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Interferons and viruses: an evolutionary arms race of molecular interactions

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Abstract

Over half a century has passed since interferons (IFNs) were discovered and shown to inhibit virus infection in cultured cells. Since then, researchers have steadily brought to light the molecular details of IFN signaling, catalogued their pleiotropic effects on cells, and harnessed their therapeutic potential for a variety of maladies. While advances have been plentiful, several fundamental questions have yet to be answered and much complexity remains to be unraveled. We explore the current knowledge surrounding four main questions: are type I IFN subtypes differentially produced in response to distinct pathogens? How are IFN subtypes distinguished by cells? What are the mechanisms and consequences of viral antagonism? Lastly, how can the IFN response be harnessed to improve vaccine efficacy?

Keywords

interferons; interferon subtype; innate immunity; viruses; viral antagonism; vaccine adjuvants

IFNs: calibrating the immune system for antiviral defense

There are three types of IFNs, types I, II, and III; they display distinct expression patterns and have myriad roles in innate and adaptive immunity. In this review we focus primarily on the ‘anti-viral’ IFNs, types I and III. An intriguing property of type I and type III IFN signaling is that an astonishingly large number of proteins signal through the same receptor complexes (Box 1, Figure 1). Evolutionary analyses of the type I IFN genes reveals that while several display signs of relaxed evolutionary constraints suggesting that they may have a redundant role, over half the family members have been subject to purifying selection, indicating that they play important and non-redundant roles in host defense [1]. Similarly, type III IFN genes have also been influenced by strong selective pressures, with evidence that distinct pathogens may have shaped their evolution [1]. These evolutionary analyses highlight two basic and important biological questions that have both puzzled and inspired researchers for decades: why are there so many type I and III IFNs, and how are they distinguished by cells? Ongoing research continues to keep these questions at the forefront. We review the current literature and further discuss the myriad mechanisms by

which viruses antagonize the IFN system. Lastly, we consider how current knowledge in this arena may inform future vaccine development.

Box 1

IFNs: a basic introduction

IFNs are a family of cytokines that are grouped into three types: I, II, and III (see Figure 1 in main text). In humans, type I IFN includes 13 IFN- α genes, and single genes for IFN- β , IFN- ϵ , IFN- κ , and IFN- ω . All 17 type I IFNs bind to and signal through a shared heterodimeric receptor complex composed of a single chain of IFNAR1 and IFNAR2, and these are present on nearly all nucleated cells (reviewed in [181,182]). In contrast to the many type I IFNs, there is only a single type II IFN, IFN- γ , which is produced mainly by immune cells. IFN- γ signals as a homodimer through a tetrameric complex composed of two subunits of IFNGR1 and two subunits of IFNGR2. Similarly to type I IFNs, the type II IFN receptors have a broad tissue distribution and, as a result, nearly all cells are capable of responding to type II IFN. The impact of type II IFN on shaping the adaptive immune response during viral, bacterial, fungal, and parasitic infections is broad and complex, and therefore beyond the scope of this review (reviewed in [183]). The type III IFNs (also known as IFN- λ s) include IFNL1, IFNL2, IFNL3, and the most recently discovered member, IFNL4 [184–186]. Type III IFNs signal through a heterodimeric receptor complex including single chains of IL-10R2 and IFNLR1. IL-10R2 is also part of a separate cytokine receptor complex, the IL-10 family receptor, which binds the cytokines IL-10, IL-22, and IL-26. Whereas IL-10R2 is widely distributed across cell types, expression of IFNLR1 is largely restricted to epithelial cells [187]. Consequentially, many cell types respond either very poorly, or not at all, to type III IFNs. Once IFNs bind to their cognate receptor on the cell surface, a signal is propagated within the cell via the JAK–STAT signaling pathway, as outlined in Figure 2 in main text.

Pattern recognition receptors and cell type: the key to unlocking specificity in type I IFN production?

Microbes contain conserved molecular features such as flagella, peptidoglycans (PGN), lipopolysaccharides (LPS), and nucleic acid structures that are either not found or are sequestered in eukaryotic hosts. These molecular features, or ‘patterns’, therefore serve as targets for host proteins known as pattern recognition receptors (PRRs), providing cells with a means to distinguish ‘self’ from ‘non-self’. The molecular targets that are recognized by PRRs are referred to as pathogen-associated molecular patterns, or PAMPs, and a variety of PRRs exist with the ability to recognize a wide range of PAMPs from fungal, bacterial, and viral origins (reviewed in [2–4]). By recognizing distinct features unique to particular infectious agents, PRRs may provide cells with a means to not only recognize a wide variety of pathogens but, to some extent, to distinguish them as well. For the purpose of this review we focus on those PRRs that specialize in detecting viral PAMPs.

Membrane-bound PRRs with the ability to recognize viral PAMPs can be located both on the cell surface and within endosomal compartments. The expression of these PRRs varies depending on cell type. In addition, intracellular nucleic acid-binding receptors that are present in most cell types detect DNA in the cytoplasm or recognize unique structures that are rare or absent in host cells, such as uncapped mRNA or double-stranded (ds) RNA (reviewed in [2–4]). The selectivity of PRRs for specific molecular features as well as the cell type that recognizes them are two factors that could potentially give rise to specificity in IFN subtype production – an early step during infection towards ultimately fine-tuning the immune response. Continuing efforts to precisely define PRR specificity and expression may ultimately provide clues to answer the question of whether the IFN response is qualitatively different in response to distinct pathogens.

Members of the RIG-I-like family of receptors (RLRs), which includes retinoic acid-inducible gene 1 (RIG-I), melanoma differentiation-associated protein 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2), specialize in detecting pathogen-derived RNA in the cytoplasm. These, together with the recently described receptor, cyclic GMP-AMP (cGAMP) synthase (cGAS), which recognizes cytoplasmic DNA in a sequence-independent fashion [5], are arguably the most relevant to virus infection and type I IFN production in non-immune cells. A more detailed description of RLR function is provided below where we discuss the strategies that viruses employ to evade their activity. Signaling initiated via these intracellular PRRs sets in motion a series of events that result in IRF3 and NF- κ B activation, both of which are required for the production of IFN- β and the release of chemokines that recruit immune cells to the site of infection.

While nearly all cells are capable of detecting intracellular PAMPs and producing IFN- β following activation of IRF3 and NF- κ B, the IFN- α subtypes are produced primarily by leukocytes [6]. In contrast to IFN- β (and IFN- α 4), the IFN- α subtypes are transcriptionally controlled primarily by IRF5 and IRF7 activation following signaling initiated through Toll-like receptors (TLRs) [3,7–10]. TLRs are membrane-bound proteins that localize to the cell surface and endosomal compartments. There are 10 TLR genes in humans, each with different specificities, with TLR3, TLR7, TLR8, and TLR9 recognizing viral nucleic acids. The expression profile of TLRs varies by cell type, as do the basal levels of IRFs and possibly the epigenetic landscape of the various IFN- α gene promoters ([11]; reviewed in [3,12]). Therefore, the resulting IFN- α milieu may depend on the natural history of the viral pathogen because this will influence which cell type recognizes the PAMP. However, testing this hypothesis has been difficult because accurately measuring IFN subtype profiles under the appropriate physiological conditions has been fraught with many challenges.

Several studies have aimed at determining whether stimulating cells with infectious agents or distinct molecular PAMPs leads to unique IFN- α subtype expression profiles, and the results have been mixed [8,13–24]. Some have shown that a broad range of IFN- α subtypes are produced following stimulation with various PAMPs, but that the relative levels of each subtype are similar [14,15,22], while others report that PAMPs differentially stimulate various IFN subtypes [13,17–21]. These discrepancies can potentially be explained by differences in experimental parameters. For example, while some studies have utilized synthetic PAMPs, others have utilized infectious agents that may preferentially infect

specific cell types and potentially antagonize cellular detection mechanisms (discussed in detail below). Some of the discrepancies may also stem from the inherent difficulty in accurately and quantitatively distinguishing between the IFN- α subtypes. In the absence of subtype-specific antibodies, researchers have relied upon measuring gene expression; however, owing to their high sequence homology, this too can be technically challenging.

Cell types and IFN production

In addition to methods of detection, the cell type chosen for study also contributes to the variability of results. Many studies to date have used preparations of peripheral blood mononuclear cells (PBMCs) – a mixed population of cells including B cells, T cells, dendritic cells, and natural killer (NK) cells, nearly all of which are capable of producing type I IFN and contributing to the overall IFN profile. While some studies have utilized cell populations that were enriched from PBMCs, such as plasmacytoid dendritic cells (pDCs), myeloid dendritic cells (mDCs), or monocytes [14,22], others did not enrich for any particular cell type [15,20,21]. It is possible that even in experiments performed without cell type enrichment, particular cell types such as pDCs may nevertheless dominate the response.

pDCs are known to be major producers of type I IFN, producing up to a hundred to a thousand times more IFN- α than other cell types [25]. They do not need to be productively infected to produce IFN and, following activation, they migrate to lymph nodes where they can potentially influence numerous cell types by their repertoire of secreted type I IFN (reviewed in [26]). For these reasons they are an attractive candidate cell type to study when seeking to determine whether IFN responses are uniquely tailored towards different pathogens.

It was recently reported that IFN responses differ depending on whether pDCs are activated by interacting with infected cells or by virus alone [27]; in a separate publication it was shown that inoculating pDCs with different infectious agents, including hepatitis C virus (HCV), influenza virus, or human immunodeficiency virus (HIV), yields drastically different levels of total IFN- α that vary with virus, time, and quantity of inoculum [23]. However, measuring differences in the relative amounts of the IFN- α subtypes was not a primary goal of the study. In another study by Szubin *et al.*, purified pDCs were stimulated with different PAMPs and the production of individual IFN- α subtypes was quantified using a qPCR-based approach. This study found that, regardless of the PAMP, the pDCs produced a highly uniform IFN- α profile [22]. These results do not support a model where individual type I IFN subtypes are selected to convey unique messages. The authors suggest the possibility that an optimized ratio of IFN subtypes was selected by evolution. Also consistent with these results is the possibility that distinct cell types produce unique IFN- α profiles. Additional work will be necessary to fully understand the kinetics and cell type-specificity of IFN- α subtype production.

Several type I IFNs are already known to be selectively produced in a tissue-specific manner. For example, IFN- κ is selectively produced in keratinocytes [28], and IFN- ϵ is produced in the genital tract. IFN- ϵ expression, however, is unique in that – unlike other type I IFNs, which contain various IRF promoter elements and are produced in response to pathogens – IFN- ϵ production is constitutive, hormonally regulated, and fluctuates

throughout the estrous cycle [29]. Recent evidence suggests that IFN- ϵ may have a unique role in mucosal immunity [30] and may play an important role in protection from sexually transmitted pathogens. A better understanding of the role that IFN- ϵ plays in mucosal immunity and how it is affected by negative feedback mechanisms are important areas for future research. For additional discussion of IFN- ϵ , the reader is referred to [189] in the current issue and to a recent review [31].

Type III IFNs also have an important role in tissue-specific IFN responses. Recent studies have shown that type III IFN signaling is crucial for protecting the epithelium. For example, type III IFN plays an important role in controlling rotavirus infection in the gut [32] and influenza infection in the respiratory tract [33]. Many of the tissue-specific effects of type III IFN can be attributed to receptor distribution; however, considering that type I and III IFNs signal through a similar intracellular pathway and induce similar genes, understanding differences in type I and III signaling may serve as a useful paradigm to further explore IFN subtype differences. For a more in-depth analysis of the recent type III IFN literature the reader is referred to a recent review [34].

Additional research and improved detection methods will no doubt help to resolve the question of whether unique type I and III IFN expression profiles are produced in response to different viral pathogens. Regardless, the evolutionary conservation of multiple IFN subtypes implies that (at least some) cells can distinguish between IFN subtypes. The following section highlights the current research aimed at understanding how IFN subtypes are distinguished by cells.

How are different IFN subtypes distinguished by cells?

Similarly to other cytokine receptor complexes, one of the two receptor chains comprising the type I IFN receptor binds its ligands with high affinity (IFNAR2), while the other receptor chain (IFNAR1) binds with low affinity [35]. Individually, the binding affinity of each type I IFN for the receptor chains varies and, as a result, the stability of the IFN–IFNAR ternary complex for the various IFNs spans a range, with IFN- β lying at the high end of the spectrum and IFN- $\alpha 1$ at the low end [36–38].

Antiviral and antiproliferative effects of type I IFN

Receptor binding affinities correlate well with the potency of the various IFN subtypes, as defined by their ability to induce two readily measurable activities: the ability to protect cells from viral infection and the ability to inhibit cell division. As a consequence, a consensus is emerging that it is the overall stability of the ternary complex that sets the IFN subtypes apart from one another. However, these two readouts have several limitations. The resolution of antiviral assays for distinguishing IFN activities is low and is further confounded by the fact that individual ISGs act on different viruses to variable degrees [39,40]. When cell type and virus are held constant, differences in the potencies of IFN subtypes for inhibiting virus infection and for activating STAT proteins (measured by tyrosine phosphorylation) are subtle – even low concentrations of weak-binding IFNs are sufficient to activate the JAK–STAT pathway (Figure 2) and induce a large number of ‘antiviral’ genes with ISRE-containing promoters [41–43]. This suggests that cells have a

low threshold for initiating an antiviral response in the presence of IFN, and argues that the initial signaling leading to the induction of ‘antiviral’ genes may be subtype-independent. Consequently, cell culture-based antiviral assays may not be suitable for making fine distinctions in IFN subtype activities.

In contrast to antiviral assays, cell anti-proliferation assays allow a greater separation of IFN potencies. These assays are strongly influenced by IFN concentration, receptor expression levels, and receptor binding affinities, indicating that additional mechanisms of gene regulation are potentially involved [38,41,44,45]. For this reason the term ‘tunable’ has been used to describe those genes whose induction correlates with antiproliferative (and likely immunomodulatory) activity [44]. Interestingly, many of these tunable genes do not contain classical ISREs within their promoters, and the details of their transcriptional regulation and cell type-specificity await further characterization [44].

Differences in the induction of tunable genes suggests that multiple factors, including IFN binding affinity and receptor density, may lead to qualitative differences in gene expression on a per cell basis. It is also possible that finely tuned receptor expression levels on specialized cells, possibly immune cells, may determine their responsiveness to IFN subtypes in an ‘all-or-none’ binary fashion.

Interestingly, a recent study by Gibbert *et al.* reported that, in mice, IFN- α 11 activates NK cells and protects mice from retroviral infections, whereas IFN- α 2 and IFN- α 5 do not [46]. This study lies at the heart of the issue discussed in this section; however, additional work will be necessary to fully understand the underlying mechanisms that led to the subtype differences observed. For example, in the Gibbert *et al.* study, IFN- α 2 and IFN- α 5 were produced by a different method (transfected cells) than IFN- α 11 (stable cells), and it is possible that these methodological differences may affect the IFN potencies, stabilities, or the immune response to the injected material. Furthermore, it will be important to determine whether the observed differences in NK activation can be explained by cell surface receptor density and whether differences in tunable gene expression are observed in NK cells following exposure to different IFN- α subtypes.

While IFN–receptor binding affinities and IFN receptor expression levels clearly have important roles in controlling the induction of tunable genes, the underlying mechanisms are less clear. Two potential mechanisms that have yet to be fully resolved include the subcellular location from where signaling occurs, and the modes by which IFNs engage their receptors.

Receptor endocytosis and IFN signaling

Following receptor engagement, the IFN–receptor complex is rapidly internalized via a clathrin-mediated pathway [47] leading to a reduction in cell surface receptor levels [48]. There is evidence for continued signaling in endosomal compartments [49], and therefore the ability to engage receptors during endocytosis likely impacts upon the quantity (and possibly the quality) of signaling. Recycling of receptor chains back to the cell surface is also influenced by IFN binding affinity. Whereas the decrease in IFNAR1 cell surface levels is similarly affected by binding of IFN- α or IFN- β , cell surface IFNAR2 levels correlate

with the stability of the IFN–IFNAR complex [43,50]. Given the strong correlation between receptor binding affinity and anti-proliferative potency, it is tempting to speculate that the ability to signal at later stages of the endosomal pathway –in other words, maintenance of the ternary complex for longer times and at low pH – may be a major factor differentiating the type I IFN subtypes.

Receptor engagement modulates the effects on IFN signaling

Another feature of IFN receptor engagement that may play a role in differentiating the IFN subtypes is the mode by which IFNs engage their receptors. Over the past few years, several X-ray crystal structures have been reported which have deepened our understanding of type I IFN–receptor interactions [43,51]. For a thorough description of the molecular details of IFN receptor engagement, the reader is referred to a review [190] in the current issue. Briefly, a structure of IFN- α 2 bound to the IFNAR1 receptor, together with a structure of the ternary complex comprising IFN- ω , IFNAR1, and IFNAR2, provides evidence that the binding interface and geometry of receptor engagement is remarkably similar between these two IFNs. An additional recent structure of IFN- β in complex with IFNAR1 shows that the overall geometry of IFN- β binding is similar; however, the IFNAR1–IFN- β interface is more extensive, and IFN- β engages (to a minor extent) an additional subunit of the IFNAR1 chain [51].

Recent evidence also suggests that the more intimate mode by which IFN- β engages IFNAR1 may have important biological consequences [51]. It has previously been shown that type I IFN signaling contributes to LPS-induced septic shock, and mice unable to respond to type I IFN signaling (specifically, IFN- β) are more resistant to LPS-induced death [52]. Surprisingly, de Weerd *et al.* found that *Ifnar2*^{−/−}, but not *Ifnar1*^{−/−} mice are sensitive to LPS-induced septic shock, suggesting that type I IFN signaling in the absence of IFNAR2 is involved [51]. Several of the genes regulated in *Ifnar2*^{−/−} mice are classical ISGs; however, many others do not bear ISRE elements or STAT binding sites within their promoters, and were not differentially expressed in wild type cells. It is possible that an alternative receptor chain is engaged in the absence of IFNAR2; however, tyrosine phosphorylation of STAT1 was not detected in cells from *Ifnar2*^{−/−} mice, suggesting that a JAK-independent activity may be involved. In future studies it will be important to determine whether the observed changes in gene expression are truly independent of JAK activity.

The role of IFN negative regulation in differentiating IFN subtype signaling

Along with the basic components of IFN signaling, ISGs themselves play an important role in modulating the IFN response [53,54]. Several ISGs desensitize cells to IFN stimulation, and the ability of the IFN subtypes to signal can be differentially affected. A noteworthy example is the role that the ISG USP18 plays in IFN signaling at later stages of the type I IFN response. USP18 is induced by JAK–STAT signaling, and the resulting protein product then competes with JAK1 for binding to the intracellular domain of IFNAR2 [54]. Interestingly, USP18 effectively blocks signaling by IFN- α 2, whereas IFN- β and a mutant version of IFN- α 2 (IFN- α 2 HEQ) that binds to IFNAR1 with an affinity similar to IFN- β remain capable of activating the JAK–STAT pathway [55,56]. In addition to effectively

increasing the threshold for signaling, it is also possible that USP18 skews cells towards an IFNAR2-independent response similar to the above mechanism, but this remains to be tested. For additional discussion of negative regulation of IFN signaling, the reader is referred to a review in the current issue [189].

Post-translational modifications of IFN pathway components

In the canonical type I IFN signaling pathway, STAT1, STAT2, and IRF9 are the main factors driving inducible gene transcription; however, many additional STAT proteins are phosphorylated following IFN signaling as well [43,57]. Interestingly, *Stat1*^{-/-} mice are partially resistant to dengue virus infection, and infected cells from these mice revealed the induction of a set of ISGs that are similarly induced in wild type mice [58]. This suggests that STAT2 and IRF9 alone, or in combination with an alternative STAT protein may compensate for the absence of STAT1. It is possible that variations in the activation of the various STATs provides a means to diversify signaling by different IFN subtypes, but efforts to quantify alternative STAT protein phosphorylation have failed to find differences among the IFN subtypes [43].

In addition to the JAK proteins that phosphorylate tyrosine residues, IFN-receptor engagement activates several serine kinases as well. Several of these kinases, such as p38 MAPK, PI3K, and AKT, have important functions in translational regulation [59–61]. Furthermore, the ISGF3 complex is modified by several post-translational modifications other than tyrosine phosphorylation, which may have important consequences for signaling. For example, STAT2 and IRF9 are substrates for acetylation, STAT1 is targeted for SUMOylation, and the phosphorylation of serine residues on STAT1 is also important for controlling ISG expression [62–65]. These are only a few examples of additional pathways that become activated following type I IFN signaling and which are often overlooked for reasons that include technical difficulty and the lack of appropriate reagents. Research in the coming years utilizing improved reagents and cutting-edge mass spectrometry techniques will no doubt shed light on this interesting aspect of signal transduction.

Viral antagonism of the IFN-induced host defense

IFNs are one of the primary defenses against all varieties of viral infections. Viruses in turn have developed a variety of IFN antagonist proteins to counteract host defense, using diverse strategies. Such strategies can be divided into two categories: inhibition of IFN induction and inhibition of IFN signaling. In this section we will primarily focus on RNA viruses and their diverse mechanisms of IFN antagonism. We illustrate this by using paramyxoviruses, which are among the best-studied viruses with respect to IFN antagonism. In addition, we describe mechanisms of IFN antagonism for a few important human pathogens from other virus families, including DNA viruses, to highlight similarities and differences in their immune evasion strategies.

Paramyxoviruses are a family of enveloped, negative-strand RNA viruses that have diverse tissue tropism and infect a variety of species. Within the paramyxovirus family the genera of *Morbillivirus*, *Rubulavirus*, and *Respiro-virus* include clinically relevant human pathogens such as measles virus (MeV), mumps virus (MuV), and parainfluenza viruses (PIV).

Recently, a new genus, *Henipavirus*, was identified, adding two highly-virulent zoonotic pathogens, Nipah virus (NiV) and Hendra virus (HeV), to the family [66].

Generally, RNA viruses are limited in their genome size given the error-prone nature of viral RNA polymerases [67]. For this reason, paramyxovirus genomes lack dedicated IFN antagonist genes (Figure 3A). Instead, IFN-antagonist proteins are typically encoded by means of co-transcriptional pseudo-templated G nucleotide insertion editing (leading to a frameshift in transcripts) and through the utilization of alternative reading frames within their conserved P genes [68–73]. Therefore, in addition to the P protein itself, several proteins with IFN-antagonizing functions that are commonly produced from the P gene include the V and C proteins and a protein variously named W, D, or I (Figure 3B). Of these, the V proteins are generally considered the principal IFN antagonists; however, there is increasing evidence that P, W/D/I, and C proteins also play important roles in IFN antagonism by distinct mechanisms. These IFN antagonist proteins broadly target several members of a select group of signaling molecules of the IFN system, including MDA5, RIG-I, IRF3, STAT1, and STAT2. Despite having common targets, the mechanisms by which these proteins interfere with the host response are relatively diverse. As discussed in detail below, these include targeting host proteins for proteasomal degradation, inhibition of protein phosphorylation, and mislocalization of host proteins to subcellular compartments where they are unable to carry out their normal functions.

Inhibition of IFN production

RNA helicases RIG-I and MDA5 detect viral RNAs and initiate a signaling cascade that results in the activation of transcription factors to promote the induction of IFN- α/β . Binding of viral RNA stimulates ATPase activity in both cellular helicases and triggers major conformational changes, allowing their homodimerization. This in turn reveals CARD domains, followed by interaction with the downstream adaptor protein Cardif/VISA/MAVS/IPS-1 (referred to as MAVS in this review). This eventually leads to the activation of both IRF3 (via IKK ϵ /TBK1) and NF- κ B (via IKK α/β), which are required for transcription from the IFN- β promoter. Both RIG-I and MDA5 act as parallel sensors for viral ssRNA and dsRNA but appear to be differentially sensitive to activation by different viruses. The lengths of the viral dsRNA and their structure determine their recognition by either RIG-I or MDA5 [74,75]. Another major PAMP is the 5'-triphosphate of viral RNA molecules, which is recognized only by RIG-I as non-self RNA [76,77] and induces the production of IFN- β . Recent work by Reis e Sousa *et al.* [78] identified in addition 5'-diphosphates of RNA molecules that also activate RIG-I. Infections performed with mammalian reo-viruses, that bear RNAs with 5'-diphosphates, are controlled in cultured cells and mice in a RIG-I dependent manner [78].

The induction of IFN can be divided into three parts: recognition of viral RNAs by RLRs, downstream signaling via the adaptor protein MAVS, and finally activation of transcription factors IRF3/7 (Figure 4). Recent studies have unveiled specific mechanisms of how paramyxovirus accessory proteins inhibit IFN production. The main PRRs that detect intracellular paramyxovirus PAMPs are members of the RLR family. To thwart detection by MDA5, paramyxovirus V proteins directly bind this receptor through a highly conserved

cysteine-rich region. This inhibits both the binding of dsRNA as well as the subsequent homo-oligomerization of MDA5 [74,79], which is necessary for signal propagation. The other two RLRs important for sensing viral RNA, RIG-I and LGP2, are also antagonized by paramyxoviruses [80,81]. Both proteins are found to associate in cells expressing the V protein, whereas their interaction is not detected in its absence [80]. LGP2 (a homolog of MDA5 and RIG-I) was previously thought to be a negative regulator of IFN signaling owing to its lack of a CARD domain and its inability to activate downstream signaling proteins. However, LGP2 was recently found to be important for the recognition of RNA viruses detected by MDA5 [82]. It has been proposed that MDA5 might require LGP2 for efficient recruitment of viral dsRNA to facilitate the initiation of signaling [82]. It therefore remains to be determined whether the main function of the V protein is to downregulate the activation of RIG-I or MDA5 through interaction with LGP2. Orthomyxoviruses (e.g., influenza A virus) are recognized by their 5'-triphosphates and therefore their antagonist proteins primarily target RIG-I [76,77]. The NS1 protein, a virulence factor of influenza A viruses [83], binds to the E3 ligases TRIM25 and Riplet, and prevents them from performing their role in the ubiquitination of RIG-I [84], which is required for optimal downstream signaling [85]. It was recently shown that NS1 acts in a species-specific fashion and preferentially binds to either avian or human TRIM25, depending on the origin of the virus [86]. However, in murine cells NS1 is unable to bind to TRIM25 but efficiently inhibits Riplet [86–88]. Interestingly, influenza A viruses of human origin have evolved to efficiently block both TRIM25 and Riplet, and therefore abrogate RIG-I signaling. These results highlight the various strategies that influenza A viruses employ to evade innate immunity in different species, and their potential for species adaptation.

The detection of viral RNAs by RLRs leads to activation of MAVS. MAVS has been shown to form functional, prion-like aggregates upon activation by RIG-I, and these potentially trigger downstream signaling to activate IRF3 [89]. In contrast to paramyxoviruses, which predominantly target the RLRs, the hepatitis viruses A, B, and C (HAV, HBV, and HCV) are well-described as antagonizing MAVS. It is intriguing to speculate why these liver-tropic viruses, despite being genetically unrelated, target MAVS and, conversely, why paramyxoviruses and orthomyxoviruses that infect first the respiratory epithelium target RLRs. HAV (picornavirus) and HCV (hepacivirus) have evolved a similar strategy of innate immune evasion because both encode proteases (3C^{pro} and NS3-4A respectively) that cleave MAVS and abrogate RLR-mediated signaling [90–92]. HBV (hepadnavirus) also antagonizes MAVS, but does so by a slightly different mechanism. Because HBV does not encode a protease, it instead utilizes its 'accessory' protein, the X protein, to target MAVS for ubiquitination and proteasomal degradation [93]. By targeting MAVS, a key adaptor protein between RLRs and IRFs, these viruses are able to disable the entire RNA-sensing pathway.

The protein components that act downstream of MAVS are also common targets for viral antagonism. For example, in addition to targeting the RLRs, paramyxovirus V proteins have been shown to inhibit IRF3 and the IRF3 kinases, IKK ϵ /TBK1, by a variety of mechanisms. These include facilitating IKK ϵ /TBK1 polyubiquitination and degradation, acting as a decoy for IKK ϵ /TBK1 to prevent IRF3 phosphorylation [94], and sequestration of IRF3 in the cytoplasm to prevent transcription of the gene encoding IFN- β [95]. The W protein of NiV

and the C protein of MeV also antagonize IRF3, but do so by a distinct mechanism. They mislocalize IRF3 inside the nucleus, thereby effectively preventing its phosphorylation and activation [96,97]. These examples clearly illustrate how different viral proteins from distinct paramyxoviruses have evolved a variety of mechanisms to antagonize this crucial step in innate immune activation (Figure 4).

Inhibition of IFN signaling

As described in detail above, binding of IFN- β to its cellular receptor triggers a signaling pathway that induces the transcription of antiviral genes. The production of IFN- β as a result of PRR activation positively feeds back on cells to upregulate the IRF7 transcription factor and increases further production of IFN [9]. Viruses have evolved numerous mechanisms antagonizing each step of this signaling cascade to inhibit the enhancement of IFN signaling (Figure 5).

Degradation of STAT proteins

STAT proteins have half-lives on the order of days, which can be greatly reduced upon infection with particular paramyxoviruses (rubulaviruses) [98–100]. The V protein of rubulaviruses targets STAT1 or STAT2 for proteasomal degradation. A V-degradation complex (VDC) containing the V protein, STAT1, STAT2, and components of an E3 ubiquitin ligase complex is assembled, and this mediates the polyubiquitination of the STAT proteins and leads to their degradation [101–104]. Interestingly, while both STAT1 and STAT2 are required for VDC formation, only one of the two STAT proteins is actually degraded, and this differs among viruses. For example, a VDC containing the PIV5 V protein leads to the loss of STAT1 [105], whereas a similar complex containing the human PIV2 (hPIV2) V protein primarily targets STAT2 for degradation [100].

In contrast to other STAT proteins, STAT2 proteins are relatively diverse across different species, and can therefore be seen as host restriction factors [106–108]. For instance, the inability of PIV5 V to interact with murine STAT2 enables infected mouse cells to respond to IFN and efficiently clear the virus [107]. Similarly, in a mouse model of dengue virus (DENV) infection, the viral NS5 protein is unable to interact with the murine STAT2 and the virus is cleared rapidly [108], suggesting STAT2 as a crucial host determinant for DENV infection. While the host tropism of both of these viruses is highly influenced by the STAT2 protein, their mechanisms for STAT antagonism are somewhat different. The DENV NS5 protein specifically targets human STAT2 for proteasomal degradation [109,110] whereas PIV5 V does not directly affect STAT2 levels, but, as described above, simply requires STAT2 for VDC formation and degradation of STAT1.

While most paramyxoviruses target STAT1 or STAT2, the V protein of MuV catalyzes proteasomal degradation of STAT1 and STAT3 [111,112]. Similarly to PIV5, STAT1 is targeted for destruction in a process involving a VDC and requires STAT2, whereas STAT3 targeting is STAT2-independent. Degradation of STAT proteins not only has implications for innate immunity pathways but also subsequently alters the adaptive immune response. The lack of STAT1 weakens signaling by types I, II, and III IFNs, whereas the lack of STAT2 will abrogate non-canonical STAT1-independent signaling by type I and III IFN. At

first, the downregulation of STAT3 seems to be counterintuitive because it is described to be a negative regulator of the type I IFN response [113]. However, the absence of STAT3 may also reduce the ability of cells to respond to inflammatory cytokines (IL-6 family) [114] and type II IFN. Upon stimulation with IFN- γ , STAT1 homodimerizes or forms STAT1–STAT3 heterodimers, which can activate transcription of genes regulated by IFN- γ -activated site (GAS) promoter elements. Intriguingly, the removal of STAT3 may have therapeutic applications for a wide range of human diseases characterized by STAT3 hyperactivity, for example cancer, arthritis, lupus, autoimmunity, dwarfism, cardiac hypertrophy, obesity, and kidney disease [115–117]. Inhibition and degradation of STAT3 leads to tumor regression and apoptosis [117], which was demonstrated using live MuV as an oncolytic virus in cancer treatment. The MuV V protein efficiently prevents the signaling of IL-6 and v-Src by degrading STAT3 [111], and therefore represents a potentially beneficial tool for targeted therapies.

Mislocalization and dephosphorylation of STAT proteins

Paramyxoviruses such as henipaviruses (NiV and HeV), morbilliviruses (MeV), and respiroviruses [Sendai virus (SeV) and human PIV1 (hPIV1)], also antagonize STAT proteins, but do so by mechanisms that do not involve protein degradation. The henipavirus V proteins disrupt IFN signaling by sequestering STAT1 and STAT2 in high molecular weight cytoplasmic complexes [118,119]. Furthermore, the henipavirus V proteins display nuclear–cytoplasmic shuttling behavior and influence the steady-state cellular distribution of STAT1 [120] by means of relocating unphosphorylated, nuclear STAT1 into the cytoplasm [118,119,121,122]. By doing so, it may antagonize the formation of a U-ISGF3 complex, which is described below. The MeV V protein is relatively divergent from the rubulavirus and henipavirus V proteins, but is nevertheless an efficient inhibitor of IFN signaling. The MeV V protein targets STAT1 and STAT2 independently through distinct N-terminal and C-terminal sites, respectively [123]. Upon binding of V proteins, STAT nuclear translocation is inhibited without affecting its phosphorylation [124]. However, it has also been demonstrated that phosphorylation of STAT1 and STAT2 is inhibited by the MeV V protein by a mechanism that involves its interaction with JAK1 [125,126]. In these studies the authors found that binding of the MeV V protein to JAK1 blocks downstream signaling to STAT proteins. In addition to binding STAT1, STAT2, and JAK1, it has been found that MeV V also interacts with STAT3 and IRF9 [124]. Similarly to MuV V protein, the V protein of MeV also prevents the transcriptional activity induced by IL-6 and v-Src [114,127] and may also impact upon type II IFN signaling. Binding of IRF9, which is absolutely required for canonical as well as non-canonical IFN signaling, may ensure complete abrogation of type I and III IFN signaling.

STAT targeting by respiroviruses differs significantly compared to other paramyxoviruses as a result of the expression of C proteins. The SeV C proteins (C', C, Y1, and Y2) affect STAT protein phosphorylation in different ways. Tyrosine phosphorylation of both STAT1 and STAT2 is inhibited [128,129], as well as serine phosphorylation of STAT1 [130]. It has also been reported that the dephosphorylation of phosphotyrosine on STAT1 is compromised [130,131], suggesting that the mechanism of IFN antagonism by SeV C proteins is based on dysregulation of STAT1 phosphorylation. Similarly to SeV, the C'

protein of hPIV1 also inhibits the phosphorylation of STAT proteins by binding and sequestering STAT1 in perinuclear aggregates [132].

Similarly to paramyxoviruses, highly pathogenic filo-viruses such as Ebola virus (EBOV) and Marburg virus (MARV) have also been described as interfering with STAT protein localization and phosphorylation regulation. The EBOV protein VP24 efficiently blocks innate immune signaling in infected cells by inhibiting nuclear translocation of phosphorylated STAT1 [133,134]. The closely related MARV in turn employs its IFN antagonist protein VP40 to inhibit IFN signaling by preventing the phosphorylation of JAK1 and the subsequent activation of STAT1 and STAT2 [135,136]. In doing so, not only are type I and III IFN signaling impaired, but type II IFN signaling is impaired as well.

Intriguingly, another layer of host defense was described recently, which may be a mechanism that hosts have evolved to counteract virus-induced dephosphorylation of STAT proteins. It has recently been shown that an unphosphorylated ISGF3 (U-ISGF3) complex is also capable of driving ISG expression [137]. Because all of the components of the ISGF3 complex are also ISGs, following initial IFN signaling the individual components of the complex are in much greater abundance. Therefore, there appear to be two phases of gene expression following IFN signaling. The initial, rapid response is driven by the classical phosphorylated form of ISGF3, which is followed by a second, more-prolonged response driven by U-ISGF3. The U-ISGF3 complex maintains the expression of a subset of the initially induced ISGs, whose protein products lead to extended resistance to virus infection [137]. Further work will be necessary to elucidate the impact that U-ISGF3-driven gene expression has on different groups of viruses in the setting of both acute and chronic infections.

DNA viruses

Similarly to RNA viruses, DNA viruses have developed sophisticated mechanisms to antagonize both IFN induction and IFN signaling. DNA viruses are recognized by absent in melanoma-2 (AIM2)-like receptors (ALRs) and the recently discovered protein cGAS. This novel PRR recognizes cytoplasmic DNA irrespective of whether it is endogenous or of viral/bacterial origin. Upon binding to DNA, cGAS catalyzes the production of cGAMP, a small molecule, which in turn binds to the adaptor protein, STING, located on the endoplasmic reticulum (ER). Activated STING interacts with TBK1, which subsequently phosphorylates IRF3. Dimerized, phosphorylated IRF3 translocates into the nucleus and promotes the transcription of IFN- β (Figure 4).

Herpesviruses replicate in the nucleus and thereby potentially evade recognition by cGAS, but they also employ several additional strategies to inhibit the induction of IFN- β . For example, ICP0, an immediate-early protein of herpes simplex virus 1 (HSV-1), as well as pp65 (ppUL83) of human cytomegalovirus (HCMV), inhibit nuclear translocation and accumulation of phosphorylated IRF3 [138,139]. Moreover, pp65 is associated with a reduced phosphorylation state of IRF3 [138]. The latency-associated nuclear antigen 1 (LANA-1) of Kaposi's sarcoma-associated herpesvirus (KSHV) competes with IRF3 for binding to the IFN- β promoter and therefore downregulates its expression [140]. In addition,

KSHV expresses a set of four IRF homologs (vIRF1, -2, -3, -4) [141–143] which may function as dominant-negative regulators of endogenous IRF1, -3, and -7. Indeed, vIRF1 has been shown to prevent IRF3-mediated transcription by binding to p300 and interfering with formation of the CBP/p300–IRF3 transcription factor complex [144,145]. BGLF4, which is expressed by another important human herpesvirus, Epstein–Barr virus (EBV), inhibits IRF3 activity by reducing the amount of active IRF3 that is recruited to DNA promoter regions [146]. In addition to inhibiting the induction of IFN, EBV also inhibits IFN signaling. For example, the oncoprotein, latent membrane protein 1 (LMP-1), interacts with TYK2 and prevents its phosphorylation and the subsequent activation of STAT2, thereby preventing transcription of ISGs [147].

Human papilloma viruses (HPV), another family of DNA viruses, have also been described to antagonize IRF3 function. In the case of HPV-16, the viral E6 oncogene directly binds to IRF3 and inhibits its transcriptional activity [148]. E6 also inhibits IFN signaling by suppressing the expression of STAT1, as reported by Hong *et al.* [149]. The IFN signaling is further diminished by downregulating phosphorylation of STAT1, STAT2, and TYK2 through direct interaction with TYK2 [150]. However, high-affinity binding of E6 to TYK2 has been described only for the ‘malignant’ HPV-18, whereas low-affinity binding is associated with the ‘benign’ HPV-11 [150]. Another HPV IFN antagonist, the E7 oncoprotein, directly interacts with IRF9 and prevents its nuclear translocation, and therefore downregulates the transcription of ISGs [151].

The adenovirus oncoprotein E1A shows functional homologies to the HPV E7 and KSHV vIRF1 protein. Similarly to vIRF1, E1A binds to the CBP/p300 complex, which subsequently prevents interaction with IRF3 and induction of IFN- β [152,153]. Furthermore, E1A is able to directly bind to and inhibit STAT1 [154] and, similarly to E7 nuclear translocation of IRF9 [62], to prevent sufficient IFN signaling.

DNA viruses have generally much larger genome sizes compared to RNA viruses and encode numerous genes, many of which remain uncharacterized. It is likely that some of these uncharacterized genes encode IFN antagonists. It would be highly beneficial for DNA viruses, especially for those with cytoplasmic replication, to counteract the function of cGAS. This key player in recognizing cytosolic DNA synthesizes a small molecule that not only triggers the production of type I IFN within the infected cell but also in neighboring cells. cGAMP migrates via gap junctions into bystander cells and induces an antiviral state as described above. It is therefore able to act in parallel with IFN- β in activating neighboring cells. cGAS was only recently discovered, and it will be of interest to identify its viral antagonists and to unravel their mechanisms of action. Doing so may identify additional drug targets and potentially facilitate the development of novel antiviral therapies.

IFN signaling and its role in vaccine development

Vaccines are by far the most cost-effective health interventions to prevent infectious diseases. Ideally, they provide the vaccinee with long-lasting adaptive immunity against the intended pathogen. Given the important role that the innate immune system plays in establishing an effective adaptive immune response, the most effective vaccines are those

that adequately engage both host systems. We describe here how our current understanding of IFN and the innate immune system may inform vaccine development.

Several types of vaccines for viral infections are currently in use, with inactivated vaccines, live-attenuated vaccines, and protein subunit vaccines being the most common. In recent years the concept of DNA vaccines has presented a major advance against infectious diseases. Plasmid DNA, encoding viral proteins under the control of a mammalian promoter, is administered and provides a very safe and effective alternative to conventional methods. The immune system is exposed to the expressed antigens and elicits strong humoral and cellular responses. DNA vaccines have been approved for West Nile virus (WNV) in horses [155]; however, low immunogenicity in higher primates and humans has been a significant obstacle.

As described above, cells are well equipped to recognize PAMPs and initiate innate immunity, and, because of this, they are often utilized as adjuvants in vaccine formulations to stimulate both cellular Th1 and humoral Th2 immunity. Ideally, adjuvants should exhibit minimal toxicity and increase the quality, breadth, magnitude, and longevity of specific immune responses to antigens. Numerous PAMP-like components triggering innate immunity are currently being investigated for their adjuvant-like properties. PAMPs such as dsRNA, ssRNA, and CpG-containing DNA are most advanced in the development of vaccines adjuvants. They are sensed and distinguished by the Toll-like receptors TLR3, TLR7, TLR8, and TLR9, which, upon activation by their respective ligands, induce potently type I IFN [156–158].

Poly-ICLC, a synthetic complex of carboxymethylcellulose, polyinosinic–polycytidylic acid, and poly-L-lysine dsRNA, is a very stable and potent TLR3 agonist that strongly induces IFN [159]. Not only can it activate TLR3 but it is also detected by intracellular MDA5. Poly-ICLC, used as vaccine adjuvant for human papilloma virus (HPV), was able to induce a profound virus-specific Th1 response in nonhuman primates which was absent in non-adjuvant control groups [160]. Transcriptional profiling revealed that in humans, poly-ICLC reliably induces many arms of innate immunity, and that many of the triggered pathways mimic what is seen with the live attenuated yellow fever vaccine 17D [159]. However, it should also be noted that activation of TLR3 has been associated with autoimmune diseases such as lupus nephritis [161].

Imidazoquinoline compounds activate immune cells via the TLR7/TLR8 MyD88-dependent signaling pathway [162]. Studies are underway utilizing these compounds for the treatment of cancer and viral infections such as HBV and HCV [163,164]. However, clinical development of several TLR7 agonists has been suspended lately due to safety concerns. Exaggerated immune stimulation [165], lymphopenia, and severe hypotension have been reported in connection to treatment with these compounds [163].

Unmethylated bacterial CpG-rich DNA, the scaffold of many DNA vaccines, is recognized by the TLR9 receptor. Short synthetic analogs of CpG DNA (CpG oligodeoxynucleotides – CpG-ODN) are functionally comparable to bacterial CpG DNA and potently stimulate a host immune response which results in the activation and proliferation of immune cells.

Natural CpG-ODNs are targeted and degraded by serum and cellular nucleases. Synthesis of CpG-ODNs using phosphorothioate backbones renders them nuclease-resistant and more stable [166]. Based on their sequence, secondary structure, and biological activity, CpG-ODNs can be grouped primarily into two classes. Class A CpG-ODNs strongly induce IFN- α secretion from pDCs, but only induce moderate pDC maturation and poor activation of B cells [167]. By contrast, class B CpG-ODNs induce poor IFN- α secretion from pDCs, but are strong activators of pDC maturation and B cells [168]. Their ability to induce strong humoral immune responses makes them ideal as vaccine adjuvants. The TLR9 agonist ISS-1018 is currently being evaluated as a vaccine adjuvant for the prevention and immunotherapy of HBV [169]. Despite having a strong activating effect, it should be noted that CpG-ODNs are implicated in triggering autoimmune responses, including rheumatoid arthritis, systemic lupus erythematosus, and diabetes [170–173].

In addition to being sensed by TLR9, cytosolic DNA, whether methylated or unmethylated, is also recognized by cGAS, a recently described nucleotidyl transferase. The activation of this DNA sensor results in the synthesis of cGAMP. Cyclic dinucleotides (CDN) secreted by bacteria are structurally distinct from cellular CDNs (3′/3′-c-di-GMP vs 2′/3′-cGAMP); however, both function as a second messenger inducing a STING-dependent type I IFN response [174,175]. The strong immunostimulatory properties of CDNs via STING/TBK1 make it an interesting target for the development of vaccine adjuvants. Although structurally unrelated to CDNs, the small molecule DMXAA was highly efficient in activating STING [176,177]. Unfortunately, this compound exhibits species specificity for murine STING while having no effect on the human ortho-log [178]. In animal studies, c-di-GMP was shown to be an effective mucosal adjuvant conferring protection against challenge with *S. pneumoniae* [179]. Another study, utilizing c-di-AMP instead, also described its high potential as an adjuvant in mucosal vaccines eliciting a strong cellular immunity [180]. Advances in understanding CDN structures, their synthesis, and the differential activation of STING will allow the development of novel vaccine adjuvants for human application.

Concluding remarks

Research conducted in the >50 years since the discovery of IFN has provided us with a basic understanding of how IFNs are produced, how they signal, and how they establish an antiviral state within cells. However, a great deal remains to be learned. Outstanding questions are listed in Box 2. Our current knowledge of pathogen recognition and IFN production provides the necessary framework for future studies that will reveal how and when the different IFN subtypes are produced. Concurrent efforts to obtain a detailed molecular understanding of the IFN–receptor complex have yielded insight into the mechanisms by which cells can differentially respond to the various IFN subtypes, and studying the ways in which viruses fight back against the IFN system has been equally illuminating. However, continued work on all of these fronts must be integrated to fully appreciate how the immune system together with non-immune tissues is affected as a whole. While this will require no small effort, the payoffs are potentially enormous – the most practical of which may be the impact that this knowledge will have on vaccine design.

Box 2**Outstanding questions**

- Does PRR specificity give rise to pathogen-specific IFN responses?
- How does type I IFN subtype expression influence the adaptive immune response?
- To what extent does negative feedback and receptor endocytosis differentiate the IFN subtypes?
- How do unphosphorylated and/or alternative STAT proteins influence the IFN response?
- To what extent do antiviral antagonism strategies influence tissue tropism?
- How do DNA viruses antagonize cGAS and the production/spread of cGAMP?
- How can PAMPs be utilized therapeutically to treat chronically infected cells (e.g., HIV, HBV, HCV, HHV-1 to -8) without over-stimulation of the immune system?

Future directions:

- Single-cell RNA-seq to characterize the induction of and response to IFN subtypes.
- Knock-in mouse strains with IFN subtype specific reporters to characterize IFN induction in response to different pathogens.

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References

1. Manry J, et al. Evolutionary genetic dissection of human interferons. *J Exp Med*. 2011; 208:2747–2759. [PubMed: 22162829]
2. Barber GN. Cytoplasmic DNA innate immune pathways. *Immunol Rev*. 2011; 243:99–108. [PubMed: 21884170]
3. Iwasaki A. A virological view of innate immune recognition. *Annu Rev Microbiol*. 2012; 66:177–196. [PubMed: 22994491]
4. Wu J, Chen ZJ. Innate immune sensing and signaling of cytosolic nucleic acids. *Annu Rev Immunol*. 2014; 32:461–488. [PubMed: 24655297]
5. Sun L, et al. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science*. 2013; 339:786–791. [PubMed: 23258413]
6. Cantell K, et al. Production of interferon in human leukocytes from normal donors with the use of Sendai virus. *Methods Enzymol*. 1981; 78:29–38. [PubMed: 6173603]

7. Au WC, et al. Characterization of the interferon regulatory factor-7 and its potential role in the transcription activation of interferon A genes. *J Biol Chem.* 1998; 273:29210–29217. [PubMed: 9786932]
8. Barnes BJ, et al. Virus-specific activation of a novel interferon regulatory factor, IRF-5, results in the induction of distinct interferon alpha genes. *J Biol Chem.* 2001; 276:23382–23390. [PubMed: 11303025]
9. Marie I, et al. Differential viral induction of distinct interferon-alpha genes by positive feedback through interferon regulatory factor- 7. *EMBO J.* 1998; 17:6660–6669. [PubMed: 9822609]
10. Perry AK, et al. The host type I interferon response to viral and bacterial infections. *Cell Res.* 2005; 15:407–422. [PubMed: 15987599]
11. Hemont C, et al. Human blood mDC subsets exhibit distinct TLR repertoire and responsiveness. *J Leukoc Biol.* 2013; 93:599–609. [PubMed: 23341538]
12. O'Neill LA, et al. The history of Toll-like receptors – redefining innate immunity. *Nat Rev Immunol.* 2013; 13:453–460. [PubMed: 23681101]
13. Baig E, Fish EN. Distinct signature type I interferon responses are determined by the infecting virus and the target cell. *Antivir Ther.* 2008; 13:409–422. [PubMed: 18572754]
14. Coccia EM, et al. Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells. *Eur J Immunol.* 2004; 34:796–805. [PubMed: 14991609]
15. Easlick J, et al. The early interferon alpha subtype response in infant macaques infected orally with SIV. *J Acquir Immune Defic Syndr.* 2010; 55:14–28. [PubMed: 20616742]
16. Genin P, et al. Differential regulation of human interferon A gene expression by interferon regulatory factors 3 and 7. *Mol Cell Biol.* 2009; 29:3435–3450. [PubMed: 19349300]
17. Hillyer P, et al. Expression profiles of human interferon-alpha and interferon-lambda subtypes are ligand- and cell-dependent. *Immunol Cell Biol.* 2012; 90:774–783. [PubMed: 22249201]
18. Hiscott J, et al. Differential expression of human interferon genes. *Nucleic Acids Res.* 1984; 12:3727–3746. [PubMed: 6427755]
19. Izaguirre A, et al. Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. *J Leukoc Biol.* 2003; 74:1125–1138. [PubMed: 12960254]
20. Palmer P, et al. Type I interferon subtypes produced by human peripheral mononuclear cells from one normal donor stimulated by viral and non-viral inducing factors. *Eur Cytokine Netw.* 2007; 18:108–114. [PubMed: 17594944]
21. Puig M, et al. TLR9 and TLR7 agonists mediate distinct type I IFN responses in humans and nonhuman primates in vitro and in vivo. *J Leukoc Biol.* 2012; 91:147–158. [PubMed: 22058422]
22. Szubin R, et al. Rigid interferon-alpha subtype responses of human plasmacytoid dendritic cells. *J Interferon Cytokine Res.* 2008; 28:749–763. [PubMed: 18937549]
23. Thomas JM, et al. Differential responses of plasmacytoid dendritic cells to influenza virus and distinct viral pathogens. *J Virol.* 2014; 88:10758–10766. [PubMed: 25008918]
24. Zaritsky LA, et al. Tissue-specific interferon alpha subtype response to SIV infection in brain, spleen, and lung. *J Interferon Cytokine Res.* 2013; 33:24–33. [PubMed: 23050948]
25. Siegal FP, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science.* 1999; 284:1835–1837. [PubMed: 10364556]
26. Colonna M, et al. Plasmacytoid dendritic cells in immunity. *Nat Immunol.* 2004; 5:1219–1226. [PubMed: 15549123]
27. Frenz T, et al. Independent of plasmacytoid dendritic cell (pDC) infection, pDC triggered by virus-infected cells mount enhanced type I IFN responses of different composition as opposed to pDC stimulated with free virus. *J Immunol.* 2014; 193:2496–2503. [PubMed: 25070849]
28. LaFleur DW, et al. Interferon-kappa, a novel type I interferon expressed in human keratinocytes. *J Biol Chem.* 2001; 276:39765–39771. [PubMed: 11514542]
29. Fung KY, et al. Interferon-epsilon protects the female reproductive tract from viral and bacterial infection. *Science.* 2013; 339:1088–1092. [PubMed: 23449591]
30. Xi Y, et al. Role of novel type I interferon epsilon in viral infection and mucosal immunity. *Mucosal Immunol.* 2012; 5:610–622. [PubMed: 22617838]

31. Wijesundara DK, et al. Unraveling the convoluted biological roles of type I interferons in infection and immunity: a way forward for therapeutics and vaccine design. *Front Immunol.* 2014; 5:412. [PubMed: 25221557]
32. Pott J, et al. IFN-lambda determines the intestinal epithelial antiviral host defense. *Proc Natl Acad Sci USA.* 2011; 108:7944–7949. [PubMed: 21518880]
33. Davidson S, et al. Pathogenic potential of interferon alphabeta in acute influenza infection. *Nat Commun.* 2014; 5:3864. [PubMed: 24844667]
34. Egli A, et al. The impact of the interferon-lambda family on the innate and adaptive immune response to viral infections. *Emerg Microbes Infect.* 2014; 3:e51.
35. Cohen B, et al. Ligand-induced association of the type I interferon receptor components. *Mol Cell Biol.* 1995; 15:4208–4214. [PubMed: 7623815]
36. Jaks E, et al. Differential receptor subunit affinities of type I interferons govern differential signal activation. *J Mol Biol.* 2007; 366:525–539. [PubMed: 17174979]
37. Lavoie TB, et al. Binding and activity of all human alpha interferon subtypes. *Cytokine.* 2011; 56:282–289. [PubMed: 21856167]
38. Piehler J, et al. Structural and dynamic determinants of type I interferon receptor assembly and their functional interpretation. *Immunol Rev.* 2012; 250:317–334. [PubMed: 23046138]
39. Schoggins JW, et al. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. *Nature.* 2014; 505:691–695. [PubMed: 24284630]
40. Schoggins JW, et al. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature.* 2011; 472:481–485. [PubMed: 21478870]
41. Levin D, et al. Stochastic receptor expression determines cell fate upon interferon treatment. *Mol Cell Biol.* 2011; 31:3252–3266. [PubMed: 21690295]
42. Moll HP, et al. The differential activity of interferon-alpha subtypes is consistent among distinct target genes and cell types. *Cytokine.* 2011; 53:52–59. [PubMed: 20943413]
43. Thomas C, et al. Structural linkage between ligand discrimination and receptor activation by type I interferons. *Cell.* 2011; 146:621–632. [PubMed: 21854986]
44. Levin D, et al. Multifaceted activities of type I interferon are revealed by a receptor antagonist. *Sci Signal.* 2014; 7:ra50. [PubMed: 24866020]
45. Moraga I, et al. Receptor density is key to the alpha2/beta interferon differential activities. *Mol Cell Biol.* 2009; 29:4778–4787. [PubMed: 19564411]
46. Gibbert K, et al. Interferon-alpha subtype 11 activates NK cells and enables control of retroviral infection. *PLoS Pathog.* 2012; 8:e1002868. [PubMed: 22912583]
47. Marchetti M, et al. Stat-mediated signaling induced by type I and type II interferons (IFNs) is differentially controlled through lipid microdomain association and clathrin-dependent endocytosis of IFN receptors. *Mol Biol Cell.* 2006; 17:2896–2909. [PubMed: 16624862]
48. Branca AA, Baglioni C. Down-regulation of the interferon receptor. *J Biol Chem.* 1982; 257:13197–13200. [PubMed: 6292180]
49. Payelle-Brogard B, Pellegrini S. Biochemical monitoring of the early endocytic traffic of the type I interferon receptor. *J Interferon Cytokine Res.* 2010; 30:89–98. [PubMed: 20028207]
50. Jaitin DA, et al. Inquiring into the differential action of interferons (IFNs): an IFN-alpha2 mutant with enhanced affinity to IFNAR1 is functionally similar to IFN-beta. *Mol Cell Biol.* 2006; 26:1888–1897. [PubMed: 16479007]
51. de Weerd NA, et al. Structural basis of a unique interferon-beta signaling axis mediated via the receptor IFNAR1. *Nat Immunol.* 2013; 14:901–907. [PubMed: 23872679]
52. Thomas KE, et al. Contribution of interferon-beta to the murine macrophage response to the toll-like receptor 4 agonist, lipopolysaccharide. *J Biol Chem.* 2006; 281:31119–31130. [PubMed: 16912041]
53. Lerner AC, et al. Transcriptional induction by interferon. New protein(s) determine the extent and length of the induction. *J Biol Chem.* 1986; 261:453–459. [PubMed: 2934388]
54. Malakhova OA, et al. UBP43 is a novel regulator of interferon signaling independent of its ISG15 isopeptidase activity. *EMBO J.* 2006; 25:2358–2367. [PubMed: 16710296]

55. Francois-Newton V, et al. USP18-based negative feedback control is induced by type I and type III interferons and specifically inactivates interferon alpha response. PLoS ONE. 2011; 6:e22200. [PubMed: 21779393]
56. Makowska Z, et al. Interferon-beta and interferon-lambda signaling is not affected by interferon-induced refractoriness to interferon-alpha in vivo. Hepatology. 2011; 53:1154–1163. [PubMed: 21480323]
57. Levy DE, Darnell JE Jr. Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol. 2002; 3:651–662. [PubMed: 12209125]
58. Perry ST, et al. STAT2 mediates innate immunity to Dengue virus in the absence of STAT1 via the type I interferon receptor. PLoS Pathog. 2011; 7:e1001297. [PubMed: 21379341]
59. Goh KC, et al. p38 MAP kinase is required for STAT1 serine phosphorylation and transcriptional activation induced by interferons. EMBO J. 1999; 18:5601–5608. [PubMed: 10523304]
60. Kaur S, et al. Role of the Akt pathway in mRNA translation of interferon-stimulated genes. Proc Natl Acad Sci USA. 2008; 105:4808–4813. [PubMed: 18339807]
61. Uddin S, et al. Interferon-alpha engages the insulin receptor substrate-1 to associate with the phosphatidylinositol 3'-kinase. J Biol Chem. 1995; 270:15938–15941. [PubMed: 7608146]
62. Tang X, et al. Acetylation-dependent signal transduction for type I interferon receptor. Cell. 2007; 131:93–105. [PubMed: 17923090]
63. Tenoever BR, et al. Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. Science. 2007; 315:1274–1278. [PubMed: 17332413]
64. Ungureanu D, et al. PIAS proteins promote SUMO-1 conjugation to STAT1. Blood. 2003; 102:3311–3313. [PubMed: 12855578]
65. Wen Z, et al. Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. Cell. 1995; 82:241–250. [PubMed: 7543024]
66. Chua KB, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science. 2000; 288:1432–1435. [PubMed: 10827955]
67. Lynch M. Evolution of the mutation rate. Trends Genet. 2010; 26:345–352. [PubMed: 20594608]
68. Chenik M, et al. Translation initiation at alternate in-frame AUG codons in the rabies virus phosphoprotein mRNA is mediated by a ribosomal leaky scanning mechanism. J Virol. 1995; 69:707–712. [PubMed: 7815533]
69. Paterson RG, Lamb RA. RNA editing by G-nucleotide insertion in mumps virus P-gene mRNA transcripts. J Virol. 1990; 64:4137–4145. [PubMed: 2166809]
70. Jacques JP, et al. Paramyxovirus mRNA editing leads to G deletions as well as insertions. EMBO J. 1994; 13:5496–5503. [PubMed: 7957115]
71. Kulkarni S, et al. Nipah virus edits its P gene at high frequency to express the V and W proteins. J Virol. 2009; 83:3982–3987. [PubMed: 19211754]
72. Vidal S, et al. Editing of the Sendai virus P/C mRNA by G insertion occurs during mRNA synthesis via a virus-encoded activity. J Virol. 1990; 64:239–246. [PubMed: 1688384]
73. Vidal S, et al. A stuttering model for paramyxovirus P mRNA editing. EMBO J. 1990; 9:2017–2022. [PubMed: 2347313]
74. Childs KS, et al. Mechanism of mda-5 inhibition by paramyxovirus V proteins. J Virol. 2009; 83:1465–1473. [PubMed: 19019954]
75. Kato H, et al. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. J Exp Med. 2008; 205:1601–1610. [PubMed: 18591409]
76. Hornung V, et al. 5'-Triphosphate RNA is the ligand for RIG-I. Science. 2006; 314:994–997. [PubMed: 17038590]
77. Pichlmair A, et al. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. Science. 2006; 314:997–1001. [PubMed: 17038589]
78. Goubau D, et al. Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5'-diphosphates. Nature. 2014; 514:372–375. [PubMed: 25119032]
79. Childs K, et al. Mda-5, but not RIG-I, is a common target for paramyxovirus V proteins. Virology. 2007; 359:190–200. [PubMed: 17049367]

80. Childs K, et al. Paramyxovirus V proteins interact with the RNA helicase LGP2 to inhibit RIG-I-dependent interferon induction. *J Virol.* 2012; 86:3411–3421. [PubMed: 22301134]
81. Parisien JP, et al. A shared interface mediates paramyxovirus interference with antiviral RNA helicases MDA5 and LGP2. *J Virol.* 2009; 83:7252–7260. [PubMed: 19403670]
82. Satoh T, et al. LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. *Proc Natl Acad Sci USA.* 2010; 107:1512–1517. [PubMed: 20080593]
83. Hale BG, et al. The multifunctional NS1 protein of influenza A viruses. *J Gen Virol.* 2008; 89:2359–2376. [PubMed: 18796704]
84. Gack MU, et al. Influenza A virus NS1 targets the ubiquitin ligase TRIM25 to evade recognition by the host viral RNA sensor RIG-I. *Cell Host Microbe.* 2009; 5:439–449. [PubMed: 19454348]
85. Gack MU, et al. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature.* 2007; 446:916–920. [PubMed: 17392790]
86. Rajsbaum R, et al. Species-specific inhibition of RIG-I ubiquitination and IFN induction by the influenza A virus NS1 protein. *PLoS Pathog.* 2012; 8:e1003059. [PubMed: 23209422]
87. Oshiumi H, et al. Riplet/RNF135, a RING finger protein, ubiquitinates RIG-I to promote interferon-beta induction during the early phase of viral infection. *J Biol Chem.* 2009; 284:807–817. [PubMed: 19017631]
88. Oshiumi H, et al. The ubiquitin ligase Riplet is essential for RIG-I-dependent innate immune responses to RNA virus infection. *Cell Host Microbe.* 2010; 8:496–509. [PubMed: 21147464]
89. Hou F, et al. MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. *Cell.* 2011; 146:448–461. [PubMed: 21782231]
90. Yang Y, et al. Disruption of innate immunity due to mitochondrial targeting of a picornaviral protease precursor. *Proc Natl Acad Sci USA.* 2007; 104:7253–7258. [PubMed: 17438296]
91. Li XD, et al. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. *Proc Natl Acad Sci USA.* 2005; 102:17717–17722. [PubMed: 16301520]
92. Meylan E, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature.* 2005; 437:1167–1172. [PubMed: 16177806]
93. Wei C, et al. The hepatitis B virus X protein disrupts innate immunity by downregulating mitochondrial antiviral signaling protein. *J Immunol.* 2010; 185:1158–1168. [PubMed: 20554965]
94. Lu LL, et al. Select paramyxoviral V proteins inhibit IRF3 activation by acting as alternative substrates for inhibitor of kappaB kinase epsilon (IKKε)/TBK1. *J Biol Chem.* 2008; 283:14269–14276. [PubMed: 18362155]
95. Irie T, et al. Inhibition of interferon regulatory factor 3 activation by paramyxovirus V protein. *J Virol.* 2012; 86:7136–7145. [PubMed: 22532687]
96. Shaw ML, et al. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J Virol.* 2005; 79:6078–6088. [PubMed: 15857993]
97. Sparrer KM, et al. Measles virus C protein interferes with beta interferon transcription in the nucleus. *J Virol.* 2012; 86:796–805. [PubMed: 22072748]
98. Haspel RL, et al. The rapid inactivation of nuclear tyrosine phosphorylated Stat1 depends upon a protein tyrosine phosphatase. *EMBO J.* 1996; 15:6262–6268. [PubMed: 8947049]
99. Lee CK, et al. Regulation of interferon-alpha responsiveness by the duration of Janus kinase activity. *J Biol Chem.* 1997; 272:21872–21877. [PubMed: 9268319]
100. Parisien JP, et al. The V protein of human parainfluenza virus 2 antagonizes type I interferon responses by destabilizing signal transducer and activator of transcription 2. *Virology.* 2001; 283:230–239. [PubMed: 11336548]
101. Andrejeva J, et al. The p127 subunit (DDB1) of the UV-DNA damage repair binding protein is essential for the targeted degradation of STAT1 by the V protein of the paramyxovirus simian virus 5. *J Virol.* 2002; 76:11379–11386. [PubMed: 12388698]
102. Leung-Pineda V, et al. DDB1 targets Chk1 to the Cul4 E3 ligase complex in normal cycling cells and in cells experiencing replication stress. *Cancer Res.* 2009; 69:2630–2637. [PubMed: 19276361]

103. Lin GY, et al. The V protein of the paramyxovirus SV5 interacts with damage-specific DNA binding protein. *Virology*. 1998; 249:189–200. [PubMed: 9740790]
104. Sarikas A, et al. The cullin protein family. *Genome Biol*. 2011; 12:220. [PubMed: 21554755]
105. Didcock L, et al. The V protein of simian virus 5 inhibits interferon signalling by targeting STAT1 for proteasome-mediated degradation. *J Virol*. 1999; 73:9928–9933. [PubMed: 10559305]
106. Paulson M, et al. Stat protein transactivation domains recruit p300/CBP through widely divergent sequences. *J Biol Chem*. 1999; 274:25343–25349. [PubMed: 10464260]
107. Parisien JP, et al. STAT2 acts as a host range determinant for species-specific paramyxovirus interferon antagonism and simian virus 5 replication. *J Virol*. 2002; 76:6435–6441. [PubMed: 12050355]
108. Ashour J, et al. Mouse STAT2 restricts early dengue virus replication. *Cell Host Microbe*. 2010; 8:410–421. [PubMed: 21075352]
109. Ashour J, et al. NS5 of dengue virus mediates STAT2 binding and degradation. *J Virol*. 2009; 83:5408–5418. [PubMed: 19279106]
110. Mazzon M, et al. Dengue virus NS5 inhibits interferon-alpha signaling by blocking signal transducer and activator of transcription 2 phosphorylation. *J Infect Dis*. 2009; 200:1261–1270. [PubMed: 19754307]
111. Ulane CM, et al. STAT3 ubiquitylation and degradation by mumps virus suppress cytokine and oncogene signaling. *J Virol*. 2003; 77:6385–6393. [PubMed: 12743296]
112. Yokosawa N, et al. C-terminal region of STAT-1alpha is not necessary for its ubiquitination and degradation caused by mumps virus V protein. *J Virol*. 2002; 76:12683–12690. [PubMed: 12438594]
113. Ho HH, Ivashkiv LB. Role of STAT3 in type I interferon responses. Negative regulation of STAT1-dependent inflammatory gene activation. *J Biol Chem*. 2006; 281:14111–14118. [PubMed: 16571725]
114. Zhong Z, et al. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science*. 1994; 264:95–98. [PubMed: 8140422]
115. Buettner R, et al. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. *Clin Cancer Res*. 2002; 8:945–954. [PubMed: 11948098]
116. Levy DE, Lee CK. What does Stat3 do? *J Clin Invest*. 2002; 109:1143–1148. [PubMed: 11994402]
117. Yu H, Jove R. The STATs of cancer – new molecular targets come of age. *Nat Rev Cancer*. 2004; 4:97–105. [PubMed: 14964307]
118. Rodriguez JJ, et al. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol*. 2002; 76:11476–11483. [PubMed: 12388709]
119. Rodriguez JJ, et al. Hendra virus V protein inhibits interferon signaling by preventing STAT1 and STAT2 nuclear accumulation. *J Virol*. 2003; 77:11842–11845. [PubMed: 14557668]
120. Meyer T, et al. Constitutive and IFN-gamma-induced nuclear import of STAT1 proceed through independent pathways. *EMBO J*. 2002; 21:344–354. [PubMed: 11823427]
121. Rodriguez JJ, et al. Identification of the nuclear export signal and STAT-binding domains of the Nipah virus V protein reveals mechanisms underlying interferon evasion. *J Virol*. 2004; 78:5358–5367. [PubMed: 15113915]
122. Shaw ML, et al. Nipah virus V and W proteins have a common STAT1-binding domain yet inhibit STAT1 activation from the cytoplasmic and nuclear compartments, respectively. *J Virol*. 2004; 78:5633–5641. [PubMed: 15140960]
123. Caignard G, et al. Inhibition of IFN-alpha/beta signaling by two discrete peptides within measles virus V protein that specifically bind STAT1 and STAT2. *Virology*. 2009; 383:112–120. [PubMed: 19007958]
124. Palosaari H, et al. STAT protein interference and suppression of cytokine signal transduction by measles virus V protein. *J Virol*. 2003; 77:7635–7644. [PubMed: 12805463]

125. Caignard G, et al. Measles virus V protein blocks Jak1-mediated phosphorylation of STAT1 to escape IFN- α /beta signaling. *Virology*. 2007; 368:351–362. [PubMed: 17686504]
126. Takeuchi K, et al. Measles virus V protein blocks interferon (IFN)- α /beta but not IFN- γ signaling by inhibiting STAT1 and STAT2 phosphorylation. *FEBS Lett*. 2003; 545:177–182. [PubMed: 12804771]
127. Yu CL, et al. Enhanced DNA-binding activity of a Stat3-related protein in cells transformed by the Src oncoprotein. *Science*. 1995; 269:81–83. [PubMed: 7541555]
128. Garcin D, et al. The amino-terminal extensions of the longer Sendai virus C proteins modulate pY701-Stat1 and bulk Stat1 levels independently of interferon signaling. *J Virol*. 2003; 77:2321–2329. [PubMed: 12551969]
129. Gotoh B, et al. The STAT2 activation process is a crucial target of Sendai virus C protein for the blockade of alpha interferon signaling. *J Virol*. 2003; 77:3360–3370. [PubMed: 12610111]
130. Saito S, et al. Dephosphorylation failure of tyrosine-phosphorylated STAT1 in IFN-stimulated Sendai virus C protein-expressing cells. *Virology*. 2002; 293:205–209. [PubMed: 11886239]
131. Komatsu T, et al. Sendai virus C protein impairs both phosphorylation and dephosphorylation processes of Stat1. *FEBS Lett*. 2002; 511:139–144. [PubMed: 11821064]
132. Schomacker H, et al. The C proteins of human parainfluenza virus type 1 block IFN signaling by binding and retaining Stat1 in perinuclear aggregates at the late endosome. *PLoS ONE*. 2012; 7:e28382. [PubMed: 22355301]
133. Reid SP, et al. Ebola virus VP24 binds karyopherin α 1 and blocks STAT1 nuclear accumulation. *J Virol*. 2006; 80:5156–5167. [PubMed: 16698996]
134. Reid SP, et al. Ebola virus VP24 proteins inhibit the interaction of NPI-1 subfamily karyopherin α proteins with activated STAT1. *J Virol*. 2007; 81:13469–13477. [PubMed: 17928350]
135. Valmas C, Basler CF. Marburg virus VP40 antagonizes interferon signaling in a species-specific manner. *J Virol*. 2011; 85:4309–4317. [PubMed: 21325424]
136. Valmas C, et al. Marburg virus evades interferon responses by a mechanism distinct from ebola virus. *PLoS Pathog*. 2010; 6:e1000721. [PubMed: 20084112]
137. Cheon H, et al. IFN β -dependent increases in STAT1, STAT2, and IRF9 mediate resistance to viruses and DNA damage. *EMBO J*. 2013; 32:2751–2763. [PubMed: 24065129]
138. Abate DA, et al. Major human cytomegalovirus structural protein pp65 (ppUL83) prevents interferon response factor 3 activation in the interferon response. *J Virol*. 2004; 78:10995–11006. [PubMed: 15452220]
139. Melroe GT, et al. Herpes simplex virus 1 has multiple mechanisms for blocking virus-induced interferon production. *J Virol*. 2004; 78:8411–8420. [PubMed: 15280450]
140. Cloutier N, Flamand L. Kaposi sarcoma-associated herpesvirus latency-associated nuclear antigen inhibits interferon (IFN) β expression by competing with IFN regulatory factor-3 for binding to IFN β promoter. *J Biol Chem*. 2010; 285:7208–7221. [PubMed: 20048166]
141. Cunningham C, et al. Transcription mapping of human herpesvirus 8 genes encoding viral interferon regulatory factors. *J Gen Virol*. 2003; 84:1471–1483. [PubMed: 12771416]
142. Neipel F, et al. Cell-homologous genes in the Kaposi's sarcoma-associated rhadinovirus human herpesvirus 8: determinants of its pathogenicity? *J Virol*. 1997; 71:4187–4192. [PubMed: 9151804]
143. Russo JJ, et al. Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc Natl Acad Sci USA*. 1996; 93:14862–14867. [PubMed: 8962146]
144. Burysek L, et al. Functional analysis of human herpesvirus 8-encoded viral interferon regulatory factor 1 and its association with cellular interferon regulatory factors and p300. *J Virol*. 1999; 73:7334–7342. [PubMed: 10438822]
145. Lin R, et al. HHV-8 encoded vIRF-1 represses the interferon antiviral response by blocking IRF-3 recruitment of the CBP/p300 coactivators. *Oncogene*. 2001; 20:800–811. [PubMed: 11314014]
146. Wang JT, et al. Epstein–Barr virus BGLF4 kinase suppresses the interferon regulatory factor 3 signaling pathway. *J Virol*. 2009; 83:1856–1869. [PubMed: 19052084]

147. Geiger TR, Martin JM. The Epstein–Barr virus-encoded LMP-1 oncoprotein negatively affects Tyk2 phosphorylation and interferon signaling in human B cells. *J Virol.* 2006; 80:11638–11650. [PubMed: 16987978]
148. Ronco LV, et al. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. *Genes Dev.* 1998; 12:2061–2072. [PubMed: 9649509]
149. Hong S, et al. Suppression of STAT-1 expression by human papillomaviruses is necessary for differentiation-dependent genome amplification and plasmid maintenance. *J Virol.* 2011; 85:9486–9494. [PubMed: 21734056]
150. Li S, et al. The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak–STAT activation by interferon-alpha. *Oncogene.* 1999; 18:5727–5737. [PubMed: 10523853]
151. Barnard P, McMillan NA. The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. *Virology.* 1999; 259:305–313. [PubMed: 10388655]
152. Juang YT, et al. Primary activation of interferon A and interferon B gene transcription by interferon regulatory factor 3. *Proc Natl Acad Sci USA.* 1998; 95:9837–9842. [PubMed: 9707562]
153. Yang XJ, et al. A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature.* 1996; 382:319–324. [PubMed: 8684459]
154. Look DC, et al. Direct suppression of Stat1 function during adenoviral infection. *Immunity.* 1998; 9:871–880. [PubMed: 9881977]
155. Davis BS, et al. West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses in vitro a noninfectious recombinant antigen that can be used in enzyme-linked immunosorbent assays. *J Virol.* 2001; 75:4040–4047. [PubMed: 11287553]
156. Goubau D, et al. Cytosolic sensing of viruses. *Immunity.* 2013; 38:855–869. [PubMed: 23706667]
157. Ishii KJ, Akira S. Innate immune recognition of nucleic acids: beyond toll-like receptors. *Int J Cancer.* 2005; 117:517–523. [PubMed: 16080197]
158. Kawasaki T, et al. Recognition of nucleic acids by pattern-recognition receptors and its relevance in autoimmunity. *Immunol Rev.* 2011; 243:61–73. [PubMed: 21884167]
159. Caskey M, et al. Synthetic double-stranded RNA induces innate immune responses similar to a live viral vaccine in humans. *J Exp Med.* 2011; 208:2357–2366. [PubMed: 22065672]
160. Stahl-Hennig C, et al. Synthetic double-stranded RNAs are adjuvants for the induction of T helper 1 and humoral immune responses to human papillomavirus in rhesus macaques. *PLoS Pathog.* 2009; 5:e1000373. [PubMed: 19360120]
161. Patole PS, et al. Viral double-stranded RNA aggravates lupus nephritis through Toll-like receptor 3 on glomerular mesangial cells and antigen-presenting cells. *J Am Soc Nephrol.* 2005; 16:1326–1338. [PubMed: 15772251]
162. Shukla NM, et al. Structure-activity relationships in human toll-like receptor 7-active imidazoquinoline analogues. *J Med Chem.* 2010; 53:4450–4465. [PubMed: 20481492]
163. Fidock MD, et al. The innate immune response, clinical outcomes, and ex vivo HCV antiviral efficacy of a TLR7 agonist (PF-4878691). *Clin Pharmacol Ther.* 2011; 89:821–829. [PubMed: 21451504]
164. Xiang AX, et al. Discovery of ANA975: an oral prodrug of the TLR-7 agonist isatoribine. *Nucleosides Nucleotides Nucleic Acids.* 2007; 26:635–640. [PubMed: 18066870]
165. Hamm S, et al. Cancer immunotherapeutic potential of novel small molecule TLR7 and TLR8 agonists. *J Immunotoxicol.* 2009; 6:257–265. [PubMed: 19848448]
166. Dalpke AH, et al. Phosphodiester CpG oligonucleotides as adjuvants: polyguanosine runs enhance cellular uptake and improve immunostimulative activity of phosphodiester CpG oligonucleotides in vitro and in vivo. *Immunology.* 2002; 106:102–112. [PubMed: 11972638]
167. Krug A, et al. Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. *Eur J Immunol.* 2001; 31:2154–2163. [PubMed: 11449369]
168. Krieg AM, et al. CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature.* 1995; 374:546–549. [PubMed: 7700380]

169. Cooper C, Mackie D. Hepatitis B surface antigen-1018 ISS adjuvant-containing vaccine: a review of HEPLISAV safety and efficacy. *Expert Rev Vaccines*. 2011; 10:417–427. [PubMed: 21506639]
170. Boule MW, et al. Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin–immunoglobulin G complexes. *J Exp Med*. 2004; 199:1631–1640. [PubMed: 15197227]
171. Christensen SR, et al. Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. *J Exp Med*. 2005; 202:321–331. [PubMed: 16027240]
172. Deng GM, et al. Intra-articularly localized bacterial DNA containing CpG motifs induces arthritis. *Nat Med*. 1999; 5:702–705. [PubMed: 10371511]
173. Zipris D, et al. TLR9-signaling pathways are involved in Kilham rat virus-induced autoimmune diabetes in the biobreeding diabetes-resistant rat. *J Immunol*. 2007; 178:693–701. [PubMed: 17202329]
174. Ablasser A, et al. cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. *Nature*. 2013; 498:380–384. [PubMed: 23722158]
175. McWhirter SM, et al. A host type I interferon response is induced by cytosolic sensing of the bacterial second messenger cyclic-di-GMP. *J Exp Med*. 2009; 206:1899–1911. [PubMed: 19652017]
176. Prantner D, et al. 5,6-Dimethylxanthenone-4-acetic acid (DMXAA) activates stimulator of interferon gene (STING)-dependent innate immune pathways and is regulated by mitochondrial membrane potential. *J Biol Chem*. 2012; 287:39776–39788. [PubMed: 23027866]
177. Roberts ZJ, et al. The chemotherapeutic agent DMXAA potently and specifically activates the TBK1–IRF-3 signaling axis. *J Exp Med*. 2007; 204:1559–1569. [PubMed: 17562815]
178. Conlon J, et al. Mouse, but not human STING, binds and signals in response to the vascular disrupting agent 5,6-dimethylxanthenone-4-acetic acid. *J Immunol*. 2013; 190:5216–5225. [PubMed: 23585680]
179. Yan H, et al. 3',5'-Cyclic diguanylic acid elicits mucosal immunity against bacterial infection. *Biochem Biophys Res Commun*. 2009; 387:581–584. [PubMed: 19615973]
180. Ebensen T, et al. Bis-(3',5')-cyclic dimeric adenosine monophosphate: strong Th1/Th2/Th17 promoting mucosal adjuvant. *Vaccine*. 2011; 29:5210–5220. [PubMed: 21619907]
181. Gibbert K, et al. IFN- α subtypes: distinct biological activities in anti-viral therapy. *Br J Pharmacol*. 2013; 168:1048–1058. [PubMed: 23072338]
182. Pestka S, et al. Interferons, interferon-like cytokines, and their receptors. *Immunol Rev*. 2004; 202:8–32. [PubMed: 15546383]
183. Schroder K, et al. Interferon- γ : an overview of signals, mechanisms and functions. *J Leukoc Biol*. 2004; 75:163–189. [PubMed: 14525967]
184. Kotenko SV, et al. IFN- λ s mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat Immunol*. 2003; 4:69–77. [PubMed: 12483210]
185. Prokunina-Olsson L, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet*. 2013; 45:164–171. [PubMed: 23291588]
186. Sheppard P, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat Immunol*. 2003; 4:63–68. [PubMed: 12469119]
187. Sommereyns C, et al. IFN- λ (IFN- λ) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. *PLoS Pathog*. 2008; 4:e1000017. [PubMed: 18369468]
188. Schneider WM, et al. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol*. 2014; 32:513–545. [PubMed: 24555472]
189. Poritt, RA.; Hertzog, PJ. Dynamic control of type I IFN signalling by an integrated network of negative regulators. *Trends Immunol*. 2015. <http://dx.doi.org/10.1016/j.it.2015.02.002>
190. Schreiber, G.; Piehler, J. The molecular basis for functional plasticity in type I interferon signaling. *Trends Immunol*. 2015. <http://dx.doi.org/10.1016/j.it.2015.01.002>

Figure 1. Human interferon (IFN) proteins. Unrooted phylogenetic tree of human IFN proteins: types I, II, and III. Type I IFN, green; type II IFN, red; type III IFN, blue. The scale bar indicates amino acid substitutions per site. The tree was generated using Geneious software [157].

Figure 2.

Interferon (IFN) signaling via the JAK–STAT pathway. Once type I or III IFNs engage their cognate receptors at the cell surface, individual receptor chains are brought into close proximity. As a result, intracellular receptor-associated tyrosine kinases of the Janus kinase (JAK) family of proteins are juxtaposed and become activated. Activated JAK proteins subsequently phosphorylate (P) members of the signal transducer and activator of transcription (STAT) family of proteins, ultimately leading to the transcriptional activation of IFN-stimulated genes (ISGs). Inasmuch as is currently known, after receptor engagement, type I and III IFNs signal through the same pathway: activation of the two JAK proteins, JAK1 and TYK2, results in the phosphorylation of conserved tyrosine residues on STAT1 and STAT2, followed by formation of a heterotrimeric complex with IFN-regulatory factor 9 (IRF9). This complex, referred to as IFN-stimulated gene factor 3 (ISGF3), translocates to the nucleus and binds to a DNA sequence known as the IFN-stimulated response element (ISRE) in the promoters of ISGs. As a result, hundreds of ISGs are transcriptionally regulated. In addition to stimulating transcription of numerous genes, IFN signaling also leads to the transcriptional repression of a variety of genes; however, the underlying mechanisms and outcomes are comparatively underexplored (reviewed in [188]). Abbreviations: GAF, γ -interferon activation factor; GAS, γ -interferon activation site.

Figure 3.

Genome organization and coding capacity of paramyxoviruses based on *Respiroviruses*. **(A)** General genome organization of paramyxoviruses. Abbreviations: F, fusion protein; H/HN/G, hemagglutinin/hemagglutinin-neuraminidase/glycoprotein (depending on virus); L, RNA-dependent RNA polymerase; M, matrix protein; N/NP, nucleocapsid protein; P/V/C, phosphoprotein and accessory proteins. **(B)** Coding capacity of the P/V/C gene of *Respiroviruses*. The P/V/C gene encodes multiple proteins by means of initiation of translation from an internal open reading frame (ORF), generating 4 C proteins (C1-4), and by means of co-transcriptional pseudo-templated G nucleotide insertion editing. Additional non-coded G nucleotides are inserted at the indicated RNA editing site causing a +1 or +2 frameshift, which results in the V and W/D proteins, respectively. The full-length P protein and the edited V and W/D proteins share the N-terminal region but have distinct C termini.

Figure 4.

Induction of interferon (IFN) and its antagonism by viral proteins. The nucleotidyltransferase cyclic GMP-AMP (cGAMP) synthase (cGAS) detects viral DNAs while the RNA helicases retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) detect viral RNAs. These recognized pathogen-associated molecular patterns (PAMP) initiate a signaling cascade that results in the activation of transcription factors to promote the induction of IFN- α/β . Binding of viral double-stranded (ds) DNA to cGAS stimulates the synthesis of cGAMP, which binds directly to the endoplasmic reticulum (ER)-located stimulator of IFN genes (STING). Upon activation, STING stimulates TANK-binding kinase 1 (TBK1) activity to phosphorylate IFN regulatory factor (IRF)3. Binding of viral single-stranded (ss)- and dsRNA stimulates ATPase activity in RIG-I-like receptors (RLRs – RIG-I, MDA5) and triggers major conformational changes, allowing their homodimerization. Upon homodimerization, RIG-I and MDA5 interact with the downstream adaptor protein mitochondrial antiviral-signaling protein (MAVS), which leads eventually to the activation of IRF3 via I κ B kinase ϵ (IKK ϵ) and TBK1. The activation of IRF3 due to viral DNAs and RNAs, together with IRF7 and nuclear factor κ B (NF- κ B), is required for transcriptional induction of the IFN- β promoter. Viral antagonist proteins disturb the signaling pathway at multiple sites with diverse mechanisms as indicated. Abbreviations: AdV, adenovirus; EBV, Epstein–Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HCV, hepatitis C virus; hPIV2, human parainfluenza virus 2; HPV-16, human papilloma virus 16; IAV, influenza A virus; ICP0, infected-cell polypeptide 0; I κ B, inhibitor of κ B; KSHV, Kaposi's sarcoma-associated herpesvirus; LANA-1, latency-associated nuclear antigen; LGP2, Laboratory of Genetics and Physiology 2; MeV, measles virus; MuV, mumps virus; NiV, Nipah virus; NS1, non-structural protein 1; NS3-4A, non-structural protein 3-4A; P, phosphorylation; PIV5, parainfluenza virus 5; pp65, phosphoprotein 65; Riplet, RING finger

protein 135; SeV, Sendai virus; TRIM25, tripartite motif-containing protein 25, Ub, ubiquitination.

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Figure 5.

Type I and III interferon (IFN) signaling and its antagonism by viral proteins. Binding of type I IFN to the IFN receptor subunits IFN α/β receptor (IFNAR)1 and IFNAR2 leads to their dimerization and activates its receptor-associated kinases tyrosine kinase 2 (TYK2) and Janus kinase 1 (JAK1). TYK2 phosphorylates IFNAR1 and enables binding of signal transducer and activator of transcription (STAT)2. STAT2 is subsequently phosphorylated (P) by TYK2 whereas STAT1 is phosphorylated by JAK1. Binding of type III IFN to the IFN- λ receptor subunits (IFNLR)1 and interleukin (IL)10 receptor (IL10R)2 leads to their dimerization, activation of the receptor kinases TYK2 and JAK1, and subsequent phosphorylation of STAT1 and STAT2. Phosphorylated STAT1 and STAT2 form a stable heterodimer, which binds to the DNA-binding subunit IFN regulatory factor (IRF) 9. This newly formed trimeric complex IFN-stimulated gene factor 3 (ISGF3) translocates into the nucleus, binds to IFN-stimulatory response elements (ISREs), and acts as an enhancer at the 5'-regulatory regions of many IFN-responsive gene promoters. STAT3 is involved in type II IFN and IL-6 cytokine signaling (pathways not shown). Viral antagonist proteins interfere with the signal transduction at multiple sites and diverse mechanisms as indicated.

Abbreviations: AdV: adenovirus; DENV: dengue virus; EBOV: Ebola virus; EBV: Epstein-Barr virus; HCMV: human cytomegalovirus; hPIV2: human parainfluenza virus 2; HPV-16/18: human papilloma virus 16/18; LMP-1: latent membrane protein 1; MARV: Marburg virus; MeV: measles virus; MuV: mumps virus; NS5: non-structural protein 5; PIV5: parainfluenza virus 5; VP24: minor viral matrix protein; VP40: viral matrix protein.