INTERFERONS AND BACTERIAL LIPOPOLYSACCHARIDE PROTECT MACROPHAGES FROM PRODUCTIVE INFECTION BY HUMAN IMMUNODEFICIENCY VIRUS IN VITRO

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Macrophages are now recognized to be important cellular targets in HIV infection. These cells can be infected with HIV in vitro (1-7) and express HIV antigens and nucleic acid in vivo (8-13). Macrophage-tropic forms of HIV can be isolated from brain, lung, and blood (4, 7, 14), and these isolates replicate for months in monocyte-derived macrophages in vitro (4). Unlike CD4 T cells, macrophages are not rapidly killed by HIV and may serve as a persistent viral reservoir. HIV-infected macrophages may also have a critical role in the immunological disturbances and encephalopathy of HIV infection (15-17). Furthermore, cultured macrophages contain reduced levels of the kinases required to activate dideoxynucleosides such as AZT, and may not be protected by these agents as efficiently as T cells (18). Thus, additional therapeutic strategies for HIV infection may be needed in order to address the special biology of HIV infection in macrophages.

Numerous studies have demonstrated the effect of various stimuli upon macrophage functions (19, 20). Endotoxin, in the form of bacterial lipopolysaccharide (LPS), profoundly affects many macrophage functions (21). Also, endogenous cytokines such as interferons may physiologically "activate" macrophages. It has been proposed that the depletion of IFN-γ-producing T cells in AIDS leads to a defect in macrophage activation (22), and consequently IFN-γ is now entering clinical trials in AIDS patients. IFN-α and IFN-β are already being tested in patients. However, the possibility that these interferons have antiviral effects against HIV in macrophages has not been examined. In this study, we demonstrate that treatment with IFN-α, IFN-β, IFN-γ, or LPS prevents the establishment of productive HIV infection in cultured macrophages.

Materials and Methods

Macrophage Culture. PBMC were isolated from the blood of healthy volunteers by centrifugation over Ficoll-Hypaque. Monocytes were initially isolated by adherence to fibronectin (23). These cells were 70-90% nonspecific esterase (NSE)1-positive (Kit 90-Al; Sigma Chem.

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1 Abbreviations used in this paper: GM-CSF, granulocyte/macrophage colony-stimulating factor; M-CSF, macrophage CSF; MDM, monocyte-derived macrophages; MOI, multiplicity of infection; NSE, nonspecific esterase; TCID50, 50% tissue-culture infectious dose.
ical Co., St. Louis, MO) and were further purified by adherence to plastic. Initially, $4 \times 10^5$ fibronectin-adherent cells were cultured in 1 ml of RPMI 1640 (Whittaker M. A. Bioproducts, Inc., Walkersville, MD or Irvine Scientific, Santa Ana, CA) containing 10% heat-inactivated autologous or AB human serum and 50 µg/ml gentamicin in the 1-cm$^2$ wells of 48-well plates. All culture materials were endotoxin-free in two assays (the *Limulus* lysate assay and a sensitive monocyte procoagulant activity assay) as previously described (22). Fibronectin-adherent cells, enriched in monocytes, were cultured at 37°C in 5% CO$_2$/95% air for 5-9 d to allow them to mature into fully adherent cells (4). An additional purification step occurred when the monolayers were washed four times at the time of infection (see below). The final adherent cells ($\sim 2 \times 10^5$/well) were 100% NSE$^+$ and are referred to as "monocyte-derived macrophages" (MDM).

**HIV Infection.** The HTLV IIIBa-L ss macrophage-tropic isolate of HIV-1 was the gift of Drs. S. Gartner and M. Popovic, National Cancer Institute, Bethesda, MD (4). The third passage of this virus in MDM under endotoxin-free conditions in our laboratory was used to make a virus pool which was aliquoted and stored at −70°C. This pool had an infectivity titer by terminal dilution assay in MDM (as determined by p24 core antigen production) of $10^6 50\%$ tissue-culture infectious doses (TCID$_{50}$)/ml. This isolate fails to replicate in T cell lines (CEM and MT-2) that support many T lymphotropic strains of HIV-1.

MDM were infected with HIV by replacing their medium with 0.2 ml per well of HIV in medium containing 10% FCS (HyClone Laboratories, Inc., Logan, UT) instead of human serum. The multiplicity of infection (MOI) ranged between 0.1 and 1 in different experiments as indicated. After at least 2 h of incubation to permit viral adsorption, unadsorbed virus and residual nonadherent cells were removed by washing each well four times with 1 ml medium. Wells were refed 1 ml medium containing FCS and the indicated treatment, and incubated for the times shown.

**Interferons, Cytokines, and LPS.** Additions to culture medium were made before infection with HIV, during infection, or after infection, as indicated. rIFN-α (also known as IFN-α2a, IFN-α2, or Roferon) (2 $\times 10^8$ U/mg) was obtained from Hoffmann-La Roche, Nutley, NJ. rIFN-β (1.8 $\times 10^8$ U/mg) was the gift of Dr. C. Budd Colby, Triton Biosciences, Inc., Alameda, CA. rIFN-γ (1.6 $\times 10^8$ U/mg) was purchased from Amgen, Thousand Oaks, CA. rIL-1β and IL-4 were obtained from Genzyme Corp., Boston, MA. rIL-2 (7.3 $\times 10^5$ U/mg) and IL-6 were obtained from Genentech, Inc., South San Francisco, CA. Recombinant granulocyte/macrophage CSF (GM-CSF) (6.7 $\times 10^6$ U/mg), M-CSF (25) (8 $\times 10^5$ U/mg), and IL-6 (5 $\times 10^6$ U/mg) were gifts from Dr. Steven C. Clark, Genetics Institute, Cambridge, MA. (GM-CSF was also obtained from Genzyme Corp. and used in preliminary experiments). rTNF-α (5.02 $\times 10^7$ U/mg) and lymphotoxin (TNF-β) (1.28 $\times 10^8$ U/mg) were gifts from Dr. H. Michael Shepard, Genentech, Inc., South San Francisco, CA. Phenol-extracted LPS from *Escherichia coli* 0111:B4 was from Calbiochem-Behring Corp., La Jolla, CA.

Crude "macrophage-activating factor" was prepared from PBMC by stimulation with Con A-Sepharose (26). After 48 h, the supernatant medium was centrifuged and stored at 4°C for no more than a week. Control supernatant was prepared in the same manner except that Con A-Sepharose was omitted.

**Viral Production and Cytopathology Assays.** Cells were incubated for the times indicated and 0.05-0.1-ml aliquots were removed without refedding and stored at −20°C for later assay of p24 core antigen by ELISA (Abbott Laboratories, N. Chicago, IL). Additional aliquots were stored at −70°C and subsequently assayed for infectious HIV by terminal dilution assay, as described above. Wells were also serially observed by phase-contrast microscopy and scored for the presence of multinucleated giant cells (≥4 nuclei per cell).

**Electron Microscopy.** Macrophage cultures in Costar (Cambridge, MA) six-well plates were fixed for 24 h in 2.5% glutaraldehyde buffered to pH 7.4 with 0.1 M phosphate, washed for 1 h in phosphate buffer, and fixed for 30 min with 2% osmium tetroxide (buffered as for the glutaraldehyde). The fixed wells were then dehydrated through a graded series of ethanol to 90% and then passed through a graded series of hydroxypropyl methylacrylate from 90% in water to 100%. Next, wells were infiltrated with sequential mixtures of hydroxypropyl
methacrylate and Epon 812, starting at three parts to one part respectively and terminating in pure Epon 812 as previously described (27). Embedding was accomplished by quickly inverting and setting a Beem capsule filled with Epon 812 over areas of the culture that contained multinucleated giant cells or normal appearing areas. After polymerization for 48 h at 60°C, the wells were frozen at −70°C and the Beem capsules were snapped to remove the cells from the plastic dishes. The embedded cultures were mounted for sectioning either parallel or perpendicular to the culture surface. Initially, blocks were thick sectioned, stained with toluidine blue, and examined by light microscopy. Areas of interest were thin sectioned with a diamond knife and stained with saturated uranyl acetate in 50% ethanol followed by bismuth oxyxynitate hydrate (28). The sections were examined on a Zeiss 10A electron microscope.

Nucleic Acid Hybridization Studies. Total cellular RNA was isolated from macrophage cultures using a modification of the single-step acid guanidinium thiocyanate-phenol-chloroform procedure (29) (RNAzol; Cinna/Biotec Laboratories International, Inc., Friendswood, TX). DNA was also obtained from this extraction and further purified using proteinase K and DNAse-free RNAse. RNA or DNA were blotted onto nitrocellulose, hybridized with a 32p-labeled HIV-1 probe, pARV-2 (30), a partial proviral clone of HIV-1SF2 (the gift of Dr. Paul Luciw, University of California, Davis, CA), and analyzed by autoradiography.

Results

Time Course of HIV Infection in Untreated Macrophages. In the absence of any treatment, MDM readily supported the replication of the HTLV-IIIb-Lai macrophage-tropic strain of HIV-1. Immediately after infection, supernatants routinely contained <30 pg/ml of p24 core antigen (the threshold of detection in this assay), indicating the effectiveness of the washing procedure used to remove free input virus (Fig. 1). By day 1, however, a small amount of p24 (100–300 pg/ml) appeared in all supernatants, and may represent residual virus that was inaccessible to the washing procedure. Thereafter, p24 antigen in the supernatant markedly increased by day 6 and reached a plateau level in ~10 d. The final levels of p24 antigen achieved varied between experiments by an order of magnitude, which may reflect differences between macrophage donors or the day of culture on which the cells were infected (data not shown).

Ultrastructure of HIV-infected Macrophages. HIV infection of untreated MDM led to the formation of multinucleated giant cells by day 6 (Fig. 2). When fixed and embedded on the culture dish and sectioned perpendicular to its surface, a polarization of virion assembly was seen. Virions were most abundant in a region characterized by intracytoplasmic vesicles containing numerous interdigitating microvilli,
reminiscent of the plasmalemma found at the surface of macrophages. These areas appeared immediately apical to a region of very densely stained cytoplasm and extensive, dilated smooth endoplasmic reticulum ~5 μm above the surface of the culture dish (Fig. 2). Proceeding apically toward the free surface of the giant cell, the intracytoplasmic vesicles became larger and the number of virions per vesicle decreased. Although virions were occasionally found budding from the plasma membrane, most budding particles were seen in small intracytoplasmic vesicles (Fig. 3, A–C). Characteristically, vesicles with the highest number of virions had an electron-dense coating (Figs. 2 and 3).

Cumulatively, more than a thousand virions were seen in sections from untreated, HIV-infected cultures. In contrast, no multinucleated giant cells or virions were seen in cultures treated with 1,000 U/ml of either IFN-α or IFN-γ, 100 ng/ml LPS, or in uninfected cultures (see below). Also, there was no evidence of incomplete virion assembly or virus accumulation inside the cells treated with interferon or LPS.

Effects of Interferons, LPS, and Cytokines on HIV Infection in Macrophages. To evaluate the effects of INF-α, IFN-γ, and LPS, these agents were added to macrophage cultures immediately after HIV infection. A single addition of each of these agents was capable of reducing p24 antigen accumulation by 1,000-fold (Fig. 1). Antigen accumulation was prevented and not merely delayed, which indicates that the production of additional virions was prevented. However, a low-level (<1,200 pg/ml) of p24 antigen was found in the media of treated cultures in all experiments. While this is compatible with the persistence of input virus not removed by the washing procedure, the possibility that some virus replication resists these treatments cannot be excluded.

A dose-response study was performed to quantitate these effects further (Fig. 4). In four experiments, treatment with 100–1,000 U/ml (30–300 pM) of IFN-α or 10–100 U/ml (25–250 pM) of IFN-γ 48 h before infection was sufficient to reduce viral production maximally, as measured by the accumulation of p24 antigen in the supernatant 14 d later. The slope of the dose-response to LPS was consistently less steep than that of the interferons, and 100 ng/ml was required to reduce p24 antigen release 10-fold. In contrast, IL-4 was much less effective, and the apparent effects at high doses could be attributed to endotoxin contamination (data not shown).

In separate experiments (Fig. 5), IFN-β at 10–100 U/ml (3–30 pM) also protected MDM from HIV. A single addition of M-CSF (from Cetus Corp.) (Fig. 5) or repeated
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additions of M-CSF (data not shown) minimally enhanced the replication of HIV. (The larger form of M-CSF from Genetics Institute also did not affect HIV replication). However, because this study used an already highly permissive in vitro infection system, the replication-enhancing effects of cytokines such as M-CSF (6) may be masked by the high background. GM-CSF partially protected MDM from HIV in two experiments (one of which is shown in Fig. 5, using GM-CSF from Genetics Institute), but had no effect in two other experiments, and actually enhanced HIV replication in one experiment (data not shown). IL-1β, IL-2, IL-6, TNF-α, and lymphotoxin (TNF-β) were inactive (Fig. 5 and Table I). In addition, unstimulated PBMC do not constitutively release into their supernatant any factor active in this experimental system, whereas the lymphokine-rich supernatant of Con A-stimulated PBMC (which contained IFN-γ; data not shown) reduced p24 accumulation ~30-fold (Table I).

Since p24 core antigen accumulation might not adequately reflect the release of infectious virus, supernatants were used to inoculate additional macrophage indicator cultures, which in turn were evaluated for multinucleated giant cells and production of p24 antigen. Whereas supernatants from untreated cultures contained >10^3 TCID₅₀/ml, no infectious virus could be detected in the supernatants of cultures pretreated with IFN-γ (1,000 U/ml), IFN-α (1,000 U/ml), or LPS (1 μg/ml). To show that this was not due to carry over of interferon or LPS into the indicator cultures, neutralizing antibodies or polymyxin B were added respectively, but still no infectious virus could be detected in the treated cultures.

Kinetics of Interferon and LPS Effects. To evaluate the stage of infection at which IFN-α, IFN-γ, or LPS acted, MDM were washed four times and treated with high doses of IFN-γ, or IFN-α or LPS initiated at increasing intervals after infection. Interferons or LPS added as late as 3 d after infection with HIV still decreased the release of p24 antigen into the supernatant (Fig. 6). By day 6 after infection, how-
Figure 5. Log-log plot of the dose-response curves of anti-HIV treatments. MDM were pretreated for 18 h with the indicated doses of IFN-α, IFN-β, IL-6, GM-CSF, and M-CSF, then infected with HIV (MOI = 0.1), washed, and the treatments re-added. Supernatants were collected for p24 core antigen assay 10 d later, and the log_{10} of the mean p24 pg/ml of duplicate cultures is shown. Multinucleated giant cells were absent in cultures treated with ≥1,000 U/ml of IFN-α and ≥10 U/ml of IFN-β, but were not prevented by IL-6, GM-CSF, and M-CSF. Data for M-CSF from Cetus Corp. are shown, and are essentially identical to data for M-CSF from Genetics Institute.

| Cytokine      | Dose (U/ml) | Log_{10} mean p24 pg/ml | Cytopathology |
|---------------|-------------|-------------------------|---------------|
| IL-1β         | 0           | 5.04                    | +             |
|               | 1           | 4.98                    | +             |
|               | 3           | 5.00                    | +             |
|               | 10          | 4.59                    | +             |
|               | 30          | 4.04                    | +             |
| IL-2          | 1           | 4.80                    | +             |
|               | 10          | 4.85                    | +             |
|               | 100         | 5.05                    | +             |
|               | 1,000       | 4.76                    | +             |
| TNF-α         | 1           | 4.79                    | +             |
|               | 10          | 4.86                    | +             |
|               | 100         | 4.55                    | +             |
|               | 1,000       | 4.45                    | +             |
| Lymphotoxin   | 1           | 4.92                    | +             |
| (TNF-β)       | 10          | 5.11                    | +             |
|               | 100         | 5.08                    | +             |
|               | 1,000       | 4.86                    | +             |
| PBMC Control  | 3%          | 4.85                    | +             |
| Supernatant   | 10%         | 4.97                    | +             |
| (Percent volume) |         | 4.80                    | +             |
| PBMC-Con A    | 3%          | 3.32                    | -             |
| Supernatant   | 10%         | 3.41                    | -             |
| (Percent volume) |         | 3.18                    | -             |

MDM were pretreated with the above agents for 18 h, infected with HIV (MOI = 0.1), and washed four times to remove inoculum virus. After replacing the treatment agents, the cultures were incubated for 12 d. p24 core antigen was measured in the culture supernatants and they are reported as the log_{10} of the mean of duplicate cultures. Cytopathology was scored by the presence or absence of multinucleated giant cells.
FIGURE 6. Semi-log plot of the time course of effectiveness of anti-HIV treatments. In the same experiment as shown in Fig. 1, MDM were infected with HIV (MOI = 1) and washed four times before adding the treatment, in order to remove p24 core antigen that may have accumulated in the medium. Cultures were treated with IFN-α (5,000 U/ml), IFN-γ (5,000 U/ml), or LPS (10 µg/ml) at the indicated times after infection with HIV. The final levels of p24 accumulation in the supernatants were measured at intervals throughout the culture period. Only the final p24 levels at 15 d after infection are shown.

However, these agents were no longer effective in preventing the accumulation of p24 antigen.

Effects of Interferons and LPS on HIV Gene Expression in Macrophages. Although IFN-α, IFN-γ, and LPS suppressed the release of p24 antigen and infectious virus from MDM into their culture media, and eliminated the formation of virions seen by electron microscopy, these techniques did not completely rule out the expression of viral genes inside the cells. Consequently, nucleic acid hybridization was used to evaluate the formation of RNA transcripts and DNA provirus within these cells. Infected MDM that were not treated with cytokines or LPS contained HIV RNA and DNA (Fig. 7). In contrast, IFN-α, IFN-γ, or LPS-treated MDM showed no evidence of viral RNA transcripts or provirus formation. Thus, in the treated cultures, the expression of nonstructural genes and the formation of proviral DNA are also prevented.

Discussion

In this report, IFN-α, IFN-β, IFN-γ, and LPS were shown to protect cultured macrophages from productive infection with a macrophage-tropic strain of HIV-1.
Since immunological reactions lead to interferon production, anti-HIV immunity (either natural or induced by a suitable vaccine) may indirectly limit the spread of HIV infection by protecting uninfected cells, thereby augmenting cytotoxic mechanisms for the removal of cells that are already infected. Since several other cytokines do not protect macrophages in this system, the protection afforded by interferons and LPS must be specifically induced.

Interferons are known to inhibit the production of infectious retroviruses by interfering with virus assembly and release (31). Consistent with these late effects, we found that IFN-α, INF-γ, or LPS are effective even when added 3 d after exposure of cells to HIV at a multiplicity of infection of 1 TCID₅₀/cell. This observation suggests that these agents act at a point in the viral life cycle beyond the early events of virus binding, penetration, and uncoating. Interference with reverse transcription and the generation of proviral DNA, the next step in the retroviral replication, are suggested by the absence of detectable viral DNA in the treated macrophages. This might occur by inhibition of viral reverse transcriptase by interferon-induced 2-5A oligonucleotides, as reported for the avian myeloblastosis virus enzyme (32). However, this conclusion cannot be supported from the data for two reasons: (a) The method used to detect proviral DNA is not sensitive enough to detect low levels of DNA provirus. (b) The definition of interferon action is best established during a single cycle of viral replication (33); for example, in HIV-infected CEM cells (a T cell leukemia cell line), virus-producing cells are reinfected by their progeny viruses, resulting in the generation of multiple copies of DNA provirus per cell, which correlates with the cytopathic effects of HIV in this cell line (34). By analogy, if macrophages become reinfected by their progeny viruses and if multiple rounds of reinfec-
tion are necessary to sustain productive infection, then interferon-induced inhibition of virus release alone would yield data indistinguishable from an effect on reverse transcription. Consequently, further studies are required to clarify the mode of action of interferon and LPS and the fate of HIV upon entry into an interferon- or LPS-treated macrophage.

Further studies are also required to determine if all HIV isolates capable of infecting macrophages are equally susceptible to interferons or LPS inhibition. It is possible that HIV adapts to culture conditions as a result of in vitro mutation and selection pressure. By preparing our viral stock under endotoxin-free conditions, we may have inadvertently selected for an interferon- and LPS-sensitive strain of HIV. We intend to test this possibility using viruses isolated by a different technique (6).

LPS and interferons (especially IFN-γ) are though to "activate" certain macrophage functions, yet these agents protected MDM in these experiments. We found no evidence that LPS worked indirectly by inducing the secretion of interferon. Although LPS is known to stimulate macrophage production of IL-6 (IFNβ2), IL-6 has no protective effect on its own. Furthermore, no interferon was detectable in the supernatants from LPS-stimulated MDM using a plaque-reduction assay for vesicular stomatitis virus on MDBK cells (data not shown). Whatever the mechanism, these results indicate that inadvertent endotoxin contamination of media and/or serum (21) may confound the results of antiviral studies in macrophages. Endotoxin contamination may contribute to the differences between our results and those recently reported by Koyanagi et al. (35). These authors studied MDM that had been isolated by adherence, exposed to activated complement, detached by EDTA and
scraping, and infected in the presence of the detergent polybrene. Endotoxin levels were not monitored in either the MDM cultures or the PBMC cultures used to grow the viral stocks that they used. In contrast, we have used essentially endotoxin-free conditions and have verified that the MDM were not artifactually activated by the culture conditions. The uninfected MDM used in these experiments did not release IL-1 or TNF detected by bioassays, and did not contain detectable mRNA for IL-1, TNF, IL-6, or tissue factor, although LPS readily induced the expression of these genes (data not shown). This pattern of gene expression is consistent with a "resting" or nonactivated macrophage phenotype. By using MDM of defined activation phenotype, an additional element of experimental control is made available. These factors may account for the >10-fold lower level of viral replication in the control cultures of Koyanagi et al. (based on p24 antigen levels) as compared with the data reported here. Suboptimal viral replication may explain the sensitivity of their assay system to viral-enhancing agents, such as colony-stimulating factors. In contrast, our experiments may have used a nearly optimal system for viral replication, accounting for the sensitivity of our assay system to inhibitory agents.

Macrophages are important host cells for lentiviruses such as HIV (1-14) and visna-maedi (36, 37). In sheep, the restricted replication of visna-maedi virus in macrophages has been associated with the production of an unusual immune interferon (36, 37). Interestingly, an unusual type of acid-labile IFN-α has been detected in the serum of HIV-infected individuals (38). A protective function for this type of interferon has not yet been demonstrated, and its presence in HIV infection may actually correlate with a worsened prognosis (39).

Neutralizing antibody to IFN-α has been reported to increase the yield of HIV in PBMC cultures from HIV-infected individuals (40). Reciprocally, the addition of IFN-α or INF-β, but not IFN-γ, has been reported to suppress the replication of T-lymphotropic strains of HIV in PBMC (41, 42). In cell lines used as models of HIV infection, IFN-α and IFN-γ are similarly active (43-47). However, in HIV-infected individuals in vivo, IFN-α does not control non-HIV viral infections effectively (48, 49), and the induction of RNAse L activity by IFN-induced 2-5A is impaired in PBMC from AIDS patients in vitro (50). Consequently, it cannot be assumed that macrophages from HIV-infected individuals will be protected from HIV by interferons as efficiently as macrophages from the uninfected donors used in our experiments. Nevertheless, it is encouraging that macrophages from AIDS patients have an appropriate enhancement of H2O2 release after IFN-γ treatment in vivo and in vitro (51). In this report, we demonstrate the activity of IFN-α, IFN-β, and IFN-γ against a macrophage-tropic strain of HIV-1 using cultured macrophages from normal donors. Since the macrophage may be the first cell type to become infected after exposure to HIV (6, 14), it would be useful to monitor the effects of interferons on monocyte/macrophage infection in clinical trials with these agents.

Summary

To determine the effects of immunomodulatory agents upon HIV replication in macrophages, cultured monocyte-derived macrophages were treated with various substances and then infected with a macrophage-tropic strain of HIV-1. Pretreatment with rIFN-α, IFN-β, and IFN-γ, or bacterial LPS prevented viral replication in macrophages. In treated cultures, little or no infectious HIV or p24 core antigen
was released into the supernatant, no virions were seen by electron microscopy, no viral RNA or DNA was detectable in the cell lysates, and no cytopathology (as determined by multinucleated giant cell formation) occurred. In contrast, pretreatment with a wide dose range of recombinant IL-1β, IL-2, IL-4, IL-6, M-CSF, TNF, or lymphotoxin failed to protect macrophages from productive infection by HIV. A consistent effect of granulocyte/macrophage-CSF on HIV replication in macrophages was not observed.

In dose response studies, pretreatment with ~100 U/ml of IFN-α, ~10 U/ml of IFN-β, or ~100 U/ml of IFN-γ was sufficient to prevent virion release maximally and to prevent cytopathology completely. In kinetic studies, IFN-α, IFN-γ, or LPS were added to the macrophage cultures either before or after infection with HIV. Even when added 3 d after infection with a multiplicity of 1.50% tissue-culture infectious dose per cell, all three treatments markedly reduced virion release, suggesting that these agents act at a point in the viral life cycle beyond the early events of virus binding, penetration, and uncoating.

These data indicate that HIV replication in previously uninfected macrophages may be regulated by an inducible host cell mechanism. These findings may explain the restricted replication of HIV in macrophages in vivo and suggest an antiviral role for interferons in the therapy of HIV infection.

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