Characterization of Immune Cells Recruited To Injectable Matrices Containing Lymphoid Stromal Cells *In Vivo*

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Statement of Purpose: Ectopic induction of lymphoid tissue-like structures can be triggered by the expression of chemokines,[1] inflammatory cytokines,[2] or the presence of immune cell types (mature dendritic cells)[3] in peripheral tissues. Formation of *de novo* lymphoid tissue near established tumor sites can lead to tumor eradication[4]. Our aim is to use injectable biomaterials carrying immune cells or soluble chemokines/cytokines to induce lymphoid tissue formation *in vivo*, as a strategy for cancer immunotherapy. As a first step, the performance of candidate materials that can be gelled *in situ* has been evaluated. Current candidates for such materials are collagen, Matrigel, sodium alginate, and a mixture of Matrigel with alginate. These biomaterials alone, mixed with a lymph node stromal cell line known as BLS4, or mixed with lymphoid tissue chemokines were injected subcutaneously in mice, and we analyzed the recruitment of immune cells into these matrices over time. This work is a first step toward in vivo engineering of lymphoid tissue.

Methods: Collagen (Inamed, Fremont, CA) was used at 1.8mg/mL, and Matrigel (BD Biosciences, San Diego, CA) was used at 60% volume concentration in PBS. Sterile alginate Pronova SLM20 (Novamatrix, Drammen, Norway) was used at 1% w/v in PBS. A 50/50 by volume mixture of Matrigel with 1% alginate solution was also prepared. Hydrogels without any cells or chemokine was injected on the left dorsal side of BALB/c mice as a control and gel mixed with BLS4 stromal cells (courtesy of T. Katakai, Kyoto Univ., Japan) or chemokine (CCL21 or CXCL13, R&D Systems) was injected on the right side. 150µ-200µL of the precursor solution was injected for each sample, and the alginate was gelled with 30µL of 1% sterile $CaCl_2$ solution as a two-stage injection. The gels were recovered 1-7 days after injection and digested with Blendzyme 3 (Roche Applied Sciences, Penzberg, Germany) and 0.02% EDTA (Sigma, Missouri, USA). The cells were then stained for TCRβ, CD45R/B220, and CD11c for flow cytometry analyses. In order to further break down the cell infiltrate types, some samples were also stained for macrophage and granulocyte cell markers, F4/80 and Ly-6G/Gr-1, respectively.

Results/Discussion: We compared the composition of immune cells infiltrating several biocompatible biomaterials injected subcutaneously in mice after 1 or 4 days, in the presence or absence of a stromal cell line from lymph nodes (BLS4), which is known to produce a number of lymphoid tissue-associated chemokines and cytokines. The frequency of B220+ (B cells) and TCRβ+ (T cell) cells infiltrating all gels was very low in the presence or absence of BLS4 stromal cells. However, the frequency of $CD11c^+$ cells (which includes a key antigen presenting cell present in lymph nodes, dendritic cells), was dependent on the composition of the injected matrix and increased for all samples significantly by day 4 (Fig.1). Notably, the addition of BLS4 stromal cells to alginate gels increased the percentage of $CD11c^+$ cells present substantially.

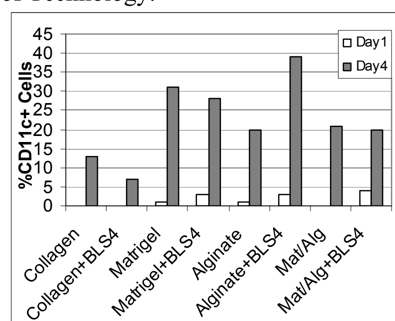

Fig. 1. %CD11c+ cells recruited for each matrix on day 1 and day 4.

Further analysis of the CD11c+ cell population by costaining to detect the cell surface marker F4/80 demonstrated the presence of both macrophages ($F4/80^+CD11c^-$ cells) and dendritic cells in the gels by day 4 (Fig.2). The presence of BLS4 cells in alginate greatly increased the fraction of $CD11c^+F4/80^{lo}$ and $CD11c^+F4/80^{high}$ cells recruited to the gel.

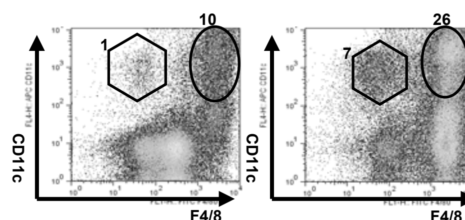

Fig. 2. FACS plots of CD11c (y) vs. F4/80 (x) for control alginate (left) and alginate+BLS4 (right) samples.

We also assessed the effect of injecting matrices containing 1 µg of either CCL21 or CXCL13, two chemokines produced constituively in lymph nodes. These molecules may be rapidly released and cleared from the matrices, as they had no effect on T or B cell recruitment into gels after 4 days.

Conclusions: The four gel types tested demonstrated different performances *in vivo*, but in all cases a $CD11c^+$ population along with macrophages and granulocytes dominated the infiltrate composition. In ongoing work, we are examining the effect of sustained chemokine/cytokine delivery from injectable matrices to drive the accumulation of immune cells associated with lymphoid tissue formation.

References:

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