Tuning Peptide Immunogenicity through Controlled Oligomerization into Nanofibers

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Statement of Purpose: Biomaterials constructed by the supramolecular self-assembly of peptides and proteins are increasingly investigated as scaffolds for regenerative medicine. However, the structural determinants of their immunogenicity have been largely unexplored, even though potential immune responses to these highly multivalent materials will ultimately determine their clinical usefulness.[1] In the present study, we investigated the immunogenicity of self-assembled peptide biomaterials in mice and discovered a surprisingly strong adjuvant activity in specific contexts. We report a fibril-forming peptide that is non-immunogenic by itself or when decorated with cell-binding RGD ligands,[2] but that induces IgG and IgM responses of equivalent strength to complete Freund's adjuvant (CFA), one of the strongest known adjuvants, when coupled to known B and T cell epitope peptides. We utilized the self-assembling peptide sequence Q11, which has previously been investigated in our laboratory for producing fibrillized gels for 3D cell culture matrices, and which can be utilized to oligomerize a wide range of functional amino acid sequences into nanofibers.[1,2] Our results indicate that self-assembling peptide biomaterials can be engineered either for non-immunogenicity or for extreme immunogenicity, both of which are useful for applications in regenerative medicine and immunotherapies, respectively.

Methods: Peptides Q11, OVA (chicken ovalbumin 323-339), a fusion of the two peptides (O-Q11), and an N-terminally biotinylated O-Q11 were synthesized on a CS Bio 136 peptide synthesizer (sequences in Figure 1a), purified to >92% by HPLC, and tested for endotoxin. Fibril morphology and epitope display was visualized with transmission electron microscopy (TEM) using biotinlated O-Q11 and streptavidin-conjugated gold nanoparticles. Epitope display was quantified with ELISA and OVA antisera from mice. C57BL/6 mice were immunized with 100 nmol of peptide with or without CFA and boosted 28 days later with 50 nmol of peptide. Sera and spleens were harvested one week following the boost. Splenocyte cultures were challenged with immunizing peptide and analyzed for the production of interferon-γ (IFN-γ), IL-2, and IL-4. Serum IgG1, IgG2a, IgG2b, IgG3, and IgM were measured by ELISA.

Results: TEM showed that O-Q11 formed nanofibers in salt containing buffers that displayed the OVA epitope on their surfaces (Fig 1b). Q11 did not raise any detectable IgG in mice (Fig 1c), even when delivered in CFA, indicating that the basic Q11 sequence was highly non-immunogenic. However, O-Q11 injected in PBS raised IgG titers as high as OVA injected in CFA, indicating that for this epitope, the Q11 sequence acted as a very strong adjuvant. Similar levels of anti-OVA antibody titers were observed when OVA/CFA and O-Q11 antisera were applied to OVA-coated plates, indicating that the OVA

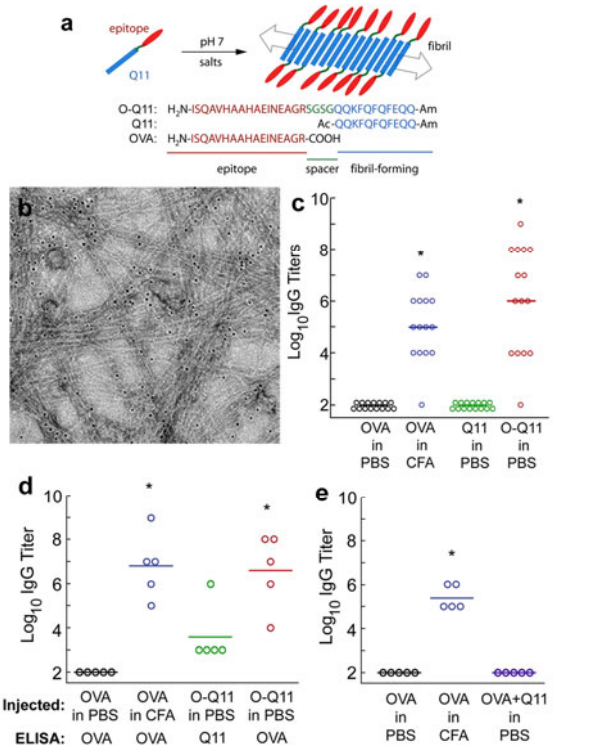

Figure 1. Schematic and sequences of self-assembled epitope-displaying fibrils (a). TEM with gold labeling of biotin-O-Q11 (b). IgG titers of peptide-immunized mice (c). Antibodies raised against O-Q11 were OVA-specific (d). Mixtures of OVA and Q11 did not raise IgG (e). *p<0.05, ANOVA with Tukey post-hoc testing.

epitope was conserved and that the high antibody titers elicited by O-Q11 were not a measurement artifact (Fig 1d). Interestingly, no antibody responses were observed for mixtures of OVA and Q11, indicating that conjugation was critical to the adjuvant activity of Q11 (Fig 1e). Peptide-specific IgG1, IgG2a, IgG2b, IgG3 and IgM were produced in O-Q11-injected mice in levels similar to OVA/CFA, but no detectable IFN-γ, IL-2, or IL-4 production was observed in peptide-challenged splenocyte cultures derived from the O-Q11 group, suggesting a possible T-independent mechanism.[3]

Conclusions: Self-assembled epitope pepitdes elicited robust and T-independent antibody titers which were comparable to those elicited by the free peptide in CFA. Antibodies raised against O-Q11 fibrils were OVA-specific and dependent on the conjugation of Q11 to the OVA epitope. These results indicate that fibrillizing pepitdes may be useful as chemically defined adjuvants, and they indicate that strong epitopes should be avoided in highly multivalent biomaterials.

References: 1. Collier JH. Soft Matter 2008;4:2310-2315.
2. Jung JP. et al. Biomaterials 2009;30:2400-2410.
3. Mond JJ. et al. Annu Rev Immunol 1995;13:655-692.