

Phenotype and function of neonatal DC

Fabienne Willems¹, Sabine Vollstedt² and Mark Suter^{3,4}

¹ Institute for Medical Immunology, Université Libre de Bruxelles, Charleroi, Belgium

² Department of Immunology, University of Tokyo, Tokyo, Japan

³ Research Department, Bavarian Nordic, Martinsried, Germany

⁴ Dekanat, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Newborns face complex physical and immunological changes before and after birth. Although the uterus is a sterile environment for the fetus, it also contains non-self material from the mother. Birth involves the transition from the sterile intra-uterine environment to an environment rich in microbes and requires rapid induction of appropriate responses to control these microbes. In this review we focus on the similarities and differences of human and murine neonatal DC and their reaction to various stimuli. A better understanding of the newborn immune system – in particular, the DC–T-cell interaction – will be beneficial for the development of improved strategies to prevent or treat infections in this vulnerable population and prepare the immune system to cope with allergens and tumors later in life.

Key words: Human • Mouse • Newborns • T-cell response • TLR



See accompanying mini-review by Demirjand and Levy

Introduction

Microbial infections are a major cause of infant mortality. During the age of 1–6 months there are more than 2 million annual deaths of human infants caused by viral and bacterial infections affecting the respiratory or intestinal tract [1]. An immature immune system, including incomplete lymphoid structure of bone marrow, spleen and LN, a low number of B and T cells, as well as the absence of memory B and T cells, is the main reason for this high mortality rate.

Furthermore in newborns, the cells of the innate immune system – in particular, DC populations – are low in absolute and relative numbers and are not capable of fully activating both the innate and adaptive immune responses for effective antigen-specific B- and T-cell responses [1–3]. In mice, these “deficiencies” in neonatal DC lead to the development of Th2 immune response to many antigenic stimuli; however, this Th2 skewing is not readily observed in humans [4]. In human newborns,

T-helper responses are often diminished in magnitude, which can affect both Th1- and Th2-cell function [5, 6]. There are, of course, exceptions and under some circumstances human neonates appear to be competent in developing mature CD4⁺ and CD8⁺ T-cell responses [1].

Here we review the recent developments in the understanding of the neonatal immune system of both humans and mice. We focus on DC, as these are important in the first line of defense and are the coordinators of the innate as well as the specific immune response. Wherever possible we compare the immune response of newborns with that of adults. An improved basic understanding of the neonatal immune system of humans and mice may open doors for improved protection of newborns and young adults against infectious agents or deviated immune responses later in life.

Development and nature of the murine and human immune system

In mice, by embryonic day 17 both conventional DC (cDC) and plasmacytoid DC (pDC) can be detected in the fetal thymus from

Correspondence: Dr. Mark Suter
e-mail: Mark.Suter@Bavarian-Nordic.com

Table 1. Total number of murine splenic cDC and pDC, subpopulations of cDC and *in vitro* activation of cells from 7-day-old mice compared with adults^{a)}

	cDC	cDC			pDC	Reference
		CD8 α^+ CD4 $^-$ CD205 $^+$	CD8 α^- CD4 $^+$ CD205 $^-$	CD8 α^- CD4 $^-$ CD205 $^-$		
Number ^{b)}	5×10^6 ; 0.25×10^6	n.t.	n.t.	n.t.	1×10^6 ; 0.12×10^6	[7–12]
Proportions ^{c)}			55;20	20;30	20;50	[7, 11, 12]
MHC II ^{d)}	↓ or =	n.t.	n.t.	n.t.	n.t.	[7–9]
CD80/86 ^{d)}	↓ or =	n.t.	n.t.	n.t.	n.t.	[8, 9, 12]
IL-12p70 ^{d)}	↓ or ↑	↓, =	↑ or ↑	n.t.	n.t.	[7, 8, 12]
IFN- γ ^{d)}	↓ or ↑	Undetectable	↓ or ↑	n.t.	n.t.	[7, 8, 12]
IFN- α ^{d)}	n.t.	n.t.	n.t.	n.t.	= or ↑	[7, 12]
TLR-9 ^{d)}	n.t.	n.t.	n.t.	n.t.	= or ↑	[7, 12]
MLR ^{d)}	↓ or =	n.t.	n.t.	n.t.	n.t.	[9, 11]
T cell ^{e)}	=	n.t.	n.t.	n.t.	n.t.	[7, 9]

^{a)} Splenic DC from adult or neonatal C57BL/6 or Balb/C mice were isolated by positive [8, 9, 12] or negative [7, 10, 11] selection and pDC or cDC subpopulations purified by cell sorting and analyzed as indicated. n.t. Not tested against adult DC, or only published once.

^{b)} Number: cell number in adults; neonate per spleen.

^{c)} Proportion: % of DC (sub)population adult; neonate.

^{d)} reduced in newborns; = similar or no major differences; ↑ increased in newborns. Values directly compared with those of adult DC taken from the literature cited.

^{e)} T cell: specific T-cell stimulation *in vitro*.

which mature T cells emerge and increase rapidly in number to reach a plateau by week 5–6 of age (Table 1) [3, 7, 12]. At birth the predominantly CD5 $^+$ B and CD4 $^+$ and CD8 $^+$ T cells seed the non-organized LN structure. Distinct B-cell follicles are formed around follicular DC and distinct T-cell areas are present by day 7 in LN [13]. Marginal zone B cells are not detected before week 1 [8]. During the first 5–6 wk the number of B and T cells increases by 10^3 – 10^4 fold [2, 3].

As in mice, haematopoiesis takes place in the human fetal liver, and cytoplasmic IgM $^+$ pre-B cells have been detected at ~8 wk of gestation and by 10–12 wk sIgM $^+$ B cells appear [14]. Greater than 90% of B lymphocytes in fetal spleen express CD5 and the CD5 $^+$ population is also the major B-cell subset in cord blood [15]. Cord blood T lymphocytes are different from naive adult T cells. They represent recent thymic emigrants reflected in their high level of expression of TCR excision circles [16]. DC-like cells that express MHC class II can be detected in human fetal thymus and liver [3]. They are identified in mesenteric LN, thymus and in non-lymphoid tissue around 12 wk of gestation, whereas fetal skin and tonsilla are populated by DC-like cells at around 23 wk [17, 18].

The development and nature of the human immune system overall is similar to that of mice but key differences should be noted. In mice, TLR-9 is critical for the control of some viral infections and is expressed on all myeloid cells, pDC and B cells, whereas in humans, TLR-9 is expressed only on B cells and pDC [19–21]. Strikingly, CpG oligonucleotides are able to induce IL-12p70 in all splenic DC subsets in mouse but not in human pDC, which nevertheless express TLR-9 [22, 23]. Moreover, naive mouse B cells constitutively express TLR-9 and proliferate in response to LPS and CpG in the absence of T-cell help. In contrast, human naive B cells do not express high levels of TLR-9

and require several stimuli or T-cell help for antigen-specific proliferation and antibody response [24, 25].

Another major difference between the human and murine immune system is the composition of immune cells in peripheral blood. The majority of human blood consists of neutrophils (70–90%), whereas in mouse blood the majority of cells are lymphocytes (70–90%). In contrast to human neutrophils, which express defensins, murine neutrophils are unable to produce defensins [26, 27]. In addition, Fc α RI, Fc γ RIIA and Fc γ RIIC are not found in mice, which may be important molecules in humans for enabling maternal human antibodies to be used efficiently by the abundant neutrophils to bind and internalize antigen–antibody immune complexes by these FcR [28, 29].

Clearly, as discussed in this section, the differences between human and mice are significant. From the dynamic increase in cell number, their changing nature and proportion, the changing anatomy of the lymphoid and non-lymphoid structures to the differences between the immune systems of a growing human and a growing mouse, all of these factors have to be taken into consideration when immune responses are studied and compared between the two species.

T-cell immune response of neonates against viruses

The immune system of neonatal mice is capable of mounting specific or even protective, mostly T-cell-based immune responses against infectious agents [2], as well as mounting protective memory responses [1, 2, 30–34]. It must be noted that although the mouse neonatal immune system is capable of mounting protective immunity, the nature and stimuli required are different

from those of the adult immune system, and the neonatal-specific immune response is orders of magnitudes lower due to the low precursor frequency of B and T cells and immature lymphoid structures in neonates. Infection with replication-competent virus is one example of stimuli that are capable of activating the neonatal immune system [2, 30, 33, 35]. Upon infection with influenza, murine leukemia, measles, lymphocytic choriomeningitis and vaccinia virus, neonates mount primary virus-specific CTL effector and antibody responses [4, 36]. The responses were generally delayed in onset and showed a shortened peak activity compared with the rapid and strong response observed in adult mice [9, 33, 35–38]. Interestingly, neonatally primed memory T cells might expand more efficiently than T cells primed at a later age [35, 39]. There are several ambiguities with these experimental systems. It is not clear what immune-modulating effects replicating viruses exert, when they are cleared and viral antigen eliminated, and at what time points priming occurs. Interestingly, a full cycle of viral gene transcription and viral particle egress without replication as for disabled infectious single cycle HSV type 1 or a full cycle without egress as for attenuated sendai virus or modified vaccinia virus Ankara (MVA) are sufficient to induce specific or even protective immune responses [31, 34, 40]. In contrast, canarypox, which is able to infect and transcribe early genes only modestly, or inactivated viruses are unable to induce a Th1 or protective immunity in neonates [34, 41, 42]. Thus, viral replication or a full cycle of viral gene transcription and translation may be required to stimulate innate immune cells in a TLR, (e.g. TLR-9)-dependent or TLR-independent manner, leading to subsequent induction of protective adaptive immune responses. Viral infection, but incomplete viral gene transcription, UV-inactivated viruses or protein antigen alone may not be sufficient to induce such responses in neonates [34, 43, 44].

Congenital or neonatal infections associated with viral or bacterial respiratory or intestinal tract infections are still a problem in less-developed countries [36, 45, 46]. Accumulating evidence now shows that *in utero* exposure to infectious pathogens in women can lead to priming of fetal T lymphocytes specific to microbial antigens [47]. A significant increase in the precursor frequency of viral-specific T cells could be detected in children who had congenital or neonatal infections with human cytomegalovirus (HCMV) or HSV; however, when compared with adults, neonatal infection with HSV induces a delayed development of HSV-specific CD4⁺ T-cell response producing IFN- γ [48, 49]. Following congenital HCMV infection, newborns have a lower quantitative CD4⁺ T-cell response to the virus compared with adults [50] but develop an adult-like quality of CD8⁺ T-cell response to HCMV [51]. These observations suggest that under the appropriate conditions of stimulation limited T-cell-mediated anti-viral immune responses can be induced in early life.

Phenotype and function of neonatal DC

DC are recognized as key cells in the innate as well as specific immune response. DC can be separated into two main groups: the

cDC and pDC [3, 52–55]. cDC and their subpopulations are the main inducers of T cells, whereas the pDC are able to produce large amounts of IFN in a very short time and are thus associated with the first line of defence against viral infection. The phenotype of cDC populations and their function in neonates is different from adult cDC and is described in detail below.

Phenotype

At birth ~0.2% of murine splenic cells are DC. The proportion of DC in the spleen increases to ~2.5% within 5–6 wk, together with changes in phenotype and function. The number of pDC in the spleen reaches adult levels by ~3 wk of age (Table 1). From birth to day 7, CD8 α ⁺CD205⁺ cDC dominate, whereas in adults CD8 α ⁺CD205⁺ represented the majority of the DC in the spleen (Table 1) [3, 7, 12]. Similar developmental kinetics of splenic DC populations were seen in irradiated adult mice, which were reconstituted with bone marrow precursor cells [56, 57].

Human DC subsets in the blood are well characterized because of their accessibility. Human blood contains at least two distinct types of DC: myeloid DC (mDC) CD11c⁺ DC and pDC that lack CD11c but express CD123 and BDCA2 [3]. Blood CD11c⁺ mDC are considered immature DC, which are in the process of migrating from the bone marrow to the peripheral tissue. The current paradigm asserts that blood CD11c⁺ mDC localize in the secondary lymphoid organs and peripheral tissues as resting interstitial DC and that they are related to the *in vitro* generated monocyte-derived DC (moDC) or CD34⁺ cell-derived interstitial DC. Blood pDC express L-selectin and migrate to the secondary lymphoid organs [52].

Studies on cord blood DC reveal that the pDC-to-mDC ratio (3:1) is reversed compared with the ratio found in adults (1:3) [58, 59]. mDC and pDC in cord blood were found to be in a more immature state than those in adult peripheral blood as they express lower levels of MHC class II, ICAM-1, CD80 and CD86 [59–63]. Using a whole blood assay, maturation of cord blood mDC induced by LPS or poly(I:C) was incomplete compared with adult cells [61]. Similar observations are made when cord blood pDC were stimulated with CpG oligonucleotides or R-848 [62, 63]. When cord blood monocytes are cultured *in vitro* in the presence of IL-4 and GM-CSF, they differentiated into immature mo-DC, which displayed reduced HLA-DR, CD80 and CD40 surface expression and underwent only partial maturation upon LPS stimulation (Table 2) [64, 65].

Function

Ex vivo isolated splenic cDC from 7-day-old (neonatal) mice express lower levels of MHC class II, CD86 and key cytokines like IL-12p70, which is important for Th1 polarization of naive T-cell precursors (Table 1) [7, 10, 11]. At this age, splenic CD8 α ⁺ and CD8 α ⁺ cDC produce equal amounts of IL-12p70 and IL-12p40, whereas in adults CD8 α ⁺ cDC are the main source of these

Table 2. Comparison of mDC and pDC in human adults and neonates^{a)}

TLR Expression in mDC and pDC					
	mDC mRNA ^{b)}	mDC mRNA ^{c)}	pDC mRNA ^{b)}	pDC mRNA ^{c)}	References
TLR-1	+	n.t.	+	n.t.	[19, 20]
TLR-2	+	=	–		[19, 20]
TLR-3	+	n.t.	–		[19, 20]
TLR-4	+	=	–		[19, 20]
TLR-5	+	n.t.	–		[19, 20]
TLR-6	+	n.t.	+	n.t.	[19, 20]
TLR-7	– or +	n.t.	+	=	[19, 20]
TLR-8	+	n.t.	–		[19, 20]
TLR-9	–		+	=	[19, 20]
TLR-10	+	n.t.	+	n.t.	[19, 20]

Surface molecule expression (adults/newborns)					
	Unstim mDC ^{d)}	LPS, PolyI:C Stim mDC ^{e)}	Unstim DC ^{d)}	CpG, R848 Stim pDC ^{e)}	References
HLA-DR	↓	= or ↓	↓	= or ↓	[58–63]
CD80	↓	↓	=	↓	[58–63]
CD86	= or ↓	= or ↓	= or ↓	↓ or =	[58–63]
CD40	↓	= or ↓	=	↓	[58–63]
CD54	=	n.t.	↓	=	[58–63]

TLR-Induced Cytokine Production (adults/newborns)			
	mDC protein ^{f)}	pDC protein ^{f)}	References
TNF-α	LPS, poly(I:C) =	R-848 ↓	[59, 61, 62, 64]
IL-6	LPS, poly(I:C) =		[64]
IL-8	LPS, poly(I:C) =		[64]
IL-12	LPS, poly(I:C) ↓↓		[61, 64, 65]
IL-23	LPS, poly(I:C), Pam ₃ CSK ₄ , R-848 ↑		[66]
IFN-α	Poly(I:C) ↓	CpG, R-848 ↓↓	[59, 61–63]
IFN-β	LPS ↓↓	CpG, R-848 ↓↓	[67]
CXCL9/10/11	LPS ↓↓		[67]

^{a)} mDC were either purified directly from adult or cord blood or derived from adult or cord blood monocytes. pDC were purified from adult or cord blood; – undetectable; + expressed; = similar or no major difference; n.t. not tested. ↓↓ strongly reduced; ↓ reduced; ↑ increased.

^{b)} mRNA of different TLR members was assessed by RT-PCR.

^{c)} Comparison of TLR expression between adult and neonatal mDC or pDC.

^{d)} The main differences in the expression of surface molecules between adult and neonatal unstimulated mDC or pDC are shown.

^{e)} The main differences in upregulation of surface molecules between adult and neonatal mDC or pDC stimulated by TLR ligands are summarized.

^{f)} The main differences in cytokine production between adult and neonatal mDC or pDC stimulated by TLR ligands are summarized.

cytokines [7, 12]. It must be emphasized that reports on the qualitative capacity of neonatal cDC to produce IL-12p70 and p40 are slightly controversial. In general, two different methods of DC enrichment protocols have been reported – negative selection *via* depletion of all lineage positive cells or positive selection by taking advantage of the murine pan-DC marker CD11c (Table 1). Upon stimulation with CpG-B, neonatal cDC produce significant amounts of IL-12p40 or p70 when selected negatively; however, after positive selection they are capable of producing the same amount of these cytokines as adult cDC (Table 1 and Vollstedt *et al.*, unpublished data). Indeed, the effect of some DC purification protocols on DC activation has been shown and discussed [8, 68]. Similar to adult cDC, IFN response factor (IRF)-5 functions as a master regulator for the induction of pro-inflammatory cytokines in cDC of neonates as shown by the impaired induction of IL-12p40 from IRF-5-deficient neonatal cDC [69] and Vollstedt *et al.* (unpublished data). TLR-9, IRF-5 and $\text{I}\kappa\text{B}\alpha$ are induced upon CpG-B stimulation in a similar manner on neonatal cDC as in cDC from adult mice (Vollstedt *et al.* unpublished data). Thus, splenic cDC of 7-day-old neonates were comparably activated by CpG as adult cDC. Whether this can be extended to other stimuli will require further investigation. LPS administration induced noticeable IL-12p70 secretion in adults but not in 3-day-old neonates while comparable serum levels of TNF- α were detected (Willems and Vollstedt *et al.* unpublished data). Whether this selective lack of LPS-inducible IL-12p70 synthesis reflects a quantitative or a qualitative defect was not determined. Importantly, the age of 7 days seems to be a critical developmental stage at which splenic $\text{CD8}^+\text{CD4}^-$ cDC accumulate with a progressive ability to produce IL-12p70 [70]. Because IRF-3 is required for efficient IL-12p35 synthesis in mouse cDC, it will be critical to compare TLR-3- and TLR-4-mediated 12p35 expression and IRF-3 induction of DC from 3-day-old or 7-day-old neonatal and adult mice [71].

In adults, pDC are well known to sense a variety of different viruses by pattern recognition receptors coupled with an extremely short lag phase to produce 10–100 times more IFN (IFN)- α/β than other immune cells [55, 72]. The importance of IFN- α/β in neonates has been confirmed by several studies [10, 40, 73, 74]. Following the first description of neonatal pDC [10], there is agreement that pDC from 7-day-old neonatal mice are capable of producing similar amounts of IFN- α as their adult counterparts, thus affirming their vital role in the first line of defense against viral infections [7, 11, 12].

Upon stimulation with CpG-A, pDC of neonatal mice use IRF-7 as the main transcription factor for IFN- α production, as demonstrated by the low amount of IFN- α produced by pDC from IRF-7-deficient neonates. This is further supported by the comparable levels of IFN- α secreted by neonatal pDC from both WT and mice deficient in other IRF (Vollstedt *et al.*, unpublished data). Similar results were found using pDC of adult mice [75]. TLR-9, IRF-7 and $\text{I}\kappa\text{B}\alpha$ are fully induced after CpG-A stimulation, though in a slightly slower time frame than in adult pDC (Vollstedt *et al.*, unpublished data).

In humans, mDC represent the major source of IL-12p70. Reduced IL-12p70 production was first observed in LPS-stimulated cord blood mononuclear cells compared with PBMC [76]. Similar findings were obtained in cord blood mononuclear cells or whole blood for other stimuli including group B streptococcus, *Staphylococcus aureus*, poly(I:C), and *Bordetella pertussis* toxin [77–79]. The cellular source of IL-12p70 in these *in vitro* cultures is most probably circulating mDC. Furthermore, cord blood moDC produced very low levels of IL-12p70 in response to LPS, poly(I:C), *B. pertussis* toxin or CD40 ligation (Table 2) [64, 65, 79]. The impaired secretion of IL-12p70 is due to a specific defect in the transcription of the IL-12p35 subunit, while the IL-12p40 subunit transcription is preserved. Adult moDC also produce IFN- β upon TLR-4 stimulation leading to expression of IFN-inducible genes. In LPS-stimulated cord blood, expressions of IFN- β and IFN-inducible genes such as CXCL9, CXCL10 and CXCL11 were selectively impaired [67]. Interestingly, addition of adult plasma did not restore the ability of neonatal blood cells to produce IFN-inducible chemokine or IL-12p70, excluding the possibility that soluble factors such as IL-10 or adenosine would be responsible for this effect, as observed for IL-12 and TNF- α , respectively [8, 80, 81]. Comparison of the profile of genes expressed by LPS-stimulated neonatal and adult moDC further confirmed a defective IFN- β production that was associated with a decreased expression of IRF-3-dependent genes but not of NF- κB -dependent genes. This finding indicates that TRIF-dependent signalling events leading to IFN- β synthesis is preferentially affected in neonatal cells despite comparable levels of TLR-4 expression in adult and neonatal moDC [82]. This is consistent with the deficient up-regulation of co-stimulatory molecules on LPS-stimulated neonatal DC as this process depends on type I IFN autocrine loop [83, 84]. Further experiments revealed that the defect in IRF-3 activation in neonatal moDC was due to altered association of IRF-3 with CBP and impaired IRF-3 DNA binding. In addition to its critical role in TLR-4-mediated IFN- β synthesis, IRF-3 was found to be directly involved in IL-12p35 gene induction [71]. Interestingly, limited IL-12p70 synthesis in neonatal moDC was related to impaired chromatin remodelling in the IL-12p35 promoter region [85]. It is therefore possible that impaired recruitment of IRF-3/CBP complex to IL-12p35 promoter could be responsible for defective synthesis of IL-12p35 subunit in LPS-stimulated neonatal moDC (Table 2).

The observation that NF- κB -dependent pathway in TLR-4 signalling is intact in neonatal moDC is consistent with their ability to produce pro-inflammatory cytokines upon LPS stimulation. Indeed, no difference was detected between adult and neonatal DC as both populations are able to produce comparable levels of TNF- α , IL-6, IL-8 in response to LPS and poly(I:C) [64]. IL-23, a member of the IL-12 cytokine family, is a heterodimeric cytokine formed by the assembly of p40 and p19 subunits [66]. IL-23 is produced by adult mDC cells in response to TLR agonists and its synthesis depends on NF- κB but not on IRF-3. Indeed, DC derived from medullar precursors of mice deficient for IRF-3 secrete IL-23 at levels comparable to WT mice [86] and Willems (unpublished data). In contrast to IL-12p35, the transcription of

IL-23p19 subunit mRNA in stimulated neonatal moDC is comparable to their adult counterparts. Moreover, the secretion of IL-23 protein is similar or higher in neonatal moDC stimulated with different ligands and compared with moDC from adults (Table 2) [66]. IL-23 is critically involved in the maintenance and amplification of Th17 T cells secreting IL-17, a main pro-inflammatory cytokine able to induce epithelial antimicrobial peptides and to mobilize phagocytes at body surfaces to resist extracellular bacilli [87]. Several studies have revealed the role of IL-23 in both protective and T-cell-mediated inflammatory disorders [87]. Neonatal DC-derived IL-23 promoted the differentiation of neonatal CD8⁺ T cells into IL-17-producing cells [88]. Although human neonatal moDC exhibit severe defects in their capacity to mature and synthesize Th1-polarizing cytokines, these data indicate that certain aspects of their activity are functional. Moreover, it appears that neonatal moDC are competent to function at adult cDC levels under some circumstances. Recombinant IFN- γ restores the capacity of neonatal LPS-activated moDC to synthesize IL-12p70 similar to the amount secreted by adult moDC [64, 78]. Gram-positive bacteria from the normal intestinal flora induced similar levels of IL-12p70 in both cord and adult cells [89]. Combined TLR-4/TLR-8 or TLR-3/TLR-8 agonists synergistically increased CD86 and CD83 expression in neonatal cord blood moDC and induced a significant up-regulation of IL-12p70 in neonates, although the level was not quite that of adult moDC [90]. Mature cord blood moDC were found to be efficient to activate antigen-specific CD8⁺ T cells leading to cytolytic activity and IFN- γ production [91]. In addition to cord blood monocytes, CD34⁺ hematopoietic progenitor cells from cord blood can differentiate into DC in response to GM-CSF and TNF- α [92]. Although these DC are able to stimulate proliferation of alloreactive naive T cells, their capacity to produce IL-12 or IL-23 needs to be explored.

There is now evidence that pDC present in human cord blood do not function as their adult counterparts [63]. Indeed, despite comparable levels of TLR-9 and TLR-7 expression in adult and neonatal pDC, neonatal pDC exhibit a severe defect in their capacity to produce IFN- α/β in response to TLR-9 as well as TLR-7 ligation or HCMV or HSV-1 exposure. This defect is detected at both the protein and mRNA levels and was associated with a reduced nuclear translocation of IRF-7 [62]. Taken together with the reduced IFN- α/β production observed in cord blood in response to TLR-3 and -4 ligands, these data indicate that deficient type I IFN synthesis is a key property of TLR-mediated responses in blood cells of human newborns [61].

The defect of neonatal pDC in response to a TLR-7 ligand, R-848, is not only limited to type I IFN production but also involves the acquisition of a mature phenotype. Indeed, cord blood pDC respond to R-848 by up-regulating CD40, CD80, CD86, CCR7, CD54 and HLA-DR molecules; however the magnitude of CD80, CD40, CCR7 and HLA-DR up-regulation was lower in neonatal pDC compared with that of adult pDC. Moreover, R-848-induced TNF- α production was found to be decreased in neonatal pDC

compared with their adult counterparts (Table 2) [62]. In human, the hematopoietic growth factor Flt3-L (FL) treatment strongly expands the numbers of cultured mDC and pDC obtained from peripheral blood of adult donors [93]. FL triggers differentiation of CD34⁺ hematopoietic progenitor cells from human fetal tissues and cord blood into pDC that are able to produce huge amounts of IFN- α/β in response to viral stimulation [94]. This suggests that FL represents a potential tool to circumvent the impaired type I IFN expression in neonatal pDC.

Flt3-L, a poietin with the potential to activate DC in neonates

FL binds to early precursor cells in fetal liver, spleen or bone marrow [95] and increases cDC and pDC two to threefold in FL-treated neonates and is associated with a hundredfold increased resistance against viral infections [10, 11, 74]. The two to threefold FL-induced increase of pDC, however, does not fully explain the hundredfold increased resistance against viral infection originally observed [10]. FL treatment increased the IFN- α production from stimulated pDC marginally but IL-12p70 from stimulated cDC populations up to fiftyfold [11]. MHC class II, CD86 and CD205 expressions on cDC and T-cell stimulatory activity of cDC or novel DC subpopulations are increased in splenic DC of FL-treated C57BL/6 or FL gene deleted (FL^{-/-}) mice [10, 74]. Therefore, in addition to increasing splenic pDC and cDC numbers, FL treatment also leads to increased cytokine production, increased T-cell stimulatory activity and the emergence of novel DC populations (with as yet unknown precursors) without affecting other cells. Indeed, administration of FL to neonatal mice selectively increases the number of DC but not CD5⁺ B cells [10, 96]. CD5⁺ B cells are the main producers of IL-10 in a type I IFN and DC-dependent manner [96]. The increased number of highly stimulatory DC relative to the unchanged number of CD5⁺ B cells might increase pro-inflammatory conditions and thus add one more variable to explain the hundredfold increased resistance of FL-treated neonates against viral infections.

The IFN-FL-pDC loop in neonates

Viral infection induces type I IFN that may have local but also distal effects. To study the effect of viral infection *per se*, the infection event has to be separated from viral multiplication and spread. Thus, attenuated viruses are required. Some live attenuated vaccine viruses are able to infect and transcribe viral genes without viral particle egress and are thus replication deficient but retain their immuno-stimulatory effect for the innate as well as the adaptive immune system. This is exemplified by MVA, which is able to increase resistance against non-related virus in newborns [40, 74]. Resistance was associated with FL-dependent increased pDC and activated cDC 7 days after the infection. Transfer of CD11c⁺ cells but not other cells into naive C57BL/6 or FL^{-/-} mice followed by viral challenge effectively

induced protection. Among the CD11c⁺ cells, pDC but not cDC were associated with this effect [40]. CpG-stimulated pDC were shown to induce IL-10 from CD5⁺ B cells *in vitro* [96]. Interestingly, in a virally driven but otherwise similar system, pDC induced only minute amounts of IL-10 from CD5⁺ B cells, indicating that host–pathogen interaction may tune DC differently compared with specific TLR engagement by CpG [96]. Indeed, MVA or disabled infectious single cycle HSV-1 activate DC in a TLR-9-dependent as well as TLR-9-independent pathway [44]. Treatment of FL^{−/−} mice at birth with recombinant (r) murine or human rFL reconstituted splenic cell number and resistance to HSV-1 infection similar to C57BL/6 mice; however, the transfer of 5×10^6 pDC to FL^{−/−} mice was ten times less protective than recombinant FL-treated mice [74]. Transfer of pDC though effective may not mimic endogenous induction of cells and the required anatomical structure to a full extent [74]. rFL or IFN-induced FL may promote the induction of pDC and additional CD11c⁺ cells or other cells [43] capable of producing IFN- α present in appropriate anatomical locations including bone marrow.

Conclusion

This review has emphasized the nature and major functional properties of DC from neonatal mice and humans. Splenic cDC from newborn mice or cord blood mDC express lower levels of MHC and CD80/86 molecules and produce decreased amounts of IL-12p70 compared with adult DC upon activation, whereas IL-6, IL-8 and TNF- α production is similar. Moreover, the secretion of IL-23 by cord-blood-derived cDC in response to TLR agonists is even higher than in adults. Neonatal DC from both species are able to stimulate CD8 T cells similar to adult cells but quantitative adult-like Th1 immune responses are not achieved. The capability of neonatal splenic mouse pDC to produce IFN I is similar to pDC from adult animals. In contrast, IFN I production from cord blood pDC is severely reduced in comparison with adult pDC. Interestingly, elevated serum FL in mice or rFL provided to cultures of cord blood pDC increases the number of pDC able to secrete adult-like amounts of IFN I after adequate stimulation.

An important question is the extent to which the mouse and human findings can be aligned in view of the differences in their immune systems. To efficiently control (viral) infections, newborns of both species depend on maternal antibodies that function in association with complement and Fc receptors. Compared with mice, humans have a wider variety of Fc receptors distributed over more cell populations, in particular neutrophils. This may render antibody-mediated protection more efficient in humans. Newborn mice may compensate for this by the effectiveness of their pDC.

Whether the qualitative differences between mouse and human DC are due to distinct intrinsic features between the two species or relies on the different experimental models used remains to be investigated. Human studies are mainly based on cord blood cells, whereas spleen DC were generally used in

newborn mouse experiments. Human moDC are now considered inflammatory DC, whereas spleen DC are classified as tissue-resident cells. The identification of mouse homologues of human blood mDC and pDC will further help to clarify the rules that govern neonatal DC responses [54, 97]. Researchers should also be aware of the dynamics of the developing immune system within a growing body that makes analysis of DC in this context a demanding process. This process can further be influenced by hormones notably corticosteroids in milk [98, 99].

Much remains to be learned about the molecular mechanisms by which the neonatal adaptive immune system may be instructed to generate protective T-helper responses while considering the fact that each infectious agent has its own agenda. Studies mentioned above suggest that defective IRF-3 activation in cord blood mDC and impaired IRF-7 translocation in cord blood pDC represent two key molecular signatures underlying the immaturity of neonatal DC in humans. Although IL-12 and type I IFN are likely to be compromised in human newborns, murine studies will allow to test the possibility that whether an efficient IL-23/IL-17 axis might be important for the protection against infectious pathogens.

Acknowledgements: We thank Drs. Hubertus Hochrein, Richard Nichols, Bavarian Nordic, Michel Goldman Université Libre de Bruxelles and Li Wu, The Walter and Eliza Hall Institute of Medical Research, Melbourne for critically reading the paper and Tadatsugu Taniguchi, University of Tokyo, Japan for sharing information. Research at the University of Zurich was supported by the Kanton of Zurich, the Swiss National Science Foundation and a grant from Bavarian Nordic Munich. The Institute for Medical Immunology (Université Libre de Bruxelles) is sponsored by the government of the Walloon region and GlaxoSmithKline Biologicals. This study was also supported by the Fonds National de la Recherche Scientifique (Belgium) and an Interuniversity Attraction Pole of the Belgian Federal Science Policy.

Conflict of interest: Mark Suter has a dual position at the University of Zurich and Bavarian Nordic, Munich.

References

- 1 Siegrist, C. A., The challenges of vaccine responses in early life: selected examples. *J. Comp. Pathol.* 2007. **137**: S4–S9.
- 2 Ridge, J. P., Fuchs, E. J. and Matzinger, P., Neonatal tolerance revisited: turning on newborn T cells with dendritic cells [see comments]. *Science* 1996. **271**: 1723–1726.
- 3 Wu, L. and Liu, Y. J., Development of dendritic-cell lineages. *Immunity* 2007. **26**: 741–750.

- 4 Lambert, P. H., Liu, M. and Siegrist, C. A., Can successful vaccines teach us how to induce efficient protective immune responses? *Nat. Med.* 2005. 11: S54–S62.
- 5 Vekemans, J., Ota, M. O., Wang, E. C., Kidd, M., Borysiewicz, L. K., Whittle, H., McAdam, K. P. et al., T cell responses to vaccines in infants: defective IFN γ production after oral polio vaccination. *Clin. Exp. Immunol.* 2002. 127: 495–498.
- 6 Gasparoni, A., Ciardelli, L., Avanzini, A., Castellazzi, A. M., Carini, R., Rondini, G. and Chirico, G., Age-related changes in intracellular TH1/TH2 cytokine production, immunoproliferative T lymphocyte response and natural killer cell activity in newborns, children and adults. *Biol. Neonate* 2003. 84: 297–303.
- 7 Dakic, A., Shao, Q. X., D'Amico, A., O'Keeffe, M., Chen, W. F., Shortman, K. and Wu, L., Development of the dendritic cell system during mouse ontogeny. *J. Immunol.* 2004. 172: 1018–1027.
- 8 Sun, C. M., Deriaud, E., Leclerc, C. and Lo-Man, R., Upon TLR9 signaling, CD5 $^{+}$ B cells control the IL-12-dependent Th1-priming capacity of neonatal DCs. *Immunity* 2005. 22: 467–477.
- 9 Dadaglio, G., Sun, C. M., Lo-Man, R., Siegrist, C. A. and Leclerc, C., Efficient *in vivo* priming of specific cytotoxic T cell responses by neonatal dendritic cells. *J. Immunol.* 2002. 168: 2219–2224.
- 10 Vollstedt, S., Franchini, M., Hefti, H. P., Odermatt, B., O'Keeffe, M., Alber, G., Glanzmann, B. et al., Flt3 ligand-treated neonatal mice have increased innate immunity against intracellular pathogens and efficiently control virus infections. *J. Exp. Med.* 2003. 197: 575–584.
- 11 Vollstedt, S., O'Keeffe, M., Odermatt, B., Beat, R., Glanzmann, B., Riesen, M., Shortman, K. and Suter, M., Treatment of neonatal mice with Flt3 ligand leads to changes in dendritic cell subpopulations associated with enhanced IL-12 and IFN- α production. *Eur. J. Immunol.* 2004. 34: 1849–1860.
- 12 Sun, C. M., Fiette, L., Tanguy, M., Leclerc, C. and Lo-Man, R., Ontogeny and innate properties of neonatal dendritic cells. *Blood* 2003. 102: 585–591.
- 13 Cupedo, T., Lund, F. E., Ngo, V. N., Randall, T. D., Jansen, W., Greuter, M. J., de Waal-Malefyt, R. et al., Initiation of cellular organization in lymph nodes is regulated by non-B cell-derived signals and is not dependent on CXC chemokine ligand 13. *J. Immunol.* 2004. 173: 4889–4896.
- 14 Dorshkind, K. and Montecino-Rodriguez, E., Fetal B-cell lymphopoiesis and the emergence of B-1-cell potential. *Nat. Rev. Immunol.* 2007. 7: 213–219.
- 15 Bhat, N. M., Kantor, A. B., Bieber, M. M., Stall, A. M., Herzenberg, L. A. and Teng, N. N., The ontogeny and functional characteristics of human B-1 (CD5 $^{+}$ B) cells. *Int. Immunol.* 1992. 4: 243–252.
- 16 Hassan, J. and Reen, D. J., Human recent thymic emigrants--identification, expansion, and survival characteristics. *J. Immunol.* 2001. 167: 1970–1976.
- 17 Hofman, F. M., Danilovs, J. A. and Taylor, C. R., HLA-DR (Ia)-positive dendritic-like cells in human fetal nonlymphoid tissues. *Transplantation* 1984. 37: 590–594.
- 18 Janossy, G., Bofill, M., Poulter, L. W., Rawlings, E., Burford, G. D., Navarrete, C., Ziegler, A. and Kelemen, E., Separate ontogeny of two macrophage-like accessory cell populations in the human fetus. *J. Immunol.* 1986. 136: 4354–4361.
- 19 Kadowaki, N., Ho, S., Antonenko, S., Malefyt, R. W., Kastelein, R. A., Bazan, F. and Liu, Y. J., Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J. Exp. Med.* 2001. 194: 863–869.
- 20 Hornung, V., Rothenfusser, S., Britsch, S., Krug, A., Jahrsdorfer, B., Giese, T., Endres, S. and Hartmann, G., Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J. Immunol.* 2002. 168: 4531–4537.
- 21 Lund, J., Sato, A., Akira, S., Medzhitov, R. and Iwasaki, A., Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J. Exp. Med.* 2003. 198: 513–520.
- 22 Uematsu, S. and Akira, S., Toll-Like receptors (TLRs) and their ligands. *Handb. Exp. Pharmacol.* 2008: 1–20.
- 23 Cao, W. and Liu, Y. J., Innate immune functions of plasmacytoid dendritic cells. *Curr. Opin. Immunol.* 2007. 19: 24–30.
- 24 Bernasconi, N. L., Onai, N. and Lanzavecchia, A., A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. *Blood* 2003. 101: 4500–4504.
- 25 Bourke, E., Bosio, D., Golay, J., Polentarutti, N. and Mantovani, A., The toll-like receptor repertoire of human B lymphocytes: inducible and selective expression of TLR9 and TLR10 in normal and transformed cells. *Blood* 2003. 102: 956–963.
- 26 Mestas, J. and Hughes, C. C., Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 2004. 172: 2731–2738.
- 27 Risso, A., Leukocyte antimicrobial peptides: multifunctional effector molecules of innate immunity. *J. Leukoc. Biol.* 2000. 68: 785–792.
- 28 Daeron, M., Structural bases of Fc gamma R functions. *Int. Rev. Immunol.* 1997. 16: 1–27.
- 29 Monteiro, R. C. and Van De Winkel, J. G., IgA Fc receptors. *Annu. Rev. Immunol.* 2003. 21: 177–204.
- 30 Nossal, G. J. V., The immunological response of fetal mice to influenza virus. *Aust. J. Exp. Biol. Med. Sci.* 1957. 35: 549–558.
- 31 Siegrist, C. A., Saddallah, F., Tougne, C., Martinez, X., Kovarik, J. and Lambert, P. H., Induction of neonatal TH1 and CTL responses by live viral vaccines: a role for replication patterns within antigen presenting cells? *Vaccine* 1998. 16: 1473–1478.
- 32 Astori, M., Finke, D., Karapetian, O. and Acha-Orbea, H., Development of T-B cell collaboration in neonatal mice. *Int. Immunol.* 1999. 11: 445–451.
- 33 Garcia, A. M., Fadel, S. A., Cao, S. and Sarzotti, M., T cell immunity in neonates. *Immunologic Res.* 2000. 22: 177–190.
- 34 Franchini, M., Abril, C., Schwerdel, C., Ruedl, C., Ackermann, M. and Suter, M., Protective T-cell-based immunity induced in neonatal mice by a single replicative cycle of herpes simplex virus. *J. Virol.* 2001. 75: 83–89.
- 35 Fadel, S. A., Ozaki, D. A. and Sarzotti, M., Enhanced type 1 immunity after secondary viral challenge in mice primed as neonates. *J. Immunol.* 2002. 169: 3293–3300.
- 36 Adkins, B., Leclerc, C. and Marshall-Clarke, S., Neonatal adaptive immunity comes of age. *Nat. Rev. Immunol.* 2004. 4: 553–564.
- 37 Belnoue, E., Fontannaz-Bozzotti, P., Grillet, S., Lambert, P. H. and Siegrist, C. A., Protracted course of lymphocytic choriomeningitis virus WE infection in early life: induction but limited expansion of CD8 $^{+}$ effector T cells and absence of memory CD8 $^{+}$ T cells. *J. Virol.* 2007. 81: 7338–7350.
- 38 Fernandez, M. A., Evans, I. A., Hassan, E. H., Carbone, F. R. and Jones, C. A., Neonatal CD8 $^{+}$ T cells are slow to develop into lytic effectors after HSV infection *in vivo*. *Eur. J. Immunol.* 2008. 38: 102–113.
- 39 Fadel, S. A., Cowell, L. G., Cao, S., Ozaki, D. A., Kepler, T. B., Steeber, D. A. and Sarzotti, M., Neonate-primed CD8 $^{+}$ memory cells rival adult-primed

- memory cells in antigen-driven expansion and anti-viral protection. *Int. Immunol.* 2006. **18**: 249–257.
- 40 Franchini, M., Hefti, H., Vollstedt, S., Glanzmann, B., Riesen, M., Ackermann, M., Chaplin, P. et al., Dendritic cells from mice neonatally vaccinated with modified vaccinia virus Ankara transfer resistance against herpes simplex virus type I to naive one-week-old mice. *J. Immunol.* 2004. **172**: 6304–6312.
 - 41 Barrios, C., Brawand, P., Berney, M., Brandt, C., Lambert, P. H. and Siegrist, C. A., Neonatal and early life immune responses to various forms of vaccine antigens qualitatively differ from adult responses: predominance of a Th2-biased pattern which persists after adult boosting. *Eur. J. Immunol.* 1996. **26**: 1489–1496.
 - 42 Zhang, X., Cassis-Ghavami, F., Eller, M., Currier, J., Slike, B. M., Chen, X., Tartaglia, J. et al., Direct comparison of antigen production and induction of apoptosis by canarypox virus- and modified vaccinia virus Ankara-human immunodeficiency virus vaccine vectors. *J. Virol.* 2007. **81**: 7022–7033.
 - 43 Hochrein, H., Schlatter, B., O'Keeffe, M., Wagner, C., Schmitz, F., Schiemann, M., Bauer, S. et al., Herpes simplex virus type-1 induces IFN- α production via Toll-like receptor 9-dependent and -independent pathways. *Proc. Natl. Acad. Sci. USA* 2004. **101**: 11416–11421.
 - 44 Samuelsson, C., Hausmann, J., Lauterbach, H., Schmidt, M., Akira, S., Wagner, H., Chaplin, P. et al., Survival of lethal poxvirus infection in mice depends on TLR9 but therapeutic vaccination provides protection. *J. Clin. Invest.* 2008. **118**: 1776–1784.
 - 45 Siegrist, C. A., Mechanisms underlying adverse reactions to vaccines. *J. Comp. Pathol.* 2007. **137**: S46–S50.
 - 46 Levy, O., Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nat. Rev. Immunol.* 2007. **7**: 379–390.
 - 47 Marchant, A. and Goldman, M., T cell-mediated immune responses in human newborns: ready to learn? *Clin. Exp. Immunol.* 2005. **141**: 10–18.
 - 48 Hayward, A. R., Herberger, M. J., Groothuis, J. and Levin, M. R., Specific immunity after congenital or neonatal infection with cytomegalovirus or herpes simplex virus. *J. Immunol.* 1984. **133**: 2469–2473.
 - 49 Burchett, S. K., Corey, L., Mohan, K. M., Westall, J., Ashley, R. and Wilson, C. B., Diminished interferon- γ and lymphocyte proliferation in neonatal and postpartum primary herpes simplex virus infection. *J. Infect. Dis.* 1992. **165**: 813–818.
 - 50 Tu, W., Chen, S., Sharp, M., Dekker, C., Manganello, A. M., Tongson, E. C., Maecker, H. T. et al., Persistent and selective deficiency of CD4⁺ T cell immunity to cytomegalovirus in immunocompetent young children. *J. Immunol.* 2004. **172**: 3260–3267.
 - 51 Marchant, A., Appay, V., Van Der Sande, M., Dulphy, N., Liesnard, C., Kidd, M., Kaye, S. et al., Mature CD8⁺ T lymphocyte response to viral infection during fetal life. *J. Clin. Invest.* 2003. **111**: 1747–1755.
 - 52 Shortman, K. and Liu, Y. J., Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol.* 2002. **2**: 151–161.
 - 53 Randolph, G. J., Angeli, V. and Swartz, M. A., Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nat. Rev. Immunol.* 2005. **5**: 617–628.
 - 54 Shortman, K. and Naik, S. H., Steady-state and inflammatory dendritic-cell development. *Nat. Rev. Immunol.* 2007. **7**: 19–30.
 - 55 Hochrein, H. and O'Keeffe, M., Dendritic cell subsets and toll-like receptors. *Handb. Exp. Pharmacol.* 2008. **183**: 153–179.
 - 56 Kamath, A. T., Pooley, J., O'Keeffe, M. A., Vremec, D., Zhan, Y., Lew, A. M., D'Amico, A. et al., The development, maturation, and turnover rate of mouse spleen dendritic cell populations. *J. Immunol.* 2000. **165**: 6762–6770.
 - 57 Wu, L., D'Amico, A., Hochrein, H., O'Keeffe, M., Shortman, K. and Lucas, K., Development of thymic and splenic dendritic cell populations from different hemopoietic precursors. *Blood* 2001. **98**: 3376–3382.
 - 58 Borrás, F. E., Matthews, N. C., Lowdell, M. W. and Navarrete, C. V., Identification of both myeloid CD11c⁺ and lymphoid CD11c⁺ dendritic cell subsets in cord blood. *Br. J. Haematol.* 2001. **113**: 925–931.
 - 59 Encabo, A., Selves, P., Carbonell-Überos, F. and Minana, M. D., The functional immaturity of dendritic cells can be relevant to increased tolerance associated with cord blood transplantation. *Transfusion* 2007. **47**: 272–279.
 - 60 Hunt, D. W., Huppertz, H. I., Jiang, H. J. and Petty, R. E., Studies of human cord blood dendritic cells: evidence for functional immaturity. *Blood* 1994. **84**: 4333–4343.
 - 61 De Wit, D., Tonon, S., Olislagers, V., Goriely, S., Boutriaux, M., Goldman, M. and Willems, F., Impaired responses to toll-like receptor 4 and toll-like receptor 3 ligands in human cord blood. *J. Autoimmun.* 2003. **21**: 277–281.
 - 62 Danis, B., George, T. C., Goriely, S., Dutta, B., Renneson, J., Gatto, L., Fitzgerald-Bocarsly, P. et al., Interferon regulatory factor 7-mediated responses are defective in cord blood plasmacytoid dendritic cells. *Eur. J. Immunol.* 2008. **38**: 507–517.
 - 63 De Wit, D., Olislagers, V., Goriely, S., Vermeulen, F., Wagner, H., Goldman, M. and Willems, F., Blood plasmacytoid dendritic cell responses to CpG oligodeoxynucleotides are impaired in human newborns. *Blood* 2004. **103**: 1030–1032.
 - 64 Goriely, S., Vincart, B., Stordeur, P., Vekemans, J., Willems, F., Goldman, M. and De Wit, D., Deficient IL-12(p35) gene expression by dendritic cells derived from neonatal monocytes. *J. Immunol.* 2001. **166**: 2141–2146.
 - 65 Langrish, C. L., Buddle, J. C., Thrasher, A. J. and Goldblatt, D., Neonatal dendritic cells are intrinsically biased against Th-1 immune responses. *Clin. Exp. Immunol.* 2002. **128**: 118–123.
 - 66 Vanden Eijnden, S., Goriely, S., De Wit, D., Goldman, M. and Willems, F., Preferential production of the IL-12(p40)/IL-23(p19) heterodimer by dendritic cells from human newborns. *Eur. J. Immunol.* 2006. **36**: 21–26.
 - 67 Aksoy, E., Albarani, V., Nguyen, M., Laes, J. F., Ruelle, J. L., De Wit, D., Willems, F. et al., Interferon regulatory factor 3-dependent responses to lipopolysaccharide are selectively blunted in cord blood cells. *Blood* 2007. **109**: 2887–2893.
 - 68 Schlecht, G., Mouries, J., Poitrasson-Riviere, M., Leclerc, C. and Dadaglio, G., Purification of splenic dendritic cells induces maturation and capacity to stimulate Th1 response in vivo. *Int. Immunol.* 2006. **18**: 445–452.
 - 69 Takaoka, A., Yanai, H., Kondo, S., Duncan, G., Negishi, H., Mizutani, T., Kano, S. et al., Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. *Nature* 2005. **434**: 243–249.
 - 70 Lee, H. H., Hoeman, C. M., Hardaway, J. C., Guloglu, F. B., Ellis, J. S., Jain, R., Divekar, R. et al., Delayed maturation of an IL-12-producing dendritic cell subset explains the early Th2 bias in neonatal immunity. *J. Exp. Med.* 2008. **205**: 2269–2280.
 - 71 Goriely, S., Molle, C., Nguyen, M., Albarani, V., Haddou, N. O., Lin, R., De Wit, D. et al., Interferon regulatory factor 3 is involved in Toll-like receptor 4 (TLR4)- and TLR3-induced IL-12p35 gene activation. *Blood* 2006. **107**: 1078–1084.
 - 72 Soumelis, V. and Liu, Y. J., From plasmacytoid to dendritic cell: morphological and functional switches during plasmacytoid pre-dendritic cell differentiation. *Eur. J. Immunol.* 2006. **36**: 2286–2292.
 - 73 Haller, O., Arnheiter, H., Gresser, I. and Lindenmann, J., Virus-specific interferon action. Protection of newborn Mx carriers against lethal infection with influenza virus. *J. Exp. Med.* 1981. **154**: 199–203.

- 74 Vollstedt, S., O'Keeffe, M., Ryf, B., Glanzmann, B., Hochrein, H. and Suter, M., The long-term but not the short-term antiviral effect of IFN- α depends on Flt3 ligand and pDC. *Eur. J. Immunol.* 2006. **36**: 1231–1240.
- 75 Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., Shimada, N. et al., IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* 2005. **434**: 772–777.
- 76 Lee, S. M., Suen, Y., Chang, L., Bruner, V., Qian, J., Indes, J., Knoppel, E. et al., Decreased interleukin-12 (IL-12) from activated cord versus adult peripheral blood mononuclear cells and upregulation of interferon- γ , natural killer, and lymphokine-activated killer activity by IL-12 in cord blood mononuclear cells. *Blood* 1996. **88**: 945–954.
- 77 Joyner, J. L., Augustine, N. H., Taylor, K. A., La Pine, T. R. and Hill, H. R., Effects of group B streptococci on cord and adult mononuclear cell interleukin-12 and interferon- γ mRNA accumulation and protein secretion. *J. Infect. Dis.* 2000. **182**: 974–977.
- 78 Upham, J. W., Lee, P. T., Holt, B. J., Heaton, T., Prescott, S. L., Sharp, M. J., Sly, P. D. and Holt, P. G., Development of interleukin-12-producing capacity throughout childhood. *Infect. Immun.* 2002. **70**: 6583–6588.
- 79 Tonon, S., Goriely, S., Aksoy, E., Pradier, O., Del Giudice, G., Trannoy, E., Willems, F. et al., *Bordetella pertussis* toxin induces the release of inflammatory cytokines and dendritic cell activation in whole blood: impaired responses in human newborns. *Eur. J. Immunol.* 2002. **32**: 3118–3125.
- 80 Levy, O., Zarembek, K. A., Roy, R. M., Cywes, C., Godowski, P. J. and Wessels, M. R., Selective impairment of TLR-mediated innate immunity in human newborns: neonatal blood plasma reduces monocyte TNF- α induction by bacterial lipopeptides, lipopolysaccharide, and imiquimod, but preserves the response to R-848. *J. Immunol.* 2004. **173**: 4627–4634.
- 81 Levy, O., Coughlin, M., Cronstein, B. N., Roy, R. M., Desai, A. and Wessels, M. R., The adenosine system selectively inhibits TLR-mediated TNF- α production in the human newborn. *J. Immunol.* 2006. **177**: 1956–1966.
- 82 Drohan, L., Harding, J. J., Holm, B., Cordoba-Tongson, E., Dekker, C. L., Holmes, T., Maecker, H. and Mellins, E. D., Selective developmental defects of cord blood antigen-presenting cell subsets. *Hum. Immunol.* 2004. **65**: 1356–1369.
- 83 Honda, K., Sakaguchi, S., Nakajima, C., Watanabe, A., Yanai, H., Matsumoto, M., Ohteki, T. et al., Selective contribution of IFN- α/β signaling to the maturation of dendritic cells induced by double stranded RNA or viral infection. *Proc. Natl. Acad. Sci. USA* 2003. **100**: 10872–10877.
- 84 Hoebe, K., Janssen, E. M., Kim, S. O., Alexopoulou, L., Flavell, R. A., Han, J. and Beutler, B., Upregulation of costimulatory molecules induced by lipopolysaccharide and double-stranded RNA occurs by Trif-dependent and Trif-independent pathways. *Nat. Immunol.* 2003. **4**: 1223–1229.
- 85 Goriely, S., Van Lint, C., Dadkhah, R., Libin, M., De Wit, D., Demonte, D., Willems, F. and Goldman, M., A Defect in Nucleosome Remodeling Prevents IL-12(p35) Gene Transcription in Neonatal Dendritic Cells. *J. Exp. Med.* 2004. **199**: 1011–1016. Epub 2004 Mar 1029.
- 86 Mise-Omata, S., Kuroda, E., Niikura, J., Yamashita, U., Obata, Y. and Doi, T. S., A proximal kappaB site in the IL-23 p19 promoter is responsible for RelA- and c-Rel-dependent transcription. *J. Immunol.* 2007. **179**: 6596–6603.
- 87 Bettelli, E., Oukka, M. and Kuchroo, V. K., T(H)-17 cells in the circle of immunity and autoimmunity. *Nat. Immunol.* 2007. **8**: 345–350.
- 88 Vanden Eijnden, S., Goriely, S., De Wit, D., Willems, F. and Goldman, M., IL-23 up-regulates IL-10 and induces IL-17 synthesis by polyclonally activated naive T cells in human. *Eur. J. Immunol.* 2005. **35**: 469–475.
- 89 Karlsson, H., Hessel, C. and Rudin, A., Innate immune responses of human neonatal cells to bacteria from the normal gastrointestinal flora. *Infect. Immun.* 2002. **70**: 6688–6696.
- 90 Krumbiegel, D., Zepp, F. and Meyer, C. U., Combined Toll-like receptor agonists synergistically increase production of inflammatory cytokines in human neonatal dendritic cells. *Hum. Immunol.* 2007. **68**: 813–822.
- 91 Salio, M., Dulphy, N., Renneson, J., Herbert, M., McMichael, A., Marchant, A. and Cerundolo, V., Efficient priming of antigen-specific cytotoxic T lymphocytes by human cord blood dendritic cells. *Int. Immunol.* 2003. **15**: 1265–1273.
- 92 Caux, C., Massacrier, C., Vanbervliet, B., Dubois, B., Durand, I., Cella, M., Lanzavecchia, A. and Banchereau, J., CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to granulocyte-macrophage colony-stimulating factor plus tumor necrosis factor α : II. Functional analysis. *Blood* 1997. **90**: 1458–1470.
- 93 Pulendran, B., Banchereau, J., Burkeholder, S., Kraus, E., Guinet, E., Chalouni, C., Caron, D. et al., Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets in vivo. *J. Immunol.* 2000. **165**: 566–572.
- 94 Blom, B., Ho, S., Antonenko, S. and Liu, Y. J., Generation of interferon α -producing predendritic cell (Pre-DC)2 from human CD34(+) hematopoietic stem cells. *J. Exp. Med.* 2000. **192**: 1785–1796.
- 95 Lyman, S. D., James, L., Vanden Bos, T., de Vries, P., Brasel, K., Gliniak, B., Hollingsworth, L. T. et al., Molecular cloning of a ligand for the flt3/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. *Cell* 1993. **75**: 1157–1167.
- 96 Zhang, X., Deriaud, E., Jiao, X., Braun, D., Leclerc, C. and Lo-Man, R., Type I interferons protect neonates from acute inflammation through interleukin 10-producing B cells. *J. Exp. Med.* 2007. **204**: 1107–1118.
- 97 O'Keeffe, M., Hochrein, H., Vremec, D., Scott, B., Hertzog, P., Tatarczuch, L. and Shortman, K., Dendritic cell precursor populations of mouse blood: identification of the murine homologues of human blood plasmacytoid pre-DC2 and CD11c+ DC1 precursors. *Blood* 2003. **101**: 1453–1459.
- 98 Groer, M. W., Davis, M. W. and Hemphill, J., Postpartum stress: current concepts and the possible protective role of breastfeeding. *J. Obstet. Gynecol. Neonatal Nurs.* 2002. **31**: 411–417.
- 99 Zahwa, H., Yorty, J. L. and Bonneau, R. H., Elevated maternal corticosterone during lactation hinders the neonatal adaptive immune response to herpes simplex virus (HSV) infection. *Brain Behav. Immun.* 2008. **22**: 339–353.

Abbreviations: cDC: conventional DC · FL: Flt3-L · mDC: myeloid DC · moDC: monocyte-derived DC · HCMV: human cytomegalovirus · IRF: IFN response factor · MVA: modified vaccinia virus Ankara · pDC: plasmacytoid DC

Full correspondence: Dr. Mark Suter, Research Department Bavarian Nordic, Fraunhoferstrasse 13, D-82152 Martinsried, Germany
Fax: +49-898565-13333
e-mail: Mark.Suter@Bavarian-Nordic.com

See accompanying mini-review:
<http://dx.doi.org/10.1002/eji.200838620>

Received: 3/4/2008
Revised: 10/10/2008
Accepted: 22/10/2008