and epithelial cells. As with αβ T cells, γδ 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 γδ T cells than αβ 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 is unclear.

In the present study we analyzed the role of 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 × 10⁴ cells/well ± 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 × 10⁴ 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
results suggest that the inhibitory activity of IFNγ was mediated via the IFNγ receptor.

The effects of other cytokines TGFβ, IL1α, IL1β, and TNFα were also assessed in the BEAS-2B cells. Alone IL1α, IL1β, and TNFα had no significant effect on BEAS-2B cell proliferation whereas TGFβ caused significant inhibition of proliferation. Similar responses to TNFα and TGFβ were seen in human peripheral airway epithelial cells. Interestingly, the combination treatment of TNFα and IFNγ significantly augmented the inhibition of proliferation induced by IFNγ alone.

To determine whether IFNγ-induced inhibition was mediated via TGFβ, cells were treated with anti-TGFβ antibodies prior to treatment with IFNγ. In addition, cells were treated with the specific nitric oxide inhibitor MMMA, to determine the role of nitric oxide secretion in IFNγ-induced inhibition of cell proliferation. There was no significant difference in proliferation following either treatment. These data suggest that IFNγ may play a direct role in the growth regulation of peripheral airway epithelium.

**Discussion**

**Dr Rennard:** In the experiments using anti-TGFβ antibodies did you have a control where you added TGFβ in some concentration known to inhibit growth and then demonstrate that the antibody could block that? Also, does the antibody that you used block all forms of TGFβ or does it have some specificity?

**Dr Kobayashi:** Yes, we did do the control that you are asking for and yes the antibody did block in that situation. The antibody we used was a polyclonal sera, however, I am not sure whether all forms of TGFβ were blocked completely. TGFβ1 was the type that was most effective at inhibiting proliferation of the BEAS-2B cells and I assumed that this would also be the case for the human peripheral epithelial cells.

**Dr Rennard:** Certainly TGFβ1 and TGFβ2 will both have similar effects on airway epithelial cells so I think that the experiments that you did are certainly appropriate. There is evidence though that the form of TGFβ that airway epithelial cells produce is TGFβ2. Some antibodies that block the active site block both β1 and β2 however this is not the case for all antibodies, so it would be interesting to know if the antibody is specific.

10. Increased Expression of IL-8 and ICAM-1 in Small Airway Epithelium from Patients with Chronic Obstructive Pulmonary Disease (COPD)


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The airway inflammatory process plays and important role in the pathogenesis of COPD. In addition, airway epithelial cells play a role in recruitment of cells, such as neutrophils, into the local airways. We attempted to determine whether human peripheral epithelial cells from patients with COPD expressed IL-8, a potent neutrophil chemoattractant.

Twenty three patients were studied in total, and 11 were diagnosed with COPD. The COPD patients included patients with chronic bronchitis, emphysema, sinusobronchial syndrome and DPB were stud-