

Variable Proliferation of Peripheral Blood Mononuclear Cells to House Dust or Mites in Patients with Bronchial Asthma

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ABSTRACT

The proliferative responses of peripheral blood mononuclear cells (PBMCs) to house dust or mites in patients with bronchial asthma tended to be higher or were significantly higher, respectively, than responses in healthy controls. Interestingly, there was a large distribution of the proliferative responses in the asthmatic group. This large distribution may be caused by increased in vivo sensitization of lymphocytes to the allergens. Therefore, proliferative responses of PBMCs to house dust or mites are useful for detecting the magnitude of in vivo sensitization of lymphocytes to the allergens in patients with bronchial asthma.

INTRODUCTION

IGE-MEDIATED HYPERSENSITIVITY plays an important role in the pathogenesis of bronchial asthma. House dust and mites are as important as the allergens. We previously reported that peripheral blood mononuclear cells (PBMCs) responded well to ovalbumin or bovine serum albumin in children with atopic dermatitis who are sensitive to hen's egg or cow's milk, which illustrates that these responses are useful for detecting sensitization of lymphocytes to each food antigen.⁽¹⁾ Many of the proliferation studies have involved the use of purified proteins from rye grass and ragweed, both of which are important seasonal allergens, but little has been reported about the responses to the house dust mite, which is a major cause of perennial allergic symptoms in many countries.⁽²⁾ In this study, we show that proliferative responses of PBMCs to house dust or mites are useful for detecting magnitude of in vivo sensitization of lymphocytes to the allergens in patients with bronchial asthma.

MATERIALS AND METHODS

Subjects

Twelve patients with bronchial asthma were selected on the basis of clinical history. Subjects ranged in age from 3 to 12 years. Bronchial asthma was rated as mild in 2 patients, moderate in 6 patients, and severe in 4 patients. Patients were instructed to discontinue all medications for 7 days before and during test-

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ing. No patients had been on systemic steroids. Six nonatopic healthy children, ranging in age from 3 to 12 years, were used as controls. We obtained informed consent from each patient or their parents.

Radioallergosorbent test (RAST) and skin testing

RAST was performed as recommended by the Phadebas RAST test kit (Pharmacia Ltd., Sweden).⁽³⁾ House dust, *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae* discs were purchased from Pharmacia. RAST results were scored from 0 to 4+ by comparison with serially diluted reference sera (Pharmacia) graded A to D (i.e., a serum less than D = 0; between D and C = 1; and greater than A = 4+). RAST scores of 2+, 3+, and 4+ were recorded as positive. Skin prick testing was performed with disposable needles.

Cell preparation and culture

PBMCs were isolated from heparinized blood by gradient centrifugation in Ficoll-Paque (Pharmacia). PBMCs were suspended to give a density of 10^6 /ml in the culture medium, which consisted of RPMI 1640 supplemented with 15% pooled human AB⁺ serum, L-glutamine 2 mM, penicillin 100 units/ml, and streptomycin 100 µg/ml. PBMCs (2×10^5) were cultured at 0.2 milliliters per well in round-bottomed microtest plates (A/S Nunc) with or without various concentrations of antigens at 37°C for 5 days in a humidified atmosphere containing 5% carbon dioxide.⁽⁴⁾

Proliferative response

DNA synthesis was measured by adding 0.5 µCi [³H] thymidine (specific activity: 43 Ci/mmol, Amersham, Japan) per well 4 hours before harvesting onto glass fiber filters. ³H incorporation was measured by liquid scintillation counting, and the results were expressed as the mean of triplicate. To compare responses between individuals, we expressed the results as stimulation index (SI).

$$SI = \frac{\text{mean CPM in cultures stimulated with antigens}}{\text{mean CPM in unstimulated cultures}}$$

Antigens

House dust (protein 310 µg/ml) and mite (*D. farinae*, protein 740 µg/ml) were purchased from Torii Yakuhin Ltd. (Japan) and used in 10^{-1} , 10^{-2} , and 10^{-3} dilutions of solution for scratch test, respectively. Each solution contains 50% glycerol and 5% NaCl.

Statistics

Statistical analysis was performed with Student's (unpaired) *t*-test.

RESULTS

RAST scores and skin testing

The RAST values for house dust, *D. pteronyssinus*, and *D. farinae* were positive (score 3 or 4 for each) and skin tests for house dust and mites (*D. farinae*) also were positive in 11 of 12 asthmatic children. The RAST values were negative in all 6 healthy controls. In another patient with bronchial asthma, the RAST scores for house dust, *D. pteronyssinus*, and *D. farinae* were 0 and skin tests for house dust and mites (*D. farinae*) were negative, respectively.

Proliferative responses of PBMCs to house dust and mites

In most patients with bronchial asthma, proliferation of PBMCs increased steadily with increasing house dust or mite dose over the range 10^{-3} , 10^{-2} , and 10^{-1} . Representative dose-responses are presented in Table 1.

VARIABLE PROLIFERATION OF LYMPHOCYTES IN ASTHMA

TABLE 1. DOSE RESPONSES OF PBMCs TO HOUSE DUST OR MITES (*DERMATOPHAGOIDES FARINAE*) FOR A PATIENT WITH BRONCHIAL ASTHMA AND A HEALTHY CONTROL

Subjects	Proliferative responses of PBMCs, SI (CPM)							
	To house dust				To Mite (D. farinae)			
	10^{-3}	10^{-2}	10^{-1}		10^{-3}	10^{-2}	10^{-1}	
Patient with asthma	1.0 (573)	2.3 (1,317)	4.5 (2,578)	20.2 (11,576)	1.0 (573)	3.1 (1,776)	4.3 (2,463)	6.2 (3,551)
Healthy control	1.0 (514)	0.8 (411)	1.0 (512)	1.1 (565)	1.0 (514)	1.1 (566)	2.1 (1,077)	2.2 (1,130)

Note: Each number shows mean of triplicate.

PBMCs = peripheral blood mononuclear cells; SI = stimulation index.

The maximal proliferative responses of PBMCs for the whole series of patients and controls are presented in Figure 1. The proliferative responses of PBMCs to house dust or mites in patients with bronchial asthma tended to be higher or were significantly higher than responses on healthy controls, respectively. The patient whose RAST scores for house dust, *D. pteronyssinus*, and *D. farinae* were 0 exhibited low responses of PBMCs to house dust or mites. Interestingly, there was a large distribution of the proliferative responses in the asthmatic group. This large distribution of the responses may be caused by the increased in vivo sensitization of lymphocytes to the allergens.

DISCUSSION

House dust and mites are important as the allergens of bronchial asthma. There have been some studies on inhaled allergen-induced proliferation of PBMCs in patients with allergic diseases.^(2,5) We have previously reported that PBMCs responded well to ovalbumin or bovine serum albumin in children with atopic dermatitis who are sensitive to hen's egg or cow's milk.⁽¹⁾ Moreover, experiments involving T-lymphocyte phenotypes have shown that the responding cells were predominantly CD4+, containing naive T cells and memory T cells.^(6,7) CD4+ T lymphocytes from atopic individuals recognize processed fragments of allergens from house dust mites bound to major histocompatibility complex class II molecules. These T cells

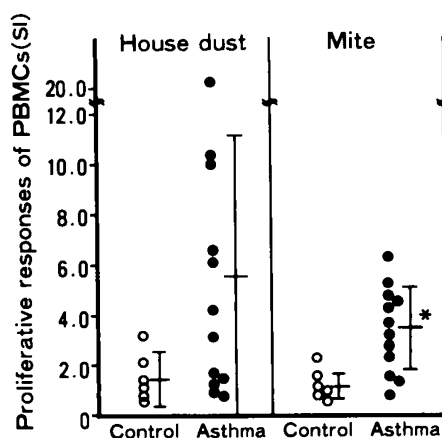


FIG. 1. Proliferative responses of peripheral blood mononuclear cells to house dust or mites in patients with bronchial asthma and healthy controls. Lines indicate mean and one standard deviation. * = the value of mites in patients was significantly ($p < 0.01$) higher than the same value in healthy controls.

become activated to induce and regulate the effector mechanisms, including the production of IgE and the activation of inflammatory cells, of allergic immune responses.⁽⁸⁾

In this study, PBMCs of asthmatic patients responded well to house dust or mites, when compared with those of healthy controls. Interestingly, there was a large distribution of the proliferative responses in asthmatic group. This large distribution may be caused by the increased in vivo sensitization of lymphocytes to the allergens. Therefore, proliferative responses of PBMCs to house dust or mites are useful for detecting magnitude of in vivo sensitization of lymphocytes to the allergens in patients with bronchial asthma.

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