

Antigen presenting cells in the nasal mucosa of patients with allergic rhinitis during allergen provocation

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Summary

Background The role of antigen presenting cells (APC) in allergic rhinitis is under-exposed. Allergen presentation to T lymphocytes is probably an important aspect of the pathophysiological mechanism of allergic rhinitis.

Objectives The aim of the study was to investigate the presence and dynamics of APC with special emphasis on Langerhans cells (LC) in the nasal mucosa of patients with an isolated grass pollen allergy during an out-of-season 2-week allergen exposure, mimicking the natural grass pollen season.

Methods Seventeen patients with isolated grass pollen allergy and four control subjects were challenged daily with allergen during a 2-week period in the winter. Biopsy specimens were obtained once before, six times during and once after the provocation period. Biopsy sections were stained with monoclonal antibodies: OKT6 (CD1a–Langerhans cells), Ki-M6 (CD68–macrophages), L25 (dendritic cells), anti-IgE, HLA-DR and HLA-DQ (Major Histocompatibility Complex Class II – antigen presenting cells), as well as staining with acid phosphatase.

Results APC with different characteristics are present in the epithelium and lamina propria of the nasal mucosa. The number of LC increased significantly in epithelium and lamina propria. IgE⁺-LC were present in the nasal mucosa and increase during provocation. HLA-DR⁺ cells with dendritic and lymphocytic morphology and HLA-DQ⁺ cells were found. The number of these cells increased during provocation in epithelium and lamina propria. The number of HLA-DR⁺ epithelial cells did not change. A significant increase in the number of Ki-M6⁺ cells (macrophages) was found in the lamina propria. However, Ki-M6⁺ cells increased to the same extent in the lamina propria in the control group.

Conclusion APC are influenced by allergen provocation. This study supports the hypothesis that (IgE⁺) LC are involved in allergic rhinitis. The role macrophages play remains doubtful.

Keywords: allergy, antigen presenting cells, biopsy, immunohistochemistry, Langerhans cells, nasal mucosa, pollen, rhinitis

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Introduction

The role and function of antigen presenting cells (APC) in allergic respiratory disease is underexposed. APC in

the respiratory mucosa may present the allergen to the T-cell system during the sensitization phase. Even more important, they may also contribute to the local activation of primed T cells during the effector phase of allergic reactions [1]. There is an increasing interest in the role of lymphocytes in IgE-mediated reactions in

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respiratory mucosa [2]. However, only a few studies have been published on antigen/allergen processing and presentation in the epithelium of the lower and upper airway, which constitutes a necessary step for T-cell activation. A number of distinct cell types, including dendritic cells (DC) [3], macrophages [4], Langerhans cells (LC) [5,6], B lymphocytes [7] and epithelial cells [8] are potentially capable of presenting antigen to T lymphocytes. Although these cell types differ markedly in their effectiveness as APC, they share certain features, including the expression of MHC class II molecules (HLA-DR).

On morphological criteria we can distinguish DC, lymphoid cells, epithelial cells and endothelial cells positive for HLA-DR. In a previous study the number of HLA-DR⁺ cells did not differ between patients with isolated grass pollen allergy and controls [9], and between patients with nasal polyps and controls [10]. Bachert *et al.*, however, described an increase in HLA-DR⁺ cells during allergen provocation [11].

The monoclonal antibody (MoAb) directed to CD1a (T6) is a specific and sensitive marker for the identification of LC and some precursors of LC called indeterminate cells [12–14]. Moreover, LC can be distinguished by the Birbeck granule, a cell organelle which seems pathognomonic for LC. In the epithelium of the nasal mucosa most DC contain Birbeck granules and are therefore LC [15]. In the lamina propria only part of the cells with dendritic morphology are LC. Other DC may be precursors of LC or LC migrated from the epithelium and matured into DC, losing their Birbeck granules and CD1a positivity [16].

LC seem to play a role in allergen presentation in atopic disease [17]. Recent investigations have shown that LC are present in nasal mucosa [9,15,18] and that the number of LC in the epithelium of the nasal mucosa of symptomatic rhinitis patients with isolated grass pollen allergy is significantly higher than in non-allergic patients with nasal polyps or normal controls [15]. The number of LC in the epithelium of the patients with isolated grass pollen allergy was significantly higher during the grass pollen season (July) than before and after the season [19]. In these patients $\pm 20\%$ of the CD1a⁺ cells in the epithelium and occasionally CD1a⁺ cells in the lamina propria were found to be positive for IgE. Bieber showed the presence of a high affinity receptor for IgE on LC [20].

To study the dynamics of APC and the IgE positivity of LC and other APC in the nasal mucosa of patients with allergic rhinitis, a provocation study was performed in patients with isolated grass pollen allergy during 2 weeks in January. During this period, three or four

biopsies were taken from the nasal mucosa of each patient. To characterize different subsets of APC, a panel MoAb directed to DC and macrophages was used. The panel consisted of MoAb directed to HLA-DR, HLA-DQ, L25, Ki-M6, CD1a and IgE. Double immunohistochemical and immunofluorescence stainings with anti-IgE was performed with some of these MoAb. Staining with acid phosphatase was also used to distinguish macrophages from other HLA class II⁺ cells.

Materials and methods

Patients and controls

Seventeen patients and four control subjects participated in this study. The patients (mean age 29 year (14–48), seven men, 10 women) had an isolated grass pollen allergy for at least 1 year, confirmed by a positive skin-prick test reaction to ALK Soluprick extract of 1 SQ/mL and no other positive skin-prick test reaction to 13 other common inhalant allergens, a median (range) radioallergosorbent test (RAST) score of 4 + (3 + –5 +) and a median (range) total IgE value of 290 IU/L (22–1900). The four control subjects (volunteers mean age 28 year (24–44), two men, two women) without nasal complaints, had no abnormalities in the nose at ENT examination and a negative skin-prick test reaction to ALK Soluprick extract of grass pollen and 13 other common inhalant allergens. None of the patients or control subjects used any medication during the study. None of the patients or control subjects had an infection of the respiratory tract or any nasal complaints during the week preceding the challenge.

None of the patients had any complications during and after the study from repetitive challenge, brushing or biopsy.

Study design

Patients and controls were challenged daily with allergen during a 2-week period. Their signs and symptoms were recorded every day. Biopsies were obtained from all patients three or four times, the controls were biopsied three times. In order to investigate a overall variability during provocation the patients were randomly divided into two groups for statistical reasons. By dividing the patients in two groups a sufficient number of different biopsy samples on different time-points could be obtained. Biopsies of the allergic patients were taken at seven different moments as shown in Table 1. The study was performed after informed consent conditions and approved by the

Table 1. Moments of biopsy. For statistical evaluation the patients were split in two groups (I and II) before and during provocation. (N = number of patients, *n* = number of biopsies)

Patient group	Moment of biopsy			
	A	B	C	
I (N = 7)	Before provocation (<i>n</i> = 7)	24–48 h (<i>n</i> = 5)	8–10 days (<i>n</i> = 6)	1–2 weeks after last provocation (<i>n</i> = 9)
II (N = 10)	$\frac{1}{2}$ h (<i>n</i> = 5) 8 h (<i>n</i> = 5)	4–6 days (<i>n</i> = 9)	14 days (<i>n</i> = 9)	

medical ethics committee of the Leyenburg Hospital, The Hague, The Netherlands.

Nasal allergen challenge

Patients and controls were challenged daily with grass pollen extract (1000, 3000, 10 000, 30 000, 100 000 SQ/mL ALK, Diephuis, Groningen, The Netherlands) with a pump spray delivering a fixed dose of $50 \pm 2 \mu\text{L}$ in each nostril. Each provocation was preceded by a 15 min period to allow the nasal mucosa to become acclimatized. On the first day of the provocation the patient was subjected to provocation with 1000 SQ/mL followed by increasing doses of allergen to establish the threshold dose. The interval between two doses was 15 min. Within 15 min of each challenge, the number of sneezes was counted and the amount of secretion, collected in pre-weighted paper tissues, was determined. The lowest concentration which resulted in either more than 0.5 g secretion or more than five sneezes in 15 min was called the threshold dose. In the following days the same concentration was used until the patient experienced serious complaints, i.e. a score of 3 on two or more items of the symptom score of the previous day, at which point the threshold dose was determined at new.

Nasal biopsies

Biopsies of nasal mucosa were taken from the lower edge of the inferior turbinate, about 2 cm posterior to the front edge using a Gerritsma forceps with a cup diameter of 2.5 mm [21]. The biopsy specimens were embedded in Tissue-Tek II OCT compound in a gelatin capsule and immediately frozen [22]. Biopsies of the allergic patients were taken before the provocation, 30 min, 8 h and 24 h

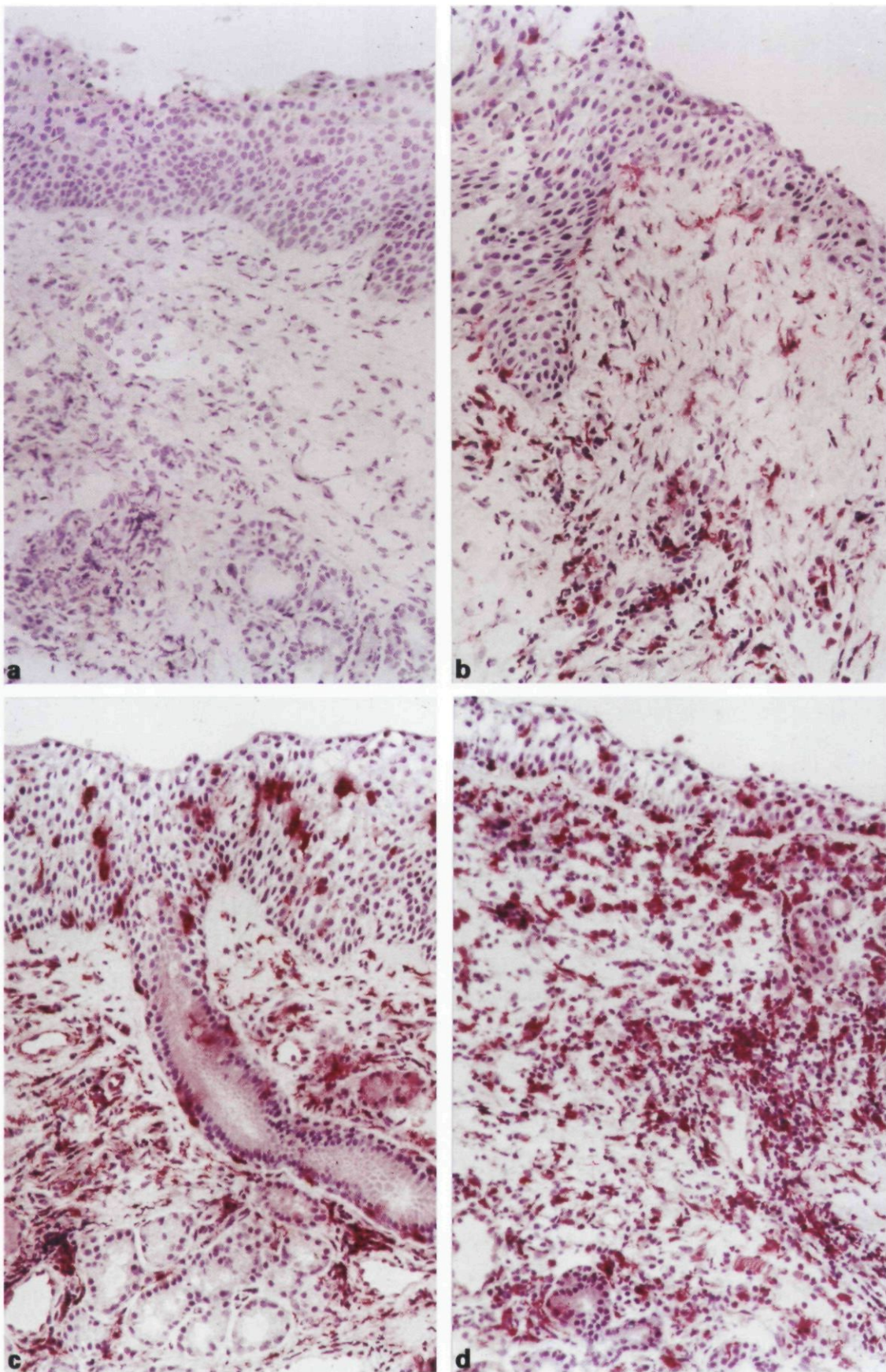
after the first provocation, 2, 4, 6, 8, 10, and 14 days after the first provocation, and 1 and 2 weeks after the last provocation (Table 1). Biopsies were always taken prior to a new provocation took place. The controls were divided into two groups and were biopsied twice. Two controls were biopsied before provocation and 12 days after the first provocation and the other two were biopsied 24 h and 12 days after the first provocation.

Staining procedure of cryostat sections

From each specimen serial 6 μm thick sections of nasal mucosa were cut on a Reichert–Jung 2800 Frigocut cryostat and transferred to gelatin-coated microscope slides. The sections were stained according to a previously described immuno-alkaline phosphatase method (AP anti-AP complex; DAKO-immunoglobulin A/S, Glostrup, Denmark) [23]. The antibodies used are listed in Table 1.

Immunofluorescence double-staining was performed by incubation with the primary MoAb, followed by incubation with goat anti-mouse Ig labelled with TRITC (GaMIg-TRITC) or FITC (GaMIg-FITC) and reincubation with another MoAb labelled with FITC or TRITC, depending on the combination of MoAb used. Each combination of MoAb was used in a few representative sections. Control staining was performed by substitution with phosphate buffered saline (PBS) and incubation with non-relevant MoAb of the same subclass.

Double-staining was not only performed by the immunofluorescence staining technique but also by immunohistochemical double-staining. FITC conjugated IgE MoAb was visualized by AP-conjugated anti-FITC polyclonal antibody. The MoAb in the second part of the double-staining was subsequently visualized using β -galactosidase conjugated polyclonal antibody.



Also staining with acid phosphatase was used to distinguish macrophages from other HLA class II⁺ DC cells. Macrophages have acid phosphatase activity throughout the cytoplasm [24] while DC only show acid phosphatase in a central spot near the lobulated nucleus [25].

Light microscopic evaluation

In two sections of each biopsy specimen the number of stained cells were counted separately in the epithelium and lamina propria. The surface area of the epithelium and lamina propria were determined by computer image analysis (Kontron's Image Analysis System Videoplan). The number of eosinophils/mm² section area in epithelium and lamina propria was calculated.

When groups of positive cells were in close proximity or showed some overlap, only the definitely positive cells were counted. When large numbers of positive cells lay closely, as after HLA-DR staining, the number of positive cells was assessed semi-quantitatively on a score from 0 to 4 with steps of 0.5, representing a range from practically no HLA-DR⁺ cells to large numbers of HLA-DR⁺ cells (Fig. 1). Moreover, based on morphology and localization, semi-quantitative assessment was made as well on a 0–4 scale of the number of HLA-DR⁺-DC and HLA-DR⁺-lymphocytic cells. Only small, round cells containing practically no cytoplasm were judged to be lymphocytes. The rest of the immigrating cells were judged to be DC/macrophages.

Statistical analysis

For the biopsy part of the study the 17 patients were split in two groups, each with its own measurement scheme: before and during provocation for statistical reasons (Table 1). Three repeated measurements were made for each of the two groups one before (A) and two during the provocation (B,C), together constituting six points on a time axis. Because the variables which were evaluated were symmetrically distributed after log transformation (i.e. with skewness tested to be non-significant) a repeated measurement analysis of variance was performed. In this analysis the six time effects on the

dependent variable are obtained as the estimates of six coefficients in a saturated model with three within-subject repetitions for the time effect (two coefficients), two between-subjects repetitions for the grouping effect (one coefficient), the interaction between repetition time and group. Relevant are significant differences in time effect and no significance of the grouping and interaction effect (two coefficients) and a constant term (one coefficient).

The differences between the end of the provocation period and the period 1–2 weeks after the last provocation were analysed with the sign test. Differences in the numbers of HLA-DR⁺ cells, L25⁺ cells and IgE⁺ LC and the percentage of IgE⁺ cells were determined by a non-parametric test against related measurements.

Results were considered to be significantly different when the *P*-value was < 0.05.

Results

General description

The sections of nasal mucosa had an average surface area of 3 mm² and usually showed a lining of ciliated columnar epithelium with or without goblet cells and/or partially stratified cuboidal epithelium. The epithelium could not be evaluated in three sections (before provocation, 6 and 8 days after the first provocation), which were excluded from the study. The lamina propria consisted usually of a looser subepithelial cell-rich layer containing most of the mucous glands and a deeper collagenous cell-poor layer. All sections were sufficiently deep to examine both layers. No microscopical differences were found between allergics and controls in terms of general characteristics.

CD1a⁺ cells The number of CD1a⁺ cells increased significantly in the epithelium (sevenfold, *P* = 0.008; Fig. 2) and lamina propria (sevenfold, *P* = 0.01; Fig. 2) during the provocation period. Already within the first 24 h after provocation the number of CD1a⁺ cells seemed to increase in the epithelium (threefold increase 30 min after provocation). However, possibly due to the small number of biopsies evaluated in this period, these changes did not reach statistical significance.

Fig. 1. Examples of scores of HLA-DR⁺ cells: (a) Nasal mucosa of patient with isolated grass pollen allergy 30 min after first provocation: practically no HLA-DR⁺ cells were seen in the epithelium (score 0.5) and lamina propria (score 0.5). (b) Nasal mucosa of patient with grass pollen allergy 4 days after first provocation: a small number of HLA-DR⁺ cells were seen in the epithelium (score 1) and lamina propria (score 1). (c) Nasal mucosa of patient with grass-pollen allergy 14 days after first provocation: quite some HLA-DR⁺ cells were seen in the epithelium (score 2) and lamina propria (score 2). (d) Nasal mucosa of patient with grass pollen allergy 4 days after first provocation: quite some HLA-DR⁺ cells were seen in the epithelium (score 2) and a large number in the lamina propria (score 3).

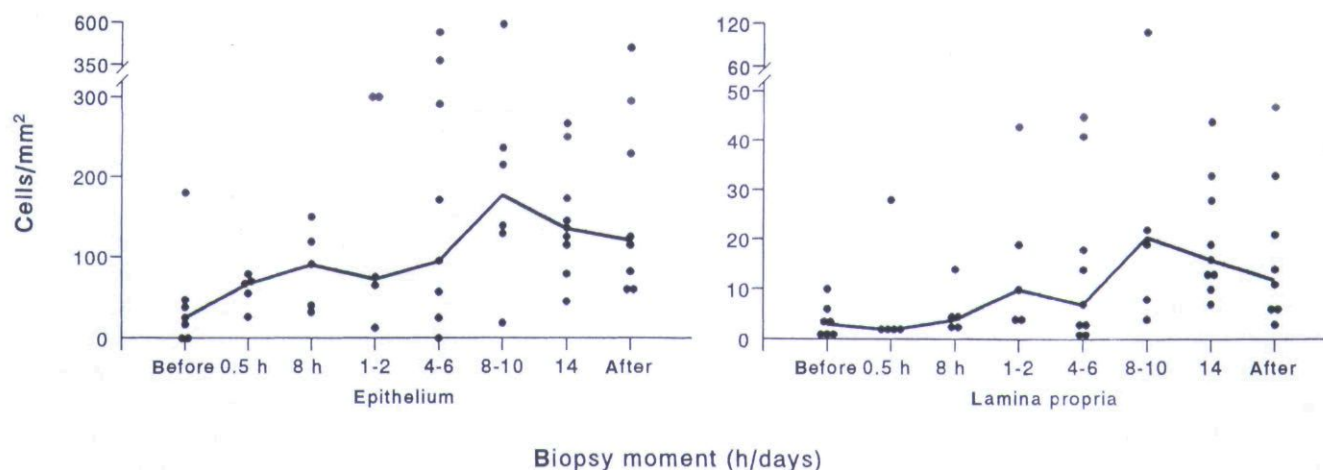


Fig. 2. Median number of CD1a⁺ cells in epithelium and the lamina propria of the nasal mucosa of patients with isolated grass pollen allergy before, during and after a 2-week provocation period. An overall significant increase in CD1a⁺ cells was found during the provocation period in epithelium ($P = 0.008$) and lamina propria ($P = 0.01$).

At the beginning of the provocation period, CD1a⁺ cells were observed in the lower layers of the epithelium and in the lamina propria, predominantly in the subepithelial layer and around the vessels. After the first provocation, CD1a⁺ cells seemed to redistribute to the epithelium.

In the second week of the provocation period, CD1a⁺ cells were found through the whole depth of the epithelium. Occasionally, CD1a⁺ cells reached the surface of the epithelium. The CD1a⁺ cells in the lamina propria were found predominantly in the subepithelial layer. In this layer, clusters of CD1a⁺ cells and lymphocytic cells were regularly observed. The number of CD1a⁺ cells in the lamina propria increased at a much slower rate than in the epithelium. In the lamina propria a significant increase was found after 24 h.

In both the epithelium and the lamina propria, the largest number of CD1a⁺ cells was found 9 days after the first provocation. This is well before the end of the provocation period. After the 9th day a small and insignificant decrease in number of LC was found in the epithelium and lamina propria. This decrease seemed to persist until 1–2 weeks after the provocation period (Fig. 2). No significant changes occurred in the control group.

HLA-DR⁺ cells HLA-DR⁺ cells were found in the epithelium and, predominantly, in the subepithelial layer of the lamina propria. The HLA-DR⁺ migratory cells showed dendritic or lymphocytic morphology. Epithelial cells in the epithelium and/or epithelial cells in the mucous glands were sometimes positive. Often large clusters (100–1000 cells) of HLA-DR⁺ cells (with dendritic and/or lymphocytic morphology) were seen in the lamina propria, mainly in the subepithelial layer. The

total number of HLA-DR⁺ cells scored on a 0–4 scale, increased significantly in epithelium ($P = 0.02$) and in the lamina propria ($P < 0.001$) during the provocation period (Fig. 3). The number of HLA-DR⁺ cells between the patients differed strongly. Some patients showed fewer HLA-DR⁺ cells at the end of the provocation period than others at the beginning. All scores from the biopsy after the provocation period (biopsy moment 3 in Fig. 3) were lower than or equal to the scores from the last biopsy during provocation (biopsy moment 2 in Fig. 3). The number of biopsy specimens evaluated was, however, too small to reach statistical significance.

The number of dendritic HLA-DR⁺ cells as well as the HLA-DR⁺ cells with lymphocytic morphology which were scored on a 0–4 scale, increased significantly in the epithelium (DC $P = 0.004$, lymphocytes $P = 0.006$) and the lamina propria (DC $P = 0.02$, lymphocytes $P = 0.001$). HLA-DR⁺ DC were already found in the epithelium and the lamina propria before provocation and their numbers increased from the beginning of the provocation period. HLA-DR⁺ cells with lymphocytic morphology were rarely seen before provocation. They usually increased in numbers in the second week of the provocation period in the epithelium and the lamina propria. The number of HLA-DR⁺ epithelial cells did not change during the provocation period. In the controls, the number of HLA-DR⁺ cells did not change during the provocation.

HLA-DQ⁺ cells HLA-DQ⁺ cells were found in the epithelium and, predominantly, in the subepithelial layer of the lamina propria. The HLA-DQ⁺ cells showed a dendritic morphology. HLA-DQ⁺ cells with a

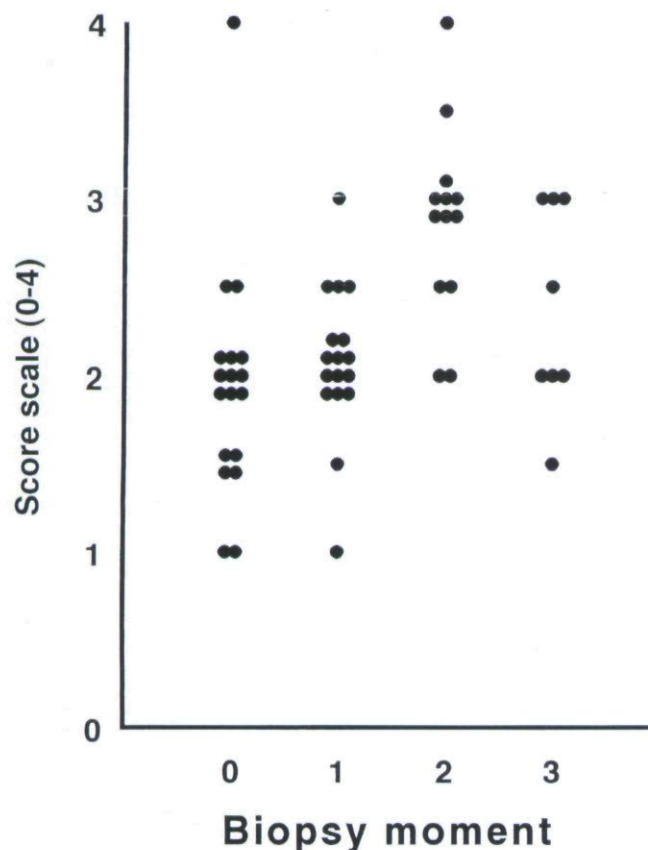


Fig. 3. Score of HLA-DR⁺ cells in the lamina propria of all patients. The null biopsy moment (0) represents biopsies taken before provocation, after 30 min or after 8 h in different patient groups. The first biopsy moment (1) represents biopsies taken 24–48 h or 4–6 days after the first provocation; the second biopsy moment (2) biopsies taken 8–10 days or 14 days after the first provocation and the third biopsy moment (3) biopsies taken 1–2 weeks after the last provocation. A significant increase was found during provocation. All scores at the biopsy after provocation (3) were lower or equal to the scores at the last biopsy during provocation.

lymphocytic morphology were not seen. The number of HLA-DQ⁺ cells was always smaller than the number of HLA-DR⁺ cells. A significant increase in the number of HLA-DQ⁺ cells was found in the epithelium ($P = 0.03$) and lamina propria ($P < 0.001$) during the provocation period (Fig. 4). Clusters of HLA-DQ⁺ cells were regularly observed.

Ki-M6⁺ cells The cells that were positive for Ki-M6, were large cells with bright cytoplasmic staining. Ki-M6⁺ cells were found in the epithelium and all layers of the lamina propria. During provocation the number of Ki-M6⁺ cells increased significantly in the lamina propria ($P = 0.003$), but not in the epithelium (Fig. 5). In the lamina propria of the controls, the

Ki-M6⁺ cells increased to the same extent. However, the results of four controls did not constitute significance.

L25⁺ cells The L25⁺ cells usually had a dendritic morphology. L25⁺ lymphocytic cell, presumably B lymphocytes, were seldomly found in the lamina propria. During the first 8 h of provocation, the number of L25⁺ cells increased significantly in the epithelium ($P = 0.03$) (Fig. 6). In the lamina propria a small, insignificant increase was found. During the rest of the provocation period a significant decrease was found in the number of L25⁺ cells in the epithelium ($P = 0.05$) and the lamina propria ($P = 0.05$) (Fig. 6). Nine days after the first provocation, hardly any L25⁺ cells were seen. Remarkably, the L25⁺ cells first disappeared from the epithelium, then from the subepithelial layers and finally from the deeper layers of the lamina propria disappeared. In the controls no changes were found in the number of L25⁺ cells.

Cells staining for acid phosphatase

Cells staining for acid phosphatase were found in the epithelium and lamina propria. Because mucus in goblet cells and in mucous glands were also positive for acid phosphatase, a reliable count of the number of cells positive for acid phosphatase was impossible.

Double-staining

Immunofluorescence double staining for CD1a and HLA-DR showed that all CD1a⁺ cells evaluated were also positive for HLA-DR and that approximately half of the HLA-DR⁺ cells in the epithelium and only a small proportion of the HLA-DR⁺ cells in the lamina propria were positive for CD1a. Most of the L25⁺ cells were also positive for HLA-DR. No L25⁺ or Ki-M6⁺ cells were found to be positive for IgE.

The number of IgE⁺ CD1a⁺ cells was determined in eight patients (29 biopsies). The mean (SD) percentage of IgE⁺ CD1a⁺ cells was 33 in the epithelium. Occasionally IgE⁺ CD1a⁺ cells were also found in the lamina propria, mainly in the subepithelial layer. During the provocation period the number of IgE⁺ CD1a⁺ cells increased significantly ($P = 0.01$). The percentage of CD1a⁺ cells which were positive for IgE, however, remained the same. The percentage of IgE⁺ CD1a⁺ cells did not change after the provocation period (Fig. 7: IgE⁺-CD1a⁺ cells).

Discussion

MHC class II positive APC are necessary for the

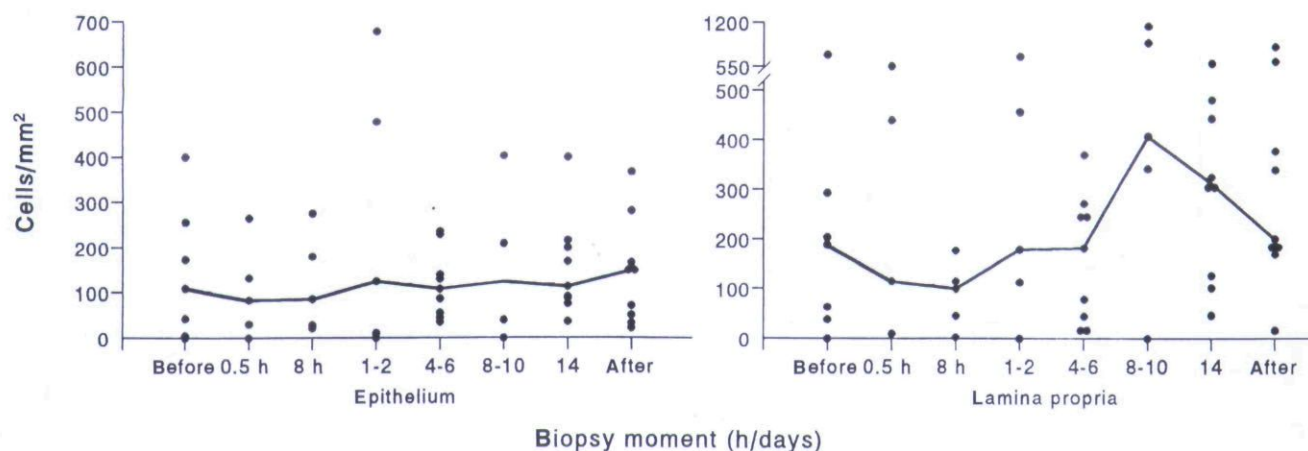


Fig. 4. Median number of HLA-DQ⁺ cells in epithelium and the lamina propria of the nasal mucosa of patients with isolated grass pollen allergy before, during and after a 2-week provocation period. An overall significant increase in HLA-DQ⁺ cells was found during the provocation period in epithelium ($P = 0.03$) and lamina propria ($P < 0.001$).

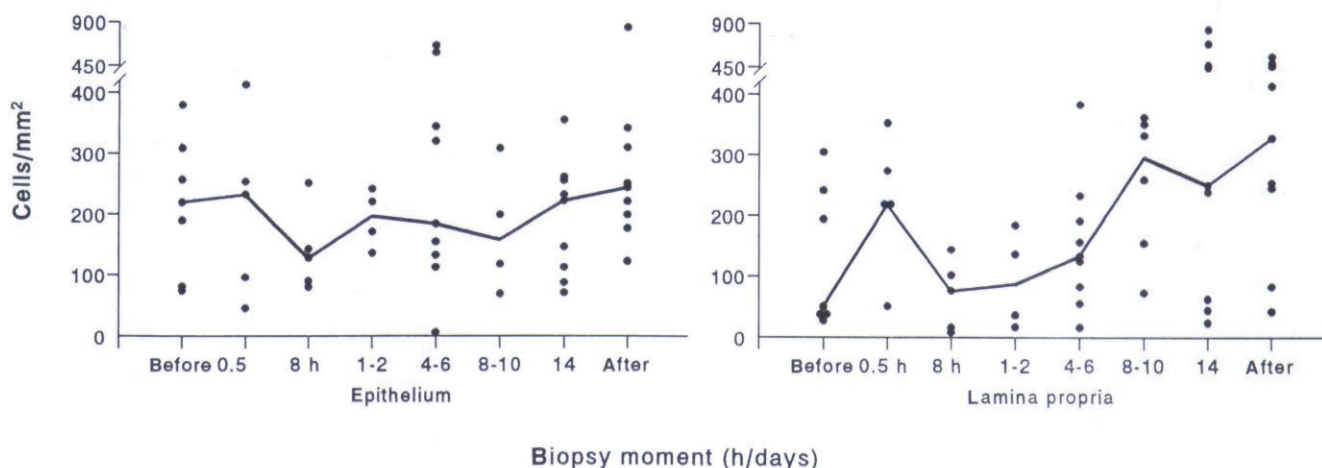


Fig. 5. Median of the number of Ki-M6⁺ cells in the epithelium and lamina propria of the nasal mucosa of symptomatic patients with isolated grass pollen allergy before, during, and after a 2-week provocation study. An overall significant increase was found in the lamina propria ($P = 0.03$). NS. Not significant.

presentation of antigen to T lymphocytes [26]. In previous studies [19,23] and also in this study, we found indications for a role of HLA class II positive LC in allergic rhinitis.

In this study we found the number of HLA-DR⁺ cells to be larger than the number of HLA-DQ⁺ cells, but both increased during allergen provocation. It is remarkable that the HLA-DQ⁺ cells, as opposed to the HLA-DR⁺ cells, all showed a dendritic morphology, suggesting that the HLA-DR⁺ DC and HLA-DQ⁺ DC do not belong to the same population. Human DC express HLA class II molecules encoded by the subloci DP, DQ and DR [26]. HLA-DQ, contrary to HLA-DR, is not concentrated at points of contact between

aggregating DC and lymphocytes and is more diffusely localized on the cell [27]. Antigen associated with DR and DQ may be handled differently [25,28,29], resulting in different biological responses.

The number of HLA-DR⁺ lymphocytic cells increased during allergen provocation. Because most of the lymphocytes in nasal mucosa are T lymphocytes [9], these HLA-DR⁺ lymphocytes will generally be activated T cells. Another matter of interest is that the extent of HLA-DR expression on epithelial cells did not increase during allergen provocation. This suggests that HLA-DR positive epithelial cells do not play an important role in allergen presentation in allergic rhinitis.

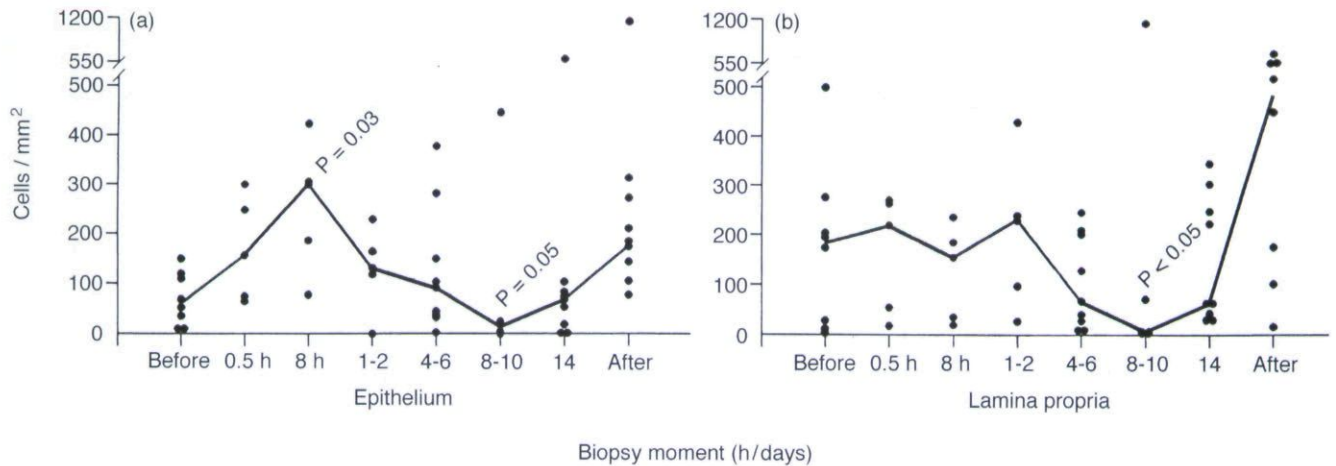


Fig. 6. Median number of L25⁺ cells in the epithelium and lamina propria of the nasal mucosa of symptomatic patients with isolated grass pollen allergy before, during and after a 2-week provocation period. A significant increase ($P = 0.03$) was found in the epithelium in the first 8 h after the first provocation. During the rest of the provocation period a significant decrease in epithelium ($P = 0.05$) and lamina propria ($P = 0.05$) was found. After the provocation period a significant increase was found in the epithelium and the lamina propria.

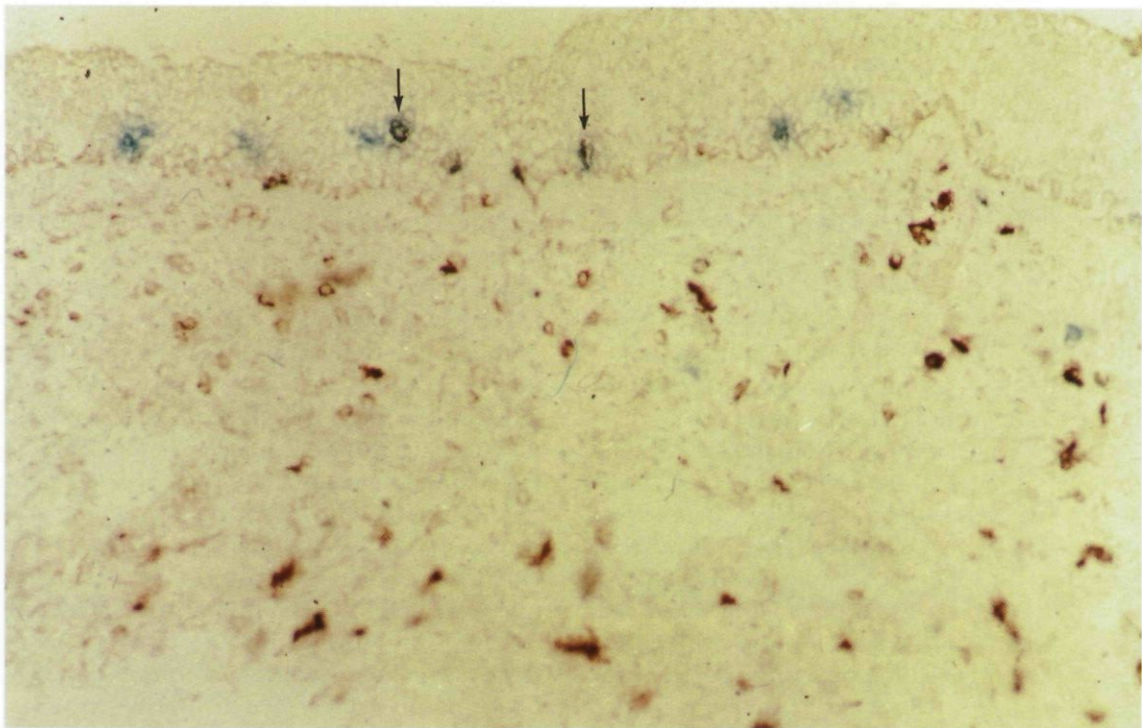


Fig. 7. Immunohistochemical double-staining using MoAb directed to CD1a and IgE. Green: CD1a⁺ cells; Brown/red: IgE⁺ cells; Green + brown/red: IgE⁺ CD1a⁺ cells (arrows).

DC are clearly more potent APC than other MHC class II positive cells (like macrophages and B cells) because of their ability to actively form cellular clusters with immunocompetent cells [30] and because DC have a

quantitatively and qualitatively superior MHC expression [3]. So far, few DC markers are available, despite the continuous efforts to make specific MoAb. In our hands acid phosphatase could not be used to distinguish

Table 2. Monoclonal antibodies used for immunohistochemistry

Monoclonal antibody	Specificity	Source
Anti-HLA-DR	MHC class II (HLA-DR)	Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands (CLB)
Leu-10	MHC class II (HLA-DQ)	Beckton Dickinson, Mountain View, CA, USA
Ki-M6	Macrophages (CD68)	Behring, Marburg, Germany
L25	B cells and dendritic cells	Y. Ishii, Tokyo, Japan [18]
OKT6	Langerhans cells (CD1a)	Orthodiagnostic
HLA-DE-TRITC	MHC class II	Becton Dickinson
Anti-IgE	IgE	CLB
Anti-IgE-FITC	IgE	CLB

between DC and macrophages in sections of nasal mucosa because mucus staining makes enumeration of the cells impossible. So, in order to get an impression of which part of the HLA-DR⁺ cells with a dendritic morphology are DC or macrophages, we used MoAb specific to DC (L25) and macrophages (Ki-M6). During provocation, the Ki-M6⁺ cells increased in the lamina propria of allergic patients and normal controls. Probably this is caused by the daily brushing of the nasal mucosa to collect surface epithelial cells in this patients. In contrast with alveolar macrophages [31], macrophages in the nasal mucosa did not express IgE.

L25, a MoAb directed against B cells and DC [32], did occur in the nasal mucosa and usually had a dendritic morphology. L25 was thought to be a marker of activated DC [33,34]. In this provocation study, however, the number of L25⁺ cells, after an initial significant increase in the first 8 h after the first provocation, decreased significantly during the rest of the provocation period. During the provocation period the depth of the area in which no L25⁺ cells could be found, increased steadily. At the end of the provocation period only a few L25⁺ cells were left in the deepest layer of the lamina propria. After the provocation period a significant increase of L25⁺ cells was found in the lamina propria. These data suggest that L25 expression on DC and/or macrophages in the nasal mucosa is inversely proportional to the state of activation of cells.

In previous studies we found that the number of LC is larger in patients with allergic rhinitis than in non-

allergic controls, and that in patients with allergic rhinitis the number of LC is higher during natural allergen provocation than without allergen provocation [19,23]. In this study we further investigated the dynamics of LC. The number of LC increased significantly in epithelium and lamina propria during the provocation period. This finding corresponds to our previous studies during natural provocation. The increase in the present study is larger than found during natural provocation, probably due to the strong allergen stimulus continuing on a daily basis. The observation of a threefold increase in LC in the first 1/2 h after provocation would suggest that the increase of LC numbers is due to migration from the lamina propria and not to mitotic activity, suggested to occur in the skin [35] apart from migration [36]. Moreover, LC seem to be able to migrate fast, i.e. several millimeters within 30 min [37]. Besides migration, LC may develop in the epithelium from indeterminate cells by obtaining Birbeck granules. Findings of Hanau [38], however, indicate that indeterminate cells first become CD1a positive, then obtain Birbeck granules by endocytosis, and finally migrate to the epidermis. This implies that indeterminate cells become LC before migrating to the epithelium. This is consistent with the finding in this study that in the first 24 h after provocation the number of LC in the epithelium increased while the number of LC in the lamina propria remained the same. The mechanism which attracts LC to the epithelium remains unclear.

The finding that in atopic dermatitis LC are positive for

IgE and that only IgE⁺ LC seem to be able to induce a T-cell proliferation to house dust mite allergen *in vitro*, suggests that IgE positivity is necessary for the allergen presenting function of LC in allergic disease [39]. The findings reported in this study [39] support the hypothesis that IgE⁺ LC play a key role in allergic disease. In respiratory allergy, part of the LC are also positive for IgE. Were we found an increase in the total number of LC in the epithelium the percentage of IgE⁺ LC remained the same, the actual number of IgE⁺ LC in the epithelium increased. To further study the role of LC in general and IgE⁺ LC in allergen provocation, functional studies are necessary. These studies should make clear whether LC in nasal mucosa are able to transport and present allergen and whether this mechanism is IgE-dependent. Other studies suggest that the principal antigen presenting cell (APC) in the lung is the dendritic cell (DC)/Langerhans cell (LC) [40,41].

In conclusion, this study shows that not only LC, as described by earlier studies, but other DC as well are found in the epithelium and lamina propria of the nasal mucosa and that the incidence of these cells increases during allergen provocation. However, no specific DC marker was found, which was expressed on DC but not on LC and/or macrophages. As a consequence it was impossible to determine more precisely the role of DC in allergen handling. Furthermore, it was impossible to detect whether other DC than LC are IgE⁺. This study does not support the hypothesis that macrophages play a role in allergic rhinitis.

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