Cytokines Alter Production of HIV-1 from Primary Mononuclear Phagocytes

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Some strains of human immunodeficiency virus type 1 (HIV-1) can infect primary monocytes and monocyte-derived macrophages in vitro. In this report, the effect of cytokines on the production of one of these strains that shows a tropism for mononuclear phagocytes, designated HIV-1_{JR-FL}, was studied. Primary peripheral blood mononuclear phagocytes infected with HIV-1_{JR-FL} were treated with the hematopoietic factors: granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), macrophage colony-stimulating factor (M-CSF), and γ-interferon (γ-IFN). The M-CSF, GM-CSF, IL-3, and γ-IFN were able to alter HIV-1 production under different conditions.

THE MAIN TARGET OF HUMAN IMMUNODEFICIENCY virus type 1 (HIV-1) appears to be the $CD4^+$ subset of T lymphocytes (*1*) and mononuclear phagocytes (*2–3*). HIV-1 can productively infect mononuclear phagocytes derived from blood, bone marrow, brain, and lung (*4*). We characterized a primary isolate of HIV-1 (HIV-1_{JR-FL}) from the brain of an acquired immunodeficiency syndrome (AIDS) patient with subacute encephalopathy that replicates efficiently in primary peripheral blood mononuclear phagocytes (*5*). This virus was isolated by infection of primary peripheral blood lymphocytes (PBL) in short-term culture and had not been passaged in cell lines, thereby avoiding genetic variation that may occur during prolonged passage in culture or through selection in cell lines. We have analyzed the kinetics of infection of this primary virus isolate in mononuclear phagocytes treated with five human cytokines.

The mononuclear phagocyte system, which includes monocytes and tissue macrophages, has important roles in immunological and inflammatory responses (*6*). Mononuclear phagocytes have surface receptors for cytokines that regulate their hematopoietic and immunologic function. The genes encoding macrophage colony-stimulating factor (M-CSF or CSF-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and γ-interferon (γ-IFN) have been molecularly cloned (*7*), and the respective biosynthetic hormones produced in quantity. These cytokines have been well studied (*6, 8*), but their effect on HIV-1 replication has not been fully characterized. We have now used a primary virus isolate and primary mononuclear phagocytes to show that M-CSF, GM-CSF, IL-3, and γ-IFN can alter HIV-1 production in infected cells under different conditions.

Human monocytes were obtained from normal healthy donors negative for antibody to HIV-1. Monocytes were purified by adherence so that they were free of contaminating T cells, and then subjected to complement-mediated T cell lysis. Twenty-four hours after infection with HIV-1_{JR-FL}, mononuclear phagocytes were treated with four hematopoietic growth factors (G-CSF, GM-CSF, M-CSF, and IL-3) continuously for up to 14 days in the first experiment. Virus production was quantified by an enzyme-linked immunosorbent assay (ELISA, Abbott) for HIV-1 p24 (core) antigen in the supernatant of the cultures. In the absence of growth factors, virus was first detected at 4 days, and peaked at 11 to 14 days. At 7 to 14 days after infection, cultures treated with GM-CSF, IL-3, and M-CSF showed large increases in amounts of HIV-1 in the cultures (Fig. 1). Granulocyte colony-stimulating factor (G-CSF) did not show an effect on HIV-1 production in mononuclear phagocytes, consistent with its biological role as an activator of neutrophils rather than mononuclear phagocytes (*9*).

Macrophages produce GM-CSF (*10*); therefore, to determine whether enhancement of HIV-1 production by M-CSF is mediated by GM-CSF, we used neutralizing GM-CSF polyclonal rabbit antiserum to M-CSF (*11*). An antiserum to GM-CSF can inhibit the induction of HIV-1 replication by GM-CSF, but not by M-CSF (Table 1). Thus, the enhancing effects of GM-CSF and M-CSF are independent phenomena, and provide a further control for the specific biological effects of GM-CSF. Neutralizing antisera to the other factors were not available to provide similar specificity controls.

The enhancement of HIV-1 production was dependent on the concentration of the growth factors in the medium (Fig. 2). HIV-1 production was greatest in the presence of those concentrations of growth factors (GM-CSF and IL-3, 100 p*M* to 1 n*M*; M-CSF, 500 p*M* to 1 n*M*) previously shown to be most active in other functional assays (*12*). Proliferation of the mononuclear phagocytes, as measured by [^{3}H]thymidine incorporation, was also correlated with the concentration of growth factor present. The increase in cell number is insufficient to

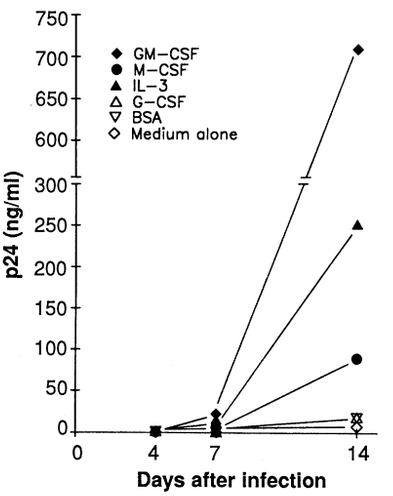

Fig. 1. Kinetics of HIV-1_{JR-FL} production from infected mononuclear phagocyte cultures treated with GM-CSF (◆), M-CSF (●), and IL-3 (▲). G-CSF (△), bovine serum albumin (▽), and medium alone (◇) are shown as negative controls. HIV-1 levels are indicated as nanograms of p24 per milliliter, based on p24 standards (Abbott). One representative experiment of seven different blood donors is shown. The range of HIV-1 enhancement by GM-CSF among different donors of mononuclear phagocytes varied from 4 to 90 times greater than that of untreated cells. Primary human mononuclear phagocytes were prepared by adherence to culture dishes with 5% human AB serum (Pel-Freeze) and 15% fetal calf serum for 3 days. On day 3, the mononuclear phagocytes were further purified by complement-mediated T cell lysis by the use of OKT3 monoclonal antibody (Ortho) and rabbit complement (Pel-Freeze) (*4*). On day 4, mononuclear phagocytes were infected by the HIV-1_{JR-FL} isolate at a dose of 500 ng of HIV-1 p24, assayed by ELISA, per 5×10^6 cells in the presence of 10 μg/ml of Polybrene for 2 hours. On day 5, mononuclear phagocytes were treated with 0.5 m*M* EDTA in phosphate-buffered saline (PBS) for 15 min at 37°C to remove cells from the plates. Mononuclear phagocytes were seeded into 96-well plates (Falcon) at a density of 3×10^4 cells per well. The growth factors (100 p*M* of GM-CSF, 1 n*M* of M-CSF, and 100 p*M* of IL-3) were added to the cells in duplicate wells. G-CSF (100 p*M*), bovine serum albumin (BSA) (0.001%), and medium alone are shown as negative controls. Medium with growth factors was changed every 3 or 4 days, and virus was assayed on days 4, 7, and 14 after infection.

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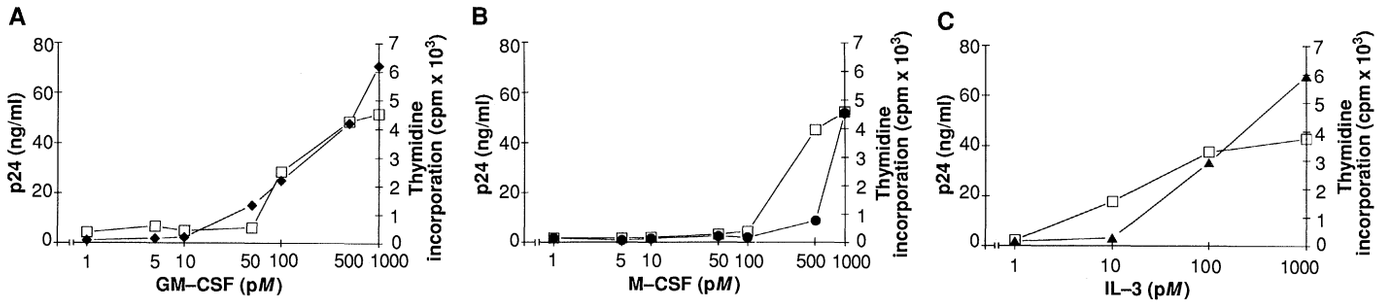

Fig. 2. Dose response of HIV-1 production and [^{3}H]thymidine incorporation to GM-CSF (**A**), M-CSF (**B**), and IL-3 (**C**). Primary human mononuclear phagocytes were prepared and treated with each hematopoietic growth factor as described in Fig. 1. GM-CSF, M-CSF, and IL-3 were added at the concentrations indicated. HIV-1 levels in supernatants from the cells at 14 days after infection treated by GM-CSF (♦), M-CSF (●), or IL-3 (▲) are represented as nanograms of p24 per milliliter, based on p24 standards. [^{3}H]Thymidine incorporation (□) during a 24-hour period was also assayed at each concentration of hematopoietic growth factor from day 13 to day 14. The experiments were repeated six times. There was about a 20-fold variation in the response of mononuclear phagocytes obtained from different blood donors, but the results were the same within each experiment.

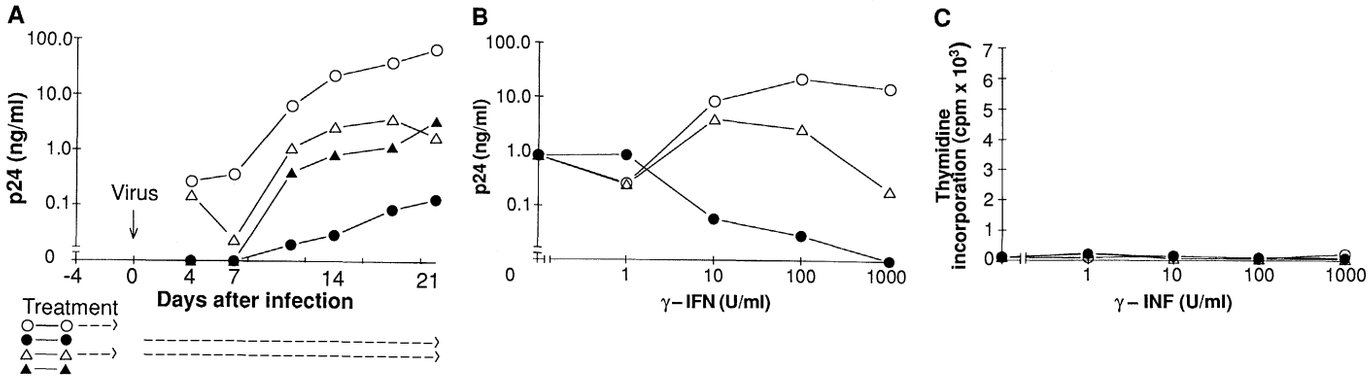

Fig. 3. Effect of γ-IFN on HIV-1 production from mononuclear phagocytes. (**A**) Kinetics of HIV-1 production from infected mononuclear phagocyte cultures treated by γ-IFN. The γ-IFN was added continuously before (○), after (●), or before and after (△) infection as indicated. HIV-1 was assayed as in Fig. 1 (note log scale). Human mononuclear phagocytes were prepared as described in Fig. 1. Mononuclear phagocytes were treated with 100 U/mol of purified recombinant γ-IFN 3 days before infection (○ and △). On the third day (day −1 after infection, as indicated on the *x*-axis), the mononuclear phagocytes were further purified by complement-mediated T cell lysis, as in Fig. 1. On the fourth day (day 0 after infection), cells were infected by the HIV-1_{JR-FL} virus at a dose of 67 ng of HIV-1 p24 per 5×10^6 cells. On the fifth day (day 1 after infection), mononuclear phagocytes were removed from plates with 0.5 m*M* EDTA buffer and seeded into 96-well plates at 3×10^4 cells per well; then 100 U of γ-IFN per milliliter was added to the cells (● and △). Medium alone (▲) is shown as a negative control. (**B**) Dose response of HIV-1 production after addition of γ-IFN. The γ-IFN was added before (○), after (●), and before and after (△) HIV-1 infection at the concentrations indicated. HIV-1 production from cells at 14 days after infection is shown as nanograms of p24 per milliliter (note log scale). (**C**) Dose response of [^{3}H]thymidine incorporation to γ-IFN. [^{3}H]Thymidine incorporation during a 24-hour period was assayed at each concentration of γ-IFN on cells treated before (○), after (●), and before and after (△) infection from day 13 to day 14. The experiments were repeated three times. There was about a tenfold variation in virus production from mononuclear phagocytes obtained from different blood donors, but the results were consistent within each experiment.

account for the large increase in virus production, because the number of cells increases at most twofold over the course of the experiments. These results do not address the question of whether other events related to cell proliferation are directly responsible for the increased viral production. In other retroviral systems, cell replication is often associated with greater levels of viral production.

We also studied the effect of γ-IFN on HIV-1 replication. γ-IFN is a potent activator of macrophage function (*6*); however, unlike the other factors studied, γ-IFN does not induce cell proliferation. The effects of γ-IFN on HIV-1 production also differed from that of the growth factors. HIV-1 replication was enhanced by treatment of the cells with γ-IFN before infection (Fig. 3A, note log scale of *y*-axis), but treatment with γ-IFN after infection resulted in a decrease of HIV-1 production. Continuous treatment with γ-IFN showed an intermediate level of virus production. Both enhanced and restricted effects on HIV-1 production were dose-dependent (Fig. 3B). However, no proliferative effects of mononuclear phagocytes by γ-IFN were observed (Fig. 3C). These data show that activation of monocytes in the absence of proliferation can also enhance HIV-1 production.

There is evidence that monocytes and macrophages play a major role in HIV-1 infection (*13*). In the central nervous system, mononuclear phagocytes appear to be the primary cell type infected with HIV-1 (*3*), and this cell type may have an important role in neurological complications of HIV-1 infection. It is therefore important to understand the processes regulating the replication of HIV-1 in mononuclear phagocytes. We have shown that hematopoietic growth factors and γ-IFN can alter HIV-1 production in primary mononuclear phagocytes. The mechanism for these effects is currently unknown. We measured the levels of CD4, the known receptor for HIV-1 (*14*), by flow cytometry on mononuclear phagocytes treated with GM-CSF and γ-IFN and found no change when compared with mock-treated cells at 7 and 13 days. Thus, if CD4 is the receptor for HIV-1 in mononuclear phagocytes, neither increased virus binding nor superinfection would account for the increased HIV-1 production. The mechanism may relate directly to the increase in proliferative capacity of the mononuclear phagocytes in the case of factors that induce growth (GM-CSF, M-CSF, and IL-3), or it may occur as a consequence of activation or differentiation (γ-IFN). It is known that immunological activation is required for replication of HIV-1 in peripheral blood T

Table 1. Induction of HIV-1 production by GM-CSF and M-CSF. Human primary mononuclear phagocytes were infected with the HIV-1_{JR-FL} strain (*5*) and continuously treated with GM-CSF or M-CSF 24 hours after infection, as described in Fig. 1 in the presence or absence of rabbit antiserum to GM-CSF (1:50) (*11*). The HIV-1–infected cells were cultured for 14 days with medium changes every 3 or 4 days. Preimmune rabbit serum (1:50) served as the negative control. On day 14 after infection, culture supernatant was assayed by HIV-1–specific ELISA for viral p24 *gag* antigens. The amounts of HIV-1_{JR-FL} p24 are indicated as nanograms of p24 per milliliter, as calculated relative to p24 standards. The rabbit serum increased the background in the ELISA assay; therefore, those samples containing rabbit serum were absorbed with protein A–Sepharose prior to the assay for p24 antigen.

Growth factor (concentration)	Treatment	HIV-1 p24
GM-CSF (100 p*M*)		708.8
GM-CSF (100 p*M*)	Antiserum to GM-CSF	22.3
GM-CSF (100 p*M*)	Preimmune serum	429.0
M-CSF (1 n*M*)		89.3
M-CSF (1 n*M*)	Antiserum to GM-CSF	66.7
M-CSF (1 n*M*)	Preimmune serum	74.7
Medium		7.6
BSA (0.001%)		19.9

lymphocytes in vitro, at least partly as a result of an increase in the effective concentrations of cellular transcription factors required for HIV-1 expression (*15*).

Recently, one group of investigators reported that GM-CSF can increase HIV-1 production (*16*) while another group reported that GM-CSF and γ-IFN decrease HIV-1 production (*17*). The interpretation of both of these studies is complicated by the fact that the investigators used a monocytoid tumor cell line, U937, and laboratory strains of HIV-1, rather than primary viral isolates and primary cells. Another explanation for apparent discrepancies is that the level of HIV-1 expression may be differentially regulated at distinct stages of mononuclear phagocyte maturation. For example, the permissiveness of mononuclear phagocytes for caprine arthritis-encephalitis virus increases with the maturation of the cells (*18*).

In vivo GM-CSF, M-CSF, and IL-3 are important regulators of hematopoiesis (*8*). γ-IFN is also a powerful regulator of immunological reactions (*6*). GM-CSF (*8*), IL-3, and γ-IFN are produced by activated T cells (*6, 7*), and GM-CSF and M-CSF are produced by activated macrophages (*10, 19*). Our results provide direct evidence that these cytokines can alter the production of HIV-1. A large number of monokines and lymphokines are involved in regulating the interaction of T cells with mononuclear phagocytes during an immune response (*6*). Because HIV-1 can infect T cells and mononuclear phagocytes, interactions between these cells by means of cytokines are likely to result in alterations in HIV-1 replication. Recently, GM-CSF was used in clinical studies as a potential therapeutic agent to augment host defense against opportunistic infections in AIDS (*20*). Our observations suggest that it may also be useful to test γ-IFN as a therapeutic agent that may not only activate macrophage function but also suppress HIV-1 replication in vivo. Because the cytokines have the potential to also increase viral production, clinical trials should incorporate appropriate laboratory assessments of the virus in treated individuals. However, in vitro observations may not reflect what happens when use of these cytokines is applied to a much more complex in vivo situation. For example, initial short-term clinical trials with GM-CSF showed dramatic increases in myeloid cell concentrations, yet viral titers were not consistently elevated.

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