



Ultrastructure of bronchial biopsies from patients with allergic and non-allergic asthma

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Summary Epithelial damage is commonly found in airways of asthma patients. The aim of this study was to investigate epithelial damage in allergic and non-allergic asthma at the ultrastructural level.

Bronchial biopsies obtained from patients with allergic asthma ($n = 11$), non-allergic asthma ($n = 7$), and healthy controls ($n = 5$) were studied by transmission electron microscopy.

Epithelial damage was found to be extensive in both asthma groups. Both in basal and in columnar cells, relative desmosome length was reduced by 30–40%. In columnar cells, half-desmosomes (i.e., desmosomes of which only one side was present) were frequently noticed. Eosinophils showing piece-meal degranulation were commonly observed in allergic asthma. Degranulating mast cells were more often observed in allergic asthma. Goblet cell hyperplasia was only found in allergic asthma. Lymphocytes were increased in both groups. In both groups, the lamina densa of the basal lamina was thicker than the control by about 40–50%. In allergic asthma the lamina densa was irregular with focal thickening.

While there was always a tendency for changes (epithelial damage, desmosomes, degranulating mast cells, basal lamina) to be more extensive in allergic asthma compared to non-allergic asthma, there was no significant difference between the

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two groups in this respect. Reduced desmosomal contact may be an important factor in the epithelial shedding observed in patients with asthma.

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Introduction

Asthma is characterized by airway inflammation and bronchial hyperreactivity. A variable degree of epithelial damage in the airways has been observed in biopsies from asthma patients.¹ Epithelial shedding is widely thought to be a major factor in the pathology of asthma and bronchial hyperresponsiveness.^{2,3} The number of ciliated epithelial cells obtained from broncho-alveolar lavage (BAL) fluid, as a representative of epithelial shedding, has been shown to correlate with the degree of airway responsiveness.⁴ A common finding in bronchial biopsies of asthmatics is a thickened basement membrane.⁵ Relatively few studies have focused on distinguishing between allergic and non-allergic asthma.^{6–10} Amin et al.,⁶ using immunohistochemistry, quantified the involvement of inflammatory cells in bronchial biopsies from allergic and non-allergic asthmatics and observed significant differences between the two groups: more eosinophils and lymphocytes, but fewer neutrophils in the allergic group. This study also showed that there was a statistically significant correlation between epithelial damage and number of eosinophils in patients with allergic asthma.⁶

The mechanism of epithelial shedding is not clear. It has been shown that failure of epithelial cells to adhere adequately to the basement membrane can result in more extensive penetration of inhaled antigens followed by mediator response.¹¹ Experimental studies in our laboratory have pointed to a possible role of desmosomes in epithelial shedding. The Th-1 cytokines (IFN- γ and TNF- α) as well as hypochlorite ions affected desmosomes and thereby cell-cell adhesion in cultured normal human bronchial epithelial cells.^{12,13} Also, the eosinophil granule-derived major basic protein (MBP) analogue poly-L-arginine caused a reduction in desmosomes in cultured human bronchial epithelial cells.¹⁴

The aim of the present study was to extract more information from the material investigated by Amin et al.⁶ by using electron microscopy to investigate details that are not apparent in the light microscope. Because of the findings in the experimental studies which showed an effect of products of inflammatory cells on desmosomes, special attention was given to desmosomes. A significant weakening of the desmosomes between epithelial

cells, both in allergic and non-allergic patients, compared to control, was observed.

Materials and methods

Patients

Bronchial biopsy specimens were taken from 23 adults, 11 of them were allergic asthmatics, seven non-allergic asthmatics and the remaining five were healthy controls. The study was conducted in accordance with the declaration of Helsinki and was approved by the ethics committee at the Faculty of Medicine at the University of Uppsala.

All patients had a clinical diagnosis of asthma, current asthma symptoms and increased responsiveness to inhaled methacholine, defined by a provocative dose of methacholine causing a 20% fall in FEV₁(PC₂₀) < 32 mg. Asthma patients were being followed as out-patients at the Department of Respiratory Medicine and Allergology at Uppsala Academic Hospital. The participants had answered a questionnaire about airway symptoms during the last 12 months before they were included in the study. The questionnaire was based on the European Community Respiratory Health Survey Questionnaire.¹⁵ None of the patients were smokers and they had been free from respiratory infections for at least 6 weeks prior to bronchoscopy and none had a history of cardiovascular disease.

Allergic asthmatics: All patients had a positive skin prick test (>3 mm) for at least one common allergen tested: birch, timothy grass (*Phleum pratense*), mugwort (*Artemisia vulgaris*), cat, dog, horse, house dust mite (*Dermatophagoides pteronyssinus*), *Cladosporium*, and *Alternaria*. All but two of the patients were on regular treatment with inhaled glucocorticosteroids (budesonide 200–800 μ g/day) and inhaled beta agonist as needed.

Non-allergic asthmatics: All had a negative skin prick test and all but two non-allergic patients were on regular treatment with inhaled glucocorticoids (budesonide or beclomethasone 200–800 μ g/day) and inhaled β_2 -agonist as required. The average use of inhaled glucocorticosteroids was similar in both asthma groups. There was no significant age difference between the two groups of asthmatics.

Controls: Controls were healthy persons who responded to a request for volunteers. No one had asthmatic symptom and all of the patients showed a negative skin prick test.

The methods for the allergic test, spirometry, metacholine test, peak flow variability and symptom score have been described in detail in Amin et al.⁶ A summary of patient characteristics is given in Table 1.

Study design

Bronchial biopsies were taken from patients with allergic asthma ($n = 11$), patients with non-allergic asthma ($n = 7$) and healthy controls ($n = 5$), and prepared for transmission electron microscopy.

The degree of epithelial damage was determined by a semi-quantitative scoring system. The relative length of the desmosomes in columnar and basal cells was determined by morphometrical techniques as (total desmosomal length/cell perimeter). The width of the basement membrane was measured quantitatively. Degranulating mast cells were counted. Presence and ultrastructure of eosinophils, neutrophils and lymphocytes was studied qualitatively. The frequency of goblet cells and the occurrence of overactive fibroblasts were also studied.

Bronchoscopy

The patients were given 10 mg of diazepam (Stesolid[®], Dumex, Copenhagen, Denmark) orally and 0.5 mg of atropin (Atropin, NM Pharma, Stockholm, Sweden) subcutaneously 30 min before the investigation. The upper airway were anaesthetized with lidocaine hydrochloride (Xylocain, Astra, Södertälje, Sweden). Using a flexible fibre bronchoscope (Olympus p 20D) with an FB 15C 2.0 mm forceps (Olympus), one or two biopsies for transmission electron microscopy were taken in the right

lung in the upper lobe bronchus immediately after the division from the main bronchus. In the first series of biopsies (five controls, five patients with allergic asthma, and three patients with non-allergic asthma) one biopsy was taken, in the second series (five patients with allergic asthma and four patients with non-allergic asthma) two biopsies were taken from each patient. The specimens were examined immediately by a light microscopy to ensure the presence of a complete mucosa and fixed as described below. The patients were instructed to take their regular asthma sprays on the morning of the bronchoscopy.

Transmission electron microscopy

Biopsies were fixed in 2.5% glutaraldehyde (Sigma, St. Louis, MO) in 0.1 M cacodylate buffer (Agar Scientific, Stansted, UK) for 1 day. After being washed in cacodylate buffer, the biopsies were post-fixed in 1% OsO₄ in cacodylate buffer for 60 min. A second wash in buffer was followed by dehydration in graded series of ethanol, before the cells were finally embedded in Agar 100 Resin (Agar Scientific). In order to avoid the damage caused by the biopsy procedure at the edges of the biopsy, only the central part of the specimen was used for transmission electron microscopy. Sections were cut and stained with uranyl acetate/lead citrate, and examined in a Hitachi H7100 transmission electron microscope (TEM) (Hitachi, Tokyo, Japan) at 75 kV. The specimens were oriented so that the sections were approximately perpendicular to the surface. Section width was approximately 150–200 µm.

Microscopic evaluation of sections

All specimens were coded and examined without any knowledge of the diagnosis. The degree of damage was judged by two-independent observers,

Table 1 Patient characteristics.

	Healthy control ($n=5$)	Allergic asthma ($n=11$)	Non-allergic asthma ($n=7$)
Age (yr)	28 (22–43)	38 (19–63)	43 (17–62)
Sex (M/F)	2/3	3/8	4/3
Inhaled steroids	0/5	9/11	5/7
FEV ₁ (% predicted)	104 (89–120)	99 (76–132)	86 (72–97)
FVC (% predicted)	99 (89–109)	91 (86–141)	88 (76–96)
Symptom score	0	3 (0–4)	1.5 (1–2)
PEF variability (%)	5 (3–9)	11 (5–22)	12 (5–20)
PC ₂₀ (mg/ml)	0	6.7 (0.32–32)	12 (0.97–32)

Definition of abbreviations: yr=year; M=male; F=female; FEV₁=forced expiratory volume in 1 s; PEF=peak expiratory flow; PC₂₀=provocation concentration of metacholine that reduces FEV₁ by 20%.

using a scale of 1–5, where 1 indicates no damage, 2 the presence of intercellular spaces between the cells, 3 detachment of a few columnar cells, 4 detachment of many, but not all columnar cells, and 5, a total loss of columnar epithelium. In those cases where two biopsies had been taken, both biopsies were examined. The results from both biopsies and both observers were averaged. There were no significant differences between the scores of the observers or between both biopsies from the same patient.

To quantify the relative length of the desmosomes 5–10 columnar and 5–10 basal cells were randomly selected for each patient for whom these parameters were quantified. The criterium for inclusion in these measurements was that the cell was attached to its neighbours. The epithelial damage in the specimens from asthma patients limited the number of cells from these biopsies that could be used for this purpose. The TEM image was transferred via a digital camera to a Synopsis (Cambridge, UK) Synapse frame capture system. The length of all desmosomes of a columnar cell, or a basal cell, respectively, was determined and divided by the perimeter of the cell. Since the absolute length of desmosomes would depend on the angle of the ultrathin section, the absolute length was related to the length of the cell membrane; the relative length obtained in this way is not dependent on the sectioning angle. The desmosomal length was determined at an original magnification of $15\,000\times$ and the perimeter of the cells was determined at an original magnification of $2000\times$. The thickness of the basal lamina was determined at an original magnification of $30\,000\times$ at 5–10 randomly selected positions along the basal lamina. The presence of eosinophils, mast cells, lymphocytes and neutrophils as well as that of goblet cells was scored qualitatively for each specimen. Degranulation of mast cells was defined as granules surrounding the cell or crossing the cell membrane.¹⁶

Scanning electron microscopy

Two biopsies in each group were, after fixation and dehydration, dried by critical point drying, and examined uncoated in a LEO (Cambridge, UK) 1530 field emission scanning electron microscope at 1 kV. For this purpose, biopsies material left over from a previous study⁶ was used.

Statistics

Differences between groups were determined by one-way analysis of variance (ANOVA) followed by

Dunnett's multiple comparison test for determination of the significance of differences between controls and the two asthma groups, as well as Student's *t*-test for determination of the significance of differences between the two asthma groups.

Results

In the healthy controls, an intact epithelium was observed by both scanning and transmission electron microscopy (Figs. 1a and 2a). Both in non-allergic patients (Figs. 1b and 2b) and in allergic patients (Figs. 1c and 2d), sites of damage were observed. In severe cases the columnar cells had been lost over larger areas and in extreme cases only a few basal cells were left covering the basement membrane (Fig. 2e). In all of the patients with non-allergic asthma, areas with flattened rather than columnar cells were observed (Fig. 2c), but such areas were not seen in patients with allergic asthma. Only in one biopsy (allergic asthma) also the basal cells were lost. A semi-quantitative scoring system confirmed that the degree of epithelial damage was significantly more extensive in allergic and non-allergic asthmatics than in controls, but that there was no significant difference between allergic and non-allergic patients (Fig. 3).

Desmosomes were present between columnar cells, between a columnar cell and a basal cell, and between basal cells. In healthy controls, desmosomes consisted of two plaques, one in each neighbouring cell (Fig. 4a). In asthmatic patients (both allergic and non-allergic) the relative length of the desmosomes was reduced (Fig. 5a). In addition, in both patient groups, many "half-desmosomes" were found (Fig. 4b), i.e., a plaque was only present in one of the neighbouring cells. In a few cases, the cell lacking the corresponding plaque appeared damaged, but in most cases neither of the cells was damaged. Compared to the columnar cells, the desmosomes of the basal cells were larger and more dense (Fig. 6). In the basal cells, the relative desmosome length was significantly reduced both in allergic and non-allergic asthma, compared to control (Fig. 5b).

In both allergic and non-allergic asthma the basal lamina was thicker compared to the control; the increase in thickness was due to an increase in the lamina dense, whereas the lamina lucida was not affected. In allergic asthma the basal lamina had a very irregular thickness with focal thickening (Figs. 7 and 8). Hemidesmosomes were observed

attaching the basal cells to the basal lamina but no obvious differences between the groups were noted in this structure.

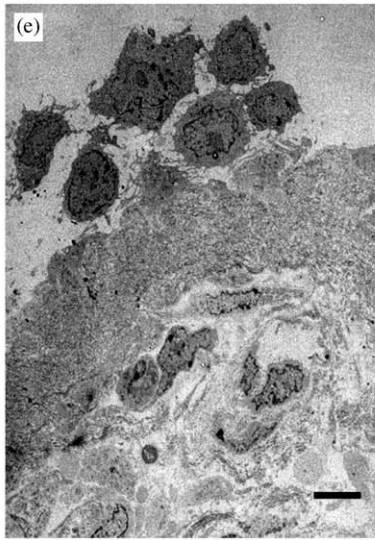
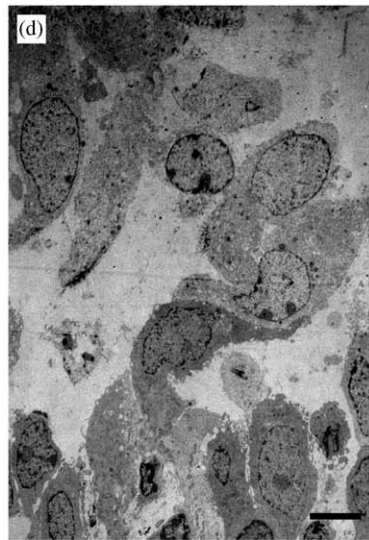
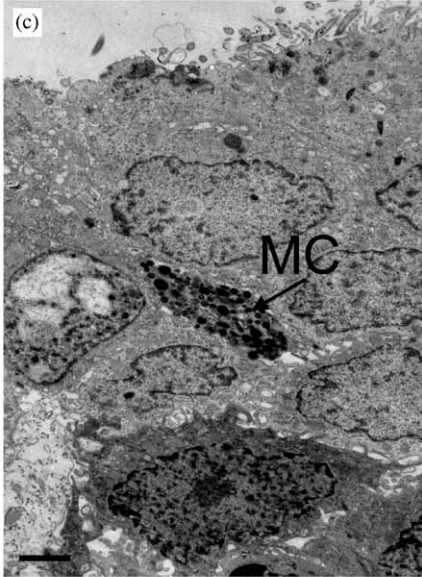
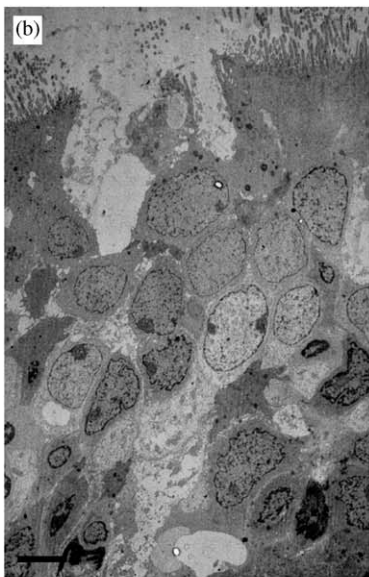


A qualitative assessment of the occurrence and structure of white blood cells was carried out (Table 2). Eosinophils were commonly observed in allergic asthma, and these cells showed mostly piece-meal degranulation (Fig. 9a). Eosinophils were most commonly observed in the subepithelial connective tissue, but some were seen penetrating the epithelium and others were seen inside the capillaries. Accumulations of eosinophils showed a spatial correlation with sites of epithelial damage (Fig. 9b). Also, in allergic asthma, about equal numbers of mast cells were degranulating and non-degranulating, whereas in non-allergic asthma most mast cells were non-degranulating, and in controls very few mast cells were found (Fig. 10). A quantitative evaluation of the number of degranulating and non-degranulating mast cells confirmed this tendency of more degranulating mast cells in the patients with allergic asthma (Fig. 11). The number of lymphocytes was increased both in allergic asthma and non-allergic asthma, and they were found both in the subepithelial layer and in the epithelium. Neutrophils were more frequently found in non-allergic asthma compared to allergic asthma. Goblet cell hyperplasia was only found in a few cases of allergic asthma (Fig. 12).

Discussion

The aim of our study was to investigate ultra-structural changes in the airway epithelium of asthma patients, and to compare changes in patients with allergic and non-allergic asthma. In the present study, both groups of asthmatics had an almost similar severity of respiratory symptoms, peak flow variability and BHR. The present study showed some marked differences between allergic and non-allergic asthma patients, namely more eosinophil accumulation and more goblet cell hyperplasia in the allergic patients. There was a tendency to more extensive epithelial damage, less desmosome connections between the epithelial cells, relatively more degranulating mast cells, and a thicker lamina densa of the basal lamina in patients with allergic asthma, but these differences were not significant, in contrast to some of the findings in the study by Amin et al.⁶ Increased

Figure 1 Scanning electron micrographs of bronchial epithelium. (a) Control, without damage. (b) Non-atopic asthma with focal damage; goblet cells (G) can be seen between the ciliated cells. (c) Atopic asthma, showing extensive loss of columnar cells, some of which are seen lying on their sides (arrows). Bar=10 μ m.



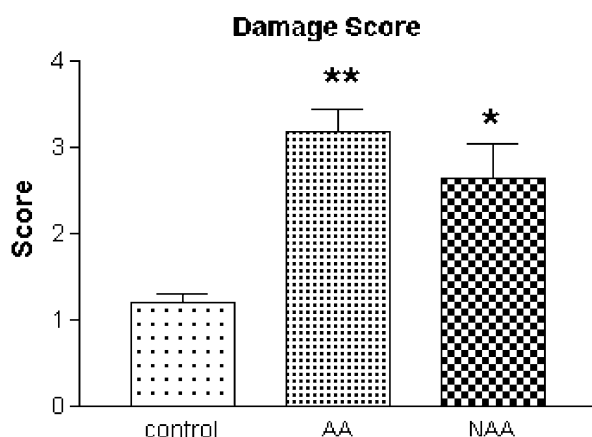


Figure 3 Scores for epithelial damage. In the scoring system, 1 means no damage, and 5 means total damage. Data show mean and standard error (thin bars). The significance of difference with the control is indicated by asterisks, * $P < 0.05$, ** $P < 0.01$.

lymphocytes were found both in allergic and non-allergic asthma.

The epithelial damage mostly comprises the columnar cells of which many were damaged or completely lost, especially in the allergic patients. This agrees with findings by us and other groups.^{1,2} In non-allergic asthmatics, we noted the occurrence of flattened rather than columnar cells, which could be part of the airway remodelling process. In the study by Amin et al.⁶ a significant difference between allergic and non-allergic patients was noted. In that study, damage was defined as the total disappearance of columnar cells, whereas in the present study also the ultrastructure of the cells was taken into account. Our scanning electron micrographs confirm that more columnar cells are actually lost in patients with allergic asthma, but transmission electron microscopy showed that damage at the ultrastructural level was extensive in both groups.

It has been pointed out that the biopsy procedure itself may cause damage to the epithelium. Söderberg et al.¹⁷ and Jeffery et al.¹⁸ showed extensive damage to the edge of the biopsies, although the epithelium in the centre appeared undamaged.¹⁸ Söderberg et al.¹⁷ studied biopsies from healthy subjects and reported that a remarkably high number of the biopsies had to be discarded because of extensive damage to the epithelium. Jeffery et

al.¹⁸ determined the extent of epithelial damage quantitatively and found that only 53% of the epithelium in healthy subjects was covered by surface epithelial cells, not significantly different from the situation in asthmatics. On the other hand, Laitinen et al.¹ reported that the control biopsy did not show damage attributable to the biopsy procedure. The biopsies investigated in the present study are part of the material investigated by Amin et al.⁶ by light microscopy, where in the healthy controls the epithelial integrity, determined over the entire length of the epithelium in the biopsy, was found to be about 70%. In the present electron microscopical study, where only the central part of the biopsy was used, the epithelial damage in the healthy subjects was relatively little, and much less than in the asthmatics (Figs. 1–3). That the central part of the biopsy would be the more intact part is in line with the results shown by Jeffery et al.¹⁸ All biopsies, be it from controls or asthma patients, were treated in the same way, and hence the difference between the epithelial damage observed between the groups must have its basis in intrinsic differences between the groups. It cannot be excluded, that some aspects of epithelial damage may be exaggerated in the material viewed in the electron microscope, if an intrinsic weakness in cell contacts in the asthma patients causes their epithelium to be damaged more by the biopsy procedure than that of healthy controls. However, the well-known fact that the bronchioalveolar lavage from asthmatics contains more cells than that from controls¹⁹ and the fact that epithelial damage is correlated with bronchial hyperresponsiveness^{6,18} support the notion that there is an in vivo difference in epithelial integrity between asthmatics and controls. This also agrees with the notion of Laitinen et al.¹ Furthermore, it is entirely implausible that observations such as the difference in length in desmosomal contacts between asthmatics and controls shown in the present study could be an artefact due to the biopsy procedure.

In one biopsy from a patient with allergic asthma where also the basal cells had been lost and the basement membrane was denuded, flattened basal cells appeared to attempt to cover up the wound, and also massive leukocyte (lymphocytes and neutrophils) infiltration was seen, as described by Erjefält and Persson²⁰ for cases of severe damage.

Figure 2 Bronchial epithelium. (a) Control, without damage. Bar=5 μ m. (b) Non-atopic asthma with focal damage and loss of ciliated cells. Bar=5 μ m. (c) Non-atopic asthma showing mast cell (MC) that has penetrated into the epithelium, and flattened surface epithelial cells. Bar=1 μ m. (d) Atopic asthma, showing extensive loss of columnar cells. Bar=5 μ m. (e) Atopic asthma, only a few basal cells are left of the entire epithelium. Bar=5 μ m.

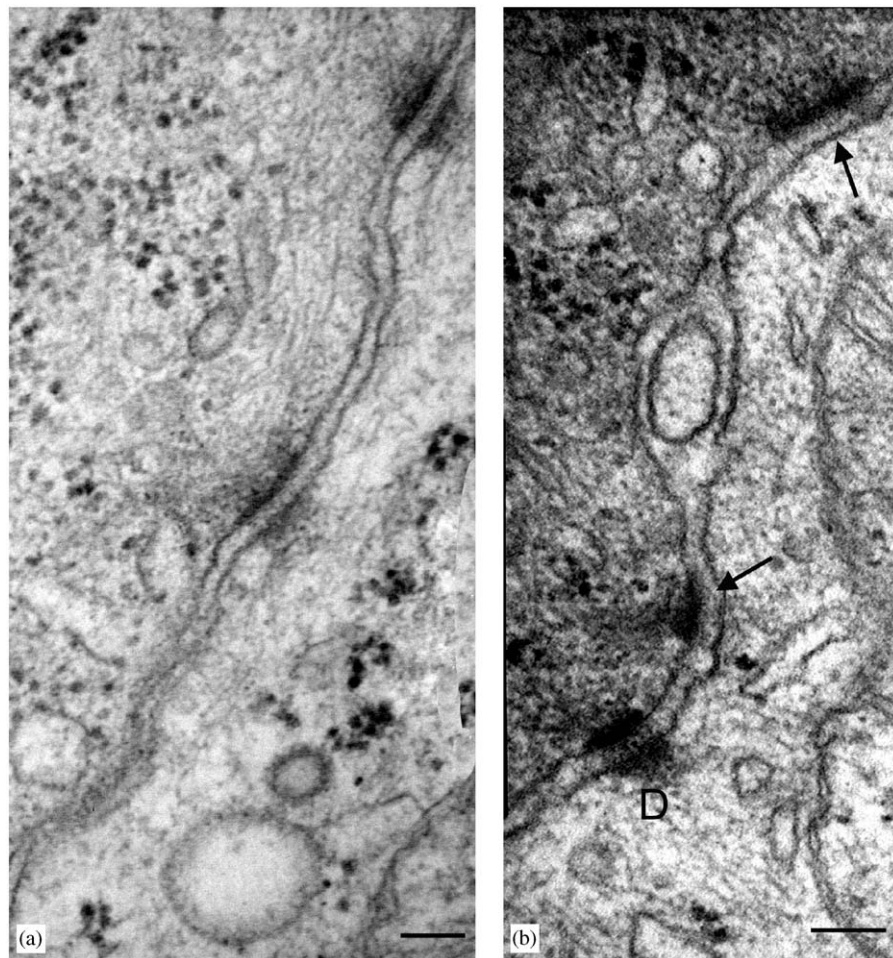


Figure 4 Desmosomes along the lateral cell membrane of the columnar cells. (a) Control. (b) Atopic asthma showing a complete desmosome (D) and two half-desmosomes (arrow). Bar (a,b)=0.1 μ m.

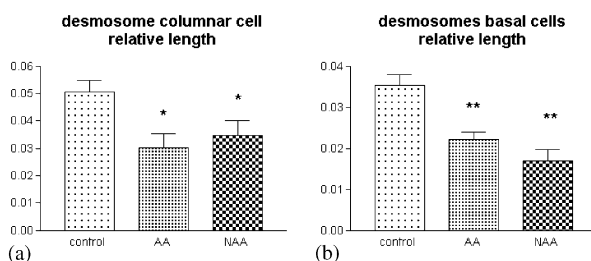


Figure 5 Relative length of desmosomes. (a) columnar cells, (b) basal cells. Data show mean and standard error (thin bars). The significance of difference with the control is indicated by asterisks, * $P < 0.05$, ** $P < 0.01$.

However, when only the columnar cells had been lost, this phenomenon was not seen.

Desmosomes

Our study found that the relative length of desmosomes was reduced in both types of asthma.

In the columnar cells, the relative length of the desmosomes was less in allergic asthma than in non-allergic asthma, and frequently, half-desmosomes were found. The desmosomes of the basal cells appear stronger than the desmosomes between columnar cells. Desmosomes are specialized major cell-cell adhesive junctions of epithelial tissue, and play an important role in maintaining tissue integrity via intermediate filaments.²¹ In addition, desmosomes may play a role in the transduction of intracellular signals that regulate cell behaviour.^{21,22} Desmosomes have to our knowledge not earlier been studied in connection with asthma. However, in experimental studies in cultured airway epithelial cells, we have shown that several factors produced by different types of leukocytes can result in the reduction of the number of desmosomes between the epithelial cells.^{12,14} Several explanations are possible for our finding. Firstly, it could be speculated that asthma patients have an intrinsic or acquired deficiency in the synthesis

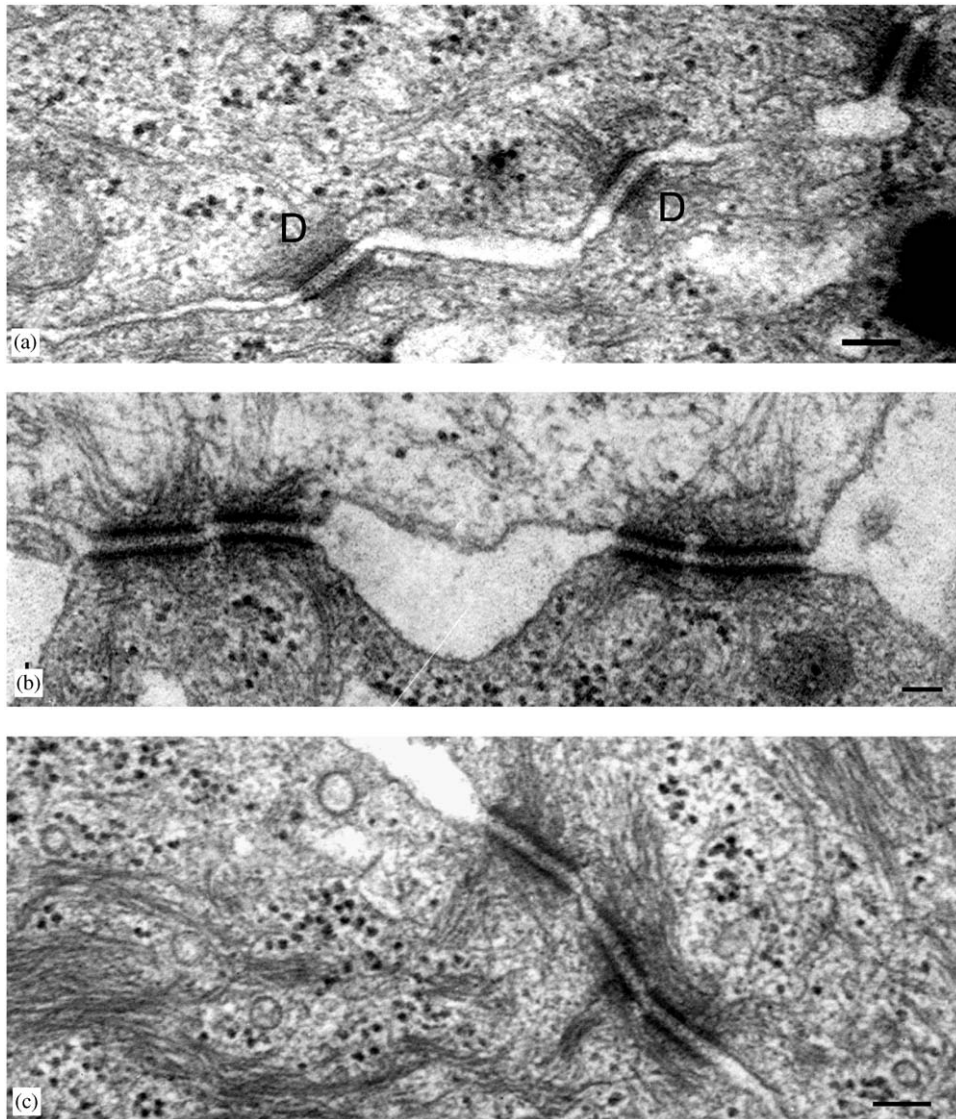


Figure 6 Desmosomes of basal cells. (a) Control, desmosomes (D) are connected to keratin filaments. (b) Non-atopic asthma. (c) Atopic asthma. Bar (a,b,c)=0.1 μ m.

of desmosomes. This would make the epithelium more prone to loss of columnar cells. Secondly, it could be speculated that inflammatory cells, in particular eosinophils, specifically accelerate the breakdown of desmosomes and/or inhibit the synthesis of desmosomes. Finally, the reduction in desmosomal contact could be a more or less unspecific effect of a decrease in metabolism in the epithelial cells, which would inhibit synthesis of desmosomes as well as other proteins. The present study does not allow a distinction between these different possibilities. It is, however, of interest that in cell pairs in which half-desmosomes were found, the cell lacking the desmosome plaque was not necessarily damaged. This would indicate that the decrease in desmosomal contacts

is not per se a result of cell death. Half-desmosomes have been observed in epithelial cells cultured in a low calcium medium, where desmosomal proteins were synthesized but not assembled, or formed partially assembled unstable half-desmosomes.²³ It has also been observed in cultured cells that half-desmosomes can be transiently formed (and then endocytosed) when neighbouring cells fail to make contact.²⁴ The half-desmosomes observed in the present study could therefore be due to a disturbance of desmosome assembly, or to lack of contact between adjoining columnar cells in damaged airway epithelium.

The basal cells observed in this study are, as expected from the biopsy site, identical to the type

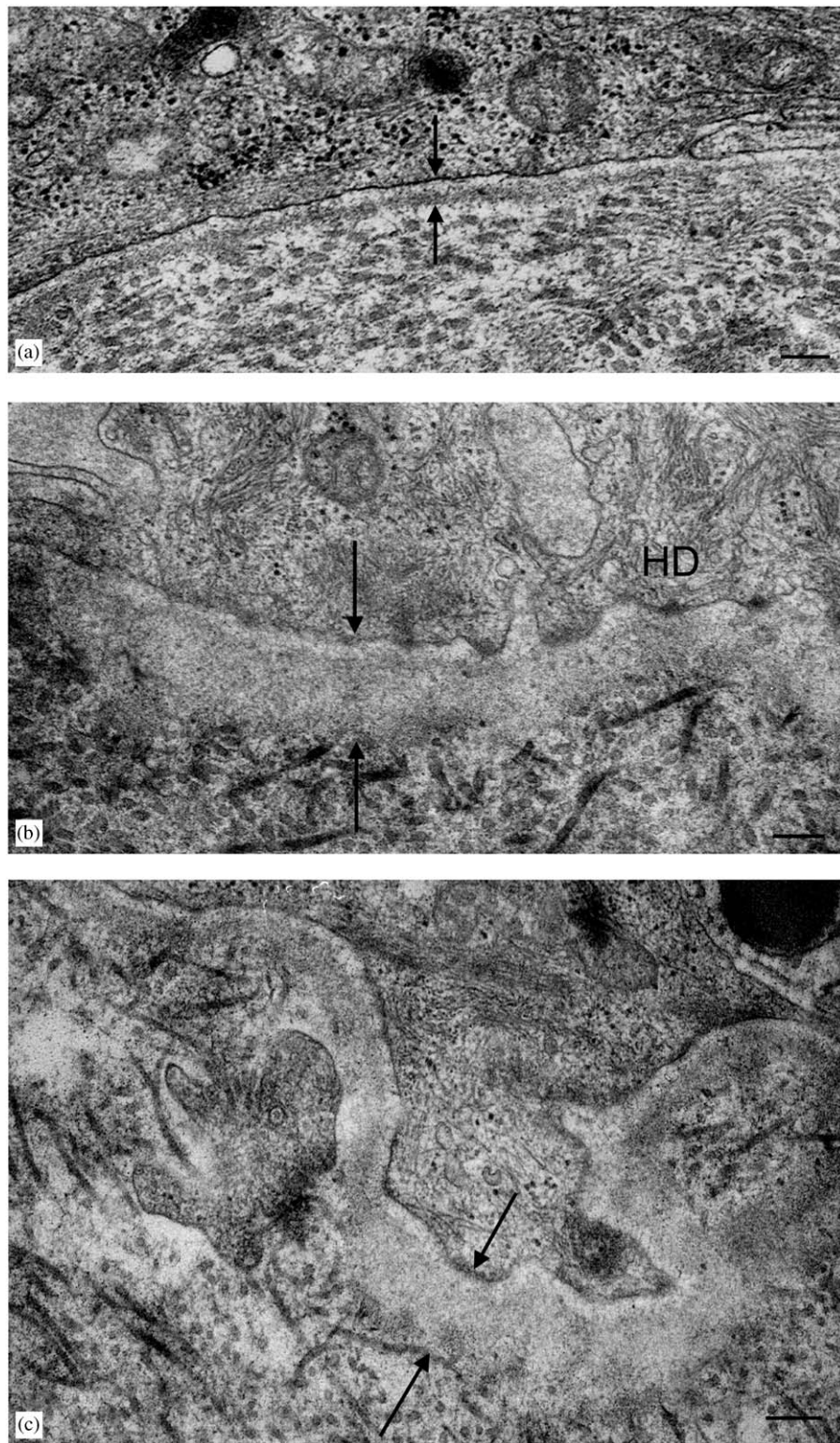


Figure 7 Basal lamina. The thickness of the basal lamina is indicated by arrows. The epithelial cells are connected to the basal lamina by hemidesmosomes (HD). (a) Control. (b) Non-atopic asthma. (c) Atopic asthma. Bar (a,b,c)=0.2 μm .

described by Nakajima et al.²⁵ with a prominent cytoskeleton and well-developed hemi-desmosomes. Even though also the desmosomes of the

basal cells are affected in asthma patients, mostly the columnar cells are lost from the epithelium, whereas the basal cells more often remain present.

This could be related to the finding that the desmosomes of the basal cells appear stronger than those of the columnar cells. From some other stratified epithelia it is known that there is a difference in the composition of desmosomes in the different layers of the epithelium.²⁶ No data are available on whether also in airway epithelium different desmosomal proteins are present in different cell types, but it may be speculated that the composition of the basal desmosomes differs from that of the columnar desmosomes. This would need further investigation.

Goblet cells

Goblet cell hyperplasia, which is responsible for increased mucus secretion was observed only in allergic cases. Mast cell chymase is thought to stimulate mucus secretion.²⁷ This could also explain increased degranulating mast cells and goblet

cell hyperplasia in allergic asthma. The finding of hyperplasia agrees with findings by other groups.²⁸

Basal lamina

Thickening of the lamina densa of the basal lamina was found in both groups of asthmatics, but more prominently in allergic asthma. In these patients, the thickness of the lamina densa showed considerable local variation. Thickened basement membranes have been described in patients with asthma, but most studies have been concerned with the reticular lamina, which can be demonstrated by light microscopy or light microscopical immunohistochemistry, and which has a thickness of about 5 μm (in controls) to about 10 μm (in asthmatics).^{16,29,30} In contrast, the basal lamina has a thickness in the order of 0.1 μm , and the changes observed in the basal lamina in asthmatic patients are hence much smaller than the changes in the reticular layer of the basement membrane. In the study by Amin et al.,⁶ a significant difference between allergic and non-allergic patients was found with regard to the thickness of the basement membrane, but this finding does not contradict that in the present study.

Inflammatory cells

The presence of inflammatory cells was only studied in a qualitative manner, since the small areas surveyed in sections for electron microscopy do not allow accurate quantitation, and a quantitative study had previously been carried out on the same patient material at the light microscopical level.⁶ The qualitative data in the present study agree with the quantitative data obtained by light microscopy.⁶

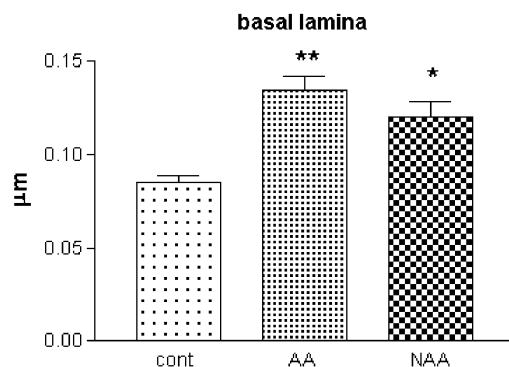


Figure 8 Quantitative determination of the basal lamina. Data shown as mean (in μm) and standard error (thin bars). The significance of difference with the control is indicated by asterisks, * $P < 0.05$, ** $P < 0.01$.

Table 2 Qualitative characteristics of the bronchial biopsies.

	Healthy					Allergic asthma											Non-allergic asthma						
	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7
Eosinophil infiltration	–	+	–	–	–	+	++	–	–	–	–	++	+++	–	–	++	–	–	–	–	+	–	–
Mast cells	+	–	–	–	–	++	+	–	–	++	+	–	+++	–	–	++	+	–	–	–	+++	+	++
Goblet cell hyperplasia	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	++	–	–	–	–	–	–	–
Lymphocyte infiltration	+	+	+	+	+	++	+	+	+	++	+	+	+++	+	+	++	+	++	+	++	+++	+	+
Neutrophils	–	–	–	–	–	–	+	–	–	+	+	–	+	–	–	+	–	–	+	+++	+	–	–
Loss of ciliated cells	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+

The qualitative characteristics are given for each biopsy included in the study. Scoring is given as (–) absent, (+) present, (++) present in large numbers, (+++) present in very large numbers.

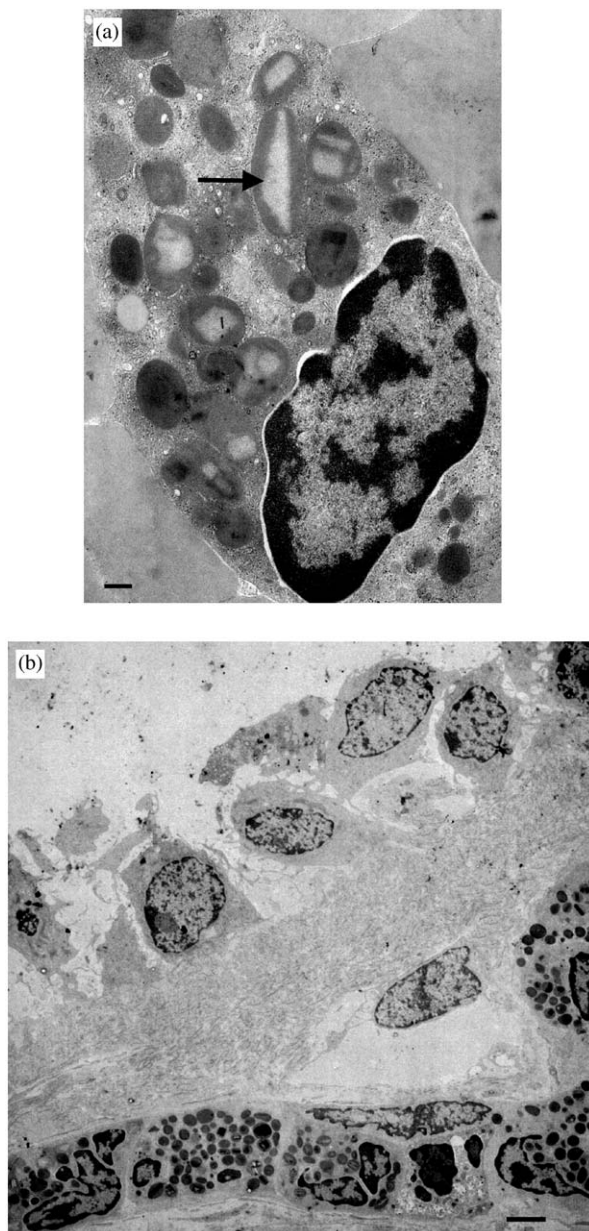


Figure 9 Eosinophil granulocytes in atopic asthma. (a) Shows piece-meal degranulation (arrow); bar=0.5 μ m. (b) Shows the relationship between eosinophils and epithelial damage in atopic asthma. Extensive infiltration of eosinophils in the bronchial capillary is associated with extensive damage to the epithelium. Bar=2 μ m.

Eosinophils: A cause of extensive epithelial damage in allergic asthma could be eosinophilic infiltration. In a previous study,⁶ we have found a statistically significant correlation between the number of eosinophils in a biopsy and the epithelial damage. Also other groups have shown a relationship between eosinophils and epithelial damage.^{31,32} In experimental studies, it was found

that one of the toxic eosinophilic proteins, or its analogue poly-L-arginine could damage airway epithelial cells in a dose- and time-dependent manner.^{14,33,34} In the present study, we found many activated eosinophils in the biopsies of nearly all allergic asthmatics, but in only one non-allergic asthmatic. A spatial correlation between the presence of eosinophils and epithelial damage was noted. The most common degranulation mechanism in vivo appears to be the so-called "piece-meal degranulation" mechanism^{35,36} but in some cases, lysis was observed as a major mode of degranulation of eosinophils in airway disease.³⁷ The fact that piece-meal degranulation was observed more commonly than lysis agrees with the literature.³⁸

Mast cells: Mast cells, which are thought to be a direct or indirect factor causing most of the pathology associated with chronic allergic asthma,³⁹ were found both in allergic and non-allergic asthma. There was a tendency for both the total number and the number of degranulating mast cells to be higher in allergic patients, but due to the individual variability no statistically significant differences between allergic and non-allergic patients could be demonstrated. One of the degranulation mechanisms for mast cells is an IgE-independent mechanism in which eosinophils stimulate mast cells to degranulate.⁴⁰ The finding that patients with allergic asthma have both more eosinophils and more degranulating mast cells fits in with this proposed mechanism.

Lymphocytes: Profuse lymphocyte infiltration was observed in both asthma groups. Other groups have not found any direct correlation between the lymphocyte infiltration and epithelial damage, but rather a correlation between the presence of lymphocyte and eosinophils suggesting a role for lymphocytes in eosinophilic infiltration and subsequent epithelial damage and airway hyperresponsiveness.² A recent review has discussed the aim of asthma therapy with regard to lymphocyte infiltration, and suggested that therapy for allergic asthma should be targeted to reduce the activity of Th-2 lymphocytes and their soluble products, which will indirectly eliminate eosinophils as well as reduce the production of IgE antibodies and other mediators of allergic and inflammatory response.⁴¹

In conclusion, we observed many similarities between allergic and non-allergic asthma. While we found differences in the pattern of inflammatory cells, such as more eosinophils in allergic asthma, confirming previous findings of this group,⁶ and while there was always a tendency for changes (epithelial damage,

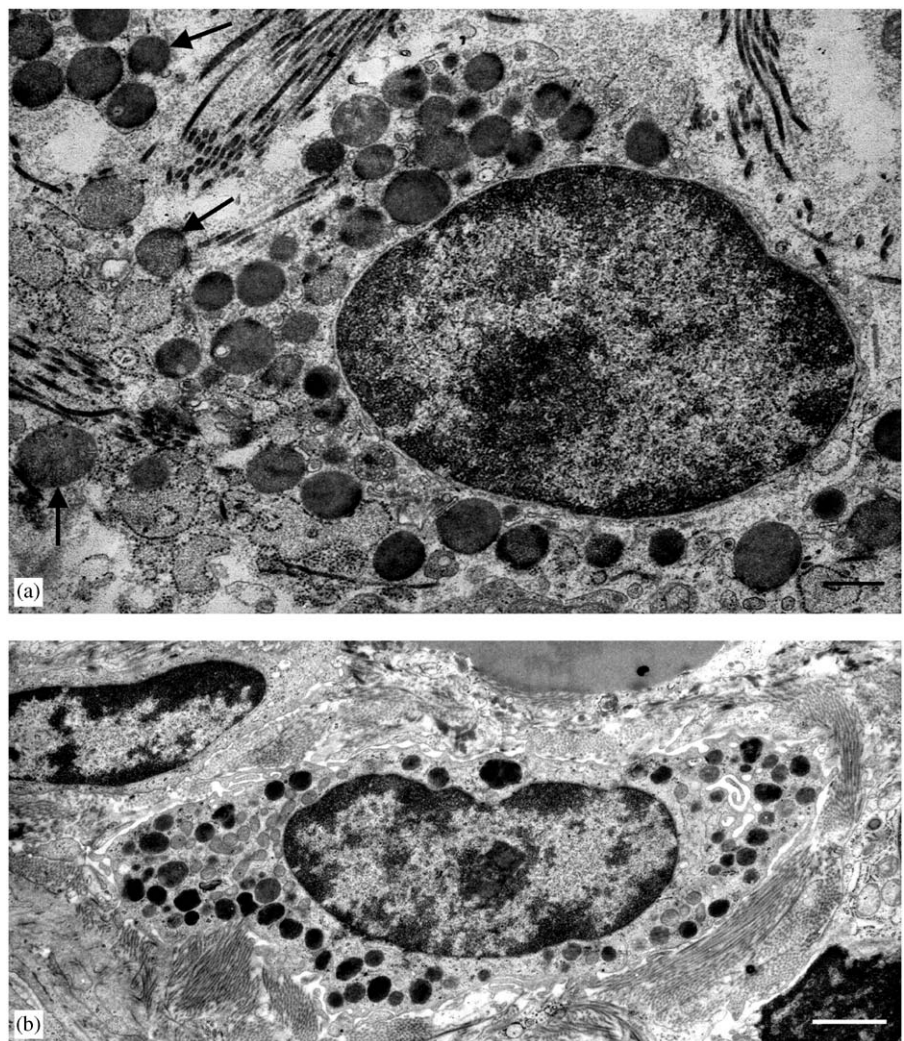


Figure 10 Mast cells. (a) Atopic asthma showing degranulation of mast cells (arrow). Bar=0.5 μ m. (b) Non-atopic asthma showing a non-degranulating mast cell. Bar=1.5 μ m.

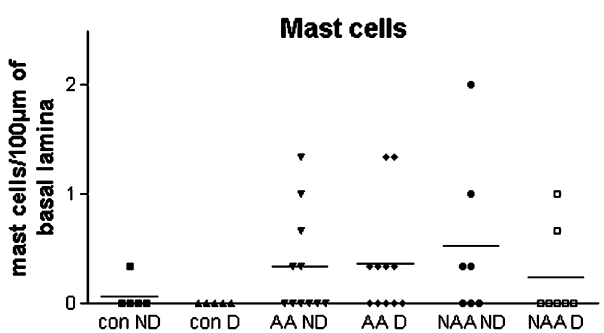


Figure 11 Quantitative evaluation of the number of (a) degranulating (D) and (b) non-degranulating (ND) mast cells in healthy controls (con), atopic asthma (AA), and non-atopic asthma (NAA). Individual data and means (horizontal bars) are shown. The data are expressed as number of cells/100 μ m of basal lamina.

desmosomes in columnar cells, basement membrane) to be more extensive in allergic asthma compared to non-allergic asthma, the differences between these two patient groups are relatively small. Goblet cell hyperplasia, and eosinophils with a piece-meal degranulation pattern were commonly observed findings in allergic cases, but not in non-allergic asthma. Mast cells were more frequently degranulating in allergic asthma than in non-allergic asthma. It could be speculated that the main difference between allergic and non-allergic asthma is the different recruitment of eosinophils and that the other differences observed are secondary. Changes in desmosomal contacts may be very relevant for the epithelial damage observed both in allergic and in non-allergic asthma.

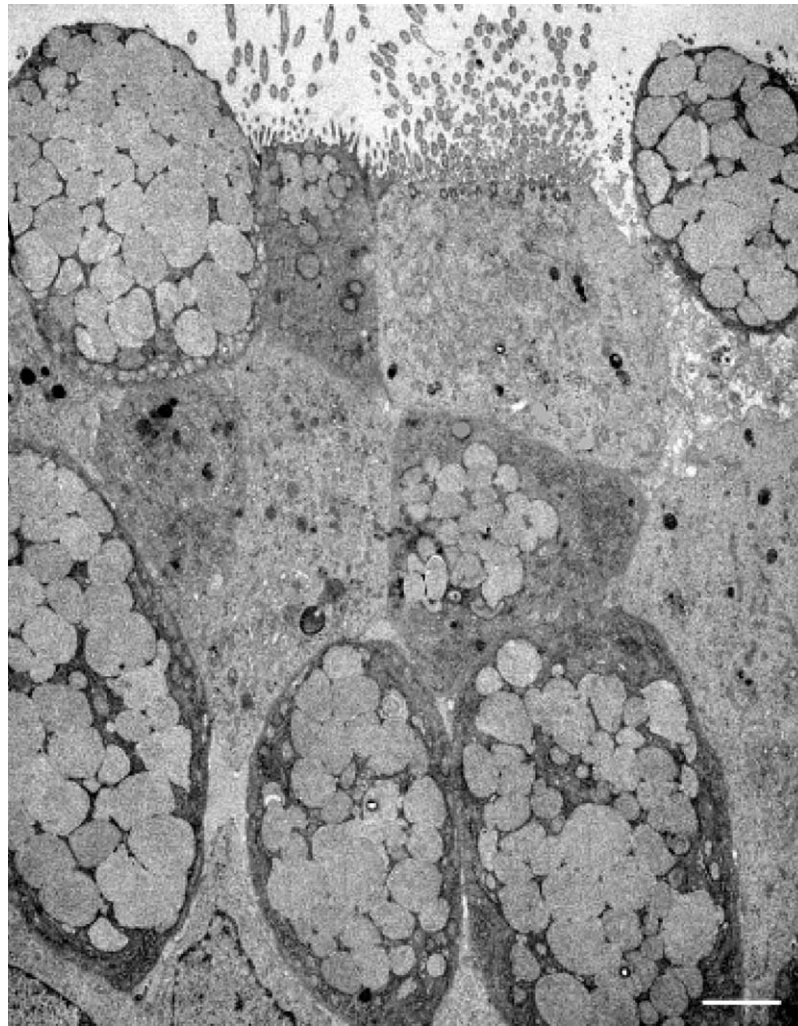


Figure 12 Goblet cell hyperplasia in a patient with atopic asthma. Bar=1 μ m.

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