***Antibodies and Reagents***

Recombinant human TGF-β1 (100-21) was purchased from Peprotech (London, UK). Recombinant human TNFα (T-0157) was purchased from Sigma (St. Louis, MO). Antibodies to vimentin (M7020), E-cadherin (M3612) S100A4 (A5114) and cytokeratin-19 (M0888) were purchased from Dako Cytochemistry (Glostrup, Denmark). Antibodies to α-smooth muscle actin (A2547), and fibronectin (F3648) were purchased from Sigma. Collagen I (sc-8783) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents unless otherwise stated were of the highest available purity from commercial sources.

***Cell Culture***

Briefly, bronchial brushings from subsegmental bronchi were suspended into sterile phosphate buffered saline. Subsequently, an equal volume of RPMI supplemented with 10% foetal calf serum (FCS) was added and the cells pelleted at 1,000 x g for 5 minutes. The pellet was re-suspended in small airway growth media (SAGM) supplemented with SAGM SingleQuots (both purchased from Lonza) and 100U/ml penicillin - 100μg/ml streptomycin and transferred to a T-25 flask pre-coated with 0.5% collagen (Nutacon). Cells were maintained at 370C in a humidified 95% air / 5% CO2 incubator until confluence. Cells were not advanced beyond P4 to ensure a uniform epithelial phenotype.

***Gelatin Zymography***

To assay for pro-MMP-9 secretion conditioned media from cells treated as indicated were separated on an 8% SDS-PAGE gel containing 0.1% gelatin. Following electrophoresis, gels were incubated in 2.5% (v/v) Triton X-100 for 30 minutes and then overnight in developing buffer (50mM Tris-HCl, 0.2M NaCl, 5mM CaCl2) at 370C. Gels were stained with Coomassie blue stain (40% methanol, 10% acetic acid, 0.05% Coomassie blue) and destained (40% methanol, 10% acetic acid) until the desired contrast was achieved.

***Human Lung Tissue Sampling***

This use of human tissue was approved by Local Research Ethics Committee.

Normal control tissue was obtained from donor lungs assessed for, but not accepted for, use in clinical lung transplant. Stable lung transplant recipients underwent transbronchial biopsy by an established technique. Tissue from recipients with OB was obtained from explanted lung at the time of retransplantation.

All lung samples were fixed in 10% formalin, embedded, sectioned and stained with H&E. Acute rejection and other pathological changes in stable patients were excluded and OB confirmed in explanted lungs by an experienced pulmonary histopathologist. Sequential sections from stable recipients, OB recipients and normal control lungs were stained with antibodies against E-cadherin, vimentin and α-smooth muscle actin using a modified immunoperoxidase method (Envision;Dako).

Areas of intact airway epithelium were identified under high power (magnification x100) as close as possible to the same area of epithelium in each section and for each marker. Five high power fields per case were evaluated. The airway epithelium was identified visually and isolated from the rest of the image using image analysis software (Adobe Photoshop CS3). The area of positive staining within the isolated epithelium was then identified using a densitometry based analysis for each field. The five high power fields were averaged for each marker in each case and used in the analysis between different patient groups.