

Transforming Growth Factor β Suppresses Human Immunodeficiency Virus Expression and Replication in Infected Cells of the Monocyte/Macrophage Lineage

By Guido Poli, Audrey L. Kinter, Jesse S. Justement, Peter Bressler, John H. Kehrl, and Anthony S. Fauci

From the Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

Summary

The pleiotropic immunoregulatory cytokine transforming growth factor β (TGF- β) potently suppresses production of the human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome, in the chronically infected promonocytic cell line U1. TGF- β significantly (50–90%) inhibited HIV reverse transcriptase production and synthesis of viral proteins in U1 cells stimulated with phorbol myristate acetate (PMA) or interleukin 6 (IL-6). Furthermore, TGF- β suppressed PMA induction of HIV transcription in U1 cells. In contrast, TGF- β did not significantly affect the expression of HIV induced by tumor necrosis factor α (TNF- α). These suppressive effects were not mediated via the induction of interferon α (IFN- α). TGF- β also suppressed HIV replication in primary monocyte-derived macrophages infected in vitro, both in the absence of exogenous cytokines and in IL-6-stimulated cultures. In contrast, no significant effects of TGF- β were observed in either a chronically infected T cell line (ACH-2) or in primary T cell blasts infected in vitro. Therefore, TGF- β may play a potentially important role as a negative regulator of HIV expression in infected monocytes or tissue macrophages in infected individuals.

A number of cytokines, including TNF- α or - β , IL-2, IL-6 and granulocyte/macrophage colony-stimulating factor (GM-CSF),¹ can upregulate the in vitro expression of human immunodeficiency virus (HIV) in infected cells (1–4). Furthermore, increased plasma and/or cerebrospinal fluid levels of both TNF- α (5, 6) and IL-6 (7, 8) have been reported in HIV-infected individuals, suggesting that these cytokines could modulate virus expression in vivo. In contrast, with the exception of IFNs (3, 9–12), no cytokines have been reported thus far to exert suppressive effects on HIV expression or replication.

Transforming growth factor β (TGF- β) is a pleiotropic cytokine well known as a bifunctional regulator of the phenotype and proliferative capacity of certain cell lines (13). It is secreted in a latent form by a variety of cells (13), including activated lymphocytes (14) and monocyte/macrophages (15, 16). Active TGF- β is a chemotactic factor for monocytes (17), and is a potent suppressive cytokine on T (18) and B (19) lymphocyte proliferation, NK cell activity (20), macrophage

activation (21), and generation of specific cytotoxic T cells (22). Furthermore, TGF- β modulates the synthesis and/or the effects of several other cytokines, such as IL-1 (18, 23), IL-2 (14), IL-3 (24), GM-CSF (24), IFN- γ (25), and TNF (23, 25, 26). Therefore, we investigated whether TGF- β could also affect the induction by certain cytokines of the expression and/or replication of HIV in cell lines as well as primary cell cultures of both T lymphocytic and monocytic lineages.

We have previously established chronically infected cell lines of promonocytic (4, 27) and T lymphocytic (28) origin. Among several infected promonocytic clones, the U1 cell line was further characterized. U1 constitutively expresses low to undetectable levels of HIV which can be potently induced by various agents, including PMA (4, 10, 27), bacterial endotoxin (LPS) (29), and certain cytokines such as GM-CSF (4, 29), TNF- α (30, 31), and IL-6 (32). Similarly, HIV production in the chronically infected T cell line ACH-2 is inducible over its low constitutive levels by PMA and TNF- α / β (10, 28, 33). In the present study, we demonstrated that the cytokine TGF- β is a suppressor of HIV expression in both chronically infected promonocytic cells as well as in primary monocyte-derived macrophages (MDM). These observations further support the hypothesis that normal homeostatic mech-

¹ Abbreviations used in this paper: Ab, antibody; GM, granulocyte/macrophage; MDM, monocyte-derived macrophages; RT, reverse transcriptase; TGF- β , transforming growth factor β .

anisms, which physiologically regulate the immune system, also play a role as modulators of HIV expression.

Materials and Methods

Induction of HIV Expression in Chronically Infected Cell Lines. The promonocytic cell line U1 and the T lymphocytic cell line ACH-2 were derived from cells surviving the acute HIV-1 infection of U937 (27) and A3.01 (28) cells, respectively. Both cell lines are characterized by a low copy number (1–2) of integrated proviruses, low constitutive expression of HIV, which can be upregulated by PMA (10, 27, 28), and certain cytokines. ACH-2-derived HIV is infectious by standard criteria (passage of ACH-2 supernatant onto PHA-stimulated PBMC (34), whereas U1-derived HIV has been found infectious with reduced efficiency exclusively for certain cell lines (35). The U937-derived chronically infected clone U33.3, which is characterized by detectable constitutive production of HIV and inducibility by PMA and cytokines (27, 31), was also used in some experiments. Typically, U1, ACH-2, or U33.3 cells were resuspended at 2.5×10^5 cells/ml in RPMI 1640 (Whitaker M.A. Bioproducts, Walkersville, MD) containing 10% FCS, plated in 96-well plates (Costar, Cambridge, MA), and stimulated with PMA (10^{-7}), rTNF- α (100 U/ml) (Genzyme, Boston, MA), rIL-6 (50–100 U/ml) (Amgen Biologicals, Thousand Oaks, CA), rGM-CSF (100 U/ml) (Genzyme), or synergistic combinations of these cytokines (32) in the presence or absence of different concentrations of purified TGF- β 1 (kindly provided by Dr. M.B. Sporn, NCI, NIH), or rTGF- β 1 (R & D Systems, Minneapolis, MN) at 37°C in 5% CO₂ for 72 h. In some experiments, U1 cells were also stimulated in the presence of rIFN- α (the generous gift of Dr. S. Petska, UMDNJ, Robert Wood Johnson Medical School, Piscataway, NJ) either alone or in combination with TGF- β . Supernatants were harvested at different times after stimulation, and tested for the presence of Mg²⁺-dependent HIV reverse transcriptase (RT) activity.

HIV Infection of Primary MDM. Monocytes obtained from Ficoll-Hypaque-separated PBMC of seronegative donors were resuspended in RPMI 1640 containing 10% FCS and purified by overnight adherence to 24-well tissue culture plates (Costar) resulting in $\sim 10^6$ monocytes/ml/well. Nonadherent cells (predominantly T lymphocytes) were eliminated by vigorous washing and pipetting. Monocytes were maintained 5–7 d before infection in IMDM (Gibco Laboratories, Grand Island, NY) containing 10% FCS, as described (32). Then, MDM were infected with a 1:100 dilution of a macrophage-tropic strain of HIV (the undiluted viral stock of AD-'87, also referred to as ADA [12]), contains $\sim 10^4$ infectious units/ml). Part of the MDM cultures were stimulated 12–16 d after infection with medium containing cytokines, such as IL-6 (100 U/ml), GM-CSF (100 U/ml), and TNF- α (100 U/ml), which we have previously shown to upregulate HIV replication in these cells (32); TGF- β (1 ng/ml); or combinations of various cytokines and TGF- β . Approximately 50% of the culture supernatants was replaced with medium or medium containing cytokines every 5 d for several weeks of culture, and supernatants were collected and tested for the presence of RT activity. No gross differences in terms of cell number and viability were observed in the different conditions used.

RT Activity Assay. 5 μ l of supernatants was added in duplicate to 25 μ l 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 μ l of the mixture was spotted onto DE81 paper, air dried, washed five times in 2×

SSC buffer, and two additional times in 95% ethanol, as previously described (36). The paper was then dried, cut, and counted on a scintillation counter (LS 5000; Beckman Instruments, Inc., Fullerton, CA). Variability of replicate cultures was always <15%.

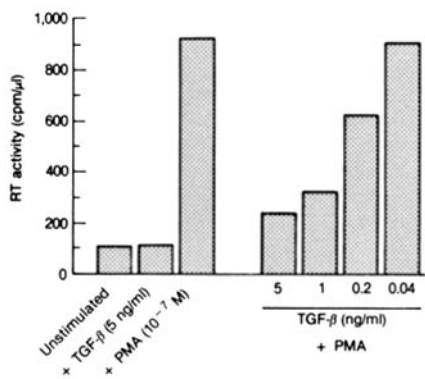
Western Blot Analysis of HIV Proteins. Cell lysates were prepared from U1 cells either unstimulated or stimulated for 40 h with PMA (10^{-7} M) in the presence or absence of TGF- β (0.5 ng/ml). 20 μ l from the lysate of 10^7 cells was added to each lane and subjected to electrophoresis through 10–20% gradient polyacrylamide gels (Integrated Sep. Sci., 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 a 1:1,000 (vol/vol) dilution of an AIDS patient serum containing high titers of anti-HIV antibody (Ab) recognizing most of the major viral proteins (10). 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.

Northern Blot Analysis of Steady State HIV RNA. Total RNA was extracted from 10^8 U1 cells by the guanidine thiocyanate phenol method with an RNA isolation kit (Stratagene, La Jolla, CA) after 24 h stimulation with PMA in the presence or absence of TGF- β (1 ng/ml). 10 μ g of total RNA was loaded per lane on a 0.8% agarose formaldehyde gel and transferred to nitrocellulose filters. The filters were baked and hybridized for 12 h with a ³²P-labeled HIV-LTR (HindIII-AvaI) probe. Filters were washed and exposed to X-ray film. The labeled probe was removed from the filters by washing at 80°C in 0.1× SSC containing 0.1% SDS. The filters were then rehybridized with a ³²P-labeled β_2 -microglobulin cDNA probe.

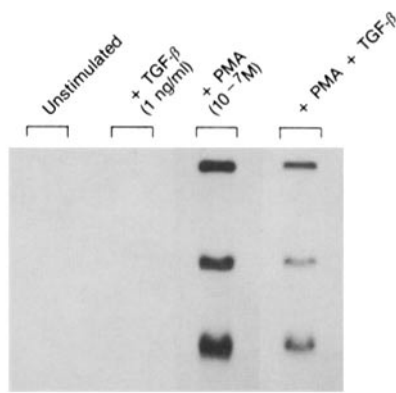
Nuclear Run-on Analysis of HIV Transcription. Nuclei from 5×10^7 U1 cells were isolated 17 h after PMA stimulation in the presence or absence of TGF- β (1 ng/ml), and nuclear run-on analysis was performed according to a published method (37). Briefly, equal amounts of ³²P-labeled RNA were hybridized to linearized plasmid DNA probes (including PUC 19, as plasmid control, pNL4-3, containing a full-length HIV genome [38]) and a human β_2 -microglobulin cDNA, immobilized on nitrocellulose filters, which were then exposed to X-ray film.

Results

TGF- β Suppresses PMA-induced Expression of HIV. U1 and ACH-2 cells were first stimulated with PMA in the presence or absence of different concentrations (0.04–5 ng/ml) of TGF- β , and culture supernatants were tested for the presence of HIV RT activity. No detectable effects were observed in U1 cells stimulated with TGF- β alone; however, U1 cells stimulated with PMA in the presence of TGF- β showed a significantly reduced production of HIV (Fig. 1 A). No significant differences were observed when U1 cells were preincubated with TGF- β for up to 4 h before the addition of PMA as compared with cells simultaneously treated with the two agents. Suppression of virus expression by TGF- β was observed throughout a 7-d culture period, and it was not attributable to cytotoxicity or decreased cell proliferation, as determined by trypan blue dye exclusion and [³H]thymidine incorporation analysis, respectively. TGF- β inhibition of RT production was correlated with a significant reduction of viral protein synthesis, as demonstrated by Western blot analysis performed on U1 cell lysates (data not shown), and with a significant reduction in the accumulation of both spliced and



A



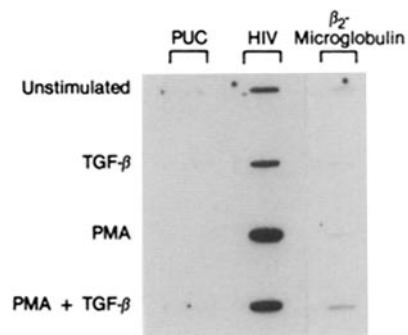
B

Figure 1. TGF- β suppression of PMA-stimulated HIV expression in U1 cells. (A) Suppression of RT production by TGF- β . Comparable effects were observed throughout a 7-d culture period after a single treatment with PMA and TGF- β . Results are representative of five independent experiments. (B) TGF- β inhibition of steady state HIV RNA accumulation. The results shown were obtained by Northern blot hybridization of total RNA extracted from U1 cells after 24-h stimulation. No significant differences were observed when RNA was extracted at different time points up to 48 h post-stimulation. Comparable amounts of RNA were loaded onto each lane, as established by reprobing the filters with a β_2 -microglobulin cDNA (data not shown).

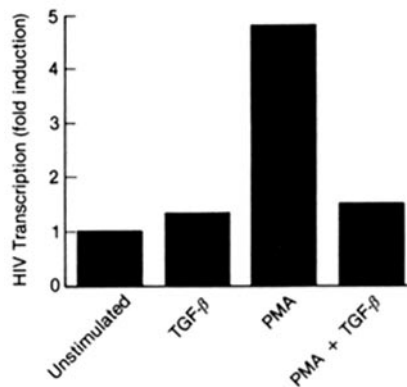
unspliced mRNA species (Fig. 1 B). Furthermore, a potent inhibition of PMA-induced HIV transcription by TGF- β treatment of U1 cells was demonstrated by nuclear run-on analysis (Fig. 2). These findings indicated that TGF- β interfered with earlier steps of virus expression in these chronically infected cells as compared with the post-translational block of HIV production previously described for IFN- α (10). In contrast to the findings with U1 cells, TGF- β showed no inhibitory effect on PMA-induced HIV expression in the chronically infected T lymphocytic cell lines, ACH-2 (data not shown).

Lack of TGF- β Effect on TNF- α -induced Expression of HIV.

A similar degree of suppression by TGF- β was observed in U1 cells stimulated with a crude cytokine-enriched supernatant obtained from primary monocytes stimulated in vitro with LPS. In this regard, we have previously demonstrated that TNF- α is one of the major HIV-inductive monokines secreted by LPS-stimulated monocytes (28). Therefore, we investigated the ability of TGF- β to suppress HIV production in U1 cells stimulated with rTNF- α . However, in con-



A



B

Figure 2. TGF- β suppression of PMA-induced HIV transcription. (A) Nuclear run-on analysis was performed on nuclei extracted from U1 cells stimulated for 17 h with PMA (10⁻⁷ M) in the presence or absence of TGF- β (1 ng/ml). The transcriptional inhibitor α -amanitin almost entirely inhibited transcription in U1 cells. (B) Densitometric laser scanning of the levels of newly synthesized HIV mRNA. Filters of the run-on experiments shown in A were subjected to laser scanning densitometric analysis (2222-10; LKB Instruments, Inc., Gaithersburg, MD). The results are shown as ratios of the areas under the curve of the scanned HIV RNA bands normalized to those of the β_2 -microglobulin controls, giving the arbitrary value of 1 to the ratio measured in unstimulated conditions. Similar results were obtained when β -actin was used as control.

trast to the inhibitory effects observed in U1 cells stimulated with PMA or LPS-monocyte supernatant, TGF- β had no suppressive effects on the induction of HIV expression by TNF- α , as measured by RT activity (Fig. 3 A) and Western blot analysis of cell-associated viral proteins. Furthermore, TGF- β did not inhibit either steady state accumulation or transcription of HIV RNA in U1 cells stimulated with TNF- α (data not shown). TGF- β inhibition of PMA-induced, but not TNF- α -induced, virus production was confirmed in a second (U33.3) chronically infected promonocytic cell line.

TGF- β Does Not Affect the PMA-induced TNF- α Autocrine Pathway in U1 Cells. We have previously reported that part of the inductive effect of PMA on U1 cells is accounted for by the triggering of an autocrine pathway of HIV expression mediated by the synthesis and release of endogenous TNF- α (31). As shown in Fig. 3 A, anti-TNF- α -neutralizing Ab par-

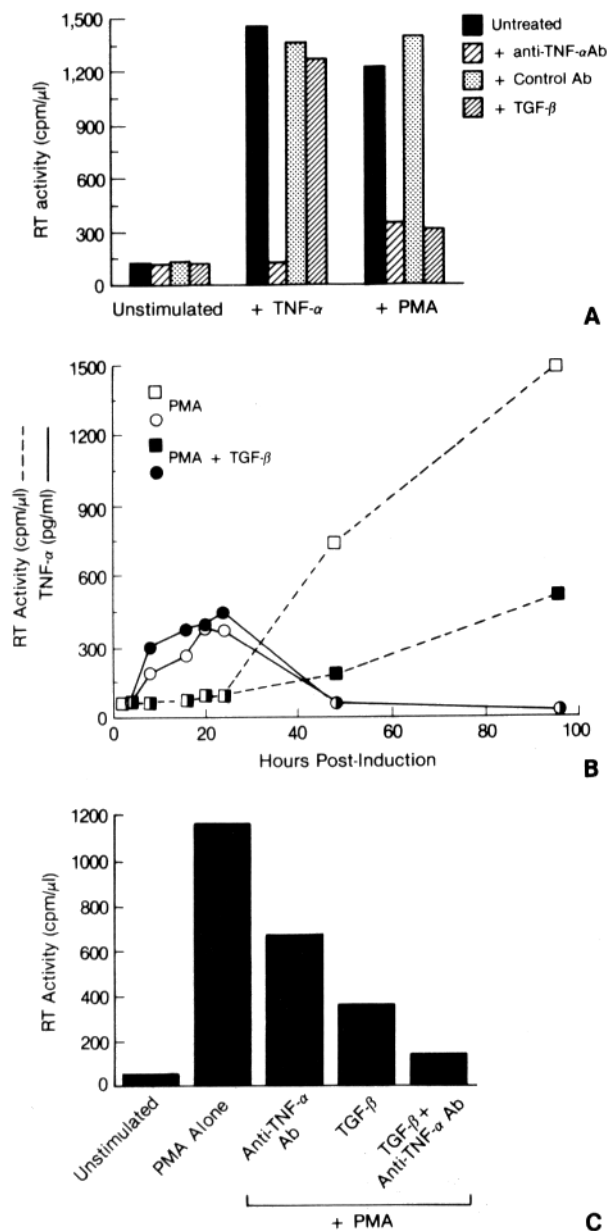


Figure 3. TGF- β does not affect either the HIV-inductive effect of exogenous TNF- α or the secretion of endogenous TNF- α in U1 cells stimulated with PMA. (A) TGF- β does not suppress TNF- α induction of HIV expression. U1 cells were stimulated with either 100 U/ml of rTNF- α or 10^{-7} M PMA in the presence or absence of a 1:100 (vol/vol) dilution of a neutralizing anti-TNF- α Ab (31), control Ab, or TGF- β (1 ng/ml). Supernatants were collected after 72 h and tested for RT activity. The results shown are representative of five independent experiments. (B) TGF- β suppresses expression of HIV, but not secretion of TNF- α in U1 cells stimulated with PMA. U1 cells were stimulated with 10^{-7} M PMA in the presence or absence of TGF- β (1 ng/ml); supernatants were collected at different times and tested for RT activity. In parallel, supernatants were also tested for the presence of TNF- α by a commercially available ELISA kit (T Cell Sciences, Inc., Cambridge, MA). No significant levels of RT activity or TNF- α secretion were detected in unstimulated conditions. (C) Cooperative suppression of PMA-induced expression of HIV in U1 cells by TGF- β and anti-TNF- α Ab. U1 cells were briefly (2 h) pretreated with 20 nU/ml of anti-TNF- α mAb (Olympus, Lake Success, NJ), TGF- β (0.4 ng/ml), or both anti-TNF- α mAb and TGF- β before stimulation with 10^{-7} M PMA. Supernatants were harvested and tested for RT activity 72 h after stimulation.

tially blocked PMA-induced HIV expression in U1 cells. Therefore, we investigated whether TGF- β could affect the ability of PMA to induce TNF- α production in U1 cells. However, as previously observed in uninfected PBMC stimulated with

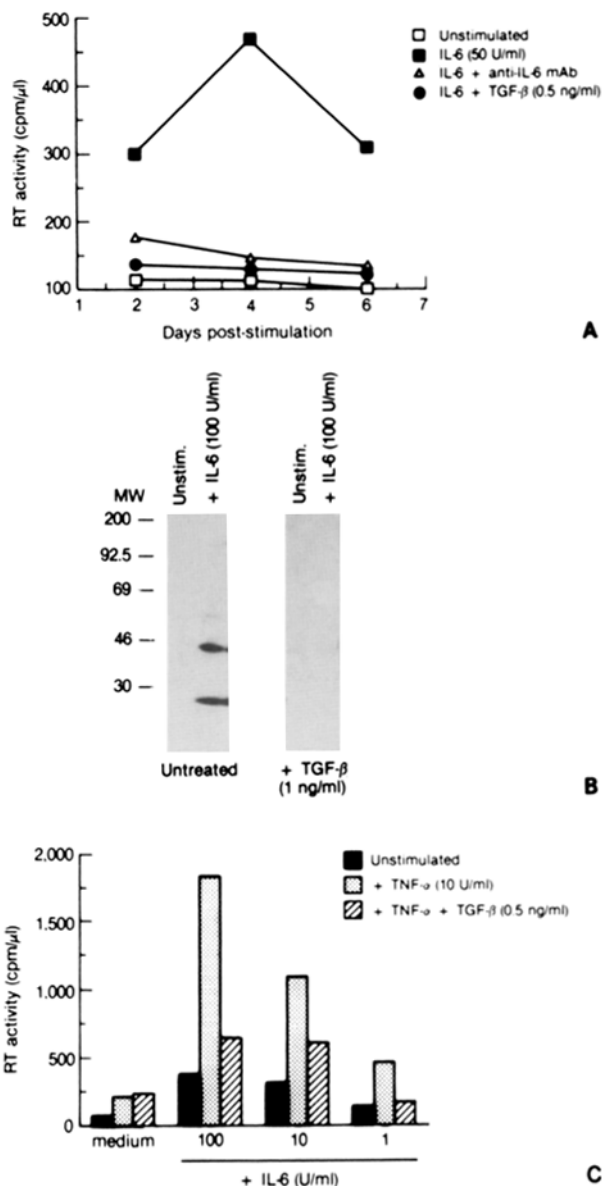


Figure 4. TGF- β suppresses IL-6 induction of HIV expression in U1 cells. (A) TGF- β suppresses HIV production in U1 cells stimulated with IL-6. Cells were stimulated with 50 U/ml of IL-6 in the presence or absence of TGF- β (0.5 ng/ml), or 1:100 dilution of anti-IL-6 mAb (R & D Systems, Minneapolis, MN). Supernatants were collected throughout 7 d of culture and were tested for the presence of RT activity. (B) TGF- β blocks the induction of HIV protein expression in U1 cells stimulated with IL-6. Western blot analysis was performed on cell lysates of U1 cells either unstimulated or stimulated for 40 h with 10^{-7} M PMA in the presence or absence of TGF- β . (C) TGF- β suppresses the synergistic induction of HIV expression in U1 cells costimulated with TNF- α (10 U/ml) and various concentrations of IL-6. Similar results were obtained when higher (100 U/ml) concentrations of TNF- α and IL-6 were used, and were also observed in U1 cells synergistically stimulated with TNF- α and GM-CSF.

PMA and PHA (23), TGF- β did not affect either the kinetics or the amount of TNF- α produced by U1 cells under these conditions (Fig. 3 B). Furthermore, TGF- β did not alter the number or the affinity of TNF receptors in PMA-stimulated cells (data not shown). Thus, TGF- β seems capable of suppressing PMA-induced HIV expression by a different mechanism other than the TNF- α autocrine pathway triggered in U1 cells by the phorbol ester. In support of this hypothesis, a virtually complete inhibition of HIV production (in the absence of toxicity) was observed when U1 cells were stimulated with PMA in the presence of both TGF- β and anti-TNF- α Ab (Fig. 3 C).

TGF- β Suppresses IL-6-induced Expression of HIV in U1 Cells. These findings suggested that HIV-inductive factors other than TNF- α were blocked by TGF- β when U1 cells were stimulated with crude cytokine preparations. In this regard, we have observed recently that IL-6, a cytokine induced by LPS stimulation of monocyte/macrophages (39), upregulates virus expression in U1 and U33.3 cells (32). In contrast to TNF- α , which upregulates virus production by activation of the cellular transcription factor NF- κ B (40–42), IL-6 induces HIV expression in these cell lines predominantly by acting at a post-transcriptional level (32). Therefore, we next investigated whether TGF- β could suppress the induction of HIV expression triggered by IL-6. As shown in Fig. 4 A, TGF- β completely suppressed the production of virus in U1 cells stimulated with IL-6, and this effect was also correlated with a reduction of viral protein synthesis to virtually undetectable levels (Fig. 4 B). Furthermore, TGF- β completely blocked both RT production and synthesis of HIV proteins in U1 cells stimulated with GM-CSF, which, similar to IL-6, affects predominantly a post-transcriptional step of HIV ex-

pression in these cells (G. Poli, A. L. Kinter, J. S. Justement, and A. S. Fauci, unpublished observations).

We have also reported that TNF- α synergizes with either IL-6 or GM-CSF in the induction of HIV expression in U1 cells (32). TGF- β significantly inhibited the production of HIV in U1 cells costimulated with TNF- α and IL-6 (Fig. 4 C), or TNF- α and GM-CSF (not shown), and these findings were confirmed by Western blot analysis. Thus, TGF- β can suppress the expression of HIV by interfering with both transcriptional (in cells stimulated with PMA), and post-transcriptional (in cells stimulated with IL-6, GM-CSF, or costimulated with both cytokines) pathways of virus activation. No significant differences between the two isoforms of TGF- β (TGF- β 1 and - β 2) (reviewed in reference 13) were observed in terms of their suppressive effects on U1 cells stimulated with various inducers; furthermore, anti-TGF- β Ab neutralized the suppressive abilities of TGF- β on HIV expression. Since neither IL-6 nor GM-CSF induce expression of HIV and ACH-2 cells, the effects of TGF- β on this system were not studied.

Cooperative Suppressive Effects of TGF- β and IFN- α on the Induction of HIV Expression in U1 Cells. We have previously reported that IFN- α exerts a potent suppressive effect on HIV production in chronically infected U1 and ACH-2 cells (10). As described in cells chronically infected with murine retroviruses (43–45), IFN- α blocked the release of mature virions from the plasma membrane of U1 and ACH-2 cells, without suppressing HIV protein synthesis (10). When U1 cells were stimulated with PMA in the presence of TGF- β and various concentrations of IFN- α , a virtually complete suppression of virus production was observed in the culture supernatant (Fig. 5). Similar results were obtained when U1 cells were stimulated with IL-6 (data not shown).

Thus, TGF- β and IFN- α affect different steps of the HIV life cycle in chronically infected U1 cells (such as transcription and translation of viral RNA vs. budding of morphologically mature viral particles from the plasma membrane, respectively). Nonetheless, we investigated whether the suppressive effect of TGF- β could be accounted for by endogenous production of IFN- α or - β . No detectable IFN- α production, as determined by vesicular stomatitis virus yield reduction assay (43–45), was observed in the different stimulatory conditions described above (P. M. Pitha, G. Poli, and A. S. Fauci, unpublished observations). Furthermore, anti-IFN- α Ab, which reversed the inhibitory effect of IFN- α on U1 cells, did not significantly affect TGF- β suppression of HIV expression (data not shown).

TGF- β Suppresses HIV Replication in Primary MDM. We further investigated whether TGF- β could also suppress HIV infection of primary cultures of MDM. In this regard, we have previously demonstrated that IL-6, as well as GM-CSF and TNF- α , significantly upregulates both the kinetics and the extent of replication of a macrophage tropic HIV strain in primary MDM (32). Consistent with our observations with chronically infected promonocytic cell lines, TGF- β also inhibited HIV replication in MDM. In MDM cultures established from certain individuals, virus production was clearly detectable in unstimulated conditions, and was significantly

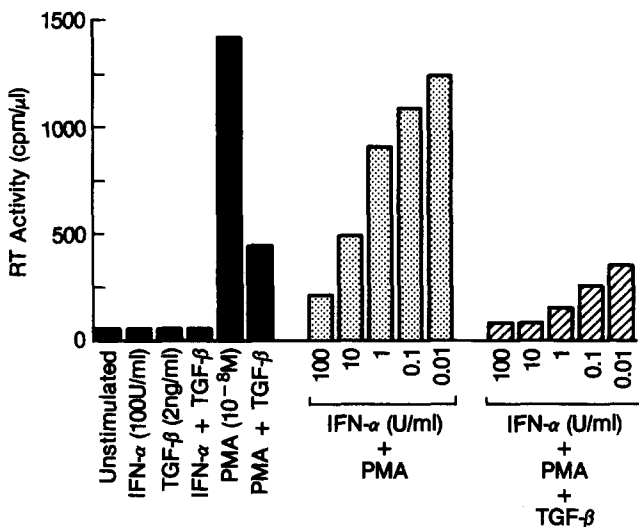


Figure 5. Suppression of PMA-induced HIV expression in U1 cells treated with TGF- β and IFN- α . A virtually complete inhibition of virus production was observed in U1 cells stimulated with PMA in the presence of TGF- β and IFN- α , whereas treatment with individual cytokines led only to a partial, although significant suppression. Additive antiviral effects were observed in PMA-stimulated U1 cells treated with suboptimal concentrations of TGF- β and IFN- α .

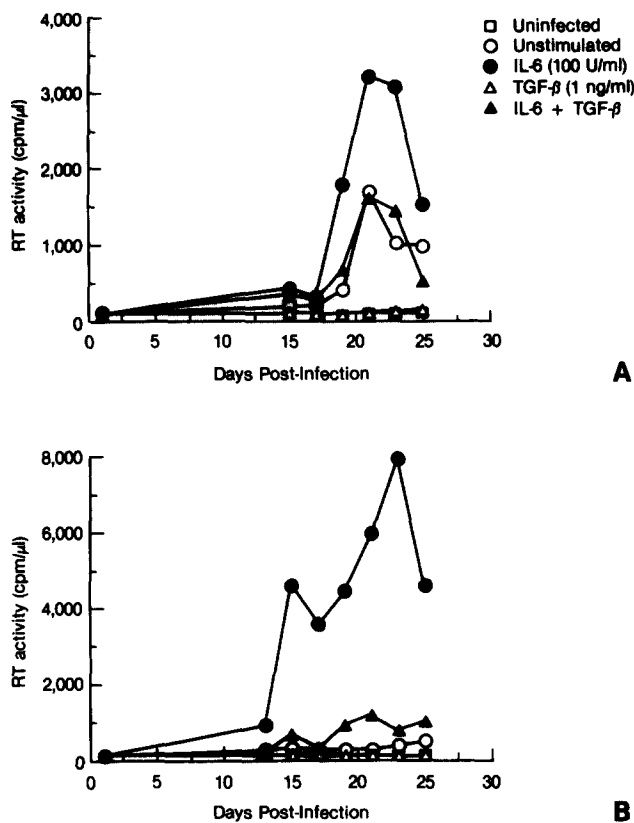


Figure 6. TGF- β inhibition of HIV replication during acute infection of primary MDM. As shown in *A*, some MDM cultures showed detectable levels of RT production even when maintained with medium in the absence of IL-6 (unstimulated). TGF- β suppressed both the constitutive (*A*) and the IL-6-induced (*A* and *B*) levels of HIV replication in MDM. The results are representative of six independent experiments.

enhanced by IL-6 (Fig. 6 *A*), whereas in cells obtained from other donors, significant levels of virus replication were observed only in the presence of IL-6 (Fig. 6 *B*). The different patterns of HIV replication observed in these cultures could not be accounted for by increased levels of cytopathicity or gross differences in the numbers of MDM, but probably reflected a significant variability among different individuals in terms of the activation state of their circulating monocytes and/or susceptibility of these cells to HIV infection, as previously observed for T lymphocytes (46). Nonetheless, TGF- β suppressed both the constitutive as well as the IL-6-stimulated production of virus (Fig. 6, *A* and *B*) in the absence of cytotoxicity or evident effects on cell adherence. Similarly, TGF- β suppressed HIV replication in MDM cultures stimulated with GM-CSF, whereas variable results (either suppression or no effect) were obtained in TNF- α -treated cultures (data not shown). Spreading of HIV in primary MDM is correlated with the accumulation of infectious virus particles and RT activity in the culture supernatants. In contrast to other retroviral proteins, such as gag and envelope, which can be shed by infected cells in culture supernatants, RT enzymatic activity is exclusively associated with released viral particles both in HIV (47, 48) and other animal retroviruses

(49, 50). We have recently confirmed this finding in our HIV-infected monocytic cell systems (Fernie, B. F., G. Poli, and A. S. Fauci, manuscript in preparation). Therefore, inhibition of the accumulation of RT activity by TGF- β observed in our primary MDM cultures (Fig. 6) directly reflects a reduced production of infectious HIV. In contrast, as previously observed with the chronically infected T cell line ACH-2, TGF- β did not significantly affect either the kinetics or the levels of HIV replication in mitogen-stimulated T cell blasts infected with a T lymphocytotropic strain of HIV (LAV) (data not shown).

Discussion

In the present study, we have demonstrated that the immunoregulatory cytokine TGF- β suppresses both virus expression in chronically infected cell lines of the monocyte/macrophage lineage and HIV replication in acutely infected primary MDM. In chronically infected cells, TGF- β affects earlier steps of the virus life cycle (such as transcription and accumulation of HIV mRNA as well as synthesis of viral proteins in the case of PMA induction of HIV) than those inhibited by IFN- α , which blocks the production of virions at a post-assembly level (10). TGF- β also suppresses the inductive ability of certain cytokines (IL-6 and GM-CSF), which alone or in synergistic combination upregulate synthesis of viral proteins and virus production predominantly at a post-transcriptional level (32). Furthermore, TGF- β is also effective in blocking the cooperation between these cytokines and TNF- α , which of itself is not affected by TGF- β . Although it is difficult to determine whether TGF- β reduces spread of infectious virus in chronically infected U1 cells, since every cell in culture already has two integrated copies of HIV provirus, TGF- β did potently block the production of infectious virus in primary MDM acutely infected with a macrophage tropic strain of HIV. Of note is the fact that others have recently reported that TGF- β upregulated HIV expression in a system of acute infection of MDM (51). Although TGF- β consistently downregulated HIV replication in our MDM cultures, we did observe a similar enhancing effect of TGF- β on the acute infection of U937 cells, which are the uninfected parental cells of U1 (G. Poli, A. L. Kinter, S. J. Justement, and A. S. Fauci, unpublished observations). Thus, under certain experimental conditions, bifunctional effects of TGF- β can be seen on HIV expression similar to the bifunctional effects of this molecule on other cell systems (reviewed in reference 13). The precise mechanisms involved in this dichotomy of effects are unclear at present.

Because both TNF- α (40–42) and PMA (52, 53) have been reported to induce expression of HIV via activation of the cellular transcription factor NF- κ B, the lack of TGF- β effect on TNF- α -stimulated cells suggests that the NF- κ B-mediated pathway is not significantly affected by TGF- β , and that TGF- β suppression of PMA-induced HIV expression may be mediated by mechanisms not directly related to NF- κ B. In support of this hypothesis, TGF- β did not inhibit either PMA or TNF- α induction of the HIV long terminal repeat (LTR),

as measured by chloramphenicol-acetyl-transferase (CAT) activity in the U1 uninfected parental cell line U937 transfected with an HIV-LTR-CAT construct (G. Poli, A. L. Kinter, P. Bressler, and A. S. Fauci, unpublished observations). Alternatively, PMA and TNF- α may trigger different pathways of NF- κ B activation in U1 cells, as recently described in uninfected K562 and Jurkat cells (54). Further studies are in progress in our laboratory to address these hypotheses.

We further investigated whether the suppressive effect of TGF- β on HIV infection in U1 cells was correlated with the induction of endogenous IFN- α (or - β), which would suppress the expression of virus. This possibility was supported by previous observations that anti-IFN- α -neutralizing Ab could upregulate the replicative capacity of HIV in both U937 cells (55) as well as in mitogen-stimulated PBMC (56). However, no evidence of IFN- α/β production or effect was found in U1 cells, regardless of the presence of TGF- β . In addition, the combination of TGF- β and IFN- α resulted in the virtually complete suppression of HIV production in U1 cells,

further indicating that these two cytokines affected different steps of HIV expression.

Of note is the fact that it has been recently reported that PBMC from HIV-infected individuals show an increased in vitro production of TGF- β (57). The present study suggests that increased production of TGF- β in infected individuals could potentially play a dichotomous role in the pathogenesis of HIV infection by both contributing to several of the immune dysfunctions described in AIDS and AIDS-associated conditions, such as decreased proliferative capacity of T lymphocytes (58), defective NK cell activity (59, 60), and monocyte functional abnormalities (61-64), while simultaneously inhibiting viral replication/expression in infected monocyte/macrophages. Finally, since other cytokines capable of inducing the expression of HIV, such as TNF- α and IL-6, have been reported to be elevated in the plasma and/or in the cerebrospinal fluid of infected individuals (5-8), the balance between inductive and suppressive cytokines may represent a critical factor in the clinical progression of HIV infection.

We thank Prof. P. M. Pitha for the determination of IFN- α presence in our culture systems, and Dr. S. K. Stanley for helpful discussions.

Address correspondence to Dr. Guido Poli, Laboratory of Immunoregulation, NIAID, Building 10, Room 11B-13, National Institutes of Health, Bethesda, MD 20892.

Received for publication 14 August 1990 and in revised form 26 November 1990.

References

1. Fauci, A.S. 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science (Wash. DC)*. 239:617.
2. Rosenberg, Z.F., and A.S. Fauci. 1990. Immunopathogenic mechanisms of HIV infection: cytokine induction of HIV expression. *Immunol. Today*. 11:176.
3. 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.
4. Folks, T.M., J.S. 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.
5. Reddy, M.M., S.J. Sorrel, M. Lange, and M.H. Grieco. 1988. Tumor necrosis factor and HIV p24 antigen levels in serum of HIV-infected populations. *J. AIDS*. 1:436.
6. Lahdevirta, J., C.P.J. 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.
7. 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.
8. Breen, E.C., A.R. Rezai, K. Nakajima, G.N. Beall, R.T. Mitsuyasu, T. Hirano, T. Kishimoto, and O. Martinez-Maza. 1990. Infection with HIV is associated with elevated IL-6 levels. *J. Immunol.* 144:480.
9. Ho, D.D., T.R. Rota, J.C. Kaplan, K.L. Hartshorn, C.A. Andrews, R.T. Schooley, and M.S. Hirsch. 1985. Recombinant human interferon alfa-A suppresses HTLV-III replication in vitro. *Lancet*. i:602.
10. 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 (Wash. DC)*. 244:575.
11. Bednarik, D.P., J.D. Mosca, N.B.K. Raji, 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.
12. Meltzer, M.S., D.R. Skillman, D.L. Hoover, B.D. Hanson, J.A. Turpin, D.C. Kalter, and H.E. Gendelman. 1990. Macrophages and the human immunodeficiency virus. *Immunol. Today*. 11:217.
13. Roberts, A.B., and M.B. Sporn. 1988. Transforming growth factor beta. *Adv. Cancer Res.* 51:107.
14. Kehrl, J.H., L.M. Wakefield, A.B. Roberts, S. Jakowlew, M. Alvarez-Mon, R. Derynck, M.B. Sporn, and A.S. Fauci. 1986. Production of transforming growth factor β by human T lymphocytes and its potential role in the regulation of T cell growth. *J. Exp. Med.* 163:1037.
15. Assoian, R., B.E. Fleurdeyls, S.C. Stevenson, P.J. Miller, D.K.

- Madtes, E.W. Raines, R. Ross, and M.B. Sporn. 1987. Expression and secretion of type beta transforming growth factor by activated human macrophages. *Proc. Natl. Acad. Sci. USA.* 84:6020.
16. Wahl, S.M., N. McCartney-Francis, and S.E. Mergenhagen. 1990. Inflammatory and immunomodulatory roles of TGF- β . *Immunol. Today.* 10:258.
 17. Wahl, S.M., D.A. Hunt, L.M. Wakefield, N. McCartney-Francis, L.M. Wahl, A.B. Roberts, and M.B. Sporn. 1987. Transforming growth factor type beta induces monocyte chemotaxis and growth factor production. *Proc. Natl. Acad. Sci. USA.* 84:5788.
 18. Wahl, S.M., D.A. Hunt, H.L. Wong, S. Dougherty, N. McCartney-Francis, L.M. Wahl, L. Ellingsworth, J.A. Schmidt, G. Hall, A.B. Roberts, and M.B. Sporn. 1988. Transforming growth factor- β is a potent immunosuppressive agent that inhibits IL-1-dependent lymphocyte proliferation. *J. Immunol.* 140:3026.
 19. Kehrl, J.H., A.B. Roberts, L.M. Wakefield, S. Jakowlew, M.B. Sporn, and A.S. Fauci. 1986. Transforming growth factor beta is an important immunomodulatory protein for human B lymphocytes. *J. Immunol.* 137:3855.
 20. Rook, A.H., J.H. Kehrl, L.M. Wakefield, A.B. Roberts, M.B. Sporn, D.B. Burlington, H.C. Lane, and A.S. Fauci. 1986. Effects of transforming growth factor β on the functions of natural killer cells: depressed cytolytic activity and blunting of interferon responsiveness. *J. Immunol.* 136:3916.
 21. Tsunawaki, S., M. Sporn, A. Ding, and C. Nathan. Deactivation of macrophages by transforming growth factor- β . *Nature (Lond.)*. 334:260.
 22. Shalaby, M.R., and A.J. Amman. 1988. Suppression of immune cell function in vitro by recombinant human transforming growth factor- β . *Cell. Immunol.* 112:343.
 23. Chantry, D., M. Turner, E. Abney, and M. Feldmann. 1989. Modulation of cytokine production by transforming growth factor- β . *J. Immunol.* 142:4295.
 24. Keller, J.R., G.K. Sing, L.R. Ellingsworth, F.W. Ruscetti. 1989. Transforming growth factor beta: possible roles in the regulation of normal and leukemic hematopoietic cell growth. *J. Cell. Biochem.* 39:175.
 25. Espevik, T., I.S. Figari, M.R. Shalaby, G.A. Lackides, G.D. Lewis, H.M. Shepard, and M.A. Palladino, Jr. 1987. Inhibition of cytokine production by cyclosporin A and transforming growth factor β . *J. Exp. Med.* 166:571.
 26. Lefer, A.M., P. Tsao, N. Aoki, and M.A. Palladino, Jr. 1990. Mediation of cardioprotection by transforming growth factor- β . *Science (Wash. DC)*. 249:61.
 27. Folks, T.M., J. Justement, A. Kinter, S.M. 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.
 28. 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.
 29. Pomerantz, R.J., M.B. Feinberg, D. Trono, D. Baltimore. 1990. Lipopolysaccharide is a potent monocyte/macrophage-specific stimulator of human immunodeficiency virus type 1 expression. *J. Exp. Med.* 172:253.
 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- κ B. *Nature (Lond.)*. 339:70.
 31. Poli, G., A. Kinter, J.S. Justement, J.H. Kehrl, P. Bressler, S. Stanley, and A.S. Fauci. 1990. Tumor necrosis factor functions in an autocrine manner in the induction of human immunodeficiency virus expression. *Proc. Natl. Acad. Sci. USA.* 87:782.
 32. Poli, G., P. Bressler, A. Kinter, E. Duh, W.C. Timmer, A. Rabson, J.S. Justement, S. Stanley, and A.S. Fauci. 1990. Interleukin 6 induces human immunodeficiency virus expression in infected monocytic cells alone and in synergy with tumor necrosis factor by transcriptional and post-transcriptional mechanisms. *J. Exp. Med.* 172:151.
 33. Folks, T.M., K.A. Clouse, J. Justement, A. Rabson, E. Duh, J.H. Kehrl, and A.S. Fauci. 1989. Tumor necrosis factor induces expression of human immunodeficiency virus in a chronically infected T-cell clone. *Proc. Natl. Acad. Sci. USA.* 86:2365.
 34. Stanley, S.K., P.B. Bressler, G. Poli, and A.S. Fauci. 1990. Heat shock induction of HIV production from chronically infected promonocytic and T cell lines. *J. Immunol.* 145:1120.
 35. Pomerantz, R.J., D. Trono, M.B. Feinberg, and D. Baltimore. 1990. Cell nonproductively infected with HIV-1 exhibit an aberrant pattern of viral RNA expression: a molecular model for latency. *Cell.* 61:1271.
 36. Willey, R.L., D.H. Smith, L.A. Lasky, T.S. Theodore, P.L. Earl, B. Moss, D.J. 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.
 37. Linial, M., N. Gunderson, and M. Groudine. 1985. Enhanced transcription of c-myc in bursal lymphoma cells requires continuous protein synthesis. *Science (Wash. DC)*. 230:1126.
 38. Adachi, A., H.E. Gendelman, S. Koenig, 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.
 39. Navarro, S., N. Debili, J.-F. Bernaudin, W. Vainchenker, and J. Doly. 1989. Regulation of the expression of IL-6 in human monocytes. *J. Immunol.* 142:4339.
 40. Osborn, L.S., S. Kunkel, and G.J. Nabel. 1989. Tumor necrosis factor and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κ B. *Proc. Natl. Acad. Sci. USA.* 86:2336.
 41. Duh, E.J., W. Maury, T.M. Folks, A.S. Fauci, and A.S. Rabson. 1989. Tumor necrosis factor activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF- κ B sites in the long terminal repeat. *Proc. Natl. Acad. Sci. USA.* 86:5974.
 42. Okamoto, T., T. Matsuyama, S. Mori, Y. Hamamoto, N. Kobayashi, N. Yamamoto, S.F. Josephs, F. Wong-Staal, and K. Shimotohno. 1989. Augmentation of human immunodeficiency virus type 1 gene expression by tumor necrosis factor alpha. *AIDS Res. Hum. Retroviruses.* 5:131.
 43. Friedman, R.M., and P.M. Pitha. 1984. The effect of interferon on membrane-associated viruses. In *Interferon: Mechanisms of Production and Action*. Vol. 3. R.M. Friedman, editor. Elsevier Science Publishing Co., Inc., Amsterdam. 319-341.
 44. Friedman, R.M., and J.M. Ramseur. 1974. Inhibition of murine leukemia virus production in chronically infected AKR cells: a novel effect of interferon. *Proc. Natl. Acad. Sci. USA.* 71:3542.
 45. Pitha, P.M., N.A. Wivel, B.F. Fernie, and H.P. Harper. 1979. Effect of interferon on murine leukemia virus infection. IV. Formation of non-infectious virus in chronically infected cells.

- J. Gen. Virol.* 42:467.
46. 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.
 47. Barré-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 (Wash. DC)*. 20:868.
 48. Popovic, M., M.G. Sarngadharan, E. Read, and R.C. Gallo. 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science (Wash. DC)*. 4:497.
 49. Coffin, J.M., and H.M. Temin. 1971. Comparison of Rous sarcoma virus-specific deoxyribonucleic acid polymerases in virions of Rous sarcoma virus and in Rous sarcoma virus-infected chicken cells. *J. Virol.* 7:625.
 50. Teramoto, Y.A., R.D. Cardiff, and J.K. Lund. 1977. The structure of the mouse mammary tumor virus: isolation and characterization of the core. *Virology*. 77:135.
 51. Lazdins, J., E. Alteri, M. Walker, K. Woods-Cok, D. Cox, G. Bilbe, R. Shipman, N. Cerletti, and G. McMaster. 1990. TGF- β upregulator of HIV replication in M Φ . *J. Leukocyte Biol.* 254:90. (Abstr.)
 52. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (Lond.)*. 326:711.
 53. Lenardo, M.J., and D. Baltimore. 1989. NF- κ B: A pleiotropic mediator of inducible and tissue-specific gene control. *Cell*. 8:227.
 54. Meichle, A., S. Schütze, G. Hensel, D. Brunsing, and M. Krönke. 1990. Protein kinase C-independent activation of nuclear factor κ by tumor necrosis factor. *J. Biol. Chem.* 265:8339.
 55. 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.
 56. 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.
 57. Kekow, J., W. Wachsman, J.A. McCutchan, M. Cronin, D.A. Carson, and M. Lotz. 1990. Transforming growth factor β and noncytopathic mechanisms of immunodeficiency in human immunodeficiency virus infection. *Proc. Natl. Acad. Sci. USA*. 87:8321.
 58. Lane, H.C., J.M. Depper, W.C. Greene, G. Whalen, T.A. Waldman, and A.S. Fauci. 1984. Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome. Evidence for a selective defect in soluble antigen recognition. *N. Engl. J. Med.* 313:79.
 59. Poli, G., M. Introna, F. Zanaboni, G. Peri, M. Carbonari, F. Aiuti, A. Lazzarin, M. Moroni, and A. Mantovani. 1985. Natural killer cells in intravenous drug abusers with lymphadenopathy syndrome. *Clin. Exp. Immunol.* 62:128.
 60. Sirianni, M.C., F. Tagliaferri, and F. Aiuti. 1990. Pathogenesis of the natural killer cell deficiency in AIDS. *Immunol. Today*. 11:81.
 61. 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. *J. Clin. Invest.* 74:2121.
 62. Poli, G., B. Bottazzi, R. Acero, L. Bersani, V. Rossi, M. Introna, A. Lazzarin, M. Galli, and A. Mantovani. 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.
 63. Prince, H.E., D.J. Moody, B.I. Shubin, and J.L. Fahey. 1985. Defective monocyte function in acquired immune deficiency syndrome (AIDS): evidence from a monocyte-dependent T-cell proliferation. *J. Clin. Immunol.* 5:21.
 64. Bender, B.S., F.A. Auger, T.C. Quinn, R. Redfield, J. Gold, and T.M. Folks. 1986. Impaired antibody-dependent cell-mediated cytotoxic activity in patients with the acquired immunodeficiency syndrome. *Clin. Exp. Immunol.* 64:166.