

Lymphocyte activation by house dust allergen in asthma: analysis with monoclonal antibodies

J. KUS

Department of Tuberculosis and Pulmonary Diseases, Institute of Tuberculosis and Chest Diseases, Warsaw, Poland

Summary

Peripheral blood mononuclear cell (MNC) response to house dust (HD) stimulation in cultures was studied in a group of 35 subjects with asthma and a multiple positive skin test (ST) reaction to inhalant allergens including HD, and in 19 healthy controls. The MNC response to allergen stimulation was assessed by interleukin-2 receptor (IL-2R) expression identified with an anti-Tac monoclonal antibody. Lymphocyte subpopulations of baseline and cultured cells were also analysed. The percentage of IL-2R presenting cells increased significantly in HD-driven cultures in the asthma group compared to controls. The increase in proportion of IL-2R⁺ cells was closely related to the increase in CD4⁺ percentage of cultured cells and was accompanied by a decrease in proportion of CD8⁺ lymphocytes and monocytes/macrophages, suggesting that HD-activated cells were CD4⁺ lymphocytes. Lymphocyte activation, measured by IL-2R expression, was significantly higher in the group of asthmatics, and positive dual (early and late) skin reaction to HD, as compared to those with single early reaction.

Clinical and Experimental Allergy, Vol. 20, pp. 165–170. Submitted 6 July 1989; revised 15 September 1989; accepted 18 September 1989.

Introduction

The abnormalities in the immune response in allergic bronchial asthma are still not clearly understood. The production of class IgE antibodies specific for inhalant allergens with subsequent release of immediate hypersensitivity mediators during the antigen–antibody reaction is the mechanism of immediate reaction [1] but it is not sufficient to be responsible for late asthmatic responses and long-lasting or permanent symptoms of asthma. It has been shown that a considerable proportion of allergic asthmatics presented late [2], delayed [3], and early phase skin reactions during intradermal allergen skin testing. Peripheral blood lymphocytes from patients with allergic asthma exposed *in vitro* to inhalant allergen recognized the allergen and responded to it by blast transformation and lymphokine production [4,5]. Recent studies of bronchoalveolar lavage (BAL) lymphocytes, recovered after bronchial allergen challenge [6], and intradermal infiltrate of the late-phase allergic skin reaction [7], showed predominantly CD4-positive lymphocyte subpopulations, supporting the theory of T-lymphocyte

involvement in the pathogenesis of allergic bronchial asthma.

In the present study monoclonal antibodies and indirect immunofluorescence techniques were used to define T-lymphocyte subpopulations and interleukin 2 receptor (IL-2R) expression on lymphocytes cultured *in vitro* in the presence of the house dust (HD) allergen. We compared the results in allergic asthmatic subjects and healthy controls and analysed the relation between *in vitro* lymphocyte response and intradermal skin test results.

Material and methods

Subjects

The study group consisted of 35 subjects with asthma and positive allergic history attending the out-patient Clinic of Institute of Tuberculosis and Chest Diseases in Warsaw for 1–10 yr. There were 21 males and 14 females with a mean ($\pm$ s.d.) age of 36.5 yr ($\pm$ 12.9). All subjects were intradermally skin tested by a trained nurse during routine diagnostic procedures and all showed multiple positive reactions to common inhalant allergens. Commercially available allergen extracts (Pasteur Institute, Paris, France) were injected (0.02 ml) on the forearms and the results were assessed after 20 min for immediate

Correspondence: Dr J. Kus, Department of Tuberculosis and Pulmonary Diseases, Institute of Tuberculosis and Chest Diseases, ul. Plocka 26, 01-138, Warsaw, Poland.

reaction and after 24 hr for late-phase cutaneous reaction (LCR). For the immediate reaction the results were graded 0 if there was no weal, + (doubtful) for a weal less than 10 mm in diameter, ++ (positive) for less than 20 mm, +++ (strongly positive) for less than 30 mm and ++++ (severe) for more than 30 mm. For the LCR the following gradation was used: 0 if the diameter of induration was less than 5 mm, + (doubtful) for induration less than 10 mm in diameter, ++ (positive) for less than 20 mm, +++ (strongly positive) for less than 30 mm and ++++ (severe) for more than 30 mm. The criteria for inclusion of the subject into the study were: (1) positive immediate skin-test reaction to HD; (2) no symptoms of asthma at the time of the study; (3) no systemic therapy with steroids; and (4) no previous hyposensitization therapy. Twenty-nine subjects had asthma alone and six had asthma with allergic rhinitis. None had allergic skin disease. Seventeen were treated with β_2 agonist inhalations, eight with β_2 agonist + beclomethasone dipropionate inhalations and the remaining 10 were on β_2 agonist + beclomethasone dipropionate inhalations + oral theophylline.

Controls

The control group comprised 19 healthy subjects recruited from the medical personnel of the Institute. All these individuals had negative allergic history and were not skin tested. The mean age in the control group was 35.1 years (± 9.1).

The study was approved by the Ethical Committee of the Institute of Tuberculosis and Chest Diseases in Warsaw.

Cultures of mononuclear cells

Venous blood was taken between September 1988 and March 1989 and mononuclear cells (MNC) were isolated by the standard technique of Boyum [8] with Ficoll-sodium diatrizoate (specific gravity 1.077) gradient centrifugation under sterile conditions. The isolated cells were washed and resuspended in RPMI 1640 medium (Gibco, Grand Island, U.S.A.) at a density of 2×10^6 cells per ml. The viability of cells checked by trypan blue exclusion was greater than 97%. The cells were cultured in 2 ml samples in RPMI 1640 supplemented with gentamycin (20 $\mu\text{g}/\text{ml}$), nystatin (20 U/ml), L-glutamine (2 mM/ml) and 10% heat-inactivated foetal calf serum (Gibco). Twenty microlitres of house dust allergen 1:500 (w/v; Pasteur Institute) were added to the allergen-stimulated cultures. Positive control samples were cultured with PHA (10 $\mu\text{l}/\text{ml}$; Difco, Detroit, U.S.A.) and negative control samples were cultured in medium supplemented with antibiotics and

foetal calf serum. Allergen-stimulated and negative control samples were cultured for 7 days and PHA-stimulated samples for 48 hr. All cultures were carried out at 37°C in humidified air containing 5% CO_2 . The antigen concentration and incubation period were determined by preliminary experiments.

Immunofluorescence staining with monoclonal antibodies

MNC separated from the peripheral blood and recovered from cultures were stained with monoclonal antibodies specific for monocytes, IL-2R and lymphocyte subpopulations. The following reagents were used: OKT3 (anti-CD3), OKT4 (anti-CD4), OKT8 (anti-CD8) (Ortho Pharmaceutical Corp., Raritan, U.S.A.) specific for mature T lymphocytes, helper/inducer and suppressor/cytotoxic lymphocytes, respectively; Mo2 (Coulter Inc., Hialeah, U.S.A.) specific for monocytes, and ClonabIL-2R (anti-Tac) monoclonal antibody (Biotest Diagnostics, Frankfurt, FRG) specific for IL-2R. Goat anti-mouse immunoglobulin labeled with fluorescein (Coulter Inc.) was used as a second antibody. The staining procedure was that recommended by the producers of monoclonal reagents and which has previously been described in detail [9,10].

The results were read under a Leitz fluorescence microscope equipped with phase contrast. Two hundred cells were counted per slide and the percentage of positive cells was calculated. The results are expressed as mean $\pm$ s.d.

Statistics

Student's *t*-test for unpaired data was used to compare the results between two groups and in the situations where the variances were not equal, Cochran's and Cox's *C*-test was applied. Student's *t*-test for paired data was used to analyse changes within groups. Correlations were tested by linear regression analysis. A probability value of 0.05 was considered significant.

Results

The baseline (before culture) peripheral blood MNC subpopulations in subjects with asthma and controls are presented in Table 1. These two groups did not differ in age, sex, percentage of monocytes, IL-2R⁺ cells and lymphocyte subpopulations, except for the percentage of CD8⁺ cells which was significantly lower in the asthmatic group (23.8 ± 5.7) compared to controls (27.0 ± 4.6) ($P < 0.05$).

Lymphocyte activation, as assessed by IL-2R expression in cultures containing PHA (positive control),

Table 1. Peripheral blood lymphocyte subpopulations (%) in subjects with asthma and controls (mean $\pm$ 1 s.d.)

Subject group	Age (yr)	Sex	CD3 ⁺	CD4 ⁺	CD8 ⁺	Mo2 ⁺	IL-2R ⁺
Asthma (n=35)	36.5 $\pm$ 12.9	21M/14F	76.9 $\pm$ 5.9	52.6 $\pm$ 5.6	23.8 $\pm$ 5.7	7.7 $\pm$ 2.7	1.09 $\pm$ 0.87
Control (n=19)	35.1 $\pm$ 9.1	11M/8F	78.2 $\pm$ 5.1	52.8 $\pm$ 4.3	27.0 $\pm$ 4.6	7.1 $\pm$ 2.0	0.89 $\pm$ 0.79
P value*	n.s.		n.s.	n.s.	<0.05	n.s.	n.s.

* Student's *t*-test for unpaired data.

medium alone (negative control) and house dust allergen, is presented in Fig. 1. The mean percentage of IL-2R⁺ in PHA-stimulated cultures did not differ between the asthmatic group and controls (68.4 $\pm$ 6.5 vs. 70.3 $\pm$ 5.7; *P*=0.31). Also, the mean percentage of IL-2R⁺ cells in negative control cultures was similar in asthma group (4.5 $\pm$ 1.6) and controls (4.1 $\pm$ 1.3) (*P*=0.41).

House dust-stimulated cultures showed a significantly higher percentage of IL-2R positive cells—11.1 $\pm$ 6.5 in the asthma group as compared to controls 4.8 $\pm$ 1.8 (*P*<0.001).

The changes in all subpopulations of MNC cultured with HD as compared to the baseline are presented in

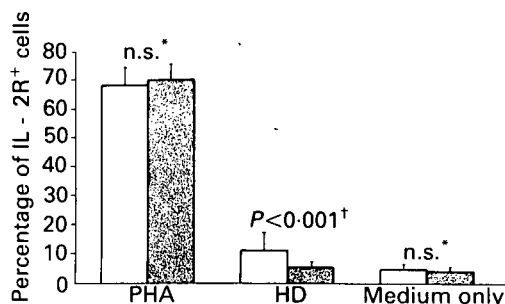


Fig. 1. IL-2R expression by lymphocytes cultured with PHA, HD and medium alone in the subjects with asthma (n=35; □) and healthy controls (n=19; ▨). *Student's *t*-test for unpaired data. †Cochran's and Cox's *C*-test.

Table 2 for the asthmatic group and in Table 3 for the control group. As can be seen in Table 2 there was no change in the mean percentage of CD3⁺ cells but the percentage of the CD4⁺ subpopulation increased from a baseline 52.6 $\pm$ 5.6 to 61.7 $\pm$ 9.0 in HD-driven cultures (*P*<0.001). Simultaneously, the CD8⁺ subpopulation and Mo2⁺-positive cells decreased from 23.8 $\pm$ 5.7 and 7.7 $\pm$ 2.7 for baseline to 19.1 $\pm$ 7.9 and 4.6 $\pm$ 2.5 for HD-stimulated cultures, respectively (*P*<0.001 for both comparisons).

The control group did not show significant changes in mean percentage of CD3⁺, CD4⁺ and CD8⁺ subpopulations in HD-stimulated MNC as compared to the baseline (Table 3). The increase in IL-2R cells from 0.89 $\pm$ 0.79 to 4.8 $\pm$ 1.8 was highly significant (*P*<0.001) but it did not differ from the percentage of IL-2R-positive cells in unstimulated (medium alone) cultures (4.8 $\pm$ 1.8 vs. 4.1 $\pm$ 1.3; *P*=0.08).

The analysis of the relationship between lymphocyte subpopulations and IL-2R⁺ cells in HD-stimulated cultures in the asthma group is presented in Table 4. The percentage of IL-2R⁺ cells did not correlate significantly with the baseline lymphocyte subpopulations but there was a significant positive correlation between CD4⁺ cells and IL-2R⁺ cells, as well as a significant negative correlation between CD8⁺ cells and IL-2R⁺ cells in HD-stimulated cultures. There was a very high positive correlation between IL-2R⁺ cells and Δ CD4⁺ calculated as the difference between the baseline percentage of

Table 2. Mononuclear cell subpopulations (%) in HD-stimulated cultures as compared to baseline in the group of 35 subjects with asthma (mean $\pm$ 1 s.d.)

Lymphocyte	CD3 ⁺	CD4 ⁺	CD8 ⁺	Mo2 ⁺	IL-2R ⁺
Baseline	76.9 $\pm$ 5.9	52.6 $\pm$ 5.6	23.8 $\pm$ 5.7	7.7 $\pm$ 2.7	1.09 $\pm$ 0.87
Cultured with HD	74.5 $\pm$ 5.1	61.7 $\pm$ 9.0	19.1 $\pm$ 7.9	4.6 $\pm$ 2.5	11.1 $\pm$ 6.5
P value*	n.s.	<0.001	<0.001	<0.001	<0.001

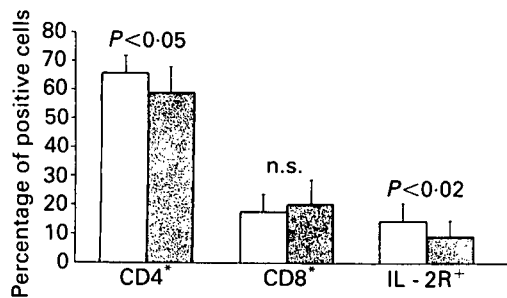
* Student's *t*-test for paired data.

Table 3. Mononuclear cell subpopulations (%) in HD-stimulated cultures as compared to baseline in the group of healthy controls (mean $\pm$ 1 s.d.)

Lymphocyte	CD3 ⁺	CD4 ⁺	CD8 ⁺	Mo2 ⁺	IL2-R ⁺
Baseline	78.2 $\pm$ 5.1	52.8 $\pm$ 4.3	27.0 $\pm$ 4.6	7.1 $\pm$ 2.0	0.89 $\pm$ 0.79
Cultured with HD	76.3 $\pm$ 3.2	54.4 $\pm$ 5.1	26.4 $\pm$ 4.2	4.2 $\pm$ 1.4	4.8 $\pm$ 1.8
<i>P</i> value*	n.s.	n.s.	n.s.	<0.001	<0.001

* Student's *t*-test for paired data.**Table 4.** Relationship between percentage of IL-2R presenting cells in HD-stimulated cultures and lymphocyte subpopulations in asthmatic subjects

Lymphocyte	Baseline			HD-stimulated cultures			
	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD4 ⁺
<i>r</i>	-0.07	-0.24	0.04	0.01	0.70	-0.65	0.91
<i>P</i>	n.s.	n.s.	n.s.	n.s.	<0.001	<0.001	<0.001

**Fig. 2.** Lymphocyte subpopulations in subjects with asthma and positive LCR to HD ($n=14$; □) as compared to those with single early ST reaction ($n=21$; ▨).

CD4⁺ and the percentage of CD4⁺ cells in HD-stimulated cultures ($r=0.91$; $P<0.001$).

In the group of 35 subjects with asthma there were 21 with single early ST reactions to HD and 14 with early and late (dual) reactions. MNC responses to HD stimulation in these two groups are presented in Fig. 2. There was a significantly higher increase in IL-2R⁺ cells and CD4⁺ cells in the group with dual skin reaction to HD as compared to those with single early reaction. The mean percentage of IL-2R⁺ cells in HD-stimulated cultures was 14.3 ± 6.6 and 9.0 ± 5.4 in groups with LCR and single early ST reaction, respectively ($P<0.02$).

Discussion

Lymphocyte response to allergen challenge *in vitro*, assessed by blast transformation [4], [³H] thymidine

incorporation [11] or lymphokine production [5] has been reported by many investigators [12,13]. The results of these studies emphasize the contribution of lymphocytes to the pathogenesis of asthma. The availability of monoclonal antibodies specific for lymphocyte subpopulations and for cell surface activation antigens make possible more detailed analysis of mononuclear cells by the immunofluorescence technique. Using this technique the subpopulations of lymphocytes isolated from subjects with allergic bronchial asthma were identified and cultured *in vitro* with house dust allergen. The experiments show that T lymphocytes, isolated from peripheral blood of subjects with allergic asthma and positive skin reaction to HD, responded to HD-allergen stimulation *in vitro* by the expression of IL-2R, identified with anti-Tac monoclonal antibody. The subpopulations which increased proportionately during culture with allergen were CD4⁺ helper/inducer lymphocytes, whereas suppressor/cytotoxic CD8⁺ subpopulations decreased (Table 2). As double staining for CD4 and IL-2R molecules of cultured MNC was not used, the relationship between the proportion of CD4⁺ cells and IL-2R positive cells, and between IL-2R⁺ cells and increase in proportion of CD4⁺ cells (Δ CD4⁺) in HD stimulated cultures was analysed. This showed linear correlation and gives indirect evidence that lymphocytes responding to HD-allergen stimulation belonged to helper/inducer CD4⁺ subpopulations.

These subjects were allergic individuals presenting multiple positive intradermal allergy skin tests. Early reaction to HD allergen skin testing was positive in all and

positive after 24 hr in 14 subjects (LCR). It was interesting to see the difference in *in-vitro* lymphocyte response to HD allergen between the group with positive skin reaction after 24 hr as compared to those with single early reactions. It appeared that the subjects presenting early and late skin reactions lasting 24 hr responded significantly higher to *in-vitro* lymphocyte stimulation with HD allergen as compared to those with early reaction only (Fig. 2). The clinical importance of LCR was mentioned by Agrawal and Zetterstrom [14] who demonstrated that all patients with positive LCR had a positive bronchial provocation test with the corresponding allergen. However, the significance of our finding is not clear. It could suggest that subjects with late skin reactions persisting for longer periods of time (24 hr) have a higher proportion of circulating antigen-specific T lymphocytes. A recent study by Frew and Kay [7] showed that lymphocytes infiltrating skin in the late-phase allergen response to intradermal allergen skin testing were predominantly of CD4⁺ phenotype and some of them also presented receptors for IL-2. The results of the experiments described here showed that the proliferative response of peripheral blood lymphocytes of subjects with asthma to allergen stimulation *in vitro* is selective and most probably restricted to CD4⁺ subpopulations. This observation is in line with other studies on lymphocyte response to allergen challenge. Rawle *et al.* [13] investigated lymphocytes from patients with various manifestations of allergy to *D. pteronyssinus* and Koponen *et al.* [15] investigated lymphocyte response to penicillin in penicillin allergy—both used double fluorescence and showed that responding cells to allergen stimulation were of the CD4⁺ phenotype. Also, Phillips *et al.* [16] showed that cloned lymphocytes specific for ambrosia antigen E were uniformly of helper/inducer CD4⁺ phenotype. Furthermore, it has been shown that activated T lymphocytes release mediators which can contribute to: (1) eosinophil activation [7,17], (2) histamine release [18,19], and (3) mast cell degranulation [20]. Thus the relationship between activation of T lymphocytes *in vivo* and exacerbation of asthma is especially interesting. The recent study of Corrigan *et al.* [23] demonstrated that patients with acute severe bronchial asthma had a significantly increased proportion of lymphocytes presenting various activation markers including IL-2R in peripheral blood, and these cells were also CD4⁺. Thus it is possible to hypothesize that T lymphocytes could play a role in asthma—not only influencing IgE synthesis [21,27]—but more importantly, as the central cell in the allergic inflammatory process.

It has been suggested that HD extract may act as a non-specific mitogen [12]. This was not evident in this study. Significant spontaneous activation of lymphocytes cultured for 7 days in medium alone were observed,

however, the increase in IL-2R⁺ cells in unstimulated cultures did not differ between the groups studied. In the control group of 19 healthy subjects the percentage of IL-2R⁺ cells in HD-stimulated cultures increased significantly as compared to baseline, but it was not different from spontaneous activation in cultures without stimulant.

In both earlier studies [10,23] and the present one, we found significantly lower CD8⁺ subpopulations in the group of subjects with asthma. These data have been confirmed by others [24,25], but some authors have found normal CD8⁺ subpopulations in asthmatics [22,26]. This discrepancy could be related to the selection of patients, season of testing and method of staining of T cell subpopulations. It has been suggested from *in-vitro* studies that the CD8⁺ subpopulation of lymphocytes negatively regulates IgE production [27,28]. We could not find any correlation between lymphocyte subpopulations and IgE level *in vivo* [23,10]. In the present study the group of 35 subjects with asthma contained five with a CD8⁺ percentage below 17.8%. This is lower than mean -2 s.d. in the control group. The response of lymphocytes in these subjects to HD allergen did not differ from these with higher CD8⁺ percentages, indicating that these 'suppressor/cytotoxic' cells did not significantly influence lymphocyte proliferation in this model.

In conclusion, we have demonstrated lymphocyte activation by HD allergen in subjects with asthma and positive ST reaction to HD. Indirect evidence shows that the responding cells were of the CD4⁺ phenotype. Our finding that lymphocytes from subjects with dual skin reactions to HD responded higher than those with single early reaction suggest that the former subjects had a higher proportion of circulating allergen specific T cells, but this hypothesis needs further study.

References

- 1 Ishizaka K, Ishizaka T. Mechanism of reaginic hypersensitivity: a review. *Clin Allergy* 1971; 1:9–24.
- 2 Frew AJ, Kay AB. The pattern of human late-phase skin reaction to extracts of aeroallergens. *J Allergy Clin Immunol* 1988; 81:1117–21.
- 3 Black PL, Marsh DG. Correlation between lymphocyte responses and immediate hypersensitivity to purified allergens. *J Allergy Clin Immunol* 1980; 66:394–401.
- 4 Girard JP, Rose NR, Kunz ML, Kobayashi S, Arbesman CE. In vitro lymphocyte transformation in atopic patients: induced by antigens. *J Allergy* 1967; 39:65–81.
- 5 Rocklin RE, Pence H, Kaplan H, Evans R. Cell mediated immune response of ragweed-sensitive patients to ragweed antigen E. In vitro lymphocyte transformation and elaboration of lymphocyte mediators. *J Clin Invest* 1974; 53:735–44.
- 6 Metzger WJ, Zavala D, Richerson HB, *et al.* Local allergen

- challenge and bronchoalveolar lavage of allergic asthmatic lungs. Description of the model and local airway inflammation. *Am Rev Respir Dis* 1987; 135:433-40.
- 7 Frew AJ, Kay AB. The relationship between infiltrating CD4⁺ lymphocytes, activated eosinophils and the magnitude of the allergen induced late phase cutaneous reaction in man. *J Immunol* 1988; 141:4158-64.
 - 8 Boyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1967; 21(suppl): 77-89.
 - 9 Kus J, Vedal S, Kijek K, Chan-Yeung M. Absence of day to day variation in lymphocyte subpopulations in humans. *Allergol Immunopathol* 1984; 12:407-9.
 - 10 Kus J, Tse KS, Enarson D, Grzybowski S, Chan-Yeung M. Lymphocyte subpopulations in patients with allergic rhinitis. *Allergy* 1984; 39:509-14.
 - 11 Broman P, Moller E. Lymphocyte transformation by grass pollen allergens: a study of atopic patients receiving immunotherapy. *Allergy* 1988; 43:321-31.
 - 12 Romagnani S, Biliotti G, Passaleva A, Ricci M. Mites and house dust allergy. III. *In-vitro* lymphocyte transformation and precipitating antibody to house dust and mite (*Dermatophagoides pteronyssinus*) extract in atopic and non-atopic individuals. *Clin Allergy* 1973; 3:51-6.
 - 13 Rawle FC, Mitchell EB, Platts-Mills TA. T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus* antigen P1: comparison of patients with asthma, atopic dermatitis and perennial rhinitis. *J Immunol* 1984; 133:195-201.
 - 14 Agrawal K, Zetterström O. Diagnostic significance of late cutaneous allergic responses and their correlation with radioallergosorbent test. *Clin Allergy* 1982; 12:489-97.
 - 15 Koponen M, Pichler W, de Weck A. T cell reactivity to penicillin: phenotypic analysis of in vitro activated cell subsets. *J Allergy Clin Immunol* 1986; 78:645-52.
 - 16 Phillips L, Chambers C, Underdown B, Zimmerman B. Lymphocyte proliferation to antigen E: demonstration of the restriction of antigen E-specific T cells to ragweed-allergic donors. *J Allergy Clin Immunol* 1987; 79:933-40.
 - 17 Parish WE, Luckhurst E. Eosinophilia VI, spontaneous synthesis of chemokinetic, chemotactic, complement receptor-inducing activities for eosinophils by bronchial T lymphocytes of asthmatic-bronchitic patients. *Clin Allergy* 1982; 12:475-88.
 - 18 Sedgwick JD, Holt PG, Turner KJ. Production of a histamine-releasing lymphokine by antigen- or mitogen-stimulated human peripheral T cells. *Clin Exp Immunol* 1981; 45:409-18.
 - 19 Thuesen DO, Speck LS, Lett-Brown MA, Grant AJ. Histamine-releasing activity (HRA). I. Production by mitogen- or antigen-stimulated human mononuclear cells. *J Immunol* 1979; 123:626-32.
 - 20 Herrman P, Schreier MH, Borel JF, Feurer C. Mast cell degranulation as a major event in the effector phase of delayed-type hypersensitivity induced by cloned helper T cells. *Int Arch Allergy Appl Immunol* 1988; 86:102-5.
 - 21 Seumura M, Kishimoto T. Regulation of human IgE response by T cells and their products. *Int Arch Allergy Appl Immunol* 1985; 77:26-31.
 - 22 Corrigan CJ, Hartnell A, Kay AB. T lymphocyte activation in acute severe asthma. *Lancet* 1988; i:1129-32.
 - 23 Kus J, Tse KS, Vedal S, Chan-Yeung M. Lymphocyte subpopulations in patients with allergic and non-allergic asthma. *Clin Allergy* 1985; 15:523-9.
 - 24 Engel P, Huguet J, Sanosa J, Sierra P, Cols N, Garcia-Calderon P. T cell subsets in allergic respiratory diseases using monoclonal antibodies. *Ann Allergy* 1984; 53:337-40.
 - 25 Bruijnzeel PLB, Hamelink ML, Prins K, Remmert G, Meyling FHJ. Blood lymphocyte subpopulations in extrinsic and intrinsic asthmatics. *Ann Allergy* 1987; 58:179-83.
 - 26 Kochman S, Bernard J, Cazabat A, Lavaud F, Lorton C, Liehn JC. Double phenotyping of immunoregulatory T cells subsets in patients with allergic asthma. *Clin Allergy* 1987; 17:579-88.
 - 27 Ohta K, Rozzo SJ, Hayward A, Harbec RJ, Kirkpatrick C. Human tonsillar IgE biosynthesis in vitro. II. Analysis of T cell regulation with monoclonal antibodies. *J Allergy Clin Immunol* 1988; 81:401-12.
 - 28 Tamir R, Castracane JM, Rocklin RE. Generation of suppressor cells in atopic patients during immunotherapy that modulate IgE synthesis. *J Allergy Clin Immunol* 1987; 79:591-8.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.