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Neonatal T Cells: A Reinterpretation

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Abstract

Neonatal $CD4^+$ and $CD8^+$ T cells have historically been characterized as immature or defective. However, recent studies prompt a reinterpretation of the functions of neonatal T cells. Rather than a population of cells always falling short of expectations set by their adult counterparts, neonatal T cells are gaining recognition as a distinct population of lymphocytes well suited for the rapidly changing environment in early life. In this review, I will highlight new evidence indicating that neonatal T cells are not inert or less potent versions of adult T cells but instead are a broadly reactive layer of T cells poised to quickly develop into regulatory or effector cells, depending on the needs of the host. In this way, neonatal T cells are well adapted to provide fast-acting immune protection against foreign pathogens, while also sustaining tolerance to self-antigens.

Keywords

neonate; immune development; immunological memory; adaptive immunity; $CD4^+$ helper T cells; $CD8^+$ cytotoxic T cells

INTRODUCTION

Immunologists have long been fascinated by the differences between $CD4^+$ and $CD8^+$ T cell functions in early life compared to adulthood. Seminal studies by Sir Peter Medawar's group in the 1950s demonstrated that fetal exposure to antigen leads to the inactivation or absence of T cells capable of mounting an immune response (1). Since this phenomenon, referred to as neonatal tolerance (2), was only observed when antigen was introduced before or during the neonatal period of life, neonatal T cells were considered uniquely susceptible to becoming unresponsive or tolerant to specific antigens. In the 1980s and early 1990s, in vitro experiments further confirmed the view that neonatal T cells were immunodeficient, or simply immature versions of their adult counterparts, based on their reduced ability to produce IL-2 and IFN-γ following activation.

Studies performed in the late 1990s proposed that $CD4^+$ T cells in neonates were not, in fact, impaired at responding to stimulation. Instead, they preferentially made T helper type 2 (Th2) cytokines (IL-4, IL-5, IL-13), which were not measured in previous studies (3–5).

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These findings led to the idea that neonatal T cells were not immunodeficient but rather an immunodeviant version of their adult counterparts. This theory better explained why individuals were more susceptible to both infection and allergies during early stages of development (6). Also, a Th2-biased immune system in early life made teleological sense, since excessive Th1 inflammation can be detrimental to the developing fetus (7). For these reasons, the immunodeviant theory was and continues to be the widely accepted paradigm in the field, with most reviews on neonatal immunity devoting a significant amount of time to discussing the cellular and molecular studies that support it.

However, accumulating evidence suggests we are in the midst of another paradigm shift in our understanding of T cell functions in the neonatal period. A new model, supported by work from numerous labs, proposes that neonatal T cells are not simply immature versions of adult T cells with blunted or deviant responses but rather a distinct population of T cells with unique functional properties well suited to perinatal life (8–12). This significant departure from the current dogma is supported by experiments showing that neonatal and adult T cells are derived from separate progenitors and exhibit distinct gene expression profiles even prior to stimulation (8–10). In fact, neonatal T cells possess a transcriptome/epigenetic landscape that makes them *more* responsive to instructive cues after activation (9, 10, 13–15). As a result, neonatal T cells appear to be poised to establish a state of tolerance to self and allogenic antigens under nonthreatening conditions. However, in the presence of life-threatening infections, this poising allows neonatal T cells to rapidly mount an effector response, albeit at the expense of forming memory, to keep the host alive.

The central question of this review is whether existing evidence supports an entirely new model whereby neonatal $CD4^+$ and $CD8^+$ T cells are neither defective nor deficient but rather uniquely suited to the purpose of protecting the host in early life. Here, I highlight the growing evidence suggesting that neonatal T cells are a distinct population of lymphocytes programmed differently than adult T cells, attempting to reconcile the differing and sometimes conflicting studies of neonatal T cell function, as well as put the new developments into historical perspective to provide a more complete picture of the biology of neonatal T cells.

NEONATAL T CELLS ARE DERIVED FROM DISTINCT PROGENITORS

To understand the biology of neonatal T cells, it is important to first trace their developmental pathway and consider their position in the broad architecture of immune development (see the sidebar titled When Is a Mouse Neonatal?). Previous work has demonstrated that the ontogeny of the immune system does not progress in a linear manner from fetal life to adulthood. Rather, the immune system is stratified into layers of distinct immune cells that develop sequentially from distinct waves of hematopoietic stem cells (HSCs) (16–19). For many years, this model, referred to as the layered immune system model (20), was only applied to different lineages of murine γδ T cells (18, 19) and B cells (16, 17), which are functionally distinct and arise in succession. $CD4^+$ and $CD8^+$ T cells are also derived from fetal liver and adult bone marrow HSCs (21–24), but they have historically been viewed as single lineages of lymphocytes that mature only after stimulation with foreign antigen. In the last 5–10 years, however, a number of groups have found compelling

evidence (in mice and humans) to extend the layered immune system model to $CD4^+$ and $CD8^+$ T cells (8, 9, 25, 26) (Figure 1). These studies have raised the provocative idea that neonatal T cells represent a distinct lineage of cells hiding in plain sight.

The first evidence for the layered immune system model came from a seminal study done in humans by the McCune group (8). They showed that in the human $CD4^+$ T cell compartment, fetal-derived $CD4^+$ T cells proliferate more rapidly than adult-derived $CD4^+$ T cells and preferentially become regulatory T cells (Tregs). This was demonstrated using an elegant humanized mouse model, where fetal and adult stem and progenitor cells (HSPCs) were injected into SCID-hu mice following stimulation with alloantigen in vitro. The authors observed a distinct transcriptome in fetal Tregs compared to their adult counterparts, suggesting that these cells are made differently in early life. More recently, studies in neonatal mice have demonstrated the propensity for $CD4^+$ T cells to exhibit rapid proliferation and differentiation in Tregs after T cell receptor (TCR) stimulation (11). As for other lineages of $CD4^+$ T cells, Adkins performed fetal thymic transplant experiments and found that fetal-derived $CD4^+$ T cells preferentially made Th2 cytokines when stimulated with low amounts of antigen (26), which recapitulated previous observations made with $CD4^+$ T cells from humans (27, 28). Collectively, these studies have provided a basis for why $CD4^+$ T cells behave differently in early life and suggest that the Treg and Th2 paths are the default tracks of differentiation for neonatal $CD4^+$ T cells.

There is also evidence for the layered immune system model in $CD8^+$ T cells. Recently, Wang et al. (25) suggested that neonatal $CD8^+$ T cells represent a distinct lineage of cells, as they exhibit a distinct gene expression profile from the time they are created in the thymus, and they maintain this difference even after being exported into the periphery (13, 29). Other compelling evidence for the existence of developmental layers in the $CD8^+$ T cell compartment was obtained by comparing the progeny of fetal and adult precursors (25). In these studies, fetal and adult progenitors were injected into an adult thymus to generate populations of $CD8^+$ T cells that developed in the same thymic and peripheral environment. Here, the fetal-derived $CD8^+$ T cells exhibited an enhanced capacity to proliferate and gave rise to more short-lived effectors after infection, whereas the adult-derived $CD8^+$ T cells responded with slower kinetics but gave rise to more memory $CD8^+$ T cells. Consistent with results observed in mice, purified subsets of naive $CD8^+$ T cells from newborn humans have also been shown to divide sooner than those from adults after in vitro stimulation (30).

The enhanced capacity of neonatal $CD4^+$ and $CD8^+$ T cells (both human and mouse) to divide after stimulation is reminiscent of other immune cells derived from fetal HSCs, such as B1 B cells. However, a question remains: Why would the immune system retain fetal HSCs during evolution to provide the host with both $CD4^+$ T cells with a bias toward becoming Tregs/Th2 cells and $CD8^+$ T cells with an inherent propensity for generating effector cytotoxic T lymphocytes? One way to reconcile these seemingly dichotomous findings is to consider the differences in experimental design of the $CD4^+$ and $CD8^+$ T cell studies. In the case of $CD4^+$ T cells, the readout for their behavior has typically been examined under noninflammatory conditions (e.g., anti-CD3/CD28, alloantigens), whereas most of the $CD8^+$ T cell work has been performed in mouse models of infections with pathogenic microbes (e.g., vaccinia virus, *Listeria monocytogenes*). Thus, it is possible that

the different fates displayed by neonatal $CD4^+$ and $CD8^+$ T cells have less to do with cell type and more to do with the different conditions under which they were stimulated. Indeed, addition of IL-12 to in vitro cultures enabled neonatal $CD4^+$ T cells to produce adult-like levels of IFN-γ (31), and vaccination of neonates with the more inflammatory BCG and whole-cell pertussis vaccines resulted in adult-like Th1 responses (32, 33). Moreover, neonatal $CD8^+$ T cells differentiate into Tregs when exposed to self-antigens in the periphery (34). Thus, it is possible that the immune system has retained fetal HSCs to generate a layer of fast-acting $CD4^+$ and $CD8^+$ T cells that can more quickly differentiate into the cells most useful to the host, depending on the circumstances in which they were primed.

NEONATAL T CELLS PERSIST INTO ADULTHOOD AND RETAIN THEIR CELL-INTRINSIC PROPERTIES

An important technological advancement that has changed our perception of neonatal T cells is the development of fate-mapping mouse models. In the past, the study of neonatal T cells was limited to how they respond in early life. However, we now have the ability to permanently label $CD4^+$ and $CD8^+$ T cells produced in neonatal mice and examine their behavior in adulthood. Studies using fate-mapping models demonstrate that the enhanced ability of neonatal T cells to rapidly differentiate into effector and regulatory cells are of significant value to the host later in life. Thus, rather than dispensing of neonatal T cells altogether, the immune system maintains this developmental layer in adulthood to serve as early effectors against foreign pathogens and sustain tolerance to self-antigens.

In the case of $CD4^+$ T cells, Yang et al. (35) used a fate-mapping model involving Foxp3-driven tamoxifen-inducible Cre (Foxp3cre-ERT2) mice to label a wave of Tregs produced at either 0–10 days of age or 35–45 days of age. They found that Tregs produced in early life are stably maintained in adulthood and exhibit a unique transcriptome. Interestingly, the genes upregulated in neonatal-derived Tregs were associated with Treg function and cell division, consistent with their more potent suppressive activity and enhanced ability to proliferate. Moreover, adoptive transfer of neonatal-derived Tregs, but not adult-derived Tregs, into newborn $Aire^{-/-}$ mice inhibited the progression of multi-organ autoimmune disease. Therefore, Tregs produced during a specific ontogenic window in early life have unique functions and are required for maintaining self-tolerance. Although more work is required to translate such findings to humans, Mold et al. (36) found evidence to suggest that human Tregs made against maternal antigens in utero persist into at least the teenage years.

For neonatal $CD8^+$ T cells, Smith et al. developed a strategy using mice with CD4-driven tamoxifen-inducible Cre (CD4cre-ERT2) to induce expression of TdTomato and permanently label or time-stamp a wave of $CD8^+$ T cells made in the thymus at the time of tamoxifen exposure (9). Using this approach, they found that $CD8^+$ T cells made near birth persist into adulthood and express higher levels of effector genes prior to stimulation, whereas $CD8^+$ T cells made later in life were enriched for genes found in naive cells. In vitro studies also demonstrated that neonatal-derived $CD8^+$ T cells retained their enhanced capacity to proliferate after TCR stimulation and underwent bystander activation in response

to innate cytokines (IL-12 and IL-18). Following infection, neonatal $CD8^+$ T cells still gave rise to terminally differentiated effectors, but $CD8^+$ T cells made later in life preferentially gave rise to long-lived memory cells. In fact, the first cells made during early stages of development were the first cells to respond to infection and become effectors in adulthood. These studies, along with recent work done by Reynaldi et al. (37), suggest that age-related differences in the $CD8^+$ T cell response to infection may be linked to the developmental composition of cells in the starting population. Collectively, these data demonstrate that neonatal $CD4^+$ and $CD8^+$ T cells are maintained in adulthood as a distinct developmental layer and have important roles in mediating immune homeostasis and protection (see sidebar titled Do Neonatal T Cells Represent a Distinct Lineage of Lymphocytes?).

NEONATAL T CELLS ARE POISED FOR RAPID DIFFERENTIATION

Another major factor that has changed our perception of neonatal T cells is the rapid growth of next-generation sequencing. In the past, immunologists tested hypotheses based upon their experience, instincts, and published data, focusing on single genes (e.g., *Nfat*) at a time. Now, with the advent of new technology, we have the ability to examine the entire transcriptome in equivalent populations of neonatal and adult T cells in an unbiased manner. Using this new technology, recent studies have shown that neonatal cells are poised for rapid differentiation (9, 10, 13–15, 25). For example, neonatal murine $CD8^+$ T cells have been shown to express more effector-like genes during thymic development (25), suggesting they follow a developmental trajectory similar to innate-like lymphocytes, such as natural killer (NK) cells, mucosa-associated invariant T (MAIT) cells, and γδ T cells, which often do not exist in a classical naive state (46). Naive neonatal $CD8^+$ T cells in the periphery exhibit a more effector-like chromatin landscape, with increased accessibility to genes that favor effector cell differentiation (9). They are essentially hardwired for rapid proliferation and differentiation. In contrast, adult T cells express genes typically associated with naive T cells (9, 25). This naive state in adult T cells is likely important for internalizing contextual information from antigen-presenting cells in the lymph node and may leave open more differentiation pathways for them to travel down. However, increased plasticity comes at the cost of time, which is a luxury that likely cannot be afforded in early life.

Mechanistically, recent work suggests that naive $CD4^+$ and $CD8^+$ T cells are transcriptionally shifted to a more effector-like state in early life because of developmental differences in miRNA expression (13, 14) (Figure 2). The miRNAs that are preferentially expressed in adult T cells may allow them to exist in a naive state by actively suppressing genes involved in differentiation. For example, in adult $CD8^+$ T cells (both mice and humans), miR-29 is enriched and represses drivers of effector cell differentiation (e.g., T-bet, Eomes) (13, 14). By contrast, in neonatal T cells, lower expression of miR-29 positions them further along the effector cell differentiation continuum and potentially explains their inherent bias toward the short-lived effector lineage after infection. Another miRNA that is upregulated in adult T cells and helps to maintain their naive phenotype is let-7 (14, 25, 47). The let-7 family of miRNAs is downregulated in fetal-derived T cells because its expression is blocked by a developmentally regulated RNA-binding protein (Lin28b) in fetal HSCs (25, 44, 48), creating a metabolic profile highly conducive to rapid growth and proliferation (47, 49, 50). A number of papers have suggested that Lin28b may be responsible for the

developmental switch between neonatal and adult T cell functions. Overexpression of Lin28b in adult murine $CD8^+$ T cells, for example, results in reduced levels of let-7 expression and increased terminal differentiation after infection, similar to neonatal $CD8^+$ T cells (25). Likewise, inhibiting Lin28b in human fetal $CD4^+$ T cells leads to the upregulation of let-7 and a reduced ability to differentiate into $FOXP3^+CD25^+$ Tregs, akin to adult $CD4^+$ T cells (51).

There are also examples of miRNAs that are upregulated in neonatal T cells (13, 14), which may keep them in a more activated state. For example, miR-181 expression plays an important role in tuning the threshold for TCR signaling during T cell development by limiting expression of multiple phosphatases (52). Thus, its expression correlates with TCR sensitivity. During T cell development, miR-181 expression is highest in $CD4^+CD8^+$ double-positive thymocytes to facilitate interaction with low-affinity ligands but is then downregulated to increase the TCR activation threshold in mature naive T cells. Neonatal $CD4^+$ and $CD8^+$ T cells express elevated amounts of miR-181 (13–15), leaving them more sensitive to TCR activation. Consistent with this idea, Palin et al. (15) reported that increased expression of miR-181 in cord blood $CD4^+$ T cells is responsible for their enhanced calcium signaling and higher Erk phosphorylation after TCR activation. In contrast, $CD4^+$ T cells from older individuals exhibit low levels of miR-181 and reduced Erk phosphorylation after TCR stimulation (53). Expression of miR-181 is also increased in tolerized T cells, as shown by Schietinger et al. (54). Thus, higher expression of miR-181 in naive neonatal T cells provides them with higher TCR sensitivity, which may enable them to respond more strongly to both self-antigens and foreign antigens.

Why would it be advantageous for neonatal T cells to exist in a more effector-like state? It seems counterintuitive that neonatal T cells are transcriptionally and epigenetically poised for differentiation during a time when they need to limit autoimmune reactions and be flexible in how they respond to different types of antigens. One possibility is that neonatal T cells are more traveled along the axis of differentiation and have a lower threshold for activation, so that they can more quickly become tolerized or activated depending on the signals encountered during priming. For example, if neonatal T cells are primed via the TCR with antigen alone, they will quickly become tolerized. However, if costimulation and inflammatory cytokines are present, neonatal T cells will rapidly differentiate into short-lived effectors. In this way, neonatal T cells are poised for both activation and tolerance, ensuring that they quickly give rise to the types of T cells most beneficial for the host.

NEONATAL T CELLS USE MORE BROADLY REACTIVE TCRs

It is well known that the recombination program for generating TCRs is different in early life (40, 55–58). In the past, the focus was on how neonatal T cells had a more restricted TCR repertoire (59), which supported the idea that neonatal T cells were immunodeficient. However, recent studies have shown that the neonatal TCR repertoire is also biased toward self-reactive TCRs, which has important bearing on how we interpret their functions. Support for a more self-reactive TCR repertoire in neonatal T cells comes from studies done in mice (60) and humans (61) that show higher amounts of CD5, a marker of TCR avidity for self-pMHC molecules, on neonatal T cells compared to their adult counterparts. These

findings are significant because the affinity between TCRs and self-pMHC molecules influences their ability to undergo homeostatic proliferation and react to foreign antigens. For example, $CD5^{hi}$ cells are able to outcompete $CD5^{lo}$ cells for self-pMHC trophic signals and proliferate more rapidly under homeostatic conditions (62, 63). Studies by Fulton et al. (64) have also demonstrated that $CD5^{hi}$ cells have enhanced reactivity to inflammatory cues and are poised to respond to infection, akin to neonatal T cells. These data are consistent with studies looking at B1 B cells, which also express a more self-reactive repertoire and respond rapidly to infection (65). In this way, the usage of different TCRs may help promote, rather than limit, immune functionality in early life.

Neonatal and adult T cells also express different TCRs due to a delay in expression of TdT, the enzyme responsible for insertion of random N-additions (55, 56). As a result, the TCR repertoire in neonatal mice is less diverse and comprised of more germ line–encoded clonotypes (66–70). It was initially assumed that TCR repertoire diversity was restricted in early life to limit pathogenic T cell responses during critical periods of growth and development. However, when Mathis and colleagues examined immune competence in adult mice lacking TdT expression, they found no major immune defects in TdT knockout mice following immunization or infection with several different viral pathogens (71). Gavin & Bevan (72) then proposed that TdT-deficient mice were not more susceptible to infection because germ line–encoded clonotypes were more cross-reactive. In their study, they showed that $CD8^+$ T cell clonotypes from TdT-deficient mice were more peptide promiscuous and capable of responding to many more different peptides than wild-type clonotypes. It appears, therefore, that restriction of TdT expression in early life benefits the host by providing the fetus/neonate with broader recognition capabilities during a time when fewer T cells are present.

Is there a cost to having a more peptide-promiscuous or self-reactive repertoire in early life? To answer this question, a number of groups have crossed TdT knockout mice to different strains of autoimmune-prone mice (73–75). Instead of an increase in autoimmune pathology, the TdT knockout mice had a lower incidence of autoimmune disease. The authors suggested that the lack of TdT conferred protection against autoimmune disease because TdT knockout mice had a more restricted TCR repertoire. However, in the presence of infection, the more peptide-promiscuous repertoire may be sufficient to elicit a protective response. Another possibility is that TdT knockout mice have a lower incidence of autoimmune disease because the germ line–encoded clonotypes are more prone to tolerance. In fact, previous studies have demonstrated that shorter TCRs are more easily tolerized (76, 77). Although this work was done in adult T cells, one recent study used an autoimmune mouse model to demonstrate that neonatal T cells are indeed more susceptible to tolerance than adult T cells (78). Thus, an additional evolutionary benefit to delaying TdT expression in early life may be that it enables the host to more rapidly fill the peripheral pool (79) with T cells best equipped to counter the many different types of antigens (self, commensal, pathogen) encountered before and after birth.

NEONATAL T CELLS HAVE AN ENHANCED ABILITY TO RESPOND TO INFLAMMATION AND DANGER SIGNALS

Our understanding of neonatal T cells has been shaped by the use of assays and metrics that work best for assessing the functions of adult T cells. Indeed, the initial characterization of neonatal T cell behavior was based largely on in vitro studies comparing the ability of cord blood T cells and adult peripheral blood T cells to produce IFN-γ after stimulation via the TCR. However, recent studies suggest that neonatal T cells may be less dependent on TCR recognition and instead have an enhanced ability to respond to inflammation and danger signals (Figure 3). For example, there is an antigen-inexperienced population of $CD8^+$ T cells (denoted virtual memory cells) that preferentially develop from fetal progenitors and can produce IFN-γ in response to innate cytokines alone (e.g., IL-12 and IL-18) (9, 25). These findings put (some) naive neonatal $CD8^+$ T cells in the same camp as other innate-like lymphocytes, such as MAIT cells, NK T (NKT) cells, γδ T cells, innate lymphoid cells, and NK cells, all of which can be activated by cytokines alone. Second, $CD4^+$ and $CD8^+$ T cells express NK cell receptors in early life (9, 25, 29, 80), some of which (CD161) have been recently shown to exhibit important regulatory functions (80). Third, neonatal $CD8^+$ T cells are capable of responding to pathogen-associated molecular patterns (PAMPs) via Toll-like receptors (TLRs). For example, human $CD8^+$ T cells isolated from cord blood have been shown to express TLR2 and TLR5, and stimulation with their respective ligands (Pam3Cys and flagellin) results in increased proliferation and production of IFN-γ (81, 82). Human neonatal $CD4^+$ T cells also respond to stimulation with TLR1/2 ligands (83, 84), raising the possibility that recognition of PAMPs may be a general feature of neonatal T cells.

Neonatal T cells may also rapidly deploy nonspecific defense mechanisms. In line with this idea, Galindo-Albarran et al. (82) found that naive $CD8^+$ T cells ($CD45RA^+CD45RO^-$) in cord blood exhibit a unique gene expression profile and chromatin landscape, which are biased in favor of innate immune response genes. Among the transcripts that are upregulated in cord blood $CD8^+$ T cells, many of them have known roles in the neutrophil activation response (e.g., antimicrobial peptides, chemokines, reactive oxygen species production) (82). These findings may help explain how neonatal $CD8^+$ T cells are able to provide innate-like immune protection against extracellular pathogens in early life (85). Neonatal $CD4^+$ T cells from human newborns have also been shown to express complement receptors (CR1 and CR2) and preferentially make IL-8 (CXCL8) after stimulation (12, 86–88). The discovery of IL-8 production by neonatal $CD4^+$ T cells was particularly surprising because this chemokine is typically made by innate immune cells (e.g., macrophages) or structural cells (e.g., epithelial cells, endothelial cells) and plays an important role in the recruitment and activation of neutrophils and γδ T cells. The secretion of IL-8 by neonatal $CD4^+$ T cells is elicited by TCR stimulation, though its production can be further enhanced by costimulation with flagellin (12). This attribute is reminiscent of other innate lymphocytes, such as marginal zone B cells, where signals generated from dual TLR and B cell receptor stimulation lead to greater amounts of antibodies (89). Lastly, ~25–30% of cord blood $CD4^+$ T cells secrete IL-8 after stimulation (12), which likely relates to their recent thymic emigrant (RTE) status and previous amounts of homeostatic proliferation (86–88). Since levels of postthymic maturation and homeostatic proliferation differ among individuals,

there is some hope that variability in IL-8 production by neonatal $CD4^+$ T cells can be used to predict future clinical outcomes (90).

One possible explanation for why neonatal T cells are biased toward recognizing danger and inflammatory signals, while adult T cells appear to be more reliant on TCR signaling for activation, relates to the different environments present during neonatal and adult T cell development. Neonatal T cells develop in a relatively sterile environment but are later exposed to both commensal antigens (during microbiome colonization) and antigens from pathogens. Since there are no intrinsic molecular features of the microbes themselves to allow T cells (or TCRs) to discriminate commensals from pathogens, the extent of TCR recognition of these foreign antigens is not helpful in determining the necessary response. In this context, danger signals in the form of tissue damage (91, 92) may be more useful for discriminating between friend and foe. By contrast, adult T cells are exposed to commensal antigens during development and develop peripheral tolerance. For these cells, commensal tolerance is acquired during maturation, and thus recognition of the foreignness of antigens by the TCR is a more reliable indicator of recent and pathogenic infection. In this way, the changing bias toward inflammatory versus TCR signals could be viewed as a useful adaptation for the different types of antigens encountered by T cells at various stages of life. If we accept that neonatal T cells are not defective but rather more danger responsive and hyperfunctional compared to adult cells in responding to inflammatory stimuli, we may be able to develop more neonate-appropriate assays to better assess their functional abilities.

NEONATAL T CELLS ARE OPTIMALLY POSITIONED FOR RAPID RESPONSES

Some of the functions of neonatal T cells have likely been overlooked because past studies have focused on T cells in the blood and lymphoid tissue. However, there is now a gaining appreciation for the roles of neonatal T cells in peripheral organs. The current dogma for conventional T cell recirculation is that naive T cells are confined to the blood and lymphatics, whereas memory cells are able to recirculate throughout the peripheral organs (93, 94). Yet these rules do not appear to apply to T cells in fetal life. Using a sheep model, Mackay et al. (95, 96) elegantly showed that naive T cells circulate throughout extralymphoid tissues during normal development of the fetal immune system. Additional studies in mice have confirmed these findings and demonstrated that peripheral organs, such as the skin (97), lung (98), and liver (9), are selectively seeded by neonatal T cells in the first few weeks of life. Although the intestine was not examined in the aforementioned studies, other reports have shown that neonatal T cells express higher levels of gut homing receptors and preferentially traffic to the small intestine (99), placing them alongside other innate-like T cells, such as $\gamma\delta$ T cells, intraepithelial lymphocytes, and MAIT cells. The propensity for neonatal T cells to localize in peripheral organs may suggest a role in organogenesis (100, 101) and help to explain why it is advantageous for them to express innate receptors in early life. In addition, the tolerance-prone neonatal T cells are ideally positioned to encounter a wide range of self-and commensal-derived antigens.

Do neonatal T cells exhibit distinct tissue distribution patterns in humans? Given the difficulty of sampling tissues from humans, our knowledge of how T cells populate peripheral organs has historically been sparse. However, key insights can be gleaned from a recent study by Farber's group comparing the phenotypes of $CD4^+$ and $CD8^+$ T cells in tissues from pediatric (0–2 y of age) and adult (>15 y of age) organ donors (102). The authors found an elevated frequency of naive $CD4^+$ and $CD8^+$ T cells across all pediatric tissues, including the lung and intestine. Also, the pediatric tissues were comprised of a high frequency of Tregs (30–40%), which declined to a relatively low number in adulthood (1–10%). Interestingly, depletion of Tregs in infant samples allowed the remaining $CD4^+$ and $CD8^+$ T cells to undergo adult-like levels of proliferation and produce significantly more cytokines after stimulation. This finding is particularly notable because previous studies have compared the behavior of neonatal and adult T cells in bulk populations and reported that neonatal T cells are hypofunctional compared to adult T cells. However, the study by Thome et al. (102), as well as other studies in mice (103), indicate that rather than being intrinsically defective, neonatal T cells may simply be more suppressed than their adult counterparts. Such data illustrate not only how location shapes immune function but also the importance of comparing purified subsets of naive T cells from neonates and adults when studying their cell-intrinsic differences.

NEONATAL T CELLS ARE IMPAIRED AT FORMING MEMORY T CELLS—A USEFUL ADAPTATION?

Our understanding of the neonatal T cell response has evolved with the use of more refined models of infection. In the past, the strategy was to directly infect neonatal and adult mice and compare the numbers and function of T cells present in both groups at various times after infection. However, it was often unclear whether the altered behavior of neonatal T cells was due to a less diverse TCR repertoire, smaller numbers of precursor cells, or an altered priming environment. Thus, a number of groups have started using adoptive cotransfer experiments to control for age-related factors when identifying cell-intrinsic differences in the neonatal T cell response to infection. These studies suggest that the ability of neonatal T cells to rapidly proliferate and respond to innate signals may come at the cost of forming memory.

In the case of $CD8^+$ T cells, Smith et al. (29) used an experimental strategy in which equal numbers of monoclonal $CD8^+$ T cells from neonatal and adult donor TCR transgenic mice were transferred into the same host prior to systemic infection with vaccinia virus or a virulent strain of *L. monocytogenes*. Neonatal donor $CD8^+$ T cells responded more quickly to infection and peaked sooner than adult donor cells but preferentially gave rise to short-lived effector cells and failed to transition into the long-lived memory pool. In contrast, the adult donor cells took longer to enter the proliferative response but preferentially became memory cells capable of responding to secondary infections. Thus, even when neonatal $CD8^+$ T cells were placed in an adult environment and provided with all the signals that adult $CD8^+$ T cells have during infection, they still rapidly proliferated and failed to form memory. Similarly, Siefker & Adkins (85) found that neonatal $CD8^+$ T cells rapidly expand during the innate phase of the response to an extracellular bacterial enteropathogen (*Yersinia*

enterocolitica), but they were not required for immune protection against secondary infection. Together, these papers suggest that the dominant function of neonatal $CD8^+$ T cells is to provide an early innate-like response during primary infections.

Neonatal $CD4^+$ T cells have also been shown to be intrinsically defective at forming memory. Using a mouse model of influenza infection, Zens et al. (10) observed a reduced number of memory $CD4^+$ T cells (and $CD8^+$ T cells) in the lungs of mice infected as infants compared to those infected as adults. The reduction of neonatal memory $CD4^+$ T cells in the lung was not due to a lack of responsiveness or proliferation but rather to an inherent propensity to become terminally differentiated and more rapidly lose their potential to transition into the long-lived tissue-resident memory pool. Importantly, adoptive transfer experiments demonstrated that the failure of neonatal $CD4^+$ T cells to persist in the lung during the memory phase of infection is due to cell-intrinsic differences and is not a function of the infant environment. These findings are reminiscent of the cell-intrinsic differences observed in $CD8^+$ T cells, suggesting that the propensity to quickly differentiate into short-lived effectors may be a general feature of neonatal T cells. From an evolutionary point of view, it is likely more beneficial for the neonate to mount a vigorous T cell response to infection than to develop immunological memory, since memory T cells are unimportant if the host fails to survive the initial infection. Also, since the neonatal T cell repertoire is extremely limited, likely it is of lesser value in the memory pool, as higher-avidity adult T cells will be made later in life, reducing the need for neonatal memory.

Compelling evidence suggests that similar principles apply to neonatal T cells in humans. For example, a study of infants experiencing viral respiratory tract infections reported an accumulation of more terminally differentiated $CD8^+$ T cells (effector memory RA^+ T cells, or TEMRAs) in the lungs of younger patients, whereas the less-differentiated tissue-resident memory $CD8^+$ T cells were more prevalent in older children (104), potentially explaining why individuals are more susceptible to repeat infections with intracellular pathogens in early life (105). There is also evidence to suggest that infants are more susceptible to persistent infection (e.g., cytomegalovirus) because neonatal $CD8^+$ T cells have an inherent propensity to become terminally differentiated and functionally exhausted (106).

In the future, it will be important to determine whether any routes of inoculation or priming conditions are favorable for the generation of neonatal memory T cells (see sidebar titled How Do Neonatal T Cells Perform in Clinical Settings?). Notably, vaccination of neonatal mice with an attenuated bacterium (ActA LM) (107) or lower doses of viral vectors (108, 109) resulted in more robust immune protection, suggesting that lower amounts of inflammation and antigen may be required for the optimal development of memory $CD8^+$ T cells in early life. Also, immunization of neonatal mice with LAIV elicits more robust immune protection compared to IIV-vaccinated mice (10), which may relate to differences in the types of memory cells generated by different modes of immunization. Collectively, these studies indicate that careful consideration of the priming conditions and routes of inoculation will need to be applied to promote durable T cell immunity in early life.

CONCLUSION AND FUTURE PERSPECTIVES

Overwhelming evidence suggests that neonatal T cells can no longer be considered immature versions of adult cells. In fact, our understanding of the biology of neonatal T cells has likely been hampered by comparing neonatal T cells to their adult counterparts. If we instead consider the developmental biology of the immune system and compare neonatal T cells to other immune cells made during the same ontogenic window, their unique functions become clearer. Like other fetal-derived lymphocytes (B1 B cells, DETCs), neonatal T cells are a distinct developmental layer of T cells evolved to perform a specialized role for the host. They are a broadly reactive layer of T cells created with a preexisting effector-like state and an ability to migrate to peripheral tissues, which enables them to quickly develop into regulatory or effector cells, depending on the needs of the host. In this way, they resemble other fast-acting innate-like populations of lymphocytes made in early life and are therefore less similar to adult T cells, which exist in a more naive state in the lymphoid tissue and respond with slower kinetics.

Viewing neonatal T cells as a discrete ontogenic layer raises a number of critical questions. First, how do environmental (e.g., microbiome, diet) and genetic factors alter the persistence of neonatal T cells in adulthood, particularly in humans? A large number of studies have suggested that early-life microbial exposures can permanently program the offspring's immune system and life-long disease risk (117–120), but whether this is accomplished by altering the developmental layering in the T cell compartment remains unclear. Second, can we predict infection outcomes and disease risk (allergies, autoimmunity) based upon the ratio of fetal-to adult-derived T cells present in the starting pool? Given that neonatal and adult T cells are long-lived and exhibit different cell-intrinsic properties, some of the interindividual variation is likely related to the layering variation of the T cell compartment. Third, can we purify or target certain subsets of naive T cells for specific therapeutic interventions? The fast-acting neonatal $CD8^+$ T cells that persist into adulthood possess many features that could be highly desirable in the context of cancer immunotherapy. Understanding which developmental layers of T cells are associated with favorable or pathological outcomes will be essential to answering this question.

There has never been a more exciting time to study neonatal T cells. We now have state-of-the-art tools to examine their behavior in a more systematic and unbiased manner at a level never before possible, leaving the future open for discovery. These studies will broaden our fundamental understanding of neonatal T cells and potentially lead to new approaches for treating and preventing human disease.

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WHEN IS A MOUSE NEONATAL?

In humans, the neonatal period extends from birth through the first month of life. In mice, the neonatal period is different, and there is often debate around finding equivalent stages of immune development in mice and humans. What we do know is that neonatal T cells in mice and humans are sculpted by the same mechanisms of immune development. Both are derived from fetal progenitors, comprised of more recent thymic emigrants, exhibit a less diverse TCR repertoire, and undergo more homeostatic proliferation. Mice have proven to be enormously helpful in providing key mechanistic insight into the biology of neonatal T cells. However, differences in the timing of key developmental events do exist between mice and humans and have led to the question of what age mice most closely resemble newborn humans. $CD4^+$ and $CD8^+$ T cells can be identified in humans at ~14 weeks gestation, for example, but sufficient numbers of peripheral T cells do not emerge in mice until 5–7 days after birth. Diversification of the TCR repertoire and the developmental switch in lymphopoiesis happen in the second trimester for humans, but they occur after birth in mice. Essentially, mice are born with a less developed immune system than humans, which is why most neonatal studies in mice are performed in 1-to 2-week-old pups.

DO NEONATAL T CELLS REPRESENT A DISTINCT LINEAGE OF LYMPHOCYTES?

Heterogeneity is a hallmark feature of the adaptive immune system in vertebrates. For B cells, separate lineages in the starting population are made during distinct windows of development and differentiate along different pathways during infection. B1 B cells are generated from fetal progenitors and are preferentially found in newborn mice (17, 38). B2 B cells arise later in life and cannot be efficiently made from fetal progenitors. These two lineages of B cells are identified by their expression of CD5 (Ly-1) (16). Is a similar developmental architecture in place for conventional $CD4^+$ and $CD8^+$ T cells? In mice, both neonatal T cells and B1 B cells are derived from fetal HSCs and express broadly reactive lymphocyte receptors that are more germ line encoded (39, 40). Prior to stimulation, both B1 B cells and neonatal T cells express a distinct transcriptome and preferentially migrate to different compartments of the body than their adult counterparts (8, 25, 41–44). Neonatal T cells and B1 B cells also exhibit more immunoregulatory properties and preferentially respond during early stages of infection, while the B2 B cells and adult T cells exhibit slower kinetics and have an enhanced ability to persist into the memory phase (9, 29, 45). Finally, neonatal T cells persist into adulthood and retain their cell-intrinsic properties (9, 35, 37), similar to B1 B cells. If neonatal T cells have a separate developmental pathway, express different genes, and exhibit distinct functional properties, why are they not considered a separate lineage of cells, akin to B1 B cells? The answer may be that no surface marker or receptor currently exists to distinguish a neonatal $CD4^+$ or $CD8^+$ T cell from its adult counterpart. Identification of a unique marker for neonatal T cells would revolutionize the field in much the same way that identification of CD5 changed our perspective of B cells.

HOW DO NEONATAL T CELLS PERFORM IN CLINICAL SETTINGS?

The unique functional attributes of neonatal T cells have not gone unnoticed by cancer researchers. Treatment of aggressive forms of hematological malignancies often requires the reconstitution of the host immune system with hematopoietic stem cells. The more tolerant-prone and fast-acting neonatal T cells are ideally suited for cancer immunotherapy. Compared to transplantations with adult bone marrow, cord blood transplantations require less stringent HLA matching and have a decreased risk of graft-versus-host disease, likely due to protolerogenic cord-derived cells (110). Cord blood T cells have been shown to rapidly expand in the periphery and mediate better graft-antitumor responses than adult-derived T cells (110–112). The precursor frequency of T cells specific for some tumor-associated antigens (e.g., PR1) is also significantly higher in cord blood compared to adult peripheral blood mononuclear cells, which may be due to incomplete central tolerance in early life (113). In addition, cord blood T cells have been found to recognize a broad pool of unconventional cytomegalovirus (CMV) epitopes, which may prove useful for preventing reactivation of CMV after transplantation (114). Lastly, cord blood T cells undergo more vigorous proliferation in the presence of IL-7 and IL-15 (115), generating a large source of long-lived memory stem T cells, which are ideal candidates for adoptive immunotherapies (116). Better understanding of the biology of neonatal T cells may ultimately direct us to more effective therapeutic strategies for treating cancer in adults.

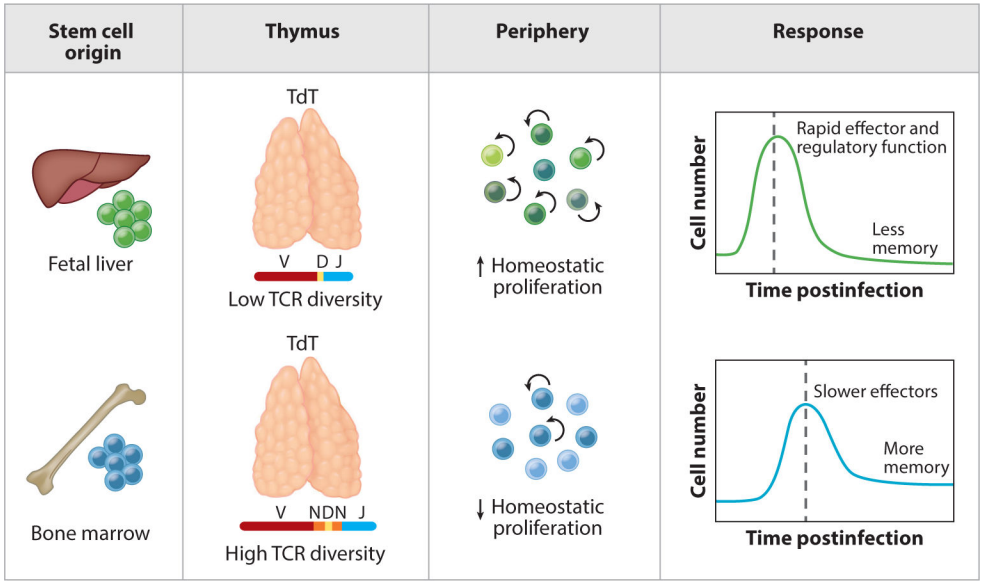

Figure 1.

Neonatal and adult T cells have different origins and functions. This figure depicts the layered immune system model for $CD4^+$ and $CD8^+$ T cells. Unlike adult T cells, neonatal T cells are derived from fetal hematopoietic stem cells, exhibit shorter and more restricted T cell receptors in the absence of TdT, and undergo higher rates of homeostatic proliferation in the periphery. Following stimulation, neonatal T cells more quickly differentiate into effector or regulatory T cells than their adult counterparts, albeit at the expense of forming long-lived memory cells. Abbreviation: TCR, T cell receptor.

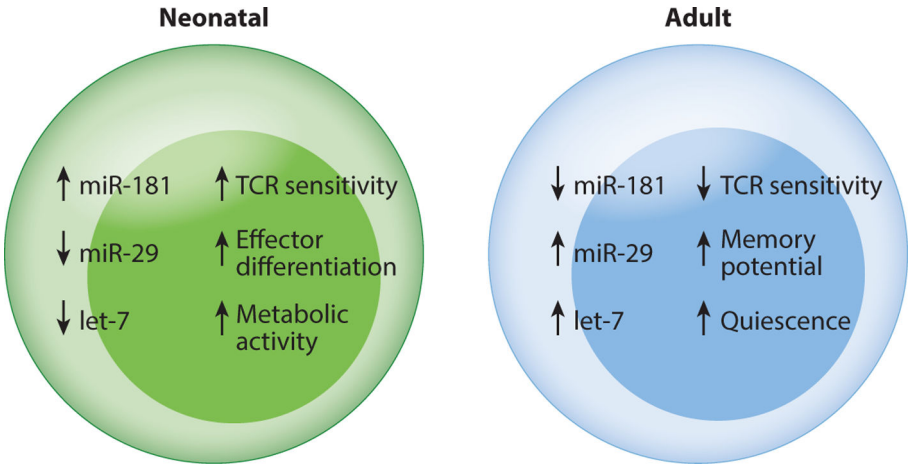

Figure 2.

Neonatal and adult T cells are programmed differently. This diagram shows how neonatal T cells are transcriptionally shifted to a more effector-like state in early life by developmentally regulated miRNAs. Importantly, these miRNAs (e.g., miR-29, miR-181, and let-7) do not function as on-off switches, but rather as fine-tuners of gene expression in naive $CD4^+$ and $CD8^+$ T cells at different stages of life. In this way, different miRNAs serve as rheostats for activation (miR-181), effector cell differentiation (miR-29), and metabolism (let-7) in T cells, adjusting various thresholds based upon the need to mount rapid effector or regulatory responses. Abbreviation: TCR, T cell receptor.

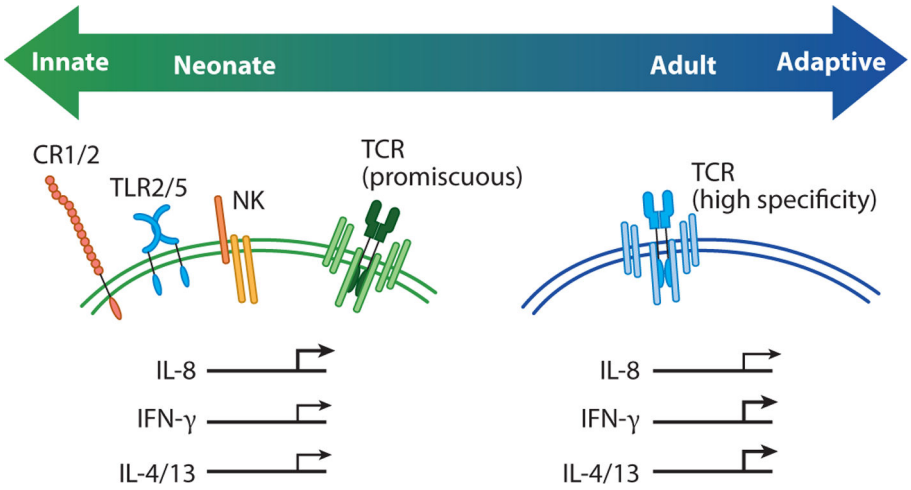

Figure 3.

Neonatal T cells exhibit innate-like functions. This diagram shows how neonatal T cells express hardwired receptors and display functions that are more typically associated with innate and innate-like T cells. For example, naive neonatal T cells can express Toll-like receptors (TLRs), complement receptors, and natural killer (NK) cell receptors, as well as a more peptide-promiscuous T cell receptor (TCR). Following TCR stimulation, neonatal T cells also rapidly produce IL-8, which is consistent with their more broadly reactive nature. In contrast, adult T cells express a higher avidity TCR and produce more conventional cytokines (IFN-γ or IL-4 and IL-13) after clonal proliferation and differentiation.