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Fluctuation of the number of CD-1(T6)-positive dendritic cells, presumably Langerhans cells, in the nasal mucosa of patients with an isolated grass-pollen allergy before, during, and after the grass-pollen season

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A monoclonal antibody against CD-1(T6) was used for studies in the nasal mucosa of patients with isolated grass-pollen allergy to determine whether the number of CD-1-positive cells, presumably Langerhans cells, depends on the season in which the nasal biopsy is performed. An earlier study had demonstrated that during the grass-pollen season, there are significantly more CD-1-positive cells in nasal mucosa of patients with isolated grass-pollen allergy than in nonallergic control subjects without nasal complaints. During the grass-pollen season, the nasal epithelium of patients with an isolated grass-pollen allergy demonstrated significantly more CD-1-positive cells than before and after the season. Before and after the season, the number of CD-1-positive cells in epithelium of the allergic patients was not significantly greater than the corresponding number in epithelium of nonallergic subjects without nasal complaints. (J ALLERGY CLIN IMMUNOL 1989;84:39-43.)

LCs are large mononuclear dendritic CD-1(T6)-positive cells containing Birbeck's granules in the cytoplasm. The role and function of LCs are incompletely known, but recent research demonstrated that they are essential antigen-presenting cells.¹⁻⁴ LCs are mesenchymal cells and derive from a precursor cell

Abbreviations used

AF: Alkaline phosphatase
LC: Langerhans cell
PBS: Phosphate-buffered saline
RaMlg: Rabbit antimouse immunoglobulin
FITC: Fluorescein isothiocyanate

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in the bone marrow.⁵ As mobile cells, they can migrate into the epidermis from a hematogenous source and, after activation, probably also migrate from the epithelium to subepithelial layers and regional lymph nodes.^{3,6} In an earlier study we found dendritic CD-1(T6), HLA-DR expressing cells, resembling LCs in the epithelium and lamina propria of nasal mucosa in patients with grass-pollen allergy and control subjects without nasal complaints. These cells were significantly more numerous in epithelium of nasal biopsy

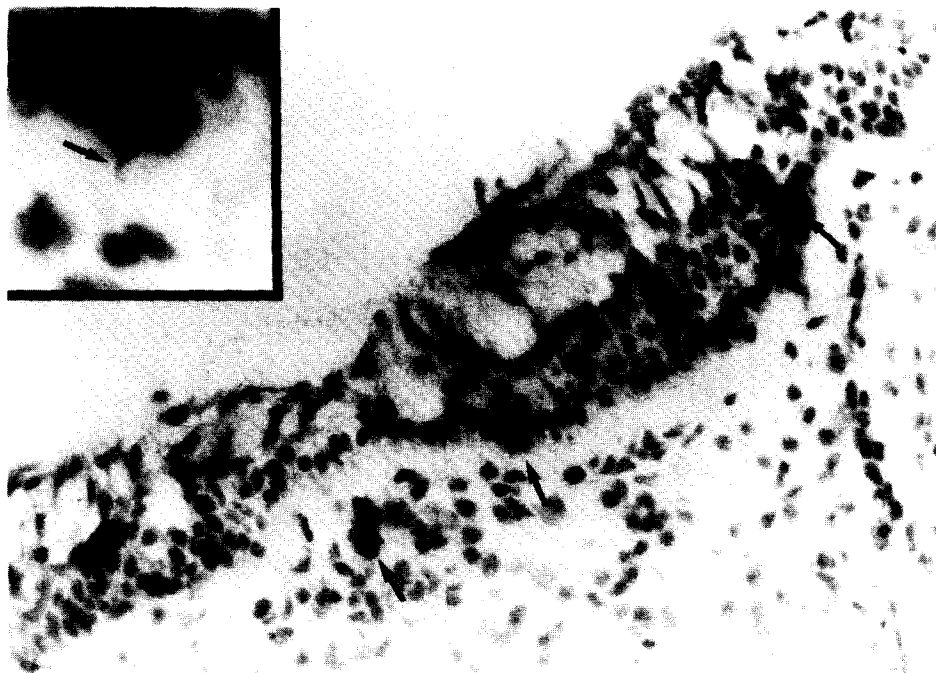


FIG. 1. Light micrograph of the nasal mucosa of a patient with an isolated grass-pollen allergy incubated with AF-labeled monoclonal anti-CD-1. (Original magnification $\times 250$.) CD-1-positive dendritic cells ($\downarrow$) in the middle and lower layers of the epithelium. Magnification of single CD-1-positive cell ($\uparrow$) (*inset*) in the epithelium clearly illustrating dendritic processes, one passing through the basal membrane.

samples of patients with grass-pollen allergy during the grass-pollen season than in epithelium of nonallergic control subjects. Using double-staining methods with CD-1 and anti-IgE FITC, we also observed IgE on CD-1-positive cells.^{7,8} Because it was not yet clear whether LCs are constitutionally more numerous or their numbers increase by migration into the epithelium during the season, further investigations were performed in patients with grass-pollen allergy to determine whether changes occur in the number of CD-1-positive cells after the grass-pollen season.

MATERIAL AND METHODS

Patients

Twelve patients with an isolated grass-pollen allergy, confirmed by skin and RAST, were selected for this study. This group consisted of eight women and four men with a mean age of 30 years (range, 17 to 51 years). The control group consisted of 12 volunteers and patients with diseases of the ear, nose, and throat not related to the nose or nasal sinuses (mean age, 28 years; range, 15 to 42 years; three men and nine women). These control subjects had no nasal complaints, no nasal abnormalities observed at ear, nose, and throat examination, and a negative RAST.

Nasal biopsy

Nasal biopsies were performed four times in each patient, that is, in July and October of 1986 and March and July of

1987. Biopsies were performed in control subjects once in July or August 1986 under informed consent conditions. The biopsy specimens were obtained from the lower edge of the inferior turbinate, about 2 cm posterior to the front edge, with a slightly modified Hartmann forceps (Aesculap Werke AG, Tuttlingen, West Germany) with a cup diameter of 2.5 mm. Local anesthesia was obtained by placing a cotton-wool carrier with 100 mg of cocaine and 3 drops of adrenalin (1:1000) under the inferior turbinate without touching the place where the biopsy specimen would be obtained. The specimens were embedded in Tissue-Tek II O.C.T. (Miles Scientific, Elkhart, Ind.) compound in a gelatin capsule and frozen immediately.^{9,10}

Antibodies

The specificity of clone 66IIC7 for the CD-1 surface marker of LC has been discussed elsewhere.¹¹

Staining procedure

The immuno-AF method used was a modification of the method described by Li et al.¹² Briefly, 6 μ m thick sections of nasal mucosa were cut on a 2800 Frigocut cryostat (Reichert-Jung GmbH, Nussloch, West Germany), transferred to gelatin-coated microscope slides, dried, fixed in acetone for 10 minutes at 20°C, rinsed in PBS, pH 7.2, and incubated with 66IIC7 (CD-1) monoclonal antibody for 30 minutes at 20°C. The sections were rinsed again in PBS for 5 minutes and incubated for 15 minutes with a RaMlg antiserum conjugated to AF (Dako-Immunoglobulin A/S,

TABLE I. Number of CD-1-positive cells per square millimeter in epithelium and lamina propria of nasal mucosa in 12 patients with isolated grass-pollen allergy before, during, and after the grass-pollen season and 11 control subjects

	Period of biopsy	Epithelium		Lamina propria	
		Mean	SD	Mean	SD
Group					
I	July 1986	55	46.9*	14	21.9
II	October 1986	31	22.0	7	5.3
III	March 1987	30	35.0	4	3.4
IV	July 1987	80	102.0*	6	5.4
Control subjects	July-August 1986	20	14.1*	4	4.0
Group comparison† (<i>p</i> value)					
I/II		0.047‡		0.165	
I/III		0.019		0.082	
I/IV		0.178		0.125	
II/III		0.445		0.007	
II/IV		0.074		0.223	
III/IV		0.047		0.072	

*In one patient in group I, in another patient in group IV, and in one control subject, the epithelium was damaged and was not included in the study.

†*p* Value from paired Student's *t* test comparing any two periods.

‡Significance, *p* < 0.05.

Glostrup, Denmark, D314) 1:20, supplemented with 10% normal human serum to saturate Fc receptors and reduce background staining (Dako-Immunoglobulin A/S, D314), then rinsed successively in PBS and Tris buffer (pH 8.0), and incubated for 30 minutes with a new fuchsin solution. This solution is made by thoroughly mixing 150 μ l of NaNO₂ 4% with 150 μ l of a solution containing 1 gm of new fuchsin (Chroma IB467, Stuttgart, West Germany) in 25 ml of HCl 2 N. Next, 60 ml 0.2 mol/L of Tris HCl buffer (pH 8.0) containing 18 mg of naphthol AS-MX phosphate (N-5000, Sigma Chemical Co., St. Louis, Mo.) dissolved in 2 ml of dimethyl formamide is added, together with 15 mg of levamisole (L-9756, Sigma Chemical Co.). The pH of the final mixture is adjusted to 8.0. Mixture was filtered and used immediately.

Finally, the sections were rinsed with distilled water, counterstained with Mayer's hematoxylin, and mounted in glycerin gelatin (Chroma).

Control sections incubated with monoclonal antibodies of the same subclass (IgG2_a), like prostate-specific antigen, were negative. Immunofluorescence double staining was performed with monoclonal CD-1 followed by incubation with goat antimouse immunoglobulin, labeled with tetramethylrhodaminoisothiocyanate, and reincubation with anti-IgE labeled with FITC (RaMIgE-FITC) or anti-HLA-DR labeled with FITC (goat antimouse HLA-DR-FITC). In addition, serial sections were incubated with anti-HLD-DR and anti-T6, alternately.

Cell quantification

The total surface area of epithelium and lamina propria in the section was estimated by superimposing a grid of 100

points (intersection of crosses, covering a surface area of 0.5 mm²) on the section with an eyepiece graticule, as described by Underwood.¹³ In the section, all large dendritic cells containing a nucleus and staining red were considered to be T6-positive cells. The total number of T6-positive cells in epithelium and lamina propria was determined, and the number of T6-positive cells per square millimeter was estimated.

Statistical analysis

For the statistical analysis, use was made of the paired Student's one-tailed *t* test for the differences between the data for the four periods and Student's two-tailed *t* test for comparison of allergic patients against control subjects. A *p* value <0.05 was considered to indicate a significant difference between the groups.

RESULTS

The sections of nasal mucosa had an average surface area of 2 mm² and usually demonstrated ciliated columnar epithelium and/or partially stratified cuboidal epithelium. All but three sections (excluded from the study) demonstrated intact or virtually intact epithelium.

All but three sections demonstrated CD-1-positive dendritic cells in the middle and lower layers of the epithelium (Fig. 1). All sections demonstrated CD-1-positive cells in the lamina propria. As a rule, the CD-1-positive cells were distributed over the basal and middle layers of the epithelium. The distribution was not always regular along the epithelium; some-

times a group of CD-1-positive cells was observed in one place, and only few such cells in the rest of the epithelium. In the lamina propria, the cells occurred in the subepithelial layer and in and around the glandular tissue.

Double immunofluorescence staining with monoclonal anti-CD-1 antibody and FITC-labeled rabbit anti-IgE demonstrated IgE on a few CD-1-positive cells in the nasal mucosa of three allergic patients investigated. Most of the CD-1-positive cells also expressed HLA-DR.

The number of CD-1-positive cells per square millimeter in nasal mucosa of the patients with isolated grass-pollen allergy in the four periods under study and of the control subjects is presented in Table I. As published earlier,⁸ the difference between the number of CD-1-positive cells in the epithelium of patients with grass-pollen allergy in the grass-pollen season and of nonallergic control subjects is significant ($p = 0.04$). The difference between these numbers before and after the grass-pollen season on one hand and control subjects on the other hand is not significant. The numbers of CD-1-positive cells were compared before, during, and after the grass-pollen season. In the epithelium the number of CD-1-positive cells in the grass-pollen season (July) was significantly higher than it was before the season (March). After the season the number of CD-1-positive cells was significantly lower than the number had been during the preceding summer. The difference in number between October 1986 and July 1987 did not quite reach significance ($p = 0.074$). The lamina propria had few CD-1-positive cells. The differences are only significant between October 1986 and March 1987.

DISCUSSION

LCs are dendritic cells capable of binding and presenting potentially antigenic molecules to T-lymphocytes and probably other cells. They are also able to secrete important immunostimulatory factors, for example, interleukin-1, exerting an influence on T cell proliferation.⁴

The highly specific and sensitive marker for LCs and indeterminate cells, CD-1, has made it possible to detect and count LCs in tissues by light microscopy. In an earlier study we found CD-1-positive cells in nasal mucosa of allergic patients and nonallergic control subjects. The finding that the number of CD-1-positive cells was higher in nasal mucosa of patients with grass-pollen allergy than of nonallergic control subjects and the demonstration of IgE on CD-1-positive cells, suggesting a role in IgE-mediated disease, led us to perform further investigations to determine whether the number of CD-1-positive cells is

influenced by the exposure to grass pollen and thus is dependent on the grass-pollen season.

We found significantly more CD-1-positive cells in epithelium of nasal mucosa of patients with isolated grass-pollen allergy during the season than before or after the season. It appears possible that these cells migrate into the epithelium during allergen provocation. A possible sequence has been suggested according to which morphologically less developed indeterminate precursor cells, containing fewer Birbeck's granules, migrate from the dermis into the epidermis in which they reach structural and perhaps also functional maturity.^{6, 13}

It has also been suggested that LCs, as antigen-presenting cells, migrate from the epidermis to lymphoid tissue.⁶ We found some small changes in the numbers of CD-1-positive cells in the lamina propria of nasal mucosa during the season. Electron microscopy studies can be expected to demonstrate whether these CD-1-positive cells are indeterminate-like precursor cells or mature LCs carrying antigen to regional lymph nodes.¹⁴ Cell division might also explain the increase of the number of CD-1-positive cells during allergen exposure, since there have been a limited number of observations of mitotic activity in LCs.^{15, 16}

The fact that a significant difference was found between the number of CD-1-positive cells in the epithelium of patients with grass-pollen allergy and nonallergic control subjects in the grass-pollen season but not before and after the season, suggests that LCs are not constitutionally more numerous in allergic patients but that they increase after allergen provocation. An interesting question remains, that is, whether CD-1-positive cells are depleted during the grass-pollen season or migrate from the epithelium after the season.

Three findings suggest a role for CD-1-positive cells in allergic disease: (1) that patients with grass-pollen allergy during the grass-pollen season have a larger number of CD-1-positive cells than nonallergic control subjects, (2) that they have a larger number of CD-1-positive cells during the season than in the non-season of the grass pollen, and (3) that such cells demonstrate IgE positivity. A role in allergen transport for these cells appears likely, although an aspecific increase caused by inflammation in allergic rhinitis cannot be excluded.

It would be interesting to determine whether CD-1-positive cells are able to bind allergen via CD-1, Fc receptors, or HLA-DR, and can therefore transport allergens through the basal membrane into the lamina propria and the regional lymph nodes. Further investigation is needed to evaluate the relationship between CD-1-positive cells, other immunologic parameters in

nasal mucosa, and the clinical features of allergic disease.

In sum, the results of this study demonstrate that (1) the number of CD-1-positive cells in nasal epithelium of patients with grass-pollen allergy is significantly higher during the grass-pollen season but not significantly higher before and after the grass-pollen season than that in nonallergic control subjects, and (2) the number of CD-1-positive cells in nasal epithelium of patients with isolated grass-pollen allergy is significantly higher in the grass-pollen season than before or after the season.

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