# Interferons as biomarkers and effectors: lessons learned from animal models


## Abstract

Interferons (IFNs) comprise type I, II and III families with multiple subtypes. Via transcription of IFN-stimulated genes (ISGs), IFNs can exert multiple biological effects on the cell. In infectious and chronic inflammatory diseases, the IFNs and their ISG sets can be potentially utilized as biomarkers of disease outcome. Animal models allow investigations into disease pathogenesis and gene knockout models have proved cause and effect relationships of molecules related to the IFN response. Sets of IFN subtypes and their ISG products provide immunological signature patterns for different viral and other diseases. In this article, we give an overview of IFNs in several virus infection models and autoimmune diseases of medical relevance. Lessons learned from animal models inform us of IFN system parameters as indicators of disease outcome and whether clinical research is warranted. Moreover, validated IFN biomarkers for prognosis enhance our understanding of therapeutic and vaccine development.



## Keywords

autoimmune disease, biomarker, innate immunity, interferon-stimulated gene, signaling pathway, therapy, type I interferon response, viral infection



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Interferon (IFN) was first discovered as a cellular secretion that protected against influenza virus infection [1]. It was ascertained that many types of IFNs comprised this cytokine family with diverse effects. There are three major types of IFNs, namely, type I IFN, consisting of IFN–α, IFN–β and other subtypes; type II IFN, consisting of IFN–γ; and the more recently discovered type III IFN, consisting of IFN-λ subtypes (IL-28 and IL-29). These IFN types are distinguished by their sequence, stimulus and cell type of production, having distinct cognate receptors. This review will focus on type I IFNs, since the majority of work in the field relevant to biomarkers has involved this IFN type. IFN-λ, which, like the type I IFNs, is induced by pathogens, will be mentioned mainly in the context of comparisons with type I IFN responses. IFN–γ is typically produced by activated T cells and NK cells and is often used as a biomarker of activation. It is a potent cytokine that plays a critical role in modulating the adaptive immune response to infectious and chronic inflammatory diseases and cancers, elucidated in numerous studies of IFN–γ receptor- or IFN–γ-deficient mice. However, because of the limited scope of this review, we will not include IFN–γ (reviewed extensively elsewhere).

The genomic revolution has provided precise information on how many IFN genes exist, potential regulatory regions and comparative genomics [2]. Indeed, type I IFNs were some of the first genes cloned using recombinant DNA technology, spawning the biotechnology industry, and were among the first recombinant proteins approved for clinical use. The measurement of specific subtypes and the inclusion of IFN-specific gene probes in microarray platforms meant that expression data on transcripts of IFN subtypes began to be gathered in databases [3]. This complemented the ability to measure IFN proteins by bioassays and immunoassays [4]. Furthermore, different subtypes could be expressed and proteins used to identify different characteristics. Correlates of IFN subtype specificity to function/potency/efficacy have highlighted their usefulness as biomarkers.

Just as the complexity of the IFN family was elucidated, our knowledge of the signal transduction pathways activated by IFNs was expanded. More than any other cytokine, IFNs have long been known to activate specific genes, known as IFN-stimulated genes (ISGs). In particular, those responsible for the definitive antiviral effects such as *2–5OAS*, *PKR* and *Mx* are well characterized and have been used as biomarkers in the blood cells of patients receiving IFN therapy. As genomic technologies have been used to research IFN signaling, so the list of ISGs has grown to nearly 2000 [5]. The use of gene targeting in animal models has conclusively correlated parameters with clinical outcome, elucidating the role of many ISGs in the pathogenesis of infectious, inflammatory and autoimmune disease, as well as cancer. Furthermore, the use of high-throughput technologies has facilitated screening assays and protocols to determine gene function *in vitro*. For example, over 300 ISGs were recently tested *in vitro* for antiviral activity against several viruses, eloquently demonstrating specific ISG signatures induced by different viruses [6]. Such comprehensive data contributes greatly to our ability to use these as biomarkers of relevant responses.

The recent explosion in knowledge of the mechanism of action of the innate immune system – the discovery of new families of receptors and sensors that drive the production of cytokines, such as type I IFNs – has increased our understanding of their importance in sensing pathogens, modulating the acute innate response and orchestrating the adaptive response. The strength, duration, timing and cellular context of these responses determine disease outcome, whether there is resolution, acute damage (e.g., septic shock) chronic disease, including inflammation, cancer, autoimmunity or even death. Thus, understanding the role of individual genes (IFN or ISG) or clusters of genes (IFN subtypes and ISG sets) in disease pathogenesis, especially through definitive animal model studies, will be instructive for their use as biomarkers of disease, therapeutic responses or vaccine efficacies. In this review, we will cover a brief introduction of the IFNs, knowledge of their mechanism of action, including illustrations of ISGs, an overview of their role in models of infectious and inflammatory

disease and how this information can be used to identify potential biomarkers, including their validation in human studies.

## IFN types

IFN–α and IFN–β are the best characterized type I IFNs, which also include IFN-τ, IFN-ε, IFN-ω and many more. For this reason, only IFN–α and IFN–β will be covered herein (Table 1). In humans, the type I IFN family has 14 genes, which encode 14 a subtypes with a high degree of amino acid homology (over 75%). In the mouse, 13 α subtypes are expressed. Human *IFNA* and *IFNB* genes are intronless, encode proteins of 166 amino acids (161–167 amino acids in mice with 80–95% identity amongst the IFN–αs) and are located at a single gene cluster on human chromosome 9 and mouse chromosome 4 [7,8]. IFNs are highly conserved across species, highlighting their evolutionary importance in host immune responses against pathogens. Murine IFN–αs have over 70% sequence identity with human IFN–αs [9,10]. However, there is only one IFN–β subtype expressed in both humans and mice [11]. Human IFN–β has 63% homology with mouse IFN–β but only 30% homology with the IFN–αs and so they are closer to each other than to the IFN–αs in their own species. Type III IFNs (IFN–λs) were first co-discovered in 2003 [12,13] and, like the type I IFNs, are antiviral (reviewed in [14]). In humans there are three IFN–λs (chromosome 19), whereas in the mouse there are only 2 λ subtypes (chromosome 7), the third being a pseudogene (Table 1). Type III IFNs are IL-10-like cytokines with five exons, contrasting with the intron-lacking type I IFN genes. Murine *IL28a* and *IL28b* genes are similar to their human counterparts and code for 174–amino acid proteins (97% identity to each other and 60% identity to human orthologs) [14].

## IFN production & induction

Most cell types in the body can produce type I and type III IFNs in response to stimuli, including viruses, with plasmacytoid dendritic cells (pDCs) being major producers [15,16]. There are numerous mechanisms of pattern recognition receptor induction of IFNs by invading viruses, involving extracellular, endosomal, cytoplasmic and nuclear receptors. Both type I and type III IFNs are induced via Toll-like receptor (TLR) activation [16,17] and cytoplasmic viral nuclei acids are sensed by RIG-I, MDA5 and LGP2. Remarkably, the IFN subtypes are not expressed equally in response to all types of invading viruses [18], and distinct type I IFN signatures have been found depending on cell type and infecting virus [6,19].

### IFN signaling

Both the IFN–αs and IFN–βs bind a single heterodimeric receptor (IFNAR composed of both IFNAR1 and IFNAR2 subunits), expressed by almost all cells in human and mouse [20–23]. Furthermore, levels of IFNAR2 in the liver have been shown to be a predictive biomarker of response to IFN treatment in chronic hepatitis C patients [24]. As the IFN–λ heterodimeric receptor is composed of IL-10R2 and IL-28aR (IFNLR1), the IFN–λs act almost exclusively on epithelial cells due to restricted expression of the IFNLR1 subunit. Human hepatocytes also express the IFN–λ receptor but this is not the case for murine hepatocytes [25]. Both expression levels of IFNAR1 and IFNAR2 subunits of the receptor along with the soluble form of IFNAR2 are important for initiation of signaling. Ternary structures of individual IFN subtypes binding to the receptor show differences in stability, impacting on signal strength. After binding to their cognate receptors, IFNs signal through the well-characterized Jak/STAT pathway, although other pathways have been found to be IFN-dependent (Figure 1). In brief, Janus kinases Jak1 and Tyk2 phosphorylate STAT1 and STAT2 proteins, which multimerize with IRF9 to form ISGF3. The ISGF3 complex translocates to the nucleus and binds to target sequences with the IFN-stimulated

response element present within the promoter regions of ISGs. Different patterns of activation of all seven STATs have been found in cells, which either homo- or hetero-dimerize, resulting in divergent pathways leading to transcriptional activation of ISGs. Other alternative pathways for IFN–λ converge in the downstream activation of ISG sets that contain both distinct and common ISGs to those induced by the type I IFNs, with IFN–λ having a narrower spectrum of mucosa-specific activities [26]. Also, type I IFNs and type II IFN overlap in their activation of ISGs. Microarray studies have shown that IFN–β drives the expression of a larger set of ISGs than IFN–γ [27]. However, a confounding factor is that certain IRFs can also induce ISGs in the absence of STAT signaling [28]. Differences in STAT activation, including tyrosine or serine phosphorylation, have been shown for individual IFN subtypes acting on a single cell type [29]. STAT phosphorylation also regulates the IFN response, mediated by reversible lysine methylation of promoter–bound transcription factors, modulating the timing and duration of ISG transcription [30]. A smaller set of ISGs are expressed in response to unphosphorylated ISGF3. As STAT phosphorylation is often critical to antiviral activity, viruses that can manipulate more unphosphorylated ISGF3 in infected cells likely gain a survival advantage.

## IFN mechanisms of action ISGs & biological effects

The IFN subtypes are not all equivalent (with examples validated in animal models (Box 1)) and collectively give rise to a myriad of functions mediated through their activation of ISG sets [31,32]. Besides antiviral actions, there are many examples of ISGs with well-known immunomodulatory mechanisms of action [33] that affect cell proliferation, maturation, differentiation, survival and migration involved in both innate and adaptive immune responses. Specialized effects on immune cells include DC activation, NK-cell activation, T-cell proliferation and survival, B-cell antibody class switching and memory T-cell maintenance (Figure 2) [34]. Select IFN–α subtypes stimulate IgG2a production in the mouse (Th1-like response) and increase survival of effector and memory T cells. Such an IFN subtype-specific drive towards Th1 polarization is likely advantageous for the host via efficient antiviral immunity. In addition, understanding the basic mechanisms of action of ISG sets can lead to better therapeutic uses of IFNs.

### Regulation of IFNs

Differences in production of IFN subtypes may be attributed to variations in sequence clustering of their promoters modulating timing of protein translation [2]. Other regulatory factors influence IFN pathways (Box 2) [22,35,36], impacting on disease outcome and validation of IFN as a biomarker. IFN expression is tightly controlled and relatively short-lived, lasting perhaps only a few days or less at most for acute lytic virus infections before returning to baseline levels. By contrast, chronic viral infections can stimulate prolonged IFN production, with more subtypes being induced, resulting in ISG sets with a longer duration of expression for many days with a disturbed baseline. As disease progresses from early through to late stage, different biomarkers may be appropriate for predictive and prognostic value, with HIV-1 infection and progression of AIDS as an example. Thus, different IFN biomarkers may be appropriate for predictive and prognostic value according to acute versus chronic infection. Metaphorically, the suite of IFN types/subtypes expressed by individuals experiencing an invading pathogen represents the shield in a coat-of–arms (Figure 3). Family heraldry may reveal differences, as specific IFN responses in each person may indeed display variations determined by the infecting virus, target cell and other factors including genetics.

A confounding factor is that some viruses evade the IFN response, encoding proteins that block IFN pathways and subsequent expression of ISGs (the examples of influenza and herpes simplex virus [HSV] are discussed later). Importantly, dysregulation of IFN production and signaling contributes to the

pathogenesis of viral infections and chronic inflammation, as has been reported for AIDS with downregulation of IFN–α receptors [37]. Host negative regulators of IFN, including SOCS, which are themselves ISGs, can also inhibit IFN signaling to promote homeostasis and return the cell to baseline [38]. Thus, measurable IFN subtype sets may either reflect immune activation or represent IFN subtypes after certain pathways have been downregulated. Understanding virus intervention and host interplay of the IFN system will assist in identifying biomarkers of infection and disease [39]. Accumulating databases of ISGs, such as the Interferome (Box 3) [5], may establish a biomarker database, comprising an IFN response signature for viral infections and other diseases.

## Clinical use

The successful use of IFN in clinical settings highlights the therapeutic importance of IFN and the availability for its measurement as a biomarker during treatment. Clinical treatment often utilizes IFN–αs derived from either human leukocytes or cultured B lymphoblastoid cells, which gives rise to a mixture of distinct subtypes that contain predominantly IFN–α2 [40]. Moreover, recombinant IFN–α2 treatments use different versions of this single subtype sourced from bacterial preparations. IFN is usually injected intramuscularly or subcutaneously and dose-related side effects are often observed with patients producing neutralizing antibodies to recombinant IFN, which limits the efficacy of treatment. Patients have been treated with IFN–α for several infections and diseases including rhinoviruses, HCV, papilloma viruses, HIV in early AIDS, Kaposi's sarcoma, hairy cell leukemia, chronic myeloid leukemia, renal cell carcinoma and multiple sclerosis (MS) [41]. Despite approved clinical treatment with type I IFNs, the mode of action has been unclear and different subtypes have not always been investigated or monitored as a biomarker.

## Animal models

Significantly, the knowledge of genetics greatly facilitated the generation of genetically modified mouse models, which could be used to identify cause and effect in different models of human disease, and thus the utility of IFNs as biomarkers. Investigations in animal models provide the perfect complementary approach to validation of biomarkers in human studies, where only correlations are readily determined, or in clinical trials, wherein efficacy and safety are generally established. Indeed, animal models have provided valuable insights into the roles of IFN subtypes and their ISG sets as biomarkers of virus infection and disease.

Comparative studies of wild-type mice with IFNAR1-knockout mice have highlighted the importance of this IFN receptor chain in the host response to virus infection [42–44], chronic inflammatory diseases, cancers and bacterial infections. Furthermore, comparative studies of wild-type mice with IFN-β-knockout mice have highlighted the importance of this IFN subtype in the host response to viral (e.g., Coxsackievirus B3 and influenza A virus [45,46]) and bacterial infection [47] and autoimmune disease [48]. Some viruses have been shown to suppress IFNs leading to a smaller set of ISGs, whilst others activate expression of specific ISGs for their benefit, including cell survival and anti-apoptotic activities allowing virus replication, persistence or even latency. Recently, the innate immune interactome has been characterized as early as 2 h post-viral infection, revealing numerous pathways leading to type I IFN production associated with possible biomarkers for disease outcome, and linked to early chemoattraction pathways [49].

### IFNs & influenza virus

Influenza viruses are segmented RNA viruses that infect humans and many animal species, associated with aerosol droplet transmission via the respiratory tract. Virulent influenza viruses can cause severe

pneumonia and death. Often, secondary bacterial infection of the lungs exacerbates asthma, chronic bronchitis, diabetes and heart disease, with kidney failure commonly being fatal in the elderly. As influenza viruses mutate rapidly, seasonal influenza virus vaccines need to be reviewed every year for improved efficacy of appropriate strain coverage. Currently, H1N1 and H3N2 are the major influenza A viruses circulating in the human population. Furthermore, high-pathogenicity avian influenza H5N1 viruses have infected humans with an average mortality rate of 60% worldwide. New reassortant viruses have recently been constructed from the contemporary H1N1 swine flu and highly pathogenic avian influenza (HPAI) H5N1 virus isolates by reverse genetics. Of the hundreds of viruses screened, one reassortant, which had the *PB2* gene from H1N1 in a constellation of other H5N1 genes, was shown to be more virulent than HPAI H5N1, with a probable increased ease of transmission from human to human [50]. Furthermore, as few as five amino acid changes in the hemagglutinin protein are likely to render the current HPAI H5N1 virus strain highly transmissible for humans [51]. Therefore, the serious threat exists for these viruses to reassort or mutate in the field allowing novel virus strains to emerge with extreme virulence and transmission of pandemic potential. Indeed, four influenza pandemics have occurred since the start of the 20th century. Current measures for controlling a devastating pandemic are limited. Strategies to improve influenza virus vaccines to offer heterosubtypic immunity with protection across many different virus strains are urgently warranted. IFNs represent a broad-spectrum antiviral control independent of virus strains and can potentially provide heterosubtypic immunity against influenza viruses [52]. Comparative studies of wild-type mice with IFN receptor-knockout mice have revealed the importance of IFN in the host response to influenza virus infection. Mice deficient in IFN signaling pathways have difficulties in resolving influenza viral disease with exacerbated pulmonary pathology [53]. Induction of IL-10 and IL-15 was shown to be type I IFN-dependent and correlated with protection. Both mice depleted of the IFNLR have been compared with wild-type mice, as well as double-knockout mice with depletion of both IFNAR1 and IFNLR. Increased pathogenicity of influenza virus in mice lacking both IFNAR and IFNLR was found compared with mice with single receptor defects [53]. An important antiviral role for the ISG expression of *MxA* has been elucidated requiring type I and type III IFN signaling pathways for induction [54]. Furthermore, IFN–β-knockout mice have also provided important information on the role of IFN–β and pDC function in respiratory infection with influenza virus. IFN–β treatment restores the balance of pDCs in the draining lymph nodes, which otherwise have a defect in antigen presentation [48,55].

TLR sensing of influenza virus occurs in pDCs, contributing to antiviral protective mechanisms via enhancement of type I IFN and type III IFN production. Stimulation of TLR2 and TLR4 with agonists, including lipopolysaccharide, provided the best protection against influenza viruses in animal models compared with other TLR3 and TLR7 agonists [56]. Early control of HPAI H5N1 virus is mediated by the type I IFN response in mice [57]. By day 1 post-infection, IFN–α and –β are produced by alveolar macrophages in the infected lung tissue, and then later by pDCs as they traffic to the lung, as measured by bioassay [58]. Thus, IFN can be used as a prognostic biomarker for acute influenza virus infection.

Furthermore, type III IFN has been found to be important for controlling influenza. Influenza virus causes pneumonia with infection of differentiated alveolar type II epithelial cells, but not infection of alveolar macrophages, and increased mRNA and protein levels for IFN–λ1 were found in virus-infected alveolar epithelial cells [59]. Treatment of mice with IFN–λ1 before virus infection significantly reduced virus replication with induction of antiviral ISGs *MX-1, 2–5OAS* and *ISG56* but not *IFN–β* [60]. Therefore, protection afforded by IFN–λ1 in control of influenza virus in these alveolar epithelial cells is independent of IFN–β-mediated mechanisms. NF-kB has been shown to negatively regulate ISG

expression and anti-influenza virus activity *in vitro*, using gene expression profiling of NF-kB-knockout cells, involving IRF1 and STAT1 binding to ISG promoters [61].

Influenza viruses have developed evasion tactics for the innate immune response, including inhibition of the IFN–β pathway using IFN antagonist NS1, an abundant nonstructural viral protein, via multiple actions [62]. The viral NS1 protein counteracts the host IFN–α/b response by blocking both IRF3 and post-transcriptional processing of cellular transcripts in a virus strain-specific manner dependent on NS1 cellular localization. This evasion strategy employed by virulent virus strains underscores the importance of the IFN system in the protective host response against influenza virus. Influenza A/PR/8/34 H1N1, a laboratory–adapted human virus strain, has a strong ability to block IRF3 and the IFN–β promoter, but does not inhibit expression of other cellular genes. However, the NS1 from reconstructed strains of influenza H1N1 that caused the Spanish flu pandemic, which killed approximately 50 million people, inhibited expression of additional ISGs and allowed virus replication in the presence of IFN. IFN–α treatment has been shown to restore inhibitory effects of NS1 from both H5N1 and H1N1 virus infections [63]. Recent focus has been directed towards the development of NS1-truncated A/Viet Nam/1203/04 strains of influenza H5N1, made by reverse genetics, to be used as live–attenuated vaccines against H5N1 highly pathogenic avian influenza [64]. Experimental vaccine candidates with *NS1*, *HA* and *PB2* viral gene mutations, tested in animal models of influenza virus vaccine/challenge, have elicited protection with production of high levels of IFN–β, demonstrating the suitability of IFN–β as a predictive biomarker.

IFN–α treatment has been shown to be important in protection against 1918 pandemic influenza virus in an animal model [58]. Intranasal administration of human IFN–α reduced lung tissue virus titers and shedding from the nasopharynx of animals challenged 1 day later with the virus. Expression of Mx protein in lung tissue correlated with protection, highlighting the relevance of IFN–α as an effective biomarker. Recently, MxA has been shown to inhibit ribonucleoprotein formation through GTPase activity during the virus replication cycle [65]. Thus, measurements of the ISG *MxA* in serum from blood samples can be a useful biomarker for survival against influenza.

In summary, influenza viruses use mechanisms to evade IFN responses and both type I and type III IFN can inhibit acute influenza virus replication, elucidating the role of IFNs as important biomarkers for monitoring disease and prognoses. Mouse models have demonstrated that TLR agonists can provide protective immunity; however, the mechanism for protection is unclear. A better understanding of the IFN response pathways and their ISG signatures in infections with influenza virus strains of different virulence will increase our knowledge for effective vaccines and therapies against lethality associated with pathogenic influenza virus strains.

### IFNs & HSVs

HSV-1 and HSV-2 are two closely related human DNA viruses and represent significant overlapping clinical infections, including genital infections, encephalitis, systemic infection and various skin manifestations. The virus infects the host directly through the mucosal membranes, where it can establish a primary lytic infection of epithelial cells and is able to spread via retrograde transport to the neurons of the sensory ganglion in the peripheral nervous system and become latent. Here, the virus can evade immune detection and alter the expression of lytic genes such that it establishes a persistent infection capable of spontaneous reactivation or following environmental cues. No successful vaccine against HSV infection has yet been uncovered, despite extensive studies on the role of the innate and adaptive host defenses. Studies from various gene knockout mice have successfully identified that the innate immune system, and type I IFNs in particular, are essential for the early

detection of virus and to provide acute antiviral protection against HSV primary infections before the development of specific effector mechanisms, as well as inhibition of virus ascending to the spinal cord and brain from the vagina, causing encephalitis.

To further complicate the development of successful treatment strategies, different strains of HSV have been shown to induce IFNs to varying extents, through different pathways [66] and different cell types [67]. HSV infection is detected by extracellular TLR and RIG-I [68], or MDA5 cytoplasmic sensing of viral DNA [69], resulting in IFN production and expression of proinflammatory cytokines, which leads to control of viral entry and replication in host cells. In humans, the TLR3 pathway for IFN production is critical for controlling primary HSV-1 replication in the CNS [70]. In mice, studies have shown that TLR2 and TLR9 recognize HSV-1 and HSV-2 in conventional DCs [71]. pDCs are rapidly recruited to the vagina within 24 h of HSV-2 infection, where they produce high levels of IFN–α in response to TLR9 recognition of CpG, which is found abundantly in HSV-2 genomes [72]. pDCs deficient in TLR9 are unable to produce IFN–α upon HSV-2 stimulation [72,73] and, *in vivo*, TLR9-/- mice infected with HSV-2 have no detectable circulating IFN–α resulting in the rapid pathology and reduced survival of these mice [74,75]. Consistent with the importance of type I IFNs in the anti-HSV response, IFNAR1-/- mice have increased susceptibility to HSV-2 infection, with elevated viral replication [76]. Similarly, STAT1-/- mice demonstrate increased HSV-1 replication in the cornea [77]. Thus, impairment in either IFN production or IFNAR signaling greatly impacts both the quality and quantity of the antiviral response and thus disease progression.

Mouse studies have shown that IFN–α1 and IFN–β were both effective in preventing mucosal HSV-1 infection, and that IFN–α2, –α4, –α5, –α6 and –α9 were ineffective [78–80]. Furthermore, IFN–β was found to be superior to IFN–α6 in preventing ocular HSV-1 infection in mice, via upregulation of ISG transcripts, *2–5OAS* in trigeminal ganglia and increased phosphorylation of STAT1 [81]. *In vitro*, IFN–β was also superior to the IFN–αs against HSV-1 infection with greater reduction of viral gene expression [79]. The downstream mechanisms of IFN regulation of HSV replication are not fully understood, although several IFN-induced pathways have been investigated. The RNase L antiviral pathway is induced by IFN–β, rather than IFN–αs, to HSV-1 in ganglia cell cultures. The anti-HSV-1 response is characterized by the induction of ISGs including *2–5OAS* and *PKR* in the ganglia of infected mice [82,83]. Disruption of the RNase L gene has previously been found to increase the susceptibility of mice to HSV-1 keratitis and viral-mediated mortality [84]. Furthermore, the absence of *PKR* increases HSV-1 replication both *in vitro* [85] and *in vivo* [86]. Interestingly, *PKR* is also the main antiviral pathway to HSV-2 activated by IFN–α1 [87]. While IFNs are effective at blocking HSV replication at the transcriptional level, HSV-1 encodes for a number of proteins that counter the activation or activity of ISGs. These include HSV late protein g134.5 that counteracts the activity of *PKR* [86,88] and ICP0 inhibition of IFN–β induction via IRF3-dependent pathways [89]. Also, ICP27 induces the secretion of a type I IFN antagonist that inhibits STAT1 phosphorylation and nuclear localization [90]. However, IFN–β, more so than IFN–α, has been reported to reduce levels of *ICP27* transcription as well as *thymidine kinase* and *glycoprotein B*, as determined by real-time PCR [78].

The ubiquitin-like molecule ISG15 is rapidly upregulated following IFN–α treatment or viral infection, and indeed mice lacking ISG15 are more susceptible to HSV-1 infection [91]. Proteomic studies have identified >300 target proteins to which ISG15 can bind [92], thus evoking potent antiviral actions impacting on multiple cellular pathways. Significantly, other viruses have evolved mechanisms to interfere with ISG15 functions in cells including the NS1 protein of influenza that blocks ISG15 conjugation [91], SARS coronavirus that encodes an ISG15 deconjugating enzyme [93] and vaccinia E3 protein, which binds to ISG15, thus blocking antiviral activity [94]. Thus, the induction of specific IFN-induced ISGylation signatures for specific antiviral responses highlight the significance of the

downstream IFN-inducible pathways, the ISGs, as measures of disease prognosis and biomarkers for viral infections.

Although HSV is in possession of several mechanisms to evade IFN responses, type I IFNs can block HSV replication at an early stage and are thus important biomarkers for monitoring disease and prognoses. Mouse models have demonstrated that TLR agonists can provide protective immunity in vaccination against HSV infection [95,96] through induction of IFN signaling pathways [76]. With vaginal administration of the TLR9 ligand, CpG oligodeoxynucleotide, mice were protected from a HSV-2 vaginal infection [75,97]; however, the mechanism for protection was unclear. Although not discussed here, type II IFN responses are crucial for the development of Th1 immunity to HSV-2 infections [76]. Elucidating the virus-specific pathways that mediate the anti-HSV mechanisms of IFN, as well as the induction of a specific IFN gene signature to define an appropriate antiviral response in HSV infection, may facilitate the design of tailored therapeutics of prognostic potential.

## IFNs & cytomegalovirus

Cytomegalovirus (CMV) is a herpesvirus causing serious disease in the immunocompromised and immunosuppressed, such as neonates and patients with AIDS. Inflammatory diseases associated with CMV infection includes hepatitis, pneumonia, encephalitis, retinitis and myocarditis, as the virus is capable of infecting a wide variety of tissues. The spleen and liver are major targets of acute virus replication, which is then followed by virus shedding in the salivary glands. The virus persists in a latent state for a lifetime and, in the immunocompetent individual, this is generally asymptomatic. However, the virus can reactivate from latency and cause pathology. CMVs have evolved to adapt to their hosts and are species-specific. Murine CMV (MCMV), a natural pathogen of mice, provides an excellent model with highly sensitive assays for viral tropism and pathology [98]. Mice lacking type I IFN receptors are significantly more susceptible to MCMV. In addition, type III IFN (IFN- 28A) has recently been shown to contribute to anti-MCMV responses in intestinal epithelial cells [99]. Both IFNs and DCs sculpt adaptive immune responses to CMV [100,101]. As pDCs produce the highest levels of type I and type III IFNs, their effective stimulation of CD4+ T-cell responses is essential for control of MCMV infection with impact on CD8+ T-cell memory [102,103]. On the other hand, CMV encodes evasion proteins, which inhibit IFN responses, and the virus has a predilection for infecting DCs. The CMV-encoded IE1 protein interacts with STAT2, downregulating transcription of ISGs such as *Mx-1* and thus manipulating the host IFN immune response [104,105].

Animal models allow investigation of the IFN response leading from virus infection to chronic inflammatory disease. Myocarditis is an inflammatory disease of heart muscle with no cure, which can lead to necrosis of myocytes and fibrosis, dilated cardiomyopathy, heart failure and death [106,107]. Pathological studies of endomyocardial biopsies have identified persistent viral infection in 66% of samples obtained from patients with 'idiopathic' dilated cardiomyopathy [108], with active myocarditis found in 9% of cases of sudden cardiac death [109] and 10% of cases of unexplained heart failure [110]. Viral myocarditis can present with fulminant and immediate life-threatening cardiac failure, which may be transient and responsive to intensive care management, although selected patient groups (most notably newborns) may have high mortality rates of up to 70%. Chronic myocardial injury beyond the acute illness can have long-term health implications, and 10-year survival rates (to transplantation or death) are in the order of 50% [111]. Indeed, cardiomyopathy that is not associated with ischemic or congenital heart disease currently accounts for 53% of all heart transplants worldwide [112].

CMV infection can induce autoimmune myocarditis [113]. Animal models have provided insights for the progression from infection to autoimmune disease. The innate immune response to viral and

self–antigens has been shown to be important in the determination of resolution of infection or progression to chronic autoimmune disease. Cardiac injury can be associated with direct viral cytopathic effects, as well as ongoing innate and adaptive immune responses that may be directed against viral or host antigenic targets [114]. BALB/c mice develop acute and chronic myocarditis, whereas C57BL/6 mice have mild disease during the first week post-infection, but do not develop chronic myocarditis [115]. Early cytokines and innate immune cells play a role in viral myocarditis. Increased TNF–α, IL-6 and IL-10 levels found in the hearts of MCMV-infected BALB/ mice suggest cytokine dysregulation in disease [116–118]. MCMV induces T-helper cells specific for peptides of cardiac myosin α heavy chain and elicits epitope-spreading autoantibodies with specific skewing towards this protein determinant [119]. Although both susceptible and resistant mouse strains develop a wide range of IgM and IgG autoantibodies to cardiac myosin, only the susceptible strain produces higher titer IgG1 autoantibodies specific for the cardiac isoform of the myosin heavy chain [120].

IFN–α and IFN–β are critically important to the development of inflammation in the heart. Susceptible BALB/c mice show a Th2 bias, whereas resistant C57BL/6 mice show a Th1 bias in anti-MCMV responses. Myocarditis can be modified by specific IFN subtypes to redirect immunity away from an autoreactive Th2 and towards a protective Th1 phenotype. IFN–α6, –α9 and –β subtypes provide protection from the development of acute and chronic heart disease [121,122]. However, identical treatment with IFN–α1, –α2, –α4 and –α5 did not protect against disease. Efficacious IFN treatment was associated with antiviral and immunomodulatory properties, cytokine expression, antibody class switching to IgG2a and homing of CD8+ T cells to the heart [116,121,122]. Moreover, IFN–α9 and IFN–β transgenes co-delivered with viral gB DNA vaccines to mice reduced virus replication in target organs and acute myocarditis [123]. IFN–α6 or IFN–α9 treatment alone prior to MCMV infection reduced CD8+ T-cell infiltrates in the heart during the acute phase, whereas IFN–β treatment reduced CD8+ T-cell numbers in the cardiac infiltrate, without affecting virus replication, and reduced chronic myocarditis. IFN–α6 therapy was associated with an increased expression of IL-18 in the heart and higher levels of serum IL-6, with lower levels of IL-10 compared with untreated control mice [116]. Furthermore, the mouse heart appears to be a target for type III IFN treatment, as coxsackievirus B3-induced myocarditis is amenable to IFN–λ2 therapy [124]. IFN response signatures, both subtypes and ISGs, need to be further studied for application as predictive and prognostic biomarkers for CMV infection and disease.

## IFNs in MS

MS is a chronic inflammatory disease of the CNS influenced by environmental, genetic and infectious factors. Genome-wide association studies using large cohorts of MS patients have found single nucleotide polymorphisms in several genes related to IFN pathways, such as *HLA-DRB1, TNFRSF1A, IRF8, TYK2, SOCS1, IL28RA* and *STAT3* [125]. However, epistasis and epigenetic factors are also important. Inflammatory lesions comprise a mixed cellular infiltrate with T cells, macrophages and B cells associated with pro-inflammatory cytokines. Although the etiological agent for MS is unknown, Epstein– Barr virus (EBV) infection, in concert with a genetic predisposition, may be a risk factor for disease development and/or progression, especially in adolescence–acquired females who have experienced symptomatic glandular fever [126]. EBNA1-specific T cells from MS patients also cross react with myelin and coproduce IFN–γ and IL-2. Furthermore, variations in the type I IFN response may influence whether the individual presents with active or inactive disease. IFN–β has been used for clinical treatment of MS for the past 18 years but the incidence of disease has greatly increased compared with the last century. Although the disease was characterized over 150 years ago, it is

becoming more apparent that there is a spectrum of different diseases, involving lesion location (cerebral versus spinal cord), with varied responses to IFN–β treatment. IFN–β levels can be measured in order to monitor patients receiving IFN therapy. Although the response to IFN–β therapy has been correlated to the MxA response, a better understanding of the action of IFN–β in MS is warranted for advancing medical needs in this field.

In an animal model, *IFNB* gene deletion augmented experimental autoimmune encephalomyelitis (EAE) induced by MBP injection of mice with more severe and chronic neurological symptoms, CNS inflammation and demyelination [127]. Furthermore, myeloid cells were required to be IFN-stimulated through IFNAR signaling in order to modulate EAE disease severity, indicating a distinct protective function of type I IFNs in the CNS [128]. In another mouse model of MS using the MOG peptide, *IFNB*-knockout female mice display more aggressive disease than male littermates, with edema in brains. The study showed that IFN–β constrains Th17 cell generation and restricts T-cell and DC trafficking into the CNS [48]. IFN–β impairs the egress of lymphocytes from lymph nodes via upregulation of CD69, which interacts with S1P1 receptor, resulting in fewer T cells invading the brain lesions [129]. IFN–β also mediates cleavage of VCAM on endothelial cells of the blood–brain barrier, inhibiting egress of T cells into the CNS. However, if the T cells have already arrived in the brain, then IFN–β can act on IFNAR–bearing macrophages and microglia by downregulating MHC class II and enhancing *PDL-2* expression, leading to reduced re-stimulation of infiltrating T cells and less pathological inflammation. Furthermore, IFN–β modulates the cytokine balance for T-helper cell differentiation through IL-12 (Th1) and IL-1b/IL-23 (Th17) by increasing IL-10 and STAT3 expression and promoting *SOCS3* transcription. IFN–β signaling also upregulates TLR3 production, since TLR3 is an ISG, allowing IRF3 and IRF7 to induce higher expression of IFN–β itself in a positive feedback loop. IFN–β activation of TLR7, via MyD88, can reduce IL-1R expression and IL-23, with increased production of IL-27, which inhibits Th17 cell differentiation, inducing IL-10 but suppressing IL-17 expression. Upregulation of *SOCS1* transcripts were found in the EAE mouse model [130] and *SOCS1*-transgenic mice develop EAE will accelerated onset [131]. SOCS1- knockout mice are resistant to development of EAE [132], whilst a *SOCS1* mimetic suppresses acute EAE and protects against relapsing–remitting EAE [133]. Taken together, IFN–β and MxA are useful biomarkers of MS. Profiles of the other IFN subtypes and ISGs, such as *SOCS*, need further elucidation for utility as biomarkers of disease prognoses, especially in relation to different MS phenotypes.

## IFNs in systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a complex autoimmune disease impacted by genetic, hormonal and environmental factors. The exact etiology of SLE is unknown, and mouse models with the development of lupus-like phenotypes have been crucial to uncovering cellular and genetic mechanisms of disease. Spontaneous (NZB/W F1, MRL/lps, BXSB/ Yaa) and induced (pristine and chronic graft-versus- host disease) mouse models of lupus have led to the identification of numerous susceptibility loci and provided identification of potential biomarkers for disease and prognosis [134]. Indeed, extensive animal and human studies have revealed a crucial role for type I IFN in the initiation and perpetuation of disease, and components of the IFN system, including transcription factors, are now putative drug targets and biomarkers in the treatment of SLE. Indeed type I IFN plays a critical role in the immunopathogenesis of SLE, since IFNAR-deleted mice fail to develop disease [135,136].

Large-scale genome-wide association studies from families with SLE and across different genetic populations have identified approximately 30 susceptibility genes for lupus, with many of these single nucleotide polymorphisms and risk alleles in type I IFN genes. In one study, two polymorphisms in particular, in *TYK2* and *IRF5*, crucial components of the type I IFN receptor signaling and IFN induction

pathways, respectively, had the strongest association with a protective affect against SLE [137]. Further studies by the same group have also mapped SNPs in *STAT4* that correlate with production of autoimmune anti-dsDNA antibodies and have an additive effect in SLE when present with two other risk alleles in *IRF5* [138]. Other risk factors included alleles in *IRF8, IFIH1, ITGAM, BLK, BANK1, PDCD1, TNFSF4, TNRAIP2* and *SPP1* [139–142]. These studies clearly exemplify the use of IFNs as biomarkers in lupus, and similar studies in other autoimmune diseases and infections may elucidate an IFN-dependent biomarker signature.

## IFN subtype therapy in animal models

Animal models have been explored for efficacy of IFN subtype treatment. Using an *in vivo* mouse model of transgene delivery of individual IFN–α subtypes, differential activities of IFN–α subtypes against several viruses were investigated. Pretreatment of mice with IFN–α5 and IFN–α6 was found to be more effective than other α-subtypes in reducing pulmonary virus titer of influenza virus A/PR/8/34, with IFNa1 being the least effective [143]. Furthermore, bodyweight loss, clinical score and pulmonary tissue damage was minimal in virus-infected mice that were pretreated with the effective IFN–α subtypes.

The capacity of individual type I IFN subtypes to enhance the efficacy of DNA vaccines encoding HA, NP and M proteins of influenza A/PR/8/34 has also been studied [143]. Lung viral titers were reduced with transgene treatment for IFN–α6 and IFN–α9 codelivered with *HA* DNA vaccine compared with mice vaccinated with HA DNA alone. Mice vaccinated with *HA/IFNA6* DNA showed the lowest clinical score, reduced body weight loss and minimal peak pulmonary tissue damage compared with HA-vaccinated mice. These findings suggest different potencies of the IFN subtypes in providing protective immunity afforded by DNA vaccines. Select IFN subtypes may be effective adjuvants for viral DNA vaccines and provide an alternative to prime–boost regimes. Thus, one standard IFN treatment using a single subtype for various viral infections is unlikely to be efficacious, and the entire subtype set should be analyzed for clinical potency [144].

In another mouse model of influenza, IFN–β protein therapy given 12 h prior to influenza A/PR/8/34 virus infection also reduced lung virus titers [55]. In addition, IFN given 14 h after influenza A/PR/8/34 infection by the intranasal route was shown to be protective and reduced lung virus titers. Single-dose prophylactic IFN–β treatment was reported to induce a Th1 response in the lungs and draining lymph nodes of the animals. Both CD4+ and CD8+ IFN–γ-expressing T cells increased in numbers along with upregulation of IL-18, IL-12R, CLL5, S1P4 and CD69 expression. Whilst the early response to influenza infection is a Th1 response, the immune response then changes to Th2 polarization at day 8 post-infection associated with eosinophilia. A better understanding of the role of IFN–α subtypes in driving a Th1 response may enable a better choice IFN subtype therapy for enhancing antiviral immunity in other models. Nonetheless, IFN prophylactic therapy may prove to be efficacious during an emerging influenza virus pandemic.

## IFNs in human therapy

As individuals acquire more microbial infections over a lifetime, constantly transforming the metagenome, there is a need to redefine health and disease status and, in particular, chronic virus infections [145]. Current knowledge of the roles of individual IFN subtypes in health and disease has not been forthcoming from clinical investigations. Both viral and host genome studies and transcriptome analyses have the potential to drive identification of clinically relevant IFN biomarkers [146]. Too often, clinical trials have not measured IFN subtypes and/or explored only limited subtypes for disease, some with poor outcomes. We will briefly discuss clinical IFN therapy for HCV and MS.

Genome-wide association clinical studies have recently revealed that variations near the *IL28B* gene, which encodes IFN–λ3, are associated with HCV infection outcome [147–149] and response to standard IFN–α and ribavirin treatment [150,151]. The protective role of this cytokine in viral pathogenesis has promising clinical impact and has led to a plethora of research reports on its mechanism of action (reviewed in [152]). Thus, expression of IFN–λ may be a marker of prognosis. Non-responders to HCV treatment have high ISGs in the liver suggesting that these are negative predictive markers for therapy [153]. HCV triggers expression of ISGs, especially *ISG15*, in a manner that is unregulated by IFN pathways. Recent IFN–λ (IL-29) innovative therapy for HCV appears promising [154]. Specific activities of recombinant IFNs in comparative studies with natural IFNs have been reviewed [155]. Both recombinant IFN–αs from natural and synthetic genes were found to bind to a common cell surface receptor and induce antiviral activity in a variety of cell lines. Similar receptor binding activities were demonstrated for consensus IFN (IFN-con1), a synthetic IFN, and IFN–α2b. However, discrete biological differences among the IFN–αs were noted. Using 12 recombinant human IFN subtypes, IFN–α17 displayed the highest HCV activity likely due to stronger activation of the Jak/STAT pathway through, in part, increased affinity for the IFNAR2 [156].

Antiviral therapies have been effective in some patients with autoimmune MS, possibly targeting epigenomic replication of EBV in B cells [157]. Production of type I IFN is found as early as 16 h post-infection of B cells. Although EBV also infects monocytes and pDC, the pDCs do not produce type I IFN due to viral evasion of the TLR induction of IFN. However, both pDCs and B cells can express latent viral genes. EBV-infected pDCs lack maturation markers CD80 and CD86 and fail to prime naive T cells, resulting in impaired antigen presentation [158]. Patients with relapsing–remitting MS show constitutive *Mx-1* expression and higher levels of the viral latency genes *EBNA-1* and *LMP2a*, compared with healthy donors. Treatment with IFN–β has been shown to reduce virus expression of the latent proteins EBNA-1 and LMP2A. IFN therapy decreases the percentage of circulating memory B cells, likely by inducing FAS-mediated apoptosis [159]. Further investigations into IFN subtypes and ISG sets may enable identification of IFN response signatures for patients with different stages of MS and tailor targeted therapy. Other forms of treatment undergoing evaluation in clinical trials, including rituximab, an anti-CD20 antibody for depletion of B cells [160], should be considered for impact on IFN responses. Measurements of IFN–β potentially provide a predictive biomarker for patients on therapy and a prognostic marker for MS patients who are not IFN-treated. However, *in situ* versus plasma/serum sources of measurable IFN–β has given disparate results. Measuring IFN–β in serum by ELISA has been shown to be more sensitive than cell bioassays based on biological activity [161]. IFN–binding factors, such as soluble IFNAR2 or neutralizing antibodies in the serum, may limit activity and impact on biomarker levels. Thus, circulating available IFN may be under-represented using some assays due to these confounding factors.

## Conclusion & future perspective

The type I and type III IFN families represent numerous subtypes, which can be induced and/ or suppressed by invading pathogens. Individual IFN subtypes display differential downstream effects on the immune system via transcriptional activation of ISG sets. Regulation of IFN signaling pathways influences disease progression. Animal models have provided valuable lessons for understanding disease pathogenesis and the validity of measuring IFN responses to infectious and autoimmune disease. Experimental studies of influenza, HSV and CMV have provided valuable insights for the use of IFN subtypes and their ISG sets as potential biomarkers of prognostic and predictive value. Appropriate levels of IFN corresponding to specific type of infection, stage of disease, sample source, treatment regime and genetic background, need to be evaluated in order to stratify individuals at risk.

Searching existing databases, such as the Interferome, may lead to the identification of IFN-specific signatures as biomarkers of infection and disease.

Further clinical investigations are required for identification of reliable and robust IFN response biomarkers for human disease. Current clinical treatments with exogenous IFN use only a few IFN subtypes, limiting the therapeutic value of the IFN family. The whole panel of IFN–$\alpha$ subtypes could be explored in human studies for exploitation of the most efficacious subtype. As some patients do not tolerate type I IFN therapy, the type III IFNs may represent an alternative option. Furthermore, the baseline immune status of the patient may impact on the response to IFN treatment, which in itself may present an opportunity to treat with a specificIFN subtype. For example, IL-17 influences the response to IFN–$\beta$ therapy in patients with MS [162]. Emerging technologies in transcriptome and genome-wide analyses are predicted to shed light on IFN response signatures as biomarkers to aid clinical diagnosis and prognosis. To conclude, a renaissance of IFN subtypes/ISGs as biomarkers and their use in clinical therapy is emerging with promises to increase our knowledge in the treatment of progressive stages of infectious and other diseases.

## Executive summary

**Interferon mechanisms of action**

- Interferons (IFNs) are potent cytokines of the immune system.
- IFN subtypes are induced by pathogens.
- Select type I IFN subtypes and IFN-stimulated genes are IFN response signatures that can be used as biomarkers of disease outcome.

**Animal models**

- Animal models allow detailed investigations of IFN response signatures for use as predictive and prognostic biomarkers.
- Confounding factors for IFN biomarkers include host regulation and virus evasion strategies.
- Viral and autoimmune diseases have been explored in experimental models.

**Future perspective**

- IFN biomarkers can tailor improved therapies and vaccine development.

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**Box 1. Interferon subtypes are not all equivalent.**

∗ In a cytomegalovirus mouse model, IFN-a6, IFN-a9 and IFN-b, but not IFN-a1, IFN-a2, IFN-a4 or IFN-a5, were protective and reduced myocarditis [116,121,122]

∗ In the influenza virus mouse model, IFN-a5 and IFN-a6 showed protection against virus infection in the lung, with IFN-a1 being the least effective [143]

∗ In a herpes simplex virus-1 retinitis mouse model, IFN-b, but not IFN-a1, IFN-a2, IFN-a4, IFN-a5, IFN-a6 or IFN-a9, was protective and reduced disease, dependent on 2–5OAS/RNase L pathways [81]

∗ In a herpes simplex virus-2 vaginitis mouse model, IFN-a1 and IFN-b, but not the other above-mentioned IFN subtypes, were protective when applied to the mucosal surface of the vagina, dependent on CD8+ T-cell responses [80]

∗ Select type I IFNs were antiproliferative and protected mice against fatal erythroleukemia via different signaling events [29]

∗ IFN-a1, IFN-a4 and IFN-a9 were protective against the development of Friend retrovirus leukemia in mice and activated both natural killer and CD8+ T cells [163]

∗ Select IFN subtypes enhance HIV-1 vaccine efficacy [164]

**Box 2. Regulation of interferon pathways.**

* Interferon (IFN) receptor density on the cell surface and concentration of soluble IFN receptor

* IFN subtype affinity of binding to receptor subunits

* IFN receptor endocytosis during progression of disease

* Inhibition of kinase phosphorylation of STATs

* Select STAT molecule activation and multimerization

* Serine versus tyrosine phosphorylation events

* Timing and duration of IFN-stimulated gene transcription mediated by reversible lysine methylation of transcription factors

* Feedback loops with select IFN-stimulated gene proteins

**Box 3. Interferome, the interferon-stimulated gene microarray data sets.**

* Tissue expression data with positive or negative regulation of ISGs have been assembled in a collective interferome database

* Dominant ISGs for antiviral activity include *PKR*, *2–5OAS*, *ISG56*, *MxA*, *RNaseL*, *IRF7*, *MHC*, *CD80*, *CD86* and *iNOS*

* Cell types used for gene expression analysis of ISGs have included B lymphoblasts, NK cells, CD4 T cells, myeloid cells, DCs, monocytes, adipocytes and a range of carcinoma and leukemic cell lines

* Tissues used for analysis of ISG subsets include whole blood, lymph node, spleen, tonsil, thymus, thyroid, prostate, lung, heart, brain, liver, adrenal gland, testis, ovary, skeletal muscle, uterus, bone marrow, salivary gland, trachea, placenta, tongue, skin, appendix, pancreas, kidney, spinal cord and ganglion from adult and fetal sources

*DC: Dendritic cell; ISG: Interferon-stimulated gene; NK: Natural killer.*

**Table 1. Interferon characteristics.**

| | IFN-α | IFN-β | IFN-λ |
|---|---|---|---|
| Genes | 14 | 1 | 3 |
| Introns | 0 | 0 | 4 |
| Chromosome | 9 (mu 4) | 9 (mu 4) | 19 (mu 7) |
| Protein | 166aa | 166aa | 174aa |
| Molecular weight | 16-27 kDa | 28-35 kDa | 20-33 kDa |
| Cell producers | Most cell types; pDCs, fibroblasts, epithelial cells, leukocytes, macrophages | Most cell types; pDCs, fibroblasts, epithelial cells, leukocytes, macrophages | Most cell types; pDCs, fibroblasts, epithelial cells, leukocytes, macrophages |
| Cell responders | Most cell types | Most cell types | Epithelial cells, (hu) hepatocytes |
| Induction | TLRs, virus, dsRNA, LPS | TLRs, virus, dsRNA, LPS | TLRs, virus |
| Signaling | IFNAR1, IFNAR2 | IFNAR1, IFNAR2 | IFNLR1, IL-10R2 |

aa: Amino acid; hu: Human; LPS: Lipopolysaccharide; mu: Murine; pDC: Plasmacytoid dendritic cell; TLR: Toll-like receptor.

**Figure 1. Interferon signaling pathways.**

The complexity of the IFN signaling pathways goes beyond the classical Jak/STAT pathway and involves use and activation of different molecules following ligand binding to cognate receptors. Dissection of pathways has shown the use of different components in explaining how cells respond to IFNs. Different adaptors, followed by activation to varying extents of the different kinases and STATs (independent or in collaboration with each other) by either tyrosine or serine phosphorylation, then in turn activation of different transcription factors, with protein binding to DNA motifs, leads to induction of ISG sets by the type I and type III IFNs, which may differ among cell types. Potentially, exposure to different IFN subtypes (individual or in combination) adds a further layer to the complexity of these IFN signaling pathways, resulting in sets of ISGs orchestrating their biological consequences upon the cell. IFN: Interferon; ISG: Interferon-stimulated gene; ISRE: Interferon-stimulated response element. Adapted with permission from [28].

**Figure 2. Pleiotropic properties of the interferon families.**

Biological activities of the IFNs, besides the antiviral, affect various stages of the immune response. IFNs affect DC activation, NK cell activation, T-cell proliferation and survival, B-cell antibody class switching, memory T-cell survival, apoptosis and angiogenesis. These pleiotropic properties reflect the set of IFN-stimulated genes that are potentially transcribed in the thousands within the cell following IFN-initiated signal transduction. DC: Dendritic cell; IFN: Interferon; NK: Natural killer.

**Figure 3. Interferon shield of defense.**

Collectively the suite of interferons (IFNs) protect the body against invading pathogens, represented as a shield with our heraldic coat of arms, the immune system. Types of IFNs relevant to this review are shown as charges of the shield divided by the cross moline into four compartments: the 14 IFN-αs and IFN-β defining the type I IFN family, IFN-γ (type II IFN family) and the three IFN-λs (type III IFN family).