Human Immunodeficiency Virus Does Not Induce Interleukin-1, Interleukin-6, or Tumor Necrosis Factor in Mononuclear Cells

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The production of interleukin-1 β (IL-1 β), IL-6, and tumor necrosis factor α (TNF- α) by fresh peripheral blood mononuclear cells was evaluated after exposure to human immunodeficiency virus (HIV) or purified recombinant HIV-1 envelope glycoprotein (rgp120). To exclude the role of contaminating endotoxin in this study, all media were subjected to ultrafiltration and reagents contained <25 pg of endotoxin per ml by *Limulus* assay. Under endotoxin-free conditions, no increases in IL-1 β , IL-6, or TNF- α mRNA or protein were detectable in cell cultures exposed to HIV-1, HIV-2, or rgp120 (0.1 to 10 μ g/ml), as compared with cytokine levels in mock-exposed cultures. However, concentrations of endotoxin (lipopolysaccharide) as low as 0.5 ng/ml induced significant production of mRNA and protein for these three cytokines. Preincubation of mononuclear cells with "shake" HIV-1 preparations and also mock-infected shake preparations prior to lipopolysaccharide stimulation resulted in a two- to threefold increase in IL-1 β and TNF- α production. This priming effect was not observed with rgp120 (0.1 to 10 μ g/ml) or standard HIV-1 or mock-infected supernatants, suggesting the presence of biologically active material independent of virus in the shake preparations. Our studies indicate that, in the absence of endotoxin, HIV-1, HIV-2, and HIV gp120 do not induce production of IL-1 β , IL-6, or TNF- α by peripheral blood mononuclear cells.

Interleukin-1 β (IL-1 β), IL-6, and tumor necrosis factor α (TNF- α) are immunoregulatory cytokines chiefly produced by monocytes and macrophages (6, 13, 21). Endotoxin is a major stimulus for production of these cytokines, but other stimuli such as viruses have been shown to induce synthesis of these molecules *in vitro* (1, 20, 37). Recently, human immunodeficiency virus type 1 (HIV-1) has been reported to induce production of IL-1 β , IL-6, and TNF- α from peripheral blood mononuclear cells (PBMC) and monocytes and macrophages (28, 32, 47). This effect was observed with live or inactivated virus as well as with purified HIV-1 envelope glycoprotein (gp120), suggesting that interaction of HIV-1 gp120 with its receptor, the CD4 molecule on the surface of monocytes, triggered the production of these cytokines (28, 32, 47). Furthermore, elevated levels of IL-1 β and TNF- α have been found in sera or in macrophage supernatants from patients with acquired immunodeficiency syndrome (5, 22, 25, 38, 49). These observations are potentially important in the pathophysiology of HIV infection since release of these cytokines could activate T cells and B cells and induce fever, cachexia, and demyelination (6, 13, 21, 36). TNF- α may also up regulate HIV expression *in vitro* by activation of the nuclear factor κ B (NF κ B), and HIV could therefore augment its own expression (10, 16, 34). We previously reported that THP-1 monocytoid cells or peripheral blood macrophages productively infected with HIV-1 did not constitutively release these cytokines but did so only after endotoxin stimulation (30; J. M. Molina, R. Schindler, R. Ferriani, M. Sakaguchi, E. Vannier, C. A. Dinarello, and J. E. Groopman, *J. Infect. Dis.*, in press). In this study, we investigated,

by using endotoxin-free conditions, the production of IL-1 β , IL-6, and TNF- α by fresh PBMC after exposure to HIV-1, HIV-2, or purified recombinant HIV-1 envelope glycoprotein (rgp120). Contrary to previous reports, no significant mRNA or protein for IL-1 β , IL-6, or TNF- α was produced by these cells in response to stimulation with HIV or its envelope glycoprotein in the absence of endotoxin.

MATERIALS AND METHODS

Cells. PBMC were obtained from healthy, HIV-seronegative donors after centrifugation of heparinized venous blood over Ficoll-Hypaque gradients (specific weight, 1.077 g/ml). The Ficoll (type 400; Sigma Chemical Co., St. Louis, Mo.) was dissolved, and Hypaque (Hypaque-M 90%; Winthrop-Breon Laboratories, New York, N.Y.) was diluted in ultrafiltered endotoxin-free water (40). RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) was subjected to ultrafiltration (40). PBMC were washed twice in RPMI 1640 medium and suspended at a final concentration of 2.5×10^6 cells per ml in ultrafiltered RPMI 1640 medium supplemented with L-glutamine (2 mM), penicillin (250 U/ml), streptomycin (250 μ g/ml), HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (10 mM), and pyrogen-free 1% heat-inactivated human serum. The cells were cultured at 37°C with 5% CO₂. After the addition of human serum, there was no detectable endotoxin in the culture medium, as tested by the *Limulus* amoebocyte lysate assay (Sigma; limit of detection of endotoxin, 25 to 50 pg/ml). The relative proportion of lymphocytes and monocytes in the PBMC preparations was determined by flow cytometry with monoclonal antibodies specific for T cells (anti-Leu4), B cells (anti-Leu16), and monocytes (anti-LeuM3) (Becton Dickinson Immunocytometry Systems, Mountain View,

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Calif.). An average of 72% of T lymphocytes, 5% of B cells, and 23% of monocytes was obtained.

Virus preparations. Highly concentrated virus preparations were made by the shaking method described by Vujcic et al. (46). H9 cells and HIV-1 IIIB (17) were originally obtained from R. C. Gallo (National Cancer Institute, Bethesda, Md.), and HIV-2 ROD (9) was provided by L. Montagnier and F. Clavel (Institut Pasteur, Paris, France). Briefly, 300×10^6 H9 cells infected with HIV-1 IIIB or HIV-2 ROD were harvested at the peak of infectivity, washed twice in ultrafiltered medium, and suspended in 5 ml of ultrafiltered RPMI 1640 medium. After 2 min of vigorous shaking, the cell debris were pelleted by centrifugation and the supernatant was filtered through a $0.45\mu\text{m}$ pore-size filter (Sartorius, Gottingen, Federal Republic of Germany) and separated into samples. Each sample had less than 25 pg of endotoxin per ml by the *Limulus* assay and was kept frozen at -70°C until further use. This virus shake preparation was highly infectious (10^6 50% tissue culture infective doses per ml, as measured by terminal dilution assays in H9 cells) and contained 10×10^6 cpm of HIV reverse transcriptase activity per ml (19). Mock-infected shake preparations were also prepared by using the same protocol.

All lots of fetal calf serum were found to be contaminated with >50 pg of endotoxin per ml. Supernatants of HIV-1 IIIB and mock-infected H9 cells were therefore cultured in ultrafiltered RPMI 1640 medium supplemented with 5% heat-inactivated human serum and were harvested at the peak of HIV reverse transcriptase activity (2×10^6 cpm/ml). These supernatants, containing <25 pg of endotoxin per ml, were separated into samples and frozen at -70°C until further use.

Purified HIV-1 IIIB rgp120. The expression of recombinant HIV-1 IIIB envelope glycoprotein in transfected Chinese hamster ovary cells and its purification by affinity chromatography have been previously described (23, 24). rgp120 is highly glycosylated, binds to CD4 with high affinity, and induces neutralizing antibodies in rodents and chimpanzees (24). The purified protein, a gift of Genentech, Inc. (San Francisco, Calif.), was provided at a final concentration of 1.8 mg/ml. There was <25 pg of endotoxin per ml in the rgp120 protein preparation at 1.8 mg/ml with the *Limulus* assay. All dilutions of rgp120 were made in ultrafiltered medium.

Binding of rgp120 to monocytes and lymphocytes. The binding of rgp120 to fresh PBMC was evaluated by flow cytometry. PBMC (10^6) were incubated for 2 h at 4°C with various concentrations of rgp120 (0 to $10\mu\text{g/ml}$). The cells were then incubated with human serum to prevent Fc receptor-mediated uptake of mouse antibodies. The cells were then incubated with anti-Leu3A monoclonal antibody (Becton Dickinson) and fluorescein-conjugated F(ab')₂ goat anti-mouse immunoglobulin G (IgG) (Cooper Biomedical, Inc., West Chester, Pa.) and analyzed by flow cytometry with a fluorescence-activated cell sorter (Becton Dickinson). Staining with an irrelevant monoclonal antibody of the same IgG subclass (IgG2a), anti-HCPA1 (CD34, human progenitor cells; Becton Dickinson), was used as a negative control. Lymphocytes and monocytes were distinguished by differences in forward light scatter and 90° light scatter, and two different gates were set (39). In the first gate, the cells were more than 90% Leu4 positive (T cells; Becton Dickinson), 5 to 10% Leu16 positive (B cells; Becton Dickinson), and less than 1% LeuM3 positive (monocytes; Becton Dickinson). Conversely, the cells were less than 1% Leu4 and Leu16

positive and more than 90% LeuM3 positive in the second gate.

Stimulation of PBMC. PBMC were cultured in 24-well plates (Costar, Cambridge, Mass.) at a concentration of 2.5×10^6 cells per ml per well and stimulated for 24 h with various stimuli as follows: (i) rgp120 (0.1 to $10\mu\text{g/ml}$); (ii) shake preparation of HIV-1 IIIB, HIV-2 ROD, or mock shake (10%, vol/vol); (iii) HIV-1 IIIB supernatant or mock supernatant (50%, vol/vol); and (iv) endotoxin (lipopolysaccharide [LPS]) (0.5 ng/ml , *Escherichia coli* serotype O55 B5; Sigma). The final amount of virus in the well, as measured by HIV reverse transcriptase activity, was the same whether a shake or supernatant virus preparation was used. For priming experiments, PBMC were first exposed to rgp120, HIV, or mock preparations for 2 h and then stimulated with 0.5 ng of LPS per ml for 24 h. Stimulation of the cells in each experiment was done in duplicate. Five independent experiments with five different PBMC donors were performed. In order to block prostaglandin release, which could inhibit cytokine production, indomethacin (5×10^{-7} M; Sigma) was added to the culture medium in two experiments.

Cytokine assays. After stimulation for 24 h, the 24-well plates were frozen and thawed three times in order to lyse the cells so that total cytokine production (cell associated and extracellular) could be measured. Previous studies have shown that this procedure was optimal for quantitation of total cytokine synthesis (14, 41, 45). The lysed cell culture preparations were kept at -70°C until assayed. Radioimmunoassays (RIA) for IL-6 and TNF- α were performed as previously described (14, 30, 41, 45). The RIA for TNF- α and IL-6 had a limit of detection of 80 pg/ml (95% confidence limit). The IL-1 β RIA was more sensitive (limit of detection, 40 pg/ml). These RIA are highly specific for the cytokine they measure and do not detect IL-1 α , alpha, beta, or gamma interferons, or granulocyte-macrophage colony-stimulating factor, and they correlate well with their respective bioassays (14, 41, 45). All samples were assayed in duplicate for each cytokine.

RNA analysis. Total cellular RNA was obtained by guanidine isothiocyanate lysis and cesium chloride gradient centrifugation from unstimulated PBMC or after a 3-h stimulation with rgp120 (1 and $10\mu\text{g/ml}$), HIV-1, or mock shake preparation (10%, vol/vol); HIV-1 or mock supernatant (50%, vol/vol); or LPS (0.5 ng/ml) (8, 15, 26). The RNA was fractionated by electrophoresis through a formaldehyde 1.2% agarose gel. Northern (RNA) blots were generated by using standard methods (26), and transcripts were visualized by autoradiography after hybridization to the following random-primed, ^{32}P -labeled probes: the IL-1 β probe, obtained from a 1,075-base-pair (*Pst*I-*Sst*I) fragment of the human IL-1 β cDNA cloned into pGEM₂ (4); the TNF- α probe, obtained from a 473-base-pair (*Eco*RI) fragment of the human TNF- α cDNA cloned into pUC9 (35); the IL-6 probe (gift of the Genetics Institute, Cambridge, Mass.), obtained from a 656-base-pair (*Eco*RI-*Xba*I) fragment of the human IL-6 cDNA clone pCSF-309 (48); and the full 1.9-kilobase cDNA clone of constitutively expressed glyceraldehyde-3-phosphate dehydrogenase (GAPD) (44) as a marker of RNA content.

Statistical analysis. Analysis of variance and two-tailed Student's *t* test were used for statistical comparisons. Results were expressed as means $\pm$ standard errors of the means.

FIG. 1. Binding of rgp120 to PBMC. PBMC (10^6) were incubated for 3 h with rgp120 (0.1 to 10 μ g/ml), stained with anti-Leu3A monoclonal antibody, and analyzed on a fluorescence-activated cell sorter. Lymphocytes and monocytes were differentiated on the basis of forward light scatter and 90° light scatter. The mean fluorescence channel intensity was measured with each concentration of rgp120 in the lymphocyte and monocyte gate. Staining with an irrelevant monoclonal antibody of the same IgG subclass, HCPA-1 (CD34), was used as a control.

RESULTS

Binding of rgp120 to lymphocytes and monocytes. The binding of rgp120 to lymphocytes and monocytes was determined by flow cytometry after staining with the anti-CD4 monoclonal antibody anti-Leu3A. Two different gates were set on the basis of forward light scatter and 90° light scatter in order to differentiate lymphocytes and monocytes (39). In the lymphocyte gate (>95% lymphocytes), 49% of the cells expressed the CD4 surface antigen with a mean channel intensity value of 145 fluorescein units (Fig. 1). After pretreatment with rgp120, a decrease in fluorescence intensity as well as a decrease in the percentage of fluorescent cells was noted. This indicated, as previously described, that rgp120 bound to the epitope of CD4 recognized by Leu3A, which appears to be the binding site for HIV-1 (24). Similar findings were observed in the monocyte gate (>90% of monocytes), where 20% of the cells expressed the CD4 surface antigen with a low mean channel intensity of fluorescence of 92 U (Fig. 1). After pretreatment with rgp120, a decrease in fluorescence intensity along with a decrease in the number of cells expressing the CD4 surface antigen was also observed (Fig. 1; data not shown). These results demonstrated that rgp120 was able to bind to the CD4 antigen on the surface of lymphocytes as well as monocytes (42).

Synthesis of IL-1 β , IL-6, and TNF- α after stimulation of PBMC with rgp120 or HIV. Purified rgp120, at concentrations ranging from 0.1 to 10 μ g/ml, failed to induce significant mRNA or protein production (less than the detection limit) for IL-1 β , IL-6, or TNF- α from PBMC as compared with medium controls (Fig. 2; Table 1). However, concentrations as low as 0.5 ng of LPS per ml induced significant amounts of mRNA as well as synthesis of protein for IL-1 β (496 ± 47 pg/ml), IL-6 (368 ± 30 pg/ml), and TNF- α ($1,766 \pm 828$ pg/ml) (Fig. 2; Table 1). Similarly, PBMC exposed for 24 h to HIV-1 or HIV-2 shake preparations or HIV-1-containing supernatant did not produce significant amounts of mRNA and protein for IL-6, IL-1 β , or TNF- α as compared with PBMC exposed to mock shake or supernatant preparations (Fig. 2; Table 1). Addition of indomethacin (5×10^{-7} M) to the culture medium did not alter these results (data not shown).

Effect of LPS on cytokine production by PBMC following

FIG. 2. Northern blot analysis of TNF- α (A), IL-1 β (B), IL-6 (C), and GAPD (D) gene expression in PBMC. A total of 50×10^6 cells were stimulated for 3 h with medium alone (lane 1), rgp120 (10 μ g/ml; lane 2), mock shake preparation (10%, vol/vol; lane 3), HIV-1 IIIB shake preparation (10%, vol/vol; lane 4), or LPS (0.5 ng/ml; lane 5). Approximately 10 μ g of total RNA was loaded per lane.

exposure to rgp120 or HIV. PBMC first treated with various concentrations of rgp120 for 2 h were then stimulated with 0.5 ng of endotoxin per ml. No priming effect of rgp120 treatment was observed in PBMC (Fig. 3). The production of IL-1 β , IL-6, or TNF- α following endotoxin stimulation was the same with or without preexposure to rgp120. We next evaluated the effect of pretreatment of PBMC with HIV-1 prior to LPS stimulation. Again, no priming effect was observed with supernatant of HIV-1 or mock-infected H9 cells (data not shown). However, pretreatment of PBMC with HIV-1 shake preparations followed by LPS stimulation

TABLE 1. Production of TNF- α , IL-1 β , and IL-6 by PBMC exposed to various stimuli^a

Stimulus and concn	Mean $\pm$ SEM concn (pg/ml) of cytokine		
	TNF- α	IL-1 β	IL-6
Control	194 $\pm$ 31	87 $\pm$ 12	108 $\pm$ 17
LPS (0.5 ng/ml)	1,766 $\pm$ 828	496 $\pm$ 47	368 $\pm$ 30
rgp120 (μ g/ml)			
0.1	102 $\pm$ 10	80 $\pm$ 8	80 $\pm$ 20
1	153 $\pm$ 34	88 $\pm$ 14	109 $\pm$ 18
10	115 $\pm$ 10	67 $\pm$ 15	105 $\pm$ 33
H9 shake			
Mock	228 $\pm$ 35	85 $\pm$ 14	148 $\pm$ 29
HIV-1	273 $\pm$ 52	79 $\pm$ 16	160 $\pm$ 41
HIV-2	164 $\pm$ 36	85 $\pm$ 15	127 $\pm$ 46
H9 supernatant			
Mock	260 $\pm$ 10	80 $\pm$ 19	130 $\pm$ 30
HIV-1	256 $\pm$ 64	93 $\pm$ 32	132 $\pm$ 28

^a PBMC were cultured in ultrafiltered RPMI 1640 medium with 1% human serum at a concentration of 2.5×10^6 cells per ml. The cells were incubated with the indicated stimulus, and TNF- α , IL-1 β , and IL-6 were measured in the cell cultures 24 h later by using RIA. Each datum point is presented as the mean $\pm$ standard error of the mean (SEM) for five separate experiments. Two experiments were performed with H9 supernatants.

FIG. 3. Production of TNF- α (A), IL-1 β (B), and IL-6 (C) by PBMC. Cells (2.5×10^6 /ml) were exposed to medium alone, rgp120 (1 μ g/ml), H9 mock shake preparation, or HIV-1 shake preparation for 2 h and then stimulated with 0.5 ng of LPS per ml for 24 h. Total cytokine production (cellular and supernatant) was measured by using RIA. Each datum point is presented as the mean $\pm$ standard error of the mean for five separate experiments (in a single experiment, the range of standard error of the mean was always within 10% of the mean). +, $P < 0.05$; *, $P < 0.01$.

resulted in a two- to threefold increase of IL-1 β and TNF- α production from these cells as compared with that from cells exposed to LPS alone (Fig. 3). However, this priming effect was also observed with the control mock shake preparations that did not contain HIV-1 (Fig. 3). No significant priming effect of virus or mock shake preparations on the production of IL-6 was noted (Fig. 3). Since the shake preparations contained <25 pg of endotoxin per ml, as determined by the *Limulus* assay, we attempted to determine if cytokines themselves were present in these preparations. We measured IL-1 β , IL-6, and TNF- α in these shake preparations as well as in the mock or HIV-containing supernatants, but they were not detectable (data not shown).

DISCUSSION

It was previously reported that HIV-1 or its purified envelope glycoprotein could induce the production of significant amounts of IL-6, IL-1 β , or TNF- α by fresh PBMC or monocytes (28, 32, 47). Our studies performed with fresh PBMC and carried out under endotoxin-free conditions are at variance with these previous data. Purified rgp120 at concentrations ranging from 0.1 to 10 μ g/ml, as well as two different preparations of HIV-1 or HIV-2, failed to induce significant mRNA or protein production for IL-1 β , IL-6, or TNF- α by PBMC (Table 1; Fig. 2).

There are several differences in experimental techniques that may account for these discrepancies. The presence of lymphocytes in addition to monocytes in our cell cultures was not likely to account for these differences since a heterogeneous population of fresh mononuclear cells is a particularly sensitive system for the detection of cytokine production (14, 41, 45). We confirmed by flow cytometry analysis (Fig. 1) that the purified rgp120 used in our experi-

ments bound to the CD4 molecule on monocytes as well as on lymphocytes (24, 42). However, despite this high affinity of gp120-CD4 binding (12, 24), no cytokine or mRNA production was observed in our system (Table 1; Fig. 2). These data, therefore, do not support the hypothesis that a signal mediated via ligand binding to surface CD4 induces production of IL-1 β , TNF- α , or IL-6 by fresh mononuclear cells. Furthermore, Wahl et al. (47) observed induction of IL-1 β production in their monocyte cultures following binding of CD4 with the murine monoclonal antibody OKT4A but not with the OKT4 monoclonal antibody, while Merrill et al. (28) reported production of IL-1 β and TNF- α with either of these monoclonal antibodies. Although we used dialyzed purified OKT4 and OKT4A (anti-Leu3A) monoclonal antibodies, during the dialysis process we found that the preparations became contaminated with endotoxin and were therefore not proper for use. Because in our system concentrations of endotoxin as low as 0.5 ng/ml were able to induce significant mRNA and protein production of these three cytokines (Table 1; Fig. 2), it is possible that the prior studies with OKT4 and OKT4A contained cytokine-inducible concentrations of endotoxin in the preparations. Our studies emphasize that even low amounts (<1 ng/ml) of endotoxin were able to stimulate the production of TNF- α , IL-1 β , or IL-6 by fresh PBMC (14, 41, 45). These data also demonstrate that the RIA we used were highly sensitive. It should also be noted that, unlike many bioassays, these RIA are not affected by the release of inhibitors of TNF or IL-1 into the culture medium. Furthermore, the addition of indomethacin to block prostaglandin-mediated inhibition of cytokine production did not affect the outcome of our experiments (data not shown).

One explanation, therefore, for the discrepancy between our data and those in previously published reports might be the presence or absence of endotoxin in the respective experimental methods. Endotoxin contamination may occur during purification of proteins (27) such as the HIV gp120 employed by Wahl et al. (47). We also found that to maintain endotoxin-free conditions in the virus preparations it was necessary to use ultrafiltered media and heat-inactivated human serum. Use of nonultrafiltered medium and/or fetal

calf serum resulted in endotoxin contamination. Although polymyxin B was used by Merrill et al. (28) in an attempt to block endotoxin in their virus preparations, this may not be sufficient since many kinds of endotoxin are not inhibited by polymyxin B (3, 7).

Another possible explanation for the differences between our data and those of other investigators may be the presence of biologically active factors, not related to HIV or endotoxin, in the virus or gp120 preparations. IL-1 production, for example, can be induced in human monocytes by many defined stimuli as well as by yet ill-defined lymphocyte factors (2, 13, 29, 31, 33). In previous reports of IL-1 production from monocytes stimulated with HIV, the virus preparations as well as the purified HIV envelope gp120 were made from T-cell lines (28, 32, 47).

We observed that, although our endotoxin-free virus shake preparations failed to induce cytokine production themselves, they primed PBMC, enhancing production of IL-1 β and TNF- α in response to subsequent LPS stimulation (Fig. 3). Although levels of endotoxin not detectable with the *Limulus* assay could exist in such shake preparations, we suspect that some other biologically active factor(s) in the preparations accounts for these results. Since a similar priming effect was seen with the mock shake preparation, it is unlikely that the virus itself has a role (Fig. 3). We tried to determine if these factors were cytokines but found no detectable amount of IL-1 β , IL-6, or TNF- α in the shake preparations as well as in the H9 supernatants. This is in agreement with a report from Tschachler et al. in which no mRNA for TNF- α was found in HIV-1-infected or uninfected H9 cells (43). Another reason for differences in observed cytokine production following HIV exposure could relate to the strain of virus used. We employed, as did Wahl et al. (47) and Nakajima et al. (32), the HIV-1 IIIB lymphotropic strain and its purified recombinant HIV-1 envelope protein (23, 24), which has been shown to be taken up by monocytes (42). Similarly, mononuclear phagocytes can be infected with this lymphocytotropic strain (11; J. M. Molina, unpublished data). Merrill et al. (28) used in their studies the monocyto-tropic strain HIV-1 JR-FL, which was grown in T cells, and obtained similar results with a lymphotropic strain, JR-CSF. It seems unlikely that the cellular tropism of the HIV strain employed explains the divergent observations regarding cytokine production since the experiments did not involve infection of the cells. This is in accordance with the findings that HIV-2 ROD preparations, which can infect monocytes as well as lymphocytes (18; J. M. Molina, unpublished observations), do not trigger cytokine production (Table 1).

Our results therefore do not support a role for HIV-1 or HIV-2 in inducing IL-1 β , IL-6, or TNF- α from fresh PBMC. These data are in agreement with previous work demonstrating that HIV-1-infected THP-1 monocytic cells or cultured peripheral blood monocytes and macrophages do not constitutively produce IL-1 β , IL-6, or TNF- α , but retain their ability to produce these cytokines following adequate stimulation by LPS or LPS plus gamma interferon (30; J. M. Molina et al., in press). It would therefore appear that the high levels of TNF- α and IL-1 β observed in sera or culture supernatants from monocytes or lymphocytes may be related to in vivo activation by endogenous LPS, infection with opportunistic pathogens, or other factors.

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