

Cat allergen-induced cytokine secretion and Fel d 1–immunoglobulin G immune complexes in cord blood

R. Casas*, M. C. Jenmalm* and B. Björkstén†

*Department of Molecular and Clinical Medicine, Division of Paediatrics, Clinical Research Centre, Faculty of Health Sciences, University Hospital, Linköping, Sweden and †Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden

Summary

Background We have recently obtained evidence for the presence of immune complexes (IC) in cord blood from allergic and non-allergic mothers. Such complexes could conceivably provide the fetus with the initial trigger for the priming of the T cell system already *in utero*.

Objective To relate the presence of Fel d 1–IgG IC to T cell cytokine production in cord blood mononuclear cells (CBMCs) after stimulation with cat allergen.

Methods CBMC obtained from babies of 15 allergic and 22 non-allergic mothers were cultured in the presence of cat allergen. The production of IFN- γ , IL-5, IL-10 and IL-13 was determined by ELISA. Furthermore, IgG1 and IgG4 antibodies to cat allergen in cord blood samples were measured by ELISA. A more sensitive ELISA was used to measure Fel d 1–IgG IC.

Results The prevalence and levels of IC were similar in cord blood from children of allergic and non-allergic mothers. The production of IL-5, IL-10, IL-13 and IFN- γ by CBMC was not influenced by maternal atopy, but IFN- γ was less commonly detected in samples with IC. There was no association between the presence of IC and any other cytokines. The levels of IgG1 and IgG4 antibodies were similar in both groups, and tended to be associated with the presence of IC.

Conclusion Immune complexes in cord blood may represent a normal mechanism for inducing primary immune responses, as the responses in babies from allergic and non-allergic mothers were largely similar. Low levels of IFN- γ seems to be related with the presence of IC in cord blood.

Keywords allergen, cord blood, cytokine, Fel d 1, IgG, immune complex

Submitted 25 March 2002; revised 20 October 2003; accepted 5 December 2003

Introduction

Increasing evidence suggests that immune responses to inhaled allergens may develop already before birth. It has been established that specific T cell responses in newborn babies can be demonstrated in cord blood, even prior to any direct exposure after birth [1–3]. This T cell priming can be demonstrated as early as 22 weeks of gestation [4]. Cord blood mononuclear cell (CBMC) responses to food and inhaled allergens are common, both in infants with and without an atopic family history [1, 5]. In contrast, immune responses to antigens to which the pregnant women are not generally exposed have not been found [5].

It has been suggested that peptides processed by the mother might access to the fetal circulation, either as free antigens or in complexes with IgG [6, 7]. We have previously reported the presence of the major cat allergen, Fel d 1, in sera from asthmatic children who are allergic to cat [8]. The allergen was mainly found in complexes with IgG and IgE antibodies.

We have also recently obtained evidence of the presence of IgG–Fel d 1 IC in cord blood from allergic and non-allergic mothers [9]. Such complexes present in the maternal circulation could provide the fetus with the initial trigger for the priming of the T cell system already *in utero*.

The cytokine milieu at the foeto-maternal interface is polarized towards the Th2 cytokine profile, as part of an evolutionary adaptative process to protect the placenta against the toxic effects of Th1 cytokines such as IFN- γ [10, 11]. Such polarization is followed by a postnatal immune deviation, as part of a natural process, of fetal Th2 skewed responses to allergens towards the Th1 cytokine response [12]. This process appears to occur less efficiently in allergic than non-allergic infants [13]. Furthermore, the postnatal maturation of T cell cytokines, in particular IFN- γ , may be delayed in children from families with an atopic history [14]. This difference appears to be present already in cord blood [15].

The objective of our work was to investigate cord blood samples from babies of allergic and non-allergic mothers for the presence of Fel d 1 in immune complexes (IC) with IgG antibodies, and their possible relationship with cytokine responses in CBMCs after stimulation with cat allergen.

Correspondence: Rosaura Casas, Department of Molecular and Clinical Medicine, Division of Paediatrics, Clinical Research Centre, Faculty of Health Sciences, Linköping University, S-581 85 Linköping, Sweden.
E-mail: rosaura.casas@imk.liu.se

Materials and methods

Study group

Pregnant women and their families were invited to participate in a prospective study of the development of allergic disease in early childhood. For the present study, cord blood samples were obtained from 37 newborn babies. The infants were selected based on availability of separated CBMCs. The atopic status of the mother (15 allergics and 22 non-allergics) was established by a typical clinical history, e.g. allergic rhinoconjunctivitis, allergic asthma or flexural itchy and the presence of circulating IgE antibodies by an Allergy Screen test (Magic Lite SQTM Allergy Screen, ALK, Hørsholm, Denmark). The parents gave their informed consent to participate in the study, and the study was approved by the Regional Ethics Committee for Human Research at the University Hospital of Linköping.

Cell culture Cord blood was collected into tubes with preservative free heparin (Becton Dickinson, Stockholm, Sweden). Cord blood mononuclear cells 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), supplemented with 20 μ M mercaptoethanol (Sigma-Aldrich). The viability of the cells was checked with trypan blue exclusion. Optimal allergen doses and time-points for cytokine secretion were determined in a pilot study including cells from six adults. Doses tested were 1000, 10 000 and 50 000 SU/mL and time-points tested were 24, 48, 72, 96, 144 and 192 h.

Aliquots of 1 mL were stimulated with 10 000 SU/mL cat allergen extract (Aquagen, ALK-Abelló, Hørsholm, Denmark). The cells were cultured at 37 °C under 5% CO₂ (Forma CO₂-incubator model 3862, Forma Scientific Inc., Marietta, OH, USA) for 24 or 144 h. The supernatants were collected after centrifugation and stored at -70 °C.

Background responses (cultures with medium only) were subtracted from the allergen-induced responses. IL-5, IL-13 and IFN- γ were analysed in the 144 h samples, while IL-10 was analysed in both supernatants collected after 24 and 144 h, as this cytokine is derived from various cellular sources [16]. The IL-10 secretion data were obtained after 24 h stimulation.

Cytokine analysis The production of IFN- γ , IL-5, IL-10 and IL-13 was assessed by ELISA. The following mouse anti-human coating antibodies were used in carbonate buffer, pH 9.6: IFN- γ (2 μ g/mL, clone no. 2571811, R&D Systems, Abingdon, UK), IL-5 (0.25 μ g/mL, clone no. TRFK 5, PharMingen, San Diego, CA, USA), IL-10 and IL-13 (dilution 1:100), coating antibodies, PeliPairTM reagent set, CLB, Amsterdam, the Netherlands). Excess free antibodies were washed off (Wellwash Ascent, Labsystems, Stockholm, Sweden) with $4 \times 250 \mu$ L phosphate-buffered saline (PBS)-Tween (0.05% Tween 20 in PBS, pH 7.4), and the wells were

incubated for 1 h at room temperature on a plate shaker (Thermostar, Labvision, Stockholm, Sweden) with 100 μ L/well of low-fat milk to block non-specific protein binding sites.

The plates were washed as described above and 50 μ L of samples or standards; recombinant human IFN- γ (R&D Systems), IL-5 (PharMingen), IL-10 and IL-13 (CLB) were added to duplicate wells. A control using AIM-V serum-free medium with 20 μ M mercaptoethanol was also included. The plates were incubated and washed as previously described.

Biotinylated detection antibodies, diluted in high-performance ELISA-dilution buffer (CLB), goat anti-human IFN- γ (0.20 μ g/mL, R&D Systems), rat anti-human IL-5 (1 μ g/mL, clone no. JES1-5A10, PharMingen), IL-10 and IL-13 (dilution 1:100), detection antibodies, CLB), were then added (50 μ L/well). This was followed by another cycle of incubation and wash.

Streptavidin-poly horseradish peroxidase (50 μ L/well, 0.1 μ M, CLB) was added, and the plates were incubated for 30 min, and then washed. Thereafter 3,3',5,5'-tetramethylbenzidine (TMB) liquid substrate (Sigma-Aldrich) was added (50 μ L/well), and incubated for 30 min in the dark. 1.8 M H₂SO₄ (50 μ L/well) was finally added to stop the reaction. The amount of substrate converted to product was thereafter detected as optical densities (ODs) at 450 nm in an Anthos ht II ELISA reader (Anthos ht II, Labdesign, Täby, Sweden). Values were expressed as pg/mL deduced from the OD of the standard curve after subtracting the blanks. The sensitivity limits for quantitative determinations were 6 pg/mL for IL-5 and IL-10, 2 pg/mL for IL-13, and 25 pg/mL for IFN- γ .

Antibody analyses Circulating IgG1 antibodies to cat allergen in cord blood samples were measured by a conventional ELISA, as previously described for the birch major allergen Bet v 1 [17]. Briefly, microtitre plates were coated with 200 μ L/well of cat extract (ALK-Abelló) at 10 mg/L in PBS. After blocking the protein binding sites with 200 μ L/well of human serum albumin (HSA) 0.5% in PBS-Tween, 200 μ L of serum samples were added. A reference curve was prepared with a standard serum. A mAb to IgG1 (clone HP6069) (Bio Zac AB, Järfälla, Sweden) was added, followed by alkaline phosphatase-conjugated rabbit-anti-mouse IgG (Sigma-Aldrich). The reaction was developed by FAST-pNPP (*p*-nitrophenyl phosphate) substrate (Sigma, St Louis, MO, USA). The OD was read at 405 nm and the values were expressed as arbitrary units (AU) deduced from the OD of the reference curve, subtracting the blanks.

A more sensitive amplified ELISA was used to measure the IgG4 antibodies to cat. The steps of coating, blocking, sample and mAb to IgG4 (clone HP6011) addition were performed as described above. As substrate, AMPAK kits (Dakopatts AB, Älvsjö, Sweden) were employed. After washing with AMPAK buffer, the plates were incubated for 20 min with NADPH in diethanolamine buffer and for 10 min with the amplifier solution. The reaction was stopped with 50 μ L/well of 0.46 M sulphuric acid. The OD was measured at 492 nm. The antibody levels were calculated from the ODs of the reference curve after subtracting the OD of the blanks, and expressed as AU.

Immune complexes An amplified ELISA was employed for the detection of Fel d 1-IgG IC, as describe elsewhere [9]. Costar 5390 microplates (Life Technologies AB) were coated with the anti Fel d 1 monoclonal antibody in ascites (KAT 1, ALK-Abelló,) diluted in carbonate-bicarbonate buffer. After blocking the free binding sites with 250 µL of 2% HSA in PBS, sera and cord blood samples diluted in PBS were added. Duplicate wells, with PBS only, were used as blanks. Subsequently, 100 µL/well of A-3150 alkaline phosphatase-conjugated goat anti-human IgG (Sigma-Aldrich) was added. As substrate, AMPAK kits (Dakopatts AB, Älvsjö, Sweden) were employed. After washing with AMPAK buffer, the plates were incubated with NADPH in diethanolamine buffer and with the amplifier solution. The reaction was stopped and OD was measured at 492 nm. A reference curve was prepared using a sample with Fel d 1-IgG IC, as confirmed by purification and Western blotting. The levels of IC in the samples were calculated from the ODs of the reference curve after subtracting the OD of the blanks and expressed as AU. Ratios higher than 2, corresponding to >30 AU/mL were considered to be positive.

Statistical analysis

As IC, cytokine and antibody levels were not normally distributed, even after logarithmic transformation, groups were compared with non-parametric tests. Comparisons between unpaired groups were performed by Mann-Whitney *U*-test. Correlations were analysed with Spearman's rank order correlation coefficient test. Fisher's exact test was used to compare proportions in unpaired groups, as the frequency for any cell was less than 5. Calculations were made by a statistical package StatView 4.5 for Macintosh (Abacus Concepts Inc., Berkeley, CA, USA).

Results

The prevalence of Fel d 1-IgG IC were similar in the cord blood from babies of allergic mothers and non-allergic mothers; i.e. 4/15 (27%) and 12/22 (54%) respectively ($P = 0.18$; Fisher's Exact test). The levels of IC were also similar in the two groups for those samples with detectable levels.

Cytokine secretion in response to stimulation with cat allergen was detected in 28/37 (76%) of the cell cultures. The prevalence of positive responses was similar in babies of allergic and non-allergic mothers, i.e. 9/15 (60%) and 18/22 (82%), respectively. Also the levels of IL-13, IFN- γ , IL-5, IL-10, IgG1 and IgG4 were similar in the two groups (Table 1).

IL-5 was detected in 19/37 samples (52%), while IFN- γ was detected in 8/37 (21%) (Table 2). There was an inverse association between the detection of IL-10 and IFN- γ , as IFN- γ was undetectable in 15/21 samples with detectable IL-10 (71%) ($P = 0.05$, Fisher's Exact test). All the samples with detectable IFN- γ also contained IL-5. Twelve of the 19 samples with detectable IL-5 secreted only this cytokine when they were stimulated with cat allergen, whereas the other seven secreted both IL-5 and IFN- γ . The cat-induced

cytokine levels of IL-5 and IL-13 correlated ($r = 0.73$; $P = 0.01$).

IFN- γ was less commonly detected in samples with than without IC, i.e. in 1/16 and 7/21 samples, respectively ($P = 0.05$; Fisher's Exact test) (Fig. 1). There was no association between the presence of IC and any of the other cytokines.

Furthermore, IgG1 antibodies to cat allergen were detected in 36/37 cord blood samples (97%), and only one sample from a baby of a non-allergic mother lacked such antibodies (Table 2). In 22 (59%) of the samples IgG4 antibodies to cat were detected. Ten of those positives corresponded to the allergic and 12 to the non-allergic groups, representing 67% and 54% of each group. The levels of IgG1 and IgG4 antibodies were similar in the two groups (Table 1), while higher levels of IgG1 and IgG4 antibodies were detected in samples with than those without IC ($P = 0.04$ and 0.01 respectively, Mann-Whitney *U*-test).

The IL-5 and IgG4 antibody levels correlated ($r = 0.7$, $P = 0.02$). A similar trend was observed for the levels of the IgG4 and IgG1 antibodies ($r = 0.64$, $P = 0.08$), as well as for the levels of IC and IgG4 antibodies to cat allergen ($r = 0.64$, $P = 0.06$; Spearman Rank correlation test).

Discussion

In this study we detected Fel d 1 major cat allergen in complexes with IgG immunoglobulins at the same frequency and levels in cord blood samples from children with mothers with and without allergic diseases, confirming the results of a previous study [9]. A recent study has proven transplacental transfer of allergens from the maternal to the fetal circulation, using an *ex vivo* placenta model, with two allergens; i.e. Bet v 1 inhalant allergen and β lactoglobulin food allergen [18]. In another study, Der p 1 allergen was detected in maternal blood, amniotic fluid and cord blood samples, providing evidence of transplacental and transamniotic transfer of allergens [19].

Immune responses to inhalant allergens are commonly detected in cord blood cells in atopic, as well as non-atopic individuals [1–3]. These responses are of fetal origin, as proven by the DNA extracted from fetal T cell clones [12]. It has been suggested that a specific T cell response by cord blood cells could be due to fetal exposure to low levels of allergenic peptides [4, 6, 12] either as free antigens, or in the form of immune complexes with maternal IgG antibodies commonly present in cord blood [17].

The presence of IC in cord blood was inversely associated with detectable levels of IFN- γ , but there was no association with the other cytokines, suggesting that IC may down-regulate IFN- γ . A decreased IFN- γ production capacity in neonatal as compared to adult T cells [20] seems to be related with a mechanism protecting the child *in utero* from the potentially harmful Th1 immunity [11]. However, neonatal immune responses in CBMC stimulated with house dust mite tend to be lower in infants who later develop atopic diseases, as compared to non-atopic babies [13]. Similarly, lower production of IFN- γ and IL-4 has been observed by polyclonally stimulated CBMC from children with a family

Table 1. Presence of Fel d 1–IgG immune complexes (IC), and IgG1 and IgG4 antibodies to cat allergen in cord blood, and cat-induced cytokine secretion in cord blood mononuclear cells (CBMC)

Allergic status of the mother	IFN- γ	IL-5	IL-13	IL-10	IC	IgG1	IgG4
Allergic	Negative	Negative	Negative	Negative	Negative	44.5	0.91
Allergic	Negative	Negative	Negative	Negative	Negative	130.2	13.5
Allergic	Negative	Negative	Negative	Negative	Negative	17.1	0.83
Allergic	Negative	Negative	Negative	Negative	Negative	15.0	6.07
Allergic	Negative	Negative	2.7	58	Negative	20.6	Negative
Allergic	Negative	Negative	Negative	Negative	Negative	22.2	5.18
Allergic	Negative	Negative	Negative	22.3	60.7	59.3	13.5
Allergic	Negative	13.6	43.7	6.5	71.9	44.3	13.5
Allergic	243.5	145.1	174.5	Negative	Negative	41.0	5.76
Allergic	33.7	62.3	18	108	Negative	28.2	Negative
Allergic	Negative	295.4	159	40.6	Negative	22.6	0.72
Allergic	81.3	75.9	50	306.5	Negative	17.3	1.05
Allergic	Negative	45.6	150	39.7	53.6	11.8	Negative
Allergic	Negative	6.8	22.9	45.5	223	14.9	Negative
Allergic	77.3	70.4	132	5.9	Negative	40.1	14.2
Non-allergic	Negative	54.7	Negative	217.1	36.3	151.2	Negative
Non-allergic	Negative	Negative	Negative	Negative	74.9	52.9	Negative
Non-allergic	Negative	13.4	17.2	–	Negative	22.7	Negative
Non-allergic	Negative	Negative	23.5	17.4	39.2	17.2	0.5
Non-allergic	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Non-allergic	Negative	Negative	5.4	19.5	Negative	49.4	6.7
Non-allergic	128.8	Negative	38.3	279.5	60.6	365	18.5
Non-allergic	Negative	Negative	Negative	Negative	41.9	28.1	5.9
Non-allergic	40.8	147	199.5	Negative	Negative	20.7	Negative
Non-allergic	Negative	12.6	3.4	19	Negative	15.7	Negative
Non-allergic	Negative	Negative	Negative	–	Negative	22.2	5.18
Non-allergic	Negative	12.9	12.1	18.8	208.6	49.1	13.5
Non-allergic	68.7	6.9	2.3	63.8	Negative	39.6	Negative
Non-allergic	Negative	Negative	23.2	37.6	255.6	72.5	Negative
Non-allergic	Negative	Negative	2	Negative	77.8	25.1	11.07
Non-allergic	Negative	17.8	57.9	–	620.5	365	13.5
Non-allergic	Negative	55	6	–	53.2	52.2	1.11
Non-allergic	Negative	98.2	77.3	–	Negative	33.1	Negative
Non-allergic	Negative	29.9	30.6	–	31	30.0	Negative
Non-allergic	Negative	23.7	35.1	67.3	Negative	31.1	Negative
Non-allergic	62.2	194.4	92	211.9	Negative	18.2	1.82
Non-allergic	Negative	Negative	6.6	41.3	37	23.1	Negative

The levels of IC and IgG1 and IgG4 antibodies are expressed in arbitrary units (AU). Levels of IL-5, IL-10, IL-13 and IFN- γ , are expressed in pg/mL.

Table 2. Presence of Fel d 1–IgG immune complexes (IC), and IgG1 and IgG4 antibodies to cat allergen in cord blood and cat-induced cytokine secretion in cord blood mononuclear cells (CBMC)

	Allergics			Non-allergics		
	<i>n</i>	Median	Range	<i>n</i>	Median	Range
IC	4/15	66	54–223	12/22	60	31–620
IgG1	15/15	23	12–130	21/22	31	16–365
IgG4	10/15	5	0.7–14	11/22	7	0.5–14
IFN- γ	4/15	79	34–243	4/22	65	41–129
IL-5	8/15	66	7–295	11/22	30	7–194
IL-10	9/14	41	6–306	11/16	41	17–279
IL-13	5/14	44	3–174	16/22	20	2–199

Number of samples (*n* = positives/total) from allergic and non-allergic mothers. The levels of IC and IgG1 and IgG4 antibodies are expressed in arbitrary units (AU). Levels of IL-5, IFN- γ , IL-10 and IL-13 are expressed in pg/mL. Median levels and ranges are given for positive samples.

history of atopy [15], as well as by allergen-stimulated CBMC from neonates who develop atopy in early childhood [21], suggesting that atopic children manifest a delayed maturation

of their cytokine responses. A reduced IFN- γ production in new-born children may play a role maintaining the Th2 responses during early postnatal life [22].

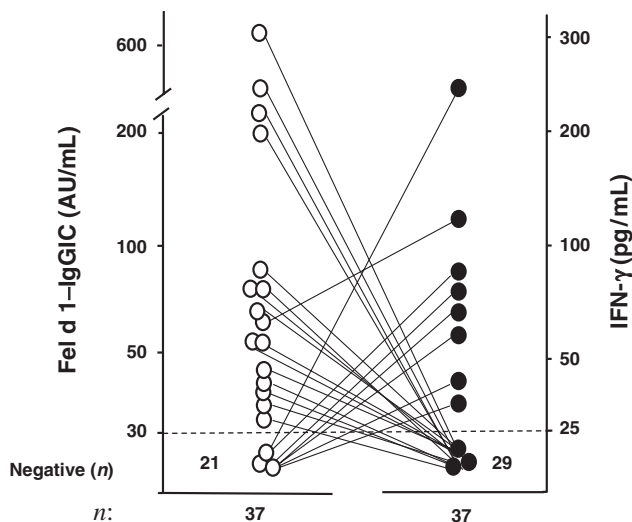


Fig. 1. Levels of immune complexes (ICs) (open circles) and IFN- γ (closed circles) in sera from allergic and non-allergic mothers. Levels of IC and IFN- γ from each sample are united by a line. The levels of ICs are expressed in arbitrary units (AU). The detection level was 30 AU/mL. Levels of IFN- γ are expressed in pg/mL. The detection level was 20 pg/mL.

Higher T cell responses to cat allergen at birth have been reported in infants whose mothers were exposed to low, rather than high concentrations of Fel d 1 during pregnancy [23]. As cat allergen is ubiquitous in Sweden [24, 25], we could not assess the effects of true lack of exposure. We have previously reported a poor relationship between exposure to cat and the presence of Fel d 1-IgG IC in cord blood, however [9].

It has previously been reported that IgG antibodies to inhalant allergens are present in virtually all neonates, both from atopic and non-atopic individuals [17, 26]. Furthermore, the levels of IgG4 to Fel d 1 are similar in allergic and non-allergic adults [27], probably due to the fact that the difference between allergic and non-allergic subjects disappears at high exposure levels [28]. Our findings indicate that higher levels of IgG1 and IgG4 antibodies were detected in samples with IC levels. Furthermore, the levels of IgG4 subclass antibodies in the maternal sera were related to the levels of IC in cord blood. We could not confirm, however, a previous reported of higher levels of IgG4 antibodies against cat allergen in cord blood from atopic as compared to non-atopic mothers [29].

Higher levels of IL-10 in amniotic fluid have been detected in atopic than non-atopic mothers [30]. We failed to demonstrate such differences in culture supernatants of CBMCs. In another study, high levels of IL-10 were detected in culture supernatants from CBMCs stimulated with house dust mite allergen, in both cells from infants with and without family history of allergy [31]. The inverse relationship between IL-10 and IFN- γ in that paper and in our study may be explained by the potent inhibitory effect of IL-10 on IFN- γ production [32]. This is consistent with other results indicating a depressed IFN- γ secreting capacity in CBMC associated to the Th2 polarization process [33].

The consequences of fetal immune responses to allergens for postnatal immunity are unknown, i.e. whether they are associated with allergy development or not. The lack of any

relationship to maternal allergy in our study would indicate that early immune responses may be part of the normal development of immunity. This does not exclude, however, other mechanisms for T cell stimulation. After birth, and under the influence of environmental factors, these T cell Th2 skewed populations can be driven along the pathways of Th2 boosting, a balanced Th1/Th2-type immunity, induction of tolerance or boosting of a Th2 skewed response associated with allergy.

In conclusion, immune complexes with allergen and maternally derived IgG antibodies in cord blood may represent a normal mechanism for inducing primary immune response.

Acknowledgements

This study was supported by the National Health and Lung Association, the National Association for The Prevention of Asthma and Allergies, the Swedish Foundation for Health Care Sciences and Allergy research, the Swedish Medical Research Council (Grant No. 7510), Sweden, and Glaxo Wellcome Ltd.

The authors wish to thank Kerstin Hagersten and Anne Marie Fornander for their technical assistance; Ing-Marie Sandberg and Lena Lindell for the helpful assistance with the serum samples.

References

- 1 Kondo M, Kobayashi Y, Shinoda S et al. Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Child* 1992; 67:1003-7.
- 2 Piccini M, Meccaci F, Sampognaro S et al. Aeroallergen Sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993; 102:301-3.
- 3 Szépfalusi Z, Nentwich I, Gerstmayr M. Prenatal allergen contact with milk proteins. *Clin Exp Allergy* 1997; 27:28-35.
- 4 Jones A, Miles EA, Warner J et al. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. *Pediatr Allergy Immunol* 1996; 7:109-16.
- 5 Holt P, O'Keefe PO, Holt BJ et al. T-cell 'priming' against environmental allergens in human neonates: sequential deletion of food antigen reactivity during infancy with concomitant expansion of responses to ubiquitous inhalant allergens. *Pediatr Allergy Immunol* 1995; 6:1-6.
- 6 Brown MA, Halonen MJ, Martínez F. Cutting the cord: is birth already too late for primary prevention of allergy? *Clin Exp Allergy* 1997; 27:4-6.
- 7 Malek A, Sager R, Schneider H. Transport of proteins across the human placenta. *Am J Reprod Immunol* 1998; 40:347-51.
- 8 Casas R, Djerf P, Häggström P et al. Circulating cat allergen and immune complexes in cat-allergic children. *Clin Exp Allergy* 1998; 28:1258-63.
- 9 Casas R, Björkstén B. Detection of Fel d 1-immunoglobulin G immune complexes in cord blood and sera from allergic and non-allergic mothers. *Pediatr Allergy Immunol* 2001; 12:59-64.
- 10 Raghupathy R. Th1-type immunity is incompatible with successful pregnancy. *Immunol Today* 1997; 18:478-82.

- 11 Wegmann T, Lin H, Guilbert L et al. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today* 1993; 14:353–6.
- 12 Prescott S, Macaubas C, Holt BJ et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; 160:4730–7.
- 13 Prescott S, Macaubas C, Smallacombe T et al. Development of allergen specific T-cell memory in atopic and normal children. *Lancet* 1999; 353:196–200.
- 14 Holt P, Clough BJ, Holt BJ. Genetic 'risk' for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992; 22:1093–9.
- 15 Rinas U, Horneff G, Wahn V. Interferon-gamma production by cord blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. *Pediatr Allergy Immunol* 1993; 4:60–4.
- 16 Macaubas C, Sly P, Burton P. Regulation of Th-cell responses to inhalant allergen during early childhood. *Clin Exp Allergy* 1999; 29:1223–31.
- 17 Jenmalm M, Holt P, Björkstén B. Maternal influence of IgG subclass antibodies to Bet v 1 during the first 18 months of life as detected with a sensitive ELISA. *Int Arch Allergy Immunol* 1997; 114:175–84.
- 18 Szépfalusi Z, Loibichler C, Pichler J et al. Direct evidence for transplacental allergen transfer. *Pediatr Res* 2000; 48:404–7.
- 19 Holloway J, Warner JO, Vance GHS et al. Detection of house-dust-mite allergen in amniotic fluid and umbilical-cord blood. *Lancet* 2000; 356:1900–2.
- 20 Holt P. Postnatal maturation of immune competence during infancy and childhood. *Pediatr Allergy Immunol* 1995; 6:59–70.
- 21 Warner J, 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.
- 22 Macaubas C, Prescott SL, Venaille TJ et al. Primary sensitisation to inhalant allergens. *Pediatr Allergy Immunol* 2000; (Suppl. 13): 9–11.
- 23 Warner J, Jones CA, Kilburn SA et al. Pre-natal sensitization in humans. *Pediatr Allergy Immunol* 2000; (Suppl. 13):6–8.
- 24 Munir AK, Björkstén B, Einarsson R et al. Cat (Fel d 1), dog (Can f 1), and cockroach allergens in homes of asthmatic children from three climatic zones in Sweden. *Allergy* 1994; 49:508–16.
- 25 Berge M, Munir A, Dreborg S. Concentrations of cat (Fel d 1), dog (Can f 1) and mite (Der f 1 and Der p 1) allergens in the clothing and school environment of Swedish school children with and without pets at home. *Pediatr Allergy Immunol* 1998; 9:25–30.
- 26 Mariani M-P, Price JF, Kemeny DM. The IgG subclass antibody response to an inhalant antigen (*Dermatophagoides pteronyssinus*) during the first year of life: evidence for early stimulation of the immune system following natural exposure. *Clin Exp Allergy* 1992; 22:29–33.
- 27 Vaughan J, McGee H, Squillace SP et al. Exposure to high concentrations of cat allergen at home is associated with increased IgG and IgG4 but not IgE to Fel d 1: IgG4 as a marker of a modified TH-2 response. *J Allergy Clin Immunol* 2000; 104 (Part 2):S363.
- 28 Aalberse RC, Van Milligen F, Tan KY et al. Allergen-specific IgG4 in atopic disease. *Allergy* 1993; 48:559–69.
- 29 Jenmalm M, Björkstén B. Cord blood levels of immunoglobulin G subclass antibodies to food and inhalant allergens in relation to maternal atopy and the development of atopic disease during the first 8 years of life. *Clin Exp Allergy* 2000; 30:34–40.
- 30 Warner J, Jones CA, Williams TJ et al. Maternal programming in asthma and allergy. *Clin Exp Allergy* 1998; 28:175–8.
- 31 Prescott S, Macaubas C, Yabuhara A et al. Developing patterns of T cell memory to environmental allergens in the first two years of life. *Int Arch Allergy Immunol* 1997; 113:75–9.
- 32 D'Andrea A, Aste-Amezaga M, Valiante NM et al. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med* 1993; 178:1041–8.
- 33 Holt P. Primary allergic sensitization to environmental antigens: Perinatal T cell priming as a determinant of responder phenotype in adulthood. *J Exp Med* 1996; 183:1297–301.