



Evaluation of CD25, CD152, Fas-ligand expression in the adenoids of allergic and non-allergic children: a pilot study

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KEYWORDS

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Summary Background: The nasal mucosa is the initial site in the upper airways of the host defence against antigen challenge. The lymphatic structure closest to the nasal mucosa is the adenoid. Apoptotic cell death is an important mechanism for maintaining homeostasis in the immune system, and for regulating the fates of lymphocytes following encounters with self and foreign antigens. This process regulates the population of lymphocytes in peripheral lymphoid organs following exposure to various allergens. **Objectives:** The objectives of this study were to find evidence for the hypothesis that the development of allergic illness depends on allergic sensitisation in the adenoid. **Methods:** In this study, cellular infiltrates in the adenoids of 12 allergic and 13 non-allergic children were evaluated. The number of positive cells for CD4, CD8, CD25, CD152, and Fas-ligand were determined using immunohistochemical stainings and imaging analysis techniques. **Results:** There was found a higher expression of CD25, CD152 and Fas-ligand in small, activated lymphocytes, especially in the interfollicular area of allergic patients than in the control group. The expression of FasL in activated lymphocytes, as well as in macrophages, was observed. Statistical analysis has shown a significant correlation between expression of CD152 and allergic disease, and a more intense correlation between CD25, CD152 and Fas-ligand and allergic disease. There was no statistically important divergence in the CD4/CD8 ratio in different samples.

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1. Introduction

The nasal mucosa is the initial site in the upper airways of the host defence system against antigen challenge. A lymphatic structure closest to the nasal mucosa is the pharyngeal tonsil (adenoid)

[1,2]. The first years of life appear to be crucial in the development of allergic diseases, but it still remains unclear which factors have the greatest effect on the manifestation and severity of the disease [3,4].

Antigen-presenting cells (APCs) are able to guide the T cell (CD4) response, skewing it toward either a predominantly Th1 or Th2 response, and influencing the development of several autoimmune diseases [5,6]. Binding the same ligand, CD28 and CTLA-4 (CD152) are receptors that regulate T cell

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activation in two different ways. CD28 is a classic co-stimulator, whereas CTLA-4 exhibits negative regulatory functions [7]. The Fas–FasL system is thought to be particularly important in the maintenance of apoptosis [8–10]. In our research, we evaluated the expression of CD 25 (r IL-2), CD152 (CTLA-4) and FasL in the pharyngeal tonsils of both allergic and non-allergic children.

2. Materials and methods

2.1. Patients and controls

In our study, we examined 37 adenoid specimens from children suffering from nasal obstruction, recurrent infections of the upper respiratory tract and recurrent otitis treated at the Department of Pediatric Otolaryngology, Medical University of Lodz, Poland. The diagnosis of allergic disease was based on the data retrieved from a questionnaire (in accordance with the International Study of Asthma and Allergies in Childhood form) completed by children's parents. The following allergens were tested (house dust mite, grass-, tree- and weed pollen, cat-, dog epithelium and moulds) and food allergens (milk, egg, fish, plain flour, peanut) by Allergopharma, Germany. Blood eosinophilia in all the children and total IgE level in those children having symptoms indicating allergy but negative results of skin tests with allergens, were estimated. The patients were subdivided into three groups:

- Group I: confirmed allergy, with signs of rhinitis and/or asthma, and positive skin-prick test.
- Group II: symptoms of rhinitis, history of bronchitis, atopic dermatitis and conjunctivitis, and both a sufficiently high total IgE for their age and a positive family history of allergy.
- Group III: no atopic features, classified as controls due to the absence of allergic symptoms as well as a negative skin-prick test, and a negative family history of allergy.

2.2. Examination tissue

Patients' adenoids were excised with an adenotome, snap-frozen in liquid nitrogen immediately after the surgery and stored at -80°C . The specimens were cut on a cryostat and then underwent immunohistochemical procedure with primary antibodies against CD25, CD152 and FasL (Novocastra and PharMingen). The number of cells expressing used markers were evaluated semi-quantitatively and qualified as low, middle and high.

2.3. Statistical analysis

The Pearson chi-square test was used for the statistical analysis and *P*-value at < 0.05 indicated a significant correlation.

3. Results

Group I consisted of 12 children (five girls and seven boys), aged from 53 to 120 months (average age 87.2 months). Seven of the patients suffered from rhinitis and/or perennial asthma. Five children were afflicted with seasonal rhinitis and/or asthma on account of grass, tree and weed allergens. Eight patients presented blood eosinophilia. Questionnaire data confirmed the diagnosis pointing to an allergic disease.

Group II included 12 children (2 girls and 10 boys), aged from 41 to 131 months. Eleven of 12 children had a positive family history of allergy. All children had symptoms of rhinitis; four of them suffered from recurrent bronchitis; five had atopic dermatitis; and four had conjunctivitis. In all of these patients a high total IgE for their age was detected. Likewise six of a total of 12 patients had blood eosinophilia. Only the children who suffered from at least two of the above-mentioned disorders were counted into this group.

Group III numbered 13 children (including six girls and seven boys), aged within the range of 51–110 months, none of whom exhibited any symptoms of allergic disease nor had a positive family history of allergy. The total IgE was correct.

Microscopically any statistically important difference in the CD4/CD8 ratio was observed, especially in the subepithelial area. The expression of CD25 in small, activated lymphocytes, mainly in the interfollicular area both in allergic patients and in the control group, was found. The number of cells with the expression of CD25 was higher in allergic patients than in other groups. The presence of CD152 (CTLA-4) was found in all the samples, mainly in the interfollicular area. The lowest intensity of the expression of this antibody was noted in non-allergic children.

The heterogeneous expression of Fas-ligand protein was observed in all groups. We also noticed an important correlation between the intensity of expression of anti-Fas-ligand antibody and the presence of allergy in our patients. There was no relevance found between a "positive" family history of allergy and a positive skin-prick test on the one hand, and eosinophilia on the other. We did notice a statistically important correlation between a

Table 1 Correlation between chosen clinical and histopathological factors

Evaluated markers	Positive history of atopy	Allergic disease	Blood eosinophils
CD 25	– ($P = 0.107$)	+ ($P = 0.045$)	– ($P = 0.405$)
CD 152	+ ($P = 0.027$)	+ ($P = 0.008$)	– ($P = 0.196$)
Fas-ligand	+ ($P = 0.002$)	+ ($P = 0.025$)	– ($P = 0.210$)

positive skin-prick test and the expression of CD25 and CD152, as well as Fas-ligand expression (Table 1).

4. Discussion

Some authors in recent publications considered the role of the adenoid in the development of allergic diseases [11,12].

Apoptosis is an exquisite cell selection mechanism that helps to maintain homeostasis of many tissues. The interaction of Fas and Fas-ligand leads to apoptosis of activated T cells, a process that is crucial for the maintenance of peripheral T cell tolerance [7].

In our study, the markers of T-cell activation and proapoptotic factors in the adenoids of allergic and non-allergic children were investigated.

Our study indicated that a more intense expression of the CD152 antibody was related to the presence of allergic diseases in examined children.

Recent data suggested that CTLA-4 plays a crucial role in the downregulation of T cell responses triggering off T cell activation in the absence of the induction of apoptotic cell death [13]. These molecules are not only involved in T cell stimulation, but also directly participate in the regulation of B cell responses [14,15].

In our research, we found a significant correlation between the numbers of CD152 positive cells and the presence of allergy, which supports this conclusion.

CD25 (IL-2 receptor) was noticed in activated T cells, activated B cells and activated macrophages [16]. More intensive expression of this protein was strongly related to the presence of allergy in our patients.

As is known, Fas-ligand is widely expressed in human tissues and is involved both in the regulation of immune homeostasis and in the regulation of a cells' life and death cycle [17] in many cell types. Nagata and Golgstein [18] and Suda et al. [19] noticed the Fas antigen expressing itself mainly in the germinal centre B cells, whereas Fas-ligand was ex-

pressed predominantly in activated T cells (interfollicular area) [20].

We also observed FasL expression in the cells mainly in the interfollicular area. The intensity of this expression was significantly correlated to the presence of allergy.

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