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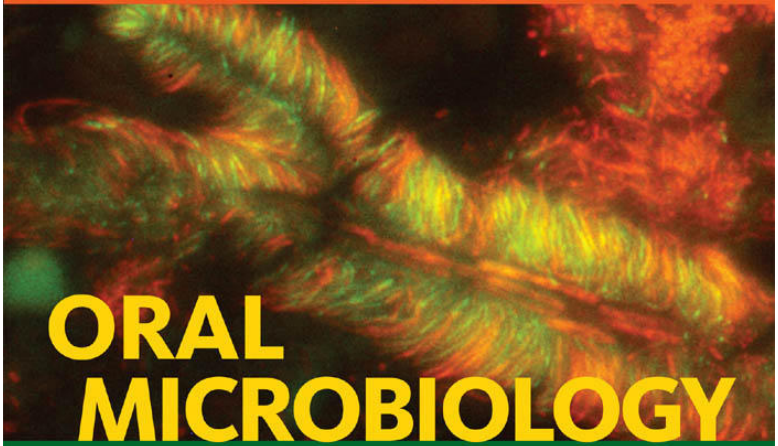
EDITED BY Richard J. Lamont, George N. Hajishengallis, and Howard F. Jenkinson



ORAL MICROBIOLOGY AND IMMUNOLOGY

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Cover image: Longitudinal view of a test-tube brush with a *Lactobacillus* sp. (red rods) as central structures. *Fusobacterium nucleatum* (green) and *Cytophaga-Flavobacterium-Bacteroides* cluster filaments radiate from the central structures. This epifluorescence image is a result of an *in situ* hybridization with fluorescently labeled specific oligonucleotide probes on thin sections of an embedded tooth. Image courtesy of V. Zijnge, M. B. M. van Leeuwen, J. E. Degener, F. Abbas, T. Thurnheer, R. Gmür, and H. J. M. Harmsen. 2010. Oral biofilm architecture on natural teeth. *PLoS ONE* 5(2):e9321. doi:10.1371/journal.pone.0009321

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Preface

In the seven years since the first edition of this book, the world of microbiology and immunology has seen incredible technological and conceptual advances. It is now almost routine to sequence the genome of a bacterium, and for that matter, a community of bacteria; the catalog of proteins for which the crystal structure is known has increased apace; knockout mice deficient in numerous components of the innate and adaptive immune system are widely available; and the regulatory interplay between the innate and adaptive arms of immunity is now better understood. Development of high resolution and 3D imaging techniques has allowed novel studies of communities growing in biofilms, as well as the more intimate interactions between microbes and host cells. High-throughput techniques and extended computer power have made population biology and epidemiology research more comprehensive. This burgeoning knowledge has changed our understanding of both the etiology of oral diseases and the nature of the pathogenic mechanisms and host responses. These changing perceptions are reflected in the updated and expanded chapters. What has (disappointingly) not improved over the last seven years is the incidence of caries and periodontal disease. It is more important than ever for dental practitioners and the clinical scientists to understand the basic science underlying oral health and disease in order for such knowledge to be translated into future health improvements.

As with the first edition, each chapter is self contained and represents the particular insights and priorities of the authors. Taken separately or together, we hope that the chapters provide the reader with the basic facts as well as with the ecological and biological context.

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About the Editors

Richard J. Lamont received a bachelor of science degree in bacteriology from the University of Edinburgh; he received a doctorate from the University of Aberdeen in 1985. After a postdoctoral fellowship at the University of Pennsylvania focusing on streptococcal adherence mechanisms, he joined the faculty at the University of Washington, in 1989. He is currently the Delta Dental Endowed Professor of oral microbiology at the University of Louisville. His research interests include the molecular mechanisms of polymicrobial synergy and the cellular interactions between oral bacteria and the host epithelium. He has taught microbiology and immunology to dental students and residents for over 25 years.

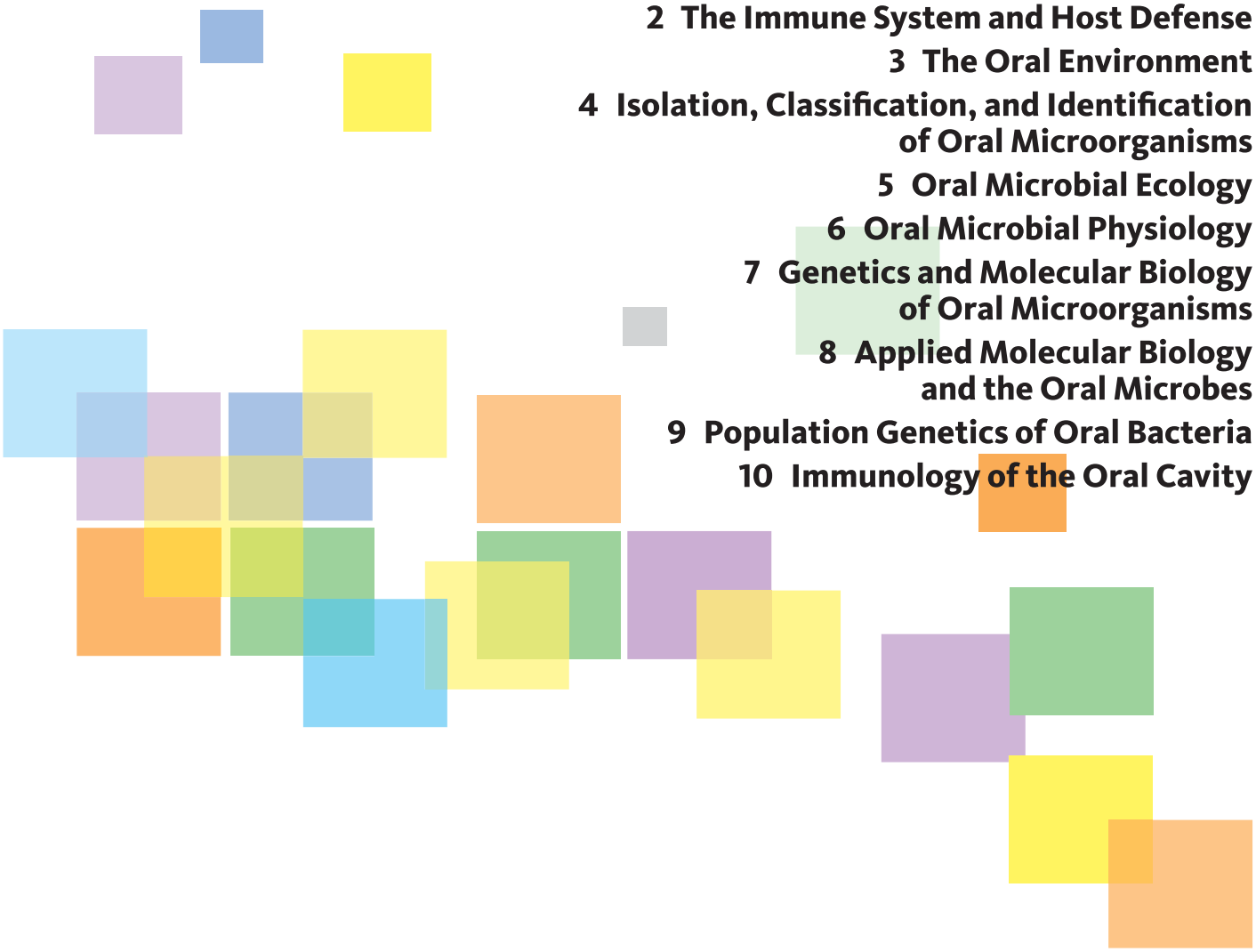
George Hajishengallis was originally trained as a dentist (DDS, 1989, University of Athens, Greece) before pursuing doctoral studies in cellular and molecular biology (PhD, 1994, University of Alabama at Birmingham). His postdoctoral training combined research in mucosal immunology (University of Alabama at Birmingham) and periodontal pathogenesis (State University of New York at Buffalo). He has held faculty appointments at the Louisiana State University, the University of Louisville, and, most recently, the University of Pennsylvania, which he joined in 2012 as a Professor of Microbiology. His field of interest lies at the host-microbe interface focusing on mechanisms of periodontal immunopathogenesis and inflammation. He has taught microbiology and immunology to dental students and residents since 1997.

Howard F. Jenkinson received his bachelor's degree in microbiology and virology from the University of Warwick, England. He completed his PhD training in 1978 at the University of Nottingham. He worked at the University of Oxford for five years as a postdoctoral researcher on the biochemistry and genetics of sporulation in *Bacillus subtilis*. He was appointed Lecturer in Oral Biology at the University of Otago, New Zealand, in 1983 and progressed through the ranks to Professor of Molecular Oral Biology at Otago (1996). He was a visiting Commonwealth

Medical Fellow at the Department of Biochemistry, University of Cambridge (1989–1990), and at the Institute of Molecular Medicine, University of Oxford (1995–1996). In 1997, he moved to the University of Bristol, England, as Professor and Chair of Oral Microbiology. His research interests include the genetics and biochemistry of microbial cell surfaces, principally streptococci and *Candida*, intermicrobial interactions, polymicrobial communities, and infective cardiovascular disease. He has taught molecular microbiology and biochemistry to dental, medical, and basic sciences students since 1983.

SECTION I

GENERAL PRINCIPLES OF ORAL MICROBIOLOGY



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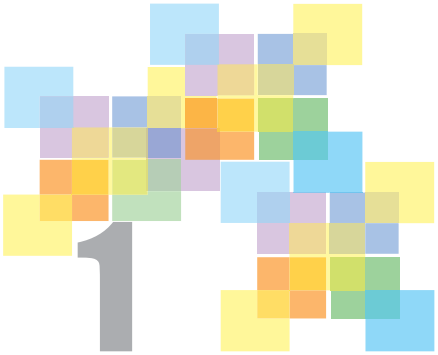

General Microbiology

Howard F. Jenkinson

INTRODUCTION

Antony van Leeuwenhoek was a Dutch scientist who is generally acknowledged as starting the discipline of microbiology. Using simple microscopes that he had fashioned in his workshop, van Leeuwenhoek made the first observations of bacteria and microorganisms, which he named "animalcules." In 1683, van Leeuwenhoek scraped material from his own teeth, describing "a little white matter, which is as thick as if 'twere batter." He continued, "I then most always saw . . . that in the said matter there were many very little living animalcules." When observing a sample from an old man who had not cleaned his teeth, van Leeuwenhoek found "an unbelievably great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time. Moreover, the other animalcules were in such enormous numbers, that all the water . . . seemed to be alive." These observations of the oral microbiota were among the first recorded sightings of live bacteria. Today, we know that the human oral cavity is a highly dynamic ecosystem that supports the growth of a tremendous number of very diverse organisms. In fact, there are roughly a million microorganisms per milliliter of saliva. The organisms that are present in saliva, mostly bacteria and fungi, are there because they are shed from the hard and soft tissues of the oral cavity and nasopharynx and they multiply in retained pools of saliva. The use of microbiological techniques, coupled with sophisticated and sensitive technologies in molecular biology, has helped us begin to gain an appreciation for the diversity of the oral microbiota. Recent estimates place the number of different species of bacteria in the oral cavity at somewhere near 700. Research into the genetics, physiology, and biochemistry of the oral microbiota has shown that the normal colonizers are a critical component in oral health and has led to an understanding of the importance of oral ecology in the development of diseases.

To fully comprehend how oral microorganisms persist and, under certain circumstances, cause disease, it is necessary to have an understanding of the structure, function, and biological activities of the oral microbiota. Why? Knowledge of the structural components of a microorganism is important because determinants on the cell surface dictate which tissues the organisms can colonize. Likewise, many components that contribute to

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the ability of the organisms to cause disease and damage host tissues are located on the cell surface. It is also important to have an appreciation for the wide variety of biological and biochemical activities that oral microorganisms possess. The metabolic capabilities of the cells—their ability to degrade the substances secreted in saliva and ingested in the diet—are of major importance in oral health and disease. How effectively organisms utilize the available nutrients determines whether an organism will establish and compete effectively at particular sites in the mouth. Moreover, the end products of metabolism of these nutrients, such as organic acids, have harmful effects on the tissues of the mouth. The following sections of this chapter highlight key features of the classification, structure, and functions of bacteria with the goal of providing a foundation for the more detailed descriptions of oral microbes, oral microbial ecology, growth of the oral microbiota, and the virulence mechanisms used by oral pathogens that are presented in the following chapters.

CLASSIFICATION SCHEMES FOR BACTERIA

The system that is commonly used for classification of life on Earth is derived from that developed by Carl Linnaeus in the 18th century. This classification scheme, originally intended for systematics of plants and animals, has been useful in accommodating new forms of life as they were discovered through the centuries. Today, life on Earth is divided into three primary domains: *Eukarya*, which are eukaryotes, and *Bacteria* and *Archaea*, which are prokaryotes, the oldest and most diverse forms of life on the planet (Table 1). Archaea, which are sometimes referred to as archaebacteria, differ genetically and metabolically from true bacteria. In fact, archaea are considered to bridge a major gap in evolution between prokaryotes and eukaryotes. Prokaryotes are distinguished from eukaryotes most notably by lack of a nuclear membrane, which separates the chromosomal DNA of the cell from the cytoplasmic contents. Eukaryotes also possess a variety of organelles and subcellular structures—like mitochondria, the Golgi apparatus, and the endoplasmic reticulum—that are

TABLE 1 General differences between prokaryotes and eukaryotes

	Domain		
Property	**Eukarya**	**Bacteria**	**Archaea**
Nuclear membrane	+	–	–
Chromosomes	>1	1	1
Chromosome organization	Linear	Circular	Circular
Murein in cell wall	–	+	–
Cell membrane lipids	Ester-linked glycerides; unbranched, polyunsaturated	Ester-linked glycerides; unbranched; saturated or monounsaturated	Ether-linked; branched; saturated
Cell membrane sterols	Present	Absent	Absent
Organelles	Present	Absent	Absent
Ribosome size	80S	70S	70S
Transcription/translation coupling	No	Yes	Yes

lacking in prokaryotes. There are a variety of other fundamental differences between these two general classes of life, some of which are summarized in Table 1. Among the more notable differences, the transcription of DNA to mRNA and the translation of RNA to protein occur in separate compartments in eukaryotes but not in prokaryotes.

Members of each of the domains can be found in the oral cavity, although the vast majority of the oral microbiota is composed of bacteria. Through highly sensitive techniques, archaea have been detected in the oral cavity, but current indications are that they appear to represent a small minority of the total organisms present on oral soft or hard tissues. Fungi, which are eukaryotic microorganisms, may also be present in the mouth, but generally, they are there in low numbers. Some of the fungi, e.g., *Candida*, flourish only when there is a restriction of access to saliva or a reduction in immunological competence. Because bacteria comprise the overwhelming majority of oral microorganisms, most of this introductory chapter focuses on bacteria.

BACTERIAL CLASSIFICATION

Most bacteria can be divided into two categories, either gram positive or gram negative, based upon a differential staining technique developed by a Danish bacteriologist, Christian Gram. The Gram stain reveals a major structural difference between the two major groups of bacteria based upon the thickness and degree of cross-linking of the cell wall. Detailed molecular studies have revealed that this relatively simple staining reaction also discloses a major evolutionary split between two major classes of bacteria. Among the bacteria, there are also organisms that cannot appropriately be classified on the basis of Gram staining, such as the agent of tuberculosis, *Mycobacterium tuberculosis*, which has a cell envelope made up of mycolic acids and waxes. Instead of Gram staining, mycobacteria can be stained by the Ziehl-Neelsen staining technique, which is also called acid-fast staining. In contrast, *Mycoplasma* species and closely related organisms are completely devoid of a cell wall, and therefore, these organisms are negative in the Gram reaction—even though genetically they are more closely related to gram-positive bacteria. Gram staining and similar techniques remain useful for bacterial identification, but the phylogenetic relationships, i.e., the evolutionary connections of bacteria, are now based almost exclusively on comparisons of nucleotide and protein sequences of organisms. Chapters 4 and 9 explain in detail many of the techniques used to assign bacteria to species, and they also outline current phylogenetic relationships of the oral microbiota.

One of the most fascinating aspects of studying microorganisms is the tremendous diversity in microbial structure, metabolic capacities, and environments in which these organisms can thrive. In nature, there are bacteria that grow optimally at pH values around 2 (acidophiles), whereas others will only grow well at pH values near 10 (alkalophiles). Some prokaryotes grow very poorly at temperatures above 15°C (psychrophiles), whereas some thrive at 100°C in hydrothermal vents miles below the surface of the ocean (thermophiles). Some microorganisms can grow with jet fuel or kerosene as the primary carbon and energy source, others create tiny internal magnets to use for directed movement, some emit light,

TABLE 2 Microorganisms of importance in the oral cavity

Gram-positive bacteria	Gram-negative bacteria
Streptococcus mutans	*Fusobacterium nucleatum*
S. sanguinis	*F. periodonticum*
S. oralis	*Haemophilus parainfluenzae*
S. mitis	*Porphyromonas gingivalis*
S. gordonii	*P. endodontalis*
S. parasanguinis	*Prevotella intermedia*
S. salivarius	*P. loescheii*
S. anginosus	*P. denticola*
Gemella morbillorum	*P. melaninogenica*
Rothia dentocariosa	*P. nigrescens*
Actinomyces naeslundii	*Tannerella forsythia*
A. gerencseriae	*Bacteroides odontolyticus*
A. odontolyticus	*Neisseria subflava*
A. oris	*Veillonella parvula*
Filifactor alocis	*Aggregatibacter actinomycetemcomitans*
Lactobacillus salivarius	*Capnocytophaga ochracea*
L. fermentum	*C. gingivalis*
L. plantarum	*Campylobacter rectus*
Bifidobacterium dentium	*C. ureolyticus*
Eubacterium nodatum	*Treponema denticola*
Parvimonas micra	*T. socranskii*
Peptostreptococcus anaerobius	*T. vincentii*
Propionibacterium acnes	

and others detoxify mercury in the environment. A variety of bacteria can corrode metals, and many, e.g., *Streptomyces*, synthesize products of significant economic importance, such as antibiotics or complex polysaccharides that are used in foods or pharmaceuticals.

Bacteria in and on the human body outnumber the cells composing the body by about 10 to 1. The number of bacteria that colonize humans is fairly small compared to the total number of known bacteria, and the number that routinely cause disease is substantially smaller still. Interestingly, the oral microbial community is among the most diverse group of organisms colonizing the various environments of a human host. To begin to become familiar with the organisms that comprise the oral microbiota in health and disease, some of the more abundant and significant oral microorganisms are listed in Table 2.

BACTERIAL ARCHITECTURE

Most bacteria are about 1 to 5 μm across the largest dimension of the cell, although there are some interesting exceptions, including a few unusual marine bacteria that are as large as 100 μm in diameter. A bacterial colony of roughly 3 mm in diameter that forms on an agar plate can contain upward of 100 million organisms. Bacteria also come in a wide variety of shapes: coccoid or spherical; bacillary or rod shaped; fusiform or long, thin rods that taper at the ends; helical or corkscrew shaped; curved; irregular; or a combination of shapes. In addition, many bacteria can form complex, multicellular structures or can differentiate into alternative shapes with clearly distinct functions and metabolic potential.

Membranes

As with all living cells, biological membranes separate the contents of the cell from the surroundings. The cytoplasmic membrane of the bacteria separates an amazingly concentrated collection of proteins, nucleic acids, lipids, and other constituents from its surroundings. The protein concentration of a typical bacterial cell is estimated at 350 mg of protein per ml; comparatively, human plasma contains only tens of milligrams of protein per milliliter. Gram-positive bacteria possess a single plasma membrane, or cytoplasmic membrane, whereas gram-negative bacteria are characterized by the presence of two membranes, a cytoplasmic (inner) membrane and an outer membrane (Fig. 1). The region between the inner and outer membranes of gram-negative bacteria is known as the periplasm, which contains the cell wall structure, proteins, and lipids. The cytoplasmic membranes of bacteria are not radically different from those of mammalian or plant cells in the sense that they consist of a phospholipid bilayer. However, unlike eukaryotic membranes, the membranes of bacteria lack sterols, such as cholesterol, and are composed primarily of saturated or monounsaturated fatty acids rather than polyunsaturated fatty acids. Membranes of archaea are also composed of a phospholipid bilayer, but the membrane lipids are attached to the glycerol moiety by ether linkages rather than the ester linkages typical of bacteria and eukaryotes. The actual composition of membranes of a given bacterial species, i.e., the number of carbons in the lipids and whether the lipids are monounsaturated or completely unsaturated, can change depending on growth conditions. However, the lipid composition of a given species remains fairly consistent when the bacteria are grown under similar conditions. On the other hand, the membrane lipid composition of different species of bacteria can vary quite a bit. Consequently, it has been possible to distinguish between even fairly closely related bacteria by comparing the composition of the lipids in the membranes of isolated bacteria grown under defined conditions.

Many biologically important proteins and enzymes are embedded in the membranes of bacteria. The cytoplasmic membrane of bacteria houses the machinery for respiration, sensing environmental signals, and transporting compounds and macromolecules into and out of the cell. Many membrane-integral or -linked proteins contribute to the virulence of the microorganisms. For example, membrane proteins can mediate adherence to the host or can have a biochemical activity that is detrimental to host

FIGURE 1 Schematic diagram illustrating the differences between the surfaces of gram-positive and gram-negative bacteria. See text for details.
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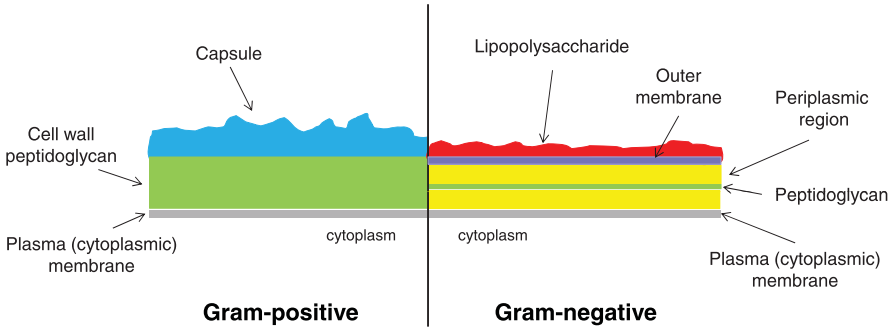

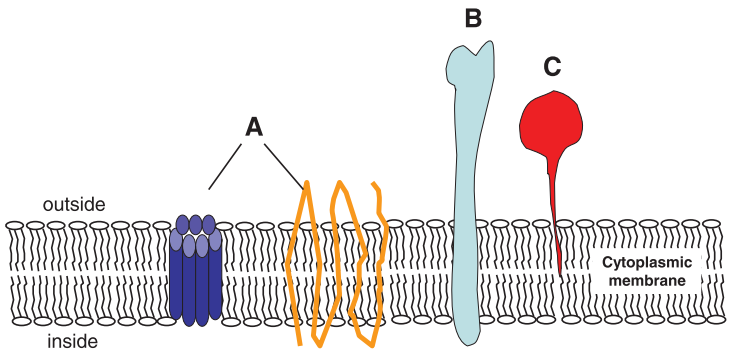

FIGURE 2 Schematic representation of a typical bacterial cytoplasmic membrane with proteins that may be involved in transport of solutes, environmental sensing, adherence, or other critical functions of the cell. See text for descriptions of A, B, and C.
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tissues, such as degradation of host proteins. The association of proteins with membranes occurs by three principal mechanisms. First, a protein can contain multiple hydrophobic domains that are able to weave their way in and out of the membrane, with hydrophilic subdomains of the protein becoming exposed alternately to the cytoplasm and external milieu (Fig. 2, structure A). Such proteins often are capable of forming pores and can be involved in the movement of solutes into or out of the cell, or they can be involved in sensing external stimuli and relaying a signal into the cell. Alternatively, membrane proteins that are in contact with the surroundings can be anchored to the cytoplasmic membrane by a single domain of the protein that is rich in hydrophobic amino acids (Fig. 2, structure B). Finally, membrane proteins can be linked to the membrane by covalent coupling to a lipid moiety, usually via a cysteine residue in the protein (Fig. 2, structure C). These covalently linked lipoproteins have many different functions, including helping bacteria adhere to target tissues.

The outer membranes of gram-negative bacteria also harbor a variety of proteins (outer membrane proteins) that serve many different functions for the organisms, although the distribution, type, and absolute number of outer membrane proteins are highly variable between genera. Porins are a general class of proteins that form pores in the outer membrane that allow nutrients and other small molecules to diffuse into the periplasm, where they can be actively transported across the cytoplasmic membrane. Porins also allow metabolic end products to diffuse out of the periplasm so they do not accumulate to toxic levels or interfere with active transport processes. The outer membrane also contains many other types of individual proteins and complex proteinaceous structures, some of which mediate adhesion to host tissues.

Lipopolysaccharides

Only gram-negative bacteria produce lipopolysaccharide (LPS), a hybrid molecule of lipid and carbohydrate that is abundant in, and adds structural integrity to, the outer membrane of the organisms. LPS consists of three major domains: a lipid A portion of the molecule, which is anchored in the outer membrane, and the core polysaccharide and O side chains, which extend from the cell into the surroundings (Fig. 3). The structure of

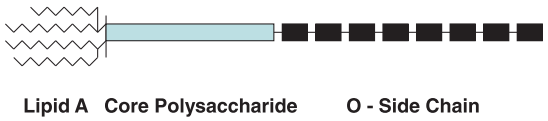

FIGURE 3 Schematic diagram of a typical LPS molecule of gram-negative bacteria.
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LPS varies considerably among gram-negative bacteria, as does the length of the O side chain. Some bacteria have "rough" LPS, which lacks a repeating O side chain, whereas bacteria with "smooth" LPS have an O side chain consisting of a fairly large and variable number of repeating subunits of carbohydrate. The rough and smooth designations do not refer to the LPS molecule directly but rather to the appearance of the colonies on agar media. Strains with long polysaccharide O side chains appear smooth and shiny on agar plates. The classification of bacterial strains by serotyping is frequently based upon the structure and composition of the core polysaccharides and O side chains.

LPS plays major roles in the ability of the organisms to elicit diseases. A listing of some of the biological properties associated with LPS, which is sometimes referred to as endotoxin, is given in Table 3. Among the more important biological effects of LPS are the ability to elicit shock, fever, and apoptosis (programmed cell death) of host cells and the ability to stimulate potent and adverse inflammatory immune reactions through a variety of pathways that ultimately result in tissue damage. Most of the detrimental biologic activities of LPS reside in the lipid A portion of the molecule. Notably, not all bacterial LPS molecules are highly toxic, nor do all elicit the reactions described in Table 3 at biologically meaningful concentrations. Instead, there is a broad spectrum of activity of LPS depending on the organism from which it is isolated. By way of example, *Porphyromonas gingivalis*, which has been implicated in human periodontal diseases, produces an LPS that strongly stimulates bone resorption, a major problem in periodontal diseases, whereas the LPS of some strains of the common intestinal bacterium *Escherichia coli* is comparatively benign in this regard. Some of the mechanisms by which LPS exacerbates periodontal diseases are covered in greater detail in chapter 14.

TABLE 3 Some relevant biological activities of LPS

Lethal toxicity
Stimulation of inflammation
Complement activation
Polymorphonuclear leukocyte activation
Macrophage activation
B-cell mitogen activity
Adjuvant activity
Pyrogenicity
Stimulation of bone resorption
Stimulation of prostaglandin synthesis
Induction of tumor necrosis factor
Hypothermia
Hypotension

Cell Wall Peptidoglycan

With few exceptions, bacteria have cell walls. The material comprising the cell wall is known as peptidoglycan or murein, which is structurally different from the cell walls of plants and fungi. Peptidoglycans consist of a repeating *N*-acetylglucosamine, *N*-acetylmuramic acid carbohydrate backbone linked to a tetrapeptide that generally contains biologically uncommon D-amino acids and diaminopimelic acid (Fig. 4). The peptides are cross-linked to various degrees, depending on the organism and growth conditions, and this cross-linking gives the peptidoglycan a meshwork-like structure that is flexible, yet strong. In gram-negative bacteria, the cell wall lies between the inner and outer membranes and is held in place by covalently bound lipoproteins that anchor the wall to the outer membrane, with the protein portion bound to the wall and the lipid portion buried in the outer membrane (Fig. 5).