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Induction of Alpha Interferon by Human Immunodeficiency Virus Type 1 in Human Monocyte-Macrophage Cultures

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The induction of interferon (IFN) by human immunodeficiency virus type 1 (HIV-1) in primary, nonstimulated monocyte-macrophage cultures was studied. HIV-1 infection, as confirmed by p24 antigen levels in the cell supernatant, led to the production of alpha interferon (IFN-α) over 7 to 21 days following infection. In two of seven experiments, the IFN detected was acid labile. Coupled reverse transcription-polymerase chain reaction analysis confirmed the induction of IFN-α mRNA in cells of HIV-1-infected cultures.

One of the immunological abnormalities in patients with human immunodeficiency virus (HIV) infections is the pathological production of various interferons, including alpha interferon (IFN-α) (15, 17, 19). In many cases, the IFN-α detected belongs to an unusual subclass characterized by lability at low pH (4, 5, 17, 18). This acid-labile IFN-α (AL-IFN-α) has gained increasing recognition as a useful prognostic indicator of the disease (1, 5, 18). However, the cellular source of the IFN-α and AL-IFN-α has not yet been identified.

Cells of the monocyte-macrophage (M/M) lineage are important hosts for HIV type 1 (HIV-1) (11, 20), and these cells also have the ability to produce IFN-α (3, 12, 19). Although a recent study failed to show significant IFN-α production by macrophage colony-stimulating factor (M-CSF)-treated M/M in response to HIV-1 infection (9, 10), we report that in unstimulated M/M cultures, infection with a virus isolate propagated in M/M ($HIV\text{-}1_{BaL}$) initiated IFN-α gene transcription and led to IFN-α production.

Peripheral blood mononuclear cells were isolated from heparinized blood of healthy volunteers or from leukapheresis units (National Institutes of Health Blood Bank) by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). Monocytes were purified from mononuclear cells either by adherence (8) or by counterflow centrifugal elutriation in pyrogen-free phosphate-buffered saline (B&B/Scott, Fiskeville, R.I.) (22, 24). These methods provide >90 to 95% pure populations of monocytes. The B-cell content of elutriated monocytes was <1%.

Monocytes were infected with HIV-1/NIH/USA/1985/$HTLV\text{-}III_{BaL}$ as described earlier (21). After infection, the cells were washed to remove free virions and seeded in 96-well Costar microtiter plates (2×10^5 cells per well) in RPMI 1640 culture medium supplemented with 20% heat-inactivated fetal calf serum and antibiotics. The cultures were refed at 4- to 5-day intervals by substituting fresh medium for half of the cell supernatant (100 μl). At indicated intervals (Fig. 1, abscissa), supernatant samples were analyzed for HIV-1 p24 antigen by an enzyme-linked immunosorbent assay (ELISA) (DuPont Co., Wilmington, Del.) and for IFN bioactivity. The controls included noninfected cells and also infected cells in which viral replication was completely inhibited by 3′-azido-3′-deoxythymidine. Alternatively, elutriated monocytes were cultured for 7 days (in Costar flasks) in RPMI 1640 medium–20% fetal calf serum–10% pooled human AB serum (5×10^6 to 10×10^6 cells per flask) and were infected with HIV-1/NIH/USA/1985/$HTLV\text{-}III_{BaL}$ as described elsewhere (6, 7).

IFN levels were determined in a bioassay using murine encephalomyocarditis virus challenge on human foreskin fibroblast target cells (25). The IFN detected was serologically classified with rabbit anti-IFN-α polyclonal antibody (National Institutes of Health [NIH] standard anti-human IFN-α) (4). For acid stability measurements, concentrated samples were incubated at pH 2 for 24 h at 4°C (4). They were then readjusted to pH 7 and tested in the IFN bioassay as described above. IFN levels were normalized to the reference human IFN-α (NIH G023-901-527) standard.

To detect IFN-α mRNA, elutriated monocytes were cultured and infected in Costar flasks as described above. Cellular RNA was extracted after the cells were detached from the wells, and IFN-α mRNA was measured by coupled reverse transcription-polymerase chain reaction (PCR) amplification of cDNAs, as described previously (10).

Figure 1 shows the IFN titers and viral p24 antigen levels in the supernatant of adherence-purified M/M. IFN was not detected on day 6 following infection, but on day 11 the HIV-infected and p24 antigen-positive samples were also positive for IFN. On day 22, IFN was still present, although the titers had decreased. IFN was not observed in noninfected cells or in infected M/M in which HIV replication was effectively inhibited by 0.22 to 2 μM 3′-azido-3′-deoxythymidine (data not shown). Hence, HIV replication, rather than mere exposure of M/M to the virus inoculum, appeared to be responsible for the IFN production seen. Table 1 summarizes the results from additional experiments, including the studies on elutriated monocytes. In the period of 7 to 21 days following infection, IFN titers varied in the 2- to 25-IU/ml range. Treatment with polyclonal rabbit anti-IFN-α antibody resulted in a reduction of IFN titers to less than 2 IU, while the control recombinant IFN-β (Cytimmune; Lee Biomolecular Research Laboratories Inc., San Diego, Calif.) lost no activity. Anti-IFN-β and anti-IFN-γ caused no decrease in IFN titer. These observations indicate that the IFN detected was IFN-α. We also treated the samples with HCl

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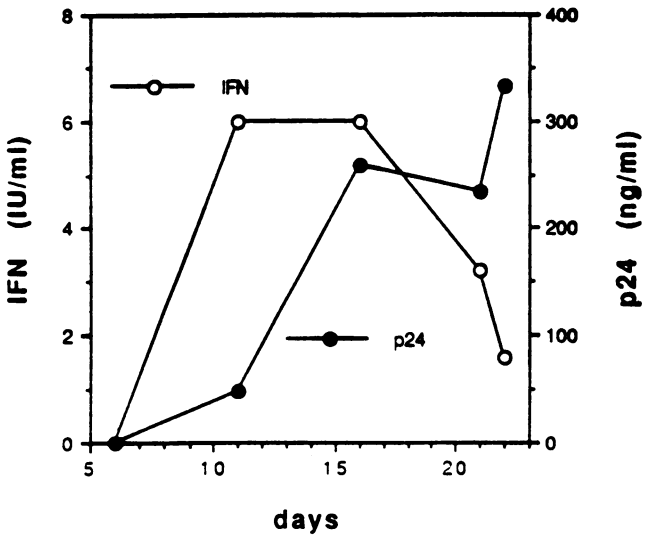

FIG. 1. IFN and p24 antigen production in HIV-infected human M/M cultures prepared from adherence-purified monocytes. IFN and viral p24 antigen production were determined by an IFN bioassay and a p24 ELISA as described in the text.

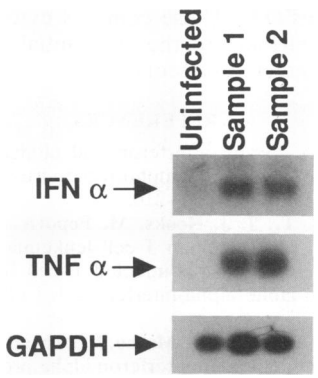

FIG. 2. Transcription of IFN-α gene in M/M cultures in vitro. The mRNAs for IFN-α, TNF-α, and the internal control glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were detected through antisense primer-directed cDNA synthesis and subsequent amplification of cDNAs by PCR (10). Reverse transcription was done with Moloney murine leukemia virus reverse transcriptase. PCR-amplified samples were Southern blotted on Nytran membranes with radiolabeled probes internal to the amplified sequences. Primer and probe sequences were given previously (10). The figure shows hybridization with IFN-α and TNF-α probes of a PCR-amplified sample 14 days postinfection; the sample had an IFN titer of 6.3 IU/ml. Positivity for TNF-α message serves as a control for infection with HIV-1, as this cytokine is known to be induced by HIV-1 (14, 16). Duplicate samples (samples 1 and 2) are shown.

at pH 2 to detect AL-IFN-α. In two of seven experiments in which this property was tested, such treatment markedly decreased the IFN titer, whereas recombinant IFN-α (Cytimmune) lost no activity after treatment with acid. However, no obvious relationship between the different conditions in our experiments and the appearance of AL-IFN-α emerged.

Next, we asked whether the HIV-elicited production of IFN-α was a consequence of induction of IFN-α gene transcription. Levels of IFN-α mRNA were estimated by coupled reverse transcription and PCR analysis (10). The Southern blot shown in Fig. 2 indicates the presence of IFN-α mRNA in the HIV-infected day 14 sample, which was also positive for tumor necrosis factor alpha (TNF-α) message and for IFN-α in the bioassay. Uninfected cells did not give positive IFN or TNF-α reactions, though they were positive for the constitutively expressed glyceraldehyde 3-phosphate dehydrogenase mRNA. These results suggest that induction of IFN-α by HIV-1 occurred at the transcriptional level.

Previous studies have implicated B cells in the production of AL-IFN-α (2, 3). The B-cell content of our elutriated monocyte preparation was <1%. Nevertheless, we cannot rule out a role for these or other contaminating cells in the observed acid-stable and acid-labile IFN-α production.

In conclusion, the results of the present study suggest that HIV infection leads to the production of detectable amounts of acid-stable and/or acid-labile IFN-α in unstimulated M/M cultures. Hence, this system may provide an in vitro model for the in vivo production of these IFNs in a high percentage of late-stage HIV-infected patients. Our observations are consistent with other studies demonstrating cytokine production by HIV-infected monocytes (transforming growth factor β, interleukin 1, and interleukin 6) (14, 16, 23) but contrast with data showing no significant induction of IFN-α in M-CSF-stimulated M/M infected with different HIV-1 isolates (9, 10). These M-CSF-stimulated cells also demonstrated a profound, specific transcriptional block of IFN-α production in response to various IFN inducers (9, 10). The difference between the results described in references 9 and 10 and our results raises the possibility that the activation state of M/M influences IFN-α production in response to HIV. This hypothesis is consistent with a recent study (12) showing that IFN production by cells of the M/M lineage is highly regulated by agents that affect the differentiation or activation state of these cells (e.g., granulocyte-macrophage colony-stimulating factor [GM-CSF] and IFN-γ). Culture of monocytes in M-CSF-containing medium resulted in cells that did not produce IFN-α in response to bacterial lipopolysaccharide (LPS), whereas cells primed with GM-CSF or IFN-γ maintained the IFN-α response to LPS (12). An inhibition of HIV-induced IFN production in M-CSF-treated M/M but not in unstimulated M/M is also consistent with the significant acceleration of HIV replication in M-CSF-stimu-

TABLE 1. IFN production in M/M cultures infected with HIV-1

Origin of cells[a]	Expt	Day[b]	IFN titer (IU/ml)[c]
Adherent	1	16	**6 (<1.6)**
Elutriated	2	12	10 (ND)
	3	7	2 (2)
	4	13	8 (8)
	5	13	8 (8)
	6	13	6 (6)
	7	14	**8 (<2)**
	8	8	25 (25)

[a] Adherent, monocytes purified by adherence to plastic; Elutriated, monocytes purified by counterflow centrifugal elutriation.

[b] Day of IFN assay postinfection.

[c] In this assay, 1 IU of IFN was equal to 2.5 laboratory units. The IFN titers in noninfected controls were <1.6 IU. Titers obtained after treatment with acid are given in parentheses. Boldface entries show titers of AL-IFN. ND, not done.

lated monocytes (13). These complex cytokine-cell interactions could contribute to the substantial variation of IFN levels in HIV-infected patients.

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