

T-lymphocyte responses to plicatic acid–human serum albumin conjugate in occupational asthma caused by western red cedar

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Background: T cells are known to play a major role in the pathogenesis of atopic allergic asthma, but it is less clear whether they are involved in occupational asthma caused by low molecular weight chemicals such as plicatic acid.

Objectives: We sought to determine whether peripheral blood T cells from patients with western red cedar asthma (WRCA) recognize plicatic acid (PA) conjugated to human serum albumin (HSA) as judged by proliferation or cytokine production and to analyze the response to PA inhalation with flow cytometry.

Results: Significant proliferative responses to PA-HSA were observed in eight of 33 patients with WRCA, none of 10 exposed nonasthmatic cedar workers, and one of 18 nonasthmatic control subjects. Two of 25 patients with WRCA also showed proliferative responses to unconjugated PA. All the WRCA responders were either currently exposed to cedar or had ceased exposure within the preceding 2 years. None of the four patients receiving oral steroids responded, but inhaled steroids did not seem to influence responsiveness. No correlations were found between the maximum stimulation response and any of the current FEV₁ values, the current PC₂₀ methacholine values, or the magnitude of the late asthmatic response to PA. Peripheral blood T-cell subset proportions and their degree of activation were similar in patients with WRCA and exposed control subjects. There was no change in T-cell phenotypes or their activation markers after PA inhalation challenge. In vitro, PA-HSA stimulation did not affect subset ratios but led to release of small amounts of IL-5 and IFN- γ , with no detectable increase in IL-4.

Conclusions: PA-HSA-specific T lymphocytes seem to be present in small numbers in the peripheral blood of patients with WRCA and may respond to antigenic exposure by producing IFN- γ and IL-5. However, the proportion of responding cells would appear to be lower than in comparable studies of atopic asthma. (*J Allergy Clin Immunol* 1998;101:841-7.)

Key words: Asthma, T-lymphocytes, cell-mediated immunity, occupational asthma, immediate hypersensitivity, cytokines, flow cytometry

Western red cedar asthma (WRCA) is caused by sensitization to plicatic acid (PA), a low molecular weight organic compound, which constitutes 40% wt/wt of cedar dust.¹ In sensitive subjects, inhalation of PA induces bronchoconstriction and elicits immediate responses, late-phase responses, or both analogous to those observed in other forms of allergic asthma.^{2,3} Mast cells and their chemical mediators have been implicated in WRCA because histamine and leukotrienes can be detected in bronchoalveolar lavage fluid after inhalation of PA.⁴ Similarly, peripheral blood basophils and bronchial mast cells from patients with WRCA release histamine when exposed in vitro to PA or to PA conjugated to human serum albumin (PA-HSA), whereas cells from atopic and nonasthmatic subjects do not.⁵ Antigen-induced histamine release from basophils or mast cells has traditionally been regarded as evidence of antigen-specific IgE,⁶ but specific IgE binding to PA-HSA is only detectable in a minority of patients with WRCA,^{5,7} and a recent series of in vitro studies failed to show any role for IgE in PA-induced basophil histamine release.⁵ In common with other (nonoccupational) forms of asthma, patients with WRCA have increased numbers of activated T-cells and eosinophils in their bronchial mucosa.⁸ It is now clear that T lymphocytes may play a central role in asthma. T lymphocytes are important not only in regulating the production of specific IgE antibodies but also may play a direct role in inducing airway inflammation through the production of cytokines.⁹ It has also been suggested that T lymphocytes play a direct role in isocyanate-induced asthma, another form of occupational asthma caused by a low molecular weight compound.¹⁰ Taken together, these studies raise the possibility that WRCA may be caused by, in part, a direct response of proinflammatory T cells to PA or to PA hapten-conjugates. In this study evidence of PA-specific T cells in the blood of patients with WRCA has been sought by using lymphocyte proliferation, flow cytometry, and in vitro cytokine production assays.

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Abbreviations used

HAS:	Human serum albumin
PA:	Plicatic acid
PBMC:	Peripheral blood mononuclear cell
WRCA:	Western Red Cedar Asthma

METHODS**Chemicals**

Unless otherwise stated, all chemicals were purchased from Fisher Scientific Ltd. (Vancouver, British Columbia, Canada). HSA, ficoll, and antibiotics were purchased from Sigma Chemical Co. (St. Louis, Mo.). PA was prepared from western red cedar dust by Forintek (Vancouver, British Columbia, Canada). PA-HSA was prepared as previously described,⁷ with approximately 25 PA molecules per albumin molecule (PA:albumin ratio, 13% wt/wt). RPMI-1640 culture medium (Gibco, Gaithersburg, Md.) with 2 mmol/L L-Glutamine was purchased from Sigma Chemical Co. and supplemented with 10% heat-inactivated human AB serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin.

Subjects

Patients with WRCA were recruited from the University of British Columbia Respiratory Clinic at Vancouver General Hospital. All had developed symptomatic asthma while working with western red cedar, and sensitivity to PA had previously been confirmed by formal bronchoprovocation testing with PA.³ Briefly, patients were asked to discontinue all medication overnight, and underwent a sham challenge with saline diluent. Provided their FEV₁ remained stable $\pm 10\%$ over the next 24 hours, they then underwent challenge with serial dilutions of PA. A positive response was defined as a 20% or greater fall in FEV₁ within 10 hours of challenge. The inhalation challenge test was terminated if the subject failed to show a fall in FEV₁ of greater than 20% after inhaling PA at a concentration of 5 mg/ml. The mean magnitude of the late asthmatic reaction in these patients was $28.5\% \pm 2.4\%$ of baseline FEV₁. Thirty-three such subjects were recruited for the proliferation assays (mean age, 42.4 years [range, 28 to 54 years]; FEV₁, $87\% \pm 3.3\%$ predicted [mean $\pm$ SEM]). A further nine patients with WRCA were recruited for the flow cytometry and cytokine assays (mean age, 32.1 years; FEV₁, $94.1\% \pm 3.2\%$ predicted). As controls for the proliferation assay, 18 nonasthmatic control subjects were recruited from laboratory personnel and hospital staff, and none had any symptoms of asthma (mean age, 28.6 years [range, 22 to 38 years]). Ten nonatopic asymptomatic individuals who were exposed to PA but not sensitized were also recruited as control subjects. The control group for the flow cytometry and cytokine assays consisted of a group of eleven such exposed asymptomatic individuals (mean age, 38.6 years; mean FEV₁, $94.8\% \pm 4.4\%$ predicted).

Serology

Total serum IgE was determined by a radioimmunoassay kit (Pharmacia, Uppsala, Sweden). Specific IgE antibodies to PA-HSA conjugate were determined by paper RAST (Pharmacia, Uppsala, Sweden).

Proliferation assay

Peripheral blood mononuclear cells (PBMCs) were prepared from heparinized venous blood by dextran sedimentation and

density gradient separation over Ficoll. The interface cells were washed twice in RPMI and adjusted to 1.33×10^6 cells/ml. One hundred fifty microliter aliquots (2.0×10^5 cells) were plated out in triplicate in 96-well round-bottomed tissue-culture plates (Corning), with the addition of 50 µl medium containing antigen or mitogen to give final concentrations of PA-HSA from 1 to 100 µg/ml; unconjugated PA of 10, 30, or 100 µg/ml; PHA of 10 and 25 µg/ml; and Con A of 10 and 50 µg/ml. Separate culture plates were cultured in a humidified CO₂ incubator (5% CO₂) for 5, 6, or 7 days. All subjects were studied at 6 days, but in some cases there were insufficient cells to allow study at the additional time points of 5 and 7 days. Proliferation was detected by the addition of 50 µl tritiated thymidine (6 µCi/ml) (Amersham) for the last 18 hours of culture. Cells were harvested onto nitrocellulose filter paper with a Brandel M-12 plate harvester (Gaithersburg, Md.) and counted in a liquid scintillation β-counter. Results were expressed as the geometric means of triplicate cultures and expressed as stimulation ratios (proliferation with mitogen or antigen divided by thymidine incorporation with buffer alone).

Lymphocyte separation and culture

Thirty milliliters of heparinized blood was taken from each subject before and 24 hours after the PA inhalation challenge test. Mononuclear cells were isolated by a standard density gradient centrifugation technique with Histopaque 1077 (Sigma). The cells were washed twice in Tyrode's buffer solution and resuspended at 1×10^6 /ml for culture in RPMI 1640 supplemented with 10% pooled AB+ serum and the following antibiotics: 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin. The cells for in vitro testing were cultured at 1×10^6 /ml in each well on flat-bottomed 10-well plates (Corning), with and without PA-HSA conjugate at 100 µg/ml, for 6 days in a 5% CO₂ humidified incubator. Con A was used as a positive control at a concentration of 25 µg/ml. Cell viability was checked before and after culture by trypan blue exclusion. The optimal concentration of PA-HSA in the culture medium was determined from the proliferation assays.

Flow cytometry

After 6 days of culture, cells were washed and resuspended in RPMI 1640 with penicillin (100 U/ml) and 2% fetal calf serum at a concentration of 2×10^6 /ml. Cells were then stained with appropriate monoclonal antibodies and analyzed on an Epics Profile flow cytometer (Coulter Electronics). Monoclonal antibodies for CD3, CD4, CD8, and CD45 were conjugated with phycoerythrin, and those for CD25 and HLA-DR were conjugated with fluorescein isothiocyanate (Becton Dickinson, San Jose, Calif.).

Determination of cytokines

Supernatants were obtained after 5 days and stored at -70°C until assayed. Cytokine assays were determined in duplicate by using an ELISA method. IL-2 and IL-4 were assayed with kits from Amersham, IL-5 with kits from R&D Systems (Minneapolis, Minn.), and IFN-γ with kits from Endogen Inc. (Boston, Mass.). The concentration of cytokines in the supernatant was determined from corresponding standard curves. The limits of detection were as follows: IL-2, 6 pg/ml; IL-4, 3 pg/ml; IL-5, 1 pg/ml; and IFN-γ, 2 pg/ml.

Ethical approval

These studies were approved by the Ethics Committee of the University of British Columbia. Informed consent was obtained from each subject before the study.

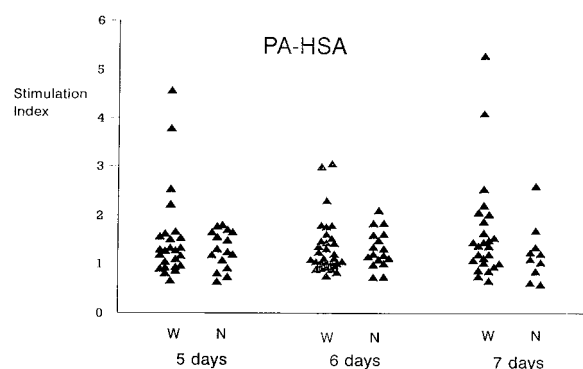


FIG. 1. Proliferative responses of PBMCs from patients with WRCA ($n = 33$) and normal control subjects ($n = 18$) stimulated for 5, 6, or 7 days, with range of concentrations of PA-HSA conjugate (1 $\mu\text{g/ml}$ to 100 $\mu\text{g/ml}$). All subjects were tested at 6 days. Where sufficient cells were available, cultures were also performed for 5 and 7 days. Values shown are maximum stimulation indices obtained for that subject on each day. W, Patients with WRCA; N, normal control subjects.

Analysis

In the proliferation assay stimulation ratios greater than 2.0 were regarded as positive, and statistical analysis was performed by Chi square procedure. Group comparisons were made by the nonparametric Mann-Whitney procedure. For correlation analysis, all values were used without transformation in a standard parametric Pearson correlation procedure. The PC_{20} methacholine results, T lymphocyte and subset numbers, and cytokine concentrations were not normally distributed. Nonparametric analyses were carried out with the Kruskal-Wallis method for comparison between multiple groups, the Mann-Whitney method for differences between two groups, and the Wilcoxon rank-sum test for comparison of paired data.¹¹

RESULTS

Proliferation assays

Among the nonasthmatic control subjects, the mean stimulation index for all doses of the PA-HSA conjugate for nonatopic and atopic control subjects was 1.09 ± 0.37 (mean $\pm$ SD). The conventional cutoff for assessing responsiveness is the control mean + 2 SD, which would be 1.83 in this instance, but for convenience, a stimulation index of 2.0 on any day was regarded as the cutoff criterion for positive response. By this criterion, PBMCs from six of 18 currently exposed patients responded to PA-HSA, whereas only one of 18 control subjects responded ($\chi^2 = 4.43$, $p < 0.05$) (Fig. 1). None of the 10 exposed asymptomatic control subjects responded. There were two responders among the eight patients who had ceased exposure within 2 years of the study, but none of the seven patients who ceased exposure more than 2 years before the study responded. A restricted analysis of current and recently exposed subjects showed no correlations between maximum proliferative response and FEV_1 values, PC_{20} methacholine values, or the magnitude of late-phase response to PA inhalation in bronchoprovocation tests (r values = 0.12, -0.09, and 0.008, respectively).

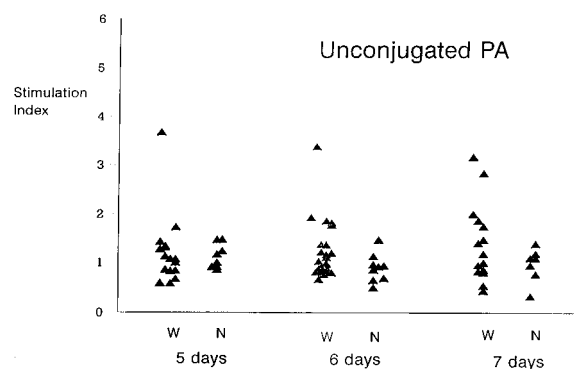


FIG. 2. Proliferative responses of PBMCs from patients with WRCA ($n = 25$) stimulated for 5, 6, or 7 days, with range of concentrations of unconjugated PA (10 $\mu\text{g/ml}$ to 100 $\mu\text{g/ml}$). Values shown are maximum stimulation indices obtained for that subject on each day. W, Patients with WRCA; N, normal control subjects.

Four of the patients with WRCA were receiving oral corticosteroids, and none of these showed proliferative responses to PA-HSA. Two of these were currently exposed, one had ceased exposure 17 months previously, and one had not been exposed for 30 months. Seven of the eight responders were taking inhaled steroids as were 16 of the 21 remaining nonresponders ($p =$ not significant). After stratifying for steroid effects, the numbers are insufficient for detailed analysis of the influence of other medication on response.

As a control for specificity, proliferative assays were also performed with unconjugated PA. Two subjects showed a proliferative response greater than 2.0 (Fig. 2). The remaining assays did not show any significant proliferative response above background, suggesting that most of the positive *in vitro* responses found with PA-HSA were due to recognition of the hapten-conjugate complex and not to recognition of unconjugated PA alone or to any mitogenic effect of PA.

Flow cytometry

Before challenge, patients had significantly more $\text{CD}3^+$ and significantly fewer $\text{CD}4^+\text{CD}25^+$ T cells compared with the control subjects (Table I). No changes in the proportions of T-cell subsets or their expression of markers were observed after inhalation challenge in patients and control subjects as compared with prechallenge values. Exclusion of atopic subjects from the analysis did not influence the results.

Subsets of T lymphocytes and their activation markers in stimulated and unstimulated cultures are shown in Table II. There were no differences between patients with WRCA and control subjects as regards the percentages of T cells expressing subset or activation markers with or without stimulation, with the exception of $\text{CD}4^+\text{CD}25^+$ and $\text{CD}8^+\text{CD}25^+$ T cells, which were both significantly lower in patients compared with control subjects. Cells from patients with WRCA showed no increase in the proportions of activated T cells within

TABLE I. Peripheral blood T-lymphocyte subsets and activation markers in patients with WRCA and exposed, nonsensitized control subjects before and 24 hours after inhalation challenge with PA

Marker	Patients (n = 9)		Control subjects (n = 5)	
	Before	After	Before	After
CD3 ⁺ (% of CD45 ⁺)	91.3 ± 5.2	91.0 ± 5.1	91.9 ± 3.3	87.6 ± 9.8
CD4 ⁺ (% of CD3 ⁺)	78.0 ± 4.6	74.2 ± 4.3	70.2 ± 4.7*	68.5 ± 14.4
CD8 ⁺ (% of CD3 ⁺)	42.4 ± 14.4	43.8 ± 10.1	40.8 ± 11.8	39.7 ± 12.2
CD4 ⁺ CD25 ⁺ (% of CD3 ⁺)	9.6 ± 4.8	10.2 ± 3.5	16.6 ± 5.0*	15.2 ± 6.3
CD8 ⁺ CD25 ⁺ (% of CD3 ⁺)	1.3 ± 0.8	1.4 ± 0.6	3.4 ± 3.2	2.2 ± 1.3
CD4 ⁺ :CD8 ⁺ ratio	1.4 ± 0.8	1.3 ± 0.6	1.7 ± 0.7	1.7 ± 0.7
HLA-DR ⁺ (% of CD45 ⁺)	18.6 ± 7.3	18.6 ± 7.7	19.5 ± 5.1	19.9 ± 3.4
CD3 ⁺ DR ⁺ (% of CD45 ⁺)	11.4 ± 5.4	10.8 ± 5.2	10.7 ± 3.0	10.3 ± 2.8

All values shown as geometric means ± SD.

**p* < 0.05 by Mann-whitney test, comparing patients and control subjects.

each subset after stimulation in vitro with PA-HSA conjugate as opposed to unstimulated control subjects. When atopic subjects from both groups were excluded from the analysis, the results were unaffected.

Profile of cytokine release

Cytokine release was examined after stimulation in vitro with PA-HSA conjugate or the polyclonal mitogen Con A (Table III). There were no significant differences between patients and control subjects in the amount of IL-4, IL-5, or IL-2 released after stimulation with Con A, but cells from patients with WRCA released more IFN- γ in these polyclonal cultures. Stimulation with the PA-HSA conjugate induced much less cytokine than was seen after Con A. The amount of IL-5 released by cells from subjects with WRCA cultured with PA-HSA was increased relative to the unstimulated controls (3.8 ± 1.3 vs 1.4 ± 0.9 pg/ml, *p* < 0.05). Compared with cells from the control subjects, cells from patients with WRCA released more IFN- γ (42.7 ± 16.7 vs 0.5 ± 0.5 pg/ml, *p* = 0.05) and more IL-2 (7.9 ± 9.9 vs 0.0 ± 0.0 pg/ml, *p* < 0.05) after culture with PA-HSA, reflecting the higher level of release of these two cytokines in the unstimulated cultures. IL-4 was not detected in supernatants from either set of cells. Once again, exclusion of atopic subjects from the analysis did not affect the results.

IL-2, IL-4, and IL-5 were not detected in the serum of patients or control subjects before or after inhalation challenge with PA.

DISCUSSION

In this study PBMCs from one third of patients with WRCA (33%) proliferated when stimulated with PA-HSA conjugate. Although the magnitude of the proliferative response was generally low, proliferation appeared to be disease-specific in that only one of 18 control subjects responded to PA-HSA. Only two of 25 patients showed a response to PA alone, consistent with previous assumptions that PA acts as a hapten and does not directly stimulate a T-cell response in its unconjugated form. No biologically significant differences were

found in the proportions of T-lymphocyte subsets in the peripheral blood or in the surface expression of activation markers before or after inhalation challenge with PA. There was, if anything, a lower degree of T-cell activation in patients with WRCA than in the control group. Stimulation with PA-HSA conjugate in vitro did not affect subset-specific expression of activation markers, but there was a small increase in IL-5 and IFN- γ production by T cells from patients with WRCA, with no difference in IL-4 or IL-2 production. Taken together, these studies suggest that there may be a small number of T cells that recognize PA-HSA in the peripheral blood of patients with WRCA and that these cells respond with production of IFN- γ and IL-5 when stimulated.

Few studies have addressed T-cell responses to low molecular weight occupational allergens. Proliferative responses have been reported to the metals nickel and cobalt, both of which can induce occupational asthma.^{12, 13} Blast transformation after culture with toluene diisocyanate-HSA has been reported in seven of eight patients with isocyanate-induced asthma,¹⁴ whereas in another study cells from nine of 15 patients with isocyanate-induced asthma produced "lymphocyte inhibitory factor" in culture.¹⁵ Three of 14 patients with anhydride-induced occupational asthma showed proliferative responses to a trimellitic anhydride-HSA conjugate,¹⁶ but animal data suggests that the acute pulmonary response to anhydrides is largely mediated by antibody.¹⁷

At first sight it may seem puzzling that only a minority of patients with WRCA should respond in the proliferation assay. Responsiveness in in vitro proliferation assays depends on several factors, including the proportion of lymphocytes recognizing the inducing antigen and their activation status, as well as technical factors such as the duration of the assay, the type of plate used, and the presence of human or heterologous sera.¹⁸ The present data suggest that, as in isocyanate-induced asthma,¹⁵ the specific reactivity of T cells declines after cessation of exposure, although the cross-sectional design does not allow a firm conclusion to be drawn. With

TABLE II. Expression of T-lymphocyte subset and activation markers in PBMCs from patients with WRCA and exposed, nonsensitized control subjects after 6 days in vitro culture with or without PA-HSA conjugate

Marker	Patients (n = 9)		Control subjects (n = 11)	
	Unstimulated	Stimulated	Unstimulated	Stimulated
CD3 ⁺ (% of CD45 ⁺)	80.8 ± 5.9	80.2 ± 6.9	85.0 ± 9.0	78.0 ± 15.3
CD4 ⁺ (% of CD3 ⁺)	58.5 ± 13.5	59.3 ± 13.7	65.8 ± 9.8	73.9 ± 28.5
CD8 ⁺ (% of CD3 ⁺)	35.2 ± 12.8	35.4 ± 13.2	34.0 ± 10.3	38.8 ± 22.5
CD4 ⁺ CD25 ⁺ (% of CD3 ⁺)	6.7 ± 2.6	6.7 ± 2.6	7.6 ± 2.2*	9.0 ± 3.5
CD8 ⁺ CD25 ⁺ (% of CD3 ⁺)	0.6 ± 0.3	0.6 ± 0.4	1.4 ± 0.9*	1.6 ± 1.7
CD4 ⁺ :CD8 ⁺ ratio	2.0 ± 1.3	2.1 ± 1.5	2.2 ± 0.9	2.2 ± 0.8
HLA-DR ⁺ (% of CD45 ⁺)	20.6 ± 6.5	21.9 ± 8.0	22.0 ± 6.2	23.8 ± 4.9
CD3 ⁺ DR ⁺ (% of CD45 ⁺)	14.8 ± 5.1	16.7 ± 6.5	15.8 ± 6.3	18.0 ± 5.6

All values shown as geometric means ± SD.

**p* < 0.05 by Mann-whitney test, comparing patients and control subjects.

low molecular weight allergens, there is also the question of the carrier used for haptentation. Recognition of haptens by B cells or T cells depends on the nature of the carrier used for haptentation because it is usually a compound determinant that is recognized, with limited cross-reactivity between different conjugates.^{14, 19-21} Most in vitro studies of low molecular weight occupational allergens have used human serum albumin for convenience, ready availability, and ease of conjugation. These reasons influenced the choice of human serum albumin in this study, and in addition, it is known that a minority of patients with WRCA have IgE antibodies in their serum directed against PA-HSA.⁷ However, it is possible that other hapten-carrier combinations arise in vivo, in which case some of the nonresponders in this study might in fact be capable of recognizing and responding to PA bound to other proteins.

The failure to demonstrate a significant shift in activated T cells by flow cytometry reflects the inherent difficulties in detecting a small responding population within a much larger nonresponding background population, even after culture with PA-HSA for 6 days in vitro. From other studies, the proportion of peripheral blood T cells that recognize airborne allergens is low, in the region of 20 to 100 per million for the house dust mite *Dermatophagoides pteronyssinus*, which contains many more antigenic epitopes than PA-HSA.²² Also, CD25⁺ T cells usually appear within 2 days of stimulation and disappear by 4 days.²³ Nevertheless, in our parallel study of patients with house dust mite allergy,²⁴ we were able to demonstrate T-lymphocyte activation in cells that had been cultured with house dust mite allergen for 6 days. Another factor to take into consideration is the effect of inhaled steroids. Most of the patients with WRCA in this study were using inhaled steroids (seven of nine), whereas in our house dust mite study the subjects were steroid-free. Finally, there is the problem of sampling a distal compartment, which may not reflect what is going on in the lung.²⁵

In our study of subjects with house dust mite allergy, we found that peripheral blood T lymphocytes of those who had a positive bronchial reaction to house dust mite

TABLE III. Cytokine release in vitro after stimulation with Con A or PA-HSA conjugate of T lymphocytes from patients with WRCA or nonasthmatic control subjects

Group	Cytokine	Unstimulated	PA-HSA	Con A
WRCA	IL-2	3.5 ± 2.0	7.9 ± 9.9*	311 ± 192
	IFN-γ	38.4 ± 16.2	42.7 ± 16.7*	3551 ± 358‡
	IL-4	0.0 ± 0.0	0.0 ± 0.0	16.2 ± 4.5
	IL-5	1.8 ± 0.8	3.8 ± 1.3†	578 ± 108
Control	IL-2	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 1.8
	IFN-γ	1.85 ± 1.36	0.5 ± 0.5	1234 ± 268
	IL-4	0 ± 0	0 ± 0	7.3 ± 3.5
	IL-5	0.0 ± 0.0	1.4 ± 0.9	453 ± 158

1 × 10⁷ PBMC, cultured for 6 days; results shown as means ± SEM (pg/ml).

**p* < 0.05, comparing patients with control subjects.

†*p* < 0.05.

‡*p* < 0.01 compared with unstimulated control.

extract had a higher proportion of CD4⁺CD25⁺ T cells when stimulated by the same allergen in vitro compared with those with a negative inhalation response.²⁴ Patients with WRCA are mostly nonatopic; among the nine patients studied by flow cytometry, only one was atopic and removing this subject from the analysis did not influence the results. In our dust mite study,²⁴ the amount of IL-5 released in the supernatant was a significant predictor of the bronchial response. The majority of T-cell clones obtained from bronchial biopsy specimens taken after inhalation challenge with toluene diisocyanate were CD8⁺, and they all produced IFN-γ, whereas 43% also produced IL-5, but only 12% produced any IL-4.²⁶ Consistent with this, CD8⁺ cells were increased in bronchial biopsy specimens from several of the patients with WRCA in our histologic study of WRCA.⁸ This association of CD8⁺ cells with occupational asthma to low molecular weight chemicals contrasts with the dominance of CD4⁺ T cells in atopic asthma.²⁷⁻³⁰

Although at first sight it may seem surprising that T cells produce IL-5 and IFN-γ, it is becoming clear that

the patterns of cytokine production by human T cells is more heterogeneous than that found in the mouse.^{31, 32} First, IL-4 and IL-5 are rarely coproduced,³³ and second, asthma is in fact associated with an increased proportion of airway T cells that produce IFN- γ protein when stimulated,³⁴ even though the mRNA profile of bronchoalveolar lavage T cells is consistent with a T_{H2}-type profile.²⁹ Furthermore, it is now becoming apparent that CD8⁺ T cells also exhibit varied cytokine patterns, with Tc1 and Tc2 phenotypes³⁵ to set alongside the established T_{H1} and T_{H2} phenotypes.³⁶ The Tc2 phenotype produces IL-5³⁵ or both IFN- γ and IL-5³⁶ and may thus be the cell type stimulated by PA-HSA in this study. Finally, animal knockout studies and various in vivo models have shown that although IL-4 (or IL-13 in human beings) is needed for B cells to switch over to make IgE,³⁷ it is IL-5 that is required for the expression of asthma and for the development of hyperreactivity after allergen challenge.³⁸ IFN- γ may also contribute to the hyperreactivity seen after allergen challenge.³⁹

In conclusion, T lymphocytes of approximately one third of patients with WRCA can recognize PA-HSA conjugates, and the PA-HSA-stimulated cells release increased amounts of IL-5 and IFN- γ , cytokines which could contribute to the bronchial mucosal inflammation seen in WRCA.⁸ Flow cytometric analysis did not reveal any fundamental differences between patients with WRCA or control subjects in their T-cell subset proportions or expression of activation markers in peripheral blood. Inhalation challenge with PA did not affect these parameters. It seems that the numbers of peripheral blood T cells recognizing PA-HSA must be relatively small, but their behavior is consistent with activation of the Tc2-like subset. Sampling of the blood carries the risk of overlooking phenomena that are principally confined to the lung.²³ Further studies will need to address which T-cell subset the responding cells belong to and whether such cell populations can be identified in the airways of patients with WRCA.

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