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Alpha Interferon Suppresses Virion but Not Soluble Human Immunodeficiency Virus Antigen Production in Chronically Infected T-Lymphocytic Cells

BRUCE F. FERNIE,[1] GUIDO POLI,[2]* AND ANTHONY S. FAUCI[2]

Division of Molecular Virology and Immunology, Department of Microbiology, Georgetown University, Rockville, Maryland 20852,[1] and Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892[2]

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Alpha interferon (IFN-α) is effective in preventing the release of human immunodeficiency virus (HIV) from chronically infected T-lymphocytic (ACH-2) and promonocytic (U1) cell lines stimulated with the phorbol ester phorbol-12-myristate-13 acetate (PMA). In the present study, we observed that together with particle production, shedding of HIV antigen ($p24^{gag}$) occurs in the T-cell line ACH-2 both constitutively and after stimulation with PMA. IFN-α, although effective in suppressing the release of HIV particles, did not inhibit shedding of $p24^{gag}$ into the culture supernatants of either unstimulated or PMA-stimulated cells. These observations may be of relevance in the evaluation of the in vivo efficacy of IFN-α treatment of HIV-infected individuals as determined by levels of p24 antigen in plasma.

Infection with the human immunodeficiency virus (HIV), the causative agent of AIDS, involves primarily, if not exclusively, the $CD4^+$ cells of the immune system, such as T lymphocytes and cells of the monocyte/macrophage lineage (25). Because both productive and latent infections of these cell types occur in vitro as well as in vivo (12, 16, 25, 26), it is important to develop therapeutic strategies that are effective in these different stages of infection. In an effort to establish in vitro models to investigate physiologic factors that may influence virus expression in cells harboring latent HIV or expressing low levels of virus, two chronically infected cell lines of T-lymphocytic (ACH-2) and promonocytic (U1) lineage were characterized in detail (2, 5). Both cell lines were obtained by limiting-dilution cloning of the population of cells surviving an acute in vitro infection with HIV type 1 (LAV strain), possess a low copy number of integrated proviruses, and do not constitutively produce significant amounts of virus (2, 5). However, a dramatic increase of HIV expression can be induced by stimulation with the phorbol ester phorbol-12-myristate-13 acetate (PMA) or by cytokines such as tumor necrosis factor (2, 4, 5, 22, 23). It was previously reported that alpha interferon (IFN-α), but not zidovudine, suppressed HIV production in PMA-stimulated ACH-2 and U1 cells (23). The IFN-α effect was correlated not with a reduced synthesis of HIV proteins but with the inhibition of HIV particle release affecting a "postbudding" step of viral maturation, as previously described for cells chronically infected with murine retroviruses (7, 21). Furthermore, it has been shown that IFN-α effectively suppressed acute HIV infection of both T-lymphocytic (9, 28) and monocytic (1, 18) cells. This effect on de novo-infected cells, in contrast to persistently infected cells, appears to be associated with reduced synthesis of viral proteins.

In the present study, we further investigated the differential effects of IFN-α on the production of virions, soluble HIV antigens (Ag), and reverse transcriptase (RT) activity in unstimulated or PMA-stimulated ACH-2 and U1 cells. The ability to address this question was made possible by the absence of HIV-mediated cytopathicity associated with HIV expression in these chronically infected cell lines (2, 5), as opposed to acutely infected cells in which release of viral Ag may be a consequence of cell lysis.

IFN-α suppressed the production of RT activity in both PMA-stimulated U1 and PMA-stimulated ACH-2 cells, and this effect was correlated in a concentration-dependent manner with the reduction of p24 Ag production in U1 promonocytic cells (Fig. 1A). In contrast, in the T-lymphocytic ACH-2 cell line, IFN-α suppression of production of RT activity was not associated with decreased levels of p24 Ag (Fig. 1B). In this regard, the RT activity detected in culture supernatants of cells infected with both HIV (24) and animal retroviruses (3) is associated almost exclusively with the virus particle, whereas capsid (*gag*) and envelope (*env*) proteins may be shed as free (soluble) Ag (10, 11, 15). We therefore investigated the possibility that the dissociation of the effect of IFN-α on RT activity versus p24 Ag production in ACH-2 cells was explained by the shedding of capsid proteins as determined by Sepharose CL-4B chromatography column fractionation analysis of culture supernatants. Sepharose chromatography is an effective method of virus purification which has been previously shown to preserve high levels of external envelope protein of murine retroviruses (17) and was also successfully used to purify from IFN-treated mouse cells murine leukemia virus particles, which are more fragile than untreated murine leukemia virus virions (20).

In PMA-stimulated ACH-2 cells, HIV type 1 p24 Ag was found both in the void volume fractions (3 and 4), which include proteins or aggregates of more than 10^7 kDa (particulate Ag), and in the total volume (fractions 8 to 11), which includes proteins with molecular masses of less than 10^5 kDa (soluble Ag) (Fig. 2A and B). RT activity was associated almost exclusively with particulate p24. The general concurrence of the p24 and RT peaks, with no significant production of soluble RT after IFN-α treatment (Fig. 2B), indicates that IFN-α did not cause rapid degradation of the released

* Corresponding author.

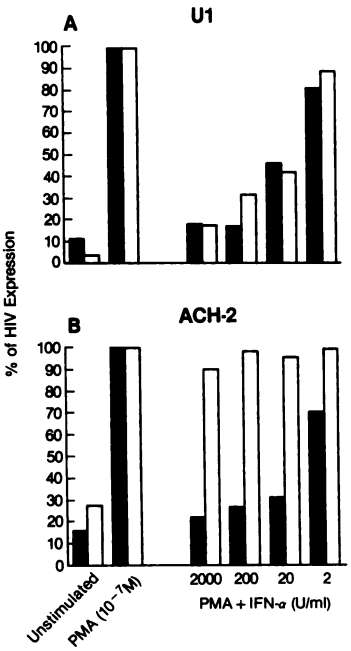

FIG. 1. Effect of IFN-α on the release of RT (closed bars) and HIV-1 p24 Ag (open bars) from PMA-stimulated U1 and ACH-2 cells. ACH-2 and U1 cells were incubated with recombinant IFN-α_2 (Roferon) in the presence (coincubation) or absence of PMA (10^{-7} M) (Sigma Chemical Co., St. Louis, Mo.). Cell culture supernatants were harvested 48 to 72 h after stimulation and tested for the presence of HIV RT activity or p24 Ag, as previously described (23). For RT activity determinations, 5 μl of ACH-2 or U1 supernatants was added in duplicate to 25 μl of a mixture containing poly(A), oligo(dT) (Pharmacia, Piscataway, N.J.), $MgCl_2$, and ^{32}P-labeled dTTP (Amersham, Arlington Heights, Ill.) and incubated for 2 h at 37°C. Then, 6 μl of the mixture was spotted onto DE81 paper (Whatman International, Maidstone, United Kingdom), which was then air dried and washed five times in 2× standard saline citrate buffer and two additional times in 95% ethanol. The paper was then dried and cut, and a count was determined with a Beckman LS 7000 scintillation counter. Variability of replicated cultures was always less than 15%. HIV Ag production in the same supernatants was measured by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit utilizing an anti-p24^{gag} monoclonal antibody (du Pont). Similar results were obtained with a p24 ELISA kit from a different source (Coulter Immunology, Hialeah, Fla.). No significant differences were observed in terms of antiviral effects in cells preincubated up to 24 h or incubated with IFN-α shortly after PMA stimulation, as previously described (23). Each value was normalized against its respective control value obtained for PMA-stimulated cells in the absence of IFN-α. The results are representative of four independent experiments.

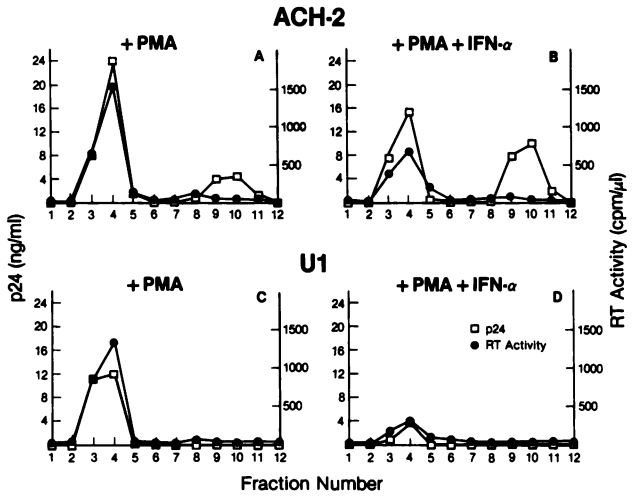

FIG. 2. Elution profile of RT activity and p24 Ag released from ACH-2 and U1 cells stimulated with PMA (10^{-7} M). Fresh, cell-free clarified supernatant fluid (0.5 ml) from U1 or ACH-2 cells was applied to a 5-ml Sepharose CL-4B (Pharmacia) column equilibrated in RPMI 1640 medium containing 10% fetal calf serum and eluted in the same buffer. Fractions (500 μl) were collected and assayed for RT activity and p24 Ag. The void volume and total volumes were determined by using dextran blue (Pharmacia) and phenol red, respectively. RT activity (corrected for background) and p24 Ag (du Pont) levels were measured for each fraction. The exclusion volume (bracket, fractions 3 and 4) was determined by using dextran blue. The total volume (bracket, fractions 9 and 10) was determined by using phenol red. ACH-2 and U1 cells were stimulated with PMA alone (A and C, respectively) or in the presence of IFN-α (200 U/ml) (B and D, respectively).

virions, which could have explained our earlier results (23), as shown in Fig. 1. Culture supernatant from PMA-stimulated U1 cells contained predominantly particulate Ag, and approximately 95% of the released RT activity was also particulate (Fig. 2C). When U1 cells were stimulated with PMA in the presence of IFN-α, a concomitant decrease of particulate Ag and RT activity was observed, as shown in Fig. 2D, which accounted for the overall effect previously described for this cell system (23). Likewise, PMA-stimulated ACH-2 cells produced a form of particulate p24 Ag which was associated with most of the RT activity. However, in contrast to the results with U1 cells, a significant amount of p24 was also detected as soluble Ag (Fig. 2A). Of interest, IFN-α treatment of ACH-2 cells caused a decrease in particulate Ag and RT activity but did not significantly affect production of soluble p24 Ag (Fig. 2B). Therefore, the discrepant results observed by direct analysis of the levels of RT activity and p24 Ag in culture supernatants of ACH-2 cells stimulated with PMA and IFN-α (Fig. 1B) can be explained by the lack of effect of IFN-α on the shedding of *gag* proteins.

In contrast to the reduced production of particulate p24 Ag, the level of soluble p24 Ag actually increased in PMA-stimulated ACH-2 cells treated with IFN-α (Fig. 2A and B). This effect was seen in separate experiments and confirmed by using two different Ag capture assays (E. I. du Pont, NEN Research Products, Boston, Mass., and Abbott Laboratories, North Chicago, Ill.). The cause of the enhancement of p24 production in IFN-α-treated cells is currently unknown. However, this effect was probably not related to disruption of virions (particulate Ag), since no concomitant increase of soluble-RT activity was observed (data not shown).

In order to verify whether these effects of IFN-α were correlated with an atypical response of ACH-2 cells to PMA, we investigated the effect of IFN-α on the constitutive production of p24 Ag. Unstimulated ACH-2 cells released both particulate and soluble p24 Ag into the culture supernatant, and PMA stimulation of ACH-2 cells increased the production of particulate and soluble p24 by 20- and 5-fold, respectively. IFN-α treatment suppressed production of particulate Ag in both unstimulated and PMA-stimulated ACH-2 cells; however, it did not inhibit the release of soluble p24 Ag under either set of conditions (data not

shown). Therefore, the difference in the effect of IFN-α on particulate versus soluble p24 Ag did not depend on PMA treatment, since the effect occurred also in unstimulated ACH-2 cells.

The present study demonstrates that both unstimulated and PMA-stimulated chronically infected ACH-2 cells, but not U1 cells, produce a significant amount of p24 Ag not associated with HIV virions. This phenomenon is not correlated with lysis of ACH-2 cells and is not accounted for by virion degradation, whereas it can be explained by shedding of viral Ag, which has been previously described for HIV p24 (15) and *env* (10, 11) proteins. Of interest, IFN-α was very effective in blocking the release of viral particles but did not interfere with the shedding of $p24^{gag}$ Ag from ACH-2 cells. We have recently observed that ACH-2 cells, but not U1 cells, also shed *env* Ag, as previously observed for other cell systems (10, 11), and IFN-α did not affect this process (data not shown). This differential effect of IFN-α on the release of particulate versus soluble p24 Ag from ACH-2 cells did not depend on the cellular response to PMA, since similar results were obtained under conditions of no PMA stimulation. Furthermore, we observed shedding of soluble *gag* Ag from two additional chronically infected T-cell lines (MOLT-4 and H9), whereas supernatants from HIV-infected primary human monocyte-derived macrophages, similar to U1 cells, did not shed significant levels of soluble Ag (data not shown).

Since the antiviral effects of IFNs reflect the sum total of complex viral and cellular effects (27), it is not unusual to observe variable efficacy of IFNs in different cell types. The increased production of soluble p24 Ag in ACH-2 cells after IFN-α treatment is unexplained. Because the existence of several species of soluble and particulate p24 has been reported (15), it is possible that different biosynthetic pathways of *gag* proteins are differentially affected by IFN-α. However, no gross alterations either in the amounts or in the relative ratios of HIV protein expression in the presence of IFN-α treatment were previously observed (23). Alternatively, it has been suggested that the ability of IFN-α to affect the release of HIV particles is a consequence of alterations of plasma membrane fluidity (6, 19). Shedding of free viral proteins may not be affected by such changes in membrane microviscosity, whereas the release of complex nucleic acid-protein-lipid aggregates in the form of virions may be more sensitive. Finally, IFN-α may affect the ability of HIV particles to be efficiently released from the cell surface by posttranslational alterations of certain HIV proteins. In this regard, it has been recently shown that the absence of a small *gag* end product (p6) in a mutagenized strain of HIV results in a pattern of budding strikingly similar to that of IFN-α-treated cells in that virus particles abnormally bud from the cell surface without being efficiently released into the culture supernatant (8).

Since circulating levels of p24 Ag can be detected in both early and late stages of AIDS (13, 14), the observations presented above may be of relevance in the evaluation of the state of HIV expression in infected individuals, particularly those undergoing therapy with IFN-α (13).

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