Developmental factors as determinants of risk for infections and atopy in childhood

P.G. Holt

ABSTRACT: The nature of the relationship between infections and allergy in children continues to be the subject of vigorous debate, in particular the issue of whether the increased frequency of wheezing respiratory infections in children is a cause or a consequence of atopy. Recent evidence suggests a subtle twist to this argument, notably that the genetic risk for susceptibility to both atopy and respiratory infections may involve a common set of genetic variations related to the efficiency of maturation of immune function in early post-natal life.

KEYWORDS: Asthma, atopy, cytokines, infancy, T-cells

The early post-natal period is recognised as a time of high risk for infection [1]. The basis for this risk is by now at least partially understood, and involves the reduced capacity for expression of a broad range of innate and adaptive immune defence mechanisms [2]. These deficiencies are broadly summarised below, and their significance in relation to susceptibility to infectious and allergic diseases is discussed.

IMMUNE COMPETENCE DURING FOETAL LIFE

The first evidence of capacity to recognise antigen-like stimuli within the developing immune system is observable at around the 15th week of gestation, at which time foetal human T-cells first express responsiveness to polyclonal mitogens, such as Phytohaemagglutinin [3]. The issue of whether these foetal T-cell interactions generate genuine T-cell memory remains controversial. On the one hand, while maternal immunisation with tetanus toxoid can induce the appearance of immunoglubulin (Ig)M within the foetus [4], this antibody cannot cross the placenta and its presence in the foetus is thus a footprint of a local primary immune response. However, studies on congenital cytomegalovirus infection [8] have convincingly demonstrated specific priming of stable major histocompatibility complex class I restricted CD8+ T-cell memory in the foetal compartment, indicating that antigenic stimuli of sufficient strength and/or persistence is capable of bypassing whatever functional deficiencies exist within the foetal immune system, and in doing so can trigger adult equivalent immunity.

The nature of the control mechanism(s) responsible for maintenance of homeostasis within the foetal immune system are multi-layered and highly complex. Assessment of the overall functional capacity of the foetal immune system reveals a pattern of generalised attenuation of adaptive immunity, accompanied by a skewing of effector functions towards the Th2 cytokine phenotype [9]. These attenuation mechanisms are most notable within the placenta, and include local production by trophoblasts and macrophages of T-cell-inhibitory metabolites of tryptophan generated via indoleamine 2,3-dioxygenase [10], accompanied by constitutive local production (particularly by trophoblasts) of interleukin (IL)-10 [11]. In addition, high levels of Fas ligand are expressed within the placenta [12], providing a mechanism for rapid elimination via apoptosis of locally activated Fas receptor-positive T-cells.

In addition to these overtly suppressive mechanisms, a second series of more subtle mechanisms coexist to deviate foetal T-cells, which escape elimination post activation, towards the Th2 pathway, thus minimising local production with...
INNATE IMMUNE FUNCTIONS IN FOETAL AND NEONATAL LIFE

Mononuclear phagocytic cells (MPC) play a key role in frontline defence against pathogens and an ancillary role in control of adaptive immune functions. Foetal and neonatal MPC appear competent with respect to phagocytic activity and bacterial killing, but exhibit diminished capacity for expression of a variety of pro-inflammatory functions including production of effector cytokines, such as IL-6 [19] and tumour necrosis factor-α [20], which may account in part for the diminished febrile response of neonates.

The recruitment of MPC into inflammatory foci in neonates is also delayed and attenuated [21], a feature of which is also seen with neutrophils in this age group [22], and in both cases this appears related to deficiencies in regulation of expression of integrins, such as macrophage 1 and L-selectin on migrating cells [22]. NK cell activity is also attenuated in neonates, particularly their capacity to respond to activation signals [23].

There is also evidence for dysregulation of eosinophil function in early life, exemplified by the increased presence of these cells in inflammatory exudates in infants [24]. In this context, ongoing studies in the current author’s laboratory (J. Denburg, McMaster University, Ontario, Canada, personal communication) suggest heightened expression of receptors for granulocyte macrophage colony stimulating factor and IL-3 on eosinophil precursors in infants; it is plausible that this may result in inflammation-driven eosinophilia in the absence of the large IL-5 signals that are required to achieve the same end in adulthood, where the precursors express predominantly IL-5R. This may explain the predilection for eosinophil recruitment to non-Th2-driven inflammatory foci that is observed in early life, as granulocyte macrophage colony stimulating factor and IL-3 are produced in virtually all classes of inflammatory responses.

An additional function of the innate immune system that is attenuated in the foetal/neonatal compartment is APC activity, particularly that of the DC population [25, 26], and some of the deficiencies in neonatal T-cell function may be a direct result of the functional immaturity of APC [27]. In particular, the generalised Th2 skewing of adaptive immune function in neonates and infants is likely to be due in part to reduced capacity of immature DC to express the IL-12p35 gene [28, 29], thus resulting in a persistent deficiency in early life in production of the major Th1 skewing stimulus IL-12p70. While epigenetic control of IL-12p35 gene transcription in neonatal DC has been demonstrated to contribute to attenuated production of IL-12, other mechanisms may also be involved. Notably, it has recently been reported in the mouse that reduced IL-12 production in neonatal DC may be partly explicable via a bystander mechanism involving hyperproduction of IL-12-inhibitory IL-10 by immature neonatal B-cells [30], which also display a range of developmental deficiencies [28].

MATURATION OF ADAPTIVE IMMUNE FUNCTIONS IN EARLY POST-NATAL LIFE: VARIATIONS IN KINETICS AS DETERMINANTS OF SUSCEPTIBILITY TO DISEASE RISK

As noted above, the newborn enters the antigenically hostile extrutemine environment ill equipped to deal with the immunological challenges that must be faced. In particular, key effector mechanisms that are required for survival of exposure to pathogens exhibit varying degrees of attenuation resulting from developmental deficiencies in a range of interrelated cellular and molecular mechanisms ranging from methylation-mediated “locking” of specific gene promoters exemplified by IFN-γ, to hyperproduction of immunosuppressive and/or immunomodulatory molecules, such as IL-10. Additionally, key effector cell populations display lineage-specific developmental deficiencies that contribute to overall diminished host resistance.

A notable example in this context is the T-cell compartment, in particular CD4+ T-cells. At birth, ≥90% of circulating naive CD4+ Th-cells express the phenotype of “recent thymic emigrants” (RTE), defined by expression of a range of surface markers, such as CD1 and CD38, and the presence of intracellular T-cell receptor excision circles. These T-cells dominate the circulating T-cell compartment of birth and are replaced slowly during infancy and early childhood by functionally mature naive T-cells. Unlike functionally mature naive T-cells, activation of these RTE though the T cell receptor triggers apoptosis, unless sufficient quantities of the common γ-chain cytokines are present to “rescue” the activated cells [31]. Additionally, it has recently been demonstrated by THORNTON et al. [32] that antigen recognition by these neonatal CD4+ T-cells lacks the specificity seen with adult naive CD4+ T-cells, and that instead the T-cell receptors on these RTE recognise a wide range of peptides and respond with a short lived intensive burst of proliferation and cytokine secretion, which is generally terminated by apoptosis [32].

The functional significance of this finding is not fully understood. However, on the basis of similar findings in the mouse relevant to CD8+ CTL function in neonates [33], the current author has postulated that this mechanism may provide the immature immune system with a mechanism for the generation of bursts of cytokines required for activation of cytolytic mechanisms with phagocytes at sites of infection, in the absence of conventional cellular immune memory [32]. These findings also provide a potential explanation for the short-lived nature of T-cell recall responses in infants after natural infection [34] or vaccination [35], i.e. many of these supposed recall responses may involve nonspecific in vitro activation of.....
short-lived naive RTE as opposed to genuine long-lived T-memory cells. The question of when and how functionally mature naive CD4+ T-cells start to play a major role in cellular immune responses in young children remains to be resolved.

This complex picture is further complicated by the generalised Th2 skewing of the adaptive immune system in infants, representing a carryover from the response pattern that dominates the foetal immune system. Surprisingly, given the central importance of Th1 cytokine production in resistance to infection, the kinetics of post-natal upregulation of Th1 function as exemplified by IFN-γ response capacity is relatively slow, and at the population level functional maturation does not start to accelerate until ~12 months post-natal [35]. The impetus for post-natal Th1 maturation is not precisely defined, but longstanding evidence from earlier studies in germ-free animals indicate that the principal stimulus is provided by signals from the microbial environment [36] transduced via TOLL receptors expressed within the innate immune system [37].

It is also clear that the kinetics of this Th1 maturation process is highly variable within the overall population, and that slow maturation may carry enhanced risk for certain diseases. One notable potential example of the latter is respiratory syncitial virus (RSV) infection. Murine studies have demonstrated that the degree of eosinophilia triggered by RSV (and by inference the intensity of ensuing airway inflammation and wheeze) is inversely related to age at infection, and thus to the degree of Th2 polarity of the infant’s immune system at that time [38]. Moreover, infections during this period carry the attendance risk of programming Th2 polarised memory, which may result in reiteration of the wheeze-associated eosinophilic host defence response upon subsequent reinfection [38, 39]. An additional important example is atopy. It is evident from a range of studies that slow kinetics of post-natal maturation of adaptive immune function (in particular Th1 function) is strongly associated with increased risk of allergic sensitisation [40]. Moreover, this risk appears to extend beyond atopy, as children with the “delayed immune maturation” phenotype also express less efficient and/or more Th2 polarised vaccine immunity [2], and are also more prone to respiratory viral infections during infancy [35, 41].

It is well recognised that both respiratory infections and allergy to inhalants are important independent triggers of intermittent wheezing symptoms in early life. Moreover, there is increasingly strong epidemiological evidence indicating that risk for the development of persistent asthma is maximal in infants subjected to airway inflammation from both inhalant allergy and respiratory infections [40, 42]; these inflammatory pathways appear to interact synergistically during this critical phase of early post-natal lung growth to alter key tissue differentiation programmes and thus to drive asthma pathogenesis (fig. 1) [40, 42].

CONCLUSIONS
The precise contribution from individual cell populations within the innate and adaptive arms of the immune system to the developmental deficiencies that underlie the increased susceptibility of young children to infections, atopy and wheeze, remain to be established. However, it is clear that children at the lower end of the immune developmental kinetics spectrum are at increased risk of a range of diseases. Why individual children become part of this at risk group remains to be determined, but recent findings provide exciting new clues. In particular, given the recognised importance of signalling through microbial pattern recognition receptors, such as CD14 and TLR2, in driving the overall maturation of the immune system, it is of particular interest to note recent findings of increased risk for atopy associated with polymorphisms in these two key pattern recognition receptors genes [43, 44]. One plausible interpretation of these findings is that children with risk-associated polymorphisms in pattern recognition receptors genes require quantitatively or qualitatively more potent microbial stimuli to efficiently drive immune maturation. This possibility merits more detailed investigation, as it may reveal novel therapeutic approaches towards atopy/asthma prevention that are readily amenable to testing.
REFERENCES

1 Wilson CB. Immunologic basis for increased susceptibility of the neonate to infection. J Pediatr 1986; 108: 1–12.
2 Holt PG, Rowe J. The developing immune system and allergy. In: Leung DY, Sampson HA, S GR, Szefler SJ, eds. Pediatric Allergy Principles and Practice. Mosby, St Louis, 2003; pp. 69–79.
3 Stites DP, Carr MC, Fudenberg HH. Ontogeny of cellular immunity in the human fetus. Development of responses to phytohaemagglutinin and to allogeneic cells. Cell Immunol 1974; 11: 257–271.
4 Gill TJ, Repetti CF, Metlay LA. Transplacental immunisation of the human fetus to tetanus by immunisation of the mother. J Clin Invest 1983; 72: 987–996.
5 McLeod R, Mack DG, Boyer K, et al. Phenotypes and functions of lymphocytes in congenital toxoplasmosis. J Lab Clin Med 1990: 116: 623–635.
6 Aase JM, Noren GR, Reddy DV, St Geme JW. Mumps-virus infection in pregnant women and the immunologic response of their offspring. New Eng J Med 1972; 286: 1379–1382.
7 Fievet N, Ringwald P, Bickii J. Malaria cellular immune responses in neonates from Cameroon. Parasite Immunol 1996; 18: 483–490.
8 Marchant A, Appay V, van der Sande M, et al. HCMV-specific CD8+ T cells are functionally active in newborns, produce anti-viral cytokines and display perforin-dependent cytolysis. J Clin Invest 2003; 111: 1747–1755.
9 Wegmann TG, Lin H, Guibert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today 1993; 14: 353–356.
10 Munn DH, Zhou M, Attwood JT, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 1998; 281: 1191–1193.
11 Roth I, Corry DB, Locksley RM, Abrams JS, Litton MJ, Fisher SJ. Human placental cytrophoblasts produce the immunosuppressive cytokine interleukin 10. J Exp Med 1996; 184: 539–548.
12 Guller S, La Chapelle L. The role of placental Fas ligand in maintaining immune privilege at maternal-fetal interface. Seminars in Reproductive Endocrinology 1999; 17: 39–44.
13 Hilkens CM, Vermeulen H, Joost van Neerjen RJ, Snijderwint FGM, Wierenga EA, Kapsenberg ML. Differential modulation of T helper type 1 (Th1) and T helper type 2 (Th2) cytokine secretion by prostaglandin E2 critically depends on interleukin-2. Eur J Immunol 1995; 25: 59–63.
14 Piccinni M-P, Giudizi M-G, Biagiotti R, et al. Progesterone favours the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. J Immunol 1995; 155: 128–133.
15 Szekeres-Bartho J, Wegmann TG. A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol 1996; 31: 81–95.
16 Young HA, Ghosh P, Ye J, et al. Differentiation of the T Helper phenotypes by analysis of the methylation state of the IFNg gene. J Immunol 1994; 153: 3603–3610.
17 White GP, Watt PM, Holt BJ, Holt PG. Differential patterns of methylation of the IFNg promoter at CpG and non-CpG sites underlie differences in IFNg gene expression between human neonatal and adult CD45RO+ T-cells. J Immunol 2002; 168: 2820–2827.
18 Rowe J, Heaton T, Kusel M, et al. High IFN-g production by CD8+ T cells and early sensitization among infants at high risk of atopy. J Allergy Clin Immunol 2004; 113: 710–716.
19 Yachie A, Takano N, Ohta K, et al. Defective production of interleukin-6 in very small premature infants in response to bacterial pathogens. Infect Immun 1992; 60: 749–753.
20 English BK, Hammond WP, Lewis DB, Brown CB, Wilson CB. Decreased granulocyte-macrophage colony-stimulating factor production by human neonatal blood mononuclear cells and T cells. Pediatr Res 1992; 31: 211–216.
21 Uhr JW, Dancis J, Neumann CG. Delayed hypersensitivity in premature neonatal humans. Nature (Lond.) 1960; 187: 1100–1101.
22 Török C, Lundahl J, Hed J, Lagercrantz H. Diversity in regulation of adhesion molecules (Mac-1 and L-selectin) in monocytes and neutrophils from neonates and adults. Arch Dis Child 1993; 68: 561–565.
23 Liebson PJ, Hunter-Laszlo M, Douvas GS, Hayward AR. Impaired neonatal NK cell activity to herpes simplex virus. J Clin Immunol 1986; 6: 216–224.
24 Bhat AM, Scanlon JW. The pattern of eosinophilia in premature infants. J Pediatr 1981; 98: 612–616.
25 Hunt DW, Huppertz HI, Jiang HJ, Petty RE. Studies of human cord blood dendritic cells: evidence for functional immaturity. Blood 1994; 84: 4333–4343.
26 Borras FE, Matthews NC, Lowdell MW, Navarrete CV. Identification of both myeloid CD11c+ and lymphoid CD11c- dendritic cell subsets in cord blood. Br J Haematol 2001; 113: 925–931.
27 Trivedi HN, Hayglass KT, Gangur V, Allardice JG, Embree JE, Plummer FA. Analysis of neonatal T cell and antigen presenting cell functions. Hum Immunol 1997; 57: 69–79.
28 Upham JW, Lee PT, Holt BJ, et al. Development of interleukin-12-producing capacity throughout childhood. Infect Immun 2002; 70: 6583–6588.
29 Goriely S, Vincart B, Stordeur P. Differentiation of the T cell repertoire. J Immunol 2001; 166: 2141–2146.
30 Sun C-M, Deriaud E, Leclerc C, Lo-Man R. Upon TLR9 Signalling, CD5+ B cells control the IL-12-dependent Th1-priming capacity of neonatal DCs. Immunity 2005; 22: 467–477.
31 Hassan J, Reen DJ. Human recent thymic emigrants-identification, expansion and survival characteristics. J Immunol 2001; 167: 1970–1976.
32 Thornton CA, Upham JW, Wikström ME, et al. Functional maturation of CD4+CD25+ CD45RA+ CD45RO+ T regulatory cells in human neonatal T cell responses to environmental allergens. J Immunol 2004; 173: 3084–3092.
33 Gavlin MA, Bevan MJ. Increased peptide promiscuity provides a rationale for the lack of N regions in the neonatal T cell repertoire. Immunity 1995; 3: 793–800.
34 Hayward AR, Groothuis J. Development of T cells with memory phenotype in infancy. Adv Exp Med Biol 1991; 310: 71–76.
Rowe J, Macaubas C, Monger T, et al. Heterogeneity in diphtheria-tetanus-acellular pertussis vaccine-specific cellular immunity during infancy: relationship to variations in the kinetics of post-natal maturation of systemic Th1 function. *J Infect Dis* 2001; 184: 80–88.

Holt PG. Environmental factors and primary T-cell sensitisation to inhalant allergens in infancy: reappraisal of the role of infections and air pollution. *Pediatr Allergy Immunol* 1995; 6: 1–10.

Ulevitch RJ. Endotoxin opens the *Tollgates* to innate immunity. *Nat Med* 1999; 5: 144–145.

Culley FJ, Pollof J, Openshaw PJ. Age at first viral infection determines the pattern of T cell-mediated disease during reinfection in adulthood. *J Exp Med* 2002; 196: 1381–1386.

Holt PG, Sly PD. Interactions between RSV infection, asthma, and atopy: unraveling the complexities. *J Exp Med* 2002; 196: 1271–1275.

Holt PG, Macaubas C, Stumbles PA, Sly PD. The role of allergy in the development of asthma. *Nature* 1999; 402: B12–B17.

Copenhaver CC, Gern JE, Li Z, et al. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. *Am J Respir Crit Care Med* 2004; 170: 175–180.

Holt PG, Sly PD. Interactions between respiratory tract infections and atopy in the aetiology of asthma. *Eur Respir J* 2002; 19: 538–545.

Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism in the 5’-flanking region of the CD14 gene is associated with circulating soluble CD14 levels with total serum IgE. *Am J Resp Cell Mol Biol* 1999; 20: 976–983.

Lauener RP, Birchler T, Adamski J, et al. Expression of CD14 and Toll-like receptor 2 in farmers’ and non-farmers’ children. *Lancet* 2002; 360: 465–466.