Injectable Hydrogels as 'Vaccination Nodes' for Anti-Tumor Immunotherapy

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Statement of Purpose: In one promising strategy for cancer immunotherapy, oncoantigen-loaded dendritic cells (DCs) are injected into patients, to allow DCs to migrate to the lymph nodes (LNs) and activate anti-tumor T cells in these organs (Steinman RM and Banchereau J. *Nature* 2007 449:419-26). However, one of the limitations of current DC vaccines is the inability for DCs in lymphoid organs to directly influence the effector phase of the immune response at distant tumor sites. To address this concern, we formulated self-gelling injectable alginate hydrogels carrying antigen-loaded dendritic cells, 'vaccination nodes' delivering exogenous dendritic cells and supporting cytokines or other factors directly to solid tumor sites. We hypothesized that activated T-cell recruitment to these gels could be exploited to focus and modulate the effector phase of the immune response at a chosen peripheral site, such as tumors or sites of infection, during the lifetime of the gel *in vivo*.

Methods: Alginate microspheres were synthesized via a water-in-oil emulsion of alginate solution (Pronova SLG20 low-endotoxin alginate, 0.01mg/mL in PBS, Novamatrix, Norway) in the presence of surfactants (Tween®80 & Span®80, Sigma-Aldrich, St.Louis, MO) crosslinked by 5% $CaCl_2$ solution. Injectable hydrogels were formulated by mixing alginate microspheres loaded with calcium ions and alginate solution (Pronova SLM20, 0.01mg/mL in PBS). For injection *in vivo*, $2x10^6$ bone marrow-derived dendritic cells were mixed with 150µL of the alginate/alginate microspheres solution for injection. The alginate carrying dendritic cells and/or IL-15/IL-15Rα complexes (R&D systems, Minneapolis, MN) were mixed with microspheres immediately prior to use and injected s.c. into the back flanks of anesthetized C57BL/6 mice. At varying time points following inoculation, injected gels were explanted and digested with 0.28WU/mL Blendzyme 3 (Roche Applied Sciences, Switzerland) and 1mg/mL alginate lyase (Sigma-Aldrich), for analysis by flow cytometry. For tumor studies, 2-$5x10^5$ B16-ova cells were injected s.c. in the back flanks of mice. Tumors were allowed to grow for 5-14 days before peritumoral immunization with alginate gels carrying DCs and/or IL-15. Tumor sizes were calculated as *axb* (*a*=long diameter [mm], *b*=short diameter [mm]).

Results: We recently described a strategy for generating 'self-gelling' alginate gels, based on using alginate microspheres as calcium reservoirs, mixed with soluble alginate solutions (Hori et al. *Biomaterials* 2008: 29(27): 3671-82). When calcium-reservoir microspheres were mixed with alginate/cell solutions, these solutions formed stable gels *in vivo* within ~60 min following injection. Strikingly, activated DCs injected in alginate elicited robust recruitment of host T-cells and dendritic cells to these gels, while empty gels or gels carrying resting DCs elicited much lower T-cell infiltrates (Figure 1A). In addition, a few DCs injected with the alginate trafficked to the regional lymph nodes (data not shown). Gels injected with SIYRYYGL peptide-loaded DCs elicited substantial accumulation of SIYRYYGL-specific T-cells to the gels, suggesting that DCs promote accumulation of activated, antigen-specific T-cells at the gel site. We tested the ability of these 'vaccination nodes' to promote anti-tumor immune responses: Small established s.c. tumors of ova-expressing B16 melanoma cells were treated 5 days after injection with alginate gels carrying activated, ova-loaded DCs and DC injection alone on the opposite flank. A majority of mice (3/4) suppressed tumor growth when treated with both a DC injection and gels carrying DCs, whereas mice receiving DC vaccination alone showed no response. We next tested the combination of the T-cell-supporting cytokine IL-15 with DC delivery in alginate gels to the site of large established B16 tumors. Superagonist complexes of IL-15 bound to its soluble IL-15Rα chain (IL-15 SA) were recently shown to promote maintenance of T-cell functions in the face of tumor-derived anergy-inducing signals (Teague et al. *Nat Med.* 2006:12(3):335-41). When mice bearing 14-day established s.c. ova-expressing B16 tumors were immunized peritumorally with ova-pulsed DCs and 30 µg IL-15 SA in self-gelling alginate, mice which exhibited signs of a weak endogenous immune response to the tumor (tumor sizes 20-50 mm^2 on the day of treatment) significantly suppressed their growth while untreated tumors of similar size grew rampantly (Figure 1B).

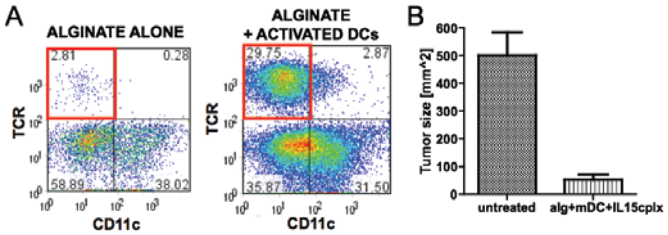

Figure 1: (A) Flow cytometry analysis of T cell (TCR^+) and DC ($CD11c^+$) infiltration of alginate gels 7 days post injection. (B) Average B16-ova tumor sizes at 28 days for untreated mice and mice receiving DC/cytokine-loaded gels on day 14.

Conclusions: Injection of self-gelling alginate carrying activated DCs induced robust T-cell trafficking to the injection site, while initiating antigen-specific immune response in the regional lymph nodes. Mice bearing established tumors treated with gels carrying both DCs and IL-15 SA were able to control tumor growth much better than control mice receiving no treatment or treatment with gels containing DCs alone. We are currently exploring strategies to further improve this treatment via combination therapies with radiation- and chemo-therapy.