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Increased occurrence of IgE⁺ and Fc_εRI⁺ cells in adenoids from atopic children

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Key words: adenoid; atopy; IgE; IgE receptor; immunohistochemistry; lymphocytes; mast cells; otitis media; plasma cells.

Background: To examine the influence of atopy on the different cell populations in adenoids, we investigated the presence of IgE⁺ cells, cells expressing the high-affinity receptor for IgE (Fc_εRI), and various other cell populations in adenoid tissue, in atopic and nonatopic children with otitis media with effusion (OME) or adenoid hyperplasia (AH).

Methods: Cryostat sections of adenoids from 14 atopic and 16 nonatopic children suffering from long-lasting OME (*n*=15) or obstructive AH (*n*=15) were investigated with immunohistochemical markers for T-cell subsets, mast cells, eosinophils, plasma cells, CD25, CD1a, IgE, and Fc_εRI.

Results: Sensitization to allergens was correlated to an increase of IgE⁺ cells in the epithelium (*P*<0.01), the extrafollicular area (*P*<0.0001), and the follicles (*P*<0.001) of the adenoids and an increase of Fc_εRI⁺ cells in the extrafollicular area (*P*<0.01). A minority of the IgE⁺ cells were plasma cells. No significant differences in cells stained for IgE, Fc_εRI, or the other markers were observed between patients with OME and AH.

Conclusions: Atopy is associated with increased numbers of IgE⁺ and Fc_εRI⁺ cells in adenoids irrespective of whether the child has OME or AH.

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The nasopharyngeal tonsil or adenoid, together with the palatine tonsils, is considered to play a central role in the induction of secretory immunity and is probably part of the mucosa-associated lymphatic tissue (MALT) (1). The adenoid is situated at the entrance of the respiratory tract and, along with the nasal mucosa, represents the first site of contact with airborne antigens before they are inhaled into the respiratory tract.

IgE production is a T-cell-dependent antibody response, and local production of IgE is rarely seen in the mucosa of the respiratory tract (2). Whether IgE is produced in the adenoid is a controversial issue (3), and it is also not known whether the adenoid is involved in the development of atopy.

The adenoid seems to be one of the most important factors in the pathophysiology of otitis media with effusion (OME), and it has clearly been shown that adenoidectomy has a significant effect on the resolution of middle-ear effusion (4, 5). Atopic diseases were more frequent among children suffering from OME than in a control group (6–8). Alterations in the microenvironment of adenoids in children with OME, explaining the role of atopy in the development of OME, have so far not been presented. However, several mechanisms have been proposed as possible explanations of the association between allergy and OME; among others, an immunologic reaction in the middle-ear mucosa to allergen inhaled through the nasopharynx, or eustachian tube dysfunction (9).

The aims of the present study were to investigate the presence of cells that stain positively for IgE and FcεRI, and the distribution of other cell populations in adenoid tissue in children with OME or adenoid hyperplasia (AH), and to relate these findings to atopy. A further aim was to analyze to what extent the IgE⁺ cells found were also plasma cells.

Material and methods

Patients

Adenoids were obtained from 30 children undergoing adenoidectomy at the Department of Otorhinolaryngology and Head and Neck Surgery, Karolinska Hospital, between February 1994 and October 1996 (Table 1). Participation was by parental informed consent, and the study was approved by the local ethics committee. A questionnaire about the child's medical, birth, and family history was answered by the parents, and all the children underwent routine ear, nose, and throat examination. All children included in the study were required to have normal, nondiseased tonsils by history and physical examination. Patients with hyperplastic tonsils (occupying more than one-third of the oropharynx) were not included in the study. Other exclusion criteria were cleft palate, known immunologic deficiency, a severe underlying disease, or treatment with antibiotics within 1 month before surgery.

Table 1. Characterization of patients included in study according to age, sex, and total serum IgE

Subjects	Age (months)*	Sex (M/F)	Total IgE† (kU/l)*
Atopic:	88 (46–154)	7/7	260 (33–2200)
OME	63 (46–114)	3/3	250 (33–520)
AH	114 (51–154)	4/4	300 (55–2200)
Nonatopic:	61 (34–151)	5/11	11 (2–95)
OME	62 (39–116)	2/7	8 (2–95)
AH	60 (34–151)	3/4	13 (5–32)

*Median and range.
†Reference value range: 2–22.3 kU/l for patients up to 4 years of age and 2–263 kU/l for 4–20-year-old patients.

Blood samples were taken during anesthesia, analyzed for total IgE (Pharmacia CAP System IgE FEIA, Pharmacia Diagnostics AB, Uppsala, Sweden), and screened with a mixture of inhalant allergens (Pharmacia CAP System Phadiatop FEIA) and a radioallergosorbent (RAST) mixture for relevant food allergens (fx5: egg, milk, peanut, soybean, fish, and wheat; Pharmacia CAP System RAST FEIA). The sera which scored positive in Phadiatop were analyzed for specific IgE antibodies against common inhalant allergens (Pharmacia CAP System RAST FEIA). All the sera positive in the mixed-food RAST were further tested with a specific RAST for milk, egg white, soybean, peanut, fish, and wheat (Pharmacia CAP System RAST FEIA).

The children were classified as atopic if they had a positive screening test with Phadiatop or the mixed-food RAST test. Out of the 30 children included in the study, 14 were classified as atopic. Eight of these were sensitized to inhalants, and six reacted to both airborne and food allergens when tested for specific IgE antibodies, with at least RAST class 2 out of six classes. The highest RAST classes among them were found for pollens and cat dander. The total serum IgE values ranged between 33 and 2200 kU/l (Table 1). Patient history revealed symptoms such as asthma, rhinoconjunctivitis, gastrointestinal discomfort, or eczema, and nine of the atopic children had previously had a positive skin prick test. Eleven out of the 14 atopic children had a first-degree relative with known atopy. At the time of surgery, four children in the atopic group had an ongoing treatment for their allergy; two were receiving steroid inhalations, one was taking oral antihistamines, and one was using a steroid ointment. None were being treated with nasal steroids.

According to the presence of middle-ear effusion, the atopic and nonatopic children were divided into two clinical groups. The OME group ($n=15$) consisted of children with

long-standing OME of at least 3 months' duration who were scheduled for tympanostomy tubes. The diagnosis of OME was based on pneumatic otomicroscopy and tympanometry showing a flat, Jerger type B tympanogram. The AH group ($n=15$), comprising children with obstructive symptoms but no history of middle-ear disease, served as a control group for the OME group. The diagnosis of AH was based on visible obstruction of the nasopharynx during clinical examination with a mirror, and a history of obstructive symptoms. The nasopharyngeal obstruction was confirmed during surgery.

Tissue handling

Adenoid specimens removed during surgery were transported in ice-cold 0.9% NaCl and within 1 h snap-frozen (after removing unrelated tissue) in chilled isopentane and stored at -70°C .

Immunohistochemistry

A monoclonal antibody (mAb) alkaline phosphatase–anti-alkaline phosphatase (APAAP) staining technique (4, 5) was applied with 6- μm -thick, acetone-fixed cryostat sections. The following mouse mAbs were used: anti-Leu-4 (reacting with CD3; working dilution 1/256, Becton Dickinson, Mountain View, CA, USA); anti-Leu-3a (reacting with CD4; 1/256, Becton Dickinson); anti-Leu-2a (reacting with CD8; 1/32, Becton Dickinson); AB 29C6 directed against the high-affinity human IgE receptor, Fc_εRI, which recognizes the receptor whether bound or unbound (1/800, a gift from Dr R. Chizzonite, Hoffman La Roche, Nutley, NJ, USA) (4, 5); an antibody against IgE (1/1200; Southern Biotechnology Associates, Birmingham, AL, USA); EG2 with specificity for eosinophil cationic protein and secreted forms of eosinophil protein X of the activated eosinophilic granulocyte (1/80, Pharmacia Upjohn, Uppsala, Sweden); an antibody against the IL-2 receptor (CD25; 1/16, Becton Dickinson); and an antibody against Langerhans' cells (CD1a; 1/40, Becton Dickinson). Rabbit anti-mouse immunoglobulin (1/40, Dakopatts, Copenhagen, Denmark) was used as the secondary antibody. The alkaline-phosphatase reaction was developed with the dye Fast Red. Mast-cell tryptase was detected with a mAb designated MAB1222 (1/1250, Chemicon International Inc, Temecula, CA, USA) and the peroxidase antiperoxidase (PAP) technique (12). The PAP reaction was developed with the dye 3-amino-9-ethylcarbazole. The sections were counterstained with hematoxylin. Specificity tests omitted the primary antibodies, and in these tests, staining was not observed. A section from each specimen

was also stained with hematoxylin and eosin for morphologic evaluation.

Two sections per antibody and specimen were examined by one of the investigators (G.P.) under coded conditions. Cell numbers were expressed semiquantitatively as follows: 0, no stained cells; +, low; ++, moderate; and +++, high occurrence of positive cells. The scale was adjusted so that the scale step "high" (+++) referred to the maximal number of cells reactive to each antibody within the specimens. The localization of the immunostained cells was related to the different compartments in the adenoid tissue: epithelium (both crypt and surface epithelium), and extrafollicular area and lymphoid follicles (with germinal centers and mantle zone).

Double immunofluorescence and confocal microscopy

Adenoids from five atopic children with the highest total serum IgE levels, above 380 kU/l, three of whom belonged to the AH group, were stained with double immunofluorescence to determine the presence of IgE⁺ plasma cells. The sections, 6 μm thick, were acetone fixed and blocked with normal goat serum (1/10), incubated with a mouse mAb (IgG2a) directed against a plasma cell antigen, PCA-1 (1/10; Coulter Immunology, Hialeah, FL, USA), followed by incubation with a Texas Red conjugated secondary antibody F(ab')₂ fragment of goat anti-mouse IgG (1/40; Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Thereafter, the sections were blocked with normal mouse serum (1/100) and incubated with FITC-conjugated mouse mAb (IgG1) anti-human IgE (1/20; Nordic Immunological Laboratories, Tilburg, The Netherlands). Single labeling of sections was performed in parallel for comparison. Specificity tests involved omission of primary antibodies or their replacement with normal mouse serum (1/100) or an irrelevant FITC-conjugated mouse IgG1 antibody, respectively. Staining was not observed in these tests. As a positive control for PCA-1, we used a specimen from a human tonsil. The samples were mounted in 0.5% *p*-phenylenediamine (PPD) in glycerol to reduce fading of immunofluorescence during microscopy (13).

A Sarastro 2000 confocal scanning laser microscope (CSLM) system (Molecular Dynamics, Sunnyvale, CA, USA) was used in dual-detector mode. This system uses a Nikon Optiphot II upright microscope and an argon ion laser. Simultaneous illumination with the 488-nm and 514-nm laser lines was used in order to excite both FITC and Texas Red efficiently. The resulting fluorescence was split into two spectral bands (FITC-detection spectral band: 535–

545 nm; Texas Red-detection spectral band: $\lambda > 600$ nm) by means of a dichroic beam-splitter (565 nm) and filters, and subsequently detected by two photomultiplier tubes (PMT). The tube voltages were adjusted for each channel to give a signal within the dynamic range of the acquisition electronics.

Statistical analysis

The chi-square test was used when comparing cell populations between clinical groups. The Mann–Whitney *U*-test was used for analysis of differences between two groups and the Kruskal–Wallis test was used for analysis of differences between three or more groups. *P* values less than 0.05 were considered statistically significant.

Results

IgE⁺ and Fc_εRI⁺ cells

IgE⁺ cells were found in the epithelium in low or moderate numbers (Figs. 1a and 2a). Twelve adenoid specimens showed high occurrence of IgE⁺ cells in the extrafollicular area (Figs. 1b and 2a). All these specimens were obtained from the atopic group. In the lymphoid follicles, IgE⁺ cells were either few or absent from all the specimens (Fig. 1c). Of the 30 adenoid specimens examined, only one was negative for IgE⁺ cells, and this came from the nonatopic group. A significantly greater number of patients in the atopic group than the nonatopic group had IgE⁺ cells in all the three different adenoid tissue compartments; i.e., the epithelium ($P < 0.01$), the extrafollicular area ($P < 0.0001$), and the follicles ($P < 0.001$) (Fig. 1a–c and Table 2).

Fc_εRI⁺ cells were most frequent in the extrafollicular area (Figs. 1d and 2b). In the epithelium, most of the specimens showed few Fc_εRI⁺ cells, and this cell type was absent from or in low numbers in the lymphoid follicles (Fig. 2b and Table 2). The majority of the nonatopic children had a moderate occurrence of Fc_εRI⁺ cells in the extrafollicular area, while the majority of the atopic subjects showed a high occurrence of positively stained cells, and this difference was statistically significant ($P < 0.01$) (Fig. 1d).

There were no statistically significant differences in the occurrence of IgE⁺ or Fc_εRI⁺ cells related to the presence of OME or AH (Table 2).

Statistically significant differences were observed when the numbers of IgE⁺ cells in the extrafollicular area were related to the total serum IgE values ($P < 0.0001$) (Fig. 3a), and

the same was true of the follicles ($P < 0.0001$) (data not shown) and the epithelium ($P < 0.01$) (data not shown). The same comparison between total serum IgE and Fc_εRI⁺ cells showed a significant difference for the Fc_εRI⁺ cells in the extrafollicular area ($P < 0.05$) (Fig. 3b) and the follicles ($P < 0.01$) (data not shown), but not in the epithelium.

Five specimens each from the atopic and the nonatopic groups were further examined with tryptase staining in order to analyze the location and number of mast cells. The distribution of the tryptase-positive cells was similar in both groups, with few cells in the extrafollicular zone except in one specimen from the child with the highest serum IgE value (2200 kU/l), in which there was a moderate occurrence of positively stained cells. Tryptase-stained cells were absent in the follicles and were found occasionally in the epithelium. The tryptase-positive cells were always outnumbered by the IgE⁺ cells in all the three different adenoid tissue compartments (Fig. 4) and were not always localized to the same area as the IgE⁺ cells.

When using double immunofluorescence and CLSM for detection of IgE⁺ plasma cells, a few double-positive cells were clearly detected in sections of an adenoid specimen belonging to the atopic child with the highest serum IgE level (2200 kU/l) (Fig. 5). Single-stained sections of the specimens showed few PCA-1⁺ cells and confirmed the occurrence of IgE⁺ cells in the adenoids seen with conventional immunohistochemistry.

T cells, eosinophils, and CD1a⁺ cells

CD3⁺ cells were most frequent in the extrafollicular area, were less frequent in the lymphoid follicles, and were scattered throughout the epithelium (Table 2). The occurrence of both CD4⁺ and CD8⁺ T cells in the different compartments showed the same pattern as for the CD3⁺ cells, and there was a predominance of CD4⁺ cells over CD8⁺ cells in all compartments. All the specimens had few CD25⁺ cells in the epithelium and the lymphoid follicles (Table 2). In the extrafollicular area, 2/3 of the specimens showed few CD25⁺ cells and 1/3 had moderate occurrence of CD25⁺ cells.

A few EG2⁺ cells were seen in the epithelium and the extrafollicular area; in some cases, with very intense staining. EG2⁺ eosinophils were absent from the lymphoid follicles (Table 2). CD1a⁺ cells were found only in the lymphoid follicles of five specimens, and only a few stained cells were seen. In all 30 cases, CD1a⁺ cells were found in the epithelium, but they were very sparse or in low numbers. In the extrafollicular zone, accumulation of this

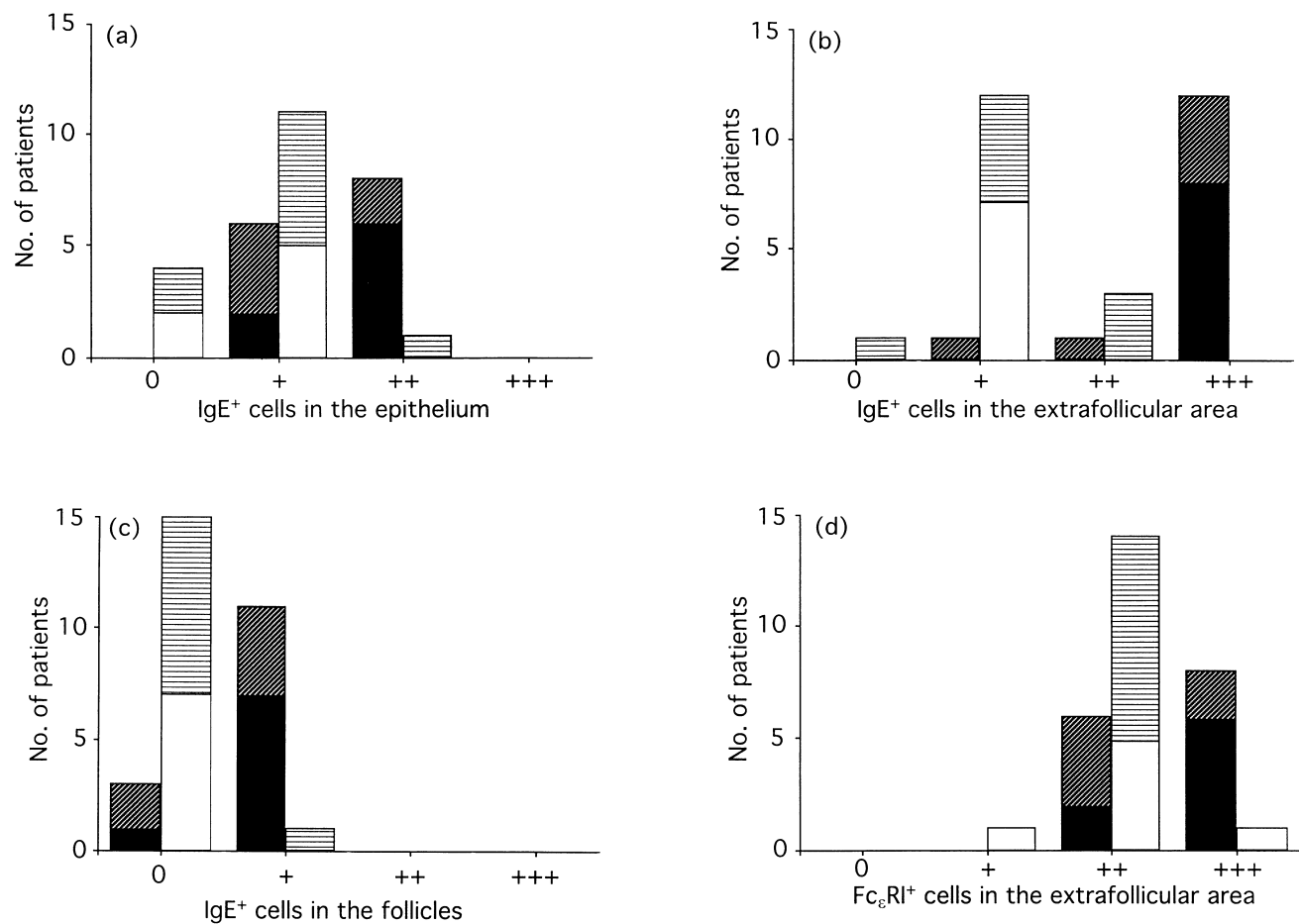


Figure 1. Occurrence of IgE⁺ and Fc ϵ RI⁺ cells in adenoids from 14 atopic (■; atopic/AH and ▨; atopic/OME) and 16 nonatopic children (□; nonatopic/AH and ▩; nonatopic/OME). Numbers of positive cells are expressed semiquantitatively: 0, no stained cells; +, low; ++, moderate; and +++, high occurrence of positive cells. a) IgE⁺ cells in epithelium, b) IgE⁺ cells in extrafollicular area, c) IgE⁺ cells in follicles, and d) Fc ϵ RI⁺ cells in extrafollicular area. Atopic vs nonatopic: a) $P<0.01$, b) $P<0.0001$; c) $P<0.001$, and d) $P<0.01$ (chi-square test).

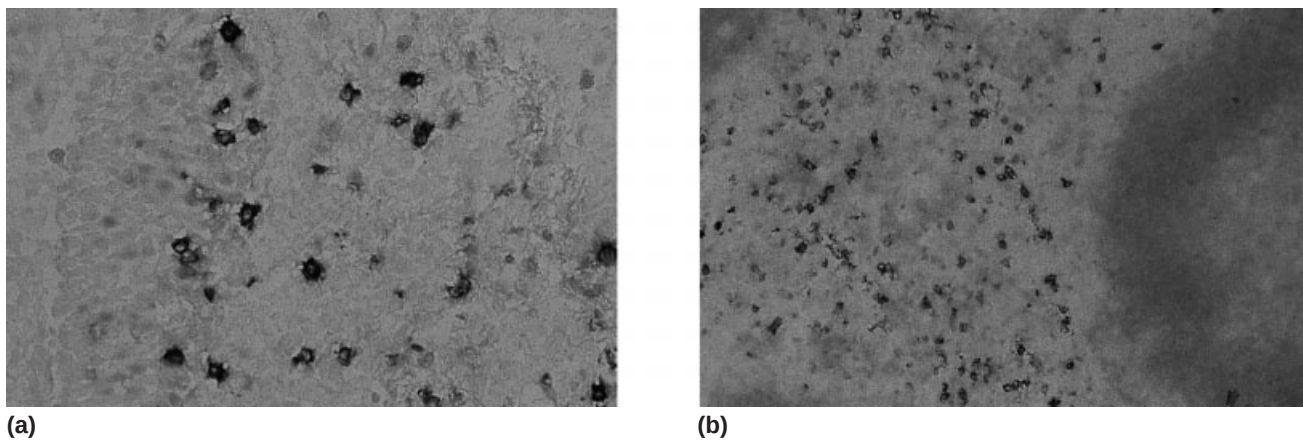


Figure 2. Immunohistochemical APAAP stainings of adenoid specimens: a) IgE⁺ cells in extrafollicular area and epithelium in atopic patient with total serum IgE level of 380 kU/l; b) Fc ϵ RI⁺ cells in extrafollicular area in same patient. Note absence of Fc ϵ RI⁺ cells in follicle in b. a) $\times 200$; b) $\times 100$.

Table 2. Medians and ranges of positive cells* in different compartments of adenoid specimens

		Atopic (14) (OME plus AH) (6+8)		Nonatopic (16) (OME plus AH) (9+7)		OME (15) (atopic plus nonatopic) (6+9)		AH (15) (atopic plus nonatopic) (8+7)	
		Median	Range	Median	Range	Median	Range	Median	Range
IgE	e	2 ^I	1–2	1 ^I	0–2	1	1–2	1	0–2
	ef	3 ^{II}	1–3	1 ^{II}	0–2	2	0–3	3	1–3
	f	1 ^{III}	0–1	0 ^{III}	0–1	0	0–1	0	0–1
Fc ϵ RI	e	1	1–2	1	1–2	1	1–2	1	1–2
	ef	3 ^{IV}	2–3	2 ^{IV}	1–3	2	2–3	2	1–3
	f	1	0–1	1	0–1	1	0–1	1	0–1
CD3	e	1	1–2	1	1–2	1	1–2	1	1–2
	ef	3	2–3	3	2–3	3	2–3	3	2–3
	f	2	1–2	1	1–2	2	1–2	2	1–2
CD4	e	1	1–2	1	1–2	1	1–2	1	1–2
	ef	3	2–3	3	3–3	3	3–3	3	1–3
	f	2	1–3	2	1–2	2	1–3	2	1–2
CD8	e	1	1–2	1	1–1	1	1–2	1	1–2
	ef	2	2–3	3	2–3	3	2–3	3	2–3
	f	1	1–2	1	1–1	1	1–2	1	1–1
CD25	e	1	1–1	1	1–2	1	1–1	1	1–2
	ef	1	1–2	1	1–2	1	1–2	1	1–2
	f	1	1–1	1	1–1	1	1–1	1	1–1
EG2	e	1	0–1	1	0–1	1	0–1	1	0–1
	ef	1	1–2	1	1–2	0	0–1	1	1–2
	f	0	0–0	0	0–0	0	0–0	0	0–0
CD1a	e	1	0–2	1	1–2	1	1–1	1	0–2
	ef	1	0–1	1	1–3	1	0–3	1	1–3
	f	0	0–1	0	0–1	0	0–1	0	0–1

*Numbers of positive cells are expressed semiquantitatively: 0, no stained cells; 1, low (+); 2, moderate (++); and 3, high occurrence (+++) of positive cells. e: epithelium, ef: extrafollicular area, f: follicles.
^IP<0.01; ^{II}P<0.0001; ^{III}P<0.001; ^{IV}P<0.01; atopic vs nonatopic (chi-square test).

cell type was observed in two cases; otherwise, CD1a⁺ cells were generally few.

No differences were shown between atopics and non-atopics or between the OME and AH groups regarding the presence of T cells, eosinophils, or CD1a⁺ cells (Table 2).

Discussion

We have found an increase of IgE⁺ and Fc ϵ RI⁺ cells in adenoid tissue to be strongly associated with atopy. Although the airway mucosa in atopic individuals contains IgE⁺ mast

cells, they seem to have been armed with IgE elsewhere, and the location of this IgE production in the upper airways is still unknown (2). Several studies of the nasal mucosa have not shown any local IgE production (14, 15), although, lately, there are some indications of locally produced IgE in the nasal mucosa (16). It has been suggested that IgE synthesis in the upper airways takes place in the lymphoid tissue of Waldeyer's ring and regional lymph nodes (14).

The majority of T cells are known to be located in the extrafollicular area of the adenoids (17), a fact which is in accordance with our findings. The majority of both the IgE⁺ cells and the Fc ϵ RI⁺ cells in our study were found in the

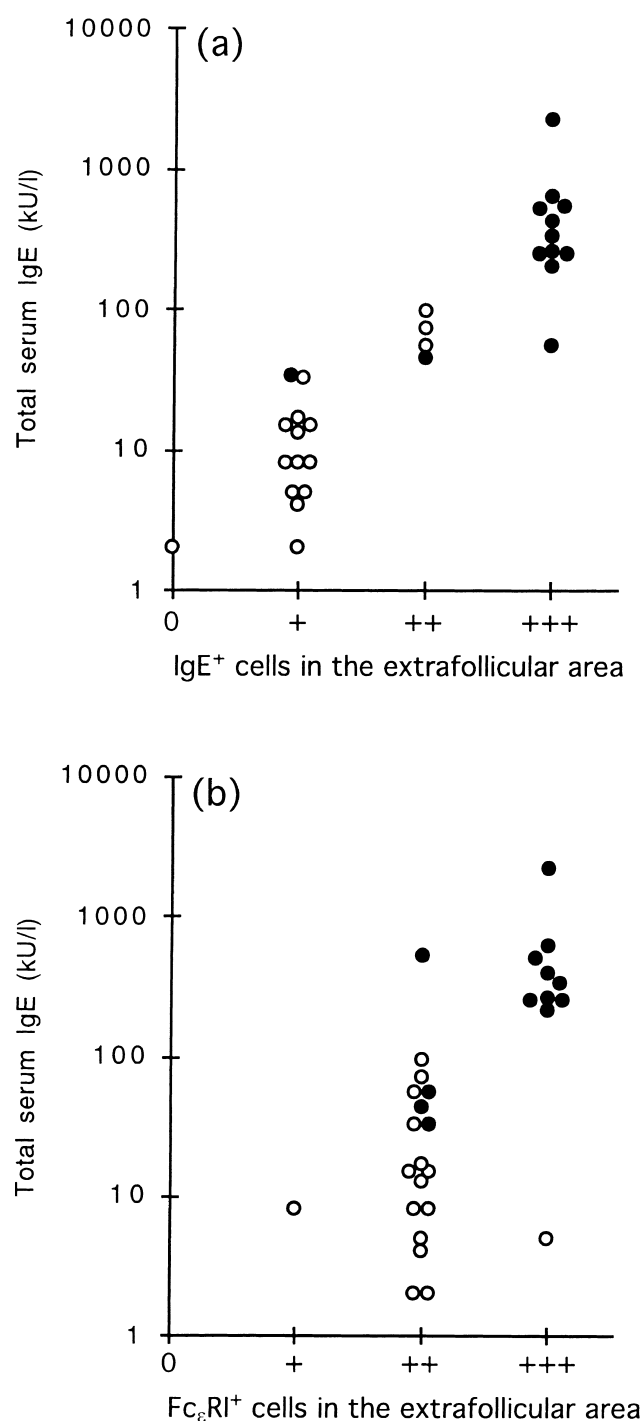


Figure 3. Total serum IgE levels related to numbers of positive cells in 13* atopic (●) and 16 nonatopic (○) children. Numbers of positive cells are expressed semiquantitatively on x-axis: 0, no stained cells; +, low; ++, moderate; and +++, high occurrence of positive cells. a) IgE⁺ cells in extrafollicular area, $P < 0.0001$; b) Fc_εRI⁺ cells in extrafollicular area, $P < 0.05$ (Kruskal-Wallis test). *Blood sample from one of 14 atopic children, who tested positive for six inhalant and food allergens with specific RAST, did not suffice for determination of total IgE.

extrafollicular area, and, although the site for production of immunoregulatory cytokines is not known for the adenoids, cytokine production in human tonsils is localized to the

extrafollicular area (18), where they also might induce the production of IgE. In a recent study, we have demonstrated a significantly higher proportion of memory T cells (CD4⁺/CD45RO⁺) in adenoids than in peripheral blood and a higher CD4⁺:CD8⁺ ratio for cells expressing CD45RO⁺ in the adenoids of atopic children (19). Therefore, it might be hypothesized that the adenoid is not only influenced by atopy, but may itself be an important factor in the development of allergy. Our finding of increased numbers of IgE⁺ and Fc_εRI⁺ cells supports the hypothesis that atopy influences immunologic reactions in the adenoid.

In contrast to our results, Fokkens et al., in a recent study, could not demonstrate any significant differences regarding IgE⁺ cells between adenoids from allergic children and controls (20). This difference could be explained by the selection of the included patients. The children classified as allergic by Fokkens et al. had a median age of only 2.2 years and probably had not yet fully developed their atopic disease and symptoms. This is reflected in lower total serum IgE levels and fewer children with specific IgE antibodies than among the atopic subjects in our study. In contrast to Fokkens et al., we also excluded patients with hyperplastic tonsils, since children not seldom have an obvious difference in size between the two organs, with a hyperplastic adenoid and small tonsils. Although the immunologic background to this condition is not known, it indicates that these lymphoid organs react in different ways, and this could possibly affect the outcome when studying atopy.

IgE⁺ cells in adenoids have previously been reported by Loesel, who showed that IgE⁺ mast cells were more strongly linked than IgE⁺ plasma cells to the presence of allergic disease (21). The study was, however, retrospective, and it is not entirely clear how the diagnosis of atopy was established. Previous findings of IgE⁺ cells in adenoids also support our results (14, 22).

The reports of IgE-producing plasma cells in both adenoids and tonsils have been questioned due to methodological problems with staining artefacts and the possibility of misinterpreting IgE⁺ mast cells as plasma cells (3). In the present study, therefore, we were also interested in identifying IgE⁺ plasma cells, which, if found, would support local IgE production. We used the mAb PCA-1 that has been claimed to identify plasma cells, although it is reported also to bind weakly to monocytes (23). Use of PCA-1 has indicated plasma cell rates in adenoids of 0.9–2.4% (24), a finding which agrees well with what has previously been reported about plasma cells in adenoid tissue (25). The results from double immunofluorescence and confocal microscopy analysis showed that few cells were PCA-1⁺

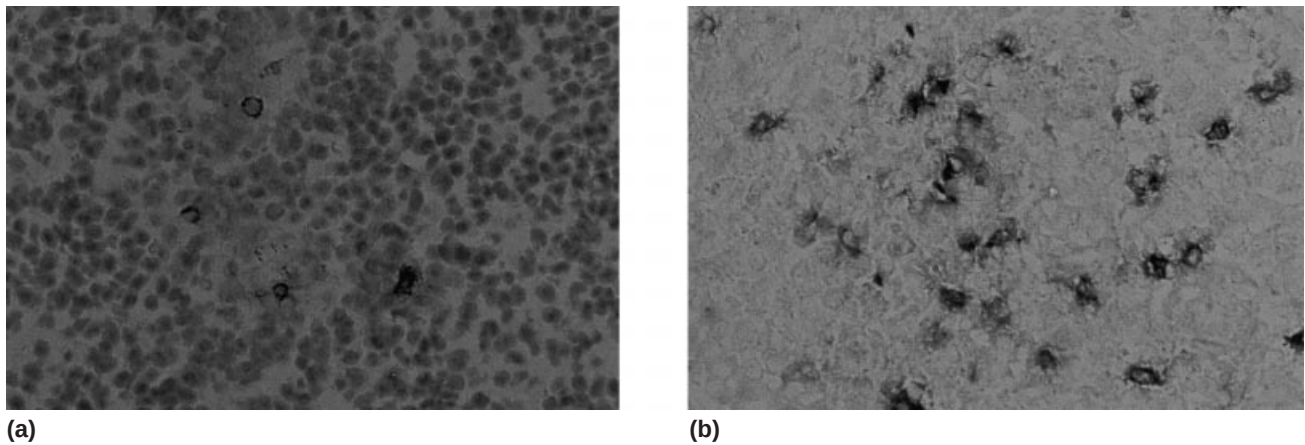


Figure 4. Immunohistochemical stainings of extrafollicular area on consecutive sections from adenoid specimen from atopic child with serum IgE level of 380 kU/l. a) PAP staining with mAb recognizing mast-cell tryptase; b) APAAP staining with mAb recognizing IgE. $\times 320$.

and only a small minority of these were IgE⁺. This may indicate that only a small amount of IgE is produced locally, but it is also possible that PCA-1 identifies only a specific subset of plasma cells.

The tryptase staining we performed indicates that mast cells can only partly be responsible for the IgE staining observed. Accumulating knowledge about the IgE receptors shows that besides plasma cells, mast cells, and basophils, several other cell types can bind IgE. Eosinophils and monocytes have been shown to express both high-affinity, Fc_εRI receptors, and low-affinity, Fc_εRII/CD23 receptors (26–28). Fc_εRII/CD23 receptors have also been shown on neutrophils (29).

In all cases, EG2⁺ cells were few, suggesting that these cells were not implicated in the staining of the majority of the IgE⁺ cells. We were also not able to detect any differences

in the distribution of EG2⁺ cells regarding the presence of atopy or ear disease.

We could not demonstrate any differences in the examined immunohistochemical markers between children with OME and AH. Subgroup analysis of IgE⁺ cells, Fc_εRI⁺ cells, or the other immunohistochemical markers used in comparing OME and AH children in the atopic and nonatopic groups, respectively, indicates that there are no differences between these groups (Table 2). Results from earlier comparative studies of T-cell phenotypes have shown an increased number of T-suppressor cells associated with AH (30), but no differences between OME and AH groups (19, 31). Increased numbers of plasma cells in the OME group have also been found (24). Although atopic diseases are more frequent among children suffering from OME, the role of allergy in the development of OME has yet to be clarified.

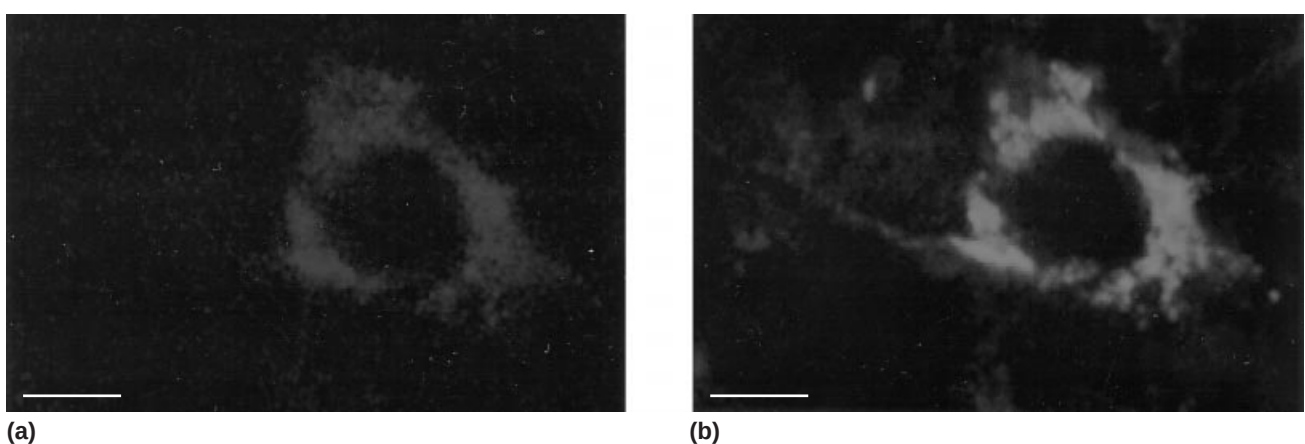


Figure 5. Cell binding both FITC-conjugated mAb against IgE (panel a) and mAb against plasma cells (PCA-1) visualized with secondary Texas Red-conjugated Ab (panel b). Cryosection, 6 μ m thick, from adenoid specimen from atopic child with total serum IgE level of 2200 kU/l was acetone-fixed and stained for double immunofluorescence. Analysis was performed with CLSM. Test scans using single-labeled cells showed that bleed through of FITC fluorescence intensity into Texas Red channel was negligible compared with fluorescence intensity in panel b. This image has been scanned with Nikon x60/1.4 oil immersion flat-field planapochromatic lens and pixel size of $0.17 \times 0.17 \mu\text{m}^2$. Scale bar is 5 μ m.

In conclusion, we have found that atopy clearly influences the number of IgE⁺ and Fc_εRI⁺ cells in adenoids regardless of the presence of OME or AH. In this study, IgE⁺ plasma cells were very few and further studies are needed to clarify to what extent the adenoid contributes to the IgE production locally. Since atopy clearly alters the microenvironment of the adenoid tissue, it seems important to take into consideration the atopic status of children in future immunologic studies of adenoids.

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