

Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms

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Background: Cytokine production at the materno-fetal interface may influence the development of atopy-predisposing immune responses. Because IL-13 possesses IL-4-like activity and may regulate the immune responses observed in atopy, it may contribute to the expression of the atopic phenotype initiated during intrauterine life.

Objective: We sought to examine IL-13 expression by fetal and neonatal cells and the placenta.

Methods: The production of IL-13 by neonatal and fetal T cells was examined by culturing the cells in the presence or absence of PHA. Production of IL-13 at term was considered in the context of the later development of atopic disease in the child. IL-13 expression in the placenta was assessed by using immunohistochemistry.

Results: IL-13 immunoreactivity within the placenta was restricted to 16 to 27 weeks' gestation (6/6 positive vs 0/10 at >27 weeks' gestation). In contrast, spontaneous release of IL-13 by fetal mononuclear cells was first observed from 27 weeks' gestation but was undetectable after 37 weeks' gestation. PHA-stimulated mononuclear cells showed increased IL-13 levels in 80% of samples. Term babies (>37 weeks' gestation) with a parental history of atopy with atopic symptoms by 3 years of age produced significantly lower concentrations of PHA-induced IL-13 when compared with babies with no parental history of atopy ($P = .034$).

Conclusion: Thus babies at risk of atopic disease in infancy display defective IL-13 production at birth. This may represent an inherent immaturity in the development of T cell-cytokine responses in babies at genetic risk for atopy or

could be a consequence of downregulation of responses by other factors. Normal pregnancy, irrespective of atopic status, is associated with the production of appreciable quantities of IL-13 initially by the placenta and subsequently by the fetus. The regulation of this production and its consequences for the mother and fetus remains to be elaborated. (*J Allergy Clin Immunol* 2000;105:951-9.)

Key words: IL-13, pregnancy, neonate, atopy

In utero priming of cells of the fetal immune system to antigen has been suggested by several investigators.¹⁻⁴ It has been shown that the development of atopic eczema in children is more strongly related to the atopic status of the mother than the father, and hence the prenatal environment is important in influencing the immune response to antigen.⁵ Pregnancy is associated with a T_{H2} bias and thereby downregulation of cell-mediated immunity that would otherwise compromise fetal survival.⁶ Lin et al⁷ observed the production of IL-4, IL-5, and IL-10 (T_{H2} -type cytokines) by the murine feto-placental unit, whereas IFN- γ production was undetectable in the second and third trimesters of pregnancy. Further investigation is required to define the balance of T_{H1} - and T_{H2} -type cytokines at the materno-fetal interface and to demonstrate the cellular source of such cytokines in humans.

T_{H2} -type cytokines are primarily involved in the induction and maintenance of IgE and IgG1 antibody production.⁸ There is evidence to suggest that the immunogenesis of atopic responses involves the clonal selection and expression of allergen-specific T_{H2} -type lymphocytes.⁹ IL-13 is a T_{H2} -type cytokine that is primarily produced by activated T cells,¹⁰ with the highest levels derived from $CD4^+$ T_{H0} and T_{H2} T-cell clones. Human basophils and mast cells are also capable of producing IL-13.^{11,12} IL-13 possesses IL-4-like activity; however, it does not act on T cells.¹³ IL-13 inhibits the production of proinflammatory cytokines derived from monocytes¹⁴ and induces B-cell proliferation and differentiation (including IgE production). Inhibition of monocyte-macrophage function by IL-13 includes suppression of IL-12 production with a concomitant reduction in T_{H1} cell differentiation. It is hypothesised that atopic mothers exhibit a supraphysiologic dominance of T_{H2} -type cytokines at the materno-fetal interface with possible promotion of a skewed atopy-predisposing immune response in the

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fetus. We aimed to investigate the production of IL-13 by fetal and neonatal mononuclear cells, as well as the placenta, during human gestation and at birth, respectively, and to relate IL-13 production at birth to the subsequent development of atopy.

METHODS

Study subjects

Blood samples were obtained from babies delivered between 24 and 34 weeks' gestation or aborted fetuses (terminated medically by using Prostin) between 15 and 23 weeks' gestation. Cord blood was obtained from the umbilical vein of the placenta after birth of premature babies, and fetal blood was obtained by means of cardiac puncture. Maternal blood was also obtained to acquire corresponding maternal plasma samples.

Cord blood samples were taken after normal vaginal deliveries of term babies (>37 weeks' gestation). The atopic status of the parents was determined by clinical history, with at least one positive skin prick test response to a common allergen and a bronchial challenge with histamine, whereby a PC₂₀ value caused by a concentration of histamine less than or equal to 8 mg/mL was regarded as an asthmatic response. The infants were clinically assessed for symptoms of atopic disease at 1 and 3 years of age, at which time skin prick tests against a panel of common allergens were also performed. All fetal and baby blood samples were acquired from the Princess Anne Hospital (Southampton, UK).

Babies with atopic parents (one or both) were regarded as being at high risk, whereas babies with nonatopic parents were placed in the low-risk control group. The high-risk babies were subdivided into the following groups: (1) the high-risk/disease group, defined as babies with an atopic parental history who had clinical atopic disease by 3 years of age; and (2) the high-risk/no disease group, defined as babies with an atopic parental history but without clinical atopic disease by 3 years of age.

Asthma was diagnosed in infants presenting with a history of two or more separate episodes of wheezing (in the absence of infection) or two or more episodes of paroxysmal nocturnal cough with sleep disturbance for 3 consecutive nights or infants receiving medication for asthma as prescribed by a physician. Eczema was diagnosed in infants presenting with erythematous, papular, microvesicular, and pruritic chronic skin eruptions, which were present for a duration of longer than 4 weeks.

Adult peripheral blood was obtained from healthy volunteers. All samples were collected with the approval of the Joint Ethics Committee of Southampton University Hospitals, and studies with fetal samples fulfilled the requirements of the Polkinghorne Committee report on the research use of fetuses and fetal tissue.

Isolation of PBMCs

PBMCs from fetal, baby, and adult heparinized blood samples were isolated by density-gradient centrifugation on Histopaque-1077 (Sigma Diagnostics) and washed in culture medium (RPMI-1640 supplemented with 2 mmol/L L-glutamine, 100 µg/mL streptomycin sulphate, and 100 U/mL benzyl penicillin).

Cell culture

PBMCs were cultured at a density of 1×10^6 cells/mL in culture medium supplemented with 10% autologous plasma in 24-well microtiter plates (Costar) at a total volume of 1 mL/well. Cells were stimulated with PHA (1 µg/mL) for 24 hours at 37°C in a humidified atmosphere with 5% CO₂ or incubated with supplemented medium alone for 24 hours. Cell-free supernatants were harvested and stored at -80°C until further analysis.

ELISA for IL-13

Concentrations of IL-13 protein in the supernatants of unstimulated and PHA-stimulated fetal, neonatal, and adult PBMC cultures were determined by using the Pelikine Compact sandwich IL-13 ELISA kit (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service). The detection limit for IL-13 was 0.5 pg/mL. The ELISA was performed according to manufacturer's instructions.

Immunohistochemistry

Placental biopsy specimens were fixed in ice-cold acetone containing 2 mmol/L phenylmethylsulphonyl fluoride and 20 mmol/L iodoacetamide overnight at -20°C. The following day the biopsy specimens were embedded into glycolmethacrylate, as described elsewhere.¹⁵ Sections (2 µm) were cut and floated onto ammonia water (0.2%) for 1 minute before mounting on poly-L-lysine slides. After blocking endogenous peroxidase (0.1% NaN₃, 0.3% H₂O₂) and nonspecific blocking, anti-human IL-13 antibody (mIgG1, R&D Systems) was applied and incubated overnight at room temperature. Antibody buffer and isotype-matched control were used as the negative control. After washing, the slides were incubated with biotinylated rabbit anti-mouse immunoglobulins (Dako) for 2 hours at room temperature. Streptavidin biotin-peroxidase complex (Streptavidin ABC, Dako) was applied after washing the slides, and the slides were incubated for 2 hours. The color was then developed by using aminoethylcarbazol with counterstaining in Mayer's hematoxylin. The sections were mounted in DPX (BD) and viewed by using light microscopy.

Statistics

Statistical analyses were performed with SPSS software (SPSS Inc). Spearman rank correlation analysis was used to compare the relationship between IL-13 levels in matched fetal cord and maternal plasma samples. The Kruskal-Wallis test (nonparametric one-way ANOVA) was used to compare IL-13 production among the low-risk, high-risk/no disease, and high-risk/disease groups. The Mann-Whitney U test was used to compare IL-13 production between pairs of groups. Statistical significance was inferred if the *P* value was less than .05.

RESULTS

IL-13 production by fetal and cord mononuclear cells during gestation and at term

The spontaneous release of IL-13 protein by unstimulated fetal and cord mononuclear cells at various time points during gestation is shown in Fig 1, A. IL-13 protein was detected in the supernatants as early as 27 weeks' gestation, with the highest level observed at 32 to 34 weeks' gestation. IL-13 production decreased to baseline concentrations by 36 weeks' gestation and remained below the limit of detection (0.5 pg/mL) up to and including term (>37 weeks' of gestation). PHA stimulation resulted in increased production of IL-13 protein in 80% of fetal and cord samples (Fig 1, B).

Concentration of IL-13 protein in paired fetal-maternal plasma samples during gestation and at term

The concentration of IL-13 protein in paired fetal cord-maternal plasma was measured to determine whether the IL-13 detectable in unstimulated mononu-

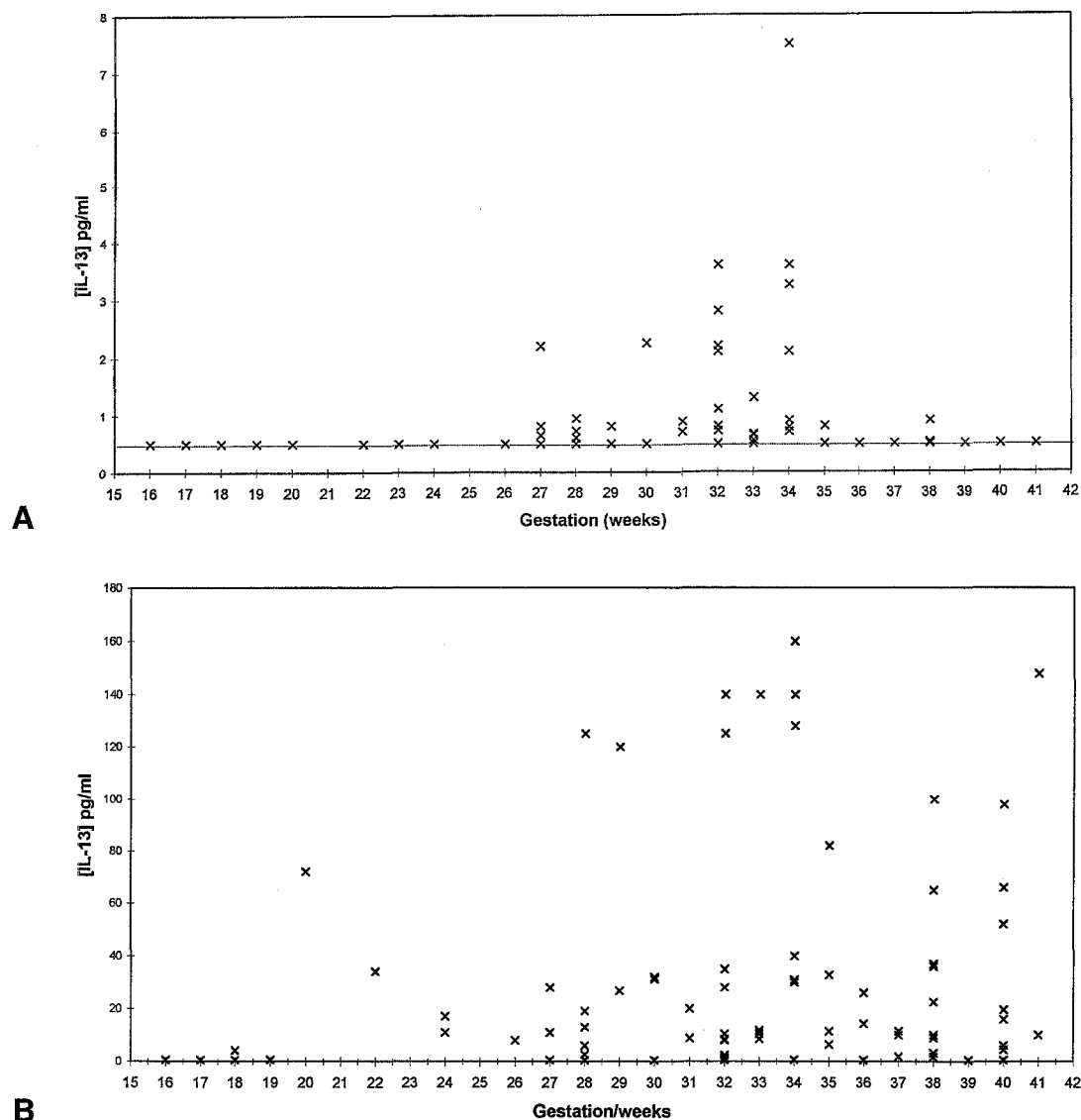


FIG 1. IL-13 production by fetal and cord blood mononuclear cells at different gestational ages to term. IL-13 produced into 24-hour culture supernatants from unstimulated (**A**) and PHA-stimulated (**B**, 1 μ g/mL) fetal and cord blood mononuclear cells was measured by using ELISA. The dotted horizontal line indicates the lower limit of detection (0.5 pg/mL), and the data from each individual is shown with a cross.

clear cell supernatants was derived from the cells per se or simply constituted the IL-13 present in the autologous plasma in which the mononuclear cells were cultured (10% of culture volume, Fig 2).

The concentrations of IL-13 in unstimulated fetal-cord mononuclear cell supernatants (Fig 1, A) could not be accounted for by the concentrations measured in autologous plasma alone (Fig 2, A) because the 10% diluted plasma used would not account for all the IL-13 seen after 24 hours' in vitro culture. Thus fetal and cord mononuclear cells spontaneously release IL-13 during the second and third trimesters of pregnancy.

Maternal plasma IL-13 concentrations showed a significant positive correlation ($r_s = 0.519$, $P = .03$; Fig 2, B) with the matched fetal/cord plasma IL-13 concentrations. Peak concentrations of IL-13 protein measured in paired

maternal-fetal cord plasma were observed at 32 weeks' gestation, with subsequent reduced concentrations occurring thereafter to term.

IL-13 production by adult and random cord mononuclear cells

The concentration of IL-13 protein in the supernatants of unstimulated and PHA-stimulated adult and random cord mononuclear cell cultures is shown in Fig 3. The spontaneous production of IL-13 by mononuclear cells was significantly higher in adults than in cord samples ($P = .001$, Mann-Whitney U test). There was no significant difference in PHA-stimulated IL-13 production between adult and cord mononuclear cells reflecting the capacity of cord mononuclear cells to produce levels of IL-13 comparable with those found in adults on mitogenic stimulation.

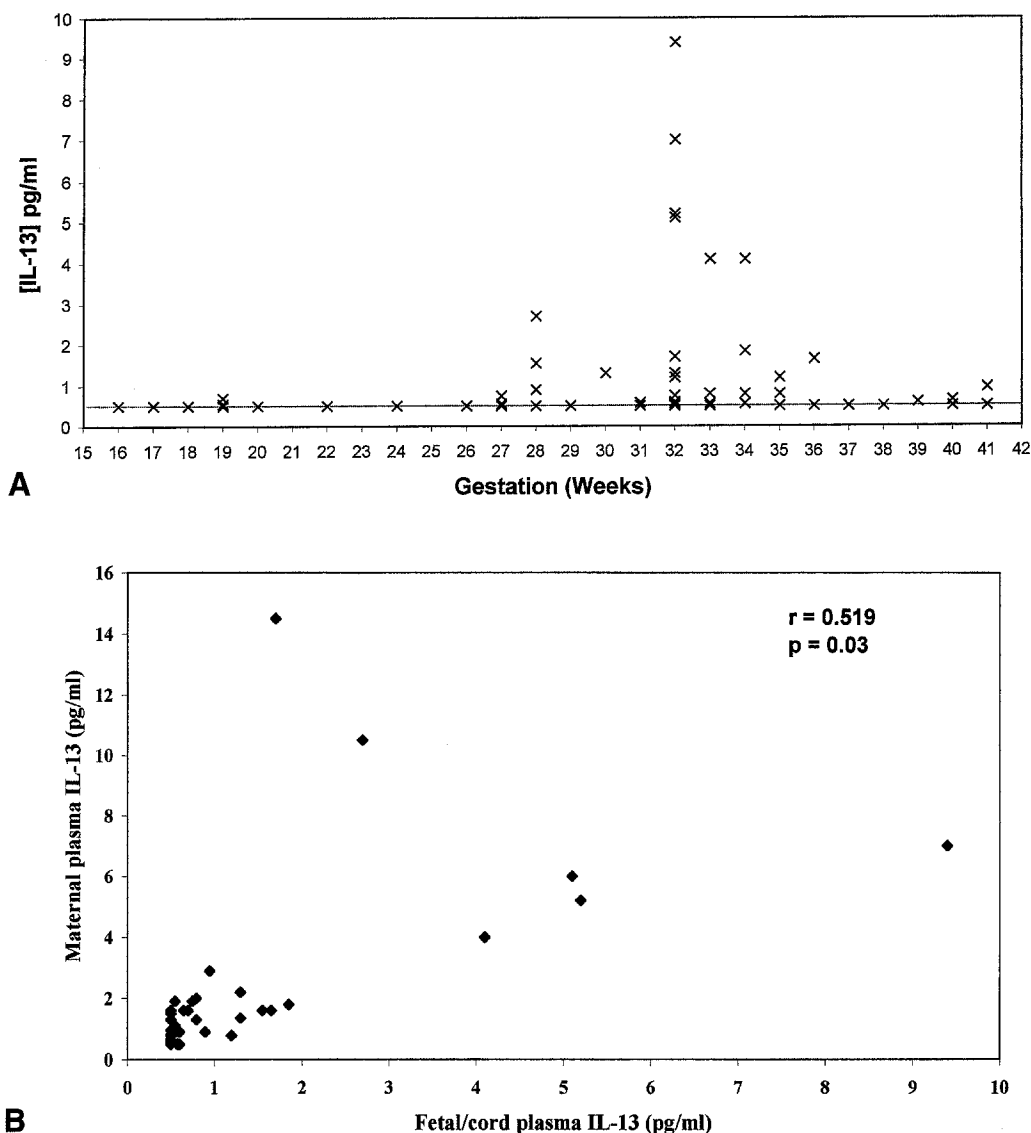


FIG 2. Concentration of IL-13 in paired fetal and maternal plasma at different gestational ages to term. A cytokine-specific ELISA was used to measure IL-13 in fetal and cord plasma (A), and these levels showed a significant positive correlation ($P = .03$) when compared with IL-13 levels in matched maternal plasma (B). The dotted horizontal line indicates the lower limit of detection (0.5 pg/mL), and individual data is shown by a cross in A.

Comparison of IL-13 production by cord mononuclear cells between babies at high risk for atopy and babies at low risk for atopy

The concentration of IL-13 protein in the supernatants of PHA-stimulated cord mononuclear cell cultures is shown in Fig 4. The Mann-Whitney U test was performed between all groups when the Kruskal-Wallis test result proved significant or almost significant. PHA-stimulated cord blood mononuclear cell cultures from babies at low risk displayed significantly increased production of IL-13 when compared with cord samples

obtained from babies at high risk for atopy that went on to have atopic disease ($P = .03$). The comparison between the low-risk group of babies and those at high risk with no development of atopic disease by 3 years of age was not significant ($P = .08$). There were no significant differences in spontaneous IL-13 production between groups (data not shown).

IL-13 immunoreactivity in the placenta

IL-13-positive cells (Fig 5) were observed in all placentas of 16 to 27 weeks' gestation ($n = 6$) but not in any of those of greater than 27 weeks' gestation, including samples to term ($n = 10$).

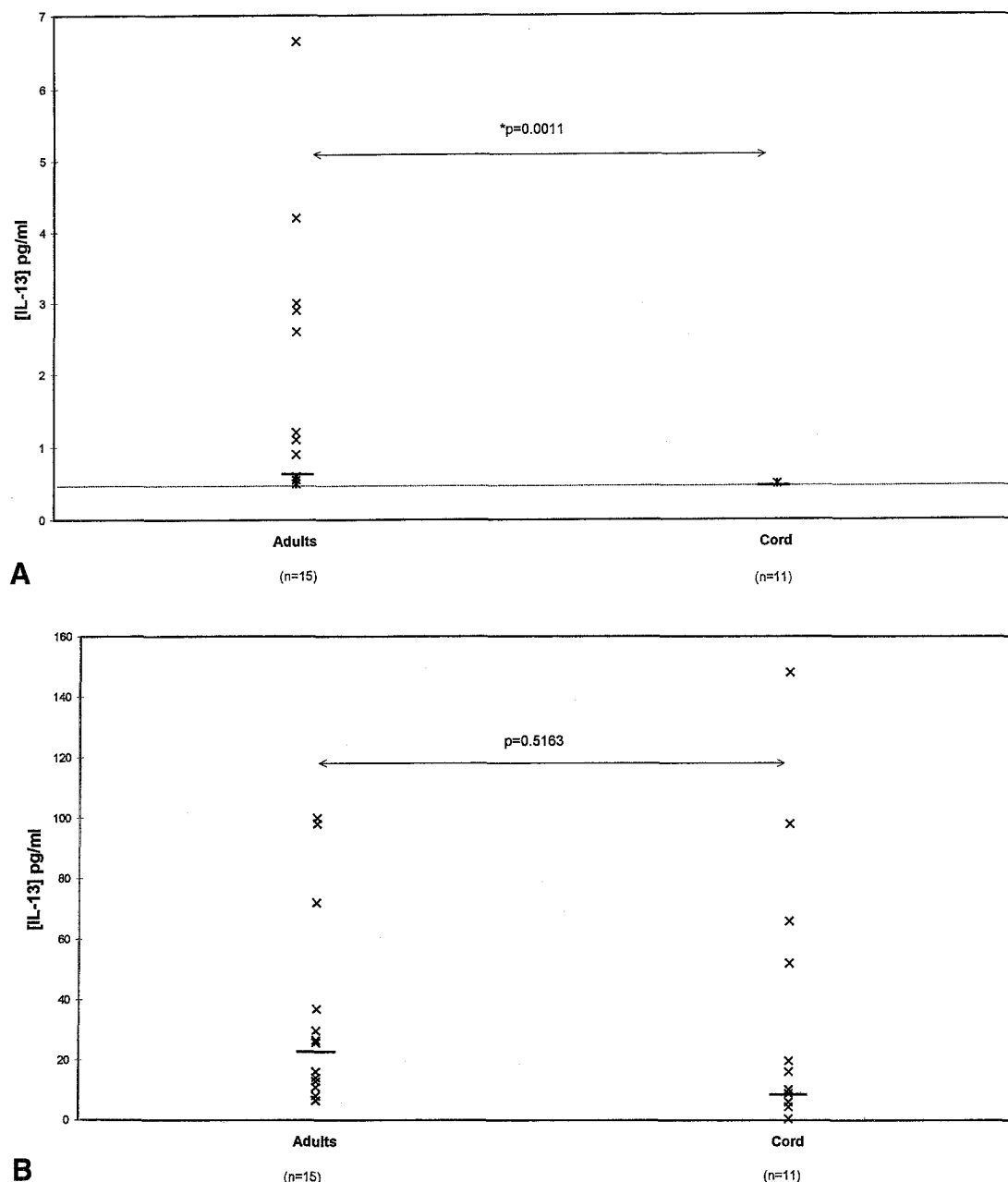


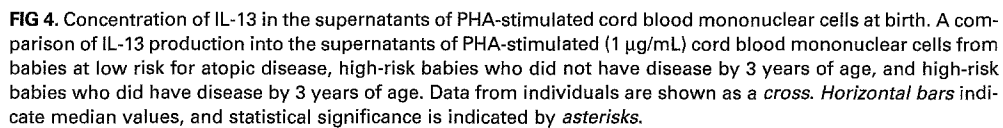
FIG 3. IL-13 production by adult and random term cord blood mononuclear cells. Levels in 24-hour culture supernatants of unstimulated (**A**) and PHA-stimulated (**B**, 1 μ g/mL) mononuclear cells are shown. The dotted horizontal line indicates the lower limit of detection (0.5 pg/mL). Median values are represented as solid horizontal bars, and data from individuals is shown as a cross.

DISCUSSION

The downregulation of T_{H1} -type immunity, and therefore the apparent T_{H2} -type skewing at the materno-fetal interface, during gestation is regarded as beneficial for the maintenance of pregnancy permitting the growing fetus to develop without the threat of harmful T_{H1} -type cytotoxic responses being mounted against the trophoblast cell population. IL-13 is a prototypic T_{H2} -type cytokine, and therefore IL-13 production by the fetal unit was investigated to develop understanding of the T_{H1}/T_{H2}

cytokine balance during gestation.

This study reports for the first time that IL-13 is spontaneously produced by fetal blood mononuclear cells during human gestation. Although the cultures were conducted in autologous plasma, which itself contains IL-13, the 10% used would not account for all the IL-13 seen after 24 hours' in vitro culture. The detection of this T_{H2} -type cytokine from as early as 27 weeks' gestation indicates that the fetal immune system is capable of cytokine synthesis and secretion from early in the third trimester and onward.



The aptness of comparing observed changes in immune responses during murine pregnancy to human pregnancy is, of course, confounded by a number of physiologic differences. However, the study by Lin et al⁷ presents findings that support the postulate that the expression of T_H2-type cytokines are favored during gestation.

This investigation of IL-13 production by the fetus and neonate is timely given the recent findings that not only does IL-13 have a central role in mediating allergic asthma,¹⁹ but it does so in an IL-4-independent manner.²⁰ To date, it is the production of IL-4 that has been the focus when considering the predominance of T_H2-type cytokines at the materno-fetal interface. Our study and that of Dealtry et al¹⁶ are the first to consider what, if any, role IL-13 has not only in the maintenance of pregnancy but in the development of the fetus itself.

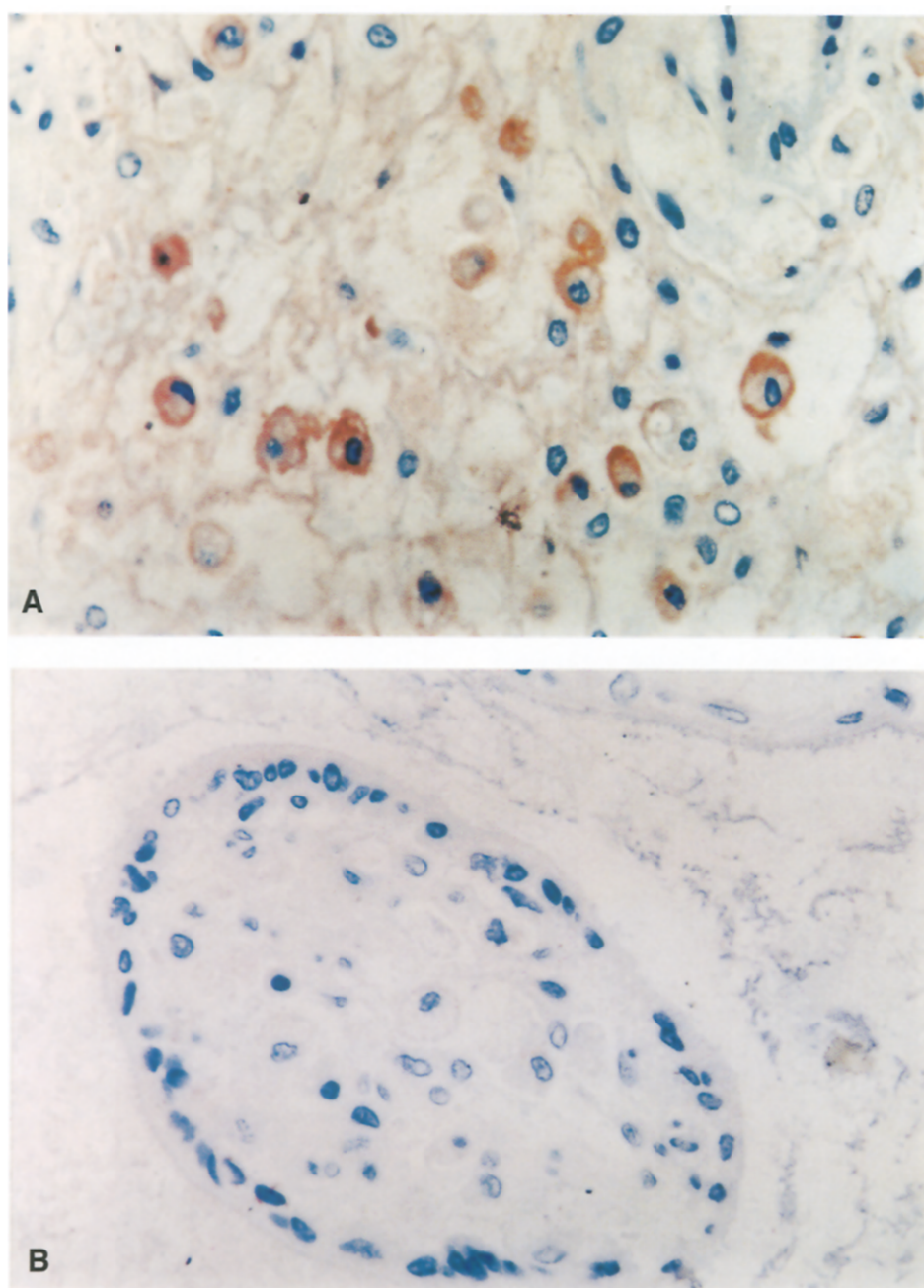


FIG 5. IL-13 immunoreactivity in the placenta. A representative example of 6 placental samples that were immunoreactive with an anti-human IL-13 mAb (A) and the isotype-matched control (B, mouse IgG1). Placentas of greater than 27 weeks' gestation ($n = 10$) did not show immunoreactivity with the anti-human IL-13 mAb.

The capacity of cord mononuclear cells to produce IFN- γ is deficient when compared with those of adults.^{21,22} An even greater reduction of PHA- and allergen-stimulated IFN- γ production is seen in cord blood cells from infants who are at increased risk of atopy because of their family history²³ or who actually do have atopic disease in infancy.²⁴ Therefore the production of IL-13 was investigated to determine the selectivity of cytokine secretion by cord mononuclear cells. Cord

mononuclear cells from term babies display reduced spontaneous IL-13 production when compared with those of adults (Fig 3); however, on PHA stimulation, IL-13 production by cord and adult mononuclear cells was not significantly different. The capacity of cord mononuclear cells to produce IL-13 is therefore not defective. This contrasts with the deficiency of IFN- γ production in stimulated cord blood mononuclear cells. Cord blood mononuclear cells have a specific capacity to secrete

individual cytokines. IL-10,²⁵ IL-4,²⁶ and IFN- γ production have been shown to be reduced relative to that found in adults, whereas reports of differences in IL-12 production have not been consistent.^{27,28} These observations can now be extended to include IL-13.

Despite the comparable production of IL-13 from PHA-stimulated adult and cord PBMCs, babies born to atopic parents and who went on to have clinical symptoms of atopic disease at 3 years of age displayed a reduced capacity to produce this cytokine when compared with babies born to nonatopic parents (Fig 4). Babies born to atopic parents who did not have atopic symptoms showed an almost significant reduction in IL-13 production on PHA stimulation when compared with low-risk babies. As similar observations regarding the deficiency in IFN- γ production in atopy-prone babies have been made,^{23,24,29} it has been anticipated that reduced levels of IFN- γ by atopy-prone babies would be associated with increased levels of T_{H2}-type cytokines, such as IL-13. This is not the case as shown in this and other studies. Prescott et al²⁹ also found significantly reduced allergen-specific IL-13 production by cord blood mononuclear cells from babies at high risk versus those at low risk of atopic disease, with IL-10 levels from cord blood mononuclear cells significantly reduced in children who had atopic disease by 2 years of age.³⁰ However, it is clear that cytokine production at birth is complex in its specificity. For example, Liao et al³¹ showed that IL-6 was increased in babies at high risk of having allergy. The polarization of the T_H phenotype at birth in normal individuals is considered to remain skewed toward the T_{H2}-type pathway until some months after birth, as demonstrated in animal models,³² with consolidation of T_{H2} responsiveness occurring over the first 2 years of life in children who have atopic disease.³⁰ The influence of postnatal allergen exposure, microbial infection, and other adjuvant factors on the development of this response are considered to be critical for directing the immune response toward a healthy phenotype.

Because IL-13 is a prototypic T_{H2}-type cytokine, these findings infer a generalized immaturity or suppression of both the T_{H1}- and T_{H2}-type immune responses at birth rather than an already existing bias toward T_{H2}-type reactivity in atopy-prone babies. Thus future therapeutic intervention in the development of atopy can now be also directed at modulating the immune response during pregnancy. A state of immune deviation could be induced with a shift from the T_{H2}- to the T_{H1}-type phenotype. The timing of such immune deviation would appear to be critical, however, because physiologic pregnancy is characterized by a baseline T_{H2}-type response. In the atopic neonate defects in the T cell and antigen-presenting cell compartments with subsequent delayed development of T_{H1}-type immunity may be more susceptible to targeted immune deviation. However, evidence of in utero priming to allergen reveals that preventative measures should be initiated earlier. Modulation of the maternal immune response, fetal immune response, or both during pregnancy may be a way forward in the prevention of allergic disease.

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