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The development of the immune system during pregnancy and early life

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It is a common misconception that the newborn is immunologically naive. However, neonatal human T cells proliferate in response to an array of antigens, including allergens (1–4), autoantigens (5), and parasite antigens (6, 7). The ability to detect antigen-specific IgE in umbilical cord blood collected at birth also indicates that neonatal T and B cells have mounted an antigen-specific response (8–10). Likewise, newborns of mothers who are vaccinated with tetanus toxoid during pregnancy have specific antibody of the IgM class in their serum, although no evidence of class switch before their own vaccination (11). The offspring of mothers infected by *Ascaris* during the pregnancy (12) also exhibit specific reactivity to this parasite at birth.

Nevertheless, fetal and newborn mammals have limited ability to mount immune responses in both quantitative and qualitative terms, relative to older age groups. This defect could reside in any combination of functions associated with mounting effective host defence, but, in some circumstances, the magnitude of the defect has been overestimated as a result of the methodology chosen to examine immune function. Most investigations of the functionality of the human fetal immune system have relied on the use of umbilical cord blood collected at birth after a full-term pregnancy (>37 weeks of gestation). Due to ethical limitations, few studies have been conducted on fetal lymphoid tissues or blood at earlier times in gestation. This review contrasts the published data obtained from studies on fetal and newborn peripheral blood mononuclear cells with the more limited information available on samples from infants and young children. In addition, the ramifications of these findings are discussed in relation to the pathogenesis of allergic disease.

Development of the fetal immune system

As in all mammals, the first stage of human fetal haemopoiesis occurs in the mesoderm of the yolk sac and the extraembryonic mesenchymal tissue. Pluripotent erythroid and granulomacrophage progenitors can be detected in the yolk sac of human embryos at 3–4 weeks of gestation. These primitive cells can then be detected in the circulation from 4 weeks of gestation as they migrate to the liver, which becomes the major site of haemopoiesis at 5–6 weeks of gestation. From 5–10 weeks, the liver undergoes a dramatic increase in size as the number of nucleated cells rises. These early progenitors are proliferating but undergoing very little differentiation, although a discrete granulocyte/macrophage population

emerges at this time. The thymus and spleen are seeded from the liver and stem cells are detectable in the bone marrow at 11–12 weeks of gestation (13). Hepatic haemopoiesis declines in the third trimester and ceases soon after birth.

The culture of fetal blood collected by fetoscopy at 12–19 weeks of gestation yields high levels of both erythroid and granulocytic/monocytic progenitor cells – monocytes comprising 42–68%, neutrophils 27–41%, and eosinophils 5–30% (14). Despite this high number of granulocyte progenitors in the circulation at this time, granulocytes are not formed in large numbers in fetuses until after birth, neutrophils being actually the last population to appear in the blood during fetal life (15).

What follows is a summary of the development of the cell populations that allergologists are familiar with from their role in the allergic response. When they were known, the functional properties of these cells have also been considered.

Macrophages and dendritic cells

Macrophages, dendritic cells, and B cells, which are discussed later, have a central role in the generation of an antigen-specific immune response, as they take up, process, and present antigens to T cells. Although dendritic cells are considered professional antigen-presenting cells because they can prime naive T cells, very little is known about them in the fetal period; therefore, they will be discussed with monocyte/macrophages, which are the first cell type to appear in the fetal circulation (15).

There are two populations of cells with a dendritic/macrophage structure in the yolk sac and mesenchyme at 4–6 weeks of age. Cells with this appearance are also evident in the prehaematopoietic liver at 5 weeks of gestation. The major population of yolk sac macrophages is MHC class II-negative, and there is a minor population that is MHC class II-positive (16). MHC class II-negative cells appear in the thymic cortex, in the marginal zones of lymph nodes, in the splenic red pulp, and in the midst of erythopoietic activity in the bone marrow. A few MHC class II-positive cells are seen in the liver at 7–8 weeks of gestation, the lymph nodes at 11–13 weeks of gestation, and the T-cell areas of the developing thymic medulla by 16 weeks of gestation, whereas thymic epithelium expresses class II at 8–9 weeks (16).

MHC class II-positive cells also occur in the skin, gastrointestinal tract, and hepatic systems. The number of hepatic sinusoidal macrophages (Kupffer cells) is low in early gestation (17 weeks was the earliest time point examined) but increases to nearly adult levels in the neonatal period. By 6 weeks of intrauterine development, the blood flow to the liver passes through the left umbilical vein and therefore comes directly from the placenta, providing a rich nutrient supply to these cells (17). HLA-DR+ Langerhans cells are detectable in the skin by 6–7 weeks of gestation. The density of these cells at days 50–100 of gestation is similar, but the cells are smaller in the earlier gestational samples, as well as less dendritic and phenotypically heterogeneous. Thus, Langerhans cells migrate into the epidermis during the first trimester and resemble the adult phenotype by the second trimester (18). There are MHC class II-positive cells in the lamina propria of the fetal gut as early as 11 weeks of gestation, but the cell type remains unidentified (19).

The only monocyte/macrophage populations that have been functionally assessed are those in the circulation collected as umbilical cord blood at term or, less frequently, preterm delivery. Term cord-blood monocytes have decreased production of a number of cytokines, including TNF-α (20), in comparison to the adult. Although cord-blood mononuclear cells can phagocytose at a level comparable to the adult, chemotaxis is reduced (21). Assessment of allogeneic responses by cord-blood mononuclear cells to adult peripheral blood leukocytes (22) has confirmed that the antigen-presenting function of cord-blood mononuclear cells is sufficiently developed to mediate a response comparable to the adult. The status of the neonatal monocyte has also been implicated in determining some aspects of T-cell function, as this cell type has a role in mediating impaired IFN-γ production by neonatal T cells (23, 24).

The one study of cord-blood dendritic cells suggests that they express relatively poor accessory function (25). Umbilical cord-blood dendritic cells in this study had lower levels of ICAM-1 and MHC classes I and II than peripheral blood dendritic cells from adults. Cord-blood dendritic cells were poor stimulators of mixed lymphocyte reactions irrespective of whether cord or adult MNC or T cells were used as the responders. In contrast, cord-blood T cells and mononuclear cells responded normally to allogeneic adult dendritic cells.

T cells

Putative prothymocytes can be identified in the fetal liver from 7 weeks of gestation as highly proliferative cells that are positive for CD7, CD45, and cytoplasmic CD3, but do not express membrane CD3, TCR b chain, or TdT (terminal deoxynucleotidyl transferase, which is involved in diversification of the DJ region of Ig heavy chain and the T-cell receptor [TCR]). Membrane CD3 is evident after week 10 of gestation, at which time the cells are less proliferative (26, 27).

CD7+ T-cell precursors from the fetal liver seed the thymus at 8–9 weeks of gestation; 60% of these are

CD2+ (cytoplasmic), only 4% are CD3+ (cytoplasmic), and none are TCR d or b positive. From 9.5 weeks to birth, TCR b+ cells increase to form over 90% of the CD7+ population (28). CD7 is an early T-lineage marker not found on myeloid or erythroid lineages and is a good marker of T cells that have not yet expressed markers of later T-cell subsets such as CD3, 4, or 8. Cells from SCID-human thymus/liver or human T cells from SCID-human peripheral blood are functionally competent. They are similar to fetal thymocytes or adult T cells, respectively (29, 30).

From 18–24 weeks of gestation, the mesenteric lymph nodes have a high percentage of CD45RA+ T cells but very few B cells or monocytes. The fetal spleen at this time has equal numbers of T cells, B cells, and monocytes/macrophages (31). Lymph-node and thymus T cells at these gestational ages do not proliferate in response to the mitogen PHA or upon anti-CD3 stimulation, although expression of CD69, an activation marker, does increase. Proliferation is observed on the addition of IL-2. In contrast, splenic T cells do proliferate to PHA and anti-CD3. T cells from fetal spleen have adult levels of CD3, CD4, and CD8, and also expressed CD2 and CD11a. Thus, the spleen is considered already fully immunocompetent by 18 weeks of gestation, having sufficient accessory cells to ensure T-cell activation, whereas the mesenteric lymph nodes are deficient in accessory cells numerically or functionally. The ability to upregulate CD69 by fetal T cells upon stimulation with anti-CD3 or PHA was comparable to the adult, whereas the response of fetal T cells to allogeneic antigen-presenting cells was much greater than the adult. The latter observation has been postulated to reflect the limited diversity of the TCR α/β repertoire of fetal T cells.

There are few memory T cells (CD45RO+) in the blood and spleen of the newborn, whereas half the T cells in adult tissues have this phenotype. Surprisingly, CD45RO+/RA– T cells are relatively abundant in the spleen and blood from premature births, about 25% and 10%, respectively, with both CD4 and CD8 subpopulations contributing. The CD4+/CD45RO+ population frequently expressed CD25 and could proliferate in response to IL-2, but not anti-CD2 or anti-CD3 (32). The investigators postulated that these cells were an embryonic population of autoreactive T-cell clones with anergic characteristics. Leakage of self-reactive T cells to the periphery before negative selection has occurred has been postulated to be greater during fetal life. CD45RO is considered a marker of memory T cells; however, a switch from CD45RO to CD45RA occurs as the final step of maturation in the thymus (33). Therefore, do these CD45RO+ cells in the fetal liver, spleen, and circulation reflect very immature T cells that have leaked from the thymus, and are thus an immature population rather than a memory population?

The fetal gastrointestinal tract may be a site of extrathymic differentiation of T cells, as has been demonstrated in the mouse (34). Human fetal intestinal mucosa has T cells detectable in the lamina propria and epithelium from 12–14 weeks of gestation (35). T cells in fetal ileum epithelium are mostly CD8+, and many of these express CD8αα. Almost half of the CD8+ cells in the lamina propria are also CD8αα, but in the Peyer's patches, when present, CD8αβ cells predominate (36). Studies in mice indicate that CD8αα cells may be thymus-independent and develop in the gut.

A substantial proportion of lamina propria lymphocytes express CD7 in the absence of CD3 and are proliferating, as indicated by Ki67 expression. There is no overlap between the gut and the blood in rearranged TCR b transcripts; therefore, the gut T cells are unlikely to be derived from blood (37). As Peyer's patches are not present until 16–19 weeks of gestation, the T cells populating the gut prior to this time are unlikely to be T cells recirculating from the Peyer's patches to the lamina propria, as occurs in adulthood. Furthermore, T cells in the fetal intestine express activation markers (HLA-DR, CD25, CD69, and low CD62L), and the majority express CD45RO (37). However, this population may also reflect thymus leakage, as thymus development is complete by the time these cells appear in the gut.

Given the recent resurgence of interest in γ/δ T cells in allergic disease (38), especially asthma, when and where does this subpopulation of T cells develop during fetal life? Rearranged TCR d genes are first seen in the liver and primitive gut between 6 and 9 weeks of gestation prior to being detectable in the thymus (39). The thymic and gut γ/δ T-cell repertoires overlap early in development but diverge and become nonoverlapping during the second trimester (40), whereas the γ/δ T-cell population in the fetal liver is distinct from the thymus, and the liver may be a site of γ/δ T-cell development in man. In the liver at 20–22 weeks of gestation, 63% of CD3+ cells are TCR α/β and 32% are TCR γ/δ. Peculiarly, a subpopulation of these liver γ/δ T cells has a CD4+ phenotype.

CD3+ T cells are detectable in the fetal circulation at about 15–16 weeks of gestation, at which time they also express CD2 and CD5 (41). Proliferation in response to PHA is first seen at 17 weeks of gestation (42). How early do antigen-specific responses occur? Umbilical cord mononuclear cells collected at birth at full term exhibit antigen-specific reactivity to allergens, including those of house-dust mite and cow's milk (1–4); parasite antigens such as those of *Plasmodium* spp. (7) and *Schistosoma* spp. (6); and autoantigens,

including myelin basic protein (5). Most of these studies have used proliferation assays, but antigen-specific cytokine production has also been observed.

Antigen-specific reactivity at earlier time points has been poorly studied. The study already cited (32) examined T-cell phenotypes in early gestation but did not investigate antigen-specific reactivity by these cells. Another study investigating allergen-specific proliferative responses demonstrated antigen-specific reactivity at 23 weeks of gestation (43). Although this is an interesting observation, much more information is required about the phenotype of cells making such responses, and the genuine specificity of such responses requires confirmation. This applies to all studies of antigen-specific reactivity at birth, given that most babies demonstrate reactivity to one or more antigens.

One of the frequently observed properties of neonatal T cells is their poor cytokine production in comparison to the adult (44, 45), particularly in relation to Th1 cytokines. The underlying mechanisms that account for this deficiency are incompletely understood, but appear to derive in part from the secretory functions of the placenta ([46] further discussion below). The relatively poor capacity of neonatal T cells to produce cytokines is thought to contribute to the impaired responses of other neonatal cell populations that rely on these factors for their functions. For example, poor IFN-γ production could help to reduce cellular cytotoxicity by NK cells (47), and reduced IL-4 has a role in reduced IgE production by neonatal B cells (48).

B cells

Pro- (CD24+/surface IgM-negative) and pre-B cells (cytoplasmic IgM+/surface IgM-negative) can be detected in the fetal liver and omentum (a long fold of peritoneal membrane which hangs down within the abdominal cavity in front of the bowels, and which is considered part of the lymphoid system because it contains loose unorganized lymphoid aggregates), but not the spleen, as early as 8 weeks of gestation. The percentage of pre-B cells in the fetal omentum and liver is similar over 8–12 weeks gestation, but the percentage of these cells decreases during weeks 13–23 in the omentum, remaining the same in the liver (49). Thus, B-cell development in the omentum is transitory. B cells become detectable in the spleen at 13–23 weeks of gestation, and CD5+ B cells can be found in the human peritoneal cavity and pleural cavity at 15 weeks of gestation (50).

The liver is an important site of B-cell differentiation in mammals (51). At 8 weeks of gestation, liver pre-B cells express the cytoplasmic m chain, and surface IgM is expressed on liver B cells by 10–12 weeks, with surface IgD being detectable from 13 weeks of gestation. CD24 expression precedes m-chain expression and is retained throughout differentiation into adulthood. Liver B cells also express CD20 but are negative for CD21 and CD22 (52).

Diffusely distributed B cells detectable in the lymph nodes from 16–17 weeks and spleen at 16–21 weeks are strongly IgM+ (50, 51). Primary nodules develop around the follicular dendritic cells of the lymph nodes from 17 weeks of gestation, and contain a virtually pure B-cell population. Germinal centre B cells are absent in the fetal lymph nodes, probably reflecting a lack of antigen. B cells are abundant in the bone marrow at 16–20 weeks of gestation. The proportion of immature B cells in the bone marrow decreases with age, and cells expressing maturity markers increase. B cells in the spleen are diffusely distributed at 22 weeks, and then form primary nodules around 24 weeks; this is later than seen in lymph nodes.

B cells emerge into the peripheral circulation at 12 weeks of gestation, and they are positive for CD19, CD20, CD21, CD22, HLA-DR, IgM, and IgD (52). The percentage of CD5+ B cells (B-1 B cells) is higher in the fetal circulation than the adult, and declines with increasing gestational age, yet even at birth most cord-blood B cells are CD5+ (B-1 B cells), in contrast to the adult, where few peripheral blood B cells express this molecule (52, 53). CD5+ B cells are largely T-independent, and CD5+ B cells produce polyreactive antibodies which may have a role in the primary immune response and be very useful in the first line of defence, a necessary function in the newborn.

Immunoglobulin production

Early IgG and IgM synthesis occurs primarily in the spleen, large amounts of both being produced by the spleen as early as 10 weeks of gestation, although levels are maximal at 17–18 weeks of gestation. Serum IgG levels slowly increase between 5.5 and 22 weeks, there is a greater increase to 26 weeks, and then there is a dramatic increase to birth. IgG of a haplotype distinct from the mother can be detected in the fetal circulation as early as 17 weeks of gestation as well as at birth, although most of the IgG is of maternal origin (54). IgG traverses the placenta throughout gestation with a marked upregulation in the transfer rate occurring from 20 weeks, and this upregulation is maximal from 32 weeks of gestation (55, 56). IgE synthesis was observed at 11 weeks of gestation in fetal liver and lung, and by 21 weeks in the spleen (57).

Despite this early burst of production in fetal life, the production of Ig isotypes at birth is impaired. Neonates have very low serum IgM and even lower IgA and IgE levels, and the IgG present is essentially of maternal origin. Polyclonal activators such as pokeweed mitogen fail to switch neonatal B cells to

IgA and IgG production. The neonatal immune system responds to a restricted array of antigens producing largely IgM of low affinity. Surface IgM and CD79 (signal transducer for membrane Ig and necessary for all IgM functions) are elevated on cord-blood B cells compared to the adult, but cord and adult B cells express similar levels of CD19, 21, 22, and 81, although CD32 is lower on cord B cells (58).

Neonatal B cells are also mature in their capacity to switch to IgE-producing cells if they are given exogenous IL-4, albeit they require levels of IL-4 higher than that required by adult B cells to switch to IgE production (48). Thus, the minimal production of IgE is not due to the immaturity of the B cells but to the lack of IL-4 produced by fetal cells, i.e., to the immature helper T-cell function. Another molecule important in directing B cells to switch to IgE production is CD40 via interaction with its ligand (CD40L) on T cells. CD40L expression is not inducible on CD3+ cells from newborn samples activated with many (59–61), but not all (62), stimuli; however, it can be readily upregulated to levels comparable to the adult at 19–28 weeks of gestation, the levels declining toward full term (59).

As IgE has a central role in the allergic response, it is worthwhile noting that despite the low levels of total IgE detectable in the circulation, specific IgE (either allergen or parasite) is detectable in cord plasma from some neonates (7–10). Furthermore, cord-blood mononuclear cells from babies delivered to helminth-infected mothers in Kenya, but not to mothers residing in North America, can spontaneously produce polyclonal and parasite antigen-specific IgE in culture. The levels induced in the cultured cells corresponded to the level of specific IgE measurable in matched cord-blood plasma (9).

Mucosal immunity

A functioning mucosal immune system is essential for survival in infancy and beyond. IgA and IgM are important in the first line of defence. In the fetal parotid gland (20–40 weeks), occasional IgM- and IgA-producing cells were observed, but no cells producing D, G, or E isotypes were seen (63). The IgA1 subclass predominates and is mostly J-chain-positive. Amylase, lysozyme, and lactoferrin were detectable and most prominent in early fetal life, whereas only small amounts of secretory component were seen. Postnatally, SC-, IgA-, and IgD-producing cells increase, probably reflecting local activation of the immune system by environmental factors (64).

Duodenal expression of secretory component, classes I and II is seen and IgA-, IgM-, and IgG-producing cells are detectable from 24–32 weeks of gestation. Only small amounts of secretory component can be visualized before week 29 of gestation, the levels increasing rapidly to adult levels by 1 week postnatally. There is some conflict in the literature about HLA-DR expression by the intestinal epithelium, but there is clearly a population of MHC class II-positive cells in the lamina propria from 11 weeks of gestation (19), and, as mentioned above, T cells are found at this site from 12–14 weeks of gestation (37). From the second postnatal week, intense expression of epithelial HLA-DR, secretory component, and IgA is seen, again reflecting modulation by environmental factors (65).

Immune responses at mucosal surfaces have an important role in the development of allergic responses and disease. Although there are very few studies of these sites during intrauterine development, it is clear that both the skin and gastrointestinal tract are relatively immunologically mature, at least structurally, prior to birth. In contrast, the airways show little evidence of population by haematopoietic cells prior to birth, and an influx is seen during the first week postnatally (66). This developmental delay in the airways may help to explain why allergic disease is first manifest in the gut and skin while the clinical symptoms of airways inflammation appear later in infancy/childhood.

Eosinophils

Eosinophil granulopoiesis occurs in the fetal liver, and eosinophilic granulocytes, identified in paraffin sections by staining with haematoxylin-eosin-azure II, are evident for the first time at 5 weeks in the hepatic laminae (67). Numbers at this site increase gradually over gestation, and then, after 20 weeks of gestation, they appear in the portal areas. The eosinophil population in the portal areas comprises a greater number of mature cells than is seen in the hepatic laminae. This was postulated to reflect increasing activity in the portal areas by the component cells that are also developing and beginning to provide growth factors.

Although eosinophilia at 3 months of age has been associated with a greater risk of the development of atopic disease at 18 months of age (68), there are no studies of eosinophil numbers and/or function at birth with regard to the development of allergic disease. Like dendritic cells, there are very few studies on either the phenotype or function of fetal and neonatal eosinophils. Interestingly, newborns have less L-selectin on their eosinophils than those of the adult, but fetal eosinophils (23–34 weeks of gestation) have adult levels of L-selectin (69, 70). As CD62L is shed from the cell surface during activation, the decreased levels of surface CD62L on newborn eosinophils may indicate activation of this population, and the process of labour itself could have had this effect. Moreover, eosinophils constituted a large proportion of the

granulocytes ($42 \pm 26\%$) in these fetal samples; however, as these samples were collected for diagnostic tests for fetal anomalies, this abundance of eosinophils may reflect fetal disorders (71).

Postnatal maturation of immune function: release from placental control

One of the long-standing enigmas of immunology has been the mechanism or mechanisms that facilitate acceptance of the fetal "allograft" by the maternal immune system. *In extremis*, failure to accept the graft, involving the active expression of T-cell immunity against potential HLA antigens expressed on fetal tissues, results in placental detachment and fetal loss, or, when reactivity is less intense, in pre-eclampsia and premature delivery.

T-cell responses in this context are heavily Th1-polarized and are dominated by IFN-γ, which is highly toxic to the placenta (46). It is now recognized that a series of overlapping control mechanisms operate at the level of the placenta, selectively downregulating Th1 immunity at the fetomaternal interface and within the fetal microenvironment itself. These include expression of FasL on fetal cells as a potential means of elimination of activated T cells (72, 73), and local production of T-cell suppressive tryptophan metabolites via indoleamine 2,3-dioxygenase, which is expressed in syncytiotrophoblasts and macrophages (74). In addition, the placenta produces high levels of a range of mediators which are Th2-trophic and/or Th1-suppressive, including IL-4 and IL-10 (75), prostaglandin E_2 (76), and progesterone (77–79). The last-named presumably maximizes the likelihood that any environmental antigens/allergens that pass across to the developing fetus via the maternal circulation will elicit Th-cell responses in the fetal immune system, which is dominated by Th2 (as opposed to Th1) cytokines (4).

Microbial stimulation and development of immune competence

As noted earlier (80), it is clear from the comprehensive literature relating to domestic and experimental animals that the principal stimuli of postnatal maturation of the immune function in mammals are signals from the microbial environment, particularly the commensal microflora of the gastrointestinal tract. Infections, particularly in the gastrointestinal and respiratory tracts, may also contribute to this process (81).

The principal focus of this late-stage maturation process is upregulation of Th1 functions, which, as noted above, are differentially dampened during fetal life. In the absence of adequate microbial stimulation during infancy, the overall balance within the adaptive immune system remains distorted toward the Th2 phenotype, resulting in blunted expression of Th1 immunity at peripheral challenge sites (82), a failure of the immune deviation mechanisms that normally regulate induction of Th2 responses at mucosal surfaces (83), and excessive class switching of immature B cells toward IgE commitment (84).

The precise cellular target(s) of these stimuli remain to be determined, but it appears likely that antigen-presenting cells (in particular, dendritic cells) play a major role (85). The nature of the molecular signalling between the microbial environment and the immune system remains to be classified; however, it may be predicted that the recently described TOLL receptors (86, 87), as well as the high-affinity receptor for bacterial lipopolysaccharide (CD14), will be found to be central in the process.

Transition from fetal to adult-equivalent immune competence: time course of changes during infancy and early childhood

Our current understanding of the postnatal maturation of immune function in man is restricted mainly to comparisons between cells taken from cord blood, as representative of fetal/neonatal life, and those from adults. Knowledge of the kinetics of the changes occurring postnatally, and associated qualitative/quantitative changes in individual cellular functions, is exceedingly sparse. However, it is becoming evident from aetiologic studies of autoimmunity and particularly allergy (88, 89) that variations in the speed of this maturation process represent important causative factors in these diseases (see below).

Of particular interest in this context are functions associated with expression of Th-cell-dependent immunity. One broad measure of these functions involves assessment of the postnatal rate of accumulation of T-memory cells in the periphery. The available studies suggest that adult-equivalent levels of T-memory cells, as demonstrated by CD45RO expression in the TcRα/β and TcRγ/δ compartments, are achieved by approximately the age of 15 years, but the rate at which this occurs within the population is extremely variable (90–93).

The generation of some aspects of T-memory is poor during infancy (94), despite apparently normal levels of initial T-cell activation, but the underlying reasons for this transient deficiency are not understood. In this context, it has been demonstrated in several laboratories that despite initially high *in vitro* responses to polyclonal stimuli, T cells from normal infants do not show the sustained proliferation typical of adults (95, 96), and do not give rise to stable clones at a frequency comparable to adults (95). Holt et al.'s (95) study was cross-sectional

and hence does not answer the key question of when incompetent T-cell precursor frequency in children reaches the adult normal range.

The related issue of age-dependent changes in cytokine production by Th cells is also not fully resolved. However, it has been reported earlier (97) that IFN-γ production in response to polyclonal stimuli rises between birth and the age of 5 years, at which time approximately adult-equivalent levels are achieved. This maturational deficit in IFN-γ production is also demonstrable at the T-cell clonal level (95). An ongoing prospective cohort study in our laboratories (98) has shown that the postnatal upregulation of IFN-γ is usually delayed until after the age of 1 year, and rises steadily thereafter; however, as reported earlier (97), we have also noted that variation within the overall population is extremely marked. We have noted too that the postnatal capacity to produce Th2 cytokines also rises postnatally, and that this rise occurs earlier (by 4 months of age) and peaks late in infancy, before declining to adult-equivalent production levels (98). This suggests that the Th1-polarization of immune function characteristic of fetal life may be normally maintained during early infancy, raising the possibility that it may have an as yet uncharacterized protective role (e.g., anti-inflammatory) during this early life phase.

In this context, it is also of interest to note that varying grades of eosinophilia, typified by the presence of these cells in the self-limiting rash erythema toxicum, are also very common in this age group (99). Furthermore, analogous to what has been reported in infant mice, human neonates can mount Th1-polarized responses to potent stimuli such as BCG (100), whereas their responses to milder stimuli (such as acellular diphtheria/pertussis/tetanus vaccine) are strongly Th2 polarized (98).

Variation in postnatal development of adaptive immune functions: implications for the pathogenesis of allergic disease

In earlier cross-sectional studies on Th-cell function in infancy, we identified a relative functional deficiency in children at high genetic risk (HR) of atopy, in comparison to their low-risk (LR) counterparts (95). This was demonstrated via limiting dilution analysis of overall immunocompetent T-cell precursor frequency, and parallel analysis of cytokine production at the T-cell clonal level. Both Th1 and Th2 cytokine production was reduced in the HR group relative to LR, but the reduction was greatest for the Th1 cytokine IFN-γ (95). Our initial interpretation of these findings (95), which has been borne out by the results of more recent studies (88), is that this deficiency in HR children is indicative of delayed kinetics in the normal transition from the fetal Th2-polarized to the adult Th1-polarized cytokine phenotype.

The potential significance of this transient maturational deficit becomes apparent when $CD4^+$ Th-cell responses to environmental allergens are examined over the same age range. These studies indicate that initial fetal responses are of the Th0/Th2 phenotype, being dominated by Th2 cytokines (4), and that "protection" against consolidation into potentially pathogenic Th2-polarized memory is (for inhalant allergens) achieved via immune deviation during infancy toward the Th1 cytokine pattern (101–103). Thus, reduced capacity to generate Th1 responses during infancy, in the form of IFN-γ and/or upstream Th1-polarizing cytokines, such as IL-12, is likely to compromise this immune deviation process, thus increasing the risk of developing allergy (88, 89). It is also of interest to note that development of atopy in childhood is associated with reduced capacity to develop immunologic memory against BCG immunization during infancy (104), and slower development of responses to diphtheria/pertussis/tetanus vaccination (105).

The mechanism or mechanisms underlying this maturational difference in immune function in HR children remain to be elucidated. The simple explanation that it represents an exaggeration of the Th2 skew which is characteristic of fetal life does not appear to be tenable, given recent findings that the magnitude of allergen-specific Th2 responses in neonates who do not develop allergy during infancy is greater than in those who do (102). However, the difference may be at least partially due to variations in capacity to recognize and/or respond to Th1-inducing signals from the extrauterine environment, as suggested by the recent finding linking intensity of atopy with a polymorphism in the CD14 gene encoding the high-affinity receptor for bacterial lipopolysaccharide (106).

Conclusion

It is becoming increasingly clear from recent studies that the seeds for expression of a variety of immunologically mediated diseases in adulthood are sown during early postnatal life. During this period, the immune system is fine-tuning a variety of key functions, in the face of direct stimulation from environmental signals not previously encountered during fetal life, and the response patterns "learned" during this period persist into adult life.

The future key to the problem of allergy may lie in comprehensive analysis of this complex maturation/education process, with the long-term aim of redirecting aberrant immune responses at an early stage of their development, before diseases such as allergy are fully expressed.

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