

Early life exposure to dietary and inhalant allergens

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Increasing evidence points to early life, including fetal life, as being a critical time period during which the infant's allergic fate may be determined. However, initial antigen priming *in utero* necessitates fetal exposure to maternally derived allergen. This article reviews our current knowledge regarding materno-fetal allergen transfer *in vivo* and reflects on mechanisms by which this exposure might exert immunomodulatory influences on the developing immune system.

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The prevalence of asthma and other allergic disorders in childhood have increased significantly in recent decades (1), with considerable repercussions for healthcare costs. Whilst the genetic basis for allergy is well established (2), genetic factors must interact with environmental influences to determine disease expression and progression (3). This complex interaction probably begins in early life, as distinctive alterations of immune responsiveness, which may predict an allergic phenotype, are apparent at birth, long before symptoms manifest (4,5).

Mechanisms of *in utero* priming

As atopy is the major risk factor for ongoing asthma, probing the mechanisms by which allergic sensitization occurs will be critical to understanding the disease. Fetal development occurs in an environment that is biased towards the T helper 2 (Th2) pathway of the immune response (6), and evidence from murine and human pregnancies reveal that T helper 1 (Th1)-type cytokines mediate pregnancy loss. The developing fetus is therefore exposed to factors that should promote a Th2 pattern of response (7), with an up-regulation of interleukin (IL)-4 and IL-10 production and concurrent down-regulation of IL-2 and interferon- γ (IFN- γ).

Why then are not all babies born allergic? There is evidence of a greater Th2-biased allergen

responsiveness in those babies destined to have allergic problems, including asthma (4,8), focusing attention on the fetus and its environment in relation to the genesis of the disease. Sensitization is known to occur in the first half of pregnancy, and T-cell precursors are sufficiently mature to undergo antigen priming by 18–22 weeks of gestation (9,10). Immunoglobulin E (IgE) and Th2 cytokines in the amniotic fluid have been demonstrated historically (11) and may have had a role in protecting the fetus from maternal parasites. This environment containing both IgE and IL-4, known to up-regulate Fc ϵ RI on mast cells, may have the same effect on dendritic cells, and their role in primary sensitization of fetal T cells with maternal IgE must be questioned. Not only is the fetal skin bathed in IgE-containing amniotic fluid, the fetus also aspirates and swallows the fluid, exposing both the respiratory and gastrointestinal tracts to IgE and other components of this fluid. It is at these sites – the skin, gut and lungs which have been exposed to IgE from at least 16 weeks of gestation – that atopic disease becomes evident after birth.

Levels of IgE as low as 10 ng/ml and allergen at 10^{-12} M enable low-affinity IgE receptor-bearing B cells to activate specific T cells in an IgE-dependent manner (12). It is probable that dendritic cells can perform this function at lower concentrations of IgE and allergen because they express the high-affinity IgE receptor (Fig. 1).

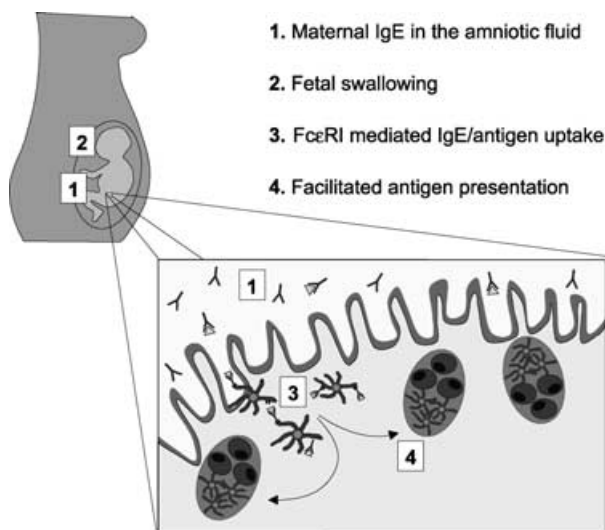


Fig. 1. Immunoglobulin E (IgE)-mediated fetal sensitization through the gut mucosa. Amniotic fluid containing allergen and maternal IgE surrounds, and is swallowed by, a fetus, here shown at 22 weeks of gestation. This fetal swallowing of amniotic fluid is the most probable route of fetal sensitization, with IgE-dependent antigen processing in the gut leading to specific T-cell responses to allergens. Whilst the antigen-presenting cell involved in fetal sensitization is not known, a probable candidate is the dendritic cell, given its unique ability to activate naive T cells. Dendritic cells (red) are ideally located at the interface of the gut with the amniotic fluid, and may be capable of facilitated presentation of allergen–IgE complexes captured by FcεRI and presented on major histocompatibility complex (MHC) class II to the T cells located in the lymphoid follicles (green).

Receptor-mediated endocytosis of the IgE–allergen complex, and processing for presentation of peptide on major histocompatibility complex (MHC) class II molecules (13), allows focusing and efficient selection, concentration and presentation of minute amounts of allergen with 100–1000-fold enhancement of activation of allergen-specific T cells (14). IgE-mediated allergen focusing by FcεRI-expressing dendritic cells may have a role in the primary immune response in the fetus because of the availability of maternally derived IgE and antigen for fetal cells. A greater understanding of the role played by FcεRI on dendritic cells, and subsequent priming of T cells to allergen during gestation, may bring to light opportunities to interfere with sensitization, a process that is known to occur during a window in the second trimester of gestation when susceptibility to later disease is maximal.

In addition, animal models have shown that the dose, timing, route of presentation and form of antigen exposure are factors regulating whether tolerance or immune priming occurs (15). Furthermore, animal studies have indicated that long-term Th1-like (tolerant) and Th2-like (allergic) memory against individual antigens occurs

over the course of the first exposures, and once consolidated is not readily reversible. Consequently, it is possible that the nature of early-life exposure to an allergen may contribute to the complex process that directs allergic sensitization and disease manifestation.

Passage of molecules to the fetus

It is well established that substances derived from the maternal environment can be found in the gestation-associated environment. Cotinine, the main metabolite of nicotine, has been found in placental tissue, coelomic and amniotic fluid and fetal serum from the first trimester when mothers have been exposed to cigarette smoke (16). Human immunodeficiency virus-1 (HIV-1) p24 antigen has been found in the amniotic fluid and fetal blood of HIV-infected mothers (17). Inulin, an inert substance with no specific transport system across the placental barrier, can also be detected in coelomic and amniotic fluid in the first trimester (18) and in the urine of term neonates (19), if administered intravenously to the pregnant woman.

As priming of the fetal immune system to environmental antigens does occur during intra-uterine development, and both inert and biological substances can pass from the mother to the fetus during pregnancy, it is necessary to demonstrate that allergen can be found in the fetal environment, in order to finally confirm that specific fetal allergen priming can occur. The demonstration of elevated umbilical cord IgE (20) and allergen-specific T-cell reactivity at birth (21) has inferred that exposure of the immunologically naïve immune system to allergens may occur antenatally, but, to date, the detection of these proteins in gestation-associated fluids has been elusive. The present studies have addressed this using a common dietary allergen – hen's-egg ovalbumin (OVA) – and an inhalant allergen to which mothers are exposed throughout pregnancy – the dust mite antigen *Dermatophagoides* p 1 (*Der* p 1) – and investigated whether these antigens might be detected in the maternal circulation, matched amniotic fluid and fetal blood samples.

The Southampton Maternal Egg-Avoidance Study

Early-life allergen exposure may be best characterized by reference to a dietary allergen, because ingestion is the main route of maternal exposure and, consequently, dietary elimination should be an effective means of reducing antigen transmission. Dietary egg is a major allergen in childhood. Egg allergy is a common cause of morbidity in

infancy (22) and is frequently the first manifestation of atopy (23). Moreover, although egg allergy usually resolves with age (24), egg sensitivity in infancy is emerging as a key indicator of later inhalant sensitization and respiratory allergic disease, which tend to persist into adult life (25). Elimination of dietary egg would therefore be an appropriate tool to investigate the parameters of early-life exposure.

Maternal-exclusion diets through pregnancy and/or breast-feeding as a means of primary allergy prevention have been studied previously by several groups (26). The prophylactic measures of these earlier studies were, in general, associated with either no improvement in allergic outcome of the intervention children, or with a benefit that was not sustained at later time-points (reviewed in ref. 27). A particularly pertinent criticism of these studies is that multiple foods ($n=2-5$) were excluded, drawing into question subject compliance and raising concerns regarding maternal and fetal nutritional wellbeing. Furthermore, the elimination diets were commenced from the third trimester of pregnancy, long after the fetus has been demonstrated to display the immunological capacity for priming (21). A lack of atopic benefit might then have resulted from the regimens missing the window of susceptibility.

The Southampton study was designed to take account of these criticisms. The MEAD study (Maternal Egg Avoidance Diet) is a randomized controlled study of egg avoidance from the second trimester of pregnancy though the duration of breast-feeding by women who have themselves, or whose partners have, a history of atopic disease. The intervention women exclude egg and all egg products. Their compliance is encouraged by the support of a dedicated study dietitian and is monitored by self-recording of accidental exposures and by the *in vitro* measurement of egg-specific immunoglobulin G (IgG) concentration through the study period, which reflects dietary intake (27). Infant allergic outcome is assessed at 3, 6, 12 and 18 months of age by a clinician or research nurse blinded to maternal study category. Atopic status is established by skin-prick testing to a panel of eight common dietary and inhalant allergens and measurement of total and specific IgE measurement at 6, 12 and 18 months of age. To date, 250 women have been recruited and the clinical results of the intervention will be analysed in the near future.

Early-life exposure to a dietary allergen

The characteristics of early-life exposure to egg allergen may be investigated by the detection

of OVA in pregnancy-associated fluids. OVA, a 44 000 kDa water-soluble glycoprotein, which by weight accounts for 58% of whole egg white, is a major egg allergen. The development of a sensitive capture enzyme-linked immunosorbent assay (ELISA) has facilitated the detection of OVA in picogram quantities in gestation-associated fluids, including amniotic fluid (28). OVA was present in three of 26 amniotic fluid samples where the corresponding maternal plasma had detectable levels (28). It is possible that this is an underestimate of the number of women with OVA present in their amniotic fluid, as the women were starved prior to the procedure and the optimal concentration of dietary OVA occurs 2 h post-ingestion (29). Analysis of the samples from the MEAD cohort may answer whether dietary exclusion measures eliminate, or reduce, fetal or newborn exposure to egg allergen and in turn reduce the risk for infant allergic sensitization, or whether the extremely low-dose, intermittent exposures may actually promote systemic priming, as has been noted for animal models (30). The conclusion of such an analysis may clearly have public health repercussions for the United Kingdom in view of the much promoted Department of Health advice for avoidance of peanuts and peanut-containing foods by pregnant women with a family history of atopy.

Early-life exposure to an inhalant allergen

The house dust mite allergen *Der p 1* is a common indoor inhalant allergen and a major source of allergen affecting atopic asthmatics (31). Mite exposure is directly associated with sensitization (32) and while intensive mite-avoidance measures are successful in reducing *Der p 1* concentration in the home (33), the ubiquitous nature of the antigen would suggest that it may have a critical role in primary sensitization in early life under normal living arrangements.

In utero exposure to *Der p 1* has been confirmed by *in vitro* detection of the allergen in pregnancy-associated fluids (34). *Der p 1* was detected in 21% of plasma samples collected from unselected pregnant women undergoing routine amniocentesis at 16–17 weeks of gestation (median concentration: 5.9 ng/ml). A total of 56% of the amniotic fluid samples matching these positive maternal plasma samples also had a detectable mite antigen (median concentration: 1.1 ng/ml). In addition, matched maternal and cord plasma samples were collected at term. In 63% of cord samples with corresponding positive maternal plasma, *Der p 1* was also detectable.

Conclusion

This work has demonstrated for the first time that allergen is present in the fetal environment, being found in the amniotic fluid at 16 weeks of gestation and in the fetal circulation at term (≥ 37 weeks). The presence of both a dietary and an inhalant allergen in the amniotic fluid and the fetal circulation suggests that there are two routes of allergen transfer to the conceptus: *trans*-amniotic and *trans*-placental. Allergen present in the amniotic fluid will gain access to the gut-associated lymphoid tissue, where Peyer's patches are structurally mature by 19 weeks of gestation, and the M cells that sample the environment are already in place (35). Recent work from our group has shown that CD83⁺ dendritic cells are present in the gut from 17 weeks of gestation (28) and T cells are present from 13 weeks of gestation (36). Likewise, allergen, either free or in complex with IgG (37), in the fetal circulation will encounter immune-competent antigen-presenting cells.

The presence of allergen and the initiation of antigen-specific responses *in utero* via the gut in the second trimester may be modified in the third trimester by the maternal environment, particularly the mother's IgE:IgG ratio, with the fetal/neonatal antigen-specific IgE responses being particularly susceptible to down-regulation (38,39). The possibility that maternal antigen-specific IgG protects against the development of IgE to the same allergen has been demonstrated recently in humans (40). Further modification of the antigen-specific immune response occurs post-natally with the acquisition of an appropriate commensal gut flora (41). Therefore, these two routes of allergen exposure *in utero* are critical to the establishment of an immune response which may later lead to the development of asthma. A greater understanding of the initiation and modification of allergen-specific responses, both before and after birth, is needed before the association between the exposure to allergen *in utero* and the subsequent disease development can be identified.

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