

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

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Peripheral blood mononuclear cells (PBMC) from the majority of the allergic patients that we tested who were skin test-sensitive to the house dust mite *Dermatophagoides pteronyssinus* (*D. pt.*) (46/67) showed significant proliferation in response to the purified major allergen Antigen P₁; PBMC from 14/15 nonsensitive controls showed no significant response. Optimal responses were seen with 10 µg Antigen P₁/ml, but 21 of 43 patients tested showed significant proliferation at 0.01 µg Antigen P₁/ml. The results of three types of experiments showed that the responding cells were T cells, predominantly of the helper phenotype. First, purified T cells in the presence of irradiated or mitomycin C-treated antigen-presenting cells showed good proliferation to Antigen P₁. Secondly, flow cytometry analysis showed a progressive increase in the percentage of viable cells bearing the Leu-3a marker—up to 88% by day 7—and demonstrated that the larger, blast-transformed cells bore this marker. Finally, interleukin 2 production was demonstrated in antigen-stimulated cultures at days 3 to 5, i.e., about 2 days before the peak of proliferation. When patients were grouped according to their disease symptoms, no clear differences were seen between the T cell responses of patients with asthma, eczema, or rhinitis, or with asthma and eczema, although patients with rhinitis alone tended to show weaker responses. Overall, there was a significant correlation between serum IgE antibody to Antigen P₁ and T cell proliferation ($r_s = 0.579$, $p < 0.001$); however, excluding individuals with no IgE antibody, the quantitative correlation was poor ($r_s = 0.26$, $p < 0.1$).

These results indicate that most patients showing immediate hypersensitivity to *D. pt.* have circulating T cells sensitized to Antigen P₁. These sensitized T cells probably act as helper cells for antibody production, but may also play a role in the pathogenesis of allergic lesions and in the delayed or chronic symptoms of these allergic diseases.

Most patients with allergic symptoms due to inhaled allergens show classical immediate hypersensitivity skin tests and have specific IgE antibodies in their sera. Although delayed hypersensitivity skin tests to allergens are not common (1), sensitized T cells are likely to be an important component of the immune response to these proteins. There is considerable evidence from experiments in both animals and man that IgE synthesis is regulated by T cells (2–4) and, from the nature of the cellular infiltrate seen in allergic lesions, it appears that some form of cell-mediated immunity may be involved in the pathogenesis of allergic diseases (5–8). Certainly, many of the symptoms of both asthma and atopic dermatitis are not only persistent but are also late in onset after allergen exposure. There is no clear correlation between the quantities of allergen-specific IgE antibody in the serum and the nature and severity of allergic symptoms; indeed, our recent studies have shown that patients may retain IgE antibodies for many years after they have ceased to suffer symptoms (9). It is therefore possible that T cell involvement is a factor in determining differences in symptoms in allergic patients who appear to be equally sensitive as judged by skin tests and serum antibody.

There have been many reports of allergen-induced proliferation of peripheral blood mononuclear cells (PBMC)³ from allergic patients (10–17). Although it is well established that the cells proliferating *in vitro* to soluble antigens such as tetanus toxoid and purified protein derivative are T cells, the evidence that this is the case for allergens is largely indirect (11, 14), and the lack of delayed hypersensitivity skin test responses to allergens suggested that confirmation was required. Many of the proliferation studies have involved the use of purified proteins from rye grass and ragweed, which are both important seasonal allergens, but little has been reported on the responses to the house dust mite *Dermatophagoides pteronyssinus* (*D. pt.*), which is a major cause of perennial allergic symptoms in many countries (13, 18, 19). Another important reason for studying T cell immunity in patients sensitive to *D. pt.* was that they suffer from several different allergic diseases. Thus, patients sensitive to *D. pt.* on the basis of skin tests or serum antibody levels suffer from rhinitis and/or asthma and/or atopic dermatitis (19). The use of the purified major allergen from *D. pt.*, Antigen P₁, allows the comparison

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³ Abbreviations used in this paper: PBMC, peripheral blood mononuclear cells; *D. pt.*, *Dermatophagoides pteronyssinus*; SRBC, neuraminidase-treated sheep red blood cells; smlg, surface membrane Ig; GM, geometric mean.

of T cell responses and humoral immunity to the same protein. The use of a purified protein also reduces the likelihood of nonspecific or mitogenic effects due to other components of mite extracts.

In these studies, we have shown that PBMC from most *D. pt.*-sensitive patients, but not from control subjects, will proliferate in response to Antigen P₁. The responding cells were shown to be T cells by separation experiments and to be predominately of the T helper (Leu-3a⁺) surface phenotype. Interleukin 2 (IL 2) production was also detected in the cultures. Good T cell proliferation to Antigen P₁ was found in patients with rhinitis, asthma, or atopic dermatitis.

MATERIALS AND METHODS

Subjects. Sixty-seven atopic subjects, selected as being sensitive to *D. pt.* on the basis of clinical history and positive immediate hypersensitivity skin prick tests (Bencard Ltd., UK), were studied; a mean wheal diameter 3 mm greater than that caused by the diluent control after 10 min was regarded as positive. The skin test reagent was made from mites grown on nonectodermal heat-denatured protein of mammalian origin; the extract contained 54 µg Antigen P₁ by radioimmunoassay and ~300,000 units of *D. pt.* allergen, as compared with the NIBSC standard 82/518 with the use of RAST inhibition. The patients were divided into groups according to their symptoms: rhinitis alone, asthma with or without rhinitis, atopic dermatitis alone, and atopic dermatitis with asthma. The majority of the patients were adults from 15 to 62 years of age, but the atopic dermatitis group included nine children from 5 to 11 years old. None of these patients had undergone hyposensitization treatment. All of the patients with atopic dermatitis were receiving local steroids for their skin lesions, and approximately one-half of the patients with asthma were receiving inhaled beclomethasone dipropionate, but none of the patients were on oral steroids at the time of the study. The control group included nine non-atopic laboratory volunteers, two atopic patients with negative skin tests to house dust and *D. pt.*, and four patients with asthma, rhinitis, or apparent food sensitivity, but with low total IgE levels and no positive skin tests to a standard battery of allergen extracts.

Antigens and antibody assays. Antigen P₁ was prepared by a modification of the technique described previously (20), with the use of *D. pt.* culture which was kindly provided by Bencard Ltd. and which was the same as that used for making the skin test reagent. This involved a 4-hr aqueous extraction of a *D. pt.* culture, precipitation with 50% saturated ammonium sulfate, Pevikon block electrophoresis (Calbiochem, La Jolla, CA), and gel filtration on a Sephadex G-100 column (Pharmacia Fine Chemicals, Piscataway, NJ). The preparation was stored freeze-dried and reconstituted in culture medium as required. Phytohemagglutinin (PHA) (Burroughs Wellcome Ltd., Research Triangle Park, NC) and concanavalin A (Con A) (Pharmacia) were used at previously defined optimal concentrations (1 µg/ml and 3 µg/ml, respectively). IgG and IgE antibodies to Antigen P₁ were measured by using a liquid-phase double antibody antigen-binding radioimmunoassay as described previously (20). The results are expressed as arbitrary units of binding activity based on a standard serum. The units are the same as those used in previous reports (19, 20).

Cell separation and culture. Blood was taken into preservative-free heparin (Paines and Byrne, Greenford, UK) (20 U/ml blood) and was separated by the standard technique with the use of Ficoll-Paque (Pharmacia). Mononuclear cells were harvested from the interface, and were washed twice in RPMI 1640 Dutch Modification (Flow Laboratories, Rockville, MD) with penicillin (100 U/ml), streptomycin (100 µg/ml), and L-glutamine (2 mM). Cells were cultured at 10⁶/ml in this medium supplemented with 10% pooled human AB⁺ serum. Six units of AB⁺ serum were screened to exclude antibodies to Antigen P₁ and nonspecific mitogenic effects before pooling. Cultures were performed in triplicate at 0.25 ml per well in flat-bottomed microtiter plates (Sterilin, UK) at 37°C in a humidified atmosphere containing 5% CO₂. Antigen or mitogen was diluted in culture medium containing serum as required, and was added in a 10-µl volume. In all experiments, PHA-stimulated cultures were included as a positive control and two cell separation experiments were rejected because there was no response to PHA. DNA synthesis was measured by adding 1 µCi [³H]thymidine (specific activity 2 Ci/mM, Amersham, UK) per well 6 hr before harvesting onto glass fiber filters. ³H incorporation was measured by liquid scintillation counting, and the results were expressed as the geometric mean (GM) of triplicates. To compare responses between individuals, the results were expressed

as stimulation index (SI) because background proliferation varied considerably.

$$SI = \frac{\text{GM cpm in cultures stimulated with Antigen P}_1}{\text{GM cpm in unstimulated cultures}}$$

T cells were purified by rosetting with neuraminidase-treated sheep red blood cells (SRBC) and by centrifugation over Ficoll-Metri-zoate (S.G. 1.090) (21, 22). Non-T cells were further separated into surface membrane Ig⁺ (smlg⁺) and Ig⁻ (smlg⁻) cells by rosetting with ox red blood cells (OxRBC) coated with a mixture of specifically purified goat anti-human Ig antibodies (anti-IgM, anti-IgG, and anti-IgE) as described previously (22). To prevent proliferation, cell populations added as accessory cells were either irradiated (2000 rad) by using a cobalt 60 source or were treated with mitomycin C (Sigma Chemical Co., St. Louis, MO) (50 µg/ml for 30 min at 37°C). Separated cells were cultured together in various proportions at an overall concentration of 10⁶/ml.

Flow cytometry. Cells were harvested from culture after various times, washed, and resuspended in RPMI 1640 (Flow Laboratories) with penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamine (2 mM), 2% fetal calf serum, and 0.2% sodium azide. They were stained with anti-Leu-3a (FITC- or Phycoerythrin-conjugated), or with FITC-conjugated anti-Leu-2a (Becton Dickinson, Sunnyvale, CA), and were analyzed by using the FACS I. Cells were also stained with propidium iodide to assess viability, and with mithramycin to estimate blast transformation.

Interleukin 2 (IL 2) assay. Culture supernatants were spun to remove cells and were stored frozen at -40°C until assayed. Cells from a mouse T cell line, CTL-D, which is highly dependent on IL 2 activity for growth, were washed thoroughly and were added at 5000 per well in 20- to 100-µl aliquots of culture supernatant dilutions in flat-bottomed microtiter plates (Sterilin). Cells were cultured at 37°C in a humidified atmosphere with 5% CO₂ for 24 hr, and were then pulsed with 1 µCi per well [³H]thymidine (Amersham; 2 Ci/mmol) and cultured for a further 5 hr before harvest. To allow comparison of results between assays, a dilution curve of a standard IL 2-containing supernatant from Con A-stimulated rat spleen cells was run in parallel with each assay. Ampules of a 4% solution of this supernatant were stored at -70°C and were used as required; this 4% solution was assigned 1000 U/ml IL 2 activity. All supernatants were assayed at a minimum of four serial twofold dilutions, in duplicate, to ensure that curves were parallel to the standard. Culture medium for these assays was RPMI (Flow Laboratories) with penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamine (2 mM), 2-mercaptoethanol (10⁻⁵ M), and 10% heat-inactivated fetal calf serum.

Statistics. Correlation coefficients were calculated by using Spearman's rank correlation. The Mann-Whitney Wilcoxon signed test was used for the comparison of SI in different groups.

RESULTS

Proliferative response of PBMC to Antigen P₁. A proliferative response to Antigen P₁ could be detected in PBMC from the majority of *D. pt.*-sensitive patients studied. This response was slower than that observed with PHA or Con A, and where studied in full, the peak proliferation was seen between days 5 and 7 (a representative time course is shown in Fig. 1a). There was a tendency for proliferation in unstimulated cultures to increase with time, and in some cases this increase was quite high by day 7 (2000 to 3000 cpm). Cultures were routinely harvested at days 5 and 7 to ensure detection of any positive response. In almost all cases, proliferation increased steadily with increasing Antigen P₁ dose over the range of 0.01 to 10 µg/ml (representative dose-response curves are shown in Fig. 1b). In general, the patients who showed a positive response at the lowest dose also gave the highest proliferation at 10 µg/ml. Thus, of the positive responders tested across the full dose range (n = 43), the 21 patients who responded at 0.01 µg/ml had a median SI at 10 µg/ml of 12.2, while the median value for those not responding at 0.01 µg/ml was 3.3 (p < 0.001). For the whole series of patients and controls, the maximal stimulation indices (day 5 or 7) at 10 µg/ml Antigen P₁ are shown in Figure 2; 46/67 *D. pt.*-sensitive patients

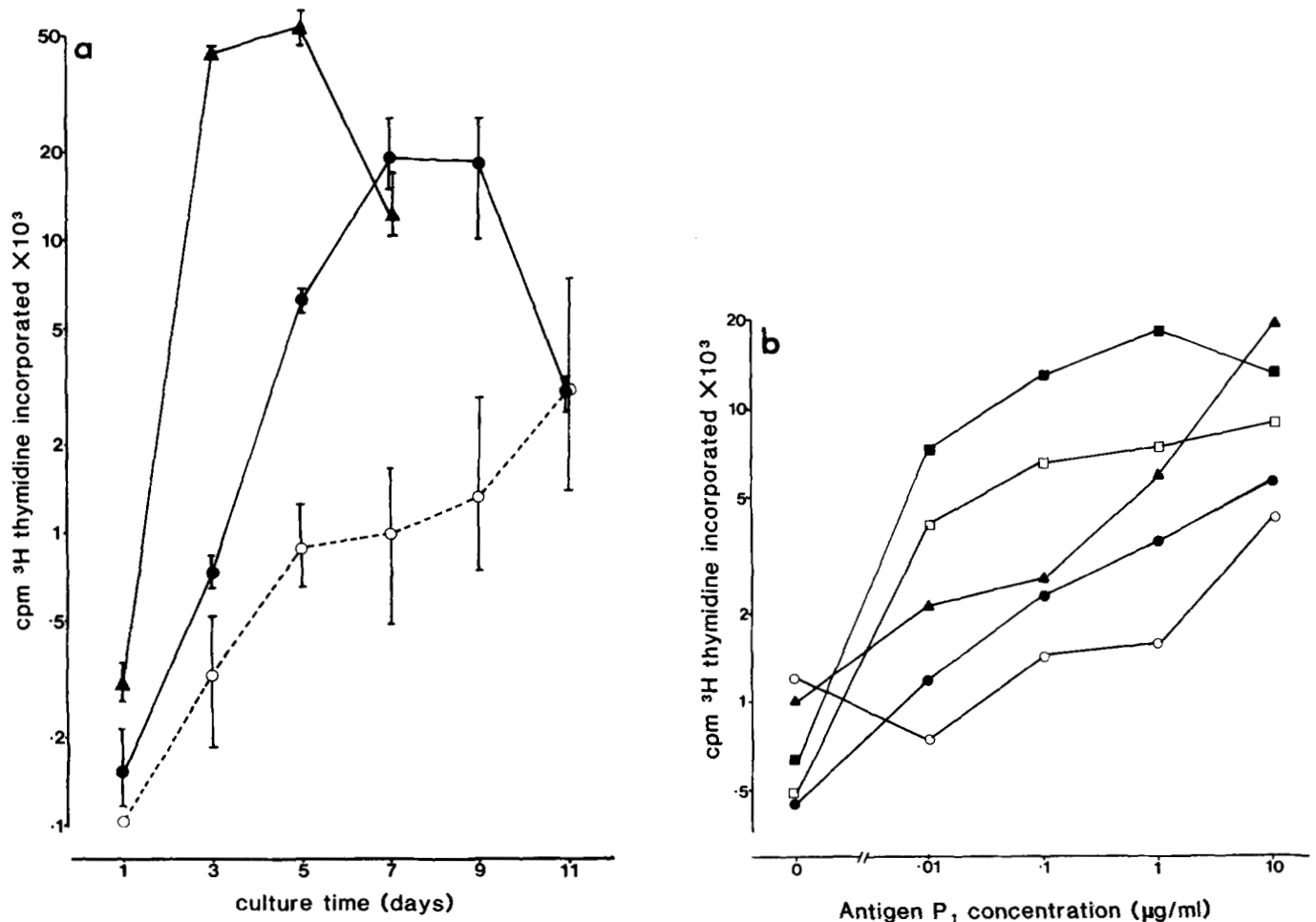


Figure 1. a, Time course of the proliferative response to Antigen P₁. Results are shown for PBMC from a single patient cultured with 10 μg/ml Antigen P₁ (●—●), Con A (▲—▲), and medium alone (○—○). Values are GM of triplicates. Bars indicate range of counts in triplicate cultures. b, Dose-response curves to Antigen P₁ for individual patients in different disease groups: atopic dermatitis (adult) ■—■; atopic dermatitis (child) □—□; asthma alone ▲—▲; asthma with atopic dermatitis ●—●; rhinitis ○—○. Values are GM of triplicates.

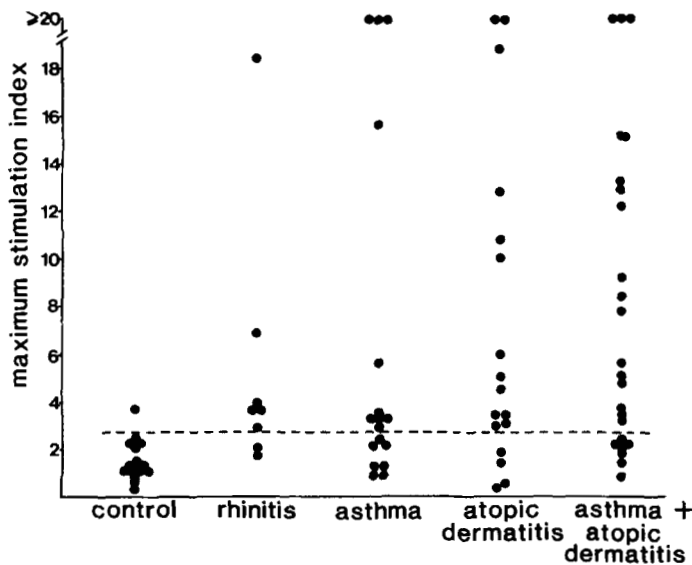


Figure 2. Maximal stimulation indices at either day 5 or day 7 with 10 μg/ml Antigen P₁ for *D. pt.*-sensitive patients grouped according to disease symptoms. Line (----) shows mean + 2 SD of 14 nonresponding controls. All patient groups significantly different from controls ($p < 0.01$ Mann-Whitney).

showed an SI of greater than 2.7, while 14/15 control subjects showed no significant response. Approximately equal proportions of patients in each disease group showed significant proliferation, but there was a tendency for rhinitis patients to show a weaker response and to respond only to higher doses of antigen. Thus, of the eight patients with rhinitis alone, only one showed an SI of greater than 2.7 when cultured with less than 1 μg/ml of Antigen P₁.

Proliferation responses by separated T cells. T cells separated by rosetting with SRBC showed excellent proliferation to Antigen P₁ when cultured with irradiated (2000 R) non-T cells. T cells alone did not proliferate in response to antigen, presumably indicating a requirement for antigen-presenting cells (Table I). Similar results were seen in 12 experiments on eight patients. Non-T cells alone showed higher levels of background proliferation, but showed no significant response to Antigen P₁. The non-T cells acted equally well as accessory cells when they were depleted of smlg⁺ cells by rosetting with anti-Ig-coated OxRBC. Treatment of the accessory populations with mitomycin C instead of with irradiation also allowed excellent proliferation (four experiments). These results indicate that the responding cells were in the T cell population separated by SRBC-rosetting.

Evidence that the proliferating cells are of the T

TABLE I
Antigen P₁-Induced proliferation^a in separated cell populations

Cell Populations	Expt. 1 ^b			Expt. 2 ^c		
	control	1 μ g/ml P ₁	10 μ g/ml P ₁	control	1 μ g/ml P ₁	10 μ g/ml P ₁
Unseparated PBMC	102	2,895	5,438	425	9,142	19,069
T cells alone ^d	58	58	323	22	52	29
Non-T cells	ND ^e	ND	ND	728	964	1,174
Irradiated (2000 R) non-T cells	ND	ND	ND	25	87	44
T + 20% irradiated non-T	202	3,946	8,609	1,454	9,837	20,771
T + 10% irradiated non-T smIg ⁻	162	3,590	7,728	1,724	4,965	12,250
T + 20% non-T (mitomycin C-treated)	851	5,217	9,989	ND	ND	ND
T + 10% non-T smIg ⁻ (mitomycin C-treated)	878	2,832	8,585	ND	ND	ND

^a Results are expressed as cpm [³H]thymidine incorporated at day 7.

^b Patient MS (asthma).

^c Patient CH (asthma + atopic dermatitis).

^d The purity of the T cells as judged by E-rosetting was >95%. Morphologically, >99% were small lymphocytes, and by rosetting with anti-Ig-coated O₂RBC, less than 1% were smIg⁺.

^e ND, not done.

helper phenotype. To determine the surface marker phenotype of the responding T cell population, Antigen P₁-stimulated PBMC cultures were harvested at various times, stained with anti-T cell monoclonal antibodies, and analyzed by flow cytometry. In two experiments, there was a marked increase in the proportion of Leu-3a⁺ cells in responding cultures; the results of one experiment are shown in detail (Fig. 3a). The increase in the percentage of viable cells bearing Leu-3a was matched by a fall in the percentage of Leu-2a⁺ cells to less than 9% by day 7. Double staining with Phycoerythrin-conjugated anti-Leu-3a and FITC-conjugated anti-Leu-2a showed no cells bearing both markers. Staining with mithramycin showed a progressive increase in the number of blast cells from day 3 onwards. The fluorescence/cell size profile with FITC anti-Leu-3a⁺ at day 7 showed the presence of large, blast-sized cells staining brightly for this marker, representing 32% of the total Leu-3a⁺ cells (Fig. 3b).

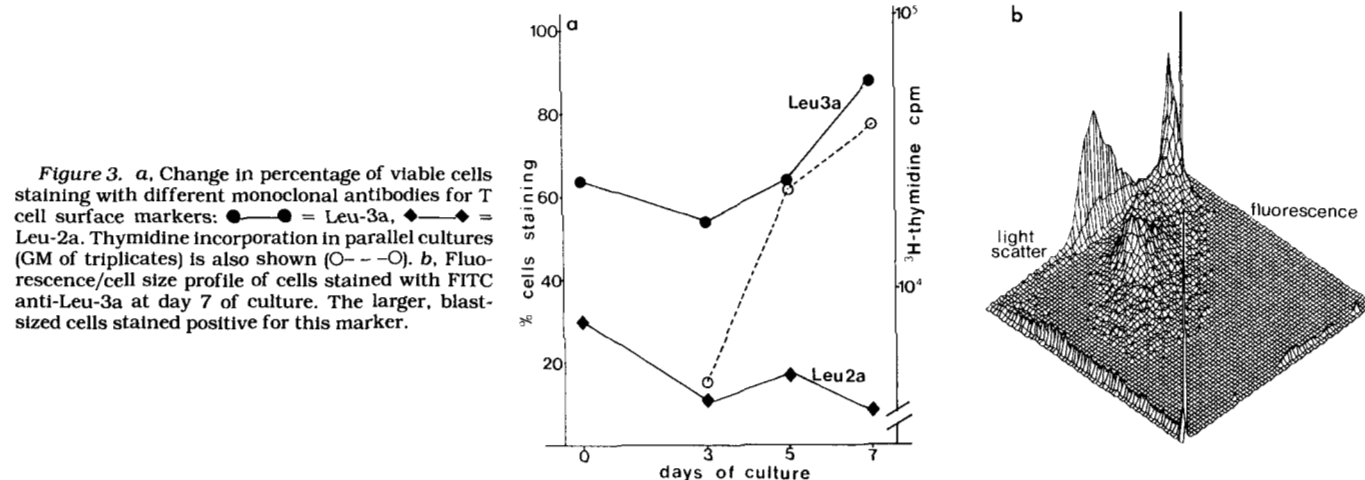
IL-2 production in Antigen P₁-stimulated PBMC cultures. Supernatants from PBMC cultures stimulated with antigen P₁ over a range of doses were tested for IL 2 activity after 1 to 7 days of culture. The dilution curves obtained with human Con A- and Antigen P₁-stimulated supernatants were parallel to those seen with the rat IL 2 (Fig. 4). IL 2 could be detected in supernatants of Antigen P₁-stimulated cultures from all six *D. pt.*-sensitive patients tested (each of whom also showed signifi-

cant proliferation in parallel cultures). Detected IL 2 levels increased with increasing doses of Antigen P₁, but were always low when compared to those detected in Con A supernatants (Table II). IL 2 production was also detected consistently later than in Con A-stimulated cultures; none were detected earlier than day 2, and maximal levels were seen at day 4 or 5. Measurable IL 2 activity was also detected in culture supernatants from some control subjects. This occurred in the presence of significant background proliferation, however, and was not affected by the presence of Antigen P₁ in the cultures.

Relationship between serum Antigen P₁-specific antibody and proliferation responses. Our patients were selected on the basis of positive skin prick tests to *D. pt.*, and most of them had IgE and IgG antibodies specific for Antigen P₁ in their serum. To compare T cell responses and humoral immunity to Antigen P₁, we examined the correlation between maximal SI at 10 μ g/ml Antigen P₁ and serum IgE antibody levels (Fig. 5). Overall, including control subjects, the correlation was significant ($r_s = 0.579$, $p < 0.001$), but for those patients with detectable IgE antibody to Antigen P₁, the quantitative correlation between levels of antibody and SI was poor ($r_s = 0.26$, $p < 0.1$).

DISCUSSION

In these experiments, we have shown that the majority of *D. pt.*-sensitive patients have T cells in their peripheral



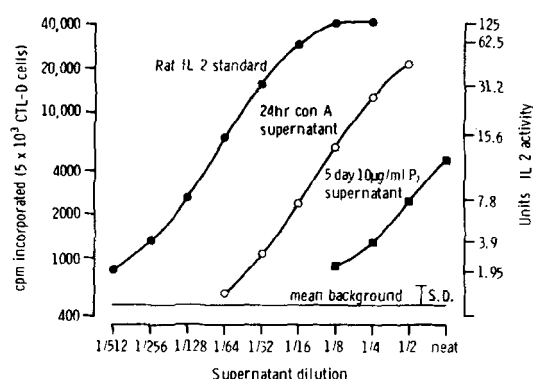


Figure 4. IL 2 assay: dilution curves of rat IL 2 standard (●—●) 24-hr supernatant from a Con A-stimulated culture (○—○) and 5-day supernatant from Antigen P₁ (10 µg/ml)-stimulated culture (■—■). Values are mean cpm in duplicate cultures. Units of IL 2 activity are arbitrarily defined in relation to the rat IL 2 standard. Line and bar (—) indicate mean and SD (n = 16) of cpm incorporated by cytotoxic T cell line-derived cells cultured without added IL 2.

TABLE II
IL 2 activity^a in culture supernatants^b

No. of Days	Control	0.01 µg/ml P ₁	0.1 µg/ml P ₁	1 µg/ml P ₁	10 µg/ml P ₁	3 µg/ml Con A
1	0	0	0	0	0	71
3	0	0	0	0	3.9	0
5	0	0	1	5.4	11.7	1
7	0	0	1	1	2	1

^a Results are given in arbitrary units (see Materials and Methods).

^b Patient ME: asthma + atopic dermatitis.

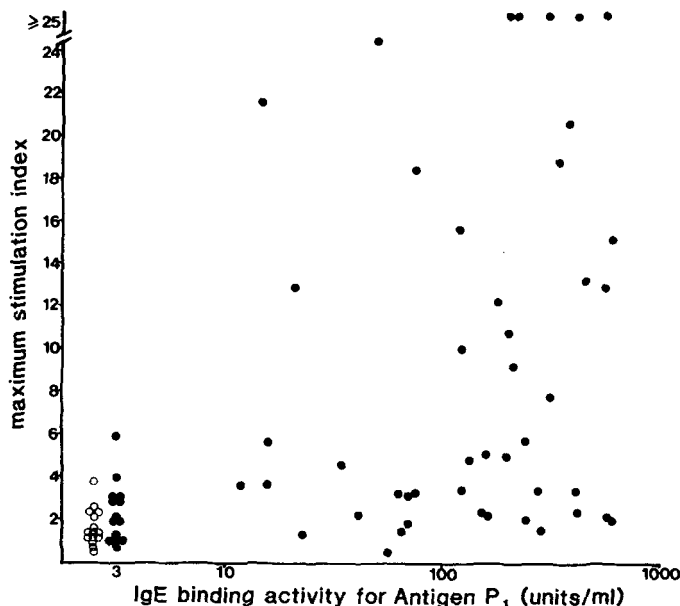


Figure 5. Correlation between maximal SI with 10 µg/ml Antigen P₁ and serum IgE antibody levels. ● = *D. pt.* skin test-positive patients. ○ = controls. Negative cut-off for the assay is 3 U/ml. Overall $r_s = 0.579$, $p < 0.001$. For patients with detectable antibody, $r_s = 0.26$, $p < 0.1$.

blood that will respond to Antigen P₁ *in vitro*. This antigen has been shown to be a major allergen of *D. pt.* in terms of the IgE antibody response (20). The time course of this proliferation is similar to those seen for other soluble antigens and allergens (10, 17). Over the dose range tested, there was no evidence that Antigen P₁ was mitogenic because control subjects showed only a minimal response. Some studies with purified ragweed proteins have shown good proliferation in skin test-negative individuals with antigen doses of around 10 to 50 µg/ml

(15, 23). In the previously reported studies on lymphocyte responses to house dust-related allergens, proliferation was observed in nonallergic subjects by using crude house dust preparations; when dust mite preparations were used, however, the response was much lower (13, 17).

Not all *D. pt.*-sensitive patients showed a significant proliferative response to Antigen P₁. In part, this may reflect the fact that not all *D. pt.*-sensitive patients respond to Antigen P₁, and in general only about 80% have easily detectable serum antibodies. A proportion (8/20) of the patients with SI of less than 2.7 had no detectable antibody to Antigen P₁. The magnitude of the proliferative response may be affected by many different factors, and a low response does not necessarily imply a lack of sensitized T cells. Allergen-specific T suppressor cells may modulate the *in vitro* response (24), and disease activity may also affect T cell function. There are reports that T cell numbers, delayed hypersensitivity skin tests, and *in vitro* T cell proliferation are reduced in patients with atopic dermatitis (25); other reports suggest that decreased functional suppressor activity and an increased T helper to suppressor ratio are features of this disease (3, 8, 26). We have observed patients whose *in vitro* proliferative responses increased with improvement in their clinical status, but this was not a consistent finding. It is possible that the number of allergen-sensitized T cells decreases in the peripheral blood during disease exacerbation.

We were interested to see whether differences could be detected in the *in vitro* T cell responses in patients with different symptoms, but we found that this was not the case. Given the very good T cell proliferative responses in patients with either asthma or atopic dermatitis, it seems unlikely that the dramatic clinical differences between these patients can be explained by differences in the number of circulating allergen-sensitized T cells (7).

A factor that could affect the magnitude of *in vitro* proliferative responses is the release of histamine from basophils in the cultures stimulated with allergen. We have shown that Antigen P₁ can release up to 70% of the total histamine from PBMC (6), and the cells used in our experiments contained 5 to 25 ng histamine per 10⁶ cells. Thus, histamine could be released at around 10⁻⁷ M in these cultures. Many investigators have reported that histamine can activate T suppressor cells; others researchers have suggested that T cells from allergic patients are particularly sensitive in this respect (27, 28), and that depletion of basophils can increase proliferation in some systems (29). We have observed 50 to 70% inhibition of proliferation with histamine added at 10⁻⁶ M to our cultures. By using the separation techniques described here, the cultures of T cells plus Smlg⁺ non-T cells contained up to 60% less histamine than PBMC cultures, but showed no increase in the proliferative response (Rawle *et al.*, manuscript in preparation).

The finding that the *in vitro* proliferative response involves cells that are predominately of the T helper surface phenotype has also been reported with the use of antigens associated with delayed hypersensitivity (e.g., tetanus toxoid and Herpes zoster) (30). Hsieh (31) reported an increase in OKT8⁺ (suppressor) cells after incubating cells from both allergic and nonallergic individuals with crude house dust extract for 1 hr, but the significance of

a change in surface markers over such a short time is unclear. Recently, Lanzavecchia *et al.* (32) described T cell lines specific for *D. pt.* that had helper function and that bore predominately T helper surface markers.

The presence of IL 2 in the culture supernatants supports the conclusions from the cell surface marker studies. IL 2 production has generally been shown to be a function of OKT4⁺ (=Leu-3a⁺) T helper cells (33, 34), although a recent report suggests that, when stimulated by Con A, OKT8⁺ (=Leu-2a⁺) cells may also produce IL 2 (35). The high levels of IL 2 detected at 24 hr with Con A stimulation, followed by a sharp fall, correspond to the results of other researchers who used mouse lymphocytes (36, 37). In human cultures stimulated with soluble antigens, IL 2 has been reported to be detectable as early as 12 hr, with peak activity at 48 hr (38), which is earlier than in our system. In other studies, "lymphocyte mitogenic factor" was reported in 3-day supernatants of PBMC cultures of allergic patients proliferating in response to grass pollen (12). The reason for the late appearance of IL 2 is not known, but it might reflect an amplification phase of the antigen-driven response, assuming that earlier stages of activation produced quantities of IL 2 insufficient to be detectable in the supernatant. In our experiments, the maximal level of IL 2 was detected approximately 2 days before the peak of proliferation; this time relationship is the same as that in Con A-stimulated cultures.

The data presented here leave little doubt that *D. pt.*-allergic patients have T cells sensitized to Antigen P₁, while most nonallergic individuals do not. The proliferation responses were at least as good as those reported with Antigen E in patients with ragweed hay fever (16). We have previously shown that most *D. pt.*-allergic patients have IgG and IgA as well as IgE antibodies to Antigen P₁, while most nonallergic individuals lack antibodies of any class (39). In addition, to the apparent lower T cell proliferative responses reported here, rhinitis patients also have lower mean levels of Antigen P₁-specific antibodies in their serum (19). The immune response to pollen allergens includes IgG and IgA antibodies, so that genetic controls over hay fever do not appear to be restricted to IgE antibody (40). Marsh *et al.* (41) have recently shown that the HLA linkage of the response to the ragweed allergen Ra5 is equally strong for IgG and IgE antibodies. The increasing evidence that the immune response to inhalant allergens includes specific T cells suggests that the factors, genetic and otherwise, which determine whether an individual responds to an allergen do not affect only humoral immunity (10–17, 32).

Basophils have been shown to be recruited into the nose during the pollen season in patients with hay fever, and into allergen-induced eczematous patch test lesions in atopic dermatitis patients (5, 6). The recruitment of basophils in both animals and man is thought to be T cell-dependent (7, 42). There is good evidence that the mononuclear cell infiltrate in the skin of patients with atopic dermatitis is largely T cells of the helper phenotype (8). Thus, it is possible that allergen-specific T cells could play a role in the pathogenesis of local allergic lesions. In experiments on guinea pigs, it appeared that the histology of passively transferred delayed hypersensitivity lesions was influenced by serum factors. Thus, sensitized T cells could transfer cutaneous basophil hypersensitivity to

nonsensitized animals, while the same cells, together with immune serum, transferred basophil-poor delayed-type hypersensitivity (43). In our experiments, we did not find a significant difference between the T cell responses in patients with strikingly different diseases, i.e., asthma and atopic dermatitis. The T cell responses reported here are at least partially similar to those reported with antigens that give rise to classical delayed hypersensitivity reactions in the skin. We recently found that passive transfer of serum from patients with atopic dermatitis can mediate allergen-induced basophil recruitment to the skin (44). Thus, it seems that the nature of symptoms caused by environmental allergens may well be influenced by a combination of IgE antibody, non-antibody humoral factors, and T cell responses.

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