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Effect of intrauterine HIV-1 exposure on the frequency and function of uninfected newborns' dendritic cells

Paula A. Velilla [a,b,c], Carlos J. Montoya [a], Alvaro Hoyos [a], Maria E. Moreno [a], Claire Chougnet [b,c,1], Maria T. Rugeles [a,*,1]

[a] *Group of Immunovirology, School of Medicine, University of Antioquia, Medellin, Colombia*
[b] *Division of Molecular Immunology, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, USA*
[c] *Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA*

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Abstract Immaturity of the neonatal immune system is considered an underlying factor for enhanced severity of infections in newborns. Functional defects of neonatal antigen-presenting cells lead to defective T-cell responses. T cells from uninfected neonates exposed *in utero* to HIV-1 (EU) exhibit phenotypic and functional alterations; however, the function of their circulating dendritic cells (DCs) has not been characterized. We hypothesized that an HIV-1-infected maternal environment may influence the infants' DC number, phenotype and function. EU exhibited a higher percentage of myeloid DCs (mDCs) than unexposed neonates, although this frequency remained lower than that observed in adults. Plasmacytoid DC (pDC) frequencies were similar in all groups, although both groups of infants tended to have lower frequencies than adults. After LPS stimulation, mDCs from EU up-regulated CD80, CD86 and B7-H1, whereas mDCs from unexposed infants upregulated B7-H1, but not CD80/CD86, and adult mDCs up-regulated mainly CD80 and CD86. IFN-α production was similar in all groups, indicating a normal pDC function. Therefore, *in utero* exposure to HIV-1 induces quantitative and qualitative changes in neonatal DCs, particularly in mDCs, which might be associated with alterations observed in T cells from these EU.

Abbreviations: APCs, Antigen-presenting cells; CB, cord blood; CBMC, cord blood mononuclear cell; CpG ODN, oligodeoxyribonucelotides with CpG motifs; DC, dendritic cells; EU, HIV-1-uninfected neonates born to HIV-1-infected mothers; MC, mononuclear cell; mDC, myeloid dendritic cell; MFI, mean fluorescence intensity; pDC, plasmacytoid dendritic cell; PB, peripheral blood; UN, unexposed neonates (born to HIV-1 negative mothers).

* Corresponding author. Group of Immunovirology, University of Antioquia, Calle 62, No. 52-29, Torre 2, Lab 532, Medellin, Colombia. Fax: +57 4 210 64 81.
E-mail address: mtrugel@catios.udea.edu.co (M.T. Rugeles).
[1] C.C. and M.T.R. contributed equally to this paper.

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Introduction

The high rate of mortality from infections in newborns is likely to arise as a consequence of the immaturity of the immune system, particularly a functional impairment of neonatal T cells has been described [1,2]. However, the fact that neonatal T cells show a normal response to TCR-independent signals and that defective TCR-dependent stimulation is restored by additional costimulation suggest that an impairment of neonatal T cell function is not only the consequence of an intrinsic deficiency of neonatal T cells but may also result from inadequate signals delivered by neonatal antigen-presenting cells (APCs).

Several studies suggest that neonatal APCs are functionally altered. The main dysfunction is detected at the level of costimulatory molecule expression (reviewed in Velilla et al. [3]). Most studies have focused on the evaluation of the costimulatory molecules CD80 and CD86 in neonatal monocyte-derived dendritic cells (MDDCs), a model of mDCs. So far, the expression of inhibitory molecules on neonatal DCs and their role in the functional alterations of these DCs has not been reported; in particular, B7-H1 expression has not yet been studied. B7-H1 is a recently identified molecule belonging to the B7 family, expressed by DCs (reviewed in [4]). It inhibits T cell function in different models [5,6].

Cytokine production by cord blood (CB) and neonatal APCs is also decreased, particularly IL-12 and IL-15 [7,8]. Plasmacytoid DCs are the main producers of Type I IFN (reviewed in [9]), and contradictory data have been published concerning the capacity of neonatal DCs to produce IFN-α [10–12].

Part of the vertically transmitted HIV-1 infection takes place *in utero*, indicating that the placental barrier is permeable to maternal HIV-1. In addition, the fetus is exposed to circulating viral proteins such as gp120. In fact, a proportion of HIV-1-uninfected neonates born to HIV-1-infected mothers (EU) exhibit HIV-1-specific T cells [13,14]. Interestingly, leukocytes, particularly T cells, of EU are functionally altered [15,16], with some of these abnormalities persisting over time [16,17]. These findings suggest that HIV-1 or its products are present in the maternal environment and that, even in the absence of infection, they can alter the development of the immune system in the EU.

Despite the fact that DCs play a critical role by providing the signals required to induce immune responses [18], their frequency, phenotype and functional activity have not been evaluated in EU. Therefore, we studied whether intrauterine exposure to HIV-1 influences the newborns' and infants' DCs number, phenotype and function, particularly the expression of the costimulatory molecules CD80 and CD86, as well as of the inhibitory molecule B7-H1.

Materials and methods

Study population

This prospective observational study enrolled 23 neonates born to HIV-1-infected mothers (EU) and 43 born to HIV-1-uninfected mothers (UN) (Table 1). All infected mothers received intravenous zidovudine (2 mg/kg weight, administered 1 h before c-section, and a zidovudine infusion at 1 mg/kg/h during labor). Twenty-two of these 23 mothers received antiretroviral therapy during pregnancy, mainly zidovudine (600 mg/day) plus lamivudine (300 mg/day), and indinavir (800 mg/every 8 h, tid) or nelfinavir (750 mg, tid) or nevirapine (400 mg/day), according to institutional criteria. The infants were not breast fed and were given HIV-1 infection prophylaxis with oral AZT for 6 weeks [19]. At the time of delivery, 40 ml of CB was collected. A sample of peripheral blood (PB) was obtained during the first 24 h after delivery, and at 1 and 6 months of age to determine whether newborns were perinatally infected by RNA virus load determination (RT-PCR —Cobas, Roche, Indianapolis, IN). At 18 months, an additional sample was taken to determine anti-HIV-1 antibodies by ELISA. In addition, we included a cohort of babies born to HIV-1-infected and -uninfected mothers (n=10 in each group) at different ranges of age (3 to 6, 6 to 9, and 9 to 12 months), to evaluate DC number and phenotype. Four of the EU neonates analyzed at birth were also included in the follow-up. PB samples from 10 healthy HIV-1-uninfected adults were used as control (median age: 23 years, range: 19–43). Six out of these 10 adult controls were nonpregnant women that were also included in the control group for pregnant women, HIV-infected or not. The remaining four individuals were males, to match the male to female ratio seen in neonates.

Institutionally approved informed consents were signed, according to Colombian government resolution 00843 of 1993 legislation.

Reagents

Fluorochrome-labeled mAb against the molecules CD11c, CD123, HLA-DR, CD80, CD86, lineage markers (Lin-1, anti-CD3, CD14, CD16, CD19, CD20 and CD56), CD34 and the corresponding isotype control antibodies was from Becton Dickinson-Pharmingen (San-Jose, CA). The anti B7-H1 mAb was from eBiosciences (San Diego, CA), while the anti-BDCA-

Table 1 Characteristics of mothers and infants studied

	Mothers				Newborns/Infants
	Age	Gestational (age in weeks)	Viral load (copies/ml)	CD4 counts (cell/μl)	HIV-1 status (*)
	Median (range)	Median (range)	Median (range)	Median (range)	
HIV+ group (n=23)	26 (18–42)	37.35 (34–39)	1370 (400–300,000)	480 (76–990)	Negative
HIV− controls (n=43)	20 (18–40)	39.05 (37–41.2)	NA	NA	Negative

Results are expressed in median (range). All HIV-infected mothers were given AZT prophylaxis. NA: not applicable.
*HIV-1 negative status was determined by viral load analysis at birth, 1 and 6 months and an ELISA test at 18 months of age.