



On the mechanisms of conjugate vaccines

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During the last three decades, the development and commercialization of conjugate vaccines against *Haemophilus influenzae* type b (Hib), pneumococcus, and serogroups C, A, W, and Y of meningococcus contributed to the virtual elimination of bacterial meningitis caused by the bacteria included in the vaccines and to the prevention of diseases that used to cause more than a million deaths annually (1, 2). Despite the great impact on public health of these vaccines, our understanding of the way these vaccines work is still limited, and we have many unanswered questions. In PNAS, Sun et al. (3) report new mechanistic insights on conjugate vaccines.

History of Conjugate Vaccines

Conjugate vaccines have been developed to induce a robust immune response against bacterial capsular polysaccharides (CPSs). CPSs are long polymers composed of many repeating units of simple sugars and serve as a protective external layer for many bacteria. Depending on the chemical composition of the repeating unit (usually composed of one to seven monosaccharides). Bacteria can synthesize hundreds of chemically and immunologically different polysaccharides. Antibodies against the polysaccharides of many pathogenic bacteria, such as meningococcus, Hib, and pneumococcus, protect people from disease. Vaccines composed of purified polysaccharides against meningococcus and pneumococcus were developed in the 1970s. Unfortunately, those vaccines, while partially immunogenic in adults, were completely unable to induce an antibody response in infants and children, the population for whom the vaccines were mostly needed. The problem was solved in the 1980s when John Robbins and Rachel Schneerson at the National Institutes of Health in Bethesda, Maryland, and David Smith and Porter Anderson in Rochester, New York, independently figured out that, in 1929, it had been reported that bacterial CPSs become very immunogenic when covalently linked to a carrier

protein (4, 5) and, thus, started working on a conjugate vaccine against Hib, which worked beautifully in infants and children. The Hib vaccine was licensed in 1990 in the United States, and John Robbins, Rachel Schneerson, Porter Anderson, and David Smith received the Albert Lasker Award for Clinical Research in 1996 for preventing meningitis in children (6). In parallel, conjugate vaccines were developed for meningococcus (7) and pneumococcus (8), and both were licensed in 1999 and 2000 in the United Kingdom and United States, respectively. Conjugate vaccines have been described also for group B *Streptococcus*, *Shigella*, *Salmonella typhi*, and *Salmonella paratyphi*. A *S. typhi* vaccine has been recently licensed in India and received World Health Organization prequalification (9).

Recently, metabolic engineering of bacteria allowed the construction of *Escherichia coli* strains that produce and export in the periplasm polysaccharides already linked to carrier proteins (10). Some of these so-called bioconjugates naturally produced in *E. coli* have already been successfully tested in several clinical trials. Bioconjugates represent a great simplification of the production process of conjugate vaccines, and they are expected to allow the production of large amounts of conjugates at a cost easier to afford for low-income countries.

Mechanistic Considerations for Conjugate Vaccines

The present knowledge of the mechanism of action of conjugate vaccines has been recently reviewed in depth (11) and is schematically summarized in Fig. 1. Briefly, after immunization, polysaccharides or conjugate vaccines are taken up by dendritic cells and transported to lymph nodes where, to induce an immune response, they need to engage both B and T cells and start the formation of germinal centers (GCs). GCs are sites within lymph nodes and the spleen where mature B cells proliferate, differentiate, and mutate their antibody genes through somatic

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conjugates. In their model (Fig. 1, circle B), the role of the conjugate is to allow loading the sugars into the MHC molecule. Briefly, following processing of the conjugates, the glycopeptides containing the junctions between the carrier protein and the polysaccharide are loaded into the MHC carrying with them the attached sugars that now are available for recognition by the T cell receptor (TCR). To prove this hypothesis, Avci et al. (12) reported the isolation of two T cell clones that recognized the polysaccharide of serotype III group B *Streptococcus*. They named these clones carbohydrate-specific CD4⁺ T cells (Tcarbs). In reality, the two mechanisms described in Fig. 1 are likely to coexist. Unfortunately, 7 years after the first publication by Avci et al. (12), no additional evidence has been reported about the existence of Tcarbs and the second mechanism of antigen presentation. No more Tcarbs have been described, and structural studies showing the junction peptide linked to the MHC or TCR have not been reported; however, the induction of Tcarb-mediated immunity has been reported also for additional conjugate vaccines such as pneumococcus (14). The paper by Sun et al. (3) in PNAS reports instead that, although three of the four conjugate vaccines studied in the paper work via Tcarbs, the conjugate vaccine made from the group C polysaccharide of *Neisseria meningitidis* (MenC) does not work via the Tcarb mechanism because the polysaccharide (which is a polymer of sialic acid) is completely depolymerized in

the endosome and no junctional glycopeptides are generated. This supports the fact that the two mechanisms of antigen presentation, peptide and peptide-Tcarb, usually coexist and that successful conjugate vaccines such as MenC do not need the Tcarb mechanism.

Conclusions

If the Tcarb hypothesis is the primary mechanism to engage T cells in conjugate vaccines, we should be able to improve their immunogenicity by increasing the number of covalent junctions between the protein and the polysaccharide. Testing this hypothesis can be an opportunity to optimize the novel chemical or biological conjugation technologies that have been recently described (10, 15). Lastly, the study by Sun et al. (3) was conducted by using, as a correlate of the Tcarb mechanism, the antibody response after priming with a polysaccharide conjugated with a protein and boosting with the same polysaccharide conjugated to a different protein. Although this correlate is likely to be correct, it would be important to nail down the presence of Tcarbs in a definitive way by isolating more Tcarbs and by determining the structure of the Tcarb bound to a glycopeptide.

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