

Fetal immune responsiveness and routes of allergic sensitization

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There is much interest in the role of early-life events in the subsequent development of atopy and/or atopic disease. Despite the ongoing debate about the intrauterine exposure of the fetus to environmental allergens and the establishment of T-cell memory, it is clear that the immunological response of the neonate at risk of atopy is more immature than that of the neonate likely to be non-atopic. The reasons for this remain unknown, but might reflect maternally transmitted signals that adapt the neonatal immune response. An inadvertent consequence of this might be an inappropriate host response to environmental signals such as those from microbial products during early post-natal life that result in an inability to dampen neonatal T helper 2-skewed responses. The developing gastrointestinal tract and the exogenous factors that impact on this, such as microbial flora and breast milk, should therefore be a focus of investigation.

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The suggestion that early life events in fetal development and early infancy influence the development of allergic disease stems from a number of observations. First, the risk of atopy is greater in the offspring of atopic mothers than atopic fathers, indicating a strong effect related to the intimate association between mother and child during pregnancy and in the first few years of life on the development of the atopic phenotype. Second, reactivity to allergens (proliferation and cytokine production), such as those of cow's milk, hen's egg and house dust mite, is evident at birth and from as early as ≈ 22 weeks of gestation (reviewed elsewhere (1)). Indeed, some studies have found an association between the extent of the proliferative response to allergen at birth and atopic outcome in infancy, while others have observed that the cytokine profile produced at birth in response to allergen or mitogen differs with respect to the subsequent development of atopic disease (2–4). Overall, it would appear that the newborn at risk of atopy (positive family history), and especially the subset of these children who do develop an atopic phenotype in infancy, have a more immature immune response at birth than their

low-risk or subsequently non-atopic counterparts. The reasons for this remain unknown, but we are beginning to understand more of the nature of the evolving immune response to allergen in early life.

Antigen exposure during intrauterine life

Although our interest in reactivity to antigens is focused on that to allergens, it is worthwhile highlighting that the neonate can mount a response to a wide array of antigens, including autoantigens and those from human immunodeficiency virus (HIV), helminth and malarial parasites (reviewed elsewhere (1)). Such reactivity has been considered surprising given the concept of neonatal tolerance, but this is now considered, in many models, to reflect a T helper 2 (Th2)-biased immune response at this time. The germ-free status of the intrauterine environment favours Th2 responses, and a Th2-skewed response at birth in the human has been demonstrated (4). The fetus is clearly capable of mounting an antigen-specific immune response, as rubella-specific and tetanus toxic-specific immunoglobulin M

(IgM) can be detected in the cord blood of infants whose mothers are infected with rubella or vaccinated with tetanus during pregnancy, respectively (5,6). Also, fetal vaccination of baboons with hepatitis B surface antigen results in specific immunoglobulin G (IgG) production by the fetus, and post-natal challenge is associated with an enhanced response (7). Therefore, the ability to detect antigen-specific responses at birth should no longer be considered extraordinary.

Analyses of neonatal responses to infectious agents (such as schistosomes, rubella and HIV) indicate that the development of fetal reactivity to such antigens is dependent on maternal exposure to the antigen. Hence, only neonates of mothers infected with schistosomiasis during pregnancy proliferate in response to schistosomal antigens, and tetanus toxoid-specific IgM is only detected in cord blood if the mother was vaccinated during pregnancy. This implies that maternal exposure to allergens should be necessary for the fetus to respond specifically to that allergen at birth.

There is little doubt that dietary antigens can be transferred from the maternal to the fetal environment. Dietary antigens can be measured in the circulation and breast milk after consumption (8), and animal models indicate that swallowed antigen passes from the maternal circulation into various fetal fluids (amniotic fluid, blood, urine) (9). The major limitation for the analysis of dietary antigens in gestation-associated compartments in humans is probably the sensitivities of our current assays. However, we have recently measured ovalbumin (OVA) in mid-gestation amniotic fluid, albeit only from the three mothers with the highest circulating levels of 26 with detectable plasma levels of OVA from a larger cohort (10). As contamination of amniotic fluid with maternal plasma during amniocentesis occurs in only 1% of samples (reflecting *trans*-placental collection, second attempt, inexperienced operator) (11) it is unlikely that the ability to detect antigen in amniotic fluid arises from maternal contamination of the sample during collection. The ability of β -lactoglobulin (BLG) to traverse the human placenta has been described recently (12), but there are as yet no reports of BLG or OVA in the fetal circulation. These preliminary observations indicate that dietary antigen can traverse both the placenta and the fetal membranes to access the fetal compartment.

What about inhalant allergens? The passage of *Berv* 1 across the human placenta has been described in an *in vitro* model (12), and there are now reports published of *Feld* 1 and *Der* p 1 in the fetal circulation and *Der* p 1 in amniotic fluid (13,14). Such studies have been criticized for the

high levels of antigen that have been measured. However, this could reflect the skewed nature of the populations chosen, as only mothers with high levels are selected for follow-up because of the low sensitivities of the current assays. Furthermore, although swallowed antigens are encountered in greater concentrations than inhaled antigens, aero-allergens will also be swallowed. Therefore, exposure to 'inhalant' allergens will occur via the respiratory and gastrointestinal tracts. Finally, it is possible that amidst the plethora of pregnancy-associated proteins there are some that cross-react with allergens. There is some evidence for this: *Feld* 1 is highly homologous to uteroglobin (15), a protein considered to protect the embryo from maternal immunological activity (16). The recent observation of birch pollen reactivity in cordocentesis and preterm samples from babies of mothers who have not been pregnant during the birch pollen season (17), also suggests that cross-reactivity might contribute to the neonatal immune response to allergens. Similarly, allergen-avoidance protocols implemented during pregnancy, although successful at reducing the allergen burden in the home (typically house dust mite) do not dramatically alter the proliferative response to allergen at birth, implying that the level of maternal exposure to allergen may not be critical to allergen reactivity of the neonate.

These observations suggest that a focus of ongoing work should be elucidation of the immunological mechanisms associated with allergen-dependent (as opposed to allergen-specific) reactivity by the neonate. There are as yet no data published on the phenotype of the antigen-responsive fetal/neonatal T cells, and little is known about the phenotype, let alone the function, of fetal/neonatal antigen-presenting cell populations, especially dendritic cells (DC). However, lower interleukin (IL)-12 production by neonatal monocyte-derived DCs compared to adult monocyte-derived DCs (18) would limit the development of antigen-specific T helper 1 (Th1) responses. Unpublished data from our own group (J. A. Holloway et al., in preparation) confirms the long speculated phenotypic immaturity (reduced expression of costimulatory molecules) of peripheral blood DCs during fetal life.

Despite the urgent need to characterize the allergen-responsive neonatal T cells in detail, it is essential that the information provided by the allergen-dependent response, observed by so many groups, is not disregarded. Even if this response turns out to be non-specific, it does reveal the immunological status of the newborn. A frequently reproduced observation is that of reduced mitogen and allergen-stimulated cytokine

[interferon- γ (IFN- γ) and IL-13] production by the newborn with an atopic family history compared to those without such a family history (3,4). In studies where clinical follow-up has been undertaken, this has been extended to be associated with those who develop atopic disease in the first few years of life compared to those who do not; thus, the newborn at risk of developing an atopic phenotype has been described as having delayed maturation (19). Whether this arises because of an inherent property of various cell populations (T cells, DCs) or via active suppression by pregnancy-associated factors, remains to be determined.

Preliminary studies highlight the importance of maternal atopy in this, as phytohaemagglutinin (PHA)-stimulated IFN- γ levels were lowest in cord blood from babies born to atopic mothers compared to atopic fathers and/or non-atopic mothers (20). These differences in the host immune response may have an important role in the post-natal development of an atopic phenotype. This possibility should be borne in mind during our search for environmental factors (especially from microbial flora) that negatively regulate the development of atopy and atopic disease, as some infants might be poor responders to such stimuli.

Sites of initiation of antigen reactivity during fetal development

Numerous lines of evidence point to ≈ 22 weeks of gestation as the time at which antigen reactivity can be initiated by the fetal immune system (17,21). There are, of course, ethical and practical limitations to the study of T cells and antigen-presenting cells during human fetal development. However, we have initiated an analysis of the phenotype and function of these populations in fetal mucosal tissues and the circulation. Our focus is the fetal gut, for two main reasons: it is structurally mature from 19 weeks of gestation with lymphoid follicles and Peyer's patches in place and M cells evident within the epithelium (22); and gastrointestinal development at this time could determine function post-natally, which could direct the host response to environmental signals, especially those from the establishing commensal flora as well as enteric pathogens. Having undertaken an extensive immunohistochemical analysis to ascertain the phenotype of antigen-presenting cells in the fetal gut from 11 weeks of gestation, we were surprised by the abundance of major histocompatibility complex (MHC) class II and CD40 expression. Further analysis indicated that T-cell priming by these

cells might be restricted to the emerging lymphoid follicles, as T cells expressing the necessary counterligand (CD40L; CD154) were only observed within these structures (10). As antigen is also present in amniotic fluid, which can be swallowed from 10 weeks of gestation (23), antigen-presenting cell populations will be exposed to antigen. Ongoing studies are focusing on the nature of the response generated at this site (Th1/Th2) and how this is modified post-natally in response to microbial products such as lipopolysaccharide (LPS) and CpG motifs. We will be able to modify this analysis as information about the nature of the reactive T cells is elucidated.

Although it will be difficult to assess if gut development differs in future atopic children, analysis of exogenous factors (amniotic fluid and breast milk) that potentially influence gut development offers a suitable alternative. To this end, we found that levels of soluble CD14 (sCD14) were significantly reduced in amniotic fluid at mid-gestation of children who were skin-prick test positive with eczema at 1 year of age compared to eczema-free, skin-prick test negative children. In a different cohort of children, we observed that sCD14 levels were lower in breast milk (3 months post-partum) fed to children who developed eczema by 6 months of age compared to breast milk fed to those who did not develop eczema by 6 months of age. This later observation was independent of the atopic status of the infant.

Our studies were prompted by the observation of lower plasma levels of sCD14 in children with elevated immunoglobulin E (IgE) and positive skin-prick tests, which were associated with a polymorphism in the CD14 promoter (24). Membrane CD14 and its soluble form facilitate the response of numerous cell types to the Gram-negative bacterial product, LPS. However, LPS-independent effects for LPS are now being described, including the inhibition of IgE production by B cells and the *trans*-cellular transport of lipids (25,26). Both LPS-dependent and LPS-independent CD14-mediated functions have a foreseeable role in regulating development of the atopic phenotype. Interestingly, the recent observation of the down-regulatory effect of probiotics on the development of eczema, while not altering skin-prick test results or specific IgE levels (27), might be explained by a probiotics-stimulated increase in sCD14 in either breast milk or at the enteric mucosal surface.

Summarizing remarks

Despite the ongoing debate about the intrauterine exposure of the fetus to environmental allergens

and the establishment of T-cell memory, it is clear that the immunological response of the neonate at risk of atopy is more immature compared to the neonate likely to be non-atopic. This altered responsiveness might reflect maternally transmitted signals that adapt the neonate for survival *ex utero*. An inadvertent consequence of this might then be an inappropriate host response to environmental signals, such as those from microbial products during early post-natal life that results in an inability to dampen neonatal Th2-skewed responses.

Acknowledgments

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