Immunodeficiency and other clinical immunology

Frequency of allergen-specific T lymphocytes in blood and bronchial response to allergen in asthma

Samuele E. Burastero, MD,[a] Daniela Fenoglio, PhD,[b] Emanuele Crimi, MD,[c] Vito Brusasco, MD,[c] and Giovanni A. Rossi, MD[d] *Milan and Genoa, Italy*

Background: *This study was designed to investigate whether the bronchial response to the sensitizing allergen in asthma is correlated with the frequency of allergen-specific T lymphocytes.*
Methods: *Twenty-three asthmatic patients sensitized to* Dermatophagoides pteryonyssinus *who had never received hyposensitizing therapy and 11 healthy control subjects were studied. Allergen-specific T lymphocytes were enumerated in peripheral blood with limiting dilution cultures. Bronchial challenge with methacholine was performed in all subjects; patients with asthma also underwent an allergen bronchial challenge. Correlations between allergen-specific T cell frequencies and nonspecific bronchial hyperresponsiveness to methacholine as independent variables and early and late bronchial responsiveness to allergen challenge as dependent variables were investigated by means of stepwise-multiple regression analysis.*
Results: *We found that the frequency of allergen-specific T lymphocytes was higher than in control subjects in both patients with asthma with ($p < 0.001$) and those without ($p < 0.05$) late-phase asthmatic response to allergen. Moreover, the provocative does of allergen necessary to produce an early 15% fall of forced expiratory volume in 1 second could be predicted in part (59%) by an equation that incorporates methacholine sensitivity and allergen-specific T cell frequency.*
Conclusions: *We conclude that allergen-specific T lymphocytes, which have an established influence on immunoglobulin E production, play an additional role in the induction of the bronchospastic response to inhaled allergen. (J ALLERGY CLIN IMMUNOL 1993;91:1075-81.)*

Key words: *Airway responsiveness, methacholine challenge, allergen challenge, house-dust mite, T lymphocytes*

Bronchial asthma is characterized by increased airway responsiveness to different stimuli.[1] In allergic asthma the inhalation of the relevant allergen plays a major pathogenic role by triggering an IgE-dependent reaction. The production of allergen-specific IgE by B lymphocytes in response to allergen in vitro is regulated by helper T cells through the release of different cytokines, which have been recently identified.[2] Therefore it can be hypothesized that allergen-specific T cells are involved in the triggering of the asthmatic reaction. Whether a relationship exists between the proportion of allergen-specific T lymphocytes and the bronchial responsiveness to allergen in vivo has not been examined.

This study was designed to investigate whether the bronchial response to the sensitizing allergen in asthma is correlated with the frequency of allergen-specific T lymphocytes. For this purpose, we analyzed the peripheral blood lymphocytes from normal control subjects and from patients with atopic asthma with isolated early or biphasic (early + late) bronchospastic response to inhaled *Dermatophagoides pter-*

From [a]Dipartimento di Biotecnologia, Hospital San Raffaele, Milan; [b]Cattedra di Immunologia, University of Genoa; [c]Dipartimento di Scienze Motorie, Cattedra di Fisiopatologia Respiratoria, University of Genoa; and [d]Divisione di Pneumologia, G. Gaslini Institute, Genoa.
Supported in part by a grant from the Italian Ministry of University and Technological and Scientific Research.
Received for publication April 20, 1992.
Revised Nov. 6, 1992.
Accepted for publication Nov. 18, 1992.
Reprint requests: Samuele E. Burastero, MD, Departement of Biotechnology, Hospital San Raffaele (DIBIT), 60, Via Olgettina, 16132 Milan, Italy.

0091-6749/93 $1.00 + .10 **1/1/44353**

Abbreviations used

Der p I:	Group I allergen *Dermatophagoides pteronyssinus*
EAR:	Early-phase asthmatic reaction
ELISA:	Enzyme-linked immunosorbent assay
FEV_1:	Forced expiratory volume in 1 second
IgE:	Immunoglobulin E
IL:	Interleukin
PBMCs:	Peripheral blood mononuclear cells
Pd_{15}:	Provocative dose causing a 15% fall of FEV_1
RAST:	Radioallergosorbent test
SI:	Stimulation index

onyssinus extract. We found that the frequency of allergen-specific T lymphocytes was abnormally elevated in patients with asthma as compared with control subjects and correlated with the severity of the bronchial response to the allergen.

METHODS

Patients

The study was performed on 22 males and 1 female outpatients (Table I) who had perennial asthma, with or without rhinitis, for the last 2 years or longer. Allergic sensitization to *D. pteronyssinus* was demonstrated in all patients by skin prick test and RAST. Criteria for patients to be included in the study were the following: to be free of symptoms at the time of study, not to have had respiratory infections for the previous 4 weeks, to have a forced expiratory volume in 1 second (FEV_1) equal to or greater than 70% of predicted value,[3] and not to be sensitized to birch pollen extract. None of the patients were currently receiving antiasthmatic treatment other than β_2-stimulants on an "as necessary" basis. These were discontinued at least 12 hours before bronchial challenges. Eleven healthy subjects with no history of allergic disease or asthma, total serum IgE levels less than 100 IU/ml; and negative results of skin prick test and RAST for dust mites were used as control subjects. All subjects were informed of the nature and scope of the study and gave written consent.

Allergen extract

The allergen extract we used for both the bronchial challenge and the T cell assays was obtained from Pharmacia (Uppsala, Sweden) in powder form. Preliminary 12% sodiumdodecylsulfate-polyacrilamide gel electrophoresis, performed according to the method of Laemmli,[4] showed in reducing condition three main bands corresponding to about 27, 29, and 40 kd as determined by silver stain analysis[5] run with proper molecular weight controls. Multiple faint bands were also visible. The amount of *Der p* I content in this extract, as quantified by ELISA with a *Der p* I-specific monoclonal antibody,[6] corresponded to 32% of its dry weight (courtesy of Dr. P. Falagiani, Lofarma Allergeni, Italy). In the allergen proliferation assay that was performed in patients with asthma (see below) a non-relevant allergen was used (purified birch extract).

The allergen extract was tested for its bacterial endotoxin content with the Lymulus amoebocytes gelification test. The assay was performed, on reconstitution of the allergen extract at 1 mg/ml in culture medium, according to the manufacturer's instructions (PBI International, Milan, Italy) and yielded 0.020 endotoxin units per milliliter; which was not significantly different from the amount found in the medium alone (not shown).

Bronchial challenges

Forced expiratory maneuvers were recorded by a wedge spirometer (Vitalograph Ltd., Buckingham, England) and the highest FEV_1 from three technically acceptable maneuvers was retained for analysis. Aerosols were delivered by an ampule dosimeter device (MEFAR, Brescia, Italy) according to methods previously described.[7] Control measures of FEV_1 were determined after inhalation of saline solution. Allergen bronchial challenge was performed with scalar dilutions of *D. pteronyssinus* reconstituted from the dried allergen, which was predosed in arbitrary units by the manufacturer (Pharmacia). Fifteen minutes were allowed between allergen inhalations and FEV_1 measurements. The allergen bronchial challenge was started from a dose of 4 arbitrary units, and the dose was increased in twofold increments until FEV_1 fell below 80% of control or up to a maximum dose of 500 arbitrary units. The twofold increments of allergen dose were obtained by inhalation of doubled allergen concentrations during 70 quiet breaths. This corresponded to an effective inhaled solution of 0.5 ml. FEV_1 was measured after challenge at 30 and 60 minutes and then hourly for 8 hours to detect the occurrence of late-phase asthmatic reaction (LAR). LAR was defined as a decrease of 15% or move in FEV_1 during the 3- to 8-hour period after the initial early-phase asthmatic reaction (EAR). For methacholine challenge, 1 mg/ml, 10 mg/ml, and 50 mg/ml solutions of methacholine in saline solution were prepared. Doubled increasing doses of methacholine were obtained by changing the number of breaths, the methacholine concentration, or both. The challenge was started from a dose of 10 μg with twofold increments until the FEV_1 that was measured 1 minute after

TABLE I. Characteristics of subjects

	Age (yr)	Sex	FEV_1 (% predicted)	RAST PRU	Pd_{15} MCh (μg)	Pd_{15} All (AU)	Max LAR (% control)
Nonatopic subjects							
1	23	100	<0.35	>5000	ND	ND	ND
2	24	F	101	<0.35	>5000	ND	ND
3	34	M	98	<0.35	>5000	ND	ND
4	32	M	89	<0.35	>5000	ND	ND
5	39	M	102	<0.35	>5000	ND	ND
6	43	M	99	<0.35	>5000	ND	ND
7	40	M	87	<0.35	>5000	ND	ND
8	25	M	105	<0.35	>5000	ND	ND
9	26	M	103	<0.35	>5000	ND	ND
10	28	F	112	<0.35	>5000	ND	ND
11	28	F	93	<0.35	>5000	ND	ND
Mean ± SEM	31 ± 2		99 ± 2	<0.35	>5000		
Asthmatic patients with isolated early response							
12	17	M	109	14	693	134	3
13	25	M	79	>17.5	31	260	0
14	19	M	100	6.5	200	46	10
15	24	M	107	17.5	2016	500	5
16	18	M	98	16	337	500	5
17	27	M	111	17.5	1760	500	2
18	19	M	95	>17.5	96	7	5
Mean ± SEM	21 ± 1		100 ± 5	15.2 ± 1	331 GM	144 GM	4 ± 1.5
Asthmatic patients with biphasic response							
19	19	F	111	>17.5	131	75	25*
20	18	M	98	>17.5	70	45	31*
21	17	M	77	>17.5	31	24	30*
22	18	M	100	>17.5	62	20	45*
23	19	M	98	>17.5	105	19	39*
24	19	M	76	17.5	205	28	40*
25	20	M	87	>17.5	20	7	35*
26	19	M	98	>17.5	41	13	28
27	18	M	97	>17.5	433	120	20
28	19	M	79	>17.5	22	20	22
29	28	M	115	3	131	500	39*
30	30	M	105	>17.5	102	46	28
31	20	M	98	14	43	7	26
32	17	M	97	>17.5	59	33	21
33	21	M	103	10	43	10	31
34	18	M	108	7.5	104	19	30*
Mean ± SEM	20 ± 3		96 ± 11	15.2 ± 1	63 GM†	28 GM†	30 ± 2‡

PRU, Peripheral resistance unit; *Max LAR*, maximum fall in FEV_1 3 to 8 hours after challenge; *MCh*, methacholine; *All*, allergen; *Au*, arbitrary units; ND, not done.

*Pharmacologically interrupted.

†$p < 0.01$ versus patients with isolated early response.

‡$p < 0.0001$ versus patients with isolated early response.

inhalation was decreased below 80% of control. The maximum dose at which methacholine challenge was stopped if patients did not respond was 5000 μg. The provocative dose causing a 15% fall of FEV_1 (Pd_{15}) was calculated by interpolation of the dose-response curves. Two hundred micrograms of inhaled albuterol was given immediately after methacholine challenge to relieve brochospasm. LARs were interrupted with

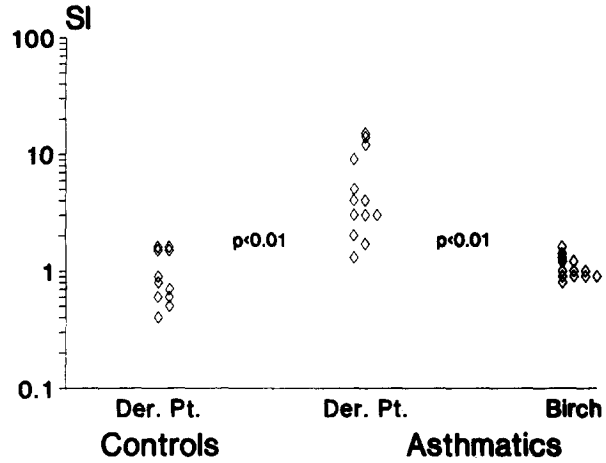

FIG. 1. Proliferation of T lymphocytes to purified extracts of *D. pteronyssinus* (*Der. Pt.*) in control subjects and patients with asthma. Patients with asthma were also tested with a nonrelevant allergen (*Birch*). The stimulation index (*SI*) in patients with asthma tested with *Der Pt* was significantly higher than those in patients with asthma tested with birch and control subjects. Similar results of statistical analysis were obtained with raw data (counts per minute incorporated by cells cultured with allergen, not corrected for spontaneous proliferation of cells cultured with medium alone). Mean values ± standard deviation of background incorporation were 240 ± 71, 274 ± 115, 226 ± 77 in Der. pt. stimulated microcultures from nonsensitized control subjects, in *D. pteronyssinus*-stimulated microcultures from patients with asthma and in birch-allergen–stimulated microcultures from patients with asthma, respectively.

β_2-stimulants and steroid treatment if required by patients or when FEV_1 fell below 60% of control. The methacholine responsiveness was always determined on the day before the allergen challenge day.

Detection and enumeration of T lymphocytes proliferating to allergen extract

Blood was drawn immediately before allergen challenge. Peripheral blood mononuclear cells (PBMCs) were isolated from 20 to 40 ml of heparinized blood on a Ficoll-HiPaque gradient (Pharmacia), then washed twice with RPMI 1640 medium (GRC International, Vienna, Va.) that contained penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine (2 mmol/L). Cells were cultured in this medium supplemented with 5% autologous plasma (hereafter referred to as a complete medium). In experiments in which allergen-specific proliferation was investigated, 0.25 ml cultures that contained 2.5×10^5 PBMCs were performed in quadruplicate in flat-bottomed microtiter plates at 37° C in a humidified atmosphere that contained 5% CO_2. Allergen or mitogen (phytohemagglutinin), used as positive control, was diluted in culture medium and added in a 10 μl volume. Optimal allergen concentration in the culture medium, as assessed in preliminary dose-response assays, was 5 μg/ml both for the *D. pteronyssinus* and the birch extract. DNA synthesis was measured by adding 1 μCi of tritiated thymidine (specific activity, 53 mCi/mmol/L, Amersham, England) per well on day 5. Cells were harvested (Skatron cell harvester, Lier, Norway) after 18-hour pulsing onto glass fiber filters. ^3H incorporation was measured in a liquid scintillation β-counter (LKB, Finland), and results were expressed as geometric mean of quadruplicates. Because of the considerable variability in background proliferation among individuals; the results were expressed as stimulation index (SI). This was defined as the ratio between the score obtained by cultures stimulated with allergen and that obtained by unstimulated cultures.

In limiting dilution experiments, scalar doses of PBMCs (2.5×10^5, 5×10^4, 10^4 2×10^3, 4×10^2 per well) were plated (one plate for each cell dose) in 96-well flat-bottom plates in complete medium that contained 5 μg/ml *D. pteronyssinus* allergen extract. Microcultures seeded with 2.5×10^5 PBMCs were pulsed with 1 μCi/well ^3H-thymidine on day 5 and those with lower cell doses were similarly pulsed on day 8 to optimize detection of allergen-specific proliferation.[8] Cultures were in all cases harvested after overnight pulsing with ^3H-thymidine. Incorporation results were considered positive when scored three times above geometric mean of ^3H-thymidine incorporated in control microcultures (PBMCs with no antigen, in quadruplicates for each cell dose). Experiments were accepted if the mean thymidine incorporation by control microcultures yielded a coefficient of variation of less than or equal to 0.10.

Statistical analysis

Analysis of the results of limiting dilution experiments was performed according to the method of the maximum likelihood.[9] For this purpose, a TI-59 calculator (Texas Instruments, Inc., Dallas, Texas), fitted with the statistic module, was used to evaluate the goodness of fit. Experimental results were accepted only if the goodness of fit test yielded $p > 0.05$.

Provocative doses of bronchial challenge tests and cell precursor frequencies were transformed in logarithmic form. Unpaired Student's t test was used to compare the frequencies of allergen-specific T cells in the different groups of subjects. Stepwise multiple regression analysis was used to assess the relationship between allergen Pd_{15} and maximum late FEV_1 fall as dependent variable and methacholine Pd_{15} and allergen-specific T lymphocytes as independent variables. The predicted values of dependent variables were ob-

TABLE II. Allergen-specific T lymphocytes (responding cells per 10,000)

Nonatopic	Isolated EAR	Biphasic
(4) 3.0	(12)10.0	(19)25.0
(5) 1.0	(13)11.0	(20) 4.0
(6) 0.8	(14)80.0	(24)15.0
(7) 0.6	(15) 4.0	(25) 5.0
(8) 0.9	(16) 1.0	(26)30.0
(9) 3.0	(17) 2.0	(27) 3.0
(10)1.0	(18)10.0	(28)10.0
(11)6.0		(29) 1.5
		(30)20.0
		(31)25.0
		(32) 6.0
		(33) 6.0
		(34)15.0
1.4	4.1*	9.0†

All values are reported as geometric means. Subject identification in parentheses. Linear regression of T cell frequency alone versus allergen-specific Pd_{15} including patients with isolated EAR and EAR + LAR yielded a significant correlation ($r = -0.55$; $p < 0.05$).

*$p < 0.05$ versus nonatopic subjects.

†$p < 0.01$ versus nonatopic subjects.

tained by solving the multiple regression equation with the smallest number of indpendent variables that removed a significant portion of variation. Data are expressed as mean values ± standard error of the mean or geometric mean when indicated.

RESULTS

Baseline values

Physiologic data, RAST results in peripheral resistance units, methacholine and allergen Pd_{15}, and maximum late FEV_1 fall are shown in Table I. In patients with biphasic reaction (EAR + LAR), both methacholine Pd_{15} and allergen Pd_{15} were significantly lower ($p < 0.01$ for both) than in patients with an isolated EAR. The maximum late FEV_1 fall in patients with a biphasic response was significantly ($p < 0.0001$) greater than that in patients with an isolated EAR. Three patients with isolated EAR and one patient with EAR + LAR had borderline reactions (patients nos. 15 and 16, 15% fall in FEV_1; patients nos. 17 and 29, 14% fall in FEV_1).

Allergen-specific T cell proliferation in allergic patients

The proliferation of T cells to *D. pteronyssinus* allergen extract, evaluated in 13 asthmatic patients (7 with EAR and 6 with EAR + LAR) was significantly higher ($p < 0.01$) than in control subjects: SI was (geometric mean) 4.36 in patients with asthma and 0.87 in control subjects (Fig. 1). In the same 13 patients, T cell proliferation to purified birch allergen extract was significantly lower compared with the proliferation to *D. pteronyssinus* (SI: 1.06; $p < 0.01$).

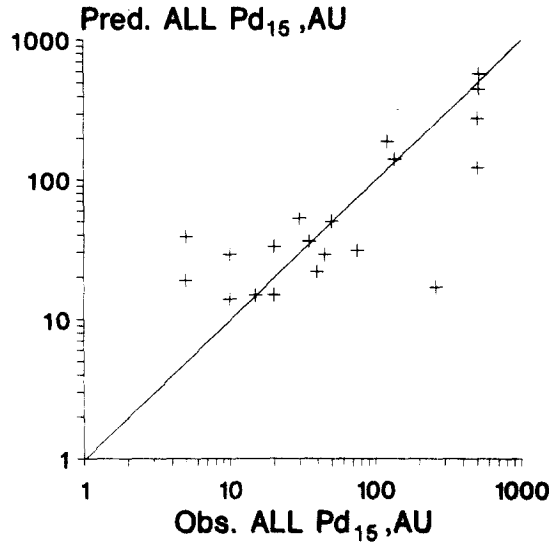

FIG. 2. Comparison between the immediate allergen sensitivity *(ALL Pd_{15})* observed in experiments and that predicted by equation 1. The line of identity is shown.

Frequencies of allergen-specific T lymphocytes

T lymphocytes specific for *D. pteronyssinus* were enumerated in 8 control subjects, 7 patients with isolated EAR, and 13 patients with EAR + LAR (Table II). The frequency of allergen-specific T lymphocytes in both patients with biphasic reaction and those with isolated EAR was higher than in control subjects ($p < 0.001$ and $p < 0.05$, respectively). No significant difference was observed between patients with EAR + LAR and patients with isolated EAR.

Correlation between allergen-specific T lymphocytes and bronchial responsiveness to allergen

In a stepwise multiple regression analysis, allergen Pd_{15} was found to be significantly correlated with methacholine Pd_{15} ($p < 0.005$) and was negatively correlated with allergen-specific T cell frequency ($p < 0.05$). Allergen Pd_{15} was partially predictable ($r^2 = 0.59$) by the following multiple regression equation:

$$\text{Log ALL } Pd_{15} \text{ (arbitrary units)} = 0.795 + 0.65 \times \text{Log MCh } Pd_{15} (\mu g) - 0.49 \times \text{Log T } (10^{-4}) \quad [1]$$

where ALL is allergen, MCh is methacholine, and T is the allergen-specific T cell frequency. A comparison

of the allergen Pd_{15} values predicted by the above model with those observed in experiments is shown in Fig. 2.

The above independent variables were not predictive of the severity of LAR. However, the complete development of LAR was not achieved in all patients, because in nine of them the severity of symptoms required treatment.

Serum-specific IgE, expressed as peripheral resistance units, according to neither single nor multiple regression analysis, was predictive of allergen Pd_{15} ($r = 0.06$; $p > 0.5$). Moreover, serum-specific IgE was not correlated with T cell frequencies ($r = 0.05$; $p > 0.8$).

DISCUSSION

In this study we have found that the frequency of allergen-specific T lymphocytes is increased in blood of patients sensitized to *D. pteronyssinus* compared with that of control subjects and is associated with the bronchial response to allergen.

The production of allergen-specific IgE in patients with atopic asthma likely depends on direct or lymphokine-mediated cooperative events between T and B lymphocytes. Allergen-specific T cells have been isolated from both healthy individuals and patients with atopic asthma.[10, 11] It is also well established that T cells can modulate IgE production by B cells in vitro.[2] However, the relative role of B lymphocytes committed to produce allergen-specific IgE and of allergen-specific T lymphocytes in relation to the response to allergen in vivo has never been studied.

The high levels of serum IgE observed in patients with atopic asthma could result from quantitative and possibly qualitative differences in the allergen-specific T cell compartment, rather than from increased frequency of allergen-specific B cells. Indeed, it was shown that T-cell—derived lymphokines may have different effects on IgE production: IL-4 is required for IgE synthesis by antigen-primed B cells[12] and IL-6 may provide an additional signal to enhance ongoing IgE synthesis,[13] whereas gamma interferon appears to be an antagonist in the cascade of events leading to IgE production.[14] In a recent report[15] allergen-specific T cell clones derived from peripheral blood of atopic donors but not of control subjects were predominantly IL-4 and IL-5 producers, when compared with T cell clones with specificity for common antigens. Moreover, IL-5–mRNA$^+$ T lymphocytes have been identified in bronchial mucosa of patients with atopic asthma, and their number has been correlated both with the severity of symptoms and the proportion of activated eosinophils.[16] As far as B lymphocytes are concerned, by using Epstein-Barr virus transformation and limiting dilution microcultures, it was found that the frequency of B cells committed to produce total and allergen-specific IgE is not different in patients with atopic asthma compared with healthy subjects[17] and among patients with asthma with and without LAR.[18]

Rawle et al.[8] showed that peripheral blood lymphocytes from patients sensitized to *D. pteronyssinus*, on in vitro allergen challenge, proliferated more than cells from control subjects, and they attributed this phenomenon to T lymphocytes. The data reported here in part confirm those results. The important new finding of this study is that the frequency of specific T lymphocytes correlates with the bronchical responsiveness to allergen in vivo. It has been previously shown[4] that the degree of allergic sensitization, as expressed by the specific IgE serum level contributes together with methacholine responsiveness to the bronchospastic response to inhaled allergen. In addition, the lymphocytes together with the eosinophils present in bronchoalveolar lavage fluid have been shown to contribute to the increase in methacholine sensitivity, which precedes the occurrence of the late-phase response.[19] The results of the present study extend these observations and show that in patients with a narrow range of IgE serum level, the allergen-specific T cells act as a further determinant in the modulation of the early bronchial response to allergen in vivo. The available data do not allow conclusive explanation of the mechanisms through which this process occurs but suggest that T cells can modulate the bronchial response to allergen in vivo through additional mechanisms other than the enhancement of specific IgE production.

Some cautions should be used in interpreting our results. First, the lymphocytes we have studied were obtained from peripheral blood and not from the target organ. However, recent reports suggest that in allergic asthma the T cell repertoire is at the same time unbalanced both in blood and at the bronchial level.[20, 21] Indeed, a recirculation of T lymphocytes from airway to peripheral blood has already been demonstrated in other hypersensitivity lung disorders such as berylliosis.[22] Second, we used a *D. pteronyssinus* preparation that contained multiple determinants as identified in polyacrylamide gel electrophoresis instead of an allergen extract that contained a single determinant. We made this decision because in patients with atopic asthma the repertoire of allergen-specific T lymphocytes likely includes specifications to multiple allergen determinants. Moreover, this preparation was clearly able to discriminate between sensitized and nonsensitizied individuals in terms of specific T cell proliferation. Finally, its extremely low

endotoxin content (0.02 IU/ml) was not different from that found in the culture medium, which makes an unpredictable effect on antigen-presenting cells of patients with atopic asthma versus control subjects unlikely.

In summary, we have demonstrated that in patients with atopic asthma the frequency of allergen-specific T lymphocytes in peripheral blood is increased and correlates with the severity of the bronchial response to allergen inhalation. These data suggest that allergen-specific T lymphocytes, together with hyperresponsiveness to methacholine, contribute to modulate the asthmatic reaction to inhaled allergen. Functional characterization of T cell clones derived from peripheral blood and from bronchial biopsy specimens or lavage may provide further insights into this issue.

REFERENCES

1. American Thoracic Society. Chronic bronchitis, asthma and pulmonary emphysema. Am Rev Respir Dis 1962;84:762-8.
2. O'Hehir RE, Garman RD, Greenstein JL, Lamb JR. The specificity and regulation of T-cell responsiveness to allergens. Ann Rev Immunol 1991;9:67-95.
3. Quanjer PH. Standardized lung function testing. Report on working party on standardization of lung function tests. European Community for Coal and Steel. ECCS Bull Eur Physiopathol Respir 1983;19(suppl 5):1-94.
4. Laemmli UK. Cleavage of structural proteins during the assembly of the bacteriophage T4. Nature 1970;227:680-5.
5. Merril CR, Goldman D, Van Kenren ML. Gel protein stains: silver stain. Methods Enzymol 1984;104:441-9.
6. Chapman MD, Heymann PW, Platts-Mill TAE. Epitope mapping of two major inhalant allergens, DerP1 and Der F1 from mites of the genus *Dermatophagoides*. J Immunol 1988; 139:1479-84.
7. Crimi E, Brusasco V, Losurdo E, Crimi P. Predictive accuracy of late asthmatic reaction to *Dermatophagoides pteronyssinus*. J ALLERGY CLIN IMMUNOL 1986;78:908-13.
8. Rawle FC, Mitchell EB, Platts-Mill TAE. 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.
9. Fazekas de St. Groth S. The evaluation of limiting dilution analysis. J Immunol Methods 1982;49:R11-23.
10. German RD, Goodwin W, Lussier AM, et al. The allergen specific T cell responses of atopic and non-atopic individuals [Abstract]. J ALLERGY CLIN IMMUNOL 1990;85:200.
11. Lanzavecchia A, Santini P, Maggi E, et al. *In vitro* selective expansion of allergen specific T cells from atopic patients. Clin Exp Immunol 1983;52:21-8.
12. Finkelman FD, Holmes J, Katona IM, et al. Lymphokine control of immunoglobulin *in vivo* isotype selection. Ann Rev Immunol 1990;8:303-33.
13. Maraguchi A, Hirano T, Tang B, et al. The essential role of B cell stimulatory factor 2 (BSF-2/IL6) for terminal differentiation of B cells. J Exp Med 1988;67:332-44.
14. Vercelli D, Jabara HH, Lauener RP, Geha RS. IL4 inhibits the synthesis of gamma IFN and induces the synthesis of IgE in human mixed lymphocyte cultures. J Immunol 1990;144: 570-3.
15. Parronchi P, Macchia D, Piccinni M-P, et al. Allergen and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. Proc Natl Acad Sci U S A 1991;88:4538-42.
16. Hamid Q, Azzawa M, Ying S, et al. Expression of mRNA for interleukin 5 in mucosal bronchial biopsies from asthmatics. J Clin Invest 1991;87:1541-6.
17. Martinez-Maza O, Guilbert B, David B, Avrameas S. The Epstein-Barr virus induced production of IgE by human B cells. Clin Immunol Immunopathol 1986;39:405-13.
18. Crimi E, Burastero SE, Brusasco V, Fenoglio D, Rossi GA. Circulating allergen-specific B and T lymphocytes in asthma [Abstract]. Presented at American Thoracic Society International Conference, May 17-20, 1992, Miami Beach, Florida.
19. Brusasco V, Crimi E, Gianiorio P, Lantero S, Rossi GA. Allergen-induced increase in airway responsiveness and inflammation in mild asthma. J Appl Physiol 1990;69:2209-14.
20. Gonzalez C, Diaz P, Galleguillos F, Ancic P, Cromwell O, Kay AB. Allergen induced recruitment of bronchoalveolar T cells (OKT4) and suppressor T cells (OKT8) in asthma: relative increases in OKT8 cells in single-early compared to late-phase responses. Am Rev Respir Dis 1987;136:600-4.
21. Gerblich AA, Salik H, Schuyler MR. Dynamic T-cell changes in peripheral blood and bronchoalveolar lavage after antigen bronchoprovocation in asthmatics. Am Rev Respir Dis 1990;143:533-7.
22. Saltini C, Helmer ME, Crystal RG. Lymphocytes. In: Crystals RB, West JB, eds. The lung: scientific foundations. New York: Raven Press, 1991:459-82.