

Late onset reactions to oral food challenge are linked to low serum interleukin-10 concentrations in patients with atopic dermatitis and food allergy

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Abstract

Background Aberrant cytokine production *in vitro* has been associated with atopic disease. No study has as yet been made of the circulating cytokine profiles in atopic patients with food allergy in response to oral allergen challenge.

Objective To assess the effect of oral allergen challenge on the serum cytokine concentrations in patients with atopic dermatitis and food allergy.

Methods Serum concentrations of interleukin (IL)-10, transforming growth factor beta 1, IL-1ra, IL-6, IL-5, IL-4 and interferon (IFN)- γ were measured before and after double-blind, placebo-controlled food challenges (DBPCFC) ($n = 73$). Before DBPCFC, combined skin prick and patch testing was performed for cow milk, egg, soybean and cereals, and production of IFN γ , IL-4, IL-10 and tumour necrosis factor alpha (TNF α) was determined in supernatants of cultures of peripheral blood mononuclear cells (PBMCs) stimulated by cow milk.

Results The oral food challenge triggered immediate onset exanthematous reactions in 22 cases and late onset eczematous reactions in 29. The late-reacting cases had more positive skin patch test and negative skin prick test reactivities with allergenic food, and they had lower serum IL10 concentrations than immediate-reacting cases. In challenge-positive cases, IL-10 concentrations increased from 2.9 (0.1–5.04) pg/mL to 3.9 (1.2–8.3) pg/mL in response to DBPCFC, $P = 0.05$, median (interquartile ranges), but not in those tolerant to cow milk. PBMCs of patients with cow milk allergy but not of those tolerant to cow milk generated TNF α in response to cow milk *in vitro*.

Conclusion These results indicate that oral allergen challenge in atopic patients with food allergy triggers systemic release of IL-10. Patients with late onset reactions were found to have lower serum IL-10 concentrations than their immediate-reacting counterparts. Considering that IL-10 is an inhibitory cytokine of delayed-type hypersensitivity, low IL-10 in late-reacting patients may explain the high frequency of their positive skin patch tests combined with negative skin prick tests.

Keywords: atopic dermatitis, food allergy, interleukin-10

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Introduction

Atopic dermatitis is a chronic inflammatory skin disease exhibiting heterogeneity in its immunological and clinical

properties. Over 80% of patients with atopic dermatitis evince immunoglobulin (Ig) E-mediated hypersensitivity to air- and food-derived allergens, as characterized by their skin prick test reactivity and specific IgE production [1]. Sensitization to more than two environmental allergens, i.e. polysensitization, occurs in a subgroup of these patients

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[2]. Another subgroup manifests contact hypersensitivity to aeroallergens or food allergens, as characterized by their skin patch test reactivity [3–6]. Immunopathologically these eczematous lesions represent delayed-type hypersensitivity reactions with an increased number and activity of T cells in the dermis [6]. Thus IgE- and cell-mediated mechanisms are contemplated in the pathophysiology of skin lesions in atopic dermatitis [3]. The immunological factors underlying this heterogeneity of atopic dermatitis remain elusive.

In patients with atopic dermatitis and food allergy, oral food challenge triggers immediate and/or late onset reactions [4,7,8]. Late onset reactions appear to be more frequent in atopic patients with positive patch test reactivity to the food responsible for the reaction at oral food challenge [4]. Late-reacting patients do not mount *in vitro* interferon (IFN)- γ response to the allergenic food in question but their immediate-reacting counterparts do [7]. This would reflect the clinical and immunological heterogeneity of atopic patients with food allergy and prompt further evaluation of cytokine profiles of patients with atopic dermatitis and food allergy following oral allergen challenge.

The present study was thus planned to evaluate whether the heterogeneity of atopic dermatitis in early childhood is related to a profile of circulating cytokines with distinct anti-inflammatory/pro-inflammatory characteristics. Atopic dermatitis and cow milk allergy have been associated with excessive IL-10 production by T cells [9–11]. IL-10, an anti-inflammatory cytokine, upregulates the IL-1 α production by antigen-presenting cells (APC), whereas it down-regulates the tumour necrosis factor alpha (TNF α), IL-6, IL-2, IFN γ production by T cells and APC [12]. In this context the concentrations of IL-10, IL-1 α , TGF β 1, IL-4, IL-5 and IL-6 in sera of patients with atopic dermatitis were determined before and after double-blind, placebo-controlled food challenge (DBPCFC) concomitantly with the *in vitro* productions of IL-10, TNF α , IFN γ and IL-4.

Methods

Patients and study design

The study involved 59 patients (21 girls and 38 boys), aged 5–46 (mean 15) months. They had been referred to a teaching dermatological or paediatric clinic for the evaluation of atopic dermatitis. All patients fulfilled the Hanifin criteria for atopic dermatitis in children [13]. Symptoms suggestive of atopic dermatitis had been manifested at a mean (range) age of 3.1 (0.1–18.0) months. The mean (range) duration of exclusive and total breastfeeding was 3.5 (0.1–11.0) and 6.5 (0.7–29.0) months, respectively. The family history of atopy was positive in 49/59 (83%) of patients. The median (interquartile, IQR) concentration of

total IgE in the sera of 59 patients was 26 (9–97) kU/L. The serum IgE antibodies specific to food, i.e. RAST $\geq$ 0.4 kU/L for cow milk and wheat, were detected in 25/59 patients. Combined skin testing with the allergenic food indicated that 6/59 were positive to skin prick test with negative skin patch test, and 25/58 showed positive reaction to skin patch test with negative skin prick test. Skin reactivity with both skin testing methods was observed in 12 patients, and 15 patients were found to have no skin test reactivity. On the basis of RAST and/or skin prick testing, 33/59 patients were identified as being sensitized to more than two allergens, i.e. polysensitized.

All patients were subjected to diagnostic DBPCFC to characterize onset time and type of reactions triggered by the relevant food, and in 14 patients DBPCFCs were repeated after 1 year of elimination diet (RAST re-determined in 4/14). The consequence of oral allergen exposure was the objective of the study. The study groups were therefore nominated on the basis of outcome of DBPCFC and involved all challenges at different time-points.

Informed consent was obtained from the parents. The study was approved by the Tampere University Hospital's Committee on Ethical Practice.

Determination of serum IgE

Solid-phase radioimmunoassay (Phadebas IgE PRIST, Pharmacia, Uppsala, Sweden) was used to estimate the concentration of serum total IgE. The radioallergosorbent test (RAST, Pharmacia, Uppsala, Sweden) was used to detect allergen-specific IgE in serum.

Skin testing

Prick testing was done on the volar aspect of the forearm, using commercially available cow milk, egg, fish, soybean and pea allergens from ALK (Allergologisk Laboratorium A/S, Horsholm, Denmark) and cereal flours (wheat, barley, rye, oat, buckwheat and soy) diluted in physiological saline. Corn was tested with fresh kernel of corn, millet and rice flakes dampened with saline. A 1-mm single-peak lancet with a shoulder to prevent deeper penetration (ALK) was used. Histamine dihydrochloride 10 mg/mL (ALK) was used as positive control and pricking with vehicle (physiological saline) was the negative control. Reactions were evaluated after 15 min and classified as negative when there was no reaction or a reaction greater than the control but smaller than half the size of the histamine or positive reaction. The classification was positive when the reaction size was at least half or equal to or twice that to histamine. The minimum size for a positive reaction was 3 mm.

Patch testing was applied as previously described [4]. Briefly, 300 mg of milk powder or 200 mg of flour of a

cereal (wheat, barley, rye, oat or soy) was mixed in 0.2 mL of isotonic saline solution. Microcrystalline cellulose was used as negative control. Approximately 20 mg of each mixture on blotting paper was set in aluminium test cups (Finn Chamber, Epitest, Hyrylä, Finland) and then attached to the patients' back with Scanpore tape (Norgeplaster A/S, Vennesla, Norway). The occlusion time was 48 h. The results were evaluated by the first 15 min after removal of the cups. If redness suggestive of irritation was observed in the test area, the area was re-examined after 30 min. A second evaluation was made after 72 h from attachment of the test cups. Observations were classified as negative when there was no visible or palpable skin reaction or there was irritation (redness strictly limited to the contact area of the cup with no infiltration and a tendency to fade away by the 72-h evaluation). Patch test results were classified as positive when redness extended beyond the area under the cup with slight infiltration (coded '+', one plus) and a tendency to attenuate by the 72-h evaluation, and when redness was apparent with oedema and eczema (coded '++', two plus).

Sensitization to multiple allergens

IgE sensitization to allergens common in this age group (egg, cow milk, grains) was defined by skin prick testing and/or serum specific IgE. Positivity in skin prick test and/or specific IgE with more than two allergens was defined as polysensitization [2].

DBPCFC

Altogether 73 DBPCFCs were performed in the study for 59 patients. Patients were on an elimination diet for 2–4 weeks prior to the oral food challenge. During the elimination diet, all received an extensively hydrolysed whey formula (Pep-tidi Tutteli, Valio Ltd, Helsinki, Finland) or a synthetic amino acid-derived formula (Neocate, Scientific Hospital Supplies International Ltd, Liverpool, United Kingdom) and five patients were partially breast-fed and mothers were advised to keep the elimination diet for the responsible foods.

Neocate was used as the placebo formula. The test formula consisted of Neocate with cow milk powder (10 mg/100 mL). In seven cases it contained wheat flour (10 mg/100 mL). The challenge protocol was carried out as previously described [4]. The challenge period was 1 week per challenge formula. Rising doses (1, 5, 10, 20, 50 and 100 mL) given at 15–30-min intervals were recorded until the adverse reaction appeared, whereafter the DBPCFC was discontinued.

Type of reaction was characterized as urticaria, exanthema, angioedema, eczema, vomiting, diarrhoea, wheezing. Onset time of reaction was defined as the time

elapsing from the consumption of the last dose eliciting the reaction. The respective cumulative values were computed from the start of challenge. Accordingly, immediate onset reactions such as pruritus, exanthema, urticaria or vomiting occurred within 1 hour after ingestion of a small dose of food and late onset reactions such as eczema, vomiting and diarrhoea occurred more than 24 h after continued ingestion of food. Cases with either immediate or late onset reactions were included in the study. Late-reacting patients manifesting immediate onset reactions were thus excluded. Patients with no reaction in response to DBPCFC were re-evaluated after one month of open food consumption, and the diagnosis of food allergy was ruled out in the absence of adverse reactions.

Samples

Three peripheral venous blood samples were collected: the first was obtained immediately before DBPCFCs on day 0, the second after the first week of challenge with test/placebo formula on day 7 and the third after the second week of challenge with placebo/test formula on day 14. Serum samples were collected and stored at -20°C .

Isolation of PBMCs and cell cultures

During 39 DBPCFCs, PBMCs containing mainly lymphocytes ($>90\%$ lymphoid cells) were isolated from heparinized blood using Ficoll-Hypaque gradient centrifugation at 400 g for 30 min at 20°C . Briefly, a total of 6.25×10^5 isolated cells in one ml of culture medium with RPMI containing antibiotics, glutamin and 10% human AB serum were cultured in a humidified 5% CO_2 atmosphere at 37°C for 48 h. The cultures contained Concanavalin A (ConA, Pharmacia, Uppsala, Sweden) at a final concentration of $50 \mu\text{g/mL}$ or a solution of dried cow milk powder (Valio Ltd, Helsinki, Finland) at a final concentration of 1 mg/mL. The supernatants were collected and stored at -70°C .

Determination of IL10, IL1ra, TNF α , IL6, TGF β 1, IL4, IFN γ , IL5

Commercial enzyme immunoassays were used: IL-10, TGF β 1 and IL-1ra were purchased from Genzyme Corporation, Cambridge, MA, USA; IL-4, IL-6, TNF α and IFN γ from CLB, Amsterdam, the Netherlands and IL-5 from R & D Systems, Inc., Minneapolis, MN, USA.

Statistics

Data are presented as means with ranges and 95% confidence intervals (CI) or medians with IQR ranges. Analysis

	Challenge-positive <i>n</i> = 51	Challenge-negative <i>n</i> = 22	Statistics (<i>P</i>)
Age at DBPCFC* (months)	14 (5–46)	16 (7–41)	0.2
Total IgE (kU/L)†	52 (15–125)	13 (7–19)	0.004
Positive IgE specific to food (≥ 0.4 kU/L)	21/46	4/15	0.24
prick + patch –‡	5/46	1/12	0.58
prick – patch +‡	21/46	4/12	0.07
Polysensitization	30/50	3/12	0.02

*Means with ranges in parantheses, ANOVA in statistical analysis. †Median with IQR in parantheses, Mann–Whitney *U*-test in statistical analysis. ‡The outcome of combined skin testing with the allergenic food is shown as prick + patch – for positive skin prick test combined with negative atopy patch test, and as patch + prick – for positive atopy patch test combined with negative skin prick test. Patients with both positive prick and patch tests and both negative prick and patch tests were excluded.

of variance (ANOVA), chi-square (χ^2) and Mann–Whitney *U*-tests were used for statistical comparisons between DBPCFCs with positive and negative outcomes. Wilcoxon-signed rank test was used for pairwise comparison of cytokine concentrations in serum samples before and after DBPCFC, and for the comparison of cytokine production in cow milk-stimulated and unstimulated cultures. Spearman's rank correlation was applied to determine the significance of associations between the concentrations of IL-10 and total IgE in the sera of patients.

Results

The outcome of DBPCFCs

Altogether 51/73 DBPCFCs were positive in 47/59 patients. Of these 51 positive challenges, 22/51 (43%) DBPCFCs were immediate onset reactions after a cumulative time of 0.6 (0.05–2.0) h to a cumulative dose of 7.8 (0.1–61) ml of the test formula, mean (range). Late onset reactions were characterized in 29/51 (57%) of 73 DBPCFCs, occurring after a cumulative time of 54 (2–192) h to a cumulative dose of 312 (40–1000) ml of the test formula, mean (range). For 14 re-challenges in 14 patients who had been on 1 year of elimination diet, immediate and late onset reactions were 2/14 and 2/14, respectively, whereas 10/14 oral allergen challenges had negative outcome.

As shown in Table 1, the challenge-positive group had more polysensitized patients than challenge-negative group. In parallel, patients in challenge-positive group had significantly greater total IgE concentrations and were frequently

Table 1. Clinical characteristics in two study groups with positive or negative outcome to double-blind, placebo-controlled food challenges (*n* = 73)

patch test positive and skin prick test negative when compared with patients with no reactions.

The cytokine production by PBMC before DBPCFC

The capacity of PBMC to produce IFN γ or IL-4 as an estimation of the functional capacity of T cells did not differ between the study groups viz. challenge-positive and -negative. In supernatants of ConA-stimulated cultures from patients with food allergy for *n* = 27 DBPCFCs, the median (IQR) IL-10, IL-4, IFN γ and TNF α concentrations were 8.2 (5.4–16.6) pg/mL, 2.4 (1.5–4.7) pg/mL, 7.6 (1.0–17.7) pg/mL and 144.8 (46.1–270.4) pg/mL, respectively, and the corresponding measurements in supernatants of cultures from patients without food allergy for *n* = 12 DBPCFCs were 15.3 (7.2–20.8) pg/mL, 2.4 (2.0–4.5) pg/mL, 6.5 (0.6–14.9) pg/mL, 85.2 (42.1–130.4) pg/mL.

As shown in Fig. 1(a), cow milk-stimulated IL-10 production by PBMCs of patients with and without cow milk allergy. In patients with cow milk allergy, the median (IQR) IL-10 concentrations in supernatants of unstimulated and cow milk-stimulated cultures for *n* = 15 DBPCFCs were 3.4 (0.3–8.0) pg/mL vs 8.5 (3.7–17.6) pg/mL, respectively, *P* = 0.001. In patients clinically tolerant to cow milk, the corresponding measurements for *n* = 9 DBPCFCs were 3.1 (0–23.2) vs 6.1 (4.8–10.0), *P* = 0.02. In patients with cow milk allergy, the median (IQR) TNF α concentrations in supernatants of unstimulated vs cow milk-stimulated cultures for *n* = 15 DBPCFCs were 170.1 (70.5–278.9) pg/mL vs 279.3 (116.4–329.2) pg/mL, *P* = 0.02 (Fig. 1b). In patients without cow milk allergy cow milk did not stimulate TNF α production *in vitro*,

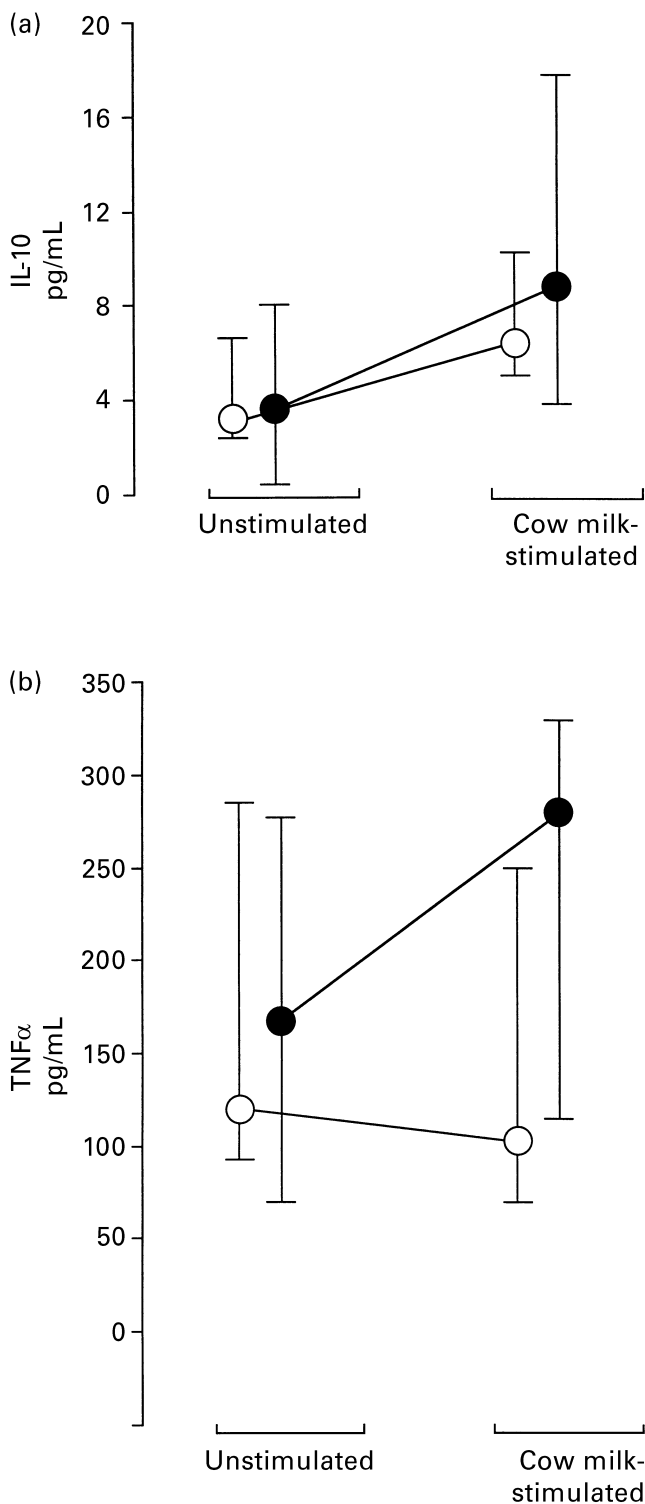


Fig. 1. The production of IL-10 (a) and TNFα (b) by PBMCs in positive challenges (●, $n=15$) and in negative challenges (○, $n=9$). Data denote the medians of measurements in unstimulated and cow milk-stimulated cell cultures. Vertical intersecting lines represent the interquartile ranges.

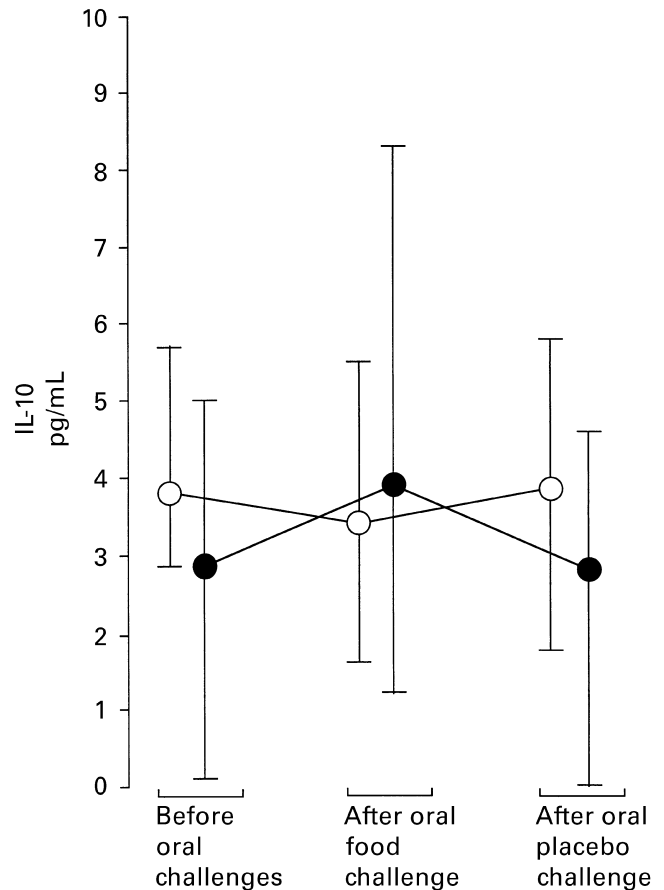


Fig. 2. Serum IL-10 concentrations in patients with atopic dermatitis and food allergy. Data denote the medians of measurements before DBPCFC and after the oral food or placebo challenge in positive challenges (●, $n=50$, one missing owing to insufficient amount of sample) and in negative challenges (○, $n=22$). Vertical intersecting lines represent the IQR ranges.

viz. the corresponding measurements in supernatants of unstimulated and cow milk-stimulated cultures for $n=9$ DBPCFCs were 121.4 (95.2–287.2) pg/mL and 102.4 (73.9–250.6), respectively.

Serum concentrations of total IgE and IL10 correlated directly in patients with food allergy ($\rho=0.376$, $P=0.004$). The correlation was consistent in patients with late onset reactions ($\rho=0.367$, $P=0.06$), with polysensitization ($\rho=0.638$, $P=0.001$) or with positive patch test combined with negative skin prick test reactivity to the allergenic food ($\rho=0.454$, $P=0.007$). The median (IQR) of serum IL-10 concentrations before the oral food challenge in DBPCFCs with late onset reactions were 1.9 (0.0–4.0) for $n=29$ DBPCFCs vs in those challenges with immediate onset reactions 4.4 (2.6–5.2) for $n=21$ DBPCFCs, $P=0.007$.

		Challenge-positive <i>n</i> = 26	Challenge-negative <i>n</i> = 10
IL-1ra (pg/mL)	<i>before</i>	481 (402.3–818.3)	321.0 (291.8–501.0)
	<i>after</i>	423 (322–596.3)	434.0 (412.3–608.8)
TGFβ1 (ng/mL)	<i>before</i>	32.3 (28.2–37.1)	32.9 (27.6–33.9)
	<i>after</i>	30.4 (24.1–43.1)	31.5 (28.8–36.6)
IL-6 (pg/mL)	<i>before</i>	0.8 (0.5–1.8)	0.6 (0.5–0.8)
	<i>after</i>	0.6 (0.5–0.7)	0.6 (0.5–0.7)
IL-4 (pg/mL)	<i>before</i>	0.9 (0–4.4)	3.8 (0.6–5.7)
	<i>after</i>	2.0 (0–4.7)	3.3 (0–5.9)

Table 2. Serum concentrations of cytokines before and after the oral food challenge in 36 DBPCFCs with positive and negative outcomes

Data presented are median of measurements with IQR in parantheses.

The effect of DBPCFC on serum cytokine profile

Figure 2 shows that the serum IL-10 concentration in patients with food allergy increased following the oral food challenge, $P=0.05$. Oral food challenge did not significantly change the serum concentration of IL-1ra, TGFβ1, IL-6, IL-4 (Table 2). Serum IL-5 and IFNγ concentrations were undetectable in the majority of the patients.

After DBPCFCs the median (IQR) of IL-10 concentrations in unstimulated vs cow milk-stimulated cultures of PBMCs in patients with cow milk allergy for $n=15$ DBPCFCs were 5.1 (1.8–7.0) vs 10.3 (6.6–18.6) pg/mL, respectively ($P=0.002$) and in patients without cow milk allergy the corresponding measurements for $n=9$ DBPCFCs were 2.9 (1.0–6.9) vs 13.0 (5.0–16.1) pg/mL, respectively ($P=0.03$). The corresponding findings for TNFα were, in the same patients with cow milk allergy for $n=15$ DBPCFCs were 196.1 (91.1–279.3) vs 219.6 (138.9–332.1), respectively ($P=0.17$), and in the patients without cow milk allergy for $n=9$ DBPCFCs 111.3 (47.1–182.5) vs 68.9 (55.1–181.2) pg/mL, respectively ($P=0.59$).

Discussion

The present study employed DBPCFC to characterize distinct clinical and immunological reactions to oral food challenge in patients with atopic dermatitis and food allergy. An attempt here was taken to rule out any interference between different food-induced reactions. To this end only immediate or late onset reactions to DBPCFC were studied. Oral food challenge increased the serum IL-10 concentration in patients with food allergy but not in patients without. This observation was consistent for 14/59 patients who had been rechallenged following 1 year of elimination diet. However, the same antigenic food enhanced the IL-10 production by PBMC in a manner comparable between atopic patients with and without food allergy, a finding in

keeping with earlier reports' [14]. Accordingly, the data presented here indicate that oral allergen challenge can trigger the systemic release of IL-10.

The upregulatory effect of oral allergen challenge on serum IL-10 may reflect a state of allergic inflammation in patients with atopic dermatitis and food allergy. Such an effect may not be directly related to the activation of macrophages/monocytes, since the serum concentrations of IL-1ra remained comparable before and after DBPCFC in patients with and without food allergy alike. Also IL-10 production by milk-stimulated PBMCs remained comparable between patients with and without food allergy. Nevertheless, it is evident that mast cells play a role in the induction of hyperresponsiveness to orally-administered dietary antigens [15]. Histamine, a mast cell-related mediator of allergic inflammation, upregulates IL-10 production by B cells of atopic patients [16] as well as by a mechanism mediated by histamine receptors on peripheral blood cells [17]. In this context it is more likely that oral allergen challenge triggers an acute state of allergic inflammation, the mediators of which may lead to a rise in serum IL-10 concentrations in patients with atopic dermatitis and food allergy.

Patients with food allergy had the capacity to generate a TNFα-mediated pro-inflammatory response to the allergenic food, as shown here and elsewhere [18]. It was also interesting to observe that after oral cow milk challenge PBMCs of patients with cow milk allergy continued to generate IL-10, but TNFα generation to cow milk *in vitro* was no longer sufficiently consistent to be statistically significant. These findings further provided indirect yet plausible evidence that elevated IL-10 concentrations in response to oral food challenge reflect a reactive state against the release of pro-inflammatory cytokines.

It has been established that up to 50% of children with atopic dermatitis and cow milk allergy manifest late onset reactions to DBPCFC [4]. So far this subgroup of atopic patients has been characterized by positive skin patch

response [4] and by defective IFN γ production *in vitro* [7,19]. Late-reacting patients were characterized by positive skin patch test and negative skin prick test results and by lower serum IL-10 concentrations than their immediate-reacting counterparts. Surprisingly, total IgE and IL-10 in sera tended to correlate directly in patients with late onset reactions and in those with positive patch tests. Although our data appear in agreement with data elsewhere [4,20] that the sensitivity of cow milk patch test exceeds that of cow milk prick test in children with cow milk allergy, no conclusion can be drawn for its use as a diagnostic test. The scope and patient population of this study do not aim at such either.

Recent work has shown that negative patch tests exhibit a cellular response *in situ* despite the absence of erythema [21]. In animal models of contact hypersensitivity, it is evident that IL-10/IL-4-producing CD4⁺ T cells take part in the downregulation of skin sensitivity to haptens [22]. IL-10, but not IL-4, is a natural suppressant of cutaneous inflammation and of hypersensitivity to contact allergens [23–25]. Atopic dermatitis involves increased monocyte activity and overexpression of IL-10 at the chronic lesional skin site [26]. Considering the suppressant role of IL-10 in delayed-type hypersensitivity, it is conceivable that IL-10 is effective in silencing skin patch test reactivity to allergen in a group of atopic dermatitis patients. Conversely, less IL-10 may enhance the skin patch test reactivity to the relevant allergen. Indeed our findings in late-reacting patients, who had low IL-10 and frequent patch test reactivity, suggest a link between IL-10 and this heterogeneity in atopic patients with food allergy.

In conclusion, these findings firstly demonstrate the upregulatory effect of oral allergen exposure on systemic IL-10 levels, and secondly, associate the role of IL-10 with the late onset reactions in patients with atopic dermatitis and food allergy.

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References

- Hoffman DR, Yamamoto FY, Geller B, Haddad Z. Specific IgE antibodies in atopic eczema. *J Allergy Clin Immunol* 1975; 55:256–67.
- Bousquet J, Knani J, Hejjoui A, Ferrando R, Cour P, Dhivert H, Michel F-B. Heterogeneity of atopy. I. Clinical and immunologic characteristics of patients allergic to cypress pollen. *Allergy* 1993; 48:183–8.
- Hanifin JM, Klas PA. The spectrum of cutaneous patch-test reactions in patients with atopic dermatitis. *Clin Rev Allergy Immunol* 1996; 14:225–40.
- Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. *J Allergy Clin Immunol* 1996; 97:9–15.
- Oranje AP, Aarsen RS, Mulder PG, van-Toorenenbergen AW, Liefwaard G, Dieges PH. Food immediate contact hypersensitivity (FICH) and elimination diet in young children with atopic dermatitis. Preliminary results in 107 children. *Acta Derm Venereol Stockh* 1992; 176:S41–S4.
- Reitamo S, Visa K, Kähönen K, Käyhkö K, Stubb S, Salo OP. Eczematous reactions in atopic patients caused by epicutaneous testing with inhalant allergens. *Br J Dermatol* 1986; 114:303–9.
- Sütas Y, Hurme M, Isolauri E. Oral allergen challenge abolishes antigen-specific interferon- γ production in the peripheral blood of children with atopic dermatitis and cow milk allergy. *Clin Exp Allergy* 1997; 27:277–83.
- Hill DJ, Firer MA, Shelton MJ, Hosking CS. Manifestations of milk allergy in infancy: clinical and immunologic findings. *J Pediatr* 1986; 109:270–6.
- Lester MR, Hofer MF, Gately M, Trumble A, Leung DYM. Downregulating effects of IL-4 and IL-10 on the IFN γ response in atopic dermatitis. *J Immunol* 1995; 154:6174–81.
- Esnault S, Benbernou N, Lavaud F, Shin HC, Potron G, Guenounou M. Differential spontaneous expression of mRNA for IL4, IL10, IL13, IL2 and interferon- γ (IFN γ) in peripheral blood mononuclear cells (PBMC) from atopic patients. *Clin Exp Immunol* 1996; 103:101–8.
- Hauer AC, Breese EJ, Walker-Smith JA, MacDonald TT. The frequency of cells secreting interferon- γ and interleukin-4, 5, and -10 in the blood and duodenal mucosa of children with cow's milk hypersensitivity. *Pediatr Res* 1997; 42:629–38.
- Borish L. Updates on cells and cytokines. IL10: Evolving concepts. *J Allergy Clin Immunol* 1998; 101:203–7.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; 92:S44–S7.
- Kroger S, Neuber K, Gruseck E, Ring J, Abeck D. Pityrosporum ovale extracts increase IL4, IL10 and IgE synthesis in patients with atopic eczema. *Acta Derm Venereol* 1995; 75:357–360.
- van Halteren AG, van der Cammen MJ, Biewenga J, Savelkoul HF, Kraal G. IgE and mast response on intestinal allergen exposure: a murine model to study the onset of food allergy. *J Allergy Clin Immunol* 1997; 99:94–9.
- Kimata H, Fujimoto M, Ishioka C, Yoshida A. Histamine selectively enhances human immunoglobulin (IgE) and IgG4 production induced by anti-CD58 monoclonal antibody. *J Exp Med* 1996; 184:357–64.
- Elenkov IJ, Webster E, Papanicolaou DA, Fleisher TA, Chrousos GP, Wilder RL. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. *J Immunol* 1998; 161:2586–93.
- Heyman M, Darmon N, Dupont C, Dugas B, Hirribaren A, Blaton MA, Desjeux JF. Mononuclear cells from infants allergic to cow's milk secrete tumor necrosis factor α , altering intestinal function. *Gastroenterology* 1994; 106:1514–23.

- 19 Hill DJ, Ball MJ, Hosking CS, Wood PR. Gamma-interferon production in cow milk allergy. *Allergy* 1993; 48:75–80.
- 20 Majamaa H, Moisiö P, Holm K, Kautiainen H, Turjanmaa K. Cow's milk allergy: diagnostic accuracy of skin prick and patch tests and specific IgE. *Allergy* 1999; 54:346–51.
- 21 Buckley C, Poulter LW, Rustin MHA. Immunohistological analysis of negative patch test sites in atopic dermatitis. *Clin Exp Allergy* 1996; 26:1057–63.
- 22 Xu H, Dilulio NA, Fairchild RL. T cell populations primed by hapten sensitisation in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon-gamma-producing (Tc1) effector CD8+ T cells and interleukin (IL) 4/IL-10-producing (Th2) negative regulatory CD4+ T cells. *J Exp Med* 1996; 183:1001–12.
- 23 Berg DJ, Leach MW, Kuhn R, Rajewsky K, Müller W, Davidson NJ, Rennick D. Interleukin-10 but not interleukin-4 is a natural suppressant of cutaneous inflammatory responses. *J Exp Med* 1995; 182:99–108.
- 24 Kondo S, McKenzie RC, Sauder DN. Interleukin-10 inhibits the elicitation phase of allergic contact hypersensitivity. *J Invest Dermatol* 1994; 103:811–4.
- 25 Ferguson TA, Dube P, Griffith TS. Regulation of contact hypersensitivity by interleukin-10. *J Exp Med* 1994; 179:1597–604.
- 26 Ohmen JD, Hanifin JM, Nickoloff BJ *et al.* Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions. *J Immunol* 1995; 154:1956–63.