

Different serum interleukin-12 and sCD30 levels in food- and pollen-sensitized children

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It has been proposed that a down-regulation of interleukin (IL)-12 and interferon (IFN)- γ might be related to susceptibility to allergy in early life. The aim of this study was to assess serum IL-12 levels in food-sensitized and pollen-sensitized children and to compare these with another activation marker, sCD30. Twenty children with pollen allergy and 22 food-sensitized children were included. The diagnosis of immunoglobulin (Ig)-E-mediated allergy, suggested by clinical symptoms, was based on skin-prick tests, serum IgE antibodies and total IgE levels. Samples from 24 non-allergic children were used as controls. IL-12 and sCD30 levels were measured by ELISA. It was found that pollen-sensitized patients had normal IL-12 and higher sCD30 levels than controls (114 vs. 63 U/ml, $p = 0.028$), but, surprisingly, food-sensitized infants showed normal sCD30 and increased serum IL-12 levels (323 vs. 118 pg/ml, $p = 0.0001$). No differences were found in patients suffering from asthma or allergic dermatitis. Levels of sCD30 and IL-12 determined in May showed a strong correlation with those obtained in November. Interleukin-12 and IgE levels had an inverse correlation ($r = -0.494$, $p = 0.0001$) whereas no correlation was found between sCD30 and IgE. Age had a strong negative influence on IL-12 levels in allergic ($Z = 4.834$, $p < 0.0005$) and in normal children ($Z = 3.00$, $p < 0.002$); by contrast, sCD30 levels were not significantly age-dependent. When IL-12 levels from the food-allergy group were compared with those from normal controls younger than 4 years of age, the difference remained significant ($p = 0.001$), ruling out an age-bias. The conclusions made in this study were that serum IL-12 and sCD30 showed different behaviors in children with food or pollen allergy. We found IL-12 and sCD30 levels in pollen-allergic patients that agree with the classical T-helper (Th) 1/Th2 paradigm of allergy. In contrast, serum IL-12 levels were increased in food-sensitized children, suggesting a different immunologic pathogenesis.

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Interleukin-12 (IL-12) is involved in immune responses against intracellular bacteria such as *Mycobacterium tuberculosis* (1) and in cell-mediated immunity (2). Interleukin-12 induces interferon (IFN)- γ production from natural killer (NK) and T cells, showing a powerful synergistic effect with IL-2 (3). It has been proposed that down-regulation of IL-12 and a deficient production of IFN- γ might be related to allergic disturbances (4–7). CD30 glycoprotein

was described as a surface antigen (Ki) expressed by Hodgkin and Reed–Sternberg cells (8). CD30-bearing cells release a soluble form of the molecule (sCD30), which can be detected in serum. This molecule is a member of the tumor necrosis factor receptor superfamily (9), which appears to be preferentially associated with T-helper (Th) 2-type responses (10,11), although not exclusively (12). High serum CD30 levels were reported in asthmatic patients (13) and

particularly in patients with allergic dermatitis (14,15). Food allergy occurs predominantly in infants, is associated with an immature immune response and has some differential follow-up characteristics. The main goal of this study was to compare IL-12 and sCD30 levels in children with food or pollen allergies.

Subjects

Pollen sensitization

Twenty pollen-sensitized children, mean age 9.8 ± 2.0 yr (range 7–13 yr), were studied. In 9/20 cases, either parents or siblings had allergic disease. All children had suffered rhino-conjunctivitis, and 11 were also asthmatic, but none of them had allergic dermatitis. All patients were asymptomatic at the time of the study, although some children suffered mild rhino-conjunctivitis during the May determination. Peak-flow measurements were performed in all pollen-sensitized children. Although none of the children suffered either acute crisis of asthma or clinical wheezing, a low peak-flow record according to the age was found in 10/20 cases in May and four of these remained low in November. Serum determinations were performed in May during the highest atmospheric concentrations of grass pollen, and were repeated in November of the same year when there was no airborne pollen. The diagnosis was based on the clinical history and skin-prick tests with allergens. Serum immunoglobulin (Ig) E-antibodies against grass pollen were class 4 (≥ 17.5 kU/l) in all children, except one who showed class 3 (≥ 3.5 kU/l). Sensitization to olive pollen was found in 12/20 cases with positive serum antibodies (class 2 to class 4). Although seven children had also serum antibodies against mites and/or mould, only one showed perennial symptoms suggesting a clinical expression of sensitization to these allergens. None of the patients received steroid treatment at the time of the study, although antihistamine drugs were administered in some cases during the May determinations. sCD30 and IL-12 measurements were also repeated in May and November on 8 non-allergic control children.

Food sensitization

After discarding nine cases with a later negative challenge test, the final food allergy group included 22 children, mean age 2 yr 3 months (range 2 months–12 yr). An open elimination-challenge test was done in all cases. Serum determinations were performed at the time of

diagnosis, before the withdrawal of the disturbing food allergen. All patients were sensitized to egg (13 cases) or cow's milk protein (11 cases), except in one child with exclusive fish sensitization. In three cases, a polysensitization to three or more allergens was present, and two children had a combined sensitization to cow's milk and egg. Coincidental sensitization to pollen and food was found in two cases. Only patients with a clear positive response to an elimination diet were included in the study, and an open food challenge was also performed after the elimination period. All 22 cases had at least one skin-prick test positive to any food allergen. Serum IgE antibodies to food allergens were detected in all cases (≥ 0.70 kU/l). Clinical symptoms included gastrointestinal disturbances (10 cases), asthma or wheezing (10 cases), allergic dermatitis (11 cases), urticaria (12 cases) and oral allergy syndrome (2 cases). None of the children showed any anaphylactic crisis. In general, patients suffered a heterogeneous clinical picture, except for one case with gastrointestinal symptoms as the unique clinical manifestation.

Controls

Samples from 33 non-allergic children, mean age 4 yr 6 months (range 5 months to 11 yr), were used as controls. A negative family history was recorded in all cases. The control group was selected from children attending the Day Paediatric Clinics for routine checkups of different ailments, such as minor surgical problems, iron-deficiency anemia, or similar processes. Children who showed levels below or above 95% confidence interval were discarded from this group in order to eliminate latent abnormal cases.

Methods

The skin-prick test was considered positive when the weal produced by the allergen was larger than the histamine weal control, used at 1 : 1.000 dilution. Measurements were performed 15–20 min post-injection and the means of the largest and smallest diameters were recorded. Specific serum IgE-antibodies were determined by the CAP system (Pharmacia), using automated equipment, and results were compared to a curve constructed with a World Health Organization (WHO)-IgE standard provided by the manufacturer. CAP results were considered positive when levels were above 0.35 kU/l, and these were graded from class 1 (≥ 0.37 kU/l) to class 6 (100 kU/l). Total serum IgE was measured by the Phadebas IgE PRIST (Pharmacia),

the results of which showed a good correlation with the CAP system IgE FEIA. Results were expressed as kU/l.

Interleukin-12 was measured by an enzyme-linked immunosorbent assay (ELISA) according to the test protocol provided by the manufacturer (Endogen, USA) and using the standards included in the kit. The sensitivity of the test was 5 pg/ml and total IL-12 (p40 + p70 forms) was also quantified. No cross-reaction with any other human cytokine was detected. Soluble CD30 (Ki-1 antigen) was also measured by an ELISA kit that uses a mouse monoclonal antibody sandwich (Dako, Denmark). The coating and conjugated monoclonal antibodies are non-competitive and they react with different epitopes on the CD30 molecule. The detection limit of the assay was estimated to be 1 U/ml.

Statistical analysis

Assuming a non-parametric distribution of serum IL-12 and sCD30 data, results are presented as median and interquartile range (25%–75%). For the same reason, differences between groups were assessed by the Mann–Whitney *U*-test. The Spearman's rank correlation coefficient was calculated in order to evaluate the relationship between two different parameters. Statistical significance was defined as a *p* value < 0.05, as it is generally assumed in clinical studies. All statistical calculations were performed using the software package StatView® v1.0 (Abacus Inc., USA).

Results

Allergic patients, as a whole, had higher serum IL-12 levels than controls (184 vs. 134 pg/ml, *p* = 0.028), whereas the increase found in serum CD30 levels was not statistically significant for a non-parametric distribution (76 vs. 63 U/ml). Nevertheless, pollen- and food-allergic groups showed a different behavior with regard to these factors. Levels of sCD30 were higher in pollen-allergic patients than in controls (114 U/ml vs. 63 U/ml, *p* = 0.031), but not in the food allergy group (63 U/ml) (Fig. 1). In contrast, IL-12 levels were increased in the food allergy group (323 pg/ml vs. 134 pg/ml, *p* = 0.0001), but no significant differences were found between pollen-allergic patients and controls (100 pg/ml vs. 134 pg/ml). These factors clearly showed an inverse trend, so the IL-12/sCD30 quotient was different in patients with food allergy as compared with patients with pollen allergy (5.72 vs. 0.96, *p* = 0.0001).

Serum IL-12 and sCD30 levels in sensitized children

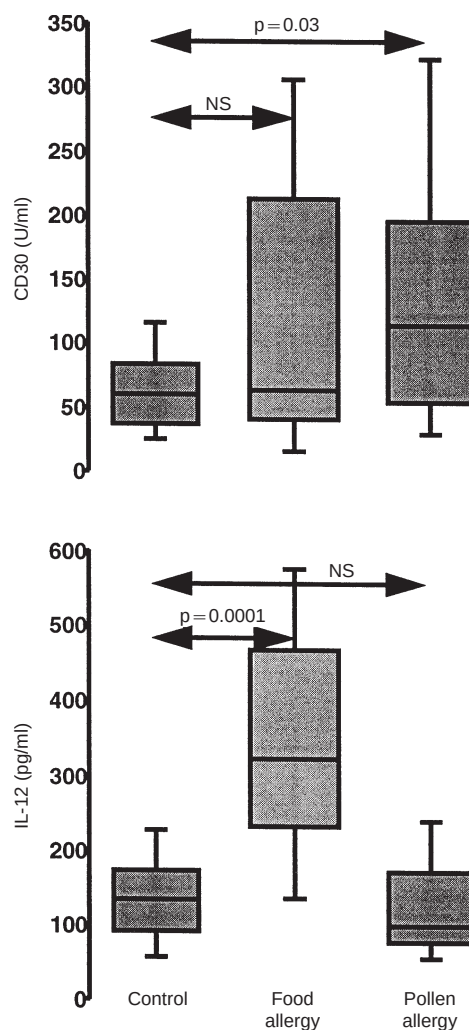


Fig. 1. Results of serum sCD30 and interleukin (IL)-12 levels showed a different pattern. sCD30 (above) was increased exclusively in the pollen-allergy group, whereas IL-12 levels (below) were increased in patients with food-sensitization but not in the former. (Data are expressed as median, interquartile values and ranges).

Interleukin-12 results were strongly influenced by age (*p* < 0.0005) within the allergic group, whereas sCD30 levels showed a great variability independent of age, although pollen-allergic patients had a rising trend without statistic significance. In non-allergic controls, both IL-12 and sCD30 levels showed a slight correlation with age, with a poor statistical significance for IL-12 levels (*p* = 0.02) (Table 1).

When IL-12 levels showed by food-allergic children were compared with data obtained from normal controls younger than 4 yr of age, the difference was still statistically significant (*p* = 0.001). In the same way, when sCD30 levels from pollen-allergic patients were compared with those found in a group of controls older than 5 yr of age, we obtained a higher statistical significance (*p* = 0.017) because sCD30

Table 1. Influence of age on sCD30 and IL-12 serum levels*

	Control group (n = 33)	Atopic patients (n = 42)
sCD30	Z = -1.92 NS (p = 0.06)	Z = -0.184 NS
Interleukin-12	Z = -3.00 p = 0.002	Z = -4.834 p < 0.0005

*Non-parametric Spearman rank correlation test.

levels decrease with age in controls and increase in pollen-sensitized patients. CD30 and IL-12 showed similar levels in patients suffering from asthma or allergic dermatitis.

Individual IL-12 and sCD30 levels obtained in May showed a strong correlation with those found in November, proving a good stability (Fig. 2). This behavior was observed in the separated study of both pollen allergic (sCD30: $r = 0.836$, $p < 0.0001$; IL-12: $r = 0.837$, $p < 0.0001$) and non-allergic controls (sCD30: $r = 0.963$, $p < 0.0001$; IL-12: $r = 0.745$, $p = 0.034$).

IL-12 levels were inversely correlated with IgE titers ($r = -0.494$, $p < 0.0005$) in the allergic group as a whole, whereas no correlation was found between IgE and sCD30 levels. On the other hand, there was no relationship between IL-12 and sCD30.

Discussion

The most useful method for the diagnosis of food allergy in children is debatable (16). A double-blind placebo-controlled food challenge was not performed in our patients; however, this procedure does not confirm the allergic nature of the reaction. In recent work, high serum titers of IgE antibodies against egg, milk or fish predicted clinical reactivity in 95% of food-allergic children, and the authors suggested that the use of the CAP System could avoid the need to perform a placebo-controlled food challenge in many patients suspected of having IgE-mediated food allergy (17).

Allergy seems to be associated with a down-regulation of IL-12 (4). A low number of IL-12 mRNA-expressing cells, and their increase after steroid treatment, was found in bronchial biopsies from adult asthmatic patients (18). In addition, a deficient IL-12 response was reported in some allergic reactions. In contrast, an enhanced production of IL-12 was found in cells from allergic patients after 4 years of successful grass pollen immunotherapy (19). Ovalbumin antigen induces IL-4 and IgE synthesis, but ovalbumin fused to IL-12 produces large quantities of IFN- γ , suggesting that IL-12 can convert a Th2-type response into a Th1-type one (20).

We observed higher serum IL-12 levels in children with food allergy than those with pollen allergy. This finding could be surprising, but an increased production of IFN- γ was also found by enzyme-linked immunosorbent spot (ELISPOT) in blood from children with cow's milk allergy (21) and different cytokine patterns in IgE-mediated allergy to food and to inhalants were previously reported (22).

Some authors proposed a possible role for IL-12 in modulating allergic inflammation (5). It is well-known that patients with allergic dermatitis improve after varicella infection, and a local expression of IL-12 mRNA was found in these patients simultaneously with an improvement in skin lesions (23). According to our results, this protective role may be present in only food-allergic and not in pollen-allergic children. Food allergy is frequently a transitory disease and symptoms and IgE-antibodies can disappear after several years on an allergen-free diet, meanwhile this situation rarely occurs in pollen-allergy. The mechanism of this tolerance is unclear and we are following up these patients in order to ascertain the clinical and immunologic outcome of patients according to the IL-12 levels at diagnosis.

Food allergy occurs early in life and these patients are always younger than children with pollen allergy. Food-allergic infants frequently

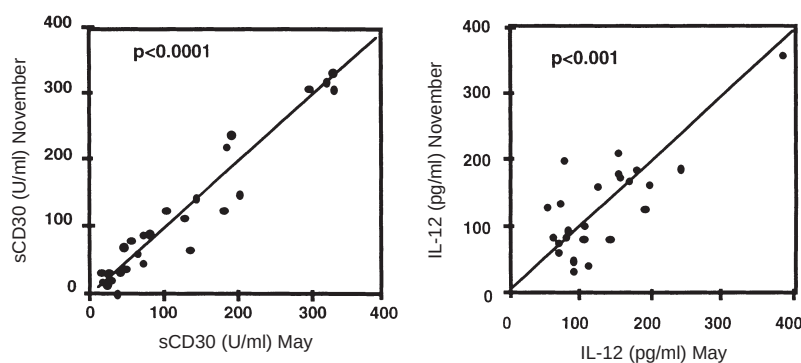


Fig. 2. May–November variation. Serum levels of sCD30 and interleukin (IL)-12 from the same patient or control case obtained in May and November showed an excellent correlation, suggesting a strong stability and a precise regulatory mechanism.

become pollen sensitized later in life. In our study IL-12 levels were age-dependent, but the age influence was stronger in allergic patients than in non-allergic controls, meaning that in pathological situations the rise was disproportionately higher in younger ($Z = -4.83$, $p < 0.0001$) than in older ($Z = 1.88$, $p = 0.06$) patients. Cytokine production by peripheral blood mononuclear cells differs in children and adults, suggesting a maturation process. When stimulated with antigens, IL-2 and IL-4 levels were lower in children than in adults whereas production of IL-6 and IFN- γ was higher (24). Precisely, macrophage IL-12 is a strong inducer of IFN- γ synthesis (25).

Interferon- γ inhibits IgE synthesis *in vitro*, but other cytokines from antigen-presenting cells, mainly IL-12, and also transforming growth factor (TGF)- β , have been shown to be more important than IFN- γ in the down-regulation of Th2 responses (26,27). We found a negative correlation between IL-12 and IgE serum levels although the interpretation must be cautious owing to the influence of age on these levels.

Concurrent with a lack of IL-12 rise, sCD30 levels were also increased in these pollen-sensitized patients, whereas food-allergic infants showed similar sCD30 values to non-allergic controls. An association between CD30 and allergic dermatitis has been reported, but not in psoriasis, seborrheic dermatitis or contact dermatitis (14,15). High sCD30 levels were also found in patients with allergic asthma (13) and CD30 could be a marker allergy after allergen exposition (28). A high number of CD30⁺ circulating cells was found in allergic patients during seasonal allergen exposure (29). Nevertheless, in our study, both sCD30 and IL-12 showed a striking steady-state level in both patients and controls, similar in May and November. This finding suggests that sCD30 and IL-12 levels are sustained by a precise regulation, although the mechanism remains unknown. In our experience the presence or absence of environmental pollen does not influence sCD30 or IL-12 levels, and an individual regulation system must be relevant. None of the pollen-allergic children suffered acute asthma at the time of the May determination, although some of them had rhino-conjunctivitis and a low peak-flow record, suggesting the presence of bronchial hyper-responsiveness and inflammation. In November, when patients were asymptomatic, IL-12 and sCD30 levels remained similar. It seems likely that non-severe symptoms and inflammation have a weak effect on serum IL-12 and sCD30 levels, but this question needs

further studies in children with acute respiratory symptoms.

sCD30 levels showed an increasing trend with age in pollen-allergic children (without reaching statistical significance), meanwhile the opposite occurred in the control group. This leads us to believe that the difference found in sCD30 levels when both groups were compared would be even greater using older, age-matched controls.

In conclusion, we found IL-12 and sCD30 levels in pollen-allergic patients that agree with the classical Th1/Th2 paradigm of allergy. In contrast, serum IL-12 levels were increased in food-sensitized children. This finding could be related to the fact that children suffering from food allergy are younger than those with pollen allergy. The rise of IL-12 in young patients could suggest a protective role and it may be related to the transitory sensitization found in some food-allergic infants.

References

1. COOPER AM, MAGRAM J, FERRANTE J, ORME IM. Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with *Mycobacterium tuberculosis*. *J Exp Med* 1997; 186: 39–45.
2. XIONG H, OHYA S, TANABE Y, MITSUYAMA M. Persistent production of interferon-gamma (IFN γ) and IL-12 is essential for the generation of protective immunity against *Listeria monocytogenes*. *Clin Exp Immunol* 1997; 108: 456–62.
3. MA X, ASTE-AMEZAGA M, GRI G. Immunomodulatory functions and molecular regulation of IL-12. In: ADORINI L, ed. IL-12. *Chemical Immunology*. Basel: Karger 1997, 68, 1–22.
4. HALONEN M, MARTINEZ FD. A deficient capacity to produce interferon-gamma: is it a risk for asthma and allergies? *Clin Exp Allergy* 1997; 27: 1234–6.
5. HAMID Q, NASEER T, MINSHALL EW, SONG YL, BOGUNIEWICZ M, LEUNG DYM. In vivo expression of IL-12 and IL-13 in atopic dermatitis. *J Allergy Clin Immunol* 1996; 98: 225–31.
6. HOEKSTRA MO, HOEKSTRA Y, DE REUS D, RUTGERS B, GERRITSEN J, KAUFFMAN HF. Interleukin-4, interferon-gamma and interleukin-5 in peripheral blood of children with moderate atopic asthma. *Clin Exp Allergy* 1997; 27: 1254–60.
7. NURSE B, HAUS M, PUTERMAN AS, WEINBERG EG, POTTER PC. Reduced interferon-gamma but normal IL-4 and IL-5 release by peripheral blood mononuclear cells from Xhosa children with atopic asthma. *J Allergy Clin Immunol* 1997; 100: 662–8.
8. SCHWAB U, STEIN H, GERDES J, LEMKE H, KIRCHNER H, SCHAADT M, DIEHL V. Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. *Nature* 1982; 299: 65–7.
9. DURKOP H, LATZA U, HUMMEL M, EITELBACH F, SEED B, STEIN H. Molecular cloning and expression of a new member of the nerve growth factor receptor family that

- is characteristic for Hodgkin's disease. *Cell* 1992; 68: 421–7.
10. DEL PRETE G, DE CARLI M, ALMERIGOGNA F, et al. Preferential expression of CD30 by human CD4+ T cells producing Th2-cytokines. *FASEB J* 1995; 9: 81–6.
11. D'ELIOS MM, ROMAGNANI P, SCALETTI C, et al. In vivo CD30 expression in human diseases with predominant activation of Th2-like T cells. *J Leukocyte Biol* 1997; 61: 539–54.
12. HAMANN D, HILKENS CMU, GROGAN JL, et al. CD30 expression does not discriminate between human Th1- and Th2-type T cells. *J Immunol* 1996; 156: 1387–91.
13. LEONARD C, TORMEY V, FAUL J, BURKE CM, POULTER LW. Allergen-induced CD30 expression on T cells of atopic asthmatics. *Clin Exp Allergy* 1997; 27: 780–6.
14. BENGSSON A, HOLM L, BACK O, FRANSSON J, SCHEYNIUS A. Elevated serum levels of soluble CD30 in patients with atopic dermatitis (AD). *Clin Exp Immunol* 1997; 109: 533–7.
15. DUMMER W, BROCKER EB, BASTIAN BC. Elevated serum levels of soluble CD30 are associated with atopic dermatitis, but not with respiratory atopic disorders and allergic contact dermatitis. *Br J Dermatol* 1997; 137: 185–7.
16. KAILA M, ISOLAURI E. Diagnosis of cow's milk allergy: open or blinded? *J Allergy Clin Immunol* 1997; 100: 714–5.
17. SAMPSON HA, HO DG. Relationship between food-specific IgE concentrations and the risk of positive food challenge in children and adolescent. *J Allergy Clin Immunol* 1997; 100: 444–51.
18. NASEER T, MINSHALL EM, MATIN RJ, et al. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. *Am J Resp Crit Care Med* 1997; 155: 845–51.
19. HAMID QA, SCHOTMAN E, JACOBSON MR, WALKER SM, DURHAM SR. Increases in (IL-12) messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997; 99: 254–60.
20. KIM TS, DEKRUYFF RH, RUPPER R, MAECKER HT, LEVY S, UMETSU DT. An ovalbumin-IL-12 fusion protein is more effective than ovalbumin plus free recombinant IL-12 in inducing a T helper cell type 1-dominated immune response and inhibiting antigen-specific IgE production. *J Immunol* 1997; 158: 4137–44.
21. HAUER AC, BREESE EJ, WALKER SMITH JA, MACDONALD TT. The frequency of cells secreting interferon-gamma and interleukin-4, -5 and -10 in the blood and duodenal mucosa of children with cow's milk. *Pediatr Res* 1997; 42: 629–38.
22. YABUHARA A, MACAUBASA C, PRESCOTT SL, et al. Th2-polarized immunological memory to inhalant allergens in atopics is established during infancy and early childhood. *Clin Exp Allergy* 1997; 27: 1261–9.
23. FUJIMURA T, YAMANASHI R, MASUZAWA M, et al. Conversion of the CD4(+) T cell profile from T-H2-dominant type to T-H1-dominant type after varicella-zoster virus infection in atopic dermatitis. *J Allergy Clin Immunol* 1997; 100: 274–82.
24. LILIC D, CANT AJ, ABINUN M, CALVERT JE, SPICKETT GP. Cytokine production differs in children and adults. *Pediatr Res* 1997; 42: 237–40.
25. CHACE JH, HOOKER NA, MILDENSTEIN KL, KRIEG AM, COWDERY JS. Bacterial DNA-induced NK cell IFN-gamma production is dependent on macrophage secretion of IL-12. *Clin Immunol Immunopathol* 1997; 84: 185–93.
26. ROMAGNANI S. Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994; 12: 227–57.
27. KRUG N, FREW AJ. The Th2 cell in asthma: initial expectations yet to be realised. *Clin Exp Allergy* 1997; 27: 142–50.
28. MOSMANN TR, SAD S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996; 17: 138–46.
29. ROMAGNANI S. The Th1/Th2 paradigm. *Immunol Today* 1997; 18: 263–6.