

Severe allergic reactions to foods are predicted by increases of CD4+CD45RO+ T cells and loss of L-selectin expression

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Objective: The aim of the study was to determine whether the clinical outcome of double-blind, placebo-controlled food challenges of patients with atopic dermatitis would be associated with changes in lymphocyte functions.

Methods: Peripheral blood mononuclear cells were prepared from 19 children with atopic dermatitis and stimulated in vitro with the suspected allergen (cow's milk, hen's egg), tetanus toxoid, and pokeweed mitogen. After 14 days in culture, quantitative and qualitative distribution of cell surface marker expression was assessed by flow cytometry, and results were compared with the clinical outcome of a subsequent oral food challenge.

Results: After stimulation with the allergen, a significant increase of CD4+CD45RO+ T cells ($p < 0.05$) was detected selectively for patients showing severe clinical reactions. This increase was not detected for patients with mild or no reactions or in six nonatopic control subjects. Increased expression of CD45RO was paralleled by a significant decrease in L-selectin expression ($p < 0.05$) for the same patient group.

Conclusion: The combined assessment of CD4+CD45RO+ and CD4+L-selectin+ expression on T cells was more sensitive for the prediction of the clinical outcome of the food challenge ($p < 0.01$) than measurement of cytokines or immunoglobulins in cell culture supernatants. These data indicate that a shift in lymphocyte functions may predict the development of severe allergic reactions in food-sensitized children with atopic dermatitis. (J Allergy Clin Immunol 1997;99:522-9.)

Key words: Atopic dermatitis, cow's milk, DBPCFC, food allergy, hen's egg, L-selectin, lymphocyte function, oral provocation

In childhood, atopic dermatitis (AD) is one of the most common and troublesome chronic diseases. In up to one third of cases, food allergy plays an important role in the pathogenesis of AD.¹ Not only eczematous reactions but also urticaria, gastrointestinal and respiratory

Abbreviations used

AD:	Atopic dermatitis
CM:	Cow's milk
DBPCFC:	Double-blind, placebo-controlled food challenge
HE:	Hen's egg
IFN- γ :	Interferon- γ
PBMCs:	Peripheral blood mononuclear cells
PWM:	Pokeweed mitogen

symptoms,² and even fatal anaphylactic reactions are reported in patients with food allergy and AD.³ Measurements of specific IgE antibodies do not always correlate with results of oral provocation tests,⁴ and there are still no objective measures that prove or predict clinical actuality of sensitization. Only double-blind, placebo-controlled food challenges (DBPCFCs) are accepted as the "gold standard" for diagnosis of food allergy in these children.⁵⁻⁷

The development of immediate-type hypersensitivity responses is T-cell-dependent. The cell infiltrate in skin lesions of AD predominantly consists of CD4+ T cells.⁸ These can be subdivided according to different states of functional activities.⁹ CD4+CD45RA+ lymphocytes represent "naive" T cells, whereas CD4+CD45RO+ lymphocytes are characterized as effector T cells. The latter group is found in almost all inflammatory skin disorders, including allergen-induced late-phase reactions.^{10, 11} Elevated numbers of CD45RO+ cells in skin lesions may be due to an increased recruitment of these cells by specialized adhesion molecules or to in situ activation of CD4+ T cells. Because T cells play a critical role in the immunopathogenesis of AD, it was the aim of this study to investigate whether in vitro lymphocyte functions and the state of T-cell activation would be possible predictors of the clinical outcome of DBPCFC in children with AD.

METHODS

Study population

Nineteen children (12 boys and 7 girls) with suspected allergy to milk and/or egg were selected for the study. Ages ranged from 5 months to 10 years (mean age, 3.2 years). All patients had AD as defined by the criteria of Sampson¹² and Seymour et al.,¹³ modified from those of Hanifin and Rajka.¹⁴ The inclusion criterion was suspicion of food-related worsening of eczema or

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TABLE I. Clinical outcome of oral food challenges

Reaction	Patient	Allergen	Specific IgE (kU/l)	Type of Reaction	Challenge dose (ml)*	Symptoms after DBPCFC
Severe	O.E.	HE	2.5	ER	30	Generalized urticaria
	J.B.	HE	9.2	ER	10	Edema, urticaria, wheezing
	T.T.	HE	27.9	ER	1	Severe urticaria
	H.C.	HE	>100.0	ER	30	Severe diarrhea, eczema
	P.H.	HE	>100.0	ER	1	Wheezing, dyspnea
	K.M.	CM	42.1	ER	10	Urticaria, wheezing
	T.T.	CM	75.0	ER	1	Severe urticaria
	P.H.	CM	>100.0	ER	3	Wheezing, dyspnea
	A.H.	HE	1.1	LR	30	Eczema
Mild	C.M.	HE	2.7	ER + LR	30	Eczema
	C.A.	HE	24.1	ER	30	Eczema
	K.M.	HE	42.8	ER	10	Eczema, urticaria
	E.R.	HE	87.1	ER + LR	10	Eczema, nausea
	A.H.	CM	0.8	ER	100	Eczema, nausea
	L.P.	CM	3.9	ER + LR	100	Eczema
	M.M.	HE	<0.35		30	
	C.B.	HE	<0.35		30	
None	D.F.	HE	<0.35		30	
	G.L.	HE	<0.35		30	
	M.M.	HE	<0.35		30	
	U.L.	HE	1.2		30	
	C.B.	HE	>100.0		30	
	M.M.	CM	<0.35		100	
	C.B.	CM	<0.35		100	
	D.F.	CM	<0.35		100	
	G.L.	CM	<0.35		100	
	M.M.	CM	<0.35		100	
	J.S.	CM	<0.35		100	
	E.R.	CM	>100.0		100	
	C.B.	CM	>100.0		100	

ER, Early reaction; LR, late phase reaction.

*Highest possible dose: HE = 30 ml and CM = 100 ml.

immediate-type clinical reactions. Patients were challenged with cow's milk (CM) and/or hen's egg (HE) according to their history (Table I).

Total IgE ranged from 6 to 85,200 kU/L (mean, 10,815 kU/L). Thirteen children had specific IgE antibodies to CM and/or HE. Anti-CM-specific IgE ranged from 0.8 kU/L to more than 100.0 kU/L (mean, 60.3 kU/L), and anti-HE-specific IgE ranged from 1.1 kU/L to more than 100.0 kU/L (mean, 41.6 kU/L) (Table I).

Six nonatopic children (3 girls and 3 boys) between the ages of 10 months and 17 years (mean age, 6.1 years) without a history of food intolerance served as control subjects. Total IgE ranged from 1 to 36 kU/L (mean, 16.3 kU/L). Results of tests for specific IgE against CM and HE and a screening test for specific IgE against aeroallergens were all negative (<0.35 kU/L).

DBPCFCs

A total of 30 oral titrated food challenges were performed in the 19 children with suspected allergy to milk and/or egg in a double-blind, placebo-controlled clinical setting as recently described.¹⁵ Patients, parents, and investigators were blinded. Nineteen allergen provocations were performed in sensitized children, and 11 were performed in nonsensitized children. Of all food challenges, 13 were performed with CM, and 17 were performed with HE according to history. For at least 5 days before provocation, patients were given a milk-free and/or

egg-free diet. Antihistamines were withdrawn at least 3 days before challenge. Topical corticosteroids were allowed twice a day in a concentration of 0.01% betamethasone. Blood was sampled before provocation.

Successive doses of native HE were 0.1, 0.3, 1.0, 3.0, 10.0, and 30.0 ml (which represents 1 full egg); successive doses for CM were identical but included additional doses of 100.0 ml and 200.0 ml. The time interval between each dose was 30 minutes. An extensively hydrolyzed casein formula with added banana flavor was used to mask the allergen (1:1 mixture). Placebo challenges were performed by administering the same volumes of the casein hydrolysate-banana flavor solution. The children were observed for up to 48 hours after each allergen or placebo challenge.

The food challenges were scored as positive if objective clinical reactions such as exacerbation of eczema, urticaria, Quincke edema, wheezing, vomiting, or diarrhea were observed. The severity of eczema was scored according to the method of Costa et al.,¹⁶ and all clinical reactions were graded (mild and severe) according to the criteria listed in Table II.

Cell preparation

Peripheral blood mononuclear cells (PBMCs) were purified from heparinized blood by density-gradient centrifugation (Lymphoprep; Biotest, Darmstadt, Germany) for 20 minutes at 2000 rpm at room temperature. Cells from the interface were

TABLE II. Grading of severity of clinical reactions on DBPCFC

	Reaction	
	Mild	Severe
AD (Costa score Δ)	5-15	>15
Urticaria (no. of wheals)	2-5	>5
Quincke edema	—	+
Respiratory symptoms	—	Wheezing, dyspnea
Gastrointestinal symptoms	Nausea	Diarrhea or vomiting

washed three times (10 minutes, 3000 rpm, at room temperature) and cultured in RPMI-1640 medium (Biochrom, Berlin, Germany) supplemented with 10% heat-inactivated fetal calf serum (Behringwerke AG, Marburg, Germany), 2 mmol/L glutamine (Biochrom), 100 IU/ml penicillin (Biochrom), 100 μ g/ml streptomycin (Biochrom), and 0.2 μ g/ml amphotericin B (Gibco/BRL, Gaithersburg, Md.).

Culture conditions

PBMCs (2×10^6) were incubated for 14 days in 24-well flat-bottom tissue culture plates (Greiner, Frickenhausen, Germany) at 37°C in a 5% CO₂ atmosphere in culture medium alone, with 50 μ g/ml ovalbumin, with 50 μ g/ml bovine casein (Sigma, Deisenhofen, Germany), with 5 μ g/ml tetanus toxoid (Behringwerke AG), or with 1 μ g/ml pokeweed mitogen (PWM). Previously performed dose-response experiments identified optimal proliferation at these concentrations (data not shown). Because of the limitations in cell numbers, one concentration was chosen for these in vitro experiments.

After 48 hours, cell-free culture supernatants were exchanged with fresh culture medium to remove antigens that would interfere with the measurement of antigen-specific immunoglobulin production in the Fluorescence Enzyme Immuno Assay and ELISA systems and to determine interleukin production in culture supernatants. On day 14, cell-free culture supernatants were removed to determine the immunoglobulin production, and cells were harvested to analyze surface marker expression by flow cytometry. All supernatants were kept frozen at -20°C until they were analyzed. The cell viability was determined by trypan blue staining.

Flow cytometric analysis of cell surface marker expression

The distribution of T-cell subpopulations was analyzed after 14 days in culture. Cells were harvested and washed with phosphate-buffered saline (20 minutes, 1500 rpm, at room temperature), resuspended in phosphate-buffered saline, and incubated with antibodies for 15 minutes at 4°C in the dark. After washing once again in phosphate-buffered saline supplemented with 0.1% sodium azide, lymphocyte subsets were analyzed on a FACScan analyzer (Becton Dickinson, Heidelberg, Germany) as previously described.¹⁷ Directly labeled monoclonal antibodies against the following cell surface molecules were used: CD45/CD14, CD4 (fluorescein isothiocyanate), CD8 (PE, Becton Dickinson), CD45RA (fluorescein isothiocyanate), L-selectin (fluorescein isothiocyanate; Immunotech, Marseille, France), CD4 (CytoChrom; PharMingen, San Diego, Calif.), and CD45RO (PE; Dako, Hamburg, Germany). The percentages of CD45RO+ and L-selectin+ (LAM-1+, LECAM-1+, Leu-8+) cells relative to total CD4+ T cells were calculated.

Determination of IL-4 and interferon- γ (IFN- γ) in culture supernatants

Commercially available ELISA kits were used to determine IFN- γ (Biosource, Camarillo, Calif.) and IL-4 (Dianova, Hamburg, Germany). The IFN- γ assay provided linear results from 4 to 1000 pg/ml, and the IL-4 assay provided results from 60 to 1000 pg/ml. To measure IL-4, cell culture supernatants were concentrated fourfold by ultrafiltration (Centricon 10 kD; Amicon, Witten, Germany).

Determination of IgG, IgA, and IgM in culture supernatants

Immunoglobulin production was measured in culture supernatants by ELISA. Ninety-six-well round-bottom microtiter plates (Greiner) were coated with affinity-purified goat anti-human IgG, IgA, and IgM antibodies (Tago, Burlingame, Calif.) diluted in 0.1 mol/L NaHCO₃ at a concentration of 3 μ g/ml, 10 μ g/ml ovalbumin, or 10 μ g/ml casein (Sigma) and left overnight at 4°C. After washing three times with washing buffer (0.1 mol/L Tris base plus Tween-20, adjusted to pH 8.2), wells were blocked with blocking buffer (1% sheep casein-Tris [wt/vol] for IgG; 1% bovine serum albumin/Tris [wt/vol] for IgA and IgM) for 2 hours at 37°C. Supernatants and human Ig standards were diluted in blocking buffer and incubated in duplicate overnight at 4°C. Alkaline phosphatase-conjugated goat anti-human IgG, IgA, and IgM antibodies (Tago) were added (final concentration, 3 μ g/ml), and plates were incubated for 2 hours at 37°C with para-nitrophenylphosphate (Sigma) as substrate. Optical density was measured in an automated Microplate ELISA reader (Dynatech, Deenkendorf, Germany) at a wavelength of 405 nm, and quantities of immunoglobulin were calculated according to the standard curves. Limits of detection in the ELISA system were 10 ng/ml for IgG and 2 ng/ml for IgA and IgM.

Determination of IgE in serum and culture supernatants

Concentrations of total and anti-CM-specific and anti-HE-specific IgE antibody titers in serum and culture supernatants were determined by FEIA with the Pharmacia CAP system (Kabi-Pharmacia, Uppsala, Sweden).¹⁸ The detection limit was 0.35 kU/ml IgE.

Statistical analysis

Nonparametric analysis (Wilcoxon signed-rank test) was applied to determine significant differences of CD4+CD45RO- and CD4+L-selectin expression on T cells between medium- and allergen-, antigen-, or mitogen-incubated T cells. Differences associated with *p* values of less than 0.05 were considered significant. The Mann-Whitney U test was used to describe significant differences between results from the allergen-challenged group of children with severe, mild, or no reactions and the control subjects. Nonparametric Spearman's rank correlation was used to test for correlation between various cellular, cytokine, and immunoglobulin measurements. The chi square test was used to describe the significant relationship between the clinical outcome of food challenges and the combination of changes in laboratory parameters after in vitro stimulation with the relevant allergen.

RESULTS

Clinical outcome of the food challenges

According to clinical criteria, 15 of 30 food challenges were judged as positive; the other 15 were judged as

negative (Tables I and II). All 15 positive reactions occurred in the group of sensitized children. Severe clinical reactions were found in eight challenges: wheezing and dyspnea developed in two cases; in five cases urticaria was observed (one child had additional wheezing and Quincke edema, and one other child had additional wheezing). The eighth child showed severe diarrhea and exacerbation of eczema. In six cases symptoms developed before administration of the highest allergen dose; all reactions were early phase. For the seven children with mild reactions, slight exacerbation of AD was the predominating symptom. In two cases mild gastrointestinal symptoms with nausea occurred; one child had urticaria. In five of seven cases symptoms did not develop before administration of the highest allergen dose. Three early-phase, three early-phase plus late-phase, and one isolated late-phase reactions were recorded in this group.

Association between clinical outcome of food challenges and changes in lymphocyte subsets

Lymphocytes were prepared from patients and control subjects before food challenges and stimulated in vitro with the suspected allergen (ovalbumin or bovine casein). The frequency of CD4+CD45RO+ T cells was measured after stimulation with the allergen and compared with the medium control. As shown in Fig. 1, a significant increase in the CD4+CD45RO+ T-cell subset was found only for patients with severe clinical reactions to the food challenge ($p < 0.05$). In seven of eight provocations, an increase in the frequency of this T-cell subset was observed. This shift was selectively detected in the patient group with severe reactions and was not observed in patients with AD and mild or no clinical reactions or in nonatopic control subjects after stimulation with the same allergen (Fig. 1).

Expression of L-selectin on T cells represents an important adhesion molecule that allows homing of T cells in the lymph node. When T cells leave regional lymph nodes as activated effector cells, they no longer express L-selectin. We therefore measured the expression of this adhesion molecule on T cells from patients undergoing food challenges to determine whether similar changes would also occur under cell culture conditions. As shown in Fig. 1, the increased frequency of CD4+CD45RO+ T cells was paralleled by a significant decrease in L-selectin expression on CD4+ T cells ($p < 0.05$), which was found only in patients with severe clinical reactions and not in those who had mild or no symptoms after provocation or in nonatopic control subjects (Fig. 2). This loss of L-selectin was observed in the CD4+CD45RO+ T cells.

To analyze whether increased CD45RO expression and decreased L-selectin expression would be a specific effect of lymphocyte stimulation with the allergen, cells of five patients and six nonatopic control subjects were also incubated with an antigen and a T-cell-dependent B-cell mitogen (PWM). Two of the five patients had severe reactions, one had a mild reaction, and two had

no reaction. Tetanus antigen was selected because all individuals were sensitized to this antigen by vaccination. CD4+ T cells of both atopic patients and nonatopic individuals responded with an increased expression of the CD45RO isoform ($p < 0.05$) (Fig. 2, A) and a decreased expression of L-selectin ($p < 0.05$) (Fig. 2, B) after stimulation with antigen and mitogen.

Table III shows the clinical outcome of food challenges and in vitro changes in T-cell marker expression. When the increased expression of CD45RO and decreased expression of L-selectin were combined, these changes predicted the development of severe clinical reactions ($p < 0.01$) and distinguished this patient group from those patients with mild or no reactions and nonatopic control subjects without any signs of food sensitivity.

Production of cytokines and immunoglobulins in cell culture

To assess whether the shift in T-cell distribution and activation would correlate with changes in cytokine and immunoglobulin production, cell culture supernatants from allergen-, antigen-, and mitogen-stimulated cells were collected and analyzed for production of IL-4 and IFN- γ . Although the clinical outcome of the food challenges did not correlate with changes in the production of IL-4 or IFN- γ , a differential pattern of IL-4 and IFN- γ production was found in cell culture supernatants from atopic and nonatopic individuals after stimulation with PWM. IFN- γ production was reduced in comparison with that of control subjects, whereas IL-4 was found to be elevated in the three of six sensitized patients with AD who had the highest level of total and allergen-specific serum IgE (46,700, 59,760, and 85,200 kU/L; anti-CM/HE IgE concentration > 100 kU/L).

Differential production of IL-4 and IFN- γ for atopic and nonatopic patients was also reflected by differences in the level of in vitro immunoglobulin production. As expected, increased production of IL-4 after PWM stimulation was paralleled by an elevation in total IgE. However, similar to the results for cytokine secretion, clinical outcome of DBPCFC did not correlate with in vitro production of total and allergen-specific IgE or of other immunoglobulin isotypes including IgA, IgM, and IgG (data not shown).

DISCUSSION

The development of severe clinical reactions after food challenges correlates with distinct changes of in vitro T-cell activation. When lymphocytes from patients with severe reactions were stimulated with the relevant allergen, an increased population of CD45RO+ T cells was detected, which was paralleled by a reduced expression of L-selectin on the same T-cell subset. These changes were specific because they were allergen-dependent and were found only in those patients who had severe clinical reactions. These results suggest that many of the clinical signs and symptoms that occur in food allergy may be triggered and controlled by T cells.

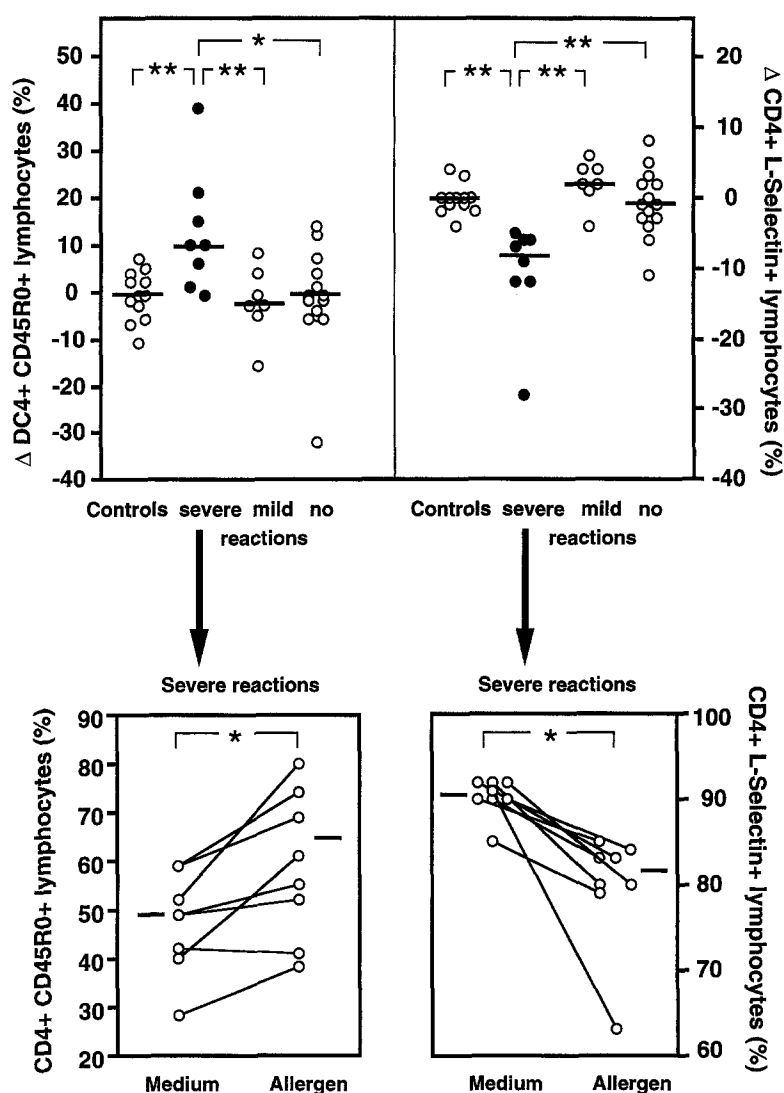


FIG. 1. Analysis of CD45RO and L-selectin expression on CD4⁺ T cells from nonatopic control subjects and from patients with AD and severe, mild, or no clinical reactions. Before food challenge, PBMCs were prepared and incubated with medium or allergen for 14 days. At the end of the culture period, cells were harvested, and expression of CD45RO isoform and L-selectin were analyzed on CD4⁺ T cells. Expressed are differences in the percentage between allergen stimulation and medium culture for each individual. In the lower panel, absolute percentages of CD45RO⁺ and L-selectin⁺ CD4⁺ T lymphocytes are shown for individual patients with severe clinical reactions on DBPCFC. * $p < 0.05$, ** $p < 0.01$.

CD4⁺ T cells are subdivided into naive, resting T cells and activated T cells with effector or memory functions.⁹ A suitable marker with which to distinguish these differences is the expression of different isoforms of the CD45 receptor family. Expression of CD45RA usually correlates with a resting state of T-cell activity. In contrast, after encounter with an antigen or allergen, activated T cells acquire the CD45RO isoform, which is paralleled by a downregulation of CD45RA expression. Effector T cells produce a variety of cytokines including IL-2, IL-4, IL-5, and IFN- γ and provide help for immunoglobulin production.¹⁹

Continuous exposure to basic food antigens, including

CM and HE, may provide a strong activation signal for T cells, resulting in polyclonal proliferation of allergen-specific lymphocytes and development of allergen-specific effector T cells. Because it is well established that casein is the main protein recognized by IgE antibodies in patients allergic to milk, we selected this protein for in vitro studies.²⁰ In addition to casein, other allergens have been identified in CM (e.g., β -lactoglobulin and α -lactalbumin). However, because of limitations in cell numbers and in order to obtain a clear response pattern, we decided to study response to casein in our patients.

Numerous studies have shown that T cells of patients allergic to egg respond well to stimulation with ovalbu-

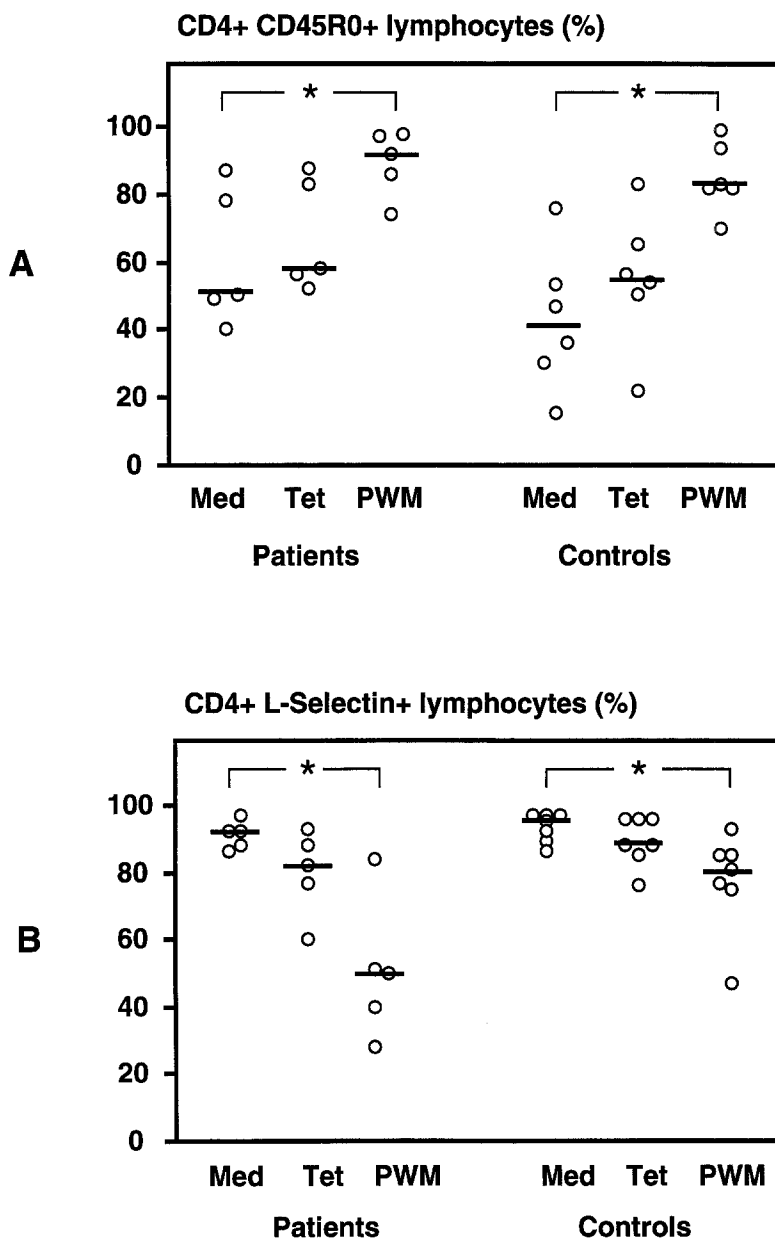


FIG. 2. Changes in CD45RO and L-selectin expression after incubation with tetanus toxoid (*Tet*) and PWM. PBMCs were prepared from five patients with AD and six nonatopic control subjects and incubated with medium (*Med*), tetanus toxoid, and pokeweed mitogen (*PWM*) for 14 days. At the end of the culture period (14 days), cells were harvested, and expression of CD45RO isoform (**A**) and L-selectin (**B**) were analyzed on CD4+ T cells. * $p < 0.05$.

min. In addition to ovalbumin, ovomucoid has recently been identified as another important egg allergen that binds to IgE antibodies.²¹ However, it is not clear how IgE-binding capacity relates to the T-cell response pattern, and information on ovomucoid as an additional important allergen was not available at the beginning of our experiments.

In vitro stimulation of circulating T cells with the allergen will result in further stimulation of the T-cell subset reflected by the growth of the CD45RO+ T-cell

population over time. Another explanation for this phenomenon could be that the changes reflect antigen-induced memory cell responses by CD45RO+ T cells because these individuals are already sensitized to these allergens.

The frequency of allergen-specific T cells in peripheral blood is low (1:50,000). To allow significant expansion of the few allergen-specific T cells present in culture, an incubation period of 14 days was chosen. Preliminary experiments indicated that a high number of cells harvested after this time were still alive. Further-

TABLE III. Prediction of development of allergic reaction by combined consideration of changes in CD4⁺ CD45RO⁺ and CD4⁺ L-selectin

	CD4 ⁺ CD45RO ⁺ ↑ and CD4 ⁺ L-selectin ↓	
	Positive	Negative
Severe clinical reaction after food challenge	7	1
Mild clinical reaction after food challenge	0	7
No clinical reaction after food challenge	4	11
Control subjects	3	9

more, the analysis of cell marker expression was performed with a life gate to further exclude analysis of dead cells.

During the development of an antigen- or allergen-specific immune response, T-cell activation occurs in local and regional lymph nodes. To migrate and home in, these tissue T cells express adhesion molecules including L-selectin.^{22, 23} Effector T cells then leave lymph nodes to travel to the local (inflammatory) tissue where they control the effector phase of the immune response. According to this concept, our *in vitro* results reflect the development of effector T cells in a fashion similar to that under *in vivo* conditions. Acquisition of the CD45RO isoform is paralleled by reduced expression of L-selectin on these cells; this loss of L-selectin allows the T cells to leave the lymph node and to migrate toward the inflammatory tissue, where expression of homing receptors may be a prerequisite for tissue-specific T-cell invasion.

Skin biopsy specimens from patients with AD have shown that the T-cell infiltrate predominantly consists of CD4⁺ T cells⁸ with a high level of CD45RO⁺ T cells.²⁴ Similar results were obtained from skin biopsy specimens of allergen-induced late-phase reactions.¹¹ To reach the skin as a target organ of allergic inflammation, a critical role is proposed for expression of a specific homing receptor, the cutaneous lymphocyte receptor. Abernathy et al.²⁵ were able to show a significant upregulation of this homing receptor in children with milk-induced eczema after *in vitro* stimulation with casein.

To further characterize these allergen-specific effector cells, cytokine secretion and immunoglobulin production were measured. Previous studies demonstrated an optimal time point for IgE determination after culturing cells for 10 to 14 days.²⁶ Our initial hypothesis was that these activated T cells would secrete increased amounts of IL-4 and provide help for IgE.²⁷ The results, however, indicate that these allergen-stimulated T cells do not produce IL-4 in a concentration detectable by our ELISA system, in spite of a fourfold concentration of the cell culture supernatants by ultrafiltration. Other authors report similar negative findings after allergen

stimulation.²⁸ Only after mitogen stimulation were we able to detect an increased production of IL-4 in cell cultures of patients with AD, which was paralleled by a decreased IFN- γ secretion compared with the control group. Such differences in cytokine production are in line with results published by several groups.²⁹⁻³² No differences in cytokine production were detected with regard to the manifestation of clinical signs and symptoms after DBPCFC. In addition, on the level of immunoglobulin production, higher amounts of IgE production were found in all atopic patients, but no differences were detected between patients with various degrees of clinical reactions and those who did not react during DBPCFC. Accordingly, T cells from patients with severe reactions provide help for IgE, but no more effectively than T cells from other atopic patients. Therefore IL-4 production and stimulation of IgE synthesis by these T cells cannot be considered as markers for predicting the development of symptoms in food allergy.

In conclusion, our data indicate that changes in lymphocyte subpopulations during *in vitro* stimulation with the relevant allergen may predict severe symptoms during food challenges in food-sensitive children. Cytokine production by T cells and the stimulation of immunoglobulin synthesis are not suitable predictors of the development of symptoms in children with food allergy. Because we studied a relatively small number of patients, we are currently not able to indicate a cutoff level of changes in T-cell distribution and activation that may correlate with such clinical reactions. The results from this study may be useful to delineate pathologic mechanisms of food allergy; however, large-scale studies—including some with ovomucoid as a stimulatory allergen—are required to evaluate how our results translate to individual laboratory test results.

REFERENCES

- Burks AW, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: clinical relevance of food hypersensitivity reactions. *J Pediatr* 1988;113:447-51.
- Sampson HA, McCasill CC. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J Pediatr* 1985;107:669-75.
- Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992;327:380-4.
- Kondo N, Agata H, Fukutomi O, Motoyoshi F, Orii T. Lymphocyte responses to food antigens in patients with atopic dermatitis who are sensitive to foods. *J Allergy Clin Immunol* 1990;86:253-60.
- Burks AW, Sampson HA. Diagnostic approaches to the patient with suspected food allergies. *J Pediatr* 1992;121:64-71.
- Sampson HA. The role of food allergy and mediator release in atopic dermatitis. *J Allergy Clin Immunol* 1988;81:635-45.
- Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an official procedure: a manual. *J Allergy Clin Immunol* 1988;82: 986-97.
- Leung DYM, Bhan AK, Schneeberger EE, Geha RS. Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. *J Allergy Clin Immunol* 1983;71:47-56.
- Sanders ME, Makgoba MW, Shaws A. Human naive and memory helper T-cells: reinterpretation of helper-inducer and suppressor-inducer subsets. *Immunol Today* 1988;9:195-9.
- Markey AC, Allen MH, Pitzalis C, Macdonald DM. T-cell inducer

- populations in cutaneous inflammation: a predominance of T-helper-inducer lymphocytes in the infiltrate of inflammatory dermatoses. *Br J Dermatol* 1990;122:325-32.
11. Frew AJ, Kay AB. UCHL1+ (CD45R0+) "Memory" T-cells predominate in the CD4 cellular infiltrate associated with allergen-induced late-phase reactions in atopic subjects. *Clin Exp Immunol* 1991;84:270-4.
12. Sampson HA. Pathogenesis of eczema. *Clin Exp Allergy* 1990;20:459-67.
13. Seymour JL, Keswick BH, Hanifin JM, Jordan WP, Illigan MC. Clinical effects of diaper types on the skin of normal infants and infants with atopic dermatitis. *J Am Acad Dermatol* 1987;17:988-97.
14. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980;92:44-7.
15. Niggemann B, Wahn U, Sampson HA. Proposals for standardization of oral food challenge tests in infants and children. *Pediatr Allergy Immunol* 1994;5:11-3.
16. Costa C, Rilliet A, Nicolet M, Saurat JH. Scoring atopic dermatitis: The simpler the better? *Acta Derm Venereol* 1989;69:41-5.
17. Remy N, Oberreit M, Thoenes G, Wahn U. Lymphocyte subsets in whole blood and isolated mononuclear leucocytes of healthy infants and children. *Eur J Pediatr* 1991;150:230-3.
18. Axen R, Drevin H, Kober A, Yman L. A new laboratory diagnostic system applied to allergy testing [abstract]. *N Engl Reg Allergy Proc* 1988;9:503.
19. De Vries JE, Gauchat JF, Aversa GG, Punnonen J, Gascan H, Yssel H. Regulation of IgE synthesis by cytokines. *Curr Opin Immunol* 1991;3:851-8.
20. Kohno Y, Honma K, Saito K, Shimojo N, Tsino H, Kanigonawa S, et al. Preferential recognition of primary protein structures of α -casein by IgG and IgE antibodies of patients with milk allergy. *Ann Allergy* 1994;73:419-22.
21. Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (*Gal d III*) compared with ovalbumin (*Gal d I*) in children with egg allergy and in mice. *J Allergy Clin Immunol* 1994;93:1047-59.
22. Mackay CR, Marston WL, Dudler L. Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J Exp Med* 1990;171:801-17.
23. Picker LJ, Butcher EC. Physiological and molecular mechanisms of lymphocyte homing. *Annu Rev Immunol* 1992;10:561-91.
24. Bos JD, Zonneveld I, Das PK, Krieg SR, Vam Der Loos CM, Kapsenberg ML. The skin immune system (SIS) distribution and immunophenotype of lymphocyte subpopulations in normal human skin. *J Invest Dermatol* 1987;88:569-73.
25. Abernathy-Carver KJ, Sampson HA, Picker LJ, Leung DJM. Milk-induced eczema is associated with the expansion of T-cells expressing cutaneous lymphocyte antigen. *J Clin Invest* 1995;95:913-8.
26. Or R, Renz H, Terada N, Gelfand EW. Regulation of B-cell immune responses: predominant role of interleukin-4 in triggering cell proliferation and Ig production in competent B cells. *Clin Immunol Immunopathol* 1994;72:141-9.
27. Gascan H, Gauchat JF, Roncarolo MG, Yssel H, Spits H, De Vriese JE. Human B-cell clones can be induced to proliferate and to switch to IgE and IgG4 synthesis by interleukin 4 and a signal provided by activated CD4+ T cell clones. *J Exp Med* 1991;173:747-50.
28. Takahashi T, Sasaki Y, Hama K, Furue M, Ishibashi Y. Production of IL-4, IL-2, IFN- γ and TNF- α by peripheral blood mononuclear cells of patients with atopic dermatitis. *J Dermatol Sci* 1992;3:172-80.
29. Reinhold U, Pawelec G, Wehrmann W, Herold M, Wernet P, Kreysel HW. Immunoglobulin E and immunoglobulin subclass distribution in vivo and relationship to in vitro generation of interferon-gamma neopterin in patients with severe atopic dermatitis. *Int Arch Allergy Appl Immunol* 1988;87:120-6.
30. Reinhold U, Pawelec G, Wehrmann W, Kukel S, Oehr P, Kreysel HW. Cytokine release from cultured peripheral blood mononuclear cells of patients with severe atopic dermatitis. *Acta Derm Venereol* 1989;69:497-502.
31. Tang M, Kamp A. Production and secretion of interferon-gamma (IFN- γ) in children with atopic dermatitis. *Clin Exp Immunol* 1994;95:66-72.
32. Renz H, Jujo K, Bradley KL, Domenico J, Gelfand EW, Leung DY. Enhanced IL-4 production and IL-4 receptor expression in atopic dermatitis and their modulation by interferon-gamma. *J Invest Dermatol* 1992;99:403-8.