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Minireview
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# Bacterial Glycans: Key Mediators of Diverse Host Immune Responses

Laurie E. Comstock[1] and Dennis L. Kasper[1,2,*]
[1]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[2]Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA
*Contact: dennis_kasper@hms.harvard.edu
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**Recent studies have shown that the synthesis of various polysaccharides by bacteria can induce immune responses that are beneficial to the bacterium, the host, or both. Here, we discuss the diverse interactions between bacterial glycans and the host immune system.**

The outermost surface of many bacteria is composed of glycans, which often represent the first interface between mammalian hosts and microbes. Our understanding of the synthesis, assembly, and secretion of bacterial capsular polysaccharides and O-antigens—which comprise the outermost layer of bacterial lipopolysaccharide—has advanced greatly in recent years. This has been accompanied by a corresponding increase in our knowledge of the many distinct ways that these molecules interact with the host immune system. In fact, some of these bacterial glycan–host interactions actually go against the established paradigm of carbohydrate-induced immune responses. In this Minireview, we explore various interactions between bacterial glycans and the host immune system.

## Polysaccharides: Heterogeneous Molecules Generating Protective Immune Responses

Bacterial capsules and O-antigens are surface polysaccharides produced by both pathogenic and nonpathogenic bacterial species. These polysaccharides are usually highly antigenic and elicit strong antibody responses. These antibody responses often confer protection against subsequent infection. Many pathogens, such as *Escherichia coli*, *Salmonella enterica*, and *Vibrio cholerae*, are classified into different serological groups based on the variability of their O-antigens and capsular polysaccharides. In fact, *E. coli* is known to synthesize at least 173 different O-type antigens and 103 different capsular polysaccharides, a diversity that results in vast intraspecies antigenic variability. Thus, infection with a bacterium of one O-antigenic or capsular type often does not elicit a protective immune response against infection with the same species of bacterium of a different O-antigen or capsular type. Lack of protection against different bacterial serogroups is illustrated by a 1991 cholera pandemic in Bengal, India. The etiologic agent was a *V. cholerae* strain, designated O139 Bengal, that produced an O-antigen unlike those of any of the 138 previously characterized *V. cholerae* O-antigen groups. The *V. cholerae* O1 strain that was endemic in the population failed to provide protection against the new O139 strain, even though the two strains were quite similar in most other respects.

The genetic basis for intraspecies diversity of O-antigens and capsular polysaccharides has been studied in many different species of bacteria. In most cases, the genes involved in the synthesis of a particular polysaccharide are clustered, often in an operon (Figure 1). Polysaccharide biosynthesis regions consist of many genes whose products are involved in the synthesis of precursor nucleotide-charged monosaccharides, glycosyltransferases that create the linkages between monosaccharides, and products involved in transport, assembly, and regulation of polysaccharide expression. These biosynthesis loci are heterogeneous within bacterial species, some with substantial genetic differences and others with an alteration in a single gene. Genes of many of these regions were acquired by multiple horizontal transfer events. Even slight genetic alterations or rearrangements can have marked structural consequences and an enormous impact on immune recognition. In addition to marked diversity in the monosaccharides composing the oligosaccharide repeating unit, other important variations contribute to a polysaccharide's biological specificity, including variations in the glycosidic linkages, the configuration of the anomeric center of each sugar, and the polymer's conformation. These variations are exceedingly important in the generation of the widely diverse structures capable of specific interactions with the immune system.

## Bacterial Glycoproteins

Once believed to be restricted to eukaryotic cells, the ability of prokaryotes to modify proteins by attaching carbohydrates was recognized in groundbreaking studies on S layer glycoproteins (Mescher et al., 1974; Sleytr and Thorne, 1976). Although the field of bacterial glycoprotein research is still young, rapid progress has been made in the last few years. The surface hair-like extensions (pili) of *Neisseria gonorrhoeae*, *N. meningitidis*, and *Pseudomonas aeruginosa* are all glycosylated, and glycosylated flagella are synthesized by *P. aeruginosa* and *Helicobacter pylori*. A general glycosylation system has also been reported in *Campylobacter jejuni* (Szymanski et al., 1999) and *Bacteroides spp.* (Coyne et al., 2005). Studies are beginning to

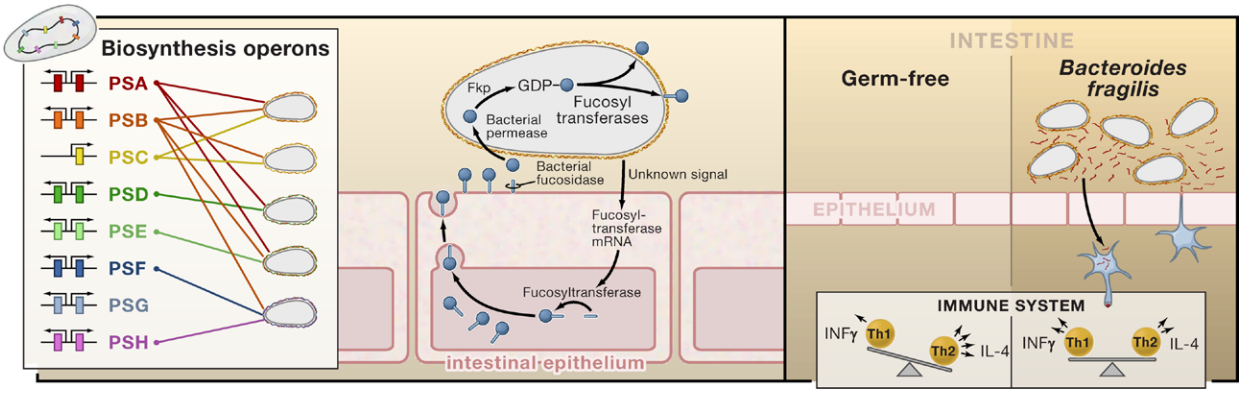


**Figure 1. *Bacteroides fragilis* Glycans in the Mammalian Intestine**

(Left) The scattered genomic distribution of the biosynthesis loci for the eight characterized capsular polysaccharides of *B. fragilis* is highlighted. *B. fragilis* modulates its surface by inverting the promoters (depicted with arrows) governing capsular polysaccharide biosynthesis. The promoters are contained within inverted repeats (black boxes), and DNA inversion between these inverted repeats is a main mechanism dictating which polysaccharides are expressed by individual bacteria. Each polysaccharide is depicted with a distinct color on the surface of the bacteria. (Middle) The bacteria and the host intestinal epithelium both express fucosylated molecules on their surfaces. *Bacteroides spp*. induce the expression of fucosylated molecules on the intestinal epithelium by somehow causing the upregulation of $\alpha$1,2 fucosyltransferase mRNA. *Bacteroides* secrete fucosidases that are able to cleave the terminal fucose moieties from host-derived glycans and internalize them through the action of a fucose permease. This fucose can be converted to GDP-fucose, which ensures its incorporation into bacterial capsular polysaccharides and glycoproteins. (Right) *B. fragilis* colonizes the gastrointestinal tract of mammals and secretes its capsular polysaccharides into the intestinal lumen. The polysaccharide is endocytosed by dendritic cells that carry the antigen to the mesenteric lymph nodes. $CD4^+$ T cells are stimulated via presentation of polysaccharides by the MHC II pathway and results in the production of Th1 cytokines such as INF$\gamma$, with concomitant downregulation of the Th2 cytokine IL-4. This Th1 response balances the Th2 skewing in germ-free animals to resemble that of conventionally colonized animals.

illuminate some of the functional properties of bacterial glycoproteins, many of which are necessary for host interactions. For example, interaction between the 45/47 kDa mannosylated Apa glycoproteins of *Mycobacterium spp.* and the host's immune system elicits an immune response (Romain et al., 1999). Immune stimulation is dependent on both the presence of the glycan component and on the extent of glycosylation (Horn et al., 1999). As research on bacterial glycoproteins continues to evolve, so too will our knowledge of important interactions mediated by these molecules during bacterial association with the host.

## Exchange of Glycans between Host and Bacterial Surfaces

A few bacteria obtain glycan molecules directly from the host and incorporate these molecules into their surface structures. These bacteria have an intimate, dependent, and exclusive association with mammalian hosts. *Haemophilus influenzae*, an important human pathogen that causes sepsis and meningitis, scavenges sialic acid from the host rather than synthesizing this sugar de novo. *H. influenzae* is able to activate this host-acquired sialic acid to CMP-sialic acid and express it on its lipopolysaccharide (reviewed in Vimr et al., 2004). Studies in a chinchilla model of middle ear infection showed that, in order to persist and cause infection, nontypable *H. influenzae* must acquire sialic acid from the host and use this host-acquired sialic acid to decorate its lipopolysaccharide (Bouchet et al., 2003). Another analogous system involves the fucosylated glycoproteins and capsular polysaccharides of the *Bacteroides spp.*, which are important human intestinal symbionts (Figure 1). *Bacteroides thetaiotaomicron* produces a signal that increases the expression of $\alpha$1,2 fucosyltransferase mRNA in intestinal epithelial cells of gnotobiotic mice (i.e., mice that are raised germ-free and then infected with specific bacteria) (Bry et al., 1996). This increased expression leads to an upregulation of fucosylated molecules on the host epithelium. *Bacteroides* organisms synthesize multiple fucosidases that cleave terminal fucose residues, which can then be internalized by the bacteria through the action of a fucose permease. Once inside the bacterial cell, the fucose can either be catabolized for energy or converted to the nucleotide-activated form (GDP-fucose) by the action of the bifunctional bacterial enzyme Fkp (Coyne et al., 2005). Activation of fucose to GDP-fucose by Fkp ensures that host-derived fucose will be incorporated into capsular polysaccharides or bacterial glycoproteins. This entire fucosylation process results in a coordination of the surface architectures of bacterium and host and probably has significant consequences for the success of *Bacteroides* in the mammalian gut. Indeed, mutant organisms that cannot incorporate fucose into their capsular polysaccharides and glycoproteins are rapidly outcompeted by wild-type bacteria in a mammalian intestinal colonization model.

## Immune Responses to Microbial Glycans

The study of bacterial infections has clearly demonstrated the importance of polysaccharides as virulence factors. In fact, capsule production is required for virulence in numerous animal models of infection. Polysaccharides contribute to virulence through several mechanisms, including antiphagocytic and antibacteriolytic activity, immune evasion, immune modulation, and biofilm production.

### Escaping Immune Surveillance

Polysaccharide-deficient bacterial strains are typically killed through the action of normal serum and polymorphonuclear leukocytes (PMNs) without specific antibodies. Killing of encapsulated bacteria by serum and PMNs requires coating these bacteria (opsonization) with both complement molecules and antibodies. Complement deposition on the organism's surface in the absence of specific antibodies is mediated by either the alternative complement pathway involving soluble multivalent lectins that activate the complement cascade, by the mannan binding lectin pathway, or by a recently described interaction with C-lectin receptors (Kang et al., 2006). Certain carbohydrate structures, such as sialic acids, which are common on the capsules of pathogens (e.g., *Neisseria meningitidis*), inhibit activation of complement in the absence of specific antibodies and thereby allow these microbes to survive in the bloodstream. The induction of specific antibodies through immunization, with consequent complement deposition on an organism, is a major factor in successful vaccination.

### Immune Evasion by Molecular Mimicry

The actual glycan composition or structure is crucial for particular interactions with the host immune system as exemplified by bacteria that synthesize polysaccharides that mimic host molecules to evade host immunity. In mammalian cells, hyaluronic acid is an important component of the extracellular matrix and is critical to many cellular processes. The hyaluronic acid capsule of group A streptococci is a clear example of a mammalian structure mimicked by a bacterial polysaccharide to evade the host immune system. A second example is provided by the sialic acid capsule of the bacterium *Neisseria meningitidis* group B. Unlike the $\alpha 2 \rightarrow 9$ linked sialic acid homopolymer that makes up the meningococcal group C polysaccharide—which is highly immunogenic in human adults and has served as a successful vaccine—the $\alpha 2 \rightarrow 8$ sialic acid homopolymer of group B has completely failed as a vaccine. Its lack of immunogenicity in humans is attributed to its structural similarity to glycoproteins in human fetal brain tissues (Finne et al., 1983). These examples illustrate how the structure of these polymers can inhibit a protective immune response, with a favorable outcome for bacteria.

### Zwitterionic Polysaccharides and Immunity

The zwitterionic polysaccharides (ZPSs; polysaccharides with both a positive and negative charge) of *B. fragilis* and type 1 *Streptococcus pneumoniae* provide a very different example of how a structural motif of a polysaccharide is essential for its biological function. This interesting class of bacterial polysaccharides has proven to be an exception to the paradigm for how carbohydrates induce immune responses. In general, polysaccharides are classic T cell–independent antigens; therefore, they do not induce activation of helper T cells that then stimulate IgG switching in B cells, nor do they elicit immunologic memory (a deficiency evidenced by a failure of booster immunization with pure polysaccharide vaccines). To enlist T cell help, polysaccharides have been coupled to protein carriers in the production of successful vaccines that induce production of IgG antibodies and memory B cells. Presumably, the carbohydrate in these glycoconjugate vaccines binds to polysaccharide-specific B cells, and then helper T cells are activated by the processing and presentation of antigenic peptides contained in the conjugate by major histocompatibility class II (MHC II) molecules.

Recently, ZPSs have been shown to be processed and presented by the MHC II pathway (Cobb et al., 2004), which had previously been thought to be reserved for proteins only. ZPSs are taken into the endosome of the antigen-presenting cell (APC) and are processed to a much smaller molecular size. ZPS processing is dependent on nitric oxide (NO) and does not involve glycosidases. The chemistry of the interactions of reactive oxygen or nitrogen species with ZPSs has not yet been delineated. Aside from the NO-dependent mechanism for reducing the molecular size, the ZPSs follow the same MHC II vesicular pathway as conventional protein antigens before being presented to the $\alpha\beta$ T cell receptor in the context of the MHC II molecule.

## Glycans and Long-Term Host-Bacterial Interactions

Clearly, polysaccharides confer advantages to bacterial pathogens in their interactions with the host but can also serve as immunodominant antigens against which the host can often mount a protective response. However, the role of bacterial polysaccharides in bacterial-host relationships that are not transient infectious interactions but rather are long-term or commensal interactions is not well defined. Production of polysaccharides by some common commensal bacteria surpasses that of many pathogenic bacteria. Of the hundreds of bacterial species that inhabit the human intestine, the *Bacteroides* are among the most abundant. Of all bacteria analyzed to date, *Bacteroides fragilis* synthesizes the greatest number of capsular polysaccharides per organism (at least eight) (Figure 1). *B. fragilis* has evolved an elaborate mechanism using "phase variation" for extensively alterating its surface whereby each polysaccharide undergoes a reversible on-off phenotype. Phase variation of seven *B. fragilis* polysaccharides is controlled by DNA inversions of the promoter regions of their biosynthesis loci, placing them in the correct or incorrect orientation for transcription of the downstream polysaccharide biosynthesis genes (Krinos et al., 2001). By this mechanism, *B. fragilis* dynamically modulates polysaccharide expression to create completely distinct surface architectures. It is possible that phase variation of such an extensive array of surface polysaccharides guarantees the long-term existence of a microbial population best fit to survive in the changing milieu of the gut, whether by immune evasion, by resistance to phage infection, by creation of a competitive advantage in colonization, or by favorable interactions with other members of the intestinal microbiota.

In addition to conferring advantages on bacteria, the capsular polysaccharides of *B. fragilis* are important in maintaining the health of the host. Polysaccharide A (PSA), the most abundant of the eight *B. fragilis* polysaccharides,

may be involved in the maturation of the host immune system (Figure 1) (Mazmanian et al., 2005). Although mice born and maintained in a germ-free environment have defects in their intestinal immune system, recent work has shown that they also have systemic immune deficiencies. Several immunologic deficits normally found in germ-free mice can be corrected by introducing *B. fragilis* ("mono-association") into these mice. The immune systems of mice mono-associated with wild-type *B. fragilis* were compared with those of mice mono-associated with the same strain that has a deletion of the genetic locus responsible for synthesizing PSA (ΔPSA). With respect to several important immunologic parameters, wild-type mono-associated mice were identical to conventionally colonized mice that were born and maintained under nonsterile conditions. In both conventionally colonized mice and wild-type mono-associated mice, about 17% of splenic lymphocytes were $CD4^+$ T cells compared to 11% for both germ-free mice and mice mono-associated with the ΔPSA deletion mutant. Splenic $CD4^+$ T cells from conventionally colonized mice and wild-type mono-associated mice produced similar levels of interferon γ, whereas there was markedly less production of this cytokine by cells from ΔPSA-mono-associated and germ-free animals. Interestingly, the conventionally colonized and wild-type mono-associated mice produced low levels of interleukin-4, whereas the ΔPSA-mono-associated and germ-free animals produced high levels of this cytokine. Therefore, the $CD4^+$ T cell subtypes of germ-free and ΔPSA-mono-associated mice were skewed toward T helper 2 (Th2) cells because of low levels of interferon γ (Th1 cytokine) and high levels of IL-4 (Th2 cytokine). In contrast, wild-type mono-associated and conventionally colonized mice have a normal balance of Th1 and Th2 cells. Finally, the spleen and thymus glands of ΔPSA-mono-associated mice exhibited histological abnormalities, whereas wild-type mono-associated mice had normal splenic and thymic histology. Remarkably, these profound effects on T cell lineages were all mediated by a carbohydrate. These findings show that bacterial carbohydrates are very important in directing the maturation of the host immune system. In fact, they appear to be the archetypal molecules of commensals that provide the molecular stimulus for symbiosis between bacteria and their hosts.

The bacterium *Helicobacter pylori* often has a long-term association with mammalian hosts—in this case, as a persistent colonizer of the stomach that sometimes causes ulcers and occasionally stomach cancer. As with *B. fragilis*, a glycan molecule of *H. pylori* appears to modulate the host's immune response. *H. pylori* produce O-antigens that are fucosylated and resemble Lewis blood group antigens. The expression of the Lewis blood group antigens on the surface lipopolysaccharides of *H. pylori* is phase variable and is dictated by slip-strand mispairing in the coding regions of fucosyltransferase genes. Therefore, a given strain of *H. pylori* will manifest as a mixed population with regard to the expression of Lewis blood group antigens. Lewis antigen-positive *H. pylori* strains bind to the C type lectin DC-SIGN that is present on host dendritic cells, and this binding inhibits naive T cells from differentiating into Th1 cells (Bergman et al., 2004). Lewis antigen-negative strains of *H. pylori* fail to bind to dendritic cells and thus promote the development of naive T cells into Th1 cells that release proinflammatory molecules, creating an unfavorable environment for colonization. The variable expression of Lewis antigens may balance the Th1 and Th2 responses to create niches where *H. pylori* can colonize the host without producing disease (Bergman et al., 2006).

In conclusion, the immense structural diversity of bacterial glycans and their presence on both pathogens and commensals inhabiting diverse host sites contribute to the many disparate interactions of these molecules with the host immune system. In addition to the role of polysaccharides as key virulence factors of bacterial pathogens, their importance for both host and bacteria in long-term commensal interactions is beginning to be appreciated. The ability not only to induce or suppress immune responses but also to modulate the mammalian immune response both systemically and locally demonstrates the great functional diversity of these important bacterial molecules.

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