

A sweet T cell response

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Although protein-polysaccharide conjugate vaccines provide notable clinical benefits, it is still not fully understood how they work. A new mechanism of action for these vaccines has been identified in which T cells can recognize sugar epitopes in the context of the major histocompatibility complex (MHC) provided they are bound to a protein ‘anchor’, which allows binding of the sugar epitope to the MHC (pages 1602–1609).

Protein-polysaccharide conjugates are one of the miracles of immunology. A bacterial capsular polysaccharide does not induce the production of antibodies in animals and is non-immunogenic in human infants, but it becomes a powerful immunogen when covalently linked (conjugated) to a protein. The history of the study of conjugates dates back to the 1920s when the pneumococcal polysaccharide was identified as target for antibodies¹, and it was shown that when conjugated to a protein, this polysaccharide could induce the production of antibodies^{2,3}. Conjugation of bacterial polysaccharides to carrier proteins was subsequently used to induce immunity against pneumococcus and *Salmonella typhimurium*^{4,5} and to study immunity to dextran⁶. In 1996, the Lasker Award was awarded to a group of researchers for the development of protein conjugate vaccines against *Haemophilus influenzae*⁷.

Today, conjugate vaccines rank among the safest and most efficacious vaccines ever developed, and every year hundreds of millions of doses of them are used to vaccinate against the pathogens *H. influenzae* type B, *Neisseria meningitidis* and pneumococcus⁸. Conjugate vaccines against group B streptococcus (GBS) and *Salmonella typhi* are currently in late-stage clinical development, whereas many other conjugate vaccines are earlier in the developmental pipeline. Conjugate vaccines induce high-affinity antibodies that bind to the bacterial polysaccharide capsule and recruit complement to the bacterial surface, which can directly kill the bacteria by complement-mediated lysis or trigger bacterial uptake by phagocytic cells in a process called opsonization. Conjugate vaccines have eliminated the diseases against which they are targeted in all countries that have implemented vaccination with them. Notably, vaccination with conjugate vaccines can also eliminate the carriage of bacteria from mucosal surfaces in vaccinated individuals, thus eliminating transmission of the pathogen to people who have not been vaccinated (a phenomenon known as herd immunity).

Despite this notable clinical success and the simplicity of the conjugation process, we are still unable to fully explain how the immune system responds to such conjugates. Current dogma states that polysaccharides function as T cell-independent antigens, as they are very hydrophilic molecules that cannot dock in the cleft of the MHC products, which evolved to bind peptides through a combination of hydrophobic and hydrophilic interactions. Therefore, polysaccharides cannot be presented to T cells in the context of the MHC on the surface of antigen-presenting cells. Consequently, B cells that recognize polysaccharides using their surface IgM cannot get help from T cells.

When B cells bind a polysaccharide linked to a carrier protein, the protein provides the T cell epitopes that engage the T cell receptor (TCR) and trigger the release of cytokines that help the B cell to proliferate, switch class from producing IgM to IgG antibodies, start the process of affinity maturation and establish memory^{9,10}. This process (Fig. 1a) implies that a B cell containing a surface IgM binds the polysaccharide part of the conjugate with low affinity, internalizes and processes the conjugate and then loads class II MHC (MHCII) with peptides derived from the carrier protein, which are then recognized by the TCR. According to this theory, the only role of the conjugate is to ensure that the polysaccharide and the carrier protein target the same B cell. However, there are no published reports showing that the co-delivery of non-covalently linked proteins and polysaccharides is able to make a polysaccharide immunogenic, suggesting that the covalent link between the protein and the sugar may have a role in engaging T cell help that is not yet understood.

A second observation that is not fully explained by the current theory of conjugate vaccines is the effect of carrier priming on the subsequent response to vaccination with a glycoconjugate. It has been reported that there is a faster antibody response to a glycoconjugate vaccine in mice and humans that had previously been primed with the carrier protein compared to those that had not been primed^{7,11}, however, this rapid response is not always seen¹². Furthermore, the literature about immune

memory induced by conjugate vaccines is unclear. ‘Memory’ is often used to describe B cell memory, and it is not known how a T cell specific for a peptide artificially conjugated to a polysaccharide can provide memory against an incoming antigen that is not linked to the same carrier protein in the pathogen. Overall, these unexplained results suggest that the identification of additional mechanisms may be necessary to fully explain how conjugate vaccines work.

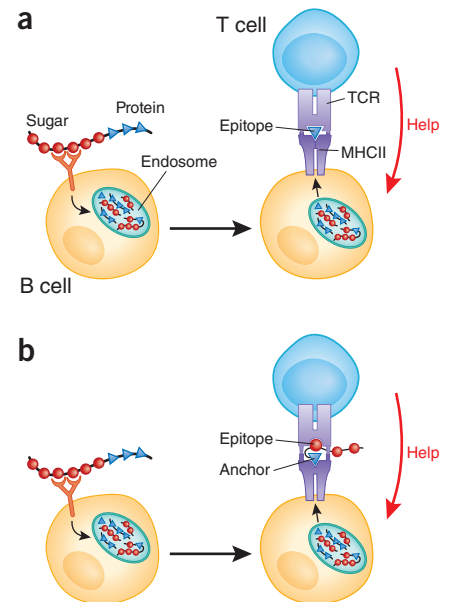


Figure 1 Two models for antigen processing and presentation of a conjugate vaccine. In a conjugate vaccine, the polysaccharide antigen (the red circles represent polysaccharide repeat units) linked to the protein (the blue triangles represent protein peptides) is taken up by a surface immunoglobulin on a B cell, internalized and processed. As the hydrophilic polysaccharide cannot enter the MHCII cavity, the polysaccharide itself cannot be presented to T cells by MHCII on the surface of the B cell in order to induce T cell help. Therefore a conjugate vaccine may use two alternative ways to induce T cell help: (a) the current accepted theory suggests that the processed peptide epitope derived from the carrier protein is presented by MHCII and recognized by a peptide-specific T cell; or (b) the new mechanism presented by Avci *et al.*¹² suggests that the peptide anchors the sugar epitope to the MHC and allows presentation of the sugar epitope to a polysaccharide-specific T cell.

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In this issue of *Nature Medicine*, Avci *et al.*¹² describe a new molecular mechanism for conjugate vaccines that may provide answers to the outstanding questions described above. The authors isolated two T cell clones that are stimulated by GBS serotype III polysaccharide (GBSIII) independently of whether the polysaccharide is conjugated to an ovalbumin, a tetanus toxoid or a lysozyme carrier protein. Their results imply that the epitope recognized by the TCR in the context of the MHC is provided by the sugar and not the peptide. This new theory (Fig. 1b) suggests a different role for the conjugate peptide than that given in the currently accepted theory, one in which it functions as an anchor that allows the sugar epitope to be presented to the TCR by the MHC.

Avci *et al.*¹² provide additional evidence to support this new model. They show that once the conjugate vaccine is internalized by B cells, it is processed into smaller pieces, and these processed sugar epitopes can be detected on the cell surface. Unconjugated polysaccharides cannot be processed in this way, suggesting that B cells have the appropriate apparatus to process and present carbohydrate epitopes in association with MHCII when they are conjugated to a peptide. The authors also show that the memory induced by conjugate vaccines is specific for the polysaccharide and not for the carrier protein. In fact, immunization with a tetanus toxoid–GBSIII conjugate induced memory that was boosted by an ovalbumin–GBSIII conjugate, but priming with unconjugated ovalbumin did not induce memory that could be boosted by the ovalbumin–GBSIII conjugate.

Given the state of the current literature, more experiments need to be done to sort out the relative roles of the models shown in Figure 1a and 1b, however, if these two mechanisms of action for conjugate vaccines coexisted, it would allow most of the outstanding questions about these vaccines to be answered.

The T cell clones found by Avci *et al.*¹² are the first to be described that recognize a pure carbohydrate epitope regardless of the peptide to which they are linked. T cells have been shown to recognize peptides, glycopeptides or lipids but not pure oligosaccharides or polysaccharides¹³. The field needs more carbohydrate-specific T cell clones to confirm the generality of the findings of Avci *et al.*¹², but their work profoundly affects our understanding of the molecular mechanisms of antigen recognition by T cells. The TCR recognizes the epitope presented by the MHC mostly through the hypervariable complementarity-determining region CDR3, which is formed by recombining the VJ and VDJ regions of the α and β chains of the TCR. In theory, this recombination could generate 10¹⁵ different TCR molecules¹⁴, and there is no reason why a TCR should not recognize all antigens, including polysaccharides. The failure to previously isolate sugar-specific T cells may have been because it was not realized that nonpeptide epitopes can be recognized by T cells, provided that they are anchored by a covalently linked peptide.

The finding that T cells can recognize polysaccharides and possibly other structures if they are covalently linked to a peptide anchor suggests that it should be possible to optimize the presentation of carbohydrate epitopes to

T cells, and this may allow for the development of new therapeutic applications using peptide-polysaccharide conjugates. As an example, Avci *et al.*¹² show that peptide-polysaccharide conjugates rationally designed to allow the presentation of multiple carbohydrate epitopes *in vivo* can be more effective vaccines in a mouse model challenged with GBS than classical conjugate vaccines. In addition, as the TCR may be able to recognize any structure linked to a peptide, T cell immunity could potentially be generated against various structures using entirely synthetic vaccines, which could be used to protect against or treat a number of diseases.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturemedicine/>.

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Stressed tumor cell, chemosensitized cancer

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miR-200 family expression results in highly proliferative ovarian cancer cells. Yet this expression is also linked to longer overall survival in women with ovarian cancer. A new study sheds light into this apparent paradox showing that two members of this family—miR-141 and miR-200a—not only boost tumor growth but also sensitize tumor cells to chemotherapy (pages 1627–1635).

The relatively well-studied miR-200 family—comprising miRs 141, 200a, 200b, 200c and 429—has been associated with the formation of cancer stem cells¹ and is intimately involved in the epithelial-to-mesenchymal

transition (EMT)². The miR-200 family is aberrantly expressed in epithelial ovarian cancer, with increased levels observed in various histotypes (serous, endometrioid and clear cell subtypes)^{3,4}; however, how these miRNAs function in ovarian cancer pathogenesis is still unclear.

Cellular oxidative stress, that is, exposure to increased levels of reactive oxygen species (ROS), elicits a miRNA response. Cellular ROS

may manifest a dual role, either promoting or suppressing carcinogenesis. In general, cells carefully regulate ROS levels, and several biochemical pathways are capable of dealing with oxidative stress, including a stress signaling network mediated by the mitogen-activated protein kinases p38 α and JNK, as well as the Keap1/Nrf2 oxidative stress response pathway^{5,6}. p38 α is a stress-activated kinase previously identified as a sensor of ROS⁷ that,

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