Anti-Cytokine Responses Elicited by Self-assembling Peptide Vaccines

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Statement of Purpose: Several chronic inflammatory conditions, including rheumatoid arthritis, psoriasis, and inflammatory bowel disease, are driven by the de-regulated action of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), Interleukin-1 beta (IL-1β), and IL-23. Current clinical therapies targeting cytokines are antibody-based inhibitors, which have limitations including high cost, side effects, and immune responses that neutralize the therapy. An alternative is to raise cytokine-neutralizing antibodies within the patient, using the patient's own immune system. Towards this goal, our lab recently described a self-assembling peptide-based system that can induce antigen-specific antibody responses without inducing detectable inflammation.[1,2] Here, we incorporated novel features to this platform to raise therapeutic responses against pro-inflammatory cytokines: 1) we incorporated B cell epitopes of TNFα to induce cytokine-specific autoantibodies, and 2) we incorporated different ratios of foreign universal CD4+ T cell epitopes to modulate the immunogenicity of the co-delivered B cell epitopes without significantly altering T cell tolerance to the cytokine.

Methods: The primary sequence of TNFα was analyzed for B/T cell epitopes using the Immune Epitope Database 2.0. The T cell epitopes used were peptides PADRE (pan-HLA DR epitope) and Vaccinia (Vac) virus-derived I1 (7-21). All peptides were 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 in water. Peptides were further diluted 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 (1mM), Q11 (1mM) ± various doses of PADRE-Q11 or Vac-Q11 T cell epitopes. Blood was collected by submandibular venipuncture, and antigen-specific IgG titers were measured by ELISA.

Results: Analysis of epitope content in the primary sequence of whole TNFα protein predicted two B cell epitopes but only one low affinity CD4 T cell epitope for Ia^b (the MHC class II haplotype present in the C57BL/6 strain), all located within the TNFα peptide under investigation. This indicated that eliciting potent antibody responses against TNF-α would require exogenous T cell epitopes. Thus, we immunized C57BL/6 mice with TNF-Q11 and combinations of PADRE or Vac peptides co-presented on Q11 nanofibers. We observed little to no antibody responses elicited in the mice immunized without exogenous T cell epitopes. Interestingly, both

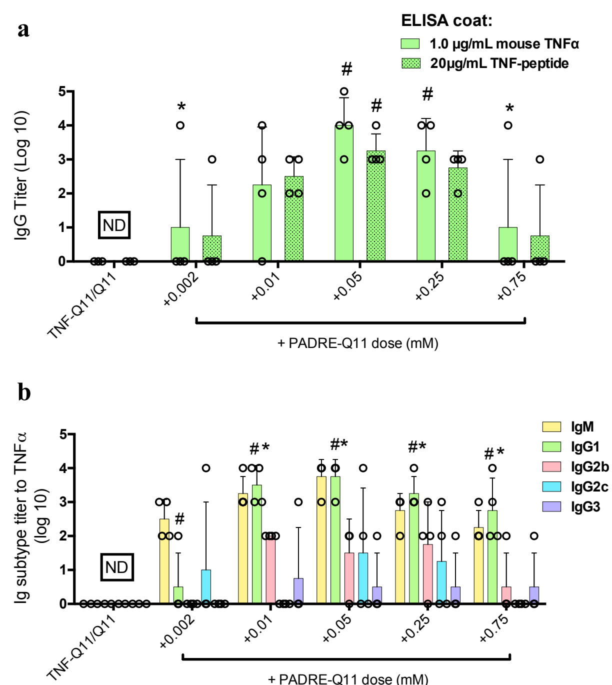

Figure 1. Anti-TNFα antibody responses elicited by co-assembled fibers displaying both TNFα B cell epitopes and PADRE T cell epitope were dependent on the amount of PADRE present in the nanofibers, showing a clear maximum at 0.05mM PADRE. Antibodies reacted against TNF peptide as well as mouse TNFα protein (a). The extent of Ig subtype class switching was also dependent on CD4+ T cell epitope content. Serum tested by ELISA against mouse TNFα (b). *, # $p<0.05$ by ANOVA, n = 4 mice per group)

PADRE-Q11 and Vac-Q11 improved the responses by increasing the titers of TNF peptide as well as TNFα protein IgG (Figure 1a), the affinity of IgG autoantibodies, and the quantity of isotype-switched autoantibodies (Figure 1b), in a dose-dependent manner. No T cell responses to the TNFα peptide were observed by ELISPOT.

Conclusions: Using self-assembling peptides containing cytokine B cell epitopes and foreign T cell epitopes to provide CD4+ T cell help, we achieved cytokine-specific antibody responses without T cell responses to the cytokine. Also, the quantity of T cell epitope in the vaccine had a critical effect on the titer, affinity, and isotype switching of the antibody response. Ongoing studies will test optimal formulations in TNFα neutralization assays and animal models of inflammatory diseases.

References: **1.** Chen J and Pompano RR. Biomaterials 2013; 34: 8776–8785. **2.** Pompano RR, Chen J, et al. Adv Healthc Mater. 2014 doi:10.1002/adhm.201400137.