

- dermatitis: evaluation of 113 patients. *J Pediatr* 1985;107:669-75.
23. Burks AW, Mallory SB, Williams LW, Shirell MA. Atopic dermatitis: clinical relevance of food hypersensitivity reactions. *J Pediatr* 1988;113:447-51.
 24. Ferguson A, Watret K. Cows' milk intolerance. *Nutr Res Rev* 1988;1:1-22.
 25. Peters T. Serum albumin. *Adv Protein Chem* 1985;37:161-245.
 26. Clement LT, Yamashita N, Martin M. The functionally distinct subpopulations of human CD4⁺ helper/inducer T lymphocytes defined by anti-CD45R antibodies derive sequentially from a differentiation pathway that is regulated by activation-dependent post-thymic differentiation. *J Immunol* 1988;141:1464-70.
 27. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone: 1. definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-57.
 28. Mosmann TR, Coffman RL. TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol* 1989;7:145-73.
 29. Paliard X, Malefijt PDW, Yssel H, et al. Simultaneous production of IL-2, IL-4, and IFN- γ by activated human CD4⁺ and CD8⁺ T cell clones. *J Immunol* 1988;141:849-55.
 30. Romagnani S, Prete GD, Maggi E, et al. Role of interleukins in induction and regulation of human IgE synthesis. *Clin Immunol Immunopathol* 1989;50:S13-23.
 31. Wierenga EA, Snoek M, Groot C, et al. Evidence for compartmentalization of functional subsets of CD4⁺ T lymphocytes in atopic patients. *J Immunol* 1990;144:4651-6.

Effect of elimination diets on food-specific IgE antibodies and lymphocyte proliferative responses to food antigens in atopic dermatitis patients exhibiting sensitivity to food allergens

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Peripheral blood mononuclear cells (PBMCs) from patients with atopic dermatitis (AD) selected as being sensitive to hen's egg or cow's milk responded to food antigens, ovalbumin, or bovine serum albumin, with significantly enhanced DNA synthesis compared with the DNA synthesis in PBMCs from nonatopic control subjects and food-sensitive patients with immediate symptoms. Patients were treated with elimination diets. Symptoms of AD had been in remission during elimination diets. The levels of specific IgE antibodies to hen's egg or cow's milk decreased during elimination diets in patients with positive radioallergen sorbent test (RAST). In patients with negative RAST, specific IgE antibodies remained negative during elimination diets. The proliferative responses of PBMCs to food antigens also decreased during elimination diets in patients with proliferative responses before elimination diets. Taken together, specific IgE antibodies to food antigens are useful indexes of the effect of elimination diets in food-sensitive patients with AD and positive RAST, and proliferative responses of PBMCs to food antigens are useful indexes of the effect of elimination diets in food-sensitive patients with AD and proliferative responses of PBMCs. (J ALLERGY CLIN IMMUNOL 1993;91:668-79.)

Key words: Atopic dermatitis, elimination diets, food allergy, lymphocyte proliferative responses, specific IgE antibodies

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Food allergy clearly seems to play an aggravating role in some patients with atopic dermatitis (AD), and the significance of IgE in the pathogenesis is discussed.^{1,2} Patients with AD caused by food allergens do not always have food-specific IgE antibodies. Wraith et al.³ have reported that 36% of foods causing "nonimmediate" symptoms give a negative radioallergosorbent test (RAST). Moreover, several investigators have reported that atopic patients have abnormal responses in cell-mediated immunity.⁴⁻⁸ Some reports exist of allergen-induced proliferation of peripheral blood mononuclear cells (PBMCs) from allergic patients.⁹⁻¹⁴ Many of the proliferation studies have involved the use of purified proteins from inhaled allergens, but little has been reported on the responses to food antigens.^{11,12} On the other hand, it is known that the levels of food-specific IgE antibodies decrease in patients with positive RAST treated with elimination diets.¹⁵ However, no index exists for the effect of elimination diets in food-sensitive patients with AD and negative RAST. In this study we showed that the levels of food-specific IgE antibodies decreased during elimination diets in food-sensitive patients with AD and positive RAST. Moreover, we showed that PBMCs from patients with AD who were sensitive to food antigens, but not from control subjects, proliferated in response to food antigens, and that proliferative responses of PBMCs gradually decreased during elimination diets in food-sensitive patients with AD. Consequently, specific IgE antibodies to food antigens were useful indexes of the effect of elimination diets in food-sensitive patients with AD and positive RAST, and proliferative responses of PBMCs to food antigens were useful indexes of the effect of elimination diets in food-sensitive patients with AD and proliferative responses of PBMCs.

MATERIAL AND METHODS

Subjects

One hundred fifty-three patients with AD were studied. Eighty-six were selected on the basis of clinical history and food challenges as being sensitive to hen's egg, and 67 were selected as being sensitive to cow's milk. The 86 patients with AD who were sensitive to hen's egg ranged in age from 3 months to 12 years (mean, 3.5 years), and the 67 patients with AD who were sensitive to cow's milk ranged in age from 3 months to 13 years (mean, 4.2 years). Diagnosis of AD was defined by the criteria of Hanifin.¹⁶ The severity of AD was graded on the basis of the food-challenge symptom scores for skin by the method of Bock et al.¹⁷: 0, healthy; absent: 1, mild; few areas of faint erythema with and without occasional scratching: 2, moderate; areas of erythema, macular and raised rash with and without scratching continuously for more than 2 minutes at a time: 3, severe; generalized marked erythema (>50%), extensive raised lesions (>25%), vesiculation and/or piloerection with and without hard continuous scratching. The cutaneous mani-

Abbreviations used

AD:	Atopic dermatitis
RAST:	Radioallergosorbent test
PBMC:	Peripheral blood mononuclear cell
BSA:	Bovine serum albumin
SI:	Stimulation index
CPM:	Counts per minute
PRU:	Phadebas RAST units

festations of all patients with AD appeared later than 2 hours after the first dose of the challenge (that is, did not appear until after the second dose). Eczematous lesions of the head and neck are prominent during infancy, whereas older children reveal lichenified lesions of popliteal and antecubital areas. The control group included 64 nonatopic healthy children without hen's egg or cow's milk sensitivity ranging in age from 4 months to 12 years (mean, 3.9 years) (control group I) and 53 patients sensitive to hen's egg or cow's milk, ranging in age from 5 months to 13 years (mean, 2.7 years), with urticaria, angioedema, acute gastroenteritis, and bronchial asthma (immediate symptoms) that appeared within 1 hour (usually within 15 minutes) after the first dose of the challenge (control group II). Both specific IgE antibodies to hen's egg or cow's milk and proliferative responses of PBMCs to ovalbumin or bovine serum albumin (BSA) were examined when diseases were diagnosed. Moreover, we investigated the courses of the severity of AD, specific IgE antibodies, and proliferative responses of PBMCs in 54 randomly selected cases of the 153 patients with AD and hen's egg or cow's milk sensitivity. Forty-three of the 54 patients with AD were treated with elimination diets excluding the offending foods, and 11 ingested the offending foods continuously. Of the 43 patients with AD treated with elimination diets, 27 were sensitive to hen's egg, ranging in age from 4 months to 12 years, and 16 were sensitive to cow's milk, ranging in age from 3 months to 12 years. Of the 11 patients with AD and continuous ingestion of the offending foods, six were sensitive to hen's egg, ranging in age from 1 year to 10 years, and five were sensitive to cow's milk, ranging in age from 8 months to 10 years. Both specific IgE antibodies to hen's egg or cow's milk and proliferative responses of PBMCs to ovalbumin or BSA were investigated several times during the course of the study for these 54 patients with AD. The severity of AD was examined every 2 weeks. Food challenges were performed mainly in the double-blind, placebo-controlled manner and in an open manner if there was a clear-cut history of major allergic skin symptoms after ingestion of a specific food or if there was a chance of systemic anaphylaxis developing. Consequently, all of the 54 patients with AD we followed and 58 of the other 99 patients with AD were diagnosed by double-blind food challenges. In double-blind, placebo-controlled food challenges, we prepared food capsules packed with dehydrated powdered whole egg (0.4 gm per capsule) or dehydrated powdered milk (0.4 gm per capsule) (Meiji Milk Products Co., Ltd., Tokyo, Japan) or placebo (potato starch). The first challenge

TABLE I. Results of proliferative responses of PBMCs to β -lactoglobulin or BSA in healthy children, patients with immediate allergic symptoms, and patients with AD who are sensitive to cow's milk

Subjects (no.)	Base CPM*	Proliferative responses of PBMCs to β -lactoglobulin		Proliferative responses of PBMCs to BSA	
		CPM*	SI*	CPM*	SI*
Healthy children (14)	332.1 $\pm$ 142.5	432.1 $\pm$ 161.0	1.36 $\pm$ 0.42	444.3 $\pm$ 123.0	1.44 $\pm$ 0.44
Patients with immediate allergic symptoms (16)	297.3 $\pm$ 117.6	422.7 $\pm$ 270.3	1.46 $\pm$ 0.79	401.0 $\pm$ 211.6	1.37 $\pm$ 0.48
Patients with AD, sensitive to cow's milk (56)	317.5 $\pm$ 142.7	787.8 $\pm$ 629.9†	2.51 $\pm$ 1.68†	1136.7 $\pm$ 1049.3‡	3.67 $\pm$ 2.17‡

Correlation between proliferative responses of PBMCs to β -lactoglobulin and the responses to BSA was calculated by Spearman rank test.

Proliferative responses to BSA correlated well ($p < 0.01$) with the responses to β -lactoglobulin.

Data were analyzed by the Wilcoxon-Mann-Whitney test.

*Mean $\pm$ 1 SD

† β -lactoglobulin-stimulated CPM and SI in patients with AD who were sensitive to cow's milk were significantly ($p < 0.005$) higher than those of healthy children and patients with immediate allergic symptoms, respectively.

‡BSA-stimulated CPM and SI in patients with AD who were sensitive to cow's milk were significantly ($p < 0.005$) higher than those of healthy children and patients with immediate allergic symptoms, respectively.

dose was one capsule, and doubled doses were continued every hour until the patient had convincing symptoms or until 16 capsules were ingested as a single challenge dose. For young children the dehydrated foods were hidden in broth or juice. We defined the time of reaction as being the time from the first dose of the challenge to the onset of symptoms. All medications were withheld for 7 days before and during challenges.

Furthermore, 10 additional patients with AD who were sensitive to both hen's egg and cow's milk and ranged in age from 8 months to 6 years (mean, 2.2 years) were studied to demonstrate changes specific to food allergen in proliferative responses of PBMCs to food allergen when only one or both of the foods were eliminated.

Cell separation and culture

Blood was taken into preservative-free heparin and was separated by gradient centrifugation with the use of Ficoll-Paque (Pharmacia, Uppsala, Sweden). PBMCs were harvested from the interface and washed three times in PBS. PBMCs were cultured at 10^6 /ml of RPMI 1640 culture medium supplemented with 15% pooled human AB⁺ serum, 10 mmol/L *N*-2-hydroxyethyl-piperazine-*N'*-2-ethane-sulfonic acid, L-glutamine (2 mmol/L), penicillin (100 IU/ml), and streptomycin (100 μ g/ml). Cultures were performed in triplicate at 0.2 ml per well in round-bottomed microtest plates (A/S Nunc, Roskilde, Denmark) with or without various concentrations of food antigens at 37°C in a humidified atmosphere containing 5% CO₂ for 3 to 5 days, because the peak proliferation was observed between days 3 and 5.¹² Food antigens, ovalbumin (Wako Pure Chemical Industries, Osaka, Japan), BSA (Wako), and β -lactoglobulin (Sigma Chemical Co., St. Louis, Mo.) were respectively used in final concentrations of 0.25 μ g/ml, 2.5 μ g/ml, and 25 μ g/ml.

Proliferative response

DNA synthesis was measured by adding 0.5 μ Ci [³H] thymidine per well 4 hours before harvesting onto glass fiber filters. [³H] thymidine incorporation was measured by liquid scintillation counting, and results were expressed as the mean of triplicates. To compare responses between individuals, results were expressed as stimulation index (SI) with the following formula (counts per minute [CPM]):

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

The value of the maximal proliferative responses of PBMCs was used for each subject. In most cases proliferation increased steadily with increasing antigen dose over the range 0.25 to 25 μ g/ml.¹² We had simultaneously investigated the proliferative responses of PBMCs to both BSA and β -lactoglobulin. The results shown in Table I indicate that considerable agreement exists between the results for proliferative responses with BSA and the results with β -lactoglobulin. Moreover, proliferative responses of PBMCs to BSA correlated well ($p < 0.01$) with the responses to β -lactoglobulin in patients with AD who are sensitive to cow's milk. Therefore we used BSA as a milk antigen. The point at which peak proliferation was seen stayed the same for SI after elimination diets as well as before elimination diets. The specificity of proliferative responses of PBMCs to ovalbumin or BSA was confirmed by a comparison of responses in healthy children and patients with AD and sensitivity to hen's egg or cow's milk. Results are shown in Table II.

Specific IgE antibodies to food antigens

The RAST was performed as recommended by the Phad-eas RAST test kit (Pharmacia).¹⁸ Hen's egg and cow's milk disks were supplied by Pharmacia. RAST results were

TABLE II. Results of proliferative responses of PBMCs to ovalbumin or BSA in healthy children, patients with AD who are sensitive to hen's egg but not to cow's milk, and patients with AD who are sensitive to cow's milk but not to hen's egg

Subjects (no.)	Base CPM*	Proliferative responses of PBMCs to ovalbumin		Proliferative responses of PBMCs to BSA	
		CPM*	SI*	CPM*	SI*
Healthy children (15)	338.1 ± 22.2	438.5 ± 130.8	1.29 ± 0.35	418.4 ± 145.8	1.24 ± 0.39
Patients with AD, sensitive to hen's egg but not to cow's milk (23)	351.7 ± 68.5	1103.7 ± 678.2†	3.09 ± 1.72†	415.3 ± 182.5	1.16 ± 0.35
Patients with AD, sensitive to cow's milk but not to hen's egg (15)	327.8 ± 76.9	352.6 ± 96.2	1.10 ± 0.33	1228.0 ± 697.9‡	3.82 ± 2.31‡

Data were analyzed by the Wilcoxon-Mann-Whitney test.

*Mean ± 1 SD

†Ovalbumin-stimulated CPM and SI in patients with AD who were sensitive to hen's egg but not to cow's milk were significantly ($p < 0.005$) higher than those of healthy children and patients with AD who were sensitive to cow's milk but not to hen's egg, respectively. There was no difference in ovalbumin-stimulated CPM and SI between healthy children and patients with AD who were sensitive to cow's milk but not to hen's egg.

‡BSA-stimulated CPM and SI in patients with AD who were sensitive to cow's milk but not to hen's egg were significantly ($p < 0.005$) higher than those of healthy children and patients with AD who were sensitive to hen's egg but not to cow's milk, respectively. There was no difference in BSA-stimulated CPM and SI between healthy children and patients with AD who were sensitive to hen's egg but not to cow's milk.

TABLE III. Results of serum IgE levels, specific IgE antibodies to hen's egg or cow's milk and proliferative responses of PBMCs to ovalbumin or BSA for control group I, control group II, and patients with AD who are sensitive to hen's egg or cow's milk

Subjects† (no.)	Age (yr)*	Serum IgE levels (U/ml)*	Specific IgE antibodies to food antigen‡ (PRU/ml)*	Base CPM*	Proliferative responses of PBMCs to food antigens§	
					CPM*	SI*
Hen's egg allergy						
Control						
Group I (32)	3.9 ± 2.1	336 ± 876	0.10 ± 0.33	331.4 ± 201.3	452.9 ± 240.7	1.40 ± 0.39
Group II (27)	2.3 ± 1.8	129 ± 119	2.95 ± 4.70**	294.1 ± 131.6	475.5 ± 164.1	1.67 ± 0.38
Patients with AD (86)	3.5 ± 3.1	860 ± 2371**	2.41 ± 4.92**	301.1 ± 158.7	1063.1 ± 654.6	3.25 ± 1.38
Cow's milk allergy						
Control						
Group I (32)	3.9 ± 3.2	397 ± 887	0.04 ± 0.15	323.2 ± 129.7	487.8 ± 211.1	1.50 ± 0.39
Group II (26)	3.0 ± 2.2	304 ± 337	1.69 ± 3.90**	294.2 ± 104.9	410.3 ± 121.5	1.43 ± 0.40
Patients with AD (67)	4.2 ± 3.3	1141 ± 2742**	1.13 ± 3.32**	306.9 ± 187.4	1283.6 ± 935.2	4.17 ± 2.63

Data were analyzed by the Wilcoxon-Mann-Whitney test.

Serum IgE levels of patients with AD were significantly (** $p < 0.01$ for each) higher than those of control groups I and II.

Specific IgE antibodies to hen's egg or cow's milk of patients with AD and of control group II were significantly (** $p < 0.01$ for each) higher than those of control group I, respectively.

Ovalbumin- or BSA-stimulated CPM and SI in patients with AD who were sensitive to hen's egg or cow's milk were significantly (|| $p < 0.005$ for each) higher than those of control groups I and II, respectively.

*Mean ± 1 SD.

†Control group I, healthy children; control group II, patients with immediate allergic symptoms.

‡Food antigens for specific IgE antibodies were hen's egg in hen's egg allergy and cow's milk in cow's milk allergy.

§Food antigens for proliferative responses of PBMCs were ovalbumin in hen's egg allergy and BSA in cow's milk allergy.

TABLE IV. Courses of the severity of AD, specific IgE antibodies to hen's egg, and proliferative responses of PBMCs to ovalbumin on 33 patients with AD who are sensitive to hen's egg

Patient	Age	Specific IgE antibodies to hen's egg (PRU/ml)		Proliferative responses of PBMCs to ovalbumin (to BSA*) (SI)		Severity of AD		Other allergic symptoms
		Before†	After‡	Before†	After‡	Before†	After‡	
Elimination diet group								
1	6 mo	0.34>	0.34>	7.48 (0.60)	2.74 (0.34)	Moderate	Mild	
2	8 mo	5.6	1.9	6.80 (1.56)	0.85 (1.45)	Moderate	Mild	
3	1 yr 0 mo	0.34>	0.34>	5.24 (1.48)	2.63 (0.89)	Moderate	Mild	
4	2 yr 5 mo	3.4	0.4	4.00 (0.97)	1.57 (0.72)	Mild	Healthy	BA
5	5 mo	1.7	0.34>	3.98 (1.19)	1.25 (1.36)	Moderate	Mild	BA
6	1 yr 10 mo	2.8	2.8	3.78	2.67	Moderate	Mild	
7	2 yr 1 mo	0.34>	0.34>	3.75 (0.85)	2.71 (0.79)	Moderate	Mild	
8	10 mo	2.4	0.9	3.51	2.02	Severe	Moderate	
9	4 yr 6 mo	0.7	0.9	3.48 (1.02)	2.39 (0.42)	Severe	Moderate	
10	1 yr 5 mo	1.4	0.4	3.27 (1.30)	1.86 (1.00)	Moderate	Healthy	
11	8 mo	7.4	2.0	3.19 (1.31)	2.27 (1.87)	Moderate	Mild	
12	6 mo	0.8	0.34>	3.15 (1.75)	2.17 (1.45)	Moderate	Mild	
13	1 yr 7 mo	17.5<	14.3	3.12	1.13	Severe	Moderate	BA
14	10 mo	17.5<	9.6	3.11	1.71	Moderate	Mild	
15	9 yr 3 mo	0.34>	0.34>	2.76 (1.75)	2.32 (0.65)	Severe	Mild	
16	8 mo	1.7	0.34>	2.54 (0.91)	1.37 (0.78)	Severe	Healthy	
17	6 mo	4.3	2.6	2.48 (1.19)	2.30 (0.91)	Moderate	Mild	
18	4 yr 9 mo	0.34>	0.34>	2.26 (0.58)	1.36 (0.70)	Mild	Healthy	UR
19	1 yr 4 mo	1.0	0.4	2.07 (1.54)	1.37 (1.01)	Mild	Healthy	DA
20	1 yr 6 mo	1.4	2.1	2.06	1.14	Mild	Healthy	BA
21	1 yr 5 mo	8.3	3.9	2.02 (1.02)	2.04 (1.47)	Mild	Healthy	
22	4 mo	5.1	0.4	2.01	1.00	Severe	Mild	
23	7 mo	3.0	0.34>	1.96 (0.87)	0.91 (0.59)	Mild	Healthy	BA
24	3 yr 3 mo	17.5<	17.4	1.25 (0.79)	1.95 (1.41)	Moderate	Mild	
25	4 mo	17.5<	0.6	1.21	2.05	Moderate	Mild	
26	12 yr 4 mo	0.7	0.34>	1.05 (1.45)	1.52 (1.13)	Severe	Moderate	BA
27	1 yr 8 mo	1.1	0.8	1.01 (1.27)	1.00 (1.29)	Mild	Healthy	
Nonelimination diet group								
28	2 yr 0 mo	4.5	6.8	4.43 (1.56)	3.71 (0.81)	Moderate	Moderate	
29	6 yr 1 mo	0.34>	0.34>	2.64	2.71	Moderate	Moderate	
30	3 yr 9 mo	3.4	3.9	2.51	2.78	Moderate	Severe	
31	1 yr 6 mo	0.34>	0.34>	2.14 (0.88)	3.05 (0.93)	Moderate	Moderate	BA
32	1 yr 6 mo	0.4	0.4	1.06	1.67	Mild	Mild	
33	10 yr 9 mo	1.6	2.2	0.72 (0.78)	3.37 (1.16)	Moderate	Severe	

BA, bronchial asthma; UR, urticaria; DA, drug allergy.

Results of statistical analysis for the data of proliferative responses of PBMCs are shown in Table VI.

*The value in each parenthesis is SI in the case in which BSA was used as a control antigen.

†Data before elimination diets or nonelimination diets were begun.

‡Data for 3 months or for more than 3 months after elimination diets or nonelimination diets were begun.

scored 0 to 4+ by comparison with serially diluted reference sera (Pharmacia) graded A to D (i.e., a serum <D = 0; between D and C = 1+; and >A = 4+). RAST scores of 2+, 3+, and 4+ (≥ 0.7 Phadebas RAST units [PRU]/ml) were recorded as positive, and RAST scores of 0 and 1+ (< 0.7 PRU/ml) were recorded as negative.

Statistics

Data were expressed as the mean $\pm$ SD. Statistical analysis was performed with use of the Wilcoxon-Mann-Whitney test, the paired Wilcoxon test, or the Spearman rank test.

TABLE V. Courses of the severity of AD, specific IgE antibodies to cow's milk, and proliferative responses of PBMCs to BSA on 21 patients with AD who are sensitive to cow's milk

Patients	Age	Specific IgE antibodies to cow's milk (PRU/ml)		Proliferative responses of PBMCs to BSA (to ovalbumin*) (SI)		Severity of AD		Other allergic symptoms
		Before†	After‡	Before†	After‡	Before†	After‡	
Elimination diet group								
1	2 yr 2 mo	0.34>	0.34>	8.44 (0.61)	1.60 (1.14)	Moderate	Mild	
2	6 yr 5 mo	0.34>	0.34>	8.29 (1.20)	1.08 (1.34)	Severe	Moderate	BA
3	1 yr 7 mo	17.5<	17.5<	5.31 (0.88)	1.30 (0.94)	Severe	Moderate	BA
4	1 yr 10 mo	0.34>	0.34>	5.10	1.68	Moderate	Mild	
5	6 mo	17.5<	9.9	5.07 (1.50)	1.61 (0.93)	Moderate	Healthy	
6	1 yr 7 mo	0.34>	0.34>	4.91 (1.27)	1.42 (0.85)	Moderate	Healthy	
7	4 yr 6 mo	1.6	0.9	4.89 (1.80)	1.30 (1.30)	Moderate	Mild	
8	3 mo	6.8	0.34>	4.84	3.78	Moderate	Mild	
9	5 yr 9 mo	0.34>	0.34>	3.62 (0.81)	2.48 (0.92)	Moderate	Moderate	
10	1 yr 8 mo	2.8	0.8	2.70 (1.13)	1.42 (0.83)	Mild	Healthy	
11	1 yr 3 mo	0.34>	0.34>	2.43	1.02	Severe	Mild	GI
12	4 yr 9 mo	0.34>	0.34>	2.32 (0.96)	1.59 (1.04)	Severe	Moderate	
13	9 yr 3 mo	0.34>	0.34>	2.12	1.33	Severe	Mild	
14	8 mo	0.34>	0.34>	1.98 (0.88)	1.33 (0.91)	Moderate	Mild	
15	12 yr 3 mo	1.7	0.34>	1.45 (0.77)	1.13 (1.13)	Severe	Mild	BA
16	1 yr 5 mo	3.0	2.6	1.19 (1.38)	1.67 (1.33)	Moderate	Mild	
Nonelimination diet group								
17	8 mo	0.34>	0.9	3.21 (1.12)	3.44 (0.96)	Moderate	Moderate	
18	1 yr 5 mo	0.34>	0.34>	2.22 (1.44)	2.71 (1.85)	Mild	Mild	BA
19	1 yr 2 mo	0.34>	0.34>	2.19	2.81	Mild	Moderate	
20	4 yr 2 mo	7.4	8.3	1.68 (0.78)	2.56 (0.81)	Moderate	Severe	
21	10 yr 9 mo	0.9	0.5	0.88	7.33	Moderate	Severe	

BA, Bronchial asthma; GI, gastrointestinal symptoms.

Results of statistical analysis for the data of proliferative responses of PBMCs are shown in Table VII.

*The value in each parenthesis is SI in the case in which ovalbumin was used as a control antigen.

†Data before elimination diets or nonelimination diets were begun.

‡Data for 3 months or for more than 3 months after elimination diets or nonelimination diets were begun.

RESULTS

Serum IgE levels, specific IgE antibodies, and proliferative responses of PBMCs to food antigens

The results of serum IgE levels, specific IgE antibodies to hen's egg or cow's milk, and proliferative responses of PBMCs to ovalbumin or BSA for control group I, control group II, and patients with AD who are sensitive to hen's egg or cow's milk are presented in Table III. Serum IgE levels of patients with AD were significantly ($p < 0.01$ for each) higher than those of control groups I and II. Specific IgE antibodies to hen's egg or cow's milk of patients with AD and of control group II were significantly ($p < 0.01$ for each) higher than those of control group I, respectively. The proliferative responses of PBMCs

to ovalbumin or BSA in patients with AD who were sensitive to hen's egg or cow's milk were significantly ($p < 0.005$ for each, Table III) higher than responses of control groups I and II, respectively. To confirm the stability of these responses, we investigated proliferative responses at least two times over 2 weeks on 36 subjects, including 10 of control group I, 10 of control group II, and 16 of patients with AD; as a result, the responses were reproducible.

Effect of elimination diets on the severity of AD, specific IgE antibodies, and proliferative responses of PBMCs to food antigens

The courses of the severity of AD, specific IgE antibodies, and proliferative responses of PBMCs were followed in 54 randomly selected patients with

TABLE VI. Results of statistical analysis for comparison of proliferative responses of PBMCs to ovalbumin or BSA between before and after elimination diets of hen's egg in 33 patients with AD who are sensitive to hen's egg

Subject (no.)		Before elimination*	After elimination*	p value
Elimination diet group				
Base CPM	(27)	351.4 ± 65.3	351.1 ± 117.4	NS
Ovalbumin-stimulated CPM	(27)	1086.2 ± 616.7	667.1 ± 283.9	<i>p</i> < 0.005
SI	(27)	3.06 ± 1.56	1.79 ± 0.60	<i>p</i> < 0.005
BSA-stimulated CPM	(20)	430.7 ± 187.2	379.0 ± 191.9	NS
SI	(20)	1.17 ± 0.35	1.01 ± 0.41	NS
Nonelimination diet group				
Base CPM	(6)	324.1 ± 57.2	373.8 ± 95.8	NS
Ovalbumin-stimulated CPM	(6)	715.5 ± 410.9	1067.7 ± 385.2	NS
SI	(6)	2.25 ± 1.32	2.88 ± 0.70	NS
BSA-stimulated CPM	(3)	312.2 ± 122.3	381.2 ± 208.8	NS
SI	(3)	1.07 ± 0.42	0.97 ± 0.18	NS

Data were analyzed by paired Wilcoxon test.

Ovalbumin-stimulated CPM and SI had significantly decreased after elimination diets compared with those before elimination diets in elimination diet group; however, no reductions occurred in nonelimination diet group. No reductions occurred in BSA-stimulated CPM and SI after elimination diets.

*Mean ± 1 SD.

AD, including the 33 patients sensitive to hen's egg and 21 patients sensitive to cow's milk. Forty-three of them were treated with elimination diets excluding the offending foods, and 11 had ingested the offending foods continuously. Of the 43 patients with AD treated with elimination diets, 27 were sensitive to hen's egg, and 16 were sensitive to cow's milk. Of the 11 patients with AD without elimination diets, six were sensitive to hen's egg, and five were sensitive to cow's milk. The courses of the severity of AD, specific IgE antibodies, and proliferative responses of PBMCs on these 54 patients with AD are shown in Table IV and Table V. In all 27 patients (no. 1 to 27 in Table IV) with AD who were sensitive to hen's egg, the severity of AD improved as a result of elimination diets of hen's egg. In all 16 patients (no. 1 to 16 in Table V) with AD who were sensitive to cow's milk except one (no. 9 in Table V) the severity of AD improved as a result of elimination diets of cow's milk. In contrast, the severity of AD in the 11 patients with AD and continuous ingestion of the offending foods did not improve or became worse (Table IV and Table V). There are no differences in the clinical improvement after elimination diets among patients with AD with or without specific IgE antibodies to hen's egg or cow's milk. However, clinical symptoms tended to improve more rapidly in patients with specific IgE antibodies to hen's egg or cow's milk than in patients without them.

The courses of specific IgE antibodies are also shown in Fig. 1, and the courses of proliferative re-

sponses are also shown in Fig. 2. The levels of specific IgE antibodies to hen's egg or cow's milk decreased gradually during elimination diets in 18 of the 22 patients with positive RAST to hen's egg and in six of the seven patients with positive RAST to cow's milk. However, in the five patients with AD and negative RAST to hen's egg and in the nine patients with AD and negative RAST to cow's milk, the levels of specific IgE antibodies remained negative during elimination diets. Furthermore, the levels of specific IgE antibodies did not decrease in six patients sensitive to hen's egg or five patients sensitive to cow's milk without elimination diets (Fig. 1). From these results it is considered that specific IgE antibodies are useful as indexes of the effect of elimination diets in patients with positive RAST to hen's egg or cow's milk. However, specific IgE antibodies are not indexes of the effect of elimination diets for patients with negative RAST. Table VI and Table VII show the results of statistical analysis for the data of the proliferative responses of PBMCs before and after elimination diets that were shown in Table IV and Table V, respectively. It is interesting to note that proliferative responses of PBMCs to ovalbumin or BSA had significantly (*p* < 0.005 for each) decreased after elimination diets compared with the proliferative responses of PBMCs before elimination diets (Table VI, Table VII, and Fig. 2). Moreover, proliferative responses of PBMCs did not decrease after elimination diets in the 11 patients with AD (6 patients sensitive to hen's egg and 5 patients sensitive to cow's milk) who continued to

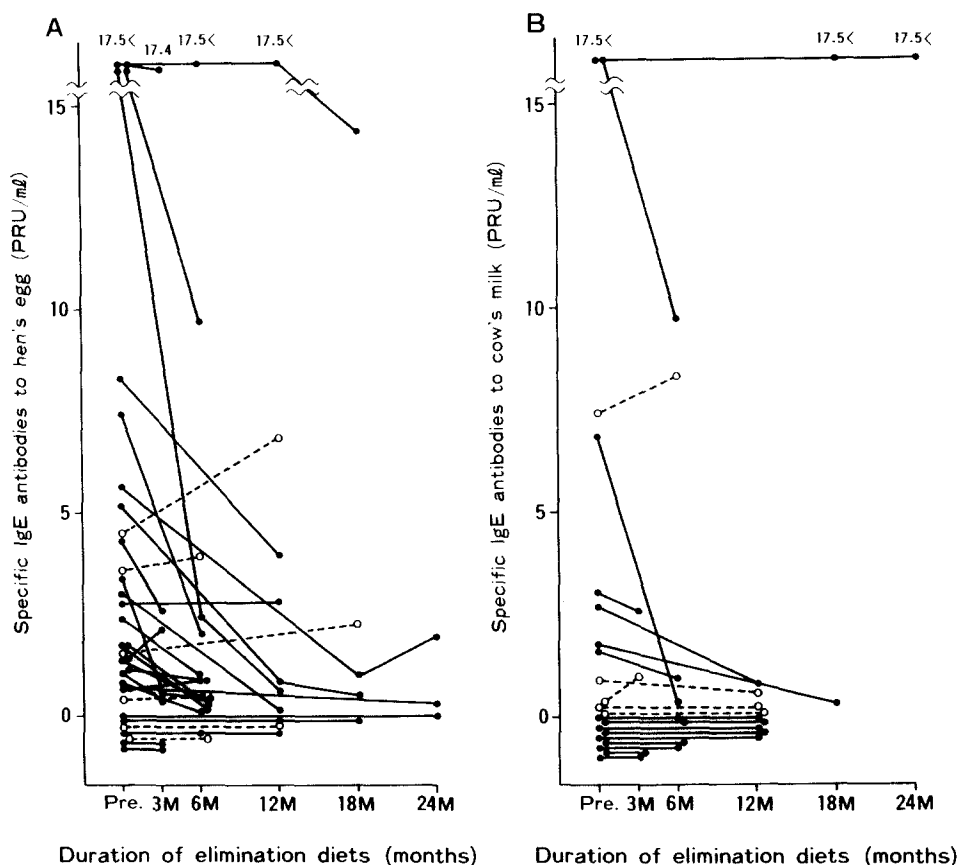


FIG. 1. A, The courses of specific IgE antibodies to hen's egg. Results illustrated in 27 patients with AD who are sensitive to hen's egg during elimination diets (●—●), and in six patients with AD who are sensitive to hen's egg with continuous ingestion of hen's egg (○—○). **B,** The courses of specific IgE antibodies to cow's milk. Results illustrated in 16 patients with AD who are sensitive to cow's milk during elimination diets (●—●) and in five patients with AD who are sensitive to cow's milk with continuous ingestion of cow's milk (○—○).

ingest the offending foods (Table VI, Table VII, and Fig. 2). Consequently, the decrease of proliferative responses of PBMCs in the elimination diet group is thought to be due to the effect of elimination diets. On the other hand, there were no reductions in proliferative responses of PBMCs to BSA in patients with AD who were sensitive to hen's egg but not to cow's milk (Table VI), and there were no reductions in proliferative responses of PBMCs to ovalbumin in patients with AD who were sensitive to cow's milk but not to hen's egg (Table VII). Thus there were no changes in the proliferative responses to the control antigen. Furthermore, we investigated the courses of proliferative responses in 10 additional patients with AD who were sensitive to both hen's egg and cow's milk and in whom only one or both of the foods were eliminated from the diet. The changes in proliferation for the eliminated or noneliminated food antigen are shown in Table VIII. When only one of the foods was eliminated in six patients (no. 1 to 6 in Table VIII), there were no changes in proliferation for the non-

eliminated food antigen, although the proliferative responses to the eliminated food antigen decreased. On the other hand, proliferative responses to both ovalbumin and BSA decreased when both hen's egg and cow's milk were eliminated in four patients (no. 7 to 10 in Table VIII). Therefore the reduction of proliferative responses of PBMCs is thought to be an antigen-specific response.

DISCUSSION

Several investigators have reported abnormal responses in cell-mediated immunity in atopic disease.⁴⁻⁸ There have been some reports about specific responses to specific antigens: for example, T-cell responses to the major allergen from *Dermatophagoides pteronyssinus*, antigen P₁, by Rawle et al.,⁹ the reaction of lymphocyte responses to ragweed antigen E by Gatien et al.,¹³ and Rocklin et al.,¹⁴ and T-lymphocyte proliferative responses induced because of potent antigen-presenting capacity of Langerhans cells after patch test of house dust mite and grass

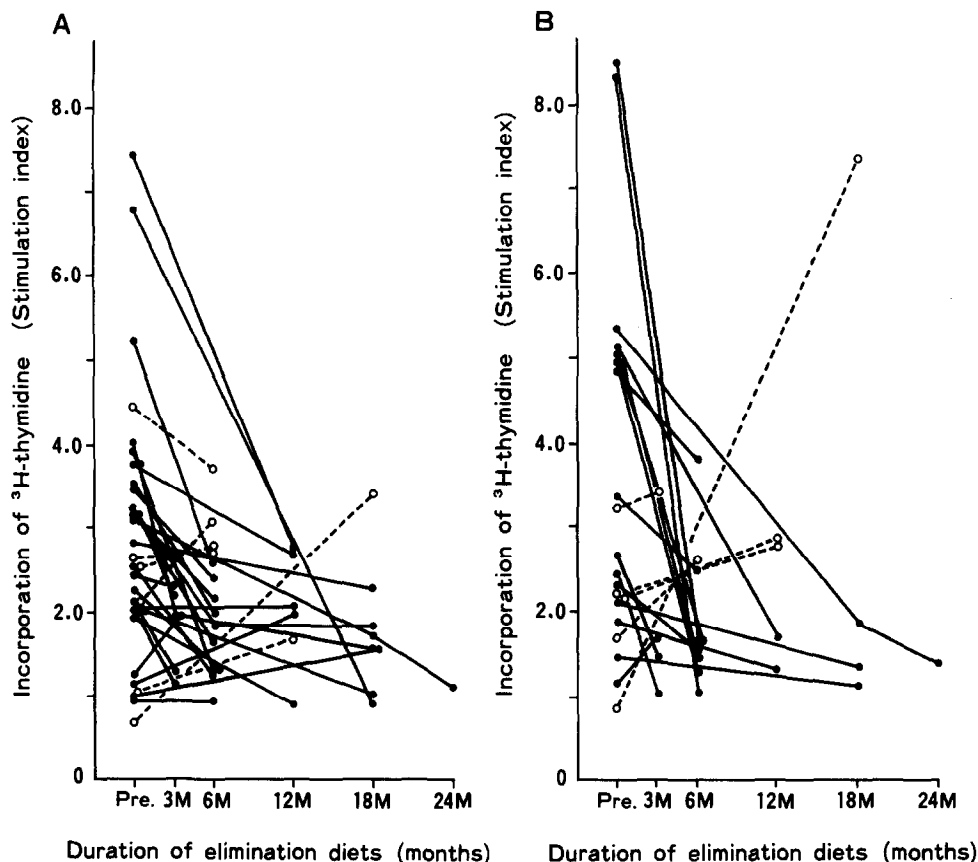


FIG. 2. A, The courses of proliferative responses of PBMCs to ovalbumin. Results illustrated for PBMCs from 27 patients with AD who are sensitive to hen's egg during elimination diets (●—●), and for PBMCs from six patients with AD who are sensitive to hen's egg with continuous ingestion of hen's egg (○—○). B, The courses of proliferative responses of PBMCs to BSA. Results illustrated for PBMCs from 16 patients with AD who are sensitive to cow's milk during elimination diets (●—●) and for PBMCs from five patients with AD who are sensitive to cow's milk with continuous ingestion of cow's milk (○—○).

pollen allergens by Bruynzeel-Kooman and Bruynzeel.¹⁹ However, little has been reported on food antigen-induced lymphocyte response.^{11, 12} In this study we investigated proliferative responses of PBMCs to food antigens, ovalbumin and BSA, in patients with AD who are sensitive to hen's egg or cow's milk and controls. We have shown that PBMCs from patients with AD sensitive to hen's egg or cow's milk have significant proliferative responses to ovalbumin or BSA. The previous study reported that patients with ragweed allergic rhinitis or ragweed hay fever had circulating lymphocytes that recognized and responded to ragweed antigen E.^{13, 14, 20, 21} Furthermore, most patients sensitive to *Dermatophagoides pteronyssinus* had T cells in their peripheral blood lymphocytes that responded to antigen P₁.⁹ In a similar manner our study with regard to food antigens suggests that specific food-sensitive AD is associated with cell-mediated immunity to specific food antigens.

An elimination diet is a good treatment for food allergy. Businco et al.⁵ reported that 12 out of 18 children with resolved allergy to cow's milk by elim-

ination diets became RAST negative, and that RAST might be useful either in the diagnosis or in the prognosis of cow's milk allergy. In our study, the levels of specific IgE antibodies to hen's egg or cow's milk decreased gradually during elimination diets. Therefore specific IgE antibodies to food antigens were useful as indexes of the effect of elimination diets in food-sensitive patients with positive RAST. However, patients with AD caused by food allergens do not always have food-specific IgE antibodies. For the patients with negative RAST, no indexes exist with which to evaluate the effect of elimination diets. Some reports on the effect of immunotherapy on lymphocyte responses have appeared previously.^{20, 22} However, it is unclear whether elimination diets in food allergy affect the proliferative responses of PBMCs to food antigens. We have here studied the effect of elimination diets on proliferative responses of PBMCs in patients with AD who are food sensitive during elimination diets. As a result, proliferative responses of PBMCs to food antigens gradually decreased during elimination diets. In these patients clinical symptoms

TABLE VII. Results of statistical analysis for comparison of proliferative responses of PBMCs to BSA or ovalbumin between, before, and after elimination diets of cow's milk in 21 patients with AD who are sensitive to cow's milk

Subjects (no.)		Before elimination*	After elimination*	<i>p</i> value
Elimination diet group				
Base CPM	(16)	326.0 ± 77.0	333.5 ± 89.4	NS
BSA-stimulated CPM	(16)	1285.3 ± 657.5	494.2 ± 163.5	<i>p</i> < 0.005
SI	(16)	4.04 ± 2.22	1.61 ± 0.65	<i>p</i> < 0.005
Ovalbumin-stimulated CPM	(12)	353.2 ± 106.0	350.7 ± 104.8	NS
SI	(12)	1.10 ± 0.35	1.06 ± 0.19	NS
Nonelimination diet group				
Base CPM	(5)	307.6 ± 45.2	356.2 ± 55.1	NS
BSA-stimulated CPM	(5)	621.2 ± 259.1	1351.7 ± 737.9	<i>p</i> < 0.025
SI	(5)	2.03 ± 0.85	3.77 ± 2.02	<i>p</i> < 0.025
Ovalbumin-stimulated CPM	(3)	350.3 ± 53.7	409.0 ± 165.6	NS
SI	(3)	1.11 ± 0.33	1.20 ± 0.56	NS

Data were analyzed by paired Wilcoxon test.

BSA-stimulated CPM and SI had significantly decreased after elimination diets compared with those before elimination diets in elimination diet group; however, no reductions occurred in nonelimination diet group. No reductions occurred in ovalbumin-stimulated CPM and SI after elimination diets.

*Mean ± 1 SD.

of AD also improved. These results suggest that elimination diets may be capable of inducing decreased responsiveness of food-sensitive lymphocytes.

In the report "Workshop on Experimental Methodology for Clinical Studies of Adverse Reactions to Foods and Food Additives,"²³ clinical trial design, specific challenge procedures, and statistical procedures are designed. Moreover, in this report it is mentioned that information including the method of challenge procedure should be presented in an article to allow the reader to assess the validity of the study.²³ In our study we defined the time of reaction in double-blind, placebo-controlled food challenges as being the time from the first challenge dose to the onset of symptoms. Hill et al.²⁴ also defined the time of reaction in the same manner. Bock et al.,¹⁷ Bock and Atkins,²⁵ Sampson,²⁶ Sampson and McCaskill,²⁷ and Burks et al.²⁸ defined the time of reaction as being the duration from the last challenge dose to the onset of symptoms. Moreover, our first challenge dose was one capsule (0.4 gm). We continued the doubled doses every hour (1, 2, 4, 8, and 16 capsules) until the patient had convincing symptoms or until 16 capsules of dried food were ingested as a single challenge dose. In the studies of Sampson²⁶ and Burks et al.²⁸ the allergen doses were increased more rapidly, because up to 8 or 10 gm dehydrated food was administered over 1 hour. Thus the speed of dose-up in our procedure is slower than the speed in their procedures. For these reasons we consider that our patients with AD seem to exhibit much later onset of clinical responses to food challenges than those reported by several other groups.^{25, 27, 28}

The measurement of proliferative responses of PBMCs to food antigens in patients with AD and hen's egg or cow's milk sensitivity is not so complicated nor time consuming in laboratories where lymphocyte proliferative responses to mitogen such as phytohemagglutinin or concanavalin A can be tested. It is not difficult to use food antigens, ovalbumin or BSA, instead of mitogens, and eight cases can be examined on one microtest plate (96 wells) simultaneously, and the data are obtained in 3 to 5 days. In our study 2×10^5 PBMCs per well (0.2 ml) were cultured, which is a greater number than the 10^5 PBMCs (0.2 ml) cultured in the studies of Hsieh²⁹ and Hiratani et al.³⁰ We used a round-bottomed microplate, although some investigators used flat-bottomed microplates.^{9, 11} When cultures were grown overnight, cells were observed gathering in the bottom of the round-bottomed microplate. Therefore cell-to-cell contact is considered to be closer in a round-bottomed microplate, and cell interaction is thought to begin sooner. Moreover, the concentration of added human pooled AB serum is 15% in our study. This percentage is higher than the 5% of the study of Buckley et al.³¹ and the 10% in other studies.^{9, 21, 29, 30} For the above reasons we consider that peak proliferation in our study seems to have occurred earlier than in other studies. Furthermore, the reagents for this measurement are not so expensive. We also examined the proliferative responses of PBMCs to β -lactoglobulin simultaneously in patients with AD who are sensitive to cow's milk. Considerable agreement existed between the results of proliferative responses with BSA and the results with β -lactoglobulin. Moreover, pro-

TABLE VIII. Courses of proliferative responses of PBMCs to ovalbumin and BSA in 10 patients with AD who are sensitive to both hen's egg and cow's milk in whom only one or both of the foods are eliminated from the diet

Patients	Age	Proliferative responses of PBMCs to ovalbumin (SI)		Proliferative responses of PBMCs to BSA (SI)	
		Before*	After*	Before*	After*
Only hen's egg eliminated					
1	2 yr	4.00†	1.49‡	2.09§	2.18
2	2 yr 8 mo	3.71†	1.25‡	2.11§	2.33
Only cow's milk eliminated					
3	10 mo	2.39§	3.12	2.40†	1.73‡
4	4 yr 7 mo	3.30§	2.25	4.29†	1.59‡
5	6 yr 5 mo	2.11§	2.82	3.00†	1.19‡
6	1 yr 2 mo	2.31§	3.65	3.92†	1.17‡
Both hen's egg and cow's milk eliminated					
7	8 mo	3.80	1.17	4.50	2.19
8	7 mo	2.65	1.13	2.34	1.10
9	2 yr 5 mo	4.00	1.13	3.85	1.12
10	8 mo	3.19	1.19	3.69	1.69

The mean $\pm$ SD SI of the value of ‡ (1.40 ± 0.23 , $n = 6$) significantly ($p < 0.05$) decreased compared with that of † (3.55 ± 0.71 , $n = 6$).

The mean $\pm$ SD SI of the value of || (2.73 ± 0.58 , $n = 6$) did not change compared with that of (2.39 $\pm$ 0.47, $n = 6$).

The mean $\pm$ SD SI of ovalbumin after elimination (1.16 ± 0.03 , $n = 4$) significantly decreased (§ $p < 0.05$) compared with that before elimination (3.41 ± 0.61 , $n = 4$) and the mean $\pm$ SD SI of BSA after elimination (1.53 ± 0.52 , $n = 4$) significantly decreased ($p < 0.05$) compared with that before elimination (3.60 ± 0.91 , $n = 4$) in the cases (patients no. 7 to no. 10) in which both hen's egg and cow's milk were eliminated.

Data were analyzed by paired Wilcoxon test.

*Before, Data before elimination diets or nonelimination diets were begun; after, data for 3 months or for more than 3 months after elimination diets or nonelimination diets were begun.

†Data on eliminated food antigen before elimination.

‡Data on eliminated food antigen after elimination.

§Data on noneliminated food antigen before elimination.

||Data on noneliminated food antigen after elimination.

liferative responses of PBMCs to BSA correlated well ($p < 0.01$) with the responses to β -lactoglobulin. In general, β -lactoglobulin is recognized as the major milk allergen.³²⁻³⁴ There are also reports that the allergenicity of BSA is recognized in cow's milk allergy.^{32, 35} Goldman et al.³² orally challenged a group of children with cow's milk allergy; 62% reacted to β -lactoglobulin and 52% reacted to BSA.³² Moreover, BSA is a single protein whose amino-acid sequence is clear.³⁶ β -Lactoglobulin has, however, three genetic variants. We are going to investigate the interaction between T-cell receptors and epitopes of the antigen, that is, a single protein, in the responses of CD4 T lymphocytes to the antigens. For the above reasons, BSA was used as a milk allergen instead of β -lactoglobulin. For measuring proliferative responses of PBMCs, patients with AD who are sensitive to only a few foods are more ideal than patients with multiple sensitivity. In this study, proliferative responses of PBMCs to ovalbumin or BSA were measured in pa-

tients with AD who were sensitive to hen's egg or cow's milk, respectively. However, this measurement is applicable to patients with AD who are sensitive to multiple foods using each purified antigen.

Taken together, specific IgE antibodies to food antigens are useful indexes of the effect of elimination diets in food-sensitive patients with AD and positive RAST, and proliferative responses of PBMCs to food antigens are useful indexes of the effect of elimination diets of food-sensitive patients with AD and proliferative responses of PBMCs.

REFERENCES

1. Sampson HA. The role of food allergy and mediator release in atopic dermatitis (Jerome Glaser lectureship). *J ALLERGY CLIN IMMUNOL* 1988;81:635-45.
2. Hoffman DR, Yamamoto FY, Geller B, Haddad Z. Specific IgE antibodies in atopic eczema. *J ALLERGY CLIN IMMUNOL* 1975;55:256-67.
3. Wraith DG, Merrett J, Roth A, Yman L, Merrett TG. Recognition of food-allergic patients and their allergens by the

- RAST technique and clinical investigation. *Clin Allergy* 1979;9:25-36.
4. Strannergard I-L, Lindholm L, Strannergard O. T Lymphocytes in atopic children. *Int Arch Allergy Appl Immunol* 1976;50:684-92.
5. Horsmanheimo M, Horsmanheimo A, Banov CH, Ainsworth SK. Cell-mediated immunity in vitro in atopic dermatitis. *Arch Dermatol* 1979;115:161-4.
6. Matloff SM, Kiselis IK, Rocklin RE. Reduced production of histamine-induced suppressor factor (HSF) by atopic mononuclear cells and decreased prostaglandin E₂ output by HSF-stimulated atopic monocytes. *J ALLERGY CLIN IMMUNOL* 1983;72:359-64.
7. Hwang K-C, Fikrig SM, Friedman HM, Gupta S. Autologous mixed lymphocyte reaction in man: VII. autologous mixed lymphocyte reaction in patients with bronchial asthma, allergic rhinitis, and atopic dermatitis. *Allergy* 1983;38:113-8.
8. Alam R, Rozniecki J, Salmaj K. A mononuclear cell derived histamine releasing factor (HRF) in asthmatic patients. Histamine release from basophils in vitro. *Ann Allergy* 1984; 53:66-9.
9. Rawle FC, Mitchell EB, Platts-Mills TAE. 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. *J Immunol* 1984; 133:195-201.
10. Girard JP, Rose NR, Kunz ML, Kobayashi S, Arbesman CE. In vitro lymphocyte transformation in atopic patients: Induced by antigens. *Allergy* 1967;39:65-81.
11. Van Sickle GJ, Powell GK, McDonald PJ, Goldblum RM. Milk and soy protein-induced enterocolitis: Evidence for lymphocyte sensitization to specific food proteins. *Gastroenterology* 1985;88:1915-21.
12. Kondo N, Agata H, Fukutomi O, Motoyoshi F, Oriti T. Lymphocyte responses to food antigens in patients with atopic dermatitis who are sensitive to foods. *J ALLERGY CLIN IMMUNOL* 1990;86:253-60.
13. Gatien JG, Merler E, Colten HR. Allergy to ragweed antigen E: effect of specific immunotherapy on the reactivity of human T lymphocytes in vitro. *Clin Immunol Immunopathol* 1975; 4:32-7.
14. Rocklin RE, Pence H, Kaplan H, Evans R. Cell-mediated immune response of ragweed-sensitive patients to ragweed antigen E.: in vitro lymphocyte transformation and elaboration of lymphocyte mediators. *J Clin Invest* 1974;53:735-44.
15. Businco L, Benincori N, Cantani A. Epidemiology, incidence and clinical aspects of food allergy. *Ann Allergy* 1984;53:615-22.
16. Hanifin JM. Atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1984;73:211-22.
17. Bock SA, Sampson HA, Atkins FM, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J ALLERGY CLIN IMMUNOL* 1988;82:986-97.
18. Chua YY, Bremner K, Llobet JL, Kokubu HL, Collins-Williams C. Diagnosis of food allergy by the radioallergosorbent test. *J ALLERGY CLIN IMMUNOL* 1976;58:477-82.
19. Bruynzeel-Kooman CAFM, Bruynzeel PLB. A role for IgE in patch test reactions to inhalant allergens in patients with atopic dermatitis. *Allergy* 1988;43:15-21.
20. Rocklin RE, Sheffer AL, Greineder DK, Melmon KL. Generation of antigen-specific suppressor cells during allergy desensitization. *N Engl J Med* 1980;302:1213-9.
21. Black PL, Marsh DG. Correlation between lymphocyte responses and immediate hypersensitivity to purified allergens. *J ALLERGY CLIN IMMUNOL* 1980;66:394-401.
22. Evans R, Pence H, Kaplan H, Rocklin RE. The effect of immunotherapy on humoral and cellular responses in ragweed hayfever. *J Clin Invest* 1976;57:1378-85.
23. Metcalfe DD, Sampson HA. Workshop on experimental methodology for clinical studies of adverse reactions to foods and food additives. *J ALLERGY CLIN IMMUNOL* 1990;86:421-42.
24. Hill DJ, Firer MA, Shelton MJ, Hosking CS. Manifestations of milk allergy in infancy: clinical and immunologic findings. *J Pediatr* 1986;109:270-6.
25. Bock SA, Atkins FM. Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. *J Pediatr* 1990;117:561-7.
26. Sampson HA. Role of immediate food hypersensitivity in the pathogenesis of atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1983;71:473-80.
27. Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J Pediatr* 1985;107:669-75.
28. Burks AW, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: clinical relevance of food hypersensitivity reactions. *J Pediatr* 1988;113:447-51.
29. Hsieh KH. Changes of lymphoproliferative response of T cell subsets to allergen and mitogen after hyposensitization in asthmatic children. *J ALLERGY CLIN IMMUNOL* 1984;74:34-40.
30. Hiratani M, Muto K, Oshida Y, Ito S, Kasai M. Lymphocyte responsiveness to *Dermatophagoides farinae* extract in mite-sensitive patients: effect of immunotherapy on cellular proliferative response and specific immunoglobulin E antibody (RAST score). *J ALLERGY CLIN IMMUNOL* 1981;68:205-11.
31. Buckley RH, Seymour F, Sanal SO, Ownby DR, Becker WG. Lymphocyte responses to purified ragweed allergens in vitro. I. Proliferative responses in normal, newborn, agammaglobulinemic, and atopic subjects. *J ALLERGY CLIN IMMUNOL* 1977;59:70-8.
32. Goldman AS, Anderson DW, Sellers WA, Saperstein S, Kniker WT, Halpern SR. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963;32:425-43.
33. Goldman AS, Sellers WA, Halpern SR, Anderson DW, Furlow TE, Johnson CH. Milk allergy. II. Skin testing of allergic and normal children with purified milk proteins. *Pediatrics* 1963;32:572-9.
34. Pearson JR, Kingston D, Shiner M. Antibody production to milk proteins in the jejunal mucosa of children with cow's milk protein intolerance. *Pediatr Res* 1983;17:406-12.
35. May CD, Remigio L, Feldman J, Bock SA, Carr RI. A study of serum antibodies to isolated milk proteins and ovalbumin in infants and children. *Clin Allergy* 1977;7:583-95.
36. Peters T. Serum albumin. *Advances in protein chemistry* 1985;37:161-245.