

REGULAR ARTICLE

CD4⁺CD25^{high} Treg cells in peripheral blood during remission and exacerbation of allergic asthma in children

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ABSTRACT

Aim: To determine the percentage of CD4⁺CD25^{high} Treg cells in peripheral blood CD4⁺ T cells of allergic asthmatic children during disease remission and exacerbation.

Methods: Peripheral blood mononuclear cells (PBMC) and serum samples were collected from 6- to 11-year-old children with mild-to-moderate allergic asthma (n = 34) and from healthy controls (n = 15). CD4⁺CD25^{high} T cells in PBMC were detected by flow cytometry. Total and specific IgE in serum were analysed by enzyme-amplified chemiluminescence, and IL-2 was measured by ELISA.

Results: There was no significant difference in CD4⁺CD25^{high} T-cell proportions between asthmatic children in exacerbation and remission as compared with controls. CD4⁺CD25^{high} T-cell percentages were not correlated with total and specific IgE. IL-2 was elevated in both disease remission and exacerbation but did not correlate significantly with CD4⁺CD25^{high} T-cell percentages.

Conclusion: CD4⁺CD25^{high} T-cell proportion in the peripheral blood of total CD4⁺ T cells is not reduced in children with allergic IgE-mediated asthma and does not differ between disease remission and exacerbation.

INTRODUCTION

The most recent data have shown the importance of regulatory T (Treg) cells in the development of allergic asthma (AA). However, the immunological basis of this disease is still controversial. CD4⁺CD25⁺ Treg cells are “naturally occurring” population of Treg cells produced by the thymus as a functionally mature T-cell subpopulation, and their role in the initiation and orchestration of immune responses has been widely studied (1–3). These cells express high levels of CD25 (CD25^{high}) and the interleukin (IL)-2 receptor α chain, circulate in blood and have been shown to suppress CD4⁺ and CD8⁺ T-cell responses to a range of stimuli, both *in vitro* and *in vivo* (4,5). Allergy and AA are linked to a Th2-skewed response to inhaled allergens; thus, a deficiency of CD4⁺CD25⁺ Treg cells has been suggested (6,7). Studies have shown that the CD4⁺CD25^{high} subset represents approximately 2–3% of total CD4⁺ T cells and exhibits the regulatory characteristics (4,8). Some reports have suggested the existence of defects in the number and/or function of Treg cells in atopic individuals (6,9–12), whereas other investigators have failed to confirm this (12,13). However, there are very few studies on the role of CD4⁺CD25⁺ Treg cells in the pathogenesis of children allergic diseases. There is considerable evidence supporting an important role of IL-2 regulating Treg-cell homeostasis in peripheral

immune tissue (14); thus, it is possible that IL-2 in serum may regulate the function of CD4⁺CD25⁺ Treg cells circulating in blood.

We hypothesized that there would be a deficiency of CD4⁺CD25⁺ Treg cells in children with asthma. Our aim was to determine whether there are quantitative changes in CD4⁺CD25^{high} Treg cells in the peripheral blood of atopic children with allergic IgE-mediated asthma during disease remission and exacerbation.

METHODS

Patients

The study group comprised 34 atopic children with allergic IgE-mediated asthma (AA) and 15 normal control (C) subjects. The demographic characteristics of the patients are summarized in Table 1. Asthma was diagnosed according to the Global Initiative for Asthma (GINA) (15)

Key notes

- CD4⁺CD25^{high} T cell proportion in the peripheral blood does not differ between children allergic asthma remission and exacerbation.

Table 1 Characteristics of the study population

	Allergic asthmatic children (exacerbation)	Allergic asthmatic children (remission)	Healthy controls
Number	19	15	15
Age in years	9.2 ± 0.5	7.1 ± 0.9	9.9 ± 0.4
Gender (M/F)	12/7	9/6	7/8
Eosinophils (%)	14.84 ± 1.72***	6.36 ± 1.62*†	2.93 ± 0.32
Total IgE (IU/mL)	538.37 ± 193.92**	419.21 ± 161.54*	33.19 ± 12.60

Data are expressed as mean ± SEM.

†p < 0.01 vs. allergic asthma in exacerbation.

*p < 0.05, **p < 0.01, ***p < 0.0001 vs. controls.

and a revised nomenclature for allergy (16). Asthmatic patients were with a positive family and personal history of atopy and had a positive skin prick test response to 1 or more of 25 common aeroallergens (including house dust mites; cat and dog dander; grass, tree and weed pollens; and moulds), at least one positive (≥ 0.35 kU/L) allergen-specific IgE and total serum IgE concentration >150 IU/mL. All patients were recruited from the National Rehabilitation Hospital. None of them was using oral (2 last months) or inhaled (2 last weeks) glucocorticoids before the study. Clinical remission of asthma was defined as reported absence of asthmatic symptoms in subjects during the last 2 months and near normal PEF and FEV₁ ($\geq 80\%$ predicted). Mild-to-moderate asthma exacerbations were defined according to GINA (15). The blood samples were taken during the first days of exacerbation within 48 h. Treatment was with short-acting inhaled beta 2-agonists. Oral glucocorticosteroids were not used. Children with uncontrolled asthma, as defined by GINA (15), before the asthma exacerbation as well as severe exacerbations of asthma were excluded. The asthma duration of all patients by documented clinical history was at least 1 year prior to the study. Exclusion criteria included evidence of acute upper and lower respiratory tract infection as well as treatment with antibiotics within previous 6 weeks, and other acute and chronic diseases. The control group was comprised of healthy children with no family and personal history of asthma or other allergic diseases and a negative skin prick test results to common aeroallergens.

Ethical aspect

The study was approved by the Lithuanian Bioethics Committee, and a written informed consent was obtained from the parents of all participating children.

Blood samples preparation

Blood samples were collected in heparinized tubes. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using Histopaque-1077 (Sigma-Aldrich, Saint Louis, MO, USA). Cells were then labelled and analysed without prior freezing at all times.

Blood samples for sera were drawn in serum tubes, clotting at room temperature for 60 min and centrifuged for

10 min at 1300 g. Serum samples were immediately frozen at -70°C until assay.

FACS analysis

For quantification of the CD4⁺CD25^{high} subset, freshly isolated PBMC were stained with a combination of fluorochrome-labelled antibodies against the following markers: anti-CD4FITC and anti-CD25PE; as isotype controls were used anti-IgG_{2a}FITC/anti-IgG₁PE (BD Pharmingen, CA, USA). Flow cytometry was performed by FACSCalibur (Becton Dickinson, Mountain View, CA, USA) with CellQuest software. Forward and side scatter properties were used to exclude dead cells and debris from analysis. At least 20 000 events were recorded from each sample. A CD25 expression profile and CD4⁺CD25^{high} population determination are shown in Figure 1. Gates were set so that the CD4⁺CD25⁻ population was based on the isotype control, while the CD25^{high} population was determined relative to the low intensity of CD25 staining found on non-CD4⁺ T cells. CD4⁺CD25^{high} cells were evaluated and expressed as a percentage of the total CD4⁺ T cells.

Measurement of IgE

Total and specific IgE concentration tests were performed according to the instructions of the manufacturer: enzyme-amplified chemiluminescence IMMULITE 2000 Allergy (DPC, Los Angeles, CA, USA).

ELISA for IL-2

IL-2 was measured in serum by ELISA. The kit was obtained from BioSource EASIA™ (BioSource Europe, Nivelles, Belgium) and used according to the manufacturer's instructions. The detection limit was 0.05 U/mL of IL-2. The absorbance was measured with ELISA autoreader "Multiscan MS" (Labsystem, Vantaa, Finland).

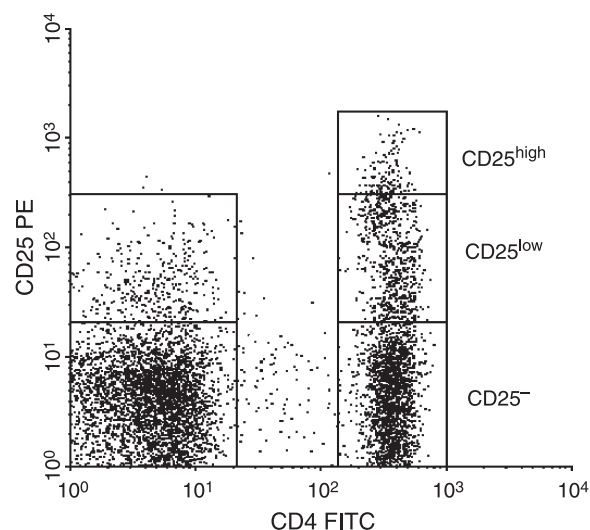


Figure 1 The determination of CD25^{high}, CD25^{low} and CD25⁻ cells. CD4⁺ cells were acquired after gating the lymphocyte population by forward- and side-scattered properties.

Statistical analysis

Values are presented as the mean $\pm$ SEM. Means were compared with two-tailed Student's *t*-test. Pearson's correlation test was used for the analysis of correlation between CD4⁺CD25^{high} Treg percentages and serum IgE and IL-2 concentrations. *p*-Values <0.05 were considered statistically significant.

RESULTS

Comparison of proportion of CD4⁺CD25^{high} T cells in peripheral blood between patient groups and healthy controls

We analysed the difference in CD4⁺CD25^{high} T-cell percentages in peripheral blood CD4⁺ T-cell population from healthy controls and children with AA during disease remission and exacerbation. As shown in Figure 2, the percentage of the CD4⁺CD25^{high} Treg cells among total CD4⁺ T cells in the PBMC of patients during disease exacerbation was $1.89 \pm 0.36\%$, in disease remission was $2.42 \pm 0.31\%$ and in controls was $2.61 \pm 0.67\%$. There was no statistically significant difference between groups (*p* > 0.05).

Association of CD4⁺CD25^{high} T-cell percentages with serum IgE

There was no significant correlation between CD4⁺CD25^{high} T-cell percentages and serum total IgE or specific IgE level in all asthmatic children.

Serum level of IL-2 and association with CD4⁺CD25^{high} T-cell proportions

Serum levels of IL-2 were significantly elevated in both remission (0.14 ± 0.04 U/mL, *p* < 0.05) and exacerbation (0.16 ± 0.05 U/mL, *p* < 0.05) of AA when compared with controls (0.05 ± 0.01 U/mL) (Fig. 3). There were no statistically significant correlations between CD4⁺CD25^{high} T-cell percentages in peripheral blood and serum IL-2 in AA remission or exacerbation.

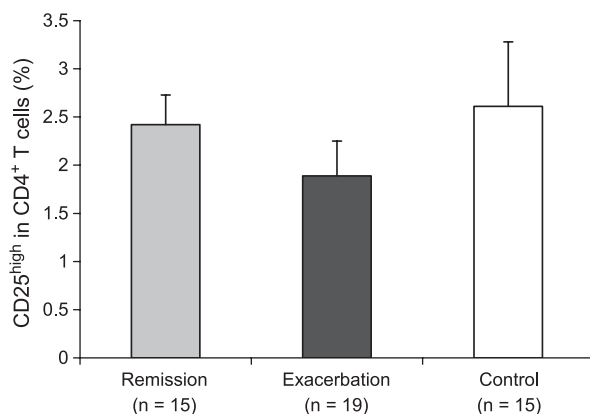


Figure 2 The percentage of CD4⁺ T cells that are CD25^{high} in peripheral blood of allergic asthmatic (AA) children in remission, during exacerbation and in normal controls (C).

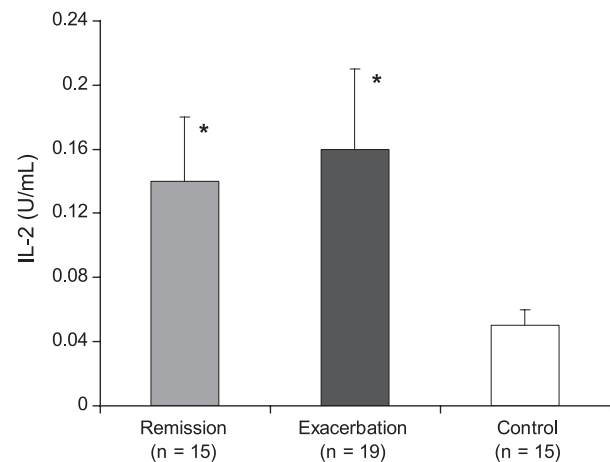


Figure 3 Serum interleukin-2 (IL-2) (U/mL) in allergic asthma (AA) remission, exacerbation and controls (C). Increase is significant in AA remission vs. C (**p* < 0.05) and AA exacerbation vs. C (**p* < 0.05).

DISCUSSION

We have shown that, contrary to our hypothesis, children with AA had no reduction in peripheral blood CD4⁺CD25^{high} T cells as compared with controls. There were no significant differences in CD4⁺CD25^{high} T-cell percentage in association with disease activity in allergic asthmatic children. The reported frequency of CD4⁺CD25^{high} Treg cells in peripheral blood from patients with allergy is inconsistent. Several independent lines of evidence indicate that the number of these cells is impaired in patients with asthma and with other allergic diseases in comparison with healthy individuals (10,12,17), whereas in other studies (6,13,18,19) differences in CD4⁺CD25⁺ Treg numbers between allergic patients and healthy controls were not found. However, data concerning the role of Treg cells in the pathogenesis of paediatric AA are scarce. Lee et al. (17) suggested that children with allergic disease had significantly fewer peripheral blood CD4⁺CD25⁺ T cells and CD4⁺CD25^{high} T cells than control subjects. However, Lin et al. (18) showed no difference in the percentage of CD4⁺CD25^{high} T cells when compared with healthy non-atopic controls. Our data are in agreement with Hartl et al. (20) who also showed no significant difference in the percentages of CD4⁺CD25^{high} cells in peripheral blood. The differences might be related to different experimental protocols, patient's age, disease status and high heterogeneity of Treg-cell families. Thus, whether the quantitative and/or functional insufficiency of natural CD4⁺CD25^{high} Treg cells plays a role in the pathogenesis of AA remains a subject of the debate, and for paediatric asthma, our data suggest that the proportion of CD4⁺CD25^{high} T cells in peripheral blood is not so important.

There were also no associations between total and specific IgE levels and percentages of CD4⁺CD25^{high} T cells in peripheral blood independently of asthma activity. Lee et al. (17) suggested that the number of Treg cells was correlated with atopic status; however in their study, the percentage of

CD4⁺CD25⁺ T cells was correlated moderately with total serum IgE level, but the percentage of CD4⁺CD25^{high} T cells did not correlate with IgE. Our data are again in agreement with Hartl et al. (20) who stressed that the proportion of CD4⁺CD25^{high} T cells in peripheral blood and bronchoalveolar lavage of children with asthma did not correlate significantly with total serum IgE level and these cell levels did not differ between atopic and nonatopic asthmatic children. It seems likely that atopy is not responsible for CD4⁺CD25^{high} T-cell quantitative changes in children with AA.

It is believed that IL-2 is specifically required for the generation and maintenance of natural Treg cells and supports the survival of these cells inside peripheral tissue (21,22). The physiological role of IL-2 could be dual, i.e. to support activated T cells and to concomitantly raise the suppressive activity and number of Treg cells (23). In our study, IL-2 concentration was increased in the serum of asthmatic children in disease remission and exacerbation as compared with controls. The results suggest T-cell activation as one of the characteristics of persistent inflammation in asthma as was shown in our previous study (24). However, no correlation was found between IL-2 and CD4⁺CD25^{high} T-cell percentages in asthma remission as well as in exacerbation. It is clear that T cells *in vivo* are not absolutely dependent on IL-2 for proliferation; moreover, Treg cells compete for IL-2 secreted by responder T cells (25), and it remains uncertain whether IL-2 is essential for the development of Treg cells in the thymus, their homeostasis in the periphery or both.

In conclusion, our data suggest that CD4⁺CD25^{high} T-cell proportion in the peripheral blood of total CD4⁺T cells is not reduced in children with allergic IgE-mediated asthma and does not differ between disease remission and exacerbation. However, the quantitative and functional characteristics of CD4⁺CD25⁺ Treg cells may differ in the airway and can be targets for future investigations.

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