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Antiviral Research 24 (1994) 205–219

Antiviral Research

Multiple effects of interferon on the replication of human immunodeficiency virus type 1

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(Received 11 November 1993; accepted 14 February 1994)

Abstract

In this review, I shall summarize the major findings about the effect of IFN on the replication of HIV-1 virus in model systems in vitro and will describe the known molecular mechanisms involved in the IFN-mediated inhibition of HIV-1 replication. Finally, I shall relate these findings to the unique features of the HIV-1 replication cycle.

Key words: Interferon; HIV-1

1. Introduction

One of the characteristic features of human immunodeficiency virus type 1 (HIV-1) infection is a long asymptomatic period between the time of the initial infection and the onset of the clinical disease. Recent studies indicate high levels of HIV-1 replication within a few weeks after the infection, when HIV-1 virus can be detected in the plasma, that is suppressed within 6 to 8 weeks after the infection. During the following clinical latency period, only 1 in 10^4 CD+ lymphocytes express HIV-1, while 1 in 100 contains HIV-1 provirus, suggesting that the virus replication is suppressed by the host-immune system. HIV-1 replication can, however, be detected even during the time of clinical latency in lymph nodes. In addition, CD4+ lymphocytes are not the only cells that can harbor HIV-1 virus; HIV-1 provirus can be detected in residential macrophages (in central nervous system and in lungs)

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SSDI 0166-3542(94)00017-3

which serve as virus reservoir. Recent evidence indicates that dendritic cells are also very permissive to HIV-1 infection; in addition, they can absorb virus and serve as an HIV-1 carrier from lymph nodes into the other organs.

The mechanism by which the immune system suppresses viral replication after the acute viremia is not fully understood. A number of humoral immune responses can be demonstrated against various virion components of HIV-1 virus, as well as against the HIV-1-encoded nonstructural, regulatory genes. Which of these components affect the long-lasting antiviral response in the infected individuals is not known. Furthermore, it is not clear whether the immunodeficiency which accompanies HIV-1 infection is a direct consequence of the HIV-1 infection or due to the virus-mediated changes in the cytokine network. Recent data indicate that the progression to AIDS is accompanied by the shift from Th-1 cytokine response and production of interleukin (IL)-2 and interferon (IFN)-γ to Th-2 response producing IL-4 and IL-10. HIV-1 infection in monocytes and macrophages was also shown to alter production of cytokines such as tumor necrosis factor (TNF)-α, IL-1β, and granulocyte macrophage stimulating factor (GM-CSF) that can in vitro up-regulate expression of HIV-1 provirus both in monocytes and T cells. It was suggested that the cytokines produced in vivo as a consequence of HIV-1 infection may contribute to the enhancement of HIV-1 replication and progression of the disease.

The cytokine system is generally a well-balanced system in which up-regulation by some cytokines can be counteracted by others. In vivo the production of IFNG by Th-1 response may play a role in the down-regulation of HIV-1 infection and a decrease in IFNG production, as a consequence of a switch from Th-1 to Th-2 response, may remove this suppression. Furthermore, it is likely that the IFNA and B, that play an essential role in the antiviral response to many viruses, also participate in the regulation of HIV-1 infection. While the HIV-1 infection does not induce IFNA and B synthesis in vitro, IFN inhibits HIV-1 replication in vitro cultured T cells, macrophages, and peripheral blood lymphocytes. Furthermore, the acid labile IFNA can often be detected in serum of AIDS patients during the later stages of disease. However, the biological role of this IFN is unknown.

2. Major regulatory steps in HIV-1 replication cycle

The HIV-1 virus particle contains the duplex RNA genome approximately 10 000 nucleotides long, protein core, and lipid membrane (rev. in Haseltine, 1991). The HIV-1 genome contains, in addition to the three major genes (*gag*, *pol* and *env*) present in all retroviruses also a number of accessory genes that affect different steps of viral replication cycle. The virus protein core contains major structural proteins as well as proteins required for virus replication, such as RNA-specific polymerase (reverse transcriptase), ribonuclease H, and integrase. The virion membrane contains at least two virus-encoded proteins. MA protein p17, that is anchored to the inner surface of the membrane through myristilic acid and the envelope viral glycoprotein. The envelope glycoprotein has two major functions: (i) it binds to cell surface receptor CD4 and facilitates the entry of the virus to the cells;

and (ii) mediates membrane fusion and allows the cell expressing this protein to fuse with the uninfected cells. The envelope mediated giant cell formation together with the virus-induced apoptosis are assumed to be the two major mechanisms of the virus-induced T cells death.

The general steps essential for the establishment of HIV-1 infection are similar to those with any other retroviruses (Fig. 1). After virus entry, the virion RNA is transcribed into DNA copy (provirus) that is integrated into cellular genome. In contrast to the viruses of retroviral group, the integration of HIV-1 provirus does not require cell replication and the transport of the provirus from the cytoplasms to nucleus is facilitated by the p17 (MA) protein.

The replication of HIV-1 provirus depends on the function of two virus-encoded accessory proteins, Tat and Rev (Fig. 2). Tat transactivates vital gene expression on transcriptional level by increasing the rate of transcription initiation as well as decreasing the polarity of transcription. This transactivation requires binding of the Tat protein to the Tat response element (TAR) present in the 5′ untranslated region of all HIV-1 mRNAs. In contrast to Tat, Rev affects expression of HIV-1 provirus at the post-transcriptional level by facilitating the transport of the genomic, unspliced 9.2-kb RNA from the nucleus to cytoplasm. Similarly as Tat, Rev is also a RNA-binding protein that recognizes the Rev responsive element localized in both the genomic and envelope mRNAs. In the absence of Rev, only the small, doubly spliced HIV-1 mRNAs can be detected in the cytoplasm. In addition to its effect on RNA transport Rev seems also to effect efficiencies of HIV-1 RNA translation.

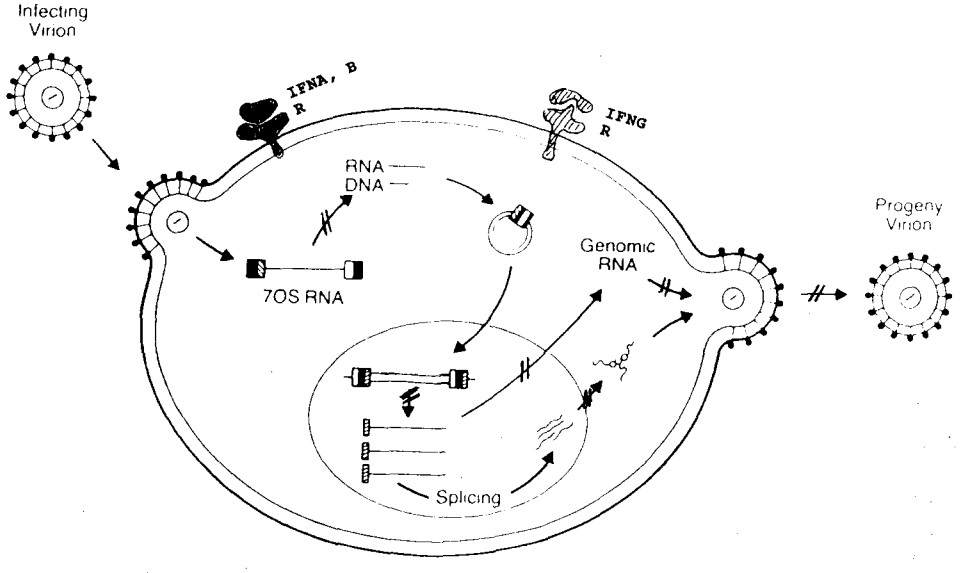

Fig. 1. Effect of IFN on HIV-1 replication cycle. IFNs upon binding to cellular receptors induce expression of a number of cellular genes and activate pre-existing transcriptional factors that can affect different steps of HIV-1 replication cycle.

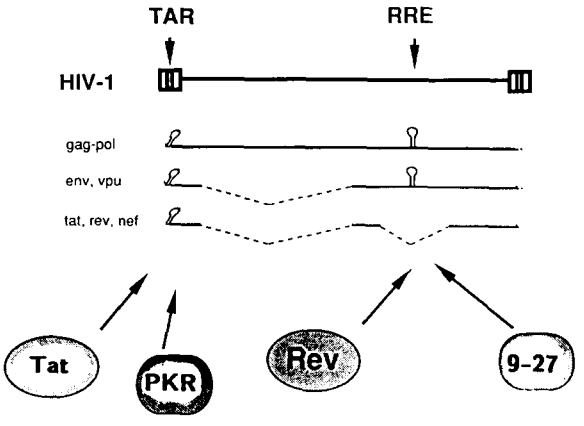

Fig. 2. Schematic diagram of action of Tat and Rev. Products of Tat and Rev genes are small proteins that interact with stem loop structure present in the 5′ end of viral RNA (TAR) or in the envelope gene (RRE). IFN-induced proteins, PKR, and *9–27* can compete with binding of Tat or Rev, respectively.

In addition to these two major transactivators, HIV-1 encodes a number of small proteins (nef, vif, vpu, and vpr) that are not essential for HIV-1 replication in vitro (cell culture) but may facilitate virus assembly and transmission as well as affect its pathology in vivo.

Thus, although the basic machinery for HIV-1 replication is similar to that of other nontransforming retroviruses, additional viral gene products enable the virus to regulate efficiently its expression and render it independent on host cell.

3. Effects of IFN on the family of viruses of the retroviral and lentiviral groups

IFNs play a major role in the early response against virus infection; they are not only part of the immune system, but also have a direct antiviral effect. It has been well established that IFN can inhibit replication of a variety of viruses, including viruses of lentivirus subgroup of the retroviral group. The inhibition of virus replication by IFNs is generally associated with the induction of several enzymatic activities, including double-stranded (ds) protein kinase (PKR), 2′,5′-oligoadenylate synthetase (2′,5′-OAS), and endonuclease L with a consequent inhibition of viral protein synthesis. In contrast to the majority of lytic viruses, the IFN-mediated inhibition of murine retroviral replication occurs at the post-translational level and affects virus assembly and release. It was shown that, in IFN-treated cells, retroviral particles accumulated at the plasma membrane and those which were released lacked the major envelope protein, gp71 and were therefore, noninfectious (rev. in Friedman and Pitha, 1984). The molecular mechanism by which IFN alters the fidelity of virus-assembly has not been clarified, but it has been suggested that alteration in virus assembly may be a result of an IFN-induced changes in physiol-

ogy of plasma membranes (Pitha, 1980).

The effect of IFN on HIV-1 replication has been extensively analyzed by several laboratories, and inhibition of HIV-1 replication was observed in IFN-treated primary cells, such as PMBC, monocytes/macrophages, in vitro-infected CD4+ T cells, and established T cells and monocytes lines (Ho et al., 1985; Brinchmann et al., 1991; Mace et al., 1988; Dolei et al., 1986; Michaelis and Levy, 1989; Yamamoto et al., 1986; Smith et al., 1991; Yamada et al., 1988; Kornbluth et al., 1989; Bednarik et al., 1989; Gendelman et al., 1990; Crespi, 1989). It has been concluded that its mode of action is similar to that observed with the murine retroviral system. There were, however, several indications, suggesting that the effect of IFN on replication of these two groups of viruses, may not be completely identical. Firstly, maximal inhibition of HIV-1 replication could be seen in de novo infection in the multiple cycle of HIV-1 replication, while the effect on persistent, chronic infection was much lower (Michaelis and Levy, 1989; Yamamoto et al., 1986; Smith et al., 1991; Yamada et al., 1988; Kornbluth et al., 1989; Gendelman et al., 1990); in the murine retroviral system, however, IFN inhibited de novo and chronic infection with the same efficiency (Pitha et al., 1976). Secondly, in the single cycle of HIV-1 infection (Shirazi and Pitha, 1992), in IFN producing T cells (Bednarik et al., 1989), and in differentiated macrophages (Kornbluth et al., 1990; Gendelman et al., 1990), IFN inhibited the early steps of HIV-1 replication and no HIV-1 transcripts could be detected in these cells. In contrast in the murine retroviral system, only a small reduction in viral RNA were detected in IFN-treated cells (Salzberg et al., 1980; Riggin and Pitha, 1982) that were not sufficient enough to explain the magnitude in the reduction of viral particle. Furthermore, upon electron microscopy examination, both the mature and immature HIV-1 particles from IFN-treated cells contain the envelope knobs, indicating that the viral glycoprotein, gp120, is assembled into HIV-1 virus (Fig. 3). Accordingly, the biochemical analysis indicated that the gp120 is present in the virions-assembled in IFN-treated cells (Rodriguez-Ortega and Pitha, unpublished results). It is likely that the differences in regulation of HIV-1 and retrovirus replication account for the observed difference in the IFN effects.

4. Differential effect of IFN on HIV-1 replication in macrophages and T cells

IFNs have been shown to inhibit HIV-1 replication in both primary cells, such as peripheral blood mononuclear cell (PBMC) and macrophages, as well as in established monocytes and T cell lines (Dolei et al., 1986; Gendelman et al., 1990; Hartshorn et al., 1987; Kornbluth et al., 1989; Kornbluth et al., 1990; Michaelis and Levy, 1989; Poli et al., 1989; Yamada et al., 1988; Yamamoto et al., 1986). The molecular nature of this inhibition, however, appears to be cell type dependent. Thus, while, in chronically infected T cells, IFN inhibited primary virus assembly and release, but did not affect viral protein synthesis (Dolei et al., 1986; Poli et al., 1989; Yamada et al., 1988), in chronically infected macrophages IFN reduced substantially the relative levels of HIV-1 RNA without affecting provirus formation (Gendelman et al., 1991). It remains to be determined whether the down-regulation of HIV-1 tran-

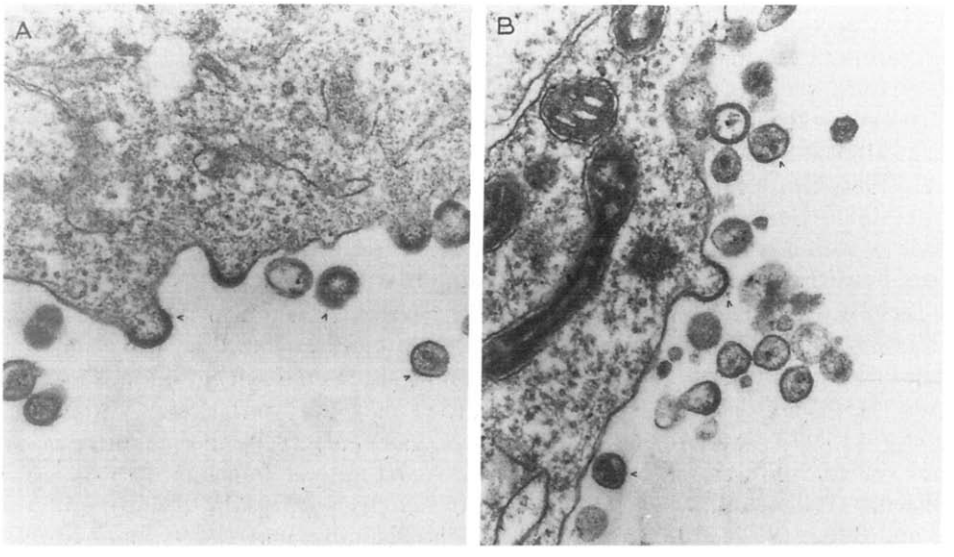

Fig. 3. Transcription electron microscopy representing section of A3.01 T cells. (A) Infected with HIV-1; (B) infected with HIV-1 and treated with human IFNA for 24 h. Arrow points out to the glycoprotein knots present on the virion particles (published through the courtesy of Dr. H. Gendelblom).

scripts in IFN-treated macrophages is a result of an IFN-induced degradation of viral RNAs or inhibition of a provirus transcription. However, this transcriptional effect seems to be unique to the primary macrophages and has not been observed in monocyte cell lines such as are U937 cells or in T cells.

The effect of IFN on acute HIV-1 infection has been analyzed extensively in both established lines and primary cells such as are PBMC and CD4+ cells. In majority of established T cell lines as well as in PBMC, IFN suppressed HIV-1 replication in a multiple infection cycle by acting at the post-translational level (Michaelis and Levy, 1989; Ho et al., 1985; Yamamoto et al., 1986; Hartshorn et al., 1987; Smith et al., 1991; Yamada et al., 1988). Hence, while IFN decreased the levels of virus released to the culture medium, it did not affect substantially the synthesis of viral RNA and proteins in infected cells. A similar effect was observed when the IFN mediated inhibition of HIV-1 replication was studied in primary blood lymphocytes or isolated CD4+ cells (Brinchmann et al., 1991). These data indicate that IFN can limit effectively the spread of HIV-1 infection through the infected culture, but does not affect the establishment of an infection. Although in most of these studies IFNA was used, IFNB seems to exert a similar effect. An exception, however, was a recent study by Brinchmann et al. (1991) who found that only IFNA, but not IFNB, could inhibit HIV-1 replication in primary CD4+ cells. This unusual finding is the first and only report that indicates a discoordinate sensitivity of the cells to these two types of IFNs. In contrast in acutely infected primary macrophages, IFN also decreased the levels of integrated HIV-1 provirus (Kornbluth et al., 1989; Kornbluth et

al., 1990). However, this early IFN block is specific for primary macrophages and has not been observed in any monocyte cell lines.

We have analyzed (Shirazi and Pitha, 1992) the effect of IFN on HIV-1 replication in a single infection cycle in the T cells (CEM-174) that are highly sensitive to the antiviral effect of IFN. In these cells, IFN significantly reduced the relative levels of HIV-1 RNAs and viral proteins. Two observations suggested that this inhibition occurred at the early stage of HIV-1 replication cycle. First, no inhibition in viral RNA and protein synthesis was observed when IFN was added to the cells in which HIV-1 infection has already been established. Secondly, the levels of integrated HIV-1 provirus were substantially decreased in IFN-treated cells. We have further shown that the inhibition in HIV-1 replication did not occur at the level of proviral integration, but that IFN treatment decreased the relative levels of extrachromosomal proviral DNA.

The early inhibition of HIV-1 replication by IFN-1 was also observed in primary macrophages, where IFN was found to inhibit provirus formation and integration (Kornbluth et al., 1989; Kornbluth et al., 1990) as well as to induce degradation of HIV-1 RNA (Gendelman et al., 1991), suggesting that the early IFN block is not limited to one cell type.

5. IFN effects the synthesis of HIV-1 provirus

The decrease in provirus formation was observed in IFN-treated T cells and it has been reported (Aboud, 1981) that IFN inhibits retroviral uptake. However, it is unlikely that the inhibition in HIV-1 provirus formation seen in the presence of IFN is a result of inhibition of virus uptake or entry since, in T cells, addition of IFN after infection was still able to inhibit virus replication (Shirazi and Pitha, 1992). Studies by Zack et al. (1990) have shown that, in infected resting PBMC, reverse transcription of HIV-1 RNA is initiated but not completed and majority of the transcripts are incomplete. These results suggest that a cellular component present in stimulated, but not in resting cells, is required for the transcription of full-length proviral DNA. Since IFN has a direct antigrowth effect, we examined whether the inhibition of provirus formation in IFN-treated cells is of a similar nature. We found, however, that, in IFN-treated cells, the formation of both the early and late reverse transcripts was inhibited, suggesting that the effect of IFN is at the initiation of transcription. Furthermore, the fact that the inhibition in provirus formation was also observed with low levels of IFN (50 units) that do not affect cell proliferation strongly suggests that the observed effect is independent on cell growth inhibition.

Interestingly, the inhibition of reverse transcription could also be observed in vitro (Shirazi and Pitha, 1993). The addition of crude extracts from IFN-treated cells, but not from the control cells, inhibited the activity of the virion-associated, as well as the recombinant, reverse transcriptase in vitro. This inhibition was specific for the HIV-1 reverse transcriptase and could not be demonstrated with murine leukemia virus reverse transcriptase. At present, the nature of this inhibition is not

known, however, we can exclude the possibility that it is due to the presence of the RNase activity selectively induced in the extracts from IFN-treated cells. Furthermore, since the activity can be removed by boiling or phenol extraction, we assume that the inhibitor is a protein and not an oligonucleotide (e.g., 2′,5′-oligoA; unpublished results). IFN has been shown to induce various RNA-binding proteins (Samuel, 1991; Constantoulakis et al., 1993) and thus, it is not unlikely that a product of IFN-induced gene(s) may affect the HIV-1 reverse transcription.

6. Effect of IFN on the activation of HIV-1 provirus

The ability of IFN to inhibit the expression of HIV-1 provirus is of primary interest since the activation of poorly expressed HIV-1 provirus may be an important step in the life-cycle of HIV-1 virus in vivo. It has been suggested that, in vivo, a major reservoir of HIV-1 are the CD4+ lymphocytes (Psallidopoulos, 1989; Schnittman, 1988), however, only a small population of these cells are expressing the HIV-1 provirus. In vitro expression of the integrated, poorly expressed HIV-1 provirus can be stimulated by extracellular stimuli, such as mitogens, cytokines, and heterologous viral infections; some of these cytokines are produced as a result of HIV-1 infection in macrophages (D'Addario et al., 1990) and the activation pathway generally involves the induction of binding of NF-κB-specific proteins. Poli et al. (1989) have shown that, both in infected T cells and monocytes, IFN inhibited the 12-*O*-tetradecanylphorbol-13-acetate (TPA) and TNF-α-stimulated HIV-1 replication. These authors suggested that, similarly as in murine retroviral system, the IFN-induced block is at the level of virus assembly and maturation. In contrast in the same cells, IFNG stimulated expression of the HIV-1 provirus.

We have analyzed the effect of IFNA on the activation of the latent tat-defective HIV-1 provirus and found that the tat-mediated transactivation of HIV-1 provirus was not affected by IFN. By contrast, stimulation of HIV-1 provirus expression or of HIV-1 LTR CAT plasmid by TPA was very ineffective in the presence of IFNA. The UV cross-linking analysis of NF-κB-specific proteins induced in TPA-treated cells in the presence and absence of IFN have shown that the binding of NF-κB-specific proteins to the enhancer region of HIV-1 LTR is altered in the presence of IFN. Mainly, the IFN-treated cells showed an absence in the binding of the p45-kDa protein that was induced by TPA in the absence of IFNA. Surprisingly, simultaneous treatment with TPA and cycloheximide activated expression of the latent HIV-1 provirus both in the presence and absence of IFN (Popik and Pitha, 1992). These results suggest that a newly synthesized or unstable protein induced in the IFNA-treated cells interferes with the binding of NF-κB-specific protein(s).

IFN was also shown to inhibit the herpes simplex virus type 1 (HSV-1)-mediated induction of HIV-1 provirus in T cells (Popik and Pitha, 1991). We have shown that the activation of HIV-1 provirus by HSV-1 infection is a result of cooperation between cellular and viral transactivators (Vlach and Pitha, 1992; 1993). HSV-1 infection induces binding of NF-κB-specific protein and a protein (HLP-1) that binds to the leader sequences (LPB-1 site) in the HIV-1 LTR. Furthermore, HSV-

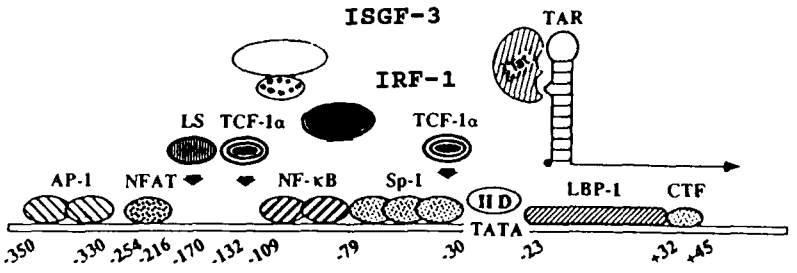

Fig. 4. Factors participating in the transcriptional regulation of HIV-1 expression. Transcriptional activation Tat that binds to TAR element in HIV-1 mRNA was shown to interact with DNA-binding factor such as Sp-1 and proteins bind to TATA box. ISGF-3 and IRF-1 are transcriptional factors that are activated in IFN-treated cells. Numbering is made in respect to the transcription start (+1).

1-encoded immediate early protein, ICPO, was shown to activate the expression of HIV-1 LTR. While IFN treatment did not affect the expression of ICPO in infected cells, a major difference in the binding pattern of NF-κ-specific proteins to the enhancer region of HIV-1 LTR was observed in IFN-treated cells. The inability of HSV-1 to activate the HIV-1 LTR in IFN-treated cells correlated with the absence of binding of p45 to the enhancer core sequence (Popik and Pitha, 1991). These results indicate that IFN activated transcriptional factors that play a role in both stimulation of IFN genes and IFN-induced genes (e.g., IRF-1, ISGF-3, or p91) may interact with the NF-κB-binding proteins, and other transactivating factors binding to the HIV-1 LTR and alter their ability to stimulate transcription (Fig. 4). These data suggest an existence of a novel, unique mechanism by which IFN can modulate expression of cellular and viral genes.

7. Post-transcriptional regulation of HIV-1 mRNA expression in IFN-treated cells

While in a majority of viral lytic infections, IFN block is at the level of viral protein synthesis, no difference on HIV-1 protein synthesis has been generally observed in IFNA-treated cells and controls. There is, however, one study that reports the down-regulation of HIV-1-specific protein synthesis in IFN-treated cells (Coccia et al., 1992). In this study, a high multiplicity of HIV-1 infection was used to infect the T cells and IFN was added to the cells shortly after the infection. The question arises why are these results principally different from the results of others? It is possible that the observed inhibition of viral protein synthesis may result from the very high moi of HIV-1 virus used for infection since the high levels of input virion RNA could induce the 2′,5′-OAS system or PKR in the presence of IFN. The in vitro experiments implicated the TAR region as a possible inducer of these two enzymatic activities.

We have recently reexamined the post-transcriptional effect of IFN by analyzing the expression of transfected HIV-1 proviral DNA (to overpass the early inhibitory effect on the level of provirus formation) in IFN-treated HeLa cells. However,

treatment of HeLa cells with IFNA and B (up to 1000 units/ml) did not result in reduction of HIV-1 RNA and protein synthesis encoded by the transfected HIV-1 proviral clone. Interestingly, IFN treatment reduced significantly the HIV-1 mRNA levels encoded by the transfected tat defective HIV-1 provirus and this inhibition could be overcome by the transfection with tat or rev expressing plasmids (Shirazi, Popik, and Pitha, in preparation). These results suggest that HIV-1-encoded Tat and Rev can evade the effect of IFN (discussed in detail in latter part of this review).

The alteration of the relative levels of spliced and unspliced HIV-1 RNAs was also observed in the IFN-treated cells in the absence of functional Tat protein. Thus, in TPA-induced cells containing integrated Tat-defective HIV-1 provirus, the major RNA species detected in cytoplasm were the 2-kb doubly spliced mRNAs, while, when these cells were transfected with the Tat expressing plasmid, all sizes of HIV-1 RNAs (9.0-, 4.2- and 2.0-kb) were present in the cytoplasm (Popik and Pitha, 1992). These data indicate that the expression of the Tat-defective HIV-1 provirus in IFN-treated cells resembles expression of the Rev defective HIV-1.

In several instances, IFN was found also to decrease levels of HIV-1 mRNA in primary macrophages, and it was implied that the stability of IFN mRNA may be decreased in IFN-treated cells (Francis et al., 1992).

8. Modulation of the IFN effect in HIV-1 infected cells; virus mimicry

In vitro infection of establishes T cell and macrophage lines as well as primary PBMC or macrophages does not induce expression of IFNA and B genes (D'Addario et al., 1990; Gendelman et al., 1990; Pitha, unpublished results). Several studies, however, suggested that HIV-1 infection can modulate the levels of two dsRNA dependent enzymes implicated in the antiviral effect of IFN. Thus, it was reported that, in T cells, HIV-1 infection induces transient expression of 2′,5′–OAS and RNase L activity as well as induction of the Mx protein (Baca, et al., 1993). Furthermore, SenGupta and Silverman (1989) have shown that the Tat responding *cis*-acting sequence (TAR) can activate in vitro both the ds-dependant protein kinase (PKR) and the 2′,5′-OAS. These authors suggested that the HIV-1 leader sequence may contribute to the IFN-induced inhibition of HIV-1 replication. It was further shown (Edery et al., 1989) that the TAR region mediated activation of PKR was able to inhibit the translation of HIV-1 mRNA and that the inhibition of protein synthesis correlated with the phosphorylation of the a subunit of the protein initiation factor 2 (eF-2). These results suggest that ds structure of HIV-1 leader sequence has the capacity to participate in the induction of at least two enzymatic systems that play a role in the antiviral effect of IFN.

Recently Constantoulakis et al. (1993) have shown that a product of IFN-induced cellular gene 9–27 is a dsRNA-binding protein that efficiently binds to the RRE of 9.0- and 4.2-kb HIV-1 RNAs. Co-expression of 9–27 with a proviral clone of HIV-1, decreased levels of unspliced and single spliced HIV-1 RNAs, while it had little effect on expression of the multiplicity spliced HIV-1 mRNAs.

Interestingly, none of the IFN-induced proteins described above seems to play a

significant role in the IFN-mediated inhibition of HIV-1 replication. It seems rather that the virus developed various mechanisms by which it can escape the IFN-induced antiviral effect. Using the Western blot analysis with antibodies specific for the different forms of 2',5'-OAS, we have not been able to detect the induction of neither the small nor the large 2',5'-OAS in acutely-infected T cells (Popik and Pitha, unpublished results). Furthermore, Roy et al. (1990) have shown that a productive HIV-1 infection results in a significant decrease in the cellular levels of PKR and found an inverse relationship between the expression of *tat* gene and PKR. These authors suggested that the inhibition of PKR activation by tat may be a consequence of tat-binding to the TAR element of HIV-1 mRNAs which may interfere with the recognition of the ds structure of the TAR region and the activation of PKR. Finally, it is also not clear whether the IFN-induced expression of *9–27* gene can inhibit HIV-1 replication in infected cells. While we were able to demonstrate 9–27-like effect in IFN-treated cells in the absence of tat this effect was easily reversed both in the presence of tat or by overexpression of rev. Both the results of the transfection experiments and the data with the Tat-defective HIV-1 indicate that the ability of the 9–27 protein to inhibit processing of the HIV-1 RNA depends on the relative levels of both Rev and 9–27 proteins. The absence of Rev negative phenotype in the IFN-treated, acutely infected cells is due to the fact that, in HIV-1-infected IFN-treated cells, the induced levels of 9–27 protein are not sufficiently high to compete the binding of Rev protein to the RRE. The ability of Tat to negate the effect of the 9–27 protein may be simply a consequence of high levels of Rev protein synthesized in the presence of Tat as well as high levels of HIV-1 transcripts that can escape the 9–27 inhibition. These results indicate that the HIV-1-encoded regulatory proteins Tat and Rev allow HIV-1 not only to replicate more effectively, but also to overcome some of the antiviral effects of IFN.

9. Multiple mechanisms of IFN inhibition; facts or artifacts

Studies of the effects of IFN on HIV-1 replication clearly indicate that IFN effects HIV-1 replication at multiple levels. Can these numerous blocks be related to the unique pathways of HIV-1 replication cycle (Fig. 1) or are they reflection of the large variability of the used systems and therefore, an experimental artifact? There is a striking similarity between the IFN-mediated effects and the mechanism by which HIV-1 regulates its expression; both systems employ the RNA-binding proteins and thus target the regulation of gene expression to the RNA levels. HIV-1 regulates its expression by encoding two RNA-binding proteins, Tat and Rev (Fig. 2). IFN as well induces dsRNA-binding proteins to mediate its antiviral effect (PKR, 2',5'-OAS, Endonuclease L 9–27, and protein). In addition, some of the IFN-induced proteins have enzymatic activities that are activated upon the binding of dsRNA. The interaction between these two systems may then lead to the numerous occasion at which the IFN-induced proteins could interfere with the basic steps of the HIV-1 replication cycle.

Thus, the inhibition of provirus formation seen in the IFN-treated cells could be

a caused by a binding of one of the IFN-induced protein to the dsRNA region formed between the tRNA primer and the template-genomic RNA; the observed inhibition of NF-κK-mediated transactivation of HIV-1 provirus in IFN-treated cells, which as shown, is associated with the altered pattern of NF-κB-binding protein is also perhaps not surprising in view of the fact that IFNs induce number of transcriptional factors such as IRF-1, ISGF3 and p91. Interaction between some of these factors (e.g., IRF-1) and NF-κB-specific proteins is likely to occur. The interaction of IFN-induced activating factors with NF-κB-specific proteins may suppress the binding and activity of NF-κB-specific proteins and result in the lack of induction of HIV-1 provirus in IFN-treated cells (Fig. 4). However, additional experiments are necessary to determine whether any of the above proposed mechanisms are really functional in IFN-treated cells.

The IFN-induced inhibition at the post-transcriptional level is much easily comprehended. The question which remain is why the inhibition of the unspliced RNA processing through the product of *9–27* gene or the inhibition of viral protein synthesis mediated either by the PKR or 2′,5′-OAS systems are not the major targets of the IFN block. As pointed above, the major reason why these systems are not efficient in HIV-1 infected cells may be the viral mimicry by which the HIV-1 virus, encoding its own transactivators, is able to mask the dsRNA regions in its genome and prevent the transactivation of PKR or 2′,5′-OAS. The overproduction of viral gene products upon tat transactivation may be then another mechanism by which the virus overcomes the IFN effect which, as shown in many viral systems, is dependent on the multiplicity of infection.

It should be pointed out that none of the above described inhibitory effects of IFN was observed in cell infected with MuLV where the major inhibition was at the level of virus assembly and maturation. In the IFN-treated, HIV-1-infected cells the inhibition of virus assembly, seen in the chronically infected cells, is much less efficient than in the chronic MuLV infection. In addition in contrast to MuLV virions which, when assembled in IFN-treated fibroblast cells, are lacking the envelope glycoprotein, HIV-1 virions assembled in the IFN-treated T cells contained the envelope glycoprotein, gp120. These results may indicate a difference in the assembly of murine retroviruses and HIV-1 or simply reflect the different changes induced by IFN in plasma membranes of various cell types (e.g., fibroblasts and T cells).

10. Conclusion

The finding that IFN can inhibit multiple steps of the HIV-1 replication cycle with different efficiencies suggest that in vivo IFN effect will be pleiotropic and IFN will have broad spectrum of action. Hence, the direct antiviral effect of IFN as observed in vitro may in vivo represent only the tip of the iceberg. Further contribution to the effect may come from the modulation of action of different cytokines that can either up-regulate HIV-1 expression or change the Th-1 and Th-2 cell population and from the enhancement of the recognition of an infected cell by the immune system.

Acknowledgments

This work was supported by Public Health Service Grant Al 26123 from the National Institute of Allergy and Infectious Diseases and a research grant 01684-13 from the American Foundation for AIDS Research.

I want to thank Dr. H. Gelderblom for his permission to use the EM pictures of HIV-1 and to the members of my laboratory, especially Drs. M. Kunzi, W. Popik, and Y. Shirazi whose work contributed to our understanding of the IFN effect on HIV-1 replication. I also thank B. Schneider for the typing of this manuscript.

References

Aboud, M., Moldovan-Levin, D. and Salzberg, S. (1981) Cytoplasmic viral DNA synthesis in exogenous infection of murine leukemia virus: effect of interferon and cycloheximide. J. Virol. 37, 836–839.

Baca, L.H., Genis, P., Kalvakolanu, D., Sen, G., Meltzer, M., Zhou, A., Silverman, R. and Gendelman, H.E. (1994) Regulation of interferon alpha inducible cellular genes in human immunodeficiency virus infected monocytes. J. Leukocyte Biol., in press.

Bednarik, D.P., Mosca, J.D., Raj, N.B.K. and Pitha, P.M. (1989) Inhibition of human immunodeficiency virus (HIV) replication by HIV-trans-activated α_2-interferon. Proc. Natl. Acad. Sci. USA 86, 4958–4962.

Brinchmann, J.E., Gaudernack, G. and Vartdal, F. (1991) In vitro replication of HIV-1 in naturally infected CD4+ T cells is inhibited by rIFNα_2 and by a soluble factor secreted by activated CD8+ T cells, but not by rIFNβ, rIFN-γ or recombinant tumor necrosis factor-α. J. AIDS 4, 480–488.

Coccia, E., Krust, B. and Hovanessian, A. (1992) Specific inhibition of protein synthesis in HIV-infected cells in response to interferon treatment. J. Interferons Res. 12 (suppl.), 588.

Constantoulakis, P., Campbell, M., Felber, B.K., Nasioulas, G., Afonina, E. and Pavlakis, G.N. (1993) Inhibition of Rev-mediated HIV-1 expression by an RNA-binding protein encoded by the interferon-inducible *9–27* gene. Science 259, 1314–1317.

Crespi, M. (1989) The effect of interferon on cells persistently infected with HIV. AIDS 3, 33–36.

D'Addario, M., Roulston, A., Wainberg, M.A. and Hiscott, J. (1990) Coordinate enhancement of cytokine gene expression in human immunodeficiency virus type 1-infected promonocytic cells. J. Virol. 64, 6080–6089.

Dolei, A., Fattorossi, A., D'Amelio, R., Aiuti, F. and Dianzani, F. (1986) Direct and cell-mediated effects of interferon-α and -γ on cells chronically infected with HTLV-III. J. Interferon Res. 6, 543–549.

Edery, I., Petryshyn, R. and Sonenberg, N. (1989) Activation of double-stranded RNA-dependent kinase (dsl) by the TAR region of HIV-1 mRNA: A novel translational control mechanism. Cell 56, 303–312.

Francis, M.L., Meltzer M.S. and Gendelman, H.E. (1992) Interferons in the persistence, pathogenesis and treatment of HIV-1 infection. AIDS Res. Human Retrovirus 8, 199–207.

Friedman, R.M. and Pitha, P.M. (1984) The effect of interferon on membrane-associated viruses. In: R.M. Friedman (Ed.), Interferons Vol. 3: Mechanisms of Production and Action, pp. 319–336. Elsevier Science, B.V./North Holland Biomedical Press, Amsterdam.

Gendelman, H.E., Baca, L.M., Turpin, J., Kalter, D.C., Hansen, A., Orenstein, J.M., Dieffenbach, C.W., Friedman, R.M. and Meltzer, M.S. (1990) Regulation of HIV replication in infected monocytes by IFN-α. Mechanisms for viral restriction. J. Immunol. 145, 2669–2676.

Gendelman, H.E., Friedman, R.M., Silverman, R. and Meltzer, M.S. (1991) The role of interferon in the persistence and pathogenicity of human immunodeficiency viral (HIV) disease. J. Interferon Res. 11 (suppl.), S73.

Haseltine, W.A. (1991) Human immunodeficiency virus (HIV) gene expression and functions. In: R.C. Gallo and G. Gay (Eds), The Human Retroviruses, pp. 69–106. Academic Press, Inc.

Hartshorn, K.L., Neumeyer, D., Vogt, M.W., Schooley, R.T. and Hirsch, M.S. (1987) Activity of interferons alpha, beta, and gamma against human immunodeficiency virus replication in vitro. AIDS

Res. Human and Retroviruses 3, 125–133.
Ho, D., Rota, T. and Kaplan, S.C. (1985) Recombinant human interferon-α suppresses HTLV-III replication in vitro. Lancet ii, 602–604.
Kornbluth, R.S., Oh, P.S., Munis, J.R., Cleveland, P.H. and Richman, D.D. (1989) Interferons and bacterial lipopolysaccharide protect macrophages from productive infection by human immunodeficiency virus in vitro. J. Exp. Med. 169, 1137–1151.
Kornbluth, R.S., Oh, P.S., Munis, J.R., Cleveland, P.H. and Richman, D.D. (1990) The role of interferons in the control of HIV replication in macrophages. Clin. Immunol. Immunopath. 54, 2 00–219.
Mace, K., Dodon, M.D. and Gazzolo, L. (1988) Restriction of HIV-1 replication in promonocytic cells: a role for IFN-α. Virology 168, 399–405.
Michaelis, B. and Levy, J.A. (1989) HIV replication can be blocked by recombinant human interferon beta. AIDS 3, 27–31.
Pitha, P.M. (1980) The effect of interferon in mouse cells infected with MuLV. Ann. NY Acad. Sci. 350, 301–313.
Pitha, P.M., Rowe, W.P. and Oxman, M.N. (1976) Effect of interferon on exogenous, endogenous and chronic murine leukemia virus infection. Virology 70, 324–338.
Poli, G., Orenstein, J.M., Kinter, A., Folks, T.M. and Fauci, A.S. (1989) Interferon-α but not AZT suppresses HIV expression in chronically infected cell lines. Science 244, 575–577.
Popik, W. and Pitha, P.M. (1992) Transcriptional activation of the Tat-defective Human Immunodeficiency virus type-1 Provirus: Effect of Interferon. Virology 189, 435–447.
Popik, W. and Pitha, P.M. (1991) Inhibition by interferon of herpes simplex virus type 1-activated transcription of tat-defective provirus. Proc. Natl. Acad. Sci. USA 88, 9573–9577.
Psallidopoulos, M.C., Schnittman, S.M., Thompson, L.M. III, Baseler, M., Fauci, A.S., Lane, H.C. and Salzman, N.P. (1989) Integrated proviral human immunodeficiency virus type 1 is present in CD4+ peripheral blood lymphocytes in healthy seropositive individuals. J. Virol. 63, 4626–4631.
Riggin, C.H. and Pitha, P.M. (1982) Effect of interferon on the exogenous friend murine leukemia virus infection. Virology 118, 202–213.
Roy, S., Katze, M.G., Parkin, N.T., Edery, I., Hovanessian, A.G. and Sonenberg, N. (1990) Control of the interferon-induced 68-kilodalton protein kinase by the HIV-1 *tat* gene product. Science 247, 1216–1219.
Salzberg, S., Bakhanashvili, M., Bari, S., Berman, I. and Abound, M. (1980) Characterization of intracellular viral RNA in interferon-treated cells chronically infected with murine leukemia virus. J. Virol. 35, 694–703.
Samuel, C.E. (1991) Antiviral actions of Interferon: Interferon-regulated cellular proteins and their surprisingly selective antiviral activities. Virology 183, 1–11.
Schnittman, S.M., Psallidopoulos, M.C., Lane, H.C., Thompson, L. Baseler, M., Massari, F., Fox, C.H., Salzman, N.P. and Fauci, A.S. (1989) The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. Science 245, 305–308.
SenGupta, D.N. and Silverman, R.R. (1989) Activation of interferon-regulated, dsRNA-dependent enzymes by HIV-1 leader RNA. Nucleic Acids Res. 17, 969–978.
Shirazi, Y. and Pitha, P.M. (1992) Alpha interferon inhibits early stages of the human immunodeficiency virus type 1 replication cycle. J. Virol. 66, 1321–1328.
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.
Smith, M.S., Thresher, R.J. and Pagano, J. (1991) Inhibition of human immunodeficiency virus type 1 morphogenesis in T cells by alpha interferon. Antimicrol. Agents Chemother. 35, 62–67.
Vlach, J. and Pitha, P.M. (1993) Differential contribution of herpes simplex virus type 1 gene products and cellular factors to the activation of human immunodeficiency virus type 1 provirus. J. Virol. 67, 4427–4431.
Vlach, J. and Pitha, P.M. (1992) Herpes simplex virus type 1-mediated induction of human immunodeficiency virus type 1 provirus correlates with binding of nuclear proteins to the NF-κB enhancer and leader sequence. J. Virol. 66, 3616–3623.
Yamada, O., Hattori, N., Kurimura, T., Kita, M. and Kishida, T. (1988) Inhibition of growth of HIV by human natural interferon in vitro. AIDS Res. Human Retroviruses 4, 287–294.

Yamamota, J.K., Barre-Sinoussi, F., Bolton, V., Pedersen, N.C. and Gardner, M.B. (1986) Human alpha- and beta-interferon but not gamma-suppress the in vitro replication of LAV, HTLV-III, and ARV-2. J. Interferon Res. 6, 143–152.

Zack, J.A., Arrigo, S.J., Weitsman, S.R., Go., A.S., Haislip, A. and Chen, I.S.Y. (1990) HIV-1 entry into quiescent primary lymphocytes: Molecular analysis reveals a labile, latent viral structure. Cell 61, 213–222.