

EDITORIAL

The significance of immune responses to allergens in early life

Since the delineation of T helper subsets in murine systems [1] and parallel differences in human adults [2], the processes which lead to either atopic (Th2-like) or non-atopic (Th1-like) responses to allergens have become a research priority. With escalating rates of allergic disease, identification of early causal pathways is essential for determining avenues of gene/environmental interaction and targets for disease prevention.

Most immunological studies in humans are limited to *in vitro* functional studies of cells derived from peripheral blood. This provides a very restricted 'viewing window' of the complex immune internet *in vivo*, and it remains important to recognize these limitations. Peripheral blood gives limited information about the immunological events initiated at mucosal surfaces, which appear central to immune development and disease expression. Despite this, studies of peripheral and cord blood have provided valuable information about the early immunological processes that lead to atopic disease. These studies include assessment of both polyclonal and allergen-specific mononuclear cell responses at different ages. As well as clear differences in established responses, there is accumulating evidence of early developmental differences in atopic individuals. This includes apparent immaturity of postnatal development as well as a number of pre-existing abnormalities at birth.

Relative immaturity of polyclonal Th1 responses at birth is one of the most consistently observed abnormalities in neonates who are at risk of atopy (positive family history) or who later develop atopic disease [3–7]. Preliminary studies indicate a direct role of maternal atopy in neonatal Th1 dysfunction [8]. Further delay in Th1 maturation in the postnatal period has also been associated with atopy [9]. It has been proposed that this leads to the failure of normal postnatal 'Th1-driven immune deviation', and persistent allergen-specific Th2 responses [10]. Longitudinal studies of allergen-specific responses in the first 2 years of life support this [11], and also indicate that differences in postnatal maturation are evident within a few months after birth. While initial immune responses normally reflect the Th2 milieu of pregnancy [12], these are short-lived in non-atopic infants [11], but appear to persist in infants with documented Th1 immaturity at birth. Antigen presenting cell [APC] immaturity may be implicated in Th1 dysfunction [10], and there is indirect evidence that APC

costimulation is defective in neonates at high risk of atopy [13]. This may affect the capacity to initiate Th1 responses to allergens in the early postnatal period. In the absence of mature APC signalling the default response appears to be Th2 differentiation [14]. Studies to determine the role of APC in allergy pathogenesis are ongoing. There is evidence of additional immunological immaturity in neonates at high atopic risk, including reduced capacity for many other cytokines (including Th2 cytokines) [15,16], and reduced expression of cytokine receptors CD34⁺ on cord blood progenitor cells [17]. The role of these functional differences is also the subject of ongoing studies.

Allergen-specific responses in the postnatal period appear to correlate with levels of early environmental exposure (reviewed in [18]). Smillie *et al.* (in this issue) have also shown a significant relationship between house dust mite (HDM) levels and the magnitude of lymphoproliferation at one year of age [19]. In the first years of life, infants at risk of atopy also appear to produce stronger Th2 cytokines in response to allergens [20]. This suggests that the genetic and environmental factors that promote atopy exert an influence in very early life.

The significance of allergen-specific responses in cord blood has been more controversial. Kondo *et al.* were the first to describe food allergen-specific mononuclear cell responses at birth [21]; a period of life when the immune system was considered 'naïve' to allergens. They and others have observed that allergens evoke higher lymphoproliferative responses and lower Th1 (IFN- γ) responses in neonates who later developed allergic disease [21–23]. Positive allergen-specific 'memory' patterns of lymphoproliferation have been noted subsequently by many other groups using different culture systems [6,16,24]. It was postulated that these responses to food allergens at birth may represent clonally expanded cell populations activated by food allergens crossing the placenta. Early studies in rodents [25], which demonstrated transplacental passage of maternally ingested food allergens to the fetal circulation and amniotic fluid, supported a similar process for 'in utero priming' in humans. This however, remains unproven despite evidence of allergen transfer across the placenta in humans [26].

The demonstration of responses to inhalant allergens by cord blood mononuclear cells (CBMC) [16,27,28] raised

yet further controversy. While there is now little doubt that CBMCs are capable of responding to inhalant allergens *in vitro*, the significance of these observations remains unclear. Although inhalant allergen (Bet v 1) can cross the human placenta in a double-sided *ex vivo* perfusion model [26], this was noted to be more selective and variable than food allergen transfer. It has been difficult to conceive how relatively low level maternal exposure to inhalants could lead to sufficient systemic levels for passage to the fetus. Our studies [16] were able to confirm that responding T cells in cord blood are (a) of fetal origin (rather than due to maternal contamination), and (b) specific for one particular allergen (and not others). However, the findings were unable to determine if these responses were derived from allergen exposure in pregnancy. We did not find any significant association between the magnitude of responses at birth and later allergic disease. It has been, quite reasonably, postulated that these aeroallergen-specific responses may arise from stimulation by cross-reacting antigens [29]. The first studies, by Piccinni *et al.* [27], that described CBMC aeroallergen responses made the simultaneous observations that while CBMCs respond to inhalants, responses to tetanus toxoid were virtually absent, suggesting that immune responses at birth were specific to exposure. This was also observed in studies by our group [16]. However, a study by Smillie *et al.* published in this issue of the journal [19] is the first to assess the relationship between environmental exposure to HDM and the magnitude of HDM-specific CBMC responses. They found no relationship. While a lack of correlation between responses and environmental exposure does not exclude *in utero* priming (as a 'dose-response' can not be assumed), positive responses in the *absence* of exposure are more convincing evidence of non-specific reactivity or cross reactivity with other antigens. We recently performed lymphoproliferative assays using diphtheria toxoid to stimulate CBMCs derived from 41 neonates with no history of exposure to this toxoid in pregnancy, and found that almost 50% ($n = 18$) showed positive responses (by previously described criteria [30]). This is supported by the observations by Smillie *et al.* [19], who observed responses to tetanus toxoid in the absence of exposure, suggesting another mechanism of 'priming' for these cells.

Regardless of whether CBMC responses are the result of direct allergen stimulation or not, exactly how these antenatal responses affect evolving responses in the early postnatal period is still of great interest. These T cells are capable of responding to allergens, and it is certainly foreseeable that the characteristics of these populations may influence responses during subsequent postnatal encounter with allergen. Moreover, observed differences in the characteristics of these allergen-responsive T cells

from neonates who subsequently develop atopy (compared to those who do not) certainly justify ongoing research in this area. Further characterization of these populations may give valuable information about underlying immune development, which clearly differs in atopic individuals.

The findings of Smillie *et al.* also have implications for allergen avoidance in pregnancy. Earlier associations between the magnitude of allergen-specific responses and allergic diseases [21–23] raised concerns that allergen exposure in pregnancy may be implicated in disease development, and allergen avoidance strategies were proposed. While Smillie *et al.* also observe higher lymphoproliferative responses to HDM in high-risk neonates, this was not related to exposure. These findings support growing evidence that allergen avoidance in pregnancy is unlikely to be beneficial (reviewed in [31]).

Thus, the study by Smillie *et al.* adds to the mounting evidence that infants at high risk of atopy have immunological abnormalities at birth unrelated to allergen exposure, but possibly the result of other underlying (genetic and environmental) influences. The recent increase in allergic disease, and the apparent immune dysfunction at birth indicate that environment changes may affect immune development very early in life. The reasons for this enhanced propensity for Th2 differentiation need to be determined urgently.

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S. L. PRESCOTT
 Department of Paediatrics
 University of Western Australia
 Princess Margaret Hospital
 PO Box D184
 Perth, Western Australia 6001
 Australia
 E-mail: susanp@ichr.uwa.edu.au