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HIV PATHOGENESIS AND TREATMENT (AL LANDAY, SECTION EDITOR)

Type I Interferon: Understanding Its Role in HIV Pathogenesis and Therapy

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Abstract Despite over 30 years of research, the contribution of type I interferons (IFN-Is) to both the control of HIV replication and initiation of immunologic damage remains debated. In acute infection, IFN-Is, likely from plasmacytoid dendritic cells (pDCs), activate NK cells and upregulate restriction factors targeting virtually the entire HIV life cycle. In chronic infection, IFN-Is may also contribute to CD4 T cell loss and immune exhaustion. pDCs subsequently infiltrate lymphoid and mucosal tissues, and their circulating populations wane in chronic infection; IFN-I may be produced by other cells. Data from nonhuman primates indicate prompt IFN-I signaling is critical in acute infection. Whereas some studies showed IFN-I administration without combination antiretroviral therapy (cART) is beneficial, others suggest that stimulating or blocking IFN-I signaling in chronic ART-suppressed HIV infection has had positive results. Here, we describe the history of HIV and IFN-I, IFN-I's sources, IFN-I's effects on HIV control and host defense, and recent interventional studies in SIV and HIV infection.

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Introduction

The association of type I interferon (IFN-I) with AIDS predates the discovery of HIV itself. Serum IFN-I activity was first described as being enriched in "homosexual men with Kaposi's sarcoma and lymphadenopathy" in 1982 [1]. Shortly thereafter, Buimovici-Klein and colleagues published a letter in *The Lancet* entitled "Is presence of interferon predictive for AIDS?" [2]. Although these studies proposed using IFN-I activity as a prognostic indicator of an eventual AIDS diagnosis in lieu of a known etiologic agent, they were the first to suggest that IFN-I may be part of the disease process. In the subsequent 30 years, the link between HIV and IFN-I has been intensely studied. Elevated plasma levels of IFN-I and interferon-stimulated genes (ISGs) in HIV-infected patients and SIV-infected monkeys have been reported many times over. These associative studies have demonstrated unequivocally that untreated infection induces widespread induction of the IFN-I system without any measurable effect on viral load. Administration of IFN-α as monotherapy or as an adjunct to antiretroviral therapy has been intensely studied (reviewed in detail below)—and the results have varied widely. Conversely, the administration of a vaccine against endogenous IFN-α to end-stage AIDS patients stabilized CD4 counts [3, 4].

More recently, evidence for a link between IFN-I and HIV pathogenesis was provided by several groups studying SIV infection in natural host monkey species that do not develop AIDS [5, 6]. After acute SIV infection, these species rapidly mute their IFN-I responses whereas disease-susceptible

macaque species maintain IFN-I signaling indefinitely. These species have several other notable differences from pathogenic species, including lack of microbial translocation, limited infection of memory cells, and absence of emergent gastrointestinal viruses [7, 8]. It is unclear whether the ability of natural hosts to shut off the IFN-I system is directly related to pathogenesis or a secondary effect. Nevertheless, the consistent observations across multiple natural host species helped invigorate interest in IFN-I as a mediator of HIV immunopathogenesis.

Cumulatively, the HIV/IFN-I field has definitively demonstrated that (i) at the host level, IFN-I production and responses are not evaded or suppressed by HIV but are insufficient to clear or control HIV and (ii) IFN-I as monotherapy is ineffective to control HIV. In the sections below, we will focus on novel data that addresses (i) IFN-I induction of antiviral responses, (ii) IFN-I driving HIV-related disease progression, and (iii) lessons from IFN-I modulation studies about how the innate system could be manipulated for treatment and prevention.

What Are the Sources of IFN-I in HIV Infection?

Antagonism of the IFN-I system remains of significant clinical interest for the treatment of HIV-related immune activation. However, sustained in vivo inhibition of the IFN-I system can be difficult to achieve. An alternative strategy to modulate the IFN-I system may be the depletion of IFN-I-producing cells. However, which immune subsets are most important to IFN-I production in HIV infection is debated.

Plasmacytoid DCs in HIV/SIV Infection

Classical Studies of Plasmacytoid DCs

Among cell types capable of making IFN-I in response to HIV, plasmacytoid dendritic cells (pDCs) are most intensely studied. Shortly after the discovery that pDCs and the elusive "natural interferon-producing cell" were, in fact, the same cellular subset, several studies documented their kinetics and activity in HIV infection (Fig. 1a) (reviewed in [9]). pDCs are found at reduced levels in chronic HIV infection compared to uninfected controls; similarly, nonhuman primate (NHP) studies have shown that the depletion of circulating pDCs occurs as early as 3 days post-infection and never completely normalizes. Numerous studies from the early 2000s reported that pDCs from HIV-infected patients have an attenuated capacity to produce IFN-α. However, recent data show that in response to some stimuli, pDCs from HIV-infected persons are hyperresponsive [10, 11].

pDCs Are Depleted from the Blood but Accumulate in Lymph Nodes and Mucosa in HIV/SIV Infection

The NHP model has been highly instructive in understanding pDC biology. pDCs accumulate in draining lymph nodes (LNs) rapidly after SIV infection, where most undergo apoptosis, contributing to the loss of circulating pDCs [12]. Similarly, subsequent groups reported a massive accumulation of pDCs in the rectal and vaginal mucosae after acute SIV infection and in chronic HIV infection (Fig. 1b) [9, 13–15], although it is unknown whether mucosal pDCs undergo apoptosis as they do in LNs. Mucosal retention of pDCs has not been observed in SIV-infected natural host species, which also avoid the persistent IFN-I signaling seen in pathogenic hosts, suggesting that altered localization of pDCs may account for continued IFN-I production in pathogenic infection [5, 6, 16•]. Overall, data from several groups have formed a consolidated model in which pDC depletion from the blood occurs due to relocation and seemingly permanent retention in lymphoid tissues.

Do pDCs Make IFN-I in Chronic HIV/SIV Infection?

pDCs have been assumed to be the primary producer of IFN-Is in HIV infection, as they secrete massive amounts of IFN-α after in vitro HIV stimulation compared to other cell types (Fig. 1c) (reviewed in [17]). Their ultimate contribution to host IFN-I production has been harder to establish in vivo. While there is a general consensus that pDCs are likely responsible for the bulk of IFN-I production in the acute phase (1–2 weeks) of infection, the role of pDCs in chronic infection is less clear. ISGs are easily detected in chronic HIV/SIV infection, but plasma IFN-α and IFNA messenger RNA (mRNA) are found at extremely low levels after acute infection, if at all [6]. This finding may be attributed to the low numbers of circulating pDCs with local IFN-α production inducing ISG expression in target cells within LNs and mucosae [16•]. A second potential explanation is that pDCs are required only in the early phase of infection to "jump-start" systemic IFN-I responses with their constitutive expression of interferon regulatory factor 7 (IRF7). Non-pDCs then upregulate IRF7 levels, which drive ISG induction via IFN-β [18, 19]. Several studies have addressed this issue in vitro recently, and the data are conflicting:

Evidence Against a Role for pDCs in Chronic IFN-I Production

Inhibition of pDC activation using TLR7/9 antagonists in SIV-infected cynomolgus macaques has minimal impact on ISG production [19]. Indeed, based on flow cytometric analysis of unstimulated pDCs taken directly ex vivo, IFN-α is detectable during acute SIV infection of cynomolgus macaques but wanes quickly. This is consistent with the finding

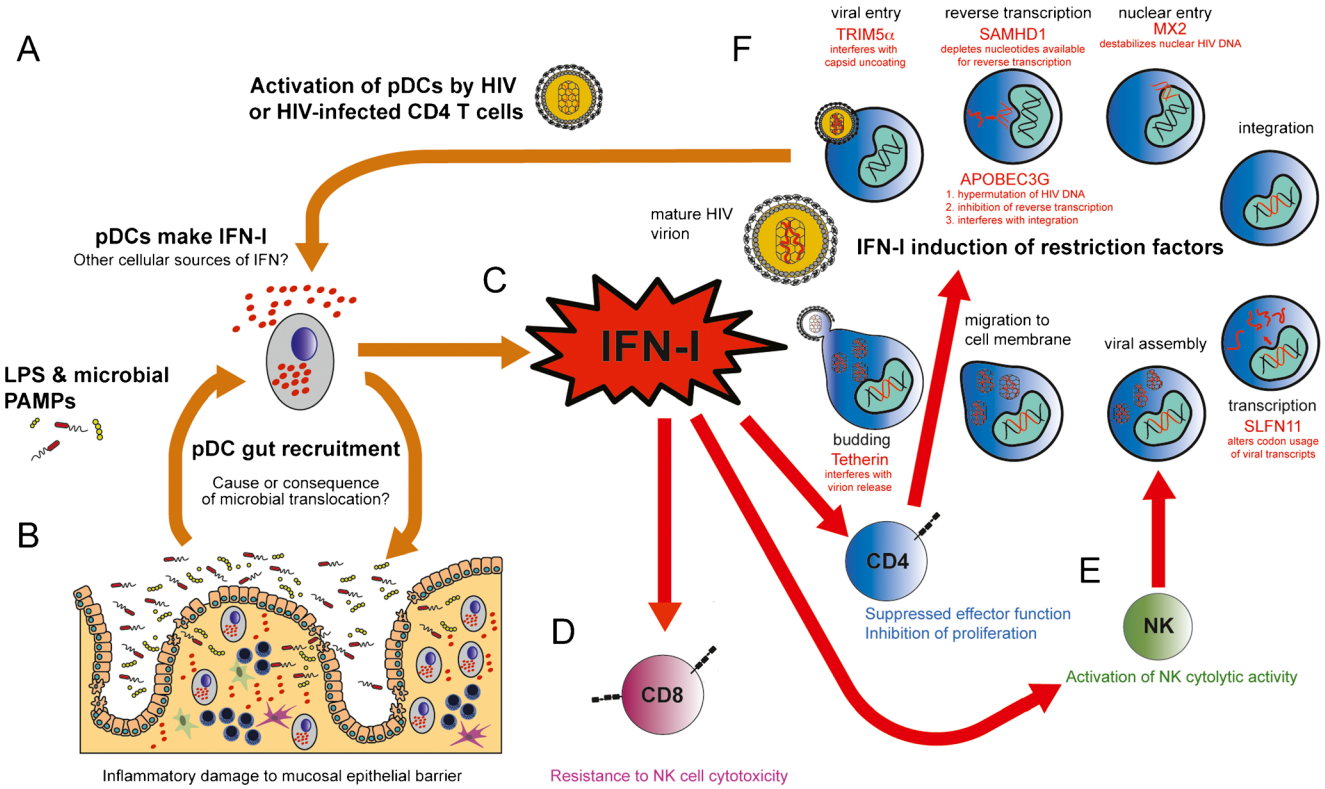

Fig. 1 Sources and effects of type I interferons in HIV infection. *A* HIV or HIV-infected CD4 T cells activate plasmacytoid dendritic cells (pDCs) to produce type I interferon (IFN-I). *B* In chronic HIV infection, pDCs are depleted from the peripheral circulation and accumulate in the rectal mucosa, where they may be activated by microbial products such as lipopolysaccharide (LPS) and other pathogen-associated molecular patterns (PAMPs) and/or create an inflammatory environment that contributes to ongoing intestinal barrier dysfunction and microbial translocation. *C* Activated pDCs produce IFN-I in acute HIV infection, but whether pDCs or other cells are the predominant IFN-I producers in chronic HIV infection remains debated. *D* IFN-I signaling stimulates CD8 T cells to upregulate MHC molecules that bind NK cell inhibitory receptors, rendering CD8 T cells resistant to NK-cell-mediated cytotoxicity. *E* IFN-I signaling facilitates the proliferation and survival of NK cells and activates NK cell cytotoxicity, which may contribute to the eradication of HIV-infected CD4 T cells. *F* IFN-I suppresses CD4 T cell effector function and inhibits CD4 T cell proliferation but also induces restriction factors that limit HIV replication at many steps of the replication cycle, including HIV entry, reverse transcription, nuclear entry/integration, transcription, and budding

that pDC-depleted mice have intact antiviral responses to local herpes simplex virus-1 infections, but they are impeded in their IFN-I responses to systemic herpesvirus infections [20].

Evidence Supporting pDCs Making Chronic IFN-I

In vivo imaging studies have shown that virtually all IFNα-expressing cells in SIV-infected monkeys also express the pDC-marker CD123 [21], and depletion of pDCs in humanized mice abolishes IFN-I and ISG responses to HIV infection [22]. We have observed in rhesus macaques that virtually all detectable IFNα made in response to in vitro stimulation with AT-2-treated SIV originated from pDCs [23]. Similar data has been seen for African green monkeys, where in vitro depletion of pDCs completely abolishes IFN-I activity [24]; however, pDC depletion only partially abrogates IFN-I production to HSV stimulation.

Given the current in vivo evidence available, it is plausible that pDCs may indeed be the primary cell type producing IFN-I in chronic infection, but their scarcity and distribution in tissues render them difficult to assess ex vivo. However, another possibility is that pDCs may only be responsible for IFN-I during the peak of viral replication, after which alternative cells are responsible. This "switch" may occur due to the emergence of new potential stimuli after acute infection, particularly microbial products [7] and novel gastrointestinal viruses [8] that are found after pathogenic infection. Antagonism of the IFN-I system remains of clinical interest in treating HIV-related residual inflammation. Thus, identifying the primary sources of IFN production has high potential for interventional strategies.

Effects of IFN on Antiviral Gene Expression

ISGs with Anti-HIV/SIV Activity

In the past decade, several ISGs that target HIV throughout its life cycle, i.e., restriction factors, have been identified (Fig. 1f).

The best characterized restriction factors are TRIM5α, which interferes with HIV-1 uncoating [25]; apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3 (APOBEC3), which induces cytidine to uridine editing of HIV cDNA, resulting in hypermutation of the plus-strand DNA [26]; and Tetherin/BST2, which is expressed on infected cell surfaces, where it binds virions and prevents their release [27–29].

Several novel IFN-I-inducible anti-HIV factors have recently been described. SAM domain and HD domain-containing protein 1 (SAMHD1) was shown to inhibit HIV reverse transcription in resting T cells [30] by removing the triphosphate from deoxynucleoside triphosphates (dNTPs), decreasing deoxynucleotide precursors [31]. MX2 prevents HIV integration into the host DNA, possibly by blocking *gag* capsid-dependent nuclear entry [32••, 33••]. Many highly exposed seronegative subjects have polymorphisms conferring increased levels of MX2 expression [34]. Similarly, elite controllers upregulate schlafen 11 (SLFN11) in CD4 T cells, especially central memory CD4 T cells, compared to viremic noncontrollers or patients on antiretroviral therapy (ART) [35•]. SLFN11 binds transfer RNAs (tRNAs) to prevent HIV's alteration of tRNA composition, which facilitates its protein production [36•]. IFN-stimulated gene 15 (ISG15) subsequently prevents HIV release from budding cells [37]. More recently, an elaborate live-cell imaging system was used to identify >50 novel factors that impact HIV replication [38••], although the majority of these remain to be confirmed by in-depth study. In sum, IFN-stimulated antiviral genes target virtually every step of the HIV life cycle.

Whether all or only some of these restriction factors are critical for controlling HIV infection in vivo remains unknown. In addition, the degree to which these restriction factors contribute to virus suppression during combination antiretroviral therapy (cART), or conversely, the extent to which their absence may contribute to low-level viremia, remains to be determined. Furthermore, the expression of restriction factors in the diverse tissues that HIV infects has not been rigorously studied but may illuminate more details about the pathogenesis of reservoir establishment.

Several ISGs Act to Amplify the IFN-I Response

More than 300 ISGs have been identified. Whereas some ISGs restrict virus replication, others enhance pathogen-associated molecular pattern (PAMP) detection and IFN signalling and amplify the IFN response. The best characterized of these ISGs is the IRF7 transcription factor [39••]. IRF7, "the master regulator" of IFN-I expression [40], is activated by innate signaling from both the TLR and RLR family of pathogen recognition receptors. In response to viral PAMPs, pDCs rapidly produce IFN-α/β that induce IRF7 transcription in neighboring cells, amplifying systemic IFN-I production. Indeed, IRF7 polymorphisms have been associated with decreased IFN-α production by pDCs in response to inactivated HIV-1 [41]. In addition to being a restriction factor, TRIM5α acts as a pattern recognition receptor for the HIV capsid and induces innate signaling [42]. The ISG cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS) stimulates IFN-I production in response to cytosolic DNA [38••, 43••]. cGAMP activates the protein "stimulator of IFN genes" (STING) to activate IκB kinase and TANK-binding kinase 1, which activate NFκB and IFN regulatory factor 3 (IRF3), culminating in IFN-I production [44••, 45••]. cGAS also triggers an IFN-I-independent antiviral gene repertoire [43••]. Another DNA binding protein, IFN-γ-inducible protein 16 (IFI16) [46], also activates STING, resulting in IFN-I production [47]. Consistent with their roles in DNA detection, cGAS and IFI16 mRNA levels are higher in viremic than suppressed HIV-infected patients [48]. IFI16 may also contribute to low CD4 T cell counts and increased T cell activation by inducing inflammasome activation and pyroptosis in quiescent CD4 T cells [49••, 50•]. Thus, numerous ISGs, once induced, act to perpetuate IFN-I production. This feed forward cycle of IFN-I induction accelerates innate responses to combat acute viral infections. However, in chronic, nonclearing infections such as HIV, the persistence of the innate response may have detrimental cytopathic effects.

Effect of IFN on Antiviral Cellular Immunity

Type I IFNs support the proliferation and survival of NK cells [51], stimulate their activation, and enhance their cytotoxic activity (Fig. 1e) [52]. Although IFN-Is may activate NK cells directly through the IFN-α receptor (IFNAR), recent data suggest IFN-I also induces NK cells and DCs to upregulate IL-15Rα, which binds and cis- or trans-presents IL-15 to NK cells, respectively [53, 54]. NK cells mediate antiviral responses primarily through the release of cytotoxic granules. However, activated NK cells modulate T cell responses by regulating antigen presentation—NK cell depletion prior to LCMV infection can actually enhance antigen-specific CD4 and CD8 T cell function [55, 56]. Intriguingly, IFN-I also renders CD8 T cells resistant to NK-cell-mediated cytotoxicity by stimulating their upregulation of MHC molecules that bind NK cell inhibitory receptors (Fig. 1d) [57•]. In the absence of IFN-I signaling, activated CD8 T cells upregulate NK cell activating ligands that render them susceptible to NK-cell-mediated cytotoxicity [58•]. Thus, in acute infections, type I IFN signaling may simultaneously activate NK cell cytotoxicity and render activated CD8 T cells resistant to NK cell attack.

Recent data from the LCMV mouse model indicate that chronic type I IFN signaling may be detrimental to antiviral CD4 T cell responses (Fig. 1). Infection with the clone 13 strain of LCMV results in a chronic infection with persistent, uncontrolled viremia, resembling HIV infection, that can model the deleterious effects of persistent TLR signaling on the immune system. Indeed, this model was used to characterize the exhaustion marker PD1 on dysfunctional CD8 T cells in unresolved LCMV infection [59]. ISGs are transiently upregulated in infection with the self-limited LCMV Armstrong (Arm) strain but persistently upregulated with the chronic LCMV clone 13 (Cl13) infection [60•], and these ISGs were upregulated in $PD1^+$ CD4 and CD8 T cells [61]. Blocking IFN-I signaling during acute LCMV-Arm infection decreased the number and function of CD8 T cells and delayed virus clearance [60•, 62••]. In contrast, IFN receptor blockade before and after establishment of chronic LCMV infection with the LCMV-Cl13 strain resulted in increased early viremia but improved virus control during chronic infection. CD4 T cell function was restored in conjunction with decreased PD-L1 and IL-10 expression on DCs [60•, 62••]. Accordingly, PD1 signaling blockade has also been shown to reduce viremia in LCMV [59], SIV [63], and HIV infection in humanized mice [64]. Collectively, these data suggest early IFN-I signaling may enhance viral control during acute infection but contribute to persistent viremia during chronic infection by inhibiting cellular immunity.

Manipulation of the IFN-I System In Vivo

In chronic infections such as HIV, it is possible that the effect of IFN-I on NK cell cytotoxicity or CD4 and CD8 T cell responses may be more important than induction of ISGs and restriction factors on host survival. Significant progress has been made in establishing humanized murine models of HIV infection so that gene-targeting studies can begin to systematically unravel the interplay of these factors. Given the complexity of the IFN-I system, in vivo manipulation is critical to understanding the relative contributions of its effects on HIV pathogenesis.

HIV infection can be envisioned as three different biological conditions: acute infection—a dynamic state with an evolving cytokine storm, upregulation of antiviral factors, establishment of the reservoir, followed by a decrease in viremia to the virus set point; chronic untreated HIV infection—with steady cytokine production, constant yet low-level ISG expression, stable viremia, and gradual CD4 T cell depletion; and chronic HIV infection with virologic suppression—with even lower cytokine production, undetectable viremia, and CD4 T cell stability or recovery. Manipulation of IFN signaling in these three stages may have differential impacts. In the sections below, we will summarize the clinical information that has been gained from in vivo manipulation of IFN-I signaling in the setting of lentivirus infection, with special emphasis on recent interventional studies in NHP/SIV models.

Early IFN-α Monotherapy Clinical Studies in HIV Infection

The first study of type I IFN administration in HIV-infected patients was published just 3 years after the initial clinical description of AIDS [65]. Subcutaneous (SQ) IFN-α administration to patients with Kaposi's sarcoma (KS) [65, 66] resulted in lower HIV burden and higher CD4:CD8 T cell ratios in some treated subjects compared to placebo (see Table 1). Subsequently, several studies evaluated SQ or intramuscular (IM) IFN-α in subjects who were asymptomatic and/or with minimal immunosuppression [67–70]. Overall, IFN-α-treated groups had a less severe CD4 decline (and in some instances an increase), lower HIV burden, fewer opportunistic infections, and slower disease progression, despite increased frequency of activated CD8 T cells.

Optimism arose from several studies of oral IFN-α reported administration in the early 1990s. In one influential study of 32 critically ill AIDS patients, 16/16 control patients died whereas 14/16 IFN-α-treated patients survived to hospital discharge [71]. A larger study from the same group reported increased Karnofsky scores and fewer symptoms in the treated subjects [72]. Whereas some studies reported an attenuation of CD4 depletion (or even an increase) with oral IFN-α [73, 74], several large, randomized, placebo-controlled, double-blind studies failed to show a statistically significant benefit [75–77]. With the unarguable efficacy of cART evident by the late 1990s, particularly in patients with lower CD4 T cell counts, and the preponderance of negative data from these rigorous studies, oral IFN-α monotherapy as an HIV treatment was abandoned.

IFN-α as an Adjunct to Antiretroviral Therapy

The approval of zidovudine and, eventually, many other antiretrovirals, renewed interest in parenteral IFN-α as an adjunct therapy. The combination of zidovudine with IM or SQ IFN-α in KS patients conferred decreased HIV burden in most studies, although the IFN-α and zidovudine doses and study duration varied widely (see Table 2) [78–80]. Subsequently, over a dozen studies have been performed in subjects on one, two, or three (or more) antiretrovirals with diverse CD4 T cell counts and HIV burdens. The majority of studies were performed in participants with detectable p24 antigen or HIV RNA and showed a decline in virus burden when IFN-α+ART was compared to ART alone [81–85]. More recently, a study of patients with hepatitis C and suppressed HIV RNA levels who received IFN-α with ribavirin showed a decrease in CD4 T cell-associated total and

Table 1 Studies of IFNα Administration in Subjects Not on Antiretroviral Therapy

Population	Intervention	Effect	Citation
SQ or IM IFNα			
37 HIV+ patients with Kaposi's sarcoma (KS)	SQ IFNα dose escalation to 36–45 MU daily×28 days	KS Responders: 1 of 4 died, ↑CD4:CD8 ratio KS Non-responders: 15 of 19 died	Krown et al., 1984 [65]
27 HIV+ patients with Kaposi's sarcoma	SQ IFNα2b 35 MU daily × 12 weeks or placebo	>75 % ↑ in antigenemia in 2/21 of the IFN group and all 6 placebo subjects	Lane et al., 1988 [66]
34 HIV+ patients, CD4≥400 cells/mm^3	SQ IFNα 35 MU daily or placebo, × ≥12 weeks	IFN: No decrease in %CD4, 7/17 HIV culture (−), no OIs Placebo: Decreased %CD4, 2/17 HIV culture (−), 5 OIs	Lane et al., 1990 [67]
162 HIV+ patients, asymptomatic	IM IFNα2b 3 MIU TIW or no treatment, indefinitely	IFN group had longer mean survival, higher survival rates, slower disease progression versus no treatment	Rivero et al., 1997 [68]
13 HIV+ patients, no ART, CD4>300 cells/mm^3, HIV RNA>5000 copies/ml	IM pegylated IFNα2a 180 ug weekly × 12 weeks	IFN: Transient ↑ in %CD4, ↓ HIV RNA levels, ↑ % HLA-DR$^+$ CD38$^+$ CD8 T cells	Asmuth et al., 2010 [69] Manion et al., 2012 [70]
Oral IFN			
32 critically ill HIV+ patients	Oral IFNα or supportive management	IFN: 14 of 16 discharged in 2–4 weeks, 2 died No treatment: All died within 4 weeks	Obel et al., 1990 [71]
199 symptomatic and 5 asymptomatic HIV+ patients	Oral IFNα 2.0 IU/kg × ≥10 weeks	Mean Karnofsky score ↑ from 60.5 to 100 Fewer HIV symptoms	Koech and Obel, 1990 [71, 72]
40 HIV+ patients	Oral IFNα 75–600 IU daily or no drug × 19–700 days	IFN: 5 died (4 due to HIV, 1 suicide); 13 had smaller CD4 count ↓ than unmatched, untreated patients	Babiuch et al., 1993 [73]
a) 252 HIV+ patients b) 40 HIV+ patients, CD4 counts 350–500 cells/mm^3 c) 38 HIV+ patients, CD4 counts >700 cells/mm^3	a) Oral IFNα 150 IU daily +/− zidovudine b) Oral IFNα 150 IU daily, zidovudine, both, or neither c) Oral IFNa 150 IU daily or nothing	a) CD4 counts ↑ in both groups b) CD4 counts were higher in IFNa group compared to zidovudine or neither groups c) IFNα group had 16 % ↑ in CD4 counts and untreated group had 11 % ↓ at 6 months	Jordan, 1994 [74]
177 HIV+ patients	Oral IFNα 0.1 or 1 or 10 IU/pound or placebo	Non-significant ↑ in CD4 count in low-dose group at 6 months and enhanced survival	Wright et al., 1998 [75]
559 HIV+ patients, WHO stage 2–4, Karnofsky score >50	Oral IFNα 150 IU daily or placebo × 28 or 60 weeks	No difference in mortality rate, disease progression, CD4 count decline, Karnofsky score, or symptom prevalence	Katabira et al., 1998 [76]
247 HIV+ patients, CD4 counts 50–350 cells/mm^3 and HIV symptoms, with or without maintenance ART	Oral IFNα 500 IU liquid or 150 IU lozenge or 200 IU tablet or placebo × 24 weeks	No significant differences in symptom burden index, weight change, CD4 counts, or Karnofsky score	Alston et al., 1999 [77]

integrated HIV DNA that persisted after stopping IFN-α and ribavirin [86]. However, none of these studies showed a statistically significant, sustained improvement in CD4 T cell recovery with the addition of IFN-α to ART, and rates of progression were unaffected by 52 weeks of IFN-α in one study with 15 years of follow-up [85]. While IFN-α administration can decrease virus rebound in the setting of a structured treatment interruption, lower CD4 T cell counts ensue [87, 88]. The net result from these clinical studies is that, despite a suppressive effect on HIV burden, adding IFN-α to an effective ART regimen does not significantly improve CD4 T cell reconstitution or clinical outcome.

IFN-α Administration in Natural Host Species of SIV

As noted above, SIV infection of disease-susceptible macaque species (rhesus, cynomolgus, pigtailed) results in an early surge of ISG expression that persists indefinitely. In contrast, SIV infection of natural host species (sooty mangabeys, African green monkeys) results in a nonpathogenic infection—and despite early expression, ISGs quickly normalize [5, 6]. To test the possibility that the resolution of ISGs was responsible for protection from disease, two studies tested the effect of IFN-α administration in natural host species. The administration of high doses of recombinant IFN-α during

Table 2 Selected studies of IFNα Modulation in Subjects on Antiretroviral Therapy

Population	Intervention	Effect	Citation
IFNα Administration			
39 HIV+ patients with KS	SQ IFNα 5–20 MU daily × 12 weeks, after 6 weeks of zidovudine 50/100/250 mg q4h	8 of 22 patients that received a stable dose of both IFNα and zidovudine had ↓ p24 antigenemia	Kovacs et al., 1989 [79]
21 HIV+ patients with KS	IM IFNα 18 MU and zidovudine 600 mg daily × 4 weeks	No decrease in HIV antigen levels	de Wit et al., 1991 [78]
17 HIV+ patients with KS	SQ IFNα 10 MU daily and zidovudine 250 mg bid × 12 weeks	No change in CD4 counts but ≥70 % ↓ of HIV p24 antigen in 7 of 10 antigenemic patients	Baumann et al., 1991 [80]
16 asymptomatic HIV+ patients	Zidovudine 250 mg bid + a) SQ IFNα 1.5 MIU TIW or b) acyclovir 800 mg bid × 12 weeks	p24 antigen ↓ in all patients with both regimens No difference between regimens	Weber et al., 1991 [102]
13 HIV+ patients on >6 weeks zidovudine	SQ IFNα 1.25-7.5 MU/m^2 TIW+zidovudine 200 mg q4h	p24 antigen ↓ by >50 % in 11/13 at 11 weeks then ↑ CD4 counts ↓ by 7.1 cells/mm^3 per week	Edlin et al., 1992 [81]
45 HIV+ symptomatic patients with CD4 counts≥150 cells/mm^3	Zidovudine 250 mg QID + SQ IFNα 3 MIU TIW or no treatment × 48 weeks	Nonsignificantly lower ↓CD4 counts and greater p24 antigen ↓ in combination group No differences in disease progression	Frissen et al., 1994 [103]
34 HIV+ symptomatic patients with CD4 counts>200 cells/mm^3	SQ IFNα 2–6 MIU and/or zidovudine 200-600 mg daily × 12 weeks then IFNα+zidovudine for 96 weeks	↓antigenemia in combination therapy at 12 weeks more than either IFNα or zidovudine alone High-dose was most effective after 96 weeks	Mildvan et al., 1996 [82]
256 HIV+ patients, CD4 counts 300–500 cells/mm^3	SQ IFNα 3 MU daily+zidovudine 200 mg TID+didanosine 0.75 mg TID or zidovudine+didanosine	Greater HIV RNA ↓ in IFNα arm ↑CD4 T cell counts in control arm High discontinuation rate, especially in IFNα arm	Haas et al., 2000 [83]
259 HIV+ patients failing current cART regimen	SQ PEG-IFNα 0.5-3 μg/kg or placebo weekly+current cART × 4 weeks then optimized cART × 24 weeks	Greater HIV RNA ↓ in IFNα arm No significant differences in CD4 T cell counts	Angel et al., 2009 [84]
180 HIV+ patients with CD4 counts≥500 cells/mm^3	SQ IFNα2b 1 MIU daily and/or zidovudine 200 mg q4h with dose escalation as tolerated × 52 weeks	Greater HIV RNA ↓ in IFNα arms Transient ↑CD4 T cell frequency in IFNα groups Similar rates in progression to AIDS after 15 years	Tavel et al., 2010 [85]
168 HIV+ patients with undetectable HIV RNA levels on cART	SQ pegylated IFNα 1.5 μg/kg from day 15 of cART interruption to day 8 after resumption or no treatment	Greater CD4 T cell ↓ in the IFNα arm ↓ HIV RNA rebound in the IFNα arm Does not prolong time to treatment resumption	Boué et al., 2011 [87]
89 primary HIV+ patients	SQ pegylated IFNα2b 1 μg/kg weekly × 14 weeks + a) cART × 36 weeks then 4 week cART interruption at weeks 36, 48, 60 with IFNα or b) cART with interruptions at weeks 36, 48, and 60 or c) continuous cART through week 72	↓ HIV RNA rebound in IFNα group but no difference in HIV RNA levels 6 months after stopping cART ↓ CD4 T cell recovery in IFNα group, but no difference by 6 months after stopping cART	Goujard et al., 2012 [88]
12 HIV+ patients with HCV co-infection and suppressed HIV RNA	SQ IFNa2a 180ug weekly and ribavirin 500–600 mg BID	2-fold decrease in CD4 T cell-associated total and integrated HIV-1 DNA during therapy that persisted after cessation	Sun et al., 2014 [86]
Vaccination against IFN			
12 HIV+ patients on ART ≥1y, CD4 counts 100–300 cells/mm^3	Adjuvanted inactivated IFNα2a +ART versus adjuvant+ART	Viral load did not increase in vaccinees; ↑/unchanged in placebo group No vaccinees but 2 placebo subjects developed AIDS	Gringeri et al., 1995 [99]
89 HIV+ with early (no ART) or advanced (ART) disease	Adjuvanted inactivated IFNα2a (N=27) or no treatment (N=62)	No clinical deterioration or ↓CD4 count in vaccinees compared to progression in untreated subjects	Gringeri et al., 1996 [4]
242 HIV+ asymptomatic patients, CD4 counts≥100 cells/mm^3	Adjuvanted inactivated IFNα2a or placebo, double-blind randomized placebo-controlled trial	Vaccine responders had ↓ HIV-related events compared to placebo or non-responders	Gringeri et al., 1999 [3]

days 9–24 of acute SIVagm infection in African green monkeys did not upregulate ISGs, decrease viremia, or affect T cell activation or CD4 T cell decline [89]. In contrast, SIV levels decreased in the one African green monkey treated during chronic infection, without changes in T cell activation [89]. Similarly, administration of recombinant IFN-α to chronically infected sooty mangabeys resulted in a modest ISG upregulation and decreased viremia, yet had no effect on cell-associated virus, CD4 T cell activation, or CD4 T cell counts [90•]. A modest, but transient, reduction of CD8 immune activation was observed. Thus, exogenous IFN-α in non-pathogenic SIV infection decreases viremia during chronic but not acute infection with minimal impact on immune activation, viremia, or disease progression.

In Vivo Inhibition of TLR Signaling in HIV/SIV Infection

As noted above, pDCs are key producers of type I IFNs. Administration of chloroquine, which inhibits TLR7 and TLR9 signaling by preventing endosomal acidification, during acute SIVmac251 infection unexpectedly increased ISG expression and decreased CD4 T cell recovery [91]. Similarly, administration of hydroxychloroquine to ART-naïve patients resulted in lower CD4 T cell counts, higher HIV RNA levels, and possibly decreased CD8 T cell activation [92, 93], whereas administration to immunologic nonresponders (lack of CD4 T cell reconstitution after ART) reduced T cell activation and inflammation and improved CD4 T cell frequency [94]. However, in another study of immunologic nonresponders, the addition of chloroquine to ART for 24 weeks increased IFN-α2 levels but had no impact on CD4 T cell counts, T cell activation, or circulating inflammatory markers [95]. Thus, indirect attempts to block IFN-I signaling thus far have had mixed success but appear to require ART suppression to exert any beneficial effect.

In Vivo Blockade of IFNAR in SIV Infection

We recently performed a comprehensive in vivo study in which we manipulated IFN-I signaling during SIV challenge and acute infection in rhesus macaques (RMs) [96••]. In one group of RMs, an antagonist of the IFNAR (IFN-1ant) was administered during acute SIVmac251 infection; pegylated IFN-α2a was given to a second cohort of RMs during acute infection, starting 1 week prior to rectal inoculation; and a third group of RMs received placebo saline during acute infection [96••]. Interfering with IFN-I signaling during acute SIV infection proved to have a profound impact on disease progression and survival. Administration of IFN-1ant from day 0 through day 28 of acute SIV infection delayed the upregulation of ISGs, including many of the aforementioned antiviral mediators, and many pattern recognition receptors by several days, but ultimately, they had comparable expression levels to placebo RMs. Nevertheless, this delay of IFN-I signaling during the first 10 days of infection proved to have a dramatic effect on the chronic phase of infection, as higher viremia, increased CD4 T cell depletion, and accelerated progression to AIDS and death ensued. In contrast, administration of pegylated IFN-α2a starting 1 week prior to rectal challenge conferred protection against systemic infection, as all RMs needed repeat exposures to become systemically infected with the high inoculum dose. This protective effect was dependent upon ISG upregulation. An IFN-I-tolerant state was established, potentially mediated by FOXO3a, ultimately resulting in delayed ISG expression during acute infection and a concomitant increase in cell-associated SIV and $CCR5^{+}$ CD4 T cell depletion [96••]. Together, these data indicate that the precise timing of antiviral gene expression during acute SIV infection in the pathogenic host can profoundly influence disease outcome. The finding that IFN-α2a prevented infection, at least temporarily, contrasts with the results of a previous study targeting transmission and acute SIV infection. Administration of IFN-α2b or the IFN-αB/D chimera to rhesus macaques starting 1 day before intravenous challenge with SIV $Delta_{B670}$ and continued through 90 days post-infection decreased peak antigenemia but did not impact disease progression [97]. The differential impact on acquisition may be attributed to the pathogenicity of SIV $Delta_{B670}$ or to the contribution of the rectal mucosal barrier, both structurally and as a consequence of local ISG upregulation and its consequences. ISGs were not evaluated in the intravenous challenge study, so whether they were persistently upregulated or whether tolerance developed is unknown. In contrast, 14 weeks of pegylated IFN-α2a administration starting during chronic SIV infection resulted in transient ISG upregulation but no impact on plasma SIV RNA levels in rhesus macaques [98]. Taken together, no NHP interventional study has demonstrated that IFN-I administration can slow progression.

Vaccination Against IFN-α in HIV-Infected Patients

In the 1990s, several clinical trials of an adjuvanted vaccine targeting endogenous IFN-α2a were pursued (see Table 1). Vaccination induced anti-IFN-α antibody production, but there was no detectable effect on CD4 T cell counts or HIV burden. However, vaccine responders had fewer HIV-related events compared to nonresponders or people who received the placebo [3, 4, 99], but the responders also had higher CD4 T cell counts, and some subjects were taking ART, rendering the data difficult to interpret.

Conclusion

Based on the combined human and animal model data, administering IFN-I during intravenous challenge, acute infection, or viremic or ART-suppressed chronic infection yielded no benefit, whereas it protected against rectal acquisition as long as IFN-I signaling was upregulated, suggesting that manipulation of the IFN system may have some potential for HIV prevention but a mucosal interface may be necessary. It is worth noting that a similar experiment has not been tested with the vaginal challenge model. Although it is an anatomical barrier, the vaginal mucosa does not contain the large population of potential target cells for HIV or SIV that are resident in the rectum. Whether a similar IFN-I-induced protective effect would be observed with a vaginal challenge is unknown, as several studies have shown that increased local inflammation may instead facilitate infection by recruiting target cells that are already present in the rectal mucosa. Indeed, increased vaginal inflammation is associated with increased susceptibility to HIV infection [100, 101]. Thus, the net result of simultaneous target cell recruitment to the vaginal mucosa and antiviral gene induction in response to IFN-I administration remains unknown, and the mode of acquisition may be necessary to consider with future vaccine platforms. In addition, the creation of an IFN-I-tolerant state with repeated IFN-α administration raises concern about vaccines that elicit ISGs. A vaccine that prevented HIV temporarily but ultimately disarmed the innate immune system and precipitated faster disease progression would be counterproductive. Elucidating the triggers of an IFN-I-tolerant state, and ultimately preventing it, will be critical when considering adjuvanted vaccines for HIV prevention. Alternatively, inducing higher but transient ISG expression during acute infection may be beneficial, but this has not been clearly demonstrated with interventions used thus far. In addition, the timing of when ISGs are beneficial, unnecessary, or detrimental in HIV infection, particularly with effective cART, remains largely unknown.

Outstanding Questions

1. Can IFN-I signaling be further increased during acute infection, and if so, would this increase virus control or increase detrimental immune activation?
2. Would adding IFN-I to cART during acute infection reduce the reservoir and slow progression more than cART alone?
3. Which cells are producing IFN-I in chronic infection?
4. Could supplementation of IFN-I to cART treatment during chronic infection reduce the viral reservoir?
5. Would blocking IFN-I signaling while administering cART during acute infection be detrimental because of the decreased restriction factor expression or advantageous because of decreased target cell recruitment and virus spread?
6. Would blocking IFN-I signaling while administering cART during chronic infection increase the reservoir because of the decreased restriction factor expression, and could it be used as a tool to reverse latency?
7. Conversely, could blockade of IFN-I in cART-suppressed HIV infection decrease end-organ disease and the reservoir because of decreased immune activation and inflammation and increased T cell responses?

Despite over 30 years of research, the exact role of IFN-I in HIV disease progression remains unclear. Numerous questions remain, and whether augmenting or blocking IFN signaling is the best strategy for prevention, treatment, and cure remains a pivotal area for HIV research.

Compliance with Ethics Guidelines

Conflict of Interest Steven E. Bosinger and Netanya S. Utay declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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