Modulation of Interleukin-1β RNA in Monocytic Cells Infected with Human Immunodeficiency Virus-1

Kenji Yamato, Ziad El-Hajjaoui, Kenneth Simon, and H. Phillip Koeffler
*Department of Medicine, Division of Hematology/Oncology, University of California
at Los Angeles School of Medicine, Los Angeles, California 90024-1678*

Abstract

The effect of HIV-1 infection on cytokine levels was studied in monocytic cells by using Northern blotting analysis. Monoblasts (THP-1, U937) did not express IL-1β RNA even if the cells were infected with HIV-1. After exposure to LPS (10 μg/ml) and 12-O-tetradecanoylphorbol-13-acetate (TPA, 100 nM) for 12 h, these HIV-1–infected monoblasts accumulated 8–15-fold greater levels of IL-1β RNA as compared with their HIV-1–uninfected counterparts that were similarly stimulated. In contrast, levels of RNAs coding for monocyte-colony-stimulating factor (M-CSF) and tumor necrosis factor-alpha (TNFα) were elevated less than twofold in the HIV-1–infected cells as compared with HIV-1–uninfected cells after their stimulation with LPS and TPA. Inhibition of new protein synthesis did not block the marked accumulation of IL-1β RNA produced by exposure to LPS and TPA in the HIV-1–infected cells. Time-course experiments showed that the maximal levels of IL-1β RNA occurred at 12 and 24 h after LPS and TPA stimulation of the HIV-1–infected and uninfected U937 cells, respectively. Studies of stability of RNA using actinomycin D showed that IL-1β RNA was equally stable in infected and uninfected U937 cells after their stimulation with TPA and LPS. Taken together, our data show that HIV-1 infection markedly augments IL-1β RNA accumulation in stimulated monocytic cells, probably through increasing rate of transcription of IL-1β. (*J. Clin. Invest.* 1990. 86:1109–1114.) Key words: HIV • IL-1β RNA • monocytic cells

Introduction

Cells of the mononuclear phagocytic lineage including their normal and leukemic progenitor cells, monocytes, and macrophages, are susceptible to infection with HIV-1 (1–6). HIV-1 is not usually cytopathic to infected mononuclear phagocytic cells. Therefore, these cells may serve as an important reservoir for persistence of HIV-1 in individuals with AIDS and they may spread HIV-1 to target organs such as lungs, bone marrow, skin, and brain, contributing to some of the pathological findings in AIDS. Monocytes and macrophages are indispensable in defense of the body against microorganisms, and

HIV-1-induced abnormalities of these cells impair host defenses (7–10).

Recently, monocytes from patients with AIDS were found often to produce higher levels of IL-1 than monocytes from normal controls (11). In addition, Folks et al. noted that a HIV-1–infected clone of the monoblastic cell line U937 produced high levels of IL-1β after exposure to conditioned media from mitogen-stimulated T lymphocytes (12). Monocytes and macrophages communicate and activate CD-4 positive T lymphocytes by presenting antigens to the cells and by producing IL-1β (13). Overexpression of IL-1β in infected macrophages may disrupt normal immunological functions of T lymphocytes and may stimulate HIV-1 production or infection in these cells. Viral latency in these cells could be abrogated by inappropriate production of IL-1. In this study we examine the effect of HIV-1 infection on monokine production; in particular, we focus on mechanisms by which HIV-1 increases the expression of IL-1β RNA.

Methods

Monoblastic leukemic cell lines (U937 and THP-1) were grown in α-MEM culture media (Flow Laboratories, Inc., Rockville, MD) with 10% fetal calf serum in a humidified atmosphere with 7% CO_2. U937 and THP-1 cells were infected with HIV-1, isolate III$_B$ (HTLV-III$_B$), and RF (HTLV-III$_{RF}$), respectively. Briefly, 5×10^6 cells were in 1 ml of stock conditioned media containing HIV-1 and 10 μg/ml of Polybrene (Sigma Chemical Co., St. Louis, MO). After 1 h incubation at 37°C, cells were washed and cultured in complete alpha-media with 10% fetal calf serum. Viability of leukemic cell lines was determined in all experimental procedures by trypan blue exclusion and was always greater than 90%. Cells were used after 2 mo of infection with HIV-1. The following were generously provided: human granulocyte-macrophage (GM-CSF)[1] was obtained from Dr. S. Clark (Genetic Institute, Cambridge, MA); α- and β-interferon (IFN) and tumor necrosis factor (TNF) from Dr. M. Shepard (Genentech, Inc., San Francisco, CA); and interleukin-1β (IL-1β) from Dr. S. Gillis (Immunex, Seattle, WA). Tetradecanoylphorbol-13-acetate (TPA) and LPS from *Escherichia coli* were purchased from Sigma Chemical Co.

Total RNA of cells was extracted by the hot phenol method (14). RNA samples were separated by a 1% agarose-formaldehyde gel and transferred to a nylon-membrane filter. Blots were probed with ^{32}P-oligolabeled (random primed) restriction fragments (15), and hybridized for 16 to 24 h at 42°C in 50% formamide, 2× SSC (1× = 150 mM sodium chloride, 15 mM sodium citrate), 5× Denhardt's, 0.1% sodium dodecyl sulfate (SDS), 10% dextran sulfate, and 100 μg/ml salmon sperm. Filters were washed to a stringency of 0.1× SSC, 65°C and exposed to Kodak XAR film. Autoradiograms were developed at different exposures and scanned by densitometry.

β-actin DNA (Eco RI-Bam HI, 700 bp) was obtained from the

---

1. *Abbreviations used in this paper:* CHX, cycloheximide; CSF, colony stimulating factor; GM-CSF, granulocyte-macrophage CSF; M-CSF, monocyte CSF; TNF, tumor necrosis factor; TPA, 12-O-tetradecanoylphorbol-13-acetate.

pHFβA-3'-ut (untranslated) plasmid (16). Monocyte-colony stimulating factor (M-CSF) cDNA was obtained from p3ACSF-RI (Xbo I–Eco RI, 2,000 bp) (17), TNF cDNA was from pSP43-4 (Eco RI, 800 bp) (18), IL-1β was from pA-26 (Pst I, 900 bp) (19) and 3'LTR (long terminal repeat) of proviral HIV-1 DNA was from pH3LTR H (Eco RI, a gift from Dr. I. Chen (UCLA, Los Angeles, CA), unpublished).

Culture supernatant was assayed for HIV-1 p24 protein by ELISA (Abbott Laboratories, North Chicago, IL). Cells were seeded at a density of 5×10^5 cells/ml and cultured for 3 d. Supernatants were harvested by centrifugation.

[^{3}H]Uridine and [^{35}S]methionine incorporation: cells were exposed to either actinomycin D (10 μg/ml) or cycloheximide (CHX, 20 μg/ml) in culture dishes (Falcon Labware, Becton Dickinson, Oxnard, CA) in triplicate per experimental point for 2 h. Cells were pulsed with 2 μCi of [^{3}H]uridine (43 Ci/mmol sp act) or 4 μCi of [^{35}S]methionine (200 mCi/mM sp act) for 1 h at 37°C, washed twice in PBS, precipitated in 5% TCA in 30 mM Na_2HPO_4 at 4°C for 1 h, filtered onto a glass microfiber membrane (Whatman Inc., Clifton, NJ; GF/F), washed in 3% TCA (30 mM Na_2HPO_4), and heated at 80°C for 1 h. Each sample was counted by liquid scintillation. Results were compared with those of untreated cells.

Results

The U937 and THP-1 cells (1×10^6/ml) infected with HIV-1 secreted in 48 h 1.2 and 1.3 μg/ml of p24 protein, respectively. Both HIV-1–infected and uninfected U937 and THP-1 cells were exposed to the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA, 100 nM) and to lipopolysaccharide (LPS, 10 μg/ml) for 12 h. RNA was extracted from cells, electrophoresed, transferred by Northern blot technique onto nylon membrane, and probed with several different ^{32}P-labeled genes. Fig. 1 A shows the results from U937 cells. Both uninfected (lane 1) and HIV-1–infected (lane 3) cells constitutively contained negligible levels of IL-1β RNA. After stimulation with TPA and LPS, the HIV-1–infected cells (lane 4) had about a 15-fold greater accumulation of IL-1β RNA as com-

pared with the uninfected U937 cells (lane 2). In contrast, equivalent levels of M-CSF RNA were present in both HIV-1–infected (lane 4) and uninfected (lane 2) U937 cells after their exposure to TPA and LPS. Fig. 1 B shows that exposure to TPA and LPS produced 10-fold greater accumulation of levels of HIV-1 RNAs in the U937 cells (lane 4) as compared with the same population of cells not exposed to TPA and LPS (lane 3).

The results using the HIV-1–infected THP-1 monoblast line were similar to those observed in the U937 cells (Fig. 2). Both the uninfected (lane 1) and HIV-1–infected (lane 3) cells constitutively contained very low levels of IL-1β RNA (Fig. 2 A). After exposure to TPA and LPS, the HIV-1–infected cells (lane 4) had about an eightfold greater accumulation of IL-1β RNA as compared with the uninfected THP-1 cells (lane 2). In comparison, less than twofold greater levels of both M-CSF and TNF RNAs were present in HIV-1–infected (lane 4) as compared with HIV-1–uninfected (lane 2) cells after their exposure to TPA and LPS. Fig. 2 B shows that exposure of HIV-1–infected THP-1 to TPA and LPS produced increased levels of the 4.3 kb (twofold) and 1.8 kb (fivefold) RNAs encoded by HIV-1 (lane 4) as compared with the same population of cells not exposed to TPA and LPS (lane 3).

Dynamics of accumulation of IL-β RNA were examined in infected and uninfected U937 cells. Fig. 3 A shows that significant accumulation of IL-1β RNA occurred in the uninfected U937 cells within 6 h of stimulation with TPA and LPS (lane 4). Levels continued to rise at 12 h (lane 5) and 24 h (lane 6). In contrast, increased levels of IL-1β RNA were detectable in the HIV-1–infected U937$_{RF}$ as early as 1.5 h (Fig. 3 B, lane 2) after exposure to TPA and LPS and reached the maximal level at 12 h (lane 5) after stimulation. Further time-response studies showed that accumulation of RNAs coding for TNFα and M-CSF paralleled each other in the HIV-1–infected U937$_{RF}$ cells after exposure to TPA and LPS (lanes 5–8) (Fig. 3 C).
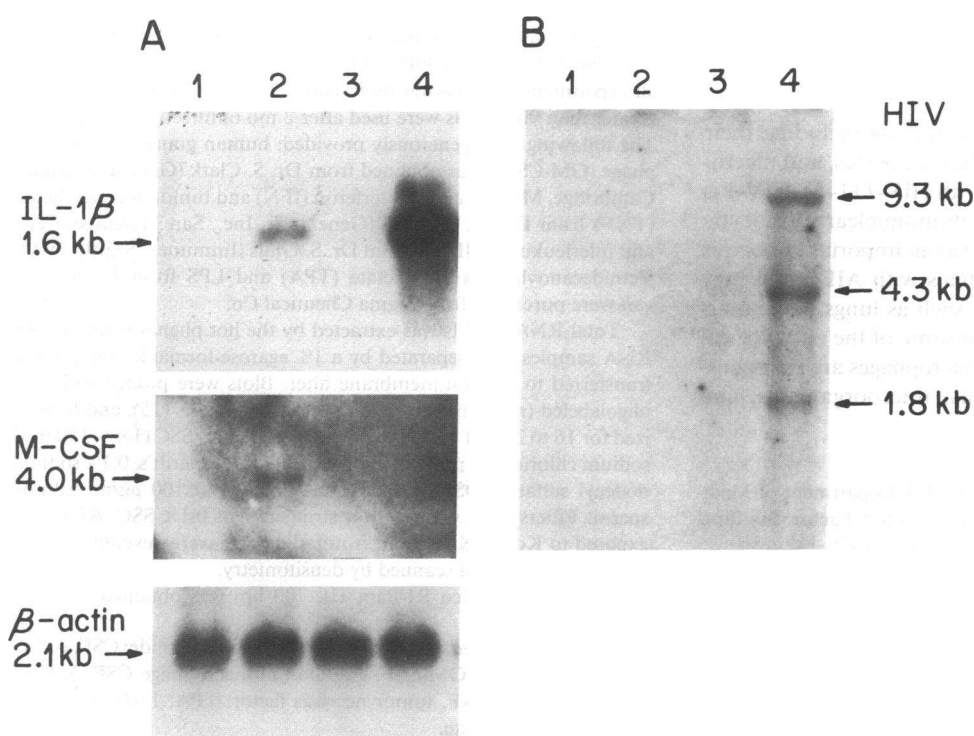
Figure 1. Expression of cytokine RNAs in uninfected and HIV-1–infected U937 cells. Uninfected (U937) and HIV-1–infected U937 (U937$_{RF}$) cells were cultured with TPA (100 nM) and LPS (10 μg/ml) for 12 h. Total RNA was extracted, size-fractionated by 1% agarose, transferred to nylon filter, and hybridized with ^{32}P-labeled IL-1β, M-CSF, β-actin RNA (A), and 3' LTR of HIV-1 proviral genome (B). (A and B) Lane 1, unstimulated U937 cells; lane 2, U937 cells stimulated with TPA and LPS; lane 3, unstimulated U937$_{RF}$ cells; lane 4, U937$_{RF}$ cells stimulated with TPA and LPS.
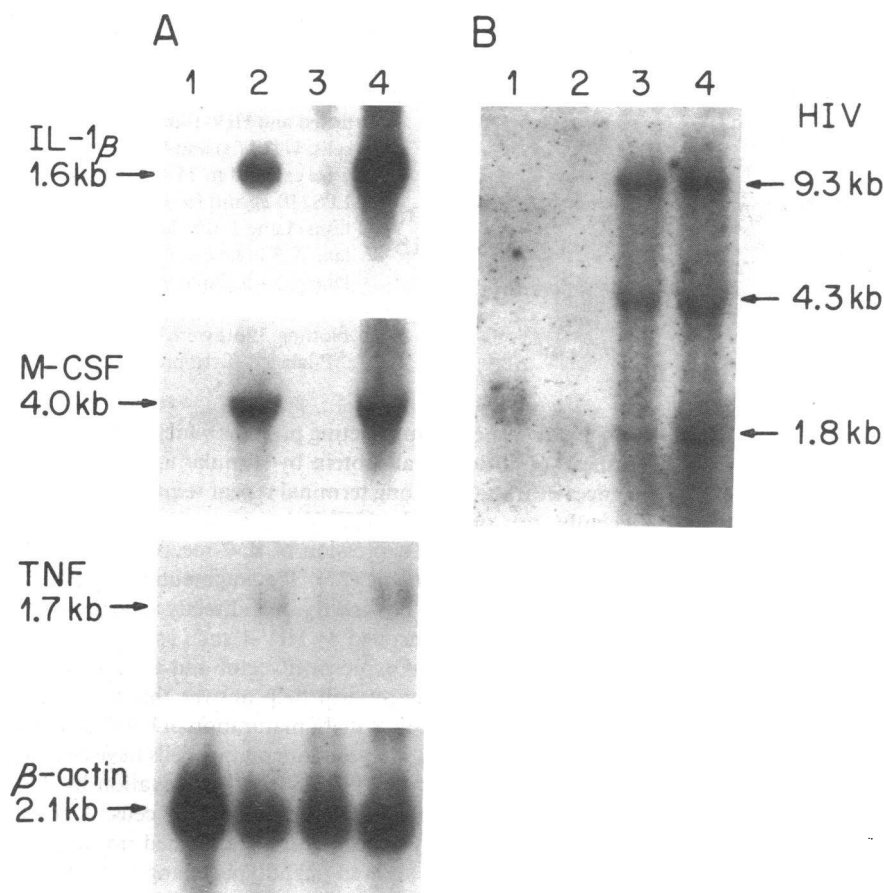
Figure 2. Expression of cytokine RNAs in uninfected and HIV-1–infected THP-1 cells. Uninfected (THP-1) and HIV-1–infected THP-1 (THP-1$_{IIIB}$) cells were exposed to TPA (100 nM) and LPS (10 µg/ml) for 12 h. Total RNA was extracted and analyzed by Northern blotting. Blot was hybridized with ^{32}P-oligolabeled IL-1β, M-CSF, β-actin DNA probes (*A*), and 3' LTR of HIV-1 proviral genome (*B*). (*A* and *B*) Lane *1*, unstimulated THP-1 cells; lane *2*, THP-1 cells cultured with TPA and LPS; lane *3*, unstimulated THP-1$_{IIIB}$; lane *4*, THP-1$_{IIIB}$ cells cultured with TPA and LPS.

Levels of TNFα and M-CSF RNAs in the HIV-1–infected cells were less than twofold greater than levels of these cytokines in the uninfected U937 and THP-1 cells after their exposure to TPA and LPS (lanes *1–4*).

The requirement of new protein synthesis for enhanced accumulation of IL-1β RNA in cells infected with HIV-1 was examined. U937$_{RF}$ cells were pretreated with CHX (20 µg/ml, protein synthesis inhibitor) for 30 min and then co-cultured with TPA, LPS, and CHX for 8 h. CHX alone slightly increased levels of IL-1β RNA (Fig. 4, lane *2*). Levels of IL-1β RNA were comparable in CHX-treated (lane *3*) and untreated (lane *4*) U937$_{RF}$ cells after their stimulation with LPS and TPA. CHX (20 µg/ml) decreased protein synthesis in U937 cells by > 95% (see Methods).

One mechanism by which HIV-1 infection might lead to increased levels of IL-1β RNA in myeloid cells is by increasing stability of the IL-1β message. In order to examine stability of IL-1β RNA, both HIV-1–infected and uninfected U937 cells were stimulated with TPA and LPS for 12 h and then cultured in the presence of actinomycin D (10 µg/ml) in order to stop new RNA synthesis. Actinomycin D decreased [^{3}H]uridine synthesis by > 90% (see Methods). Cells were harvested at different times, RNA extracted, electrophoresed, Northern blotted, and probed for levels of IL-1β RNA. The IL-1β RNA was very stable in both uninfected and infected cells with no change in the message levels of IL-1β, even after 12 h of exposure to actinomycin D (Fig. 5). Longer exposure of both cell types to actinomycin D markedly decreased viability of the cells (data not shown). These Northern blots were rehybridized

with ^{32}P-labeled c-*myc* (Fig. 5, bottom); half life ($t_{1/2}$) of RNA coding for this protooncogene was less than 3 h in both the HIV-1–infected and uninfected cells.

Discussion

Our study found that U937 and THP-1 monoblastic cell lines infected with HIV-1 had higher levels of IL-1β RNA than did their uninfected counterparts after stimulation with TPA and LPS. In contrast, levels of RNAs coding for several other cytokines (M-CSF, TNF) were less than twofold elevated in the infected cells as compared with the uninfected ones. Folks et al. (12) showed that conditioned media from PHA-stimulated mononuclear cells from peripheral blood enhanced expression of membrane-bound IL-1β on cells of a subclone of HIV-1–infected U937 as compared with uninfected parental cells. The stimulatory material in the PHA-conditioned media was not identified. TPA and LPS stimulate transcription of IL-1, and TPA stabilizes IL-1 mRNA (20, 21); but why TPA and LPS enhanced production of IL-1β RNA in our HIV-1–infected myeloid cells as compared with the uninfected cells is unclear. In addition, we found that recombinant GM-CSF (200 pM), α- and β-IFN (1,000 U/ml), TNF (25 ng/ml), or IL-1β (50 U/ml) did not enhance levels of IL-1β RNA in either HIV-1–infected or uninfected U937 cells (data not shown).

Time-response experiments showed that the accumulation of IL-1β RNA in HIV-1–infected cells occurred more quickly and was of a greater magnitude than in uninfected cells after their stimulation with TPA and LPS. Half-life experiments
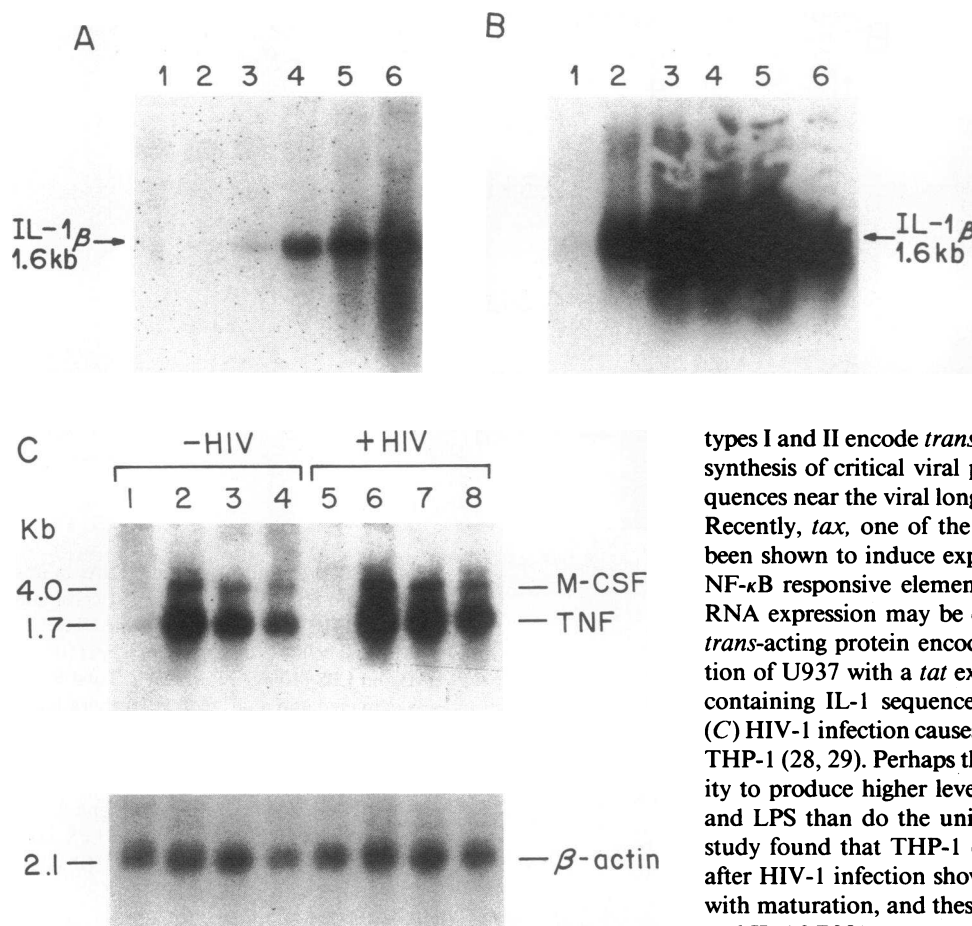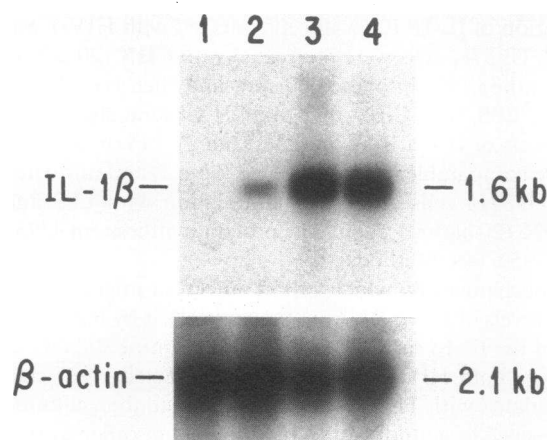
Figure 3. (A and B). Time-response of levels of IL-1β mRNA in uninfected and HIV-1–infected U937 cells. U937 (A) and U937$_{RF}$ (B) were exposed to TPA (100 nM) and LPS (10 μg/ml) for various durations. Lane 1, 0 h; lane 2, 1.5 h; lane 3, 3 h; lane 4, 6 h; lane 5, 12 h; lane 6, 24 h. Total RNA was extracted and analyzed by Northern blotting. Blots were hybridized with ^{32}P-labeled IL-1β probe.

Figure 3 C. Time-response of levels of TNFα and M-CSF RNAs in uninfected and HIV-1–infected U937 cells. U937 (lanes 1–4) and U937$_{RF}$ (lanes 5–8) were exposed to TPA (100 nM) and LPS (10 μg/ml) for various durations. U937 uninfected: lane 1, untreated cells; lane 2, 3 h; lane 3, 6 h; lane 4, 12 h. U937$_{RF}$: lane 5, untreated cells; lane 6, 3 h; lane 7, 6 h; lane 8, 12 h. Total RNA was extracted and analyzed by Northern blotting and hybridization with ^{32}P-labeled TNFα, M-CSF, and β-actin DNA probes.

types I and II encode *trans*-acting proteins which initiate RNA synthesis of critical viral protein by stimulating *cis*-acting sequences near the viral long terminal repeat sequences (25, 26). Recently, *tax*, one of the HTLV-1 *trans*-acting proteins, has been shown to induce expression of IL-2 receptors through a NF-κB responsive element (27). The augmentation of IL-1β RNA expression may be directly or indirectly mediated by a *trans*-acting protein encoded by HIV-1 such as *tat*. Transfection of U937 with a *tat* expression vector and a reporter gene containing IL-1 sequences will help answer this hypothesis. (C) HIV-1 infection causes slight maturation of U937 cells and THP-1 (28, 29). Perhaps these more mature cells have a capacity to produce higher levels of IL-1β after activation by TPA and LPS than do the uninfected, less mature cells. A recent study found that THP-1 cells that were studied immediately after HIV-1 infection showed phenotypic changes compatible with maturation, and these cells had increased levels of TNFα and IL-1β RNA as compared with uninfected cells (29). With chronic infection, the cell population became less mature and no longer produced increased levels of these cytokines. Our HIV-1 chronically infected populations of U937 and THP-1 cells are morphologically the same maturity as the uninfected

showed that IL-1β RNA was equally stable in both the HIV-1–infected and uninfected cells after their stimulation. This suggests that accumulation of IL-1β RNA in these cells is enhanced, not by increasing stability of IL-1β mRNA but, probably, by increasing the rate of transcription of the IL-1β gene. Transcriptional run-on experiments are necessary to confirm this impression.

How HIV-1 might enhance the rate of IL-1β gene transcription in U937 and THP-1 cells is not clear from our experiments. Possibilities include: (a) Several recent reports suggest that HIV-1 viral particles can trigger expression of IL-1, TNF, and IL-6 genes in macrophages by binding to CD4 molecules (22–24). This effect does not require viral expression in the cells and can be reproduced by using recombinant gp120 (envelop protein) of HIV-1; stimulation is probably by the gp120 protein binding to CD4 (23). In our experiments, CHX probably inhibited viral production but was unable to inhibit the enhanced accumulation of IL-1β RNA in the HIV-1–infected U937 cells stimulated with TPA and LPS. (b) Human retroviruses including HIV-1 and human T cell leukemia virus

Figure 4. Effect of CHX on accumulation of IL-1β mRNA. U937$_{RF}$ cells were pretreated with CHX (20 μg/ml) for 30 min. LPS and TPA were added and cells were cultured for an additional 8 h. Total RNA was extracted and analyzed by Northern blotting. Lane 1, untreated U937$_{RF}$ cells; lane 2, cells cultured with CHX (20 μg/ml) for 8.5 h; lane 3, cells exposed to TPA (100 nM) and LPS (10 μg/ml) for 8 h; lane 4, cells pretreated with CHX (20 μg/ml) for 30 min and then exposed to TPA (100 nM) and LPS (10 μg/ml) in the presence of CHX (20 μg/ml) for 8 h.
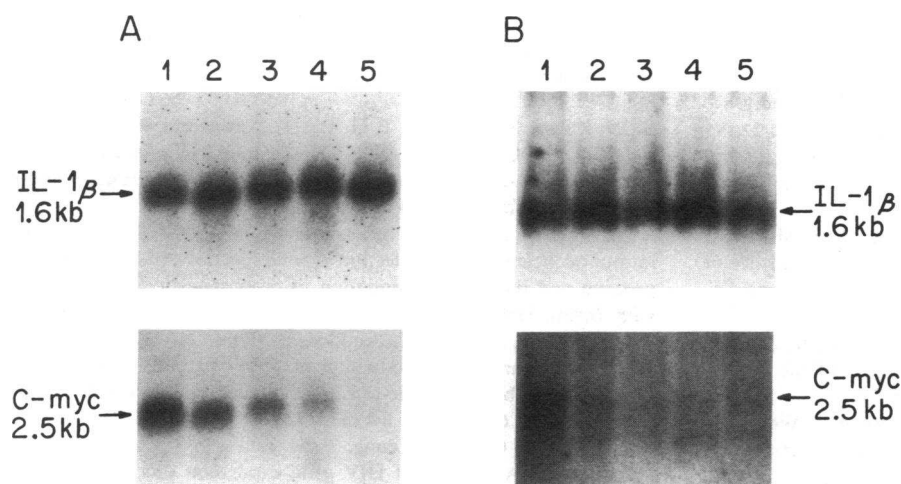
Figure 5. Stability of IL-1β mRNA in HIV-1–uninfected and infected U937 cells. U937 (*A*) and U937$_{RF}$ (*B*) cells were cultured with TPA and LPS for 12 h and then exposed to actinomycin D (10 μg/ml), and total RNA was extracted at different durations of culture. Northern blots were probed with ^{32}P-labeled IL-1β and c-*myc* cDNA. Lane *1*, 0 h; lane *2*, 1.5 h; lane *3*, 3 h; lane *4*, 6 h; lane *5*, 12 h after exposure to actinomycin D.

cells (data not shown). Further studies are required to determine if they are functionally more mature.

The HIV-1–infected THP-1 cells constitutively produce easily detectable levels of HIV-1 RNA (Fig. 2 *B*) as compared with HIV-1–infected U937 cells (Fig. 1 *B*). Expression of different levels of HIV-1 RNAs by these two cell lines may be explained by their differences in maturation. THP-1 cells are more mature than U937 cells. In addition, THP-1 cells express high levels of NF-κB; U937 cells do not, but their expression of NF-κB can be induced by TPA (30). HIV-1 gene expression in the monocytic lineage is regulated in part by cellular levels of NF-κB (30).

Production of IL-1 may be important in pathogenesis of AIDS and AIDS-related complex. IL-1 stimulates production of T cell growth factor and its receptor in T lymphocytes, stimulating their proliferation. Activation by IL-1 may also stimulate expression of NF-κB causing viral production in latently infected T lymphocytes (31). Furthermore, IL-1 is indispensable for immunological interactions between T lymphocytes and monocytes/macrophages during antigen presentation (32). Overexpression of IL-1 may disrupt this dialogue between these cells. In addition, IL-1 can support the growth of Kaposi's sarcoma cells (33, 34). Enhanced expression of IL-1β in skin macrophages may contribute to pathogenesis of this HIV-1–related malignancy.

Acknowledgments

References

1. Folks, T. M., S. W. Kessler, J. M. Orenstine, J. S. Justement, E. S. Jaffe, and A. S. Fauci. 1988. Infection and replication of HIV-1 in purified progenitor cells of normal human bone marrow. *Science (Wash. DC).* 242:919–922.

2. Ho, D., T. Rota, and M. Hirsch. 1986. Infection of monocytes/macrophages by human T-lymphotropic virus type III. *J. Clin. Invest.* 77:1712–1715.

3. Gartner, S., P. Markovits, M. H. Kaplan, R. C. Gallo, and M. Popovic. 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science (Wash. DC).* 233:215–219.

4. Salahuddin, S., R. Rose, J. Groopman, P. Markham, and R. C. Gallo. 1986. Human T-lymphotropic virus type III infection of human alveolar macrophages. *Blood.* 68:281–284.

5. Levy, J., J. Shimabukuro, T. McHugh, C. Casavant, D. Stites, and L. Oshino. 1985. AIDS-associated retroviruses (ARV) can productively infect other cells besides human T helper cells. *Virology.* 147:441–448.

6. Popovic, M., and S. Gartner. 1987. Isolation of HIV-1 from monocytes but not T lymphocytes. *Lancet.* ii:916.

7. Smith, P. D., K. Ohura, H. Masur, H. C. Lane, A. S. Fauci, and S. M. Wahl. 1984. Monocyte function in the acquired immunodeficiency syndrome: defective chemotaxis. *J. Clin. Invest.* 74:2121–2128.

8. Prince, H. E., D. J. Moody, B. I. Shubin, and J. L. Fahey. 1985. Defective monocyte function in acquired immunodeficiency syndrome (AIDS): evidence from a monocyte-dependent T-cell proliferation system. *J. Clin. Immunol.* 5:21–25.

9. Poli, G., B. Bottazzi, R. Acero, and L. Bersani. 1985. Monocyte function in intravenous drug abusers with lymphadenopathy syndrome and in patients with acquired immunodeficiency syndrome: selective impairment of chemotaxis. *Clin. Exp. Immunol.* 62:136–142.

10. Fauci, A. S. 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science (Wash. DC).* 239:617–622.

11. Lepe-Zuniga, J., P. W. A. Mansell, and E. M. Hersch. 1987. Idiopathic production of intereleukin-1 in acquired immunodeficiency. *J. Clin. Microbiol.* 25:1695–1700.

12. 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 cell line. *Science (Wash. DC).* 238:800–802.

13. Unaue, E. R., and P. M. Allen. 1987. The basis for immunoregulatory role of macrophages and other accessory cells. *Science (Wash. DC).* 236:551–557.

14. Maniatis, T., E. P. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 194 pp.

15. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6–13.

16. Ponte, P., P. Gunning, H. Blau, and L. Kedes. 1983. Human actin genes are single copy for alpha-skeletal and alpha-cardiac actin but multicopy for β- and -cytoskeletal genes: 3′ untranslated regions are isotype specific but are conserved in evolution. *Mol. Cell. Biol.* 3:1783–1791.

17. Wong, G. G., P. A. Temple, A. C. Leary, J. S. Wite-Giannotti, Y. C. Yang, A. B. Ciarletta, M. Chung, P. Murths, R. Kriz, R. J.

Kaufman, et al. 1987. Human CSF-1: molecular cloning and expression of 4-kd cDNA encoding the human urinary protein. *Science (Wash. DC).* 235:1504–1508.

18. Pennica, D., G. E. Nedwin, J. S. Hayflick, P. H. Seeburg, R. Derynek, M. A. Palladino, W. J. Kohr, B. B. Aggarwal, and D. V. Goeddel. 1984. Human tumor necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature (Lond.).* 312:724–729.

19. Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolf, and C. A. Dinarello. 1984. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. Natl. Acad. Sci. USA.* 81:7907–7911.

20. Fenton, M. J., B. D. Clark, K. L. Collins, A. C. Webb, A. Rich, and P. E. Auron. 1987. Transcriptional regulation of the human prointerleukin 1β gene. *J. Immunol.* 138:3972–3979.

21. Fenton, M. J., M. W. Vermeulen, B. D. Clark, A. C. Webb, and P. E. Auron. 1988. Human Pro-IL-1β gene expression in monocytic cells is regulated by two distinct pathways. *J. Immunol.* 140:2267–2273.

22. 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-β_2) production by HIV-1. *J. Immunol.* 142:532–536.

23. Wahl, L. M., M. L. Corcoran, P. W. Pyle, L. O. Arthur, A. Harel-Bellan, and W. L. Farrar. 1989. Human immunodeficiency virus glycoprotein (gp120) induction of monocyte arachidonic acid metabolites and interleukin 1. *Proc. Natl. Acad. Sci. USA.* 86:621–625.

24. Merrill, J. E., Y. Koyanagi, and I. S. Y. Chen. 1989. Interleukin-1 and tumor necrosis factor α can be induced from mononuclear phagocytes by human immunodeficiency virus type 1 binding to the CD4 receptor. *J. Virol.* 63:4404–4408.

25. Sodroski, J., C. Rosen, W. C. Goh, and W. Haseltine. 1985. Transcriptional activator protein encoded by x-*lor* region of the human T-cell leukemia virus. *Science (Wash. DC).* 228:1430–1434.

26. Dayton, A. I., J. G. Sodroski, C. A. Rosen, W. C. Goh, and W. A. Haseltine. 1986. The *trans*-activator gene of the human T-cell lymphotropic virus type III is required for replication. *Cell.* 44:941–947.

27. Ruben, S., H. Poteat, T. H. Tan, K. Kawakami, R. Roeder, W. Haseltine, and C. A. Rosen. 1988. Cellular transcriptional factors and regulation of IL-2 receptor gene expression by HTLV-1 *tax* gene product. *Science (Wash. DC).* 241:89–92.

28. Petit, A. J. C., F. G. Terpstra, and F. Miedema. 1987. Human immunodeficiency virus infection down-regulates HLA class II expression and induces differentiation in promonocytic U937 cells. *J. Clin. Invest.* 79:1883–1889.

29. Molina, J. M., D. T. Scadden, R. Bym, C. A. Dinarello, and J. E. Groopman. 1989. Production of tumor necrosis factor α and interleukin 1β by monocytic cells infected with human immunodeficiency virus. *J. Clin. Invest.* 84:733–737.

30. Griffin, G. E., K. Leung, T. M. Folks, S. Kunkel, and G. J. Nabel. 1989. Activation of HIV gene expression during monocyte differentiation by induction of NF-κB. *Nature (Lond.).* 339:70–73.

31. Osborn, L., S. Kunkel, and G. J. Nabel. 1989. Tumor necrosis factor α and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κB. *Proc. Natl. Acad. Sci. USA.* 86:2336–2340.

32. Unanue, E. R., and P. M. Allen. 1987. The basis for the immunoregulatory role of macrophages and other accessory cells. *Science (Wash. DC).* 236:551–557.

33. Ensoli, B., S. Nakamura, S. Z. Salahuddin, P. Biberfeld, L. Larsson, B. Beaver, F. Wong-Staal, and R. C. Gallo. 1989. AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and aparcrine growth effects. *Science (Wash. DC).* 243:223–226.

34. Nakamura, S., S. Z. Salahuddin, P. Biberfeld, B. Ensoli, P. D. Markham, F. Wong-Staal, and R. C. Gallo. 1988. Kaposi's sarcoma cells: long term culture with growth factor from retrovirus-infected CD4+ T cells. *Science (Wash. DC).* 242:426–430.