Cytokine-modulating self-assembling peptide materials

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Statement of Purpose: Cytokines modulate the strength and phenotypes of adaptive immunity. We recently described a self-assembling peptide-based system to display peptide or protein antigens on nanofibers and elicit potent antibody responses without inducing inflammation.[1-3] Here, we developed two strategies to influence the cytokines associated with these responses: 1) We developed self-assembling peptides capable of eliciting anti-cytokine antibodies, and 2) we employed cytokine mimicking peptides to enhance the immunogenicity of co-delivered peptide antigens. In the first case, we produced self-assembled nanofibers containing an active fragment of TNFα (residues 4-23), along with a universal CD4 T helper epitope PADRE (pan-HLA DR epitope, aKXVAAWTL KAa, where a is D alanine, and X is L-cyclohexylalanine) to elicit neutralizing antibodies against TNFα, towards the treatment of inflammatory diseases. In the second strategy, we co-assembled a peptide fragment from human IL-1β (residues 163-171) for its previously reported non-inflammatory, adjuvanting properties, and assessed its ability to improve the magnitude or quality of antibody responses to a model antigen.

Methods: All peptide sequences were analyzed for B cell and T cell epitope content using the Immune Epitope Database 2.0. Each peptide was synthesized in tandem with the self-assembling fibrillization domain, Q11 (QQKFQFQFEQQ), by standard Fmoc-based solid phase peptide synthesis, purified to > 90% by HPLC, and their identities confirmed by MALDI-MS. 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. C57Bl/6 mice were immunized subcutaneously with co-assembled TNF-Q11 (2mM) ± 0.05mM PADRE-Q11 or with OVA-Q11 + Q11 ± IL1β-Q11 (1mM, 0.95-1mM, various doses, respectively). Blood was collected weekly by submandibular venipuncture, and peptide-specific Ig titers were measured by ELISA. To quantify T cell responses, cells from the draining lymph nodes were harvested 7 d after the booster and challenged *in vitro* with peptide (5 or 10 μM); secretion of interferon-γ (IFN-γ) and interleukin-4 (IL-4) were measured by ELISPOT after 48 hr.

Results: Analysis of epitope content in the TNF-α peptide predicted two B cell epitopes but only one low affinity CD4 T cell epitope for $I-A^b$. This indicated that eliciting potent antibody responses against the TNF peptide would require exogenous T cell epitopes. Thus, we immunized C57BL/6 mice with TNF and PADRE peptides presented on Q11 nanofibers. We observed antibody responses elicited in the mice immunized with TNF-Q11 + PADRE-Q11 but not with TNF-Q11 alone (Figure 1a). As expected from the epitope analysis, the T cell responses were focused on PADRE, not TNF (Figure 1b). For the IL-1β peptide no T cell or B cell epitopes were predicted. We tested adjuvant formulations that contained 0.1mM, 0.1μM, or 0.1nM of IL-1β peptide co-assembled with 1mM OVA-Q11 and 1mM Q11. We observed that only the mid dose of IL-1β peptide significantly increased pOVA Ig titers for up to two weeks after the first booster injection (Figure 1c) without having a detectable effect on the T cell responses to pOVA (figure 1d). As expected from the epitope analysis, no antibodies (data not shown) or T cell responses (figure 1d) were focused towards IL-$1\beta_{163-171}$.

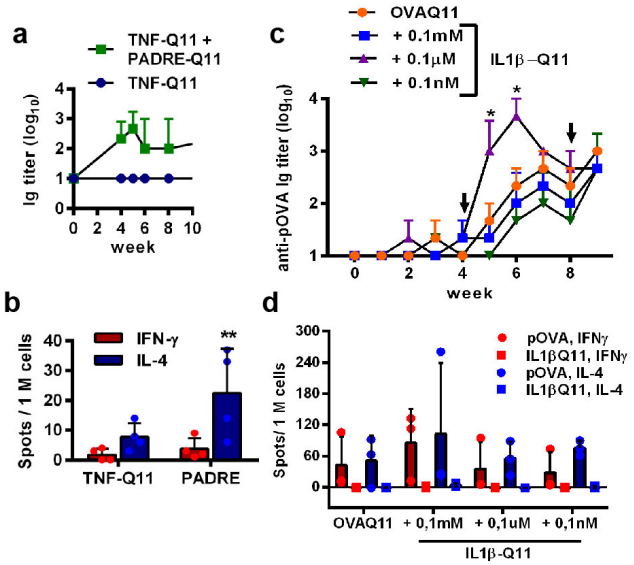

Figure 1. Adaptive immune responses elicited by self-assembling peptide vaccines modified with selected cytokine peptides. Fibers containing murine TNF_{4-23} and the T epitope PADRE raised antibodies against the cytokine (a) but T cell responses were focused on the PADRE sequence (b). Immunization with co-assembled fibers containing 0.1μM IL-$1\beta_{163-171}$ peptide temporarily increased the titers after a booster immunization (c) but did not change the T cell responses to the model $OVA_{323-339}$ peptide. (d) */** $p<0.05$

Conclusions: In this study, we tested initial formulations of self-assembling peptides incorporating selected features from cytokines based on their epitope content. Using cytokine peptides as antigens, we achieved cytokine-specific antibodies without T cell responses. We also tested co-assembled fibers containing different doses of an adjuvanting fragment derived from an inflammatory cytokine. Future studies will involve testing the first strategy in models of inflammatory diseases and the second strategy as a non-inflammatory vaccine adjuvant.

References: 1. Rudra JS. PNAS USA. 2010; 10:622-627. 2. Hudalla GA. Adv. Healthc. Mater. 2013; 2:1114-1119. 3. Chen J and Pompano RR. Biomaterials 2013;34: 8776–8785.