# Antigen Presentation and T Cell Response in Umbilical Cord Blood and Adult Peripheral Blood

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**Abstract:** The efficiency of the immune response is well-known to be decreased in the perinatal period compared to adulthood. Several factors may play a role in this finding, including immaturity of adaptive immune responses, as well as alterations in the prevalence and functionality in elements of humoral and cellular immune reactions compared to adult-type immunity. The process of antigen presentation and adequate T cell function are cornerstone features in coordinating the immune response already at this early age. Over the recent decades, several studies have revealed remarkable details that contribute to these alterations. However, many aspects of the exact mechanisms are still not fully understood.

In this review, we aim to summarize current knowledge of studies of altered cell prevalence and functionality that contribute to differences of antigen presentation and the T cell immune response between the perinatal and adult periods. Decreased level of antigen presentation, lower expression of costimulatory molecules, lower Th1 and Th17 response, and deficient function of regulatory elements are the most important differences in CB compared with adult peripheral blood. These differences are of practical importance from two distinct aspects. First, the decreased efficiency of the immune response plays an important role in the development of several diseases affecting preterm and term neonates, as well as in a higher incidence of infections compared to adults. Second, umbilical cord blood (UCB)-derived hematopoietic stem cells are widely used in the treatment of different hematological and immunological disorders. The prevalence of graft-versus-host disease (GVHD) is lower upon UCB-derived stem cell transplantation compared to adult peripheral blood or bone marrow-derived stem cells. Therefore, the deeper understanding of the mechanisms contributing to a decreased T cell response is of importance in improving therapeutic efficiency in related disorders.

**Keywords:** APC, cord blood, neonate, T cell, Th17, Treg.

## INTRODUCTION

The process of antigen presentation and adequate T cell function are cornerstone features in coordinating the immune response already in the perinatal period. Over the recent decades, several studies have revealed remarkable details that contribute to these alterations observed in cord blood in comparison to adult peripheral blood. In this review, we aim to summarize current knowledge of studies of altered cell prevalence and functionality that contribute to the differences of antigen presentation and the T cell immune response between the perinatal and adult periods.

These differences are of practical importance from two distinct aspects. First, the decreased efficiency of the immune response plays an important role in the development of several diseases affecting preterm and term neonates, as well as in a higher incidence of infections compared to adults. Second, umbilical cord blood-derived hematopoietic stem cells are widely used in the treatment of different hematological and immunological disorders. The prevalence of graft-versus-host disease (GVHD) is lower upon cord blood stem cell transplantation compared to adult peripheral blood or bone marrow-derived stem cells. Therefore, the deeper understanding of the mechanisms contributing to a decreased T cell response is of importance in improving therapeutic efficiency in related disorders.

## ANTIGEN PRESENTING CELLS

Monocytes are one of the most fundamental antigen presenting cells (APCs). They play a role in phagocytosis, cytokine production, and induction of cytotoxic effector cells. Circulating monocytes undergo tissue-specific differentiation into mononuclear macrophages and dendritic cells. They are first present in fetal blood at 18-20 weeks of gestation and increase with gestational age [1]. Experimental evidence suggests functional defects of neonatal APCs. Their incomplete activation and maturation contribute to a decreased function of cord blood (CB) T cells due to alterations in presenting costimulatory signals [2].

CB monocytes have low baseline expression of CD86 (B7-2) and CD40 costimulatory molecules. Potent activators, such as the combination of IFN-g and CD40 ligand (CD40L, CD154) do not up-regulate the expression of these molecules [3]. CB DCs also show signs of immaturity as they exhibit low or no basal expression of CD40, CD80 (B7-1) or CD86 [4,5]. In another study, neonatal plasmacytoid DCs (pDCs)

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showed incomplete maturation after stimulation with the TLR9 agonist CpG, as demonstrated by the lower expression of CD80, CD83, CD86 and CD40 in comparison to stimulated adult pDCs [6]. Similarly, upregulation of CD40 and CD80 on CB myeloid DCs (mDCs) in response to LPS and the TLR3 agonist poly (I:C) was lower compared to that of adult mDCs [7].

Pérez *et al*. described the response of monocytes upon stimulation from CB of healthy neonates of different gestational ages (very-preterm, preterm, full-term). CD14+ monocytes cultured with lipopolysaccharide (LPS) or LPS and IFN-g showed lower HLA-DR expression for the very-preterm group in both unstimulated and LPS-stimulated cells. No differences were detected for CD40+ cell percentages between very-preterm and preterm or full-term neonates. An increase in CD80 and a decrease in CD86 within all groups were described upon stimulation. The production of IL-12 was lower in very-preterm neonates. Adhesion capability of neonatal monocytes was similar and independent of gestational age. Their results demonstrate developmental differences in the sensitivity of neonatal monocytes to stimulation: very-preterm neonatal monocytes do not fully respond to LPS and have diminished functions compared with preterm or full-term neonates [1].

Darmochwal-Kolarz *et al*. determined the expression of CD274 (B7-H1), B7-H4, CD200, and CD200R co-stimulatory molecules on myeloid BDCA-1+ and lymphoid BDCA-2+ DCs, monocytes and B lymphocytes in CB of healthy full-term neonates compared to PB of healthy adults [8]. They showed that the expression of these molecules are significantly lower on CB DCs compared to adults. However, the expression of B7-H4 as well as CD200 and CD200R were significantly higher on CD14+ monocytes in CB. The interaction between CD200 and CD200R suppresses mast cell degranulation and decrease cytokine production, mainly that of IL-13, TNF-a, IFN-g, and IL-17 [9]. Therefore, the increased percentages of CD200 and CD200R expressing monocytes may contribute to the decrease of cytokine production by CB monocytes.

Other investigations also demonstrated that CB monocytes and macrophages produce low levels of pro-inflammatory cytokines such as TNF-a, IL-1b or IL-12 upon LPS stimulation [10,11]. Lower levels of cytokine production by CB mDCs in response to LPS, combined or not with IFN-g, have also been reported and affect TNF-a, IL-1, IL-6 and IL-12 production [12,13]. Interestingly, the latter study showed that the number of cytokine expressing cells was equivalent, but the level of expression per individual cell was lower.

IL-27 is produced primarily by APCs and is immunosuppressive towards a variety of immune cell types. Kraft *et al*. demonstrated that IL-27 gene expression is elevated in CB macrophages compared to macrophages of healthy adults. Furthermore, they evaluated the duration over which elevated IL-27 gene expression and intracellular cytokine expression may impact immune responses in mice. IL-27 remained elevated throughout infancy and then declined in adult mice. Dose-responsive gene expression to progesterone was demonstrated in macrophages, suggesting that IL-27 may be induced during pregnancy to contribute to the immunosuppressive environment at the fetal-maternal interface. Neutralization of IL-27 in neonatal macrophages improved the ability of these cells to limit bacterial replication. Moreover, neutralization of IL-27 during incubation with the Mycobacterium bovis bacillus Calmette-Guérin vaccine augmented the level of IFN-g elicited from allogeneic CD4+ T lymphocytes [14].

Some data support a defect of CB monocytes in TLR signaling. Although neonatal monocytes express similar levels of mRNA for different TLRs compared to adult monocytes [15,16], these data do not exclude possible differences in the protein expression of these receptors. The upregulation of TLR4 and CD14 expression upon LPS stimulation was not observed in CB monocytes [15,17]. Additionally, TLR4 protein expression was lower on monocytes from premature newborns compared to those from full-term neonates [16].

Gold *et al*. investigated the capacity of CB DCs to process and present antigen to CD8 cells. They used human CD8 cell clones to compare the ability of CB and adult monocyte-derived DCs to present or process and present antigen *via* the MHC class I pathway. They assessed the ability of DCs to present antigenic peptide, present an HLA-E restricted antigen, process and present an MHC class I restricted antigen through the classical MHC class I pathway, and cross present cell-associated antigen *via* MHC class I. They discovered no deficiency in CB DCs to perform any of these processing and presentation functions and concluded that, unlike TLR signaling or cytokine production, the MHC class I antigen processing and presentation pathway is functional in CB DCs and therefore may not explain the diminished control of pathogens [18].

When the capacity of CB DCs to stimulate allogeneic CB T cells was analyzed, weak proliferation was observed in pDC stimulated cultures compared to total CB DC stimulated ones [5], which was linked to an increased proportion of apoptotic T cells in the former cultures. Mechanisms underlying these data have not been elucidated and could involve either a direct apoptotic signal given by CB pDCs or a failure in the induction of rescuing signals.

Interestingly, the phagocytotic function of CB monocytes also appears to be compromised. CB monocytes exhibit a trend towards lower phagocytosis of E. coli compared to adult monocytes. This alteration is even more pronounced in fetuses younger than 30 weeks of gestation [19].

Finally, another mechanism that may account for the dysfunction of CB APCs is the inhibitory effect by regulatory T cells (Tregs). The direct inhibitory effect exerted by naturally occurring Tregs on DC function has recently been demonstrated in several murine models [20,21]. In addition, nTregs could indirectly inhibit APC function as a result of their inhibition of effector T cells and through the secretion of anti-inflammatory cytokines such as IL-10 and TGF-b [22]. Since nTregs are prevalent and functionally active in human CB, Treg-mediated inhibition could constitute an additional mechanism for APC dysfunction.

## INTERACTION BETWEEN APCs AND T LYMPHOCYTES

Further details of functional maturation of DCs, especially regarding their interaction with T cells, have recently been described. Langrish *et al*. demonstrated that immature DCs from CB showed the same marker expression when compared to immature DCs derived from adults [23]. Both adult and CB DCs have high expression of CD11 and HLA-DR, as well as little or no expression of CD25 and CD83 maturation markers and moderate expression of CD86. Following stimulation with LPS, adult DCs adopted a mature phenotype in contrast to CB DCs, which remained phenotypically immature, failing to upregulate HLA-DR and only minimally increasing CD86 expression. This is in line with the findings of De Wit *et al*. that CB pDCs had lower expression of CD86 than adult pCBs upon TLR9 stimulation [6]. Another study even described a decrease in CD86 expression following LPS stimulation in CD14+ monocytes [1]. This failure to mature is not in itself linked to an inability to respond to LPS, as TLR4 is expressed on CB DCs at equivalent levels, according to data by Langrish *et al* [23]. In contrast, other findings describe a lack of TLR4 upregulation upon LPS stimulation in CB monocytes [15].

CB DCs were also unable to produce IL-12p70 and to down-regulate the expression of the chemokine receptor CCR5 and induced lower levels of IFN-g production from allogeneic naive CD4+ T cells than their adult counterparts. CB T cells stimulated in the same system produced lower amounts of IFN-g compared to adult T cells, but CB DCs were again less able to stimulate them than those derived from adult blood. In contrast, the kinetics of the production of TNF-a and IL-10 in response to LPS stimulation was comparable to adult DCs. The decreased ability of CB DCs to attain a fully mature adult phenotype, and to activate naive CD4 T cells to produce IFN-g suggests that they are intrinsically preprogrammed against the generation of a Th1 immune response [23].

The CD40L-CD40 interaction is important in regulating the cellular immune response. Han *et al*. studied the function of the CD40L-CD40 interaction between T cells and B cells or monocytes in CB compared with adult blood *in vitro*. In whole blood, adult monocytes, unlike neonatal monocytes, were activated following T cell activation. However, the activation of adult monocytes was not dependent on the CD40L-CD40 interaction. Using the CD40L trimer (Lt), they demonstrated that CB B cells have comparable responses to CD40 ligation to those of adult B cells. Monocytes, both_CB and adult-derived, do not respond as well as B cells and this is probably related to low expression of CD40. However, IFN-g upregulated CD40 on adult monocytes but not on CB monocytes. This potentiated the adult monocyte response to CD40 ligation by CD40Lt. Their findings suggest that the neonatal CD40L-CD40 interaction is immature in the cellular immune response involving monocytes and that IFN-g fails to activate neonatal monocytes for a response to CD40L [3].

Elliott *et al*. found that CD40 was expressed at similar levels on resting B cells from adults, young children (2-20 months of age) or CB. CD40 expression was higher on CB B cells compared to adult B cells after stimulation with PMA and ionomycin, but similar on adult and CB B cells activated by CD3-stimulated T cells. CB T cells stimulated with PMA and ionomycin expressed adult levels of CD40L initially, but this expression was more transient on CB T cells. When adult and CB mononuclear cells were stimulated with CD3 mAb, T cells from some CB samples showed different kinetics of CD40L expression compared with

adult T cells. However, other CB samples showed adult patterns of T cell CD40L expression. When mononuclear cells were depleted of B cells and monocytes before stimulation with CD3 mAb, the MFI and percentage of T cells expressing CD40L increased, with adult and CB T cells showing similar patterns of expression. These results demonstrate some differences in expression of CD40 and CD40L between neonatal and adult lymphocytes, but do not directly account for the relative deficiencies of humoral immunity in neonates [24].

## PREVALENCE AND FUNCTION OF T LYMPHOCYTES

Decreased functionality of neonatal T cells is a widely recognized experimental and clinical phenomenon. Besides reduced functioning, differences in the prevalence of distinct lymphocyte subsets have been described. The majority of CB lymphocytes are naive (CD45RA) cells in contrast to adults, where effector (CD45RO) cells dominate [25]. The higher prevalence of naive cells (CD4+ CD45RA+ CCR7+) was confirmed by our results, whereas that of central (CD4+ CD45RA- CCR7+) and effector memory T cells (CD4+ CD45RA- CCR7-) was lower in CB compared with adults [26]. The percentage of CD4 lymphocytes was significantly higher in newborns compared to adults, while that of CD8 cells was lower, resulting in a gradual decline of the CD4/CD8 ratio [27]. The prevalence of NK cells is also lower in CB [26].

Both Th1 and Th2 responses are required for comprehensive immune function. Neonatal mice and humans show a strong bias towards Th2 polarization [23,28], which is observed at all phases from the generation of primary effectors to memory responses [29]. However, both mouse and human neonatal T cells retain the ability to mount protective Th1 responses under specified conditions [30,31].

García Vela *et al*. analyzed intracellular cytokine production in PMA-ionomycin-brefeldin A stimulated CB and adult peripheral blood samples. Compared to adults, CB T lymphocytes produced less IL-2, IL-4 and IFN-g. In CB, these cytokines were produced largely by CD4 cells. In adults, IL-2 and IL-4 were also produced predominantly by CD4 cells compared with CD8 lymphocytes, however, IFN-g was produced by both CD4 and CD8 T cells [25].

Gasparoni *et al*. measured intracellular IL-2, IL-4, IFN-g and IL-10 production in T cells in response to Con A in 12 very preterm, 12 preterm and 20 term neonates, 10 children and 10 adults. Immunoproliferation to Con A was lower in CB than in children or adults. The percentage of IL-2-producing CD4 and CD8 cells was higher in all newborn groups than in children and adults, while the percentage of IL-4-producing cells was higher for CD8 and lower for CD4 cells in CB than in children and adults. Neonates had lower percentages of CD4+ and CD8+ IFN-g-producing cells. A significant negative correlation was observed between gestational age and CD4+ IFN-g-, CD8+ IL-2-, and CD4+ IL-10-producing cells. In addition, a positive correlation was found between gestational age and CD8+ IL-10-producing cells [27].

Nitsche *et al*. compared induced secretion of cytokines on the level of proteins and mRNA in CB and adult blood by various stimuli. While IL-2 levels were similar in CB and adults, the induction of IFN-g was lower in CB. Production of IL-4, IL-5, and IL-13 and the hematopoietic cytokine IL-3 was decreased in CB versus adults after T cell receptor (TCR)-mediated stimulation, whereas production of GM-CSF was comparable. The lower levels of Th1 and Th2 cytokines were maintained in CB during a 4-day time course study, while after 12 hours IL-3 and GM-CSF reached similar levels in CB to those in adults. For all cytokines except IFN-g, the inhibitory IC50 values by cyclosporin A were similar in CB and adults. In contrast, the expression and activation of transcription factors were lower in CB. Activation of NF-κB by TPA/ionomycin was detected in adult blood but not in CB. Furthermore, the expression of the Th subset-specific transcription factors T-bet and c-maf was lower in CB than in adults, whereas GATA-3 expression was similar. Expression of T-bet and c-maf correlated with expression of Th1 and Th2 cytokines, respectively. Time course experiments revealed that T-bet expression was stimulated in both cell types, whereas c-maf and GATA-3 were induced only in adult blood. The authors concluded that diminished capability of CB to synthesize cytokines is probably due to decreased activation of NF-κB, whereas differences in Th subsets are due to differences in regulation of Th lineage-specific transcriptions factors [32].

Using different methods, Kilpinen *et al*. obtained different results when analyzing the activation of NF-κB in human CB T lymphocytes. In their study, the activity was tested by quantifying the nuclear proteins binding to an oligonucleotide containing the consensus kappa B binding sequence using electrophoretic mobility shift assay. The data obtained demonstrate that phorbol dibutyrate/calcium ionophore A23187 combination

induced a clearly higher nuclear translocation of NF-κB in neonatal than adult T cells, restricted to the CD4 cell subset. Analysis of the nuclear extracts with antibodies directed against the major components of NF-κB, the p50 and RelA (p65) proteins, indicated that the composition of NF-κB was similar in CB and adult cells. These results suggest that neonatal T cells are exposed to oxidative stress-inducing signals during delivery and/or are intrinsically more sensitive to NF-κB activating signals than adult T cells [33].

The ability of neonates to deviate from the Th1 responses and expand Th2 immune responses may, at least in part, be explained by reduced expression of IL-12 [34]. Upon the addition of IL-12 in both mouse and human neonatal systems, T cell responses are redirected towards a Th1 profile [35]. The inability of CB DCs to produce IL-12 may therefore be essential to the establishment of a Th2 bias, perhaps through the action of other dominantly acting Th2 cytokines such as IL-10 [23].

On the other hand, Schultz *et al*. demonstrated that the anti-inflammatory response is also deficient in CB. After anti-CD3/anti-CD28 costimulation, IL-10 production of neonatal T lymphocytes was strongly reduced. IL-10 receptor expression was diminished on neonatal T lymphocytes compared to adults, but was comparable on neonatal B lymphocytes and monocytes [36].

Negative control of CB IFN-g production is generally attributed to the Th1-antagonistic effect of mediators produced by the placenta, but there is evidence for additional and more direct transcriptional regulation. White *et al*. report that CB CD3+ CD45RO- T cells, in particular the CD4+ CD45RO- subset, are hypermethylated at CpG and non-CpG (CpA and CpT) sites within and adjacent to the IFN-g promoter compared to adult cells. In contrast, CpG methylation patterns in CB IFN-g-producing CD8+ CD45RO- T cells and CD56+ CD16+ CD3- NK cells did not differ significantly from those in adults. Consistent with this finding, IFN-g production by stimulated naive cord blood CD4 cells is reduced 5- to 10-fold relative to adult CD4 cells, whereas production in neonatal and adult CD8 cells is of a similar level. Evidence of significant CpA and CpT methylation was not discovered in promoter sequence from other cytokines (IL-4, TNF-a, or IFN-gR alpha-chain) [37].

Antigen exposure is necessary to induce maturation of the adaptive immune system. Thus, neonatal lymphocytes are predominantly naive and have a resting phenotype, as exposure to foreign antigen in utero is uncommon in uncomplicated pregnancies. Among preterm infants, however, T cells are often activated as reflected by increased proportions of cells expressing the activation markers CD25, CD69, and HLA-DR as compared with T cells of term infants. This effect is independent of the presence of chorioamnionitis. Additionally, gestational age at the time of spontaneous birth is inversely correlated with T cell activation in the absence of chorioamnionitis. These findings support the concept that activation of the fetal adaptive immune system in utero is closely associated with preterm labor. Due to the ongoing infection, increased T cell activation was of course also demonstrated in term neonates delivered following chorioamnionitis [38].

In adults, the principal stimulus for naive T cell activation and maturation is considered to be TCR triggering by neoantigenic peptides. Crespo *et al*. demonstrate, however, that neonatal naive T cells can upregulate expression of the maturation antigen CD45RO through TLR-dependent mechanisms. Using selected TLR ligands, they found consistent upregulation of CD45RO by neonatal T cells, while this response was not present in adult naive T cells [39]. Furthermore, the proportion of CD45RO+ T cells was higher in preterm versus term CB, probably reflecting premature maturation of these cells in response to premature exposure to microbial elements. A consequence of this exposure may be increased migration of T cells to non-gastrointestinal sites (eg. lungs, skin, or brain) that may underlie the systemic inflammatory organ dysfunction often seen in premature infants. This notion is supported by interesting results. The homing molecule α4β7 promotes T cell entry into intestinal sites and CCR4 supports T cell chemotaxis and entry into non-gastrointestinal sites [40]. Preterm CB T cells had lower expression of the α4β7 integrins and higher expression of CCR4 as compared with term CB. The expression of CD45RO and α4β7 receptors was higher in the presence of clinical chorioamnionitis, independently from gestational age [39].

The expression of CD80, CD86, CD28, and CD152 (CTLA-4) were also examined by Elliott *et al*. on lymphocytes isolated from CB, young children (2-20 months of age) and adults. There was no difference in the expression of CD80 or CD86 between adult and neonatal B cells, either resting or activated. A higher proportion of resting T cells expressed CD28 in CB and young children compared to adults. CD28 expression

was similar on adult and CB T cells activated with PMA and ionomycin. However, CD28 was expressed at greater intensity on a higher percentage of neonatal T cells than adult T cells stimulated with CD3. CD152 expression was lower on CB T cells than adult T cells stimulated with PMA and ionomycin and undetectable on CB T cells stimulated with CD3. In contrast, intracellular CD152 was equivalent in adult and neonatal T cells stimulated with PMA and ionomycin, suggesting that trafficking of CD152 to the cell surface may be differentially regulated in neonatal T cells. Since the T cell response is determined by the balance of signals received from CD28 and CD152, high levels of CD28 expression and lower surface expression of CD152 on CB T cells may represent increased likelihood to promote activation in neonatal T cells [41].

Schelonka *et al*. questioned whether limitations in the T cell repertoire contribute to the lower responsivity noted in CB. To describe developmental changes of the T cell repertoire, cDNA segments corresponding to third complementarity regions (CDR3) of CB TCRs from gestational age of 24-41 weeks were amplified with TCR family-specific probes. The resulting amplified CDRs were visualized by fingerprinting and single strand conformation polymorphism (SSCP) analysis. At 24 weeks of gestation there were no limitations in TCRBV (T cell receptor beta-chain variable) family usage, and the degree of CDR3 size heterogeneity was not different from the adult. However, earlier in gestation, CDR3s were shorter for all families and gradually increased in size until term. The degree of oligoclonal expansion observed in CB was greater than in adult peripheral blood. T cell oligoclonal expansion was greatest at 29-33 weeks of gestation and declined toward term. Expansions were detectable in both CD4 and CD8 subpopulations. Their findings indicate that the genetic mechanisms of repertoire diversification appear intact as early as 24 weeks of gestation, but repertoire diversity is limited as a result of smaller CDR3 sizes [42].

In line with earlier findings, Chen *et al*. reported that CB naive CD4 T cells had impaired activation and early Th1 differentiation compared with adult peripheral blood naive CD4 T cells following stimulation by allogeneic DCs derived from adult monocytes. Early Th1 polarization was dependent on IL-12 and CD154, and CB CD4 T cell/DC co-cultures had impaired expression of both proteins. CB naive CD4 T cells had low basal levels of signal transduction and activation of transcription 4 mRNA and protein, and, after alloantigen stimulation, reduced IL-12-induced signal transduction and activation of transcription 4 tyrosine phosphorylation, compared with adult naive T cells. FoxP3 protein expression, a marker for Tregs, was lower for naive CD4 T cells of CB compared with those of adult peripheral blood, which argued against increased Treg activity as a mechanism for the decreased Th1 differentiation of CB CD4 T cells [43].

The early adequate function of Tregs may critically influence healthy immune maturation. Schaub *et al*. investigated T cell responses to innate (lipid A and peptidoglycan) and adaptive (phytohemagglutinin, PHA) stimuli at birth compared to adult immune responses [44]. They demonstrated that Tregs were generally present and functional in CB following PHA stimulation, consistent with studies demonstrating functional Tregs in cord blood [45]. However, both adaptive and innate stimulation induced generally a less mature Treg response in comparison to adults. One explanation is that a majority of Tregs mature after birth, which is in parallel with data from Wing *et al*. demonstrating that CB CD4+ CD25+ cells do not inhibit responses to self-antigens, compared with adult Tregs [46]. Secondly, the expression of Treg markers was compared in CB and adult blood. Similar results were found demonstrating present but lower expression of CD4+ CD25high cells and FoxP3 expression in CB compared with adults following innate stimulation, in line with results from Chen *et al*. [43]. This indicates that lower expression of CD4+ CD25high cells and FoxP3 may contribute to a less suppressive capacity of Tregs in CB compared with adults.

Furthermore, Th17 cells were expressed at low concentrations following innate stimulation in CB, and the correlation with Th1/Th2 cytokines observed in adult samples was also conserved. Interestingly, IL-17 secretion was restricted to innate stimulation in CB and PHA had no effect. Thus, efficient innate responses have an impact on the development of subsequent adaptive responses [44]. One potential explanation for low IL-17 in CB is a delayed maturation of Th17 cells after birth as described for Tregs [47]. Another possibility is that Th17 cells require additional specific stimulation to enhance potent activation. However, activation with anti-CD3/ant-CD28 and IL-6 plus TGF-b did not induce significant IL-17 secretion. In summary, the combination of low Th17 and Th1 cells, increased Th2 cytokine secretion in addition to less efficient suppression by Tregs in cord blood may set the stage for a vulnerable immune system early in life. Innate immune stimuli such as high microbial exposures may be potential candidates driving the early immune system.

Chang *et al*. reported results on Tregs [48] that contradict the above studies by Chen *et al*. [43] and Schaub *et al* [44]. They compared CB CD4+ CD25- and CD4+ CD25+ T cells, either naïve or antigenic stimulated, to their counterparts in unmobilized adult peripheral blood with respect to genetic expression patterns, immunophenotype, suppressive activity, and mechanisms of suppression. Both naïve CB and adult CD4+ CD25+ T cells expressed comparably elevated mRNA levels of GITR, FoxP3, CD25, CD152, as well as elevated protein levels of CTLA-4 and GITR. However, only naïve adult peripheral blood but not CB CD4+ CD25+ T cells suppressed allogeneic responses. Stimulation of CD4+ CD25- T cells by MUTZ-3-specific immature dendritic cells (MUTZ-iDC) elicited amplification of these genes and potent suppression of CD4+ CD25- T cell proliferation induced by MUTZ-iDC but not by unrelated stimulators. Compared to that from unmobilized adult blood, a significantly higher percentage of CD4+ CD25+ CD152+ Treg cell subsets were induced from CB CD4+ CD25- T cells following allogeneic stimulation. These results suggest that CB CD4+ CD25+ Treg cells, which are induced at a higher rate by allogeneic stimulation when compared to unmobilized adult blood, can readily function as potent allogeneic immune suppressors and may in part contribute to the decrease in CB alloantigen recognition and activation of CB CD4+ CD25- T cells.

In an investigation by Black *et al*., naïve CD4 cells from extremely preterm infants, term infants, and adults were assayed for their capacity to develop into Th17 effector cells *in vitro* [49]. Instead of developmental limitations in Th17 cell capacity observed by Schaub *et al*. *in vivo* [44], they found that Th17 cell lineage capacity inversely correlated with age such that T cells from extremely preterm infants had the greatest tendency to develop into Th17 cells, followed by term infants, and then by adult naive T cells which demonstrated little or no ability to become Th17 cells. This was in contrast to Th1 cell responses in which adults showed a significant Th1 cell bias compared with infants. Neonates expressed higher levels of IL-23R, ROR-gt, and STAT3 prior to activation and showed a significant Th17 cell bias after activation. In contrast, adult cells expressed more TBX21 with a corresponding Th1 cell bias.

Cosmi *et al*. reported that cord blood Th17 cells develop primarily from a CD161+ CD4+ precursor population and that this population is largely absent in adult naive cells [50]. Given that preterm T cells generate equivalent or higher levels of IL-17 and IL-21 and show more Th17 cells by flow cytometry after culture under Th17 cell conditions, it was presumed that preterm infants might have higher numbers of the CD161+ Th17 cell precursor population. Preterm and term cells were analyzed by flow cytometry to determine the CD161+ fraction within the live CD3+ CD4+ T cell population. In spite of higher capacity for Th17 cell differentiation, preterm infants had significantly fewer CD161+ Th17 cell precursor cells prior to stimulation, suggesting that Th17 cells can also develop from CD161- precursors in preterm infants. Although the results of Black *et al*. show that CB CD4 cells have the capacity to become Th17 cells *in vitro*, they may fail to do so *in vivo* as a result of inadequate antigen presentation and cytokine signals.

Lee *et al*. recently reported a novel function of progesterone in regulation of naive CB T cell differentiation into key Treg subsets [51]. Progesterone drives allogeneic activation-induced differentiation of CB naive, but not adult peripheral blood T cells into immune-suppressive Tregs, many of which express FoxP3. Compared with those induced in the absence of progesterone, the FoxP3+ T cells induced in the presence of progesterone highly expressed memory T cell markers. In this regard, the Treg compartment in progesterone-rich CB is enriched with memory-type FoxP3+ T cells. Moreover, CB APCs were more efficient than their adult counterparts in inducing FoxP3+ T cells. Another related function of progesterone discovered was to suppress the differentiation of CB CD4 cells into inflammation-associated Th17 cells. Progesterone enhanced activation of STAT5 in response to IL-2, whereas it decreased STAT3 activation in response to IL-6, which might provide a potential mechanism for the selective activity of progesterone in generation of Tregs versus Th17 cells. Additionally, progesterone has a suppressive function on the expression of the IL-6 receptor by T cells. These results identified a novel role of progesterone in regulation of fetal T cell differentiation for promotion of immune tolerance.

Palin *et al*. investigated the hypothesis that impaired neonatal CD4 T cell immunity was due to reduced signaling by naive CD4 cells after engagement of the αβ-TCR/CD3 complex and CD28 [52]. Calcium influx following engagement of CD3 was significantly higher in CB naive CD4 cells compared with adult CD4 cells. Neonatal naive CD4 cells also had higher Erk phosphorylation following activation. The microRNA miR-181a, which enhances activation-induced calcium influx in murine thymocytes [53], was expressed at

significantly higher levels in CB naive CD4 cells compared with adult cells. Overexpression of miR-181a in adult naive CD4 cells increased activation-induced calcium flux, implying that the increased miR-181a levels of CB naive CD4 cells contributed to their enhanced signaling. In contrast, AP-1-dependent transcription, which is downstream of Erk and required for full T cell activation, was lower in CB naive CD4 cells compared with adult cells. Thus, CB naive CD4 cells have enhanced activation-dependent calcium flux, indicative of the retention of a thymocyte-like phenotype. Enhanced calcium signaling and Erk phosphorylation are decoupled from downstream AP-1-dependent transcription, which is reduced and likely contributes to limitations of CB CD4 cell immunity.

Our own investigation regarding calcium influx kinetics of CB T cells revealed different results, probably due to different methodology and investigated cell subsets. We aimed to characterize calcium influx kinetics of activated T lymphocytes in CB and to test the functionality and expression of Kv1.3 and IKCa1 lymphocyte potassium channels, important regulators of calcium influx [54]. We measured the calcium influx kinetics applying a novel flow cytometry approach in the Th1, Th2, CD4 and CD8 T lymphocyte subsets activated with PHA. With the exception of the CD4 subset, calcium influx kinetics was decreased upon activation in CB T lymphocytes compared with adults. Neonatal T lymphocytes were found to be less sensitive to the specific inhibition of Kv1.3 and IKCa1 potassium channels. The expression of Kv1.3 channels was higher on major T lymphocyte subsets of newborns except for Th1 lymphocytes. Our findings suggest that the characteristics of short-term activation of major neonatal T lymphocyte subsets are decreased compared with adults. The altered function of neonatal lymphocyte potassium channels may contribute to this phenomenon.

Galectin-1 (Gal-1) plays a key role in the regulation of inflammatory responses and immune tolerance with strong immunosuppressive properties [55]. It supports the survival of naive T cells and has antiproliferative effects on both naïve and activated T cells [55,56]. Gal-1 induces apoptosis of CD8 and Th1 cells, while Th2 cells are protected from Gal-1-mediated cell death [57]. Gal-1 also suppresses the secretion of the proinflammatory cytokine IL-2 [58] and favors the secretion of the anti-inflammatory IL-10 [59]. Gal-1 is also regarded as a very plausible effector molecule for the function of CD4+ CD25high regulatory T cells [60]. Interestingly, in contrast with the well-known immunosuppressive effect of Gal-1, we found that the prevalence of Gal-1-expressing CD3, CD4, CD8, Treg, and NK lymphocytes are lower in CB than in adult peripheral blood [26]. Lower Gal-1+ cell proportion might be due to the naivety and lower level of long-term stimulation of CB lymphocytes. This is supported by the demonstrated higher prevalence of naive, but lower prevalence of central and effector memory T cells in comparison to adults, and the positive correlation between the number of CD3 lymphocytes expressing intracellular Gal-1 and the prevalence of memory T cells. The amount of intracellular Gal-1 is comparable in UCB to that in adult peripheral blood, and suggests that the Gal-1-producing capability of these cells is mature. The intracellular expression of Gal-1 may be downregulated in neonatal lymphocytes due to the already reduced immune reactivity of CB. In contrast with previous findings, our results indicate that the administration of exogenous Gal-1 failed to decrease the rate of proliferation in T lymphocytes isolated from either adult blood or CB. This suggests that Gal-1-expressing lymphocytes are unlikely to play a major role in mitigating the immune reactivity of CB.

## CONCLUSION

As demonstrated by the above investigations, certain aspects of antigen presentation and T cell function are immature in CB compared to adults, while others are comparable in the two groups. In several cases, immature aspects in CB can reach similar level of functionality to that seen in adult blood under distinguished conditions. A sequence in the level of immaturity can often be recognized when blood samples from different age groups are examined, ranging from preterm infants *via* term neonates and children to adults.

Decreased level of antigen presentation, lower expression of costimulatory molecules (summarized in Table **1**), lower Th1 and Th17 response, and deficient function of regulatory elements are the most important differences in CB compared with adult peripheral blood. Stimuli from microbial exposure or preterm birth may drive the early immune system to ‘catch up’ with the above deficiencies and reach comparable level of function to adult immunity.

Understanding differences between CB and adult blood immunity is important to improve vaccination strategies, fight the high prevalence of infection in newborns, decipher the pathomechanism of immune-related disorders in neonates, including diseases that primarily affect preterm infants. Finally, improving the

**Table 1: Alteration of CD Markers in Cord Blood (CB) Compared to Adult Peripheral Blood**

| Marker | Cell type | Function | Alteration in CB | References |
|---|---|---|---|---|
| CD25 | T lymphocyte | Activation marker | Higher expression in preterm than in term CB | [38] |
| CD28 | T lymphocyte | Stimulatory receptor for CD80, CD86 | Higher expression | [41] |
| CD40 | monocyte, DC | Costimulation | Lower baseline expression | [3-5] |
| CD45RA | T lymphocyte | Naive T lymphocyte | Higher expression | [25] |
| CD69 | T lymphocyte | Activation marker | Higher expression in preterm than in term CB | [38] |
| CD80 | DC | B7-1, costimulation | Lower baseline expression | [4, 5] |
| CD86 | monocyte, DC | B7-2, costimulation | Lower baseline expression | [3-6] |
| CD152 | T lymphocyte | Inhibitory receptor for CD80, CD86 | Lower expression | [41] |
| CD 274 | DC | B7-H1, costimulation | Lower expression | [8] |
| CD200, CD200R | DC | Costimulation, decrease cytokine production | Lower expression | [8] |
| CD200, CD200R | monocyte | Costimulation, decrease cytokine production | Higher expression | [8] |

outcome of cord blood transplantation and decreasing the rate of GVHD also relies on a better understanding of distinct features of cord blood immunity.

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