

II. Environmental and inflammatory mechanisms

Prenatal origins of allergic disease

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The prevalence of asthma and related allergic disorders has increased considerably over the last 25 years. Because genetic stock has not changed, environmental factors must have influenced the phenotype. Infants who experience the development of allergy already have an altered immune response at birth. We have investigated the development of immune responses during gestation and the effect of maternal allergen exposure during pregnancy and infant exposure in the first month of life on the development of allergy and disease. There was higher specific peripheral blood mononuclear cell proliferation to house dust mite and birch pollen in the third trimester compared with the second trimester, with the first positive responses seen at 22 weeks gestation. Maternal exposure to birch pollen after 22 weeks resulted in higher infant peripheral blood mononuclear cell responses to birch pollen at birth. Infants born at term, with at least 1 atopic parent with asthma, who experienced the development of allergic symptoms and positive skin prick test by 1 year of age had raised proliferative responses to house dust mite at birth compared with those infants with no symptoms. In genetically predisposed individuals, antenatal factors including maternal and thereby fetal exposure to allergens and materno-placental-fetal immunologic interactions are active in determining whether an allergic predisposition is manifested as disease. (*J Allergy Clin Immunol* 2000;105:S493-6.)

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There is ever increasing evidence to support the theory that many aspects of health and disease are determined prenatally and that subsequent alterations to lifestyle and diet are secondary to the effect of the programming that occurs during pregnancy.¹ This is directly applicable to allergic disease where immune responses at birth implicate intrauterine exposure in primary sensitization.^{2,3} Intervention immediately after birth to reduce allergen exposure has largely resulted in a delay in the onset of disease rather than its prevention,^{4,5} indicating that immunologic memory has been established before birth and is retained during the first months of life. Evidence that children are more likely to inherit allergic disease

Abbreviation used

LGL: Large granular lymphocyte

from the mother than the father further supports the importance of prenatal influences.⁶

ALTERED IMMUNE RESPONSES AT BIRTH

There is a distinct need to further elucidate the factors that lead to the early development of immune responses associated with an increased risk of the development of allergic disease. It is often difficult to separate the effect of environmental conditions occurring during pregnancy from those in existence during the first months of life because they are rarely significantly different. An obvious example is cigarette smoking. Most mothers who smoke during pregnancy will continue to do so after the baby is born,⁷ although some might refrain during pregnancy and recommence after the birth. Antenatal smoking has been implicated in the increase of cord blood IgE⁸ and in the increase of the prevalence of eczema and higher IgE levels up to 3 years of age.⁹ The existence of a parameter that can be measured directly at the time of birth gives an indication that allergic mechanisms have already been put in place, but it is likely to be the continuation of the environmental influence in the first months of life that determines whether this results in disease.

Another immune response that can be shown to be altered at birth is the capacity of PBMCs to produce IFN- γ . Several groups have shown that babies with a family history of allergy are born with PBMCs that are deficient in the production of the T_{H1} cytokine, IFN- γ , when compared with babies from nonatopic families.^{10,11} We have shown that this deficiency can be specific for individual allergens that subsequently trigger disease² which provides evidence that this is not purely an inherited characteristic but that it can be influenced during gestation.

How early can specific responses be detected?

We have shown that fetuses are able to demonstrate positive PBMC responses to allergens from 22 weeks of pregnancy and that maternal exposure to birch pollen allergen from this point onwards results in increased infant responses to birch pollen extract at birth.¹² There is also a significant correlation between the expression of the marker CD45RO by T cells and increasing gestational age (Fig 1), indicating that fetal immune memory

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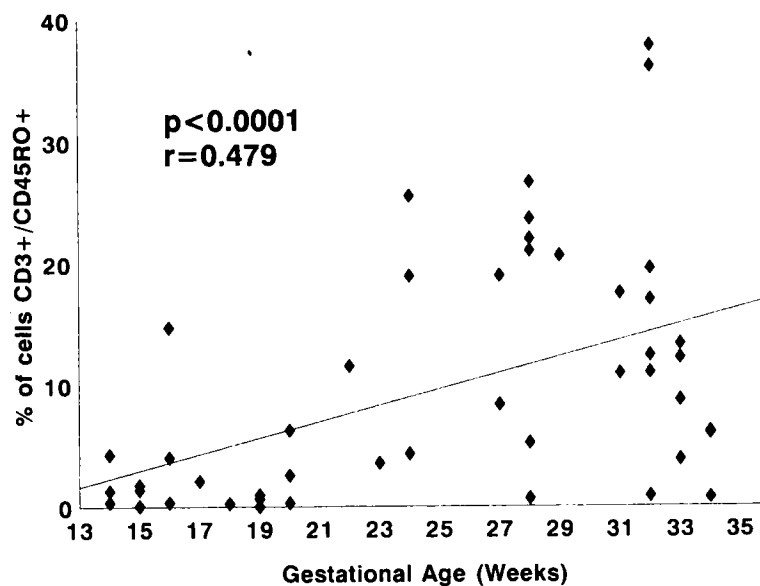


FIG 1. The percentage of T cells in umbilical cord blood expressing the marker CD45RO at varying stages of gestation.

develops as gestation progresses. These findings suggest that antigen passage is occurring across the placenta. This may be determined in part by the mother's own immune response to that antigen and her atopic status. We have also shown that there is a negative correlation between the concentration of specific maternal IgG to house dust mite and the infant PBMC proliferative response to house dust mite at birth.¹³ Therefore it appears that the immune response of infants with an inherited tendency to a reduced IFN- γ production (which can probably originate from either the maternal or paternal line) may be influenced still further by maternal allergen exposure and immune response to that allergen during pregnancy, resulting in the existence of a T_{H2} -biased response in the infant, even at birth. This hypothesis is supported additionally by the work demonstrating that the immune interactions at the materno-placental-fetal interface are distinctly biased toward T_{H2} -like responses.

Pregnancy is an allergic disease

Despite the intimate contact between fetal-derived allogeneic tissue and maternal-derived decidual tissue, normally the immunocompetent mother does not reject the fetus. Numerous mechanisms have been suggested, which create an intrauterine environment that favors the continuation of pregnancy. Recent evidence would suggest that cytokines play an important role in regulating the maternal immune response to the fetal allograft, and potentially acting as growth factors for the placenta and fetus.

In the murine model, it is known that the fetoplacental unit produces IL-4, IL-5, and IL-10 during all 3 trimesters of pregnancy.¹⁴ Jones et al¹⁵ have shown that IL-4 is produced by the human amnion epithelium in the first and third trimester of pregnancy (second trimester not tested);

Cadet et al¹⁶ in 1995 described the presence of messenger RNA coding for IL-10 at term in human placental tissue.

Wegmann et al¹⁴ in 1993 suggested that this switch in the T-helper-cell population of the fetoplacental unit to produce T_{H2} cytokines (IL-4, IL-5, IL-10) inhibits maternal T_{H1} cytokine production (IL-2, IFN- γ) and TNF- β . Therefore whether or not the mother is allergic herself, the fetus is exposed to conditions that would favor the development of T_{H2} -like responses.

To evaluate the importance of each of these factors on the developing immune response of the fetus, it is necessary to first understand their roles in the progression of a normal pregnancy.

IL-2. IL-2 is suggested to be a pregnancy-compromising molecule when produced by human placenta¹⁷ and decidual tissue¹⁸ and it can be detectable in amniotic fluid.¹⁹ In human models, IL-2 can increase proliferation of uterine large granular lymphocytes (LGLs), creating lymphokine-activated killer cells with enhanced cytotoxic activity.²⁰ In vitro, culture with IL-2 increases the proportion of decidual LGLs that express CD16 and turns decidual LGLs incapable of killing first trimester cultured trophoblast cells into effective cytotoxic cells. Thus IL-2 expression, and thereby decidual LGL activation, may compromise the invasiveness of trophoblasts during implantation and placental growth, making suppression of IL-2 production an important mechanism for successful pregnancy.

IL-4, IL-5, and IL-10. IL-4, IL-5, and IL-6 are involved in various phases of B-cell development, while IL-10 can switch off the cytokine synthesis of T_{H1} -like cells. IL-4 appears to have a particularly important role in the immunologic maintenance of pregnancy, for example if IL-1 is a stimulus for parturition, IL-4 (which can inhibit IL-1 production by human monocytes)²¹ may pre-

vent untimely expression of IL-1 bioactivity. IL-4 and IL-4 receptor expression have been identified in both fetal and maternal endothelial cells within the uteroplacental tissue.²² IL-4 inhibits natural killer-cell adhesion to endothelial cells and their subsequent recruitment and activation.²³ IL-4 is known to stimulate cell growth and differentiation; therefore its presence at the fetomaternal interface is not surprising. It has also been shown to upregulate MHC class II molecules.²⁴ Both placental villous macrophages at term and decidual macrophages strongly express class II MHC molecules. Thus IL-4 may contribute to the upregulation of class II molecules on both fetal and maternal macrophages and thereby assist with activation of antigen-presenting cells involved in antimicrobial or allotypic immune responses during pregnancy. IL-4 is detectable in amniotic fluid.

IL-10 is a product of various cell types (including monocytes, B cells and T cells) and is associated with the differentiation of T_{H2} cells. IL-10 inhibits cytokine production, particularly of IFN- γ , by T cells and natural killer cells.²⁵ It has been suggested that IL-10 prevents the production of the IFN- γ -inducing cytokine, IL-12, by accessory cells. One possible mechanism for the findings of reduced IFN- γ production by neonatal T cells is an imbalance of IL-10 and IL-12 production.

Recently it has been shown that villous cytotrophoblasts spontaneously release IL-10²⁶ in all 3 trimesters of pregnancy and that the IL-10 is bioactive as determined by its ability to suppress IFN- γ production in an allogeneic mixed lymphocyte reaction. It therefore can be suggested that cytotrophoblast IL-10 is an important factor that contributes to maternal tolerance of the allogeneic fetus by damping the inflammatory immune response. IL-10 is also present in amniotic fluid, and we have evidence that significantly higher concentrations of IL-10 are found in the amniotic fluid of atopic women compared with nonatopic women, which may affect the development of immune responses of the fetus as amniotic fluid is swallowed.

IFN- γ . IFN- γ is an abortifacient, whose effect may be mediated through the activation of natural killer cells.²⁷ Indeed IFN- γ not only promotes the development of cytotoxic lymphocytes and natural killer cells but also inhibits the B-cell development induced by cytokines from T_{H2}-like cells²⁸ and inhibits proliferation of T_{H2}-like cells. However, IFN- γ stimulates HLA-G expression by trophoblasts,²⁹ which may allow these cells to avoid cytotoxicity by decidual LGLs. Trophoblast populations do not express MHC class II molecules nor the highly polymorphic, classical HLA-A, B, C antigens, but they do express a nonclassical, nonpolymorphic product encoded by the HLA-G locus. The level of expression is associated with the degree of contact with the maternally derived decidual tissue and is highest in the first trimester, declining toward term.³⁰ It seems therefore that the effect of IFN- γ on maternal cells at the fetal interface is pregnancy compromising, but production by fetal cells may be important in early fetal immunologic development and protection.

Our own preliminary results show that PBMCs from most fetuses spontaneously release IFN- γ during the second and third trimesters of pregnancy but that there are some that do not release this T_{H1} cytokine even when stimulated with PHA. Other groups have shown that fetal plasma concentrations of IFN- γ are higher in the first compared with the third trimester,³¹ which may be associated with the need to avoid a T_{H2}-biased, fetal immune response. At the time of birth, a significant negative association has also been found between the presence of mRNA coding for IL-4 in stimulated cells and the concentration of IFN- γ in the supernatant.¹³

FURTHER INVESTIGATIONS

We are currently investigating the presence of mRNA coding for IFN- γ , IL-4, IL-5, IL-10, IL-12, and IL-13 and their protein products in the fetal-placental-maternal human system in the second and third trimesters of pregnancy to determine how, and when, maternal and placental cellular responses affect the development and control of infant responses to allergens. We have preliminary evidence of CD68⁺ monocytes in both the maternal and fetal circulations within the placenta, indicating active antigen presentation to be occurring. Intracellular immunohistochemistry has demonstrated cells that produce IL-13 within the placental unit, further supporting the T_{H2}-biased environment. We also have data to suggest that the way fetuses downregulate the effect of exposure to increased T_{H2}-type cytokines on the development of their own immune response is to only be able to generate very low levels of mRNA coding for IL-4 or IL-13 before 36 weeks of gestation. In PHA-stimulated fetal PBMCs collected before 36 weeks of pregnancy, either very faint signals or no signal at all could be demonstrated for these two cytokines.

CONCLUSIONS AND HYPOTHESES

The intrauterine environment in which a fetus develops is highly biased toward T_{H2}-like immune responses. The systems of babies with an inherited tendency toward a deficient production of IFN- γ are less likely to be able to counteract the effect of this imbalance on their own developing immune responses, particularly if the mother is also allergic and is exposed to the allergens that trigger her own allergic disease during the pregnancy. This may cause a further increase in the concentrations of circulating T_{H2}-like cytokines, which could cross the placenta and influence the fetal immune response. The normal pattern of fetal cytokine production appears to be T_{H1} biased until shortly before the time of normal term delivery, when all babies demonstrate IL-4 production. Holt et al³² showed that cord blood responses to ovalbumin and house dust mite stimulation demonstrated a dominance of mRNA production coding for IL-4 over IFN- γ , which would fit with the finding that fetal plasma concentrations of IFN- γ decreased toward term³¹ as a normal phenomenon.

Maternal antigen exposure during pregnancy is likely to lead to the development of tolerance mechanisms in the infant, except when the exposure occurs against a background of heightened T_H2 -like activity when it could lead to the establishment of allergic-type responses. These responses may then be reinforced by the exposure of the infant to the same antigens in the first months of life, leading to persistent IgE responses.

It is now necessary to determine whether modulation of maternal immune responses during pregnancy, either by altering the environment or controlling allergic reactions by therapeutic intervention, can prevent a T_H2 response from becoming persistent in the infant.

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