# Defective antigen-presenting cell function in human neonates

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## Abstract

Immaturity of the immune system has been suggested as an underlying factor for the high rate of morbidity and mortality from infections in newborns. Functional impairment of neonatal T cells is frequently quoted as the main underlying mechanism for such immaturity. However, recent studies suggest that neonatal antigen-presenting cells (APCs) also exhibit functional alterations, which could lead to secondary defects of adaptive T cell responses. In this review, we summarize what is known on the functionality of APC at birth and during early childhood. Compared to adults, neonatal APCs display markers of immaturity and produce low levels of cytokines. Multiple factors could be involved in neonatal APC alteration, such as intrinsic immaturity, defective interaction between APCs and T cells, and regulatory T cell-mediated inhibition. Characterization of the relative contribution of each mechanism is clearly needed to better understand the functional capability of the neonatal immune system.

### Keywords

Neonate; Immune System; Antigen-presenting cells; Dendritic cells; Regulatory T cells; Macrophages; Monocytes; Childhood; Costimulatory molecules; Cytokines

## Introduction

The world Health Organization estimates that approximately 7.1 million infants between 1 and 12 months of age die annually of infections [1]. The most common causes of early death are acute respiratory and diarrheal diseases, caused by bacterial and viral pathogens [1-3]. Such infections are rarely fatal in older children and adults. The immaturity of the neonatal immune system has been suggested as an underlying factor for such high rate of morbidity and mortality [2]. Defects in the immune system during early life also influence the responses to neonatal immunizations, frequently resulting in poor antibody responses [4,5]. Indeed, with the exception of BCG, most vaccines administered during the first six months of life require several doses to induce protection, and the ability of vaccines to induce protection is directly correlated with the progressive maturation of the infant's immune system [5].

It has been speculated that the immaturity of the immune system in early life is due to a functional impairment of T cells. Neonatal T cells exhibit intrinsic deficiencies that prevent them from becoming fully activated [6,7], which are summarized in Table 1. However, recent studies suggest that the immaturity of the neonate's immune system implies more than

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alterations in T cell activation. In particular, neonatal antigen-presenting cells (APCs) also exhibit functional alterations (summarized in Table 1), which could lead to secondary defects of adaptive T cell responses. The immaturity of APC system in murine neonates has been extensively documented (for review, see [8,9]. However, due to the well-established differences in development between human and murine neonates [5], we have focused this review on the findings obtained with human APCs.

## Antigen-presenting cells

APCs are key players during innate immune responses, and are responsible for the induction of adaptive immune responses. The immunological outcome after an antigenic challenge depends on several factors, including the type of APC. Naïve T cell activation requires at least two signals provided by APCs: one is antigen-dependent, TCR-mediated (signal 1) and the other one is antigen-independent and depends on co-stimulation (signal 2). Recently, antigen-independent signals have themselves been divided into a signal 2, which corresponds to cognate interaction with co-stimulatory molecules, and a signal 3 constituted by the action of soluble mediators such as cytokines. It is also known that the interactions between APC and activated T cells constitute a two-way dialog that leads to APC maturation on one hand, and the generation of fully-activated effector T cells on the other hand [10,11]. Both quantitative and qualitative alterations in such interactions promote the development of tolerance in effector T cells [11-14].

## Neonatal monocytes and macrophages

Functional defects of neonatal mononuclear phagocytes have been documented, compared to their adult counterparts. Such defects include evidence of immaturity, decreased production of cytokines and altered phagocytosis (see Table 1). In particular, neonatal monocytes exhibit low baseline expression of the costimulatory molecules CD86 and CD40. Expression of these molecules is not up-regulated by potent activators, such as the combination of IFN-γ and CD40 Ligand (CD40L) [15]. In addition, neonatal monocytes and macrophages produce low levels of proinflammatory cytokines such as TNF-α, IL-1β or IL-12, in response to bacterial lipopolysaccharide (LPS, a TLR4 ligand) [16-18], and other TLR ligands such as triacylated BLP (TLR1/2 ligand), mycoplasma-associated lipopeptide (TLR2/6 ligand) and imiquimod (TLR7 ligand) [19]. These alterations probably result from defects in the signaling pathways downstream of TLR engagement, but the exact nature of such defect(s) is not yet elucidated. Although neonatal monocytes express similar levels of mRNA for different TLRs than adult monocytes [20,19], these data do not exclude possible differences in the protein expression of such receptors. Some data also support a defect in TLR signaling: (1) the up-regulation of TLR4 and CD14 expression upon LPS stimulation is not observed in neonatal monocytes [19,21]; and (2) the protein expression of MyD88, an important adaptor molecule involved in TLR-mediated signaling, is decreased in neonatal monocytes [21], although MyD88 mRNA levels are similar in neonatal and adult monocytes [19]. It should be noted that the expression of several key molecules involved in TLR signaling, such as TIRAP and IRAK-4, has not been carefully examined. Similar levels of mRNA have been reported for those 2 molecules, but protein expression has not been determined [19]. Finally, interestingly, TLR4 protein expression is lower on monocytes from premature babies compared to those from full-term babies [20]. This difference could constitute an underlying mechanism for the exacerbated susceptibility to infections exhibited by premature babies, and suggest a regulation of TLR expression in relation to fetal development.

Adding to the complexity, differential defects were reported depending on the TLR ligand used to stimulate neonatal monocytes. In particular, phosphorylation of p38 MAPK and TNF-α production was decreased in neonatal monocytes upon LPS stimulation, while it was normal

after R-848 stimulation, a TLR 7/8 ligand [19]. This observation may reflect either the presence in neonate monocytes of factors interfering specifically with TLR4 signaling, or the existence of alternative pathways to activate p38 MAPK.

Neonatal macrophages also exhibit decreased responsiveness to IFN-γ, which is linked to a marked alteration in STAT-1 phosphorylation [22,23]. Importantly, this latter finding is associated with deficient killing of intra-cellular pathogens by IFN-γ-stimulated neonatal macrophages [24], which could have broad implications in vivo.

Decreased production of IL-12 by CBMC stimulated by *Staphylococcus aureus* Cowan (SAC) has also been described [25,17]. Since monocytes are the main producers of IL-12 in SAC-stimulated PBMC, these results also point towards defective activation of neonatal monocytes.

Phagocytosis constitutes a major function of monocytes. In this regard, neonatal monocytes exhibit a trend towards lower phagocytosis of *E. coli* compared to adult monocytes, this alteration being even more pronounced in fetuses younger than 30 weeks of gestation [26]. However, pinocytosis appears functional in neonatal monocytes, since an intact ability to take up proteins such as bovine serum albumin has been reported [27]. Finally, the capacity of monocytes to differentiate into DCs is also altered, as suggested by differences in the morphology of neonatal monocyte-derived dendritic cells (MDDCs) compared to MDDCs from adults [28].

In summary, although defects in TLR activation cascade are clearly present in neonatal monocytes/macrophages, a better definition of both the extent of such defects and their *in vivo* relevance has not yet been achieved. Those signaling pathways converge in the activation of NF-kB and MAP-Kinases. Alternative pathways leading to the activation of these key molecules also appear to exist, but their regulation and function in neonatal monocytes/ macrophages are not yet understood. A better molecular understanding of those pathways is clearly required to better delineate the exact nature of the functional defects of neonatal monocytes.

## Cord blood monocyte-derived dendritic cells

Due to the low frequency of DCs in peripheral blood, most studies of neonatal DCs have been carried out using in vitro monocyte-derived dendritic cells (MDDCs). Defective functions of cord blood MDDCs (CB-MDDC) compared to adult MDDCs have been reported (see Table 1). In particular, CB-MDDCs exhibit signs of immaturity, including a low basal expression of MHC Class II and costimulatory molecules (CD80, CD40). Expression of CD1a molecules, which are important for the presentation of lipopeptidic antigens, is also lower on CB-MDDC [28,29]. Following LPS or necrotic cell stimulation, CB-MDDCs retain an immature phenotype, demonstrated by their failure to up-regulate HLA-DR, CD86 and CD83 [30,31]. As discussed earlier for neonatal monocytes, this incomplete DC maturation may be a consequence of defective responses to TLR signaling. Of interest, mRNA levels of molecules downstream with TLR4 signaling such as MAPKKK, NF-KB and TANK, are markedly decreased in LPS-stimulated CBMDDCs compared to adult MDDCs [32]. However, it is important to underline that the response to other TLRs expressed in this cellular population, TLR1, 2, 3, 6, and 8 and possibly TLR5 [33], has not yet been reported in neonate cells.

CB-MDDC exhibit additional evidence of functional immaturity, including defective endocytosis, which could result from their decreased expression of the mannose receptor [28, 31], and decreased IL-12 production in response to both LPS and CD40L [29]. This IL-12 defect appears to mainly stem from alterations in the expression of the p35 chain, following transcriptional repression at the chromatin level [34], and it can be restored by addition of recombinant IFN-γ [29].

The weak co-stimulation delivered by neonatal APCs may be responsible for altered function of neonatal T cells. Consistent with this hypothesis, CB-MDDCs are poor inducers of proliferation or production of IFN-γ by allogeneic CB T cells, compared to adult MDDCs [28,29,35]. In contrast, mature CB-MDDCs can efficiently prime antigen-specific CD8^{+} T cells [36].

Considered together, those data suggest that neonatal MDDCs require a higher level of activation than adult DCs but, once activated, they are fully competent for the induction of adaptive effector responses. However, the detailed molecular analysis of activation pathways in these cells remains too sketchy to draw definitive conclusions on the functionality of those cells.

## Cord blood dendritic cells

Early studies isolated cord blood DCs (CB-DCs) by fractionation of CBMCs by T-cell rosetting, plate adherence and metrizamide density gradient, and identified them by morphology. Such CB-DCs were poor stimulators of both neonatal and adult allogeneic T cells [37,38], potentially as a consequence of decreased expression of MHC Class II, Class I and ICAM-1 molecules on CB-DCs [37]. Following the discovery of markers that allow a better identification of DC subsets, it was recently shown that CB-DCs (defined as Lineage[−]/HLA-DR[+]) represent 0.3% of the CB mononuclear cells [27]. Interestingly, the majority of the CB-DCs in this study showed no expression of CD11c, the classical marker of myeloid DC (mDCs), but did express CD123, a marker widely used to characterize plasmacytoid DCs (pDCs) [27]. In general, an increased number and proportion of pDCs was reported in CB compared to adult peripheral blood. Widely-different pDC:mDC ratios in CB have been reported by different investigators, ranging from 1:1 to 3:1 [39-43]. Nevertheless, since the usual pDC:mDC ratio in adults is 1:2, a consensus towards increased pDC:mDC ratio in CB is emerging from these studies. However, it should be noted that the use of different combinations of markers to identify pDC and mDC among the studies makes it difficult to directly compare the published data.

CB-DCs appear to be immature, as they exhibit low or no basal expression of CD40, CD80 or CD86 [27,39]. In the latter study, DCs were identified as Lin[−]/HLA-DR^{+}, and differential evaluation of costimulatory molecules on pDCs vs mDCs was not done. In another study, neonatal pDCs (Lin^{−}/HLA-DR^{+}/CD11c^{−}/CD123^{+}) exhibited 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 [44]. Similarly, upregulation of CD40 and CD80 on CB-mDCs in response to LPS and the TLR3 agonist poly (I:C) was significantly lower compared to that of adult mDCs [45]. Of note, in contrast to their lower level on neonatal monocytes, protein expression of TLR2 and TLR4 is normal in neonatal mDCs [41]. However, the regulation of critical molecules involved in TLR signaling has not yet been evaluated in neonatal DCs, and could account for the relative unresponsiveness of neonatal DCs to TLR stimulation.

Neonatal pDCs fail to upregulate IFN-α mRNA expression in response to CpG stimulation [44]. Interestingly, defective production of virus-stimulated IFN-α by unfractionated CB mononuclear cells had been reported in earlier studies [46,47]. Because pDCs were subsequently identified to be the major cellular source of IFN-α in virus-stimulated mononuclear cells, decreased IFN-α production by unfractionated CB mononuclear cells is likely the reflection of defective production by CB-pDCs. Lower levels of cytokine production by CB-mDCs in response to LPS, combined or not with IFN-γ, have been observed and affect TNF-α, IL-1, IL-6, and IL-12 production [40,41]. Interestingly, the latter study showed that the number of expressing cells was equivalent, but the level of expression per individual cell

was lower. In addition, IFN-α secretion by unseparated CB cells is decreased in response to the TLR3 agonist poly (I:C) [45]. Since the expression of TLR3 is limited to mDCs, this result suggests again a defect in the response of these cells to viral products.

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 [39], which was linked to an increased proportion of apoptotic T cells in the former cultures. Mechanisms underlying such 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. The ability of CB-DCs to capture antigenic protein is also altered, suggesting that those cells may in fact not function properly as APCs [27]. However, this hypothesis is challenged by the fact that unfractionated CB-DCs induce strong allogeneic responses [39], suggesting a complex picture in terms of the functionality of neonatal DCs. In that regard, data obtained in babies who have been exposed in utero to viruses bring some important information.

Several cohorts of babies exposed to Human Immunodeficiency Virus-1 (HIV) and human cytomegalovirus (CMV) have been analyzed. Although congenitally HIV-infected infants present both a more severe course of disease and lower T cell reactivity than infected adults [48], it has also been shown that CB T cells from some uninfected neonates born to HIV-infected mothers are strongly responsive to peptides derived from the HIV envelop glycoprotein (env), as detected by IL-2 production after *in vitro* restimulation [49]. In that study, cells from 8 out of 23 neonates responded to two or more env peptides. Also, the proportion of both activated and memory CD4+ T cells (defined as HLA-DR$^+$CD38$^+$ and CD45RO$^+$, respectively) was increased in these neonates compared to neonates born to HIV-uninfected mothers [50]. Moreover, HIV-1-specific CTL precursors have been detected in CB from both uninfected (2/22) and infected (1/3) neonates born to HIV-infected mothers [48, 51]. Similarly, during congenital CMV infection, expansion and differentiation of CMV-specific CD8$^+$ T cells have been observed [52]. These data suggest that priming of both CD4$^+$ and CD8$^+$ T cells can occur in utero, arguing against the hypothesis that neonatal DC are completely defective. However, it should be noted that both HIV and CMV can infect DCs. Since it has been shown in murine models that viral infection of DCs can override the need for CD4$^+$ T cell costimulation to induce CD8$^+$ responses [53], a similar mechanism could be at play for in utero infection by HIV and CMV. Consequently, activation of specific T cell responses may have been induced despite the partial immaturity of the neonatal APC system.

## Mechanisms underlying APC dysfunction

The following mechanisms can be proposed to explain the dysfunction of neonatal APCs (Fig. 1):

1. Intrinsic immaturity of these cells. As mentioned above, neonatal APCs appear to require a higher level of activation to reach functional status than adult APCs do. Experimental evidence suggest that the signaling cascades downstream of TLR engagement are affected in several neonatal APC populations.

2. Defective interaction between APCs and T cells. Since optimal activation of APCs involves crosstalk with activated CD4+ T cells [54,55], defective neonatal T cell activation will induce defective function of neonatal APCs.

3. Inhibition by regulatory T cells. The direct inhibitory effect exerted by natural regulatory T cells on DC function has recently been demonstrated in several murine models [56,57]. In addition, nT$_{reg}$ could indirectly inhibit APC function as a result of their inhibition of effector T cells and/or through secretion of anti-inflammatory cytokines such IL-10 and TGF-β [58-60]. Since nT$_{reg}$ are prevalent and functionally

active in human CB (see below), $T_{reg}$-mediated inhibition could constitute an additional mechanism for APC dysfunction.

## Natural Treg in neonates

Natural regulatory T cells ($nT_{reg}$) have a thymic origin [61] and have proved essential in the establishment and maintenance of immunological tolerance [58]. These cells are characterized by the constitutional expression of CTL-associated antigen 4 (CTLA-4) and of the high affinity IL-2 receptor chain, CD25. They also express high levels of the transcription factor from the forkhead family, FOXP3 [58]. The mechanisms by which $nT_{reg}$ exert their immunosuppressive function are not clearly established, but APCs are one of their cellular targets. An interesting piece of evidence is the $nT_{reg}$ -induced up-regulation of the tryptophancatabolizing enzyme indoleamine 2,3-dioxigenase (IDO) in APCs, mainly DCs [62,63]. Since tryptophan is required for T cell function, its increased catabolism would induce T cell suppression. Increased IDO expression by APCs is mediated through the interactions of CTLA-4, constitutively expressed by $nT_{reg}$, with B7 molecules on DCs. These data have led to propose the concept of IDO-expressing,"tolerogenic" APCs. It has also been observed that the interactions of $nT_{reg}$ and DCs result in the down-regulation of the expression of CD80, CD86 and MHC Class II molecules on DC, which also converts these DCs into "tolerogenic" APCs [57,64]. Finally, $nT_{reg}$ directly interact with DCs during in vivo suppression, further suggesting a role for these cells in the down-regulation of DC function [65]. However, results from in vitro and in vivo studies indicate that $nT_{reg}$ also directly inhibit effector T cell function, something that would indirectly affect DC function. In addition, the role played by the $T_{reg}$ secretion of immunosuppressive cytokines such IL-10 and TGF-β is still debated, since in vivo and in vitro models provide somewhat discrepant results [58-60]. There is also evidence supporting the effect of membrane-associated TGF-β as a mechanism underlying nTreg activity [66]. Finally, it has been postulated that Treg could function as an IL-2 "sink", since they are the only cells expressing at homeostasis the high-affinity IL-2 receptor. Interestingly, FOXP3 has been shown to upregulate both *CTLA-4* and *CD25* genes [67], which could explain why ectopic expression of FOXP3 transforms non-$T_{reg}$ into functional inhibitory $T_{reg}$ [68].

In the neonatal context, $nT_{reg}$ play an important role during pregnancy, maintaining maternal tolerance to the fetus [69]. CTLA-4-expressing $nT_{reg}$ are present in high numbers in the decidua [70,71]. They may be crucial in the high IDO level detected at the fetal-maternal interface, which has been proposed as a major factor in the lower immune reactivity of this compartment [71-75].

At birth, Hara group's recently reported a high proportion of CD4$^+$CD25$^+$ $nT_{reg}$ in CB, which is particularly marked in premature babies compared to full-term babies [76]. These cells express the $T_{reg}$ markers CTLA-4 and FOXP3, but, unlike the adult $nT_{reg}$, they exhibit a naïve phenotype evidenced by CD45RA expression and high levels of T cell receptor recombination excision circles (TREC) [76]. Neonatal $nT_{reg}$ exert potent immunosuppressive activity, and suppress T effector proliferation and cytokine production following stimulation with either mitogens, alloantigens or specific antigens [76-79]. The mechanism(s) by which these $nT_{reg}$ cells exert their suppressive activity in neonates is not fully established but they appear similar to those exerted by adult $nT_{reg}$, since neonatal $nT_{reg}$ act in vitro through contact-dependent, but IL-10- and TGF-β-independent mechanism(s) [76,77]. It has also been observed that CB-MDDCs induce neonatal and adult T cells to produce low levels of IFN-γ but high levels of IL-10, which is reminiscent of a regulatory T cell-type 1 (Tr1) profile [35]. However, the existence of additional inhibitory mechanisms mediated by neonatal $nT_{reg}$ should be further evaluated.

Together, these findings suggest a model whereby elevated numbers of $T_{reg}$ ($nT_{reg}$ or induced Tr1) in neonates are critical in maintaining homeostasis and preventing autoimmunity, as

suggested by the low incidence of graft-versus-host disease observed after CB transplantation [80,81]. Conversely, functional neonatal $T_{regs}$ could play a detrimental role, by impairing the capacity of the neonatal immune system to control infections, in particular through their down-modulation of APC function.

## The immune response in early childhood years

Despite the fact that the immune competence of neonates increases with age, several alterations in the number and function of APCs are present during early childhood. Percentages of circulating mDCs are decreased in 12 month-old children compared to adults [82]. A progressive decrease in the absolute number of pDCs occurs in the first year of life, but adult levels are reached only around 5 years of age [83]. An age-related increase in the capacity of PBMC to synthesize IL-12 in response to LPS or SAC stimulation has also been reported, but levels of production of such cytokine in 12 month-old children remain low [17,25]. A complex picture is emerging from different studies concerning the capacity of APC from young children to stimulate T cells, depending on the functional readout. Indeed, studying responses in children older than a year, Clerici et al. reported adult-levels of T cell proliferation after stimulation with alloantigens or vaccine antigens [84]. In contrast, decreased production of cytokines in response to vaccine antigens was described for T cells from 12-month-old infants [82]. These apparently discrepant results could come from the differential activation signals involved in different T cell functions.

Most of the studies of APC functionality during early childhood have been done in relation to the development of asthma/atopy, and have focused on Th2 versus Th1 pattern. Interestingly, neonates appear to exhibit a predisposition toward production of Th2 cytokines after exposure to environmental allergens [85,2], followed by an age-dependent change toward Th-1-like responses [86,87]. This Th2 bias is usually considered to be a major underlying mechanism for the development of atopy in early life, and could again be a consequence of APC defects. Supporting this concept, low numbers of IL-12-producing cells have been observed in neonates with inherited atopic disease [88,89], as well as low LPS-induced production of IL-12 in children who develop atopic diseases compared to children who do not [90]. The baseline expression of HLA Class II on CB monocytes is lower in children in whom allergic diseases developed within the first two years of life [91]. Children with allergic asthma have lower pDC numbers compared to healthy children. Interestingly, children with atopic dermatitis exhibited increased numbers of another DC population (Lin$^-$/HLA-DR$^+$/CD11c$^-$/CD123$^{dim}$), which are considered to be less differentiated DCs and are also present in high numbers in cord blood [92]. Considered together, these results suggest a role of DC immaturity/dysfunction in the susceptibility to atopic diseases during early life. However, further studies that evaluate the phenotype and function of APCs are clearly required to define the extent of APC defects during early childhood, as well as to better characterize the kinetics of maturation of these cells.

## Conclusions

As reviewed herein, strong experimental evidence suggest functional defects in APC from neonates and young infants. Such defects are expected to contribute to the functional immaturity of the immune system of human newborns, in addition to the intrinsic defects on neonatal T cells. However, the use of different experimental settings has made difficult to compare, and thus validate, the results obtained in different studies. The few studies carried-out with primary DCs, and not in vitro monocyte-derived cells, probably best reflect the *in vivo* phenotype and function of neonatal DCs. However, due to the possibility that maternal factors present in cord blood may influence the characterization of neonatal DCs, the ideal setting would be to study peripheral blood neonatal DCs. Unfortunately, this approach is very difficult, due to the amount of blood required to study such rare cellular populations. Despite

such limitations, the available data strongly suggest a defect of APCs in delivering costimulatory signals to T cells, as a consequence of their incomplete activation and/or maturation. Considering that TLR engagement is one of the major pathways for DC activation, the study of molecules involved in the signaling cascades downstream of TLRs should be a priority for research.

Another mechanism of APC dysfunction in neonates could arise from the activity of $nT_{reg}$. The role played by such cells in the failure of the immune system to control infections on one hand, and in contrast, in controlling autoimmune pathological processes, has emerged only recently. It is expected that the role played by $nT_{reg}$ number and/or activity in many childhood diseases will be recognized in the future. Of great interest as well, several alternative mechanisms mediating $nT_{reg}$ suppressive activity have been postulated, in adults and neonates, including a direct effect on the level of activation of APC. However, the real contribution of each of these mechanisms to neonatal immune dysfunction is currently unknown, and their elucidation will without doubt become a vibrant field of research for immunologists interested in pediatric disorders.

Finally, a better understanding of the kinetics of maturation of APCs during early childhood is clearly necessary to improve the design of vaccination strategies as well as to prevent the development of autoimmune diseases and atopic disorders.

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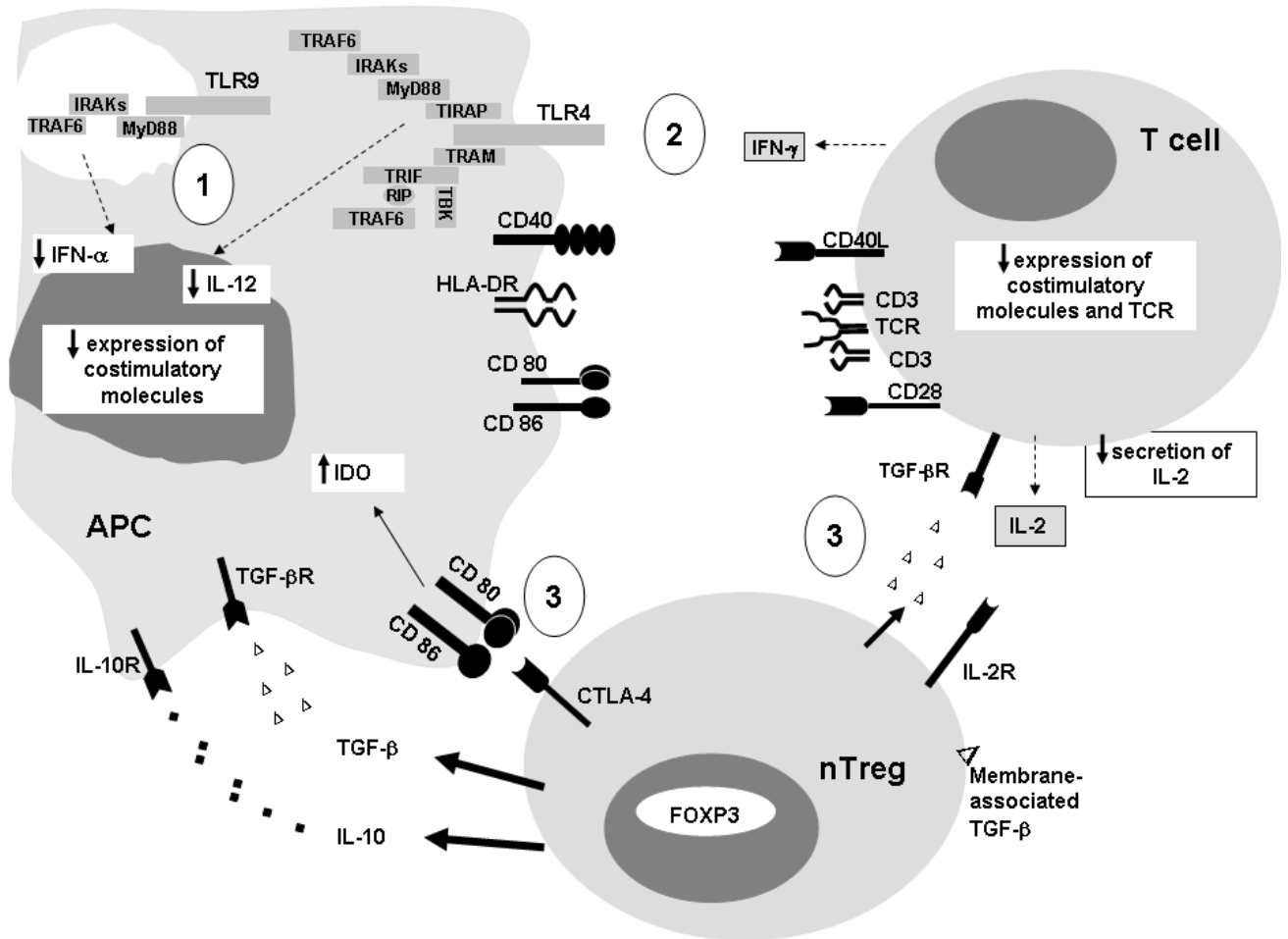
**Figure 1.**
**Potential mechanisms underlying dysfunction of neonatal dendritic cells.** Several mechanisms can be proposed to explain APC dysfunction in neonates:

1. **Intrinsic immaturity of neonatal DCs.** Basal expression of costimulatory molecules is decreased in neonatal APCs. In addition, alterations in the TLR complex, affecting principally TLR4 and TLR9, have been described in neonatal APCs. This could lead to defects in up-regulation of costimulatory molecule expression, as well as cytokine secretion (IL-12, IFN-α) following stimulation with bacterial and/or viral products.

2. **Defective interaction between neonatal APCs and T cells.** Defects in activation levels of neonatal T cells could lead to alterations in APC functions, since T cell-mediated signals play a major role in APC maturation/activation. Among the described T cell defects are alterations in IFN-γ and IL-2 secretion in response to TCR-dependent stimulation, decreased expression of CD40L at basal condition and after stimulation, and decreased expression of the TCR complex.

3. **Inhibition by nTreg of both neonatal APCs and T cells.** Neonatal nTreg can down-modulate the function of both APCs and T cells through direct and indirect mechanisms. Among these mechanisms are interaction between the CTLA-4 molecule expressed by Treg and the CD80/CD86 molecules expressed by APCs, leading to increased IDO expression; secretion of immunosuppressive cytokines, IL-10 and TGF-β, and expression of membrane-associated TGF-β; consumption of

IL-2 by the high-affinity IL-2R expressed by nTreg; and finally, FOXP3-mediated mechanisms that have not yet been identified.

**Table 1**

Findings supporting defective function of cord blood (CB) T cells and neonatal APCs

**Findings that support defective function of CB T cells**
- Low baseline expression of TCR/CD3 complex and adhesion molecules

- Blunted up-regulation of CD40L expression

- Defects in the production of cytokines

- Limited CD8 cytotoxic activity

**Findings that support defective function of CB monocytes and macrophages**
- Low basal levels of expression of costimulatory molecules by monocytes

- Unresponsiveness to LPS and IFN-γ of both monocytes and macrophages

- Reduced capacity of monocytes to differentiate into DCs

- Altered IL-12 production by PBMC

- Decreased phagocytic activity of CB macrophages

**Findings that support defective function of CB dendritic cells**
- Low basal expression of costimulatoy molecules by CB-MDDC, CB mDCs and CB pDCs

- Altered maturation of CB-MDDC, CB mDCs and CB pDCs in response to TLR or CD40 signaling

- Defective production of cytokines by CB-MDDCs in response to TLR or CD40 signaling

- Decreased ability of CB-MDDCs and CB pDCs to stimulate allogeneic responses

- Reduced endocytic activity of CB-MDDCs.