Original article

Increased expression of T-helper cell activation markers in peripheral blood of children with atopic asthma.

Background: *Activated T-helper (CD4) cells have been implicated to contribute to the pathogenesis of bronchial asthma. However, the profile of circulating CD4 subsets in relation to disease activity and asthma severity is unclear.*

Objective: *To study the dynamic changes in peripheral blood CD4 cells expressing the activation markers naïve/memory (CD45RA/CD45RO) and interleukin–2 light chain receptor (CD25) in asthmatic children during and after resolution of acute asthma attacks and to determine whether the expression of these activation markers would be of value in monitoring asthma severity and the response to glucocorticoid inhalation.*

Methods*: Peripheral blood samples were obtained from 20 asthmatic children aged between 0.5 and 9 years (mean±SD: 4.37±2.37 years) with acute asthma attacks, 10 children with lower respiratory tract infection and 20 healthy, age-matched subjects. CD4 cells expressing CD45RA, CD45RO, CD45RA+RO+ and CD25 were analyzed by dual flow cytometry and serum IgE was measured by ELISA. In asthmatic children, the measurements were repeated after the resolution of acute attacks.*

Results: *During acute asthma attacks, the percentages of CD45RA, CD45RO, CD45RA+RO+ and CD25 were significantly increased as compared to the control group (p<0.05 for CD45RA and <0.0001 for the other 3 subsets). After resolution of asthma attacks, a significant reduction of all subsets was noticed and the percentages of CD45RA and CD45RO decreased to normal values while those of CD45RA+RO+ and CD25 remained significantly higher than the controls (p<0.05 for each marker). Unlike healthy children and patients with acute lower respiratory infections, asthmatic children showed increased CD45RO/CD45RA ratio (>1) and a significant increase of the percentage of CD45RA+RO+. During acute asthma attacks, patients with severe persistent asthma showed the highest percentages of all T- helper subsets when compared to those with moderate or mild persistent asthma. Positive correlations were found between serum IgE levels and both CD45RO and CD25 (r = 0.962, p<0.001 and 0.882, p<0.05 respectively) during acute asthma attacks and these correlations remained significant in remission (r = 0.632, p<0.05 and 0.589, p<0.05 respectively). Glucocorticoid inhalation therapy induced a significant reduction in the percentage of CD45RO, CD45RA+RO+ and CD25.*

Conclusion: *Peripheral blood T-helper cell activation markers are reliable indicators for monitoring disease activity and severity of asthma. The reversed ratio of memory/ naïve T-helper cells together with the presence of a clone of cells co-expressing both naive and memory surface markers feature atopic asthma from acute lower respiratory infections. Glucocorticoid inhalation therapy induces a significant inhibition of peripheral blood T-helper cell activation markers.*

Key words: Children, atopic asthma, T-helper cell subsets, glucocorticoid inhalation, lower respiratory infections, CD45RO, CD45RA, CD25.

**Shereen M. Reda,
Afaf A. Mostafa*,
Kawther M. Abdou*,
Abeer A. Shehab*.**

*From the Departments of Pediatrics
and Clinical Pathology*,
Ain Shams University,
Cairo, Egypt.*

Correspondence:
*Assist. Prof. Shereen Reda
Department of Pediatrics,
Faculty of Medicine,
Ain Shams University,
Abbassiah, Cairo, Egypt.
Phone (202) 4187440
E-mail: Shereen_reda
@hotmail.com*

INTRODUCTION

Asthma is a common and increasingly prevalent disease affecting 5% of adults and more than 10% of children [1]. Although it is one of the most common diseases in childhood, its cellular and molecular pathology have not been studied frequently in children.

In adults, asthma is accompanied by chronic inflammation of the bronchial mucosa, in which selective eosinophil influx and local T-cell activation are characteristic (and almost universal) features [2]. It is thought that toxic eosinophil products (lipid mediators and basic granule proteins) damage the bronchial mucosa in asthma, resulting ultimately in the clinical manifestations (variable airway obstruction and bronchial hyperresponsiveness). Evidence, accumulated over the past two decades, suggests that selective eosinophil recruitment and activation in asthmatic bronchial mucosa is mediated, at least in part, by the actions of eosinophil-active cytokines derived predominantly from activated CD4 T-cells, of which interleukin-5 (IL-5) is uniquely eosinophil-specific [3,4].

T-helper cells are a heterogeneous group of cells that can be divided into subpopulations on the basis of the expression of the cell surface markers. The expression of the RA and RO isoforms of the leukocyte common antigen (CD45) molecule, define two subpopulations. Broadly, CD45RA+ T-cells include the "naïve" phenotype and CD45RO+ T cells include the "memory" phenotype. Naïve CD4 T cells are essential for responses to new foreign antigens and have different antigen-presenting cell requirements than memory T-cells while memory T cells have in vitro recall response to antigens, preferential locomotion in response to attractants and mitogens, and migrate toward inflammatory sites [5]. The in vitro studies with human peripheral blood suggest that a subset of CD45RA T-helper cells provides helper function for IgE synthesis in synergy with the CD45RO T-helper cells [6].

Bronchial biopsy specimens from adults with symptomatic asthma revealed increased numbers of lymphocytes expressing CD25 (the α chain of IL-2 receptor), which is suggestive of T-cell activation [2]. Also, the bronchoalveolar lavage fluid after endobronchial segmental allergen challenge showed increased numbers of CD4 T-helper cells expressing the activation marker CD25 [7]. Additional evidence of T lymphocyte activation was demonstrated by the increased expression of CD25 in the peripheral blood of adults with acute severe asthma [8].

Because peripheral blood is the reservoir of the lymphocytes that are recruited into the bronchial tree during asthma, it is interesting to investigate the profile of lymphocyte subsets and T-cell activation in the peripheral blood of asthma patients. Although it has been reported that activated T-cells were significantly increased in the peripheral blood of subjects with symptomatic atopic asthma [9], other investigators have shown that T-cell activation occurred only within the airway [10]. The reports in this field contain controversial

information, and for pediatric asthma, the data are even fewer. The relative lack of knowledge about the molecular immunopathology of asthma in children stems partly from the practical and ethical problems with obtaining access to the bronchial mucosa.

Fortunately, studies on the properties of peripheral blood T-cells in adult asthma have suggested that the properties of these T-cells, reflect those of cells in the bronchial mucosa, owing perhaps to a "spillover" or recirculation of the activated T cells into the peripheral blood [11]. In this study, we used this observation to demonstrate the dynamic changes of CD4 cells expressing naïve/ memory (CD45RA/CD45RO) and the activation marker (CD25) in the peripheral blood of asthmatic children during and after resolution of acute asthma attacks in order to determine the characteristics of T-helper cell activation in atopic asthma and to correlate the expression of these markers with asthma severity. Also, we studied the changes in the expression of activation markers that associate the use of inhaled glucocorticoid therapy in asthmatic children.

METHODS

This case-control study included 20 asthmatic children (11 boys and 9 girls) ranging in age from 0.5 to 9 years (mean ± SD: 4.37 ±2.37 years) who were followed up at the Pediatric Allergy and Immunology Unit of Ain Shams University Children's Hospital, Cairo, Egypt. The diagnosis of asthma was established according to the American Thoracic Society criteria [12]. These patients were evaluated during acute asthma attacks and followed up until the attacks subsided clinically and were then reevaluated. The asthma exacerbations were triggered by exposure to food allergens in 12 children, upper respiratory tract infections in 2, and both factors together in 3 subjects. In three patients the triggering agent was not clear. The patients were sub-classified into 6 patients with mild persistent, 7 with moderate persistent, and 7 with severe persistent asthma according to clinical data and peak expiratory flow (PEF) measurements [13]; the latter was measured in cooperative children older than 5 years.

At entry of the study, all patients were receiving inhaled B2 agonists "as required"; while 9 patients were on regular oral B2 agonist (dosage: 0.1-0.3 mg/kg/day) and 12 patients were taking regular sodium cromoglycate inhalation (30-40mg/day).

The age-and sex- matched control group comprised 20 healthy children (10 boys, 10 girls) ranging in age between 0.75 to 9 years (4.18 ±2.64 years). These children had no personal or family histories of asthma or allergic diseases. In addition, 10 non-asthmatic non-atopic children (6 boys, 4 girls) from one to 7 years of age (3.6 ±1.8 years) with acute

lower respiratory tract infections were enrolled in the study for comparison. These patients had no personal or family histories of asthma or other allergic disorders, and were life-long free of asthma symptoms and their chest auscultation findings as well as chest radiographic images were consistent with the diagnosis of bronchiolitis in 2 patients, lobar pneumonia in 3, and bronchopneumonia in 5 patients.

An informed consent was obtained from the parents of each child before enrollment.

Study Design
All asthmatic children underwent detailed history taking and medical examination in order to determine the degree of asthma severity and the severity of the attack. Patients who were maintained on regular inhaled and/or oral corticosteroids were excluded from the study. At this stage, peripheral blood samples were obtained and medications were subscribed according to asthma severity. All recruited asthmatic patients were commenced or continued B2 agonist inhalation and sodium cromoglycate. Owing to the severity of bronchospasm, 15 patients started de novo inhaled corticosteroid therapy at dosages ranging from 100 to 300ug/day. All children were followed up clinically until remission was achieved and a second blood sample was then obtained from each patient. The interval between the first and second sample ranged from 7 to 14 days.

Peripheral blood sampling, detailed history and medical examination were performed only once on the healthy controls and those with acute lower respiratory tract infection.

Methods
Five milliliters (5ml) of venous blood were collected from each subject under aseptic conditions and were divided into: (i) Three ml were dispensed gently into a tube containing K-EDTA as anticoagulant (1mg/ml) and were used immediately for complete blood counting, differential counting (using Coulter counter T660 -Coultronics, France) and flow cytometric analysis. (ii) Two ml were collected into a plain tube to obtain serum used for the determination of immunoglobulin (Ig) E level. Sera collected were kept frozen at –20ºC till analysis.

Flow cytometric analysis
Optimal dilutions of antibodies labeled with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were used. Labeled antibodies under study were CD4-FITC, CD4-PE, CD45RO-PE, CD45RA-FITC and CD25-PE (Becton- Dickinson, San Jose, CA, USA). Negative isotype-matched controls FITC and

PE labeled were used to determine the non-specific binding of monoclonal antibodies.

Red cells in EDTA samples were lysed using whole blood lysing technique (ammonium chloride 1.5 mmol/L, potassium biphosphate 100 mmol/L, and tetra sodium EDTA 10 mmol/L). The total leukocyte count (TLC) was adjusted in each sample to $5\text{-}10\text{x}10^3$ cell/μl. One hundred μl of each sample were added to corresponding tubes containing 10 μl of each of the monoclonal antibodies studied for comparison. This was followed by gentle vortex and incubation in the dark for 15 minutes. Two ml of working lysing solution were added to each tube, vortexed and incubated again in the dark for 5-10 minutes, centrifuged at 3000 rpm for 5 minutes and the supernatant was discarded. Tubes were then washed 3 times with phosphate buffered saline (PBS) and lastly cells were resuspended in 500 μl of PBS and analyzed using Coulter Epics XL flow cytometer (Profile Instrument, Coulter electronics, Hielach, FL, USA). Lymphocytes were electronically gated using light scatter parameters (forward and side scatter). The results were expressed as percentage of cells positively coexpressing the predetermined markers in comparison to isotype matched controls.

Estimation of serum IgE levels
Immunoglobulin E in stored serum samples was measured by enzyme linked immunosorbent assay (ELISA) (Pathozyme-IgE, Omega Diagnostics Limited, UK). The value of IgE used for data analysis was the percentage from the highest normal for age [13] (Patient's actual level / Highest normal value for age X 100).

Statistical Analysis
Comparisons among studied groups were made by Student's t test for normally distributed data and Mann-Whitney U-test for non-parametric data. Paired data were analyzed by paired t test. Correlation coefficients and their statistical significance were determined with Spearman's rank correlation analysis. Statistica for Windows version 5 (Stat software, Tulsa, Ok, USA) was used for all statistical analyses. Probability values of less than 0.05 were regarded as statistically significant.

RESULTS
T helper cell subsets in the studied groups
As shown in table 1, the percentages of CD45RA, CD45RO, CD45RA+RO+, and CD25 in patients with acute asthma attacks were significantly increased as compared to control children ($P<0.05$ for CD45RA and <0.0001 for the other 3 subsets). Also, the percentages of CD45RA+RO+ and CD25 during acute asthma

attacks were higher than those of children with lower respiratory tract infections (P<0.0001 for each subset). After the acute attacks had resolved by therapy, the percentages of CD45RA and CD45RO became comparable to those of normal controls while those of CD45RA+RO+ and CD25 were still significantly higher than normal ranges (P<0.005, <0.05 respectively). The analyses with paired t test showed a significant reduction of the percentages of CD45RA, CD45RO, CD45RA+RO+, and CD25 cells when patients were in remission (P<0.05 for CD45RA and <0.0001 for the other 3 subsets).

Concerning patients with lower respiratory tract infections, a significant increase in the proportion of CD45RA and CD45RO cells as compared to normal children was observed (P<0.0001 and <0.05 respectively). Meanwhile, no significant differences in the percentages of CD45RA+RO+ and CD25 were found between both groups.

When the distribution from naïve to memory T-helper cells was analyzed by means of calculating the RO/RA ratio, patients with lower respiratory infections showed RO/RA ratio that was comparable with the control ratio (<1) while a reversed RO/RA ratio (>1) was seen in asthmatic children.

Table (1): The percentages of peripheral blood T-Helper (CD4) cells expressing naïve (RA)/memory (RO) and interleukin–2 light chain receptor (CD25) in the studied groups

% Of CD4 T-cell Expressing	Asthma (n:20)		LRI (n:10)	Controls (n:20)
	During attacks	In remission		
CD45RA				
Range	10.5 - 69.5	12.5 - 50.8	41.7 - 59.7	17 - 33.5
Mean ± SD	36.56 ± 19.77	25.67 ± 9.81	50.36 ± 6.2	22.36 ± 4.97
Paired t	2.418 *			
Z	2.326*	1.217	4.399**	
●Z	1.539	4.0034*		
CD45RO				
Range	13.5 - 81.7	5.49 - 24.2	15.6 - 21.3	10.7 - 20.8
Mean ± SD	37.17 ± 25.48	12.82 ± 5	18.55 ± 2.07	13.72 ± 2.81
Paired t	4.809**			
Z	4.341**	1.0008	3.629*	
●Z	1.363	4.09*		
CD45RO/RA ratio				
Range	0.465 - 2.585	0.509 - 0.789	0.261- 0.462	0.367 - 0.914
Mean ± SD	1.05 ± 0.52	0.509 ± 0.133	0.373 ± 0.62	0.63 ± 0.16
Paired t	4.619**			
Z	3.029*	2.569	4.926**	
●Z	4.339**	2.903*		
CD45RA⁺RO⁺				
Range	11.9 - 79.8	0.16 - 49.2	0.6 - 3.1	1.2 - 4.1
Mean ± SD	45.19 ± 22.93	13.19 ± 15.53	2.07 ± 0.72	2.24 ± 0.78
Paired t	7.598**			
Z	5.41**	3.029*	0.593	
●Z	4.339**	2.463*		
CD25				
Range	5.21- 49.1	5.57 - 20.4	2.1- 6.7	1.68 - 6.8
Mean ± SD	24.5 ± 13.97	6.66 ± 4.21	4.17 ± 1.46	4.25 ± 1.47
Paired t	7.021**			
Z	6.255**	1.961*	0.149	
●Z	6.443**	1.561		

LRI: Lower respiratory tract infection.
Mann-Whitney test was employed, Z: Vs controls, ●Z: Vs LRI
*: Significant (P<0.05), **: Highly significant (P<0.0001).

T helper subsets and asthma severity
During attacks of acute asthma, patients with severe persistent asthma showed the highest percentages of all studied T helper subsets (CD45RA, CD45RO, CD45RA+RO+ and CD25) when compared to those with moderate or mild persistent asthma. However, after resolution of the acute attacks, the percentages of CD45RO and CD25 were still significantly elevated in severe persistent asthma when compared with the other two categories of asthma (table2).

Table (2): The mean percentages of peripheral blood T-helper (CD4) cells expressing naïve (RA) / memory (RO) and interleukin–2 light chain receptor (CD25) according to asthma severity.

Mean % of CD4 cells expressing	Mild (n: 6) M ± SD	Moderate (n: 7) M ± SD	t value	Severe (n: 7) M ± SD	t value	●t value
During Attacks CD45RA	23.46 ± 7.28	29.71 ± 18.28	0.781	54.62 ± 15.88	4.404*	2.721*
CD45RO	14.61 ± 0.82	25.88 ± 6.35	4.285*	67.78 ± 16.23	7.96**	6.357**
CD45RA+RO+	14.82 ± 3.06	48.47 ± 7.41	10.335**	67.92 ± 7.98	15.27**	4.725*
CD25	7.15 ± 2.11	24.37 ± 2.71	12.59**	39.5 ± 6.62	15.27**	5.59*
In Remission CD45RA	24.0 ± 5.85	21.45 ± 6.68	0.722	31.32 ± 13.06	1.263	1.77
CD45RO	9.98 ± 2.29	10.59 ±3.96	0.33	17.49 ± 4.45	3.711*	3.058*
CD45RA+RO+	3.35 ± 4.63	9.45 ± 7.12	1.79	25.36 ± 20.25	2.589*	1.961
CD25	3.99 ± 1.35	5.09 ± 2.12	1.08	10.49 ± 4.8	3.188*	2.718*

Student t- test was employed, t: versus mild, ●t: versus moderate.
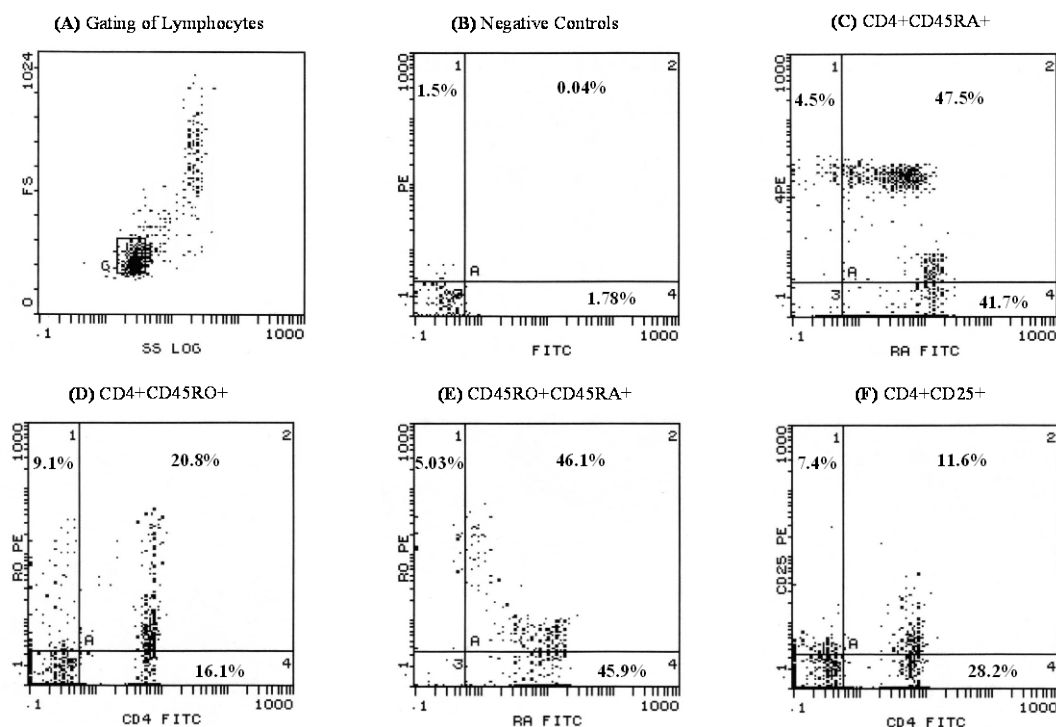***: Significant (P<0.05); **: Highly significant (P<0.0001)**

Figure 1: A set of histograms that represent flow cytometric analysis of the percent (%) coexpression of T cell surface markers in an asthmatic patient understudy. The first one (A) shows gating of lymphocytes depending on forward (FS) and side scatters (SS). In the second histogram (B), quadrants are set on the basis of negative controls. Percent of coexpression is represented on the upper right quadrant, % expression of FITC labeled markers on lower right quadrant, and % expression of PE labeled markers on upper left quadrant. Histograms "C" through "F" show coexpression of different markers in succession CD45RA+ CD4+ (47.5%), CD45RO+CD4+ (20.8%), CD45RO+CD45RA+ (46.1%), and CD4+CD25+ (11.6%).

Correlation analyses

Positive correlations were found between T helper cells expressing CD45RO and those expressing CD25 in patients with acute asthma attack (r = 0.873,P<0.05) and in remission (r = 0.873, P<0.05).

The co-expression of naïve/memory surface markers (CD45RA+RO+) on T helper cells was positively correlated with CD45RO and CD25 cells during asthma attacks (r = 0.797, P<0.05 and 0.902, P<0.001 respectively) and in remission (r = 0.722, P<0.05 and 0.840, P<0.001 respectively). A strong positive correlation was found between the percentage of T helper cells expressing the CD45RO and serum IgE levels during acute asthma attacks (r = 0.962, P<0.001). This correlation remained significant during remission (P< 0.05). A similar positive correlation was also seen between serum IgE values and the percentage

of T helper cells expressing CD25 in patients with acute asthma attacks (r = 0 .882,P<0.05) and during remission (r = 0.589, P<0.05) (Figure 2). Meanwhile, the eosinophil percentage in the peripheral blood of asthmatic patients did not show any significant correlation with either CD45RO or CD25 during asthma attack (r = 0.346, P>0.05 and 0.371, P>0.05 respectively) or in remission (r = 0.266, P>0.05 and 0.316, P>0.05 respectively).

Effect of glucocorticoid inhalation on the peripheral blood T-helper cell subsets

In asthmatic children, the introduction of glucocorticoid inhalation therapy was associated with a significant reduction in the mean percentages of peripheral blood T cells expressing CD45RO, CD45RA+RO+ and CD25 (Figure 3).
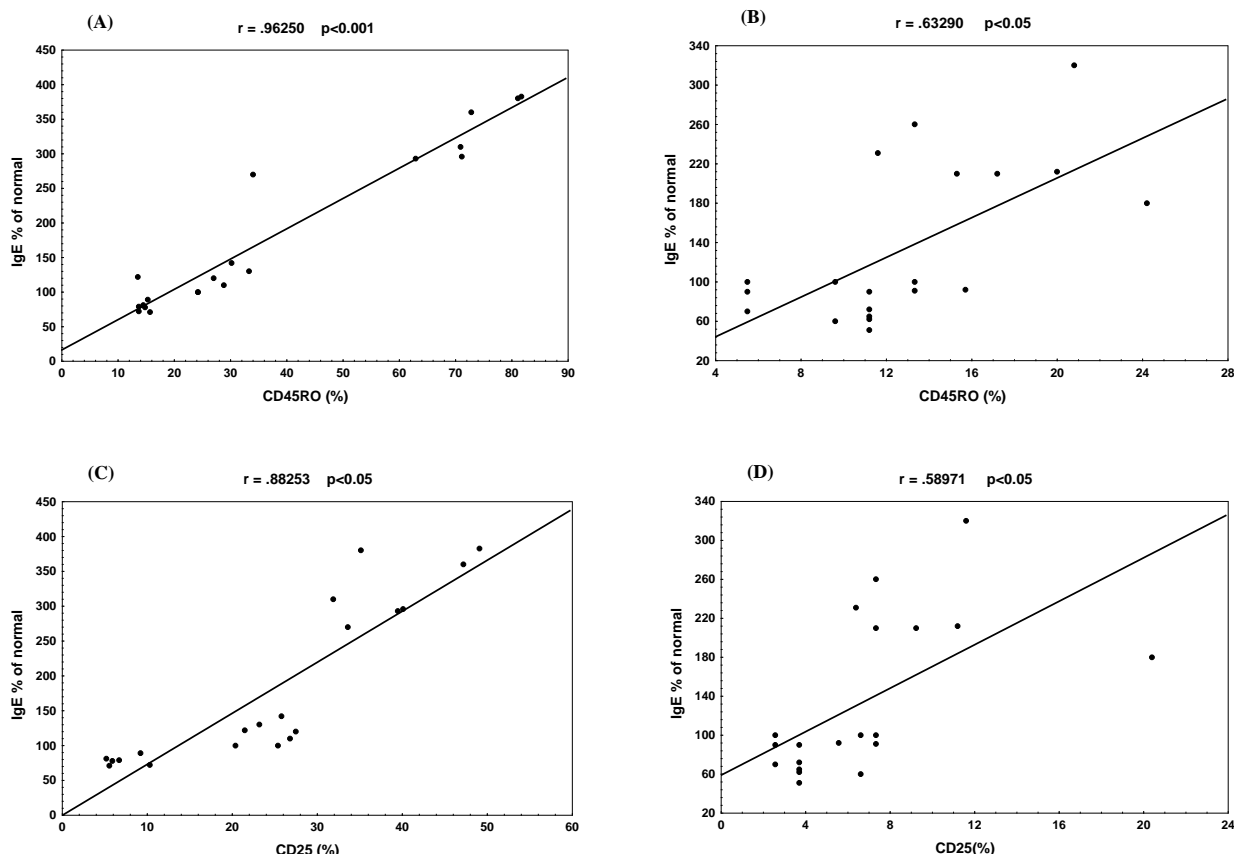

Figure 2: Positive correlations were found between the percentages of peripheral blood memory T-helper cells (CD45RO) and serum IgE (% of normal) among asthmatic children during acute attacks (A) and after resolution (B). Also, similar relationships were found between the percentages of peripheral blood interleukin–2 light chain receptor (CD25) and serum IgE (% of normal) during acute attacks of asthma (C) and in remission (D).
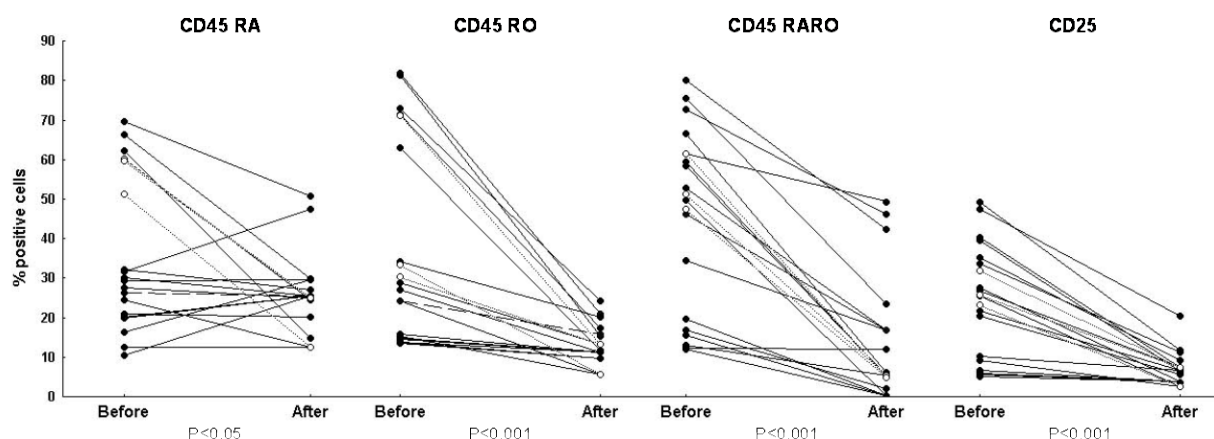
Figure 3: Percentages of peripheral blood CD4 cells expressing the activation markers CD45RA, CD45RO, CD45RARO and CD25 in atopic asthmatic children at the beginning of the study (before) and after introduction of inhaled glucocorticoid therapy (after). P values by paired t test. Open circles = mild asthmatics; closed circles = moderate and severe asthmatics.

DISCUSSION

In this study, using dual flow cytometry, we examined the kinetics of CD4 cells expressing CD45RA, CD45RO, CD45RA+RO+ and CD25 surface molecules in asthmatic children during and after resolution of acute asthma attacks that developed spontaneously in response to allergen exposure. Our results revealed that the percentages of these surface markers in the peripheral blood of children with acute asthma attacks were significantly higher than those of healthy subjects. Previous studies reported increased expression of CD45RO and CD25 on T-helper cells in both atopic and non-atopic asthmatics as compared to their respective controls [14-16]. Also, the increased expression of CD25 on the T-helper cells was shown to correlate positively with the levels of plasma soluble interleukin-2 receptor in acute asthma attacks [17]. Moreover, our results showed significant reduction in the percentages of CD45RA, CD45RO, CD45RARO and CD25 after resolution of asthma attacks denoting that the expression of these activation markers was up-regulated during acute exacerbation of asthma. The remarkable decrease of the peripheral blood T-helper cell activation markers after resolution of acute asthma attacks might be due to the potential homing tendency of memory T cells (CD45RO) to the bronchial mucosa of atopic patients during remission [18], and to the nature of IL-2Rα (CD25), which is expressed upon T cell activation and fades away when cells return to the resting state [17]. However, it is worth mentioning that during remission the percentages of CD45RA+RO+ and CD25 were still higher than those of the controls denoting that after resolution of asthma attack there might be some degree of T-helper cell stimulation that signals an ongoing subclinical allergic lung inflammation.

The concurrent lower respiratory tract infections in bronchial asthma are often impossible to distinguish from spontaneous exacerbations of asthma [7]. The question of whether or not the presence of airway inflammation in asthma would increase the activation markers of T-helper cells was of major interest to us. For this purpose, we included ten patients with lower respiratory tract infection in whom the diagnosis of atopic asthma was ruled out by medical history, chest auscultation and radiographic findings. Among these patients, the expression of CD45RA and CD45RO on T-helper cells were significantly elevated as compared to healthy children and were comparable to those with acute attacks of asthma. In view of the fact that the RA isoform of CD45 is expressed on newly formed T cells that have not encountered specific antigen while during activation by exposure to specific antigen, the RA isoform is stably modulated to RO [19], the observed elevation in the expression of CD45RA and CD45RO in patients with lower respiratory tract infections could be due to pathogen-induced stimulation of T-helper. Consequently, one might suggest that the increased expression of CD45 RA and CD45RO during asthma would be due to the ongoing inflammation rather than being an inherent feature of atopy. In fact, this suggestion was refuted when the distribution of T-helper cell activation marker from RA to RO was further analyzed and a reversed RO/RA ratio (>1) was seen in asthmatic children as compared to those with acute lower respiratory infections and the control subjects. The possible explanation of this finding relies on the fact that in normal non- atopic individuals, the naive T-helper cell (CD45RA) switches upon activation to memory cells (CD45RO), and because the rate of apoptosis of the RO isoform is faster than that of the RA isoform [20] the uniformity of RO/RA ratio is

always kept <1. According to our findings, the reversed RO/RA ratio in atopic subjects might represent a state of increased T-helper cell activation that is accompanied by decreased rate of memory cell (CD45RO) apoptosis.

Another important finding in this study was the isolation of a clone of T-helper cells that co-expressed both RA and RO isoforms of CD45. Although, the exact function of cells expressing both isoforms is unknown, yet it has been suggested that these cells are transitional cells progressing from naïve to memory phenotypes or from resting to recently primed cells [18]. Also, in vitro studies have shown that the transition from CD45RA to CD45RO is accompanied by cell activation and proliferation [21]. Compatible with this view, our data showed that the mean percentage of T-helper cells expressing both isoforms was 45.19% during acute asthma attacks and declined significantly to 13.19% after the resolution of asthma, the latter percentage was still significantly higher than that of healthy children (2.24%) and those with lower respiratory tract infections (2.07%). Therefore, we suggest that the observed increase of T-helper cells expressing both RA and RO isoforms might reflect the hyperactivity and the dynamic nature of T-helper cell in atopic asthma. This suggestion was further consolidated by the significant positive correlation found between the percentages of CD4 cells expressing both isoforms and those expressing either CD45RO or CD25 in asthmatic children.

Studies conducted on lung biopsies and bronchoalveolar lavage fluids of patients with atopic asthma have shown significant numbers of activated T-helper cells (CD25) following allergen challenge indicating that the degree of T-helper cell activation is positively correlated with the degree of broncho-spasm[2,22]. Also, peripheral blood CD25 T-helper cell counts were positively correlated with the increase in airway obstruction in patients with severe asthma [7,8]. Moreover, our results showed that the degree of T-helper cell activation was influenced by the frequency of asthma attacks (asthma severity) since the percentages of CD45RO, CD45RARO and CD25 were significantly elevated among cases with severe persistent asthma as compared to the corresponding values observed in those with moderate and with mild persistent asthma. These observations denote that the rate of T-helper cell activation reflects both the severity and frequency of bronchospasm.

Elevated IgE in serum is one of the most important events in the pathogenesis of atopic asthma. It has been reported that in atopy, the stimulated memory T-helper cells (CD45RO) provide contact-dependent activation signal to B cells to induce IgE switching via secretion of IL-13 [23]. In our study, the serum IgE values

correlated positively with the percentage of T-helper cells expressing CD45RO in asthmatic patients during acute attacks of asthma and in remission. Also, similar to others [16], our results revealed a significant positive correlation between the percentages of CD25 and serum IgE in atopic asthmatic children denoting that T cell activation is associated with increased IgE production.

It is well known that eosinophils also, play an important role in the inflammation of the airway in asthma. In addition, T-helper 2 lymphocytes stimulated by specific allergens secrete IL-5 that induces proliferation, and prolonged survival of eosinophils [4]. In addition, several groups reported a positive correlation between activated memory CD4 T cells and the percentage of eosinophils in bronchoalveolar lavage fluid of asthmatics after bronchial allergen challenge[2,24,25]. Contrary to what was expected, the peripheral blood eosinophil percentages did not correlate significantly with either CD45RO or CD25 percentages in the peripheral blood. This might be due to the recruitment of eosinophil to the bronchial mucosa during exacerbation of asthma [2,7,22], or because the eosinophils stimulated by cytokines have a longer survival time [4] unlike the transient expression of the activation markers on T-helper lymphocytes.

Glucocorticoids are the most effective therapy for the control of asthma symptoms and reversal of bronchial hyperresponsiveness. Previous studies have suggested that they do this, at least partly, by reducing T cell activation and concomitant cytokine production [15,26]. In addition, inhaled glucocorticoids reduce the expression of the activation markers in both T lymphocytes recovered from bronchoalveolar lavage and peripheral blood, along with improvement of symptoms[27,28]. In this study, among the 15 patients who achieved clinical remission by inhaled glucocorticoid therapy, the expression of T-helper cell activation markers decreased significantly after treatment, though not reaching values of healthy children. Of note, the majority of these patients had either moderate or severe persistent asthma, thus, the possibility that the sustained expression of T-helper stimulation markers was influenced by asthma severity cannot be ruled out. This lends an additional support to the concept that the alteration of T-helper cell stimulation is an inherent feature of asthma and current therapies may alleviate the clinical manifestations of acute bronchospasm but do not induce complete down regulation of T-helper stimulation. Therefore, we suggest that monitoring of T-helper cell activation markers during therapy would provide some knowledge about the degree of control of T-helper cell activity. In clinical practice, this would help in the adjustment of glucocorticoid inhalation therapy,

especially during quiescence of asthma symptoms, in order to provide proper control of subclinical lung inflammation with consequent airway remodeling.

In conclusion, peripheral blood T-helper cell activation markers are reliable indicators for monitoring disease activity and severity of asthma. The reversed ratio between naïve and memory T-helper cells together with the presence of a clone of T-helper cells co-expressing both naive and memory isoforms characterize atopic asthma from acute respiratory infections. Glucocorticoid inhalation therapy induces a significant inhibition of T-helper cell hyperactivity in childhood atopic asthma.

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