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Selective Decrease in Human Immunodeficiency Virus Type 1 (HIV-1)-Induced Alpha Interferon Production by Peripheral Blood Mononuclear Cells during HIV-1 Infection

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We previously reported that human immunodeficiency virus type 1 (HIV-1), herpes simplex virus (HSV), and Sendai virus induce higher levels of alpha interferon (IFN-α) in blood dendritic cells than in monocytes of healthy donors. In the present study, the levels of IFN-α induced by T-cell tropic (IIIb and RF) and monocytotropic (BaL) strains of HIV-1 and by HSV were significantly decreased in peripheral blood mononuclear cells (PBMCs) derived from subjects with asymptomatic and symptomatic HIV-1 infection. In contrast, Sendai virus, a paramyxovirus that induces proportionally more IFN-α in monocytes and B cells than do either HIV-1 or HSV, stimulated normal levels of IFN-α in PBMCs from the HIV-1-infected men. The IFN-α produced by PBMCs from the HIV-1-seropositive subjects was partially acid labile, whereas the IFN-α produced by PBMCs from the HIV-1-seronegative donors was acid stable. We hypothesize that there is a selective defect in IFN-α production by peripheral blood dendritic cells, whereas the host retains the IFN-α-producing capacity of monocytes and B lymphocytes. The loss of IFN-α production in response to HIV-1, herpesviruses, and possibly other pathogens could contribute to the progression of HIV-1 infection and to the development of AIDS.

Alpha interferon (IFN-α) is a cytokine produced by mononuclear leukocytes and is important in innate immunity to viral infections (7). This cytokine inhibits viral replication through induction of antiviral factors and by modulation of cytotoxic immune responses. Recent evidence obtained from mice with genetically deficient IFN-α systems confirms that IFN-α is critical in the control of acutely lytic and relatively noncytopathogenic viruses (28). In particular, infection of these mice with lymphocytic choriomeningitis virus, an arenavirus with chronic pathogenic properties similar to those of human immunodeficiency virus type 1 (HIV-1) (27), results in enhanced, disseminated disease.

Several lines of evidence suggest that HIV-1 replication, which causes chronic, persistent infection leading to AIDS, may be controlled in part by IFN-α. These include the association between primary viremia with the appearance of IFN-α in the blood of individuals with acute HIV-1 infection (1, 41) and the anti-HIV-1 effects of IFN-α in vivo (9, 21) and in vitro (33). The production of IFN-α by peripheral blood mononuclear cells (PBMCs) in vitro in response to HIV-1 (34), herpesvirus (herpes simplex virus [HSV]) (19, 22, 36, 37), paramyxovirus (Newcastle disease virus) (36), and rhabdovirus (vesicular stomatitis virus) (36, 42) decreases in association with the progression of HIV-1 infection. Presumably, this is due to a dysfunction in or a quantitative loss of peripheral blood dendritic cells, which we have recently shown are the predominant producers of IFN-α in response to HIV-1, HSV, and Sendai virus (a paramyxovirus) (11). In addition, abnormalities in IFN-α production by blood monocytes, which also produce IFN-α after stimulation with HIV-1, herpesvirus, and paramyxovirus (7, 11, 13, 15), could be involved in this defective cytokine response.

In the present study we investigated the IFN-α production induced by T-cell tropic and monocytotropic strains of HIV-1, HSV, and Sendai virus in PBMC cultures from subjects with a range of durations of HIV-1 infections. The data show that PBMCs were defective in the production of IFN-α in response to HIV-1 and HSV beginning in the early, asymptomatic phases of HIV-1 infection. In contrast, the capacity of PBMCs to produce IFN-α in response to Sendai virus was not significantly altered, even in the advanced stages of HIV-1 infection. This dichotomy in IFN-α production may be due to a selective defect in the function of circulating dendritic cells during HIV-1 infection.

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MATERIALS AND METHODS

Subjects. Anticoagulated blood (7 U of heparin per ml) was collected by venipuncture from randomly selected HIV-1-seronegative healthy donors and HIV-1-seropositive men in the Pitt Men's Study. The Pitt Men's Study, one of four sites in the Multicenter AIDS Cohort Study, is an ongoing natural history investigation of HIV-1 infection in homosexual and bisexual men (20). All blood specimens were processed within 6 h of collection on Ficoll-Hypaque density gradients (Sigma, St. Louis, Mo.) as described previously (11).

Viruses. Stock aliquots of HIV-1 IIIb and HIV-1 RF were propagated in the U937 promonocytic cell line (CRL 1593; American Type Culture Collection [ATCC], Rockville, Md.) by standard methods (11, 17, 34). The titers of these pools were $10^{8.5}$ 50% tissue culture infective doses ($TCID_{50}$s) per ml for HIV-1 IIIb and $10^{6.5}$ $TCID_{50}$s/ml for HIV-1 RF, as determined by cocultivation in phytohemagglutinin-stimulated PBMCs. A monocytotropic isolate of HIV-1, BaL, was propagated in primary peripheral blood monocytes/macrophages. The titer of this virus pool was $10^{5.25}$ $TCID_{50}$s/ml, as determined by assay in primary monocyte/macrophage cultures.

The Cantell strain of Sendai virus (VR-907; ATCC) was prepared by inoculation of the allantoic fluid of 11-day-old embryonated chicken eggs (White Longhorn chicken eggs; Penn State Poultry Farm, University Park, Pa.) (11). The titer of the Sendai virus in allantoic fluid was 2×10^4 hemagglutinating units per ml.

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HSV type 1 strain 75-0876 was derived from a liver specimen obtained at autopsy from a patient with a disseminated herpesvirus infection and was propagated in human foreskin fibroblasts (11). The virus titer was 1.2×10^8 PFU/ml, as determined by plaque assay in Vero cells.

The virus preparations and cell lines were negative for mycoplasma (DNA probe; Genprobe, San Diego, Calif.) and endotoxin (*Limulus* amebocyte lysate assay; Sigma). Virus preparations were cryopreserved in single-use aliquots to maintain comparability among the experiments. On the day that the viruses were added to PBMCs for IFN-α induction, they were thawed in a 37°C water bath, partially purified by pelleting in a Beckman microcentrifuge at $12,000 \times g$ for 2 h at 5°C, and resuspended in RPMI 1640 medium (Whittaker M.A. Bioproducts, Walkersville, Md.) supplemented with 10% heat-inactivated fetal calf serum (FCS; Hyclone Laboratories, Logan, Utah).

IFN-α induction. Induction of IFN-α was done by culturing 0.1 ml of virus with an equal volume of PBMCs in round-bottom 96-well polystyrene tissue culture plates (Corning Glass Works, Corning, N.Y.). The viruses were added to the PBMCs at the following concentrations in 0.1 ml each: HIV-1 IIIb, $1 \times 10^{7.5}$ $TCID_{50}$s; HIV-1 RF, $1 \times 10^{5.5}$ $TCID_{50}$s; HIV-1 BaL, $1 \times 10^{5.0}$ $TCID_{50}$s; Sendai virus, 2×10^1 hemagglutinating units; HSV-1, 1.2×10^5 PFU. The PBMCs were infected with 0.1 ml of each viral preparation at a concentration of 2.5×10^6 cells per ml in RPMI 1640 medium supplemented with 10% heat-inactivated FCS. Mock-stimulated PBMCs from the same subjects were included in each experiment and did not show evidence of IFN-α production. Culture supernatants were harvested for IFN-α determinations after 18 to 24 h of incubation at 37°C in 5% CO_2. The supernatants were centrifuged in a Beckman microcentrifuge at 12,000 $\times g$ for 2 h at 5°C prior to testing in order to remove virus or cellular components that might interfere with the IFN-α bioassay.

Flow cytometric analysis. A monoclonal antibody cocktail of anti-HLA DR fluorescein isothiocyanate (FITC), anti-CD3 phycoerythrin (PE), anti-CD19 PE, anti-CD16 PE, anti-CD56 PE, and anti-CD14 PE was used to determine the proportion of dendritic cells in the blood of healthy donors and HIV-1-infected volunteers (11). Flow cytometric analysis of $CD4^+$ and $CD8^+$ T-cell subpopulations was done with monoclonal antibody combinations of either CD3 FITC-CD4 PE, CD3 FITC-CD8 PE, or immunoglobulin G2 FITC-immunoglobulin G1 PE (Becton Dickinson, San Jose, Calif.). In these determinations, PBMCs in 50 μl of whole blood were labelled with the appropriate monoclonal antibodies, and an ammonium chloride buffer was used to lyse the erythrocytes in the specimens. The stained PBMCs were subsequently preserved in 1% formaldehyde (Polysciences, Warrington, Pa.) until analysis. Flow cytometric quantitation of $CD4^+$ and $CD8^+$ T-cell subsets was done in an EPICS Profile II flow cytometer (Coulter Cytometry, Hialeah, Fla.), and quantitation of dendritic cells was done in an EPICS Elite flow cytometer (Coulter Cytometry) by standard methods (11).

IFN-α bioassay and characterization. IFN titers were determined by a bioassay with WISH cells (CCL 25; ATCC) as indicator cells and encephalomyocarditis virus (VR-129B; ATCC) as a cytopathic challenge virus (11). IFN titers were calculated as the 50% inhibition of cytopathic effect. The data were adjusted to international units by correction against a recombinant IFN-α laboratory standard (Accurate Chemical, Westbury, N.Y.) which was previously calibrated to the international reference standard, Ga23-902-530 (National Institutes of Health, Bethesda, Md.), in our laboratory.

Characterization of the IFN samples was achieved by neutralization with type-specific antiserum (i.e., horse anti-human IFN-α polyclonal antiserum; Boehringer Mannheim, Indianapolis, Ind.) at a 10-fold excess concentration. The specimens were incubated with the anti-IFN-α antibodies at room temperature for 1 to 2 h prior to assay. Acid stability was tested by the stepwise addition of 1.5 M HCl (J. T. Baker, Phillipsburg, N.J.) to the specimens. Colorimetric pH indicator paper (Fisher Scientific, Pittsburgh, Pa.) was used to verify that the samples were adjusted to pH 2. Approximately 2 to 5 μl of HCl was required to acidify 100 μl of each of the samples. These samples were incubated at pH 2 overnight at 5°C, returned to pH 7 by the stepwise addition of 1 M NaOH (Fisher Scientific), and assayed. The portion of antiviral activity in the samples that was sensitive to acid treatment was calculated as follows: percent acid sensitive = 100 − [(antiviral titer after treatment with acid/antiviral titer of mock control) × 100]. The portion of antiviral activity neutralized by anti-IFN-α antibody was calculated as follows: percent neutralized = 100 − [(antiviral titer after treatment with antibody/antiviral titer of mock control) × 100]. Recombinant IFN-α (Accurate Chemical) and recombinant IFN-γ (Boehringer Mannheim) were included as positive and negative controls in these assays, respectively. As expected, only IFN-α was neutralized (>95%) by treatment with type-specific antiserum and only IFN-γ was neutralized (>95%) by treatment with acid (data not shown).

β_2-Microglobulin and neopterin assays. The levels of β_2-microglobulin and neopterin in serum were determined as markers of disease progression in studies of IFN-α production in HIV-1-infected individuals. β_2-Microglobulin and neopterin were measured by radioimmunoassay according to the manufacturers' instructions (Pharmacia, Fairfield, N.J., and Henning, Berlin, Germany, respectively). Serum specimens were stored at −70°C until the day of the assay.

TABLE 1. Clinical characteristics and IFN-α titers in sera of HIV-1-infected homosexual men

Donor no.	Duration of HIV infection (mo)[a]	$CD4^+$ cell no./mm^3	IFN-α titer in serum (log_{10} IU/ml)	Medications[b]
17	>99[c]	745	<1.0	None
16	14	722	<1.0	None
15	>100[c]	693	1.2	None
14	31	624	<1.0	None
13	43	496	<1.0	None
12	98	439	1.2	ZDV
11	48	437	1.3	Fluconazole
19	105	430	Not done	ZDV
10	13	429	2.2	ZDV
9	>94[c]	412	<1.0	ZDV; ddI
8	97	388	<1.0	None
7	38	386	<1.0	ZDV
6	87	334	Not done	None
5	>89[c]	292	Not done	None
4	74	289	<1.0	None
3	17	254	Not done	ZDV, Bactrim
2	>90[c]	206	1.9	ZDV, ddC, fluconazole, GCV, DAP
1	95	143	<1.0	ZDV, Bactrim
21	109	138	2.1	ZDV, ddI, ACV, PENT
20	83	46	1.4	Bactrim
22	103	35	1.9	D4T, DAP, PENT, IFN-α
23	55	29	<1.0	ZDV, DAP, PENT, GCV, Bactrim
18	111	22	1.3	ZDV, ddC, INH, PENT
24	78	15	1.7	ZDV, ddC, ddI, DAP

[a] The seroconversion date was estimated as the midpoint between the last visit when the subject was seronegative for HIV-1 and first visit when the subject was seropositive for HIV-1.
[b] ZDV, zidovudine; ddC, dideoxycytosine; GCV, ganciclovir; DAP, dapsone; ddI, dideoxyinosine; INH, isoniazid; PENT, pentamidine; ACV, acyclovir; D4T, stavudine; Bactrim, trimethoprim-sulfamethoxazole.
[c] HIV-1-seroprevalent subject at study entry.

RESULTS

Clinical status of HIV-1-infected subjects. The cohort consisted of five subjects who were HIV-1 seropositive on entry into the natural history study in 1984 and who had a duration of infection of >89 months at the time of these experiments and 19 subjects with a median duration of infection of 78 months (range, 13 to 111 months) after documented seroconversion (Table 1). The absolute numbers of blood $CD4^+$ T cells in these men ranged from 15 to 745 cells per mm^3, with a mean ± standard error of 334 ± 46 cells per mm^3. The cohort comprised 16 asymptomatic individuals and 8 men with AIDS-related symptoms. Most of the subjects (13 of 24) had reported use of at least one antiretroviral agent.

Production of IFN-α by PBMCs from HIV-1-infected subjects. PBMCs were tested for their ability to produce IFN-α in response to in vitro stimulation with HIV-1 IIIb, HIV-1 RF, HIV-1 BaL, Sendai virus, and HSV. These determinations revealed that many subjects had a marked inability to produce IFN-α in response to HIV-1 IIIb, HIV-1 RF, HIV-1 BaL, and HSV (Fig. 1). These viruses are very potent inducers of IFN-α in dendritic cells and relatively weak to moderate inducers of IFN-α in monocytes (11). In contrast to these data, normal levels of IFN-α were produced in response to Sendai virus, which is a strong inducer of IFN-α in both dendritic cells and monocytes and to a lesser degree in B lymphocytes. In additional analyses, the number of dendritic cells in PBMCs as defined by the HLA DR^+, $CD3^-$, $CD19^-$, $CD16^-$, $CD56^-$,

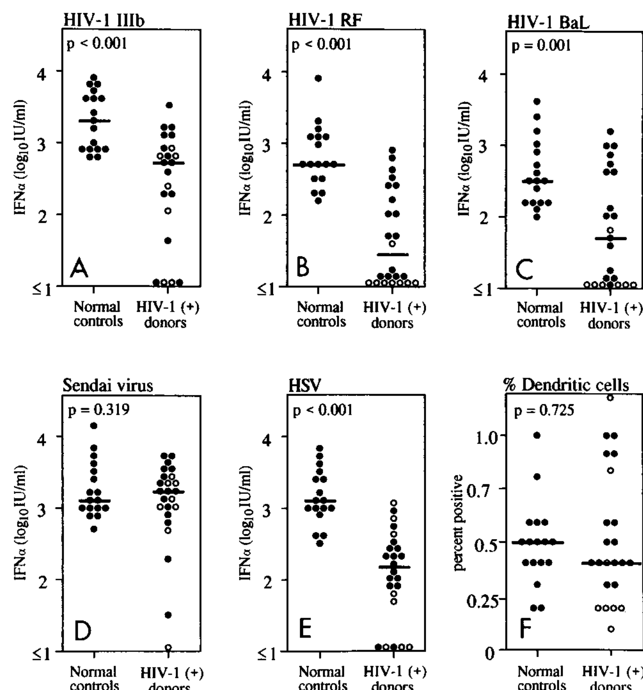

FIG. 1. IFN-α production by PBMCs from HIV-1-seronegative and HIV-1-seropositive subjects induced by HIV-1 T-cell tropic (IIIb [A] and RF [B] and monocytotropic (BaL [C]) strains, Sendai virus (D), and HSV type 1 (E) and the percentage of blood dendritic cells (F). The closed circles refer to asymptomatic subjects, and the open circles refer to subjects with <200 $CD4^+$ T cells per mm^3. Median values are depicted by bars. Differences between HIV-1-infected donors and controls were determined by one-way analysis of variance.

$CD14^-$ phenotype was estimated by flow cytometry (11). These determinations showed that the proportion of dendritic cells was not decreased in the PBMCs from these HIV-1-infected donors.

The relevance of defective IFN-α production was studied in the context of other parameters of HIV-1 infection (Table 2). Regression analysis revealed that defective production of IFN-α was significantly correlated with the duration of HIV-1 infection. The IFN-α titers produced in response to all viruses decreased by approximately 1 IU/ml for every month of HIV-1 infection in the cohort (range, 1.03 to 1.04 IU/ml).

Association of IFN-α production in vitro with serologic parameters of HIV-1 infection. Regression analysis of serum and lymphoid markers of HIV-1 infection was performed (Table 2). Among the serum markers, the levels of β_2-microglobulin and neopterin increased as a function of the duration of HIV-1 infection. IFN-α in serum was evaluable in 20 of 24 HIV-1-infected subjects (Table 1). A significant increase in the IFN-α titer in serum was observed as a function of the duration of infection (Table 2) ($P = 0.03$). Analysis of the percentages and absolute numbers of $CD4^+$ and $CD8^+$ T cells showed trends toward decreasing numbers of $CD4^+$ T cells and increasing numbers of $CD8^+$ T cells in these subjects.

We also examined correlations of changes in the in vitro production of IFN-α to changes in β_2-microglobulin, neopterin, IFN levels in serum and T-cell subset numbers in blood. The defects of IFN-α production were distinguished from these other parameters of HIV-1 infection in that IFN-α production in vitro was not significantly correlated to changes in any of these markers (data not shown).

Characterization of IFN. The antiviral activity in the supernatants of PBMC cultures stimulated with the five viruses was characterized as IFN-α by neutralization of ≥92% of the antiviral activity with specific antiserum (data not shown). In PBMCs from HIV-1-infected subjects, approximately 40 to 50% of the antiviral activity induced by HIV-1 IIIb and Sendai virus was sensitive to treatment at low pH (Table 3); this loss of IFN-α activity was significantly more than that noted in PBMC cultures from the HIV-1-seronegative controls. In contrast, the IFN-α induced by HIV-1 BaL was partially acid labile in cultures from both HIV-1-seronegative and HIV-1-seropositive subjects (Table 3). The IFN-α produced by stimulation with the other inducers, however, was predominantly acid stable in the cultures from both the HIV-1-seronegative and the HIV-1-seropositive subjects. No association was found between the production of acid-labile IFN-α in vitro and the presence of acid-labile IFN-α in the sera of the same individuals (data not shown).

TABLE 2. Regression analysis of parameters of HIV-1 infection as a function of duration of infection

Disease parameter	*r* value	Slope	*P* value
HIV-1 IIIb-induced IFN-α	0.393	−1.030	0.07
HIV-1 RF-induced IFN-α	0.572	−1.040	0.004
HIV-1 BaL-induced IFN-α	0.525	−1.040	0.01
Sendai virus-induced IFN-α	0.488	−1.030	0.02
HSV-induced IFN-α	0.470	−1.030	0.02
$CD3^+$ cell no.	0.017	0.273	0.94
$CD3^+$ cell percent	0.062	0.018	0.78
$CD4^+$ cell no.	0.300	−2.100	0.15
$CD4^+$ cell percent	0.430	−0.167	0.04
$CD8^+$ cell no.	0.121	1.390	0.57
$CD8^+$ cell percent	0.410	0.161	0.50
Dendritic cell percent	0.254	0.003	0.24
Serum neopterin level	0.374	0.112	0.07
Serum β_2-microglobulin level	0.501	0.015	0.01
Serum IFN-α level	0.488	0.011	0.03

DISCUSSION

The results of the study show that there was a significant defect in the production of IFN-α in vitro by PBMCs from HIV-1-seropositive subjects in response to T-cell tropic and monocytotropic strains of HIV-1 and to HSV. Production of IFN-α by PBMCs from HIV-1-seropositive subjects in response to a paramyxovirus, Sendai virus, however, was comparable to that of by PBMCs from healthy HIV-1-seronegative subjects. These data suggest that there is a selective impairment in the IFN-α response during HIV-1 infection that is dependent on the specific type of viral inducer.

We postulate that the selective decrease in IFN-α production relates to a defect in dendritic cell function in HIV-1-infected individuals. These cells are the major producers of IFN-α in response to HIV-1 and HSV infection in vitro, accounting for 50- to 60-fold and 16-fold more IFN-α than monocytes, respectively, on an equal-cell basis (11). The present results suggest that the number of circulating dendritic cells as defined by the $HLA\text{-}DR^+$ $CD3^-$ $CD19^-$ $CD16^-$ $CD56^-$ $CD14^-$ phenotype (38) was not decreased in the blood of homosexual men with either asymptomatic or symptomatic HIV-1 infection. These have also been defined as dendritic cells in our laboratory by their veiled, ruffled morphologies (11) and their potent stimulation of an allogeneic mixed leu-

TABLE 3. Characterization of IFN-α by acid treatment

Subject	Mean ± SE % (no. of subjects) antiviral activity sensitive to pH 2				
	HIV-1 IIIb	HIV-1 RF	HIV-1 BaL	Sendai virus	HSV
HIV-1 seronegative	4 ± 8 (17)	5 ± 7 (17)	30 ± 11 (17)	10 ± 8 (14)	15 ± 12 (14)
HIV-1 seropositive	51 ± 7 (16)[a]	15 ± 23 (7)	39 ± 12 (11)	38 ± 5 (19)[b]	21 ± 14 (9)

[a] $P < 0.0001$ compared with the HIV-1-seronegative subjects (Student's t test).
[b] $P = 0.003$ compared with the HIV-1-seronegative subjects (Student's t test).

kocyte response (34a). Thus, IFN-α production was defective in subjects with normal percentages of blood dendritic cells. In this regard, Howell et al. (19) showed that patients with AIDS had a reduced frequency of IFN-α-producing PBMCs and low levels of IFN-α produced on a per-cell basis in response to HSV infection in vitro. More refined experiments with flow cytometry-sorted preparations of dendritic cells from HIV-1-infected subjects will be needed to define the mechanism of defective IFN-α production.

The nature of the defect in IFN-α production by PBMCs in response to HIV-1 and HSV is unknown. IFN-α is actually a heterogeneous family of proteins encoded by multiple genes of the IFN-α superfamily (32). Activation of the IFN-α supergene family may occur through transmembrane signaling initiated by membrane-bound virus or after early steps in viral replication that involve viral or host double-stranded RNA. In this regard, induction of IFN-α by HIV-1 and HSV requires adsorption to their respective cell receptors but does not require complete virus replication (11–13). Moreover, increased levels of production of IFN-α occur at higher cell concentrations (5, 11), suggesting that an accessory factor is needed for the optimal synthesis of IFN-α mRNA and protein. Thus, the selective defect in IFN-α production during HIV-1 infection could be at either the primary or the accessory cell level. A further possibility is that persistent HIV-1 infection in vivo could hyperstimulate the production of IFN-α by dendritic cells, thus rendering them hyporesponsive to stimulation by viruses in vitro (39). Gendelman et al. (14) have in fact demonstrated decreased IFN-α production by cultured monocytes/macrophages in response to stimulation with poly(I):poly(C), Newcastle disease virus, and HSV.

The normal IFN-α response to Sendai virus in HIV-1-seropositive men may be due to retention of the IFN-α-producing capacities of monocytes and B lymphocytes. This is supported by our previous results which showed that Sendai virus induces more IFN-α in these PBMC subpopulations than does HIV-1 or HSV, even though dendritic cells produce the most IFN-α in response to all three viruses (11). Alternatively, a subpopulation of dendritic cells could maintain its IFN-α-producing capacity in response to Sendai virus. Of interest is that other investigators have reported that human blood dendritic cells are heterogeneous, consisting of at least two subsets that could differ in their functional capacities (30, 31).

A portion of the IFN-α produced by PBMCs from the HIV-1-seropositive subjects in vitro was acid labile. This may be related to the well-documented presence of circulating acid-labile IFN-α in AIDS patients (8, 10, 33). In contrast to other reports (3, 4, 26), we detected a substantial amount (30%) of acid-labile IFN-α only in PBMCs from HIV-1-seronegative donors that were stimulated with HIV-1 BaL. Our data suggest, therefore, that production of acid-labile IFN-α by PBMCs in vitro is primarily linked to HIV-1 infection in the intact host and is not a predominant characteristic of the IFN-α produced by the PBMCs of healthy donors. Finally, we found no association between the production of acid-labile IFN-α in vitro and the presence of acid-labile IFN-α in the HIV-1-infected subjects. Although the cellular origin and physicochemical state of acid-labile IFN-α have yet to be determined, there is evidence that acid-labile IFN-α is a conjugate of IFN-α and either an uncharacterized protein (43) or IFN-γ (4).

The role of IFN-α produced by dendritic cells in host immunity remains to be elucidated. We hypothesize that the cytokine is important in the early phase of the innate immune response to HIV-1 and other viral infections. IFN-α may operate in an autocrine and paracrine fashion to inhibit HIV-1 replication in dendritic cells and other cellular targets of the retrovirus (33). Diminution of IFN-α production could also lead to a decrease in natural killer cell lysis of HIV-1-infected targets (34). Therefore, loss of the IFN-α-producing capacity of dendritic cells, and possibly monocytes/macrophages, may result in enhanced HIV-1 replication.

A new concept is that the IFN-α produced by dendritic cells is important to their antigen-presenting function. It has been well-established that dendritic cells are highly effective stimulators of the primary responses of naive T cells to HIV-1 (24, 25) and other antigens (23, 38, 40). IFN-α regulates the expression of major histocompatibility class I and II molecules that are required for presentation of antigenic peptides to $CD8^+$ and $CD4^+$ T cells (7). Recently, IFN-α has been reported to upregulate IFN-γ (2) and interleukin 2 (18) production, presumably by type 1 $CD4^+$ T-helper cells. Thus, a defect in IFN-α production by dendritic cells, and possibly monocytes/macrophages, could have a significant effect on antiviral T-cell immune responses that are regulated by T-helper cells. It is pertinent that both the loss of IFN-α production (19, 22, 34, 36, 37, 42) and type 1 T-helper cell function (6, 16, 29, 35) have been associated with the progression of HIV-1 infection.

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REFERENCES

1. **Abb, J., R. Zachoval, V. Zachoval, and F. Deinhardt.** 1987. HIV antigen, HIV antibody and serum interferon in a patient with encephalopathy. Infection **15:**425–426.
2. **Brinkmann, V., T. Geiger, S. Alkan, and C. H. Heusser.** 1993. Interferon α increases the frequency of interferon γ-producing human $CD4^+$ T cells. J. Exp. Med. **178:**1655–1663.
3. **Capobianchi, M. R., F. DeMarco, P. DiMarco, and F. Dianzani.** 1988. Acid-labile human interferon alpha production by peripheral blood mononuclear cells stimulated by HIV-infected cells. Arch. Virol. **99:**9–19.
4. **Capobianchi, M. R., P. Mattana, F. Mercuri, G. Conciatori, F. Ameglio, H. Ankel, and F. Dianzani.** 1992. Acid lability is not an intrinsic property of interferon-α induced by HIV-infected cells. J. Interferon Res. **12:**431–438.
5. **Cederblad, B., and G. V. Alm.** 1990. Infrequent but efficient interferon-α-producing human mononuclear leukocytes induced by herpes simplex virus

in vitro studied by immuno-plaque and limiting dilution assays. J. Interferon Res. **10:**65–73.

6. **Clerici, M., F. T. Hakim, D. J. Venzon, S. Blatt, C. W. Hendrix, T. A. Wynn, and G. M. Shearer.** 1993. Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals. J. Clin. Invest. **91:**759–765.
7. **DeMaeyer, E., and J. DeMaeyer-Guignard (ed.).** 1988. Interferons and other regulatory cytokines. John Wiley & Sons, Inc., New York.
8. **DeStefano, E., R. M. Friedman, A. E. Friedman-Kien, J. J. Goedart, D. Henriksen, O. T. Preble, J. A. Sonnabend, and J. Vilcek.** 1982. Acid-labile human leukocyte interferon in homosexual men with Kaposi's sarcoma and lymphodenopathy. J. Infect. Dis. **146:**451–455.
9. **deWit, R., J. K. M. E. Schattenkerk, C. A. B. Boucher, P. J. M. Bakker, K. H. N. Veenhof, and S. A. Danner.** 1988. Clinical and virological effects of high-dose recombinant interferon-α in disseminated AIDS-related Kaposi's sarcoma. Lancet **ii:**1214–1217.
10. **Eyster, M. E., J. J. Goedart, M. C. Poon, and O. T. Preble.** 1983. Acid-labile alpha interferon. A possible preclinical marker for the acquired immunodeficiency syndrome in hemophilia. N. Engl. J. Med. **309:**583–586.
11. **Ferbas, J. J., J. F. Toso, A. J. Logar, J. S. Navratil, and C. R. Rinaldo, Jr.** 1994. $CD4^+$ blood dendritic cells are potent producers of IFN-α in response to in vitro HIV-1 infection. J. Immunol. **152:**4649–4662.
12. **Fitzgerald-Bocarsly, P.** 1993. Human natural interferon-α producing cells. Pharmacol. Ther. **60:**39–62.
13. **Francis, M. L., and M. S. Meltzer.** 1993. Induction of IFN-alpha in monocyte-enriched PBMC requires gp120-CD4 interaction but not virus replication. J. Immunol. **151:**2208–2216.
14. **Gendelman, H. E., R. M. Friedman, S. Joe, L. M. Baca, J. A. Turpin, G. Dveksler, M. S. Meltzer, and C. Dieffenbach.** 1990. A selective defect of interferon α production in human immunodeficiency virus-infected monocytes. J. Exp. Med. **172:**1433–1442.
15. **Grage-Griebenow, E., D. Lorenzen, R. Fetting, H.-D. Flad, and M. Ernst.** 1993. Phenotypical and functional characterization of Fcγ receptor I (CD64)-negative monocytes, a minor human monocyte subpopulation with high accessory and antiviral activity. Eur. J. Immunol. **23:**3126–3135.
16. **Graziosi, C., G. Pantaleo, K. R. Gantt, J.-P. Fortin, J. F. Demarest, O. J. Cohen, R. P. Sékaly, and A. S. Fauci.** 1994. Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals. Science **265:**248–252.
17. **Gupta, P., R. Balachandran, K. Grovit, D. Webster, and C. Rinaldo, Jr.** 1987. Detection of human immunodeficiency virus by reverse transcriptase activity, antigen capture assay, and radioimmunoassay. J. Clin. Microbiol. **25:**1122–1125.
18. **Holáň, V., K. Kohno, and J. Minowada.** 1991. Natural human interferon-α augments interleukin-2 production by a direct action on the activated IL-2-producing T cells. J. Interferon Res. **11:**319–325.
19. **Howell, D. M., S. B. Feldman, P. Kloser, and P. Fitzgerald-Bocarsly.** 1994. Decreased frequency of functional natural interferon-producing cells in peripheral blood of patients with the acquired immune deficiency syndrome. Clin. Immunol. Immunopathol. **71:**223–230.
20. **Kaslow, R. A., D. G. Ostrow, R. Detels, J. P. Phair, B. F. Polk, and C. R. Rinaldo, Jr.** 1987. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. Am. J. Epidemiol. **126:**310–318.
21. **Lane, H. C., J. A. Kovacs, J. Feinberg, B. Herpin, V. Davey, R. Walker, L. Deyton, J. A. Metcalf, M. Baselar, N. Salzman, J. Manischewitz, G. Quinnan, H. Masur, and A. S. Fauci.** 1988. Antiretroviral effects of interferon-α in AIDS-associated Kaposi's sarcoma. Lancet **ii:**1218–1222.
22. **Lopez, C., P. A. Fitzgerald, and F. P. Siegal.** 1983. Severe acquired immune deficiency syndrome in male homosexuals: diminished capacity to make interferon-α in vitro associated with severe opportunistic infections. J. Infect. Dis. **148:**962–966.
23. **Macatonia, S. E., C.-S. Hsieh, K. M. Murphy, and A. O'Garra.** 1993. Dendritic cells and macrophages are required for Th1 development of $CD4^+$ T cells from αβ TCR transgenic mice: IL-12 substitution for macrophages to stimulate IFN-γ production is IFN-γ-dependent. Int. Immunol. **5:**1119–1128.
24. **Mahta-Damani, A., S. Markowicz, and E. G. Engleman.** 1994. Generation of antigen-specific $CD8^+$ CTLs from naive precursors. J. Immunol. **153:**996–1003.
25. **Manca, F., G. Li Pira, D. Fenoglio, S. P. Fang, A. Habeshaw, S. C. Knight, and A. G. Dalgleish.** 1994. Dendritic cells are potent antigen-presenting cells for in vitro induction of primary human $CD4^+$ T-cell lines specific for HIV gp120. J. Acquired Immune Defic. Syndr. **7:**16–23.
26. **Matsuoka, H., Y. Kakui, A. Tanaka, Y. Cho, and J. Imanishi.** 1985. Acid-labile interferon produced in human peripheral leukocytes by induction with Sendai virus. J. Gen. Virol. **66:**2491–2494.
27. **Moskophidis, D., F. Lechner, H. Pircher, and R. Zinkernagel.** 1993. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature (London) **362:**758–761.
28. **Müller, U., U. Steinhoff, L. F. L. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet.** 1994. Functional role of type I and type II interferons in antiviral defense. Science **264:**1918–1921.
29. **Murray, H. W., D. A. Scavuzzo, C. D. Kelly, B. Y. Rubin, and R. B. Roberts.** 1988. $T4^+$ cell production of interferon gamma and the clinical spectrum of patients at risk for and with acquired immunodeficiency syndrome. Arch. Intern. Med. **198:**1613–1616.
30. **O'Doherty, U., M. Peng, S. Gezelter, W. J. Swiggard, M. Betjes, N. Bhardwaj, and R. M. Steinman.** 1994. Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. Immunology **82:**487–493.
31. **Patterson, S., J. Gross, P. Bedford, and S. C. Knight.** 1991. Morphology and phenotype of dendritic cells from peripheral blood and their productive and non-productive infection with human immunodeficiency virus type 1. Immunology **72:**361–367.
32. **Pestka, S., J. A. Langer, K. C. Zoon, and C. E. Samuel.** 1987. Interferons and their actions. Annu. Rev. Biochem. **56:**727–777.
33. **Pitha, P. M.** 1991. Multiple effects of interferon on HIV-1 replication. J. Interferon Res. **11:**313–318.
34. **Rappocciolo, G., J. F. Toso, D. J. Torpey III, P. Gupta, and C. R. Rinaldo, Jr.** 1989. Association of alpha interferon production with natural killer cell lysis of U937 cells infected with human immunodeficiency virus. J. Clin. Microbiol. **27:**41–48.

34a. **Rinaldo, C., et al.** Unpublished data.

35. **Rinaldo, C., P. Piazza, Y. Wang, J. Armstrong, P. Gupta, M. Ho, S. Petteway, D. Reed, D. Lyter, and L. Kingsley.** 1988. HIV-1-specific production of IFN-γ and modulation by recombinant IL-2 during early HIV-1 infection. J. Immunol. **140:**3389–3393.
36. **Rossol, S., R. Voth, H. P. Laubenstein, W. E. G. Müller, H. C. Schröder, K. H. Meyer zum Büschenfelde, and G. Hess.** 1989. Interferon production in patients with HIV-1. J. Infect. Dis. **159:**815–821.
37. **Siegal, F. P., C. Lopez, P. A. Fitzgerald, K. Shah, P. Baron, I. Z. Leiderman, D. Imperato, and S. Landesman.** 1986. Opportunistic infections in acquired immune deficiency syndrome result from synergistic defects of both the natural and adaptive components of cellular immunity. J. Clin. Invest. **78:** 115–123.
38. **Steinman, R. M.** 1991. The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. **9:**271–296.
39. **Stringfellow, D. A.** 1981–1982. Interferon induction: hyporesponsiveness and prostaglandins. Texas Rep. Biol. Med. **41:**116–121.
40. **Thomas, R., L. S. Davis, and P. E. Lipsky.** 1993. Comparative accessory cell function of human peripheral blood dendritic cells and monocytes. J. Immunol. **151:**6840–6852.
41. **von Sydow, M., A. Sönnerborg, H. Gaines, and O. Strannegård.** 1991. Interferon-alpha and tumor necrosis factor-alpha in serum of patients with various stages of HIV-1 infection. AIDS Res. Hum. Retroviruses **7:**375–380.
42. **Voth, R., S. Rossol, K. Klein, G. Hess, K. H. Schütt, H. C. Schröder, K.-H. Meyer zum Büschenfelde, and W. E. G. Müller.** 1990. Differential gene expression of IFN-α and tumor necrosis factor-α in peripheral blood mononuclear cells from patients with AIDS related complex and AIDS. J. Immunol. **144:**970–975.
43. **Yee, A. M. F., J. P. Buyon, and Y. K. Yip.** 1989. Interferon α associated with systemic lupus erythematosus is not intrinsically acid labile. J. Exp. Med. **169:**987–993.