

Atopic asthma: differential activation phenotypes among memory T helper cells

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Summary

Background T cells have been implicated in the pathogenesis of atopic asthma. We have previously shown that memory T helper cells (CD4⁺CD45RO⁺) are preferentially activated relative to naïve T helper cells (CD4⁺CD45RA⁺) after bronchial allergen challenge. However, specific T helper subpopulations that are activated in atopy and/or asthma remain undefined.

Objective To determine the T helper subpopulations and activation phenotypes relevant to acute and stable asthma that may be common with or distinct from atopy.

Methods Two groups of atopic asthmatics (ten acute and nine stable asthmatics) and two non-asthmatic groups (14 non-asthmatic atopics and eight normal non-atopic controls) were analysed. Ten acute asthmatics were assessed in the emergency room during an acute episode (FEV₁ 43.6% ± 18.4). Nine stable asthmatics were assessed during a symptom-free period (FEV₁ 85% ± 6). Using multiple colour flow cytometry we analysed T cell subpopulations and the expression of IL-2-receptor (IL-2R) and MHC-class II antigens (MHC II) on naïve and memory T helper cells in the peripheral blood of asthmatic and non-asthmatic groups.

Results Atopic asthmatics (acute and stable) had an increased percentage of memory T helper cells expressing IL-2R compared with normal non-atopics (mean SD 16.1 ± 6%, 12.4 ± 2% and 7.7 ± 1.8%, $P < 0.05$) but not compared with non-asthmatic atopics (10 ± 3.5%). Naïve T helper cells had low expression of IL-2R and MHC II in all four groups. MHC II antigen expression was increased in memory T helper cells of asthmatics (acute and stable) compared with normal non-atopics (13.9 ± 7.5, 10.6 ± 5 and 4.9 ± 2.5, $P < 0.05$) but not compared with non-asthmatic atopics (7.92 ± 4). A novel finding was that IL-2R and the MHC II molecules were mainly expressed in non-overlapping populations and coexpression was found predominantly on memory T helper cells. Asthmatics (acute and stable) had higher proportion of double positive memory T helper cells (IL-2R⁺MHC II⁺) compared with both non-asthmatic groups ($P < 0.05$).

Conclusions We demonstrate a differential expression of IL-2R⁺ and MHC II⁺ on CD45RO⁺ T helper cells that would suggest that there are three subsets of activated memory T helper cells in asthmatics. Two non-overlapping IL-2R⁺ or MHC II⁺ CD45RO⁺ T helper cells and a third subpopulation of activated cells that coexpress IL-2R and MHC II (double positives). This latter subpopulation is significantly higher in asthmatics (acute or stable) compared with both non-asthmatic groups, suggesting a specific T helper activation phenotype distinct to atopic asthmatics as compared with atopic non-asthmatics.

Keywords: asthma, CD45, T helper cells, IL-2R, MHC-class II

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Table 1. Characteristics of study subjects

Subjects	<i>n</i>	Age	Sex	FEV ₁ (L)	% pred.
*Acute asthmatics	10	33.6 ± 7.8	6 F, 4 M	1.7 ± 0.88	43.6 ± 18.4
*Stable asthmatics	9	27.6 ± 8.1	5 F, 4 M	3.25 ± 0.7	84.4 ± 5.6
*Non-asthmatics	14	33.3 ± 6.6	6 F, 8 M	3.6 ± 0.4	104.9 ± 10.6
†Normals	8	31.4 ± 7.1	7 F, 1 M	3.5 ± 0.8	110.9 ± 7.1

Values are represented as mean ± SD. *All three groups (acute, stable and non-asthmatics) are atopics, as described in Materials and methods. †Normal controls are non-atopic, as described in Materials and methods.

Introduction

Airway inflammation in asthma has been associated with activated T cells and the cytokines they secrete [1]. Current understanding indicates that T cells play a central role in the initiation and mediation of the airway inflammatory response in asthma [2]. Activated T cells have been found in the peripheral blood of asthmatics during exacerbation [3,4], after bronchial allergen challenge [5] and at the level of bronchial biopsy [6]. However, the distinct activation phenotypes of memory and naïve T helper cells specific to asthma have not yet been fully identified.

CD4⁺ T helper cells can be further subdivided based on the expression of distinct isoforms of the CD45R (leucocyte common antigen) molecule [7]. The CD45 molecule has tyrosine phosphatase activity in its cytoplasmic portion [8] and is associated with T cell activation [9]. Based on the expression of two different isoforms of CD45 – CD45RA and CD45RO – produced by alternative splicing [10], CD4⁺ T helper cells can be further divided into two subpopulations – the CD4⁺CD45RA⁺ and the CD4⁺CD45RO⁺ – that include naïve and memory phenotypes, respectively.

We have previously characterized the T cell activation phenotype in stable asthmatics after *in vivo* allergen challenge [11]. Our analysis of two lymphocyte activation markers, IL-2R and MHC II, indicates that IL-2R is preferentially increased in memory T helper cells after allergen challenge. Although prior observations demonstrate increased IL-2R in acute asthmatics [3,4], the exact T helper subpopulation that may be activated during an acute clinical setting has not been well characterized. To extend our observations [11] and those of others [4,12], we analysed activation phenotypes in naïve and memory T helper cells of acute asthmatics (acute) during emergency room visits in comparison with stable asthmatics (stable) to assess immunological changes in relationship to exacerbation of symptoms. Additionally, to assess changes in T cell activation phenotypes due to asthma and not atopy, two additional non-asthmatic groups were analysed: atopic

non-asthmatics (non-asthmatics) and non-atopic normal controls (normals). Our findings indicate a specific T helper activation phenotype associated with atopic asthma and not with non-asthmatic atopy.

Materials and methods

Subjects

The Brigham and Women's Hospital Human Research Committee approved the study and written informed consent was obtained from all participating subjects.

Acute atopic asthmatics (acutes) Ten atopic asthmatics (6 F, 4 M; mean age 33.6 ± 7.8 years), admitted to the emergency room of the Brigham and Women's Hospital for asthma exacerbation were enrolled in the study (Table 1). Inclusion criteria were: (i) FEV₁ < 75% of predicted; (ii) non-smokers; and (iii) therapy consisting only of β-2 agonists or inhaled steroids (maximum eight puffs/day) as regular treatment. Exclusion criteria were: (i) oral or systemic steroid therapy within the last month; (ii) temp > 100° F; (iii) immunotherapy within the last 3 months; (iv) any other concomitant systemic or lung disease; and (v) pregnancy.

A medical questionnaire was administered to determine clinical history and spirometry was performed to measure the severity of exacerbation. Blood samples were withdrawn before the administration of any additional medication other than the subject's outpatient therapy. All patients had a clinical history of allergic asthma since early childhood with the exception of one patient who had onset in adolescence. All the patients were on β-2 agonist for control of symptoms, two patients had additional regular treatment with inhaled steroids (less than eight puffs/day). Spirometry was performed on admission prior to any additional medication. The mean FEV₁ was 1.7 ± 0.88 L (43.6 ± 18.4% of predicted), and the mean FEF 25–75%

was 1.2 ± 0.93 L ($26.7.1 \pm 21.8\%$ of predicted) consistent with moderate to severe obstruction [13].

Stable atopic asthmatics (stables) Nine atopic asthmatics, asymptomatic when enrolled in the study (5 F, 4 M; mean age 27.6 ± 8.1 years), were analysed from the Asthma Center outpatient clinic at the Brigham and Women's Hospital. Inclusion criteria were: (i) $FEV_1 > 75\%$ of predicted, off all medication; (ii) non-smokers; and (iii) only on β -2 agonist for symptomatic relief. Exclusion criteria were: (i) requirement of regular steroid treatment with either inhaled or oral steroids therapy within the last 3 months; (ii) immunotherapy within the last 3 months; (iii) any other concomitant systemic or lung disease; and (iv) pregnancy. A medical questionnaire was administered to determine clinical history. Spirometry and blood samples were collected during a symptom-free period. The patients had history of mild asthma [13] and allergy. All patients had at least one positive skin test to one environmental allergen of the New England region. The patients were non-smokers and had an $FEV_1 > 75\%$ of predicted, and were off all medications except β -2 agonists, which were withheld 8 h prior to spirometry screening. The mean FEV_1 was 3.25 ± 0.7 L ($85 \pm 6\%$ of predicted). These patients participated in a previous study [11] but the data analysed in this research have not been previously published.

Non-asthmatic atopics (non-asthmatics) A group of 14 healthy age-matched atopic (6 F, 8 M; mean age 33.3 ± 6.6 years) volunteers were enrolled. Inclusion criteria were: (i) a positive skin test to at least one environmental allergen; (ii) no clinical history of asthma, lung disease or atopic dermatitis; (iii) non-smokers; and (iv) no history of any systemic disease or pregnancy. All subjects had a positive skin test to at least one of the allergen extracts mentioned below. Seven subjects had a history of allergic rhinitis but were asymptomatic at the time of the blood withdrawal and lung function testing and had not received immunotherapy within the last 3 months. The other seven had a positive family history but no personal history of allergies. The mean FEV_1 was 3.6 ± 0.4 L ($104.9 \pm 10.6\%$ of predicted). This group was used to assess differences in T cell activation due to atopy and not to asthma.

Non-atopic normal controls (normals) Eight normal (7 F, 1 M, mean age 31.4 ± 7.11 years) age-matched healthy volunteers with no clinical history of atopy or asthma and a negative skin test to all the allergen extracts were enrolled. The mean FEV_1 was 3.5 ± 0.77 L ($110.9 \pm 7.06\%$ of predicted).

The stable atopic asthmatics, non-asthmatic atopics and

non-atopic controls had spirometry values within the normal range [14].

Allergic skin tests

Skin prick test was performed with a panel of common aeroallergens extracts (glycerol diluent) of the New England area: maple, birch white, oak, grass mix, timothy, ragweed grass/*Ambrosia*, dust mix, *Dermatophagoids f.*, *Dermatophagoids pt.*, *Hormodendrum c.*, *Alternaria temis*, *Aspergillus fumigatus*, cockroach, dog hair, cat hair (Fel d I), cat pelt (Fel d I) (Miles). The positive and negative controls were histamine and glycerol diluent, respectively. A weal of 3 mm above the negative control of the longest and orthogonal diameter at 15 min was considered a positive response.

Lung function tests

The pulmonary function parameters studied were: FEV_1 , FVC and FEF 25–75%. Three forced expiratory manoeuvres were performed with a spirometer (Puritan-Bennet PS 600, Puritan-Bennet Corp, Lenexa, KS, USA) in a standing position. Only the means of the FEV_1 values for each study group are shown (Table 1). For acute asthmatics, spirometry was performed before administration of medications during the emergency room visit, and in the case of the other groups, during their screening visit, in a symptom-free period. Predicted values [15] were recorded according to age, sex and size. The best of the three manoeuvres with the highest FEV_1 value was recorded.

Monoclonal antibodies

Monoclonal antibodies (mAb) specific for CD3 (T cells), CD25 (IL-2R), CD45RA (leucocyte common antigen) (2H4), CD8 (cytotoxic T cells), MHC II monomorphic antigen for HLA-DR, HLA-DQ and HLA-DP molecules (I3), were obtained from Coulter Corporation (Miami, FL, USA). Biotinylated anti-CD45RO (UCHL1) and biotinylated anti-CD25 were obtained from Dako Corporation (Carpinteria, CA, USA). The mAb for $CD4^+$ (T helper) was purified by a protein A column and eluted with 125 mM acetate in distilled water. Purified mAb was brought to a final concentration of approximately 1 mg/mL, and conjugated to Cy5 (80 nmoles) (Biological Detections Systems Inc., Pittsburgh, PA, USA) at room temperature for 30 min. Free dye was separated from Cy5-labelled mAb by elution through a gel permeation column (G50 sephadex) using phosphate buffered saline (PBS) + 0.02% NaN_3 . Streptavidin Red-613 was obtained from Life Technologies Inc. (Grand Island, NY, USA). Each antibody was titrated to determine the highest dilution

Table 2. Analysis of T lymphocytes subpopulations

Cell phenotype	Acute asthmatics (<i>n</i> = 10)	Stable asthmatics (<i>n</i> = 9)	Non-asthmatics (<i>n</i> = 14)	Normals (<i>n</i> = 8)
†CD3 ⁺	61.7 ± 9	72.3 ± 4	67.9 ± 9	71.5 ± 4
†CD8 ⁺	16.5 ± 6	21.6 ± 8	23 ± 7	20.8 ± 5
†CD4 ⁺	45.9 ± 9	50.2 ± 9	44.4 ± 6	50.4 ± 4
‡IL-2 ⁺	16 ± 4*	9.7 ± 2	9 ± 1	7.9 ± 1
‡MHC-II ⁺	11.4 ± 3†	7.9 ± 2	10.2 ± 2†	5.6 ± 2

Values are represented as Mean ± SD. **P* < 0.001, acute asthmatic vs. stable asthmatics, and the two non-asthmatic groups (atopic non-asthmatics and non-atopic normal controls). ***P* < 0.05, atopics (acute asthmatics and non-asthmatic) vs. non-atopic normal controls. †% of lymphocytes. ‡% of CD4⁺ T cells.

to give an optimal fluorescent signal. Murine isotype negative controls were used to establish background fluorescence for each fluorochrome.

Peripheral blood mononuclear cells isolation

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll-Hypaque gradient [16]. Briefly, 20 cc of whole blood were diluted 2 : 1 in PBS and placed on a Ficoll-Hypaque gradient (Pharmacia, Uppsala, Sweden) (density 1070 ± 0.001 g/mL) and centrifuged at 800 *g* for 30 min. The interface was recovered, washed twice and resuspended in PBS with 3% fetal calf serum (FCS) (Life Technology, Calsbad, CA, USA).

Flow cytometry

For immunofluorescence staining, PBMC, isolated as described above, were incubated in aliquots of 5 × 10⁵ cells in 50 L of PBS containing 3% FCS and 0.01% of NaN₃ (PBS-FCS), with saturating concentrations of fluorochrome-labelled mAb for 30 min at 4° C. The samples were washed twice with 1 mL of the same buffer and resuspended in 300 L of PBS-FCS. Those suspensions incubated with biotinylated antibodies requiring a second step were incubated for 20 min in 50 L of PBS-FCS with saturating concentrations of streptavidin-Red 613 and washed twice and resuspended in 300 L of the same buffer. Samples were analysed without fixation on a Coulter Epics Elite fluorescence activation cell sorter using 488 (FITC, PE and R613) and 633 (Cy5) excitation wavelengths. Lymphocytes were gated according to size and granularity in a forward and side scatter plot. Fluorescence was detected at 525 (FITC), 590 (PE), 613 (R613) and 670 (Cy5) nm.

The CD3⁺, CD4⁺ and CD8⁺ T cells were analysed and proportions were calculated based on the lymphocyte

population. The distributions of the other markers (CD25, CD45RA, CD45RO and MHC II) were analysed for coexpression with CD4⁺ T cells in a two parameter log scale histogram gating on the CD4⁺ T cells and values are expressed as percentage of CD4⁺ T cells. When analysing CD45RO⁺ cells together with CD4⁺ T cells, 'high' and 'low' fluorescent populations were identified. The proportion of CD4⁺CD45RO⁺ T cells were calculated based on the 'high' population.

Flow cytometry data were analysed with the Listmode Coulter Elite software. Graphics were prepared using WinMDI software (Joseph Trotter, Scripps Institute, La Jolla, CA, USA).

Statistical analysis

Statistical analyses of unpaired data were made using the Student's *t*-test or Mann-Whitney test. The Kruskal-Wallis ANOVA and Dunn's method were used for multiple comparison analysis. Sigma Stat statistical software (Jandel Scientific/SPSS Science, Chicago, IL, USA) was used for all of the statistical analyses.

Results

Analysis of T lymphocyte subpopulations

T cell surface markers were analysed: CD3 (pan T cell), CD4 (helper T cell) and CD8 (cytotoxic T cell). The values obtained are expressed as percentage of total lymphocytes (Table 2). Expression of IL-2R and MHC II as markers of activation were also analysed in total T helper cells expressed as a percentage of CD4⁺ T cells.

We analysed CD4⁺ T cells for expression of the T cell activation markers, IL2-R and MHC II. Our findings confirm and extend prior observations [3,4]. CD4⁺IL-2R⁺ T cells are significantly increased in acute asthmatics

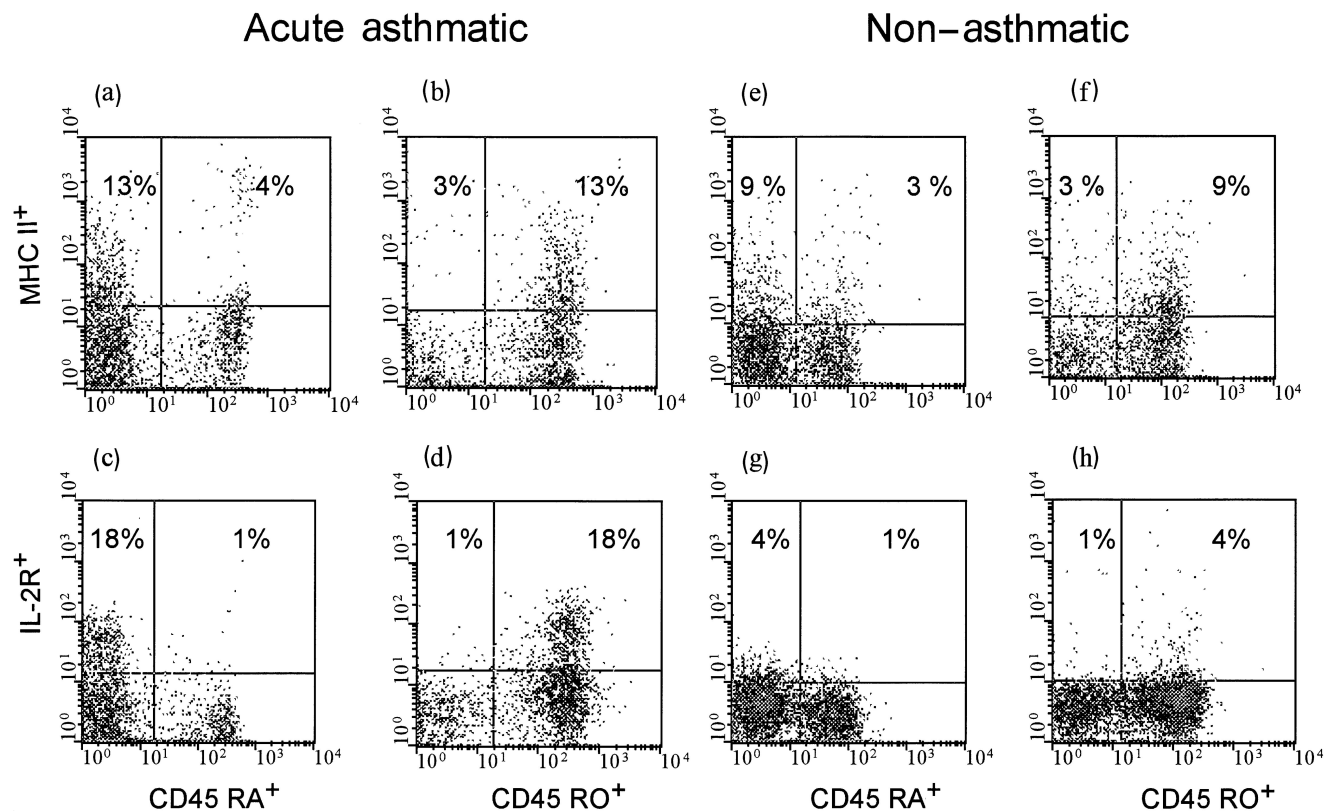


Fig. 1. Three-colour flow cytometry analysis of activated T cells. Shown is a representative experiment of the analysis of the coexpression of IL-2R and MHC II with CD45RA (naïve) and CD45RO (memory) surface molecules on helper T cells. The cells were gated on the CD4⁺ T cells. Shown are two-parameter histograms of CD4⁺ T cells from an acute asthmatic (a–d) and from a non-asthmatic (e–h). The y-axis represents MHC II⁺ (upper panels) or IL-2R⁺ cells (lower panels). The x-axis represents CD45RA⁺ or CD45RO⁺ cells. The upper right quadrant represents the proportion of CD4⁺ T cells that coexpress MHC II-CD45RA (a and e), MHC-II-CD45RO (b and f) and IL-2R-CD45RA (c and g) or IL-2R-CD45RO (d and h).

compared with stable asthmatics (16 ± 4 vs. 9.7 ± 2) as well as compared with two other groups, non-asthmatic atopics (9.0 ± 1) and normals (7.91) ($P < 0.001$). In contrast, differences among CD4⁺ MHC II⁺ T cells in acute asthmatics compared with stable asthmatics and non-asthmatic atopics (11.4 ± 3 , 7.92 , 10.2 ± 2) are not significant. However, compared with normals, acute asthmatics and non-asthmatics have increased proportion of CD4⁺ MHC II⁺ T cells (11.4 ± 3 and 10.2 ± 2 vs. 5.6 ± 2 , $P < 0.05$).

Differential distribution of IL-2R and MHC II expression among T helper subpopulations via three-colour flow cytometry analysis

To determine which CD4⁺ T cells express the IL-2R and MHC II activation markers, we analysed the expression of these activation markers on naïve and memory T helper cells. A representative experiment of three-colour flow

cytometry analysis of PBMC from an acute asthmatic (Figs 1a–d) and from a non-asthmatic atopic (Figs 1e–h) is shown. The histograms represent plots of PBMC that were gated on CD4⁺ T lymphocytes, showing the predominant coexpression of IL-2R and MHC II with CD45RO molecules. Increased activation markers expression among the CD4⁺CD45RO⁺ vs. the CD4⁺CD45RA⁺ T cells is shown for both samples. Increased expression of MHC II and IL-2R on the memory T cell is shown in the acute asthmatic subject in comparison to the non-asthmatic (Figs 1b and d vs. f,h, respectively).

Analysis of the total expression of IL-2R (Fig. 2a) and MHC II (Fig. 3a) among naïve T cells in acute asthmatics, stable asthmatics, non-asthmatics and normals demonstrated the following: there was no significant difference in the percentage of naïve (CD4⁺CD45RA⁺) T cells expressing IL-2R (Fig. 2a) among all four groups analysed (4.07 ± 1.9 , 4.38 ± 1.56 , 4.09 ± 1.8 and 2.6 ± 0.5 , respectively). The percentage of CD4⁺CD45RA⁺ T cells

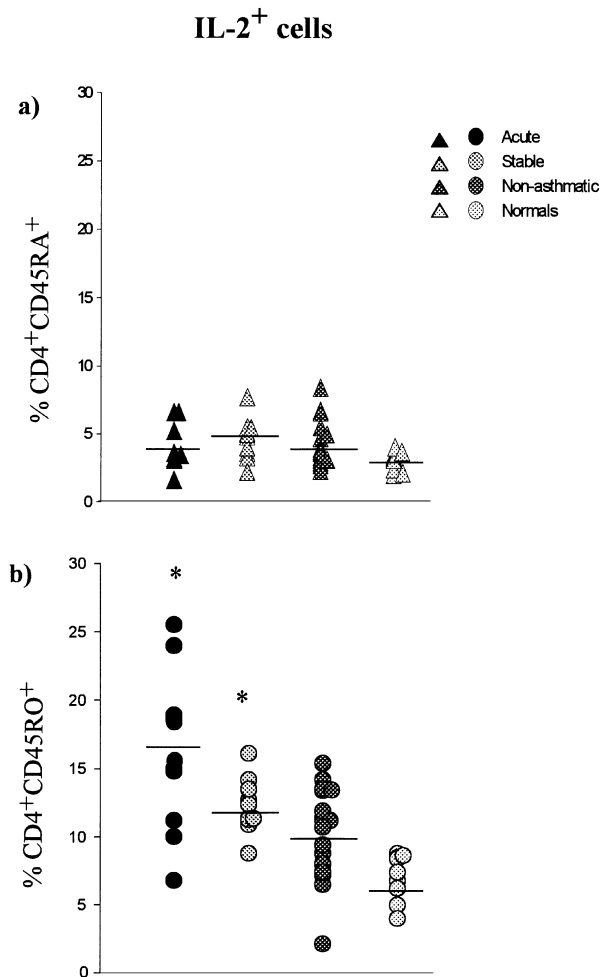


Fig. 2. (a) IL-2R expression on naïve T helper cells from acute asthmatics ($n = 7$), stable asthmatics ($n = 9$), non-asthmatics ($n = 14$) and normals ($n = 7$). Shown are the percentages of CD4⁺CD45RA⁺ expressing IL-2R molecules. (b) IL-2R expression on memory T helper cells from acute asthmatics ($n = 10$), stable asthmatics ($n = 9$), non-asthmatics ($n = 14$) and normals ($n = 8$). Shown are the percentages of CD4⁺CD45RO⁺ expressing IL-2R. The lines in each plot represent the mean for each group. * $P < 0.05$, acute and stable asthmatics vs. normals. The lines in each plot represent the mean for each group.

expressing MHC II (Fig. 3a) was also similar among the four groups (6.27 ± 3.7 , 5.26 ± 0.99 , 5.9 ± 2.3 and $2.81.6$).

In the same groups, analysis of the total expression of IL-2R (Fig. 2b) and MHC II (Fig. 3b) among memory T cells demonstrated the following: the asthmatics (acute and stable) had an increased proportion of CD4⁺CD45RO⁺ T cells that expressed IL-2R compared with normals (16.1 ± 5.9 and 12.4 ± 2.1 vs. 4.9 ± 2.5 , $P < 0.05$). A gradient effect was observed in terms of increased IL-2R

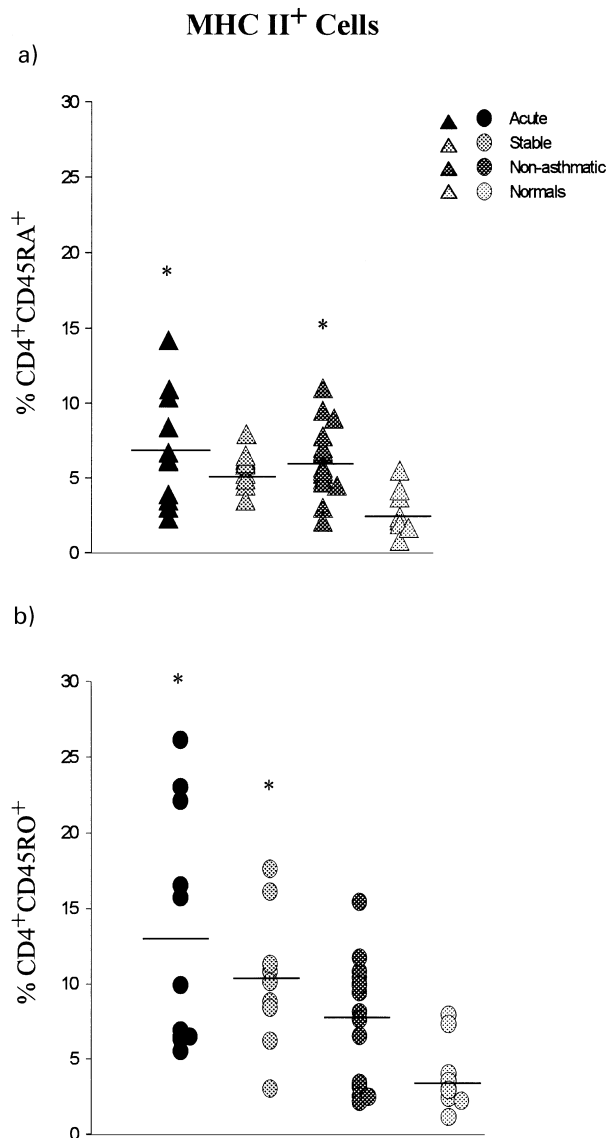


Fig. 3. (a) MHC II expression on naïve T helper cells from acute asthmatics ($n = 10$), stable asthmatics ($n = 9$), non-asthmatics ($n = 14$) and normals ($n = 7$). Shown are the percentages of CD4⁺CD45RA⁺ expressing MHC II molecules. The lines in each plot represent the mean for each group. * $P < 0.05$, acute asthmatics and non-asthmatics vs. normals. (b) MHC II expression on memory T helper cells from the same groups as described in Fig. 2(b). Shown are the percentages of CD4⁺CD45RO⁺ expressing MHC II molecules. The lines in each plot represent the mean for each group. $P < 0.05$, acute and stable asthmatics vs. normals.

expression in memory T helper cells in acute asthmatics relative to stable asthmatics and non-asthmatic atopics (16.1 ± 5.9 , 12.4 ± 2.1 and 10.1 ± 3.5). The proportion of memory T helper cells expressing MHC II was

Table 3. Coexpression of MHC II and IL-2R among naïve (CD45RA⁺) and memory (CD45RO⁺) T helper cells

Subjects	MHC II ⁺ IL-2R ⁺ †	MHC II ⁺ IL-2R ⁺ ‡	Subjects	MHC II ⁺ IL-2R ⁺ †	MHC II ⁺ IL-2R ⁺ ‡
Acute asthmatics			Non-asthmatics		
1A	nd	nd	1C	0.3	2.1
2A	0.5	0.6	2C	0.2	1.2
3A	< 0.1	4	3C	0.3	0.5
4A	1	10	4C	< 0.1	1.7
5A	1	1.3	5C	< 0.1	1.4
7A	< 0.1	3.1	6C	0.2	0.5
8A	< 0.1	2.3	7C	0.3	1.8
10A	0.3	2	8C	< 0.1	0.6
11A	nd	7.7	9C	0.9	0.5
12A	nd	5.6	10C	< 0.1	0.3
			11C	0.2	4.3
			12C	0.2	1.9
			13C	nd	nd
			14C	nd	nd
Mean	0.7	4.1*	Mean	0.3	1.4
Stable asthmatics			Normals		
1B	0.2	1.5	1D	< 0.1	1
2B	0.7	5.0	2D	nd	0.5
3B	0.4	3.0	3D	1	0.5
4B	0.3	nd	4D	< 0.1	0.7
5B	0.5	2.9	5D	< 0.1	2.2
6B	0.3	2.8	6D	< 0.1	1
7B	0.3	3.0	7D	< 0.1	0.8
8B	nd	3.1	8D	nd	nd
9B	nd		3.1		
Mean	0.4	3.1*	Mean	1.0	1.0

†% CD45RA⁺ T helper cells. ‡% CD45RO⁺ T helper cells. * $P < 0.05$ compared with the two non-asthmatic groups (atopic non-asthmatics and non-atopic normal controls). nd = not determined.

significantly higher in asthmatics (acute and stable) vs. normals (13.9 ± 8 and 10.6 ± 5 vs. 4.9 ± 2.5 , $P < 0.05$). For MHC II, there were no significant differences between asthmatics relative to non-asthmatic atopics (7.92 ± 4). Memory T helper cells in all four groups, as anticipated, had a higher proportion of IL-2R than the reciprocal naïve T helper population (note change of scale) ($P < 0.05$, Fig. 2a compared with Fig. 2b). In contrast, MHC II expression in memory T helper cells was higher only in both asthmatic groups and not in the non-asthmatic groups ($P < 0.05$, Fig. 3a compared with Fig. 3b).

Differential distribution of IL-2R and MHC class II expression among T helper subpopulations via four-colour flow cytometry analysis

To determine the proportion of CD4⁺CD45RA⁺ and CD4⁺CD45RO⁺ cells that expressed IL-2R and/or MHC II molecules, cells were gated on the CD4⁺CD45RO⁺ T

memory cells or CD4⁺CD45RA⁺ T naïve cells, and the proportion of IL-2R and MHC II was determined in a two-parameter histogram. A representative four-colour analysis of memory T helper cells from an acute asthmatic, a stable asthmatic, a non-asthmatic atopic and a non-atopic normal control is shown (Figs 4a–d, respectively). The CD4⁺CD45RO⁺ T memory cells positive for MHC-class II (y-axis) and IL-2R (x-axis) are shown, indicating the cells that coexpress IL-2R and MHC II.

Coexpression of IL-2R and MHC II on CD4⁺CD45RA⁺ (naïve) and CD4⁺CD45RO⁺ (memory) T cells

Table 3 shows the differences in coexpression of IL-2R and MHC II molecules among naïve and memory T helper cells of the asthmatics (acute and stable) vs. non-asthmatics and normals. Based on our four-colour analysis (Fig. 4) we determined the proportion of CD4⁺CD45RA⁺ and CD4⁺CD45RO⁺ T cells that coexpress IL-2R and MHC

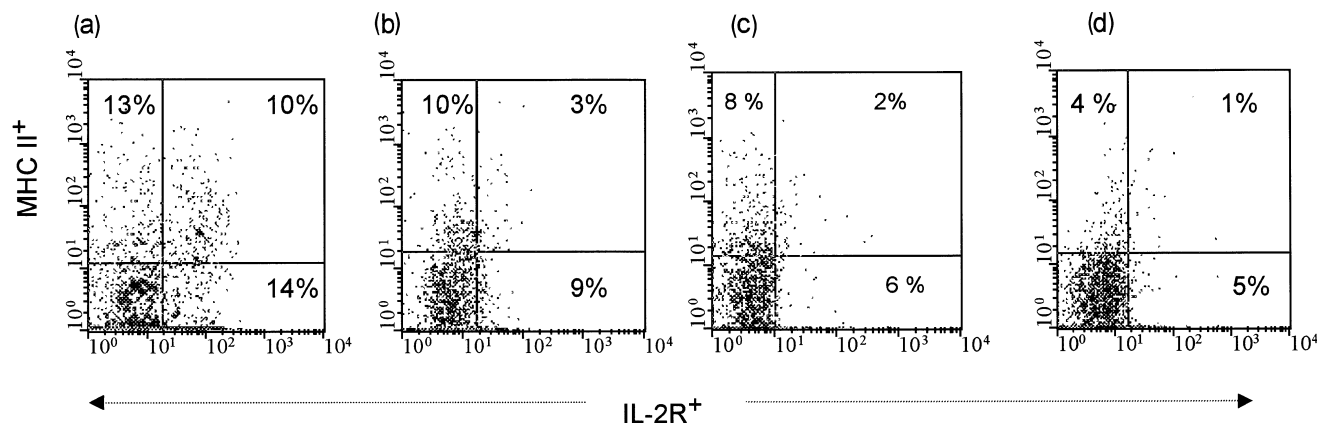


Fig. 4. Four-colour flow cytometry analysis of activated T cells. Shown is a representative experiment of the analysis of the expression of IL-2R and MHC II molecules on memory T helper cells. Samples obtained from an acute asthmatic (a), stable asthmatic (b), non-asthmatic (c) and a normal control (d) were gated on the $CD4^+CD45RO^+$ T cell population and were analysed. The y-axis represents the MHC II⁺ memory T helper cells and the x-axis represents the IL-2R⁺ cells. The upper right quadrant represents the memory T helper cells that coexpress MHC II molecule and the IL-2 receptor. The upper left quadrant and the lower right quadrant represent the populations that are either MHC II⁺ IL-2R⁻ or MHC II⁻ IL-2R⁺, respectively.

II (Table 3). The four groups have comparable percentages of $CD4^+CD45RA^+$ T cells coexpressing IL-2R and MHC II (0–1%).

In contrast, the $CD4^+CD45RO^+$ T cells of both acute and stable asthmatics had a significant increase in the proportion of cells coexpressing IL-2R and MHC II compared with the non-asthmatic atopics (mean SD 4.1 ± 3 and 3.1 ± 2 vs. 1.4 ± 1.1 , $P < 0.05$) and normals (4.1 ± 3 and 3.1 ± 2 vs. 1 ± 0.5 , $P < 0.05$).

Taken together, these data indicate that the T cells coexpressing IL-2R and MHC II from asthmatics are exclusively distributed among $CD4^+CD45RO^+$ subpopulations and represent approximately 20–30% of the activated cells among the acute or stable asthmatics (Figs 2b, 3b and Table 3).

Discussion

In order to determine the T cell activation phenotypes relevant to atopy or asthma, we analysed stable and acute atopic asthmatics in comparison with atopic non-asthmatics and non-atopic normal controls. We have characterized the T helper cell differential activation phenotype in peripheral blood among this spectrum of atopic subjects using multiple colour flow cytometry.

Our data confirm and expand previous reports that have identified activated $CD4^+$ T cells in the peripheral blood of asthmatics [3–5,16,17]. In addition to demonstrating increased proportion of activated T helper cells in peripheral blood, we determined that activated T helper cells from acute and stable atopic asthmatics are predominantly of the memory T helper phenotypes mainly

expressing IL-2R ($CD4^+CD45RO^+IL-2R^+$). These results are in agreement with our previous report analysing peripheral blood following allergen bronchoprovocation in asthmatics [11], demonstrating differential distribution of IL-2R and MHC II molecules among $CD4^+CD45RA$ (naïve) and $CD4^+CD45RO^+$ (memory) T cells.

In this study, simultaneous analysis of the expression of IL-2R and MHC II surface molecules on naïve and memory T helper cells showed that IL-2R and MHC II are usually expressed in non-overlapping subpopulations of T helper cells in non-asthmatic atopics and non-atopic normal controls. In contrast, asthmatics (acute and stable) have higher proportions of T helper cells coexpressing both IL-2R and MHC II, predominantly of the memory phenotype, compared with both non-asthmatic groups' T cells. We speculate that different activated subpopulations e.g. IL-2R⁺ MHC II⁻ or MHC II⁺ IL-2R⁻ memory T helper cells, may represent different stages of activation of the same cells or different populations of cells following distinct activation pathways.

Our analysis of $CD4^+$ T cells from peripheral blood of the acute asthmatics indicated upregulation of MHC II and increased expression of IL-2R on the memory T cells. However, further four-colour analysis revealed that these markers were coexpressed only in a small percentage of memory T helper cells and the majority were in distinct populations. In contrast, non-asthmatic atopics have a divergence from this phenotype, i.e. up-regulation of MHC II expression without an increase in IL-2R expression on their memory T cells, and minimal coexpression of these markers among the $CD4^+CD45RO$ T cells.

Although the function of MHC II expression on

activated T cells is unclear, reports suggest a role for negative signalling in T cell activation [18]. Interestingly MHC II antigens are upregulated on T cells by interferon- γ [19], which is known to play a down-regulatory effect on IgE synthesis [20]. We speculate whether CD4⁺MHC II⁺ T cells may play a role in the ability of non-asthmatic atopics to control pulmonary inflammation while maintaining an allergen-specific IgE immune response.

A relationship between activated T helper cells and asthma severity has been previously noted. Our results are consistent with prior reports [17] indicating that an increased proportion of CD4⁺IL-2R⁺ T cells is associated with asthma exacerbation and is correlated with low FEV₁ values (not shown). Improvement in FEV₁ after glucocorticoid therapy is associated with a decreased proportion and number of CD4⁺ T cells expressing IL-2R [17]. Of interest, while we observed a trend towards increased expression of CD4⁺MHC II⁺ molecules in acute compared with stable asthmatics, there were no differences with respect to non-asthmatic atopics. Thus, in this study, MHC II does not seem to be an activation marker associated with asthma activity. Our results are in agreement with previous reports that show no correlation between improvement of FEV₁ and changes in the proportion of CD4⁺ T cells expressing MHC II [4,17].

In other atopic diseases, such as atopic dermatitis, increased proportion of CD4⁺ T cells expressing IL-2R and MHC II has also been associated with severity of the disease [21]. In allergic rhinitis, activated T cells expressing IL-2R have been identified in the lamina propria [22] and in nasal submucosa, after local allergen challenge [23].

Our findings, and those of others [3,4], suggest that activated T helper cells expressing IL-2R are a common feature of asthma exacerbation. However, increased proportions of T helper cells expressing MHC II appear to be related to asymptomatic, non-asthmatic atopy and not to atopic asthma exacerbation, as shown in our analysis of non-asthmatic atopics. In addition to the naïve and memory function of the CD4⁺ T cells that express CD45RA vs. CD45RO isoforms, respectively [24–26], their specific roles remain unclear. However, prior reports indicate that each isoform may have distinct roles in the induction of IgE production [27]. Other potential roles for CD4⁺CD45RA⁺ T cells include a suppressor-inducer function [28]. Our findings indicate that activated T helper cells during asthma exacerbation are predominantly of the CD45RO phenotype, which is consistent with the notion that memory and/or primed T helper cells are included in the pool of cells bearing this surface marker [24–26].

Thus, our multiple marker flow cytometry *ex vivo* analysis indicates the presence of two activation phenotypes among T helper cells (IL-2R⁺ memory T helper cell and MHC II⁺ memory T helper cell) in atopic asthmatics

(acute and stable). The MHC II⁺ memory T helper phenotype is found in non-asthmatic atopics. There is a gradient effect: IL-2R⁺ memory T helper cells are present at higher proportions in acute asthmatics, followed by stable asthmatics, non-asthmatic atopics and normals, suggesting a correlation with clinical manifestation of atopy. We have identified a discrete group of activated memory T cells among asthmatics (both acute and stable) which have coexpression of IL-2R and MHC II molecules (CD4⁺CD45RO⁺IL-2R⁺MHC II⁺), indicating a significant phenotypic difference between atopic asthmatics and asymptomatic atopic subjects. Taken together, our data suggest that differences among T helper subpopulations may play a role in the association of atopy with asthma and warrant further investigation.

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