

# Overcoming Dendritic Cell Tardiness to Triumph Over IL-13 Receptor: A Strategy for the Development of Effective Pediatric Vaccines

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**Abstract:** Neonatal exposure to antigen gives rise to a primary response comprising both T helper 1 (Th1) and T helper 2 (Th2) lymphocytes. However, re-encounter with the same antigen yields an indubitably biased response with minimal Th1 but excessive Th2 cells. Since Th1 cells combat microbes while Th2 cells react to allergens, the neonate faces susceptibility to both microbial infections and allergic reactions. The Th1/Th2 imbalance of neonatal immunity stems from a delayed maturation of dendritic cells that yields limited IL-12 cytokine during the neonatal stage. Th1 cells developing under these circumstances up-regulate the IL-13R $\alpha$ 1 chain that physically associates with the IL-4R $\alpha$  chain, forming a potentially hazardous heteroreceptor. During re-challenge with antigen, IL-4 from Th2 cells utilizes the heteroreceptor to signal the death of Th1 cells, leading to the Th2 bias of neonatal immunity. Our view to overcome Th1 deficiency is to supplement neonatal immunizations with toll-like receptor ligands that could stimulate maturation of dendritic cells and augment IL-12 production to counter IL-13R $\alpha$ 1 up-regulation. This regimen would yield Th1 cells devoid of the heteroreceptor and resistant to IL-4-induced apoptosis. Accordingly, the neonate

would have balanced Th1/Th2 immunity and withstand both microbes and allergens. Such approaches could open new avenues for better pediatric vaccines and allergy therapies. [*Discovery Medicine* 9(49):554-559, June 2010]

## Overview of the Neonatal Immune System

The immune system provides protection against microbes and tumors, but this function is not well adapted in the newborn, leading to high susceptibility to infections and ineffective vaccination of infants and children. Landmark experiments performed by Sir Peter Medawar and colleagues in the 1950s demonstrated that exposure to antigen, the immunogenic entity of the microbe, during the neonatal period leads to unresponsiveness or tolerance to such antigen during a later encounter (Billingham et al., 1953). These observations led to the view that the neonatal period is a window during which tolerance is feasible, and it was mostly exploited to investigate tolerance to alloantigens as it relates to organ transplantation. The initial findings on the mechanism underlying neonatal tolerance were revealed in the 1980s and suggested that deletion/inactivation of T cells is the lead factor for the unresponsiveness (Gammon et al., 1986). This postulate, however, was short lived, and investigation of the immune response in organs other than the lymph nodes, the usual site of inflammation, revealed immune responses in the spleen of mice exposed to antigen during the

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neonatal period and challenged with the same antigen as adults (Forsthuber et al., 1996; Singh et al., 1996). The perception that neonatal antigen yields tolerance then shifted to the view that the newborn is capable of developing immunity (Ridge et al., 1996; Sarzotti et al., 1996). However, cellular analyses of the responses revealed rather surprising findings as the neonatal immunity was dominated by T helper 2 (Th2) lymphocytes rather than the usual proinflammatory Th1 cells (Adkins et al., 2000; Chen and Field, 1995; Forsthuber et al., 1996; Min et al., 2000; Powell and Streilein, 1990; Singh et al., 1996). Given that Th1 cells produce mostly interferon  $\gamma$  (IFN $\gamma$ ), 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, it was logical to associate this Th1/Th2 imbalance with the neonate's vulnerability to microbial infections and allergic reactions. Despite the relevance of neonatal immunity to pediatric vaccines and control of children allergies, investigation of the mechanisms underlying the imbalance of the Th subsets was rather sluggish due to the limitations associated with the newborn (Arulanandam et al., 1999; Barrios et al., 1996). Animal models adaptable for studying the neonatal immune system have recently been developed and began to shed light on the Th2 shift of neonatal immunity (Li et al., 2004). Recent reports indicated that during the initial exposure to antigen the newborn, in fact, develops a balanced Th1/Th2 immunity (Li et al., 2004). However, re-exposure of these primary T cells to the antigen yields secondary responses that display a deficit in Th1 cells but an excess of Th2 cells. This was intriguing as the Th1 cells may have undergone a state of unresponsiveness or apoptosis during re-encounter with antigen. Indeed, recent findings indicated that antigen recognition marks the neonatal Th1, but not Th2 cells, for susceptibility to apoptosis (Li et al., 2004; Lee et al. 2008). This was the result of delayed maturation of a specific subset of antigen presenting cells (APCs) that could not provide sufficient environmental factors required to counter marking of Th1 cells for apoptosis (Li et al., 2004; Lee et al., 2008). This communication highlights the current understanding of neonatal immunity and proposes strategies to offset the deficiencies associated with maturation of APCs and improve pediatric vaccines and treatments for allergies (Andersson et al., 2004; Siegrist, 2001).

### **IL-13R $\alpha$ 1, a Death Switch for Neonatal Th1 Cells**

In neonates, like in adults, the innate immune system

serves as the first line of defense against pathogens. Upon microbial infection, innate cells of the immune system initiate a myriad of defense mechanisms devised according to pathogen associated pattern recognition molecular motifs. The receptors for these motifs, known as toll-like receptors (TLRs), serve to sense the microbe's molecular imprint and gauge a measured response to incapacitate the microbe. The consequence of the TLR-initiated response manifests in the form of an environment hostile for the survival of microbes. In most instances, the microbe is engulfed by the innate cells and fragmented by multiple enzymatic reactions. The significance of this process is multidimensional, as the pathogen is incapacitated and pieces of its proteins (or peptides) associate with major histocompatibility complex (MHC) molecules for display on the surface of the innate cells. The peptide-MHC complexes serve as an imprint for activation of microbe-specific adaptive immunity in the event of persistence. Dendritic cells are well-equipped for this task and can trigger specific immunity adapted by TLR-induced factors to counter microbial persistence effectively. For instance, bacterial or viral infections yield mostly Th1 responses while parasites and allergens trigger mostly Th2 responses. In the neonates, an initial encounter with antigen induces primary responses that include both Th1 and Th2 cells. However, when a rechallenge with the same antigen is made, the response produces mostly Th2 cells, even for antigens defined to trigger Th1 responses in adults. This is puzzling as to the fate of Th1 cells and the excess of Th2 lymphocytes and raises the possibility that the Th1 cells are incapacitated and have become anergic and/or have undergone apoptosis as a consequence of the second antigen encounter. Addition of IL-2 during the second exposure did not restore Th1 responses, thereby excluding the anergy postulate (Min et al., 1998). However, analysis of apoptotic markers indicated that Th1 cells undergo cell death during rechallenge with antigen (Li et al., 2004). This is even more intriguing because the same antigen that induced differentiation and growth of Th1 cells during the initial exposure to antigen leads to apoptosis during the second encounter of the same antigen. Since Th2 cells were maintained in the secondary responses, one would envision that these cells reconditioned the environment unfavorably for Th1 survival through exuberant production of IL-4. In fact, neutralization of IL-4 during rechallenge with antigen abolished apoptosis and restored vigorous Th1 secondary responses (Li et al., 2004). Given that Th1 cells do not express the conventional IL-4 receptor (IL-4R) (Huang and Paul, 1998), a search for a surrogate receptor was initiated and yielded a rather significant find-

ings. Indeed, the primary neonatal Th1 cells up-regulated IL-13R $\alpha$ 1 chain which was able to heterodimerize with IL-4R $\alpha$  chain and produced a type II (IL-4R $\alpha$ /IL-13R $\alpha$ 1) receptor through which both IL-4 and IL-13 cytokine can signal (Murata et al., 1998; McKenzie et al., 1999). A search for IL-13 in the sera during rechallenge with antigen revealed no measurable cytokine and this suggested that the excess of IL-4 from the Th2 cells binds to the heteroreceptor on Th1 cells and induces their apoptosis. This is a likely scenario because the Th2 cells which express the conventional IL-4R, but not the heteroreceptor, do not undergo apoptosis. In fact, blockade of the heteroreceptor by either anti-IL-4R $\alpha$  or anti-IL-13R $\alpha$ 1 antibodies nullifies apoptosis of Th1 cells in vitro (Li et al., 2004). Also, injection of either antibody during rechallenge with antigen restores significant Th1 responses. This was even more surprising as IL-4 is not viewed as an apoptotic cytokine, but blockade of the apoptotic pathway with Z-VAD peptide, a substrate analogue for caspase 3, inhibited IL-4-mediated apoptosis of Th1 cells (Li et al., 2004).

Naïve neonatal T cells do not express IL-13R $\alpha$ 1 chain, but injection of antigen which leads to differentiation of these cells to both Th1 and Th2 specifically leads to up-regulation of the chain on the former. Thus, up-regulation of IL-13R $\alpha$ 1 is antigen-dependent and restricted to Th1 cells. Furthermore, this phenomenon represents a developmental trait, as adult T cells driven by the same antigen to Th1 differentiation could not up-regulate IL-13R $\alpha$ 1. Moreover, the other fascinating aspect of this IL-13R $\alpha$ 1 imprint for Th1 death arises from the fact that IL-4 is produced by the primary Th2 cells but does not trigger apoptosis of the Th1 counterparts. Yet, during rechallenge with antigen, IL-4 drives death of the secondary Th1 cells. Given that IL-13R $\alpha$ 1 is up-regulated on the primary Th1 cells even on day 1 of antigen exposure, it is likely that rapid and robust production of IL-4 during rechallenge with antigen leads to excess cytokine that can drive death of Th1 cells. In summary, upon exposure to antigen, neonatal Th1 cells up-regulate IL-13R $\alpha$ 1 chain, which associates with IL-4R $\alpha$  to form an IL-4R $\alpha$ /IL-13R $\alpha$ 1 heteroreceptor that serves as a Th1 death switch driven by an excess of IL-4 emanating from secondary Th2 cells.

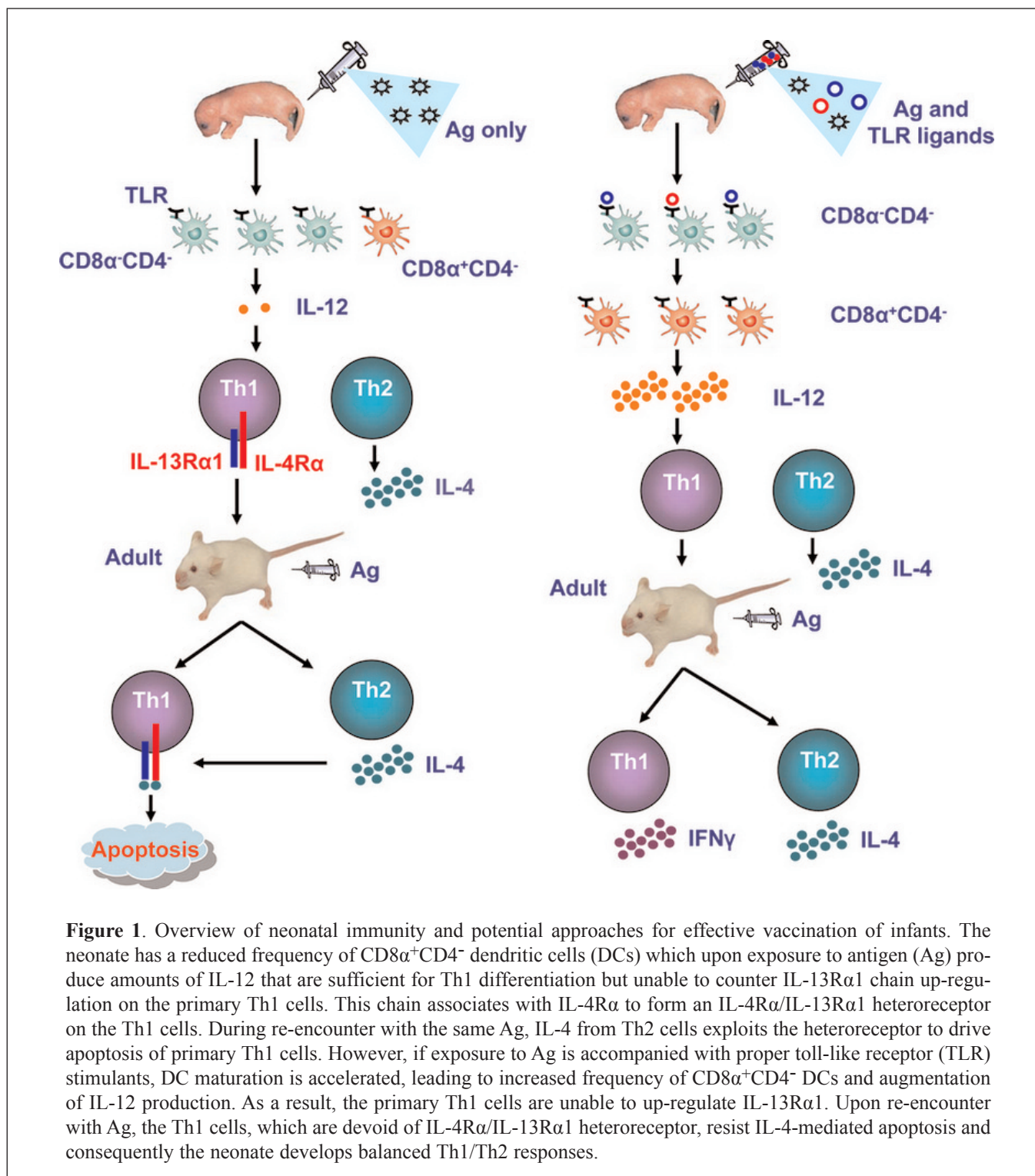
### **Delayed Maturation Undermines DC Mandate in Neonatal Immunity**

Upon exposure to antigen, both neonatal and adult naïve T cells differentiate into Th1 lymphocytes (Li et

al., 2004). Given that Th1 differentiation requires IL-12 from the presenting cells, one would assume that the neonatal environment provides sufficient cytokine for such naïve to Th1 transition to occur. The difference, though, is that neonatal Th1 cells up-regulate IL-13R $\alpha$ 1 but the adult T cells do not (Li et al., 2004). It is thus logical to envision that differential make-up of presenting cells among adult and neonatal animals may be responsible for IL-13R $\alpha$ 1 up-regulation, particularly that most of neonatal APCs are monocyte/macrophages while the adult APCs comprise more and diverse DCs with a mature phenotype (Li et al., 2001). The fact that enrichment of the neonatal environment with adult DCs nullified up-regulation of IL-13R $\alpha$ 1 by the neonatal T cells indicates that DCs, but not the abundant monocytes, are the presenting cells that can control the expression of IL-13R $\alpha$ 1 on neonatal T cells (Lee et al., 2008). This bodes well with the fact that DCs are able to produce IL-12, as transfer of IL-12-deficient DCs does not counter IL-13R $\alpha$ 1 expression on the Th1 cells. Moreover, since DCs arise as CD8 $\alpha$ <sup>-</sup>CD4<sup>-</sup> precursors that mature into CD8 $\alpha$ <sup>+</sup>CD4<sup>-</sup>, and CD8 $\alpha$ <sup>-</sup>CD4<sup>+</sup> (Shortman, 2000), we envisioned that IL-13R $\alpha$ 1 up-regulation may be the result of different presenting function among the subsets (Lee et al., 2008). Again neonatal enrichment with CD8 $\alpha$ <sup>+</sup>CD4<sup>-</sup> DCs, but not CD8 $\alpha$ <sup>-</sup>CD4<sup>+</sup> DCs nullified IL-13R $\alpha$ 1 up-regulation on newborn Th1 cells (Lee et al., 2008). On the day of birth, most of the dendritic cells display an immature CD8 $\alpha$ <sup>-</sup>CD4<sup>-</sup> phenotype, but by day 6 after birth, ongoing maturation leads to significant accumulation of CD8 $\alpha$ <sup>+</sup>CD4<sup>-</sup> and CD8 $\alpha$ <sup>-</sup>CD4<sup>+</sup> subsets (Lee et al., 2008). Coincidentally, exposure to antigen on day 6 yields neonatal Th1 cells with minimal up-regulation of IL-13R $\alpha$ 1 (Lee et al., 2008). These observations led to the conclusion that delayed maturation of DCs is responsible for the gradual accumulation of CD8 $\alpha$ <sup>+</sup>CD4<sup>-</sup> DCs which function as neonatal presenting cells (Lee et al., 2008). Primary Th1 cells develop when the frequency of CD8 $\alpha$ <sup>+</sup>CD4<sup>-</sup> DCs is limited (day 1) or optimal (day 6). The difference, though, is that day 1 Th1 cells display up-regulated IL-13R $\alpha$ 1 expression while day 6 T cells have minimal IL-13R $\alpha$ 1 expression (Lee et al., 2008). The issue then is how a higher frequency of CD8 $\alpha$ <sup>+</sup>CD4<sup>-</sup> DCs nullifies IL-13R $\alpha$ 1 expression on day 6 Th1 cells. As indicated above, the CD8 $\alpha$ <sup>+</sup>CD4<sup>-</sup> DC present the antigen and produce IL-12 to drive differentiation to Th1 cells. When low frequencies of cells are available for presentation, more antigen is loaded per DC and high avidity interactions with T cells could occur, leading to differentiation and IL-13R $\alpha$ 1 up-regulation. Alternatively, the less frequent

DCs produce lower amounts of IL-12 that suffice to drive Th1 differentiation, but not adequate to counter IL-13R $\alpha$ 1 up-regulation. Given that monocytes/macrophages as well as DCs take up the antigen, the avidity postulate may not hold true. However, by pro-

viding exogenous IL-12 on day 1 alongside exposure to antigen, the up-regulation of IL-13R $\alpha$ 1 is nullified despite the fact that the frequency of CD8 $\alpha$ <sup>+</sup>CD4<sup>-</sup> DCs remains low and antigen loading is unchanged. It is thus the quantity of IL-12 available in the neonatal



environment that controls IL-13R $\alpha$ 1 expression by the primary Th1 cells. This is supported by the observation that the amount of IL-12 was 4-fold higher on day 6 due to the increased frequency of the presenting DC subset (Lee et al., 2008). The point here is that maturation of DCs, which controls the frequency of presenting cells and their ability to produce the cytokine IL-12, regulates IL-13R $\alpha$ 1 expression. In sum, delayed maturation of DCs is responsible for low IL-12 in the environment leading to up-regulation of IL-13R $\alpha$ 1 on Th1 cells, which marks the cells for apoptosis during a later encounter with antigen.

## Conclusion

Due to susceptibility to microbial infections, the neonate is viewed as one of immunocompromised populations. However, the vulnerability of the newborn to allergic reactions presents a challenge to this stance. The imbalanced Th1/Th2 response that usually develops at the neonatal stage rationalizes these dual nuances. Indeed, as indicated in the model illustrated in Figure 1, delayed maturation of the dendritic cells leads to a diminished frequency of CD8 $\alpha$ <sup>+</sup>CD4<sup>+</sup> DCs that do present antigen but furnish the environment with sub-optimal IL-12 sufficient for Th1 differentiation, but not enough to counter IL-13R $\alpha$ 1 up-regulation (left panel). Due to heterodimerization with IL-4R $\alpha$  chain, the Th1 cells are flagged with a death IL-4R $\alpha$ /IL-13R $\alpha$ 1 heteroreceptor through which IL-4 can signal. The neighboring Th2 cells, which can expeditiously produce IL-4 upon re-encounter with antigen, readily take advantage of the heteroreceptor to drive apoptosis of the Th1 cells. The expense for the newborn is defective Th1 responses and excessive Th2 cells, hence the susceptibility of the neonate to both infections and allergic reactions. The roots for these burdens stem from the limited IL-12 in the neonatal environment which is the result of delayed maturation of dendritic cells. The fact that few vaccines are effective in the neonate suggests that the immunization compound could be hastening the maturation of presenting cells and/or triggering increased IL-12 secretion by the innate cells. In fact, in some experimental models incorporation of CpG, a TLR-9 ligand, leads to the induction of Th1 responses (Demirjian and Levy, 2009; Millan et al., 1998). It is therefore reasonable to envision that the choice of vaccines be based on strains that trigger TLR function or DC maturation. Alternatively, a more practical approach would be to supplement vaccines with defined and safe TLR ligands that could stimulate DC maturation and IL-12 production as illustrated in the

right panel of our model. An increase in the frequency of presenting cells would magnify the number of responding cells. In addition, amplified IL-12 would drive Th1 differentiation and counter IL-13R $\alpha$ 1 up-regulation. The resulting Th1 cells would not express the death IL-4R $\alpha$ /IL-13R $\alpha$ 1 heteroreceptor and resist the otherwise toxic IL-4 cytokine from the Th2 counterparts. Similar suggestions were made by others (Li et al., 2004), and the insights made on to the role of dendritic cells in neonatal immunity (Lee et al., 2008) support the idea of TLR-ligand supplemented vaccination.

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