Self-assembling adjuvants that raise strong antibody responses against protein antigens

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Statement of purpose: Conventional adjuvants such as alum or oil emulsions are poorly defined in terms of their physicochemical properties, which makes it challenging to study their mechanisms of action and engineer better adjuvants. As a novel chemically defined adjuvant system, we have recently found that decorating the self-assembling peptide QQKFQFQFEQQ (Q11) with peptide antigens provides robust self-adjuvanting vaccines that elicit long-term antibody production in a T cell-dependent manner.[1] Due to natural variability in HLA haplotyes, however, designing short peptide antigens with equal immunogenicity across a broad population is challenging, which may ultimately limit the applicability of this strategy. Here we demonstrate that self-assembled fibrils can also be utilized to elicit strong antibody responses to protein antigens. A model protein antigen was conjugated to self-assembled Q11 fibrils with orientational precision using a chemoselective, site-directed conjugation strategy (Fig. 1A).[2] Specifically, a phosphonate-presenting Q11 derivative was synthesized, assembled into fibrils, and used as a capture ligand to covalently bind fusion proteins containing the enzyme cutinase (Fig. 1A). This approach provided control over antigen dose, which was achieved by varying the ratio of phosphonate to cutinase present during the reaction. The chemical definition of the self-assembling adjuvant, the ability to adjust antigen dose, and the strong antibody responses raised indicate that this strategy may be an attractive platform for the development of vaccines against a wide variety of pathogens.

Methods: Peptides were synthesized using standard solid phase peptide synthesis protocols. His-tagged cutinase-GFP was expressed in E. coli. Endotoxin was removed from the recombinant fusion protein using Triton X-114 cloud-point precipitation, and all endotoxin levels were below 0.15 EU by LAL chromogenic endpoint assay. Green fluorescent protein (GFP) was used as an easily characterized model antigen. Cutinase-GFP (C-GFP) was conjugated to phosphonate-Q11 (phos-Q11) by mixing 1 mM fibrillized peptide in 1x PBS (pH 7.4) with C-GFP and allowing the reaction to proceed overnight at room temperature. Unreacted protein was removed by serial centrifugation and washing. C-GFP conjugation was measured by GFP fluorescence and by the release of P-nitrophenol, which occurs upon cutinase conjugation. C57BL/6 mice were immunized subcutaneously with 9.3 μg C-GFP conjugated to 1 mM Q11:phos-Q11 (99:1 molar ratio) (C-GFP-Q11). Positive and negative controls were 9.3 μg C-GFP in complete Freund's adjuvant (CFA), and PBS, respectively. Boosting with half the primary dose was performed after 4 weeks (+ control boosted in incomplete Freund's adjuvant). Total serum IgG was measured by ELISA.

Results: Absorbance measurements at 403 nm indicated that p-nitrophenol generated by the conjugation reaction increased as a function of time (Fig. 1B), and the final concentration of conjugated C-GFP was controlled by initial reactant ratios (Fig. 1C). By week 2, significant levels of antigen-reactive IgG were detected in sera of mice immunized with C-GFP-Q11 and C-GFP in CFA when compared to mice immunized with C-GFP in PBS (Fig. 2). By week 4, total IgG decreased in mice immunized with C-GFP-Q11 and C-GFP in CFA, but antibody responses could be restored in both groups with a booster immunization. In contrast, no booster response was observed in mice immunized with GFP-cutinase in PBS. The IgG present in serum from mice immunized with C-GFP-Q11 (predominantly IgG1 but containing detectable IgG2a, IgG2b, IgG3 and IgM as well) was similarly reactive towards ELISA plates coated with C-GFP-Q11 or C-GFP, suggesting that the antibodies are primarily raised against the protein antigen, not the peptide adjuvant.

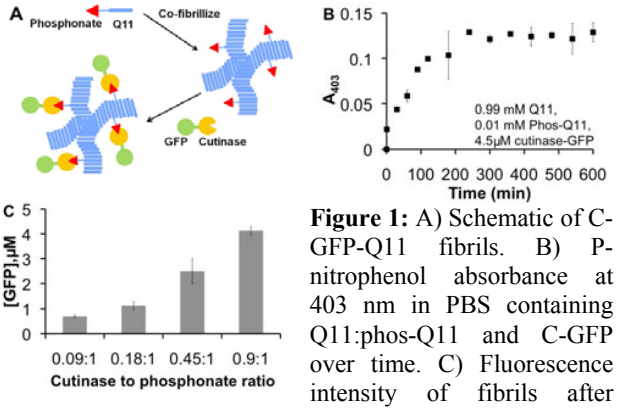

Figure 1: A) Schematic of C-GFP-Q11 fibrils. B) P-nitrophenol absorbance at 403 nm in PBS containing Q11:phos-Q11 and C-GFP over time. C) Fluorescence intensity of fibrils after reacting different amounts of C-GFP with 1 mM Q11:phos-Q11 (99:1 molar ratio).

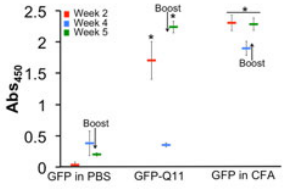

Figure 2: Total IgG produced in mice immunized with C-GFP-Q11, C-GFP in CFA, or C-GFP in PBS at various time points. (mean ± sd dev, * $p<0.05$ by ANOVA compared to neg ctrl, Tukey post-hoc).

Conclusions: Using a self-assembled peptide adjuvant, strong antibody responses were raised against a model protein antigen. Antigen dose could be controlled during the material's formulation, and immunized mice raised antibodies reactive towards the C-GFP fusion protein, suggesting future work in which this platform could be applied towards a variety of pathogen-derived proteins in order to provide robust, self-adjuvanting vaccines.

References: 1) Rudra, J. S. et al, PNAS 2009 107:622-627. 2) Hodneland, C. D. et al, PNAS 2002 99:5048-5052.