JOURNAL OF VIROLOGY, Oct. 1989, p. 4404–4408
0022-538X/89/104404-05$02.00/0

Vol. 63, No. 10

Interleukin-1 and Tumor Necrosis Factor α Can Be Induced from Mononuclear Phagocytes by Human Immunodeficiency Virus Type 1 Binding to the CD4 Receptor

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Received 20 April 1989/Accepted 7 July 1989

Cytokines such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNFα) are important in normal immune processes. In this study, we demonstrate that human immunodeficiency virus type 1 (HIV-1) virions induce normal peripheral blood mononuclear phagocytes to produce both IL-1 and TNF within a few hours after their exposure to virus. The induction of these cytokines by HIV-1 does not require a productive infection. Blocking studies with soluble CD4 indicate that the effect is mediated through the CD4 molecule. In addition, the treatment of mononuclear phagocytes with OKT4A monoclonal antibody mimics the effects of HIV-1. Thus, these results indicate that induction of IL-1 and TNFα can occur via signals mediated through the CD4 molecule on mononuclear phagocytes. TNF has been shown by other investigators to induce HIV-1 expression. Therefore, TNFα may play a role in autocrine and paracrine regulation of HIV-1 expression. In addition, the induction of IL-1 and TNF by HIV-1 may also contribute to some of the neurologic and physiologic disorders associated with acquired immunodeficiency syndrome.

Human immunodeficiency virus (HIV) infects cells which bear its receptor, the CD4 molecule (10, 22). These cells include CD4-positive T lymphocytes, as well as mononuclear phagocytes, peripheral blood monocytes, and tissue macrophages. Infection of mononuclear phagocytes by HIV has been demonstrated in vitro by using strains of HIV which are tropic for mononuclear phagocytes. By using in situ immunohistochemical and direct virus isolation techniques, infection has also been demonstrated in patients (16, 24). The regions of the body where HIV infection of macrophages is particularly evident are the lung (alveolar macrophages) and the brain (either blood-derived macrophages or brain microglial cells) (15, 16, 24). We have investigated the effects of HIV infection on mononuclear phagocyte function, specifically on the release of monokines known to be produced by activated mononuclear phagocytes.

Two of the cytokines produced by activated mononuclear phagocytes as well as brain microglia are interleukin-1 (IL-1) and tumor necrosis factor alpha (TNFα) (12, 19). These cytokines are produced in response to normal immune stimuli such as immune complexes, lipopolysaccharides, and phorbol esters (1, 6). These cytokines produce a broad spectrum of biological effects. In the immune inflammatory response, they produce such pleiotropic effects as activation of neutrophil and macrophage cytotoxic potential, induction of prostaglandin E (PGE) and subsequent production of fever, increase in vascular permeability, increase in expression of major histocompatibility complex antigens, and induction of interleukin-2 (IL-2) receptor expression followed by T cell growth (12, 29, 32). In the central nervous system, IL-1 and TNF are sleep and fever inducers. IL-1 induces activation and proliferation of astrocytes; TNF, on the other hand, contributes to necrosis of cerebral blood vessels and possibly to demyelination (20, 30).

After treatment of mononuclear phagocytes with a monocytic strain of HIV-1 ($HIV\text{-}1_{JR\text{-}FL}$), we find that IL-1 and TNF are both induced to high levels. Furthermore, we provide evidence that this effect does not require viral replication and is mediated through the binding of HIV-1 to its receptor, the CD4 molecule.

MATERIALS AND METHODS

Mononuclear phagocyte preparation. Fresh human mononuclear phagocytes from healthy HIV-1 seronegative donors were separated on Ficoll-Hypaque gradients and by adherence to 25-mm culture dishes. After purification of mononuclear phagocytes by complement lysis with OKT3 monoclonal antibody (gift of Patrick Kung) to remove contaminating T-cell populations, the mononuclear phagocytes (1×10^6 cells) were infected with $HIV\text{-}1_{JR\text{-}FL}$ (400-ng p24 antigen as measured by the HIV-specific enzyme-linked immunosorbent assay [Abbott Laboratories]) or with human T-cell leukemia viruses type II (HTLV-II) (at the same protein concentrations as $HIV\text{-}1_{JR\text{-}FL}$) for 1 h in the presence of Polybrene (10 μg/ml) at 37°C. Cells were placed in Iscove's medium supplemented with 0.25% bovine serum albumin and transferrin (1 μg/ml), and supernatants from infected or uninfected mononuclear phagocytes were tested for IL-1, TNF, and virus production at indicated time points.

Preparation of virus. $HIV\text{-}1_{JR\text{-}FL}$-containing culture supernatants from infected peripheral blood T cells were clarified by low-speed centrifugation (2,100 rpm, 20 min). HIV-1 virions were pelleted by high-speed centrifugation (22,000 rpm, 1.5 h) through a 20% sucrose cushion in TEN (10 mM Tris hydrochloride [pH 7.4], 1 mM EDTA, 0.1 M NaCl) to remove growth factors from activated T cells. Pelleted virus was suspended in RPMI 1640 medium with 0.25% bovine serum albumin and stored at −70°C. Mock material was prepared from uninfected T cells of the same donors in an identical way. $HTLV\text{-}II_{MO}$ virus (7, 34) from 729 pH6neo cell lines was also prepared by the same procedure. Heat inactivation of HIV-1 was performed at 56°C for 30 min to remove infectivity.

IL-1 and TNF bioassays. IL-1 was measured by the method

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of Conlon, the LBRM 331A5 conversion assay (9). Supernatants were diluted 1:4 or 1:8 and were incubated overnight on LBRM cells (10^5 per well, in a Falcon microdilution plate) with 10 μg of phytohemagglutinin P (Difco Laboratories) per ml. These cell-free supernatants were harvested, and IL-2 production was assessed by the method of Gillis et al. (18). CTLL-2 cells were suspended to 4×10^4 cells per ml in Click's medium (Altick Associates), and 100 μl was added to each well of a 96-well flat-bottomed plate (Falcon, Becton Dickinson Labware). IL-2-containing sample supernatants were added in 100-μl quantities at doubling dilutions from 1/8 to 1/512. The plates were incubated for 24 h at 37°C, at which time they were pulsed with 0.5 μCi of [^{3}H]TdR per well (Dupont, NEN Research Products). The plates were harvested after an additional 18 h. The amount of incorporation was measured with a Beckman liquid scintillation counter (Beckman Instruments, Inc.). In every assay, the [^{3}H]TdR uptake by CTLL2 cells after response to test samples was compared to the CTLL2 response to standards by using a probit analysis (18). The dilution point where the standard gave 30% maximal proliferation ([^{3}H]TdR uptake) was considered to be 100 U/ml. The IL-1 activity is expressed as units of IL-2 per ml (U/ml) according to the equation: units of IL-2 = reciprocal titer of test material at 30% maximal counts per minute of standard/reciprocal titer at 30% maximal counts per minute of standard × 100.

TNF was measured by a modification of the method of Aggarwal et al. (2). The murine L929 cell line was retrieved in log growth phase by trypsinization, washed several times, chromated in a total volume of 1.0 ml with 0.25 mCi of ^{51}Cr (Amersham Corp.) for 1 h, and washed three times in medium. Chromated target cells were plated with the test sample in 96-well U-bottomed plates (Costar) at a concentration of 5×10^4 cells per 0.2 ml per well with 5 μg of dactinomycin per ml. After 18 h, the plates were centrifuged at 1,500 rpm and 0.1 ml of supernatant was aspirated and counted in a gamma counter. Experimental values were read off a standard curve generated by using recombinant TNF standards, the generous gift of Cetus Corp.

Treatment with antibodies and soluble CD4. OKT4 (mouse monoclonal antibody, immunoglobulin G2b) and OKT4A (mouse monoclonal antibody, immunoglobulin G2a) antibodies were the gift of Patrick Kung. OKT8 and anti-HLA-DR (both mouse monoclonal antibodies, immunoglobulin G2a) were purchased from Ortho Diagnostic, Inc. Soluble CD4 was the gift of David Ho. In these experiments, HIV-1 was used at 200 ng, a p24 antigen level corresponding to approximately 1×10^5 infectious units. Virus, antibodies (used at 1.25 μg/ml), and mock supernatant were preincubated with soluble CD4 (15 μg/ml) or without soluble CD4 for 1 h at 4°C and then added to mononuclear phagocytes (4×10^5 cells).

RESULTS

We have previously identified an isolate of HIV-1, termed HIV-$1_{JR\text{-}FL}$, isolated from brain tissue of an individual with severe acquired immunodeficiency syndrome (AIDS) dementia, which replicates well in mononuclear phagocytes (25). By using this isolate, we investigated the role of HIV-1 in induction of IL-1 and TNF production by mononuclear phagocytes. We assayed the induction of IL-1 and TNF in normal peripheral blood mononuclear phagocytes exposed to the HIV-$1_{JR\text{-}FL}$ strain by using two sensitive and specific bioassays (2, 9, 18). A burst of IL-1 was detected between 2 and 6 h after exposure of mononuclear phagocytes to HIV-1 (Fig. 1). IL-1 can induce PGE production which can feed back and suppress cytokine production, including IL-1 (23). However, the addition of indomethacin to inhibit PGE production had no effect, indicating that PGE was either not induced or not inhibitory to IL-1 in this system. Concentrations of virion equivalent to those in IL-1-containing samples were used in the LBRM conversion assay (9) directly and gave 4 U of IL-1 activity per ml. Treatment of positive supernatants with anti-IL-1 (1:50, Cistron) reduced IL-1 activity to ≤7 U/ml.

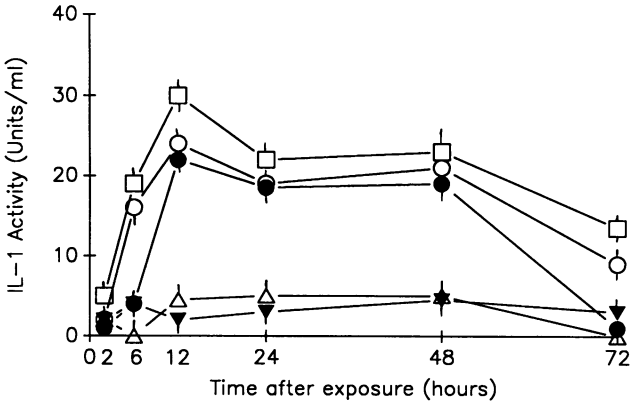

FIG. 1. Kinetics of IL-1 production after exposure of mononuclear phagocytes to retrovirus. ○, HIV-$1_{JR\text{-}FL}$-exposed mononuclear phagocytes; ●, heat-inactivated HIV-$1_{JR\text{-}FL}$-exposed mononuclear phagocytes; ▼, HTLV-II-exposed mononuclear phagocytes; △, mock supernatant-exposed mononuclear phagocytes. Each of these was tested in the presence of polymyxin B, 15 μg/ml. □, HIV-$1_{JR\text{-}FL}$ as above but without polymyxin B. The data presented are means plus or minus the standard error of a single representative experiment out of four experiments performed.

TNF production from 10 to 25 nM was also significantly stimulated between 2 and 12 h after HIV-1 exposure, 100- to 1,000-fold higher than mock and untreated mononuclear phagocytes. A significantly greater amount of TNF continued to be released in subsequent intervals up to 48 h, but the peak production was in the 6 to 12 h interval (Fig. 2). In this regard, HIV-1 acts in a manner very similar to other stimuli, such as phorbol myristate acetate, lipopolysaccharide, or

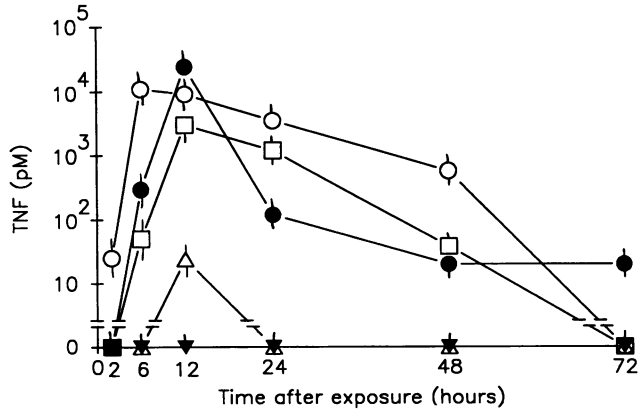

FIG. 2. Kinetics of TNF production after exposure of mononuclear phagocytes to retrovirus. See the legend to Fig. 1 for symbols. Preparation of mononuclear phagocytes, virus, and cytokine-containing supernatants was identical to the methods described in the legend to Fig. 1. The data presented are means plus or minus the standard error of a single representative experiment out of three experiments performed.

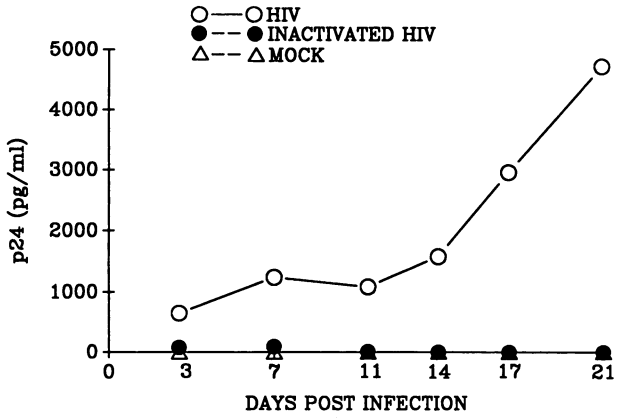

FIG. 3. Production of HIV-1 from mononuclear phagocytes. Mononuclear phagocytes were infected with HIV-1_{JR-FL} (○) or exposed to heat-inactivated HIV-1_{JR-FL} (●) (cells were the same as described in the legends to Fig. 1 and 2). HIV-1 production was assayed by p24 antigen measurements in a specific enzyme-linked immunosorbent assay (Abbott Laboratories).

Sendai virus (1, 6). Treatment of positive supernatants with anti-TNFα (1:100, Endogen) reduced TNF activity by 85%.

We have previously demonstrated that a second isolate of HIV-1 (HIV-1_{JR-CSF}) from the spinal fluid of the same individual from whom HIV-1_{JR-FL} was isolated was less efficient at productively infecting mononuclear phagocytes (25). Nevertheless, we found that HIV-1_{JR-CSF} was as effective at inducing IL-1 and TNF as was HIV-1_{JR-FL}. This result and the rapid kinetics of induction suggested that productive infection by HIV-1 may not be necessary for IL-1 or TNF induction. We tested this possibility by inactivating HIV-1_{JR-FL} by heat. HIV-1_{JR-FL} production was assayed by measurements of p24 antigen (*gag* gene product of HIV-1) in a specific enzyme-linked immunosorbent assay (Abbott Laboratories); there was no detectable p24 antigen following heat-inactivated HIV-1_{JR-FL} preparations (Fig. 3). The heat-inactivated HIV-1_{JR-FL} was as effective in inducing IL-1 and TNF as was infectious HIV-1_{JR-FL} (Fig. 1 and 2). Thus, the induction of IL-1 and TNF by HIV-1 is dependent upon exposure of mononuclear phagocytes to HIV-1, but productive infection by the virus is not required. Polymyxin B had no significant effect on IL-1 or TNF production, ruling out effects of endotoxin in our HIV preparation and assay (Fig. 1 and 2). To control for allogeneic effects between T cells, the source of virus used, and the mononuclear phagocyte targets, we performed the experiments with autologous T cells and mononuclear phagocytes and obtained identical findings (data not shown). HTLV-II is a human retrovirus distinct from HIV-1 (7, 34). HTLV-II_{MO} virions were used as a further control for the specificity of the induction of IL-1 and TNF by HIV-1. We tested whether HTLV-II_{MO} virions would activate mononuclear phagocytes to produce IL-1 and TNF in the same manner as HIV-1. Our results demonstrate that HTLV-II does not induce IL-1 or TNF, and, thus, the induction of IL-1 and TNF is specific to HIV-1 (Fig. 1 and 2).

The CD4 molecule is the receptor for HIV infection and is present on both T cells and mononuclear phagocytes. We determined whether the mechanism of induction of IL-1 and TNF by HIV-1 in these cells was through the CD4 receptor. Since soluble CD4 inhibits binding of HIV-1 to the receptor (11, 14, 21, 35), we tested soluble CD4 in our induction assays. As demonstrated in Fig. 4A and B, soluble CD4

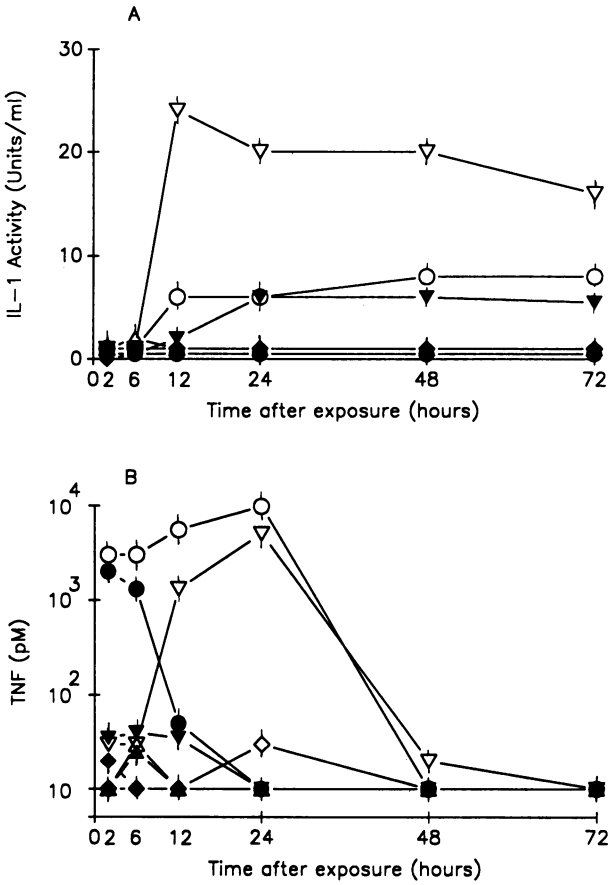

FIG. 4. A and B. Effect of OKT4, OKT4A, and soluble CD4 on IL-1 and TNF production. HIV-1_{JR-FL}-exposed mononuclear phagocytes with (●) or without (○) soluble CD4; OKT4A-exposed mononuclear phagocytes with (▼) or without (▽) soluble CD4; OKT4-exposed mononuclear phagocytes with (◆) or without (◇) soluble CD4; mock supernatant-exposed mononuclear phagocytes with (▲) or without (△) soluble CD4. Preparation of mononuclear phagocytes, virus, and assays of cytokine-containing supernatants was as described in the legends to Fig. 1 and 2. The data are means plus or minus the standard error of a single representative experiment of four experiments performed.

could abrogate HIV-1_{JR-FL} induction of IL-1 and TNF in a dose-dependent fashion, with maximal inhibition at 15 μg/ml. Soluble CD4 at 1.5 μg/ml had no inhibitory effect, while 150 μg/ml inhibited to the same extent as 15 μg/ml did (data not shown). TNF induction at the earliest time points (2 and 6 h) (Fig. 4B) was not consistently abrogated. We speculate that induction events, independent of HIV-1/CD4 interaction, may occur at these times. The epitope necessary for HIV-1 binding is recognized by the monoclonal antibody OKT4A (10, 22). OKT4A and antibodies to other monocyte and T-cell surface antigens were tested for direct activity on the mononuclear phagocytes. OKT4, OKT8, and antiHLA-DR had no effect (data not shown). However, OKT4A by itself induced IL-1 and TNF production. That the antibody is not activating cells through their Fc receptor is controlled for by virtue of the fact that OKT4A, OKT8, and anti-HLA-DR are all immunoglobulin G2a. This induction by OKT4A was inhibited by soluble CD4 (Fig. 4A and B). We conclude that signals mediated via the CD4 molecule result in the production of the cytokines IL-1 and TNF.

DISCUSSION

Recent studies have shown that supernatants from human monocytes or macrophages stimulated with bacterial lipopolysaccharides contained a factor which was able to induce HIV-1 replication in infected T cell clones; this factor was inhibited by antibody to TNFα (8). These results, in combination with our own, suggest that TNFα and HIV-1 can each induce expression of the other, resulting in a positive feedback loop for HIV-1 replication. Thus, infection of mononuclear phagocytes with HIV-1 can induce TNFα, which then might induce HIV-1 in latently infected T cells or mononuclear phagocytes. In addition, from the work described here, we would predict that IL-1 would also participate in this process by inducing TNF or by direct T-cell activation, a process necessary for HIV-1 replication (12, 29, 32, 36). We have previously shown that granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3 produced by activated T cells can induce HIV-1 replication in infected primary mononuclear phagocytes (26). Thus, the regulation of HIV-1 is a complex process involving first the infection of both mononuclear phagocytes and T cells. Subsequently, paracrine and autocrine regulation of HIV-1 replication via factors from mononuclear phagocytes (TNFα, IL-1, and macrophage CSF) and T-cell factors (GM-CSF and IL-3) would occur (26).

The CD4 molecule, in addition to serving as a receptor for HIV-1 on T cells and mononuclear phagocytes, has a number of normal physiologic functions. It has been shown to interact with major histocompatibility complex class II molecules on the surface of accessory cells during antigen presentation to T cells. It has also been shown to function as a cell adhesion molecule and, more recently, has been implicated in signal transduction during T-cell activation by its association with the CD3 T-cell-receptor complex (4, 5, 31, 33). Since stimulation of CD4 receptors by either HIV-1 or OKT4A antibody results in the release of TNFα and IL-1, signal transduction through CD4 by as yet unknown ligands under normal physiologic circumstances may also result in the release of TNFα and IL-1 from activated macrophages. Such release of cytokines is obviously critical for initiation of the immune response, since both TNFα and IL-1 enhance a variety of T-cell functions. The studies presented here, therefore, suggest a new function for the CD4 molecule on mononuclear phagocytes.

Clinical manifestations of AIDS include both immunologic and neurologic disorders. The virus has been demonstrated by in situ hybridization, immunohistochemistry, and direct isolation to be present in mononuclear phagocytes and in the brain and the blood of AIDS patients. Production of factors such as TNF and IL-1 from mononuclear phagocytes after stimulation with HIV-1 may result in some of the features of AIDS. Fever, cachexia, and aseptic meningitis are associated with AIDS. These may be due to the pyrogenic effects of IL-1 and TNFα as a result of interactions between HIV-1 and microglia or blood-derived mononuclear phagocytes (15–17, 24). Sensory and motor dysfunction may be the result of sensitivity to increased temperature of nerve fibers of AIDS patients as a result of fever. In addition, cerebral vasculitis and vasculopathies are associated with central nervous system lymphomas in AIDS patients (3, 13), possibly related to TNF release (20). A subset of patients infected with HIV-1 have been identified to have high levels of PGE, IL-1, and TNFα in serum (27, 28). Further examination of mononuclear phagocytes from patients will be important in determining contributions of these cytokines to the disease process.

ACKNOWLEDGMENTS

We thank Jia-Qi Zhao and Amadou Diagne for technical assistance and Richard Burger for preparation of the manuscript.

This work was partially supported by University-Wide Task Force on AIDS, University of California and by Public Health Service grant RO-1 NS26983 from the National Institutes of Health (J.E.M.) and NS 25508 (I.S.Y.C.).

ADDENDUM IN PROOF

Recent studies show that HIV-1 can induce IL-1, IL-6, and arachidonic acid metabolites from mononuclear phagocytes (30a, 35a).

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