

T-cell reactivity for a peanut-derived epitope in the skin of a young infant with atopic dermatitis

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A role for food allergen-specific T cells in the pathogenesis of atopic dermatitis (AD) in infants with food allergy is indicated from studies performed with peripheral blood lymphocytes.¹ However, no reports exist that describe the isolation of food allergen-specific T cells from the skin of infants with AD. We investigated the presence of cow's milk- and peanut-specific T cells in the skin of the described subject.

CASE REPORT

A 6-month-old male patient with AD was taken to our outpatient clinic. His father had had allergic asthma. Because his two brothers had already had food allergy (including peanut allergy) and AD, his mother never consumed peanut- or nut-containing foods. During the third trimester of pregnancy and during lactation, her diet was also free from cow's milk and hen's egg white proteins. Until the age of 3 months, the patient was exclusively breast-fed. Then a whey protein hydrolysate was introduced, after which he developed diarrhea. The hydrolysate was replaced by a soy-based formula for 2 months; gastrointestinal symptoms disappeared, but the patient had severe AD. Almost all disease symptoms disappeared when the formula was omitted and an extensively hydrolyzed casein-based formula was introduced. Cow's milk allergy was diagnosed by elimination of and provocation with cow's milk. Cow's milk challenge induced an urticarial reaction.

METHODS

After informed consent was obtained from the patient's mother, 5 ml of heparinized blood and a 3 mm skin-punch biopsy specimen from lesional AD skin were obtained at the same time. Serum was obtained at the time of sampling and 3 months later. Specific serum IgE was analyzed by RAST (DPC, Los Angeles, Calif.).

Abbreviations used

AD:	Atopic dermatitis
CPE:	Crude peanut extract
CSE:	Crude soy extract
IFN:	Interferon
PBMC:	Peripheral blood mononuclear cell
PNA:	Peanut agglutinin

A skin-cell suspension was prepared by enzymatic digestion, according to the method described by Sager et al.² To select for food allergen-specific T cells, the suspension was cocultured with 10⁶ irradiated (20 Gy) peripheral blood mononuclear cells (PBMCs) in culture medium (RPMI 1640; Gibco, Grand Island, N.Y.) supplemented with 10% human AB serum (Bloedbank, Utrecht, The Netherlands), IL-2 and IL-4 (both 50 U/ml; kindly provided by Novartis Research Institute, Vienna, Austria), and food allergen. For selection of cow's milk-specific T cells, a mixture of total casein, α -lactalbumin, and β -lactoglobulin (each at 50 μ g/ml; kindly provided by Dr. E. C. H. van Beresteijn, Netherlands Institute for Dairy Research, Ede, The Netherlands) was added, and for selection of peanut-specific T cells, 50 μ g/ml of a crude peanut extract (CPE; kindly provided by Dr. E. C. de Jong, TNO Nutrition and Food Research Institute, Zeist, The Netherlands) was introduced. CPE contained the major allergens Ara h 1 and Ara h 2, as well as the lectin peanut agglutinin (PNA) in a ratio similar to that found in peanuts. The polyclonal T-cell line SE1, isolated by stimulation with CPE, was subcloned by limiting dilution as described previously.³

Allergen specificity in PBMCs, SE1, and the T-cell clones derived from SE1 was examined in lymphocyte stimulation tests.³ Allergens tested were total casein, α -lactalbumin, β -lactoglobulin, and CPE. Specificity of T-cell clone SE1.41 was further analyzed in lymphocyte stimulation tests with purified Ara h 1, Ara h 2, PNA, and a crude soy extract (CSE). Ara h 1, Ara h 2, and PNA had an estimated purity of more than 95% as judged by Coomassie Brilliant Blue-stained polyacrylamide gel electrophoresis. Molecular weights were 63 kd for Ara h 1, 14 to 17 kd for Ara h 2, and 30 kd for PNA. Each allergen was tested in lymphocyte stimulation tests for its capacity to induce T-cell proliferation in PBMCs from an individual sensitized to that allergen.

To assess the T_H phenotype of T-cell clone SE1.41, its cytokine secretion profile was determined. Therefore in culture medium with 10% human AB serum, 10⁶ T cells were cocultured with 10⁶ autologous, irradiated (40 Gy), Epstein Barr Virus-transformed B cells, which had been preincubated overnight both with and without 50 μ g/ml of CPE.³ The presence of IL-4 and interferon- γ (IFN- γ) in the culture supernatants was examined by ELISA (CLB, Amsterdam, The Netherlands).

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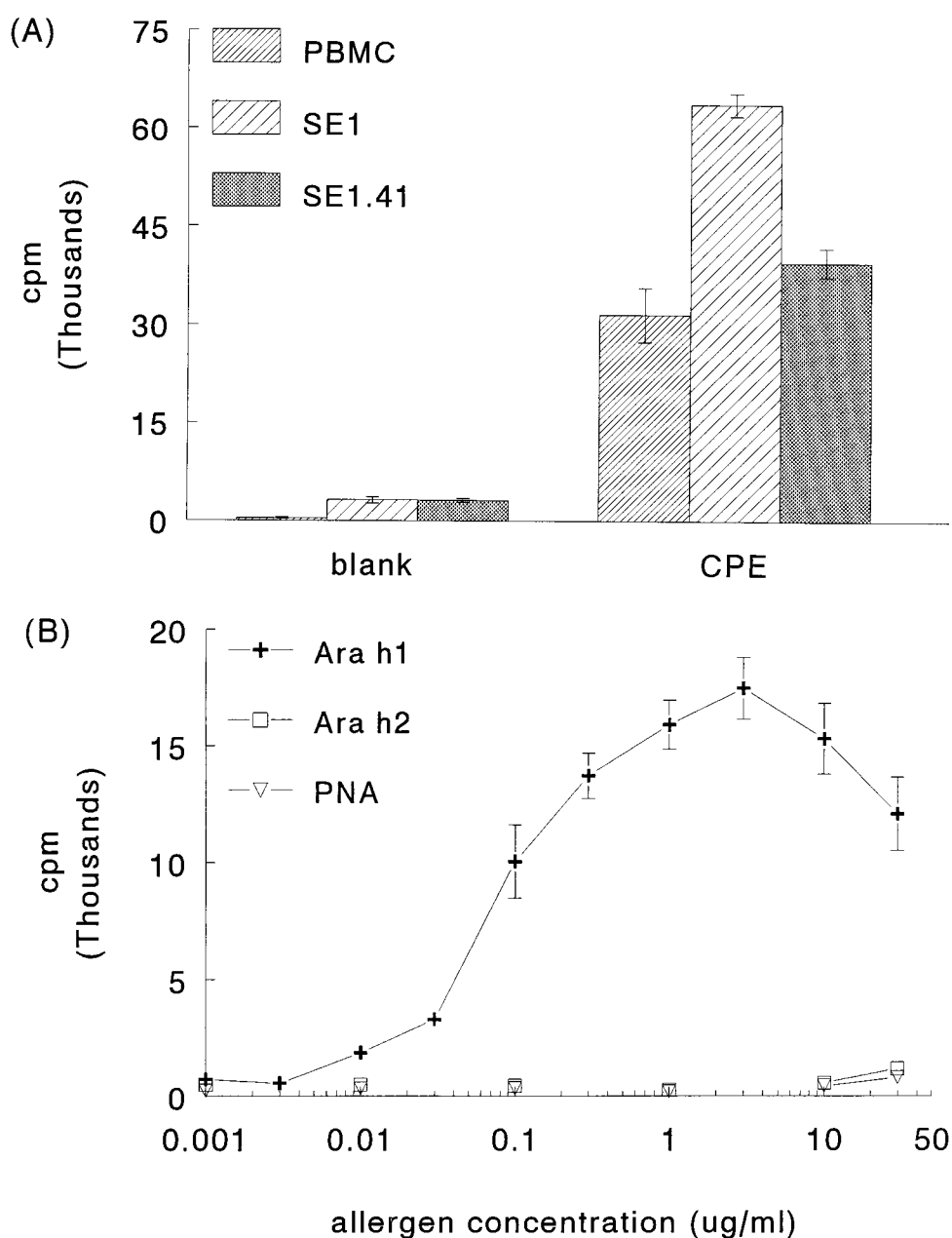


FIG. 1. A, Freshly isolated T cells from PBMCs, a skin-derived polyclonal T-cell line (SE1), and a T-cell clone (SE1.41) isolated from SE1 all respond to 50 μ g/ml of CPE. B, T-cell clone SE1.41 recognizes the major peanut allergen Ara h 1 but not Ara h 2 or PNA.

Control supernatants never contained detectable amounts of IL-4 or IFN- γ .

RESULTS

At the time of sampling, a total serum IgE of 289 kU/L was measured, and specific serum IgE was detected for cow's milk (RAST class 3) and soy (class 1); peanut-specific IgE was not detectable. In PBMCs, responses ($\pm$ SD) to cow's milk allergens were low

compared with the blank stimulation with medium alone (385 ± 92 cpm), casein (2721 ± 1433 cpm), α -lactalbumin (1307 ± 456 cpm), and β -lactoglobulin (1519 ± 104 cpm). Culturing skin-derived T cells in the presence of the above mentioned cow's milk allergens did not produce a viable cow's milk-reactive polyclonal T-cell line, and no reactivity to cow's milk allergens was present in the skin-derived polyclonal T-cell line SE1, which was propagated in the presence of CPE. In contrast, marked

specificity for CPE was detected in both PBMCs and SE1 (Fig. 1, *A*). The CPE-reactive, skin-derived T-cell clone SE1.41 did not respond to CSE (not shown), thus excluding the possibility that SE1.41 was selected by ingestion of soy. Further analysis of SE1.41 demonstrated that it was specific for the major peanut allergen Ara h 1 (Fig. 1, *B*). SE1.41 exhibited a T_{H0} phenotype of cytokine production after stimulation with CPE (first stimulation, 1770 pg/ml IL-4 and 590 pg/ml IFN- γ ; second stimulation, 656 pg/ml IL-4 and 1421 pg/ml IFN- γ). Three months after referral, total IgE was increased to 467 kU/L and positive RAST results were seen for cow's milk (class 3) and peanut (class 3) but not for soy.

DISCUSSION

No significant T-cell reactivity for either casein, α -lactalbumin, or β -lactoglobulin was found in the patient's skin. Successful isolation of cow's milk-reactive T cells may have been impeded by a sampling error. This may have been the result of a relatively low number of cow's milk-specific T cells in chronically inflamed AD skin, as opposed to early, allergen-triggered, eczematous reaction sites. The relevance of cow's milk sensitization for this child's AD is, however, indicated by the high titer of cow's milk-specific serum IgE, the presence of cow's milk allergen-specific T cells in the blood, and the positive diagnosis for cow's milk allergy.

Until the time of testing, peanuts and peanut-derived products were thoroughly omitted from the patient's environment. For this reason, the isolation of Ara h 1-specific T_{H0} clone SE1.41 from the patient's skin is noteworthy, even though the clinical relevance of peanut sensitization in this case is not evident. Support for the existence of a causal relationship between T-cell recog-

nition of Ara h 1 and the presence of peanut-specific serum IgE is twofold. First, SE1.41 was isolated 3 months before detectable humoral sensitization to peanut. Second, SE1.41 did not respond to CSE, indicating that at least part of the peanut-binding IgE in this infant results from T-cell recognition of peanut and not soy.⁴ Thus we favor the hypothesis that unwitting exposure to very low doses of peanut allergens, rather than exposure to higher concentrations of cross-reactive allergens, causes T-cell reactivity and subsequent production of IgE specific to peanut in infants.

In conclusion, we demonstrate that food allergen-specific T cells can be isolated from the skin of an infant with AD, even before detectable food-specific serum IgE. Moreover, our results indicate that T-cell reactivity for a peanut-derived epitope may develop in a young infant despite thorough avoidance of peanut proteins.

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