Self-assembled Nanomaterials Elicit T Cell-dependent Immune Responses

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Statement of Purpose: Nano-biomaterials based on self-assembling peptides are being developed as synthetic matrices for applications in regenerative medicine [1]. The factors that render these highly multivalent materials therapeutic, or immunogenic will determine their clinical usefulness. In a recent report, we demonstrated that the non-immunogenic self-assembling peptide Q11, when conjuagted to a peptide containing CD4 and B cell determinants ($OVA_{323-339}$), acted as a powerful adjuvant and elicited robust anti-OVA antibody responses [2]. These findings are broadly important for biomaterials design and demonstrate a novel method for adjuvanting short peptide epitopes in a chemically defined manner. In the present work, to understand the mechanism and longevity of the immune response against self-assembled peptide antigens, we used $OVA_{323-339}$-Q11 (OVA-Q11) and a disease relevant peptide from the circumsporozoite protein (CS) of malaria conjugated to Q11 (CS-Q11) to investigate long term antibody prodution, the role of T cells, and local tissue responses. Understanding the mechanism of action of peptide adjuvants and their applicability to disease-relevant epitopes is important for their development as immunotherapies.

Methods: Biotinylated Q11 (Biotin-QQKFQFQFEQQ), OVA-Q11 and the malaria epitope CS-Q11, were synthesized, purified by HPLC, and tested for endotoxin. Wild type B6 mice or B6 lacking T cell receptors mice were immunized subcutaneously with 100 nmol of OVA-Q11 or CS-Q11 and antibody responses were evaluated by ELISA. For adoptive transfer studies, 5×10^5 carboxyfluorescien succinimidyl ester (CFSE) labeled OTII cells (T cells expressing receptors specifically for OVA) were transferred into mice prior to immunization with OVA-Q11. Proliferation of the transferred cells was quantified using flow cytometry after 5 days. Peptide distribution in vivo was analyzed by immunizing mice intramuscularly with 10 % Biotin-Q11 mixed with OVA-Q11. At different time points tissue and draining lymph nodes were extracted, processed, and stained for biotin.

Results: TEM data showed that OVA-Q11 formed nanofibers in salt containing buffers (Fig. 1a) and mice immunized with OVA-Q11 elicited detectable antibody responses for 36 weeks after a single boost at week 4 (Fig. 1b). A spike in the antibody levels was detetcted after the boost and antibody levels reached their maximum at 6 weeks. Mice immunized with CS-Q11 also exhibited similar long-lived antibody responses (data not shown).

OVA- and CS-Q11 when injected either separately or as co-assembled fibrils raised equivalent levels of antibodies against both epitopes without altering immune responses to each other (data not shown). Adoptively transferred OTII cells proliferated robustly in OVA-Q11 immunized mice (Fig. 1d) and the proliferation was comparable to mice immunized with free $OVA_{323-339}$ in complete Freund's adjuvant (data not shown). No proliferation was observed in Q11 immunized mice (Fig. 1c). No antibodies were observed in T cell receptor knock out mice immunized with OVA- or CS-Q11 (data not shown). Together this data demonstrated that T cells are required for antibody production against self-assembled antigens. Furthermore, the antibodies raised against OVA-Q11 (Fig. 1e) and CS-Q11 (data not shown) were capable of binding whole ovalbumin and CS proteins respectively. OVA-Q11 co-assembled with biotin-Q11 was observed in the draining lymph nodes of immunized mice as early as 1 week and persisted for 6 weeks as confirmed with immunofluoresence staining (Fig. 1f).

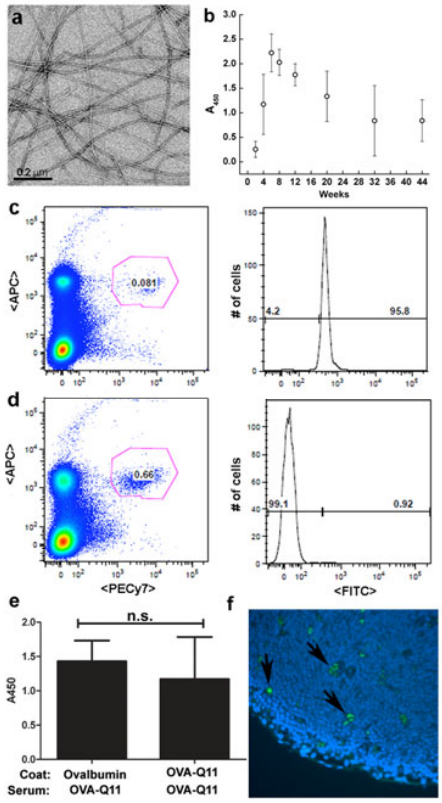

Figure 1. (a) Self-assembled fibrils of OVA-Q11 and (b) antibody responses in mice immunized with OVA-Q11 (○ represents mean of 5 mice). Proliferation of OTII cells in mice immunized with (c) Q11 and (d) OVA-Q11. (e) Antibodies against OVA-Q11 react with whole ovalbumin and (f) biotin-Q11 (green) in the lymph nodes at wk 6.

Conclusions: Self-assembled antigenic peptides elicited long-lived and T cell-dependent antibody responses. The antibodies were reactive against whole proteins and the peptide assemblies were localized to the lymph nodes. The results demonstrate that peptide antigen assemblies can generate robust T-dependent antibody responses without frequent boosting or additional adjuvants.

References: 1. Collier JH. et al. Chem Soc Rev 2010; 39:3413-3424. 2. Rudra J. et al. PNAS 2010;107:622-627.