

# The evolving role of interferons in viral eradication strategies

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## Abstract

Interferons (IFNs) are a family of pleiotropic cytokines that are released when viral infection is sensed by pattern recognition receptors. They induce an antiviral state in target cells through influencing the expression of hundreds of genes termed IFN-stimulated genes (ISGs), which interfere with the replication of viruses in wide-ranging ways, and they have stimulatory effects on antiviral cell-mediated immunity. Although the role of therapeutic IFNs in the management of infectious diseases has predominantly been restricted to the treatment of chronic hepatotropic viruses, IFNs have effects on the replication of diverse families of viruses in cell culture models, and the potential to harness our endogenous defence system through therapeutic modulation of IFN pathways remains a tantalising prospect for both the broad-spectrum and tailored treatment of viral infections. Additionally, the study of the IFN system has become crucial to our understanding of host/pathogen molecular interactions, which provides plentiful targets for small molecule inhibitors of infection. Although the emergence of directly acting antivirals (DAAs) has resulted in the displacement of pegylated IFN $\alpha$  (pegIFN $\alpha$ ) for the treatment of HCV, recent findings have suggested potential roles for IFNs and IFN-related therapies in HIV and HBV eradication strategies, opening up a new avenue of research for this important family of cytokines.

Keywords: interferons, IFN, HIV, HCV, HBV

## Introduction

There are three families of IFNs: type 1 IFNs, containing IFN $\alpha$  (of which there are at least 12 subtypes) and IFN $\beta$ ; type 2 IFN, containing IFN $\gamma$ ; and type 3 IFNs, containing IFN $\lambda$ 1–4 [1]. Type 1 IFNs and the lesser-studied type 3 IFNs are the families involved primarily in antiviral responses. The trigger for IFN release is the sensing of viruses by pattern recognition receptors (PRRs), which detect conserved features of pathogenic microbes termed pathogen-associated molecular patterns (PAMPs). Well-studied PRRs include the toll-like receptors (TLRs), which are membrane-associated receptors, and the retinoic acid inducible gene I (RIG-I)-like receptors (RLRs), which are found in the cytosol. Type 1 IFNs signal via the heterodimeric type 1 IFN receptor (composed of IFNAR1 and IFNAR2), leading to activation of the JAK/STAT pathways, activation of IFN regulatory factor (IRF)-associated transcription factor complexes and expression of ISGs. As well as genes that appear to function by direct interaction with components of the virus to reduce viral replication, ISGs include genes involved in such diverse cellular processes as transcription, translation, lipid metabolism and apoptosis [2]. Large-scale screens for antiviral activity among these genes have become experimentally feasible over the past decade and have characterised both broadly antiviral ISGs and those that act on specific viral families [3]. IFNs also have effects on cell-mediated immunity through the IL-15-dependent activation of natural killer cells and are important for the development and survival of antiviral responses in CD8+ effector T cells.

## Viral clearance and IFN responsiveness

PegIFN $\alpha$ , co-administered with ribavirin, had been the standard of care for the treatment of HCV for some years prior to the introduction of DAAs. The rates of virological cure are heavily influenced by viral genotype and range from 40% to 85% in non-cirrhotic treatment-naïve patients treated for 24–48 weeks [4]. PegIFN $\alpha$  is also a contemporary option for the treatment of chronic HBV infection and, when used in the context of HBV e antigen (HBVeAg)-positive disease for 48 weeks, achieves HBVeAg

seroconversion rates of 20–35% at 6 months post treatment [5]. While only a small proportion of HBVeAg seroconverters lose HBV surface antigen (representing clearance of virus from blood), the rate is higher than for nucleos(t)ide-based therapy [6]. Hepatitis delta virus (HDV) infection is also treated primarily with pegIFN $\alpha$ , which produces sustained virological responses in around 23%, although recent reports suggest that these rates are substantially compromised by late recurrences [7]. Correlations of treatment success with IFN $\alpha$  are well studied in the context of chronic HCV infection and include age, sex, ethnicity, HCV viral genotype, pre-therapy viral load, IFNL3/IFNL4 genotype and the presence of hepatic fibrosis [5].

At the cellular and molecular level, the extent of the increase in expression of ISGs after IFN $\alpha$  administration is a strong predictor of virological responses, as shown through analysis of liver biopsy specimens taken before and (6 hours) after initial IFN $\alpha$  doses [8]. Increases in natural killer cell activation markers also reproducibly correlate with virological responses across different study contexts for a range of viral infections, whereas this has not been true of CD8+ T cell responses [9–11]. Interestingly, the ability to upregulate ISG expression appears to correlate inversely with baseline ISG levels, such that patients with higher baseline ISG expression show less dramatic changes after dosing and have poorer virological outcomes [8]. These findings are also borne out in studies of IFN $\alpha$ -induced suppression of HIV-1 plasma viral load, where high baseline ISG levels and weaker ISG upregulation are associated with absence of HIV-1 viral load decline [12]. The fact that lack of virological efficacy appears to relate to lack of response at the *cellular* level (i.e. failure of the host to *mount* the IFN-induced response), which may ultimately relate to higher baseline IFN signalling, may be important for future optimisation of IFN-based treatment strategies. Markers for IFN responsiveness would be beneficial to distinguish lack of host response from lack of anticipated virological response in clinical trials. Eradication-centred IFN-treatment strategies should also take account of the potential immunosuppressive effects of *long-term* IFN signalling on cell-mediated immunity suggested by many models of persistent viral infection [1].

## Emerging mechanisms for HIV/HBV cure efforts

Observations from both cell culture models of viral infection and clinical studies of IFN $\alpha$  in humans have reignited interest in IFNs

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for viral eradication efforts, perhaps particularly for HBV and HIV. HBV is a hepadnavirus possessing a relaxed circular, partially double-stranded DNA genome, which is translocated to the nucleus of infected hepatocytes where it is converted to covalently closed circular DNA (cccDNA). In addition to subgenomic RNAs, the cccDNA gives rise to the viral pre-genomic RNA, which is ultimately reverse-transcribed to produce the relaxed circular, partially dsDNA that is incorporated into the nascent virus particle. The relatively stable cccDNA intermediate is thought to be the major barrier to HBV eradication and is a target for HBV therapeutics in patients who cannot clear HBV surface antigen from serum despite sustained suppression of HBV DNA over many years of long-term nucleos(t)ide-based therapy. IFN-induced epigenetic regulation and silencing of HBV cccDNA had been described as a means of controlling HBV replication [13]. In 2014, Lucifora *et al.* reported that IFN $\alpha$  treatment in cell culture models of HBV infection results in degradation of cccDNA [14]. This occurred through the upregulation of members of the APOBEC3 family of cytidine deaminases, specifically APOBEC3A, which, through interaction with the HBV core protein, induced cytidine deamination of cccDNA and its subsequent degradation. Lymphotoxin B receptor activation led to the same downstream results via APOBEC3B. This suggests a mechanism whereby IFN $\alpha$ , or another ISG-inducing cytokine, could eradicate cccDNA from the liver. A number of trials have since been designed using varying combinations of nucleos(t)ide analogues followed by or with IFN $\alpha$  and although current protocols have yet to yield exciting results, these findings have opened the way for therapeutic exploitation of this mechanism for HBV cure efforts.

In 2013, Azzoni *et al.* reported the results of a strategic treatment interruption trial using pegIFN $\alpha$  monotherapy in HIV-1-positive patients [15]. Subjects who were stably suppressed on conventional antiretroviral therapy (ART) were co-administered pegIFN $\alpha$  for 5 weeks followed by discontinuation of ART and maintenance on IFN $\alpha$  monotherapy. Forty-five percent of patients maintained HIV-1 plasma viral load suppression to <400 HIV-1 RNA copies/mL on IFN $\alpha$  monotherapy 12 weeks after ART discontinuation. Additionally, in patients who maintained virological suppression, a decrease in integrated HIV DNA in CD4+ T cells from baseline was apparent. This suggested that IFN $\alpha$  was having effects on the cellular HIV-1 DNA reservoir. A modest effect of IFN $\alpha$  on the level of HIV-1 DNA in CD4+ T cells of patients who underwent pegIFN $\alpha$  and ribavirin therapy for HCV co-infection (in the absence of any analysis of host IFN responsiveness) has also been described [16]. HIV-1 latency is considered a major mechanism of viral persistence on ART, although there is also evidence for the contribution of ongoing HIV-1 replication in lymphatic tissues, where penetration of ART is suboptimal [17]. The mechanism of the IFN $\alpha$ -induced drop in cellular HIV-1 DNA is currently unclear and a number of ongoing clinical trials aim to characterise these effects in detail.

## Pattern recognition receptor agonists

As mentioned above, the release of IFNs is the result of recognition of pathogenic microbes via pattern recognition receptors. The downstream effects of this process may involve multiple subtypes of type 1 and type 3 IFNs. Although, for IFN families, the type 3 IFN receptor is known to have a different cellular distribution from the type 1 receptor, probably resulting in slightly divergent physiological effects, the functional differences between the range of type 1 IFN subtypes and type 3 IFN subtypes are incompletely understood. Particular type 1 IFN subtypes have been shown to induce somewhat distinctive signalling cascades based on their structural interactions and affinity for the type 1 IFN receptor, and

are thought to work in fairly complex cyclical cascades *in vivo*, which may have consequences for effective pathogen clearance [18]. A newer strategy to harness the IFN effector response is the upstream activation of pattern recognition receptors through PRR agonists. Although the antiviral effect of these molecules is thought to be mediated primarily through the induction of IFN $\alpha$ , one theoretical advantage of this strategy is that it may be able to induce the release of more physiologically effective IFN subtypes and the broader cytokine and immune cell interaction responses necessary to engage antiviral adaptive immunity. The orally available TLR7 agonist, GS-9620, showed promising results in a woodchuck model of HBV infection with impressive reductions in woodchuck hepatitis virus (WHV) serum viral load, persistent suppression of viral DNA after treatment and WHV surface antigen clearance [19]. Decreases in HBV DNA were also seen in a small number of HBV-infected chimpanzees treated with GS-9620, although seroconversion to surface antigen was not observed [20]. Although earlier TLR7 agonists developed for the treatment of HCV infection were associated with concerning side effect profiles, recent Phase 1 studies of GS-9620 in humans show the molecule to be well tolerated if administered once weekly and capable of inducing upregulation of ISGs in peripheral blood cells [21]. GS-9620 is also undergoing studies in non-human primate models of HIV infection to characterise effects on lentiviral reservoirs.

The effect of CPG 7909, a TLR9 agonist, on the HIV-1 DNA reservoir in peripheral blood and HIV-1-specific T cell responses has been investigated in a *post hoc* analysis of a randomised trial of CPG 7909 adjuvanted versus non-adjuvanted pneumococcal vaccination in HIV-1-positive patients [22]. Small differences in the level of total HIV-1 DNA in PBMCs were detectable pre and post later vaccination time-points, concurrent with differences in HIV-1-specific CD8+ T cell activation markers. Consequently, the orally available TLR9 agonist MGN1703 is undergoing further clinical investigation to identify potential effects on cellular HIV-1 DNA reservoirs.

## Conclusions

IFNs induce a highly diverse antiviral response involving multiple arms of immunity, from which we can learn greatly about potential routes to pathogen clearance. Although targeting of virus-encoded enzymes through small molecule inhibitors has revolutionised the treatment of medically important viruses, alternative approaches may be required for eradicating persistent infections. The capability of IFNs to influence viral sensing and to target persistent intermediates of viral replication, which are unaffected by contemporary antiviral therapy, warrants attention for future treatment strategies.

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## Conflicts of interest

The author has no conflict of interest to declare.

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