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In Vivo Effects of HIV-1 Exposure in the Presence and Absence of Single-Dose Nevirapine on Cellular Plasma Activation Markers of Infants Born to HIV-1–Seropositive Mothers

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Summary

Short-course antiretroviral drug regimens reduce the risk of mother-to-child transmission of HIV-1, but mechanisms affording protection of such interventions remain poorly defined. Because T-cell activation is an important factor in productive HIV-1 infection, we tested the hypothesis that single-dose nevirapine (NVP) reduces immune activation, which in turn reduces the likelihood of transmission. We compared concentrations of cord and maternal blood plasma immune activation markers, neopterin, β_2-microglobulin, and soluble $_L$-selectin, in 2 groups of HIV-1–exposed newborns whose mothers either received NVP at the onset of labor or who only received NVP as postexposure prophylaxis within 72 hours of birth and among HIV-unexposed controls. In utero exposure of the infant to HIV-1, regardless of NVP exposure, led to demonstrable increases in immune activation markers, this being most notable in the presence of preexisting infection. Contrary to what was hypothesized, immune activation was increased by prebirth exposure to single-dose NVP, with this effect being enhanced in infants already infected at birth. Our data suggest that reductions in immune activation do not explain transmission prevention effects of single-dose NVP. Our data also suggest a biological explanation for why HIV-1–infected infants exposed perinatally to antiretroviral drugs might experience hastened disease progression, namely, in some HIV-1–infected individuals, NVP may synergize with HIV-1 to enhance an environment that favors increased HIV-1 replication.

Keywords

plasma immune activation markers; newborn; nevirapine; HIV-1–seropositive mothers

Antiretroviral drugs, even if given in short and simple regimens, can dramatically reduce the risk of mother-to-child HIV-1 transmission.[1-5] The simplest regimen involves only 1 dose of nevirapine (NVP) to the mother at the onset of labor and 1 dose to the infant within 72 hours of birth.[2,6] Although consistently observed, the efficacy of these regimens is surprising

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because short regimens of monotherapy are expected to have, at best, minor effects on maternal viral load. The reduction in maternal plasma viral load achieved with even the longest of these regimens explained only a fraction of the transmission reduction caused by the regimen.[7]

There is general consensus that productive replication of the HIV-1 viral genome in $CD4^+$ T lymphocytes depends on cellular activation.[8,9] However, exposure to HIV-1 in the absence of seroconversion has been shown to induce HIV-specific, cell-mediated immune responses,[10] a process which, in its own right, would require T-cell activation. If HIV-specific, cell-mediated immune responses are truly protective, then we have the paradoxical situation that protective immunity can successfully occur in an immune environment that ordinarily may be considered to favor HIV-1 replication. Some antiretroviral drugs, for example, the protease inhibitor indinavir,[11] have been shown to have immunomodulatory consequences distinct from benefits attributable to control of viral replication. One consequence observed in vivo after a short-course zidovudine-lamivudine (AZT-3TC) regimen was reduced memory T-cell responses to HIV.[12] These findings raise the question of whether there may be immune-suppressive effects of antiretroviral drugs given to prevent maternal-infant HIV transmission. Characterization of immunomodulatory influences of antiretroviral drugs may help explain why drugs are effective to reduce transmission.

In this study, we tested the hypothesis that the efficacy of NVP to reduce maternal-infant HIV-1 transmission may in part be a consequence of its ability to reduce immune activation as measured by decreased levels of 1 or more of the immune activation markers, neopterin, β_2-microglobulin (β_2-m), and soluble $_L$-selectin (s_L-selectin), all soluble factors which have been used to quantify levels of T-cell activation in vivo.[13,14]

METHODS

Study Population

The study population included HIV-seropositive and HIV-seronegative women delivering live-born infants at Chris Hani Baragwanath Hospital, Soweto, South Africa. Women who had not received any antiretroviral drugs before the infant's birth either for prevention of mother-to-child HIV-1 transmission or for HIV treatment (n = 124) were recruited as part of a postexposure prophylaxis (PEP) trial.[15] Women were eligible for the trial if they tested HIV positive for the first time after delivery and were drug naive usually because they received no prenatal care or had attended prenatal clinics where HIV testing was not available at the time. Their infants were randomized to receive either AZT or NVP to reduce the risk of vertical transmission. Drug-exposed, HIV-positive women (n = 78) were recruited among women delivering at the hospital around the same time who had attended prenatal clinics at Chris Hani Baragwanath Hospital, had received HIV counseling and testing there, and had accepted NVP as part of a demonstration of antiretroviral therapy (DART) initiative. NVP was given, as previously described,[2] as a single maternal 200-mg oral dose at the onset of labor and a single 0.6-mL infant dose within 72 hours of birth. A control group of pregnant, HIV-seronegative women (n = 30) was recruited at the same site over the same period.

The infants of these mothers were followed up prospectively after birth to determine their HIV status until 3 months or until at least 4 weeks after all breast-feeding had stopped. HIV-1 DNA polymerase chain reaction (PCR) was performed on infant peripheral blood samples collected at 6 weeks of age (Amplicor, Roche Diagnostic Systems, Inc, Branchburg, NJ). Samples collected on the day of birth were tested if the 6 weeks' PCR was positive to establish the timing of infection. A positive result on the day of birth was used to infer intrauterine (IU) transmission, and a negative result at birth with a positive result at 6 weeks or later was used to infer intrapartum transmission (IP). Children negative at 6 weeks or older were included as

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exposed-uninfected (EU). Children born to HIV-seronegative women (control group) were not followed up after birth.

We selected for this nested, case-control study all 18 HIV-infected infants (4 IU and 14 IP) and a random sample of 63 uninfected infants from the cohort of 124 infants born to HIV-seropositive mothers who did not receive any antiretroviral drugs before delivery (PEP). We also selected all 7 HIV-infected infants (4 IU and 3 IP) and a random sample of 54 uninfected infants from the cohort of 78 infants born to HIV-seropositive mothers who received single-dose NVP before delivery (DART). Samples were insufficient for this analysis for 1 of the IP infected infants (PEP group).

Laboratory Methods

Small aliquots (3–5 mL) of cord blood were obtained by cordocentesis immediately after delivery of the placenta, and 10 mL peripheral blood was obtained from each mother within 24 hours of delivery. Blood samples were drawn into EDTA tubes, and plasma samples were processed by standard procedures within 24 hours of collection and stored at −70°C until testing.

As levels of the plasma immune activation markers, neopterin, β_2-m, and s_L-selectin, are considered early and reliable markers of immune activation and response reflecting the activation of a variety of different cell types (neopterin: T cells/monocytes, β_2-m: $CD8^+$ T cells, and sL selectin: T lymphocytes, monocytes, and neutrophils) as well as being commonly used as diagnostic/prognostic tools of HIV-1 disease, a reduction in any of these markers was thought to best demonstrate an associated reduction in cellular immune activation. Maternal and infant (cord blood) plasma levels of the immune activation factors, β_2-m and s_L-selectin, were determined using the commercially available Quantikine ELISA assay kits obtained from R&D Systems, Inc (Minneapolis, MN), as described by the manufacturer. β_2-m levels were determined using undiluted plasma, whereas s_L-selectin determination required a 100-fold dilution of the samples. Neopterin was quantitated in undiluted plasma using an Immunotech ELISA system (Beckman Coulter, Marseille, France). The minimum detectable dose of β_2-m is less than 0.2 μg/mL; for s_L-selectin, less than 0.3 ng/mL; and for neopterin, 0.2 ng/mL.

Plasma HIV RNA levels were measured using the Roche Amplicor RNA Monitor assay (Roche Diagnostic Systems, Inc) with a lower detection limit of 400 RNA copies/mL. CD4 T-cell counts were determined using the commercially available FACSCount System from Becton Dickinson (San Jose, CA).

Statistical Analysis

Concentrations of the activation markers in plasma were compared between the groups using the nonparametric Mann-Whitney *U* test. Spearman rank correlation coefficient was used to describe associations between continuous variables. Differences between mothers' and infants' activation markers were tested using the Wilcoxon signed rank test for paired data. Statistical analyses were performed using SPSS software (version 11.0, SPSS, Inc, Chicago, IL). All statistical tests were 2-tailed and considered significant at *P* values of less than 0.05. No statistical corrections were applied for the multiple comparisons.

RESULTS

Clinical Characteristics of HIV-1–Seropositive Mothers and Their Infants

Clinical characteristics of the mother-infant participants who were included in this nested, case-control study are presented in Table 1. Viral loads were significantly higher among mothers

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who had not received any antiretroviral drugs before delivery (PEP) compared with mothers given NVP at the onset of labor (DART).

Infants Exposed to or Infected With HIV-1 Demonstrate Greater Immune Activation at Birth Than Control Uninfected Infants

To establish if exposure to and/or infection with HIV-1 leads to increased immune activation as evidenced by raised levels of cord-blood plasma activation markers, infants born to HIV-1–infected mothers were stratified on the basis of their infection outcomes as EU, IP (acquired infection IP, birth PCR negative, and 6-week PCR positive), and IU (acquired infection in utero and birth PCR positive) and compared with control infants born to HIV-1–uninfected mothers. As markers are measured in cord blood, the IP infants are uninfected at delivery and so do not indicate consequences of productive infection at this time point.

Neopterin levels were elevated to more than control levels among infected (IP and IU) and uninfected infants born to HIV-positive mothers regardless of whether mothers received antiretroviral drugs (ie, in both the DART and PEP groups) (Fig. 1A, i and iv). Exposure to HIV-1 resulted in moderately elevated levels compared with HIV-seronegative controls of β_2-m and s_L-selectin that attained significance among EU infants whose mothers received NVP (Fig. 1A, v–vi). Infants who became infected during delivery (IP) did not show any difference from EU infants in immune activation markers. In contrast, in utero infection (IU) resulted in substantial and significant increases in all the activation markers (Fig. 1A, i–vi).

Single-Dose Nevirapine Contributes to Immune Activation in Infants Born to HIV-1 Seropositive Mothers, Particularly Among Infants Infected In Utero

Having established that infants born to HIV-positive mothers have increased immune responsiveness as evidenced by raised levels of plasma activation markers, we next questioned if single-dose NVP might reduce immune reactivity in the presence of exposure or infection with HIV-1. Figure 1B shows a direct comparison of plasma immune activation markers of PEP and DART infants within each of the infection outcome groups (EU, IP, and IU). Contrary to our hypothesis, NVP exposure significantly increased neopterin ($P = 0.002$) and s_L-selectin ($P = 0.016$) levels of EU infants (Fig. 1B, i and iii) as well as increased neopterin ($P = 0.021$) and β_2-m ($P = 0.021$) levels of infants who were infected in utero (IU) (Fig. 1B, vii and viii).

Immune Activation in the Infant Is Modulated Independently of the Mother's Viral Load and CD4 T-Cell Count and Is Not Influenced By Gestation Period

Given that infants exposed to HIV-1 or infected with HIV-1 demonstrated enhanced immune activation, we wanted to ascertain whether the mother's viral load would directly impact on the levels of the immune activation factors of the PEP and DART infants. Only neopterin from the EU DART infants was weakly correlated to maternal viral load ($P = 0.028$, $r = 0.298$). Given that PEP mothers had significantly higher viral loads compared with DART mothers ($\log_{10}$ 4.78 and $\log_{10}$ 4.35, respectively; $P = 0.016$), we might have expected that activation markers would be lower in the NVP-exposed group (DART) in contrast to what we observed. After adjusting for maternal viral load, the association between NVP exposure and higher levels of neopterin ($P = 0.035$) and s_L-selectin ($P = 0.016$) remained statistically significant in the EU group, indicating that differences in viral load between the groups did not account for the increased immune activation that we observed. No statistically significant correlations were demonstrated between maternal $CD4^+$ T-cell counts and infant (CB) neopterin, β_2-m, and s_L-selectin levels for the PEP infants (EU, IP, or IU). It would be expected that the same would hold true for relationships between DART mothers and infants, although this was not tested, given that maternal CD4 T-cell counts were not determined for this group.

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Infants born before 37 weeks' gestation (preterm infants) might be expected to have a reduced ability to respond to antigen compared with term infants; however, no significant differences in levels of neopterin, β_2-m, and s_L-selectin levels were observed between preterm or term infants of either the PEP or DART group (data not shown).

Single-Dose Nevirapine Does Not Modulate Immune Activation Markers of IP Transmitting and Nontransmitting HIV-1–Infected Mothers

The immune activation markers tended to be higher among HIV-1–infected mothers than among uninfected control mothers (Fig. 2A). However, in contrast to the findings among the children, NVP exposure was not associated with differences in activation markers among nontransmitting mothers or mothers who transmitted IP (Fig. 2B). There was an intriguing increase in neopterin among IU transmitting mothers given NVP (Fig. 2B, vii).

Immune Activation in the Infant Is Modulated Independently of that in the Mother

As it is known that a range of low-molecular-weight compounds (<500 d) is actively or passively transferred through the placenta to the fetus,[16] we sought to examine whether immune activation in the infant might be modulated independently of that in the mother by determining differences between mothers and infants in their immune activation markers. Interestingly, whereas levels of neopterin did not differ appreciably between infants and mothers, mother-child differences are notable for β_2-m and s_L-selectin with infants presenting with significantly higher levels of these markers in all groups, including the controls. The differences between mothers and infants were largest for infants infected in utero in both the PEP and DART groups. Within the control group, there were no significant correlations between mothers' and infants' (n = 22) levels of neopterin ($r = 0.401$, $P = 0.064$), β_2-m ($r = 0.150$, $P = 0.504$), or s_L-selectin ($r = 0.068$, $P = 0.764$), and infants had significantly increased levels of neopterin ($P = 0.013$), β_2-m ($P = 0.001$), and s_L-selectin ($P < 0.001$) compared with their mothers, further supporting independent immune activation modulation in the infant.

DISCUSSION

Previous studies have described nonspecific and HIV-1–specific immune responses in uninfected infants born to HIV-seropositive mothers,[17-25] indicative of some immune challenge in utero. Based on previous demonstration of reduced T-helper cell reactivity to HIV envelope peptides in cord blood of infants born to HIV-1–seropositive mothers in the presence of AZT-3TC[12] and single-dose NVP[26] given to the mother, we hypothesized that other markers of immune activation would accordingly be reduced. This current study was therefore designed using the plasma markers, neopterin, β_2-m, and s_L-selectin, to assess the extent of immune activation in infants born to HIV-1–seropositive mothers in the presence (DART group) and absence (PEP group) of single-dose NVP administered to the mother at the start of labor.

Our results have shown that (i) in support of earlier studies, there was substantial immune activity in response to exposure to virus in utero (that did not result in infection) or as a result of exposure to other immune consequences of having an HIV-infected mother, and as might be expected, this was most elevated in infants infected in utero; (ii) in contrast to what we had hypothesized, there was evidence of further increased immune activation in the cord blood of infants exposed to NVP when compared with their drug-unexposed counterparts, and most notably, existing infection at birth was marked by substantially elevated levels of all immune activation markers in the presence of NVP; (iii) levels of peripheral blood immune activation markers were higher in infants than their mothers (HIV negative and HIV positive), particularly for β_2-m and s_L-selectin, indicating that their immune systems develop independently of their mothers; (iv) immune activation in the infant was modulated independently of maternal viral

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load; and (v) elevated immune activation in NVP-exposed, HIV-infected infants relative to drug-unexposed, HIV-infected infants (IU group) suggests an apparent synergy between HIV-1 and NVP in increasing overall immune activation. This latter phenomenon was also apparent among the IU mothers.

How does one explain attenuated HIV-specific, T-helper cell activity[26] yet increased levels of immune activation markers in plasma through a brief exposure (the period of labor and delivery) to NVP? That NVP shows consequences of immune stimulation in such a short period would suggest that it may mediate its effects predominantly on cells of the innate immune response as these respond rapidly to stimuli. Plasma levels of immune activation markers are indicative of events that have already occurred, whereas detecting recall to HIV peptides (T-helper cell reactivity) involves activation of T cells after exposure in vivo to NVP. Because NVP has already increased activation of cells in vivo, as evidenced by increased levels of soluble immune activation markers, T cells may be more anergic on subsequent stimulation with peptides and therefore unable to respond in vitro. This is reminiscent of what occurs in HIV-infected individuals where spontaneous release of cytokines is often enhanced because of in vivo priming, whereas induced release of cytokines is impaired relative to uninfected controls. Because T-cell responses in newborns are very weak, even a slight reduction in responsiveness would reduce most responses to less than the level of detection. Alternatively, among T cells, immune responsiveness in the presence of NVP may be different, for example, increased levels of immune activation markers may be derived from CD8 T cells, whereas CD4 T cells are deactivated under these same circumstances. Although inflammatory markers may also be increased in infants born to HIV-infected mothers as a result of exposure and possible infection with agents other than HIV, it is unlikely that infections would be increased in the NVP-exposed group as opposed to the drug-unexposed group that could account for our results. Our 2 HIV groups would be expected to be comparable in this regard, and the increase in immune activation demonstrated is clearly caused by single-dose NVP exposure.

Levels of immune activation markers, neopterin, β_2-m, and s_L-selectin, reflect physiologic and pathologic conditions, and in HIV-1 infection, the former two are correlates of stage of disease and prognosis,[13,14,27] whereas increased levels of s_L-selectin have been reported in HIV-infected infants.[28] One could question the extent of maternal transfer of these factors to the infants because studies concur that substances can be transferred from maternal blood to the fetus. There is no documented evidence that passive or active transfer of neopterin (253 d) occurs. Furthermore, fetal neopterin[29] and β_2-m[30] concentrations have been reported to change during gestation with neopterin levels being reported to be substantially greater than maternal levels and not being correlated significantly with paired maternal levels, demonstrating that, during gestation, there is a progressive increase in fetal cell-mediated immunity and monocyte-macrophage activation.[29,30] The immune activation marker levels measured in control uninfected infants of HIV-uninfected mothers in this study suggest an association to neonatal immune system development.

Neopterin production is associated with early T-cell responses[14] and serves as an indirect measure of oxidative stress and therefore apoptosis (T-cell anergy).[31] Elevated levels of β_2-m have been associated with an increased turnover of immune cells, especially lymphocytes; however, high concentrations can trigger a cascade of signaling events that exert a negative effect on the immune system, including impaired antigen-presentation capacity of dentritic cells.[32] Enhanced apoptosis has been described in cord-blood T lymphocytes of HIV-exposed newborns, with the one infected newborn tested in this study demonstrating the highest levels of $CD4^+$ and $CD8^+$ apoptosis.[33] It stands to reason that raised levels of these markers would have consequences on the immune capability of newborn infants because increased apoptosis of T cells and reduced functional capacity of dendritic cells with a diminished ability to activate T cells would compromise antigen-specific, T-cell responses. Thus, although exposure to

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HIV-1 may result in priming of the immune system (nonspecific and HIV-1–specific immune responses), the presence of single-dose NVP may be sufficient to further drive the immune system into an anergic/immunodeficient state. Single-dose NVP can mediate its antiviral activity on the one hand by directly binding to the HIV-1 reverse transcriptase and, on the other hand, induce an anergic state in cells that may also be beneficial in preventing replication of HIV-1.

It is intriguing that levels of immune activation markers are higher in infants than in their mothers among all groups, even the HIV-seronegative controls. This would support the idea that (i) immune development is accompanied by signs of immune activation; (ii) levels of activation markers in plasma may serve to dampen immune responsiveness early in life and may account for the general T-cell anergy that exists in early life; and (iii) these factors are likely to play very specific roles in immune responsiveness in the infant and are thus unlikely to merely be waste products of immune responses. Furthermore, on the one hand, there appeared to be an independence of the infant's developing immune system from the mother, but on the other, there were similarities in how infants and mothers responded to common factors in both environments. The mothers' HIV-1 status appears to influence the infants' ability to counteract infectious agents either (i) through altered maturation of the infant's immune responsiveness through exposure to HIV-1 from the mother or (ii) through deficient signaling that may occur through lack of provision of essential factors to the fetus that may be necessary for early immune development.

Our findings challenge, in particular, the way one might view the role of immune activation in promoting the establishment and augmentation of HIV-1 replication in the infant. Our data do not argue against the antiviral effects of short-course treatment, rather we propose, in addition, that not all immune activation is necessarily deleterious and, if caused in vivo by NVP, may well assist infants to prevent establishment of infection. However, for infants already infected when exposed to NVP (ie, those with in utero–acquired infection), the augmented immune activation that occurs in the presence of both NVP and already established infection at birth may well be deleterious. This brings to mind those studies that have indicated that infants who become infected despite perinatal AZT prophylaxis may have a more rapid course of the HIV-1 disease and higher mortality compared with infants who become infected without drug exposure.[34-37] However, this has not been observed in all studies[38,39] and may be confounded by the severity of maternal disease. Our data would provide a biological explanation for how this may occur, and whether disease progression is more rapid among infected children with NVP exposure should be investigated. What is further thought provoking is the effect of NVP on levels of activation markers in the IU transmitting mothers, a phenomenon that did not occur in the absence of NVP (IU PEP mothers). This would suggest the existence of some factor/s unique to this group of mothers that may also relate to why these mothers were more likely to transmit HIV-1 to their fetuses during pregnancy. We propose that NVP is able to synergize with HIV-1 to increase immune activation either through a mechanism whereby NVP directly acts to activate cells harboring HIV-1 or indirectly by NVP acting on bystander uninfected cells which in turn produce cytokines/factors that increase immune responsiveness or increase HIV-1 replication which in turn generates elevations in levels of activation markers. A question that is raised is what is different about these IU mothers compared with other transmitting (IP) and nontransmitting mothers. The HIV-1 strain infecting these mothers may be much more conducive to increased replication through immunomodulatory effects of NVP, or alternatively, NVP may be metabolized differently in these women, resulting in different immunomodulatory effects to the other women. It will be important to establish if other antiretroviral drugs or drug combinations used to prevent mother-infant HIV-1 transmission also enhance immune activation in some individuals. Given that immune factors such as neopterin are excreted renally,[40] we cannot exclude the possibility

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that reduced clearance may occur in the presence of NVP and so give rise to increased levels when compared with drug-unexposed individuals.

In conclusion, this study has demonstrated that HIV-1 exposure and short-course antiretroviral prophylaxis impact on the developing immune system of the infant. Short-course NVP exposure may be beneficial with respect to driving immune activation before the establishment of an infection in an infant exposed to HIV-1, but may have some detrimental consequences in the case of existing infection acquired in utero. Understanding the synergistic interaction between HIV-1 infection and NVP in substantially enhancing immune activation in some individuals but not in others will provide important insights that could lead to a means to identify mothers who present with this phenomenon, as these are the mothers who are likely to transmit HIV-1 during pregnancy.

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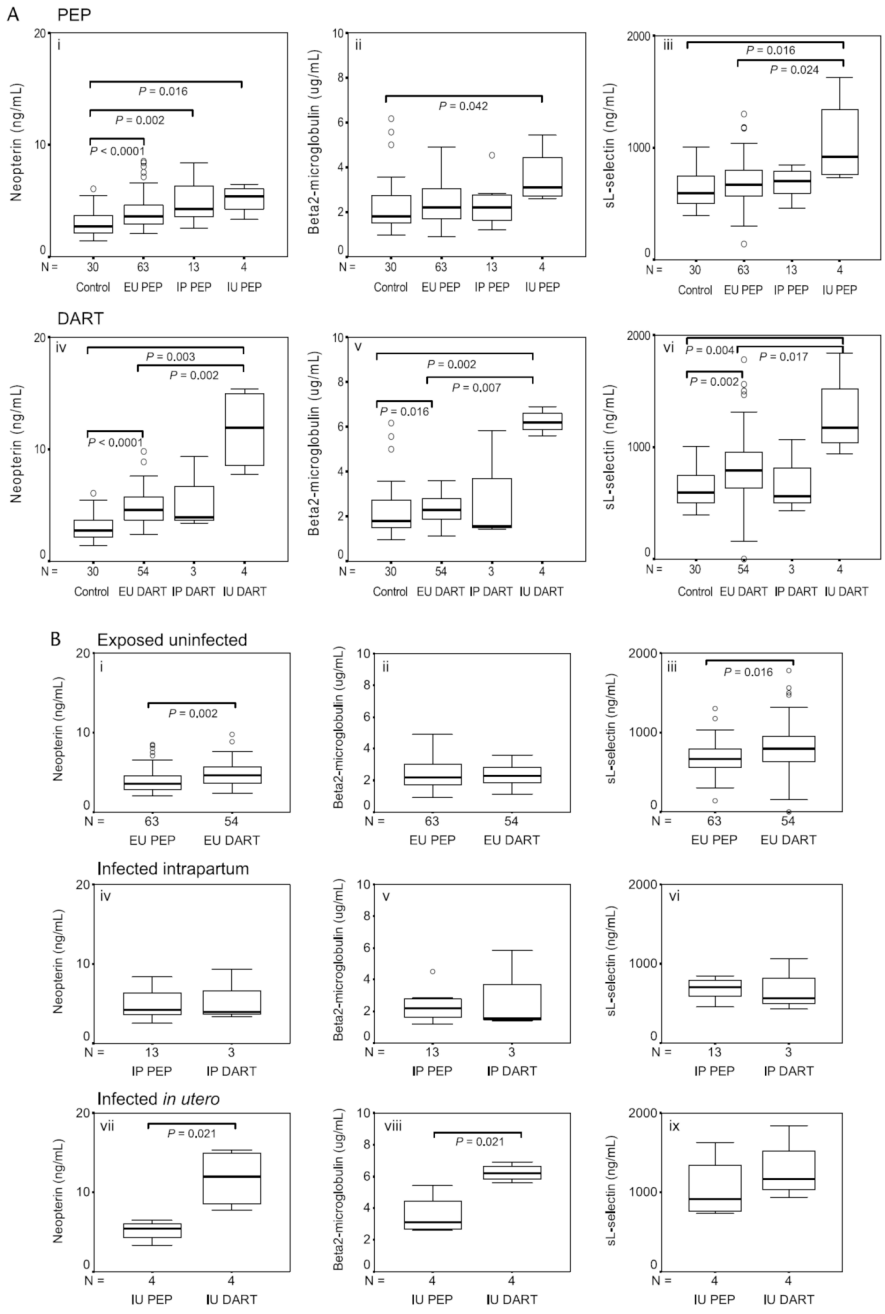

FIGURE 1.

Levels of soluble immune activation markers in plasma of infants born to HIV-seronegative and HIV-seropositive mothers. A, Levels of neopterin (ng/mL) (i and iv), β_2-m (μg/mL) (ii and v), and s_L-selectin (ng/mL) (iii and vi) of infants born to HIV-1 seropositive mothers in the absence (PEP) and in the presence (DART) of a single-dose of NVP, respectively. Immune activation marker levels measured in uninfected (control) infants are included. B, This panel depicts the effects of single-dose NVP exposure (DART) versus absence of NVP exposure (PEP) on the levels of neopterin (ng/mL), β_2-m (μg/mL), and s_L-selectin (ng/mL) of infants who remained uninfected (EU) (i, ii, and iii, respectively) or who become infected IP (iv, v, and vi, respectively) or in utero (IU) (vii, viii, and ix, respectively). Data are presented as

medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars). Significant differences between groups and sample numbers per group are indicated.

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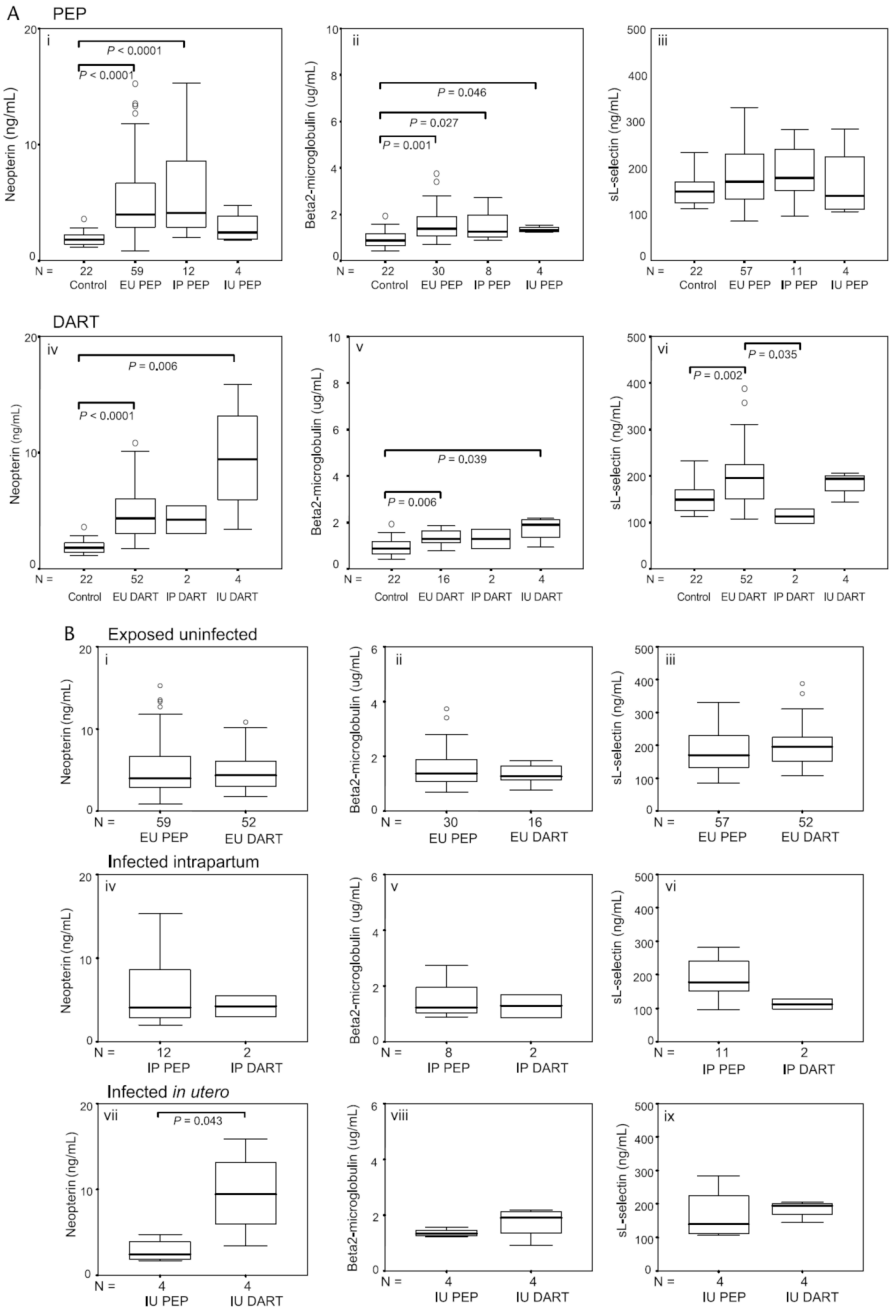

FIGURE 2.

Levels of soluble immune activation markers of HIV-1–infected mothers grouped according to infection status of the infant and NVP exposure. A, Levels of neopterin (ng/mL), β_2-m (μg/mL), and s_L-selectin (ng/mL) in uninfected (control) mothers and HIV-1–seropositive mothers who did not receive NVP (PEP group) and those who did receive NVP at the start of labor (DART group), grouped according to infection outcome of the infant (EU, IP, and IU). B, Comparison of the effects on levels of neopterin (ng/mL), β_2-m (μg/mL), and s_L-selectin (ng/mL) of single-dose NVP exposure (DART) versus absence of NVP exposure (PEP) in HIV-1–seropositive mothers grouped according to infection status of the infant (EU, IP, or IU). Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th

percentiles (bars). Significant differences between groups and sample numbers per group are indicated.

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TABLE 1

Clinical Characteristics of the HIV-Seropositive Mothers and Their Infants

	No Antiretroviral Drugs Given Before Birth (PEP)	Single-Dose NVP Given Before Birth (DART)	Total (PEP and DART)
N	80	61	141
Mean (SD)			
Mothers' $CD4^+$ T-cell count	477 ± 259	ND	477 ± 259
Mothers'age(y)	26 ± 5	26 ± 5	26 ± 5
Infant birth weight (g)	2919 ± 453	3039 ± 409	2964 ± 438
Median (IQR*)			
Mothers' viral load (log_{10})†	4.8 (4.1 – 5.2)	4.4 (3.7 – 4.9)	4.6 (3.9 – 5.1)
% (n/N)			
Infant sex (male)	56 (40/72)	50 (29/58)	53 (69/130)
Primiparity	34 (25/74)	23 (13/56)	29 (38/130)
Vaginal delivery	100 (74/74)	91 (49/54)	96 (123/128)
Preterm (<37 wk)	24 (17/71)	9 (5/55)	17 (22/126)
Infants HIV infected IP‡	11 (14/124)	4 (3/78)	8 (17/202)
Infants HIV infected IU‡	3 (4/124)	5 (4/78)	4 (8/202)
Infants ever breast-fed	49 (37/75)	12 (7/58)	33 (44/133)

*25th and 75th percentiles.

†Significant differences between PEP and DART groups are indicated ($P = 0.016$).

‡Transmission rates for PEP and DART study.

IQR indicates interquartile range; ND, not determined.