results suggest that the inhibitory activity of IFNγ was mediated via the IFNγ receptor.

The effects of other cytokines TFGβ, 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 TFGβ 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, sinusibronchial syndrome and DPB were stud-

ied. The results from these patients were compared to data obtained from six current smokers and six individuals who had never smoked (non-smokers). After collecting samples using the ultrathin fiberscope analysis was performed by PCR, immunocytochemistry (to detect protein expression) and culture of collected epithelial cells to follow IL-8 production.

An average of 1×10^6 cells were collected with a viability of approximately 60%. Almost all the cells were non-ciliated and keratin-positive, indicating that they were epithelial in origin. RT-PCR for IL-8 and β-actin was carried out using 30 amplification cycles to obtain a liner range of amplification for accurate sample comparison. The relative intensity of IL-8 mRNA transcripts, corrected for β-actin, was 1.43 in COPD patients, 0.74 in control smoker patients and 0.15 in control non-smoker patients. The difference the COPD group and the non-smokers was significant but there was no statistically significant difference between the COPD group and the current smoker group.

Using immunocytochemical staining, cells from COPD patients were 90% positive for IL-8 whereas 61% of the cells from current smokers were positive and only 21% from the non-smokers. Thus, there was a statistically significant difference in the number of IL-8 expressing cells from the COPD patients compared to the smoker and non-smoker control groups.

These findings were supported by results obtained from culturing peripheral airway epithelial cells *in vitro* and subsequent analysis of IL-8 production. In conclusion, small airway epithelial cells from COPD patients express greater levels of IL-8 message and protein compared to non- and current smokers.

Discussion

Dr Hogg: It would be very interesting to look at the cells from the COPD patients that are expressing high levels of IL-8 and ICAM-1 and see if they are expressing any E-18 protein.

Dr Takizawa: Yes.