

Intestinal inflammation in children with atopic eczema: faecal eosinophil cationic protein and tumour necrosis factor- α as non-invasive indicators of food allergy

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Summary

Background Food allergy is contemplated in atopic eczema. Early recognition of food allergies is difficult and the diagnosis is often missed because of the non-specificity of symptoms. New non-invasive tests are clearly needed.

Objective and methods We measured the concentrations of tumour necrosis factor- α , eosinophil cationic protein and α -1 antitrypsin in faeces as indicators of intestinal inflammation induced by double-blind placebo-controlled oral cow's milk challenge in infants and young children with atopic eczema.

Results An increased α -1 antitrypsin concentration ($> 2 \text{ mg/g}$) after cow's milk challenge was detected in 43% of the infants positive as compared with 11% of the infants negative to challenge, $P=0.02$. The concentration of eosinophil cationic protein in faeces increased after cow's milk challenge in patients positive to challenge ($P=0.02$) but not in those negative to challenge ($P=0.79$). The concentration of eosinophil cationic protein was enhanced particularly in patients manifesting immediate-type reactions to the cow's milk challenge. The concentration of tumour necrosis factor- α increased after cow's milk challenge in patients positive to challenge ($P=0.005$) but not in those negative to challenge ($P=0.25$). The concentration of tumour necrosis factor- α in faeces was enhanced particularly in patients manifesting delayed-type reactions to the cow's milk challenge.

Conclusion We conclude that in children with atopic eczema food allergy is associated with intestinal inflammation indicating that more general immunologic disturbances than previously thought take place in these patients. We further suggest that faecal eosinophil cationic protein, tumour necrosis factor- α and α -1 antitrypsin distinctly indicate various reaction types of food allergy. Parallel testing with eosinophil cationic protein and tumour necrosis factor- α may significantly enhance the accuracy in diagnosis of food allergy in patients with atopic eczema.

Keywords: atopic eczema, eosinophil cationic protein, tumour necrosis factor- α , cow's milk allergy, intestinal inflammation

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Introduction

In patients who have atopic eczema, IgE antibodies are readily produced in response to ordinary exposure to

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common environmental antigens. Early in life dietary antigens predominate [1] and allergic reactions to food are common [2,3]. The type of immune response to food is determined by the gut antigen handling [4]. Impairment of the intestine's barrier to dietary antigen entry [5] may be the key determinant in exaggerated immune

responses. Excessive antigenic stimulation may result in overproduction of proinflammatory cytokines.

T-helper cells, TH₁ cells and TH₂ cells, reciprocally regulate immunologic events occurring in antigen exposure [6]. The cytokine milieu generated by TH₂ cells, which is characteristic of atopic diseases, may convert an immune response to dietary antigens into an allergic signal [7]. Tumour necrosis factor- α (TNF α) has a dual role depending on the cytokine milieu [8,9]. There is evidence for its disease-aggravating role in inflammatory bowel disease [10] and a protective role in bacterial and parasitic infections [9]. In infants allergic to cow's milk, TNF α has been shown to disturb the gut mucosal barrier directly [11]. Particularly in atopic infants the immune responses induced by allergens may reveal deleterious TNF α secretion. It has been shown that TNF α enhances the cytotoxicity of eosinophils [12], which appear to be the major tissue-destroying cells in allergic inflammation [13].

We sought to ascertain whether intestinal inflammation can be demonstrated in atopic eczema patients who have cow's milk allergy proven by double-blind placebo-controlled cow's milk challenge. For this purpose the concentration of eosinophil cationic protein (ECP) in serum and faeces and the concentrations of TNF α and α -1 antitrypsin in faeces were assessed before and after the challenge period. In addition, TNF α release by activated peripheral blood mononuclear cells (PBMC), isolated before and after challenge, was measured. Specifically, we aimed to establish whether these markers could be used as indicators of food allergy in infants with atopic eczema.

Materials and methods

Patients and samples

The study material comprised 71 infants, aged 3–60 (median 14) months, fulfilling the Hanifin criteria [14] for atopic eczema in children. They had been referred to a paediatric clinic on the basis of suspected cow's milk allergy and were allocated to double-blind placebo-controlled cow's milk challenge. Before challenge the patients were 4 weeks on a milk elimination diet. There was a family history of atopic disorders in 50 (70%) of the cases. None of the patients was receiving systemic corticosteroid therapy. Antihistamine medication was discontinued for periods of 3 days to 6 weeks before challenge depending on the drug's duration of action.

Blood and faecal samples were collected before the first challenge (day 1) and on day 8 (before the second challenge) and on day 15 of challenge, corresponding to

the placebo and cow's milk challenge periods. All faecal samples were handled in the same way: immediately after spontaneous defecation the sample was cooled and stored at +6°C for transporting. Within a maximum of 12 h sample was delivered in a cold-transport box, frozen and stored at –70°C until analysis. The serum total IgE concentration, cow's milk-specific IgE (RAST, Pharmacia, Uppsala, Sweden), and skin-prick tests (Allergologisk Laboratorium A/S, Horsholm, Denmark) were determined before challenge.

The study protocol was approved by the Ethical Review Committee of Tampere University Hospital. Informed consent was obtained from the parents.

Double-blind placebo-controlled cow's milk challenge protocol

The placebo formula was the synthetic amino acid-derived Neocate (SHS Int. Ltd, Liverpool, UK) and the test formula consisted of Neocate and 10 g of cow's milk powder/100 mL. The formulae were prepared in the hospital's milk bank. As previously described [15], a randomization scheme was used to fix the sequence of the challenges, so that the nursing staff, attending paediatricians, parents and investigators were unaware of the administered formula's nature. The challenge period for each formula was 7 days. On the first day of the oral challenge with placebo or test formula, rising doses (1, 5, 10, 20, 50, 100 mL) were given at 30-min intervals, until milk intake appropriate for age was reached. If no reaction occurred within 8 h in hospital the challenge was continued at the patient's home. The parents recorded any clinical symptoms, including skin eruptions, pruritus, vomiting, diarrhoea and irritability. When a clinical reaction appeared the patient was examined in hospital and the challenge was discontinued. Onset of reaction was defined as the time between the latest dose and the specific symptom as well as from the start of the challenge. In like manner, the dose eliciting the symptom and the cumulated dose from the start of the challenge were determined. All patients were examined on days 8 and 15 of the challenge, corresponding to the placebo and cow's milk challenge period, and the code was thereafter opened. Cow's milk allergy was defined as an unequivocal adverse reaction to cow's milk challenge. To judge long-term tolerance and reveal any false-negative result of challenge, all patients negative to challenge continued to consume cow's milk. The patients positive to cow's milk challenge consumed a tolerated substitute formula. All patients were seen 1 month after commencement of challenge, and the diagnosis was confirmed (by E.I.).

Determination of ECP in serum specimens

The quantitative determination of ECP in serum was performed by a radioimmunoassay (Pharmacia ECP RIA; Pharmacia Diagnostics AB, Uppsala, Sweden), as instructed by the manufacturer. The radioactivity was determined using a 1470 Wizard gamma counter (Wallac Ltd, Turku, Finland), and the ECP concentrations were read from a standard curve obtained using standards from 1 to 200 µg/L.

Determination of ECP and TNFα in faecal specimens

Deep-frozen faecal specimens were thawed at room temperature, suspended 1:1 (w/v) in physiological saline and allowed to sediment. Of the supernatant, 0.5–1.0 mL was transferred to an Eppendorf tube and centrifuged at 25 000 *g* for 10 min. The supernatant was then used for the determination of ECP and TNFα. The determination of faecal ECP was performed as described above for serum specimens. A commercial enzyme immunoassay (Human TNFα ELISA Kit, Endogen Inc., Boston, Massachusetts, USA) was used, as instructed for serum specimens, for the determinations of faecal TNFα.

Determination of α-1 antitrypsin in faecal specimens

Frozen faecal specimens were thawed at room temperature and homogenized. Approximately 1 g was transferred to a glass tube and lyophilized. The resulting dry material was ground and 50 mg transferred to an Eppendorf tube. One millilitre of 0.15 M NaCl solution was added, and α-1 antitrypsin was extracted by vigorous mixing in a Vortex mixer for 20 min at room temperature. The resulting suspension was centrifuged at 25 000 *g* for 10 min to remove the debris, and the supernatant was used for the determination of α-1 antitrypsin using a Behring BNA nephelometer according to the manufacturer's instructions. The results are given as mg/g dry weight of lyophilized faeces.

Determination of TNFα production during lymphocyte induction

Mononuclear cells containing mainly lymphocytes were isolated by Ficoll-Paque gradient centrifugation at 400 *g* for 30 min at 20° C. To induce lymphocytes, 6.25×10^5 isolated cells in 1 mL of RPMI 1640 containing antibiotics, glutamin and 10% human AB serum were cultured in a humidified 5% CO₂ atmosphere at 37° C for 48 h together with Concanavalin A, (Con A, Pharmacia, Uppsala, Sweden), at a final concentration of 50 µg/mL,

and cow's milk protein solution (skimmed milk powder, Valio Finnish Co-operative Dairies' Association, Helsinki, Finland) at a final protein concentration of 1 mg/mL. A control cell population was generated with RPMI 1640 containing antibiotics, glutamin and 10% human AB serum. The supernatants were collected and stored frozen at -70° C, until the determination of TNFα, which was performed as described above. The results from different runs were equalized by employing comparison of standard curves, and are expressed as pg/L.

Statistics

The data are presented as medians with upper and lower quartiles, or as means with 95% confidence intervals. Because of skewed distribution of serum total IgE concentration, logarithmic (ln) transformation was used. The Friedman test, the Kruskal–Wallis test, the Student's *t*-test and χ^2 test were used in statistical comparisons. The indices of sensitivity, specificity and likelihood ratios for positive and negative test results were calculated using the ROC (receiver operating characteristic) curve. ROC curves for the inflammatory markers were constructed to choose the optimal cut-off point, defined as the closest point where sensitivity is 100% and specificity is 100%.

Results*Clinical data*

Of the total 71 cow's milk challenges, 39 (55%) were positive. The clinical data on the infants positive or negative to challenge are shown in Table 1. Of the positive reactions, 22 involved acute-onset pruritus, urticaria or exanthema. In 17 patients, delayed-onset reactions of eczematous type appeared (Table 2). Additional gastrointestinal symptoms occurred in 13 patients. Vomiting and diarrhoea occurred in three patients with immediate-type reactions and loose stools or diarrhoea in 10 patients with delayed-type reactions.

The concentration of α-1 antitrypsin in faeces

The concentration of α-1 antitrypsin increased in patients positive to challenge ($P=0.04$), but not in those negative to challenge ($P=0.96$); Friedman test (Fig. 1). Before challenge an increased concentration of α-1 antitrypsin (>2 mg/g) in faeces was detected in 16% of the infants. After the challenge with cow's milk, 43% of the infants positive as compared to 11% of those negative to challenge had an increased concentration of α-1 antitrypsin in faeces; $P=0.02$, χ^2 test. After the

Table 1. Clinical data on infants and young children with atopic eczema positive or negative to double-blind placebo-controlled cow's milk challenge

	Positive challenge (<i>n</i> = 39)	Negative challenge (<i>n</i> = 32)	<i>P</i>
Age at onset of symptoms (months)	4 (3–5)	5 (3–6)	0.52
Duration of exclusive breastfeeding (months)	4 (3–5)	3 (2–4)	0.42
Duration of total breastfeeding (months)	7 (6–9)	7 (5–8)	0.61
S-IgE (kU/L)	36 (22–58)	33 (15–73)	0.87
Positive serum IgE for cow's milk (>0.4 kU/L)	53%	35%	0.16
Positive skin-prick test for cow's milk	46%	19%	0.04
Multiple food allergy (> 3 foods)	36%	31%	0.68

Data denote mean (95% confidence interval) or percentage of patients.

placebo challenge, none of those positive and 13% of those negative to cow's milk challenge had an increased concentration of α -1 antitrypsin. The sensitivity of α -1 antitrypsin to detect cow's milk allergy was 43% and the specificity was 84%. The likelihood ratio for positive test result was 2.75 and the likelihood ratio for negative test result was 0.67.

The concentration of ECP in faeces

Figure 2 depicts the changes in faecal ECP concentrations before challenge, after placebo challenge and after cow's milk challenge. The concentration of ECP in faeces

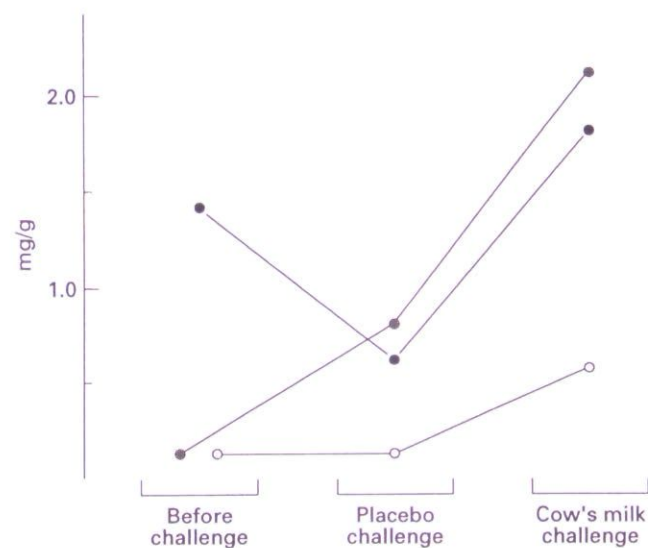
Table 2. The dose inducing symptoms and time elapsing from the dose until reaction

	Reaction type	
	Immediate (<i>n</i> = 22)	Delayed (<i>n</i> = 17)
Dose inducing the symptoms (mL)	16 (6–27)	86 (70–104)
Time from last dose until reaction (h)	0.3 (0.1–0.4)	16 (6–27)
Cumulative dose until reaction (mL)	27 (8–47)	200 (129–270)
Time from commencement of challenge until reaction (h)	2 (0.1–5)	31 (15–46)

Data denote mean (95% confidence interval).

increased after cow's milk challenge in patients positive to challenge ($P=0.02$) but not in those negative to challenge ($P=0.79$); Friedman test. When faecal ECP concentration of 80 ng/g was chosen as the cut-off point in the ROC curve, the sensitivity to disclose cow's milk allergy was 54% and the specificity 64%. The likelihood ratio for positive test result was 1.49 and the likelihood ratio for negative test result was 0.72.

The median (lower quartile–upper quartile) concentration of ECP after cow's milk challenge was significantly

**Fig. 1.** The median concentration of α -1 antitrypsin in faeces of atopic eczema patients negative to cow's milk challenge or manifesting immediate-type or delayed-type clinical reaction to cow's milk challenge. ●, Immediate. ●, Delayed. ○, Negative.

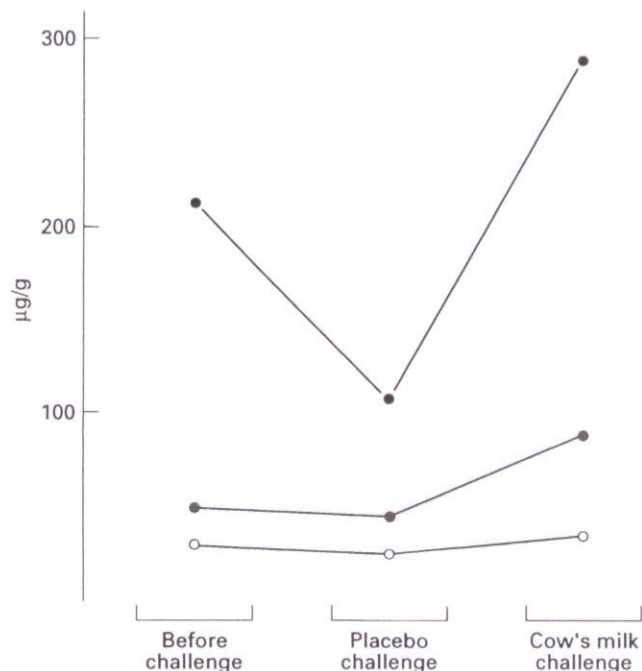


Fig. 2. The median concentration of eosinophil cationic protein (ECP) in faeces of atopic eczema patients negative to cow's milk challenge or manifesting immediate-type or delayed-type clinical reaction to cow's milk challenge. ●, Immediate. ●, Delayed. ○, Negative.

different in patients manifesting immediate-type or delayed-type clinical reaction and patients negative to challenge: 284 (135–473), 85 (61–257), 34 (27–84) ng/g, respectively; $P = 0.01$, Kruskal–Wallis test.

The concentration of TNF α in faeces

As shown in Fig. 3, the concentration of TNF α increased after cow's milk challenge in patients positive ($P = 0.005$) but not in those negative to challenge ($P = 0.25$); Friedman test. When faecal TNF α concentration of 100 pg/g was chosen as the cut-off point in the ROC curve, the sensitivity to disclose cow's milk allergy was 71% and the specificity 65%. The likelihood ratio for positive test result was 2.02 and the likelihood ratio for negative test result was 0.45.

The median (lower quartile–upper quartile) concentration of TNF α was significantly different after cow's milk challenge in patients manifesting immediate-type or delayed-type clinical reaction and patients negative to challenge: 165 (92–374), 265 (165–772), 84 (20–119) pg/g, respectively; $P = 0.04$, Kruskal–Wallis test.

Parallel testing with faecal ECP and TNF α increased the sensitivity to detect cow's milk allergy. The sensitivity was 86% and the specificity 42%. The likelihood ratio

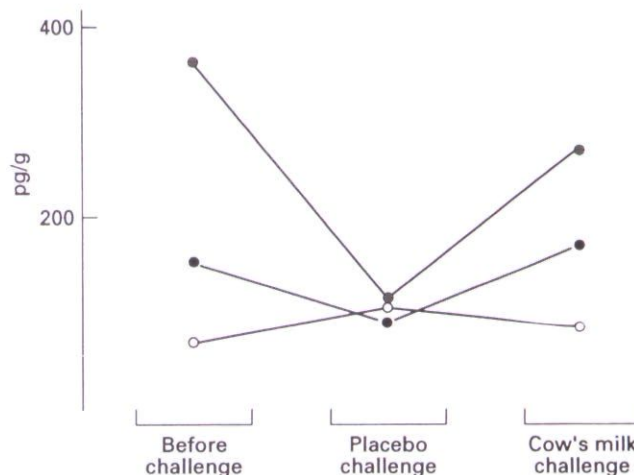


Fig. 3. The median concentration of tumour necrosis factor- α (TNF α) in faeces of atopic eczema patients negative to cow's milk challenge or manifesting immediate-type or delayed-type clinical reaction to cow's milk challenge. ●, Immediate. ●, Delayed. ○, Negative.

for positive test result was 1.48 and the likelihood ratio for negative test result was 0.33.

The concentration of ECP in sera

In patients manifesting immediate-type reaction the median (lower quartile–upper quartile) concentrations of ECP in sera were 5.8 (3.8–13.8), 6.9 (5.3–15.8), 6.7 (4.5–11.4) μ g/L before challenge, after placebo challenge and after cow's milk challenge, respectively; $P = 0.70$, Friedman test. In patients manifesting delayed-type reaction the concentration of ECP in sera was 6.4 (5.2–10.6), 7.7 (4.9–13.6), 7.4 (4.1–11.5) μ g/L before challenge, after placebo challenge and after cow's milk challenge, respectively; $P = 0.15$, Friedman test.

TNF α release in peripheral blood mononuclear cell culture supernatants

There were no differences in the concentrations of TNF α in the peripheral blood mononuclear cell culture supernatants after placebo or cow's milk challenge. In patients manifesting immediate-type reaction the concentrations of TNF α were 310 (242–429), 263 (217–323), 249 (138–560) pg/mL before challenge, after placebo challenge and after cow's milk challenge, respectively; $P = 1.00$, Friedman test. In patients manifesting delayed-type reaction the concentrations of TNF α were 209 (76–309), 322 (236–489), 339 (235–421) pg/mL before challenge, after placebo challenge and after cow's milk challenge, respectively; $P = 0.21$, Friedman test. There was no difference

in TNF α release induced by RPMI or Con A between the patients positive and negative to challenge, either.

Discussion

Hypersensitivity reactions to dietary antigens make an important contribution in the pathogenesis of atopic eczema in infants [2,3]. Demonstrating that certain foods exacerbate atopic eczema is, however, laborious. Allergen-specific IgE and skin-prick tests may not be positive early in life [16,17]. Consequently, these cannot be used in early recognition of food allergy. Moreover, these tests detect only IgE-mediated reactions [15,18,19]. New non-invasive tests are clearly needed in disclosing food allergy in patients with atopic eczema.

In the present study we found that faecal ECP and TNF α distinctly indicate allergic inflammation in the intestine related to cow's milk allergy: immediate-type reactions were associated with an enhanced ECP concentration in faeces and delayed-type reactions with an enhanced TNF α concentration. Our results indicate that, firstly, in infants with atopic eczema, food allergy is associated with intestinal inflammation and secondly, that faecal ECP, TNF α and α -1 antitrypsin as non-invasive inflammatory markers may provide a useful tool in detecting food allergy.

Immediate adverse reactions to food occur soon after ingestion of small amounts of food. The dominance of pruritus, urticarial skin eruptions and exanthema after milk ingestion has been noted in immediate-type reactions [15,20–23]. In experimental animals, immediate hypersensitivity reactions in the small intestine are associated with mucosal oedema and increased intestinal permeability [24]. Increased numbers of eosinophils have been found in the duodenal mucosa of patients with cow's milk allergy [25]. Patients with atopic eczema frequently show raised blood eosinophil counts [26] and serum ECP [27] and deposition of eosinophil-derived major basic protein in eczematous skin [28]. In the present study we found increased concentrations of ECP in the faeces of patients with the immediate-type clinical reaction to cow's milk challenge.

Disclosing delayed-type clinical reactions consisting of chronic eczematous eruptions, chronic diarrhoea and vomiting is particularly difficult. The result may be sustained allergic inflammation and gut mucosal dysfunction [29]. Assessment of the lymphocyte stimulation and cytokine generation upon incubation of lymphocytes with the offending antigen has shown some promise in detecting delayed-type reactions to food [30]. TNF α is a proinflammatory cytokine mainly produced by monocytes and macrophages. It has been shown to play an important effector role in immunologically mediated

enteropathy [31]. Increased TNF α release by peripheral blood mononuclear cells in infants with cow's milk allergy [11] and an increased plasma TNF α concentration in atopic eczema [32] have been found. These data suggest that TNF α is involved in the pathogenesis of atopic eczema and food allergy. In the present study, we observed an increased concentration of TNF α in faeces of patients positive to cow's milk challenge, particularly in those responding with delayed-type reactions.

The intestinal inflammation found in atopic eczema patients with skin-confined reactions to cow's milk challenge indicates that more general immunological disturbances than previously thought take place in these patients. Intestinal inflammation was characterized by increased concentrations of α -1 antitrypsin in faeces, which indicates protein loss in the intestine, and by increased concentrations of ECP and TNF α in faeces. We suggest that ECP, TNF α and α -1 antitrypsin as noninvasive indicators of intestinal inflammation and might be used in early identification of patients who would benefit from the strict avoidance of dietary allergens and who could then be subjected to oral food challenge. However, this needs further investigation and standardization.

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References

- 1 Björkstén B. Risk factors in early childhood for the development of atopic diseases. *Allergy* 1994; 49:400–7.
- 2 Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: Evaluation of 113 patients. *J Pediatr* 1985; 107:669–75.
- 3 Burks AW, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: Clinical relevance of food hypersensitivity reactions. *J Pediatr* 1988; 113:447–51.
- 4 Sanderson IR, Walker WA. Uptake and transport of macromolecules by the intestine: Possible role in clinical disorders (an update). *Gastroenterology* 1993; 104:622–39.
- 5 Majamaa H, Isolauri E. Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J Allergy Clin Immunol*, in press.
- 6 Fishman MA, Perelson AS. Th1/Th2 cross regulation. *J Theor Biol* 1994; 170:25–56.
- 7 Powrie F, Coffman RL. Cytokine regulation of T-cell function: potential for therapeutic intervention. *Immunol Today* 1993; 14:270–4.
- 8 Hernandez-Pando R, Rook GAW. The role of TNF- α in

- T-cell-mediated inflammation depends on the Th1/Th2 cytokine balance. *Immunology* 1994; 82:591–5.
- 9 Vassalli P. The pathophysiology of tumor necrosis factors. *Ann Rev Immunol* 1992; 10:411–52.
 - 10 MacDonald TT, Hutchings P, Choy M-Y, Murch S, Cooke A. Tumour necrosis factor- α and interferon- γ production measured at the single cell level in normal and inflamed human intestine. *Clin Exp Immunol* 1990; 81:301–5.
 - 11 Heyman M, Darmon N, Dupont C et al. Mononuclear cells from infants allergic to cow's milk secrete tumor necrosis factor α , altering intestinal function. *Gastroenterology* 1994; 106:1514–23.
 - 12 Silberstein DS, David JR. Tumor necrosis factor enhances eosinophil toxicity to *Schistosoma mansoni* larvae. *Proc Natl Acad Sci USA* 1986; 83:1055–9.
 - 13 Nutman TB. The eosinophil and eosinophilia. *Immunol Allergy Clin North Am* 1987; 7:355–70.
 - 14 Hanifin JM. Epidemiology of atopic dermatitis. *Monogr Allergy* 1987; 21:116–31.
 - 15 Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. *J Allergy Clin Immunol*, in press.
 - 16 Zeiger RS. Atopy in infancy and early childhood: Natural history and role of skin testing. *J Allergy Clin Immunol* 1985; 75:633–9.
 - 17 Sigurs N, Hattevig G, Kjellman 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.
 - 18 Fukutomi O, Kondo N, Agata H et al. Timing of onset of allergic symptoms as a response to a double-blind, placebo-controlled food challenge in patients with food allergy combined with a radioallergosorbent test and the evaluation of proliferative lymphocyte responses. *Int Arch Allergy Immunol* 1994; 104:352–7.
 - 19 Isolauri E, Suomalainen H, Kaila M et al. Local immune response in patients with cow milk allergy: Follow-up of patients retaining allergy or becoming tolerant. *J Pediatr* 1992; 120:9–15.
 - 20 Hill DJ, Firer MA, Shelton MJ, Hosking CS. Manifestations of milk allergy in infancy: clinical and immunologic findings. *J Pediatr* 1986; 109:270–6.
 - 21 Jalonen T. Identical intestinal permeability changes in children with different manifestations of cow's milk allergy. *J Allergy Clin Immunol* 1991; 88:737–42.
 - 22 Suomalainen H, Soppi E, Isolauri E. Lymphocyte response to cow's milk proteins in patients with cow's milk allergy: relationship to antigen exposure. *Pediatr Allergy Immunol* 1994; 5:20–6.
 - 23 Suomalainen H, Soppi E, Isolauri E. Immunologic disturbances in cow's milk allergy I: delayed maturation of suppressor activity. *Pediatr Allergy Immunol* 1993; 4:196–202.
 - 24 Bloch KJ, Walker WA. Effect of locally induced intestinal anaphylaxis on the uptake of a bystander antigen. *J Allergy Clin Immunol* 1981; 67:312–6.
 - 25 Challacombe DN, Wheeler EE, Campbell PE. Morphometric studies and eosinophil cell counts in the duodenal mucosa of children with chronic nonspecific diarrhoea and cow's milk allergy. *J Pediatr Gastroenterol Nutr* 1986; 5:887–91.
 - 26 Leiferman KM. Eosinophils in atopic dermatitis. *Allergy* 1989; 44 (Suppl. 9):20–6.
 - 27 Czech W, Krutmann J, Schöpf E, Kapp A. Serum eosinophil cationic protein (ECP) is a sensitive measure for disease activity in atopic dermatitis. *Br J Dermatol* 1992; 126:351–5.
 - 28 Ott NL, Gleich GJ, Peterson EA et al. Assessment of eosinophil and neutrophil participation in atopic dermatitis: Comparison with the IgE-mediated late-phase reaction. *J Allergy Clin Immunol* 1994; 94:120–8.
 - 29 Heyman M, Grasset E, Ducroc R, Desjeux J-F. Antigen absorption by the jejunal epithelium of children with cow's milk allergy. *Pediatr Res* 1988; 24:197–202.
 - 30 Kondo N, Fukutomi O, Agata H et al. The role of T lymphocytes in patients with food-sensitive atopic dermatitis. *J Allergy Clin Immunol* 1993; 91:658–68.
 - 31 Garside P, Mowat A. Natural killer cells and tumour necrosis factor- α -mediated enteropathy in mice. *Immunology* 1993; 78:335–7.
 - 32 Sumimoto S, Kawai M, Kasajima Y, Hamamoto T. Increased plasma tumour necrosis factor- α concentration in atopic dermatitis. *Arch Dis Child* 1992; 67:277–9.

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