

High Sensitivity and Specificity of Proliferative Responses of Lymphocytes to Food Antigens for Detection of the Offending Food in Patients with Food-Sensitive Atopic Dermatitis

NAOMI KONDO, M.D., HIDEO KANEKO, M.D., TOSHIYUKI FUKAO, M.D.,
RYOSUKE INOUE, M.D., TAKAYUKI TERAMOTO, M.D.,
HIDEAKI TASHITA, M.D., and YUKARI YOKOYAMA

ABSTRACT

The sensitivities of radioallergosorbent test (RAST) scores for hen's egg or cow's milk in patients with hen's egg- or cow's milk-sensitive immediate symptoms were high, whereas the sensitivities of RAST scores for hen's egg or cow's milk in patients with hen's egg- or cow's milk-sensitive atopic dermatitis (AD) were not high. The specificities of the RAST scores in patients with food-sensitive AD were high. In contrast, both the sensitivity and the specificity of proliferative responses of peripheral blood mononuclear cells (PBMCs) to ovalbumin (OVA) or bovine serum albumin (BSA) were high in patients with hen's egg- or cow's milk-sensitive AD, but the sensitivity of proliferative responses of PBMCs to OVA or BSA were not high in patients with hen's egg- or cow's milk-sensitive immediate symptoms. Moreover, by combining the sensitivity and the specificity values, it was determined that a stimulation index (SI) greater than 2.0 (positive) served as a convenient method to mark a specific offending food in food-sensitive AD. Proliferative responses of PBMCs to food antigens may be more useful than RAST scores for detection of offending food(s) in food-sensitive AD.

INTRODUCTION

THE ROLE OF IGE-MEDIATED FOOD HYPERSENSITIVITY in the pathogenesis of atopic dermatitis (AD) is controversial.^(1,2,3) In contrast, from the nature of the cellular infiltrate seen in eczematous lesions, it appears that some form of cell-mediated immunity may be involved in the pathogenesis of AD.⁽⁴⁾ Certainly, many of the symptoms of AD are not only persistent but are also late in onset after food ingestion. It was also reported that the histology of an eczematous lesion is more indicative of a type IV cell-mediated response.⁽⁵⁾ Wraith et al.⁽³⁾ reported that the percentage of positive food radioallergosorbent tests (RASTs) in patients with "non-immediate" allergic symptoms after food ingestion (reactions appearing after 1 hour) was lower than that in patients with "immediate" allergic symptoms after food ingestion (reactions occurring in less than 1 hour). The RAST is a sensitive procedure for the diagnosis of IgE-mediated hypersen-

Department of Pediatrics, Gifu University School of Medicine, Tsukasa-machi 40, Gifu 500, Japan.

sitivity but not for other types of hypersensitivity. We have previously reported that the proliferative responses of peripheral blood mononuclear cells (PBMCs) to ovalbumin (OVA) or bovine serum albumin (BSA) in children with AD who are sensitive to hen's egg or cow's milk were significantly higher than those of healthy children and hen's egg- or cow's milk-sensitive children with immediate symptoms.⁽⁶⁾ In this study, we show that the proliferative responses of PBMCs to each food antigen may be useful for detection of allergens in food-sensitive AD.

MATERIALS AND METHODS

Subjects

One hundred thirty-seven patients with AD, selected as being sensitive to hen's egg or cow's milk on the basis of clinical history and double-blind, placebo-controlled food challenges, were studied. The diagnosis of AD was defined by the criteria of Hanifin.⁽⁷⁾ Subjects ranged in age from 4 months to 9 years. In double-blind, placebo-controlled food challenges, each dehydrated powdered food (Meiji Milk Products Co., Tokyo, Japan) selected for testing was placed in a white opaque capsule (0.4 gm per capsule). Our first challenge dose was one capsule; doubled doses were then continued every hour either until the patient had convincing symptoms or until 16 capsules were ingested as a single-challenge dose. For young children, the dehydrated powdered foods were hidden in broth or juice. Patients were instructed to discontinue all medications for 7 days before and during testing. No patients had been on systemic steroids. The control groups included 42 nonatopic healthy children (control group I), ranging in age from 5 months to 9 years, who did not have hen's egg- or cow's milk sensitivity and 69 patients (control group II), ranging in age from 5 months to 9 years, who had acute urticaria or angioedema (immediate symptoms). Blood samples were obtained when diseases were active.

RASTs

RASTs were performed as recommended by the Phadebas RAST test kit (Pharmacia, Uppsala, Sweden).⁽⁶⁾ Hen's egg and cow's milk discs were purchased from Pharmacia. RAST results were scored on a scale from 0 to 4⁺ by comparing them with serially diluted reference sera (Pharmacia) graded on a scale from A to D (i.e., a serum <D = 0, a serum between D and C = 1, and a serum >A = 4⁺). RAST scores of 2⁺, 3⁺, and 4⁺ were recorded as positive.

Cell preparation and culture

PBMCs were isolated from heparinized blood by gradient centrifugation in Ficoll-Paque solution (Pharmacia). PBMCs were suspended to a density of 10⁶/mL in the culture medium consisting of RPMI 1640 supplemented with 15% pooled human AB⁺ serum, L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 µg/mL). PBMCs (2 × 10⁵) were cultured 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 for 5 days in a humidified atmosphere containing 5% CO₂.⁽⁶⁾

Proliferative response

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

$$SI = \frac{\text{mean counts per minute (CPM) in cultures stimulated with food antigens}}{\text{mean CPM in unstimulated cultures}}$$

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Antigens

OVA and BSA were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and used in concentrations of 0.25, 2.5, and 25 $\mu\text{g/mL}$, respectively.

Statistics

Frequencies were compared by Fisher's exact test (2×2 contingency tables).

RESULTS

RAST scores

The RAST values for hen's egg were positive in 26 of 30 patients (86.7%) with immediate symptoms who were sensitive to hen's egg, whereas the values were positive in only 44 of 84 patients (52.4%) with AD who were sensitive to hen's egg and in only 2 of 42 healthy controls (4.8%) (Table 1). The RAST values for cow's milk were positive in 31 of 39 patients (79.5%) with immediate symptoms who were sensitive to cow's milk, whereas the values were positive in only 18 of 53 patients (34.0%) with AD who were sensitive to cow's milk and in only 1 of 42 healthy controls (2.4%) (Table 2). As shown in Tables 1 and 2, the sensitivities of RAST scores in patients with immediate symptoms were high (86.7% for hen's egg sensitivity, 79.5% for cow's milk sensitivity) although the sensitivities of RAST scores in patients with AD

TABLE 1. RADIOALLERGOSORBENT TEST (RAST) SCORES FOR HEN'S EGG AND PROLIFERATIVE RESPONSES OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs) TO OVALBUMIN IN HEALTHY CONTROLS AND PATIENTS WITH HEN'S EGG-SENSITIVE IMMEDIATE SYMPTOMS OR ATOPIC DERMATITIS (AD)

Subjects	RAST scores ^a		Proliferative responses of PBMCs (stimulation index)					
	— ^b	+ ^c	<1.5	≥ 1.5	<2.0	≥ 2.0	<2.5	≥ 2.5
Hen's egg-sensitive immediate symptoms								
Healthy controls (N = 42)	40	2	26	16	40	2	41	1
Patients with immediate symptoms (N = 30)	4	26 ^d	17	13	25	5	28	2
Sensitivity	86.7%		43.3%		16.7%		6.7%	
	(26/30)		(13/30)		(5/30)		(2/30)	
Specificity	95.2%		61.9%		95.2%		97.6%	
	(40/42)		(26/42)		(40/42)		(41/42)	
Hen's egg-sensitive AD								
Healthy controls (N = 42)	40	2	26	16	40	2	41	1
Patients with AD (N = 84)	40	44 ^d	10	74 ^d	14	70 ^d	31	53 ^d
Sensitivity	52.4%		88.1%		83.3%		63.1%	
	(44/84)		(74/84)		(70/84)		(53/84)	
Specificity	95.2%		61.9%		95.2%		97.6%	
	(40/42)		(26/42)		(40/42)		(41/42)	

^aRAST scores for hen's egg.

^b—, scores 0 and 1.

^c+, scores 2, 3, and 4.

^d $P < 0.001$ compared with healthy controls.

TABLE 2. RADIOALLERGOSORBENT TEST (RAST) SCORES FOR COW'S MILK AND PROLIFERATIVE RESPONSES OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs) TO BOVINE SERUM ALBUMIN IN HEALTHY CONTROLS AND PATIENTS WITH COW'S MILK-SENSITIVE IMMEDIATE SYMPTOMS OR ATOPIC DERMATITIS (AD)

Subjects	RAST scores ^a		Proliferative responses of PBMCs (stimulation index)					
	— ^b	+ ^c	<1.5	≥1.5	<2.0	≥2.0	<2.5	≥2.5
Cow's milk-sensitive immediate symptoms								
Healthy controls (N = 42)	41	1	33	9	39	3	41	1
Patients with immediate symptoms (N = 39)	8	31 ^d	23	16	34	5	38	1
Sensitivity	79.5%		41.0%		12.8%		2.6%	
	(31/39)		(16/39)		(5/39)		(1/39)	
Specificity	97.6%		78.6%		92.9%		97.6%	
	(41/42)		(33/42)		(39/42)		(41/42)	
Cow's egg-sensitive AD								
Healthy controls (N = 42)	41	1	33	9	39	3	41	1
Patients with AD (N = 53)	35	18	6	47 ^d	8	45 ^d	20	33 ^d
Sensitivity	34.0%		88.7%		84.9%		62.3%	
	(18/53)		(47/53)		(45/53)		(33/53)	
Specificity	97.6%		78.6%		92.9%		97.6%	
	(41/42)		(33/42)		(39/42)		(41/42)	

^aRAST scores for cow's milk.^b—, scores 0 and 1.^c+, scores 2, 3, and 4.^d*P* < 0.001 compared with healthy controls.

were not high (52.4% for hen's egg sensitivity, 34.0% for cow's milk sensitivity). The specificities were 95.2% for hen's egg sensitivity and 97.6% for cow's milk sensitivity.

Proliferative responses of PBMCs to food antigens

In most patients with food-sensitive AD, proliferation of PBMCs increased steadily with increasing OVA or BSA dose over the range of 0.25 to 25 $\mu\text{g/mL}$. For convenience, subjects were divided into two groups according to the SI values: greater than 1.5, 2.0, or 2.5 (positive) and less than 1.5, 2.0, or 2.5 (negative). We evaluated three sets of possible criteria for a positive result (<1.5 or ≥ 1.5 , <2.0 or ≥ 2.0 , <2.5 or ≥ 2.5). The sensitivities and the specificities are shown in Tables 1 and 2. In the cases in which the SI was greater than 1.5, 2.0, or 2.5, results were recorded as positive. As shown in Table 1, an SI sensitivity greater than 1.5, 2.0, or 2.5 in proliferative responses of PBMCs to OVA of 88.1%, 83.3%, or 63.1%, respectively, was present in patients with hen's egg-sensitive AD. An SI specificity greater than 1.5, 2.0, or 2.5 in proliferative responses of PBMCs to hen's egg of 61.9%, 95.2%, or 97.6% respectively, was present in patients with hen's egg-sensitive AD. In contrast, an SI sensitivity greater than 1.5, 2.0, or 2.5 in proliferative responses of PBMCs to OVA of 43.3%, 16.7%, or 6.7%, respectively, was present in patients with hen's egg-sensitive immediate symptoms. As shown in Table 2, an SI sensitivity greater than 1.5, 2.0, or 2.5 in proliferative responses of PBMCs to BSA of 88.7%, 84.9%, or 62.3%, respectively, was present in patients with cow's milk-sensitive AD. An SI specificity greater than 1.5, 2.0, or 2.5 in proliferative responses of PBMCs to BSA of 78.6%, 92.9%, or 97.6%, respectively, was present in patients with cow's

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milk-sensitive AD. In contrast, an SI sensitivity greater than 1.5, 2.0, or 2.5 in proliferative responses of PBMCs to BSA of 41.0%, 12.8%, or 2.6%, respectively, was present in patients with cow's milk-sensitive immediate symptoms. These results show that both the sensitivity and the specificity of proliferative responses of PBMCs to OVA or BSA are high in patients with hen's egg- or cow's milk-sensitive AD, but the sensitivity of proliferative responses of PBMCs to OVA or BSA are not high in patients with hen's egg- or cow's milk-sensitive immediate symptoms. When an SI greater than 2.0 was recorded as positive in patients with food-sensitive AD, both the sensitivity and the specificity were over 80% (see Tables 1 and 2). Therefore, an SI greater than 2.0 was recorded as positive and served as a marker of a specific offending food in food-sensitive AD.

DISCUSSION

In our previous study, it was shown that the proliferative responses of PBMCs to OVA or BSA in children with AD who are sensitive to hen's egg or cow's milk were significantly higher than those of healthy children and hen's egg- or cow's milk-sensitive children with immediate symptoms. However, in patients with AD there were no significant correlations between the proliferative responses of PBMCs and the RAST values.⁽⁶⁾ Moreover, we have reported that the responding cells were also shown, through experiments involving T-lymphocyte phenotypes, to be predominantly CD4⁺ cells.⁽⁸⁾ Mosmann et al.⁽⁹⁾ have reported that mouse helper T-cell clones fall into two main groups (Th1 and Th2), defined primarily by differences in the pattern of lymphokines synthesized. Th1 clones synthesize interleukin-2 (IL-2), interferon- γ (IFN- γ), and lymphotoxin, whereas these lymphokines are not detectably expressed in Th2 clones. Conversely, only Th2 clones synthesize detectable amounts of interleukin-4 (IL-4) and interleukin-5 (IL-5). Th1 clones also cause effective delayed-type hypersensitivity reactions. IL-4 causes increased IgE production as well as increased levels of Fc ϵ RII on B lymphocytes. Romagnani et al.⁽¹⁰⁾ have reported that IFN- γ and IL-4 could be produced by different T-helper cells in humans, as has been shown in mice, but that they could also be the product of the same T-cell clones. Moreover, Wierenga et al.⁽¹¹⁾ have presented evidence for the presence of distinct functional subsets of CD4⁺ T cells in man on the basis of the production of IL-4 and IFN- γ . In our previous study, IL-2 activity and IFN- γ concentrations in culture supernatants of OVA-stimulated PBMCs from hen's egg-sensitive patients with AD were significantly higher than those of healthy children and hen's egg-sensitive patients with immediate symptoms.⁽⁸⁾ Expression of Fc ϵ RII on B lymphocytes in OVA-stimulated PBMC cultures from hen's egg-sensitive patients with AD was significantly greater than that of healthy children, but it tended to be less than that of hen's egg-sensitive children with immediate symptoms. These results suggest that in patients with AD who are sensitive to food, the CD4⁺ T lymphocytes stimulated by food antigens secrete lymphokines such as IL-2 and IFN- γ that are secreted from Th1 clones in mice in addition to expression of Fc ϵ RII on B lymphocytes that is induced by IL-4 secreted from Th2 clones in mice. As a result, cell-mediated immunity may also occur in addition to IgE-mediated hypersensitivity. Moreover, in patients with AD who have the low specific IgE antibody to the offending food antigen and whose symptoms are late in onset after food ingestion, IgE production of B cells may be suppressed by IFN- γ and, consequently, cell-mediated immunity may predominantly occur as compared to IgE-mediated hypersensitivity.

In this study, the sensitivities of RAST scores in patients with food-sensitive immediate symptoms were high, whereas the sensitivities of RAST scores in patients with food-sensitive AD were not high. The specificities of RAST scores in patients with food-sensitive AD were high. In contrast, both the sensitivity and the specificity of proliferative responses of PBMCs to OVA or BSA were high in patients with hen's egg- or cow's milk-sensitive AD, but the sensitivity of proliferative responses of PBMCs to OVA or BSA were not high in patients with hen's egg- or cow's milk-sensitive immediate symptoms. Moreover, by combining the sensitivity and the specificity values, it was determined that an SI greater than 2.0 (positive) served to mark a specific offending food in food-sensitive AD. Taken together, for detection of offending food in food-sensitive AD, proliferative responses of PBMCs to food antigens may be more useful than RAST scores. Rosen et al.⁽¹²⁾ reported on the evaluation of skin testing in food allergies. Moreover, MacPhee et al.⁽¹³⁾ used the skin window chamber technique for evaluation of T-cell responses. Additional experiments along these lines are now under way.

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Address reprint requests to:
Naomi Kondo, M.D.
Department of Pediatrics
Gifu University School of Medicine
Tsukasa-machi 40, Gifu 500
Japan