Directing Naïve T cell Migration Via Patterned Nanoparticles Carrying Immobilized Chemokine

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Statement of Purpose: Chemokine-mediated T cell migration within lymphoid tissues plays an important role in lymph node organization and immune responses[1]. Chemokines, including the key lymphoid tissue chemokine CCL21, can bind to extracellular matrix (ECM) or cell surfaces via glycosaminoglycans such as heparan sulfate; it has thus been postulated that chemokines may act in the form of soluble gradients or bound to matrix/cells. To determine how lymphocytes respond to chemokine presented from the solid phase, we prepared cationic nanoparticles (NPs) that could be coated with heparan sulfate (thought to be the native substrate for chemokine binding to ECM *in vivo*) followed by CCL21, and patterned NPs into defined areas of 2D glass surfaces. We seeded naïve T cells either directly in contact with chemokine-bearing NPs or near 2D patterned regions of the particles, and assessed the migration of the cells over time.

Methods: Poly(methyl methacrylate) (PMMA) NPs (236 ± 12 nm diameter) with surface amine groups were synthesized by surfactant-free emulsion polymerization of methyl methacrylate and 2-aminoethyl methacrylate following previously published methods[2]. The NPs were then biotinylated with biotin-NHS (Pierce, Rockford IL) following the manufacturer's instructions. To coat the NPs with polysaccharide, nanoparticles were incubated with heparin sulfate (HS, Sigma) for 1 hr at room temperature. Coating with the polysaccharide was monitored by measuring the zeta potential as a function of coating concentration using a Malvern Zetasizer. To bind chemokine to the HS-coated NPs, the particle suspension was resuspended in 2ug/ml CCL21 (R&D Systems), for 2 hr at 20°C then washed 4X. Chemokine release from the particles was measured by ELISA in RPMI 1640 medium at 37°C. To deposit the chemokine-bearing NPs in defined 2D regions on glass coverslips, we patterned the nanoparticles using a biotinylated photoresist copolymer (poly(o-nitrobenyl methacrylate-*r*-methyl methacrylate-*r*-PEG methacrylate, PR) following a method we previously reported for protein patterning[3]. Briefly, 2D patches were defined on PR-coated glass coverslips by photolithography; UV-exposed regions of the PR film were selectively dissolvable. Streptavidin was linked to the surfaces from solution, followed by immobilization of the chemokine-bearing nanoparticles via their biotin groups. Nanoparticles binding to the surface outside the defined patches were removed by dipping surfaces in neutral pH PBS, which dissolved the UV-exposed regions of the PR film. Finally, the adhesion molecule vascular cell adhesion molecule-1 (VCAM-1, R&D Systems) was coated on the entire surface via adsorption. Naïve T cells were isolated from the spleen of C57Bl/6 mice using MACS® (Miltenyi) separation columns. T cells were incubated on surfaces at 37°C and tracked live by time-lapse videomicroscopy to determine the migration paths of individual cells in different regions of the surface.

Results/Discussion: The zeta potential of polysaccharide-coated nanoparticles was examined by varying the concentration of heparin sulfate from 0-1mg/ml during adsorption. The zeta potential of the nanoparticles decreased and reached a maximum negative zeta potential at a heparan sulfate concentration of 0.5mg/ml, indicating saturated coverage of the particles (data not shown). CCL21 release from the particles as measured by ELISA occurred slowly, with ~10% of the bound chemokine released after 10 hrs at 37°C (data not shown). We monitored the migration of T cells in contact with surfaces where defined 2D regions were coated with the CCL21-bearing NPs. Within NP-coated regions on the surface where the NPs were evenly distributed, T cells displayed random migration patterns in the presence of chemokine (Fig. 1A) but were nonmotile in the absence of CCL21. Near the edge of these NP-coated regions, 42% of the cells originally located in the NP-free area displayed directional migration towards the region where chemokine-bearing particles were patterned ~2 hrs post seeding on the surface (Fig. 1B), indicating a contribution of soluble chemokine released from the NP region creating a chemotactic gradient for the cells.

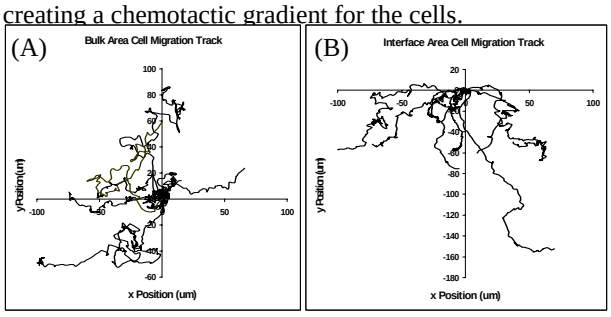

Figure 1. 2D migration paths of individual naïve T cells in (A) NP-coated regions and (B) 50 µm away from the edge of an NP-coated region of patterned surfaces. Edge of the NP-coated region was located in the –x direction.

Conclusions: Here we have shown that naïve T cell migration can be triggered by a combination of soluble- and solid-phase signaling on 2-D surfaces diplaying polysaccharide-bound chemokines. The nanoparticles developed here can also be conjugated with different chemokines in three-dimensional gels and may serve as a system to guide cell migration in tissue engineering scaffolds.

References:

1. Cyster JG. Immunological Reviews 2003; 195:5-14.
2. Banerjee, DJ. J. Biomed. Mater. Res. 2000; 50:331.
3. Doh J. J. Am. Chem. Soc.; 2004; 126:9170-9171.