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Antiviral Research 24 (1994) 221–233

Antiviral Research

Interferons in the pathogenesis and treatment of human immunodeficiency virus infection

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(Received 19 January 1994; accepted 14 February 1994)

Abstract

There still remains several unanswered questions concerning the pathogenesis of human immunodeficiency virus (HIV) infection. Interferons (IFNs), as well as other cytokines, are both dysregulated in HIV infection and serve as effector molecules that modulate the replicative capacity of HIV. Acid-labile IFN-α, an aberrant form of interferon earlier described in certain autoimmune diseases, has been detected in HIV-infected individuals. Conversely, a deficient expression of IFN-α may occur usually associated with HIV disease. Although conflicting findings have been reported on whether IFN-γ, a product of activated T and natural killer (NK) cells, is elevated in the peripheral blood (PB) compartment, high levels of its expression have been observed in the germinal centers of the lymph nodes during HIV disease. IFN-α and IFN-β have shown potent anti-retroviral effects in several in vitro systems of both acute and chronic HIV infection. These findings have served as the basis of the rationale for their therapeutic application, resulting in some positive effects at least in those patients with relatively high $CD4^+$ T cell counts and healthy immune functions. Furthermore, IFN-α has shown important therapeutic effects on HIV-associated Kaposi's sarcoma (KS). Both suppressive and inductive effects on HIV replication in vitro have been described for IFN-γ, whereas no clear clinical benefits have been reported following its administration to HIV-infected individuals. In conclusion, IFNs are involved in several pathogenic aspects of HIV infection and AIDS, and certain IFNs may serve as important tools to limit the spread of the virus and the progression of disease.

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SSDI 0166-3542(94)00018-4

Key words: Interferon; HIV; Anti-retroviral effect; Pathogenesis

1. Introduction

Since the recognition that the human immunodeficiency virus (HIV) causes a progressive deterioration of the human immune system culminating in the acquired immunodeficiency syndrome (AIDS), the search for factors that could control the replication of the virus has begun. In addition to regulatory proteins encoded by HIV itself, such as Tat and Rev (reviewed by Weiss, 1993), several host-related factors have been shown to be capable of up- or down-regulating the replication of HIV, including chemical and physical agents and several cytokines (reviewed by Poli and Fauci, 1993). Within the complex and redundant cytokine network, certain molecules such as tumor necrosis factor alpha (TNF-α) and -β have clearly shown the ability to induce HIV replication in vitro. In contrast, interferon-α (IFN-α) is a potent suppressor of multiple steps of the virus life cycle; similar results have been obtained for IFN-β. The potential role played by IFN-γ in HIV expression is complex manifesting both positive and negative regulatory effects: the same is true for certain other cytokines, such as Transforming Growth Factor beta (TGF-β) and IL-4 (Poli and Fauci, 1993). Our discussion will focus on the best characterized effects of different IFNs on HIV replication, that may serve as the basis for current and future clinical use of these agents.

1.1. IFN-α

High levels of IFN-α have been reported in HIV-infected individuals (Krown et al., 1991; Grunfeld et al., 1991; von Sydow et al., 1991), although a defect in the synthesis of IFN-α mRNA and protein was found in their PB mononuclear cells (PBMC) (Voth et al., 1990). This apparent discrepancy is likely to be explained by the presence of the so-called "acid labile IFN-α" (Rossol et al., 1989; Lau and Livesey, 1989; Rinaldo et al., 1990; Lau et al., 1991; Capobianchi et al., 1992a), that was previously described in autoimmune diseases such as systemic *lupus erythematosus* and rheumatoid arthritis. Acid-labile IFN-α may represent a dysfunctional IFN that may lead to immune suppression by inhibiting the production or effect of normal IFN (Hess et al., 1991). Although it is still unclear whether a molecular form corresponding to "acid-labile IFN-α" truly exists, it has been shown in HIV-infected individuals that it is actually a mixture of both IFN-α species and IFN-γ (Capobianchi et al., 1992a).

The HIV envelope component gp 120 has been shown to induce IFN-α in vitro from PBMC or monocytes of seronegative donors upon interaction with the CD4 molecule (Capobianchi et al., 1992b; Francis and Meltzer, 1993). However, a deficient synthesis and production of IFN-α, reminiscent of the ex vivo findings with PBMC (Voth et al., 1990), has been shown to occur as a consequence of productive HIV infection of monocyte-derived macrophages (MDM) (Gendelman et al., 1990a).

Addition of IFN-α to phytohemagglutinin (PHA)-stimulated PBMC infected in vitro with HIV-1 has resulted in a profound suppression of virus production, in association with decreased synthesis of viral proteins (Ho et al., 1985); similar findings have been observed in acutely infected $CD4^+$ T cell lines (Yamamoto et al., 1986; Hartshorn et al., 1987; Yamada et al., 1988; Bednarik et al., 1990; Shirazi and Pitha, 1992; Popik ans Pitha, 1991; Popik and Pitha, 1992).

At the molecular level, IFN-α causes a block in the life cycle of HIV occurring after viral entry and before or at the beginning of the reverse transcription process in acutely infected T cells (Shirazi and Pitha, 1992). In addition, endogenous IFN-α suppressed the activation of the HIV-1-LTR-CAT in studies where cells lines were permanently or transiently transfected with an IFN-α gene driven by the HIV-1 LTR. This effect was correlated with the inhibition of binding of a 45 kD NF-kB complex to the core/enhancer region of the viral LTR (Popik and Pitha, 1991; Popik and Pitha, 1992). IFN-α-mediated suppression of the HIV LTR has been also observed in neuroblastoma and glioma cell lines (von Briesen et al., 1991). In MDM, IFN-α is a potent inhibitor of HIV replication, likely acting at multiple steps of the virus life cycle, including reverse transcription, de novo transcription and post-transcriptional events (Kornbluth et al., 1989; Gendelman et al., 1990b; Gendelman et al., 1990c; Meyelan et al., 1993).

Certain IFN-α effects on HIV-infected cells are similar to those earlier described for cells infected with other viruses and retroviruses (Friedman and Pitha, 1984). For example, IFN-α-treated cells appear to produce a significantly less progeny of infectious virions than untreated control cells (Kornbluth et al., 1989; Gendelman et al., 1990c; Hansen et al., 1992). Furthermore, IFN-α has been shown to suppress the production of new virions from cell lines chronically infected with HIV by affecting the very late phase of particle release from the plasma membrane (Poli et al., 1989; Yasuda et al., 1990; Gendelman et al., 1990b; Smith et al., 1991; Fernie et al., 1991; Biswas et al., 1992). This "post-budding" effect was not correlated with the inhibition of viral antigen shedding from the plasma membrane (Fernie et al., 1991).

IFN-α is the first cytokine for which an autocrine/paracrine role as negative regulator of HIV replication has been reported. Addition of anti-IFN-α neutralizing antibodies (Ab) to mixed cultures of patients' PBMC with allogeneic PHA-blasts has been shown to increase both the levels of HIV replication and the frequency of positive viral isolation (Gallo et al., 1983; Markham et al., 1986). Similar effects have been described during acute in vitro infection of the U937 cell line, where treatment with anti-IFN-α or anti-IFN-β Ab caused an accelaration of the kinetics of viral replication compared to control cells (Macè et al., 1989; Gazzolo and Macé, 1990; Locardi et al., 1990).

Based on its ability to block different steps in HIV replication in vitro as well as its relative deficiency in HIV-infected individuals, IFN-α has been administered both alone and in combination with other agents for therapeutic purposes. In vitro synergy between IFN-α and anti-reverse transcriptase nucleoside analogs, such as AZT, has been described and reviewed elsewhere (Dubreuil et al., 1990; Schuitemaker et al., 1990; Krown, 1990; Francis et al., 1992), providing the basis for the combined use of these two agents in clinical trials. The clinical use of IFN-α resulted

in an anti-neoplastic effect against HIV-associated KS, where partial and even complete remissions were achieved (Lane et al., 1988; Lane, 1989; Lane et al., 1990; and Francis et al., 1992). In one of these studies, a decreased in HIV-p24 antigenemia was also observed, indicating an in vivo anti-viral effect of IFN-α (Lane et al., 1988). These effects occurred in patients with $CD4^+$ cell counts above 150 per mm^3, indicating that the anti-viral effect of IFN-α could have been caused by its immunostimulatory properties on a relatively healthy immune system. In this regard, earlier in vitro studies have suggested that the ability of IFN-α to block the spreading of infection in primary PBMC was correlated with the induction of cell-mediated cytotoxicity (Dolei et al., 1986).

Therefore, IFN-α represents a potentially important anti-retroviral agent in the search for better strategies to block the spreading of HIV infection in individuals and the progression of disease.

1.2. IFN-β

IFN-α and IFN-β have been shown to exert similar effects and limited studies have been conducted comparing these two molecules in the context of HIV infection. In vitro restoration of the defective NK cell activity of HIV-infected patients (Poli et al., 1985) and suppression of HIV replication in primary MDM (Kornbluth et al., 1989; Meyelan et al., 1993) and cell lines (Williams et al., 1989) have been reported for IFN-β. Furthermore, as reported for IFN-α, addition of anti-IFN-β Ab to infected cultures resulted in increased levels of virus replication (Locardi et al., 1990), indicating that IFN-β may act as a suppressive endogenous cytokine. A derivative of the U937 cell line that contained a stable IFN-β transgene under the control of a murine MHC gene has shown resistance to HIV infection, an effect that could be reversed by addition of anti-IFN-β neutralizing Ab to the cultures (Macè et al., 1991).

A limited number of clinical trials have used IFN-β in HIV infected patients. No significant changes in the number of HIV proviral copies present in PBMC have been reported after treatment of patients with this agent (Oka et al., 1989; Oka et al., 1992). However, an increased plasma half-life of AZT has been observed in regimens of combination therapy with IFN-β (Nokta et al., 1991). Rapid disease progression was observed in 3 out of 4 patients with AIDS-associated KS treated with IL-2 and IFN-β (Krigel et al., 1989), although it is more likely that these adverse effects were attributable to IL-2 and/or IL-2-induced IFN-γ rather than to IFN-β (Mc Elrath et al., 1990; Nokta et al., 1991).

1.3. IFN-γ

Several studies have described increased levels of IFN-γ and/or its functionally related molecule neopterin (a product of IFN-γ-activated macrophages) in the plasma/serum of HIV-infected individuals (Fuchs et al., 1988; Fuchs et al., 1989; Rinaldo et al., 1990; Reddy et al., 1990; Krown et al., 1991). Increased constitutive secretion of IFN-γ has been observed from PBMC of HIV-infected patients as a function

Table 1
Effects of IFNs on HIV life cycle

HIV life cycle	IFN-α (IFN-β)	IFN-γ	Key references
CD4 binding/entry	*	*	
Pre-integration events, reverse transcription	↓	*	Shirazi et al. (1992)
Integration	*	*	
Transcription from proviral DNA	↓	↑↓	Gendelman et al. (1990); Kornbluth et al. (1989); Meyelan et al. (1993); Popik et al. (1991), (1992)
Translation of HIV proteins	↓	↑↓	Gendelman et al. (1990); Ho et al. (1985); Kornbluth et al. (1989); Meyelan et al. (1993)
Assembly and release of progeny virions	↓	↑↓	Biswas et al. (1992); Fernie et al. (1991); Poli et al. (1989); Smith et al. (1991); Yasuda et al. (1990)

*No evidence of IFN effect or no studies available.

of the stage of disease (Vyakarnam et al., 1991). Freshly isolated PBMC from patients, similar to $CD8^+$ CTL clones, produced IFN-γ when challenged in vitro with a proper antigenic peptide (Jassoy et al., 1993). Earlier studies have shown that PBMC obtained from HIV-infected individuals produce less IFN-γ in response to different stimuli (Murray et al., 1984; Murray et al., 1985; Rook et al., 1985; Lane et al., 1985; Cauda et al., 1987; Nokta et al., 1990). Similarly, mononuclear cells isolated from the lamina propria of the large bowel showed a decreased constitutive and inducible capacity to secrete IFN-γ (Kotler et al., 1993). It is likely that some of these discrepancies were due to a variable state of in vivo priming/activation of patients' cells (Capsoni et al., 1992) and/or to the fact that IFN-γ can be secreted by several cell types, such as $CD8^+$ T cells, T_H0 and T_H1 $CD4^+$ T cells, and NK cells. Lung alveolar macrophages obtained from HIV-infected individuals, but not AM obtained from control healthy donors expressed mRNA for the IFN-γ-inducible gene IP-10 (Buhl et al., 1993). These cells also showed an activated phenotype, as indicated by increased levels of cell surface Class II antigens, and by their constitutive ability to secrete TNF-α and superoxide anion (Buhl et al., 1993). Increased expression of IFN-γ and neopterin in the cerebrospinal fluid have been reported as stable markers of disease progression in HIV-infected individuals, particularly those with AIDS-associated disorders of the central nervous system (Griffin et al., 1991). Lymph nodes of HIV-infected individuals showed expression of IFN-γ mRNA in the germinal centers at levels much more abundant than those found in reactive lymph nodes obtained of HIV-seronegative individuals (Emilie et al., 1990), a finding confirmed by other studies (Boyle et al., 1993; C. Graziosi et al., unpublished results). The possibility of a direct physical association between HIV and IFN-γ has been suggested by in vitro studies showing that the p17 gag protein of HIV-1 could specifically bind IFN-γ (Caruso et al., 1989).

IFN-γ has been broadly investigated as an immunostimulating agent capable of restoring some of the defective immune functions associated with HIV infection, including monocyte, NK, and T cell functions (Poli et al., 1985; Rook et al., 1985; Murray et al., 1987; Murphy et al., 1988). Furthermore, IFN-γ either alone or in combination with TNF-α or other agents has been administered to HIV-infected patients, with unclear clinical results (Ganser et al., 1986; Agosti et al., 1992).

Addition of IFN-γ to cell cultures undergoing acute in vitro infection has resulted in dichotomous results. IFN-γ has generally shown a less pronounced anti-HIV activity than that exerted by IFN-α or IFN-β (Yamamoto et al., 1986; Hartshorn et al., 1987; Yamada et al., 1988). Suppression of HIV replication has been demonstrated in primary MDM (Kornbluth et al., 1989; Meyelan et al., 1993) and in the monocytic THP-1 and U937 cell lines (Tsunetsugu-Yokota and Honda, 1990; Hammer et al., 1986) treated with IFN-γ. Similar results were obtained with lung AM obtained from healthy macaques and infected in vitro with simian immunodeficiency virus, that causes an AIDS-like syndrome in macaques (Walsh et al., 1991). In one study, both suppressive and inductive effects of IFN-γ on HIV replication were observed in MDM depending on whether serum was present in the culture medium, an effect that was explained by enhancement of Fc-dependent infection mediated via up-regulation of cell surface Fc receptors by IFN-γ (Degre et al., 1992). Enhancement, no effect, or suppression of HIV replication has been reported earlier as a function of whether MDM were treated with IFN-γ before, at the same time, or after infection (Koyanagi et al., 1988); however, no explanation was provided for these opposite effects. A dichotomous effect of IFN-γ on HIV expression has also been demonstrated in chronically infected U1 cells, where IFN-γ caused a major redirection of the primary cellular site of virion production from the plasma membrane to intracytoplasmic Golgi-derived compartments (Biswas et al., 1992).

IFN-γ likely represents an important endogenous factor sustaining HIV replication in an autocrine/paracrine manner in PBMC (Vyakarnam et al., 1990; A.L. Kinter, G. Poli, L.M. Fox, and A.S. Fauci, unpublished results), in contrast to IFN-α and IFN-β that can serve as endogenous negative regulators of HIV replication (see above).

Finally, it has also been shown that IFN-γ can inactivate free infectious virus by production of reactive oxygen intermediates (ROI) (Ennen and Kurth, 1993). However, in light of the fact that ROI can lead to increased HIV expression via activation of NF-kB (reviewed by Staal et al., 1992), it is likely that a multifactorial balance of pro- and anti-viral effects is the basis of the conflicting reports on the role of IFN-γ as a regulator of HIV replication.

2. Perspectives

A natural distinction needs to be drawn between Class I (IFN-α and IFN-β) and Class II (IFN-γ) IFNs, in terms of their potential impact on HIV disease. IFN-α and -β interfere in vitro with multiple steps of HIV life cycle, from early pre-integration events (Shirazi and Pitha, 1992; Gendelman et al., 1990b) until the very late stage of

virion detachement from the cell surface (Poli et al., 1989; Yasuda et al., 1990; Fernie et al., 1991; Smith et al., 1991) in a variety of cell types studied, including primary PBMC and macrophages. Therefore, these IFNs should have the potential capacity of blocking HIV spreading in vivo, and some evidence of this effect has been reported (Lane et al., 1988). Perhaps the most important question is why IFN-α and IFN-β have not shown more impressive benefits as anti-retroviral agents. No definitive explanations can be provided. However, several hypotheses can be formulated. First, these IFNs are efficacious as an anti-retroviral agent, but do not achieve sufficient concentrations in sites where important levels of virus replication occur, for example in the lymphoid tissue (Fauci, 1993; Pantaleo et al., 1993; Graziosi et al., 1993; Embretson et al., 1993) or brain (Koenig et al., 1986). Second, the presence of the so-called "acid-labile IFN-α" may cause an interference with the signal transduction pathway(s) necessary for IFN-α to exert its anti-HIV effect. Third, it is possible that IFN-resistant strains of HIV develop during treatment (an observation that has been anecdotally reported, but not confirmed), as now clearly observed with other classes of anti-retroviral agents, from reverse transcriptase to protease inhibitors (reviewed by Richman, 1993). Finally, it is possible that a combination of all of the above mentioned possibilities is the explanation for the lack of broad efficacy of IFN-α treatment in HIV disease. It is noteworthy in this regard, that the most encouraging results have been obtained in individuals with a relatively healthy immune system and more than 150 $CD4^+$ T cells per mm^3 (Lane et al., 1988), perhaps indicating that the most relevant in vivo effect of IFN-α is exerted via the potentiation of anti-HIV immune responses. However, it is also possible that combination of IFN-α or IFN-β with other anti-retroviral agents result in a synergistic anti-viral effect in individuals.

Unlike IFN-α and IFN-β, IFN-γ has not shown clear-cut anti-retroviral effects in vitro. Both dichotomous and clear up-regulatory effects on HIV expression have been reported, particularly in cells of monocytic lineage (Koyanagi et al., 1988; Biswas et al., 1992). IFN-γ is present at very high levels in the lymph nodes of HIV-infected individuals, particularly in association with the areas of the germinal centers infiltrated by $CD8^+$ T cells (Emilie et al., 1990). Furthermore, $CD8^+$ T cells express IFN-γ on their cell surface (Caruso et al., 1990). Both IFN-γ and its related marker neopterin have been reported to be elevated in the PBC of HIV-infected individuals (reviewed in Fuchs et al., 1988). These findings do not support an obvious rationale for IFN-γ administration in HIV-infected individuals. In this regard, the clinical trials that have been attempted thus far that included IFN-γ have not shown clear efficacy, and have also provided some evidence of exacerbation of disease progression (Ganser et al., 1986; Agosti et al., 1992). However, the recent hypothesis that HIV disease is associated with a dysregulation of T_H1 versus T_H2 dependent immune responses (Clerici and Shearer, 1993) proposes a new potential role for IFN-γ in the pathogenesis and treatment of this disease. In particular, T_H1 $CD4^+$ T cells are characterized by the preferential production of IFN-γ and preferentially potentiate cell-mediated immunity, whereas T_H2 cells secrete mostly IL-4 and support Ab-mediated responses (reviewed by Romagnani, 1992). However, it should be remarked that findings different from those originally described by Cler-

ici and Shearer have been reported on cytokine profiles in HIV-infected individuals (S. Romagnani, personal communication; C. Graziosi, G. Pantaleo and A.S. Fauci, unpublished results) and that it is unclear at the present time whether a "shift" from T_H1 to T_H2 cytokine profiles occurs during progression of HIV disease. Because, regulation of T_H1 and T_H2 function has profound influences on several microbiological diseases, further studies are required in order to clarify whether a similar role exists in HIV disease and AIDS.

Finally, it has been recently shown that IFN-treated cells express a set of cellular gene products that are capable of suppressing HIV replication by interference with the binding of the HIV-encoded protein Rev to its RNA target sequence, RRE (Costantoulakis et al., 1992). Rev, like Tat, represents an essential protein for HIV in order to efficiently replicate in human cells (reviewed in Weiss, 1993). Previously, it has been shown that the RNA-Tat binding region of HIV (Tar) can activate the double-stranded RNA-dependent kinase, one of the well defined pathway of action of IFNs (Edery et al., 1989). In this regard, current protocols of gene therapy for HIV involve the use of Tar decoy molecules as tool for interference with virus replication (Sullenger et al., 1991). Therefore, it will be important to investigate whether the observed anti-viral effects are actually due to a direct interference of the decoy molecule with Tat-mediated transactivation of the viral LTR, or involve the induction of IFN-dependent pathways.

In conclusion, IFNs have been involved both as target genes of HIV during the infection of the host, as well as potential therapeutic molecules for the treatment of HIV disease and of AIDS-associated neoplasms. Further in vitro as well as in vivo studies are indicated in order to maximize the potential beneficial effects of these molecules in the treatment of HIV disease and its complications.[1]

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[1] *Note:* Synergistic induction of HIV expression and cell death has been shown to occur in chronically-infected U1 cells co-stimulated with TNF-α and IFN-γ.

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