

INTERFERON AS A POSSIBLE PATHOGENETIC FACTOR IN HIV INFECTION

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ABSTRACT

Widely recognized as an important defensive mechanism against viral infections, interferons (IFN), especially when present in the body for prolonged periods of time, may be occasionally associated to pathological conditions such as autoimmune diseases or degenerative disorders. More recently prolonged IFN activation has been found in advanced HIV infection at titers commensurate with the disease activity. This IFN, that is antigenically identical to human alpha IFN but is unstable at low pH, is actually a mixture of alpha IFN with other acid labile cytokines, including gamma IFN, whose simultaneous action gives rise to a synergistic activity that is not longer observed after inactivation of IFN gamma. *In vitro* this IFN type can be induced by HIV infected cells or by purified HIV glycoprotein gp120. In fact, peripheral blood mononuclear cells (PBMC) cultivated in the presence of gp120 show enhanced production of IFN alpha and gamma and of IL-6, TNF, IL-1, IL-10, and IL-8. Moreover, a significant activation of B cells has been observed, leading to the enhancement of both IgG and IgM release. Furthermore a significant proportion of gp120 stimulated PBMC undergo apoptosis. While high concentrations of IFN alpha inhibit HIV replication and reduce infectivity of HIV from chronically infected cells, IFN gamma determines enrichment of adhesion molecules on HIV envelope, leading to widening of HIV host spectrum and infection of cells lacking, the major CD4 receptor and to facilitated cell mediated transmission. These data argue for a possible pathogenetic role of IFN, at least under certain conditions of HIV infection.

Key words: IFN, alpha interferon, gamma interferon, acid-labile, HIV, AIDS

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RESUMEN

Ampliamente reconocidos como un mecanismo defensivo importante contra infecciones virales, los interferones (IFN), sobre todo cuando están presentes en el organismo por períodos de tiempo prolongados, pueden asociarse a condiciones patológicas como enfermedades autoinmunes y degenerativas. Más recientemente, en la infección por el Virus de la Inmunodeficiencia Humana (VIH) avanzada se ha hallado activación prolongada de IFN a títulos que se corresponden con la actividad de la enfermedad. Este IFN, que es antigénicamente idéntico a IFN alfa humano pero es inestable a pH bajo, es realmente una mezcla de IFN alfa con otras citoquinas ácido-lábiles, incluyendo IFN gamma, cuya acción simultánea da lugar a una actividad sinérgica que desaparece al inactivar el IFN gamma. *In vitro* se puede inducir este tipo de IFN por células infectadas con VIH o por la glicoproteína gp120 del VIH purificada. De hecho, células mononucleares periféricas (PBMC), cultivadas en presencia de gp120, muestran aumento de la producción de IFN alfa y gamma y de IL-6, TNF, IL-1, IL-10, e IL-8. También se ha observado activación significativa de células B, con el incremento de la liberación tanto de IgG como de IgM. Además, una proporción significativa de las PBMC estimuladas por gp120 sufre apoptosis. Mientras concentraciones altas de IFN alfa inhiben la replicación del VIH y reduce la infectividad de VIH proveniente de células infectadas crónicamente, el IFN gamma determina el enriquecimiento de la cubierta del VIH en moléculas de adhesión, lo que conduce a ampliar el espectro de hospederos del VIH, a la infección de células carentes del receptor CD4 y a una facilitación de la transmisión mediada por células. Estos datos argumentan a favor de un posible papel patogénico del IFN en la infección por VIH, al menos bajo ciertas condiciones.

Palabras claves: IFN, alpha interferon, gamma interferon, acid-labile, HIV, AIDS

Introduction

Interferons (IFNs) are cytokines that are expressed as an early response to viral infections. Soon after its discovery, it became evident that in viral infections the activation of the IFN system, at a time when immune response is not yet activated, is largely responsible for recovery (1, 2). In fact, sev-

eral studies in animal models, as well as in humans, have supported this conclusion (3).

However IFNs have other important activities, including antiproliferative capacity, modulation of cell differentiation, stimulation of the expression of cell surface molecules, such as receptors for growth factors

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and major histocompatibility complex (MHC) antigens, and regulation of several immune functions (1-4).

Furthermore IFNs can interact with other regulatory factors (such as cytokines, growth factors and hormones), to affect multiple body functions. This pleiotropic activity suggests a role for IFNs as homeostatic agents involved in the control of growth and differentiation, in addition to a pivotal role in the defense mechanisms against viral infections. This is not surprising, since viral infections, causing, in infected cells, a shift of genomic control from cell DNA to viral genome, probably represent the highest degree of cellular dedifferentiation.

IFNs are a family of proteins divided into two main classes: type I IFN, including IFN- alpha, -beta, -omega and -tau, and type II IFN, including IFN-gamma. Several subtypes of IFN-alpha have been identified, which differ molecularly and, to a certain extent, also biologically (5). This redundancy probably has a role in facilitating a fine regulation of the entire system, at both induction and action level, and is also common to other molecules involved in the immune regulation, such as the chemokine family.

During acute infections the activation of type I IFN rapidly occurs, but is transient, so that after a few days IFN is no longer detectable in body fluids, and the organism is, for some time, refractory to restimulation. The feedback mechanisms involved in shutting off IFN activation are not completely understood, but there is no doubt that down regulation is essential, and that prolonged activation of IFN system could be potentially harmful for the host (6, 7). The first suggestion that prolonged IFN activation could be, at least in part, responsible for some aspects of the virus-induced pathology, came from the early studies conducted in mice infected with lymphochoriomeningitis virus (LCMV). In newborn animals inoculated with LCMV, virus replication is not restricted, probably due to the incompetence of the host to mount an adequate immune response. The animals develop an early syndrome consisting of growth retardation and damage of several organs, and a late syndrome due to renal failure characterized by the deposition of immune complexes in glomeruli. Both early and late syndromes could be mimicked by administering IFN to uninfected newborn animals (8); on the other hand, both syndromes could be prevented in infected animals by inoculating antibody preparations capable of neutralizing IFN activity (9). These results indicate that not the virus replication itself, but the virus-induced IFN is actually responsible for the pathogenetic damage.

Soon after these observations, it became evident that persistent IFN activation is present in a number of human and animal diseases, some overtly associated to chronic viral infections, others of unknown origin, such as autoimmune diseases (9). Particularly, IFN is present in the serum of goats and mice

infected with lentiviruses, and in the sinovial fluid of goats with retrovirus-induced arthritis, and is spontaneously released by leukocytes from diseased tissue (10-13). Furthermore in a rat model of juvenile diabetes the appearance of the pancreatic damage is subsequent to type I IFN overexpression in islets, and the administration of IFN to EMC-infected mice after the acute phase of the infection has an exacerbating effect on the pathogenesis of virus-induced diabetes (14). In humans, IFN has been detected in the serum of HIV-infected patients, as well as in the serum of patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren syndrome, systemic and cutaneous vasculitis, scleroderma, as well as in the sinovial fluids of RA patients and in the fluids of psoriatic lesions, also containing retrovirus-like particles (15-23). In addition, the serum IFN detected in most of these cases was initially thought to represent a new species or an aberrant form of some already recognized molecule belonging to the type I IFN family (24, 25), since it is neutralized by antibodies to IFN-alpha, but is partially sensitive to inactivation at low pH, a property that is peculiar of type II IFN. Due to these characteristics, the IFN present in AIDS and in autoimmune diseases has been termed acid-labile IFN-alpha. As a consequence of the persistent activation of the IFN system in these diseases, several markers of the IFN activity are also detected in the blood stream, such as elevated levels of neopterin and β 2-microglobulin, increased activity of 2-5A oligo A synthetase, increased expression of activation markers on leukocytes, etc. (26-28). Whatever the origin and function of IFN in these diseases, it is undoubtful that there is a correlation between the titer of circulating IFN and disease activity (29-32). This observation gave rise to the hypothesis that IFN could participate in the pathogenetic events involved in the disease progression, supported also by previous observations showing that in a mouse model with a lupus-like syndrome (New Zealand Black mice) the administration of either type I or type II IFN accelerated the course of the disease and increased the severity of tissue damage (33), whereas antibodies to IFN resulted in a significant remission of the autoimmune disease (34).

In this review we will report our experience on this topic, particularly concerning the characterization and possible origin of circulating IFN in HIV-infected patients, and the possible contribution to the pathogenesis of the disease.

Induction of acid-labile IFN by HIV-infected cells and by virus coat glycoprotein

Experimental support for the hypothesis that the circulating acid-labile IFN-alpha in HIV-infected individuals is induced by the virus or by virus-related structures is based on the following observations.

The cocultivation of normal peripheral blood mononuclear cells (PBMC) with cells chronically or acutely infected with HIV-1 and fixed with glutaraldehyde leads to the release in the supernatants of an antiviral activity, that is maximal at an effector/inducer cell ratio ranging from 3 to 10, and reaches the titer of 1: 30.000 (35). This antiviral activity is almost abrogated by treatment with antibodies to IFN-alpha (Figure 1), has an apparent molecular weight of 20.000 Da. and is partially destroyed by exposure to acidic pH (30-90 % inhibition of titer). All these characteristics are also exerted by the antiviral activity found in the serum of HIV-infected patients (acid-labile IFN-alpha). A biochemical characterization of the *in vitro* produced acid-labile IFN-alpha has shown that, differently from all known IFN-alpha subtypes, glycosylation is important for the activity of this IFN, and that also acid-lability is strictly dependent on glycosylation (Table 1) (36).

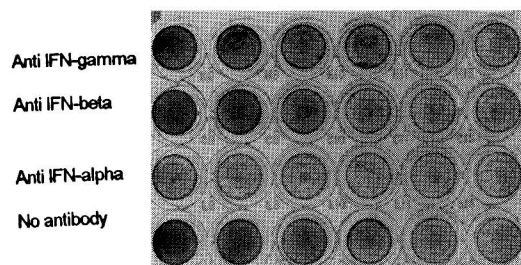


Figure 1. Neutralization of IFN induced by HIV-1 infected cells fixed with glutaraldehyde by antiserum to IFN-alpha, and not by antiserum to IFN-gamma and -beta. Ten units of IFN induced by HIV-1 infected cells fixed with glutaraldehyde were serially diluted on base 2, then exposed to the various antibodies at a fixed concentration (40 Neutralization Units/mL). After an incubation at 37 °C for 30 min, the mixtures were transferred to WISH cells to measure the residual antiviral activity, in a conventional assay, by using Sindbis virus as challenge, as described in 35.

Purification of IFN-alpha molecules by means of affinity chromatography with specific antibodies have indicated that the IFN-alpha molecules retained by the affinity column are acid stable, suggesting that some glycosylated molecules different from IFN-alpha could be responsible for the acid-lability of the antiviral activity. Furthermore, during this separation a substantial amount of antiviral activity is lost. Subsequently we have found that minute amounts of IFN-gamma are induced in this experimental system, but are undetectable by conventional biological assays such as neutralization of antiviral activity. The removal of IFN-gamma by affinity chromatography with specific antibodies reduced the antiviral activity by 30-90 %, similarly to the exposure to acid. Reconstitution experiments performed by recombining the fractions separated by affinity chromatography showed that in fact the starting acid-labile antiviral activity can be restored

Table 1. Effect of tunicamycin on acid-lability of IFN induced by HIV-infected cells.

Tunicamycin	IFN titer (U/mL)		Acid-lability (%)
	pH 7	pH 2	
Exp. 1	-	1.000	70
	+	300	0
Exp. 2	-	10.000	97
	+	300	0

Normal PBMC were cocultivated for one day with H9 cells chronically infected with HIV-1 and fixed with glutaraldehyde, in the presence or in the absence of the glycosylation inhibitor tunicamycin. The supernatants were tested for IFN content, by measuring its antiviral titer before and after acid treatment (36).

by recombining the two IFN types separated by chromatography, indicating that the synergistic effect of IFN-alpha and -gamma determines an antiviral activity more potent than that expected on the basis of the amount of either IFN type, and that exposure to acid pH abrogates such potentiation (Table 2). Since similar experiments performed on the IFN present in the serum of HIV-infected individuals gave substantially similar results, we concluded that the exposure of circulating PBMC to HIV-infected cells triggers the production of multiple, conventional IFN types, and that the simultaneous action of the different IFN molecules gives rise to a synergism of action, that is no longer observed after the inactivation of the IFN-gamma component with acid pH (37). More importantly, in the mixtures of IFN-alpha and gamma the resulting enhancement of biological activity could not be abolished by antibodies to IFN gamma (38), yet are responsible for the synergism with IFN-alpha, suggesting that the interaction of different IFN types in biological systems can have as yet unanticipated effects.

The mechanisms of IFN induction by HIV-infected cells and the main PBMC subpopulations involved were investigated by using recombinant molecules, synthetic peptides and monoclonal antibodies (MAbs) on one hand, and PBMC fractionated by adherence followed by antibody coated magnetic bead separation.

Table 2. Acid-labile potentiation of IFN fractions separated by anti-IFN-alpha affinity column.

Fraction	IFN measured total acid-stable	IFN expected total acid-stable	Ratio measured/expected	
Unglycosylated retained (a)	4.000	4.000		
Glycosylated excluded (b)	240	<30		
Glycosylated retained (c)	500	400		
a + b	4.000	2.000	2.120	2.000
a + c	2.000	2.000	2.250	2.250
b + c	800	240	370	250

The IFN produced by normal PBMC induced with HIV-infected cells was partially purified by ammonium sulphate precipitation, and separated in glycosylated and unglycosylated fractions by affinity chromatography on a ConA-sepharose column. The two fractions were then applied on an affinity column with anti-IFN-alpha antibodies. The fractions excluded or retained by the antibodies were then titrated, either alone, or after their recombination, and their acid-lability established (37).

While for IFN-beta the actual induction stimulus in a virus infection is represented by the viral nucleic acid, several reports in the past have indicated that nucleic acid-free viral envelopes, or viral glycoproteins, can induce IFN-alpha even in the absence of any detectable viral replication (5, 39-44). By using recombinant HIV-1 glycoproteins we could demonstrate that the HIV-specific structure responsible for the induction of both IFN-alpha and -gamma in normal PBMC is the external glycoprotein gp120, or its precursor gp160 (45, 46). The interaction of gp120 with cellular CD4 is required, since either MAb to CD4, or sCD4 decreased the amount of IFN induced by either HIV-infected cells or gp120 (47), as has been also observed in a similar experimental system by others (48).

However the gp120 interaction with the membrane of the induced cells appears to be rather complex, and to involve additional interaction sites, since not only MAb to the CD4-interacting domains of gp120, but also MAb to the third variable domain (V3 loop), and synthetic peptides representing the V3 loop, dose-dependently inhibited IFN production (Table 3). Furthermore, besides CD4, other cell membrane components proved to be involved in the interaction with gp120, namely the galacto-cerebroside (gal-cer) and its sulfate derivatives (sulfatides), since both MAb to gal-cer and soluble sulfatides inhibited IFN induction (49, 50).

In addition to the stimulation of IFN-alpha and -gamma, and of several functions correlated with IFN activity, such as β 2-microglobulin and neopterin, gp120 was able to upregulate the production of a large number of other cytokines. In fact, in PBMC cultivated in the presence of gp120 we found a stimulation of IL-6, TNF, IL-1-alpha and -beta, confirming the results obtained by others, and showed, for the first time, a stimulation of IL-10 and IL-8 (51-53).

Furthermore, a significant activation of B cells has been observed, leading to the enhancement of both IgG and IgM release in gp120-induced PMBC cultures (45). Gp120 does not seem to indiscriminately activate all PBMC functions, since some cytokines, such as IL-4 (Th type-2) and IL-2 (Th type-1), are not found in the supernatants of

gp120-stimulated cultures. The absence of IL-2 stimulation is paralleled by the lack of activation of DNA synthesis in gp120, stimulated PBMC (47), while the gp120-stimulated PBMC express increased IL-2 receptors, and can be induced by exogenous IL-2 to proliferate (54), suggesting that they are in a state of at least partial activation. According to this hypothesis, other activation markers, both early (such as CD69), and late (such as CD45RO and CD71), are induced by gp120, although to lower extent and with different kinetics as compared to PHA (54). Furthermore a significant, although low proportion of PBMC stimulated with gp120 undergo apoptosis (Table 4).

Table 4. Apoptosis in gp120-treated PBMC.

Treatment	% + cells (mean \pm sem)	p
None	3.3 \pm 0.49	
Gp120	8.0 \pm 0.76	< 0.001
Anti Fas	11.2 \pm 1.02	< 0.001

Normal PBMC were incubated for one day with either recombinant HIV-1 IIIIB gp120 or anti Fas MAb. The apoptotic cells were visualized by immunocytochemical staining of nuclei containing fragmented DNA (TUNEL).

Taken together, these results clearly show that HIV, or its soluble products such as gp120, can modify several PBMC functions, by inducing a number of cytokines and a partial state of immune activation. It is therefore possible that the gp120-driven changes of PBMC functions *in vivo* can participate in the immunopathologic events responsible for disease progression. Moreover HIV replication can be affected by the virus-induced cytokines, leading to the conclusion that in the body the overall effects can result not only from the multifactorial balance of pro- and anti-viral actions of each single cytokine, but it can also be derived from the synergistic interaction of several cytokines. Therefore it would be of great interest to dissect the actions of the various cytokines, to identify the individual pieces of such a composite puzzle. The possible involvement of IFNs in the outcome of HIV infection is discussed in the subsequent parts of this view.

Table 3. Inhibition of IFN production by synthetic peptides.

Peptide	Residues	IC ₅₀ (μ M)
CTRPNNNTRKSIRIQRGPGRAFVTIGKIGNMRQAHCN	266-302	4
NTRKSIRIQRGPGRAFVTIG	272-291	5
VEINCTRPNNNTRKSIRIQ	262-280	> 25
RIQRGPGR	278-285	> 50
QRGPGRAF	280-287	> 50
GPGRAFVT	282-289	> 50
GRAFVTIGK	284-292	> 50

Normal PBMC were exposed to recombinant HIV-1 IIIIB gp120 in the presence or in the absence of graded amounts of the indicated peptides, covering to a variable extent the V3 loop region of the glycoprotein molecule, as indicated. The antiviral activity present in the supernatants after one day of culture was then measured to determine the peptide concentration which is able to define a 50 % inhibition of the IFN yield as compared to control cultures (49).

IFN-alpha determines a decreased infectivity of HIV progeny from chronically infected cells

As compared to conventional cytopathic viruses, HIV seems to be less sensitive to the inhibitory effects of IFN-alpha. However circulating IFN-alpha appears to confer a selective advantage to IFN-resistant HIV variants, since the isolation of such variants is more common in the advanced stages of the disease, as compared to early stages (55). This suggests that endogenous IFN-alpha can be an important determinant of the virus phenotype, possibly involved in pathogenic events.

The effects of IFN-alpha on HIV replication *in vitro* has been extensively investigated in different experimental systems, including both primary cells, such as PBMC, monocytes/macrophages, isolated CD4+ T cells, and established T lymphoblastoid and monocytoid cell lines. Furthermore both acute and persistent HIV infection have been analyzed. From these studies it can be concluded that, despite the conflicting, but generally modest results obtained with the exogenous administration of IFN *in vivo* (56-58), in the *in vitro* experiments HIV shows a certain degree of sensitivity to IFN, particularly during exogenous infections involving multiple cycles of replication (59-70). The effects on chronic infections seems to be much less pronounced. Generally speaking, IFN seems to exert on HIV the same effects observed in the early studies on animal retroviruses (71, 72). In fact, in acute infections the IFN seems to act by inhibiting early steps of virus replication, such as reverse transcription, but also viral RNA and protein synthesis can be inhibited (73-75). On the contrary, in persistently infected cell cultures IFN-alpha seems to act by inhibiting late steps involving maturation events (76-80).

To better define the mechanisms of action of IFN-alpha on the late events of HIV multiplication, we used promyelocytic cells (U937) infected in our laboratory with the III_B strain of HIV, and persistently releasing detectable amounts of infectious virus. In this cell line IFN treatment determines a dose-dependent inhibition of virus release, but the synthesis of viral components (mainly p24) is not grossly inhibited, in contrast to what has been observed in similar experimental systems by others (81). However, the assembly of virions is impaired, leading to the formation of tear-drop shaped virus particles, and the infectivity of both cell-associated and released virus is strongly reduced in IFN-treated cultures (Table 5). Neither the proteolytic processing of virus *gag* protein, nor its phosphorylation seems to be affected, and the amount of virionic RNA appears to be normal in IFN-treated cultures. However, the amount of gp120 incorporated by budding virions is decreased, accounting for a reduced ability of such virions to bind to and infect CD4 positive target cells (82). Similar results have been obtained by others in a T-cell system (83), but the mecha-

nisms determining the alteration of gp120 incorporation into released virions have not yet been elucidated.

Table 5. Infectivity of HIV released by IFN-alpha-treated U937.

Treatment	Ratio infectivity/p24*
None	587
IFN 100 U/mL	59
IFN 1000 U/mL	19

*TCID₅₀/ng p24

Chronically infected U937 cells were exposed to IFN alpha at the indicated concentrations, and after three days the virus progeny was collected and purified by sucrose density centrifugation. Virus infectivity was then measured and referred to the amount of p24 present in the virus preparations.

IFN-gamma determines enrichment of adhesion molecules on progeny virus

It has been shown that HIV maturation and release occur at restricted cell membrane sites, coincident with those regions involved in the contact with other cells (84). Furthermore it has been demonstrated that during the budding process from the cell membrane, the HIV envelope captures a number of cellular proteins, including cytokine receptors such as IL-2R, adhesion molecules such as LFA-1, ICAM-1, -2 and -3, HLA Class I and II, as well as cell lineage markers such as CD3 and CD19, reflecting, like a fingerprint, the antigenic properties of its host cell (85). These molecules are exposed on the outer surface of virions, since specific MAbs immobilized to a plastic surface capture infectious virions (86). Furthermore, the same MAbs, in a soluble form, display the typical behavior of neutralizing antibodies (Figure 2). Although it is possible that the blockade of these molecules on the target cells can actually determine the inhibition of virus growth as the result of interference with intercellular communication events. The possibility that the cellular molecules present on the virus envelope can participate in the binding to the target cells is strongly supported by the following observations.

Since IFN-gamma is able to upmodulate the expression of several adhesion molecules, we used this cytokine to establish whether the changes in the cellular expression of membrane molecules are reflected in the virus progeny.

Diploid fibroblasts chronically infected with HIV-1 do not express HLA DR, and the surface of released virions is negative for this molecule. However, in cultures exposed to IFN-gamma, together with other HIV-upregulatory cytokines to compensate for the inhibitory effect of IFN on virus production, the progeny virus acquires HLA DR, as a result of the induction of this antigen on its host cells (87).

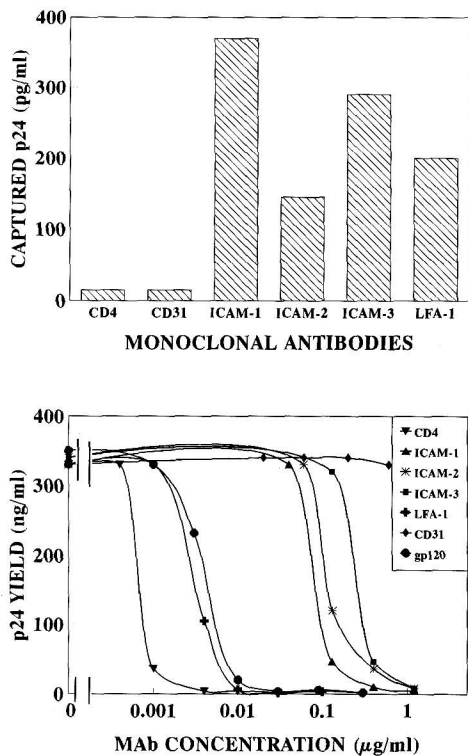


Figure 2. Upper panel: antigenic profile of HIV-1 progeny (IIIB strain) from chronically infected lymphoblastoid H9 cells, as determined by immobilized antibody capture (86). Lower panel: effect of MABs against cellular antigens on the virus yield by CD4 positive (C8166) cells infected at low MOI (0.01 TCID₅₀/cell) with the HIV-1 preparation characterized in the upper panel.

Such findings have been confirmed and expanded in U937 cells persistently infected with HIV. In fact in this system IFN-gamma determines a stimulation of the expression of both HLA DR and ICAM-1, whereas the expression of LFA-1 remains unchanged. Virus progeny from IFN-treated cells also showed increased uptake of both HLA DR and ICAM-1, while LFA-1 levels were unaffected, as determined by immunocapture of purified virus progeny by immobilized specific MABs (88). The increased amount of ICAM-1 was also detected by measuring virus associated molecules by ELISA in virus lysates, as shown in Table 6.

Since it has been hypothesized that the virus-associated adhesion molecules can function as alternative or additional ligands for virus adsorption to target cells, we tested the ability of adhesion molecules-enriched virions obtained from IFN-gamma treated U937 cells to infect host cells negative for the major HIV receptor CD4, but expressing the ICAM-1 ligand LFA-1, such as Lymphoblastoid B cell lines. The results indicated that, while control virions displaying few ICAM-1 mole-

Table 6. Effect of IFN-gamma on the incorporation of ICAM-1 by HIV-1.

Treatment	ICAM-1 molecules/virion mean ± sem)	HIV-1 capture by anti-ICAM-1 (ratio to irrelevant MAb) (mean ± sem)
None	1.3 ± 0.4	2.4 ± 0.2
IFN	6.2 ± 0.7	5.6 ± 0.3
p	0.003	0.017

Virus progeny from U937 cells chronically infected with HIV-1 IIIB, either untreated or treated for three days with IFN-gamma (100 U/ml), was purified by sucrose density centrifugation, and the amount of virion bound ICAM-1 was measured by ELISA on virus lysates. The amount of ICAM-1 exposed on the outer surface of virions was determined by immobilized antibody capture (86), and expressed as the ratio between the HIV captured by anti-ICAM-1 MAb-coated wells and the HIV captured by irrelevant MAB-coated well (84-88).

cules are unable to infect such cell lines, the virus progeny from IFN-gamma treated U937 cells, displaying increased ICAM-1 on its surface, is able to generate a productive infection in the two tested B cell lines (88).

These results suggest that ICAM-1 molecules exposed on the virion surface can confer to HIV the ability to infect cells lacking the major receptor CD4, allowing virus binding to surrogate receptors such as cognate ligands expressed by the target cells.

In conclusion, these data show that the presence of cytokines may enable HIV to expand its host cell spectrum to CD4-negative cells.

IFN-gamma facilitates cell-mediated transmission of HIV

It is generally accepted that the ability of IFN-gamma to stimulate ICAM-1 expression could influence the intercellular communication events involved in immune cross-talking and in the leukocyte-endothelium multistep interactions (89). We obtained evidence that intercellular adhesion molecules are also involved in the cell-mediated transmission of HIV infection, and that increased ICAM-1 expression is at least in part responsible for the stimulation of HIV transmission, in an original system of cell-to-cell mediated infection (90-92). Namely, we developed an experimental system involving human umbilical vein endothelial cells (HUVEC). In these cells, HIV causes an abortive infection, since virus DNA is synthesized, but progeny virus is not detected; however, the addition of lymphoblastoid or native PHA activated T cells permits the transmission of the infection to permissive cells, and infectious progeny is released, accompanied by a massive cytopathic effect (Figure 3). The membrane interaction between HIV-infected HUVEC and T cells is very intimate and involves partial fusion of the cell membranes at distinct points, whereas in cocultures of uninfected HUVEC with T cells, fusion between the two adjacent cell membranes could not be observed (91, 92). When HUVEC are treated with IFN-gamma, a dose-dependent increase of the HIV yield in the

subsequent cocultures with T cells is observed. This phenomenon does not appear to depend on the stimulation of early events of HIV infection of HUVEC, but, rather, it seems to be due to a more extensive membrane interaction with the T cells, since more fusion points can be detected by the transmission electron microscopy analysis of the cocultures. Since IFN-gamma treated HUVEC show enhanced ICAM-1 expressions and are more sensitive to the inhibitory effect of anti ICAM-1 MAb on virus transmission to T cells, it is likely that the enhancement of HIV infection by IFN-gamma in this experimental system is a direct consequence of the stimulation of ICAM-1 expression by HUVEC. Since endothelial cells lining the blood vessels are important components of the blood-tissue barriers, it has to be considered that they can function as transient reservoirs of HIV infection, and can transmit the virus to circulating PBMC during the intercellular communication events involved in the leukocytes trafficking and, possibly, extravasation. It is also possible that through this mechanism HIV may be able to cross the anatomical barriers and to invade otherwise inaccessible body districts, such as the central nervous system. Inflammatory cytokines such IFN-gamma, upmodulating the expression of adhesion molecules by endothelial cells, may be involved in this circuit, facilitating the HIV transmission from endothelial cells to circulating cells, thus contributing to virus invasion and spread through the organism.

Conclusions

Persistent IFN activation has been hypothesized to be not only an epiphenomenon of HIV infection but to be involved in the pathogenetic events underlying AIDS development (56, 57, 93, 94). To this respect, it is noteworthy that HIV itself, HIV-infected cells, as well as shed gp120, represent a chronic stimulus to IFN production by circulating cells (94, 96), but, as already established for many retroviruses, virus replication appears to be poorly sensitive to the antiviral effects of either IFN types. Particularly, in persistently infected cells, that can be considered as virus reservoirs *in vivo*, the virus cycle stages sensitive to type I IFN are those involved in virus maturation, while the infection is not cleared by IFN.

This can represent an escape mechanism by the virus, that, although inhibited by type I IFN in its possibility to generate an infectious cycle in permissive cells, could persist in long-living cell reservoirs, despite high levels of circulating IFN. On the other hand, IFN-gamma seems to facilitate alternative modes of virus transmission by mechanisms involving upregulation of the expression of the cell membrane molecules involved in intercellular adhesion.

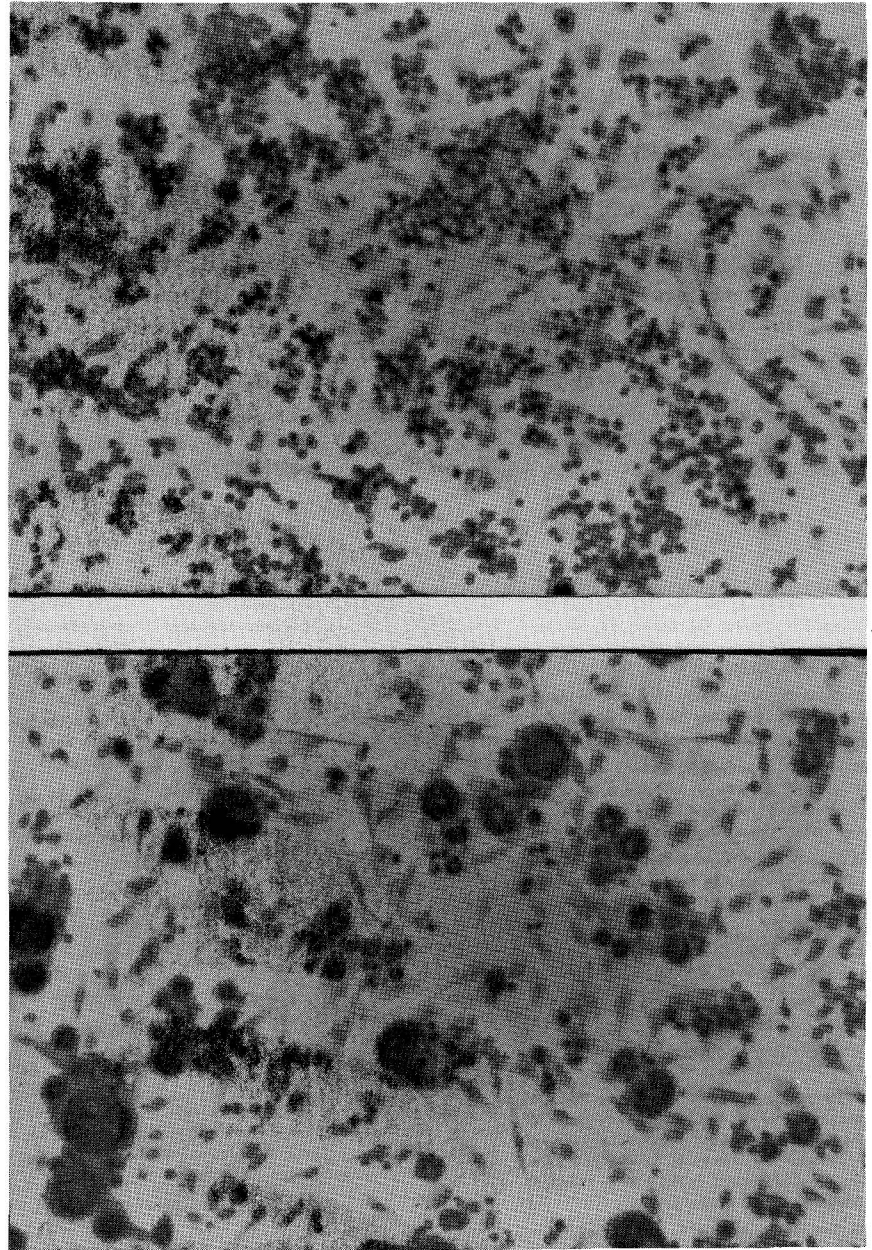


Figure 3. Cocultures of uninfected (upper panel) or HIV-1 infected (lower panel) HUVEC with CD4 positive C8166 cells. Freshly isolated HUVEC were infected with a HIV-1 isolate obtained from an advanced AIDS patient (strain P1), and cocultivated with rescuer C8166 cells. Cocultures were stained with crystal violet at day 4 of infection. Long shaped HUVEC are surrounded by small lymphoblastoid cells (upper), or by large syncytia (lower), as it is commonly found in productively infected CD4 positive cell cultures.

As shown in our experiments, the virus progeny from cells exposed to IFN-gamma captures, during budding, an increased amount of these cellular molecules, that can be used as alternative ligands to infect target cells not infectable through the CD4 receptor. A direct consequence is the widening of the host cell spectrum for a given virus population.

On the other hand, the increased membrane interactions occurring in cells exposed to IFN- γ can facilitate the HIV transmission from reservoirs to T cells, that have repeatedly shown to be poorly sensitive to the antiviral effects of IFN, resulting in an increased spread of HIV, and, possibly, in the bypass of anatomical barriers.

On the whole, although the examples described here are far from elucidating the interplay between IFNs, HIV and its potential target cells, they suggest that such interactions are very complex and not unidirectional, being able to either limit or favor virus infection. Therefore it is conceivable that a potent host defense system, such as IFN, can even play a role as a pathogenetic factor in the infection of HIV, depending on the circumstantial conditions of the microenvironment where it acts, and, possibly, also

on the balance of the actions of other factors able to up- or down-modulate HIV infectivity and cell susceptibility to its infection (97).

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