

CD-1 (T6), HLA-DR-expressing cells, presumably Langerhans cells, in nasal mucosa

W. J. FOKKENS,¹ T. M. VROOM,² E. RIJNTJES¹ & P. G. H. MULDER³

¹Dept. of Otorhinolaryngology, Leyenburg Hospital, The Hague, ²Dept. of Pathology, Slotervaart Hospital, Amsterdam and

³Dept. of Biostatistics, Erasmus University, Rotterdam, The Netherlands

In the skin, epidermal Langerhans cells (LC) constitute a major population of antigen-presenting cells. These cells are characterized by the expression of both CD-1 (T6) and HLA-DR on the cell membrane. We wanted to know whether similar CD-1/HLA-DR-positive cells occur in the nasal mucosa of patients with an isolated grass pollen allergy and in non-allergic controls. CD-1/HLA-DR-positive dendritic cells were found in columnar and cuboidal epithelium and the lamina propria of the nasal mucosa. These CD-1/HLA-DR-positive cells presumably correspond with LC in the skin. We also found significantly more CD-1-positive cells in nasal biopsy samples of allergic than in those of the non-allergic controls. In the allergic patients some of the CD-1-positive cells were found to be surface IgE-positive, possibly due to passive adherence of IgE to Fc receptors.

Key words: Langerhans cell; nasal mucosa; CD-1 (T6)-positive cells; allergic patients.

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CLINICAL ASPECTS

A crucial aspect of allergic rhinitis, the transportation of allergens into the nasal mucosa, is virtually undocumented. Recent studies suggest that Langerhans cells (LC), known as antigen-presenting cells, play a role in atopic dermatitis. This study, for the first time, shows the presence of CD-1-positive cells, presumably LC, in the nasal mucosa. Possibly these LC also play a role in transporting allergens into the nasal mucosa. The impression exists that LC are more abundant in the grass pollen season than before and after it. Research is in progress in order to confirm this impression and to discover more data about the transport function of allergens.

Langerhans cells (LC) are large mononuclear dendritic CD-1 (T6)-positive cells with Birbeck granules in their cytoplasm. The role and function of LC remained obscure but recent research showed them to be essential antigen-presenting cells (20, 21, 24). LC are mesenchymal cells and derive from a precursor cell in the bone marrow (10). Being mobile cells, they are able to migrate into the epidermis from a haematogenous source and probably also, when activated, from the epidermis to the dermis and regional lymph nodes (16, 24). LC express Fc-receptors, C3-receptors, and class II major histocompatibility complex (MHC) molecules,

designated HLA-DR in man (13, 18, 22, 23). In patients with atopic dermatitis, Bruynzeel-Koomen et al. (3) found IgE molecules on epidermal LC. Outside the epidermis, cells resembling LC ultrastructurally are present in a variety of tissues. These cells are called indeterminate cells, veiled cells, or interdigitating cells, depending on their localization. Such LC-like cells have been demonstrated in various squamous epithelia, normal dermis, lymphatics and draining lymph nodes, reviewed by Braathen (2), and Peyer's patches of the gut (27). In bronchial epithelium LC have been reported by Richard (17), but Sertl et al. (19) could not

demonstrate CD-1-positive cells although they found HLA-DR-positive dendritic cells.

Similarities in the expression of cell-surface receptors and of the antigens present on these cells suggest that they belong to the same lineage of antigen-presenting cells (4, 25). The precise relationship between these subpopulations is not yet fully understood, but indeterminate cells are thought to be the direct precursors of LC. The monoclonal anti-CD-1 (T6) antibody provides a specific and sensitive probe for the identification of LC and indeterminate cells (5, 7, 9, 14). Outside the thymus, other CD-1-positive cells besides LC and indeterminate cells are only found in neoplastic diseases, such as histiocytosis X and T-cell lymphoma (15). Unlike HLA-DR, CD-1 expression by Langerhans cells in the skin is not, or only slightly, reduced by local corticosteroid therapy (1).

LC-like cells have not been described in columnar epithelium of the upper airways. The present study was performed to find out whether CD-1/HLA-DR-positive cells occur in the nasal mucosa, where antigenic stimulation is so frequent.

MATERIAL AND METHODS

The 35 patients and controls selected for this study, 15 males and 20 females ranging in age

from 17 to 65 years, were divided into three groups. The first group was composed of 11 patients (mean age 30 years) with an isolated grass pollen allergy confirmed by skin and radio-allergo-sorbent tests (RAST). The second group (mean age 27 years) consisted of 12 controls (volunteers and patients with diseases of the ear, nose and throat (ENT) not related to the nose or nasal sinuses) without nasal complaints, no nasal abnormalities seen at ENT examination, and a negative RAST. The third group (mean age 38 years) consisted of 12 patients with nasal and/or sinusal polyps and a negative RAST, undergoing an operation to remove the polyps.

Nasal biopsies were performed between July and November in 1986 under informed consent conditions. The biopsies of the patients with isolated grass pollen allergy were all taken in July, during the grass pollen season. The specimens of nasal mucosa were taken from the lower edge of the inferior turbinate, about 2 cm posterior to the front edge, with a Gerritsma forceps, a modified Hartmann forceps with a cup diameter of 2.5 mm. Local anaesthesia was obtained by placing a cotton-wool carrier with 100 mg cocaine and 3 drops of adrenaline (1:1000) under the inferior turbinate without touching the place where the biopsy specimen would be taken (8). The specimens were embedded in Tissue-Tek II

Table 1
Antibodies and antisera used in the present study

Antibody	Type	Specificity	Source
66IIC7 (CD-1)	M	CD-1	Monosan, Sanbio, Uden, The Netherlands
Anti HLA-DR	M	MHC Class II antigen	Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam (CLB)
Anti-IgE-FITC	PR	epsilon heavy chain	CLB
Anti-mouse Ig-TRITC	PG	mouse immunoglobulins	Cappel, Conchranville, USA
Anti-mouse Ig-Per	PR	mouse immunoglobulins	Dakopatts, Copenhagen, Denmark
Anti-prostate antigen	M	prostate epith. cells	Eurodiagnostics, Oss, The Netherlands

M = mouse monoclonal, P = polyclonal, R = rabbit, G = goat, FITC = fluorescein-iso-thiocyanate, Per = peroxidase, TRITC = tetra-methyl-rodamine-isothiocyanate



Fig. 1. Light micrographs of the nasal mucosa of a patient with an isolated grass pollen allergy incubated with AF-labeled monoclonal anti-CD-1 ($\times 250$). CD-1-positive dendritic cells ($\downarrow$) in the middle and lower layers of the epithelium. Inset: Single CD-1-positive cell ($\downarrow$) in the epithelium; clearly showing dendritic processes.

O.C.T. compound in a gelatin capsule and frozen immediately (6).

The antibodies used are listed in Table 1. The specificity of clone 66IIC7 for the CD-1 (T6) marker used to label LC in the epidermis has been described elsewhere (11).

The immuno alkaline phosphatase (AF) staining method used was a modification of the method described by Li (12). Briefly, 6 μ -thick sections of nasal mucosa were cut on a Reichert-Jung 2800 Frigocut cryostat, transferred to gelatin-coated microscope slides, dried and fixed in acetone for 10 min at 20°C, rinsed in phosphate-buffered saline (PBS, pH 7.2) and incubated with 66IIC7 (CD-1) monoclonal antibody for 30 min at 20°C. The sections were rinsed again in PBS for 5 min and incubated for 15 min with a rabbit anti-mouse immunoglobulin antiserum conjugated to alkaline phosphatase (DAKO D314) 1:20 supplemented with 10% normal human serum (NHS) to saturate Fc-receptors and reduce background staining (DAKO D314), then rinsed successively in PBS and TRIS buffer (pH 8.0), and incubated for 30 min with a New Fuchsin solution.

This solution is made by thoroughly mixing 150 μ g NaNO_2 4% with 150 μ g of a solution

containing 1 g New Fuchsin (Chroma 1B467, Stuttgart, FRG) in 25 ml HCl 2N. Next 60 ml 0.2 M TRIS-HCl buffer (pH 8.0) containing 18 mg Naphthol-AS-MX phosphate (Sigma N-5000, St. Louis, MO, USA) dissolved in 2 ml dimethyl formamide, is added together with 15 mg levamisole (Sigma L-9756). The pH of the final mixture is adjusted to 8.0. Filter before use. 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 (IgG2a) like prostate specific antigen were negative. Immunofluorescence double-staining was performed with monoclonal CD-1 followed by incubation with goat anti-mouse immunoglobulin labeled with TRITC (GaMIg-TRITC) and reincubation with anti-IgE labeled with FITC (RaMIgE-FITC) or anti-HLA-DR labeled with FITC (GaM-HLA-DR-FITC). In addition serial sections were incubated with anti-HLA-DR and anti-T6 alternately.

The total surface area of epithelium and lamina propria in the section was estimated by superimposing a grid of 100 points (interaction of crosses, covering a surface area of 0.5 mm²) on the section

using an eyepiece graticule as described by Underwood (26). 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 mm² estimated.

Statistical analysis

Since the frequency distribution of the number of T6-positive cells per mm² in epithelium and lamina propria was not symmetrical and the variances were unequal, Kruskal-Wallis one-way analysis of variances was used for calculating the overall *P*-value. For paired comparison we used two-tailed Mann-Whitney U-test. A *P*-value < 0.05 was considered to indicate a significant difference between groups.

RESULTS

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

All but two sections showed CD-1-positive dendritic cells in the middle and lower layers

of the epithelium (cf. Fig. 1). All sections showed CD-1-positive cells in the lamina propria. The distribution was not always regular along the epithelium; in some cases a group of CD-1-positive cells was seen in one place and only few such cells in the rest of the epithelium. In the lamina propria such cells were seen mainly 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 showed 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.

Table 2 gives the numbers of CD-1-positive cells/mm² surface area of the epithelium and the lamina propria in the nasal mucosa for the three groups. The number of CD-1-positive cells varied significantly over the three groups in the epithelium and lamina propria (*P* = 0.047/*P* = 0.046).

The number of CD-1-positive cells in the epithelium of allergic patients was significantly higher than the number in patients without nasal complaints and patients with nasal and/or sinusal polyps (*P* = 0.018). The number of CD-1-positive cells in the lamina

Table 2
Numbers of CD-1-positive cells/mm² in epithelium and lamina propria of nasal mucosa in patients with isolated grass pollen allergy, patients without nasal complaints, and patients with nasal and/or sinusal polyps.

Group	Patients (<i>n</i> = 35)	CD-1-positive cells	
		Epithelium mean (SD)	Lamina propria mean (SD)
I	with grass pollen allergy (<i>n</i> = 11*)	55 (49.5)	14 (22.0)
II	without nasal complaints (<i>n</i> = 12)	20 (14.1)	4 (4.0)
III	with nasal and/or sinusal polyps (<i>n</i> = 12)	28 (23.1)	10 (10.4)
Kruskal-Wallis overall <i>P</i> -value:		0.047	0.046
Mann-Whitney:			
<i>P</i> -value I vs. II/III		0.018	0.224
<i>P</i> -value II vs. I/III		0.074	0.013

*In one of these patients the epithelium was damaged; this epithelium was not included in the study. A difference between two patient groups was accepted as significant when the *P* value was < 0.05.

propria were small. A significant difference was found between the numbers of such cells in lamina propria of patients without nasal complaints compared with allergic patients and patients with nasal and/or sinusal polyps ($P = 0.013$).

DISCUSSION

Langerhans cells are dendritic cells capable of binding and presenting potentially antigenic molecules to T lymphocytes. The finding that CD-1 is a highly specific and sensitive marker for Langerhans and indeterminate cells means that LC in tissues can be detected and counted by light microscopy. Until now, epithelial Langerhans cells were believed to occur only in squamous epithelium. The present results show that CD-1-positive cells, presumably LC, also occur in columnar epithelium and lamina propria of the nasal mucosa. Electron-microscopical studies will show whether Birbeck granules – which are pathognomonic for LC – are present in these cells.

We found significantly more CD-1-positive cells in the epithelium of nasal biopsy samples of allergic than in those of non-allergic patients. Although the number of CD-1-positive cells in the lamina propria were small and results should be interpreted carefully, we found a significant difference between the number of these cells in the lamina propria of controls without nasal complaints compared to allergic patients and patients with nasal and/or sinusal polyps. So far, an explanation for this finding has not been found.

We used immunofluorescence methods and double staining with CD-1 and anti-IgE, and found IgE on CD-1-positive cells in the nasal epithelium of patients with grass pollen allergy. In studies of atopic dermatitis, Bruynzeel-Koomen (3) also found IgE molecules on epidermal LC and postulated that this binding occurred via the Fcε-receptors on LC. It is not clear why only some of the CD-1-positive cells showed surface IgE. Bruynzeel-Koomen (3) also reported that some LC in the skin failed to show surface IgE, and suggested that this was to be ascribed to a variation of FcεR expression,

as has been described for other cell populations such as eosinophils, monocytes, and alveolar macrophages.

Although the possibility of an aspecific increase of CD-1-positive cells due to inflammation in allergic rhinitis cannot be excluded, the finding of surface IgE on these cells points towards a possible role in the allergic mechanism. If in allergic rhinitis CD-1-positive cells, presumably LC, do indeed play a role in allergen presentation, it remains an interesting question whether these cells are constitutionally more numerous in nasal mucosa of patients with allergic rhinitis or whether they migrate to the epithelium during the exposition.

Studies in patients with grass pollen allergy are in progress to find out whether the number of CD-1-positive cells in the nasal mucosa is dependent on the grass pollen season.

In sum, the results of the present study show that:

1. CD-1-positive cells are present in epithelium and lamina propria of nasal mucosa;
2. the number of CD-1-positive cells is higher in nasal epithelium of patients with a grass pollen allergy than in non-allergic patients without nasal complaints or with nasal and/or sinusal polyps;
3. the number of CD-1-positive cells is lower in nasal lamina propria of controls without nasal complaints compared with allergic patients and patients with nasal and/or sinusal polyps; and
4. IgE is present on CD-1-positive cells in the nasal epithelium of patients with a grass pollen allergy.

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REFERENCES

- Berman, B., France, D. S. & Martinelli, G. P.: Modulation of expression of epidermal Langerhans cell properties following in situ exposure to glucocorticoids. *J. Invest. Dermatol.* **80**, 168-171, 1979.
- Braathen, L. R., Bjercke, S. & Thorsby, E.: The antigen presenting function of human Langerhans cells. *Immunobiology* **168**, 301-321, 1984.
- Bruynzeel-Koomen, C., van Wichen, D. F., Toonstra, J. & Bruynzeel, P. L. B.: The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. *Arch. Dermatol. Res.* **278**, 199-205, 1986.
- Drexhage, H. A., Mullink, H., De Groot, J., Clarke, J. & Balfour, B. M. A.: Study of cells present in peripheral lymph of pigs with special reference to a type of cell resembling Langerhans cells. *Cell. Tissue Res.* **202**, 407-430, 1979.
- Dubertret, L., Picard, O., Bagot, M., et al.: Specificity of monoclonal antibody anti-T6 for Langerhans' cells in normal human skin. *Br. J. Dermatol.* **106**, 287-289, 1982.
- Feltkamp-Vroom, Th.M. & Boode, J. H. M.: An embedding and sectioning technique for immunohistochemical studies of minute specimens of tissue. *J. Clin. Pathol.* **23**, 188-189, 1970.
- Fithian, E., Kung, P., Goldstein, G., Rubinfeld, M., Fenoglio, C. & Edelson, R.: Reactivity of Langerhans cells with hybridoma antibody. *Proc. Natl. Acad. Sci. USA* **78**, 2541-2548, 1981.
- Fokkens, W. J., Rijntjes, E., Vroom, Th.M., Gertsma, V.: A mild biopsy method to obtain high-quality specimens of nasal mucosa. *Rhinology* (accepted for publication).
- Harrist, T. J., Muhlbauer, J. E., Murphy, G. F., Mihm, M. C. & Bahn, A. K.: T6 is superior to Ia (HLA-Dr) as a marker for Langerhans cells and indeterminate cells in normal epidermis: a monoclonal antibody study. *J. Invest. Dermatol.* **80**, 100-103, 1983.
- Katz, S. I., Tamaki, K. & Sachs, D. H.: Epidermal Langerhans cells are derived from cells originating in bone marrow. *Nature (Lond.)* **282**, 324-326, 1979.
- Lerch, P. G., van de Rijn, M., Schrier, P. & Terhorst, C.: Biochemical comparison of the T6 antigen and HLA-A, B antigens. *Hum. Immunol.* **6**, 13-30, 1983.
- Li, C. Y., Yam, L. T. & Crosby, W. H.: Histochemical characterization of cellular and structural elements of the human spleen. *J. Histochem. Cytochem.* **20**, 1049-1058, 1972.
- Loning, T., Gaselitz, J., Seifert, G., Weber, K. & Osborn, M.: Identification of Langerhans cells: simultaneous use of sera to intermediate filaments, T6 and HLA-DR antigens on oral mucosa, human epidermis and their tumours. *Virchows Arch.* **398**, 119-128, 1982.
- Murphy, G. F., Bhan, A. K., Sato, S., Harrist, T. J. & Mihm, C. M. Jr.: Characterization of Langerhans cells by the use of monoclonal antibodies. *Lab. Invest.* **45**, 465-468, 1981.
- Murphy, G. F., Bhan, A. K., Sato, S., Mihm, M. C., Harrist, T. J.: A new immunologic marker for human Langerhans cells. *N. Engl. J. Med.* **304**, 791-792, 1981.
- Murphy, G. F., Fonferko, E., Flotte, T., Bhan, A. K.: Association of basal-lamina defects with epidermal and dermal T6-positive cells: evidence of Langerhans-cell migration. *Arch. Dermatol. Res.* **278**, 126-132, 1985.
- Richard, S., Barbey, S., Pfister, A., Scheinmann, P., Jaubert, F. & Nezelof, C.: Mise en evidence de cellules de Langerhans dans l'epithelium bronchique humain. *C. R. Acad. Sci. Paris, t. 305, serie III*, 35-39, 1987.
- Rowden, G., Phillips, T. M. & Lewis, M. G.: Ia antigens on indeterminate cells of the epidermis: Immunoelectronmicroscopic studies of surface antigens. *Br. J. Dermatol.* **100**, 531-542, 1979.
- Serfl, K., Takemura, T., Tschachler, E., Ferrans, V. J., Kaliner, M. A. & Schevach, E. M.: Dendritic cells with antigen-presenting capability reside in airway epithelium, lung parenchyma, and visceral pleura. *J. Exp. Med.* **163**, 436-451, 1986.
- Silberberg, I.: Apposition of mononuclear cells to Langerhans cells in contact allergic reactions. *Acta Derm. Venerol. (Stockh.)* **53**, 1-12, 1973.
- Silberberg, I., Baer, R. L. & Rosenthal, S. A.: The role of Langerhans cells in allergic contact hypersensitivity. A review of findings in man and guinea pig. *J. Invest. Dermatol.* **66**, 210-217, 1976.
- Stingl, G., Katz, S. I., Shevach, E. M., Wolff-Schreiner, E. & Green, I.: Detection of Ia antigens on Langerhans cells in guinea pig skin. *J. Immunol.* **120**, 570-578, 1978.
- Stingl, G., Wolff-Schreiner, E. C., Pichler, W. J., Gschnait, F., Knapp, W. & Wolff, W.: Epidermal Langerhans cells bear Fc and C3 receptors. *Nature* **268**, 245-246, 1977.
- Streilein, J. W., Lonsberry, L. W. & Bergstresser, P. R.: Depletion of epidermal Langerhans cells and Ia immunogenicity from tapestripped mouse skin. *J. Exp. Med.* **155**, 863-871, 1982.
- Thorbecke, G. J., Belsito, D. V., Bienenstock, A. N., Possick, L. E. & Baer, R. L.: The Langerhans cell, as a representative of the accessory cell system, in health and disease. *Immunobiology* **168**, 313-324, 1984.
- Underwood, J. C. E.: Induction to biopsy interpretation and surgical pathology. Springer Verlag, Berlin 1981.
- Wilders, M. M., Drexhage, H. A., Weltvreden, E. F., Mullink, H., Duijvestijn, A. & Meuwissen, S. G. M.: Large mononuclear Ia-positive veiled cells in Peyer's patches. *Immunology* **43**, 453-460, 1983.

Address:

W. J. Fokkens

Department of Otorhinolaryngology

University Hospital Rotterdam

Dr. Molewaterplein 40

3015 GD Rotterdam

The Netherlands

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