

From Division of Allergy and Clinical Immunology, Department of Medicine,
University of Iowa and Veterans Administration Hospitals,
Iowa City, Iowa, U.S.A.

A STUDY OF DUST-INDUCED LYMPHOCYTE BLASTOGENESIS *

By

T. J. YOO, C. Y. KUO & C. HEATH

For more than half a century house dust has been generally accepted and verified as one of the most important causative allergens in human atopic disease. At present, clinical history and intradermal skin testing for immediate hypersensitivity are the usual methods for evaluating house dust allergies. Specific IgE antibody study also provides useful information for evaluating allergic diseases (12). Studies of allergen-stimulated lymphocyte blastogenesis in ragweed, grass and mold allergy suggest that an examination of *in vitro* lymphocyte transformation also might be helpful for an understanding of house dust allergic states (1, 5, 10, 11). The clinical significance of delayed or cell-mediated (type IV) hypersensitivity in the symptomatology of dust allergy is unknown. Delayed onset skin reactions to atopic allergens are well documented (4, 6). It is the purpose of this paper to examine the *in vitro* lymphocyte response to dust extract as a measure of cell-mediated immunity, a subject that has not been previously studied in this allergy.

* Supported partly by National Foundation March of Dimes and Veterans Administration research grants.

MATERIAL AND METHODS

Selections of Patients and Controls

Twelve asymptomatic healthy adults were randomly selected from the general population to provide a normal control. In addition, 32 patients diagnosed by skin test and clinical history as dust allergic were selected from the University of Iowa and the Veterans Administration Hospital Allergy Clinic (from September, 1975 to March, 1976). Preliminary skin tests and clinical evaluations were done on all 44 subjects. Clinical evaluations included symptoms of nasal stuffiness, rhinorrhea and episodes of sneezing. Skin tests were made with commercial extracts of dust allergen (10,000 PNU, Hollister-Stier—Spokane, Washington) used in the Allergy Clinic. Skin tests (interpreted as positive when weal size was at least 6 to 8 mm) were graded from negative to 3+.

The 44 subjects were divided into three groups: (I) 12 asymptomatic subjects from the general population who served as normal control, (II) 14 patients who were allergic to dust but who had never received dust immunotherapy, and (III) 18 patients who were allergic to dust and had received routine immunotherapy (serial subcutaneous injections of 500 PNU aqueous dust extract (Hollister-Stier) that were maintained at 3-week intervals) during the year prior to this study.

Lymphocyte Culture

Twenty to thirty milliliters of heparinized venous blood was obtained from all 44 subjects for lymphocyte cultures. Lymphocytes were isolated by centrifuging the whole blood to recover the buffy coat. They were applied to a 1.078 specific gravity Ficoll-Hypaque gradient. The lymphocytes were then washed three times in minimal essential medium (MEM) (GIBCO) and resuspended at a concentration of 5×10^5 lymphocytes/ml of MEM stock solution (composed of MEM, L-glutamine (GIBCO), and pen-streptomycin (GIBCO)) with 20 per cent pooled human sera. Preliminary tests were done to determine the number of cells and the antigen dose for this blastogenesis study.

The resuspended lymphocytes were cultured by microculture techniques using flat plastic culture plates (Falcon). Each culture contained 5×10^4 cells (this cell number was chosen by preliminary study and was the optimum lower number for antigen-stimulated lymphocyte blastogenesis (microculture) in most patients studied) in 0.1 ml of cell suspension and allergen or cell suspension without allergen (control). The cultures were incubated for 7 days at 37° C in 5 per cent CO₂. As a reference positive control, 0.1 ml of cell suspension with phytohemagglutinin (PHA) was incubated for 3 days at 37° C in 5 per cent CO₂. All cultures, in triplicate, were pulsed with 1 μ Curie of tritiated thymidine (New England Nuclear) for 7 hours at 37° C in 5 per cent CO₂.

Following pulse, cells were harvested by a cell harvester (Biomedical Research Institute, Model M 24, V) and counted on a liquid scintillation counter. The results were expressed in a stimulation index calculated by dividing the radio-

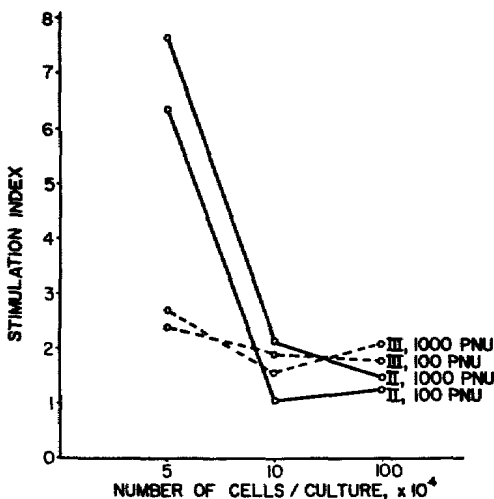


Fig. 1.

Determination of the optimal cell number in lymphocyte cultures. One patient from groups II and III was tested at two dust allergen concentrations (100 and 1000 PNU/ml).

active counts per minute (ct/min) of the dust culture by the ct/min of the MEM control culture. The ct/min of the dust and control cultures were corrected by subtracting background ct/min.

RESULTS

The results of lymphocyte cultures that were made to determine the number of lymphocytes per culture are shown in Fig. 1. Two subjects are shown, one who had received immunotherapy (from group III) and one who had not (from group II). Both concentrations of cells were set up with 100 and 1000 PNU/ml of dust extract. Representative dust lymphocyte culture results are tabulated in Table 1. As the stimulation index was seen to be significantly affected by varying the cell concentration, 5×10^4 cells per culture were chosen to be used for further study. Preliminary study to

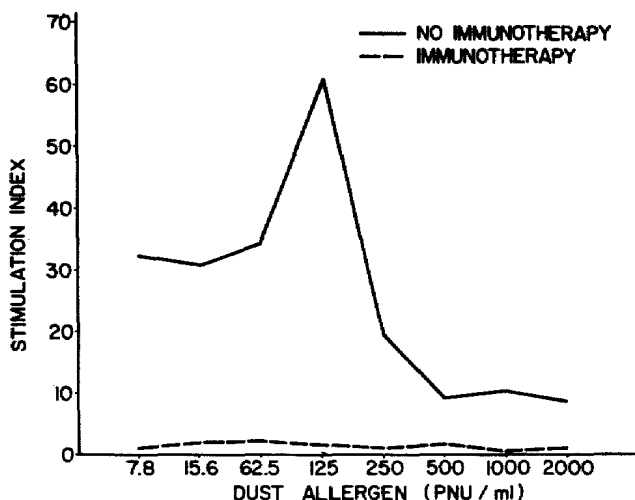


Fig. 2.

A representative dose response curve of dust lymphocyte cultures. 5×10^4 lymphocytes per culture was used.

determine optimum antigen dose ranges showed that there were no significant changes in lymphocyte response with the normal control (group I) and the patient group III between antigen doses of 7.8 PNU/ml to 2000 PNU/ml. Fig. 2 shows the results of this preliminary study and represents a typical representative curve for the individuals from groups II and III. In all patients studied, the highest stimulation was between 100 and 1000 PNU/ml. Therefore, throughout this study, dust allergen concentrations of 100, 250, 500 and 1000 PNU/ml were used.

The average stimulation index of each group at each allergen concentration was calculated, and the results of these studies are shown in Fig. 3. Compared are normal control and those patients receiving and not receiving immunotherapy. There was an increase in the stimulation index in allergy patients who had never received immunotherapy (group II) as

compared with the control group (I). Group III individuals, receiving immunotherapy, had an average stimulation index compared with the control group (I). Group III individuals,

TABLE I
Representative Dust Lymphocyte Cultures with Varying Cell Numbers

Trial*	Number of cells per culture	L.S.I.**		Ct/min†	
		Dust dose = 1000	Dust dose = 100	MEM control	PHA"
1	5×10^4	4.62	4.19	96.65	
	1×10^5	18.67	1.72	290.72	14,651
	1×10^6	0.64	6.80	2,433.00§	
2	5×10^4	6.36	7.66	50.21	
	1×10^5	1.08	2.14	360.04	30,663
	1×10^6	1.27	1.48	516.09	
3	5×10^4	1.56	1.16	391.90	
	1×10^5	4.17	1.35	605.10	64,375
	1×10^6	1.31	3.21	152.77	
4	5×10^4	2.72	2.40	252.17	
	1×10^5	1.57	1.90	435.55	44,499
	1×10^6	2.12	1.79	255.54	

* Patients of group II were tested in trials 1 and 2; group III in trials 3 and 4.

** L.S.I.: lymphocyte stimulation index.

† Ct/min: radioactive counts per minute.

" PHA: phytohemagglutinin.

§ This unusually high number is probably the result of contamination.

T-test analysis demonstrated that there is no significant difference between the normal control group (I) and the group receiving immunotherapy (II) at the level of 0.05. However, there is a significant difference between the normal control group (I) and the group (III) not receiving immunotherapy. There is also a significant difference between those patients receiving (III), and those not receiving (II), immunotherapy.

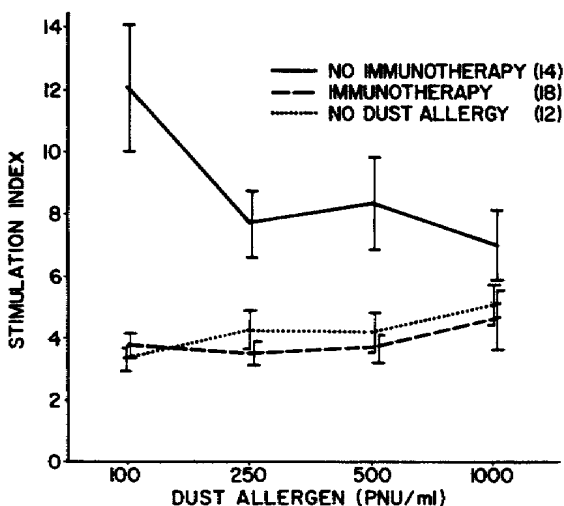


Fig. 3.

Lymphocyte stimulation to dust allergen. The average stimulation index of each group was compared at four dust allergen concentrations (100, 250, 500 and 1000 PNU/ml). Bar indicates standard error. The numbers in parentheses are the number of subjects in each group.

DISCUSSION

The aqueous extracts of house dust are commonly regarded as belonging to the most useful and relevant clinical reagents in the treatment of clinical dust allergy. In many clinical allergy departments, immunotherapy by hyposensitization schedule with house dust extracts is used for an extended period of time. Dust allergy has been shown to be a reaginic immune response; but despite investigation, the precise character of the principal allergen(s) has not been well characterized. The failure to characterize dust allergen(s) is probably due to their extremely heterogeneous nature (2).

In this study, while using commercially available dust allergens, the lymphocyte blastogenic response in dust allergy pa-

tients was higher than normal control. These findings indicate that the dust allergy patient had a broad immune response to dust allergen. It is a generally accepted idea that immediate hypersensitivity is related to the presence of reagenic antibody. The detection of significant *in vitro* lymphocyte proliferation in response to dust allergen is not clearly understood, but T-lymphocyte is acknowledged as a helper to the B-lymphocyte in the production of specific antibody (3). It has been shown that T-lymphocytes are necessary to initiate IgE response and regulate IgE production (3, 9). It is not clear whether heightened helper T-cell activity also accompanies the increased effector T-cell function, but a suggestion that this is so was seen when rats were challenged with ascaris antigen to produce reagin and their T-cell response to PHA was heightened (8). In any event, the meaning of this increased lymphocyte response in dust allergy patients is not clear. If the increase reflects patients' dust allergy, we would expect to observe some correlation between the severity of symptoms and the lymphocyte stimulation index. There is no evidence that this is so, and this point should be further investigated.

Past studies with human lymphocytes have demonstrated both a proliferative response to AgE and diminution in proliferative response after specific immunotherapy in ragweed allergic individuals (5, 11). Similar results were obtained with individuals who were alternaria-sensitive (1). It was speculated in the above studies that specific immunotherapy for immediate hypersensitivity diseases may induce tolerance in T-cell responses (1, 5, 11). Our results in this paper on dust allergy are compatible. More precise measurements of subpopulations of T-cell markers would enable us to better evaluate the changes in cell-mediated immune responses associated with immunotherapy.

SUMMARY

The *in vitro* measurement of dust allergen-stimulated lymphocyte blastogenesis by the incorporation of H^3 -thymidine can be a useful measurement for the evaluation of the cellular immune function in dust allergy patients. Dust allergy patients receiving immunotherapy had lower lymphocyte responses than those not receiving immunotherapy. Additionally, dust allergy patients as a whole showed higher lymphocyte stimulation indices than normal control.

REFERENCES

1. Anderson, J. A., Lane, S. R., Howard, W. A., Leiken, S. & Oppenheim, J. J. (1974): The effect of hyposensitization of alternaria-induced lymphocyte blastogenesis. *Cell. Immunol.* 10, 442-449.
2. Berrens, L. (1971): The chemistry of atopic allergens. Monogr. in Allergy, vol. 7. S. Karger, Basel.
3. Claman, H. N., Chaperon, E. A. & Triplett, R. F. (1966): Thymus-marrow cell combinations. Synergism in antibody production. *Proc. Soc. exp. Biol. (N.Y.)* 122, 1167-1171.
4. Dolovich, J., Hargreave, F. E., Chalmers, R., Shier, K. J., Gauldie, J. & Bienestock, J. (1973): Late cutaneous allergic responses in isolated IgE-dependent reactions. *J. Allergy clin. Immunol.* 52, 38-46.
5. Gaten, J. G., Merler, E. & Colten, H. R. (1975): Allergy to ragweed antigen E: effect of specific immunotherapy on the reactivity of human T lymphocytes *in vitro*. *Clin. Immunol. Immunopath.* 4, 32-37.
6. Green, G. R., Zweiman, B., Beerman, H. & Hildreth, E. A. (1967): Delayed skin reactions to inhalant antigens. *J. Allergy* 40, 224-236.
7. Kishimoto, T. & Ishizaka, K. (1973): Regulation of antibody response *in vitro*. V. Effect of carrier-specific helper cells on generation of hapten-specific memory cells of different immunoglobulin classes. *J. Immunol.* 111, 1-51.
8. Kuo, C. Y. & Yoo, T. J. (1976): The effect of allergenic stimulation on the growth of rat hepatoma. *Proc. Amer. Ass. Cancer Res.* 17, 197 (Abstract).
9. Okumura, K. & Tada, T. (1971): Regulation of hemocytotropic antibody formation in the rat. III. Effect of thymectomy and splectomy. *J. Immunol.* 106, 1019-1025.
10. Petrunov, B. & Konstantinova, D. (1972): Application of the lymphocyte transformation test (LTT) to the diagnosis of pollen allergy. *Acta allergol.* 27, 229-235.

11. Rocklin, R. E., Pence, H., Kaplan, H. & Evans, R. (1974): Cell-mediated immune response of ragweed-sensitive patients to ragweed antigen E. *In vitro* lymphocyte transformation and elaboration of lymphocyte mediation. *J. clin. Invest.* 53, 735-744.
12. Wide, L. (1973): Clinical significance of measurement of reaginic (IgE) antibody by RAST. *Clin. Allergy* 3 (Suppl.), 583-595.

Authors' address:

T. J. Yoo, M.D., Ph.D.
Associate Professor of Medicine
Division of Allergy and Clinical
Immunology
Department of Internal Medicine
University of Iowa and
Veterans Administration Hospital
Iowa City, Iowa 52242
U.S.A.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.