Biomimetic Thymic Niche for Generating Functional, Antigen-Specific T cells from Stem Cells: Controlling Notch and T cell receptor Signaling in 3D Scaffolds

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Statement of Purpose: Current T cell adoptive transfer technology is limited due to difficulties in autologous or donor cell isolation and expansion, as well as morbidity and limited availability of donor cells[i]. Multipotency of stem cells makes them an attractive, alternative source of cells from which functional T cells can be generated by in vitro differentiation. It is well established in the T cell development literature that two key signals, presented in the thymic niche, in a highly controlled and sequential manner, play crucial roles in generating functional T cells: (a) Delta-like ligands(DLL, Notch ligands)-Notch receptor signaling[ii] and (b) Major Histocompatibility Complex (MHC)-T cell receptor (TcR) signaling[iii]. DLL-transduced stromal cells, and also microbeads with surface-immobilized Notch ligands have been able to efficiently direct hematopoietic stem cells into the early T cell lineage[iv,v]. However, most efforts to date, focuses on mimicking these signals in 2D environments. We have developed a 3D microenvironment that can efficiently present these ligands. We hypothesize that this biomaterial-based, biomimetic thymic niche can provide a platform to present varying ligand densities to induce Notch and MHC signaling of varying strength, in a stromal cell-free system. We also show that in addition to Notch-ligand induced early T cell generation, antigen-loaded MHC-tetramer mediated TcR signaling can further direct stem cells into functional, antigen-specific T cells.

Methods: Antigen-specific T cells were first differentiated from embryonic stem cells in a 2D system, where both Notch and MHC signaling was presented. R1 mouse ES cells were cultured on OP9 bone marrow cells transfected to express Delta-like-ligand 1 (OP9-DL1). After 9 days of co-culture, non-adherent single cells were isolated and c-kit+ sca-1+ hematopoietic progenitors were further cultured with LCMV GP34 tetramer added on day 16. Cells were harvested at day 26, for flow cytometry analysis of antigen-specific CD8+ T cells. In order to translate this signaling system into a 3D, stromal cell-free system, PEG-based scaffolds with defined pores was fabricated by using the inverse opal, or inverted-colloidal crystal method. 40% (w/v) PEG diacrylate polymer solution was added with photoinititor onto an ordered template of PMMA microspheres. Either, biotin-PEG-acrylate or protein A-PEG-acrylate was added to the polymer solution. This would allow ligand binding to the scaffold via biotin-streptavidin and protein A-Fc interactions. The polymer solution was then UV-polymerized, and PMMA microspheres were dissolved in acetic acid. The biotin-PEG acrylate and protein A-PEG acrylate were prepared by mixing 1:1 moles biocytin, or protein A, respectively, with acrylate-PEG-succinimidyl valerate. Scaffolds were stained with either fluorescently tagged streptavidin or fluorescently tagged secondary antibody to test if biotin and protein A are still functional.

Results: R1 ES cells cultured under above conditions to generate antigen-specific T cells were analyzed by flow cytometry at day 26. Figure 1 shows flow cytometry plots of CD4 and CD8 expression, as well as percentage of CD8+GP34tet+ cells in the presence (+) and absence (-) of GP34 peptide-loaded tetramers (GP34tet) or OP9-DL1 cells. Only conditions that employed MHC/TCR signaling generated high levels (23%; 41%) of GP34-specific CD8+ T cells. For 3D scaffolds, figure 2 shows fluorescent microscope images of (a),(c) plain PEG, (b) biotinylated, and (d) protein A-immobilized scaffolds. (a) and (b) were stained with streptavidin-PE and (c) and (d) were stained with IgG-FITC. The results confirm that both biotin and protein A remain functional and able to bind to streptavidin and Fc, respectively.

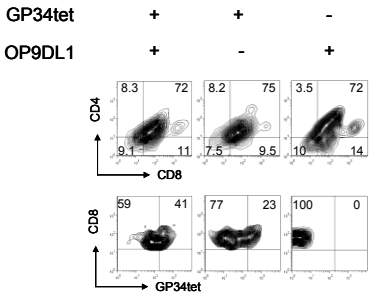

Figure 1. Flow cytometry plots of differentiated R1 ES cells at day 26.

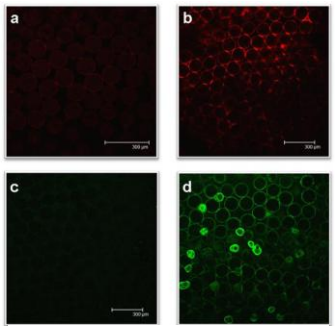

Figure 2. Fluorescence microscopy images of functionalized, inverse opal scaffolds.

Conclusions: In this study, we show that GP34-MHC-tetramer-mediated TCR signaling, together with Notch signaling, can direct differentiation of ES cells into antigen-specific CD8+ T cells. We have also shown that a hydrogel scaffold with interconnected pores can be fabricated by using the inverse opal method. We were able to immobilize biomolecules onto the scaffold, providing binding spots for ligands such as biotinylated, or Fc-conjugated Delta ligands and biotinylated MHC molecules. Studies are in progress to evaluate the effect of 3D scaffolds on T cell differentiation and elucidate the effect of ligand density on Notch and TcR signaling.

References:
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