

# Activated peripheral blood CD4 and CD8 T-lymphocytes in child asthma: correlation with eosinophilia and disease severity

Gemou-Engesaeth V, Kay AB, Bush A, Corrigan CJ. Activated peripheral blood CD4 and CD8 T-lymphocytes in child asthma: correlation with eosinophilia and disease severity.

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There now exists compelling evidence of a role for cell-mediated immunity in the pathogenesis of adult asthma, but little information is available as to what extent this process participates in the pathogenesis of childhood asthma. We hypothesised that asthma in children is associated with the activation of T-lymphocytes whose products regulate, at least in part, the mobilisation and recruitment of eosinophils and thereby disease severity. Our aims, therefore, were to compare the expression of activation markers, including CD45 isoforms, on peripheral blood T-lymphocytes from asthmatic and non-asthmatic, allergic control children matched for age and atopic status, and to attempt to correlate the percentages of activated T-lymphocytes in the asthmatics with the numbers of peripheral blood eosinophils and with disease severity. Seventeen children with moderate to severe chronic asthma were compared with 8 non-asthmatic, allergic children matched for age and atopic status. Expression of the activation markers CD25, HLA-DR and VLA-1 and the CD45 isoforms CD45RA and CD45RO on peripheral blood CD4 and CD8 T-lymphocytes was measured using dual fluorescence flow cytometry. Peripheral blood eosinophils were measured using an automated laser cytometer. Asthma severity was assessed by a symptom score, spirometry and measurement of histamine PC<sub>20</sub>. The absolute numbers of eosinophils in the peripheral blood of the asthmatics were elevated as compared to the non-asthmatic, allergic controls ( $p < 0.01$ ), whereas the absolute numbers of both CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes were not significantly different. The percentages and absolute numbers of CD4<sup>+</sup> T-lymphocytes expressing CD25 and HLA-DR and CD8<sup>+</sup> T-lymphocytes expressing CD25 also were significantly elevated in the asthmatics as compared with the controls ( $p < 0.04$  in each case). However, the percentages and absolute numbers of CD4<sup>+</sup> T-lymphocytes expressing VLA-1 and CD8<sup>+</sup> T-lymphocytes expressing HLA-DR and VLA-1 did not significantly differ between the asthmatics and controls. Similarly, the percentages and absolute numbers of both CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes expressing the CD45 isoforms CD45RA and CD45RO were not significantly different in the asthmatics and controls. In the asthmatics, the numbers of peripheral blood eosinophils correlated with disease severity as measured by histamine PC<sub>20</sub> ( $p = 0.02$ ). The percentages of CD4<sup>+</sup> T-lymphocytes expressing HLA-DR correlated both with the numbers of peripheral blood eosinophils and with disease severity (histamine PC<sub>20</sub> ( $p < 0.03$ )). In addition, the percentages of CD8<sup>+</sup> T-lymphocytes expressing CD25 correlated positively with disease severity as measured by a symptom and therapy score ( $p = 0.03$ ). Finally, the percentages of CD8<sup>+</sup> T-lymphocytes expressing HLA-DR correlated with histamine PC<sub>20</sub> ( $p = 0.03$ ). These observations are consistent with the hypothesis that activated T-lymphocytes play a role in the pathogenesis of childhood asthma at least in part through their influence on eosinophil recruitment.

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There is increasing evidence that the bronchial mucosal inflammation characteristic of adult asthma represents a specialised form of cell-mediated immunity, in which cytokine products of activated T-lymphocytes orchestrate the accumulation and activation of granulocytes, particularly eosinophils (1). Eosinophils, through their release of basic proteins and lipid mediators, are strongly implicated in mucosal damage and the mechanisms which underlie bronchial hyperreactivity.

T-lymphocyte activation is accompanied by the enhanced expression of several surface markers including CD25 (the interleukin-2 receptor), HLA-DR (class II histocompatibility antigen) and  $\beta_1$ -integrins such as VLA-1 which are involved in binding of cells to extracellular matrix proteins. In addition, activated T-lymphocytes *in vitro* show a change in CD45 isoform expression from CD45RA (thought to be a marker of "naïve" T-lymphocytes) to CD45RO ("memory" T-lymphocytes) (2, 3). Recent immunocytochemical studies of bronchial biopsies taken from adult patients with asthma (4–7) showed that activated (CD25<sup>+</sup>) cells can be detected in the bronchial mucosa and their numbers can be correlated with both the numbers of local activated eosinophils and with disease severity. Double staining experiments demonstrated that the majority of these cells are T-lymphocytes (8). Activated (CD25<sup>+</sup>, HLA-DR<sup>+</sup>, VLA-1<sup>+</sup>) CD4 T-lymphocytes were also detected in the peripheral blood of adult patients with acute severe asthma (9, 10), and their numbers were reduced following therapy to a degree which correlated with clinical improvement. Finally, elevated numbers of activated CD4 and CD8 T-lymphocytes were observed in bronchoalveolar lavage (BAL) fluid of adult asthmatics, and correlations were again observed with the numbers of BAL fluid eosinophils and with disease severity (11, 12).

While these studies provide compelling evidence for a role for cell-mediated immunity in the pathogenesis of adult asthma, there is at present very little information as to what extent these processes participate in the pathogenesis of asthma in children. We hypothesised that asthma in children is associated with the activation of T-lymphocytes whose products regulate, at least in part, the mobilisation and recruitment of eosinophils and thereby disease severity. Our aims, therefore, were to compare the expression of activation markers, including CD45 isoforms, on peripheral blood T-lymphocytes from asthmatic and non-asthmatic, allergic control children matched for age and atopic status, and to attempt to correlate the percentages of activated T-lymphocytes in the asthmatics with the numbers of peripheral blood eosinophils and with disease severity.

## Material and methods

### Patients

Seventeen children between 7 and 16 years of age with moderate to severe chronic asthma and eight normal age-matched controls were included in this study (Table 1). The children with asthma fulfilled the criteria for the definition according to the American Thoracic Society (13). Sixteen of the asthmatics were taking inhaled glucocorticoids at the time of the study (0.4 to 1.6 mg divided twice daily) while the remaining patient was taking inhaled sodium cromoglycate (40 mg divided four times daily). In addition, all asthmatics were taking inhaled  $\beta_2$ -agonists as required. The nature of this prophylactic therapy was in accordance with disease severity as recommended in a current clinical statement (14). None of the patients or controls had had any acute infection in the three weeks prior to the study and none had taken systemic glucocorticoids in the preceding two months. All patients and controls were characterised for their atopic status. Subjects were designated atopic if they demonstrated one or more positive skin prick tests, defined as a wheal reaction at 15 min, at least as large as the positive histamine control in the presence of a negative medium control, and/or one or more positive RAST tests ("Phadebas" RAST, Pharmacia, Uppsala, Sweden) to a range of eight common aeroallergens (house dust mite – *Dermatophagoides pteronyssinus*, grass pollen *Phleum pratense*, mugwort – *Artemisia vulgaris*, three trees, silver birch – *Betula verrucosa*, cat dander, dog dander and *Aspergillus fumigatus*) ("Soluprick", ALK, Hørsholm, Denmark). Five of the asthmatic patients and three of the control patients had allergic rhinitis, while three of the asthmatics and three of the controls had mild eczema.

The study was carried out in accordance with the amended Declaration of Helsinki, and ethical approval was granted by the Royal Brompton National Heart & Lung Hospital Ethics Committee. All sub-

Table 1. Demographics and therapy of asthmatic and non-asthmatic, allergic control children

| Variable             | Asthmatics                                                      | Controls                       |
|----------------------|-----------------------------------------------------------------|--------------------------------|
| No. of patients      | 17                                                              | 8                              |
| Gender               | 14M, 3F                                                         | 3M, 5F                         |
| Age <sup>1</sup>     | 10 (7–15)                                                       | 10 (7–16)                      |
| Therapy <sup>2</sup> | 16/17 inhaled BDP<br>(0.4–1.6 mg)<br>1/17 inhaled SC<br>(40 mg) | 1/8 intranasal SC<br>(41.6 mg) |
| Atopy                | 16/17                                                           | 7/8                            |

<sup>1</sup> = median and range (yr); <sup>2</sup> = BDP = beclomethasone dipropionate; SC = sodium cromoglycate; doses represent total daily doses which were divided twice (BDP) or four times daily (SC). All asthmatic patients were taking inhaled  $\beta_2$ -agonists "as required".

jects and their parents were given written information concerning the purpose of the study and the trial procedure, and written consent was obtained from parents allowing participation of their children.

#### Clinical protocol

Asthmatic patients attended the hospital clinic between 10 a.m. and midday, two to four hours after taking any inhaled medication. Patients (with the help of their parents as necessary) completed a disease severity score based on that described by Aas (15) which was previously shown to correlate with the severity of bronchial inflammation in adult asthmatics (6). Lung function was measured using a compact spirometer (Vitalograph, England) and bronchial reactivity measured using a Wright nebuliser and a tidal breathing method (16). The nebuliser output was standardised at 0.13 ml/min and histamine inhaled for 2 min at each doubling concentration. The results were expressed as the provocative concentration of histamine that caused a 20% decrease in FEV<sub>1</sub> compared with the FEV<sub>1</sub> after an initial inhalation challenge with 0.9% sodium chloride (histamine PC<sub>20</sub>). Skin prick tests were performed to extracts of 8 common aeroallergens. Prior to lung function testing, 10–15 ml of venous blood were collected into preservative-free heparin for isolation of mononuclear cells. In addition, serum was isolated for RAST testing and a full blood count including differential leukocyte count performed using an automated laser cytometer. Peripheral venous blood for isolation of mononuclear cells, serum for RAST testing and full blood count and leukocyte differential were similarly obtained from the control patients. Skin tests, histamine PC<sub>20</sub> and spirometry were also performed on the control patients.

#### Isolation of peripheral blood mononuclear cells

For isolation of peripheral blood mononuclear cells, heparinised peripheral venous blood was mixed with an equal volume of RPMI-1640 medium (Gibco, Paisley, Scotland). Aliquots (30 ml) were layered onto aliquots (20 ml) of Ficoll-Paque (Pharmacia) in sterile conical tubes ("Falcon", Becton Dickinson, Cowley, England). After centrifuging at 1000 g for 20 min at 20°C, peripheral blood mononuclear cells were removed from the plasma/Ficoll interface using gentle suction, transferred to sterile polystyrene universal containers and washed twice with RPMI-1640. Cells were subsequently maintained at 4°C during further experimental manipulations.

#### Flow cytometry

Peripheral blood mononuclear cells were double-stained for T-lymphocyte phenotypic and activation markers using the following murine IgG monoclonal antibodies: anti-CD4 and anti-CD8 coupled to phycoerythrin (PE), anti-CD45RA and irrelevant IgG1 control coupled to fluorescein isothiocyanate (FITC) (Becton Dickinson); anti-CD25, anti-HLA-DR and anti-CD45RO coupled to FITC (Dako, High Wycombe, England); anti-VLA-1 coupled to FITC (T-cell Sciences, Cambridge, MA, USA). Aliquots of 500,000 cells in 50 µl of phosphate buffered saline containing 0.1% wt/vol sodium azide and 0.5% wt/vol bovine serum albumin ("PAB" medium) were used for staining. All steps were performed at 4°C. To measure the expression of CD25, HLA-DR, VLA-1, CD45RA and CD45RO on CD4 and CD8 T-lymphocytes, aliquots of cells were incubated (10 min) with 10 µl of one of each of the FITC-labelled antibodies (including the irrelevant IgG1-FITC control antibody) along with 10 µl of anti-CD4-PE or anti-CD8-PE. After washing once in "PAB" medium, the cells were resuspended in 500 µl of phosphate buffered saline alone and maintained at 4°C pending flow cytometric analysis.

Flow cytometry was performed on a FACS Analyzer equipped with a FACSLite argon laser (Becton Dickinson) and calibrated using standard techniques. For each sample 10,000 PBMC were counted after gating on volume/90° light scatter plots to exclude contaminating erythrocytes and debris. Further gates were then set on red fluorescence (PE) histograms to isolate CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes. Monocytes, which stain weakly with anti-CD4 and NK cells, which stain weakly with anti-CD8, were excluded from analysis. Green fluorescence (FITC) histograms of these phenotypic subsets were then analysed to detect surface expression of activation markers. The percentages of positively stained cells were obtained by subtracting the fluorescence histogram of cells stained with the control IgG1-FITC antibody from those of the specifically stained cells.

#### Statistical analysis

Comparisons of unpaired data were made using the Mann Whitney U test. Probability values for linear correlations were calculated from the Spearman rank correlation co-efficient. The Bonferroni correction was applied where appropriate. Analysis was performed using Minitab software (Minitab, State College, USA). All ranges are quoted as the median and semi-interquartile range.

Table 2. Lung function tests and peripheral blood eosinophil and mononuclear cell counts of asthmatic and non-asthmatic, allergic control children

| Variable <sup>1</sup>                       | Asthmatics      | Controls           | p value <sup>2</sup> |
|---------------------------------------------|-----------------|--------------------|----------------------|
| FEV <sub>1</sub>                            | 74 (68.5–81)    | 86.5 (86.75–95.75) | 0.005                |
| FEF <sub>25</sub>                           | 71 (59–81)      | 102.5 (79.5–109.8) | 0.02                 |
| FEF <sub>50</sub>                           | 59 (48–71)      | 86.5 (78.5–113.5)  | 0.005                |
| FEF <sub>75</sub>                           | 42 (33–72)      | 79.5 (62.7–111.5)  | 0.02                 |
| Histamine PC <sub>20</sub><br>(mg/ml)       | 1.3 (0.45–3.80) | 9.6 (3.0–32.0)     | 0.01                 |
| Total eosinophils<br>( $\times 10^{-9}/l$ ) | 0.7 (0.45–0.9)  | 0.25 (0.125–0.45)  | 0.01                 |
| Total MNC<br>( $\times 10^{-9}/l$ ) (NS)    | 3.2 (2.55–3.5)  | 2.65 (2.05–3.56)   | 0.52                 |

<sup>1</sup> = lung function tests are expressed as a percentage of the predicted value (median and semi-interquartile range); MNC = mononuclear cells; FEV<sub>1</sub> = forced expiratory volume in 1 s; FEF<sub>25</sub> = forced expiratory flow at 25% of vital capacity, etc.; <sup>2</sup> = Mann-Whitney U test, asthmatics compared with controls; NS = not significant.

## Results

Whereas the asthmatic children showed evidence of significant airways obstruction and bronchial hyper-reactivity to histamine challenge (median histamine PC<sub>20</sub> = 1.3 mg/ml at the time of blood sampling), corresponding measurements in the atopic non-asthmatic control children encompassed the normal range (Table 2). The total numbers of peripheral blood eosinophils in the asthmatic children were significantly elevated as compared with the controls ( $p=0.01$ , Table 2), but there were no significant differences in the numbers of peripheral blood

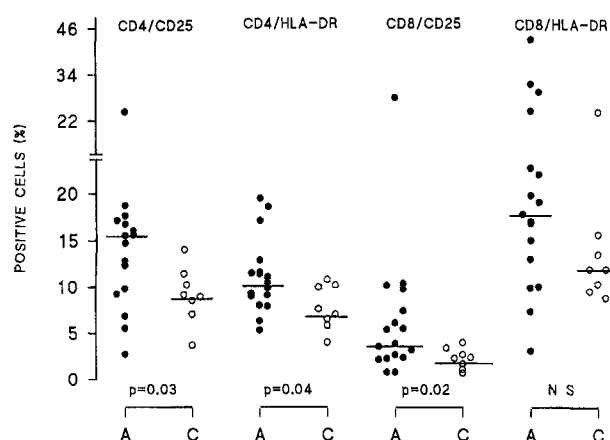


Fig. 1. Percentages of CD4 and CD8 peripheral blood T-lymphocytes expressing the activation markers CD25 and HLA-DR in a group of 17 asthmatic children (A) and 8 non-asthmatic controls (C). Bars correspond to median values. Statistical comparisons (asthmatics compared with controls in each case) were made using the Mann-Whitney U test. NS = not significant.

mononuclear cells (lymphocytes and monocytes) (Table 2).

The percentages of both CD4 and CD8 T-lymphocytes comprising the total peripheral blood mononuclear cells were not significantly different in the asthmatics as compared with the controls (Table 3). The absolute numbers of these cells per unit volume of blood were also not significantly different (Table 4). The percentages of peripheral blood CD4 T-lymphocytes expressing the activation markers CD25 and HLA-DR were significantly elevated in the asthmatics as compared with the controls ( $p=0.03$ ,  $0.04$  respectively) (Table 3, Fig. 1). Similarly, the per-

Table 3. Percentages of CD4 and CD8 T-lymphocytes and percentages of CD4 and CD8 T-cells expressing activation markers in the peripheral blood of asthmatic and non-asthmatic, allergic control children

|                                                                  | Asthmatics |           | Controls |           | p value <sup>1</sup> |
|------------------------------------------------------------------|------------|-----------|----------|-----------|----------------------|
|                                                                  | Median     | SIQR      | Median   | SIQR      |                      |
| CD4 and CD8 T-cells (% total peripheral blood mononuclear cells) |            |           |          |           |                      |
| CD4 <sup>+</sup>                                                 | 32.5       | 28.2–38.4 | 35.3     | 30.1–42.2 | NS                   |
| CD8 <sup>+</sup>                                                 | 18.4       | 13.8–22.7 | 21.3     | 17.8–23.9 | NS                   |
| Activation markers (% total CD4 or CD8 T-lymphocytes)            |            |           |          |           |                      |
| CD4/CD25 <sup>+</sup>                                            | 15.6       | 9.6–17.5  | 9.1      | 7.5–11.2  | 0.03                 |
| CD4/HLA-DR <sup>+</sup>                                          | 10.6       | 8.6–12.4  | 7.4      | 6.1–10.3  | 0.04                 |
| CD4/VLA-1 <sup>+</sup>                                           | 2.6        | 2.0– 3.2  | 2.0      | 1.2– 3.0  | NS                   |
| CD4/CD45RA <sup>+</sup>                                          | 70.2       | 63.4–76.0 | 72.4     | 65.9–78.5 | NS                   |
| CD4/CD45RO <sup>+</sup>                                          | 56.4       | 43.0–74.1 | 42.8     | 36.5–56.0 | NS                   |
| CD8/CD25 <sup>+</sup>                                            | 4.0        | 2.5– 8.7  | 2.4      | 1.9– 2.7  | 0.02                 |
| CD8/HLA-DR <sup>+</sup>                                          | 17.9       | 11.6–26.0 | 12.0     | 9.8–15.2  | NS                   |
| CD8/VLA-1 <sup>+</sup>                                           | 3.4        | 2.4–12.3  | 3.1      | 2.0– 5.3  | NS                   |
| CD8/CD45RA <sup>+</sup>                                          | 90.6       | 87.0–93.0 | 85.5     | 82.4–91.2 | NS                   |
| CD8/CD45RO <sup>+</sup>                                          | 41.4       | 34.0–56.0 | 35.4     | 31.3–39.6 | NS                   |

<sup>1</sup> = Mann-Whitney U test, asthmatics compared with controls; NS = not significant; SIQR = semi-interquartile range.

Table 4. Absolute counts of total CD4 and CD8 T lymphocytes and CD4 and CD8 T lymphocytes expressing activation markers in the peripheral blood of asthmatic and non-asthmatic allergic control children

|                                                                 | Asthmatics |          | Controls |          | p value <sup>1</sup> |
|-----------------------------------------------------------------|------------|----------|----------|----------|----------------------|
|                                                                 | Median     | SIQR     | Median   | SIQR     |                      |
| CD4 and CD8 T-cells (cells/mm <sup>3</sup> whole blood)         |            |          |          |          |                      |
| CD4 <sup>+</sup>                                                | 800        | 700–1050 | 800      | 725–1000 | NS                   |
| CD8 <sup>+</sup>                                                | 800        | 600– 850 | 800      | 600– 875 | NS                   |
| Activation markers (positive cells/mm <sup>3</sup> whole blood) |            |          |          |          |                      |
| CD4/CD25 <sup>+</sup>                                           | 140        | 73–163   | 92       | 47–101   | 0.03                 |
| CD4/HLA-DR <sup>+</sup>                                         | 114        | 70–137   | 73       | 38– 93   | 0.01                 |
| CD4/VLA-1 <sup>+</sup>                                          | 21         | 16– 26   | 16       | 10– 24   | NS                   |
| CD4/CD45RA <sup>+</sup>                                         | 562        | 507–608  | 579      | 527–628  | NS                   |
| CD4/CD45RO <sup>+</sup>                                         | 451        | 344–593  | 342      | 297–448  | NS                   |
| CD8/CD25 <sup>+</sup>                                           | 36         | 20– 91   | 17       | 5– 34    | 0.03                 |
| CD8/HLA-DR <sup>+</sup>                                         | 147        | 64–255   | 82       | 54–174   | NS                   |
| CD8/VLA-1 <sup>+</sup>                                          | 27         | 19– 98   | 25       | 16– 42   | NS                   |
| CD8/CD45RA <sup>+</sup>                                         | 725        | 696–744  | 684      | 659–730  | NS                   |
| CD8/CD45RO <sup>+</sup>                                         | 331        | 272–448  | 283      | 250–317  | NS                   |

<sup>1</sup> = Mann-Whitney U test, asthmatics compared with controls; NS = not significant; SIQR = semi-interquartile range.

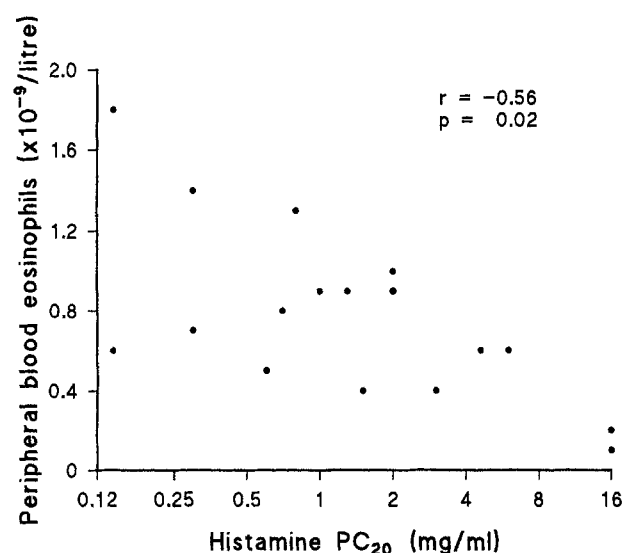


Fig. 2. Correlation (Spearman rank correlation co-efficient) of histamine PC<sub>20</sub> (mg/ml) with the numbers of peripheral blood eosinophils in a group of 17 asthmatic children.

percentages of CD8 T-lymphocytes expressing CD25 were significantly elevated in the asthmatics ( $p = 0.02$ ), and while increased percentages of CD8 T-lymphocytes expressing HLA-DR were observed in the majority of the asthmatic children as compared with the controls, this difference did not attain statistical significance (Table 3, Fig. 1). Similar differences were observed on comparing the absolute numbers of CD4 and CD8 T-lymphocytes expressing CD25 and HLA-DR in the peripheral blood of the asthmatics and controls (Table 4). The absolute numbers of peripheral blood CD4 and CD8 T-lymphocytes expressing the activation marker VLA-1 and the CD45 isotypes CD45RA and CD45RO did not differ in the asthmatic and non-asthmatic, allergic control children (Tables 3, 4).

Considering the asthmatic children as a group, a negative correlation was observed between the absolute numbers of eosinophils in the peripheral blood of these patients and their histamine PC<sub>20</sub> (whose absolute value is inversely proportional to the degree

of bronchial hyperreactivity) ( $p = 0.02$ , Fig. 2). In addition, the percentages of both CD4 and CD8 peripheral blood T-lymphocytes expressing the activation markers CD25 and HLA-DR in these patients, which were elevated as compared with the non-asthmatic controls, in each case correlated positively with the numbers of eosinophils in their peripheral blood and their disease severity as measured by a symptom and medication score, and negatively with histamine PC<sub>20</sub>, although not all of these correlations attained statistical significance (Table 5, Fig. 3). No significant correlations were observed between the percentages of peripheral blood CD4 and CD8 T-lymphocytes expressing CD25 and HLA-DR and the extent of airways obstruction as measured by FEV<sub>1</sub> (forced expiratory volume in 1 s) (data not shown).

## Discussion

The data presented in this paper represent the first demonstration, to our knowledge, that elevated numbers of activated CD4 and CD8 T-lymphocytes can be detected in the peripheral blood of children in association with asthma. These changes may reflect a "spill-over" effect whereby activated T-lymphocytes in the bronchial mucosa in asthma migrate into the peripheral circulation. The fact that the percentages and absolute numbers of peripheral blood CD4 T-lymphocytes expressing the activation markers CD25 and HLA-DR, and of CD8 T-lymphocytes expressing CD25 were elevated in asthmatics as compared with a control group well matched for age and atopic status suggests that T-lymphocyte activation may be physiologically associated with asthma and not simply with the atopic state itself (although it does not exclude the possibility that a lesser degree of peripheral blood T-lymphocyte activation might be associated with atopy and atopic diseases such as atopic rhinitis). It was for this reason that we compared atopic asthmatics with atopic, and not non-atopic, controls.

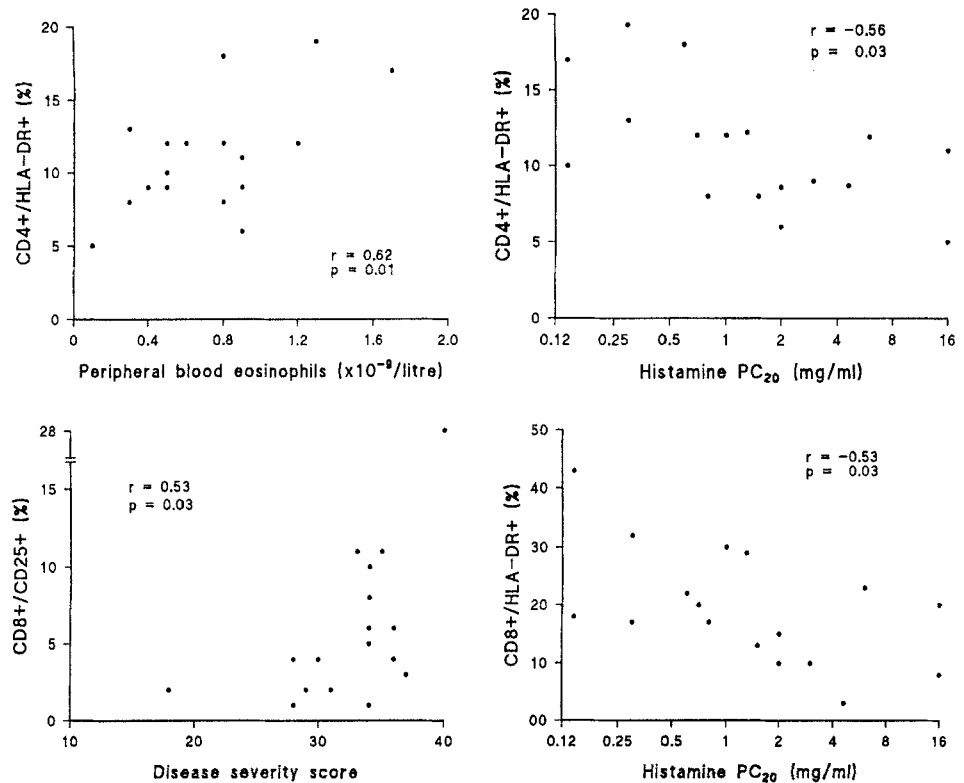
One principal difference between the asthmatic and control groups is that the former were being treated at the time of blood sampling with inhaled glucocorticoids or sodium cromoglycate, raising the possibility that the observed T-lymphocyte activation might have been caused by the therapy itself. Since we have shown previously (9, 10) that glucocorticoid therapy of adult patients with acute severe asthma was associated with a reduction in the extent of peripheral blood T-lymphocyte activation to a degree which correlated with the degree of clinical improvement, it seems more likely that the T-lymphocyte activation observed in our children with asthma was not a consequence of glucocorticoid therapy but rather reflected ongoing T-lymphocyte activation. The possibility of short-term variability

Table 5. Correlation of the percentages of peripheral blood CD4 and CD8 T-lymphocytes expressing the activation markers CD25 and HLA-DR with the numbers of peripheral blood eosinophils and disease severity (severity score and histamine PC<sub>20</sub>) in 17 asthmatic children

| Activated T-cells       | Eosinophils    |                | Histamine PC <sub>20</sub> |      | Severity Score |      |
|-------------------------|----------------|----------------|----------------------------|------|----------------|------|
|                         | r <sup>1</sup> | p <sup>1</sup> | r                          | p    | r              | p    |
| CD4/CD25 <sup>+</sup>   | 0.25           | NS             | -0.18                      | NS   | 0.09           | NS   |
| CD4/HLA-DR <sup>+</sup> | 0.62           | 0.01           | -0.56                      | 0.03 | 0.12           | NS   |
| CD8/CD25 <sup>+</sup>   | 0.42           | NS             | -0.42                      | NS   | 0.53           | 0.03 |
| CD8/HLA-DR <sup>+</sup> | 0.37           | NS             | -0.53                      | 0.03 | 0.13           | NS   |

<sup>1</sup> r = rank correlation coefficient; p = statistical significance of rank correlations. Statistically significant correlations are illustrated in Fig. 3; NS = not significant.

Fig. 3. Correlations (Spearman rank correlation co-efficients) of the percentages of peripheral blood CD4 T-lymphocytes expressing the activation marker HLA-DR with the numbers of peripheral blood eosinophils (*top left*) and histamine PC<sub>20</sub> (*top right*), the percentages of CD8 T-lymphocytes expressing the activation marker CD25 with disease severity score (*bottom left*) and the percentages of CD8 T-lymphocytes expressing HLA-DR with histamine PC<sub>20</sub> (*bottom right*) in a group of 17 asthmatic children (see also Table 5).



in the expression of T-lymphocyte activation markers in relation to inhaled glucocorticoid therapy was minimised by standardising the time of blood sampling in relation to the timing of this therapy.

Because we had hypothesised that asthma in childhood is associated with the activation of T-lymphocytes whose products regulate, at least in part, the mobilisation and recruitment of eosinophils and thereby disease severity, we sought a possible correlation in the asthmatic patients between the numbers of peripheral blood eosinophils and disease severity (Fig. 2). The close correlation observed between these two variables supports our hypothesis and is in line with similar findings in adult asthmatics (17). We also sought possible correlations in the asthmatics between the percentages of CD4 and CD8 T-lymphocytes expressing the activation markers CD25 and HLA-DR, which we found to be elevated in these patients as compared with controls, and measurements of disease severity (symptom and medication scores and histamine PC<sub>20</sub>) and the numbers of peripheral blood eosinophils (Table 5, Fig. 3). In fact, the percentages of both CD4 and CD8 peripheral blood T-lymphocytes expressing CD25 and HLA-DR in each case correlated positively with the numbers of peripheral blood eosinophils and disease severity score and negatively with histamine PC<sub>20</sub>. The fact that these correlations did not all attain statistical significance is perhaps not surprising in view of the relatively narrow spec-

trum of disease severity and the possible confounding effect of variable doses of inhaled glucocorticoid therapy.

The activation status of peripheral blood CD4 and CD8 T-lymphocytes in adult asthmatics has been the subject of several recent studies (9–12, 18, 19). In these studies, the pattern of expression of activation markers and the phenotypes of the T-lymphocytes expressing these markers varied. These different observations may reflect variability in the pathogenesis of the disease, particularly the presence or absence of co-existing atopy, variable "spill-over" of activated T-lymphocytes from the bronchial mucosa into the peripheral circulation and confounding influences such as comparisons with differing control groups and the possible effects of therapy. One particular feature relevant to childhood asthma may be the increased incidence of coexisting viral infections in exacerbations of the disease, which might result in the activation of CD8 T-cells in addition to CD4 T-cells.

The physiological significance of the expression of the CD45 isoforms CD45RA and CD45RO appears to be related to previous antigenic exposure. CD45RA and CD45RO are expressed on peripheral blood T-lymphocytes in a reciprocal fashion. CD45RA "high" cells are CD45RO "low", and vice versa. CD45RO "high" T-lymphocytes in the laboratory show enhanced help for immunoglobulin synthesis, enhanced responses to recall antigens and

greater cytotoxicity as compared to CD45RA "high" cells (3). For these reasons, CD45RO "high" cells are thought to be "memory" cells which have previously been activated by exposure to specific antigen. Consistent with this hypothesis is the observation that the numbers of T-cells expressing CD45RO in the peripheral blood increase with age, whereas the numbers of T-cells expressing CD45RA decrease. Since in the present study the asthmatic and non-asthmatic control children were matched for age, this variability would not have confounded the data. In a group of severe asthmatics, it was shown that, compared with both atopic and non-atopic, non-asthmatic controls, these patients had elevated percentages of peripheral blood CD4 T-lymphocytes, but not CD8 cells expressing the "memory" CD45 isoform CD45RO and reduced percentages of CD4 but not CD8 T-cells expressing the reciprocal "naive" isoform CD45RA (18). This observation is consistent with the hypothesis that severe exacerbations of asthma are associated with the mobilisation of "memory" CD4 T-cells which are in the process of being activated by specific antigen. The fact that such changes were not observed in the present study may reflect the fact that these asthmatic children had milder disease in which "spill-over" of activated T-lymphocytes into the peripheral blood is less marked. In addition, it is possible to speculate that, in children, who have had less exposure to antigens including allergens, the "memory" T-cell response may be less marked. These hypotheses are also consistent with the demonstration (20) that the percentages of peripheral blood CD4 T-lymphocytes expressing CD45RA in a group of children with mild asthma were not significantly different from those in groups of atopic non-asthmatic patients and patients with atopic dermatitis. All three groups did however show increased expression of CD45RA as compared with non-atopic controls. These data suggest that, at least in children, elevated percentages of peripheral blood CD4 T-lymphocytes expressing CD45RA might be associated with the atopic state rather than specifically with asthma, although why elevated numbers of "naive" CD4 T-lymphocytes should be present in the peripheral blood of children with atopy is not clear.

In summary, we have demonstrated that activated CD4 and CD8 T-lymphocytes can be detected in the peripheral blood of children with asthma, and we have provided circumstantial evidence to suggest that their products might regulate eosinophil mobilisation and asthma severity. In this sense, the pathogenesis of childhood asthma shows a number of similarities with that of the adult disease. Our observations suggest that novel therapeutic strategies directed at inhibition of T-lymphocyte function might

have an important role to play in the management of asthma in children and adults alike.

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