

Review article

Does atopic disease start in foetal life?

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The prevalence of IgE-mediated allergies, such as eczema, hay fever, and asthma, has increased dramatically over the past few decades. The short time over which this change has occurred and continues to occur indicates that the environment plays a central role in these disorders. That there is an important inherited component in these diseases is well established, and numerous genes have been identified as having a possible role (1). However, a complex interplay between the environment and our genes is likely to determine disease development and progression. The environmental factors that affect the inherited susceptibility have been reviewed elsewhere (2). Here we will consider the contribution of pregnancy and foetal development in this issue.

Introduction

Interest in the contribution of the gestation-associated environment to the development of atopic disease has been generated and sustained by three main factors. Firstly, elevated umbilical cord-blood IgE was identified as a specific marker of later atopic disease (3–6), although, unfortunately, this has not proved to be a sensitive marker of subsequent disease development, as was initially hoped. However, the observation indicates that foetal exposure to allergens occurs with priming *in utero*, which may be significant in the development of disease. Allergen-specific reactivity at birth has been demonstrated to a range of common allergens, such as those of house-dust mite (HDM), cow's milk, and hen's

egg (7–10). Such reactivity supports the contention that foetal exposure occurs to both dietary and, perhaps more surprisingly, inhalant allergen, and that the foetal immune system attains a level of maturity enabling the initiation of antigen-specific immune responses before birth.

Finally, the increased risk of atopic disease, particularly in early infancy, in the offspring of atopic mothers compared to those of atopic fathers suggests that pregnancy may be a critical time in determining subsequent disease development (11, 12). Caution in the interpretation of these observations must always be exercised, as mothers have been found to underrate disease occurrence in their partners (13); therefore, a self-reported history should always be obtained from the fathers to avoid this. In a study where self-reporting of both parents was conducted (14), the investigators found a positive association between recurrent wheezing in the first 2 years of life and both asthma and allergy in the mother, but not the father.

In the past, attempts have been made to explain this association between atopic disease in early life and a maternal history of such diseases by the "Carter effect" – the more rarely affected sex has a higher threshold for disease manifestation requiring more predisposing genes, and therefore imparting more disease-related genetic material to the offspring (15). More recent data suggest that this association results from foetal programming – development during critical periods of foetal growth may have lifelong effects on structure, physiology, and metabolism (16) – a concept to be returned to later.

Foetal production of IgE

Although cord-blood total IgE levels are very low, its detection indicates that the foetus is able to produce IgE, as this immunoglobulin type does not cross the placenta (17). Foetal production of IgE occurs from as early as 11 weeks of gestation, the liver being the sole source at this stage. During the following 10 weeks, the foetal lung and spleen commence IgE production (18). Cord-blood lymphocytes from infants delivered to helminth-infected mothers in Kenya, but not to mothers residing in North America, could spontaneously produce polyclonal and parasite antigen-specific IgE in culture (19). Moreover, the level of parasite antigen-specific IgE induced in culture correlated with the level of specific IgE measurable in the matched cord-blood plasma. *Filaria*-specific IgE has also been demonstrated in 58% of cord-blood samples from infants delivered in Madras, India (20). In neither of these investigations did the foetus itself have microfilaria and/or circulating filarial antigen; therefore, the route of parasite antigen exposure remains undefined. Importantly, studies such as this indicate that the foetus is perfectly able to mount an antigen-specific IgE response before birth, and, at birth, stimulation via CD40 and IL-4 induces IgE levels like those of adult peripheral blood mononuclear cells (PBMC) stimulated in the same fashion (21). It is interesting to note that the measurement of HIV-specific IgE in cord blood has been proposed as a means of identifying neonates that are actually HIV infected, as the measurement of HIV-specific IgG does not provide this information because of its maternal origin (22).

Studies of Western populations have identified allergen-specific IgE in relatively few cord-blood samples, although a correlation between maternal IgE and cord-blood IgE is well documented (12, 23). It is imperative in these later studies that maternal contamination always be ruled out, and, although this is not always done, it is likely that this relationship reflects the measurement of foetally derived IgE in the cord blood. This association may be explained by the report of maternal inheritance of atopic IgE responsiveness on chromosome 11q (24) or by as yet undefined mechanisms.

Antigen-specific reactivity by the foetus and neonate

Antigen-specific PBMC reactivity at birth has now been demonstrated by many laboratories using a range of allergens, both dietary and inhalant (7–10). The rate of positive responders to a given antigen varies from laboratory to laboratory, probably reflecting different antigens and different levels of exposure in geographically discrete populations, as well as variations in the methods used by each group.

Until recently, it was considered that antigenic challenge during neonatal life is tolerogenic rather than immunogenic. That this is not necessarily the case has been demonstrated by a number of groups (25–27). For example, Ridge et al. (25) concluded that tolerance is not an intrinsic property of the neonatal immune system but that tolerance or immunization upon antigenic challenge in the neonate is determined by the “nature of the antigen-presenting cell” and the antigen dose. Clearly, the studies of allergen-specific responses at birth represent responses initiated *in utero* rather than by the neonate. Thus, a more realistic model of how the human foetus might respond to antigens would be provided by studies conducted in germ-free animals.

A particularly relevant study in this respect is that of Sudo et al. (28). Using germ-free mice, they examined the role of the gastrointestinal flora in oral tolerance induction to IgE responsiveness. Th2-mediated responses were maintained and Th1 responses abrogated in germ-free mice, exposed orally and then systemically challenged with ovalbumin (OVA). Most interesting of all was the restoration of the susceptibility of both Th1 and Th2 responses to oral tolerance induction by reconstituting the gastrointestinal flora of the germ-free mice with *Bifidobacterium infantalis*, an organism common to their normal commensal flora. This effect was seen only if reconstitution was done in the neonatal period.

Studies such as those described above suggest that the outcome of exposure to allergens (or any other antigen) during foetal life should be predominantly Th2 biased. Furthermore, low antigen dose, as is likely to occur in the foetus, also favours Th2 reactivity (29, 30). This arises from reduced ligation of the T-cell receptor by MHC molecules containing antigen-derived peptide and subsequent limited phosphorylation of intracytoplasmic kinases and calcium mobilization, resulting in IL-4 production. In addition, the cytokine environment in which antigen presentation to T cells occurs may also influence outcome with regard to Th1 and Th2 reactivity. The presence of IL-12 at this time is particularly critical in the generation of antigen-specific Th1 cells (31). Both IL-4 (31) and PGE₂ (32) have been described as favouring Th2 reactivity.

Thus, the conditions under which the human foetus encounters antigen are likely to favour Th2 reactivity. Recent work has identified “universal skewing” of allergen-specific responses at birth to a Th2-predominated profile (10). It is unfortunate that IL-4, IL-9, and IFN- γ protein levels were not measurable in this study, but the abundance of IL-10, IL-6, and IL-13 from allergen-specific clones (HDM and OVA) in the absence of detectable IFN- γ led to this conclusion. Most importantly of all, it was shown that the allergen reactive T-cell clones were of foetal, and not maternal, origin, laying to rest the often levelled criticism of

studies such as these that the response being detected reflects contamination of the sample with maternal PBMC. This was clearly not the case.

Relevance to atopic disease

As is often the case, deeper analysis of an initial observation, in this case the phenomenon of allergen-specific reactivity at birth, raises more questions than it answers. From the above, it would appear that many foetuses are exposed to and develop Th2-biased responses to allergen, prompting the question – why doesn't everybody suffer atopic disease? Is there a difference in the nature or degree of response to allergens in children at risk of developing atopic disease (at least one first-degree relative with disease) compared to their low-risk counterparts, and, even more importantly, do those children who develop disease irrespective of their family history have a demonstrably different response to allergen either before or at birth?

The development of atopic eczema with positive skin prick tests to cow's milk at 1 year of age, where the symptoms improved on a milk-free diet, was significantly associated with raised cord-blood mononuclear cell (CBMC) proliferative responses to β -lactoglobulin (7). Allergen-specific CBMC proliferative responses tended to be higher in neonates with an immediate family history of atopy than in their low-risk counterparts, although this was significant only for responses to the cat allergen Fel d 1 (10, 33).

Despite the ongoing debate as to what information proliferative responses may give us beyond clearly identifying those foetuses that have been immunologically primed to allergen, the investigation of cytokine responses has been rewarding. It has been well documented that neonates in general have reduced IFN- γ production in comparison to adults (34–36). However, IFN- γ production at birth is even further decreased in infants at risk of atopic disease (37) and those who do develop disease. This observation has now been demonstrated by numerous groups for both mitogen- and allergen-stimulated responses (7, 10, 38, 39). Downregulated IFN- γ production by β -lactoglobulin-stimulated CBMC has been described in infants who developed cow's milk-mediated allergic disease at 1 year of age (7) and a group of children in Japan who also later developed atopic disease (38, 39). HDM- and OVA-specific IFN- γ (mRNA) was also reduced at birth in children who developed atopic disease at 2 years of age (33).

Thus, it has been postulated that downregulation of the prototypic Th1 cytokine IFN- γ at birth may be associated with the subsequent development of atopic disease, a Th2-mediated disorder. Recent work supports this by showing that at-risk children who developed disease continued to be poor producers of

IFN- γ over the first 2 years of life while consolidating Th2 cytokine production (33). Additionally, one of the most commonly described features of PBMC from adults and children with atopic dermatitis is decreased IFN- γ production in comparison to controls who do not suffer from this disease (40, 41). An inability to mount an adequate IFN- γ response may therefore be a central defect in many atopic disorders, a defect evident in early foetal life. Unfortunately, although this defect has been recognized for many years and many studies have been conducted to identify the underlying mechanism(s), the cause remains to be elucidated.

Further complicating the situation in the neonate is the observation that IFN- γ is not the only cytokine downregulated. PHA-stimulated CBMC from high-risk children who developed atopic disease by 3 years of age had significantly reduced IL-13 levels in addition to lower IFN- γ levels (42). Allergen-specific IL-6, IL-10, and IL-13 protein levels and IL-4 mRNA were significantly reduced at birth in children who later developed atopic disease (33). Thus, the paradox exists that although there is "universal skewing" toward Th2 reactivity at birth, this response is not enhanced at birth in those infants who later develop a Th2-mediated disease but is actually dampened in comparison to neonates who later show no evidence of disease. Downregulation of numerous immunologic functions may be a common feature of children with a family history of atopic disease. For example, opsonization (43) and IgA levels (44) are lower in high-risk neonates. Whether these observations reflect downregulation or delayed maturation (45) remains to be seen. Delayed maturation may explain why a large number of infants develop atopic dermatitis which they "outgrow" because their immune response finally catches up with that of their counterparts (Fig. 1).

Does atopic disease start in foetal life?

From the above, it can be seen that not only is the foetus exposed to allergens to which it can mount an antigen-specific response but also that this could entail initiation of an antigen-specific IgE response. However, intrauterine allergen exposure is probably very common, especially for dietary antigens, as reflected by the large proportion of neonates who have positive proliferative responses to allergens such as those of cow's milk (9). Numerous questions are raised by this observation. What is the pathway of allergen exposure in the foetus? Where does the priming of the foetal immune system occur? Does the antigen-specific response generated during foetal life have a role to play in later development of atopic disease? What are the underlying mechanisms of the downregulated responses exhibited at birth by children who develop atopic disease?

Foetal allergen exposure

The most commonly postulated pathway for allergen exposure of the foetus is transplacental. This would be particularly relevant to IgG/allergen immune complex transfer from the mother to the foetus. IgG is well known to traverse the placenta, with different isotypes showing preferential transfer (IgG1 > IgG3 > IgG4 > IgG2) (46). The transport pathway across the first cellular layer with which maternal IgG comes into contact (foetally derived syncytiotrophoblast) has been recently described, and it is dependent upon the neonatal Fc receptor (FcRn) (47, 48). Small amounts of IgG cross the placenta from early in gestation, and the levels increase rapidly from 20 weeks of gestation to become maximal from 32 weeks of gestation until birth (49). Although evidence of foetal priming to allergens has been known for a number of years, the passage of allergen, either free or complexed, to the foetus has not been demonstrated. A number of groups have established the experimental models to examine this, and the necessary investigations are under way.

As PBMC allergen-specific responses can be demonstrated as early as 23 weeks of gestation, it seems unlikely that allergen exposure occurs via IgG complexes crossing the placenta. An alternative route of allergen exposure, postulated by our group, may be across the foetal membranes where the maternal (decidua) and foetal tissues are in intimate contact. The passage of proteins from the maternal to the foetal side of this membrane is already known to occur and is best demonstrated by the detection of decidualy derived prolactin in the amniotic fluid (50). We have also shown that IgE is detectable in amniotic fluid at

16–17 weeks of gestation, as well as at term, in levels that correlate with maternal levels (51), suggesting that IgE also passes from the vascularized decidua to the amniotic fluid. Interestingly, IgG is also found in the amniotic fluid, and epithelial cells in the human foetal gastrointestinal tract also express FcRn (52). We have also recently demonstrated that HDM allergen is detectable in amniotic fluid at 16–17 weeks of gestation (Holloway et al., unpublished observation).

The major advantage of transamniotic transfer of allergen and other factors that may regulate the ensuing response is the access to the gut-associated lymphoid tissue. Peyer's patches are structurally mature by 19 weeks of gestation with the M (manifold) cells that sample the environment already in place by this time (53). Recent work from our group has shown that there are CD83+ dendritic cells within this tissue from as early as 17 weeks of gestation (Jones et al., unpublished observation), and T cells are known to be present as early as 13 weeks of gestation (54).

The gastrointestinal tract of the human foetus is exposed to amniotic fluid by foetal swallowing, which can begin as early as 10 weeks of gestation (55). This process accounts for 70% of the daily protein turnover in the amniotic fluid (56). Likewise, the skin is exposed to the components of amniotic fluid, and to a much lesser extent the airways are exposed because a small amount of amniotic fluid aspiration occurs due to foetal respiratory movements.

Although the above is an attractive hypothesis, much work remains to be done, as with the transplacental pathway, to elucidate the contribution made by either or both pathways to foetal exposure and response to allergen.

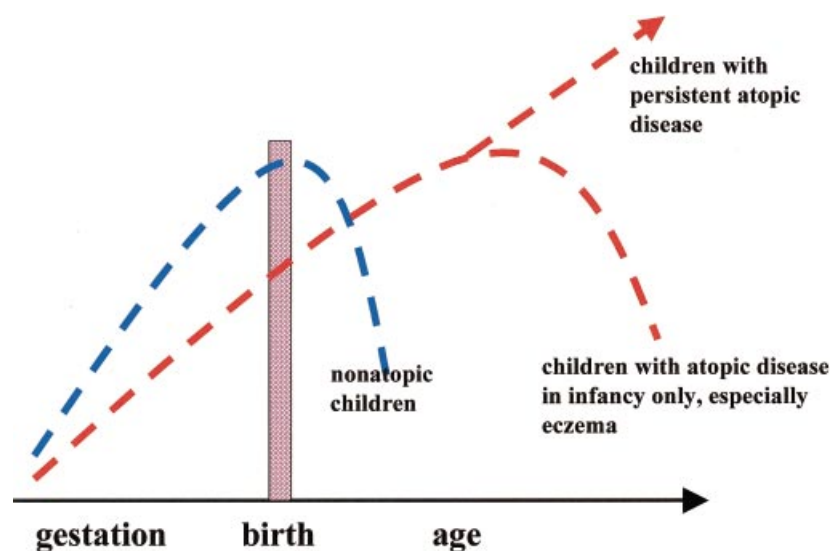


Figure 1. Th2-type reactivity before and after birth. Th2 responses predominate at birth but are dampened in children who later develop disease. Consolidation of Th2 responses occurs postnatally in children who develop atopic disease but is downregulated in those who do not.

Foetal programming

The interaction of the foetus with the gestation-associated environment, be that via the amniotic fluid or nutritional factors at the placental interface, may lead to foetal programming. Thus, a susceptibility to later disease development, in this case atopic disease, would be acquired. Evidence that this may be happening has been provided by a number of studies. Godfrey et al. (57) showed that raised IgE concentration in adulthood was associated with disproportionate foetal growth as demonstrated by increased head circumference at birth. More recently, the association between greater head circumference and raised IgE levels *ex utero* has been observed in children (58), although no consistent relationship was found between head circumference and skin prick test positivity or clinical asthma. Large head circumference at birth has also been associated with raised cord-blood IgE (59) and with an increased risk of asthma.

Hypotheses to explain this association have focused on maternal nutrition, but before conclusions can be drawn from such studies larger cohorts need to be examined prospectively. Moreover, the identification of the underlying mechanisms – for example, altered thymus growth, as suggested by the investigators in these studies – needs to be made.

Priming the human foetus to antigen

Allergens are not the only antigens to which the human foetus shows evidence of priming. Proliferation responses at birth are seen to schistosomal antigens in neonates of schistosomal-infected, but not uninfected, mothers (60), and to malarial antigens in neonates born in Cameroon (61). In the latter study, the percentage of cord bloods with a measurable proliferative response to malarial antigens was only marginally reduced compared to adults, whereas antigen-specific IFN- γ production by cord-blood cells was, not surprisingly, highly reduced. Furthermore, the number of cells expressing cytokine mRNA (IFN- γ , TGF- β , and IL-4) after culture with autoantigens such as acetylcholine receptor, myelin basic protein, and peripheral myelin protein P2 was higher in cord blood than in peripheral blood from healthy adult donors (62). Thus, priming of the foetal immune response may occur to a vast array of antigens.

How early in gestation might priming of the foetal immune system occur? Allergen-specific responses are demonstrable from 23 weeks of gestation (63), and birch-pollen-specific reactivity at birth is more likely if maternal exposure to the birch-pollen season occurred during months 5–7 of pregnancy (20–28 weeks of gestation) (8). Interestingly, it has been shown that although CD45 RO+/RA- “memory” cells are seldom detected in neonatal blood and spleen, they are

relatively abundant in foetal blood and spleen (14–21 weeks of gestation) (64). A similar observation has been made for CD40 ligand (CD40L). Levels of CD40L induced by PHA on foetal cells at birth are negligible in comparison to the adult; however, at 21 weeks of gestation, the levels induced are similar to the adult (earlier gestational ages were not studied), with the level of upregulation of this costimulatory molecule declining toward term (65). Thus, there may be a period of foetal life when the foetus is exquisitely sensitive to initiation of antigen-specific reactivity. This response is likely to be to autoantigens because of their relative abundance. However, if exogenous antigen is present at this time, foetal priming to this may result.

One of the many questions raised by this hypothesis is, why don't we all suffer from autoimmune and atopic diseases, given the high degree of reactivity at birth to the antigens that can trigger these diseases? The best way to answer this is to consider the adult immune response to allergens. Few adults show detectable antigen-specific reactivity to dietary antigens, such as those of cow's milk, to which upward of 80% of neonates show reactivity (9), whereas reactivity to inhalant allergens, such as those of HDM, by adults are frequently seen despite the absence of HDM-mediated disease in most adults. This different level of responsiveness reflects the deletion of food allergen-reactive T cells from the repertoire by anergy or apoptosis as a consequence of high-dose antigen exposure in the gut and the development of oral tolerance. In contrast, low-dose exposure to inhalant allergens results in inappropriate immune deviation in those who suffer disease mediated by these allergens (66, 67).

When does this modulation occur in the neonate? It probably begins very early postnatally, as food antigen-reactive T cells must be anergized/apoptosed from the repertoire very early – they are seldom detected after 12 months of age (33). Babies at risk of developing atopic disease are commonly described as having impaired function/delayed maturation of various immunologic responses, including opsonization and cytokine production. Perhaps these infants also have immature/down-regulated anergic and apoptotic functions. That this is likely is shown by the fact that most infants “outgrow” their food-mediated allergic disease, and egg- and milk-specific IgE is no longer detectable in the circulation. Inhalant allergen-mediated disease may reflect the delayed maturation/suppression of immune deviation, as has been suggested elsewhere (45).

Suppression or delayed maturation of anergic or apoptotic activity by neonates may have drastic consequences for autoimmune disease as well, given the high degree of reactivity to autoantigens evident at birth (62). Indeed, the prevalence of autoimmune disease is also increasing in Western countries (68). Does a similar mechanism explain both phenomena?

The gastrointestinal flora

A critical step in postnatal regulation of the antigen-specific reactivity seen at birth may be the acquisition of the appropriate commensal gut flora. The importance of the commensal flora is best demonstrated by the above study of germ-free mice in which colonization during the neonatal period was essential for immune deviation of Th2 responsiveness (28). Moreover, the gastrointestinal flora is considered a prerequisite for the development of oral and thereby systemic tolerance.

Is there any evidence that colonization of the gastrointestinal tract is altered or impaired in populations experiencing an increased prevalence of allergic disease? Studies comparing the gut commensal flora of children in Sweden (high prevalence of disease) with that of children from Estonia (low prevalence of disease) indicate that there may be differences. There was more intensive colonization with lactobacilli in Estonian children (1-year-olds) whereas clostridia were more frequent in Swedish children (69). Further investigations in children in both populations who developed atopic disease found that these children were less often colonized with lactobacilli and had higher counts of aerobic organisms (70).

Alternatively, there may be impaired recognition and response to bacterial products derived from the commensal flora in children who develop atopic disease. That this may be the case is demonstrated by the poor responses to DPT vaccination observed in children who subsequently developed atopy (71), a response that may explain the observation of an

inverse association between tuberculin responses and atopic disease (72).

The importance of the gut flora is highlighted by the apparent requirement of the commensal gut flora, especially Gram-negative organisms, for successful oral tolerance. This is postulated to reflect the requirement for cytokines such as IL-1 and TNF- α that are produced by tissue macrophages upon stimulation with Gram-negative bacterial products such as lipopolysaccharide (LPS). This response is mediated primarily by the "LPS receptor", CD14. The possible role of both membrane and soluble CD14 has recently come under scrutiny (73, 74) and further studies will determine whether this molecule has a role in prevention of the development and progression of atopic disease.

Prenatal modification of allergen-specific immune responses

The effect of the commensal flora is postnatal, but are there mechanisms that function before birth that may regulate postnatal activity of antigen-reactive T cells? A number of studies suggest that IgG may have a role. Proliferation responses to HDM by umbilical cord-blood cells are inversely correlated with levels of cord-blood HDM-specific IgG (75). Furthermore, raised maternal anti-Bet v 1 IgG at birth was associated with a reduced prevalence of allergic disease at 18 months of age (76). Most interesting of all are studies in rodents in which maternal IgG was found to suppress IgE responses in the neonate (77). This was antigen-specific and persisted for the life of the offspring.

The observation of IgE in amniotic fluid (52) has led us to postulate that priming of the foetal immune

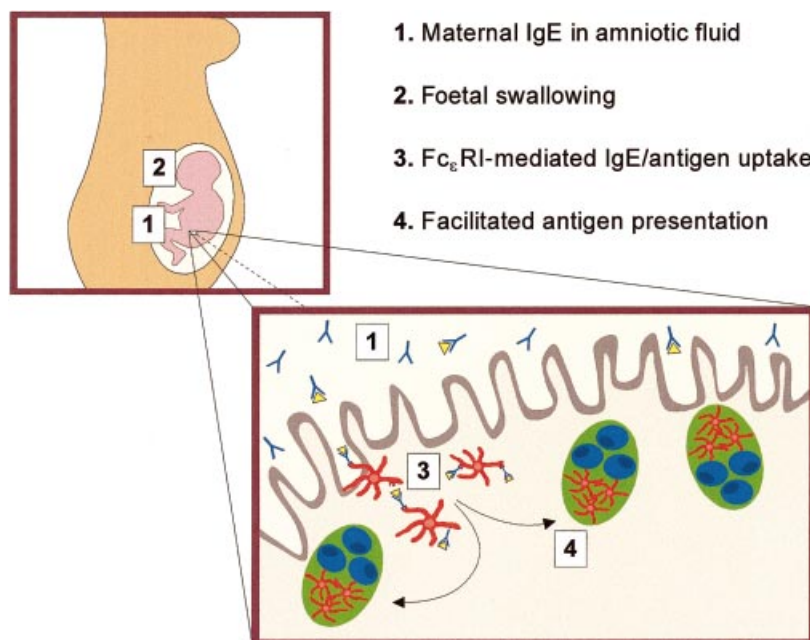


Figure 2. Potential pathway of IgE-mediated antigen focusing occurring via interaction of foetal gastrointestinal tract and amniotic fluid.

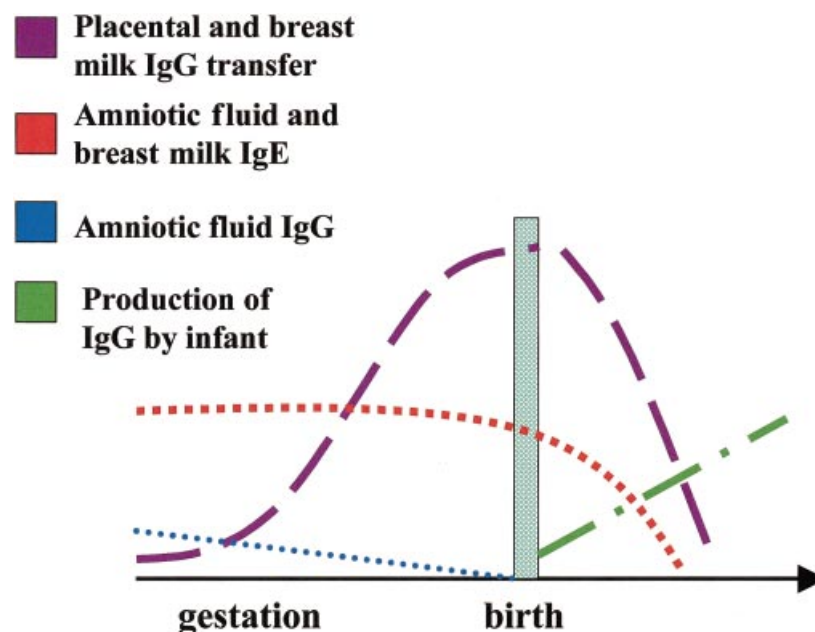


Figure 3. IgE and IgG passage to foetus and neonate.

response may occur in an IgE-mediated fashion (Fig. 2). IgE-mediated antigen-focusing has been described to occur via both the high- (78) and low- (CD23) (79) affinity IgE receptors on dendritic cells and B cells, respectively. It allows antigen presentation at a 100–1000-fold lower level of antigen than in the absence of IgE and requires only small amounts of IgE, being able to bind the IgE receptors at levels below which IgG would not bind to its receptors. That raised IgG anti-IgE is associated with a reduced prevalence of atopic disease at 18 months of age in a high-risk population (80) provides tantalizing evidence that IgE exposure of the human foetus during gestation may play an important role in determining disease outcome.

If an IgE-mediated mechanism, as postulated above, is indeed functional at the maternofetal interface during pregnancy, it may ultimately be the IgE/IgG ratio of the mother that has the greatest consequences for the offspring. As most mothers will have circulating IgE throughout the pregnancy, the foetus will be exposed to IgE via the amniotic fluid for this time period. This IgE may function in two ways. Firstly, it may initiate antigen-specific responses via IgE-mediated antigen focusing, as has been described for B cells and dendritic cells. Secondly, it may function as a more general signal of the environment to be encountered postnatally; i.e., exposure to high levels indicates, historically, a parasite burden in the mother and therefore inevitable postnatal exposure of the neonate.

IgG passage across the placenta may provide a regulatory step in this response, as would appear to be the case in rodents. IgG traverses the placenta throughout pregnancy, with a marked upregulation in the transfer rate occurring from 20 weeks of gestation,

and becoming maximal from 32 weeks of gestation (Fig. 3) (46, 49). IgG4, commonly elevated with IgE, is very poor at both crossing the placenta and binding to IgG receptors. Thus, historically, in a parasitic infestation, high maternal antigen-specific IgE and IgG4 would maintain the neonatal IgE response. However, if antigen-specific IgG1 or IgG3 is abundant, a down-regulatory signal is delivered to the foetus. Thus, the relative abundance of IgE and each of the IgG isotypes, as well as of the IgG receptors, may determine disease outcome. Interestingly, if the birch-pollen season coincides with the last 2 months of pregnancy when IgG transfer across the placenta is maximal, the neonate does not show birch-pollen-specific reactivity at birth, unlike exposure to the birch-pollen season in the preceding 3 months (8).

Conclusions

Much of the evidence available shows foetal life to be critical in the subsequent development of atopic disease. In fact, it might be more correct to say that IgE-mediated antigen-specific responses can be initiated *in utero*. The nature of this response may be regulated by the maternal environment, particularly the mother's IgE/IgG ratio, with these responses being susceptible to downregulation. Further modification occurs postnatally, and the gastrointestinal commensal flora has been identified as an important contributor. Understanding the underlying mechanisms whereby allergen-specific responses are initiated and modified both before and after birth will offer alternative targets for therapeutic intervention.

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