

Bacterial and Viral Infections

Victor Nizet and Jeffrey D. Esko

This chapter illustrates some key mechanisms by which glycans influence the pathogenesis of bacterial and viral infections and describes examples of opportunities for therapeutic intervention.

BACKGROUND

Infectious diseases remain a major cause of death, disability, and social and economic disorder for millions of people throughout the world. Poverty, poor access to health care, human migration, emerging disease agents, and antibiotic resistance all contribute to the expanding impact of infectious diseases. Prevention and treatment strategies for infectious diseases derive from a thorough understanding of the complex interactions between specific viral or bacterial pathogens and the human (or animal) host.

Just as glycans are major components of the outermost surface of all animal and plant cells, so too are oligosaccharides and polysaccharides found on the surface of all bacteria and viruses. Thus, most (if not all) interactions of microbial pathogens with their hosts are influenced to an important degree by the pattern of glycans and glycan-binding receptors that each expresses. This holds true at all stages of infection, from initial colonization of host epithelial surfaces, to tissue spread, to the induction of inflammation or host-cell injury that results in clinical symptoms. The microbial molecules responsible for disease manifestations are known as virulence factors.

In a complex environment with many microbial threats, higher organisms have evolved systems of immunity that can discriminate between potential pathogens and mount appropriate antimicrobial responses to block systemic spread and reduce damage to their cells and tissues. Glycan-receptor interactions play crucial roles in microbial pattern recognition as well as in the regulatory signals that govern the normal activities of immune cells. One important reason why certain microbes cause disease is that they have evolved to display their own sugars and receptors in a fashion that mimics or interferes with host glycan-based immune functions.

BACTERIAL SURFACE GLYCANS AS VIRULENCE FACTORS

Polysaccharide Capsules

A human in good health is colonized by as many as 10^{14} bacteria on their skin and mucosal surfaces (particularly in the gastrointestinal tract), a number that exceeds by an order of magnitude the number of cells in our own body. Despite all these direct encounters, only a small handful of bacterial species are known to spread into the body to produce serious infections. Although not restricted to pathogenic species, one feature that these disease-causing agents share in common is the presence of a polysaccharide capsule that covers the bacterial surface (see Chapter 20). Capsule expression by the bacteria poses a particular challenge to immune clearance.

Effective killing of bacteria by phagocytes such as neutrophils or macrophages requires opsonization, a process in which the bacterial surface is tagged with complement proteins or specific antibodies. Phagocytes express receptors for activated complement or antibody Fc domains, which allow host defense cells to bind, engulf, and kill the bacteria. Anionic sugars such as sialic acid present in bacterial surface capsules (e.g., those of the neonatal pathogens, group B *Streptococcus* [GBS] and *Escherichia coli* K1) can bind the host regulatory protein factor H, thereby attenuating the activity of the alternative complement pathway. Through their surface polysaccharide capsules, bacteria also cloak protein structures on their surfaces to which antibodies might be directed.

Generally, humans can generate good antibody responses against bacterial polysaccharide capsules, but this ability is diminished at extremes of age, so that infants and the elderly are particularly prone to invasive infection with encapsulated pathogens. Certain bacteria avoid antibody defenses through molecular mimicry of common host glycan structures, masquerading as "self" to avoid immune recognition. An example is the leading pathogen, group A *Streptococcus* (GAS), which expresses a nonimmunogenic capsule of hyaluronan, identical to the nonsulfated glycosaminoglycan so abundant in host skin and cartilage (see Chapter 15). The contribution of capsule-based host mimicry to bacterial immune evasion is also well illustrated by the homopolymeric sialic acid capsules of *Neisseria meningitidis* (meningococcus), an important cause of sepsis and meningitis. The group C meningococcal capsule is composed of an α 2-9-linked sialic acid polymer that is a unique bacterial structure. In contrast, the group B meningococcal capsule is composed of an α 2-8-linked sialic acid polymer that resembles a motif present on neural cell adhesion molecules (NCAMs) found in human neural tissues (see Chapter 14). The group C capsule has proven to be a successful vaccine antigen in human populations, whereas the group B capsule is essentially nonimmunogenic.

Another challenge posed to host immunity by certain pathogens is the great diversity of capsular structures, which is reflected in the different compositions and linkages of repeating sugar units that are produced by different strains of the same bacterial species. Often, these structures are immunologically distinct, allowing classification of different capsule "serotype" strains; for example, there are five major capsule serotypes of meningococcus (A, B, C, Y, and W-135), six different capsule serotypes of the respiratory pathogen *Haemophilus influenzae* (A-F), nine capsule serotypes of GBS (Ia, Ib, and II-VIII), and more than 90 different serotypes of *Streptococcus pneumoniae* (pneumococcus), which is a leading cause of bacterial pneumonia, sepsis, and meningitis. Antibodies generated by the host against the capsule of one serotype strain typically do not provide cross-protective immunity. Thus, individuals can be repeatedly infected over their lifetime by different serotype strains of the same bacterial pathogen. Figuratively, the strategy of capsular molecular mimicry (e.g., GAS) provides the pathogen with invisibility to the immune system and the strategy of antigenic diversity of capsule types presents a moving target to the immune system. Genetic exchange of capsule biosynthetic genes among serotype strains of an individual species (e.g., the polysialyltransferase gene of meningococcus) can lead to capsule switching in vivo, which provides another means of pathogen escape from protective immunity.

Streptococcus pneumoniae (SPN; "pneumococcus")

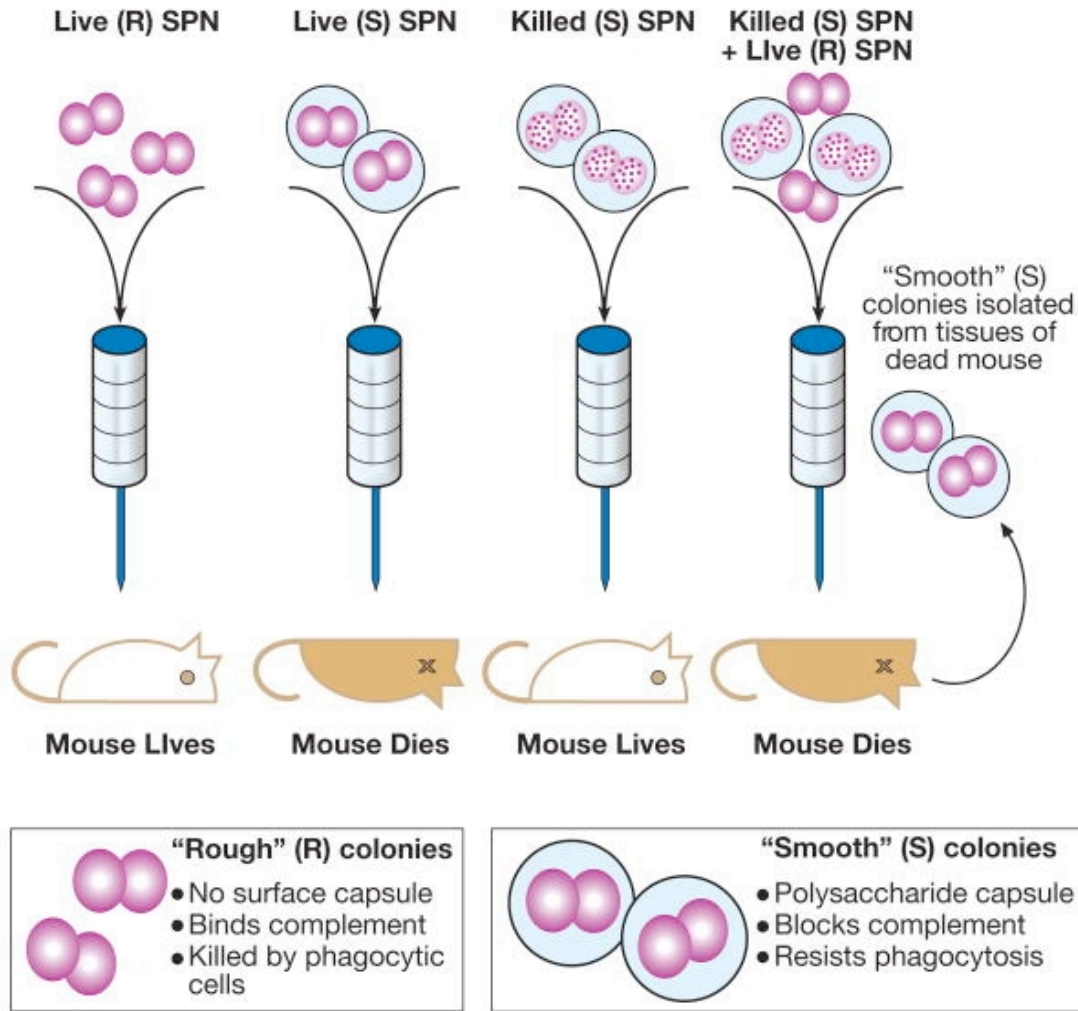
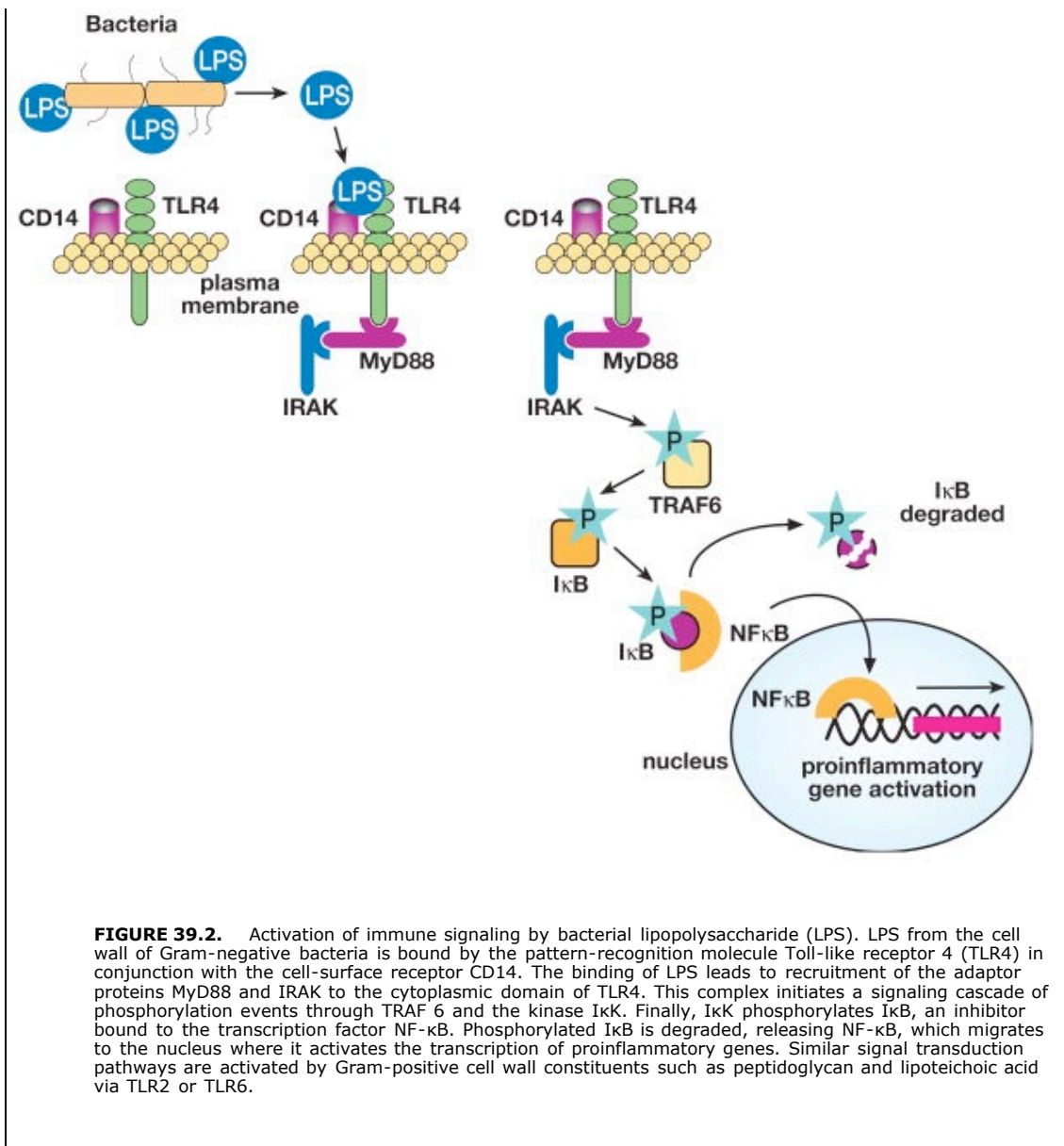


FIGURE 39.1. Classical experiments on the role of the pneumococcal polysaccharide capsule in virulence. *Streptococcus pneumoniae* (SPN) strains can be identified with either a "rough" (R) or a "smooth" (S) phenotype, the latter being due to expression of a thick polysaccharide capsule on their surface. In 1928, Frederick Griffith found that (R) SPN strains were avirulent for mice, whereas (S) strains were highly lethal. Heat-killed (S) strains did not produce disease, but when mixed with live (R) bacteria, the mouse died, and the recovered bacteria expressed the (S) phenotype. Thus, the live (R) strains had been "transformed" to (S) strains by a factor present in the heat-killed preparation of (S) SPN. The factor later proved to be DNA, providing the first evidence that DNA was the basis of the genetic code.

The key role of the capsule in the virulence of multiple bacterial pathogens has been demonstrated through genetic mutagenesis of essential biosynthetic genes and infectious challenge in small animal models of disease. Compared to the wild-type parent bacterial strains, isogenic capsule-deficient mutants of GAS, GBS, pneumococcus, *H. influenzae*, meningococcus, *Salmonella typhi* (typhoid fever), *Bacillus anthracis* (anthrax), and several other important human pathogens are rapidly cleared from the bloodstream by opsonophagocytosis and they are unable to establish systemic infections. Perhaps the most historically significant bacterial virulence factor of all is the pneumococcal capsule, because in classical experiments by Frederick Griffith in 1928, the disease-causing capacity of virulent, encapsulated (smooth) strains was transferred to nonvirulent, nonencapsulated (rough) strains, thereby providing the framework for the subsequent discoveries of Oswald Avery, Colin MacLeod, and Maclyn McCarty that demonstrated DNA to be the genetic material (Figure 39.1).

Lipopolysaccharide

Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria (see Chapter 20). LPS contains a lipid A moiety, which is embedded in the outer membrane, and two carbohydrate components that extend outward: a core oligosaccharide containing sugars not found in vertebrates (such as ketodeoxyoctulonate [Kdo] and heptose) and a polysaccharide side chain known as the O-antigen that can vary from strain to strain within an individual species. The biosynthesis of LPS is described in Chapter 20. Many mucosal pathogens such as *H. influenzae* and *Neisseria gonorrhoeae* lack true O-antigens; instead, they produce lipooligosaccharides (LOSs) that contain a recognizable core structure from which one or more monosaccharide or short oligosaccharide branches extend.



LPS is a pathogen-associated molecular pattern (PAMP) that is recognized by the innate immune system and stimulates inflammatory responses to clear bacteria that have breached the barrier defenses of the skin or mucosal epithelium. Soluble LPS released by invading bacteria, and particularly its lipid A component, interacts with the opsonic receptor CD14 and the membrane protein Toll-like receptor 4 (TLR4) to initiate the immune signaling process (Figure 39.2). TLR4 belongs to an evolutionarily conserved family of receptors (TLRs) that can distinguish closely related microbially derived ligands. For example, although Gram-positive bacteria lack LPS, the TLR2 receptor can recognize peptidoglycan or lipoteichoic acid derived from their cell walls. TLR intracellular signaling is regulated by a group of inter-leukin-1 (IL-1) receptor-associated kinases (IRAKs), which bind to the TLR intracellular TIR (Toll/interleukin-1 receptor) domain, a process that requires the presence of adapter proteins (e.g., MyD88). A signaling cascade ultimately leads to the activation of the transcription factor NF-κB and its translocation to the nucleus, where it positively regulates the promoters for genes encoding several proinflammatory cytokines (e.g., TNF-α and IL-1).

Although TLR4 detection of LPS is a critical element in triggering host innate immunity, a dangerous syndrome known as sepsis can develop in the setting of overwhelming infection, where patterns of immune activation become dysregulated and spin out of control. Symptoms include fever, low blood pressure, rapid heart rate, abnormal white blood cell counts, and dysfunction of multiple organ systems that may lead to lung or kidney failure and death. Original studies by Richard Pfeiffer in the 1890s revealed that a purified heat-stable component of Gram-negative bacteria was itself sufficient to trigger sepsis in experimental animals; this compound was later discovered to be LPS, which consequently is often referred to as bacterial "endotoxin." In the 1960s, it was discovered that a particular strain of mice (C3H/He) was completely resistant to the sepsis-inducing properties of LPS. In the 1990s, a mutation in these mice was mapped to the *TLR4* gene, thereby identifying TLR4 as the functional receptor for LPS in mammals.

Many Gram-negative bacteria vary or modify their LPS to interfere with host immune defense mechanisms. For example, by incorporating modifications that reduce the overall negative charge of LPS, bacteria can repel cationic host antimicrobial peptides (e.g., defensins) away from their cell wall target of action. *Salmonella* species produce gastroenteritis and systemic infections including typhoid fever in humans. The relative resistance of *Salmonella* to antimicrobial peptide killing has been studied extensively in the model organism *S. enterica* serovar Typhimurium. A prominent *S. enterica* LPS modification that confers resistance to antimicrobial peptides is the addition of 4-aminoarabinose to the phosphate group of the lipid A backbone, a genetically controlled phenotype that can be up-regulated 3000-fold during infection. During its adaptation to chronic colonization of the airway of cystic fibrosis patients, *Pseudomonas aeruginosa* has been shown to synthesize a unique hexa-acylated lipid A containing palmitate and 4-aminoarabinose that confers resistance to antimicrobial peptides and stimulates increased cytokine release, thereby perpetuating the chronic pneumonia and lung inflammation that characterize this inherited disorder.

In response to a shift in growth temperature, the plague bacillus *Yersinia pestis* changes the number and type of acyl

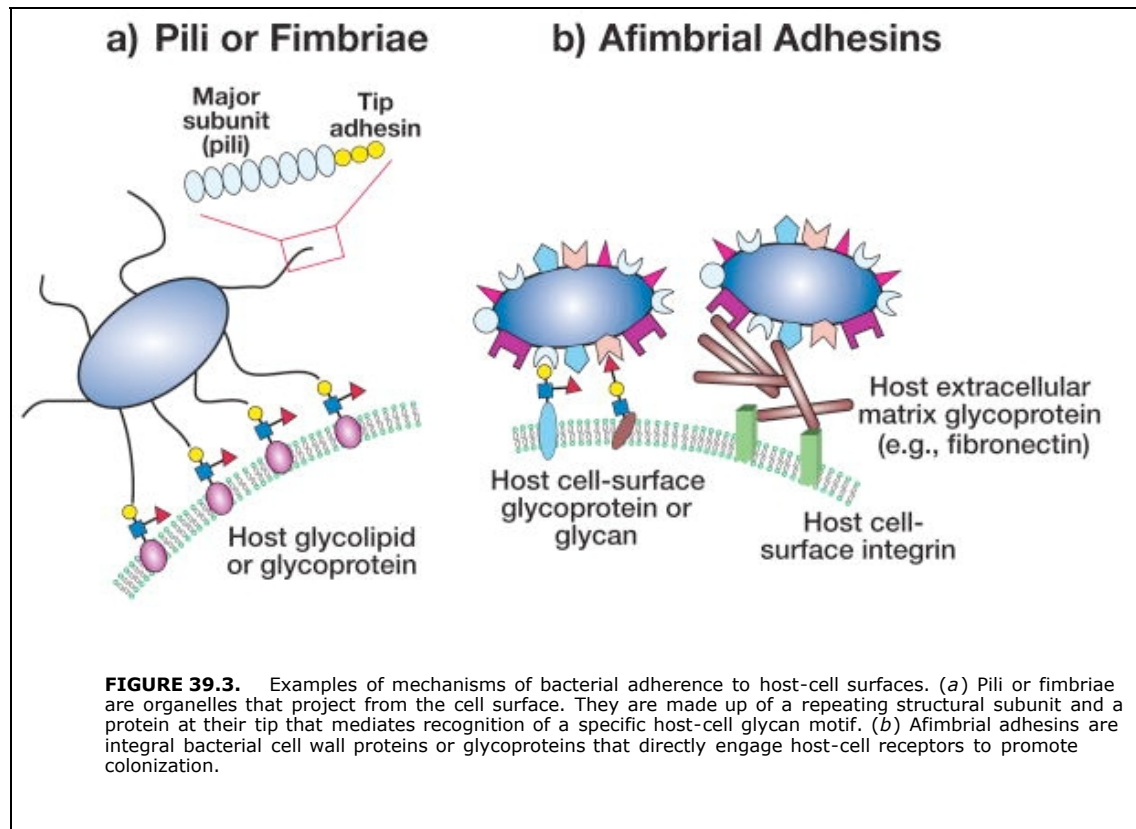
groups on the lipid A of its LPS. At environmental temperatures (21°C), *Y. pestis* expresses predominantly hexa-acylated lipid A, whereas at the body temperature of the mammalian host (37°C), the pathogen expresses mostly tetra-acylated lipid A. The more complicated hexa-acylated version of the LPS strongly induces cytokine release from host cells, which suggests that the production of a less immunostimulatory form of LPS upon entry into the mammalian host might represent a virulence mechanism to avoid immune detection. Recently, this hypothesis was validated by genetically engineering *Y. pestis* to force it to make the potent TLR4-stimulating (hexa-acetylated) version of its LPS even at 37°C. When tested in animal models, the modified *Y. pestis* was completely avirulent even at high-challenge doses.

LOS plays a pivotal role in infection produced by several mucosal pathogens. For example, the sexually transmitted bacterium *N. gonorrhoeae* can express several antigenically distinct types of LOS and can switch from one type to another by an unknown mechanism as a means of immune evasion. During infection, autolysis of the gonococci releases LOS, which stimulates the release of tumor necrosis factor, proteases, and phospholipases from mucosal cells of the genital tract, and these agents contribute to the pathogenesis of pelvic inflammatory disease, a major cause of female infertility. Gonococcal LOS is also involved in the resistance of *N. gonorrhoeae* to the bactericidal activity of normal human serum. Specific LOS oligosaccharide types are known to be associated with serum-resistant phenotypes of *N. gonorrhoeae*. More specifically, the pathogen can directly and efficiently transfer sialic acid from the traces of CMP-sialic acid found in host body fluids to modify its LOS, converting a serum-sensitive organism to a serum-resistant organism. There are also considerable structural similarities between gonococcal LOS and glycosphingolipid antigens present on human red blood cells; this is a form of molecular mimicry that may hamper immune detection.

MECHANISMS OF COLONIZATION AND INVASION

Adhesins and Receptors

Adherence to skin or mucosal surfaces is a fundamental characteristic of the normal human microflora and also an essential first step in the pathogenesis of many important infectious diseases (see Chapter 34). Most microorganisms express more than one type of adherence factor or "adhesin." A large fraction of microbial adhesins are lectins that bind directly to cell surface glycoproteins, glycosphingolipids, or glycosaminoglycans; adhesion may be mediated through terminal sugars or internal carbohydrate motifs. In other cases, the bacteria express adhesins that bind matrix glycoproteins (e.g., fibronectin, collagen, or laminin) or mucin, providing a form of attachment to the mucosal surface. The specific carbohydrate ligands for bacterial attachment on the animal cell are often referred to as adhesin receptors and they are quite diverse in nature. The tropism of individual bacteria for particular host tissues (e.g., skin vs. respiratory tract vs. gastrointestinal tract) is effectively determined by the array of available adhesin-receptor pairs (see Table 34.2).



In a number of cases, the key adhesive factor is an assembly of protein subunits that project from the bacterial surface in hair-like threads known as pili or fimbriae (Figure 39.3a). Such pili are usually composed of a repeating structural subunit providing extension and a different "tip adhesin" that actually mediates the host-cell interaction. Often the structural genes and enzymes for pilus assembly are encoded in a bacterial operon. Lateral mobility of pili structures in the bacterial membrane provides a Velcro-like binding effect to epithelial surfaces. Certain strains of *E. coli* express pili that bind avidly to P-blood group-related glycosphingolipids in the bladder epithelium, leading to urinary tract infection. Pathogenic strains of *Salmonella* produce pili that facilitate adherence to human intestinal cell mucosa, thereby causing food poisoning and infectious diarrhea. In other cases, a surface-anchored protein (afimbrial adhesin) expressed by the bacteria represents a critical colonization factor (Figure 39.3b). For example, the filamentous hemagglutinin (FHA) of *Bordetella pertussis* promotes strong attachment of the bacteria to the ciliated epithelial cells of the bronchi and trachea, triggering local inflammation and tissue injury that results in the syndrome of "whooping cough." FHA is a component of modern pertussis vaccines given in infancy and early childhood to block infection.

For certain pathogens, epithelial attachment is a two-step process, in which a microbial glycosidase acts upon a target

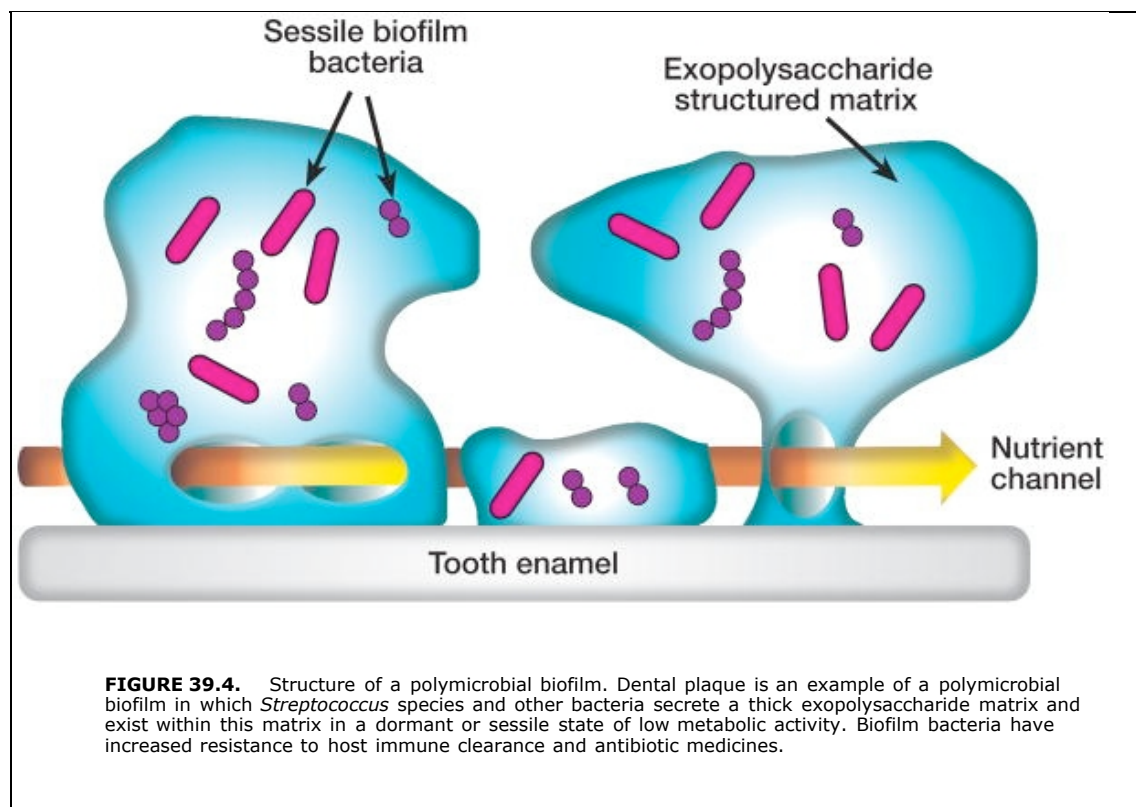
cell polysaccharide to modify its structure into a novel glycan, which then serves as the adhesin receptor. For example, a secreted *P. aeruginosa* neuraminidase produces increased numbers of asialoglycolipid receptors, which may promote colonization of the cystic fibrosis airway. Likewise, the neuraminidase of *S. pneumoniae* removes sialic acid from respiratory epithelial cells to expose underlying *N*-acetylglucosamine and galactose residues to which the bacterium binds with higher affinity.

Invasion Factors

Glycan-lectin interactions play pivotal roles in enabling certain pathogens to penetrate or invade through epithelial barriers, whereupon they may disseminate through the bloodstream to produce deep-seated infections. *S. enterica* serovar Typhi causes typhoid fever in humans, a process that begins with intracellular invasion of intestinal epithelial cells. The outer core oligosaccharide structure of the serovar Typhi LPS is required for internalization in epithelial cells. Removal of a key terminal sugar residue on the outer core markedly reduces the efficiency of bacterial uptake. *Streptococcus pyogenes*, the common cause of strep throat but also an agent of serious invasive infections, attaches to human pharyngeal and skin epithelial cells through specific recognition of its hyaluronan capsular polysaccharide by the hyaluronan-binding protein CD44 (see Chapter 15). This binding process induces marked cytoskeletal rearrangements manifested by membrane ruffling and opening of intercellular junctions that allow tissue penetration by GAS through a paracellular route.

The human malaria parasite *Plasmodium vivax* is completely dependent on interaction with the Duffy blood group antigen for invasion of human erythrocytes. The Duffy blood group antigen is a 38-kD glycoprotein with seven putative transmembrane segments and 66 extracellular amino acids at the amino terminus. The binding site for *P. vivax* has been mapped to a 35-amino-acid segment of the extracellular region at the amino terminus of the Duffy antigen. Unlike *P. vivax*, *P. falciparum* does not use the Duffy antigen as a receptor for invasion. A 175-kD *P. falciparum* sialic-acid-binding protein (also known as EBA-175 [erythrocyte-binding antigen-175]) binds sialic acid residues on glycophorin A during invasion of the erythrocyte. Some *P. falciparum* laboratory strains use sialic acid residues on alternative sialoglycoproteins such as glycophorin B as invasion receptors, with binding being mediated by other EBA family members. The use of multiple invasion pathways may provide *P. falciparum* with a survival advantage when faced with host immune responses or receptor heterogeneity in host populations.

Biofilms



Biofilm formation is another mechanism that promotes bacterial attachment to host surfaces, often in the form of a polymicrobial community. For example, oral biofilms comprise, in total, about 1000 species, only half of which are culturable and the remaining species can only be identified by nucleic acid detection methods. *Streptococcus* species predominate (60–90%), but *Eikenella*, *Haemophilus*, *Prevotella*, and *Propionibacterium* species can also be found. Dental plaque represents an oral biofilm in which dense, mushroom-like clumps of bacteria pop up from the surface of the tooth enamel, interspersed with bacteria-free channels filled with extracellular polysaccharide (EPS) produced by the bacteria that can serve as diffusion channels (Figure 39.4). Bacteria within biofilms communicate with one another through soluble signaling molecules in a process known as “quorum sensing” to optimize gene expression for survival. In biofilms, bacteria live under nutrient limitation and in a dormant state in which defense molecules (e.g., antimicrobial peptides) produced by the immune system and pharmacologic antibiotics are less effective. Moreover, the EPS matrix can bind and inactivate these same agents, contributing to the persistence of the biofilm and difficulty in medical treatment of biofilm infections, such as those that arise on catheters and other medical devices.

The EPS synthesized by biofilm bacteria vary greatly in their composition and in their chemical and physical properties. The majority of EPS types are polyanionic because of the presence of either uronic acids (D-glucuronic, D-galacturonic, or D-mannuronic acids) or ketal-linked pyruvate. Inorganic residues, such as phosphate or sulfate, also contribute to the negative charge. In rare cases, EPS is polycationic, as exemplified by the adhesive polymer obtained from *Staphylococcus epidermidis* strains that produce biofilms on catheters. Ordered secondary configuration frequently takes the form of aggregated helices. In some of these polymers, the backbone composition of sequences of β 1-4 or β 1-3 linkages confers rigidity (as is seen in the cellulosic backbone of xanthan from *Xanthomonas*), whereas the β 1-2 or β 1-6 linkages found in many dextrans provide more flexible structures. It is thought that the EPS itself can serve as the

primary carbon reserve for biofilm microorganisms during substrate deprivation.

Exploiting Heparan Sulfate Proteoglycans

A number of bacteria utilize heparan sulfate proteoglycans such as syndecans (see Chapter 16) as attachment factors for host epithelia, including the genitourinary pathogens *Chlamydia trachomatis* and *N. gonorrhoeae*. Another mechanism by which pathogens exploit host glycan structures is illustrated by the common bacterial pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*, each of which is capable of producing infections in a number of body sites, especially in hospitalized patients. Neither organism binds to syndecans; rather, they release factors that promote the shedding of the syndecan ectodomain from the surface of the host epithelial cell. The negatively charged heparan sulfate side chains of the shed ectodomain bind tightly to cationic molecules such as antimicrobial defensin peptides and lysozyme, neutralizing their antibacterial activities. Thus, these organisms have evolved ways to co-opt the host-cell shedding machinery to release surface proteoglycans, neutralize innate host defenses, and promote their own survival and pathogenicity.

VIRAL INFECTION

The specific binding of a virus particle to a target receptor on the host-cell surface is a prerequisite for viral entry and the subsequent intracellular replication steps in the viral life cycle. As most cell-surface constituents are glycoconjugates, it is not surprising that most viral receptors are mapped to the glycan components of cell-surface glycoproteins, glycolipids, or proteoglycans. These virus-glycan interactions are responsible for species and tissue tropism (see Table 34.1), as is illustrated here with examples provided by the important human infectious agents influenza virus, herpes simplex virus, and human immunodeficiency virus.

Influenza Virus

In humans, influenza viruses are common pathogens of the upper respiratory tract, and seasonal epidemics affect 10–20% of the general population. However, the virus can also be deadly; the influenza pandemic of 1918–1919 killed up to 40 million people worldwide. Influenza virus subtypes are designated by a nomenclature that is based on their surface glycoproteins—namely, hemagglutinin (H) and neuraminidase (N). The first human influenza viruses to be isolated in the 1930s were designated H1N1 based on serological reactions, and this subtype included the pandemic 1918 strain. In 1958, an antigenic shift resulted in the emergence of human H2N2 viruses, and in 1968, a shift to H3N2 viruses occurred; the latter strains have remained the most prevalent in recent years. Emergence of new influenza strains in the human population occurs via transmission from other animal species, especially poultry. Typically, human and avian influenza viruses are different and are not infectious for both species. However, pigs can become infected with both types of viruses, and act as a “mixing vessel” to produce recombinant viruses capable of transmission to humans. Occasionally, direct avian-human transmission can occur, often with enhanced pathogenicity, as demonstrated by the emergence of the recent H5N1 avian influenza in many countries throughout Southeast Asia; human-to-human spread of H5N1 avian influenza has not been conclusively documented.

Interactions between influenza virus hemagglutinin and sialic acid determine the tissue and species tropism of the virus. Human influenza viruses bind to host cell-surface targets that contain *N*-acetylneuraminic acid; however, in the pig, *N*-glycolylneuraminic acids can be used. Binding to sialic acids occurs via a shallow depression near the tip of the hemagglutinin glycoprotein. Viruses that infect humans bind preferentially to terminal sialic acids containing α -6 linkages, whereas others (such as the bird influenza viruses) favor binding to α -3-linked sialic acid, a receptor-binding specificity that correlates with a specific amino acid at position 226 of hemagglutinin. Hemagglutinins that have leucine at position 226 selectively bind to α -6 sialic acid and occur preferentially in human strains; hemagglutinins that have glutamine at position 226 are specific for α -3 linkages and occur mostly in avian strains of the virus. Pigs can become infected with both avian and human strains because both α -3- and α -6-linked sialic acids occur in the trachea of swine. However, a direct binding switch from avian to human specificity can apparently also occur, as happened with the 1918 influenza virus (see cover figure of this book).

The cell-surface receptor(s) for influenza viruses is widely considered to be sialic acid linked to either glycoprotein or glycosphingolipid. However, recent data suggest that the process by which influenza virus enters cells may have further levels of complexity that also depend on protein-glycan interactions. For example, in influenza infection of macrophages, the viruses undergo an additional lectin-like interaction with host mannose receptors after the initial sialic acid binding. In addition, recent experiments in cell lines deficient in terminal *N*-linked glycosylation (due to a mutation in the *N*-acetylglucosaminyltransferase I gene) showed deficient cell entry, even though the initial sialic-acid-dependent interactions occurred normally. These results suggest that influenza virus requires one or more *N*-linked glycoproteins as a cofactor for cell entry after the sialic acid efficiently promotes the initial attachment. In this sense, influenza would share similarities with adenoviruses, another common cause of upper respiratory tract infections, in which host cell-surface integrins function as internalization coreceptors following initial sialic-acid-dependent binding.

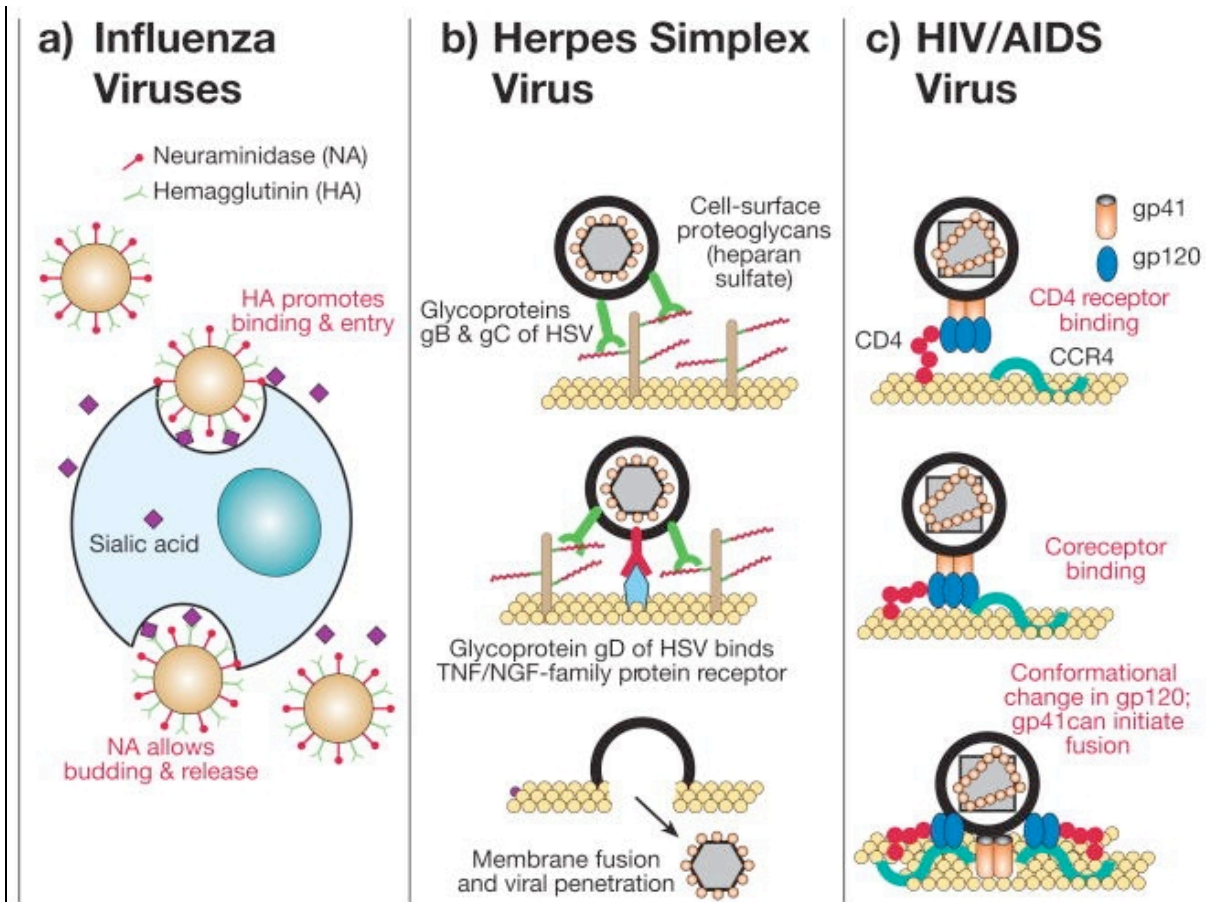


FIGURE 39.5. Mechanisms of viral entry into host cells. (a) Influenza virus initiates host cell contact and entry by binding to cell-surface sialic acid receptors through its surface glycoprotein hemagglutinin. After intracellular replication, a cell-surface neuraminidase cleaves sialic acid from the surface of the host cells, allowing viral escape. (b) Herpes simplex virus (HSV) engages host cells first through a low-affinity engagement of heparan sulfate proteoglycans via its surface glycoproteins gB and gC. Subsequently, a higher-affinity binding of viral protein gD to a member of the tumor necrosis factor–nerve growth factor (TNF/NGF) receptor family promotes membrane fusion. (c) Human immunodeficiency virus (HIV) surface glycoprotein gp120 binds sequentially to the CD4 receptor on T cells and then to a coreceptor such as chemokine receptor CCR4. The latter interaction triggers a conformational change in gp120, which exposes gp41, the HIV factor capable of initiating membrane fusion.

Upon completing its cellular replication cycle, the final release of influenza virus from an infected cell surface relies on the action of the viral neuraminidase, which acts to remove sialic acid (the viral receptor) from the surface of the host cells. Without this step, the newly forming virus particles would immediately rebound to their receptor and not be efficiently released into the extracellular space, remaining attached to the cell in large clumps. Thus, the establishment of a productive influenza virus infection is dependent on both neuraminidase and hemagglutinin and a delicate balance between the functions of the two glycoproteins (Figure 39.5a). It is also possible that the neuraminidase assists in evading host mucosal soluble mucin decoys during the process of infection.

Herpes Simplex Virus

Herpes simplex viruses-1 and -2 (HSV-1 and HSV-2) are human pathogens capable of infection and spread in a number of human cell types, with establishment of latent, recurrent infections. The most common infections include recurrent cold sores of the mouth and lips (usually HSV-1) or sexually transmitted genital ulcer disease (usually HSV-2). HSV infection is mediated by a family of ten viral envelope glycoproteins, which have a variety of specific, and sometimes redundant, functional roles in the establishment of cell infection and spread throughout the host. HSV infection first requires virus attachment to the cell-surface membrane before penetration and entry of the nucleocapsid into the cytoplasm can occur. HSV glycoproteins B (gB) and C (gC) have been shown to be involved in the initial attachment phase through the interaction of positively charged residues with negatively charged heparan sulfate (HS) of cell-surface proteoglycans. This HS-dependent attachment facilitates a second high-affinity attachment in which glycoprotein D (gD) binds to a member of the tumor necrosis factor–nerve growth factor (TNF/NGF) receptor family. Following attachment, the virus penetrates the cell by fusion of the virus envelope with the cell plasma membrane (Figure 39.5b).

Several lines of evidence identified HS as the critical initial receptor for HSV infection. HS proteoglycans are commonly found on the surface of most vertebrate cell types susceptible to HSV infection, and removal of HS from the cell surface by enzymatic treatment or by selection of mutant cell lines defective in HS expression renders the cells resistant to HSV infection by reducing virus attachment to the cell surface. Heparin, a more heavily modified form of HS, can inhibit viral infection by masking the HS-binding domain on the virus envelope. Immobilized HS columns bind to the principal mediators of virus attachment (gB and gC), and HSV deletion mutants lacking gB and gC glycoproteins exhibit impaired virus binding to the cell surface. The gC glycoprotein also binds to the C3b component of human complement, which aids in immune evasion by protecting against antibody-independent neutralization.

Human Immunodeficiency Virus

Human immunodeficiency virus (HIV) is a retrovirus and the etiologic agent of the acquired immunodeficiency syndrome (AIDS), a pandemic disease now affecting nearly 40 million people worldwide, especially in sub-Saharan Africa. The genome of HIV contains only three major genes (*env*, *gag*, and *pol*) that direct the formation of the basic components of the virus. Glycoprotein products of the *env* gene include an envelope precursor protein gp160 (which undergoes proteolytic cleavage to form the outer envelope glycoprotein gp120 that is responsible for the cellular tropism of the virus) and transmembrane glycoprotein gp41 (which catalyzes fusion of HIV to the target cell's membrane). The first step in HIV infection involves the high-affinity attachment of the CD4-binding domains of gp120 to CD4, a receptor present on certain T cells, macrophages, dendritic cells, and microglial cells. Once gp120 is bound to the CD4 protein, the HIV envelope undergoes a structural change, exposing additional binding domains of gp120 that interact with a cell-surface chemokine coreceptor (CCR5 or CXCR4). This more stable two-pronged attachment allows gp41 to penetrate the cell membrane, bringing the virus and cell membranes in close approximation for fusion and subsequent entry of the viral capsid containing the replication enzymes reverse transcriptase, integrase, and ribonuclease into the cell (Figure 39.5c). Meanwhile, the very heavy N-glycosylation of gp120/41 poses a challenge to the host immune system, as potentially immunogenic peptides are blocked from processing for presentation by major histocompatibility complex (MHC) class II and/or blocked from recognition by antibodies.

The differences in chemokine coreceptors present on cells can also explain how different strains of HIV may infect cells selectively. Strains of HIV known as T-tropic strains selectively interact with the CXCR4 chemokine coreceptor to infect lymphocytes, whereas M-tropic strains of HIV interact with the CCR5 chemokine coreceptor to infect macrophages. Natural resistance to a viral disease may occur in a human subpopulation due to the presence of a CCR5 mutation (CCR5Δ32) in certain individuals. CCR5 is also posttranslationally modified by O-linked glycans and both CCR5 and CXCR4 contain sulfated amino-terminal tyrosines. Sulfated tyrosine residues contribute to the binding of CCR5 to gp120/CD4 complexes and to the ability of HIV-1 to enter cells.

Syndecans also have a role in HIV pathogenesis. Primary isolates of HIV from CD4⁺ T cells can bind to all members of the syndecan family via their HS side chains. By binding to syndecans, the fragile HIV-1 virion avoids rapid degradation in the bloodstream. HIV can exploit syndecan binding as an attachment receptor for macrophage entry; in addition, HIV captured by syndecans on nonpermissive cells for replication (e.g., epithelial or endothelial cells) can be transmitted in *trans* to CD4⁺ T cells. In effect, the syndecan-rich endothelial surface provides a microenvironment that amplifies the kinetics of HIV-1 replication in T cells.

SOME BACTERIAL TOXINS ARE GLYCOSYLTRANSFERASES

The disease symptoms associated with many important bacterial infections can largely be attributed to the action of secreted exotoxins that initiate their action by binding to glycosphingolipid receptors on host cells to alter the function of host cells. These include cholera toxin, botulinum toxin, and tetanus toxin as reviewed in Chapter 34. The family of large clostridial cytotoxins exemplifies another glycan-dependent pathogenic effect of bacterial toxins. This family includes toxin A and toxin B from *Clostridium difficile* (agents that cause diarrhea and colitis, especially in patients whose normal flora has been altered by previous antibiotic therapy) and the hemorrhagic and lethal toxins of *C. sordellii* (agents that cause gas gangrene in wound infections that receive soil contamination). These bacterial products turn out to be glucosyltransferases, and this enzymatic activity is critical to their mechanism of toxic action.

In *C. difficile*, the genes encoding toxin A and toxin B are encoded on a pathogenicity locus, along with negative and positive regulators of their expression. Following release from the bacterium, the two toxins translocate to the cytoplasm of target cells and inactivate a series of small GTP-binding proteins, including Rho, Rac, and Cdc42. Inactivation of these substrates occurs through monoglucosylation of a single reactive threonine with UDP-glucose as a cosubstrate. This threonine lies within the effector-binding loop of the target protein and is responsible for coordinating a divalent cation that is critical to binding GTP. By glucosylating small GTPases, toxin A and toxin B cause actin condensation and cell rounding, activation of the transcription of a number of inflammatory genes such as cytokines, and ultimately the death of the cell through apoptosis. In the intestine, these changes lead to neutrophil infiltration, disruption of tight junctions, fluid loss, and diarrhea.

GLYCAN-BASED INTERACTIONS OF HOST AND GUT MICROFLORA: COMMENSALS AND PATHOGENS

The nature of the relationship between microbes and the human host spans the spectrum from mutually beneficial (symbiotic), to benefiting the microbe without harming the host (commensal), to benefiting the microbe at the expense of the host (pathogenic). One of the key factors determining the placement of any particular microbe-host interaction along this continuum of potential outcomes is the repertoire of glycans expressed on host-cell surfaces.

Bacteroides thetaiotaomicron is an anaerobic bacterium that is one of the most abundant members of the normal colonic microflora in mice and humans. This interesting microbe has evolved a mechanism to establish and maintain a nonpathogenic, mutually beneficial relationship with its mammalian host. A clue to this relationship came from examination of the gut epithelium of mice that are raised germ-free and then infected with specific bacteria. Without bacterial exposure, the intestinal epithelium lacks expression of fucosylated glycoconjugates; when normal colonic bacteria are present, Fuca1-2Gal glycan expression is abundant on the surface of these host cells. *B. thetaiotaomicron* preferentially utilizes fucose both as an energy source and for incorporation into its own surface capsule and glycoproteins, phenotypes that are required for successful colonization. When dietary fucose is low, the bacterium sends a signal to the intestinal cells that increases their expression of mRNA for an α1-2-fucosyltransferase, resulting in incorporation of fucose into surface Fuca1-2Gal glycoconjugates on the epithelial lining. *B. thetaiotaomicron* also expresses multiple fucosylidases to cleave these terminal fucose residues and a fucose permease for uptake of the released sugar. Thus, the gut commensal has a system for engineering the production of its own nutrient source from its host; but because the system is regulated for use only in times of need, the host in turn only has to synthesize enough fucosylated glycans to support the maintenance of this important member of its normal microflora. Moreover, the well-adapted *B. thetaiotaomicron* has evolved additional elaborate systems for regulating its expression of particular polysaccharide-binding proteins and glycosidases to forage and consume hexose sugars from the host's dietary intake when abundant, or to switch over to glycosidases in the host mucus lining when sufficient polysaccharides are missing from the diet.

Helicobacter pylori infects nearly half the world's population, but it triggers chronic gastritis and stomach ulcers (conditions that are known to increase the risk of stomach cancer) in only a small subset of these individuals. Patterns of glycan expression in both host and microbe appear to help determine whether *H. pylori* persists as a benign commensal or triggers disease pathology. *H. pylori* expresses an adhesin (BabA) that can interact directly with gastric epithelium expressing glycans that terminate with the Lewis^b blood group antigen. Expression shows that no binding of *H. pylori* occurs to similar glycans modified to express the Lewis^a antigen. Lewis^b expression in human intestine is limited to mucus-producing pit cells in the gastric epithelium. Transgenic mice engineered to express Lewis^b show enhanced binding of *H. pylori* to their gastric epithelium, which triggers an enhanced cellular immune response and more severe gastritis. This microenvironment of immune activation appears to set the stage for a glycan-based process

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