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Review

Chemical Biology Approaches to Designing Defined Carbohydrate Vaccines

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Carbohydrate antigens have shown promise as important targets for developing effective vaccines and pathogen detection strategies. Modifying purified microbial glycans through synthetic routes or completely synthesizing antigenic motifs are attractive options to advance carbohydrate vaccine development. However, limited knowledge on structure-property correlates hampers the discovery of immunoprotective carbohydrate epitopes. Recent advancements in tools for glycan modification, high-throughput screening of biological samples, and 3D structural analysis may facilitate antigen discovery process. This review focuses on advances that accelerate carbohydrate-based vaccine development and various technologies that are driving these efforts. Herein we provide a critical overview of approaches and resources available for rational design of better carbohydrate antigens. Structurally defined and fully synthetic oligosaccharides, designed based on molecular understanding of antigen-antibody interactions, offer a promising alternative for developing future carbohydrate vaccines.

Carbohydrate antigens expressed by pathogens are often structurally unique, and are thus potential targets for developing vaccines and diagnostics. Vaccines based on capsular polysaccharides (CPS) against multiple pathogenic bacteria have been part of routine vaccinations for many years (Ada and Isaacs, 2003; Schumann et al., 2013; U.S. Department of Health and Human Services, 2012). These vaccines are prepared using CPS purified from bacterial cultures (European Medicines Agency, 2009; World Health Organization, 2003; Costantino et al., 2011). However, the presence of impurities that are coisolated, such as cell-wall polysaccharides, have been associated with side effects and hyporesponsiveness (Esposito et al., 2010; Goldblatt et al., 1992; Musher et al., 1990; Poolman and Borrow, 2011; Sen et al., 2005). Since isolated polysaccharides are structurally heterogeneous, multiple purification and quality control steps are required before an antigen can be formulated in a vaccine (European Medicines Agency, 2009; World Health Organization, 2000, 2003, 2005, 2006, 2009, 2012b). Efforts to improve the efficacy and safety of these vaccines are important to achieve comprehensive vaccination and eradication of the respective pathogens. Synthetic oligosaccharides based on the repeating units of CPS can be an attractive option to furnish vaccines free of contaminants that have predictable clinical outcomes (Seeberger and Werz, 2007). Designing vaccines based on synthetic oligosaccharides is not straightforward and proves to be scientifically challenging. The identification of an epitope that will eventually induce protective immunity in vivo is a major bottleneck. Innovative methodologies that can aid the design of epitopes will render this process faster and less cumbersome.

Vaccines Based on Cell-Surface Glycans

Glycotopes or glycan B cell epitopes are segments of antigenic polysaccharides recognized by the binding sites of membrane-bound immunoglobulin (Ig) molecules (B cell receptor, BCR) on B cells. Polysaccharides are T cell-independent antigens that can induce a short-term, IgM-dependent immune response, but fail to efficiently elicit immunological memory (Mazmanian and Kasper, 2006; Mond et al., 1995; Schumann et al., 2013; Stein, 1992). Conjugation to a carrier protein converts glycans to T-cell-dependent antigens that can induce the formation of a long-lasting memory response. A T-cell-dependent immune response is accompanied by the differentiation of polysaccharide-specific B cells to plasma cells. Reinfection in the case of a pathogen or boosting in the case of a vaccine then results in proliferation of plasma cells and affinity maturation of secreted antibodies (Abbas et al., 2012).

Glycoconjugate vaccines have been highly successful in preventing infectious diseases caused by *Neisseria meningitidis*, *Haemophilus influenza*, and *Streptococcus pneumoniae* (Garegg and Maron, 1979; Johnson et al., 2010). Despite the effectiveness of CPS-based vaccines, certain disadvantages are encountered in vaccine manufacture. One major bottleneck is the process of isolation and purification of pure capsular polysaccharides from pathogenic bacteria. Not all bacterial strains can be cultured on sufficient scale (Rappuoli et al., 2011). The production of polysaccharides requires efforts to optimize growth conditions (Cimini et al., 2010; Gonçalves et al., 2002; Jang et al., 2008; Jin et al., 2009). Furthermore, certain CPSs are unstable and degrade during isolation or formulation processes (Pujar et al., 2004; Sturgess et al., 1999). After isolation, the purification of polysaccharides must be monitored on multiple levels, as specified by guidelines set up by the regulatory authorities (World Health Organization, 2000, 2003, 2005, 2006, 2009, 2012b). For instance, the existence of contaminants, such as other cellular polysaccharides, proteins, and nucleic acid, must be excluded. The structural integrity of the polysaccharide is assessed by wet chemical and instrumental analyses, such as nuclear magnetic resonance (NMR) spectroscopy

(Jones, 2008; Jones and Currie, 1991; Jones et al., 1991; Talaga et al., 2002). After limited depolymerization, chemical activation, and conjugation, the absence of reactive groups on the glycan fragments must be demonstrated. These and other steps of quality control must be performed frequently during glycoconjugate manufacture, raising the cost per dose of the final vaccine. Furthermore, it can be expected that vaccine formulations containing chemically derivatized polysaccharides display artificial, nonprotective epitopes that render the vaccine inefficient (Wessels et al., 1998).

An important step toward the design of an effective vaccine is the elucidation of the right antigenic epitope(s) that confer(s) the production of antibodies that can protect the host from a pathogen (so-called protective epitopes). Even if information on a putative protective epitope is available, it is nearly impossible to purify the respective polysaccharide fragment to homogeneity in order to maximize vaccine efficiency.

Synthetic Oligosaccharide-Based Vaccines

Synthetic oligosaccharides representing the repeating units of CPS can be an attractive alternative to isolated polysaccharides to produce structurally defined vaccines free of impurities (Schumann et al., 2013). Since synthetic oligosaccharides can be easily characterized, the manufacture of vaccines based on these antigens is highly reproducible. In addition, access to molecularly defined antigenic components may result in more efficient final formulations while potentially lowering the costs for vaccine development.

Recent years have seen extensive research in the field of oligosaccharide synthesis that have led to the design of highly potent antigenic targets (Berkin et al., 2002; Chong et al., 1997; Costantino et al., 2011; Kudryashov et al., 2001; Martin et al., 2013b; Morelli et al., 2011; Parameswar et al., 2009; Safari et al., 2008; Schofield et al., 2002; Verez-Bencomo et al., 2004; Zhu et al., 2009). The potency of synthetic oligosaccharide-based vaccines is exemplified by the promising protective effects of a *Haemophilus influenzae* type b (Hib) vaccine based on synthetic oligosaccharides that is marketed in Cuba (Verez-Bencomo et al., 2004).

Despite the progress concerning synthetic oligosaccharide-based vaccines, designing the respective antigens remains challenging. As isolated polysaccharides are structurally complex, the corresponding glycoconjugates most likely contain the "right" B cell epitopes needed to confer protective immunity. Synthetic oligosaccharides, in contrast, are much smaller in size; hence, the presence of protective epitopes in such an antigen is not ensured. Identification of the right epitope is a time-consuming step. Antigen design has traditionally been an iterative process: synthetic targets are chosen based on the chemical structure of repeating units and, after conjugation to a carrier protein, evaluated in immunization experiments in animals (Robbins et al., 2009; Safari et al., 2012). If the resulting antibody response does not target the pathogen, different antigenic constructs will have to be synthesized. Since the procurement of synthetic oligosaccharides in ample quantities for mouse immunization experiments (typically 10–20 mg of the deprotected glycan) is resource intensive, this trial-and-error process in vaccine development is inefficient. Once the structure of a promising antigen is known, however, chemical synthesis can be optimized to provide large amounts of material (Kabanova et al., 2010; Pozsgay et al., 2012). Recently, a chemoenzymatic method was devised to generate gram amounts of tumor-associated hexasaccharide antigens Globo H and SSEA-4, both of which are interesting targets in the development of antitumor vaccines (Tsai et al., 2013). Globo H is a good example of developing carbohydrate vaccines based on basic biochemical and clinical findings and further optimizing the structure to a fully synthetic epitope (Bremer et al., 1984; Gilewski et al., 2001). Glycoconjugates based on such a fully synthetic glycotope have been advanced to early stages of clinical trials (Gilewski et al., 2001).

Thus, understanding the structure of a protective B cell epitope is vital before immunization experiments are performed.

The currently available tools to map carbohydrate epitopes (see below) can make use of small amounts of glycans for screening purposes. Thus, the efforts of organic chemists can be shifted from providing ample amounts of few structures to generating whole libraries of structurally diverse glycans in smaller quantities. Multiple approaches have been undertaken to facilitate this process of generating diversity, such as the programmable one-pot glycosylation technique, which employs the sequential activation of different glycosylating agents based on reactivity differences to furnish oligosaccharides in one reaction vessel without intermediate purification (Lee et al., 2006; Wu and Wong, 2011). Enzymatic oligosaccharide syntheses have been developed to generate a variety of complex glycan structures (Nycholat et al., 2013; Tsai et al., 2013; Wang et al., 2013b), although this approach is still somewhat limited to the more common monosaccharides found in glycans from eukaryotes.

Automated solid-phase oligosaccharide synthesis has been used to generate a plethora of synthetic oligosaccharides of different complexities. Since the process of oligosaccharide assembly is fully automated, target oligosaccharide structures can be tailored toward the needs of the experimentalist using a limited set of monosaccharide building blocks (Calin et al., 2013; Plante et al., 2001; Seeberger and Werz, 2005, 2007). It is thus conceivable that a large variety of potential antigens can be generated much faster than by using traditional solution-phase oligosaccharide assembly.

Structural Attributes of Carbohydrate B Cell Epitopes

B cell epitopes can be classified into two groups: sequential (continuous) epitopes and conformational (discontinuous) epitopes. A sequential B cell epitope is recognized as the primary structure of an antigen, while a conformational B cell epitope consists of residues that may be distantly separated in the primary structure and are recognized due to the close proximity within the folded 3D structure (Arnon and Van Regenmortel, 1992). The nature of an epitope is of utmost importance during antigen design: conformational epitopes are associated with a distinct secondary structure of the glycan, a feat that may only be achieved in longer sequences comprising multiple repeating units. Thus, the synthetic effort compromises the applicability of these long glycans as synthetic antigens. For instance, CPS of group B streptococci (GBS) type III adopts a helical structure above a length of five repeating units (González-Outeiriño et al., 2005; Jennings, 2012; Woods and Yongye, 2012). Polysaccharide fragments that are smaller than five repeating units fail to elicit an efficient immune response (Jennings, 2012; MacKenzie