

EDITORIAL

Cord blood memory responses: are we being naïve?

In the simplest terms, the recent dramatic increase in allergic disease is due to altered host immune responses to a changing environment. Although it is still widely thought that the 'modern' environment is more 'allergenic' to the host, it seems more likely that environmental changes have had other direct effects on intrinsic host immunity to promote allergic patterns of response. In keeping with this, substances that typically evoke allergic responses (common foods, pollens, dander and house dust) have probably changed relatively little during this period. Rather, other environmental changes are 'loading the gun' in favour of 'Th2' allergic responses to allergens which might otherwise evoke 'Th1' responses in the same individuals under other conditions. Hence, allergens are increasingly viewed as the 'target' of these responses, rather than the primary cause of allergic disease.

More significantly, the modern environment consists of many exposures which have potential immunological consequences. In addition to changes in dietary patterns and microbial exposure, the human immune system has recently been exposed to a myriad of completely novel chemical compounds which contaminate modern homes, foods and water supply. Many of these organic toxins are measured in humans tissues and fluids including cord blood, the placenta and breast milk. It is possible that alterations in dietary composition (antioxidants, polyunsaturated fats) [1,2], enteric flora (probiotics) [3], and other exposures including modern contaminants (including phthalates, polychlorinated biphenyl compounds (PCBs), organochlorines) [4] could all alter the intrinsic 'propensity' for Th2 responses. The effects of these and other environmental changes are not well understood, but are the focus of recent research by a number of groups including our own and that of Devereux *et al.* [5] discussed later in this issue.

As with many other diseases, it is clear that the expression of allergic diseases is the result of complex interplay between 'genetic' predisposition and 'environmental' influences. However, this is not a static equation. Any potential influence is likely to be greater during early development, rather than in the mature state. Thus, the concept of 'timing' adds another vital level to the concept of 'nature vs. nurture'. Moreover, timing effects may also apply equally to gene expression as to environmental exposure (Fig. 1). This may in part explain the complexity and heterogeneity of disease expression.

Accordingly, the impact of environmental change is likely to be greatest on the forming immune system, particularly during gestation and in the early postnatal period. While there has been much conjecture about the significance of fetal T cell responses to allergen, less attention has been directed to the more fundamental question of possible adverse environmental influences during this formative period.

Thus while discussion of '*in utero*' T cell responses to allergens is of great interest, we wish to emphasize the need to broaden this discussion to include the more generalized effects of the modern environment on the developing immune system. A better understanding of the underlying factors which are 'stacking the deck' in favour of persistent Th2 responses to allergens, will more reliably lead to prevention strategies (than will an ongoing debate about whether the fetus is 'sensitized' *in utero* or not).

Lymphoproliferation and cytokine responses to allergens have been readily detected in cord blood. Although aspects of these responses may vary subtly with atopic risk (family atopy) and later clinical disease, there has been no clear relationship between levels of allergen exposure and the patterns of neonatal response [6]. This has raised further questions about the significance of the cord blood mononuclear 'memory' responses measured to allergens at birth, and has already been discussed recently in this journal [6,7]. In this current issue Devereux and colleagues [5] address the capacity of CD45RO⁺ 'memory' and CD45RA⁺ naïve cord blood T cells to respond to various antigens and allergens.

As noted by these investigators the expression of CD45RA and CD45RO are generally considered by immunologists to identify unsensitized (naïve) and sensitized (memory) T cells, respectively. These different isoforms of CD45R are generated as splice variants that are detected at the cell surface as CD45RA (high molecular weight) and CD45RO (low molecular weight). For the antigen-experienced adult, CD45RO⁺ T cells comprise around 50% of T cells in peripheral blood with the proportion increasing with age to be about 80% of circulating T cells in centenarians [8]. In contrast, numerous investigators have shown that the percentage of CD45RO⁺ T cells is dramatically lower in the neonatal circulation (umbilical cord blood) comprising around 10% of T cells [8,9].

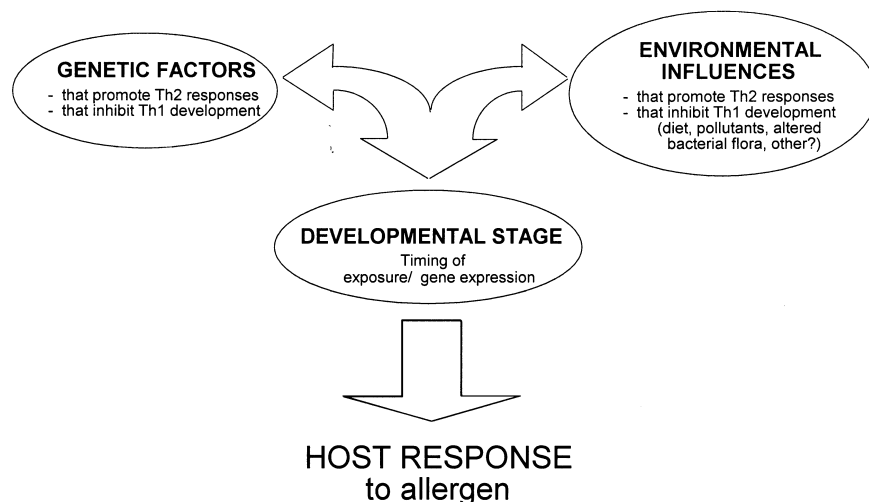


Fig. 1. The effects of both genetic and environmental factors are considerably greater in early development. Regardless of the timing of first allergen encounter, the capacity of other maternal environmental exposures to affect patterns of fetal immune development should not be underestimated.

Immunologists have utilized the relatively discrete expression of CD45RA and CD45RO to ascertain if responsiveness to an antigen or peptide resides in the naïve or memory T cell compartment. Until recently most of these studies have been conducted by simple depletion experiments as used in the accompanying paper. However, non-T cell populations in mononuclear cell preparations, especially dendritic cells, also express CD45RA and CD45RO [10] so inadvertent depletion of antigen-presenting cell populations could have dramatic consequences for the functional read-out chosen, typically proliferation assays. Recent studies that have utilized methodologies to combat the loss of non-T cells have yielded surprising results. Proliferative responses to immunodominant epitopes of myelin basic protein (MBP) were obtained more frequently from both CD4⁺ CD45RA⁺/RO⁻ cells than from CD4⁺ CD45RA⁻/RO⁺ cells of normal subjects and those with multiple sclerosis [11]. Similarly, nickel-reactive T cells from non-nickel allergic patch test-negative individuals [12] were obtained from the CD4⁺ CD45RA⁺ subset. In both of these studies the recall response to tetanus toxoid was in the CD45RO⁺ subset.

It has been suggested that instead of CD45RA⁺ expression by T cells being a marker for naïve T cells that it might rather identify resting T cells. This would explain the propensity to identify antigen-specific MBP-specific and nickel reactive T cells in the CD45RA⁺ subset from subjects without multiple sclerosis and individuals without nickel allergy, respectively. Animal studies have indicated that reversion from CD45RO⁺/RA⁻ to RO⁻/RA⁺ is possible. Studies of this nature are limited in humans but it was postulated that if reacquisition of the high molecular weight isoform CD45RA, was to occur, CD45RO expression would be retained [13]. Indeed, a prospective analysis of recipients of T cell-depleted bone marrow indicates that

although RA expression can be reacquired, albeit at lower levels, concomitant loss of RO expression does not occur [14]. Therefore, by our current definitions CD45RA⁺/RO⁻ cells in the human must be antigen-inexperienced rather than resting memory cells (although as MBP and nickel responsiveness occurs in this subset we probably need to re-evaluate this definition).

From the above it would seem that by observing antigen-specific proliferation by CD45RO⁺ cells in 50% of the timothy grass responders (as studied in detail in the accompanying paper [5]) would seem indicative of memory in these neonates. But why do the remaining 50% of responders have reactivity that resides in the CD45RA⁺ compartment? It is important that we understand a little about the immunology of the neonatal T cell, which is in fact about all that is currently known about these cells. The expression of CD38 by greater than 95% of neonatal T cells (reviewed in [15]) makes this population more akin to mature thymocytes than peripheral T cells. Moreover, it is unclear whether neonatal CD4⁺ CD45RA⁺ T cells are indeed precursors of the adult CD4⁺ CD45RA⁺ T cells given the many functional differences between these two populations [16]. So can we assume that the neonatal CD4⁺ CD45RO⁺ T cell is equivalent to the adult T cell of the same phenotype?

The main problem with using CD45RO expression to identify memory cells in the neonatal period is that the majority of thymocytes also express CD45RO. These cells generally do not survive negative selection so do not emerge into the periphery [17] but CD45RO⁺ cells present in the fetal/neonatal circulation might represent thymic 'leakage'. Fetal spleen at 14–21 weeks of gestation contains a large population of CD45RO⁺ RA⁻ cells. Around 27% of splenic T cells are CD45RO⁺ at the peak of expression at 15–17 weeks of gestation which declines

with increasing gestational age, and the percentage of CD45RO⁺ cells in the circulation also decreases over gestation [18]. If CD45RO⁺ cells in the circulation are immature thymocytes that have evaded negative selection in the thymus it would be anticipated that this decreases with increasing gestational age. Most T cells in the human fetal gut (14–27 weeks of gestation) express CD45RO which, in conjunction with the expression of other markers of developing thymocytes, is considered to indicate extrathymic origin [19]. Thus, CD45RO⁺ cells in the peripheral circulation of the neonate might also identify T cells that are extrathymic in origin. To add further complication CD4⁺ CD45RA⁺ RO⁺CD25⁺ cells that contained CD45RO transcripts have been described [20]. It was postulated that this subpopulation had a functionally transitional nature into memory cells [20] but data published over the past few years would suggest that these cells are putative T regulatory or suppressor T cells [21].

Devereux *et al.* [5] are the first to publish data that attempts to elucidate the issue of the development of T cell memory subsequent to intrauterine exposure to allergen. While there is an urgent need to clarify this, it is imperative that we first improve our understanding of neonatal T cell function. Specifically, it needs to be ascertained if CD45RO expression at birth is equivalent to that of the adult and what the pattern of T cell memory acquisition is postnatally. Can long-term T cell memory be established in the absence of the microbial signals so pivotal to the development of immunological tolerance?

Expression of clinical allergic disease occurs within months of birth in many infants. Regardless of when allergen-specific memory first develops, poorly defined factors appear to be operating early in immune development to predispose to persistent Th2 responses. The timing of first exposure to individual allergens is likely to be variable (before and after birth) but generally occurs when the immune system is immature and more vulnerable to other influences. We need to understand why the ambient 'conditions' during this early formative period are predisposing to Th2 responses to encountered allergens, and how we may modify these to prevent allergic diseases.

Acknowledgements

Special thanks to Laura, Jack, Bronte and Grace who helped with the cover illustration.

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