341c Adhesion of Hematopoietic Stem Cells and Acute Myeloid Leukemia Cell Lines to Functionalized Surfaces of P-Selectin and Cd34 Antibody

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The inflammatory response, stem cell homing and tumor metastasis all occur through very similar mechanisms. In each case cells cross from the bloodstream to a region of interest via four distinct events: (1) initial contact with the endothelium, (2) rolling, (3) firm adhesion and (4) transmigration. The group of glycoproteins called selectins mediate the rolling event through their transient interactions with sialyl Lewis x derivatives on the adjacent surface, and in vivo experiments have shown that P-selectin, the largest of the selectin molecules, is of particular importance.

We investigated the effects of immobilized P-selectin chimera in the presence and absence of antibodies on the rolling velocities of normal cells (neutrophils, hematopoietic stem cells) and leukemic cell lines (Kg1a and HL60 cells). Our aims were to compare the behavior of the leukemic cell lines to normal cells on P-selectin alone, then to selectively reduce the rolling velocities of cells expressing CD34 antigen on their surface (hematopoietic stem cells and Kg1as) using a combination of immobilized monoclonal antibodies against CD34 and P-selectin. We used a parallel plate flow chamber assembly, where a suspension of the cells in question were perfused at physiologic shear rates over the functionalized surfaces. Movies of the rolling cells were digitized and subsequently analyzed using a MATLAB program to determine the rolling velocity of each cell.

Cells of the same relative sizes (hematopoietic stem cells and neutrophils; Kg1as and HL60s) rolled at similar rolling velocities on various concentrations of the immobilized P-selectin. The rolling velocities of cells expressing CD34 antigen (hematopoietic stem cells and Kg1as) were significantly reduced in the presence of the antibody and P-selectin while the velocities of cells that do not express CD34 were unaffected – the antibody alone did not support rolling. The success of these experiments suggests the application of using a functionalized selectin/antibody surface to facilitate cell separation. We have successfully isolated Kg1as from a mixture of Kg1as and HL60s based on their differential rolling velocities on immobilized P-selectin and anti-CD34, and are currently developing separation protocols for a mixture of hematopoietic stem cells and neutrophils. Through continued research, this technology may have clinical and research applications as a low stress method for purifying cells without significantly affecting their surface antigen profile, or to facilitate cell isolation for stem cell therapies.