

Enhanced IL-4 but normal interferon-gamma production in children with isolated IgE mediated food hypersensitivity

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Atopic disorders such as atopic dermatitis and asthma have been characterised by an imbalance in interferon-gamma (INF- γ) and IL-4. Whether similar imbalances are found in atopic disorders with different clinical manifestations, such as IgE mediated immediate food hypersensitivity, is not clear. We have examined the *in vitro* production of INF- γ and IL-4 in peripheral blood mononuclear cells (PBMC) following phytohaemagglutinin stimulation from children with isolated immediate IgE mediated food hypersensitivity (egg, milk, "nut"), children with moderate and severe atopic dermatitis, and normal children. Children with immediate food reactions were excluded if they had a history or evidence of atopic dermatitis or asthma. PBMC from children with IgE mediated food hypersensitivity produced significantly more IL-4 ($p=0.013$) but equivalent INF- γ ($p=0.26$) compared to PBMC from control children. In contrast, PBMC from children with atopic dermatitis produced significantly less INF- γ ($p<0.001$) and more IL-4 ($p<0.008$) than PBMC from normal children. In addition, there was no difference in IL-4 ($p=0.74$) but significantly less INF- γ ($p<0.001$) produced by PBMC from the children with atopic dermatitis than food hypersensitivity. We demonstrate that children with IgE mediated food hypersensitivity and no other manifestation of atopic disease have enhanced IL-4 production without the defect in INF- γ production observed in childhood AD and asthma. We postulate that isolated IL-4 enhancement promotes the development of IgE mediated hypersensitivity disorders such as food allergy, whilst the combination of defective INF- γ and enhanced IL-4 production promotes inflammatory atopic disorders such as AD and asthma.

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Cytokine imbalances contribute to the development of atopic disease. Increased production of the Th2 cytokine IL-4 and decreased production of the Th1 cytokine INF- γ have been described in atopic diseases such as asthma and atopic dermatitis (1–4). Whether these imbalances are found in all atopic disorders is not clear. Cytokine imbalances can precede the development of clinical atopy, as indicated by the finding of diminished INF- γ production from cord blood cells of infants who subsequently develop atopic disease (5, 6). IL-4 promotes the development of IgE antibodies which are a characteristic of the atopic state (7). In addition to the development of specific IgE antibodies, atopic diseases such as atopic dermatitis (AD) and asthma are also charac-

terised by chronic inflammation (8, 9). The contribution of the specific cytokine abnormalities in Th2 type cytokine environment, with enhanced IL-4 and decreased INF- γ , to the development of chronic inflammation in atopic disease is unclear. It is possible that differing manifestations of the atopic state might be determined by different cytokine imbalances. If this were so, an atopic disease characterised primarily by specific IgE production may be associated with a raised IL-4 alone, while atopic disease with a more complex inflammatory reaction may demonstrate in addition reduced INF- γ .

To examine these possibilities we have studied children who have presented with IgE mediated immediate food hypersensitivity as their sole manifes-

tation of atopy and have compared their production of INF- γ and IL4 in response to the T cell mitogen phytohaemagglutinin (PHA) to that of children with atopic dermatitis and healthy non-atopic controls.

Subjects

Children presenting with a history of immediate reactions to food were included if the reactions (see classification below) had occurred within 60 minutes of ingestion and they had evidence of specific IgE to the relevant food. Specific IgE was defined as a positive skin prick to the relevant food, or in cases where skin prick testing was not performed, a positive radioallergosorbent test (RAST) to the relevant food. All children were examined by a paediatric allergist/immunologist and were excluded if physical examination revealed evidence of asthma or atopic dermatitis or if they had a past history of treatment for atopic dermatitis or asthma. The clinical reactions to food were classified into the following three grades of severity (10): Grade 1, immediate symptoms of urticaria/angiodema and erythema, localised to the face; Grade 2, generalised immediate symptoms of urticaria/angiodema and erythema, and /or immediate onset of vomiting; and Grade 3, generalised immediate symptoms of urticaria/angiodema and erythema plus respiratory symptoms of wheeze and/or stridor.

Fourteen children with isolated IgE mediated immediate food allergy were identified for the study. Heparinized blood samples were obtained from the children when blood was taken for IgE and/ or full blood count examination at their initial consultation. The mean age of the children was 25 months and mean serum IgE 252 IU/ml.

Peripheral blood mononuclear cells (PBMCs) were obtained from 15 children with atopic dermatitis, all of whom had attended a paediatric allergist/immunologist for management of their atopic dermatitis. Heparinized blood was obtained from these children when blood was being taken for IgE, RAST or/and full blood examination. These children were included irrespective of a history of food sensitivity. All were requiring daily topical corticosteroid treatment, and had moderate or severe atopic dermatitis according to the criteria of Hanifin and Rajka (11). The mean age of the children was 48 months and mean IgE 9305 IU/ml.

Control samples were obtained from 15 healthy non-atopic children admitted for elective surgery at the Royal Children's Hospital (for herniotomies, orchidopexies, circumcisions, squint repairs). These children had no history of atopy and had serum IgE in the normal age range. Heparinized blood was obtained immediately on anaesthetic induction for control subjects following parental consent. The

mean age of the children was 27 months and mean IgE 29 IU/ml. Blood collection and investigations performed were approved by the Royal Children's Hospital Ethics Committee.

Materials and methods

Reagents used were: AIM-V medium (Gibco, Grand Island, NY) supplemented with 5×10^{-5} M β -mercaptoethanol (β -ME, ICN, Costa Mesa, CA.); PHA, avidin-peroxidase (Sigma Chemical Co., St Louis, MO); Ficoll-Hypaque (Pharmacia, Uppsala, Sweden); Monoclonal antibodies (Mab)-anti-INF- γ , biotinylated anti INF- γ , anti IL-4 biotinylated anti IL-4 and rhINF- γ , rhIL-4 (Pharmingen, CA), TMB substrate (KPL, Gaithersburg, M. D.).

Rast

Testing was performed using the Kallestad Allercoat EAST Conjugate Pack (Sanofi Diagnostics Pasteur, Inc. MN, USA) in accordance with manufacturers instructions. Results are expressed as allercoat ELISA allergosorbent units (AEU/ml). Levels of allergen specific IgE antibodies correspond to Very High (4 AEU/ml), High (3 AEU/ml), Moderate (2 AEU/ml), Low (1 AEU/ml), Equivocal (1 AEU/ml) and Absent (0 AEU/ml).

Cell cultures

PBMC were separated from heparinized blood by Ficoll-Hypaque density gradient centrifugation. PBMC layer was washed three times with sterile PBS. PBMC were cultured in sterile falcon culture tubes (Becton Dickinson) at a concentration of 1×10^6 /ml (1 ml) in AIM-V with β -ME at 37°C in humidified atmosphere with 5% CO₂. Cultures were stimulated with 10ug/ml PHA or left unstimulated for control. Supernatants were collected at 48 hours after brief centrifugation, and were stored at -70°C until required for cytokine assay.

Cytokine production

Time course experiments showed PHA induced IL-4 production maximal at 24-48 hours and PHA induced INF- γ production maximal at 48-72 hours. IL-4 and INF- γ were assayed by sandwich ELISA as previously described (12). Briefly, high binding ELISA plates were coated with purified Mab at 1 ug/ml and incubated overnight at 4°C. Plates were blocked for 2 hours, washed and samples incubated at room temperature for a further 4 hours. After washing, biotinylated Mab at 0.5ug/ml was added and incubated at room temperature for 45 minutes, after which avidin-peroxidase (1:400 of 1mg/ml)

Table 1. Type of food and severity of reaction in 14 children with IgE mediated food hypersensitivity

Clinical reaction to:	Number of cases	Grade 1*	Grade 2*	Grade 3†
Egg	4	2	1	1
Egg and nut	6	2	3	1
Nut	3		1	2
Milk	1			1
Total	14	4	5	5

*Grade 1, immediate symptoms of urticaria/angioedema and erythema, localized to the face; †Grade 2, generalised immediate symptoms of urticaria/angioedema and erythema, and/or immediate onset of vomiting; ‡Grade 3, generalised immediate symptoms of urticaria/angioedema and erythema plus respiratory symptoms of wheeze and/or stridor.

was added. After final washing TMB substrate (KPL, Gaithersburg, M. D.) was added and reaction stopped by addition of H_2SO_4 . Plates were read at 450 nm on spectrophotometer. All samples and standards were performed in duplicate, and data was analysed using Biomek 1000 data reduction software (Beckman). Minimum detection levels were calculated by Biomek 1000 software and were: IL-4 -2 pg/ml, INF- γ -10 pg/ml. IL-4 detection was confirmed by the ability to detect IL-4 at 2 pg/ml when spiked into media and assayed.

Statistical methods

Analyses were performed using Mann-Whitney non-parametric *U*-test after Kruskal-Wallis test showed significant differences between the three groups. Chi-squared test was used for group comparison of IL-4 secretion.

Results

Subjects

The clinical features of the 14 children with IgE mediated food hypersensitivity (egg and "nut" -6, egg-4, "nut" -3 and milk -1) are summarised in Table 1. The relevant "nut" was peanut in five cases and cashew in one case in children with egg and "nut" reactions, and peanut in two cases and cashew in one case in the children with "nut" only reactions. Twelve children had positive skin test to the suspected food (mean wheal $\pm$ s.d.; 9mm $\pm$ 4 mm). Two children did not have skin tests performed and had positive RAST's (case 1-6.9/3.9 AEU/ml, egg and peanut respectively, case 2-1.46 AEU/ml, egg) to the relevant food. Ten of the 14 children had systemic reactions including generalised urticaria to the ingested food, and the remaining four children had urticaria and swelling localised to the face.

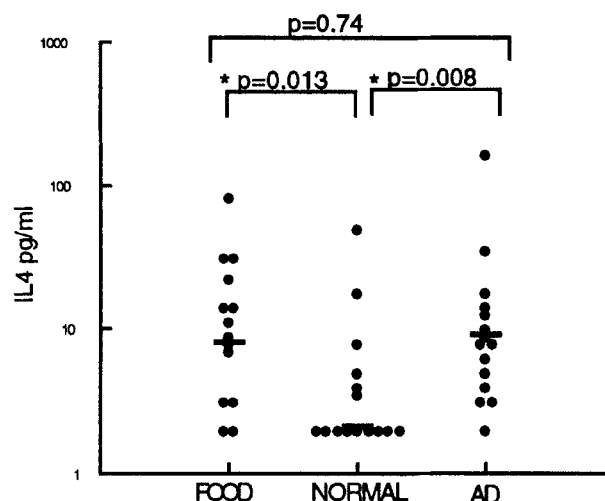


Fig. 1. IL-4 production from 1×10^6 peripheral blood mononuclear cells (PBMC)/ml obtained from children with food IgE mediated food hypersensitivity (FOOD), atopic dermatitis (AD) and normal controls (NORMAL). PBMCs cultured for 48 hours culture with phytohaemagglutinin (10 μ g/ml). Bold line represents median values.

IL-4 production

PBMC from children with food hypersensitivity produced significantly more IL-4 than PBMC from control children ($p=0.013$, median 8pg/ml and 1pg/ml respectively) (Fig. 1). Twelve of the 14 children with food hypersensitivity were found to produce detectable IL-4 in culture supernatants ($p<0.05$) compared with six of 15 non-atopic children. In addition PBMC from children with AD produced more IL-4 (median 10pg/ml) than PBMC from normal children ($p=0.008$). There was no statistical difference in IL-4 production in children with AD and food hypersensitivity ($p=0.74$). In addition there was no difference in the numbers of children with detectable IL-4 in culture supernatants in these two groups.

Children with grade 1 food hypersensitivity produced less IL-4 than those children with either grade 3 hypersensitivity ($p<0.014$), or than children who had systemic reactions (grades 2 and 3) ($p<0.034$) (Fig. 2). No such difference was observed in serum IgE; grade 1 vs grade 3 ($p=0.46$), grade 1 vs grade 2+3 ($p=0.52$).

INF- γ production

No significant difference in PBMC INF- γ production was observed between normal and food hypersensitive children ($p=0.26$, median 3123pg/ml and 3030pg/ml, respectively) (Fig. 3). In contrast PBMC from children with AD produced significantly less INF- γ ($p<0.001$, median 660pg/ml) than PBMC from both normal children and children with food

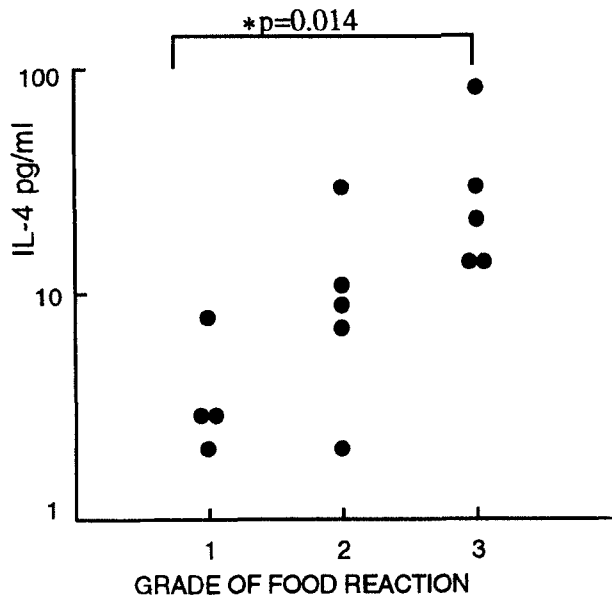


Fig. 2. IL-4 production from 1×10^6 peripheral mononuclear cells (PBMC)/ml obtained from children with food IgE mediated food hypersensitivity graded according to severity of reaction. Grade 1-Mild, Grade 2-Moderate, Grade 3-Severe (see Table 1 for details). PBMCs cultured for 48 hours culture with phytohaemagglutinin ($10 \mu\text{g/ml}$).

hypersensitivity.

When graded according to severity of food hypersensitivity, no difference in INF- γ production was observed in food hypersensitivity group; grade 1 vs grade 3 ($p=0.22$), grade 1 vs grade 2+3 ($p=0.16$).

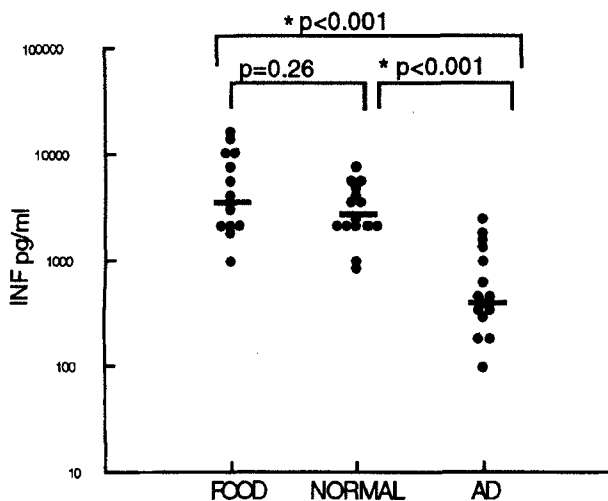


Fig. 3. INF- γ production from 1×10^6 peripheral blood mononuclear cells (PBMC)/ml obtained from children with food IgE mediated food hypersensitivity (FOOD), atopic dermatitis (AD) and normal controls (NORMAL). PBMCs cultured for 48 hours with phytohaemagglutinin ($10 \mu\text{g/ml}$). Bold line represents median values.

Discussion

We have shown that PBMC from children with IgE mediated food hypersensitivity with no clinical manifestations of other atopic disease have enhanced IL-4 production but normal INF- γ production in response to mitogenic stimuli. Studies examining PBMC from children and adults with atopic dermatitis and asthma have shown combined enhanced IL-4 and diminished INF- γ production in response to mitogens such as PHA (4), ionomycin (2) and Concanavalin A (3). We have also observed in the current and previous studies (4, 12) enhanced IL-4 and diminished INF- γ production in PHA stimulated PBMC from children with moderate-severe atopic dermatitis. This contrasts with our present findings in children with isolated food hypersensitivity, where increased IL-4 production but unimpaired INF- γ production was observed. As mitogens stimulate T cells in an unrestricted polyclonal manner, our observations point to an underlying dysregulation of IL-4 but not of INF- γ in children with isolated IgE mediated food hypersensitivity.

Previous studies examining cytokine production in allergen stimulated PBMC from subjects with IgE mediated food hypersensitivity have been unable to detect IL-4 in culture supernatants (13, 14) and reports of INF- γ responses to allergens have been conflicting. Both increased and decreased PBMC INF- γ production have been observed in response to ovalbumin in egg sensitive children with atopic dermatitis (14-17) and decreased INF- γ in response to peanut allergen stimulation in peanut sensitive children (13). These studies did not specifically examine isolated food allergy as we have done, but rather food allergy in association with other atopic conditions such as asthma and atopic dermatitis. Thus findings of diminished INF- γ in response to allergens may have been confounded by the presence of atopic dermatitis or asthma, both conditions in which decreased INF- γ responses to allergens have been observed (5, 18). Furthermore, as *in vitro* cytokine response to allergens reflect both the innate predisposition to generate that cytokine and the frequency of sensitised cells, it is possible that the inherent decrease in INF- γ secretion by atopic cells may be counterbalanced by increased numbers of T cells sensitised to specific allergen in atopic subjects.

As immediate food hypersensitivity reactions are an IgE mediated disease, and IL-4 is a principal determinant of IgE production, it is of interest that the clinical severity of the food reaction significantly correlated with IL-4 production *in vitro*. This suggests that the enhancement of *in vitro* IL-4 may have a direct relationship to the development of IgE mediated disease *in vivo*. In support of such a notion, enhanced IL-4 has been previously correlated with

IgE production in atopic children (19), and size of skin prick test is significantly associated with the occurrence of IgE mediated food reactions (20). Of additional interest, we found no correlation between severity of food reaction and INF- γ production, indicating a dissociation of IL-4 and INF- γ production in isolated IgE mediated food hypersensitivity.

The interrelation between INF- γ and IL-4 in the development of atopic disease is of interest. INF- γ downregulates IL-4 (21) while IL-4 can exert a negative feedback on INF- γ production (22). It has been unclear to what extent the imbalances of IL-4 and INF- γ observed in atopic disorders result from separate intrinsic defects of production or secretion or are determined by mutual interaction of these cytokines. Our findings demonstrate increased IL-4 production independently from reduced INF- γ production and imply that decreases of the latter are not necessarily due to down regulation by enhanced IL-4. This suggests that "atopy" may be heterogeneous with respect to imbalances of INF- γ and IL-4.

We have shown that children with food hypersensitivity alone when compared with children with atopic dermatitis have differences in cytokine profiles from mitogen stimulated PMBC. The numbers in this study were relatively small and further studies would be of interest. The existing studies with food allergen stimulation are conflicting and it would also be of interest to examine cytokine production in response to relevant food allergens in children with isolated food hypersensitivity. Our data suggest that intrinsic differences in cytokine profiles may determine different manifestations of atopic disease.

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References

1. REINHOLD U, PAWELEC G, WEHRMANN W, et al. Cytokine release from cultured peripheral blood mononuclear cells of patients with severe atopic dermatitis. *Acta Derm Venerol* 1989; 69: 497-502.
2. ROUSSET F, ROBERT J, ANDARY M, et al. Shifts in interleukin-4 and interferon-gamma 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: 59-69.
3. JUJO K, RENZ H, ABE J, et al. Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. *J Allergy Clin Immunol* 1992; 90: 323-6.
4. TANG M, KEMP A, VARIGOS G. IL4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunol* 1993; 92: 120-4.
5. WARNER JA, MILES EA, JONES AC, et al. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994; 24: 423-30.
6. TANG ML, KEMP AS, THORBURN J, HILL DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994; 344: 983-5.
7. PENE J, ROUSSET F, BRIERE F, et al. IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-gamma. *J Immunol* 1988; 141: 1218-24.
8. BEASLEY R, ROCHE WR, ROBERTS JA, HOLGATE ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989; 139: 806-17.
9. LEUNG DY, BHAN AK, SCHNEEBERGER EE, GEHA RS. Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. *J Allergy Clin Immunol* 1983; 72: 47-50.
10. KEMP A, VAN AP, MUKHI A. Measles immunization in children with clinical reactions to egg protein. *Am J Dis Child* 1990; 144: 33-5.
11. HANIFIN JM. Standardized grading of subjects for clinical research studies in atopic dermatitis: workshop report. *Acta Derm Venereol* 1989; 144 Suppl: 28-30.
12. CAMPBELL DE, KEMP AS. Proliferation and production of interferon-gamma and IL-4 in response to *Staphylococcus aureus* and *Staphylococcal* superantigen in childhood atopic dermatitis. *Clin Exp Immunol* 1997; 107: 392-7.
13. DORION BJ, BURKS AW, HARBECK R, et al. The production of interferon-gamma 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.
14. NOMA T, YOSHIZAWA I, AOKI K, et al. Cytokine production in children outgrowing hen egg allergy. *Clin Exp Allergy* 1996; 26: 1298-307.
15. REEKERS R, BEYER K, NIGGEMANN B, et al. The role of circulating food antigen-specific lymphocytes in food allergic children with atopic dermatitis. *Br J Dermatol* 1996; 135: 935-41.
16. SHINBARA M, KONDO N, AGATA H, et al. Interferon-gamma and interleukin-4 production of ovalbumin-stimulated lymphocytes in egg-sensitive children. *Ann Allergy Asthma Immunol* 1996; 77: 60-6.
17. KONDO N, FUKUTOMI O, SHINBARA M, ORII T. Inhibition of interferon-gamma and interleukin-2 production from lymphocytes stimulated with food antigens by an anti-allergic drug, Tranilast, in patients with food-sensitive atopic dermatitis. *Biotherapy* 1994; 8: 19-22.
18. WIERENGA EA, SNOEK M, DE GROOT C, et al. Evidence for compartmentalization of functional subsets of CD2+ T lymphocytes in atopic patients. *J Immunol* 1990; 144: 4651-6.
19. TANG ML, COLEMAN J, KEMP AS. Interleukin-4 and interferon-gamma production in atopic and non-atopic children with asthma. *Clin Exp Allergy* 1995; 25: 515-21.
20. HILL D, DUKE A, HOSKINGS C, HUDSON I. Clinical manifestations of cow's milk allergy in children. II. The diagnostic value of skin tests and RAST. *Clin Allergy* 1988; 18: 481-90.
21. PENE J, CHRETIEN I, ROUSSET F, et al. Modulation of IL-4-induced human IgE production *in vitro* by IFN-gamma and IL-5: the role of soluble CD23 (s-CD23). *J Cell Biochem* 1989; 39: 253-64.
22. VERCELLI D, JABARA HH, LAUENER RP, GEHA RS. IL-4 inhibits the synthesis of IFN-gamma and induces the synthesis of IgE in human mixed lymphocyte cultures. *J Immunol* 1990; 144: 570-3.