Since the discovery of the human immunodeficiency virus in 1983 much has been uncovered concerning its biology and mechanisms of pathogenesis. HIV is a lentivirus and like other viruses in this class are linked by similarities in genetic structure, mechanisms of replication, persistence, and biology of host-cell interactions. Lentivirus infection in susceptible hosts induce slow disease and replicate at accelerated rates during disease progression. Furthermore, all lentiviruses can productively infect cells of macrophage lineage. This is typified by studies of virus replication in the central nervous system during disease. In the (AIDS) dementia complex characteristic mononucleated and multinucleated macrophages contain abundant virus particles within cytoplasmic organelles and being released from the cell surface. Macrophage-lineage cells producing HIV gene products of progeny virions has also been identified in spinal cords, lungs and lymphatics of infected individuals. Furthermore, macrophages are major interferon alpha producing cells in man. If IFN is to be considered as a therapeutic agent in HIV-infected patients, then the interactions of the virus, the macrophage and IFN must be thoroughly evaluated.
Interactions Between Interferon and the Human Immunodeficiency Virus

HOWARD E. GENDELMAN,1,2 LISA M. BACA,1 JIM A. TURPIN,1 D. CHESTER KALTER,1,2 CARL DIEFFENBACH,3 ROBERT M. FRIEDMAN,3 and MONTE S. MELTZER1

1HIV Immunopathogenesis Program, Department of Cellular Immunology, Walter Reed Army Institute of Research, Washington DC 20307
2Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockville, MD 20850
3Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Since the discovery of the human immunodeficiency virus in 1983 (1) much has been uncovered concerning its biology and mechanisms of pathogenesis. HIV is a lentivirus and like other viruses in this class are linked by similarities in genetic structure, mechanisms of replication, persistence, and biology of host-cell interactions (2). Lentivirus infection in susceptible hosts induce slow disease and replicate at accelerated rates during disease progression. Furthermore, all lentiviruses can productively infect cells of macrophage lineage (3,4). This is typified by studies of virus replication in the central nervous system during disease. In the brains of patients with the acquired immune deficiency syndrome (AIDS) dementia complex characteristic mononucleated and multinucleated macrophages contain abundant virus particles within cytoplasmic organelles and being released from the cell surface (Figure 1). Macrophage-lineage cells producing HIV gene products or progeny virions has also been identified in spinal cords, lung and lymphatics of infected individuals (3,4). Furthermore, macrophages are major interferon alpha (IFNα) producing cells in man. If IFN is to
Figure 1. Ultrastructural (electron microscopic) features of HIV infection in the central nervous system. Numerous progeny HIV virions are seen associated with a CNS macrophage of a patient who died of the acquired immune deficiency syndrome (AIDS) dementia complex. Myelin debris are found in the center of this illustration.

If HIV were considered as a therapeutic agent in HIV-infected patients, then the interactions of the virus, the macrophage and IFN must be thoroughly evaluated.

To study the interactions between the HIV and the macrophage we devised tissue culture systems to permit long term culture and propagation of these cells. The use of macrophage colony stimulating factor (MCSF) allowed the successful long term culture and permitted the use of monocyte-derived macrophages for use as recipient cell monolayers for the isolation and propagation of HIV (5,6). When peripheral blood mononuclear cells from HIV-infected individuals
Figure 2. Cytopathic effects in HIV-infected monocytes. PBMC purified to > 98% monocytes and cultured 7 days as adherent monolayers in medium with human serum and MCSF were exposed to the HIV isolate, ADA, at a multiplicity of infection of 0.1 infectious virus/cell. Seven days after infection, 500 IU/ml recombinant IFNα was added and maintained at this concentration throughout the culture interval. Wright-stained monolayers represent: uninfected monocytes at 7 days (left panel) and HIV-infected monocytes at 7 days with typical HIV-induced multinucleated giant cells (right panel).

were added to cultures of MCSF-stimulated monocytes progeny virus recovery was successful in 93% of attempts regardless of the stage of disease (7). This served as both a reproducible and sensitive isolation technique and useful in studies of the biologic properties of this particular class of virus. Furthermore, HIV that demonstrate preferential tropisms for cells of macrophage and/or lymphocyte lineage can be recovered simultaneously from infected individuals.

HIV infection of monocytes at a multiplicity of infection (MOI=0.1) with the ADA strain results in cell fusion (Figure 2) coincident with the peak of virus production. Multinucleated giant cell formation and is often followed by cell degeneration 4-7 days later. The viral life cycle is different in infected cultures of CD4+ lymphoblasts. Here, inoculation of cells with ADA at an MOI=0.1
results in cell fusion and degeneration within 1-2 days of peak viral production. In CD4+ lymphoblasts maturation of viral particles occurs by budding from the plasma membrane in marked contrast to the intracellular assembly and accumulation seen in macrophages (Figure 3).

The tropism of HIV to CD4+ T cells and monocytes must be considered both in the understanding of disease pathogenesis and interesting potential interventions to treat and prevent HIV infection in man. We employed both cell systems to study the regulation and antiviral effects of IFN, a cytokine with known ability to suppress the replication of many viruses. Furthermore, IFN is produced in vivo...
during advanced HIV disease. As both T cells and macrophages are potential sources for the IFNα detected during disease we investigated whether HIV-infection of these target cells resulted in IFN production. Analyses of tissue culture supernatant fluids from monocytes and lymphoblasts productively infected individually with 15 different viral strains demonstrated no IFN activity (inhibition of murine encephalomyocarditis virus-induced cytopathic effects in human foreskin fibroblasts). When HIV-infected macrophages were treated with poly(I)-poly(C) or Newcastle disease virus (NDV), both potent IFNα inducers, little or no IFN was produced (<10 U/ml) through 5 days of cell culture. Control uninfected monocytes stimulated with either poly(I)-poly(C) or NDV produced ≥1000 U/ml of IFNα. Investigations of HIV-infected monocytes 8, 12 and 24 hrs after addition of poly(I)-poly(C) and analyses of RNA prepared from cell lysates by reverse transcription coupled polymerase chain reaction (PCR) amplification of cellular RNA failed to demonstrate IFNα-specific RNAs (Figure 4). These results confirm the near absence of IFN activity in culture fluids of poly(I)-poly(C)-treated HIV-infected monocytes and localize this defect at the level of transcription.

The preceding results present an apparent paradox. During progressive HIV infection in man high levels of IFNα are produced yet HIV infection of its principal target cells in vitro does not result in IFNα activity or IFN production upon induction with double stranded synthetic or viral RNAs. This paradox may be resolved through analogy with visna-maedi virus, a lentivirus which produces chronic neurologic and pulmonary disease in infected ruminants. As is described with HIV infection, visna-maedi virus replication in sheep macrophages fails to elicit IFN production. After addition of PBMCs to these infected macrophages high levels of IFN are produced (8). Preliminary experiments performed in our laboratory support this mechanism for IFN regulation. High levels of
Figure 4. Induction of IFNα mRNA in HIV-infected monocytes by poly (I).poly (C). Monocytes cultured 7 days as adherent monolayers were exposed to HIV at a MOI of 0.01 infectious virus/target cell. Two wks after infection, 100 ug/ml poly (I).poly (C) was added to HIV-infected and uninfected control cultures for 4 hrs. All cultures were washed and refed with fresh medium. RNA from cell lysates was extracted and mixed with antisense primers. After reverse transcription, cDNA was amplified by PCR and the products of 25 cycles analyzed by Southern blot hybridization with a IFNα-specific probe. Coupled reverse transcription/PCR amplification products from cell lysates of monocytes at various times after poly (I).poly (C) treatment for uninfected cells are shown in lanes 1 (0 hr), 3 (8 hr), 5 (12 hr), and 7 (24 hr), and for HIV-infected cells in lanes 2 (0 hr), 4 (8 hr), 6 (12 hr), an 8 (24 hr).

IFNα are produced through the interactions of HIV-infected macrophages and PBMCs. Further experiments are in progress to identify the IFN-producing cell.

The block in IFNα-induced defect in the HIV-infected monocyte is specific. Replicate experiments of poly(I)-poly(C) treated (8, 12 and 24 hr) HIV-infected and uninfected monocytes using the coupled reverse transcription/PCR analysis of mRNA showed indistinguishable levels of TNFα, IL-6 and IL-1β (Figure 5). High
Figure 5. Induction of cytokine mRNA in HIV-infected monocytes by poly (I).poly (C). Monocytes cultured 7 days as adherent monolayers were exposed to HIV at a MOI of 0.01 infectious virus/target cell. Two wks after infection, 100 ug/ml poly (I).poly (C) was added to HIV-infected and uninfected control cultures for 4 hrs. All cultures were washed and refed with fresh medium. RNA from cell lysates was extracted and mixed with antisense primers. After reverse transcription, cDNA was amplified by PCR and the products of 25 cycles analyzed by Southern blot hybridization with cytokine-specific probes. Coupled reverse transcription/PCR amplification products from cell lysates of monocytes at various times after poly (I).poly (C) treatment for uninfected cells are shown in lanes 1 (0 hr), 3 (8 hr), and 5 (12 hr) and for HIV-infected cells in lanes 2 (0 hr), 4 (8 hr), 6 (12 hr), 7 (24 hr) and 8 (48 hr).
levels of these mRNAs were associated with equally high levels of the identical cytokines detected by ELISA. Furthermore, other species of IFN, IFNβ and IFNα, produced at low levels by monocytes show equal levels of mRNA expression and kinetics of induction in HIV-infected and uninfected monocytes after poly(I):poly(C) treatment. These results provide yet another important mechanism of how HIV replicates continuously in vivo in cells of mononuclear phagocyte origin, cells whose function revolves around the ingestion and destruction of microbial pathogens. Other known mechanisms of virus escape from host immune surveillance include: enhanced infection in macrophages by non-neutralizing antibodies, antigenic drift and variation of the viral envelope, sequestration of virions in endocytic vacuoles and resistance to inactivation by proteolytic enzymes. The selective defect in interferon production may also explain opportunistic infections found with normal CD4+ T cells. Here, activation of a variety of microbial pathogens may occur in the HIV-infected monocytes and macrophages in brain, lung and other target tissues.

Although, IFNα induction was blocked in HIV-infected macrophages further investigations were performed in order to determine if the poly(I):poly(C) treatment of these infected cells affected viral replication. Poly(I):poly(C) induces a strong antiviral activity in several cell types mediated through the production of IFN, and can also elicit a direct antiviral activity in treated cells. The first mechanism was apparently blocked in the HIV-infected monocyte, however, the direct pathway for antiviral activity may be intact. We examined this possibility through analyses of viral gene expression in HIV-infected monocytes after poly(I):poly(C)-treatment. HIV mRNA was readily detected in cell lysates of infected monocyte cultures by coupled reverse transcription/PCR analysis with both LTR and gag primers. After poly(I):poly(C) treatment, levels of HIV mRNA decreased to baseline by 72 h. This reduction in viral mRNA
was coincident with an equivalent reduction in cytopathicity and RT activity and an increase in 2'-5'(A) oligoadenylate synthetase in the same cell cultures. Increased levels of HIV mRNA were detected in untreated infected monocyte cultures during this exact 72 h time interval. It is possible that this antiviral activity was mediated by the low levels of IFNβ and IFNα produced or through the direct induction of 2'-5'(A) oligoadenylate synthetase, independent of IFN.

IFNα restricts HIV replication in infected monocytes and T-cells by distinct mechanisms. The effect in T cells is moderate. Pretreatment of lymphoblasts with 500 U/ml of recombinant human IFNα and continual addition of IFNα during subsequent infection by HIV ADA MOI=0.1 did not alter the levels of HIV mRNA, proviral DNA or p24 Ag in cells or released in culture fluids when compared to the untreated HIV infected cell cultures (Figure 6A). However, numbers of progeny HIV virions as measured by reverse transcriptase activity in and limiting dilution titrations in culture fluids was reduced 2-3 fold (Figure 6B). A concomitant increase of 2-3 fold of intracellular reverse transcriptase was observed following lysis of the IFN-treated HIV-infected cells. These data suggest that the mechanism of action of IFNα on the replication of HIV in lymphoblasts is directed at virus assembly or release.

In contrast to these results, IFNα effects on HIV infection of macrophages are quite dramatic. Addition of 50 to 500 U/ml of recombinant human IFNα 24 hr before, at the time of, or 24 hours following HIV-infection and continuous exposure of monocytes to IFN thereafter abrogated virus infection (Figure 7). No proviral DNA, mRNA, p24 Ag or RT activity was detected in cell or culture fluids of IFN-treated infected monocytes up to 2 weeks after viral challenge. In this system IFN interrupted an early stage of the virus replication cycle: virus-receptor binding, penetration, uncoating and/or reverse transcription. The exact mechanism(s) for the antiviral activity are not yet known but may include: (a) changes in virus receptor
Figure 6. Effect of IFN on replication of HIV in lymphoblasts. PHA/IL-2 treated lymphoblasts were exposed to ADA, a monocyte tropic HIV isolate, at a multiplicity of infection of 0.01 infectious virus/cell with and without 50 or 500 U/ml IFNα. IFN was maintained at this concentration throughout the culture interval. All cultures were refed with fresh medium every 2 to 3 days. Levels of p24 Ag (A) in culture fluids were determined by ELISA. Levels of RT activity in culture fluids are shown in (B).

number or distribution (IFN-treated monocytes show alterations in levels of CD4, FcR, CD11a, and mannosylated protein receptors, each of which is implicated in the uptake of HIV into monocytes); (b) changes in the monocyte plasma membrane that interrupt fusion or uptake of the virion into the cell; and (c) changes in subcellular compartments or cytosolic milieu that prevent or alter reverse transcription (IFN-treated cells synthesize 2'5'-oligonucleotides that induce RNases, and directly inhibit reverse transcription).

HIV-infected monocytes exposed to recombinant IFNα 3 or 7 days after infection showed a gradually decrease in both p24 Ag and RT
Figure 7. Effect of IFN on replication of HIV in monocytes. Monocytes cultured 7 days as adherent monolayers in medium with MCSF were exposed to ADA, a monocyte tropic HIV isolate, at a multiplicity of infection of 0.01 infectious virus/cell with 500 U/ml IFNα added 1 day before, at the time of and 1 day after infection and maintained at this concentration throughout the culture interval. All cultures were refed with fresh medium every 2 to 3 days. Levels of p24 Ag in culture fluids were determined by ELISA.

activity levels through 2 weeks of culture (Figure 8). By 3 weeks, little or no p24 Ag or reverse transcriptase activity was detected in culture fluids or cell lysates of any IFN-treated monocyte culture. HIV-induced multinucleated giant cells which were evident 7 days after infection with a frequency of about 10 to 20% of total cells, also decreased in frequency in the IFN-treated cultures to <5% total cells at 3 weeks.

In the absence of detectable levels of viral proteins (p24 Ag or RT activity), or of gross morphologic evidence for virus infection, proviral DNA was detected by polymerase chain reaction analyses 14 days after virus challenge in monocyte cultures treated with 500
Figure 8. Effect of IFN on chronic HIV-infection of monocytes. Monocytes cultured 7 days as adherent monolayers in medium with MCSF were exposed to ADA, a monocyte tropic HIV isolate, at a multiplicity of infection of 0.01 infectious virus/cell with 500 U/ml IFNα added 3 or 7 days after infection and maintained at this concentration throughout the culture interval. All cultures were refed with fresh medium every 2 to 3 days. Levels of p24 Ag in culture fluids were determined by ELISA.

U/ml IFN for 7 days. Furthermore, HIV mRNA as detected by Northern blot analysis in the IFN-treated, HIV-infected monocytes was reduced > 90% of that in control infected cells. Analysis of these same cell populations by in situ hybridization (Figure 9) for HIV-specific mRNA showed productive infection in 244 of 1164 untreated HIV-infected cells (a frequency of 21 ± 3% infected cells in triplicate samples) versus 9 of 861 IFN-treated HIV-infected monocytes (a frequency of 1 ± 1% infected cells). These data in toto document potent transcriptional restriction (at the level of viral RNA synthesis or accelerated degradation) of virus replication in IFN-treated, HIV-infected monocytes, a restriction that approaches true microbiological latency.
Figure 9. Effect of IFN on HIV-specific mRNA in infected monocytes. Monocytes were exposed ADA, a monocyte tropic HIV isolate, at a multiplicity of infection of 0.01 infectious virus/cell. Seven days after infection, 500 U/ml IFNα was added and maintained at this concentration throughout the culture interval. The frequency of cells that express HIV-specific mRNA as detected by in situ hybridization in control infected monocyte cultures 14 days after HIV infection is shown on the upper panel. Cells in the lower panel were treated with 500 U/ml IFN 7 days after HIV infection and cultured with IFN for an additional 7 days.
Infection by lentiviruses is followed by an persistence of the viral pathogen in cells of macrophage lineage. The mechanism(s) that underlie the maintenance of this infection in cells with strong microbicidal capabilities remains uncertain. Macrophages are also important for viral dissemination because they are present in large numbers in inflammatory exudates and in bodily secretions. Thus, the adaptive mechanisms for replication and persistence by the virus to this host cell is extraordinarily important in an understanding of disease pathogenesis. The dramatic response of the HIV-infected macrophages to IFN and an equally dramatic dysregulation of the IFN in the cell may underlie mechanisms of both HIV persistence and replication in its human host.

Acknowledgments. The authors thank Ms. Tze-Jou Yeh for IFN assays and members of the Walter Reed Retroviral Research Group for continued support and excellent patient management. Dr. 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

REFERENCES


