Thermally stable self-adjuvanting vaccines via self-assembling peptides

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Statement of Purpose: A new approach to vaccine design is needed to prevent problems associated with vaccine distribution. At present all commercially available vaccines depend on storage between 2 - 8 °C, and those that are inadvertently stored above or below this range suffer from reduced immunogenicity. The proteins in existing subunit vaccines are commonly denatured at high temperatures, while low temperatures can precipitate their adjuvants. Distribution to locations without the resources for temperature regulation, especially in the developing world, poses a major challenge to maintaining the quality of current subunit and conjugate vaccines. As an alternative, we have sought to develop highly thermostable self-adjuvanting vaccine preparations through supramolecular chemistry. In our previous findings, we showed that short peptide epitopes synthesized in tandem with an 11-amino acid peptide, Q11 (QQKFQFQFEQQ), self-assemble into nanofibers that raise strong, specific antibody responses in mice, without the need for additional adjuvants [1,2]. Here, our hypothesis was that self-assembled peptide-based vaccines would be stable to heat and cold, as they do not depend on specific folding of their antigens. To investigate this, we tested these materials' thermal stability in both wet and dry conditions, using model antigens (OVA) and a tuberculosis antigen, ESAT6, comparing immunogenicity in mouse models.

Methods: The model antigen $OVA_{323-339}$ (ISQAVHAAHAEINEAGR) and the tuberculosis antigen $ESAT6_{51-70}$ (YQGVQQKWDATATELNNALQ) were synthesized alone or in tandem with a self-assembling beta-sheet domain, Q11 (QQKFQFQFEQQ-Am), using standard Fmoc-based solid phase peptide synthesis methods. Peptides were purified to > 90% by HPLC and their identities confirmed by MALDI. 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. Peptides in powder form or dissolved in solution were heated or stored frozen for 1 – 7 days. Anesthetized C57Bl/6 mice or CBA/J (H-2k) mice were immunized subcutaneously (s.c.) with 100 µL of 2 mM OVA-Q11 or ESAT-Q11. Blood samples were collected weekly by submandibular venipuncture, and OVA-specific or ESAT-specific IgG titers in the serum were measured by ELISA.

Results: After s.c. immunization, both heated and unheated OVA-Q11 induced strong OVA-specific IgG responses after a single immunization, with a further increase after a boost with a half-dose of the nanoassemblies (Fig. 1A). Even peptides heated to 80°C for 24h raised strong responses, though they were slightly diminished in the case of dry heating. Similarly, ESAT-specific IgG responses were seen starting at week 2 in all groups, with similar titers regardless of the heating condition and a sustained response over time. Antibody responses were not seen when the ESAT peptide was delivered without conjugation to Q11 (Fig. 1B).

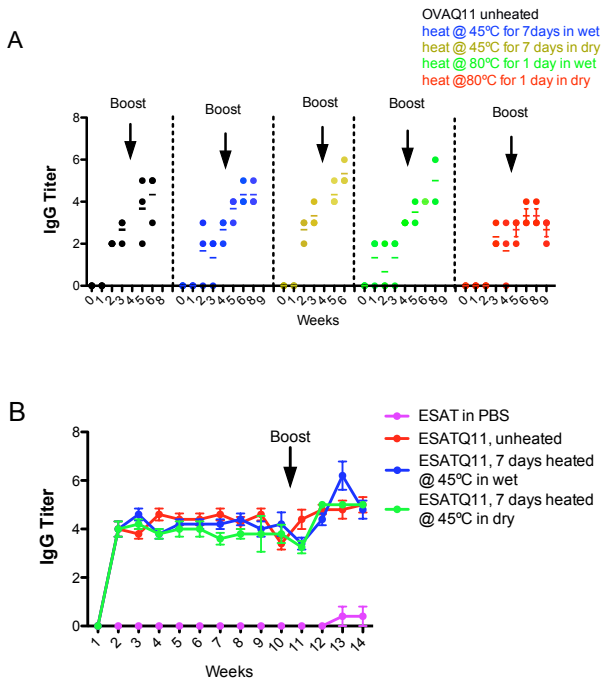

Figure 1. A) Antigen-specific IgG titers measured by ELISA in sera from mice immunized s.c. at weeks 0 and 4 with unheated or heated OVA-Q11. B) Antigen-specific IgG titers measured by ELISA in serum from mice immunized s.c. at weeks 0 and 10 with unheated or heated ESAT-Q11 or $ESAT6_{51-70}$ in PBS.

Conclusions: A thermally stable self-assembled peptide vaccine system was designed to address the current challenges to vaccine distribution. Our findings demonstrate that self-assembled peptide antigens were thermally stable, raising durable antibody responses for OVA and a TB (ESAT6) antigen in mice even after sustained exposure to high temperatures. Moving forward, we plan to compare the thermal stability of these assemblies to that of standard vaccination systems (e.g. an ESAT-conjugate vaccine with adjuvant), by measuring antibody titers and protective efficacy against TB infection in mouse models.

References: [1] Rudra JS, et al, PNAS 2010, 107(2):622-627. [2] Rudra JS, et al, Biomaterials 2012, 33(27):6476-84.