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Interferon Responses in HIV Infection: From Protection to Disease

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Abstract

Interferons, induced early during viral infections, represent important regulators of both innate and adaptive immune responses, and provide protective effects against a wide range of pathogens, including HIV. Several* in vitro *studies and some* in vivo *data from HIV-exposed seronegative cohorts indicate that interferons and interferon-mediated immune responses are crucial in preventing early HIV replication. Following establishment of HIV infection, the uncontrolled (aberrant) activation of the immune system, in part regulated by interferon levels, contributes to HIV-1-induced immune activation and disease progression. Modulation of interferon responses prior to and during HIV infection shows promise for development of novel therapeutics to prevent HIV transmission, clear HIV infection, and dampen chronic immune activation. In this review we discuss the role that interferons play in protection from HIV infection, acute infection, and their role in HIV pathogenesis and disease progression. Lastly, we review recent advances in modulating interferon responses for purposes of developing novel HIV therapeutic approaches. **(AIDS Rev. 2014;16:43-51)**

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Key words

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Introduction

Interferons (IFN), commonly known as antiviral cytokines, have existed in early chordates for around 500 million years[1], and represent important regulators of both innate and adaptive immune responses, carcinogenesis, and immune cell function. Induced early during viral infections, IFNs provide protective effects against a range of viral pathogens, including influenza, hepatitis C (HCV), herpes simplex, vaccinia, and HIV. Due to their antiviral properties, IFNs are currently used for treatment of various diseases, including HCV infection. However, millions of years of concurrent viral/IFN evolution have led to the development of viral evasion mechanisms, minimizing the therapeutic potential of IFNs and improving viral replication and survival. In this review, we discuss the role of IFNs in the protection against establishment of HIV infection and early viral replication as well as their role in HIV pathogenesis and disease progression. Finally, we discuss recent advances in developing IFN treatment for HIV-infected patients.

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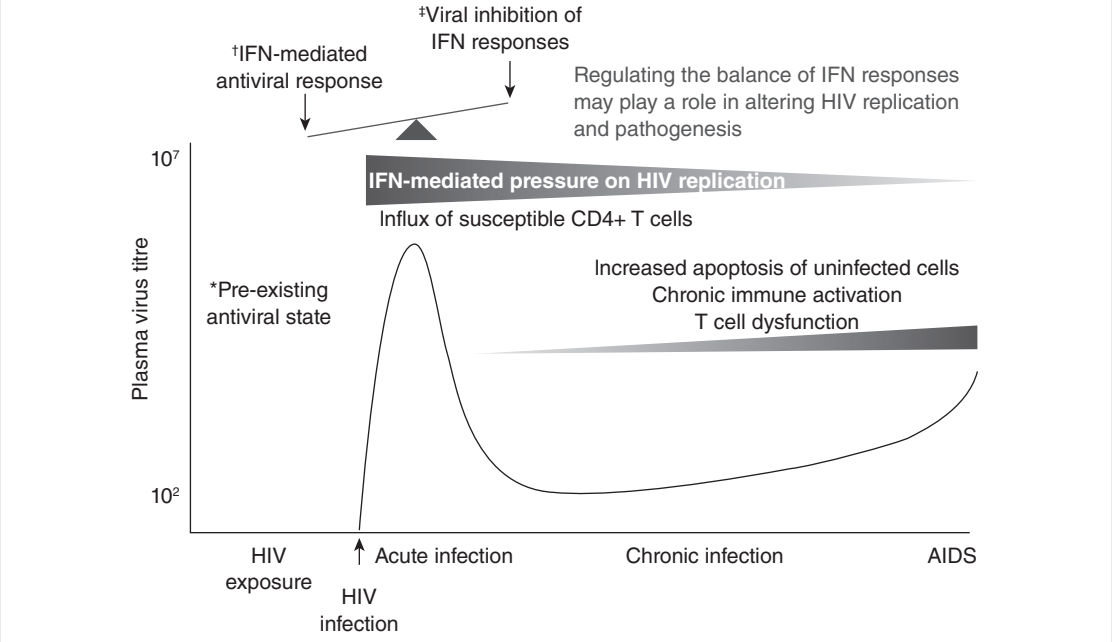
Overview of interferon responses

Three types of IFN have been identified so far: type I (α, β, δ, ε, κ, τ, ω, ζ), II (γ) and III (λ). Type I IFNs contain nine subtypes: IFN-α (with 13 known members), IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-τ, IFN-ω, and IFN-ζ(limitin)[2]. Not all type I subtypes are found in humans, with IFN-δ found only in pigs, IFN-τ found only in ruminant animals, and IFN-ζ identified in the murine



*Interferon responses have been linked with natural resistance to HIV infection. This includes protective genetic factors, enhanced IFN-γ production by NK cells, and increased expression of viral restriction factors (APOBEC3G, ISGs).
†Interferon-mediated antiviral response includes induction of antiviral restriction factors (APOBEC3G, TRIM5-α, Tetherin etc.), apoptosis of HIV-infected cells, and the enhancement of innate and adaptive immune responses.
‡Viruses can block nearly every aspect of the IFN responses, including IFN production, signaling, and the antiviral actions of ISGs.

Figure 1. *Role of interferon responses during HIV infection. Innate antiviral responses play an important role in preventing the establishment of HIV infection. Once infection is established, protective and damaging effects of IFNs are often seen in parallel. IFN-induced immune responses in the early stages of infection restrict viral replication and spreading through upregulation of antiviral restriction factors, apoptosis of HIV-infected cells, and enhancement of innate and adaptive immune responses. While evidence suggests that IFN antiviral responses continue during the chronic stage of infection, they also contribute to increased apoptosis of uninfected cells, T-cell dysfunction, and chronic immune activation. IFN: interferon; ISG: IFN-stimulated gene; NK: natural killer.*

IFN gene cluster[2]. Type I IFNs are acid stable, a property that has eased the process of their identification and characterization[1]. Type II IFNs consist of only one member, IFN-γ, distinguished from type I IFNs by its acid liable properties. The most recent addition to the IFN family, type III or IFN-λ, consist of three members: IFN-λ1, IFN-λ and IFN-λ3, previously known as IL28A, IL28B, and IL29, respectively. Structurally, type III IFNs are different from both type I and type II IFNs and exhibit a high structural homology with IL10/IL22[3].

Most cells in the body are capable of producing type I IFNs, including leukocytes, fibroblasts, and endothelial cells[4]. Originally recognized for their ability to confer antiviral resistance, type I IFNs have a range of immunomodulatory and antitumor properties as well[5]. Primary producers of IFN-γ are natural killer (NK), NKT, $CD8^+$ T and Th1 $CD4^+$ T-cells[6]. In addition to antiviral immune responses, type II IFN is important in allergic responses, tumor control, and a broad range of immune responses to pathogens other than viruses[3]. While type III IFNs are produced by a wide range of cell types, they primarily act on epithelial cells and are suspected to play an important role in preventing viral invasion through skin and mucosal surfaces[7]. Induction of IFNs commonly involves interferon regulatory factors (IRF) that, together with nuclear factor kappa B (NFκB), bind IFN promoters and initiate transcription of IFN genes. The strength and nature of IFN response depends on the stimulus and the cell type involved; human plasmacytoid dendritic cells (pDC), the most potent producers of type I and III IFNs in the body, produce 1,000-times more IFN-α than other cell types in response to stimulation by DNA or RNA viruses[8,9]. Following induction, IFNs exert their function by binding to their respective cell-surface receptors: type I IFN (IFNAR1 and IFNAR2), type II IFN (IFNGR1 and IFNGR2), and

type III IFN (IFNLT1 and IL10RB)[3]. Altering the responsiveness of the target cells through regulation of receptor surface expression is one of the major mechanisms of regulating the strength and duration of the IFN responses[3]. Binding of IFNs to their respective receptors triggers intracellular signaling pathways (mainly the JAK-STAT pathway), ultimately resulting in transcriptional activation of IFN-stimulated genes (ISG), which are the primary factors responsible for antiviral and immunomodulatory IFN properties. Over 400 ISGs have been identified[4], some of which have direct antiviral function. These antiviral ISGs include proteins that catalyze cytoskeletal remodeling, induce apoptosis of infected cells, and regulate posttranscriptional and posttranslational modifications[10]. Both Mx proteins and nitric oxide (NO) are some of the better-studied ISGs, known to inhibit a wide range of viruses. The Mx proteins inhibit early stage replication of a wide range of RNA viruses, including influenza and members of a bunyavirus family[11]. Interferon-γ-mediated induction of NO synthase limits the replication of several viruses, including vaccinia and herpes simplex-1[12]. In addition, IFNs regulate both adaptive and innate immune response, acting directly or indirectly on NK cells, B-cells, T-cells, DCs, and phagocytic cells[13]. As viruses evolve under immune selection pressure, they develop new ways of controlling and exploiting protective IFN responses to promote viral pathogenesis and survival. Viruses can block nearly every aspect of the IFN response[14,15], including inhibition of IRF and NFκB functions and mechanisms that target the antiviral actions of ISGs. For example, human herpes virus-8 encodes for IRF homologues (vIRF), that inhibit host IRFs, such as IRF1 and IRF3, thereby interfering with IFN production[16,17]. Together, the intensive interaction between the host antiviral responses and viral defensive mechanisms has been the main driving force for their evolution over millions of years. This review will focus on the interaction between IFNs and HIV.

Interferons and their role in natural protection against HIV infection

It is generally thought that HIV infection arises from a single or relatively few founder viruses, establishing a single focus of infected mucosal $CD4^+$ T-cells[18]. The failure of most infected foci to develop into an established infection site could in part be explained by the effects of IFN-induced antiviral factors that prevent early HIV replication and viral dissemination. Interferon induction leads to activation of numerous ISGs, some of which are antiviral restriction factors. APOBEC3G, an IFN-α-regulated gene, acts to restrict HIV replication in newly infected cells by editing C→U in HIV DNA negative strand, introducing premature stop codons and inhibiting reverse transcription and chromosomal integration[19-21]. Other IFN-induced HIV restriction factors include TRIM5-α, tetherin (BST-2) and SAMHD1, which act to block uncoating of the incoming virion, block release of enveloped viruses, and inhibit HIV replication in myeloid cells, respectively[22,23]. Several *in vitro* studies have demonstrated IFN's ability to restrict HIV replication. Exposure of cells to IFN-α, -β, and -γ prior to infection induces an antiviral state and prevents productive viral infection[24,25]. Treatment of cells with IFN-α inhibited Vpu-deficient HIV replication through activation of tetherin, an antiviral restriction factor that prevents virion release. Treatment with IFN-λ was shown to inhibit HIV-1 infection of macrophages through upregulation of extracellular CC chemokines and activation of intracellular innate immune responses (the induction of other IFNs and APOBEC3G/3F)[26]. Together, this *in vitro* evidence suggests that IFN-mediated antiviral innate immunity against HIV infection plays an important role in containing and controlling the dissemination of HIV in the early stages of infection.

Unfortunately, the therapeutic potential of the described HIV inhibitors is lessened due to HIV's numerous evasion mechanisms. HIV-1 counteracts IFN responses by inhibiting IFN-driven intrinsic restriction factors (such as APOBEC3G and tetherin) via the action of viral accessory proteins (including Vif and Vpu) and by inhibiting upregulation of other antiviral proteins encoded by ISGs in target cells[22,27]. However, IFNs and IFN-mediated responses have been proposed to play an important role in natural protection against HIV infection in HIV-exposed seronegative (HESN) individuals. Cohorts of individuals who remain uninfected with HIV despite repeated exposure have been identified around the world and include different at-risk populations: commercial sex workers, men who have sex with men, infants born to HIV-positive mothers, injection drug users, hemophiliacs, and discordant couples[28]. Expression of IFN-α-induced APOBEC3G was significantly higher in peripheral blood mononuclear cells (PBMCs) and in cervical tissues of HESN individuals exposed to HIV through sexual intercourse[29]. This increase in APOBEC3G expression correlated with a reduced susceptibility of PBMCs to *in vitro* infection with $HIV\text{-}1_{Ba\text{-}L}$ R5 strain. It would seem that a potent IFN-induced antiviral immune response including factors such as APOBEC3G could offer a strong barrier against HIV infection, both systemically and mucosally.

Table 1. Clinical studies examining the effect of altering interferon responses on HIV infection

Study	Treatment	Patient group	Results
Gringeri, et al.[75]	IFN-α-2b (250 µg) was injected intramuscularly 3-times at 1-month intervals. Priming injections were followed by booster injections administered every 3 months	242 asymptomatic patients with $CD4^+$ counts 100-634 cells/mm^3, both ART treated and untreated	Low immunogenicity of the vaccine, reduced risk of occurrence of AIDS-related symptoms in patients with rise of anti-IFN-α antibody
Piconi, et al.[79]	Hydroxychloroquine* 400 mg/day for 6 months	20 ART-treated patients with absolute CD4 count < 200 cells/µl during the last 12 months of therapy with suppressed viremia (< 37 HIV RNA copies/ml)	Decreased immune activation
Murray, et al.[78]	250 or 500 mg of chloroquine* daily, or placebo for 2 months	13 patients not on ART with CD4 T-cell count > 250 cells/ml	Reduced systemic T-cell immune activation
Paton, et al.[80]	Hydroxychloroquine* 400 mg or matching placebo once daily for 48 weeks	83 patients not on HAART with CD4 cell count > 400 cells/µl and viral load > 1,000 copies/ml	Greater decline in CD4 cell count and increased viral replication
Yola, et al.[82]	Standard TB treatment + IFN-γ injection at a dose of 500,000 i.u. subcutaneously 3-times weekly for 8 weeks	51 TB/HIV coinfected patients with CD4 cell count > 350 cells/ml not previously on HAART	Increase in $CD4^+$ lymphocyte counts and decrease in plasma HIV RNA concentrations
Azzoni, et al.[81]	180 or 90 µg/week of PEG-IFN-α2a + ART; after 5 weeks, ART was interrupted, and PEG-IFN-α2a was continued for 12-24 weeks	23 HIV-1-infected patients on ART with plasma HIV RNA load < 50 copies/ml and $CD4^+$ T-cell count > 450 cells/µl	Control of HIV replication and decreased HIV-1 integration

*Chloroquine, an endosomal inhibitor used in the treatment of malaria and autoimmune disorders for its ability to reduce chronic immune activation, was shown to block IFN-α production, resulting in reduced T- and pDC-cell activation and blocking of negative modulators of T-cell function, indoleamine 2,3-dioxygenase, and programmed death ligand 1[77]. ART: antiretroviral therapy; IFN: interferon; PEG-IFN: pegylated interferon; TB: tuberculosis.

Natural killer cells, major producers of IFNγ and a critical component of the host innate immune responses, have been identified to play a pivotal role in natural protection against HIV-1 infection. Expression of IFN-γ by phorbol myristate acetate/ionomycin-activated NK cells was significantly elevated in a HESN cohort in individuals exposed to HIV through sexual intercourse with a known HIV-positive partner[30]. Similar observations were made in Vietnamese HESN intravascular drug users, showing increased percentages of NK cells producing IFN-γ and tumor necrosis factor (TNF)-α and -β chemokines (CCL3, CCL4 and CCL5), either after *in vitro* activation or without stimulation compared to healthy controls and HIV-positive individuals[31]. Additionally, NK cells expressing the protective killer cell immunoglobulin-like receptor 3DS1, associated with delayed progression to AIDS[32] and reduced risk of HIV-1 infection[33], have been shown to produce more IFN-γ in unstimulated state[34] and mediate strong inhibition of HIV-1 replication *in vitro*[35]. Together, these studies further indicate that IFN-γ production by innate immune cells, particularly NK cells, may play a major role in natural protection from HIV infection.

Interferon regulatory factor-1, a major transcriptional regulator of IFN responses, was identified as one of the key correlates of protection in the Puwmani sex worker cohort in Nairobi, Kenya. The IRF1 plays a crucial role in host antiviral immune response as well as HIV replication. Specific polymorphisms within the IRF1 gene were shown to be associated with reduced likelihood of seroconversion[36,37]. Interestingly, PBMCs from individuals with these protective IRF1 polymorphisms exhibited lower basal IRF1 expression and responsiveness to IFN-γ stimulation[37], and reduced ability to support HIV transcription and replication when infected with a single-cycle VSV-G pseudotyped HIV-1 virus ($HIV\text{-}_{VSV\text{-}G}$)[38]. As with the majority of innate antiviral responses, this protective effect was restricted to the initial stages of HIV-1 infection and did not provide any benefits once infection was established[37,39]. Recent data from the same cohort has shown that early robust but transient IRF1 responses may be one of the key factors in preventing the establishment of HIV infection[40]. Overall, these results indicate that IFN-γ production and IFN-γ-mediated immune responses by innate immune cells seem to be an important

factor in controlling the establishment of HIV-1 infection in HESN individuals. It seems conceivable that a robust but transient antiviral immune response, together with the protective physical barrier, could potentially be sufficient in restricting early HIV replication, while at the same time preventing over-activation of the immune system.

Interferons and acute HIV infection

It is generally accepted that the early innate immune responses against HIV-1 are likely a crucial factor in determining the clinical course of the disease. Following mucosal transmission of HIV, viral RNA is undetectable in the circulation for a period of about 10 days. This is referred to as the "eclipse period", or the period from viral entry into a cell to the production of new virions[41]. Virus levels increase exponentially following the eclipse period, to reach the peak at around 21-28 days postinfection. One of the first signs of immune response to HIV infection is the surge in expression of inflammatory cytokines, including IFN-α and IFN-γ[42]. This coincides with a steep rise in HIV-1 viral load and contributes to symptoms of acute retroviral syndrome[41]. This increase in viremia was shown to be associated with rapid and transient increase in IFN-α and a slow and sustained increase in IFN-γ as well as other proinflammatory cytokines[42]. Dendritic cells, specifically pDCs which are recruited to the infection site by virus-mediated induction of MIP-3α/CCL20 in endocervical epithelium, are thought to be the major producers of IFN-α[41], while increased NK and NK T-cell activation during acute infection[43,44] might explain the observed increase in IFN-γ. There is considerable controversy whether or not this early IFN induction is beneficial or detrimental to the host. Increased immune activation leads to influx of other immune cells, including macrophages and T-cells, to the site of the infection, transforming the submucosal environment into an ideal place for viral replication and expansion of the HIV-1 founder population. However, the early induction of IFNs and IFN-mediated immune responses may prevent or partially control HIV from spreading through activation of intrinsic antiviral factors, as well as induction of apoptosis of HIV-1-infected cells[45]. The HIV replication in pDCs is significantly restricted, and this viral restriction could be removed by antibodies against IFN-α, reflecting the crucial role early IFN responses play in the control of viral replication[46]. The notion that IFNs and IFN-mediated mechanisms contribute to HIV control *in vivo* is further supported by observations made in HIV elite controllers, a group of HIV-infected patients who are able to maintain undetectable viral loads in absence of antiretroviral therapy. Machmach, et al. showed preserved pDC count and IFN-α production in elite controllers[47]. Additionally, pDCs from elite controllers had higher capacity to reduce HIV production and induce HIV-infected T-cell apoptosis compared to viremic patients. Type I IFN-mediated antiviral activity exerts selective pressure on the transmitted virus pool, with virus isolates from patients during acute HIV infection being more resistant to *in vitro* control by IFN-α than viruses isolated from the same patients during chronic infection[48]. The establishment of systemic HIV-1 infection by relatively IFN-α-resistant founder viruses indicates that IFN-α and IFN-α-mediated host responses play a crucial role in restricting early HIV replication.

While optimal IFN responses before the establishment of infection can be protective, once infection is established the early innate responses can lead to recruitment of additional susceptible T-cells to the infection site, perpetuating viral replication and spread. The IFNα responses induced in acute HIV infection are also thought to contribute to acute-phase $CD4^+$ T-cell apoptosis. The first evidence of infection-induced apoptosis is the increased expression of TNF-related apoptosis-inducing ligand (TRIAL) and FAS ligand in plasma, and this increase in TRAIL coincides with the increase in IFN-α levels[49]. In response to stimulation by cell-free HIV and HIV-infected cells, pDCs produce a range of IFN-α subtypes as well as IFN-β, IFN-κ, IFN-ω, and IFN-λ[50]. Recently Zhou, et al. have shown that autophagy, a catabolic process of degradation and recycling of cellular components through the lysosomal machinery, is critical for HIV-induced production of IFN-α by pDCs and that the signaling process involves TLR7[51]. This was shown to be the case with both infectious and noninfectious virus, indicating that viral replication is not necessary for robust induction of IFN-α by HIV-1. Both infectious and noninfectious HIV-1 induce IFN-α, which activates TRAIL expression on pDCs, leading to selective apoptosis of uninfected $CD4^+$ T-cells and IFN-α induced immunopathogenesis[52]. The virus replicates rapidly and spreads into other lymphoid tissues, including gut-associated lymphoid tissue where major loss of $CD4^+$ T-cells (80%) occurs within the first three weeks of HIV-1 infection[53,54]. In HIV infection, protective and damaging effects of IFN-α are frequently seen in parallel. It seems likely that IFN-induced immune responses in the early stages of infection may in fact limit HIV replication and spreading to some degree. However paradoxically, these same protective responses contribute to overall immune activation and enable local expansion and lymphatic dissemination of the virus. Interferon responses during acute HIV infection may be the last attempt by the host

immune response to curtail HIV replication, with the balance eventually tipping in favor of HIV, which utilizes this elevated immune activation to result in increased viral replication and ultimately disease progression.

Role of interferons in HIV pathogenesis and disease progression

The progression of HIV disease is marked by a progressive loss of CD4+ T-cells, leading to AIDS and ultimately death. Several mechanisms have been proposed to lead to this gradual loss of CD4+ T-cells, including chronic immune activation and cytopathic effects of HIV on CD4+ T-cells. Elevated levels of IFN-ω[55], IFN-β[56], IFN-γ[57], and IFN-α in patients with AIDS have been reported as early as 1982[58]. Interferon-α has been identified as one of the key cytokine markers of HIV pathogenesis. Interferon-α, TRIAL-mediated destruction of uninfected CD4+ T-cells continues beyond acute infection, and leads to the destruction of lymph node structure in advanced stages of HIV-1 infection. Persistent activation of pDCs and associated IFN-α production during chronic infection likely contributes to systemic inflammation and the overall AIDS-associated immune activation. Paradoxically, HIV-1 disease progression is marked by loss of pDCs from the blood due to cell death and redistribution to the lymph nodes[59]. However, pDCs from lymph nodes of HIV-infected patients acquire an activated but immature phenotype and express significantly higher levels of IFN-α before dying[59]. Plasma type I IFN levels, which is thought to be primarily derived from pDCs in lymph nodes, correlates positively with plasma HIV-1 RNA levels and inversely with CD4+ T-cell count, and type I IFN levels are attenuated in antiretroviral-treated individuals with suppressed HIV-1 replication[60]. Furthermore, type I IFNs derived from HIV-activated pDCs induce the production of immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO), resulting in T-cell dysfunction[61,62] and favoring the development of immunosuppressive regulatory T-cells[63].

Additionally, IFN responses contribute to the immunopathology associated with microbial translocation. Massive destruction of CD4+ T-cells in the gastrointestinal tract leads to translocation of the intestinal bacteria and associated induction of proinflammatory cytokines and type I IFNs, all of which are thought to contribute to chronic immune activation[64]. Further evidence for a link between IFNs and HIV disease comes from pathogenic simian immunodeficiency virus (SIV) infection of rhesus macaques, where chronic IFN-α production associates with disease progression[65]. Interestingly, while both pathogenic SIV infection of rhesus macaques and nonpathogenic SIV infection of natural hosts induce strong IFN-α responses during acute infection, only pathogenic rhesus macaques that progress to AIDS show persistent decrease in circulating pDC levels and an increase in IFN-α over the course of chronic infection[65,66]. In nonpathogenic SIV infection, there is a rapid resolution of type-I IFN responses and an establishment of immune quiescent state during the course of chronic infection. These studies emphasize the damaging effects of persistent IFN-α in the later stages of HIV infection.

Information on the role of IFN in HIV disease progression and pathogenesis has so far been mostly limited to the effects of IFN-α, and very little is known about the potential roles other IFN subtypes may play during the course of infection. Interferon-γ-induced NO was shown to be highly increased in the serum of patients infected with HIV, especially in patients with low CD4 counts[67-69]. Further studies are required to determine whether this increase is due to increased viral replication or increased incidence of opportunistic infections, or both[70]. Nitric oxide induced by HIV-tat has been implicated in neurotoxicity and the pathogenesis of HIV-associated dementia[71-73]. Hence, IFN-γ and perhaps other IFN-mediated host antiviral responses (e.g. NO production) during chronic HIV infection may contribute to HIV pathogenesis.

While robust but transient IFN expression in the initial stages of HIV infection can restrict viral replication, the HIV-mediated overstimulation of IFN responses during chronic infection fuels pathogenesis and disease progression. Despite over 30 years of research on the HIV-IFN interaction, numerous questions still remain about the roles of different IFN subtypes, cells that produce them, and the mechanisms that regulate their cellular production during HIV infection. Ongoing research will undoubtedly continue to reveal new interactions between IFNs and HIV and could allow for the generation of IFN-mediated therapy in HIV infection.

Interferons and HIV therapy

Given the small founder population in HIV transmission, the time of exposure and the first few days following infection are the period of greatest vulnerability of the virus and potentially represent a window of opportunity for IFN-mediated intervention. Anti-HIV properties of IFN action offer a unique advantage for HIV-1 prevention and therapy, as it would be difficult for HIV to develop resistance to both intracellular and extracellular antiviral factors. The challenge remains how to minimize the

immunopathological HIV-induced IFN effects, while optimizing the beneficial antiviral IFN effects. Several studies have looked at benefits of both the use of IFN inhibitors in an attempt to reduce IFN-mediated immunopathogenesis as well as the induction of IFN responses in order to strengthen antiviral immunity.

Due to IFN-α's role in HIV immunopathogenesis, IFN-α inhibitors have been tested in several clinical trials. In an attempt to elicit immune responses against IFN-α, asymptomatic HIV-infected patients were vaccinated against IFN-α in a phase I/II study and then again in a double-blind, placebo-controlled, phase II/III clinical trial[74,75]. Despite low immunogenicity, individuals that responded to the anti-IFN-α vaccine had decreased levels of circulating IFN-α and lower rates of occurrence of HIV-1-related events. In another approach to dampen elevated chronic IFN-α levels, chloroquine, an endosomal inhibitor used in the treatment of malaria and autoimmune disorders for its ability to reduce chronic immune activation[76], was used to treat chronic HIV-infected patients. In an *in vitro* model, chloroquine mediated the blockade of IFN-α, which lead to reduced T- and pDC cell activation and blocking of negative modulators of T-cell function, IDO, and programmed death ligand 1[77]. Chloroquine treatment was shown to reduce T-cell immune activation in a double-blind, randomized, placebo-controlled trial in antiretroviral therapy (ART)-naive patients with CD4 T-cell counts > 250 cells/ml[78]. Another study looking at the effects of chloroquine treatment in ART-treated patients with $CD4^+$ T-cell counts < 200 cells/μl showed decreased immune activation, measuring several immunological parameters, including: decreased circulating LPS levels, decreased T-cell activation, reduced production of inflammatory cytokines, reduced IFN-α-producing pDCs, and increases in T_{reg} numbers[79]. However, a recent randomized controlled trial looking at the effect of chloroquine treatment in ART-naive patients with $CD4^+$ T-cell counts > 400 cells/μl found that the use of chloroquine did not reduce T-cell activation and actually led to a greater decline in $CD4^+$ T-cell count and increased viral replication[80]. This study shows that at earlier stages of infection, in ART-naive patients with high CD4 counts, chloroquine treatment results in enhanced HIV replication and accelerated CD4 decline due to inhibition of IFN-mediated antiviral responses. On the other hand, later in the infection in patients with lower CD4 counts, the benefits of inhibiting immune activation might outweigh the suppression of the IFN-mediated antiviral responses. This could be in part due to the fact that the majority of antiviral effector cells (pDCs, NK, and T-cells) are destroyed in later stages of infection and with them the ability to mount any IFN-mediated HIV control. The benefit of IFN responses depends on the stage of HIV infection and the immune status of the patient.

Results from a small study by Azzoni, et al. demonstrate that providing excess IFN in HIV patients using pegylated (PEG)-IFN-α2a monotherapy induced a durable suppression of HIV-1 replication and decreased viral integration following ART interruption[81]. The study analyzed the effect of PEG-IFN-α2a treatment in 23 HAART-treated patients with CD4 counts > 450 cells/μl and HIV plasma RNA levels of < 50 copies/ml. All subjects received PEG-IFN-α2a therapy in addition to HAART for five weeks; following that, ART was discontinued and PEG-IFN-α2a therapy was maintained for 12-24 weeks. Results show that 45% of treated patients were able to maintain viral control and showed significantly lower ratios of integrated HIV DNA copies/$CD4^+$ T-cell between endpoint and baseline. This study provides the first clinical evidence showing that natural host defense mechanisms can successfully reduce circulating HIV reservoirs and control HIV replication without continued ART. Despite its success in suppressing HIV replication and reducing HIV reservoirs, the therapeutic effects of PEG-IFN-α2a need to be studied further, perhaps in combination with other immunotherapeutic regimen(s) to achieve HIV functional cure in HIV-infected patients. Taking into consideration the protective role of IFNs in early stages of HIV replication and the potential to reduce circulating HIV reservoirs, IFN therapy could be an important part of the postexposure prophylaxis.

Treatment with IFNs has been evaluated in the context of several HIV coinfections for its ability to control viral replication, generating mixed results. Treatment with IFN-γ in patients coinfected with HIV and tuberculosis with median CD4 counts > 350 cells/ml was shown to be safe, with improved clinical outcomes, and resulted in increased $CD4^+$ T-cell counts and decreased plasma viral load[82]. Administration of IFN-α2b in combination with didanosine[83] or HAART[84] in HIV-positive patients with AIDS-associated Kaposi's sarcoma (KS), has shown no effects on survival or durable clearance of KS herpesvirus[83,84]. Pegylated IFN-α-2a/ribavirin combination therapy was shown to be successful in generating a high and sustained virological response against acute HCV infection in HIV-coinfected patients[85]. More recent research shows that PEG-IFN-λ/ribavirin combination treatment exhibits potent antiviral effects against HCV and shows an improved tolerability profile compared to IFN-α[86]. Together, these mixed results of modulating IFNs or IFN responses in

HIV treatment highlight the urgency for better understanding of IFN's cellular sources and the mechanisms of IFN action during different stages of HIV infection, and potential effects on other viral and microbial infections. It is important to note that adverse side effects associated with IFN therapy and mode of administration could provide further challenges for utilization of IFNs in HIV treatment and prevention. Having said that, advances in drug-delivery research may allow the administration of IFNs via a slow-releasing pill. As discussed previously, different stages of HIV disease may require completely different treatment approaches. Patients at the later stages of HIV infection may benefit from reduction of IFNs or IFN effects resulting in reduced immune activation achieved through the use of IFN inhibitors. Induction of IFN responses prior to and at the early stages of HIV infection could restrict viral replication and allow for HIV clearance.

Conclusion

Thirty years after the discovery of HIV virus as the causative agent of AIDS, with 2.5 million new infections in 2011, the search for an effective vaccine remains a global priority. While ART is excellent at controlling viral replication in HIV-infected patients, a "functional cure" cannot be achieved due to the establishment of viral reservoirs and highlights the need for alternative treatment options. *In vitro* studies and data from HESN cohorts indicate that IFN responses are crucial in preventing initial HIV replication and establishment of infection. Interferons act as a double-edged sword in protection against and exacerbation of HIV infection. While IFNs can restrict HIV replication at the point of exposure and control viral spread during acute infection, IFNs contribute to persistent immune activation and $CD4^+$ T-cell depletion during chronic infection. Overproduction of IFNs at the late stages of the disease leads to active viral replication and, consequently, worsened disease outcome. Modulation of IFN responses prior to and during HIV infection has the potential to prevent HIV transmission, clear HIV infection, and dampen chronic immune activation. The utilization of IFNs alone or in combination with other therapeutic regimens in HIV treatment represents a great potential, inciting the urgency for further studies. The benefit of IFN therapy, however, will depend on the stage of HIV infection and the immune status of the patients (e.g. CD4 counts and viral loads). Therefore, this urges a need for a detailed mechanistic understanding of the interactions between IFNs and HIV. A better understanding of the IFN antiviral immune responses at the initial site of infection and during disease progression is crucial to the design of successful preventive and therapeutic strategies.

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