

Oral cow milk challenge abolishes antigen-specific interferon- γ production in the peripheral blood of children with atopic dermatitis and cow milk allergy

Y. SÜTAS, M. HURME* and E. ISOLAURI

Department of Pediatrics, and Universities of Tampere and Turku *Department of Microbiology and Immunology, Tampere University Medical School and Tampere University Hospital, Tampere, Finland

Summary

Background A major feature of atopic dermatitis (AD) is the propensity to generate IgE to environmental antigens. Despite extensive information on IgE dysregulation in AD, the nature of immune responses to ingested allergens is poorly characterized.

Objective To determine the clinical and immunological responses to food in AD patients.

Methods To characterize the type and timing of clinical reactions to oral cow milk, 83 AD patients aged 2 to 60 months were subjected to double-blind placebo-controlled food challenge (DBPCFC). IFN- γ and IL-4 production by their peripheral blood mononuclear cells (PBMC) was determined before and after DBPCFC.

Results Of 50 patients positive to DBPCFC, 46% manifested exanthematous-type immediate-onset reactions and 54% eczematous-type late-onset reactions. In either group, the production of IL-4 and IFN- γ by Concanavalin A-stimulated PBMC was comparable before and after DBPCFC. For immediate-reacting patients, the median IFN- γ production by milk-stimulated PBMC was 11.5 (4.2–17.2) pg/mL as against 2.3 (0.2–5.7) pg/mL by unstimulated PBMC, $P = 0.0008$ before DBPCFC, and 4.6 (2.8–10.3) pg/mL vs 4.2 (1.7–9.0) pg/mL, $P = 0.40$, correspondingly after DBPCFC.

Conclusion Before DBPCFC, immediate-reacting but not late-reacting patients were found to be capable of allergen-specific IFN- γ production *in vitro*, indicating the heterogeneity in AD patients. After DBPCFC, the IFN- γ generation abolished, indicating the effect of oral allergen exposure on IFN- γ -producing responses of AD patients.

Keywords: atopic dermatitis, food allergy, interferon-gamma, tolerance

Clinical and Experimental Allergy, Vol. 27, pp. 277–283. Submitted 15 January 1996; revised 23 April 1996; accepted 16 September 1996.

Introduction

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease [1], the pathogenesis of which is under intense investigation [2]. High serum IgE concentrations to common environmental allergens are found in up to 80% of patients [3,4], indicating dysregulation of B-cell responses in AD. Allergen-specific CD4⁺ T-cells that produce IL-4 but not IFN- γ predominate in skin lesions and peripheral

blood of AD patients [5,6], disclosing functional impairment of T-cell responses. IL-4 promotes IgE production [7] and IFN- γ down-regulates both IL-4- and allergen-induced IgE production [8,9], suggesting that aberrant cytokine production profiles play a central role in the pathogenesis.

Clinical and immunological observations indicate that AD patients evince heterogeneity in the severity of cutaneous symptoms [10,11], in the type and number of sensitizing allergens [12,13], in serum total and allergen-specific IgE concentrations, and in skin test positivity to allergens [14]. Studies investigating the cytokine production by peripheral blood mononuclear cells (PBMC) have disclosed a positive

Correspondence: Erika Isolauri MD, Department of Paediatrics, University of Turku, 20520 Turku, Finland.

correlation between IL-4 production and the severity of AD and/or risk of sensitization to multiple allergens [11,13]. It is as yet uncertain whether the allergen-specific IFN- γ production differs in relation to the heterogeneity of AD.

The objective of this study was to investigate clinical and immunological characteristics of children with AD in relation to oral allergen challenge. To establish the type and onset-time of reactions to an oral allergen, infants with AD were subjected to double-blind placebo-controlled food challenge (DBPCFC) with cow milk, the major food allergen at this age [12,15]. To characterize the nature of immune responsiveness in patients with or without clinical reactions to DBPCFC and in patients with immediate or late-onset reactions, skin prick tests for cow milk and serum cow milk-specific IgE concentrations were evaluated before DBPCFC, and the production of IFN- γ and IL-4 by PBMC was assessed before and after DBPCFC.

Methods

Patients

Altogether 83 children aged 2 to 60 (mean 16) months were studied. The patients had been referred to teaching clinics of paediatrics and dermatology for evaluation of atopic dermatitis. All patients fulfilled the criteria of Hanifin for atopic dermatitis in children [16]. The mean duration of exclusive breast feeding was 3.5 (95% confidence interval, CI, 3–4) months. The mean age at onset of eczema was 4.5 (95% CI, 4–5) months. There was a family history of atopic disorders in 63 (76%) patients. In 12 (14%) patients, there were recurrent respiratory symptoms such as wheezing, and in seven (8%) patients, gastrointestinal symptoms such as loose stools, vomiting and diarrhoea. None of the patients was receiving systemic corticosteroid therapy during the study and antihistamine medication was discontinued for 3 days to 6 weeks before skin testing and oral cow milk challenge, depending on the drug's duration of action.

Patients were enrolled on a cow milk protein-free diet for 2–4 weeks before DBPCFC and received an extensively hydrolyzed whey hydrolysate formula and/or calcium supplement. Patients with a relevant history of allergy were also skin-tested for egg white, cereals (wheat, oat, barley, rye), citrus fruits, fish, soy and/or animal danders. Patients were designated multiple allergic when SPT and specific IgE were positive for two or more allergens (21 patients to food allergens, eight to food allergens and animal danders). The severity of dermatitis was mild in 76 and moderate in seven patients before DBPCFC.

Informed consent was obtained from the parents and the study was approved by the Committee on Ethical Practice in Tampere University Hospital.

DBPCFC

DBPCFC was performed as previously described [17,18]. The placebo formula was a synthetic amino acid-derived formula, Neocate (SHS Int., Liverpool, UK) and the test formula consisted of Neocate and 100 g skimmed cow milk powder/litre. Briefly, on the first day of challenge, increasing amounts (1, 5, 10, 50 and 100 ml) of the allocated formula were given at 30-min intervals until intake appropriate to age was reached. The challenge period was 1 week for both placebo and test formula. In the case of a clinical reaction, the challenge was discontinued and the patient examined. All patients were clinically assessed weekly after each challenge, corresponding to days 8 and 15 of the challenge. On day 15 of the challenge, the clinical outcome was determined as positive or negative, and thereafter the code of the challenge formula was opened.

Severity of skin symptoms

The total clinical severity score was defined at the commencement of DBPCFC as the sum of the individual scores, graded as 0 (none), 1 (mild), 2 (moderate) and 3 (severe) for each of six parameters (pruritus, erythema, oedema/papulation, lichenification, scaling, and erosion/weeping) [19]. The extent of skin involvement was estimated using the rule of nines [19]. AD was severe when the total severity score was more than 7 and the extent of lesions more than 20% of the body surface area [19].

Determination of IgE

The concentrations of serum total IgE and cow milk-specific IgE were determined before DBPCFC by solid-phase radioimmunoassay (Phadebas IgE Prist, Pharmacia, Uppsala, Sweden) and by radioallergosorbent test (RAST, Pharmacia, Uppsala, Sweden) according to the manufacturers' instructions.

Skin testing

Skin prick testing (SPT) for cow milk was done to elicit IgE-mediated hypersensitivity. Prick testing was done on the volar aspect of the forearm, using a commercially available cow milk allergen ALK (Allergologisk Laboratorium, Horsholm, Denmark) and histamine dihydrochloride 10 g/ml (ALK) as positive control. To prevent deeper penetration, a 1 mm single-peak lancet with shoulder was used (ALK). Reactions were read after 15 min, and a response half of the histamine reaction or more was recorded as positive when the mean diameter of the wheal was at least 3 mm and the negative control at the same time, 0 mm.

Table 1. Clinical characteristics of AD patients who had positive or negative reactions to double-blind placebo-controlled cow milk challenge

	Positive (n = 50)	Negative (n = 33)	Statistics
Total IgE* (kU/L)	27 (14–51)	28 (18–44)	$F = 0.02, P = 0.89$
Positive milk-specific IgE† (>0.4 kU/L)	40%	14%	$\chi^2 = 5.71, P = 0.02$
Positive skin prick test† for cow milk	34%	15%	$\chi^2 = 2.45, P = 0.12$
Multiple allergy†	58%	31%	$\chi^2 = 5.56, P = 0.02$

* Mean (95% confidence intervals)

† Percentage of patients in each group

Blood samples and cell cultures

Blood (5 ml) from an antecubital vein was drawn in the morning and heparinized. Altogether three samples were obtained from each patient immediately before commencement of the challenge (day 1), and on day 8 and day 15 of the challenge. Cell cultures were prepared from each of these three samples. Briefly, peripheral blood mononuclear cells (PBMC) containing mainly lymphocytes (>90% lymphoid cells) were isolated by Ficoll-Hypaque gradient centrifugation at 400 g for 30 min at 20°C. A total of 6.25×10^5 isolated cells in one ml of RPMI 1640 containing antibiotics, glutamin and 10% human AB serum (culture medium) were cultured in a humidified 5% CO₂ atmosphere at 37°C for 48 h. The cultures contained one of the following: Concanavalin A (Con A, Pharmacia, Uppsala, Sweden) at a final concentration of 50 µg/ml, a solution of dried cow milk powder (Valio Finnish Co-operative Dairies' Association, Helsinki, Finland) at a final concentration of 1 mg/ml. A control cell population was also prepared containing the culture medium alone. The supernatants were collected and stored at –70°C for cytokine assays.

Cytokine assays

IFN-γ and IL-4 in frozen culture supernatants for day 1 and the challenge day corresponding to a week after the oral cow milk challenge were determined by commercially available ELISA kits according to manufacturer's instructions (IFN-γ: E-IFNG, Endogen Inc., Cambridge, MA, USA and IL-4: CLB, Compact Human Interleukin-4 ELISA Kit, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). The results from different runs were equalized employing comparison of standard curves and were expressed as pg/ml. The sensitivities of the assays were 5 pg of IFN-γ per millilitre and 2.3 pg of IL-4 per millilitre.

Statistical analysis

Data presented are the means of measurements with 95% confidence intervals, CI, or the median of measurements within the interquartile range, IQR, from the 25th to the 75th percentile. Because of the skewed distribution of serum total IgE concentration, logarithmic transformations were used. One-way analysis of variance, ANOVA, chi-square test and Wilcoxon signed-rank test were used for statistical comparisons. The level of significance was $P < 0.05$. Spearman rank correlation test was applied in determining correlation between the concentration of milk-specific IgE in sera of patients and milk-specific IFN-γ production by their PBMC before DBPCFC.

Results

Clinical data

Altogether 50 (60%) of the AD patients manifested positive cutaneous reactions to DBPCFC. Of challenge-positive AD patients, 46% had pruritus, urticaria and/or exanthema appearing within 15 min of the challenge after a mean cumulative intake of 27 (95% CI, 8–45) ml test formula; these were designated immediate-onset reactions. Late-onset reactions of eczematous type occurred in 54% of the challenge-positive patients 38 (95% CI, 25–50) hours after a mean cumulative intake of 260 (190–330) ml test formula. Gastrointestinal symptoms, including diarrhoea, loose stools and vomiting, were recorded in 24 (48%) patients; these appeared within 31 (95% CI, 18–45) hours after a cumulative intake of 212 (95% CI, 130–290) ml test formula. During the placebo challenges, patients showed no exacerbation of skin condition. Three patients were treated with a single dose of antihistamine medication due to their acute reactions at the commencement of the challenge. As shown in Table 1, a significantly greater

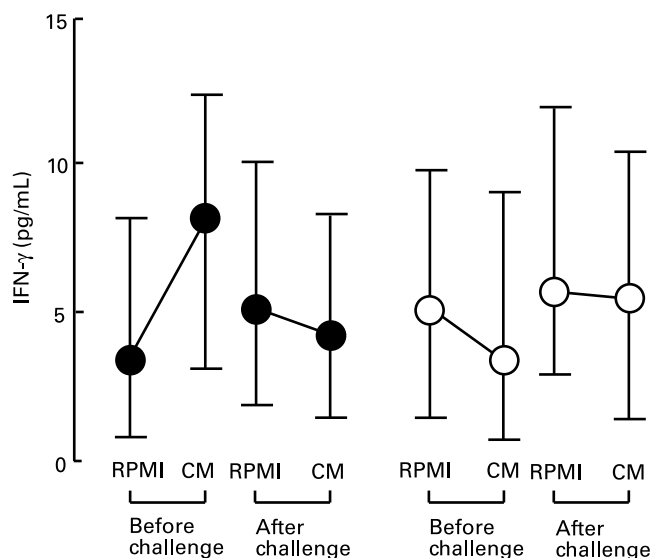


Fig. 1. Comparison of IFN- γ production between unstimulated (RPMI) and milk-stimulated (CM) peripheral blood mononuclear cells before and after the double-blind placebo-controlled cow milk challenge. Filled circles (●) represent the median of measurements of 42 patients who had positive reactions to the challenge, and empty circles (○) represent 26 patients with negative reactions to the challenge. Intersecting lines represent the interquartile range for the median value.

percentage of patients in the challenge-positive group had elevated serum milk-specific IgE concentrations and multiple allergy in comparison to the challenge-negative group.

IFN- γ production by PBMC in AD patients with positive or negative reactions to DBPCFC

In challenge-positive patients, IFN- γ production by PBMC in milk-stimulated cultures was significantly greater than in unstimulated cultures before DBPCFC, $P = 0.004$ (Fig. 1, Wilcoxon signed-rank test). In contrast, after DBPCFC, their IFN- γ production in milk-stimulated cultures was comparable to that in unstimulated cultures, $P = 0.27$. In challenge-negative patients, the IFN- γ production in milk-stimulated cultures remained comparable to that in unstimulated cultures before and after DBPCFC, $P = 0.12$ and $P = 0.55$, respectively. A significant inverse correlation was found between milk-specific IgE concentration in sera of patients and IFN- γ production in milk-stimulated cultures before DBPCFC, $P < 0.0001$, $\rho = 0.95$ (Spearman rank correlation).

In ConA-stimulated cultures, there was no significant difference in IFN- γ production before and after DBPCFC in either group. In challenge-positive patients, the median of IFN- γ production before the challenge was 15.9 (IQR, 7.0–25.4) pg/ml and after DBPCFC, 10.8 (IQR, 7.4–31.3) pg/ml, $P = 0.58$, and in challenge-negative patients, 16.3

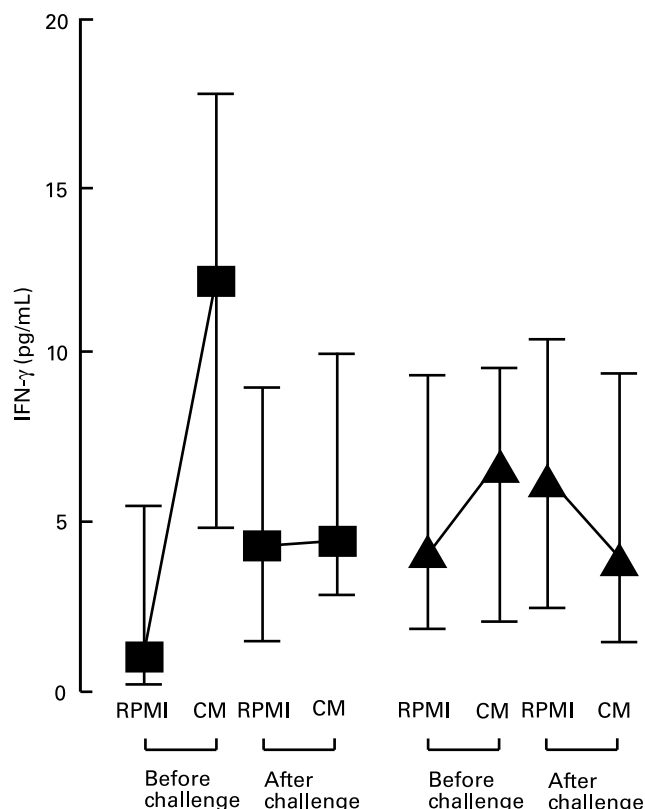


Fig. 2. Comparison of IFN- γ production between unstimulated (RPMI) and milk-stimulated (CM) peripheral blood mononuclear cells before and after the double-blind placebo-controlled cow milk challenge. Filled squares (■) represent the median of measurements of 17 patients with immediate-onset reactions to the challenge, and filled triangles (▲) represent 25 patients with late-onset reactions to the challenge. Intersecting lines represent the interquartile range for the median value.

(IQR, 6.4–28.8) pg/ml and 14.7 (IQR, 8.4–33.7) pg/ml, respectively, $P = 0.20$.

IFN- γ production by PBMC in AD patients who had immediate- or late-onset reactions to DBPCFC

In AD patients who had immediate-onset reactions, the IFN- γ production in milk-stimulated cultures was significantly greater than in unstimulated cultures before DBPCFC, $P = 0.0008$ (Fig. 2, Wilcoxon signed-rank test). After DBPCFC, their IFN- γ production in milk-stimulated cultures was comparable to that in unstimulated cultures, $P = 0.40$. In AD patients who had late-onset reactions, the IFN- γ production in milk-stimulated cultures remained comparable to that in unstimulated cultures before DBPCFC, $P = 0.65$. After DBPCFC, the IFN- γ production in milk-stimulated cultures from these patients tended to be less than in unstimulated cultures, $P = 0.07$. When milk-specific IgE concentration in sera of patients with

immediate- and late-onset reactions were separately correlated to the concentration of IFN- γ in milk-stimulated cultures before DBPCFC, a significant inverse correlation was found in patients with late-onset reactions but not in patients with immediate-onset reactions, $P = 0.0005$, $\rho = 0.70$ and $P = 0.58$, $\rho = 0.13$, respectively (Spearman rank correlation).

IL-4 production by PBMC in AD patients

In unstimulated and milk-stimulated cultures, IL-4 production by PBMC in all 82 patients was below the level of sensitivity of the assay, i.e. < 2.3 pg/ml. In ConA-stimulated cultures, IL-4 production by PBMC was detected above 2.3 pg/ml in 42/82 (51%) patients. IL-4 production by ConA-stimulated PBMC before and after DBPCFC remained comparable in all patient groups. In challenge-positive patients, the median IL-4 production before and after DBPCFC was 3.1 (IQR, 2.6–4.9) pg/ml and 4.2 (2.9–6.6) pg/ml, $P = 0.25$ (Wilcoxon signed-rank test), and in challenge-negative patients, 3.2 (IQR, 2.8–4.6) pg/ml and 3.9 (3.1–4.3) pg/ml, $P = 0.25$ (Wilcoxon signed-rank test).

Discussion

This study determined clinical and immunological characteristics of AD patients during a cow milk elimination diet and after DBPCFC. Our clinical data corroborate earlier observations on clinical reactions to oral cow milk in a subgroup of AD patients [20], and heterogeneity in the type and timing of these reactions [21]. Our immunological data indicate that allergen-specific IFN- γ production is recruitable before DBPCFC in AD patients with immediate-onset reactions but not in patients with late-onset reactions or in those tolerant to cow milk. These results demonstrate that AD patients manifest distinctive characteristics according to the type and timing of their reactions during DBPCFC, and their IFN- γ -producing immune responses.

Previous data clearly show that the capacity of PBMC to produce IFN- γ in children is less than in adults [22], and allergic patients generate lower levels of IFN- γ than age-matched healthy children [22–25]. However, the earlier studies did not assess the IFN- γ production in direct relation to oral allergen challenge. Our results indicate that allergic patients are capable of generating allergen-specific IFN- γ during allergen elimination, i.e. before DBPCFC but not after DBPCFC. In our study, the IFN- γ production capacity detected in ConA-stimulated cultures remained within ranges similar to those in cow milk allergic patients [22], and the oral allergen challenge did not influence it.

In atopic patients, studies with group I allergen from *Lolium perenne* pollen (*Lol p I*) have indicated less allergen-specific IFN- γ production by PBMC than in non-atopics on natural exposure to pollen [26]. Our results agree with these

observations, showing down-regulation of allergen-specific IFN- γ production after DBPCFC. In parallel to reports on peanut allergens [27], we could not detect any allergen-specific IL-4 production using a commercial ELISA. The explanation may be the low frequency of allergen-specific IL-4 producing cells in the peripheral blood [27]. Our prechallenge data showing allergen-specific IFN- γ production in patients with immediate-onset reactions but not in patients with late-onset reactions are at variance with other investigators showing the reversed pattern of allergen-specific IFN- γ production [23]. The variance appears to be due to the differences in patients' characteristics including age, presence of atopic dermatitis and IgE hypersensitivity to multiple allergens.

In earlier work [23], patients with late-onset reactions were of older age, indicating higher capacity to generate IFN- γ . Only half of their patients manifested eczema and few had skin prick test reactions to cow milk allergen. Thus IFN- γ production in the previous study was reflecting characteristics of patients at extreme ends of clinical IgE hypersensitivity to cow milk as with or without skin prick test reactivity to cow milk allergen. In contrast our study consisted of a younger population of patients with atopic dermatitis. More than half of patients with late-onset reactions were shown to have IgE hypersensitivity to multiple allergens by means of skin prick testing and allergen-specific IgE in serum. A significant inverse correlation was found between milk-specific IgE in sera and milk-specific IFN- γ production in prechallenge cultures of late-reacting patients but not in patients with immediate-onset reactions. Thus IgE-mediated mechanisms cannot be excluded in our patients with cow milk allergy and atopic dermatitis even though they manifested late-onset reactions during DBPCFC.

In the present study, we did not assess the lymphocyte proliferation to cow milk before and after DBPCFC. Previously, oral cow milk challenge has been shown to abrogate the allergen-specific lymphocyte proliferation in cow milk-allergic patients with predominantly gastrointestinal symptoms but not in patients with skin symptoms, mainly of immediate-onset reactions [28]. These results were in keeping with results on gluten sensitivity in patients with coeliac disease, showing cell-mediated immunity to gluten during gluten-free diet but not after gluten ingestion [29,30]. It has been suggested that after allergen ingestion, sensitized lymphocytes may localize in the small bowel, being thus present in fewer numbers in the peripheral blood [30]. In AD patients, *in-situ* IFN- γ mRNA expression at the time of severe exacerbation of skin symptoms has been found greater than at the time of 50% reduced severity of skin symptoms [31]. On the basis of these observations, the diminished allergen-specific IFN- γ production after DBPCFC in our patients may be explained by the migration

of allergen-specific T-cells out of the circulation after allergen ingestion.

Another explanation for the down-regulatory effect of oral allergen challenge on allergen-specific IFN- γ production may involve the nature of immune responses in the induction of oral tolerance, i.e. systemic hyporesponsiveness to an ingested antigen [32]. As described in rodents, tolerance to antigens is composed of a T-cell compartment with clonal anergy and active suppression as well as a B-cell compartment with IgG and IgA responses [33–35]. It has been established in animal studies that feeding high doses of antigen would result in anergy with increased T-helper-2(Th-2)-like responses, i.e. IL-4 production and antibody responses [36], whereas feeding low doses would result in active suppression of T-helper-1(Th1)-like responses, i.e. down-regulation of delayed-type hypersensitivity [36]. These studies indicated a gradient of sensitivity to tolerance induction, with Th1>Th2>B cells, depending on dose and frequency of antigen intake. Studies employing the transfer of Peyer's patches from tolerized animals to naive animals further indicate that the induction of active suppression occurs primarily in the gut, whereas anergy is recruited both systemically and locally [37].

For tolerance induction in humans, antigen feeding reduces the antigen-specific T-cell proliferation and diminishes the delayed skin test reactivity to that antigen [38], showing confinement of oral tolerance to the Th1 compartment. Indeed the evidence that IL-4-producing Th2 responsiveness in the peripheral blood is not essential for the induction of oral tolerance [39] underlines the importance of Th1-regulation in tolerance induction. AD has been shown to engage with the increased transfer of intact allergens from gut to peripheral blood [40], which may influence the dose-dependent mechanisms of tolerance induction. The frequency of patch test reactivity in AD patients with immediate-onset reactions to oral allergen challenge tends to be lower than in those with late-onset reactions [41], suggesting that different mechanisms contribute to the clinical responsiveness in allergic patients. In the light of such recent evidence, it remains to be investigated whether the diminished allergen-specific IFN- γ production in AD patients reflects their capability to regulate Th1-like responses to oral allergens.

References

- Hanifin JM. Atopic dermatitis. *J Allergy Clin Immunol* 1984; 73:211–22.
- Bos JD, Kapsenberg ML, Sillevs-Smitt JH. Pathogenesis of atopic eczema. *Lancet* 1994; 343:1338–41.
- Hoffman DR, Yamamoto FY, Geller B, Haddad Z. Specific IgE antibodies in atopic eczema. *J Allergy Clin Immunol* 1975; 55:256–67.
- Sigurs N, Hottevig G, Kjellmann B et al. Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. *J Allergy Clin Immunol* 1994; 94:757–63.
- Van der Heijden F, Wierenga E, Bos J, Kapsenberg M. High frequency of IL-4- producing CD4+ allergen specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 1991; 97:389–94.
- Wierenga EA, Snoek M, De Groot C et al. Evidence for compartmentalization of functional subsets of CD4+T lymphocytes in atopic patients. *J Immunol* 1990; 147:2942–9.
- Del Prete G, Maggi E, Parronchi P et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 1988; 140:4193–7.
- Rousset F, Robert J, Andary M et al. Shifts in interleukin-4 and interferon- γ production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis. *J Allergy Clin Immunol* 1991; 87:58–69.
- Pène J, Rousset F, Brière F et al. IgE production by normal human lymphocytes is induced by IL-4 and suppressed by IFN- γ and prostaglandin E2. *Proc Natl Acad Sci USA* 1988; 85:6880–4.
- Tang M, Kemp A, Varigos G. IL-4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunol* 1993; 92:120–4.
- Hamid Q, Boguniewicz M, Leung DYM. Differential *in situ* cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994; 94:870–6.
- Sampson HA. The role of allergens in atopic dermatitis. *Clin Rev Allergy* 1993; 11:471–90.
- Pène J, Rivier A, Lagier B, Becker WM, Michel FB, Bousquet J. Decreases in IL-4 release by PBMC are related with heterogeneity of atopy. *Immunology* 1994; 81:58–64.
- Sampson HA, Albergo R. Comparison of results of skin tests, RAST, and double-blind placebo-controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 1984; 74:26–33.
- Schrander JJP, van den Bogart JPH, Forget PP et al. Cow's milk protein intolerance in infants under 1 year of age: a prospective epidemiological study. *Eur J Pediatr* 1993; 152:640–4.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; 92(Suppl.):44–7.
- Bock SA, Sampson HA, Atkins FA. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol* 1988; 82:986–97.
- Isolauri E, Sütas Y, Mäkinen-Kiljunen S et al. Efficacy and safety of hydrolysed cow milk and amino acid-derived formulas in infants with cow milk allergy. *J Pediatr* 1995; 127:550–7.
- Rajka G. Diagnosis and grading (severity). In: Rajka G. ed. *Essential aspects of atopic dermatitis*. Berlin: Springer-Verlag, 1989:216–22.
- Sampson HA, McCaskill CC. Food hypersensitivity in atopic dermatitis: an evaluation of 113 patients. *J Pediatr* 1985; 107:669–75.
- Hill DJ, Shelton MJ, Hosking CS. Manifestations of milk

- allergy in infancy: clinical and immunologic findings. *J Pediatr* 1986; 109:270–6.
- 22 Suomalainen H, Laine S, Soppi E, Isolauri E. Immunologic disturbances in cow's milk allergy II: evidence for defective interferon-gamma generation. *Pediatr Allergy Immunol* 1993; 4:203–7.
- 23 Hill DJ, Ball MJ, Hosking CS, Wood PR. Gamma-interferon production in cow milk allergy. *Allergy* 1993; 48:75–80.
- 24 Tang M, Kemp A. Production and secretion of interferon-gamma (IFN- γ) in children with atopic dermatitis. *Clin Exp Immunol* 1994; 95:66–72.
- 25 Tang MLK, Kemp AS, Thorburn J, Hill DJ. Reduced interferon- γ secretion in neonates and subsequent atopy. *Lancet* 1994; 344:983–5.
- 26 Gagnon R, Akoum A, Hébert J. LpI-induced IL-4 and IFN- γ production by peripheral blood mononuclear cells of atopic and nonatopic subjects during and out of the pollen season. *J Allergy Clin Immunol* 1993; 91:950–6.
- 27 Dorion BJ, Burks WA, Harbeck R et al. Allergens, IgE, mediators, inflammatory mechanisms. The production of interferon- γ in response to a major peanut allergy, Ara h II, correlates with serum levels of IgE anti-Ara h II. *J Allergy Clin Immunol* 1994; 93:93–9.
- 28 Suomalainen H, Soppi E, Isolauri E. Lymphocyte proliferation to cow's milk proteins in patients with cow milk allergy: relationship to antigen exposure. *Pediatr Allergy Immunol* 1995; 5:20–6.
- 29 Howdle PD, Bullen AW, Losowsky MS. Cell-mediated immunity to gluten within the small intestinal mucosa in coeliac disease 1982; 23:115–22.
- 30 Bullen AW, Losowsky MS. Cell-mediated immunity to gluten fraction III in adult coeliac disease. *Gut* 1978; 19:126–31.
- 31 Grewe M, Gyufko K, Schöpf E, Krutmann J. Lesional expression of interferon- γ in atopic eczema. *Lancet* 1994; 343:25–6.
- 32 Chalacombe SJ, Tomasi TB. Systemic tolerance and secretory immunity after oral immunization. *J Exp Med* 1980; 153:1459.
- 33 Melamed D, Friedman A. In vivo tolerization of Th1 lymphocytes following a single feeding with ovalbumin: anergy in the absence of suppression. *Eur J Immunol* 1994; 24:1974–81.
- 34 Friedman A, Weiner HL. Induction of oral tolerance or active suppression following oral tolerance is determined by antigen dosage. *Proc Natl Acad Sci USA* 1994; 91:6688–92.
- 35 Fuller KA, Pearl D, Whitacre CC. Oral tolerance in experimental autoimmune encephalitis: serum and salivary antibody responses. *J Neuroimmunol* 1990; 64:581–4.
- 36 Hoyne GF, Thomas WR. T-cell responses to orally administered antigens. Study of the kinetics of lymphokine production after single and multiple feeding. *Immunology* 1995; 84:304–9.
- 37 Takahashi I, Nakagawa I, Kiyono H et al. Mucosal T cells induce systemic anergy for oral tolerance. *Biochemical and Biophysical Research Communications* 1995; 206:414–20.
- 38 Husby S, Mestecky J, Moldoveanu Z, Holland S, Elson CO. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. *J Immunol* 1994; 152:4663–70.
- 39 Garside P, Steel M, Worthey EA et al. T helper 2 cells are subject to high dose tolerance and are essential to its induction. *J Immunol* 1995; 154:5649–55.
- 40 Majamaa H, Isolauri E. Evaluation of gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J Allergy Clin Immunol* 1996; 97:985–90.
- 41 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.