

REVIEWS

Interferons at age 50: past, current and future impact on biomedicine

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Abstract | The family of interferon (IFN) proteins has now more than reached the potential envisioned by early discovering virologists: IFNs are not only antivirals with a spectrum of clinical effectiveness against both RNA and DNA viruses, but are also the prototypic biological response modifiers for oncology, and show effectiveness in suppressing manifestations of multiple sclerosis. Studies of IFNs have resulted in fundamental insights into cellular signalling mechanisms, gene transcription and innate and acquired immunity. Further elucidation of the multitude of IFN-induced genes, as well as drug development strategies targeting IFN production via the activation of the Toll-like receptors (TLRs), will almost certainly lead to newer and more efficacious therapeutics. Our goal is to offer a molecular and clinical perspective that will enable IFNs or their TLR agonist inducers to reach their full clinical potential.

The discovery and molecular understanding of the cellular mechanisms and clinical use of interferons (IFNs) have been a major advance in biomedicine over the past 50 years. This family of secreted autocrine and paracrine proteins stimulates intracellular and intercellular networks that regulate resistance to viral infections, enhance innate and acquired immune responses, and modulate normal and tumour cell survival and death. After their discovery in 1957 (REF. 1), it was soon appreciated that IFNs were critically important to the health of animals and humans and that the IFN system had potential as therapies for infections for both RNA and DNA viruses (TIMELINE).

However, advances in molecular biology a decade later and into the 1970s were required before the promise could be realized. The 1980s saw their introduction into the clinic as the first pharmaceutical products of the budding biotechnology industry, and, importantly, as a demonstration of the effectiveness of IFNs not only for viral diseases and cancer but also for [multiple sclerosis](#) (MS). The 1990s were marked by an expansion in their clinical applications with regulatory approvals worldwide and a further understanding of molecular events influencing biological actions. Current studies have led to new insights into IFNs as a fundamental component of the innate immune system. Additionally, studies have revealed how IFN production is induced through Toll-like receptors (TLRs), the actions of IFN-stimulated genes (ISGs), the identification of viral mechanisms that

resist actions of this potent protein, and how mutation and suppression of gene products of the IFN system in and by malignant cells may affect the initiation and progression of cancer.

After binding to high-affinity receptors, IFNs initiate a signalling cascade through signalling proteins that can also be activated by other cytokines, which were first identified through studies of IFNs. Cellular actions are mediated through specific ISGs, which underlie the antiviral effects, as well as immunoregulatory and antitumour effects. Future drugs that could act as molecular activators for ISGs, many of which exist in a latent state or as agonists for TLRs, might be expected to have potent antiviral, antitumour and/or immunomodulatory effects.

From more than 100,000 published papers, we offer a perspective with a focus on human IFNs to stimulate the future investigation of important questions. Although IFNs function as an integrated system, conceptually it helps to consider their production, which is mediated through TLR activation, and their action, which is mediated through JAK/STAT (Janus kinase/Signal Transducers and Activators of Transcription) and other signalling pathways. The production of IFNs is important for understanding the role of IFNs in innate immunity, while their effects relate to dissecting underlying and future mechanisms of action and application. Highlighting initial pivotal discoveries and more recent findings of conceptual importance, we review how IFNs

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are induced, the cellular actions of IFNs and ISGs, human therapeutic applications, and summarize important questions for biomedicine and drug and clinical development initiatives.

How synthesis is induced

Production of IFNs, both *in vitro* and *in vivo*, is transient and requires stimulation by viruses, microbial products or chemical inducers. In the course of the discovery of IFNs, either live or heat-inactivated influenza viruses were initially identified as inducers¹. Subsequently other microbial products, including those of bacteria, protozoa, and RNA and DNA viruses, were also recognized to induce IFN². It was also shown that microbial nucleic acids, lipids, polysaccharides or proteins trigger induction of IFNs through activation of TLRs (FIG. 1). An early pivotal discovery identified double-stranded (ds) RNAs, both natural and synthetic, as potent inducers³, leading to the simplistic paradigm that viruses induce IFNs by producing dsRNA; in reality, it is only one of the viral gene products responsible for induction. Nonetheless, dissection of cellular responses that lead to induction were spearheaded by the analysis of dsRNA-mediated signalling pathways.

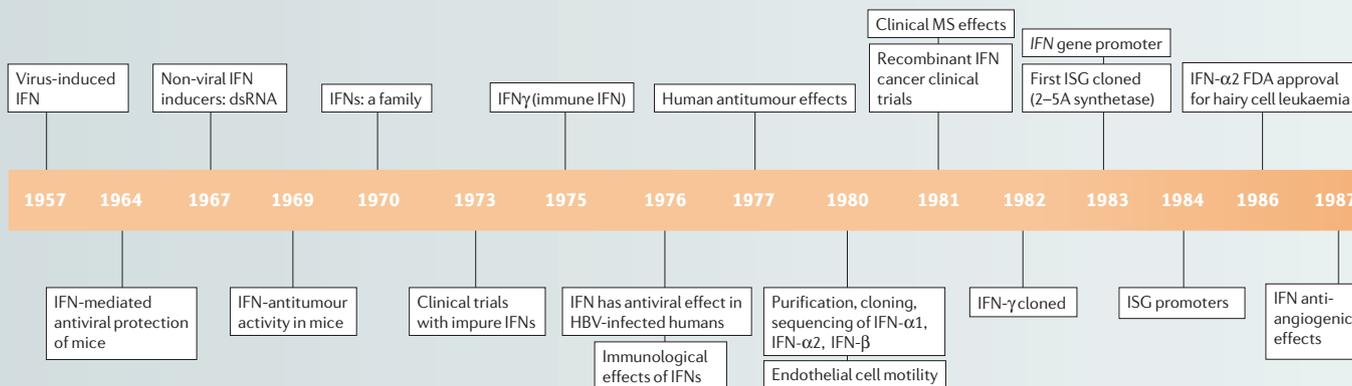
dsRNA is recognized by TLR3, which is present mostly in endosomal membranes⁴, and also by two cytoplasmic RNA helicases, retinoic acid-inducible gene I (RIG-I) and melanoma differentiation associated protein 5 (MDA5)⁵ (FIG. 2). The cytoplasmic proteins can also recognize single-stranded (ss) RNAs but only if they have 5'-triphosphates. Mice in which the *TLR3* (REF. 6), the *RIG-I*⁷ or the *MDA5* (REF. 8) gene has been disrupted are more susceptible to virus infection; however the relative importance of the three proteins for inhibitory activity varies for the immune defence against different viruses. Surprisingly, the presence of TLR3 can enhance pathogenesis in mice infected with influenza A virus⁹ or West Nile virus¹⁰. An adaptor protein for TLR3 signalling is TLR adapter molecule 1 (TRIF), whereas

the mitochondrial protein IFN- β -promoter stimulator 1 (IPS1; also known as VISA) is an adaptor for RIG-I and MDA5; both TRIF and IPS1 recruit inhibitor of nuclear factor- κ B (NF κ B) kinase (IKK) and TANK-binding kinase (TBK1), the common activator kinases¹¹. Other nucleic acids such as ssRNA, acting through TLR7 and TLR8, and bacterial oligodeoxyribonucleotides, acting through TLR9, are also potent inducers¹². Bacterial lipopolysaccharides induce IFNs through TLR4 and also recruit TRIF; viral glycoproteins bind and activate different TLRs. An additional cytoplasmic receptor exists for recognizing viral DNA¹³.

IFN genes, which are normally transcriptionally silent, are induced by the binding of TLR-activated transcription factors to their promoters. Transcriptional induction of IFN- β has been a model experimental system for defining interactions of transcription factors as an enhanceosome multiprotein complex with DNA¹⁴. The most important transcription factors for induction are proteins of the IFN regulatory factor (IRF), specifically IRF3 and IRF7, and NF κ B families^{15,16}. IRFs are activated by the kinases TBK1 or IKK ϵ ; activated IRFs then dimerize and translocate to the nucleus¹⁵. The IKK protein kinase complex phosphorylates I κ B and releases it from NF κ B; NF κ B is then further activated by phosphorylation by other kinases¹⁷.

To evade the IFN system, viruses have evolved many mechanisms to block IFN synthesis and actions — acting at almost every step of the signalling pathway^{18,19}. For example, a hepatitis C virus (HCV)-encoded protease can cleave IPS1 off the mitochondrial membrane and block RIG-I/MDA5-mediated signalling²⁰. Hepatitis B virus (HBV) ORF-C and terminal proteins can also block induction. Influenza virus, Ebola virus, papilloma viruses and the human herpes Kaposi's sarcoma-associated virus (KSHV) encode proteins that interfere with IRF activation or induction and actions of IFNs²¹. For example, KSHV can downregulate one of the receptor chains for IFN- γ , and the NS1 protein of influenza viruses prevents

Timeline | Major milestones and discoveries in 50 years of interferon research



The molecular understanding and clinical use of interferons (IFNs) has been a major advance in biomedicine over the past half century. Discovery of IFNs evolved from studies of viral interference beginning in 1935 with evidence for inhibition by one virus with replication of a second. 1980 marked a particularly 'big year', which saw the purification, cloning and sequencing of IFN- α 1, IFN- α 2 and IFN- β . FDA, Food and Drug Administration; HBV, hepatitis B virus; HCV, hepatitis C virus; IRF, interferon regulatory factor; ISG, interferon-stimulated gene; JAK, Janus kinase; MS, multiple sclerosis; STAT, signal transducer and activator of transcription.

CpG oligonucleotides

Bacterial DNA oligodeoxynucleotide sequences that include a cytosine–guanosine sequence and certain flanking nucleotides that have been found to induce innate immune responses through interaction with Toll-like receptor 9.

DMXAA

5,6-dimethylxanthenone-4-acetic acid. An experimental anticancer drug currently in clinical trials for lung and prostate cancer. It is classified as a vascular disruption agent, causing apoptosis (death) of vessel endothelial cells and the release of vasoactive molecules, which inhibit the formation of new tumour blood vessels.

Antiviral unit

An antiviral unit is the concentration of an interferon required to inhibit virus replication *in vitro* by 50%; an international WHO standard provides a reference base for each major interferon type.

establishment of an antiviral state through the interaction with RIG-I^{22,23}. Conversely, patients with defects in the production of type I IFNs, due to mutation of the *UNC93B* gene, are highly susceptible to *herpes simplex virus 1* (HSV-1) encephalitis²⁴.

Development of small-molecule activators of induction are only beginning; however, delineation of the responsible signalling pathway has identified many target proteins. CpG oligonucleotides are activators of TLR9 (REF. 25); the quinolinamine imiquimod²⁶ and its analogues activate TLR7 (REFS 25,26); and DMXAA induces IFN synthesis through a TLR-independent pathway²⁷. Development of chemical modulators that selectively activate IFN synthesis or block the synthesis of inflammatory cytokines could have a broad therapeutic potential^{28,29}.

IFN proteins and their receptors

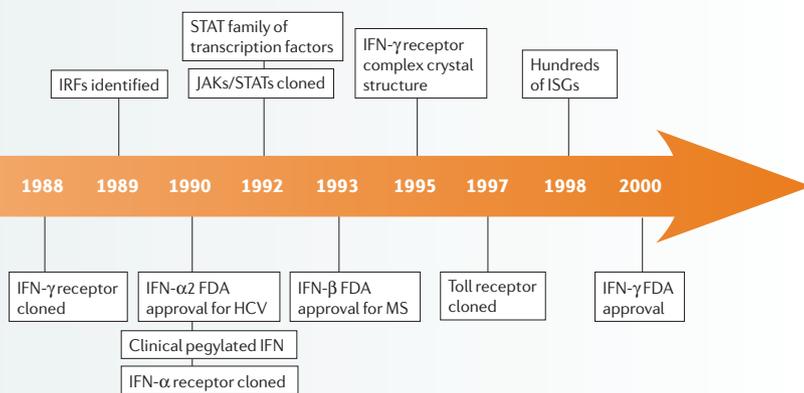
The realization that IFNs constitute a protein family arose initially from the definition of antigenic differences between human fibroblast (IFN-β) and leukocyte IFNs (IFN-α)³⁰. Although protein purification studies suggested the potential multigenic nature of IFN-α, this was only firmly established by the cloning of IFN-α1, IFN-α2 and IFN-β^{31–33}, which, when accomplished, helped solidify the financial future of the nascent biotechnology industry. The number of functional genes identified that encode type I IFNs has grown subsequently: 17 non-allelic genes have now been described in humans. All lack introns and cluster on chromosome 9 (REFS 34,35). Of the type I IFNs, there are 13 IFN-αs (plus an additional synthetic consensus sequence and also additional minor allelic variants), whereas there is only one type of IFN-β, IFN-ω, IFN-ε or IFN-κ (FIG. 3). Among mammals, the number of type I IFN genes is variable; some have unique types (for example, IFN-δ occurs only in pigs and IFN-τ only in ruminants) and others are devoid of a particular type (for example, IFN-ω in mice). Consistent with the universal biological definition of IFNs (that is, proteins inducing relatively species-specific antiviral effects), all

type I IFNs, which are mostly non-glycosylated proteins of 165–200-plus amino acids, share homologies that range from 30–85% within a species. Essentially all have relatively high specific potencies (1 × 10⁷ to 1 × 10⁹ antiviral units per mg protein).

Although type I IFNs have qualitative and quantitative differences in their antiviral and other actions^{33–40}, the reason for origin and maintenance through evolution of these related proteins is unknown. All mammalian species have retained, however, at least one IFN-α and one IFN-β⁴¹. In humans, expression of IFN-κ and IFN-ε seems tissue specific^{35,42}, but all cells are able to produce other IFNs. In monocyte-derived dendritic cells, in which viral infection induces expression of the 15 IFN-α/IFN-β/IFN-ω subtypes, stimulation of TLR3 or TLR4 induces mostly IFN-β and IFN-α1, which emphasizes the differences in the promoter sequences for the *IFN-αs*, *IFN-ω*, and *IFN-β* genes that govern the response to different inducers⁴³.

Type I IFNs belong to the helical cytokine family with secondary structures of a five α-helix bundle held in position by two disulphide bonds⁴⁴. They act through a cell-surface receptor composed of two ubiquitously expressed transmembrane proteins, IFN (α, β and ω) receptor 1 (IFNAR1) and IFNAR2 (the genes for which are clustered on chromosome 21), and are associated with two cytoplasmic tyrosine kinases, TYK2 and JAK1 (REF. 39) (FIG. 3). Formation of the IFN-receptor complex involves one side of the IFN protein interacting with IFNAR2 in a region forming the hinge between the two fibronectin type III (FnIII) domains (FIG. 3); binding affinity is in the nanomolar range³⁹. IFNAR1 binds IFNs with an affinity 1,000-fold weaker than that of IFNAR2, with a binding site located opposite to the IFNAR2 binding site. Binding studies are consistent with the ternary complex between IFNAR1, IFN and IFNAR2 having a 1:1:1 stoichiometry, and a similar if not identical architecture for all type I IFNs. Ternary complex assembly is a two-step process; the ligand binds first to one IFNAR and then recruits the second with no identified interaction between the two IFNARs³⁹.

As affinities for IFNAR2 are generally much higher than for IFNAR1, a binding pathway in which IFNs bind first to IFNAR2 and then IFNAR1 should have a higher probability. However, with IFN-α1 having a low affinity for IFNAR2, the relevance of the reverse-binding pathway, which could lead to differing cellular effects, has been confirmed⁴⁵. If differences in the structures of the IFN-receptor complexes cannot account for the differential activities of type I IFNs, then a body of argument — which includes studies on the activities of engineered IFNs — suggests that differential affinities for IFNARs and thus, ternary complex stability, govern differential biological activities^{39,46–48}. The cell-surface concentration of IFNARs and their lateral organization into microdomains could also be important cellular parameters that shape responsiveness to individual IFNs⁴⁹. Similar or other changes in receptor organization may also account for the increased susceptibility to HBV infection that occurs with polymorphisms in class II cytokine receptor genes⁵⁰.



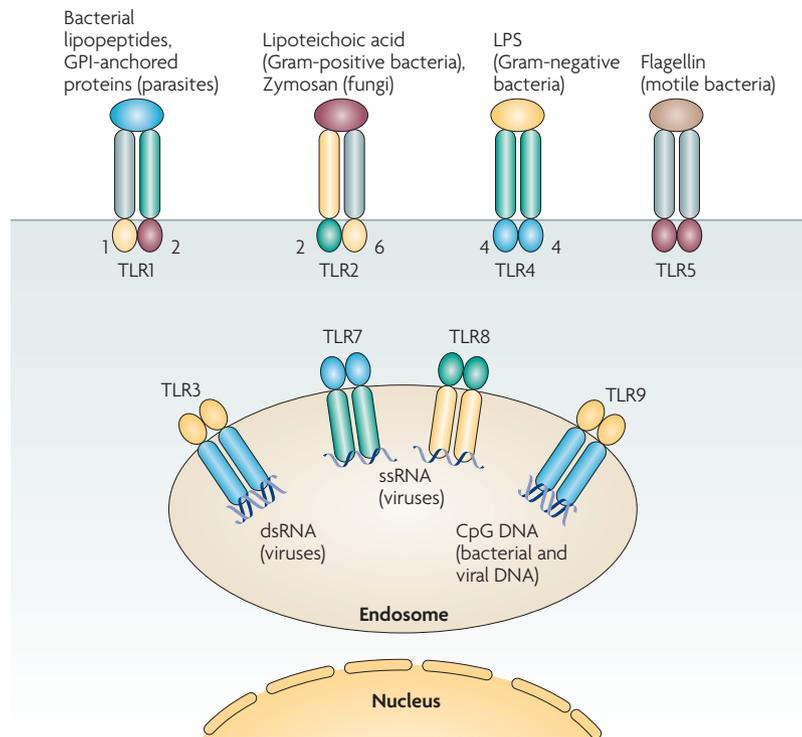


Figure 1 | Mammalian Toll-like receptors (TLRs) and their ligands. TLR1, TLR2, TLR4, TLR5 and TLR6 are located on the cell surface. Their extracellular domains (depicted as rods) bind specific microbial products that act as ligands and the intracellular domains (depicted as spheres) signal via specific cytoplasmic signalling proteins. TLRs function as homodimers or heterodimers. The ligands specific for several such dimers are listed at the top of the figure. Several other TLRs, such as TLR3, TLR7/8 and TLR9, recognize specific nucleic acids that are often produced by viruses. They span the endosomal membrane with the ligand-binding domains inside the lumen and the signalling domains in the cytoplasm. They also function as dimers and recognize double-stranded (ds) RNA, single-stranded (ss) RNA or dsDNA containing CpG sequences. GPI, glycosylphosphatidylinositol; LPS, lipopolysaccharide.

IFN- γ is a single glycosylated protein of 140 amino acids that is designated as a type II IFN because of its distant amino-acid sequence homology with type I IFNs, and its production by natural killer (NK) or activated T cells. Like type I IFNs, it binds to two class II cytokine receptor proteins; when ligand bound it forms a complex of two of each of the receptor proteins linked to an antiparallel homodimer of IFN- γ ⁵¹ (FIG. 3). IFN- γ receptor 1 (IFNGR1) maps to chromosome 6 and has a JAK1 binding domain and a STAT1 docking site. IFNGR2 contains a JAK2 binding domain and maps to chromosome 21q22.1 in a cluster that also contains IFNAR1, IFNAR2 and interleukin 10 receptor 2 (IL10R2; also known as IL10RB)⁵². Although IFNGR1 is constitutively present on all cells, IFNGR2 is tightly regulated and less widely expressed. Promoter polymorphisms and/or mutations within both chains have been associated with increased susceptibility to malaria and mycobacteria — in a few patients these defects have been reconstituted by marrow transplantation⁵³. The type III IFN family with three subtypes of IFN- λ , which are co-produced with IFN- β , activate the same main signalling pathway as type I IFNs but have evolved a completely different receptor structure⁵⁴ (FIG. 3).

How cells respond to IFNs

Initial studies identified several genes that were induced by type I IFNs and analysis of their promoters identified conserved DNA elements^{55–58}. Proteins bound to these elements after treatment with type I IFNs were purified and identified as STAT1, STAT2 and IRF9 (REFS 59–62). To identify other components of IFN-dependent signalling cascades, the promoter element of the 6–16 gene was used to drive IFN-dependent expression of guanine phosphoribosyl transferase; cells that did not respond to IFNs were then selected with 6-thioguanine. Following chemical mutagenesis, several mutant cell lines were obtained, each lacking a protein essential for signalling. For example, mutant *UIA* was shown by complementation to lack the tyrosine kinase TYK2 (REFS 60,62). Subsequently seven STAT and four JAK family members were identified; these transcription factors and tyrosine kinases have been shown to be essential for responses not only to IFNs but also to other cytokines and growth factors as well⁶¹.

Minimum requirements for a response to type I IFNs are the heterodimeric IFN receptor; the tyrosine kinases TYK2 and JAK1, which reciprocally transphosphorylate the receptor chains when activated; STAT1 and STAT2, which are phosphorylated in response to signalling; and the unphosphorylated IRF9 (FIG. 3). Transcription in response to IFN-dependent signalling is initiated by high-affinity binding to specific palindromic promoter sequences of the trimeric complex of STAT1, STAT2 and IRF9. The response to IFN- γ requires only the two receptor proteins, the kinases JAK1 and JAK2, and STAT1. STAT1 and STAT3 bind competitively to the same phosphotyrosine residue of IFNGR1, with the binding of STAT1 greatly favoured⁶³. Although initial work was carried out primarily in human fibroblasts, recent studies have identified additional complexity that allows individual cell types to respond by activating different STATs in response to the same IFN (reviewed in REFS 64,65). Analysis of defects in the IFN system has identified germline mutations in humans that result in deficiencies of STAT1 or TYK2, with enhanced susceptibility to infection by viruses^{61,66}. Although the mouse has been a useful model, the human defects are not always identical to the effects that result from the targeted deletions of these genes in mice⁶¹.

Upon activation of receptors, the JAKs undergo autophosphorylation and transphosphorylation to increase their activity, and then phosphorylate the IFN receptors and finally STATs. However, the kinase activities of the JAKs are not sufficient to explain all nuances of signalling. Tissue-specific differences in activating additional protein kinases probably contribute to the differential responses of various cells to a single type of IFN. In at least some cell types, the p85 subunit of phosphatidylinositol 3-kinase (PI3K) is associated with IFNAR1. The activation of p85 by IFN leads to AKT phosphorylation and expression of the chemokine (C-X-C motif) ligand 11 (CXCL11) gene, encoding an important chemokine⁶⁷. Type I IFNs also activate p38, and inactivation of p38 blocks induction by IFN- β of CXCL11 and TNFSF10 (encoding tumour

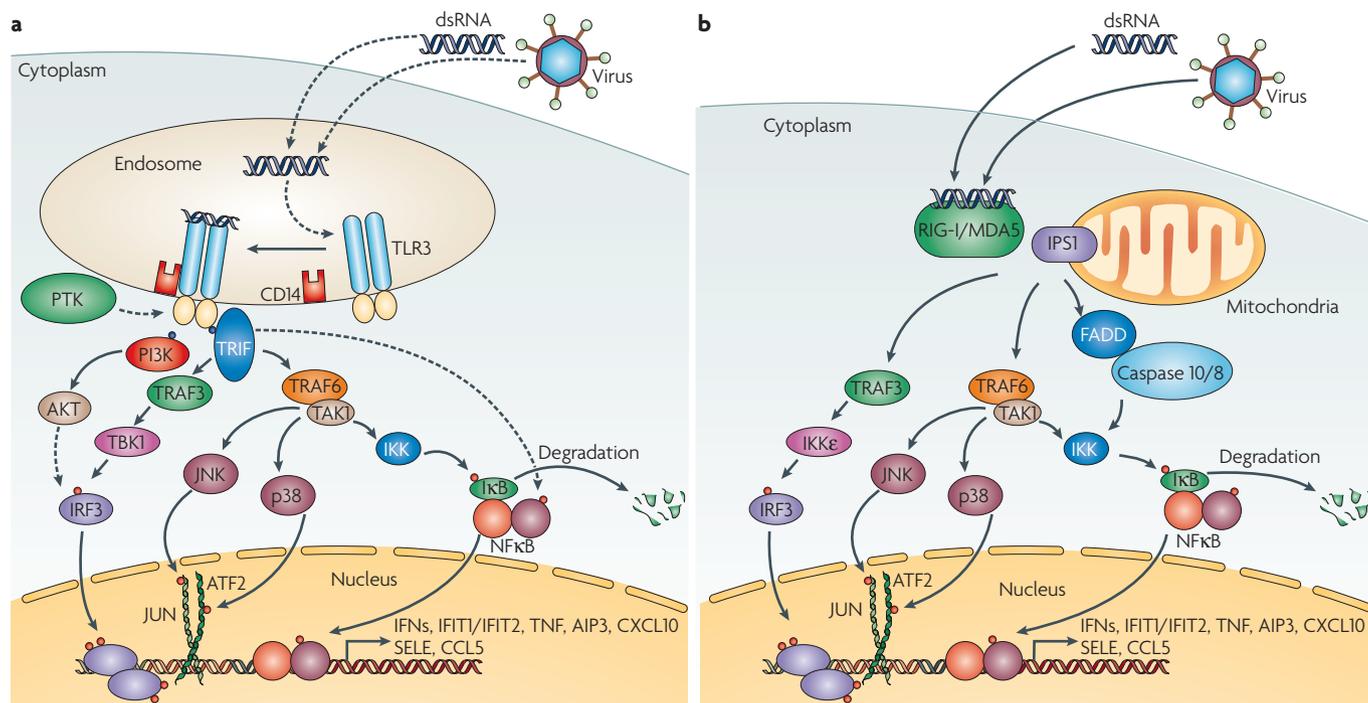


Figure 2 | Different interferon (IFN) signalling pathways activated by dsRNA and viruses. Extracellular double-stranded (ds) RNA or intracellular dsRNA produced during viral replication can activate different signalling pathways triggered by either membrane-bound Toll-like receptor 3 (TLR3) or cytoplasmic retinoic acid-inducible gene I (RIG-I; also known as DDX58) or melanoma differentiation associated protein 5 (MDA5; also known as IFIH1). **a** | TLR3 recognizes dsRNA in the lumen of the endosome, which causes phosphorylation of specific tyrosine residues in TLR3 by an unidentified protein tyrosine kinase (PTK). TLR3 dimerizes, binds to CD14 and activates the signalling complex assembled by TLR adaptor molecule 1 (TRIF). Two major pathways bifurcate from TRIF. One, composed of tumour necrosis factor (TNF) receptor-associated factor 3 (TRAF3) and TANK-binding kinase (TBK1/IKKε), leads to phosphorylation of the transcription factor IFN regulatory factor 3 (IRF3). IRF3 requires further phosphorylation by the phosphatidylinositol 3-kinase (PI3K)/AKT pathway for its full activation, which is initiated by binding PI3K to phosphorylated TLR3. The other branch acts through TRAF6 and transforming growth factor-β-activated kinase 1 (TAK1; also

known as MAP3K7) leading to the activation of nuclear factor-κB (NFκB), JUN and activating transcription factor 2 (ATF2) transcription factors. The activated transcription factors translocate from the cytoplasm to the nucleus, bind to the cognate sites in the promoters of the target genes and singly or in combinations induce their transcription. **b** | The cytoplasmic RNA helicases RIG-I and MDA5 recognize dsRNA or 5' triphosphorylated single-stranded (ss) RNA and use the mitochondrial membrane-bound protein IFN-β-promoter stimulator 1 (IPS1; also known as VISA) as the specific adaptor. IPS1 functions like TRIF and activates the same transcription factors leading to the induction of similar genes. In addition, they cause apoptosis by activating caspases 8 and 10 through the interaction of FADD with IPS1. Solid arrows denote steps that have been fully delineated, stippled arrows show steps that contain as yet unknown intermediaries. AIP3, atrophin-1 interacting protein 3; CCL5, chemokine (C-C motif) ligand 5; CXCL10, chemokine (C-X-C motif) ligand 10; IFIT1/2, interferon-induced protein with tetratricopeptide repeats 1/2; IKK, inhibitor of NFκB kinase; SELE, selectin E (endothelial adhesion molecule 1).

necrosis factor-related apoptosis-inducing ligand, APO2L/TRAIL)⁶⁸ and of CXCL10 (encoding the chemokine IP-10; also known as IFN-γ-induced peptide, 10 kDa) in primary leukocytes⁶⁹.

An important function of the activation of protein serine kinases such as p38 and protein kinase C (PKC) in response to IFN-dependent signalling is phosphorylation, directly or indirectly, of transcription factors⁷⁰. Serine 727 of STAT1 is phosphorylated in response to IFN-γ by the kinase cascade PI3K—AKT—PKC—MKK4—p38 (MKK4, mitogen-activated protein kinase kinase 4; also known as MAP2K4), with some variation in the activation of different PKC or MKK proteins in different cells⁶⁵. IFN-dependent activation of PI3K, extracellular response kinases (ERKs) and p38 stimulates the phosphorylation of NFκB (but not IκB), AP-1 and possibly PU.1, respectively. These activated transcription factors may then either drive gene expression independently of

activated STATs or cooperate with activated STATs on certain promoters (FIG. 4). Conversely, transcription initiated by phosphorylated STATs does not proceed indefinitely; homeostasis and balance result from the actions of phosphatases such as SHP1 and SHP2 and a family of ISGs, the suppressor of cytokine signalling (SOCS) proteins^{71,72}. SOCS inhibit receptor signalling both by directly inhibiting JAKs and by targeting the receptor complex for proteasomal degradation⁷¹.

Prior exposure to other cytokines conditions how a cell will respond and, conversely, IFNs condition responses to other cytokines^{64,65,73}. An excellent example of such an effect is prior exposure of human macrophages to IFN-γ, which changes the response to IL10 from activation of STAT3 to activation of STAT1 (REF. 74). Although, as reductionist scientists, we tend to study the responses of cells in culture to treatment with IFNs alone, the situation *in vivo* is obviously much more complex.

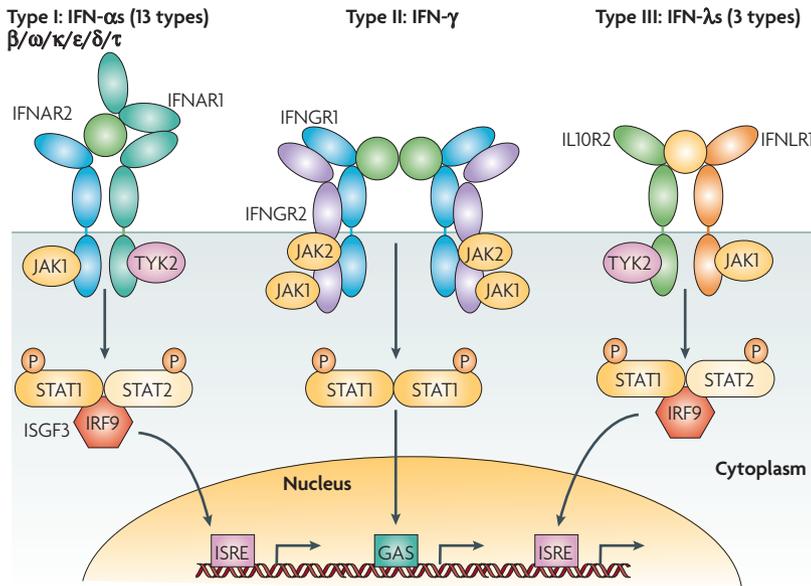


Figure 3 | Receptor activation or ligand–receptor complex assembled by type I, type II or type III interferons. Type I interferons (IFNs) (α , β , ω , κ , ϵ , δ (pigs), τ (ruminants)) interact with IFN (α , β and ω) receptor 1 (IFNAR1) and IFNAR2; type II IFN- γ with IFN- γ receptor 1 (IFNGR1) and IFNGR2; and type III IFN- λ s with IFN- λ receptor 1 (IFNLR1; also known as IL28RA) and interleukin 10 receptor 2 (IL10R2; also known as IL10RB). Type II IFN- γ is an antiparallel homodimer exhibiting a two-fold axis of symmetry. It binds two IFNGR1 receptor chains, assembling a complex that is stabilized by two IFNGR2 chains. These receptors are associated with two kinases from the JAK family: JAK1 and TYK2 for type I and III IFNs; JAK1 and JAK2 for type II IFN. All IFN receptor chains belong to the class 2 helical cytokine receptor family, which is defined by the structure of the extracellular domains of their members: approximately 200 amino acids structured in two subdomains of 100 amino acids (fibronectin type III modules), themselves structured by seven β -strands arranged in a β -sandwich. The 200 amino-acids domain usually contain the ligand binding site. IFNAR2, IFNLR1, IL10R2, IFNGR1 and IFNGR2 are classical representatives of this family, while IFNAR1 is atypical as its extracellular domain is duplicated. GAS, IFN- γ -activated site; IRF9, IFN regulatory factor 9; ISGF3, IFN-stimulated gene factor 3, refers to the STAT1–STAT2–IRF9 complex; ISRE, IFN-stimulated response element; P, phosphate; STAT1/2, signal transducers and activators of transcription 1/2.

Both STAT1 and STAT3 have activities in addition to their roles as cytokine-activated transcription factors. *STAT1* is activated in response to both type I and type II IFNs and *STAT3* is activated in response to gp130 cytokines such as IL6. As *STAT1* and *STAT3* drive essentially opposite biological responses, large signal-dependent changes in their concentrations will affect their relative activation by a further signal. Indeed, an increase in the ratio of *STAT1*–*STAT3* after IFN- α 2 treatment of patients with melanoma correlated with survival⁷⁵. Another consequence of cytokine-dependent increases in *STAT* expression is that unphosphorylated *STAT1* and *STAT3* have important functions that are quite distinct from those of the phosphorylated proteins^{76,77}. For example, unphosphorylated *STAT3* activates a subset of κ B-dependent genes by forming a complex with NF κ B⁷⁸. Thus the role of *STATs* in signalling after receptor binding has expanded from kinase-activated transcription factors to proteins that, even in the absence of ligand activation, activate transcription and participate in cell-type specificity, resulting in diverse patterns of ISG induction in different cell types in response to a single IFN.

ISGs: molecular mechanisms of antiviral action

ISGs are a diverse group of more than 300 genes (which and how many are a function of cell-type signalling variations as discussed above) that mediate the biological and therapeutic effects of IFN stimulation^{79,80} (TABLE 1). Studies of their mode of action have resulted in fundamental discoveries concerning translational control, regulation of RNA stability and editing, and protein transport and turnover¹⁸. Furthermore, proteins that are induced upon IFN stimulation, especially those that can be activated or inhibited *in vivo*, are targets for high-throughput screening for identification of new modulators of the IFN system.

Examples for this are 2',5'-oligoadenylate synthetases (OASs) and ribonuclease L (RNASEL), which inhibit a broad range of RNA viruses⁸¹. Viral dsRNA can directly activate one of several human OAS proteins to produce a unique 2'-to-5' linked oligoadenylate of 3–6 bases (2–5A) from ATP⁸². The only well-established function of 2–5A is activation of the ubiquitous, latent enzyme, RNASEL⁸³. 2–5A binding to RNASEL induces monomeric, inactive RNASEL to dimerize into a potent endoribonuclease that cleaves single-stranded regions of RNA on the 3' side of UpUp and UpAp dinucleotides^{84–86}. The OAS–RNASEL pathway can inhibit the replication of encephalomyocarditis virus, Coxsackie virus B4, West Nile virus, some retroviruses and HCV⁸¹. Furthermore, degradation of cellular mRNA and rRNA by RNASEL damages the host cell machinery that is required for viral replication and can result in apoptosis, contributing to both antiviral and antitumour actions^{87–89}. RNASEL also cleaves self-RNA into small degradation products that activate the recognition receptors, RIG-I and MDA5, to induce IFN- β , similar to that of non-self viral RNA⁹⁰, thus perpetuating and amplifying the production of IFN- β . A high-throughput screen has resulted in the identification of small molecules that can activate RNASEL and produce broad-spectrum antiviral effects⁹¹.

The dsRNA-activated protein kinase (PKR) and OAS were the first enzymes identified that uniquely respond to IFNs^{92,93}. PKR is a serine/threonine kinase that mediates translational and transcriptional control in response to dsRNA and other signals^{92–96}. In addition, the cellular protein PACT (also known as PRKRA) activates PKR in the absence of dsRNA⁹⁷. PKR mediates translational control by phosphorylating the protein synthesis initiation factor EIF2 α , resulting in an inactive complex between EIF2–GDP and the recycling factor, EIF2B. These events produce global inhibition of protein synthesis that blocks further viral replication and full amplification of the viral-induced cellular stress response. Many viruses, however, evade PKR through a range of strategies such as binding and sequestering dsRNA, thus depriving PKR of its activator or inhibition of its kinase activity⁹⁸.

Another ISG family that influences translation is the strongly induced p56-related proteins (*IFIT* gene products). Two of these, p56 and p54, inhibit protein synthesis by blocking the action of the translation initiation factor EIF3 (REF. 99). p56 and p54 bind to different subunits

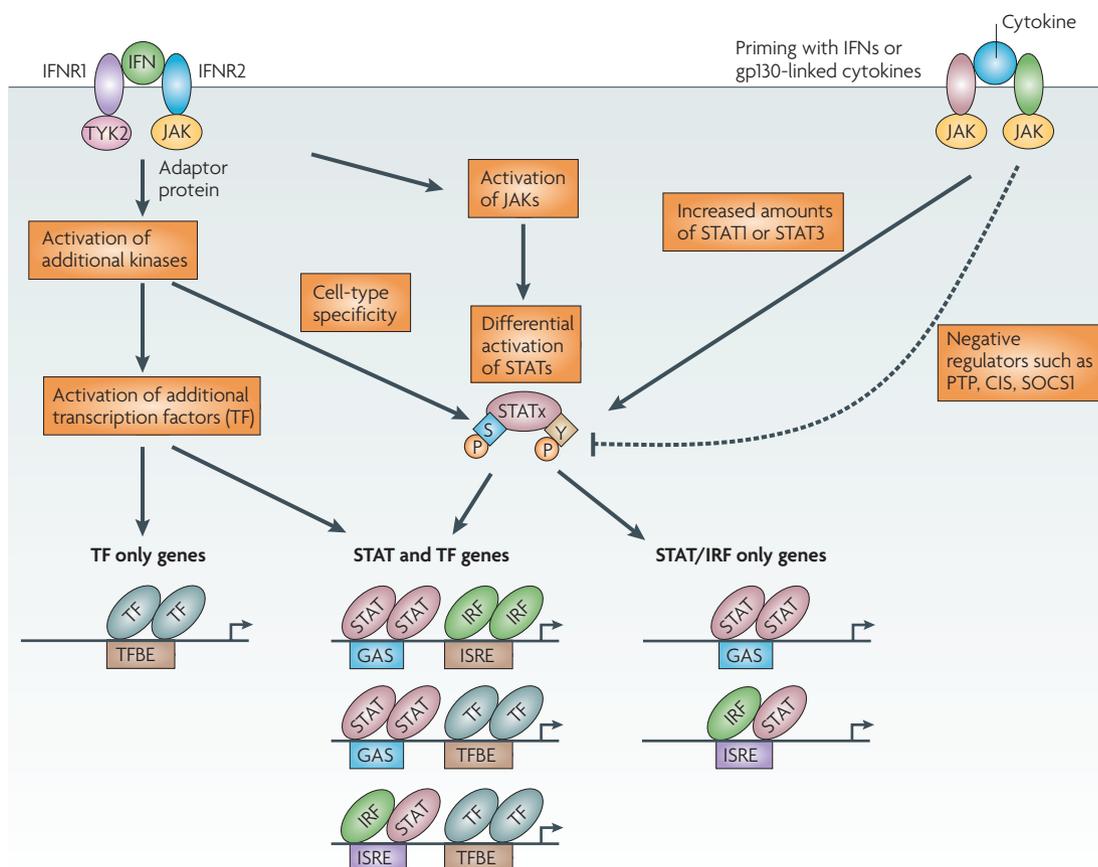


Figure 4 | Complexity of the signalling response. Different types of cells respond differentially to a single type of interferon (IFN) by varying the activation of specific signal transducers and activators of transcription (STATs), additional transcription factors (TFs) and kinases in addition to the Janus kinases (JAKs). Priming of cells by pre-treatment with another cytokine modulates the response further by increasing the amounts of negative regulators and by modulating other processes. Most genes require STATs, with or without additional TFs, and several genes respond only to activated TFs and not to STATs. The STATs bind to IFN- γ -activated site (GAS) elements or, together with IFN regulatory factor (IRF) proteins, to IFN-stimulated response elements (ISREs), and the TFs bind to specific binding elements (TFBE). CIS, cytokine inducible SH2-containing protein; PTP, protein tyrosine phosphatase; SOCS1, suppressor of cytokine signalling 1.

of EIF3 and block some of its diverse functions. HCV mRNA translation is inhibited more strongly by p56 than by cellular mRNAs, because its initiation is internal ribosome entry site (IRES)-mediated, and not CAP-mediated; thus it selectively inhibits viral protein synthesis¹⁰⁰.

The ISG-encoded antiviral protein Mx was identified because of the resistance of mouse strain A2G to influenza A viruses (*Mx1*, orthomyxovirus resistance gene 1)^{101–103}. Mx proteins are large (~80 kDa) GTPases in the dynamin superfamily that self-assemble and bind viral nucleocapsids. This interferes with intracellular trafficking and activity of viral polymerases, thus inhibiting replication of many RNA viruses including influenza and measles viruses¹⁰⁴. The human homologue, MXA, is a cytoplasmic protein that associates with intracellular membranes.

ISG15 encodes a ubiquitin-like, 15 kDa protein that modifies more than 100 proteins through a process known as ISGylation^{105–107}. ISG15 inhibits HIV-1 release from cells, mimicking the effect of IFN¹⁰⁸ and infections by influenza, herpes and Sindbis viruses¹⁰⁹. The antiviral mechanism of ISG15 *in vivo* is unknown but could relate

to its cytokine-like properties or to its ability to conjugate and modify the function of cellular or viral proteins. *ISG15* is also a target gene for dysregulation of the p53 and ISG pathways that occurs in many types of cancer, suggesting an additional role in tumorigenesis¹⁰⁷.

Phospholipid scramblase 1 (PLSCR1), a protein implicated in Ca²⁺-dependent reorganization of plasma membrane phospholipids¹¹⁰, either inserts into the plasma membrane or binds DNA in the nucleus depending on its palmytoylation¹¹¹. PLSCR1 has antiviral activity that possibly results from the enhanced transcription of a subset of ISGs¹¹². TRAIL/APO2L is an ISG that contributes to apoptosis and therefore probably to both the antiviral and antitumour effects of IFNs^{113–115}. Although many ISGs promote apoptosis, some promote cell survival. For example, the ISG *GIP3* (or 6–16)^{116,117} localizes to mitochondria and has anti-apoptotic actions, including inhibition of caspase-3 (REF. 118). A related ISG, encoded by *IFI27* (*ISG12*)¹¹⁹, promotes an age-dependent resistance to alphavirus encephalitis in mice without affecting either levels of apoptosis or viral yields¹²⁰. Additional ISGs with

Caspase

A group of enzymes that have a role in promoting apoptosis (that is, programmed cell death). Inhibition of such enzymes might be useful for combating cell and tissue damage in conditions such as myocardial infarction, stroke, inflammatory diseases and neurodegenerative diseases. Augmentation of such enzymes, through the production of pro-apoptotic proteins, might be useful for combating proliferative conditions, such as cancer.

Table 1 | ISGs in the antiviral and anticancer effects of IFNs

Gene	Protein function	Mechanism of action	Refs
OAS, RNASEL	RNA cleavage	Degrades viral and cellular RNA, induces IFN- α/β	81–91
PKR	EIF2 α phosphorylation	Blocks protein synthesis, transcriptional signalling	94–98
p56	Binds EIF3	Blocks protein synthesis	99,100
Mx1	Wraps around viral nucleocapsids	Interferes with intracellular trafficking	101–104
ISG15	ISGylation	Cytokine-like, protein modification	105–107
PLSCR1	Phospholipid migration, DNA binding	Enhances expression of some ISGs	110–112
TRAIL/APO2L	Ligand of death receptor	Apoptosis	113–115
XAF1	Blocks inhibitor of apoptosis (XIAP)	Apoptosis	247
G1P3 (6–16)	Inhibits caspase 3	Anti-apoptotic	116,117
ISG12	Not known	Antiviral	119,120
GBP1	GTPase	Antiviral, angiogenesis inhibitor	121,268
ISG20	3'-exonuclease for RNA and DNA	Antiviral	122
PML	Not known	Antiviral, antitumor	123
ADAR1	Adenosine deaminase for dsRNA	RNA editing, altered translation	124
Viperin (cig5)	Not known	Antiviral	125
iNOS	Nitric oxide synthase	Antiviral	126
Nup98/Nup96	Nucleoporin, RNA and protein transporters	Antiviral	127,270
IRF7, RIG-I, MDA5, STAT1	Signalling to IFN- α/β genes or to ISGs	Induction of type I IFNs	79,80

ADAR1, adenosine deaminase, RNA-specific; dsRNA, double-stranded RNA; EIF2 $\alpha/3$, eukaryotic initiation factor 2 $\alpha/3$; G1P3, interferon, α -inducible protein; GBP1, guanylate binding protein 1, interferon-inducible, 67kDa; iNOS, inducible nitric oxide synthase; IRF7, interferon regulatory factor 7; ISGs, interferon-stimulated genes; MDA5, melanoma differentiation associated protein 5 (also known as IFI1); Mx1, myxovirus (influenza virus) resistance 1, interferon-inducible protein p78; Nup98/Nup96, nucleoporin 98/96 kDa; OAS, 2'-5'-oligoadenylate synthetase; PKR, protein kinase R; PLSCR1, phospholipid scramblase 1; PML, promyelocytic leukaemia; RIG-I, retinoic acid-inducible gene 1 (also known as DDX58); RNASEL, ribonuclease L; STAT1, signal transducer and activator of transcription 1, 91 kDa; TRAIL/APO2L, tumour necrosis factor-related apoptosis-inducing ligand (also known as TNFSF10); Viperin (cig5), also known as RSAD2; XAF1, X-linked inhibitor of apoptosis-associated factor 1; XIAP, X-linked inhibitor of apoptosis protein (also known as BIRC4).

probable or confirmed antiviral activities include the guanylate-binding protein 1 (GBP1); a 3',5'-exonuclease encoded by *ISG20*; the promyelocytic leukaemia protein (PML); adenosine deaminase (ADAR1); the endoplasmic reticulum-associated protein Viperin (cig5) that can inhibit human cytomegalovirus; inducible nitric oxide synthase (iNOS); and the nucleoporins Nup98 and Nup96 (REFS 121–128).

Finally, many IFN-pathway signalling proteins are themselves ISGs, thus providing an autocrine loop that amplifies IFN responses; examples are IRF7, RIG-I, MDA5 and STAT1. As ISGs with high levels of transcriptional induction are still poorly characterized functionally^{79,80,128}, some could prove to be critical mediators of antiviral and other actions. Because all biological effects of IFNs are mediated through the action of ISGs (TABLE 1), further research into understanding the functions of the protein products of these may lead to more efficacious antiviral and other therapeutics.

Innate and adaptive immunity

In addition to direct inhibition of viral replication by ISGs, a second level of IFN action augments adaptive and acquired immune responses. Early warning of pathogen presence is delivered by tissue-associated and circulating dendritic cells, one type of which, the plasmacytoid

dendritic cell, is the circulating type I IFN-producing cell¹²⁹. In addition to TLR activation on cells at the sites of pathogen invasion or replication, this response culminates with dendritic cell-mediated presentation to CD4⁺ T cells of pathogen-derived peptide fragments that are bound to surface major histocompatibility complex (MHC) class II molecules. MHC class II proteins are selectively upregulated by IFN- γ , whereas type I IFNs fail to do so owing to the STAT2-dependent induction of SOCS1 (REF. 130). Infected cells that display peptide fragments associated with MHC class I molecules on the surface are recognized and subsequently eliminated by CD8⁺ T cells, thereby clearing the virus. Either type I IFNs or IFN- γ can markedly upregulate MHC class I-dependent antigen presentation. In addition to MHC molecules, other ISGs involved in antigen processing include the lysosomal membrane permeabilization (LMP) components of proteasomes and transporters for antigen processing (TAPs), which shuttle peptides into the endoplasmic reticulum for loading onto nascent MHC class I proteins^{131–135}.

IFNs also promote accumulation of leukocytes at sites of pathogen invasion; specifically, IFNs (along with cytokines such as TNF α and IL1 β), strongly promote the expression of vascular adhesion molecules including intracellular adhesion molecule 1 (ICAM1). Furthermore, IFNs induce the production of chemotactic

Inducible nitric oxide synthase

(iNOS). An inducible haem-containing enzyme that produces nitric oxide in response to inflammatory signals.

Nucleoporins

Proteins that function in the nuclear transport of protein and RNA.

cytokines (chemokines), which participate in leukocyte recruitment. As examples, three closely related chemokines involved in accumulation of activated T cells and macrophages are the ISGs CXCL9 (also known as MIG, monokine induced by IFN- γ); CXCL10 (also known as IP-10, IFN- γ 10 kD inducible protein); and CXCL11 (also known as I-TAC, interferon-inducible T-cell α -chemoattractant)^{136–139}. True to their names, these chemokines are not expressed in the absence of IFN signalling.

In the development of an adaptive immune response, IFN- γ is produced by an early warning NK cell, or by activated T cells¹⁴⁰. IFN- γ governs expression of class II transactivator (CIITA), a master regulator of transcription of the MHC class II molecules themselves, as well as the associated invariant chain, which helps stabilize MHC class II heterodimers. HLA-DM catalyses the displacement of the invariant chain from the MHC class II peptide binding site as the mature MHC class II-peptide conjugate is finalized for insertion into the plasma membrane^{141–149}. Finally, of substantial importance in the host response to the virus is the IFN-mediated activation of cytotoxic effector function among cells of innate and adaptive immunity including NK cells, dendritic cells, macrophages and T cells. Indeed, the property of stimulating macrophages contributed substantially to the recognition of IFN- γ as a biologically important lymphokine and as a ‘different’ IFN¹⁵⁰.

Human therapeutic applications

Based on preclinical studies of broad spectrum inhibition of virus replication, IFNs were initially investigated as antivirals with activity against RNA and DNA viruses. Clinical effectiveness for both has now been established. But development of relatively specific, low molecular mass antivirals has largely supplanted broad application except for HBV and HCV chronic infections. The first US Food and Drug Administration (FDA) approval for IFN- α 2, however, was not for virus infection but for cancer, which was driven by interest created by publicity resulting from its effectiveness in American Cancer Society trials. Subsequently, placebo-controlled randomized trials established the effectiveness of IFN- β for relapsing, remitting MS — an apparent paradox in terms of the mechanistic understanding of IFN actions, as IFNs, as discussed above, are generally immune augmenting rather than immunosuppressive. Presently, a number of drugs are being or have been designed to target different components of the IFN system for different therapeutic indications (FIG. 5).

Viruses. The recognition that HBV often caused a chronic infection leading to cirrhosis and hepatocellular carcinoma suggested that infected patients might benefit from IFNs¹⁵¹. Initial clinical trials of impure IFN- α ¹⁵² suggested benefit but studies with impure IFN- β were less promising¹⁵³. These low-dose studies were followed, however, by higher doses of recombinant IFNs, when they became available, which then confirmed beneficial effects¹⁵⁴.

HBV chronic infection evolves with hepatitis e antigen (HBeAg)-positive quiescent viruses escaping inhibition during conversion of an immunotolerant

to an immunoactive phase, with enhanced immune elimination of infected hepatocytes^{155,156}. In many, this immune response causes suppression of viral replication and HBeAg loss. A quiescent phase or ‘healthy carriage’ may ensue but disease reactivation is common (HBeAg-negative disease¹⁵⁷). In HBeAg-positive early HBV infection, IFNs have not been particularly effective. However, in the immunoactive chronic phase, HBV is sensitive to IFN- α 2 and the ongoing immune response is augmented, leading to quiescent HBeAg-negative disease in up to 40% of patients. IFN- α 2 (Roferon-A, Hoffmann-LaRoche; Intron-A, Schering-Plough), now usually in the form of a long-acting pegylated version, has been widely used to treat HBeAg-positive HBV infections¹⁵⁸.

IFN- α 2 has also been used in the HBeAg-negative disease that develops when viral mutations permit viral reactivation following HBeAg loss. IFNs reduce viraemia (usually by over 90%) and induce host responses, but drug withdrawal often leads to disease recurrence; however, a proportion of patients (approximately 10–15%) have a prolonged period of viral suppression¹⁵⁹. Therapy for chronic HBV infection illustrates the two complementary activities of IFNs: in HBeAg-positive disease IFN increases an immune response, whereas in HBeAg-negative disease IFNs act as direct antivirals.

In the late 1980s ‘non-A, non-B hepatitis’ or ‘post-transfusion hepatitis’ was effectively treated with IFNs¹⁶⁰. Subsequent studies identified the causative agent as HCV¹⁶¹. Initial clinical studies of IFN- α 2 resulted in sustained and curative virological responses in up to 20% of patients¹⁶². Response rates to monotherapy with IFN- α 2 for chronic HCV infection were transformed in the mid-1990s by combined use with the weak antiviral ribavirin — over 40% of patients responded¹⁶³. These results mimic studies for HSV keratitis in which therapy with a combination of IFNs and a weak antiviral agent (acyclovir) were synergistic¹⁶⁴. Therapy for chronic HCV infection has now evolved and current regimes commonly use a long-acting pegylated IFN- α 2 plus ribavirin with cure of up to 60% of patients. Possibly as a result of selection against RNASEL cleavage sites in its genome, or inhibition of PKR, effectiveness is much less for genotype 1 than for genotypes 2 and 3 of HCV^{162,271,272}.

In addition to HBV and HCV infections, other chronic viral infections have been effectively treated. Both systemic and topical IFN- α s and IFN- β have reduced virus titres and decreased clinical manifestations of herpes zoster, HSV and cytomegalovirus infections^{165–169}. Almost simultaneous introduction of acyclovir and its analogues, however, which proved to have greater clinical efficacy and reduced side effects, ended clinical development of IFNs for these indications. Papilloma virus infections of skin, larynx and genitals were found to respond with regression of warts upon either intralesional or systemic administration of IFN- α s and IFN- β ^{170–175}. When compared with placebo, useful therapeutic effects resulted for patients with extensive or refractory disease, but permanent eradication was infrequent. These studies did, however, establish a basis for the use of the TLR7 IFN-inducing agonist imiquimod topically for genital warts with decreases in HPV DNA and with complete

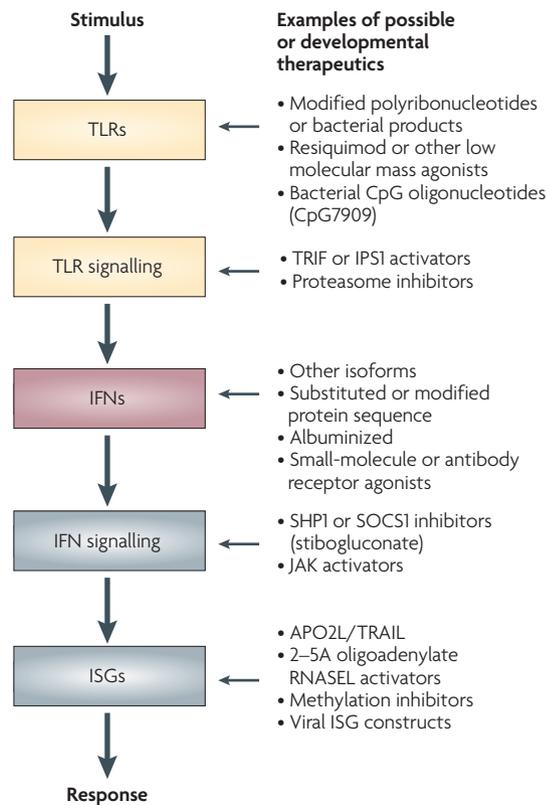


Figure 5 | Potential drug targets in the interferon (IFN) system. Examples of potential or developmental drugs targeted at different steps in the pathways are presented. IPS1, IFN- β promoter stimulator 1 (also known as VISA); ISG, IFN-stimulated gene; JAK, Janus kinase; RNASEL, ribonuclease L; SOCS1, suppressor of cytokine signalling 1; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand (also known as APO2L); TRIF, TLR adapter molecule 1.

response frequencies of approximately 50% compared with 5% in placebo controls^{176–178}. In studies of HIV, virus recovery and clinical manifestations of both early and late stages were reduced¹⁷⁹. However, effectiveness of azidothymidine or protease inhibitors was not enhanced when IFNs were used in combination¹⁸⁰.

Once adequate quantities of human IFNs became available through recombinant DNA production, prophylaxis and treatment of acute respiratory virus infections were assessed. Reduction in virus yield, infection frequency and symptom scores resulted from administration of intranasal IFNs in experimental challenge infections with rhinoviruses, influenza viruses and coronaviruses^{181–184}. Prophylactic efficacy for natural rhinovirus colds in family and work settings was also identified^{185–187}. However, as a result of the nasal erosions and bleeding resulting from mucosal irritation by IFN- α 2 and IFN- β , symptom scores under field conditions were significantly higher in treated patients when compared with placebo. Case-based use in the setting of the severe acute respiratory syndrome (SARS) epidemic suggested clinical effectiveness of the consensus sequence type I IFN against the coronavirus aetiological agent¹⁸⁸. These findings and the preclinical and clinical antiviral effectiveness with topical

and/or high-dose administration suggest that IFNs might have prophylactic or therapeutic effectiveness in SARS or in an influenza or other virus pandemic.

Multiple sclerosis (MS). In about 85% of patients with MS, an inflammatory demyelinating disorder of the central nervous system, disease begins with approximately annual episodes of transient neurological dysfunction (relapsing–remitting MS or RR-MS). Initial studies of IFNs in the 1970s followed tissue-culture studies suggesting that cells from MS patients secreted less IFN-like activity following viral induction than did controls. These findings, combined with a notion that a slow or chronic viral infection might be causative, resulted in the evaluation of using an intrathecal, impure IFN- β as therapy that identified a reduction in relapses¹⁸⁹; however, subsequent clinical trials were either inconclusive (IFN- α 2) or detrimental (IFN- γ)^{190–192}. But in 1993, recombinant IFN- β given subcutaneously in a randomized placebo-controlled trial for RR-MS reduced relapses by about a third and resulted in marked reductions in subclinical disease, as assessed by magnetic resonance imaging (MRI)^{193,194}. This report ushered in the modern age of MS therapeutics: by showing that the natural history of MS could be modified; by documenting that IFN- β was clinically beneficial; and by the demonstration of MRI lesions as a useful surrogate of clinical effectiveness, now widely used in MS drug development. It is now common clinical practice to initiate IFN- β (Betaferon/Betaserom, Bayer Schering/Chiron; Avonex, Biogen Idec; Rebif, Merck Serono (or medications of comparable efficacy)) at the time of diagnosis¹⁹⁵. Attacks decrease by about 30%, numbers of new and active MRI lesions (which reflect inflammation) are often reduced as soon as 1-month after initiation, and long-term clinical benefits are now considered plausible¹⁹⁶. Although it has unequivocally represented a breakthrough, improvements on IFN- β are needed as it is only partially effective and is expensive for the life-long, non-curative use^{195,197}.

Pathogenesis of MS remains unknown but evidence implicates genetic–environmental interactions with critical timing of exposures to initiating factors. Epidemiological studies highlight Epstein–Barr virus and low plasma levels of vitamin D, and genetic studies implicate several polymorphic variants of immune-response genes^{198–201}. The most obvious clinical outcome from IFN- β is a reduction in MRI lesions^{202–204}, and protein products of ISGs probably mediate these effects.

As one example, an IFN- β ISG product, CD69, forms an inhibitory association with a sphingosine 1-phosphate receptor (S1PR). The consequence *in vivo* is suppressed lymphocyte exit from lymph nodes and restriction in numbers of circulating lymphocytes available to cross the blood–brain barrier²⁰⁵. Reduction in expression of matrix metalloproteinase 9 (MMP9) in activated lymphocytes and increased soluble vascular cell adhesion molecule (sVCAM) levels in plasma have also been identified and assigned putative roles in the beneficial effects of IFN- β for patients with MS^{202,206,207}. Expression-array studies and candidate gene evaluations have been applied, without

MRI lesions

Regions of abnormal signals in the brain or spinal cord, detected by magnetic resonance imaging (MRI), and indicative of tissue changes related to the multiple sclerosis pathogenic process. Depending on the imaging technique, these MRI changes can reflect inflammation, demyelination, axonal destruction or scarring.

Box 1 | Important research questions for the future

- What specific roles do the multiple isoforms of interferons (IFNs) play in host defences?
- What new roles will be identified for the IFN system and their protein products in the crosstalk between innate and cellular immunity?
- How do cellular gene products avoid activating Toll-like receptor (TLR) pathways while microbial gene products activate them very efficiently?
- What are cell type differences in other alternative signalling pathways interacting with JAK/STAT?
- What are cell and tissue specific differences in molecular responses?
- How do responses differ in cells of different maturities (for example, stem cells, senescent cells)?
- What are the functions of the still many uncharacterized IFN-stimulated genes (ISGs)?
- Which of the ISGs are most important for the specific cellular and clinical effects?
- Will ways of activating the IFN system prove therapeutically useful for the newly human papilloma virus (HPV)-associated squamous neoplasms of upper airways?
- What mechanisms cause the difficult spectrum of clinical side effects of fatigue and anorexia?
- Do IFNs play a bystander or pathogenic role in lupus erythematosus, rheumatoid arthritis or aplastic anaemia?
- Do low or high-affinity antibodies to IFNs or their protein products influence innate immunity?
- What are the molecular causes of resistance to IFNs in viral diseases? Multiple sclerosis? Cancer?
- Will helicases or proteases augment activity of IFNs for hepatitis B virus infections?
- Will anti-angiogenic or promoter methylation inhibitors increase antitumour activity?
- Can novel receptor agonists and antagonists be engineered for specific functions?
- Can a physicochemical rather than biological standard be developed for scientific and regulatory comparison?
- Will small-molecule activators of IFN-inducible proteins have antiviral and/or antitumour effects?
- How effective will components of the IFN system prove as immunological adjuvants?
- Can effective oral systemic inducers be identified?

success, in attempts to identify molecular biomarkers of the therapeutic effect of IFN- β in MS²⁰⁸. Development of validated outcome measures for treatment success (and failure) will aid in this process²⁰⁹.

Cancer. Based on the reduction in disease morbidities^{210,211}, initial regulatory approvals for the marketing of IFN- α 2 for a chronic B (hairy) cell leukaemia occurred within 5 years from clinical introduction as a result of close collaboration between academic institutions, government and industry. In hairy cell leukaemia and chronic myelogenous leukemia (CML), IFN- α 2 decreased marrow infiltration with malignant cells and normalized peripheral haematological parameters^{210,212–214}. In CML, in addition to reductions in leukaemic cell mass, a decrease resulted in cells with the abnormal, activated BCR-ABL kinase^{212–214}. Over 90% of patients with CML with complete cytogenetic response were in remission at 10 years²¹³. However, the survival advantage for IFN- α 2, when compared with chemotherapy for CML, has now been exceeded by the even greater effectiveness of the targeted inhibitor of the activated BCR-ABL kinase, such as imatinib (Gleevec; Novartis) and now other newer tyrosine kinase inhibitors.

In addition to hairy cell leukaemia and CML, therapeutic effectiveness of IFN- α 2 in causing at least partial disease regression has been identified in more than a dozen other malignancies including myeloma, lymphomas, melanoma, renal cell and bladder carcinoma, and Kaposi's sarcoma²¹⁵. For example, in lymphomas of various histologies and of both B-cell and T-cell phenotypes, IFN- α 2 has been effective in inducing tumour regressions in almost half of the patients involved in the study, and even in patients previously treated with chemotherapy^{215,216}. Prolonged disease-free and overall survival in intermediate prognosis lymphomas has resulted from IFN- α 2 in combination with chemotherapy, even given for limited periods, in randomized multicenter trials^{215,216}. International Phase III trials have been conducted with survival impact confirmed in metastatic renal carcinoma, but like in CML, the orally active, targeted tyrosine kinase inhibitors have changed the natural history of renal carcinoma, extending survival in metastatic disease more than the injectable IFN- α 2.

Cure of metastatic malignancies can result when micrometastases are eliminated in patients at highest risk for recurrence after surgical removal of a primary tumour. Effectiveness as a surgical adjuvant for murine tumours provided the rationale leading to pioneering clinical studies that suggested benefit of impure IFN- α when given after surgery for osteosarcoma^{217,218}. This surgical adjuvant approach was the basis for evaluation of IFN- α 2 in patients at high risk for recurrence of melanoma. Initial beneficial effects of significant prolongation of disease-free survival have now largely been validated by combined analyses of multi-institutional trials, by subsequent studies that have included evaluation of pegylated IFN- α 2 and by meta-analyses^{219–221}.

Like other potent physiological mediators such as glucocorticoids, IFNs have toxicities when administered with pharmacological intent^{222,223}. These have been dose related and particularly difficult at the high dose used for melanoma. With the initial dose, malaise, fever and chills, which last for a few hours, dominate but tachyphylaxis occurs with subsequent injections. Fatigue and anorexia, the aetiology of which is not understood, are often dose-limiting with chronic administration for cancer or MS; at higher doses weight loss occurs and may be significant (>10%). Reversible elevation of hepatic transaminases may occur, as may haematological effects, most markedly granulocytopenia.

Like in MS, failure to fully understand the mechanism(s) of antitumour action has slowed further development. Suppression, mutation and polymorphisms of IFNs and their signalling mechanisms in and by malignant cells are emerging as important contributors to cancer development^{224–234}. Mutations in RNASEL have been associated with prostate carcinoma and with presence of the retrovirus XMRV^{235,273–275}. Epigenetic and genetic silencing of IFN-signalling or ISG expression may also influence tumour development²³⁶. Reversal of these effects are likely to be the basis for effectiveness of IFNs and/or inducers in murine carcinogen-induced tumours, and may contribute to effectiveness in advanced disease, and provides a rationale for developing TLR agonists for

Glucocorticoids

A group of compounds that belongs to the corticosteroid family. These compounds can either be naturally produced (hormones) or synthetic. They affect metabolism and have anti-inflammatory and immunosuppressive effects. Many synthetic glucocorticoids (for example, dexamethasone) are used in clinical medicine as anti-inflammatory drugs.

chemoprevention^{237–239}. Indeed, TLR agonists appear to be effective and are already establishing a role in treatment of malignancy with the proven effectiveness of the TLR7 agonist imiquimod used topically for basal cell carcinomas as an example. Furthermore, relative clinical safety has been established for phosphorothioate oligoribonucleotide agonists for TLR9.

Induction of apoptosis by the ISG products APO2L/TRAIL and Fas has been identified in many malignant cell types, as has induction of APO2L/TRAIL on immune effector cell surfaces, thus sensitizing tumour cells to T-cell, NK cell and macrophage-mediated cytotoxicity^{240–243} (TABLE 1). Intralesional administration of IFN- α into basal cell carcinomas increased *Fas* expression and correlated with regression²⁴⁴. IFN- γ has increased susceptibility to apoptosis by Fas activators and cytotoxic chemotherapies in many cell types including melanoma and colorectal carcinoma^{245,246}. Through interactions with p53 and the inhibitor of apoptosis, XIAP, the ISG product XAF1 may allow APO2L/TRAIL to fully activate downstream caspases^{247,248}. In addition, the ISG product IRF1 can suppress another anti-apoptotic protein, Survivin²⁴⁹.

Antitumour activity *in vivo* may also be mediated by augmented lytic activity of immune effector cells and by enhanced immunogenicity of tumour cells. Both T-cell and NK-cell trafficking, expansion and lytic activity can be promoted by IFNs and ISGs; furthermore, IFN- γ is secreted from these activated cells into the tumour micro-environment^{250–255}. In addition to stimulating immune effector cells, IFNs have critical roles in antigen processing and presentation, as discussed above, both by T cells and dendritic cells²⁵⁶. In addition, IFN- γ can upregulate the tumour-associated antigens, carcinoembryonic antigen and TAG72, both *in vitro* and *in vivo*²⁵⁷.

IFNs can also inhibit angiogenesis by altering the stimuli from tumour cells and by directly inhibiting endothelial cells — indeed, they were the first angiogenic inhibitor identified²⁵⁸. Endothelial cells are inhibited in motility²⁵⁹, undergo coagulation necrosis *in vitro* and inhibition of angiogenesis occurs *in vivo* within 24 hours of tumour cell inoculation^{260,261}. Suppression of basic fibroblast growth factor (bFGF; also known as FGF2) correlated with reduced vascularization and tumour growth^{262,263}. IFNs also inhibit vascular endothelial growth factor (VEGF) mRNA and protein expression by regulating its promoter²⁶⁴. IL8, a mediator of angiogenesis, was inhibited *in vitro* and *in vivo* by IFN- α 2b and IFN- β ; other angiogenesis inhibitory members of the chemokine family, CXCL9, CXCL10 and CXCL11, are ISGs^{265–267}.

In endothelial cells, the ISG product guanylate binding protein 1, interferon-inducible, 67 kDa (GBP1), functioned as an inflammatory response factor inhibiting endothelial cell proliferation and angiogenesis in part through MMPs²⁶⁸. Clinically, IFN- α 2 has proved effective in the treatment of infantile haemangiomas, haemangioblastomas, giant cell tumour of the mandible and Kaposi's sarcoma^{215,269}. Thus, induction of ISGs that function as angiostatic inhibitors, coupled with secondary downregulation of angiogenic factors, may contribute to antitumour effects²⁶⁹.

Perspective

IFNs provide fundamental cellular defence mechanisms against viral infections and cancer and are thus critically important to the health of animals and humans. Because of their clinical effectiveness in limiting virus replication, reducing tumour cell mass, controlling disease symptoms and prolonging survival, IFNs are now licensed worldwide for the treatment of various viral, malignant and immune disorders; market sales approach US\$4 billion. As part of the innate immune response, IFNs are not only a principal cytokine that blocks viral replication through the action of specific ISGs, but also (particularly IFN- γ) mediate critical elements of the cellular immune response for recurring bacterial infections in chronic granulomatous disease and for mycobacteria. Because all biological effects of IFNs are mediated through the action of ISGs, understanding the functions of these genes may lead to more efficacious anticancer and antiviral therapeutics. For example, certain IFN-regulated proteins, such as OAS, RNASEL and PKR, exist in either latent inactive or active states, which could be targeted for potent antitumour and/or antiviral effects (FIG. 5).

IFNs have therefore more than reached the effectiveness anticipated by early virologists: they are not only an antiviral with a spectrum of clinical effectiveness against both RNA and DNA viruses, but have been the prototypical biological response modifiers for oncology, and have proved to have effectiveness in suppressing manifestations of MS. The study of IFNs has resulted in fundamental insights into cellular signalling mechanisms and innate and acquired immunity. In addition, their therapeutic use has improved the quality and quantity of life for millions of patients worldwide. However, to fully realize their potential, many questions remain unanswered (BOX 1). As exemplified by recent publications^{276–278}, further investigations will only enable IFNs to have even greater impacts in biomedicine.

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Competing interests statement
The authors declare **competing financial interests**: see web version for details.

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