

## Structural characterization of bronchial mucosal biopsies from healthy volunteers: a light and electron microscopical study

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*Structural characterization of bronchial mucosal biopsies from healthy volunteers: a light and electron microscopical study. M. Söderberg, S. Hellström, T. Sandström, R. Lundgren, A. Bergh.*

**ABSTRACT:** Bronchial mucosal biopsies were collected by flexible fibroptic bronchoscopy (FFB) from normal healthy volunteers. The volunteers were not receiving any medication and had been free from respiratory infection for at least five weeks prior to the bronchoscopy. Biopsies with a minimum of 115  $\mu\text{m}$  of undamaged perpendicularly sectioned epithellum were investigated by light and transmission electron microscopy. The height of the epithellum varied between 22–62.5  $\mu\text{m}$ , with a median value of 41.5  $\mu\text{m}$ . The volume density of the various epithelial cells in relation to the total epithellum was 61% for the ciliated and non-ciliated, non-secretory luminal cells, 6% for the goblet cells and 32% for the basal cells. The width of the lamina reticularis of the basement membrane ranged between 3–17.5  $\mu\text{m}$ , with a median value of 8.5  $\mu\text{m}$ . Blood vessels occupied 2.8% of the subepithelial tissue. Only a few inflammatory cells were seen in the connective tissue, volume density occupied by lymphocytes and polymorphonuclear granulocytes, 0.6% and 0.2% respectively. This study shows that structural damage is a common finding in biopsies collected by FFB. Mechanical damage of bronchial biopsies caused by the forceps should be considered before alterations in the epithellum are attributed to pulmonary disease.

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Flexible fibroptic bronchoscopy (FFB) has opened a new field in the diagnosis of pulmonary disease [1–3]. This rapid, relatively simple and harmless procedure enables direct inspection of the lower respiratory tract. Small biopsy forceps allow collection of mucosal biopsies from well defined areas. These biopsies are valuable in the diagnosis of carcinoma [2, 3], other respiratory diseases, such as sarcoidosis [4], and in studying the effect of airway irritants. Recently, changes in the bronchial mucosa, such as epithelial shedding have attracted interest in attempts to understand the pathogenesis of asthma [5]. However, in order to interpret the various findings in disease, it is first necessary to have a detailed knowledge of the normal structure of the bronchial mucosa, and to determine whether the procedure of tissue removal by forceps [6] or tissue preparation gives rise to artefacts. There appear to be no extensive reports on the morphology of bronchial mucosal biopsies from healthy volunteers.

### Materials

Bronchial mucosal biopsies were collected from 37 healthy nonsmoking volunteers, aged 22–44 yrs, mean age 29.2. Two were women. The vital capacity (VC) and forced expiratory volume in one second (FEV<sub>1</sub>) of all volunteers was >70% of the predicted normal value. The subjects did not have any signs of upper or lower respiratory disease for at least five weeks before the FFB and were not receiving any medication. Bronchial mucosa was biopsied from the second or third order bronchi of the right or left upper or lower lobes. Two to five biopsies were taken from each subject, a total of 120 in all. Biopsies with at least 115  $\mu\text{m}$  of undamaged perpendicularly sectioned epithellum and a depth from the mucosal surface of approximately 60  $\mu\text{m}$  were used for quantitative studies. Thirty-nine biopsies from 27 subjects were examined by light microscopy (LM) and transmission electron microscopy (TEM), and 16

biopsies from 10 subjects with scanning electron microscopy (SEM). The study was approved by the Ethical Committee of the University of Umeå.

### Methods

The volunteers were premedicated with 0.25–0.75 mg atropine or 0.25–0.75 ml morphine-scopolamine (morphine 10 mg·ml<sup>-1</sup> and scopolamine 0.4 mg·ml<sup>-1</sup>) subcutaneously. Topical anaesthesia, induced by 250–280 mg of lignocaine was sprayed through the mouth, dripped into the larynx and trachea with a syringe, and applied into the bronchi *via* the bronchoscope. The bronchoscope was introduced through the mouth without intubation and with the subject in supine position.

The bronchoscopes used were Olympus BF1T or BF1T10 and the biopsy forceps were Olympus FB20C, FB21C, FB22C or FB24K (Olympus, Tokyo, Japan). The biopsies were taken by three trained bronchoscopists.

#### *Light microscopy and transmission electron microscopy*

All biopsies were collected into physiological saline and transferred to 2.5% buffered glutaraldehyde within half an hour. After at least 24 hours fixation, the biopsies were rinsed in a 0.1 M solution of sodium cacodylate buffer (pH 7.2) for 30 minutes and then postfixed in 1% osmium tetroxide in the same buffer, overnight at 4°C. After another rinse, the specimens were dehydrated in a graded series of acetone and then embedded in an epoxy-resin Polybed 812. Sections were cut at 1 µm for light microscopy and 70–80 nm for TEM. The 1 µm sections were stained with toluidine blue, examined by light microscopy and photographed. For TEM, the sections were contrasted with uranylacetate and lead citrate.

#### *Morphometric analyses*

Using a light microscope with a drawing tube, the height of the epithelium was measured on a digitizing table connected to an MOP Videoplan Image Analyzer (Kontron AG, West Germany). The measurements were made on sections cut perpendicular to the ciliated epithelium at a magnification of 40×. In each section, the height of the epithelium was determined at three to five different points, 60–115 µm apart. The thickness of the lamina reticularis of the basement membrane (for nomenclature [7]), the collagenous noncellular layer beneath the lamina densa of basement membrane, was also measured. By a point counting method, the volume density of goblet cells, ciliated and non-ciliated, non-secretory luminal cells and basal cells was measured in relation to the total epithelial volume (fig. 1). The volume density of polymorphonuclear granulocytes, lymphocytes, erythrocytes and vessels in the connective tissue was also determined to a depth of 60–300 µm from the mucosal surface.



Fig. 1. – Light micrograph of normal human bronchial mucosa. The grid exemplifies how the morphometric analysis was performed by a point-counting method. The intersections are the points. Toluidine blue staining. Bar=20 µm.

#### *Scanning electron microscopy*

The biopsies were gently washed in physiological saline to remove mucus mechanically, fixed in 2.5% glutaraldehyde, critical point dried, mounted on holders and coated with gold [6]. The specimens were examined in a scanning electron microscope at a magnification of 600–12,000 times.

#### Statistics

The Spearman rank correlation was used to test the correlation between epithelial height and volume density of basal cells.

#### Results

All biopsies had a round or oval shape, often with a little tail attached. The mark made by the needle biopsy forceps BF24K was easily recognized in some of the biopsies (fig. 2).

#### *Light microscopy*

The biopsies contained connective tissue, lined with a pseudostratified, ciliated, columnar epithelium. Irrespective of the type of biopsy forceps, the epithelium exhibited damaged areas in which epithelial cells were lacking or in which the epithelial height was considerably reduced (fig. 2b). Such damaged areas were not evaluated quantitatively. The height of the epithelium varied between 22–62.5 µm with a mean±SD of 42±5.41 µm, median value 41.5 (n=39). Within one biopsy, the epithelial height was fairly uniform but could occasionally differ up to 32 µm from one point to another.

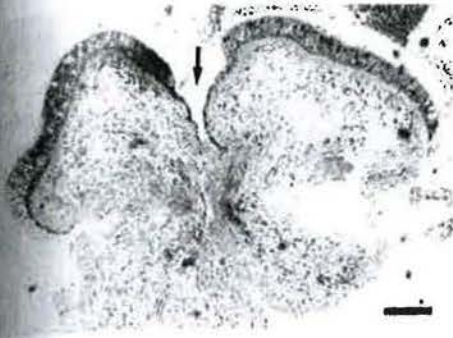


Fig. 2. - a) Light micrograph of a bronchial biopsy from a healthy volunteer. The needlemark of the biopsy-forceps is seen in the centre (arrow). Toluidine blue staining. Bar=100  $\mu$ m. Left: Biopsy-forceps (10x24K) (approximately normal size). b) Detail of needle impression shown in a). Note the successive flattening of the epithelium, leaving only one layer of cells in the bottom of the needle mark. LM. Bar=20  $\mu$ m.

The epithelium consisted of columnar ciliated cells, and occasional non-ciliated, non-secretory luminal cells intermingled with a few goblet cells. In most cases, two layers of basal cells were apparent (fig. 3). The basal cells occupied 32% of the epithelium with a range between 16-52% (table 1). There was a positive correlation between the volume of the basal cells and the height of the epithelium. Some transitional cells resembling both basal and ciliated cells were observed. The intercellular space was narrow, except near areas where the epithelium had been torn off the underlying stroma.

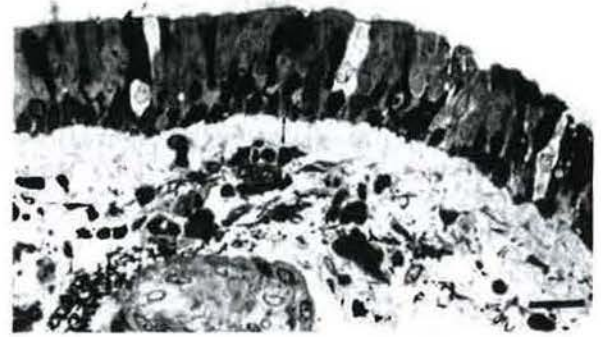


Fig. 3. - Light micrograph of a sectioned normal human bronchial epithelium. Arrows indicate the thickness of the subepithelial lamina. Toluidine blue staining. Bar=20  $\mu$ m.

Table 1. - Volume density of epithelial cells. The values are given as mean $\pm$ SD (n=39)

%	mean $\pm$ SD	range
Ciliated and non-ciliated, non-secr. luminal cells	61 $\pm$ 9.5	33-80
Basal cells	32 $\pm$ 8.6	16-52
Goblet cells	6 $\pm$ 6.3	0-25

Immediately below the lamina densa of the basement membrane, was the lamina reticularis with densely packed collagenous fibres (fig. 3). The thickness of the lamina reticularis ranged between 4.5-17.5  $\mu$ m with a mean $\pm$ SD of 8 $\pm$ 2.82  $\mu$ m, median value 8.5  $\mu$ m (n=39). The intra-individual difference ranged between 1-13.5  $\mu$ m. The inner surface of the lamina reticularis faced a loose connective tissue, 2.8% of which was occupied by the blood vessels (table 2). Some polymorphonuclear granulocytes and lymphocytes and a few extravasated erythrocytes were seen in the connective tissue. Occasional mast cells also occurred. There was no apparent difference in the stromal composition between the superficial and deeper parts of the biopsies.

Table 2. - Volume density of blood cells and vessels in the stroma. The values are given as mean $\pm$ SD (n=39)

	mean $\pm$ SD %	range
Lymphocytes	0.56 $\pm$ 1.0	0-4
Polymorphonuclear leucocytes	0.15 $\pm$ 0.4	0-1.6
Erythrocytes	1.34 $\pm$ 2.6	0-9
Vessels	2.81 $\pm$ 3.2	0-17

### Transmission electron microscopy

The intercellular spaces were generally narrow but widened intercellular spaces appeared in some specimens, either just beneath the epithelial surface or immediately above the basement membrane (fig. 4). The widened intercellular spaces occurred in intact epithelium and near sites of disrupted epithelium.

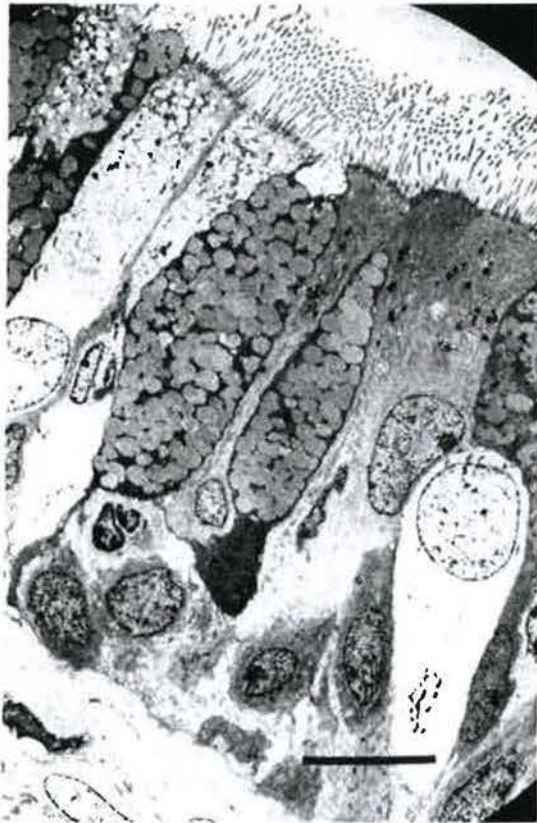


Fig. 4. - Electron micrograph of normal human bronchial epithelium. The intercellular space is narrow except between the basal cells just above the basement membrane. Bar=10  $\mu$ m.

Microvilli were not only apparent on the ciliated cells but also on the non-ciliated cells. Some ciliated cells showed numerous rounded or oval cytoplasmic vacuoles. The mitochondria were mainly distributed in the upper parts of the epithelial cells.

The goblet cells varied in appearance. Secretory granules often completely filled the upper portion of the cell, which bulged laterally towards neighbouring cells or protruded into the bronchial lumen (fig. 4). Despite analysis of numerous sections only single endocrine type cells with dense core granules were seen.

The lamina reticularis was composed of densely packed collagenous fibres (figs 5 and 6). A few bundles of nerves were observed and occasionally features resembling nerve terminals were identified in between the basal portion of the epithelial cells.

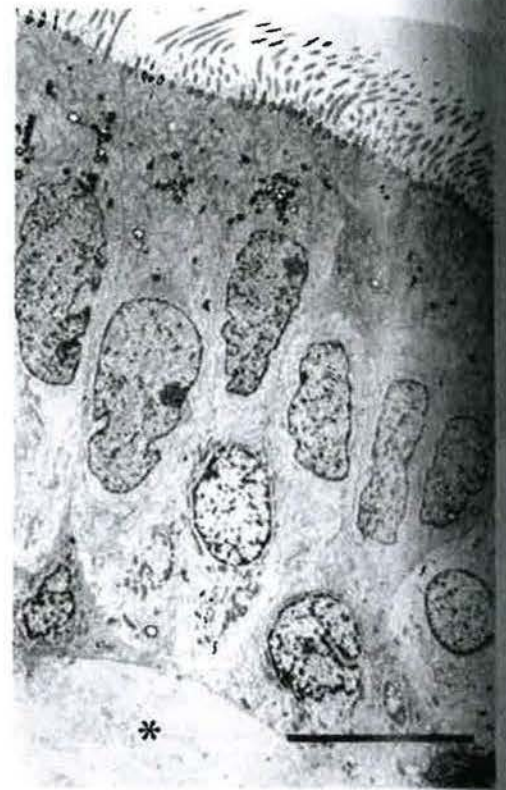


Fig. 5. - Electron micrograph of normal human bronchial epithelium. Goblet cells are lacking in this area. Note the narrow intercellular space and the reticular lamina of the basement membrane (\*). Bar=10  $\mu$ m.



Fig. 6. - Electron micrograph showing details of the subepithelial lamina reticularis (sl), of the basement membrane (arrows). The reticular lamina is composed of bundles of collagenous fibres. Bar=1  $\mu$ m.

### Scanning electron microscopy

The biopsies were covered by a thick carpet of cilia interrupted by goblet cells appearing like small holes. In a few biopsies, there were small areas of non-ciliated epithelial cells with or without microvilli.

## Discussion

One third of all biopsies collected were included in the study. Many of these biopsies showed an extensively damaged epithelium. The number of discarded biopsies is remarkably high despite the fact that they were collected by experienced bronchoscopists reflecting the difficulties of this procedure. The major cause of this damage has to be mechanical. It is reasonable to assume that the epithelial injury was caused by the forceps, since the bronchoscope hardly touched the bronchial wall before the biopsy was collected. This assumption is supported by the shape of the biopsies taken with the forceps BF24K in which the damaged epithelium directly reflects the design of the instrument (fig. 2).

In earlier investigations, shedding of epithelial cells has been interpreted as representing an integral compound of the disorder studied [8], but this study shows that the extent to which bronchial biopsies are damaged by the forceps should be considered before epithelial injuries are attributed to disease. Mechanical damage is even more likely if abnormal-looking epithelium in an apparently damaged area is found adjacent to normal-looking epithelium. To exclude such interpretation problems in our biopsies, only those with an intact epithelial surface covering at least 115  $\mu\text{m}$  were analysed qualitatively and quantitatively. Though mechanical damage appears to be the major cause of distortion, specimen preparation might also affect the tissue. This is particularly important when estimating the width of the intercellular spaces, which are strongly influenced by the osmolarity of the fixative.

The ratio of the goblet cells to the ciliated and non-ciliated, non-secretory luminal cells was approximately 1:10. This figure is lower than that reported for both trachea, 1:5 [9] and bronchi, 1:3 [10]. Compared to trachea, it seems that the number of goblet cells decreases peripherally in the bronchial tree. The relatively high ratio of goblet cells in bronchi found by McDowell *et al.* [10] can be explained by the fact that these investigators collected some of their specimens from heavy smokers, which is known to induce hyperplasia of mucus-producing cells [11]. In our study all subjects were nonsmokers. Occasional epithelial cells with a cytoplasmic density and a configuration resembling ciliated cells but without cilia were noted in the TEM. These intriguing cells also lacked basal bodies. The function of this type of cell is not known. Brush cells have been reported in the bronchial epithelium [12, 13], but we did not find any in our study. Although most epithelial cells appeared normal, certain cells exhibited cytoplasmic vacuoles, which could be a result of the preparative procedure or indicate pathological or age-dependent changes.

The epithelial height and volume density of basal cells showed a positive correlation. The physiological significance of this is unknown, but has been previously referred [14]. To avoid variations in thickness caused by the sectioning procedure, the reticular lamina was measured in sections in which the epithelium had been cut perpendicularly. Despite this, the thickness of the

subepithelial lamina varied considerably. In fact, the thickness of the lamina reticularis in some of our healthy subjects is comparable to dimensions reported for lamina reticularis in patients suffering from asthma [15, 16] and asymptomatic asthmatics [15]. MEINL and BRUNNER [17] suggested that the thickness of the basement membrane increases with age. The present results do not favour this suggestion as laminae reticulares measuring up to 17.5  $\mu\text{m}$  were seen in our young or middle-aged subjects.

The bronchial microvasculature has attracted attention following suggestions that it participates in the pathophysiology of asthma. In the present study, the bronchial microvasculature occupied almost 3% of the connective tissue. It has been speculated that inflammatory and/or neurohumoral mediators cause contraction to endothelial cells [18]. Plasma escapes through widened interendothelial gaps, and then filters through the basal membrane that seem to offer little hindrance to plasma proteins [8]. The mediator-induced microvascular leakage will cause increased airway resistance by mucosal and submucosal oedema.

In summary, extensive epithelial damage of bronchial biopsies collected in healthy subjects by FFB was commonly found. Mechanical damage caused by the biopsy technique should be considered prior to associating tissue change with a diseased condition. The ratio of the goblet cells to ciliated cells was 1:10. The thickness of the lamina reticularis of the basement membrane varied considerably and did not increase with age. The number of polymorphonuclear leucocytes, lymphocytes and mast cells was remarkably low. A complete absence of or a presence of a negligible number of such inflammatory cells could be a reliable criterion to differentiate healthy from diseased individuals. We hope that the measurements made of the various components will subsequently be compared with the same measurements in biopsies from patients with asthma and other respiratory diseases. Such work is in progress in our laboratory.

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*Caracterisation structural des biopsies de la muqueuse bronchique chez des volontaires sains etude au microscope optique et electronique. M. Söderberg, S. Hellstrom, T. Sandstrom, Lundgren, A. Bergh.*

RÉSUMÉ: Des biopsies de la muqueuse bronchique ont été prélevées chez 39 sujets par fibroscopie. Les sujets n'avaient pas eu d'infection des voies aériennes pendant au moins 3 semaines avant la bronchoscopie. Aucun d'entre eux n'était fumeur et aucun ne prenait de médicaments. Trente-neuf biopsies avec au minimum 115 µm d'épithélium intact ont été examinées en microscopie optique et en microscopie électronique à transmission. Dix-huit biopsies ont été examinées en microscopie électronique à balayage. La hauteur de l'épithélium variait entre 22–62.5 µm, valeur moyenne 41.5 (médiane 41.5). La proportion des différentes cellules par rapport à l'épithélium total était respectivement: 61% (33–80) pour les cellules ciliées et non ciliées, 6% (0–25) pour les cellules à mucus et 32% (16–52) pour les cellules basales. La membrane basale était soustendue par une couche de fibres de collagène d'une épaisseur de 3–17.5 µm, moyenne 8.0 (médiane 8.5). Les vaisseaux sanguins occupaient 2.8% (0.17) du chorion et il y avait peu de cellules inflammatoires: 0.6% de lymphocytes et 0.2% de neutrophiles. Cette étude montre que les lésions épithéliales sont très fréquentes dans les biopsies prélevées par fibroscopie bronchique. Par conséquent, avant de considérer les lésions épithéliales comme la manifestation de pathologies pulmonaires il faut envisager la possibilité d'altérations dues aux pinces du bronchoscope. *Eur Respir J.*, 1990, 3, 261–266.