the folding-competent PrP is efficiently exported to the cell surface also in the absence of Tmp21. Thus, the authors conclude that Tmp21 is specifically required for PrP* solubility and export under conditions of ER stress. The authors show that not only PrP* but also other misfolded GPI-anchored proteins such as CD59 undergo this new export mechanism, and they identify the GPI anchor and a misfolded domain as prerequisites. In the absence of stress, a small portion of the misfolded GPI-anchored proteins is cleared from the ER by this pathway, but this clearance is strongly enhanced upon ER stress induction. Therefore, the authors termed this new mechanism rapid ER stress–induced export (RESET). RESET is enhanced only before UPR activation and is deactivated when the UPR is able to sufficiently cope with the stress.

Satpute-Krishnan *et al.*[2] contribute to a better understanding of the post-translational processes that are in place to reestablish ER proteostasis during ER stress in the absence of UPR activation. As the main focus of ER stress research has been laid on transcriptional responses, this new line of research adds valuable information to the field. It will be of high interest to extend the findings of Satpute-Krishnan *et al.*[2] to a broader set of disease-associated misfolded GPI-anchored proteins. As RESET represents a mechanism to increase chaperone availability in the initial phase of acute ER stress, its pharmacological activation by small-molecule UPR inducers may be a promising strategy to combat conformational diseases[8]. Furthermore, the tools established in this work might facilitate studying the elusive mechanism of plasma membrane quality control of misfolded GPI-anchored proteins.

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## Competing financial interests

The authors declare no competing financial interests.



VACCINE DEVELOPMENT

# NKT-cell adjuvants in conjugate

Two studies demonstrate that natural killer T-cell adjuvants, covalently attached to either carbohydrate or peptide epitopes, yield effective vaccines.

Paul B Savage

As the understanding of immune responses to vaccination has increased, the nature of vaccines has changed. Where once whole infectious organisms (attenuated or inactivated) were used exclusively, now substructures of these organisms and even individual molecular epitopes are used. Using simple molecular epitopes can decrease issues related to infections with live organisms, unproductive responses to vaccine components and impure and uncharacterized substructures from whole organisms. A key component of simpler vaccines is often an adjuvant that elicits an immune response to the vaccine epitope, which leads to long-lasting, protective memory responses. Two new studies develop natural killer T (NKT)-cell adjuvants covalently coupled to vaccine epitopes and show that they are useful in generating immune responses to both carbohydrates and peptides[1,2]. NKT cells are a subset of T cells, and they regulate immune responses to infection among other activities.

Many types of vaccine adjuvants are in use or are being developed. These include simple aluminum salts (alum), saponins and Toll-like receptor (TLR) agonists. The latter mimic molecular patterns associated with infectious agents, and recognition by various forms of TLRs leads to production and release of inflammatory cytokines[3]. These inflammatory messengers can substantially improve responses to vaccination. NKT cells also recognize molecular patterns associated with infectious agents; however, the mechanism by which they recognize molecular patterns is distinct from that of TLRs. TLRs bind to and recognize their agonists directly, whereas NKT cells require antigen presentation by antigen-presenting cells, such as dendritic cells[4]. Antigen presentation to NKT cells results in cytokine release and direct interaction between cells, which promotes antigen-presenting cell maturation and an increase in their ability to present antigens to T and B cells. Consequently, there has been interest in using NKT-cell antigens as vaccine adjuvants[5].

*In vivo* development of high-affinity, protective antibodies for a vaccine epitope is one of the key gauges of vaccine effectiveness. Multiple events are required, including antibody class switching (for example, IgM to IgG) and affinity maturation to give antibodies with dissociation constants for the targeted epitope, which are often in the nanomolar range. T-cell interactions (or 'help') with B cells are required for development of this type of antibody, and initial work with NKT-cell agonists suggested that NKT-cell help might not result in generation of high-affinity antibodies and long-lasting responses to vaccines[6,7]. Typical T-cell help given to B cells requires T-cell activation through peptide recognition, whereas NKT-cell help involves stimulation via glycolipid presentation.

As most cell types, including bacteria, are coated with distinctive and essential carbohydrates, vaccines based on carbohydrate epitopes offer an attractive means of generating antibody responses to potential pathogens[8]. Carbohydrate vaccines in clinical use are composed of the carbohydrate epitope linked to an antigenic peptide. Cavallari *et al.*[1] reasoned that a carbohydrate epitope linked to an NKT-cell antigen would yield a vaccine capable of eliciting the necessary immune responses for generation of

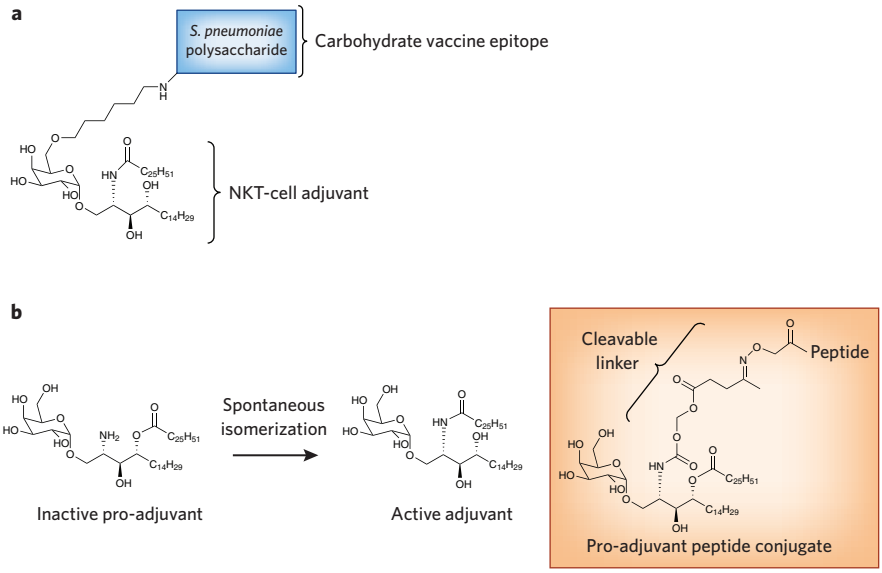


**Figure 1 |** Structures of NKT-cell adjuvant-vaccine epitope conjugates. (**a**) Glycolipid–*S. pneumoniae polysaccharide* conjugate. (**b**) Mechanism of pro-adjuvant to adjuvant transformation and structure of pro-adjuvant–peptide conjugate.

protective, high-affinity antibodies to the targeted epitope. The authors covalently attached the capsular polysaccharide found on *Streptococcus pneumoniae* to a potent NKT-cell agonist at a site shown to not interfere with presentation and stimulation of NKT cells (**Fig. 1a**). When administered to mice, they saw the hallmarks of effective vaccination: class switching from IgM to IgG, affinity maturation (via several rounds of mutation) and generation of epitope-specific memory B cells. Because the vaccine did not include peptide, NKT cells provided the help required for these processes. Furthermore, Cavallari *et al.*[1] demonstrated that vaccination resulted in protection against a lethal challenge of *Streptococcus pneumoniae* in mice.

The basic structure of antigens for NKT cells is relatively stable; however, Anderson *et al.*[2] observed a rearrangement of the ceramide portion of a NKT-cell antigen under acidic conditions, which resulted in generation of a fatty acid ester. Under neutral or basic conditions, this ester spontaneously reorganized, within hours, to give the original fatty acid amide structure required for NKT-cell antigen activity (**Fig. 1b**). On the basis of this observation, the authors devised a clever strategy to generate a pro-adjuvant (as in pro-drug) carrier for peptide antigens to deliver both peptide and adjuvant to the same antigen-presenting cells. The antigens were attached to the pro-adjuvant via an oxime linkage. Esterase activity revealed an amine group, and oxygen-to-nitrogen acyl migration gave the active adjuvant (**Fig. 1b**). Vaccination with the pro-adjuvant–peptide conjugate led to generation of peptide-specific cytotoxic lymphocytes (CTLs) in mice, whereas coadministration of the peptide antigen with the glycolipid adjuvant (unconjugated) gave only very weak responses.

Immunogenic peptides can trigger allergic responses and allergic airway inflammation, and one strategy shown to limit these responses is to generate CTLs specific for the peptide allergen[9]. Anderson *et al.*[2] sensitized mice to a well-used, antigenic peptide from ovalbumin and subsequently vaccinated the mice with the pro-antigen–ovalbumin peptide conjugate. Vaccination resulted in ovalbumin-specific CTLs, and later challenge with the ovalbumin peptide resulted in a marked decrease in the responses associated with allergic airway inflammation.

This work[1,2], along with other reports, demonstrate that NKT cells can provide T-cell help and stimulate protective memory responses to vaccines. The authors have demonstrated a new means of combining vaccine epitopes with NKT-cell adjuvants to give effective conjugates. These relatively simple, well-characterized vaccines appear well suited for generating specific immune responses for treatment of human disease. ■

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