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Hanson, Brian D.; Nara, Peter L.; Maheshwari, Radha K.; S. Sidhu, Girmel; Bernbaum, John G.; Hoekzema, David; Meltzer, Monte S.; and Gendelman, Howard, "Loss of Infectivity by Progeny Virus from Alpha Interferon- Treated Human Immunodeficiency Virus Type 1-Infected T Cells Is Associated with Defective Assembly of Envelope gp120" (1992). *Virology Papers*. 92.
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Journal of Virology, Dec. 1992, p. 7543–7548
0022-538X/92/127543-06$02.00/0
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Vol. 66, No. 12

Loss of Infectivity by Progeny Virus from Alpha Interferon-Treated Human Immunodeficiency Virus Type 1-Infected T Cells Is Associated with Defective Assembly of Envelope gp120

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Received 2 June 1992/Accepted 7 August 1992

Levels of human immunodeficiency virus (HIV) DNA, RNA, or p24 antigen and reverse transcriptase activity in T-cell cultures treated with 500 IU of recombinant alpha interferon (rIFNα) per ml were comparable to those in control cultures. Radioimmunoprecipitation analysis of proteins in lysates of IFN-treated T cells documented a marked accumulation of HIV proteins. Localization of gp120 by immunofluorescence showed a diffuse pattern in IFN-treated cells quite distinct from the ring pattern in untreated control cells. That large quantities of gp120 in aberrant cell compartments might affect HIV morphogenesis was confirmed in infectivity studies: virions from IFN-treated cells were 100- to 1,000-fold less infectious than an equal number of virions from control cells. Direct examination of IFN-treated and control HIV-infected cells by transmission electron microscopy showed little difference in the number or distribution of viral particles. However, quantitation of gp120 by immunogold particle analysis revealed a marked depletion of envelope glycoprotein in virions released from IFN-treated cells. This defect in gp120 assembly onto mature viral particles provides a molecular basis for this loss of infectivity.

Clinical and experimental observations support a central role for alpha interferon (IFN-α) in the regulation of human immunodeficiency virus (HIV) replication (2, 5–7, 9–11, 14–16, 23, 25, 26, 28, 31, 32, 34, 35). High-titer viremia with HIV present in both plasma and cells is evident during acute infection. Within weeks of the onset of symptoms, HIV viremia falls to low levels. IFN levels in plasma directly correlate with viral load during this acute viral syndrome (32, 35). Clinical trials of exogenous IFN-α in early HIV disease show a significant reduction in the level of p24 antigen (Ag) in the plasma of treated subjects and less AIDS-associated opportunistic infection (15). Such preliminary evidence for antiviral efficacy in patients is matched by other reports that document the potent antiviral activity of IFN in HIV-infected T-cell and monocyte cultures (7, 9, 10, 14, 23, 25, 30). HIV infection of monocytes is completely inhibited by IFN-α administered prior to or at the time of viral challenge (9, 14). HIV-infected monocytes treated with IFN-α after infection show little or no viral gene expression. However, and in contrast to the antiviral effects seen in monocytes, the effects of IFN-α on HIV replication in T cells are less dramatic (7, 9, 25, 30, 33, 34). A number of studies show no changes in viral gene expression following IFN-treatment of HIV-infected T cells. Although most investigators agree that the principal effect of IFN in T cells is on the terminal stage of the HIV life cycle, there is little accord as to the exact nature of this effect (7, 9, 23, 25, 30, 33). We describe in this report an IFN-associated defect in the assembly of gp120 onto mature viral particles that accounts for significant loss of virion infectivity. This assembly defect provides a novel molecular basis for IFN-associated antiviral effects on HIV replication in T cells.

To measure the effect of IFN-α on levels of HIV DNA and RNA in infected T cells, peripheral blood mononuclear cells isolated from whole blood by Ficoll-diatrizoate density gradient centrifugation were cultured in RPMI 1640 (GIBCO, Grand Island, N.Y.) with 1 μg of phytohemagglutinin (PHA; Sigma Chemical Co., St. Louis, Mo.) per ml, 10% partially purified human interleukin-2 (IL-2; Advanced Biotechnologies Inc., Columbia, Md.), and 15% heat-inactivated fetal calf serum (Sterile Systems, Inc., Logan, Utah) for 3 days. PHA/IL-2-treated T cells were exposed to HIV-$1_{HTLV-IIIB}$ (Advanced Biotechnologies) at a multiplicity of infection of 0.01 infectious virus per target cell with and without 500 IU of rIFN-α_{2b} per ml (a generous gift from Schering-Plough Research Laboratories, Inc., Kenilworth, N.J.). All virus stock and reagents were free of mycoplasma (Gen-probe II; Gen-probe Inc., San Diego, Calif.) and bacterial endotoxin contamination. Culture medium was half exchanged every 2 to 3 days for 2 weeks. Cell lysates of HIV-infected T cells were extracted with phenol and chloroform-isoamyl alcohol, and the DNA was precipitated with ethanol. Polymerase chain reaction amplification of HIV-specific DNA sequences with nucleotide primers from the 5′ long terminal repeat and *gag* genes and 2.5 U of *Taq* polymerase (Cetus Corp., Emeryville, Calif.) per ml was performed on 1 μg of total

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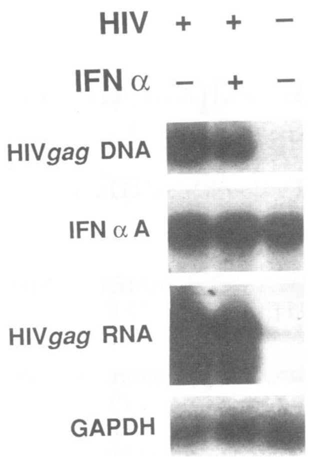

FIG. 1. Effect of IFN-α on levels of HIV DNA and RNA in 14-day HIV-$1_{HTLV-IIIB}$-infected T cells. HIV *gag* DNA was amplified by the polymerase chain reaction. Levels of DNA for the IFN-α_A gene in cell lysates served as a reference for comparison between samples. HIV *gag* RNA was subjected to reverse transcription and polymerase chain reaction amplification of the cDNA transcripts. mRNA for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference to allow analysis of RNA between different samples.

cellular DNA with an automatic cycler (Perkin Elmer-Cetus, Emeryville, Calif.). The products of 25 cycles were analyzed by Southern blot hybridization after agarose gel electrophoresis with a radiolabeled DNA probe specific for a *gag* sequence internal to the primer pairs. Levels of DNA for the IFN-α_A gene in cell lysates served as a reference for comparisons between samples (9). Total RNA was extracted from cell lysates with acidic guanidinium isothiocyanate-phenol-chloroform. Levels of viral and cellular RNA were estimated after reverse transcription with antisense primers and polymerase chain reaction amplification of the cDNA transcripts. The mRNA for the cellular enzyme, glyceraldehyde 3-phosphate dehydrogenase served as internal reference to allow analysis of RNA between different samples.

Initial studies showed that HIV-1 replication in T cells was only minimally affected by exposure to recombinant IFN-α (rIFN-α). We and others show that even concentrations of rIFN-α as high as 10,000 IU/ml will not prevent infection of primary T cells or T-cell lines by HIV (9, 30, 33). Furthermore, levels of HIV DNA and RNA in 14-day HIV-1-infected T cells treated with 500 IU of rIFN-α per ml at the time of infection and throughout 2 weeks of culture were no different from those in untreated control cells (Fig. 1). Similarly, levels of p24 Ag and reverse transcriptase (RT) activity in the same cultures are comparable (<twofold difference). Levels of p24 Ag and RT activity in T-cell cultures treated with 500 IU of rIFNα per ml were 89% ± 2% and 74% ± 6% (mean ± standard error of the mean [SEM] for three different experiments), respectively, of those in control cultures 12 to 15 days after HIV infection.

More detailed analysis of p24 Ag and RT activity levels in IFN-treated cells revealed a marked difference in the localization of these viral proteins (Table 1). Less than 10% of p24 Ag in cultures of HIV-infected T cells was found in cell lysates 2 weeks after infection (7% ± 3% [mean ± SEM for three experiments]). In contrast, levels of p24 Ag in lysates of HIV-infected T cells treated continuously with rIFN-α for 2 weeks were 24% of the total p24 Ag level in these cultures (23% ± 1% [mean ± SEM for three experiments]). Indeed, although there was no difference in total p24 Ag levels between IFN-treated and control cells, the level of cell-

TABLE 1. Effect of IFN-α on levels of HIV p24 Ag and RT activity in culture fluids and lysates of infected T cells[a]

Culture fraction	Level (%) of p24 Ag (ng/ml) in:		RT activity (%) (10^5 cpm/ml) in:	
	Control cells	Cells with IFN-α	Control cells	Cells with IFN-α
Culture fluids	78 (90%)	67 (76%)	34.4 (96%)	27.3 (84%)
Cell lysate	9 (10%)	21 (24%)	1.3 (4%)	5.2 (16%)
Total	87	88	35.7	32.5

[a] PHA/IL-2-treated T cells infected with HIV-$1_{HTLV-IIIB}$ were cultured with and without 500 IU of rIFN-α per ml for 2 weeks. Levels of p24 Ag in culture fluids and freeze-thaw cell lysates were determined in triplicate by enzyme-linked immunosorbent assay (E. I. Du Pont de Nemours & Co., Billerica, Mass.). RT activity was estimated by incorporation of [^{3}H]dTTP into DNA transcripts of polyr(A) (9). Radioactivity was estimated by liquid scintillation spectroscopy. HIV-1 stock served as positive control for p24 Ag and RT activity.

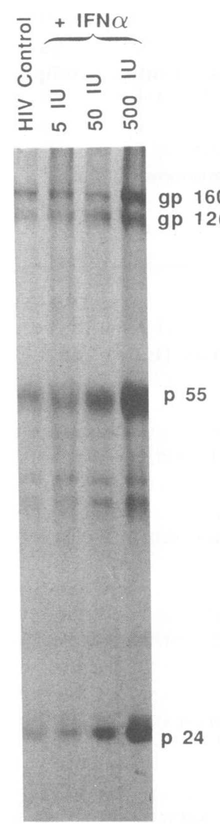

FIG. 2. Effect of IFN-α on levels of HIV proteins in infected T cells. PHA/IL-2-treated T cells infected with HIV-$1_{HTLV-IIIB}$ were cultured with and without 0 to 500 IU of rIFN-α per ml for 2 weeks. All cultures were exposed to 250 μCi of [^{35}S]methionine for 3 h and then washed. Radiolabeled HIV-specific proteins were isolated from cell lysates with pooled HIV-seropositive sera bound to protein A-Sepharose CL-4B (Pharmacia). The immune complexes were washed and boiled; immunoprecipitated proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography (1, 32).

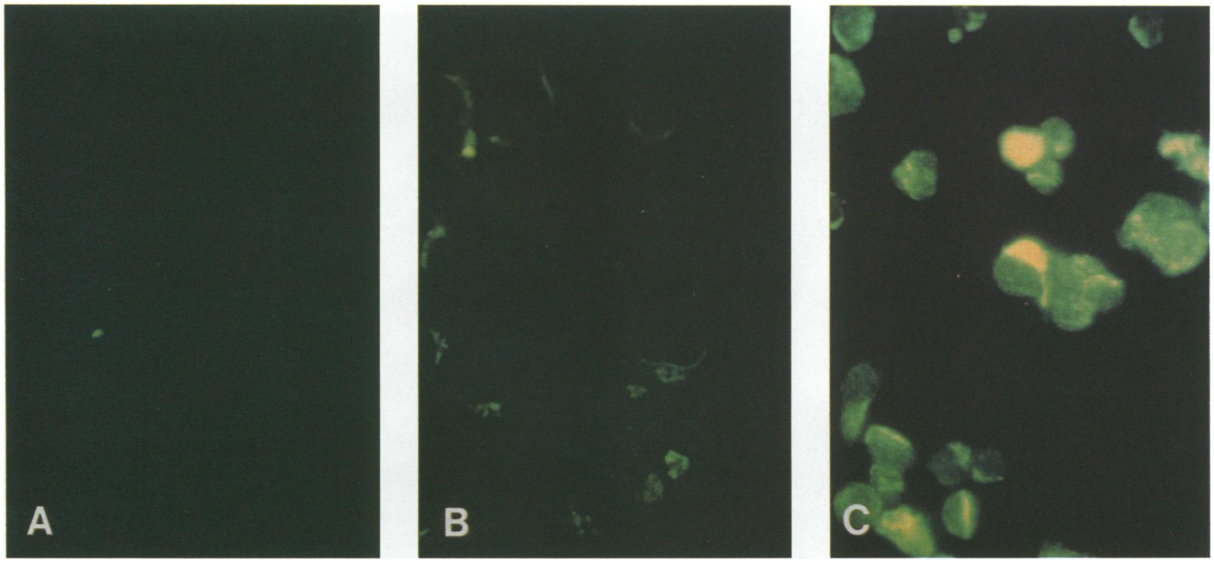

FIG. 3. Effect of IFN-α on the distribution of gp120 in HIV-infected T cells. PHA/IL-2-treated T cells infected with HIV-$1_{HTLV\text{-}IIIB}$ were cultured with and without 500 IU of rIFN-α per ml for 2 weeks. Cells were washed, fixed in acetone, and exposed to goat anti-HIV-1_{SF2} gp120 (12) (provided by N. Haigwood, Chiron Corp.). Treated cells were washed after a reaction time of 1 h on ice and then resuspended for another 1 h in fluorescein isothiocyanate-conjugated rabbit anti-goat immunoglobulin G (Sigma). Washed cells were examined by epi-illumination fluorescence microscopy (18). (A) Uninfected control cells. (B and C) HIV-infected T cells cultured with (panel C) and without (panel B) IFN-α. Magnification, ×250.

associated p24 Ag in IFN-treated cultures was threefold higher than that in control cultures. Localization of RT activity in cultures of IFN-treated cells followed a similar pattern. Again, only 3% of RT activity in cultures of HIV-

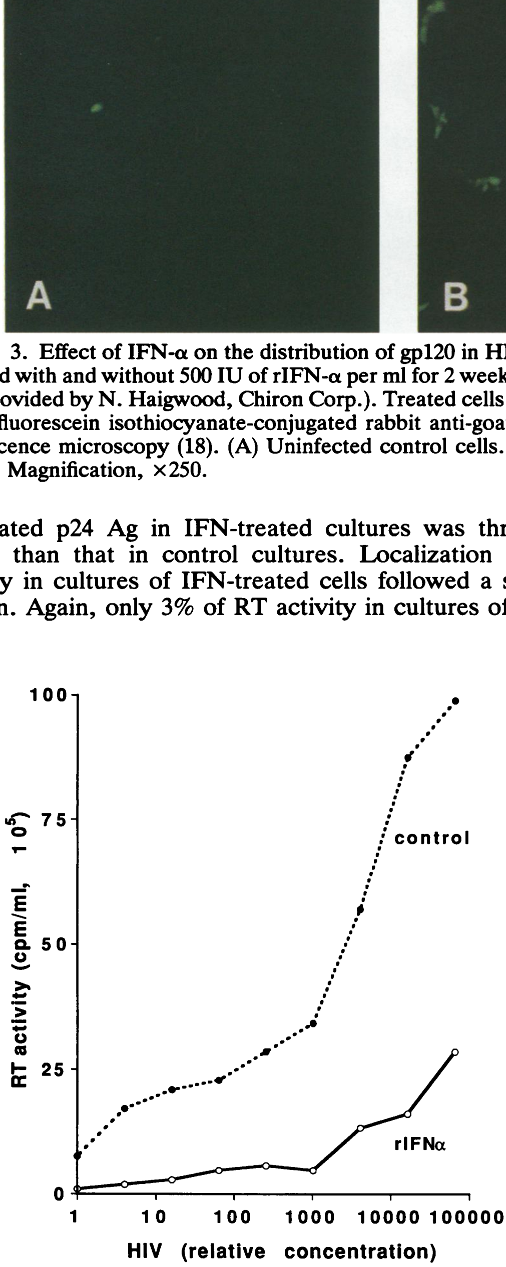

FIG. 4. Effect of IFN on infectivity of progeny virus from HIV-infected primary T cells. PHA/IL-2-treated T cells infected with HIV-$1_{HTLV\text{-}IIIB}$ at a multiplicity of infection of 1 were cultured with and without 500 IU of rIFN-α per ml. Seven days after infection, virus in fluids of control and IFN-treated cultures was pelleted by ultracentrifugation and adjusted to equal levels of RT activity. Fourfold viral dilutions were added to uninfected PHA/IL-2-treated T cells. Levels of RT activity in culture fluids (mean of triplicate determinations) are shown 10 days after infection. The SEM for the triplicate determinations for each point was less than 10% of the mean value.

infected T cells was found in cell lysates 2 weeks after infection (3% ± 1% [mean ± SEM for three experiments]). Most RT activity was released into the culture fluid. Virtually all of this RT activity was virion associated and was recovered in the pellet after ultracentrifugation. Levels of RT activity in lysates of T cells treated continuously with rIFN-α for 2 weeks were 15% of the total RT activity in these cultures (15% ± 1% [mean ± SEM for three experiments]). Cell-associated RT activity in IFN-treated cultures was four- to fivefold higher than that in control cultures.

The preceding data documented an accumulation of viral proteins and virions within IFN-treated T cells. This accumulation was confirmed by radioimmunoprecipitation analysis of viral proteins in cell lysates 2 weeks after infection (Fig. 2). The total amount of HIV protein in lysates of IFN-treated cells increased directly with the IFN concentration. It is important to note that processing of HIV proteins (relative levels of gp160 and gp120 or p55 and p24) in IFN-treated and control cells at this level of analysis was comparable (Fig. 2).

Further evidence that IFN treatment of infected T cells induced accumulation of viral proteins within the cell is strikingly illustrated in immunofluorescence studies for gp120 (Fig. 3). HIV-infected T cells showed a characteristic ring pattern for gp120; virtually all of this processed HIV envelope glycoprotein was at or on the plasma membrane. In contrast, the identical cells treated continuously with IFN for 2 weeks showed a bright and diffuse pattern for gp120. Previous reports show that about 10% of gp160 in T cells is cleaved to produce gp120. Most gp160 (85 to 95%) is transported to and degraded in lysosomes (32). In the absence of IFN treatment, gp120 is transported to the plasma membrane without degradation and assembled into mature virions. IFN treatment markedly affects this transport process by mechanisms presently unknown, so that gp120 or unprocessed gp160 becomes diffusely localized throughout the cell. Interestingly, radioimmunoprecipitation

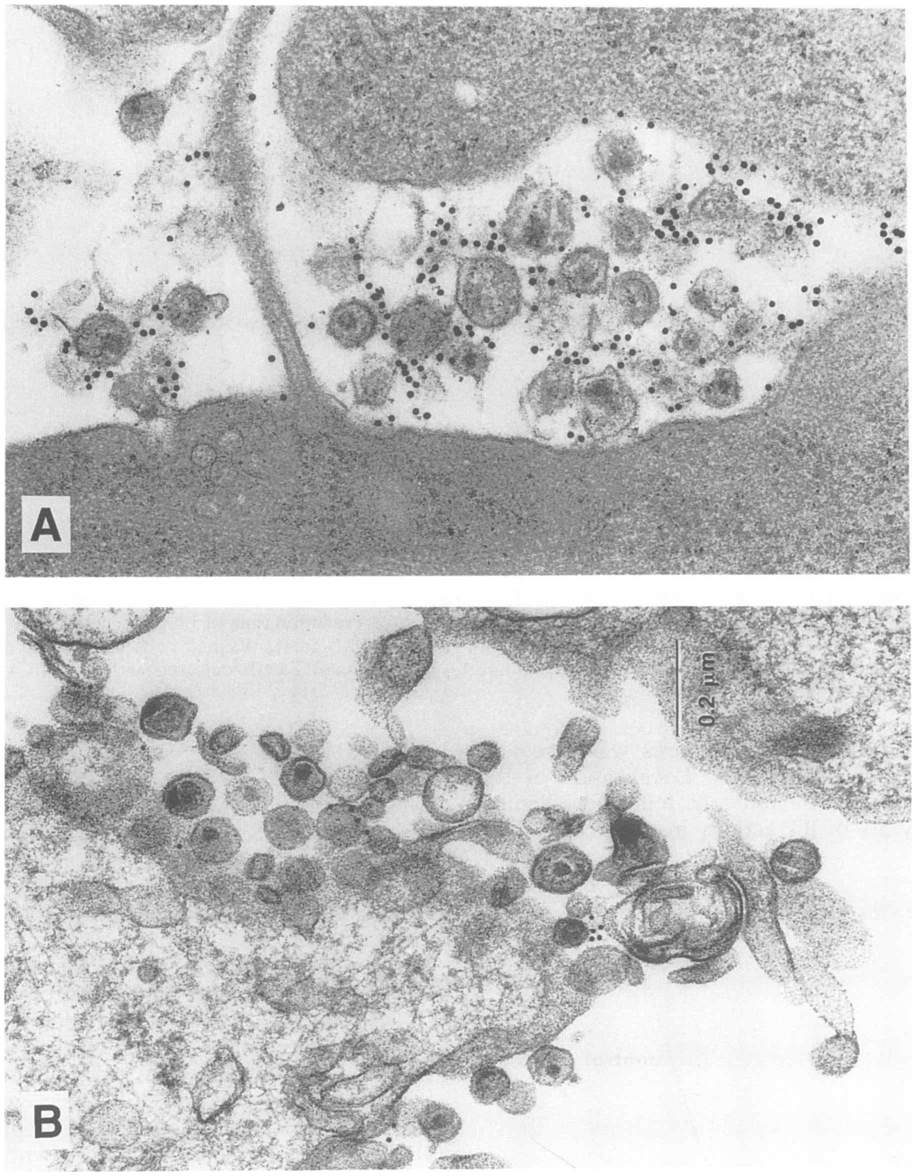

FIG. 5. Effect of IFN-α on envelope gp120 of virions from HIV-infected H9 cells. H9 cells (provided by R. C. Gallo, National Institutes of Health) infected with HIV-$1_{HTLV-IIIB}$ were cultured with and without 500 IU of rIFN-α per ml for 2 weeks and then exposed to goat anti-HIV-1_{SF2} gp120 (12) for 2 h at room temperature. Antibody-treated cells were washed and exposed to rabbit anti-goat immunoglobulin G-gold conjugate (10-nm gold particles; Amersham International, Amersham, United Kingdom) for 2 h at room temperature. Unbound gold particles were removed after washing. Cells were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, en block stained, dehydrated in ethanol, and embedded in SPURR's plastic resin. After polymerization, blocks were cut, and 50- to 70-nm sections were placed on copper grids and poststained with lead citrate (22). Grids were examined in a JEOL 100B electron microscope. (A) Virions from control HIV-infected H9 cells. (B) Virions from IFN-treated cells. Magnification, ×73,000.

analysis showed that the relative amounts of gp160 and gp120 were not changed by IFN treatment. This suggests that gp120 is not degraded in its altered cellular compartment.

Accumulation of gp120 in alternative cell compartments during IFN treatment suggested that this envelope component may not be freely available for virus assembly. We examined this hypothesis by performing a direct analysis of virions released from IFN-treated T cells. Progeny virions in culture fluids of IFN-treated and control cells 2 weeks after virus infection were adjusted to equal levels of RT activity and assayed for infectivity on PHA/IL-2-treated T cells (Fig. 4). Virions from IFN-treated cells were at least 1,000-fold less infectious than an equal number of virions from control cells. Such loss of infectivity was confirmed in three replicate experiments and in a syncytial plaque assay with CEM-SS cells (20). Again, virions from IFN-treated cells were 100- to 1,000-fold less infectious than an equal number of virions from control cells.

The most direct evidence that virions from IFN-treated T cells were deficient in envelope gp120 came from transmission electron-microscopic studies with immunogold-labeled

anti-gp120 (Fig. 5). HIV virions were numerous at the plasma membrane 12 days after virus infection. The immunogold label delineated obvious virion-associated gp120 on most viral particles. In a survey of 225 individual virions, the average number of gold particles per virion was 5.5 ± 0.4 (mean ± SEM). In contrast, virions from the identical cells treated continuously with IFN showed a marked reduction in the amount of gp120. There was no appreciable change in the number or distribution of virions at the plasma membrane of IFN-treated cells, but the number of gold particles per virion was 0.5 ± 0.1 (mean ± SEM for 122 virions), a reduction of 91%. A replicate experiment showed 10.5 gold particles per virion with control T cells 11 days after HIV infection versus 0.9 gold particles per virion (a reduction of 91%) from the same cells treated with 500 IU of rIFN-α per ml. Experiments with H9 cells, a continuous T-cell line, infected with HIV-$1_{HTLV-IIIB}$ and cultured with and without rIFN-α for 10 days showed changes in virion-associated gp120 of similar magnitude. The number of gold particles per virion in cultures of H9 cells was 1.4 ± 0.2 (mean ± SEM for 140 virions). Virions from IFN-treated H9 cells had only 0.2 ± 0.1 gold particle per virion (mean ± SEM for 82 virions), a reduction of 86%. Another experiment with H9 cells showed 4.5 gold particles per virion in control cells 8 days after HIV infection versus 0.7 gold particle per virion (a reduction of 85%) in cells treated with 500 IU of rIFN-α per ml. These studies document a profound and selective depletion of HIV envelope gp120 on the virions released from IFN-treated infected T cells and provide a molecular basis for the loss of infectivity.

A major antiviral effect of IFN for inhibition of HIV replication in T cells operates in the stages of virus assembly and release (30, 33). There is general agreement that the rate and extent of viral protein synthesis change little during IFN treatment of chemically HIV-infected T cells (7, 9, 30, 33). Other antiviral effects of rIFN-α were demonstrated during acute infection of T cells with HIV-1 (12a, 28). However, the numbers of progeny virus released from infected cells are reproducibly decreased for both primary T cells from blood and continuous T-cell lines (7, 9, 28, 30, 33). Certain investigators find decreased numbers of virions budding into extracellular spaces (30); others report a marked accumulation of viral particles at the cell surface (33). We confirm this effect of IFN on HIV replication in T cells. Synthesis of HIV DNA, RNA, and protein was minimally affected, even by high concentrations of IFN administered continuously throughout the culture interval. Indeed, the morphology and number of virions at the plasma membrane in IFN-treated and control HIV-infected cells were indistinguishable by transmission electron microscopy. However, these analyses underestimate the potent effects of IFN on HIV morphogenesis. Virions released from IFN-treated T cells were 100- to 1,000-fold less infectious than an equal number of virions from control cells. The basis for this dramatic change resides in an assembly defect for gp120 onto the mature viral particle.

The effects of IFN on HIV morphogenesis in T cells has precedent in other viral systems (8, 17–19, 24). Progeny vesicular stomatitis virus (a rhabdovirus) and murine leukemia virus (a retrovirus) from infected cells treated with IFN-α are much less infectious than are virions from control cells (3, 13, 17–19, 21, 24, 27, 29). Analysis of progeny virus from IFN-treated vesicular stomatitis virus-infected cells by transmission electron microscopy documents a marked decrease in glycoprotein envelope spikes (17–19). Transport of the vesicular stomatitis virus envelope glycoprotein to the plasma membrane is inhibited by IFN-α. Immunofluorescence studies show accumulation of envelope glycoprotein in Golgi complexes: endoglycosidase digestion experiments suggest an IFN-α-associated block in envelope glycoprotein transport through the *trans* Golgi (4, 29). Similarly, changes in the infectivity of murine leukemia viruses are directly related to IFN-α-induced alterations of viral envelope glycoprotein processing and assembly (3). These fundamental observations provide a sound basis for exploration of IFN-associated defects in gp120 assembly in HIV-infected T cells. Studies directed at mechanisms of gp160 cleavage, transport of gp120 through the Golgi complex, and assembly of gp120 onto the core virion at or near the plasma membrane are directed at key areas of investigation. Careful definition of the site and mechanism of IFN action should allow better design of alternate therapeutic agents that act at this vulnerable stage of the HIV life cycle.

We thank Robert M. Friedman, Uniformed Services University of the Health Sciences, and James E. Whitman, Jr., for helpful discussions and Victoria Hunter for excellent graphics.

H. E. Gendelman is a Carter-Wallace Fellow of the Johns Hopkins University School of Public Health and Hygiene in the Department of Immunology and Infectious Diseases. This work was supported in part by the Henry M. Jackson Foundation for the Advancement of Military Medicine.

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