Eur Respir J
1990, 3, 1023–1028

Bronchial epithelium in humans recently recovering from respiratory infections caused by influenza or Mycoplasma

M. Söderberg*, S. Hellström**, R. Lundgren*, A. Bergh***

Bronchial epithelium in humans recently recovering from respiratory infections caused by influenza or Mycoplasma. M. Söderberg, S. Hellström, R. Lundgren, A. Bergh.

ABSTRACT: Sixty three bronchial mucosal biopsies were collected from 12 patients recovering from respiratory infections with influenza virus (n=8) and Mycoplasma (n=4). The bronchoscopy was performed within 1–6 wks from the onset of symptoms. Of the 63 biopsies prepared for light microscopy, 27 biopsies from the influenza patients and 10 from the Mycoplasia patients were further analysed at the light and electron microscope level. The epithelium and the underlying stroma was analysed morphometrically and the results were compared to those of 39 biopsies from 27 healthy subjects. A slightly raised bronchial reactivity was noted in two patients. In the influenza patients the epithelial height was greater compared to that of the healthy subjects and subepithelial lymphocytosis was noted. Areas with damaged epithelium, epithellum shedddlng, and occasionally a thickened basement membrane, considered typical for asthma, occurred in both infected groups, and in the biopsies of the healthy subjects. *Eur Respir J., 1990, 3, 1023–1028.*

Depts of * Lung Medicine, ** Anatomy and *** Pathology, University Hospital, Umeå, Sweden.

Correspondence: M. Söderberg, Dept of Lung Medicine, University Hospital, S-901 85 Umeå, Sweden.

Keywords: Biopsy; bronchial mucosa; bronchial reactivity; influenza; morphometry; Mycoplasma.

Received: October 9, 1989; accepted after revision May 9, 1990.

This study was supported by grants from the Swedish Heart Lung Foundation and the Swedish Association against Allergic Diseases.

Influenza and Mycoplasma infections are sometimes associated with the onset or aggravation of asthma [1–4]. It has been speculated that asthma is evoked secondary to epithelial damage caused by the microorganisms, or induced by mediators released from the damaged epithelium, or inflammatory cells, or induced by other mechanisms [5]. Injuries to the bronchial epithelium; epithelial shedding and mucosal oedema are thought to be some of the mechanisms involved in the pathogenesis of asthma [6–9].

The aim of the present study was to examine whether the bronchial mucosa of patients, recovering from these two infections, exhibit epithelial changes similar to those observed in asthmatics. Furthermore, we wanted to elucidate whether there was an increased reactivity in the post-infectious period, and if there was a correlation between this reactivity and the degree of epithelial damage.

Materials and methods

Biopsies from the bronchial mucosa were collected from 12 patients, 7 women and 5 men, aged 23–51 yrs, who had recently recovered from a respiratory infection caused by either an influenza virus; Influenza A (n=6), Influenza B (n=1), Parainfluenza (n=1) or *Mycoplasma pneumoniae* (n=4). The diagnosis was established by a fourfold increase in the antibody titre, which is considered to be diagnostic for the disease (table 1); the testing being performed by a complement binding technique. Six of the patients had more than two positive reactions when skin prick tested with a range of common allergens; three not being prick tested and three being negative. Three subjects were smokers. One patient had been free from asthma for at least 10 yrs, and the others had no history of previous respiratory disease (table 1). The biopsies were compared to 39 biopsies from 27 healthy nonsmoking volunteers, analysed in a previous study [10]. The study was approved by the Ethical Committee of the University of Umeå.

Bronchoscopy

Bronchoscopy was performed using a flexible fibreoptic bronchoscope within 1–6 wks after the onset of the symptoms. The patients were premedicated with morphine-scopolamine (10 $mg \cdot ml^{-1}$ + 0.4 $mg \cdot ml^{-1}$) 0.5–1 ml or atropine sulphate 0.5–0.75 mg. Lidocaine up to 300 mg was used for local anaesthesia. The bronchoscopes used were Olympus BF 1T and BF 10T, and the examination was performed without intubation, with the patient in the supine position. The biopsies were taken, from the second or third order bronchi, from the right or left upper or lower lobes (table 1), by two experienced bronchoscopists.

Table 1. – Data of each patient with influenza or Mycoplasma infection

Pt no.	Sex/age	Type infection/ antibody titre	Heredity of asthma/smoker	Skin prick test	PC_{20} $mg \cdot ml^{-1}$	Bronchoscopy at time after onset wks	No. and sites of biopsies
1*	F/42	My/256	N/Y	Neg	5.8–12	4	add
2	M/46	I.A/128	N/N	Not done	14.5	1	aaac
3	F/38	My/256	N/N	Neg	>16	4	aa
4	F/52	My/64	N/N	Pos	>16	6	aabb
5	F/27	I.A/128	Y/N	Pos	5.6–9.8	4	aabbb
6	M/51	I.A/128	N/N	Neg	>16	4	aabccd
7	F/35	PI/128	Y/Y	Pos	>16	3	aaabbccd
8	F/48	I.A/32**	N/N	Pos	>16	4	abbbcdd
9	F/41	I.B/128	Unknown/Y	Pos	>16	5	aaaccccd
10	M/34	My/256	N/N	Not done	>16	6	aacccd
11	M/26	I.A/128	N/N	Pos	8.5	4	aaabcc
12	M/27	I.A/128	N/N	Not done	Not done	6	aaabb

My: Mycoplasma; I: Influenza (A or B); PI: Parainfluenza; N: no; Y: yes; M: male; F: female; *: all biopsies lacked epithelium; **: direct positive immunofluorescence; Neg: negative; Pos: positive; a: right upper lobe; b: right lower lobe; c: left upper lobe; d: left lower lobe. The biopsies underlined are included for morphometry. PC_{20}: provocative concentration producing a 20% fall in forced expiratory volume in one second.

Light microscopy (LM) and transmission electron microscopy (TEM)

The biopsies were collected into saline and then transferred into 2.5% glutaraldehyde within 15 min. After immersion in the fixative for at least 24 h, the specimens were rinsed in a 0.1 M solution of sodium cacodylate buffer (pH 7.2) for 30 min and then postfixed overnight at 4°C in 1% osmium tetroxide in the same buffer. After another rinse, the specimens were dehydrated in a graded series of acetone and embedded in an epoxy resin, Polybed 812. Sections were cut at 1 μm for LM and 70–80 nm for TEM. The 1 μm sections were stained with toluidine-blue and examined by LM. For TEM, the sections were contrasted with uranyl acetate and lead citrate.

Morphometrical analyses

All of the biopsies were studied in the light microscope but only those biopsies which had a surface of at least 115 μm of continuous undamaged epithelium, were included in the morphometrical analysis [10]. From a total of 63 biopsies, obtained from the 12 diseased subjects, 39 (62%) were included, 27 of 48, (56%) influenza biopsies, and 10 of 15, (67%) Mycoplasma biopsies. From the 27 healthy subjects, 39 biopsies out of 77 (51%) were included. In 11 biopsies from the diseased subjects, the subepithelial tissue adjacent to damaged epithelium was compared with that adjacent to undamaged epithelium.

Using a light microscope with a drawing tube, the height of the epithelium and the thickness of the lamina reticularis of the basement membrane was measured on a digitizing table, connected to an MOP Videoplan Image Analyser (Kontron AG, West Germany) as described earlier [10]. The measurements were made on sections cut perpendicular to the surface of the

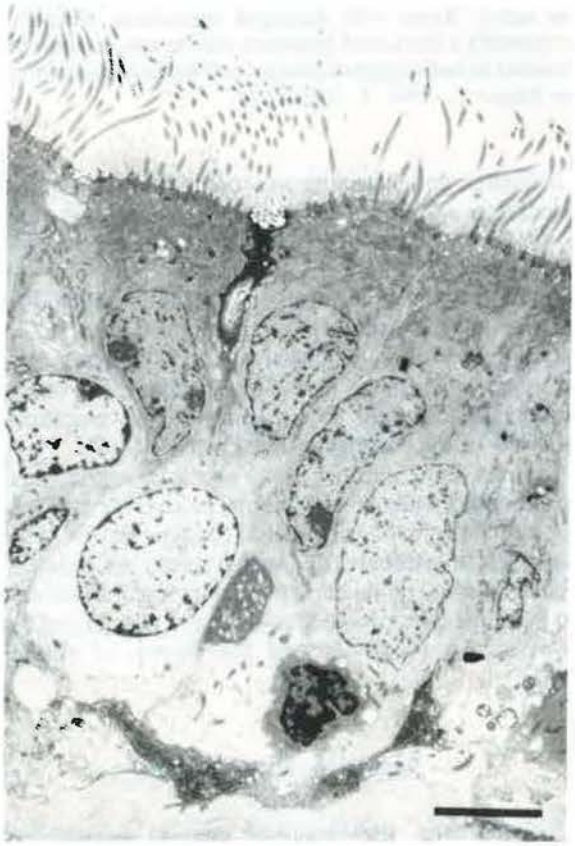

Fig. 1. – Electron micrograph of a human bronchial biopsy collected from a patient 6 wks after onset of symptoms of Mycoplasma infection. Bar=5 μm.

epithelium and at 3–5 different points, 60–115 μm apart at a magnification of 400×. In six biopsies, the thickness of the lamina reticularis of the basement membrane in areas covered by an intact epitheluim, was compared to that in areas of damaged epithelium. By a point counting method, using a square lattice in the light microscope at the same magnification, the volume density of ciliated and non-ciliated non-secretory luminal cells, goblet cells and basal cells was measured in relation to the total epithelial volume. Volume density of polymorphonuclear granulocytes, lymphocytes and vessels in the subepithelial tissue was measured to a depth of 60–180 μm from the mucosal surface. The results were compared to those obtained earlier from biopsies from the healthy volunteers [10]. Differences were tested by the Mann-Whitney test and considered significant when $p<0.05$.

Histamine and methacholine tests

Within one week after the bronchoscopy, bronchial reactivity to inhaled histamine or methacholine (concentrations up to 16 $mg \cdot ml^{-1}$) was measured according to the method described by HARGREAVE *et al.* [11]. The provocation concentration causing a fall in the forced expiratory volume in one second (FEV_1) of 20% was expressed as PC_{20}. One person was not tested due to the geographical distance between home and hospital.

Results

Except for a cough in five cases, the patients were free of other symptoms at the time of the bronchoscopy. All had a vital capacity (VC) and FEV_1 of more than 75% of the predicted normal values. Bronchial reactivity was slightly raised in two subjects. One month later, it had returned to normal. The X-ray findings were normal, except in one of the Mycoplasma patients (no. 10) who had minor interstitial infiltration in the inferior part of the left lung.

At bronchoscopy, the bronchial mucosa looked normal or had a slight reddish appearance. In the light microscope, the majority of biopsies from the patients were similar to those from the healthy subjects (fig. 1). Areas of intact epithelium were often interrupted by smaller or larger areas of partially destroyed epithelium or a denuded basement membrane (fig. 2).

The epithelial height was greater in the influenza patients than the controls (table 2). In these patients the median value was 50 μm and the upper and lower quartiles were 45–51 μm (n=8); the corresponding values in the healthy subjects being 41 μm and 38–45 μm. The epithelial height in the Mycoplasma patients had a median value 45 μm (n=3), range 42–55 μm. Occasionally an increased intercellular space was noted but this did not differ from that of the healthy subjects

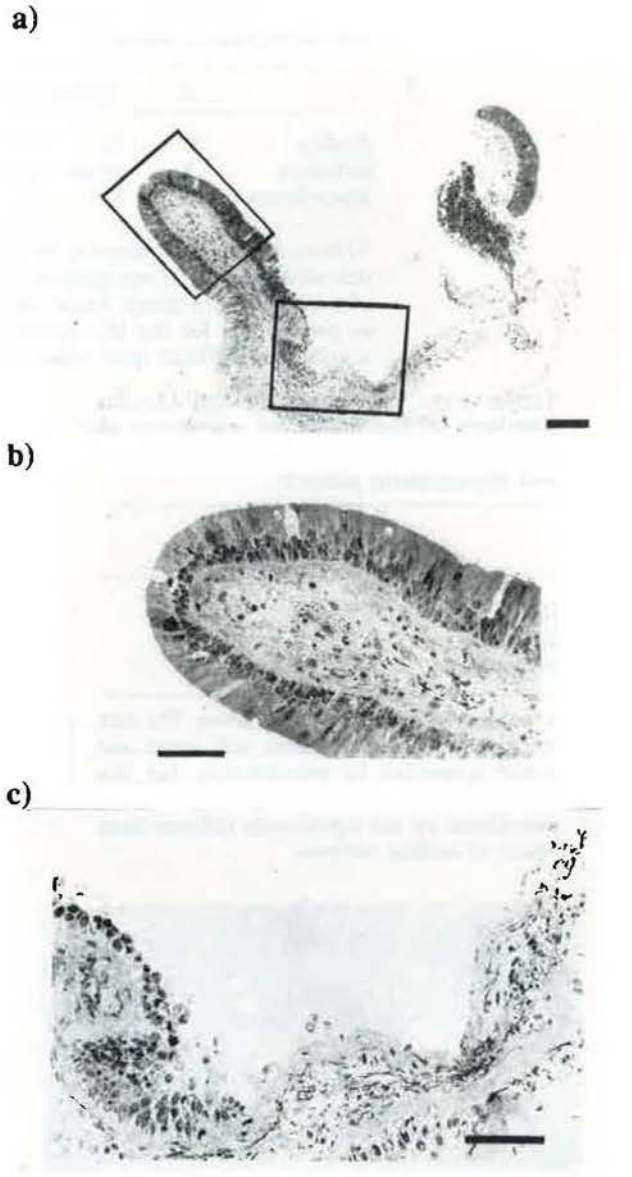

Fig. 2. – a) Light micrograph of a bronchial biopsy obtained from a patient 6 wks after onset of symptoms of Mycoplasma infection. The bronchial mucosa appears normal except for the damage caused by the biopsy forceps. Toluidine blue staining. Bar=100 μm. b) Detail of intact epithelium in (a). Bar=50 μm. c) Detail of denuded basement membrane in (a). Bar=50 μm.

Table 2. – The height of the surface epithelium in the bronchial biopsies of influenza and Mycoplasma patients

	n	Height μm	
Healthy	27	41	(38–45)
Influenza	8	50*	(45–51)
Mycoplasma	3	45	(42–55)

n: number of subjects in each group. Data are given as median values with upper and lower quartiles in parenthesis, for the Mycoplasma patients with range in parenthesis. *: $p<0.05$ when compared to the healthy subjects.

Table 3. – Composition of bronchial epithelial cells in biopsies from influenza and Mycoplasma patients

	n	Cil/non-cil		Goblet		Basal	
Healthy	27	61	(54–67)	4	(0–8)	32	(30–36)
Influenza	8	64 NS	(60–66)	3 NS	(2–5)	34 NS	(32–35)
Mycoplasma	3	63	(61–77)	3	(0–4)	34	(23–35)

Volume density is expressed as % of epithelial volume occupied by ciliated (cil) and non-ciliated (non-cil) non-secretory luminal cells, goblet cells, basal cells. n: number of subjects in each group. Values are given as median with upper and lower quartiles in parenthesis, for the Mycoplasma patients with range in parenthesis. NS: not significantly different from values of the healthy subjects.

Table 4. – Thickness of the lamina reticularis of the basement membrane of bronchial epithelium obtained from influenza and Mycoplasma patients

	n	Thickness µm	
Healthy	27	8	(7–9)
Influenza	8	7 NS	(7–7)
Mycoplasma	3	5 NS	(5–8)

n: number of subjects in each group. The data are given as median values with upper and lower quartiles in parenthesis, for the Mycoplasma patients with range in parenthesis. NS: not significantly different from values of healthy subjects.

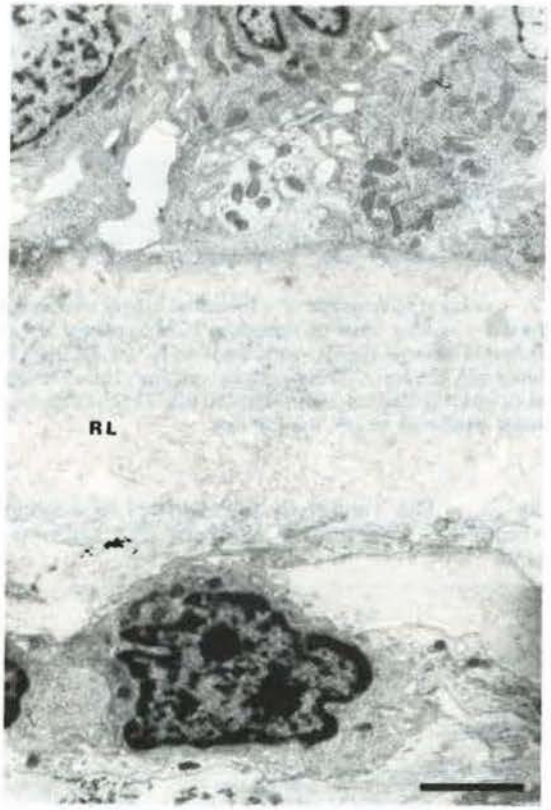

Fig. 3. – Electron micrograph of the reticular lamina (RL), of the basement membrane of human bronchial epithelium in a biopsy from a patient one week after the onset of symptoms of influenza. Bar=2 µm.

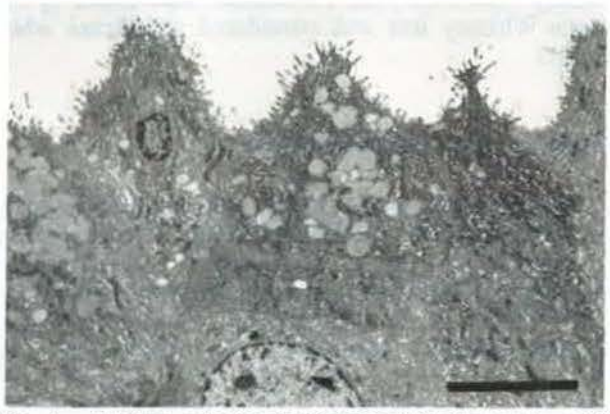

Fig. 4. – Electron micrograph of regenerating human bronchial epithelium. Bronchial biopsy collected from a patient one week after onset of symptoms of influenza infection. Note the absence of cilia. Bar=5 µm.

[10]. Moreover, the volumes occupied by the various cell types in the epithelium did not vary (table 3). The thickness of the lamina reticularis of the basement membrane was not increased (table 4) (fig. 3). The thickness of the basement membrane in areas covered by an intact epithelium did not differ from that in areas with a denuded basement membrane, in the six biopsies containing large areas of both intact epithelium and denuded basement membrane. The volume density of the subepithelial elements is shown in table 5. A slight but significant increase in the number of subepithelial lymphocytes was noted in the influenza patients.

The volumes occupied by the measured components of the subepithelial tissue, not covered by an intact epithelium, did not differ from that of the areas with an intact epithelium or from that of the healthy subjects. Neither did the smokers differ from the nonsmokers in this respect.

In one of the biopsies from an influenza patient (no. 2), obtained one week after the onset of symptoms, areas of a lower epithelium without any or with a reduced number of cilia were observed (fig. 4). Also, in the other biopsies from this patient in areas of a higher epithelium only occasional ciliated cells were seen. In all the other patients and in the healthy subjects the non-ciliated cells were sparse.

Table 5. – Inflammatory cells and vessels in the subepithelial tissue of the bronchial mucosa in influenza and Mycoplasma patients

	n	Lymph		Poly		Vessels	
Healthy	27	0	(0–1.1)	0	(0–0)	1.9	(1.2–3.3)
Influenza	8	2*	(0.8–3)	0 NS	(0–0)	3 NS	(1.8–3.3)
Mycoplasma	3	1	(1–2)	1	(0–1)	2	(1–5)

Volume density is expressed as % volume occupied by lymphocytes (Lymph), polymorphonuclear leucocytes (Poly) and vessels. n: number of subjects in each group. Data are given as median values with upper and lower quartiles in parenthesis, for the Mycoplasma patients with range in parenthesis. *: $p<0.05$; NS: not significantly different from values of healthy subjects.

Discussion

The present study showed no major differences either in degree of epithelial damage, or in the thickness of basement membrane between the biopsies from healthy and those from the diseased subjects. These findings are in contrast to previous studies [12, 13] in which patients suffering from influenza have been reported to exhibit a reddened mucosa, epithelial shedding and sometimes a denuded basement membrane [13]. However, the patients in the study by HERS [12] had been analysed during active disease whilst in the present study the patients had recovered and had no symptoms. Nevertheless, tracheobronchial clearance has been reported to remain reduced up to 15 months after Mycoplasma infection [14, 15], and mucociliary clearance in the nose being reduced up to 32 days after the onset of symptoms of a common cold. However, a long-lasting dysfunction of the bronchial mucociliary apparatus does not necessarily mean a damaged structure of the epithelium.

The biopsies from the patients recovering from the infections exhibited damaged areas which were considered as artifacts of the biopsy procedure; the damaged areas being similar to those reported for biopsies from healthy subjects [10]. The relative number of biopsies with at least 115 μm of intact epithelium in the present study was greater than that in the study of the healthy subjects. Despite standardization of techniques used by experienced bronchoscopists, the number of discarded biopsies was great. Although epithelial damage caused by the infections cannot be excluded, we regard most of the epithelial damage as being related to the biopsy procedure. We are at present in the process of investigating modifications in the biopsy procedure.

The only significant difference between the influenza patients and the healthy subjects, was that in the influenza patients the height of the surface epithelium was greater and a slight lymphocytosis in the subepithelial tissue was noted. The increased epithelial height may indicate that epithelial repair and regeneration had taken place after damage caused by the infection. Similar changes of the epithelium, caused by infection, have been reported at other sites of the respiratory tract after infection [16, 17]. Epithelial changes caused by influenza appear to be short-lasting, since regenerating epithelium was observed in the patient examined in the first week after the onset of disease (fig. 4). Epithelial repair after influenza infection had been described in man [12, 18] and mice [19]. In infected mice, regeneration of epithelium was already obvious within five days. After ten days the epithelium was low but contained both ciliated and non-ciliated cells [19]. This might correspond to the assumed phase of regeneration, noted as areas of a low epithelium and absence of cilia, in the biopsies in the present study collected one week after the onset of symptoms from the patients with influenza.

In the study by PEDERSEN *et al.* [20] on 26 humans with common colds, nasal epithelial scrape biopsies were collected within 24–32 h from the onset of symptoms, and at regular intervals for 32 days. The number of ciliated cells in relation to all columnar cells in the scrapes were lowest at day four, and gradually increased to reach normal values within 5–7 wks [20]. In children, nasal epithelium has been reported to return to normal 2–10 wks after influenza and other viral infections [18].

An increased thickness of the basement membrane has been considered pathognomonic for asthma. The thickness of the lamina reticularis of the basement membrane in our study was similar to values noted in asthmatics [21], but there was no significant difference between the biopsies from the patients and those from the healthy subjects. Furthermore, the thickness of the basement membrane did not differ between areas covered by an intact epithelium and areas with a denuded basement membrane. It is doubtful whether a thickened basement membrane is an indicator of bronchial disease. The results also stress the importance of having extensive and valid control material before any conclusions from material in a diseased condition can be made.

Bronchial reactivity was normal in 10 of the 12 patients, probably because of the fairly long interval between onset of disease and examination. Bronchial reactivity during influenza infection has been described to rise initially, but to normalize within seven weeks [22]. The slight but significantly raised number of inflammatory cells in the subepithelial tissue of the influenza patients observed in the present study was probably evidence of a bronchial inflammation.

Conclusions

In previous investigations influenza and Mycoplasma infections have been associated with the onset of asthma, a condition morphologically characterized by epithelial shedding and a thickened basement membrane [6–8]. The present study showed no persistent structural changes in the bronchial mucosa except an increase in the height of the epithelium and a slight lymphocytosis in the influenza patients. The study does not support the hypothesis that influenza and Mycoplasma infections induce epithelial changes associated with asthma. It is possible though that the epithelium in patients with a persistent bronchial hyperreactivity after these infections has a different appearance.

References

1. Beasly R, Coleman ED, Hermon Y, Holst PE, O'Donnel TV, Tobias, M. – Viral respiratory tract infection and exacerbations of asthma in adult patients. *Thorax*, 1988, 43, 679–683.
2. Berkovich S, Millian SJ, Snyder RD. – The association of viral and Mycoplasma infections with recurrence of wheezing in the asthmatic child. *Ann Allergy*, 1970, 28, 43–49.
3. Huhti E, Mokka T, Nikoskelainen J, Halonen P. – Association of viral and Mycoplasma infections with exacerbations of asthma. *Ann Allergy*, 1974, 33, 145–149.
4. Minor TE, Dick EC, Baker JW, Oulette JJ, Cohen M, Read CE. – Rhinovirus and Influenza type A infections as precipitants of asthma. *Am Rev Respir Dis*, 1976, 113, 149–153.
5. Smith TF, Remigio LK. – Histamine in nasal secretions and serum may be elevated during viral respiratory tract infection. *Int Arch Allergy Appl Immunol*, 1982, 67, 380–383.
6. Dunnill MS. – The pathology of asthma with special reference to changes in the bronchial mucosa. *J Clin Pathol*, 1960, 13, 27–33.
7. Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. – Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am Rev Respir Dis*, 1976, 113, 131–139.
8. Laitinen LA, Heino M, Laitinen A, Kava T, Haathela T. – Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis*, 1985, 131, 599–606.
9. Persson CGA. – Leakage of macromolecules from the tracheobronchial microcirculation. *Am Rev Respir Dis*, 1987, S135, 71–75.
10. Söderberg M, Hellström S, Sandström T, Lundgren R, Bergh A. – Structural characterization of bronchial mucosal biopsies from healthy volunteers. A light and electronmicroscopical study. *Eur Respir J*, 1990, 3, 261–266.
11. Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF, Dolovich J. – Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol*, 1981, 68, 347–355.
12. Hers JF. – Disturbances of the ciliated epithelium due to influenza virus. *Am Rev Respir Dis*, 1966, 93, 162–171.
13. Walsh JJ, Dietlein LF, Low FN, Burch GE, Mogabgab WJ. – Bronchotracheal response in human influenza. *Arch Intern Med*, 1961, 108, 376–388.
14. Camner P, Jarstrand C, Philipsson K. – Tracheobronchial clearance 5–15 months after infection with *Mycoplasma pneumoniae*. *Scand J Infect Dis*, 1978, 10, 33–35.
15. Jarstrand C, Camner P, Philipsson K. – *Mycoplasma pneumoniae* and tracheobronchial clearance. *Am Rev Respir Dis*, 1974, 110, 415–419.
16. Hermansson A, Prellner K, Hellström S. – Persistent structural changes in the middle ear mucosa of the rat after an experimentally induced episode of pneumococcal otitis media. *Acta Otolaryngol (Stockh)*, (in press).
17. Widemar L, Hellström S, Stenfors LE. – Different structural changes in membrane Schrapnelli in serous and purulent otitis media. *Acta Otolaryngol (Stockh)*, 1986, 102, 266–273.
18. Carson JL, Collier AM, Hu SS. – Acquired ciliary defects in nasal epithelium of children with acute viral upper respiratory infections. *N Engl J Med*, 1985, 312, 463–468.
19. Ramphal R, Fischlschweiger W, Shands JW Jr, Small PA Jr. – Murine influenzal tracheitis: a model for the study of infleunza and tracheal epithelial repair. *Am Rev Respir Dis*, 1979, 120, 1313–1324.
20. Pedersen M, Sakakura Y, Winther B, Brofeldt S, Mygind N. – Nasal mucociliary transport, number of ciliated cells, and beating pattern in naturally acquired common colds. *Eur J Respir Dis*, 1983, 64 (Suppl. 128), 355–364.
21. Cuts E, Levison H, Cooper DM. – Ultrastructure of airways in children with asthma. *Histopathology*, 1978, 2, 407–421.
22. Little JW, Hall WJ, Douglas RG Jr, Mudholkar GS, Speers DM, Patel K. – Airway hyperreactivity and peripheral airway dysfunction in influenza A infection. *Am Rev Respir Dis*, 1978, 118, 295–303.

L'épithélium bronchique chez les patients peu après la guérison d'infections respiratoires dues à l'Influenza ou au Mycoplasme. M. Söderberg, S. Hellström, R. Lundgren, A. Bergh.

RÉSUMÉ: Nous avons étudié 63 biopsies des muqueuses bronchiques prélevées chez 12 patients en convalescence après infection respiratoire au virus de l'Influenza (n=8) ou au Mycoplamse (n=4). La bronchoscopie a été exécutée entre une et six semaines après le début des symptômes. Des 63 biopsies examinées en microscopie optique, 27 provenant de patients atteints d'Influenza et 10 de patients atteints de Mycoplamse ont été analysées ultérieurement au microscope optique et électronique. L'épithélium et le stroma sous-jacent ont fait l'objet d'une analyse morphométrique, et les résultats ont été comparés à ceux de 39 biopsies provenant de 27 sujets bien portants. L'on a noté chez 2 patients une discrète augmentation de la réactivité bronchique. Chez les patients atteints d'Influenza, l'épaisseur de l'épithélium est augmentée, par comparaison à celle des sujets normaux, et l'on note une lymphocytose sous-épithéliale. Des zones d'atteinte épithéliale, de desquamation épithéliale, et parfois un épaississement des membranes basales, ont été considérés comme typiques pour l'asthme, se sont retrouvés dans les deux groupes infectés, ainsi que dans les biopsies provenant de sujets bien portants. *Eur Respir J., 1990, 3, 1023–1028.*