

Selective Dendritic Cell Activation by Non-inflammatory Peptide Assemblies

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Statement of Purpose: To initiate an adaptive immune response, vaccines must activate three functions of antigen presenting cells (APCs): (1) uptake of the antigen, (2) surface expression of co-stimulatory molecules, and (3) presentation to antigen-specific T cells. Traditional vaccines accomplish this by using inflammatory adjuvants such as Alum (aluminum hydroxide microparticles), which non-specifically activate nearby APCs but can cause pain, fever, and allergy. We recently showed that self-assembled peptides raise antibody and T cell responses to peptide antigens without the local inflammation associated with alum¹. To gain insight into how immune responses develop against non-inflammatory biomaterials, here we tested the extent to which APCs were activated by self-assembled peptides.

Methods: The peptide antigen OVA₃₂₃₋₃₃₉ was synthesized in tandem with the self-assembling beta-sheet domain, Q11 (QQKFQFQFEQQ-Am), by standard Fmoc-based solid phase peptide synthesis (OVAQ11). Peptides were purified to > 90% by HPLC and their identities confirmed by MALDI. OVAQ11 was labeled at the N-terminus with AlexaFluor-647-SE (Invitrogen). Peptides were dissolved and allowed to self-assemble in phosphate-buffered saline. The endotoxin levels of these solutions were 1 EU/mL as measured by a Limulus amoebocyte lysate assay. C57Bl/6 mice were immunized with $100 \mu\text{L}$ of 2 mM peptide. For APC activation experiments, lavage fluid was collected 20 h after intraperitoneal (i.p.) injection, spun down, and the cells were analyzed by flow cytometry. Cells were identified as dendritic cells (CD11c⁺ MHC⁺ F480⁻), monocytes (CD11c⁻ CD11b^{med} F480^{med}), or macrophages (CD11c⁻ CD11b⁺ F480⁺). For T cell proliferation experiments, mice were immunized subcutaneously (s.c.) at the shoulders before receiving an intravenous infusion of CFSE-labeled OVA-specific T cells (OTII). Draining lymph nodes were collected 5 days later, and OTII proliferation was analyzed by flow cytometry.

Results: To test antigen uptake and activation of surface markers, mice were immunized i.p. with fluorescently-labeled OVAQ11, phosphate buffered saline (PBS), or OVAQ11 with Imject Alum (Pierce). After 20 h, cells collected from the lavage fluid were analyzed by flow cytometry. In OVAQ11-immunized mice, a significant fraction of the dendritic cells (DCs, 29%), monocytes (72%), and macrophages (Mac, 11%) had acquired the antigen (Fig. 1a). Of the two primary types of APCs, only the DCs – not the macrophages – had upregulated the co-stimulatory molecules CD80 and CD86 (also called B7.1 and B7.2, respectively) (Fig. 1b). Furthermore, these markers were selectively upregulated on only the DCs that had acquired OVAQ11, not on the DCs in the same samples that had not acquired the peptide. In contrast, Alum-immunization elicited broad activation: the average population of DCs expressed high levels of CD86, and

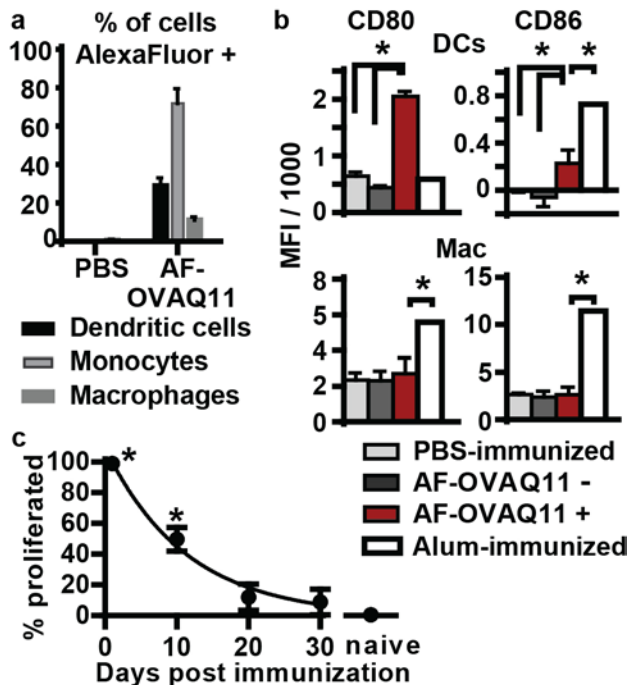


Figure 1. APC activation by self-assembled peptides. Analysis of (a) uptake of labeled peptide and (b) mean fluorescent intensity (MFI) of activation markers on antigen presenting cells 20 h after i.p. immunization. $N = 3$ mice per group. * $p < 0.01$ by 2-way ANOVA, Tukey's multiple comparisons. (c) Proliferation of OTII cells that were transferred 1 – 30 days after s.c. immunization with OVAQ11. Curve fitted with 1-phase exponential decay. $N = 4$ mice per group. * $p < 0.0001$ compared to naïve mice by 1-way ANOVA, Dunnett's multiple comparisons.

macrophages expressed both markers at high levels. We also verified that cells from the lavage fluid of OVAQ11-immunized mice could drive OTII proliferation after transfer into naïve mice (not shown), indicating that these cells are indeed functionally activated. Finally, we tested the timescale of activation of antigen presentation. OVA-specific T cells proliferated when transferred 1 or 10 days after immunization with OVAQ11, but not 20 or 30 days after (Fig. 1c). The half-life of antigen presentation was 6 – 10 days (95% confidence interval), similar to or slightly longer than with Alum-adjuvanted OVA protein².

Conclusions: Non-inflammatory self-assembled peptides provided antigen uptake by APCs and a uniquely selective expression of co-stimulatory molecules on only the DCs that acquired the fibers. This activation was sufficient to drive proliferation of antigen-specific T cells for 10 days after a single immunization. This work highlights that activation pathways distinct from those of alum can be exploited to develop potent biomaterials vaccines.

[1] Chen, Pompano. Biomaterials 2013; 34:8776-85.

[2] Hutchison. FASEB J 2012; 26:1272-79.