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Neonatal immunity: faulty T-helpers and the shortcomings of dendritic cells

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Abstract

Immunity in the newborn is characterized by minimal Th1 function but an excess of Th2 activity. Since Th1 lymphocytes are important to counter microbes and Th2 cells favor allergies, the newborn faces susceptibility to microbial infections and allergic reactions. Delayed maturation of certain dendritic cells leads to limited IL-12 production during the neonatal period. The Th2 cytokine locus of neonatal CD4⁺ T cells is epigenetically poised for rapid and robust production of IL-4 and IL-13. Together, these circumstances lead to efficient differentiation of Th2 cells and the expression of an IL-4R α /IL-13R α 1 heteroreceptor on Th1 cells. Upon rechallenge, Th2 cells rapidly produce IL-4 which utilizes the heteroreceptor to drive apoptosis of Th1 cells yielding the Th2 bias of neonatal immunity.

Introduction

Landmark experiments by Sir Peter Medawar's group in the 1950s demonstrated that neonatal exposure to antigen (Ag) leads to a lack of responsiveness to the same Ag during a later encounter [1]. Deletion or inactivation of T cells was believed to be the underlying mechanism for such unresponsiveness [2]. Since that time, the neonatal period has been viewed as a 'window of opportunity' for inducing unresponsiveness to specific Ag. By analogy to T cell tolerance to self Ag (Box 1), this neonatally-induced unresponsiveness was referred to as neonatal tolerance [3]. This interpretation fits well with the known susceptibility of newborns to microbial infection [4], however it is insufficient to explain the vulnerability of infants and children to immune-mediated allergic reactions [5]. A turning point in immunology came about in the 1980s when it was recognized that T cells could be classified into subsets based on cytokine production and associated effector function [6]. Th1 cells produce mostly interferon γ (IFN γ), an inflammatory cytokine important in responses against microbial infections, while Th2 cells secrete IL-4 and IL-13 which participate in immunity against parasites but also play major roles in allergic reactions. Shortly thereafter, it was reported that the newborn is indeed

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capable of raising an immune response [7,8], however, the Th component of this response manifests a strong bias toward Th2 function [9–14]. Since Th2 cells do not elicit the usual inflammatory reactions associated with Th1 cells but nevertheless constitute responsiveness, the dogma of ‘neonatal tolerance’ shifted to one of ‘neonatal immunity’ (Box 2). While unbalanced Th1:Th2 responses explain the susceptibility of the newborn to both infections and allergic reactions, they raise the intriguing question as to how such a shift comes about in the first place, particularly since potent neonatal Th1 responses can develop under specific circumstances including infection with particular microbes [8,9,15–19]. In fact, a great deal of interest has been devoted lately towards defining regimens that could promote neonatal Th1 immunity [20–22]. The current understanding of neonatal immunity suggests that delayed developmental maturation of accessory cells as well as T cell intrinsic epigenetic factors play major roles in the asymmetrical Th1:Th2 immunity in the newborn [23–25].

Box 1

T cell tolerance to self antigens

T cell tolerance refers to unresponsiveness to self antigens. It can be acquired during T cell maturation in the thymus (“central tolerance”) or during circulation in the periphery (“peripheral tolerance”). Central tolerance involves mainly apoptosis of self-reactive T cells. However, other mechanisms such as receptor editing which diminishes reactivity to self and conversion of potentially aggressive thymocytes to suppressor T cells contribute to central tolerance [52]. AIRE, a transcription factor that controls expression of self antigens in the thymus, is an important mediator of central tolerance and recent studies indicate it is particularly critical in establishing tolerance to self in early life [53]. Peripheral tolerance involves mostly inadequate presentation of self antigens under non-inflammatory conditions, inactivation of self reactive lymphocytes by Treg or suppressor T cells, and to a lesser extent apoptosis by antigen overdose [54]. All these mechanisms lead to unresponsiveness to self-antigen, i.e. T cell tolerance of self.

Box 2

A new way of looking at things: the shift in “neonatal tolerance” to “neonatal immunity”

For decades, neonatal tolerance was defined as the unresponsiveness of newborns to antigens that are otherwise immunogenic in adults [1–3]. Most neonatal studies involved allogeneic or microbial antigens known to induce Th1 type responses in adults. The early investigations on neonatal responses were usually focused on searching for Th1 cells and their inflammatory responses. Th1 responses were often undetectable in neonates, leading to the idea that the neonatal immune system was somehow functionally deficient. Later, however, extensive analysis of neonatal responses revealed the presence of Th2 responses [9–12] that were not observed in the earlier studies because they do not display the inflammatory reactions or the cytokine profiles associated with Th1 cells nor do they counter microbes or alloantigens as effectively or as measurably as Th1 cells. The observation of neonatal Th2 responses, however, overturned the notion of unresponsiveness or tolerance *per se* and shifted the terminology to neonatal immunity. This is further reinforced by observations that certain microbes and alloantigens under particular circumstances can indeed induce neonatal Th1 responses [8,18–20,49].

Primary neonatal responses comprise both Th1:Th2 responses

The size limitations associated with murine neonates restricted the initial studies to analysis of secondary responses that arise upon re-challenge with Ag during adult life. The lymph nodes, which are the usual sites of interest for investigation of immune responses were initially found to be unresponsive in adult mice which had been primed with Ag neonatally leading to the belief that T cell deletion/inactivation was the chief mechanism for the lack of adult secondary responses (Figure 1). In fact, responses do develop in animals that were exposed to Ag at the neonatal stage but these comprised mostly of Th2 cells instead of the usual inflammatory Th1 lymphocytes [9–14]. These findings greatly impacted the field of neonatal immunology and the idea that the newborn is able to develop immune responses was accepted [26]. Further results indicated that the dose and form of Ag can influence the quality of neonatal responses with Th1 cells being observed alongside Th2 lymphocytes though still with great disparity [8,9,27]. Given that these studies relied solely on analyses of secondary responses, the question then was whether the unbalanced Th1:Th2 responses reflect an impediment in mounting primary Th1 responses due to exuberant Th2 deviation, an inability in maintaining Th1 effectors, or a dampening of Th1 function during rechallenge with Ag. Addressing this question was made possible by the development of a neonate-to-neonate T cell receptor (TCR) transgenic adoptive transfer system [28] where the frequency of responding cells is increased and can be traced and analyzed *in vivo* upon exposure to Ag at birth (Figure 2). This was accomplished using CD4⁺ T cells from DO11.10 mice (H-2^d haplotype) which express the ovalbumin (OVA)323-339-specific TCR as a transgene [29] and are detectable by the TCR-OVA-specific anti-clonotypic antibody KJ1-26 [30]. Accordingly, DO11.10 T cells from 1 day old newborns were transferred to one day old Balb/c mice (H-2^d) and the hosts were given Ag in the form of Ig-OVA, a genetically engineered immunoglobulin (Ig) incorporating OVA323-339 within the heavy variable region, which internalizes into APCs via FcγRs and increases peptide presentation significantly [28]. The transgenic cells were then traced with KJ1-26 antibody and their primary responses were analyzed *ex vivo* [23]. The studies performed using this model revealed that both Th1 and Th2 CD4⁺ cells develop in the primary neonatal response (Figure 2). Importantly, similar results were obtained in wild type neonates [31]. However, when the animals were rechallenged with Ag as adults, only Th2 responses were observed both in the TCR transgenic system and in normal mice [23,31]. It remains to be defined whether similar unbalanced CD8 T cell responses develop in newborns.

Neonatal Th1 immunity: imprinted attributes and the inability to withstand re-challenge with antigen

The fact that neonatal primary responses are comprised of both Th1 and Th2 cells but that the secondary responses are biased towards Th2 cells raises intriguing questions as to the fate of the primary Th1 cells and the mechanism underlying their unresponsiveness during recall with Ag [23,25]. From a practical point of view, the diminished ability of the neonate to produce Th1 responses imparts vulnerability to microbial infections and the bias towards Th2 cells confers susceptibility to allergic reactions. Thus, understanding the mechanisms underlying the simultaneous Th1 unresponsiveness and robust Th2 function of the neonate represents a prerequisite for the development of pediatric vaccines and strategies against allergies [32,33]. As there is no specific surface marker which can unambiguously distinguish Th1 from Th2 cells, we used the neonate-to-neonate T cell transfer model described above to trace primary Th1 and Th2 cells and analyzed their phenotypes upon initial contact with Ag as well as their fate upon re-exposure to Ag (Figure 2) [23]. These studies yielded novel observations that significantly contributed to our understanding of the Th1:Th2 imbalance of neonatal immunity. As described above, the neonate develops both Th1 and Th2 cells in the primary response and these cells arise with similar frequencies [23]. Surprisingly, however, if the cells are recalled by Ag stimulation *in vitro* or rechallenge *in vivo*, the Th2 cells respond but the Th1 cells undergo

apoptosis (Figure 3). Intriguingly, the death of Th1 cells is driven by IL-4 produced by the Th2 secondary cells and neutralization of such IL-4 prevents Th1 apoptosis and sustains IFN γ responses [23,25]. However, IL-4 did not appear to be signaling through the conventional IL-4 receptor which is expressed by adult Th1 cells but is deficient in signaling. [34]. In the face of this dilemma, primary Th1 cells were isolated and probed by gene array for cytokine receptor expression. The findings clearly showed the specific induction of IL-13R α 1 in primary Th1 cells in response to the initial exposure to Ag (Figure 3). Furthermore, this receptor chain associates with the IL-4R α chain to form an IL-4R α /IL-13R α 1 heteroreceptor [35] through which IL-4 from the Th2 cells can trigger apoptosis of the Th1 cells, hence the Th2 bias of both recall and secondary neonatal responses [25]. Thus, developmental up-regulation of IL-13R α 1 represents an imprinted attribute of Th1 cells, leading to apoptosis instead of responsiveness to re-challenge with Ag.

Neonatal Th2 immunity: developmental-specific epigenetic marks facilitate Th2 function

Murine neonatal CD4⁺ lymph node cells produce high levels of both IL-4 and IL-13 within 24 hr of activation [24]. This rapid Th2 cytokine production is independent of cell cycle entry. In striking contrast, adult CD4⁺ T cells do not secrete substantial levels of Th2 cytokines until at least 3 days of Th2 differentiation and only after extensive proliferation. This distinction raised the question as to the mechanism underlying the capacity of neonates to rapidly produce Th2 cytokines. In adults, a great deal is known about the acquisition of high level Th2 cytokine expression during differentiation [36,37]. The *Il5*, *Il13*, and *Il4* genes are arranged in tandem within a chromosomal region referred to as the Th2 cytokine locus. In naïve adult CD4⁺ cells, the Th2 locus is in an inaccessible, quiescent state. Following activation under Th2 conditions, multiple epigenetic events occur. DNase I hypersensitivity sites appear rapidly and this is followed by histone modifications that are permissive for gene expression. Lastly, CpG residues become demethylated, initially at important regulatory regions and eventually, throughout the locus. These epigenetic modifications are thought to be essential for the acquisition of high level Th2 gene transcription. The rapid Th2 cytokine production by neonatal CD4⁺ cells might occur if the Th2 locus in neonates had pre-existing epigenetic characteristics similar to those in adult Th2 effector cells. Initial experiments indicated that there may be differential methylation of the Th2 locus in neonatal and adult T cells. The demethylating agent 5-aza-deoxycytidine greatly augmented IL-4 production by adult, but not neonatal, CD4⁺ cells, suggesting that the neonatal Th2 locus may be relatively hypomethylated [24]. Indeed, in neonates hypomethylation was observed at CNS-1, an enhancer and co-regulator of Th2 cytokine gene expression. Since demethylation of CNS-1 in developing adult Th2 cells is critical for the acquisition of high level IL-4 production [38], its hypomethylation in neonates almost certainly contributes to the rapid and robust Th2 function of neonatal CD4⁺ cells (Figure 4). Furthermore, the hypomethylated state of CNS-1 in neonates did not require homeostatic proliferation since it was hypomethylated in thymic as well as naïve peripheral CD4⁺ cells. Lastly, the hypomethylated state of CNS-1 was greatly expanded in IL-4-producing CD4⁺ thymocytes [24]. These observations indicate that neonatal Th cells are epigenetically poised for the immediate production of Th2 cytokines and the rapid development into Th2 effector cells. Naïve human cord blood Th cells also produce significant amounts of the Th2 cytokine, IL-13, [39] and DNase I hypersensitivity sites upstream and two hypomethylated regions downstream of the *IL13* gene were detected [40], suggesting that similar epigenetic features might be operative in human neonates.

The epigenetic readiness of neonates might explain the ease with which newborns develop Th2 primary responses against Ag which would otherwise be defined to trigger Th1 responses in adults. Also, since IL-4 signals apoptosis of neonatal Th1 cells through the IL-4R α /IL-13R α 1 heteroreceptor, the epigenetic readiness of the Th2 cytokine locus and its consequent rapid

production of IL-4 by neonatal Th2 cells likely contributes to the unbalanced secondary immunity in the newborn.

Sluggish maturity of accessory cells compromises neonatal Th1 immunity

The primary line of defense against microbes includes, among other mechanisms, the capture and destruction of pathogens by cells of the innate immune system. During this process fragments of microbial constituents bind to MHC molecules and the complexes are displayed on the surface of the accessory cells. A persistent display of the complexes alerts the lymphocytes of the adaptive immune system to develop a comprehensive, robust and durable immunity against microbes. Thus, the accessory cells serve as a primary line of defense and function as instructional Ag presenting cells (APC) that educate lymphocytes for the development of adaptive immune responses. In this capacity it is reasonable to envision neonatal APCs as important players in the regulation of IL-13R α 1 on Th1 cells and the consequent unbalanced neonatal immunity. Initial studies have shown that naïve T cells taken from an adult mouse, transferred and primed with Ag in a newborn mouse differentiate into Th1 cells that do not upregulate IL-13R α 1 or undergo apoptosis [23]. However, if the same naïve T cells originate from newborn instead of adult mice, the resulting Th1 lymphocytes up-regulate IL-13R α 1 and die by apoptosis during re-challenge with Ag [23]. These observations indicate that up-regulation of IL-13R α 1 on Th1 cells is developmentally regulated. Kinetic analysis of Ag-induced up-regulation of IL-13R α 1 expression indicated that the level of IL-13R α 1 was maximal on day 2 after birth and began to decline on day 3 [23]. Day 6 post-partum was a turning point when the primary Th1 cells no longer expressed IL-13R α 1 and supported the development of Th1 secondary responses when re-challenged with Ag at a later time [25]. These findings indicate that the developing primary Th1 cells gradually lose the ability to up-regulate IL-13R α 1 expression upon exposure to Ag [23,25]. Further studies have similarly highlighted the mechanisms by which developing neonatal Th1 cells “grow out of” IL-13R α 1 expression [25].

Earlier investigations have shown that secondary neonatal Th1 responses develop if exogenous IL-12 is supplied during re-challenge with Ag [13]. It was therefore hypothesized that IL-12 interferes with IL-13R α 1 up-regulation to nullify IL-4 mediated-apoptosis and sustain Th1 secondary responses [25]. This hypothesis proved correct because IL-12 suppresses both IL-13R α 1 expression and apoptosis of primary Th1 cells and sustains secondary Th1 responses (Figure 5). Since differentiation of naïve T cells into Th1 alongside Th2 cells requires IL-12 and the primary neonatal responses contain Th1 cells, some IL-12 must have been available to drive differentiation but not sufficient for suppression of IL-13R α 1 expression. This was indeed the case because by day 6 after birth, the neonates produced sufficient IL-12 and the primary Th1 cells no longer express IL-13R α 1 or undergo apoptosis, but rather support secondary Th1 responses [25]. Neonatal human cells also produce limited amounts of IL-12 [41] and this could drive similar mechanisms for biased human neonatal immunity.

It is known that dendritic cells (DCs) are the main producers of IL-12. Likewise, it is also known that neonates have lower frequencies of DCs compared to other accessory cells [28]. Thus, it is possible that the inability of the neonate to produce sufficient IL-12 is due to the reduced frequency of DCs [28] or altered function (e.g. Toll-like receptor [TLR] signaling) and/or IL-10 from neonatal B cells [42–44]. This premise was tested and when neonates were enriched with IL-12-sufficient (IL-12^{+/+}) adult splenic DCs and primed with Ag, the primary Th1 cells did not up-regulate IL-13R α 1 expression or undergo apoptosis [25]. Instead, they were able to develop secondary responses and produce their signature cytokine IFN γ (Figure 5) [25]. In fact, when the enrichment was comprised of IL-12 insufficient (IL-12^{-/-}) adult splenic DCs, the Th1 cells expressed IL-13R α 1, were apoptotic, and did not develop secondary Th1 responses. Given that murine DCs can be divided into different subsets [45], based on

their expression of CD4 and CD8, similar enrichment experiments with IL-12^{+/+} and IL-12^{-/-} cells were performed to determine the subset(s) responsible for IL-12 production in the neonate. These experiments identified the CD8 α ⁺CD4⁻ DC subset as the specific neonatal accessory cell that is responsible for limited IL-12 production and the bias of neonatal immunity towards Th2 cells [25]. Specifically, most DCs are CD8 α ⁻CD4⁻ double-negative cells with minimal CD8 α ⁺CD4⁻ and CD8 α ⁻CD4⁺ subsets. However, maturation gradually increases CD8 α ⁺CD4⁻ and CD8 α ⁻CD4⁺ populations and by day 6 after birth the accumulation of these accessory cells reaches a significant frequency [25]. At this time point the neonate becomes able to produce sufficient amounts of IL-12 to minimize expression of IL-13R α 1 on Th1 cells and promote Th1 secondary responses (Figure 5). Given that only CD8 α ⁺CD4⁻ DC were able to suppress IL-13R α 1 expression and restore Th1 secondary responses, it is likely that this subset functions as a major neonatal APC and produces sufficient IL-12 to suppress IL-13R α 1 expression and restore Th1 responses (Figure 5). Also, the delayed maturation of this subset is most likely responsible for insufficient production of IL-12 during Ag presentation. This delay leads to sustained IL-13R α 1 up-regulation on primary Th1 cells and expression of IL-4R α /IL-13R α 1 heteroreceptor (Figure 5). Upon rechallenge with Ag which stimulates both Th1 and Th2 cells, the robust IL-4 from Th2 utilizes the heteroreceptor to drive apoptosis of the activated neighboring Th1 cells and unbalance secondary neonatal immunity.

Conclusions

The neonatal model illustrated in Figure 6 highlights the points which we believe control the Th2 bias of murine neonatal immunity. Due to delayed maturation of DC, the newborn possesses a minimal number of the Ag presenting, IL-12-producing CD8 α ⁺CD4⁻ subset. Consequently, very little IL-12 is available to oppose up-regulation of IL-13R α 1 on primary Th1 cells and the readiness of the Th2 locus for prompt differentiation of Th2 cells. Rechallenge with Ag will trigger Th2 cells to produce IL-4 rapidly, which drives the death of Th1 cells through their IL-4R α /IL-13R α 1 heteroreceptor expressed during the primary response. However, by day 6 after birth significant numbers of the CD8 α ⁺CD4⁻ DC subset accumulate, leading to the production of IL-12 in sufficient quantities to counter up-regulation of IL-13R α 1 during exposure to Ag. Consequently, the Th1 cells lack the IL-4R α /IL-13R α 1 heteroreceptor and will not undergo apoptosis, but rather develop secondary responses during rechallenge with Ag. Some of the key questions remaining to be addressed include: a) the mechanism underlying up-regulation of IL-13R α 1 expression on neonatal cells and how IL-12 counters such expression, b) since adult T cells do not up-regulate IL-13R α 1 expression when primed within the neonatal environment there must be a time point and a mechanism by which T cells lose susceptibility to Ag-induced IL-13R α 1 expression, c) the pathway that triggers death of neonatal Th1 cells by IL-4 signaling through IL-4R α /IL-13R α 1 heteroreceptor, d) whether the heteroreceptor plays similar role in the development of reduced Th17 neonatal immunity [46,47], e) whether a similar heteroreceptor controls neonatal immunity in humans, and f) how the epigenetic readiness of the Th2 locus in neonates comes about. On the other hand, the observations to date raise intriguing questions as to the efficacy of pediatric vaccines and treatment of allergies in infants. Consideration should be given to compounds such as microbial TLR ligands that trigger maturation of DCs or restore activation of other APCs [48] and augment IL-12 production. This could sustain development of Th1 cells by counter regulation of IL-13R α 1 expression which could account for the Th1 neonatal immunity observed with certain microbes [18–20,49] and by opposing Th2 differentiation which would limit allergies. Finally, the role that T regulatory (Tregs) or suppressor cells might play in neonatal immunity needs to be more clearly defined [50,51] and this might help prevent their interference with the effectiveness of pediatric vaccines.

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Figure 1. Exposure to antigen at birth leads to Th2 responses that preserve tolerance

Early studies showed that when newborns are given antigen (Ag) on the day of birth and challenged with the same Ag as adults, tolerance develops (left). However, if newborn littermates are not given the Ag at birth but are challenged with the same regimen, Th1 responses develop and provide immunity (right). Subsequent studies however showed that the newborns given Ag on the day of birth and challenged with the same Ag as adults do in fact respond but rather produce Th2 responses (left panel). Since Th2 cells do not manifest inflammatory reactions to Ag like the Th1 cells, this response has been historically viewed as tolerance.

Figure 2. Primary neonatal responses are comprised of both Th1 and Th2 cells

Primary neonatal immune responses can be tracked using a neonate-to-neonate T cell receptor (TCR) transgenic adoptive transfer system. T cell receptor (TCR) transgenic T cells specific for ovalbumin (OVA)323-339 peptide are harvested from the spleens of DO11.10 neonatal mice, pooled, and transferred to Balb/c newborns to increase the frequency of responding neonatal cells. The hosts were exposed to Ag shortly thereafter and a clonotype-specific anti-TCR antibody was used to trace the responding cells for analysis of their cytokine responses to determine whether they are Th1- or Th2-type cells. This model has revealed that a balanced Th1:Th2 primary response develops in the neonates but the Th1 cells express the IL-13Rα1 chain.

Figure 3. IL-4 utilizes the IL-4 α /IL-13R α 1 heteroreceptor to drive apoptosis of Th1 cells during re-challenge with Ag and biases adult secondary immunity towards Th2 cells
 IL-13R α 1 expressed on primary Th1 cells heterodimerizes with IL-4R α and forms an IL-4 α /IL-13R α 1 heteroreceptor. When neonatally primed mice are rechallenged as adults with specific antigen (Ag), IL-4 from Th2 cells binds to the heteroreceptor on Th1 cells and drives their apoptosis resulting in a bias of secondary responses towards Th2 cells.

Figure 4. Neonatal epigenetic readiness sustains rapid production of IL-4

The neonatal Th2 cytokine locus is epigenetically poised for rapid Th2 cytokine production because CNS-1, a key Th2 regulatory region, pre-exists in a relatively hypomethylated (white circles) state in naïve neonatal CD4⁺ cells. This leads to a default state in which there is rapid production of Th2 cytokines under neutral conditions (i.e. not biased with cytokines and blocking antibodies to produce Th2 cells). In contrast, CNS-1 is hypermethylated (black circles) in naïve adult CD4⁺ cells and the production of high levels of Th2 cytokines requires longer stimulation times under Th2-promoting conditions. Thus, the pre-existing epigenetic modifications in neonates provide them with the competency to produce high levels of Th2 cytokines under neutral conditions and more rapidly than adult cells, even when the latter are activated under Th2-promoting conditions.

Figure 5. $CD8\alpha^+CD4^-$ dendritic cell subset reaches optimal frequency on day 6 after birth and produces sufficient IL-12 to suppress IL-13R α 1 up-regulation on neonatal Th1 cells
 Most dendritic cells (DCs) in newborn mice spleens are of the $CD8\alpha^-CD4^-$ phenotype. Maturation begins soon after birth and by day 6 both $CD8\alpha^+CD4^-$ and $CD8\alpha^-CD4^+$ reach optimal frequency. If antigen (Ag) is given on day 1, the low numbers of Ag presenting $CD8\alpha^+CD4^-$ DCs, results in very little IL-12 being produced. While this level of IL-12 is sufficient for Th1 differentiation, it is not enough to suppress IL-13R α 1 up-regulation on Th1 cells. By day 6 due to maturation and increase in the $CD8\alpha^+CD4^-$ DC subset, IL-12 production is augmented and sufficient to suppress IL-13R α 1 up-regulation on Th1 cells.

Figure 6. An updated model of neonatal immunity

The frequency of the $CD8\alpha^+CD4^-$ dendritic cell (DC) subset in the neonatal environment is minimal on the day of birth. Exposure to antigen (Ag) during the neonatal period leads to reduced production of IL-12 by the Ag-presenting $CD8\alpha^+CD4^-$ DCs which is not sufficient to downregulate IL-13R α 1 expression on the developing primary Th1 cells. As a result, IL-13R α 1 associates with IL-4R α to form a type II IL-4R α /IL-13R α 1 heteroreceptor. Upon rechallenge of the adult mice with the same antigen, IL-4 produced by Th2 cells signals through the heteroreceptor and causes apoptosis of Th1 cells. However, by day 6 after birth, DC maturation yields a significant number of $CD8\alpha^+CD4^-$ DCs which produce sufficient amounts of IL-12 to downregulate IL-13R α 1 expression on Th1 cells. Consequently, IL-4R α /

IL-13R α 1 heteroreceptor formation becomes minimal and IL-4 produced by Th2 cells during re-challenge with antigen can no longer drive apoptosis of Th1 cells and secondary Th1 responses develop.