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Minireview

Carbohydrate based vaccines

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Abstract In the past decades, a gradual increase in the resistance to antibiotics has been observed, leading to a serious thread for successful treatment of bacterial infections. This feature in addition to difficulties in developing adequate drugs against (tropical) diseases caused by parasites has stimulated the interest in vaccines to prevent infections. In principle, various types of cell surface epitopes, characteristic for the invading organism or related to aberrant growth of cells, can be applied to develop vaccines. The progress in establishing the structure of carbohydrate immuno-determinants in conjunction with improvements in carbohydrate synthesis has rendered it feasible to develop new generations of carbohydrate-based vaccines.

Keywords: Glycoconjugate vaccines; Cell surface antigens; Infections; Cancer; Oligosaccharide synthesis

1. Introduction

The development of vaccines based on carbohydrates has a long history. As early as 1923, Heidelberger and Avery [1] described a 'soluble specific substance', of pneumococci to consist most likely of polysaccharides (PSs) and being typical for the serotype. Francis and Tillett [2] noted that intradermal injections of type-specific polysaccharides induced the development of circulating antibodies for heterologous types of Pneumococci. Later, Heidelberger et al. [3] established that pneumococcal capsular polysaccharides could be used as vaccines, providing a long lasting immunity. Despite the potential to apply such compounds as vaccines, the development of chemotherapeutics and antibiotics has led to a loss of interest in this application. Renewed interest for preventive vaccination was induced by the steady increase in resistance towards antibiotics. In 1983, Pneumovax™ was introduced, being a capsular polysaccharide vaccine derived from 14 *pneumonia* serotypes. Subsequently, Pneumovax™ 23 was presented containing isolated polysaccharides from 23 serotypes out of the about 90 known. This vaccine gives in healthy adults (short term) protection for about 90% of the infections by these microorganisms. However, polysaccharides are poorly immunogenic in persons of high-risk groups: (i) neonates and children until the age of two; (ii) elderly and chronically ill people; (iii) splenectomised patients; (iv) immuno-compromised people e.g. HIV infected. The age-related response to plain polysaccharides may also be structure dependent: in contrast to other capsular polysaccharides, those of group A *Neisseria. meningitidis* and pneumococci type 3 and 18C are good immunogens in infants from 3 to 6 months. They induce protective IgG antibodies. In fact vaccines were prepared from their capsular polysaccharides, e.g. against meningococcal infections vaccines containing capsular polysaccharides from the meningococcal types: A + C, A + C + W135 or A + C + Y + W135. Polysaccharides are considered to give an immune response independent of T cells; they stimulate B-cells to produce antibodies without the involvement of T-cells. However, some zwitterionic capsular polysaccharides can activate $CD4^+$ T cells. These polysaccharides are processed to low molecular weight carbohydrates by a nitric oxide-mediated mechanism and presented to T cells through the MHCII endocytic pathway [4]. Furthermore, tolerance could become a problem.

In contrast to polysaccharides, glycoproteins are T-cell dependent (TD) antigens, having a larger immune response for the same antigens. Already in 1931, Avery and Goedel [5] reported that covalent attachment of carbohydrates to a suitable protein induced an enhanced immunogenicity compared to the polysaccharides as such. In general, immunization with neoglycoproteins consisting of capsule-derived carbohydrates coupled to an immunogenic protein provides a long lasting protection to encapsulated bacteria for adults as well as for persons at high risk and young children (for reviews see: [6–8]). In addition to isolated polysaccharides, oligosaccharides derived from the capsular polysaccharides by degradation or obtained by total synthesis are effective compounds for the preparation of glycoconjugate vaccines. Such oligosaccharides have the advantage that they can be purified to single molecular species and thereby being good starting compounds for the preparation of well-defined vaccines. In general polysaccharides are intrinsically polydisperse in molecular mass. Conjugation of these macromolecules will yield heterogeneous mixtures of compounds.

In this mini review, the emphasis will be on some of the main aspects of glycoconjugate vaccines, rather than comprising all developments in this fast moving area.

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Abbreviations: CRM_{197}, cross-reactive material of diphtheria toxoid; KLH, key hole limpet emocyanin; PS, polysaccharide; GPIs, glycosylphosphatidylinositol anchors; CEA, carcino-embryonic antigen; STn, Sialyl Tn; LPG, lipophosphoglycan

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2. Preparation of neoglycoproteins

2.1. Carrier protein

Any protein, to be used in conjugation procedures for the preparation of vaccines or medical treatment, has as a general prerequisite that it should be allowed and safe for human administration. Proteins that have been applied to couple with carbohydrates are e.g. tetanus and diphtheria toxoids, cross-reactive material of diphtheria toxoid (CRM_{197}) [9], key hole limpet hemocyanin (KLH) and the outer membrane complex of *Neisseria meningitides* [10]. A few other bacteria-derived proteins were so far studied in the laboratory, only.

2.2. Coupling

For coupling of polysaccharides to a protein, chemical activation of the polysaccharide and sometimes of both compounds is necessary. The procedure to activate the carbohydrate depends on the structure e.g.: reactive aldehyde groups can be created from *vicinal* hydroxyl groups by periodate oxidation; carboxyl groups can be activated with carbodiimide followed by appropriate conversion; *N*-acetylamino functions can be (partially) de-*N*-acetylated followed by activation with nitrous acid. Most of these reactions give rise to a random creation of reactive centers in the polysaccharide. Main reactive groups in proteins are the terminal amino group and the ε-amino functions of lysine. Random activation of the reaction partners will allow the formation of conjugates, however the structure of the reaction product may be rather undefined.

To create neoglycoconjugates having well defined structures, the introduction of the linkage between carbohydrate and protein should be as specific as possible. To avoid steric hindrance between protein and glycan and to expose the immunogenic epitopes, bifunctional spacer molecules can be introduced in one or both of the reaction partners. Spacer molecules may contain as reactive functions amino, carboxyl or thiol groups [11].

In general, polysaccharides rarely present a singe molecular species, but rather a family of closely related compounds, differing in degree of polymerization. Since the immunogenic epitope comprises only part of the molecule, carbohydrate chains as short as oligosaccharides can be used for the preparation of effective vaccines. Oligosaccharides can be obtained in pure state through organic synthesis or by degradation of polysaccharides. Coupling of oligosaccharides to proteins can be performed in a rather specific route, affording well-defined conjugates in a reproducible way [6–8].

3. Glycoconjugate vaccines against bacterial infections

For several types of bacterial infections glycoconjugate vaccines can be based on fragments of capsular polysaccharides. In particular, the following bacteria should be mentioned:
Streptococcus pneumoniae
Neisseria meningitidis
Haemophilus influenzae
Salmonella typhi
Shigella dysenteriae
Group B Streptococcus
Klebsiella pneumoniae

The availability of methods to prepare specific oligosaccharide structures opened the possibility to explore the relation between the oligosaccharide chain length and the potency of the glycoconjugate as vaccine. We investigated the potency of synthetic polysaccharide type-3 related di- tri- and tetrasaccharide-CRM_{197} conjugates to provide protection against *S. pneumoniae* type 3 infection. To this end the synthetic oligosaccharides were coupled via the squarate method [12] to cross-reactive material (CRM_{197}) of modified diphtheria toxin in various oligosaccharide/protein ratios. The products were analyzed for protein and carbohydrate content. The protective immunity in mice was investigated in mice after two subcutaneous challenges with a three-week interval of 2.5 μg oligosaccharide per mouse. All mice immunized with the tri- or tetrasaccharide conjugates had IgG antibodies that bound the capsular polysaccharide. After intraperitoneal injection of a dose *S. pneumonia* lethal for control mice, all immunized mice having formed polysaccharide type 3 specific antibodies survived. Here, a correlation was found between polysaccharide specific antibodies and protective capacity of the conjugates [13] (see Table 1).

The results fit well to previous studies [14,15], wherein it was shown that a hexasaccharide derived from polysaccharide type 3 coupled without spacer as a pentasaccharide to KLH was capable of inducing protective immunity against type 3 pneumococci in mice.

In a study on the immunogenicity and protective capacity of *S. pneumoniae* 6B capsular polysaccharide derived di-(Rhaα1-4-Rib-ol-5*P*-), tri- (Rib-ol-5*P*-2Galα1-3Glc-) and tetrasaccharide (Rhaα1-4-Rib-ol-5*P*-2Galα1-3Glc-) conjugated to KLH, we have found that the di- and tetrasaccharide (one repeating unit) conjugates contain already epitopes capable of inducing 6B-specific, fully protective antibodies in rabbits and mice, respectively [16].

In an investigation of a vaccine against *S. dysenteriae* type 1, a series of specific, synthetic oligosaccharides was prepared on the basis of the tetrasaccharide repeating unit of the O-specific polysaccharide. The oligosaccharides were coupled to human serum albumin and the conjugates were applied as vaccines [17]. A clear influence was observed of chain lengths. The octa-, dodeca-, and hexadeca-saccharides were immunogenic, without adjuvant. Interestingly, the oligosaccharide conjugates induced higher anti O-specific IgG levels than the conjugate of the native O-specific polysaccharide. However, the tetrasaccharide representing a single repeating unit was not effective. Also oligosaccharide loading of the protein is a relevant factor, it should not be too high, nor too low.

The development of a synthetic conjugate vaccine against *H. influenzae* type B provides an interesting example of the potency of present day methodology. Peeters et al. [18] had shown that conjugates of tri- and tetrameric 3-β-D-ribose-(1-1)-ribitol-5-phosphate and tetanus or diphtheria toxoid afforded anti capsular-polysaccharide responses with an increasing IgG/IgM ratio in adult mice and monkeys. Chong et al. [19] have chosen a different approach. They coupled oligosaccharides consisting of repeating units of the *H. influenzae* type B polysaccharide to synthetic peptides containing potent T-helper cell determinants and B-cell epitopes. The conjugate provided T-help and the carbohydrate hapten became T-cell-dependent. In an infant rat model, the raised antibodies were protective against *H. influenzae* type B infection. Optimal results were obtained for the trimeric repeating unit. An break-