Review

The yin and yang of viruses and interferons

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Interferons (IFNs)-α/β are critical effectors of the innate immune response to virus infections. Through activation of the IFN-α/β receptor (IFNAR), they induce expression of IFN-stimulated genes (ISGs) that encode antiviral proteins capable of suppressing viral replication and promoting viral clearance. Many highly pathogenic viruses have evolved mechanisms to evade an IFN response and the balance between the robustness of the host immune response and viral antagonistic mechanisms determines whether or not the virus is cleared. Here, we discuss IFNs as broad-spectrum antivirals for treatment of acute virus infections. In particular, they are useful for treatment of re-emerging virus infections, where direct-acting antivirals (DAAs) have limited utility due to DAA-resistant mutations, and for newly emerging virus strains in which the time to vaccine availability precludes vaccination at the onset of an outbreak.

IFNs-α/β: host-derived broad spectrum antivirals

Virus infections range from mild and benign to highly virulent epidemics and pandemics, and significantly affect global health. DAAs, which target specific steps of virus replication, and vaccines, are currently the most effective therapeutic intervention strategies used against virus infections. Newly emerging or re-emerging viruses that have undergone mutations may, however, be resistant to the effects of DAAs, whereas vaccines require that the virus strain be identified before vaccine production, precluding their use at the onset of any new virus infection outbreak. Broad-spectrum antivirals, capable of modulating the innate immune response regardless of the infecting virus, present as ideal candidates as a first-line treatment for acute virus infections such as respiratory tract or sexually transmitted infections.

IFNs-α/β are produced by plasmacytoid dendritic cells (pDCs), macrophages, fibroblasts and endothelial cells, and are critical effectors in an innate immune response to virus infections [1]. IFNs-α/β are induced following pattern recognition receptor (PRR) activation by viruses (Box 1, as reviewed in [2]) and target many different stages of viral replication: for example, viral entry, envelope uncoating, genome replication, protein assembly, and release of viral progeny [3,4]. IFNs-α/β also activate different cell types in the immune system to promote viral clearance and induce apoptosis of cells to prevent viral replication [1,3]. As host-derived innate immune response factors, IFNs are, therefore, broad-spectrum antivirals, crucial for the primary host response to viral infection.

IFNs-α/β bind to and activate the IFNAR complex, resulting in the rapid induction of transcription and translation of ISGs (Figure 1, as reviewed in [3]). Notably, IFNARs are ubiquitously expressed on all cell lineages; probably an evolutionary consequence of different viruses being able to target and infect different cell types. IFNAR expression on any and all cell types ensures that an IFN response to a virus may be induced upon infection.

As an IFN response is central to a robust innate immune response, viruses have evolved a variety of mechanisms to interfere with IFN production and signaling, to disrupt innate host antiviral factors. Successful viral clearance is determined by the balance between virus-encoded molecules that antagonize the host innate immune response and the robustness of the host innate immune response. Here, we discuss how IFN therapy presents as a viable treatment option for a range of acute virus infections that target a variety of tissues, including respiratory tract infections by severe acute respiratory syndrome coronavirus (SARS-CoV) and influenza A viruses, infections of the liver by hepatitis C virus (HCV) and hepatitis B virus (HBV), and mucosal infections by herpes simplex virus (HSV). This may be particularly important given the paucity of broad-spectrum antivirals for treating newly emerging and re-emerging virus infections, which present a major threat to human health.

Yin: IFN activates the immune system

In addition to the induction of ISG expression in all cell types, IFNs shape the landscape of the immune system in response to virus infection by promoting neutrophil survival [3,5] and the activation of macrophages [6], natural killer cells [7], DCs [1,8], B cells [9] and $CD8^+$ T cells [1], and T helper (Th)1 polarization of effector $CD4^+$ T cells [8] (Figure 2). IFN therapy therefore has the advantage over DAA treatments in that, in addition to stimulating genes that block viral replication in infected cells, IFNs activate other innate and adaptive immune responses to combat the virus.

Polymorphisms in genes encoding factors involved in different stages of the IFN response can lead to marked differences in susceptibility to virus infection and severity of disease, and can also serve as predictive markers for the outcome of IFN treatment. For example, polymorphisms in host genes encoding proteins associated with regulation of

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 1471-4906/$ – see front matter doi:10.1016/j.it.2012.01.004 Trends in Immunology, April 2012, Vol. 33, No. 4

Box 1. IFN-α/β induction by viral pathogen-associated molecular patterns (PAMPs)

PRRs, including membrane-associated Toll-like receptors (TLRs), and cytosolic RNA and DNA sensors such as RIG-I, are able to detect both extracellular, endosomal or cytosolic viral PAMPs: viral genomic material. The primary outcome of this nonspecific surveillance system that detects any and all viruses, regardless of the target cell or tissue tropism of the virus, and independent of where the virus is located, is the transcriptional activation of IFN-α/β genes and the rapid production and secretion of these IFNs. Upon viral PAMP recognition, PRRs are able to trigger a phosphorylation-dependent signaling cascade to activate IRF3 and/or IRF7. For instance, endosomal RNA/DNA-sensing TLR7 and TLR9 activate IRF7 via myeloid differentiation primary response gene 88 (MyD88), whereas endosomal dsRNA-binding TLR3 and RIG-I, are able to activate IRF3 and IRF7 through TBK1 and inhibitor of IKKε. IRF3 and IRF7 activation results in their nuclear translocation where they act as transcription factors and up-regulate IFN-α/β gene expression.

an IFN response such as interferon receptor α-chain (IFNAR1) [10], the IFN-inducible myxovirus resistance GTPase protein, Mx [11], the IFN-inducible 2′,5′-oligoadenylate synthetase (OAS) [12] and the suppressor of cytokine signaling (SOCS) 3 associated with regulation of an IFN response [13], are predictive markers linked with the rate of sustained virological response (SVR) to HCV infection following IFN-α treatment. This highlights the importance of an intact IFN response during viral infection and indicates that genetic variations among patients can present as a challenge for optimizing IFN therapy.

Yang: virulence factors antagonize the IFN response

It is not surprising that many pathogenic viruses, including SARS-CoV, influenza A viruses, HCV and HSV, have developed mechanisms to disrupt and limit the IFN-α/β response (Table 1). Many viruses are able to evade the innate immune system by directly targeting pathways required for the induction of IFN-α/β production [14–18]. These viruses are also able to inhibit an IFN response, by interfering with effectors in IFN-inducible signaling cascades [19–21]. Understanding the basis of these antagonistic mechanisms is essential for optimizing the timing and dosage of IFN treatments as a viable therapy for acute virus infections.

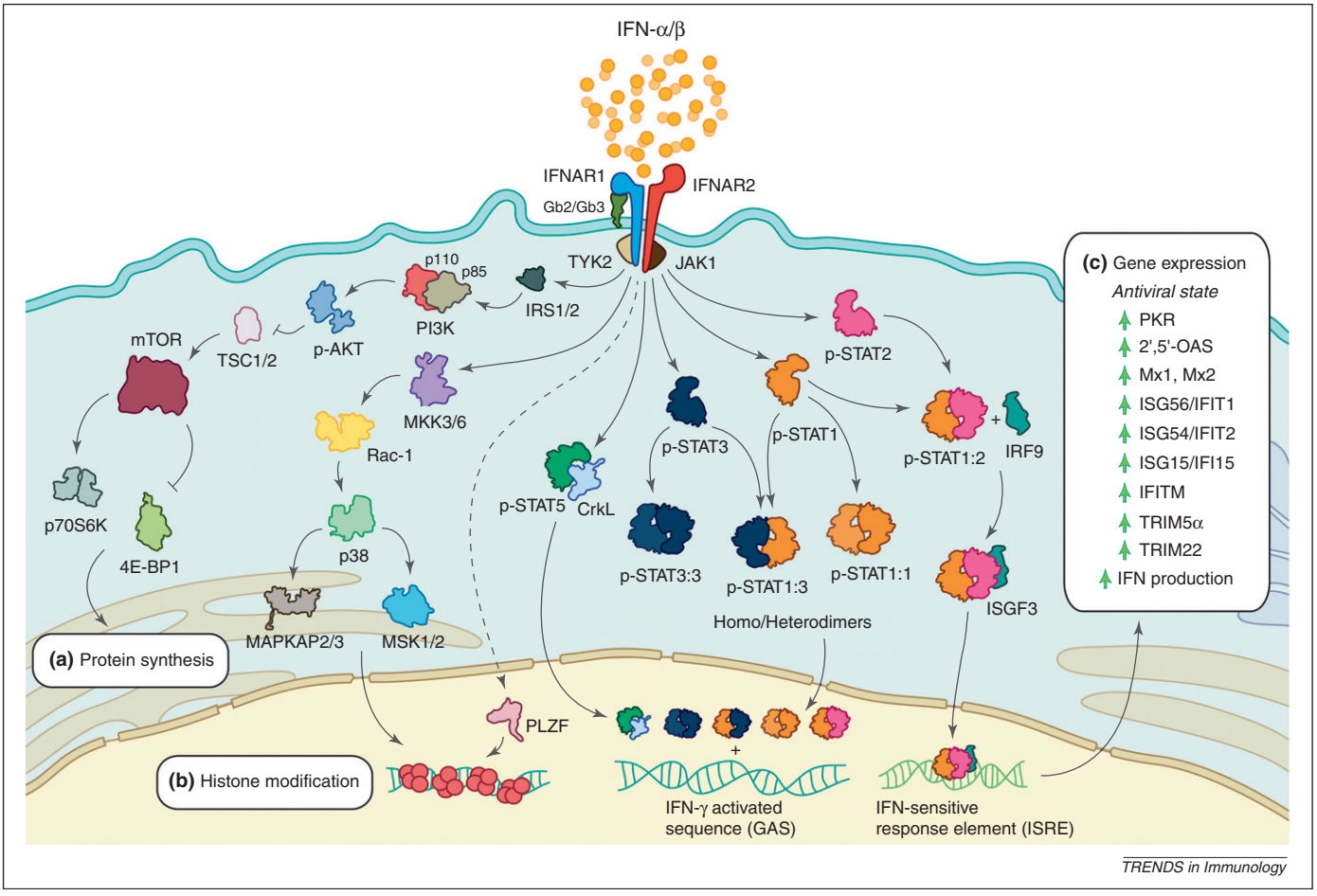

Figure 1. IFNs-α/β induce an antiviral state by regulating gene expression and protein translation. IFNs-α/β bind with high affinity to the IFNAR complex, composed of an α-chain, IFNAR1, which is structurally modified by cell membrane glycosphingolipids, galabiosylceramide (Gb2) and globotriaosylceramide (Gb3) to promote efficient IFN binding [71,72], and a β-chain, IFNAR2. IFN binding to IFNAR induces phosphorylation of the receptor-bound tyrosine kinases, tyrosine kinase 2 (TYK2) and JAK1, leading to the subsequent regulation of: **(a)** protein synthesis via the activation of PI3K and mammalian target of rapamycin (mTOR); **(b)** histone modification, via the activation of p38 MAPK; and **(c)** gene expression via the phosphorylation of STAT proteins and MAPK activation. TYK2- and JAK1-mediated activation of insulin receptor substrate (IRS) 1 and IRS2 is required for recruitment and activation of PI3K. PI3K phosphorylates AKT, which inactivates inhibitors of mTOR, tuberous sclerosis protein (TSC) 1 and TSC2 [73]. mTOR activates the serine/threonine kinase p70S6K, and inactivates eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), to upregulate cap-dependent mRNA translation and protein synthesis. p38 is activated downstream of MAPK kinases 3 and 6 (MKK3/6) and regulates histone modification and gene expression through mitogen- and stress-activated protein kinase (Msk) 1 and Msk2. In addition, IFN signaling invokes promyelocytic leukemia zinc finger (PLZF) protein-mediated histone modification to regulate ISG expression [74]. Phosphorylation of STAT proteins results in their dimerization and IFN-stimulated gene factor (ISGF) formation. STAT complexes translocate to the nucleus and bind to specific gene elements in the promoters of ISGs, IFN-γ activated sequence (GAS) and IFN-sensitive response element (ISRE), to induce expression of antiviral genes.

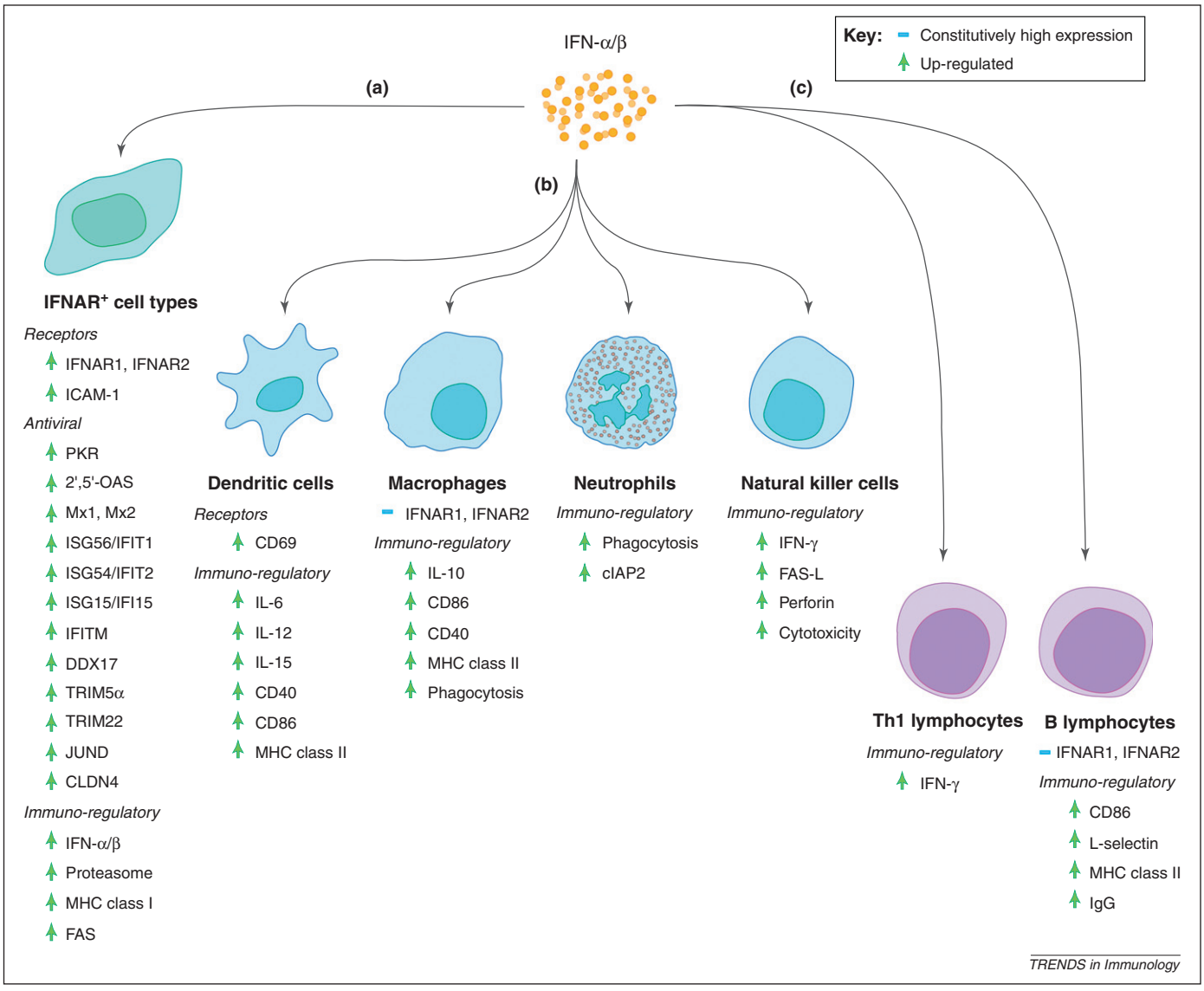

Figure 2. IFNs-α/β invoke the activation of immune cells. IFN-α/β signaling modulates the innate and adaptive immune response to virus infection. **(a)** IFNAR$^+$ cells: IFN-α/β signaling results in the expression of key antiviral factors: PKR, 2′,5′-OAS, Mx1, Mx2, ISG56/IFN-induced protein with tetratricopeptide repeats (IFIT) 1, ISG54/IFIT2 and ISG15/IFN-activated gene 15 (IFI15) [75]. Furthermore, IFNs-α/β upregulate the expression of IFN-inducible trans-membrane protein (IFITM), an inhibitor of influenza A viruses, SARS-CoV, dengue virus and West Nile virus infections [4,76], tripartite motif-containing protein 5α (TRIM5α) and TRIM22, which are antiviral factors that limit HIV-1 infection [77], and transcription factor jun-D (JUND) and claudin 4 (CLDN4) [78]. IFN treatment primes cells for apoptosis by modulating the expression of proteasome subunits, major histocompatibility complex (MHC) class I, and FAS receptor (CD95) [79–82]. IFNs-α/β also contribute to the activation and differentiation of cells involved in the **(b)** innate and **(c)** adaptive immune responses to virus infection. IFN-α/β induces production of interleukin (IL)-6, IL-12, and IL-15 by DCs, and IL-10 by macrophages to modulate B and T cell differentiation (Th1 polarization) and activation [79]. IFN-β signaling in pDCs leads to altered CD69 and sphingosine-1-phosphate 4 (S1P4) receptor expression, thereby affecting pDC retention in lymph nodes [83]. IFNs-α/β increase MHC class II, CD40 and CD86 expression on antigen presenting cells. IFN-α/β treatment induces macrophage and neutrophil phagocytosis [79,84]. Moreover, IFNs-α/β promote neutrophil survival by activating cellular inhibitor of apoptosis 2 (cIAP2) [5]. Natural killer (NK) cells respond to IFNs-α/β with increased FAS ligand (FASL) and perforin expression, and IFN-γ production [85,86]. In response to IFNs-α/β, B cells upregulate L-selectin and IgG production [9,79].

IFN therapy as a first-line treatment against newly emerging or re-emerging virus outbreaks: SARS-CoV

The SARS-CoV outbreak originated in Hong Kong in late 2002–2003 and resulted in >8000 cases of disease worldwide, with a 9.6% mortality rate between November 2002 and July 2003 (http://www.who.int/csr/sars/country/table2004_04_21/en/index.html). SARS-CoV is a single-stranded RNA virus that encodes in its genome virulence factors that antagonize the IFN-α/β response. In infected host cells, SARS-CoV expresses the nonstructural protein (Nsp) 1 and Nsp3. Nsp1 suppresses host gene expression by disrupting mRNA translation and by upregulating mRNA degradation [14,22]. Immunoprecipitation and luciferase reporter studies have shown that Nsp1 directly associates with the 40S ribosomal subunit to inhibit its translational activity [22]. In addition, the Nsp1–40S complex is able to modify mature 5′-capped RNAs to limit translation and promote degradation [22]. In the context of an IFN response, these antagonistic mechanisms of Nsp1 on host gene expression and protein synthesis inhibit IFN-α/β expression and production [14]. *In vitro*, Nsp1 also inhibits signal transducer and activator of transcription (STAT) 1 protein phosphorylation induced by IFN-α treatment [23]. Both Nsp1 and Nsp3 inhibit interferon regulatory factor (IRF) 3 and IRF7 activation to downregulate IFN production in response to viral infection [23,24]. Specifically, Nsp3 inhibits IRF3 in human bronchial epithelial cells via its papain-like protease (PLP) domain, which

Table 1. Virus-encoded proteins that antagonize the IFN response.

Virus	Protein	Function(s)	References
SARS-CoV	Nsp1	Associates with the 40S ribosomal subunit to inhibit its translational activity. Promotes mRNA degradation by modifying mature 5′-capped RNAs and inhibits IRF3 and IRF7 activation, and IFN-inducible STAT1 phosphorylation.	[14,22,23]
	Nsp3	Inhibits IRF3 phosphorylation and nuclear translocation via its papain-like protease (PLP) domain.	[24]
	ORF6	Localizes to the ER and binds to nuclear import factors to prevent the nuclear translocation of phosphorylated STAT1 dimers.	[19]
	M	Inhibits the function of RIG-I and signaling effectors TBK1, IKKε and TRAF3.	[25]
Influenza A viruses	NS1	Inhibits the function of RIG-I, cleavage and polyadenylation specific factor 30 kDa (CPSF30), poly(A)-binding protein II (PABPII), protein kinase RNA-activated (PKR), and 2′,5′-OAS/RNaseL, via its dsRNA-binding domain or protein-binding domain. Disrupts IFN-inducible signaling events by downregulating the surface expression of IFNAR1 and upregulating SOCS1 expression, leading to a reduction in STAT phosphorylation and nuclear translocation. Interacts with the internal SH2 domain of p85β, the inhibitory subunit of PI3K to promote cell survival during early stages of infection.	[15,20]
HCV	NS3/4A	Targets mitochondrial antiviral signaling (MAVS) proteins required for RIG-I-mediated IRF3 activation.	[16]
	NS5A	Associates with intracellular membranes and inhibits the function of PKR.	[55]
	IRES	Binds to PKR before viral dsRNA binding, to prevent PKR activation.	[57]
	E2	Blocks PKR activation via its eIF-2α phosphorylation homology domain.	[87]
HBV	Pol	Blocks IRF signaling and TBK1/IKKε activity by interacting with the host DEAD box RNA helicase, DDX3.	[17]
HSV	ICP0	Inhibits IRF3 activity.	[18]
	ICP27	Inhibits STAT phosphorylation and nuclear translocation.	[21]
	ICP34.5	Dephosphorylates eIF-2α to reverse PKR-mediated inactivation of eIF-2α.	[63]
	US11	Inhibits the function of PKR and 2′,5′-OAS via its dsRNA-binding domain.	[64]

interacts with IRF3 to inhibit IRF3 phosphorylation and nuclear translocation [24]. In addition to Nsp1 and Nsp3, the open reading frame 6 (ORF6) and matrix (M) proteins of the SARS-CoV also inhibit an IFN response [19,25]. ORF6 localizes to the host endoplasmic reticulum (ER) and blocks the transcription factor function of phosphorylated STAT1, by binding to nuclear import factors to prevent its translocation to the nucleus [19]. The M protein interacts with RNA sensor retinoic acid-inducible gene 1 (RIG-I), an RNA helicase and key intracellular PRR associated with induction of IRF-dependent IFN production following detection of viral RNAs. The M protein also interacts with the signaling effectors serine/threonine-protein kinase 1 (TBK1), inhibitor of nuclear factor-κB kinase subunit ε (IKKε), and tumor necrosis factor (TNF)-associated factor 3 (TRAF3), again associated with IFN gene induction [25]. Thus, there are multiple mechanisms by which SARS-CoV might inhibit the host IFN response. The implications are that an IFN response to SARS-CoV infection must be dramatically limited for virus replication to proceed, suggesting that the dominant immune response is the IFN response.

Different recombinant IFN-αs and IFN-β are now approved for various clinical indications, including the treatment of chronic HCV infections [26,27]. Through a comprehensive analysis of how structural features in the IFN-α/β molecules – crucial clusters of amino acids – affect the sensitivity of target cells to IFN-induced biological responses, specific epitopes on the exposed surface of the IFN molecule have been identified that are associated with receptor recognition [28–39]. Accumulating evidence suggests that the affinity of a particular IFN-α/β subtype for IFNAR determines the biopotency of the IFN, specifically in the context of antiviral and antiproliferative responses [28,39]. A direct consequence of this was the design and development of a synthetic IFN-α, IFN alfacon-1, that exhibits optimized affinity for IFNAR [40–43].

Initially, treatment for SARS-CoV infection focused on the use of a DAA, ribavirin, in combination with corticosteroid therapy [44,45]. However, in a pilot clinical study, the therapeutic potential of IFN alfacon-1 was evaluated in individuals infected with SARS-CoV and hospitalized in Toronto, Canada [46].

IFN alfacon-1 treatment together with corticosteroids is associated with reduced disease-associated impaired oxygen saturation, more rapid resolution of radiographic lung abnormalities and lower levels of disease-associated creatine kinase. *In vitro* studies to examine the mechanism of action of IFN against the SARS-CoV have revealed that IFN-inducible Janus kinase 1 (JAK1), protein kinase C (PKC)-δ and p38 mitogen-activated protein kinase (MAPK) activation mediate IFN antiviral protection. Target genes downstream of activation of these kinases are differentially expressed in the peripheral blood cells of SARS patients treated with IFN alfacon-1 compared with patients not treated with IFN, and functionally these genes are associated with antimicrobial activity [47]. Treatment of a human bronchial epithelial cell line, Calu-3, with IFN alfacon-1 before infection with the SARS-CoV results in inhibition of virus infection and a reduction in overall virus yield, further supporting the idea that IFN alfacon-1 demonstrates antiviral activity against the SARS-CoV [40]. These data demonstrate that despite the inherent ability of the SARS-CoV to inhibit IFN production and limit an IFN response, treatment with exogenous IFN-α overrides these inhibitory effects. These results support the further evaluation of IFN alfacon-1 as a first-line treatment for acute SARS-CoV infection and approved

randomized clinical trial protocols are in place in the USA and Canada should there be outbreaks of SARS-CoV.

IFN therapy for influenza A virus infections

Seasonal influenza A virus infections are a considerable health burden and vaccine programs are currently implemented in most developed countries. Vaccines, however, are not relevant during an outbreak involving an emergent variant. The 2009 H1N1 swine-origin influenza A virus is a prime example of how quickly a pandemic can develop given the potential for genetic shift and mutation of influenza A viruses among natural hosts. During the 2009 H1N1 pandemic, DAAs such as the neuraminidase inhibitors oseltamivir and zanamivir were widely used before a vaccine became available [48]. Not surprisingly, however, DAA-resistant variants of pandemic H1N1 emerged [48,49]. Avian H5N1 influenza virus outbreaks, now affecting populations throughout Asia and Europe, are associated with mortality rates around 60% [50]. Notably, a number of H5N1 strains are resistant to oseltamivir [51]. To date, there have been no reported cases of human-to-human transmission of this lethal H5N1 influenza virus infection, but if a newly emerging strain capable of human-to-human transmission appears, DAA resistance will develop and until a vaccine becomes available – probably 4–6 months – populations will be at risk in the absence of access to broad-spectrum antivirals.

NS1 is the primary virulence factor encoded by influenza A viruses and it is expressed in host cells during the earliest stages of infection [15]. In comparison with SARS-CoV Nsp1, influenza virus NS1 has both overlapping functions as well as unique mechanisms to inhibit the IFN response. NS1 acts both in the nucleus and cytoplasm of an infected cell, and is the primary antagonist of the host innate immune response. Remarkably, NS1 has evolved to inhibit virtually all stages of the IFN response to virus infection, including inhibition of IFN production, interference with IFN signaling events, and inhibiting the function of antiviral factors induced by IFN signaling. NS1 inhibits the activity of RIG-I (Box 1) where the NS1 dsRNA-binding domain interacts directly with RIG-I [15]. Within the nucleus of an infected cell, NS1 inhibits the processing and synthesis of host mRNAs, including IFN-α/β mRNAs, by binding to and inhibiting both cleavage and polyadenylation specific factor 30 kDa (CPSF30) and poly(A)-binding protein II (PABPII), via its protein-binding domain [15]. The expression of avian H5N1 NS1 disrupts IFN signaling events by downregulating the surface expression of one of the IFNAR subunits, IFNAR1, and by upregulating SOCS1 protein expression, leading to a reduction in IFN-inducible STAT phosphorylation and STAT homo/heterodimer nuclear translocation [20]. NS1 is able to block directly the antiviral activities of IFN-inducible antiviral proteins such as protein kinase RNA-activated (PKR) and 2′,5′-OAS/RNaseL, via its protein-binding domain and dsRNA-binding domain, respectively [15]. The Src homology 2 (SH2)-binding domain within the protein-binding region of NS1 permits interaction with the internal SH2 domain of p85β, the inhibitory subunit of phosphoinositide 3-kinase (PI3K). This leads to activation of the PI3K–AKT pathway [15]. Activation of PI3K, a downstream target of IFN-α/β signaling, by a specific NS1 promotes cell survival during the early stages of infection, illustrating the complex interplay between virus encoded factors and the IFN-α/β response [15]. Remarkably, distinct highly pathogenic respiratory viruses, namely influenza viruses and the SARS-CoV, encode non-structural proteins in their genomes that function as virulence factors that specifically target the host innate IFN response, further emphasizing the importance of IFNs as broad-spectrum antivirals.

A recently completed randomized controlled trial has examined the safety and efficacy of recombinant IFN-α (rIFN-α) treatment, administered in the form of a nasal spray, in military recruits, in the context of protection from respiratory virus infections [52]. Serum IgM levels were measured as evidence of virus infection. Subjects receiving rIFN-α had lower concentrations of serum IgM specific for H3N2 influenza A virus, influenza B virus, adenovirus (species B), and parainfluenza virus types 1, 2 and 3 [52]. Specifically with regard to influenza A virus, only 30 recruits treated with rIFN-α had detectable levels of influenza A virus IgM compared with 104 recruits in the untreated control group [52]. No adverse events were reported in the treatment group, the data demonstrating that IFN was well tolerated and was effective in preventing a variety of common viral respiratory infections. Thus as for the SARS-CoV, the implications are that treatment with IFN-α can override the inhibitory effects of NS1 on an IFN response during influenza A virus infection.

IFN therapy for highly pathogenic and oncogenic viral infections: HBV and HCV

Worldwide, >170 million people are infected with HCV, resulting in approximately 350 000 deaths each year (http://www.who.int/mediacentre/factsheets/fs164/en/). More than an estimated 350 million people are chronically infected with HBV, resulting in approximately 600 000 deaths each year (http://www.who.int/mediacentre/factsheets/fs204/en/). HCV and HBV target the liver and cause both acute and chronic infections, resulting in liver cirrhosis and eventually, hepatocellular carcinoma [16,53].

The current approved standard-of-care treatment for HCV infection comprises daily ribavirin in combination with weekly pegylated IFN-α (peg-IFN-α). The covalent linkage of polyethylene glycol to IFN-α increases the half-life of IFN-α in the circulation. Common side effects associated with IFN therapy include a range of flu-like symptoms (fatigue, fever, myalgia), that often diminish spontaneously during the first few weeks of therapy. More severe neuropsychiatric disturbances including sleep disturbances and depressive mood changes have their onset within the first months of IFN therapy. Hematological disturbances such as neutropenia or anemia may occur and are responsive to IFN dose reduction or treatment [granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), respectively]. This combination IFN/ribavirin therapy has been very successful in patients infected with HCV genotypes 2 or 3, and 70–90% of patients go on to achieve an SVR, characterized by undetectable HCV RNA following 24 weeks of treatment [53]. The rate of SVR falls to 40–60% in patients infected with HCV genotypes 1 or 4,

following 48 weeks of treatment with peg-IFN-α and ribavirin [53].

The incomplete response to IFN treatment is partially attributable to virally encoded virulence factors that interfere with an IFN response: NS3/4A and NS5A. NS3/4A is an HCV serine protease that targets mitochondrial antiviral signaling (MAVS) proteins required for RIG-I-mediated IRF3 activation and subsequent IFN production [16]. Recently, the secondary structure of the HCV genotype 1b NS3 N-terminal region was identified as a predictive marker for the virological response in patients who had received IFN and ribavirin combination therapy for 48 weeks. Specifically, polymorphisms in the secondary structure of the NS3 amino-terminal region segregate HCV genotype 1b infected individuals into two groups and are predictive of the virological response to peg-IFN plus ribavirin therapy [54]. NS5A associates with intracellular membranes and its expression is vital for HCV genome replication. NS5A is able to interact with IFN-inducible PKR to evade an IFN-induced antiviral response. Polymorphisms in amino acid residues 70 and 91 in the HCV core and in NS5A are also predictive markers of the virological response in patients receiving IFN and ribavirin therapy [55]. Notably, NS5A is a target of IFN, because the IFN-activated gene 15 (IFI15)/interferon stimulated gene (ISG15) encoding a 17-kDa protein, promotes ISGylation of NS5A to enhance its degradation, thereby inhibiting HCV replication [56].

In addition to HCV NS5A, both the HCV envelope protein E2 and the internal ribosome entry site (IRES) are able to inhibit IFN-inducible PKR activity [57]. E2 contains a eukaryotic translation initiation factor 2 (eIF-2α) phosphorylation homology domain through which it is able to interact with PKR, whereas the HCV IRES binds to PKR, precluding dsRNA binding, thereby preventing PKR activation [57]. Despite these potent inhibitory effects of HCV-encoded factors on an IFN response, clinical data provide direct evidence that peg-IFN-α treatment in combination with ribavirin is effective at limiting HCV infection and, dependent on the HCV genotype, may invoke a SVR.

The mechanisms by which HBV evades an innate immune response are less well understood. The HBV polymerase (Pol) blocks IRF signaling and subsequent IFN production by inhibiting TBK1/IKKε activity, associated with PRR signaling [17]. This inhibition is mediated by direct protein–protein interactions between Pol and the host DEAD box (D-E-A-D amino acid sequence motif) RNA helicase, DDX3, that enhances TBK1/IKKε activity [17]. Peg-IFN-α is an effective treatment for HBV infection, again suggesting that IFN treatment can overcome virus-imposed inhibition of the innate immune response, specifically IFN production. Peg-IFN-α is an effective treatment option for hepatitis B e core antigen (HBeAg)-positive disease, where detection of HBeAg in the blood is indicative of viral replication. Up to 40% of HBeAg-positive patients treated with peg-IFN-α are able to develop HBeAg-specific antibodies (seroconversion) by 6 months after the end of treatment. This percentage rises to 60% at 5 years after the end of treatment [58]. In comparison to monotherapy with the DAA lamivudine, which can lead to the emergence of mutant lamivudine-resistant HBV strains, peg-IFN-α alone or in combination with lamivudine is up to 50% more effective for inducing HBeAg seroconversion, although more side effects are reported in patients receiving peg-IFN-α [59]. In contrast to HBeAg-positive disease, peg-IFN-α, alone or in combination with ribavirin, has limited effect in patients with late stage HBeAg-negative disease, where the HBV mutation has resulted in loss of HBeAg expression [60]. In a randomized clinical trial, the percentage of HBeAg-negative patients with HBV DNA levels <10 000 copies/ml, receiving peg-IFN-α monotherapy, dropped from 36% to 20%, from the end of treatment to 24 weeks later [60]. The stage of viral disease can therefore affect the efficacy of IFN therapy and the timing of treatment contributes to the capacity to resolve an infection. Moreover, as for influenza viruses and SARS-CoV, despite HBV and HCV encoding viral factors that antagonize an IFN response, exogenous IFN therapy has proven to be an effective treatment for establishing an SVR.

IFN therapy for highly transmissible viral infections: HSV-1

HSV-1 is a highly contagious virus, prevalent among sexually transmitted infections. HSV-1 is able to establish a latent infection in immunocompetent individuals by evading the immune system and is only reactivated when the host immune system is weakened [61,62].

The HSV-1 genome encodes a number of virulence factors, namely infected cell protein (ICP) 34.5, ICP0 and ICP27, which are associated with immunoevasion and suppression of the innate immune response to virus infection [18,21,63,64]. Specifically, ICP34.5 dephosphorylates eIF-2α to reverse PKR-mediated inactivation of eIF-2α [63], ICP0 localizes to the cytoplasm and inhibits IRF3 activity [18], and ICP27 blunts the IFN-inducible JAK–STAT signaling pathway by inhibiting IFN-inducible STAT1 phosphorylation and nuclear translocation [21]. Furthermore, HSV structural protein US11, which has a dsRNA-binding domain, disrupts the activation of the IFN-inducible antiviral proteins 2′,5′-OAS and PKR [64].

For immunocompromised individuals infected with HSV-1, viral pathogenesis can lead to serious life-threatening disease; more so in the context of emergent drug-resistant HSV-1 strains [65–67]. Different DAAs have been used to control HSV-1 infection, including acyclovir, penciclovir and foscarnet, resulting in the emergence of DAA-resistant HSV-1 strains [65–67]. IFN-γ is able to exert antiviral activity by stimulating a T cell response. However, IFN-γ alone may have limited efficacy in immunocompromised HSV-1-infected individuals lacking a robust adaptive immune response. Recent studies have shown that when immunocompromised nude mice are infected with a DAA (acyclovir)-resistant HSV-1 variant and treated with IFN-β in combination with IFN-γ, viral infection is reduced [65]. These preliminary data are in further support of the broad-spectrum antiviral activities of IFNs-α/β.

Shifting the balance to favor the host innate immune response: the future of IFN antiviral therapy

Mechanisms for viral evasion of the host immune response include both the expression of many virulence factors by a

single virus to target different stages of the IFN response, or the expression of a single, highly specialized molecule that alone targets multiple facets of an IFN response. *A priori*, the widespread existence of these virally encoded virulence factors that target an IFN response highlights the critical role of a robust IFN response to limiting virus infection. The ability of IFNs-α/β to target multiple types of viruses at different stages of viral replication, and the ubiquitous expression of IFN receptors on cells that are susceptible to different virus infections with different tissue tropisms, as well as the ability of IFNs to activate innate immune cells and influence the adaptive immune response, emphasizes the relevance of IFNs-α/β as broad-spectrum antivirals.

Understanding the viral strategies for evasion of an IFN will permit the design of strategic IFN treatment regimens to both protect from and clear virus infections. The opportunity to limit virus infections even in the absence of characterizing the specific infecting virus, a reality during an outbreak of unknown etiology, or during a pandemic of a newly emerging or re-emerging virus strain, has profound implications for global health. Indeed, early data indicate that IFN therapy may be effective in treating West Nile virus [68], hemorrhagic yellow fever virus [69] and Ebola virus infections [70]. Moreover, short-term IFN therapy for an acute virus infection may not invoke the debilitating side effects associated with long-term IFN therapy for chronic infections such as HBV and HCV. Preliminary data from pilot clinical trials of IFN treatment for SARS-CoV and for influenza A viruses showed this to be the case [46,52]. Cognizance of the yin and yang of viruses and IFNs opens the door to the widespread clinical application of these broad-spectrum antiviral IFNs.

Acknowledgment
E.N.F. is a Tier 1 Canada Research Chair.

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