

CASE REPORT

Immunological Aspects of Oral Desensitization in Food Allergy

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The therapeutic approach to food allergy is still very controversial. When it is not possible to eliminate from the diet responsible allergens either because the food is essential (eg, milk, egg), or because the foods to avoid would be too numerous, symptoms can be treated pharmaceutically. The best drugs in such cases are membrane stabilizers with a prophylactic effect, such as sodium chromoglycate and ketotifen. When these drugs are unsuccessful, specific immunotherapy (SIT) is advisable (1).

Recent studies showed a spontaneous desensitization in patients who had been following an elimination diet for one or more years [reported in 19–44% of the cases (2–5)] but not associated with a reduction of serum specific IgE titre. Subcutaneous hyposensitizing treatment has not proven to be very successful (6–10), even though subcutaneous rush desensitization is reported for patients with peanut allergy (11–13). The enzyme-potentiated desensitization proposed by some also needs further study (14). Oral desensitization, despite some negative opinions (8, 10, 15, 16) on its efficacy, has been successfully attempted by some authors (17). In particular, in a recent study conducted with 14 children who were allergic to various foods (milk, egg, fish, apple), our group obtained complete desensitization in as many as 12 cases (18).

Considering our previous experiences, we report the case of a desensitization to milk according to a standardized protocol (18), in which we found very interesting immunological modifications.

CASE REPORT

We report the case of a 6-year-old child, who had been presenting with bronchial asthma and vomiting a few minutes after the ingestion or inhalation of milk, starting from the age of 1 month. Such symptoms and their time of onset put this patient in the first group according to the classification of the Melbourne Milk Allergy Study (19). Family and personal history were taken accurately and physical examination was made, with negative results, including dermatographism. The diagnosis of milk allergy was made through prick tests, both with standardized allergens: α -lactalbumin (α -LA), β -lactoglobulin (β -LG), casein (CS), and crude milk. Tests were considered positive when a wheal ≥ 4 mm in diameter appeared. All skin tests were positive. Total and specific IgE to α -LA, β -LG, and CS were positive (α -LA class 4, β -LG class 3, CS class 2); specific IgG and IgA to the same allergens were also measured (Pharmacia CAP FEIA).

An oral provocation test was eventually conducted in a double-blind placebo-controlled food challenge (DB-PCFC). Oral provocation test was attempted with increasing doses of crude milk diluted in vanillin (ml 0.01, 0.1, 0.5, 1, 5, 10, 15, 30 with 1-hr intervals). The test was considered positive, as vomiting, generalized erythema, urticaria, pruritus, and abdominal pain were reported a few minutes after the ingestion of a 30-ml dose of milk, requiring therapy with corticosteroids and antihistamines. No reaction at all was reported after placebo administration (vanillin alone). The patient then underwent an oral desensitization according to a standardized protocol (18). According to such a protocol, milk is administered, first diluted, then undiluted, in progressively increasing doses, up to a maximum daily dose of 100 ml of undiluted milk (Table 1).

Before starting desensitization, baseline ECP, tryptase in serum, IL-4 and IFN- γ in serum and in the supernatant of peripheral blood mononuclear cells incited by phytohemagglutinin (PHA) and with phorbol myristate acetate (PMA, SIGMA Milan Italy) or with β -LG and lymphocyte subsets were assessed.

Allergy evaluation and immunological measurements were then reassessed at 1, 3, 6, 12, and 18 months after the beginning of the treatment.

Lymphocyte subsets were analyzed as follows: 100 μ l of EDTA-anticoagulated whole blood was incubated for 30

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TABLE 1. PROTOCOL OF DESENSITIZATION TO MILK ALLERGY*

	Days	Daily dose (drops)
Starting dilution*	1-3	4
	4-6	6
	7-9	10
	10-12	12
		Drops
Pure milk	13-15	1
	16-18	2
	19-21	4
	22-24	6
	25-27	10
	28-30	16
	31-33	32
	34-36	48
	37-40	40 × 2
	41-44	40 × 3
	45-48	40 × 4
	49-52	50 × 4
	53-56	60 × 4
		ml
	57-60	4.5 × 3
	61-64	5 × 3
	65-68	6 × 3
	69-72	5 × 4
	73-76	6 × 4
	77-80	7 × 4
	81-84	9 × 4
	85-88	12 × 4
	89-92	15 × 4
	93-96	15 × 5
	97-100	30 × 3
	101-104	50 × 2

* 10 drops of milk in 10 ml of water.

min at 4°C with the following FITC- or PE-conjugated monoclonal antibodies: CD45 (LCA), CD14, CD3 (Leu-4), CD19 (Leu-12), CD4, CD8, CD16 (Leu-11c), CD56 (NKH-1), HLA-DR, CD25, CD23 (Becton Dickinson) and CD30 (Ber-H2 clone, Dako A/S, Glostrup, Denmark). After erythrocyte ammonium chloride lysis, cell pellets were washed twice in PBS supplemented with 0.1 mM ethylene diamine tetraacetate (EDTA) and 0.02% NaN₂ (PBS-EDTA) and kept on ice until examination; 3000 events were acquired in list mode. Lymphocyte gate purity always exceeded 98% as evaluated by CD45^{bright} staining. Quadrant analyses were performed with CELLQuest software (BD). The absolute numbers of circulating T (CD3⁺), T helper (CD4⁺), T suppressor (CD8⁺), B (CD19⁺) lymphocytes and natural killer cells (CD3⁺/CD16⁺CD56⁺) were determined by multiplying their frequency by lymphocyte counts.

Mononuclear Cell Isolation. The last aliquot (4 ml) of blood sample was placed into heparinized sterile tubes for leukocyte isolation. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque gradient centrifugation and then washed three times with Ca²⁺- and Mg²⁺-free phosphate-buffered saline (PBS). Thereafter PBMC were resuspended at concentration of 1 × 10⁶ cells/ml in culture medium (CM) (RPMI 1640; Flow Laboratories,

Irvine, Scotland), supplemented with antibiotics (100 units/ml of penicillin and 100 mg/ml of streptomycin; Flow Laboratories), L-glutamine (2 mmol/liter, Gibco Laboratories, Grand Island, New York), and 10% autologous human serum.

Lymphocyte Transformation T-Cell (LTT) and Lymphokine Production. Into 96-well, flat-bottom tissue plates (Falcon 3070, Becton Dickinson Labware) 2.5 × 10⁵ PBMC in 250 µl of CM were placed. The cells were incubated at 37°C in a humidified atmosphere with 5% CO₂ for three days, in presence of one of the following mitogens or antigens added to triplicate wells: PHA (100 µg/ml, M form, Gibco) plus PMA (1 ng/ml) and β-LG (100 µg/ml). Negative control wells received CM without mitogens or antigens.

The supernatants of the stimulated cultures were harvested after 72 hr of incubation and stored at -20°C until testing for lymphokine production. The final volume of 250 µl was reconstituted with an equal quantity of fresh CM, without antigen or mitogen, and the incubation was continued. During the last 16 hr of incubation, the cells were pulsed with 0.5 µCi/well of [methyl-³H]thymidine (6.7 Ci/mmol, NEN Products, Boston, Massachusetts). The incorporation of thymidine into DNA was determined by liquid scintillation counter (Beckmann LS 7000) and radioactivity expressed as mean of doubled cultures of counts per minute (cpm) and stimulation index (SI: mean cpm of stimulated cultures/mean cpm of unstimulated cultures). With regards to the stimulation with β-LG, a positive proliferative response was defined as SI ≥ 3.

In the culture supernatants IL-4 and IFN-γ were quantified by ELISA kits [InterTest-4, Genzyme Corporation, Cambridge, Massachusetts; (minimum sensitivity 3 pg/ml IL-4) and InterTest-γ (minimum sensitivity 3 pg/ml IFN-γ)].

ECP plasma concentrations were evaluated by means of an immunoenzymatic method (Pharmacia, Uppsala, Sweden), while tryptase serum levels were quantified employing RIA assays (Pharmacia).

RESULTS

Desensitization was accomplished in four months, without any relevant side effect. The only trouble the patient had, on the first administration of undiluted milk, was "itching of the mouth and throat and nausea," which receded spontaneously. During the fourth month of treatment, the patient took 200 ml of undiluted milk without any problem, and after six months was able to ingest dairy products and foods containing milk (fresh and fermented cheeses, milk chocolate, sweets, etc).

Skin tests, strongly positive at the beginning to milk, β-LG, α-LA, and CS, turned completely negative after seven months. At the same time, the level of specific IgE to β-LG decreased from 6.7 to 2.5 KU/liter and that to α-LA from 19.8 to 2.82 and to CS from 83.8 to 13.7 (Figure 1).

Specific IgG and IgA to β-LG, α-LA, and CS

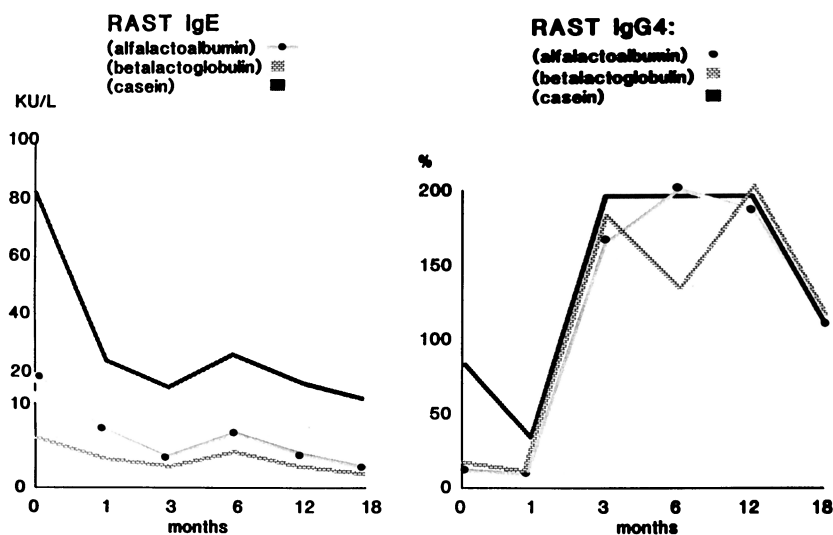


Fig 1. Immunologic changes induced by desensitization in milk allergy.

showed a progressive increase within 18 months (Figures 1 and 2). Total IgE, which was elevated at the beginning and after an initial further increase, returned to baseline levels after 3 months of treatment (Figure 3) a progressive increase of ECP up to a peak of 50 $\mu\text{g/liter}$ at the end of treatment also was observed, and then gradually fell to within normal range (Figure 3). No significant variation in tryptase level was observed. As for cytokine levels in the supernatant of cell cultures, we observed different trends of the two cytokines. *In vitro* IL-4 production decreased to a very low level, as did the level in serum. At the same time the IFN- γ level in serum increased, and an increase of its production was observed, both sponta-

neously and when induced by mitogen and by β -LG (Figure 4).

The IFN- γ level 18 months after the end of treatment returned to baseline, even though that resulting from the induction by β -LG remained a bit higher.

When analysing the kinetics of lymphocyte subsets, no significant modifications of percent CD3⁺ (total T lymphocytes), CD19⁺ (total B lymphocytes), CD4⁺ (T helper/inducer lymphocytes), CD8⁺ (T suppressor/cytotoxic lymphocytes), activated T lymphocytes (CD3⁺/HLA-DR⁺), and NK cells were observed at the different time points.

DISCUSSION

Few studies have been done on the pathophysiology of oral hyposensitizing treatment. Hanson et al in

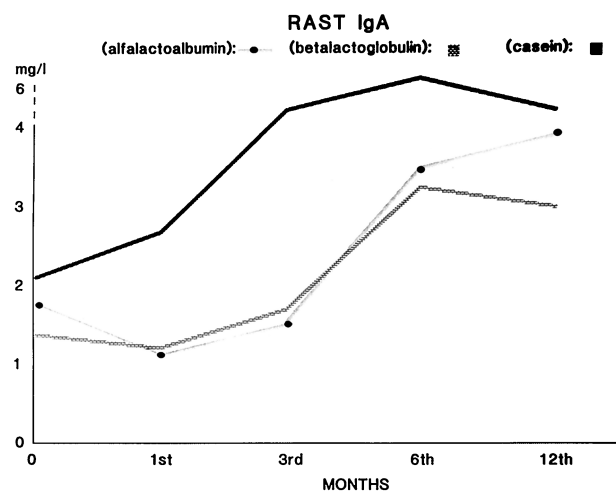


Fig 2. Immunologic changes induced by desensitization in milk allergy.

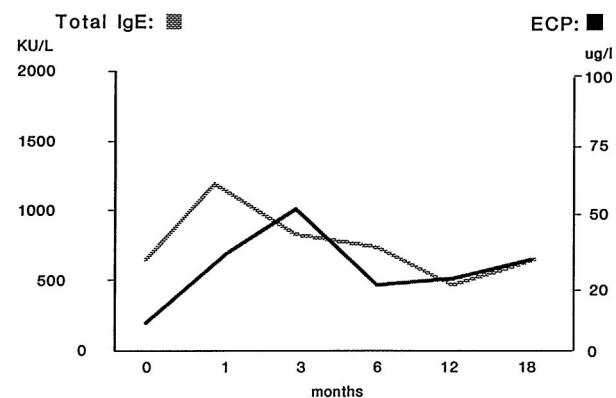


Fig 3. Immunologic changes induced by desensitization in milk allergy.

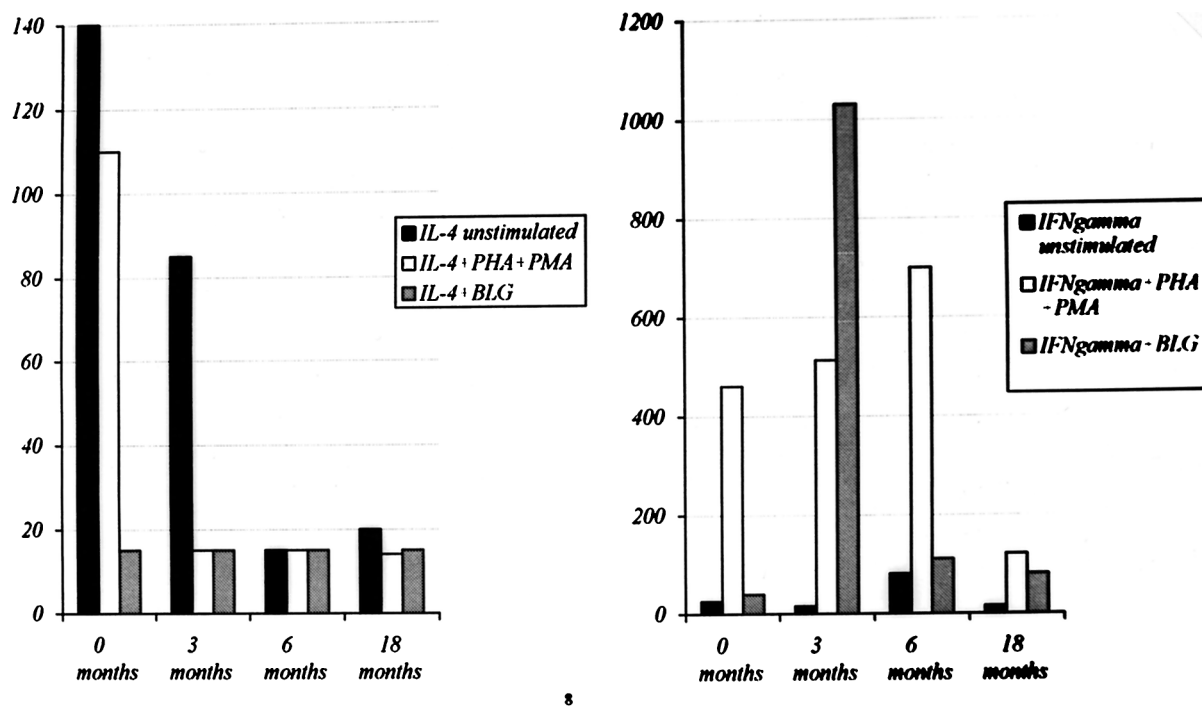


Fig 4. IL-4 and IFN- γ modifications during desensitization to milk.

1979 (20) suggested that oral administration of antigenic proteins induces a suppression of both IgE and IgG. This suppression, according to some authors (21) is antigen specific; it is probably mediated by a serum transferable factor (22) and/or by suppressor T lymphocytes according to Ngan et al (23).

It is likely that secretory IgA production could be induced, but we cannot exclude the involvement of immunological tolerance (24). However, there are no certainties on this matter. It is known that in allergic patients there is an imbalance between Th-1 and Th-2 lymphocyte subsets, which produce cytokines that ultimately regulate Ig production. A prevalence of Th-2 lymphocytes with an increased IL-4 level (which results in promotion of IgE synthesis) and a decrease of Th-1 lymphocytes and decreased IFN- γ production (which results in IgE synthesis inhibition) is observed, accompanied by IgE overproduction (25–27).

In atopic patients exposure to the allergen provokes a Th-2 response, with increased production of IL-3, IL-4, IL-5, and IL-13, which, on the one hand stimulate IgE production and on the other, eosinophil activation, which results in the release of phlogistic proteins, such as ECP, EPX, MBP, etc.

It was suggested that in respiratory allergy, hypo-sensitizing treatment could induce a switch from a

prevalent Th-2 to a prevalent Th-1 response, and an increase of IL-2 and IFN- γ may follow. A direct consequence could be a decreased activation of eosinophils and of correlated phlogistic indexes (eg, ECP).

In this case we report negativization of prick tests; the decrease of specific IgE to β -LG, α -LA, and CS; and an increase of specific IgG4, surprisingly correlated to findings found in hyposensitizing treatment in respiratory allergies.

The decrease of IL-4 serum level and *in vitro* production and the accompanying increase of IFN- γ serum level and *in vitro* production that we observed lead us to suggest that during desensitization there is a shift from a Th-2 (IL-4 producer) to Th-1 (IFN- γ producer) lymphocyte subset, as is observed in respiratory allergy. No such report has been found in literature to date.

We have no explanation for the increase in ECP during the treatment. This finding seems to be contradictory with the increase of Th-1 and the decrease of Th-2. With decreasing Th-2 function, a lower IL-5 secretion is expected (IL-5 being the most important cytokine in the activation of eosinophils), and thus also a decrease of the production of the proteins secreted by eosinophils, particularly ECP. That is exactly the opposite to what we observed. It is, how-

ever, true that the ECP level increase could be due to a response of the patient to the bulk doses of allergen; but it is also true that in similar conditions during hyposensitizing treatment in respiratory allergy ECP usually falls. With no previous report in literature, this finding remains to be explained. Only the increase of IL-5 could justify eosinophil activation. The fact that we also noticed a large increase of serum-specific IgA in all three antigenic milk molecules, which could coincide with an increase in secretory IgA (although we have no proof of this as yet), seems to us to be very interesting. Consistent with the previously documented IL-5 role in IgA production (28), the finding of an increase in specific IgA, parallel to that of ECP, could be accounted for as ultimately resulting from increased IL-5 secretion. This remains only a supposition, however; we are going to attempt to verify this through a study we have already designed. Further studies are underway to explain such aspects.

We may conclude that the possibility to attempt oral desensitization should always be considered in children, for whom elimination from the diet of important foods such as milk or egg could result in important nutritional problems.

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