

Patterns of food allergen-specific cytokine production by T lymphocytes of children with multiple allergies

T. H. Scott-Taylor*, J. B. Hourihane*†, J. Harper‡ and S. Strobel*§

*Division of Immunobiology, Institute of Child Health, London, UK, †Division of Infection, Inflammation and Repair, University of Southampton, Southampton University Hospitals NHS Trust, Southampton, UK, ‡Department of Dermatology, Great Ormond Street Hospital NHS Trust, London, UK and §Institute of Biomedical and Clinical Science, Peninsula Medical School and Peninsula Postgraduate Health Institute, Plymouth, Devon, UK

Abstract

Background The contribution of different T cell subsets to the overall measured cytokine response to food allergens is largely unexplored.

Method The patterns of cytokine production of peripheral blood-derived T cells after allergen stimulation were studied in 22 children with multiple food allergies and in 20 non-allergic children as controls, using flow cytometry.

Results Proportions of T cells of food-sensitized children spontaneously secreting IFN- γ and IL-10 (without antigen stimulation) were lower than non-atopic children and adult controls ($P \leq 0.001$). The proportions of IL-4-producing cells *in vitro* were significantly increased ($P \leq 0.04$) and IFN- γ -producing cells were significantly reduced ($P \leq 0.05$) in sensitized children after incubation with and without dendritic cell presentation of peanut extract, β -lactoglobulin and ovalbumin. The reverse pattern was found in non-sensitized children and adult controls. IL-4 secretion in allergic children to sensitizing allergens was mainly restricted to the CD4⁺ CD45 RO⁺ population while in non-atopic controls both CD4⁺ and CD8⁺ CD45 RO⁺ cells produced mostly IFN- γ . Food-specific IgE values did not correspond with cytokine responses but IL-4 production and IFN- γ reduction relative to normal children were closely associated with total IgE levels.

Conclusion Food-allergic children's IL-4 cytokine response to their relevant allergens is predominantly from a memory population of CD4⁺ CD45 RO⁺ cells, whereas IL-4 and IFN- γ secretion of non-allergic controls was predominantly from mixed CD4⁺ and CD8⁺ CD45 RO⁺ populations.

Keywords allergy, CD45 RO, IFN- γ , IL-2, IL-4, IL-10, T cells

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Introduction

The development and persistence of atopic disease [1–3] is affected by events very early in life [4]. The establishment of imbalanced T cell cytokine secretion, with reduced IFN- γ and increased IL-4, IL-5 and IL-13 secretion, is an underlying feature common to a number of atopic diseases [5–7]. IFN- γ production is lowest in children with a genetic background of atopy, and the normal postnatal increase in IFN- γ production is delayed in newborns who go on to develop atopy [8–10]. T cells of individuals with atopic diseases secrete IL-4 in response to specific sensitizing agents [5, 11–13]. Clonal cell lines derived from sensitized children mostly comprise CD4⁺ T cells that secrete mostly IL-4 and/or IL-5 and little IFN- γ [14–16].

Food-allergic individuals have a cytokine response pattern with raised IL-4 and reduced IFN- γ production similar to individuals with eczema [6, 17] and asthma [7]. This pattern is seen after mitogenic stimulation and after CD3 cross-linking of circulating lymphocytes [18, 19]. The phenotypes of T lymphocytes after allergen-specific stimulation and a T-helper type 2 (Th2)-polarized cytokine response in food-allergic (FA) children have not been examined in detail, although there are some reports addressing this issue [20, 21]. Our prospective study in 22 children with multiple allergies examines which T lymphocyte populations contribute significantly to T-helper type 1 (Th1) and Th2 cytokine production in allergic children and whether the response is related to sensitization, clinical reactivity or total and allergen-specific IgE.

Materials and methods

This study was approved by the local Medical Ethics Committee, and informed consent was obtained from all participants and parents. Twenty-two FA patients (10 female

Correspondence: Tim Scott-Taylor, Department of Clinical Immunology, Royal Free Hospital NHS Trust, Pond Street, Hampstead, London NW3 2QG, UK.

E-mail: t.scott-taylor@medsch.ucl.ac.uk

and 12 male patients), 10 age- and sex-matched children without any history of allergy and 10 healthy adults (controls) were studied. The FA group were all recruited in a specialist paediatric allergy clinic. They were considered FA if they had a strong recent history of reactivity to the food and supportive skin tests and allergen-specific IgE measurements [22]. Formal food challenges were not routinely performed.

Skin prick tests were performed with standardized extracts (Alk Abello, Hungerford). Histamine hydrochloride (10 mg/mL) was used as the positive control and phosphate-buffered saline (PBS)/0.1% glycine as the negative control. The test was regarded as positive when the mean weal diameter exceeded 3 mm above the negative control. Total serum IgE and specific IgE to egg, cow's milk and peanut protein were measured with Pharmacia Cap[®] RAST (Upsala, Sweden) according to the manufacturer's recommendations. Samples above 0.35 kU/L were considered positive.

Monoclonal antibody hybridomas and reagents

Hybridoma OKT3 (anti-CD3, American Type Culture Collection (ATCC) CRL-8001), (anti-human CD14, ATCC HB-247), L242 (anti-HLA DR, ATCC HB-171) and BU12 (anti-human CD19, donated by Dr D Hardie, University of Birmingham) were cultured to a high density in 20% fetal calf serum/hybricare medium (ATCC, Cat. No. 46-X), and the supernatant was collected, filtered (0.4 µm) and frozen. Peanut protein antigen (PN, defatted peanut protein, SPF Inc., Austin, TX, USA), bovine serum albumin (BSA), β-lactoglobulin (BLG), ovalbumin (OVA) and ovomucoid (Sigma B6917, A5503, L3908 and AS625) (Sigma, Gillingham, UK) were tested for lipopolysaccharide (LPS) content by the Limulus Amoeba Lysate kit (Quadrant Ltd, Epsom, Surrey, UK). The highest levels of LPS contamination were 0.03 ng/mL for BSA, 1.6 ng/mL for OVA, 4.7 ng/mL for BLG and <6 pg/mL for PN. Preliminary tests indicated that IFN-γ and IL-4 production was not affected by peripheral blood mononuclear cell (PBMC) incubation in concentrations of LPS (Sigma L-2903) lower than 100 ng/mL, more than 10 times lower than the highest LPS concentrations present in the antigen solutions.

Cell culture

Blood was collected with 5 U/mL of heparin, diluted in an equivalent volume of Roswell Park Memorial Institute (RPMI) 1640 medium culture, and monocytic cells (PBMCs) were collected over a cushion of Lymphoprep[™] (Axis-Shield ProC As, Oslo, Norway, density 1.077 g/mL) after 30 min centrifugation at 280 g. The upper layer (50/50 plasma/RPMI medium) was collected, clarified twice at 320 and 700 g for 10 min and used as a growth medium supplement. Functional dendritic cells (DCs) were generated from adherent peripheral blood monocytes with culture in 20% autologous plasma/RPMI supplemented with 100 ng/mL human granulocyte macrophage-colony stimulating factor (Insight Biotech JR-1900, Wembley, UK) and 50 ng/mL human IL-4, (Insight Biotech IB-1016), as described previously [23].

Immature DCs were incubated with anti-CD19, anti-CD3 and anti-CD14 hybridoma supernatants (350, 200 and 400 µL, respectively, per 1×10^6 cells in 2 mL of RPMI for

15 min at 4 °C) and depleted of contaminating cells by magnetic separation with 10 µL of sheep anti-mouse IgG-coated beads (DynaL Biotech., Bromborough, UK). Purified DCs were cultured with food antigens (100, 10 and 1 µg/mL) for 24 h on days 6 and 9 of culture, washed twice in RPMI and added to autologous T cells in a 1:10 ratio. Non-adherent PBMCs were suspended for 15 min in 2 mL of RPMI in a mixture of anti-CD19, anti-CD14 and anti-HLA-DR supernatants. Non-T cells were depleted with magnetic beads coated with anti-mouse IgG in 20% autologous plasma/RPMI and 300 000 cells per well dispensed in 96-well plates. T cells were pulsed with BSA, BLG, OVA and PN at final concentrations of 100 and 10 µg/mL or incubated with two successive aliquots of antigen-pulsed DCs, 30 000 cells per well, 10:1 T:DC ratio, 72 h apart. Brefeldin A (Sigma B6751), ionomycin (Sigma IO634) and phorbol myristate acetate (Sigma P8139) were added at final concentrations of 10, 1 and 10 µg/mL, for the last 2 h of incubation. Intracellular cytokine expression was examined 48 h after antigen incubation or addition of the second DC aliquot.

Antibody staining and flow cytometry

Antibody staining was performed in 96-well plates with two washes by decanting and blotting the plate between each staining cycle. Cells were fixed in 25 µL of a fixative solution (reagent A, Serotec BUFO9B, Kidlington, UK) at room temperature for 15 min. Cells were then stained with three antibodies simultaneously in a permeabilizing agent (Leukoperm, Serotec BUFO9B) for 1 h at room temperature for the following antigens: CD3 fluorescein isothiocyanate (FITC) (Pharmingen 30108X), CD8 Cy-Chrome (Serotec MCA1226F), CD45 RO Cy-Chrome (31308X Pharmingen), IL-2 (BD 340450), IL-4 (BD 340451), IL-10 (Serotec MCA1531PE) or IFN-γ (BD 340452) conjugated to phycoerythrin (PE). Controls were stained with mouse isotype control antibodies IgG1 FITC, IgG1 PE and IgG2 Cy-Chrome (BD 349041, 349043 and 349047), anti-CD8 FITC, anti-IFN-γ PE and anti-CD45 RO or CD3 Cy-Chrome. Finally, cells were washed twice with 200 µL RPMI/0.01% sodium azide, resuspended in 100 µL PBS and analysed on a Becton Dickinson FACS Calibur analyser, Cowley, UK.

The measure of percentage change related to percentage of intracellular cytokine expression in response to food antigens was derived by dividing the (% of cytokine-positive cells after addition of antigen by the % of cytokine-positive values without antigens $\times 100$)–100.

Statistical analyses

Statistical analyses were performed using SPSS for Windows (version 8.0) and Microsoft Excel. The results were tested using an independent sample two-tailed *t*-test. The mean and standard deviation of group stimulation indices were compared using the *F*-test. Correlation was assessed using Spearman's rank correlation coefficient. *P*-values <0.05 were considered significant.

Results

The age range of the FA patients was 3 years, 4 months to 12 years 11 months (Table 1). The total serum IgE ranged from

Table 1. Major clinical features of food-sensitized children

	Age (years, months)	Total IgE (kU/L)	Specific IgEs (kU/L)			Major symptoms and allergies	Other IgEs* (other SPTs †)
			Peanut	Milk	Egg		
S1	8, 5	14600	> 100	> 100	> 100	Eczema; autism: M, E, P	9 (ND)
S2	6, 8	6768	> 100	> 100	> 100	Eczema; GI; C18 del; M, P [‡] , E [‡]	11 (7)
S3	10, 6	5748	> 100	12.4	4.97	Eczema; P, mT	15 (7)
S4	6, 7	4647	54.0	> 100	> 100	Eczema; C18 del; M, E, P [‡]	11 (6)
S5	11, 4	3319	9.4	10.7	26.4	Asthma; E, P [‡]	8 (4)
S6	9, 7	3099	29.9	10.2	7.27	Eczema; E, P [‡]	8 (8)
S7	7, 5	1949	14.6	6.92	> 100	Eczema; E, P [‡]	13 (7)
S8	9, 7	1081	2.1	2.91	10.8	Food allergy (GI); M, E, P	4 (8)
S9	8, 0	1010	46.5	8.1	42	Asthma; M, E, P*	8 (8)
S10	6, 8	1000	52.9	3.84	7.31	Food allergy (GI); M, E, P [‡]	9 (7)
S11	3, 4	868	< 0.35	23.6	< 0.35	Eczema; E, S [‡]	3 (4)
S12	9, 2	692	9.8	1.87	0.94	Asthma; P	10 (8)
S13	11, 5	569	35.9	4.7	< 0.35	Eczema; P, E, mr	16 (4)
S14	12, 11	478	> 100	< 0.35	< 0.35	Asthma; P	5 (4)
S15	6, 8	444	< 0.35	0.72	< 0.35	Food allergy (GI); B [‡]	13 (6)
S16	3, 7	325	< 0.35	0.57	< 0.35	Asthma, E	4 (7)
S17	3, 5	286	3.44	5.17	4.99	Food allergy (GI); M, E, P	7 (5)
S18	5, 8	212	5.06	3.82	1.1	Asthma; E, P	1 (4)
S19	8, 2	146	1.8	0.79	< 0.35	Food allergy (GI); mR	3 (3)
S20	11, 3	128	3.9	0.71	< 0.35	Eczema; mR	2 (3)
S21	4, 6	122	28.6	6.1	4.9	Food allergy (GI); M, E	9 (6)
S22	5, 3	11.6	0.8	< 0.35	< 0.35	Atopy; no food allergy	1 (2)
S23	7, 0	11.4	< 0.35	< 0.35	< 0.35	Urticaria; no food allergy	2 (0)

ID, immunodeficiency; GI, gastrointestinal symptoms such as diarrhoea, vomiting and abdominal pain; C18q del; chromosome 18q deletion syndrome; E, egg; P, peanut; M, milk; mT, milk tolerated; mr, resolving milk allergy; mR, resolved milk allergy; S, sesame; B, brazil nut; SPTs, skin prick tests; ND, not done.

*Other specific IgEs > 5 kU/L. †Other skin prick tests > 3 mm diameter. ‡Anaphylaxis.

11.4 to 14 600 kU/L with a median of 2386 kU/L. Five of 22 children were allergic to only one of the three foods in the study, five to two foods and 12 of 22 were allergic to all three foods. The clinical symptoms and the range of allergic diseases are summarized in Table 1.

Interleukin-4 and interferon- γ secretion in sensitized and non-sensitized children

Antigen-specific changes in IL-4 (top panel) and IFN- γ (bottom panel) production are shown in Fig. 1 for sensitized and non-sensitized children. The proportion of cytokine-positive cells increased initially to 48 h and then decreased to baseline levels after 4 days. In egg-sensitized children, the initial proportion of IL-4-positive cells, without antigen stimulation, was high in comparison with non-allergic children and reached a peak 48 h after stimulation. Maximum values for an antigen-induced IFN- γ response were reached between 24 and 48 h in non-atopic children. IFN- γ -positive cells of egg-allergic children were low at baseline level and decreased further in response to OVA (Fig. 1, bottom panel). In contrast, the non-allergic T cells responded to OVA with a decrease in IL-4 and an increase in IFN- γ -producing T cells. Changes in cells were similar for CD4 and CD8 T cells in sensitized and non-atopic children (data not shown).

Cytokine production of lymphocytes of allergic and non-allergic children and adults

Milk-, egg- and peanut-sensitized children displayed different levels of cytokine-positive circulating T cells compared with

non-allergic children or adults (Fig. 2). The frequency of IL-10- and IFN- γ -secreting cells in FA children was significantly lower than in controls ($P < 0.001$). IL-2 showed no differences between the groups. IL-4 values were raised only in FA children, and levels did not differ between the control groups. Allergic CD45 RO⁺ T cells (memory activated) contained elevated proportions of IL-4-producing cells compared with non-atopic children but less than adult levels ($P \leq 0.001$) (Fig. 2b).

Food antigen T cell cytokine stimulation

The pattern of IL-4 and IFN- γ responses of CD3 cells and CD45 RO⁺ T cells to food antigen to which children were allergic was markedly different from controls (Fig. 3). While IL-2 and IL-10 responses were unrelated to food sensitization, the IL-4-positive T cell percentage increased in FA children after stimulation, and non-sensitized children exhibited a lower proportion of IL-4-positive T cells in response to food antigen. Peanut-, milk- and egg-sensitized children demonstrated an overall reduction in the proportion of T cells producing IFN- γ , while non-sensitized children generally showed higher production in response to food antigens. Differences between sensitized and non-sensitized children for both IL-4 and IFN- γ were significant ($P \leq 0.01$) for all three food allergens tested.

Allergen presentation by in vitro pulsed dendritic cells

DC-driven IL-4 and IFN- γ changes were similar to T cell cytokine responses observed in PBMC culture, but the proportion of T cells responding was generally considerably

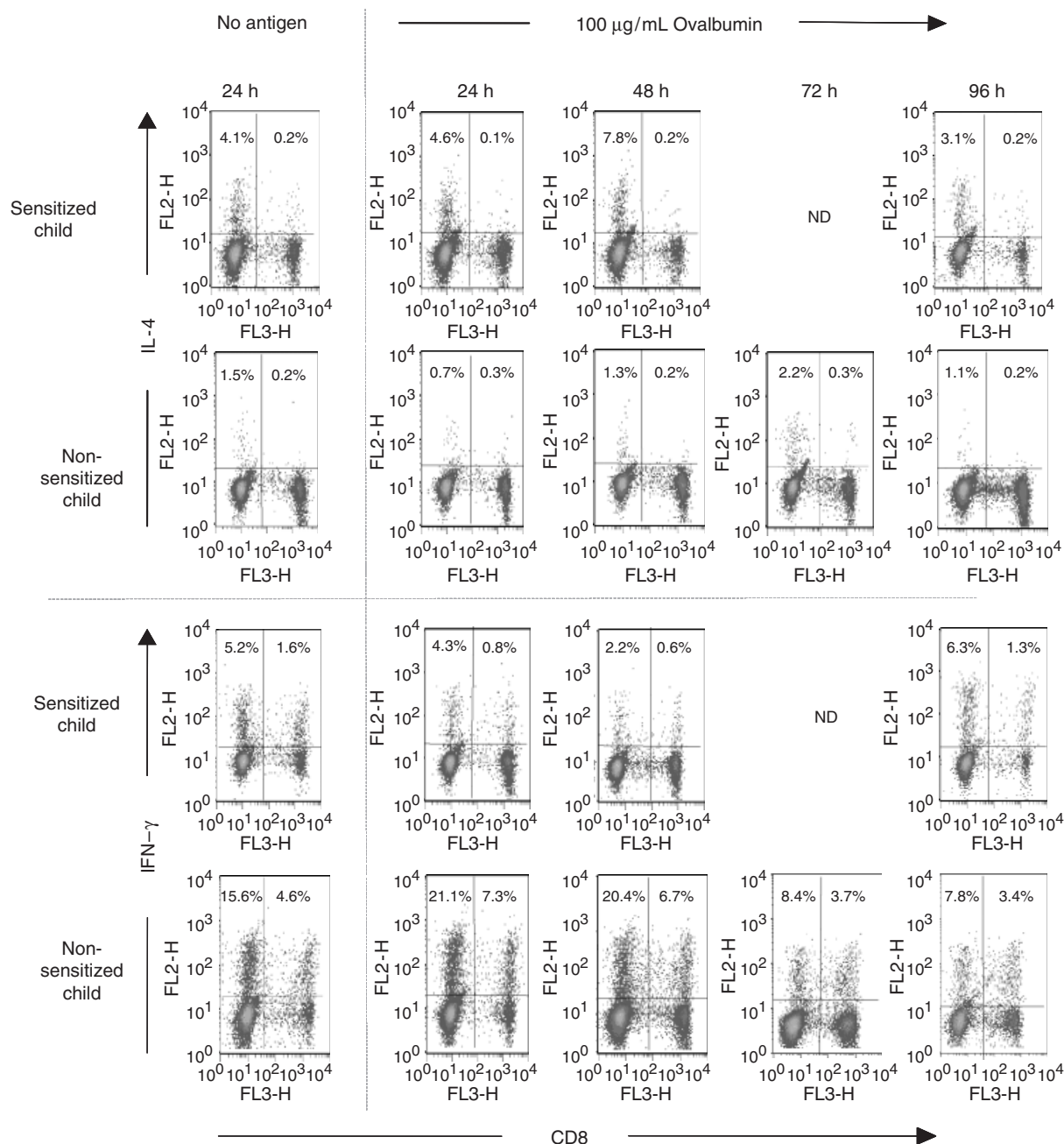


Fig. 1. IL-4- and IFN- γ -producing cells in egg-sensitized children and controls. Percentage of IL-4- and IFN- γ -positive cells in unstimulated and ovalbumin (OVA)-stimulated peripheral blood mononuclear cells plotted over 4 days in egg-allergic children and controls. The percent of cytokine-positive cells was determined by FACS analysis of control and egg-sensitized children for 4 days (24–96 h) with and without additional antigen 100 μ g/mL of OVA. The IL-4 cytokine (sensitized) or IFN- γ (non-sensitized) responses increase over 2 days in culture and decrease thereafter. The figure is representative of three separate experiments. ND, not done.

higher (Fig. 3, middle panels). IL-2 and IL-10 levels were not related to food concentration or sensitivity (data not shown). Changes in IL-4-producing T cells of FA children differed significantly from controls after exposure to PN-, BLG- and OVA-pulsed DC ($P = 0.02$, $P = 0.001$ and $P = 0.04$). Control children produced more IFN- γ after DC pulsing, compared with FA children, for all food allergens tested ($P \leq 0.04$). T cell IL-4 production to DC-mediated antigen presentation in FA children was disproportionately confined to CD4⁺ CD45 RO⁺ cells in allergic children ($P < 0.01$). Both CD4⁺ and CD8⁺ cells of non-sensitized children had slightly higher

proportions of IFN- γ -secreting populations in culture compared with allergic children after PN, BLG and OVA presentation ($P \leq 0.07$).

Antigen-specific cytokine secretion and correlation with total immunoglobulin E levels

Individual IL-4 and IFN- γ responses of peanut-, milk- and egg-sensitized children are shown in Fig. 4 relative to the total serum IgE. Increases in IL-4 secretion, with a concomitant decrease in IFN- γ , were seen in all food-sensitized individuals

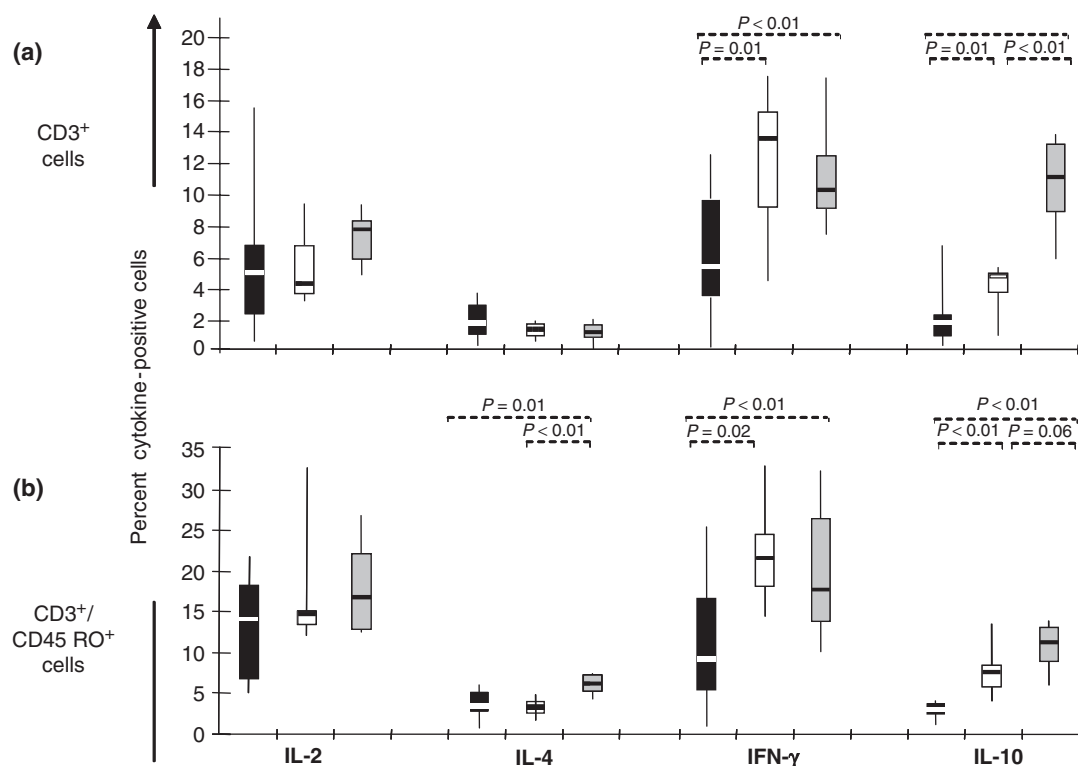


Fig. 2. Populations of IL-2-, IL-4-, IFN- γ - and IL-10-positive T cells in sensitized children, control children and non-allergic control adults without antigen stimulation: (a) CD3⁺ cells and (b) CD45 RO⁺ CD3⁺ cells. Data displayed as median, complete range and 25–75% quartile ranges of sensitized children ($\blacksquare$, $n = 22$), non-sensitized children ($\square$, $n = 10$) and non-sensitized adults ($\blacksquare$, $n = 10$) for each cytokine. (a) Cytokine-positive cells within the population of CD3⁺ cells. (b) Cytokine-positive cells within the CD45 RO⁺ CD3⁺ population. P -values ≤ 0.05 are displayed.

and this inversion of the normal cytokine response was associated with a high total IgE but unrelated to the level of food-specific IgE (see Table 2). IL-4 and IFN- γ responses to food antigens were inversely correlated ($P < 0.001$).

Individual cytokine response and correlation with resolving allergy

Peanut-sensitized children with an IL-4 decrease in response to PN tended to be those with a low total IgE ($n = 5$) (A, Fig. 4). Non-sensitized children with decreased IFN- γ to PN ($n = 2$) (B, Fig. 4) tended to be those allergic children with a higher total IgE and more widespread allergies (Table 1), although this did not reach statistical significance. Four patients with clinically resolving milk allergy were included in the study (S3, S13, S19, S20). Where milk was tolerated clinically but specific IgE and high total IgE were present, IL-4 production of T cells decreased after BLG stimulation (T, Fig. 4) similar to those children without IgE and resolved milk allergy (R, Fig. 4). Where symptoms persisted in the absence of specific IgE (r, Fig. 4), IL-4 secretion increased and IFN- γ decreased in response to BLG.

Discussion

Our prospective study has demonstrated that T cells of multiple FA children respond with a reduced IFN- γ and an increased IL-4 secretion *in vitro* when challenged with the three major allergens: peanut extract, BLG and OVA.

The marked changes in cytokine secretion patterns were more closely related to the overall atopic state, assessed by a high total serum IgE level and clinical symptoms, than to the levels of antigen-specific IgE levels. Cytokine responses induced by specific food stimulation, in both normal and allergic individuals, far outweighed proliferative responses to the same antigens (unpublished observation, 2004). Reduced IFN- γ secretion and enhanced IL-4 secretion in FA children in response to food allergens to which they are sensitized were measured in discrete allergen-specific T cell populations. Clinically tolerated food allergens evoked an *in vitro* response that was similar to non-sensitized children or non-atopic adults. *In vitro* antigen presentation through pulsed DCs enhanced the magnitude of the responses, but did not affect the overall response pattern. Allergen-specific T cell responses in both FA children and in controls showed that the major responding cell is within the CD45 RO⁺ memory or activated cell population.

The cytokine secretion by blood-derived lymphocytes of children with multiple food sensitizations showed altered proportions of Th1- and Th2-like cells even in the absence of allergen-specific stimulation. The proportion of cells producing IL-10 was elevated, and the proportion of cells producing IFN- γ was reduced. IL-2-producing cell populations were unaffected and did not differ in any of the groups studied. IL-2 has been reported to be normal in atopic children [9, 19], while IL-10 levels decreased in multiple sensitized FA children and in children at risk of developing allergies [24]. Similar changes have been observed in responses to LPS [25] and influenza infection [26] in allergic

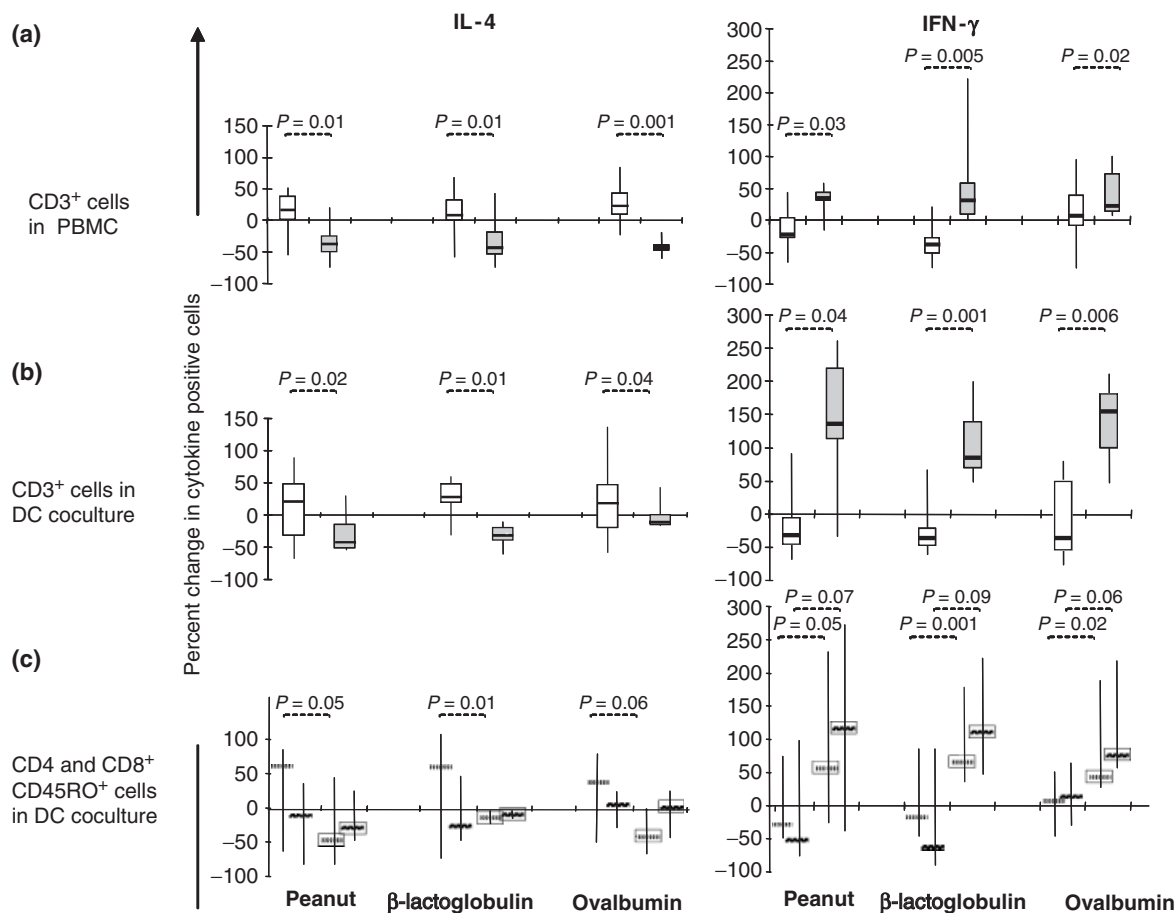


Fig. 3. Percentage change of T cells positive for IL-4 and IFN-γ in (a) peripheral blood mononuclear cells (PBMCs). Data displayed as median, complete range and 25–75% quartile ranges of sensitized (□), non-sensitized (■) children for each cytokine. (b) CD3⁺ T cells in response to dendritic cell (DC) presentation of food antigens. (c) CD45 RO⁺ CD4⁺ and CD45 RO⁺ CD8⁺ cells. Data displayed as complete range and median for CD4⁺ and (—) for CD8⁺ cells. Median values (—) of sensitized children are boxed (□). Changes in cytokine production were calculated as (% cytokine-positive cells with antigen/% cytokine-positive cells without antigen) – 1 × 100. P-values ≤ 0.09 are displayed.

Table 2. Correlation of IL-4 and IFN-γ with specific and total IgE

	Specific IgE	Total IgE
Peanut		
IL-4	0.08	0.02*
IFN-γ	0.23	0.10
Milk		
IL-4	0.24	0.04*
IFN-γ	0.38	0.001**
Egg		
IL-4	0.64	0.08
IFN-γ	0.63	0.04*

* $P < 0.05$, ** $P < 0.001$.

individuals. Increased IL-10 responses to cat and birch allergens have been described by Jenmalm et al. [11]. IL-10 inhibits both Th1 and Th2 cell populations and interferes with IgE synthesis and eosinophil survival [27, 28], and may facilitate the development of atopic disease by a failure to down-regulate initial stages of the allergic sensitization. Hypothetically, an increase in IL-10 secretion following allergen exposure could be related to acquisition tolerance, which is a common occurrence for milk and egg allergies in childhood.

IL-4 and IFN-γ cytokine responses were highly inversely correlated and there is some evidence of a counter-regulatory pathway in multiple-allergic children [29, 30]. IL-4 in food-sensitized children was maximally produced within 48 h, similar to the kinetics of the normal IFN-γ response. IL-4 and IFN-γ promoters may alternatively acquire stable hypoacetylation histone in polarized Th1 and Th2 cells [31, 32], which could allow the persistence of polarized cytokine responses in populations of allergen-specific cells.

Food allergen-induced IFN-γ production was predictably low in FA children compared with controls. This low IFN-γ production in culture was not increased by attempts to enhance the food allergen presentation by autologous DCs pulsed with allergen. Mitogenic IFN-γ responses were unaffected and proliferative responses to mitogens in allergic children and controls were not different (data not shown), indicating a more specific regulatory defect in response to allergen exposure in these patients. This is consistent with the model of delayed maturation of Th1-associated functions as a primary risk factor for manifestation of atopic disorders [8–10, 33].

FA children in our study showed an allergen-specific Th2 cytokine response in the CD4⁺ lymphocyte population, and a normal allergen-specific Th1 response to clinically tolerated

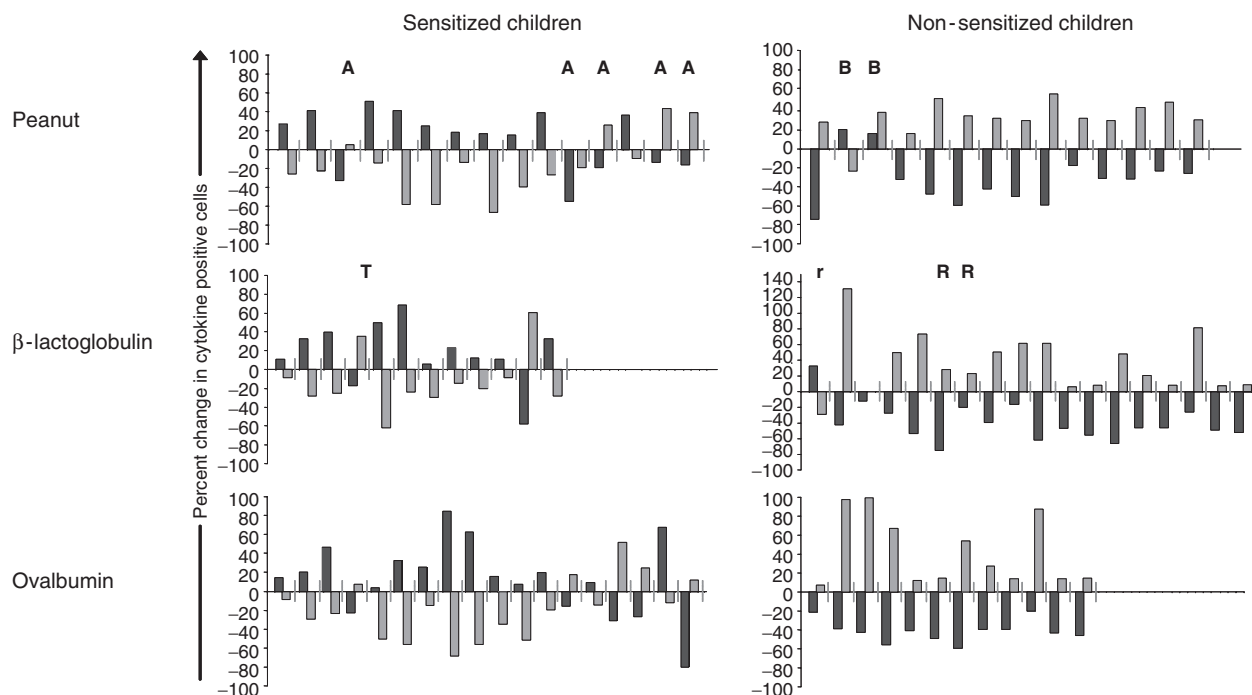


Fig. 4. Individual percentage change in % IL-4- and IFN- γ -positive cells in peripheral blood mononuclear cells of sensitized and non-sensitized children cultured with peanut extract, β -lactoglobulin (BLG) and ovalbumin (OVA). (■) = IL-4. (▒) = IFN- γ . 'A' indicates a peanut extract-sensitized child who responded with a decrease in IL-4 production after peanut extract stimulation. 'B' indicates a peanut extract-tolerating child who responded with increased IL-4 T cell production to peanut extract stimulation. 'r' indicates clinically resolving allergy. 'R' indicates resolved milk allergy. The majority of sensitized children showed an increase in IL-4 and a decrease in IFN- γ on exposure to allergens. Cells of non-sensitized children responded almost uniformly with an increase in IFN- γ production when exposed to peanut extract, BLG and OVA. Sensitized and non-sensitized children have been arranged on the X-axis, with those with the highest serum concentration of total IgE on the left.

food antigens in over 90%. Food allergens in children with resolved allergy evoked an *in vitro* response similar to non-sensitized children or non-atopic adults. IFN- γ was mainly derived from CD4⁺ and CD8⁺ CD45 RO⁺ cell populations, CD8⁺ IFN- γ -producing cells being particularly reduced in FA. The majority of allergic children showed increased IL-4 production restricted to their CD4⁺ CD45 RO⁺ cell population in response to allergen-pulsed DCs. IL-4 production was similarly restricted to CD45 RO⁺ T cells in mitogenic responses of children with multiple allergies [18], atopic dermatitis [10] and eczema [22]. Allergic children who generated a Th1 response to a sensitizing antigen tended to be those with low total IgE and diverse allergies, while non-sensitized children generating a Th2 response had high total IgE and more allergies. Children with a clinically resolving milk allergy had persisting Th2 cytokine responses. Resumption of Th1 cytokine production was only seen after complete resolution of allergy, as described for peanut- [20] and egg-sensitized [34] children. Longitudinal studies of antigen-specific cytometry and analysis of promoter histone acetylation of memory T lymphocytes could further indicate how closely associated cytokine polarization of CD45 RO⁺ cells is involved in the transition to tolerance and resolution of allergy.

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