Supplemental Information

Epithelial Stress and Structural Remodelling in Childhood Asthma

*I A Fedorov, S J Wilson, D E Davies, S T Holgate
Division of Infection, Inflammation and Repair, School of Medicine, Southampton General Hospital, Southampton SO16 6YD, UK and *City Clinical Hospital #1, Chelyabinsk, Russia.

Immunohistochemical analysis

For each biopsy specimen, the entire intact epithelium in two non-serial sections was systematically assessed based on red, blue, green (RGB) colour balance; non-specific immunostaining (that is, staining that was also present with isotype control antibodies) which was sometimes evident in the brush border was excluded from all quantitation. The amount of staining for collagen III within the lamina reticularis was also assessed using this approach. To ensure reproducibility of analysis, the image analysis system was standardised using a section of bronchial mucosa stained for EGFR by the same method, as previously described.[1] For EG2 stained sections the numbers of positive cells were counted in the lamina propria excluding areas of glands, muscle or damage. The area of the section was then measured using computer assisted image analysis. The thickness of the lamina reticularis was also determined by computerised image analysis. In brief, an image of the lamina reticularis was captured and delineated. A Euclidean distance transformation was applied; this identified the central line within the lamina reticularis. A grey level coding was then assigned to each pixel between the central line and the outer
boundary, relative to the distance (that is, the higher the grey level the greater the distance [thickness]). An appropriate scaling for the magnification used was then applied and the distance in microns calculated. All measurements were performed by an observer who was blinded to the clinical group from which the biopsy specimen was derived.


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