

Short communication

Demonstration of the high-affinity IgE receptor (Fc_εRI) on Langerhans' cells of diseased nasal mucosa

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Langerhans' cells in the skin have recently been shown to bind IgE molecules via the high-affinity IgE receptor (Fc_εRI). Using two highly specific antibodies against the antibody-binding α -chain of this receptor, 29C6 and 6F7, we demonstrate by immunohistochemistry and immunoelectron microscopy that Langerhans' cells of diseased nasal mucosa can express the Fc_εRI. Tissue sections from hyperplastic nasal conchae and nasal polyps of atopic and nonatopic patients have shown no basic differences in epithelial Fc_εRI-bearing cells. Only a few cells expressed the low-affinity IgE receptor (Fc_εRII) (T $\bar{u}$ 1 antibody) in some sections. These findings suggest that Langerhans' cells play an important role in the induction of transepithelial IgE-mediated allergy and in the mediation of inflammation of the nasal mucosa via their Fc_εRI.

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Langerhans' cells (LCs) in the respiratory epithelium are MHC class II+ cells which may bind and present respiratory allergens. Membrane-bound IgE has been demonstrated on LCs of nasal mucosa, and there is evidence that LCs carrying IgE play a role in antigen-specific lymphocyte responses (1).

Receptors for the Fc fragments of IgE on cell membranes are of either the high- (Fc_εRI) or the low-affinity type (Fc_εRII). The functional significance of Fc_εRII has not yet been well elucidated. The structurally distinct Fc_εRI consists of a transmembrane α -chain, a β -chain with four transmembrane domains, and two disulfide-linked transmembrane γ -chains.

Recently, the Fc_εRI has been identified on LCs of normal and diseased epidermis (2, 3). In the present investigation, using specific antibodies against the α -subunit of Fc_εRI and against the Fc_εRII, we demonstrate that, like the oral mucosa (4), LCs of nasal mucosa primarily express the Fc_εRI and, to a minor extent, the Fc_εRII.

Material and methods

Patients and tissue

Nasal biopsies were obtained from six patients with nasal polyps and six patients with hyperplastic nasal conchae requiring surgical treatment. These patients were selected on the basis of atopy and nonatopy. Atopy was established by at least one positive skin prick test performed with 10 common inhalant allergens, combined with a positive specific serum IgE determined by a multiallergen RAST disk (Phadiatop test). In all patients, unspecific nasal hyperreactivity was determined by acetylcholine (ACh) provocation (4 mg of ACh was delivered to one nostril, and the reaction was measured by rhinomanometry and development of nasal symptoms such as blockage, sneezing, and hypersecretion). The control was 0.9% saline. This test was considered positive if airflow decreased by more than 30% and the symptom score increased by 6 points.

Three of the patients with nasal polyps were atopic and three were nonatopic. Their peripheral

blood eosinophil count range was 1–18%; nasal hyperreactivity was absent. Three of the patients with hyperplastic nasal conchae were also atopic, and three were nonatopic. Their peripheral blood eosinophil count range was 2–3%; nasal hyperreactivity was moderate.

Methods

Immunohistochemical staining methods. The following monoclonal antibodies were used in the APAAP method: T6 (Dako, Denmark) specific for the CD1a epitope on LCs; Tü1, against the Fc_εRII, evaluated for specificity by the myeloid workshop and kindly provided by Prof. Dr A. Ziegler, FU, Berlin, Germany; a polyclonal anti-IgE antibody (DAKO, Denmark); and two monoclonal antibodies against the antigen-binding α -chain of the Fc_εRI (donated by Dr R. Chizzonite, Nutley, NJ, USA), one (6F7) binding competitively with IgE, i.e., only to the unoccupied receptor, and the other (29C6) binding to all Fc_εRI. Immunohistochemistry was performed on frozen, 4- μ m-thick cryostat sections, as previously described in detail (5). Unspecific staining was ruled out in each case by omission of the specific antibody and by its replacement with an irrelevant, isotype-matched monoclonal antibody.

Histochemical staining methods. Frozen sections were air-dried and incubated for 12 h in toluidine blue solution (pH 0.5, 0.1% toluidine blue) at room temperature, washed with water, dehydrated twice with alcohol, cleared with xylol, and mounted with Vitro Clud.

Methods for assessment of slides. Cell counts were performed on serial tissue sections. The same tissue areas were identified by corresponding microscopic structures. Nucleated, positively stained cells were counted in 3–5 adjacent microscopic fields at $\times 400$ magnification by two blinded, independent observers. The area of one field counted was 0.196 mm². The fields to be counted were selected as follows:

- 1) in the epithelium, by beginning with the first field in the center of the section and continuing the counts to both sides
- 2) in the stroma, by counting the corresponding subepithelial fields.

Results are expressed as mean counts $\pm$ standard deviation/microscopic field.

Immunoelectron microscopic methods. The ultrastructural localization of the Fc_εRI was analyzed by a pre-embedding immunoperoxidase method, as described in detail elsewhere (6). After fixation in Nakane's paraformaldehyde solution (7), the

cryostat-sectioned specimens were incubated either with the monoclonal anti-Fc_εRI antibody 29C6, or with equal amounts of nonimmune mouse IgG for control purposes. The specimens were postfixed in osmic acid, dehydrated in a graded ethanol series, and embedded in Araldite. The ultrathin sections of the tissue were examined with a Zeiss electron microscope EM9, the double condenser system being used.

Results

Fig. 1 shows the mean number of positively stained epithelial cells. Nearly all specimens of nasal mucosa contained dendritic T6+ LCs in the basal and middle part of the epithelium (Fig. 2). Fc_εRI was strongly expressed on these LCs (Fig. 3). The total number of epithelial 29C6+ cells was more than twice that of LCs. With the antibody against IgE, fewer than half of the 29C6+ cells were shown to have bound IgE. Only a small percentage of 29C6+ epithelial cells reacted with 6F7. The specific antibody against the Fc_εRII, Tü1, showed an even lower expression on these cells. Mast cells were rare within the epithelium as determined by both methods, toluidine blue staining and electron microscopy. Reactivity to the markers studied was not significantly different between atopic and non-atopic patients. However, there was less reactivity to IgE on hyperplastic nasal conchae, than polyps.

Fig. 4 shows the immunoelectron microscopic localization of the anti-Fc_εRI antibody on an epithelial LC. The cell membranes were labeled by electron-dense peroxidase deposits, and the cyto-

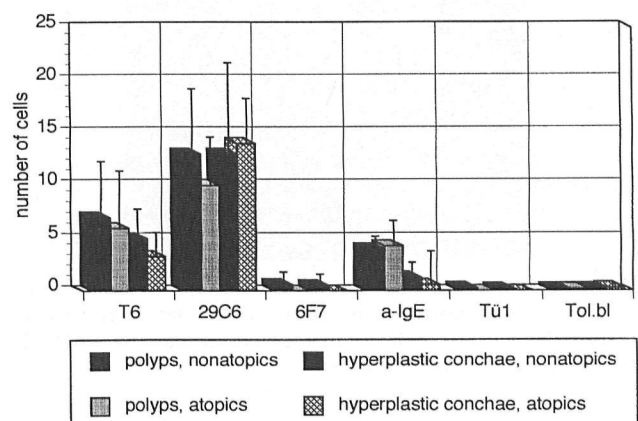


Fig. 1. Reactivity of nasal epithelium to different antibodies. Number of cells in nasal mucosal epithelium staining positively with T6 (Langerhans' cells), 29C6 (Fc_εRI, high-affinity IgE receptor), 6F7 (Fc_εRI, nonoccupied receptor), anti-IgE, Tü1 (Fc_εRII, low-affinity IgE receptor), and toluidine blue (Tol.bl) (mast cells). Data are expressed as mean counts $\pm$ standard deviation/high-power microscopic field. $n = 3$ for each patient category.

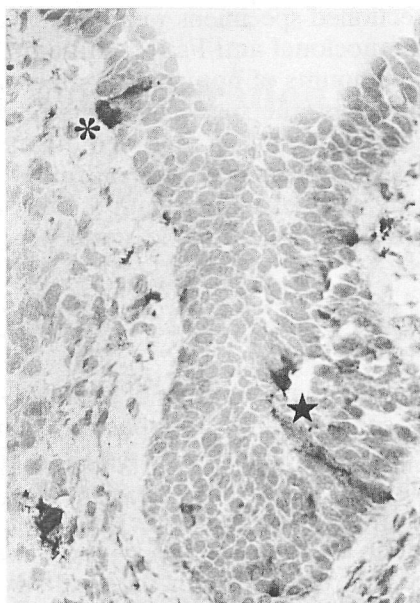


Fig. 2. Langerhans' cells in nasal mucosa. Scattered CD1a+ dendritic cells in epithelium (★) and below basal membrane (*) (polyp of atopic patient, APAAP, monoclonal antibody T6, $\times 100$).

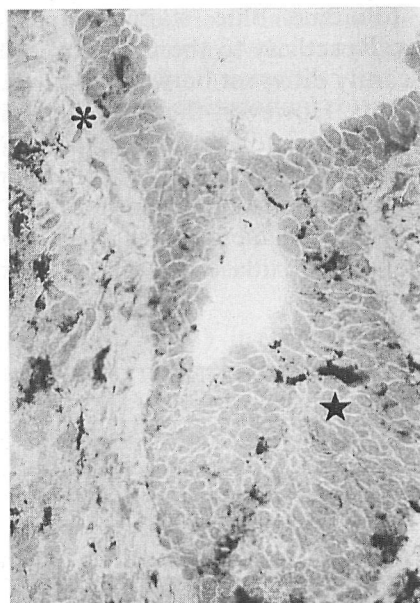


Fig. 3. Fc ϵ RI-bearing cells in nasal mucosa. On serial sections, Fc ϵ RI-bearing dendritic cells (*) could be identified as CD1a+ Langerhans' cells (polyp of atopic patient, APAAP, monoclonal antibody 29C6, $\times 100$).

plasm of the cells contained typical tennis racket-shaped Birbeck granules.

Fig. 5 shows the number of positively stained stromal cells. There were only a few T6+ LCs (Fig. 2), whereas many nondendritic cells stained with 29C6 (Fig. 3) and about half as many with anti-IgE and toluidine blue. As in the epithelium, only

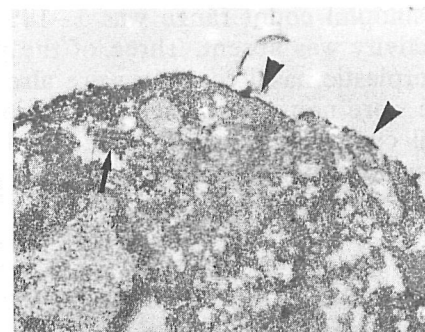


Fig. 4. Immunoelectron microscopic localization of anti-Fc ϵ RI antibody on epithelial LC. Cell membrane is labeled by electron-dense peroxidase deposits (arrowheads). Cytoplasm of cell contains Birbeck granules (arrow) ($\times 23\,600$).

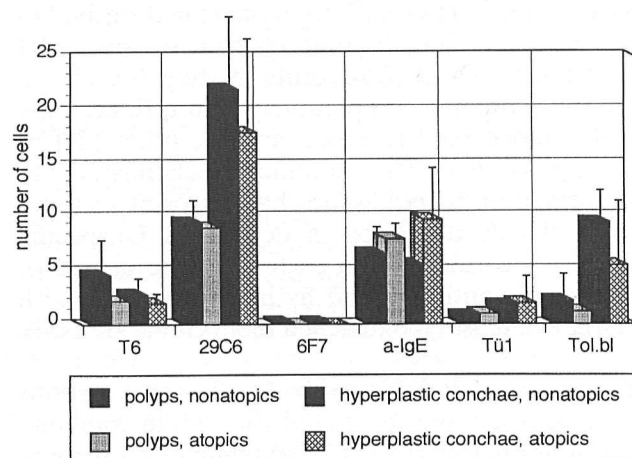


Fig. 5. Reactivity of nasal submucosa to different antibodies. Number of cells in nasal stroma staining positively with T6 (Langerhans' cells), 29C6 (Fc ϵ RI, high-affinity IgE receptor), 6F7 (Fc ϵ RI, nonoccupied receptor), anti-IgE, Tü1 (Fc ϵ RII, low-affinity IgE receptor), and toluidine blue (Tol.bl) (mast cells). Data are expressed as mean counts $\pm$ standard deviation/high-power microscopic field. $n = 3$ for each patient category.

a few 6F7+ and Tü1+ cells were noted. Reactivity to the markers studied was not significantly different in atopic and nonatopic patients. However, polyps clearly showed fewer 29C6+ cells and toluidine blue+ cells than hyperplastic nasal conchae. The latter cells were more numerous and larger in hyperplastic nasal conchae and contained more cytoplasmic granules.

Discussion

Little is known about the differences in the cell infiltrate in nasal mucosa of atopic and nonatopic patients, but cell counts reported from various groups vary considerably (8). Generally, cell counting in the epithelium in polyps and hyperplastic nasal conchae is difficult because epithelial cell shedding in inflamed mucosa can lead to different

degrees of epithelial thickness. In accordance with other reports (8), we did not find a basic difference in T6+ (CD1a+) LC counts between nonatopic and atopic subjects.

We demonstrated, for the first time, that LCs in the nasal epithelium bear the Fc_εRI. Fc_εRI-bearing LCs were identified in serial sections in comparison of dendritic T6+ and 29C6+ epithelial cells. The 29C6 labeling of LCs was confirmed by immunoelectron microscopy. Furthermore, we showed that LCs in the nasal mucosa react little with 6F7, which binds only to nonoccupied Fc_εRI. This finding, together with the low number of IgE-bearing epithelial cells, parallels our previous observations in the lesional skin of atopic dermatitis (3). The difference between the numbers of IgE+ cells and 6F7+ cells, on the one hand, and 29C6+ cells, on the other hand, in both epithelium and stroma can be explained by a failure of the 6F7 marker to recognize all nonoccupied IgE receptors in the nasal mucosa.

The nasal epithelium definitely contained more 29C6+ than T6+ cells (Fig. 1). This suggests that Fc_εRI-bearing cells other than LCs, such as mast cells and/or basophils, are present (9). Recently, Fc_εRI-bearing monocytes have been demonstrated in atopic patients, and these may also contribute to the 29C6+ cells in our sections (10). Since the number of toluidine blue+ cells in the epithelium of our sections was very low, we speculate that mast cells migrate into the nasal epithelium, degranulate (11), and thus decrease in stainability for the most part.

Nasal stroma contained few T6+ LCs and many more 29C6+ cells in hyperplastic conchae (Fig. 5), probably mast cells and/or basophils, since the stromal tissue harbored considerably higher numbers of IgE+ and toluidine blue+ cells than the epithelium.

The sections studied here yielded hardly any reactivity with the Fc_εRII (CD23), except for a few epithelial and stromal cells. This agrees with our findings in keratinizing the epithelium (3).

One may speculate that the function of Fc_εRI on LCs in nasal mucosa may be twofold: it may

provide a means for classical antigen presentation, and it may function as a barrier against potential allergens in atopic and nonatopic patients. Since these observations were done on diseased mucosa, they should be followed up by observations on normal mucosal biopsies.

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