CONJUGATE VACCINES USING SYNTHETIC CARBOHYDRATE ANTIGENS: A TOOL FOR ANTI-TUMOR THERAPEUTICS VACCINES

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Glycoconjugates represents an important class of compounds involved in many recognition functions (1). They surround the cells as capsularpolysaccarides or lipopolysaccharide in bacteria or as glycoproteins andglycolipids in the eukaryotic cells glycocalix. They are thus frequently the recognition site of the immune system. Carbohydrates are usually related with a T-independent response, devoid of immunological memory and producing low affinity antibodies. This situation was dramatically improved for the manufacture of anti-bacterial vaccines by the introduction in the 80's of the chemical conjugation process to a protein carrier, as a way to abtain a T-dependent response with immunological memory. The effectiveness of this process was firmly established in animals for many carbohydrates (2). In humans the most remarkable example is the vaccine against *Haemophilus influenzae* type b (3-6).

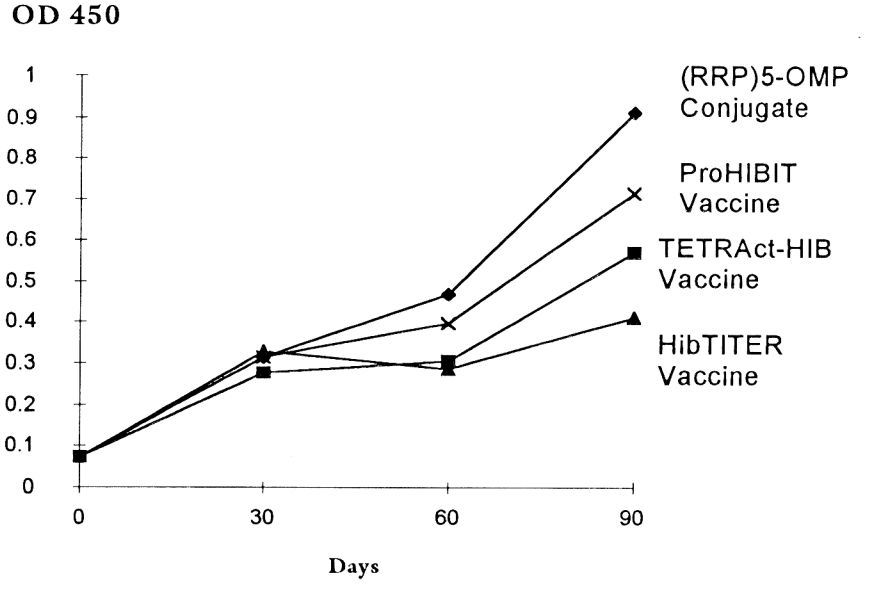
The advances in the last 20 years have made the synthesis of oligosaccharides for vaccine development a reality, although, in each case the size of the desired oligosaccharide as well as their complexity, determines the chances of synthetic compound to compete with the natural analog in 1080 we started a project for the development of a conjugated vaccine aginst H. influenzae type b using a synthetic decasaccharide. As the result of seven years of work we developed a highly competitive process for the synthesis of the disaccharide monomer (7) for the decasaccharide $(RRP)_5$ assembly methodology and for the conjugation process with meningococcal outer membrane protein complex (OMP) (8).

The antibody titer raised after immunization of mice with the synthetic $(RRP)_5$ couplet to meningococcal OMP were measured against the natural polysaccharide (Figure 1) and compared with antibody titer obtained against commercially available vaccines. This preliminary results stimulated us to develop this model as a human vaccine. The use of a natural modified polysaccharide or a synthetic oligasaccharide made feasible the production of conjugated against bacterial pathogens after the choice of several parameters among the most important are the fragment size and the conjugation process. However, for tumor therapeutic vaccines the problem is more complex, because in addition to theknown T-independence, carbohydrate tumor associated antigens behave as self antigen then is necessary to bypass the tolerance.

To obtain a good anti-carbohydrate responce, the epitope included in the vaccine, the spacer and the method of conjugation must be selected very carefully otherwise the neoepitopes formes durig conjugation will become dominants in the immune response.

We have investigated the possible use of carbohydrates associated with liposomes carriers to avoind the formation of neoepitopes during the conjugation process with proteins. Gregoriadis (9) suggested that B-Cell and T-cell epitope entrapped in the same liposome afford the same results as conjugation.

Figure 1. Antibody titer against the Hib capsular polysaccharide.



1. Varki A. Biological roles of oligosaccharides: all of the theories are correct. Glycobiology 1993;97-130.

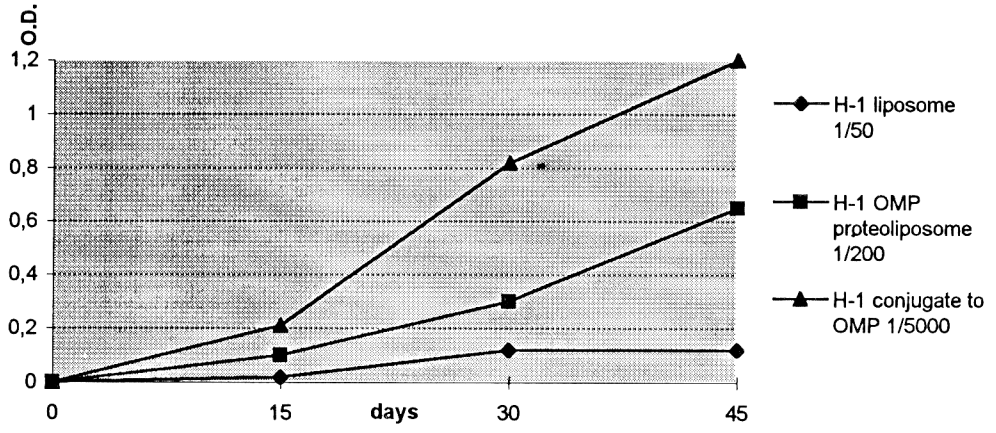
2. Robbin JB, Shneerson R. Polysaccharide-protein conjugates: a new generation of vaccines. J Infect Dis 1990;161:821-832.

3. Schneerson R, Barrera O, Sutton A, Robbins JB. Preparation characterization and immunogenicity of *H. influenzae* type b polusaccharide-protein conjugates. J Exp Med 1980;152:361.

4. Anderson PW, Picheiro ME, Insel RA. Immunogens consisting of aligosaccharides from the capsule of *H. influenzae* type b coupled to diphtheria toxoid or the toxin protein CRM_{197}, J Clin Invest 1985;76:52-59.

5. Marburg S, Jom D, Tolman RL, Arison B, Mccauley J, Kniskem PJ *et al.* J Am Chem Soc 1986;108:5282-7.

Figure 2. Antibody titer elicited against H-1 (BSA conjugate) using several combination of H-1 neoglycolipids.



We synthesized the H-1 trisaccharide and coupled through a spacer-arm to different lipid anchor. The trisaccharide was then immunologically expressed either coating simple HIV loposomes or meningococcal aouter membrane proteoliposome. The conjugate with the same protein was also studied as a positive control. The results are shown in Figure 2. The titer of antibodies elicited by simple liposomes were the lowest.

The H-1 neoglycolipid coating the proteoliposome induce higher titer but the response was mainly IgM and significantly lower than those obtained for the parent conjugate.

The lack of T-dependence in the response against the carbohydrate portion of a neoglycolipidid and their targeting effect to cells receptor lectins, offer the opportunity to entrapped carbohydrate antigens conjugated to proteins in liposomes coated with neoglycolipids for targeting the antiden to specific cells or tissue.

The structure of the carbohydrate portion of the neoglycolipid will direct the liposome to specific cells and will probably modulate the immune response against the encapsulated antigen.

For this purpose we synthesized the neoglycolipids represented in Figure 3.

The effect of the targeting properties of this neoglycoplipids will be studied in the model of tumor-associated sialyl-Tn disaccharide coupled to meningococcal Outer Membrane Protein.

6. Weinberg GA, Granoff DM. Polysaccharide-protein conjugate vaccines for the prevention of *H. influenzae* type b disease. J Pediatr 1988;113:621-631.

7. Chiu Machado I, Madrazo Alonso O, Vérez Bencomo V. A new approach to the ribosyl-ribitol unit for the synthesis of *Haemophilus influenzae* type b oligosaccharides. J Carbohydr Chemistry 1994;13:465-74.

8. Chiu Machado I, Castro Palomino J, Lopetegui C, Madrazo Alonso O, Vérez Bencomo V. Syntnesis of riboturanosides by catalyst with Lewis acids. Glycoslation *versus* transacetylation. J Carbohydrate Chem 1995;14:W551-561.

9. Gregoriadis G, Wang Z, Barenholz Y and Francis MJ. Liposome-entrapped T-cell peptide provides help for a co-entrapped B-cell peptide to overcome genetic restriction in mice and induce immunological memory. Immunology 1993;80:535-540.

Figure 3 Synthetic neoglycolipids.