Eur Respir J, 1993, 6, 1257–1263
Printed in UK - all rights reserved

Copyright ©ERS Journals Ltd 1993
European Respiratory Journal
ISSN 0903 - 1936

The distribution of adhesive mechanisms in the normal bronchial epithelium

S. Montefort*, J. Baker*, W.R. Roche**, S.T. Holgate*

The distribution of adhesive mechanisms in the normal bronchial epithelium. S. Montefort, J. Baker, W.R. Roche, S.T. Holgate. ©ERS Journals Ltd 1993.

ABSTRACT: The integrity of the bronchial epithelium is dependent on various adhesion mechanisms that serve to hold the composite structure of the epithelium together and anchor it to the underlying basement membrane. Using immunohistochemistry we wanted to map out a number of these junctional and non-junctional adhesion mechanisms in the normal human bronchial epithelium.

The β1-associated integrin subunit α2 was immunolocalized to all of the epithelial intercellular spaces, whilst α6 and β4 were strongly evident at the basal cell layer basement membrane junction. The α1 and α5 integrin subunits were not detected anywhere in the epithelium.

Monoclonal antibodies (MoAbs) to tight junction polypeptides and the E-cadherin, liver cell adhesion molecule (LCAM), immunolocalized to the apicolateral portions of the intercellular junctions between all neighbouring columnar cells, with LCAM extending further along the lateral cell membrane. Desmosomal protein (dp) 1 and 2 MoAbs gave a punctate pattern between all of the suprabasal cells, and exhibited the greatest intensity of staining at the junction between the columnar and basal cell layers.

In conclusion, there is an organized distribution of adhesive mechanisms within the normal human bronchial epithelium, which may be targeted by the various insults which lead to epithelial shedding.

Eur Respir J., 1993, 6, 1257–1263.

Depts of *Immunopharmacology and **Pathology, University of Southampton, Southampton, UK.

Correspondence: S. Montefort
Dept of Immunopharmacology
Medicine 1, Level D, Centre Block
Southampton General Hospital
Tremona Road
Southampton SO9 4XY
UK

Keywords: Adhesion
bronchial epithelium

Received: October 14 1992
Accepted after revision May 8 1993

The normal bronchial epithelium is a complex, pseudostratified, ciliated columnar cell structure [1], the best known function of which is to serve as a protective barrier to the underlying submucosa [2]. It fulfils this function by impeding the access of irritant stimuli to the sensory intraepithelial and submucosal nerves, and also by trapping and excluding pollutants/allergens present in the bronchial lumen in its mucociliary escalator [3]. In addition, it forms the supporting matrix for many different cell types involved in immune regulation, detoxification and maintenance of periciliary fluid homeostasis. Apart from these structural functions, the bronchial epithelium is capable of producing proinflammatory mediators, such as leukotrienes [4, 5], endothelin [6], bronchoconstrictor neuropeptides [7], and cytokines, and bronchodilatory substances, such as prostaglandin E_2 and the epithelial cell derived relaxant factor [8, 9]. These varied functions emphasize why epithelial shedding is so important in conditions such as asthma, where focal damage to this barrier and bronchial hyperresponsiveness have been associated [10].

The widely-accepted hypothesis for the cause of epithelial shedding in asthma is that insults, such as ozone [11], viruses [12–14], and eosinophil granule arginine-rich proteins, produce a cytotoxic attack against epithelial cells, leading to disruption and shedding of damaged cells [15, 16]. However, the finding of viable epithelial cells in bronchoalveolar lavage fluid and or "damaged" cells still attached to the bronchial wall [17] suggests that site-directed cytoxicity is not the only mechanism leading to epithelial cell shedding in bronchial asthma. Our recent observation of a potential plane of cleavage between the suprabasal and basal cell layers of the epithelium [18] suggests that disturbance to the intraepithelial adhesive mechanisms may be important in epithelial disruption.

The integrity of the bronchial epithelium, like that of other epithelia, is dependent on various adhesive mechanisms, that serve to hold the composite structure together and bind it to the underlying basement membrane. These adhesive mechanisms can be broadly subdivided into junctional and non-junctional types. Some of these adhesive structures have dual roles, such as the tight junction (zona occludens), which, in addition to maintaining the surface seal of the epithelium, also serves to maintain selectivity over epithelial permeability [19], and gap junctions (nexus), which, in addition to binding adjacent epithelial cells, are also involved in intercellular ion exchange [20].

The best characterized epithelial junctional adhesive structures are desmosomes (macula adherens) [21], and hemidesmosomes [22], being important in cell-to-cell and cell-to-basement membrane adhesion, respectively. Although studied extensively in human skin, their distribution has not been extensively investigated in the human bronchial epithelium. Desmosomes exist as discrete structures, visible on electronmicroscopy by the presence of dense plaques on

each side of a 25–35 nm wide intercellular space, and tonofilaments extending from the plaques into the cellular cytoplasm. Desmosomes are comprised of four desmosomal proteins (dp1– dp4), which are mostly localized within the plaque and around the tonofilaments, and three desmosomal glycoprotein s (dg1–dg3), which are mostly found in the extracellular region [23]. Hemidesmosomes are composite structures, which in the skin [24] and cornea [25] have been shown to be largely comprised of α6β4 integrin aggregates, which probably interact closely with basement membrane components, such as laminin. In addition, some of the β1 integrin heterodimers, which are known to interact with matrix proteins [26], have also been immunolocalized in the normal human bronchus [27, 28], where they have an extensive distribution. This might be expected, since many of these adhesion molecules are receptors to most of the basement membrane components [29], and form components of junctional adhesive mechanisms in other tissues [24, 25].

Since knowledge concerning adhesive mechanisms which maintain the integrity of the human airway epithelium is rudimentary, we have used immunohistochemical and electronmicroscopic produces to map the distribution of a number of important adhesive mechanisms in normal bronchial epithelium, to provide a database with which to compare diseased states where epithelial damage occurs, such as asthma.

Methods

Bronchial tissue

Four different samples of bronchial tissue were obtained from the segmental bronchi of fresh lobectomy specimens, obtained from two patients undergoing surgery for lung malignancies. One patient had no history of smoking, whilst the other had ceased to smoke 18 months prior to surgery. Neither of the patients suffered from any other illness. The small pieces of bronchial wall were taken well away from the tumour margin. Each bronchial sample (n=8) was divided into three; one for transmission electronmicroscopy, one for frozen sectioning, and one for embedding in glycolmethacrylate (GMA) resin, a water soluble resin which has the advantage over other resins of preserving many immunoreactive epitopes [30].

Electronmicroscopy

The tissue sample was immediately transferred into 3% glutaraldehyde fixative for 2 h, and then washed in caccodylate/sucrose buffer overnight. The biopsy was then post-fixed in 2% osmium tetroxide in caccodylate buffer for 2 h, rinsed in millipored double distilled water and incubated in 2% uranyl acetate for 1 h. It was then dehydrated through graded alcohols, cleared in Histosol for 20 min and infiltrated with 50:50 Histosol/ Spurr resin for 30 min. Finally, it was infiltrated in 100% Spurr overnight. The specimen was then embedded in Spurr in a small tube, spun and polymerized at 60°C for 16 h. Ninety nanometre sections were cut on an ultramicrotome (C. Reichart, Austria) and mounted on uncoated copper grids. Sections were examined using a Hitachi H7000 microscope (Hitachi, Tokyo, Japan).

Immunohistochemistry

Table 1 displays the range of monoclonal antibodies (MoAbs) used in this study.

Frozen sections

The tissue samples were snap-frozen in liquid nitrogen. Cryostat sections of 5 μm were cut, mounted onto a glass slide coated with 0.01% poly-L-lysine as an adhesive, dried for 30 min at room temperature and stored a -20°C until used. For immunostaining the unfixed frozen sections were incubated with the primary monoclonal antibody (MoAb) for 30 min at room temperature. After washing

Table 1. – Monoclonal antibodies (MoAbs) used for immunohistochemistry

Adhesion protein	MoAb	Source	Cryostat	GMA
α1 (CD49a)	TS2-7 mouse IgG1	D. Garrod Manchester, UK	+	+
α2 (CD49b)	CLB-4 mouse IgG1	A. Sonnenberg The Netherlands	-	+
α5 (CD49e)	B1E5 mouse IgG1	D. Hall S. Francisco, US	+	+
α6 (CD49f)	GoH3 rat IgG1	A. Sonnenberg The Netherlands	+	+
β4	5B5 mouse IgG1	J. Aplin Manchester, UK	+	+
LCAM	Decma-1 rat IgG1	Sigma Chem. Poole, UK	+	+
Dp1 + 2	11.5F mouse IgG1	D. Garrod Manchester, UK	+	+
Tight Junction polypeptide	ZO1 mouse IgG1	T. Fleming Southampton, UK	+	-

GMA: glycolmethacrylate resin; IgG: immunoglobulin G; Dp: desmosomal protein; CD: cluster designation; LCAM: liver cell adhesion molecule.

three times in phosphate buffered saline, (PBS) pH 7.4, the appropriate second layer antibody linked to the fluorochrome was added for 30 min. The sections were rinsed and mounted in a mixture of PBS/glycerol in a ratio of 1:9. They were viewed under a Zeiss photomicroscope, fitted with an ultraviolet filtered mercury bulb.

Glycolmethacrylate (GMA) sections

The tissue sample was placed in ice-cooled acetone, containing the protease inhibitors phenylmethyl-sulphonyl fluoride (2 nM) and iodoacetamide (2 nM), and rapidly cooled to -20°C overnight. The following day the sample was transferred into acetone at room temperature for 15 min, and then into methyl benzoyl for a further 15 min. The tissue was immersed in GMA monomer (Polysciences, Northampton, UK) at 4°C for 7 h, during which time the GMA solution was changed three times. On completion, the tissue was embedded in GMA resin (prepared by mixing GMA monomer, N,N-dimethy-laniline in polyethylene glycol (PEG) 400 and benzoyl peroxide) and polymerized overnight at 4°C. The GMA blocks were stored in airtight containers at 20°C until immunostaining.

Sections were cut on an ultramicrotome at 2 μm thickness and floated onto ammonia water (1 in 500), and then picked onto 0.01% poly-L-lysine glass slides, where they were allowed to dry at room temperature for 1–6 h. Endogenous peroxidases were blocked using a solution of 0.1% sodium azide and 0.3% hydrogen peroxide; this was followed by three rinses in Tris buffered saline (TBS) adjusted to pH 7.6. Undiluted culture supernatant, consisting of Dulbecco's minimum essential medium (MEM) + 10% foetal calf serum + 1% bovine serum albumin, was applied for 30 min and, after draining, the primary antibodies were added. The sections were incubated with the primary antibodies overnight at 37°C. After rinsing, biotinylated rabbit anti-mouse immunoglobulin G (IgG) antigen-binding fragment (Fab) (Dako Ltd, High Wycombe, UK) was applied for 2 h and followed by the streptoavidin-biotin-horse-radish-peroxidase complex (Dako Ltd, High Wycombe, UK) for 2 h. After rinsing in TBS, (amino-ethyl carbamazole (AEC) in acetate buffer (pH 5.2) and in hydrogen peroxide, was used as substrate to develop a peroxide-dependent colour reaction. The sections were then counterstained with Mayer's haematoxylin.

Results

No differences in the distribution of any of the adhesive mechanisms studied were noted between the eight different tissue samples.

Electronmicroscopy (EM)

Transmission EM showed intercellular junctions between adjacent columnar cells to be similar to those noted in other epithelia, with tight junctions, intermediate junctions and desmosomes quite easily identified (fig. 1). The



Fig. 1. – Electronmicrograph of intercellular junction between two columnar epithelial cells showing tight junction (tj), intermediate junction (ij), and desmosomes (d). (Original magnification ×20,000. Bar = 1 μm).

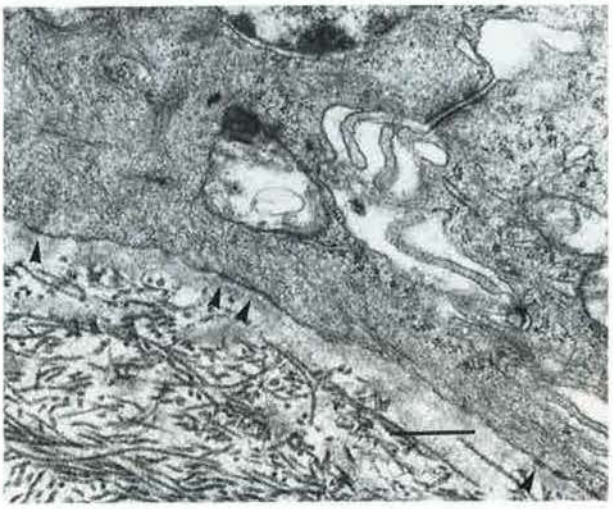


Fig. 2. – Multiple hemidesmosomes (arrowheads) in basal cell basement membrane interface. (Original magnification ×20,000. Bar = 1 μm).



Fig. 3. – Immunostaining for α2 integrin in bronchial epithelium on GMA-embedded biopsy. GMA: glycolmethacrylate resin. (Original magnification ×400. Bar = 10 μm).

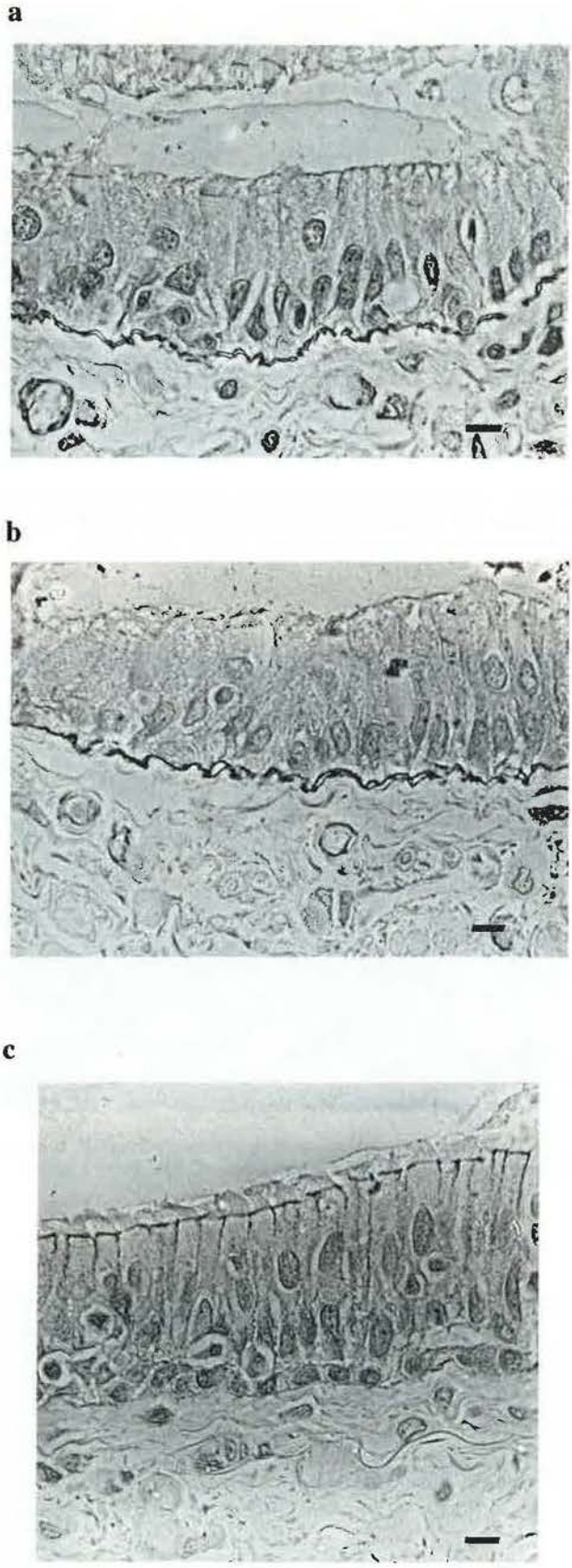


Fig. 4. – Immunostaining in bronchial epithelium on GMA-embedded biopsy for a) α6 integrin b) β4 integrin and c) E-cadherin. GMA: glycolmethacrylate resin. (Original magnification for a) and b) ×400, and for c) ×450. Bar = 10 μm).



Fig. 5. – Immunofluorescent punctate staining for desmosomal proteins 1 and 2 on frozen section of bronchial epithelium. (Original magnification ×400. Bar = 10 μm).

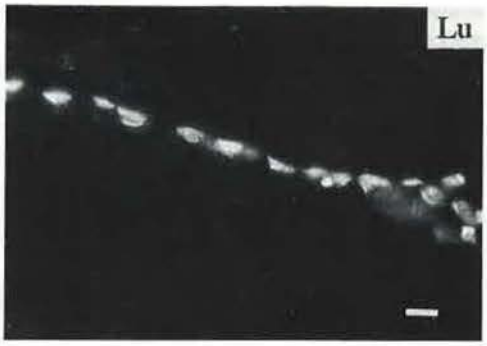


Fig. 6. – Immunofluorescent staining for tight junctions in bronchial epithelium on frozen section. Lu: bronchial lumen. (Original magnification ×400. Bar = 10 μm).

desmosomes noted were similar in structure and dimensions to those reported in other epithelia. Hemidesmosomes were observed as multiple electron-dense areas (fig. 2) where the basal cells impinged upon the underlying basement membrane. However, these structures were not as prominent as reported in other human tissues such as skin.

Immunohistochemistry

Glycolmethacrylate. GMA had the advantage of enabling thin sections to be cut, whilst preserving morphology and localization of immunostaining to specific cellular structures. We failed to demonstrate any epithelial staining with the MoAb to the α1 subunit or the α5 subunits. In the case of the α2 subunit, immunostaining was observed around all of the bronchial epithelial cells including the basal cells (fig. 3). The α6 and β4 showed similar patterns of distributions, being only expressed in the sub-basal region of the epithelium as a clear linear pattern and less strongly around submucosal vessels (figs 4a and 4b). The MoAb to the E-cadherins immunostained the apical portions of the intercellular junctions found between the columnar epithelial cells (fig. 4c), and to a lesser degree, occasional parts of the sub-basal cell area.

Frozen sections. Cryo-immunohistochemistry was most helpful in the localization of dp1 and dp2 in desmosomes.

A punctate pattern was observed along the lateral border of the suprabasal cells, with a denser distribution at the interface between the suprabasal and basal cell layers (fig. 5). There was a complete absence of staining below the basal cell layer. Immunostaining with the MoAb against tight junctions, ZO1, was localized to the apical part of the intercellular borders but did not extend down as far as the E cadherins (fig. 6). The cryo-immunofluorescent technique confirmed the GMA findings for the α1, α2, α6 and β4 subunits, with the latter two again being found at the basal cell basement membrane junction (figs 7a and 7b). The E-cadherin was seen in the intercellular borders between neighbouring columnar cells, immunostaining most intensely in the apical region of these junctions (fig. 7c). In cross-section the cadherin immunostaining was seen to surround the individual epithelial cells (fig. 7c).

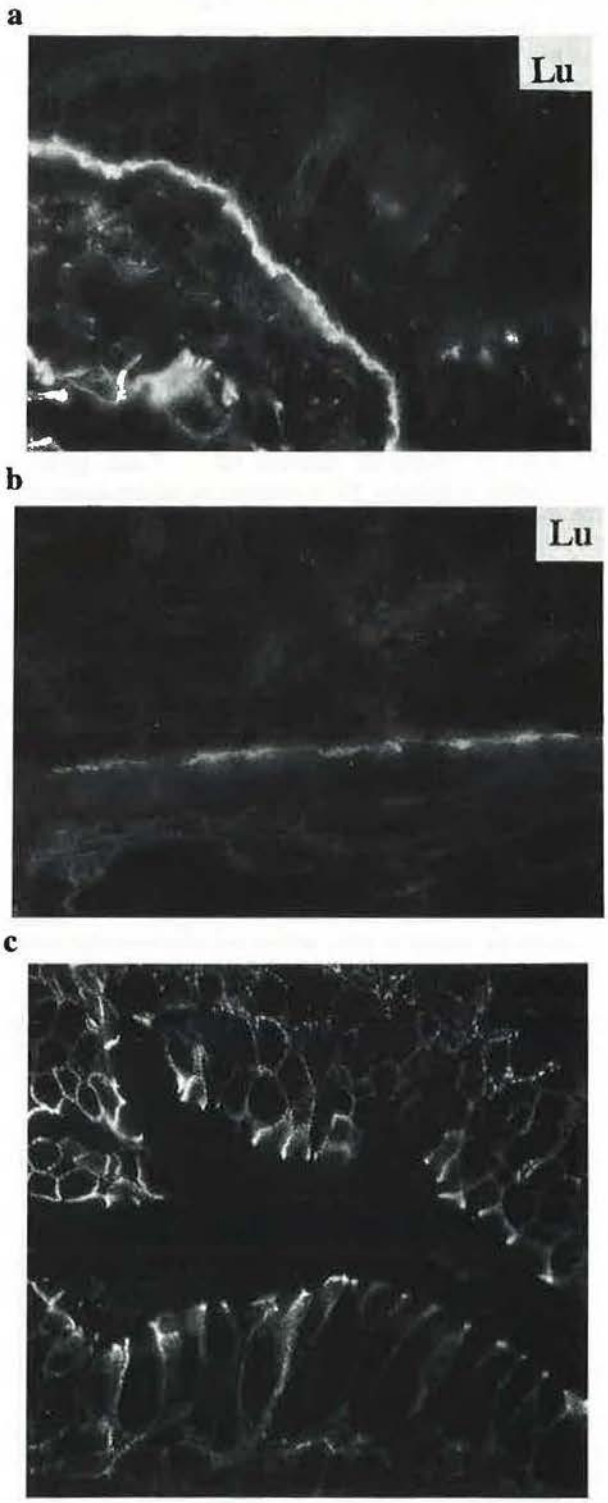


Fig. 7. – Immunostaining on frozen section for a) α6 integrin b) β4 integrin and c) E-cadherin. Lu: bronchial lumen. (Original magnification for a) and b) ×400 and for c) ×450. Bar = 10 μm).

Discussion

Using a combination of immunohistochemistry and electronmicroscopy it has been possible to build-up a picture of the structures important in maintaining the structural integrity of the bronchial epithelium. Of particular novelty in this study was the clear demonstration of tight junctions and E-cadherin, probably present within intermediate junctions. Whilst these structures may be important in regulating paracellular transport, attachment of the epithelium to its basement membrane appears to rely heavily on the expression of hemidesmosomes, containing the α6 and β4 integrin subunits. In contrast to hemidesmosomes, the structures upon which the majority of the epithelial integrity depends are the desmosomes found along the lateral and inferior borders of the suprabasal cells. An increased number of these structures along the apical border of the basal cells indicates an important site of epithelial stress, and corresponds to the site of cleavage of epithelial cells especially in asthma. These observations indicate the complexity of the normal bronchial epithelium and the potential importance of these structures as a target for disruption in inflammatory airway disorders.

The epithelium in the normal human bronchus, is described as pseudostratified, ciliated and columnar [1]. It appears that the basal cells provide a foundation upon which the suprabasal cells attach. In human skin [24], and cornea [25], the basal cells are fixed firmly to the basement proteins *via* hemidesmosomes with their constituents α6β4 integrins. However, while hemidesmosal structures were visualized in the bronchial epithelium by transmission electronmicroscopy, they seemed less distinct than described by others in the skin. The presence of strong immunostaining for the α6 and β4 components of hemidesmosomes along the inferior border of the basal cells adds further evidence to the important role played by these hemidesmosomal integrins in anchoring the epithelium to the basement membrane. Neither of these integrin subunits were evident immunohistochemically above this level, although some staining was observed around submucosal blood vessels. Thus, in addition to forming a laminin receptor with β1, α6 is also able to interact with β4 to form a further integrin receptor for laminin present in vascular basement membrane [31].

The α2 integrin subunit, which comprises a component of one of the β1 collagen/laminin receptors, immunolocalized to the basal cell/basement membrane interface, where it would be expected to interact with basement membrane

components. The immunostaining of this integrin also extended between most of the epithelial cells, confirming the recently published findings of DAMJANOVICH *et al.* [27]. We have failed to demonstrate any immunostaining for either α1 or α5, which forms a component of the classical fibronectin receptor, in the intact normal epithelium, despite the reports of α5 being expressed in cultured human bronchial epithelial cell monolayers [28]. It is possible that these integrin components are only expressed under specific conditions or in the presence of specific growth factors.

It is frequently stated that the integrity of the bronchial epithelium is dependent upon the tight junction, a complex adhesive structure found to surround suprabasal cells. However, this seems unlikely, since immunostaining with the tight junction MoAb, ZO1, was restricted to a discrete structure at the apex of the suprabasal epithelial cells intercellular borders - confirming the findings of GODFREY *et al.* [32], who utilized a freeze-fracture technique for studying tight junctions. The complexity of the tight junction, together with its apical localisation, makes it a strong candidate for controlling paracellular transport, although how this is achieved is not known. A second region of electro-density is observed just below the tight junctions and has been designated the intermediate junction. The distribution of immunostaining with the anti E-cadherin MoAb suggests identity with the intermediate junction. This is in keeping with the finding of BOLLER *et al.* [33], who similarily localized E-cadherin to the intermediate junction of the adult mouse intestinal epithelium. Along with the tight junctions, it is likely that intermediate junctions contribute to the selectively of paracellular transport, possibly by the epithelial cell regulation of extracellular Ca^{++} concentration. E-cadherin may also contribute towards intercellular adhesion between adjacent suprabasal cells, since immunostaining for this molecule was present in a graded fashion below the site of the intermediate junctions.

The most important adhesion mechanism which operates in epithelial structures is the desmosome. This composite structure was easily identified in the bronchial epithelium, between adjacent epithelial cells and the characteristic plaque and tonofilament structures, as previously described in skin. Using a MoAb directed against shared epitopes expressed on desmosomal proteins 1 and 2, which are situated mainly in the desmosomal plaque, the distribution of desmosomes within the epithelium could clearly be seen as punctate immunofluorescent structures, when viewed by ultraviolet microscopy. Punctate staining was present along the lateral and inferolateral borders of the suprabasal cells. This was also evident between adjacent basal cells, but not along their inferior border. There was a particularly dense population of desmosomes at the junction between suprabasal and basal cell layers, suggesting that a strong intercellular bond is needed in this plane to maintain epithelial integrity. It is possible that a disturbance of desmosomal adhesion could account for the epithelial fragility in asthma, especially since this seems to occur between the suprabasal and basal cells [18]; the site of high desmosomal expression. An alternative explanation is that neutral proteases, either from inflammatory cells *per se*, *e.g.* tryptase, elastase, or induced by a cognate interaction between eosinophils and epithelial cells [34], could result in enzymatic degradation of desmosomes, with a subsequent reduction in their capacity to anchor epithelial cells.

Whatever the mechanisms of epithelial damage in asthma and other disorders, the widespread distribution of desmosomes and other adhesive structures within the bronchial epithelium may be the target of inflammatory responses in which the epithelium becomes damaged. Thus, understanding of factors controlling their expression, function and degradation is of clear importance in airway homeostasis and the mechanisms by which it may be disturbed.

References

1. McDowell EM, Beals T. – Biopsy Pathology of the Bronchus. London, Chapman and Hall Ltd, 1986.
2. Dunnill MS. – The pathology of asthma with specific reference to changes in the bronchial mucosa. *J Clin Pathol* 1960; 13: 27–33.
3. Forrest JB, Lee RM. – The bronchial wall: integrated form and function. *In*: Crystal & West, eds. The Lung: Scientific Foundations. New York, Raven Press Ltd, 1991; pp. 729–739.
4. Bigby TD, Goetzl EL, Holtzman MJ. – Canine epithelial cells generate leukotriene B4 in response to calcium ionophore. *Clin Res* 1985; 33: 76A.
5. Holtzman MJ, Aizawa H, Nadel JA, Goetzl J. – Selective generation of leukotriene B4 by tracheal epithelial cells from dogs. *Biochem Biophys Res Comun* 1983; 144: 1071–1076.
6. Springall DR, Howarth PH, Counihan H, Djukanovic R, Holgate ST, Polak JM. – Endothelin immunoreactivity of airway epithelium in asthmatic patients. *Lancet* 1991; 337: 69–72.
7. Barnes PJ. – Asthma as an axon reflex. *Lancet* 1986; 242–245.
8. Vanhoutte PM. – Epithelium-derived relaxing factor(s) and bronchial hyperreactivity. *Am Rev Respir Dis* 1988; 138 (Suppl.): S24–S30.
9. Barnes PJ, Cuss FM, Palmer JB. – The effect of airway epithelium on smooth muscle contractility in bovine trachea. *Br J Pharmacol* 1985; 86: 685–691.
10. Beasley R, Roche WR, Roberts JA, Holgate ST. – Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989; 139: 806–817.
11. Fabbri LM, Aizawa H, Alpert SE, *et al.* – Airway hyperresponsiveness and changes in cell counts in bronchoalveolar lavage after ozone exposure in dogs. *Am Rev Respir Dis* 1984; 129: 288–291.
12. Ramphal R, Fischlschweiger W, Shands JWM, Small PA. – Murine influenzal tracheitis: a model for the study of influenza and tracheal epithelial repair. *Am Rev Respir Dis* 1979; 120: 1313–1318.
13. Hers JFP. – Disturbances of the ciliated epithelium due to influenza virus. *Am Rev Respir Dis* 1966; 93: 162–171.
14. Walsh JJ, Dietlein LF, Low FN, Burch GE, Mogabgab WJ. – Bronchotracheal response in human influenza. *Arch Intern Med* 1961; 108: 376–388.
15. Frigas E, Gleich GJ. – The eosinophil and the pathophysiology of asthma. *J Allergy Clin Immunol* 1986; 77: 527–537.
16. Motojima S, Frigas E, Loegering DA, Gleich GJ. – Toxicity of eosinophil cationic proteins for guinea-pig tracheal epithelium *in vitro*. *Am Rev Respir Dis* 1989; 138: 801–805.
17. Montefort S, Djukanovic R, Holgate ST, Roche WR. – Ciliated cell damage in the bronchial epithelium in asthmatics and nonasthmatics. *Clin Exp Allergy* 1992; 23: 185–189.
18. Montefort S, Roberts JA, Beasley CR, Holgate ST, Roche

WR. – The site of disruption of the bronchial epithelium in asthmatics and nonasthmatics. *Thorax* 1992; 47(7): 499–503.
19. Gumbiner B. – Structure, biochemistry and assembly of epithelial tight junctions. *Am J Phys* 1987; 253 (Cell Physiol 22): C749–C758.
20. McNutt MS, Weinstein RS. – Membrane ultrastructure at mammalian intercellular junctions. *Prog Biophys Mol Biol* 1973; 26: 45–101.
21. Garrod DR, Parrish EP, Mattey DL, Martson JM, Measures HR, Vilela MJ. – *In*: Edelman GM, Cunningham BA, Thierry JP, eds. Desmosomes from Morphoregulatory Molecules. John Wiley & Sons Inc., 1990.
22. Montefort S, Herbert CA, Robinson C, Holgate ST. – The bronchial epithelium as a target for inflammatory attack in asthma. *Clin Exp Allergy* 1992; 22(5): 511–521.
23. Miller K, Mattey D, Measures H, Hopkins C, Garrod D. – Localisation of the protein and glycoprotein components of bovine nasal epithelial desmosomes by immuno-electron-microscopy. *EMBO* 1987; 6(4): 885–889.
24. Sonnenberg A, Calafat J, Jansen H, *et al.* – Integrin α6β4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell basement membrane adhesion. *J Cell Biol* 1991; 113: 907–917.
25. Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK. – α6β4 integrin heterodimer is a component of hemidesmosomes. *Proc Natl Acad Sci USA* 1990; 87: 8970–8974.
26. Albelda SM. – Endothelial and epithelial cell adhesion molecules. *Am J Respir Cell Mol Biol* 1991; 4: 195–203.
27. Damjanovich L, Albelda SM, Mette SA, Buck CA. – Distribution of integrin cell adhesion receptors in normal and malignant lung tissue. *Am J Respir Cell Mol Biol* 1992; 6(2): 197–206.
28. Sapsford RJ, Devalia JL, McAulay AE, d'Ardenne AJ, Davies RJ. – Expression of α1–6 integrin cell surface receptors in normal human bronchial biopsies and cultured bronchial epithelial cells. *J Allergy Clin Immunol* 1991; 87 (Suppl.): A303.
29. Yurchencko PD, Schittny JC. – Molecular architecture of basement membrane. *FASEB J* 1990; 4: 1577–1590.
30. Britten KM, Howarth PH, Roche WR. – Immunohistochemistry on resin sections, a comparison of resin embedding techniques for small mucosal biopsies. *Biotechnic Histochem* 1992; (submitted).
31. Lotz MM, Korzelius CA, Mereurio AM. – Human colon carcinoma cells use multiple receptors to adhere to laminin: involvement of α6β4 and α2β1 integrins. *Cell Regul* 1991; 1: 249–257.
32. Godfrey RWA, Severs NJ, Jeffery PK. – Freeze-fracture morphology and quantification of human bronchial epithelial tight junctions. *Am J Respir Cell Mol Biol* 1992; 6: 453–458.
33. Boller K, Vestweber D, Kemler R. – Cell-adhesion molecule, uvomorulin, is localised in the intermediate junctions of adult intestinal epithelial cells. *J Cell Biol* 1985; 100: 327–332.
34. Herbert CA, Edwards D, Boot JR, Robinson C. – *In vitro* modulation of the eosinophil-dependent enhancement of the permeability of the bronchial mucosa. *Br J Pharmacol* 1991; 104: 391–398.