

Asthma

The early life origins of asthma and related allergic disorders

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A focus on the way the disease evolves in early life

Pharmacological treatment of established asthma is highly effective in controlling symptoms and improving quality of life. However, no treatment has been hitherto shown to modify the natural course of the disease and no cure has been identified. Indeed the best predictors of continuing asthma into adulthood are: early age of onset; sensitisation to house dust mites; reduced lung function; and the presence of bronchial hyperresponsiveness in childhood, irrespective of therapy.¹ This suggests that we have made little progress in understanding the basic causes of the disease than was the case in the 12th century when Moses Ben Maimon, Jewish physician and philosopher, wrote in his handbook for his patient, the Sultan Saladin's son, "I have no magic cure to report. Asthma has many aetiological aspects and should be treated according to the various causes that bring it about".² Under such circumstances, attention should continue to focus on understanding the way in which the disease evolves in early life, in order to identify targets for primary preventive therapy.

FETAL RESPONSES TO ALLERGENS

It should not be surprising to any paediatrician that the fetus is capable of mounting sophisticated immune responses. All will be familiar with the antenatal immune response in relation to the detection of IgM antibodies from as early as 20–26 weeks gestation in fetuses of mothers infected with rubella in early pregnancy.³ Tetanus toxoid specific IgM can be detected in the cord blood of infants whose mothers were immunised during pregnancy.⁴ Direct immunisation of fetal baboons with recombinant hepatitis B surface antigen results in specific IgG detectable in the fetal but not maternal circulation, and subsequent postnatal immunisation results in an enhanced production of antibodies.⁵ These studies show that the entrenched paradigm that early antigen exposure induces tolerance is not consistently the case. Indeed, stem cells are present in the human yolk sac at 21 days of gestation with the first

lymphocyte seen in the thymus at the end of the ninth week of gestation. The lymphocytes can be seen in a range of organs, including the lungs and gut, from 14 weeks; and by 19–20 weeks, circulating B cells have detectable surface immunoglobulin M.⁶ This implies that the full sensitisation process must have occurred from antigen presentation through T cell proliferation to B cell stimulation and antibody production. It is even possible to show that cultured cord blood cells from neonates born to parasitised mothers will produce IgE when stimulated with parasite antigens.⁷

It is clear that there is virtually universal priming to environmental antigens which has occurred before birth, but in those infants destined to be allergic and develop diseases such as asthma, the responsiveness is altered. At its most extreme, a small percentage of infants destined to have allergic disease already has raised cord blood total and specific IgE. This has proved to be a highly specific but very insensitive marker of later disease.^{8–12} A number of studies have shown that the pattern of peripheral blood mononuclear cell responsiveness to allergens in neonatal blood samples has predicted the subsequent development of allergic disease.^{13–15} Proliferative responses of cord blood cells to allergens is a universal phenomenon; in one study proliferation occurred to house dust mite in 46% of samples, to purified major allergen of house dust mite in 73%, and ovalbumin from hen's egg in 42%. This was not due to maternal cross-contamination of the neonatal blood sample because genotyping of T cell clones from the cord blood established their fetal origin.¹⁴ My group have shown that peripheral blood mononuclear cell responses to allergen can be detected from as early as 22 weeks gestation.¹⁶ While there is universal priming to allergens, enhanced responsiveness is associated with a higher risk of allergy. Furthermore, a number of studies have shown differences in cytokine profiles from allergen and mitogen stimulated peripheral blood mononuclear cells in

those neonates destined to be allergic.¹⁷ All studies suggest that there is a disturbance of the balance between cytokines which suppress an allergic response as characterised by T helper-1 (Th-1) phenotypic responses compared with allergy promoting Th-2 responses. In the former, the characteristic cytokines are interleukin 12 (IL-12) and interferon gamma (IFN- γ), and in the latter, IL-4, 5, and 13. While Th-1 and Th-2 phenotypic responses have been viewed as mutually exclusive with reciprocal suppression orchestrated by IFN- γ and IL-4, the concepts are now changing as it has become clear that Th-1 diseases characterised by autoimmune disorders, such as insulin dependent diabetes mellitus and Th-2 mediated atopic diseases, such as hay fever, eczema, and asthma respectively, coexist more frequently than might be expected by chance.¹⁸ It is now clear that there is at least a third group of T helper cells known as regulatory T cells, generating cytokines IL-10 and transforming growth factor beta (TGF β). These have a suppressive influence on both Th-1 and Th-2 cells. It is likely that perturbations of regulatory processes as well as of balance between Th-1 and Th-2 activities underlies the susceptibility to having an allergically biased response to antigen.

ROUTE OF FETAL ALLERGEN EXPOSURE

Two potential routes of exposure have been identified. The first route is via the fetal gut, by swallowing allergen in amniotic fluid. It has been possible to detect significant quantities of the major allergen of house dust mite, Der p 1, and ovalbumin in amniotic fluid.¹⁹ The protein turnover in amniotic fluid occurs at a rate of 70% each day at term, with much of this removal being via fetal swallowing.²⁰ Clearly the fetus also aspirates amniotic fluid into the respiratory tract and in addition has direct exposure via a highly permeable skin. However, while there are no mature antigen presenting cells or T cells within the skin or airways, the fetal gut has mature immune active cells and is probably the principle route by which sensitisation can occur.²¹ There are antigen presenting cells detectable within the fetal gut from as early as 11–12 weeks gestation. These are shown to be HLADR+ and constitute a mixed bag of macrophages, dendritic cells, and B cells. By 14–16 weeks gestation, rudimentary lymphoid follicles have formed within the gut, and at that time it is possible to show mature dendritic cells which have picked up antigen, T cells, and B cells, all co-segregating within the lymphoid follicles. The surface markers

on these cells suggest that they have all the necessary costimulatory signalling pathways available to facilitate antigen presentation to T lymphocytes.²¹ The antigen presenting cells also have detectable high and low affinity IgE as well as IgG receptors at this early stage in the second trimester of pregnancy.²²

The second route of exposure is by direct transfer of allergen across the placenta. It has now been shown that such transfer is mostly in complexed form with immunoglobulin G.²³ Thus the exposure to allergen complexed with IgG will occur optimally in the third trimester of pregnancy when there is active transport of IgG across the placenta.

PREGNANCY AS AN ALLERGIC PHENOMENON

The fetoplacental unit generates fetopaternal antigens which should in principle lead to a maternal immune rejection of the pregnancy. It is well recognised that in murine models, the fetoplacental unit produces Th-2 cytokines such as IL-4 and IL-5 throughout pregnancy.²⁴ These cytokines inhibit maternal Th-1 activity which in turn protects the pregnancy. Indeed, maternal generation of IFN- γ , IL-2, and tumour necrosis factor beta (TNF- β) is directly associated with compromise of the pregnancy.²⁵ IL-2 promotes proliferation of uterine large granular lymphocytes, creating lymphokine activated killer cells with enhanced cytotoxic activity.²⁶ IL-2 in vitro increases the lymphocyte expression of CD16, which in turn is associated with increased killing of cultured trophoblast cells. This will compromise the invasiveness of trophoblast during implantation and placental growth.²⁷ IFN- γ is an abortifacient, mediating its effect by activation of natural killer (NK) cells.²⁸

While there is rather less information available in relation to human pregnancy, it has been shown that IL-4 is produced by human amnion epithelium, both in the first and third trimesters of pregnancy,²⁹ and IL-13 is produced by the placenta predominantly during the second trimester of pregnancy.³⁰ There is also an increased production of the regulatory cytokines IL-10³¹ and TGF- β .³² It is virtually never possible to detect any IL-2, IL-12, or IFN- γ . Thus there is a marked bias of exposure of maternal lymphocytes at the placental interface to Th-2 promoting Th-1 suppressing cytokines (fig 1). It is perhaps not surprising that the fetus also is exposed to such cytokines, and indeed all of the above Th-2 and T regulatory cytokines are detectable in the amniotic fluid.³³ This will tend to bias the fetal immune response towards a Th-2 phenotype,

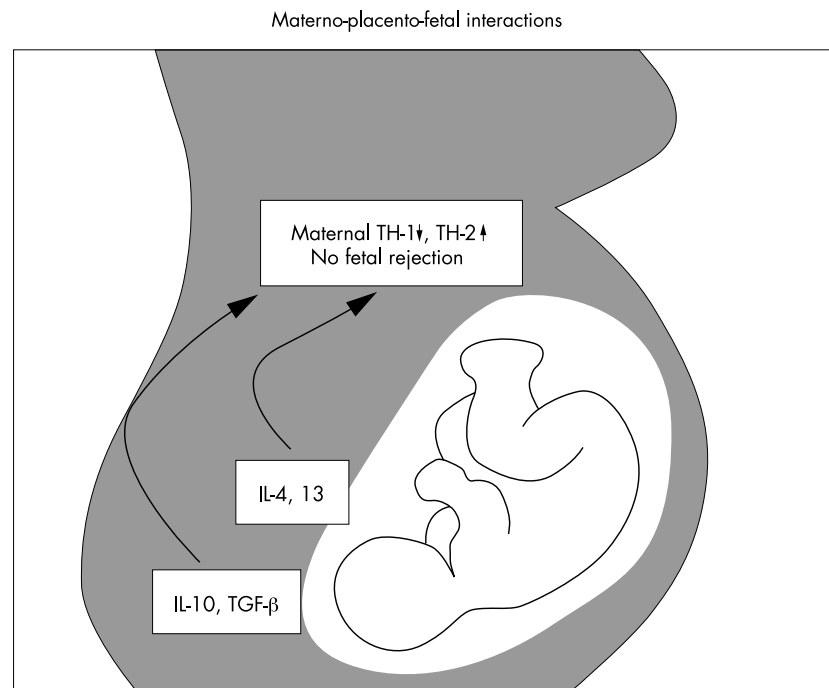


Figure 1 One of the explanations for maintenance of a successful pregnancy is that both Th-2 (IL-4 and IL-13) and T regulatory (IL-10 and TGF- β) cytokines are generated by the conceptus to down-regulate maternal Th-1 immune responses to fetopaternal antigens which might otherwise lead to fetal rejection.

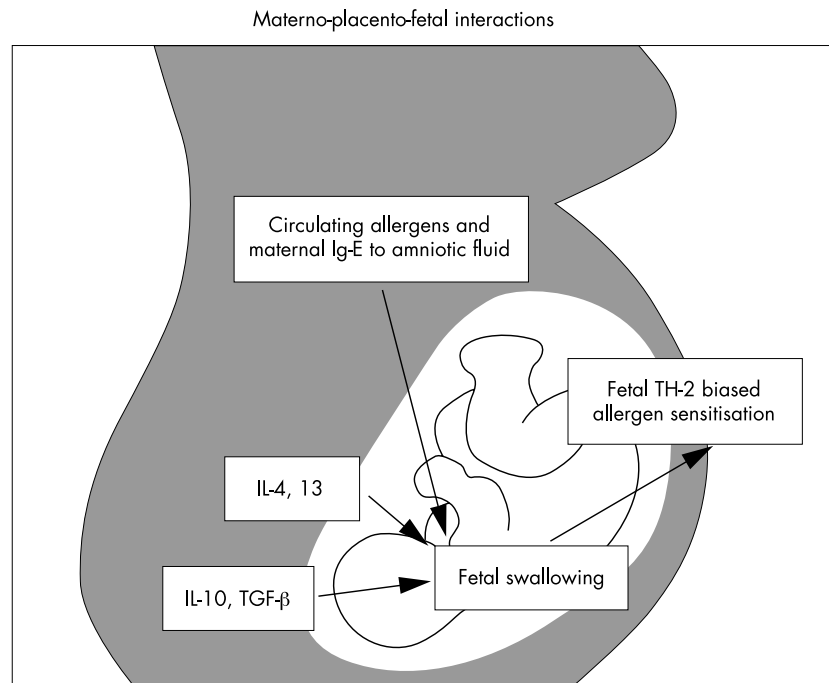


Figure 2 The consequence of protection of the pregnancy by regulation of maternal Th-1/Th-2 balance is that the fetus is also exposed to a high concentration of Th-2 and T regulatory cytokines. These are present in the placenta, together with maternal IgE and allergens which have reached the amniotic fluid via the maternal circulation, leading to the potential for fetal allergic sensitisation, particularly as a consequence of swallowing and the generation of gut priming of the immune response. Antigen presenting cells, T cells, and B cells are mature within the fetal gut from as early as 16 weeks gestation.

and indeed this is usually the case¹⁴ (fig 2). However, the fetus does to a certain extent balance up the response

by the spontaneous release of IFN- γ . Indeed, fetal plasma IFN- γ concentrations are high in the first trimester of

pregnancy and subside slowly during the course of pregnancy.³⁴ The Th-2 and T regulatory cytokines have additional critical functions in stimulating cell growth and differentiation. Indeed, TGF- β has a pivotal role in relation to lung airway development.³⁵

Thus there is a fine balance of cytokine production between mother, placenta, and the fetus, orchestrating a down-regulation of maternal immune responses to feto-paternal antigens, at the same time as encouraging normal fetal growth and immunological responsiveness. It is, therefore, predictable that relatively minor perturbations of this balance can have an impact either decreasing or increasing a risk of subsequent allergic and/or airway disease.

FACTORS MODIFYING ANTENATAL ALLERGEN SPECIFIC IMMUNE RESPONSES

Maternal atopy confers a far higher risk of the early expression of atopic disease in the offspring than paternal atopy. This suggests that an atopic mother creates a more allergy promoting environment for her fetus and young baby. Amniotic fluid contains IgE at levels proportionate to the maternal circulating IgE levels. Thus mothers with high levels who are themselves atopic, expose their fetuses to higher quantities of IgE through the amniotic fluid, even though IgE does not cross the placenta into the fetal circulation.³⁶ It is reasonable to hypothesise that the combination of IgE in the amniotic fluid and IgE receptors on antigen presenting cells in the fetal gut creates an environment whereby sensitisation is facilitated to exceedingly low levels of antigen. This occurs by a phenomenon of antigen focusing whereby the IgE facilitates antigen presenting cell responses.³⁷ This sophisticated mechanism could have evolved to facilitate enhanced neonatal host response to the inevitable exposure which will occur to maternal helminths. Indeed, infants born to helminth infected mothers have specific Th-2 biased immune responses to helminth antigen and IgE antibodies to these antigens.³⁸ Now that we have very low parasite exposure, it is likely that molecules present in sensitising allergens have molecular counterparts to parasite antigens, leading to stimulation of the same immune response.³⁹

In addition to differences in IgE levels in amniotic fluid of atopic mothers, there are also higher levels of IL-10. This regulatory cytokine will suppress both Th-1 and Th-2 cells. This is consistent with the finding that infants born to atopic mothers have not only significantly lower levels of Th-1 cytokines such as IFN- γ from stimulated

cord blood mononuclear cells,^{9-11 13} but also reduced levels of Th-2 cytokines such as IL-13.³⁰ However, the balance is still biased towards greater suppression of Th-1 than Th-2 activity.

A number of studies have suggested that IgG from the mother may have a modulating effect on fetal immune responses. Thus, there is an inverse relation between cord blood house dust mite specific IgG antibodies and cord blood mononuclear responses to house dust mite.⁴⁰ There is also an inverse relation between cat IgE antibodies in children of 8 years of age and the levels of IgG cat antibodies the same child had at birth.⁴¹ This very much mirrors older murine studies in which maternal IgG was found to suppress IgE responses in neonates.⁴² Levels of specific IgG antibody to allergen in the cord blood reflect those of maternal IgG, which in turn is likely to be a reflection of maternal allergen exposure. This suggests rather paradoxically that high allergen exposure in the mother could potentially have a protective effect against the development of allergy. Indeed, children of mothers who received rye-grass immunotherapy during pregnancy have fewer positive skin tests to rye-grass 3-12 years later, than infants born to mothers who stopped their immunotherapy during pregnancy.⁴³

Immunotherapy among other immune mechanisms generates IgG antibodies which might have their blocking effect by antigen neutralisation, preventing access to antigen presenting cells. It is, however, possible that IgG antibody directly confers an inhibitory signal to antigen presenting cells via an immunoreceptor tyrosine based inhibition motif on Fc γ RIID receptors which specifically bind IgG 1 and 3, which are preferentially transported across the placenta.⁴⁴

Asthma prevalence has increased in parallel with increasing affluence; one hypothesis is that this relates to the influence of changing diet. There has been a declining rate of consumption of fresh fruit and vegetables which has paralleled the rise in atopic disease.⁴⁵ Such foods are associated with antioxidant activity and could be implicated in protecting against the development of airway inflammation. However, there may be additional impacts on Th-1/Th-2 balance in that retinoids have been shown to inhibit IL-4 dependent IgE production by murine B cells.⁴⁶ It is possible that this effect would have its major impact ante- rather than post-natally.

Other nutrients which have been shown to have some influence in established asthma have been imbalances in fatty acid intakes. Diets rich in linolenic

acid promote prostaglandin E2 production which in turn promotes IL-4 production.⁴⁷ It is well established that fish oil supplementation produces decreases in pro-inflammatory mediators such as leukotrienes.⁴⁸ The effects on clinical features of asthma are modest or non-existent.⁴⁹ However, it is possible that variations in lipid intakes during pregnancy could have a greater impact on the developing immune response. A large birth cohort study has shown a remarkable association between frequent consumption of products high in cow milk fat at 2 years of age and a reduced risk of asthma at 3 years.⁵⁰ To what extent this might reflect a high intake in the mother during pregnancy remains to be established.

There have been some intriguing associations between large head circumference at birth and levels of total IgE at birth,⁵¹ in childhood,⁵² and in adulthood.⁵³ Furthermore, large head circumference at birth is strongly associated with asthma and particularly asthma requiring medical attention.⁵⁴ It is possible that large head size is representative of a rapid fetal growth trajectory which in turn is a consequence of very good maternal nutrition. However, towards the end of pregnancy, neither maternal nutrient intake nor placental capacity to delivery nutrients to the fetus can be sustained. The head and brain continue to grow as a consequence of a brain sparing reflex, leading to a baby of normal birth weight with a large head. The poor nutrition to the body in the latter stages of the pregnancy will have its major effect on rapidly dividing tissues such as those in the immune system which could subtly alter balances of Th-1 and Th-2 activity.

It is, of course, important to recognise that variations in nutrient delivery to the fetus will also have an impact on airway growth and maturation. It has long been known that vitamin A plays a major role in lung development, with mild deficiency having effects on airway branching and lung epithelial cell differentiation.⁵⁵

Maternal smoking in pregnancy clearly has a significant impact on airway growth development, leading to differences in lung function of the newborn compared with those born to non-smoking mothers.⁵⁶⁻⁵⁸ Such infants are more likely to develop wheezing illnesses in the first year of life.⁵⁷ However, while pregnancy smoking increases wheezing illnesses in infancy, there is little evidence based on meta-analyses that maternal pregnancy smoking has any effect on allergic sensitisation.⁵⁹ Thus if maternal pregnancy smoking has any impact, it is likely to be increasing the severity of

disease in those who have additional factors promoting the development of allergic sensitisation.

POSTNATAL IMMUNE MODULATION AND THE HYGIENE HYPOTHESIS

Numerous studies have suggested that there is an inverse relation between early exposure to microbial products and a reduced risk of developing allergy and allergic disease. As all babies are born with a weakly Th-2 biased immune response, there must be a rapid down-regulation postnatally. Exposure to microbial products has been suggested as one important event achieving this effect. A number of studies have shown an inverse relation between the prevalence of atopy and tuberculin responsiveness,⁶⁰ hepatitis A,⁶¹ and measles infection⁶² in early childhood. These observations have led to a whole programme of research to develop Th-1 immuno-adjuvants as treatment for allergic disease. BCG and heat killed listeria monocytogenes are currently the main focus of work.⁶³ However in the long term, DNA vaccines may prove to be more effective.⁶⁴ The difficulty is to establish whether the infections associated with less allergy truly down-regulate the response or whether they are a marker of some other component of hygiene. There are inconsistencies in that some studies, particularly focused on more developed countries, have failed to show an inverse relation between measles and atopy.⁶⁵ Furthermore, while some studies have shown an inverse relation between frequent use of antibiotics and an increased risk of asthma, hay fever, and eczema,⁶⁶ others have failed to show this, and indeed the only prospective study showed no association with either allergy or asthma.⁶⁷

It is perhaps more credible that infant gut colonisation by appropriate microbial flora will have a more important immunomodulating effect.⁶⁸ The original proposers of the gut flora hypothesis have shown that children from a country with a low prevalence of atopy, namely Estonia, have a very different intestinal bacterial flora to those infants from countries with a higher prevalence of atopy, namely Sweden. Furthermore the group have shown that both in Estonia and Sweden, allergic children are less often colonised with lactobacilli compared with non-allergic children but have higher counts of aerobic micro-organisms, such as coliforms and *Staphylococcus aureus*.⁶⁹ It has been suggested that the much lower prevalence of allergy and asthma among the children of farmers who have been born on farms and have been exposed to raw

and sometimes unpasteurised milk, which obviously has a higher microbial load, particularly of lactobacilli, is consistent with the gut flora hypothesis.^{70 71} The anthroposophic lifestyle, which avoids immunisations and antibiotics but includes consumption of a diet of fermented vegetables rich in lactobacilli, is associated with significantly less atopy and skin test positivity in childhood.⁷²

This has led inevitably to a study employing lactobacillus GG given prenatally to mothers who had at least one first degree relative or partner with atopic disease and to their infants for the first six months of life. Atopic eczema occurred less frequently up to 2 years of age in the actively treated compared with the placebo treated infants.⁷³ However, there was no difference in the above study in the prevalence of raised IgE or positive skin prick tests. The group have subsequently shown that administration of the probiotic to mothers modified concentrations of one of the regulatory cytokines, TGF- β 2, in breast milk.⁷⁴ This latter observation suggests that the main effect of early microbial exposure is not specifically upregulating Th-1 responses but more in upregulating the activity of Th-3 or regulatory cells which will have a controlling effect both on Th-1 and Th-2 activity. This would be consistent with the observation that Th-1 and Th-2

diseases can coexist more frequently than by chance.¹⁸

One additional explanation for reduced prevalence of atopic disease among children of farmers and also, indeed, those who have been exposed to a cat and/or dog in the home in early infancy, is that this increases exposure to endotoxin.⁷⁵ Indeed, there is an inverse relation between house dust endotoxin concentrations and skin test positivity to allergens.⁷⁶ Endotoxin or lipopolysaccharide combines with the CD14 molecule on macrophages and monocytes, or with soluble (s) CD14 in biological fluids, and thereby signals through toll-like receptor 4 on antigen presenting cells such as dendritic cells to stimulate production of IL-12 and IL-18 which, in turn, promotes IFN- γ production by T cells. My group has recently shown that sCD14 in the amniotic fluid of pregnancies where the infant has subsequently developed positive skin tests and eczema, is lower than in those pregnancies where the infant has not developed any atopic problems. Similar differences in sCD14 levels were also observed in the breast milk of mothers whose babies subsequently had atopic eczema.⁷⁷ Thus maternal IgG antibodies and sCD14 levels, together with the fetus's capacity to generate IFN- γ , provide the main counter-balances to fetal and infant allergic sensitisation (fig 3).

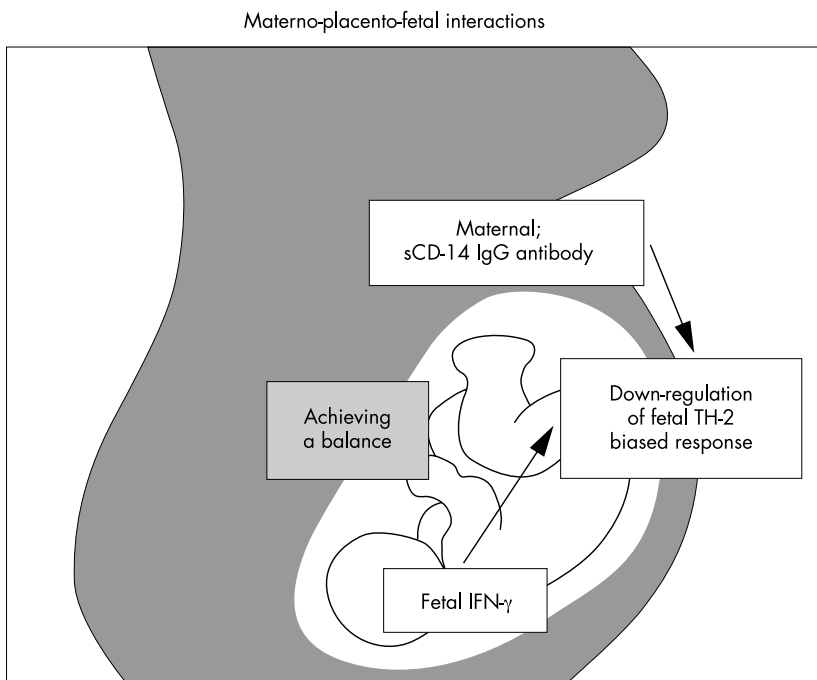


Figure 3 A number of factors counter-balance the drive to a fully committed Th-2 response to allergens in the fetus. These include the fetal generation of IFN- γ and a maternal supply of soluble CD14 which, in combination with endotoxin, will lead to stimulation of IL-12 and, therefore, IFN- γ production. Maternal IgG antibody which is actively transported across the placenta may block fetal allergen sensitisation.

POTENTIAL PREVENTION STRATEGIES

Insights into these early events indicate that primary prophylaxis to prevent allergic sensitisation and tendency to allergic disease will be focused on the fetus. Here, optimisation of nutrition, particularly in the third trimester of pregnancy, perhaps with lipid, antioxidant, and vitamin A supplementation, should have particular value. Strategies focusing on either high allergen exposure to increase IgG antibodies or complete allergen avoidance will need to await further research. Clearly avoidance of maternal smoking during pregnancy will reduce wheezing illnesses but not necessarily atopy.

Secondary prevention after sensitisation which has often occurred in fetal life will focus on the newborn infant and young child up to 12–24 months of age. A high risk group could be identified by a persistent Th-2 biased response in the first months of life, and the strategies may involve Th-1 immunoadjuvants, DNA vaccines, or seeding the gut with appropriate intestinal flora. The focus of this therapy may rest on the mother, thereby improving delivery of appropriate immune modulators in breast milk.

Once the allergic march has commenced, the ability to interfere with progression diminishes appreciably. However, tertiary prophylaxis with pharmacotherapy utilising cetirizine has been shown to delay the development of asthma, at least in those infants who were sensitised to aeroallergens.⁷⁸

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