A Selective Defect of Interferon α Production in Human Immunodeficiency Virus-infected Monocytes

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Summary

Interferon α (IFN-α) induces significant antiretroviral activities that affect the ability of human immunodeficiency virus (HIV) to infect and replicate in its principal target cells, CD4^{+} T cells and macrophages. A major endogenous source of IFN-α during any infection is the macrophage. Thus, macrophages have the potential to produce both IFN-α and HIV. In this study, we examined the production of IFN-α and other cytokines by macrophage colony-stimulating factor (M-CSF)-treated cultured monocytes during HIV infection. Tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6, IFN-ω, or IFN-β were not detected nor was the mRNA expressed in either uninfected or HIV-infected monocytes. However, both uninfected and HIV-infected monocytes produced high levels of each of these cytokines after treatment with synthetic double-stranded RNA [poly(I)·poly(C)]. Uninfected monocytes also produced high levels of IFN-α after treatment with poly(I)·poly(C), Newcastle disease virus, or herpes simplex virus. In marked contrast to the preceding observations, HIV-infected monocytes produced little or no IFN-α before or after treatment with any of these agents. The absence of detectable IFN-α activity and mRNA in poly(I)·poly(C)-treated HIV-infected monocytes was coincident with high levels of 2',5' oligoadenylate synthetase and complete ablation of HIV gene expression. The antiviral activity induced by poly(I)·poly(C) may be a direct effect of this synthetic doubled-stranded RNA or secondary to the low levels of IFN-β and IFN-ω produced by infected cells. The markedly diminished capacity of HIV-infected monocytes to produce IFN-α may reflect a specific adaptive mechanism of virus to alter basic microbicidal functions of this cell. The inevitable result of this HIV-induced cytokine dysregulation is virus replication and persistence in mononuclear phagocytes.

Macrophages play a central role in maintenance of the steady state, in body defense against infectious or neoplastic challenge, and in control of inflammation largely through the secretion of soluble factors or cytokines (1, 2). Paradoxically, these scavenger cells also represent a major cellular reservoir for many microbial pathogens, including HIV (3). The HIV-infected macrophage continuously produces progeny virus through both subclinical infection and overt disease, often in the face of a vigorous, virus-induced, host immune response. There is strong evidence that resident tissue macrophages of the central nervous system (4–6), lung (7), lymph nodes (8), and blood monocytes (9–11) each harbor HIV, and support its replication. How HIV infects macrophages and contributes to clinical disease is mediated in part by the complex interactions between the mononuclear phago-

cyte of each tissue and the other cell types which result in the production of injurious secretory products or cytokines. IL-1, TNF-α, and IL-6 have each been implicated in the pathogenesis of disease symptoms during HIV infection (12). HIV can also directly induce cell dysfunction and alter host immune responses to affect the virus–target cell interaction and disease pathogenesis (13–15).

The mechanisms for persistence by HIV in mononuclear phagocytes, cells whose prime function evoked for destruction of foreign pathogens, are poorly understood. One possibility involves the dysregulation of normal cell differentiation and microbicidal function induced by virus. Many of these macrophage functions are mediated by autocrine and paracrine cytokines. One such cytokine, IFN-α, is particularly important as it is involved in both cell differentiation

and in defense against viral and nonviral infectious pathogens. Indeed, IFN-α can significantly suppress or prevent the replication of many animal lentiviruses including HIV (16). In a recent placebo-controlled clinical trial, IFN-α showed signficant antiviral activity in HIV-infected patients treated early in the course of disease (17). The replication of visna-maedi virus in sheep macrophages is also restricted in vivo by IFN (18). Restricted lentivirus replication induced by IFN in macrophages and/or CD4$^+$ lymphocytes is a consequence of specific and selective transcriptional blocks in expression of viral genes and also interference with the mechanisms for viral assembly and maturation during the virus life cycle (19–21). Characterization of IFN regulation in the HIV-infected macrophages may permit insight into the virus–host cell adaptive mechanisms that result in HIV survival and persistent replication in cells of macrophage lineage.

Materials and Methods

Isolation and Culture of Monocyte Target Cells. Monocytes were recovered from PBMC of HIV and hepatitis B–seronegative donors after leukapheresis and purified by countercurrent centrifugal elutriation of mononuclear leukocyte-rich fractions of blood cells. Cell suspensions were >98% monocytes by criteria of cell morphology on Wright-stained cytosmears, by granular peroxidase, and by nonspecific esterase. Monocytes were cultured as adherent cell monolayers (7.5 × 10^5 cells/24 mm plastic culture well) in 0.5 ml DMEM (formula 78-176AJ; Gibco Laboratories, Grand Island, NY) with 10% heat-inactivated A+ human serum, 50 μg/ml gentamicin, and 1,000 U/ml highly purified (<0.01 ng/ml endotoxin) recombinant human macrophage CSF (M-CSF)[1] (FAP-809, a generous gift from the Cetus Corp., Emeryville, CA) (22). All culture reagents were screened and found negative for endotoxin contamination.

HIV Infection of Monocyte Targets. M-CSF-treated monocytes were exposed at a multiplicity of infection (MOI) of 0.01 infectious virus/target cell to the ADA HIV strain originally isolated and passaged in monocytes (22). HTLV-IIIB/H9 (contributed by Dr. R. Gallo) was obtained from the AIDS Research and Reference Reagent Program, AIDS Progam, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD). All viral stocks were tested and found free of mycoplasma contamination (Gen-probe II; Gen-probe Inc., San Diego, CA). M-CSF-treated monocytes were cultured as adherent monolayers 7–10 d before use as target cells. All cultures were refed with 50% fresh medium every 2–3 d. Levels of p24 antigen (Ag) in culture fluids were determined by ELISA (E. I. Dupont de Nemours & Co., Billerica, MA). For reverse transcriptase (RT) activity, replicate samples of culture fluids were added to a reaction mixture of 0.05% NP-40 (Sigma Chemical Co., St. Louis, MO), 10 μg/ml poly(A), 0.25 U/ml oligo(dT) (Pharmacia Fine Chemicals, Piscataway, NJ), 5 mM dithiothreitol (Pharmacia), 150 mM KCl, 15 mM MgCl$_2$, and [^{3}H]dTTP (2 Ci/mmol; Amersham Corp., Arlington Heights, IL) in pH 7.9 Tris-HCl buffer for 24 h at 37°C. Radiolabeled nucleotides were precipitated with cold 10% TCA and washed with 5% TCA and 95% ethanol in an automatic cell harvester (Skatron Inc., Sterling, VA) on glass filter discs. Ra-

dioactivity was estimated by liquid scintillation spectroscopy (23). HTLVIIIB served as positive control for both p24 Ag and RT activity.

Growth of Macrophage Variant HIV in Macrophage Targets. Macrophage variant HIV initially isolated onto M-CSF-treated monocytes by cocultivation with PBMC from HIV-seropositive patients was serially passaged on monocyte target cells. Such macrophage variant HIV infects both monocyte and T cell targets (24). For the HIV isolate ADA, infection of monocyte targets at an MOI of 0.01 infectious virus/target cell induced: (a) typical cytopathic effects (multinucleated giant cells and lysis), (b) p24 Ag levels initially detected in culture fluids at 3–5 d with maximum levels ≥50 ng/ml, (c) levels of RT activity in culture fluids ≥60 × 10^6 cpm/ml, (d) proviral HIV DNA detected 12 h after infection by PCR amplification with peak levels at 10 d, and (e) infectious titers for macrophages of 1 × 10^4 TCID$_{50}$/ml.

Detection of HIV-specific DNA by PCR Amplification and Southern Blot Hybridization. Cell lysates of HIV-infected monocytes were extracted twice with phenol and chloroform/isoamyl alcohol and the DNA was precipitated with ethanol. PCR amplification of HIV-specific DNA with nucleotide primers from the LTR and *gag* genes and 2.5 U/ml Taq polymerase (Cetus Corp.) was performed with an automatic cycler (Perkin Elmer-Cetus, Emeryville, CA). The products of 40 cycles (5 min at 90°C initial denaturation, then 2.5 min at 94°C, 3 min at 55°C, and 2 min at 72°C) 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 (25 and Table 1). λDNA-negative and HXB2 proviral DNA-positive controls were included with each assay.

In Situ Hybridization with HIV RNA Probes. Single-stranded HIV [^{35}S]RNA probes were synthesized from recombinant DNA plasmids containing SP6/T7 promoters (Promega Biotec, Madison, WI). Cytosmears of cells on silanated glass slides were fixed in 4% paraformaldehyde. Specimens were prehybridized in 10 mM Tris, pH 7.4, 0.3 M NaCl-0.03 M sodium citrate, pH 7.4, Denhardt's solution (0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% BSA), and 200 μg/ml yeast tRNA at 45°C for 2 h and hybridized in this solution with 10% dextran sulfate, 5 μM dithiolthreitol and 1 × 10^6 cpm ^{35}S-labeled HIV RNA (Oncor, Inc., Gaithersburg, MD). Slides were serially washed in solutions with RNase to reduce binding of nonhybridized probe. Autoradiography was performed in absolute darkness (26).

Dot Blot Analysis of mRNA. Total RNA was extracted from cell lysates with acidic guanidinium isothiocyanate/phenol/chloroform and analyzed by Northern blot hybridization on nylon membranes (Nytran; Schleicher & Schuell, Keene, NH) with a radiolabeled 2′,5′ oligoadenylate synthetase probe (27).

Induction of Cytokines in HIV-infected Cells. At various times after HIV infection, monocytes were treated with 100 μg/ml poly(I)·poly(C) (Sigma Chemical Co.) for 4 h. Cultures were washed free of poly(I)·poly(C) and refed with fresh medium. Newcastle disease virus was prepared in hen's eggs (20) and used at a MOI of 1 infectious virus/target cell to infect HIV-infected and control monocytes. HSV type-1 was prepared from stock in Vero cells and provided as a generous gift from Dr. D. Gangemi, Columbia, SC. HSV was used at a MOI of 1 infectious virus/target cell to infect monocyte cultures.

Quantitation of Cytokine Activity. Culture fluids from control and HIV-infected monocytes were analyzed by ELISA for the human cytokines TNF-α, IL-1β, IL-6, and IL-4 (Quantikine Immunoassay; Research and Diagnostics Systems, Minneapolis, MI). IFN activity in culture fluids was assayed by inhibition of cytopathic effects in-

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[1] *Abbreviations used in this paper:* Ag, antigen; M-CSF, macrophage colony-stimulating factor; MOI, multiplicity of infection; RT, reverse transcriptase.

Table 1. *Nucleotide Primers in Detection of Cytokines and HIV Gene Products*

Amplification product size	Nucleotide position	Primer	Sequence
TNF-α			
237 bp	503–527	Sense	GAGCTGAGAGATAACCAGCTGGTG
	740–716	Antisense	CAGATAGATGGGCTCATACCAGGG
	588–608	Probe	CCCTCCACCCATGTGCTCCTC
IL-1β			
179 bp	480–500	Sense	AAAAGCTTGGTGATGTCTGG
	659–638	Antisense	TTTCAACACGCAGGACAGG
	549–567	Probe	ATGGAGCAACAAGTGGTG
IL-6			
159 bp	317–337	Sense	GTGTGAAGCAGCAAAGAAGC
	476–455	Antisense	CTGGAGGTACTCTAGGTATAC
	399–420	Probe	GGATTCAATGAGGAGACTTGC
IFN-α			
274 bp	240–259	Sense	TCCATGAGATGATCCAGCAG
	514–492	Antisense	ATTTCTCGCTCTGACAACCTCCC
	433–454	Probe	AAATACTTCCAAAGAATCACT
IFN-β			
186 bp	343–364	Sense	GATTCATCTAGCACTGGCTGG
	529–509	Antisense	CTTCAGGTAATGCAGAATCC
	379–400	Probe	GAGAACCTCCTGGCTAATGTC
IFN-ω			
147 bp	238–258	Sense	AACATGAACCTCCTAGACCA
	385–366	Antisense	TCCCTGGAAGTACCTCCTCA
	341–360	Probe	GCAATTAGCAGCCCTGCACT
GAPDH			
195 bp	199–217	Sense	CCATGGAGAAGCTGGGGG
	394–374	Antisense	CAAAGTTGTCATGGATGACC
	280–299	Probe	CTAAGCATGTGGTGGTGCA
HIV LTR/gag			
370 bp	435–455	Sense	AGCTGCTTTTTGCCTGTACT
	805–782	Antisense	GACGCTCTCGCACCCATCTCTCTC
	551–570	Probe	GACCTGAAAGCGAAAGGGAA

duced by murine encephalomyocarditis virus in FS-4 human fore-skin fibroblasts (28).

Coupled Reverse Transcription/PCR Detection of Cytokine and HIV-specific RNA. Levels of cytokine or viral RNA were estimated after reverse transcription with antisense primers and PCR amplification of the cDNAs. The mRNA for the cellular enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), served as an internal control to allow analysis and comparison of RNA species between different samples. Briefly, 2.0 μg total cellular RNA in 0.025 ml was mixed with 0.3 μg of the antisense primers for GAPDH (29), TNF-α (30), IL-1β (31), IL-6 (32), IFN-α (33), IFN-ω (34), IFN-β (35), and HIV *LTR/gag* (25). Table 1 lists the primer sequences used. The mixture was heated at 70°C for 5 min, cooled

on ice, and treated with 500 U Moloney murine leukemia virus RT (Bethesda Research Laboratories, Bethesda, MD) and 0.5 mM each of all four deoxynucleotide triphosphates. Reverse transcription reactions were at 37°C for 15 min then stopped by heating at 95°C for 10 min. For PCR amplification of the cDNA products, reaction mixtures were divided into equal aliquots and mixed with 0.5 μg sense and antisense primers, 0.5 mM deoxynucleotide triphosphates, and 2 U Amplitaq (Cetus Corp.). The products of 25 cycles (1.5 min at 94°C, 1.5 min at 50°C, and 2.0 min at 72°C) were analyzed by Southern blot hybridization (36). The oligo-nucleotides were synthesized on a DNA synthesizer (Applied Biosystems, Inc., Foster City, CA) and checked for purity by polynucleotide kinase labeling and sequence gel analysis. Oligonu-

cleotides were typically 95% pure. Hybridization analyses of HIV LTR/gag amplified cDNAs were also performed with random primer-labeled 370-bp HIV DNA internal to the primer pairs.

Results

Cytokine Activities in Culture Fluids of HIV-infected Monocytes. No IFN activity (inhibition of murine encephalomyocarditis virus-induced cytopathic effects in human foreskin fibroblasts) was detected in culture fluids of uninfected or HIV-infected monocytes with any of 15 different HIV isolates at any time through 3 wk of culture. Similarly, levels of TNF-α, IL-1β, or IL-6 in culture fluids of uninfected and HIV-infected monocytes as quantified by ELISA were ⩽1 pg/ml for all times examined through 2 wk. Fluids from monocyte cultures were examined for cytokine activities every 2 d.

Induction of IFN Activity in Culture Fluids of Uninfected and HIV-infected Monocytes by poly(I)·poly(C) or NDV. Culture
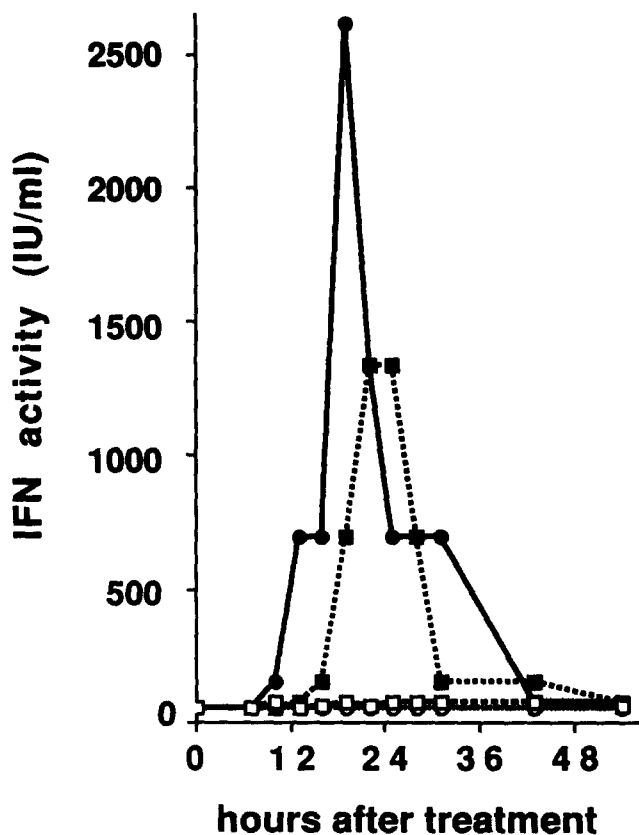

Figure 1. Time course for IFN production by HIV-infected monocytes treated with poly(I)·poly(C) and NDV. Monocytes cultured 7 d as adherent monolayers were exposed to HIV at an MOI of 0.01 infectious virus/ target cell. 2 wk after infection, 100 μg/ml poly(I)·poly(C) (*circles*) or NDV (*squares*) at an MOI of 1 infectious virus/target cell was added to HIV-infected (*open symbols*) and uninfected control (*closed symbols*) cultures for 4 h. All cultures were washed free of poly(I)·poly(C) and NDV and refed with fresh medium. IFN activity in culture fluids was assayed at 3-h intervals by inhibition of murine encephalomyocarditis virus-induced cytopathic effects in human fibroblasts. Each data point represents the mean of duplicate determinations in one of four replicate experiments.

fluids from uninfected control monocytes treated with poly(I)·poly(C) or NDV contained high levels of IFN activity (Fig. 1). Analysis of the time course for production of IFN activity at 3 h intervals revealed maximum levels (>1,000 U/ml) 20–24 h after treatment. Neutralization studies with class-specific anti-IFN antibodies showed that >98% of antiviral activity was IFN-α. By 43 h, all IFN activity in these culture fluids returned to baseline levels. In striking contrast, culture fluids from HIV-infected monocytes treated with these identical agents had little IFN activity at any time through 54 h. HIV-infected monocytes were treated with poly(I)·poly(C) or NDV 2 wk after virus inoculation: ∼45–60% of monocytes in these cultures were positive for HIV mRNA by in situ hybridization; levels of p24 Ag in culture fluids were >50 ng/ml. In more than 10 replicate experiments with 3 different HIV isolates (strains ADA, 24, and 36), little IFN activity (⩽ 10 U/ml) was detected in culture fluids of HIV-infected monocytes treated with 10–500 μg/ml poly(I)·poly(C), NDV, or HSV type-1 12–18 days after HIV inoculation.

The preceding data documents a profound defect in the production of IFN-α by monocytes infected with HIV after treatment with any of several different IFN inducers. Further experiments explored the relationship between time after HIV infection and the onset of this functional defect. Monocytes cultured 7 d as adherent monolayers were exposed to the HIV isolate ADA at an MOI of 0.01 infectious virus/target cell. At 1, 3, 5, 7, and 14 d after virus infection, HIV-infected monocytes were treated with 100 μg/ml poly(I)·poly(C) for 4 h. IFN activity in culture fluids of HIV-infected and matched uninfected control monocytes was measured 24 h after poly(I)·poly(C) treatment. IFN activity in culture fluids of uninfected poly(I)·poly(C)-treated monocytes was 1250 IU/ml. HIV infection had no effect on poly(I)·poly(C)-induced IFN levels in monocyte culture 1 d after virus infection. By 3 d after HIV infection, the levels of IFN activity in these monocyte cultures were reduced by 50%. The reduction in poly(I)·poly(C)-induced IFN activity in culture fluids of HIV-infected monocytes increased with time with infection: 80% by day 5, 90% by day 7, and ⩾98% by day 14.

Induction of Cytokine Gene Expression in HIV-infected Monocytes by poly(I)·poly(C). To further quantitate the extent and specificity of the HIV-induced defect in IFN production by poly(I)·poly(C)-treated, HIV-infected monocyte cultures, we examined IFN-α-specific mRNA using coupled reverse transcription/PCR analysis. Levels of mRNA for the constitutive cellular enzyme GAPDH were also examined as a reference transcript to confirm the presence of cellular RNA and the efficiency of PCR amplification for all samples (Fig. 2). GAPDH mRNA amplification products were present at equivalent levels with the cell lysates of all cultures examined. No IFN-α mRNA amplification products were detected with cell lysates from uninfected or HIV-infected monocyte cultures not treated with poly(I)·poly(C) (lanes 1 and 2). The predicted 274-bp amplification product of IFN-α mRNA was evident with cell lysates of uninfected monocyte cultures 8, 12, and 24 h after poly(I)·poly(C) treatment (lanes 3, 5, and 7). In contrast, this IFN-α mRNA amplification product
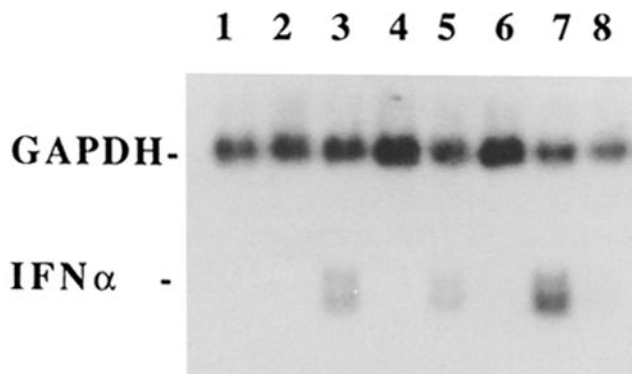
Figure 2. Induction of IFN-α mRNA in HIV-infected monocytes by poly(I)·poly(C). Monocytes cultured 7 d as adherent monolayers were exposed to HIV at an MOI of 0.01 infectious virus/target cell. 2 wk after infection, 100 μg/ml poly(I)·poly(C) was added to HIV-infected and uninfected control cultures for 4 h. All cultures were wash 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 were analyzed by Southern blot hybridization with a IFN-α–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 h), *3* (8 h), *5* (12 h), and *7* (24 h), and for HIV-infected cells in lanes *2* (0 h), *4* (8 h), *6* (12 h), and *8* (24 h).

was not detected with any cell lysate of HIV-infected monocyte cultures treated with poly(I)·poly(C) under identical conditions and for identical time intervals after induction (lanes *4*, *6*, and *8*). Replicate experiments with primer pairs specific for the mRNA transcripts of two other members of the IFN-α family of genes (IFN-α1 and IFN-α2) yielded significant levels of amplification products with cell lysates of uninfected poly(I)·poly(C)–treated monocytes, but not with the lysates of similarly treated HIV-infected cells. These results confirm the near absence of IFN activity in culture fluids of poly(I)·poly(C)–treated, HIV-infected, monocyte cultures described in the preceding experiments and localize this HIV-associated defect to a transcriptional block.

The specificity of this HIV-associated transcriptional block in the expression of IFN-α genes was explored by quantitation of mRNA for several other cytokines induced in monocytes by poly(I)·poly(C) (Fig. 3). Coupled reverse transcription/PCR analysis of mRNA for IL-1β, IL-6, and TNF-α in cell lysates of uninfected and HIV-infected monocyte cultures showed no or little (IL-1β in HIV-infected monocytes) cytokine mRNA amplification products. These results are consistent with the absence of these cytokines as detected by ELISA in the monocyte culture fluids. After poly(I)·poly(C) treatment, however, high and indistinguishable levels of amplification products for mRNA of IL-1β (179-bp product), IL-6 (159-bp product), and TNF-α (237-bp product) were identified with cell lysates from both uninfected and HIV-infected monocyte cultures. These relatively high levels of mRNA products were associated with similarly high concentrations of these same cytokines in monocyte culture fluids as detected by ELISA (Table 2). Thus, strong HIV-associated
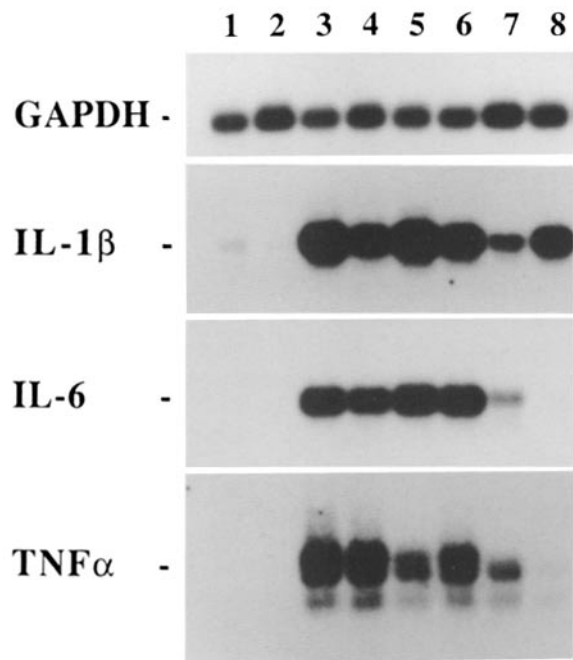

Figure 3. Induction of cytokine mRNA in HIV-infected monocytes by poly(I)·poly(C). Monocytes cultured 7 d as adherent monolayers were exposed to HIV at an MOI of 0.01 infectious virus/target cell. 2 wk after infection, 100 μg/ml poly(I)·poly(C) was added to HIV-infected and uninfected control cultures for 4 h. 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 were 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 h), *3* (8 h), and *5* (12 h) and for HIV-infected cells in lanes *2* (0 h), *4* (8 h), *6* (12 h), *7* (24 h), and *8* (48 h).

Table 2. *Cytokine Levels in Culture Fluids of Control and HIV-infected Monocytes Treated with poly(I)·poly(C)*

Cytokine	Monocyte culture	Cytokine levels with time after treatment:				
		0 h	12 h	24 h	48 h	72 h
				pg/ml		
TNF-α	Control	0	600	1,100	800	600
	HIV-infected	0	1,150	1,650	1,800	1,000
IL-6	Control	0	380	900	550	1,300
	HIV-infected	0	100	300	200	220
IL-1β	Control	0	10	10	20	10
	HIV-infected	0	40	70	40	50

Monocytes cultured 7 d as adherent monolayers were exposed to HIV at a MOI of 0.01 infectious virus/target cell. 2 wk after infection, 100 μg/ml poly(I)·poly(C) was added to HIV-infected and uninfected control cultures for 4 h. All cultures were washed free of poly(I)·poly(C) and refed with fresh medium. Cytokine levels in culture fluids were determined by ELISA.
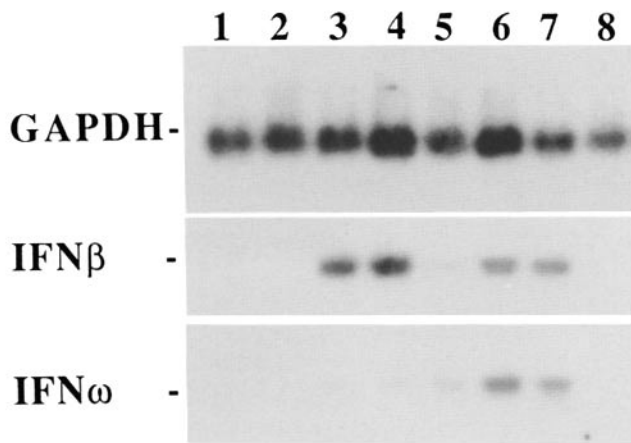
Figure 4. Induction of IFN-ω and IFN-β mRNA in HIV-infected monocytes by poly(I)·poly(C). Monocytes cultured 7 d as adherent monolayers were exposed to HIV at an MOI of 0.01 infectious virus/target cell. 2 wk after infection, 100 μg/ml poly(I)·poly(C) was added to HIV-infected and uninfected control cultures for 4 h. 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 were analyzed by Southern blot hybridization with a IFN-ω– and IFN-β–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 h), *3* (8 h), *5* (12 h), and for HIV-infected cells in lanes *2* (0 h), *4* (8 h), *6* (12 h), *7* (24 h), and *8* (48 h).
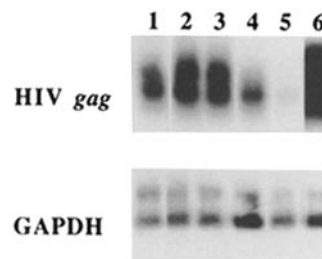

Figure 5. Detection of *gag*-specific RNA in HIV-infected monocytes treated with poly(I)·poly(C). Monocytes cultured 7 d as adherent monolayers were exposed to HIV at an MOI of 0.01 infectious virus/target cell. 2 wk after infection, 100 μg/ml poly(I)·poly(C) was added to HIV-infected cultures for 4 h. 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 were analyzed by Southern blot hybridization with a HIV *gag*-specific probe between primer pairs. Coupled reverse transcription/PCR amplification products from cell lysates of HIV-infected monocytes at various times after poly(I)·poly(C) treatment are shown in lanes *1* (0 h), *2* (12 h), *3* (24 h), *4* (48 h), and *5* (72 h), and of matched untreated HIV-infected cells at 72 h shown in lane *6*.

transcriptional block in the expression of IFN-α genes was not evident in the expression of several other cytokine genes.

While IFN-α is the predominant IFN activity produced by monocytes, it is not the only IFN expressed by these cells. Quantitation of poly(I)·poly(C)–induced IFN-α (IFN-α$_{II}$1), and IFN-β mRNA by coupled reverse transcription/PCR analysis in cell lysates of uninfected and HIV-infected monocytes showed that the HIV-associated transcriptional block in the expression of IFN-α genes was not extended to the expression of other IFN genes (Figure 4). Neither uninfected nor HIV-infected monocytes expressed IFN-β and IFN-ω mRNA without poly(I)·poly(C) treatment (lanes *1* and *2*). Both uninfected and HIV-infected monocyte cultures expressed these IFN mRNA after poly(I)·poly(C) treatment: no differ-

ences in the amount or time course for appearance of the predicted 166- and 147-bp amplification products for IFN-β and IFN-ω mRNA were evident with cell lysates of uninfected or HIV-infected monocytes. It is likely that these minor IFN species represent most if not all of the low level IFN activity detected in HIV-infected monocyte cultures treated with poly(I)·poly(C) or NDV (see Fig. 1). These results, in toto, document a profound and highly specific transcriptional block in the expression of IFN-α in monocytes infected with HIV.

HIV Gene Expression in Virus-infected Monocytes Treated with poly(I)·poly(C). Poly(I)·poly(C) induces strong antiviral activity in a variety of target cells through at least two different mechanisms. This synthetic double-stranded RNA induces endogenous IFN production in several cell types, but it also directly stimulates antiviral activity in treated cells in the absence of IFN. The first mechanism is apparently blocked in the HIV-infected monocyte; however, the alternative direct pathway for antiviral activity may be intact. We examined this possibility by analysis of viral gene expression in HIV-infected monocytes after poly(I)·poly(C)-treatment (Fig. 5). HIV gene products (viral mRNA and DNA) were readily detected in cell lysates of infected monocyte cultures by coupled reverse transcriptase and/or PCR analyses with both LTR/*gag* and *env* primers (lane *1* and not shown). After
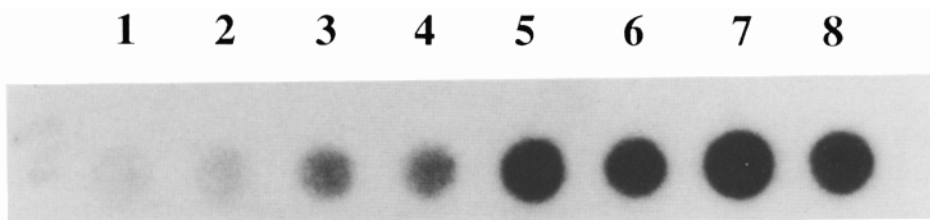

Figure 6. Detection of 2′,5′ oligoadenylate synthetase mRNA in HIV-infected macrophages treated with poly(I)·poly(C). Monocytes cultured 7 d as adherent monolayers were exposed to HIV at an MOI of 0.01 infectious virus/target cell. 2 wk after infection, 100 μg/ml poly(I)·poly(C) was added to HIV-infected cultures for 4 h. All cultures were washed and refed with fresh medium. Cell lysates of HIV-infected monocytes were extracted with acidic guanidinium isothiocyanate/phenol/chloroform and the RNA was precipitated with isopropanol. RNA was analyzed by Northern blot hybridization with a radiolabeled 2′, 5′ oligoadenylate synthetase-DNA probe. Autoradiographs show samples from poly(I)·poly(C)–treated uninfected monocytes in lanes *1* (0 h), *3* (8 h), *5* (12 h), and *7* (24 h) or HIV-infected monocytes in lanes *2* (0 h), *4* (8 h), *6* (12 h), and *8* (24 h).

poly(I)·poly(C) treatment, levels of HIV mRNA decreased to baseline by 72 h (lanes 2–5). This reduction in viral mRNA was coincident with an equivalent reduction in p24 Ag and RT activity levels in the same cultures. In contrast, levels of HIV mRNA in infected monocyte cultures not treated with poly(I)·poly(C) increased over the same time interval (lane 6).

It is possible that this antiviral activity was mediated by the low levels of IFN-β and IFN-ω ($\leqslant$10 U/ml antiviral activity) produced by infected cells. However, poly(I)·poly(C) also induces 2',5' oligoadenylate synthetase in several different cells independently of IFN-α This induced enzyme binds to dsRNA and catalyzes the conversion of ATP to 2',5' oligoadenylate, the activator of 2',5' oligoadenylate-dependent RNase L. The RNase, in turn, cleaves and inactivates single-stranded viral RNAs. Treatment of uninfected and HIV-infected monocytes with poly(I)·poly(C) induced a potent and indistinguishable transcriptional amplification of 2',5' oligoadenylate synthetase as detected by dot blot analysis of mRNA for this enzyme (Fig. 6). Thus, in the face of a stringent, HIV-associated, transcriptional block in the expression of IFN-α in the infected monocyte, poly(I)·poly(C) increased 2',5' oligoadenylate synthetase mRNA and elicited an effective antiviral reaction.

Discussion

The role of IFN in HIV disease is both complex and seemingly paradoxical. In vitro, IFN-α, IFN-β, and IFN-γ each have potent antiretroviral activity (16). While IFN-α does not prevent infection of HIV in CD4+ T cells, this cytokine significantly restricts virus replication in infected cells (20, 21, 25). The mechanism for this antiviral effect is through induction of a partial and reversible block in the assembly and/or release of progeny virions (19, 25). In macrophages, the antiviral action of IFN-α is more effective than that in T cells and operates through different mechanisms. Monocytes treated with IFN-α at the time of virus challenge show no evidence of HIV infection. IFN-α interrupts one or more early events in the virus replication cycle before formation of proviral DNA. Monocyte cultures infected with HIV before IFN-α treatment show a gradual decrease in levels of p24 Ag and RT activity to baseline. HIV-induced cytopathic changes are markedly reduced, as is HIV-specific mRNA, and the frequency of productively infected cells is ≤1%. Virus particles released 24 h after IFN-α treatment are 1,000-fold less infectious than equal numbers of control virions. But, levels of proviral DNA in the IFN-α-treated and control HIV-infected cells are indistinguishable. Large quantities of proviral DNA in cells with little or no evidence for active transcription documents a situation approaching true microbiological latency (25).

In HIV-infected patients, administration of IFN-α alone for 12 wk to asymptomatic subjects decreases ability to isolate virus from blood leukocytes and reduces p24 antigenemia. In follow-up studies, none of the IFN-α-treated patients developed AIDS-associated opportunistic infection compared with 30% of the placebo-treated group (17).

In contrast to these promising therapeutic interventions with exogenous IFN-α, the appearance of endogenous IFN in blood during the natural history of HIV disease is perhaps more ominous. Even though there is no evidence for direct induction of IFN by HIV itself, serum of patients with late stage HIV disease has high levels of an acid-labile IFN-α, a poor prognostic index that predicts onset of opportunistic infection (37, 38). The cell source for this acid-labile IFN-α is not known. Indeed, production of all IFN types during HIV infection is suppressed. Production of IFN-γ by PBMC of HIV-infected patients is significantly reduced (39). Transcription of the IFN-γ gene, but not the IL-2 gene, is impaired in HIV-infected T cells (40). Similarly, monocytes and PBMC from HIV-infected patients show a markedly reduced ability to secrete IFN-α or express IFN-α mRNA after exposure to vesicular stomatitis virus, influenza A virus, or HSV type 1 infected fibroblasts (41–43). What is most remarkable about these latter observations, is that they occur coincident with a frequency of HIV-infected cells in patient blood that is exceedingly low. About 1% of blood leukocytes harbor HIV DNA (44–46). Of the total number of infected cells, <0.01% show active expression of HIV genes (47). Yet production of IFN-α in PBMC of HIV-infected patients, cells that are at least 99% virus-free, is 1,000-fold less than that in PBMC of controls (43).

We document in this report a profound and highly selective defect in the expression of IFN-α genes by HIV-infected monocytes that was evident under a wide range of experimental conditions and inducing agents. The expression of other cytokine genes (TNF-α, IL-1β, IL-6) was apparently not affected. Indeed, expression of other IFN genes (IFN-ω, IFN-β) was also unaffected by HIV-infection. The inability of HIV-infected monocytes to express IFN-α may represent an adaptive response by this virus to ensure its survival and unimpeded replication in monocyte target cells. It is notable that at least some antiviral mechanisms normally induced by IFN-α can be induced in the HIV-infected monocyte by poly(I)·poly(C). This synthetic double-stranded RNA induced equivalent levels of 2',5' oligoadenylate synthetase mRNA in both control and HIV-infected monocytes. More significantly, HIV-infected monocytes treated with poly(I)·poly(C) showed a dramatic decrease in both p24 Ag and RT activity levels and HIV-specific mRNA. The activation of antiviral pathways in poly(I)·poly(C)-treated, HIV-infected monocytes may be mediated by a direct effect of this synthetic double-stranded RNA or by the low levels of IFN-β or IFN-ω produced by infected cells. Indeed, other cytokines induced by poly(I)·poly(C), such as TNF-α, may interact with these minor species of interferons to achieve an effective antiviral reaction. Thus, even though HIV-induces a selective block in the expression of IFN-α genes, the pathways induced by this cytokine can still be exploited for antiviral therapy. Such pathways of antiviral activity may be important not only for HIV, but also for the many other viral infections (herpes simplex, herpes zoster, cytomegalovirus) coincident with HIV disease. For example, patients infected with HIV develop persistent, localized, and slowly progressive herpes zoster infections refractory to acyclovir therapy (48). The HIV-associated

transcriptional block in IFN-α gene expression in the macrophages of these lesions may underlie the basic pathophysiology of this disease manifestation.

The mechanism for the transcriptional block in IFN-α gene expression in HIV-infected, M-CSF-treated, cultured monocytes is not yet known. Remarkably, HIV-infected monocyte cultures that exhibited this near absolute IFN-α defect showed a frequency for productively infected cells of 30–60%. If the IFN-α defect is a direct sequela of infection, then all cells must harbor the HIV provirus, which in turn is expressed in only half of the monocytes. Alternatively, the IFN-α defect may be mediated by a soluble factor released by HIV-infected cells that affects the entire cell population. This latter hypothesis is consistent with observations on PBMC of HIV-infected patients in which a cell population that is 99% virus-free shows a 1,000-fold decrease in ability to produce IFN-α (43). The experimental system described in this report should allow molecule identification of this putative soluble factor.

The dissociation between the expression of IFN-α and IFN-ω in HIV-infected monocytes is of special interest. The human IFN-α gene family consists of at least 20 nonallelic members, among which are the IFN-αII genes (49, 53). In cows, the more than 30 IFN-αII genes represent the major source of IFN-α and encode proteins that are acid-labile (50). In man, the IFN-αII gene subfamily has a single functional gene that encodes IFN-ω, and three or four other pseudogenes (49–53). Very little is known about the regulation of human IFN-αII genes or its encoded protein. IFN-ω is six amino acids longer at the COOH terminus than other IFN-α. Excluding these additional amino acids, IFN-ω shares a 60% homology with IFN-α proteins (homologies between other IFN-α proteins exceed 80%) and a 30% homology with IFN-β (51). Certain investigators suggest IFN-αII is a distinct IFN species, perhaps a vestigial link between IFN-α and IFN-β (50). Since the bovine IFN-αII proteins are acid-labile, it is tempting to speculate that IFN-ω is the entity responsible for the acid-labile IFN-α in late-stage HIV disease. That expression of all IFN-α genes is blocked at the transcriptional level in our in vitro system, and in cells from patients infected with HIV, and expression of IFN-ω in HIV-infected monocytes is intact supports this speculation. However, definite proof of IFN-ω as the acid-labile IFN-α in HIV disease, must await sequence data for the circulating activity.

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