
Primary sensitization in infants

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Objective: It has become increasingly clear that the mechanisms by which an allergic immune response is generated are complex and may begin even before a baby is born. Genetic, environmental, nutritional, and immunologic factors acting during pregnancy all play a role in determining whether or not a baby is born with a propensity to develop allergic sensitization and subsequent allergic disease. The objective of the research described in this article is to determine whether manipulation of any or all of these could lead to prevention of disease.

Data Sources: The database used was Medline up to and including 1997. The Conference Proceedings of the XVth World Congress of Asthmology in Montpellier 1996 are quoted. Many of the research hypotheses are generated from work performed in our own laboratories.

Study Selection: The criteria used to select studies for review centered around a necessity for the data to have been collected in very early life with, if possible, a follow-up period to determine disease progression, or for the data to have been collected during pregnancy to elucidate primary mechanisms.

Results: It has been shown that a fetus is able to mount a proliferative response to a common allergic trigger (beta-lactoglobulin, house dust mite, etc) as early as 22 weeks of pregnancy. Maternal exposure to allergens influences her own IgG production which modulates the allergen exposure of the fetus resulting in either primary sensitization of T cells or "tolerance" to the allergen. Atopic mothers create a more Th2-orientated environment for the developing fetus than non-atopic mothers.

Conclusions: Manipulation of the maternal immune response during pregnancy, either by altering her environment or controlling her allergic reactions, may be a method of preventing the development of allergic disease in infants.

Ann Allergy Asthma Immunol 1999;83:426–430.

INTRODUCTION

Answering the question 'How early in life does sensitization occur?' is currently one of the key research areas in the prevention of allergic disease. It is particularly important to determine at which stage of 'sensitization' is the point at when the process cannot be arrested and disease becomes inevitable. 'Sensitization' has been used to describe many processes from the priming of immune cells, to the devel-

opment of a positive allergy skin test or RAST, to the manifestation of symptoms. However, 'primary sensitization' is more precisely the antigen-specific cellular mechanisms that eventually lead to IgE production. Current knowledge would suggest that intervention at this point could prevent the progression into clinical disease.

THE INCREASING PROBLEM

Prevention of allergic disease has become increasingly important as the prevalence has continued to rise despite the introduction of new, more effective medications. This increased prevalence of allergic diseases^{1,2} reported by epidemiologic investigations cannot be accounted for by a change in genetic stock, which implicates environmental influences in inducing a shift from potential to active disease. Exactly what these environmental influences are, and at what time point

they are active, has not yet been fully elucidated, but there is strong evidence which points towards very early life as the critical period for sensitization. Determining the constituent factors that lead to primary allergic sensitization is a vital step in finding the most effective way of arresting the process.

THE FIRST YEAR OF LIFE

There is a large body of evidence which implicates environmental influences in the first year of life in the development of allergic sensitization and disease. As early as 1983 it was reported that asthmatic children were significantly more likely to demonstrate a positive bronchial challenge response to dog if a dog had been present in the home during the child's first year of life than if it had been introduced after the first year.³ We have further confirmed this relationship by studying cat exposure and the development of sensitivity to cat in a prospective study of the development of allergy and asthma in 150 high risk babies. By 2 years of age 35% of those exposed to cat from birth had a positive skin prick test to cat compared with 3% of those without exposure.⁴ Other information that environmental aeroallergen exposure may be important in infancy comes from month of birth studies, particularly in relation to birch pollen allergy. Bjorksten et al⁵ correlated the highest skin reactivity to birch pollen in infants born just before the peak of the birch pollen season. This exposure influenced the development of pollen allergy for 20 years. It was estimated that allergy to birch pollen could be reduced by 28% if individuals were not born in the months of February to April. Other studies have suggested that exposure to high concentrations (>2 ug/g) of the house dust mite allergen Der p1 in domestic dust in the second year of life is related to the subsequent development of sensi-

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The National Asthma Campaign, The British Lung Foundation, The David and Frederick Barclay Foundation, The British Allergy Foundation, The European Society for Pediatric Allergy and Immunology, Sandoz Pharmaceuticals, Pharmacia and National Power have provided funding for the work described in this article.

Received for publication March 15, 1998.

Accepted for publication in revised form March 1, 1999.

tization to house mites⁶ and that the higher the exposure the earlier the onset of wheeze in those infants who developed asthma. Most recently, however, it has been shown that there appears to be no absolute threshold concentration that could function as a target to provide zero risk of sensitization.⁷ Children with a positive family history of atopy were susceptible to even very low levels of exposure and the same group demonstrated sensitization to mite at 3 years of age associated with a median exposure to Der p1 + Der f1 of 700 ng/g dust in the first 18 months of life, but found some sensitization occurring at levels of ~100 ng/g. This is in agreement with our finding that exposure to Der p1 concentrations lower than 500 ng/g is less likely to be associated with sensitization to mite,⁸ and both studies indicate that when considering prevention of allergic sensitization we should be aiming for the lowest levels that can possibly be achieved.

ENVIRONMENTAL INTERVENTION AFTER BIRTH

Several groups have performed studies to investigate the effect of limited changes to the environment of a baby immediately following birth on the development of allergic sensitization. The majority have been changes to diet with little work being performed on inhalant allergens. Maternal avoidance of certain foods during lactation results in a delay in onset of atopic dermatitis in the infant,^{9,10} however concerns have been raised that the delay is followed by more severe disease than would have been the case with a normal diet due to a lack of the development of tolerance mechanisms.¹¹ Two studies have been able to demonstrate a reduced prevalence of atopy in groups of high risk infants fed a whey hydrolysate formula.^{12,13} Both showed an approximate halving of allergic disorders compared with their control groups. The periods of follow-up were 18 months and 36 months. One study has combined either infant feeding from birth with an extensively hydrolyzed collagen/soya formula or mater-

nal food avoidance (milk and dairy products, eggs, fish, nut, and soya) during lactation with house dust mite reduction measures and compared the development of allergic manifestations in this group with a control group on a normal diet and no environmental alterations in the home.¹⁴ They showed a significant reduction in eczema and overall allergic disease, but this was strongest at 1 and 2 years, becoming less so at 4 years, again suggesting a delaying rather than a preventive outcome.

IS BIRTH TOO LATE?

The studies described above all worked with the hypothesis that the vulnerable time period for primary sensitization does not begin until immediately after birth and continues for an ill-defined period throughout the first year of life. Two studies have attempted to modify the development of allergy by restricting the mother's diet in the latter stages of pregnancy. The first of these which continued the avoidance postnatally demonstrated¹⁵ a reduction in the incidence of atopic eczema, but this was not confirmed by the second¹⁶ where mothers from allergy prone families avoided milk, dairy products, and eggs from the 28th week of pregnancy to delivery and not thereafter. After 5 years of follow-up, no significant difference in atopy (as defined by skin testing and IgE measurements), asthma, eczema or rhinoconjunctivitis was seen between the intervention group and a control group. These results have promoted the idea that there is not a vulnerable period during fetal life which is a possible target for the prevention of allergic disease. This view is now challenged by evidence of intrauterine programming revealed by several studies and supported by the evidence that an infant is more likely to inherit atopy from its mother than its father.¹⁷

It is now accepted that high risk babies and babies who subsequently develop allergic disease have an altered immune response at birth compared with babies with no family history of allergy who do not develop disease. Holt et al¹⁸ originally demon-

strated that, while the interferon gamma-(IFN- γ)-producing capacity of CD4+ T cell clones from children aged 1 month to 4 years is reduced relative to adults, it is significantly lower still in babies born to families with a history of allergic disease. It has also been demonstrated that IFN- γ production by cord blood mononuclear cells is reduced in newborns with a family history of atopic disease.¹⁹ This was independent of cord blood IgE levels. We have demonstrated that the development of atopic eczema with positive allergy skin prick tests to cow's milk and egg at 1 year of age, where the symptoms improved on an egg-free and milk-free diet, was significantly associated with raised proliferative responses and defective IFN- γ production to stimulation with β -lactoglobulin and a trend for similar responses to ovalbumin.²⁰ This was not observed in those who did not develop atopic eczema or those with atopic eczema not associated with foods. Responses to cat fur extract were not significantly different between those with and without atopic eczema. Similar (but non-specific) responses to phytohaemagglutinin were reported by Tang et al.²¹ This has important implications for the prediction at birth, not only of the probability of atopy, but also of the specific allergens likely to cause problems.

THE INTRA-UTERINE ENVIRONMENT

The presence of these responses at birth is indicative of a process which begins during the gestational period and which, therefore, has the potential to be affected by the mother's own immune responses and possibly by her exposure to environmental allergens. The transfer of immunologically active proteins (eg, cytokines, IgG antibodies, and antigens) across the placenta from mother to fetus could influence the developing fetal immune response in such a way as to result in the Th1/Th2 imbalance in response to a specific antigen which can be demonstrated at birth.

The suggestion that pregnancy is a Th2 phenomenon²² has fueled a great deal of interest in the materno-placental-fetal environment as a place where "primary sensitization" (used here in the context of immune cell priming) could take place.

Despite the intimate contact between fetally derived allogeneic tissue and maternally-derived decidual tissue, normally the immunocompetent mother does not reject the fetus. Numerous mechanisms have been suggested to 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, as well as potentially acting as growth factors for the placenta and fetus.

Studies in rodent models have suggested that the Th2 cytokines, interleukins 4 and 10 (IL-4 and IL-10), favour the continuation of pregnancy, whereas the Th1 cytokines interleukin 2 (IL-2) and IFN- γ are detrimental.^{23,24} In humans the relationship does not appear to be so clear cut as in human gestation associated tissue, both Th1 and Th2 type cytokines are expressed.²⁵ The most noticeable feature of cytokine ex-

pression in human uteroplacental tissues is that, whereas most other cytokines are constitutively expressed by one or more cell types, there is an almost complete absence of IL-2. It has been suggested that in humans, local blockade of the Th1 type response producing IL-2 is the most important promoter of maternal tolerance, and that this is a major reason for the increase seen in Th2 type responses.²⁶

When present at the materno-placental-fetal interface, IL-2 increases the proliferation of uterine large granular lymphocytes in the decidua, creating killer cells that express CD16 and are capable of killing placental trophoblast cells thereby blocking implantation and placental growth.²⁷ Interleukin-4 on the other hand, appears to have a particularly important role in the maintenance of pregnancy as it inhibits NK cell adhesion to endothelial cells and their subsequent recruitment and activation.²⁸ It is also known to stimulate cell growth and differentiation.

Interleukin-10 is also produced by the placental cytotrophoblasts²⁹ and is important as it can switch off the cytokine synthesis of Th1 cells, in particular blocking IFN- γ production in the

placenta, where it is an abortifacient due to its activation of NK cells and cytotoxic lymphocytes.³⁰

FETAL IMMUNE RESPONSES

In the light of the above information it becomes interesting to speculate whether the interactions between the Th1 and Th2 cytokines during pregnancy could be influential in determining the type of immune response which develops in the fetus to antigens it may be exposed to via the mother (Fig 1).

The first lymphocytes are seen in the thymus of the developing fetus at 8 to 9 weeks gestation and the 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 phytohaemagglutinin (PHA) and histocompatibility antigens.^{31,32}

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 to 16 weeks and is likely to be critical to the development of immune competence as most B cell antigen responses require T cell help.³³ This would suggest that fetal T cells could be primed to common antigens during the second trimester of pregnancy, which might explain why the studies of maternal allergen avoidance in the third trimester were ineffective, being too late.

HOW EARLY DOES PRIMARY SENSITIZATION OCCUR?

It is possible to investigate the time course of the development of circulating immune responses by studying peripheral blood mononuclear cell responses from the umbilical cord blood of babies born prematurely or those where it has been necessary to terminate the pregnancy very early (due to fetal abnormalities which do not affect the immune response).

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 -lac-

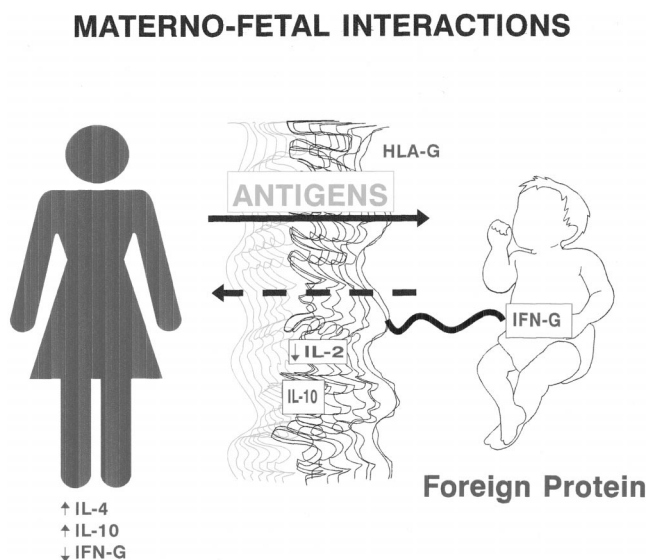


Figure 1. A diagrammatic representation of some of the materno-fetal interactions present during pregnancy, showing cytokine production by the mother, placenta and fetus and the potential route of primary sensitization.

toglobulin, and ovalbumin) and that the proliferation ratios correlate with gestational age.³⁴ 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. The only other feasible explanation could be that the responses are anti-idiotypic reactions to specific maternal IgG, however this is extremely unlikely as at birth infant lymphocyte responses are not seen to tetanus toxoid³⁵ against which all mothers possess IgG antibodies.

We have also demonstrated that infant cells stimulated at birth with birch tree pollen showed a significantly ($P = .01$) higher proportion of positive responders in the group whose mothers had been exposed to birch pollen beyond the 22nd week of pregnancy compared with those who were not exposed in this time period.³⁴ This would add support to the concept that maternal antigen exposure during the second and third trimesters of pregnancy results in fetal T cell priming.

The mother's own immune response can also regulate the developing fetal reactions as the concentration of maternal IgG specific for house dust mite is negatively associated with the infant PBMC proliferative response to house dust mite at the time of birth.³⁶ There is preliminary evidence that an atopic mother creates an even more Th2-biased environment for her fetus than that necessary to prevent fetal rejection as there are significantly higher concentrations of IL-10 in the amniotic fluid of atopic women during pregnancy than non-atopic women.³⁷

This could be part of the explanation for the greater risk of inheritance of maternal rather than paternal allergy.

IS PRIMARY PREVENTION AN ATTAINABLE GOAL?

The strong evidence implicating environmental exposure in the first year of life in the development of allergic sensitization suggested convincingly that avoidance of allergens or a reduction in allergen concentrations during this period might result in reduced rates of

sensitization. The findings of most studies, that this approach produced a delay in the onset of symptoms, but not a complete prevention of disease, was disappointing. With the recent developments demonstrating altered immune responses at birth, perhaps this outcome was not surprising as "primary sensitization" had already occurred and therefore the mechanisms leading to clinical manifestations were already established and would be triggered whenever infant allergen exposure did occur.

It is possible that the concentration of perennial allergen exposure experienced by the infant during the first year of life also applies to the mother during pregnancy and that it is this exposure which is more related to primary sensitization in susceptible individuals.

If a primed immune response is not in existence at birth and allergen levels remain low during the first year of life can the process of sensitization with eventual progression to disease be prevented? The answer will only be found by performing studies of allergen avoidance commencing in early pregnancy with long-term follow-up of clinical symptoms. The question of what 'dose' of allergen results in tolerance rather than sensitivity is one which has a great bearing on the outcome of such studies and needs very careful monitoring. It is possible to conceive of a raised specific maternal IgG being a way of blocking fetal PBMC proliferative responses, but the mechanism of this still requires much more elucidation.

On the basis of already existing knowledge, it is possible to hypothesize that controlling the environment of an allergic mother during pregnancy and thereby reducing her symptoms and Th2 cytokine production, as well as reducing the allergen reaching the fetus, will prevent the infant being born with an immune response already switched to a Th2-like response to a specific allergen. A low allergen environment present during the infant's first year of life could then have a better chance of resulting in prevention rather than postponement of atopic dis-

ease. It is also possible to conceive of a high-dose environment postnatally being able to promote the Th1 response established by the antenatal avoidance. This would therefore confine the avoidance to a relatively short period, making it a more practical procedure. Which of these hypotheses, if either, is eventually proved correct remains to be seen, but primary sensitization and its prevention will no doubt continue to be the focus of much research in the future.

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