ORIGINAL ARTICLE

Oral Immunotherapy With Egg and Milk: Changes in Peripheral Serum Cytokines Are Not Predictive Factors for Severe Adverse Reactions or for the Final Report

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■ **Abstract**

Introduction: Oral immunotherapy (OIT) is a new approach in patients with food allergy. Various immunological mechanisms underlie the reversal of food allergy. In this paper, we study possible changes in peripheral cytokine patterns during OIT.
Methods: Determinations of cytokines in peripheral blood were made in children who had milk or egg allergy and who received OIT. The determinations were made before and after OIT, and again following a final repeat oral challenge a month after a diet excluding the culprit food.
Results: No significant changes were registered in the cytokines studied (IL-2, IL-4, IL-6, IL-10, IL-12, IL-17, IFNγ, and TNF) at any of the 3 time points. Similarly, no differences in cytokine pattern were observed between children who had presented anaphylaxis during OIT and those who overcame or did not overcome the final oral challenge.
Discussion: Peripheral cytokines do not undergo significant changes during the OIT process. They are not predictors of serious adverse reactions or the final result of the OIT.

Key words: Egg allergy. Milk allergy. Oral immunotherapy. Cytokines.

■ **Resumen**

Introducción: Se ha introducido la inmunoterapia oral frente a alimentos como una nueva terapia en pacientes con alergia alimentaria. Diferentes mecanismos inmunológicos han sido descritos en un intento de explicar la reversibilidad de esta situación de alergia alimentaria. En este artículo, estudiamos los posibles cambios en el patrón de citoquinas en sangre periférica a lo largo del proceso de la inmunoterapia oral.
Métodos: Se realizó determinación de citoquinas en sangre periférica en tantos niños con alergia a leche o huevo que realizaron inmunoterapia oral. Las determinaciones se realizaron tanto de forma previa como tras la finalización de la OIT, así como tras una reprovocación final, un mes después de seguir una dieta exenta del alimento implicado.
Resultados: No se registraron cambios significativos en las citokinas estudiadas (IL-2, IL-4, IL-6, IL-10, IL-12, IL-17, IFNγ y TNF) entre ninguna de las tres determinaciones temporales. Tampoco existieron diferencias en el patrón de citokinas entre los niños que habían presentado anafilaxias durante la OIT ni entre los que superaron o no superaron la provocación final.
Discusión: Las citokinas periféricas no sufren cambios significativos a lo largo del proceso de OIT. No son factores predictivos de reacciones adversas graves ni del resultado final de la OIT.

Palabras clave: Alergia a la leche. Inmunoterapia oral. Citoquinas.

Introduction

Food allergy is emerging as a "second wave" of the allergy epidemic, replacing the "first wave", which was dominated by respiratory allergy [1,2]. In the first years of life, eggs and milk are the foods that most often produce this type of reaction, which can affect up to 8% of children [3].

Allergy to eggs and milk can cause anaphylaxis and even death. Treatment involves strict avoidance of the culprit food, thus decreasing the variety of foods that can be consumed and the quality of life of patients and their families. Patients are also at risk of serious reactions due to cross contamination and dietary transgressions.

Oral immunotherapy (OIT) has been used to treat food allergy for several years. By gradually introducing increasing amounts of the culprit foods into the diet [4,5], children tolerate the intake of usual amounts of these foods. This normalization of intake also reduces the risk of anaphylaxis.

Various immunological mechanisms underlie the reversal of food allergy, including a decrease in specific IgE [6], an increase in specific IgG4 [7], and a decrease in the antigen-specific response of basophils [8,9]. Evidence has also been reported for the modification of the cellular immune response in OIT, with a decrease in the T_H2 response [10,11] and in the generation of T cells with a nonreactive phenotype [12]. Consequently, the response observed in these children changes from their preferred T_H2 pattern [13] to a T_H1/T_H2 balance pattern. In the present article, we analyze possible changes in the pattern of peripheral cytokines throughout the OIT process.

Patients and Methods

Participants

The study population comprised 48 children (33 boys and 15 girls) with egg allergy (n=29) or milk allergy (n=19). Their mean (SD) age was 7.5 (2.3) years. Twenty-seven children had multiple food allergies, while the rest were allergic to egg or milk alone. Thirty-six children had bronchial asthma, all due to dust mite allergy, and 21 had atopic dermatitis. To be included in the study the children had to fulfil all 4 of the following diagnostic criteria: (1) a clinical history of immediate allergic reaction to egg or cow milk; (2) positive in vitro specific IgE to egg white, ovalbumin, and ovomucoid in egg-allergic patients or to cow milk, α-lactalbumin (ALA), ß-lactoglobulin (BLG), or casein in milk-allergic patients; (3) positive skin test results to egg white, ovalbumin, and ovomucoid in egg-allergic patients or cow milk, ALA, BLG and casein in milk-allergic patients; and (4) a positive open oral challenge test with scrambled egg or cow milk. This fourth criterion was not required in 12 children who had developed anaphylaxis following the ingestion of egg or milk in the previous months.

The study was approved by the local ethics committee, and all the parents provided their informed consent before the children were enrolled in the study.

In Vivo Tests

Skin prick tests were performed with commercial extracts of egg white (10 mg/mL), ovalbumin (10 mg/mL), ovomucoid (10 mg/mL), cow milk (5 mg/mL), ALA (5 mg/mL), BLG (1 mg/mL), and casein (20 mg/mL) (Laboratorio BIAL) following the recommendations of the European Academy of Allergy and Clinical Immunology [14]. The skin test results were regarded as positive if the wheal diameter was ≥3 mm. A positive control of histamine 10 mg/mL and a negative control of saline 0.9% were included.

The initial open food challenges were carried out 15 days before the OIT protocol was started. The challenges were carried out with incremental doses of scrambled egg or milk, starting with a quantity of 1/1000 of egg or 0.1 mL of cow milk and ending, 8 steps later, with the ingestion of a whole egg or 200 mL of cow milk. All the doses were administered at 20-minute intervals in the hospital.

OIT with egg consisted of the administration of incremental doses of dehydrated egg whites (OVO DES NM, Nutrición Médica) in 9 steps or milk according to the protocol of SEICAP [4,5]. The egg could be administered in water or orange juice. The first dose was administered in the hospital, and the subsequent doses were administered at home on a daily basis for 1 week. The dose was increased every week, and this increased dose was administered in the hospital. Finally, tolerance to 1 scrambled egg or 200 mL of milk was verified. Subsequently, the children had to continue consuming 1 scrambled egg at least 3 days a week or 200 mL of cow milk in addition to any amount of other foods containing egg (eg, cakes, omelettes) or milk (eg, cakes, sweets, yoghourt).

After 1 year of being able to consume egg or milk, without presenting symptoms on intake (eg, oral allergy syndrome, urticaria, abdominal pain), parents were advised to ensure that their children followed a diet totally free of milk or egg for 1 month followed by subsequent readministration based on the same protocol as the initial OIT. This repeat oral challenge was not carried out in cases in which the patients presented symptoms after the ingestion of egg or milk despite regular consumption and in cases where the parents did not give their consent. Egg or milk was eventually readministered to 26 children.

In Vitro Tests

Specific IgE

Specific IgE to egg white, ovalbumin, ovomucoid, cow milk, ALA, BLG, and casein was measured using the ImmunoCAP FEIA system (Thermo Fisher Scientific). Values higher than 0.35 kU_A/L were considered positive.

Cytokines

The cytokines IL-2, IL-4, IL-6, IL-10, TNF, IFNγ, and IL-17 were assessed by flow cytometry using patient serum (BD Cytometric Bead Array [CBA] Human $T_H1/T_H2/T_H17$ Cytokine Kit, BD Sciences). The determination was carried out prior to the start of OIT, immediately after the end of OIT in all patients and immediately before performing the final repeat oral challenge test only in those patients in whom it was performed.

Statistical Analysis

The statistical analysis was carried out using SPSS (SPSS Inc). Quantitative data were expressed as median and interquartile range and qualitative data as frequencies. Means were compared using the Wilcoxon test for paired data. The comparisons were 2-tailed in all cases, with a P value of <.05 considered to be statistically significant.

Results

OIT was administered to 29 children (20 boys, 9 girls) with egg allergy and 19 children (13 boys, 6 girls) with milk allergy. In all children, tolerance was reached to 1 complete egg and 200 mL of milk. The values of specific IgE prior to OIT for egg and milk were as follows: egg white, 6.6 kU$_A$/L (2.2-12.6); ovalbumin, 5.7 kU$_A$/L (1.2-11.7); ovomucoid, 4.4 kU$_A$/L (2.1-11.5); cow milk, 30.8 kU$_A$/L (10.0-59.8); ALA, 6.2 kU$_A$/L (0.9-17.9); BLG, 3 kU$_A$/L (1.3-9.9); and casein, 19.6 kU$_A$/L (8.8-37.4). Nine children experienced anaphylaxis during the procedure (5 with egg and 4 with milk). A repeat oral challenge was performed in 26 cases with the food involved after 1 month of an exclusion diet (16 in egg and 10 in milk). In the case of egg, 8 patients tolerated the reintroduction of egg. In the case of milk, 7 children tolerated reintroduction.

In the study population as a whole, there were no significant differences in cytokine values at baseline, completion of OIT, and the repeat oral challenge (Table). Similarly, no differences were observed between the 3 time points. No significant differences in cytokine values were recorded before the repeat oral challenge in patients who tolerated or did not tolerate this oral challenge 1 month after following a diet free of food (Table). This absence of significance extends to the other 2 points when cytokine values were determined (baseline and after completion of OIT) (data not shown).

Similarly, no significant differences were observed between the children who developed anaphylaxis and those who did not during OIT in relation to the baseline cytokine values (Table).

No statistically significant values were observed when the results were analyzed according to the food involved (milk or egg, data not shown).

Discussion

As with sublingual and parenteral immunotherapy with aeroallergens, OIT tries to reverse the imbalance in the T_H1/T_H2 response in atopic patients. This reversal of the predominance of the T_H2 response is observed indirectly as a decrease in specific IgE values [6,8], a decrease in the results of skin tests with the allergens used in immunotherapy [3,4,8], and an increase in specific IgG4 values [7]. Changes in the specific immune response to allergens should be mediated through a change in the cytokine profile, so that there is a decrease in T_H2 cytokines (IL-4, IL-6, and IL-10) in favor of a predominance of T_H1-dependent cytokines (IL-2, TNF, and IFNγ). Several studies support that this type of treatment induces the creation of specific regulatory T cells that release, along with other cytokines, IL-17, IL-10, TGFα, and TGFß [10,15,16]. Together, these immunological changes lead a high percentage of patients to acquire tolerance or desensitization to the food with which OIT is administered.

In our series, we did not detect significant changes in the serum values of any of the cytokines studied. Our data are similar to those published by other authors [17] in children with cow milk OIT, although in this group, there were significant decreases in the values of platelet-derived growth factor and vascular endothelial growth factor, neither of which cytokines was assessed in our study. However, in another series of patients with OIT with egg, significant decreases were observed in several of the T_H1 and T_H2 cytokines in peripheral blood at

Table. Cytokine Values (pg/mL) at the 3 Study Time-Points, Anaphylaxis During Oral Immunotherapy, and Tolerance to Oral Egg Rechallenge

	IL-2	IL-4	IL-6	IL-10	IL-17	TNF	IFNγ
Baseline	0.10 (0-1.8)	0.54 (0-1.4)	2.2 (0.82-3.28)	0.72 (0.13-1.28)	2.69 (0-6.7)	0.48 (0-1.26)	0.27 (0-0.96)
Post-OIT	0.12 (0-1.8)	0.68 (0-1.5)	1.22 (0.82-3.08)	0.55 (0-1.35)	1.63 (0-6.6)	0.38 (0-1.63)	0.32 (0-1.09)
Before rechallenge	0.22 (0-1.5)	0.43 (0-1.1)	1.87 (0.67-2.41)	0.66 (0-0.98)	2.92 (0-5.55)	0.6 (0-1.23)	0.34 (0-1.18)
Anaphylaxis During OIT							
Yes	0.89 (0.04-2.29)	0.62 (0.17-1.61)	2.27 (0.71-3.67)	0.22 (1.02-1.53)	2.7 (0.02-6.4)	0.65 (0.34-1.55)	0.28 (0-1.50)
No	0.1 (0 -1.9)	0.51 (0-1.38)	2.18 (0.86-3.32)	0.69 (0.07-1.15)	2.04 (0-6.84)	0.28 (0-0.96)	0.25 (0-0.91)
Oral Egg Rechallenge Tolerance							
Tolerance	0.31 (0-1.54)	0.43 (0-1.42)	1.98 (0.88-2.43)	0.66 (0-1.06)	3.79 (1.67-7.11)	0.72 (0-1.36)	0.72 (0-1.36)
No Tolerance	0.13 (0-1.86)	0.44 (0-1.02)	0.81 (0.51-1.96)	0.66 (0-0.95)	1.5 (0-5.5)	0.12 (0-1.54)	0 (0-0.72)

the end of OIT, although these decreases were not reproduced in our series [12].

There are several explanations for our findings. The technique used may not be sufficiently sensitive, although it is the same as that used by other authors [17]. In addition, the changes motivated by desensitization to a single allergen are not sufficiently significant to produce significant changes in peripheral blood, although a decrease in antigen-specific production of IL-5 and IL-13 was observed in studies of peripheral blood mononuclear cells stimulated with antigen after OIT with peanut [10,11], milk [18], or egg [19]. This absence of modification in peripheral blood cytokines may be due to the fact that the patients were atopic, 56% were sensitized to multiple foods, 75% had bronchial asthma due to sensitization to dust mites, and 44% had atopic dermatitis. The predominance of the T_H2 response was of such magnitude that desensitization to a single allergen is not sufficient to induce significant changes in the cytokine values in peripheral blood. Given that the proportion of antigen-specific T lymphocytes in peripheral blood is less than 0.05%, a change in the clones of lymphocytes that affect the antigens involved in the allergic response to milk or egg will be of little quantitative importance in the modification in the cytokine release pattern that leads to significant changes in peripheral blood.

Similarly, no statistically significant differences were observed in cytokine values in patients who experienced anaphylaxis during OIT compared with patients who did not present it. Thus, the pattern of cytokines in peripheral blood does not seem to differ between these populations, with the result that its determination at the beginning of OIT cannot predict the need for more cautious administration in specific patients. Other analytical parameters, such as specific IgE values, wheal diameter, and basophil activation can predict which patients have a greater risk of developing anaphylaxis during OIT [4,5,8].

Initially, we assumed that the modification in this cytokine pattern would be different in patients who, after successfully finishing OIT, continued with a regular intake of the food for at least 1 year and were still able to tolerate the food after a 1-month exclusion diet. These children were eventually considered able to tolerate the food. In contrast, children who did not tolerate intake were considered to be desensitized. Although the degree of food sensitization seems to be clearly different in both populations, there are no differences in their peripheral blood cytokine pattern immediately before the repeat oral challenge or in any previous one. While we can suppose that the process underlying the immune response to the food differs from the initial situation before OIT as opposed to that observed in tolerant children, this immune change is not observed in their peripheral cytokines. A study of changes in cytokine values would probably require us to focus on cell populations with an immune function after stimulation with the antigen.

Finally, determination of the cytokines studied in peripheral blood did not reveal significant differences before or after the end of the OIT processes with milk or egg. Consequently, their values cannot identify patients at risk of developing anaphylaxis during OIT or patients with sustained desensitization to the food with which the OIT has been performed.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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