Plasmacytoid Dendritic Cells Engagement by Influenza Vaccine as a Surrogate Strategy for Driving T-Helper Type 1 Responses in Human Neonatal Settings

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Background. The elicitation of T-helper type 1 (Th1) cellular immunity to eradicate intracellular pathogens is a challenging task because of the interleukin 12 (IL-12) deficit observed in early infancy.

Methods. Screening cord blood responses to various pediatric vaccines and Toll-like receptor (TLR) agonists for innate responses and CD4+ T-cell differentiation.

Results. We identified that nonadjuvanted inactivated trivalent influenza vaccine (TIV) was able to cosignal T cells for the production of interferon γ (IFN-γ) in a neonatal setting. This process includes the mobilization of neonatal plasmacytoid dendritic cells (pDCs) as antigen-presenting cells (APCs) that efficiently engage Th1 cells in an IL-12-independent but type I IFN-dependent manner. In addition, cord blood pDCs efficiently cross-presented antigen to CD8+ T cells. Importantly, activation by TIV mainly requires TLR7; however, R848/TLR7- and CpG/TLR9-activated pDCs, which poorly produced IFN-α, induce neonatal Th2 responses.

Conclusions. TLR pathway engagement in pDCs is necessary but not sufficient for a successful neonatal Th1 outcome. We provide evidence of a mature and functional neonatal immune system at the level of APCs and T cells and propose to implement the IFN-α/IFN-γ axis in pediatric vaccination as a surrogate for the defective IL-12/IFN-γ axis.

Keywords. Plasmacytoid dendritic cells; Newborn; Th1; Influenza Vaccine.

Vaccination formulation has shifted from early empirical approaches to late, rationally designed strategies that integrate molecularly defined antigens, and adjuvants into vaccines. On this winding road, systems biology approaches have initiated the definition of a comprehensive picture, using high-throughput immune system analyses, which more accurately define protection correlates [1]. The rationale of vaccine design is, however, facing a paucity of immune system knowledge for the main target population that is receiving immunizations: neonates and infants [2]. How the immune system in early life responds to vaccines is barely known and requires assessment to elaborate the next generation of vaccines.

Cellular immune responses to vaccines remain the major knowledge gap and are generally ignored during evaluations of adult vaccination efficacy. This situation is even worse following pediatric vaccination, for obvious practical and ethical reasons. Despite the efficacy of the BCG vaccine in the induction of T-helper type 1 (Th1) responses [3], newborns are viewed with limited cellular immunity, possibly due to the reduced inflammation to Toll-like receptor (TLR) activation [4-7] and defective antigen-presenting cell (APC) functions [8].
TLR7/8 agonists may behave appropriately [9], although their impact on neonatal interleukin 12 (IL-12) and dendritic cell (DC) functions remains unknown. Emerging subunit vaccines seek molecularly defined, powerful adjuvants with predictable side effects; TLR agonists enter this category but are misfit in the neonatal context, as studies show progressive maturation with age of the IL-12/interferon γ (IFN-γ) response [7, 10].

We and others have shown that neonatal dendritic cells in the mouse model are not intrinsically defective for innate responses [11, 12]. In humans, epigenetic regulation of neonatal pDCs were obtained following receipt of written consent from the mothers of participating infants. Cord blood samples were obtained from the Établissement Français du Sang.

Cord blood monocyte-derived DCs (MoDCs) [13]. CpG/TLR9 activation of neonatal plasmacytoid DCs (pDCs) could be defective for IFN-α production [14]. To what degree the human neonatal innate and adaptive immune system is immature and/or dysfunctional to elicit IFN-γ cellular immunity is unknown. Exploiting our recent demonstration that isolated neonatal pDCs can efficiently respond to a variety of viruses, including herpes simplex virus type 1, human immunodeficiency virus type 1 (HIV-1), and influenza A virus, we [15] investigated their potential contribution in driving IFN-γ–associated T-cell responses. Under these conditions, but also following CpG activation, neonatal pDCs can produce various chemokines and cytokines, including IFN-α. We thus evaluated their capacity to be mobilized to promote neonatal T-helper cell differentiation into Th1 effectors in vitro. Screening neonatal cord blood responses to various pediatric vaccines, we identified the inactivated trivalent influenza vaccine (TIV) as a Th1-driving adjuvant through pDCs activation in a neonatal setting. On the basis of our findings, we propose that vaccination strategies promoting cellular immunity in early life could be based on the IFN-α/IFN-γ axis rather than on the traditional IL-12/IFN-γ pathway, which is hardly mobilized in infants.

**METHODS**

**Blood**

Heparinized cord blood samples were collected from healthy full-term neonates from Maternity Port Royal and Bichat between 2009 and 2012. Buffy coats from 18–60-year-old donors were obtained from the Établissement Français du Sang. Approval from the Institut Pasteur Review Board for Medical Research and the Regional Ethics Committee CPP IDF IV was obtained to conduct the present study. Cord blood samples were obtained following receipt of written consent from the mothers of participating infants.

**Cell Purification**

pDCs were purified from cord blood mononuclear cells (CBMCs) or adult peripheral blood mononuclear cells, using anti–BDCA-4 magnetic beads and an autoMACS Pro apparatus (Miltenyi Biotec). Enriched pDCs were then FACS sorted (>99% pure) as CD123<sup>hi</sup>CD45RA<sup>−</sup>CD11b<sup>−</sup>CD11c<sup>−</sup> on a FACS Aria cell sorter (BD). The gating strategy for pDC purification was as we previously published [13]. pDC depletion was performed using BDCA4 magnetic beads, which led to approximately 80% pDC depletion from the CBMC cell fraction. CD4<sup>+</sup> naive cord and adult T cells were sorted as 99% of CD4<sup>+</sup>CD45RA<sup>−</sup>CD45RO<sup>−</sup>CD25<sup>−</sup>.

**Media and Reagents**

All antibodies were from eBioscience, except those against BDCA-2 (AC144), BDCA-4 (AD5–17F6), and IFN-α (LT27:295), which were from Miltenyi Biotec.

Complete medium consisted of Roswell Park Memorial Institute 1640 containing 5%–10% fetal calf serum and antibiotics (GIBCO BRL). R848 and LPS were from Invivogen. CpG-A (2216; 5′-GGGACGATCGTCGGGCGG-3′) and CpG-B (2006; 5′-TCGTCGTTTGTGTTTGTGTT-3′) were synthesized by Sigma. Human IFN-α was a mixture of 12 subtypes from PBL InterferonSource. Neutralizing anti-IFNAR antibody (MMHAR-2; Cat: 21 385–1), anti–IFN-α (rabbit antibody; Cat: 31 130–1), anti–IFN-β (rabbit polyclonal antibody; Cat: 31 410–1) were from PBL InterferonSource. Normal rabbit immunoglobulin G (IgG; Cat: 2729S) was from Cell Signaling. Neutralizing anti–interleukin 6 (IL-6; Cat: 554 541), anti–IL-12 (Cat: 554 659), anti–tumor necrosis factor α (TNF-α; Cat: 554 508), anti CCR5 (Cat: 555 990), anti-CXCR3 (Cat: 557 184), and control antibodies (mIgG1 [Cat: 553 447] and mlgG2a [Cat: 553 453]) were from BD. Influenza A virus strain A/PR8/1958 (H1N1) was kindly provided by Dr N. Naffakh (Paris, France). HIV MN AT-2 inactivated viral particles were kindly gifted by J. D. Lifson (Frederick, MD). Vaccines were procured as follows: polio vaccine (Imovax) was from Sanofi Pasteur; hepatitis B vaccine (Engerix B, based on hepatitis B virus surface antigen [HBsAg] with Al(OH)<sub>3</sub>) was from GSK; pentavalent vaccine (which included poliovirus; HBsAg; diphtheria; tetanus, and pertussis toxoids; and Bordetella pertussis pertactin and hemagglutinin) plus Haemophilus influenzae b vaccine (Infanrix Hex) were from GSK; measles, mumps, rubella vaccine (Priorix) was from GSK; 2010/2011 and 2011/2012 seasonal TIVs (Vaxigrip, based on influenza A virus subtypes H1N1 and H3N2 and influenza B virus) were from Sanofi Pasteur; and BCG vaccine was from Sanofi Pasteur.

**In Vitro T-Cell Differentiation**

A total of 250 µL of fresh heparinized cord or adult blood was plated in 96-well flat-bottomed plates and stimulated with the indicated reconstituted vaccines at a ratio of vaccine to blood volume of 1:10. Twenty-four hours later, plasma was collected and tested for cytokines. To determine the effect of vaccine stimulation on T-cell polarization, mononuclear cells from the vaccine-exposed cord blood indicated above were separated by
Lymphoprep and were further cultured with Dyna anti-CD3 beads at a ratio of 1:1 for 6 days. The differentiated T cells were restimulated by plate-bound anti-CD3 (10 µg/mL; clone OKT3) and soluble anti-CD28 (0.5 µg/mL; clone CD28.2) for 24 hours.

Isolated pDCs were stimulated for 24 hours and washed twice, and 10^6 pDCs were cocultured with 5 × 10^5 purified allogeneic-naïve CD4^+ T cells from cord blood. Six days later, T cells were restimulated with PMA/Ionomycin for intracellular staining or with plate-bound anti-CD3 and soluble anti-CD28 for 24 hours to test for secreted cytokines.

For the T-cell differentiation using medium conditioned by virus-activated pDCs, 10^4 neonatal pDCs were stimulated with 40 HAU/mL H1N1/A/PR/8 for 24 hours, and the supernatants were collected as conditioned medium. Then, 5 × 10^4 naïve CD4^+ T cells were cultured with Dyna aCD3/aCD28 beads at a ratio of 1:1 by using 50% volume of conditioned medium. Six days later, T cells were restimulated with PMA/Ionomycin for intracellular staining.

**Cytokine Measurement**

After stimulation, supernatants were harvested and stored at −20°C for further analysis. Cytokines and chemokines were measured using Luminex kits (Biosource) according to the manufacturer’s instructions. Prepared samples were run on a Luminex X100 machine and analyzed using Starstation software (Applied Cytometry). Interleukin 22 (IL-22), IL-12p70, and Luminex X100 machine and analyzed using Starstation software (Applied Cytometry). Interleukin 22 (IL-22), IL-12p70, and Luminex X100 machine and analyzed using Starstation software (Applied Cytometry). Interleukin 22 (IL-22), IL-12p70, and the supernatants were collected as conditioned medium.

**Statistical Analysis**

Unpaired t tests were used to compare 2 groups of data. Data are presented as the mean value ± SD. P values of <.05 were considered statistically significant.

**RESULTS**

**TIV Can Drive a Neonatal Th1 Response in a pDC-Dependent Manner**

Neonatal blood was previously shown to be defective for IL-12 in response to LPS [10]. When exposed to LPS/TLR4 and R848/TLR7 and stimulation with 8 different vaccine formulations, neonatal blood showed a decreased production of IL-12p70, whereas similar amounts of IL-23 were produced (Supplementary Figure 1A and 1B). In these conditions, TLR-dependent IL-12–driven neonatal Th1 differentiation is strongly compromised. BCG vaccine has been shown to be one of the rare vaccines to induce neonatal Th1 responses and, additionally, to induce bystander adjuvant effects that enhance Th1 responses to other neonatal vaccines [16]. We have used a surrogate method to study the adjuvant effects of a panel of vaccines on neonatal T-cell differentiation. Whole cord blood was first stimulated with different pediatric vaccines for 24 hours, then CBMCs were separated and further stimulated with aCD3 beads for 6 days. As expected, BCG vaccine induced IFN-γ–producing T cells in correlation with its capacity to trigger innate IL-12 responses in cord blood (Figure 1A and 1B). Despite the lack of detectable IL-12p70 in BCG-stimulated whole cord blood, this is in agreement with the well-described Th1-inducing capacity of BCG vaccine. One possibility could be that very low levels of IL-12p70 were produced and rapidly consumed by other cells.

Interestingly, among the other vaccines, only TIV induced a Th1 differentiation, but it did so independently of innate IL-12 responses (Figure 1A and 1B and Supplementary Figure 2). In addition, no innate IFN-γ response was observed in TIV-stimulated cord blood (Figure 1B). In addition, we observed a similar T-cell cytokine profile following whole cord blood conditioning by TIV and BCG vaccine, including IFN-γ, TNF-α, granulocyte macrophage colony-stimulating factor, and IL-22 (Figure 1A). Assessing the innate inflammatory responses of neonatal whole blood, we found that IFN-α but neither IL-12 nor innate IFN-γ was found in response to TIV (Figure 1B).

First, we determined the innate response of the IL-12 family to vaccines and then to differentiated T cells by analyzing a large panel of secreted cytokines. Importantly, any of the vaccines was not able to induce substantial amount of IL-12p70 required for Th1 induction (Supplementary Figure 1C–1E). BCG vaccine but not other vaccines induced detectable amounts of IL-12p40, IL-23, and IFN-γ (Supplementary Figure 1C–1F). Similar to adult blood, BCG vaccine induced IL-12p40 and IL-23 but not IL-12p70 in neonatal blood (Supplementary Figure 1C–1F).
Because pDCs are a primary source of IFN-α in response to influenza virus, which is expressed in a TLR7-dependent manner [17], we exposed pDC-depleted or total CBMCs to TIV and BCG vaccine. IL-12 secretion remained unchanged for BCG vaccine, but the IFN-α response to TIV was strongly reduced for pDC-depleted CBMCs (Figure 1C), correlating with a decrease in expression of T cell-derived IFN-γ (Figure 1D). We previously reported the production of IL-12p40 by a small subset of CD2−CD5+ pDCs in response to live H1N1 PR/8 influenza virus [13], but we failed to detect any IL-12p40 with CBMCs stimulated by TIV. We conclude that, in addition to BCG vaccine, TIV is able to promote neonatal Th1 responses, albeit with a pDC requirement.

**Influenza Virus Vaccine Formulations Can Educate Neonatal pDCs to Promote Th1 Responses**

The results described above showed that pDC depletion from CBMCs greatly reduced the IFN-α response to TIV and decreased expression of T-cell–derived IFN-γ, which strongly suggests that pDCs play a critical role in driving the Th1 response in the context of TIV stimulation. Next, we directly addressed the APC functions of neonatal pDCs by studying their effect on
the T-helper cell differentiation of naive allogeneic CD4 T cells. Following influenza virus activation, neonatal pDCs promoted the differentiation of IFN-\(\gamma\)- but not IL-4-producing T cells when various live strains of H1N1 or TIV were used (Figure 2A and Supplementary Figure 3A). Together with IFN-\(\gamma\), a few T cells producing IL-22 and IL-10 but not IL-17 were also induced by TIV-activated neonatal pDCs. These results were confirmed by cytokine detection in culture supernatants (Supplementary Figure 3B). Importantly, there was no difference between neonatal and adult pDCs in the capacity to induce Th1

\[ \text{Figure 2. Virus-activated neonatal plasmacytoid dendritic cells (pDCs) act as antigen-presenting cells driving T-helper type 1 (Th1) responses.} \]

A. A total of 10\(^4\) adult or neonatal pDCs were activated with H1N1/A/PR/8 (40 HAU/mL), H1N1/A/California (40 HAU/mL), and trivalent inactivated influenza vaccine (TIV; 1:100 dilution) in a volume of 125 \(\mu\)L for 24 hours. Then, 10\(^4\) pDCs were cocultured with 5 \times 10\(^4\) allogeneic naive neonatal CD4\(^+\) T cells in a volume of 250 \(\mu\)L for 6 days. Intracellular interferon-\(\gamma\) (IFN-\(\gamma\)) and interleukin 4 (IL-4) were determined following PMA/Ionomycin restimulation.

B. Neonatal pDCs were activated by TIV and then cocultured with allogeneic naive neonatal (n = 3) or adult (n = 3) CD4\(^+\) T cells for CD4 T-helper cell differentiation assessed by intracellular staining for the indicated cytokines. The frequency of cytokine-producing cells is expressed as the means ± SD of values from 3 donors. Pie charts display the relative frequency of the indicated cytokine-producing T cells. Abbreviations: IFN, interferon; IL-10, interleukin 10; IL-17, interleukin 17; IL-22, interleukin 22.

C. Graphs illustrating the percentage of cytokine-positive T cells in neonatal (Neo) and adult (Adu) pDC coculture conditions.
differentiation following H1N1 stimulation (Figure 2A), consistent with the notion that innate responses of neonatal pDC are intact following certain viral stimulations, as we recently demonstrated [15]. Besides, neonatal pDCs can drive comparable Th1 responses, using either neonatal or adult naive T cells (Figure 2B).

It should be mentioned that in the absence of innate activation, neonatal pDCs rapidly underwent apoptosis and could not drive any T-helper cell differentiation. These results clearly indicate that both live and inactivated influenza viruses can activate neonatal pDCs and efficiently drive a Th1 response.

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**Figure 3.** Qualitative difference in Toll-like receptor (TLR)–mediated activation of neonatal plasmacytoid dendritic cells (pDCs) leads to predominant T-helper type 1 (Th1) or Th2 responses. Trivalent inactivated influenza vaccine (TIV; 1:100 dilution), H1N1/A/PR/8 (40 HAU/mL), TLR9 agonists (CpGA and CpGB; 5 μg/mL each), or TLR7/8 agonist (R848; 1 μg/mL)–activated neonatal pDCs were used to prime neonatal allogeneic CD4 T-helper cell differentiation, which was analyzed by intracellular staining for interferon γ (IFN-γ), interleukin 4 (IL-4), interleukin 17 (IL-17), interleukin 22 (IL-22), and IL-10. A, Results shown as mean ± SD for TIV (n = 2), H1N1/A/PR/8 (n = 5), and R848 (n = 5). B, Dot plots are shown for all conditions, and pie charts display the relative frequency of the indicated cytokine-producing T cells under the conditions described above.
TLR Triggering of pDCs Can Lead to Either Neonatal Th1 or Th2 Responses

Because TIV activates leukocytes via TLR7 [18], we next compared the capacity of the TLR7 and TLR9 pathways to activate neonatal pDCs for the induction of Th1 responses. Similar Th1 differentiation was obtained with neonatal pDCs activated by CpGA/TLR9 (Figure 3). However, CpGB/TLR9, as well as R848 (a TLR7/8 agonist [19]), mainly induced Th2 differentiation and low levels of IFN-γ, clearly showing that TLR activation of pDCs itself was not sufficient for Th1 priming (Figure 3). pDCs were recently shown to promote Th22 cells [20]. In our experimental conditions, only CpGB efficiently induced Th22 cells, but all stimuli failed to induce Th17 cells (Figure 3). Similar results were obtained with adult pDCs (Supplementary Figure 3). Analysis of costimulatory molecule expression showed that all stimuli induced similar upregulation of CD40 and

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Figure 4. Modulation of neonatal plasmacytoid dendritic cell (pDC) activation by various agonists triggering an identical Toll-like receptor. A, Neonatal pDCs were activated with CpGA (5 μg/mL each), CpGB (5 μg/mL), R848 (1 μg/mL), H1N1/A/PR/8 (40 HAU/mL), or trivalent inactivated influenza vaccine (TIV; 1:100 dilution) for 24 hours. Costimulatory molecule CD40, CD80, CD86, and HLA-DR expression were analyzed by fluorescence-activated cell-sorter analysis. B, Interferon α (IFN-α) and tumor necrosis factor α (TNF-α) were detected in the supernatants at 24 hours, and levels are expressed as the means ± SD of values for 3–6 donors. For panel A, one of 3 representative experiments is shown. *P < .05 for the comparison of the R848 response to TIV. Abbreviation: ND, not detected.
Figure 5. Type I interferons (IFNs) regulate neonatal T-helper type 1 (Th1) differentiation. A, A total of 5 × 10^4 naive CD4+ T cells were stimulated with aCD3/CD28 beads at a ratio of 1:1, and, as indicated, recombinant IFN-α (10^3 U/mL) was added to the culture in the presence or absence of 10 μg/mL neutralizing anti-type I IFN receptor antibody or isotype control antibody. Intracellular IFN-γ and interleukin 4 (IL-4) expression was determined following PMA/Ionomycin restimulation. B–D, H1N1/A/PR/8-stimulated plasmacytoid dendritic cell–conditioned medium was used for the differentiation of the aCD3/aCD28 bead-stimulated naive CD4+ T cells. Neutralizing antibodies against type I interferon receptor, IFN-α/β, interleukin 12 (IL-12), tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), CCR5, CXCR3, or isotype control (Iso mAb; 10 μg/mL for each) was added into the culture. At day 6, differentiated T cells were tested for intracellular IFN-γ and IL-4 expression following PMA/Ionomycin restimulation (B). Alternatively, differentiated T cells were stimulated with plate-bound aCD3 and soluble anti-CD28, and IFN-γ and IL-4 expression was determined from the supernatants (D). The ratio of IFN-γ–positive cells from each condition against the isotype antibody control condition was calculated (C). The results are the means ± SD of values for 5 (C) or 4 (D) independent donors. *P < .05 for the comparison with the isotype-control-treated group.
HLA-DR, whereas limited CD80/CD86 upregulation was found following CpGA, H1N1, and TIV stimulation (Figure 4A). Th2 conditions were associated with a high level of CD80/CD86 expression and low IFN-α production (Figure 4B) following R848 and CpGB activation of pDCs. Under the various conditions described above, pDCs produced similar amounts of TNF-α (Figure 4B). Therefore, successful neonatal Th1 was associated with high IFN-α and CD40 induction and poor CD80/CD86 expression on neonatal pDCs.

**Influence of IFN-α on Neonatal Th1 Polarization**

We next assessed the mechanism involved in IFN-γ cellular immunity induced by activated neonatal pDCs. We first evaluated the influence of type I IFN by differentiating naive neonatal T cells activated by CD3/CD28 beads and cultured with or without IFN-α (Figure 5A). Under these conditions, IFN-α shifted T-cell differentiation from IL-4- to IFN-γ-producing T cells, which was blocked by the addition of an anti-type I IFN receptor (IFNAR) antibody. Medium conditioned by virus-activated pDCs also induced neonatal Th1 responses (Figure 5B–5D). Following pDC activation by viruses, pDCs are known to produce IFN-α, TNF-α, IL-6, CXCL10, CCL3, and CCL4, but among the blocking antibodies tested, only IFNAR blockade inhibited Th1 priming capacity. We previously showed that CD28–CD5+ pDCs were producing IL-12p40 by intracellular staining, but IL-12p40 was not detected in the supernatant of activated pDCs, most likely reflecting the paucity of this subset among neonatal pDCs [13]. Consistent with this, IL-12–blocking antibodies failed to modulate the outcome of Th1 differentiation, demonstrating the IL-12 independence of the phenomenon (Figure 5B–5D). We conclude that the innate cytokine environment following neonatal pDC activation by viruses shaped Th1 responses through type I IFN.

**Neonatal pDCs Efficiently Cross-present Viral Particles to CD8+ T Cells**

The induction of cytotoxic T-cell responses via antigen cross-presentation by human pDCs has also emerged as a new target for vaccine design [21]. When neonatal pDCs were loaded with HIV MN AT-2–inactivated viral particles, they were able to promptly cross-present antigen to an HLA-A2–restricted Gag-specific CD8+ T-cell clone, as is seen with adult pDCs, clearly demonstrating the ability of cord blood pDCs to perform cross-presentation, as well (Figure 6). Overall, neonatal pDCs behave as efficient APCs that are capable of activating T-cell immunity and of driving IFN-γ–producing T cells.

**DISCUSSION**

The age-dependent maturation of the IL-12 response in the context of TLR activation requires the development of alternative strategies for the induction of inflammatory T-cell responses to vaccines and pathogens. After screening for vaccines administered to young infants that could favor Th1 responses, we showed that TIV, which is a poor inducer of neutralizing antibodies in early life, displayed adjuvant properties allowing the differentiation of neonatal T cells into IFN-γ–producing T cells. Initial screen of pediatric vaccines was performed on whole blood to take into account neonatal cell-extrinsic and cell-intrinsic factors. TIV but not other viral preparations, such as live MMR vaccine, was able to condition neonatal blood cells for IFN-γ production. Because pDCs are not permissive to measles virus infection, it is possible that a first round of infection of CD46-positive permissive cells would be necessary to produce any effect, which might explain why effects were not seen in our experimental conditions [22, 23]. Induction of neonatal Th1 responses could be achieved through the mobilization of neonatal pDCs and was dependent on type I IFN production, which occurs in response to contaminating RNA material in the commercial TIV preparation.

pDCs can promote a large variety of T-helper cell differentiation patterns, depending on their activation context. Early studies showed that human pDCs treated with interleukin 3 and CD40L drive Th2 responses [24], whereas treatment with CD40L plus influenza A virus can induce Th1 responses in an IL-12– and IFN-α–dependent manner [25]. More recently, thymic stromal lymphopoietin–treated pDCs were shown to promote Foxp3+ T-regulatory cells [26], while CpGB-activated pDCs also could induce Th22 differentiation in an IL-6– and TNF-α–dependent manner [20], and TLR7 was shown to activate pDCs to support memory Th17 cells through IL-1β and IL-23 [27]. With regard to influenza A virus [17], TIV activates the immune system through TLR7 and in a pDC-dependent manner [18]. However, we show that the activation of pDCs through

Figure 6. Neonatal plasmacytoid dendritic cells (pDCs) are competent for cross-presentation. Adult and neonatal pDCs pulsed with human immunodeficiency virus (HIV) MN AT-2 inactivated viral particles were cultured with a Gag-specific CD8+ T-cell clone, and interferon γ (IFN-γ)–producing T cells were analyzed by enzyme-linked immunosorbent spot analysis. The results are expressed as the means ± SD and are representative of 3 experiments.
TLR7 by TIV and R848 leads to dominant Th1 and Th2 responses, respectively. Similar results were observed when neonatal DCs are activated by CpGA and CpGB, leading to Th1 and Th2 differentiation, respectively. Under our experimental conditions, we have identified that, following the stimulation of TIV or CpGa, pDC-derived type I IFN was important in priming the differentiation of IFN-γ-producing T cells. On the other hand, CpGB and R848, with the induction of very low levels of IFN-α, mainly promoted neonatal Th2 responses. Interestingly, we show the impairment of R848 in inducing neonatal IL-12p70, which suggests that its incapacity to trigger the IL-12/IFN-γ–pathway in conventional DCs, although this needs to be fully investigated. Under all conditions, neonatal pDCs also induced some IL-22–producing T cells, as was previously reported for adult pDCs [20].

Finally, antiviral immunity relies critically on the induction of cytotoxic CD8+ T-cell responses requiring direct antigen presentation, as well as cross-presentation by DCs [28]. DCs derived from neonatal monocytes can perform major histocompatibility complex I presentation [29]. The early demonstration of the capacity for pDC cross-presentation in the context of HIV [21] led us to investigate this issue with circulating neonatal pDCs. Although the exact contribution of pDCs in the overall cross-presentation process remains to be clarified [30], our careful evaluation has shown that neonatal pDCs are fully competent for cross-presentation. Because TIV can induce CD8+ T-cell responses in children as well, cross-presentation is likely to play an important part [31].

We demonstrated that neonatal pDCs can potently participate in innate and adaptive immunity in the context of viral infection, indicating that pDCs may play an active role in protecting neonates from infections. In contrast to early data indicating that pDCs would be poorly effective in early life, we recently showed potent IFN-α responses of neonatal pDC to a large variety of viruses [15]. In line with these observations, we provide evidence that the current inactivated influenza vaccine is able to trigger pDCs to efficiently induce Th1 differentiation. Importantly, we evaluated commercially available vaccines and not laboratory preparations. The Th1 priming capacity of influenza virus–activated neonatal pDCs was correlated with the production of high levels of IFN-α, and we demonstrated with influenza virus that Th1 differentiation occurred in a type I IFN–dependent but IL-12–independent manner. Poliovirus vaccine, as well as other viral vaccines, did not show such potency, in agreement with the Th2 response observed in response to oral poliovirus vaccination [32]. Although replicating viruses were more potent in terms of immune responses, systems biology studies applied to seasonal influenza vaccination highlighted that both TIV and a live attenuated vaccine (LAIV) were able to activate pDC functions in vivo [1]. Adult and >5-year-old children can both develop influenza virus–specific IFN-γ–producing CD4+ T cells [31]. LAIV always demonstrates superior efficacy over TIV [31, 33]. Nevertheless, at ages as young as 6 months, TIV was also shown to induce significant IFN-γ T-cell responses [31]. The limited capacity of TIV in inducing neutralizing antibodies in the very young can be thus distinguished from the adjuvanticity of the vaccine preparation in shaping T-cell responses. Whereas LAIV cannot be administered to very young children, recent safety studies show that TIV is well tolerated starting from 2 months of age [34]. BCG vaccine has been shown to induce bystander adjuvant effects by boosting immune response to other vaccines [16], but systematic BCG vaccination has been stopped in many developed countries. All other pediatric vaccines fail to promote IFN-γ T-cell responses. The ability of TIV to induce CD4 Th1 responses in very young vaccinated children could be investigated as a proof of concept, using TIV as an adjuvant to potently boost pediatric vaccination toward cellular immunity for other vaccines.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank F. Goffinet (CIC Mère-Enfant), for cord blood sample collection; G. Badre, for coordination; Nadia Naftakh (Institut Pasteur), for the H1N1/A/California 2009 virus; R. Golub, for critical reading of the manuscript; and the Center for Human Immunology at Institut Pasteur, for support in conducting these studies.

Financial support. This work was supported by the ANR (grant ANR-09-MIEN-017; support to X. Z. and S. L.); the Fondation pour la Recherche Médicale (grant DEQ20120323719); the French government’s Investissement d’Avenir program, Laboraatoire d’Excellence in Integrative Biology of Emerging Infectious Diseases (grant ANR-10-LABX-62-IBED); the European Commission FP7 ADITEC program (HEALTH-F4-2011-280873 to X. Z. and S. L.); the Shanghai Rising Star program (grant 12QA1403600 to X. Z.); the Pasteur–Paris University International PhD program (to B. M.); Institut Carnot (to B. M.); DIM Malinf et region 1df (to D. Z.); ANRS (to O. S.), Sidaction (to O. S.), and Fondation Areva (to O. S.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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