

Review article

Maternofetal interaction and allergy

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**J. A. Warner, A. C. Jones,
E. A. Miles, B. M. Colwell,
J. O. Warner**

Child Health, University of Southampton,
Southampton, UK

Dr Jill A. Warner
Child Health
Level G, Centre Block
Southampton General Hospital
Tremona Road
Southampton SO16 6YD
UK

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The heritability of allergic disease was first described by Cooke & Vander Veer in 1916 (1). Analysis of their allergic patients demonstrated a familial tendency with atopy in 50% of children with one allergic parent and in 80% of those with two allergic parents. More recently, Ruiz et al. (2) showed that significantly more infants with an allergic mother developed allergic eczema than those with an allergic father. In 1992, Cookson et al. (3) reported that the transmission of atopy at the chromosome 11q locus was detectable only through the maternal line. These studies suggest that an atopic mother can modify the development of the infant immune response toward the allergic phenotype. It is possible that this could be brought about by the antibody and cytokine profiles experienced by the fetus *in utero*.

The placenta is embryonic in origin, and contains genetic material from both the mother and the father. It has the potential to express paternally derived antigens and, therefore, to be treated as foreign tissue by the mother. This was first described in 1954 by Medawar (4), who suggested that mechanisms to prevent rejection of the placenta by the maternal immune system must exist for pregnancy to be successful. One of these mechanisms involves placental trophoblasts in that they express a unique, slightly modified MHC class I antigen which does not trigger the maternal nonself rejection mechanisms (5). However, fetal cells can pass into the maternal circulation, thereby enabling priming to occur in other locations in the mother's immune system. Maternal immune suppression at the placen-

tal interface is mediated by the production of immunoregulatory factors by trophoblasts and decidual suppressor cells which reduce lymphocyte activation (6–8). Systemic immune suppression involves both serum suppressor factors (9–11) and changes in peripheral blood cell populations (12, 13), of which one of the most notable is a reduction in the number of circulating CD4-positive T cells toward term (14).

Cell-mediated immunity (particularly natural killer [NK] cell activity) is detrimental to the pregnant state, and many groups (mostly in animal models) have reported a damping of this response in the maternal system with a concurrent move toward humoral immunity. In 1993, Wegmann et al. (15) suggested that this is mediated by a switch in the T helper cell population of the fetoplacental unit to produce Th2 cytokines (interleukins 4, 5, and 10 [IL-4, IL-5, IL-10]) which inhibit maternal Th1 cytokine production – IL-2, interferon-gamma (IFN- γ), and tumor necrosis factor-beta (TNF- β). These are deleterious cytokines for pregnancy, as IL-2 and IFN- γ induce NK cell activity (16). NK cells have been shown to have a role in spontaneous abortion and may attack the trophoblast (17). Additionally, IFN- γ is an abortifacient, whose effect may be mediated through the activation of NK cells (18). Indeed, IFN- γ not only promotes the development of cytotoxic lymphocytes and NK cells, but also inhibits the B-cell development induced by cytokines from Th2-like cells (19) and inhibits proliferation of Th2-like cells. IL-4, IL-5, and IL-6 are involved in various phases of B-cell develop-

ment, while IL-10 can switch off the cytokine synthesis of Th1-like cells. Therefore, by producing Th2-like cytokines, the fetoplacental unit blocks the maternal cell-mediated responses which would compromise fetal survival.

It would seem that either a humoral or a cell-mediated response mounted against the fetus could be damaging, but the placenta adsorbs large amounts of antifetal MHC antibody (20). However, antibodies directed against antigens other than those of the placenta or fetus are transferred across the placenta and can be employed by the neonate as passive immune protection.

The questions which arise are as follows:

- 1) Can the production of Th2-like cytokines by the placenta direct the fetal Th0-like cells toward the Th2 rather than the Th1-like phenotype?
- 2) At what stage in pregnancy might this occur?
- 3) Does maternal allergen exposure affect the development of specific T-cell responses in the fetus?
- 4) Does an atopic mother who already has circulating Th2 cytokines exert a greater influence on the Th1/Th2 switch in the fetus than a nonatopic mother?

Before any of these questions can be answered, it is necessary to know the normal time course of T-cell development in the fetus. The first lymphocytes are seen in the thymus of the developing fetus at 8–9 weeks' gestation, and the thymic cortex is distinguishable from the medulla at 10 weeks. At this stage, the more mature T cells in the thymus can demonstrate proliferative responses to the mitogen phytohemagglutinin (PHA) and histocompatibility antigens (21, 22).

T-cell numbers increase in the fetal spleen and liver between the 12th and 23rd week of gestation. The migration of the T cells to the circulation occurs at approximately 15–16 weeks and is likely to be critical to the development of immune competence as most B-cell antigen responses require T-cell help (23). Until recently, little was known about the functional maturity of the circulating T cells at this stage. However, we have performed FACS analysis of fetal T cells and shown that while CD25+ T cells were virtually undetectable at 15 weeks' gestation, at 22 weeks they were approximately 50% of the levels at full term (Table 1). Peripheral blood mononuclear cell (PBMC) proliferative responses to PHA were first detected at 17 weeks. These proliferative responses continued to increase, were significantly higher in the third trimester than the second trimester ($P < 0.0002$), and correlated with gestational age. Even more importantly, we have demonstrated the same increase in proliferative response to specific inhalant and

Table 1. Fetal peripheral blood T-cell responses and cell-surface markers during gestation period (15–22 weeks)

Gestation (weeks)	Presence of positive proliferative response to PHA	% of lymphocytes CD3+	% of T cells CD25+
15–16 (n=5)	—	3%	<10%
17–18 (n=9)	+	7%	20%
19–20 (n=8)	+	22%	30%
21–22 (n=5)	+	27%	50%

ingestant allergens, supporting the theory of intra-uterine priming (24).

Normal T-cell proliferation depends on IL-2. IL-2 has been shown to be produced in amounts similar to adult cells in response to mitogenic and allogenic stimulation of cells from newborn babies (25, 26). However cord-blood cell production of other cytokines is lower than adult cells. Normal healthy cord-blood T cells produce approximately 10% IFN- γ , less than 10% IL-4, and 20–50% TNF- β , IL-3, and granulocyte/macrophage colony-stimulating factor (GM-CSF) of that produced by adult cells (27). In 1992, Holt et al. (28) showed that production of IFN- γ by mitogen-stimulated CD4+ T-cell clones, while being low in babies and young children (aged 1 month to 4 years), was significantly lower still in babies born to families with a history of allergic disease. Rinas et al. (29) showed this to be true also of stimulated cord-blood PBMCs, with the cells of babies born to families at high risk of allergy producing less IFN- γ than those of low-risk babies. We have found raised cord-blood T-cell proliferative responses and defective IFN- γ production after stimulation with specific allergens at birth in babies who have subsequently developed allergic problems in relation to those allergens (30), and these observations have been partly confirmed by others (31). However, very little is known about the capability of human fetal T cells to proliferate and produce cytokines in response to mitogens or antigens during gestation.

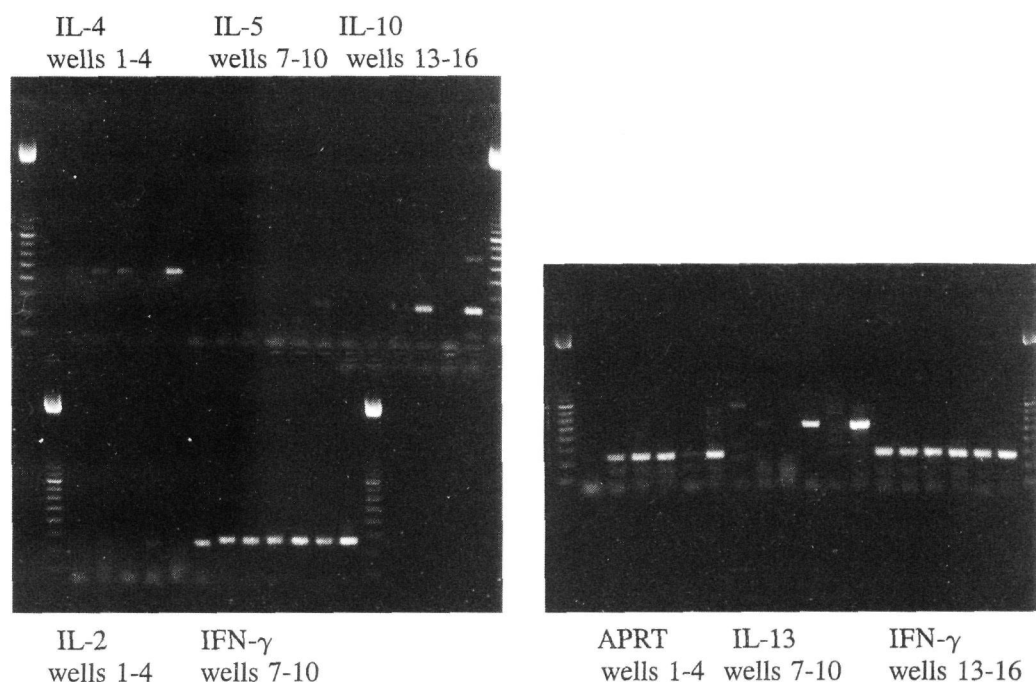
In our own studies, we have shown that from 22 weeks of gestation fetal T cells are able to produce positive proliferative responses (>2 times background) to a range of allergen sources (house-dust mite, cat fur, birch tree pollen, β -lactoglobulin, and ovalbumin) and that the proliferation ratios correlate with gestational age. This is strong evidence that maternal allergen exposure is important in the priming of fetal T cells such that at birth they already respond to environmental allergens. We are currently investigating whether those T cells are of

the Th1 or Th2 phenotype and what determines this outcome.

Babies at high risk at birth of developing allergy with a raised proliferative response to β -lactoglobulin and deficient IFN- γ production were those who went on to develop food-associated eczema with a positive skin prick test to milk (30). It is important to know at what stage in pregnancy this priming occurred with its related downregulation of IFN- γ . Positive proliferative responses can be seen from 22 weeks' gestation, and there are circulating T cells from 15 weeks' gestation; therefore, it can be hypothesized that the 2nd trimester of pregnancy is the vital time period. It has been shown that maternal food-allergen avoidance in the 3rd trimester of pregnancy is ineffective in the prevention of infant allergy (32). This may have been too late because fetal sensitization had already occurred. We have also shown that infant cells stimulated at birth with birch tree pollen showed a significantly ($P=0.01$) higher proportion of positive responders in the group whose mothers had been

exposed to birch pollen beyond the 22nd week of pregnancy than in those who were not exposed in this time period. This would suggest that maternal avoidance of trigger factors needs to be established before 22 weeks' gestation to prevent fetal T-cell priming. It remains to be seen whether the positive responders go on to develop allergic disease, because it is equally possible that this is a normal phenomenon and may even be involved in the induction of tolerance of these antigens.

In the murine model, it is known that the fetoplacental unit produces IL-4, IL-5, and IL-10 during all three trimesters of pregnancy (15). Jones et al. (33) have shown that IL-4 is produced by the human amnion epithelium in the 1st and 3rd trimesters of pregnancy (2nd trimester not tested), and, in 1995, Cadet et al. (34) described the presence of messenger RNA coding for IL-10 at term in human placental tissue. We are currently investigating the presence of mRNA coding for IFN- γ , IL-4, IL-5, IL-10, IL-12, and IL-13, as well as their protein products in the fetal-placental-maternal human



LANE ORDER	SAMPLE TYPE	INFANT GESTATION (wks)	CYTOKINE SIZE (base pairs)						
			IL-4 450	IL-5 257	IL-10 230	IL-2 255	IFN- γ 270	APRT 246	IL-13 500
1	Unstimulated	23	-	-	-	-	+	-	-
2	PHA-stimulated	23	+	-	-	-	+	+	+
3	Unstimulated	31	+	-	-	-	+	+	-
4	PHA-stimulated	31	+	-	+	+	+	+	+
5	N/A	N/A	-	-	-	-	+	+	-
6	N/A	N/A	+	+	+	+	+	+	+

Fig. 1. Amplified DNA from RNA coding for interleukins 2, 4, 5, 10, and 13, interferon-gamma, and APRT (positive control) for unstimulated and PHA-stimulated fetal and premature baby PBMC cultures. Samples in lanes 5 and 6 are unrelated samples which were run on same gel.

system in the 2nd and 3rd trimesters of pregnancy, to determine how, and when, maternal and placental cellular responses affect the development and control of infant responses to allergens.

Preliminary results show that PBMCs from most fetuses spontaneously release IFN- γ during the 2nd and 3rd trimesters of pregnancy, but that there are some which do not release this Th1 cytokine even when stimulated with PHA. By the time positive proliferative responses are seen to antigens (>22 weeks' gestation), mRNA coding for the Th2 cytokines IL-4, IL-10, and IL-13 can be detected in fetal PBMCs (Fig. 1). We have also shown IL-10 protein in placental cell lysates in the 2nd and 3rd trimesters.

We hypothesize that the role of nonspecific fetal PBMC IFN- γ production is to counteract the effects of IL-4 and IL-10 produced by the placenta and/or mother. A mechanism such as this would be essential to prevent an allergic (Th-2) phenotype in all newborns. Failure of this mechanism may underlie the development of atopy.

Currently, we are not aware of any reports of the ability of maternal allergy-promoting cytokines to cross the placenta; however, amniotic fluid has been shown to contain granulocyte colony-stimulating factor (G-CSF), GM-CSF, TNF- α , IL-1, IL-6, and IL-8 (35). This does not necessarily indicate passage from the mother, as they may all be placentally derived; however, further investigation is needed. We have been able to detect IL-10 in amniotic fluid.

Conclusions

We have been able to demonstrate that the PBMCs of some fetuses are unable to produce IFN- γ , and that fetal PBMCs do possess mRNA coding for IL-4, IL-10, and IL-13. It is still too large a jump to suggest that exposure to placental and maternal Th2 cytokines causes this phenomenon, but investigation is continuing.

It appears that the 2nd trimester of pregnancy is the most important time period for the initiation of these events, and maternal allergen exposure does affect the T-cell responses of the fetus. As yet, there is no work to prove that an increased inheritance of atopy from the mother compared to the father is due to maternal Th2 cytokines crossing the placenta and influencing the fetus, but we hope to elucidate this in the future.

There is one remaining question. If successful pregnancy is a Th2 phenomenon, are atopic mothers more fertile than nonatopic mothers and could this be related to the increase in the prevalence of allergic disease!

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