MIGRATION AND ADHESION OF LYMPHOCYTES CULTURED UNDER LOW SHEAR CONDITIONS

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ABSTRACT: Mechanical forces have an important effect on lymphocyte migration and adhesion. Culturing Jurkat cells (a T lymphoblastoid cell line) in a low-shear environment has a profound effect on the migratory behavior of the cells, but no significant effect on their adhesion strength to fibronectin-coated surfaces. Using a 2-D cell migration assay, we determined that cells cultured in a low shear reactor system for 4-10 days had a significantly higher cell speed than cells cultured statically. Analysis of cell trajectories using the persistent random walk also revealed a higher RMS cell speed and random motility coefficient for cells cultured in the low shear reactor. One possible explanation for these differences is an increased integrin alpha-5 subunit expression on cells cultured in the low shear environment.

INTRODUCTION: Activation of T lymphocytes requires a specific sequence of cell signaling and intracellular events that are triggered through interactions with monocytes or other antigen-presenting cells. Since migration and adhesion are two of the most significant properties modulating these interactions, our efforts aim at elucidating the effect of mechanical forces on lymphocyte adhesion and motility. This study compares the migratory behavior of Jurkat cells cultured statically and in a rotating cell culture system (RCCS). This system is a rotating wall reactor with an inner core used for oxygenating the cell suspension. RCCS presents a low-shear environment for cell culture [1].

METHODS: Jurkat cells were maintained in either static cell suspension culture or in the RCCS reactor. Cell adhesion experiments on fibronectin-coated polystyrene surfaces were carried out using a parallel plate flow chamber with varying flow rates. Trajectories of cells migrating on fibronectin were determined and used to calculate cell migration speeds. The persistent random walk model was used for further data analysis [2]. Receptor quantification and DNA cell cycle analysis were carried out using standard flow cytometry methods.

RESULTS: Jurkat cells cultured in the RCCS showed significantly higher measured cell speeds than cells cultured statically as shown in Fig. 1. The persistent random walk model yielded larger RMS cell speeds and random motility coefficients as shown in Figure 2, but lower persistence times for cells cultured in the RCCS. No significant changes were measured between the two groups in cell-substrate adhesion strength, and integrin beta-1 or alpha-4 subunit expression. Cell cycle analysis was also performed.

DISCUSSION: Cells cultured in the RCCS show higher speeds of migration without any changes in adhesiveness to fibronectin. These differences in migratory behavior may be due to changes in intracellular signaling. Literature results indicate that culture in the RCCS affects lymphocyte activation and intracellular signaling [1].

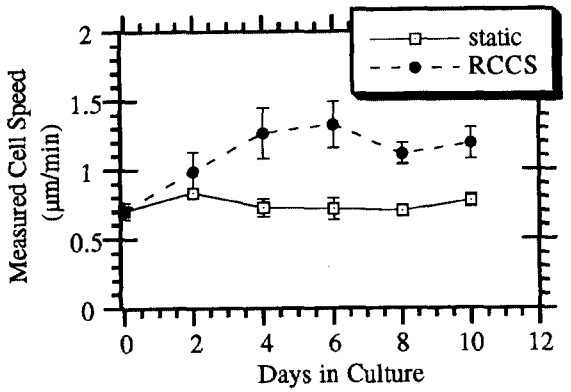

Figure 1: Changes in cell speed with time in culture.

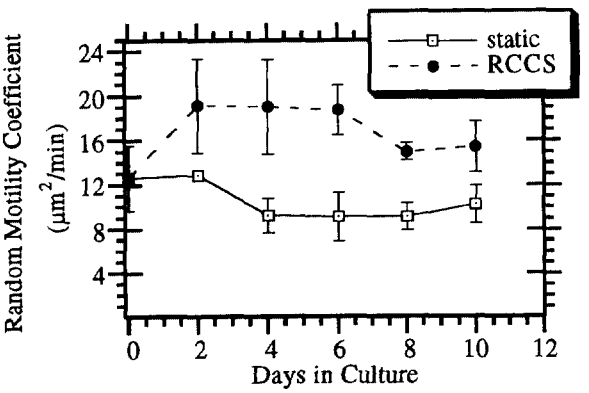

Figure 2: Changes in random motility coefficient with time in culture.

CONCLUSIONS: Our studies show that mechanical forces play an important role in modulating cell migration speeds independent of cell-substrate adhesion strength. Also, culture in a low shear environment yields cells that migrate longer distances over large periods of time than cells cultured statically.

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