

Double phenotyping of immunoregulatory T cell subsets in patients with allergic asthma

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Summary

In order to determine whether the dissection of helper/inducer (CD4+) and suppressor/cytotoxic (CD8+) lymphocyte subsets with Leu 8 reagent would reveal any differences between allergic asthma patients and non-atopic controls, we compared in both groups the 'true helper' T cell subset (Leu 8 – CD4+), responsible for the major helper effect, and one of the suppressor T cell subpopulations (Leu 8 – CD8+). Peripheral blood mononuclear cells from sixty-nine individuals, including nineteen extrinsic asthmatics, fifteen intrinsic asthmatics, seventeen patients with chronic obstructive lung disease and eighteen healthy controls, were comparatively analysed. Although total CD4+ cells and total CD8+ cells were similar for all groups, we found in the extrinsic asthma patients group a significant increase in the number of 'true helper' T cell sublineage (Leu 8 – CD4+) and of suppressor cells expressing Leu 8 – CD8+ phenotype. Such imbalances may be implicated in the pathogenesis of atopic asthma.

Introduction

Ever since Buckley and Becker's report [1], which suggested that elevated serum IgE in atopic patients may be associated with a lack of suppressor T lymphocytes in peripheral blood, suppressor T cell abnormalities have been implicated by some investigators [2–18] but not by others [19–27] in the pathogenesis of allergy. Despite these conflicting results about such a defect in suppressor T cell regulation, there is general agreement that proportions of helper T cells are normal in allergic diseases [2–27].

Recent dissection of immunoregulatory T cell subpopulations using the anti Leu 8 monoclonal antibody [28–30] has demonstrated heterogeneity within both helper/inducer (CD4+) and suppressor/cytotoxic (CD8+) lymphocytes: numerically minor

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Leu 8- CD4+ subpopulation, the so-called 'true helper' cell subset, is exclusively responsible for the helper effect by interleukin 2 secretion [28,29] whereas T cells expressing Leu 8- CD8+ phenotype interact with Leu 8+ CD8+ subset to suppress immune response in man [28,30]. The present study was initiated to determine whether the dissection of CD4+ and CD8+ subsets with Leu 8 reagent, using a double phenotyping method, could reveal any differences between patients with extrinsic asthma and control groups. For this purpose, peripheral blood of nineteen patients suffering from extrinsic asthma with elevated serum IgE were comparatively analysed with fifteen non-atopic asthmatics, seventeen patients suffering from chronic obstructive lung disease and eighteen normal individuals.

Subjects and methods

Subjects

Five groups of subjects totalling sixty-nine individuals were studied.

Extrinsic asthmatics. The demographic and clinical data on these nineteen patients in this study are shown in Table 1. They were similar in age, family and personal history of allergy, and all suffered from allergic asthma. The atopic nature of the disease was ascertained by skin-prick tests, positive inhalation tests and measurements of total serum IgE and IgE specific to the relevant allergen. The patients were tested during asymptomatic periods. None of them had received any drugs during the week preceding T cell subset evaluation.

Intrinsic asthmatics. Fifteen non-atopic asthmatics, seven males and eight females, 20-49 years old, having reversible airway constriction, a negative history of allergy and negative allergy skin-test reactions, were included in this group. Six of them were treated with an inhalant β_2 -bronchodilator which was suspended at least 3 days before the study.

Patients with chronic obstructive lung disease. Seventeen patients with irreversible chronic obstructive lung disease were recruited for the study. The ten males and seven females, 42-51 years old, were chosen on the basis of FEV₁: FVC ratio of less than 60% with less than a 15% improvement in FEV₁ after the administration of an inhaled bronchodilator. All patients were ex-smokers and each was clinically stable. Their usual medications, which included aminophylline ($n=17$) and an inhaled beta-agonist ($n=14$) were suspended at least 3 days before the study was performed. None of the patients was receiving either systemic or inhaled corticosteroids. None of them had family or personal history of either allergy or asthma and none had positive skin-prick test reactions.

Healthy controls. The eighteen healthy individuals, nine males and nine females, had negative skin tests, no personal or family history of atopic diseases, and ages ranging from 20 to 45 years.

Isolation of lymphoid cells

Twenty millilitres of heparinized venous blood were obtained at the same time of day to eliminate the effects of circadian rhythm. Total cell count and percentage of lymphocytes, monocytes and polymorphonuclear leucocytes were determined. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque (Flow

Table 1. Demographic and clinical data of the extrinsic asthma patients

Subject no.	Sex/age	Relevant antigen	Skin-prick test*	FEV ₁ (% predicted)†	PC ₂₀ ‡	Total serum IgE§	Specific IgE¶
1	F/29	House dust mite	2+	90	0.98	280	3.20
2	F/36	House dust mite	4+	97	0.55	490	> 17.50
3	F/26	House dust mite	3+	95	6.55	340	> 17.50
4	F/38	House dust mite	2+	104	0.49	270	2.85
5							
M/35	House dust mite	2+	101	3.37	270	3.40	
6	M/27	Grass pollen	4+	100	2.52	600	> 17.50
7	M/30	Grass pollen	3+	91	1.03	720	> 17.50
8	M/29	House dust mite	2+	80	0.16	360	10.40
9	F/31	Grass pollen	3+	76	0.41	1300	> 17.50
10	F/34	House dust mite	2+	92	0.54	430	8.50
11	M/28	House dust mite	3+	115	2.27	600	> 17.50
12	F/35	House dust mite	3+	82	0.49	260	> 17.50
13	M/31	House dust mite	3+	75	0.99	600	> 17.50
14	F/37	Grass pollen	4+	77	1.05	570	> 17.50
15	M/29	House dust mite	3+	90	3.02	620	> 17.50
16	M/34	House dust mite	2+	92	4.18	260	7.40
17	F/36	House dust mite	3+	105	0.28	290	> 17.50
18	M/25	Grass pollen	4+	83	0.57	780	> 17.50
19	F/29	Grass pollen	3+	78	0.10	420	12.30

* Skin-prick test performed and graded according to Pepys J. In: Gell PGH, Coombs RRA, Lachmann PJ, eds. Clinical aspects of immunology. Oxford: Blackwell Scientific Publications, 1975:55-80.

† Forced expiratory volume in 1 sec, percentage predicted according to the proposed standards of the European Comity for Coal and Steel (Bull Euro Physiopathol Respir 1983; 19 (suppl 5):65-86).

‡ Concentration of methacholine provoking a 20% fall in forced expiratory volume in 1 sec. The value is taken from the log-dose-response curve by linear extrapolation of the last two points.

§ Paper radio-immunosorbent test (Phadebas PRIST), results are expressed in Ku/litre.

¶ Radio-allergo-sorbent test (Phadebas RAST), results are expressed in PRU/ml.

Laboratoires, Irvine, U.K.) gradient centrifugation [31]. Cell suspensions were washed twice in Hank's solution, then suspended in RPMI 1640 supplemented with 10% foetal calf serum (complete medium).

Reagents

Monoclonal mouse hybridoma antisera were CD4 and IOT4 (helper/inducer T cells), CD8 and IOT8 (suppressor/cytotoxic T cells) purchased respectively from Ortho Pharmaceutical Corporation (Ruritan, NJ, U.S.A.) and Immunotech (Marseille, France), and anti-Leu 8 from Becton Dickinson (Franklin Lake, U.S.A.). Indicator erythrocytes were prepared by coupling sheep anti-mouse Ig antibodies purified by affinity chromatography to ox red blood cells using benzoquinone as the cross-linking agent [32].

Identification of T cell subpopulations

CD4, CD8 and Leu 8 antigen-bearing cells were identified by immunofluorescence assay and the analytical rosetting method described in detail elsewhere [32]. This latter method, which is as sensitive as the complement-mediated cytotoxicity [32] and

Table 2. Comparative analysis of rosetting tests and immunofluorescence assays for detection of lymphocyte antigen phenotypes

Subjects†	CEA*		Anti CD4		Anti CD8		Anti Leu 8	
	RT‡	IF§	RT	IF	RT	IF	RT	IF
a	0§	5	50	55	36	33	65	65
b	0	4	45	46	19	22	67	62
c	0	3	34	38	20	21	73	68
d	0	5	60	55	22	25	76	77
e	0	4	39	41	29	25	70	75
f	0	2	46	52	25	28	69	66
g	0	1	42	45	23	23	61	65
h	0	0	44	40	27	24	70	67

* CEA: Carcinoembryonic antigen, unrelated monoclonal antibody used as control antibody.

† Subjects: a to d were volunteer normal subjects, e and f were patients with extrinsic asthma, g was a patient with intrinsic asthma and h was a patient with chronic obstructive lung disease.

‡ Rosetting test.

§ Immunofluorescence microscopic assay.

indirect immunofluorescence methods (Table 2), was used to allow further isolation of the rosette-forming cells by subsequent centrifugation on Ficoll cushion [33]. Briefly, 5×10^4 lymphoid cells in 0.02 ml of complete medium were incubated for 30 min at room temperature with 5 μ l monoclonal antibody diluted 1:10 in medium. After two washes, the cells were suspended in 50 μ l of complete medium and then 20 μ l indicator erythrocytes (0.2% v/v) were added. The tubes were spun down for 5 min at $200 \times g$ and left at room temperature for 30 min. The pellet was gently suspended and rosettes, defined as cells binding four or more ox erythrocytes, were counted on more than 300 cells.

Dissection of helper/inducer and suppressor/cytotoxic T cell subsets

'True helper' cells (Leu 8- CD4+) and the suppressor subset expressing Leu 8- CD8+ phenotype were evaluated by a two-step method [34-36]. Leu 8+ and Leu 8- subpopulations were first identified using the rosetting technique described above, then the rosetted mixture was layered over Ficoll-Hypaque and centrifugated for 5 min at $500 \times g$. The Leu 8+ cell pellet as well as the Leu 8- cell interface were recovered. Analysis of purity showed that pellet cells yielded more than 95% Leu 8+ rosette-forming cells and interface cells less than 1% Leu 8+ rosette-forming cells with a total cell recovery higher than 75%. In the second step, the interface cells (Leu 8-) were identified as CD4+ or CD4- and CD8+ or CD8- using a subsequent rosetting test. Finally, the proportions of Leu 8- CD4+ and Leu 8- CD8+ lymphocytes were expressed as percentages of PBMC.

Statistics

In Tables 3 and 4, values are expressed as mean and standard error of the mean. In Figs 1 and 2, bars show mean and one standard deviation. The degree of significance of the differences between the populations was determined using the χ^2 test for gender and the Mann-Whitney rank sum test for the other characteristics and the results [37].

Table 3. Study population characteristics

Symptomatology	Extrinsic asthma	Intrinsic asthma	COLD*	None
No. subjects	19	15	17	18
Sex (M:F)	9:10	7:8	10:7	8:10
Total IgE†	498 ± 256‡	62 ± 58	57 ± 61	53 ± 62
FEV ₁ (% predicted)§	90.68 ± 11.27	80.4 ± 12.76	61.06 ± 8.06	—
PC ₂₀ ¶	1.55 ± 1.69	1.60 ± 1.41	2.99 ± 3.71	—
WBC**	6589 ± 357	6732 ± 512	6952 ± 533	6783 ± 424
Lymphocytes (per mm ³)	2043 ± 115	2102 ± 151	2088 ± 492	1933 ± 91
Lymphocyte (%)	31.3 ± 1.5	30.5 ± 1.4	29.0 ± 1.9	30.1 ± 1.6

* Chronic obstructive lung disease.

† Paper radio-immunosorbent test (Phadebas PRIST), results are expressed in Ku/litre.

‡ Figures represent mean ± standard deviation.

§ Forced expiratory volume in one second, percentage predicted.

¶ Concentration of methacholine provoking a 20% fall in FEV₁.

** White blood cell count.

Table 4. Analysis of T cell subset percentages in extrinsic asthma patients as compared to other groups*

	Symptomatology			
	Extrinsic asthma	Intrinsic asthma	COLD†	None
Total CD4+	39.5 ± 2.2‡	44.6 ± 2.5 n.s.§	42.8 ± 2.4 n.s.	40.7 ± 1.9 n.s.
Total CD8+	25.4 ± 1.6	26.8 ± 3.9 n.s.	27.0 ± 1.7 n.s.	27.7 ± 1.4 n.s.
Total Leu 8+	55.8 ± 3.6	75.9 ± 1.77 <i>P</i> < 0.001	72.6 ± 1.47 <i>P</i> < 0.001	76.2 ± 2 <i>P</i> < 0.001
Leu 8- CD4+	16.0 ± 1.4	8.8 ± 0.8 <i>P</i> < 0.001	9.8 ± 0.7 <i>P</i> < 0.002	8.6 ± 0.8 <i>P</i> < 0.001
Leu 8- CD8+	14.5 ± 1.0	9.5 ± 0.8 <i>P</i> < 0.002	10.4 ± 0.6 <i>P</i> < 0.004	9.3 ± 0.9 <i>P</i> < 0.002

* Each control group is compared with the extrinsic asthma group. The degree of significance of the differences is determined by the Mann-Whitney rank sum test.

† Chronic obstructive lung disease.

‡ Figures represent percentage of PBMC ± standard deviation.

§ n.s. = Not significant

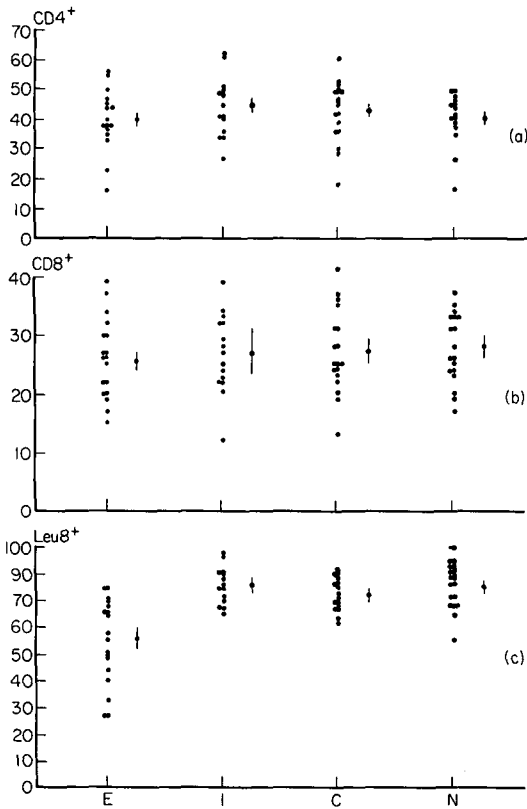


Fig. 1. Comparison of the percentages of (a) helper/inducer T lymphocytes CD4+, (b) suppressor/cytotoxic T lymphocytes CD8+ and (c) T lymphocytes expressing Leu 8+ phenotype in groups of extrinsic asthma patients (E), intrinsic asthma patients (I), chronic obstructive lung disease patients (C) and normal controls (N). Bars show mean $\pm$ standard deviation.

Results

We studied nineteen extrinsic asthmatics and a group of control subjects including fifteen non-atopic asthmatics, seventeen patients suffering from chronic obstructive lung disease and eighteen normal individuals. Study population characteristics are summarized in Table 3. There were no significant differences between the groups in white blood cell count, nor in lymphocyte percentage and number. Therefore, lymphocyte subsets were evaluated on the basis of proportions of PBMC, using the analytical rosetting method, and values for extrinsic asthmatic patients were compared with the three control subgroups. As shown in Fig. 1, no significant differences were observed for either the proportions of total helper/inducer CD4+ lymphocytes or for the suppressor/cytotoxic CD8+ cells. However, extrinsic asthma patients had significantly lower numbers of Leu 8+ cells than each of the control subgroups (Fig. 1). In order to determine the cell phenotypes responsible for such abnormality, we dissected CD4+ and CD8+ lymphocytes using the Leu 8 reagent and we were able to evaluate the proportions of true helper cells (Leu 8– CD4+) and of suppressor cells expressing Leu 8– CD8+ phenotype (Fig. 2). The number of Leu 8– CD4+ true helper cells was not significantly different between any of the groups except for

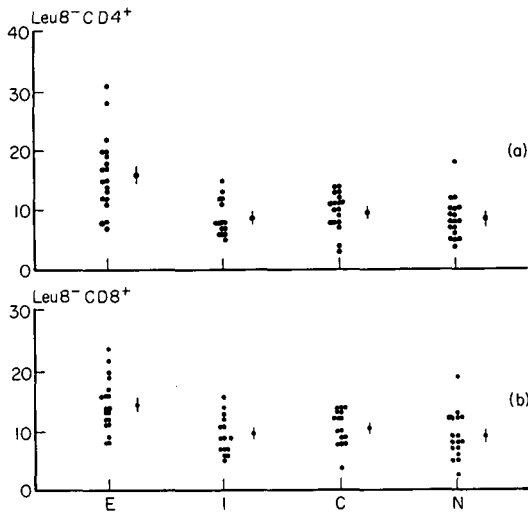


Fig. 2. Comparison of the percentages of (a) true helper T lymphocytes Leu 8- CD4+ and (b) suppressor subset expressing Leu 8- CD8+ phenotype in groups of extrinsic asthma patients (E), intrinsic asthma patients (I), chronic obstructive lung disease patients (C) and normal controls (N). Bars show mean $\pm$ standard deviation.

extrinsic asthma patients where it was significantly higher than in each of the other groups. Likewise, extrinsic asthma patients had higher values of suppressor cells expressing Leu 8- CD8+ phenotype than each of the other groups. Furthermore, no significant differences were found between intrinsic asthma patients, chronic obstructive lung disease patients and healthy controls either for true helper lymphocytes or for Leu 8- CD8+ suppressor cells. Mean values and *P* values for these different T cell subsets as evaluated in each group are shown in Table 4.

Discussion

It has been postulated that the pathogenesis of atopy is associated with defective immune regulation, inducing an excessive production of IgE against a variety of allergens [38,39]. It is well established in rats that IgE production is regulated, like other immunoglobulin classes, by T helper as well as T suppressor lymphocytes [40,41]. Evidence for a similar mechanism in man is only circumstantial. Kikkawa *et al.* [42] reported association between very high levels of IgE and T cell deficiencies, suggesting that defective T cell regulation may play some part in the development of allergy.

The availability of monoclonal antibodies has been an important advance in the study of human regulatory T cell pool using CD4 and CD8 differentiation antigens to discriminate helper/inducer and suppressor/cytotoxic subsets, respectively [43,44]. The present work shows that extrinsic asthma patients had similar numbers of helper and suppressor T cells as compared with patients suffering from non-atopic pulmonary diseases and normal subjects. These findings agree with a general consensus that proportions of total helper CD4+ cells are normal in allergic diseases and with some investigators who found normal numbers of total suppressor CD8+ cells [19,20,22,26], even though such abnormalities have been implicated by others [3,5,7-10,16,18]. It is not clear why different groups have reported different results. This may in part be due to patient selection of atopic individuals, with eczema [5,7,18] or allergic

rhinitis and asthma [3,8–10,20], or to other variables such as the age of the patients and possible influence of immunotherapy [9]. The different experimental techniques used may also contribute to these discrepancies [26].

The development of technologies capable of discriminating and continuously propagating clonal populations of human T lymphocytes *in vitro* has provided new basis for phenotypic identification and functional analysis of these cells. Accordingly, we used an anti-Leu 8 monoclonal antibody to dissect both CD4+ and CD8+ lymphocytes of peripheral blood of patients suffering from extrinsic asthma with the aim of obtaining a more representative picture of the cell-mediated immune system in these patients with high IgE levels. This reagent is of considerable potential in the context of a double phenotyping [45] since it provides a means to subdivide both helper CD4+ and suppressor CD8+ lymphocytes although it reacts with a majority of leucocytes when used alone [46,47]. Therefore, we identified the 'true helper' T lymphocyte subset (Leu 8 – CD4+) responsible for the major helper effect [28] and we found a significant increase in this sublineage in the peripheral blood of extrinsic asthma patients as compared with patients suffering from non-atopic pulmonary diseases and normal subjects. These true helper cell subpopulations may represent the T lymphocyte subset that secretes IgE-enhancing factors as described by Ishizaka [48] and Katz [49]. Besides, by the dissection of suppressor lymphocytes, we revealed in atopic patients a significant increase in the Leu 8 – CD8+ subset implicating a concomitant decrease in the Leu 8 + CD8+ cells. Such imbalances of both suppressor T cell subsets might account for functional abnormalities of these cells, which have been found in atopic patients as inability to generate suppressor activity with concanavalin A [50] or deficiency of cell-mediated cytotoxicity [51]. However, it should be stressed that there is currently neither information on the nature of the cells that suppress the triggering of IgE synthesis nor on the status of such cells in allergic donors.

All in all, imbalances of both true helper and suppressor subpopulations may account for the rise in IgE antibodies in patients with allergic asthma and suggest a relationship between cellular abnormalities and allergic diseases associated with elevated IgE levels. We do not know, however, whether these findings reflect a primary defect inherent in the atopic diathesis or are secondary to the disease.

References

- 1 Buckley RH, Becker WG. Abnormalities in the regulation of human IgE synthesis. *Immunol Rev* 1978; 41:288–314.
- 2 Anfosso F, Alcaraz G, Vervloet D, Charpin J. Variations in T cell subsets during hyposensitization of grass-sensitive patients with formaldehyde modified extracts. *Allergy* 1982; 37:613–5.
- 3 Boyer J, Sohaley G, Frobert Y, Veyseyre C, Cornillon J, Touraine R. Typage des sous-populations lymphocytaires T par les anticorps monoclonaux dans l'allergie respiratoire. *Rev Fr Allergol* 1983; 2:97–8.
- 4 Brostoff J, Pack S, Lydyard PM. Histamine suppression of lymphocyte activation. *Clin Exp Immunol* 1980; 39:739–45.
- 5 Butler M, Antherton D, Levinsky RJ. Quantitative and functional deficit of suppressor T cells in children with atopic eczema. *Clin Exp Immunol* 1982; 50:92–8.
- 6 Canonica GW, Mingari MC, Melioli G, Colombatti M, Moretta L. Imbalances of T cell subpopulations in patients with atopic diseases and effect of specific immunotherapy. *J Immunol* 1979; 123:2669–72.
- 7 Chandra RK, Backer M. Numerical and functional deficiency of suppressor T cells precedes development of atopic eczema. *Lancet* 1983; ii:1393–4.

- 8 Engel P, Huguet J, Sierra P, Cols N, Garcia-Calderon PA. T cell subsets in allergic respiratory disease using monoclonal antibodies. *Ann Allergy* 1984; 53:337-44.
- 9 Kus J, Tse KS, Enarson D, Grzybowski S, Chon Yeung M. Lymphocyte subpopulations in patients with allergic rhinitis. *Allergy* 1984; 39:509-14.
- 10 Kus J, Tse KS, Vedal S, Moira CY. Lymphocyte subpopulations in patients with allergic and non-allergic asthma. *Clin Allergy* 1985; 15:523-30.
- 11 Rivlin J, Kuperman O, Freier S, Godfrey S. Suppressor lymphocyte activity in wheezy children with and without treatment by hyposensitization. *Clin Allergy* 1981; 11:353-6.
- 12 Rola-Pleszczynski M, Blanchard R. Abnormal suppressor cell function in atopic dermatitis. *J Invest* 1981; 76:279-83.
- 13 Schuster DL, Bongiovanni BA, Pierson DL, Barbaro JF, Wong DTO, Levinson AL. Selective deficiency of a T cell subpopulation in active atopic dermatitis. *J Immunol* 1980; 124:1662-7.
- 14 Shohat B, Volovitz B, Varsano I. Induction of suppressor T cells in asthmatic children by theophylline treatment. *Clin Allergy* 1983; 13:487-93.
- 15 Strannegard IL, Strannegard O. Increased sensitivity of lymphocytes from atopic individuals to histamine-induced suppression. *Scand J Immunol* 1977; 6:1225-31.
- 16 Thomson LF, Mellon MH, Zeiger RS, Spiegelberg HL. Characterization with monoclonal antibodies of T lymphocytes bearing Fc receptors for IgE (T_h cells) and IgG (T_g cells) in atopic patients. *J Immunol* 1983; 131:2772-6.
- 17 Valverde E, Vich JM, Huguet J, Garcia-Calderon PA. An in vitro study of lymphocytes in patients with atopic dermatitis. *Clin Allergy* 1983; 13:81-8.
- 18 Zachary CB, MacDonald DM. Quantitative analysis of T-lymphocyte subsets in atopic eczema using a monoclonal antibody and flow cytofluorimetry. *Br J Dermatol* 1983; 108:411-22.
- 19 Armistead JG, Ewan PW. Suppressor T cell numbers and function in atopic patients with high serum IgE levels. *J Clin Lab Immunol* 1983; 12:179-85.
- 20 Dominguez MA, Sanz ML, Lobera T, Oehling A. T helper and T suppressor subpopulations in pollinosis, effect of specific immunotherapy. *Allergol Immunopathol* 1983; 11:415-20.
- 21 Gupta S, Fikrig S, Good RA. Subpopulations of human T lymphocytes. XIII. T cell subpopulations in children with bronchial asthma. *Int Arch Allergy appl Immunol* 1980; 61:293-8.
- 22 Ilfeld DN, Mazar A, Garty M *et al.* Effect of oral colchicine on T cell subsets, monocytes and concanavalin A-induced suppressor cell function in asthmatic patients. *Clin Allergy* 1986; 16:407-16.
- 23 Martinez JD, Santos J, Stechschulte J, Abdou NL. Nonspecific suppressor cell function in atopic subjects. *J Allergy Clin Immunol* 1979; 64:485-90.
- 24 Ogden BE, Krueger GG, Hill HR. Lymphocyte suppressor activity in atopic eczema. *Clin Exp Immunol* 1979; 35:269-75.
- 25 Schuster DL, Pierson D, Bongiovanni B, Levinson AF. Suppressor cell function in atopic dermatitis associated with elevated immunoglobulin E. *J Allergy Clin Immunol* 1979; 64:139-43.
- 26 Schuyler M, Gerblin A, Urda G. Atopic asthma: T lymphocyte subpopulations. *Clin Allergy* 1985; 15:131-8.
- 27 Wang SR, Zweiman B. Histamine suppression of human lymphocyte responses to mitogens. *Cell Immunol* 1978; 36:28-36.
- 28 Gatenby PA, Kansas GS, Chen Yu X, Evans RL, Engleman EG. Dissection of immunoregulatory subpopulations of T lymphocytes within the helper and suppressor sublineages in man. *J Immunol* 1982; 129:1997-2000.
- 29 Goldstein H, Volkman DJ, Ambros JL, Fauci AS. Characterisation of a T4+ /Leu8+ T cell clone that directly help B cell Ig production by secreting B cell differentiation factor. *J Immunol* 1985; 135:339-43.
- 30 Reinherz EL, Morimoto C, Fitzgerald K, Hussey RE, Daley JF, Schlossman SF. Heterogeneity of human T4+ inducer T cells defined by a monoclonal antibody that delineates two functional subpopulations. *J Immunol* 1982; 128:463-8.
- 31 Boyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968; suppl: 77-89.
- 32 Bernard J, Ternynck T, Zagury D. A general method for the cytochemical and ultrastructural studies of human lymphocyte subsets defined by monoclonal antibodies. *Immunol Lett* 1982; 4:65-73.
- 33 Bernard J, Dufer J. Cytochemical analysis of human peripheral blood lymphocyte subsets defined by monoclonal antibodies. *Scand J Immunol* 1983; 17:89-93.
- 34 Kochman S, Bernard J, Schwartz H *et al.* Failure to distinguish ultrastructurally between T4+ (helper) and T8+ (suppressor/cytotoxic) T cell subsets. *Scand J Immunol* 1984; 19:165-73.

- 35 Bernard J. All human 'true' helper cells (Leu 3+ 8-) express acid alpha naphthyl acetate esterase. *Br J Hematol* 1984; 57:381-2.
- 36 Dufer J, Bernard J. Surface phenotype and cytochemistry of T cells. *Blood* 1985; 65:246-7.
- 37 Conover WJ. *Practical non parametric statistics*. New York: John Wiley and Sons, 1971.
- 38 Geha RS. Suppressor T cell in human allergic disease. *J Allergy Clin Immunol* 1979; 64:477-8.
- 39 Strannegard O, Strannegard IL. In vitro differences between the lymphocytes of normal subjects and atopics. *Clin Allergy* 1979; 9:637-43.
- 40 Okumura K, Tada T. Regulation of homocytotropic antibody formation in rat. II Effect of thymectomy and splenectomy. *J Immunol* 1971; 106:1019-25.
- 41 Michael G, Bernstein JL. Thymus dependence of reaginic antibody formation in mice. *J Immunol* 1973; 111:1600-1.
- 42 Kikkawa Y, Kanimura K, Sekiguchi T. Thymic alymphoplasia with hyper IgE globulinaemia. *Paediatrics* 1973; 51:690-6.
- 43 Reinherz EL, Kung PC, Breard JM, Goldstein G, Schlossman SF. T cell requirements for generation of helper factor(s) in man: analysis of the subsets involved. *J Immunol* 1980; 124:1883-7.
- 44 Reinherz EL, Kung PC, Breard JM, Goldstein G, Schlossman SF. A monoclonal antibody reactive with the human cytotoxic/suppressor T cell subset previously defined by hetero-antiserum termed TH2. *J Immunol* 1980; 124:1301-7.
- 45 Lanier LL, Loken MR. Human lymphocyte subpopulations identified by using three color immunofluorescence and flow cytometry analysis: correlation of Leu 2, Leu 3, Leu 7, Leu 8, and Leu 11 cell surface antigen expression. *J Immunol* 1984; 132:151-6.
- 46 Kansas GS, Wood GS, Fishwild DM, Engleman EG. Functional characterization of human T lymphocyte subsets distinguished by monoclonal anti-Leu 8. *J Immunol* 1985; 134:2995-3002.
- 47 Kansas GS, Wood GS, Engleman EG. Maturation and functional diversity of human B lymphocytes delineated with anti-Leu 8. *J Immunol* 1985; 134:3003-6.
- 48 Ishizaka K. Twenty years with IgE: from the identification of IgE to regulatory factors for the IgE response. *J Immunol* 1985; 135:I-X.
- 49 Katz DH. Immunologic alteration related to expression of the allergic phenotype. *J Allergy Clin Immunol* 1986; 78:980-7.
- 50 Geha RS, Reinherz EL, Leung D, Mekee KT, Schlossman S, Rosen FS. Deficiency of suppressor T cells in the hyperimmunoglobulin E syndrome. *J Clin Invest* 1981; 68:783-91.
- 51 Leung D, Wood N, Dubey D, Rhodes AR, Geha RS. Cellular basis of defective cell mediated lympholysis in atopic dermatitis. *J Immunol* 1983; 130:1678-82.

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