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Recent advances in carbohydrate-based vaccines

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Vaccinations provide an efficient and cost-effective way to combat devastating human diseases. Besides pathogenic protein markers, cell surface carbohydrates from biological sources are widely used as vaccines. Recently, synthetic immunogenic carbohydrate–protein conjugates have been advanced to vaccine candidates. Progress in the chemical synthesis of oligosaccharides and conjugation methods stimulated the development of novel carbohydrate-based vaccine candidates.

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Introduction

Carbohydrates play a crucial role in many biological processes [1–3]. Carbohydrate analysis, synthesis, and medical applications have developed significantly over the past decades [4,5,6•,7]. Today, carbohydrates are used as antibiotics, anticoagulants, and to treat diabetes. Purified carbohydrates from bacteria, when conjugated to carrier proteins, have been shown to be efficient vaccines against *Neisseria meningitides* [8] and *Streptococcus pneumoniae* [9]. Still, purified carbohydrates display several shortcomings as vaccines. The isolation of carbohydrates from biological material is a tedious process often yielding scarce amounts of oligosaccharide mixtures, and it is limited to organisms that can be cultured. Advances in the chemical synthesis of neoglycoconjugates [10,11] ushered in a new era of well-defined carbohydrate-based vaccines building upon promising results for *Haemophilus influenzae* type b and malaria [12,13].

In the past, carbohydrate-based vaccine development suffered from the low inherent immunogenicity of this family of molecules. Various methods have now been developed to overcome this serious problem. The use of adjuvants, covalent attachment to immunostimulants or integration into complex multicomponent vaccines has helped deciphering strategies for the efficient use of carbohydrates in vaccination. For bacterial and viral diseases, oligosaccharide-based vaccines present advantages compared to conventional protein-based vaccines. First, pathogenic cell surface molecules typically carry distinct characteristic carbohydrate portions which are crucial for the interaction with host structures. Furthermore, genetic variation in pathogens because of selective pressure does not directly result in a change of the cell surface oligosaccharides since the expression of carbohydrates is not under direct genetic control. In the following, we will overview the recent development of carbohydrate-based vaccines starting with synthesis strategies, proceeding to ways for efficient stimulation of the immune system and finally discussing specific examples.

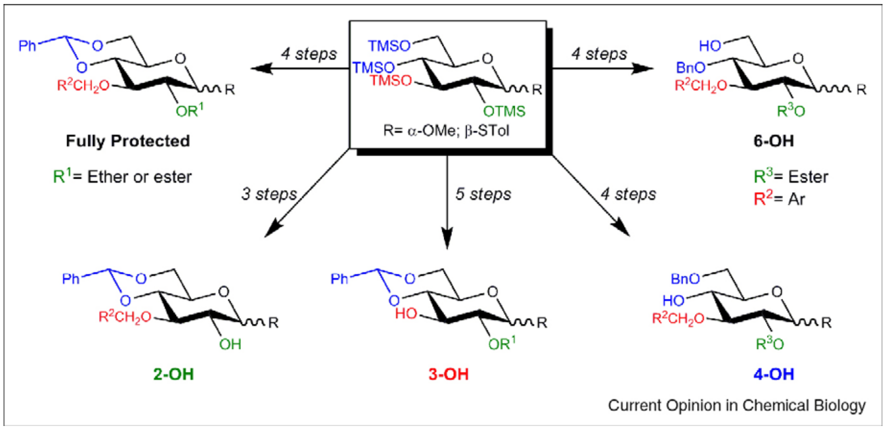
Recent advances in carbohydrate synthesis

Obtaining pure and well-defined carbohydrate-based pharmaceuticals asks for efficient chemical syntheses. Besides implementation of automated methods in the synthesis of oligosaccharides [14,15], methodological progress in carbohydrate chemistry has focused on novel protecting groups and glycosylation methods leading to increased yields and selectivities [14,16]. One-pot protection of monosaccharides (Figure 1) [17••] and *de novo* strategies [18–20] allow to access bulk quantities of building blocks. 2,3,4,6-Tetra-*O*-trimethylsilylated glycosides can be transformed into various synthetically useful building blocks in one pot [17••]. *De novo* strategies in contrast, use linear preprotected fragments that are connected through stereoselective C–C bond forming reactions to provide the desired, differentially protected, glycosylating agents.

Enhancing the immunogenicity of carbohydrates

Since many pathogenic oligosaccharide structures resemble host glycans, they are often not able to elicit an effective immune response by themselves [22]. Therefore, carbohydrates are typically conjugated, that is they are covalently attached *via* crosslinkers, to immunogenic protein carriers such as tetanus toxoid (TT). Figure 2 shows different ways how carbohydrates can be coupled to peptides. Some promising examples include vaccine candidates against *Haemophilus influenzae* type b [12], malaria [13], cancer [23••,24], anthrax [25], shigellosis

Figure 1



One-pot synthesis of different building blocks starting from persilylated glucose. Figure adapted from [21].

[26], and HIV [27–30]. These vaccine candidates feature variable numbers of oligosaccharide copies resulting in heterogeneous mixtures of immunogens. To obtain well-defined neoglycoproteins, novel, efficient methods for the site-specific attachment of carbohydrates to proteins have been developed [31•,32].

Adjuvants, particularly alum, are compounds that do not confer immunity against the antigen but significantly enhance the immune response of a patient against a coadministered antigen. One highly potent experimental adjuvant is the natural product saponin QS-21 isolated from the *Quillaja saponaria* (QS) tree. Two adjuvant-active components of the heterogeneous QS-21 have been identified: QS-21-Api and QS-21-Xyl, which differ in one carbohydrate unit (xylose and arabinose, respectively). Recently, both isomers QS-21-Api [33••] and QS-21-Xyl [34••] were synthesized by Gin and coworkers. Immunological tests showed that the efficiency of synthetic QS-21 was comparable to that of naturally isolated QS-21 [33••,34••]. The use of synthetic QS-21 (sQS-21) for a phase-I clinical trial with a bivalent melanoma vaccine has been approved [34••].

HIV vaccines

At present, more than 30 million people are infected with human immunodeficiency virus (HIV) and effective vaccines are urgently needed. The main target for vaccine development and prophylactic viral agents is the heavily glycosylated viral surface, in particular, glycoprotein gp120. The immunogenic protein part of gp120 is masked by a glycan shield suggesting a vaccine that consists of gp120 oligosaccharide structures. In the early nineties, a set of neutralizing antibodies against a broad range of HIV-1 isolates were purified from HIV-1 infected individuals. Vaccination with this panel of antibodies was sufficient to protect animals from viral challenge. The antibody set included 2G12, an IgG_1 immunoglobulin that recognizes multiple high-mannose structures on gp120 through a novel domain-exchanged assembly of two 2G12 molecules [36]. Numerous attempts toward synthetic mimetics of the epitope recognized by 2G12 have been made [27–30,37]. Recently, a dendrimer decorated with synthetic oligomannoses was shown to inhibit 2G12 binding to gp120 with an IC_{50} in the nanomolar range, thus highlighting the importance of a multivalent display of the carbohydrate antigens [38•]. A fully synthetic multivalent glycopeptide that strongly interacted with 2G12 was also developed [39]. Nonetheless, this glycoconjugate failed to neutralize HIV-1 virions in guinea pigs and rhesus macaques [40]. To date, no synthetic glycoconjugate has succeeded in inducing antibodies that effectively crossreact with the carbohydrate portion of gp120.

Anticancer vaccines

Tumor cells often display a surface glycosylation pattern that differs from normal cells. Overexpression of tumor-associated carbohydrate antigens (TACA) is commonly observed. Lipid-anchored Globo-H, GM2, GD3, and glycolipids containing an *N*-acetyl galactosamine (TN antigen) part have been identified, among others, as TACA. Often, they are involved in adhesion and invasion processes of malignant cells and thereby in the formation of metastases. Immunization against tumor antigens is difficult since they are self-antigens that are tolerated by the immune system. Furthermore, the immune system of cancer patients is often in an attenuated state. Boons and coworkers [23••] have recently synthesized a construct that induces the production of IgG antibodies against a glycopeptide from human mucin (MUC1). Their construct consists of three parts that are covalently linked to each other (Figure 3): a TACA B cell epitope, a T helper epitope, and a Toll-like receptor (TLR) ligand. The