How Bacterial Carbohydrates Influence the Adaptive Immune System

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Abstract
The capsular polysaccharides (CPSs) of most pathogenic bacteria are T cell–independent antigens whose conjugation to carrier proteins evokes a carbohydrate-specific response eliciting T cell help. However, certain bacterial CPSs, known as zwitterionic polysaccharides (ZPSs), activate the adaptive immune system through processing by antigen-presenting cells and presentation by the major histocompatibility complex class II pathway to CD4+ T cells. This discovery was the first mechanistic insight into how carbohydrates—a class of biological molecules previously thought to be T cell independent—can in fact activate T cells. Through their ability to activate CD4+ T cells, ZPSs direct the cellular and physical maturation of the developing immune system. In this review, we explore the still-enigmatic relations between CPSs and the adaptive immune machinery at the cellular and molecular levels, and we discuss how new insights into the biological impact of ZPSs expand our concepts of the role of carbohydrates in microbial interactions with the adaptive immune system.
INTRODUCTION

Most pathogenic bacteria express large-molecular-weight (large-MW) surface polysaccharides, usually in the form of a capsule that coats the bacterial surface. A quick PubMed search on bacterial capsular polysaccharides (CPSs) indicates that an awareness of these molecules existed in the early 1920s. When the pneumococcal type XIV CPS was purified in 1938 (1), it was described as a polysaccharide composed of glucose, amino sugars, and acetyl groups, with an absence of uronic acids. Three decades after its first purification, this polysaccharide was shown to comprise a tetrasaccharide repeating unit, \( \rightarrow 4\beta\text{-Glc(1→6)}\beta\text{-GlcNAc(1→3)}[\beta\text{-Gal1→4}]\beta\text{-Gal1→} \) (2). Thanks to advances in structural biochemistry and carbohydrate chemistry, more than 90 Strepoccus pneumoniae CPSs and other bacterial polysaccharides have been chemically characterized to date (3). Structural knowledge of these CPSs has allowed investigators to uncover mechanisms by which the human immune system deals with many bacterial pathogens.

CPSs offer substantial protection to bacterial pathogens against phagocytosis by migrating phagocytes and tissue-fixed macrophages—cells constituting a crucial host defense mechanism that limits microbial growth and spread and that probably accounts for much of the host’s ability to ward off microorganism-induced diseases (4). This resistance to phagocytosis is conferred primarily by preventing innate host opsonins, such as C3 and its degradation fragments, from coating microorganisms. Complement deposition promotes bacterial uptake by phagocytes via complement receptors. CPSs inhibit this process by several mechanisms (4): (a) Some capsules physically mask subcapsular components of the cell that activate the alternative complement pathway independent of antibody; (b) capsules containing sialic acid cause preferential binding of serum proteins that specifically inhibit amplification of the alternative pathway of complement (5); (c) some capsules lessen the microorganisms’ binding affinity for factor B compared with that of unencapsulated strains; therefore, amplification of C3B deposition via the alternative complement pathway is not promoted, and complement deposition is insufficient for phagocytosis. Induction of antibodies to CPS by immunization is an elegant strategy that has been applied for more than half a century to protect hosts against bacterial invasion by enhancing bacterial phagocytosis (6, 7).

Specific polysaccharide structures functioning in different ways to affect bacterial interactions with the immune system represent a paradigm for the way fine structures of carbohydrates dictate the characteristics—and indeed the very nature—of immune responses (see sidebar, Nomenclature of Monosaccharides, and Figure 1). Certain structural features of CPSs, such as variations in repeating unit composition, ring forms, glycosidic linkage positions, anomeric-center configurations, and conformations, contribute to differences in the immune response to these polysaccharides. The way in which slight structural differences can give rise to distinct immune responses is illustrated by the group B and C polysaccharides of Neisseria meningitidis. The meningococcal group B polysaccharide is composed of \( \rightarrow 8\alpha\text{-D-NeuAc(2→} \) repeating units, whereas the group C polysaccharide has the repeating unit structure \( \rightarrow 9\alpha\text{-D-NeuAc(2→} ; \) the only difference is the \( \alpha\text{-D-NeuAc(2→} \) versus \( \alpha\text{-D-NeuAc(2→} \) glycosidic

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Figure 1

The \( \alpha \) and \( \beta \) configurations of glucopyranose (hexacyclic glucose). In the \( \alpha \) anomer, the \(-\text{OH}\) substituent on the anomeric carbon, rests on the opposite side (trans) of the ring from the \( \text{CH}_2\text{-OH} \) side branch, and in the \( \beta \) anomer, the anomeric hydroxyl sits on the same side (cis) of the plane of the ring.
linkages between the sialic acid repeating sugars (8). Amazingly, the group C polysaccharide is an immunogenic molecule in human adults, whereas the group B polysaccharide is completely nonimmunogenic, failing to induce an antibody response. This difference may be due to the existence of structures identical to the repeating unit of the group B polysaccharide in mammalian tissues; thus, the group B molecule is not recognized as foreign by the immune system.

Group B Streptococcus (GBS) CPSs of types Ia and Ib serve as striking examples of how small differences in the structure of polysaccharides influence immune recognition. In this case, each polysaccharide has five identical sugars in its repeating units, all linked identically except for a single $\beta_1 \rightarrow 4$ versus $\beta_1 \rightarrow 3$ linkage of Galp to GlcNAc in the side chain of the repeating sugar structure (Figure 2). This single linkage difference leads to distinct antibody responses that are not cross-protective (9). Structural features of most bacterial polysaccharides have been reviewed in detail by Ovodov (10).

Over the past two decades, we have defined another structural attribute—charge motif—that significantly influences immunologic responses to CPSs (11–13). Certain CPSs—e.g., polysaccharide A (PSA) of the common intestinal gram-negative obligate anaerobe Bacteroides fragilis—activate the innate immune system through Toll-like receptors (TLRs), and the innate immune system works in conjunction with the adaptive immune system in responding to these molecules (11–14). PSA is processed by antigen-presenting cells (APCs) and presented through the major histocompatibility complex class II (MHCII) pathway to CD4$^+$ T cells, which are consequently activated (14). Biochemical analysis of purified PSA established that its novel immunologic activities result from a unique structural feature: a zwitterionic charge motif (12).

In this review, we explore the molecular and cellular mechanisms by which bacterial CPSs regulate the adaptive immune system, focusing on the structural features and immunologic relevance of glycoconjugate vaccines and zwitterionic polysaccharides (ZPSs).

**NOMENCLATURE OF MONOSACCHARIDES**

Monosaccharides are polyhydroxy carbonyl compounds composed of two or more hydroxyl groups and a carbonyl group that can be either a ketone (called ketose) or an aldehyde (called aldose). Monosaccharides are classified according to several characteristics; one is the size of the ring when they are in cyclic form, and another is the stereocenters (stereocenter is any point in a molecule bearing groups such that an interchanging of any two groups leads to a stereoisomer). A monosaccharide ring with five atoms is called a furanose, and a ring with six atoms is called a pyranose. Monosaccharides are also classified with the stereocenters they contain. The letters D or L assigned to the monosaccharides arise from the orientation of the asymmetric carbon furthest from the carbonyl group. During the conversion from straight-chain form to cyclic form, the carbon atom containing the carbonyl oxygen, called the anomeric carbon, may take two possible configurations called anomers (see Figure 1).

Representatives of the monosaccharide building blocks discussed in this review include GlcpNAc, N-Acetylglucopyranose; Neu5Ac, N-Acetylneuraminic acid; AATp, 2-acetamido-4-amino-2,4,6-trideoxygalactopyranose; and Galf, Galactofuranose.

**GLYCOCONJUGATE VACCINES: A MILESTONE IN PROTECTION AGAINST ENCAPSULATED BACTERIA**

Most bacterial CPSs are T cell–independent antigens (15–20) that induce specific IgM responses, with minimal IgG class switching. Immunization with pure CPSs fails to induce a booster response because of a lack of sustained T cell memory. Although these bacterial surface molecules have been identified as potent vaccine candidates, their inability to induce the activation of adaptive immune machinery is a major impediment to their use in pure form. CPSs cannot stimulate the immune responses of people in high-risk age groups, such as children less than two years old and the elderly. In the early 1980s, inspired by the hapten-carrier protein conjugation strategy (21, 22), CPSs of bacterial relevance of glycoconjugate vaccines and zwitterionic polysaccharides (ZPSs).

**MHCI:** major histocompatibility complex class II

**ZPS:** zwitterionic polysaccharide

**Hapten:** a molecule that can elicit an immune response only when attached to a large carrier such as a protein
Figure 2

Chemical structures of the repeating units of group B streptococcal (GBS) capsular polysaccharides of types Ia and Ib. These two polysaccharides differ only in the glycosidic linkages between the side chain galactose and N-acetyl glucosamine residues ($\beta 1 \rightarrow 4$ versus $\beta 1 \rightarrow 3$, respectively, as highlighted by red shading in the figure) (9).

GBS Ia polysaccharide

GBS Ib polysaccharide

Structural Characteristics of Glycoconjugate Vaccines

Glycoconjugate vaccine design has traditionally been highly empirical. Relatively little systematic work has been done on the carbohydrate portion of these vaccines or on the coupling technology used in their development, nor have other variables (e.g., molar ratio optimization, epitope size, chain length, and carbohydrate modification) been systematically studied. The conjugation of carrier proteins to the polysaccharide has been accomplished by a number of techniques, including the reaction of amino side chains on the lysine or arginine residues of the targets were coupled to carrier proteins possessing T cell peptide epitopes (3, 9, 23, 24). Immunization with glycoconjugate vaccines relies on the elicitation of T cell help for polysaccharide antigens, with promotion of polysaccharide-specific IgM-to-IgG switching, long-lived responses, and immunogenicity in children and the elderly. Over the past 20 years, several glycoconjugate vaccine constructs have been introduced into clinical use (25) and have played an enormous role in preventing infectious diseases caused by highly virulent pathogens such as Haemophilus influenzae, S. pneumoniae, and N. meningitidis (3, 25).
proteins either with the activated carboxylate groups on polysaccharide chains (i.e., carbodiimide coupling) or with aldehyde groups generated by the oxidation of 1,2-diols on the sugar chains (i.e., reductive amination). These coupling reactions yield highly complex, matrix-like structures that are extremely hard to characterize. As we discuss below, efforts have been made over the past decade to improve the immunogenicity of glycoconjugates by designing and synthesizing new generation vaccines.

Traditionally, the quality of vaccine constructs has been evaluated only by their immunologic properties and not by their physicochemical properties. However, an increasing number of sophisticated analytical techniques, including nuclear magnetic resonance (NMR) spectroscopy, are being applied to the quality control of glycoconjugate vaccines. The applications include but are not limited to identification and identity testing of the glycan component, identification and quantification of impurities, quantification of polysaccharide-protein ratios, and detection and quantification of polysaccharide degradation and depolymerization after conjugation (26). Recently, NMR spectroscopy has been used for systematic physicochemical characterization of meningococcal vaccines, with determination of the identity and O-acetyl distribution of conjugated polysaccharides; size-exclusion chromatography coupled to multi-angle laser light scattering detection (SEC-MALLS) has been used for evaluation of the molecular size and distribution of different sized polymers; circular dichroism and fluorescence microscopy have been used for the conformational analysis of carrier proteins; and liquid chromatography coupled to mass spectrometry (LC-MS) has been used to assess the covalent linkages between the polysaccharide and the carrier protein (27).

Cooperation of B and T Lymphocytes: A Critical Factor in Recruitment of T Cell Help by Glycoconjugate Vaccines

In the early 1970s, Mitchison (21, 22), conducting studies in which mice were immunized with various hapten-carrier conjugates and boosted either with different conjugates or with transferred lymphocytes from primed mice, made an important discovery about hapten-carrier protein conjugates. These studies identified two kinds of cells, one recognizing the hapten and the other recognizing the carrier; these cells, which were then called antibody-forming cell precursor (AFCP) and helper cells, are now known as B cells and helper T cells, respectively. This discovery, called the “cellular cooperation hypothesis,” laid the groundwork for our understanding of how B and T cells collaborate to generate the humoral immune response.

The current understanding of the activation of the adaptive immune system by glycoconjugate vaccines is based on research with haptens conjugated to carrier proteins. The traditional explanation for the mechanism of induction of polysaccharide-specific antibodies by glycoconjugates is that these vaccines generate T cell help through several steps. Polysaccharide-protein conjugates bind to the B cell receptor (BCR) of polysaccharide-specific pre-B cells and are taken into the endosome. Once inside the cell, the protein portion is digested by proteases to release peptide epitopes, which bind to MHCII by replacing the self-peptide. The peptide from the vaccine carrier protein is presented to the αβ receptor of CD4+ T cells in the context of the MHCII molecule. Peptide/MHCII-activated T cells release cytokines to stimulate B cell maturation and induce immunoglobulin class switching from IgM to polysaccharide-specific IgG (Figure 3). Although efforts have been made to test this hypothesis at the cellular level (20), the precise molecular mechanisms underlying glycoconjugate processing and presentation in the MHCII pathway have not yet been fully dissected. It will be critical to learn what happens to the carbohydrate in the endosome. The polysaccharide is covalently linked to the carrier protein, usually by a very strong chemical bond such as a secondary amine. The chemical conditions in the endolysosome may be sufficient to oxidize the carbohydrate or enzymatically digest the protein but are not sufficient to break

NMR spectroscopy:

nuclear magnetic resonance spectroscopy
Immunization with a glycoconjugate vaccine results in bidirectional activation of B cells and T cells. As we currently understand the process, the glycoconjugate enters the B cell by binding to the polysaccharide-specific B cell receptor (BCR). The T cell epitope of the carrier protein generated in the endosome then binds to the MHCII molecule to be presented on the cell surface. Recognition of the peptide epitope by the αβ TCR on the CD4+ T cell, along with the interaction of costimulatory molecules CD80/86 with CD28, permits the activation of the T cell, leading to the secretion of IL-2 and upregulation of the IL-2 receptor on the T cell. Finally, the activated T cell secretes IL-4 and upregulates CD40L to activate the B cell through CD40-CD40L binding and signaling via the IL-4 receptor, giving rise to polysaccharide-specific, high-affinity antibody (IgG) secretion by the B cell (20).

As a model glycoconjugate vaccine, a GBS type III polysaccharide–tetanus toxoid conjugate (GBSII–TT) was used. The results revealed a cellular signaling pathway for the induction of a polysaccharide-specific adaptive immune response. Thus, T cell help is recruited through presentation of processed antigen to TCRs by APCs in the context of MHCII molecules (signal 1), interaction of costimulatory molecules CD80 and CD86 on APCs with CD28 on T cells (signal 2), and B cell stimulation through interaction of CD40 with CD40L on activated T cells (signal 3). The interaction of CD80/86 with CD28 induces secretion of IL-2 by T cells; IL-2 then stimulates the proliferation of T cells through binding with the IL-2 receptor on the T cell surface. As a consequence of CD40-CD40L interaction between T and B cells, IL-4 is secreted by T cells and...
stimulates B cells by binding to the IL-4 receptor on the B cell surface. Stimulation of B cells by T cells induces IgM-to-IgG class switching on B cells, resulting in polysaccharide-specific IgG secretion by B cells.

The influence of B cell–T cell interaction on the quality of the immune response generated by conjugate vaccines has been documented by Perez-Melgosa and colleagues (28). Conjugate vaccines based on H. influenzae type b CPS (PRP) and various carrier proteins elicit protective antibody responses to PRP in infants only when multiple doses are given; the sole exception to this rule is for PRP conjugated to meningococcal Outer-membrane proteins (Omps), of which a single dose is sufficient to induce protection. The mechanism underlying this observation is the enhancement of CD40 ligand–mediated, T cell–dependent antibody production by Omps.

Characterizing the influence of carrier proteins on the immunogenicity of glycoconjugate vaccines is crucial for a better understanding of the mechanism of action of these vaccines and to the design of more immunogenic vaccines, as exemplified by the conjugation of PRP with Omps (28). In the conjugate vaccines that have been clinically applied thus far, the chosen carriers have been highly immunogenic modified bacterial proteins, including tetanus toxoid (TT), mutated diphtheria toxin (CRM197), or the Omps of N. meningitidis. The logic behind this choice is that most individuals have previously been immunized with these proteins, and therefore the response to the polysaccharide can potentially be improved by preexisting immunity to the carrier protein. However, this logic is not valid in some situations (29, 30). In a phenomenon called carrier-induced epitope suppression (CIES), individuals previously immunized with a carrier protein may show inhibited immune responses to the polysaccharide when immunized with a glycoconjugate vaccine containing that protein. CIES has been observed primarily with peptide haptens rather than with polysaccharide haptens and thus is thought to arise from competition among peptide haptens bound to carrier proteins for a limited number of carrier-specific helper T cells. Carrier-specific suppressor T cells also play a role in CIES (30). The impact of consecutive or concurrent administration of the same carrier proteins has not yet been dissected in mechanistic terms. Both enhancement and inhibition of polysaccharide-specific immune responses attributable to carrier proteins have been reported; however, because of a lack of understanding of these interactions at a molecular level, the immunologic basis of carrier responses remains unexplained (30).

**Current Trends in Glycoconjugate Design**

Although the value of glycoconjugate vaccines that are in clinical use has been decisively established, the synthesis of structurally well-defined conjugates is necessary not only to improve current vaccines but also to determine the structural features that govern antibody response. Current-generation glycoconjugate vaccines are typically prepared by polysaccharide modification at multiple sites, with subsequent coupling to proteins randomly in a cluster form. Consideration of critical factors such as the nature of the carrier, the ratio of carrier to polysaccharide, and the optimal types of linkages between these molecules would greatly enhance the immunologic properties of glycoconjugate vaccines. The past decade has seen progress from empirical toward rational design of conjugate vaccines. Studies have focused mainly on two aspects of these vaccines: conjugation chemistry and the nature of the carrier protein.

As has been mentioned, current conjugation chemistry requires polysaccharide modifications (e.g., random oxidation of the sugar chain) that alter natural epitopes, with consequent generation of low-affinity antibodies to the native polysaccharide. In addition, random conjugation between activated groups along the length of multiple repeating units of a polysaccharide and random active sites on a protein (e.g., all lysine residues) is difficult to reproduce from batch to batch in a clinical
vaccine preparation. To overcome these obstacles, glycoconjugate vaccines have been prepared by chemically controlled coupling of polysaccharides or of the oligosaccharides derived from CPSs to carriers through their reducing ends via linker molecules (31–33). These conjugates have proven valuable not only as new vaccine candidates for clinical use but also as molecular probes used in the mapping of antigenic determinants of CPSs (to optimize vaccine configuration) and in studies of the molecular mechanisms of antibody and cellular responses to conjugate vaccines (33).

Linker technology also affects particular peptide epitopes in carrier proteins in conjugate vaccine constructs. Presentation of a conjugate-vaccine peptide generated in APCs to T cells is critical to the induction of an immune response to the polysaccharide. Thus, optimization of peptide presentation could potentially increase the immunogenicity of conjugate vaccines. The best approach is to couple polysaccharides to specific peptide epitopes of carrier proteins rather than to intact proteins, thereby allowing the presentation of a significantly larger number of peptides to T cells than in the intact protein conjugates. Moreover, carrier-specific antibody generation might be avoided with this technique. There are numerous examples of successful conjugation of peptides to polysaccharides through linker molecules (32, 34–36). One clever strategy has been to use recombinant carrier proteins constructed to contain strings of 6, 10, or 19 human CD4+ T cell epitopes from various antigenic proteins (36). In each construct, separation of epitopes by the Lys-Gly spacer provides flexibility to the polypeptide and allows conjugation of the polysaccharide to the amino side chains of the lysine spacers. Thus, these synthetic polypeptide carriers can be recognized by most human MHCII haplotypes, and glycoconjugate vaccines prepared with these carriers elicit strong protective antibody responses and do not induce carrier-specific suppression of antibodies to the polysaccharide. Finally, rapid and efficient expression of these polypeptide carriers in Escherichia coli makes them especially attractive for use in glycoconjugate vaccine production.

**Preliminary Evidence for MHCII Presentation of Carbohydrates in Conjugate Vaccines**

In glycoconjugate vaccines, the type of response generated to T helper epitopes is a critical factor in improving immunogenicity over that of unconjugated polysaccharides. A recent study involving only microscopy techniques yielded preliminary data suggesting that the carbohydrate portion of a glycoconjugate vaccine may be presented on the surface of APCs in the context of the MHCII molecule (37). To elucidate this possibility, we review the relevant data on how one group of polysaccharides—the ZPSs—is processed and presented by APCs and recognized by CD4+ T cells.

**Zwitterionic Polysaccharides: Carbohydrates Recognized by T Cells**

The adaptive arm of the immune system facilitates immunity through T cell recognition of foreign antigens. Although T cells have evolved to possess a repertoire of receptors that recognize epitopes of all biopolymers, the traditional paradigm has long dictated that only peptide epitopes of protein antigens are presented to and recognized by T cells. Over the past decade, studies have shown that peptides are not, in fact, the only antigens capable of being recognized by T cells. Several reports have described glycopeptide and glycolipid processing and MHC/MHCII/CD1 presentation and recognition by T cells (38–44). MHCII binds to the glycopeptides—and not just the peptide portion—of these types of epitopes. In addition, TCR binding to processed glycopeptides depends on contact of the receptor with the epitope formed by both the glycan and the peptide that is bound to MHC (38). For instance, Holmdahl and colleagues (42, 43) showed that epitope glycosylation plays
a critical role in T cell recognition of type II collagen (CII). These investigators found that the immunodominant T cell epitope in healthy joint cartilage of humans and rats is O-glycosylated. Studies of glycopeptide epitopes derived from tumor antigen mucin-like glycoprotein 1 (MUC1) are particularly important (39). In tumor cells, protein glycosylation forms tumor-specific glycopeptide epitopes that can be recognized by CD4+ T cells (40, 41). The MHC-like CD1 molecule also presents lipids and glycolipids to γδ TCR-positive and invariant natural killer T (iNKT) cells (44). These studies illustrate that when a glycopeptide or a glycolipid epitope is introduced to the adaptive immune machinery by APCs, it is specifically recognized by T cells. The ZPSs are the newest (and surprising) members of the new generation of antigens with T cell epitopes.

High-resolution NMR studies revealed the chemical composition of PSA and demonstrated both positively and negatively charged motifs on each repeating unit (51). PSA, a tetrasaccharide consisting of repeating units composed of −3)-α-D-AATp-(1–4)[β-D-Galp(1–3)]−α-D-GalpNac(1–3)-β-D-Galp(1– (Figure 4a), has a molecular size of ~110 kDa (51).

The predominant feature shared by MHCII-binding carbohydrates is their zwitterionic structure (Figure 4a and 4b). As detailed below, ZPSs are depolymerized in the endosomes of APCs into molecules of ~10–15 kDa; these molecules bind to MHCII proteins (14, 48). Studies of PSA showed that removal of either its negative or its positive charge eliminates its MHCII binding and thus its recognition by T cells (12, 14). These exciting findings have prompted discussions about the nature of the interaction/binding of processed ZPSs with MHC proteins and about the interaction of the MHCII-bound carbohydrate epitope and the αβ TCR. Several studies have suggested explanations for ZPS-MHCII interactions (52–54); the activation of TCRs by ZPSs is not yet understood at the molecular level.

Peptides bind to MHCII molecules in an extended conformation along the binding groove of MHCII, which is formed by two parallel α helices sitting atop a β sheet. Because the binding groove of the MHCII molecule is open at both ends, MHCII-bound peptides are not limited in size. Thus, peptides that bind to MHCII molecules are variable in length. Peptides fit into the binding groove of MHCII in a linear, unfolded fashion through their anchor residues at various distances from the ends of the peptide (4). The common anchor residues of MHCII-binding peptides include an acidic (negatively charged) amino acid at position 4 and a basic (positively charged) amino acid at position 6 (with positions designated according to the order of amino acids in contact with the binding groove of MHCII) (4). An obvious issue is the degree to which ZPS-MHCII and peptide-MHCII complexes are similar. It is well
established that both negative and positive charges are required for ZPS binding to MHCII. Alteration of the zwitterionic charge motif on these antigens by N-acetylation (which eliminates the positive charge) or carbodiimide reduction (which eliminates the negative charge) results in the loss of T cell stimulatory activity (11). A recent study showed, by confocal microscopy, that both N-acetylated PSA (negatively charged) and carbodiimide-treated PSA (positively charged) are taken up into the endosome of APCs but that, unlike zwitterionic PSA, neither of these charge-modified polysaccharides is presented on the surface of the APC (Figure 5) (55). It remains unclear whether the zwitterionic charge motif is solely responsible for binding (electrostatically) to MHCII or is needed for a particular three-dimensional (3-D) conformation that facilitates the binding of processed ZPSs to MHCII.

The 3-D conformations of two ZPSs that are very different from each other in primary structure (e.g., monosaccharide composition)—Sp1 of S. pneumoniae and PSA2 of B. fragilis—were determined by a combination of NMR spectroscopy experiments and molecular dynamics calculations (52, 53). The two ZPSs have almost identical 3-D conformations: an extended right-handed helix with eight monosaccharide residues per turn and a pitch of 20 Å (Figure 4c and 4d). Positive and negative charges alternate...
(a–d) Confocal microscopy images illustrate the role of zwitterionic charges in the uptake and presentation of PSA by antigen-presenting cells (APCs) (Raji B cell line) in the context of MHCII (55). PSA (a) and its derivatives missing negative, positive, or both charges (b, c, and d, respectively) are labeled in red; MHCII (a–d) is labeled in green; and yellow areas show colocalization of the two. All polysaccharides are taken up by the APCs (endosomal colocalization in a–d). Although depolymerized PSA colocalizes with MHCII both in the endosomes and on the cell surface (a), none of the charge-modified PSA derivatives—negatively charged N-acetylated PSA (b), positively charged carboxylate-reduced PSA (c), or N-acetylated, carboxylate-reduced neutral PSA (d)—colocalizes with the MHCII molecule on the APC surface. (e) White area of trilocalization (arrows) of PSA (blue), MHCII (red), and the αβ TCR (green). The αβ TCR interacts in a cognate fashion with PSA in the context of MHCII (12). The images in this figure are reproduced, with permission, from Cell and Glycobiology, References 12 and 55.
conformations. These researchers also demonstrated that changes in 3-D conformation of PSA correlate with its binding to MHCII. Using an ELISA-based technique, they demonstrated that PSA derivatives smaller than three repeating units and derivatives with neutral, negative, or positive charges do not bind to MHCII, whereas zwitterionic PSA fragments ranging in size from 3 to 30 kDa do. It is interesting that higher-MW PSA fragments bind to MHCII very modestly compared with fragments of 3–30 kDa. The authors stated that the less efficient binding of high-MW PSA to MHCII is not attributable to a lack of helical structure but perhaps is due to masking of the binding domain of PSA by a higher-order structure that has not yet been identified. This study strongly supports the hypothesis that PSA must maintain a helical content to enable MHCII binding and that the zwitterionic charge motif is critical for the formation of a helical structure. These observations correlate well with the in vivo binding of processed zwitterionic PSA (10–15 kDa) with MHCII in the endosome of APCs (12, 13).

In addition to providing the right 3-D conformation for binding, zwitterionic charges on PSA may facilitate binding to MHCII through electrostatic interactions. Cobb & Kasper (55) used various concentrations of sodium chloride to inhibit the interactions of MHCII molecules and various antigens. Unlike the MHCII-bound myelin basic protein T cell epitope (MBPp) or the superantigen staphylococcal enterotoxin A (SEA), both of which were unaffected by the addition of salt, PSA was inhibited significantly (by 60%) in terms of MHCII binding by the addition of salt, which blocked electrostatic PSA-MHCII interactions (55). In addition, an acidic medium favored MHCII binding of PSA. The binding of MHCII with PSA, peptide (MBPp), and superantigen (SEA) was compared at pH 5.0 and pH 7.3. Although MBPp and SEA binding affinity was similar at the two pH values, that of PSA was sensitive to pH, with a fivefold higher binding affinity in acidic medium. The requirements for an acidic environment for processing into smaller-MW polysaccharides suggest that PSA, like protein, is processed within the endolysosome of the MHCII pathway and is bound to MHCII in the acidic endosome. These experiments and several other published studies eliminated the possibility that ZPSs are superantigens (13, 14, 56, 57).

Among the human MHCII proteins, HLA-DR was shown to be responsible for presenting processed PSA to CD4+ T cells (12). Further exploration of allelic selectivity showed that preprocessed PSA (chemically depolymerized into 15-kDa fragments) binds to HLA-DR2 with an affinity ($K_d = 0.31 \mu M$) sixfold and threefold higher, respectively, than what it shows for HLA-DR1 ($K_d = 1.9 \mu M$) or HLA-DR4 ($K_d = 1.0 \mu M$) (55). The binding affinity of PSA was ~15-fold higher than that of both MBPp and SEA. Of even greater interest, PSA bound to HLA-DR with 1:1 stoichiometry (just like the peptide antigen MBPp), whereas the superantigen SEA had a binding stoichiometry of 1–2:1. To elucidate the binding position of PSA on the MHCII molecule, competition experiments were performed wherein increasing concentrations of PSA were added to MHCII preloaded with either peptide (MBPp) or superantigen (SEA). Remarkably, with regard to MHCII binding, PSA outcompeted peptide and SEA by ~75% and 80%, respectively. These findings may suggest that PSA binds both in the MHCII groove (like peptides) and outside the groove (mimicking superantigens). Another possible explanation for this observation is that PSA induces a conformational shift in MHCII that disrupts peptide and superantigen interactions.

A conclusive and direct explanation for the molecular interactions of ZPSs and MHCII can be obtained only by crystallography experiments. A cocrystal of a processed ZPS bound to an MHCII molecule would reveal all the molecular requirements for MHCII-ZPS binding. Crystallization of biomolecules is achieved by the use of structurally well-defined single molecules. There are extremely few examples of cocrystals of carbohydrates with their binding
proteins. Current knowledge of carbohydrate chemistry is very limited, and chemical synthesis of high-MW complex carbohydrate structures is extremely challenging. So far, investigators have succeeded in synthesizing one fully protected tetrasaccharide repeating unit of PSA (58). Because of the obstacles encountered in synthesizing pure, single-molecule ZPS epitopes that can be recognized by T cells, co-crystals of MHCII-processed carbohydrate complex have not yet been created.

**Processing and Presentation of ZPSs by the MHCII Pathway**

As described above, at the molecular level, PSA shows similarities to peptides in binding to MHCII. At the cellular level, PSA is handled by the MHCII pathway in a manner similar to that documented for traditional protein antigens (12). This process involves several steps: (a) PSA is taken into the endosome of APCs; (b) PSA is processed into low-MW T cell epitopes in the endolysosome; (c) endolysosomes containing processed PSA fuse with exocytic vesicles containing self-peptide (CLIP)-bound HLA-DR (in humans), creating the MIIC (MHCII-containing) vesicle; (d) acidification of MIIC initiates HLA-DM-mediated exchange of CLIP with processed PSA on HLA-DR; and (e) PSA-loaded HLA-DR is presented on the surface of an APC to be recognized by a CD4+ T cell (Figure 6a). We next discuss these steps in more detail.

The first step is the uptake of antigen by APCs. PSA is endocytosed by professional APCs such as dendritic cells (DCs), B cells, and macrophages (12, 13, 59). Confocal microscopy experiments have shown that PSA is taken up by APCs with rapid kinetics and colocalizes with the endosomal marker lysosome-associated membrane protein 1 (LAMP-1) (12). No published data elucidate PSA uptake by APCs. However, preliminary data obtained in our laboratory indicate that a receptor-independent mechanism, most likely macropinocytosis, is involved (M. Kazmierczak, unpublished observations).

Once in the endosome, PSA is depolymerized by a novel mechanism that is quite distinct from that used in the processing of protein antigens. As is well established, peptides presented by MHCII are generated in acidified endocytic vesicles. As the protein is engulfed by the cell, a progressive decrease in the pH of the endosome activates the acid proteases (e.g., cathepsins) that reside in these vesicles, and these enzymes degrade the protein to generate peptide epitopes (4). The tenets of classic cell biology would dictate that eukaryotic APCs do not possess a sufficient number of highly specific glycosidic enzymes (or any other means) to process the diverse carbohydrates presented by microorganisms within an APC. However, as we have shown for ZPSs, chemical degradation of these molecules in endosomes can produce smaller derivatives (13, 48). PSA is depolymerized from ∼110 kDa to ∼15 kDa in the endosomes. Incubation of radiolabeled PSA ([3H]PSA) with various APCs and extraction of the endosomal compartments yield degraded PSA detectable by gel filtration chromatography.

Monosaccharide composition and glycosidic linkages in bacterial carbohydrates are generally very different from and frequently more complex than in the polysaccharide structures of eukaryotic cells. Bacterial ZPSs are therefore resistant to depolymerization by eukaryotic glycosidases. PSA processing was found to be independent of enzymatic degradation (13). After careful exploration of possible environmental factors in PSA depolymerization (e.g., pH, reducing or oxidizing conditions), investigators concluded that PSA and other polysaccharides are susceptible to oxidative cleavage in vitro by certain reactive oxygen species and reactive nitrogen species. To determine the role of oxidation on PSA processing for presentation to T cells in vivo, PSA was introduced into knockout mice lacking NADPH oxidase (the enzyme responsible for the generation of superoxide) and knockout mice lacking inducible nitric oxide synthase [iNOS, the enzyme responsible for the generation of nitric oxide (NO)]. As
Figure 6
(a) Schematic of ZPS processing and presentation by the MHCII pathway (12). Extracellular ZPS is endocytosed by the antigen-binding cell (APC) and processed into smaller-molecular-weight polysaccharides (~15 kDa) by inducible nitric oxide synthase (iNOS)-mediated oxidative depolymerization (chemical reaction is shown in b). The endosome containing processed ZPS then fuses with the resident lysosome and the exocytic vesicle to form the MIIC vesicle containing HLA-DR, HLA-DM, LAMP-1 (lysosome-associated membrane protein 1), and processed polysaccharide. Finally, the processed polysaccharide is loaded onto HLA-DR with the aid of HLA-DM and is presented on the surface of the APC to be recognized by the αβ TCR. (b) Deamination of the AATp residue in one repeating unit of PSA, leading to depolymerization of PSA in the endosomes of APCs (13). The positively charged amino group is lost by this reaction only at the cleavage site corresponding to one repeating unit in each processed molecule (containing ~16 repeating units).
The activation of CD4<sup>+</sup> T cells by PSA (11) (explained in more detail below). In this model, PSA is administered intraperitoneally, along with sterile cecal contents, and sterile abscesses form if PSA is processed and activates CD4<sup>+</sup> T cells. When injected with PSA, all NADPH<sup>−/−</sup> mice formed abscesses, whereas iNOS<sup>−/−</sup> mice did not. When injected with preprocessed (15-kDa) PSA, however, the iNOS<sup>−/−</sup> mice did develop abscesses. These results clearly demonstrate that APCs need iNOS for processing of PSA and for activation of CD4<sup>+</sup> T cells (13).

After exposure of APCs to microbial products, iNOS is upregulated and catalyzes the oxidation of L-arginine to form NO (60). These events in response to PSA served as evidence for the role of NO in PSA processing (13). Further studies defined the mechanism of NO-dependent PSA processing (13), using CD11c<sup>+</sup> DCs as APCs. First, DCs from wild-type and iNOS<sup>−/−</sup> mice were incubated either with PSA or with N-acetyl PSA (in which positively charged free amino groups had been blocked with N-acetylation). PSA was depolymerized to a much greater extent in DCs from wild-type mice than in DCs from iNOS<sup>−/−</sup> mice; however, N-acetyl PSA was degraded minimally in both wild-type and iNOS<sup>−/−</sup> DCs. This experiment suggested that the depolymerization of PSA is mediated by NO and that free amino groups are necessary for NO degradation. These studies also showed that NO-independent depolymerization of PSA occurs through a deamination reaction. It is well established that NO-derived deaminative degradation of polysaccharides requires free amino or N-sulfo groups on sugar units, whereas N-acetyl sugars are resistant to deamination (61).

Deamination is also crucial for the activation of T cells by PSA. In vitro, PSA induces the proliferation of CD4<sup>+</sup> T cells when T cells from either wild-type or iNOS<sup>−/−</sup> mice are incubated with PSA in the presence of DCs from wild-type mice. CD4<sup>+</sup> T cells do not proliferate when incubated with the polysaccharide in the presence of iNOS<sup>−/−</sup> DCs. However, if T cells are stimulated with preprocessed PSA (in vitro, NO degrades PSA into fragments of ~15 kDa), they proliferate to the same extent upon coculture with either iNOS<sup>−/−</sup> or wild-type DCs. This in vitro experiment correlated with the in vivo abscess formation experiment described above.

The [1H]NMR spectra of PSA and the NO-degraded product (PSA-NO) are identical. Thus, deamination of PSA does not alter its repeating unit structure except at cleavage sites. Furthermore, critical charges on amino and carboxylate groups remain intact after NO depolymerization. In short, processing of PSA in the endosomes of APCs is based on a chemical reaction called deaminative cleavage, and this reaction is mediated by iNOS-generated NO (Figure 6b). This study served as the first demonstration of a mechanism for bacterial polysaccharide processing through the MHCII pathway (13).

Once PSA is processed in the endosome of the APC, endosomes are fused with lysosomes and exocytic vesicles to form the MIIC vesicle carrying HLA-DR and the accessory molecule HLA-DM. HLA-DM plays a critical role in peptide presentation by MHCII, facilitating dissociation of the MHCII self-peptide CLIP, which permits binding of processed endosomal peptides. Cobb and colleagues (12, 55) described the role of HLA-DM in PSA presentation as very similar to that in peptide presentation. In vitro, PSA binding of MHCII was greatly catalyzed by the presence of HLA-DM. In an ex vivo PSA presentation assay, MHCII complexes of processed PSA were immunoprecipitated from either wild-type or HLA-DM<sup>−/−</sup> primary splenocytes. Significantly less carbohydrate was detected in immunoprecipitates from HLA-DM<sup>−/−</sup> cells. Finally, in an in vivo T cell activation assay, abscess induction by PSA was reduced in mice lacking the HLA-DM protein (55).

The presentation of PSA-loaded MHCII on the surface of APCs was demonstrated by both confocal microscopy and...
coimmunoprecipitation. Confocal images of APCs incubated with fluorescently labeled PSA showed that this polysaccharide colocalizes with the MHCII protein on the surface of APCs (Figure 5). When these APCs are treated with drugs blocking the MHCII pathway at different stages (e.g., with colchicine, which inhibits endocytosis by blocking microtubule polymerization, or with bafilomycin A1, which inhibits loading of PSA on MHCII by blocking acidification of the MHC vesicle—an event required for HLA-DM-mediated removal of self-peptide CLIP on MHCII), presentation of PSA on the cell surface was dramatically diminished. Although confocal microscopy images showing colocalization of PSA with MHCII suggested that PSA is bound to MHCII, these images could not definitively confirm binding on the cell surface because the resolution in fluorescence microscopy is not high enough to reveal molecular interactions.

To resolve this issue, a coimmunoprecipitation experiment was done. After incubation of APCs with radiolabeled PSA, surface-bound MHCII molecules were precipitated with an antibody to the MHCII protein, and a PSA fragment of ∼15 kDa was shown to be bound to these surface-bound MHCII molecules (13).

Finally, engagement of surface MHCII proteins of PSA-treated APCs with the αβ TCR on the surface of CD4+ T cells was documented by confocal microscopy (Figure 5e). Trilocalization of MHCII, PSA, and the αβ TCR suggested that PSA induces APC–T cell engagement through binding to both MHCII and the αβ TCR. These results illustrate how ZPSs can be presented by MHCII molecules to activate CD4+ T cells in a paradigm that represents an alternative to conventional protein-antigen processing and presentation by the MHCII pathway. Although the direct interaction of ZPSs with αβ T cells is an established fact, our knowledge of the molecular nature of ZPS–T cell engagement is very limited. So far, there have been two attempts to generate ZPS-specific T cell clones (56, 62). PSA- and Sp1-reactive T cell hybridomas were generated by cell fusion of in vitro-activated T cells and a mouse thymoma cell line (62). These polyclonal T cells are reactive with a variety of other ZPSs but not with non-ZPSs, a result indicating cross-reactivity between ZPSs. In addition, ZPS-reactive T cell hybridomas were transferred to recipient rats, and the function of the ZPS-reactive T cell hybridomas was evaluated in vivo. Recipient rats were challenged with B. fragilis, and protection against intraabdominal abscess formation was assessed. This work confirmed the modulatory role of ZPS-specific T cells in abscess formation.

More recently, investigators showed that intraperitoneal Sp1 injection into mice results in the accumulation of Th1- and Th17-polarized CD4+CD44highCD62lowCD25neg memory T cells (56). Furthermore, stimulation of CD4+ T cells with Sp1 induces oligoclonal expansion of these cells within the TCR β chain variable region (TCR BV) families. Sp1-mediated CD4+ T cell proliferation (like that following stimulation of CD4+ T cells with classical protein antigens) yields a nonrestricted Vβ repertoire in vivo and in vitro. In contrast, superantigens induce a polyclonal response within a restricted number of specific TCR BV families (56). Oligoclonal expansion of polysaccharide-activated CD4+ T cells suggests ZPS recognition by the TCR antigen-binding domain.

**Chemical Modification of T Cell–Independent Polysaccharides**

After the discovery of MHCII-dependent CD4+ T cell activation by ZPSs, a follow-up study showed that chemical introduction of zwitterionic motifs into anionic bacterial polysaccharides generates chemically modified ZPSs that execute biological functions quite similar to those performed by natural ZPSs (63). The naturally anionic GBS type 1b and type III polysaccharides were chemically modified to possess a zwitterionic charge motif. These chemically derived ZPSs upregulated the expression of MHCII in APCs through the TLR2 receptor in APCs and induced the proliferation of CD4+ T cells. These activities were similar to what has been described for natural ZPSs.
Removal of either charge on these molecules resulted in the termination of T cell–dependent activity. This study expanded the observations made with natural ZPSs such as PSA.

Immunologic Significance of T Cell Activation by PSA

Intraabdominal abscess formation is one of the most common problems encountered after surgery involving the peritoneum. In a rat model simulating human intraabdominal sepsis, B. fragilis, in contrast to other anaerobic bacterial species, has the distinct ability to induce experimental abscesses when implanted into the peritoneal cavity along with sterile cecal contents. The organism alone does not induce abscesses (64, 65). However, prophylactic or therapeutic subcutaneous administration of PSA (by itself, without sterile cecal contents) aborts the formation of intraabdominal abscesses in rats challenged with B. fragilis or with other intestinal bacteria capable of synergistically stimulating abscess formation (66). Studies aimed at understanding the cellular basis of this protection against abscess formation demonstrated that splenic T cells from PSA-treated animals confer protection when transferred to animals challenged with these abscess-inducing bacteria (67–69). These studies demonstrated that PSA possesses novel immunomodulatory properties affecting T cell function. A review by Mazmanian & Kasper (70) explains in detail the T cell–dependent immunomodulatory role of PSA in intraabdominal abscess formation.

In an effort to understand whether the mechanism of protection by ZPSs (PSA and Sp1) against abscess formation would also apply to surgical adhesion formation in vivo, Ruiz-Perez and colleagues (71) studied a rodent model of fibrosis. They found that a distinct population of CD4+ CD45RBlo T cells produces IL-10 in response to ZPSs and is responsible for protection in the fibrosis model. Moreover, in this study, IL-10 was shown to be responsible for ZPS-mediated protection against the formation of intraabdominal abscesses.

ZPSs are key contributors to symbiosis during commensalism. Commensalism is critical to the development of a balanced immune system. PSA—produced by B. fragilis, a prominent component of the gut microflora—is the archetypical molecule that stimulates the immune system to balance its CD4+ T cell lineages (59). Intestinal monocolonization of previously germ-free mice with PSA—expressing B. fragilis directs maturation of the immune system. Monoassociation of germ-free mice with a PSA-bearing B. fragilis strain (wild type) corrects systemic T cell deficiencies, redresses Th1/Th2 imbalances, and directs lymphoid organogenesis (Figure 7) (59). These effects are not found in germ-free mice monoassociated with an isogenic B. fragilis mutant incapable of synthesizing PSA. Identified as a TLR2 agonist, PSA coordinates an innate and adaptive immune response.
that results in the production of interferon-γ, a key factor in the Th1 differentiation observed in colonization studies (14). An important and as yet unresolved issue is whether a single type or multiple types of CD4+ T cells are responsible for inducing both Th1 and IL-10 cytokine production. It remains unclear whether PSA activates the same cell to do different things (depending on other environmental signals) or whether it activates different cells through either the same or different mechanisms.

Very recently, PSA has been used as a carrier molecule to induce an adaptive immune response to Tn-hapten—a tumor-associated carbohydrate antigen—in an effort to develop a novel cancer immunotherapy (73). This study demonstrates the design, synthesis, and
immunologic evaluation of an entirely carbohydrate vaccine candidate.

SUMMARY AND CONCLUSION

Virtually all biological functions are regulated directly or indirectly by proteins. While an understanding of biological phenomena requires the investigation of proteins, the best-studied and most appreciated biopolymers, scientists have increasingly realized that biological events cannot be explained solely by the functions of proteins. Fine tuning of the system requires the involvement of other biomolecules, such as carbohydrates and lipids. This broadened perspective has stimulated investigations of these previously underappreciated bioregulators. The biological significance of carbohydrates—both in pure form and in association with other molecules—is now quite clear. In this article, we have reviewed several newly discovered aspects of carbohydrate interactions with the adaptive immune system.

In the past decade, paradigm-shifting observations have been made by investigators studying the unique bacterial capsules called ZPSs. Contrary to prior concepts, the ZPSs are now known to be processed and presented by APCs and to be recognized by T cells of the adaptive immune system. We have learned that, after entering APCs, carbohydrates are processed to smaller molecular size within the endosome via chemical mechanisms that are initiated with the oxidative burst and depend on reactive oxygen species and reactive nitrogen species. ZPSs such as PSA from *B. fragilis* are able to bind to MHCII within the endosome through electrostatic forces. When binding to MHCII takes place, these carbohydrates can be presented to and activate T cells, eliciting important immune responses. As discussed at length in this review, significant progress has been made in understanding the molecular requirements for ZPS stimulation of the adaptive immune system. However, many points remain to be clarified, including the precise physical-chemical and structural characteristics of the interactions of ZPSs, MHCII molecules, and TCRs. Crystal structures of MHCII-bound ZPS epitopes in contact with the TCR will help elucidate the exact nature of these interactions, but the ability of carbohydrate chemists to synthesize the molecules necessary for these studies is limited at this point. ZPS-specific T cell clones could also reveal the characteristics of ZPS activation of T cells. However, the creation of such clones is particularly challenging given their predilection to induce IL-10, which downregulates IL-2 and therefore turns off the T cell's proliferative capacity.

ZPSs play a unique role in regulating intraabdominal abscess formation. On the one hand, ZPSs are required for the induction of abscesses when injected along with sterile cecal contents (12). On the other hand, when given alone to rodents that are subsequently challenged with live bacteria, regardless of species, they protect against the induction of abscesses (66). This protective effect is also seen in models of surgical adhesion formation (71) and inflammatory bowel disease (72). These polysaccharides can also stimulate the development of a well-balanced immune system. An in-depth understanding of the interactions of this unique class of biological molecules with the immune system is likely to provide new opportunities to combat infections through regulation of immune responses (74). Most pathogens are coated with carbohydrates (e.g., bacterial CPSs), which are major virulence factors and excellent vaccine targets. To enable the adaptive immune system to recognize carbohydrates, scientists developed glycoconjugation. In glycoconjugate vaccines, peptides generated from the protein portion are presented to CD4+ T cells; thus, T cell help is recruited and the production of IgG antibody to the polysaccharide by B cells is induced. Despite substantial progress in the prevention of infectious diseases, the success of current glycoconjugates varies with the population being immunized and with the characteristics of the specific vaccine. Persistent problems include (a) the need for booster doses, (b) poor immunogenicity in the elderly and in patients with underlying B lymphocyte defects,
The relatively short duration of immunity, instances in which nonprotective antibodies—but not most opsonic IgG subclasses—are produced, and the highly complicated heterogeneous structures of conjugates, which can result in much less efficient immunity to the polysaccharide and undesired immunity to the carrier protein (3, 7). Construction of glycoconjugates has been a random process in which two molecules (a carbohydrate and a protein) have been linked without optimization of the design on the basis of solid scientific principles. An understanding of the basic mechanisms governing glycoconjugate processing and presentation may be crucial for the creation of a new generation of glycoconjugate vaccines whose chemical and physical properties are specifically designed to enhance immunogenicity markedly.

FUTURE CONSIDERATIONS

Advances in the biology, chemistry, and immunology of carbohydrates have yielded novel insights into how this important class of biological molecules interfaces with the immune system in ways that were not previously appreciated. In this article, we have reviewed some of the data showing that ZPSs are actually processed and presented by the MHCII pathway and that these events result in the induction of T cell responses that are important in the development of a mature and well-balanced immune system as well as in the prevention of immune-mediated inflammatory diseases. The recognition of carbohydrates by T cells and the biologically important responses that result present an opportunity to study other functions of these biomolecules, such as the way in which the immune system recognizes glycoproteins on infectious organisms and the mechanisms by which the immune system responds to glycoconjugate vaccines. It has become clear in recent years that many bacteria (as well as viruses, fungi, and parasites) are decorated with carbohydrates. There are large gaps in our knowledge of how these molecules regulate the response to microorganisms. A better understanding of these interactions is likely to facilitate the development of drugs and vaccines for the treatment and prevention of infectious and autoimmune diseases.

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