

Allergen-induced cytokine secretion in relation to atopic symptoms and immunoglobulin E and immunoglobulin G subclass antibody responses

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There are few studies on allergen-induced cytokine production in allergic children, and little is known of antigen-specific cytokine regulation of human immunoglobulin (Ig) G subclass antibody responses. An association with T-helper 1 (Th1)-like immunity and complement-activating antibodies remains to be demonstrated in humans. We have previously observed that atopic symptoms are associated with high levels of IgG subclass, especially IgG₄, antibodies to birch and β -lactoglobulin. The differences were seen early in life for the food allergen and increased with age for the inhaled allergen. The aim of this study was to investigate the association between atopic symptoms, birch allergen-, and β -lactoglobulin-induced cytokine production in peripheral blood mononuclear cells (PBMC), and serum IgE and IgG subclass antibody responses to these allergens in children in order to further clarify the role of Th1- and Th2-like immunity in responses to various antigens. PBMC from 55 eight-year old children, who had been followed prospectively from birth, were stimulated with birch- and β -lactoglobulin. Production of interleukin (IL)-5, IL-6, IL-10, IL-13 and interferon (IFN)- γ was analysed by ELISA and expression of IL-4 and IL-9 mRNA by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). IgG subclass antibody levels to birch- and β -lactoglobulin in serum were determined by ELISA, and IgE antibodies by Magic-LiteTM and CAP-RASTTM, respectively. Birch-induced expression of IL-4, but not of the other cytokines, was associated with IgE antibodies to birch. Furthermore, the IL-4 expression and IL-6 production correlated with serum IgG₄ antibody levels to this allergen, and IFN- γ secretion with IgG₁ antibody responses. There were no correlations between β -lactoglobulin-stimulated cytokine production and IgG subclass antibody levels to that allergen, except for a negative association between β -lactoglobulin-stimulated IL-4 expression and IgG₁ antibodies. Atopic children tended to have high levels of birch and β -lactoglobulin-induced IL-5, IL-6 and IL-10 secretion. Birch-induced IL-4 expression may be the major factor in determining IgE antibody formation to that allergen, while allergen-induced IL-5, IL-6 and IL-10 secretion in PBMC is associated with atopic symptoms. Th1-like immunity to inhaled allergens could be associated with production of the opsonizing and complement-activating IgG₁ antibody subclass, and Th2-like immunity with IgG₄ antibody responses.

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Allergic diseases are believed to be due to T-helper 2 (Th2)-like immunity to allergens in affected tissues (1). The Th1/Th2-concept was originally described in murine models (reviewed in (2)), where immune responses to allergens delivered to the respiratory mucosa are characterized by a cross-regulation between interferon (IFN)- γ -secreting Th1 and Th2 cell populations producing interleukin (IL)-4, the latter thereby stimulating immunoglobulin (Ig) E production (3). The Th1/Th2 dichotomy was later also described in humans (4). Allergen-specific T-cell clones from atopic adults appear to be polarized towards a Th2 (4) or Th0 (5) phenotype. More recently, atopy was shown to be associated with increased levels of IL-4 (6,7), IL-5 (8,9) and IL-13 (8) accompanied by decreased (6) or similar (8,9) levels of IFN- γ in allergen stimulated bulk cultures with peripheral blood mononuclear cells (PBMC).

Relatively little is known of cytokine regulation of human IgG subclass antibody responses, and antigen-specific responses in particular. In mice, IL-4 stimulates production of non-complement-activating IgG₁ antibodies, whereas IFN- γ inhibits IgG₁ responses and enhances synthesis of complement-activating and opsonizing IgG_{2a} antibodies (10). Therefore, it has been suggested that Th1-like immunity stimulates antibody responses important for phagocyte-dependent defence. In humans, this is less clear. The production of the complement-activating and opsonizing IgG subclasses, i.e. IgG₁ and IgG₃, was upregulated by IL-10 in anti-CD40-stimulated naive IgD + B cells (11). Another study showed that IFN- γ inhibited PWM-stimulated PBMC IgG₁ antibody synthesis, whereas spontaneous IgG₂ antibody synthesis in PBMC was enhanced (12).

There is more evidence that IL-4 stimulates IgG₄ antibody production. This has been shown with PBMC (13–16), as well as with purified B cells costimulated via the CD40 pathway (17). IL-4-induced IgG₄ production from PBMC is inhibited by IFN- γ (13,15,16), whereas this cytokine has no effect in the B cell–CD40 system (17). Anti-IL-4 antibodies reduced antigen-specific IgG₄ antibody responses in helminth-stimulated PBMC from travellers to endemic areas, whereas anti-IFN- γ antibodies enhanced the synthesis of all antigen-specific IgG subclasses (18,19). Induction of synthesis of IgG₄ antibodies to bee venom phospholipase A₂ in allergen-stimulated PBMC in the presence of soluble CD40 ligand was also shown to be dependent on IL-4, whereas once switching had occurred, Th1-inducing conditions (CD2 stimu-

lation) enhanced IgG₄, but inhibited IgE antibody expression (20). *In vivo* studies of immune responses to parasites in humans also support Th2-like immunity (21) being associated with IgG₄ antibody production (22), whereas patients producing antigen-specific IFN- γ show IgG₁, IgG₂ and IgG₃ antibodies to helminths.

In a cohort of 96 children followed prospectively from birth to 8 years of age, we have previously observed that atopic symptoms were associated with high IgG subclass, especially IgG₄, antibodies to birch- and β -lactoglobulin, with the differences seen early in life for the food allergen and increasing with age for the inhaled allergen (23) (see reference (48)). The aim of this study was to investigate the association between atopic symptoms, birch- and β -lactoglobulin-induced cytokine production in PBMC, and serum IgE and IgG subclass antibody responses to the allergens in 55 of those 8-year-old children in order to further clarify the role of Th1- and Th2-like immunity in responses to various antigens.

Materials and methods

Study group

The study comprised 55 8-year-old children followed from birth with questionnaires mailed at 3, 6, 9 and 12 months and then yearly up to 8 years. At 6 months, 18 months and 8 years, a medical examination focusing on signs of atopic disease, an interview with one or both parents, a venous blood sample and skin-prick tests were performed. The children were selected based on availability of separated PBMC from an original cohort of 96 children, which has been described in detail previously (23). The PBMC were collected from October 1995 to November 1996, with cells from similar numbers of atopic and non-atopic children collected before, during and after the birch pollen season.

Atopic dermatitis was defined as pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution, as suggested by Hanifin and Rajka (24). Asthma was defined as three or more episodes of bronchial obstruction, at least once verified by a physician. Allergic rhinoconjunctivitis was defined as rhinitis and conjunctivitis appearing at least twice after exposure to a particular allergen and not related to infection. Urticaria was defined as allergic if it appeared at least twice within one hour after exposure to a particular allergen. Probable atopy was used when some, but not all of the diagnostic features described

above were present. Twenty-two of the children were diagnosed as atopic during the 8-year follow-up period. Six children were classified as probably atopic and 27 children did not develop any allergic manifestations. Six children were sensitized to birch, i.e. they had positive skin-prick tests or circulating IgE antibodies to birch (range 1.4–92.2 SU/ml). Six of the children had been sensitized to milk early in life, and two children were still sensitized at 8 years of age.

Cell culture

At 8 years of age, venous blood was drawn into tubes with preservative-free heparin (Becton Dickinson, Stockholm, Sweden). PBMC were isolated on Ficoll Paque density gradient (Pharmacia Biotech, Sollentuna, Sweden), and washed three times in RPMI-1640 (Life Technologies AB, Täby, Sweden) containing 2% fetal calf serum (Life Technologies AB). They were then cryopreserved by standard methodology in 10% DMSO (Sigma-Aldrich, Stockholm, Sweden), 50% fetal calf serum and 40% RPMI-1640. After thawing, the cells were resuspended at 1×10^6 viable cells/ml in AIM-V serum-free medium (Life Technologies AB, Mulgrave, Australia) supplemented with 20 mM mercaptoethanol (Sigma-Aldrich, Castle Hill, Australia). One-ml aliquots were cultured at 37°C with 5% CO₂ with medium alone, 10 kSQU/ml Aquagen birch extract (ALK, Hørsholm, Denmark) or 10 µg/ml β-lactoglobulin (Sigma-Aldrich). Optimal doses and time points for cytokine secretion were determined in a pilot study including cells from five adults. After 24 and 96 h, the samples were centrifuged at 2000 g for 5 min, the supernatants were aspirated and stored at –20°C and the cell pellets were treated as below. In samples with limited number of cells, the 24 h cultures had a higher priority than 96 h cultures, and stimulation with birch had a higher priority than stimulation with β-lactoglobulin.

Semiquantitative reverse transcriptase–polymerase chain reaction (RT–PCR) for β-actin, IL-4 and IL-9

Total RNA was extracted and cDNA were synthesized as described in (25). cDNA was amplified for IL-4, which was not detectable with ELISA, IL-9, to which no antibodies are available, and β-actin as an internal control for cDNA levels. The PCRs were performed according to (25), except that *Platinum Taq* DNA polymerase (Life Technologies) was used. The number of cycles was determined according to the detection level and linear phase of the reaction, and were 30 for β-actin and 38 for IL-4 and IL-9.

The primers were obtained from Life Technologies. The sequences for the β-actin and IL-4 primers have been described (25), and the IL-9 primer sequences were 5' GGG ATC CTG GAC ATC AAC TT 3' and 5' CAG AAG ACT CTT CAG AAA TG 3' (annealing temperature 56°C, expected product size 307 bp). Analysis of the amplified fragment size (by agarose gel and ethidium bromide staining) and nucleotide sequencing confirmed that the correct PCR products were amplified.

Following denaturation with sodium hydroxide, PCR products were blotted onto nylon membranes (Hybond N+, Amersham, UK) using a slot-blotter (Hoefer Scientific Instruments, San Francisco, CA, USA). The membranes were hybridized overnight with double-stranded probes as described in (25), and the binding was visualized by chemiluminescence using a commercial kit (ECL, Amersham) according to the manufacturer's instructions. The membranes were exposed to Hyperfilm (Amersham) and developed. After scanning the membranes, the intensity of each slot was determined using a densitometer (Scan Analysis 2.02, Biosoft, Cambridge, UK) and the results expressed as a ratio of cytokine to β-actin density after subtraction of background responses (cultures with medium only).

ELISA for the detection of IL-4, IL-5, IL-6, IL-10, IL-13 and IFN-γ

The levels of IL-4, IL-6, IL-13 and IFN-γ were determined by commercially available ELISAs (CLB Pelikine Compact™, Murex, Sydney, Australia), as described by the manufacturer. IL-5 levels were assayed with ELISA-kits (Endogen, Haem, Melbourne, Australia) according to the recommendations of the manufacturer, except that, for increased sensitivity, poly streptavidin-horseradish peroxidase from CLB was used instead of the streptavidin-horseradish peroxidase conjugate from Endogen. An in-house ELISA was used for determination of IL-10 levels, using rat-IgG₁-antihuman IL-10 monoclonal antibody (clone JES3–9D7, Pharmingen, Becton Dickinson, Sydney, Australia) for coating on Costar 3690 microtiter plates and biotinylated rat IgG_{2a}-antihuman-IL-10 monoclonal antibody (clone JES3–12G8, Pharmingen) for capture, and recombinant human IL-10 (CLB) for the standard curve. For detection, poly streptavidin-horseradish peroxidase conjugate (CLB) was added, followed by Neogen K-Blue® substrate (Geographic Scientific, Brisbane, Australia). Allergen-induced cytokine levels are given after

subtraction of background responses (cultures with medium only). The sensitivity limit for quantitative determinations were 2 pg/ml for IL-4 (for this cytokine, the cytokine levels in the samples were below the limit of detection), 6 pg/ml for IL-5, 2 pg/ml for IL-6, 10 pg/ml for IL-10, 3 pg/ml for IL-13, and 20 pg/ml for IFN- γ .

Conventional ELISA for the detection of IgG, IgG₁ and IgG₄ antibodies to rBet v 1 and β -lactoglobulin

The determination of IgG subclass antibodies to rBet v 1 and β -lactoglobulin was performed as described in detail elsewhere (26). Briefly, Costar 3590 microtitre plates (Life Technologies AB, Täby, Sweden) were coated with 10 μ g/ml β -lactoglobulin (Sigma-Aldrich, Stockholm, Sweden) or 1 μ g/ml rBet v 1 (kindly provided by Prof D Kraft, Institute of General and Experimental Pathology, AKH, University of Vienna, Austria) in PBS. Free binding sites were blocked with human serum albumin (HSA) (Chemicon, Stockholm, Sweden). Human serum samples or buffer without added serum (blanks) were added to duplicate wells, and then monoclonal antibodies to the human IgG subclasses (IgG (clone HP6064), IgG₁ (clone HP6069) and IgG₄ (clone HP6011), Bio Zac AB, Järfälla, Sweden) were added. These clones were all recommended by WHO/IUIS (27). Alkaline-phosphatase-conjugated rabbit antimouse IgG (Sigma-Aldrich, Stockholm, Sweden) was added, and p-nitrophenyl phosphate (pNPP) (Sigma-Aldrich) was used as substrate. The optical density (OD) was read at 405 nm. Values were expressed as arbitrary units (AU) deduced from the ODs of the reference serum curve, after subtracting the blanks. The reference sera, which were calibrated according to a semiquantitative method as described previously (26), were from a patient undergoing birch immunotherapy and an individual with known high antibody titres to β -lactoglobulin, respectively. The titres in the reference sera were, for antibodies to rBet v 1: IgG₁ 413 AU, IgG₂ 115 AU, IgG₃ 1.0 AU and IgG₄ 1845 AU, and for antibodies to β -lactoglobulin: 2300 AU for IgG₁, 1900 AU for IgG₂, 180 AU for IgG₃ and 8300 AU for IgG₄.

Enzyme-amplified ELISA for the detection of IgG₂, IgG₃ and IgG₄ antibodies to rBet v 1 and β -lactoglobulin

A more sensitive enzyme amplified ELISA was employed for the measurement of IgG₂ and IgG₃ antibodies and for samples where IgG₄ antibodies were not measurable with the conventional ELISA. Coating, blocking and addition of human serum samples, monoclonal antibodies

to IgG₂ (clone HP6014), IgG₃ (clone HP6050) and IgG₄, and alkaline-phosphatase-conjugated rabbit antimouse IgG were as described above. For the substrate reaction, AMPAK kits (Dakopatts AB, Älvsjö, Sweden) were used. NADPH in diethanolamine buffer was allowed to react with the phosphatase for 20 min followed by 10 min incubation with amplifier solution. OD was measured at 492 nm. Values were expressed as arbitrary units (AU) deduced from the ODs of the reference serum curve, after subtracting the blanks. Generally, intra-assay coefficients of variation were < 5% and interassay coefficients of variation were < 15% in the conventional and enzyme amplified ELISA.

Determination of IgE antibodies to birch and β -lactoglobulin

The levels of IgE antibodies to birch were analyzed with the high sensitivity protocol (incubation of serum samples over night) of Magic-LiteTM, a commercial chemiluminometric method, according to the recommendations of the manufacturer (ALK, Hørsholm, Denmark). The levels of IgE antibodies to β -lactoglobulin were determined by CAP-RASTTM, as described by the manufacturer (Pharmacia Diagnostics AB, Uppsala, Sweden).

Statistics

As the cytokine and antibody levels were not normally distributed even after logarithmic transformation, non-parametric tests, corrected for ties, were used. Comparisons between unpaired groups were analysed using the Mann-Whitney *U*-test, comparisons between paired groups with Wilcoxon signed rank test, and correlations were analysed with Spearman's rank order correlation coefficient test. The chi-squared test was employed for categorical variables, and Fisher's exact test was used when the expected frequency for any cell was less than five. A probability level of < 5% was considered to be statistically significant. Calculations were performed with a statistical package StatView 4.5 for Macintosh (Abacus Concepts Inc., Berkeley, California, USA).

Ethics

The study was approved by the Regional Ethics Committee for Human Research at the University Hospital of Linköping.

Table 1a. IgG subclass antibodies to birch and β -lactoglobulin in children with ($n = 22$) and without ($n = 27$) atopic symptoms. Median levels (AU) and the 10th and 90th percentiles are indicated. The levels in children with and without atopic symptoms were compared using the Mann–Whitney U -test

Allergen	Subclass	Atopic symptoms	No atopic symptoms	p
Birch	IgG ₁	20.8 (6.6–127.8)	13.6 (3.5–53.6)	ns
	IgG ₂	nd (nd–5.5)	nd (nd–2.5)	ns
	IgG ₃	nd (nd–0.2)	nd (nd)	ns
	IgG ₄	nd (nd–21.1)	nd (nd –2.1)	< 0.05
β -lactoglobulin	IgG ₁	79.2 (nd–1415.4)	73.7 (nd–670.5)	ns
	IgG ₂	16.9 (nd–394.9)	21.8 (nd–202.0)	ns
	IgG ₃	7.1 (nd–44.6)	4.1 (nd–40.3)	ns
	IgG ₄	71.2 (nd–3045.6)	87.2 (nd–2925.3)	ns

nd, not detectable. ns, not significant.

Results

IgG subclass antibody levels

Atopic symptoms were associated with high levels of IgG₄ antibodies to birch (Table 1a), with no differences for the other subclasses and for antibodies to β -lactoglobulin. Children who were, as compared to those who were not, sensitized to birch had higher levels of IgG, especially IgG₄, subclass antibodies to birch (Table 1b).

Frequency and kinetics of cytokine responses

The frequency of cytokine responses to birch (Table 2a) and β -lactoglobulin was similar. The levels of birch-stimulated IL-6 were similar at 96 and 24 h, whereas β -lactoglobulin-induced IL-6 production was higher at 96 h than 24 h ($p < 0.01$). IL-10 production was higher at 24 h than 96 h for both birch ($p < 0.001$) and β -lactoglobulin ($p < 0.01$), whereas the levels of IL-5, IL-13 and IFN- γ increased from the early to the late time-point for both allergens ($p < 0.001$ – $p < 0.01$). The levels at 24 and 96 h correlated for all cytokines ($r = 0.44$ – 0.90 , $p < 0.001$, $p < 0.05$).

Correlation between cytokines

As shown in Table 2b, the birch-induced IL-5 levels and IL-9 expression correlated with each other, as well as with the IL-10 and IL-13 levels, whereas IL-4 expression appeared to be independently regulated. The levels of IL-10 and IL-13,

as well as IL-6, also correlated with the IFN- γ levels. A similar pattern was observed for β -lactoglobulin-induced cytokines, except that for this antigen the IL-4 expression and IL-13 levels correlated.

Cytokine production in relation to sensitization

Four of the six children who were sensitized to birch (positive skin-prick tests and/or detectable IgE antibodies) expressed birch-induced IL-4 mRNA, as compared with five of 49 non-sensitized children (Fisher's exact test, $p < 0.01$), with no significant differences for the other cytokines. The numbers of cow's-milk-sensitized children were too small for a meaningful statistical analysis.

Cytokine production in relation to IgG subclass antibody levels

Three of five children with high levels of IgG₄ antibodies to rBet v 1 (> 10 AU) expressed birch-induced IL-4 mRNA, as compared with six of 50 children with low IgG₄ antibody levels (Fisher's exact test, $p < 0.05$). Similarly, increased birch-induced IL-6 production at 24 h was associated with high levels of IgG₄ antibodies to rBet v 1 ($p < 0.05$). In contrast, high levels of birch-induced IL-10 ($p < 0.01$) and IFN- γ at 96 h (Fig. 1) were associated with high levels of IgG₂ and IgG₁ antibodies to rBet v 1, respectively. There were no correlations between β -lactoglobulin-stimulated cytokine production and IgG subclass antibodies to that allergen,

Table 1b. IgG subclass antibodies to birch in children who were ($n = 6$) or were not ($n = 49$) sensitized to birch. Median levels (AU) and the 10th and 90th percentiles are indicated. The levels in sensitized and non-sensitized children were compared using the Mann–Whitney U -test

Allergen	Subclass	Birch-sensitized	Not birch-sensitized	p
Birch	IgG ₁	52.3 (17.1–456.3)	13.8 (4.6–42.5)	< 0.05
	IgG ₂	3.2 (nd–16.1)	nd (nd–3.5)	< 0.01
	IgG ₃	nd (nd–0.4)	nd (nd)	< 0.01
	IgG ₄	11.1 (1.1–102.4)	nd (nd –2.5)	< 0.001

nd, not detectable; ns, not significant.

Table 2a. Frequency of birch-induced cytokine responses. The percentage of positive responses are shown at 24 (n = 55) and 96 h (n = 33)

	IL-4	IL-5	IL-6	IL-9	IL-10	IL-13	IFN- γ
24 h	16%	0%	98%	23%	93%	60%	35%
96 h	nd	25%	97%	nd	64%	85%	61%

except for a negative association between IL-4 mRNA expression and IgG₁ antibody levels ($r = -0.38$, $p < 0.05$).

Cytokine production in relation to atopic symptoms

Birch- and β -lactoglobulin-stimulated cytokine production in children with, as compared to without, atopic symptoms during the first eight years of life, is shown in Figs 2 and 3. The results were closely similar in the subgroup of children with symptoms during the 12-month period preceding the 8-year follow up. Atopic symptoms were associated, and tended to be associated, with high levels of β -lactoglobulin and birch-induced IL-5 (Fig. 2) and IL-6 (Fig. 3a), respectively. Similarly, birch- and β -lactoglobulin-induced IL-10 production were higher in children with, as compared to without, atopic symptoms (Fig. 3b). High allergen-induced IL-9 expression and IL-13 secretion, as well as low IFN- γ secretion, also tended to be associated with atopic symptoms (data not shown). Children who had both atopic symptoms and were sensitized (positive skin-prick tests or detectable IgE antibodies to allergens at any age, $n = 6$) tended to have lower levels of β -lactoglobulin-stimulated IL-10 production at 24 h ($p = 0.07$) than the non-sensitized children with atopic symptoms ($n = 8$).

Discussion

The similar frequency of cytokine responses to the inhaled allergen birch and the food allergen β -lactoglobulin in 8-year-old children may seem to be in contrast to studies showing more proliferative (28) and cytokine (25) responses to

house dust mite than to ovalbumin after infancy, as responses to that food allergen are down-regulated after birth while responses to the inhalant allergen increase with age. There are, however, no data on the development of T-cell responses to birch or cow's milk proteins. We have recently observed that the IgG subclass antibody responses to β -lactoglobulin peak in early infancy and are then downregulated, although antibodies to the cow's milk protein are still detectable in the majority of children at 8 years of age (23).

As the levels of allergen-induced IL-5, IL-13 and IFN- γ , but not IL-6 and IL-10, increased from the early to the late time-point, the cellular sources of the cytokines may be different. Although not investigated in this study, $\geq 90\%$ of house dust mite- and ovalbumin-stimulated IL-6 production in PBMC from children has previously been shown by us to come from non-T-cells, and approximately 50% of IL-10 was also from the non-T-cell population (Macaubas et al., unpublished observations). Therefore, it is likely that monocytes are activated to secrete IL-6 and IL-10 early in the cultures, possibly via allergen cross-linking of antibodies bound to Fc γ R. It is possible that T cells may contribute even more to the IL-10 response at the late time-point, as it has also been shown that this cytokine is produced after, and is dependent on, IL-2 (29). It has been suggested that IL-10 synthesis is stimulated by proinflammatory cytokines to dampen the immune response as part of a negative-feedback mechanism (30).

There was a correlation between the Th2-like cytokines IL-5 and IL-9. Furthermore, the IL-10 and IL-13 levels correlated with those cytokines,

Table 2b. Correlation between birch-induced cytokines (Spearman's rank, corrected for ties, r values). The levels at 96 h ($n = 33$) are used for IL-5, IL-13 and IFN- γ and the levels at 24 h ($n = 55$) for the remaining cytokines

	IL-4	IL-5	IL-6	IL-9	IL-10	IL-13	IFN- γ
IL-4	—						
IL-5	ns	—					
IL-6	ns	ns	—				
IL-9	ns	0.34*	ns	—			
IL-10	ns	0.40*	0.50**	0.27*	—		
IL-13	ns	0.57**	ns	0.41*	0.55**	—	
IFN- γ	ns	ns	0.49**	ns	0.46**	0.57**	—

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

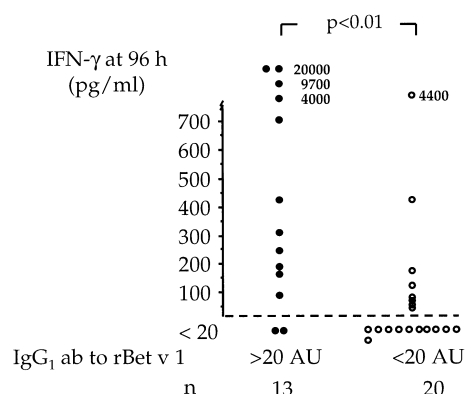


Fig. 1. Birch-induced IFN- γ secretion in children with high (●) and low (○) titres of IgG₁ antibodies to rBet v 1.

and also with IFN- γ , consistent with the suggestion that these cytokines are not strictly Th2-like in humans (5,31). The correlation between IL-6 and IL-10 was possibly a result of monocyte activation, resulting in IFN- γ production.

It is intriguing that IL-4 was the only cytokine that was related to the presence of circulating IgE and/or positive skin-prick tests to birch. Although the number of birch-sensitized individuals in this study was small and the lack of association with some of the other cytokines may be due to type-II errors, the findings suggest that IL-4 may be the major stimulant for IgE synthesis. IL-13 induces IgE switching independently of IL-4 in human B-cells, although less potently (32). The latter interleukin is also a less typical Th2 cytokine, as it is produced by both Th1 and Th2 clones. Furthermore, it does not induce Th2 differentiation because T cells lack functional IL-13 receptors (33).

Previous studies of allergen-induced IL-5 and IL-13 secretion in PBMC were performed in atopic individuals who were both symptomatic and sensitized to the studied allergens, while the controls were neither symptomatic nor sensitized (8,9). Therefore, it was not possible to determine whether high levels of those cytokines were related to atopic symptoms or to sensitization to the specific allergen. In another study (7), PBMC from birch-sensitized patients secreted IL-4 after stimulation with birch, but not cat, extract. The only other studied cytokine was IFN- γ .

Birch-induced IL-4 was also associated with high levels of IgG₄ antibodies to rBet v 1, and IFN- γ with high levels of IgG₁ antibodies. A similar cytokine regulation of IgG subclass antibody responses to parasites has been reported. Our findings would support the concept that Th1-like immunity stimulates complement-activating and opsonizing human IgG subclasses,

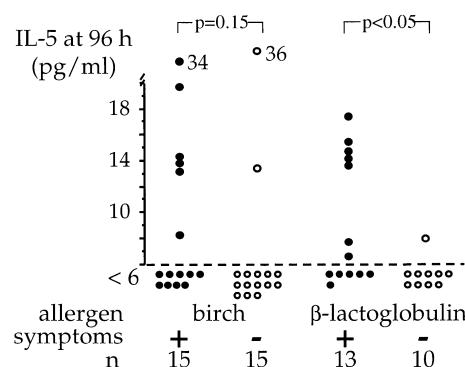


Fig. 2. Birch and β -lactoglobulin-induced IL-5 secretion in children with (●) and without (○) atopic symptoms during the first eight years of life.

as previously described in mice (10). High levels of IL-6 and IL-10 were associated with increased IgG₄ and IgG₂ antibody levels, respectively. These cytokines are important for isotype-committed B-lymphocyte differentiation and antibody secretion (34,35).

There was not a similar association between β -lactoglobulin-induced cytokine production and IgG subclass antibody levels to this cow's milk

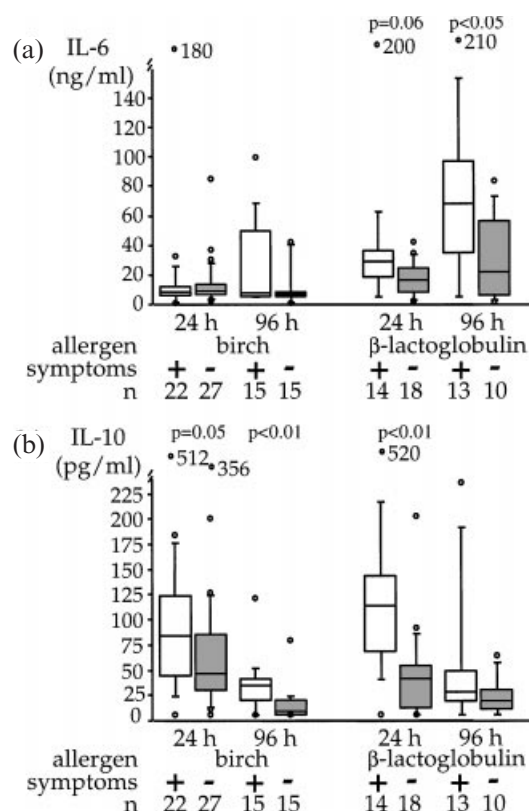


Fig. 3. Birch and β -lactoglobulin-induced IL-6 (a) and IL-10 (b) secretion in children with and without atopic symptoms. The 10th, 25th, 50th, 75th and 90th percentiles are indicated, as well as outliers.

protein. This may be due to differences in dose and route of exposure.

Atopic symptoms were associated, or tended to be associated, with high levels of birch- and β -lactoglobulin-induced IL-5, IL-6 and IL-10 secretion. IL-5 causes allergic inflammation by promoting eosinophil maturation, adhesion to endothelium, priming for chemotactic responses, activation, degranulation, cytotoxicity and survival (36). Enhanced expression of this cytokine has been demonstrated in the affected tissue in asthma (1), allergic rhinoconjunctivitis (37) and atopic dermatitis (38) as well as after allergen stimulation of PBMC of atopic individuals (8,9).

One of the many effects of the pleiotropic cytokine IL-6, which is produced by several different cell types after stimulation during infection, trauma or immunologic challenge, is to enhance humoral responses by promoting B-lymphocyte differentiation (34). The role of IL-6 in allergic disease is not well-studied, but it is released as part of the cutaneous response to allergen in a blister-chamber model (39). As IL-6 enhances secretory IgA responses (40,41), this cytokine may also have a role in downregulation of inflammation.

The involvement of IL-10 in allergic disease is controversial. This cytokine has both anti-inflammatory (30,42) and humoral immunity stimulating properties (35). Atopic patients show increased spontaneous mRNA expression in skin lesions and PBMC, and increased PHA/PMA-stimulated release of IL-10 (43,44). Atopic asthma has also been associated with decreased bronchoalveolar lavage IL-10 levels and decreased spontaneous and LPS-stimulated IL-10 production (45). The data on IL-10 regulation of IgE synthesis are also conflicting. The IL-4-induced IgE synthesis in the B-cell system is enhanced by IL-10 (35), whereas IL-10 reduces IL-4-stimulated IgE synthesis and enhances IgG₄ synthesis in PBMC (46,47). In contrast, IgG₄ production is enhanced by IL-10. A tempting explanation to these apparently contradictory results could be that IL-10 is upregulated by proinflammatory cytokines in inflamed tissues, although it may also be important in controlling type-I hypersensitivity reactions. We recently observed that house dust mite-induced IL-10 responses were negatively correlated with wheal size in sensitized children (Macaubas et al., unpublished observations). On the other hand, allergen-induced IL-10 secretion correlated with atopic symptoms in this study, even though the sensitized symptomatic children tended to produce less IL-10 than the non-sensitized children after β -lactoglobulin stimulation. Moreover, in

another study we observed anti-CD3-induced IL-10 secretion in PBMC to be higher in skin prick test negative than in skin prick test positive 18-month-old children with atopic eczema and the IL10/IL-4 ratio was negatively correlated with total serum IgE levels (Jenmalm et al., unpublished observations).

In conclusion, birch-induced IL-4 expression may be the major factor in determining IgE antibody formation to that allergen, while allergen-induced IL-5, IL-6 and IL-10 secretion in PBMC is associated with atopic symptoms. Th1-like immunity to inhaled allergens appears to be associated with production of the opsonizing and complement-activating IgG₁ antibody subclass, and Th2-like immunity with IgG₄ antibody responses.

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