JOURNAL OF VIROLOGY, Dec. 1990, p. 6080–6089
0022-538X/90/126080-10$02.00/0

Vol. 64, No. 12

Coordinate Enhancement of Cytokine Gene Expression in Human Immunodeficiency Virus Type 1-Infected Promonocytic Cells

MARIO D'ADDARIO, ANNE ROULSTON, MARK A. WAINBERG, AND JOHN HISCOTT*

Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, and Department of Microbiology and Immunology and McGill AIDS Centre, McGill University, Montreal, Quebec H3T 1E2, Canada

Received 1 June 1990/Accepted 18 September 1990

A promonocytic cell model was used to investigate cytokine gene transcription in U937 and U9-IIIB cells chronically infected with human immunodeficiency virus type 1 (HIV-1). The production of interferon (alpha-1 interferon [IFN-α1], IFN-α2, and IFN-β), interleukin (interleukin 1α [IL-1α], IL-1β, and IL-6), and tumor necrosis factor α (TNF-α) mRNA was characterized by quantitative polymerase chain reaction mRNA phenotyping in U937 and U9-IIIB cells following coinfection with Sendai paramyxovirus or stimulation with lipopolysaccharide (LPS). Chronic HIV-1 infection of U9-IIIB cells resulted in a low constitutive level of transcription of TNF and IL-1 genes but not IFN genes; however, when the cells were coinfected with Sendai virus, 10- to 20-fold higher levels of IFN-β, IL-1β, IL-6, and TNF-α mRNA were observed in U9-IIIB cells than in similarly induced U937 cells. The enhanced levels of cytokine RNA in virus-infected U9-IIIB cells were also accompanied by higher levels of IFN antiviral activity and TNF secretion than in U937 cells. Transcript levels for IFN-α1 and IFN-α2 were equivalently induced in virus-infected U937 and U9-IIIB cells, indicating that a generalized derepression of cytokine gene expression did not occur as a consequence of HIV-1 infection. When LPS was used as an inducer, a distinct pattern of cytokine gene expression was detected in U9-IIIB cells. TNF-α and IL-1β but not IFN-α or IFN-β transcripts were induced by LPS. These results suggest that HIV-1 infection of promonocytic cells may prime or sensitize cells such that subsequent antigenic challenge leads to coordinate enhancement of cytokine gene expression.

Cells of the monocyte/macrophage lineage bearing the CD4 receptor (1, 12, 32) are subject to infection by human immunodeficiency virus type 1 (HIV-1) (2, 21, 57) at a high frequency (20, 30, 39, 61); however, these cells are not subject to the extreme cytopathic effects observed in HIV-1-infected T lymphocytes (17). Because of the lack of cytopathic effect, monocytes may serve as intracellular reservoirs for HIV and disseminate the virus to other organs, including the lungs, brain, skin, and lymph nodes (22–24, 33, 53, 56, 58, 66). In both HIV-1-infected T cells and monocytes/macrophages, viral DNA persists in association with the infected host cell and may remain in a latent, inactive state for extensive periods, only to be reactivated by a variety of stimuli, including mitogens, cytokines, and other infectious viruses (11, 17, 71).

HIV infection of myeloid cells results in the impairment of crucial functions in host defense, such as antigen presentation, chemotaxis, cell killing, and cytokine production (41, 60, 64). Cytokines are polypeptide hormones released by monocytes/macrophages and lymphocytes as a nonadaptive response to viruses and other antigens. Cytokines play a pivotal role in activation of T- and B-cell response during pathogenic infections and in regulation of immunocyte maturation during normal hematopoietic hemostasis (5, 9, 14, 51). HIV-1-infected monocytes in some cases spontaneously secrete cytokines such as interleukin 1 (IL-1) and tumor necrosis factor (TNF), which may account in part for fevers and the wasting syndrome common to patients with acquired immunodeficiency syndrome (AIDS) (5, 14, 43). TNF and IL-1 may also stimulate HIV-1 gene expression and multiplication via induction of transcription factor NF-κB, which binds to the enhancer element of the HIV long terminal repeat (15, 19, 31, 46, 49, 54). Similarly, increased IL-1 and IL-6 expression by infected cells may augment inflammatory responses in HIV-infected patients (7, 14, 47). Secretion of cytokines such as interferon (IFN) may exert an opposing antiviral effect on HIV-1 pathogenesis by restricting viral multiplication (3, 40, 55; M. Dubreuil, L. Sportza, M. D'Addario, J. Lacoste, R. Rooke, M. A. Wainberg, and J. Hiscott, Virology, in press); for example, addition of anti-IFN antibody either to lymphocyte cultures derived from patients with AIDS or to promonocytic cells during HIV-1 infection increased the kinetics of p24 antigen expression and virus production (2, 40, 42).

The objective of the present study was to investigate the effects of HIV infection on the induction of cytokine gene expression in the promonocytic U937 cell model (6, 40, 50, 52, 65). A polymerase chain reaction (PCR) assay (45), standardized with the endogenous glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene and exogenously added SV2CAT DNA (25), was used to measure the relative amounts of different monocyte-derived cytokine mRNAs produced in cells chronically infected with HIV (U9-IIIB). In general, U937 and U9-IIIB cells did not constitutively express high levels of IFN, IL-1, or TNF RNA; however, antigenic challenge of these cells by paramyxovirus (Sendai virus) coinfection or by treatment with lipopolysaccharide (LPS) increased 10- to 20-fold the steady-state levels of mRNA for several cytokines, including TNF-α, IL-1β, IL-6, and beta interferon (IFN-β). Enhanced mRNA levels were accompanied by a 7-fold increase in the secretion of TNF-α and a 10-fold increase in antiviral activity. These results indicate that HIV-infected monocytic cells may be primed or sensitized as a consequence of HIV-1 infection such that

* Corresponding author.

subsequent antigenic challenge leads to increased production of specific monocyte-derived cytokines.

MATERIALS AND METHODS

Cell culture and inductions. U937 cells (a $CD4^+$ promonocytic cell line) (65) and U9-IIIB cells (6) (U937 cells chronically infected with HIV-1 strain IIIB) were maintained at 37°C and 5.0% CO_2 in RPMI 1640 medium (GIBCO) supplemented with 5.0% calf serum, 1.0% glutamine, 1,000 IU of penicillin per ml, 20 μg of streptomycin per ml, and 1 μg of gentamicin per ml. Induction of exponentially growing cells was performed as described for the individual experiments; in general promonocytic cells (grown to a density of 10^6 cells per ml) were treated with recombinant IFN-α2 (a gift from Schering Canada) (250 IU/ml) for 4 h and then infected with Sendai virus (500 hemagglutinating units per ml (28). LPS (Sigma) was added to a final concentration of 1 μg/ml.

PCR. Total cellular RNA was isolated from U937 and U9-IIIB cells at different times after induction by a modified guanidinium isothiocyanate procedure (8) and then treated with 3 U of RNase-free DNase (RQ1 DNase; Promega) for 30 min at 37°C in 40 mM Tris hydrochloride (pH 7.9)–10 mM NaCl–6 mM $MgCl_2$. The RNA was phenol extracted, ethanol precipitated, and stored at −70°C. Reverse transcription was performed on total RNA (1 μg) with 100 U of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) and 10 pmol of primer B (3′ end) in a total volume of 50 μl in reverse transcription buffer containing 50 mM Tris hydrochloride (pH 8.3), 75 mM KCl, 10 mM dithiothreitol, and 3 mM $MgCl_2$. PCR assays were performed in a total volume of 100 μl (with 25 μl of reverse transcription product) in PCR buffer containing 100 mM Tris hydrochloride (pH 8.4), 500 mM KCl, 15 mM $MgCl_2$, 100 μg of bovine serum albumin per ml, 2 mM each of the four deoxynucleoside triphosphates (Pharmacia), 10 pmol of primer A (5′ end), 10 pmol of primer B (3′ end), and 2.5 U of *Taq* DNA polymerase (Promega). After reverse transcription, the reverse transcription products were held at 95°C for 2 min, annealed at 55°C for 1 min, extended at 72°C for 1 min, and denatured at 95°C for 1 min. Twenty-five cycles of amplification were used. In preliminary experiments, reverse transcription and PCR amplification were performed on twofold dilutions of RNA from 0.05 to 2.0 μg for 20, 25, or 30 cycles. These experiments showed that with 25 cycles of amplification, differences in PCR product signal were quantitatively related to input RNA. The PCR products were analyzed on a 5% denaturing polyacrylamide gel and autoradiographed overnight at −70°C. The primers were radiolabeled with [γ-^{32}P]ATP (ICN) and polynucleotide kinase. The marker (pAT153 plasmid cleaved with *Hae*III) was treated with phosphatase and 5′ end labeled with [γ-^{32}P]ATP.

Quantitation of PCR products. SV2CAT DNA in quantities ranging from 10^{-3} to 10^{-9} μg was added to the PCR mixtures at the end of the reverse transcriptase step. Opposing primers for SV2CAT and cytokine genes were added, and the amplification reactions were performed as described above. At intervals between 18 and 30 cycles, the PCR products were analyzed on a 5% polyacrylamide gel and quantified by scintillation counting of counts per minute in the band to generate a coamplification rate. The rate of SV2CAT amplification was 0.16 ± 0.01 cpm per cycle; the rates of IFN-β, TNF-α, and IL-1β amplification were 0.18, 0.17, and 0.2 cpm per cycle respectively. The cytokine RNA present in 0.5 μg of total RNA was measured in five or six independent reaction mixtures, and the average number of counts per minute (and standard deviation) in the PCR product was determined. These reaction mixtures contained different amounts of SV2CAT DNA ranging from 1.8×10^4 to 3.6×10^8 molecules (equivalent to 0.5 to 10,000 copies per cell), and, assuming equivalent coamplification rates, the cytokine RNA copy number was determined by using the following equation:

$$x = \frac{W/(M \times A)}{C} \times \frac{y_1}{y_0}$$

where x is the number of copies of cytokine RNA per cell, W is the mass of SV2CAT DNA in grams, M is the molecular mass of SV2CAT (3.3×10^6 g/mol), A is Avogadro's number (6.023×10^{23} molecules per mol), C is the number of cell equivalents, y_1 is the number of counts per minute in the cytokine cDNA product, and y_0 is the number of counts per minute in the SV2CAT DNA product. C is derived by dividing the total RNA yield by the number of cells.

S1 mapping analysis. The S1 mapping procedure was performed essentially as described previously (29) with 20 to 50 μg of total RNA from Sendai virus-induced U937 cells or U9-IIIB cells chronically infected with HIV-1.

Assays for TNF and IFN. Supernatants from uninduced and induced cells were assayed for TNF-α by a radioimmunoassay (Genzyme Inc., Kneeland, Mass.) according to the manufacturer's specifications. IFN antiviral activity was assayed by a cytopathic effect inhibition assay using GM-2767 cells and vesicular stomatitis virus as the challenge virus (18).

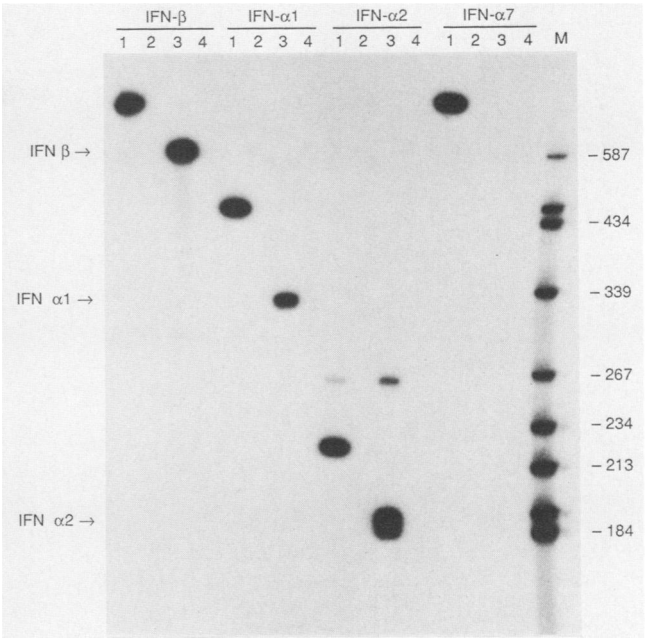


FIG. 1. Analysis of IFN gene transcripts in U937 cells infected with Sendai virus or chronically infected with HIV-1. Total RNA (50 μg) was analyzed by S1 mapping using probes for IFN-α1, IFN-α2, IFN-α7, and IFN-β (29). Lanes: 1, probe alone; 2, uninfected U937 RNA; 3, RNA from U937 cells induced with Sendai virus for 6 h; 4, RNA from HIV-1-infected U937 cells (U9-IIIB). The arrows on the left indicate the positions of the DNA-RNA hybrids; the nucleotide positions of marker fragments (pAT153 × *Hae*III) in lane M are indicated on the right.

TABLE 1. Oligonucleotides used in cytokine mRNA phenotyping

Transcripts	Location of primers in oligonucleotide sequences[a]		PCR fragment sizes (bp)	
	5′ primer	3′ primer	Full length	Cleavage products
IFN-α1	911–930	1573–1554	622	*Bgl*II (280, 382)
IFN-α2	1181–1200	1460–1440	280	*Pvu*II (96, 184)
IFN-β	−61 – −42	577–558	638	*Pst*I (265, 373)
IFN-γ	402–421	5001–4982	860	*Taq*I (140, 283, 437)
IL-1α	1–21	480–460	480	*Hin*dIII (192, 278)
IL-1β	1–21	540–520	540	*Hin*dIII (140, 400)
IL-6	4121–4141	4320–4299	201	*Pst*I (68, 64, 48, 20)
TNF-α	2175–2199	2820–2801	412	*Pvu*II (233, 94, 85)
GAPDH	371–388	546–565	196	*Mbo*I (125, 71)
SV2CAT	40–17	4780–4757	303	*Rsa*I (168, 135)

[a] The sequence data and nucleotide position numbering were derived from references 4, 13, 25, 27, 48, 63, and 70.

RESULTS

Type 1 IFN gene expression in U937 cells. Initial S1 nuclease protection experiments were performed to determine whether HIV-1 infection of U937 cells induced constitutive IFN mRNA production (Fig. 1). IFN mRNA corresponding to the major type 1 IFN species, IFN-α1, IFN-α2, and IFN-β, was detected in Sendai virus-induced U937 cells (Fig. 1, lane 3 in each group); however, no IFN transcripts were identified in U9-IIIB cells chronically infected with HIV (Fig. 1, lanes 4), indicating that IFN gene expression was not constitutively stimulated by HIV-1 infection. This observation is in agreement with the results of Molina et al., who demonstrated that HIV-1 infection did not induce expression of other cytokines, such as TNF, IL-1, or IL-6, in mononuclear cells (44). Recently we demonstrated that IFN gene expression was nonetheless inducible in HIV-infected cells by Sendai virus superinfection (Dubreuil et al., in press).

Cytokine mRNA phenotyping. To examine the inducibility of IFNs and other monocyte-derived cytokines with greater sensitivity, a series of oligonucleotide primers was produced for PCR amplification of cytokine RNA (45). The cytokine primers, their locations within the nucleotide sequence of the gene, and the sizes of the expected PCR products and their restriction enzyme cleavage products are listed in Table 1. The expression of cytokine gene mRNA was examined in U937 and U9-IIIB cells 8 h after Sendai paramyxovirus coinfection (Fig. 2). Sendai virus was chosen as an inducing agent, since its cytokine-activating potential in U937 cells has been characterized previously (28; Dubreuil et al., in press). TNF-α, IL-1β, and IFN-β transcripts were not detected in unstimulated U937 cells (Fig. 2A through C, lanes 1); Sendai virus infection of U937 cells induced IFN-β transcription (Fig. 2C, lane 2) but only weakly stimulated

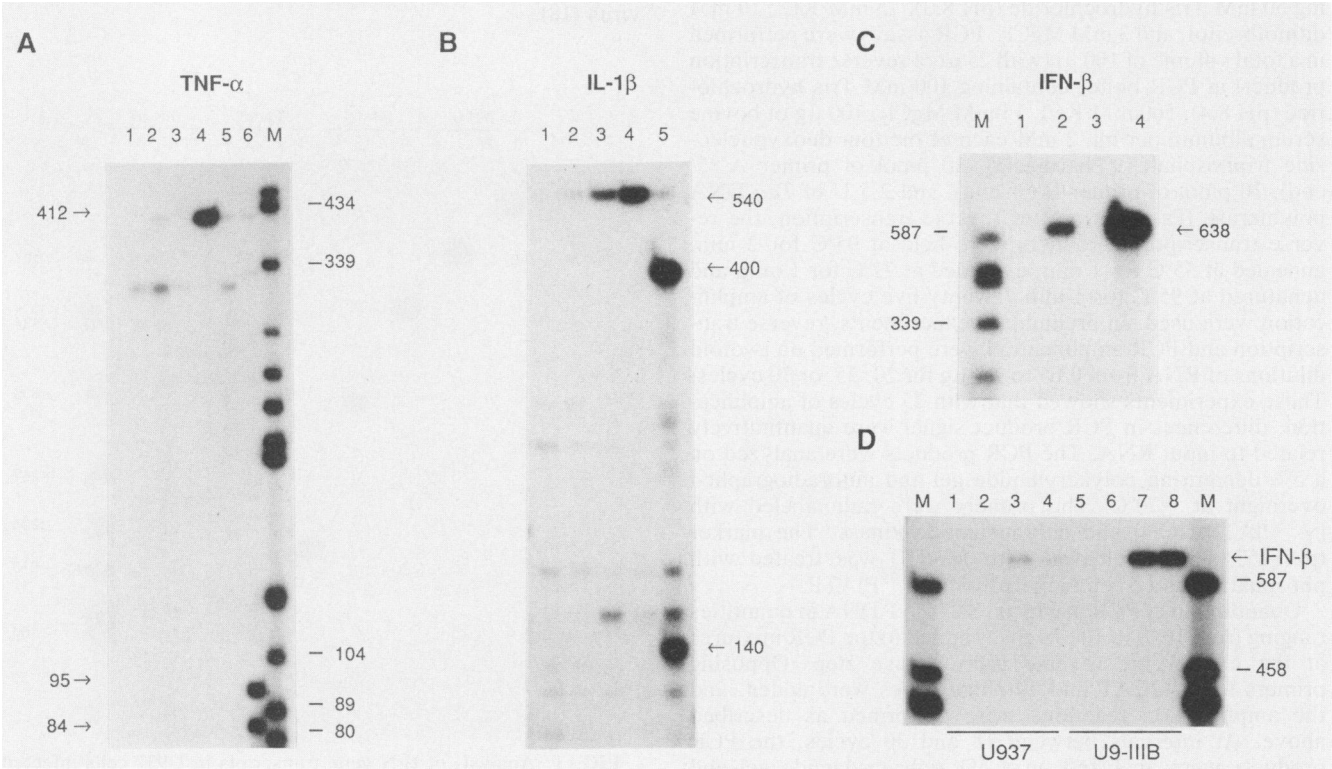


FIG. 2. Detection of increased cytokine RNA levels in HIV-1-infected cells by PCR amplification. Total RNA (1 μg) from U937 (lanes 1 and 2) or U9-IIIB (lanes 3 and 4) cells was analyzed on a 5% polyacrylamide gel for TNF-α (A)-, IL-1β (B)-, and IFN-β (C)-specific RNA by mRNA phenotyping 8 h after Sendai virus infection. Lanes 1 and 3 contain RNA from untreated U937 and U9-IIIB cells, respectively; lanes 2 and 4 contain RNA from U937 and U9-IIIB cells treated with IFN-α2 (250 IU/ml) for 4 h and then infected with Sendai virus (500 hemagglutinating units per ml) for 8 h. (A) Lane 5 is a duplicate of lane 2; lane 6 contains the *Pvu*II-cleaved TNF-α PCR product. (B) Lane 5 is the *Hin*dIII-cleaved IL-1β PCR product. (D) Total RNA (20 μg) from U937 cells (lanes 1 to 4) or U9-IIIB cells (lanes 5 to 8) was analyzed in duplicate for IFN-β-specific RNA by S1 mapping. Lanes: 1, 2, 5, and 6, uninduced; 3, 4, 7, and 8, recombinant IFN-α2 treated and Sendai virus infected; M, Marker plasmid pAT153 cleaved with *Hae*II and end labeled. The arrows indicate the PCR products.

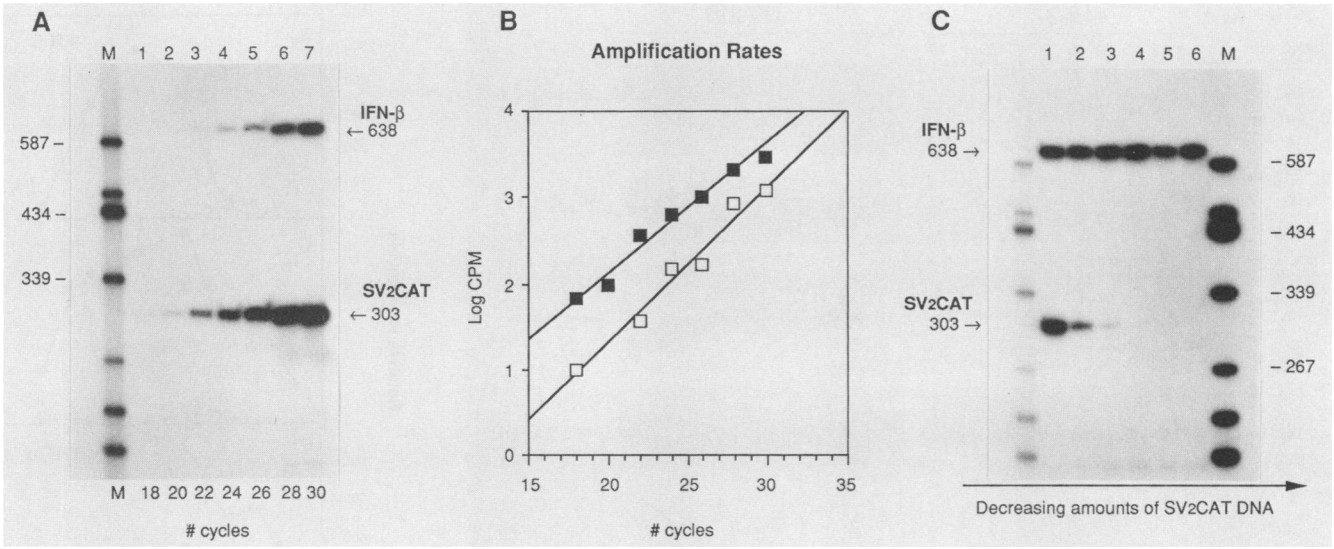


FIG. 3. Coamplification efficiencies and quantitative titration of IFN-β cDNA and SV2CAT DNA. (A) Reverse transcription was performed on total RNA (0.5 μg) from Sendai virus-induced U9-IIIB cells as described in Materials and Methods. SV2CAT (2 pg) was added to the reaction mixture, and the IFN-β and SV2CAT templates were coamplified by PCR for 18 to 30 cycles and analyzed on a 5% denaturing polyacrylamide gel (lanes 1 to 7). The nucleotide positions of IFN-β and SV2CAT PCR products are indicated by arrows. (B) The log of the cpm incorporated into the IFN-β (□) and SV2CAT (■) products (minus background cpm) was plotted against the number of cycles to give relative amplification rates. (C) A constant amount of total RNA (0.5 μg) from Sendai virus-induced U9-IIIB cells was mixed with 10-fold dilutions of SV2CAT DNA (from 2×10^{-5} to 2×10^{-10} μg) (lanes 1 to 6) to titrate IFN-β cDNA relative to known amounts of SV2CAT as described in Materials and Methods. Lane M, Marker plasmid pAT153 cleaved with *Hae*III and end labeled.

TNF-α and IL-1β genes (Fig. 2A and B, lanes 2). In contrast, a distinct pattern of cytokine gene activation was observed in U9-IIIB cells; IL-1β RNA was constitutively expressed (Fig. 2B, lane 3), and after virus infection, TNF-α, IL-1β, and IFN-β RNA levels were increased 10- to 50-fold over the levels in Sendai virus-induced U937 cells. The differential activation of IFN-β RNA in U937 and U9-IIIB cells was also confirmed independently by S1 mapping analysis (compare Fig. 2C, lanes 2 and 4, with Fig. 2D, lanes 3, 4, 7, and 8). This experiment suggested that the IFN-β gene, while not constitutively activated in HIV-1-infected cells, may be more inducible after antigenic stimulation of an HIV-infected cell population. Similarly, TNF-α gene transcription was strongly induced by Sendai virus infection of U9-IIIB but not U937 cells; a low constitutive level of TNF-α RNA was detected in U9-IIIB cells (35). The IL-1β gene appeared to be expressed constitutively as a consequence of HIV-1 infection of promonocytic cells and was also stimulated by coinfection with a different virus.

To quantify these observations, conditions for mRNA

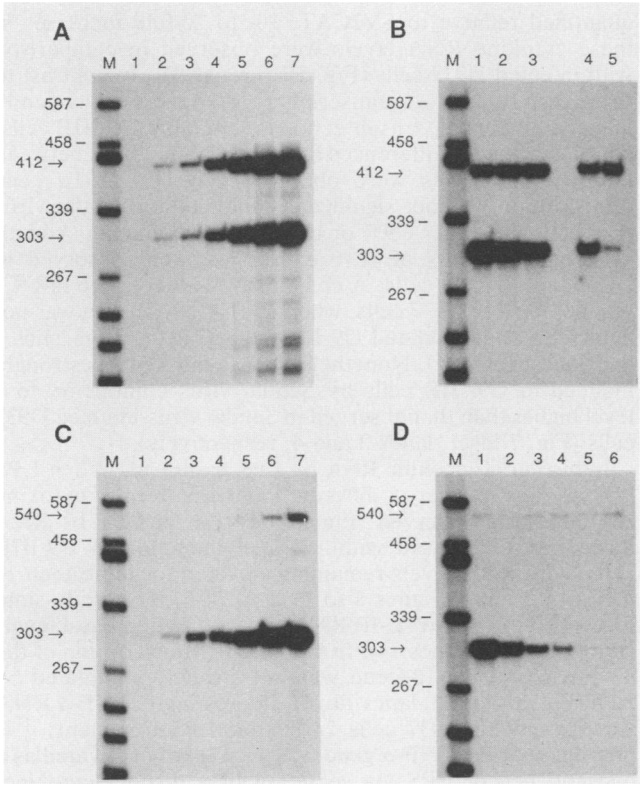
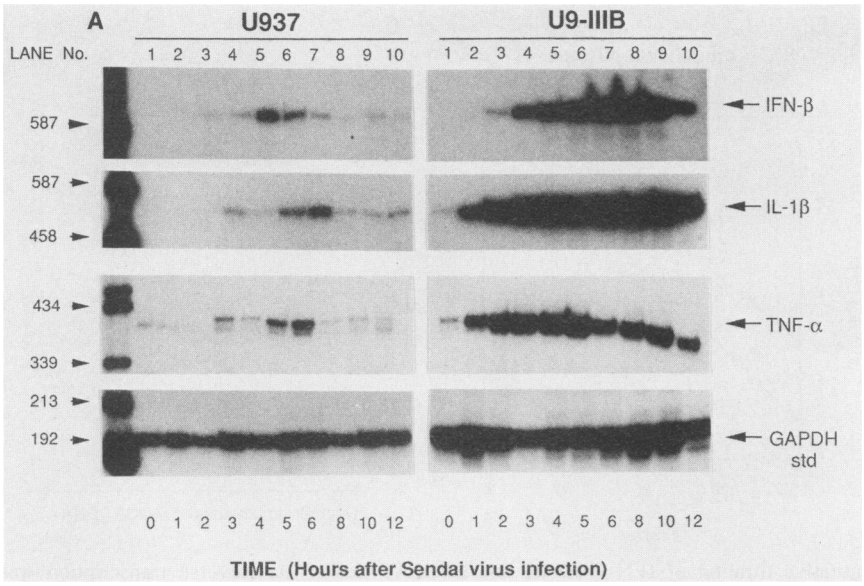


FIG. 4. Quantitative titration of TNF-α and IL-1β PCR products from U9-IIIB cells. Total RNA (0.5 μg) from IFN-α2-treated, Sendai virus-infected (8 h) U9-IIIB cells was analyzed for TNF-α (A and B)- or IL-1β (C and D)-specific RNA by PCR amplification. (A and C) SV2CAT DNA (2 pg) was added to the reverse transcription reaction mixture and coamplified together with TNF-α (A) or IL-1β (C) for 18 (lane 1), 20 (lane 2), 22 (lane 3), 24 (lane 4), 26 (lane 5), 28 (lane 6), or 30 (lane 7) cycles of PCR amplification. (B) A constant amount of total RNA (0.5 μg) from Sendai virus-induced U9-IIIB cells was mixed with the following dilutions of SV2CAT DNA to titrate TNF-α products relative to SV2CAT: 2×10^{-3} μg (lane 1), 1×10^{-3} μg (lane 2), 2×10^{-4} μg (lane 3), 2×10^{-5} μg (lane 4), and 1×10^{-5} μg (lane 5). (D) Total RNA from Sendai virus-induced U9-IIIB cells was mixed with 10-fold dilutions of SV2CAT DNA (2×10^{-5} to 2×10^{-10} μg) (lanes 1 to 6) to titrate IL-1β PCR products relative to SV2CAT. Lanes M, Fragments of the marker plasmid pAT153 cleaved with *Hae*III. The correctly sized (in base pairs) PCR products for TNF-α, IL-1β, and SV2CAT are indicated by arrows.



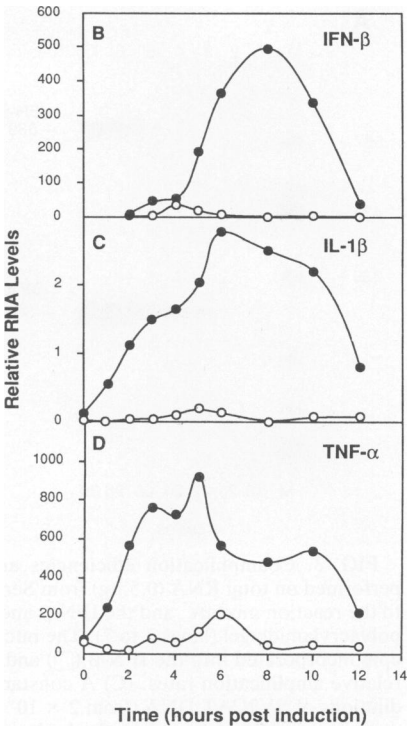


FIG. 5. Kinetic analysis of cytokine RNA production in U937 and U9-IIIB cells. (A) Total RNA (0.5 μg) was isolated from U937 and U9-IIIB cells 0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 h after Sendai virus infection (lanes 1 to 10, respectively) and analyzed for IFN-β-, IL-1β-, TNF-α-, and GAPDH-specific RNA by PCR amplification. The positions of marker fragments (pAT153 × *Hae*III) from representative gels are indicated on the left; the correctly sized (in base pairs) PCR products are indicated by the arrows on the right. The GAPDH signal was coamplified together with that of TNF-α. IFN-β (B), IL-1β (C) and TNF-α (D) RNA signals obtained in U937 (○) and U9-IIIB (●) cells as shown in panel A were quantified by determining the amount of radioactivity in counts per minute in the correctly sized PCR fragments. The amount of specific cytokine RNA in the peak sample was titrated relative to SV2CAT as described in the legends to Fig. 3 and 4, and other RNA values were normalized relative to the peak value by using the counts per minute values as reference.

phenotyping were standardized with two types of reference primers: the GAPDH gene as an endogenous RNA control and SV2CAT DNA as an exogenously added standard (Table 1). Figure 3A illustrates the accumulation of coamplified SV2CAT DNA and IFN-β cDNA with increasing amplification cycles; Fig. 3B demonstrates that the rates of the two independent amplifications were very similar (0.16 versus 0.18 cpm per cycle, respectively). Thus, a quantitative estimate of the amount of IFN-β cDNA can be made on the basis of the amount of exogenous SV2CAT DNA added to the reaction mixture. An example of such an evaluation is shown (Fig. 3C); in this experiment, a constant amount of total RNA (0.5 μg) from IFN-β-expressing cells was coamplified together with different 10-fold dilutions of SV2CAT DNA ranging from 2×10^{-5} μg (Fig. 3C, lane 1) to 2×10^{-10} μg (Fig. 3C, lane 6). On the basis of SV2CAT standardization, the IFN-β product observed in Fig. 3C corresponds to 500 RNA copies per cell. Figure 4 illustrates the same quantitative approach for TNF-α RNA in Sendai virus-induced U9-IIIB cells (Fig. 4A and B) and for IL-1β RNA (Fig. 4C and D). The IL-1β signal was equivalent to 2 RNA copies per cell, while the TNF-α RNA was equivalent to approximately 1,000 copies per cell.

Induction of cytokine RNA by paramyxovirus coinfection. The steady-state levels of IFN-β, TNF-α, and IL-1β mRNA were coordinately increased in Sendai virus-infected U9-IIIB cells during a 12-h kinetic analysis of cytokine gene transcription (Fig. 5). In U9-IIIB cells, the peak RNA levels for IFN-β, TNF-α, and IL-1β occurred 6 to 8 h after induction; when the levels were normalized to GAPDH and quantified relative to SV2CAT, 10- to 20-fold increases in these cytokine RNA levels were observed in comparison with infected U937 cells (Fig. 5B through D). In contrast to these results, IFN-α1 transcripts (Fig. 6) were equivalently induced by Sendai virus infection in U937 and U9-IIIB cells, but no significant differences in the induction levels between the two cell types were observed (Fig. 6). IL-1α gene transcription was not significantly induced by Sendai virus coinfection in either U937 or U9-IIIB cells (data not shown).

Distinct patterns of IL-6 expression were observed in U937 and U9-IIIB cells. A constitutive level of IL-6 mRNA was detected in U937 cells, whereas IL-6 expression was not detected in unstimulated U9-IIIB cells (Fig. 7, inset lanes 1 and 3, respectively). Nonetheless, IL-6 mRNA was strongly induced in U9-IIIB cells by Sendai virus coinfection to a level higher than that observed in Sendai virus-induced U937 cells (Fig. 7 inset, lanes 2 and 4, respectively).

Induction of cytokine RNA by LPS treatment. When LPS was used as inducer, a different kinetic pattern of cytokine response was observed (Fig. 8). TNF-α and IL-1β RNA levels were increased within 2 h of induction in U9-IIIB cells, with RNA levels remaining elevated for more than 10 h (Fig. 8A and B, lanes 8 to 13); by 24 h, the steady-state levels of TNF-α and IL-1β RNA had returned to basal levels (Fig. 8A and B, lanes 14). In U937 cells, transcription of the TNF-α or the IL-1β gene was not strongly stimulated by LPS (Fig. 8A and B, lanes 1 to 6). Interestingly, TNF-α RNA was present in U937 cells 24 h after LPS treatment; it is possible that the TNF-α gene was secondarily activated as a consequence of LPS via secretion of a distinct cytokine.

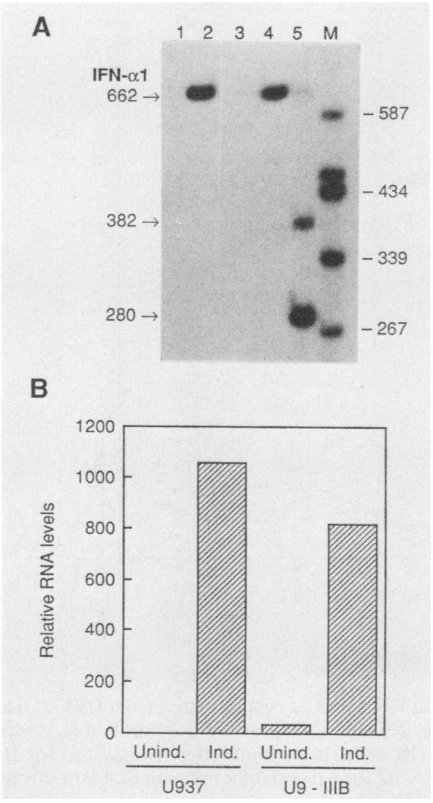


FIG. 6. Equivalent induction of IFN-α1 RNA in Sendai-virus induced U937 and U9-IIIB cells. (A) Total RNA (5 μg) from U937 cells (lanes 1 and 2) and U9-IIIB cells (lanes 3 and 4) was analyzed for IFN-α1-specific RNA; the cells were unstimulated (lanes 1 and 3) or treated with IFN-α2 for 4 h and infected with Sendai virus for 8 h (lanes 2 and 4). Lane 5 contains the *Bgl*II-cleaved PCR product from Sendai virus-infected U9-IIIB cells (lane 4). Lane M, Marker plasmid pAT153 cleaved with *Hae*III. The correctly sized PCR products are indicated by arrows on the left. (B) The relative levels of IFN-α1 RNA were determined by densitometric scanning of appropriately exposed gels. Unind., Uninduced; Ind., induced.

This possibility is currently being examined. Transcription of the IFN-α and IFN-β genes was not induced in either cell type by LPS treatment. Similarly, a low basal level of IL-1α transcription was observed in U937 cells and was not elevated in U9-IIIB cells (data not shown). Taken together, these experiments demonstrate that expression of specific cytokine transcripts such as TNF-α, IL-1β, and IFN-β was potentiated in HIV-infected cells by paramyxovirus coinfection. Similarly, in LPS-treated U9-IIIB cells, transcription of IL-1 and TNF-α but not IFN was stimulated.

Enhanced secretion of type 1 IFN and TNF. To determine whether the increased expression of TNF-α mRNA in U9-IIIB cells was accompanied by an increase in secreted protein, TNF-α levels in supernatants from uninduced or Sendai virus-induced U937 and U9-IIIB cell cultures were measured; in addition, the TNF levels in sera from three HIV-positive patients with AIDS and two seronegative males were measured. The results of the radioimmunoassay shown in Fig. 9 demonstrate that U9-IIIB cells coinfected with Sendai virus produced seven times more TNF-α than similarly induced U937 cells. A higher level of TNF-α was also demonstrated in the sera of patients with AIDS than in the sera of HIV-negative individuals, as previously demonstrated (36, 68). Similarly, supernatants from U9-IIIB cells coinfected with Sendai virus contained 10-fold-higher levels of IFN antiviral activity (30,000 IU/ml) than induced U937 cells contained (3,000 IU/ml), as determined by a cytopathic effect inhibition assay (18). Therefore, the increased steady-state levels of TNF and IFN-β mRNA observed by PCR amplification are reflected in higher levels of TNF and IFN secretion.

DISCUSSION

The results of the present study demonstrate that HIV-1-infected promonocytic U9-IIIB cells express increased levels of IFN-β, IL-1, TNF, and IL-6 mRNA following coinfection with Sendai virus. HIV-1 infection may produce a signal that alters cytokine activation mechanisms and coordinately increases the response to other stimuli that induce cytokine secretion. Preliminary experiments using other myeloid leukemic cell lines and HIV-1-infected derivatives suggest that these observations are not restricted to the U937 model but may be relevant to other HIV-1-infected myelomonocytic cells.

Several HIV regulatory proteins have been characterized that could potentially modulate cellular gene transcription in monocytic cells (reviewed in reference 11). Of these, the 15-kDa Tat protein is known to interact with the stem-loop structure at the 5′ end of viral RNA and *trans* activate expression of all sequences linked to the HIV long terminal repeat (11, 59). As yet, however, no cellular genes which are activated by the unique *trans*-activation mechanism of the *tat* gene have been identified. Nevertheless, transgenic mice expressing the Tat protein develop dermal lesions resembling Kaposi's sarcoma (KS), suggesting a role for *tat* in the etiology of KS (67). Cells isolated from KS lesions constitutively release several growth factors and cytokines which are thought to stimulate autocrine and paracrine growth effects leading to tumor formation (16). Since *tat* mRNA is not found in KS tumor cells, Langerhans macrophages (58, 66) may serve as cellular targets for the expression of the *tat* gene, which in turn stimulates these cells to secrete cytokines and growth factors (67). *tat* may play an analogous role in the HIV-infected promonocytic cell model used in this study.

Recently, a novel HIV-1 regulatory gene, *vpr*, which increased the rate of replication and accelerated the cytopathic effect of the virus in T cells was characterized (10). *vpr* was also capable of *trans* activating the HIV long terminal repeat as well as other heterologous promoters. This initial characterization of *vpr* suggests that cells chronically infected with HIV may constitutively express a *trans*-activator protein capable of enhancing transcription of specific cellular genes.

HIV infection of monocytic progenitor cells has been shown to induce a partially differentiated cellular phenotype which can alter the pattern of virus expression and multiplication (50, 52). HIV-1-induced differentiation could lead to activation of signal transduction pathways which modulate cellular transcription factors. One such pathway may lead to induction of transcription factor NF-κB (26, 38). NF-κB can be activated from a latent cytoplasmic non-DNA-binding form to a nucleus-localized DNA-binding protein by activation signals such as double-stranded RNA, phorbol esters, and LPS (reviewed in reference 38). NF-κB is involved in transcription activation of several genes involved in immune responsiveness and inflammation, including multiple cytokines such as IL-2, IFN-β, TNF, IL-6, and granulocyte-macrophage colony-stimulating factor; also, NF-κB binds

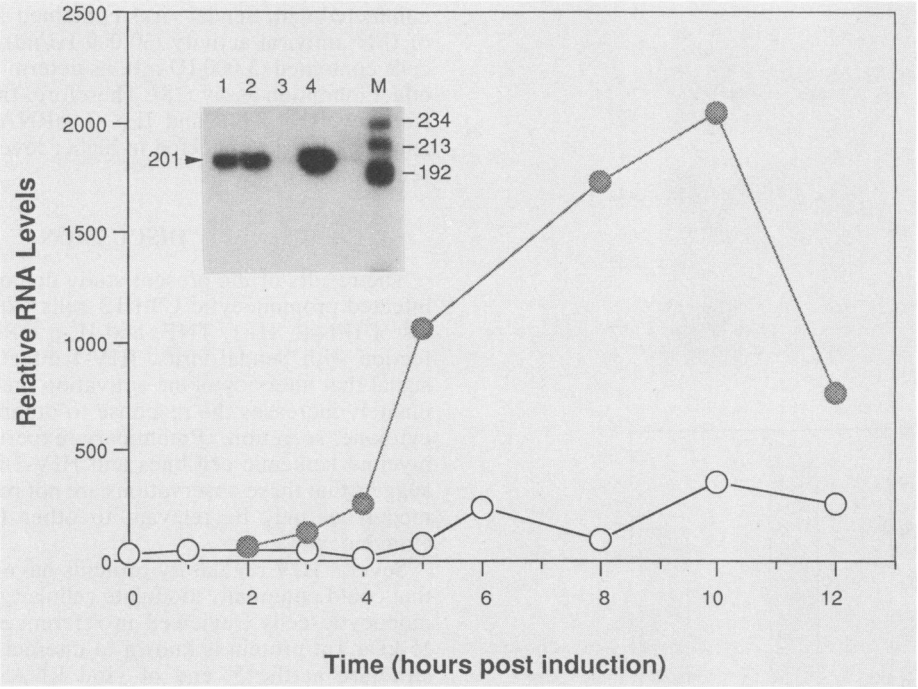


FIG. 7. Kinetic analysis of IL-6 mRNA production in U937 and U9-IIIB cells. Total RNA (0.5 μg) was isolated from U937 (○) and U9-IIIB (●) cells 0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 h after Sendai virus infection; IL-6-specific RNA was analyzed and quantified as described in the legend to Fig. 5. (Inset) Total RNA (2 μg) from U937 cells (lanes 1 and 2) and U9-IIIB cells (lanes 3 and 4) was analyzed for IL-6 mRNA, indicated by the arrowhead. Cells were unstimulated (lanes 1 and 3) or treated with IFN-α2 for 4 h and infected with Sendai virus for 8 h (lanes 2 and 4). Lane M, nucleotide positions of marker fragments from pAT153 cleaved with *Hae*III.

and activates the HIV-1 enhancer (38). Since virus infection acts as a potent activator of NF-κB (28, 37, 38), constitutive NF-κB activity may be present in cells chronically infected with HIV in a form capable of inducing gene transcription. Partial monocytic differentiation induced by HIV infection (50, 52) may result in NF-κB induction (26) and constitute a priming signal. Despite the presence of NF-κB, inducibility of cytokines genes may be maintained by the requirement for concomitant interaction with other regulatory proteins (37, 38). Thus, subsequent antigenic stimulation would also be required to specifically induce cytokine gene expression.

Voth et al. (68) demonstrated recently that mononuclear cells from patients with AIDS-related complex or AIDS produced higher levels of TNF mRNA than control mononuclear cells produced, because of a posttranscriptional increase in mRNA half-life. The relative contributions of increased transcriptional initiation and mRNA stability to the coordinate enhancement of cytokine gene expression are currently being examined (A. Roulston, unpublished results). Many transiently expressed genes encoding cytokines and growth factors, and certain proto-oncogenes, possess AU-rich sequences in the 3′ untranslated region of mRNA

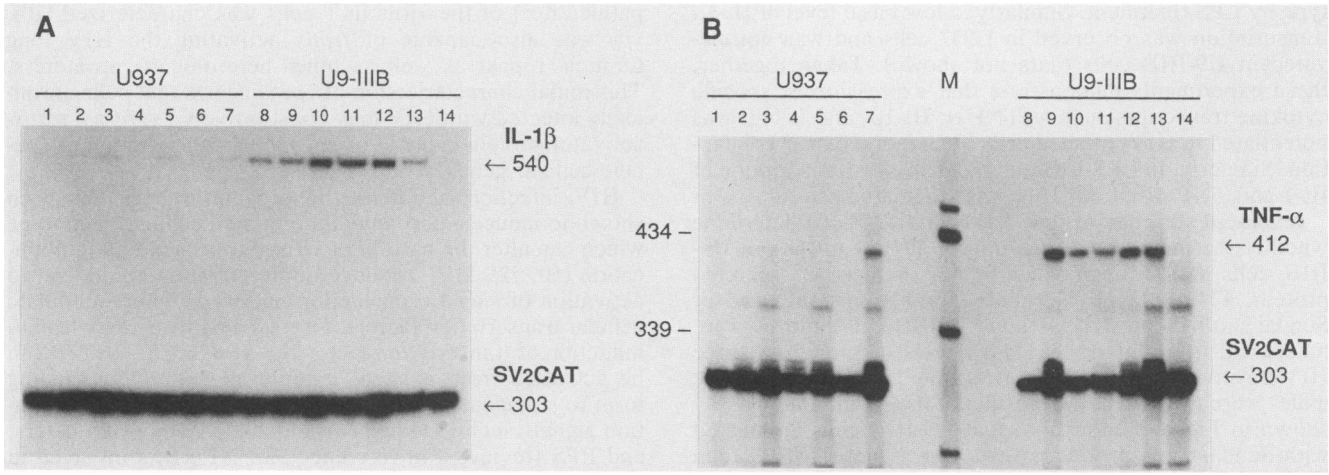


FIG. 8. Induction of TNF-α and IL-1β transcription by LPS. Total RNA (1 μg), isolated from U937 (lanes 1 to 7) or U9-IIIB (lanes 8 to 14) cells 0, 2, 4, 6, 8, 10, and 24 h after LPS treatment (1 μg/ml), was analyzed for IL-1β (A)- and TNF-α (B)-specific RNA by mRNA phenotyping. A constant amount of SV2CAT (2 pg) was added to each reaction after reverse transcription and coamplified. (B) Lane M, pAT153 × *Hae*III marker. The correctly sized PCR products are indicated by the arrows.

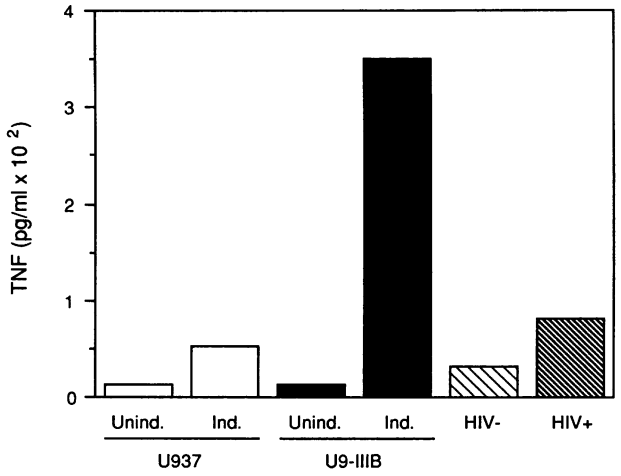


FIG. 9. Secretion of TNF-α from U937 and U9-IIIB cells. Cell culture supernatants were tested for the presence of TNF-α both under unstimulated conditions and after 24-h exposure to Sendai virus by a radioimmunoassay (Genzyme). Supernatants were obtained from U937 cells (□), U9-IIIB cells (■), sera from two seronegative individuals (▧), and sera from three patients with AIDS (▨). The values are the averages of duplicate measurements.

which decrease the half-lives of these specific mRNAs by a poorly defined mechanism (62). The AU-rich instability sequences of multiple cytokine RNA species represent a distinct stage of RNA metabolism at which these genes could be coordinately regulated as a consequence of HIV infection.

The importance of these observations for AIDS pathogenesis may lie in the fact that unscheduled cytokine production by HIV-1-infected monocytic or lymphocytic cells may generate stimulatory or inhibitory effects on HIV-1 expression and on the course of virus infection (34, 69). Secretion of immunoregulatory cytokines may contribute to the reactivation from a latent viral state (71) to a productive infection. Recent experiments have demonstrated that TNF and IL-1 act as physiological activators of HIV-1 transcription and multiplication via binding of NF-κB to the HIV enhancer (49, 54); secretion of these cytokines may not only increase the viral burden in patients with AIDS but also contribute to cachexia and fever. Similarly, IL-6 production may contribute to inflammatory and acute-phase reactions in patients infected with HIV (7, 47). In summary, our experiments describe the coordinate enhancement of cytokine gene expression in HIV-infected monocytic cells in response to nonspecific antigenic stimulation. Secretion of these immunoregulatory hormones may influence the course of HIV pathogenesis and contribute to reactivation of latent virus.

ACKNOWLEDGMENTS

M.D. and A.R. were equal contributors to the work reported in this paper.

This work was supported by research grants from Health and Welfare Canada, the Medical Research Council, and the Cancer Research Society, Inc. J.H. is the recipient of a senior scholarship from the Fonds de la Recherche en Santé du Québec.

We thank C. Dieffenbach, Uniformed Services University of the Health Sciences, Bethesda, Md., for providing the sequence information concerning the GAPDH primers and D. Howell, University of Medicine and Dentistry of New Jersey, for performing the IFN assays. We also thank Rhona Rosenzweig and Sandy Fraiberg for typing and editing the manuscript.

LITERATURE CITED

1. **Asjo, B., I. Ivhed, M. Gidlund, S. Fuerstenberg, E. M. Fenyo, K. Nilsson, and H. Wigzell.** 1987. Susceptibility to infection by the human immunodeficiency virus (HIV) correlates with T4 expression in a parental monocytoid cell line and its subclones. Virology **157**:359–365.
2. **Barre-Sinoussi, F., J.-C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220**:868–871.
3. **Bednarik, D. P., J. D. Mosca, B. N. K. Raj, and P. M. Pitha.** 1989. Inhibition of human immunodeficiency virus (HIV) replication by HIV-*trans*-activated α_2-interferon. Proc. Natl. Acad. Sci. USA **86**:4958–4962.
4. **Beuni, G., R. Rangei, E. Palla, V. Carinci, D. T. Buonamassa, and M. Melli.** 1987. Human interleukin 1 beta gene. Gene **52**:95–101.
5. **Beutler, B., and A. Cerami.** 1988. Tumor necrosis, cachexia, shock, and inflammation: a common mediator. Annu. Rev. Biochem. **57**:505–518.
6. **Boulerice, F., S. Bour, R. Geleziunas, A. Lvovich, and M. A. Wainberg.** 1990. High frequency of isolation of defective human immunodeficiency virus type 1 and heterogeneity of viral gene expression in clones of infected U-937 cells. J. Virol. **64**:1745–1755.
7. **Breen, E., A. Rezai, K. Nakajima, G. Beall, R. Mitsuyasu, T. Hirano, T. Kishimoto, and O. Martinez-Maza.** 1990. Infection with HIV is associated with elevated IL-6 levels and production. J. Immunol. **144**:480–484.
8. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162**:150–159.
9. **Clark, S. C.** 1989. Regulation of the acute phase and immune response: interleukin-6. Ann. N.Y. Acad. Sci. **557**:438–443.
10. **Cohen, E. A., E. Terwilliger, Y. Jalinoos, J. Proulx, J. Sodroski, and W. A. Haseltine.** 1990. Identification of HIV-1 vpr product and function. J. Acquired Immune Defic. Syndr. **3**:11–18.
11. **Cullen, B. R., and W. C. Greene.** 1989. Regulatory pathways governing HIV-1 replication. Cell **58**:423–426.
12. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312**:763–767.
13. **Devos, R., H. Cheroutre, Y. Taya, W. Degrave, H. Van Heuverswyn, and W. Fiers.** 1982. Molecular cloning of human interferon-γ cDNA and its expression in eukaryotic cells. Nucleic Acids Res. **10**:2487–2501.
14. **Dinarello, C. A.** 1989. Interleukin-1 and its biologically related cytokines. Adv. Immunol. **44**:153–204.
15. **Duh, E. J., W. J. Maury, T. M. Folks, A. S. Fauci, and A. B. Rabson.** 1989. Tumor necrosis factor α activates human immunodeficiency virus-1 through induction of nuclear factor binding to the NF-κB sites in the long terminal repeat. Proc. Natl. Acad. Sci. USA **86**:5974–5978.
16. **Ensoli, B., S. Nakamura, S. Z. Salahuddin, P. Biberfeld, L. Larsson, B. Beaver, F. Wong-Staal, and R. Gallo.** 1989. AIDS-Kaposi's sarcoma derived cells express cytokines with autocrine and paracrine growth effects. Science **243**:223–226.
17. **Fauci, A. S.** 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science **239**:617–622.
18. **Fitzgerald-Bocarsly, P., M. Feldman, M. Mendelsohn, S. Curl, and C. Lopez.** 1988. Human mononuclear cells which produce interferon-alpha during NK(HSV-FS) assays are HLA-DR positive cells distinct from cytolytic natural killer effectors. J. Leukocyte Biol. **43**:323–334.
19. **Folks, T. M., K. A. Clouse, J. Justement, A. Rabson, E. Duh, J. H. Kerhl, and A. S. Fauci.** 1989. Tumor necrosis factor alpha induces expression of human immunodeficiency virus in a chronically infected T-cell clone. Proc. Natl. Acad. Sci. USA **86**:2365–2368.
20. **Folks, T. M., S. W. Kessler, J. M. Orenstein, 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 242:919–922.
21. Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M.Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, F. White, R. Foster, and P. D. Markham. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science 224:500–503.
22. Gartner, S., D. M. Markovitz, R. F. Markovitz, R. F. Betts, and M. Popovic. 1988. Virus isolation from and identification of HTLV-III/LAV-producing cells in brain tissue from a patient with AIDS. J. Am. Med. Assoc. 256:2365–2370.
23. Gartner, S., R. F. Markovitz, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic. 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. Science 233:215–219.
24. Gendelman, H. E., O. Narayan, S. Molineaux, J. E. Clements, and Z. Gho. 1985. Slow, persistent replication of lentivirus: role of tissue macrophages and macrophage precursors in bone marrow. Proc. Natl. Acad. Sci. USA 82:7086–7090.
25. Gorman, C., L. F. Moffat, and B. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. 2:1044–1051.
26. Griffin, B., K. Leung, T. Folks, S. Kunkel, and G. Nabel. 1989. Activation of HIV gene expression during monocyte differentiation by induction of NF-κB. Nature (London) 339:70–73.
27. Henco, K., J. Brosius, A. Fujisawa, J. I. Fujisawa, J. R. Haynes, J. Hochstadt, T. Kovacic, M. Pasek, A. Schambock, J. Schmid, K. Todokoro, M. Walchli, S. Nagata, and C. Weissmann. 1985. Structural relationship of human interferon-α genes and pseudogenes. J. Mol. Biol. 185:227–260.
28. Hiscott, J., D. Alper, L. Cohen, J. F. Leblanc, L. Sportza, A. Wong, and S. Xanthoudakis. 1989. Induction of human interferon gene expression is associated with a nuclear factor that interacts with the NF-κB site of the human immunodeficiency virus enhancer. J. Virol. 63:2557–2566.
29. Hiscott, J., K. Cantell, and C. Weissmann. 1984. Differential transcription of human interferon genes. Nucleic Acids Res. 12:3727–3746.
30. Ho, D. D., T. R. Rota, and M. S. Hirsch. 1986. Infection of monocytes/macrophages by human T lymphotropic virus type III. J. Clin. Invest. 77:1712–1715.
31. Israel, N., U. Hazan, J. Alcami, A. Munier, F. Arenzana-Seisdedos, F. Bachelerie, A. Israel, and J. L. Virelizier. 1989. Tumor necrosis factor stimulates transcription of HIV-1 in human T lymphocytes, independently and synergistically with mitogens. J. Immunol. 143:3956–3960.
32. Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier. 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature (London) 312:767–768.
33. Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci. 1986. Detection of AIDS virus in macrophages in brain tissue from patients with encephalopathy. Science 233:1089–1092.
34. Koyanagi, Y., W. A. O'Brien, J. Q. Zhao, D. W. Golde, J. C. Gasson, and I. S. Y. Chen. 1989. Cytokines alter production of HIV-1 from primary mononuclear phagocytes. Science 241: 1673–1675.
35. Lacoste, J., M. D'Addario, A. Roulston, M. A. Wainberg, and J. Hiscott. 1990. Cell-specific differences in activation of NF-κB regulatory elements of human immunodeficiency virus and beta interferon promoters by tumor necrosis factor. J. Virol. 64: 4726–4734.
36. Lahdevirta, J., C. P. Maury, A. M. Teppo, and H. Repo. 1988. Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. Am. J. Med. 85:289–295.
37. Leblanc, J.-F., L. Cohen, M. Rodrigues, and J. Hiscott. 1990. Synergism between distinct enhanson domains in viral induction of the human beta interferon gene. Mol. Cell. Biol. 10:3987–3993.
38. Lenardo, M., and D. Baltimore. 1989. NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell 58:227–229.
39. Levy, J. A., J. Shimabukuro, T. McHugh, C. Casavant, D. Stites, and L. Oshiro. 1985. AIDS-associated retroviruses (ARV) can productively infect other cells besides human T helper cells. Virology 147:441–448.
40. Mace, K., M. Duc-Dodon, and L. Gazzolo. 1989. Restriction of HIV-1 replication in promonocytic cells: a role for IFN-α. Virology 168:399–405.
41. Mann, D. L., S. Gartner, F. Le Sane, H. Buchow, and M. Popovic. 1990. HIV-1 transmission and function of virus-infected monocytes/macrophages. J. Immunol. 144:2152–2158.
42. Markham, P. D., S. Z. Salahuddin, K. Veren, S. Orndorff, and R. C. Gallo. 1986. Hydrocortisone and some other hormones enhance the expression of HTLV-III. Int. J. Cancer 37:67–72.
43. 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.
44. Molina, J.-M., D. T. Scadden, C. Amirault, A. Woon, E. Vannier, C. A. Dinarello, and J. E. Groopman. 1990. Human immunodeficiency virus does not induce interleukin-1, interleukin-6, or tumor necrosis factor in mononuclear cells. J. Virol. 64:2901–2906.
45. Mullis, K. B., and F. A. Faloona. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol. 155:335–350.
46. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (London) 326:711–713.
47. 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) production by HIV. J. Immunol. 142:531–536.
48. Nedwin, G. E., S. L. Naylor, A. Y. Sakaguchi, D. Smith, J. J. Nedwin, D. Pennica, D. V. Goeddel, and P. W. Gray. 1985. Human lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization. Nucleic Acids Res. 13:6361–6373.
49. 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.
50. Pauza, C. D., J. Galindo, and D. D. Richman. 1988. Human immunodeficiency virus infection of monoblastoid cells: cellular differentiation determines the pattern of virus replication. J. Virol. 62:3558–3564.
51. Pestka, S. 1987. Interferons and their actions. Annu. Rev. Biochem. 56:727–777.
52. Petit, A. J. C., F. 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.
53. Plata, F., B. Autran, L. P. Martins, S. Wain-Hobson, M. Raphael, C. Mayaud, M. Denis, J.-M. Guillon, and P. Debre. 1987. AIDS virus specific cytotoxic T lymphocytes in lung disorders. Nature (London) 328:348–350.
54. Poli, G., A. Kinter, J. S. Justement, J. H. Kerhl, P. Bressler, S. Stanley, and A. S. Fauci. 1990. Tumor necrosis factor α functions in an autocrine manner in the induction of human immunodeficiency virus expression. Immunology 87:782–785.
55. Poli, G., J. M. Orenstein, A. Kinter, T. M. Folks, and A. S. Fauci. 1989. Interferon-α but not AZT suppresses HIV expression in chronically infected cell lines. Science 244:575–577.
56. Popovic, M., and S. Gartner. 1987. Isolation of HIV-1 from monocytes but not T-lymphocytes. Lancet ii:916–920.
57. Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo. 1984. Detection, isolation, and continuous production of cytopathic human T lymphotropic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. Science 224:497–500.
58. Rappersberger, K., S. Gartner, P. Schenk, G. Stingl, V. Groh, E. Tschachler, D. L. Mann, K. Wolff, K. Konrad, and M. Popovic.

1988. Langerhans cells are an actual site of HIV-1 replication. Intervirology **29**:185–190.

59. **Roy, S., U. Delling, C.-H. Chen, C. A. Rosen, and N. Sonenberg.** 1990. A bulge structure in HIV-1 TAR RNA is required for Tat binding and Tat-mediated *trans*-activation. Genes Dev. **4**:1365–1373.
60. **Roy, S., L. Fitz-Gibbon, L. Poulin, and M. A. Wainberg.** 1988. Infection of human monocytes/macrophages by HIV-1: effect on secretion of IL-1 activity. Immunology **64**:233–238.
61. **Salahuddin, S. Z., R. M. Rose, J. E. Groopman, P. D. Markham, and R. C. Gallo.** 1986. Human T-lymphotropic virus type III infection of human alveolar macrophages. Blood **68**:281–284.
62. **Shaw, G., and R. Kamen.** 1986. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell **46**:659–667.
63. **Shigeo, O., and T. Taniguchi.** 1981. Structure of chromosomal gene for human interferon-β. Proc. Natl. Acad. Sci. USA **78**:5305–5309.
64. **Smith, P. D., K. Ohura, H. Masur, H. C. Lane, A. S. Fauci, and S. M. Wahl.** 1984. Monocyte function in the acquired immune deficiency syndrome. Defective chemotaxis. J. Clin. Invest. **74**:2121–2128.
65. **Sundstrom, C., and K. Nilsson.** 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int. J. Cancer **17**:565–577.
66. **Tschachler, E., V. Groh, M. Popovic, D. L. Mann, K. Konrad, B. Safai, L. Eron, F. Veronese, K. Wolff, and G. Stingl.** 1987. Epidermal Langerhans cells: a target for HTLV/LAV infection. J. Invest. Dermatol. **88**:233–240.
67. **Vogel, J., S. Hinrichs, R. K. Reynolds, P. Lucin, and G. Jay.** 1988. The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. Nature (London) **335**:606–611.
68. **Voth, R., S. Rossol, K. Klein, G. Hess, K. Schütt, H. Schröder, K. Buschfelde, and W. Müller.** 1990. Differential gene expression of IFN-α and tumor necrosis factor α in peripheral blood mononuclear cells from patients with AIDS related complex and AIDS. J. Immunol. **144**:970–975.
69. **Wong, G., J. Krowka, D. Stites, and D. Goeddel.** 1988. *In vitro* anti-human immunodeficiency virus activities of tumor necrosis factor-α and interferon-γ. J. Immunol. **140**:120–124.
70. **Yasukawa, K., T. Hirano, Y. Watanabe, K. Muratani, T. Matsuda, S. Nakai, and K. Kishimoto.** 1987. Structure and expression of human B-cell stimulatory factor-2 (BSF-2/IL-6) gene. EMBO J. **6**:2939–2945.
71. **Zack, J. A., S. J. Arrigo, S. R. Weitzman, A. S. Goo, A. Haislip, and I. S. Y. Chen.** 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile latent viral structure. Cell **61**:213–222.