Biomimetic Thymic Niche for Generating Functional, Antigen-Specific T cells from Stem Cells: Quantitative Control of Notch and T cell receptor Signaling through Biomaterials

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Statement of Purpose: Multipotency of stem cells makes them an attractive, alternative source of cells from which functional T cells can be generated by in vitro differentiation. Two key signals presented in the thymic niche play crucial roles in generating functional T cells: (a) Delta-like ligands(DLL, Notch ligands)-Notch receptor signaling and (b) Major Histocompatibility Complex (MHC)-T cell receptor (TcR) signaling. However, most efforts to date, focuses on mimicking these signals in 2D environments[i,ii]. We have developed a 3D microenvironment that can efficiently present these ligands in a controlled manner, at varying ligand densities in a stromal cell-free system. We have shown that antigen-loaded MHC tetramers can induce TCR signaling to further direct stem cells into antigen-specific T cells[iii]. We hypothesize that the generated 3D microenvironment can present antigen-loaded MHC in a 3D manner as well.

Methods: Antigen-specific T cells were first differentiated from embryonic stem cells in a 2D system. R1 mouse ESCs were cultured on OP9 cells transfected to express Delta-like-ligand 1 (OP9-DL1). After 9 days of co-culture, cells were further cultured with LCMV GP34 tetramer added on day 16. Cells were harvested at day 26, for flow cytometry analysis. Hydrogel scaffolds with defined pores were fabricated by using the inverse opal method. PEG diacrylate polymer solution with either biotin-PEG-acrylate or protein A-PEG acrylate was added with photoinitiator onto an ordered template of PMMA microspheres. After UV-polymerization the PMMA microspheres were dissolved in acetic acid to leave behind ordered pores. Scaffolds were stained and imaged to test if biotin and protein A are still functional. In order to quantify the density of ligands that can be immobilized onto scaffolds, biotinylated scaffolds were incubated in different concentrations of fluorescently tagged streptavidin. Similarly, protein A scaffolds were incubated with IgG. Unbound ligands were washed out and quantified by using a fluorescence plate reader. The scaffold ligand density was compared with density of ligands coated onto 2D tissue culture polystyrene (2D data not shown). DLL4, a known Notch ligand was biotinylated and immobilized onto biotinylated scaffolds via streptavidin linker. Fc-DLL1, another Notch ligand, was immobilized onto protein A scaffolds. C2C12 mouse myoblasts were seeded onto the scaffolds and expression of Hes-1, a known downstream Notch gene, was determined by quantitative RT-PCR. and compared with that in cells seeded onto DLL-immobilized TCPS (2D data not shown).

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 plots of CD4 and CD8 expression, as well as percentage of CD8+GP34tet+ cells in the presence (+) and absence (-) of GP34 peptide-loaded tetramers or OP9-DL1 cells. Only conditions that employed MHC/TCR signaling generated high levels (23%; 41%) of GP34-specific CD8+ T cells. Figure 2 shows fluorescent microscopy 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. Figure 3 (a)-(b) shows the density of (a) streptavidin or (b) IgG on biotinylated, or protein A scaffolds, respectively. The density of ligand bound to the scaffold could be varied by varying the initial incubation concentrations. Figure 3 (c)-(d) shows Hes-1 gene expression in (c) biotin-DLL4 immobilized scaffolds and (d) Fc-DLL1-immobilized scaffolds.

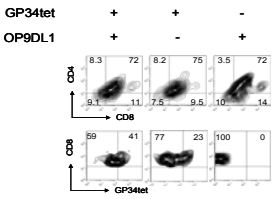

Figure 1. Flow cytometry plots of differentiated R1 ES cells at day 26.

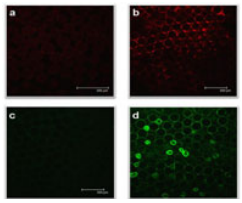

Figure 2. Fluorescence microscopy images of functionalized scaffolds.

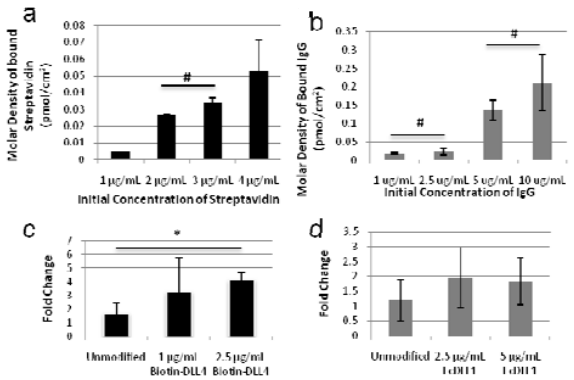

Figure 3. (a)-(b) Molar density of bound ligands (all groups other than # $p<0.05$) (c)-(d) Hes-1 gene expression. (* $p<0.05$)

Conclusions: In this study, we show that MHC-tetramer-mediated TCR signaling, together with Notch signaling, can direct differentiation of ES cells into antigen-specific CD8+ T cells. We have fabricated a hydrogel scaffold and immobilized biomolecules onto the scaffold that allows immobilization of Notch or MHC ligands. We have also shown that density of ligands can be varied on these scaffolds, and that Notch signaling can be induced in cells seeded onto the ligand-immobilized scaffolds.

[i] Schmitt TM, et al. Immunity. 2002:17:749-56.
[ii] Taqvi S, et al. J Biomed Mat Res A. 2006:79:689-97.
[iii] Lin J, et al. Tissue Eng Part A. 2010:16(9):2709-20