Cytokine-Induced Expression of HIV-1 in a Chronically Infected Promonocyte Cell Line

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A model system for cytokine-induced up-regulation of human immunodeficiency virus type 1 (HIV-1) expression in chronically infected promonocyte clones was established. The parent promonocyte cell line U937 was chronically infected with HIV-1 and from this line a clone, U1, was derived. U1 showed minimal constitutive expression of HIV-1, but virus expression was markedly up-regulated by a phytohemagglutinin-induced supernatant containing multiple cytokines and by recombinant granulocyte/macrophage colony-stimulating factor alone. Recombinant interleukin-1 (IL-1), IL-2, interferon-γ, and tumor necrosis factor-α did not up-regulate virus expression. Concomitant with the cytokine-induced up-regulation of HIV-1, expression of membrane-bound IL-1β was selectively induced in U1 in the absence of induction of other surface membrane proteins. This cytokine up-regulation of IL-1β was not seen in the uninfected parent U937 cell line. These studies have implications for the understanding of the mechanism of progression from a latent or low-level HIV-1 infection to a productive infection with resulting immunosuppression. In addition, this model can be used to delineate the potential mechanisms whereby HIV-1 infection regulates cellular gene expression.

THE COMMON DENOMINATOR OF the immunosuppressed state resulting from infection with the human immunodeficiency virus type 1 (HIV-1) and leading to the acquired immune deficiency syndrome (AIDS) is the infection and destruction of the helper-inducer subset of T lymphocytes that express the CD4 phenotypic marker (*1*). Of particular interest is the fact that the time from initial infection with HIV-1 to clinically observed immunosuppression and disease is often measured in years (*2*), suggesting that a state of latent or chronic, low-level HIV-1 infection occurs in most infected individuals. The mechanisms of conversion from a latent or chronic HIV-1 infection to a productive infection are unclear, but activation signals are thought to play a major role in this process. Studies in vitro support this concept in that nonproductive infections of normal T cells with HIV-1 can be established in the absence of exogenous activation signals (*3*); however, HIV-1 expression can be induced in these and similar systems by activation with mitogens (*4*) or antigens (*5*) or by simultaneous cotransfection of heterologous viral DNAs with the HIV-1 long terminal repeat (LTR) and the *tat*-3 gene into susceptible cell lines (*6*). In addition, we have recently reported that wild-type virus can be chemically induced from an HIV-1–infected nonproducing T cell line (*7*). It is unclear what role, if any, physiological stimuli, such as cytokines that participate in the normal immune response, play in the regulation of HIV-1 expression in infected cells.

Recent studies indicate that the monocyte/macrophage probably has an important role in the initiation and propagation of HIV-1 infection. The presence of virus in these cells in vivo, in several different tissues, has been documented by such techniques as in situ hybridization, the demonstration of reverse transcriptase (RT), and the isolation and subsequent passage of virus (*8, 9*). We and others have shown that the monocyte/macrophage is the major cell containing HIV-1 in the brains of individuals with AIDS encephalopathy (*9, 10*). Furthermore, a number of studies have shown that monocyte/macrophages can be infected with HIV-1 in vitro (*11*).

An important aspect of infection of monocyte/macrophages with HIV-1 is the fact that, in contrast to its effect on the $CD4^+$ T lymphocyte, the virus is not readily cytopathic for these cells (*8, 9, 11*). Thus, the monocyte may well serve as a major reservoir of infection with HIV-1, carrying virus to the brain (*8–10*) as well as to susceptible $CD4^+$ T lymphocytes. It is therefore important to understand the mechanisms regulating the expression of virus in infected monocyte/macrophages. Because of the technical difficulties inherent in the use of primary cultured monocytes, such as terminal differentiation and lack of cell division, investigators have turned to monocyte tumor lines to study HIV-1 infection. For example, the promonocyte cell line U937 has been used as an in vitro model of monocyte infection (*12*). In this system, tumor-promoting agents can induce cellular differentiation that results in down-regulation of expression of the cellular receptor for HIV-1 (the CD4 molecule) as well as suppression of HIV-1 expression itself in acutely infected cells (*13*). In addition, by using U937 cells that have been acutely or chronically infected with HIV-1, others have shown that cytokines can suppress HIV-1 expression in these cells (*14*).

We established a line of U937 cells chronically infected with HIV-1 and from this line derived a panel of clones by limiting dilution (*15*). One of these clones, U1, which shows minimal constitutive expression of HIV-1, served as a model of an inducible monocyte line. We demonstrated that certain cytokines can induce expression of HIV-1 in the U1 clone and that, concomitant with the cytokine-induced up-regulation of HIV-1, the expression of the monokine interleukin-1β (IL-1β) is selectively up-regulated.

We initially stimulated human mononuclear cells with phytohemagglutinin (PHA) and used the partially purified culture supernatant (sup) from which lectin had been removed to study the effect on the expression of HIV-1 in the U1 clone. The supernatant induced significant RT activity in the

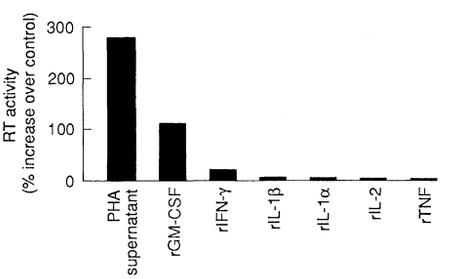

Fig. 1. Effect of recombinant cytokines on HIV-1 expression in U1 cells. U1 cells (5×10^5) cultured in duplicate in medium (RPMI 1640 with 10% fetal calf serum) were incubated with 20% PHA-sup (Electronucleonics) or 10 U of recombinant cytokines (rIL-1β and rIL-1α; Cistron Biotechnology and Biogen; rIL-2, Cetus; rTNF and rIFN-γ, Genentech; and rGM-CSF, Immunex) for 48 hours at 37°C. Culture supernatants were harvested and stored at −70°C. For RT assays we used a modification of the procedure described by Willey *et al.* (*26*). Briefly, 10 μl of supernatant containing RT was added in duplicate to a mixture containing poly(A), oligo(dT) (Pharmacia), $MgCl_2$, and ^{32}P-labeled deoxythymidine 5′-triphosphate (dTTP) (Amersham) and incubated for 2 hours at 37°C. Ten microliters of the mixture was then spotted onto DE81 paper, dried, and washed in 1× standard saline citrate buffer. The paper was then dried, cut, and counted on a Beckman LS 7000 scintillation counter. PHA alone had no effect on the induction of RT. Variability of replicate cultures was always less than 10%. Induction of HIV-1 in the U1 cell line with cytokine-enriched PHA-sup or recombinant cytokines was observed in over 15 experiments.

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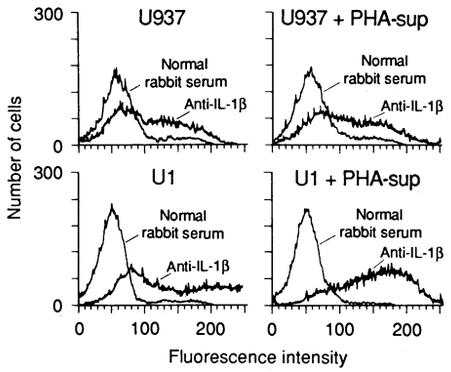

Fig. 2. Cytokine induction of membrane-bound IL-1β. Uninfected U937 cells and U1 cells were activated with PHA-sup as described in Fig. 1. After a 48-hour culture period, cells were washed twice in sorter buffer (Hanks buffer containing 1% bovine serum albumin and 0.05% sodium azide) at 4°C. Normal human serum (1:50) was incubated with cells for 30 minutes at 4°C to block Fc receptors. Rabbit antiserum (1:100) to recombinant IL-1β (*24*) or normal rabbit serum (1:100) was added for 30 minutes at 4°C. The cells were than washed twice in sorter buffer and incubated with goat antiserum to rabbit immunoglobulin G (IgG) conjugated with fluorescein isothiocyanate (1:100) (Jackson Laboratory) for 30 minutes at 4°C. Cells were then washed twice and fixed in 1% paraformaldehyde and analyzed for intensity of fluorescence by flow cytometry using a fluorescence-activated cell sorter-analyzer (Becton Dickinson). Data were collected as $\log_{10}$ fluorescence units and expressed on a linear scale. Histograms shown represent data from two experiments.

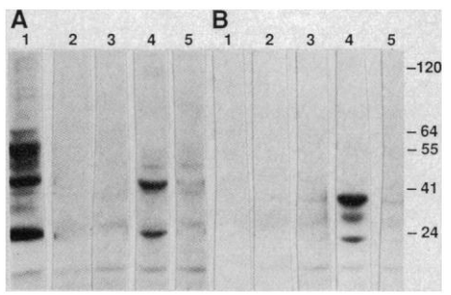

Fig. 3. Immunoblot of cytokine-induced infected U1 and uninfected U937 cells. U937 cells and U1 cells were induced with PHA-sup as described in Fig. 1. A3.01 cells were infected with HIV-1 and harvested at peak of virus production. Twenty microliters from total cell lysates containing approximately 2×10^8 viable cells per milliliter from infected A3.01 cells (lane 1), U1 cells (lane 2), U937 uninfected (lane 3), U1 + PHA-sup (lane 4), and U937 uninfected + PHA-sup (lane 5) were added to each lane and subjected to electrophoresis through 3 to 27% gradient polyacrylamide gels (Integration Separation Sciences) for 6 hours and transferred overnight onto nitrocellulose filters. After blocking, nitrocellulose filters were incubated overnight with an AIDS patient's serum (1:1000) (**A**) or rabbit antiserum to recombinant IL-1β (1:500) (**B**). Filters were then washed and incubated with ^{125}I-labeled protein A (200,000 dpm/ml), washed, air-dried, and exposed to x-ray film for 6 hours (*27*). Immunoblots shown represent data from four separate cytokine-induction experiments.

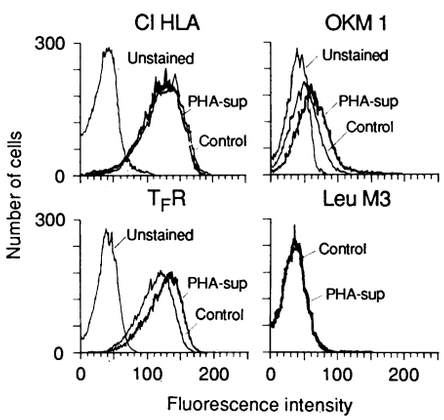

Fig. 4. Effect of cytokines on U1 cell-surface protein expression. Cells were cultured as described in Fig. 1. After two washes in sorter buffer, the cells were incubated for 30 minutes at 4°C with 2 μl of the monoclonal antibodies Leu M3, transferrin receptor (T_FR), class I HLA (CI HLA) (Becton Dickinson), or OKM 1 (CR3) (Ortho Diagnostics Systems). Cells were then washed, and goat antibody to mouse IgG coupled with fluorescein isothiocyanate (1:50) (Jackson Laboratory) was added as a second antibody for 30 minutes. The stained cells were then washed, fixed, and analyzed as described in Fig. 2. Histograms shown represent data from two experiments.

U1 cells at 20 to 40% concentration of the sup. Although the PHA-sup was partially purified for IL-2, it was unclear which cytokines were responsible for the induction of virus expression. We thus tested a number of recombinant cytokines for their ability to induce HIV-1 expression (Fig. 1). The only cytokine to have an effect on HIV-1 expression in U1 was recombinant granulocyte/macrophage colony-stimulating factor (rGM-CSF). With rIL-1α, rIL-1β, recombinant interferon-γ (rIFN-γ), or recombinant tumor necrosis factor-α (rTNF), there was no stimulatory effect, and there was no additive or synergistic effects when rGM-CSF was used with these other cytokines. There was also no effect when rIL-1 and rIL-2 were tested together.

We had determined by restriction-enzyme analysis that the U1 cell contained two integrated copies of proviral DNA (*16*). Because we had shown that a cytokine could up-regulate HIV-1 expression in U1, it was of interest to determine if the presence of integrated provirus in the cell would affect the induction of cellular genes. In this regard, IL-1β has been described as a membrane-bound cytokine that is expressed on the surface of monocytes after cellular activation (*17*). Thus, we examined whether there was a relation between the induction of HIV-1 expression by cytokines as described above and the surface expression of IL-1β. We determined by flow cytometric analysis the induction by PHA-sup of expression of membrane-bound IL-1β on chronically infected U1 cells compared to the parental uninfected U937 cells (Fig. 2). Approximately 50% of the U937 cells and the U1 cells constitutively expressed low levels of membrane-bound IL-1β. There was no increase in this expression in the U937 cells after exposure to PHA-sup. However, a significant shift in the intensity of fluorescence was observed in the U1 cells with over 80% of cells showing enhanced fluorescence after induction with PHA-sup.

We further demonstrated that IL-1β was co-expressed with HIV-1 proteins after induction of U1 cells with cytokines by performing immunoblotting experiments using serum from an AIDS patient containing antibodies to the standard HIV-1 proteins and a rabbit antiserum to rIL-1β (Fig. 3). The expression of HIV-1 proteins in U1 (lanes A2 and A4) was compared to that of an HIV-1–infected T cell line (*18*) (lane A1). The predominant viral bands observed in the U1 line after induction with cytokine were p24, gp41, and gp120; viral bands were not detected in the uninduced U1 cells. Likewise, when U1 cells were tested for IL-1β proteins, only the U1 line expressing HIV-1 antigens after induction with cytokines produced detectable IL-1β proteins (lane B4). Three bands with molecular weights of approximately 18,000, 26,000, and 36,000 were detected in the cytokine-stimulated U1 cells. These weights are consistent with those of secreted, membrane-bound, and precursor forms of IL-1β, respectively. No IL-1β proteins were observed in uninfected U937 cells after cytokine induction (lane B5).

The co-expression of HIV-1 and membrane-bound IL-1β in U1 cells after induction with cytokines was not the manifestation of a global or pleiotropic effect on protein expression. To determine if the expression of other surface membrane proteins was up-regulated after induction of U1 cells with cytokines, we analyzed the cells by flow cytometry using monoclonal antibodies against four cell-surface proteins (class I HLA, CR3, the transferrin receptor, and Leu M3) that are generally displayed on cells of monocyte lineage. As shown in Fig. 4, the surface densities of these proteins on U1 cells were essentially unchanged after treatment with the PHA-sup.

Thus, we have shown that physiologically and immunologically relevant molecules can enhance the expression of virus in chronically infected U1 cells, and that cells of monocyte lineage that are noncytopathically infected with HIV-1 can be induced to express virus by these molecules. These findings have important implications in our understanding of the mechanisms by which an HIV-1–infected individual may progress from an asymptomatic carrier state to a state of immunosuppression leading to clinical AIDS.

Multiple cytokines have been reported to activate monocytes and modulate their function (*19*). Two of these, GM-CSF and IFN-γ, have been reported to inhibit the replication of HIV-1 in U937 cells (*14*). Since the study of the latter was carried out with randomly infected cells, the results probably reflect the net effect of these cytokines on the total population of infected cells. The present study, with a single clone, reflects what one would expect to see from a single cell isolated from an infected culture. It is reasonable to believe that infected cells would respond to various cytokines by either up-regulation or down-regulation of HIV-1 infection, depending on the type of cytokine used for induction and on the genomic integration site of the HIV-1 provirus. The up-regulation of HIV-1 expression by cytokines, specifically GM-CSF, may be a result of the induction of a cellular gene that in turn induces the promoter of HIV-1 either through a cis mechanism that depends on the integration site of the provirus or by transactivation via a DNA-binding protein. In this regard, a DNA-binding protein (NF-kB) has been described that binds to the HIV-1 LTR in infected T cells and up-regulates virus expression (*20*).

Interleukin-1 is a well-characterized molecule with many physiological properties, including the induction of fever and other acute phase responses (*21*). This cytokine also has immunoregulatory functions on T and B cells (*22*). Recent reports have indicated that IL-1 may exert much of its effect at the membrane level (*23*). Our finding of enhanced expression of membrane-bound IL-1β after cytokine induction in the U1 clone suggests the possibility that, under certain circumstances, infected monocytes can activate T cells via IL-1 and thus increase the susceptibility of the T cells to infection with HIV-1. It is also possible that enhanced monocyte production of IL-1 may serve an autocrine function (*24*) enabling the monocyte to remain activated and thus support viral replication.

The effect of HIV-1 infection on expression of IL-1 remains to be clarified. However, it is possible that the HIV-1 *tat* gene product (*25*) either directly or indirectly regulates IL-1 gene expression via DNA-binding proteins that bind to sites within the IL-1 promoter region. Alternatively, as mentioned above, the uniqueness of the U1 clone could relate to the integration site of the proviral DNA into the cellular genome and thus represent a clone atypical to infected monocytes in general.

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Biological Invasion by *Myrica faya* Alters Ecosystem Development in Hawaii

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The exotic nitrogen-fixing tree *Myrica faya* invades young volcanic sites where the growth of native plants is limited by a lack of nitrogen. *Myrica* quadruples the amount of nitrogen entering certain sites and increases the overall biological availability of nitrogen, thereby altering the nature of ecosystem development after volcanic eruptions.

BIOLOGICAL INVASIONS BY EXOTIC species can alter the composition of natural and agricultural communities substantially (*1, 2*). Analyses of such effects have been used to test theories in population and community ecology (*3*) and have contributed to discussions of potential environmental effects of releases of genetically altered organisms (*4*). A demonstration that biological invasions by exotic species can also alter fluxes of energy, water, and nutrients could be similarly valuable; it would illustrate that whole ecosystems can be regulated by the populations and properties of individual species (*5*). A more general understanding of such effects would also be useful to conservation biology—exotic species that alter ecosystem-level properties not only compete with or consume native species, but also alter the underlying nature of the area they invade.

Recent reviews (*5, 6*) show that some biological invasions (especially by animals) alter ecosystems, but systematic studies have been few. We studied ecosystem-level consequences of invasion by *Myrica faya*, a small tree with a nitrogen-fixing symbiosis, into ecosystems developing in young volcanic substrates in Hawaii Volcanoes National Park. Such sites contain no native plants with nitrogen-fixing symbioses. Indeed, the actinorrhizal symbionts (*7*) that *Myrica* pos-

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