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Interferon-β-Induced Human Immunodeficiency Virus Resistance in CD34$^+$ Human Hematopoietic Progenitor Cells: Correlation with a Down-Regulation of CCR-5 Expression

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To explore the possibility of conferring a long-term resistance against human immunodeficiency virus (HIV) by a low continuous production of interferon-β (IFN-β) in hematopoietic progenitor cells, we transduced the human CD34$^+$ TF-1 cells with a retroviral vector ensuring IFN-β production. The IFN-β-transduction of TF-1 cells resulted in resistance to infection with HIV-LAI, as shown by the selective survival of IFN-β-transduced CD4$^+$ cells and the protection against HIV-induced apoptosis. A similar response against HIV-LAI infection was obtained after pretreatment with 100 U/ml of recombinant IFN-α2b or IFN-β. In contrast, after the addition of macrophage cell tropic (M cell-tropic) HIV strain, a treatment with exogenous IFN-α2b resulted in a $\geqq$10-fold lower protection compared with exogenous IFN-β or IFN-β transduction. This specific effect of IFN-β on M cell-tropic HIV strains was correlated with a down-regulation of the CCR-5 chemokine receptor expression, corresponding to a novel antiviral effect of IFN-β.

INTRODUCTION

Several defects in hematopoiesis have been reported in human immunodeficiency virus (HIV)-infected individuals, leading to hematological disorders, including anemia, granulocytopenia, thrombocytopenia, and myelodysplastic/hyperplastic alterations of the bone marrow (Scaden *et al.,* 1989). Both direct killing and indirect apoptotic effects on CD34$^+$ hematopoietic progenitor cells have been observed in the presence of HIV. These hematopoietic progenitor cells are potential target cells for HIV-1 because 25–50% of CD34$^+$ cells express the CD4 cell surface marker, which serves as high-affinity receptor for HIV-1 (Louache *et al.,* 1994), and *in vitro,* the presence of HIV proviral sequences has been detected in the ensuing colonies of erythroid and myeloid lineages generated from HIV-infected CD34$^+$ cells (Chelucci *et al.,* 1995).

To establish an HIV resistance in CD34$^+$ progenitor cells, we investigated a somatic cell gene therapy based on the multiple antiretroviral activities of interferon (IFN)-β, which affects multiple stages of the HIV life cycle, including penetration, transcription, virion assembly, and budding (De Maeyer and De Maeyer-Guignard, 1998; Francis *et al.,* 1992; Michaelis *et al.,* 1989). Clinical trials with high concentrations of IFN-α have been conducted on patients with acquired immune deficiency syndrome, but only transient effects on the disease have been obtained (Lane *et al.,* 1990). Moreover, type I IFNs have induced frequent side effects in individuals, including fever, malaise, rashes, and headache, that sometimes result in interruption of the treatment. IFN-β has been described to be less toxic than IFN-α on hematopoietic progenitor cells (Broxmeyer *et al.,* 1983). Our approach consists of establishing an anti-HIV state in cells through the low continuous synthesis of IFN-β (Macé *et al.,* 1991). For this purpose, we constructed the HMB-K^{b}HuIFNβ retroviral vector carrying the human IFN-β coding sequence under the constitutive expression control of a murine H-2K^b gene promoter fragment (Vieillard *et al.,* 1997). We have previously shown that the IFN-β transduction of peripheral blood lymphocytes (PBLs) from uninfected or from HIV-infected donors inhibited virus replication, favored survival of CD4$^+$ T cells, enhanced Th-1-like cytokine production, and improved the proliferative response of CD4$^+$ T cells to recall antigens (Vieillard *et al.,* 1997). These observations prompted us to investigate the possibility of obtaining a long-term antiviral state in all possible target cells of HIV, including T cells, monocytes/macrophages, and dendritic cells, by transduction of CD34$^+$ hematopoietic progenitor cells with the HMB-K^{b}HuIFNβ retroviral vector.

To investigate the effects of a low constitutive expression of IFN-β on the HIV resistance of human CD34$^+$ cells, we used the CD34$^+$ TF-1 cell line as a model of hematopoietic progenitor cells (Kitamura *et al.,* 1989). We analyzed the HIV-1 resistance of IFN-β-transduced TF-1 cells and compared the effect of the addition of exogenous IFN-α2b or IFN-β on TF-1 cells, challenged with

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TABLE 1

Characteristics of Untransduced, Neo-transduced-, and IFN-β-transduced TF-1 Cells 4 Days after Onset of HIV-LAI Infection

	Percent transduction	IFN production (U/10^5 cells/48 h)	Percent of $CD4^+$-expressing cells
Experiment 1			
UT/UI		<3	44
UT/HIV		<3	18
IF-T/UI	34	25	45
IF-T/HIV	64	44	41
Experiment 2			
Neo-T/UI	42	<3	56
Neo-T/HIV	51	<3	18
IF-T/UI	39	32	45
IF-T/HIV	63	66	38

Note. UT, untransduced cells; IF-T, IFN-β-transduced cells; Neo-T, neo-transduced cells; UI, uninfected cells; HIV, HIV-LAI-infected cells.

either T cell-tropic or M cell-tropic HIV strains. We report a protective effect of IFN-β transduction against HIV-LAI infection, accompanied by a survival advantage of IFN-β-transduced TF-1 cells, resulting from a resistance to the apoptosis induced by HIV. In addition, our results demonstrate that IFN-β treatment blocked the infection of TF-1 cells by M cell-tropic HIV strains specifically and more efficiently than IFN-α2b; this was correlated with an inhibition of the CCR-5 chemokine receptor expression.

RESULTS

Enhancement of HIV-LAI resistance in IFN-β-transduced TF-1 cells. The effect of a low, constitutive expression of IFN-β on HIV-1 resistance in $CD34^+$ hematopoietic cells was examined using the $CD34^+$ TF-1 cell line transduced by the HMB-K^bHuIFNβ retroviral vector. As shown in Table 1, in two experiments, the cells were transduced with the IFN-β transgene with an efficacy of ~35% and produced a low level of IFN corresponding to ~28 U/10^5 cells/48 h, measured 4 days after the IFN-β transduction (Table 1). The analysis of cell surface markers revealed that 100% of untransduced, *neo*-transduced-, and IFN-β-transduced cells were strongly positive for the CD34 marker (data not shown), and ~50% of TF-1 cells expressed low levels of the CD4 marker (Table 1).

One day after IFN-β transduction, the cells were challenged with the T cell-tropic HIV-LAI strain at a m.o.i. close to 0.01. In untransduced control cells, the cell viability steadily decreased until day 4 and then increased at the same level as that for uninfected cells (Fig. 1A). This slight decrease in cell viability was correlated with an increase of HIV DNA copy number per cell, which steadily increased to reach an average copy number of 0.3 on day 4 and then declined progressively to disappear completely within 12 days (Fig. 1B). At this

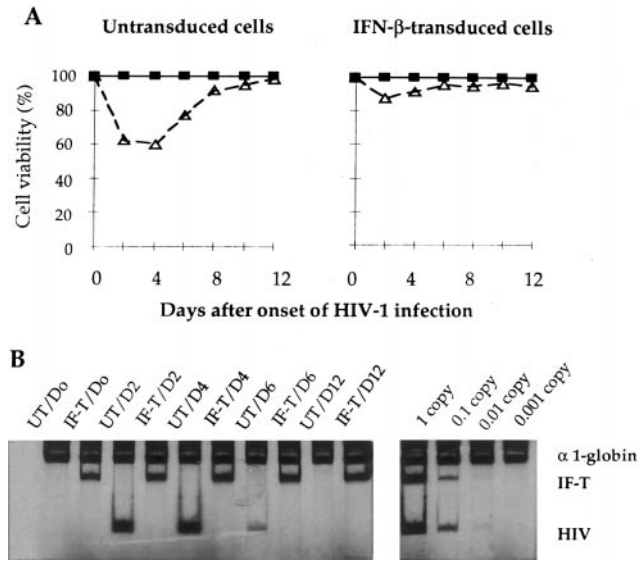

FIG. 1. Kinetics of the HIV resistance of IFN-β-transduced TF-1 cells. (A) Cell viability of untransduced and IFN-β-transduced cells. The percentage of cell viability was calculated every 2 days by dividing the total number of cells in the population infected by HIV-LAI (△) by the number of cells present in uninfected cultures (■), taken to be 100%. (B) Evolution of HIV DNA copies (HIV) and HMB-K^bHuIFNβ integrations (IF-T) in untransduced (UT) and IFN-β-transduced cell populations, measured at days (D) 0, 2, 4, 6, and 12 after HIV-LAI infection.

time, there was a ~2-fold decrease in the percentage of $CD4^+$-expressing cells (Table 1). The p24 production in these control cells remained close to background level (data not shown) (i.e., inferior to 30 pg/ml), indicating that HIV-LAI infection of TF-1 cells is not productive. As shown in Fig. 2, similar results were obtained with *neo*-

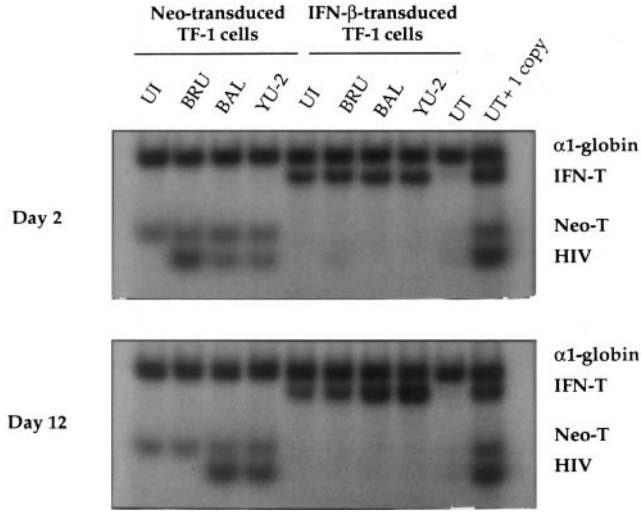

FIG. 2. Analysis of resistance against T cell-tropic (BRU) and M cell-tropic (BAL, YU-2) HIV strains of *neo*-transduced (neo-T) and IFN-β-transduced (IFN-T) TF-1 cells at days 2 and 12 after onset of HIV infection. UI indicates uninfected cells; HIV, HIV-infected cells; UT, untransduced cells; UT + 1 copy, one copy of *neo* transgene, IFN-β transgene, and HIV DNA.

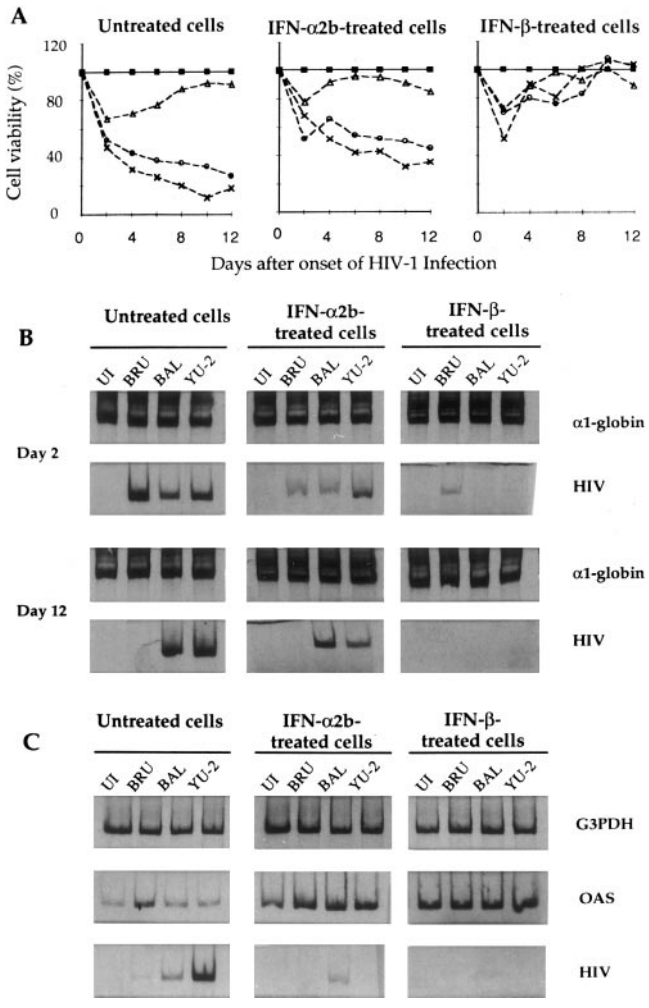

FIG. 3. Kinetics of resistance against T cell-tropic (BRU) and M cell-tropic (BAL, YU-2) HIV strains of untreated, IFN-α-treated, or IFN-β-treated TF-1 cells. (A) Cell viability of untreated, IFN-α2b-treated, and IFN-β-treated cells. The percentage of cell viability was calculated every 2 days by dividing the total number of cells in the population infected by HIV-LAI (△), HIV-BAL- (○) or HIV-YU-2- (X) infected cells by the number of cells present in uninfected cultures (■), taken to be 100%. (B) Evolution of HIV DNA copies (HIV) 2 and 12 days after onset of HIV addition. (C) RT-PCR analysis to detect the human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) transcripts, which serves as a quantitative control in this experiment, the OAS transcripts (Vieillard *et al.,* 1994), and the HIV-*gag* (HIV) messengers, quantified 4 days after the onset of HIV infection.

transduced TF-1 cells after the addition of the HIV-LAI infectious particles. We observed a temporary increase of the HIV DNA copy number per cell, to reach an average of 0.6 on day 2 and then to disappear at day 12.

In the IFN-β-transduced cells exposed to HIV-LAI, the cell viability was not altered (Fig. 1A), the number of HIV DNA copies per cell remained inferior to 0.01 (Figs. 1B and 2), and the percentage of $CD4^+$ cells remained at the level of ~55% observed in the uninfected cells (Table 1). Treatment with IFN-α2b or with IFN-β induced in TF-1 cells a similar resistance against the T cell-tropic HIV-LAI strain. Compared with untreated cells, we observed a low, temporary decrease in the cell viability (Fig. 3A), correlated with a number of HIV DNA copy per cell ~10-fold lower than that observed in untreated cells, 2 days after the onset of infection (Fig. 3B). All of these data indicated that a low production of IFN-β was sufficient to induce an antiviral state in TF-1 cells against the T cell-tropic HIV-LAI strain.

Interestingly, at 4 days after HIV-LAI infection, in both experiments, we observed in the population containing IFN-β-transduced cells a ~2-fold increase of IFN-β production (Table 1), correlated with an increase in the average number of HMB-K^bHuIFNβ integrations per cell close to 2 (Figs. 1B and 2), indicating a selective survival of the IFN-β-transduced cells among HIV-LAI-surviving cells.

IFN-β-transduction protects HIV-LAI-infected TF-1 cells from apoptosis. To further elucidate whether IFN-β transduction protects TF-1 cells against HIV-LAI strain in the absence of a productive infection, we investigated the presence and frequency of apoptotic cells. The exposure of untransduced TF-1 cells to HIV-LAI resulted in a significant increase in the percentage of apoptotic cells; 36% and 15% of apoptotic cells were detected, 7 and 15 days, respectively, after the addition of HIV-LAI compared with ~5% of apoptotic cells detected in uninfected control cells (Table 2). On the contrary, in IFN-β-transduced cells exposed to HIV-LAI, the percentage of apoptotic cells remained at a steady level of 6% (Table 2), indicating that the low, constitutive expression of IFN-β by TF-1 cells conferred protection against HIV-LAI-mediated apoptosis. To determine the effect of IFN-β-transduction on apoptosis, we used annexin V as a specific marker for the early events of apoptosis (Koopman *et al.,* 1994). As shown in Fig. 4 in *neo*-transduced control cells, we observed a high level of apoptotic cells (22%) 1 day after the addition of heat-inactivated HIV-LAI particles, suggesting that infection was not required for the induction of apoptosis. In contrast, in IFN-β-transduced cells, the level of early apoptotic events remained as low as that in the control cells (8%) (Fig. 4), confirming the

TABLE 2

Percentages of Apoptotic Untransduced and IFN-β-transduced TF-1 Cells after HIV-LAI Infection

	Percent of FITC-positive cells[a]	
	Day 7	Day 15
UT UI	4	5
UT HIV	36	15
IF-UI	4	2
IF-T HIV	6	6

Note. UT, untransduced cells, IF-T, IFN-β-transduced cells; UI, uninfected cells; HIV, HIV-LAI-infected cells.

[a] Days after HIV infection.

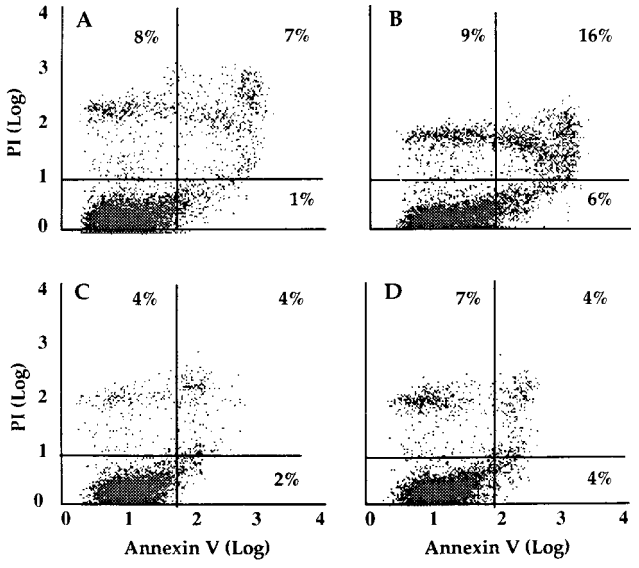

FIG. 4. Flow cytometric analysis of early events of apoptosis in *neo*- (A and B) and IFN-β- (C and D) transduced cells 1 day after the addition of heat-inactivated HIV-LAI particles (B and D). Cells were directly stained with annexin-V-FITC and propidium iodide (PI). The percentages of apoptotic cells corresponds to the total of the percentages of the upper and lower right quadrants.

protective effect of IFN-β against HIV-mediated apoptosis.

IFN-β induces high resistance against M cell-tropic HIV stains compared with IFN-α. We also investigated the effect of exogenously added IFN-α2 or IFN-β on the M cell-tropic HIV strains. To this purpose, TF-1 cells were cultivated in the presence of 100 U/ml IFN-α2b or IFN-β and infected with the HIV-BAL or HIV-YU-2. In both cases, the treatment with IFN-α2b or IFN-β resulted in a similar induction of the (2′-5′-oligoadenylate synthetase (OAS) expression (Fig. 3C). In untreated cells, we observed a dramatic decrease of the cell viability after infection with HIV-BAL or HIV-YU-2 (Fig. 3A). This decrease of cell viability was correlated with an increase in HIV DNA copy number per cell. Twelve days after the addition of the virus, ~42% of the cells were infected by HIV-BAL or HIV-YU-2, as shown by PCR analysis (Fig. 3B). In addition, a high level of HIV-BAL- or HIV-YU-2-*gag* RNA transcripts was detected 4 days after the onset of HIV infection (Fig. 3C), and a p24 production of ~200 pg/10^5 cells (Fig. 5A) was detected 6 days after the addition of the virus. This high HIV replication in TF-1 cells infected with M cell-tropic HIV strain was correlated with the presence of a large proportion of HIV infectious particles (Fig. 5B). In IFN-α2b-treated cells infected by HIV-BAL or HIV-YU-2, we observed a strong decrease in the cell viability (Fig. 3A). In contrast, the cell viability remained at a steady level of ~80% in IFN-β-treated cells (Fig. 3A). Moreover, in IFN-α2b-treated cells, we observed that ~14% or 6% of the cells were HIV infected, 12 days after the addition of HIV-BAL or HIV-YU-2, respectively (Fig. 3B), and low levels of HIV-*gag* transcripts were also detected in HIV-BAL-infected cells (Fig. 3C). In contrast, in IFN-β-treated cells, the levels of HIV DNA copies and of HIV RNA transcripts remained close to the background level (Figs. 3B and 3C). Furthermore, after the addition of 100 U/ml of IFN-β, the level of p24 release and the production of HIV infectious particles were ~5-fold lower compared with IFN-α2b-treated cells (Figs. 5A and 5B). We also observed in IFN-β-transduced cells a strong resistance against M-tropic HIV strains. As shown in Fig. 2, at 12 days after the addition of the virus, the level of HIV DNA copies remained close to background values. In addition, the level of p24 production remained inferior to 10 pg/10^5 cells (data not shown).

This demonstrated that the treatment of hematopoietic progenitor TF-1 cells with a low dose of IFN-β was more efficient than a treatment with a comparable amount of IFN-α2b, specifically against M cell-tropic HIV strains and suggested that the block of HIV infection by IFN-β occurred at a stage of viral DNA integration or before it.

Down-regulation of the CCR-5 chemokine receptor expression by IFN-β. To determine whether IFN-β-mediated resistance to M cell-tropic HIV strains resulted from a block that occurred at an early step of the HIV infectious cycle, we analyzed by RT-PCR the expression of two β-chemokine receptors identified as cofactors for the entry of HIV-1 into target cells. The CXCR-4 chemokine receptor was shown to act as a coreceptor for T cell-tropic strains and the CCR-5 as a coreceptor for M

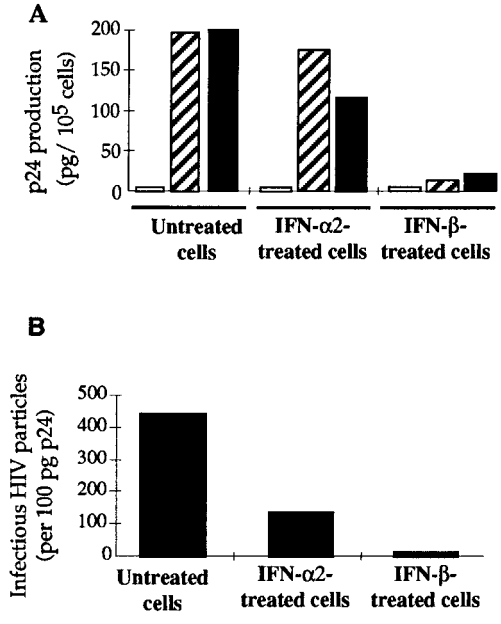

FIG. 5. Inhibition of HIV replication in IFN-α2b- and IFN-β-treated TF-1 cells 6 days after the onset of HIV addition. (A) p24 production in the culture medium of cells challenged with HIV-LAI (□), HIV-BAL (▨), or HIV-YU-2 (■) strains. (B) Titration of infectious HIV particles released by untreated, IFN-α2b-treated, or IFN-β-treated cells at 6 days after the onset of HIV-YU-2 infection, determined on P4.2 HeLa cells.

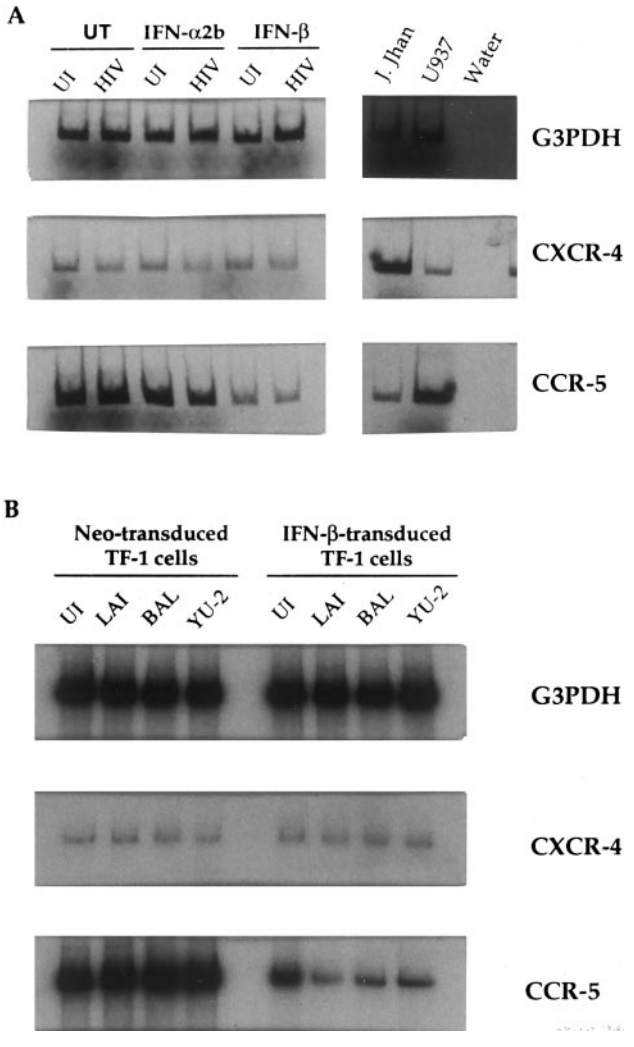

FIG. 6. Detection of CXCR-4 and CCR-5 mRNA by RT-PCR analysis. (A) Untreated (UT), IFN-α2b-treated, and, IFN-β-treated TF-1 cells, 4 days after the onset of HIV-YU-2 infection. For CXCR-4 mRNA, the T cell line J. Jhan served as positive control and water served as negative control. For CCR5 mRNA, the U937 promonocytic cell line served as positive control and water served as negative control. (B) *neo*-transduced and IFN-β-transduced TF-1 cells 4 days after the onset of HIV infection.

cell-tropic viruses (Berger *et al.,* 1998). Deichmann *et al.,* (1997) observed that CD34$^+$ hematopoietic progenitor cells expressed both the CXCR-4 and the CCR-5 coreceptors. As shown in Fig. 6A, TF-1 cells exhibited low levels of CXCR-4 transcripts, ~6.8-fold lower compared with the level of expression obtained in the J. Jhan CD4$^+$ T lymphocytic cell line. In addition, the level of CXCR-4 expression was slightly modified after a treatment with IFN-α2b or IFN-β (Fig. 6A). On the contrary, the CCR-5 receptor was highly expressed on TF-1 cells, and its expression was significantly decreased in IFN-β-treated cells. We observed 7.1-fold less abundant transcripts in IFN-β-treated cells compared with untreated cells and 4.7-fold lower compared with IFN-α2b-treated cells. In IFN-β-transduced TF-1 cells, we also detected a 7.0-fold decrease in the CCR-5 transcripts compared with *neo*-transduced TF-1 cells (Fig. 6B). As shown in Fig. 7, flow cytometry then confirmed that compared with control cells, in both IFN-β-transduced and IFN-β-treated cells, there was a ~4-fold decrease in CCR-5 cell surface expression. Furthermore, the effect of IFN-α2b on CCR-5 expression was 2-fold less pronounced than that of IFN-β.

These results demonstrate that IFN-β was more efficient than IFN-α2b to inhibit the infection with M cell-tropic HIV stains by the drastic decrease of the CCR-5 expression.

DISCUSSION

The aim of the present study was to investigate the possibility of conferring an antiviral state to CD34$^+$ TF-1 cells, as a model of hematopoietic progenitor cells, through a low constitutive expression of IFN-β and to elucidate mechanisms by which type I IFNs confer HIV resistance to these cells. We found that CD34$^+$ TF-1 cells can be transduced by the HMB-K^{b}HuIFNβ retroviral vector and that low constitutive expression of IFN-β offers significant protection against HIV-LAI infection, as shown by the survival of the CD4$^+$ fraction of TF-1 cells and the selective advantage of IFN-β-expressing cells among the IFN-β-transduced cell population. Moreover, the engagement of the TF-1 cells in the myeloid pathway could explain that a productive HIV infection occurred only in cells infected with M cell-tropic HIV strains (Kitamura *et al.,* 1989). Several studies suggest that the non-M-tropic strains can enter into macrophages, resulting into very low level virus replication (Schmidtmayerova *et al.,* 1992; Valentin *et al.,* 1990). The mechanism of this very low level infection has been related to a block in

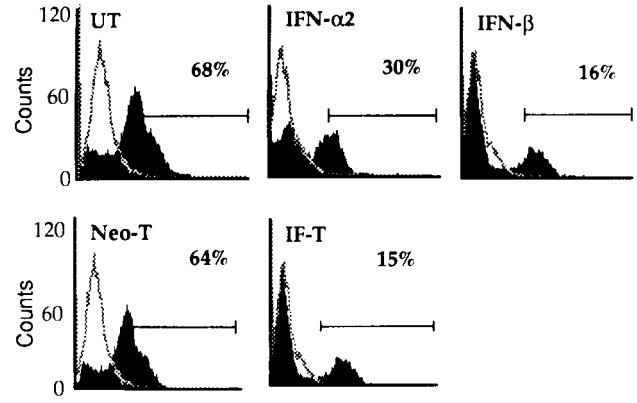

FIG. 7. Detection of CCR-5 expression by flow cytometry in untreated (UT), IFN-α2b-treated, and IFN-β-treated TF-1 cells 2 days after the addition of 100 U/ml recombinant IFN, as well as in *neo*- and IFN-β-transduced TF-1 cells, 2 days after transduction. The percentage of CCR-5 positive cells was determined by single color analysis. Cells were stained with anti-CCR-5-FITC monoclonal antibody; 10,000 events were acquired. White histograms show the isotype-matched controls.

reverse transcription. The reason for this observation is not known but could involve intracellular events influenced by the viral envelope and cytokine production (Schrier *et al.,* 1990).

In untransduced cells infected with the T cell-tropic HIV-LAI strain, we observed only a transitory infection, as shown by the presence of HIV DNA copies in cells, detectable during ~4 days after the addition of the virus, and the decrease in the percentage of the $CD4^+$ cells in the culture. Several studies have reported that indirect mechanisms acting on $CD34^+$ cells may be responsible for the AIDS-associated hematopoietic suppression (Donahue *et al.,* 1987; Molina *et al.,* 1990; Scadden *et al.,* 1989). This hypothesis is reinforced by analysis of the apoptosis induced by HIV. In untreated cells challenged with HIV-LAI, we observed a strong induction of apoptosis, as previously described by Zauli *et al.* (1994). In IFN-β-transduced cells, a similar level of apoptosis was detected in uninfected and HIV-LAI-infected cells. This prevention of apoptosis in IFN-β-transduced TF-1 cells could contribute to their high resistance to HIV-1 infection.

Additional experiments were performed to compare the effects of IFN-α2b and IFN-β on HIV resistance against M cell-tropic HIV strains. In cells infected by M cell-tropic HIV strains, we observed a higher HIV resistance conferred by IFN-β compared with IFN-α2b. After an IFN-α2b treatment, the number of HIV DNA copies and the production of HIV particles were not significantly different from untreated cells, but the infectivity of HIV-YU-2 particles was less pronounced. In cells treated with IFN-β, the virus production remained at a very low level. The inhibitory effect of type I IFNs on HIV replication has been shown to affect both early and late steps of the virus life cycle. Many studies have shown that in T cells and in monocytes, antiviral effects of IFN-α and -β take place at early steps of the HIV life cycle, resulting in a reduced viral DNA, RNA, and protein synthesis (Baca-Regen *et al.,* 1994; Meylan *et al.,* 1993). Shirazi *et al.* (1993) demonstrated that the inhibition of HIV replication was detected only when T cells were treated with IFN-α2b before HIV infection, indicating that IFN-α interferes with an early step of the HIV infectious cycle that occurs before the integration of the proviral DNA. In a previous report, we demonstrated that the inhibition of HIV replication in lymphocytes by IFN-β transduction occurred at a stage before reverse transcription (Vieillard *et al.,* 1994). For numerous viruses, including HIV, attachment to the receptor is followed by a redistribution or capping of the virus particles on the cell surface. IFN-β treatment induces an inhibition of the lateral mobility of the cell surface receptors for the lectin concanavalin A, resulting in a significant reduction in capping (Pfeffer *et al.,* 1980). In addition, some results suggest an important relationship between the lipid composition of the cell membrane and infection with some viruses. In this respect, it may be relevant that IFNs can block fusion of the HIV envelope protein and the cellular membrane (Aloia *et al.,* 1993). In addition, IFN-α2 exerts an effect on the terminal stage of the HIV life cycle. In IFN-α2-treated cells, the released HIV particles were 100- to 1000-fold less infectious compared with particles released from untreated cells because of a defect in gp120 assembly into the mature viral particle (Hansen *et al.,* 1992).

Our study provides evidence that the protection of TF-1 by IFN-β against M cell-tropic strains is partially mediated by down-regulation of the CCR-5 expression. The regulation of chemokine receptor expression by cytokines may be important for the regulation of chemokine action. Interleukin-2 has been reported to induce both CCR-1 and CCR-2 expression in T and NK cells (Loetscher *et al.,* 1996; Polentarutti *et al.,* 1997), and granulocyte colony-stimulating factor have been shown to up-regulate CXR-1 and CXR-2 expression in polymorphonuclear leukocytes (Lloyd *et al.,* 1995). In contrast, IFN-γ and tumor necrosis factor-α down-regulated CCR-2 and CXCR-2 expression, respectively (Asagoe *et al.,* 1998; Penton-rol *et al.,* 1998), and IFN-α down-regulated CXCR-4 expression on peripheral blood mononuclear cells (PBMCs) (Shirazi *et al.,* 1998). Of the chemokine receptors, CCR-5 is the most important for viral infection because it is the principal receptor for primary macrophage-tropic viruses, and individuals homozygous for a defective CCR-5 allele (delta32/delta32) are highly resistant to infection with HIV-1 (Dean *et al.,* 1996; Liu *et al.,* 1996; Samson *et al.,* 1996). In addition, anti-CCR-5 antibodies inhibit the infection of PBMCs by M cell-tropic HIV-1 strains (Wu *et al.,* 1997). Novel strategies are being implemented to examine mechanisms to sequester or prevent the expression of chemokine receptors to make cells resistant to infection with HIV (Cairns and D'Souza, 1998; Moore, 1997).

Gene therapy is based on the premise that insertion of anti-HIV genes into target cells will render them resistant to HIV infection and/or replication (Bridges and Sarver, 1995; Yu *et al.,* 1994). Many technological difficulties exist in adapting this strategy to large-scale clinical testing, including inefficient ability to target viral reservoirs, expensive, and labor-intensive *ex vivo* manipulations of target cells. Some of these obstacles may be resolved with the use of pluripotent stem cells with self-renewal capacity, which will permit the entry of gene therapy into the mainstream of therapeutic options available for infected individuals (Bahner *et al.,* 1996). Autologous or allogeneic transplantation with IFN-β-transduced progenitor cells thus might allow the permanent repopulation of all hematopoietic lineages of the immune system with intracellularly immunized cells. However, we observed that the HIV protection against M cell-tropic strains was $\geqq$10-fold less pronounced when HIV infection took place before IFN-β transduction (data not shown), suggesting that the antiviral effect of IFN-β is

more efficient in uninfected cells. This is in line with our previous results that demonstrated that efficient HIV resistance was obtained only in PBLs derived from HIV-infected donors with ≧200 $CD4^+$ T cells/mm^3 of peripheral blood (Vieillard *et al.,* 1997).

The present results, together with those previously reported, are encouraging for the further exploration of a gene transfer program using the IFN-β transduction of hematopoietic $CD34^+$ progenitor cells.

MATERIALS AND METHODS

Cell lines and IFNs. Human $CD34^+$ TF-1 cells (Kitamura *et al.,* 1989) were cultured at a density of 10^5 cells/ml in RPMI-1640 medium (Gibco Life Technologies, Cergy-Pontoise, France) supplemented with 10% fetal calf serum (FCS; ATGC Biotechnologie, Noisy le Grand, France) and 3 μg/ml human recombinant interleukin-3 (IL-3; R&D Systems, Abingdon, UK). Human P4-2 HeLa cells (Charneau *et al.,* 1994) were cultured in DMEM (Gibco) supplemented with 10% FCS. Recombinant human IFN-α2b (Introna, Schering Plough) and *Escherichia coli*-derived recombinant IFN-β were used at 100 U/ml.

HIV strains. HIV-LAI stock was prepared as described previously (Vieillard *et al.,* 1995) and corresponded to a reverse transcriptase activity of 2×10^6 cpm/ml with an infectivity of 3×10^6 $TCID_{50}$. HIV-YU-2 and HIV-BAL stocks were prepared by passage of the virus in human PBL cultures. At days 10–15 after infection, supernatant from the HIV-infected cultures were clarified by centrifugation, aliquoted, and stored at −80°C. Virus stocks were titered by triplicate end point dilution in PBMC cultures, using p24 antigen production. HIV-YU-2 stock contained 40 ng/ml p24 with an infectivity of 2.5×10^5 $TCID_{50}$. HIV-BAL stock contained 100 ng/ml p24. Inactivated HIV-LAI particles were obtained by heating at 59°C for 45 min as described previously (Zauli *et al.,* 1996).

IFN-β transduction of TF-1 cells. One million TF-1 cells were transduced with the HMB-K^bHuIFNβ retroviral vector by coculturing the cells for 3 days on Ψ-CRIP-HMB-K^bHuIFNβ packaging cells (Vieillard *et al.,* 1994; 1997) in IMDM medium (Gibco) supplemented with 10% FCS, 3 μg/ml IL-3, and 10 μg/ml protamine sulfate (Sigma-Aldrich, Saint Quentin Fallavier, France). The *neo*-transduced control cells consisted of TF-1 cells cocultured for 3 days on Ψ-CRIP-HMB-Neo packaging cells (Hawley *et al.,* 1989). The transduction efficacy was estimated by PCR amplification, and the absence of murine packaging cells was verified by PCR analysis with a murine α-globin set primer, as described previously (Vieillard *et al.,* 1997).

IFN titrations. IFN titrations were carried out on human WISH cells in microtiter plates (40,000 cells/well), using VSV as the challenge virus. Cells were incubated overnight in 0.15 ml with 2-fold IFN dilutions and then infected with vesicular stomatitis virus at 10^{-2} PFU/cell. One IFN unit corresponds to the reciprocal of the dilution protecting 50% of the cells.

Fluorescence-activated cell sorter analysis. TF-1 cells were incubated with fluorescein-isothiocyanate-conjugated anti-CD4 (Becton Dickinson, Le Pont de Claix, France), anti-CD34 (Becton Dickinson), or anti-CCR-5 (R&D Systems) monoclonal antibodies for 30 min. The cells were then washed three times with PBS/1% BSA, resuspended in PBS, and analyzed by flow cytometry on a FACScan (Becton Dickinson). Isotype-matched Ig served as negative controls (Becton Dickinson).

Quantification of HIV replication. Two million of untransduced, IFN-β-transduced, or IFN-treated TF-1 cells were infected at an m.o.i. of 0.01, with the T cell-tropic HIV-LAI strain or with the M cell-tropic HIV-BAL or HIV-YU-2 strains, during 2 h in the presence of 10 μg/ml protamine sulfate. After washing in PBS, the cells were resuspended in culture medium. Uninfected cell populations were run in parallel. Every 2 days, culture supernatants were collected and frozen at −80°C for subsequent assays, and the cells were resuspended in fresh culture medium. We determined the cell viability, the number of HIV DNA copies by PCR amplification, the presence of HIV RNA transcripts by RT-PCR, and virus released in the culture supernatants by an enzyme-linked immunosorbent assay for HIV p24 antigen (Dupont de Nemours, Les Ulis, France). The production of infectious HIV particles was determined on P4.2 HeLa cells, as described previously (Charneau *et al.,* 1994).

PCR analysis of HIV infection and of transduction efficacy. DNA extracts were prepared as described previously (Seif *et al.,* 1991). One hundred nanograms of DNA were amplified by PCR for 30 cycles in the presence of 1 μM [α-^{33}P]dCTP (10 mCi/mM; NEN Life Science Products, Le Blanc Mesnils, France). The following primers were used: a HIV *gag* primer set, 5′-ATCAAGCAGCCATGCAAAT-3′ and 5′-CTTTTGGTCCTTGTCTTATGTC-3′; a HMB-K^bHuIFNβ transgene primer set, 5′-GTTCAGGCAAAGTCTTAGTC-3′ and 5′-GATGATAGACATTAGCCAGG-3′; HMB-*neo* sequences, 5′-ATGGATTGCACGCAGGTTCT-3′ and 5′-CTCGCTCGATGCGATGTTTC-3′; and a α_1-globin primer set, 5′-ACCATGGTGCTGTCTCCTGC-3′ and 5′-GCATGGCCACGAGGCTCCA-3′. The PCR products were detected by autoradiography after electrophoresis on 4% nondenaturing polyacrylamide gels. DNA of each band was quantified using a PhosphorImager (Molecular Dynamics, Sevenoaks, UK). Serial 10-fold dilution of DNA preparations from HIV-LAI-infected J. Jhan cells and from plasmid-transfected U937 cells containing one copy of HIV or one copy of IFN-β-transgene per cell, respectively (Macé *et al.,* 1991; Vieillard *et al.,* 1995) were used as standards and amplified in each reaction to determine interassay variability and sensitivity.

Flow cytometric analysis of apoptotic cells. Detection of apoptotic cell death was realized using the terminal

deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) methodology (Piqueras *et al.,* 1996). TF-1 cells were fixed in a PBS solution containing 4% paraformaldehyde (PFA) for 1 h at 4°C washed three times in PBS/1% BSA and resuspended during 1 h at −20°C in 70% ethanol. The cells were incubated in buffer reaction containing dUTP fluorescein-conjugated (Promega, Charbonnières, France) for 30 min at 4°C. Ten units of TdT (Promega) were added for 1 h at 37°C, and 0.5 M EDTA was added to stop the reaction. The cells were washed three times in PBS/1% BSA, fixed 10 min in a PBS/1% PFA solution at 4°C, resuspended in PBS, and analyzed with the FACScan. Analysis of early events of apoptosis was realized with the Annexin-V apoptosis detection kit (R&D Systems) at day 1 after the addition of heat-inactivated HIV-BRU particles as described previously (Koopman *et al.,* 1994).

Chemokine receptor expression. Total RNAs were isolated using the RNA isolation kit (Stratagene, Montigny-le-Bretonneux, France). cDNA products were obtained from 1 μg of total RNA treated with DNase I (Promega) using the First Strand Synthesis kit (Pharmacia Biotech, Orsay, France). One-sixteenth of the cDNA products was amplified by PCR for 30 cycles in the presence of 1 μM [α-^{33}P]dCTP (10 mCi/mM; NEN Life Science Products) to detect the human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) transcripts as a quantitative control. To estimate chemokine receptor expression, cDNA products were amplified by PCR for 35 cycles, using a CXCR-4 primer set 5′-ACGTCAGTGAGGCAGATG-3′ and 5′-GATGACTGTGGTCTTGAG-3′ and a CCR-5 primer set 5′-GTCCAATCTATGACATCA-3′ and 5′-GGTGTAATGAAGACCTTC-3′. The reaction products were detected by autoradiography after electrophoresis on 4% nondenaturing polyacrylamide gels and were quantified using the PhosphorImager.

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REFERENCES

Aloia, R. C., Tian, H., and Jensen, F. C. (1993). Lipid composition and fluidity of the human immunodeficiency virus envelope and host cell plasma membrane. *Proc. Natl. Acad. Sci. USA* **90,** 5181–5185.

Asagoe, K., Yamamoto, K., Takahashi, A., Suzuki, K., Maeda, A., Nohgawa, M., Harakawa, N., Takano, K., Mukaida, N., Matsushima, K., Okuma, M., and Sasada, M. (1998). Down-regulation of CXCR2 expression on human polymorphonuclear leukocytes by TNF-α. *J. Immunol.* **160,** 4518–4525.

Baca-Regen, L., Heinzinger, N., Stevenson, M., and Gendelman, H. E. (1994). Alpha interferon-induced antiretroviral activities: restriction of viral nucleic acid synthesis and progeny virion production in human immunodeficiency virus type 1-infected monocytes. *J. Virol.* **68,** 7559–7565.

Bahner, I., Kearns, K., Hao, Q. L., Smogorzewska, E. M., and Kohn, D. B. (1996). Transduction of human CD34$^+$ hematopoietic progenitor cells by a retroviral vector expressing an RRE decoy inhibits human immunodeficiency virus type 1 replication in myelomonocytic cells produced in long-term culture. *J. Virol.* **70,** 4352–4360.

Berger, E. A., Doms, R. W., Fenyo, E. M., Korber, B. T., Littman, D. R., Moore J. P., Sattentau, Q. J., Schuitemaker, H., Sodroski, J., and Weiss, R. A. (1998). A new classification for HIV-1 *Nature* **391,** 240.

Bridges, S. H., and Sarver, N. (1995). Gene therapy and immune restoration for HIV disease. *Lancet* **345,** 427–432.

Broxmeyer, H. E., Lu, L., Platzer, E., Feit, C., Juliano, L., and Rubin, B. Y. (1983). Comparative analysis of the influences of human gamma, alpha and beta interferons on human multipotential (CFU-GEMM), erythroid (BFU-E) and granulocyte-macrophage (CFU-GM) progenitor cells. *J. Immunol.* **131,** 1300–1305.

Cairns, J. S., and D'Souza, M. P. (1998). Chemokines and HIV-1 second receptors: the therapeutic connection. *Nature Med.* **4,** 563–568.

Charneau, P., Mirambeau, G., Roux, P., Paulous, S., Buc, H., and Clavel, F. (1994). HIV-1 reverse transcription: A termination step at the center of the genome. *J. Mol. Biol.* **241,** 651–662.

Chelucci, C., Hassan, H. J., Locardi, C., Bulgarini, D., Pelosi, E., Mariani, G., Testa, U., Federico, M., Valtieri, M., and Peschle, C. (1995). In vitro human immunodeficiency virus-1 infection of purified hematopoietic progenitors in single-cell culture. *Blood* **85,** 1181–1187.

Dean, M., Carrington, M., Winkler, C., Huttley, G. A., Smith, M. W., Allikmets, R., Goedert, J. J., Buchbinder, S. P., Vittinghoff, E., Gomperts, E., Donfield, S., Vlahov, D., Kaslow, R., Saah, A., Rinaldo, C., Detels, R., and O'Brien, S. J. (1996). Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. *Science* **273,** 1856–1862.

Deichmann, M., Kronenwett, R., and Haas, R. (1997). Expression of the human immunodeficiency type 1 coreceptors CXCR-4 (fusin, LESTR) and CKR-5 in CD34$^+$ hematopoietic progenitor cells. *Blood* **89,** 3522–3528.

De Maeyer, E., and De Maeyer-Guignard, J. (1988). "Interferons and Other Regulatory Cytokines." John Wiley and Sons Ltd., Chichester.

Donahue, R. E., Johnson, M. M., Zon, L. I., Clark, S. C., and Groopman, J. E. (1987). Suppression of in vitro haematopoiesis following human immunodeficiency virus infection. *Nature* **326,** 200–203.

Francis, M. L., Meltzer, M. S., and Gendelman, H. E. (1992). Interferons in the persistence, pathogenesis, and treatment of HIV infection. *AIDS Res. Hum. Retroviruses* **8,** 199–207.

Hansen, B. D., Nara, P. L., Maheshwari, R. K., Sidhu, G. S., Bernbaum, J. G., Hoekzema, D., Meltzer, M. S., and Gendelman, H. E. (1992). Loss of infectivity by progeny virus from alpha interferon-treated human immunodeficiency virus type 1-infected T cells is associated with defective assembly of envelope gp120. *J. Virol.* **66,** 7543–7548.

Hawley, R. G., Sabourin, L. A., and Hawley, T. S. (1989). An improved retroviral vector for gene transfer into undifferentiated cells. *Nucleic Acids Res.* **17,** 4001.

Kitamura, T., Tange, T., Terasawa, T., Chiba, S., Kuwaki, T., Miyagawa, K., Piao, Y. F., Miyazono, K., Urabe, A., and Takaku, F. (1989). Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3, or erythropoietin. *J. Cell. Physiol.* **140,** 323–334.

Koopman, G., Reutelingsperger, C. P. M., Kuijten, G. A. M., Keekhen, R. M. J., Pals, S. T., and van Oers, M. H. J. (1994). Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood* **84,** 1415–1420.

Lane, H. C., Davey, V., Kovacs, J. A., Feinberg, J., Metcalf, J. A., Herpin, B., Walker, R., Deyton, L., Davey, R. T., and Faloon, J. (1990). Interferon-alpha in patients with asymptomatic human immunodeficiency virus (HIV) infection. *Ann. Intern. Med.* **112,** 805–811.

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R.,

MacDonald, M. E., Stuhlmann, H., Koup, R. A., and Landau, N. R. (1996). Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **86,** 367–377.

Lloyd, A. R., Biragyn, A., Johnston, J. A., Taub, D. D., Xu, L. L., Michiel, D., Sprenger, H., Oppenheim, J. J., and Kelvin, D. J. (1995). Granulocyte-colony stimulating factor and lipopolysaccharide regulate the expression of interleukin 8 receptors on polymorphonuclear leukocytes. *J. Biol. Chem.* **270,** 28188–28192.

Loetscher, P., Seitz, M., Baggiolini, M., and Moser, B. (1996). Interleukin-2 regulates CC chemokine resceptor expression and chemotatic responsiveness in T lymphocytes. *J. Exp. Med.* **184,** 569–577.

Louache, F., Debili, N., Marandin, A., Coulombel, L., and Vainchenker, W. (1994). Expression of CD4 by human hematopoietic progenitors. *Blood* **84,** 3344–3355.

Macé, K., Seif, I., Anjard, C., De Maeyer-Guignard, J., Duc Dodon, M., Gazzolo, L., and De Maeyer, E. (1991). Enhanced resistance to HIV-1 replication in U937 cells stably transfected with the human IFN-β gene behind an MHC promoter fragment. *J. Immunol.* **147,** 3553–3559.

Meylan, P. R. A., Guatelli, J. C., Munis, J. R., Richman, D. R., and Kornbluth, R. S. (1993). Mechanisms for the inhibition of HIV replication by interferon-α, -β, and -γ in primary human macrophages. *Virology* **193,** 138–148.

Michaelis, B., and Levy, J. A. (1989). HIV replication can be blocked by recombinant human interferon beta. *AIDS* **3,** 27–31.

Molina, J. M., Scadden, D. T., Sakaguchi, M., Fuller, B., Woon, A., and Groopman, J. E. (1990). Lack of evidence for infection or of effect on growth of hematopoietic progenitor cells after *in vivo* or *in vitro* exposure to human immunodeficiency virus. *Blood* **76,** 2476–2482.

Moore, J. P. (1997). Coreceptors: Implications for HIV pathogenesis and therapy. *Science* **276,** 51–52.

Penton-rol, G., Polentarutti, N., Luini, W., Borsatti, A., Mancinelli, R., Sica, A., Sozzani, S., and Mantovani, A. (1998). Selective inhibition of expression of the chemokine receptor CCR2 in human monocytes by IFN-γ. *J. Immunol.* **160,** 3869–3873.

Pfeffer, L. M., Wang, E., and Tamm, I. (1980). Interferon inhibit the redistribution of the cell surface components. *J. Exp. Med.* **152,** 469–474.

Piqueras, B., Autran, B., Debre, P., and Gorochov, G. (1996). Detection of apoptosis at single-cell level by direct incorporation of fluorescein-dUTP in DNA strand breaks. *Biotechniques* **20,** 634–640.

Polentarutti, N., Allavena, P., Bianchi, G., Giardina, G., Basile, A., Sozzani, A., Mantovani, A., and Introna, M. (1997). IL-2-regulated expression of the monocyte chemotactic protein-1 receptor (CCR2) in human NK cells: Characterization of a predominant 3.4 kb transcripts containing CCR2B and CCR2A sequences. *J. Immunol.* **157,** 2689–2694.

Samson, M., Libert, F., Doranz, B. J., Rucker, J., Liesnard, C., Farber, C. M., Saragosti, S., Lapoumeroulie, C., Cognaux, J., Forceille, C., Muyldermans, G., Verhofstede, C., Burtonboy, G., Georges, M., Imai, T., Rana, S., Yi, Y., Smyth, R. J., Collman, R. G., Doms, R. W., Vassart, G., and Parmentier, M. (1996). Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* **382,** 722–725.

Scadden, D. T., Zon, L. I., and Groopman, J. E. (1989). Pathophysiology and management of HIV-associated hematologic disorders. *Blood* **74,** 1455–1463.

Schmidtmayerova, H., Bolmont, C., Baghdiguian, S., Hirsch, I., and Chermann, J. C. (1992). Distinctive pattern of infection and replication of HIV-1 strain in blood-derived macrophages. *Virology* **190,** 124–133.

Schrier, R. D., McCutchan, J. A., Venable, J. C., Nelson, J. A., and C. A. Wiley (1993). T-cell-induced expression of human immunodeficiency virus in monocytes/macrophages. *J. Virol.* **64,** 3280–328.

Seif, I., Lauret, E., and Riviere, I. (1991). Isolation of DNA from cells and from enzyme reactions. *Methods Mol. Cell. Biol.* **2,** 216–217.

Shirazi, Y., and Pitha, P. M. (1993). Interferon α-mediated inhibition of human immunodeficiency virus type 1 provirus synthesis in T-cells. *Virology* **193,** 303–312.

Shirazi, Y., and Pitha, P. M. (1998). Interferon downregulates CXCR4 (fusin) gene expression in peripheral blood mononuclear cells. *J. Hum. Virol.* **1,** 69–76.

Valentin, A., Albert, J., Fenyo, E. M., and Asjo, B. (1990). HIV-1 infection of normal human macrophage cultures: Implication for silent infection. *Virology* **177,** 790–794.

Vieillard, V., Lauret, E., Rousseau, V., and De Maeyer, E. (1994). Blocking of retroviral infection at a step prior to reverse transcription in cells transformed to constitutively express interferon β. *Proc. Natl. Acad. Sci. USA* **91,** 2689–2693.

Vieillard, V., Lauret, E., Maguer, V., Jacomet, C., Rozenbaum, W., Gazzolo, L., and De Maeyer, E. (1995). Autocrine interferon-β synthesis for gene therapy of HIV infection: Increased resistance to HIV-1 in lymphocytes from healthy and HIV-infected individuals. *AIDS* **9,** 1221–1228.

Vieillard, V., Cremer, I., Lauret, E., Rozenbaum, W., Debré, P., Autran, B., and De Maeyer, E. (1997). Interferon β transduction of peripheral blood lymphocytes from HIV-infected donors increases Th1-type cytokine production and improves the proliferative response to recall antigens. *Proc. Natl. Acad. Sci. USA* **94,** 11595–11600.

Wu, L., Paxton, W. A., Kassam, N., Ruffing, N., Rottman, J. B., Sullivan, N., Choe, H., Sodroski, J., Newman, W., Koup, R. A., and Mackay C. R. (1997). CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. *J. Exp. Med.* **185,** 1681–1691.

Yu, M., Poescha, E., and Wong-Staal, F. (1994). Progress towards gene therapy for HIV infection. *Gene Therapy* **1,** 13–26.

Zauli, G., Vitale, M., Carla Re, M., Furlini, G., Zamai, L., Falcieri, E., Gibellini, D., Visani, G., Davis, B. R., Capitani, S., and La Placa, M. (1994). In vitro exposure to human immunodeficiency virus type 1 induces apoptotic cell death of the factor-dependent TF-1 hematopoietic cell line. *Blood* **83,** 167–175.

Zauli, G., Vitale, M., Gibellini, D., and Capitani, S. (1996). Inhibition of purified CD34+ hematopoietic progenitor cells by human immunodeficiency virus 1 or gp120 mediated by endogenous transforming growth factor β1. *J. Exp. Med.* **183,** 99–108.