Allergic rhinitis and house dust mite sensitization determine persistence of asthma in children.

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Abstract

Background: MAS study revealed that chronic course of asthma characterised by airway hyper-responsiveness and impairment of lung function at school age is determined by continuing allergic airway inflammation which begin in the first 3 years of life. Respiratory tract infections early in life, childhood allergic comorbidities, exposure to environmental determinants of asthma and active smoking may all contribute to increased risk of allergic disorders. While these correlations have been established, there is little understanding of the immunological mechanisms by which asthma develops into a persistent disease, or by which symptoms regress.

Methods: Eighty patients were included into the analysis. Clinical and immunoregulatory predictors of asthma were analyzed.

Results: Presence of mAPI criteria as well as the presence of house dust mite-allergy and allergic rhinitis at present, were associated with a reduced prevalence of asthma remission. The presence of increased eosinophil blood count in API (Asthma Predictive Index) criteria was associated with a lower expression of CD25 positive cells. Current presence of HDM-allergy was associated with a higher FeNO level and higher expression of CD25CD71 positive cells. Allergic rhinitis was associated with a higher expression of PPAR and CD25CD71 positive cells; in the same group of patients a lower expression of GARP positive cells were observed.

Conclusions: Probability of persistence of childhood asthma was largely determined by the presence of allergic rhinitis and sensitization to HDM. Our results suggest natural remission of clinical symptoms of asthma in children are not fully related to immunoregulation processes. Additionally, API criteria are related to asthma persistence.

Introduction

Asthma symptoms beginning in adults might have originated in childhood. About 3–5% of individuals will develop wheeze symptoms that persist from early childhood into adulthood (1). Multicentre Allergy study revealed that the chronic course of asthma characterized by airway hyper-responsiveness and impairment of lung function at school age is determined by continuing allergic airway inflammation which begins in the first 3 years of life. However, children with a non-atopic wheezing phenotype lose their symptoms over school age and retain normal lung function at puberty (2). Respiratory tract infections early in life, childhood allergic comorbidities, exposure to environmental determinants of asthma and active smoking in adolescence and later in life, all may contribute to increased risk of allergic disorders (1,3). While these correlations have been established, there is little understanding of the immunological mechanisms by which asthma develops into a persistent disease, or by which symptoms regress. The mechanisms by which immune responses to antigens lead to either allergy or tolerance involve complex interactions between genetic background and the exposure.

A wide range of cell types and suppressive molecules are involved in induction and maintenance of tolerance. The forkhead transcription factor (Foxp3) expression in regulatory T cells (Tregs) plays a crucial role in maintaining immunological tolerance (4,5). The IL-2 receptor (CD25) and transferrin receptor (CD71) are activation markers of T and B cells, and their increased expression might reflect an increased activation state (3,6). Activation of transcription factors, such as the suppressor of cytokine signalling (SOCS), and peroxisome proliferator-activated receptor gamma (PPAR γ), also influence the course of inflammation (PPAR γ exerts anti-inflammatory effects) (6,7). The expression of suppressor of cytokine signaling 3 (SOCS3) is induced by various cytokines, including IL6, IL10, and interferon (IFN)-gamma (8,9). Glycoprotein A repetitions predominant (GARP) is involved in the regulation of peripheral tolerance (10). Our hypothesis was that some cells' mediators might play more prominent role in these processes than others. Therefore, we assessed the relationship between the expressions of above parameters expression and asthma remission in children.

For further understanding of the natural history of asthma, the current study was designed with the aim to investigate the clinical and immunological parameters which may be associated with the resolution of asthma symptoms. Children with continuous asthma and those in whom childhood asthma symptoms had resolved were compared with respect to a number of criteria including: clinical data, API criteria (Asthma Predictive Index), and immunoregulatory parameters production by peripheral blood mononuclear cells (PBMC).

Patients And Methods

Patients

The study was a prospective study based on 80 asthmatic children, aged 7 – 10, who were diagnosed with bronchial asthma <5 years of age based on mAPI (modified API) (Asthma Predicted Index) criteria (11,12). Children were registered, diagnosed and followed up in our outpatient allergy outpatient clinic. Patients with other chronic diseases such as congenital or acquired heart and lung diseases, rheumatologic diseases and immunodeficiency were excluded from the study.

Parents were requested to attend the clinic with their children by phone call. They were interviewed personally. At the first visit they were informed about the purpose of the study, how to score allergy and asthma symptoms and underwent skin testing. Demographic data regarding sex, type of residence, exposure to molds, animals at home, exposure to tobacco, perinatal history (type of delivery, APGAR score, birth weight) as well as family and medical history were taken, patients were classified as atopic based on history and positive skin prick testing. In patients unable to undergo skin testing, a serum specific IgE for a specific allergen was employed. At this visit children were divided into two groups: *remission group* - children in whom asthma treatment was discontinued at the last visit to the allergy clinic due to the absence of clinical symptoms of the disease and *persistent group* - children who remained on anti-asthmatic therapy due to asthma symptoms. Patients were recruited to each study group until the number of 40 children in each group was reached. The second visit took place six months

after the first one, and the third visit took place 12 months from the first visit to confirm asthma status. During each visit lung function and exhaled nitric oxide concentration were measured and the patients' diaries were evaluated. Additionally, at the third visit, blood samples were obtained from all patients to assess immunological parameters.

Asthma diagnosis in early childhood

The childhood asthma was defined as positive by using the modified Asthma Predicted Index (mAPI) which requires 4 or more wheezing episodes in the last year, in addition to one of three major criteria: physician-diagnosed parental asthma, physician-diagnosed child eczema, sensitization to ≥ 1 aeroallergen or two out of three minor criteria: wheezing apart from colds, blood eosinophils $\geq 4\%$, sensitization to ≥ 1 food allergen (11, 12).

Asthma remission

Clinical remission of asthma in our patient was defined as the absence of asthma symptoms for at least 12 months without the use of inhaled corticosteroids and short acting beta2 agonists. In the cases with clinical remission, the spirometry and reversibility test confirmed complete remission (13).

Allergen sensitization

Skin prick testing to standard allergen extracts was performed using Allergopharma, Reinbek, Germany. Reaction $>3\text{mm}$ in diameter above the negative control recorded at 15 min were considered positive.

The measurements of serum specific IgE was applied, if necessary. Allergen sensitization was defined as specific IgE of ≥ 0.35 KU/L for at least one of tested allergens (chemiluminescence method (CLIA), Immulite 2000, XPI, Siemens, Germany). For the purpose of the study we defined allergy as the presence of serum IgE of ≥ 0.35 KU/L specific for allergens: i.e. dust mites, moulds, cat and dog dander, grasses, wild grasses, and tree pollen.

Symptom-medication score

The combined symptom-medication score (SMS) was calculated as a sum of symptom score (SS) and the medication score (MS) recorded by the patients on a daily basis between the visits (14). SS was recorded with the use of a point score system for the most common symptoms of rhinitis (rhinorrhea, sneezing, itching, and nasal congestion), conjunctivitis (ocular pruritus, watery eyes, itching), and respiratory disorders (cough, wheeze, and dyspnea). The results of the score helped to document asthma remission/asthma symptoms as well as allergic rhinitis (AR).

Lung function, body plethysmography (sR_{tot} , R_{occ})

All pulmonary function tests were performed with a Master Screen unit (Erich Jaeger GmbH-Hochberg, Germany), as described elsewhere, in accordance with the ATS/ERS guidelines (15,16). The highest of 3

successful measurements of spirometry was taken and analysed. The results were expressed as the percentage of a predicted value.

Nitric oxide (NO) measurement

Fractional exhaled nitric oxide (FeNO) was measured with a chemiluminescence analyser (Sievers NOA 280i, CO, USA). Exhalations were performed in accordance with the ATS/ERS guidelines (17). Children had to maintain a constant expiratory flow of 50 mL/s for at least 3 seconds and exhalations were repeated until three measurements were within 5% of the mean.

Immunological assessment

The following panel of antibodies conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin-chloro-phyll-protein (Per-CP), or allophycocyanin (APC), was used for the following assays: PPARG-FITC, CD11c-PE, CD 25 FITC, CD 4 PerCP, CD 71 PE, CD 73, PerCP, Anti- GARP APC, FOXP3 PE, (all antibodies from Becton Dickinson, San Diego, CA, USA) and SOCS3 (from Abbexa, Cambridge, UK). All procedures were carried out according to the manufacturer's instructions. The detailed description of the immunological tests and cells preparation is included in an appendix.

The study was approved by the Medical Ethics Committee of the Medical University of Lodz. All parents or legal guardians gave their oral and written consent for the evaluation of data from the medical documentation of their children.

Statistical methods

Statistical analysis was conducted in three steps. The first and second step comprised the logistic regression analysis in univariate followed by the multivariate model. Significant ($p < 0,1$) predictors of asthma remission defined in univariate models were included into the final multivariate model. During the third step, immunological parameters were compared in relation to the presence or absence of previously defined clinical predictors of asthma remission. The significance threshold of p-level was set at 0.05. The statistical analysis was performed with the STATISTICA 13.1 (StatSoft Poland, Kraków).

Results

Eighty patients were included into the analysis. Clinical characteristics of study groups are presented in Table 1.

Clinical predictors of asthma remission

In the first step all available clinical data as independent variables were included into the univariate model of logistic regression analysis together with asthma remission as a dependent variable. The results are shown in Table 2. The presence of mAPI criteria such as inhalant allergy, wheezing apart from colds, an increased eosinophil blood count, as well as the presence of house dust mite (HDM)-allergy and allergic rhinitis; were associated with a reduced prevalence of asthma remission.

Inflammatory/immunoregulatory predictors of asthma remission

In the second step inflammatory/immunoregulatory data (categorized according to lower and upper quartiles) as independent variables were included into the univariate model of logistic regression analysis together with asthma remission as dependent variable. Only the FeNO level was associated with asthma remission (Table 3). The second versus the first quartile of FeNO increased the prevalence of asthma remission, but the third and fourth did not affect it.

Finally, all statistical predictors were included into the one model of multivariate logistic regression analysis. The results showed that only the current allergic rhinitis independently decreased the probability of asthma remission (OR=0,15; 95%CI:0,039 to 0,56).

Associations between clinical and inflammatory/immunoregulatory predictors of asthma remission

The presence of increased eosinophil blood count in API (Asthma Predictive Index) criteria was associated with a lower expression of CD25 positive cells (Table 4). Current HDM-allergy was associated with a higher FeNO level and a higher expression of CD25CD71 positive cells. Allergic rhinitis was associated with a higher expression of PPAR and CD25CD71 positive cells; in the same group of patients a lower expression of GARP positive cells were observed.

Allergy to house dust mite allergens and the presence of allergic rhinitis symptoms determine the expression of CD25 + CD71 + cells in patients with/without asthma. Figure 1.

Discussion

There is little understanding of the immunological mechanisms by which asthma develops into a persistent disease, or by which symptoms regress (18,19). A proportion of children with asthma will “outgrow” their disease, and become symptom-free as adults, even though many of them remain hyperresponsive (20-22). Our study was performed to better understand the mechanism why some patients with pre-school asthma show persistent asthma at school age whereas others grow out of this condition. The results provide novel insights into the differences between children whose asthma has resolved spontaneously and those in whom it remains symptomatic. In the presented study only allergic rhinitis was a strong predictor of asthma persistence into adulthood. Hypersensitivity to house dust mite and possibly higher level of exhaled NO (weak association) as well as mAPI criteria, such as sensitization to aeroallergens, blood eosinophils, wheezing apart from colds, were associated with persistent disease. The expression of all immunological parameters between both groups (asthma remission vs asthma persistence) was comparable which suggests that natural remission of clinical symptoms of asthma in children is not related to immunoregulatory processes and only follows the clinical and allergic status. However, immunoregulatory parameters such as CD25, CD25CD71, PPAR, GARP and Foxp3 positive cells are related to the presence of allergic rhinitis, hypersensitivity to dust mites, as well as elevated numbers of eosinophils and wheezing apart from colds in API, which suggest their possible role in the regulation of peripheral tolerance.

In our study, especially the combination of rhinitis and sensitization to HDM predicted the persistence of childhood asthma into school age children. Many studies addressed predictors of remission or persistence of asthma from early childhood through adulthood (23-26). Immunoregulatory parameters related to the outcome of childhood asthma have been widely discussed, however are not yet well established (4,7,10,19,27). Specific observations in the present study, regarding immunology parameters, were surprising. Presence of allergic rhinitis correlated with PPAR, CD25CD71 and GARP. Glycoprotein A repetitions predominant (GARP) has strong anti-inflammatory and regulatory effects on human cells in vitro as well as in vivo. It is involved in the regulation of peripheral tolerance (10) and plays an essential role in mediating suppressive effects of Treg (10). Authors showed that sGARP had a synergistic effect together with Treg by preventing inflammation (10). In our study we showed higher expression of GARP positive cells in patients without AR. Khare et al demonstrated that CD11c-specific PPAR γ deficiency impairs de novo Foxp3 expression in CD4 $^{+}$ T cells and augments the expression of pro-inflammatory cytokines in CD11c $^{+}$ cells, therefore impairing airway tolerance induction (28). In the latest study by Chen et al. PPAR- γ also as a factor that drives type 2 responses in allergy (7). CD71 increased expression might reflect an increased activation state (6). In our study we revealed a higher expression of PPAR γ and CD25CD71 in peripheral blood mononuclear cells of patients with allergic rhinitis vs children without AR. Additionally, we found a higher expression of CD25CD71 in children with hypersensitivity to house dust mite. We revealed the association between asthma remission, HDM allergy, the presence of allergic rhinitis and CD25CD71 expression (Figure 1). It is clearly visible that HDM allergy and the presence of allergic rhinitis rather than asthma remission determine CD25CD71 expression. Naturally occurring Tregs, express the FoxP3, which is needed to maintain the suppressive activity of mature peripheral Tregs and is a specific molecular marker of Tregs in human peripheral blood. The Foxp3 expression in Tregs plays a crucial role in maintaining immunological tolerance. In our study, API parameters: wheezing apart from colds correlated with the Foxp3 expression and the blood eosinophils count correlated with higher level of CD25 positive regulatory T cells. The suppressor of cytokine signaling 3 (SOCS3) silencing attenuates eosinophil functions, key inflammatory cells in asthma, however in our study it did not correlate with studied clinical factors.

Our study is the first showing immunological profile of regulatory cells in asthmatic children with asthma persistence or remission. We can hypothesize that the modification of immune regulation in asthmatic children takes place under different interactions.

Our study has some limitations. We have recognized that asthma in childhood is a heterogeneous phenotype, however, our study was not powered to differentiate asthma on phenotype only to characterized factors that contribute to asthma persistence or remission. Next, all immunological measurements were ~~done~~ performed when the children were older therefore all findings are related to the present clinical outcomes. Our study has a limited time of follow up; a longer time of observation would allow us to confirm long-term remission.

Our conclusion is that the probability of persistence of childhood asthma was largely determined by the presence of allergic rhinitis and sensitization to HDM. Our results suggest that natural remission of

clinical symptoms for asthma in children are not fully related to immunoregulation processes. Additionally, API criteria such as wheezing apart from colds, elevated blood eosinophils count and sensitization to aeroallergens are related to asthma persistence. The underlying mechanisms for the allergy or tolerance and remission/relapse of asthma symptoms remain unclear, and therefore, future research on asthma should be longitudinal and integrate standardized molecular approaches in identical for both paediatric and adult populations (29).

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Tables

Due to technical limitations, full-text HTML conversion of the tables could not be completed. However, the the tables can be downloaded and accessed as PDFs.

Figures

Figure 1

Allergy to house dust mite allergens (HDM) and the presence of allergic rhinitis (AR) symptoms determine the expression of CD25 + CD71 + cells in patients with/without asthma.

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