Modular B-cell and T-cell Epitope Assembly in a Peptide-based Vaccine

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Statement of Purpose: Modern vaccine development is focused on raising responses to well-defined subunit antigens that enhance specificity but lack the immunogenicity of killed or inactivated microbes. Traditionally, short peptide, hapten, and carbohydrate antigens are conjugated to large carrier proteins and formulated with a suitable adjuvant to increase immunogenicity. The carrier protein provides both high-density display of the antigen for B-cell activation and T-cell epitopes for T-cell help. However, large protein carriers are difficult to optimize because each mol ratio of each antigen must be separately conjugated and tested with a suitable adjuvant, and they require extensive purification from endotoxin if expressed in bacteria.

We recently developed an adjuvant platform based on self-assembled peptide nanofibers of the peptide Q11, which elicit strong antibody responses in mice to antigens presented at high density along the fibers[1,2]. Fibers formed by co-assembly of multiple antigens raised a strong antibody response to each[3]. Here, we tested whether modular Q11-based co-assembly could be used in a different way, to combine target peptide antigens containing only a B-cell epitope with a universal T cell epitope in a self-adjuvanting vaccine, thus eliminating the need for carrier proteins and additional adjuvants.

Methods: Antigens were analyzed for B-cell and T-cell epitope content using the Immune Epitope Database 2.0. The B antigen was a proprietary antigen from a pharmaceutical company; the T-cell epitope was PADRE (pan-HLA DR epitope, aKXVAAWTLKAa, where a is D alanine, and X is L-cyclohexylalanine). Peptide antigens were synthesized in tandem with the self-assembling beta-sheet domain, Q11 (QQKFQFQFEQQ-Am), by standard Fmoc-based solid phase peptide synthesis. Peptides were purified to > 90% by HPLC and their identities confirmed by MALDI. For co-assembly, dry peptides were mixed by vortexing before dissolution. Peptides were dissolved in phosphate-buffered saline, and the endotoxin levels of these solutions were ≤ 1 EU/mL as measured by a Limulus amebocyte lysate assay. Co-assembled fibers were imaged by transmission electron microscopy (TEM). C57Bl/6 mice (Ia^b) were immunized subcutaneously (s.c.) with B-Q11 (2 mM), a 1:1 v/v mixture of B-Q11 (4 mM) with Imject Alum (Thermo Scientific, Rockford, IL), or co-assembled B-Q11 + Q11 + PADRE-Q11 (1, 0.95, 0.05 mM, respectively). Blood samples were collected weekly by submandibular venipuncture, and B-specific Ig titers in the serum were measured by ELISA. To quantify the T cell response, cells from the draining lymph nodes were harvested 7 d after a booster immunization and challenged in vitro with the B and/or T peptide epitopes (5 μM); secretion of interferon-γ (IFN-γ) and interleukin-4 (IL-4) were measured by ELISPOT assays after 48 hr.

Results: A putative 18-mer peptide antigen from *Staphylococcus aureus* was analyzed for B-cell and T-cell epitope content. This antigen was previously identified as protective in mice when conjugated to a diphtheria toxin mutant carrier protein and adjuvanted in alum. Epitope analysis confirmed that the sequence, denoted B, contained a B-cell epitope but no T-cell epitopes with affinity < 5 μM for the Ia^b MHC-II haplotype. Therefore, we co-assembled B-Q11 with PADRE-Q11; PADRE is a powerful CD4+ T helper epitope for Ia^b and most human HLA molecules. The co-assembled nanofibers (Fig. 1a) raised B-specific IgG responses in mice that reached a titer of 10^4 after a single boost (Fig. 1b). Consistent with a lack of T-cell help, no response was raised by B-Q11 alone or even in alum adjuvant. Furthermore, the T-cell cytokine response was focused solely upon the T epitope (PADRE-Q11), with no T cells reactive against B and no synergy between the epitopes (Fig. 1c).

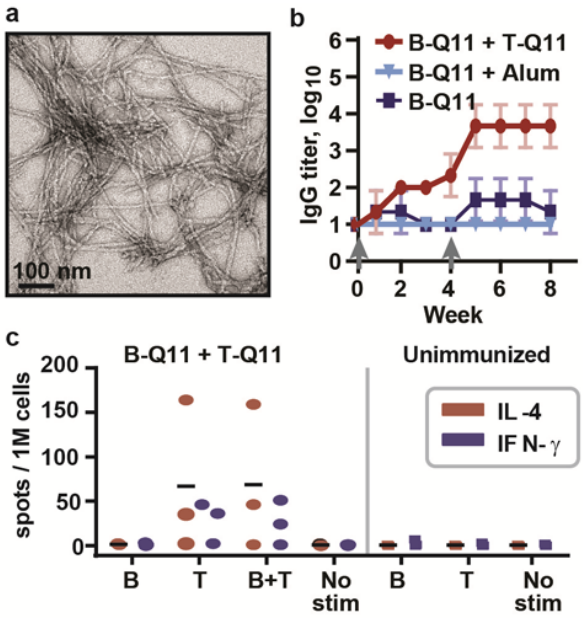

Figure 1. Immunization with co-assembled B-Q11+T-Q11 fibers, shown by TEM (a), raised B-specific antibody responses in mice, measured by ELISA (b), while the T-cell cytokine responses were specific only for PADRE (the T epitope), as measured by ELISPOT (c).

Conclusions: Modular self-assembly of antigen-presenting Q11 fibers was used to combine a peptide antigen containing only a B cell (antibody) epitope from *Staphylococcus aureus* with a separate universal T cell epitope peptide. Co-assembled fibers generated a high-titer antibody response to the B epitope and a T-cell cytokine response to PADRE. Thus, this straightforward co-assembly approach provides an alternative to conjugation of peptide antigens to carrier proteins and will enable rapid testing of various T/B epitope ratios in the future.

References: **1.** Rudra, JS. PNAS USA. 2010; 10:622-627. **2.** Rudra JS. ACS Nano. 2012; 6:1557-1564. **3.** Rudra JS. Biomaterials, 2012; 33: 6476-6484.