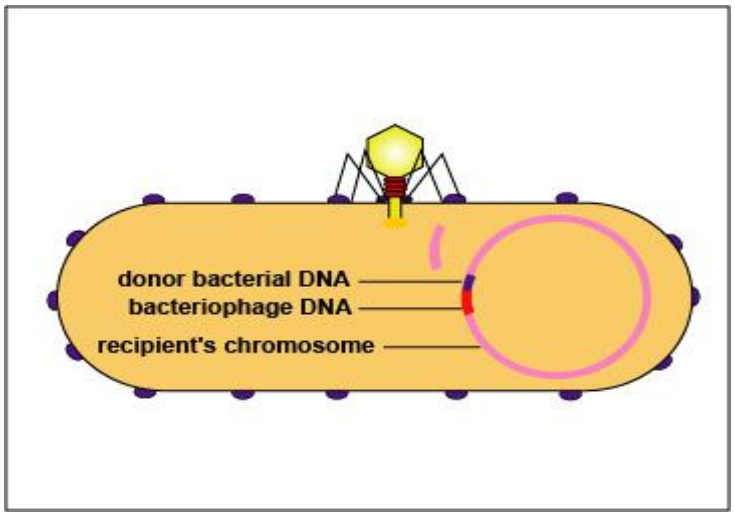


Figure 3.4: Specialized Transduction by Temperate Bacteriophage. Step 1: A temperate bacteriophage adsorbs to a susceptible bacterium and injects its genome. Step 2: The bacteriophage inserts its genome into the bacterium's chromosome to become a prophage. Step 3: Occasionally during spontaneous induction, the DNA is excised incorrectly and a small piece of the donor bacterium's DNA is picked up as part of the bacteriophage's genome in place of some of the bacteriophage DNA that remains in the bacterium's chromosome. Step 4: As the bacteriophage replicates, the segment of bacterial DNA replicates as part of the bacteriophage's genome. Every bacteriophage now carries that segment of bacterial DNA. Step 5: The bacteriophage adsorbs to a recipient bacterium and injects its genome. Step 6: The bacteriophage genome carrying the donor bacterial DNA inserts into the recipient bacterium's chromosome.

Conjugation

Genetic recombination in which there is a transfer of DNA from a living donor bacterium to a living recipient bacterium by cell-to-cell contact. In Gram-negative bacteria it typically involves *a* conjugation *or* sex pilus.

Conjugation is encoded by plasmids or transposons. It involves a donor bacterium that contains a conjugative plasmid and a recipient cell that does not. A conjugative plasmid is self-transmissible, in that it possesses all the necessary genes for that plasmid to transmit itself to another bacterium by conjugation. Conjugation genes known as *tra* genes enable the bacterium to form a mating pair with another organism, while *oriT* (origin of transfer) sequences determine where on the plasmid DNA transfer is initiated by serving as the replication start site where DNA replication enzymes will nick the DNA to initiate DNA replication and transfer. In addition, mobilizable plasmids that lack the *tra* genes for self-transmissibility but possess the *oriT* sequences for initiation of DNA transfer may also be transferred by conjugation if the bacterium containing them also possesses a conjugative plasmid. The *tra* genes of the conjugative plasmid enable a mating pair to form, while the *oriT* of the mobilizable plasmid enable the DNA to moves through the conjugative bridge (Figure 3.5).

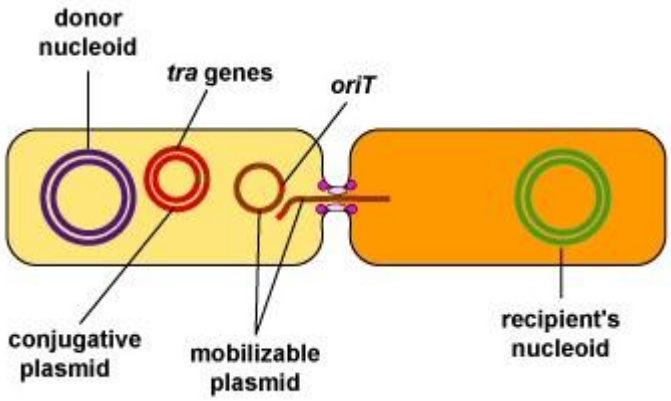


Figure 3.5: Transfer of Mobilizable Plasmids During Conjugation. Mobilizable plasmids, that lack the tra genes for self-transmissibility but possess the oriT sequences for initiation of DNA transfer, may also be transferred by conjugation if the bacterium containing them also possesses a conjugative plasmid. The tra genes of the conjugative plasmid enable a mating pair to form while the oriT quences of the mobilizable plasmid enables the DNA to move through the conjugative bridge.

Transposons ("jumping genes") are small pieces of DNA that encode enzymes that enable the transposon to move from one DNA location to another, either on the same molecule of DNA or on a different molecule. Transposons may be found as part of a bacterium's chromosome (conjugative transposons) or in plasmids and are usually between one and twelve genes long. A transposon contains a number of genes, such as those coding for antibiotic resistance or other traits, flanked at both ends by insertion sequences coding for an enzyme called transpoase.

Transpoase is the enzyme that catalyzes the cutting and resealing of the DNA during transposition.

Conjugative transposons, like conjugative plasmids, carry the genes that enable mating pairs to form for conjugation. Therefore, conjugative transposons also enable mobilizable plasmids and nonconjugative transposons to be transferred to a recipient bacterium during conjugation.

Many conjugative plasmids and conjugative transposons possess rather promiscuous transfer systems that enables them to transfer DNA not only to like species, but also to unrelated species. The ability of bacteria to adapt to new environments as a part of bacterial evolution most frequently results from the acquisition of large DNA sequences from another bacterium by conjugation.

a. General mechanism of transfer of conjugative plasmids by conjugation in Gram-negative bacteria

In Gram-negative bacteria, the first step in conjugation involves a conjugation pilus (sex pilus or F pilus) on the donor bacterium binding to a recipient bacterium lacking a conjugation pilus. Typically the conjugation pilus retracts or depolymerizes pulling the two bacteria together. A series of membrane proteins coded for by the conjugative plasmid then forms a bridge and an opening between the two bacteria, now called a mating pair.

Using the rolling circle model of DNA replication, a nuclease breaks one strand of the plasmid DNA at the origin of transfer site (*oriT*) of the plasmid and that nicked strand enters the recipient bacterium. The other strand remains behind in the donor cell. Both the donor and the recipient plasmid strands then make a complementary copy of themselves. Both bacteria now possess the conjugative plasmid. This process is summarized in Figure 3.6).

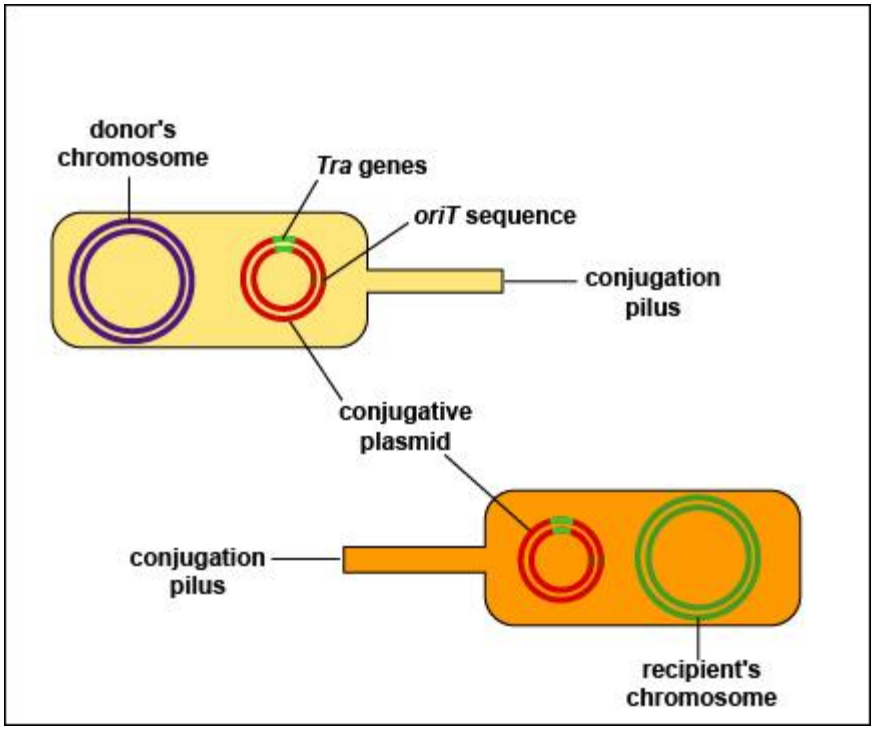


Figure 3.6: Transfer of Conjugative Plasmids. Step 1: In Gram-negative bacteria, the first step in conjugation involves a conjugation pilus (sex pilus or F pilus) on the donor bacterium binding to a recipient bacterium lacking a conjugation pilus. Step 2: Typically the conjugation pilus retracts or

depolymerizes pulling the two bacteria together. A series of membrane proteins coded for by the conjugative plasmid then forms a bridge and an opening between the two bacteria, now called a mating pair. Step 3: Using the rolling circle model of DNA replication, a nuclease breaks one strand of the plasmid DNA at the origin of transfer site (oriT) of the plasmid. The nuclease also has helicase activity and unwinds the strand that is going to be transferred. Step 4: The nicked plasmid strand enters the recipient bacterium. The other strand remains behind in the donor cell. Step 5: Both the donor and the recipient plasmid strands then make a complementary copy of themselves. Step 6: Both bacteria now possess the conjugative plasmid and can make a conjugation pilus.

This is the mechanism by which resistance plasmids (R-plasmids), coding for multiple antibiotic resistance and conjugation pilus formation, are transferred from a donor bacterium to a recipient. This is a big problem in treating opportunistic Gram-negative infections such as urinary tract infections, wound infections, pneumonia, and septicemia by such organisms as *E. coli, Proteus, Klebsiella, Enterobacter, Serratia,* and *Pseudomonas,* as well as with intestinal infections by organisms like *Salmonella* and *Shigella*.

There is also evidence that the conjugation pilus may also serve as a direct channel through which single-stranded DNA may be transferred during conjugation.

b. F^+ conjugation

This results in the transfer of an F^+ plasmid possessing *tra* genes coding only for a conjugation pilus and mating pair formation from a donor bacterium to a recipient bacterium. One strand of the F^+ plasmid is broken with a nuclease at the origin of transfer (*oriT*) sequence that determines where on the plasmid DNA transfer is initiated by serving as the replication start site where DNA replication enzymes will nick the DNA to initiate DNA replication and transfer. The nicked strand enters the recipient bacterium while the other plasmid strand remains in the donor. Each strand then makes a complementary copy. The recipient then becomes an F^+ male and can make a sex pilus.

In addition, mobilizable plasmids that lack the *tra* genes for self-transmissibility but possess the *oriT* sequences for initiation of DNA transfer, may also be transferred by conjugation. The *tra* genes of the F^+ plasmid enable a mating pair to form and the *oriT* sequences of the mobilizable plasmid enable the DNA to moves through the conjugative bridge (Figure 3.1.5).

c. Hfr (high frequency recombinant) conjugation

Hfr conjugation begins when an F^+ plasmid with *tra* genes coding for mating pair formation inserts or integrates into the chromosome to form an Hfr bacterium. (A plasmid that is able to integrate into the host nucleoid is called an *episome*.) A nuclease then breaks one strand of the donor's DNA at the origin of transfer (*oriT*) location of the inserted F^+ plasmid and the nicked strand of the donor DNA begins to enter the recipient bacterium. The remaining non-nicked DNA strand remains in the donor and makes a complementary copy of itself.

The bacterial connection usually breaks before the transfer of the entire chromosome is completed so the remainder of the F^+ plasmid seldom enters the recipient. As a result, there is a transfer of some chromosomal DNA, which may be exchanged for a piece of the recipient's DNA through homologous recombination, but not the ability to form a conjugation pilus and mating pairs.

Evolution in bacteria was previously viewed as a result of mutation or genetic drift. Today, genetic exchange, or gene transfer is viewed as a major driving force in the evolution of prokaryotes. This driving force has been widely studied in organisms like E. coli. Bacteria reproduces asexually, where daughter cells are clones of the parent. This clonal nature leads to random mutations that occur during DNA replication that potentially helps bacteria evolve. It was originally thought that only accumulated mutations helped bacteria evolve. In contrast, bacteria also import genes in a process called homologous recombination, first discovered by the observation of mosaic genes at loci encoding antibiotic resistance. The discovery of homologous recombination has made an impact on the understanding of bacterial evolution. The importance of evolution in bacterial recombination is its adaptivity. For example, bacterial recombination has been shown to promote the transfer of multi drug resistance genes via homologous recombination that goes beyond levels purely obtained by mutation.

• Viral genome replication.

A virus must undergo the process of **replication** to create new, infectious virions that are able to infect other cells of the body or subsequent hosts. After gaining entry into the body, a virus makes physical contact with and crosses the plasma membrane of a target cell. Inside, it releases and replicates its genome while facilitating the manufacture of its proteins by host ribosomes. Virus particles are assembled from these newly synthesized biological molecules and become infectious virions. Finally, the virions are released from the cell to continue the process of infection.

The seven stages of virus replication are categorized as follows:

- 1. Attachment
- 2. Penetration
- 3. Uncoating
- 4. Replication
- 5. Assembly
- 6. Maturation
- 7. Release

A mnemonic to remember the stages of virus replication is the sentence "**A** **PUR**ple **A**pple **M**ight **R**edden." The letters in bold are the first letters of the names of the seven stages in order.

All viruses must perform the seven stages in order to create new virions. Some stages may take place simultaneously with other stages, or some stages may take place out of order, depending upon the virus. This chapter describes the details of what occurs during each stage of viral replication.

1. Attachment

A cell interacts with the extracellular world at the plasma membrane, and it is at this location that a virus first makes contact with a target cell. As the plasma membrane of the cell is composed of a phospholipid bilayer that has numerous proteins protruding from the membrane. These surface proteins have a variety of functions that include transporting ions and molecules, facilitating the binding of one cell to another, or acting as receptors for incoming proteins. The majority of plasma membrane proteins are **glycosylated**, meaning that they have been modified with sugars and carbohydrates. To infect a cell, it is critical that a virus initiates **attachment**—the binding of the virus to the host cell. This interaction is specific: the virus contains a **virus attachment protein** that adsorbs to a **cell surface receptor** on the cell (Table 1). The target receptor molecules on the cell surface are normal molecules required for cellular functions that viruses have evolved to exploit, usually glycoproteins or the sugar/carbohydrate residues present on glycoproteins or the plasma membrane. For instance, rhinovirus binds a protein known as intercellular adhesion molecule 1 (ICAM-1), involved in the attachment of one cell to another. Influenza A virus strains bind to the sialic acid sugars found at the ends of cellular carbohydrate chains, and herpes simplex viruses (HSV) reversibly bind to glycosaminoglycans (GAGs), such as heparan sulfate, in order to bind to the herpesvirus entry mediator protein or nectins on the cell surface (Fig. 1).

Table 1. Cell Surface Receptors for Attachment of Human Viruses

Virus	Cell surface receptor(s)
Rhinoviruses	Intercellular adhesion molecule 1 (ICAM-1) (90%), low-density lipoprotein receptor (10%)
Poliovirus	Poliovirus receptor (PVR) CD155
Human immunodeficiency virus	CD4 (receptor); CCR5 or CXCR4 (coreceptors)
Influenza A virus	Sialic acid
Measles virus	CD46, CD150
Herpes simplex virus-1	Heparan sulfate, HVEM, Nectin-1
Dengue virus	DC-SIGN
Hepatitis B virus	Sodium taurocholate–cotransporting polypeptide
Human papillomavirus	Heparan sulfate, integrins

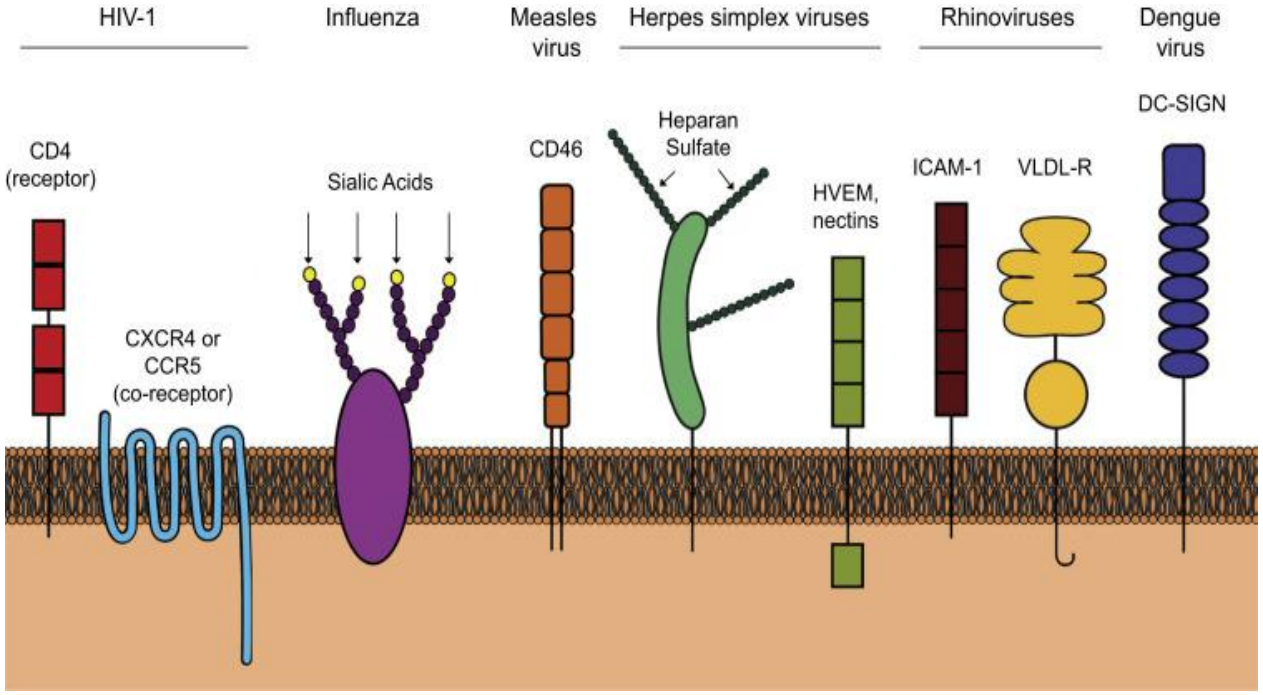


Figure 1. Cell surface receptors.
Different viruses use specific cell surface receptors for attachment. HIV-1 requires CD4 as a receptor and chemokine receptors CCR5 or CXCR4 as coreceptors. Influenza viruses bind to terminal sialic acid residues found on cell surface glycoproteins. Laboratory strains of measles virus bind CD46 (although CD150 is also a receptor for the virus). Herpes simplex virus-1 initially binds to heparan sulfate on GAGs in order to specifically bind entry receptors, such as HVEM or nectins. Ninety percent of rhinoviruses use ICAM-1 as a receptor, while 10% use the VLDL receptor. Dengue virus attaches using DC-SIGN. Note the different structures and types of receptors that viruses use for entry. The tropism of the virus is determined by which cells in the body express the cell surface receptor.

Some viruses also require **coreceptors** to infect cells. HIV initially binds to a protein known as CD4 on the surface of T lymphocytes ("T cells") but requires one of two coreceptor proteins to continue the process of infection. As humans that have a modified version of CCR5, one of these coreceptors, are largely resistant to infection with HIV because the virus cannot use the modified CCR5 as a coreceptor and so infection is blocked. Infection of a cell can be prevented if attachment of the virus can be inhibited, and virus attachment proteins are the target of many antiviral drugs in use and in development.

Attachment involves opposing electrostatic forces on the virus attachment protein and the cell surface receptor. The virus attachment protein is located in the outermost portion of the virus, since this is where contact with the cell occurs. The attachment protein protrudes from the envelope of an enveloped virus, whereas nonenveloped viruses have one or more capsid proteins that interact with the cell surface receptor. The viral attachment proteins can extend from the surface of the virion or can be within "canyons" formed by capsid proteins.

For example, 90% of human rhinovirus serotypes bind to ICAM-1 on the surface of cells. Instead of binding to the outside of the rhinovirus capsid, the molecule docks into a deep canyon formed by the rhinovirus VP1, VP2, and VP3 proteins (Fig. 2A). In contrast, 10% of human rhinoviruses attach to the very low-density lipoprotein (VLDL) receptor. This interaction does not occur in canyons formed by the viral proteins, however. Instead, several VP1 proteins at the vertices of the icosahedral capsid bind to the receptor (Fig. 2B). Even if the binding affinity between the VP1 protein and the VLDL receptor is low, the multiple VP1 proteins increase the total binding strength of the interaction. This example also illustrates that different strains of the same virus can take advantage of different cell surface receptors for attachment.

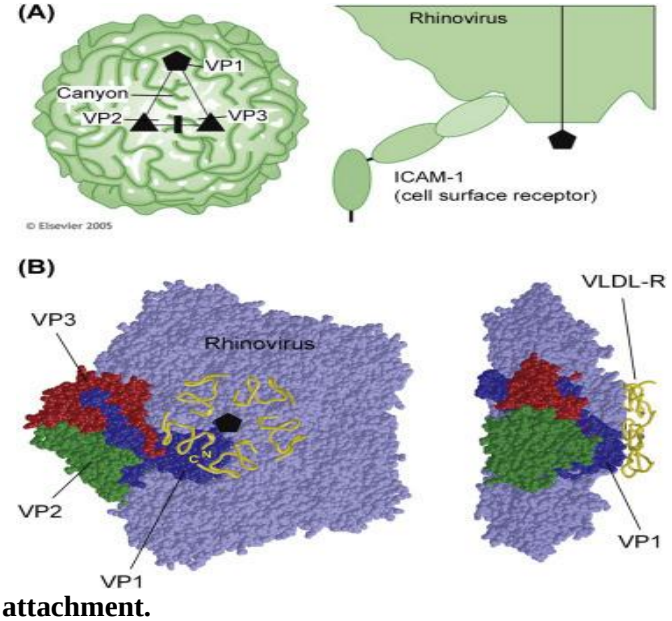


Figure 2. Rhinovirus attachment.

(A) 90% of rhinovirus serotypes use ICAM-1 as a cell surface receptor. The cellular protein binds into a canyon formed by capsid proteins VP1, VP2, and VP3. (B) 10% of rhinovirus serotypes bind the very low-density lipoprotein receptor (VLDL-R). In contrast to ICAM-1 binding, the binding of these rhinovirus serotypes occurs on the fivefold axis at the vertex of the capsid icosahedron, formed by repeating VP1 proteins. This space-filling model shows the surface of the rhinovirus capsid (gray) with one structural unit highlighted, formed by VP1 (blue), VP2 (green), and VP3 (red). The gold molecules represent the VLDL receptors, showing where they bind to rhinovirus protein VP1.

2. Penetration

Following attachment, successful viruses quickly gain entry into the cell to avoid extracellular stresses that could remove the virion, such as the flow of mucus. Penetration refers to the crossing of the plasma membrane by the virus. In contrast to virus attachment, penetration requires energy, although this is contributed by the host cell, not the virus.

Several different mechanisms are utilized by viruses to gain entry into a cell (Fig. 3, Table 2). One of these takes advantage of a normal host process: endocytosis. As described in Chapter 3, “Features of Host Cells: Cellular and Molecular Biology Review,” cells are able to import molecules through the process of endocytosis.

Receptor-mediated endocytosis occurs when receptors on the cell surface are bound by their ligands and internalized in clathrin-coated pits or caveolae that become endocytic vesicles. Eventually, these vesicles lose their clathrin or caveolin coating and fuse with "early endosomes," slightly acidic vesicles (pH of 6.0–6.5) that become "late endosomes" as their acidity increases (pH of 5.0–6.0). Late endosomes deliver materials to lysosomes, larger vesicles full of digestive enzymes.

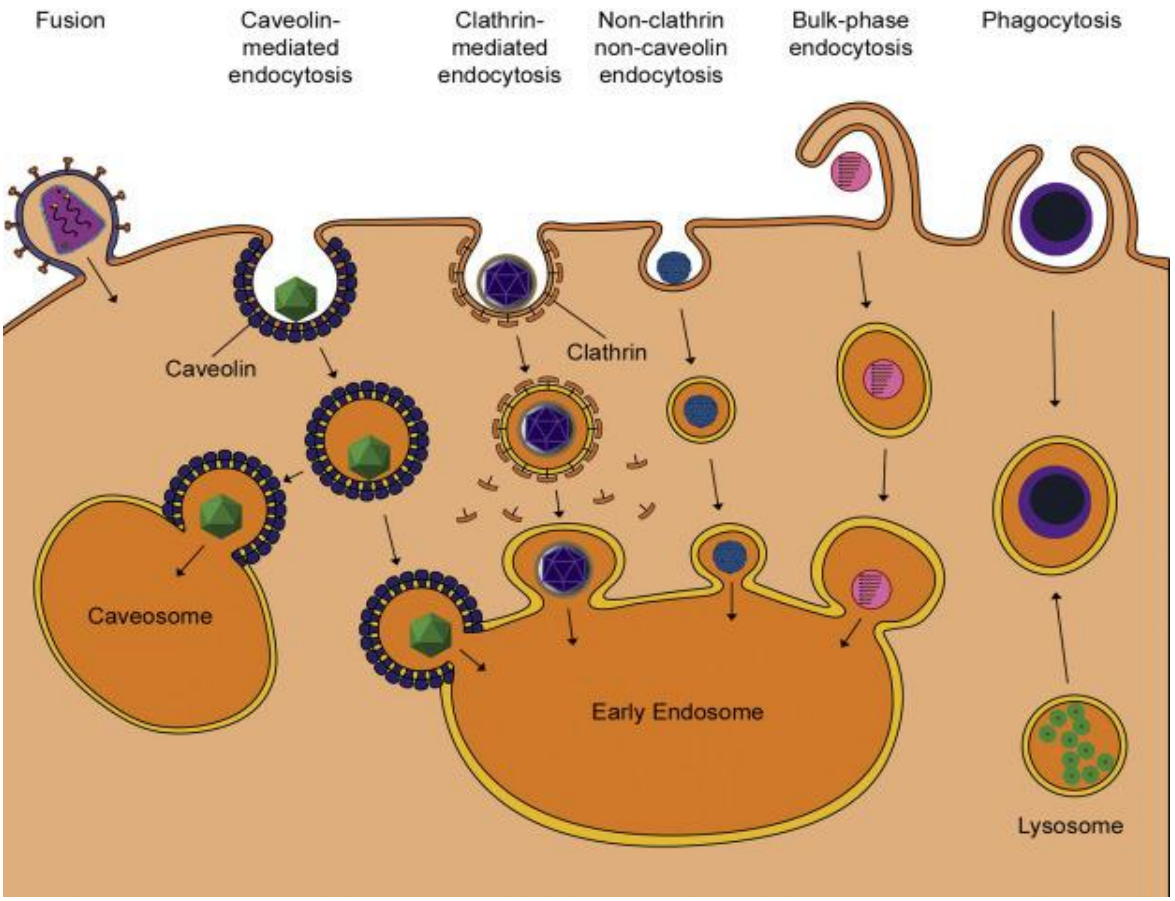


Figure 3.Viral penetration into the cell.
Different viruses take advantage of various cellular mechanisms to gain entry into the cell after binding their specific cell surface receptors. Some enveloped viruses undergo fusion, which fuses the viral envelope with the plasma membrane. Both enveloped and nonenveloped viruses take advantage of receptor-mediated endocytosis in caveolin- or clathrin-coated pits to gain entry into the cytoplasm of the cell. Still other viruses undergo receptor-mediated endocytosis that is independent of both clathrin and caveolin. Bulk-phase endocytosis and phagocytosis are also utilized by viruses to gain entry into the cell.

Table 2. Methods of Penetration for Select Human Viruses

Type of penetration (entry)	Virus examples
Clathrin-mediated endocytosis	Dengue virus, hepatitis C virus, reovirus, adenovirus, parvovirus B19, West Nile virus
Caveolin-mediated endocytosis	Human papillomavirus, SV40, hepatitis B virus
Fusion	HIV, influenza, respiratory syncytial virus, herpes simplex viruses, dengue virus, Ebola virus

Receptor-mediated endocytosis is commonly used by viruses to penetrate the plasma membrane. As the pH of the endosome drops, the viral proteins change configuration, which allows them to escape from the endosome. Depending upon the virus, this can happen in early endosomes, late endosomes, or lysosomes. Both enveloped and nonenveloped viruses take advantage of receptor-mediated endocytosis to gain entry into the cytoplasm of the cell (Fig. 3). Most types of viruses use clathrin-mediated endocytosis to enter the cell, including dengue virus, hepatitis C virus, and reoviruses. A few well-known viruses that infect humans, such as SV40 and papillomaviruses (that cause warts or cervical cancer), use caveolae-mediated endocytosis; this was discovered by using a drug that inhibited the formation of caveolae. Blocking clathrin-mediated endocytosis did not prevent.

The entry of these viruses into cells. Still other viruses undergo receptor-mediated endocytosis that is independent of both clathrin and caveolin.

Other forms of endocytosis, such as bulk-phase endocytosis and phagocytosis, are also exploited by viruses to enter the cell. In bulk-phase endocytosis, the cell forms a vesicle that engulfs whatever molecules are present in the extracellular fluid, including viruses. **Phagocytosis** is a form of receptor-mediated endocytosis that is used by specialized cells to engulf entire cells. Recently, two large DNA viruses, HSV-1 and mimivirus, were shown to enter cells through phagocytosis-like pathways.

A method of penetration that is used exclusively by enveloped viruses is **fusion**. Fusion of the viral envelope can occur at the cell membrane or within endocytosed vesicles, such as the endosome, and is mediated by the same viral protein that is used by the virus for attachment or by a different viral protein, depending upon the virus. For instance, HIV has a protein known as gp120 that binds to

CD4 and one of the two coreceptors for entry, CCR5 or CXCR4. Once this occurs, a different viral protein, gp41, fuses the virus envelope with the cell membrane, releasing the nucleocapsid into the cytoplasm.

3. **Uncoating**

Uncoating refers to the breakdown or removal of the capsid, causing the release of the virus genome into the cell to wherever genome replication and transcription will take place. Uncoating can be separated from or tightly linked with penetration, and viruses achieve uncoating in a variety of different ways (Fig. 4.5). For example, rhinoviruses are taken into the cell by receptor-mediated endocytosis in clathrin-coated vesicles. Within the acidic endosome, the virus expands in size about 4%, and one of the capsid proteins, VP1 (viral protein 1), forms pores in the endosome that allow the release of the rhinovirus RNA genome. On the other hand, influenza virus has a viral protein known as hemagglutinin (HA) embedded into the virus envelope. HA binds to sialic acid residues found on the surface of respiratory epithelial cells, and penetration occurs through receptor-mediated endocytosis. The low pH of the endosome causes a conformational change in the viral HA protein, revealing a fusion peptide that brings the two membranes close together and fuses the viral envelope with the endosomal membrane. In this case, the HA protein facilitates both attachment and uncoating of the virus. The released viral RNA genome segments are transported to the nucleus and enter through nuclear pores. Other viral capsids, such as those of poliovirus, have been thought to not enter the cell at all: the binding of the poliovirus capsid to the cell surface receptor causes a conformational change in the virion that creates a pore in the cell membrane through which the viral RNA is released into the cytoplasm. In contrast, many viruses remain largely intact after penetration. Reoviruses do not completely uncoat within the cytoplasm, providing a “home base” for genome replication.

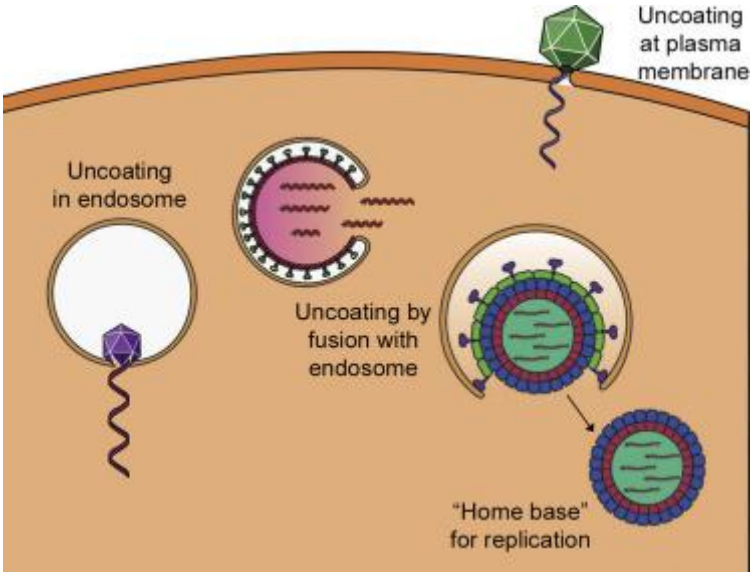


Figure 4. Uncoating of virion capsids.

Certain viruses, including rhinoviruses, expand to form pores in the endosome through which the viral genome can escape. Like influenza virus, other viruses induce fusion of the virion envelope

with the endosomal membrane, releasing the viral genome. Historically, it has been though that poliovirus capsids do not enter the cell at all: binding of the capsid to the cell surface receptor induces a conformation change that creates a pore in the membrane through which the genome is transported. Many viruses maintain a partially intact capsid in the cytosol that acts as a “home base” for replication, like reoviruses do.

Many herpesviruses infect neurons but must replicate in the nucleus, which can be quite a distance from their site of entry at the plasma membrane. After fusion of the viral envelope with the plasma membrane, the intact nucleocapsids of HSV-1 are transported along microtubules to the nucleus. HSV proteins bind to dynein, a host cell protein that “walks” vesicles of cargo along microtubules. At the nucleus, the HSV capsid docks at a nuclear pore and its viral DNA is transported into the nucleus (Fig. 5). Still other capsids are small enough to pass through nuclear pores: the hepatitis B capsid, with a diameter around 30 nm, may be imported intact through a nuclear pore to uncoat within the nucleus.

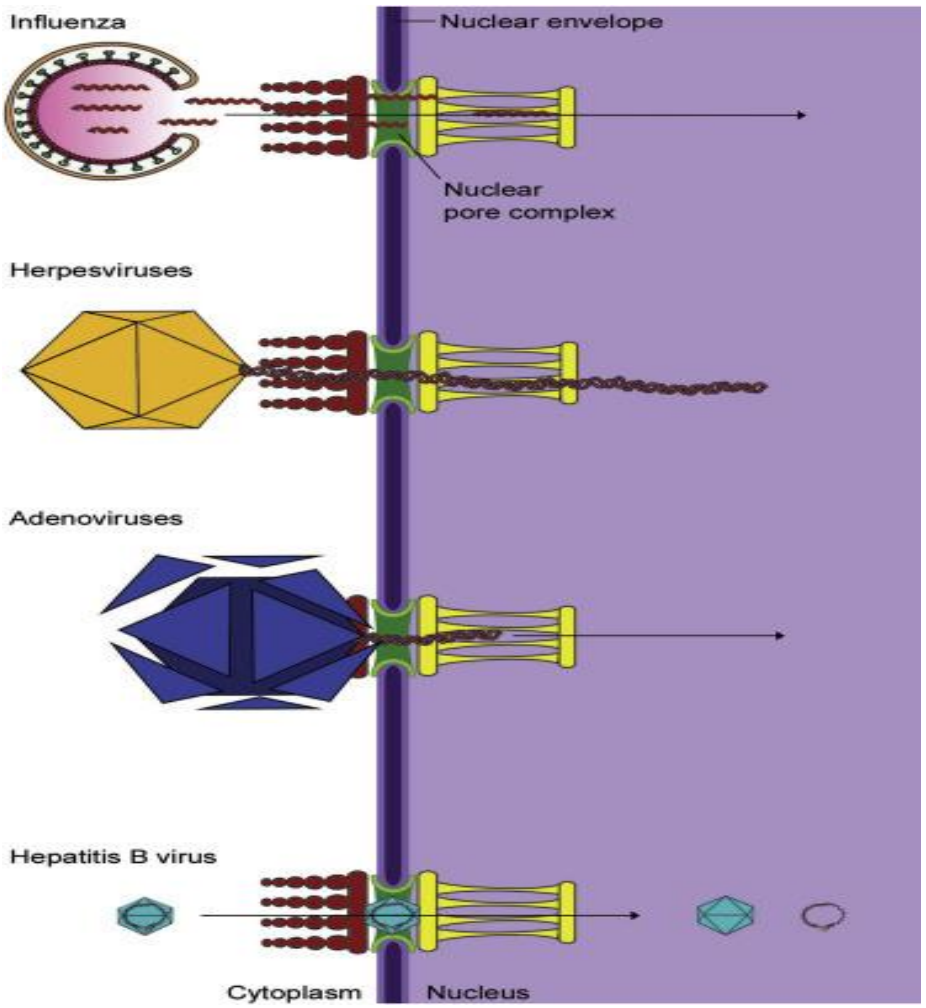


Figure 5.Transport of viral genomes into the nucleus.
Several viruses must transport their genomes into the nucleus for viral transcription and/or replication to occur. Influenza genome segments are transported through the nuclear pore into the nucleus. Herpesvirus capsids are transported along microtubules to the nuclear pore, where uncoating occurs. Adenovirus capsids disassemble at the nuclear pore and the viral DNA is transported into the nucleus. Other viruses, including hepatitis B virus, are small enough that the entire capsid might be able to pass through the nuclear pore.

4. Replication

In the same way that our DNA encodes the information to manufacture our proteins, a virus's genome acts as the instructions for the synthesis of virus proteins. To create new virions, the proteins that will be incorporated into the virion are made through expression of viral genes, and the virus genome is copied through the process of replication. The replication strategy of a virus is generally dependent upon the type of nucleic acid genome it contains (Fig. 6). Many classes of viruses exploit cellular proteins to replicate their genomes. The location of these proteins will therefore generally dictate where the replication of the viral nucleic acid will take place.

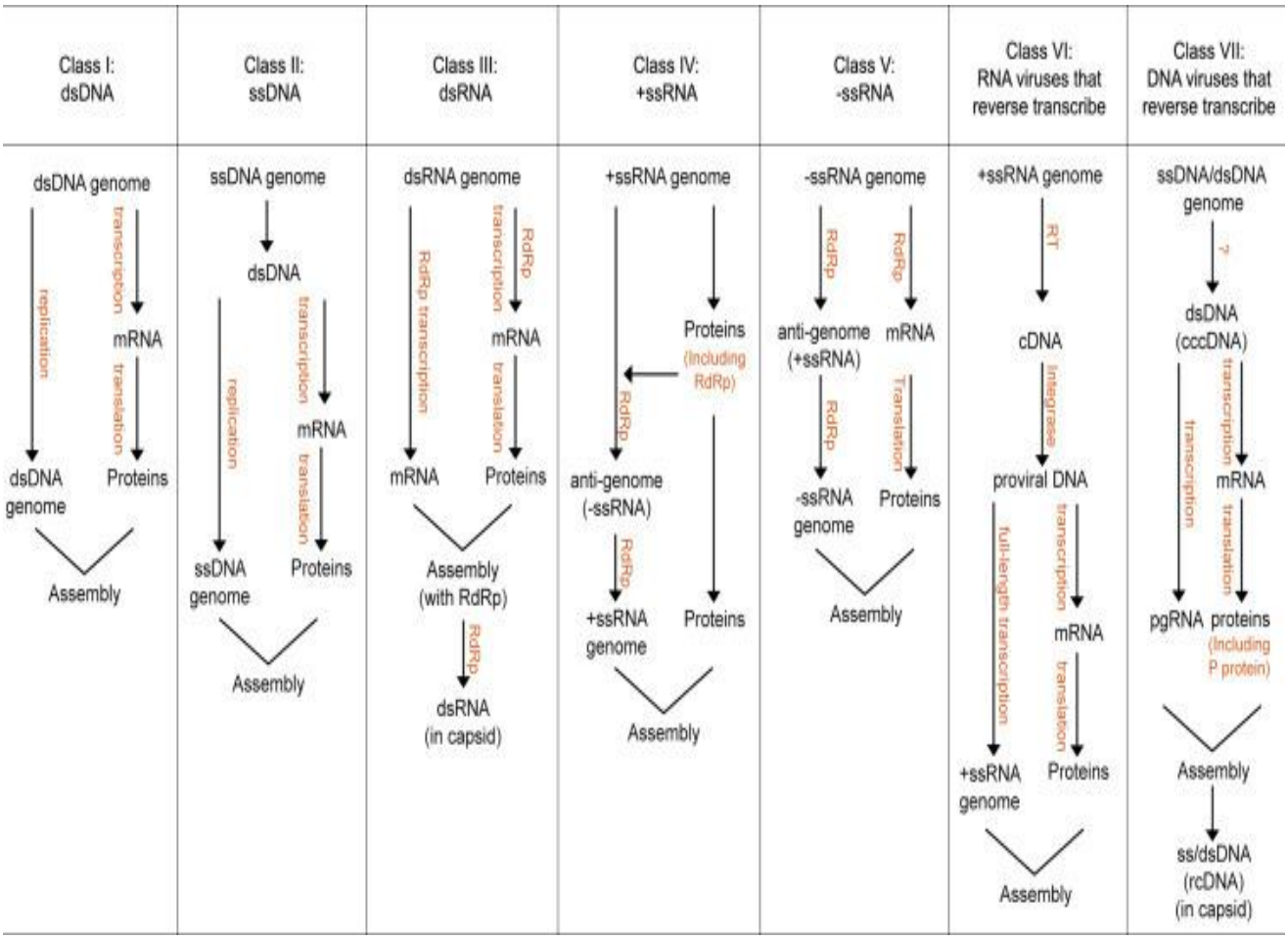


Figure 6. The replication strategies of the viruses in the Baltimore replication classes.
All viruses, regardless of their type of nucleic acid genome, must copy the genome and translate proteins in order to assemble nascent virions.

All living organisms have genomes composed of double-stranded (ds) DNA, but viruses are unique in that their genomes can be made of single-stranded (ss) DNA, as well as dsRNA or ssRNA, which can be positive sense or negative sense. The Baltimore classification system, as discussed in Chapter 2, "Virus Structure and Classification," is useful when discussing the general replication strategies of viruses because it categorizes the viruses into seven classes based upon their type of genome:

1. Double-stranded DNA viruses
2. Single-stranded DNA viruses

3. Double-stranded RNA viruses
4. Positive-sense RNA viruses
5. Negative-sense RNA viruses
6. RNA viruses that reverse transcribe
7. DNA viruses that reverse transcribe

As the replication strategies of these classes are discussed, it may be helpful to refer to Table 4.3 and Fig. 2.11 to keep track of which vertebrate-infecting viral families are found in each class.

Table3. **Families of Human Viruses Within Each Replication Class**

Family	**Virus examples**
Class I: dsDNA viruses	
Adenoviridae	Adenovirus
Herpesviridae	Herpes simplex virus, Epstein–Barr virus, varicella zoster virus
Papillomaviridae	Human papillomavirus
Polyomaviridae	JC polyomavirus, BK polyomavirus, SV40
Poxviridae	Variola, vaccinia
Class II: ssDNA viruses	
Parvoviridae	Parvovirus B19
Anelloviridae	Torque teno virus
Class III: dsRNA viruses	
Picobirnaviridae	Human picobirnavirus
Reoviridae	Rotavirus
Class IV: +ssRNA viruses	
Astroviridae	Human astrovirus
Caliciviridae	Norwalk virus
Coronaviridae	Human coronavirus

Viral nucleic acids are found in a variety of configurations. They can be linear or circular, and they can be **segmented** into several smaller pieces within the virion, as occurs with influenza viruses, or **nonsegmented** like rabies virus, containing one molecule of nucleic acid that encodes all necessary genes. Longer molecules are more subject to breaking, but segmented viruses must package all genome segments into a virion for it to be infectious. Regardless of the structure of their nucleic acid, all viruses need to express their viral proteins and replicate their genome within the cell in order to create new virions.

1. *Class I: dsDNA Viruses*

All living organisms have double-stranded DNA genomes. Viruses with dsDNA genomes therefore have the most similar nucleic acid to living organisms and often use the enzymes and proteins that the cell normally uses for DNA replication and transcription, including its DNA polymerases and RNA polymerases. These are located in the nucleus of a eukaryotic cell, and so all dsDNA viruses that infect humans (with the exception of poxviruses) enter the nucleus of the cell, using the various mechanisms of entry and uncoating mentioned above. Many recognizable human viruses have dsDNA genomes, including herpesviruses, poxviruses, adenoviruses, and polyomaviruses.

Transcription of viral mRNA (vmRNA) must occur before genome replication if viral proteins are involved in replicating the virus genome. In addition, certain translated viral proteins act as **transcription factors** to direct the transcription of other genes. As discussed in Chapter 3, "Features of Host Cells: Cellular and Molecular Biology Review, transcription factors bind to specific sequences within the **promoters** of cellular genes immediately upstream of the transcription start site to initiate transcription of those genes. **Enhancers**, regulatory sequences also involved in transcription, are located farther away from the transcription start site and can be upstream or downstream. dsDNA viruses also have promoter and enhancer regions within their genomes that are recognized not only by viral transcription factors but by host transcription factors, as well. These proteins initiate transcription of the viral genes by the host RNA polymerase II.

Processing of viral precursor mRNA (also known as posttranscriptional modification) occurs through the same mechanisms as for cellular mRNA. Viral transcripts receive a 5′-cap and 3′-poly(A) tail, and some viruses' transcripts are spliced to form different vmRNAs. For example, the genes of herpesviruses are each encoded by their own promoter and are generally not spliced, but the human adenovirus E genome has 17 genes that encode 38 different proteins, derived by alternative splicing of vmRNA during RNA processing.

The dsDNA viruses transcribe their viral gene products in waves, and the **immediate early** and/or **early** genes are the first viral genes to be transcribed and translated into viral proteins.

These gene products have a variety of functions, many of which help to direct the efficient replication of the genome and further transcription of the **late** genes that encode the major virion structural proteins and other proteins involved in assembly, maturation, and release from the cell. The replication of the viral genome requires many cellular proteins; having the late genes transcribed and translated after the virus genome has been replicated ensures that the host enzymes needed for replication are not negatively affected by the translation of massive amount of virion structural proteins.

To create new virions, viral proteins must be translated and the genome must also be copied. With the exception of poxviruses, the genome replication of all dsDNA viruses takes place within the nucleus of the infected cell. Eukaryotic DNA replication, also reviewed in more detail in Chapter 3, "Features of Host Cells: Cellular and Molecular Biology Review," is also carried out by DNA polymerases and other proteins within the nucleus. DNA polymerases, whether they are cell derived or virus derived, cannot carry out de novo synthesis, however. They must bind to a short primer of nucleic acid that has bound to the single-stranded piece of DNA, forming a short double-stranded portion that is then extended by DNA polymerase (Fig. 4.8A). Primase is the enzyme that creates primers during cellular DNA replication, and some viruses, such as polyomaviruses and some herpesviruses, take advantage of the cellular primase enzyme to create primers on their dsDNA genomes during replication. Other herpesviruses, such as HSV-1, provide their own primase molecule, although this process occurs less commonly. Still other viruses, such as the adenoviruses, encode a viral protein primer that primes its own viral DNA polymerase (Fig. 4.8B). Cellular DNA polymerases are used by polyomaviruses and papillomaviruses, while all other dsDNA viruses encode their own DNA polymerases to replicate the viral genome. Many other cellular enzymes and proteins are required for DNA synthesis, and viruses are dependent on these to varying degrees, depending upon the specific virus. The poxviruses are a notable exception to this: they encode all the proteins necessary for DNA replication. In fact, they also encode the proteins needed for transcription of RNA, and so, unlike all other dsDNA viruses, they do not need to gain entry into the nucleus of a host cell for either genome replication or transcription and processing of viral genes, allowing their replication to take place entirely in the cytoplasm.

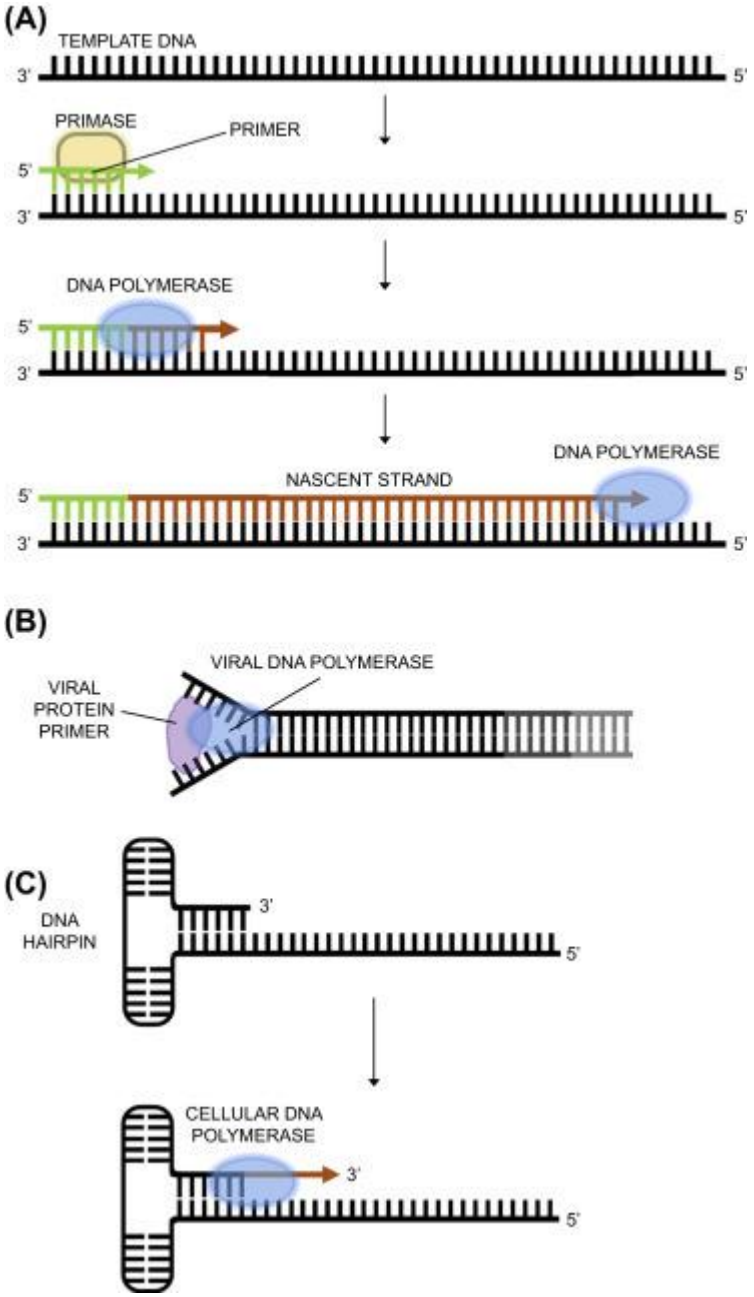


Figure 4.8 DNA priming.
DNA polymerases cannot carry out de novo synthesis and so need a primer in order to replicate DNA. Some viruses take advantage of the cellular primase in order to create primers (A), while other viruses, such as adenoviruses, encode a protein primer that primes its own DNA polymerase (B). In the process of self-priming, the ssDNA genomes of parvoviruses fold back upon themselves to form hairpin ends that act as a primer for host DNA polymerase (C).

2. *Class II: ssDNA Viruses*

Viruses with ssDNA genomes infect primarily bacteria and plants, although two families, Anelloviridae and Parvoviridae, infect humans. These viruses are some of the smallest known viruses, with nonenveloped icosahedral capsids of 18–30 nm in diameter, and correspondingly small genomes of 4000–6000 nucleotides.

Because they encode only a few genes, they are completely dependent on host cell enzymes for genome replication and transcription.

During replication, the ssDNA genome enters the nucleus of the host cell, where the ssDNA is converted to dsDNA by DNA polymerase during S phase of the cell cycle. This occurs because the ssDNA genome of parvoviruses has "hairpin" ends that fold back and complementarily bind to the ssDNA (Fig. 4.8C). This process, known as **self-priming,** creates a primer for DNA polymerase to extend. After the ssDNA genome becomes double-stranded, RNA polymerase II is able to transcribe the viral genes, which are then translated into viral proteins, and DNA polymerase replicates the genome so assembly of nascent virions can occur.

3. Class III: dsRNA Viruses

dsRNA viruses are all nonenveloped and possess icosahedral capsids. They have segmented genomes, and two families of dsRNA viruses infect humans. Viruses in the *Reoviridae* family include rotavirus, so named because the virion looks like a wheel (*rota* means "wheel" in Latin; Fig. 4.9). Rotavirus has 11 genome segments and is the major cause of childhood diarrhea. Picobirnaviruses are another family of dsRNA viruses that infect humans, but they are bisegmented, only having two genome segments that together are around 4.2 kb in length (the name of the viral family means "small two-RNA viruses," referring to the two dsRNA genome segments). Human picobirnaviruses have been isolated from diarrhea, although the association of the virus as a cause of a specific disease is currently unclear.

Unlike DNA viruses, viruses with RNA genomes do not usually enter the nucleus of an infected cell. Because they do not have a DNA intermediate, none of the host enzymes involved in DNA replication are required for the replication of the RNA genome. However, RNA viruses must still transcribe vmRNA so that viral proteins can be translated by host ribosomes and new virions can be formed. Cellular mRNA is transcribed by a **DNA-dependent RNA polymerase** called RNA polymerase II. As the name suggests, a DNA-dependent RNA polymerase requires a DNA template to make RNA, so it cannot transcribe mRNA from an RNA genome. Therefore, cells do not contain the enzymes necessary to transcribe mRNA from an RNA template, and so all RNA viruses must carry *or encode* their own **RNA-dependent RNA polymerase** (RdRp) to transcribe viral mRNA (Fig. 4.10A). dsRNA viruses contain an RdRp that is carried into the cell within the virion.

As mentioned in Section 4.3, reoviruses do not completely uncoat within the cytoplasm of the cell, providing a "home base" for transcription. In fact, free viral dsRNA or mRNA is not observed within the cytoplasm of the cell. The RdRp is closely associated with the partially uncoated capsid, which contains pores through which the transcribed mRNA passes to enter the cytoplasm of the cell, where the mRNA associates with host ribosomes.

Only one of the two RNA strands within the rotavirus genome, the negative strand, is used as a template by the virus's RdRp to transcribe mRNA. The viral mRNA is translated by host ribosomes to produce structural and nonstructural viral proteins. Each genome segment is transcribed into mRNA that is **monocistronic**, meaning that each mRNA transcript encodes one protein only, as is the case with eukaryotic mRNA transcripts. As new capsids are forming, a viral mRNA from each genome segment becomes enclosed within the capsid, along with the RdRp protein. Within the capsid, the RdRp synthesizes along each mRNA transcript just once to create the complementary negative strand, thereby forming the dsRNA genome in the newly formed capsid.

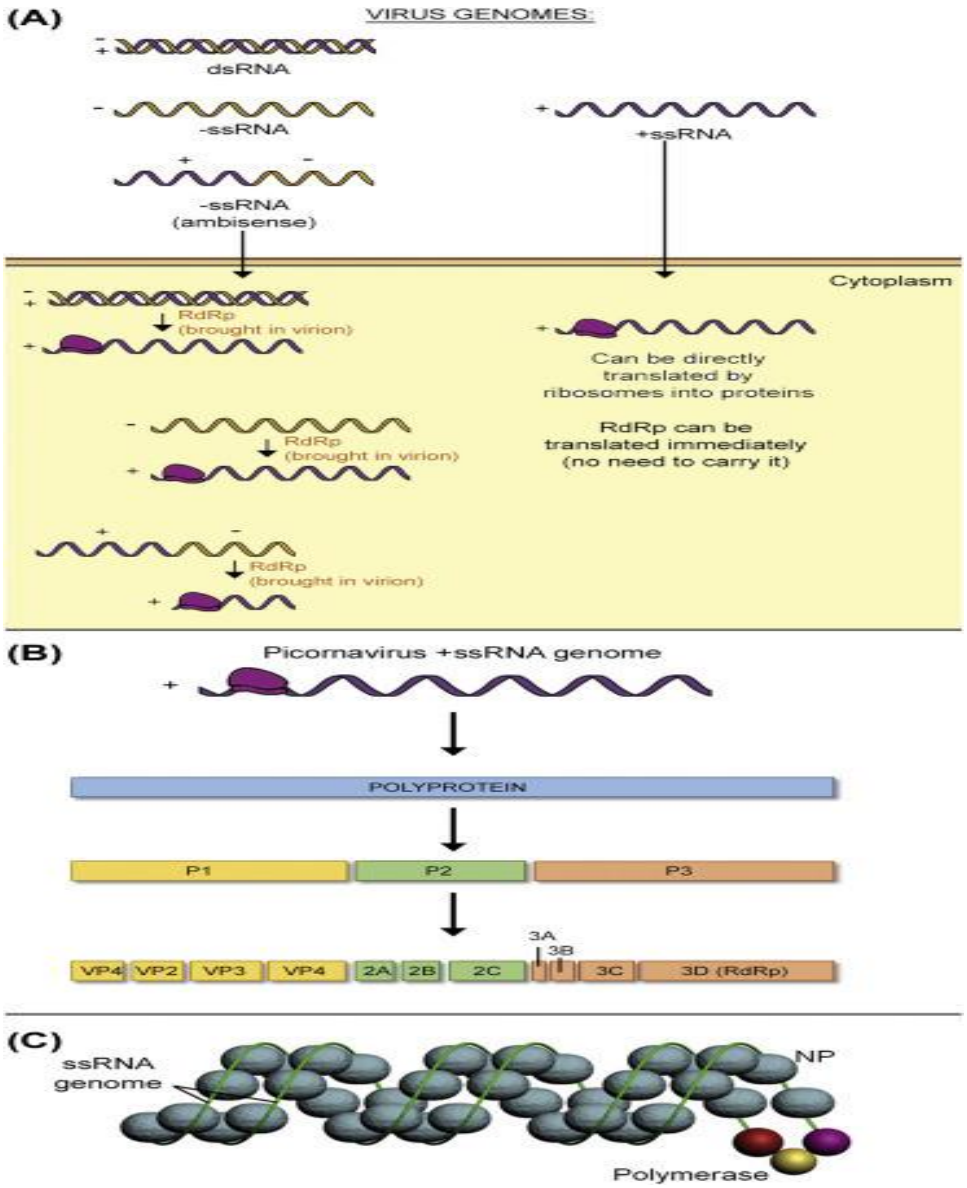


Figure 4.10 Details of RNA virus replication.
(A) Like mRNA, +ssRNA viruses have infectious genomes that can immediately be translated by ribosomes. Consequently, they do not need to bring an RdRp into the cell. RNA viruses with dsRNA, −ssRNA, or ambisense genomes must carry their own RdRp protein into the cell in order for transcription to occur. (B) The +ssRNA picornaviruses encode a single polyprotein that is cleaved several times to create all the proteins necessary for replication. (C) The ribonucleoprotein complex of helical viruses, such as this one from influenza A virus, is composed of the −ssRNA genome, the protecting nucleocapsid protein (NP), and associated proteins, including the RdRp complex.

4. *Class IV: +ssRNA Viruses*

RNA viruses are unique in that their genetic information is encoded using RNA, not DNA. As we have seen, this can occur as dsRNA, but many ssRNA viruses also exist. Viruses with ssRNA genomes that can act directly as mRNA are known as **positive-sense** RNA viruses (abbreviated +ssRNA). Similarly, ssRNA viruses with genomes that are not able to be immediately translated by ribosomes are known as **negative-sense** RNA viruses (abbreviated -ssRNA). Negative-sense RNA must be copied into positive-sense RNA by a viral RdRp before it can be translated by ribosomes (Fig. 4.10A). The terms *positive strand* and *negative strand* are also used interchangeably with these two terms.

+ssRNA viruses are more abundant than any other class of viruses and infect a wide range of host species. They include seven different human viral families, including the coronaviruses, flaviviruses, and picornaviruses, that cause significant disease in humans (Table 4.3). Their abundance indicates that +ssRNA viruses have been very successful evolutionarily.

Because the genome of +ssRNA viruses acts as mRNA, these viruses have genetic information that is **infectious**. Their genomes are translatable by host ribosomes and have 5′-caps (or proteins that act similarly to a 5′-cap) and often contain poly (A) tail sequences at the 3′-end. Experiments that delivered only the genome of poliovirus into the cytoplasm of a cell resulted in new virions being formed, because translation of the genome is the first activity that takes place with +ssRNA genomes. This produces all the viral proteins necessary for orchestrating the remainder of the replication cycle. Where dsRNA viruses must carry an RdRp within the virion, +ssRNA viruses *encode* an RdRp within their +ssRNA genome. The RdRp protein is produced immediately upon entry into the cell by translation of the viral genome. It is important to note, however, that even though the virus encodes its own RdRp protein, cellular proteins are often also required for replication to take place. For example, despite that the poliovirus genome is infectious, it is not replicated when injected into a *Xenopus* frog oocyte (ovum/egg) unless the cytoplasm from a human cell is injected alongside the genome, indicating that at least one human cellular component is required for poliovirus genome replication.

A common characteristic of +ssRNA viruses is that their infectious genome encodes a **polyprotein**, meaning that the genome is translated by ribosomes into a long chain of amino acids that is then cleaved into several smaller proteins. This provides an economical method of deriving several proteins from the translation of only one piece of mRNA. In the case of picornaviruses, the positive-strand genome is translated in its entirety, and then proteases cleave the polyprotein in different locations to create several different proteins (Fig. 4.10B). Alternative cleavage of certain sections results in additional proteins.

Other +ssRNA viruses, such as the togaviruses that include rubella virus, begin by translating only a portion of the +ssRNA genome to create an initial set of proteins that direct the later replication of the genome and translation of other viral proteins. This allows for the creation of "stages" of virus replication, similar to what is observed with immediate early, early, and late gene transcription of certain DNA viruses. Creation of polyproteins also commonly accompanies this method of translation, and termination suppression results in the production of different polyprotein chains. This happens at a low rate (approximately 10% of proteins initially synthesized from the togavirus +ssRNA genome) but results in the generation of important viral proteins, including the viral RdRp.

As the replication proteins of +ssRNA viruses are synthesized, they tend to gather at or within certain membranes in the cell, creating **replication complexes**. For example, the viral proteins of poliovirus remain bound to rough ER (rER) membranes or secretory vesicles. Poliovirus is a nonenveloped virus, so the function of this appears to be to concentrate viral proteins in one location of the cell to better facilitate replication processes. Another reason this may have evolved is to shield the viral ssRNA from intracellular immune responses, discussed further in Chapter 6, "The Immune Response to Viruses."

The viral genome of +ssRNA viruses is used to create a complementary negative strand, the **antigenomic RNA**, that is used as a template to create many copies of the +ssRNA genome. Along with viral protein production, copying of the viral genome is a necessary step in generating the required elements for creating new virions.

5. *Class V: −ssRNA Viruses*

In contrast to +ssRNA viruses, negative-sense RNA viruses (−ssRNA viruses) have genomes that do not act as mRNA. Therefore, like their dsRNA counterparts, they must carry an RdRp within the virion into the cell. There exist six −ssRNA virus families that include some of the most well-known disease-causing viruses, including Ebola virus, Marburg virus, measles virus, mumps virus, rabies virus, and influenza virus. These viruses have enveloped, helical capsids and can have segmented genomes (like influenza) or nonsegmented genomes (like rabies virus).

Viruses with −ssRNA genomes generally do not enter the nucleus (although the −ssRNA influenza viruses are a notable exception to this rule). The −ssRNA genomes are not capped and do not have poly(A) tails, because the −ssRNA does not function as mRNA. Instead, the genome must first be transcribed by the viral RdRp into mRNA, which is then translated. −ssRNA viruses have helical nucleocapsids, where the viral RNA is coated with a repeating nucleocapsid protein, termed NP or N. In addition, the viral RdRp and other proteins necessary for transcription also associate with the nucleocapsid protein and viral RNA. Together, the complex of viral RNA and proteins is termed the viral **ribonucleoprotein** complex, because it contains RNA and viral proteins (Fig. 4.10C).

Within the cell, the −ssRNA is immediately transcribed into viral mRNAs by the viral RdRp and any other required helper proteins to produce the virus's proteins.

As with +ssRNA viruses, antigenomic RNA is created to act as a template for replication of the genome. In the case of −ssRNA viruses, the complementary antigenomic RNA is +ssRNA. This antigenome is not identical to the positive-sense viral mRNAs produced during infection, however, since viral mRNAs are capped and polyadenylated. At some point during viral replication, a switch occurs so the RdRp drives genome replication over mRNA transcription. This can occur because certain translated viral proteins bind to the −ssRNA genome at sites that would normally stop the polymerase, allowing it to continue copying the entire genome into the antigenome. In some cases, newly translated viral proteins join the RdRp complex to promote genome replication over viral mRNA transcription.

Certain RNA viruses, termed **ambisense** viruses, have genomes that are partially negative sense and partially positive sense. They are still considered within the class of −ssRNA viruses, however, because the positive-sense portion of their genome is not directly infectious: it must first be copied into an antigenome segment that is used to create the viral mRNA. The arenaviruses are the only ambisense viruses that infect humans (although plant viruses in the *Tospovirus* genus within the *Bunyaviridae* family also have an ambisense genome). The arenaviruses, which include Lassa virus and lymphocytic choriomeningitis virus, have two genome segments, a long (L) segment and short (S) segment, which are each ambisense. Positive-sense viral mRNA is transcribed by the RdRp from the negative-sense portions of these segments. A complementary antigenome is also transcribed for each segment, and this is used to create the viral mRNA from the positive-sense portions of the genome segments (Fig. 4.11).

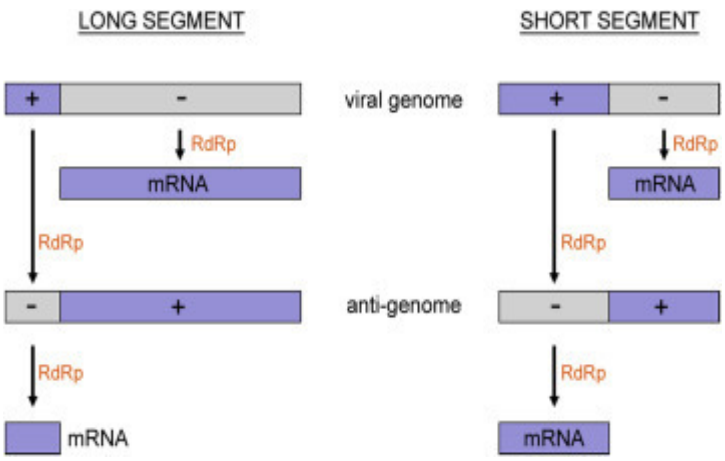


Figure 4.11Replication of ambisense genomes.
Ambisense genomes are composed of both −ssRNA and +ssRNA. The viral RdRp transcribes the mRNA from the −ssRNA portion. The +ssRNA portion is not directly translatable by ribosomes and must first be transcribed into the antigenome, which has the opposite sense as the ambisense genome. The −ssRNA portion of the antigenome is then transcribed into mRNA by the viral RdRp.

RNA viruses are more prone to mutation than DNA viruses. All polymerases, whether they use DNA or RNA as a template, introduce errors as they incorporate an incorrect nucleotide, but DNA-dependent DNA polymerases have **proofreading** ability: they can remove an incorrectly placed nucleotide and replace it with the correct one. RdRps, on the other hand, do not have proofreading ability. This raises the overall error rate of the enzyme, from 1 error per 10^9 bases for a DNA polymerase to greater than 1 error per 10^5 bases for an RdRp, which results in lower enzyme **fidelity**, or accuracy. RNA viruses have some of the highest mutation rates of all biological entities. Mutations generated by RdRps may result in mutated viral proteins and, subsequently, slightly different strains of the virus that may survive better under environmental pressures.

When more than one strain of virus enters a cell, **recombination** can occur. Recombination is the process by which a virus exchanges pieces of its genetic material with another strain of the same virus. This process, which can occur in dsRNA, +ssRNA, or −ssRNA viruses, occurs during genome replication when the RdRp, while copying one RNA genome template, switches to the template of another strain of the virus and continues replicating, thereby creating a hybrid genome that is different from either parent strain. The switching of templates has been shown to occur at random sequences or at complementary sequences on the two genome templates that base pair with each other.

Segmented viruses can also undergo **reassortment** when two strains of virus with segmented genomes enter and replicate within the same cell. When the genome segments are copied, segments from one virus may mix with segments from another virus when they are being packaged into new virions, creating a new strain of virus. This can be potentially dangerous when two strains of viruses from different subtypes reassort to create a viral strain that has not previously circulated within the human population.

6. *Class VI: RNA Viruses That Reverse Transcribe*

The first event that occurs after a +ssRNA or −ssRNA virus enters a host cell is translation or transcription, respectively. **Retroviruses** also have RNA genomes, but must **reverse transcribe** their genome before using host enzymes to transcribe it. In human cells, DNA is used as a template to create mRNA. Retroviruses, on the other hand, encode and carry within their virions an enzyme called **reverse transcriptase** (RT) that is an RNA-dependent DNA polymerase. Reverse transcriptase is able to reverse transcribe the ssRNA genome into a linear strand of double-stranded complementary DNA (cDNA), which is then integrated into a host chromosome.

There exist only a handful of retroviruses, and even fewer that infect humans (Table 4.3). The most well-studied human retrovirus is HIV, the virus that causes AIDS. Within the body, HIV infects and causes the slow decline of T lymphocytes, unarguably one of the most important immune system cells in the defense against pathogens of all kinds. People with HIV are diagnosed with AIDS when

the number of T lymphocytes in the blood falls below a certain number, indicating that the person's immune system is severely compromised (hence the origin of "immune deficiency syndrome" in the name of the disease). Without a functioning immune system, people with AIDS often succumb to **opportunistic infections** that healthy people would manage. It is often these opportunistic infections that ultimately cause death in people infected with HIV.

Retroviruses are unlike any other viruses because their genome is diploid, meaning that two copies of the genome are present within the virion. Other viruses are segmented and have their genomes in several segments, but the segments all encode different viral genes. The retrovirus genome is +ssRNA, although it does not serve as mRNA, like the genomes of +ssRNA viruses do. They are also unique among the RNA viruses because their genome will be copied by cellular enzymes, rather than an RdRp.

The retrovirus genome has several different domains that are of importance during viral replication. The two ends of the genome are flanked by redundant sequences, termed R. Inside of the R domain is the U5 (unique to the 5′) and U3 (unique to the 3′) domains on the 5′- and 3′-ends of the RNA, respectively. These domains will end up forming **long terminal repeats** (LTRs) on each side of the cDNA that will be important for **integration** of the HIV cDNA into the host's DNA.

Because the HIV reverse transcriptase enzyme is a target for drugs against HIV, it has been extensively studied and is the paradigm among retrovirus reverse transcriptases. RT is a heterodimer composed of two different-sized polypeptide chains, although one chain is just a slightly shorter version of the other. It is a unique enzyme because it can perform several enzymatic functions. It acts as an *RNA-dependent* DNA polymerase, a *DNA-dependent* DNA polymerase, and it also has RNase H activity, meaning that it is able to degrade RNA when bound to DNA. Although it is a DNA polymerase, it does not have proofreading ability, and is as error-prone as the RdRps, introducing an incorrect nucleotide once every 10^5 bases. It is also slow, about a 10th of the speed of DNA polymerase.

Only one of the two viral ssRNA strands is reverse transcribed by RT, although recombination can occur if the reverse transcriptase jumps to the other strand of viral ssRNA during reverse transcription. Initiation of reverse transcription requires a primer, which is provided by one of around 100 tRNAs (most of which are specific for lysine or proline) that the virus obtained from the previous host cell. The tRNA partially unwinds and complementary base pairs to 18 nucleotides within the **primer-binding site** (PBS), located toward the 5′-end of the viral RNA (Fig. 4.12). RT binds to the primer and extends its sequence, adding DNA nucleotides complementary to the viral RNA, until it reaches the end. This forms what is referred to as the **negative-strand strong-stop DNA**, because the RT has extended the negative strand and it stops when it reaches the end of the RNA template. The U5 and R sequences are copied in this segment.

The RNase H activity of RT degrades the RNA from the RNA–DNA hybrid, leaving the ssDNA U5 and R sequences. Because the R sequence in the DNA is complementary to the R sequence found on the 3′-end of the viral RNA, the two R sequences bind and the RT is able to continue extending the negative strand until the end, where the PBS sequence is found. After being reverse transcribed, the viral RNA is degraded by the RNase H activity of the enzyme, although a small portion resists being digested. This **polypurine tract (PPT)** is composed of purine nucleotides, namely adenine and guanine, and it acts as a primer for RT to begin reverse transcribing the DNA positive strand. This PPT is extended by RT in the 5′ → 3′ direction, through the U3, R, and U5 domains, until it reaches the tRNA primer, where it finishes the strand by copying 18 nucleotides into the tRNA primer. (You will recall that these 18 nucleotides of the tRNA initially unwound and bound to the PBS. By copying this segment of the tRNA, another PBS sequence has been created.) This segment is termed the **positive-strand strong-stop DNA**, because it is the positive DNA strand and came to a stop because it reached the end of the viral DNA template.

The tRNA is digested by the RT RNase H, which leaves available the PBS sequence. This anneals to its complementary sequence found at the end of the newly copied negative strand. This acts as the primer for RT to complete the positive strand. Similarly, the negative-strand is extended to the end of the positive-strand strong-stop DNA.

The resulting DNA is double stranded with repeated ends, termed LTRs, that are composed of the U3, R, and U5 domains (Fig. 4.12). These LTRs are important during the integration of this **proviral DNA** into the genome of the host cell. **Integrase** (IN), another necessary retroviral enzyme found within the virion, carries out the process of integration (Fig. 4.13). Having removed two base pairs from each end of the proviral DNA, IN creates a nick in the host chromatin and joins the proviral DNA to the host DNA. DNA repair enzymes within the cell seal the break.

At this point, the integrated viral DNA is like any other cellular gene and will be transcribed by the host RNA polymerase II. Promoter sequences within the U3 region are bound by host transcription factors that are recruited within activated cells, causing the production of several viral mRNAs through splicing and ribosomal frameshifting. Full-length mRNA is produced, complete with a 5′-cap and 3′-poly(A) tail, and two copies are packaged into **nascent** (newly formed) virions as the diploid viral genome.

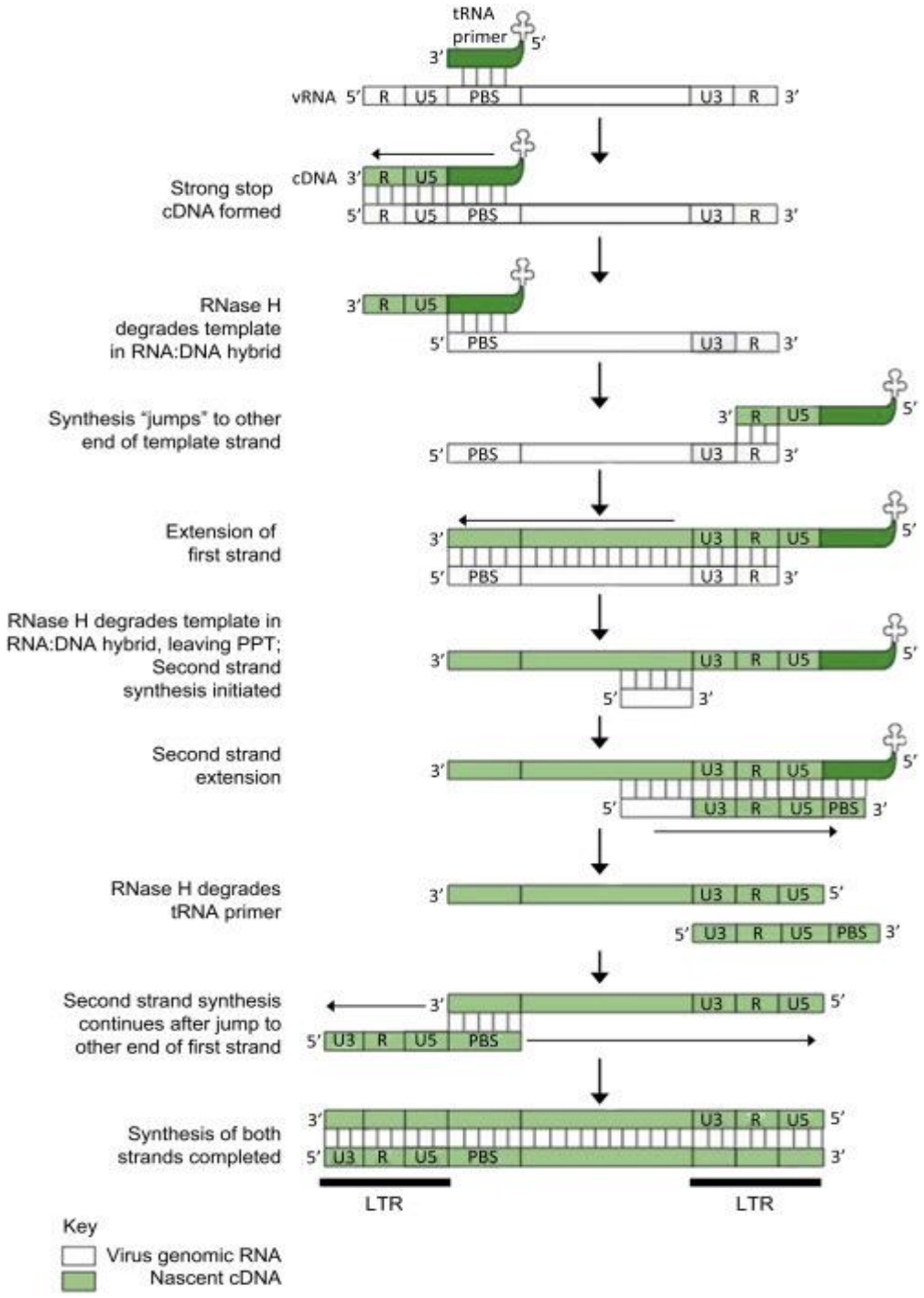


Figure 4.12 Retrovirus reverse transcription.

Reverse transcription begins with a cellular tRNA binding to the primer-binding site (PBS) in the genomic ssRNA. RT binds to and adds DNA nucleotides to the primer in the 5′ → 3′ direction, copying the U5 and R sequences and forming the "negative-strand strong-stop cDNA." RT RNase H activity degrades the RNA of the RNA:DNA section. The strong-stop cDNA is transferred to the 3′-end of the RNA and binds it because of the complementary R sites. Synthesis of DNA continues in the 5′→3′ direction, completing the entire negative-sense DNA strand. RNase H activity of RT degrades the RNA template, with the exception of the polypurine tract (PPT), which acts as a primer for the synthesis of the positive-sense DNA strand. RT binds to the PPT and extends it through the U3, R, and U5 domains, completing 18 nucleotides into the tRNA primer to create the new PBS. The tRNA is digested. This "positive-strand strong-stop cDNA" is transferred to the other end of the DNA template; RT completes replication of both strands, creating long terminal repeats (LTRs) on both ends of the double-stranded cDNA. (It is likely that the RNA template circularizes during reverse transcription, which facilitates the "transfer" of the strong-stop cDNAs to the other end of the strand.)

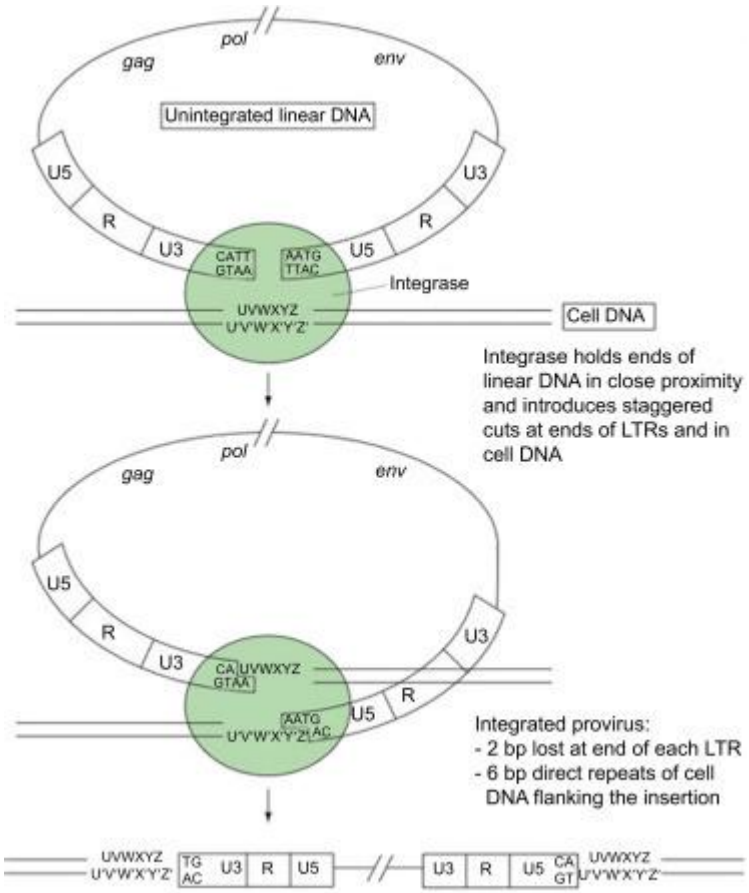


Figure 4.13 Integration of the retrovirus genome into the host chromosome.
Viral integrase removes two base pairs from each end of the proviral DNA, creates a nick in the host chromatin, and joins the proviral DNA to the host DNA. Cellular DNA repair enzymes seal the nick in the sugar–phosphate backbone of the DNA.

7. *Class VII: DNA Viruses That Reverse Transcribe*

Retroviruses are RNA viruses that reverse transcribe, but two families of DNA viruses also undergo reverse transcription during their replication within the cell. However, in contrast to retroviruses that undergo reverse transcription as one of the first events in the cell, DNA viruses that reverse transcribe do so at the end of their replication cycle in order to generate their DNA genomes.

Whether RNA or DNA, any virus that reverse transcribes is termed a **retroid virus**. Two DNA virus families fall into this category, *Caulimoviridae*, which infect plants, and the *Hepadnaviridae*, which infect animals. The only human virus within this family is hepatitis B virus (HBV). The virus infects the liver and can cause **hepatitis**, inflammation of the liver. Approximately 5% of infections with HBV become a long-term, **chronic** infection that can lead to liver scarring (cirrhosis) and even liver cancer.

Like other DNA viruses, the genome of hepadnaviruses must also be transported into the nucleus. The DNA genome is partially double stranded and partially single stranded. The complete strand is the negative-sense DNA, and the incomplete strand is positive-sense DNA (Fig. 4.15A).

This **relaxed circular DNA** (rcDNA) is transported into the nucleus, where the gapped segment is repaired into double-stranded DNA by a yet unidentified enzyme, completing the **covalently closed circular DNA** (cccDNA). The cccDNA is ligated and maintained as an **episome**, meaning that the circular dsDNA does not integrate into the host DNA but remains as a separate entity within the nucleus.

From the negative strand of episomal cccDNA, host RNA polymerase II transcribes viral mRNAs that leave the nucleus and are translated by host ribosomes to create viral proteins. RNA polymerase II also creates an RNA **pregenome** that leaves the nucleus. Interestingly, it functions as mRNA but also acts as the template for reverse transcription. Within the cccDNA are two identical sequences of 12 nucleotides, termed DR1 (direct repeat 1) and DR2 (direct repeat 2) (Fig. 4.15A). The pregenomic RNA (pgRNA) begins being translated on the negative strand of the DNA at a site upstream of DR1, and RNA polymerase II transcribes the entire negative strand, including the DR2 site. The dsDNA episome is circular, however, and RNA polymerase II continues past its initial starting point, terminating downstream of DR1 at a polyadenylation signal. The result is a long pgRNA that has repeating sequences at both ends that each include a copy of DR1. Part of the repeated sequence folds into a physical structure, known as epsilon (ε), that serves as the initial start of reverse transcription.

The pgRNA and the recently translated P protein are packaged into forming capsids. Similarly to the RT protein of retroviruses, the **P protein** functions as an RNA-dependent DNA polymerase, a DNA-dependent DNA polymerase, and an RNase H, removing RNA from RNA–DNA hybrids. The P protein binds to the ε structure and reverse transcribes a few base pairs from it (Fig. 4.15B). This functions as a primer that binds to the positive-sense pgRNA and reverse transcribes it, creating the complete negative strand of the DNA genome. The RNase H activity of P protein degrades the pgRNA from this strand, leaving a short RNA segment at the end that includes the repeated DR1 sequence. This RNA primer relocates to the other end of the DNA and binds to the identical DR2 sequence there (Fig. 4.15C). P protein extends this positive-sense DNA strand to the end of the negative-sense template. This creates the shorter positive-sense DNA strand within the HBV genome, which bridges the gap between the 5′- and 3′-ends of the negative-sense strand using complementary sequences. This completes genome replication and the creation of the rcDNA.

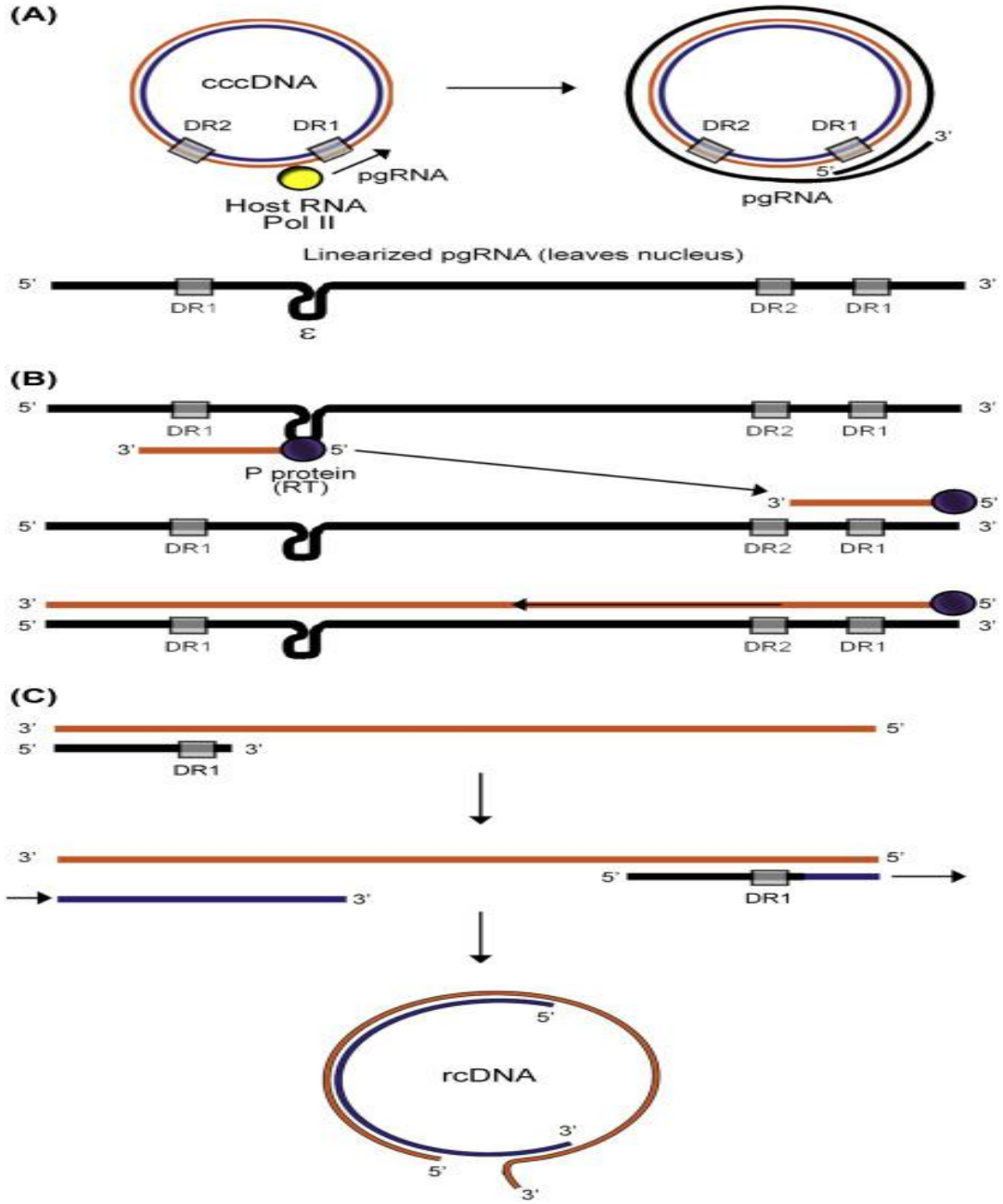


Figure 4.15 Reverse transcription of the HBV genome.

The HBV genome has identical repeated sequences termed DR1 and DR2. Once the rcDNA is repaired into closed circular DNA (cccDNA), cellular RNA polymerase II transcribes an RNA pregenome (A). It starts upstream of the DR1 site and completes a full circle, ending past the DR1 site where it began. This results in an RNA pregenome that has a 5′-DR1 site and 3′-DR2 and DR1 sites. In the cytoplasm, the pregenomic RNA (pgRNA) is encapsidated. The HBV reverse transcriptase, known as P protein, binds to the ε site and reverse transcribes DNA in the 5′→3′ direction (B). This small piece acts as a DNA primer, which jumps to the other end of the pgRNA, binding to the complementary sequence found there. P protein complex the reverse transcription of the negative DNA strand and degrades most of the RNA:DNA duplex. (C) The remaining RNA segment is transferred to the other end of the negative-sense DNA strand, binding to the complementary DR2 sequence found there. It continues synthesizing DNA in the 5′→3′ direction; because of complementary sequences, the genome circularizes and the P protein completes the synthesis of the shorter positive-sense DNA strand.

4.5. **Assembly**

Viruses are created from newly synthesized components, and to be released from the cell, those components must be collected at a particular site of the cell and undergo assembly to form an immature virus particle. In the same way that penetration and uncoating are difficult to separate in the cycle of some viruses, assembly can often occur alongside maturation and release.

The location of virion assembly will depend upon the particular virus. It can take place within the nucleus of the cell, at the plasma membrane, or at a variety of intracellular membranes, such as the Golgi complex. Most nonenveloped DNA viruses assemble their nucleocapsid in the nucleus, since that is the site of genome replication. Viral proteins are imported through nuclear pores to reach the site of assembly. When assembled, most DNA viruses are too large to fit through nuclear pores, however. At this point, some viruses are able to traverse the double-membraned nuclear envelope, while others induce cell lysis or apoptosis to escape the nucleus. On the other hand, viruses with envelopes derived from the plasma membrane usually assemble there.

The nucleic acid genome of a helical virus is protected by repeating capsid proteins. Because of this, capsid proteins can begin wrapping the genome as soon as it is copied (or vice versa, depending upon the virus: the genome can be wrapped around capsid proteins; Fig. 4.16). In contrast, some icosahedral viruses nearly complete the assembly of their capsids before the nucleic acid genome is inserted.

Spontaneous assembly of the capsid, termed “self-assembly,” occurs with the capsid proteins of simple icosahedral viruses, such as the picornaviruses and parvoviruses. The assembly of viruses with more complex architecture is orchestrated by a variety of viral chaperone proteins called scaffolding proteins. Herpesviruses and adenoviruses are examples of large icosahedral viruses that assemble with scaffolding protein assistance.

6. **Maturation**

After the nucleic acid genome and other essential proteins are packaged within the capsid, which was assembled from one or several translated viral proteins, the final steps of virus replication occur: maturation and release. Up to this point, the virion had been in the process of forming, and if the cell were broken open at this point, the virions would not be able to initiate infection of new cells. Maturation refers to the final changes within an immature virion that result in an infectious virus particle. Structural capsid changes are often involved, and these can be mediated by host enzymes or virus-encoded enzymes. A good example involves the influenza HA protein. It is involved in attachment to the cell’s sialic acid, as described above, and the HA protein is able to bind sialic acid after being glycosylated (via posttranslational modification).

However, the HA protein must be cleaved into two portions, HA1 and HA2, to become infectious, because although the HA1 portion binds the cell surface receptor, the HA2 portion is what fuses the viral envelope to the endosomal membrane to release the virus into the cytoplasm. This cleavage of HA into HA1 and HA2 is carried out by cell proteases (enzymes that cleave proteins). In contrast, the HIV core particle is composed of proteins encoded by the gag gene. The gene is translated into a polyprotein that is cleaved by the viral protease to form the capsid, matrix, and nucleocapsid proteins of the virion. In this case, maturation occurs after the virion has been released from the cell surface (Fig. 4.17) and is required to form an infectious virion. Discussed in Chapter 8, "Vaccines, Antivirals, and the Beneficial Uses of Viruses," several anti-HIV drugs work by inhibiting the action of the HIV protease, thereby preventing the cleavage of the polyprotein and subsequent formation of an infectious virion.

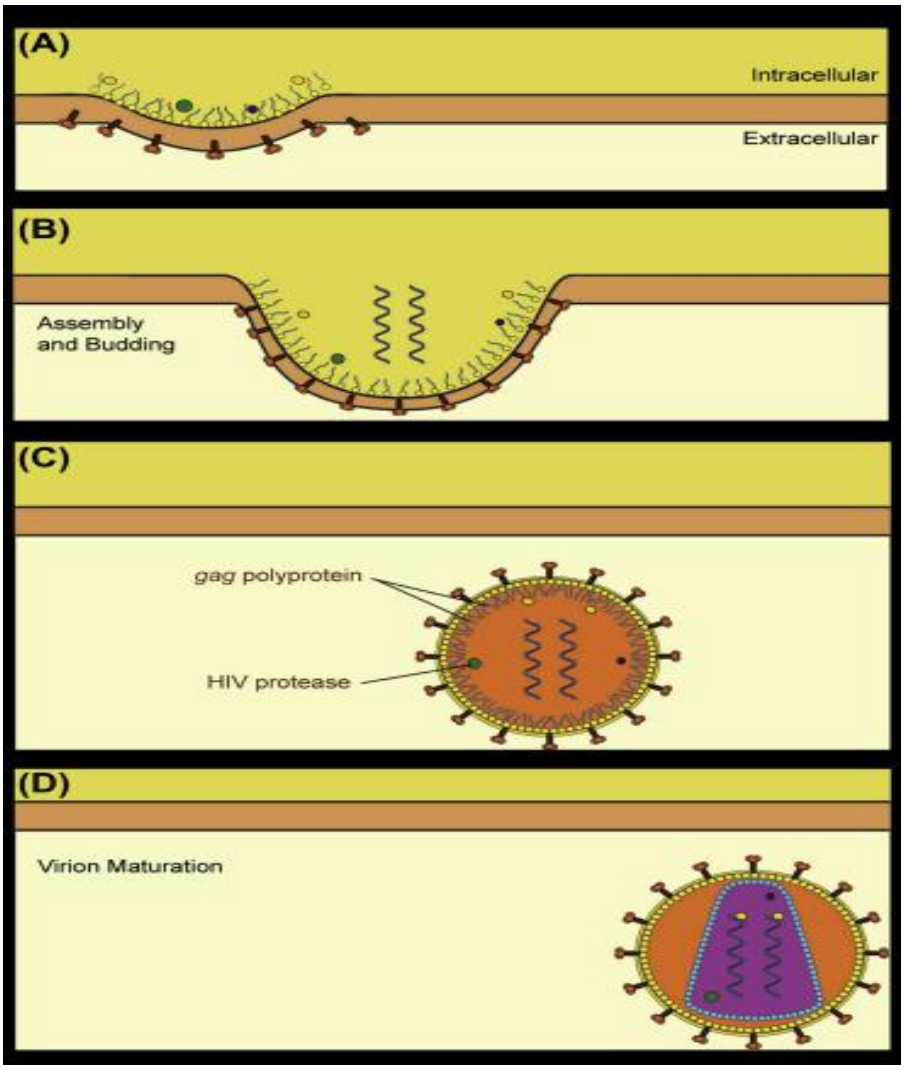


Figure 4.17 Assembly, release, and maturation of HIV virions.

(A) HIV proteins congregate at the plasma membrane of the cell, causing a bud to form in the membrane. The diploid RNA genome is packaged into the assembling capsid (B). The virus is released from the cell membrane, but the Gag polyprotein has not yet been cleaved to separate the capsid and matrix proteins of the virion (C). The HIV protease cleaves the polyprotein, allowing the proteins to complete the infectious virion architecture (D).

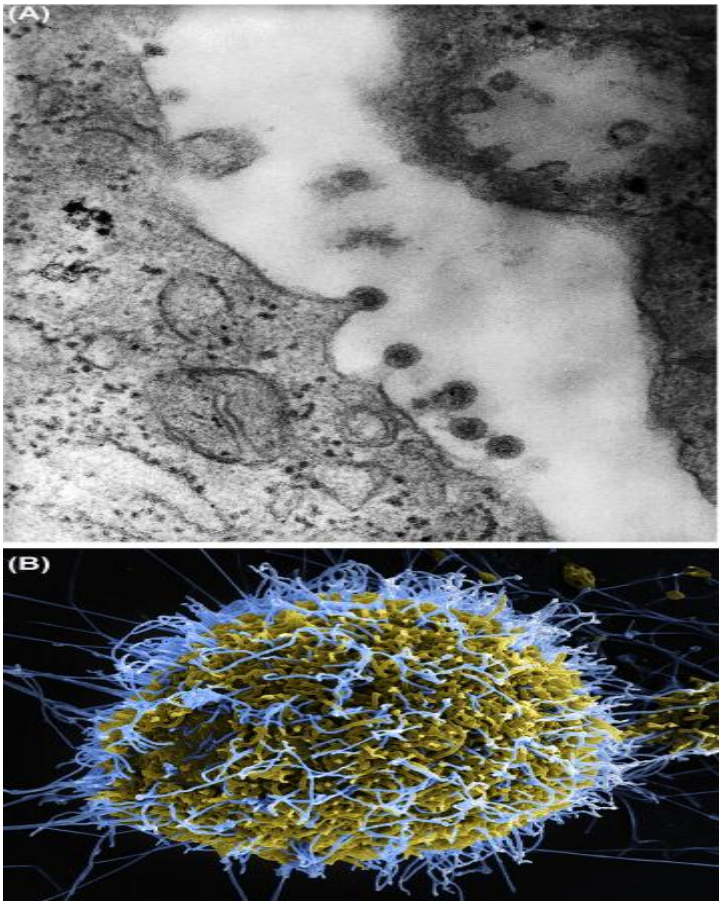
7. Release

The final step in the virus replication cycle is release of the virion into the extracellular environment, where it can continue the cycle of infection with new cells. Release can occur in several different manners, depending upon the virus. Viruses that obtain their envelope from the plasma membrane generally assemble on the inside layer of the plasma membrane, embedding their envelope proteins into the plasma membrane. As the viral capsid proteins interact, the membrane-associated viral proteins cause the plasma membrane to begin curving around the capsid. This continues until the plasma membrane is completely wrapped around the virus, which leaves the cell. This process is known as budding (Fig. 4.17A and B; Fig. 4.18).

Viruses can bud from any of the membrane systems within the cell, including the rER, Golgi complex, or even the nuclear envelope. In this case, the already enveloped virion does not need to bud through the plasma membrane. It generally undergoes exocytosis to leave the cell.

Nonenveloped viruses can also exit the cell via exocytosis. **Lytic** viruses, however, disrupt the plasma membrane and cause the **lysis**, or bursting, of the cell. This releases the nascent virions to infect new cells. Many nonenveloped human viruses are released through cell lysis.

The processes of assembly, maturation, and release are closely linked, but all are required to create progeny infectious virions able to continue the cycle of infection.



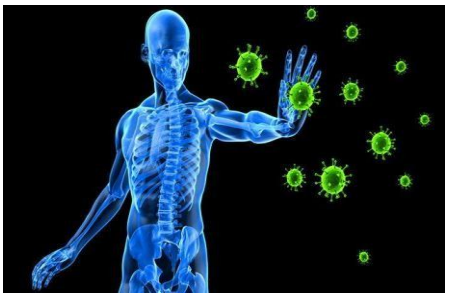
Virion budding.
(A) Rubella virus virions are observed budding from the host plasma membrane in this transmission electron micrograph. (B) In this digitally enhanced pseudocolored scanning electron micrograph, helical Ebolavirus virions (blue) are budding from an infected cell (yellow).

5. Immunology:

- Overview of the immune system.
- Innate immunity and adaptive immunity, major histocompatibility complex (MHC) and their role in antigen presentation, cytokines.
- Antigen- chemical nature, types; hapten, adjuvant.
- Monoclonal and polyclonal antibodies.
- Antigen-antibody reaction.
- Hypersenitivity and allergy.
- Vaccines and vaccination.
- Immunological techniques- ELISA, RIA, Immunofluorescence, Immuno electrophoresis, Flow cytometry, Fluorescence-Activated Cell Sorting (FACS).

Part-1: Introduction: overview of the immune system:

The immune system is a host defence system comprising many biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, known as pathogens, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue.



In many species, there are two major subsystems of the immune system: the innate immune system and the adaptive immune system. Both subsystems use humoral immunity and cell-mediated immunity to perform their functions. In humans, the blood–brain barrier, blood– cerebrospinal fluid barrier, and similar fluid–brain barriers separate the peripheral immune system from the neuroimmune system, which protects the brain.

Pathogens can rapidly evolve and adapt, and thereby avoid detection and neutralization by the immune system; however, multiple defense mechanisms have

also evolved to recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess a rudimentary immune system in the form of enzymes that protect against bacteriophage infections. Other basic immune mechanisms evolved in ancient eukaryotes and remain in their modern descendants, such as plants and invertebrates.

These mechanisms include phagocytosis, antimicrobial peptides called defensins, and the complement system. Jawed vertebrates, including humans, have even more sophisticated defence mechanisms, including the ability to adapt over time to recognize specific pathogens more efficiently. Adaptive (or acquired) immunity creates immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination.

Part- 2: Cells and organs of Immune system:

i. **Hematopoietic stem cells:** All functionally specialized, mature blood cells (red blood cells, granulocytes, macrophages, dendritic cells, and lymphocytes) arise from a single cell type, the hematopoietic stem cell (HSC). The process by which HSCs differentiate into mature blood cells is called hematopoiesis. Two primary lymphoid organs are responsible for the development of stem cells into mature immune cells: the bone marrow, where HSCs reside and give rise to all cell types; and the thymus, where T cells complete their maturation.HSCs are rare—fewerthan one HSC is present per 5 $x10^4$ cells in the bone marrow—and their numbers are strictly controlled by a balance of cell division, death, and differentiation. Under conditions where the immune system is not being challenged by a pathogen (steady state or homeostatic conditions), most HSCs are quiescent. A small number divide, generating daughter cells. Some daughter cells retain the stem-cell characteristics of the mother cell—that is, they remain self-renewing and able to give rise to all blood cell types. Other daughter cells differentiate into progenitor cells that lose their self-renewal capacity and become progressively more committed tparticular blood cell lineage. As an organism ages, the number of HSCs decreases, demonstrating that there are limits to an HSC's self-renewal potential. When there is an increased demand for hematopoiesis (e.g., during an infection or after chemotherapy), HSCs display an enormous proliferative capacity. This can be demonstrated inmice whose

hematopoietic systems have been completely destroyed by a lethal dose of x-rays (950 rads). Such irradiated mice die within 10 days unless they are infused with normal bone marrow cells from a genetically identical mouse.

Although a normal mouse has 3108 bone marrow cells, infusion of only 104 to 105 bone marrow cells from a donor is sufficient to completely restore the hematopoietic system, which demonstrates the enormous capacity of HSCs for self-renewal. Our ability to identify and purify this tiny subpopulation has improved considerably, and investigators can now theoretically rescue irradiated animals with just a few purified stem cells, which give rise to progenitors that proliferate rapidly and populate the blood system relatively quickly.

Hematopoiesis Is the Process by Which Hematopoietic Stem Cells Develop into Mature Blood Cells. An HSC that is induced to differentiate (undergo hematopoiesis) loses its self-renewal capacity and makes one of two broad lineage commitment choices (see Figure 2-1). It can become a common myeloid-erythroid progenitor (CMP), which gives rise to all red blood cells (the erythroid lineage), granulocytes, monocytes, and macrophages (the myeloid lineage), or it can become a common lymphoid progenitor (CLP), which gives rise to B lymphocytes, T lymphocytes, and NK cells. Myeloid cells and NK cells are members of the innate immune system, and are the first cells to respond to infection or other insults. Lymphocytes are members of the adaptive immune response and generate a refined antigen specific immune response that also gives rise to immune memory. As HSCs progress along their chosen lineages, they lose the capacity to contribute to other cellular lineages. Interestingly, both myeloid and lymphoid lineages give rise to dendritic cells, antigen- presenting cells with diverse features and functions that play an important role in initiating adaptive immune responses. The concentration and frequency of immune cells in blood are listedin Table 2-1.

TABLE 2-1 Concentration and frequency of cells in human blood

Cell type	Cells/mm^3	Total leukocytes (%)
Red blood cells	5.0×10^6	
Platelets	2.5×10^5	
Leukocytes	7.3×10^3	
Neutrophil	$3.7–5.1 \times 10^3$	50–70
Lymphocyte	$1.5–3.0 \times 10^3$	20–40
Monocyte	$1–4.4 \times 10^2$	1–6
Eosinophil	$1–2.2 \times 10^2$	1–3
Basophil	$<1.3 \times 10^2$	<1

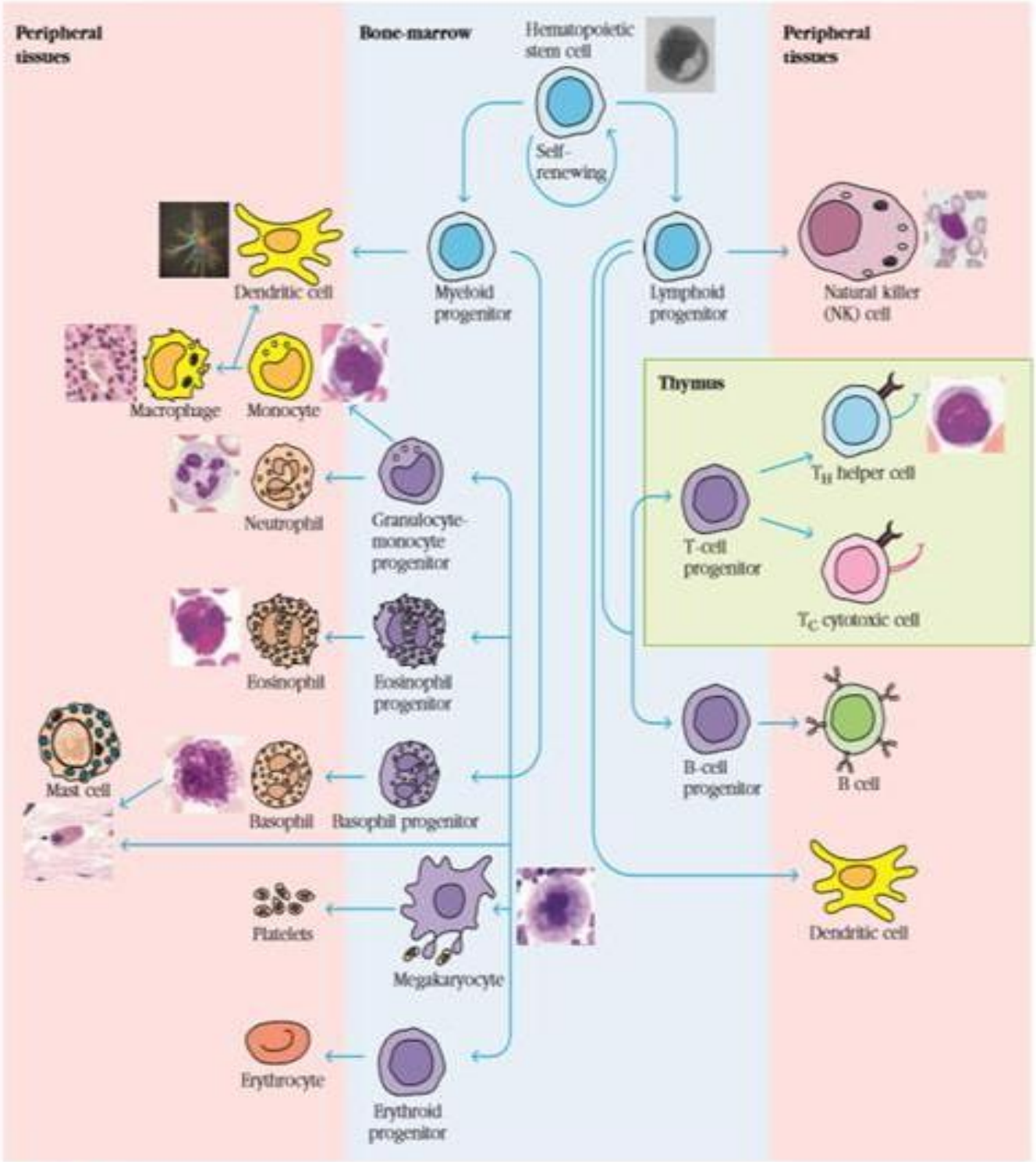
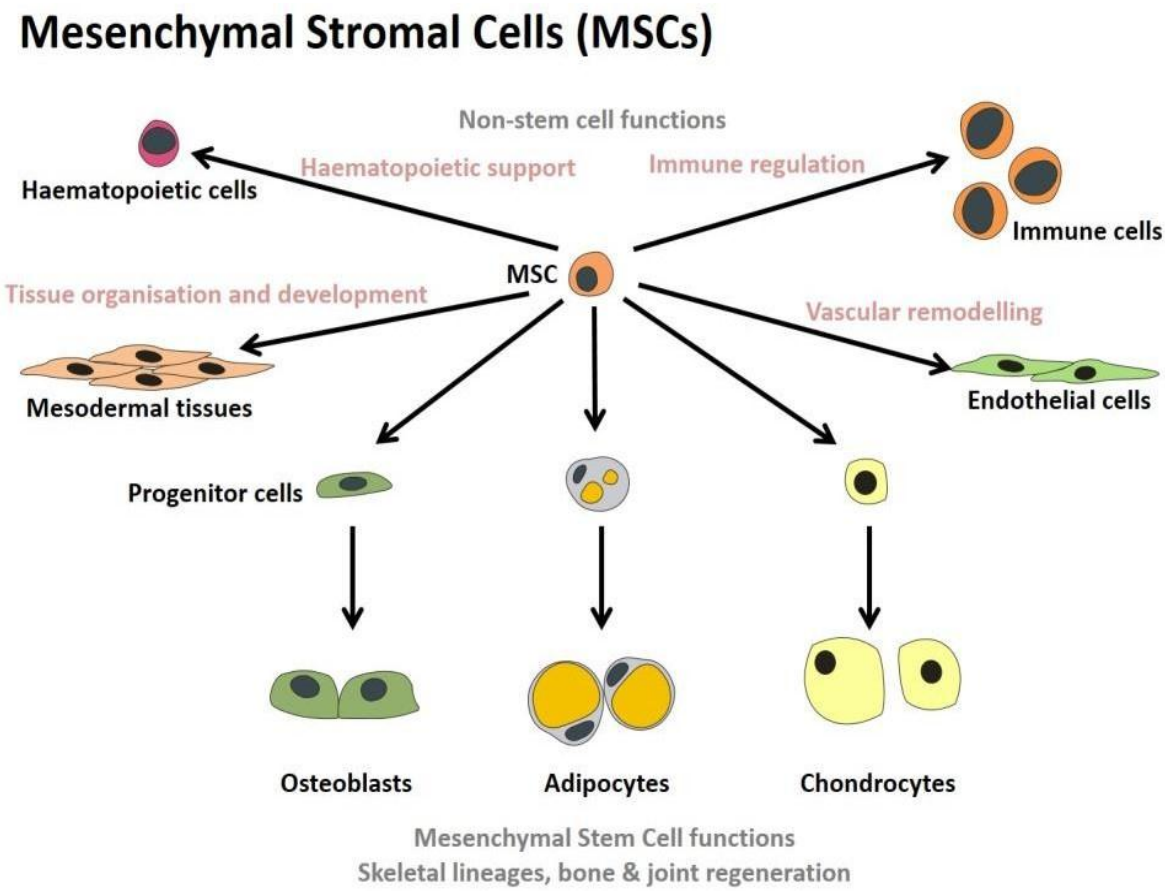


FIGURE 2-1 Hematopoiesis. Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors. Most immune cells mature in the bone marrow and then travel to peripheral organs via the blood. Some, including mast cells and macrophages, undergo further maturation outside the bone marrow. T cells develop to maturity in the thymus.

ii. Stromal cell:

Stromal cells are connective tissue cells of any organ, for example in the uterine mucosa (endometrium), prostate, bone marrow, lymph node and the ovary. They are cells that support the function of the parenchymal cells of that organ. The most common stromal cells include fibroblasts and pericytes. The term stromal comes from Latin stromat-, "bed covering", and Ancient Greek strôma, "bed". The interaction between stromal cells and tumor cells is known

to play a major role in cancer growth and progression. In addition, by regulating local



cytokine networks (e.g. M-CSF, LIF), bone marrow stromal cells have been described to be involved in human haematopoiesis and inflammatory processes. Stromal cells (in the dermis layer) adjacent to the epidermis (the top layer of the skin) release growth factors that promote cell division. This keeps the epidermis regenerating from the bottom while the top layer of cells on the epidermis are constantly being "sloughed" off the body. Additionally, stromal cells play a role in inflammation responses, and controlling the amount of cells accumulating at an inflamed region of tissue. Certain types of skin cancers (basal cell carcinomas) cannot spread throughout the body because the cancer cells require nearby stromal cells to continue their division. The loss of these stromal growth factors when the cancer moves throughout the body prevents the cancer from invading other organs.Stroma is made up of the non-malignant cells, but can provide an extracellular matrix on which tumor cells can grow. Stromal cells may also limit T-cell proliferation via nitric oxide production, hindering immune capability.

iii. Hematopoietic growth factors:

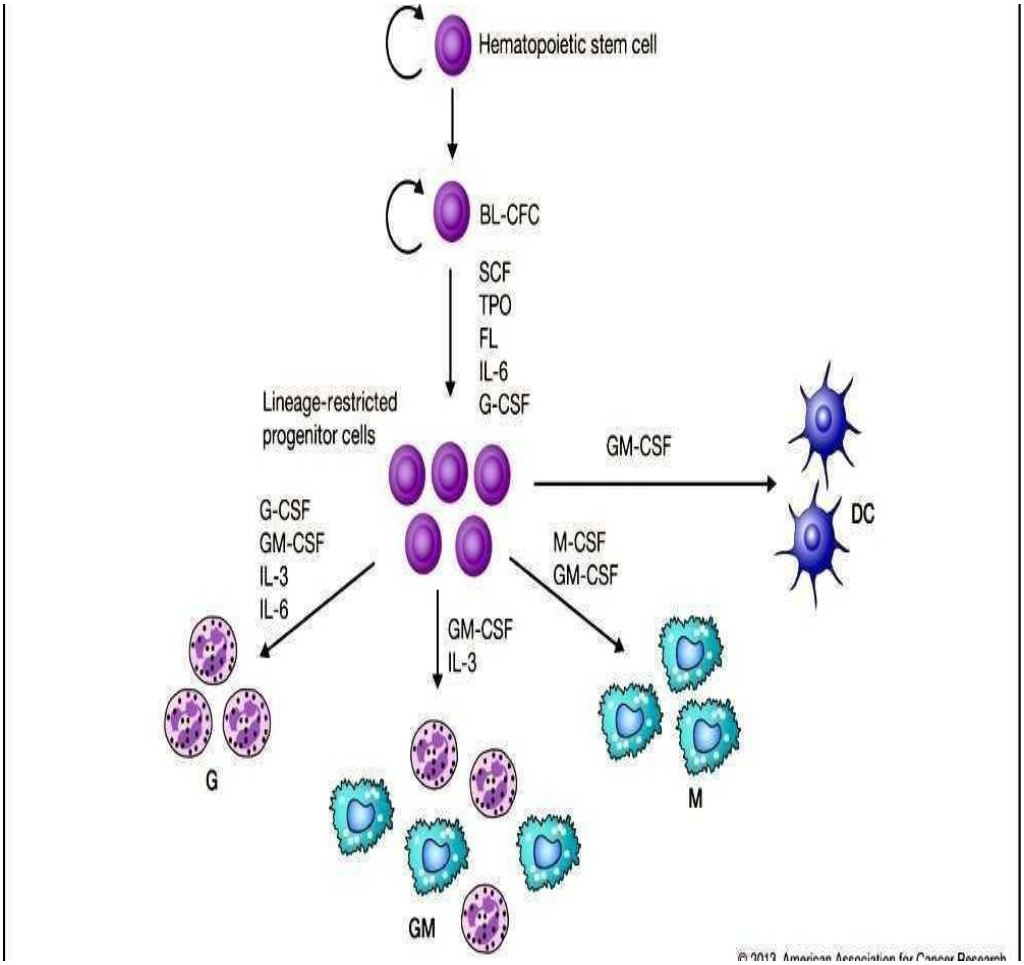
Hematopoietic growth factors are a family of cytokines that interact with specificreceptors on hematopoietic cells. Thesemolecules regulate the functional activation of the specific cells with which theyinteract and are required for the survival,proliferation, and differentiation of

hematopoietic progenitors. The development of recombinant DNA technologyhas made it possible to synthesize and purify pharmacologic doses of a variety ofhematopoietic growth factors. A numberof growth factors have been studied inclinical trials since the mid-1980s, including granulocyte colony-stimulatingfactor (G-CSF), granulocyte-macrophagecolony-stimulating factor (GM-CSF),macrophage colony-stimulating factor(M-CSF), erythropoietin (EPO), interleukin-3 (IL-3), and, more recently, themolecule putatively thought to representthrombopoietin.

G-CSF:G-CSF acts primarily on the neutrophil component of the blood. Its action occurs by a variety of mechanisms, including stimulation of granulocyte colonies, differentiation of progenitor cells toward neutrophil lineage, and stimulation of neutrophil maturation. Overall, it increases the number of neutrophils capable of fighting bacteria. G-CSF is available in various formulations throughout the world. In the United States, it is available as filgrastim. G-CSF can be administered subcutaneously or intravenously. Depending on the specific use, the dose ranges from 5 to 10 μg/kg per day. It should not be administered 24 hours prior to or 24 hours after chemotherapy. G-CSF is usually continued until the absolute neutrophil count has been greater than 500 cells/mm3 (500,000 cells/mL) for at least 3 days. G-CSF is clinically indicated for use in:

- Chemotherapy-induced neutropenia
- Collection of stem cells for transplantation
- Bone marrow or peripheral stem cell transplantation
- Congenital neutropenia

The major side effect of G-CSF is bone pain (due to the expansion of the cell population within the marrow). Transient minor side effects that have been reported with relative frequency include fever, hyperuricemia, and skin rash. Rarely, severe reactions such as anaphylaxis, capillary leak syndrome, and diffuse alveolar haemorrhage have been reported, although a causal relationship between these reactions and filgrastim administration is yet to be determined.

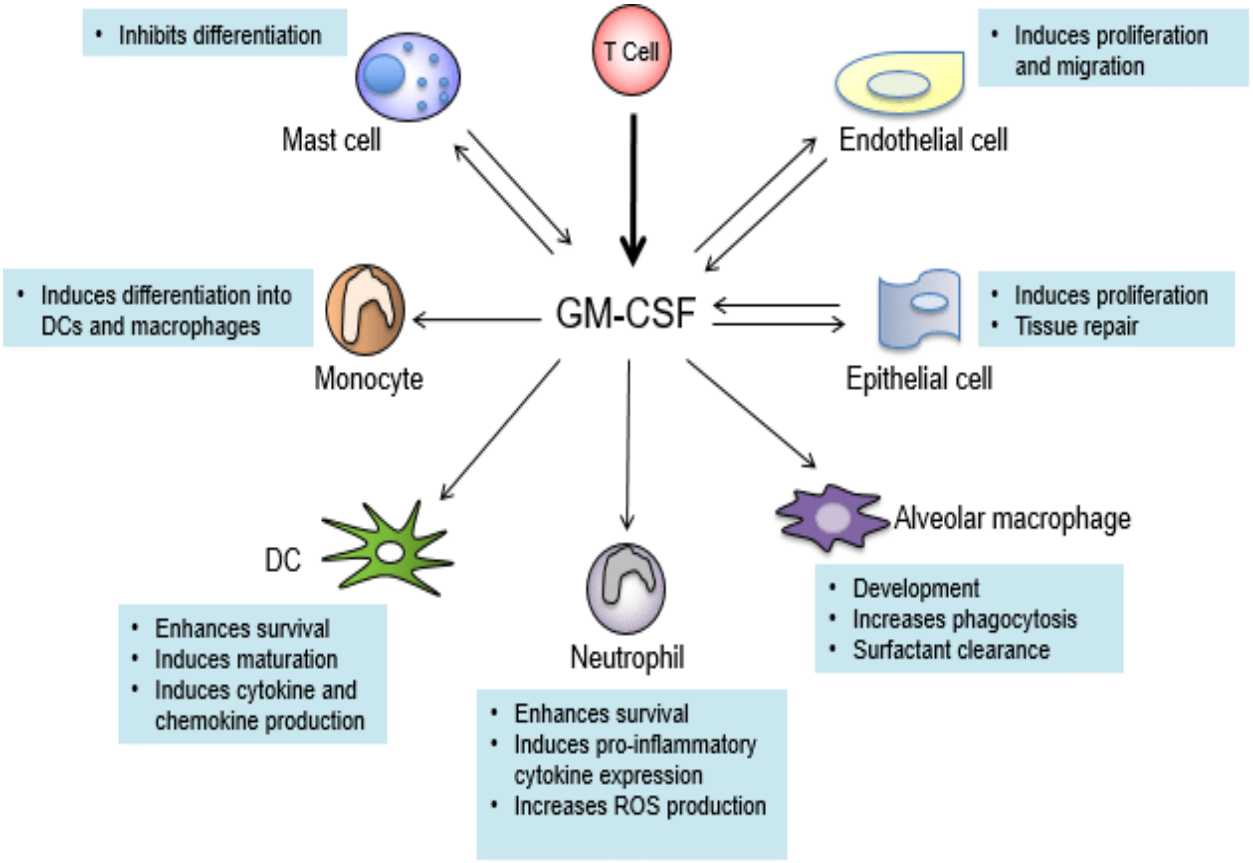


GM-CSF:

GM-CSF exerts its effect via stimulation of colonies containing neutrophils, eosinophils, and monocytes (Fig 1). In the clinical setting, the effects of GM-CSF include increasing the number of neutrophils, eosinophils, and monocytes and improving the function of mature neutrophils, eosinophils, and monocytes. GM-CSF is available as sargramostim in the United States. The dose of GM-CSF varies from 250 to 500 µg/m2, depending on the specific use.5 It is approved for use in:

- Chemotherapy-induced neutropenia
- Bone marrow or peripheral stem cell transplantation
- Collection of stem cells for transplantation

GM-CSF has many side effects similar to GCSF, although the frequency of these may be higher due to its effect on inflammatory cytokines. These side effects include skin rash, diarrhoea, fever, malaise, chills, and headaches.

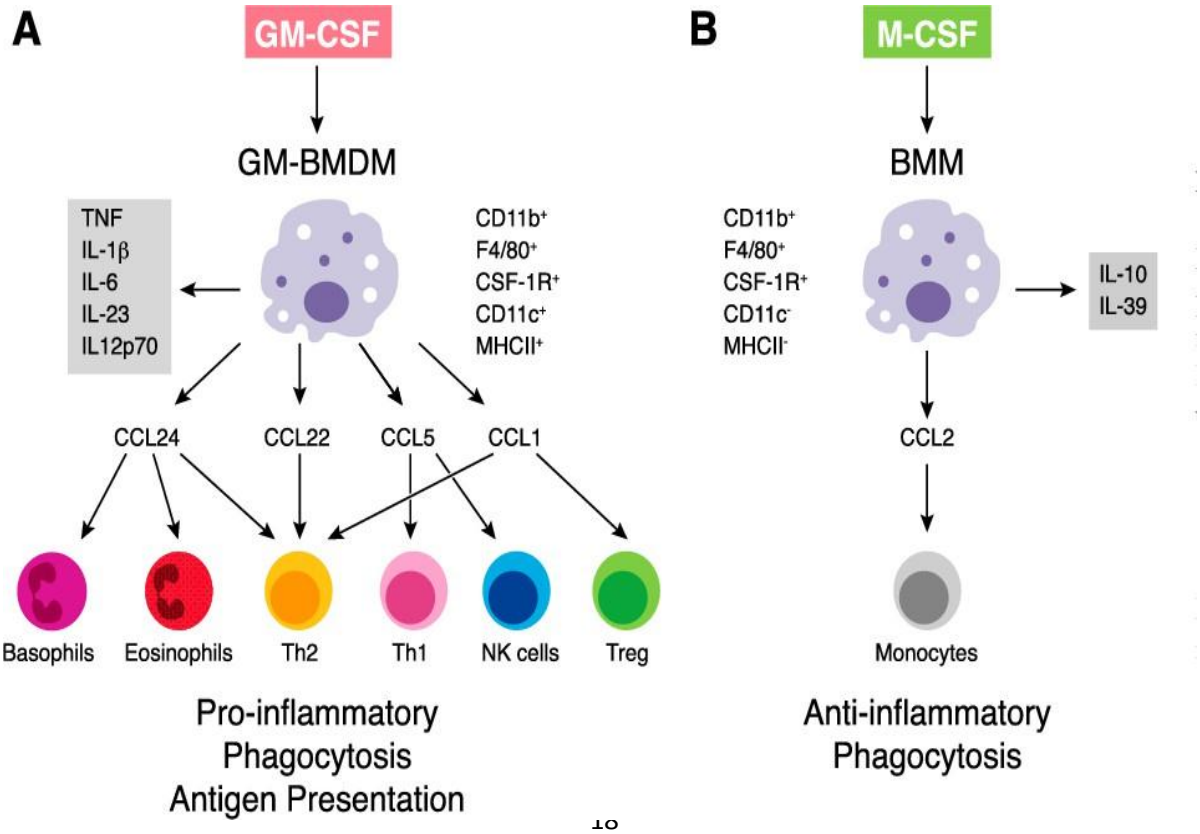


M-CSF: Macrophage colony-stimulating factor (M-CSF), is a secreted cytokine which causes hematopoietic stem cells to differentiate into macrophages or other related cell types. Eukaryotic cells also produce M-CSF in order to combat intercellular viral infection. It is one of the three experimentally described colony-stimulating factors. M-CSF binds to the colony stimulating factor 1 receptor. It may also be involved in development of the placenta.

M-CSF is a hematopoietic growth factor that is involved in the proliferation, differentiation, and survival of monocytes, macrophages, and bone marrow progenitor cells. M-CSF affects macrophages and monocytes in several ways, including stimulating increased phagocytic and chemotactic activity, and increased tumour cell cytotoxicity. The role of M-CSF is not only restricted to the monocyte/macrophage cell lineage. By interacting with its membrane receptor (CSF1R or M-CSF-R encoded by the c-fms proto-oncogene), M-CSF also modulates the proliferation of earlier hematopoietic progenitors and influence numerous physiological

processes involved in immunology, metabolism, fertility and pregnancy. M-CSF released by osteoblasts (as a result of endocrine stimulation by parathyroid hormone) exerts paracrine effects on osteoclasts. M-CSF binds to receptors on osteoclasts inducing differentiation, and ultimately leading to increased plasma calcium levels—through the resorption (breakdown) of bone. Additionally, high levels of CSF-1 expression are observed in the endometrial epithelium of the pregnant uterus as well as high levels of its receptor CSF1R in the placental trophoblast. Studies have shown that activation of trophoblastic CSF1R by local high levels of CSF-1 is essential for normal embryonic implantation and placental development. More recently, it was discovered that CSF-1 and its receptor CSF1R are implicated in the mammary gland during normal development and neoplastic growth.

Locally produced M-CSF in the vessel wall contributes to the development and progression of atherosclerosis. M-CSF has been described to play a role in renal pathology including acute kidney injury and chronic kidney failure. The chronic activation of monocytes can lead to multiple metabolic, hematologic and immunologic abnormalities in patients with chronic kidney failure. In the context of acute kidney injury, M-CSF has been implicated in promoting repair following injury, but also been described in anopposing role, driving proliferation of a pro-inflammatory macrophage phenotype.



Erythropoietin (EPO): Erythropoietin stimulates stem cells toward the production of RBCs. Its clinical result is usually not seen before 7 days after initiating therapy and may take as long as 14 days. It can be administered intravenously, intramuscularly, or subcutaneously. Prior to initiating therapy, one should ascertain that the patient has adequate iron stores to sustain the increased haemoglobin production. Erythropoietin is indicated for treating patients with anaemia from a variety of causes, including:

- chronic renal failure (with or without dialysis)
- AIDS
- Chemotherapy
- Neonatal anaemia due to prematurity
- Rheumatoid arthritis

It is also indicated to prevent or reduce the need for allogeneic blood transfusions in surgical patients. The dose of erythropoietin ranges from 50-150U/kg 3 times weekly for most patients, although in chemotherapy patients and those undergoing elective surgery, the dose can be increased to as high as 300 U/kg. Studies have demonstrated that erythropoietin can be effective at 40,000 U once a week. This dose schedule has received approval from the Food and Drug Administration. Once the haematocrit has reached the 30% to 36% (0.30-0.36) range, the erythropoietin should be reduced to a maintenance dose. The side effects of erythropoietin are minimal with the predominantly reported adverse events being bone pain, headaches, hypertension, and, rarely, thrombocytosis with venous fistula occlusion.

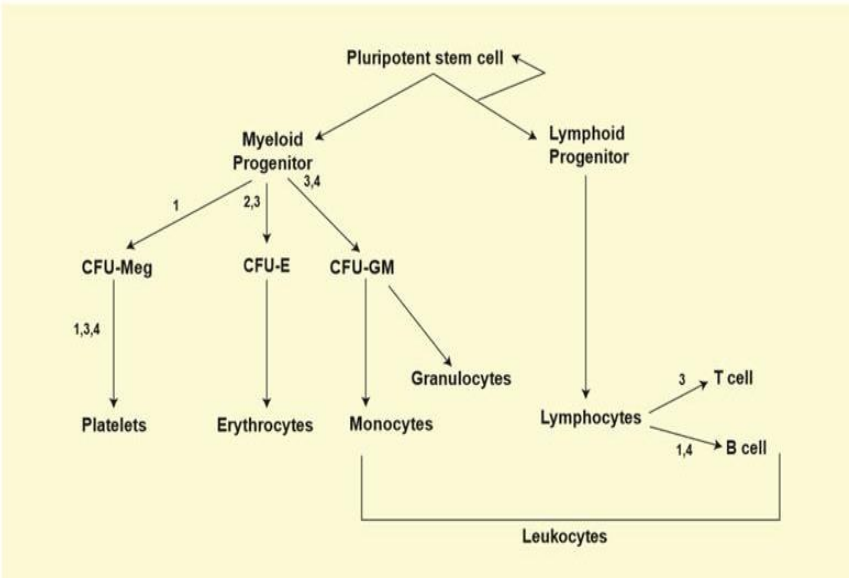


Fig 1. Differentiation of the pluripotent stem cell with the major sites of action of the various growth factors. CFU, colony-forming units; CSF, colony-stimulating factor; E, erythrocyte; G, granulocyte; GM, granuloctye-macrophage; IL, interleukin; Meg, megakaryocyte; 1, IL-11; 2, erythropoietin; 3, GM-CSF; 4, G-CSF.

Hematopoietic Growth Factors Currently Approved for Clinical Use

Growth Factor	Synonyms	Indications
Granulocyte colony-stimulating factor	G-CSF, rG-CSF, rhG-CSF, filgrastim	Chemotherapy-induced neutropenia; stem cell collection for transplantation; bone marrow and/or peripheral stem cell transplant; congenital neutropenia, idiopathic neutropenia, cyclic neutropenia
Granulocye-macrophage colony-stimulating factor	GM-CSF, sargramostim, rGM-CSF, rhGM-CSF	Chemotherapy-induced neutropenia; stem cell collection for transplantation; bone marrow and/or peripheral stem cell transplant
Erythropoietin	Epoetin alfa, epoetin beta	Anemia associated with chronic renal failure, chemotherapy, AIDS, prematurity, rheumatoid arthritis, and prior to elective surgery to reduce need for allogeneic blood transfusions
Oprelvekin interleukin-11	rhIL-11, rIL-11, recombinant human	Prevention of chemotherapy-induced severe thrombocytopenia

CSF indicates colony-stimulating factor; G, granuloctye; GM, granuloctye-macrophage; IL, interleukin.

iv. Lymphoid organs:

Lymphoid organs, such as the spleen, thymus, and bone marrow, are innervated. This fact indicates that the CNS is in permanent contact with the IS. In addition, neurotransmitters and neuropeptides regulate inflammation and immunity.The mediators involved may stimulate or inhibit immune and inflammatory processes. Substance P, neurokinin A and B (collectively known as tachykinins), calcitonin gene-related peptide (CGRP), and vasoactive intestinal peptide (VIP) are proinflammatory mediators capable of eliciting an inflammatory response. These mediators also enhance various immune responses. In contrast, somatostatin and galanin are anti-inflammatory and immunosuppressive mediators.

➢ Primary Lymphoid Organs—Where Immune Cells Develop:

The ability of any stem cell to self-renew and differentiate depends on the structural organization and cellular function of specialized anatomic microenvironments known as stem cell niches. These sequestered regions are typically populated by a supportive network of stromal cells. Stem cell niche stromal cells express soluble and membrane-bound proteins that regulate cell survival, proliferation, differentiation, and trafficking. The organs that have microenvironments that support the differentiation of hematopoietic stem cells actually

change over the course of embryonic development. However, by mid to late gestation, HSCs take up residence in the bone marrow, which remains the primary site of haematopoiesis throughout adult life. The bone marrow supports the maturation of all erythroid and myeloid cells and, in humans and mice, the maturation of B lymphocytes (as described in Chapter 10). HSCs are also found in blood and may naturally recirculate between the bone marrow and other tissues. This observation has simplified the process used to transplant blood cell progenitors from donors into patients who are deficient (e.g., patients who have undergone chemotherapy). Whereas once it was always necessary to aspirate bone marrow from the donor—a painful process that requires anesthesia—it is now sometimes possible to use enriched hematopoietic precursors from donor blood, which is much more easily obtained. Unlike B lymphocytes, T lymphocytes do not complete their maturation in the bone marrow. T lymphocyte precursors need to leave the bone marrow and travel to the unique microenvironments providedby the other primary lymphoid organ, the thymus, in order to develop into functional cells.

- *The Bone Marrow Provides Niches for Hematopoietic Stem Cells to Self- Renew and Differentiate into Myeloid Cells and B Lymphocytes:*

The bone marrow is a primary lymphoid organ that supports self-renewal and differentiation of hematopoietic stem cells (HSCs) into mature blood cells. Although all bones contain marrow, the long bones (femur, humerus), hip bones (ileum), and sternum tend to be the most active sites of haematopoiesis. The bone marrow is not only responsible for the development and replenishment of blood cells, but it is also responsible for maintaining the pool of HSCs throughout the life of an adult vertebrate. The adult bone marrow (Figure 2-5), the paradigmatic adult stem cell niche, contains several cell types that coordinate HSC development, including (1) osteoblasts, versatile cells that both generate bone and control the differentiation of HSCs, (2) endothelial cells that line the blood vessels and also regulate HSC differentiation, (3) reticular cells that send processes connecting cells to bone and blood vessels, and, unexpectedly, (4) sympathetic neurons, which can control the release of hematopoietic cells from the bone marrow. A microscopic cross-section reveals that the bone marrow is tightly packed with stromal cells and hematopoietic cells at every stage of differentiation. With age, however, fat cells gradually replace 50% or more of the bone marrow compartment, and the efficiency of haematopoiesis decreases. The choices that an HSC makes depend largely on the environmental cues it receives. The bone marrow is packed with hematopoietic cells at all stages of development, but it is likely that the precursors of each myeloid and lymphoid subtype mature in distinct environmental micro-

niches within the bone marrow. Our understanding of the microenvironments within the bone marrow that support specific stages of haematopoiesis is still developing. Evidence suggests, however, that the endosteal niche (the area directly surrounding the bone and in contact with bone-producing osteoblasts) and the vascular niche (the area directly surrounding the blood vessels and in contact with endothelial cells) play different roles (see Figure 2-5c). The endosteal niche appears to be occupied by quiescent HSCs in close association with osteoblasts that regulate stem cell proliferation. The vascular niche appears to be occupied by HSCs that have been mobilized to leave the endosteal niche to either differentiate or circulate. In addition, the more differentiated a cell is, the farther it appears to migrate from its supportive osteoblasts and the closer it moves to the more central regions of the bone. For example, the most immature B lymphocytes are found closest to the endosteum and osteoblasts, while the more mature B cells have moved into the more central sinuses of the bone marrow that are richly served by blood vessels. Finally, it is important to recognize that the bone marrow is not only a site for lymphoid and myeloid development but is also a site to which fully mature myeloid and lymphoid cells can return. Mature antibody-secreting B cells (plasma cells) may even take up long-term residence in the bone marrow. Whole bone marrow transplants, therefore, do not simply include stem cells but also include mature, functional cells that can both help and hurt the transplant effort.

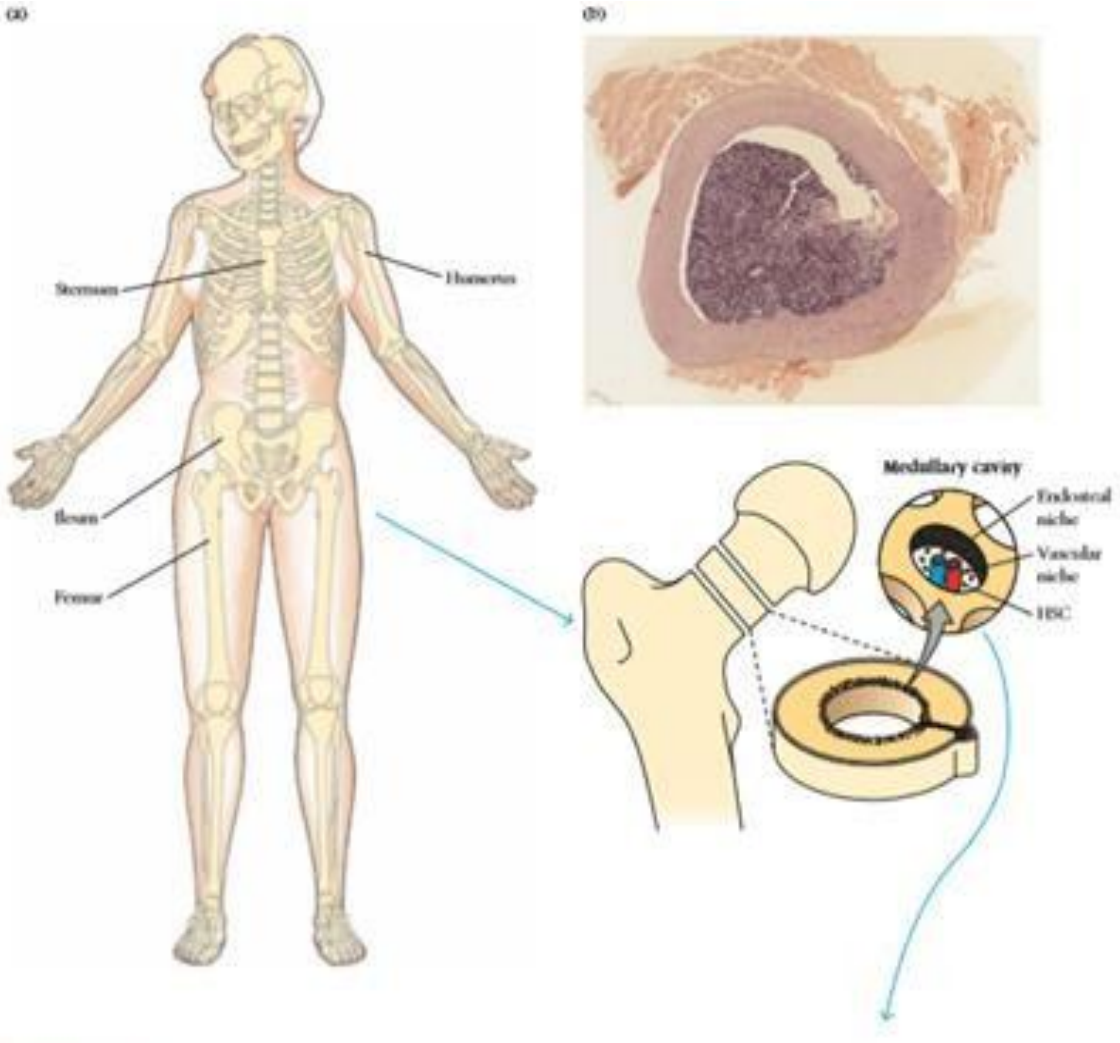
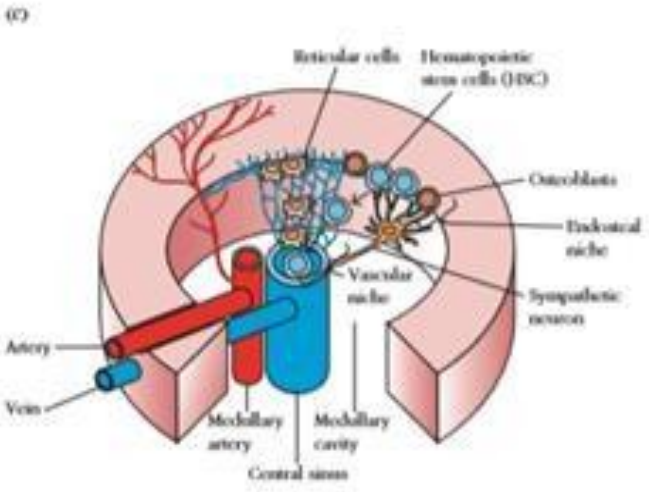


FIGURE 2-5 The bone marrow microenvironment. (a) Multiple bones support hematopoiesis, including the hip (ileum), femur, sternum, and humerus. (b) This figure shows a typical cross-section of a bone with a medullary (marrow) cavity. (c) Blood vessels (central sinus and medullary artery) run through the center of the bone and form a network of capillaries in close association with bone and bone surface (endosteum). Both the cells that line the blood vessels (endothelium) and the cells that line the bone (osteoblasts) generate niches that support hematopoietic stem cell (HSC) self-renewal and differentiation. The most immature cells appear to be associated with the endosteal (bone) niche; as they mature, they migrate toward the vascular (blood vessel) niche. Fully differentiated cells exit the marrow via blood vessels. (J. Xu, Courtesy of Indiana University School of Medicine.)



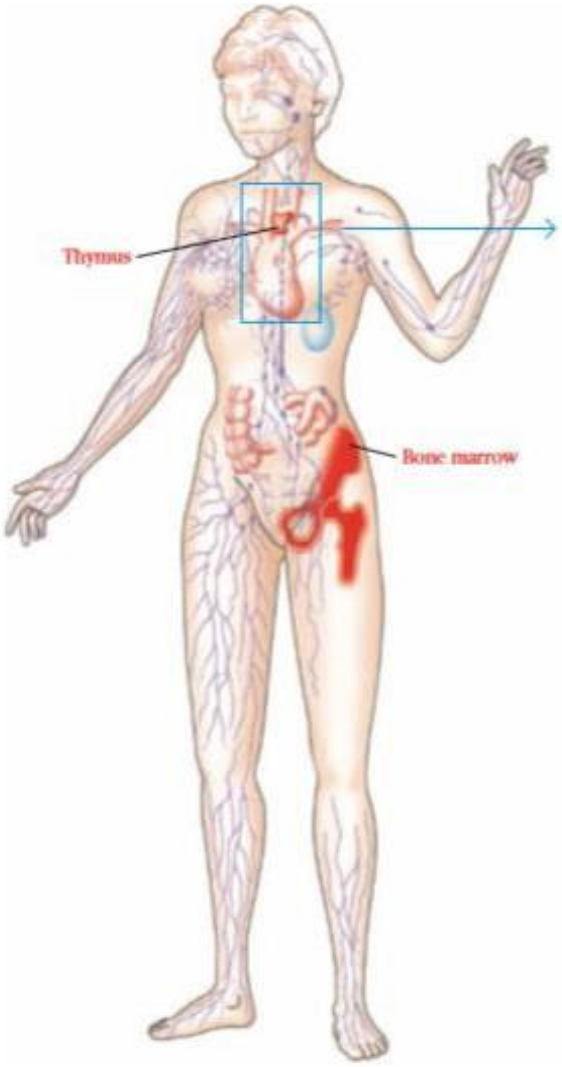
- ***The Thymus Is a Primary Lymphoid Organ Where T Cells Mature:***

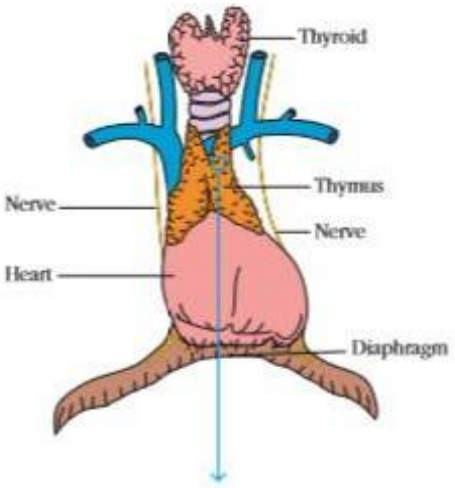
T-cell development is not complete until the cells undergo selection in the thymus (Figure 2-6). The importance of the thymus in T-cell development was not recognized until the early 1960s, when J.F.A.P. Miller, an Australian biologist, worked against the power of popular assumptions to advance his idea that the thymus was something other than a graveyard for cells. It was an underappreciated organ, very large in prepubescent animals, that was thought by some to be detrimental to an organism, and by others to be an evolutionary dead-end. The cells that populated it—small, thin-rimmed,featureless cells called thymocytes—looked dull and inactive. However, Miller proved that the thymus was the all-important site for the maturation of T lymphocytes. T-cell precursors, which still retain the ability to give rise to multiple hematopoietic cell types, travel via the blood from the bone marrow to the thymus. Immature T cells, known as thymocytes (thymus cells) because of their site of maturation, pass through defined developmental stages in specific thymic microenvironments as they mature into functional T cells. The thymus is a specialized environment where immature T cells generate unique antigen receptors (T cell receptors, or TCRs) and are then selected on the basis of their reactivity to self MHC-peptide complexes expressed on the surface of thymic stromal cells. Those thymocytes whose T-cell receptors bind self MHC-peptide complexes with too high affinity are induced to die (negative selection), and those thymocytes that bind self MHC-peptides with an intermediate affinity undergo positive selection, resulting in their survival, maturation, and migration to the thymic medulla. Most thymocytes do not navigate the journey through the thymus successfully; in fact, it is estimated that 95% of thymocytes die in transit. The majority of cells die because they have too low an affinity for the self-antigen MHC combinations that they encounter on the surface of thymic epithelial cells and fail to undergo positive selection. These developmental events take place in several distinct thymic microenvironments (see Figure 2-6). T-cell precursors enter the thymus in blood vessels at the corticomedullary junction between the thymic cortex, the outer portion of the organ, and the thymic medulla, the inner portion of the organ. At this stage thymocytes express neither CD4 nor CD8, markers associated with mature T cells.

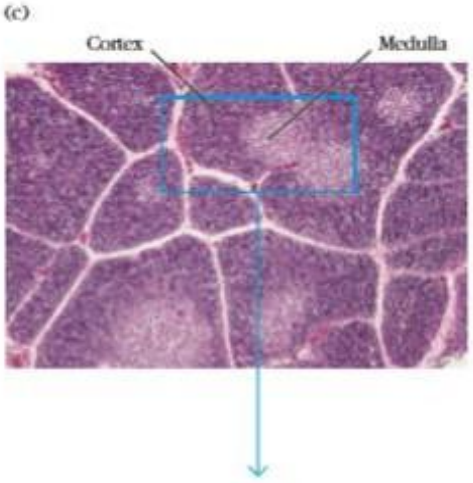
They are therefore called double negative (DN) cells. DN cells first travel to the region under the thymic capsule, a region referred to as the subcapsular cortex, where they proliferate and begin to generate their T-cell receptors. Thymocytes that successfully express TCRs begin to express both CD4 and CD8, becoming double positive (DP) cells, and populate the cortex, the site where most (85% or more) immature T cells are found. The cortex features a distinct set of stromal cells, cortical thymic epithelial cells (cTECs), whose long processes are

perused by thymocytes testing the ability of their T-cell receptors to bind MHC-peptide complexes (Video 2-1). Thymocytes that survive selection move to the thymic medulla, where positively selected thymocytes encounter specialized stromal cells, medullary thymic epithelial cells (mTECs). Not only do mTECs support the final steps of thymocyte maturation, but they also have a unique ability to express proteins that are otherwise found exclusively in other organs. This allows them to negatively select a group of potentially very damaging, auto reactive T cells that could not be deleted in the cortex.

Mature thymocytes, which express only CD4 or CD8 and are referred to as single positive (SP), leave the thymus as they entered: via the blood vessels of the corticomedullary junction. Maturation is finalized in the periphery, where these new T cells (recent thymic emigrants) explore antigens presented in secondary lymphoid tissue, including spleen and lymph nodes.







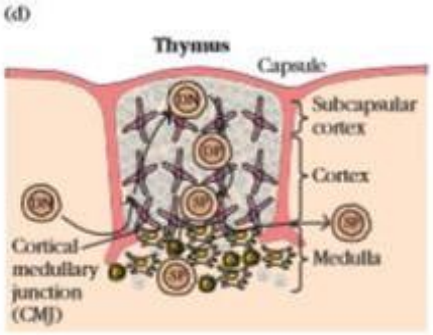


FIGURE 2-6 The structure of the thymus. The thymus is found just above the heart (a, b) and is largest prior to puberty, when it begins to shrink. Panel (c) depicts a stained thymus tissue section and (d) a cartoon of the microenvironments: the cortex, which is densely populated with DP immature thymocytes (blue) and the medulla, which is sparsely populated with SP mature thymocytes. These major regions are separated by the corticomedullary junction (CMJ), where cells enter from and exit to the bloodstream. The area between the cortex and the thymic capsule, the subcapsular cortex, is a site of much proliferation of the youngest (DN) thymocytes. The route taken by a typical thymocyte during its development from the DN to DP to SP stages is shown. Thymocytes are positively selected in the cortex. Autoreactive thymocytes are negatively selected in the medulla; some may also be negatively selected in the cortex. [2-6c: Dr. Gladden Willis/Getty Images.]

➢ Secondary Lymphoid Organs—Where the Immune Response Is Initiated:

As just described, lymphocytes and myeloid cells develop to maturity in the primary lymphoid system: T lymphocytes in the thymus, and B cells, monocytes, dendritic cells, and granulocytes in the bone marrow. However, they encounter antigen and initiate an immune response in the microenvironments of secondary lymphoid organs (SLOs).

- ***Secondary Lymphoid Organs Are Distributed Throughout the Body and Share Some Anatomical Features:***

Lymph nodes and the spleen are the most highly organized of the secondary lymphoid organs and are compartmentalized from the rest of the body by a fibrous capsule. A somewhat less organized system of secondary lymphoid tissue, collectively referred to as mucosa-associated lymphoid tissue (MALT), is found associated with the linings of multiple organ systems, including the gastrointestinal (GI) and respiratory tracts. MALT includes tonsils, Peyer's patches (in the small intestine), and the appendix, as well as numerous lymphoid follicles within the lamina propria of the intestines and in the mucous membranes lining the upper airways, bronchi, and genitourinary tract (Figure 2-7). Although secondary lymphoid organs vary in their location and degree of organization, they share key features. All SLOs include anatomically distinct regions of T-cell and B-cell activity, and all develop lymphoid follicles, which are highly organized microenvironments that are responsible for the development and selection of B cells that produce high-affinity antibodies.

- ***Lymphoid Organs Are Connected to Each Other and to Infected Tissue by Two Different Circulatory Systems: Blood and Lymphatics:***

The immune cells are the most mobile cells in a body and use two different systems to traffic through tissues: the blood system and the lymphatic system. The blood has access to virtually every organ and tissue and is lined by endothelial cells that are very responsive to inflammatory signals. Hematopoietic cells can transit through the blood system—away from the heart via active pumping networks (arteries) and back to the heart via passive valve-based systems (veins) within minutes. Most lymphocytes enter secondary lymphoid organs via specialized blood vessels, and leave via the lymphatic system.

The lymphatic system is a network of thin walled vessels that play a major role in immune cell trafficking, including the travel of antigen and antigen-presenting cells to secondary lymphoid organs and the exit of lymphocytes from lymph nodes.

Lymph vessels are filled with a protein-rich fluid (lymph) derived from the fluid component of blood (plasma) that seeps through the thin walls of capillaries into the surrounding tissue. In an adult, depending on size and activity, seepage can add up to 2.9 litres or more during a 24-hour period. This fluid, called interstitial fluid, permeates all tissues and bathes all cells. If this fluid were not returned to the circulation, the tissue would swell; causing edema that would eventually become life threatening. We are not afflicted with such catastrophic edema because much of the fluid is returned to the blood through the walls of venules. The remainder of the interstitial fluid enters the delicate network of primary lymphatic vessels. The walls of the primary vessels consist of a single layer of loosely apposed endothelial cells. The porous architecture of the primary vessels allows fluids and even cells to enter the lymphatic network. Within these vessels, the fluid, now called lymph, flows into a series of progressively larger collecting vessels called lymphatic vessels (see Figures 2-7b and 2-7c).

All cells and fluid circulating in the lymph are ultimately returned to the blood system. The largest lymphatic vessel, the thoracic duct,empties into the left subclavian vein. It collects lymph from all of the body except the right arm and right side of the head. Lymph from these areas is collected into the right lymphatic duct, which drains into the right subclavian vein (see Figure 2-7a).

By returning fluid lost from the blood, the lymphatic system ensures steady-state levels of fluid within the circulatory system. The heart does not pump the lymph through the lymphatic system; instead, the slow, low-pressure flow of lymph is achieved by the movements of the surrounding muscles. Therefore, activity enhances lymph circulation. Importantly, a series of one-way valves along the lymphatic vessels ensures that lymph flows in only one direction. When a foreign antigen gains entrance to the tissues, it is picked up by the lymphatic system (which drains all the tissues of the body) and is carried to various organized lymphoid tissues such as lymph nodes, which trap the foreign antigen. Antigen-presenting cells that engulf and process the antigen also can gain access to lymph. In fact, as lymph passes from the tissues to lymphatic vessels, it becomes progressively enriched in specific leukocytes, including lymphocytes, dendritic cells, and macrophages. Thus, the lymphatic system also serves as a means of transporting white blood cells and antigen from the connective tissues to organized lymphoid tissues, where the lymphocytes can interact with the trapped antigen and undergo activation. Most secondary lymphoid tissues are situated along the vessels of the lymphatic system. The spleen is an exception and is served only by blood vessels. All immune cells that traffic through tissues, blood, and lymph nodes are guided by small molecules known as chemokines. These proteins are secreted by stromal cells, antigen presenting cells, lymphocytes, and granulocytes, and form gradients that act as attractants and guides for other immune cells, which express an equally diverse set of receptors for these chemokines.

The interaction between specific chemokines and cells expressing specific chemokine receptors allows for a highly refined organization of immune cell movements.

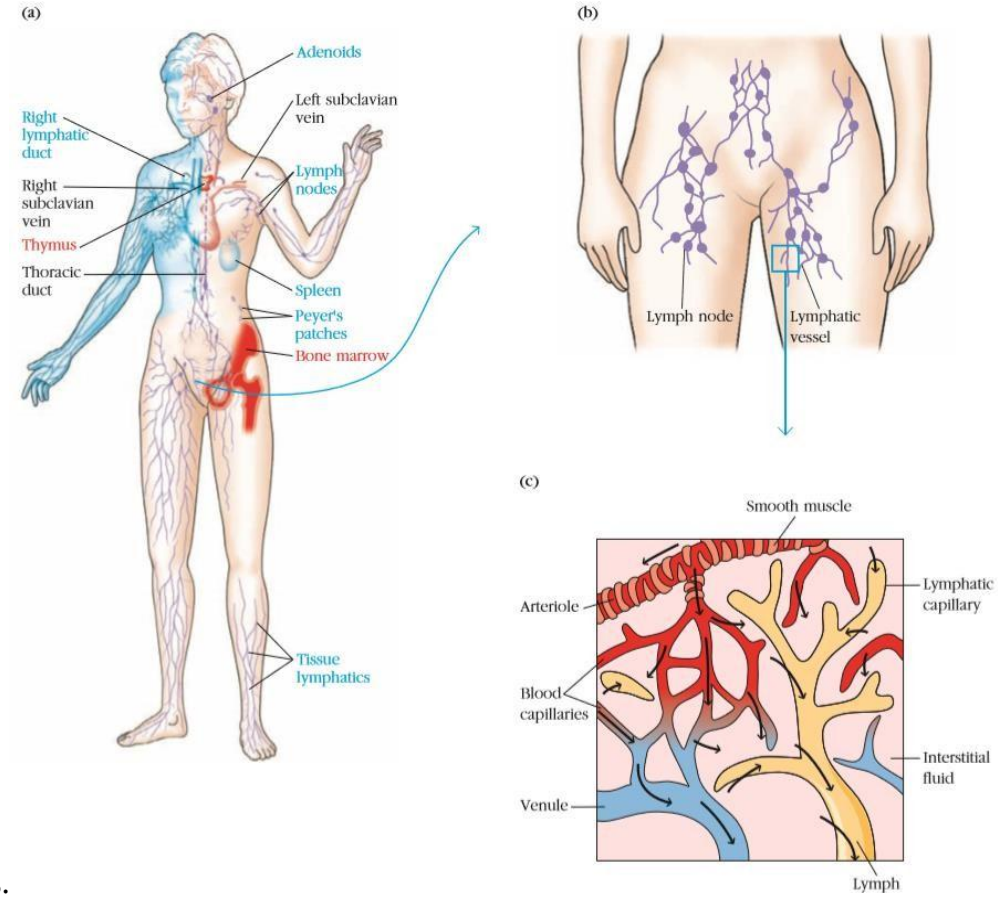


FIGURE 2-7 The human lymphoid system. The primary organs (bone marrow and thymus) are shown in red; secondary organs and tissues, in blue. These structurally and functionally diverse lymphoid organs and tissues are interconnected by the blood vessels (not shown) and lymphatic vessels (purple). Most of the body's lymphatics eventually drain into the thoracic duct, which empties into the left subclavian vein. However, the vessels draining the right arm and right side of the head (shaded blue) converge to form the right lymphatic duct, which empties into the right subclavian vein. The inset (b) shows the lymphatic vessels in more detail, and (c) shows the relationship between blood and lymphatic capillaries in tissue. The lymphatic capillaries pick up interstitial fluid, particulate and soluble proteins, as well as immune cells from the tissue surrounding the blood capillaries (see arrows). *[Part (a): Adapted from H. Lodish et al., 1995,* Molecular Cell Biology, *3rd ed., Scientific American Books, New York.]*

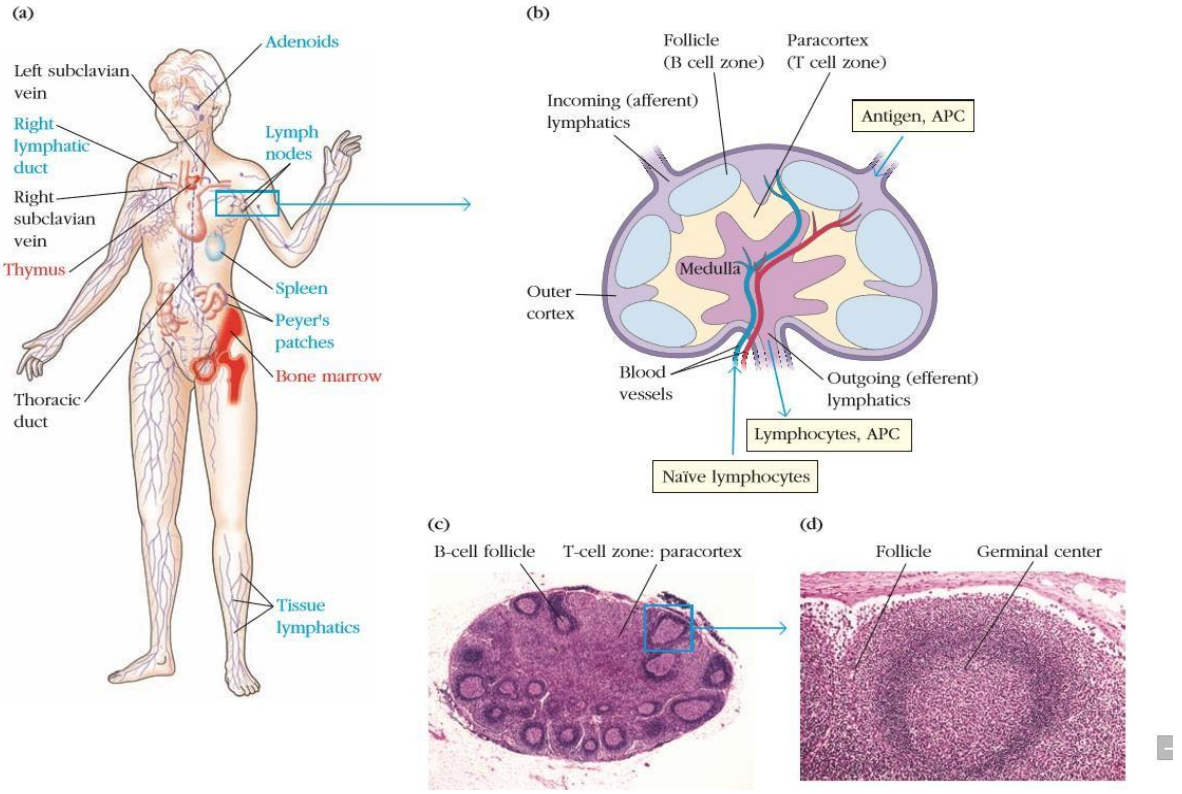
- ***The Lymph Node Is a Highly Specialized Secondary Lymphoid Organ:***

.Lymph nodes (Figure 2-8) are the most specialized SLOs. Unlike the spleen, which also regulates red blood cell flow and fate, lymph nodes are fully committed to regulating an immune response. They are encapsulated, bean-shaped structures that include networks of stromal cells packed with lymphocytes, macrophages, and dendritic cells. Connected to both blood vessels and lymphatic vessels, lymph nodes are the first organized lymphoid structure to encounter antigens that enter the tissue spaces. The lymph node provides ideal microenvironments for encounters between antigen and lymphocytes and productive, organized cellular and humoral immune responses.

Structurally, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each of which supports a distinct microenvironment (see Figure 2-8).

The outermost layer, the cortex, contains lymphocytes (mostly B cells), macrophages, and follicular dendritic cells arranged in follicles. Beneath the cortex is the paracortex, which is populated largely by T lymphocytes and also contains dendritic cells that migrated from tissues to the node. The medulla is the innermost layer, and the site where lymphocytes exit (egress) the lymph node through the outgoing (efferent) lymphatics. It is more sparsely populated with lymphoid lineage cells, which include plasma cells that are actively secreting antibody molecules.

Antigen travels from infected tissue to the cortex of the lymph node via the incoming (afferent) lymphatic vessels, which pierce the capsule of a lymph node at numerous sites and empty lymph into the sub capsular sinus (see Figure 2-8b). It enters either in particulate form or is processed and presented as peptides on the surface of migrating antigen presenting cells. Particulate antigen can be trapped by resident antigen-presenting cells in the subcapsular sinus or cortex, and it can be passed to other antigen-presenting cells, including B lymphocytes. Alternatively, particulate antigen can be processed and presented as peptide-MHC complexes on cell surfaces of resident dendritic cells that are already in the T-cell-rich



paracortex

Mononuclear Cells:

Mononuclear cells refer to blood cells that have a single, round nucleus, such as lymphocytes and monocytes. When isolated from circulating blood, they are called peripheral blood mononuclear cells (PBMC), but other sources exist, such as the umbilical cord, spleen, and bone marrow. The established method for separating mononuclear cells from blood is through density gradient centrifugation using the polysaccharide, Ficoll. Upon spinning, the cells collect in a layer called the buffy coat, which comprise about 1% of the total sample volume. From this fraction, more specific cell types can be further isolated by purification methods that target specific cell surface proteins: CD4 for T helper cells, CD8 for cytotoxic T cells, CD19 for B cells, CD14/CD16 for monocytes, among others. Mononuclear cells have been essential in the research areas of disease, therapeutics, vaccines, immunology, diagnostics and sequencing.

➢ Granulocytic cells:

Granulocytes are at the front lines of attack during an immune response and are considered part of the innate immune system. Granulocytes are white blood cells (leukocytes) that are classified as neutrophils, basophils, mast cells, or eosinophils on the basis of differences in cellular morphology and the staining of their characteristic cytoplasmic granules (Figure 2-2). All granulocytes have multi-lobed nuclei that make them visually distinctive and easily distinguishable from lymphocytes, whose nuclei are round. The cytoplasm of all granulocytes is replete with granules that are released in response to contact with pathogens. These granules contain a variety of proteins with distinct functions: Some damage pathogens directly; some regulate trafficking and activity of other white blood cells, including lymphocytes; and some contribute to the remodelling of tissues at the site of infection. See Table 2-2 for a partial list of granule proteins and their functions.

Neutrophils: Constitutethe majority (50% to 70%) of circulating leukocytes (see Figure 2-2a) and are much more numerous than eosinophils (1%–3%), basophils (1%), or mast cells (1%). After differentiation in the bone marrow, neutrophils are released into the peripheral blood and circulate for 7 to 10 hours before migrating into the tissues, where they have a life

span of only a few days. In response to many types of infections, the number of circulating neutrophils increases significantly and more are recruited to tissues, partially in response to cues the bone marrow receives to produce and release more myeloid cells. The resulting transient increase in the number of circulating neutrophils, called leukocytosis, is used medically as an indication of infection. Neutrophils are recruited to the site of infection in response to inflammatory molecules (e.g.,chemokines) generated by innate cells (including other neutrophils) that have engaged a pathogen. Once in tissues, neutrophils phagocytose (engulf) bacteria very effectively and also secrete a range of proteins that have antimicrobial effects and tissue remodelling potential. Neutrophils are the dominant first responders to infection and the main cellular components of pus, where they accumulate at the end of their short lives. Although once considered a simple and "disposable" effector cell, the neutrophil has recently inspired renewed interest from investigations indicating that it may also regulate the adaptive immune response.

Basophils are nonphagocytic granulocytes (see Figure 2-2b) that contain large granules filled with basophilic proteins (i.e., they stain blue in standard H&E staining protocols). Basophils are relatively rare in the circulation, but can be very potent. In response to binding of circulating antibodies, basophils release the contents of their granules. Histamine, one of the best known proteins in basophilic granules, increases blood vessel permeability and smooth muscle activity. Basophils (and eosinophils, below) are critical to our response to parasites, particularly helminths (worms), but in areas where worm infection is less prevalent, histamines are best appreciated as the cause of allergy symptoms. Like neutrophils, basophils may also secrete cytokines that modulate the adaptive immune response.

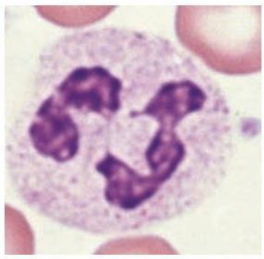
TABLE 2-2 Examples of proteins contained in neutrophil, eosinophil, and basophil granules

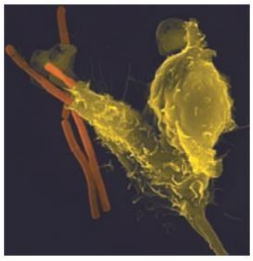
Cell type	Molecule in granule	Examples	Function
Neutrophil	Proteases	*Elastase, Collagenase*	Tissue remodeling
	Antimicrobial proteins	*Defensins, lysozyme*	Direct harm to pathogens
	Protease inhibitors	*α1-anti-trypsin*	Regulation of proteases
	Histamine		Vasodilation, inflammation
Eosinophil	Cationic proteins	*EPO*	Induces formation of ROS
	Ribonucleases	*MBP*	Vasodilation, basophil degranulation
	Cytokines	*ECP, EDN*	Antiviral activity
	Chemokines	*IL-4, IL-10, IL-13, TNFα*	Modulation of adaptive immune responses
		RANTES, MIP-1α	Attract leukocytes
Basophil/Mast Cell	Cytokines	*IL-4, IL-13*	Modulation of adaptive immune response
	Lipid mediators	*Leukotrienes*	Regulation of inflammation
	Histamine		Vasodilation, smooth muscle activation

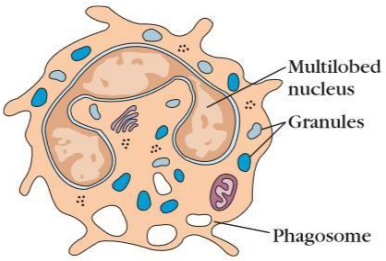
Eosinophils, like neutrophils, are motile phagocytic cells (see Figure 2-2d) that can migrate from the blood into the tissue spaces. Their phagocytic role is significantly less important than that of neutrophils, and it is thought that they play their most important role in the defense against multicellular parasitic organisms, including worms. They can be found clustering around invading worms, whose membranes are damaged by the activity of proteins released from eosinophilic granules. Like neutrophils and basophils, eosinophils may also secrete cytokines that regulate B and T lymphocytes, thereby influencing the adaptive immune response. In areas where parasites are less of a health problem, eosinophils are better appreciated as contributors to asthma and allergy symptoms.

- **Mast cells:** (see Figure 2-2c) are released from the bone marrow into the blood as undifferentiated cells; they mature only after they leave the blood. Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts. Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active substances. Mast cells also play an important role in the development of allergies.

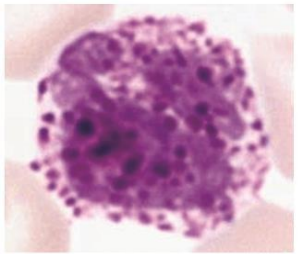
(a) Neutrophil

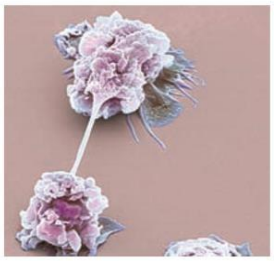


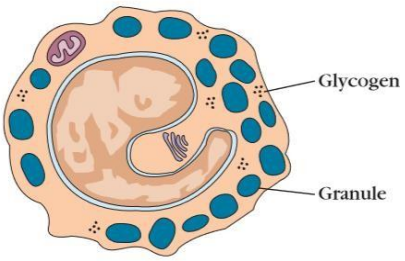




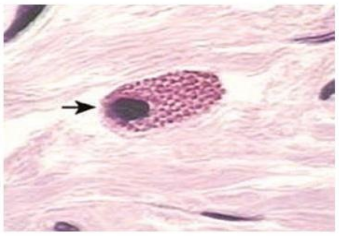
(b) Basophil

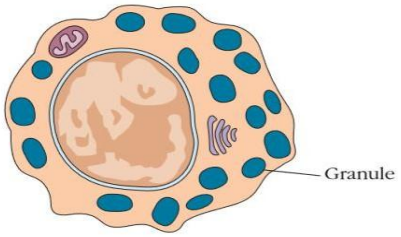




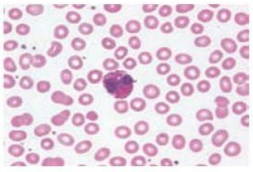


(c) Mast cell





(d) Eosinophil



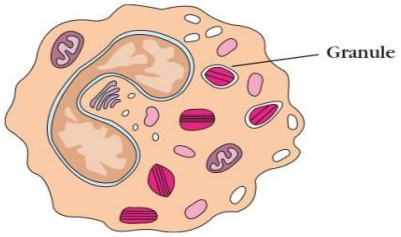


FIGURE 2-2 Examples of granulocytes. (a, b, c, d) Hematoxylin and eosin (H&E) stains of indicated cells in blood smears. (a, middle) Neutrophil engulfing bacteria visualized by scanning electron microscopy (SEM) and colorized digitally. (b, middle) SEM of activated granulocytes (colorized). Each image is accompanied by a cartoon depicting the typical morphology of the indicated granulocyte. Note differences in the shape of the nucleus and in the number, color, and shape of the cytoplasmic granules. [2-2a, left: Science Source/Getty Images; 2-2a, right: Creative Commons, http://es.wikipedia.org/wiki/Archivo:Neutrophil_with_anthrax_copy.jpg; 2-2b, left: Dr. Gladden Willis/Visuals Unlimited, Inc.; 2-2b, right: Steve Gschmeissner/Photo Researchers; 2-2c, left: Courtesy Gwen V. Childs, Ph.D., University of Arkansas for Medical Sciences; 2-2d, left; Pathpedia.com.]

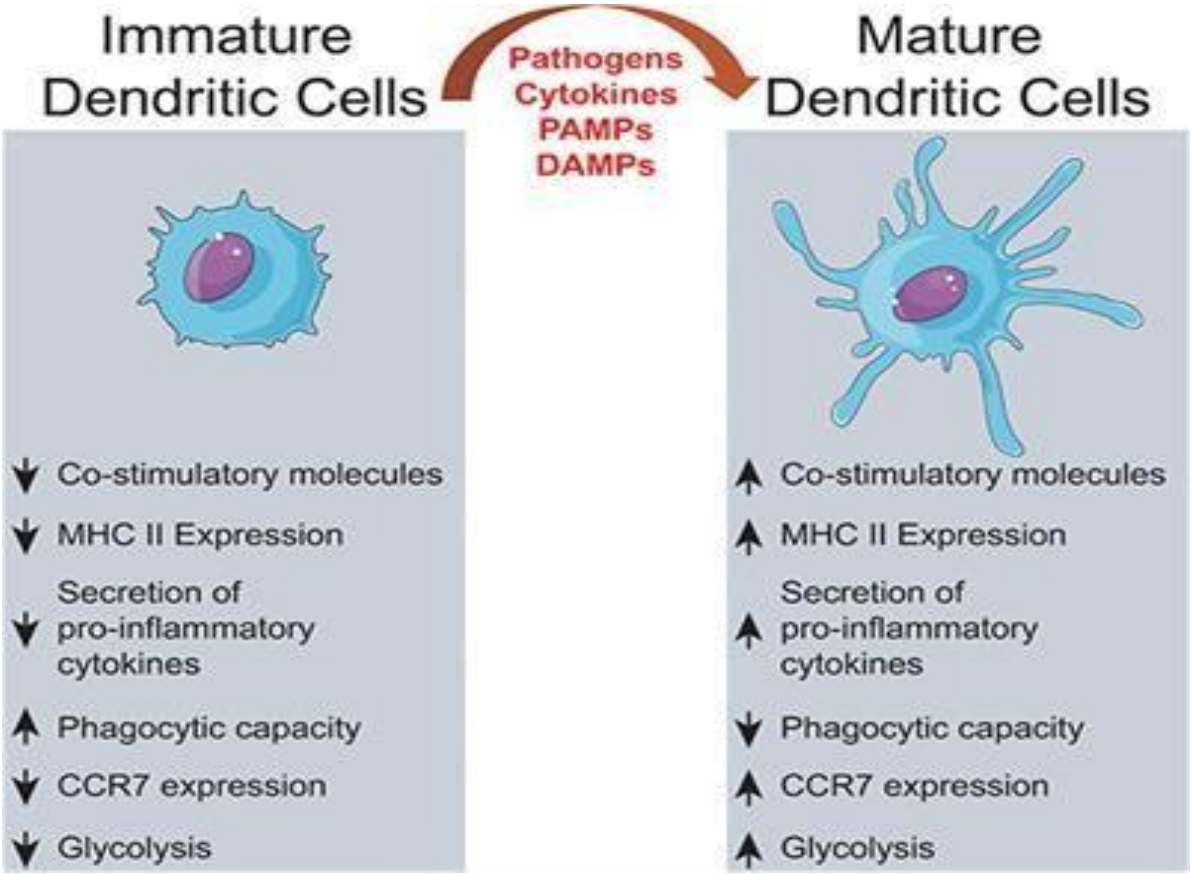
- **Dendritic Cell:**

The discovery of the dendritic cell (DC) by Ralph Steinman in the mid-1970s resulted in awarding of the Nobel Prize in 2011. Dendritic cells are critical for the initiation of the immune response and acquired their name because they are covered with long membranous extensions that resemble the dendrites of nerve cells and extend and retract dynamically,increasing the surface area available for browsing lymphocytes. They are more diverse a population of cells than once was thought, and seem to arise from both the myeloid and lymphoid lineages of hematopoietic cells. The functional distinctions among these diverse cells are still being clarified and are likely critically important in tailoring immune responses to distinct pathogens and targeting responding cells to distinct tissues.

Dendritic cells perform the distinct functions of antigen capture in one location and antigen presentation in another. Outside lymph nodes, immature forms of these cells monitor the body for signs of invasion by pathogens and capture intruding or foreign antigens. They

process these antigens, then migrate to lymph nodes, where they present the antigen to naïve T cells, initiating the adaptive immune response.

When acting as sentinels in the periphery, immature dendritic cells take on their cargo of antigen in three ways. They engulf it by phagocytosis, internalize it by receptor-mediated endocytosis, or imbibe it by pinocytosis. Indeed, immature dendritic cells pinocytose fluid volumes of 1000 to 1500 m3 per hour, a volume that rivals that of the cell itself. Through a process of maturation, they shift from an antigen-capturing phenotype to one that is specialized for presentation of antigen to T cells. In making the transition, some attributes are lost and others are gained. Lost is the capacity for phagocytosis and large-scale pinocytosis. However, the ability to present antigen increases significantly, as does the expression of costimulatory molecules that are essential for the activation of naïve T cells. After activation, dendritic cells abandon residency in peripheral tissues, enter the blood or lymphatic circulation, and migrate to regions of the lymphoid organs, where T cells reside, and present antigen.



It is important to note that, although they share a name, follicular dendritic cells do not arise in bone marrow and have completely different functions from those described for

the dendritic cells discussed above. Follicular dendritic cells do not function as antigen-presenting cells for TH-cell activation. These dendritic cells were named for their exclusive location in organized structures of the lymph node called lymph follicles, which are rich in B cells.

✓ Part- 3:*Types of immunity:*

- **Innate immunity:** Innate immunity can be seen to comprise four types of defensive barriers: anatomic, physiologic, phagocytic, and inflammatory(Table 1-2).

TABLE 1-2 Summary of nonspecific host defenses

Type	Mechanism
Anatomic barriers	
Skin	Mechanical barrier retards entry of microbes. Acidic environment (pH 3–5) retards growth of microbes.
Mucous membranes	Normal flora compete with microbes for attachment sites and nutrients. Mucus entraps foreign microorganisms. Cilia propel microorganisms out of body.
Physiologic barriers	
Temperature	Normal body temperature inhibits growth of some pathogens. Fever response inhibits growth of some pathogens.
Low pH	Acidity of stomach contents kills most ingested microorganisms.
Chemical mediators	Lysozyme cleaves bacterial cell wall. Interferon induces antiviral state in uninfected cells. Complement lyses microorganisms or facilitates phagocytosis. Toll-like receptors recognize microbial molecules, signal cell to secrete immunostimulatory cytokines. Collectins disrupt cell wall of pathogen.
Phagocytic/endocytic barriers	Various cells internalize (endocytose) and break down foreign macromolecules. Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill, and digest whole microorganisms.
Inflammatory barriers	Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity, and influx of phagocytic cells into the affected area.

- *The Skin and the Mucosal Surfaces Provide Protective Barriers Against Infection:*

Physical and anatomic barriers that tend to prevent the entry of pathogens are an organism's first line of defense against infection. The skin and the surface of mucous membranes are included in this category because they are effective barriers to the entry of most microorganisms. The skin consists of two distinct layers: a thinner outer layer—the **epidermis** and a thicker layer—the **dermis**. The epidermis contains several layers of tightly packed epithelial cells.

The outer epidermal layer consists of dead cells and is filled with a waterproofing protein called keratin. The dermis, which is composed of connective tissue, contains blood vessels, hair follicles, sebaceous glands, and sweat glands. The sebaceous glands are associated with the hair follicles and produce an oily secretion called **sebum**. Sebum consists of lactic acid and fatty acids, which maintain the pH of the skin between 3 and 5; this pH inhibits the growth of most microorganisms. A few bacteria that metabolize sebum live as commensals on the skin and sometimes cause a severe form of acne. One acne drug, isotretinoin (Accutane), is a vitamin A derivative that prevents the formation of sebum.

Breaks in the skin resulting from scratches, wounds, or abrasion are obvious routes of infection. The skin may also be penetrated by biting insects (e.g.,mosquitoes, mites, ticks, fleas, and sand flies); if these harbor pathogenic organisms, they can introduce the pathogen into the body as they feed. The protozoan that causes malaria, for example,is deposited in humans by mosquitoes when they take a blood meal. Similarly, bubonic plague is spread by the bite of fleas, and Lyme disease is spread by the bite of ticks.

The conjunctivae and the alimentary, respiratory, and urogenital tracts are lined by mucous membranes, not by the dry, protective skin that covers the exterior of the body. These membranes consist of an outer epithelial layer and an underlying layer of connective tissue. Although many pathogens enter the body by binding to and penetrating mucous membranes, a number of nonspecific defense mechanisms tend to prevent this entry. For example,saliva,tears,and mucous secretions act to wash away potential invaders and also contain antibacterial or antiviral substances.The viscous fluid called mucus,which is secreted by epithelial cells of mucous membranes, entraps foreign microorganisms. In the lower respiratory tract, the mucous membrane is covered by cilia, hairlike protrusions of the epithelial-cell membranes. The synchronous movement of cilia propels mucus-entrapped

microorganisms from these tracts. In addition, non-pathogenic organisms tend to colonize the epithelial cells of mucosal surfaces. These normal flora generally outcompete pathogens for attachment sites on the epithelial cell surface and for necessary nutrients.

- *Physiologic Barriers to Infection Include General Conditions and Specific Molecules:*

The physiologic barriers that contribute to innate immunity include temperature, pH, and various soluble and cell associated molecules. Many species are not susceptible to certain diseases simply because their normal body temperature inhibits growth of the pathogens. Chickens, for example, have innate immunity to anthrax because their high body temperature inhibits the growth of the bacteria. Gastric acidity is an innate physiologic barrier to infection because very few ingested microorganisms can survive the low pH of the stomach contents. One reason new borns are susceptible to some diseases that do not afflict adults is that their stomach contents are less acid than those of adults.

A variety of soluble factors contribute to innate immunity, among them the soluble proteins lysozyme, interferon, and complement. **Lysozyme**, a hydrolytic enzyme found in mucous secretions and in tears, is able to cleave the peptidoglycan layer of the bacterial cell wall. **Interferon** comprises a group of proteins produced by virus-infected cells. Among the many functions of the interferon is the ability to bind to nearby cells and induce a generalized antiviral state. **Complement**, is a group of serum proteins that circulate in an inactive state. A variety of specific and nonspecific immunologic mechanisms can convert the inactive forms of complement proteins into an active state with the ability to damage the membranes of pathogenic organisms, either destroying the pathogens or facilitating their clearance. Complement may function as an effector system that is triggered by binding of antibodies to certain cell surfaces, or it may be activated by reactions between complement molecules and certain components of microbial cell walls. Reactions between complement molecules or fragments of complement molecules and cellular receptors trigger activation of cells of the innate or adaptive immune systems. Recent studies on **collectins**indicate that these surfactant proteins may kill certain bacteria directly by disrupting their lipid membranes or, alternatively, by aggregating the bacteria to enhance their susceptibility to phagocytosis.

Many of the molecules involved in innate immunity have the property of pattern recognition, the ability to recognize a given class of molecules. Because there are certain types of molecules that are unique to microbes and never found in multicellular organisms, the ability

to immediately recognize and combat invaders displaying such molecules is a strong feature of innate immunity. Molecules with pattern recognition ability may be soluble, like lysozyme and the complement components described above, or they may be cell-associated receptors. Among the class of receptors designated the toll-like receptors (TLRs), TLR2 recognizes the lipopolysaccharide (LPS) found on Gram-negative bacteria. It has long been recognized that systemic exposure of mammals to relatively small quantities of purified LPS leads to an acute inflammatory response (see below). The mechanism for this response is via a TLR on macrophages that recognizes LPS and elicits a variety of molecules in the inflammatory response upon exposure. When the TLR is exposed to the LPS upon local invasion by a Gram-negative bacterium, the contained response results in elimination of the bacterial challenge.

- *Cells That Ingest and Destroy Pathogens Make Up a Phagocytic Barrier to Infection:*

Another important innate defense mechanism is the ingestion of extracellular particulate material by phagocytosis. Phagocytosis is one type of endocytosis, the general term for the uptake by a cell of material from its environment. In phagocytosis, a cell's plasma membrane expands around the particulate material, which may include whole pathogenic microorganisms, to form large vesicles called phagosomes (Figure 1-3). Most phagocytosis is conducted by specialized cells, such as blood monocytes, neutrophils, and tissue macrophages. Most cell types are capable of other forms of endocytosis, such as receptor-mediated endocytosis, in which extracellular molecules are internalized after binding by specific cellular receptors, and pinocytosis, the process by which cells take up fluid from the surrounding medium along with any molecules contained in it.

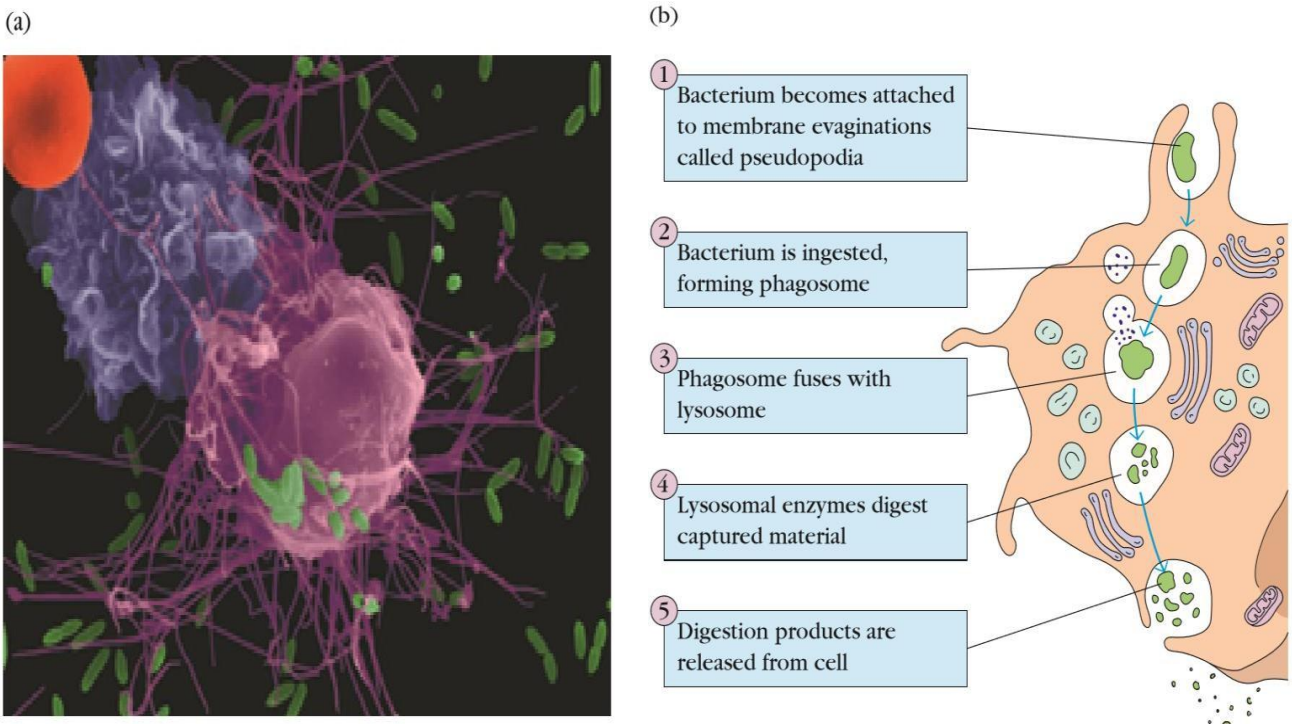


FIGURE 1-3 (a) Electronmicrograph of macrophage (pink) attacking *Escherichia coli* (green). The bacteria are phagocytized as described in part b and breakdown products secreted. The monocyte (purple) has been recruited to the vicinity of the encounter by soluble factors secreted by the macrophage. The red sphere is an erythrocyte. (b) Schematic diagram of the steps in phagocytosis of a bacterium. [*Part a, Dennis Kunkel Microscopy, Inc./Dennis Kunkel.*]

- *Inflammation Represents a Complex Sequence of Events That Stimulates Immune Responses:*

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the inflammatory response. As described above, a molecular component of a microbe, such as LPS, may trigger an inflammatory response via interaction with cell surface receptors. The end result of inflammation may be the marshalling of a specific immune response to the invasion or clearance of the invader by components of the innate immune system. Many of the classic features of the inflammatory response were described as early as 1600 BC, in Egyptian papyrus writings. In the first century AD, the Roman physician Celsus described the "four cardinal signs of inflammation" as rubor (redness), tumor (swelling), calor (heat), and dolor (pain). In the second century AD, another physician, Galen, added a fifth sign: functiolaesa (loss of function). The cardinal

signs of inflammation reflect the three major events of an inflammatory response (Figure 1-4):

1. Vasodilation—an increase in the diameter of blood vessels—of nearby capillaries occurs as the vessels that carry blood away from the affected area constrict, resulting in engorgement of the capillary network.The engorged capillaries are responsible for tissue redness (erythema) and an increase in tissue temperature.
2. *An increase in capillary permeability* facilitates an influx of fluid and cells from the engorged capillaries into the tissue.The fluid that accumulates (exudate) has much higher protein content than fluid normally released from the vasculature.Accumulation of exudate contributes to tissue swelling (**edema**).
3. *Influx of phagocytes* from the capillaries into the tissues is facilitated by the increased permeability of the capillaries.The emigration of phagocytes is a multistep process that includes adherence of the cells to the endothelial wall of the blood vessels (margination), followed by their emigration between the capillaryendothelial cells into the tissue (diapedesis or extravasation),and,finally,their migration through the tissue to the site of the invasion (chemotaxis).As phagocytic cells accumulate at the site and begin to phagocytose bacteria, theyrelease lytic enzymes,which can damage nearby healthy cells.The accumulation of dead cells, digested material, and fluid forms a substance called pus.

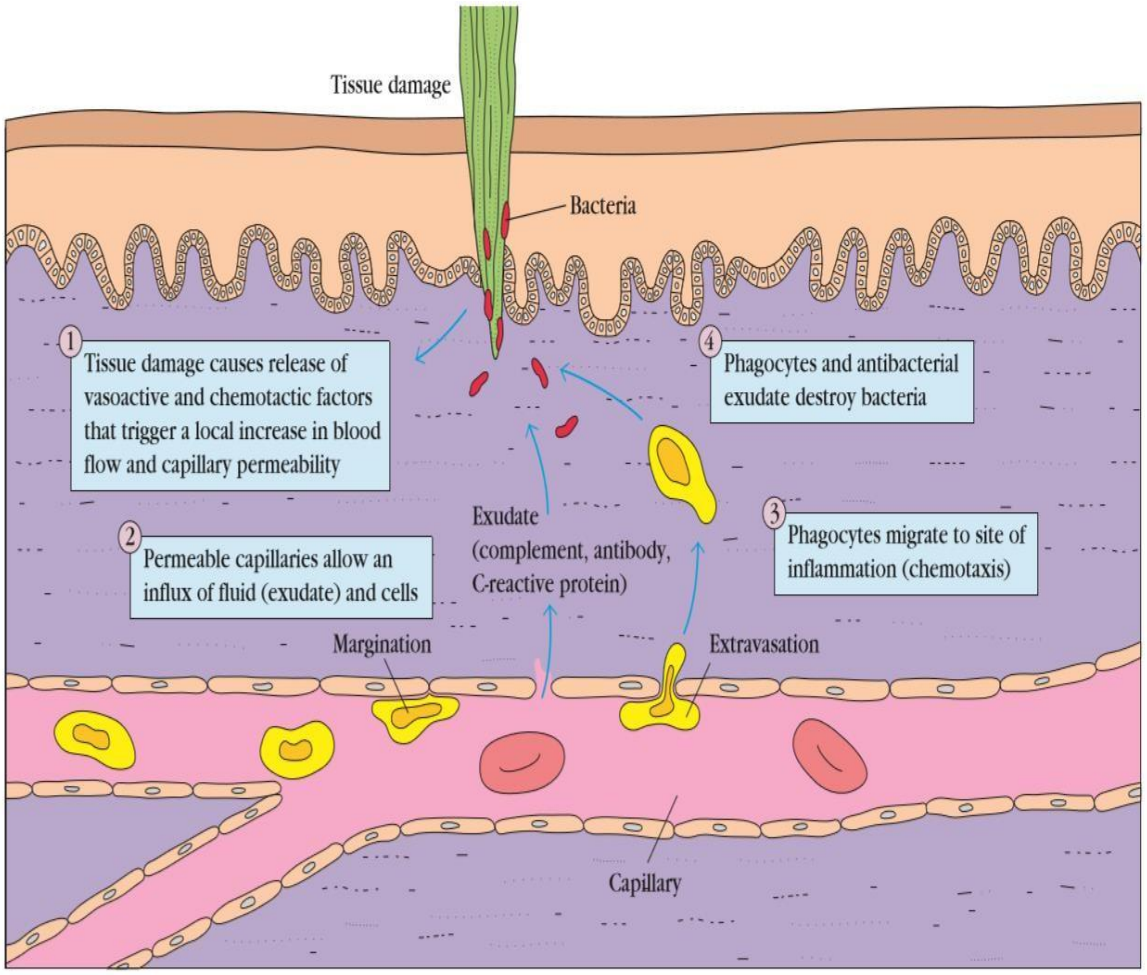


FIGURE 1-4 Major events in the inflammatory response. A bacterial infection causes tissue damage with release of various vasoactive and chemotactic factors. These factors induce increased blood flow to the area, increased capillary permeability, and an influx of white blood cells, including phagocytes and lymphocytes, from the blood into the tissues. The serum proteins contained in the exudate have antibacterial properties, and the phagocytes begin to engulf the bacteria, as illustrated in Figure 1-3.

The events in the inflammatory response are initiated by a complex series of events involving a variety of chemical mediators whose interactions are only partly understood. Some of these mediators are derived from invading microorganisms, some are released from damaged cells in response to tissue injury, some are generated by several plasma enzyme systems, and some are products of various white blood cells participating in the inflammatory response.

One of the principal mediators of the inflammatory response is histamine, a chemical released by a variety of cells in response to tissue injury. Histamine binds to receptors on nearby capillaries and venules, causing vasodilation and increased permeability. Another important group of inflammatory mediators, small peptides called kinins, are normally present in blood plasma in an inactive form. Tissue injury activates these peptides, which then cause vasodilation and increased permeability of capillaries. A particular kinin, called bradykinin, also stimulates pain receptors in the skin. This effect probably serves a protective role, because pain normally causes an individual to protect the injured area.

Vasodilation and the increase in capillary permeability in an injured tissue also enable enzymes of the blood-clotting system to enter the tissue. These enzymes activate an enzyme cascade that results in the deposition of insoluble strands of fibrin, which is the main component of a blood clot. The fibrin strands wall off the injured area from the rest of the body and serve to prevent the spread of infection.

Once the inflammatory response has subsided and most of the debris has been cleared away by phagocytic cells, tissue repair and regeneration of new tissue begins. Capillaries grow into the fibrin of a blood clot. New connective tissue cells, called fibroblasts, replace the fibrin as the clot dissolves. As fibroblasts and capillaries accumulate, scar tissue forms.

- Adaptive immunity:

Adaptive immunity is capable of recognizing and selectively eliminating specific foreign microorganisms and molecules (i.e., foreign antigens). Unlike innate immune responses, adaptive immune responses are not the same in all members of a species but are reactions to specific antigenic challenges. Adaptive immunity displays four characteristic attributes:

- **Antigenic specificity**
- **Diversity**
- **Immunologic memory**
- **Self/nonself recognition**

The antigenic specificity of the immune system permits it to distinguish subtle differences among antigens. Antibodies can distinguish between two protein molecules that differ in only a single amino acid. The immune system is capable of generating tremendous diversity in its recognition molecules, allowing it to recognize billions of unique structures on foreign antigens. Once the immune system has recognized and responded to an antigen, it exhibits immunologic memory; that is, a second encounter with the same antigen induces a heightened state of immune reactivity. Because of this attribute, the immune system can confer life-long immunity to many infectious agents after an initial encounter. Finally, the immune system normally responds only to foreign antigens, indicating that it is capable of self/nonself recognition. The ability of the immune system to distinguish self from nonself and respond only to nonself molecules is essential, for, as described below, the outcome of an inappropriate response to self-molecules can be fatal.

Adaptive immunity is not independent of innate immunity. The phagocytic cells crucial to nonspecific immune responses are intimately involved in activating the specific immune response. Conversely, various soluble factors produced by a specific immune response have been shown to augment the activity of these phagocytic cells. As an inflammatory response develops, for example, soluble mediators are produced that attract cells of the immune system. The immune response will, in turn, serve to regulate the intensity of the inflammatory response. Through the carefully regulated interplay of adaptive and innate immunity, the two systems work together to eliminate a foreign invader.

- *The Adaptive Immune System Requires Cooperation Between Lymphocytes and Antigen-Presenting Cells:*

An effective immune response involves two major groups of cells: T lymphocytes and antigen-presenting cells. Lymphocytes are one of many types of white blood cells produced in the bone marrow by the process of hematopoiesis. Lymphocytes leave the bone marrow, circulate in the blood and lymphatic systems, and reside in various lymphoid organs. Because they produce and display antigen binding cell-surface receptors, lymphocytes mediate the defining immunologic attributes of specificity, diversity, memory, and self/nonself recognition. The two major populations of lymphocytes—**B lymphocytes (B cells)** and **T lymphocytes (T cells)**.

B lymphocytes:

B lymphocytes mature within the bone marrow; when they leave it,each expresses a unique antigen-binding receptor on its membrane (Figure 1-5a). This antigen-binding or B-cell receptor is a membrane-bound antibody molecule. Antibodies are glycoproteins that consist of two identical heavy polypeptide chains and two identicallight polypeptide chains.Each heavy chain is joined with a light chain by disulfide bonds,and additional disulfide bonds hold the two pairs together.The amino-terminal ends of the pairs of heavy and light chains form a cleft within which antigen binds.When a naive B cell (one that has not previously encountered antigen) first encounters the antigen that matches its membranebound antibody, the binding of the antigen to the antibody causes the cell to divide rapidly;its progeny differentiate into memory B cells and effector B cells called plasma cells. Memory B cells have a longer life span than naive cells, and they express the same membrane-bound antibody as their parent B cell. Plasma cells produce the antibody in a form that can be secreted and have little or no membrane-bound antibody.Although plasma cells live for only a few days,they secrete enormous amounts of antibody during this time. It has been estimated that a

single plasma cell can secrete more than 2000 molecules of antibody per second. Secreted antibodies are the major effector molecules of humoral immunity.

T-lymphocytes:T lymphocytes also arise in the bone marrow.Unlike B cells, which mature within the bone marrow,T cells migrate to the thymus gland to mature. During its maturation within the thymus,the T cell comes to express a unique antigen-binding molecule,called the T-cell receptor, on its membrane.

Unlike membrane-bound antibodies on B cells,which can recognize antigen alone, T-cell receptors can recognize only antigen that is bound to cell-membrane proteins called **major histocompatibility complex (MHC)** molecules. MHC molecules that function in this recognition event,which is termed "antigen presentation,"are polymorphic (genetically diverse) glycoproteins found on cell membranes. There are two major types of MHC molecules: Class I MHC molecules,which are expressed by nearly all nucleated cells of vertebrate species, consist of a heavy chain linked to a small invariant protein called 2-microglobulin. Class II MHC molecules,which consist of an alpha and a beta glycoprotein chain,are expressed only by antigen-presenting cells.

When a naive T cell encounters antigen combined with a MHC molecule on a cell, the T cell proliferates and differentiates into memory T cells and various effector T cells.

There are two well-defined subpopulations of T cells: T helper (TH) and T-cytotoxic (TC) cells. Although a third type of T cell, called a T suppressor (TS) cell, has been postulated, recent evidence suggests that it may not be distinct from TH and TC sub populations. T helper and T cytotoxic cells can be distinguished from one another by the presence of either CD4or CD8membrane glycoproteins on their surfaces (Figure 1-5b, c). T cells displaying CD4 generally function asTH cells, whereas those displaying CD8 generally function as T_C cell.

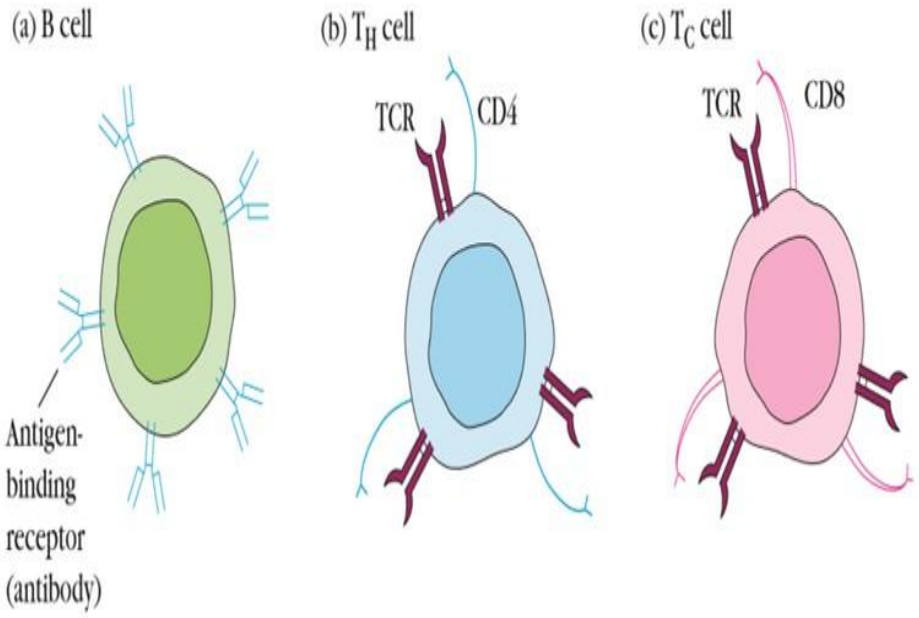


FIGURE 1-5 Distinctive membrane molecules on lymphocytes. (a) B cells have about 10^5 molecules of membrane-bound antibody per cell. All the antibody molecules on a given B cell have the same antigenic specificity and can interact directly with antigen. (b) T cells bearing CD4 ($CD4^+$ cells) recognize only antigen bound to class II MHC molecules. (c) T cells bearing CD8 ($CD8^+$ cells) recognize only antigen associated with class I MHC molecules. In general, $CD4^+$ cells act as helper cells and $CD8^+$ cells act as cytotoxic cells. Both types of T cells express about 10^5 identical molecules of the antigen-binding T-cell receptor (TCR) per cell, all with the same antigenic specificity.

- *ANTIGEN-PRESENTING CELLS:*

Activation of both the humoral and cell-mediated branches of the immune system requires cytokines produced by TH cells. It is essential that activation of TH cells themselves be carefully regulated, because an inappropriate T-cell response to self-components can have fatal autoimmune consequences. To ensure carefully regulated activation of TH cells, they can recognize only antigen that is displayed together with class MHC II molecules on the surface of antigen-presenting cells (APCs). These specialized cells, which include macrophages, B lymphocytes, and dendritic cells, are distinguished by two properties:

1. They express class II MHC molecules on their membranes,

2. They are able to deliver a co-stimulatory signal that is necessary for TH-cell activation.

Antigen-presenting cells first internalize antigen, either by phagocytosis or by endocytosis, and then display a part of that antigen on their membrane bound to a class II MHC molecule. The TH cell recognizes and interacts with the antigen–class II MHC molecule complex on the membrane of the antigen-presenting cell (Figure 1-6). An additional costimulatory signal is then produced by the antigen-pr the TH cell.

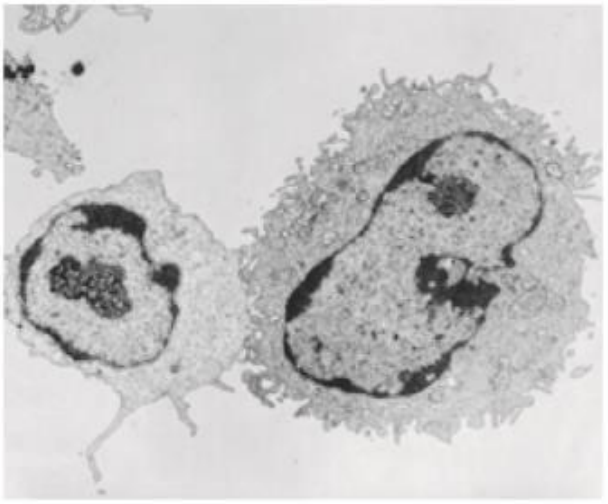


FIGURE 1-6 Electron micrograph of an antigen-presenting macrophage (*right*) associating with a T lymphocyte. [*From A. S. Rosenthal et al., 1982, in* Phagocytosis—Past and Future, *Academic Press, p. 239.*]

- *Antigen Is Recognized Differently by B and T Lymphocytes:*

Antigens, which are generally very large and complex, are not recognized in their entirety by lymphocytes. Instead, both B and T lymphocytes recognize discrete sites on the antigen called antigenic determinants, or epitopes. Epitopes are the immunologically active regions on a complex antigen, the regions that actually bind to B-cell or T-cell receptors. Although B cells can recognize an epitope alone, T cells can recognize an epitope only when it is associated with an MHC molecule on the surface of a self-cell (either an antigen- presenting cell or an altered self-cell).Each branch of the immune system is therefore uniquely suited to recognize antigen in a different milieu. The humoral branch (B cells) recognizes an enormous variety of epitopes: those displayed on the surfaces of bacteria or viral particles, as well as those displayed on soluble proteins, glycoproteins, polysaccharides, or lipopolysaccharides that have been released from invading pathogens. The cell-mediated branch (T cells) recognizes protein epitopes displayed together with MHC molecules on self- cells, including altered self-cells such as virus-infected self-cells and cancerous cells. Thus, four related but

distinct cell-membrane molecules are responsible for antigen recognition by the immune system:

- **Membrane-bound antibodies on B cells**
- **T-cell receptors**
- **Class I MHC molecules**
- **Class II MHC molecules**

Each of these molecules plays a unique role in antigen recognition, ensuring that the immune system can recognize and respond to the different types of antigen that it encounters.

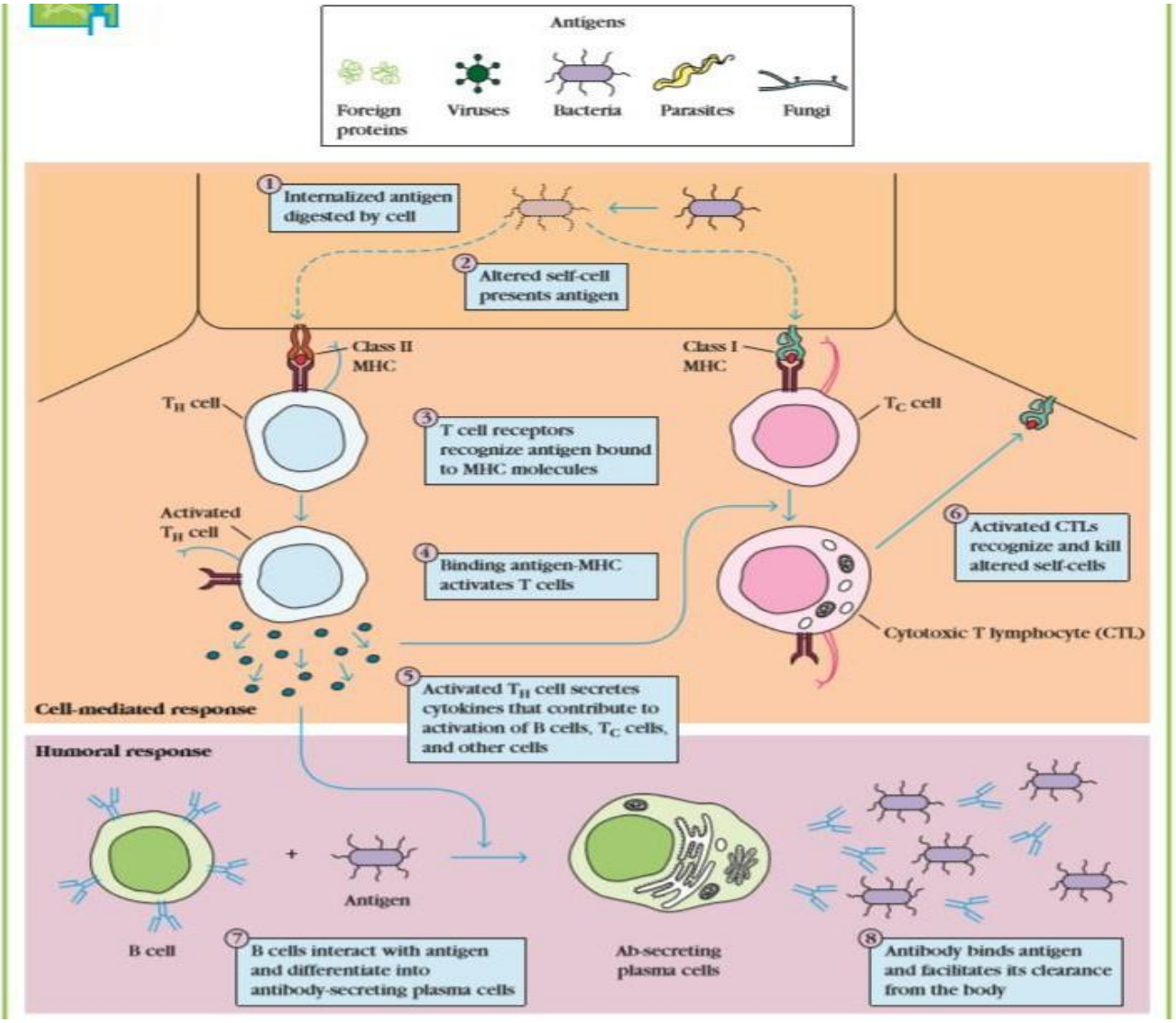


FIGURE 1-7 Overview of the humoral and cell-mediated branches of the immune system. In the humoral response, B cells interact with antigen and then differentiate into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body. In the cell-mediated response, various subpopulations of T cells recognize antigen presented on self-cells. T_H cells respond to antigen by producing cytokines. T_C cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self-cells (e.g., virus-infected cells).

- *The Major Histocompatibility Molecules Bind Antigenic Peptides:*

The major histocompatibility complex (MHC) is a large genetic complex with multiple loci.The MHC loci encode two major classes of membrane-bound glycoproteins:class Iand class II MHC molecules. As noted above, TH cells generally recognize antigen combined with class II molecules,whereas TC cells generally recognize antigen combined with class I molecules (Figure 1-8).

MHC molecules function as antigen-recognition molecules, but they do not possess the fine specificity for antigen characteristic of antibodies and T-cell receptors.Rather,each MHC molecule can bind to a spectrum ofantigenic peptides derived from the intracellular degradation of antigen molecules. In both class I and class II MHC molecules the distal regions (farthest from the membrane) of different alleles display wide variation in their amino acid sequences. These variable regions form a cleft within which the antigenic peptide sits and is presented to T lymphocytes (see Figure 1-8). Different allelic forms of the genes encoding class I and classII molecules confer different structures on the antigen-binding cleft with different specificity.Thus the ability to present an antigen to T lymphocytes is influenced by the particular set of alleles that an individual inherits.

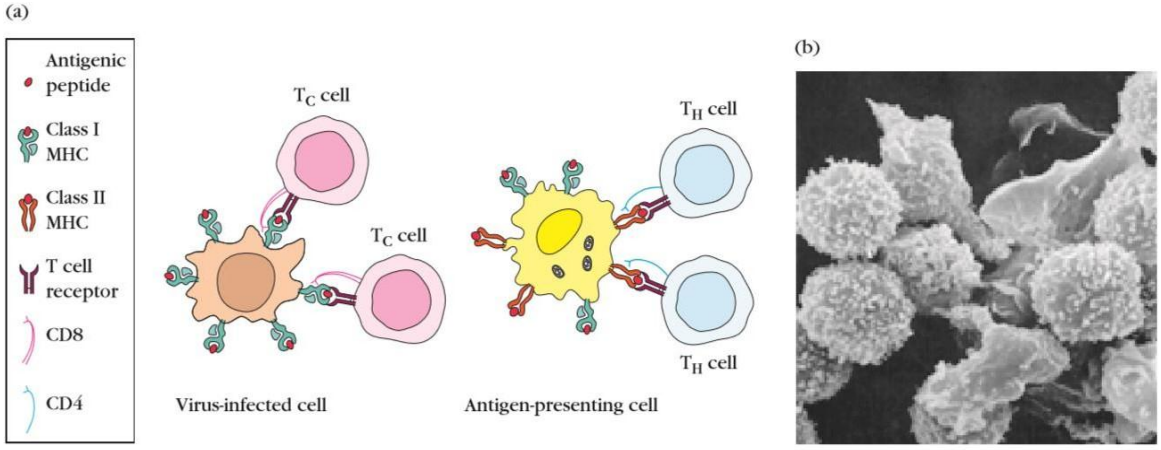


FIGURE 1-8 The role of MHC molecules in antigen recognition by T cells. (a) Class I MHC molecules are expressed on nearly all nucleated cells. Class II MHC molecules are expressed only on antigen-presenting cells. T cells that recognize only antigenic peptides displayed with a class II MHC molecule generally function as T helper (T_H) cells. T cells that recognize only antigenic peptides displayed with a class I MHC molecule generally function as T cytotoxic (T_C) cells. (b) This scanning electron micrograph reveals numerous T lymphocytes interacting with a single macrophage. The macrophage presents processed antigen combined with class II MHC molecules to the T cells. [*Photograph from W. E. Paul (ed.), 1991,* Immunology: Recognition and Response, *W. H. Freeman and Company, New York; micrograph courtesy of M. H. Nielsen and O. Werdelin.*]

- *Complex Antigens Are Degraded (Processed) and Displayed (Presented) with MHC Molecules on the Cell Surface:*

Exogenous antigen is produced outside of the host cell and enters the cell by endocytosis or phagocytosis. Antigen presenting cells (macrophages, dendritic cells, and B cells) degrade ingested exogenous antigen into peptide fragments within the endocytic processing pathway. Experiments suggest that class II MHC molecules are expressed within the endocytic processing pathway and that peptides produced by degradation of antigen in this pathway bind to the cleft within the class II MHC molecules. The MHC molecules bearing the peptide are then exported to the cell surface. Since expression of class II MHC molecules is limited to antigen-presenting cells, presentation of exogenous peptide– class II MHC complexes is limited to these cells. T cells displaying CD4 recognize antigen combined with class II MHC molecules and thus are said to be class II MHC restricted. These cells generally function as T helper cells.

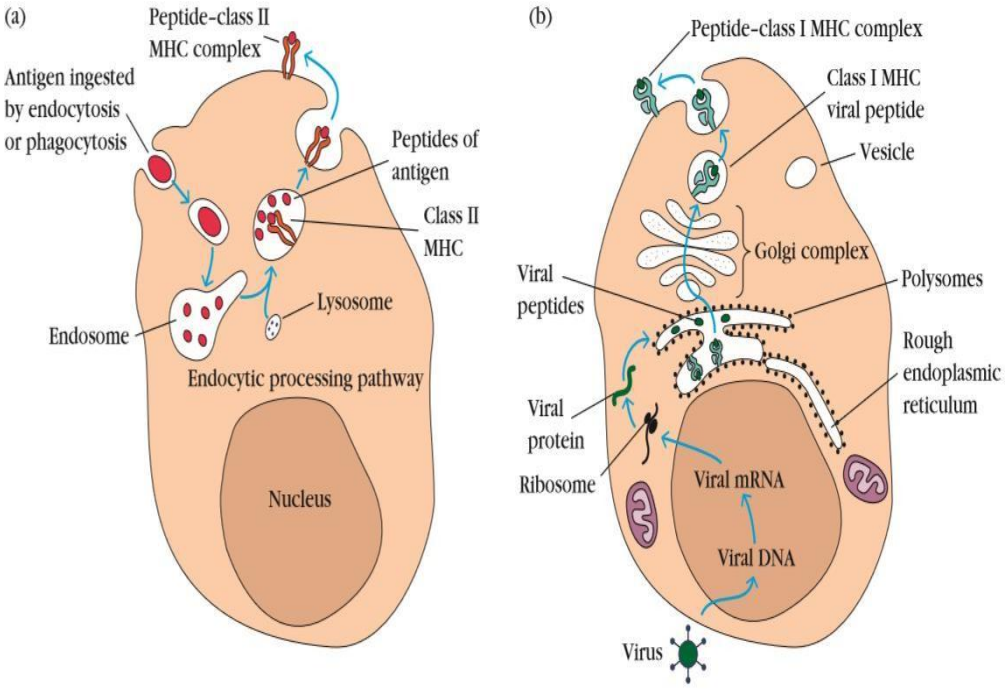


FIGURE 1-9 Processing and presentation of exogenous and endogenous antigens. (a) Exogenous antigen is ingested by endocytosis or phagocytosis and then enters the endocytic processing pathway. Here, within an acidic environment, the antigen is degraded into small peptides, which then are presented with class II MHC molecules on the membrane of the antigen-presenting cell. (b) Endogenous antigen, which is produced within the cell itself (e.g., in a virus-infected cell), is degraded within the cytoplasm into peptides, which move into the endoplasmic reticulum, where they bind to class I MHC molecules. The peptide–class I MHC complexes then move through the Golgi complex to the cell surface.

Endogenous antigen is produced within the host cell itself. Two common examples are viral proteins synthesized within virus-infected host cells and unique proteins synthesized by cancerous cells. Endogenous antigens are degraded into peptide fragments that bind to class I MHC molecules within the endoplasmic reticulum. The peptide– class I MHC complex is then transported to the cell membrane. Since all nucleated cells express class I MHC molecules, all cells producing endogenous antigen use this route to process the antigen. T cells displaying CD8 recognize antigen associated with class I MHC molecules and thus are said to be class I MHC restricted. These cytotoxic T cells attack and kill cells displaying the antigen–MHC class I complexes for which their receptors are specific.

- *Cytokines:*

After a TH cell recognizes and interacts with an antigen–MHC class II molecule complex, the cell is activated—it becomes an effector cell that secretes various growth factors known collectively as cytokines. The secreted cytokines play an important role in activating B cells, TC cells, macrophages, and various other cells that participate in the immune response. Differences in the pattern of cytokines produced by activated TH cells result in different types of immune response.Under the influence of TH-derived cytokines, a TC cell that recognizes an antigen– MHC class I molecule complex proliferates and differentiates into an effector cell called a cytotoxic T lymphocyte (CTL). In contrast to the TC cell, the CTL generally does not secrete many cytokines and instead exhibits cell-killing or cytotoxic activity. The CTL has a vital function in monitoring the cells of the body and eliminating any that display antigen, such as virus-infected cells, tumor cells, and cells of a foreign tissue graft. Cells that display foreign antigen complexed with a class I MHC molecules are called altered self-cells; these are targets of CTLs.

Part- 4:

Antigens: Substances that can be recognised by the immunoglobulin receptor of B cells, or by the T-cell receptor when complexed with MHC, are called Antigens.

The molecular properties of antigens and the way in which these properties ultimately contribute to immune activation are central to our understanding of the immune system.

1. *Immunogenicity Versus Antigenicity:*

Immunogenicity and antigenicity are related but distinct immunologic properties that

sometimes are confused. Immunogenicityis the ability to induce a humoral and/or cell mediated immune response:

B cells + antigen -> effector B cells + memory B cells

‖

(plasma cells)

T cells + antigen ->effector T cells + memory T cells

‖

(e.g.,CTLs, THs).

Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an immunogen.

Antigenicity is the ability to combine specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors). Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called haptens, are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity.

2. *Factors That Influence Immunogenicity:*

To protect against infectious disease, the immune system must be able to recognize bacteria, bacterial products, fungi, parasites, and viruses as immunogens. In fact, the immune system actually recognizes particular macromolecules of an infectious agent, generally either proteins or polysaccharides. Proteins are the most potent immunogens, with polysaccharides ranking second. In contrast, lipids and nucleic acids of an infectious agent generally do not serve as immunogens unless they are complexed with proteins or polysaccharides. Immunologists tend to use proteins or polysaccharides as immunogens in most experimental studies of humoral immunity (Table 3-1). For

TABLE 3-1 Molecular weight of some common experimental antigens used in immunology

Antigen	Approximate molecular mass (Da)
Bovine gamma globulin (BGG)	150,000
Bovine serum albumin (BSA)	69,000
Flagellin (monomer)	40,000
Hen egg-white lysozyme (HEL)	15,000
Keyhole limpet hemocyanin (KLH)	>2,000,000
Ovalbumin (OVA)	44,000
Sperm whale myoglobin (SWM)	17,000
Tetanus toxoid (TT)	150,000

cell-mediated immunity, only proteins and some lipids and glycolipids serve as immunogens. These molecules are not recognized directly.

Proteins must first be processed into small peptides and then presented together with MHC molecules on the membrane of a cell before they can be recognized as immunogens. Recent work shows that those lipids and glycolipids that can elicit cellmediated immunity must also be combined with MHC-like membrane molecules called CD1.

Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters. The next two sections describe the properties that most immunogens share and the contribution that the biological system makes to the expression of immunogenicity.

3. *Haptens and the Study of Antigenicity:*

The pioneering work of Karl Landsteiner in the 1920s and 1930s created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope on a complex protein antigen. Landsteiner employed various haptens, small organic molecules that are antigenic but not immunogenic. Chemical coupling of a hapten to a large protein, called a carrier, yields an immunogenic hapten-carrier conjugate. Animals immunized with such a conjugate produce antibodies specific for:

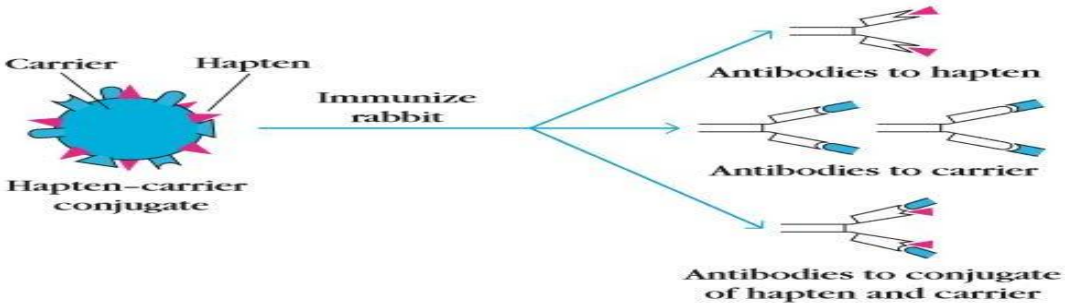
(1) The hapten determinant,

(2) Unaltered epitopes on the carrier protein, and

(3) New epitopes formed by combined parts of both the hapten and carrier

By itself, a hapten cannot function as an immunogenic epitope. But when multiple molecules of a single hapten are coupled to a carrier protein (or nonimmunogenichomopolymer), the

hapten becomes accessible to the immune system and can function as an immunogen.



Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti–BSA
Hapten–carrier conjugate (DNP-BSA)	Anti–DNP (major) Anti–BSA (minor) Anti–DNP/BSA (minor)

FIGURE 3-10 A hapten-carrier conjugate contains multiple copies of the hapten—a small nonimmunogenic organic compound such as dinitrophenol (DNP)—chemically linked to a large protein carrier such as bovine serum albumin (BSA). Immunization with DNP alone elicits no anti-DNP antibodies, but immunization with DNP-BSA elicits three types of antibodies. Of these, anti-DNP antibody is predominant, indicating that in this case the hapten is the immunodominant epitope in a hapten-carrier conjugate, as it often is in such conjugates.

4. *Epitops:*

Immune cells do not interact with, or recognize, an entire immunogen molecule; instead, lymphocytes recognize discrete sites on the macromolecule called epitopes, or antigenic determinants. Epitopes are the immunologically active regions of an immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. Studies with small antigens have revealed that B and T cells recognize different epitopes on the same antigenic molecule. For example, when mice were immunized with glucagon, a small human hormone of 29 amino acids,antibody was elicited to epitopes in the aminoterminal portion, whereas the T cells responded only to epitopes in the carboxyl-terminal portion. Lymphocytes may interact with a complex antigen on several levels of antigen structure.An epitope on a protein antigen may involve elements of the primary,secondary,tertiary, and even quaternary structure ofthe protein (see Figure 3-1). In polysaccharides, branched chains are commonly present, and multiple branches may contribute to the conformation of epitopes.

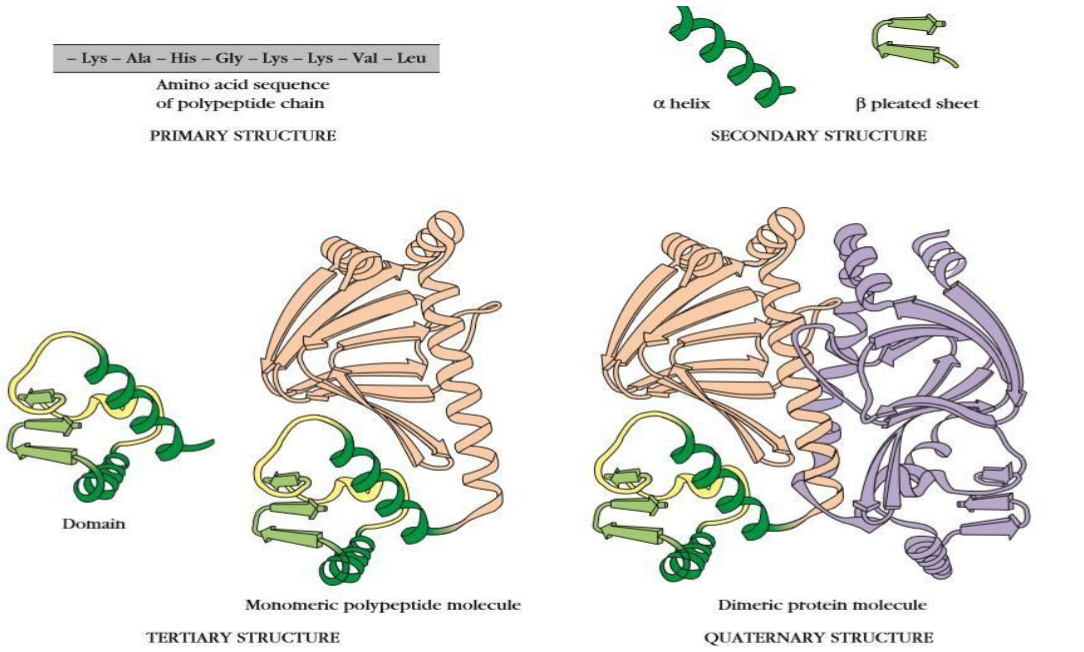


FIGURE 3-1 The four levels of protein organizational structure. The linear arrangement of amino acids constitutes the primary structure. Folding of parts of a polypeptide chain into regular structures (e.g., α helices and β pleated sheets) generates the secondary structure. Tertiary structure refers to the folding of regions between secondary features to give the overall shape of the molecule or parts of it (domains) with specific functional properties. Quaternary structure results from the association of two or more polypeptide chains into a single polymeric protein molecule.

The recognition of antigens by T cells and B cells is fundamentally different (Table 3-4).B cells recognize soluble antigen when it binds to their membrane-bound antibody. Because B cells bind antigen that is free in solution, the epitopes they recognize tend to be highly

TABLE 3-4 Comparison of antigen recognition by T cells and B cells

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules

accessible sites on the exposed surface of the immunogen. As noted previously, most T cells recognize only peptides combined with MHC molecules on the surface of antigen-presenting cells and altered self-cells; T-cell epitopes, as a rule, cannot be considered apart from their associated MHC molecules.

5. *ADJUVANTS:*

Adjuvants (from Latin adjuvare, to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available. For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant. Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects (Table 3-3):

- Antigen persistence is prolonged.
- Co-stimulatory signals are enhanced.
- Local inflammation is increased.
- The nonspecific proliferation of lymphocytes is stimulated.

Aluminum potassium sulfate (alum) prolongs the persistence of antigen. When an antigen is mixed withalum, the salt precipitates the antigen. Injection of this alum precipitate results in a slower release of antigen from the injection site, so that the effective time of exposure to the antigen increases from a few days without adjuvant to several weeks with the adjuvant. The alum precipitate also increases the size of the antigen, thus increasing the likelihood of phagocytosis.

Water-in-oil adjuvants also prolong the persistence of antigen. A preparation known as Freund's incomplete adjuvant contains antigen in aqueous solution, mineral oil, and an

TABLE 3-3 Postulated mode of action of some commonly used adjuvants

Adjuvant	POSTULATED MODE OF ACTION			
	Prolongs antigen persistence	Enhances co-stimulatory signal	Induces granuloma formation	Stimulates lymphocytes nonspecifically
Freund's incomplete adjuvant	+	+	+	−
Freund's complete adjuvant	+	++	++	−
Aluminum potassium sulfate (alum)	+	?	+	−
Mycobacterium tuberculosis	−	?	+	−
Bordetella pertussis	−	?	−	+
Bacterial lipopolysaccharide (LPS)	−	+	−	+
Synthetic polynucleotides (poly IC/poly AU)	−	?	−	+

emulsifying agent such as mannidemonooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection. This preparation is based on Freund's complete adjuvant, the first deliberately formulated highly effective adjuvant, developed by Jules Freund many years ago and containing heat-killed Mycobacteria as an additional ingredient. Muramyl dipeptide, a component of the mycobacterial cell wall, activates macrophages, making Freund's complete adjuvant far more potent than the incomplete form. Activated macrophages are more phagocytic than unactivated macrophages and express higher levels of class II MHC molecules and the membrane molecules of the B7 family.

The increased expression of class II MHC increases the ability of the antigen-presenting cell to present antigen to TH cells. B7 molecules on the antigen presenting cell bind to CD28, a cell-surface protein on TH cells, triggering co- stimulation, an enhancement of the T-cell immune response. Thus, antigen presentation and the requisite co-stimulatory signal usually are increased in the presence of adjuvant.

Alum and Freund's adjuvants also stimulate a local, chronic inflammatory response that attracts both phagocytes and lymphocytes. This infiltration of cells at the site of the adjuvant injection often results in formation of a dense, macrophage-rich mass of cells called a

granuloma. Because the macrophages in a granuloma are activated, this mechanism also enhances the activation of TH cells. Other adjuvants (e.g., synthetic polyribonucleotides and bacterial lipopolysaccharides) stimulate the nonspecific proliferation of lymphocytes and thus increase the likelihood of antigen-induced clonal selection of lymphocytes.

✓ Part- 5:

1. *Immunoglobulins:*

Immunoglobulins, also known as antibodies, are glycoprotein molecules produced by plasma cells (white blood cells). They act as a critical part of the immune response by specifically recognizing and binding to particular antigens, such as bacteria or viruses, and aiding in their destruction. The antibody immune response is highly complex and exceedingly specific. The various immunoglobulin classes and subclasses (isotypes) differ in their biological features, structure, target specificity and distribution. Hence, the assessment of the immunoglobulin isotype can provide useful insight into complex humoral immune response. Assessment and knowledge of immunoglobulin structure and classes is also important for selection and preparation of antibodies as tools for immunoassays and other detection applications.

The first evidence that antibodies were contained in particular serum protein fractions came from a classic experiment by A.Tiselius and E.A.Kabat,in 1939.They immunized rabbits with the protein ovalbumin (the albumin of egg whites) and then divided the immunized rabbits' serum into two aliquots.Electrophoresis of one serum aliquot revealed four peaks corresponding to albumin and the alpha (α),beta (β), and gamma (γ) globulins. The other serum aliquot was reacted with ovalbumin, and the precipitate that formed was removed; the remaining serum proteins,which did not react with the antigen, were then electrophoresed. A comparison of the electrophoretic profiles of these two serum aliquots revealed that there was a significant drop in the γ-globulinpeak in the aliquot that had been reacted with antigen (Figure 4-1).Thus, the γ-globulin fractionwas identified as

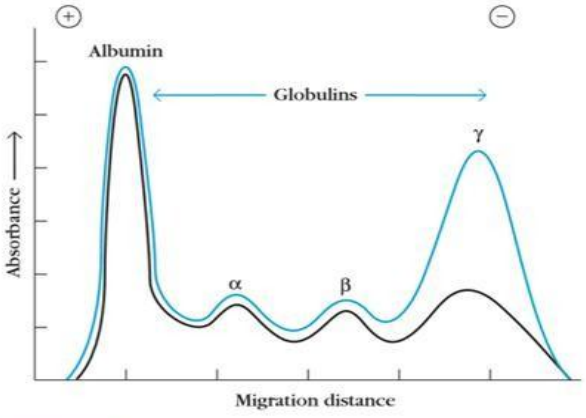


FIGURE 4-1 Experimental demonstration that most antibodies are in the γ-globulin fraction of serum proteins. After rabbits were immunized with ovalbumin (OVA), their antisera were pooled and electrophoresed, which separated the serum proteins according to their electric charge and mass. The blue line shows the electrophoretic pattern of untreated antiserum. The black line shows the pattern of antiserum that was incubated with OVA to remove anti-OVA antibody and then electrophoresed. *[Adapted from A. Tiselius and E. A. Kabat, 1939,* J. Exp. Med. ***69:****119, with copyright permission of the Rockefeller University Press.]*

containing serum antibodies, which were called immunoglobulins,to distinguish them from any other proteins that might be contained in the γ-globulin fraction.

2. *Immunoglobulins Are Heterodimers:*

Antibody molecules have a common structure of four peptide chains (Figure 4-2). This structure consists of two identical light (L) chains, polypeptides of about 25,000 molecular weight, and two identical heavy (H) chains, larger polypeptides of molecular weight 50,000 or more. Like the antibody molecules they constitute, H and L chains are also called immunoglobulins. Each light chain is bound to a heavy chain by a disulfide bond,and by such noncovalent interactions as salt linkages,hydrogen bonds,and hydrophobic bonds,to form a heterodimer (H-L).Similar noncovalent interactions and disulfide bridges link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain $(H\text{-}L)_2$ antibody structure, a dimer of dimers. The first 110 or so amino acids of the amino-terminal region of a light or heavy chain vary greatly among antibodies of different specificity. These segments of highly variable sequence are called V regions: V_L in light chains and V_H in heavy. The regions of relatively constant sequence beyond the variable regions have been dubbed C regions, C_L on the light chain and C_H on the heavy chain.

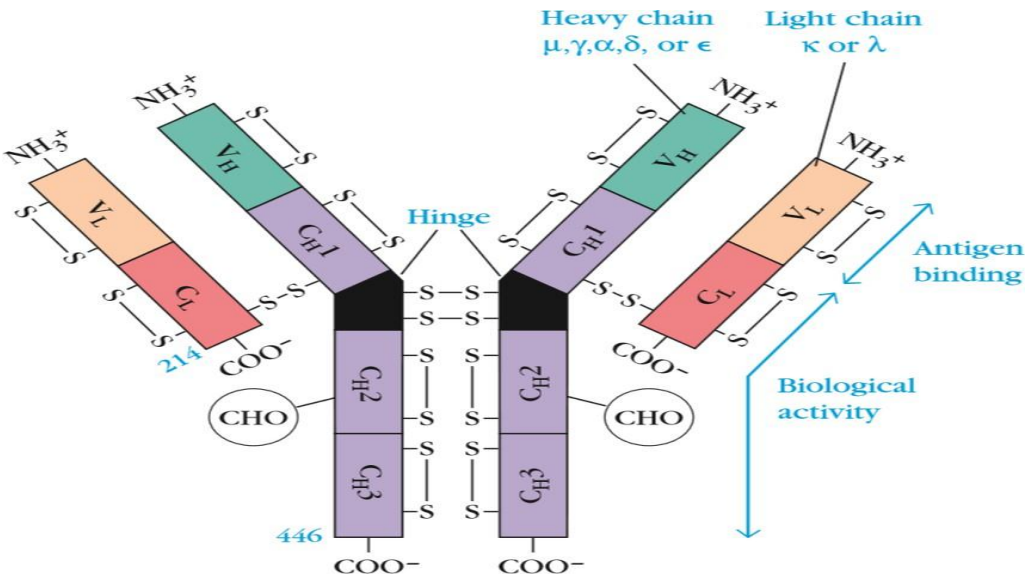


FIGURE 4-2 Schematic diagram of structure of immunoglobulins derived from amino acid sequencing studies. Each heavy and light chain in an immunoglobulin molecule contains an amino-terminal variable (V) region (aqua and tan, respectively) that consists of 100—110 amino acids and differs from one antibody to the next. The remainder of each chain in the molecule—the constant (C) regions (purple and red)—exhibits limited variation that defines the two light-chain subtypes and the five heavy-chain subclasses. Some heavy chains (γ, δ, and α) also contain a proline-rich hinge region (black). The amino-terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the other domains. The μ and ε heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule.

3. *Light-Chain Sequencing Revealed That Immunoglobulins Have Constant and Variable Regions:*

When the amino acid sequences of several Bence-Jones proteins (light chains) from different individuals were compared, a striking pattern emerged. The amino-terminal half of the chain, consisting of 100–110 amino acids, was found to vary among different Bence-Jones proteins. This region was called the variable (V) region. The carboxyl-terminal half of the molecule, called the constant (C) region, had two basic amino acid sequences. This led to the recognition that there were two light chain types, kappa (κ) and lambda (λ). In humans, 60% of the light chains are kappa and 40% are lambda, whereas in mice, 95% of the light chains are kappa and only 5% are lambda. A single antibody molecule contains only one light chain type, either or, never both.

4. *Heavy-Chain Sequencing Revealed Five Basic Varieties of Heavy Chains:*

For heavy-chain sequencing studies, myeloma proteins were reduced with mercaptoethanol and alkylated, and the heavy chains were separated by gel filtration in a denaturing solvent. When the amino acid sequences of several myeloma protein heavy chains were compared,a pattern similar to that of the light chains emerged. The amino-terminal part of the chain, consisting of 100–110 amino acids, showed great sequence variation among myeloma heavy chains and was therefore called the variable (V) region.

TABLE 4-1 Chain composition of the five immunoglobulin classes in humans

Class	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	γ1, γ2, γ3, γ4	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n = 1$ or 5
IgA	α	α1, α2	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n = 1, 2, 3,$ or 4
IgE	ε	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

The remaining part of the protein revealed five basic sequence patterns, corresponding to five different heavy-chain constant (C) regions (μ, δ, γ, ε, and α).Each of these five different heavy chains is called an isotype. The length of the constant regions is approximately 330 amino acids for δ, γ, and α and 440 amino acids for μ and ε.The heavy chains of a given antibody molecule determine the class of that antibody: IgM(μ), IgG(γ), IgA(α), IgD(δ), or IgE(ε). Each class can have either κ or λ light chains.

A single antibody molecule has two identical heavy chains and two identical light chains,H_2L_2,or a multiple $(H_2L_2)_n$ of this basic four-chain structure (Table 4-1).

Minor differences in the amino acid sequences of the α and γheavy chains led to further classification of the heavy chains into subisotypes that determine the subclass of antibody molecules they constitute. In humans, there are two subisotypes of αheavy chains- α1 and α2 (and thus IgG3, and IgG4). In mice, thereare four subisotypes, γ1, γ2a, γ2b,and γ3,and the corresponding subclasses.

Careful analysis ofthe amino acid sequences ofimmunoglobulin heavy and light chains showed that both chains contain several homologous units of about 110 amino acid residues. Within each unit, termed a domain, an intrachaindisulfide bond forms a loop of about 60 amino acids.

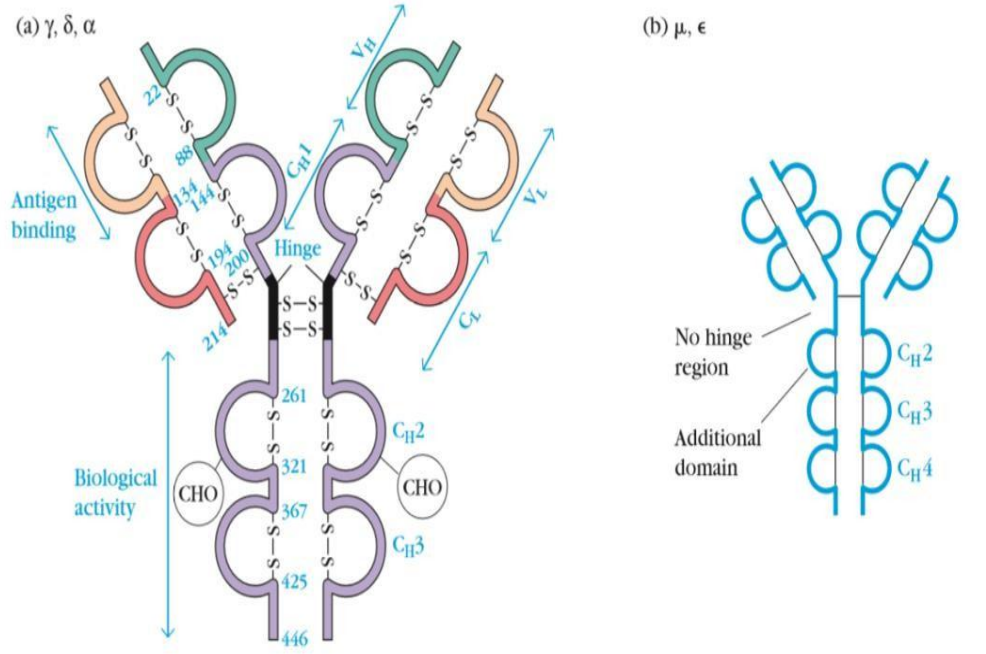


FIGURE 4-6 (a) Heavy and light chains are folded into domains, each containing about 110 amino acid residues and an intrachain disulfide bond that forms a loop of 60 amino acids. The amino-terminal domains, corresponding to the V regions, bind to antigen; effector functions are mediated by the other domains. (b) The μ and ε heavy chains contain an additional domain that replaces the hinge region.

Light chains contain one variable domain (V_L), and one constant domain (C_L);heavy chains contain one variable domain (V_H),and either three or four constant domains two subclasses,IgA1 and IgA2)—and four subisotypes of γ heavy chains: γ1, γ2, γ3, and γ4 (therefore four subclasses, IgG1, IgG2, (C_H1, C_H2, C_H3, and C_H4),depending on the antibody class (Fig 4-6).

5. *Antibody Classes and Biological Activities:*

i. Immunoglobulin G (IgG):

IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two γ heavy chains and two κ or two λ light chains (see Figure 4-13a).There are four human IgG subclasses,distinguished by differences in γ-chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3,and IgG4 (Table 4-2).

TABLE 4-2 Properties and biological activities* of classes and subclasses of human serum immunoglobulins

Property/Activity	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM‡	IgE	IgD
Molecular weight†	150,000	150,000	150,000	150,000	150,000–600,000	150,000–600,000	900,000	190,000	150,000
Heavy-chain component	γ1	γ2	γ3	γ4	α1	α2	μ	ϵ	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In vivo serum half life (days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+/−	++	−	−	−	+++	−	−
Crosses placenta	+	+/−	+	+	−	−	−	−	−
Present on membrane of mature B cells	−	−	−	−	−	−	+	−	+
Binds to Fc receptors of phagocytes	++	+/−	++	+	−	−	?	−	−
Mucosal transport	−	−	−	−	++	++	+	−	−
Induces mast-cell degranulation	−	−	−	−	−	−	−	+	−

*Activity levels indicated as follows: ++ = high; + = moderate; +/− = minimal; − = none; ? = questionable.

†IgG, IgE, and IgD always exist as monomers; IgA can exist as a monomer, dimer, trimer, or tetramer. Membrane-bound IgM is a monomer, but secreted IgM in serum is a pentamer.

‡IgM is the first isotype produced by the neonate and during a primary immune response.

The amino acid sequences that distinguish the four IgG subclasses are encoded by different germ-line C_H genes, whose DNA sequences are 90%–95% homologous. The structural characteristics that distinguish these subclasses from one another are the size of the hinge region and the number and position of the interchaindisulfide bonds between the heavy chains (Figure 4-14).

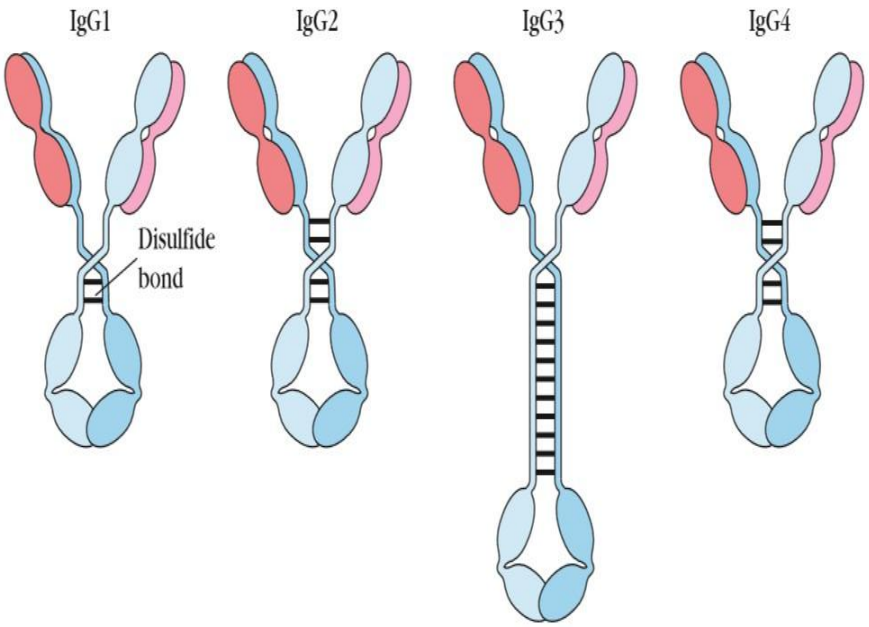


FIGURE 4-14 General structure of the four subclasses of human IgG, which differ in the number and arrangement of the interchain disulfide bonds (thick black lines) linking the heavy chains. A notable feature of human IgG3 is its 11 interchain disulfide bonds.

The subtleamino acid differences between subclasses of IgG affect the biological activity of the molecule:

- IgG1,IgG3,and IgG4 readily cross the placenta and play an important role in protecting the developing fetus.
- IgG3 is the most effective complement activator, followed by IgG1; IgG2 is less efficient,and IgG4 is not able to activate complement at all.
- IgG1 and IgG3 bind with high affinity to Fc receptors on phagocytic cells and thus mediate opsonisation.IgG4 has an intermediate affinity for Fc receptors,and IgG2 has an extremely low affinity.

ii. Immunoglobulin M (IgM):

IgM accounts for 5%–10% of the total serum immunoglobulin, with an average serum concentration of 1.5 mg/ml. Monomeric IgM, with a molecular weight of 180,000, is expressed as membrane-bound antibody on B cells.IgM is secreted by plasma cells as a pentamer in which five monomer units are held together by disulfide bonds that link their carboxyl-terminal heavy chain domains (Cμ4/Cμ4) and their Cμ3/Cμ3 domains (see Figure 4-13e). The five monomer subunits are arranged with their Fc regions in the center of the pentamer and the ten antigen-binding sites on the periphery of the molecule.

Each pentamer contains an additional Fc-linked polypeptide called the J (joining) chain, which is disulfide- bonded to the carboxyl-terminal cysteine residue of two of the ten μ chains. The J chain appears to be required for polymerization of the monomers to form pentameric IgM; it is added just before secretion of the pentamer.

IgM is the first immunoglobulin class produced in a primary response to an antigen, and it is also the first immunoglobulin to be synthesized by the neonate. Because of its pentameric structure with 10 antigen-binding sites,serum IgM has a higher valency than the other isotypes. An IgM molecule can bind 10 small hapten molecules; however, because of steric hindrance,only 5 or fewer molecules of larger antigens can be bound simultaneously. Because ofits high valency,pentamericIgM is more efficient than other isotypes in binding antigens with many repeating epitopes such as viral particles and red blood cells (RBCs). It takes 100 to 1000 times more molecules of IgG than of IgM to achieve the same level of agglutination.A similar phenomenon occurs with viral particles:less IgM than IgG is requiredto neutralize viral infectivity. IgM is also more efficient than IgG at activating complement. Complement activation requires two Fc regions in close proximity,and the pentameric structure ofa single molecule of IgM fulfills this requirement.

Because of its large size, IgM does not diffuse well and therefore is found in very low concentrations in the intercellular tissue fluids. The presence of the J chain allows IgM to bind to receptors on secretory cells,which transport it across epithelial linings to enter the external secretions that bathe mucosal surfaces. Although IgA is the major isotype found in these secretions,IgM plays an important accessory role as a secretory immunoglobulin.

iii. ImmunoglobulinA (IgA):

Although IgA constitutes only 10%–15% of the total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary,and digestive tracts.In serum,IgA exists primarily as a monomer, but polymeric forms (dimers, trimers, and some tetramers) are sometimes seen, all containing a J-chainpolypeptide (see Figure 4-13d). The IgA of external secretions,called secretory IgA, consists of a dimer or tetramer,a J-chain polypeptide, and a polypeptide chain called secretory component (Figure 4-15a).The J-chain polypeptide in IgA is identical to that found in pentamericIgM and serves a similar function in facilitating the polymerization of both serum IgA and secretory IgA.The secretory component is a 70,000-MW polypeptide produced by epithelial cells of mucous membranes. It consists of five immunoglobulin-like domains that bind to the Fc region domains of the IgA dimer. This interaction is stabilized by a disulfide bond between the fifth domain of the secretory

component and one of the chains of the dimeric IgA.

The daily production of secretory IgA is greater than that of any other immunoglobulin class. IgA-secreting plasma cells are concentrated along mucous membrane surfaces.Breast milk contains secretory IgA and many other molecules that help protect the newborn against infection during the first month of life. Because the immune system of infants is not fully functional,breast-feeding plays an important role in maintaining the health of newborns.

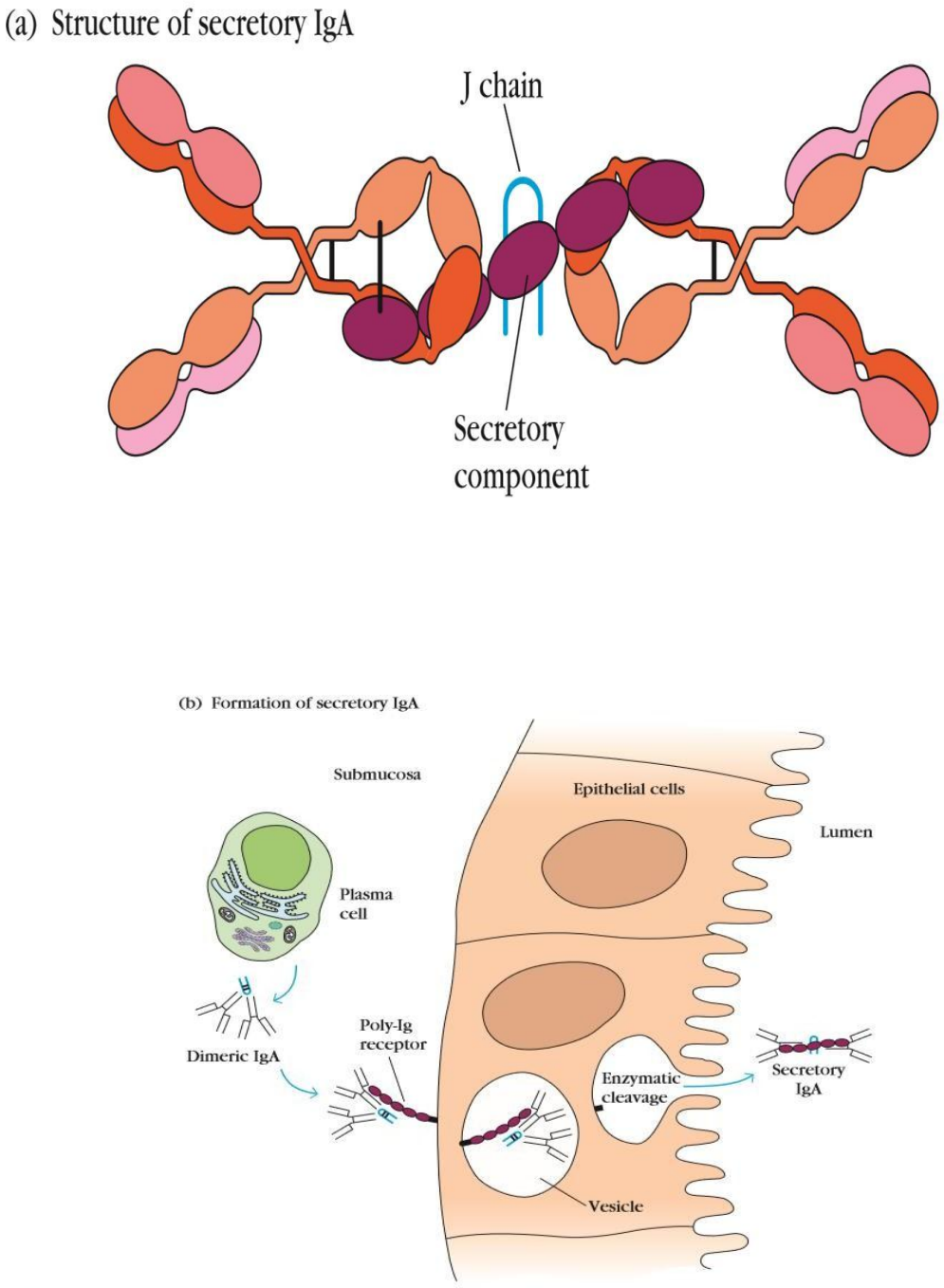


FIGURE 4-15 Structure and formation of secretory IgA. (a) Secretory IgA consists of at least two IgA molecules, which are covalently linked to each other through a J chain and are also covalently linked with the secretory component. The secretory component contains five Ig-like domains and is linked to dimeric IgA by a disulfide bond between its fifth domain and one of the IgA heavy chains. (b) Secretory IgA is formed during transport through mucous membrane epithelial cells. Dimeric IgA binds to a poly-Ig receptor on the basolateral membrane of an epithelial cell and is internalized by receptor-mediated endocytosis. After transport of the receptor-IgA complex to the luminal surface, the poly-Ig receptor is enzymatically cleaved, releasing the secretory component bound to the dimeric IgA.

Secretory IgA serves an important effector function at mucous membrane surfaces, which are the main entry sites for most pathogenic organisms. Because it is polymeric, secretory IgA can cross-link large antigens with multiple epitopes. Binding of secretory IgA to bacterial and viral surface antigens prevents attachment of the pathogens to the mucosal cells, thus inhibiting viral infection and bacterial colonization. Complexes of secretory IgA and antigen are easily entrapped in mucus and then eliminated by the ciliated epithelial cells of the respiratory tract or by peristalsis of the gut. Secretory IgA has been shown to provide an important line of defense against bacteria such as *Salmonella, Vibrio cholerae,*and *Neisseria gonorrhoeaeand* viruses such as polio, influenza,andreovirus.

iv. ImmunoglobulinE (IgE):

The potent biological activity of IgE allowed it to be identified in serum despite its extremely low average serum concentration (0.3 µg/ml).IgE antibodies mediate the immediate hypersensitivity reactions that are responsible for the symptoms of hay fever,asthma,hives,and anaphylactic shock.The presence of a serum component responsible for allergic reactions was first demonstrated in 1921 by K. Prausnitz and H. Kustner, who injected serum from an allergic person intra-dermally into a nonallergic individual. When the appropriate antigen was later injected at the same site, a wheal and flare reaction (analogous to hives) developed there. This reaction, called the P-K reaction (named for its originators,Prausnitz and Kustner),was the basis for the first biological assay for IgE activity.

IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells.

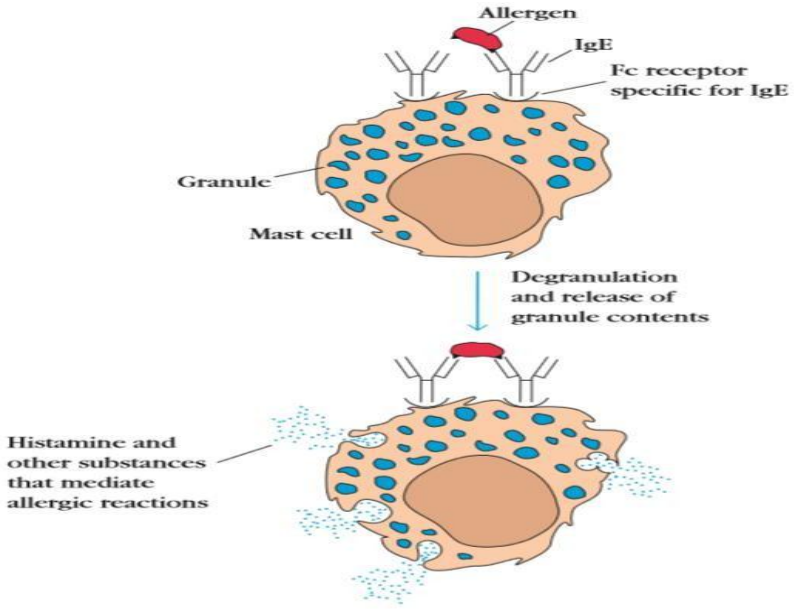


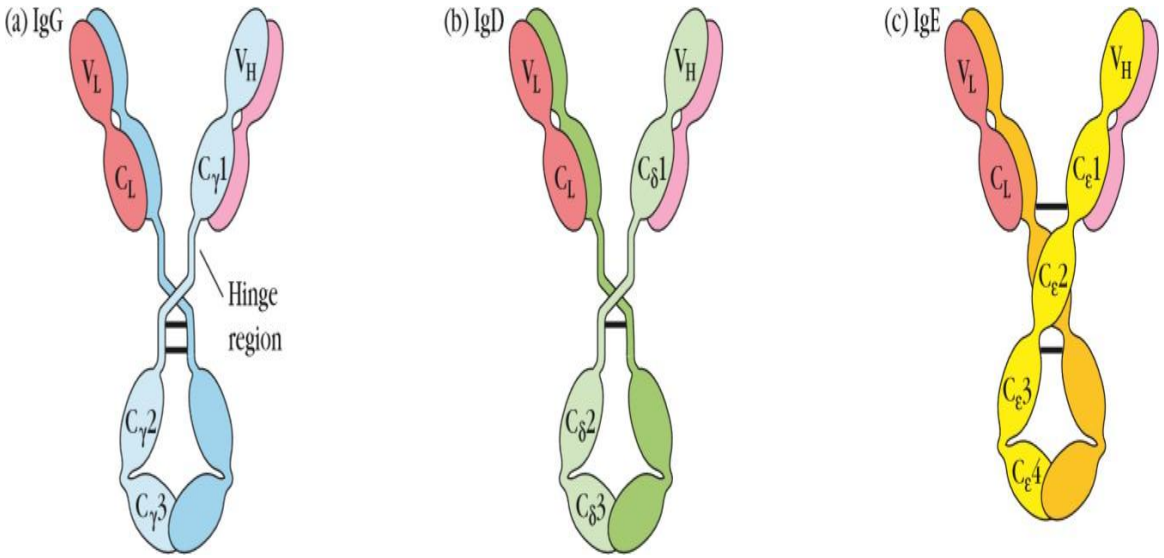
FIGURE 4-16 Allergen cross-linkage of receptor-bound IgE on mast cells induces degranulation, causing release of substances (blue dots) that mediate allergic manifestations.

Cross- linkage of receptorboundIgE molecules by antigen (allergen) induces basophils and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment, a process known as degranulation. As a result, a variety of pharmacologically active mediators are released and give rise to allergic manifestations (Figure 4-16).

Localized mast-cell degranulation induced by IgE also may release mediators that facilitate a buildup of various cells necessary for antiparasitic defense.

v. Immunoglobulin D (IgD):

IgD was first discovered when a patient developed a multiple myeloma whose myeloma protein failed to react with antiisotype antisera against the then-known isotypes: IgA, IgM, and IgG. When rabbits were immunized with this myeloma protein,the resulting antisera were used to identify the same class of antibody at low levels in normal human serum. The new class,called IgD,has a serum concentration of 30 g/ml and constitutes about 0.2% of the total immunoglobulin in serum. IgD, together with IgM, is the major membraneboundimmunoglobulin expressed by mature B cells,and its role in the physiology of B cells is under investigation. No biological effector function has been identified for IgD.



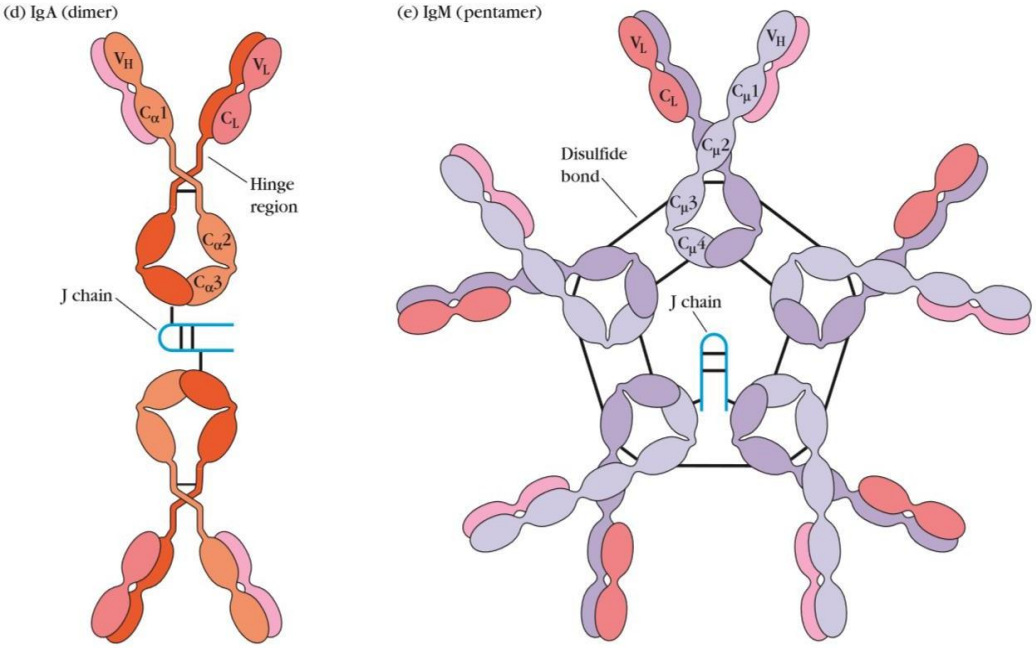


FIGURE 4-13 General structures of the five major classes of secreted antibody. Light chains are shown in shades of pink, disulfide bonds are indicated by thick black lines. Note that the IgG, IgA, and IgD heavy chains (blue, orange, and green, respectively) contain four domains and a hinge region, whereas the IgM and IgE heavy chains (purple and yellow, respectively) contain five domains but no hinge region. The polymeric forms of IgM and IgA contain a polypeptide, called the J chain, that is linked by two disulfide bonds to the Fc region in two different monomers. Serum IgM is always a pentamer; most serum IgA exists as a monomer, although dimers, trimers, and even tetramers are sometimes present. Not shown in these figures are intrachain disulfide bonds and disulfide bonds linking light and heavy chains (see Figure 4-2).

6. *Monoclonal Antibody:*

Most antigens offer multiple epitopes and therefore induce proliferation and differentiation of a variety of B-cell clones,each derived from a B cell that recognizes a particular epitope.The resulting serum antibodies are heterogeneous, comprising a mixture of antibodies, each specific for one epitope (Figure4-21).Such a polyclonal antibodyresponse facilitates the localization,phagocytosis,and complement-mediated lysis of antigen; it thus has clear advantages for the organism in vivo. Unfortunately, the antibody heterogeneity that increases immune protection in vivo often reduces the efficacy of an antiserum for various in vitro uses. For most research, diagnostic, and therapeutic purposes, monoclonal antibodies, derived from a single clone and thus specific for a single epitope, are preferable.

Direct biochemical purification of a monoclonal antibody from a polyclonal antibody preparation is not feasible. In 1975, Georges Köhler and Cesar Milstein devised a method for preparing monoclonal antibody, which quickly became one of immunology's key technologies. By fusing a normal activated,antibody-producing B cell with a myeloma cell (a cancerous plasma cell),they were able to generate a hybrid cell, called a hybridoma, that possessed the immortalgrowth properties of the myeloma cell and secreted the antibody produced by the B cell.The resulting clones of hybridoma cells, which secrete large quantities of monoclonal antibody, can be cultured indefinitely. The development of techniques for producing monoclonal antibodiesgave immunologists a powerful and versatile research tool. The significance of the work by Köhler and Milstein was acknowledged when each was awarded a Nobel Prize.

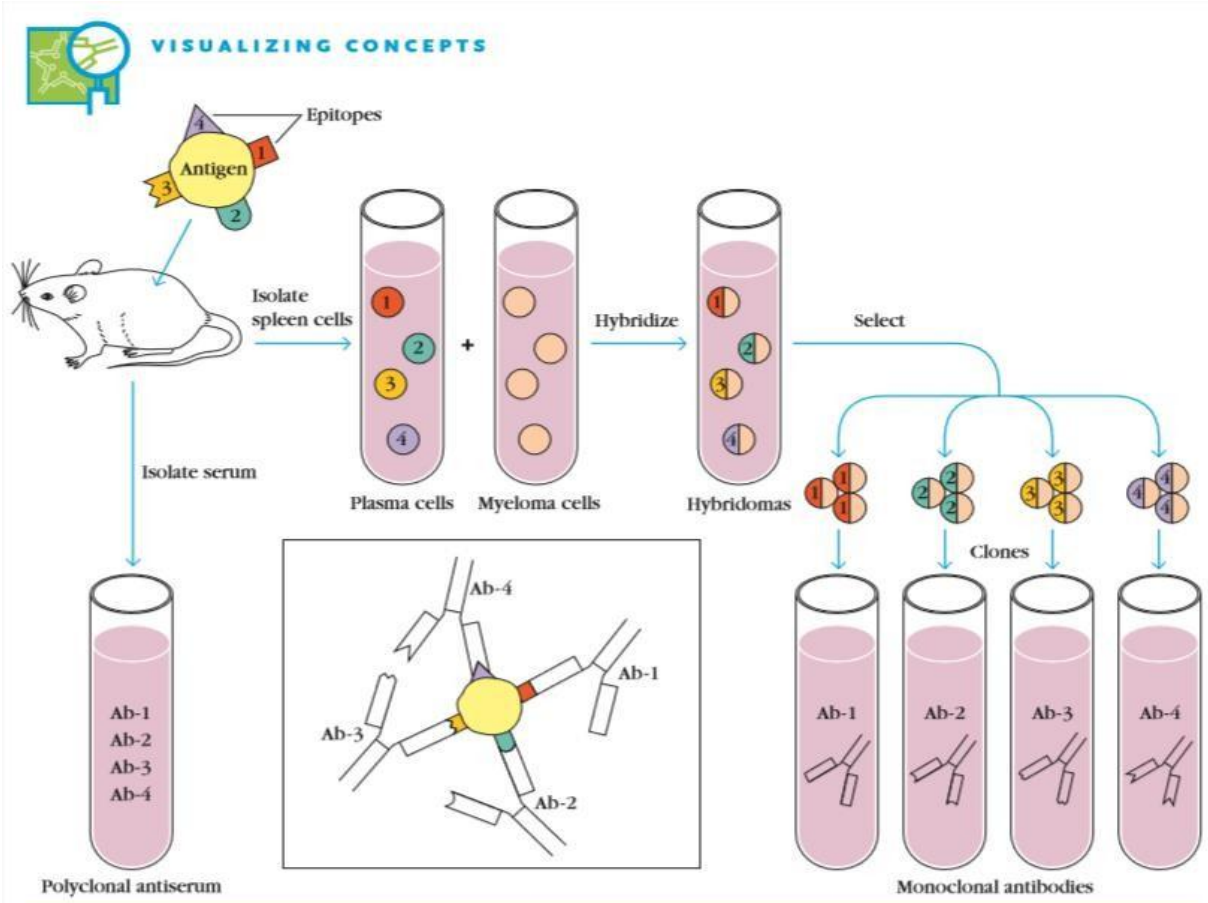


FIGURE 4-21 The conventional polyclonal antiserum produced in response to a complex antigen contains a mixture of monoclonal antibodies, each specific for one of the four epitopes shown on the antigen (inset). In contrast, a monoclonal antibody, which is derived from a single plasma cell, is specific for one epitope on a complex antigen. The outline of the basic method for obtaining a monoclonal antibody is illustrated here.

- *Monoclonal Antibodies Have Important Clinical Uses:*

Monoclonal antibodies are proving to be very useful as diagnostic, imaging, and therapeutic reagents in clinical medicine.Initially,monoclonal antibodies were used primarily as in vitro diagnostic reagents. Among the many monoclonal antibody diagnostic reagents now available are products for detecting pregnancy, diagnosing numerous pathogenic microorganisms, measuring the blood levels of various drugs, matching histocompatibility antigens, and detecting antigens shed by certain tumors.

Radiolabeled monoclonal antibodies can also be used in vivo for detecting or locating tumor antigens,permitting earlier diagnosis of some primary or metastatic tumors in patients. For example, monoclonal antibody to breast-cancer cells is labeled with iodine-131 and

introduced into the blood to detect the spread of a tumor to regional lymph nodes.This monoclonal imaging technique can reveal breast-cancer metastases that would be undetected by other, less sensitive scanning techniques.

Immunotoxins composed of tumor-specific monoclonal antibodies coupled to lethal toxins are potentially valuable therapeutic reagents.The toxins used in preparing immunotoxins include ricin, Shigella toxin, and diphtheria toxin, all of which inhibit protein synthesis.

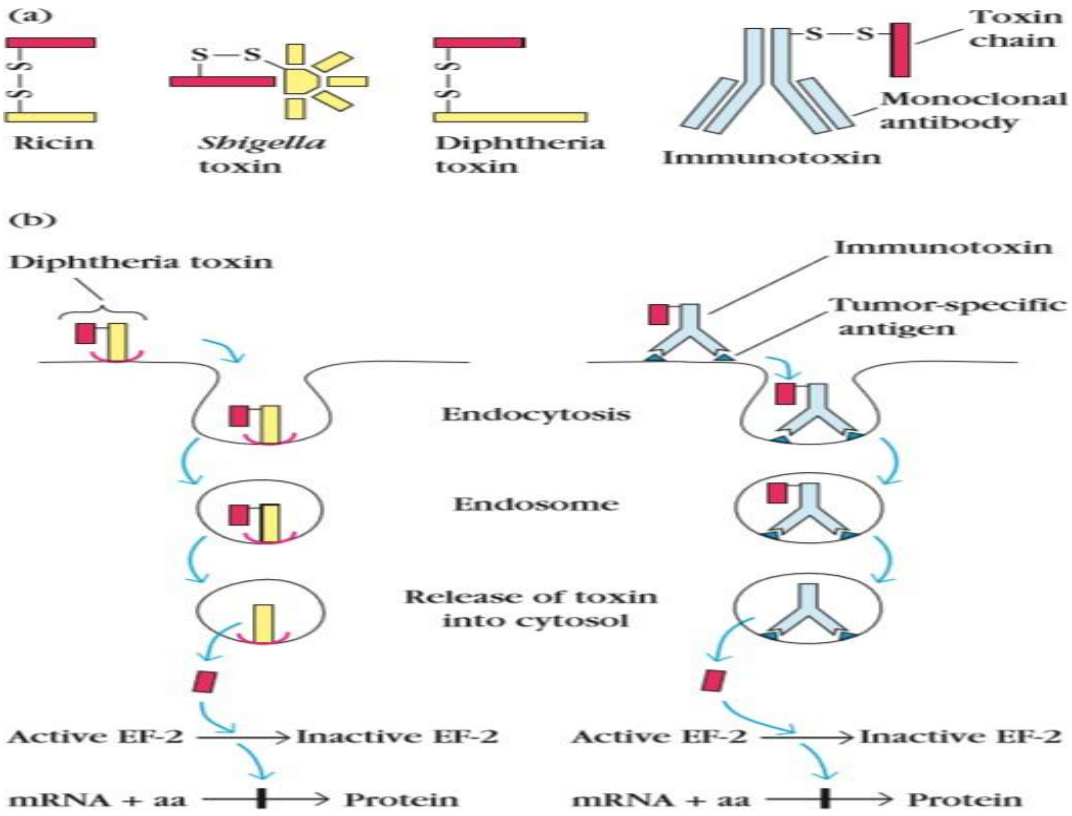


FIGURE 4-22 (a) Toxins used to prepare immunotoxins include ricin, *Shigella* toxin, and diphtheria toxin. Each toxin contains an inhibitory toxin chain (red) and a binding component (yellow). To make an immunotoxin, the binding component of the toxin is replaced with a monoclonal antibody (blue). (b) Diphtheria toxin binds to a cell-membrane receptor (*left*) and a diphtheria-immunotoxin binds to a tumor-associated antigen (*right*). In either case, the toxin is internalized in an endosome. The toxin chain is then released into the cytoplasm, where it inhibits protein synthesis by catalyzing the inactivation of elongation factor 2 (EF-2).

These toxins are so potent that a single molecule has been shown to kill a cell. Each of these toxins consists of two types of functionally distinct polypeptide components, an inhibitory (toxin) chain and one or more binding chains,which interact with receptors on cell surfaces; without the binding polypeptide(s) the toxin cannot get into cells and therefore is harmless.An immunotoxin is prepared by replacing the binding polypeptide(s) with a monoclonal antibody that is specific for a particular tumor cell (Figure 4-22a). In theory, the attached monoclonal antibody will deliver the toxin chain specifically to tumor cells, where it will cause death by inhibiting protein synthesis (Figure 4-22b). The initial clinical responses to such immunotoxins in patients with leukemia, lymphoma, and some other types of cancer have shown promise,and research to develop and demonstrate their safety and effectiveness is underway.

✓ Part- 6:

1. *Complement: Components, function, mode of action:*

The complement system, also known as complement cascade, is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promote inflammation, and attack the pathogen's cell membrane. It is part of the innate immune systemwhich is not adaptable and does not change during an individual's lifetime. The complement system can, however, be recruited and brought into action by antibodies generated by the adaptive immune system.

- *Components:*

The complement system consists of a number ofconstitutive serum small proteins that are synthesized by the liver, and circulate in the blood as inactive precursors. When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages. The end result of this complement activation or complement fixation cascade is stimulation of phagocytes to clear foreign and damaged material, inflammation to attract additional phagocytes, and activation of the cell-killing membrane attack complex. Over 30 proteins and protein fragments make up the complement system, including serum proteins, and cell membrane receptors. They account for about 10% of the globulin fraction of blood serum.The proteins and glycoproteins

composing the complement system are synthesized largely by liver hepatocytes, some by blood monocytes, tissue macrophages and epithe-lial cells of the gastro-intestinal and genito-urinary tracts.

i. The proteins that form the complement system are labelled numerically with the prefix C (e.g., C1 –C9).

ii. Some complement components are designated by letter symbols (e.g., factor B, D, P) or by trivial names (e.g., homologous factor).

iii. There are at least 19 of these components; they are all serum proteins and together they make up about 10% globulin fraction of serum.

iv. The molecular weights of the complement components vary between 24 kDa for factor D and 460 kDa for C19.

v. Serum concentration in humans varies between 20 μg/ml of C2 and 1300 μg/ml of C3.

vi. Complement components are synthesized at various sites like C2, C3, C4, C5; B, D, P and I are from macrophages, C3, C6, C8 and B from liver (Table 7.1 and7.2).

Table 7.1: Complement components

Name	MW (kDa)	Serum concentration (μg/ml)
Classical pathway		
C1q	460	80
C1r	83	50
C1s	83	50
C4	200	600
C2	102	20
C3	185	1300
Alternative pathway		
D	24	1
B	90	210
Terminal components		
C5	204	70
C6	120	65
C7	120	55
C8	160	55
C9	70	60
Control proteins		
C1-INH	105	200
C4-bp	550	250
H	150	480
I	88	35
P	4 × 56	20
Vitronectin	83	500

Table 7.2: The components of the complement system and their functions

Functionally distinct classes of complement protein	
Function	**Protein**
Binding to antigen; antibody complexes	C1q
Activating enzymes	C1r C1s C2b Bb D
Membrane-binding proteins and opsonins	C4b C3b
Peptide mediators of inflammation	C5a C3a C4a
Membrane attack proteins	C5b C6 C7 C8 C9

- ***Mode of action:***

  Mainly two biochemical pathways activate the complement system:

1. The classical complement pathway:

The classical pathway is triggered by activation of the C1-complex. The C1-complex is composed of 1 molecule of C1q, 2 molecules of C1r and 2 molecules of C1s, or $C1qr^2s^2$. This occurs when C1q binds to IgM or IgGcomplexed with antigens. A single pentamericIgM can initiate the pathway, while several, ideally six, IgGs are needed. This also occurs when C1q binds directly to the surface of the pathogen. Such binding leads to conformational changes in the C1q molecule, which leads to the activation of two C1r molecules. C1r is a serine protease. They then cleave C1s (another serine protease). The $C1r^2s^2$ component now splits C4 and then C2, producing C4a, C4b, C2a, and C2b (historically, the larger fragment of C2 was called C2a but is now referred to as C2b). C4b and C2a bind to form the classical pathway C3-convertase (C4b2a complex), which promotes cleavage of C3 into C3a and C3b. C3b later joins with C4b2a to make C5 convertase (C4b2a3b complex).

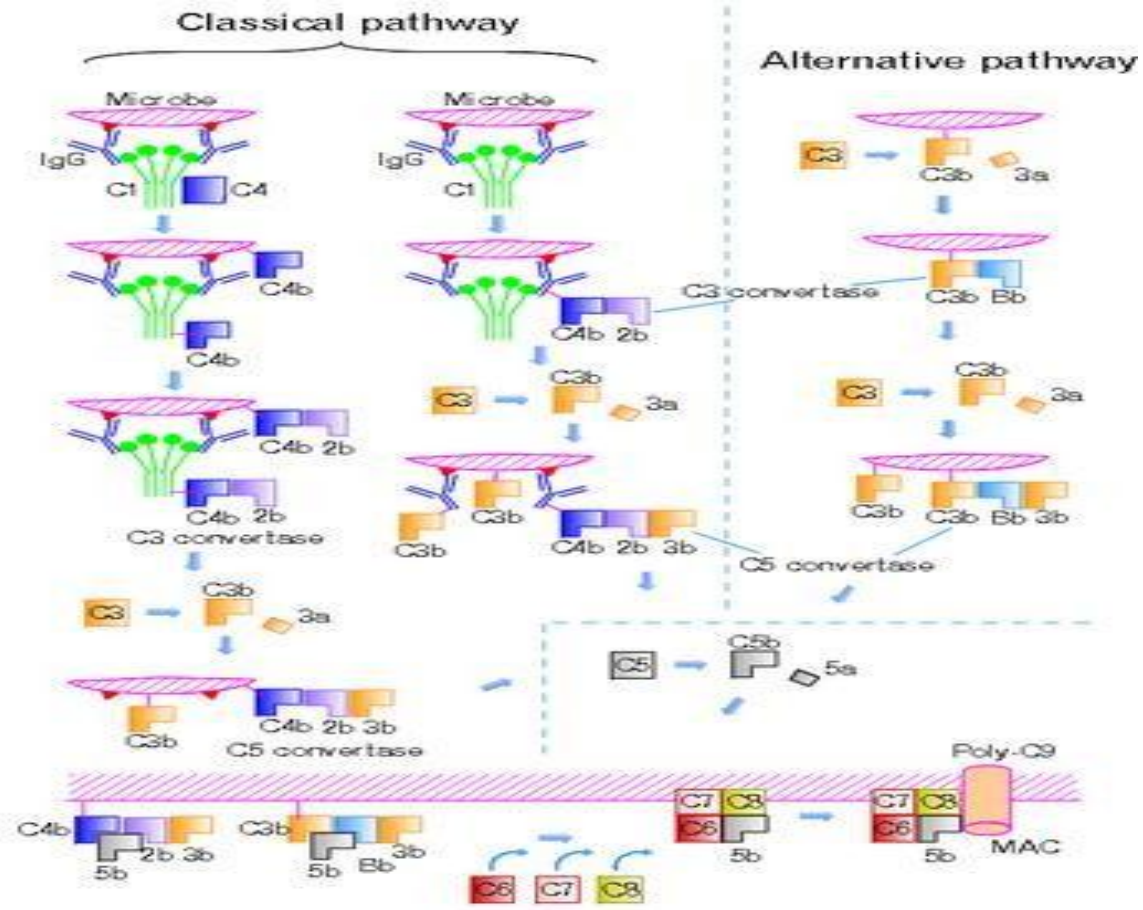
2. The alternative complement pathway:

The alternative pathway is continuously activated at a low level, analogous to a car engine at idle, as a result of spontaneous C3 hydrolysis due to the breakdown of the internal thioester bond (C3 is mildly unstable in aqueous environment). The alternative pathway does not rely on pathogen-binding antibodies like the other pathways. C3b that is generated from C3 by a C3 convertase enzyme complex in the fluid phase is rapidly inactivated by factor H and factor I, as is the C3b-like C3 that is the product of spontaneous cleavage of the internal thioester. In contrast, when the internal thioester of C3 reacts with a hydroxyl or amino group of a molecule on the surface of a cell or pathogen, the C3b that is now covalently bound to the surface is protected from factor H-mediated inactivation. The surface-bound C3b may now bind factor B to form C3bB. This complex in the presence of factor D will be cleaved into Ba and Bb. Bb will remain associated with C3b to form C3bBb, which is the alternative pathway C3 convertase.

The C3bBb complex is stabilized by binding oligomers of factor P (properdin). The stabilized C3 convertase, C3bBbP, then acts enzymatically to cleave much more C3, some of which becomes covalently attached to the same surface as C3b. This newly bound C3b recruits more B, D and P activity and greatly amplifies the complement activation. When complement is activated on a cell surface, the activation is limited by endogenous complement regulatory proteins, which include CD35, CD46, CD55 and CD59, depending on the cell. Pathogens, in

general, don't have complement regulatory proteins (there are many exceptions, which reflect adaptation of microbial pathogens to vertebrate immune defenses). Thus, the alternative complement pathway is able to distinguish self from non-self on the basis of the surface expression of complement regulatory proteins. Host cells don't accumulate cell surface C3b (and the proteolytic fragment of C3b called iC3b) because this is prevented by the complement regulatory proteins, while foreign cells, pathogens and abnormal surfaces may be heavily decorated with C3b and iC3b. Accordingly, the alternative complement pathway is one element of innate immunity.

Once the alternative C3 convertase enzyme is formed on a pathogen or cell surface, it may bind covalently another C3b, to form C3bBbC3bP, the C5 convertase. This enzyme then cleaves C5 to C5a, a potent anaphylatoxin, and C5b. The C5b then recruits and assembles C6, C7, C8 and multiple C9 molecules to assemble the membrane attack complex. This creates a hole or pore in the membrane that can kill or damage the pathogen or cell.



- ***Function:***

1. **Opsonization and phagocytosis:**

   C3b, bound to immune complex or coated on the surface of pathogen, activate phagocytic cells. These proteins bind to specific receptors on the phagocytic cells to get engulfed.

2. **Cell lysis:**

   Membrane attack complex formed by C5b6789 components ruptures the microbial cell surface which kills the cell.

3. **Chemotaxis:**

   Complement fragments attract neutrophils and macrophages to the area where the antigen is present. These cell surfaces have receptors for complements, like C5a, C3a, thus, run towards the site of inflammation, i.e. chemotaxis.

4. **Activation of mast cells and basophils and enhancement of inflammation:**

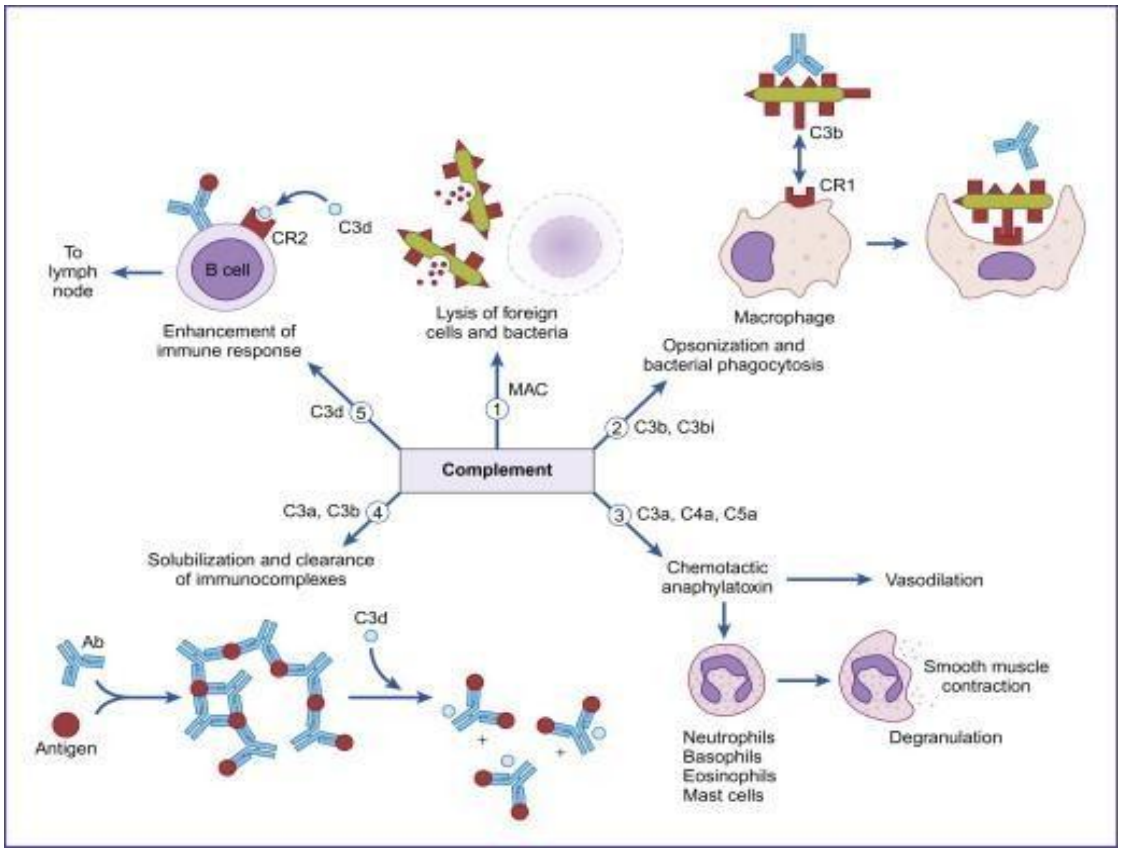
   The proteolytic complement fragments, C5a, C4a, and C3a induce acute inflammation by activating mast cells and neutrophils. All three peptides bind to mast cells and induce degranulation, with the release of vasoactive mediators such as histamine. These peptides are also called anaphylatoxins because the mast cell reactions they trigger are characteristic of anaphylaxis. Binding to specific complement receptors on cells of the immune system, they trigger specific cell functions, inflammation, and secretion of immunoregulatory molecules.

5. **Production of antibodies:**

   B cells have receptor for C3b. When C3b binds to B-cell, it secretes more antibodies. Thus C3b is also an antibody producing amplifiers which converts it into an effective defense mechanism to destroy invading microorganism.

6. **Immune clearance:**

   The complement system removes immune complexes from the circulation and deposits them in the spleen and liver. Thus it acts as anti-inflammatory function. Complement proteins promote the solubilization of these complexes and their clearance by phagocytes.



✓ Part- 7:*Antigen – Antibody interactions: Agglutination, precipitation, immune diffusion, immuunoelectrophoresis.*

i. Strength of Antigen-Antibody Interactions:

The noncovalent interactions that form the basis of antigen-antibody (Ag-Ab) binding include hydrogen bonds, ionic bonds,hydrophobic interactions,and van der Waals interactions (Figure 6-1). Because these interactions are individually weak (compared with a covalent bond), a large number of such interactions are required to form a strong Ag-Ab interaction. Furthermore, each of these noncovalent interactions operates over a very short distance, generally about $1x10^{-7}$ mm (1 angstrom, Å); consequently, a strong Ag-Ab interaction depends on a very close fit between the antigen and antibody. Such fits require a high degree of complementarity between antigen and antibody, a requirement that underlies the exquisite specificity that characterizes antigen-antibody interactions.

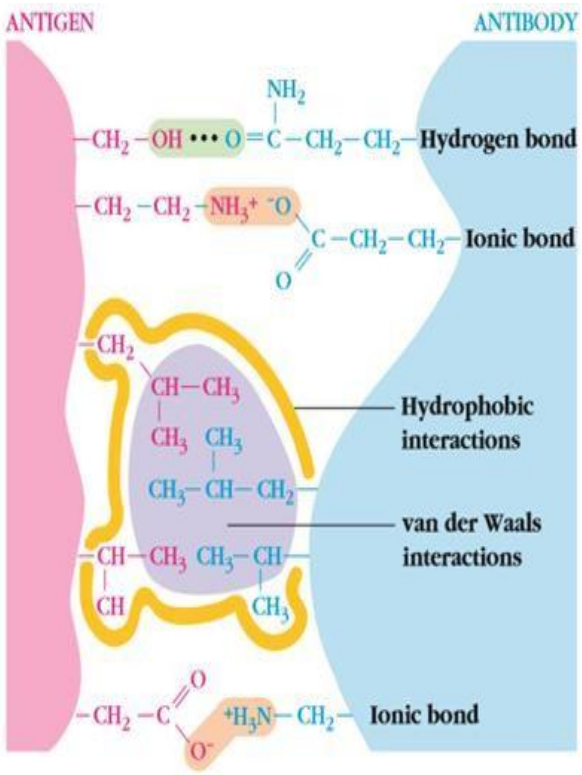


FIGURE 6-1 The interaction between an antibody and an antigen depends on four types of noncovalent forces: (1) hydrogen bonds, in which a hydrogen atom is shared between two electronegative atoms; (2) ionic bonds between oppositely charged residues; (3) hydrophobic interactions, in which water forces hydrophobic groups together; and (4) van der Waals interactions between the outer electron clouds of two or more atoms. In an aqueous environment, noncovalent interactions are extremely weak and depend upon close complementarity of the shapes of antibody and antigen.

ii. *Precipitation Reactions:*

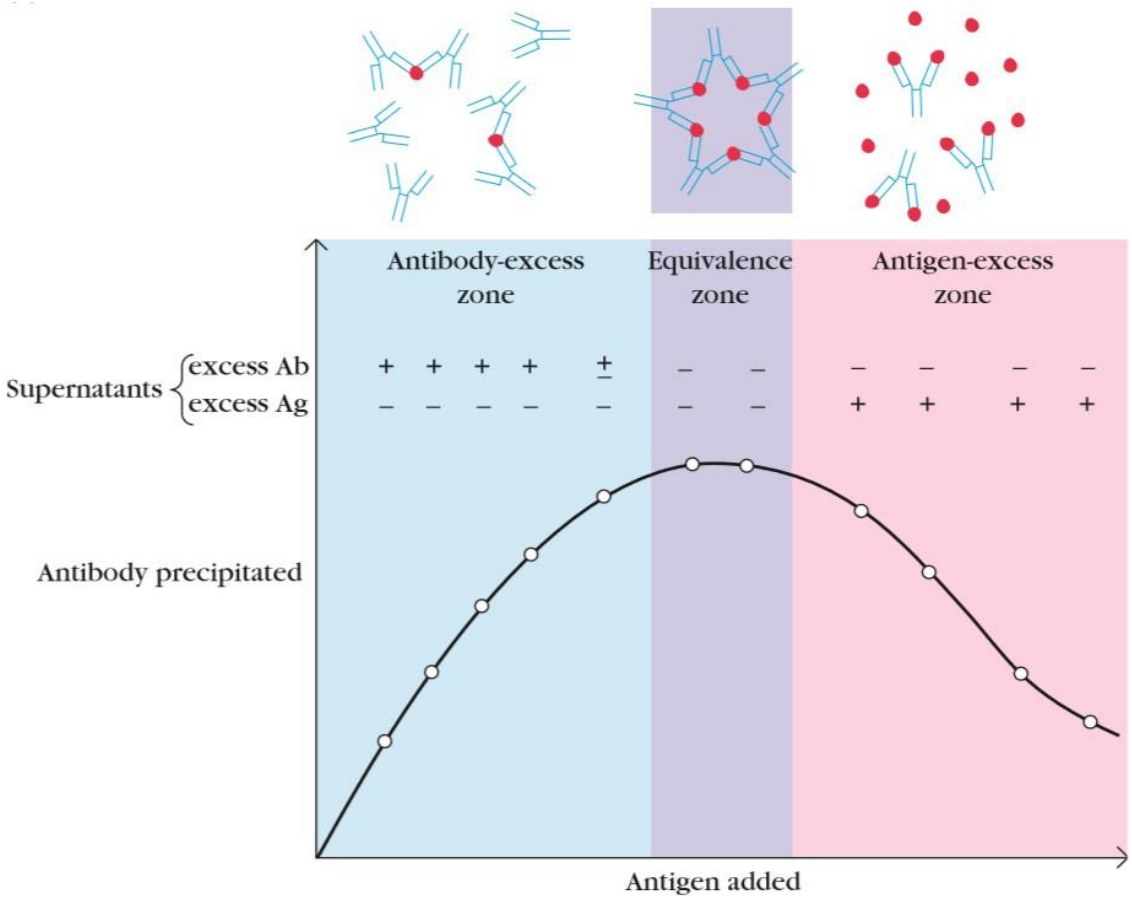
Antibody and soluble antigen interacting in aqueous solution form a lattice that eventually develops into a visible precipitate. Antibodies that aggregate soluble antigens are called precipitins. Although formation of the soluble Ag-Ab complex occurs within minutes, formation of the visible precipitate occurs more slowly and often takes a day or two to reach completion.

Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen:

- The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments.
- The antigen must be either bivalent or polyvalent; that is, it must have at least two copies of the same epitope, or have different epitopes that react with different antibodies present in polyclonal antisera.

- ***Precipitation Reactions in Fluids Yield a Precipitin Curve:***

A quantitative precipitation reaction can be performed by placing a constant amount of antibody in a series of tubes and adding increasing amounts of antigen to the tubes. At one time this method was used to measure the amount of antigen or antibody present in a sample of interest. After the precipitate forms, each tube is centrifuged to pellet the precipitate, the supernatant is poured off, and the amount of precipitate is measured. Plotting the amount of precipitate against increasing antigen concentrations yields a precipitin curve. As Figure shows, excess of either antibody or antigen interferes with maximal precipitation, which occurs in the so-called equivalence zone, within which the ratio of antibody to antigen is optimal. As a large multimolecular lattice is formed at equivalence, the complex increases in size and precipitates out of solution. As shown in Figure under conditions of antibody excess or antigen excess, extensive lattices do not form and precipitation is inhibited. Although the quantitative precipitation reaction is seldom used experimentally today, the principles of antigen excess, antibody excess, and equivalence apply to many Ag-Ab reactions.



- *Precipitation Reactions in Gels Yield Visible Precipitin Lines:*

Immune precipitates can form not only in solution but also in an agar matrix.When antigen and antibody diffuse toward one another in agar,or when antibody is incorporated into the agar and antigen diffuses into the antibody-containing matrix, a visible line of precipitation will form.As in a precipitation reaction in fluid, visible precipitation occurs in the region of equivalence,whereas no visible precipitate forms in regions of antibody or antigen excess. Two types of immune diffusion reactionscan be used to determine relative concentrations ofantibodies or antigens,to compare antigens,or to determine the relative purity of an antigen preparation. They are radial immune diffusion (the Mancini method) and double immunodiffusion(the Ouchterlony method);both are carried out in a semisolid medium such as agar.In radial immune diffusion, an antigen sample is placed in a well and allowed to diffuse intoagar containing a suitable dilution of an antiserum.

As the antigen diffuses into the agar, the region of equivalence is established and a ring of precipitation, a precipitin ring, forms around the well (Figure 6-5,upper panel).

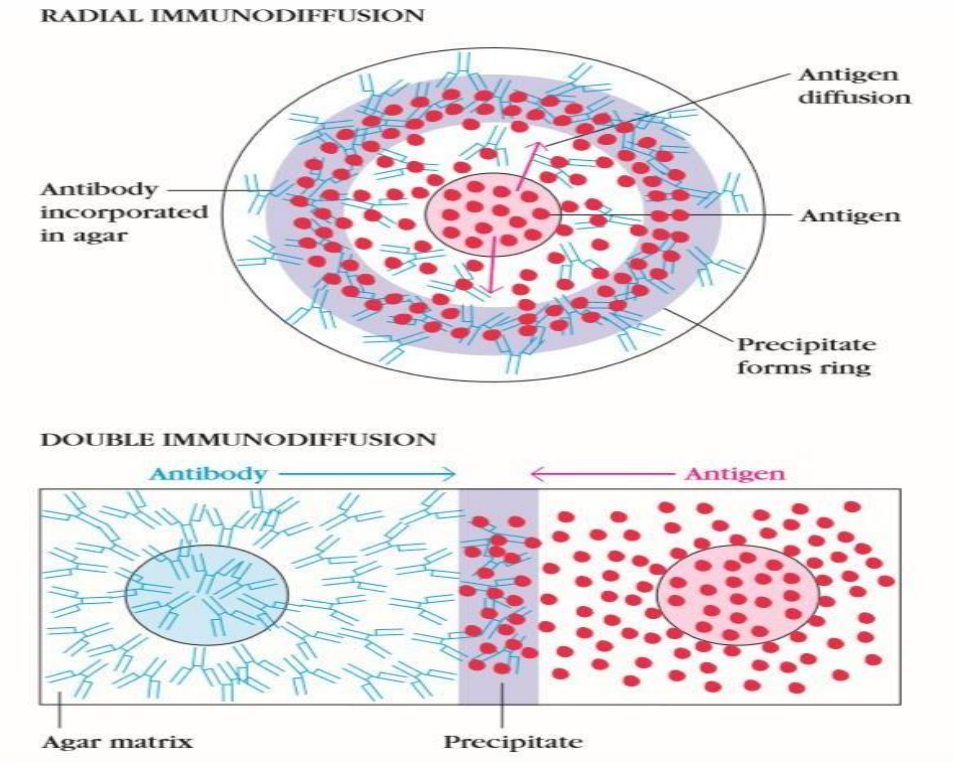


FIGURE 6-5 Diagrammatic representation of radial immunodiffusion (Mancini method) and double immunodiffusion (Ouchterlony method) in a gel. In both cases, large insoluble complexes form in the agar in the zone of equivalence, visible as lines of precipitation (purple regions). Only the antigen (red) diffuses in radial immunodiffusion, whereas both the antibody (blue) and antigen (red) diffuse in double immunodiffusion.

The area of the precipitin ring is proportional to the concentration of antigen.By comparing the area ofthe precipitin ring with a standard curve (obtained by measuring the precipitin areas ofknown concentrations of the antigen),the concentration of the antigen sample can be determined. In the Ouchterlony method, both antigen and antibody diffuse radially from wells toward each other,thereby establishing a concentration gradient.As equivalence is reached, a visible line of precipitation, a precipitin line,forms (Figure 6-5,lower panel).

- *Immunoelectrophoresis Combines Electrophoresis and Double Immunodiffusion:*

In immunoelectrophoresis,the antigen mixture is first electrophoresed to separate its components by charge. Troughs are then cut into the agar gel parallel to the direction ofthe electric field, and antiserum is added to the troughs. Antibody and antigen then diffuse toward each other and produce lines of precipitation where they meet in appropriate proportions (Figure 6-6a). Immunoelectrophoresis is used in clinical laboratories to detect the presence or absence of proteins in the serum. A sample of serum is electrophoresed, and the individual serum components are identified with antisera specific for a given protein or immunoglobulin class (Figure 6-6b).This technique is useful in determining whether a patient produces abnormally low amounts of one or more isotypes, characteristic of certain immunodeficiency diseases. It can also show whether a patient overproduces some serum protein, such as albumin, immunoglobulin, or transferrin. The immunoelectrophoretic pattern of serum from patients with multiple myeloma, for example, shows a heavy distorted arc caused by the large amount of myeloma protein, which is monoclonal Ig and therefore uniformly charged (Figure 6-6b). Because immune electrophoresis is a strictly *qualitative* technique that only detects relatively high antibody concentrations (greater than several hundred g/ml), it utility is limited to the detectionof quantitative abnormalities only when the departure from normal is striking, as in immunodeficiency states and immune proliferative disorders.

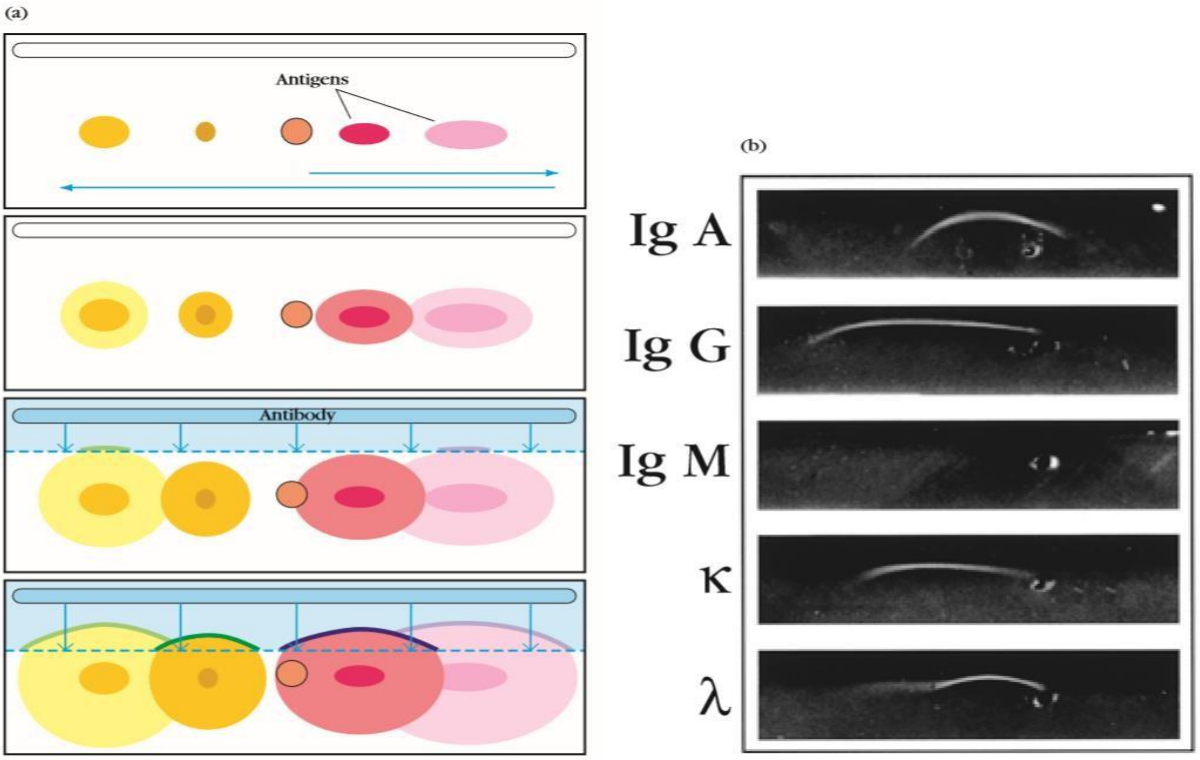


FIGURE 6-6 Immunoelectrophoresis of an antigen mixture. (a) An antigen preparation (orange) is first electrophoresed, which separates the component antigens on the basis of charge. Antiserum (blue) is then added to troughs on one or both sides of the separated antigens and allowed to diffuse; in time, lines of precipitation (colored arcs) form where specific antibody and antigen interact. (b) Immunoelectrophoretic patterns of human serum from a patient with myeloma. The patient produces a large amount of a monoclonal IgG (λ-light-chain-bearing) antibody. A sample of serum from the patient was placed in the well of the slide and electrophoresed. Then antiserum specific for the indicated antibody class or light chain type was placed in the top trough of each slide. At the concentrations of patient's serum used, only anti-IgG and anti-λ antibodies produced lines of precipitation. *[Part(b), Robert A. Kyle and Terry A. Katzman, Manual of Clinical Immunology, 1997, N. Rose, ed., ASM Press, Washington, D.C., p. 164.]*

iii. *Agglutination Reactions:*

The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions are called agglutinins. Agglutination reactions are similar in principle to precipitation reactions; they depend on the crosslinking of polyvalent antigens. Just as an excess of antibody inhibits precipitation reactions, such excess can also inhibit agglutination reactions; this inhibition is called the prozone effect. Because prozone effects can be encountered in many types of immunoassays, understanding the basis of this phenomenon is of general importance.

- *Hemagglutination Is Used in Blood Typing:*

Agglutination reactions (Figure 6-7) are routinely performed to type red blood cells (RBCs). In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood-group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient RBCs is the basis for matching blood types for transfusions.

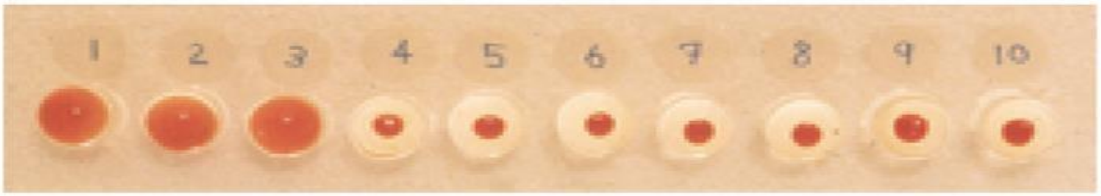


FIGURE 6-7 Demonstration of hemagglutination using antibodies against sheep red blood cells (SRBCs). The control tube (10) contains only SRBCs, which settle into a solid "button." The experimental tubes 1–9 contain a constant number of SRBCs plus serial two-fold dilutions of anti-SRBC serum. The spread pattern in the experimental series indicates positive hemagglutination through tube 3. *[Louisiana State University Medical Center/MIP. Courtesy of Harriet C. W. Thompson.]*

- *Bacterial Agglutination Is Used To Diagnose Infection:*

A bacterial infection often elicits the production of serum antibodies specific for surface antigens on the bacterial cells. The presence of such antibodies can be detected by bacterial agglutination reactions. Serum from a patient thought to be infected with a given bacterium is serially diluted in an array of tubes to which the bacteria is added. The last tube showing visible agglutination will reflect the serum antibody titer of the patient. The agglutinin titer is defined as the reciprocal of the greatest serum dilution that elicits a positive agglutination reaction. For example, if serial twofold dilutions of serum are prepared and if the dilution of 1/640 shows agglutination but the dilution of 1/1280 does not, then the agglutination titer of the patient's serum is 640. In some cases serum can be diluted up to 1/50,000 and still show agglutination of bacteria. The agglutinin titer of an antiserum can be used to diagnose a bacterial infection. Patients with typhoid fever, for example, show a significant rise in the agglutination titer to *Salmonella typhi.* Agglutination reactions also provide a way to type bacteria. For instance, different species of the bacterium *Salmonella* can be distinguished by agglutination reactions with a panel of typing antisera.

- *In Agglutination Inhibition, Absence of Agglutination Is Diagnostic of Antigen:*

A modification of the agglutination reaction, called agglutination inhibition, provides a highly sensitive assay for small quantities of an antigen .For example, one of the early types of home pregnancy test kits included latex particles coated with human chorionic gonadotropin (HCG) and antibody to HCG (Figure 6-8). The addition of urine from a pregnant woman, which contained HCG, inhibited agglutination of the latex particles when the anti-HCG antibody was added; thus the absence of agglutination indicated pregnancy.

Agglutination inhibition assays can also be used to determine whether an individual is using certain types of illegal drugs, such as cocaine or heroin. A urine or blood sample is first incubated with antibody specific for the suspected drug. Then red blood cells (or other particles) coated with the drug are added. If the red blood cells are not agglutinated by the antibody, it indicates the sample contained an antigen recognized by the antibody, suggesting that the individual was using the illicit drug. One problem with these tests isthat some legal drugs have chemical structures similar to those of illicit drugs, and these legal drugs may cross-react with the antibody, giving a false-positive reaction. For this reason a positive reaction must be confirmed by a nonimmunologic method.

Agglutination inhibition assays are widely used in clinical laboratories to determine whether an individual has been exposed to certain types of viruses that cause agglutination of red blood cells. If an individual's serum contains specific antiviral antibodies, then the antibodies will bind to the virus and interfere with hemagglutination by the virus. This technique is commonly used in premarital testing to determine the immune status of women with respect to rubella virus. The reciprocal of the last serum dilution to show inhibition of rubella hemagglutination is the titer of the serum. A titer greater than 10 (1:10 dilution) indicates that a woman is immune to rubella, whereas a titer of less than 10 is indicative of a lack of immunity and the need for immunization with the rubella vaccine.

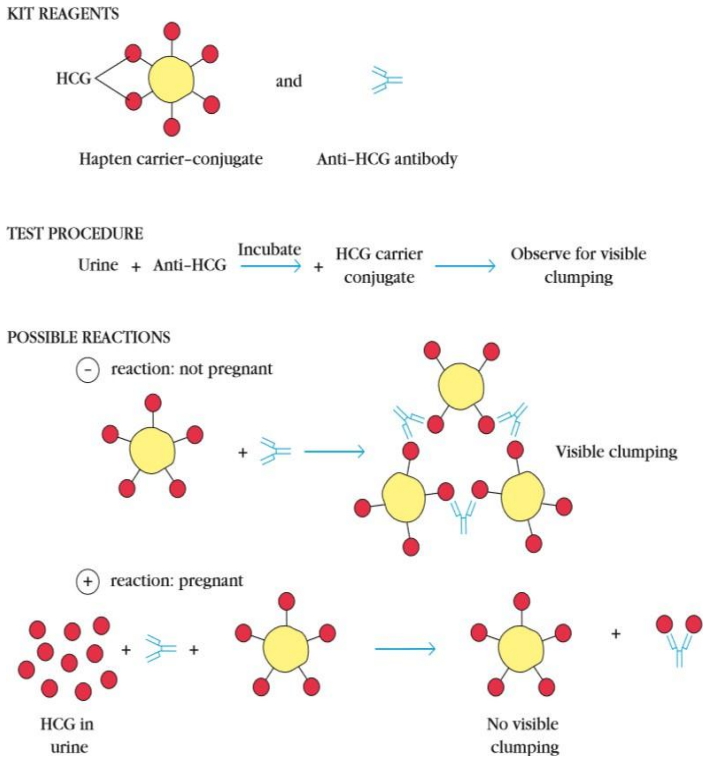


FIGURE 6-8 The original home pregnancy test kit employed hapten inhibition to determine the presence or absence of human chorionic gonadotropin (HCG). The original test kits used the presence or absence of visible clumping to determine whether HCG was present. If a woman was not pregnant, her urine would not contain HCG; in this case, the anti-HCG antibodies and HCG-carrier conjugate in the kit would react, producing visible clumping. If a woman was pregnant, the HCG in her urine would bind to the anti-HCG antibodies, thus inhibiting the subsequent binding of the antibody to the HCG-carrier conjugate. Because of this inhibition, no visible clumping occurred if a woman was pregnant. The kits currently on the market use ELISA-based assays (see Figure 6-10).

✓ Part- 8:Hypersensitivity: Definition, types, examples

Hypersensitivity (also called hypersensitivity reaction or intolerance) refers to undesirable reactions produced by the normal immune system, including allergies and autoimmunity. They are usually referred to as an over-reaction of the immune system and these reactions may be damaging, uncomfortable, or occasionally fatal. Hypersensitivity reactions require a pre-sensitized (immune) state of the host. The Gell and Coombs classification of hypersensitivity is the most widely used, and distinguishes four types of immune response which result in bystander tissue damage.Anaphylactic reactions within the humoral branch initiated by antibody or antigen-antibody complexes as immediate hypersensitivity, because

the symptoms are manifest within minutes or hours after a sensitized recipient encounters antigen. Delayed-type hypersensitivity (DTH) is so named in recognition of the delay of symptoms until days after exposure.

- ***Types:***

  The response of the host to the presence of foreign substances can trigger four types of hypersensitivity reactions:

  i. *IgE-Mediated (Type I) Hypersensitivity(Anaphylactic Reaction):*

A type I hypersensitive reaction is induced by certain types of antigens referred to as allergens, and has all the hallmarks of a normal humoral response.That is, an allergen induces a humoral antibody response by the same mechanismsfor other soluble antigens, resulting in the generation of antibody-secreting plasma cells and memory cells. What distinguishes a type I hypersensitive response from a normal humoral response is that the plasmacells secrete IgE.This class of antibody binds with high affinity to Fc receptors on the surface of tissue mast cells and blood basophils. Mast cells and basophils coated by IgE are said to be sensitized. A later exposure to the same allergen cross-links the membrane-bound IgE on sensitized mast cells and basophils, causing degranulation of these cells (Figure 16-2).The pharmacologically active mediators released from the granules act on the surrounding tissues. The principal effects—vasodilation and smooth-muscle contraction—may be either systemic or localized, depending on the extent of mediator release.

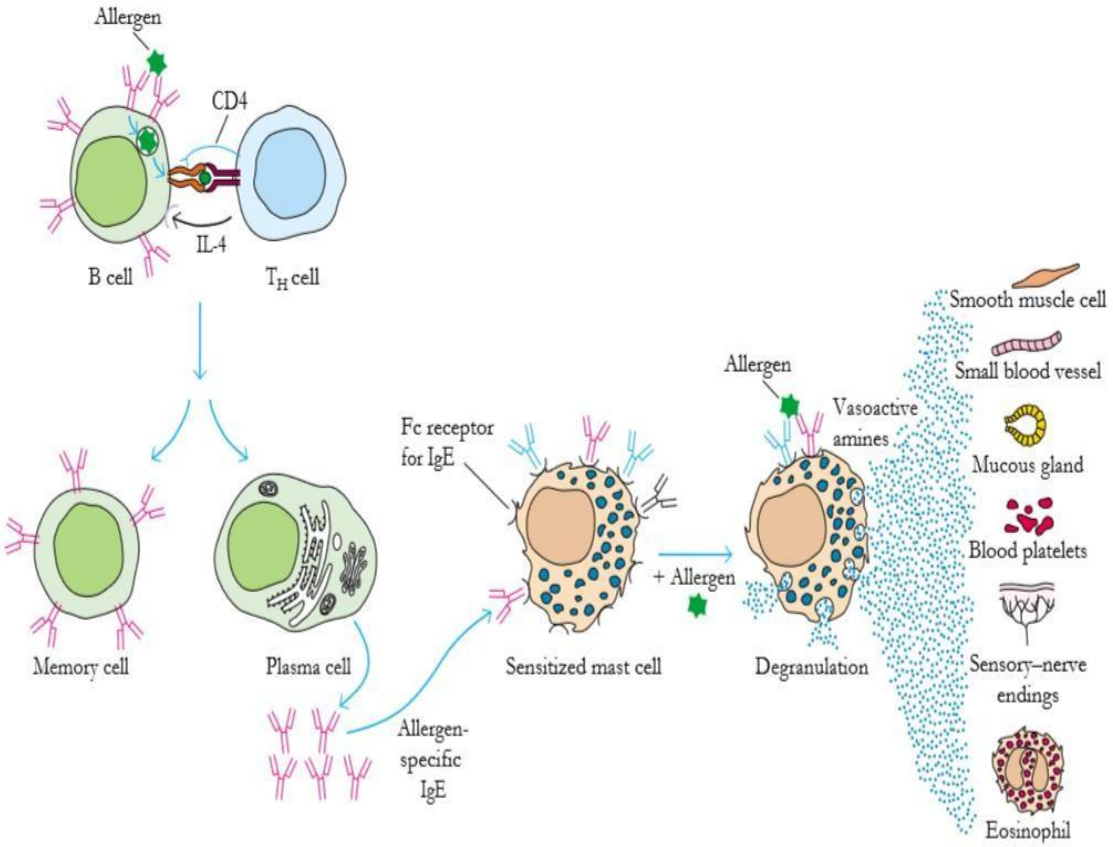


FIGURE 16-2 General mechanism underlying a type I hypersensitive reaction. Exposure to an allergen activates B cells to form IgE-secreting plasma cells. The secreted IgE molecules bind to IgE-specific Fc receptors on mast cells and blood basophils. (Many molecules of IgE with various specificities can bind to the IgE-Fc receptor.) Second exposure to the allergen leads to crosslinking of the bound IgE, triggering the release of pharmacologically active mediators, vasoactive amines, from mast cells and basophils. The mediators cause smooth-muscle contraction, increased vascular permeability, and vasodilation.

These allergic reactions are systemic or localized, as in allergic dermatitis (e.g., hives, wheal and erythema reactions). The reaction is the result of an antigen cross-linking with membrane-bound IgE antibody of a mast cell or basophil. Histamine, serotonin, bradykinin,and lipid mediators (e.g., platelet activating factor, prostaglandins, and leukotrienes) are released during the anaphylactic reaction. These released substances have the potential to cause tissue damage.

TABLE 16-1 Common allergens associated with type I hypersensitivity

Proteins	**Foods**
Foreign serum	Nuts
Vaccines	Seafood
	Eggs
Plant pollens	Peas, beans
Rye grass	Milk
Ragweed	
Timothy grass	**Insect products**
Birch trees	Bee venom
	Wasp venom
Drugs	Ant venom
Penicillin	Cockroach calyx
Sulfonamides	Dust mites
Local anesthetics	
Salicylates	**Mold spores**
	Animal hair and dander

TABLE 16-4 Mechanism of action of some drugs used to treat type I hypersensitivity

Drug	Action
Antihistamines	Block H_1 and H_2 receptors on target cells
Cromolyn sodium	Blocks Ca^{2+} influx into mast cells
Theophylline	Prolongs high cAMP levels in mast cells by inhibiting phosphodiesterase, which cleaves cAMP to 5′-AMP*
Epinephrine (adrenalin)	Stimulates cAMP production by binding to β-adrenergic receptors on mast cells*
Cortisone	Reduces histamine levels by blocking conversion of histidine to histamine and stimulates mast-cell production of cAMP*

*Although cAMP rises transiently during mast-cell activation, degranulation is prevented if cAMP levels remain high.

ii. *Antibody-Mediated Cytotoxic (Type II) Hypersensitivity:*

Type II hypersensitive reactions involve antibody-mediated destruction of cells. Antibody can activate the complement system, creating pores in the membrane of a foreign cell (see Figure 13-5), or it can mediate cell destruction by antibodydependent cell-mediated cytotoxicity (ADCC). In this process,cytotoxic cells with Fc receptors bind to the Fc region of antibodies on target cells and promote killing of the cells. Antibody bound to a foreign cell also can serve as an opsonin, enabling phagocytic cells with Fc or C3b receptors to bind and phagocytose the antibody-coated cell.

In a cytotoxic reaction, the antibody reacts directly with the antigen that is bound to the cell membrane to induce cell lysis through complement activation. These antigens may be intrinsic or "self" as in autoimmune reactions or extrinsic or "non-self." Cytotoxic reactions are mediated by IgG and IgM. Examples of cytotoxic reaction are the Rh incompatibility of a newborn, blood transfusion reactions, and autoimmune diseases like Pemphigus Vulgaris, Bullous Pemphigoid, autoimmune hemolyticanemia and Goodpasture's syndrome to name a few.

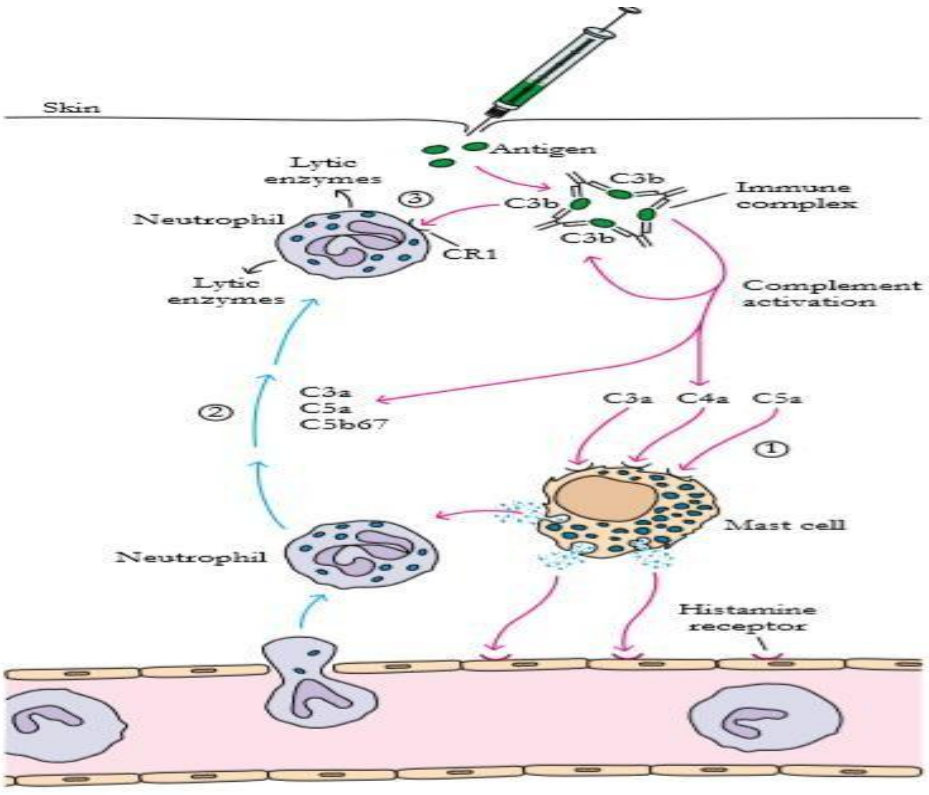


FIGURE 16-15 Development of a localized Arthus reaction (type III hypersensitive reaction). Complement activation initiated by immune complexes (classical pathway) produces complement intermediates that (1) mediate mast-cell degranulation, (2) chemotactically attract neutrophils, and (3) stimulate release of lytic enzymes from neutrophils trying to phagocytose C_3b-coated immune complexes.

iii. *Immune Complex–Mediated (Type III) Hypersensitivity:*

Type III hypersensitive reactions develop when immune complexes activate the complement system's array of immune effector molecules. The C3a, C4a, and C5a complement split products are anaphylatoxins that cause localized mast-cell degranulation and consequent increase in local vascular permeability. C3a, C5a, and C5b67 are also chemotactic factors for neutrophils, which can accumulate in large numbers at the site of immune-complex deposition.Larger immune complexes are deposited on the basement membrane of bloodvessel walls or kidney glomeruli, whereas smaller complexes may pass through the basement membrane and be deposited in the sub-epithelium.The type of lesion that results depends on the site of deposition of the complexes.

Much of the tissue damage in type III reactions stems from release of lytic enzymes by neutrophils as they attempt to phagocytose immune complexes. The C3b complement component acts as an opsonin, coating immune complexes. A neutrophil binds to a C3b-coated immune complex by means of the type I complement receptor, which is specific for C3b. Because the complex is deposited on the basementmembrane surface, phagocytosis is impeded, so that lytic enzymes are released during the unsuccessful attempts of the neutrophil to ingest the adhering immune complex. Further activation of the membrane-attack mechanism of the complement system can also contribute to the destruction of tissue. In addition, the activation of complement can induce aggregation of platelets, and the resulting release of clotting factors can lead to formation of microthrombi.

IgG and IgM bind antigen, forming antigen-antibody (immune) complexes. These activate complement, which results in PMN chemotaxis and activation. PMNs then release tissue damaging enzymes. Tissue damage present in autoimmune diseases (e.g., systemic lupus erythematosus), and chronic infectious diseases (e.g., leprosy) can be attributed, in part, to immune complex reactions.

iv. *Type IV or Delayed-Type Hypersensitivity (DTH):*

When some subpopulations of activated TH cells encounter certain types of antigens,they secrete cytokines that induce a localized inflammatory reaction called delayed-type hypersensitivity (DTH). The reaction is characterized by large influxes of nonspecific inflammatory cells,in particular,macrophages. This type of reaction was first described in 1890 byRobert Koch, who observed that individuals infected with Mycobacterium

tuberculosis developed a localized inflammatory response when injected intradermally with a filtrate derived from a mycobacterial culture.He called this localized skin reaction a "tuberculin reaction." Later, as it became apparent that a variety of other antigens could induce this response (Table 16-6),its name was changed to delayed-type or type IV hypersensitivity in reference to the delayed onset of the reaction and to the tissue damage (hypersensitivity) that is often associated with it.

TABLE 16-6 Intracellular pathogens and contact antigens that induce delayed-type (type IV) hypersensitivity

Intracellular bacteria	Intracellular viruses
Mycobacterium tuberculosis	Herpes simplex virus
Mycobacterium leprae	Variola (smallpox)
Listeria monocytogenes	Measles virus
Brucella abortus	
Intracellular fungi	Contact antigens
Pneumocystis carinii	Picrylchloride
Candida albicans	Hair dyes
Histoplasma capsulatum	Nickel salts
Cryptococcus neoformans	Poison ivy
Intracellular parasites	Poison oak
Leishmania sp.	

The term hypersensitivity is somewhat misleading, for it suggests that a DTH response is always detrimental.Although in some cases a DTH response does cause extensive tissue damage and is in itself pathologic, in many cases tissue damage is limited, and the response plays an important role in defense against intracellular pathogens and contact antigens. The hallmarks of a type IV reaction are the delay in time required for the reaction to develop and the recruitment of macrophages as opposed to neutrophils, as found in a type III reaction. Macrophages are the major component of the infiltrate that surrounds the site of inflammation.

Cell-mediated reactions are initiated by T-lymphocytes and mediated by effector T-cells and macrophages. This response involves the interaction of antigens with the surface of lymphocytes. Sensitized lymphocytes can produce cytokines, which are biologically active substances that affect the functions of other cells. This type of reaction takes 48-72 hours, or longer, after contact with the antigen to fully develop. Many chronic infectious diseases, including tuberculosis and fungal infections, exhibit delayed hypersensitivity.

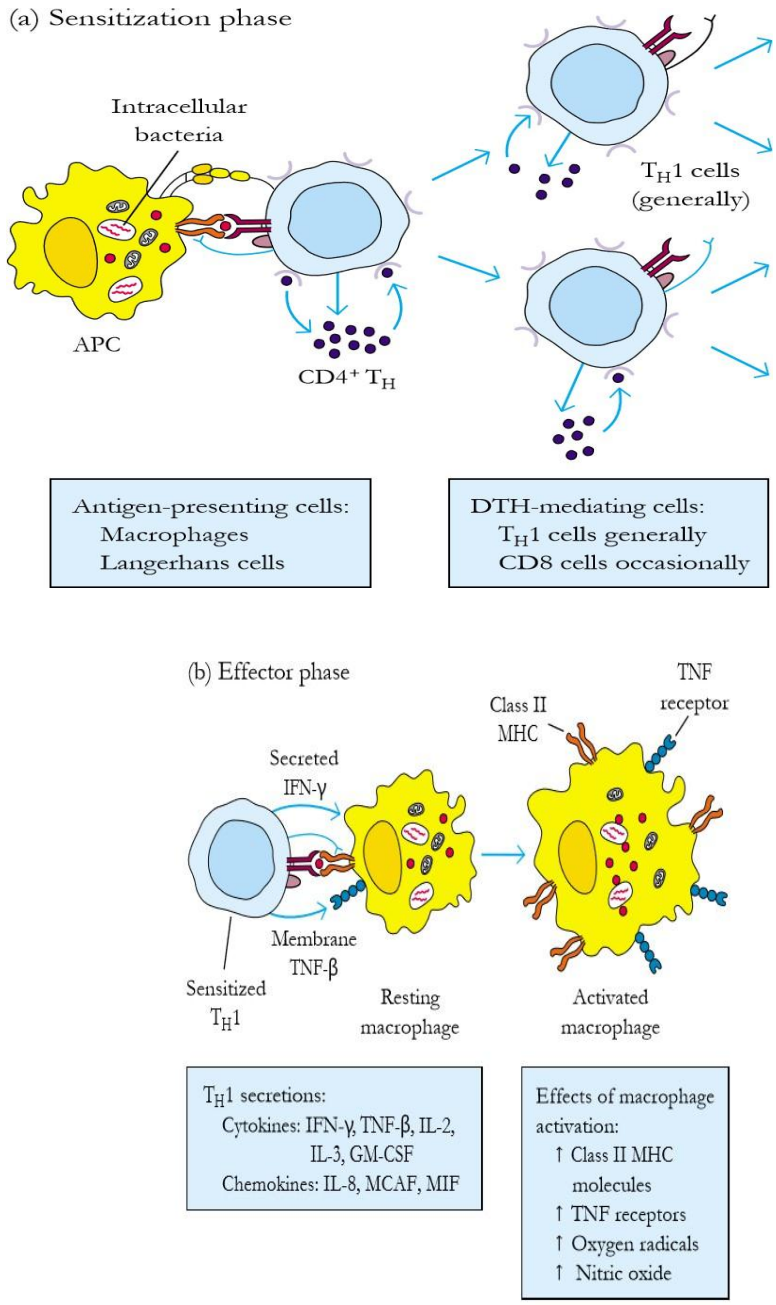


FIGURE 16-17 Overview of the DTH response. (a) In the sensitization phase after initial contact with antigen (e.g., peptides derived from intracellular bacteria), T_H cells proliferate and differentiate into T_H1 cells. Cytokines secreted by these T cells are indicated by the dark blue balls. (b) In the effector phase after subsequent exposure of sensitized T_H1 cells to antigen, the T_H1 cells secrete a variety of cytokines and chemokines. These factors attract and activate macrophages and other nonspecific inflammatory cells. Activated macrophages are more effective in presenting antigen, thus perpetuating the DTH response, and function as the primary effector cells in this reaction.

✓ **<u>Part- 9:</u>Vaccines: Active and passive immunization (definition, characteristics, functions, examples).**

1. Active and passive immunization:

Immunity to infectious microorganisms can be achieved by active or passive immunization. In each case, immunity can be acquired either by natural processes (usually by transfer from mother to fetus or by previous infection by the organism) or by artificial means such as injection of antibodies or vaccines (Table 18-1). The agents used for inducing passive immunity include antibodies from humans or animals, whereas active immunization is achieved by inoculation with microbial pathogens that induce immunity but do not cause disease or with antigenic components from the pathogens. This section describes current usage of passive and active immunization techniques.

TABLE 18-1 Acquisition of passive and active immunity

Type	Acquired through
Passive immunity	Natural maternal antibody
	Immune globulin*
	Humanized monoclonal antibody
	Antitoxin†
Active immunity	Natural infection
	Vaccines‡
	Attenuated organisms
	Inactivated organisms
	Purified microbial macromolecules
	Cloned microbial antigens
	Expressed as recombinant protein
	As cloned DNA alone or in virus vectors
	Multivalent complexes
	Toxoid§

*An antibody-containing solution derived from human blood, obtained by cold ethanol fractionation of large pools of plasma; available in intramuscular and intravenous preparations.

†An antibody derived from the serum of animals that have been stimulated with specific antigens.

‡A suspension of attenuated live or killed microorganisms, or antigenic portions of them, presented to a potential host to induce immunity and prevent disease.

§A bacterial toxin that has been modified to be nontoxic but retains the capacity to stimulate the formation of antitoxin.

- *Passive immunizationInvolves Transfer of Preformed Antibodies:*

Jenner and Pasteur are recognized as the pioneers of vaccination, or induction of active immunity, but similar recognition is due to Emil von Behring and HidesaburoKitasato for their contributions to passive immunity. These investigators were the first to show that immunity elicited in one animal can be transferred to another by injecting it with serum from the first.

Passive immunization, in which preformed antibodies are transferred to a recipient, occurs naturally by transfer of maternal antibodies across the placenta to the developing fetus. Maternal antibodies to diphtheria, tetanus, streptococci, rubeola, rubella, mumps, and poliovirus all afford passively acquired protection to the developing fetus. Maternal antibodies present in colostrum and milk also provide passive immunity to the infant. Passive immunization can also be achieved by injecting a recipient with preformed antibodies. In the past, before vaccines and antibiotics became available,passive immunization provided a major defense against various infectious diseases. Despite the risks incurred by injecting animal sera,usually horse serum,this was the only effective therapy for otherwise fatal diseases. Currently, there are several conditions that warrant the use of passive immunization. These include:

- Deficiency in synthesis of antibody as a result of congenital or acquired B-cell defects, alone or together with other immunodeficiencies.
- Exposure or likely exposure to a disease that will cause complications (e.g., a child with leukemia exposed to varicella or measles), or when time does not permit adequate protection by active immunization.
- Infection by pathogens whose effects may be ameliorated by antibody. For example, if individuals who have not received up-to-date active immunization against tetanus suffer a puncture wound, they are given an injection of horse antiserum to tetanus toxin. The preformed horse antibody neutralizes any tetanus toxin produced by *Clostridium tetani* in the wound.

Passive immunization is routinely administered to individuals exposed to botulism, tetanus, diphtheria, hepatitis, measles,and rabies (Table 18-2).Passively administered antiserum is also used to provide protection from poisonous snake and insect bites.Passive immunization can provide immediate protection to travelers or health-care workers who will soon be exposed to an infectious organism and lack active immunity to

it. Because passive immunization does not activate the immune system, it generates no memory response and the protection provided is transient.

For certain diseases such as the acute respiratory failure in children caused by respiratory syncytial virus (RSV), passiveimmunization is the best preventative currently available. A monoclonal antibody or a combination of two monoclonal antibodies may be administered to children at risk for RSV disease.

TABLE 18-2 Common agents used for passive immunization

Disease	Agent
Black widow spider bite	Horse antivenin
Botulism	Horse antitoxin
Diphtheria	Horse antitoxin
Hepatitis A and B	Pooled human immune gamma globulin
Measles	Pooled human immune gamma globulin
Rabies	Pooled human immune gamma globulin
Respiratory disease	Monoclonal anti-RSV*
Snake bite	Horse antivenin
Tetanus	Pooled human immune gamma globulin or horse antitoxin

- *Active Immunization Elicits Long-Term Protection:*

Whereas the aim of passive immunization is transient protection or alleviation of an existing condition,the goal of active immunization is to elicit protective immunity and immunologic memory. When active immunization is successful,a subsequent exposure to the pathogenic agent elicits a heightened immune response that successfully eliminates the pathogen or prevents disease mediated by its products. Active immunization can be achieved by natural

infection with a microorganism,or it can be acquired artificially by administration of a vaccine (see Table 18-1). In active immunization, as the name implies, the immune system plays an active role—proliferation of antigen-reactive T and B cells results in the formation of memory cells. Active immunization with various types of vaccines has played an importantrole in the reduction of deaths from infectious diseases,especially among children.

- Vaccination of children is begun at about 2 months of age. The recommended program of childhood immunizations in this country, updated in 2002 by the American Academy of Pediatrics. The program includes the following vaccines:
- Hepatitis B vaccine
- Diphtheria-pertussis (acellular)- tetanus (DPaT) combined vaccine
- Inactivated (Salk) polio vaccine (IPV); the oral (Sabin) vaccine is no longer recommended for use in the United States
- Measles-mumps-rubella (MMR) combined vaccine
- Haemophilusinfluenzae (Hib) vaccine
- Varicella zoster (Var) vaccine for chickenpox
- Pneumococcal conjugate vaccine (PCV); a new addition to the list.

In addition, hepatitis A vaccine at 18 months and influenza vaccines after 6 months are recommended for infants in high-risk populations.

- ✓ **Diagnostic immunology: ELISA, RIA, Immunofluorescence, Flow cytometry, Fluorescence activated cell sorting (FACS).**

1. ELISA:

Enzyme-linked immunosorbent assay, commonly known as ELISA (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. Such a substrate is called a chromogenic substrate. A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and β-galactosidase. These assays approach the sensitivity of RIAs and have the advantage of being safer and less costly.

- **INDIRECT ELISA:**

Antibody can be detected or quantitatively determined with an indirect ELISA (Figure 6-10a).Serum or some other sample containing primary antibody (Ab1) is added to an antigen-coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab_1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab_2), which binds to the primary antibody. Any free Ab_2 then is washed away,and a substrate for the enzyme is added.The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers,which can measure the absorbance of all of the wells of a 96-well plate in seconds.

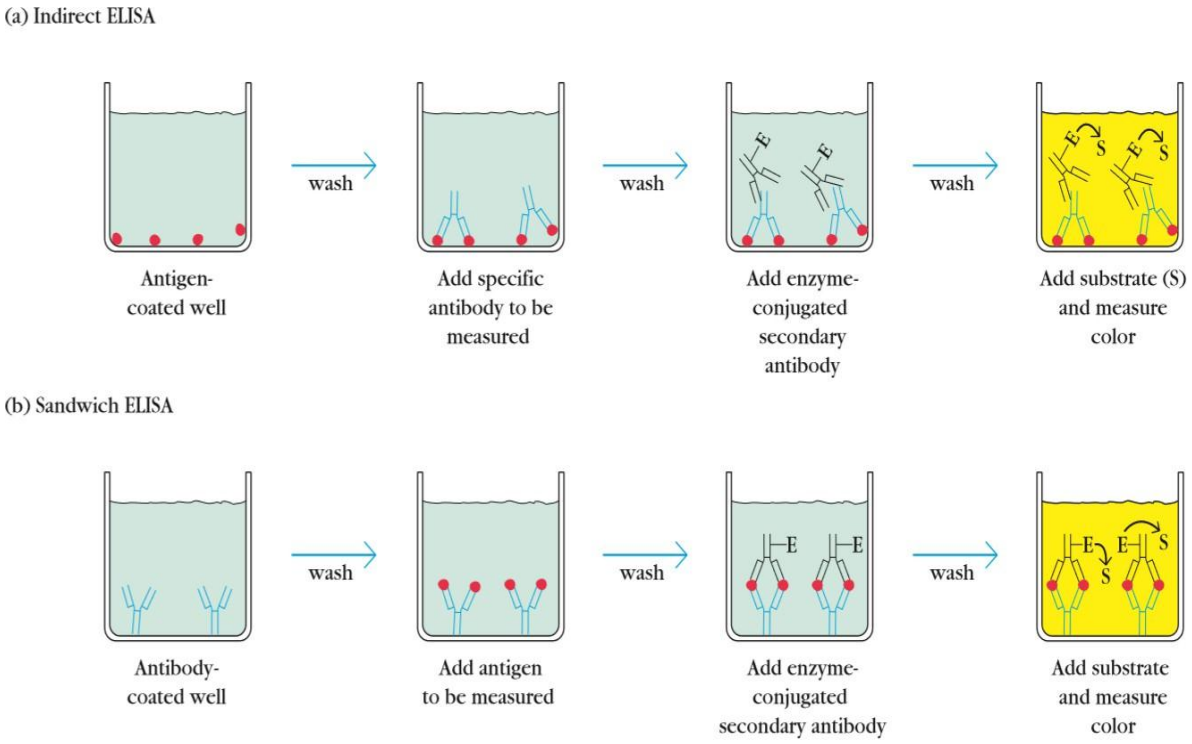
Indirect ELISA is the method of choice to detect the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. In this assay, recombinant envelope and core proteins of HIV are adsorbedas solid-phase antigens to microtiter wells. Individuals infected with HIV will produce serum antibodies to epitopes on these viral proteins. Generally, serum antibodies to HIV can be detected by indirect ELISA within 6 weeks of infection.

- **SANDWICHELISA:**

Antigen can be detected or measured by a sandwich ELISA (Figure 6-10b). In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody.After the well is washed,a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen.After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.

- **COMPETITIVEELISA:**

Another variation for measuring amounts of antigen is competitive ELISA (Figure 6-10c). In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigencoated microtiter well.The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well.Addition of an enzyme-conjugated secondary antibody (Ab_2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. In thecompetitive assay, however, the higher the concentration of antigen in the original sample,the lower the absorbance.



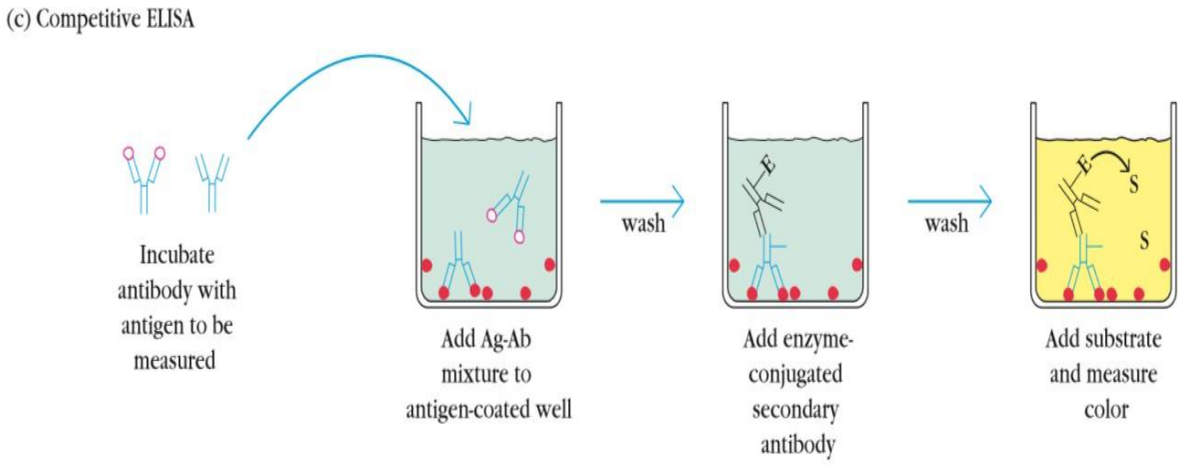


FIGURE 6-10 Variations in the enzyme-linked immunosorbent assay (ELISA) technique allow determination of antibody or antigen. Each assay can be used qualitatively, or quantitatively by comparison with standard curves prepared with known concentrations of antibody or antigen. Antibody can be determined with an indirect ELISA (a), whereas antigen can be determined with a sandwich ELISA (b) or competitive ELISA (c). In the competitive ELISA, which is an inhibition-type assay, the concentration of antigen is inversely proportional to the color produced.

2. Radioimmunoassay (RIA):

One of the most sensitive techniques for detecting antigen or antibody is radioimmunoassay (RIA). The technique was first developed in 1960 by two endocrinologists,S.A.Berson and Rosalyn Yalow, to determine levels of insulin–anti-insulin complexes in diabetics. Although their technique encountered some skepticism, it soon proved its value for measuring hormones,serum proteins,drugs,and vitamins at concentrations of 0.001 micrograms per milliliter or less. In 1977,some years after Berson's death,the significance of the technique was acknowledged by the award of a Nobel Prize to Yalow.

The principle of RIA involves competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody. The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody. Then test samples of unlabeled antigen of unknown concentration are added in progressively larger amounts. The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody.As the concentration of unlabeled antigen increases, more labeled antigen will be displaced from the binding sites.The decrease in the

amount of radiolabeled antigen bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample.

The antigen is generally labeled with a gamma-emitting isotope such as ^{125}I,but beta-emitting isotopes such as tritium (^{3}H) are also routinely used as labels. The radiolabeled antigen is part of the assay mixture; the test sample may be a complex mixture, such as serum or other body fluids,that contains the unlabeled antigen.The first step insetting up an RIA is to determine the amount of antibody needed to bind 50%–70% of a fixed quantity of radioactive antigen (Ag∗) in the assay mixture. This ratio of antibody to Ag∗ is chosen to ensure that the number of epitopes presented by the labeled antigen always exceeds the total number of antibody binding sites.Consequently,unlabeled antigen added to the sample mixture will compete with radiolabeled antigen for the limited supply of antibody. Even a small amount of unlabeled antigen added to the assay mixture of labeled antigen and antibody will cause a decrease in the amount of radioactive antigen bound,and this decrease will be proportional to the amount of unlabeled antigen added.To determine the amount of labeled antigen bound, the Ag-Ab complex is precipitated to separate it from free antigen (antigen not bound to Ab), and the radioactivity in the precipitate is measured.A standard curve can be generated using unlabeled antigen samples of known concentration (in place of the test sample), and from this plot the amount of antigen in the test mixture may be precisely determined.

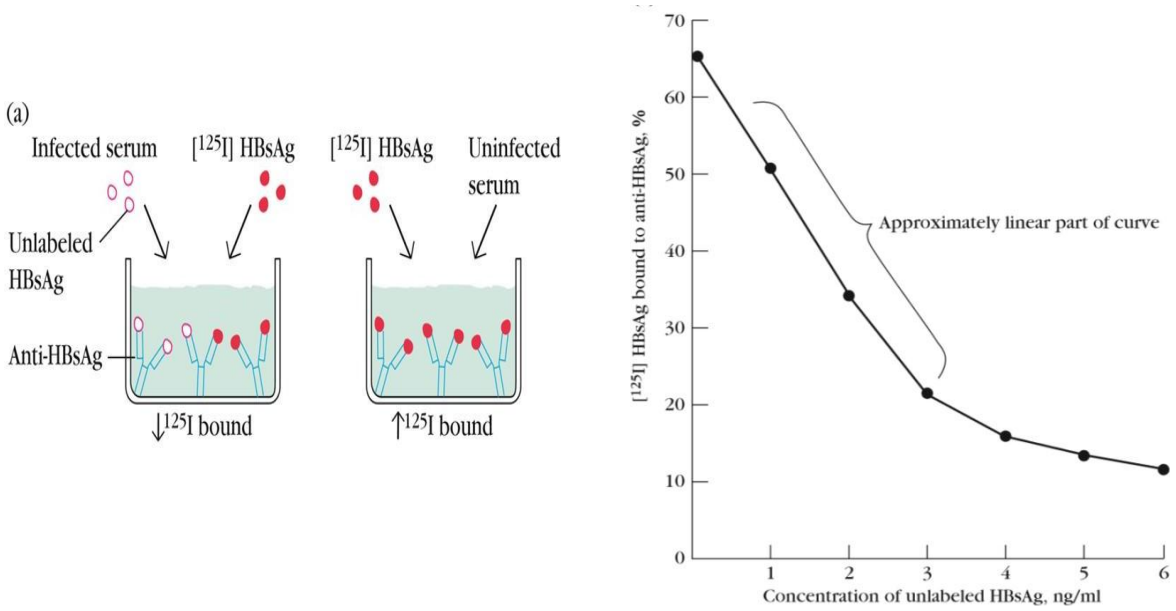


FIGURE 6-9 A solid-phase radioimmunoassay (RIA) to detect hepatitis B virus in blood samples. (a) Microtiter wells are coated with a constant amount of antibody specific for HBsAg, the surface antigen on hepatitis B virions. A serum sample and [^{125}I]HBsAg are then added. After incubation, the supernatant is removed and the radioactivity of the antigen-antibody complexes is measured. If the sample is infected, the amount of label bound will be less than in controls with uninfected serum. (b) A standard curve is obtained by adding increasing concentrations of unlabeled HBsAg to a fixed quantity of [^{125}I]HBsAg and specific antibody. From the plot of the percentage of labeled antigen bound versus the concentration of unlabeled antigen, the concentration of HBsAg in unknown serum samples can be determined by using the linear part of the curve.

3. Immunofluorescence:

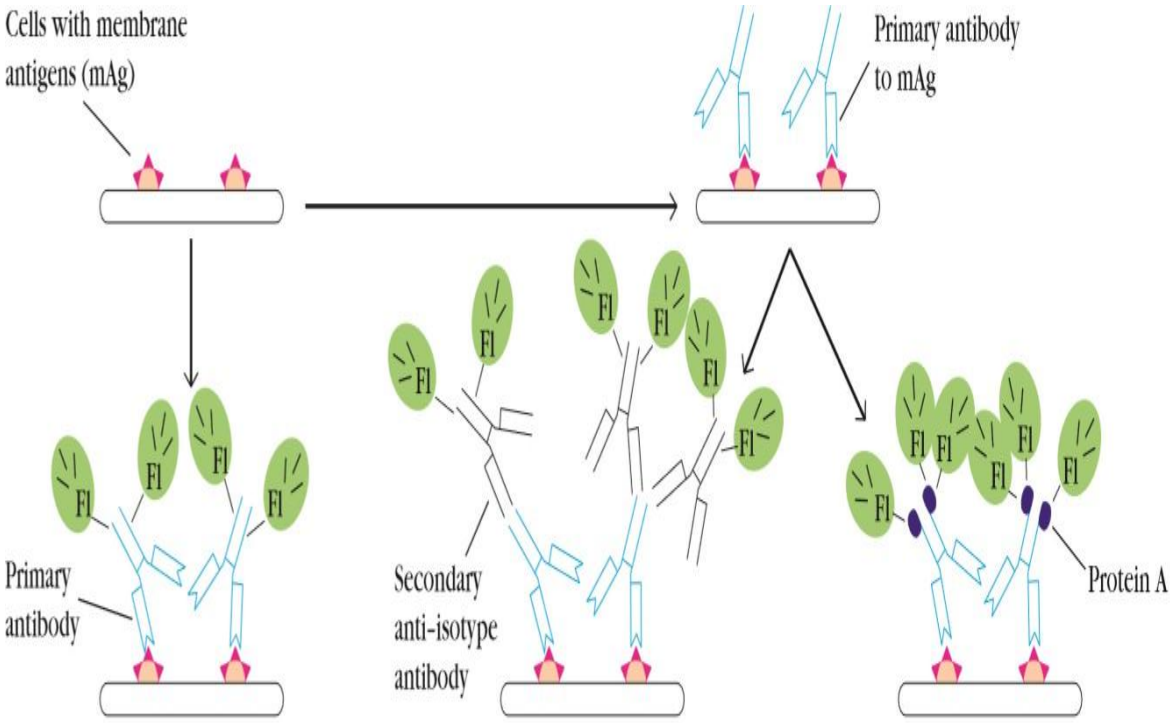
In 1944, Albert Coons showed that antibodies could be labeled with molecules that have the property of fluorescence. Fluorescent molecules absorb light of one wavelength(excitation) and emit light ofanother wavelength (emission). If antibody molecules are tagged with a fluorescent dye, or fluorochrome,immune complexes containing these fluorescently labeled antibodies (FA) can be detected by colored light emission when excited by light of the appropriate wavelength.Antibody molecules bound to antigens in cells or tissue sections can similarly be visualized.The emitted light can be viewed with a fluorescence microscope,which is equipped with a UV light source. In this technique, known as immunofluorescence, fluorescent compounds such as fluorescein and rhodamine are in common use, but other highly fluorescent substances are also routinely used,such as phycoerythrin, an intensely colored and highly fluorescent pigment obtained from algae. These molecules can be conjugated to the Fc region of an antibody molecule without affecting the specificity of the antibody. Each of the fluorochromes below absorbs light at one wavelength and emits light at a longer wavelength:

- Fluorescein, an organic dye that is the most widely used label for immunofluorescence procedures,absorbs blue light (490 nm) and emits an intense yellow-green fluorescence (517 nm).
- Rhodamine, another organic dye,absorbs in the yellow-green range (515 nm) and emits a deep redfluorescence (546 nm).Because it emits fluorescence at a longer wavelength than fluorescein, it can be used in two-color immunofluorescence assays. An antibody specific to one determinant is labeled with fluorescein, and an antibody recognizing a different antigen is labeled with rhodamine. The location of the fluorescein-tagged antibody will be visible by its yellow-green color,easy to distinguish from the red color emitted where the rhodamine-tagged antibody has bound.By conjugating fluorescein to one antibody and rhodamine to another antibody,one can,for example, visualize simultaneously two different cell-membrane antigens on the same cell.
- Phycoerythrin is an efficient absorber of light (~30-fold greater than fluorescein) and a brilliant emitter of red fluorescence,stimulating its wide use as a label for immunofluorescence.

Fluorescent-antibody staining of cell membrane molecules or tissue sections can be direct or indirect (Figure 6-14). In direct staining, the specific antibody (the primary antibody) is directly conjugated with fluorescein; in indirect staining, the primary antibody is unlabeled and is detected with an additional fluorochrome-labeled reagent. A number of reagents have been developed for indirect staining.The most common is a fluorochrome-labeled secondary antibody raised in one species against antibodies of another species, such as fluorescein-labeled goat anti-mouse immunoglobulin.

Indirect immunofluorescence staining has two advantages over direct staining. First, the primary antibody does not need to be conjugated with a fluorochrome.Because the supply of primary antibody is often a limiting factor,indirect methods avoid the loss of antibody that usually occurs during the conjugation reaction. Second, indirect methods increase the sensitivity of staining because multiple molecules of the fluorochrome reagent bind to each primary antibody molecule,increasing the amount of light emitted at the location of each primary antibody molecule.

Immunofluorescence has been applied to identify a number of subpopulations of lymphocytes, notably the $CD4^+$ and $CD8^+$ T-cell subpopulations.The technique is also suitable for identifying bacterial species, detecting Ag-Ab complexes in autoimmune disease, detecting complement components in tissues, and localizing hormones and other cellular products stained in situ.Indeed,a major application of the fluorescent-antibody technique is the localization of antigens in tissue sections or in subcellular compartments. Because it can be used to map the actual location of target antigens,fluorescence microscopy is a powerful tool for relating the molecular architecture of tissues and organs to their overall gross anatomy.



(d)

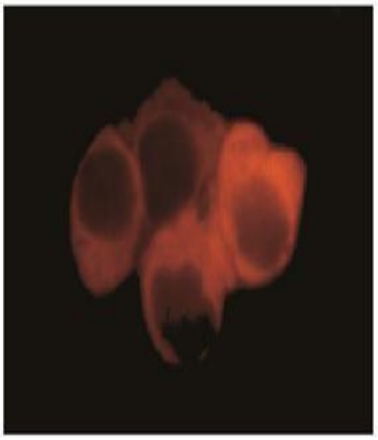


FIGURE 6-14 Direct and indirect immunofluorescence staining of membrane antigen (mAg). Cells are affixed to a microscope slide. In the direct method (a), cells are stained with anti-mAg antibody that is labeled with a fluorochrome (Fl). In the indirect methods (b and c), cells are first incubated with unlabeled anti-mAg antibody and then stained with a fluorochrome-labeled secondary reagent that binds to the primary antibody. Cells are viewed under a fluorescence microscope to see if they have been stained. (d) In this micrograph, antibody molecules bearing μ heavy chains are detected by indirect staining of cells with rhodamine-conjugated second antibody. *[Part (d), H. A. Schreuder et al., 1997, Nature* ***386:****196, courtesy H. Schreuder, Hoechst Marion Roussel.]*

4. Flow cytometry:

The fluorescent antibody techniques described are extremely valuable qualitative tools, but they do not give quantitative data. This shortcoming was remedied by development of the flow cytometer, which was designed to automate the analysis and separation of cells stained with fluorescent antibody. The flow cytometer uses a laser beam and light detector to count single intact cells in suspension (Figure 6-15).Every time a cell passes the laser beam,light is deflected from the detector, and this interruption of the laser signal is recorded. Those cells having a fluorescently tagged antibody bound to their cell surface antigens are excited by the laser and emit light that is recorded by a second detector system located at a right angle to the laser beam. The simplest form of the instrument counts each cell as it passes the laser beam and records the level of fluorescence the cell emits; an attached computer generates plots of the number of cells as the ordinate and their fluorescence intensity as the abscissa. More sophisticated versions of the instrument are capable of sorting populations of cells into different containers according to their fluorescence profile. Use of the instrument to determine which and how many members of a cell population bind fluorescently labeled antibodies is called analysis;use of the instrument to place cells having different patterns of reactivity into different containers is called cell sorting.

The flow cytometer has multiple applications to clinical and research problems.A common clinical use is to determine the kind and number of white blood cells in blood samples. By treating appropriately processed blood samples with a fluorescently labeled antibody and performing flow cytometric analysis, one can obtain the following information:

- How many cells express the target antigen as an absolute number and also as a percentage of cells passing the beam.For example,if one uses a fluorescent antibody specific for an antigen present on all T cells,it would be possible to determine the percentage of T cells in the total white blood cell population.Then,using the cell-sorting capabilities of the flow cytometer,it would be possible to isolate the T-cell fraction of the leukocyte population.
- The distribution of cells in a sample population according to antigen densities as determined by fluorescence intensity.It is thus possible to obtain a measure of the distribution of antigen density within the population of cells that possess the antigen.This is a powerful feature of the instrument,since the same type of cell may

express different levels of antigen depending upon its developmental or physiological state.

- The size of cells.This information is derived from analysis of the light-scattering properties of members of the cell population under examination.

Flow cytometry also makes it possible to analyze cell populations that have been labeled with two or even three different fluorescent antibodies. For example, if a blood sample is reacted with a fluorescein-tagged antibody specific for T cells,and also with a phycoerythrin-tagged antibody specific for B cells, the percentages of B and T cells may be determined simultaneously with a single analysis.Numerous variations of such "two-color"analyses are routinely carried out, and "three-color"experiments are common.Aided by appropriate software, highly sophisticated versions of the flow cytometer can even perform "five-color"analyses.

Flow cytometry now occupies a key position in immunology and cell biology,and it has become an indispensable clinical tool as well. In many medical centers, the flow cytometer is one of the essential tools for the detection and classification of leukemias (see the Clinical Focus). The choice of treatment for leukemia depends heavily on the cell types involved,making precise identification of the neoplastic cells an essential part of clinical practice. Likewise, the rapid measurement of T-cell subpopulations, an important prognostic indicator in AIDS, is routinely done by flowcytometric analysis. In this procedure, labeled monoclonal antibodies against the major T-cell subtypes bearing the CD4 and CD8 antigens are used to determine their ratios in the patient's blood. When the number of CD4 T-cells falls below a certain level, the patient is at high risk for opportunistic infections.

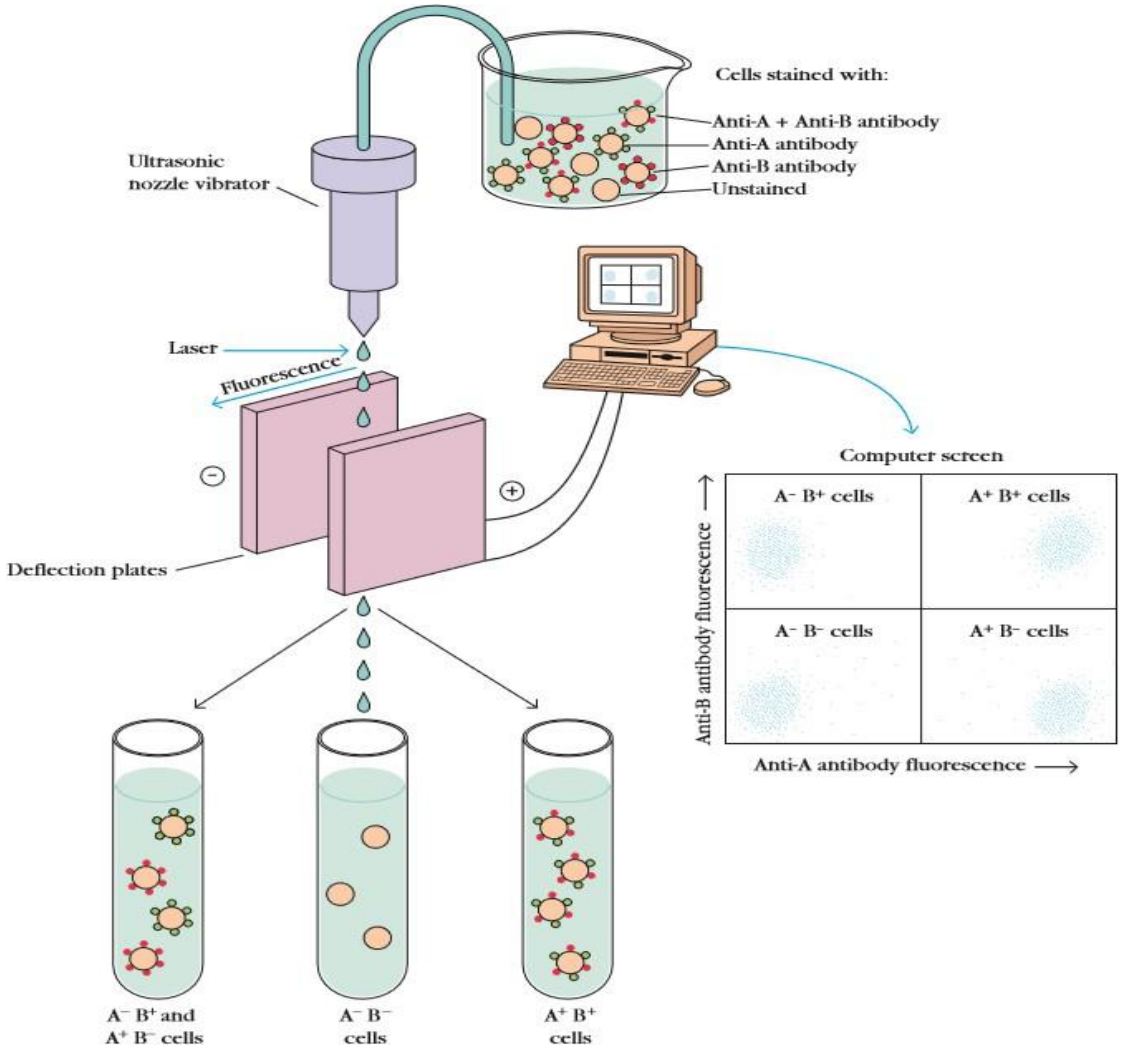


FIGURE 6-15 Separation of fluorochrome-labeled cells with the flow cytometer. In the example shown, a mixed cell population is stained with two antibodies, one specific for surface antigen A and the other specific for surface antigen B. The anti-A antibodies are labeled with fluorescein (green) and the anti-B antibodies with rhodamine (red). The stained cells are loaded into the sample chamber of the cytometer. The cells are expelled, one at a time, from a small vibrating nozzle that generates microdroplets, each containing no more than a single cell. As it leaves the nozzle, each droplet receives a small electrical charge, and the computer that controls the flow cytometer can detect exactly when a drop generated by the nozzle passes through the beam of laser light that excites the fluorochrome. The intensity of the fluorescence emitted by each droplet that contains a cell is monitored by a detector and displayed on a computer screen. Because the computer tracks the position of each droplet, it is possible to determine when a particular droplet will arrive between the deflection plates. By applying a momentary charge to the deflection plates when a droplet is passing between them, it is possible to deflect the path of a particular droplet into one or another collecting vessel. This allows the sorting of a population of cells into subpopulations having different profiles of surface markers.

In the computer display, each dot represents a cell. Cells that fall into the lower left-hand panel have background levels of fluorescence and are judged not to have reacted with either antibody anti-A or anti-B. Those that appear in the upper left panel reacted with anti-B but not anti-A, and those in the lower right panel reacted with anti-A but not anti-B. The upper right panel contains cells that react with both anti-A and anti-B. In the example shown here, the A^-B^-—and the A^+B^+—subpopulations have each been sorted into a separate tube. Staining with anti-A and anti-B fluorescent antibodies allows four subpopulations to be distinguished: A^-B^-, A^+B^+, A^-B^+, and A^+B^-.

5. Fluorescence activated cell sorting (FACS):

Some cell types differ sufficiently in density that they can be separated on the basis of this physical property. White blood cells (leukocytes) and red blood cells (erythrocytes), for instance, have very different densities because erythrocytes have no nucleus; thus these cells can be separated by equilibrium density-gradient centrifugation. Most cell types cannot be differentiated so easily, so other techniques, such as flow cytometry, must be used to separate them.

To separate one type of cell from a complex mixture, it is necessary to have some way to mark and then sort out the desired cells. As we will see below, it is possible to mark cells by expressing a fluorescent protein in them, but if only a few cells in the population express the protein, how can we sort them from the nonfluorescent ones? The cells can be analyzed in a flow cytometer. This machine flows cells past a laser beam that measures the light that they scatter and the fluorescence that they emit; thus it can quantify the cells expressing the fluorescent protein in a mixture. A fluorescence-activated cell sorter (FACS), which is based on flow cytometry, can both analyze the cells and select the few fluorescent cells from thousands of others and sort them into a separate culture dish (Figure 4-2). To achieve this, the cells are mixed with a buffer and forced through a vibrating nozzle to generate tiny droplets. The concentration of cells is adjusted so that most of the droplets do not contain cells, and the ones that do contain only one. Just before the nozzle, the stream of cells passes through a laser beam so that the presence and size of a cell can be recorded from the scattered light using one detector, and the amount of fluorescent light emitted can be quantified using a second, fluorescent light detector. If a cell is present in a droplet, the droplet is given a negative electric charge as it emerges from the nozzle. The stream of droplets then passes through two plates that generate an electric field proportional to the fluorescence detected from the cell in the droplet. This field generates a force that moves charged droplets out of the stream of uncharged droplets and into a collection tube. Since the amount of force applied is proportional to the fluorescence emitted by the cell in the droplet, cells with different levels of fluorescence can be collected. Having been sorted from other cells, the selected cells can be grown in culture.

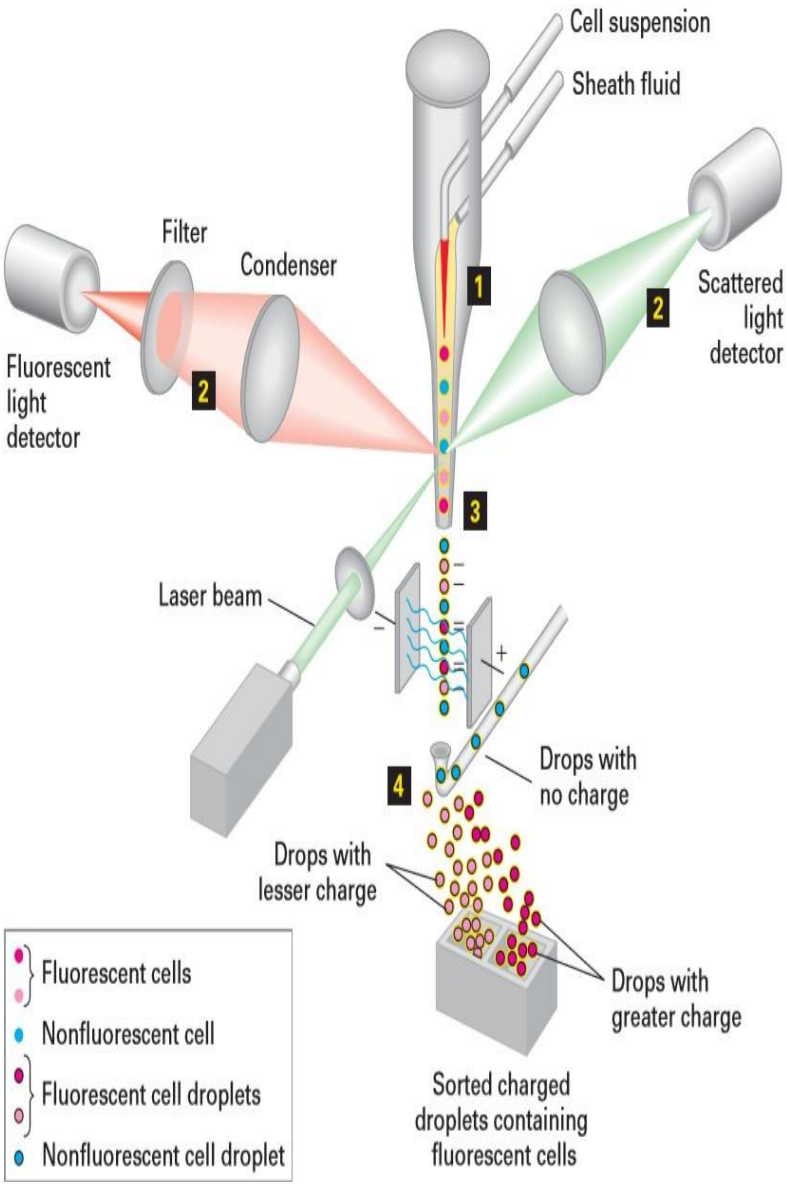
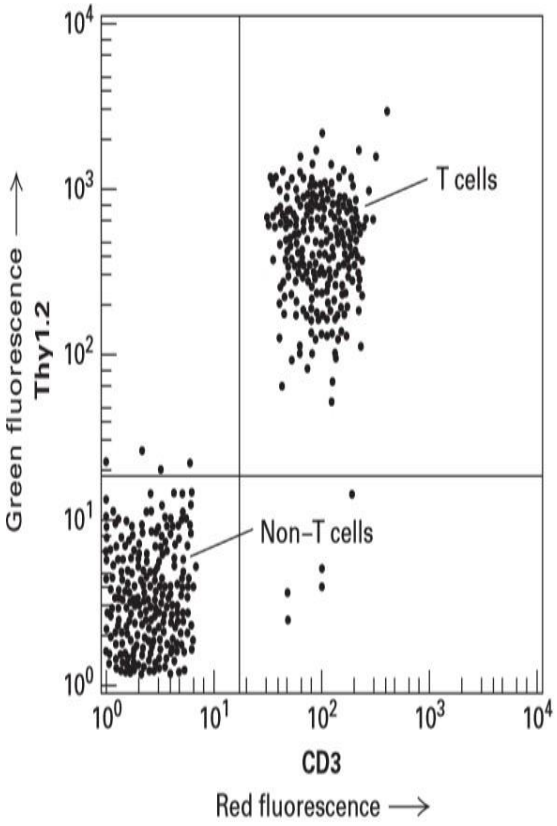


FIGURE 4-2 A fluorescence-activated cell sorter (FACS) separates cells having different levels of fluorescence. Step 1: A concentrated suspension of labeled cells is mixed with a buffer (the sheath fluid) so that the cells pass single file through a laser light beam. Step 2: Both the fluorescent light emitted and the light scattered by each cell are measured; from measurements of the scattered light, the size and shape of the cell can be determined. Step 3: The suspension is then forced through a nozzle, which forms tiny droplets containing at most a single cell. At the time of formation at the nozzle tip, each droplet containing a cell is given a negative electric charge proportional to the fluorescence of that cell determined from the earlier measurement. Step 4: Droplets now pass through an electric field, so that those with no charge are discarded, whereas those with different electric charges are separated and collected. Because it takes only milliseconds to sort each droplet, as many as 10 million cells per hour can pass through the machine.

The FACS procedure is commonly used to purify the different types of white blood cells, each of which bears on its surface one or more distinctive proteins and so will bind monoclonal antibodies specific for its proteins. If a cell mixture is incubated with a fluorescent dye linked to the antibody to a specific cell-surface protein, only the desired cells will be fluorescent. Only the T cells of the immune system, for instance, have both CD3 and Thy1.2 proteins on their surfaces. The presence of these surface proteins allows T cells to be separated easily from other types of blood cells or spleen cells (Figure 4-3).



EXPERIMENTAL FIGURE 4-3 T cells bound to fluorescence-tagged antibodies to two cell-surface proteins are separated from other white blood cells by FACS. Spleen cells from a mouse were treated with a red fluorescent monoclonal antibody specific for the CD3 cell-surface protein and with a green fluorescent monoclonal antibody specific for a second cell-surface protein, Thy1.2. As the cells were passed through a FACS, the intensity of the green and red fluorescence emitted by each cell was recorded. Each dot represents a single cell. This plot of green fluorescence (vertical axis) versus red fluorescence (horizontal axis) for thousands of spleen cells shows that about half of them—the T cells—express both CD3 and Thy1.2 proteins on their surfaces (upper-right quadrant). The remaining cells, which exhibit low fluorescence (lower-left quadrant), express only background levels of these proteins and are other types of white blood cells. Note the logarithmic scale on both axes. [Data from Chengcheng Zhang, Whitehead Institute.]

Other uses of flow cytometry include the measurement of a cell's DNA and RNA content and the determination of its general shape and size. The FACS can make simultaneous measurements of the size of a cell (from the amount of scattered light) and the amount of DNA that it contains (from the amount of fluorescence emitted from a DNA-binding dye). Measurements of the DNA content of individual cellsare used to follow replication of DNA as the cells progress through the cell cycle.

5. Medical Microbiology: Principle of epidemiology; Air borne diseases, water borne diseases, food borne diseases, arthropod borne diseases, Sexually transmitted diseases, Respiratory diseases.

Principle of epidemiology

Introduction- Recently, a news story described an inner-city neighborhood's concern about the rise in the number of children with asthma. Another story reported the revised recommendations for who should receive influenza vaccine this year. A third story discussed the extensive disease-monitoring strategies being implemented in a city recently affected by a massive hurricane. A fourth story described a finding published in a leading medical journal of an association in workers exposed to a particular chemical and an increased risk of cancer. Each of these news stories included interviews with public health officials or researchers who called themselves epidemiologists. Well, who are these epidemiologists, and what do they do? What is epidemiology? This lesson is intended to answer those questions by describing what epidemiology is, how it has evolved and how it is used today, and what some of the key methods and concepts are. The focus is on epidemiology in public health practice, that is, the kind of epidemiology that is done at health departments.

Definition of Epidemiology

The word epidemiology comes from the Greek words epi, meaning on or upon, demos, meaning people, and logos, meaning the study of. In other words, the word epidemiology has its roots in the study of what befalls a population. Many definitions have been proposed, but the following definition captures the underlying principles and public health spirit of epidemiology: Epidemiology is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems. Key terms in this definition reflect some of the important principles of epidemiology.

Study

Epidemiology is a scientific discipline with sound methods of scientific inquiry at its foundation. Epidemiology is data-driven and relies on a systematic and unbiased approach to the collection, analysis, and interpretation of data. Basic epidemiologic methods tend to rely on careful observation and use of valid comparison groups to assess whether what was observed, such as the number of

cases of disease in a particular area during a particular time period or the frequency of an exposure among persons with disease, differs from what might be expected. However, epidemiology also draws on methods from other scientific fields, including biostatistics and informatics, with biologic, economic, social, and behavioral sciences. In fact, epidemiology is often described as the basic science of public health, and for good reason. First, epidemiology is a quantitative discipline that relies on a working knowledge of probability, statistics, and sound research methods. Second, epidemiology is a method of causal reasoning based on developing and testing hypotheses grounded in such scientific fields as biology, behavioral sciences, physics, and ergonomics to explain health-related behaviors, states, and events. However, epidemiology is not just a research activity but an integral component of public health, providing the foundation for directing practical and appropriate public health action based on this science and causal reasoning.

Distribution

Epidemiology is concerned with the **frequency** and **pattern** of health events in a population:

Frequency refers not only to the number of health events such as the number of cases of meningitis or diabetes in a population, but also to the relationship of that number to the size of the population. The resulting rate allows epidemiologists to compare disease occurrence across different populations.

Pattern refers to the occurrence of health-related events by time, place, and person. Time patterns may be annual, seasonal, weekly, daily, hourly, weekday versus weekend, or any other breakdown of time that may influence disease or injury occurrence. Place patterns include geographic variation, urban/rural differences, and location of work sites or schools. Personal characteristics include demographic factors which may be related to risk of illness, injury, or disability such as age, sex, marital status, and socioeconomic status, as well as behaviors and environmental exposures. Characterizing health events by time, place, and person are activities of descriptive epidemiology, discussed in more detail later in this lesson.

Determinants

Epidemiology is also used to search for determinants, which are the causes and other factors that influence the occurrence of disease and other health-related events. Epidemiologists assume that illness does not occur randomly in a population, but happens only when the right accumulation of risk factors or determinants exists in an individual. To search for these determinants, epidemiologists use analytic epidemiology or epidemiologic studies to provide the “Why” and

"How" of such events. They assess whether groups with different rates of disease differ in their demographic characteristics, genetic or immunologic make-up, behaviors, environmental exposures, or other so-called potential risk factors. Ideally, the findings provide sufficient evidence to direct prompt and effective public health control and prevention measures.

Health-related states or events

Epidemiology was originally focused exclusively on epidemics of communicable diseases3 but was subsequently expanded to address endemic communicable diseases and non-communicable infectious diseases. By the middle of the 20th Century, additional epidemiologic methods had been developed and applied to chronic diseases, injuries, birth defects, maternal-child health, occupational health, and environmental health. Then epidemiologists began to look at behaviors related to health and well-being, such as amount of exercise and seat belt use. Now, with the recent explosion in molecular methods, epidemiologists can make important strides in examining genetic markers of disease risk. Indeed, the term healthrelated states or events may be seen as anything that affects the well-being of a population. Nonetheless, many epidemiologists still use the term "disease" as shorthand for the wide range of health-related states and events that are studied.

Specified populations

Although epidemiologists and direct health-care providers (clinicians) are both concerned with occurrence and control of disease, they differ greatly in how they view "the patient." The clinician is concerned about the health of an individual; the epidemiologist is concerned about the collective health of the people in a community or population. In other words, the clinician's "patient" is the individual; the epidemiologist's "patient" is the community. Therefore, the clinician and the epidemiologist have different responsibilities when faced with a person with illness. For example, when a patient with diarrheal disease presents, both are interested in establishing the correct diagnosis. However, while the clinician usually focuses on treating and caring for the individual, the epidemiologist focuses on identifying the exposure or source that caused the illness; the number of other persons who may have been similarly exposed; the potential for further spread in the community; and interventions to prevent additional cases or recurrences.

Application

Epidemiology is not just "the study of" health in a population; it also involves applying the knowledge gained by the studies to community-based practice. Like the practice of medicine, the

practice of epidemiology is both a science and an art. To make the proper diagnosis and prescribe appropriate treatment for a patient, the clinician combines medical (scientific) knowledge with experience, clinical judgment, and understanding of the patient. Similarly, the epidemiologist uses the scientific methods of descriptive and analytic epidemiology as well as experience, epidemiologic judgment, and understanding of local conditions in "diagnosing" the health of a community and proposing appropriate, practical, and acceptable public health interventions to control and prevent disease in the community.

Summary

Epidemiology is the study (scientific, systematic, data-driven) of the distribution (frequency, pattern) and determinants (causes, risk factors) of health-related states and events (not just diseases) in specified populations (patient is community, individuals viewed collectively), and the application of (since epidemiology is a discipline within public health) this study to the control of health problems.

Historical Evolution of Epidemiology

Although epidemiology as a discipline has blossomed since World War II, epidemiologic thinking has been traced from Hippocrates through John Graunt, William Farr, John Snow, and others. The contributions of some of these early and more recent thinkers are described below.

Circa 400 B.C. Epidemiology's roots are nearly 2500 years old. Hippocrates attempted to explain disease occurrence from a rational rather than a supernatural viewpoint. In his essay entitled "On Airs, Waters, and Places," Hippocrates suggested that environmental and host factors such as behaviors might influence the development of disease.

1662 Another early contributor to epidemiology was John Graunt, a London haberdasher and councilman who published a landmark analysis of mortality data in 1662. This publication was the first to quantify patterns of birth, death, and disease occurrence, noting disparities between males and females, high infant mortality, urban/rural differences, and seasonal variations.

1800 William Farr built upon Graunt's work by systematically collecting and analyzing Britain's mortality statistics. Farr, considered the father of modern vital statistics and surveillance, developed many of the basic practices used today in vital statistics and disease classification. He concentrated his efforts on collecting vital statistics, assembling and evaluating those data, and reporting to responsible health authorities and the general public.

1854 In the mid-1800s, an anesthesiologist named John Snow was conducting a series of investigations in London that warrant his being considered the “father of field epidemiology.” Twenty years before the development of the microscope, Snow conducted studies of cholera outbreaks both to discover the cause of disease and to prevent its recurrence. Because his work illustrates the classic sequence from descriptive epidemiology to hypothesis generation to hypothesis testing (analytic epidemiology) to application, two of his investigations will be described in detail. Snow conducted one of his now famous studies in 1854 when an epidemic of cholera erupted in the Golden Square of London.

He Introduction to Epidemiology Page 1-8 began his investigation by determining where in this area persons with cholera lived and worked. He marked each residence on a map of the area, as shown in Figure 1.1. Today, this type of map, showing the geographic distribution of cases, is called a spot map.

19th and 20th centuries In the mid- and late-1800s, epidemiological methods began to be applied in the investigation of disease occurrence. At that time, most investigators focused on acute infectious diseases. In the 1930s and 1940s, epidemiologists extended their methods to noninfectious diseases. The period since World War II has seen an explosion in the development of research methods and the theoretical underpinnings of epidemiology. Epidemiology has been applied to the entire range of health-related outcomes, behaviors, and even knowledge and attitudes. The studies by Doll and Hill linking lung cancer to smoking and the study of cardiovascular disease among residents of Framingham, Massachusetts are two examples of how pioneering researchers have applied epidemiologic methods to chronic disease since World War II.

During the 1960s and early 1970s health workers applied epidemiologic methods to eradicate naturally occurring smallpox worldwide.8 This was an achievement in applied epidemiology of unprecedented proportions. In the 1980s, epidemiology was extended to the studies of injuries and violence.

In the 1990s, the related fields of molecular and genetic epidemiology (expansion of epidemiology to look at specific pathways, molecules and genes that influence risk of developing disease) took root. Meanwhile, infectious diseases continued to challenge epidemiologists as new infectious agents emerged (Ebola virus, Human Immunodeficiency virus (HIV)/ Acquired Immunodeficiency Syndrome (AIDS)), were identified (Legionella, Severe Acute Respiratory Syndrome (SARS)), or changed (drug-resistant Mycobacterium tuberculosis, Avian influenza). Beginning in the 1990s and

accelerating after the terrorist attacks of September 11, 2001, epidemiologists have had to consider not only natural transmission of infectious organisms but also deliberate spread through biologic warfare and bioterrorism.

Today, public health workers throughout the world accept and use epidemiology regularly to characterize the health of their communities and to solve day-to-day problems, large and small.

Uses

Epidemiology and the information generated by epidemiologic methods have been used in many ways. Some common uses are described below. Assessing the community's health Public health officials responsible for policy development, implementation, and evaluation use epidemiologic information as a factual framework for decision making. To assess the health of a population or community, relevant sources of data must be identified and analyzed by person, place, and time (descriptive epidemiology).

• What are the actual and potential health problems in the community?

• Where are they occurring?

• Which populations are at increased risk?

• Which problems have declined over time?

• Which ones are increasing or have the potential to increase?

• How do these patterns relate to the level and distribution of public health services available?

More detailed data may need to be collected and analyzed to determine whether health services are available, accessible, effective, and efficient. For example, public health officials used epidemiologic data and methods to identify baselines, to set health goals for the nation in 2000 and 2010, and to monitor progress toward these goals.

Making individual decisions

Many individuals may not realize that they use epidemiologic information to make daily decisions affecting their health. When persons decide to quit smoking, climb the stairs rather than wait for an elevator, eat a salad rather than a cheeseburger with fries for lunch, or use a condom, they may be influenced, consciously or unconsciously, by epidemiologists' assessment of risk. Since World War II, epidemiologists have provided information related to all those decisions. In the 1950s, epidemiologists reported the increased risk of lung cancer among smokers. In the 1970s, epidemiologists documented the role of exercise and proper diet in reducing the risk of heart disease. In the mid-1980s, epidemiologists identified the increased risk of HIV infection associated

with certain sexual and drug-related behaviors. These and hundreds of other epidemiologic findings are directly relevant to the choices people make every day, choices that affect their health over a lifetime.

Completing the clinical picture

When investigating a disease outbreak, epidemiologists rely on health-care providers and laboratorians to establish the proper diagnosis of individual patients. But epidemiologists also contribute to physicians' understanding of the clinical picture and natural history of disease. For example, in late 1989, a physician saw three patients with unexplained eosinophilia (an increase in the number of a specific type of white blood cell called an eosinophil) and myalgias (severe muscle pains). Although the physician could not make a definitive diagnosis, he notified public health authorities. Within weeks, epidemiologists had identified enough other cases to characterize the spectrum and course of the illness that came to be known as eosinophilia-myalgia syndrome.13 More recently, epidemiologists, clinicians, and researchers around the world have collaborated to characterize SARS, a disease caused by a new type of coronavirus that emerged in China in late 2002. Epidemiology has also been instrumental in characterizing many non-acute diseases, such as the numerous conditions associated with cigarette smoking — from pulmonary and heart disease to lip, throat, and lung cancer.

Searching for causes

Much epidemiologic research is devoted to searching for causal factors that influence one's risk of disease. Ideally, the goal is to identify a cause so that appropriate public health action might be taken. One can argue that epidemiology can never prove a causal relationship between an exposure and a disease, since much of epidemiology is based on ecologic reasoning. Nevertheless, epidemiology often provides enough information to support effective action. Examples date from the removal of the handle from the Broad St. pump following John Snow's investigation of cholera in the Golden Square area of London in 1854, to the withdrawal of a vaccine against rotavirus in 1999 after epidemiologists found that it increased the risk of intussusception, a potentially life-threatening condition.15 Just as often, epidemiology and laboratory science converge to provide the evidence needed to establish causation. For example, epidemiologists were able to identify a variety of risk factors during an outbreak of pneumonia among persons attending the American Legion Convention in Philadelphia in 1976, even though the Legionnaires' bacillus was not identified in the laboratory from lung tissue of a person who had died from Legionnaires' disease until almost 6

months later.

Core Epidemiologic Functions

In the mid-1980s, five major tasks of epidemiology in public health practice were identified: public health surveillance, field investigation, analytic studies, evaluation, and linkages. A sixth task, policy development, was recently added. These tasks are described below.

Public health surveillance

Public health surveillance is the ongoing, systematic collection, analysis, interpretation, and dissemination of health data to help guide public health decision making and action. Surveillance is equivalent to monitoring the pulse of the community. The purpose of public health surveillance, which is sometimes called "information for action," is to portray the ongoing patterns of disease occurrence and disease potential so that investigation, control, and prevention measures can be applied efficiently and effectively. This is accomplished through the systematic collection and evaluation of morbidity and mortality reports and other relevant health information, and the dissemination of these data and their interpretation to those involved in disease control and public health decision making.

Morbidity and mortality reports are common sources of surveillance data for local and state health departments. These reports generally are submitted by health-care providers, infection control practitioners, or laboratories that are required to notify the health department of any patient with a reportable disease such as pertussis, meningococcal meningitis, or AIDS. Other sources of health-related data that are used for surveillance include reports from investigations of individual cases and disease clusters, public health program data such as immunization coverage in a community, disease registries, and health surveys.

Field investigation

As noted above, surveillance provides information for action. One of the first actions that results from a surveillance case report or report of a cluster is investigation by the public health department. The investigation may be as limited as a phone call to the healthcare provider to confirm or clarify the circumstances of the reported case, or it may involve a field investigation requiring the coordinated efforts of dozens of people to characterize the extent of an epidemic and to identify its cause. The objectives of such investigations also vary. Investigations often lead to the identification of additional unreported or unrecognized ill persons who might otherwise continue to spread infection to others. For example, one of the hallmarks of investigations of persons with

sexually transmitted disease is the identification of sexual partners or contacts of patients. When interviewed, many of these contacts are found to be infected without knowing it, and are given treatment they did not realize they needed. Identification and treatment of these contacts prevents further spread.

Analytic studies

Surveillance and field investigations are usually sufficient to identify causes, modes of transmission, and appropriate control and prevention measures. But sometimes analytic studies employing more rigorous methods are needed. Often the methods are used in combination — with surveillance and field investigations providing clues or hypotheses about causes and modes of transmission, and analytic studies evaluating the credibility of those hypotheses. Clusters or outbreaks of disease frequently are investigated initially with descriptive epidemiology. The descriptive approach involves the study of disease incidence and distribution by time, place, and person. It includes the calculation of rates and identification of parts of the population at higher risk than others. Occasionally, when the association between exposure and disease is quite strong, the investigation may stop when descriptive epidemiology is complete and control measures may be implemented immediately. John Snow's 1854 investigation of cholera is an example.

More frequently, descriptive studies, like case investigations, generate hypotheses that can be tested with analytic studies. While some field investigations are conducted in response to acute health problems such as outbreaks, many others are planned studies. The hallmark of an analytic epidemiologic study is the use of a valid comparison group. Epidemiologists must be skilled in all aspects of such studies, including design, conduct, analysis, interpretation, and communication of findings.

• Design includes determining the appropriate research strategy and study design, writing justifications and protocols, calculating sample sizes, deciding on criteria for subject selection (e.g., developing case definitions), choosing an appropriate comparison group, and designing questionnaires.

• Conduct involves securing appropriate clearances and approvals, adhering to appropriate ethical principles, abstracting records, tracking down and interviewing subjects, collecting and handling specimens, and managing the data.

• Analysis begins with describing the characteristics of the subjects. It progresses to calculation of

rates, creation of comparative tables (e.g., two-by-two tables), and computation of measures of association (e.g., risk ratios or odds ratios), tests of significance (e.g., chi-square test), confidence intervals, and the like. Many epidemiologic studies require more advanced analytic techniques such as stratified analysis, regression, and modeling.

• Finally, interpretation involves putting the study findings into perspective, identifying the key take-home messages, and making sound recommendations. Doing so requires that the epidemiologist be knowledgeable about the subject matter and the strengths and weaknesses of the study.

Evaluation Epidemiologists, who are accustomed to using systematic and quantitative approaches, have come to play an important role in evaluation of public health services and other activities. Evaluation is the process of determining, as systematically and objectively as possible, the relevance, effectiveness, efficiency, and impact of activities with respect to established goals.

• Effectiveness refers to the ability of a program to produce the intended or expected results in the field; effectiveness differs from efficacy, which is the ability to produce results under ideal conditions.

• Efficiency refers to the ability of the program to produce the intended results with a minimum expenditure of time and resources.

The evaluation itself may focus on plans (formative evaluation), operations (process evaluation), impact (summative evaluation), or outcomes — or any combination of these. Evaluation of an immunization program, for example, might assess the efficiency of the operations, the proportion of the target population immunized, and the apparent impact of the program on the incidence of vaccine-preventable diseases. Similarly, evaluation of a surveillance system might address operations and attributes of the system, its ability to detect cases or outbreaks, and its usefulness.

Linkages

Epidemiologists working in public health settings rarely act in isolation. In fact, field epidemiology is often said to be a "team sport." During an investigation an epidemiologist usually participates as either a member or the leader of a multidisciplinary team. Other team members may be laboratorians, sanitarians, infection control personnel, nurses or other clinical staff, and, increasingly, computer information specialists. Many outbreaks cross geographical and

jurisdictional lines, so co-investigators may be from local, state, or federal levels of government, academic institutions, clinical facilities, or the private sector. To promote current and future collaboration, the epidemiologists need to maintain relationships with staff of other agencies and institutions. Mechanisms for sustaining such linkages include official memoranda of understanding, sharing of published or on-line information for public health audiences and outside partners, and informal networking that takes place at professional meetings.

Policy development

The definition of epidemiology ends with the following phrase: "...and the application of this study to the control of health problems." While some academically minded epidemiologists have stated that epidemiologists should stick to research and not get involved in policy development or even make recommendations, public health epidemiologists do not have this luxury. Indeed, epidemiologists who understand a problem and the population in which it occurs are often in a uniquely qualified position to recommend appropriate interventions. As a result, epidemiologists working in public health regularly provide input, testimony, and recommendations regarding disease control strategies, reportable disease regulations, and health-care policy.

The Epidemiologic Approach

As with all scientific endeavors, the practice of epidemiology relies on a systematic approach. In very simple terms, the epidemiologist:

- Counts cases or health events, and describes them in terms of time, place, and person;
- Divides the number of cases by an appropriate denominator to calculate rates; and
- Compares these rates over time or for different groups of people.

Airborne Transmission of Disease:

Aerosols, such as those generated by a human sneeze, are important vehicles for person-to-person transmission of many infectious diseases. Respiratory diseases are spread in this fashion. For example, Mycobacterium tuberculosis, the bacterium that causes the disease tuberculosis, has spread in this way to infect at least one-third of the world's population. In addition, respiratory spread of influenza and cold viruses is so efficient that virtually everyone has been infected, sometimes several times a year, as in the case of colds.

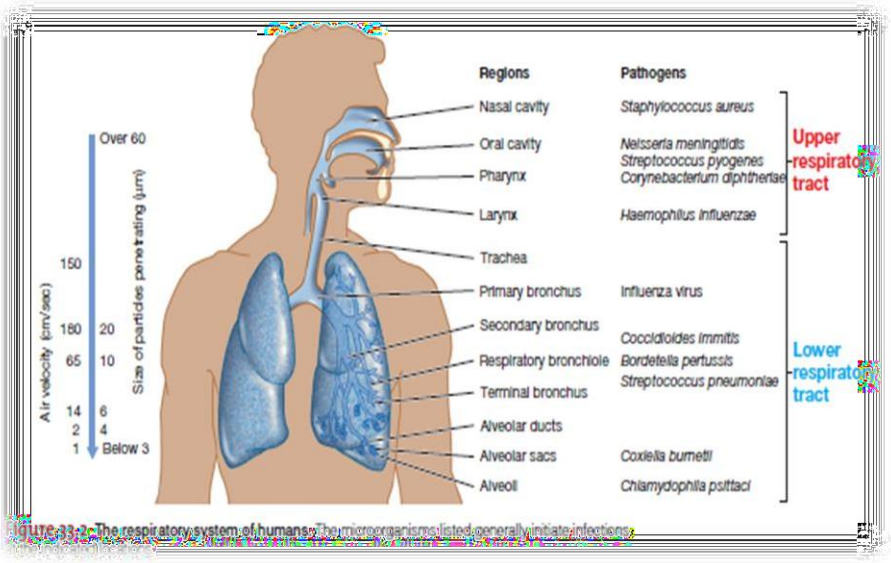
Fig 33.1High-speed photograph sneezing unstifled sneeze.

Airborne Pathogens

Microorganisms found in air are derived from soil, water, plants, animals, people, and other sources. In outdoor air, soil organisms predominate. Indoors, the concentration of microorganisms is considerably higher than outdoors, especially for organisms that originate in the human respiratory tract. Most microorganisms survive poorly in air. As a result, pathogens are effectively transmitted among humans only over short distances. Certain pathogens, however, survive under dry conditions and can remain alive in dust for long periods of time. Because of their thick, rigid cell walls, gram-positive bacteria (Staphylococcus, Streptococcus) are generally more resistant to drying than gram-negative bacteria. Likewise, the waxy layer of Mycobacterium cell walls resists drying and promotes survival. The endospores of endospore-forming bacteria are extremely resistant to drying but are not generally passed from human to human in the endospore form.

Large numbers of moisture droplets are expelled during sneezing (Figure 33.1), and a sizable number are expelled during coughing or simply talking. Each infectious droplet is about 10 µm in diameter and may contain one or two microbial cells or virions. The initial speed of the droplet movement is about 100 m/sec (more than 325 km/h) in a sneeze and ranges from 16 to 48 m/sec during coughing or shouting. The number of bacteria in a single sneeze varies from 10,000 to 100,000. Because of their small size, the moisture droplets evaporate quickly in the air, leaving behind a nucleus of organic matter and mucus to which bacterial cells are

attached.



Respiratory Infections Humans breathe about 500 million liters of air in a lifetime, much of it containing microorganism-laden dust. The speed at which air moves through the respiratory tract varies, and in the lower respiratory tract the rate is quite slow. As air slows down, particles in it stop moving and settle. Large particles settle first and the smaller ones later; only particles smaller than 3 _m travel as far as the bronchioles in the lower respiratory tract (**Figure 33.2**). Of course, most pathogens are much smaller than this, and different organisms characteristically colonize the respiratory tract at different levels. The upper and lower respiratory tracts offer decidedly different environments, favoring different microorganisms.

Bacterial and Viral Pathogens

Most human respiratory pathogens are transmitted from person to person because humans are the only reservoir for the pathogens; pathogen survival thus depends on person-to-person transmission. Here we discuss some of the pathogens that are transmitted primarily via the respiratory route. However, many of these such as Streptococcus spp., cold viruses, and influenza can also be transmitted via direct contact or on fomites. A fewrespiratory pathogens such as Legionella pneumophila (legionellosis,or Legionnaires' disease) are transmitted primarily fromwater or soil and thus do not require person-to-person propagation. Bacterial and viral respiratory infections, serious in themselves, often initiate secondaryproblems that can be life-threatening. Thus, accurate and rapiddiagnosis and treatment of respiratory infections can limit hostdamage. Many bacterial and viral pathogens can be controlled byimmunization. Most respiratory bacterial pathogens respondreadily to antibiotic therapy, but antiviral drug treatment

optionsare generally limited.

Streptococcal Diseases The bacteria Streptococcus pyogenes and Streptococcus pneumonia (**Figure 33.3**) are important human respiratory pathogens;both organisms are transmitted by the respiratory route.S. pneumoniae is found in the respiratory flora of up to 40% ofhealthy individuals. Although endogenous strains do not causedisease in most normal individuals, they can cause severerespiratory disease in compromised individuals.Streptococci are nonsporulating, homofermentative, aerotolerant, anaerobic gram-positive cocci. Cells ofS. pyogenes (Figure 33.3a) typically grow in elongated chains, as domany other members of the genus. Pathogenicstrains of S. pneumoniae typically grow in pairs or short chains,and virulent strains produce an extensive polysaccharide capsule(Figure 33.3b).

***Streptococcus pyogenes*: Epidemiologyand Pathogenesis**

Streptococcus pyogenes, also called group A Streptococcus(GAS)(Figure 33.3a), is frequently isolated from the upper respiratorytract of healthy adults. Although numbers of endogenousS. pyogenes are usually low, if host defenses are weakened or a new, highly virulent strain isintroduced, acute suppurative(pus-forming) infections are possible. S. pyogenes is the cause of streptococcal pharyngitis, or "strep throat." Most isolates fromclinical cases of streptococcal pharyngitis produce a toxin thatlyses red blood cells in culture media, a condition called beta-hemolysis. Streptococcal pharyngitis is characterizedby a severe sore throat, enlarged tonsils with exudate,tender cervical lymph nodes, a mild fever, and general malaise.S. pyogenes can also cause related infections of the middle ear(otitis media), the mammary glands (mastitis), infections of thesuperficial layers of the skin (pyoderma or impetigo) (impetigocan also be caused by Staphylococcus aureus) (**Figure 33.4**),erysipelas, an acute streptococcal skin infection (**Figure 33.5**),necrotizing fasciitis, an infection of subcutaneous tissue, andseveral conditions linked to the after effects of streptococcalinfections.

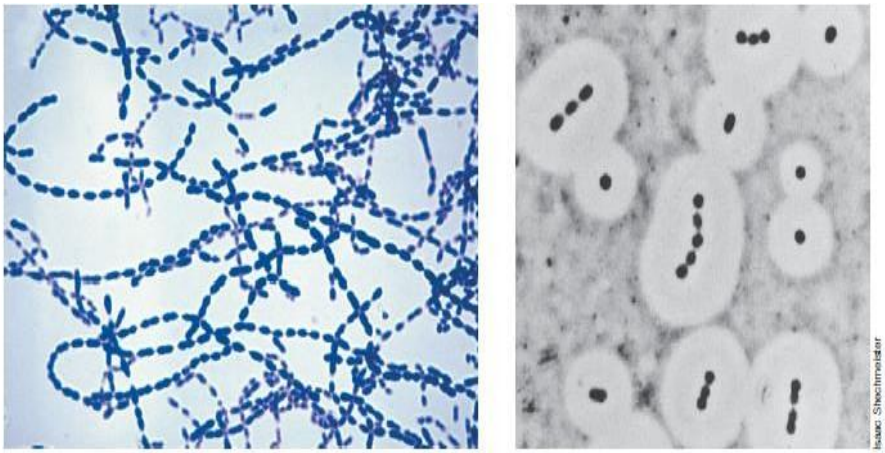


Figure 33.3 ***Streptococcus*** **pathogens.** *(a) Streptococcus pyogenes* grows in chains. The cells range in size from 1 to 2 μm in diameter. *(b)* India ink negative stain of *Streptococcus pneumoniae*. An extensive capsule surrounds the cells, which are about 0.5–1.2 μm in diameter.

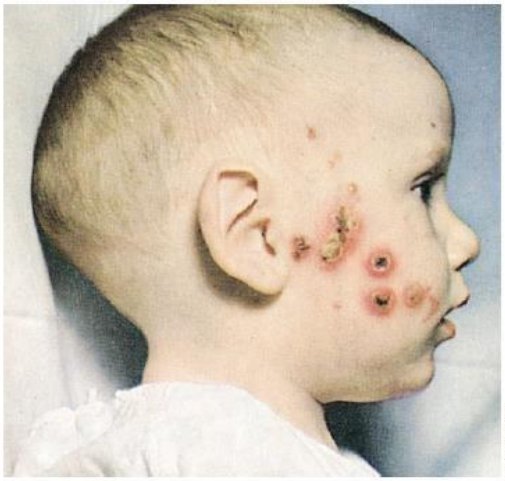


Figure 33.4 **Typical lesions of impetigo.** Impetigo is commonly caused by *Streptococcus pyogenes* or *Staphylococcus aureus*.

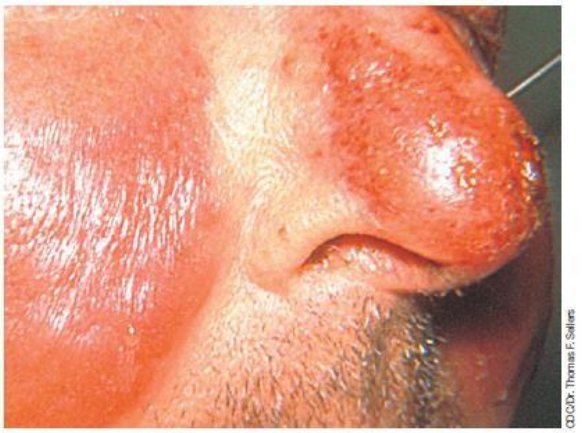


Figure 33.5 **Erysipelas.** Erysipelas is a *Streptococcus pyogenes* infection of the skin, shown here on the nose and cheeks, characterized by redness and distinct margins of infection. Other commonly-infected body sites include the ears and the legs.

About half of the clinical cases of severe sore throat are due to Streptococcus pyogenes, with most others due to viral infections. An accurate, rapid determination of the cause of the sore throat is important. If the sore throat is due to S. pyogenes, rapid, complete treatment of streptococcal sore throat is important because untreated streptococcal infections can lead to serious diseases such as scarlet fever, rheumatic fever, acute glomerulonephritis, and streptococcal toxic shock syndrome. On the other hand, if the sore throat is due to a virus, treatment with antibacterial drugs (antibiotics) will be useless, and may promote antimicrobial drug resistance. Certain GAS strains carry a lysogenic bacteriophage that encodes streptococcal

pyrogenic exotoxin A (SpeA), SpeB, SpeC, and SpeF. These exotoxins are responsible for most of the symptoms of streptococcal toxic shock syndrome (STSS) and **scarletfever.** Streptococcal pyrogenic exotoxins are superantigens that recruit large numbers of T cells to the infected tissues. Toxic shock results when the activated T cells secrete cytokines, which in turn activate large numbers ofmacrophages and neutrophils, causing local and systemic inflammationand tissue destruction.Occasionally GAS causes fulminant (sudden and severe) invasivesystemic infection such as cellulitis, a skin infection in subcutaneouslayers, and necrotizing fasciitis, a rapid and progressivedisease resulting in extensive destruction of subcutaneous tissue,muscle, and fat. Necrotizing fasciitis is responsible for the dramaticreports of "flesh-eating bacteria." In these cases, SpeA, SpeB,SpeC, and SpeF, as well as the bacterial cell surface M protein,function as superantigens. These diseases cause inflammationresulting in extensive tissue destruction.Invasive streptococcal disease including cellulitis, necrotizingfasciitis, scarlet fever, and STSS occur in an estimated 11,000patients per year. Death occurs in up to 15% of these patients(about 50% in STSS). In all of these cases, timely and adequatetreatment of the GAS infection stops production of the superantigenand its effects.

Other Streptococcal Diseases

Untreated or insufficiently treated S. pyogenes infections may leadto other serious diseases, even in the absence of active infection.These severe nonsuppurative (non-pus-forming) poststreptococcaldiseases usually occur about 1 to 4 weeks after the onset of astreptococcal infection. The immune response to the invadingpathogen produces antibodies that cross-react with host tissueantigens on the heart, joints, and kidneys, resulting in damage tothese tissues. The most serious of these diseases is **rheumaticfever** caused by rheumatogenic strains of S. pyogenes. Thesestrains contain cell surface antigens that are similar to heart valveand joint antigens. Rheumatic fever is an autoimmune disease;antibodies directed against streptococcal antigens also react withheart valve and joint antigens, causing inflammation and tissue Destruction. Damage to host tissues may bepermanent, and is often exacerbated by later streptococcal infectionsthat lead to recurring bouts of rheumatic fever.Another nonsuppurative disease is acute poststreptococcalglomerulonephritis, a painful kidney disease. This immune complexdisease develops following infection with S. pyogenes due tothe formation of streptococcal antigen–antibody complexes inthe blood. The immune complexes lodge in the glomeruli (filtrationmembranes of the kidney), causing inflammation of the kidney(nephritis)

accompanied by severe pain. Within several days,the complexes are usually dissolved and the patient returns tonormal. Unfortunately, even timely antibacterial treatment maynot prevent glomerulonephritis. Only a few strains of S. pyogenes,so-called nephritogenic strains, produce this painful disease, butup to 15% of infections with nephritogenic strains cause glomerulonephritis. Because infection induces strain-specific immunity, reinfectionby a particular S. pyogenes strain is rare. However, there maybe up to 150 different strains defined by distinct cell surface Mproteins. Thus, an individual can be infected multiple times bydifferent S. pyogenes strains. There are no available vaccines toprevent S. pyogenes infections.

Diagnosis of *Streptococcus pyogenes*

Several rapid antigen detection (RAD) systems have been developedfor identification of S. pyogenes. Surface antigens are firstextracted by enzymatic or chemical means directly from a swabof the patient's throat. The antigens are then detected using antibodiesspecific for surface proteins of S. pyogenes with immunologicalmethods such as latex bead agglutination, fluorescentantibody staining, and enzyme immunoassay (EIA). Using these methods, clinical specimenscan be quickly processed, sometimes in just a few minutes.Rapid diagnostic tests allow the physician to initiate appropriateantibiotic therapy to treat GAS infections and prevent more seriousdisease.

A more accurate confirmation of GAS infection is a positiveculture from the throat or lesion grown on sheep blood agar. Although the RAD tests are nearly as specific asthroat cultures, they can be up to 40% less sensitive, leading tofalse-negative reports. Throat cultures take up to two days toprocess, hence the popularity of the RAD tests. Serology tests arethe most sensitive tests available for identifying recent streptococcalinfections. Patients are examined for the presence orincrease of antibodies (rise in titer) to streptococcal antigens.The detection of new antibodies or an increase in the quantity ofexisting antibodies confirms a recent streptococcal infection.

Streptococcus pneumoniae

The other major pathogenic streptococcal species, Streptococcus pneumoniae (Figure 33.3b), causes invasive lung infections that often develop as secondary infections to other respiratory disorders. Strains of S. pneumoniae that are encapsulated are particularly pathogenic because they are potentially very invasive. Cells invade alveolar tissues (lower respiratory tract) in the lung, where the capsule enables the cells to resist phagocytosis and elicit a strong host inflammatory response. Reduced lung function, called pneumonia, can result from accumulation

of recruited phagocytic cells and fluid. The S. pneumoniae cells can then spread from the focus of infection as a bacteremia, sometimes resulting in bone infections, middle ear infections, and endocarditis. Untreated invasive pneumococcal disease has a mortality rate of about 30%. Even with aggressive antimicrobial treatment, individuals hospitalized with pneumococcal pneumonia have up to 10% mortality. Laboratory diagnosis of S. pneumoniae is based on the culture of gram-positive diplococci from either patient sputum or blood. There are over 90 different serotypes (antigenic capsule variants), and, as for S. pyogenes, infection induces immunity to only the infecting serotype of S. pneumoniae.

Prevention and treatment

Effective vaccines are available for prevention of infection by the most common strains of S. pneumoniae. A vaccine for adultsconsists of a mixture of 23 capsular polysaccharides from the most prevalent pathogenic strains. The vaccine is recommendedfor the elderly, healthcare providers, individuals with compromised immunity, and others at high risk for respiratory infections.A vaccine containing seven capsular polysaccharides conjugated to diphtheria protein is recommended for children, age 2–23 months, to prevent ear infections. Novaccine is available for GAS.

Both GAS and S. pneumoniae can be treated with antibiotics. Penicillin G and its many derivatives are the agents of choice for treating GAS infections. Erythromycin and other antibacterial drugs are used in individuals who have penicillin allergies. S. pneumoniae infections respond quickly to penicillin G therapy, but up to 30% of pathogenic isolates now exhibit resistance to penicillin. Erythromycin and cefotaxime resistance is also found in some strains, and a few strains exhibit multiple drug resistance. Thus, each pathogenic isolate must be tested for antibiotic sensitivity. All strains are sensitive to vancomycin. Invasive disease such as pneumonia caused by drug-resistant S. pneumonia is now a reportable disease in the United States; more than 3000 cases are reported annually.

Diphtheria and Pertussis

Corynebacterium diphtheriae causes diphtheria, a severe respiratorydisease that typically infects children. Diphtheria is preventable and treatable. C. diphtheriae is a gram-positive, nonmotile, aerobic bacterium that forms irregular rods that may appear asclub-shaped cells during growth (**Figure 33.7*a***). **Pertussis** or **whooping cough** is a serious respiratory disease caused by

infection with Bordetella pertussis, a small, gramnegative, aerobic coccobacillus that is a member of the Betaproteobacteria (**Figure 33.8**). Pertussis affects mostly children butcan cause seriousrespiratory disease for anyone. The disease ispreventable and curable.

Diphtheria Epidemiology, Pathology, Prevention, and Treatment

Diphtheria was once a major childhood disease, but it is nowrarely encountered because an effective vaccine is available. Inthe United States and other developed countries, the disease is virtually unknown. Worldwide, over 5000 fatal cases of diphtheriaoccur per year, largely because of a lack of effective immunization programs in less developed countries.*Corynebacterium diphtheriae* enters the body, infecting thetissues of the throat andtonsils. The organism spreads fromhealthy carriers or infected individuals to susceptible individualsby airborne droplets. Previous infection or immunization provides resistance to the effects of the potent diphtheria exotoxin. Throattissues respond to C. diphtheriae infection by forming a characteristiclesion called a pseudomembrane (Figure 33.7b), which consists of damaged host cells and cells of C. diphtheriae. Pathogenic strains of C. diphtheriae lysogenized by bacteriophage β produce a powerful exotoxin called diphtheria toxin that inhibitseukaryotic protein synthesis, leading to cell death. Death from diphtheria is usually due to a combination of the effects of partial suffocation and tissue destruction by exotoxin. In untreated infections, the toxin can cause systemic damage to the heart (about 25% of diphtheria patients develop myocarditis), kidneys, liver, and adrenal glands. C. diphtheria isolated from the throat is diagnostic for diphtheria. Nasal or throat swabs are used to inoculate blood agar, tellurite medium, or the selective Loeffler's medium that inhibits the growth of most other respiratory pathogens. Prevention of diphtheria is accomplished with a highly effective toxoid vaccine, part of the DTaP (diphtheria toxoid, tetanus toxoid, and acellular pertussis) vaccine. Penicillin, erythromycin, and gentamicin are generally effective for stopping C. diphtheriae growth and further toxin production, but do not alter the effects of preformed toxin. Diphtheria antitoxin (an antiserum produced in horses) contains neutralizing antibodies, but is available only for serious acute cases of diphtheria. Early administration of both antibiotics and antitoxin is necessary for effective treatment of the acute disease.

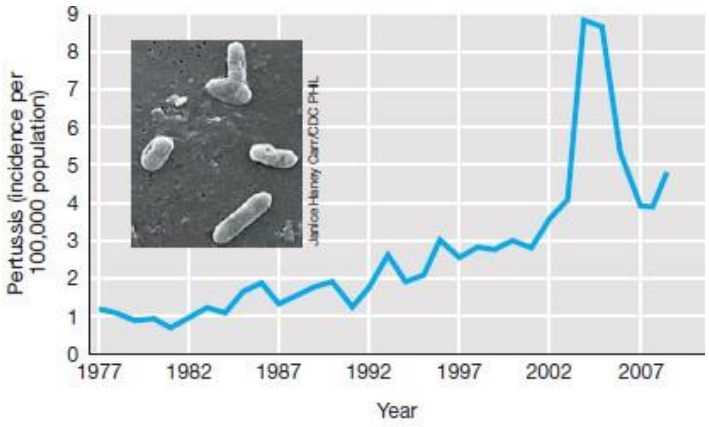


Figure 33.8 ***Bordetella*** **and pertussis.** The scanning electron micrograph (inset) shows the coccobacillus *Bordetella* sp. The variably shaped organisms range from 0.2 to 0.5 μm in diameter and are up to 1.0 μm in length. The graph shows the incidence of pertussis per 100,000 population caused by respiratory infection with *Bordetella pertussis*. There were 25,616 cases of pertussis in 2005, mostly in infants and school-age children, triple the number of 2001. After 2005, the incidence declined significantly, but was rising again by 2009. Data are from the Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Pertussis Pertussis, also known as whooping cough, is an acute, highly infectious respiratory disease now observed frequently in children under 19 years of age. Infants less than 6 months of age, who are

too young to be effectively vaccinated, have the highest incidence of disease and also have the most severe disease. B. pertussis attaches to cells of the upper respiratory tract by producing a specific adherence factor called filamentous hemagglutinin antigen, which recognizes a complementary molecule on the surface of host cells. Once attached, B. pertussis grows andproduces pertussis exotoxin. This potent toxin induces synthesis of cyclicadenosinemonophosphate (cyclic AMP), which is at least partially responsible for the events that lead tohost tissue damage. B. pertussis also produces an endotoxin, which may inducesome of the symptoms of whooping cough. Clinically, whoopingcough is characterized by a recurrent, violent cough that can lastup to 6 weeks. The spasmodic coughing gives the disease itsname; a whooping sound results from the patient inhaling deepbreaths to obtain sufficient air. Worldwide, there are up to 50million cases and over 250,000 pertussis deaths each year, mostin developing countries. B. pertussis is endemic worldwide andpertussis remains a problem, even in developed countries, usuallydue to inadequate immunization.

Pertussis Epidemiology In the United States there has been an upward trend of B. pertussisinfections and disease since the 1980s, reversing a trend thatstarted with the introduction of an effective pertussis vaccine.

In 1976, the year of lowest prevalence and incidence, therewere only 1010 reported cases of pertussis. By contrast, in 2005,there were 25,616 cases. Although the numbers of infections have declined in recent years compared to the peak incidence in2004–2005, the incidence is still significantly higher than in the1990s (Figure 33.8). In the United States pertussis causes about 14 deaths per year. About 60% of recent cases were in adolescentsand adults of all ages who lacked appropriate immunity. About13% of cases were in children less than 6 months of age who hadnot yet received all of the recommended doses of pertussis vaccine.Up to 32% of coughs lasting 1 to 2 weeks or longer may becaused by B. pertussis. Pertussis is an endemic disease; incidencerises cyclically as populations become susceptible and areexposed to the pathogen. Lack of appropriate immunization at allages may be adding to the overall higher incidence of pertussis ascompared to recent decades.

Pertussis Diagnosis, Prevention, and Treatment Diagnosis of whooping cough can be made by fluorescent antibodystaining of a nasopharyngeal swab specimen or by actual culture of the organism. For best recovery of B. pertussis, a nasopharyngealaspirate is inoculated directly onto a blood–glycerol–potatoextract agar plate (although not selective, this rich medium supportsgood recovery of B. pertussis). The β-hemolytic coloniescontaining small gram-negative coccobacilli are tested forB. pertussis by a latex bead agglutination test or are stained witha fluorescent antibody specific for B. pertussis for positive identification.A polymerase chain reaction (PCR) test is consideredthe most sensitive and preferred diagnostic test. Improveddiagnostic and reporting techniques may be one reason for therecent observed increase in pertussis cases in the UnitedStates, but the disease may still be underreported, especially inadolescents and adults.A vaccine consisting of proteins derived from B. pertussis ispart of the routinely administered DTaP vaccine. This vaccine is normally given to children at appropriate intervals beginning soon after birth. The acellular pertussis vaccine has fewer side effects than the older pertussis vaccines and has caused no deaths. It is also recommended for adolescents and certain populations of adults (healthcare and childcare workers)as well as young children. Worldwide, immunization programs should be targeted tochildren, but immunization of adolescents and adults should alsobe a priority because vaccinated individuals lose effective immunity within 10 years and can

transmit B. pertussis to youngchildren. Vaccination of a large percentage of the population isnecessary to build herd immunity.Cultures of B. pertussis are killed by ampicillin, tetracycline, anderythromycin, although antibiotics alone do not seem to be sufficientto kill the pathogen in vivo: A patient with whooping coughremains infectious for up to 2 weeks following commencement ofantibiotic therapy, indicating that the immune response may bemore important than antibiotics for eliminating B. pertussis fromthe body.

***Mycobacterium*, Tuberculosis, and Hansen's disease:** Tuberculosis (TB) is caused by the gram-positive, acid-fast bacillus Mycobacterium tuberculosis. The Germanmicrobiologist Robert Koch isolated and described the causative agent in 1882. A related Mycobacterium species,Mycobacterium leprae, causes Hansen's disease (leprosy). All mycobacteria share acid-fast properties due to the waxy mycolicacid constituent of their cell wall. Mycolic acid allows theseorganisms to retain carbol-fuchsin, a red dye, after washing in 3% hydrochloric acid in alcohol (**Figure 33.9**).

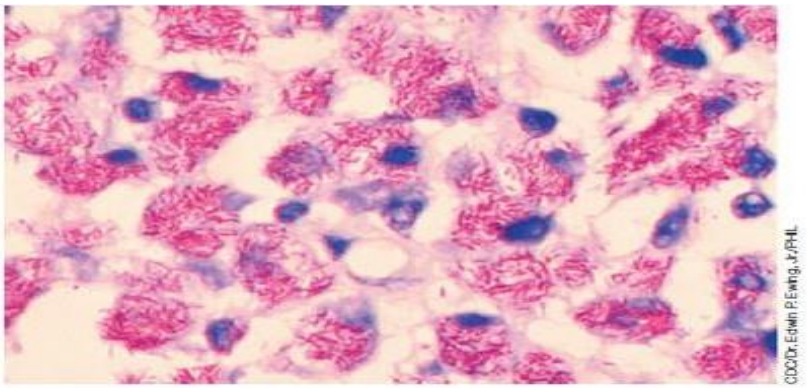


Figure 33.9 Mycobacteria. Here an acid-fast stained lymph node biopsy from a patient with HIV/AIDS displays *Mycobacteroum avium*. Multiple bacilli, stained red with carbol-fuchsin, are evident inside each cell. The individual rods are about 0.4 μm in diameter and up to 4 μm in length.

Tuberculosis Epidemiology

Mycobacterium tuberculosis is easily transmitted by the respiratory route; even normal conversation can spread the organism from person to person. At one time, TB was the most important infectious disease of humans and accounted for one-seventh of all deaths worldwide. Presently, over 13,000 new cases of TB and over 600 deaths occur each year in the United States. Worldwide, TB still accounts for over 1.4 million deaths per year, almost 11% of all deaths due to infectious disease. About one-third of the world's population has been infected with M. tuberculosis. Many new TB cases in the United States occur in acquired

immunodeficiency syndrome (AIDS) patients.

Tuberculosis Pathology

The interaction of the human host and the bacterium M. tuberculosisis determined both by the virulence of the strain and the resistance of the host. Cell-mediated immunity plays a critical role inthe prevention of active disease after infection. TB can be a primaryinfection (initial infection) or postprimary infection (reinfection).Primary infection typically results from inhalation of droplets containing viable M. tuberculosis bacteria from an individualwith an active pulmonary infection. The inhaled bacteria settle inthe lungs and grow. The host mounts an immune response toM. tuberculosis, resulting in a delayed-type hypersensitivity reaction
and the formation of aggregates of activatedmacrophages, called tubercles. Mycobacteria often survive and grow within the macrophages, even with anongoing immune response. In individuals with low resistance, the bacteria are not controlled and the pulmonary infection becomes acute, leading to extensive destruction of lung tissue,the spread of the bacteria to other parts of the body, and death.In these cases, M. tuberculosis survives both the low pH andthe effects of the oxidative antibacterial products found in thelysosomes of phagocytes such as macrophages.In most cases of TB, however, an obvious acute infection doesnot occur. The infection remains localized, is usually inapparent,and appears to end. But this initial infection hypersensitizes the individual to the bacteria or their products and consequentlyalters the response of the individual to subsequent or post primary infections by M. tuberculosis. A diagnostic skin test, calledthe **tuberculin test**, can be used to measure this hypersensitivity.

In a hypersensitive individual, tuberculin, a protein extract from M. tuberculosis, elicits a local immune inflammatory reactionwithin 1–3 days at the site of an intradermal injection. The reactionis characterized by induration (hardening) and edema (swelling). An individual exhibiting this reactionis said to be tuberculin-positive, and many healthy adults showpositive reactions as a result of previous inapparent infections.A positive tuberculin test does not indicate active disease, butonly that the individual has been exposed to the organism in thepast and has generated a cell-mediated immune response againstM. tuberculosis.For most individuals, this cell-mediated immunity is protective and lifelong. However, some tuberculin-positive patients developpostprimary tuberculosis through reinfection from outsidesources or as a result of reactivation of bacteria that haveremained dormant in lung macrophages, often for years. For example, advanced age, malnutrition, overcrowding, stress, andhormonal changes may reduce

effective immunity in untreatedindividuals and allow reactivation of dormant infections. Becauselatent M. tuberculosis can become activated many years after theinitial exposure and immune response, individuals who have apositive tuberculin test are treated with antimicrobial agents forlong periods of time. Postprimary mycobacterial infections oftenprogress to chronic infections that result in destruction of lungtissue, followed by partial healing and calcification at the infectionsite. Chronic postprimary TB often results in a gradualspread of tubercular lesions in the lungs. Bacteria are found inthe sputum in individuals with active disease, and areas of destroyed tissue can be seen in X-rays (**Figure 33.10**).

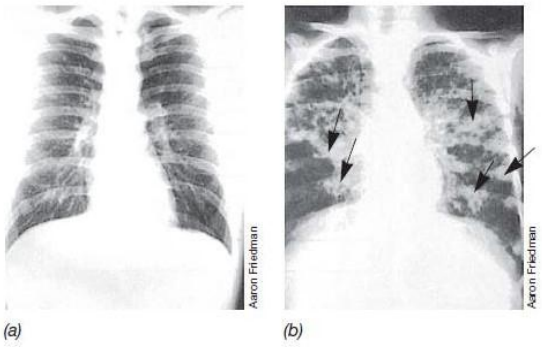


Figure 33.10 Tuberculosis X-ray. *(a)* Normal chest X-ray. The faint white lines are arteries and other blood vessels. *(b)* Chest X-ray of an advanced case of pulmonary tuberculosis; white patches (arrows) indicate areas of disease. These patches, or tubercles as they are called, may contain viable cells of *Mycobacterium tuberculosis*. Lung tissue and function is permanently destroyed by these lesions.

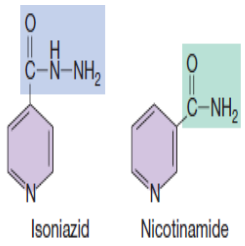


Figure 33.11 Structure of isoniazid (isonicotinic acid hydrazide). Isoniazid is an effective chemotherapeutic agent for tuberculosis. Note the structural similarity to nicotinamide.

Tuberculosis Prevention and Treatment

Individuals who have active cases of TB may spread the diseasesimply by coughing or speaking. Because TB is highly contagious,the U.S. Occupational Safety and Health Administration has stringent requirements for the protection of healthcare workerswho are responsible for TB patient care. For example, patientswith infectious tuberculosis must be hospitalized in negativepressurerooms. In addition, healthcare workers who havepatient contact must be provided with personally fitted facemasks having high-efficiency particulate air (HEPA) filters toprevent the passage of M. tuberculosis cells in sputum or on dustparticles.

Antimicrobial therapy of TB has been a major factor in controlof the disease. Streptomycin was the first effective antibiotic, butthe real revolution in treatment came with the discovery of isonicotinicacid hydrazide, called isoniazid (INH) (**Figure 33.11**). Thisdrug, specific for mycobacteria, is effective, inexpensive, relativelynontoxic, and readily absorbed when given

orally. Although themode of action of isoniazid is not completely understood, itaffects the synthesis of mycolic acid by Mycobacterium. Mycolicacid is a lipid that complexes with peptidoglycan in the mycobacterialcell wall.Isoniazid probably functions as a growth factor analog of thestructurally related molecule, nicotinamide. As such, isoniazidwould be incorporated in place of nicotinamide and inactivateenzymes required for mycolic acid synthesis. Treatment ofmycobacteria with very small amounts of isoniazid (as little as 5picomoles [pmol] per 10^9 cells) results in complete inhibition ofmycolic acid synthesis, and continued incubation results in lossof outer areas of the cell wall, a loss of cellular integrity, and death. Following treatment with isoniazid, mycobacteria losetheir acid-fast properties, in keeping with the role of mycolic acidin this staining property.Treatment is typically achieved with daily doses of isoniazidand rifampin for 2 months, followed by biweekly doses for a total of 9 months. This treatment eradicates the pathogen and preventsemergence of antibiotic-resistant organisms. Failure tocomplete the entire prescribed treatment may allow the infection to be reactivated, and reactivated organisms are often resistant tothe original treatment drugs. Incomplete treatment encouragesantibiotic resistance because a high rate of spontaneous mutationsin surviving M. tuberculosis promotes rapid acquisition ofresistance to single antibiotics. To ensure treatment and thus discouragedevelopment of antibiotic-resistant organisms, directobservation of treatment may be necessary for noncompliantindividuals. In populations such as hospitals and nursing homes,where resistant mycobacterial strains are most likely to be present,patients are routinely treated with up to four drugs for2 months, followed by rifampin–isoniazid treatment for a totalof 6 months. Multiple drug therapy reduces the possibility thatstrains having resistance to more than one drug will emerge.Resistance of M. tuberculosis to isoniazid and other drugs,however, is increasing, especially in AIDS patients. A number ofstrains that are resistant to both isoniazid and rifampin havealready emerged. Treatment of these strains, called multidrug-resistant tuberculosis strains (MDR TB), requires the use ofsecond-line tuberculosis drugs that are generally more toxic, lesseffective, and more costly than rifampin and isoniazid. A WorldHealth Organization (WHO) survey indicated that up to 20% ofMDR TB strains are extensively drug-

resistant (XDR TB) strains.XDR TB strains have resistance to virtually all TB drugs, includingthe second-line drugs. Preventing emergence of these strains requires better diagnostic and drug susceptibility tests in addition to new anti-TB treatment drugs and regimens.In many countries, immunization with an attenuated strain of

Mycobacterium bovis, the Bacillus Calmette-Guerin (BCG) strain,is routine for prevention of TB. However, in the United Statesand other countries where the prevalence of *Mycobacterium tuberculosis* infection and disease is relatively low, immunizationwith BCG is discouraged. The live BCG vaccine induces adelayed-type hypersensitivity response, and all individuals who receive it develop a positive tuberculin test. *This compromisesthe tuberculin test as a diagnostic and epidemiologic indicatorfor the spread of M. tuberculosis infection.*

Mycobacterium leprae **andHansen's Disease (Leprosy)**

Mycobacterium leprae, discovered by the Norwegian scientistG.A. Hansen in 1873, causes Hansen's disease, also known asleprosy. M. leprae is the only Mycobacterium species that has notbeen grown on artificial media. The armadillo is the only experimentalanimal that has been successfully used to grow M. lepraeand achieve symptoms similar to those in the human disease.The most serious form of Hansen's disease is lepromatousleprosy, characterized by folded, bulblike lesions on the body,especially on the face and extremities (**Figure 33.12**). The lesionsare due to the growth of M. leprae cells in the skin and maycontain up to 109 bacterial cells per gram of tissue. Like othermycobacteria, M. leprae from the lesions stain deep red with carbol-fuchsin in the acid-fast staining procedure, providing arapid, definitive demonstration of active infection. Lepromatousleprosy has a very poor prognosis. In severe cases the disfiguring lesions lead to destruction of peripheral nerves and loss of motor function. Many Hansen's disease patients exhibit less-pronounced lesions from which no bacterial cells can be recovered.

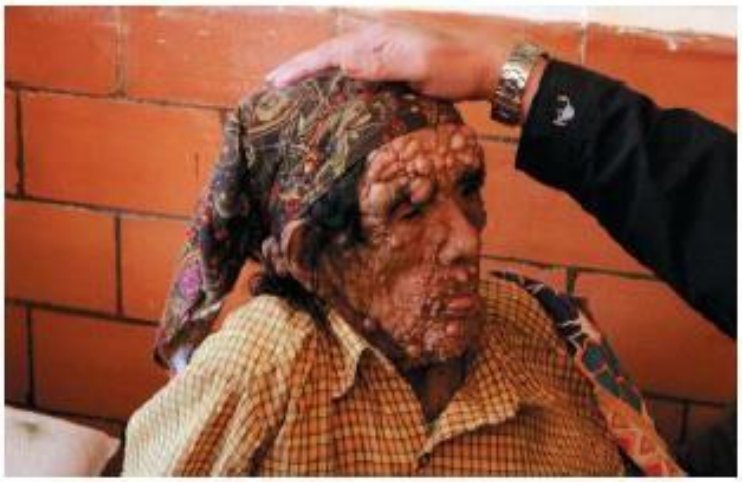


Figure 33.12 Lepromatous leprosy lesions on the skin. Lepromatous leprosy is caused by infection with *Mycobacterium leprae*. The lesions can contain up to 10^9 bacterial cells per gram of tissue, indicating an active uncontrolled infection with a poor prognosis.

These individuals have the tuberculoid form of the disease. Tuberculoid leprosy is characterized by a vigorous delayed-type hypersensitivity response and a good prognosis for spontaneous recovery. Hansen's disease of either form, and the continuum ofintermediate forms, is treated using a multiple drug therapy(MDT) protocol, which includes some combination of dapsone(4,4'-sulfonylbisbenzeneamine), rifampin, and clofazimine. As inTB, drug-resistant strains have appeared, especially after inadequatetreatment or treatment with single drugs. Extended drugtherapy of up to 1 year with a MDT protocol is required foreradication of the organism.The pathogenicity of M. leprae is due to a combination ofdelayed hypersensitivity and the invasiveness of the organism.Transmission is by direct contact as well as respiratory routes, but Hansen's disease is not as highly contagious as TB. The timefrom exposure to onset of disease varies from several weeks toyears, or even decades. During this time, M. leprae cells growwithin macrophages, causing an intracellular infection that canresult in large numbers of bacteria within the skin, leading to thecharacteristic lesions.In many areas of the world, the incidence of Hansen's disease isvery low. Worldwide, however, over 750,000 new cases of the diseaseare reported each year. About 100 cases are reported annuallyin the United States, mostly in southern states, or amongimmigrants from the Caribbean islands or Central America.

Ninety percent of worldwide cases are in Madagascar, Mozambique,Tanzania, and Nepal. Up to 2 million people are permanentlydisabled as a result of Hansen's disease, but because of the chronic nature and long latent period of the disease, it may beunrecognized and unreported in as

many as 12 million people.

Other Pathogenic *Mycobacterium* Species

A common pathogen of dairy cattle, Mycobacterium bovis ispathogenic for humans as well as other animals. M. bovis entershumans via the intestinal tract, typically from the ingestion of unpasteurized milk. After a localized intestinal infection, theorganism eventually spreads to the respiratory tract and initiatesthe classic symptoms of TB. M. bovis is a different organism from M. tuberculosis, although the genomes of the two organisms arevery similar. There is no observed difference in their infectivityand pathogenesis in humans, although the genome of M. bovishas several gene deletions compared with that of M. tuberculosis.Pasteurization of milk and elimination of diseased cattle haveeradicated bovine-to-human transmission of TB in developedcountries.

A number of other Mycobacterium species are also occasionalhuman pathogens. For example, M. kansasii, M. scrofulaceum,M. chelonae, and a few other mycobacterial species can cause disease.Respiratory disease due to the Mycobacterium avium complexof organisms (including M. avium and M. intracellulare)is particularly dangerous in AIDS patients or other immunecompromisedindividuals; these opportunistic pathogens rarelyinfect healthy individuals.

***Neisseria meningitidis*, Meningitis,and Meningococcemia**

Meningitis is an inflammation of the meninges, the membranesthat line the central nervous system, especially the spinal cordand brain. Meningitis can be caused by viral, bacterial, fungal, orprotist infections. Here we will deal with infectious bacterialmeningitis caused by Neisseria meningitidis and a related infection,**meningococcemia**.Neisseria meningitidis, often called *meningococcus*, is a gramnegative,nonsporulating, obligately aerobic, oxidase-positive,encapsulated diplococcus, about0.6–1.0 _m in diameter. At least 13 pathogenic strains of *N. meningitidis* are recognized. Antigenic differences in capsularpolysaccharides distinguish each strain.

Epidemiology and Pathology

Meningococcal meningitis often occurs in epidemics, usually inclosed populations such as military installations and college campuses. It typically strikes older school-age children and youngadults. Up to 30% of individuals carry N. meningitidis in thenasopharynx with no apparent harmful effects. In epidemic situations,the prevalence of carriers may rise to 80%. The trigger forconversion from the asymptomatic carrier state to pathogenicacute infection is unknown.In an

acute meningococcus infection, the bacterium is transmittedto the host, usually via the airborne route, and attaches to thecells of the nasopharynx. Once there, the organism gains access tothe bloodstream, causing bacteremia and upper respiratory tractsymptoms. The bacteremia sometimes leads to fulminantmeningococcemia, characterized by septicemia, intra vascular coagulation, shock, and death in over 10% of cases. *Meningitis* is another possible serious outcome of infection. Meningitis is characterizedby sudden onset of headache, vomiting, and stiff neck,and can progress to coma and death in a matter of hours. Up to 3%of acute meningococcal meningitis victims die.In the United States, there were 1057 cases of serious meningococcaldisease in 2008, the lowest number since 1977. The longterm decreased incidence indicates the success of widespreadvaccination in susceptible populations. However, the mortalityrate in recent years was over 10%.

Diagnosis, Prevention, and Treatment

Specimens isolated from nasopharyngeal swabs, blood, or cerebrospinalfluid are inoculated onto modified Thayer–Martin medium, a selective medium that suppressesthe growth of most normal flora, but allows the growth of thepathogenic members of the genus, *N. meningitidis* and Neisseriagonorrhoeae. Colonies showing gram-negative diplococcus morphologyand a positive oxidase test are presumptively identifiedas Neisseria. Due to the rapid onset of life threatening symptoms, preliminary diagnosis is often based onclinical symptoms and treatment is started before culture testsconfirm infection with *N. meningitidis*.Penicillin G is the drug of choice for the treatment of*N. meningitidis* infections. However, resistant strains have beenreported. Chloramphenicol is the accepted alternative agent fortreatment of infections in penicillin-sensitive individuals. Anumber of broad-spectrum cephalosporins are also effective.Naturally occurring strain-specific antibodies acquired by subclinicalinfections are effective for preventing infections in mostadults. Vaccines consisting of purified polysaccharides or polysaccharidesfrom the most prevalent pathogenic strains conjugatedto diphtheria toxin are available and are used to

immunizesusceptible individuals. The vaccines are used to prevent infectionin certain susceptible populations such as military recruitsand students living in dormitories. In addition, rifampin is oftenused as a chemoprophylactic antimicrobial drug to eradicate thecarrier state and prevent disease in close contacts of infectedindividuals.

Other Causes of Meningitis

A number of other organisms can also cause meningitis. Acutemeningitis is usually caused by one of the pyogenic bacteria suchas Staphylococcus, Streptococcus, or *Haemophilus influenzae*.*H. influenzae* primarily infects young children. An effective vaccinefor preventing *H. influenzae* meningitis is available and isrequired in the United States for school-age children. Several viruses also cause meningitis. Among these are herpessimplex virus, lymphocytic choriomeningitis virus, mumps virus,and a variety of enteroviruses. In general, viral meningitis is lesssevere than bacterial meningitis.

Viruses and Respiratory Infections

The most prevalent human infectious diseases are caused byviruses. Most viral diseases are acute, self-limiting infections, butsome can be problematic in healthy adults. We begin here by describing measles, mumps, rubella, and chicken pox, all common,endemic viral diseases transmitted in infectious droplets byan airborne route.

Measles

Measles (rubeola or 7-day measles) affects susceptible children asan acute, highly infectious, often epidemic disease. The measlesvirus is a paramyxovirus, a negative-strand RNA virus that enters the nose and throat by airborne transmission,quickly leading to systemic viremia. Symptoms startwith nasal discharge and redness of the eyes. As the disease progresses,fever and cough appear and rapidly intensify, followed bya characteristic rash (**Figure 33.14**); symptoms generally persistfor 7–10 days. Circulating antibodies to measles virus are measurableabout 5 days after initiation of infection; the serum antibodiesand T-cytotoxic lymphocytes combine to eliminate thevirus from the system. Possible postinfection complicationsinclude middle ear infection, pneumonia, and, in rare cases,measles encephalomyelitis. Encephalomyelitis has a mortalityrate of nearly 20% and can cause neurological disorders includinga form of epilepsy. Of the 131 measles cases that occurred in 2008, 15 of the infected individuals were hospitalized.

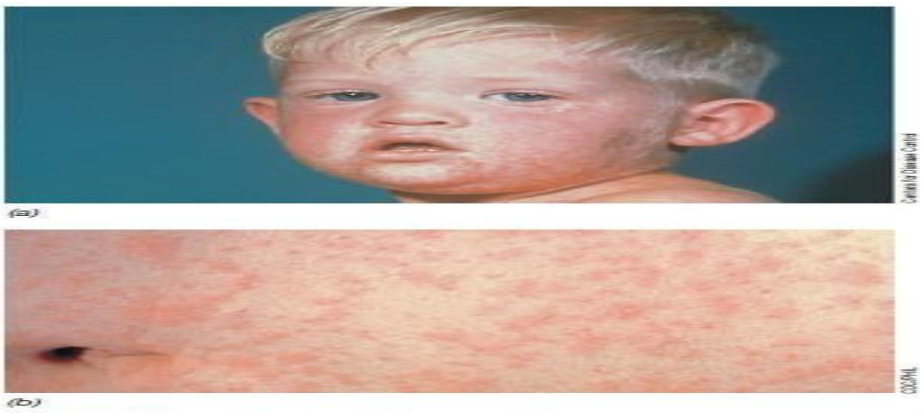


Figure 33.14 Measles in children. *(a)* The light pink rash starts on t head and neck, and *(b)* spreads to the chest, trunk, and limbs. Discrete papules coalesce into blotches as the rash progresses for several days

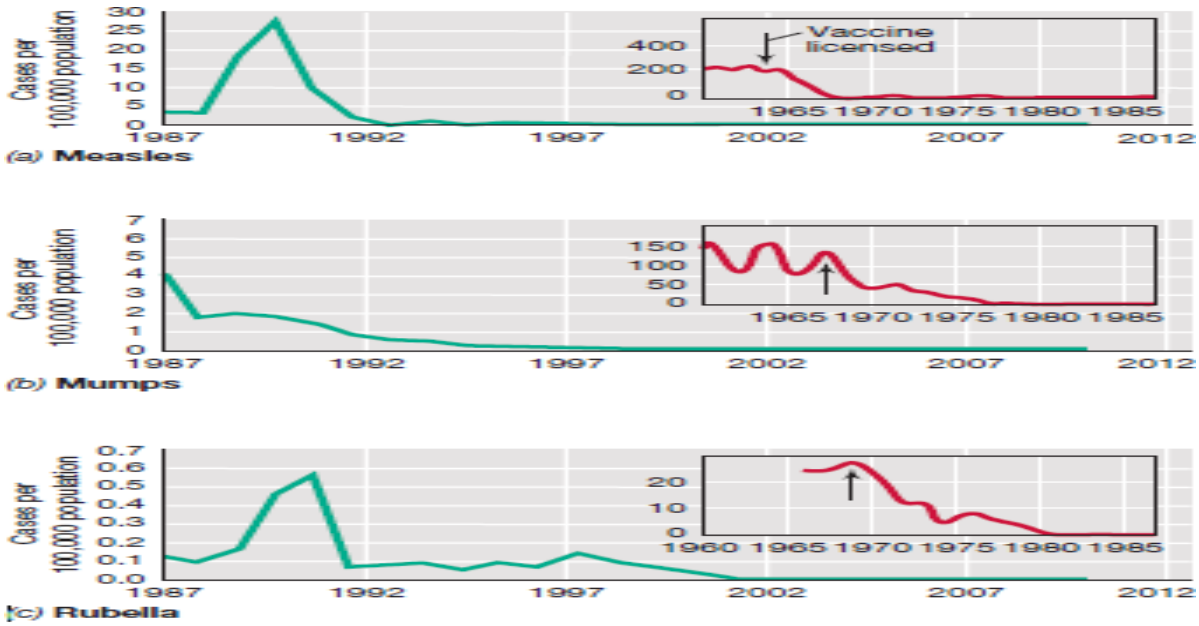


Figure 33.15 Viral diseases and vaccines. Major childhood viral diseases are now controlled by the MMR (measles, mumps, rubella) vaccine in the United States. Data are from the Centers for Disease Control and Prevention, Atlanta, Georgia.

Although once a common childhood illness, measles is generallylimited now to rather isolatedoutbreaks in the United Statesbecause of widespread immunization programs begun in the mid-1960s (**Figure 33.15*a***). Outbreaks generally occur only inpopulations that were not immunized or were inadequatelyimmunized. Over 90% of the cases were either acquired outsidethe United States or were associated with contact with travelersto foreign countries. Worldwide, measles remains endemic andstill causes over 400,000 annual deaths, mostly in children.Because the disease is highly infectious, all public school systemsin the United States require proof of immunization before a childcan enroll. Active immunization is done with an attenuated viruspreparation as part of the MMR (measles, mumps, rubella) vaccine. A childhood case of measles generallyconfers lifelong immunity to reinfection.

Mumps

Mumps, like measles, is caused by a paramyxovirus and is alsohighly infectious. Mumps is spread by airborne droplets, and thedisease is characterized by inflammation of the salivary glands,leading to swelling of the jaws and neck (**Figure 33.16**). The virusspreads through the bloodstream and may infect other organs, including the brain, testes, and pancreas. Severe complicationsmay include encephalitis and, very rarely, sterility. The hostimmune response produces antibodies to mumps virus surfaceproteins, and this generally leads to a quick recovery and lastingimmunity to reinfection. An attenuated vaccine is highly effectivefor preventing mumps. Hence, the prevalence of mumps in developedcountries is usually very low, with disease generally restrictedto individuals who did not receive the MMR vaccine (Figure33.15b). In 2006, however, an outbreak centered in the MidwesternUnited States involved more than 5000 cases, significantly up froma normal number of less than 300 cases per year since 2001. The outbreak affected mainly young adults (18–34). As a result, recommendationsfor immunizations were revised to target school-agechildren, healthcare workers, and adults at high risk.

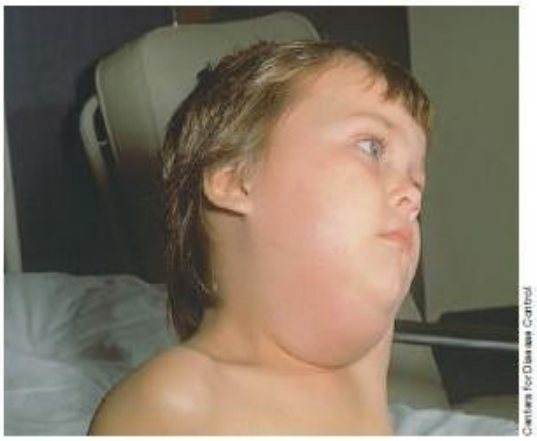


Figure 33.16 Mumps. Glandular swelling characterizes infection with the mumps virus.

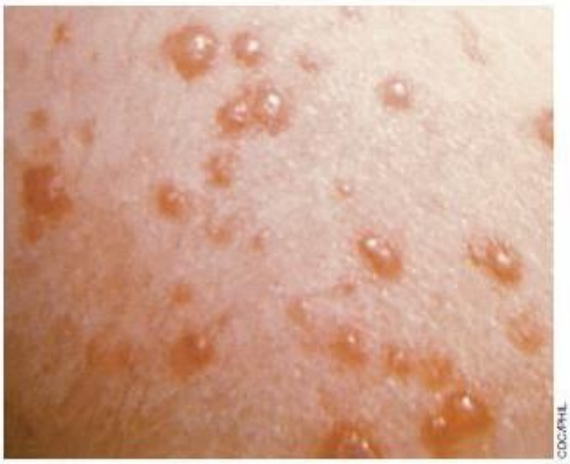


Figure 33.17 Chicken pox. Mild papular rash associated with the infection by varicella-zoster virus (VZV), the herpesvirus that causes chicken pox.

Rubella

Rubella (German measles or 3-day measles) is caused by a singlestranded,positive-sense RNA virus of the togavirus group. Disease symptoms resemble measles but are generallymilder. Rubella is less contagious than measles, and thus asignificant proportion of the population has never been infected.During the first three months of pregnancy, however, rubellavirus can infect the fetus by placental transmission and cause serious fetal abnormalities including stillbirth,

deafness, heart and eye defects, and brain damage. Thus, women should not be immunized with the rubella vaccine or contract rubella duringpregnancy. For this reason, routine childhood immunizationagainst rubella should be practiced. An attenuated virus isadministered as part of the MMR vaccine. The low incidence of cases since 2001, coupled with the high degree ofprotection from the vaccine and the relatively low infectivity of the virus, suggest that rubella is no longer endemic in the United States (Figure 33.15c).

Chicken Pox and Shingles

Chicken pox (varicella) is a common childhood disease caused by the varicella-zoster virus (VZV), a DNA herpesvirus. VZV is highly contagious and is transmitted by infectious droplets, especially when susceptible individuals are in close contact. In schoolchildren, for example, close confinement during the winter months leads to the spread of VZV through air bornedroplets from infected classmates and through contact with contaminated fomites. The virus enters the respiratory tract, multiplies,and is quickly disseminated via the bloodstream, resultingin a systemic papular rash that quickly heals, rarely leaving disfiguring marks (**Figure 33.17**). An attenuated virus vaccine is nowused in the United States. The reported annual incidence of chicken pox, now about 40,000 cases per year, is about one fourth of the number of cases reportedprior to 1995, the year thevaccine was licensed for use. Since 2003, VZV infections have been nationally notifiable, resulting in an increased number ofreported cases.VZV establishes a lifelong latent infection in nerve cells. The virus occasionally migrates from this reservoir to the skin surface,causing a painful skin eruption referred to as shingles(zoster). Shingles most commonly strikes immune suppressedindividuals or the elderly. The prophylactic use of human hyperimmuneglobulin prepared against the virus is useful for preventingthe onset of symptomsof shingles. Such therapy is advisedonly for patients for whom secondary infections such as pneumoniaor encephalitis, occasionally associated with shingles, maybe life-threatening. To prevent shingles, a vaccine is recommendedfor individuals over 60 years of age. The vaccine stimulatesantibody and T-cytotoxic cell immunity to VZV, keepingVZV from migrating out of nerve ganglia to skin cells.

Colds Colds are the most common of infectious diseases.People acquire about ten colds for every other infectious disease, except influenza. Colds are viral infectionsthat are transmitted via droplets spread from person to person incoughs, sneezes, and respiratory secretions. Colds are usually of short duration, lasting 1 week or less, and the symptoms aremilder than other

respiratory diseases such as influenza. **Table33.1** compares the symptoms of colds and influenza.Each personaverages more than three colds per year throughouthis or her lifetime (Figure 33.18). Coldsymptoms includerhinitis (inflammation of the nasal region, especially the mucousmembranes), nasal obstruction, watery nasal discharges, and ageneral feeling of malaise, usually without fever. Rhinoviruses,positive-sense, single-stranded RNA viruses of the picornavirus group (**Figure 33.19*a*** and Section 21.8), are the most commoncauses of colds. At least 115 differentrhinoviruses havebeen identified. About 25% of colds are due to infections with other viruses. Coronaviruses (Figure 33.19b) cause 15% of allcolds in adults. Adenoviruses, coxsackie viruses, respiratory syncytialviruses (RSV), and orthomyxoviruses are collectively responsible for about10% of colds. Each of these viruses may alsocause more serious disease. For example, one adenovirus strainproduces a severe and sometimes lethal respiratory infection.

Colds generally induce a specific, local, neutralizing IgA antibodyresponse. However, the number of potential infectious agents makes immunity due to previous exposure very unlikely.The sheer numbers of viruses that might cause a cold also precludethe development of useful vaccines.Aerosol transmission of the virus is probably the major meansof spreading colds, although experiments with volunteers suggestthat direct contact and fomite contact are also methods of transmission.Most antiviral drugs are ineffective against the commoncold, but a pyrazidine derivative (**Figure 33.20*a***) has shownpromise for preventing colds after virus exposure. In addition,new experimental antiviral drugs are being designed based oninformation derived from three-dimensional structures. For example, the antirhinovirus drug WIN 52084 (Figure 33.20b)binds to the virus, changing its three-dimensional surface configurationand disrupting rhinovirus binding to the host cell receptorICAM-1 (intercellular adhesion molecule-1), thus preventinginfection. Alpha interferon, a cytokine, is also effective in preventingthe onset of colds. Thus, there are several experimentalpossibilities for cold prevention and treatment, although noneare widely accepted as effective and safe. Because colds are generally

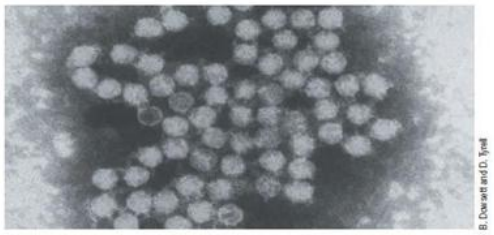
brief and self-limiting, treatment is aimed at controllingsymptoms, especially nasal discharges, with antihistamine andde congestant drugs.

Influenza

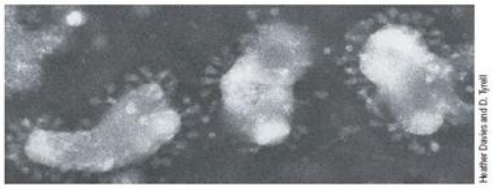
Influenza is caused by an RNA virus of the orthomyxovirus group. Influenza virus is a single-stranded, negativesense,helical RNA genome surrounded by an envelope made up ofprotein, a lipid bilayer, and external glycoproteins (**Figure 33.21**).There are three different types of influenza viruses: influenza A,influenza B, and influenza C. Here we consider only influenza A because it is the most important human pathogen.

Table 33.1 *Colds and influenza*

Symptoms	*Cold*	*Influenza*
Fever	Rare	Common (39–40°C); sudden onset
Headache	Rare	Common
General malaise	Slight	Common; often quite severe; can last several weeks
Nasal discharge	Common and abundant	Less common; usually not abundant
Sore throat	Common	Less common
Vomiting and/ or diarrhea	Rare	Common in children



(a)



(b)

Figure 33.19 **Common cold viruses.** Transmission electron micrographs. *(a)* Human rhinovirus. Each rhinovirus virion is about 30 nm in diameter. *(b)* Human coronavirus. Each coronavirus virion is about 60 nm in diameter.

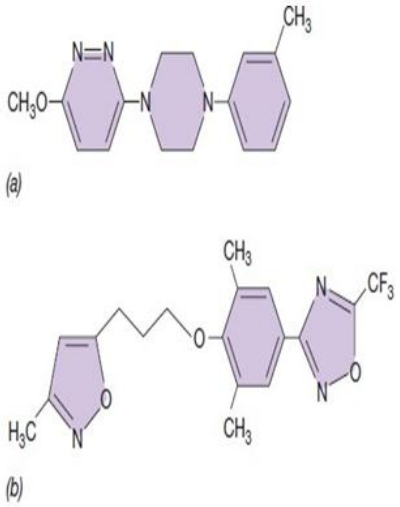


Figure 33.20 **Experimental antirhinovirus drugs.** *(a)* The structure of 3-methoxy-6-[4-(3-methylphenyl)]-1-piperazinyl. *(b)* The structure of WIN 52084, a receptor-blocking drug.

Influenza Antigens and Genes

Each strain of influenza A virus can be identified by a unique setof surface glycoproteins. These glycoproteins are hemagglutinin(HA or H antigen) and neuraminidase (NA or N antigen). Each virus will have one type of HA and one type of NA on its surface.HA is important in the attachment of virus to the host cells. NAis instrumental for release of virus from host cells

(Figure 33.21).Infection or immunization with an influenza strain results inproduction of IgA antibodies that are reactive with the HA andNA glycoproteins. When antibody binds to HA or NA, the virusis blocked from either attaching or releasing, and is neutralized,stopping the infection process. Over time, the HA and NA glycoproteinantigens acquire minor antigenic changes due to pointmutations in the RNA coding sequences. These changes alter oneor more amino acids in the glycoprotein, altering their ability tobe recognized by antibody. Thus, these mutations create slightlyaltered antigens, a phenomenon called **antigenic drift**. As aresult, immunity to a given virus strain diminishes as the strainmutates, and reinfection with the mutated strain can occur. The influenza A virus genome issingle-stranded RNA. The RNA genome is arranged in a highlyunusual manner; the genome is segmented, with single-stranded RNA genes found on each of eight distinct segments. During virus maturation in the host cell, the viral RNA segments are packaged randomly. To be infective,a virus must be packaged so it contains one copy of each of theeight gene segments.Occasionally more than one strain of influenza infects a singleanimal at one time. In such a case, the two strains could infect a single cell, and gene segments from both viruses would be reproduced.When packaging occurs, the segments from the twostrains may be mixed; an individual virus is likely to be a mosaic of the two infecting viruses, containing some, but not all, of thegenes from each virus. In effect, the mixed-genome virusinstantly becomes a new virus strain. This mixing of gene fragments between different strains of influenza virus is calledreassortment.Unique reassortant viruses result in **antigenic shift**, a majorchange in an antigen resulting from the total replacement of anRNA segment. Antigenic shift can immediately and completelychange one or both of the major HA and NA viral glycoproteinsand any of the other viral genes.

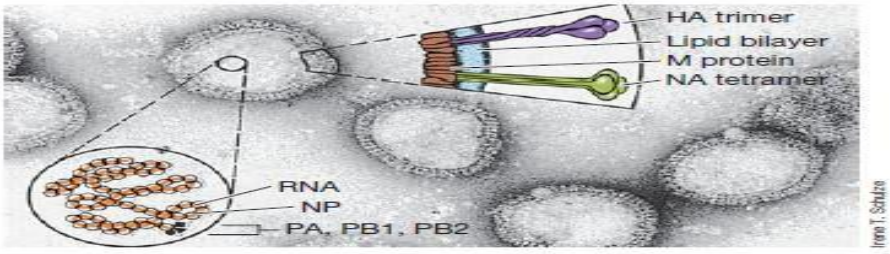


Figure 33.21 Electron micrograph of influenza virus. The photo shows the location of the major viral coat proteins and the nucleic acid. Each virion is about 100 nm in diameter. HA, hemagglutinin (three copies make up the HA coat spike); NA, neuraminidase (four copies make up the NA coat spike); M, coat protein; NP, nucleoprotein; PA, PB1, PB2, other internal proteins, some of which may have enzymatic functions.

Influenza Epidemiology

Human influenza virus is transmitted from person to personthrough the air, primarily in droplets expelled during coughingand sneezing. The virus infects the mucous membranes of the upper respiratory tract and occasionally invades the lungs. Symptomsinclude a low-grade fever lasting 3–7 days, chills, fatigue,headache, and general aching (Table 33.1). Recovery is usuallyspontaneous and rapid. Most of the serious consequences of influenza infection occur from bacterial secondary infections inpersons whose resistance has been lowered by the influenza infection. Especially in infants and elderly people, influenza isoften followed by bacterial pneumonia; death, if it occurs, is usuallydue to the bacterial infection. Annually, influenza causes 3–5million cases of severe illness and is implicated in 250,000–500,000 deaths worldwide.Most infected individuals develop protective immunity to theinfecting virus, making it impossible for a strain of the same antigenictype to cause widespread infection—an epidemic—untilthe virus encounters another susceptible population. Immunityis dependent on the production of secretory IgA antibodies andT-cytotoxic lymphocytes directed at HA and NA glycoproteins.Influenza exists in human populations as an endemic viral disease,and severe localized influenza outbreaks occur every year from late autumn through the winter. Each year, antigenic driftresults in some reduction of immunity in the population and isresponsible for the recurrence of epidemics, severe widespreadoutbreaks, which occur in a 2- to 3-year cycle.

Table 33.2 *Influenza pandemics*

Year	*Name*	*Strain*
1889	Russian	H2N2
1900	Old Hong Kong	H3N8
1918	Spanish	H1N1
1957	Asian	H2N2
1968	Hong Kong	H3N2
2009	Swine	H1N1

Influenza Pandemics

Pandemics, worldwide epidemics, are much less frequent thanoutbreaks and epidemics, occurring from 10 to 40 years apart(**Table 33.2**). They result from antigenic shift involving

reassortmentof viruses from two or more species. Virtually all of themajor pandemics resulted from reassortment of avian influenzaviruses and human influenza viruses in swine (**Figure 33.22**).Swine cells have receptors for both avian and human orthomyxovirusesand can bind and propagate both avian and humaninfluenza strains. If swine are infected with both human and avian strains at the same time, the two unrelated viruses can reassort,resulting in antigenically unique viruses (antigenic shift)that can infect many humans because of a lack of host immunity. Reassortment with animal strains and

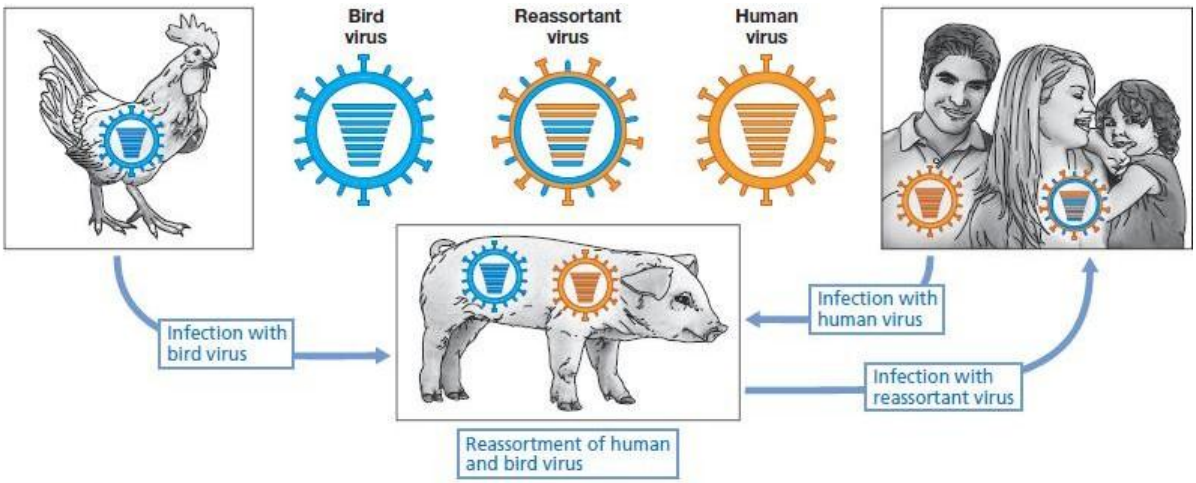


Figure 33.22 Influenza virus reassortment. Reassortments take place in swine. Influenza strains that originate in birds and humans can infect pigs. If a pig is infected at the same time with a bird virus and a human virus, the viruses can reassort. The reassortant virus may then infect humans. If the reassortant contains antigens that are unique, infections may cause pandemics.

infection into humans occurs periodically but unpredictably, continually raising thepossibility of a rapidly emerging, highly virulent influenza strainfor which there is no preexisting immunity in the human population.Worldwide deaths due to the influenza A "Spanish flu" pandemicof 1918 was about 50 million, with some estimates as highas 100 million people worldwide; up to 2 million deaths occurredin the United States (Figure 32.1). Although there have beenseveral pandemics during the last 130 years (Table 33.2), nonehas been as catastrophic as the 1918 flu. The virulence of the1918 influenza is not fully understood, but appears to be due tothe host response to the novel pathogen. This pathogen apparentlystimulated production and release of large amounts of inflammatory cytokines, resulting in systemic inflammation anddisease in susceptible individuals.

The 1957 outbreak of the so-called Asian flu also developed into a pandemic. The pandemic strain was a virulentmutant virus, differing antigenically from all previousstrains. Immunity to

this strain was not present, and the virusspread rapidly throughout the world. It first appeared in the interiorof China in February 1957 and by April had spread to HongKong. From Hong Kong, the virus infected sailors on naval shipsand emerged in San Diego, California. In May, an outbreakoccurred in Newport, Rhode Island, on a naval vessel. From thattime, outbreaks occurred continuously in various parts of theUnited States. The peak incidence occurred in October, when22 million new cases developed.Pandemic influenza A (H1N1) 2009 spread much more rapidlythan Asian flu, starting from an original focus of infection inMexico and spreading quickly to the United States, Europe, andCentral and South America. The pandemic influenza A (H1N1) 2009 virus is sometimes called "swine flu" because the reassertedvirus apparently developed in pigs (Figure 33.22). It is a reassortantvirus consisting of RNA segments derived from human andbird influenza, and reassorted in swine. From the swine reservoir,it emerged to infect humans. First recognized in March 2009, thevirus was declared a pandemic on June 11, 2009. By September2009, the virus had spread worldwide. During the flu season in the Southern Hemisphere (May–October 2009), pandemic influenzaA (H1N1) 2009 spread rapidly, causing widespread disease.Although pandemic influenza virus did not seem to be extraordinarilyvirulent, the pandemic was widespread even during thenon-influenza-season summer months of 2009 (June–August) inNorthern Hemisphere countries in Europe and North America,demonstrating that it is fully adapted to humans and can spreadvery easily. Even though the infection was prevalent in2009–2010, the overall mortality rate for this pandemic strain wasrelatively low, an estimated 0.1–0.2%, perhaps only slightly higherthan seasonal influenza mortality. A vaccine was made available inOctober 2009 to slow the advance of the pandemic.A potentially devastating avian influenza, the influenza AH5N1 strain, also called avian influenza, appeared in Hong Kongin 1997, apparently jumping directly from the avian host tohumans without the pig intermediate. H5N1 has now beenreported in birds throughout Asia, Europe, the Middle East, andNorth Africa; it has not yet spread to birds in the Americas, Australia,or Antarctica. The H5N1 virus has reemerged severaltimes over the last decade; the most recent outbreaks occurred inEgypt and Indonesia. Since 2003, 495 cases of human H5N1infections have been confirmed worldwide, resulting in 292deaths, an overall mortality rate of almost 60%. H5N1 is spread directly from avian species, usually domestic chickens or ducks, to humans through prolonged contact or the eating of infected birds. At this time, avian influenza can be spread human tohuman only after prolonged close contact, but some reports indicate that H5N1

has infected swine. This event could set the stage for reassortment with human influenza strains that also infects wine. Such a reassortment could create a new and highly infective virus for which there is no immunity in humans, startinganother influenza pandemic. Plans are in place both nationallyand internationally to provide appropriate vaccines and supportfor potential pandemics initiated by this and other emergentinfluenza strains. A recombinant vaccine for the H5N1 virus isavailable on a limited basis.

Influenza Prevention and Treatment

Influenza epidemics can be controlled by immunization. However,the selection of appropriate strains for vaccines is complicated by the large number of existing strains and the ability of existingstrains to undergo antigenic drift and antigenic shift. When newstrains evolve, vaccines are not immediately available, but throughcareful worldwide surveillance, samples of the major emergingstrains of influenza virus are usually obtained before there are epidemics.In the United States, immunization preparations are reformulatedannually to target current prevalent strains. The targetedstrains, chosen at the end of each influenza season, are grown inembryonated eggs and inactivated. The inactivated viral strains(two influenza A and one influenza B) are mixed to prepare a vaccineused for immunization prior to the next influenza season.

In general, influenza immunization is recommended for thoseindividuals most likely to acquire the disease and develop serioussecondary illnesses. Influenza immunization is currently recommendedfor everyone over 50 years of age, for those suffering from chronic debilitating diseases (for example, AIDS patients,chronic respiratory disease patients, and so on), and for healthcareworkers. Effective artificial immunity from the inactivatedinfluenza vaccine lasts only a few years and is strain-specific. Anattenuated live-virus vaccine is recommended for young adults,and may confer longer-lasting immunity. Influenza A may also be controlled by use of antiviral drugs.The adamantanes, amantadine and rimantadine, are syntheticamines that inhibit viral replication. The neuraminidaseinhibitors oseltamivir (Tamiflu) and zanamivir (Relenza) block release of newly replicated virions of influenzaA and B and H5N1 avian virus. Thesedrugs are used to treat ongoing influenza and shorten the course and severity of infection.

They are most effective when given very early in the courseof the infection. The adamantanes and oseltamivir also preventthe onset and spread of influenza.Drug resistance has alreadyoccurred in some of the most dangerousinfluenza strains. Neither pandemic influenza A (H1N1)2009 nor the H5N1 avian influenza is susceptible to the adamantanes.Although most influenzaviruses are

susceptible to theneuraminidase inhibitors, a few isolates of pandemic influenza A (H1N1) 2009 are resistant to oseltamivir.Treatment of influenza symptoms with aspirin,especially inchildren, is not recommended. Aspirin treatment of influenza hasbeen linked todevelopment of Reye's syndrome, a rare but occasionally fatal complication involving the centralnervous system.

Water borne Microbial Disease

Common-source infectious diseases are caused by microbialcontamination of materials shared bya large number of individuals.The most important common source of infectious disease is contaminated water; the failure of a single step in thedrinking water purification process may result in the exposure ofthousands or even millions of individuals to an infectious agent.Common-source waterborne diseases are significant sources of morbidity and mortality, especially in developing countries. Evenin developed countries, breakdowns in water treatment plantsor the lack of access to clean water in times of emergency cancontribute to the development of a waterborne disease outbreak.Bacteria, viruses, and protists cause waterborne infectiousdiseases. Waterborne diseases begin as infections. Contaminated water may cause infection even if only a small number of microorganismsare present. Whether or not exposure toa pathogencauses disease is a function of the virulence of the pathogen andthe general ability of the host to resist infection.

Sources of Waterborne Infection

Human pathogens can be transmitted through untreated orimproperly treated water used for drinking and cooking. Anothercommon source of disease transmission is through pathogencontaminatedwater used for swimming and bathing.

Potable Water Because everyone consumes water through drinking and cooking,water is a common source of pathogen dissemination andhas a very high potential for the catastrophic spread of epidemic disease. As we have already discussed, water supplies in developedcountries usually meet rigid quality standards, limiting thespread of waterborne diseases. Waterborne disease outbreaks, however, occasionally occur in developing countries due tolapses in water quality. Isolated outbreaks affecting low numbersof individuals also occur from consumption of contaminated water from nonregulated sources (such as private wells) or fromconsumption of untreated water from streams or lakes. Thesesources may be contaminated by fecal material from humans or animals.Microorganisms transmitted in drinking water generally growin the intestines and leave the body in feces, which may in turnpollute water. If a new host consumes the water, the pathogenmay colonize the host's intestine and cause disease. From 1974 to2006 in the UnitedStates,

729 drinking water–associated diseaseoutbreaks occurred—an average of about 23 outbreaks per year(**Figure 35.11*a***). Bacterial, viral, and protist pathogens are occasionally transmitted in drinking water (**Table 35.1**).

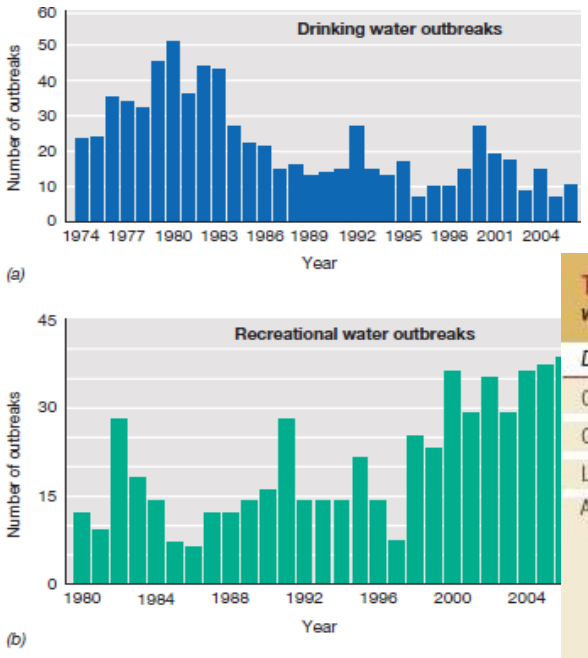


Figure 35.11 Waterborne disease outbreaks. Data were provided b the Centers for Disease Control and Prevention, Atlanta, Georgia, USA. *(a)* Reported drinking water disease outbreaks from 1974 to 2006. Of 72 outbreaks, about 90% were due to biological agents (bacteria, viruses, and protists). *(b)* Reported recreational water outbreaks from 1980 to 2006. Of 544 total outbreaks, almost all were due to biological agents.

Table 35.1 *Reported infectious disease outbreaks associated with drinking water in the United States, 2005–2006*[a]

Disease	*Agent*	*Outbreaks*	*Cases*
Cryptosporidiosis	*Cryptosporidium*	1	10
Giardiasis	*Giardia*	1	41
Legionellosis	*Legionella*	10	43
Acute gastrointestinal illness	*Escherichia coli* and *Campylobacter jejuni*	1	60
	Campylobacter jejuni	1	32
	Norovirus and *Campylobacter jejuni*	1	139
	Norovirus	2	196
	Hepatitis A	1	16
	Unknown[b]	2	75

Recreational Water Recreational waters include freshwater recreational areas such asponds, streams, and lakes, as well as public swimming and wadingpools. Recreational waters can also be sources of waterborne disease,and historically cause disease outbreaks at levels roughlycomparable to those caused by drinking water (Figure 35.11b).The operation of public swimming and wading pools is regulatedby state and local health departments. The United StatesEPA establishes limits for bacteria in recreational freshwaters(monthly geometric mean for all samples of,33/100 ml for enterococcior ,126/100 ml for E. coli) and marine waters (,35/100 mlfor enterococci). Local and state governments have the authorityto set standards above or below these guidelines, and many statesuse a single-sample maximum as well as the geometric

mean forsetting standards and defining levels of contamination thatconstitute violations. For example, the state of Indiana standardis 125 E. coli cells per 100 ml as a geometric mean, with a singlesamplemaximum of 235/100 ml. Thus, waters that exceed 235E. coli, even if their geometric mean count was not greater than125, would be in violation of Indiana's water standards. Private swimming pools, spas, and hot tubs are unregulated and areoccasional sources of outbreaks of waterborne diseases.Over a 27-year period (1980–2006), 544 waterborne diseaseoutbreaks were from recreational waters in the United States, orabout 20 outbreaks per year (Figure 35.11b). **Table 35.2** categorizesrecreational water outbreaks according to the infectiousdiseases occurring in recent years.

Waterborne Infections in Developing Countries

Worldwide, waterborne infections are a much larger problem thanin the United States and other developed countries. Developingcountries often have inadequate water and sewage treatmentfacilities, and access to safe, potable water is limited. As a result, diseases such as cholera, typhoid fever, and amebiasisare important public health problems in the developingworld.

Cholera

Cholera is a severe diarrheal disease that is now largely restricted to the developing world. Cholera is an example of a major water borne disease that can be controlled by application of appropriate public health measures for water treatment.

Table 35.2 *Reported infectious disease outbreaks associated with recreational water in the United States, 2005–2006*[a]

Agent[b]	*Outbreaks*	*Cases*
Bacteria		
Campylobacter jejuni	1	6
Escherichia coli	3	10
Legionella	8	124
Leptospira	2	46
Pseudomonas aeruginosa	9	101
Shigella sonnei	4	41
Parasites		
Cryptosporidium	31	3751
Giardia intestinalis	1	11
Cryptosporidium and *Giardia*	1	55
Naegleria fowleri	1	2
Schistosoma	2	4
Virus		
Norovirus	5	99

[a]Data provided by the Centers for Disease Control and Prevention, Atlanta, Georgia. In all, 68 outbreaks occurred over 2 years.
[b]*Campylobacter jejuni*, *Escherichia coli*, *Shigella sonnei*, *Cryptosporidium*, *Giardia*, and norovirus outbreaks cause gastroenteritis. *Legionella* causes acute respiratory disease. *Leptospira* causes systemic infections and aseptic meningitis. *Pseudomonas aeruginosa* causes dermatitis. The amoeba *Naegleria fowleri* causes meningoencephalitis; all cases were fatal. *Schistosoma*, a helminth parasite, causes schistosomiasis, a disease characterized chiefly by parasitic infestations of venous vessels in the intestines and liver.

Biology and Epidemiology : Cholera is typically caused by ingestion of contaminated watercontaining Vibrio cholerae, a gram-negative, curved rod–shaped Proteobacterium (**Figure 35.12)**;As with many waterborne diseases, cholera can also be associated with food consumption.

For example, in the Americas, consumption of rawshellfish and raw vegetables has been associated with cholera.

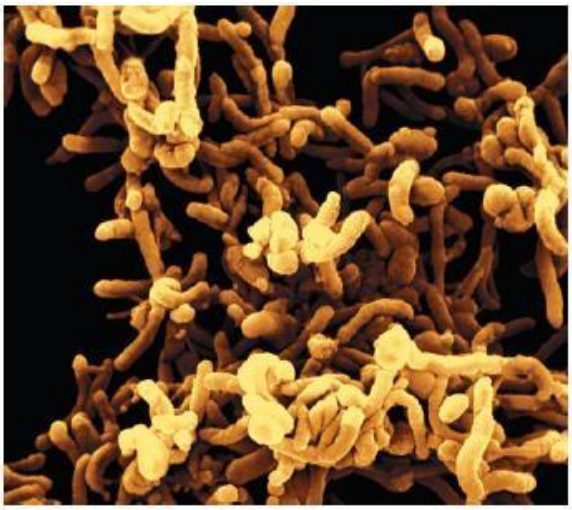


Figure 35.12 Cells of *Vibrio cholerae*. This colorized scanning electron micrograph shows a rod to curved rod morphology. The organism is about 0.3 μm in diameter and up to 2 μm in length.

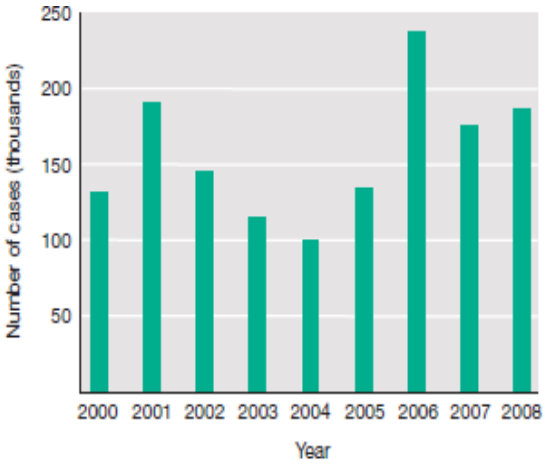


Figure 35.13 Cholera cases. The reported cholera cases from 2000 to 2008 show a generally increasing trend. Up to 90–95% of cholera cases are unreported. Over 95% of all reported cases occur in Africa. Data were provided by the World Health Organization.

Presumably, vegetables washed in contaminated water and shellfishbeds contaminated by untreated sewage transmitted thedisease.Since 1817, cholera has swept the world in seven majorpandemics. Two distinct pandemic strains of V. cholerae are recognized,known as the classic and the El Tor biotypes. TheV. cholerae O1 El Tor biotype started the seventh pandemic inIndonesia in 1961, and its spread continues to the present. Thispandemic has caused over 5 million cases of cholera and morethan 250,000 deaths and continues to be a major cause of morbidityand mortality, especially in developing countries; as istypical for infectious diseases, the highest prevalence of cholerais in developing countries, especially in Africa. In 1992, a geneticvariant known as V. cholerae O139 Bengal arose in Bangladeshand caused an extensive epidemic. V. cholerae O139 Bengal hascontinued to spread since 1992, causing several major epidemics,and may be the agent of an eighth pandemic.Cholera is endemic in Africa, Southeast

Asia, the Indian subcontinent,and Central and South America. Epidemic choleraoccurs frequently in areas where sewage treatment is either inadequateor absent. Worldwide, there were 190,130 reported casesand 5143 deaths reported in 2008, with over 98% of all reportedcases occurring in Africa. About 100,000 cases or more havebeen reported annually since 2000, with a low of 95,560 cases in2004, and a high of 236,896 cases in 2006 (**Figure 35.13**). TheWorld Health Organization estimates that only 5–10% of choleracases are reported, so the total incidence of cholera exceeds1 million cases per year. Even in developed countries, the diseaseis a threat. A handful of cases are reported each year in theUnited States, rarely caused by drinking water. Many recent casesare imported, often in food. A few cases are possibly from endemic sources; raw shellfish seems to be the most commonvehicle, presumably because V. cholera may be free-living incoastal waters in endemic areas, where the pathogen adheres to the marine microflora ingested by the shellfish (**Figure 35.14**).

Pathogenesis

The ingestion of 10^8–10^9 cholera vibrios is generally required to cause disease. The ingested V. cholerae cells attach to epithelial cells in the small intestine where they grow and release cholera toxin, a potent enterotoxin. Studies in human volunteers have shown that stomach acidity is responsible for the large inoculum needed to initiate cholera; human volunteers given bicarbonate to neutralize gastric acidity developed cholerawhen given as few as 10^4 cells. Even lower cell numbers can initiateinfection if V. cholerae is ingested with food, presumably becausethe food protects the vibrios from destruction by stomach acidity.Cholera enterotoxin causes severe diarrhea that can resultin dehydration and death unless the patient is given fluid andelectrolyte therapy. The enterotoxin causes fluid losses of up to20 liters (20 kg or 44 lb) per day. The mortality rate from\ untreated cholera is typically 25–50% and can be much higher under conditions of severe crowding and malnutrition.

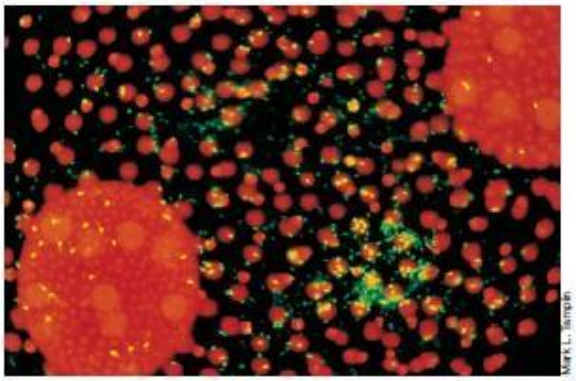


Figure 35.14 Cells of *Vibrio cholerae* attached to the surface of *Volvox*, a freshwater alga. The sample was from a cholera-endemic area in Bangladesh. The *V. cholerae* cells are stained green by a monoclonal antibody to bacterial cell surface proteins. The red color is due to the fluorescence of chlorophyll a in the algae.

Cholera is diagnosed by the presence of the gram-negative,comma-shaped V. cholerae bacilli in the "rice water" stools (nearlyliquid feces) of patients with severe diarrhea.Immunization is not normally recommended for cholera prevention,but a whole-cell oral vaccine directed against the El Torbiotype is currently available for use in high-risk situations, suchas after natural disasters that compromise water treatment andpurification systems. The vaccine, as well as natural infection,provides effective but short-lived immunity. No vaccine protectsagainst the new V. cholerae O139 Bengal serotype. Public healthmeasures such as adequate sewage treatment and a reliablesource of safe drinking water are the most important measuresfor preventing cholera. V. cholerae is eliminated from wastewaterduring proper sewage treatment and drinking water purificationprocedures. For individuals traveling in cholera-endemic areas,attention to personal hygiene and avoidance of untreated wateror ice, raw food, and raw or undercooked fish or shellfish offerprotection against contracting cholera.

Treatment of Cholera : Cholera treatment is simple, effective, and inexpensive. Intravenousor oral liquid and electrolyte replacement therapy [20 g of glucose,4.2 g of sodium chloride (NaCl), 4.0 g of sodium bicarbonate(NaHCO3), and 1.8 g of potassium chloride (KCl) dissolved in1 liter of water] is the most effective means of cholera treatment.Oral treatment is preferred because no special equipment orsterile precautions are necessary. Effective fluid and electrolytereplacement reduces mortality to about 1%. Streptomycin ortetracycline may shorten the course of infection and the sheddingof viable cells, but antibiotics are of little benefit without simultaneous fluid and electrolyte replacement.

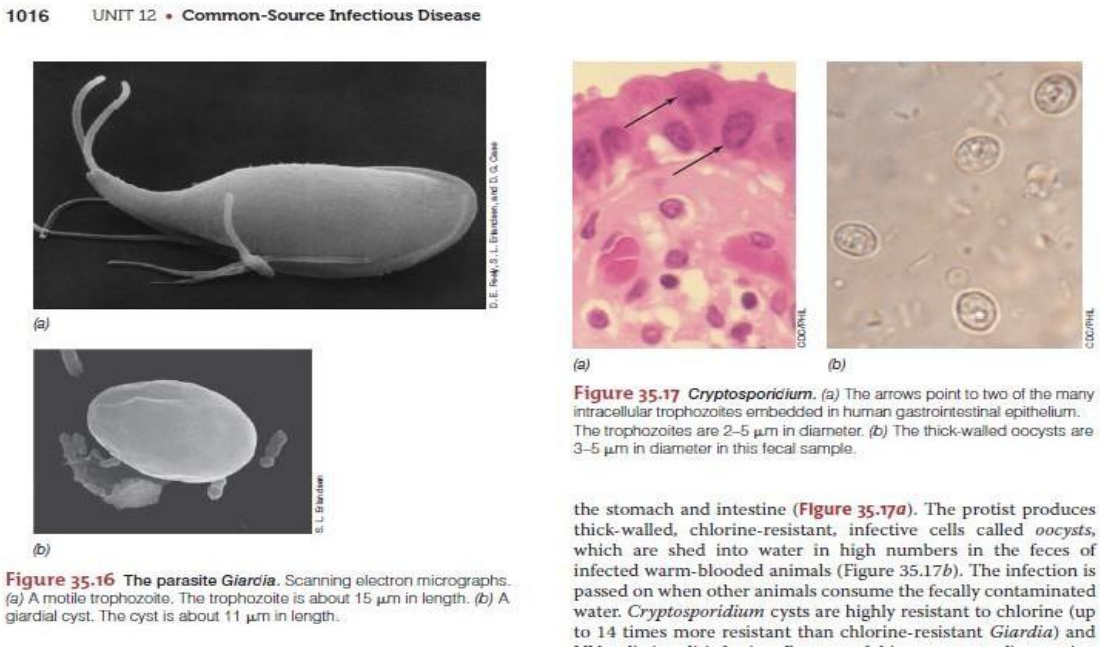
Giardiasis and Cryptosporidiosis

Giardiasis and cryptosporidiosis are diseases caused by the protists Giardia intestinalis and Cryptosporidium parvum, respectively.These organisms continue to be problematic even in well-regulatedwater supplies because they are found in nearly all surface watersand are resistant to chlorine disinfection.

Giardiasis

Giardia intestinalis, also called Giardia lamblia, is a flagellatedprotist that is usually transmitted to humans infecally contaminated water, although foodborne and sexual transmission of giardiasis have also been documented. Giardiasisis an acute gastroenteritis caused by this organism. The protest cells, called trophozoites (**Figure 35.16*a***), produce a resting stagecalled a **cyst** (Figure 35.16b). The cyst has a thick protective wallthat allows the pathogen to resist drying and chemical disinfection.After a person ingests the cysts in contaminated water, the cystsgerminate, attach to the intestinal wall, and cause the symptomsof giardiasis: an explosive, foul-smelling, watery diarrhea, intestinalcramps, flatulence, nausea, weight loss, and malaise. Symptomsmay be acute or chronic. The foul-smelling diarrhea and theabsence of blood or mucus in the stool distinguish giardiasisfrom bacterial or viral diarrheas. Many infected individualsexhibit no symptoms but act as carriers; *G. intestinalis* can establishitself in a stable, symptom-free relationship with its host.*G. intestinalis* was the infectious agent in 1 of the 20 recentdrinking water infectious disease outbreaks in the United States. The thick-walled cysts are resistant to chlorine, and most outbreaks have been associated with water systems thatused only chlorination as a means of water purification. Watersubjected to proper clarification and filtration followed by chlorinationor other disinfection is generally free ofGiardia cysts.Giardiasis can also be contracted from ingestion of water frominfected swimming pools or lakes (Table 35.2). Giardia cysthave been found in 97% of surface water sources (lakes, ponds,and streams) in the United States. Isolated cases of giardiasishave been associated with untreated drinking water in wildernessareas. Beavers and muskrats are frequent carriers of *Giardia* andmay transmit cells or cysts to water supplies, making the water apossible source of human infection. As a safety precaution, waterconsumed from rivers and streams, for example, during a campingor hiking trip, should be filtered and treated with iodine orchlorine, or filtered and boiled. Boiling is the preferred methodto ensure that water is free of pathogens.Laboratory diagnostic methods include the demonstration ofGiardia cysts in the stool or the demonstration

of Giardia antigensin the stool using a direct EIA (enzyme immunosorbentassay). The drugs quinacrine, furazolidone, and metronidazoleare useful for treating acute giardiasis.



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(a)

(b)

Figure 35.16 **The parasite *Giardia*.** Scanning electron micrographs. *(a)* A motile trophozoite. The trophozoite is about 15 μm in length. *(b)* A giardial cyst. The cyst is about 11 μm in length.

(a) (b)

Figure 35.17 ***Cryptosporidium.*** *(a)* The arrows point to two of the many intracellular trophozoites embedded in human gastrointestinal epithelium. The trophozoites are 2–5 μm in diameter. *(b)* The thick-walled oocysts are 3–5 μm in diameter in this fecal sample.

the stomach and intestine (**Figure 35.17*a***). The protist produces thick-walled, chlorine-resistant, infective cells called *oocysts*, which are shed into water in high numbers in the feces of infected warm-blooded animals (Figure 35.17*b*). The infection is passed on when other animals consume the fecally contaminated water. *Cryptosporidium* cysts are highly resistant to chlorine (up to 14 times more resistant than chlorine-resistant *Giardia*) and

Cryptosporidiosis

The protist *Cryptosporidium parvum* lives as a parasite in warmblooded animals. The protists aresmall, round cellsthat invade and grow intracellularly in mucosal epithelial cells ofthe stomach and intestine (**Figure 35.17*a***). The protist producesthick-walled, chlorine-resistant, infective cells called oocysts,which are shed into water in high numbers in the feces ofinfected warm- blooded animals (Figure 35.17b). The infection ispassed on when other animals consume the fecally contaminatedwater. Cryptosporidium cysts are highly resistant to chlorine (upto 14 times more resistant than chlorine-resistant Giardia) andUV radiation disinfection. Because of this property, sedimentationand filtration methods must be used to remove *Cryptosporidium*from water supplies. From 2005 through 2006, Cryptosporidiumwas responsible for 31 of the 68 recreational waterborne diseaseoutbreaks (Table 35.2).*C. parvum* was responsible for the largest single commonsourceoutbreak of a waterborne disease ever recorded in theUnited States. In the spring of 1993 in Milwaukee, Wisconsin,USA, over 403,000 people in the population of 1.6 million developeda diarrheal illness that was traced to the municipal watersupply. Spring rains and runoff from surrounding farmland haddrained into Lake Michigan and overburdened the water purificationsystem,

leading to contamination by *C. parvum*. Theprotist is a significant intestinal parasite in dairy cattle, the likelysource of the outbreak.Cryptosporidiosis is usually a self-limiting mild diarrhea thatsubsides in 2 weeks or less in normal individuals. However,individuals with impaired immunity, such as that caused by HIV/AIDS, or the very young or old can develop serious complications.In the Milwaukee outbreak, about 4400 people requiredhospital care, and 50–100 died of complications from the disease,including severe dehydration.The Milwaukee outbreak highlights the vulnerability of water purification systems, the need for constant water monitoring andsurveillance, and the consequences of the failure of a large watersupply system. In addition to the toll of human morbidity andmortality, the epidemic cost an estimated $96 million in medicalcosts and lost productivity.

Laboratory diagnostic methods for cryptosporidiosis includethe demonstration ofCryptosporidium oocysts in the stool.Treatment is unnecessary for those with uncompromised immunity.For individuals undergoing immunosuppressive therapy(for example, prednisone), discontinuation of immunosuppressivedrugs is recommended. Immunocompromised individuals should be given supportive therapy such as intravenous fluids andelectrolytes.

Legionellosis (Legionnaires' Disease)

Legionella pneumophila, the bacterium that causes legionellosis,is an important waterbornepathogen normally transmitted inaerosols rather than through drinking or recreational waters.

Biology and Epidemiology

Legionella pneumophila was first discovered as the pathogen thatcaused an outbreak of pneumonia during an American Legionconvention in Philadelphia, Pennsylvania, USA, in the summer of1976. *L. pneumophila* is a thin, gram-negative, obligately aerobicrod (**Figure 35.18**) with complex nutritional requirements,including an unusually high iron requirement. The organism canbe isolated from terrestrial and aquatic habitats as well as fromlegionellosis patients.*L. pneumophila* is present in small numbers in lakes, streams,and soil. It is relatively resistant to heating and chlorination, so itcan spread through water distribution systems. It is commonlyfound in large numbers in cooling towers and evaporative condensersof large air conditioning systems. The pathogen grows inthe water and is disseminated in humidified aerosols. Humaninfection is by way of airborne droplets, but the infection is notspread from person to person. Further

evidence for this is thefact that annual outbreaks of legionellosis tendto peak in mid-tolate summer months when air conditioners are extensively used.*L. pneumophila* has also been found in hot water tanks andwhirlpool spas, where it can grow to high numbers in warm(35–45°C), stagnant water. Epidemiological studies indicate that *L. pneumophila* infections occur at all times of the year, primarilyas a result of aerosols generated by heating/cooling systems andcommon practices such as showering or bathing. Overall, theincidence of reported cases of legionellosis had been about 4–6cases per million in the United States, but in the last several yearsthe incidence has risen to nearly 8 cases per million. In 2007,there were 2716 reported cases (**Figure 35.19**). The increase inreported cases may be a result of an actual increase in infectionsor an increase in recognition and reporting; formerly, up to90% of actual cases were probably not diagnosed or properlyreported. Prevention of legionellosis can be accomplished byimproving the maintenance and design of water-dependent coolingand heating systems and water delivery systems. The pathogencan be eliminated from water supplies by hyperchlorination or byheating water to greater than 63°C.

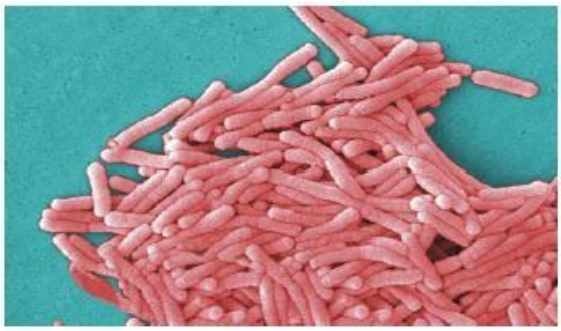


Figure 35.18 ***Legionella pneumophila***. Colorized scanning electron micrograph of *L. pneumophila* cells. Cells are 0.3–0.6 μm in diameter and up to 2 μm in length.

Figure 35.19 **Incidence of Legionnaire's disease in the United States.** In 2007, there were 2716 reported cases. Data are from the Centers for Disease Control and Prevention, Atlanta, Georgia.

Pathogenesis

In the body, *L. pneumophila* invades and grows in alveolar macrophages and monocytes as an intracellular parasite. Infectionsare often asymptomatic or produce a mild cough, sorethroat, mild headache, and fever. These mild, self-limiting cases,called Pontiac fever, are generally not treated and resolve in 2–5days. Elderly individuals whose resistance has been previouslycompromised, however, often acquire more serious infectionsresulting in pneumonia. Certain serotypes of *L. pneumophila*(more than 10 are known) are strongly associated with the pneumonicform of the infection. Prior to the onset of pneumonia,intestinal disorders are common, followed by high fever, chills,and

muscle aches. These symptoms precede the dry cough and chest and abdominal pains typical of legionellosis. Death,usually due to respiratory failure, occurs in up to 10% of pneumoniacases.

Diagnosis and Treatment

Clinical detection of *L. pneumophila* is usually done by culturefrom bronchial washings, pleuralfluid, or other body fluids.Serological (antibody) tests are used as retrospective evidence for Legionella infection. As an aid in diagnosis, *L. pneumophila*antigens can sometimes be detected in patient urine. *Legionella pneumoph*ila can be treated with the antibiotics rifampin anderythromycin. Intravenous administration of erythromycin is thetreatment of choice.

Typhoid Fever and Other Waterborne Diseases

Various bacteria, viruses, and protists can transmit common source water borne diseases. These diseases are a significant source of morbidity, especially in developing countries.

Typhoid Fever

On a global scale, probably the most important pathogenicbacteria transmitted by the water route are **Salmonella** enteric serovar Typhi, the organism causing typhoid fever, and *Vibrio cholerae*, the organism causing cholera, which we discussedpreviously.Although *S. enterica ser. typhi* may also be transmitted bycontaminated food and by direct contact frominfected individuals, the most common and serious means oftransmission worldwide is through water. Typhoid fever has beenvirtually eliminated in developed countries, primarily due to effective water treatment procedures. In the United States, thereare fewer than 400 cases in most years, but, typhoid fever was a major public health threatbefore drinking water was routinely filtered and chlorinated. However, breakdown of water treatment methods,contamination of water during floods, earthquakes, and otherdisasters, or cross-contamination of water supply pipes fromleaking sewer lines can propagate epidemics of typhoid fever,even in developed countries.

Viruses

Viruses can also be transmitted in water and cause human disease.Quite commonly, enteroviruses such as poliovirus, norovirus,and hepatitis A virus are shed into the water in fecal material.The most serious of these is poliovirus, but wild poliovirus hasbeen eliminated from the Western Hemisphere and is endemiconly in Nigeria, Afghanistan, Pakistan, and India. Althoughviruses can survive in water for relatively long periods, they areinactivated by disinfection

with agents such as chlorine.

Amebiasis

Certain amoebae inhabit the tissues of humans and other vertebrates,usually in the oral cavity or intestinal tract, and someof these are pathogenic. We discussed the general properties of amoeboid protists. Worldwide, *Entamoeba histolytica* is a common pathogenicprotist transmitted to humans, primarily by contaminated waterand occasionally through contaminated food. *E. histolytica* isan anaerobic amoeba; the trophozoites lack mitochondria (**Figure 35.20**). Like *Giardi*a, the trophozoites of *E. histolytica*produce cysts. Cysts ingested by humans germinate in the intestine,where amoebic cells grow both on and in intestinal mucosalcells. Many infections are asymptomatic, but continued growthmay lead to invasion and ulceration of the intestinal mucosa,causing diarrhea and severe intestinal cramps. With furthergrowth the amoebae can invade the intestinal wall, a conditioncalled dysentery, characterized by intestinal inflammation, fever,and the passage of intestinal exudates, including blood and mucus.If not treated, invasive trophozoites of *E. histolytica* can invadethe liver and occasionally the lung and brain. Growth in thesetissues can cause severe abscesses and death. Worldwide, up to100,000 individuals die each year from invasive amebic dysentery.The disease is extremely common in tropical and subtropicalcountries worldwide, with at least 50 million people developingsymptomatic diarrhea annually and up to 10-fold more havingasymptomatic disease. In the United States, several hundredcases occur each year, mostly near international borders in the Southwest. *E. histolytica* amebiasis can be treated with the drugs dehydroemetinefor invasive disease and diloxanide furoate for certain asymptomatic cases, as in immunocompromised individuals, butamoebicidal drugs are not universally effective. Spontaneouscures do occur,suggesting that the host immune system plays arole in ending the infection. However, protective immunity is not an outcome of primary infection, and reinfection is common. The disease is kept at very low incidence in regions that practice adequate sewage treatment. Amoebic infestation due to exposure to improperly treated sewage and the use of untreated surface waters for drinking purposes are the usual causes of amebiasis.

Demonstration of *E. histolytica* cysts in the stool, trophozoites in tissue, or the positive results forantibodies to *E. histolytica* in the blood from an EIA (enzyme immunoassay) are used for the laboratory diagnosis of amebiasis.

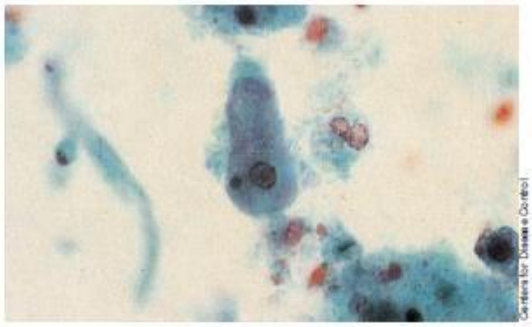


Figure 35.20 The trophozoite of *Entamoeba histolytica*, the amoeba that causes amebiasis. Note the discrete, darkly stained nucleus. The small red structures are red blood cells. The trophozoites range from 12 to 60 μm in length.

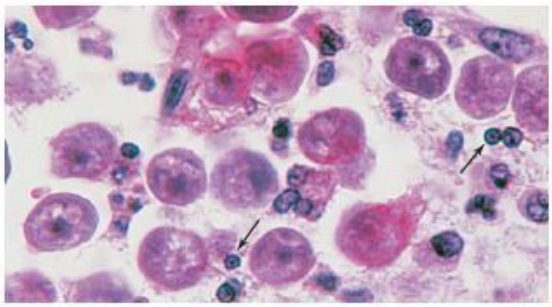


Figure 35.21 Trophozoites of *Naegleria fowleri* in brain tissue. This amoeba causes meningoencephalitis. Oval to round and amoeboid (irregularly shaped) trophozoites (arrows) are present as dark-stained structures with densely stained nuclei. There is extensive destruction of the surrounding brain tissue. Individual trophozoites are 10–35 μm long.

Naegleria fowleri can also cause amebiasis, but in a very different form. *N. fowleri* is a free-living amoeba found in soil and in water runoff. N. fowleri infections usually result from swimming or bathing in warm, soil-contaminated water sources such as hotsprings or lakes and streams in the summer. This free-livingamoeba enters the body through the nose and burrows directlyinto the brain. Here, the organism propagates, causing extensivehemorrhage and brain damage (**Figure 35.21**). This condition is called **meningoencephalitis**. Death usually results within aweek. From 1999 to 2003, there were 12 outbreaks, each a singleindividual who was infected by swimming or wading in a lake, pond, or stream in summer. All cases resulted in death. Prevention can be accomplished by avoiding swimming inshallow, warm freshwater, such as farm ponds and shallow lakes and rivers in summer. Swimmers are advised to avoid stirring upbottom sediments, the natural habitat of the pathogen. Diagnosis of *N. fowleri* infection requires observation of the amoebae in the cerebrospinal fluid. If a definitive diagnosis can be done quickly, the drug amphotericin B is used to treat infections.

Foodborne Disease

If food is not decontaminated or preserved, pathogens may growin it and cause foodborne diseases with significant morbidityand mortality. Like waterborne diseases, foodborne illnesses arecommon-source diseases. A single contaminated food source from a food-processing plant ora restaurant may affect a large numberof people. In 2010, chicken feed contaminated with Salmonellaused at two egg production farms in Iowa infected eggs distributed nationally, and caused over 1500 infections. Each year in theUnited States, there are an estimated 25,000foodborne disease outbreaks. As many as 76 million Americans are affected, an estimated 13 million acquire significant illnesses, 325,000 are hospitalized, and 5000 people die from foodborne diseases each year. Most outbreaks are due to improper food handling and preparationby consumers and affect small numbers of individuals, usually in the home. Occasional outbreaks affect large numbers of individuals because they are caused by breakdowns in safe food handling and

preparation at food-processing and distribution plants. Most foodborne illnesses are unreported because the connection between food and illness is not made. Foodborne illness is largely preventable; appropriate monitoring of food sources and disease outbreaks provides the basis for protecting consumers. The food industry and the government set standards and monitor food sources to control and preventfoodborne disease.

Foodborne Disease and Microbial Sampling The most prevalent foodborne diseases in the United Statesare classified as food poisonings (FP) or food infections (FI); somediseases fall intoboth categories. **Table 36.6** lists the microorganismsthat cause these diseases. Special microbial samplingtechniques are necessary to isolate and identify the pathogensand toxins responsible for foodborne diseases, and a variety ofgrowth-dependent, immunological, and molecular techniquesare used. Foodborne illnesses and outbreaks are reported to theCenters for Disease Control and Prevention through PulseNet and FoodNet reporting systems.

Foodborne Diseases

Food poisoning, also called **food intoxication**, is disease thatresults from ingestion of foods containing preformed microbialtoxins. The microorganisms that produced the toxins do not have to grow in the host and are often not alive at the time the contaminated food is consumed; the ingestion and action of a bioactive toxin causes the illness. We previously discussed some of these toxins, notably the exotoxin of *Clostridium botulinum*. and the superantigen toxins of *Staphylococcus*and *Streptococcus*. **Food infection** is ingestionof food containing sufficient numbers of viable pathogens tocause infection and disease in the host. We discuss major foodborneinfections.

Microbial Sampling for Foodborne Disease

Along with nonpathogenic microorganisms that cause spoilage,pathogenic microorganisms may be present in fresh foods. Rapiddiagnostic methods that do not require pathogen growth or culture have been developed to detect important food pathogenssuch as *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus*,and *Clostridium botulinum*. Molecular and immunology- basedtests are used to identify both toxin and pathogen contamination of foods and other products such as drugs and cosmetics. The presence of afoodborne pathogen or toxin is not sufficient to link a particularfood to a specific foodborne disease outbreak; the suspectpathogenor toxin must be isolated and identified to establish itsrole in a foodborne illness.Isolation and growth of pathogens from nonliquid foods usuallyrequire preliminary treatment to suspendmicroorganismsembedded or entrapped within the food. A standard methoduses a specialized blender called a stomacher (**Figure 36.10**). Thestomacher processes a wide

variety of solid and semisolid samplessuch as fresh and processed meat, dry fruits, cereals, grains, seeds, cheese, cosmetics, and for biomedical applications, pharmaceuticalproducts and tissue samples. The sample is sealed in a sterilebag. Paddles in the stomacher crush, blend, and homogenize the samples under conditions that prevent contamination by otherorganisms. Although a traditional blender could also be used toprocess samples, the sealed bag stomacher systemprevents contaminationfrom outside sources, eliminates cleanup betweeneach sample run, and eliminates generation of aerosols. Thehomogenized samples can then be analyzed in various ways.Foods sampled for microorganisms or toxins should be examinedas soon after processingas possible; if examination cannotbegin within 1 hour of sampling, the food should be refrigerated.Frozen food should be thawed in the original container in arefrigerator and examinedor cultured as soon as thawing iscomplete. In addition to identifying pathogens in food, diseaseinvestigators must obtain foodborne pathogens from the diseaseoutbreak patients to establish a cause-and-effect relationshipbetween the pathogen and the illness. In many cases, fecal samplescan be cultured to recover suspected foodborne pathogens.Food or patient samples can be inoculated onto enriched media, followed by transfer to differential or selective media for isolationand identification, as described for the isolation of human pathogens. Final identificationof foodbornepathogens is based on growth characteristics and biochemicalreaction patterns. The use of molecular and genetic methods. such as the polymerase chain reaction, enzyme immunoassays, nucleic acid probes, nucleic acid sequencing, pulsed-field gel electrophoresis (PFGE), and ribotyping may be used to identify specific organisms.

Figure 36.10 A stomacher. Paddles in this specialized blender homogenize the solid food sample in a sealed, sterile bag. The sample is suspended in a sterile solution.

Foodborne Disease Epidemiology

There are often clusters of cases of a foodborne disease in aparticular place because microorganisms from a single commoncontaminated food, such as salads or hamburgers served from ahome, school cafeteria, college dining hall, restaurant, or mess hall,are ingested by many individuals. In addition, central processingplants and central food

distribution centers provide opportunitiesfor contaminated foods to cause multiple disease outbreaksin far-flung locations, as when contaminated spinach grown inCalifornia caused outbreaks across the United States. We shall seehow the food epidemiologist tracks outbreaks and determinestheir source, often down to the field, processing plant, or pointof-preparation facility in which the food was contaminated.

Spinach and *Escherichia coli* O157:H7

In 2006 an outbreak of illness associated with *Escherichia coli*O157:H7 occurred in the United States and was linked to the consumption of ready-to-eat packaged fresh spinach. The outbreak was quickly traced to a food-processing facility in California.First linked to the spinach product in September, the outbreakcaused at least 199 infections. Of these, 102 individuals were hospitalizedand 31 developed hemolytic uremic syndrome. At leastthree deaths were attributed to the outbreak.The remarkably short duration and rapid end to this epidemic—the first case was confirmed in late August and the last reportedin early October—is a testament to efficiency and cooperationamong public health facilities across the country. In this case, two of these networks—FoodNet and PulseNet—were used to define the source and stop the outbreak. The contaminatedspinach was distributed nationwide from the Californiaprocessing plant, but most disease cases were not in the West. Thetwo states affected most were Wisconsin, with 49 cases, and Ohio,with 25 cases; there were only 2 cases in California.Because *E. coli* O157:H7 (**Figure 36.11**) has been well studied,public health officials were able to identify the strain found in thebagged spinach and determine its origin. They conclusively linkedthe outbreak to the bagged spinach, traced it back to the processingplant, and eventually traced it to an agricultural field in thevicinity of the processing plant. DNA from the organisms isolatedfrom regional outbreakswas typed using pulsed-field gel electrophoresis(PFGE), a form of gel electrophoresis thatbetterdistinguishes between large molecules and is used in pathogenidentification. The patterns obtained were then compared; theresults showed that the same strain was responsible for the diseasein various parts of the country. The common thread in the geographicallyisolated outbreaks was consumption of the suspectedlots of bagged spinach originating from a single California facility.The precise source of the outbreak, although it has been tracedto a field near the processing plant, remains unknown. Feral pigsand domestic cattle are present in the vicinity of the identifiedfield, and contaminated wells or surface waters used for irrigationmay have introduced the pathogen into the fields and eventuallyinto the spinach. The original source was almost certainly animalin origin, as E. coli is an enteric organism found naturally only inthe intestine of animals.The spinach epidemic, although serious and even deadly forsome, was discovered, contained, and stopped very quickly. However, this

incident also shows how centralized food-processingfacilities can quickly spread disease to large and distant populations.

Food hygiene standards and surveillance must be maintainedat the highest possible level in central food-processing anddistribution facilities.

Food Disease Reporting

In the United States foodborne outbreaks are reportable to theCenters for Disease Control and Prevention through FoodNet.Identification of particular organisms responsible for foodbornedisease outbreaks is particularly important. A reporting systemcalled PulseNet International is an international molecular subtypingnetwork for foodborne disease surveillance. It consists ofnational and regional PulseNet organizations from the UnitedStates, Canada, Europe, Asia, Latin America, the Caribbean, andthe Middle East. The organization collects and shares molecularsubtyping data from PFGE DNA fingerprints of organisms implicatedin foodborne disease outbreaks.

Figure 36.11 ***Escherichia coli*** **O157:H7.** The cell, about 1 μm in diameter, as it appears in a colorized transmission electron micrograph showing peritrichous flagella.

Food Infection

Food infection results from ingestion of food containing sufficientnumbers of viable pathogensto cause infection anddisease in the host. Food infection is very common, and we begin with a common bacterial cause, Salmonella. Manyfood infection agents can also cause waterborne diseases.

Salmonellosis

Salmonellosis is a gastrointestinal disease typically caused by foodborne*Salmonella* infection. Symptoms begin after the pathogencolonizes the intestinal epithelium. *Salmonella* are gram- negative,facultatively aerobic, motile rods related to *Escherichia coli* and other enteric bacteria. Salmonella normallyinhabits the animal intestine and is thus found in sewage.The nomenclature of the Salmonella spp. is based on taxonomicthat differentiate strains by virtue of biochemical, serological,and molecular (nucleic acid–

based) characteristics. Theaccepted species name for the pathogenic members of the genusis *Salmonella enterica*. Based on nucleic acid analyses, there areseven evolutionary groups or subspecies of *S. enterica*. Mosthuman pathogens fall into group I, designated as a single subspecies,*S. enterica* subspecies *enterica*. Finally, each subspeciesmay be divided into serovars (serological variations, also calledserotypes). Thus, the organism formally named *Salmonella enteric*subspecies *enterica* serovar *Typhi* is usually called *Salmonella enterica* serovar *Typhi* and is often abbreviated to *Salmonella Typhi. S. enterica ser. Typhi* causes the serious human diseasetyphoid fever but is rare in the United States. Most of the 500 orso annual foodborne cases caused by *S. enterica ser. Typhi* areacquired outside the United States.A number of other S. enterica serovars also cause foodborne gastroenteritis.

In all, over 1400 Salmonella serovars cause disease inhumans. *S. enterica serovarsTyphimurium*

and *Enteritidis* arethe most common agents of foodborne salmonellosis in humans.

Epidemiology and Pathogenesis

The incidence of salmonellosis has been steady over the lastdecade, with about 40,000–45,000 documented cases each year(**Figure 36.15**). However, less than 4% of salmonellosis cases are probably reported, so the incidence of salmonellosis is probablyover 1 million cases every year. The ultimate sources of the foodborne salmonellas are theintestinal tracts of humans and other warm-blooded animals, and there are several routes by which these bacteria may enterthe food supply. The bacteria may reach food through fecal contaminationfrom food handlers. Food production animals such aschickens, pigs, and cattle may harbor *Salmonella* serovars that arepathogenic to humans, and the bacteria may be carried throughto finished fresh foods such as eggs, meat, and dairy products.Salmonella food infections are often traced to products such as custards, cream cakes, meringues, pies, and eggnog made withuncooked eggs. Other foods commonly implicated in salmonellosisoutbreaks are meats and meat products such as meat pies,cured but uncooked sausages and meats, poultry, milk, and milkproducts.

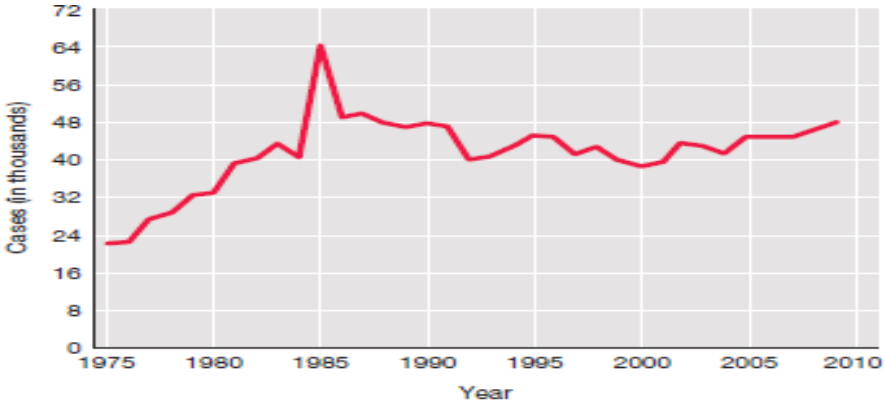


Figure 36.15 Reported cases of salmonellosis in the United States. Most cases of salmonellosis are foodborne. The total number of reported cases in 2007 was 47,995. The high incidence in 1985 was caused by contamination of pasteurized milk that was mixed with raw (unprocessed) milk in a dairy processing plant in Illinois. Data are from the Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

The most common *salmonellosis* is enterocolitis. Ingestion offood containing viable Salmonella results in colonization of the small and large intestine. Onset of the disease occurs 8–48 hours after ingestion. Symptoms include the sudden onset of headache, chills, vomiting, and diarrhea, followed by a fever that lasts a fewdays. The disease normally resolves without intervention in 2–5days. After recovery, however, patients may shed *Salmonella* infeces for several weeks. Some patients recover and remainasymptomatic, but shed organisms for months or even years;they are chronic carriers. A few serovars ofSalmonella may also cause septicemia (a blood infection) andenteric or typhoid fever, a disease characterized by systemicinfection and high fever lasting several weeks. Mortality canapproach 15% in untreated typhoid fever.The pathogenesis of *Salmonella* infections starts with uptakeof the organisms from the gut. Salmonella ingested in food orwater invades phagocytes and grows as an intracellular pathogen,spreading to adjacent cells as host cells die. After invasion, pathogenicSalmonella uses a combination of endotoxins, enterotoxins,and cytotoxins to damage and kill host cellsMicrobial Sidebar "Virulence in *Salmonella*"), leading to the classicsymptoms of salmonellosis.

Diagnosis, Treatment, and Prevention

Foodborne salmonellosis is diagnosed from observation of clinicalsymptoms, history of recent food consumption, and culturing theorganism from feces. Selective and differential media are used toidentify *Salmonella* and discriminate it from other gram-negative Rods. Tests for the presence of Salmonella arecommonly used on animal food products, such as raw meat,poultry, eggs, and powdered milk. Salmonella has also beenfound, however, in nonmeat and nondairy food, including produce(cantaloupes and tomatoes) and peanut butter. Tests forSalmonella in food include several rapid tests, but even rapidtests rely on

culture-based enrichment procedures to increaseSalmonella numbers to testable levels. The established standardused by PulseNet for epidemiological investigations is pulsedfieldgel electrophoresis. This moleculartyping technique can discriminate between various Salmonellaserovars.For enterocolitis, treatment is usually unnecessary, and antibiotictreatment does not shorten the course of the disease or eliminate the carrier state. Antibiotic treatment, however, significantlyreduces the length and severity of septicemia and typhoidfever. Mortality due to typhoid fever can be reduced to less than1% with appropriate antibiotic therapy. Multi-drug-resistantSalmonella are a significant clinical problem.Properly cooked foods heated to at least 70 ^{0}C are generally safeif consumed immediately, held at 50 ^{0}C, or stored immediately at48°C. Any foods that become contaminated by an infected foodhandler can support the growth of *Salmonella* if the foods areheld for long periods of time, especially without heating or refrigeration.Salmonella infections are more common in summerthan in winter, probably because warm environmental conditionsgenerally favor the growth of microorganisms in foods.Although local laws and enforcement vary, because of thelengthy carrier state, infected individuals are often banned fromwork as food handlers until their feces are negative for Salmonellain three successive cultures.

Pathogenic *Escherichia coli*

Most strains of *Escherichia coli* are common members of theenteric microflora in the human colon and are not pathogenic. A few strains, however, are potential foodborne pathogens. There are about 200 known pathogenic *E. coli* strains, all of which acton the intestine. Several are characterized by their production ofpotent enterotoxins and may cause life-threatening diarrheal disease and urinary tract infections. The pathogenic strains aredivided based on the type of toxinthey produce and the specificdiseases they cause.

Shiga Toxin–Producing *Escherichia coli* (STEC)

Shiga toxin–producing *Escherichia coli* (STEC) produce verotoxin,an enterotoxin similar to the Shiga toxin produced by Shigella dysenteriae. Formerly known as enterohemorrhagicE. coli(EHEC), the most widely distributed STEC is E. coliO157:H7 (Figure 36.11). Up to 90% of allSTEC infections arecaused by E. coli O157:H7. After a person ingests food or water containingSTEC, the bacteria grow in the small intestine andproduce verotoxin. Verotoxin causes bothhemorrhagic (bloody)diarrhea and kidney failure. *E. coli* O157:H7 causes an estimated60,000 infections and 50 deaths from foodborne disease in the United States each year. STEC strains arethe leadingcause of hemolytic uremic syndrome and kidney failure, with 292cases reported in2007, about half in children under 5 years of age.About 40% of STEC infections are caused bythe

consumptionof contaminated uncooked or undercooked meat, particularlymass-processed ground beef. *E. coli* O157:H7 is a member of thenormal microbiome in healthy cattle; it canenter the human foodchain if meat is contaminated with intestinal contents duringslaughter andprocessing. In several major outbreaks in theUnited States caused by *E. coli* O157:H7, infectedground beeffrom regional distribution centers was the source of contamination. Infected meat products caused disease in several states. Anotheroutbreak was caused by processed and cured, but uncooked beefin ready-to-eat sausages. The source of contamination was thebeef, and the E. coli O157:H7 probably originated from slaughteredbeef carcasses.In 2003, the Food Safety and Inspection Service of the UnitedStates Department of Agriculture reported 20 positive results of 6584 samples (0.03%) of ground beef analyzed for *E. coli* O157:H7. *E. coli* O157:H7 has also been implicated in food infectionoutbreaks from dairy products, fresh fruit, and raw vegetables. Contamination of the fresh foods by fecal material, typically fromcattle carrying the *E. coli* O157:H7 strain, has been implicated inseveral of these cases.

Because *E. coli* O157:H7 grows in the intestines and is found in fecal material, it is also a potential source of waterborne gastrointestinal disease. Several outbreaks have also occurred in day-care facilities, where the presumed route of exposure is oral–fecal contamination.

Other Pathogenic *Escherichia coli*

Children in developing countries often contract diarrheal diseasecaused by *E. coli*. *E. coli* can also be the cause of "traveler's diarrhea,"a common enteric infection causing watery diarrhea in travelers to developing countries. The primary causal agents arethe enterotoxigenic *E. coli* (ETEC). The ETEC strains usuallyproduce one of two heat-labile, diarrhea-producing enterotoxins.In studies of United States citizens traveling in Mexico, the infectionrate with ETEC is often greater than 50%. The prime vehiclesare foods such as fresh vegetables (for example, lettuce in salads)and water. The very high infection rate in travelers is due to contaminationof local public water supplies. The local population is usually resistant to the infecting strains, presumably becausethey have acquired resistance to the endemic ETEC strains.

Secretory IgA antibodies in the bowel prevent colonization ofthe pathogen in local residents, but the organism readily infectsthe nonimmune travelers and causes disease.Enteropathogenic E. coli (EPEC) strains cause diarrheal diseasesin infants and small children but do not cause invasive disease orproduce toxins. Enteroinvasive E. coli (EIEC) strains cause invasivedisease in the colon, producing watery, sometimes bloody diarrhea.The EIEC strains are taken up by phagocytes, but escape lysisin the

phagolysosomes, grow in the cytoplasm, and move intoother cells in much the same way as pathogenic Salmonella strains.This invasive disease causesdiarrhea and is common in developingcountries.

Diagnosis and Treatment

Illness from *E. coli* O157:H7 and other STEC strains is areportable infectious disease in the United States. The generalpattern established for diagnosis, treatment, and prevention ofinfectionby *E. coli* O157:H7 reflects current procedures used forall of the pathogenic E. coli strains. Laboratory diagnosis requiresculture from the feces and identification of the O (lipopolysaccharide)and H (flagellar) antigens and toxins by serology. Identificationof strains is also done using DNA analyses such asrestriction fragment length polymorphism and PFGE. *E. coli*O157:H7 outbreaks are reported through FoodNet and PulseNetto the Centers for Disease Control and Prevention.Treatment of *E. coli* O157:H7 and other STEC infectionsincludes supportive care and monitoring of renal function, bloodhemoglobin, and platelets. Antibiotics may be harmful becausethey may cause the release of large amounts of verotoxin fromdying *E. coli* cells. For other pathogenic *E. coli* infections, treatmentusually includes supportive therapy and, for severe cases and invasivedisease, antimicrobial drugs to shorten and eliminate infection.

Prevention

The most effective way to prevent infection with foodborneSTEC is to make sure that meat is cooked thoroughly, whichmeans that it should appear gray or brown and juices should be clear. As we discussed above, the United Stateshas approved the irradiation of ground meat as an acceptablemeans of eliminating or reducing food infection bacteria,largely because *E. coli* O157:H7 has been implicated in severalfoodborne epidemics. To process foods such as ground beef,large-scale production plants may mix and grind meat fromhundreds or even thousands of animals together; the grindingprocess could distribute the pathogens from a single infectedanimal throughout the meat. Short of cooking, penetratingradiation is considered the only effective means to ensuredecontamination.In general, proper food handling, water purification, andappropriate hygiene prevent the spread of pathogenic *E. coli*.Raw foods should be washed thoroughly. Traveler's diarrhea canbe prevented by avoiding consumption of local water and uncooked foods.

Campylobacter

Species of *Campylobacter* are the most common reported causeof bacterial foodborne infections in the United States. Cells of*Campylobacter* species are gram-negative, motile, curved rods to spiral-shaped bacteria that grow at reduced oxygen tension

asmicroaerophiles. Several pathogenic species,*Campylobacter jejuni* (**Figure 36.16**), *C. coli, and C. fetus, are recognized.*

C. jejuni and *C. coli* account for almost 2 million annualcases of bacterial diarrhea (Table 36.6). C. fetus is a major cause ofsterility and spontaneous abortion in cattle and sheep.

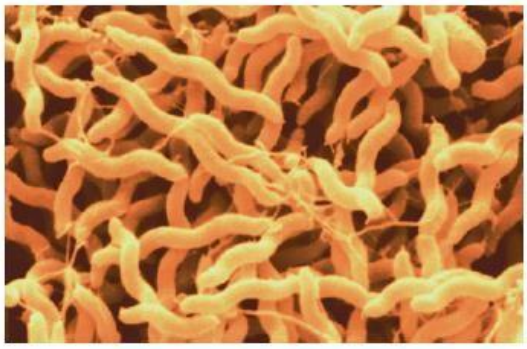


Figure 36.16 ***Campylobacter jejuni.*** The gram-negative curved rods shown in this colorized scanning electron micrograph are about 1 μm in diameter.

Epidemiology and Pathology

Campylobacter is transmitted to humans via contaminated food,most frequently in poultry, pork, raw shellfish, or in surfacewaters. *C. jejuni* is a normal resident in the intestinal tract ofpoultry; virtually all chickens and turkeys are normally colonizedwith this organism. According to the United States Departmentof Agriculture, up to 90% of turkey and chicken carcasses and over30% of hog carcasses may be contaminated with Campylobacter.Beef, on the other hand, is rarely a vehicle for this pathogen.*Campylobacter* species also infect domestic animals such asdogs, causing a milder form of diarrhea than that observed inhumans. Campylobacter infections in infants are frequentlytraced to infected domestic animals, especially dogs.After a person ingests cells of Campylobacter, the organismmultiplies in the small intestine, invades the epithelium, andcauses inflammation. Because *C. jejuni* is sensitive to gastric acid,cell numbers as high as 104 may be required to initiate infection.However, this number may be reduced to less than 500 if thebacteria are ingested in food, or are ingested by a person takingmedication to reduce stomach acid production. Campylobacterinfection causes a high fever (usually greater than 104°F or40°C), headache, malaise, nausea, abdominal cramps, and profusediarrhea with watery, frequently bloody, stools. The disease subsides in about 7–10 days. Spontaneous recovery from Campylobacter infections is often complete, but relapses occur inup to 25% of cases.

Diagnosis, Treatment, and Prevention

Diagnosis of *Campylobacter* food infection requires isolation ofthe organism from stool samples and identification by growthdependenttests, immunological assays, or

molecular tests. Serious

C. jejuni infections are often seen in infants. In these cases, diagnosisis important; selective media and specific immunologicalmethods have been developed for positive identification of this organism. Erythromycin and quinolone treatment may be usefulearly in severe diarrheal disease. Adequate personal hygiene, properwashing of uncooked poultry (and any kitchenware coming incontact with uncooked poultry), and thorough cooking of meateliminate *Campylobacter* contamination.As with other foodborne infections, epidemiologic investigations are based on PFGE analysis of recovered organisms. Data sharedon PulseNet are used to track the spread of Campylobacter anddetermine its origin.

Listeriosis

Listeria monocytogenes causes **listeriosis**, a gastrointestinal foodinfection that may lead to bacteremia and meningitis. L. monocytogenesis a short, gram-positive, nonsporulating coccobacillus that is acid-, salt- and cold-tolerant and facultatively aerobic(**Figure 36.17**).

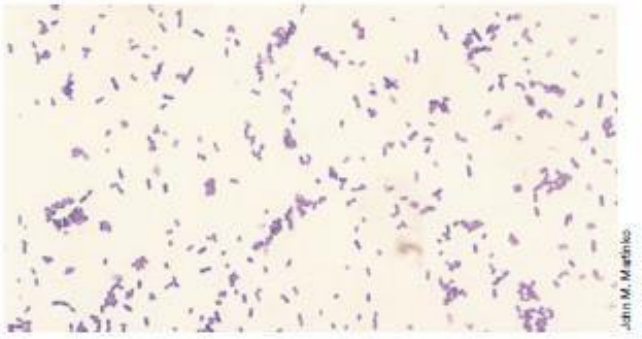


Figure 36.17 ***Listeria monocytogenes***. This Gram stain shows gram-positive coccobacilli, about 0.5 µm in diameter.

Epidemiology and Pathology

L. monocytogenes is found widely in soil and water; virtually nofood source is safe from possible

L. monocytogenes contamination.Food can become contaminated at any stage during foodproduction or processing. Food preservation by refrigeration,which ordinarily slows microbial growth, is ineffective in limitinggrowth of this psychrotolerant organism. Ready-to-eat meats,fresh soft cheeses, unpasteurized dairy products, and inadequatelypasteurized milk are the major food vehicles for thispathogen, even when foods are properly stored at refrigerator temperature (48°C).*L. monocytogenes* is an intracellular pathogen. It enters thebody through the gastrointestinal tract in contaminated food.Phagocytes take up the pathogen in a phagolysosome.

Thistriggers production of listeriolysin O, which lyses the phagolysosome and releases *L. monocytogenes* into the cytoplasm. Here itmultiplies and produces ActA, a surface protein that induceshost cell actin polymerization, which moves the pathogen to the cytoplasmic membrane. At the cytoplasmic membrane, the complexpushes out, forming protrusions called filopods. The filopodsare then ingested by surrounding cells and the cycle starts again.This mechanism allows *L. monocytogenes* to move from cell tocell without exposure to antibodies, complement, or neutrophils.Specific immunity to *L. monocytogenes* is through cell-mediatedTH1 inflammatory cells. Particularly susceptiblepopulations include the elderly, pregnant women, newborns, andimmunosuppressed individuals [for example, transplant patientsundergoing steroid therapy and acquired immunodeficiencysyndrome (AIDS) patients]. Although exposure to *L. monocytogenes* is undoubtedly verycommon, there are only about 2500 estimated cases of clinicallisteriosis each year, and fewer than 1000 are reported. Nearly all diagnosed cases require hospitalization. Acute listeriosis is rareand is characterized by septicemia, often leading to meningitis,with a mortality rate of 20% or higher. About 30–40 listeriosisdeaths are reported annually in the United States.

Diagnosis, Treatment, and Prevention

Listeriosis is diagnosed by culturing *L. monocytogenes* from theblood or spinal fluid. *L. monocytogenes* can be identified in foodby direct culture or by molecular methods such as ribotyping andthe polymerase chain reaction. Clinical isolates are analyzed byPFGE to determine molecular subtypes. The subtype patternsare reported to PulseNet at the Centers for Disease Controland Prevention. Intravenous antibiotic treatment with penicillin, ampicillin, or trimethoprim plus sulfamethoxazole is recommendedfor invasive disease.

Prevention measures include recalling contaminated foodand taking steps to limit L. monocytogenes contamination at thefood-processing site. Because *L. monocytogenes* is susceptible toheat and radiation, raw food and food-handling equipment can bereadily decontaminated. However, without pasteurizing or cookingthe finished food product, the risk of contamination cannot beeliminated because of the widespread distribution of the pathogen.

Individuals who are immunocompromised should avoid unpasteurizeddairy products and ready- to-eat processed meats.Pregnant women should also avoid foods that may transmit*L. monocytogenes* because spontaneous abortion is a frequentoutcome of listeriosis.

Other FoodborneInfectious Diseases

Over 200 other microorganisms, viruses, and other infectiousagents such as prions contribute tofoodborne diseases.

Bacteria

*Yersinia enterocolitica*is commonly found in the intestines of domestic animalsand causes foodborne infections due to contaminated meat anddairy products. The most serious consequence of *Y. enterocolitica*infection is enteric fever, a severe life-threatening infection. Bacilluscereus produces two enterotoxins that cause diarrhea and vomiting.The organism grows in foods such as rice, pasta, meats, orsauces that are cooked and left at room temperature to coolslowly. Endospores of this gram-positive rod germinate and toxinis produced. Reheating may kill the B. cereus, but the toxin mayremain active. B. cereus may also cause a food infection similar tothat caused by *Clostridium perfringens*. *Shigella* species can causesevere invasive gastroenteritis called shigellosis. About 20,000cases of shigellosis are reported each year in the United States,with up to 150 million cases worldwide. Most *Shigella* infectionsare the result of fecal– oral contamination, but food and waterare occasional vehicles. Several members of the *Vibrio* genuscause food poisoning in persons who consume contaminatedshellfish.

Viruses : The largest number of annual foodborne infections is thought tobe caused by viruses. In general, viral foodborne illness consistsof gastroenteritis characterized by diarrhea, often accompanied by nausea and vomiting. Recovery is spontaneous and rapid,usually within 24–48 hours (“24-hour bug”). Noroviruses (**Figure36.18**) are responsible for most of these mild foodborne infections in the United States, accounting for over 9 millionof the estimated 13 million annual cases of foodborne disease.Rotavirus, astrovirus, and hepatitis A collectively cause 100,000 cases of foodborne disease each year. These viruses inhabit thegut and are often transmitted to food or water with fecal matter.As with many foodborne infections, proper food handling, handwashing,and a source of clean water to prepare fresh foods areessential to prevent infection.

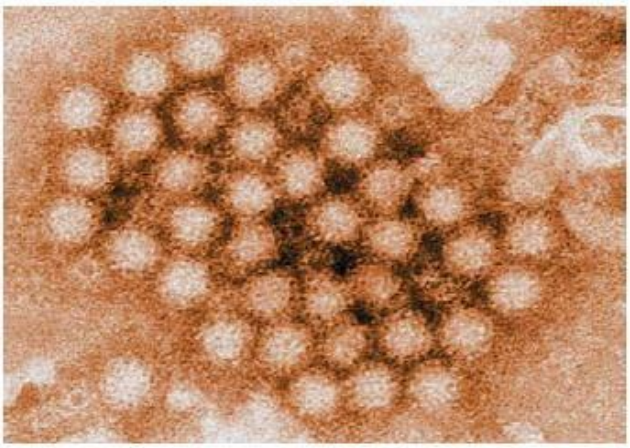
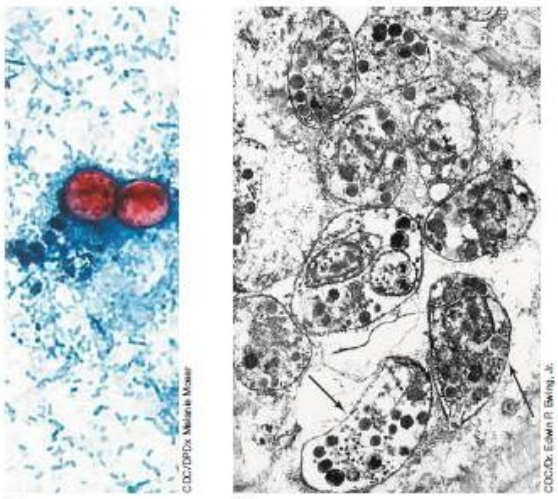


Figure 36.18 Human norovirus. The virus was isolated from a patient with diarrhea. Individual norovirus particles have an indistinct rough outer edge and are about 27 nm in diameter.

Protists Important foodborne protist diseases are listed in Table 36.6. Protists including *Giardia intestinalis*, *Cryptosporidium parvum* and *Cyclospora cayetanensis* (**Figure 36.19*a***) can be spread in foods contaminated by fecal matter in untreated water used to wash, irrigate, or spray crops. Fresh foods such as fruits are often implicated as the source of these protists. Cyclosporiasis is an acute gastroenteritis and is an important emerging disease. In the United States, most cases are acquired by eating fresh produce imported from other countries. *Toxoplasma gondii* is a protist spread through cat feces, but is also found in raw or undercooked meat. In most individuals, toxoplasmosis is a mild, self-limiting gastroenteritis. However, prenatal infection of the fetus can lead to serious acute toxoplasmosisresulting in tissue involvement, cyst formation, andcomplications such as myocarditis, blindness, and still birth.

Immunocompromised individuals such as people with acquiredimmunodeficiency syndrome (AIDS) may develop acute toxoplasmosis.*T. gondii* grows intracellularly and forms structures called tachyzoites (Figure 36.19b) that eventually lyse the cell andinfect nearby cells, resulting in tissue destruction. Tachyzoitescan cross the placenta and infect the fetus. Toxoplasma infectionsin compromised hosts can be treated with the antiprotistdrug pyrimethamine.



(a) (b)

Figure 36.19 Protists transmitted in food. *(a) Cyclospora cayetanensis* oocysts in a stool sample from an affected patient. The oocysts, stained red with safranin, are about 8–10 μm in diameter. *(b)* Tachyzoites of *Toxoplasma gondii*, an intracellular parasite. In this transmission electron micrograph, the tachyzoites (arrows) are in a cystlike structure in a cardiac myocyte. Tachyzoites are generally elongated to crescent in form, about 4–7 μm long by 2–4 μm wide.

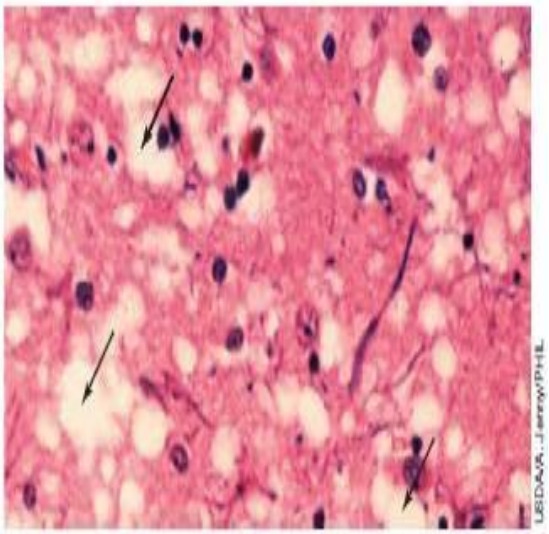


Figure 36.20 A brain section from a cow with bovine spongiform encephalopathy (BSE). The vacuoles, appearing as holes (arrows), giv the brains of infected animals a distinct spongelike appearance.

Prions, BSE, and nvCJD : Prions are proteins, presumably of host origin, that adopt novelconformations, inhibiting normal protein function and causing degeneration of neural tissue. Human priondiseases are characterized by neurological symptoms includingprogressive depression, loss of motor coordination, and dementia.A foodborne prion disease in humans known as new variantCreutzfeldt–Jakob Disease (nvCJD) has been linked to consumptionof meat products from cattle afflicted with bovine spongiformencephalopathy (BSE), a prion disease commonly called "mad cow disease." A slow-acting degenerative nervous system disorder,nvCJD has a latent periodthat may extend for years after exposureto the BSE prion. Nearly 200 people in Great Britainandother European countries have acquired nvCJD. However, nvCJDlinked to domestic meat consumption has not been observedin the United States. BSE prions consumed in meat products from affected cattle trigger human protein analogs to assume analtered conformation, resulting in protein dysfunction and disease. The terminal stages of both BSE andnvCJD are characterized by large vacuoles in brain tissue, givingthe brain a "spongy" appearance, from which BSE derivesitsname (**Figure 36.20**).

In the United Kingdom and Europe, about 180,000 cattle werediagnosed with BSE and destroyedin the 1990s. Brains of slaughteredanimals are routinely tested for BSE in the United States,and several cattle with BSE have been found in Canadian andU.S. herds. In Europe and North America, all cattle known orsuspected to have BSE have been destroyed. Bans on cattle feedscontaining cattle meat and bone meal

appear to have stopped thedevelopment of new cases of BSE in Europe and have kept theincidence of this disease very low in North America. The infectingprions were probably transferred to food production animalsthrough meat and bone meal feed derived from infected cattle orother animals not approved for human consumption.

Diagnosis of BSE is done by testing using a prion-susceptiblemouse strain or by immunehistochemical or micrographic analysisof biopsied neural tissue.

Food Borne Diseases:

Foodborne illness can occur if you eat foods that are contaminated with harmful pathogens such as bacteria, viruses, and fungi.

The World Health OrganizationTrusted Source estimates that 1 in 10 people worldwide develop a foodborne illness each year. That's equivalent to 600 million new cases per year. In the United StatesTrusted Source, however, the annual infection rate is slightly higher, with an estimated 1 in 6 people experiencing a foodborne illness each year. Some foodborne illnesses are mild, whereas others can lead to long-term health complications or death if left untreated.

Is there a difference between foodborne illness and food poisoning?

Although people may use the terms "foodborne illness" and "food poisoning" interchangeably, there are minor differences between the two.

"Foodborne illness" is an umbrella term that describes any illness caused by consuming foods or beverages contaminated with harmful pathogens — such as bacteria, viruses, and fungi — or their toxins.

A foodborne illness may be due to an infection or intoxication.

A foodborne infection can occur if you eat foods that contain live bacteria or other pathogens. These pathogens can later grow in your gut and cause symptoms such as abdominal cramping, diarrhea, and vomiting.

On the other hand, intoxication — also called food poisoningTrusted Source — can happen if you consume foods containing toxins that harmful pathogens release. The live pathogens themselves do not need to be present in the food.

Therefore, food poisoning is a type of foodborne illness.

What causes foodborne illnesses?

Foodborne illnesses may result from any of the following sources:

• **Bacteria:** These may be present in raw and undercooked meat, fish, and poultry; unpasteurized dairy products; contaminated fruits and vegetables; and contaminated drinking water.

• **Viruses:** Viruses are transmitted to the body through food contaminated by viral particles.

• **Parasites:** Contaminated water and soil can transmit harmful parasites to fresh produce, seafood, meat, poultry, and other foods.

• **Prions:** These infectious proteins are associated with "mad cow disease" and can come from eating parts of cattle, such as the brain tissue.

• **Naturally occurring chemicals:** Naturally occurring toxins in mushrooms, staple foods such as corn and cereal, and mold on grains can cause long-term health complications.

• **Environmental pollutants:** Byproducts of plastic production and waste management, as well as heavy metals such as lead and mercury found in water and soil, can contaminate foods and lead to foodborne illnesses.

What are some examples of foodborne illnesses?

Here are some common foodborne illnesses and their symptoms. Symptoms may take anywhere from 1 week to several months to develop.

How are foodborne illnesses treated?

Treatment for foodborne illnesses may involve a combination of at-home remedies and over-the-counter and prescription medications.

However, the types of medications that healthcare professionals prescribe will depend on the type of pathogen responsible for the foodborne illness and the severity of symptoms. Severe cases may require hospitalization.

A healthcare professional may recommend that you:

• drink extra fluids to stay hydrated if you have diarrhea or vomiting

• get extra rest if you are feeling fatigued

• take antibiotics, if prescribed

• take antitoxin as administered

• consider surgery for some parasitic and toxic cases

What can you do to prevent foodborne illness?

Preventing foodborne illness is an important public health task. The U.S. Department of Agriculture and the CDCTrusted Source have issued food safety guidelines to help you avoid becoming sick with a foodborne illness.

They recommend:

- **Washing your hands:** Wash your hands often and thoroughly with warm, soapy water for at least 20 seconds before and after handling raw or cooked foods, using the bathroom, handling pets, or tending to anyone who is ill.
- **Cleaning items well:** Clean food surfaces, utensils, and cutting boards with hot, soapy water after each use. Learn how to clean your wooden cutting board.
- **Separating foods:** Keep raw meat, poultry, seafood, and eggs separate from cooked and ready-to-eat foods, including fruits and vegetables, to avoid cross contamination.
- **Cooking food thoroughly:** Cook foods to a safe internal temperature to avoid undercooking and reduce foodborne illness risk. Use this detailed cooking temperature list to guide you.
- **Avoiding raw beverages:** Avoid drinking raw and unpasteurized dairy and juice products.
- **Storing food properly:** Keep foods out of the temperature danger zone of 40–140°F (5–60°C) by thawing frozen food safely in the refrigerator and refrigerating foods within 2 hours of cooking.
- **Isolating when you're sick:** Stay at home if you're feeling unwell and avoid preparing food for others during this time, even for several days after your symptoms have subsided.

Most common bacterial foodborne pathogens are:

- *Campylobacter jejuni* which can lead to secondary Guillain–Barré syndrome and periodontitis[9]
- *Clostridium perfringens*, the "cafeteria germ"
- *Salmonella* spp. – its *S. typhimurium* infection is caused by consumption of eggs or poultry that are not adequately cooked or by other interactive human-animal pathogens
- *Escherichia coli O157:H7* enterohemorrhagic (EHEC) which can cause hemolytic-uremic syndrome

Other common bacterial foodborne pathogens are:

- *Bacillus cereus*
- *Escherichia coli*, other virulence properties, such as enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroaggregative (EAEC or EAgEC)
- *Listeria monocytogenes*

- *Shigella* spp.
- *Staphylococcus aureus*
- *Streptococcus*
- *Vibrio cholerae*, including O1 and non-O1
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Less common bacterial agents:

- *Brucella* spp.
- *Corynebacterium ulcerans*
- *Coxiella burnetii* or Q fever
- *Plesiomonas shigelloides*

Enterotoxins

Botulism

In addition to disease caused by direct bacterial infection, some foodborne illnesses are caused by enterotoxins (exotoxins targeting the intestines). Enterotoxins can produce illness even when the microbes that produced them have been killed. Symptom onset varies with the toxin but may be rapid in onset, as in the case of enterotoxins of *Staphylococcus aureus* in which symptoms appear in one to six hours. This causes intense vomiting including or not including diarrhea (resulting in staphylococcal enteritis), and staphylococcal enterotoxins (most commonly staphylococcal enterotoxin A but also including staphylococcal enterotoxin B) are the most commonly reported enterotoxins although cases of poisoning are likely underestimated. It occurs mainly in cooked and processed foods due to competition with other biota in raw foods, and humans are the main cause of contamination as a substantial percentage of humans are persistent carriers of *S. aureus*. The CDC has estimated about 240,000 cases per year in the United States.

- *Clostridium botulinum*
- *Clostridium perfringens*
- *Bacillus cereus*

The rare but potentially deadly disease botulism occurs when the anaerobic bacterium *Clostridium botulinum* grows in improperly canned low-acid foods and produces botulin, a powerful paralytic toxin.

Pseudoalteromonas tetraodonis, certain species of *Pseudomonas* and *Vibrio*, and some other bacteria, produce the lethal tetrodotoxin, which is present in the tissues of some living animal species rather than being a product of decomposition.

Emerging foodborne pathogens*[edit]*

- *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas sobria*

Scandinavian outbreaks of *Yersinia enterocolitica* have recently increased to an annual basis, connected to the non-canonical contamination of pre-washed salad.

Preventing bacterial food poisoning

Proper storage and refrigeration of food help in the prevention of food poisoning.

The government is primarily responsible for the prevention of food-borne illnesses. This is achieved through the implementation of strict hygiene rules and a public veterinary service that monitors animal products throughout the food chain, from farming to delivery in shops and restaurants. This regulation includes:

- traceability: the origin of the ingredients (farm of origin, identification of the crop or animal) and where and when it has been processed must be known in the final product; in this way, the origin of the disease can be traced and resolved (and possibly penalized), and the final products can be removed from sale if a problem is detected;
- enforcement of hygiene procedures such as HACCP and the "cold chain";
- power of control and of law enforcement of veterinarians.

In August 2006, the United States Food and Drug Administration approved phage therapy which involves spraying meat with viruses that infect bacteria, and thus preventing infection. This has raised concerns because without mandatory labeling, consumers would not know that meat and poultry products have been treated with the spray.[20]

At home, prevention mainly consists of good food safety practices. Many forms of bacterial poisoning can be prevented by cooking food sufficiently, and either eating it quickly or refrigerating it effectively.[2] Many toxins, however, are not destroyed by heat treatment.

Techniques that help prevent food borne illness in the kitchen are hand washing, rinsing produce,[21] preventing cross-contamination, proper storage, and maintaining cooking temperatures. In general, freezing or refrigerating prevents virtually all bacteria from growing, and heating food sufficiently kills parasites, viruses, and most bacteria. Bacteria grow most rapidly at the range of temperatures between 40 and 140°F (4 and 60°C), called the "danger zone". Storing food below or above the "danger zone" can effectively limit the production of toxins. For storing leftovers, the food must be put in shallow containers for quick cooling and must be refrigerated within two hours. When food is reheated, it must reach an internal temperature of 165°F (74°C) or until hot or steaming to kill bacteria.

Mycotoxins and alimentary mycotoxicoses

The term alimentary mycotoxicosis refers to the effect of poisoning by mycotoxins through food consumption. The term mycotoxin is usually reserved for the toxic chemical products produced by fungi that readily colonize crops. Mycotoxins sometimes have important effects on human and animal health. For example, an outbreak which occurred in the UK during 1960 caused the death of 100,000 turkeys which had consumed aflatoxin-contaminated peanut meal. In the USSR in World War II, 5,000 people died due to alimentary toxic aleukia (ALA). The common foodborne Mycotoxins include:

- Aflatoxins – originating from *Aspergillus parasiticus* and *Aspergillus flavus*. They are frequently found in tree nuts, peanuts, maize, sorghum and other oilseeds, including corn and cottonseeds. The pronounced forms of aflatoxins are those of B1, B2, G1, and G2, amongst which Aflatoxin B1 predominantly targets the liver, which will result in necrosis, cirrhosis, and carcinoma. In the US, the acceptable level of total aflatoxins in foods is less than 20 μg/kg, except for Aflatoxin M1 in milk, which should be less than 0.5 μg/kg.
- The official document can be found at FDA's website.
- Altertoxins – are those of alternariol (AOH), alternariol methyl ether (AME), altenuene (ALT), altertoxin-1 (ATX-1), tenuazonic acid (TeA), and radicinin (RAD), originating from *Alternaria* spp. Some of the toxins can be present in sorghum, ragi, wheat and tomatoes. Some research has shown that the toxins can be easily cross-contaminated between grain commodities, suggesting that manufacturing and storage of grain commodities is a critical practice.
- Citrinin
- Citreoviridin
- Cyclopiazonic acid

- Cytochalasins
- Ergot alkaloids / ergopeptine alkaloids – ergotamine
- Fumonisins – Crop corn can be easily contaminated by the fungi *Fusarium moniliforme*, and its fumonisin B1 will cause leukoencephalomalacia (LEM) in horses, pulmonary edema syndrome (PES) in pigs, liver cancer in rats and esophageal cancer in humans.[33][34] For human and animal health, both the FDA and the EC have regulated the content levels of toxins in food and animal feed.
- Fusaric acid
- Fusarochromanone
- Kojic acid
- Lolitrem alkaloids
- Moniliformin
- 3-Nitropropionic acid
- Nivalenol
- Ochratoxins – In Australia, The Limit of Reporting (LOR) level for ochratoxin A (OTA) analyses in 20th Australian Total Diet Survey was 1 µg/kg, whereas the EC restricts the content of OTA to 5 µg/kg in cereal commodities, 3 µg/kg in processed products and 10 µg/kg in dried vine fruits.
- Oosporeine
- Patulin – Currently, this toxin has been advisably regulated on fruit products. The EC and the FDA have limited it to under 50 µg/kg for fruit juice and fruit nectar, while limits of 25 µg/kg for solid-contained fruit products and 10 µg/kg for baby foods were specified by the EC.
- Phomopsins
- Sporidesmin A
- Sterigmatocystin
- Tremorgenic mycotoxins – Five of them have been reported to be associated with molds found in fermented meats. These are fumitremorgen B, paxilline, penitrem A, verrucosidin, and verruculogen.[40]
- Trichothecenes – sourced from *Cephalosporium*, *Fusarium*, *Myrothecium*, *Stachybotrys*, and *Trichoderma*. The toxins are usually found in molded maize, wheat, corn, peanuts and rice, or animal feed of hay and straw. Four trichothecenes, T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), and deoxynivalenol (DON) have been most commonly encountered by humans and animals. The consequences of oral intake of, or dermal exposure to, the toxins will result in alimentary toxic

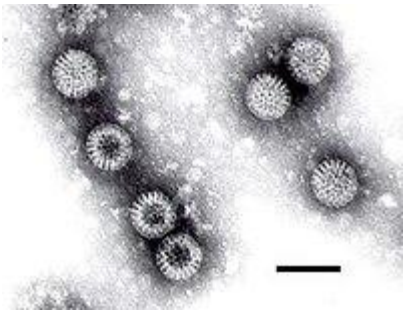
aleukia, neutropenia, aplastic anemia, thrombocytopenia and/or skin irritation. In 1993, the FDA issued a document for the content limits of DON in food and animal feed at an advisory level. In 2003, US published a patent that is very promising for farmers to produce a trichothecene-resistant crop.

- Zearalenone
- Zearalenols

Viruses[edit]

Viral infections make up perhaps one third of cases of food poisoning in developed countries. In the US, more than 50% of cases are viral and noroviruses are the most common foodborne illness, causing 57% of outbreaks in 2004. Foodborne viral infection are usually of intermediate (1–3 days) incubation period, causing illnesses which are self-limited in otherwise healthy individuals; they are similar to the bacterial forms described above.

- Enterovirus
- Hepatitis A is distinguished from other viral causes by its prolonged (2–6 week) incubation period and its ability to spread beyond the stomach and intestines into the liver. It often results in jaundice, or yellowing of the skin, but rarely leads to chronic liver dysfunction. The virus has been found to cause infection due to the consumption of fresh-cut produce which has fecal contamination.[48][49]
- Hepatitis E
- Norovirus



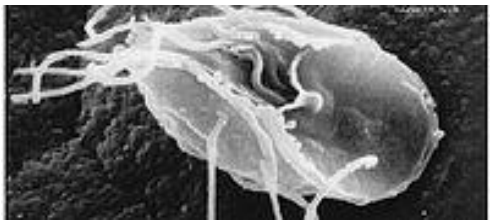
RotavirusRotavirus

Parasites

Most foodborne parasites are zoonoses.

- Platyhelminthes:[*citation needed*]
  - *Diphyllobothrium* sp.
  - *Nanophyetus* sp.
  - *Taenia saginata*

- *Taenia solium*
- *Fasciola hepatica*
- See also: Tapeworm and Flatworm

- Nematode:[51]
  - *Anisakis* sp.
  - *Ascaris lumbricoides*
  - *Eustrongylides* sp.
  - *Toxocara*
  - *Trichinella spiralis*
  - *Trichuris trichiura*
- Protozoa:[*citation needed*]
  - *Acanthamoeba* and other free-living amoebae
  - *Cryptosporidiosis*
  - *Cyclospora cayetanensis*
  - *Entamoeba histolytica*
  - 
  - *Giardia lamblia* *Giardia lamblia*
  - *Sarcocystis hominis*
  - *Sarcocystis suihominis*
  - *Toxoplasma*

Natural toxins

Several foods can naturally contain toxins, many of which are not produced by bacteria. Plants in particular may be toxic; animals which are naturally poisonous to eat are rare. In evolutionary terms, animals can escape being eaten by fleeing; plants can use only passive defenses such as poisons and distasteful substances, for example capsaicin in chili peppers and pungent sulfur compounds in garlic and onions. Most animal poisons are not synthesised by the animal, but acquired by eating poisonous plants to which the animal is immune, or by bacterial action.

- Alkaloids
- Ciguatera poisoning
- Grayanotoxin (honey intoxication)

- Hormones from the thyroid glands of slaughtered animals (especially triiodothyronine in cases of *hamburger thyrotoxicosis* or *alimentary thyrotoxicosis*)
- Mushroom toxins
- Phytohaemagglutinin (red kidney bean poisoning; destroyed by boiling)
- Pyrrolizidine alkaloids
- Shellfish toxin, including paralytic shellfish poisoning, diarrhetic shellfish poisoning, neurotoxic shellfish poisoning, amnesic shellfish poisoning and ciguatera fish poisoning
- Scombrotoxin
- Tetrodotoxin (fugu fish poisoning)

Some plants contain substances which are toxic in large doses, but have therapeutic properties in appropriate dosages.

- Foxglove contains cardiac glycosides.
  - Poisonous hemlock (conium) has medicinal uses.[*citation needed*]

Other pathogenic agents

- Prions, resulting in Creutzfeldt–Jakob disease (CJD) and its variant (vCJD)

"Ptomaine poisoning" misconception

Ptomaine poisoning was a myth that persisted in the public consciousness, in newspaper headlines, and legal cases as an official diagnosis, decades after it had been scientifically disproven in the 1910s.

In the 19th century, the Italian chemist Francesco Selmi, of Bologna, introduced the generic name *ptomaine* (from Greek *ptōma*, "fall, fallen body, corpse") for alkaloids found in decaying animal and vegetable matter, especially (as reflected in their names) putrescine and cadaverine. The 1892 *Merck's Bulletin* stated, "We name such products of bacterial origin ptomaines; and the special alkaloid produced by the comma bacillus is variously named Cadaverine, Putrescine, etc." While *The Lancet* stated, "The chemical ferments produced in the system, the... ptomaines which may exercise so disastrous an influence." It is now known that the "disastrous... influence" is due to the direct action of bacteria and only slightly due to the alkaloids. Thus, the use of the phrase "ptomaine poisoning" is now obsolete.

At a Communist political convention in Massillon, Ohio, and aboard a cruise ship in Washington, D.C., tainted potato salad sickened hundreds in separate incidents during a single week in 1932, drawing national attention to the dangers of so-called "ptomaine poisoning" in the pages of the American news weekly, *Time*. In 1944,

another newspaper article reported that over 150 people in Chicago were hospitalized with ptomaine poisoning, apparently from rice pudding served by a restaurant chain.

India

In India, *Entamoeba* is the most common cause of food illness, followed by *Campylobacter* bacteria, *Salmonella* bacteria, *E. coli* bacteria, and norovirus. According to statistics, food poisoning was the second most common cause of infectious disease outbreak in India in 2017. The numbers of outbreaks have increased from 50 in 2008 to 242 in 2017.

Organizations[edit]

The World Health Organization Department of Food Safety and Zoonoses (FOS) provides scientific advice for organizations and the public on issues concerning the safety of food. Its mission is to lower the burden of foodborne disease, thereby strengthening the health security and sustainable development of Member States. Foodborne and waterborne diarrhoeal diseases kill an estimated 2.2 million people annually, most of whom are children. WHO works closely with the Food and Agriculture Organization of the United Nations (FAO) to address food safety issues along the entire food production chain—from production to consumption—using new methods of risk analysis. These methods provide efficient, science-based tools to improve food safety, thereby benefiting both public health and economic development.

International Food Safety Authorities Network (INFOSAN)

The International Food Safety Authorities Network (INFOSAN) is a joint program of the WHO and FAO. INFOSAN has been connecting national authorities from around the globe since 2004, with the goal of preventing the international spread of contaminated food and foodborne disease and strengthening food safety systems globally.

This is done by:

1. Promoting the rapid exchange of information during food safety events;
2. Sharing information on important food safety issues of global interest;
3. Promoting partnership and collaboration between countries; and
4. Helping countries strengthen their capacity to manage food safety risks.

Membership to INFOSAN is voluntary, but is restricted to representatives from national and regional government authorities and requires an official letter of designation. INFOSAN seeks to reflect the multidisciplinary nature of food safety

and promote intersectoral collaboration by requesting the designation of Focal Points in each of the respective national authorities with a stake in food safety, and a single Emergency Contact Point in the national authority with the responsibility for coordinating national food safety emergencies; countries choosing to be members of INFOSAN are committed to sharing information between their respective food safety authorities and other INFOSAN members. The operational definition of a food safety authority includes those authorities involved in: food policy; risk assessment; food control and management; food inspection services; foodborne disease surveillance and response; laboratory services for monitoring and surveillance of foods and foodborne diseases; and food safety information, education and communication across the farm-to-table continuum.

Prioritisation of foodborne pathogens

The Food and Agriculture Organization of the United Nations and The World Health Organization have published a global ranking of foodborne parasites using a multicriteria ranking tool concluding that *Taenia solium* was the most relevant, followed by *Echinococcus granulosus*, *Echinococcus multilocularis*, and *Toxoplasma gondii*. The same method was used regionally to rank the most important foodborne parasites in Europe ranking *Echinococcus multilocularis* of highest relevance, followed by *Toxoplasma gondii* and *Trichinella spiralis*.

Sexually Transmitted Diseases:

Sexually transmitted infections (**STIs**), also referred to as **sexually transmitted diseases** (**STDs**), are infections that are commonly spread by sexual activity, especially vaginal intercourse, anal sex and oral sex. Many times STIs initially do not cause symptoms. This results in a greater risk of passing the disease on to others. Symptoms and signs of disease mayinclude vaginal discharge, penile discharge, ulcers on or around the genitals, and pelvic pain. STIs can be transmitted to an infant before or during childbirth and may result in poor outcomes for the baby. Some STIs may cause problems with the ability to get pregnant.

More than 30 different bacteria, viruses, and parasites can be transmitted through sexual activity. Bacterial STIs include chlamydia, gonorrhea, and syphilis.] Viral STIs include genital herpes, HIV/AIDS, and genital warts. Parasitic STIs include trichomoniasisWhile usually spread by sex, some STIs can be spread by non-sexual contact with donor tissue, blood, breastfeeding, or during childbirth. STI diagnostic tests are usually easily available in the developed world, but this is often not the case in the developing world.

The most effective way of preventing STIs is by not having sex. Some vaccinations

may also decrease the risk of certain infections including hepatitis B and some types of HPV. Safer sex practices such as use of condoms, having a smaller number of sexual partners, and being in a relationship where each person only has sex with the other also decreases the risk. Circumcision in adult males may be effective to prevent some infections. During school, comprehensive sex education may also be useful. Most STIs are treatable or curable. Of the most common infections, syphilis, gonorrhea, chlamydia, and trichomoniasis are curable, while herpes, hepatitis B, HIV/AIDS, and HPV are treatable but not curable. Resistance to certain antibiotics is developing among some organisms such as gonorrhea

In 2015, about 1.1 billion people had STIs other than HIV/AIDSAbout 500 million were infectedwith either syphilis, gonorrhea, chlamydia or trichomoniasisAt least an additional 530 million people have genital herpes and 290 million women have human papillomavirus STIs other than HIV resulted in 108,000 deaths in 2015 In the United States there were 19 million new cases of sexually transmitted infections in 2010. Historical documentation of STIs date back to at least the Ebers papyrus around 1550 BC and the Old Testament. There is often shame and stigma associated with these infections. The term *sexually transmitted infection* is generally preferred over *sexually transmitted disease* or *venereal disease*, as it includes those who do not have symptomatic disease.

SIGN AND SYMPTOMS : Not all STIs are symptomatic, and symptoms may not appear immediately after infection. In some instances a disease can be carried with no symptoms, which leaves a greater risk of passing the disease on to others. Depending on the disease, someuntreated STIs can lead to infertility, chronic pain or death.

The presence of an STI in prepubescent children may indicate sexual abuse.

Transmission

A sexually transmitted infection present in a pregnant woman may be passed on to the infantbefore or after birth.

	Known risks	Possible
Performing oral sex on a man	• Throat chlamydia[17] • Throat gonorrhea[17] (25–30%) • Herpes (rare) • HPV[18] • Syphilis[17] (1%)[19]	• Hepatitis B (low risk)[20] • HIV (0.01%)[21] • Hepatitis C (unknown)
Performing oral sex on a woman	• Herpes • HPV[18]	• Throat gonorrhea[17] • Throat chlamydia[17]
Receiving oral sex—man	• Chlamydia • Gonorrhea[17] • Herpes • Syphilis[17] (1%)[19]	• HPV
Receiving oral sex—woman	• Herpes	• HPV • Bacterial vaginosis[17] • Gonorrhea[17]

Vaginal sex—man	• Chlamydia (30–50%)[20] • Crabs • Scabies • Gonorrhea (22%)[22] • Hepatitis B • Herpes (0.07% for HSV-2)[23] • HIV (0.05%)[21][23] • HPV (high: around 40–50%)[24] • Mycoplasma hominis infection[30][31][32][33][34] • *Mycoplasma genitalium*[35][36][37] • Syphilis • Trichomoniasis • *Ureaplasma infection*[38][39][34]	• Hepatitis C
Vaginal sex—woman	• Chlamydia (30–50%)[20] • Crabs • Scabies • Gonorrhea (47%)[25] • Hepatitis B (50–70%) • Herpes • HIV (0.1%)[21] • HPV (high;[20] around 40–50%)[24] • Mycoplasma hominis infection[30][31][34] • Syphilis • Trichomoniasis • Ureaplasma infection[38][39][34]	• Hepatitis C

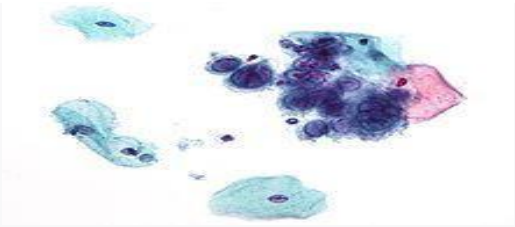
Bacterial

- Chancroid (*Haemophilus ducreyi*)
- Chlamydia (*Chlamydia trachomatis*)
- Gonorrhea (*Neisseria gonorrhoeae*), colloquially known as "the clap"
- Granuloma inguinale or (*Klebsiella granulomatis*)
- *Mycoplasma genitalium*
- *Mycoplasma hominis*
- Syphilis (*Treponema pallidum*)

- Ureaplasma infection

Fungal

- Candidiasis (yeast infection)



Micrograph showing the viral cytopathic effect of herpes (ground glass nuclear inclusions, multi-nucleation). Pap test. Pap stain.

Viral

- Viral hepatitis (Hepatitis B virus)—saliva, venereal fluids.(Note: H transmission.
- Herpes simplex (Herpes simplex virus 1, 2) skin and mucosal, transmissible with or without visible blisters
- HIV (*Human Immunodeficiency Virus*)—venereal fluids, semen, breast milk, blood
- HPV (*Human Papillomavirus*)—skin and mucosal contact. 'High risk' types of HPV cause almost all cervical cancers, as well as some anal, penile, and vulvar cancer. Some other typesof HPV cause genital warts.
- Molluscum contagiosum (molluscum contagiosum virus MCV)—close contact

Parasites

- Crab louse, colloquially known as "crabs" or "pubic lice" (*Pthirus pubis*). The infestation andaccompanying inflammation is Pediculosis pubis
- Scabies (*Sarcoptes scabiei*)
- Trichomoniasis (*Trichomonas vaginalis*), colloquially known as "trich"

Main types:

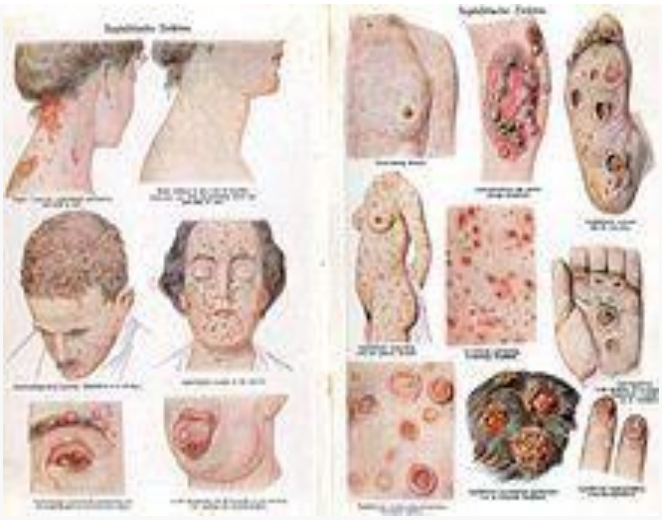
Sexually transmitted infections include: Chlamydia is a sexually transmitted infection caused bythe bacterium *Chlamydia trachomatis*. In women, symptoms may include abnormal vaginal discharge, burning during urination, and bleeding in between periods, although most women do not experience any symptoms. Symptoms in men include pain when urinating, and abnormal discharge from their penis. If left untreated in both men and women, Chlamydia can infect the urinary tract and potentially lead to pelvic inflammatory disease (PID). PID can cause serious problems during pregnancy and even has the potential to cause infertility. It can

cause a woman to have a potentially deadly ectopic pregnancy, in which the egg implants outside of the uterus. However, Chlamydia can be cured with antibiotics.

- The two most common forms of herpes are caused by infection with herpes simplex virus (HSV). HSV-1 is typically acquired orally and causes cold sores, HSV-2 is usually acquired during sexual contact and affects the genitals, and however either strain may affect either site. Some people are asymptomatic or have very mild symptoms. Those that do experience symptoms usually notice them 2 to 20 days after exposure which last 2 to 4 weeks. Symptoms can include small fluid-filled blisters, headaches, backaches, itching or tingling sensations in the genital or anal area, pain during urination, Flu like symptoms, swollen glands, or fever. Herpes is spread through skin contact with a person infected with the virus. The virus affects the areas where it entered the body. This can occur through kissing, vaginal intercourse, oral sex or anal sex. The virus is most infectious during times when there are visible symptoms, however those who are asymptomatic can still spread the virus through skin contact. The initial infection and symptoms are usually the most severe because the body does not have any antibodies built up. After the primary attack, one might have recurring attacks that are milder or might not even have future attacks. There is no cure for the disease but there are antiviral medications that treat its symptoms and lower the risk of transmission (Valtrex). Although HSV-1 is typically the "oral" version of the virus, and HSV-2 is typically the "genital" version of the virus, a person with HSV-1 orally CAN transmit that virus to their partner genitally. The virus, either type, will settle into a nerve bundle either at the top of the spine, producing the "oral" outbreak, or a second nerve bundle at the base of the spine, producing the genital outbreak.
- The human papillomavirus (HPV) is the most common STI in the United States. There are more than 40 different strands of HPV and many do not cause any health problems. In 90% of cases the body's immune system clears the infection naturally within 2 years. Some cases may not be cleared and can lead to genital warts (bumps around the genitals that can be small or large, raised or flat, or shaped like cauliflower) or cervical cancer and other HPV related cancers. Symptoms might not show up until advanced stages. It is important for women to get pap smears in order to check for and treat cancers. There are also two vaccines available for women (Cervarix and Gardasil) that protect against the types of HPV that cause cervical cancer. HPV can be passed through genital-to-genital contact as well as during oral sex. It is important to remember that the infected partner might not have any symptoms.

• Gonorrhea is caused by bacterium that lives on moist mucous membranes in the urethra, vagina, rectum, mouth, throat, and eyes. The infection can spread through contact with the penis, vagina, mouth or anus. Symptoms of gonorrhea usually appear 2 to 5 days after contact with an infected partner however, some men might not notice symptoms for up to a month. Symptoms in men include burning and pain while urinating, increased urinary frequency, discharge from the penis (white, green, or yellow in color), red or swollenurethra, swollen or tender testicles, or sore throat. Symptoms in women may include vaginal discharge, burning or itching while urinating, painful sexual intercourse, severe pain in lowerabdomen (if infection spreads to fallopian tubes), or fever (if infection spreads to fallopian tubes); however, many women do not show any symptoms.

• There are some antibiotic resistant strains for Gonorrhea but most cases can be cured with antibiotics.



Secondary syphilis

• Syphilis is an STI caused by a bacterium. Untreated, it can lead to complications and death. Clinical manifestations of syphilis include the ulceration of the urogenital tract, mouth or rectum; if left untreated the symptoms worsen. In recent years, the prevalence of syphilis has declined in Western Europe, but it has increased in Eastern Europe (former Soviet states). A high incidence of syphilis can be found in places such as Cameroon, Cambodia, Papua New Guinea. Syphilis infections are increasing in the United States.

• Trichomoniasis is a common STI that is caused by infection with a protozoan parasite called *Trichomonas vaginalis*. Trichomoniasis affects both women and men, but symptoms are more common in women. Most patients are treated with an antibiotic called metronidazole, which is very effective.

- HIV (human immunodeficiency virus) damages the body's immune system, which interferes with its ability to fight off disease-causing agents. The virus kills CD4 cells, which are white blood cells that help fight off various infections. HIV is carried in body fluids, and is spread by sexual activity. It can also be spread by contact with infected blood, breast feeding, childbirth, and from mother to child during pregnancy. When HIV is at its most advanced stage, an individual is said to have AIDS (acquired immunodeficiency syndrome). There are different stages of the progression of and HIV infection. The stages include primary infection, asymptomatic infection, symptomatic infection, and AIDS. In the primary infection stage, an individual will have flu like symptoms (headache, fatigue, fever, muscle aches) for about 2 weeks. In the asymptomatic stage, symptoms usually disappear, and the patient can remain asymptomatic for years. When HIV progresses to the symptomatic stage, the immune system is weakened, and has a low cell count of CD4+ T Cells. When the HIV infection becomes life-threatening, it is called AIDS. People with AIDS fall prey to opportunistic infections and die as a result. When the disease was first discovered in the 1980s, those who had AIDS were not likely to live longer than a few years. There are now antiretroviral drugs (ARVs) available to treat HIV infections. There is no known cure forHIV or AIDS but the drugs help suppress the virus. By suppressing the amount of virus inthe body, people can lead longer and healthier lives. Even though their virus levels may be low they can still spread the virus to others.

Viruses in semen

Twenty-seven different viruses have been identified in semen. Information on whether or not transmission occurs or whether the viruses cause disease is uncertain. Some of these microbesare known to be sexually transmitted. Those found in semen are listed by the CDC.

Microbes known to be sexually transmissible (but not generally considered STIs) include:

- Marburg virus – Virus in semen for seven weeks after clinical recovery.
- HTLV (both types 1 and 2) – Sexually transmissible, consumption of breastmilk brea

Pathophysiology

Many STIs are (more easily) transmitted through the mucous membranes of the penis, vulva, rectum, urinary tract and (less often—depending on type of infection) the mouth, throat, respiratory tract and eyes. The visible membrane covering the head of the penis is a mucous membrane, though it produces no mucus (similar to the lips of the mouth). Mucous membranes differ from skin in that they allow

certain pathogens into the body. The amount of contact with infective sources which causes infection varies with each pathogen but in all cases, a disease may result from even light contact from fluid carriers like venereal fluids ontoa mucous membrane.

Some STIs such as HIV can be transmitted from mother to child either during pregnancy or breastfeeding.

Healthcare professionals suggest safer sex, such as the use of condoms, as a reliable way of decreasing the risk of contracting sexually transmitted diseases during sexual activity, but safer sex cannot be considered to provide complete protection from an STI.

The transfer of and exposure to bodily fluids, such as blood transfusions and other blood products, sharing injection needles, needle-stick injuries (when medical staff are inadvertently jabbed or pricked with needles during medical procedures), sharing tattoo needles, and childbirth are other avenues of transmission. These different means put certain groups, such as medical workers, and haemophiliacs and drug users, particularly at risk.

It is possible to be an asymptomatic carrier of sexually transmitted diseases. In particular, sexually transmitted diseases in women often cause the serious condition of pelvic inflammatory disease.

Diagnosis

Testing may be for a single infection, or consist of a number of tests for a range of STIs, including tests for Syphilis, Trichomonas, Gonorrhea, Chlamydia, herpes, hepatitis and HIV. No procedure tests for all infectious agents.

STI tests may be used for a number of reasons:

- as a diagnostic test to determine the cause of symptoms or illness
- as a screening test to detect asymptomatic or presymptomatic infections
- as a check that prospective sexual partners are free of disease before they engage in sex without safer sex precautions (for example, when starting a long term mutually monogamoussexual relationship, in fluid bonding, or for procreation).
- as a check prior to or during pregnancy, to prevent harm to the baby
- as a check after birth, to check that the baby has not caught an STI from the mother
- to prevent the use of infected donated blood or organs
- as part of the process of contact tracing from a known infected individual
- as part of mass epidemiological surveillance

Early identification and treatment results in less chance to spread disease, and for some conditions may improve the outcomes of treatment. There is often a window period after

initial infection during which an STI test will be negative. During this period, the infection may be transmissible. The duration of this period varies depending on the infection and the test. Diagnosis may also be delayed by reluctance of the infected person to seek a medical professional. One report indicated that people turn to the Internet rather than to a medical professional for information on STIs to a higher degree than for other sexual problems.

PREVENTION

Strategies for reducing STI risk include: vaccination, mutual monogamy, reducing the number ofsexual partners, and abstinence. Behavioral counseling for all sexually active adolescents and for adults who are at increased risk. Such interactive counseling, which can be resource intensive, is directed at a person's risk, the situations in which risk occurs, and the use of personalized goal-setting strategies.

The most effective way to prevent sexual transmission of STIs is to avoid contact of body parts or fluids which can lead to transfer with an infected partner. Not all sexual activities involve contact: cybersex, phonesex or masturbation from a distance are methods of avoiding contact.

Proper use of condoms reduces contact and risk. Although a condom is effective in limiting exposure, some disease transmission may occur even with a condom.

Both partners can get tested for STIs before initiating sexual contact, or before resuming contact if a partner engaged in contact with someone else. Many infections are not detectable immediately after exposure, so enough time must be allowed between possible exposures and testing for the tests to be accurate. Certain STIs, particularly certain persistent viruses like HPV, may be impossible to detect.

Some treatment facilities utilize in-home test kits and have the person return the test for follow- up. Other facilities strongly encourage that those previously infected return to ensure that the infection has been eliminated. Novel strategies to foster re-testing have been the use of text messaging and email as reminders. These types of reminders are now used in addition to phone calls and letters. After obtaining a sexual history, a healthcare provider can encourage risk reduction by providing prevention counseling. Prevention counseling is most effective if provided in a nonjudgmental and empathetic manner appropriate to the person's culture, language, gender, sexual orientation, age, and developmental level. Prevention counseling for STIs is usually offered to all sexually active adolescents and to all adults who have received a diagnosis, have had an STI in

the past year, or have multiple sex partners.

Vaccines

Vaccines are available that protect against some viral STIs, such as Hepatitis A, Hepatitis B, and some types of HPV. Vaccination before initiation of sexual contact is advised to assure maximal protection. The development of vaccines to protect against gonorrhea is ongoing.

Condoms

Condoms and female condoms only provide protection when used properly as a barrier, and only to and from the area that they cover. Uncovered areas are still susceptible to many STIs.

In the case of HIV, sexual transmission routes almost always involve the penis, as HIV cannot spread through unbroken skin; therefore, properly shielding the penis with a properly worn condom from the vagina or anus effectively stops HIV transmission. An infected fluid to broken skin borne direct transmission of HIV would not be considered "sexually transmitted", but can still theoretically occur during sexual contact. This can be avoided simply by not engaging in sexual contact when presenting open, bleeding wounds Other STIs, even viral infections, can be prevented with the use of latex, polyurethane or polyisoprene condoms as a barrier. Some microorganisms and viruses are small enough to pass through the pores in natural skin condoms, but are still too large to pass through latex or synthetic condoms.

Proper male condom usage entails:

- Not putting the condom on too tight at the tip by leaving 1.5 centimetres (0.6 in) roomfor ejaculation. Putting the condom on too tightly can and often does lead to failure.
- Wearing a condom too loose can defeat the barrier
- Avoiding inverting or spilling a condom once worn, whether it has ejaculate in it or not
- If a user attempts to unroll the condom, but realizes they have it on the wrong side, then this condom may not be effective
- Being careful with the condom if handling it with long nails
- Avoiding the use of oil-based lubricants (or anything with oil in it) with latex condoms, as oil can eat holes into them
- Using flavored condoms for oral sex only, as the sugar in the flavoring can lead to yeast infections if used to penetrate

In order to best protect oneself and the partner from STIs, the old condom and its contents are to be treated as infectious and properly disposed of. A new condom is used for each act of intercourse, as multiple usage increases the chance of breakage, defeating the effectiveness as a barrier. In case of female condoms, the device consists of two rings, one in each terminal portion.The larger ring should fit snugly over the cervix and the smaller ring remains outside the vagina, covering the vulva. This system provides some protection of the external genitalia.

Other

The cap was developed after the cervical diaphragm. Both cover the cervix and the main difference between the diaphragm and the cap is that the latter must be used only once, using a new one in each sexual act. The diaphragm, however, can be used more than once. These two devices partially protect against STIs (they do not protect against HIV).
Researchers had hoped that nonoxynol-9, a vaginal microbicide would help decrease STI risk. Trials, however, have found it ineffectiveand it may put women at a higher risk of HIV infection.

Epidemiology

In 2008, it was estimated that 500 million people were infected with either syphilis, gonorrhea, chlamydia or trichomoniasis. At least an additional 530 million people have genital herpes and 290 million women have human papillomavirus. STIs other than HIV resulted in 142,000 deaths in 2013. In the United States there were 19 million new cases of sexually transmitted infectionsin 2010.

In 2010, 19 million new cases of sexually transmitted infections occurred in women in the United States. A 2008 CDC study found that 25–40% of U.S. teenage girls has a sexually transmitted disease. Out of a population of almost 295,270,000 peoplethere was were 110 million new and existing cases of eight sexually transmitted infections.

Over 400,000 sexually transmitted infections were reported in England in 2017, about the same as in 2016, but there were more than 20% increases in confirmed cases of gonorrhoea and syphilis. Since 2008 syphilis cases have risen by 148%, from 2,874 to 7,137, mostly among men who have sex with men. The number of first cases of genital warts in 2017 among girls aged 15– 17 years was just 441, 90% less than in 2009 – attributed to the national human papilloma virus immunisation programme.

AIDS is among the leading causes of death in present-day Sub-Saharan Africa. HIV/AIDS is transmitted primarily via unprotected sexual intercourse. More than 1.1

million persons areliving with HIV/AIDS in the United States, and it disproportionately impacts African Americans.Hepatitis B is also considered a sexually transmitted disease because it can be spread through sexual contact. The highest rates are found in Asia and Africa and lower rates are in the Americas and Europe. Approximately two billion people worldwide have been infected with the hepatitis B virus.

Respiratory Diseases:

Respiratory diseases, or **lung diseases**, are pathological conditions affecting the organs and tissues that make gas exchange difficult in air-breathing animals. They include conditions of the respiratory tract including the trachea, bronchi, bronchioles, alveoli, pleurae, pleural cavity, the nerves and muscles of respiration. Respiratory diseases range from mild and self-limiting, such as the common cold, influenza, and pharyngitis to life-threatening diseases such as bacterial pneumonia, pulmonary embolism, tuberculosis, acute asthma, lung cancer, and severe acute respiratory syndromes, such as COVID-19. Respiratory diseases can be classified in many different ways, including by the organ or tissue involved, by the type and pattern of associated signs and symptoms, or by the cause of the disease.

The study of respiratory disease is known as pulmonology. A physician who specializes in respiratory disease is known as a pulmonologist, a chest medicine specialist, a respiratory medicine specialist, a respirologist or a thoracic medicine specialist.

Obstructive lung disease:

Asthma, chronic bronchitis, bronchiectasis and chronic obstructive pulmonary disease (COPD) are all obstructive lung diseases characterised by airway obstruction. This limits the amount of air that is able to enter alveoli because of constriction of the bronchial tree, due to inflammation. Obstructive lung diseases are often identified because of symptoms and diagnosed with pulmonary function tests such as spirometry. Many obstructive lung diseases are managed by avoiding triggers (such as dust mites or smoking), with symptom control such as bronchodilators, and with suppression of inflammation (such as through corticosteroids) in severe cases. One common cause of COPD including emphysema, and chronic bronchitis, is tobacco smoking, and common causes of bronchiectasis include severe infections and cystic fibrosis. The definitive cause of asthma is not yet known.

Restrictive lung diseases:

Restrictive lung diseases are a category of respiratory disease characterized by a loss

of lung compliance, causing incomplete lung expansion and increased lung stiffness, such as in infants with respiratory distress syndrome. Restrictive lung diseases can be divided into two categories: those caused by intrinsic factors and those caused by extrinsic factors. Restrictive lung diseases yielding from intrinsic factors occur within the lungs themselves, such as tissue death due to inflammation or toxins. Conversely, restrictive lung diseases caused by extrinsic factors result from conditions originating from outside the lungs such as neuromuscular dysfunction and irregular chest wall movements.

Chronic respiratory disease

Chronic respiratory diseases (CRDs) are long-term diseases of the airways and other structures of the lung. They are characterized by a high inflammatory cell recruitment (neutrophil) and/or destructive cycle of infection, (e.g. mediated by *Pseudomonas aeruginosa*). Some of the most common are asthma, chronic obstructive pulmonary disease, and acute respiratory distress syndrome. CRDs are not curable; however, various forms of treatment that help dilate major air passages and improve shortness of breath can help control symptoms and increase the quality of life.

Telerehabilitation for chronic respiratory disease

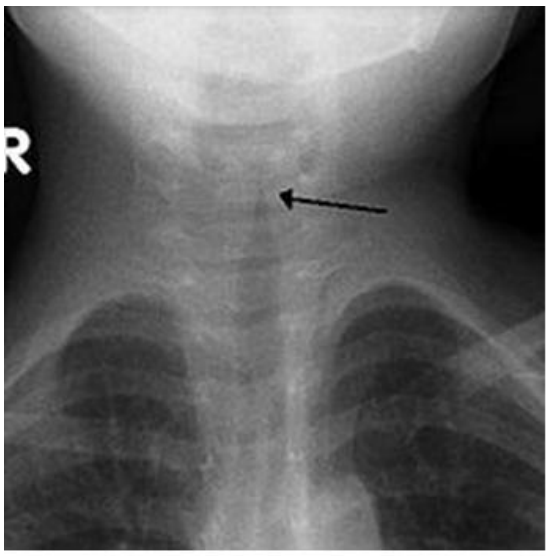
The latest evidence suggests that primary pulmonary rehabilitation and maintenance rehabilitation delivered through telerehabilitation for people with chronic respiratory disease reaches outcomes similar to centre-based rehabilitation. While there are no safety issues identified, the findings are based on evidence limited by a small number of studies.

Respiratory tract infections

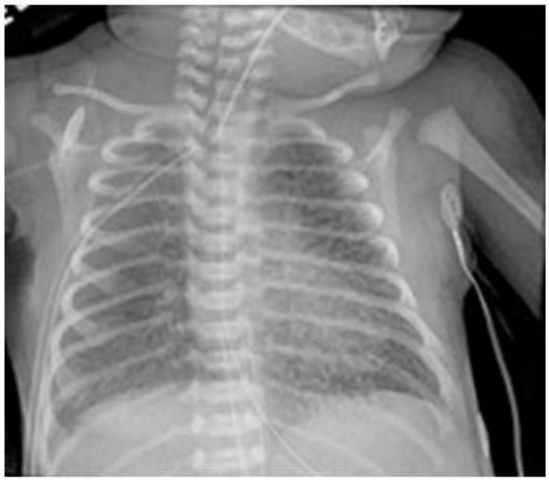
Infections can affect any part of the respiratory system. They are traditionally divided into upper respiratory tract infections and lower respiratory tract infections.

Upper respiratory tract infection

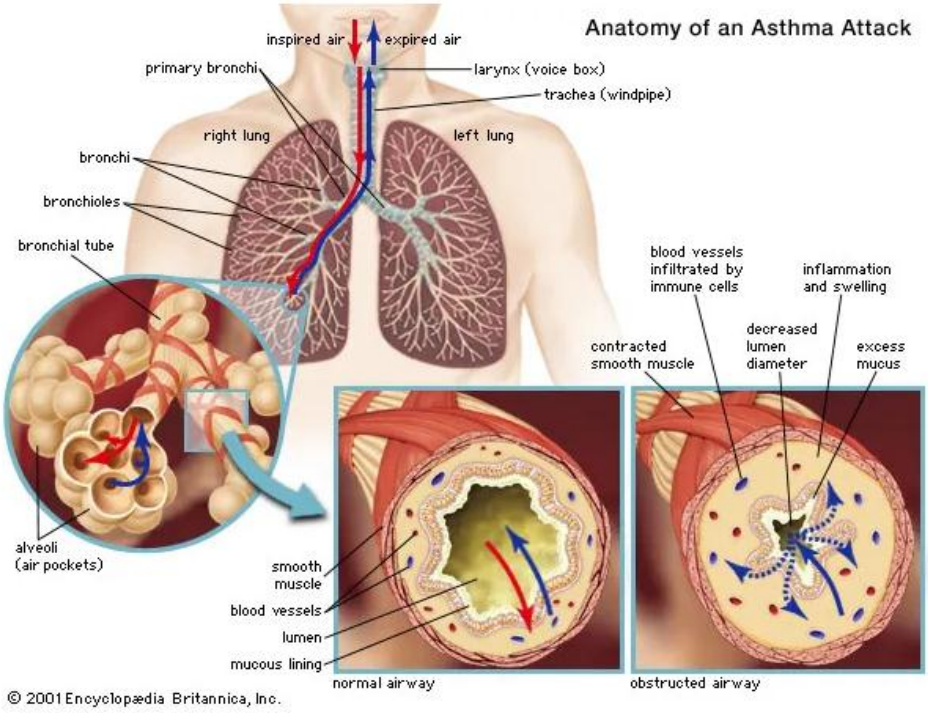
The upper airway is defined as all the structures connecting the glottis to the mouth and nose. The most common upper respiratory tract infection is the common cold. However, infections of specific organs of the upper respiratory tract such as sinusitis, tonsillitis, otitis media, pharyngitis and laryngitis are also considered upper respiratory tract infections.



Classic steeple sign indicating croup



Pulmonary interstitial emphysema



Epiglottitis is a bacterial infection of the larynx which causes life-threatening swelling of the epiglottis with a mortality rate of 7% in adults and 1% in children. *Haemophilus influenzae* is still the primary cause even with vaccinations. Also Streptococcus pyogenes can cause epiglottitis. Symptoms include drooling, stridor, difficulty breathing and swallowing, and a hoarse voice.

Croup (Laryngotracheobronchitis) is a viral infection of the vocal cords typically lasting five to six days. The main symptom is a barking cough and low-grade fever. On an X-ray, croup can be recognized by the "steeple sign", which is a narrowing of the trachea. It most commonly occurs in winter months in children between the ages of 3 months and 5 years. A severe form caused by bacteria is called bacterial tracheitis.

Tonsillitis is swelling of the tonsils by a bacterial or viral infection. This inflammation can lead to airway obstruction. From tonsillitis can come a peritonsillar abscess which is the most common upper airway infection and occurs primarily in young adults. It causes one swelling of one of tonsils pushing the uvula to the unaffected side. Diagnosis is usually made based on the presentation and examination. Symptoms generally include fever, sore throat, trouble swallowing, and sounding like they have a "hot potato" in their mouth.

Lower respiratory tract infection

The most common lower respiratory tract infection is pneumonia, an infection of the lungs which is usually caused by bacteria, particularly *Streptococcus pneumoniae* in Western countries. Worldwide, tuberculosis is an important cause of pneumonia. Other pathogens such as viruses and fungi can cause pneumonia, for example severe acute respiratory syndrome, COVID-19 and pneumocystis pneumonia. Pneumonia may develop complications such as a lung abscess, a round cavity in the lung caused by the infection, or may spread to the pleural cavity.

Poor oral care may be a contributing factor to lower respiratory disease, as bacteria from gum disease may travel through airways and into the lungs.

Upper and lower respiratory tract infection

Primary ciliary dyskinesia is a genetic disorder causing the cilia to not move in a coordinated manner. This causes chronic respiratory infections, cough, and nasal congestion. This can lead to bronchiectasis, which can cause life-threatening breathing issues.

Diagnosis

Respiratory diseases may be investigated by performing one or more of the following tests:

- Biopsy of the lung or pleura
- Blood test
- Bronchoscopy
- Chest X-ray
- CT scan, including high-resolution computed tomography
- Culture of microorganisms from secretions such as sputum
- Ultrasound scanning can be useful to detect fluid such as pleural effusion
- Pulmonary function test
- Ventilation–perfusion scan

Epidemiology

Respiratory disease is a common and significant cause of illness and death around the world. In the US, approximately one billion common colds occur each year. A study found that in 2010, there were approximately 6.8 million emergency department visits for respiratory disorders in the U.S. for patients under the age of 18. In 2012, respiratory conditions were the most frequent reasons for hospital stays among children.

In the UK, approximately 1 in 7 individuals are affected by some form of chronic lung disease, most commonly chronic obstructive pulmonary disease, which includes asthma, chronic bronchitis and emphysema. Respiratory diseases (including lung cancer) are responsible for over 10% of hospitalizations and over 16% of deaths in Canada.

In 2011, respiratory disease with ventilator support accounted for 93.3% of ICU utilization in the United States.

6. Mathematical approach for microbiologists:

Introduction

Life on Earth can be divided into several kingdoms, with the prokaryote domains of the Archaea and the Bacteria standing out by dint of their vast metabolic diversity and versatility which is belied by the paltry morphological variation encountered in these domains (Figure 1). By contrast, a stunning variation in morphology is exhibited by the eukaryote kingdoms Plantae and Animalia, but there it is coupled

Figure 1 *Shapes of unicellular bacteria. The basic shapes are bacilli (rods,* i.e. *cylinders capped by hemispheric domes), cocci (actually short rods), vibrios (curved rods), and spirilla ('corkscrews'). If cells remain attached they can form stacked or grape-like clusters, or elongated filaments (known as trichomes, called streptococci when the individual cells are cocci). Cellular inclusions can distort the bacillus into a club-shape (far right).*

to a range of metabolic variability which seems quite modest when compared to that of the prokaryotes: fungi, plants, and animals make do with a fairly standard core metabolism (and even so, owe much of their metabolic versatility to endosymbiontic prokaryotes), while bacteria are not only able to derive their molecular building blocks from a huge range of compounds, organic as well as inorganic, but also to catalyse reduction–oxidation reactions with a wide range of substrates, deriving energy from inorganic salts, light, or organic compounds. Equally impressive is the ability of microbial life to adapt to extremes in temperature and salinity.

The biochemical versatility of microbes is not only apparent as we survey different species, but also often occurs (albeit in a more restricted form) within the individual cell, which is typically able to adapt its metabolism to the prevailing conditions, often to a far greater extent than are cells in multicellular organisms[4]. We shall return to this contrast in Section 6 and meanwhile fix our attention on the dynamic metabolic adaptability of the individual microbial cell.

Our ultimate goal is to be able to describe quantitatively how the cell responds to changing environmental conditions, not only in terms of the biochemical compounds it produces (which comprise the material substance of their own cells as well as substances secreted into the ambient medium), but also in terms of the compounds that are removed from the environment in the process. To appreciate the importance of this goal, one need only consider the role played by micro-organisms in tempering the impact of global climate

change, for instance their involvement in sequestration of carbon dioxide. Moreover, achieving the goal of accurate quantitative description can aid the optimisation of bioreactors and thus improve the efficiency of biotechnological-industrial applications.

The *dynamic allocation* approach

One way of understanding both the challenges the microbe faces and the ways in which it opposes these challenges, is to conceive of the cell as an agent that is constantly solving an *allocation problem*: that of distributing the molecular building blocks which it has at its disposal among the various kinds of catalytic machinery that are encoded in its genome. At the molecular-mechanistic level, this distribution is dependent on the rates at which the various genes are being transcribed. Thus, to respond in an adaptive manner to changes in the environment, such as increases or decreases in the availability of nutrients, effectively means adjusting these rates of expression (and hence the building block allocation among different types of molecular machinery) to the prevailing conditions. Moreover, we should also expect these gene expression rates to be adjusted in response to the internal status of the cell, in particular the levels of intracellular stores of building blocks.

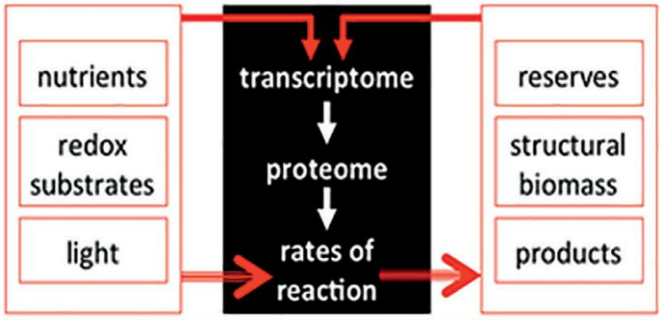


Figure 2 *Flow of information in a microbial cell*

Combining these ideas, we arrive at the information flow diagram shown in Figure 2. Ambient (external) conditions, listed on the left, serve as inputs to gene expression rates, which govern the transcriptome, which determines the proteome, which determines the rates at which the cell's biochemical reactions proceed. Indicated here are the *availability* conditions, which themselves are affected by additional environmental factors such as temperature, pH, water activity, salinity, and so on[2]. In addition to expression-level regulation, there is also a more locally acting mode of regulation in which the activity of the machinery is regulated, often by covalent addition of moieties such as phosphoryl groups.

The realised catalytic activities of the cell result in bioproduction of three major kinds of compounds: reserves; 'structural biomass'; and products that are secreted into the

ambient medium. The densities of the reserves (*i.e.* reserves normalised by structural biomass) also serve as information inputs to this central pathway, closing a regulatory loop.

The meaning of 'structural biomass' may not be immediately clear. Intuitively, it comprises everything inside the cell except the reserves – but this makes for a circularity when we define reserves, in turn, as components that do not belong to the structural biomass. The latter step is not necessary in those cases where reserves can be clearly distinguished on the basis of their biochemical identity, and often also on the basis of the fact that they occur in discrete sub-cellullar structures called *inclusions*, examples of which are shown in Figure 3. However, there are also awkward cases that are less clear-cut and this prompts us to define reserves formally as 'non-structural'. To avoid logical circularity, we then define the structural biomass as the bare minimum compatible with life, corresponding to a state that can be induced experimentally by starving the cell of all essential nutrients. Droop introduced the idea of subsistence biomass (or cell composition, *etc.*) to express the same idea; thus, subsistence biomass and functional-structural biomass are two names for the same concept.

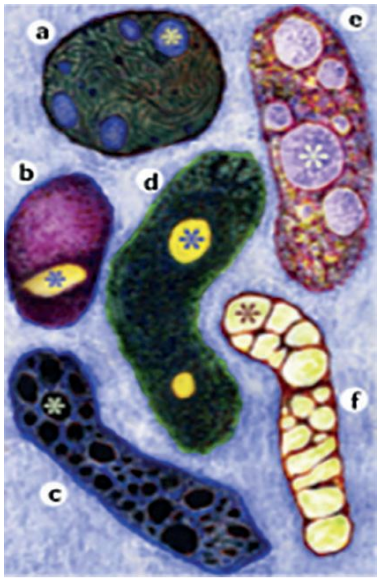


Figure 3 Reserve inclusions. In each cell depicted, an asterisk indicates one of the storage inclusions. *(a) Cyanophycin,* Anabaena variabilis. *(b) Sulfur,* Thermoanaerobacter sulfurigignens. *(c) Glycogen,* Methylacidiphilum fumariolicum. *(d) Polyphosphate,* Campylobacter jejuni. *(e) Polyhydroxybutyrate,* Rhodovibrio sodomensis. *(f) Triacylglycerol,* Rhodococcus opacus

The central regulatory pathway in Figure 2 has been depicted in a 'black box' to call to mind that these steps have traditionally not been readily observable. By contrast, the quantities to the left and the right were more easily measured, and hence classic models of microbial growth and metabolism have always focussed on interrelating these 'white

box' quantities. With the advent of 'high through- put' molecular-biological techniques, this black box has become more transparent, although there remain serious limitations as regards the achievable resolution, *e.g.* spatial, temporal, and in terms of molecular speciation.

Our general goal is to formulate mathematical models that accurately describe the relationships between the quantities enumerated in Figure 2. Our discussion thus far suggests that we sharpen this goal with the following objectives: first, we wish to have a theory that is sufficiently flexible to accommodate (and take advantage of) any data that may be available regarding what has been depicted as a 'black box' in Figure 2; second, we do not want to lose contact with the classic models that deal with 'white box' quantities only; and finally, we wish to have a theory that satisfies fundamental physicochemical laws and constraints, such as conservation principles and biochemical stoichiometry.

We believe that approaching the subject as an allocation problem is a natural and elegant way of fulfilling all of these desiderata. At the heart lies a simple principle, expressed by the following equation:

$$\alpha_i = r_i / r_\Sigma \qquad (1)$$

where α_i is the fraction of molecular building blocks devoted to the synthesis of machinery of type *i*, r_i is a *regulatory law* (or *r-function*, for short), and $r_\Sigma = \sum_i r_I$ is a normalising constant which assures that $\alpha_i \in [0, 1]$ and $\sum_i \alpha_i = 1$. This approach based on the perspective of 'allocation' or 'investment' has been gaining widespread acceptance in recent years, no doubt prompted by advances in high-throughput transcriptomics and proteomics which make this point of view seem almost self- evident.

The indexing in Eqn (1) can be fine-grained, with *i* corresponding to one particular protein, or more coarse-grained, with *i* corresponding to a co-regulated class of proteins, all involved in a particular metabolic or physiological function. The quantity r_i may be a function of one or more quantities describing the cell's internal state, or its ambient medium. These functions may be chosen so as to let the resulting overall relationships accord with the classic models, or alternatively, r_i may be linked to transcriptomic data.

The latter connection is made explicit if we imagine r_i as the amount of mRNA (per cell) encoding machinery if type *i*; Eqn (1) then describes the fraction of 'ribosome time' being dedicated to machinery of type *i*. This correspondence is, incidentally, rather imperfect, since different mRNA species have different turnover times, different affinities for the ribosome, and so on. These effects tend to skew the effective allocation fraction and we

should really think of r_i as the mRNA concentration after we have adjusted for such distortions.

Since the quantities on which the r_i are assumed to depend are in general allowed to be time-varying, we refer to this approach as the *dynamic allocation theory*. We enunciated three desiderata: the possibility of connecting with transcriptomics/ proteomics; the possibility of connecting with classic models; and accordance with conservation principles and suchlike. The first two have been accounted for; as regards the latter, it turns out that the recipe 'physicochemical principles plus *r*-functions' suffices in many cases to specify a model completely. In the next

section, we will review various aspects of microbial physiology that justify and motivate the dynamic allocation theory.

Selected aspects of microbial physiology

Microbial cells exhibit an astonishing degree of adaptability in the face of an often harsh environment. Responsiveness to changing conditions and an exquisitely optimised investment in internal stores are among the key traits that underpin this adaptability. We presently review these traits in more detail.

Nutrient uptake

In order to be processed by the cell's metabolic pathways and become available as fuel or molecular building blocks, nutrients must first traverse the cellular envelope. There are two basic ways in which this envelope is organised in prokaryotes (Figure 4), traditionally named for the way in which they respond to the Gram stain[26], which remains an important tool in microbial microscopy to the present day. In Gram- positive cells, a rigid polysaccharide layer called peptidoglycan forms the bulk of the

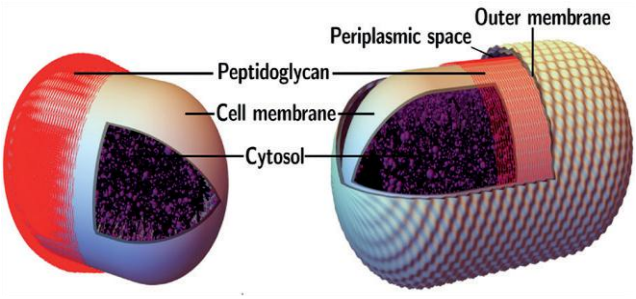


Figure 4 *General structure of Gram-positive (left) and Gram-negative (right) bacteria.*

cell wall, whereas in Gram-negative bacteria the peptidoglycan layer is diaphanous and there is a second bilipid membrane called the *outer membrane*. The area between outer and cytoplasmic membranes is occupied by a special substance with a high protein concentration, which is called the *periplasm*.

Although some hydrophobic molecules can pass through the membrane by diffusion, most nutrients require a dedicated pathway to be translocated across the membrane. The latter is mediated by means of membrane-spanning transport proteins that function individually or in complex assemblies, depending on the mode of transport they effectuate (Figure 5). The substrates may be transferred by themselves (*uniport*), or along with another substance, which may undergo net translocation in the same direction as the target molecule (*symport*) or in the opposite direction (*antiport*). Transport may be driven by the ΔG derived from the gradient of the substrate itself (*i.e.* facilitated diffusion in uniport) or that of the co-transported species (in symport and antiport), whereas in more complex systems the required ΔG is derived from group translocation or hydrolysis of adenosine-5-triphosphate (ATP); group translocation involves the chemical modification of the transported substances during their uptake across the membrane.

Gram-negative bacteria have to overcome a special challenge, since the nutrient molecule has to negotiate both an outer and an inner (cytoplasmic) membrane. This challenge is met by the so-called *ATP binding cassette (ABC) system*. Transport across the outer membrane is mediated by passive uniport through *porins*; once inside the periplasm the nutrient is swiftly bound by high-affinity binding proteins which deliver their cargo to an ATP-hydrolysing transporter.

Regulation of gene expression

Microbial cells regulate the expression levels of their uptake machinery so as to make (near-) optimal use of their limited supply of molecular building blocks. Regulation at the level of transcription is typically mediated by *regulatory proteins* that bind the DNA. Binding of such a protein may stimulate the expression of the gene, or inhibit it; examples of both modes of regulation are shown in Figure 6. In Figure 6a, it can be seen that the presence of the nutrient substrate maltose leads to expression of the enzymes that constitute a biochemical pathway specifically dedicated to feeding this molecule into the pathways of core metabolism. In Figure 6b, on the other hand, it is the presence of the product of a biosynthetic pathway, in this case arginine, that represses the production of enzymes involved in its synthesis. A design feature that these and many other examples have in common is that DNA- binding proteins serve as an adaptor between the substrate (maltose) or the product (arginine) to transform the signal (*i.e.* the concentrations of those compounds) into the propensity with which RNA polymerase initiates transcription.

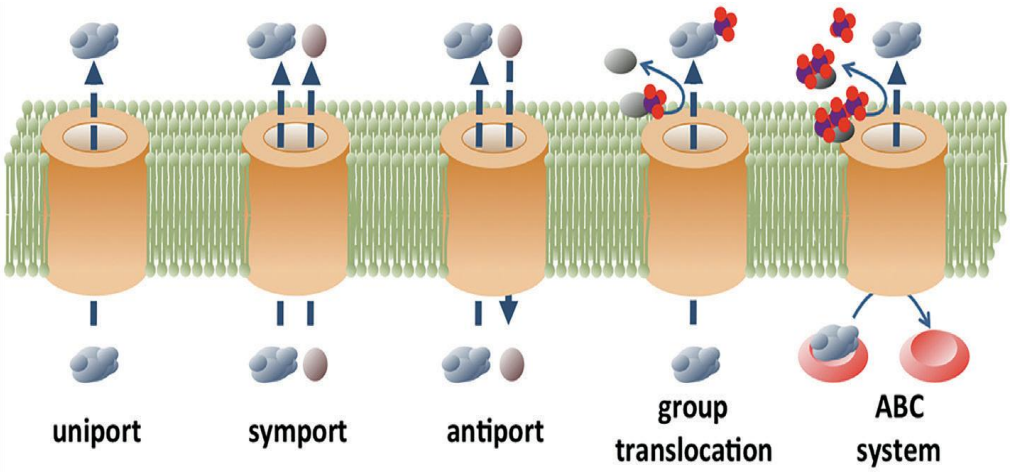


Figure 5 *General modes of action in nutrient uptake machinery.*

In both these examples, a single control point governs the expression of a suite of enzymes, which makes functional sense since the enzymes catalyse subsequent steps in a particular biochemical pathway, and are hence needed (or not needed) in unison. A collection of protein-encoding domains of DNA (cistrons) that is under common regulatory control is called an *operon*. This modular organisation of gene expression underpins the notion that the index i in Eqn (1) denotes functional units rather than individual proteins. There will also be enzymes that will be shared by several pathways, in which case their regulation may be more complex (or indeed more simple, when expression is *constitutive*, *i.e.* always 'on').

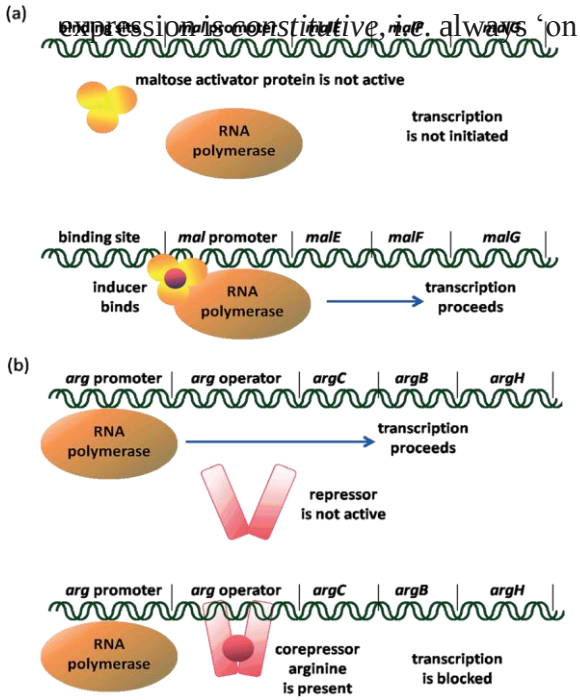


Figure 6 *Examples of regulatory mechanisms in bacteria. (a) Transcription of the maltose transport system gene is initiated when the inducer maltose binds the activator protein, which engages the RNA polymerase and allows it to start transcription. (b) Transcription of the arginine biosynthetic pathway gene is blocked when the corepressor arginine binds to the repressor protein, leading to cessation of transcription.*

The mechanisms of induction and repression shown in Figure 6 can be generalised to the notions of positive feedforward and negative feedback, as illustrated in Figure 7a. This

figure also shows negative feedforward, in which two alternate substrates inhibit one another's processing pathways (a phenomenon known as *catabolite repression*[34]). Thus ample availability of one of them, say A, favours the expression of the enzymes that are dedicated to the utilisation of A. These various modes can operate together, mediated *via* several regions (interaction sites) upstream from the promoter. Thus, several activating and repressing proteins may simultaneously interact with the DNA and modify the rate of assembly of the RNA polymerase at the promoter.

Each of these positive or negative interactions can be realised in two different ways, according to the familiar algebraic rules + × + = +, – × – = +, – × + = –, and + × – = –. The somewhat byzantine biological terminology (glossed in Figure 7b) tends to obscure these elegant basic principles.

There are additional modes of regulation that come into play under conditions of extreme stress. Nutrient shortage in the environment induces an increase in the number of uncharged tRNAs in the cell, and as the ratio of uncharged to charged tRNA increases, the ribosome wastes a greater portion of its time interacting with uncharged tRNA, which leads to a stall of the ribosome, along with a production of small nucleotides called *alarmones*. These initiate the so-called *stringent response* characterised by a cessation of rRNA and tRNA synthesis and ribosome production, as well as by a decrease in protein and DNA synthesis and amino acid production. Another stress response involves *heat shock proteins* that help the cell to recover from physicochemical challenges such as high temperatures, radiation damage, or exposure to corrosive chemicals.

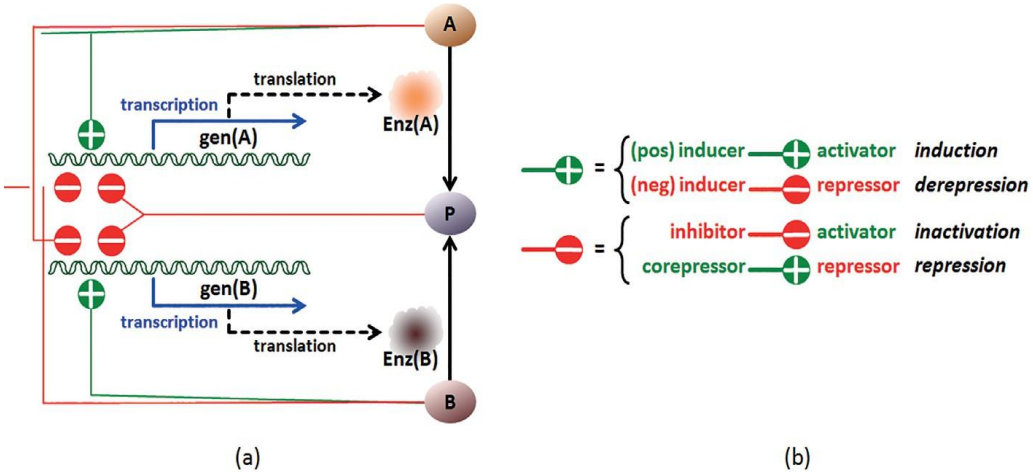


Figure 7 Regulatory mechanisms in bacteria. (a) Diagram illustrating modes of control in prokaryotic gene regulation. Two alternative substrates (A and B) are both converted to a common product (P), via reactions catalysed by specific enzymes [enz(A) and enz(B)] which are encoded by genes [gen(A) and gen(B)]. Both positive and negative control are possible, as indicated. (b) Modes of regulation of gene expression. Stimulation of the expression of a given gene can be mediated by activation, in which an activator protein interacts with the upstream regulating region of the gene;

this involves interaction with an inducer. Stimulation may also be effected via derepression, in which a repressor (an inhibitory transcription factor) is rendered less effective by an inducer. Inhibition of gene expression can likewise be achieved in two ways, either via inactivation of the activator protein via interaction with an inhibitor, or via activation of the repressor by binding to a corepressor. Inducers, inhibitors, and corepressors are often metabolites (substrates or products) but can also be components of an intracellular signalling cascade.

Cell division and growth

Bacteria multiply by division, and exhibit exponential growth curves which microbiologists call logarithmic. Prior to cell division, the cell elongates to approximately twice its original length while its genetic material is replicated. Once this is complete, filamentous temperature sensitive (Fts) proteins, chief among which is FtsZ, assemble to form the cell division apparatus (divisome) by polymerising and thus forming the so-called Z-ring, which localises to the middle of the cell. New cell envelope material is synthesised and the two chromosomes are pulled apart. The Z-ring subsequently depolymerises and a partition called the *septum* is formed, which divides the cell in two approximately equal parts.

The time required to produce a single generation may be referred to as the generation time T_g; it depends on environmental conditions as well as genetic traits. During a time span over which T_g is constant, growth is said to be *exponential*: after a period of time equal to $t=nT_g$, there will have been n doublings and the number of cells will be $N=N_0 2^n$ where N_0 and N denote, respectively, initial and final number of cells. If W denotes the biomass of the population, then $\dot{W} \stackrel{\text{def}}{=} dW/dt$ is its growth rate and $\dot{W}/W \equiv \mu$ is the *specific growth rate*. Exponential growth is characterised by the condition, $N/N_0 = W/W_0$ where μ is a constant. This yields

If we assume that T_g, we find $2^n = e^{\mu t} = e^{\mu n T_g}$ and so $T_g = (\ln 2)/\mu$.

Classic experimental methods and models

As intimated in Section2, the classic mathematical models for microbial growth and metabolism are closely tied to what can be readily observed in the standard laboratory systems for microbial growth. Accordingly, we briefly review the latter before discussing these models in more detail.

Culture systems

There are various ways of cultivating micro-organisms in bioreactors. The main methods of cultivation are *batch cultivation* and *chemostat cultivation*.

Batch culture

A batch culture is an enclosed vessel in which micro-organisms are able to grow as they consume substrate[40]. The biomass-specific substrate consumption rate q per unit of biomass of the population in the batch culture can be described as a function of the nutrient concentration $[N]$ by means of the Michaelis–Menten equation:

$$q_S = q_{s,max}\left(1 + \frac{K_m}{[N]}\right)^{-1} \quad (2)$$

where $q_{S,max}$ represents the maximum substrate consumption rate and K_m denotes the concentration of the substrate at the half-maximal consumption rate. The Pirt equation[42] describes the relation between q_S and the specific growth rate μ:

$$q_S = \frac{\mu}{Y_{s,max}} + m_S \quad (3)$$

where $Y_{S,max}$ is the maximum yield of biomass, and m_S is a maintenance coefficient. With $\mu = \frac{\dot{W}}{W}$ (*cf.* Section 3.3), it follows that:

$$\dot{W} = Y_{s,max}(q_s - m_s)W \quad (4)$$

and together with Eqn (2) this yields:

$$\dot{W} = Y_{s,max}\left(q_{s,max}\left(1 + \frac{K_m}{[N]}\right)^{-1} - m_s\right)W. \quad (5)$$

The following conservation law applies if intracellular reserves do not vary:

$$[N]_0 = [N] + \frac{W}{Y_{s,max}V} + (m_s/V)\int_0^\tau W(\tau)d\tau \quad ,$$

where $[N]_0$ expresses the initial nutrient concentration and V is the volume of the medium in which the culture is grown. This allows us to obtain a system of differential equations for W and $[N]$.

Continuous culture

In sundry applications, it is advantageous if cultures can be maintained in constant environmental conditions for long periods of time, which is not possible with closed batch

cultures, which are continually undergoing dynamical change as the culture matures and eventually runs out of fresh substrate. However, such steady-state conditions can be achieved in a continuous-flow culture, or chemostat (Figure 8). In such a device, there is a continuous supply of fresh medium combined with the withdrawal of an equal flux of cultivation broth, allowing the cultivation volume to remain constant[43]. Although much can be gleaned from transient dynamic behaviour, the tradition has been to wait until this transient dies out and the system attains an equilibrium, which is then investigated in detail.

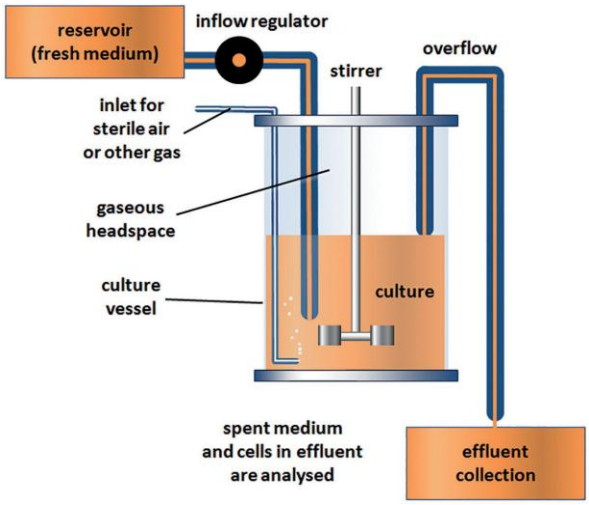


Figure 8 *Continuous culture system, 'chemostat'. The main vessel contains a well-stirred culture medium, which is replenished from the reservoir. The culture vessel maintains a constant volume of culture, since the inflow of a fresh medium from the reservoir equals the outflow of spent medium (overflow) from the vessel.*

In the chemostat, both the growth rate and the population density can be controlled independently and simultaneously. To this end, the experimenter manipulates two key parameters: (*i*) the dilution rate $D = F/V$ where V is the volume of the main vessel, and F is the volumetric rate at which fresh medium is supplied; and (*ii*) the concentration of (limiting) nutrient $[N]_R$ in the reservoir. Varying D, different growth rates can be achieved, with an eventual standing stock dictated by $[N]$. We can see this by pursuing the following analysis.
We begin by considering biomass balance:

$$\frac{dW}{dt} = \mu W - \frac{F}{V} W$$

as well as a similar balance equation for the nutrient concentration $[N]$:

$$\frac{d[N]}{dt} = \frac{F}{V}([N]_R - [N]) - \tilde{\sigma}_W \mu W,$$

where $\tilde{\sigma}_W$ is a stoichiometric coefficient. Equilibrium is defined by the conditions $\frac{dW}{dt}$ =0 and $\frac{d[N]}{dt}$ =0. Hence we obtain the following steady-state chemostat equations:

$$\mu = D; \qquad [N] = [N]_R - \tilde{\sigma}_W W. \tag{8}$$

The first equation shows that by setting the dilution rate *D* of the chemostat to a certain value, we are able to cultivate micro-organisms at a specific growth rate equal to *D*. This is why the chemostat is such an important laboratory tool, affording a view of the physiology of microbes under well-defined conditions (constant growth rate, constant environmental conditions) or to examine how the growth rate affects, for instance, the rate of product formation. Specifically, if we assume the Monod equation[44], $\mu([N])= (1+K/[N])^{-1}$ we find:

$$W = \left([N]_R - \frac{DK_S}{\hat{\mu}-D}\right)\tilde{\sigma}_W{}^{-1}; Y = D\left([N]_R - \frac{DK_S}{\hat{\mu}-D}\right)\tilde{\sigma}_W{}^{-1}$$

$$[N]= \frac{DK_S}{\hat{\mu}-D}$$

These relationships have been plotted in Figure 9; we can see that, as *D* approaches $\hat{\mu}$, a 'wash-out catastrophe' occurs.

Chemostat systems allow us to maintain exponential growth for a long period of time, as well as to repeat experiments under the same conditions, which facilitates the study of competitiveness of different strains and species under the same environmental conditions. Competition between different strains of a single species can be exploited to eliminate all but one, and thus the chemostat can be used as a tool to isolate specific types of bacteria, which can subsequently be studied in more detail[17]. Moreover, under certain specific conditions, such as nutrient shortage, long-term chemostat cultivation leads to adaptation of the cells to these conditions, which suggests that the chemostat might also be used in 'evolutionary' engineering.

Contemporary approaches: microscopic models

In sharp contrast to the classic whole-organism descriptions, which have few dynamic degrees of freedom, stand the models that explicitly represent individual molecular species; biochemistry being what it is, the natural consequence of this approach is $p \gg 1$; for instance, with $p \sim 10^8$ molecules inside the cell, a naive 'molecular dynamics' type of approach would result in computationally highly demanding and analytically barely tractable models. Thus, the construction and analysis of models capable of describing the cell as a whole system is challenging. One good example is the model of bacterial metabolism, where the state variables are metabolite concentrations, gene expression levels, transcription factor activities, metabolic fluxes, and biomass concentration[23]. However, in many cases the aim is not to describe the whole organism but instead to focus on specific subsystems of the cell, such as the assembly of the Z-ring, or the electron transport chains of mitochondria and purple non-sulfur bacteria.

Depending on the specific properties of the given biological network under consideration, different formalisms can be employed to simulate its dynamic behaviour. Signalling and regulatory networks concern signal flows, whereas metabolic networks deal with mass and

energy flows. Metabolic networks have been analysed using flux balance analysis, metabolic flux analysis, pathway analysis by elementary modes, or extreme pathways. Signalling networks can likewise be addressed *via* a variety of techniques, ranging from ODEs to Boolean networks in global cellular models.

The dynamic allocation theory as a bridge between classic and contemporary approaches

In Section 2, we advocated the dynamic allocation approach as a generalisation of classic approaches that would be more amenable to modern 'big data' settings. The approach rests on the principle expressed by Eqn (1), but it stands to reason that this equation does not suffice by itself to meet these desiderata. We need to augment the allocation principle with dynamic compositional reckoning in terms of stoichiometric components.

Stoichiometric components

A stoichiometric component is a fixed linear combination of chemical species (which may be molecules, ions, functional groups), up to a multiplicative constant. By definition, such a component has a fixed empirical formula. To fix the multiplicative constant, we can normalise by the dominant chemical element [*e.g.* it is customary to think of carbohydrates as multiples of $C(H_2O)_m$, where m□□; the number of multiples then counts as a number of *C-moles*]. Alternatively, when the component corresponds to a single chemical species, it is more natural to think in terms of the number of

moles of the discrete particles (molecules) of this species. Taking such stoichiometric components as a starting point has the great advantage that the dynamical behaviour of the model is almost self-evident in terms of the stoichiometric components; all that is really required is careful book-keeping, which is linear by its very nature.

Models with p=0, such as Monod and Pirt–Herbert–Marr, represent an extreme case in which the biomass is accounted for as a single component. To make this connection, we have to impute to these models the assumption of strict compositional homeostasis, so that biomass, as such 'all-included', has a fixed empirical formula. In the next step up, for the Droop–Caperon model, we regard the various reserves as separate components, along with a 'subsistence biomass' and each of these components is assumed to have a fixed empirical formula. The *conical hull* (in the linear algebra sense) of these empirical formulas is the set of allowed chemical compositions at the level of the whole organism. In 'microscopic' models with $p \gg 1$, the components correspond to single molecules or aggregates of molecules that co- vary in terms of their copy numbers per cell, as will be the case, for example, with enzymes encoded by a single operon (Section 3.2).

The choice of the number of components is thus tied up with, on the one hand, the assumptions

regarding compositional homeostasis one is willing to entertain, and, on the other hand, the data available, in particular as regards the observed variation of the studied organism as regards its chemical composition. If a component i takes the form $€=_k k_{ik}\ P_k$, where the coefficient k_{ik} represents the predominance of protein

P_k in component i, then the bridge to proteomics is immediate, and the bridge to transcriptomics takes the general form $r_i = €_k k_{ik} €_l \varsigma_{kl} R_l$, where R_l is the level of the lth species of mRNA and the coefficient ς_{kl} is a conversion factor.

A sensible default position halfway between the extremes can be negotiated by combining Grover's *Variable-Internal-Stores* approach with a fairly coarse- grained partition of catalytic machinery as suggested by Scott and co-workers[60,61]; the resulting model is represented schematically in Figure 10 for the case of two essential nutrients, here shown as red and green dots.

Regulatory laws

To tie the stoichiometric dynamics to the allocation principle, Eqn (1), we need to postulate regulatory laws which we call *r*-functions. A generic example is shown in Figure 11: a decreasing sigmoid function which transforms the density of a nutrient x_i into the term r_i corresponding to allocation α_i of building blocks toward the machinery needed to acquire the nutrient corresponding to reserve i.

The precise mathematical form of this sigmoid is relatively unimportant: the salient point is that such a sigmoid can be parametrised by a midpoint parameter ξ

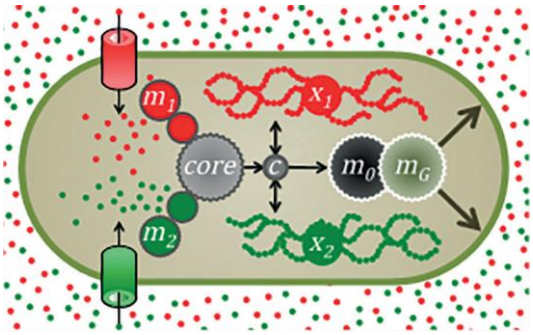


Figure 10 *Model diagram. Transporters and dedicated processing machinery* (m_1 *and* m_2) *feed the material into core metabolism, which leads to a central hub* (c) *that communicates with reserve polymers* (x_1 *and* x_2) *and feeds biosynthesis which iscarried out by synthetic machinery* m_O*; growth is driven by growth machinery* m_G.

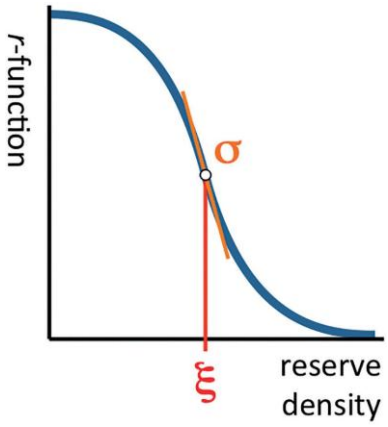


Figure 11 Generic r-function. The simplest type of r-function depends on the density of one selected reserve, and governs the expression of the machinery required for the uptake and processing of the nutrient stored in that reserve. The sigmoid curve has two key parameters, location of the midpoint (ξ) and slope at the midpoint (σ); the values of these parameters determine if the model behaves more 'Monod- like' or 'Droop-like'

and a slope parameter σ. It can be shown that the model will behave like the Monod/ Pirt models, or like the Droop model, depending on the values that are selected for these parameters.

The configuration in which reserve densities govern the regulatory law corresponds to what we identified as negative feedback control in Section 3.2, with the internal store (x_i in Figure 10) corresponding to the product **P** in Figure 7. The formalism proposed here extends effortlessly to any number of reserves, and to the inclusion of maintenance costs and how the dynamics change when these costs can no longer be covered by the available reserves or external supplies. However, the reserves have to correspond to essential (non-substitutable) nutrients, and there are many known cases where alternative (substitutable) nutrients converge on a common reserve polymer. The regulation of assimilatory machinery for such alternative substrates is more involved. The general pattern is that the organism's gene regulation is set up to utilise a 'preferred' compound in the first instance, and only express transporters and/or enzymes dedicated to 'less preferred' compounds when the preferred one is no longer available, giving rise to characteristic multi- phasic growth curves $W(t)$ (in batch cultures) in which the bacteria are inoculated together with two or more alternative substrates, but regulate their machinery in such a way as to deplete these nutrients one after the other[34]; in the case of two nutrients this growth curve is called *diauxic*. Of course, these terms do not express actual states of mind of bacteria, but rather for which nutrient the overall yield (efficiency of converting nutrient into biomass) is greatest.

The genetic circuit underlying this behaviour was shown in Figure 7, where alternative substrates (A and B in Figure 7) exert crossed negative feedforward control over their respective operons. To represent this behaviour, the environmental concentrations of the alternate nutrients have to be sensed somehow, either directly as the intracellular concentration reflects the ambient one, as with the maltose operon depicted in Figure 7, or else *via* an intracellular signalling cascade; the so-called two-components systems, for instance, which constitute a large group of signalling pathways in prokaryotes[62]. In either

case, the functional behaviour can be captured by an *r*-function of the form shown in Figure 12: besides depending on the reserve density in the original sigmoid fashion, there is also a change-over dependence on the ratio of environmental concentrations. Thus, the regulatory law in this case acquires characteristics of both feedback and feedforward, and can be interpreted as a smooth (or 'fuzzy') rendition of a Boolean operation.

Outlook: living together as one

As the cells absorb nutrients and redox substrates from the ambient medium and secrete products back into this medium, they change the conditions they are faced with and to which they are adapting. It is precisely this causal loop which necessitates the careful analysis of culture conditions (both batch and chemostat) which we briefly reviewed in Section 4.1.

In its crudest form, this environmental feedback loop has a detrimental, competitive character (*Verelendung*). However, when species with different

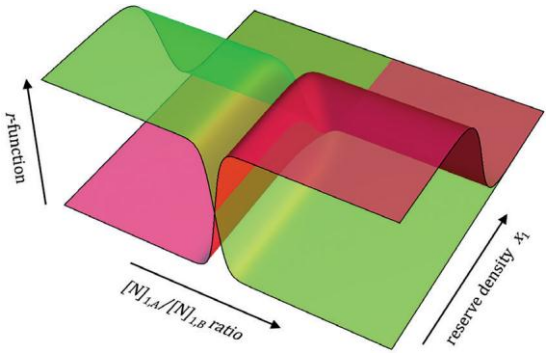


Figure 12 *Cross-regulation. Two substitutional nutrients with ambient concentrations* $[\mathrm{N}]_{1,\mathrm{A}}$ *and* $[\mathrm{N}]_{1,\mathrm{B}}$ *are converted into reserve type 1. The corresponding* r*-functions* $\mathrm{r}_{1,\mathrm{A}}$ *and* $\mathrm{r}_{1,\mathrm{B}}$ *are functions of* $\frac{[N]_{1,A}}{[N]_{1,B}}$ *and of* x_1*. The red surface is a graph of* $\mathrm{r}_{1,\mathrm{A}}$ *and the green surface is a graph of* $\mathrm{r}_{1,\mathrm{B}}$.

metabolic capabilities live together, one species' product can be another species' substrate: symbiotic cycles arise that allow the agglomeration of species to thrive. Microbial mats, for instance, are consortia of several species with distinct biochemical functionalities, undertaking intricate co-operative interactions to harvest energy and matter from the environment.

This differentiation into complementary, metabolic capabilities can also occur within the colony, that is, within the group of descendants of a common progenitor cell. Cyanobacterial heterocysts (Figure 13), for instance, arise in the absence of combined nitrogen (*e.g.* nitrate, ammonia) in the environment, and express *nitrogenase*, which allows them to convert dinitrogen into ammonia and supply the vegetative cells

with fixed nitrogen in the form of glutamate and β-aspartyl-arginine; the latter fix carbon dioxide and supply the heterocysts with carbohydrates such as sucrose.

This division of labour allows both nitrogen and carbon fixation to occur at the same time and nearly the same place; cyanobacteria employ oxygenic photosynthesis to fix carbon, and

nitrogenase is inactivated by oxygen. The cells exchange organic molecules *via* a shared fluid phase, comprising the unstirred water layer surrounding the filament, the glycocalyx, and possibly a shared periplasmic space. Moreover, the heterocysts secrete the pentapeptide PatS that inhibits heterocyst differentiation, thus ensuring a regular spacing of heterocysts rather than random clustering.

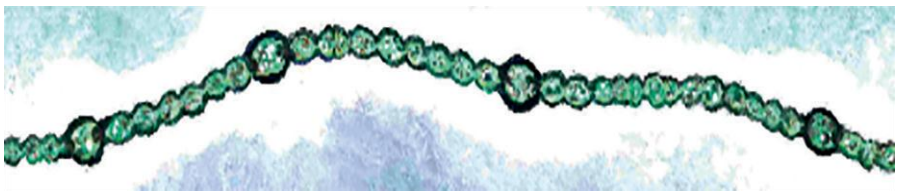


Figure 13 *Diazotrophic filament of* Anabaena *OCC7120. The difference between the photosynthetic vegetative cells (small) and nitrogen-fixing heterocysts (large) is conspicuous.*

These are the hallmarks of true multicellularity: (*i*) cells are exposed to a milder version of the ambient medium, as a result of metabolites they excrete into this medium, as part of (*ii*) their differentiation toward specialised metabolic or physiological tasks, among which are (*iii*) growth and reproduction. The metabolites in the shared *interstitial* medium have beside their 'raw' biochemical function, also a 'token' function in mediating cell fate (*e.g.* PatS); these tokens are often modified intermediaries of energy metabolism, such as ppGpp (an alarmone) or cAMP.

The phylogenetic development of multicellularity is merely an elaboration of these traits: differentiation into more subtypes, a delicate conditioning of the interstitial medium, and gradual shift towards the 'token' function as hormones and cell-adhesion molecules co-ordinate the organisation into tissues (which drives the morphological richness we evoked in Section 1).

Given the success of the "physicochemical principles plus *r*-functions" approach in free-living unicellulars, we may well wonder if these principles transfer to the whole-organism level in multicellular organisms. There are several key differences (Figure 14). As a result of homeostasis of the interstitium, individual cells are no longer adapting to a harsh medium; their gene expression is governed, to a far greater degree, by stimuli from first messengers; and the accumulation of internal stores has become one of the specialised functions.

We should like to retain the idea of starting from physicochemical principles, and augment it with appropriate regulatory laws, which must now be concerned with the composition of the interstitium, the status of the storage tissues, and the control of somatic growth and development *versus* germline reproduction.

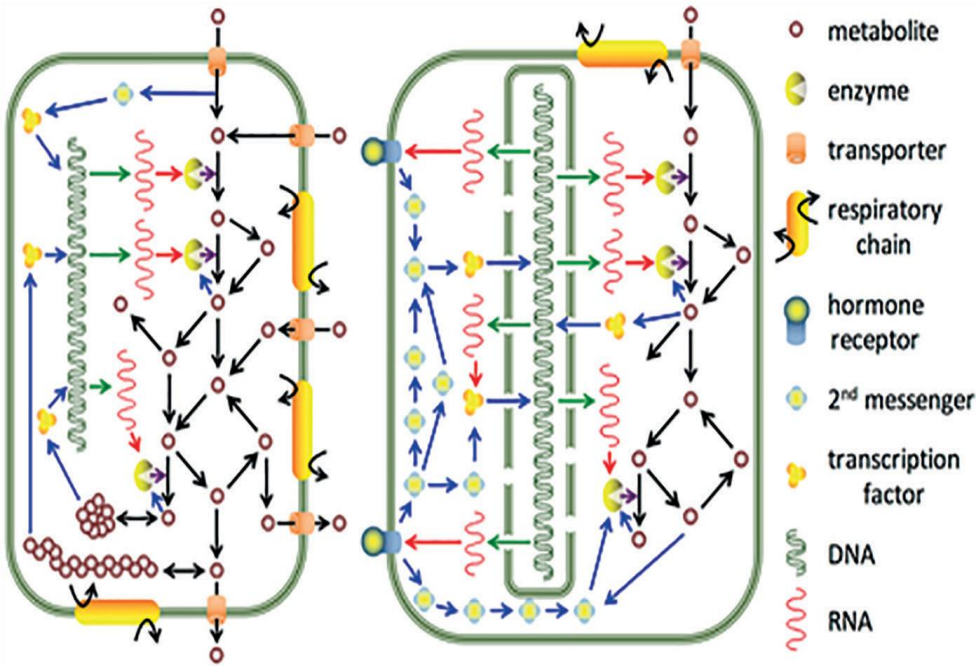


Figure 14 Uni- versus multicellular lifestyles. Left: a free-living unicellular organism; it has a high degree of metabolic versatility and adaptability, and the signalling pathways that control gene expression are predominantly geared to availability of nutrients and substrates in the ambient environment; on the right, a cell that is part of a multicellular conglomerate: such a cell is shielded from some of the environment's fluctuations due to the organism's homeostasis of the milieu intérieur and accordingly is metabolically less versatile; signalling pathways controlling gene expression are dominated by extracellular 'first messenger' signals that arise elsewhere in the organism and co-ordinate the cell's specialised activities within the multicelled whole.

BASIC METHODS FOR MICROBIAL ENUMERATION:

There are a number of different methods to enumerate the microorganisms that are present

in a given population. Each method has its own peculiarities to transform the data obtained (colony forming units, total microorganisms, etc...) in microbial density of the sample. A simple method for the enumeration of bacteria and fungi is based on the quantification of colonyforming units (CFU) per ml or g of sample. For this, we must prepare serial dilutions of the sample, plate the diluted suspensions and count the number of colony forming units. Figure 1 shows a scheme of the method.

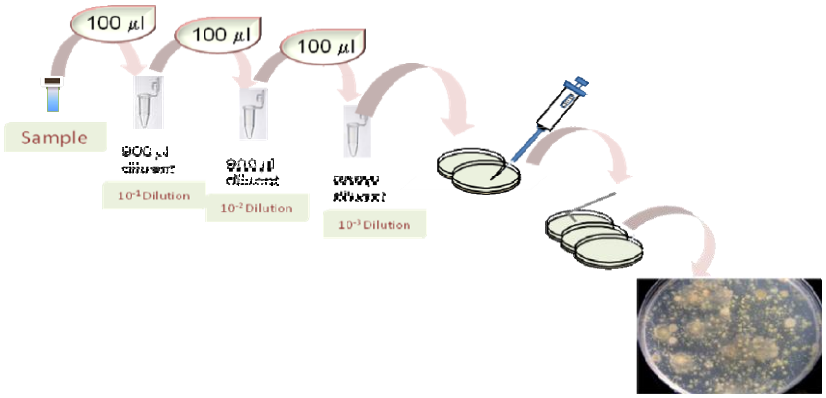


Figure 1. CFU count method scheme.

In the previous chapter, we have solved some problems based on the use of this method. However, it should be noted that, normally, 3 or 5 plates per dilution are used.

If we perform different decimal dilutions and inoculate more than one plate per dilution, we have different possibilities to interpret the results. It is important that the number of colonies developing on the plates not be too large or too small. The usual practice, which is the most valid statistically, is to count colonies only on plates that have between 30 and 300 colonies. For determining the number of bacteria per mL in the original solution, we have to take into account the average colonies of the selected dilution, the dilution factor and the volume plated.

$$\text{CFU/ml} = \frac{\textbf{A}\ \text{colonies (average)}}{\textbf{B}\ \text{volume plated (ml)}}\ \text{X}\ \ \textbf{DF}\ \text{(Dilution factor)}$$

$$\text{CFU/ml} = \frac{\textbf{A}\ \text{ecolonies (average)}}{\textbf{B}\ \text{volume plated (ml)}}\text{X}\ \ \frac{1}{\textbf{CF}\ \text{(Concentration factor)}}$$

With the insights acquired in the previous chapter, you should resolve the following problem:

2.1. Calculate the bacterial density, expressed as CFU/ml, for the three different samples presented in

the table. The dilutions used and the number of CFU obtained in each case are also presented in the table. In all cases, the spread volume was 100 µl/plate.

Sample	Dilution	Plate 1	Plate 2	Plate 3
1	10^{-3}	1816	1698	1885
	10^{-4}	180	159	186
	10^{-5}	16	19	10
2	10^{-2}	475	477	480
	10^{-3}	45	48	51
	10^{-4}	5	10	4
3	10^{0}	335	328	324
	10^{-1}	32	28	29
	10^{-2}	5	3	2

An interesting question arises when microbial density is below the detection limit, the lowest number of CFU that can be detected by the method used. How must be the results expressed in this situation? Try to solve the following problem:

2.2. Which is the detection limit of the method if we inoculate 3 plates of a sample with 100 µl per plate?

A variant of the standard CFU method is the quantification in microdrops. The main differences are that, in this case, the plated volume is lower (usually, 10 or 20 µl) and it is not extended in the plate. The method is schematically described in Figure 2.

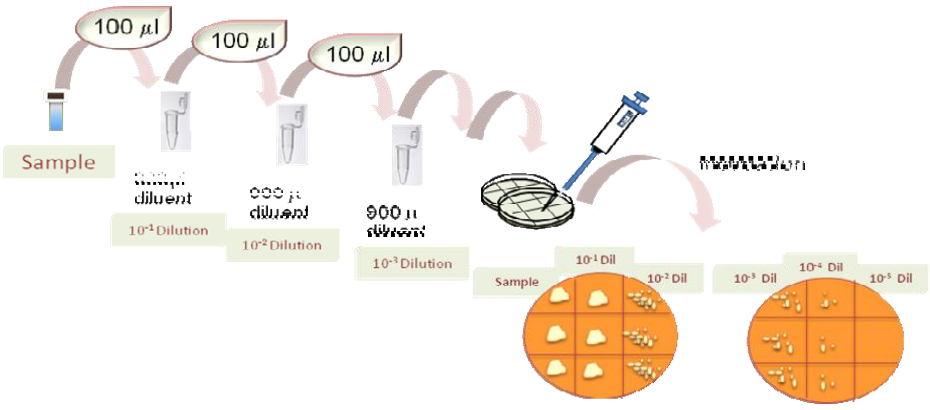


Figure2. Scheme of the microdrop method for CFU count.

2.3. The microdrop method was used to determine the bacterial density of a suspension. The dilutions employed and the results are shown in the table. The plated volume was 10 µl.

Dilution	Count 1	Count 2	Count 3
10^{-3}	180	159	186
10^{-4}	16	19	10

What is the bacterial density of the sample?

Another usual technique to enumerate microbes is the **Most Probable Number (MPN) method**. This is an enumeration method based on the statistic, and it allows estimating the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in serial dilutions. First, decimal dilutions of the sample are prepared and then 1 ml of each dilution is inoculated into 3 broth culture tubes (different numbers of replicates and dilution series can be used). After incubation, tubes are examined for turbidity and those that are positive are recorded for each dilution. For example, if all tubes show growth, the results will be noted as 333. Once the results have been noted, the MPN table (MacGrady table) should be used to determine the most probable number of microorganisms for ml or g.

Solve the following problems using the MacGrady table attached below.

2.4. The 1:10 and 1:100 dilutions have been prepared from a water sample. Moreover, 3 sets of 3 tubes were prepared, each containing 10 ml of nutrient broth. The first set of tubes was directly inoculated with the water sample; the second set was inoculated with the 1:10 dilution and the third, with the 1:100 dilution. The inoculated volume was 1 ml in all cases. The tubes were incubated for 48 h at 20°C and, after this period, each tube was examined for turbidity (growth). From the results shown in the table, what is the bacterial density of the water sample?

Procedure	**Results***		
Sample	G	G	G
1:10 Dilution	G	G	G
1:100 Dilution	NG	G	NG

*G = growth = turbidity; NG = not growth = transparent

2.5. Using the example given above, if growth was not detected in any tube, which would be the bacterial density?

MacGrady Table (1 ml/tube, 3 tubes/dilution, 3 ten-fold dilutions). **Results expressed as bacteria/ml**

Number of positive tubes	MPN	Number of positive tubes	NMP	Number of positive tubes	NMP
000	-	**201**	1.4	**302**	6.5
001	0.3	**202**	2.0	**310**	4.5
010	0.3	**210**	1.5	**311**	7.5
011	0.6	**211**	2.0	**312**	11.5
020	0.6	**212**	3.0	**313**	16.0
100	0.4	**220**	2.0	**320**	9.5
101	0.7	**221**	3.0	**321**	15.0
102	1.1	**222**	3.5	**322**	20.0
110	0.7	**223**	4.0	**323**	30.0
111	1.1	**230**	3.0	**330**	25.0
120	1.1	**231**	3.5	**331**	45.0
121	1.5	**232**	4.0	**332**	110.0
130	1.6	**300**	2.5	**333**	>140.0
200	0.9	**301**	4.0		

Other alternatives to determine the total number of microorganisms in a sample are the **enumeration methods based in microscopy** (photonics, epifluorescence, ...).

The relatively large organisms such as yeast or protists can be enumerated using counting chambers (or haemocytometer) (Figure 3). In this procedure, the number of cells in a given volume of liquid culture is counted directly in various microscope fields. The main disadvantage of this method is that usually it is not possible to distinguish between live and dead cells.

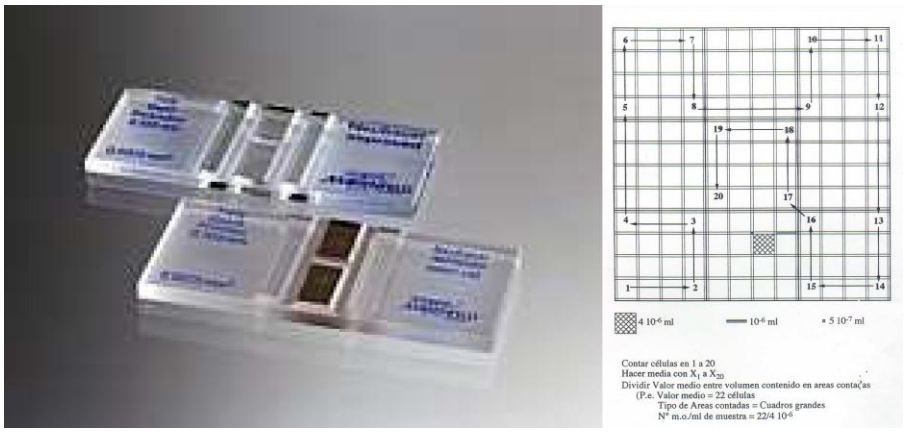


Figure 3. Some cells counting chambers and scheme to select grids.

The different types of counting chambers differ in the counting grids and in the depth of the chambers. The volume of each grid must be known, in order to determine the microbial concentration. Moreover,

prior to the enumeration the sample can be diluted or concentrated, so the equations to be used are:

$$\text{Microorganisms/ml} = \frac{\text{Average number of microorganisms/grid}}{\text{Grid volume (ml)}} \text{ X Dilution factor}$$

$$\text{Microorganisms/ml} = \frac{\text{Average number of microorganisms/grid}}{\text{Grid volume (ml)}} \text{ X } \frac{1}{\text{Concentration factor}}$$

Attention should be given to the units of each factor in the equation. The final result is expressed as number of organisms per volume of sample.

2.6. To determine the yeast concentration in a dense suspension, this suspension was diluted 1,000 times before preparing the counting chamber. Then, 20 grids were counted and the results were recorded in the table that appears below. If the chamber factor given by the manufacturer is 0.25 10^{-6} ml/grid, could you determine the number of yeasts per ml of suspension?

Grid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
No. cells	12	14	12	11	12	14	15	12	9	11	12	14	15	10	11	9	10	11	12	14

For several decades, **epifluorescence microscopy** has allowed the visualization and enumeration of microorganisms previously stained with fluorochromes. This is a technique valid for quantification of bacteria (Figure 4).

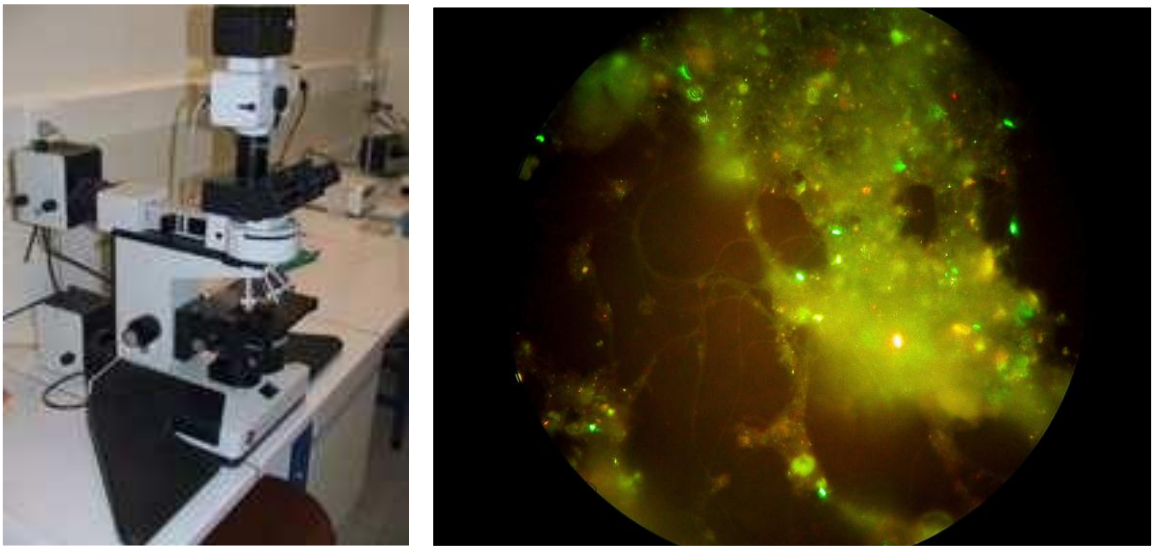


Figure 4. Epifluorescence microscope and image of a sample stained with acridine orange.

In order to determine the microbial density in a given sample it is necessary to know the microscope factor (number or fields per filter). The equation used to convert the data into a microbial density is the following one:

$$\text{Microorganisms/ml} = \frac{\text{Average number of microorganisms/field}}{\text{filtered (ml)}} \text{ X Microscope factor X Dilution factor Volume}$$

2.7. To estimate the bacterial density of a water sample, 100 µl of this sample was stained with acridine orange and filtered through a membrane filter of 0.2 µm pore diameter. The filter was mounted on a slide and observed using an epifluorescence microscope. 20 fields were counted and number of bacteria obtained by field was recorded in the following table:

Fields	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
No. cells	15	12	11	9	21	16	13	13	15	20	22	13	14	12	18	17	9	22	18	18

If the microscope factor is 30,954 fields/filter, what is the bacterial density of the sample? What would the bacterial density be if 1 ml of the 10^{-2} dilution had been filtered?

Finally, a classic and very simple method for microbial quantification is based on the **measurement of the absorbance** (at a given wavelength) of a microbial suspension and the transformation of this value into a number of cells (or CFU) per ml. Obviously, this method requires a previous study in which various dilutions of the microbial suspension are prepared and measured (absorbance) in order to get an equation that correlates both parameters. In future studies, this equation can be used to determine the microbial density from absorbance data.

2.8. From a dense suspension of *Escherichia coli* we have prepared different suspensions in which absorbance and CFU/ml were determined. The results obtained are given in the following table:

Suspension	**Absorbance (550 nm)**	**No. bacteria/ml**
1	0.020	$3.55\ 10^{6}$
2	0.052	$2.04\ 10^{7}$
3	0.102	$5.65\ 10^{7}$
4	0.164	$1.95\ 10^{8}$
5	0.213	$4.75\ 10^{8}$
6	0.264	$6.90\ 10^{8}$

If we have a sample with an absorbance of 0.18, what is the bacterial density?

SOLUTIONS

2.1. CFU/ml? Volume spread = 100 µl/plate

Sample	Dilution	Plate 1	Pla7e 2	Plate 3	CFU/ml
1	10^{-3}	1816	1698	1885	
	10^{-4}	180	159	186	$1.75\ 10^{7}$
	10^{-5}	16	19	10	
2	10^{-2}	475	477	480	
	10^{-3}	45	48	51	$4.8\ 10^{5}$
	10^{-4}	5	10	4	
3	10^{0}	335	328	324	
	10^{-1}	32	28	29	$2.97\ 10^{3}$
	10^{-2}	5	3	2	

2.2. Detection limit for CFU method: 100 µl/plate, 3 plate/sample

	0.1 ml	0.1 ml	0.1 ml
Real case (colonies enumerated):	0	0	0
Detection limit (assumption):	0	0	1

$$\frac{0.33 \text{ colonies}}{0.1 \text{ ml plated}} = 3.3 \text{ CFU/ml}$$

Detection limit (assumption): 1 colony/3 plates = 3.3 CFU/ml

Real case: 0 colonies/3 plates = Detection limit = <3.3 CFU/ml

Detection limit for this situation: 100 µl /plate, 5 plates/sample

Detection limit = <2 CFU/ml

2.3. Microdrop method (10 µl/drop). CFU/ml?

Dilution	Count 1	Count 2	Count 3	CFU/ml
10^{-3}	180	159	186	
10^{-4}	16	19	10	$1.5\ 10^{7}$

2.4. MPN. Bacterial density?

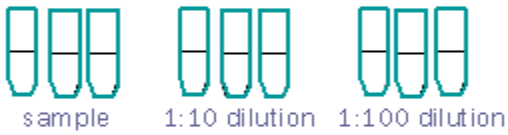
Inoculated volume = 1 ml of sample or dilution

Incubation: 48 h at 20ºC

Check growth (turbidity)

45 bacteria/ml

2.5. MPN. Detection limit?



Real case (tubes positives): 0 0 0

Detection limit: 1 0 0

Detection limit <0.3 bacteria/ml

0.3 bacteria/ml

2.6. Yeast suspension. Diluted 1,000 times. Chamber Factor = 0.25 10^{-6} ml/grid

No. yeasts/ml of suspension?

Grid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
No. cells	12	14	12	11	12	14	15	12	9	11	12	14	15	10	11	9	10	11	12	14

2.7. Epifluorescence microscopy. Volume filtered = 100 µl. Microscope Factor = 30,954 fields/filter. Bacterial density?

Field	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
No. cells	15	12	11	9	21	16	13	13	15	20	22	13	14	12	18	17	9	22	18	18

$$\frac{15.4 \text{ bacteria/field} \times 30{,}954 \text{ fields/filter}}{0.1 \text{ ml/filter}} = 4.77\ 10^{6} \text{ bacteria/ml}$$

Volume filtered = 1 ml. Dilution 10^{-2}. Microscope Factor = 30.954 fields/filter. Bacterial density?

$$\frac{15.4 \text{ bacteria/field} \times 30{,}954 \text{ fields/filter} \times 100}{1 \text{ ml/filter}} = 4.77\ 10^{7} \text{ bacteria/ml}$$

2.8. Density of a suspensión of *E. coli* with an absorbance (550) of 0.18?

Suspension	Absorbance (550 nm)	Bacteria/ml	Log bacteria/ml

Log bacteria/ml = 8.982 Abs + 6.683 (r = 0.97)

$1.994\ 10^{8}$ bacteria/ml

7. Suggested Readings

1. Microbiology by M.J. Pelczar Jr., E.C.S. Chan and N.R. Krieg. TMH
2. General Microbiology by R.Y. Stanier, E.A. Adelberg, J.L. Ingram . MacMillan
3. Brock biology of microorganisms by M.T. Madigan, J.M. Martinko, J. Parker. PHI
4. Bacterial metabolism by G. Gottschalk. Springer
5. Microbial physiology by A.G. Moat, J.W. Foster. John Wiley
6. Industrial microbiology by L.E. Cassida
7. Medical microbiology by Greenwood

8. Assignment

1. Compare endotoxin with exotoxin. Discuss the mode of action of diphtheria toxin. What are the factors that contribute to make a pathogen virulent? (2+3+5)
2. Define chemical defence mechanism. Briefly discuss the role of interferon and complement in host defence mechanism. (2+8)
3. Discuss the process of microbial pathogenicity, in term of infection and disease in man.
4. What are antigen and antibody? Describe the different type of antigen -antibody interaction. Clonal selection theory. (2+4+4)
5. Distinguish between nitrogen fixation, nitrification and denitrification. How do these processes contribute to the nitrogen cycle in nature? (5+5)
6. Describe with labelled diagram the structure of Ig G. How the structure could be ascertained? Describe the genetic basis of variability of IgG molecules formed in the body. (3+3+4)
7. Name one example each of human cancer caused by dsDNA virus and ssRNA virus. Discuss the mechanism of induction of cancer by ssRNA virus. (2+8)
8. What is chemotherapy? Give a brief account of development of chemotherapy. What are the qualities for a good chemotherapeutic agent? (2+5+3)
9. Describe the general structure of immunoglobulin. Mention the roles of T-cells and B-cells in the immunological activity in human body. (4+6)

10. What is Plasmids and how are they classified? Give a brief account of replication, transfer and recombination of plasmids.
11. Describe the molecular structure of immunoglobulins.
12. How many polypeptide chains are present in each antibody molecule? How many antigen sites are present per antibody? How many different antibodies are produced in each mature B lymphocyte?
13. What are three different sources of antibody variability?
14. In what ways the structures of antibodies and T-cell antigen receptor similar?
15. Write short notes on any four of the following:

a) Vermi-culture b) Inflammation c) Oncogenic Virus
d) Presumptive test e) T-cells f) Humoral immunity
g) auxotroph h) Vector i) Biogas
j) Antibiosis k) Endotoxin l) Rhizosphere effect
m) Cytotoxic T-cells n) Stirred-tank bioreactor
o) Third generation penicillinsp) Biomining q) Hypersensitivity
r) Operon models) Clonal theory for antibody formation
t) Biopesticidesu) BOD & COD v) Bioleaching w)RIA
x) ELISA y) Allergy z) Structure of Antibody

16. List what takes place at each of the seven steps of viral replication.
17. Considering that each virus must bind to a specific cell surface receptor for attachment, explain how you would create a drug that prevents viral attachment.
18. Focusing on the nucleic acids and enzymes involved, draw out the replication strategies of the seven classes of viruses.
19. Regardless of the type of nucleic acid, what are the general requirements for a virus to create functional nascent virions?
20. Make a chart that lists the location of transcription for each of the seven classes of viruses.
21. Explain why +ssRNA viruses do not have to carry their own RdRp within their virions.
22. What is the difference between recombination and reassortment?
23. List the steps involved in the reverse transcription and integration of a retrovirus genome.
24. Describe the steps involved in replicating the genome of HBV.
25. Both HIV and HBV use reverse transcription. Explain how reverse transcription is used differently in the replication of these two viruses.

26. What generally determines whether or not a virus needs to gain entry into the nucleus to replicate?

27. Make a table of the seven classes of viruses and list what the first event is that occurs after the virus gains entry into the cell. Transcription? Reverse transcription? Translation?

28. Which of the cellular processes described in this chapter are limited only to enveloped viruses compared to nonenveloped viruses?

29. Which classes of viruses are more prone to introducing mutations during genome replication?

30. What would be the result of interfering with the maturation of virions?

31. Looking at the one-step growth curves, extracellular virus disappears because the virus enters the cell. Why does the virus initially disappear from the intracellular samples, too?

All the materials are self-written and collected from ebook, journals and websites.