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Carbohydrate Vaccines- A burgeoning field of Glycomics

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ABSTRACT

Glycomics is the study that deals with the structures and functions of carbohydrates. The discovery Of novel and increasing number of numerous biological roles of carbohydrates , glycomics has explored the field of carbohydrate vaccines. Glycoconjugate vaccines in which the cell surface carbohydrate from a microorganism is covalently attached to a carrier protein are proving to be highly effective in generating protective immune responses to prevent a wide range of diseases. The carbohydrate based agents – glycoproteins and polysaccharides can be difficult to isolate from natural sources and the natural isolates can have heterogeneity and contamination. So, the alternative would be to identify antigenic carbohydrates and then synthesize them in the laboratory. Novel chemical and enzymatic oligosaccharide techniques are making it possible to envision a new generation of carbohydrate based vaccines. Carbohydrate vaccines have leading roles in cancer, *haemophilus influenza* B, malaria, candidiasis, AIDS etc. The present article focuses on the potential of carbohydrate vaccines, thus paving the way for development in the field of glycomics.

Key words: vaccines, glycobiology, micro-organisms,proteomics.

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INTRODUCTION

Vaccination is the most cost efficient and powerful medical intervention in control, prevention and eradication of many diseases that affect human population .Vaccines are agentsthat boost the immune system by stimulating antibodies or immune cells to combat against infection and diseases. Vaccines commonly made from weakened or attenuated pathogens or from immunogenic proteins, glycoproteins, or polysaccharides obtained from microorganisms. Carbohydrates are regarded as darlings of the biotechnology industry. Evolutionarily studies indicate that carbohydrates are more stable than proteins and have multiple roles in physiology and pathophysiology. Carbohydrates are involved in inflammation, cell interactions, pathogen host adhesion, signal transduction development and a myriad of other processes. In the last decade, carbohydrates have been used as targets for effective vaccines against bacteria, and have also been developed as adjuvants as well as vaccine carriers for protein antigens for immunotherapy. Better understanding of the broad range of biological activities, carbohydrates are now gaining recognition as a class of biopolymers .Carbohydrate microarray development has vastly accelerated the potential for biochemical characterization of carbohydrates. Thus, following DNA and proteins, carbohydrates are gaining recognition as a third class of biopolymers that play a vital role in a broad range of biological activities. Complex carbohydrates are the next frontier in understanding the secret molecular messages that rule the life of our cells. Carbohydrates determine blood type, regulate plant growth, and have roles in cancer, diabetes and human development.Vaccines derived from totally synthetic carbohydrate antigens have been shown to elicit an immune response in both preclinical and clinical settings. Carbohydrate based vaccines including protein conjugates with bacterial polysaccharides have been a major focus of commercial ventures. Carbohydrates based drugs involve interaction with specific antibodies.

Land marks that led to the development of carbohydrate vaccines

1. By 1970 it was recognized that antibiotics would not be the ultimate solution.
2. Numerous failures due to the rise of resistant forms of the disease organisms.
3. Advances in the understanding of the immune system .
4. Structural determination of numerous carbohydrates leading to the development of defined glycoconjugates

Glycoconjugates

Glycoconjugates are found in cell surfaces, in extracellular matrices surrounding cells and in connective tissues .Glycoconjugatesare the basis for the development of many new vaccines to protect us against diseases. The study of structure of glycoconjugates is called as Glycomics. Glycoconjugates of different molecular configurations are a part of many vital biological processes including molecular recognition, surface adhesion and cellular signaling. Functional molecular display on surfaces is a prerequisite for conclusive determination of glycoconjugate function and for the integration of bioactive carbohydrate structures. Thin film coats of glycan- based linker polymers allow for single- step covalent attachment of glycoconjugates, glycans and proteins. Glycoconjugate vaccines provide effective prophylaxis against bacterial infections. Bacterial polysaccharides are T-cell independent antigens.The multivalency and large size of these antigens causes the B-cell receptors to cluster and induce immunoglobulin synthesis .Thus, booster injections bring falling antibody levels back to original post-immunization levels, but fail to produce a massive response In contrast, proteins are univalent and require T-cell participation to induce antibody synthesis . This is accomplished by covalently binding carbohydrate antigens to proteins, resulting in preparation of glycoconjugate vaccines .Glycans , either alone or asglyconjugates, have great potential for use as drugs .Complex glycans are intrinsically more stable than protein based drugs . They are more easily formulated for drug delivery .Sugars are highly specific and potentially less immunogenic than proteins or RNA based strategies.

Advantages of Glycoconjugate vaccines

The descriptions of glycan structure-function relationships that are yielded by the burgeoning field of glycomics differ from those produced by proteomics and genomics. Glycoconjugatevaccines, in which a cell surface carbohydrate from a microorganism is covalently attached to an appropriate carrier protein are proving to be the most effective means to generate protective immune responses to prevent a wide range of diseases .Conjugate vaccines are so – called because their conjugation of the polysaccharide antigen converts the T-cell independent carbohydrate antigen into T-cell dependent antigen with benefits in immunological response. Conjugate vaccines with oligosaccharides coupled to carrier proteins are proving to be highly effective for e.g.Haemophilus influenza type B carbohydrate vaccine.

WHY CARBOHYDRATE VACCINES?

1. Polysaccharides (PS), on killer cells or purified , produce protective immune responses.
2. Infants and young children do not produce sufficient antibodies from actual disease, hence the need for synthetic carbohydrate vaccines.
3. Specific PS molecules characteristic of the particular strain (or type) produce the protective response.
4. Vaccination with PS reduces the need to vaccinate with the organism itself.
5. When the PS is coupled to a protein , it produces a much higher titer (in rabbits).

CLASSIFICATION OF CARBOHYDRATE VACCINES

There are two major types of carbohydrate vaccines :

1. Natural carbohydrate vaccines
2. Synthetic carbohydrate vaccines

Natural carbohydrate vaccines are a heterogenous mixture and include small amounts of impurities and contaminants. In contrast, synthetic carbohydrate based vaccines have low cost of production, consistent composition and homogeneity and purity ; with little or no batch- to- batch variation . Classification of synthetic carbohydrate vaccinesis given in the **Table 1.**

Table 1 : Classification of carbohydrate synthetic conjugated vaccines.

Source of Carbohydrate and targeted bacterium	*Protein Carrier*	***Conjugation***
*Haemophilus infuenza*type bCPS	DTd, TTd	**Isourea bond**
Haemophilus infuenza type b CPS	OMP	**Thioether bond**
Haemophilus influenza type b CPS	OMP, TTd	**Disulfide**
Streptococcus pneumoniae 14 ,19F CPS	BSA , TTd	**Amine**
Streptococcus pneumoniae 4 CPS	TTd	**Amide bond**
Neisseria meningitidis A CPS	TTd	**Isourea bond**
Neisseria meningitidis A , C, W 135 , Y , Z CPS	BSA , TTd	**Amide bond**
Neisseria meningitidis A , B	BSA , TTd	**Amine**

, N –Pr , B , C CPS		
Neisseria meningitidis B , C , CPS	BSA , CRM ,197 , TTd	**Amide bond**

BSA : Bovine serum albumin ; CRM 197 : nontoxic mutant diptheria toxin; DTd : diptheria toxoid ; OMP : outer membrane protein ; TTd : tetanus toxoid.

There are two approaches to synthesize carbohydrate vaccines:

1. **Semisynthetic approach**: Carrier protein is combined with naturally derived carbohydrate antigen. The first semisynthetic vaccine was discovered against *Haemophilus influenzae* type b that is efficacious in human beings.

2. **Synthetic approach**: Carrier protein is linked with synthesized carbohydrate antigen. Carbohydrate antigens are expressed by human cancer cells .Carbohydrate cancer vaccines are the best vaccines obtained from synthetic approachbecause of the presence and abundance of epitopes on the surface of tumor cells.

METHODS OF SYNTHESIS

1. Programmable one pot solution phase synthesis: Pre-synthesized carbohydrate building blocks are combined sequentially in a single reaction vessel with assistance of computer program.

2. Automated solid phase synthesis: The first step involves the attachment of the starting material (carbohydrate antigen) to an insoluble support such as a polymer or resin by a linker . The reaction is continued to completion by addition of oligosaccharides .The linker is linked with appropriate reagent and target polysaccharide is set free. The development of an automated oligosaccharide synthesizer provides rapid access to biologically relevant compounds.

BIOLOGICAL APPLICATIONS

1. *Haemophilus influenza* type b vaccine: In November 2003, the first synthetic human vaccine was discovered in CubaHaemophilus*influenzae* type b causes diseases such as meningitis , epiglottitis , septicemia , facial cellulitis , pneumonia , arthritis etc. *H. influenzae* strains are mostly non-encapsulated and a number of them do not have a carbohydrate polysaccharide structure .This lack of protection suggested the need for development of carbohydrate polysaccharide conjugate vaccines.In this process for the carbohydrate component – a polyribosylribitolphosphate – a one pot solution – phase oligomerization is used and then coupled with carrier protein. This vaccine protects infants and young children from pneumonia and meningitis .*Streptococcuspneumoniae* causes a number of serious illnesses in young children around the world. This bacterium is the leading cause of pneumonia , meningitis , sinusitis and acute otitis media in children less than 5 years of age. *S. pneumoniae* produces a capsule to protect itself from our immune system. It makes a carbohydrate polymer and the bacteria place this carbohydrate polymer on the surface of their cells. This capsule inhibits white blood cell phagocytosis. Carbohydrate based vaccines against *Haemophilus influenzae* type b, *Neisseria meningitides*, *Streptococcuspneumoniae* and *Salmonella typhi* are already licensed .

2. Malaria vaccine: Malaria has been affecting approximately 300 million to 500 million people across different countries .The malarial toxin produced by *Plasmodium falciparum* contains a carbohydrate moiety that could presumably be mimicked to create a vaccine. The vaccine is based on a class of glycolipids called glycosyl-phosphatidylinositols (GPIs). They are a key to malarial activity and vaccines based on these molecules could trigger anti-GPI antibodies to neutralize the toxin. Mice immunized with chemically synthesized *P. falciparum* GPI showed high degree of protection as compared with control group. This vaccine will be soon tested in monkeys.

3. Candidiasis vaccine: Candidiasis is one of the most common hospital acquired infections. *Candida ablicans*causes fungalinfections that affect skin , mucous membrane and blood stream . A β-1,2mannan component is the main glycoprotein in the Candida cell- wall which is synthesized and teethered to protein to make it immunogenic. β-1,2 mannan is an important cell- wall antigen capable of inducing antibodies that could protect mice against Candida infection.

4. AIDS vaccine: AIDS is a major disease affecting human population across different nations. The human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome (AIDS). HIV type 1 (HIV-1) virus is responsible for the current global pandemic of HIV and AIDS .Carbohydrates , which are having strong defense against host immune attack can serve as targets for vaccines. As such no vaccine is still available for AIDS .However Institute of Glycomics, Griffith Universty, Queensland , Australia is making immense efforts to launch the vaccine soon . An antigen for AIDS ,gp120 , which is a glycoprotein of viral envelope of HIV has been discovered. The viral surface presents a glycoprotein,gp120 which plays a prominent role in penetration of the virus into cells of immune system . gp120 –associated molecules interact with CD4 proteins and chemokine receptors on T-lymphocytes, macrophages and dendritic cells to initiate internalization of HIV by the host cell. Four gp120 binding proteins which possess anti-HIV effects have been isolated. They are :

1. 2G12 - a human monoclonal antibody .

2. DC-SIGN - a dendritic cell lectin and

3.Two proteins viz. cyanovirin and scytovirin from cyanobacteria Human antibody called 2G12 binds the carbohydrate part of gp120 and people who make that antibody can survive HIV infection for a long time . The carbohydrate moiety of gp120 is synthesized and included into 2G12 – type antibodies and conjugated to carrier.

This vaccine is presently undergoing trials in guineapigs and monkeys.

5.Cancer vaccine : The field of cancer vaccines is as vibrant as ever. Over the last few years, anti-cancer immunotherapy has emerged as a new and exciting area for controlling tumors. Immunisation is carried out with tumor- associated antigens aiming at stimulating specific immune response against cancer cells. Carbohydrate antigens are potential targets for such immune interventions since they are exposed at the surface of tumor cells where they are hidden on normal cells. Several carbohydrate based vaccines are under development to treat cancer.

These vaccines are based on carbohydrate haptens conjugated to a protein carrier. Carbohydrate based antitumor vaccine is a long standing ambition in the prevention and treatment of cancer .Vaccines based on exposed core protein, which contains major histocompatibility complex unrestricted epitopes, and carbohydrate structures are targets for the immunotherapy of cancers of epithelial origin. A vaccine formulated using synthetic sialyl-Tn has proven to be highly target – specific in human trials, and the induction of high anti-sTn antibody titres correlated with prolonged surival of breast cancer patients. Clinical experiments using vaccine preparations based on whole tumor cell walls have met with very little success and one possible explanation is that the vaccines are not sufficiently immunogenic. However, conjugates of synthetic tumor – related antigens such as Tn, Thomson-Friedenreich and sialyl-Tn (sTn) with an antigenic carrier protein , such as keyhole limpet haemocyanin (KLH) , are much more immunogenic and have been successfully applied in active immunotherapy of tumor- bearing hosts . Patients given the vaccine developed an anti-carbohydrate immunoresponse and had significantly highersurival rates than patients who did not receive treatment. Furthermore , levels of sTn antibodies showed an inverse correlation with growth in measurable tumors. Many of the carbohydrate antigens are really altered self antigens and for this reason sometimes in cancer patients the body does not react to them immunologically. Immune reponses against carbohydrate antigens have been categorized as TI (Thymus Independent) in nature because they do not require cognate interactions between antigen specific B and T cells. These antigens are known to stimulate an antibody response in athymic mice. TI antigens are divided into TI-1 and TI-2 types.However , carbohydrate epitopes over-expressed on the surfaces of cancer cells may evoke a B cell response when introduced in an appropriate fashion to a host's immune system. HexasaccharideGlobo-H, first synthesized in 1996 is the most advanced monomeric vaccine. It is currently undergoing clinical trials in investigating treatment for cancer of the breast , prostate and ovar. The carbohydrate antigen Globo-H is a potential target for vaccine therapy .In this the complex carbohydrate molecule- Globo-H, the hexasaccharide has been synthesized , conjugated to keyhole limpet hemocyanin and administered with the immunologic adjuvant QS-21 as a vaccine for patients with prostate cancer who relapsed after primary therapies such as radiation or surgery. The structure of Globo-H is given in **figure 1**.

Another carbohydrate based vaccine called GMK contains ganglioside GM2. GMK vaccination induces AntiGM2 antibodies that target melanoma cells . The vaccine is currently in phase-3 trials for malignant melanoma . To circumvent the drawbacks associated with the use of traditional carbohydrate-protein conjugates, multiple antigenic glycopeptides (MAG) based on lysine core extended with peptidic arms displaying carbohydrate antigens were developed. This synthetic immunogen, MAG, displays a high antigen density at the surface of an inert lysine core, thus limiting irrelevant antibody production. MAGs have high purity and accurate chemical definition which are essential features for a safe vaccine. Tn antigen acts as a carbohydrate tumor marker in the MAG, since it represents a good target for developing therapeutic vaccines against lung, colon or breast cancer, The MAG strategy could also apply to the development of anti-bacterial vaccines.

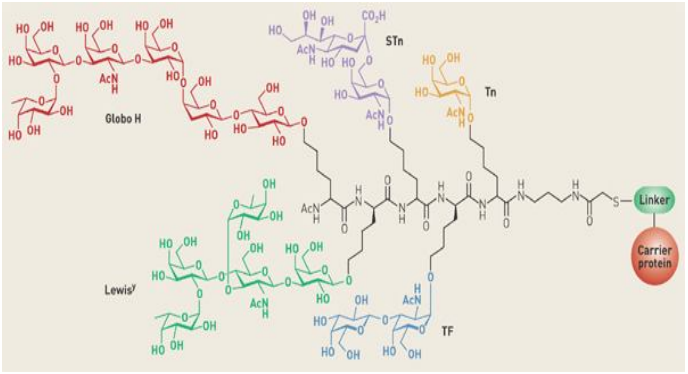

Figure 1. Structure of Globo-H Hexasaccharide

CONCLUSION

Thus carbohydrate based vaccines are stirring an excitement in medical circles. Hence , once a covalent link is established in carbohydrate moiety it opens up new horizons for glycomics . Carbohydrate conjugate vaccines prove to be versatile basis for novel vaccines. The advent of synthetic carbohydrate vaccines herald a new age for vaccines. They bring the possibility of preventing common bacterial infections for which vaccines with older technology are ineffective against infants and individuals with short term protection. They are been used very successfully to protect humans against various pathogens and diseases.

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