

Lymphocyte responses to purified ragweed allergens in vitro

I. Proliferative responses in normal, newborn, agammaglobulinemic, and atopic subjects

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To evaluate cell-mediated immune responsiveness to pollen allergens in atopic subjects, we studied the deoxyribonucleic acid (DNA) synthetic responses of their cultured lymphocytes to purified ragweed antigens E, K, and Ra-3. Since lymphocytes from some highly ragweed-sensitive subjects gave poor proliferative responses when harvested on day 6, we undertook a series of dose-response and time-course studies in atopic and control subjects. Surprisingly, vigorous DNA synthetic responses to antigen E occurred with lymphocytes from all 45 subjects, including 19 highly ragweed-sensitive atopic adults (8 immunotherapy treated, 11 untreated); 13 nonatopic controls; 4 newborns, and 9 agammaglobulinemic patients. The geometric mean of peak response counts per minute in all 45 subjects was 21,163 and in unstimulated cultures was 2,416 ($p = < 0.0001$). The mean day on which the maximal responses occurred was 8.7, and the mean dose eliciting the maximum responses was 59 $\mu\text{g/ml}$. Statistical comparisons of the stimulated culture data revealed no significant intergroup differences. The finding of vigorous responsiveness to these purified pollen allergens by lymphocytes from nonatopic normal, newborn, and agammaglobulinemic subjects suggests that ragweed pollen antigens are either ubiquitous and lead to cell-mediated responsiveness in all subjects with intact cell-mediated immunity, or that they may have mitogenic properties in addition to their known antigenic properties.

Prompted by the results of recent studies in rodents showing an important role for T cells in regulating IgE antibody responses to antigens,^{1, 2} we undertook studies to evaluate parameters of cell-mediated immunity in atopic human subjects. As a part of this investigation we sought to determine whether the deoxyribonucleic acid (DNA) synthetic response of atopic individuals' peripheral blood lymphocytes to allergens differed from that of nonatopic normal subjects. In the limited number of published studies evaluating human lymphocyte responsiveness to pol-

len allergens, conflicting results were obtained.³⁻¹² Most reported an overall greater responsiveness by lymphocytes from atopic-sensitive persons than by those from nonatopic controls.^{3-6, 8, 9, 11, 12} In some, however, responses were greater in untreated than in treated subjects^{9, 11}; in others, treatment had no effect³; and in still others, lymphocytes from normal subjects responded to pollen antigens.^{7, 10}

In our initial studies we encountered the unexpected finding that lymphocytes from some highly ragweed-sensitive atopic subjects, when harvested on the sixth or seventh day of culture, showed poor proliferative responses to a wide range of doses of purified ragweed antigen E. Finding this, we then initiated a series of time-course and dose-response studies in allergic and nonallergic individuals. The present report summarizes the results of these studies in which vigorous DNA synthetic responses to purified ragweed antigens occurred in lymphocytes not only from atopic subjects but also from nonatopic normal subjects, newborn infants, and agammaglobulinemic patients. Moreover, the degree of re-

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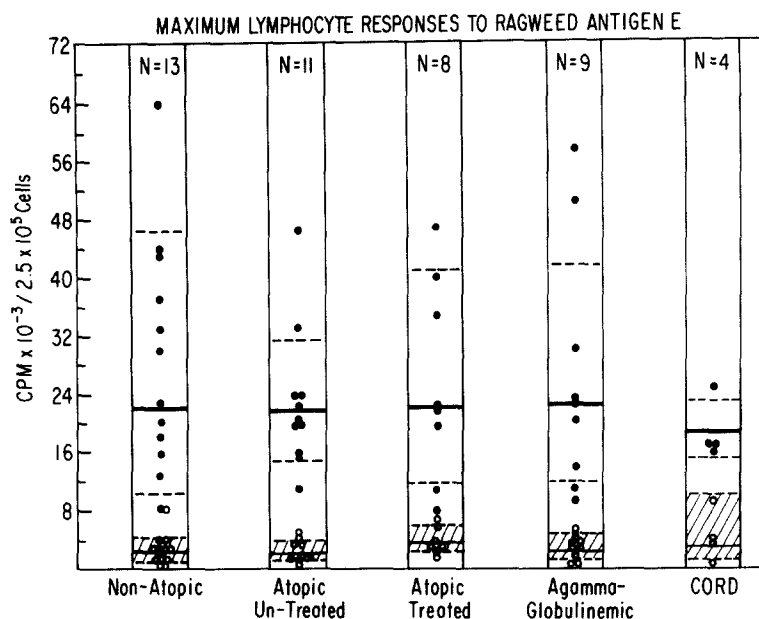


FIG. 1. Maximum lymphocyte responses to ragweed antigen E in the five subject categories. Data presented are the geometric means of results of quadruplicate cultures expressed in counts per minute per 2.5×10^5 lymphocytes. Responses in stimulated cultures of individual subjects are represented by the closed circles and in unstimulated cultures by the open circles. The geometric means of the maximal responses for each group are indicated by the solid horizontal lines, and the antilogs of the mean logs ± 1 SD for each group are shown by the broken horizontal lines.

TABLE I. Summary of lymphocyte stimulation data: Ragweed antigen E

Group	No.	Mean day of max. resp.*	Mean dose (γ) elic. max. resp.*	Means* of max. cpm/ 2.5×10^5 cellst	
				Unstim.†	Ag E stim. †
Nonatopic, normal	13	8.9 ± 1.3	54.8 ± 33.7	7.59 ± 0.81 (1,969)	9.99 ± 0.75 (21,858)
Atopic, untreated	11	8.8 ± 1.6	51.6 ± 29.7	7.61 ± 0.67 (2,028)	9.97 ± 0.38 (21,377)
Atopic, treated	8	8.1 ± 0.64	73.8 ± 28.3	8.21 ± 0.39 (3,692)	9.99 ± 0.63 (21,839)
Agammaglobulinemic	9	8.7 ± 0.87	66.7 ± 33.1	7.65 ± 0.74 (2,103)	10.02 ± 0.62 (22,439)
Newborn	4	9.0 ± 1.63	48.1 ± 27.6	7.89 ± 1.33 (2,677)	9.82 ± 0.20 (18,328)
All subjects	45	$8.7 \pm .35$	59.0 ± 10.8	$7.79 \pm .26$ (2,416)	9.96 ± 0.08 (21,163)

*Lymphocyte stimulation data are expressed as means $\pm$ SDs of logarithms of the data. Figures in parentheses are the geometric means expressed in counts per minute.

†No statistically significant intergroup differences found by Student's *t* test except as indicated by bracketed data, where $p = 0.0353$.

sponsiveness by lymphocytes from the various subject groups did not differ statistically. These studies suggest that purified ragweed pollen antigens are either ubiquitous and lead to cell-mediated responsiveness in all subjects with intact cell-mediated immunity, or that they may have mitogenic properties in addition to their known antigenic properties.

MATERIALS AND METHODS

Subjects

Lymphocytes from 45 individuals were studied for their responsiveness to purified ragweed antigen E; responses to antigens K and Ra-3 were also evaluated in 6 of them. The 45 subjects included 19 highly ragweed-sensitive atopic adults, 8 of whom had received perennial immunotherapy

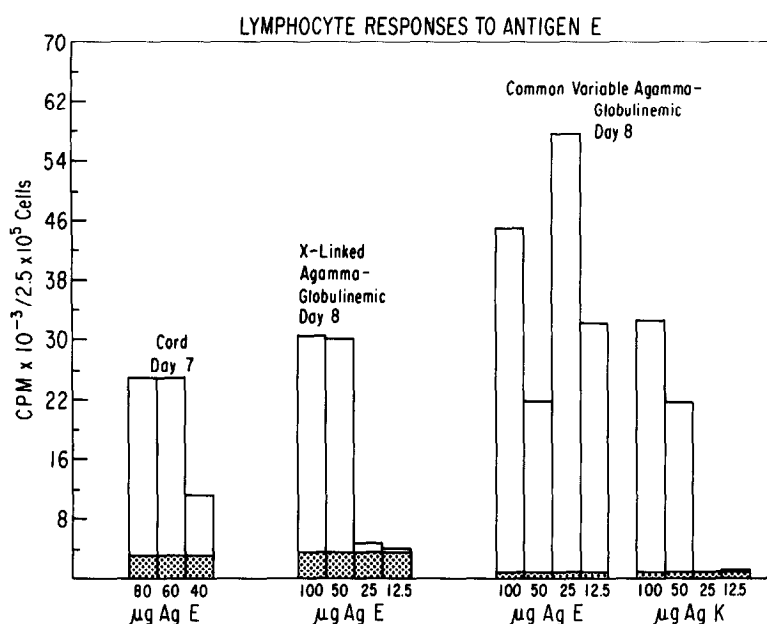


FIG. 2. Representative antigen E stimulation experiments with lymphocytes from cord blood of newborn infant and from two agammaglobulinemic subjects. Open bars represent the geometric means of results of quadruplicate cultures expressed in counts per minute per 2.5×10^5 lymphocytes; dotted bars are the means of the unstimulated cultures harvested on the same day.

with whole ragweed extract for several years and 11 of whom were untreated; 13 nonatopic normal adult volunteers, 3 of whom were foreign postdoctoral fellows (including 1 who had never been exposed to ragweed as he had just arrived from Japan); 4 newborn infants; and 9 agammaglobulinemic patients, 3 lacking surface Ig-positive B lymphocytes (X-linked) and 6 with Ig-positive B lymphocytes (common variable type). Informed consent was obtained from all subjects and the studies were conducted with the approval of the Duke University Committee on Human Investigation. The ragweed-sensitive subjects had strongly positive immediate skin test reactions to short ragweed extract, whereas the nonatopic normal and agammaglobulinemic subjects were skin test negative.

Mice

Six- to eight-week-old BALB/c female mice from the Duke mouse colony were sacrificed to obtain spleen cells for antigen E stimulation. The cells from the spleens of 5 mice were teased into sterile RPMI 1640 and pooled for each of these experiments. Culture conditions were similar to those described subsequently for the human lymphocytes except that 20 mg/100 ml L-arginine was added to the medium, 2% normal human serum was used to supplement the medium in lieu of 20% human AB plasma, and each culture contained 10^6 spleen cells per well.

Antigens

Purified antigens E, K, and Ra-3 were supplied by Dr. John Nutter of the Research Resources Branch of the National Institutes of Allergy and Infectious Diseases. *Limulus* assays on the two different lots of antigen E employed re-

vealed the presence of 100 and 500 ng endotoxin, respectively (Dr. Nutter, personal communication). Purified *Salmonella minnesota* endotoxin R 595, donated by Dr. Robert Wheat of the Department of Microbiology and Immunology, Duke University School of Medicine, was prepared by chloroform:methanol (4:1) extraction¹³ and freed of phospholipids by methanol precipitation.¹⁴

Lymphocyte stimulation studies

Lymphocyte cultures were performed in microtiter plates (Linbro Chemical Co., New Haven, Conn.) according to a modification of the method of Hartzmann and associates.¹⁵ One volume of sterile heparinized venous blood was mixed with 3 volumes of sterile 0.95% sodium chloride, layered over Ficoll-Hypaque, and centrifuged at 400 g for 30 min at room temperature to obtain a purified lymphocyte-monocyte cell suspension. The cells were washed 3 times at 4° C in sterile 295 milliosmolar phosphate-buffered saline (PBS), pH 7.2, supplemented with 5% pooled human AB plasma, and then resuspended in RPMI 1640 medium supplemented with 5,000 U penicillin G, 5,000 µg streptomycin, and 2 mM L-glutamine/100 ml, 2.5 mM Hepes buffer, and 20% pooled AB plasma from male donors. The cells were added to the wells in 0.1 ml volumes containing 2.5×10^5 lymphocytes, and varying concentrations of antigen in media or media alone were added to the wells in 0.1 ml volumes, making the final volume 0.2 ml per well; all cultures were done in quadruplicate. Concentrations of the purified ragweed antigens or endotoxin employed ranged from 10 ng to 100 µg/ml; the cultures were incubated at 37° C in 5% CO₂ in a humidified atmosphere for varying periods of time ranging from 5 to 12 days. Each culture was

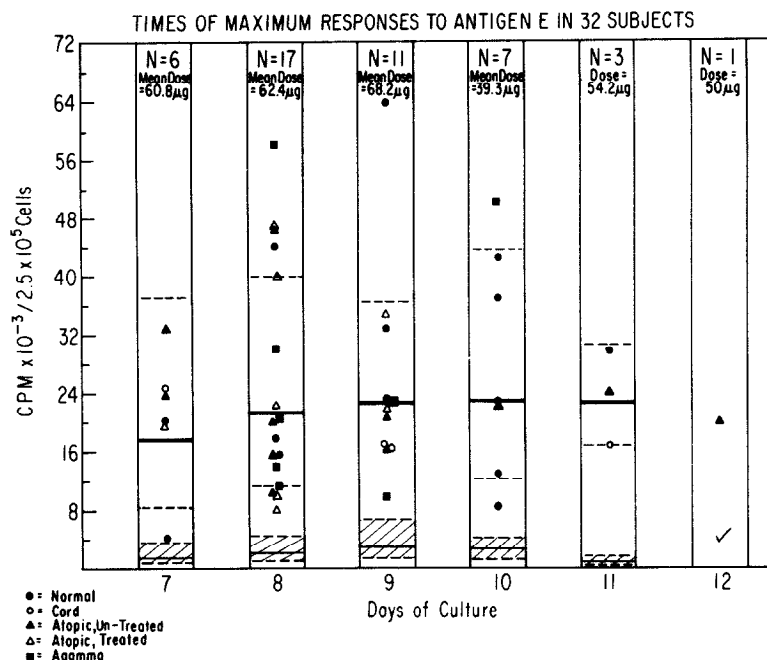


FIG. 3. Maximum lymphocyte responses to ragweed antigen E in all 45 subjects grouped according to the day on which the maximal responses occurred. See legend to Fig. 1 for explanation of data. Individual subject responses in stimulated cultures are indicated by various symbols representing the group from which the subjects were derived. The crosshatched areas at the bottom of each bar represent the geometric means ± 1 SD of the unstimulated culture data for those subjects on that day. The mean dose of antigen E eliciting the maximum response on day 9 was significantly higher ($p = 0.0362$) than the mean dose eliciting that response on day 10, and the unstimulated culture mean on day 9 was significantly ($p = 0.02$) higher on day 9 than on day 11; all other intergroup comparisons by Student's *t* test revealed no significant differences.

pulsed with $2 \mu\text{Ci}$ of ^3H -thymidine (New England Nuclear, Boston, Mass., 2 Ci/mmol) 6 hr prior to harvest. The cultures were harvested with an automated harvester (Otto Hiller, Madison, Wis.) onto glass-fiber filter paper, and counted in Bray's scintillation fluid. The mean ($\pm$ SE) of the coefficients of variation of the quadruplicate sets of data presented in the paper was $20.46\% \pm 1.79\%$. Data in counts per minute were transformed logarithmically for statistical analysis.

RESULTS

Lymphocyte stimulation with antigen E

The results of these studies were surprising. First, we found that peak proliferative responses to antigen E occurred later than we had expected, with the average time of maximum response in all subjects being 8.7 days (Table I). Second, we found, on comparing the peak responses, that lymphocytes from the nonatopic normal controls responded as well or better than those from the highly ragweed-sensitive subjects (Fig. 1, Table I). Indeed, the geometric mean of all 45 subjects' responses to antigen E (21,163 cpm) was 40% of the geometric mean of maximal responses to phytohemagglutinin (53,201 cpm) in 53 determinations on a normal population, using the same culture conditions. The peak geometric mean counts per minute in

quadruplicate cultures of lymphocytes from the nonatopic nonragweed-sensitive foreign postdoctoral fellow newly arrived from Japan was 30,038 and occurred on day 11 of culture; the geometric mean counts per minute of his unstimulated cultures harvested on the same day was 904. Third, and in contrast to the findings of Gaten and associates¹¹ with purified T cells from atopic subjects, we did not find a biphasic dose-response curve with unfractionated lymphocytes from atopic subjects. Lymphocytes from immunotherapy-treated atopic ragweed-sensitive patients responded as well as untreated ragweed-sensitive patients, and the dose eliciting maximum responses in the treated subjects was only slightly higher ($74 \mu\text{g}/\text{ml}$) than in the untreated group ($51.6 \mu\text{g}$, $t = 1.64$, $p = > 0.10$).

The finding of similar stimulation of DNA synthesis in lymphocytes from normal nonatopic and in those from ragweed-sensitive atopic subjects led us to study responses by lymphocytes from individuals who would not be expected to have an antibody response to antigen E, i.e., newborn infants and agammaglobulinemic patients. The results of three representative experiments done in these patient groups are presented in Fig. 2. The peak geometric mean counts per minute per 2.5×10^5 cells in quadrupli-

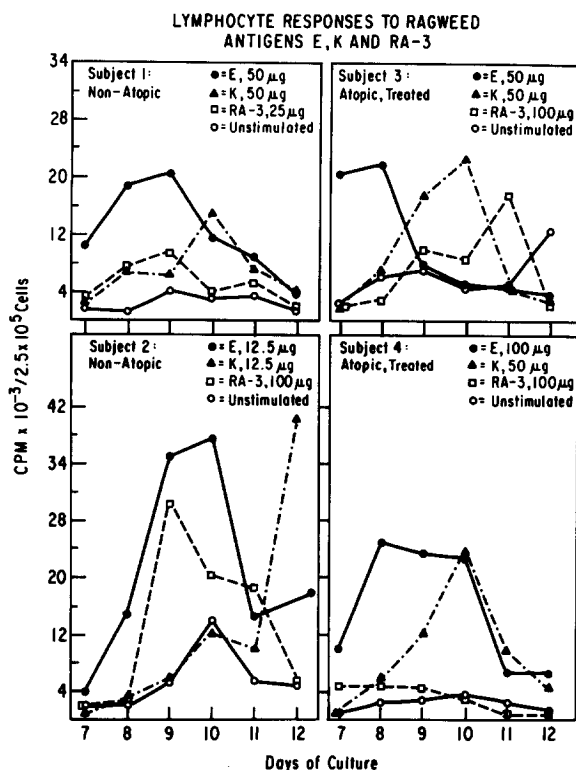


FIG. 4. Results of time-course and dose-response studies with ragweed antigens E, K, and Ra-3 in four subjects. See legend to Fig. 1 for full explanation of the data shown, which represent only the time-courses for the maximal responses. Antigen doses eliciting these responses are indicated in the insets; three other doses of each antigen were evaluated in each subject on each day of the experiment.

cate cultures of antigen E-stimulated lymphocytes from the cord blood of a newborn infant was 24,692, compared with a geometric mean of 3,483 cpm in unstimulated cultures harvested on the same day; the maximum response occurred on day 7 at a dose of 80 $\mu\text{g}/\text{ml}$ of antigen E. Lymphocytes from an X-linked agammaglobulinemic patient responded maximally with 30,072 cpm to 100 $\mu\text{g}/\text{ml}$ of antigen E on day 8 of culture, and those from a patient with common variable agammaglobulinemia with 57,876 cpm to 25 $\mu\text{g}/\text{ml}$ of antigen E on day 8. The serum IgE concentration in the X-linked patient was 13.8 IU/ml, and in the common variable agammaglobulinemic patient, 7.2 IU/ml.¹⁶

The maximum responses of all 45 subjects' lymphocytes are depicted graphically in Fig. 1 and summarized in Table I. The geometric means of counts per minute per 2.5×10^5 cells in stimulated cultures of the five different subject categories ranged from a low of 18,328 in the newborns to 22,439 in the agammaglobulinemic subjects. The means of the stimulated cultures from the other three subject

categories, i.e., normal nonatopic, atopic untreated, and atopic treated, were intermediate and extremely close, with all being in the 21,000 range. In contrast, the geometric mean counts per minute in the unstimulated cultures ranged from 1,962 to 3,692. The *t* tests performed between the mean logs of the stimulated culture data from the five subject groups did not show any of the means to differ significantly from each other. The same was true when intergroup comparisons were made of the means from unstimulated cultures with the exception that the counts per minute in unstimulated cultures of atopic treated subjects were barely ($p = 0.0353$) statistically higher than counts per minute in unstimulated cultures from atopic untreated subjects. The mean dose of antigen E eliciting maximum responses in all subject groups was 59 $\mu\text{g}/\text{ml}$, and there were no statistically significant intergroup differences in either the mean dose eliciting those maximum responses or in the mean time at which the responses occurred (Table I).

In Fig. 3 the maximum responses of all subjects' lymphocytes are presented according to the day on which that response occurred. None of the peak responses occurred on day 5 or 6. Twenty-eight of the 45 peak responses occurred on days 8 and 9, six on day 7, seven on day 10, three on day 11, and one on day 12. It can also be seen that peak responses by individuals in the five patient categories are scattered throughout the 6-day period, in keeping with the finding of no statistically significant intergroup differences in the mean time of peak response (Table I). Thus there was no tendency for the responses of atopic subjects' lymphocytes to peak at a different time than did those of other subjects. The mean dose of antigen E eliciting the maximum response on any given day ranged from 39.3 μg on day 10 to 68.2 μg on day 9; *t* tests comparing these doses showed that only the day 9 and day 10 doses differed significantly from each other ($t = 2.26$; $p = 0.0362$).

Lymphocyte stimulation with antigens K and Ra-3

We next investigated whether other purified ragweed antigens have stimulatory properties similar to those of antigen E. Fig. 4 presents the results of concurrent dose-response and time-course studies of lymphocyte responses to ragweed antigens E, K, and Ra-3 in two nonatopic and two atopic treated subjects. Again, note the tendency for peak responses to antigen E to occur on days 8, 9, or 10. In contrast, the response to antigen K occurred later—on day 10 in three subjects and on day 12 in the other. Lymphocytes from an atopic untreated subject, not shown here, responded to 100 μg of antigen K on day 9 but

his response to Ra-3 was not evaluated. The peak response to Ra-3 tended to follow that of antigen E in the two nonatopic subjects, but occurred on day 11 in atopic subject No. 3. Little or no response occurred with Ra-3 stimulation of lymphocytes from atopic subject No. 4. In the only agammaglobulinemic patient whose lymphocyte response to antigens other than E has been studied thus far, a very brisk response was noted to 100 μ g of antigen K on day 8 of culture (Fig. 2).

Evaluation of possible effects of heparin

Because of Weisberg and co-workers' report⁷ that lymphocyte responses to a variety of crude pollen extracts seemed to be greater in plasma-supplemented than in serum-supplemented medium, we evaluated their suggestion that the results could be due to interaction of the pollen antigens with heparin. In these experiments, Ficoll-purified mononuclear cells were washed 5 times in phosphate-buffered saline (PBS) at 4° C; then one-half were resuspended in heparin-free RPMI 1640 medium, as described previously, except that 20% autologous serum was used to supplement the medium rather than 20% AB plasma. The other half of the washed cell suspension was resuspended in RPMI 1640 supplemented with 20% heparinized AB plasma. The results of such an antigen E stimulation experiment with a normal nonatopic subject's lymphocytes are shown in Fig. 5. As can be seen, there was very little difference in the results of the parallel experiments. The peak response occurred on day 9 in the plasma-supplemented cultures and on day 8 in the serum-supplemented cultures. The dose eliciting the maximum response in each case was 100 μ g. Thus omission of heparin from the cultures did not abolish this normal nonatopic subject's response to antigen E.

Evaluation of possible effects of endotoxin

When it was learned that the antigen E being supplied from the Research Resources Branch of the National Institute of Allergy and Infectious Diseases contained nanogram quantities of endotoxin, it became important to determine whether the observed effects of the antigen preparations could have been due to endotoxin. Even though previous investigations have shown that human peripheral blood lymphocytes, unlike mouse spleen cells, fail to proliferate in response to endotoxin,¹⁵ we re-evaluated this under the conditions used for all of the lymphocyte stimulation experiments with the purified ragweed antigens in this study. Two types of experiments were conducted: in the first, lymphocytes from one atopic untreated and from one nonatopic subject were cultured in the presence of purified *Salmonella* endotoxin in

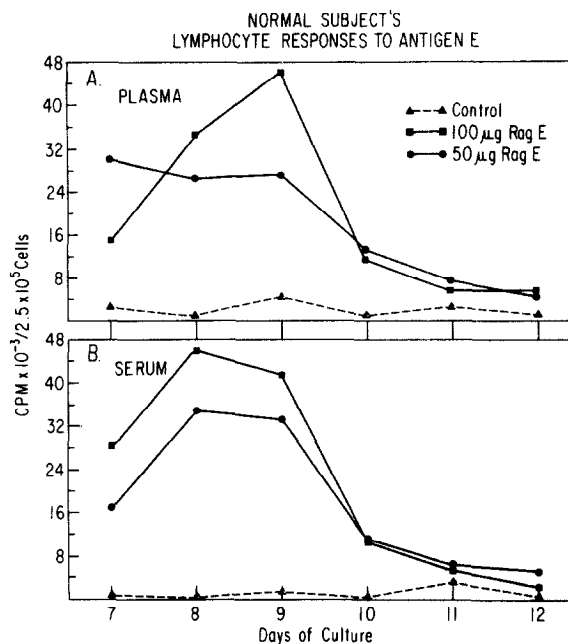


FIG. 5. Time-course and dose-responses to antigen E by a normal subject's lymphocytes when cultured in plasma-supplemented or serum-supplemented medium. Not shown are dose-response and time-course curves for 25 and 12.5 μ g and 100 ng of antigen E, to which very little response was noted.

concentrations ranging from 12.5 ng to 100 μ g and harvested on days 4, 5, and 7 to 11 of culture; and in the second, lymphocytes from a nonatopic normal subject were cultured with either antigen E alone in concentrations ranging from 12.5 ng to 100 μ g or with a combination of ragweed antigen E over the same dosage range and either 100 μ g or 500 ng/ml of *Salmonella* endotoxin and harvested on days 7 to 12. No stimulation was observed with any dose of *Salmonella* endotoxin in any of the subjects on any day of harvest. Moreover, the responses in antigen E-stimulated cultures with added *Salmonella* endotoxin did not differ significantly from those of cultures containing antigen E alone.

Antigen E stimulation of mouse spleen cells

Experiments were conducted to evaluate lymphocyte responsiveness of mouse spleen cells to purified ragweed antigen E (1) in order to determine whether the stimulation noted with human lymphocytes might be due to a nonspecific mitogenic property of antigen E and (2) because mouse spleen cells are known to be responsive to endotoxin. As stated previously, the two lots of antigen E used in these experiments contained nanogram concentrations of endotoxin; studies by previous investigators have shown that microgram concentrations of endotoxin are needed to elicit significant responses by mouse spleen cells.¹⁵ When

spleen cells were pooled from five mice and incubated with antigen E in concentrations ranging from 12.5 ng to 100 μ g in each of two separate experiments, no significant responses were obtained in cultures harvested on days 2 to 4 and 6 to 11.

DISCUSSION

The finding that maximum lymphocyte proliferative responses to purified ragweed antigen E occurred on days 8 or 9 of culture in a majority of the subjects tested was unexpected. In several recently published studies of human lymphocyte responsiveness to ragweed antigen E, the cultures were harvested only on day 6^{6, 11, 12} or on day 7^{8, 9}; in the present study no subject's peak responsiveness to ragweed antigen E occurred on day 6. Only one previously published study reported examining the kinetics of the in vitro response to pollen allergens.⁵ In that study, Richter and Nasipz compared lymphocyte responses to dialyzed ragweed extract on days 5, 7, and 10 and concluded that maximum transformation occurred on day 7; responses on days 8 and 9 were not evaluated. Thus the major reason for divergent results among published studies appears to be that in most studies cultures were harvested prior to the average time of peak lymphocyte responsiveness to pollen allergens in vitro. In this laboratory, under the same experimental conditions, the average time of peak response to other soluble antigens such as diphtheria and tetanus toxoids is on the sixth or seventh day of culture, although some normal subjects' lymphocytes have responded maximally to *Candida* antigen on day 8.

Even more surprising was the finding that lymphocytes from all normal, atopic, and agammaglobulinemic subjects tested in these dose-response and time-course studies showed significant proliferative responses to stimulation with purified ragweed allergens. These findings are in keeping with those of Weisberg and co-workers,⁷ who observed that a variety of crude pollen extracts, as well as extracts of the leaves, seeds, stems, and roots of ragweed, produced significant stimulation of lymphocytes from nonatopic as well as atopic subjects in lymphocyte cultures harvested on day 8. Those investigators felt, however, that the apparent nonspecific stimulation they found was possibly artifactual and due to an interaction of heparin with the pollen extracts. In the studies in the present report, however, lymphocytes from normal nonatopic subjects responded equally well to purified ragweed antigen E whether cultured in a heparin-free serum-supplemented medium or in a plasma-supplemented medium.

The finding that the two lots of antigen E used for our studies contained nanogram concentrations of en-

dotoxin raised the question of whether the apparent nonspecific stimulation obtained was due either to endotoxin alone or to endotoxin acting in synergy with antigen E, as reported for phytohemagglutinin and concanavalin A.¹⁸ Studies in the present report present two lines of evidence against the possibility that endotoxin in the purified antigen E preparations was responsible for the stimulation seen: (1) highly purified endotoxin in doses ranging from nanograms to micrograms failed either to stimulate human lymphocytes under the same time-course and dose-response conditions used for antigen E or to augment the stimulation caused by antigen E alone in synergy experiments and (2) mouse spleen cells did not respond to the antigen E preparations used in prolonged time-course and dose-response studies.

The nature of the stimulation caused by antigens E, K, and Ra-3 is not resolved by the studies presented here. While the finding that lymphocytes from subjects having no IgE antibody response to antigen E (i.e., newborns and agammaglobulinemic patients) indicates that reaginic antibody production does not invariably accompany cellular proliferative responsiveness to this agent, these observations do not allow differentiation between an antigenic versus a mitogenic effect of the purified pollen antigens. Agammaglobulinemic subjects who have intact T cell function have been shown to have normal lymphocyte proliferative responses to other antigens such as tetanus and diphtheria toxoids and *Candida* antigen.¹⁹ Similarly, Leiken and associates²⁰ found a 31% incidence of in vitro lymphocyte responsiveness of cord blood lymphocytes to various ubiquitous antigens. Thus it is entirely possible that the response noted by lymphocytes from all subject groups in the present study is that to a ubiquitous antigen. Even though ragweed does not grow in many areas of the world, there may be cross-reacting antigens in those locales that would explain responsiveness by lymphocytes from subjects such as the newly arrived foreign post-doctoral fellow from Japan in the present study. On the other hand, the stimulation properties of the purified ragweed antigens in the present study are not inconsistent with those of a nonspecific mitogen. The known mitogens are all plant-derived,²¹⁻²³ and the molecular weights of the purified mitogens of concanavalin A (25,500) and pokeweed (22,000 to 32,000) are close to the molecular weight of purified ragweed antigen E (37,800).²¹⁻²⁴ Moreover, peak responsiveness of human peripheral blood lymphocytes to pokeweed can occur very late, with lymphocytes from some normal subjects responding as well on day 10 as on day 7.²⁵ In general, however, responses to other well-studied plant mitogens occur earlier than

those to specific antigens, most peak responses to the latter occurring on day 7.²² The lack of a response to antigen E in the mouse spleen cell experiments would tend to argue against but not to exclude a nonspecific mitogen effect, since interspecies differences in responsiveness to mitogens have been documented.²²

The observations reported here may relate to factors influencing the development of the atopic response, particularly if allergens other than ragweed antigens E, K, and Ra-3 prove to have similar properties. Weisberg and co-workers' findings⁷ of similar broad stimulatory properties with a variety of crude pollen extracts suggest that the latter may be the case. In addition to weeds, grasses, and trees, legumes constitute another major category of plants whose allergens often lead to marked IgE antibody responses; a majority of the known phytomitogens are derived from bean plants.²¹ In this regard, it is of interest that occupational exposure to dry ground bean powder has induced IgE-mediated hypersensitivity to components of such powders.²⁶ Similar occupationally induced IgE-mediated allergic reactions have been reported in investigators or pharmacists working with dry ragweed, grass, and tree pollen powders.²⁶ As pointed out by Kanellakes and Mathews,²⁶ however, the lack of occupationally induced allergy to aqueous extracts of such pollens suggests that the route of exposure in addition to the plant-derived nature of the material may both be crucial factors in the induction of such sensitivity, the powder being more likely to gain access to mucous membrane surfaces. Finally, if substances that readily induce blastogenesis in lymphocytes are more likely to be allergenic, additional factors must come into play before IgE antibody production occurs.

If a variety of common allergens prove to have nonspecific mitogenic properties, the lymphocyte subpopulations they stimulate as well as the actions they have on those cells will be important to delineate. Concanavalin A has been shown to be primarily a T cell stimulant²⁵ and is known to induce the formation of T cells that elaborate a factor capable of suppressing antibody synthesis by B cells.²⁷ In contrast, one of the five major fractions of pokeweed mitogen stimulates both T and B cells, inducing the latter (with the necessary help of some T cells) to differentiate into immunoglobulin-producing and -secreting cells.²⁸ Ishizaka and co-workers²⁹⁻³¹ have shown that either intact or urea-denatured antigen E or its alpha and beta chains are capable of priming T helper cells for IgE antibody production in mice. The pronounced DNA synthesis induced by ragweed antigen E in the present study's agammaglobulinemic patients (who all had intact T cell function) suggests that

the DNA synthetic response to antigen E is at least a response of T cells. Additionally, it has been shown that T cells are the predominant, if not sole, subpopulation of human lymphocytes giving proliferative responses to antigens in *in vitro* cultures.³² Gatien and co-workers¹¹ have reported that antigen E stimulates DNA synthesis in T cell-enriched fractions of allergic subject's lymphocytes but were unable to elicit proliferative responses in B cell-enriched populations; however, their cultures were harvested only on day 6. If, as the present study suggests, antigen E stimulates T cells from a majority if not all immunocompetent human subjects to undergo DNA synthesis, the factors determining which individuals will develop pronounced IgE antibody responses may involve other actions on the stimulated or even on the unstimulated lymphocyte subpopulations.

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