Interleukin 6 Induces Human Immunodeficiency Virus Expression in Infected Monocytic Cells Alone and in Synergy with Tumor Necrosis Factor α by Transcriptional and Post-transcriptional Mechanisms

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Summary

The immunoregulatory cytokine interleukin 6 (IL-6) directly upregulates production of human immunodeficiency virus (HIV) in acutely as well as in chronically infected cells of monocytic lineage. In addition, IL-6 synergizes with tumor necrosis factor α (TNF-α) in the induction of latent HIV expression. Unlike TNF-α, upregulation of viral expression induced by IL-6 alone does not occur at the transcriptional level and it is not associated with accumulation of HIV RNA. However, when IL-6 and TNF-α synergistically stimulate HIV production, accumulation of HIV RNA and increased transcription are observed, indicating that IL-6 affects HIV expression at multiple (transcriptional and post-transcriptional) levels.

HIV, the causative agent of AIDS, can infect and persist in the CD4+ cells of the immune system, such as T lymphocytes and mononuclear phagocytes (1). A number of mechanisms have been recently delineated whereby virus expression is upregulated in infected cells (reviewed in reference 1). Of particular interest is the fact that the virus can utilize a number of cell-derived soluble factors or cytokines for this purpose. In this regard, it has recently been demonstrated that cytokine-rich crude supernatants of mononuclear cells (2, 3), as well as recombinant cytokines such as TNF-α (4, 5) and -β (4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)1 (2, 6), can induce expression of virus in HIV-infected cells. It is noteworthy that this virus, which utilizes a functionally important molecule (CD4) on the surface of immune competent target cells as its receptor for cell entry (7), has also evolved cellular mechanisms for the upregulation of its expression by certain cytokines operative in the normal homeostatic control of the immune system. Furthermore, the molecular pathway(s) whereby HIV upregulation is accomplished for certain cytokines such as TNF is similar to the molecular mechanisms that modulate the expression of cellular gene function (8, 9). Specifically, TNF induction of HIV expression utilizes the pleiotropic cellular transcription factor NF-κB (9), which binds to the core enhancer region of the HIV long terminal repeat (LTR) (10-13).

Of particular interest in the human immunoregulatory network is the cytokine IL-6, which has a broad range of effects on the regulation of the immune system (14, 15). IL-6 is also known as B cell stimulatory factor 2 (16), hybridoma/plasmacytoma growth factor (17), hepatocyte-stimulating factor (18), IFN-β2 (19), and 26kD protein (20). Synthesis of IL-6 is inducible in many cell types (reviewed in references 14 and 15), including mononuclear phagocytes and T lymphocytes. Among the several conditions whereby synthesis of IL-6 is induced, infection of T lymphocytes with the human T lymphotropic virus 1 (HTLV-1) (20), and cell stimulation by cytokines such TNF-α (21) and IL-1 (22), are of particular interest. Among its several functions (14, 15), IL-6 induces terminal differentiation of lymphocytes to plasma cells (23) and proliferation of precursor and mature T cells (24), and acts as an autocrine growth factor for multiple myelomas/plasmacytomas (25, 26).

With regard to the relationship of this cytokine to HIV, in vitro infection of normal monocyte/macrophages with HIV-1 has been found to induce gene expression and secretion of IL-6 (27). In addition, increased levels of IL-6 have recently been reported both in the serum (28) and in the cerebrospinal fluid (29) of HIV-infected patients. Thus, it is important to determine whether this cytokine is capable of directly affecting the expression of HIV in infected cells and/or
of modulating the inducibility of this virus in response to other cytokines.

In the present study we demonstrate that IL-6 directly stimulates HIV replication in primary human macrophages acutely infected in vitro. In addition, IL-6 induces HIV expression in chronically infected promonocytic cells and synergizes with TNF-α in this effect. Furthermore, at the molecular level, we provide evidence that IL-6 induction of HIV expression involves multiple and diverse mechanisms.

Materials and Methods

Infection of Primary Monocyte-derived Macrophages (MDM).
Heparinized venous peripheral blood from healthy seronegative donors was diluted 1:4 with cold PBS and subjected to Ficoll-Hypaque gradient (550 g for 30 min at 4°C) in order to separate the mononuclear cell fraction (PBMC). PBMC were carefully resuspended in cold PBS and washed twice (350 g for 10 min at 4°C) in order to reduce platelet contamination. Finally, PBMC were resuspended in Iscove’s Modified Dulbecco’s Medium (IMDM; Gibco Laboratories, Grand Island, NY) containing 10% FCS (basic medium). The bacterial endotoxin (LPS) content of the medium and FCS was <3 pg/ml, and <400 pg/ml, respectively. Then, ~2 x 10^5 cells/ml well were plated in 24-well plastic tissue culture plates (Costar, Cambridge, MA). After overnight incubation at 37°C in humidified, 5% CO2 atmosphere, nonadherent cells (highly enriched in T lymphocytes) were removed by vigorous pipetting. The remaining adherent cells (predominantly monocytes) were maintained in culture in the presence or absence of 100 U/ml of rIL-6, or TNF-α, or GM-CSF for an additional 5–7 days before infection with a macrophage-tropic strain of HIV (AD-87) (30). The LPS contents of these cytokine concentrations were <0.1 µg/ml. No significant differences in comparison to adherence-purified monocytes were observed when monocytes were purified by countercurrent centrifugal elutriation and infected with HIV. Approximately 50% of the culture supernatants was replaced with basic medium or medium containing cytokines every 5–7 days after HIV infection for several weeks. In parallel experiments, the autologous nonadherent (T lymphocyte–enriched) cells were maintained and infected with HIV in similar culture conditions. PHA-stimulated T cell blasts were also infected with a T lymphocytic HIV strain (LAV) and were maintained in culture in medium containing IL-2 (31) plus additional cytokines.

HIV-infected Cell Lines. The chronically HIV-infected U1 cell line was obtained by limiting dilution cloning of promonocytic U937 cells that survived an acute HIV-1 infection (32). U1 cells possess two integrated copies of HIV proviral DNA (Maury, W., and A. Rabson, personal communication) and constitutively express low levels of virus as determined by immunofluorescence, cellular and supernatant-associated reverse transcriptase (RT) activity, HIV p24 antigen capture, Western blot and Northern blot analysis (RT Assay). Aliquots of supernatants from cytokine-stimulated and unstimulated cultures were tested for the presence of HIV RT by the method of Willey et al. (37). 10 µl of supernatants were added in duplicate to 50 µl of a mixture containing poly(A), oligo(dT) (Pharmacia Fine Chemicals, Piscataway, NJ), MgCl2, and 32P-labeled deoxythymidine 5'-triphosphate (dTTP) (Amersham Corp., Arlington Heights, IL), and incubated for 2 h at 37°C. 6 µl of the mixture was spotted onto DE81 paper (Whatman International, Maidston, England), air-dried, washed five times in 2 x SSC buffer, and two additional times in 95% ethanol. The paper was then dried, cut, and counted on a scintillation counter (No. LS 7000; Beckman Instruments, Fullerton, CA).

Western Blot Analysis. Cell lysates were prepared from U1 cells incubated for 72 h in the presence or absence of 100 U/ml of IL-6, 100 U/ml of TNF-α, or the combination of both cytokines. 20 µl of the lysate of 10^5 cells was added to each lane and subjected to electrophoresis through 10–20% gradient polyacrylamide gels (Integration Separation Sciences, Hyde Park, MA) for 6 h. The migrated proteins were then transferred overnight onto nitrocellulose filters. After saturation with a 5% milk solution, filters were incubated for 2 h with 1:1,000 vol/vol dilution of an AIDS patient serum containing high titers of anti-HIV Ab recognizing most of the major viral proteins (33). Filters were then washed and incubated for 90 min with 125I-labeled protein A (200,000 dpm/ml), washed, air-dried, and exposed overnight to x-ray film, as previously described (33).

Northern Blot Analysis. U1 cells were resuspended at 2.5 x 10^6 cells/ml and maintained in RPMI 1640 with 10% FCS in the presence or absence of various cytokines. Total RNA was extracted by the guanidinium thiocyanate phenol method with an RNA Isolation Kit (Stratagene, La Jolla, CA) from 10^6 U1 cells after 24, 48, and 72 h of cytokine stimulation. 10 µg of total RNA was loaded per lane on a 0.8% agarose formaldehyde gel and transferred to nitrocellulose by Northern blotting. The filters were baked and hybridized for 12 h with a 32P-labeled HIV-LTR (HindIII-AvaI) probe. Filters were washed and exposed to radiographic film. The labeled probe was removed from the filters by washing at 80°C in 0.1 x SSC, containing 0.1% SDS and then rehybridized with a 32P-labeled β-actin cDNA probe. The results were confirmed by RNA slot blot analysis (12).

Nuclear Run-On Analysis. Nuclei from 5 x 10^6 U1 cells were isolated 17 h after cytokine stimulation and nuclear run-on analysis were performed according to a published procedure (38). Briefly, equal amounts of 32P-labeled RNA (10' cpm/0.8 ml) were hybridized to linearized plasmid DNA probes immobilized on nitrocellulose filters. Probes included PBR 322 or PUC 19 as plasmid controls, pNL4-3, which is a plasmid containing a full-length HIV genome (39), and human β-actin cDNA. Filters were exposed to x-ray film for various times up to 7 days and densitometric laser scanning was performed on the different autoradiograms using an LKB Kit (Stratagene, La Jolla, CA) from 10^6 Ul cells after 24, 48, and 72 h of cytokine stimulation. 10 µg of total RNA was loaded per lane on a 0.8% agarose formaldehyde gel and transferred to nitrocellulose by Northern blotting. The filters were baked and hybridized for 12 h with a 32P-labeled HIV-LTR (HindIII-AvaI) probe. Filters were washed and exposed to radiographic film. The labeled probe was removed from the filters by washing at 80°C in 0.1 x SSC, containing 0.1% SDS and then rehybridized with a 32P-labeled β-actin cDNA probe. The results were confirmed by RNA slot blot analysis (12).

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Results

Different Cytokines Upregulate HIV Replication in Acutely Infected Primary MDM. MDM cultures exposed to various concentrations of certain recombinant cytokines showed an increase in HIV replication. As previously reported (6), GM-CSF enhanced HIV production in primary infected MDM (Fig. 1A). Similarly, both TNF-α (Fig. 1A) and IL-6 (Fig. 1A and B) were effective in upregulating HIV replication in MDM. Worthy of note is the fact that a significant degree of variability was observed between different monocyte donors in terms of susceptibility to HIV infection (with levels of peak HIV production in the absence of exogenous cytokine stimulation ranging between 300 and 12,000 cpn/μl), probably reflecting different levels of in vivo activation and/or differences in individual susceptibility to HIV infection, as previously observed for T lymphocytes (31). Nonetheless, IL-6, and to a lesser extent TNF-α and GM-CSF, reproducibly increased HIV expression in MDM. 5 of 5 donors infected MDM showed higher peak RT levels and accelerated kinetics of viral replication as compared with unstimulated cells, as shown in Fig. 1B. In contrast, IL-6 did not show any significant effect on HIV infection of primary T lymphocytes either when cells were maintained in culture conditions similar to MDM or when stimulated with PHA, cultivated in the presence of IL-2, and infected with a T lymphocytotropic isolate of HIV (data not shown).

Thus, IL-6 represents a new HIV-inductive cytokine for infected cells of the monocytic lineage, which have been previously described as important in vivo targets of infection (1). Because of the broad range of susceptibilities to HIV infection observed with MDM obtained from different donors, we further investigated the inductive effects of IL-6 on HIV expression by using the chronically infected monocytic cell line U1, which in our experience gave much more consistent results from experiment to experiment (32-34). Similar to the primary infected macrophages, HIV expression is inducible in U1 cells by TNF-α (34, 35) and by GM-CSF (2).

IL-6 Induction of HIV Expression in Chronically Infected Promonocytic Cells. The chronically infected U1 cells were exposed to various concentrations (from 0.1 to 1,000 U/ml) of rhIL-6; cell viability and virus production were monitored daily up to 12 d after treatment with an individual cytokine. Maximal stimulation of HIV production was observed with 100 U/ml of IL-6 occurring between 72 and 96 h after stimulation (Fig. 2). As little as 1 U/ml of IL-6 was capable of inducing viral expression in U1 cells (Fig. 2), whereas cytokine concentrations higher than 100 U/ml did not result in a further increase of HIV production. No cellular toxicity was observed in IL-6–treated U1 cells at any IL-6 concentrations tested, as determined by trypan blue dye exclusion criteria (>95% viable cells throughout the 7-d culture period). Furthermore, IL-6 upregulated viral expression from U1 cells to levels comparable to those induced by TNF-α (Fig. 2) and GM-CSF (not shown). A second chronically infected promonocytic cell line, U33.3, unlike U1 cells, constitutively expresses detectable amounts of virus (500-1,000 cpn/μl of
RT activity) (34). HIV expression in U33.3 cells was also inducible by IL-6 and TNF-α (data not shown). As we previously observed in infected primary T cells, IL-6 (up to 1,000 U/ml) was not capable of affecting HIV production by the T lymphocytic cell line ACH-2 (data not shown).

**IL-6 Synergizes with TNF-α in the Upregulation of HIV Expression in U1 Cells.** Having established that multiple cytokines were capable individually of upregulating HIV expression in chronically infected promonocytic cells, we investigated the possibility that simultaneous stimulation of U1 cells with IL-6 and TNF-α (co-stimulation) could result in further increases in virus production. We observed that co-stimulation of these cells with 100 U/ml of IL-6 and TNF-α resulted in a synergistic induction of HIV expression (Fig. 3 A). Incubation of U1 cells for 72 h in the presence of 100 U/ml of IL-6 and 100 U/ml of TNF-α resulted in ~65-fold stimulation (up to 100-fold in other experiments) of HIV expression over the constitutive levels, whereas individual incubation of U1 cells with the two cytokines led to a ~10-fold RT increase (Fig. 3 A). In addition to RT, both IL-6 and TNF-α induced synthesis of specific HIV proteins that were virtually undetectable in unstimulated conditions, as determined by Western blot analysis (Fig. 3 B). Co-stimulation of U1 cells with 100 U/ml each of IL-6 and TNF-α resulted in an increased intensity of the expressed bands as well as in the appearance of additional bands corresponding to specific HIV proteins (Fig. 3 B), reflecting the synergistic induction of RT activity in the culture supernatants. Suboptimal concentrations of IL-6 (1 U/ml) were still effective in synergizing with TNF-α. Both the direct and the synergistic effects of these cytokines on HIV expression were confirmed by indirect immunofluorescence and by neutralization studies which indicated that polyclonal and monoclonal anti-IL-6 and anti-TNF-α Ab were capable of suppressing both the direct and the synergistic effects of IL-6 and TNF-α stimulation on HIV expression (data not shown).

We have also investigated whether combinations of cytokines could further enhance the levels of HIV replication in acutely infected primary MDM cultures, as compared with single cytokine treatments. We have not observed significant enhancement of HIV production in these cells by any combination of exogenous cytokines, thus far. However, it has been previously reported that HIV infection of MDM induces secretion of both TNF-α (36, 40) and IL-6 (27), which could act both synergistically with exogenous cytokines as well as in an autocrine/paracrine manner (14, 15, 34, 41), in maximizing the levels of HIV production in these culture conditions. In this regard, we have also observed production of IL-6 in long-term (more than 30-d-old) MDM-infected cultures as well as reduced HIV replication in MDM treated with anti-IL-6 or anti-TNF-α Ab (Poli, G., J. S. Justement, A. Kinter, and A. S. Fauci, unpublished observations).

**IL-6 Induction of Viral Expression in U1 Cells Is Not Associated with Increased Levels of Steady-State HIV RNA.** To investigate whether induction of HIV expression by IL-6 alone was mediated by an increased transcription of viral RNA, as previously demonstrated for TNF-α (11–13), we compared the levels of total HIV RNA present in U1 cells stimulated by these cytokines. TNF-α induced substantial amounts of HIV RNA (Fig. 4 A), whereas IL-6 did not induce a significant accumulation of HIV RNA over the constitutive levels. However, HIV protein expression was induced to comparable levels by IL-6 and TNF-α (Fig. 4 B). In contrast, when U1 cells were co-stimulated with IL-6 and TNF-α, a synergistic accumulation of HIV RNA was observed in association with enhanced HIV protein production (Fig. 4, A and B). RNA slot blot analysis confirmed both the lack of induction of HIV RNA following stimulation with IL-6 alone and the synergistic accumulation of viral RNA in U1 cells co-stimulated with IL-6 and TNF-α (data not shown).
Synergistic induction of HIV RNA by co-stimulation of U1 cells with IL-6 and TNF-α. (A) Northern blot analysis of total HIV RNA extracted from: (Lane 1) unstimulated cells; (lane 2) cells stimulated with IL-6; (lane 3) cells stimulated with TNF-α; (lane 4) cells co-stimulated with IL-6 and TNF-α. HIV RNA was detectable after 72 h of stimulation exclusively in U1 cells that had been co-stimulated with IL-6 and TNF-α (lane 4), but not with TNF-α alone (lane 3). Similar results were obtained when lower concentrations (100 U/ml) of TNF-α were used alone or in combination with IL-6 (data not shown). (B) Direct IL-6 induction of viral proteins, but not of HIV RNA. The relative amounts of RNA are represented as areas (mm²) under the curve obtained by densitometric laser scanning of the HIV specific RNA bands shown in Fig. 3 A after 24 h of stimulation. The amounts of p24 released in the culture supernatants 72 h after cytokine stimulation were calculated by interpolation of the optical density (OD) values of the culture supernatants with a standard antigen curve, both measured with the same ELISA apparatus.

Costimulation of U1 cells with IL-6 and TNF-α resulted in a synergistic production of HIV p24 antigen that saturated the maximal optical capacity (equivalent to ~400 ng/ml) of the ELISA reader.

Discussion

In the present study we have demonstrated that the cytokine IL-6, which plays an important immunoregulatory role at multiple levels of the immune response (14, 15), directly upregulates the production of HIV in primary infected MDM as well as in chronically infected monocytic cells. In contrast, IFN-γ was not effective in modulating either the susceptibility of primary T cells to HIV infection or the induction of virus expression in chronically infected T cell lines. In addition, IL-6 synergizes in the induction of viral expression in U1 cells co-stimulated with TNF-α, which was previously described as a potent HIV inductive cytokine (3–5, 11-13, 34–36). Synergy between cytokines is a well-recognized mechanism of modulation of the normal immune response (14, 41). The present observation that certain of these cytokines can synergistically induce HIV expression in chronically infected cells of HIV transcription, nuclear run-on analysis was performed on U1 cells stimulated with various cytokines alone or in combination. TNF-α stimulation induced transcription of new HIV RNA three-fourfold over the unstimulated control, whereas IL-6 treatment alone did not significantly change the baseline transcriptional levels (Fig. 5). However, co-stimulation of U1 cells with IL-6 and TNF-α resulted in a synergistic increase of HIV transcription (~10-fold over the unstimulated controls). To verify that these results were attributable to actual transcriptional changes, parallel experiments were performed with nuclei isolated from stimulated U1 cells that were incubated with the transcriptional inhibitor α-amanitin, which almost entirely suppressed transcription.

IL-6 and TNF-α Synergize at the Transcriptional Level to Upregulate HIV Expression. To determine whether the synergistic increase in HIV expression detectable at the protein, RT, and mRNA levels was correlated with an increased rate of HIV transcription, nuclear run-on analysis was performed on U1 cells stimulated with various cytokines alone or in combination. TNF-α stimulation induced transcription of new HIV RNA three-fourfold over the unstimulated control, whereas IL-6 treatment alone did not significantly change the baseline transcriptional levels (Fig. 5). However, co-stimulation of U1 cells with IL-6 and TNF-α resulted in a synergistic increase of HIV transcription (~10-fold over the unstimulated controls). To verify that these results were attributable to actual transcriptional changes, parallel experiments were performed with nuclei isolated from stimulated U1 cells that were incubated with the transcriptional inhibitor α-amanitin, which almost entirely suppressed transcription.

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indicates that HIV mimics the normal homeostatic mechanisms utilized by the immune system to upregulate its own gene expression. In this regard, cytokines such as IL-6 and TNF-α are known to function in an autocrine/paracrine manner to modulate the state of activation of certain immune competent cells (14, 15, 41). Of interest, we have recently observed that TNF-α can induce expression of HIV in chronically infected T lymphocytic and monocytic cells in an autocrine fashion (34). We are currently investigating whether a similar mechanism is demonstrable for IL-6.

At the molecular level, we and others have demonstrated that the inductive effect of TNF-α on HIV expression involves the activation of the cellular transcription factor NF-kB (10-13), which is a pleiotropic mediator of tissue-specific and cellular gene function (8, 9). In contrast, IL-6 stimulation induces expression of viral proteins and production of RT activity to levels comparable to that of TNF-α, but unlike TNF-α, does not increase significantly the levels of steady-state viral mRNA over the constitutive levels. However, co-stimulation of U1 cells with IL-6 and TNF-α results in a synergistic enhancement of HIV expression measurable both at the RNA and at the protein/RT level. Nuclear run-on analysis demonstrates that this synergistic effect occurs, at least in part, at the transcriptional level. However, U1 stimulation with IL-6 alone did not lead to a significant increase of HIV transcription. Therefore, it is likely that IL-6 affects the expression of HIV by at least two mechanisms. In the presence of TNF-α, IL-6 increases the levels of viral transcription and of steady-state RNA, resulting in a synergistic enhancement of HIV expression. In the absence of TNF-α, IL-6 acts post-transcriptionally, increasing the expression of HIV proteins and RT activity without significantly inducing new transcription or accumulation of HIV RNA. In this regard, it has been recently shown that IL-6 can affect the synthesis of immunoglobulins at both the transcriptional and post-transcriptional level (42).

The observation that IL-6 upregulates the production of HIV in vitro both directly and in synergy with other cytokines provides a potential model to investigate the physiologic as well as pathogenic mechanisms that influence the degree of HIV expression in vivo. On the other hand, it has been reported recently that in vitro infection of monocytes with HIV induces synthesis and secretion of IL-6 (27). In fact, we have also observed IL-6 production in infected primary macrophage cultures (Poli, G., J. S. Justement, A. Kinter, and A. S. Fauci, unpublished observations). Furthermore, increased levels of IL-6 have been demonstrated both in the plasma (28) and in the cerebrospinal fluid (29) of HIV-infected individuals. Further understanding of the role of IL-6 and other cytokines in the regulation of HIV expression in infected cells may provide important insight into phenomena such as the progressive increase in HIV expression and ultimately viral burden that invariably occurs in infected individuals, and which are associated with deterioration of immune function and clinical progression towards AIDS (43).

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References

1. Fauci, A. S. 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science (Wash. DC). 239:617.
2. Folks, T. M., J. Justement, A. Kinter, C. A. Dinarello, and A. S. Fauci. 1987. Cytokine-induced expression of HIV-1 in a chronically infected promonocyte line. Science (Wash. DC). 238:800.
3. Clouse, K. A., D. Powell, I. Washington, G. Poli, K. Strebel, W. Farrar, P. Barstad, J. Kovacs, A. S. Fauci, and T. M. Folks. 1989. Monokine regulation of human immunodeficiency virus-1 expression in a chronically infected human T cell clone. J. Immunol. 142:431.
4. Folks, T. M., K. A. Clouse, J. Justement, A. Rabson, E. Duh, J. H. Kehrl, and A. S. Fauci. 1989. Tumor necrosis factor α induces expression of human immunodeficiency virus in a chronically infected T cell clone. Proc. Natl. Acad. Sci. USA. 86:2365.
5. Matsuyama, T., Y. Hamamoto, G.-I. Soma, D. Mizuno, N. Yamamoto, and N. Kobayashi. 1989. Cytocidal effect of tumor necrosis factor on cells chronically infected with human immunodeficiency virus (HIV): enhancement of HIV replication. J. Virol. 63:2504.
6. Koyanagi, Y., W. A. O'Brien, J. Q. Zhao, D. W. Golde, J. C. Gasson, and I. S. Y. Chen. 1988. Cytokines alter production of HIV-1 from primary mononuclear phagocytes. Science (Wash. DC). 241:1673.
7. Klatzmann, D., F. Barre-Sinoussi, M. T. Nugeyre, C. Danquet, E. Vilmer, C. Griscelli, F. Brun-Vezinet, C. Rouzioux, J. C. Gluckman, and J. C. Chermann. 1984. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science (Wash. DC). 225:59.
8. Greene, W. C., E. Bohmlein, and D. W. Ballard. 1989. HIV-1, HTLV-I and normal T-cell growth: transcriptional strategies and surprises. Immunol. Today. 10:272.
9. Lenardo, M. J., and D. Baltimore. 1988. NF-kB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell. 8:227.
10. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (Lond.). 326:711.
13. Okamoto, T., T. Matsuyama, S. Mori, Y. Hamamoto, N. Kobayashi, N. Yamamoto, S. F. Josephs, F. Wong-Staal, and K. Shimotomoto. 1989. Augmentation of human immunodeficiency virus type 1 gene expression by tumor necrosis factor alpha. *AIDS Res. Hum. Retroviruses.* 5:131.

14. Kishimoto, T. 1989. The biology of interleukin-6. *Blood.* 74:1.

15. Wong, G. G., and S. C. Clark. 1988. Multiple actions of interleukin 6 within a cytokine network. *Immunol. Today.* 9:137.

16. Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, S. Tsunawasa, F. Sakiyama, H. Matsui, Y. Takahara, T. Taniguchi, and T. Kishimoto. 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature (Lond.)* 324:73.

17. Van Damme, J., G. Opdenakker, R. J. Simpson, M. R. Rubira, S. Cayphas, A. Vink, A. Billius, and J. Van Snick. 1987. Identification of the human 26-kD protein, interferon beta-2 (IFN-beta-2), as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor. *J. Exp. Med.* 165:914.

18. Gauthie, J., C. Richards, D. Harnish, P. Landsdorp, and H. Bumann. 1987. Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc. Natl. Acad. Sci. USA.* 84:7251.

19. Zilberstein, A., R. Ruggieri, J. H. Korn, and M. Revel. 1986. Structure and expression of cDNA and genes for human interferon-beta-2, a distinct species inducible by growth-stimulatory cytokines. *EMBO J.* 5:5259.

20. Haegeman, G., J. Content, G. Volckaert, R. Deryckere, J. Tavernier, and W. Fiers. 1986. Structural analysis of the sequence coding for an inducible 26-kDa protein in human fibroblasts. *Eur. J. Biochem.* 159:625.

21. Waltho, Z., L. T. May, and P. B. Seegah. 1988. Transcriptional regulation of the interferon-beta 2/B cells differentiatation factor BSF-2/hepatocyte-stimulating factor gene in human fibroblasts by other cytokines. *J. Immunol.* 140:974.

22. Sironi, M., F. Breviario, P. Prosperpio, A. Biondi, A. Vecchi, J. Van Damme, E. Dejana, and A. Mantovani. 1989. IL-1 stimulates IL-6 production in endothelial cells. *J. Immunol.* 142:549.

23. Muraguchi, A., T. Kishimoto, Y. Miki, T. Kuritani, T. Kaieda, K. Yoshizaki, and Y. Yamamura. 1981. T cell-replacing function (TRF) induced IgG secretion in a human B blastoid cell line and demonstration of acceptors for TRF. *J. Immunol.* 127:412.

24. Lotz, M., J. H. Vaughan, and D. A. Carson. 1988. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. *Science (Wash. DC).* 241:1218.

25. Nordan, R. P., and M. Potter. 1986. A macrophage-derived factor required by plasmacytomas for survival and proliferation in vitro. *Science (Wash. DC).* 233:566.

26. Kawano, M., T. Hirano, T. Matsuda, T. Taga, Y. Horii, K. lwato, H. Asaoku, B. Tang, O. Tanabe, H. Tanaka, A. Kuramoto, and T. Kishimoto. 1988. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. *Nature (Lond.)* 332:83.

27. Nakajima, K., O. Martinez-Maza, T. Hirano, E. C. Breen, P. G. Nishanian, J. F. Salazar-Gonzalez, J. L. Fahey, and T. Kishimoto. 1989. Induction of IL-6 (B cell stimulatory factor-2/IFN-B) production by HIV. *J. Immunol.* 142:531.

28. Breen, E. C., A. R. Rezai, K. Nakajima, G. N. Beall, R. T. Mitsuysu, T. Hirano, T. Kishimoto, and O. Martinez-Maza. 1990. Infection with HIV is associated with elevated IL-6 levels. *J. Immunol.* 144:480.

29. Gallo, P., K. Frei, C. Rordorf, J. Lazdins, B. Tavolato, and A. Fontana. 1989. Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system: an evaluation of cytokines in cerebrospinal fluid. *J. Neuroimmunol.* 23:109.

30. Endelman, H. E., J. M. Orenstein, M. A. Martin, C. Perrus, R. Mitra, T. Phipps, L. A. Wahl, H. C. Lane, A. S. Fauci, D. S. Burke, D. Skillman, and M. S. Melitzer. 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. *J. Exp. Med.* 167:148.

31. Folks, T. M., J. Kelly, S. Benn, A. Kinter, J. Justement, J. Gold, R. Redfield, K. W. Sell, and A. S. Fauci. 1986. Susceptibility of normal human lymphocytes to infection with HTLVIII/LAV. *J. Immunol.* 136:4049.

32. Folks, T. M., J. Justement, A. Kinter, S. Schnittman, J. Orenstein, G. Poli, and A. S. Fauci. 1988. Characterization of a promonocyte clone chronically infected with HIV and inducible by 13-phorbol-12-myristate acetate. *J. Immunol.* 140:1117.

33. Poli, G., J. M. Orenstein, A. Kinter, T. M. Folks, and A. S. Fauci. 1989. Interferon-alpha but not AZT suppresses HIV expression in chronically infected cell lines. *Science (Wash. DC).* 244:575.

34. Poli, G. A. Kinter, J. S. Justement, J. H. Kehrl, P. Bressler, S. Stanley, and A. S. Fauci. 1989. Tumor necrosis factor-alpha functions in an autocrine manner in the induction of HIV expression. *Proc. Natl. Acad. Sci. USA.* 87:782.

35. Griffin, G. E., K. Leung, T. M. Folks, S. Kunkel, and G. J. Nabel. 1989. Activation of HIV gene expression during monocye differentiation by induction of NF-kB. *Nature (Lond.)* 339:70.

36. Clouse, K. A., P. B. Robbins, B. Fernie, J. M. Ostrove, and A. S. Fauci. 1989. Viral antigen stimulation of the production of human monokines capable of regulating HIV-1 expression. *J. Immunol.* 143:470.

37. Willey, R. L., D. H. Smith, L. A. Lasky, T. S. Theodore, P. L. Earl, B. Moss, D. Capon, and M. A. Martin. 1988. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. *J. Virol.* 62:139.

38. Linial, M., N. Gounderson, and M. Groudine. 1985. Enhanced transcription of c-myc in bursal lymphoma cells requires continuous protein synthesis. *Science (Wash. DC).* 230:1126.

39. Adachi, A., H. E. Gendelman, S. Koening, T. M. Folks, R. Willey, A. Rabson, and M. A. Martin. 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J. Virol.* 59:284.

40. Merrill, J. E., Y. Koyanagi, and I. S. Y. Chen. 1989. Interleukin-1 and tumor necrosis factor alpha can be induced from mononuclear phagocytes by human immunodeficiency virus type 1 binding to the CD4 receptor. *J. Virol.* 63:4404.
41. Sherry, B., and A. Cerami. 1988. Cachectin/tumor necrosis factor exerts endocrine, paracrine, and autocrine control of inflammatory responses. *J. Cell Biol.* 107:1269.

42. Raynal, M. C., Z. Liu, T. Hirano, L. Mayer, T. Kishimoto, and S. Chen-Kiang. 1989. Interleukin 6 induces secretion of IgG1 by coordinated transcriptional activation and differential mRNA accumulation. *Proc. Natl. Acad. Sci. USA.* 86:8024.

43. Schnittman, S. M., C. Psallidopoulos, H. C. Lane, L. Thompson, M. Baseler, F. Massari, C. H. Fox, N. P. Salzman, and A. S. Fauci. 1989. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science (Wash. DC).* 245:305.