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Differential Effects of Human Immunodeficiency Virus Type 1 Envelope Protein gp120 on Interferon Production by Mononuclear Cells from Adults and Neonates

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While considerable progress in examining the course of human immunodeficiency virus (HIV) infection in adults has been made, a better understanding of the natural history of perinatal HIV infection remains to be obtained. Dysregulation of the production and functions of various cytokines, especially the interferons (IFNs), during HIV infections has been reported. Using an in vitro model system, we examined the effects of the HIV type 1 envelope protein, gp120 (10, 50, and 100 ng/ml), on gamma IFN (IFN-γ) and IFN-α production by lymphocytes from neonates and adults and also examined the potential regulatory effects of gp120 on phorbol 12-myristate acetate (PMA)- and Sendai virus-induced IFN-γ and IFN-α production by lymphocytes. PMA at a concentration of 50 ng/ml plus 50 ng of calcium ionophore A23187 per ml was used to induce IFN-γ, while 150 hemagglutinating units of Sendai virus was used to induce IFN-α production. The antiviral activity of both IFN-α and IFN-γ in leukocyte culture supernatants was assayed on BG-9 cells by a dye uptake technique using vesicular stomatitis virus as a challenge virus. Placental cord blood leukocyte (CBL) samples from healthy, term infants and adult peripheral blood leukocytes (APBL) produced no IFN in response to gp120. However, CBL produced significantly decreased levels of IFN-γ compared with APBL in response to PMA plus ionophore. gp120 significantly suppressed both Sendai virus-induced IFN-α and PMA-induced IFN-γ production by both CBL and APBL in a dose-dependent manner. However, gp120-induced suppression of IFN-α and IFN-γ was significantly greater with CBL than with APBL. Treatment of CBL and APBL with gp120 did not induce any phenotypic alteration of the CD45 RO subset. Increased suppression of IFN-α and IFN-γ production by gp120 in neonates may partially explain their apparent increased susceptibility to the clinical progression of HIV infections compared with that of adults.

Neonates appear to have increased susceptibility to infections in general (41) and, more specifically, infections with the human immunodeficiency virus (HIV) (7). Earlier studies showed that the immune system of the newborn is deficient in several aspects of humoral and cellular immunity (17), including decreased cytokine production (28) and cytotoxic functions (35). The clinical course of HIV infection appears to differ significantly between adults and infants and children. A more comprehensive understanding of the natural history of perinatal HIV infection remains to be obtained. Previous studies have shown that B-lymphocyte abnormalities frequently accompany HIV infections, especially in children. Specific-antibody production by HIV-infected children has been reported to be significantly depressed compared with that of infected adults (2). Progressive encephalopathy early in the course of the disease also has been considered to be a distinguishing feature of HIV infections in children (40). It has been shown previously that cortisol, a stress hormone whose levels are often elevated in infected patients, produced selective suppressive effects on the natural killer (NK) cell activity of neonatal lymphocytes compared with cells from adults (34). Furthermore, it was demonstrated that the ability of placental cord blood leukocytes (CBL) to produce gamma interferon (IFN-γ) in vitro was significantly less than that of adult peripheral blood leukocytes (APBL) (35).

Although HIV infection is associated with dysregulation of various cytokine levels in the serum and cerebrospinal fluid of infected subjects (6, 9, 12, 18, 20, 22, 23, 26, 30), the source of these cytokines and the specific factors that affect their regulation are the subject of considerable current interest. It is now evident that one of the important consequences of HIV infection is deficiency in the production of the IFNs that otherwise help to prolong the asymptomatic period of infection or assist in clearance of the virus (32). Previous studies have shown that infection of neonatal cells from the umbilical cord and adult lymphocytes with human T-cell leukemia virus 1 (HTLV-1) in vitro can be controlled by treatment with IFN-α, -β, and -γ (16, 42). Since the extent of immune deficiency observed in HIV-infected subjects does not necessarily correlate with the number of infected lymphocytes, it is reasonable to assume that circulating noninfectious products of the HIV genome might contribute to the immune deficiency observed, particularly in perinatally infected subjects. In the present study, we have examined the effects of the HIV type 1 (HIV-1) envelope protein, gp120, on IFN production by neonatal CBL in comparison with APBL. Our results may provide a better understanding of the pathogenic mechanisms underlying the differential courses of HIV disease in neonates and adults.
gp120 inhibits Sendai virus-induced IFN-α production by lymphocytes. Data presented in Table 1 show the dose-response effects of gp120 on Sendai virus-induced IFN-α production by CBL and APBL. gp120 at 10, 50, and 100 ng/ml produced 37%, 60%, and 75% suppression of IFN-α production, respectively, by CBL, whereas suppression of IFN-α production by APBL was relatively less (22%, 39%, and 58% suppression for 10, 50, and 100 ng of gp120 per ml, respectively). Since 50 ng/ml consistently yielded significant suppression of IFN production by both CBL and APBL, that concentration was used for all subsequent studies.

As shown in Fig. 1, gp120 mediated greater inhibition of Sendai virus-induced IFN-α production by CBL than by APBL. CBL cultured with 150 HAU of Sendai virus alone produced a mean value (± standard error [SE]) of 1,773 ± 570 IU of IFN-α compared with 1,087 ± 446 IU produced by APBL under the same experimental conditions. IFN-α production varied for both CBL and APBL, with ranges of 60 to 6,400 and 60 to 10,000 IU, respectively, for CBL and APBL samples. Although CBL produced a slightly increased level of Sendai virus-induced IFN-α compared with that of APBL, the levels were not statistically different between the two groups (distinguished sample variance ratio \[F = 1.04; P < 0.45\]). At a

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**FIG. 1.** IFN-α production by adult and umbilical cord blood lymphocytes. CBL and APBL were cultured (3 × 10^7/ml) in RPMI 1640 complete medium with or without 150 HAU of Sendai virus in the presence or absence of different concentrations of gp120 at 37°C. After 24 h of incubation, the supernatant fluid was collected and centrifuged to remove cell debris and traces of virus as described in Materials and Methods. IFN-α activity in the culture fluids was measured on BG-9 cells by dye uptake assay using VSV as a challenge virus as described in Materials and Methods. Values are mean titers of triplicate determinations. Horizontal lines define the means ± SE of the values. A total of 14 CBL and 22 APBL samples from separate donors were examined, and assays were done simultaneously. The statistical significance of differences was calculated by Fisher’s two-way variance analysis test.
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**gp120 does not induce phenotypic alteration of CD45 RO**

subsets. CD45 RO+ lymphocytes are memory cells that produce Th-1 cytokines, including IFN-γ. Earlier reports (28) showed that the neonatal lymphocyte pool contains a majority of CD45 RA- (naive) cells and fewer CD45 RO+ cells. We investigated the possibility that reduced IFN-γ production may be due to a lower number of CD45 RO+ cells in the neonatal pool. Our results with flow cytometry analysis and specific monoclonal antibodies showed that although the neonatal pool contains a significantly lower level of CD4 RO+ cells (10.5%) than APBL (21.0%), this proportion did not change significantly upon treatment with gp120 (data not shown). Although gp120 did not induce any observed phenotypic alterations, it is possible that it may suppress the activity of the CD4 RO+ subpopulation, resulting in a lower level of production of IFN-γ. Alternatively, it is possible that in addition to the CD45 RO+ subset, other lymphocyte subsets capable of producing IFN-γ may be affected by gp120.

**DISCUSSION**

The present study was designed to examine the differential immunoregulatory roles of gp120 for immunologically active cells from neonates compared with cells from adults. This investigation addresses the question of what the pathogenic mechanisms underlying the apparent increased susceptibility of neonates to HIV infections and their progression are. It has been shown (31, 35) that neonatal cells produce significantly lower levels of IFN-γ than adult lymphocytes. Recently, it was reported that HIV envelope peptides selectively suppress the proliferative responses of lymphocytes from neonates in comparison with those of adults (43). However, the mechanisms of HIV peptide-induced suppression of immune responses in neonates remain to be elucidated. The present investigation shows that IFN-α and IFN-γ production by both adult and neonatal mononuclear cells was suppressed significantly by treatment with gp120. However, suppression of both IFN-α and IFN-γ production was greater with neonatal cells. Further, in CBL, suppression of IFN-γ production was greater than suppression of IFN-α production. Although IFN-γ was detectable in all supernatants from neonatal cell cultures, the concentrations were generally <50% of adult levels. In contrast, the level of IFN-α production by neonatal cells in response to Sendai virus was slightly higher than that of adult lymphocytes, but the difference was not statistically significant.

The type of IFN-α produced by HIV-infected patients as well as induced in vitro by gp120-treated peripheral blood mononuclear cells from healthy donors was observed to be both acid labile and stable at pH 2 (5, 38). In our investigation, we also observed both acid-stable and acid-labile IFN-α; the levels of acid-labile IFN-α were approximately 20 to 30% (data not presented).

A recent study (19) showed that IFN-α-producing subsets that are affected in response to HIV infection in vitro have been found to be CD4+ , HLA-DR+ , CD3+ , and CD19+ . Ongoing studies are attempting to more rigorously define the differential effects of other lymphocyte subpopulations similar to these. Previous studies have shown that both Th-1- and Th-2-derived cytokines play a complex role in the pathogenesis of HIV infection (10–13). Mikovits et al. (33) showed that interleukin 4 (IL-4) and IL-13 exert divergent effects on HIV...
expression in monocytes. Lathey et al. (29) reported that tumor necrosis factor alpha and IL-1β are differentially upregulated during asymptomatic infections, whereas the activity fades away as the disease progresses. IFNs have been shown to inhibit HIV replication in both acutely and chronically infected cells, while IFN-γ has been shown to either induce or suppress viral replication (45). High levels of IFN-γ also have been reported to be present in HIV-infected subjects (21, 27), and HIV infection causes a low level of secretion of IFN-α (24, 46). Capobianchi et al. (5) showed that recombinant gp120 obtained from a baculovirus expression system induced IFN-α production by healthy peripheral blood mononuclear cells in a dose-dependent manner. However, Capobianchi et al. used gp120 at an extremely high concentration of 5 to 20 μg/mL. In this report, we present evidence that gp120 obtained from the same expression system at very low concentrations (10 to 100 ng/mL) by itself did not induce either IFN-α or IFN-γ production (data not presented). The differences in the production of IFN-α may be due to the different concentrations of gp120 used in the two studies. Interestingly, gp120 at these lower concentrations significantly suppressed IFN-α production induced by PMA plus ionophore A23187 and Sendai virus-induced IFN-γ production by both APBL and CBL. Furthermore, gp120-induced suppression of PMA- and Sendai virus-induced IFN-γ and IFN-α production was significantly greater with CBL than with APBL.

It is reasonable to assume that synthesis and shedding or secretion of HIV-derived peptides into the circulation during HIV infection may result in suppression of production of various cytokines by effector cells. The amount of HIV-derived products shed during HIV infection may vary from picograms to nanograms per milliliter of serum (36). In the present in vitro study, gp120 at levels similar to the concentrations in serum in acutely infected patients showed significant inhibitory effects on PMA- and Sendai virus-induced IFN production by APBL and CBL, and the suppression was significantly higher with CBL. The degree of IFN suppression is consistent with earlier reports (1, 9) which suggest that IFN production and its progression to AIDS.

REFERENCES


mRNA for various cytokines on a per-cell basis and that these effects vary further during the progression of the disease. Decreased IL-2 and IFN-γ production in asymptomatic patients has been reported (14, 39). Haraguchi et al. (25) showed that a synthetic heptadecapeptide corresponding to a highly conserved domain of the immunosuppressive retroviral envelope protein, p15E, demonstrated significant suppression of staphylococcal enterotoxin B-induced IFN-γ by healthy human peripheral blood mononuclear cells. Tyring et al. (44) showed that certain synthetic peptides corresponding to specific sequences of HIV envelope gp41 and gp120 enhanced in vitro production of IL-1 and tumor necrosis factor but suppressed the production of IFN-α, IFN-γ, and IL-2 by APBL.

Our observation that gp120 selectively suppressed IFN-γ production by neonatal lymphocytes is consistent with the above findings and correlates with an earlier report of decreased immune responses of neonates to HIV peptides (45). Although the exact mechanisms underlying the selective suppression of both IFN-α and IFN-γ production by gp120 in neonates are not fully understood, the suppression may explain, in part, the unique immunologic status of the perinatal period with the increased susceptibility of the fetus and neonate to HIV infection and its progression to AIDS.

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