

ORIGINAL ARTICLE

IgE-positive plasma cells are present in adenoids of atopic children

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Abstract

Conclusions. We demonstrated the presence of IgE⁺ plasma cells in the adenoids of atopic children. Our data suggest that adenoids are capable of local production of IgE and support the role of adenoids as an inductive site for allergic reactions. **Objective.** We have previously demonstrated increased numbers of IgE⁺ cells in the adenoids of atopic children and also found support for an IL-4-induced class switch to IgE production in adenoids. In search of further evidence of adenoids being involved in IgE-mediated sensitization, we investigated the distribution of plasma cells and macrophages positive for IgE in adenoids. **Material and methods.** Adenoid tissue from atopic and non-atopic children was examined using immunohistochemical markers for IgE, plasma cells (CD138) and macrophages (CD68). The distribution of positive cells was determined in the extrafollicular area and in the follicles of adenoids. Co-localization of IgE and CD138⁺ plasma cells and CD68⁺ macrophages was examined using immunohistochemical double-staining methods. **Results.** Non-atopic adenoids contained no or very few IgE⁺ cells. In contrast, IgE⁺ cells were found in high numbers in atopic adenoids, mainly in the extrafollicular area. Higher numbers of IgE⁺ plasma cells and IgE⁺ macrophages were also found in the adenoids of atopic children.

Keywords: Adenoid, atopy, IgE, immunohistochemistry, macrophage, plasma cell

Introduction

With every breath taken, the nasal mucosa and adenoids are exposed not only to pathogens, but also to different air-borne allergens. As part of Waldeyer's lymphatic ring, the adenoids are probably the site of antigen-induced priming of B cells and generation of antigen-specific lymphoid memory cells and effector B cells, which thereafter migrate to the upper respiratory tract [1,2].

The local source of IgE-producing cells in the respiratory tract has been debated and remains largely unknown. Although plasma cells or co-localization of IgE to B cells were not identified in previous studies of the nasal mucosa [3], recent findings [4] support the concept of local IgE synthesis in the nasal mucosa of allergic rhinitis patients. Kleinjan et al. [5] detected IgE⁺ B and IgE⁺ plasma cells in the mucosa of patients with seasonal and perennial allergic rhinitis, and IgE synthesis has been

demonstrated in cultured nasal mucosa explants from patients with seasonal rhinitis [6].

The situation in the adenoid tissue with regard to IgE⁺ cells is less well characterized. We have demonstrated [7] that epsilon germ-line transcript levels in adenoids are dependent on IL-4 mRNA expression and serum IgE levels, suggesting local IgE switching. An atopy-related alteration of T cell subsets has also been shown with higher values for the ratio CD4⁺/CD45RO⁺: CD8⁺/CD45RO⁺ in adenoids of atopic children [8]. We have also reported [9] an association between increased numbers of IgE⁺ and IgE receptor⁺ (FcεRI⁺) cells in the adenoids of children and atopy patients. The IgE⁺ cells were mainly neither mast cells nor eosinophils [9], but were not further classified.

Given that the adenoids may potentially play a role in local IgE production, we aimed to further identify IgE⁺ cells and their localization within the adenoid tissue of children. This was done in order to further

test the hypothesis of the adenoids being involved in IgE-mediated sensitization, resulting in local production of IgE in the adenoid tissue. We used immunohistochemical double-staining methods for IgE and CD138 and CD68 to identify and quantify IgE⁺ plasma cells and IgE⁺ macrophages, respectively in the adenoids of atopic and non-atopic children.

Material and methods

Subjects

Adenoids were obtained from 12 children, as described previously [9]. In brief, the adenoid tissue was obtained during adenoidectomy, which was performed either for adenoidal hyperplasia with secondary nasal obstruction or for long-standing otitis media with effusion. Informed consent was obtained from the parents of the subjects. The study was performed with the approval of the ethical committee of Karolinska University Hospital.

The parent(s) completed a questionnaire focusing on the child's medical history, including allergic symptoms, medication and family history of the following atopic diseases: asthma, rhinoconjunctivitis and atopic eczema dermatitis syndrome (AEDS) [10].

Before surgery, all children underwent a routine ENT examination. A blood sample was obtained during the operation and analyzed for total IgE (Pharmacia CAP System IgE FEIA; Pharmacia Diagnostics AB, Uppsala, Sweden) and screened with Phadiatop[®], a mixture of inhalant allergens (including birch pollen, mugwort, timothy grass, Dermatophagoides pteronyssinus, Cladosporium herbarum, cat, dog and horse; Pharmacia CAP System Phadiatop FEIA), and a mixture of common food allergens (fx5; egg, milk, peanut, soybean, fish and wheat; Pharmacia CAP System Specific IgE FEIA). The sera that scored positive on the Phadiatop test (≥ 0.35 kU/l) were further analyzed for IgE antibodies against common inhalant allergens (Pharmacia CAP System Specific IgE FEIA). Children were classified as atopic if they had a positive Phadiatop test.

Part of the adenoid tissue of these 12 children had been used in 2 previous studies and the rest of the

adenoids had been stored at -70°C . We selected adenoids from six of the children with the highest serum IgE values and the most pronounced allergic symptoms. All six atopic children underwent adenoidectomy between September and October. As controls, six adenoids from non-atopic children were selected. The non-atopic children had no symptoms of allergy and had negative Phadiatop and fx5 tests.

The six atopic children were all sensitized to inhalant allergens and four of them were also sensitized to food allergens (fx5). They had symptoms of rhinitis or rhinoconjunctivitis and/or asthma. Five of them also had AEDS. At the time of surgery, five of the atopic children were receiving treatment for their allergy: one with inhaled steroid, two with a short-acting β_2 -agonist inhalant and five with topical steroid ointment.

Patient characteristics are presented in Table I.

Immunohistochemical staining protocol

Acetone-fixed, 6- μm thick cryostat sections of the biopsy specimens were used for single and double enzymatic staining. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 and 0.1% NaN_3 in phosphate-buffered saline (PBS) for 15 min [11]. The sections were treated with 10% normal horse serum (Vector Laboratories Inc., Burlingame, CA) and subsequently with an avidin-biotin blocking kit (Vector Laboratories). For single staining, these steps were followed by incubation with the primary antibodies, anti-CD68 (0.1 $\mu\text{g}/\text{ml}$; Dako A/S, Glostrup, Denmark), anti-CD138 (0.9 $\mu\text{g}/\text{ml}$; Dako) and anti-IgE (0.5 $\mu\text{g}/\text{ml}$; Southern Biotechnology Associates, Inc., Birmingham, AL), for 30 min. After washing in PBS the sections were incubated with biotinylated anti-mouse IgG, made in horse (Vector Laboratories), for another 30 min. Finally, after repeated washing in PBS, the sections were incubated with horseradish peroxidase-conjugated avidin-biotin complex (Vectastain[®] elite ABC Kit; Vector Laboratories) for 30 min. The peroxidase reaction was developed with 3,3'-diaminobenzidine (DAB Substrate Kit; Vector Laboratories).

The washing solution was then changed to Tris-buffered saline (TBS) containing 0.05% Tween 20. The sections were treated with 2% normal mouse

Table I. Clinical characteristics of the study subjects.

Characteristic	Atopic children	Non-atopic children
No. of patients	6	6
Age (months); mean (SD)	98 (± 39)	89 (± 42)
No. of males/females	4/2	4/2
Serum IgE (kU/l); median (range)	450 (260–2200)	8 (2–15)

serum (Dako), followed by 30 min of incubation with the second-step primary antibody, biotinylated anti-IgE (2 µg/ml; Southern Biotechnology Associates). The sections were washed and then incubated with alkaline phosphatase-conjugated avidin-biotin complex (Vectastain ABC-AP Kit; Vector Laboratories) for 30 min. The alkaline phosphatase reaction was developed with 5-bromo-4-chloro-3-indolyl phosphate-nitroblue tetrazolium (BioRad Laboratories, Hercules, CA). Endogenous alkaline phosphatase activity was blocked with the addition of levamisole (Vector Laboratories).

No staining was observed when the primary antibodies were substituted with an irrelevant control mouse IgG₁ (Dako). This control was performed for all biopsies in the single-staining protocol (1 µg/ml) and for the first- (1 µg/ml) and second-step (2 µg/ml) primary antibody in the double-staining protocol.

Evaluation of the immunohistochemical stainings

Two sections per monoclonal antibody and specimen were stained and examined blindly by 2 independent investigators (G. P. and C. H.) using a Nikon Eclipse 400 microscope at a magnification of $\times 400$. The adenoid specimens were only evaluated for the presence of cells in the follicles and extrafollicular area, as CD138 is also expressed on epithelial cells [12]. Cells positive for CD68, CD138 and IgE, as well as double positive cells (IgE⁺CD68⁺ and IgE⁺CD138⁺), were counted. Five fields were counted in the extrafollicular area at a magnification of $\times 400$ and the total number of positive cells per square millimeter was calculated. The number of positive cells in the total area occupied by five different follicles in

each specimen was counted at a magnification of $\times 400$.

Inter-observer variation between cell counts was $<10\%$ in $>90\%$ of cases.

Results

Single-stained cells

IgE⁺ cells. Cells stained for IgE were mainly found in the extrafollicular area of adenoids from atopic children (Table II). The IgE⁺ cells were not evenly distributed throughout the adenoid tissue but appeared in clusters, while other areas of the same specimen had few or no IgE⁺ cells present. The 5 high-power fields ($\times 400$) were therefore selected in the areas where the IgE⁺ cells predominated. The non-atopic adenoids had no or very few IgE⁺ cells, all of which were weakly stained, and the few cells that were observed were mainly located in the follicles. The follicles of atopic adenoids contained both weakly and strongly stained IgE⁺ cells but these were less numerous than in the extrafollicular area.

CD138⁺ cells. The CD138⁺ cells were evenly distributed throughout the extrafollicular area and follicles, with higher concentrations in the former area (Figure 1a). No differences were observed between the atopic and non-atopic groups.

CD68⁺ cells. Cells that stained for CD68 were diffusely distributed in the extrafollicular area and to a smaller degree in the follicles. Many of the CD68⁺ cells in the follicles were much larger than those in the extrafollicular area (Figure 1b). The

Table II. Numbers of positive cells in the extrafollicular area and follicles in the adenoids of atopic and non-atopic children. Mean values are shown, with ranges in parentheses.

Cell type	Atopic children	Non-atopic children
Extrafollicular area ^a		
IgE ⁺ cells	211 (63–509)	4 (0–25)
CD138 ⁺ cells (plasma cells)	310 (42–779)	376 (126–636)
CD68 ⁺ cells (macrophages)	632 (408–939)	622 (442–758)
IgE ⁺ plasma cells	38 (13–134)	2 (0–13)
IgE ⁺ macrophages	103 (38–147)	2 (0–13)
Follicles ^b		
IgE ⁺ cells	8 (0–36)	3 (0–9)
CD138 ⁺ cells (plasma cells)	17 (0–95)	21 (0–60)
CD68 ⁺ cells (macrophages)	45 (14–94)	44 (22–90)
IgE ⁺ plasma cells	2 (0–13)	1 (0–5)
IgE ⁺ macrophages	3 (0–7)	2 (0–8)

^aNumber of positive cells per square millimeter.

^bNumber of positive cells in the total area of the follicles.

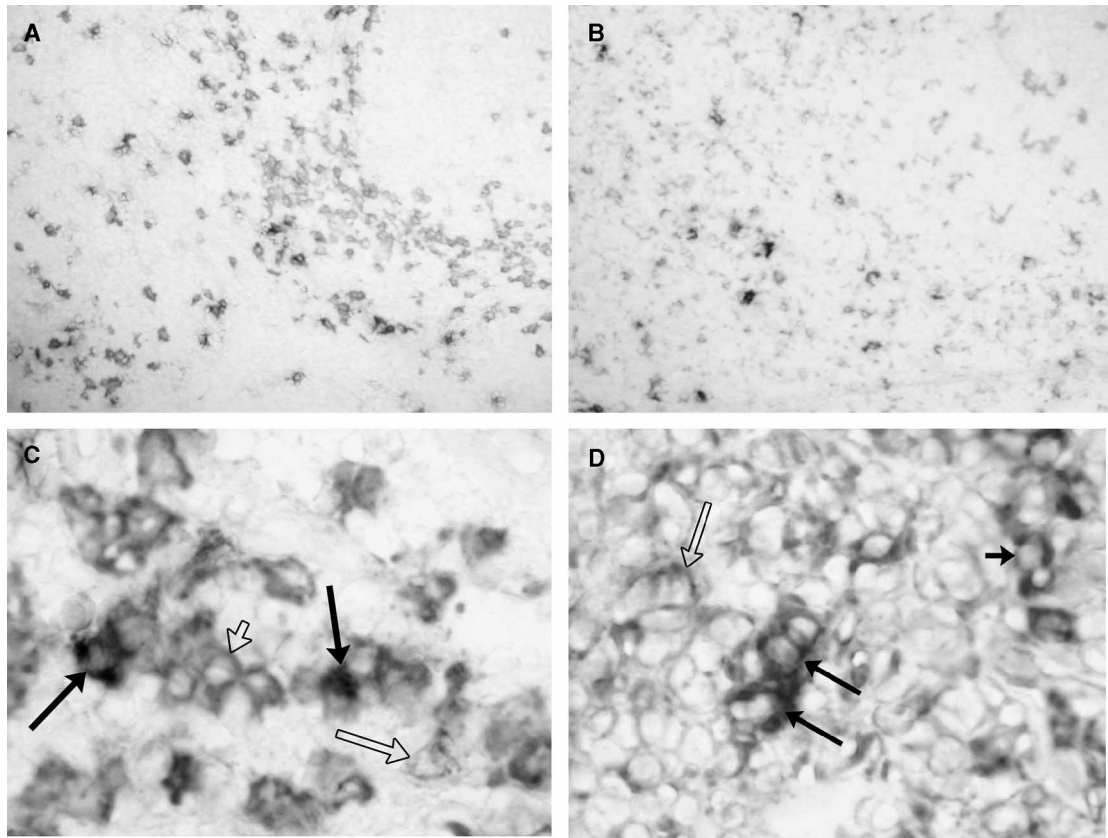


Figure 1. Adenoid tissue from an atopic child. Serial sections double-immunostained with monoclonal antibodies against (a) IgE and CD138 (plasma cells) or (b) IgE and CD68 (macrophages). Open long arrows indicate single-immunostained IgE⁺ cells (c and d), open short arrows plasma cells (c) and solid short arrows macrophages (d); solid long arrows indicate double-immunostained cells, IgE⁺ plasma cells (c) and IgE⁺ macrophages (d).

number of CD68⁺ cells in the follicles and extra-follicular area did not differ between atopic children and controls.

Double-stained cells

IgE⁺/CD138⁺ cells. Double staining resulted in blue-colored IgE⁺ cells and brown-colored CD138⁺ cells. Cells that were double-positive (IgE⁺ plasma cells) were stained with both colors (brown and blue) (Figure 1a and 1c). Like the single-positive cells for IgE, the IgE⁺ plasma cells appeared in clusters. The number of IgE⁺ plasma cells differed considerably between the two groups of children, with increased numbers among the atopic children. These double-positive cells were mainly located in the extrafollicular area, where >10% of the plasma cells were IgE⁺ (Table II).

IgE⁺/CD68⁺ cells. Double staining resulted in blue IgE⁺ cells, brown CD68⁺ cells and mixed-color (brown and blue) IgE⁺ macrophages (Figure 1b and 1d).

The atopic children had increased numbers of the IgE⁺ macrophages compared to non-atopic children

and the double-stained cells were predominantly located in the extrafollicular area, where approximately one in six macrophages were positive for IgE (Table II).

Discussion

The presence of IgE⁺ plasma cells in the adenoids of atopic children has been clearly demonstrated in this study. This finding gives further evidence in support of the hypothesis of local IgE production in the lymphatic tissue of adenoids from atopic children.

The present data are in line with those of a previous immunohistochemical study from our laboratory [9], in which we found the presence of IgE⁺ and IgE receptor⁺ cells in adenoids to be highly associated with the atopic status of the children examined. In that study we could exclude mast cells and eosinophils as the major source of the IgE⁺ cells found.

The nasal mucosa has recently been suggested to be a site of local IgE production [4]. The evidence provided in support of this concept is IgE synthesis by B cells in the mucosa of allergic rhinitis patients, with high specificity for the provoking allergen [6],

and the observation that IgE⁺ B and IgE⁺ plasma cells are found in significantly higher numbers in the nasal mucosa of allergic patients compared with non-allergic controls [5]. The origin of these IgE⁺ plasma cells is not known but it has been speculated that cervical lymph nodes and the lymphatic tissue of Waldeyer's ring are involved in IgE synthesis [13].

The nasal mucosa and adenoid tissue constitute two separate organs in the upper airways and are purported to play different roles in the immune response. It is therefore interesting to compare our data on IgE⁺ cells, plasma cells and IgE⁺ plasma cells in adenoids with data for the nasal mucosa.

The findings of Kleinjan *et al.* [5] are based on the same immunohistochemical technique for double staining of cells as used in our study. Furthermore, the cell density per square millimeter was calculated and could thus be compared with values from the extrafollicular area in this study. While IgE⁺ cells were more abundant in the nose than in the adenoids (ratio 2:1), both plasma cells and IgE⁺ plasma cells were more numerous in the adenoids than in the nose (ratio 1:7 and 1:4, respectively). These differences between the nasal mucosa and adenoid tissue become more striking if one bears in mind that patients with allergic rhinitis were all studied during seasonal exposure to the allergens involved [5]. The abundance of IgE⁺ cells in the nasal mucosa compared to the adenoids could be a consequence of this difference. The IgE⁺ cells in the nasal mucosa may have another origin, for example mast cells and eosinophils. This assumption is supported by the fact that the nasal mucosa functions as a target organ for allergic manifestation in the upper airways, generating IgE-dependent symptoms of rhinitis, whereas the immunological role of the adenoid is to provide the upper airways, including the nasal mucosa, with immunoreactive cells and IgE. It is less likely that allergen exposure can explain the increased numbers of single- and double-positive plasma cells in the adenoids, as our subjects were studied during a period in which there was low or no exposure to the aeroallergens involved. The abundance of plasma cells in adenoids is not surprising, bearing in mind the putative role of the adenoids as a source of immunocompetent cells for the upper airways. It is intriguing to speculate that the IgE⁺ plasma cells in adenoids are intended for dispersion throughout the upper respiratory tract for production of IgE in different target organs, rather than for local secretion of IgE in the adenoid tissue only. This assumption is also supported by our finding of an increase in adenoid tissue of the proportion of IgE⁺ cells that are plasma cells, compared with the findings in the nasal mucosa [5].

Although adenoidal follicles are densely populated with B cells and plasma cell precursors, the follicles in our study contained lower numbers of IgE⁺ plasma cells than the extrafollicular area, where the majority of both plasma cells and IgE-producing plasma cells were located. A plausible explanation for this distribution is that the extrafollicular area is rich in both T and B cells, which is a prerequisite for an antigen-specific immune response followed by an isotype switching of B cells to Ig-producing plasma cells [2]. The number of Ig-producing cells, all Ig classes included, has also been found to be higher in the extrafollicular area of adenoids than in follicles [14].

Using the monoclonal antibody CD138 we were able to identify more IgE⁺ plasma cells compared with our previous results using the plasma cell antigen-1 (PCA-1) antibody [9]. To our knowledge no comparison has been made between the plasma cell markers CD138 and PCA-1 in adenoids or other normal lymphoid tissue. However, great variation in the expression of CD138 and PCA-1 has been reported when different human cell lines from patients with plasma cell myelomas were analyzed [15]. This is one plausible reason why the present finding of double-stained IgE⁺ plasma cells is not fully congruent with our previous results [9].

Approximately half of the IgE⁺ cells in the extrafollicular area of atopic adenoids were CD68⁺ macrophages, which means that approximately one in six macrophages were IgE⁺. One plausible explanation could be that the macrophages express the high-affinity receptor for IgE, FcεRI. Findings from the nasal mucosa of atopic subjects support this assumption, as 20% of CD68⁺ macrophages express FcεRI [16]. Although CD68 has recently been observed on dendritic cells of human tonsil [17] we do not believe that this finding has any significant impact on the interpretation of our data, as we previously found only very few of these cells in the adenoid [9].

In summary, we have demonstrated the presence of IgE⁺ plasma cells in the adenoids of atopic children. We hypothesize that the adenoids are involved in IgE-mediated sensitization, with local differentiation of IgE-producing plasma cells constituting a probable source of mucosal B cells for the upper airways. Further paired studies of adenoids and nasal mucosa could give increased insight into the integrated role of these two organs in the immunological reactions that follow allergen exposure.

Acknowledgements

We thank Catharina Johansson and Anne Svensson for their excellent technical assistance. This work

was supported by grants from the Solstickan Foundation, the Swedish Asthma and Allergy Association, the Swedish Research Council, the Swedish Foundation for Health Care Sciences and Allergy Research, the Hesselman Foundation and the Karolinska Institutet.

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