8. Flow Cytometrical Analysis of T Cell Subsets Adhesiveness to Vascular Endothelial Cells and Airway Epithelial Cells

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We are interested in the cellular and molecular mechanisms for leukocyte trafficking into the airway lumen. We and others have studied neutrophil influx into the lumen induced by smoking. B_2 integrin, LFA-1 and MAC-1 expressed on the neutrophil and ICAM-1 on the epithelial and endothelial cells play a central role in the adherence of circulating neutrophils to the endothelium, migration along the epithelial cell surface and migration through the subendothelial matrix into the airway lumen.

Although the pathway for neutrophil adhesion is well established little is know about the mechanism of T cell adherence to epithelial cells and migration. In particular, the mechanism of T cell adhesion to epithelial cells in unknown. We therefore set out to study the process of T cell—epithelial/endothelial cell adhesion.

Adhesion of unstimulated T cells is very high and we believe that this may be due to the purification and labelling procedures carried out during the adhesion assay. Our method was a post-labelling method using anti-CD3 monoclonal antibody that was FITC-conjugated. Therefore we used flow cytometry to measure T cell adhesion.

This the flow cytometry autoadherent assay. The x-axis shows cell size and the y-axis shows granularity. This cell population includes everything; monolayer cells, T cells, B cells, macrophages and dead cells. Gating of FITC-positive cells was difficult due to autofluorescence of dead cells. On the third graph using FITC and PI-red in which we could gate out dead cells. FITC-positive cells were adherent T cells. On the final graph we can get a population of cells that are just adherent T cells, and can count the number of these cells.

This slide shows the representative results of T cell adherence to endothelial cells from our flow cytometry-based method compared to traditional techniques. Endothelial cells were treated with and without IL1β, IFNγ, and TNFα and T cells were activated with phorbol dibutyrate which is a PKC activator. The results indicate that the background level of autoadherence, using the flow cytometry method, was significantly lower than that seen using the conventional purification and labelling procedure. TNFα was found to be a potent stimulator of T cell adhesion to endothelial cells while T cell adherence to epithelial cells was induced in the presence of IFNγ, but not IL1β or TNFα.

We then assessed the role of the adhesion molecules ICAM-1, LFA-1, VCAM-1 and VLA-4 by treating the cells with blocking monoclonal antibodies. T cell autoadherence to endothelial cells was inhibited 60～70% in the presence of antibodies to ICAM-1 or LFA-1, 30% in the presence of anti-VLA-4 and nearly 100% with a combination of anti-LFA-1 and anti-VLA4 antibodies. Adherence to epithelial cells was blocked almost completely by antibodies to ICAM-1 or LFA-1 and was not effected by antibodies to VLA-4. We found that the level of expression of ICAM-1 and VCAM-1 was increased on endothelial cells in the presence of TNFα but IFNγ was more potent for epithelial cells. These results were confirmed by immunostaining and immunoprecipitation techniques and directly parallel our results with T cell autoadherence.

Analysis of autoadherence by $\gamma\delta$ T cells, which comprise only 5% of the T cell population, indicated that autoadherence was 2 times more efficient than conventional $\alpha\beta$ T cells to both endothelial cells

and epithelial cells. As with $\alpha\beta$ T cells, $\gamma\delta$ T cell adherence to epithelial cells was inhibited by antibodies to LFA-1 and ICAM-1. Interestingly, the expression of LFA-1, VLA-4 and VLA-5 was greater on $\gamma\delta$ T cells than $\alpha\beta$ T cells. In conclusion, the difference in cytokine sensitivity may indicate a role for localization of T cells and especially for accumulation of T cells under different conditions in the inflammatory response in the airway.

Discussion

Dr Rennard: Have you any evidence that the lymphocytes become activated after adhesion to either endothelial or epithelial cells?

Dr Nakajima: Yes. I have measured LFA-1 and VLA-5 expression on autoadherent leukocytes and these remain the same before and after autoadhesion. I think that avidity after stimulation by such an adhesion molecule will be different.

Dr Rennard: How long is the adhesion assay?

Dr Nakajima: 20 min.

Dr Rennard: Have you looked to see if adhesion is reversible?

Dr Nakajima: No.

9. The Growth Inhibition of IFNγ in Airway Bronchial Epithelial Cells

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It has been accepted that airway mucosal injury is closely linked to airway hyperresponsiveness, which is an important phenomenon found in bronchial asthma. Once epithelial damage occurs its recovery is largely dependent on replication of bronchial epithelial cells. In addition, it is known that growth factors and cytokines play a critical role in the regulation and proliferation of these cells, however it is not clear whether lymphocyte-derived growth factors have any influence on the replication of airway epithelial cells. IFNγ is a potent lymphokine produced by activated T cells, especially Th1 cells, and plays a prominent role in airway inflammatory and immune interactions, however, its role in airway repair in unclear.

In the present study we analyzed the role if IFNγ on proliferation of the human bronchial epithelial cell line BEAS-2B and on peripheral airway epithelial cells. We assessed the effect of IFNγ on proliferation of the cell line by direct cell counting and by colormetric assay. BEAS-2B cells were plated at a density of 2×10^4 cells/well $\pm$ IFNγ for 7 days. On days 3, 5, and 7 trypsin-EDTA was added to each well to release the cells. For colormetric assays BEAS-2B cells were cultured at a density of 0.5×10^4 cells/well in the absence or presence of IFNγ for 7 days. On day 7 cells were then treated with MTT labeling reagents and absorbance at 650 nm was determined using an ELISA plate reader.

IFNγ significantly inhibited the growth of BEAS-2B cells, as determined by absolute cell number, in a dose-dependent fashion. Similar results were obtained using the colormetric assay.

We then compared the effects of IFNγ on the human peripheral epithelial cells. As seen with the BEAS-2B cells, IFNγ inhibited the growth of peripheral airway epithelial cells in a dose-dependent manner. Preincubation of the cells for 6 hr with anti-IFNγ receptor antibodies and subsequent incubation of the cells with 15 ng/ml IFNγ, significantly reduced the inhibitory effect of the cytokine alone. These