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# Genetic Lesions of Type I Interferon Signalling in Human Antiviral Immunity

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The concept that type I interferons (IFN-I) are essential to antiviral immunity derives from studies on animal models and cell lines. Virtually all pathogenic viruses have evolved countermeasures to IFN-I restriction, and genetic loss of viral IFN-I antagonists leads to virus attenuation. But just how important is IFN-I to antiviral defence in humans? The recent discovery of genetic defects of IFN-I signalling illuminates this and other questions of IFN biology, including the role of the mucosa-restricted type III IFNs (IFN-III), informing our understanding of the place of the IFN system within the concerted antiviral response. Here we review monogenic lesions of IFN-I signalling pathways and summarise the organising principles which emerge.

## Is IFN-I Essential to Human Antiviral Immunity?

**Interferon (IFN**, see [Glossary](#)) was discovered more than 60 years ago by Isaacs and Lindemann [1]. This soluble factor, produced by virally infected cells in culture, conferred an **antiviral state** when applied to uninfected naïve cells prior to infection. Its discovery led to the clinical application of **IFN-I** (IFN $\alpha$ ) as the first host-directed antiviral therapy [2]. Over the intervening six decades, the molecular basis of IFN-I activity has been carefully dissected ([Box 1](#)) and its essential function in antiviral defence has been confirmed in model organisms [3]. In parallel with these discoveries has come the realisation that virtually all human viral pathogens encode strategies to evade and/or subvert the antiviral activity of IFN-I [4,5]. Furthermore, loss of viral IFN-I antagonists leads to virus attenuation and is being exploited for the development of novel live viral vaccines [6]. The ‘arms race’ between host and virus plays a decisive role in viral pathogenesis, driving viral evolution and restricting interspecies transmission [4,5].

## The IFN System

IFN-I does not operate in isolation. Additional IFNs have been identified; namely, IFN $\gamma$  (type II IFN) [7] and the mucosa-restricted IFNs (IFN $\lambda$ 1–4, or type III IFNs) [8–10]. These IFN types differ in their range of activity in tissues and their tendency to cause immunopathology and can be considered part of an integrated ‘IFN system’. **IFN-III** is produced by most cells but acts mainly at epithelial surfaces, due to constrained receptor expression [11]. IFN-III restricts virus replication at the point of initial encounter without inducing systemic immune activation or immunopathology [10–16] – a relatively high-yield, low-cost outcome. By contrast, most tissues respond to IFN-I, which assumes greater importance when viruses breach epithelial barriers (e.g., invading into the lymphatics, bloodstream, or brain), but with an inherently greater risk of toxicity, particularly for the brain [17]. Finally, **type II IFN (IFN-II)** (IFN $\gamma$ ) is an extremely potent immunostimulatory cytokine, with the greatest potential for immunopathology. While expression of the IFN $\gamma$  receptor (IFNGR) is, like that of the IFN-I receptor (IFNAR), widespread, the production of IFN $\gamma$  is tightly controlled and restricted to specialised immune cells [18]. Since all IFNs share the ability to induce an antiviral state in responding cells, there is scope for compensation if one or more IFN type is disabled. The brain parenchyma is a notable exception since it cannot respond to IFN-III, and cells capable of making IFN $\gamma$  are generally absent from the brain.

## Highlights

Inborn errors of immunity affecting all key molecular components of the interferon (IFN)-I signalling pathway [IFN-alpha/beta receptor (IFNAR)1, IFNAR2, Janus kinase 1 (JAK1), tyrosine kinase 2 (TYK2), signal transducer and activator of transcription (STAT)1, STAT2, and interferon regulatory factor 9 (IRF9)] have been identified in humans.

Deficiency of IFNAR results in potentially fatal susceptibility to live-attenuated viral vaccines, but without general susceptibility to common childhood viral diseases. Clinically evident vulnerability to a broader spectrum of viral diseases, including respiratory viruses such as influenza as well as live-attenuated viral vaccines, often accompanies deficiency of STAT2 and IRF9. These molecules transduce signals downstream of IFN-I and IFN-III, suggesting that the latter provides compensatory antiviral defence in IFNAR-deficient patients.

Children with defects in IFN-I and IFN-III signalling are not particularly susceptible to viruses such as cytomegalovirus (CMV), suggesting that this virus has successfully evolved mechanisms to overcome IFN-I/III restriction.

STAT1-deficient patients, who lack signalling in response to all types of IFN (I, II, and III), show the widest viral susceptibility of all.

Pathological dissemination of parenterally delivered live-viral vaccines in otherwise healthy children should signify an inborn error of IFN-I immunity until proved otherwise.

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**Box 1. Canonical IFN-I Signalling**

In the current paradigm, the type I IFNs (IFN-I) – comprising 13 subtypes of IFN $\alpha$  and one of IFN $\beta$ , IFN $\epsilon$ , IFN $\kappa$ , and IFN $\omega$  – all signal at the single heterodimeric IFN alpha/beta receptor (IFNAR) expressed by all nucleated cells. By contrast, responses to the type III IFNs (IFN $\lambda$ 1–4, IFN-III), identified first in 2003 [8–10], are constrained by the restricted expression of the IFN lambda receptor (IFNLR) on epithelial cells, (human) hepatocytes, and some immune cell subsets [11,16,60,61,71]. In the canonical IFN-I pathway, IFN-I binding initiates an intracellular signalling cascade in which reciprocal transphosphorylation of the receptor-associated kinases JAK1 and TYK2 is accompanied by phosphorylation of the signal transducers and activators of transcription STAT1 and STAT2 [59,83,95]. The majority of the transcriptional response to IFN-I is attributable to the heterotrimer comprising phosphorylated STAT1 and 2 together with IRF9 [56,95,96]. This complex, known as ISGF3, translocates to the nucleus where it interacts with an ISRE [97] in the promoter of multiple ISGs [97]. A fraction of the phosphorylated STAT1 also homodimerises to form the GAF [98], agonising a distinct but overlapping set of genes bearing GAS elements, typically associated with type II IFN (IFN $\gamma$ ) signalling [98]. A broadly similar pathway is activated in response to IFN-III [8–10]. This simplified model omits multiple STAT-dependent and STAT-independent signalling pathways activated downstream of IFNAR, some of which involve unphosphorylated ISGF3 components assembled in distinct transcriptional complexes (e.g., STAT2:IRF9, U-ISGF3) [99].

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Given the ability of viruses to evade IFN-I in their natural hosts, how important is this response for antiviral immunity in humans? This question has added relevance given that dysregulation of IFN-I immunity may promote viral pathogenesis [17,19]. In humans, **inborn errors of immunity** that compromise the expression and/or function of genes such as *TLR3*, *IFIH1*, *IRF3*, and *IRF7* involved in synthesis of IFN-I, IFN-III, and other proinflammatory mediators in response viral infection, are associated with heightened clinical susceptibility to viral disease [20–30]. These disorders underline a central principle: that innate immunity makes an essential contribution to viral defence in humans. However, the fact that these pathways engage a range of innate immune effector mechanisms beyond the IFN-I response means that the precise involvement of IFN-I in this process remains uncertain. To dissect this, we turn to defects of the cellular response to IFN-I itself.

Within this group of autosomal recessive (AR) diseases are loss-of-expression variants negating the response to IFN-I alone (*IFNAR1* or *IFNAR2*), responses to both IFN-I and IFN-III (i.e., *STAT2*, *IRF9*), and *STAT1* variants, which negate responses to all IFNs (Figure 1). These inborn errors of immunity produce a phenotype of vulnerability to severe and/or recurrent viral disease (Table 1), the clinical significance of which depends on the extent of compromise to the IFN system as a whole. However, variable expressivity is also recognised; for example, ranging from death in infancy to survival into adulthood with no apparent phenotype in some signal transducer and activator of transcription (STAT)2-deficient patients [31]. This may, among other factors, be due to environmental differences in the range and dose of viruses encountered by individual patients and/or the effectiveness of compensatory immune pathways against specific pathogens [32].

**IFNAR Deficiency**

Deficiency of IFNAR acts as the most specific readout of the involvement of IFN-I in human antiviral defence, revealing an essential but surprisingly narrow function attributable to IFN-I. IFNAR comprises two subunits, IFNAR1 and IFNAR2. Homozygosity for a nonsense *IFNAR2* variant was first reported in two siblings, coming to light when the proband developed fatal encephalitis, complicated by **haemophagocytic lymphohistiocytosis (HLH)**, following receipt of the live-attenuated measles, mumps, and rubella (MMR) vaccine [33]. Analysis of patient dermal fibroblasts revealed undetectable IFNAR2 protein expression accompanied by absent transcriptional responses to IFN $\alpha$ /IFN $\beta$ . This resulted in failure to mount an antiviral state in response to recombinant IFN $\alpha$  and an inability to control the replication of genetically modified IFN-sensitive viruses *in vitro*. These defects were rescued by complementing patient cells with wild-type (WT) *IFNAR2*. Notably, and surprisingly, prior to receipt of MMR the proband had been healthy with no apparent phenotype of heightened sensitivity to common childhood viral diseases. This was true despite

serological testing revealing evidence of prior infection with the herpesviruses Epstein–Barr virus (EBV) and cytomegalovirus (CMV). Supporting this unexpectedly narrow vulnerability to viral disease, the patient's IFNAR2-deficient sister remained well without a clinically apparent defect of viral resistance (MMR was withheld). It was suggested that this narrow phenotype might reflect residual IFNAR1-dependent signalling, as reported in *Ifnar2*<sup>-/-</sup> mice [34]; however, a similar clinical phenotype was recognised in two unrelated children bearing homozygous nonsense mutations in *IFNAR1* [35]. Their clinical phenotype – healthy until exposure to live-attenuated viruses – recapitulated IFNAR2 deficiency. Both suffered life-threatening dissemination of live viral vaccines, either MMR or yellow fever vaccine [35]. In cells (dermal fibroblasts and EBV-transformed B cells) from these cases, the IFNAR1 protein and transcriptional response to IFN $\alpha$ /IFN $\beta$  was absent, conferring a defect of antiviral state induction to recombinant IFN $\alpha$  or IFN $\beta$ , rescued by complementation with *IFNAR1*. As in IFNAR2-deficient cases, IFNAR1 deficiency was not associated with clinically evident vulnerability to viruses encountered in the natural environment such as influenza, other common respiratory viruses, or herpesviruses including CMV, despite serological evidence of past infection [35]. Thus, unlike in mice, the clinical and molecular consequences of human IFNAR1 and IFNAR2 deficiency appeared to be indistinguishable. Responses to IFN $\gamma$  were intact in both IFNAR1- and IFNAR2-deficient fibroblasts *in vitro* [33,35], again contrasting with findings in murine cells [36,37]. Although not systematically assessed, none of the defects of development or homeostasis reported in *Ifnar1*<sup>-/-</sup> mice [38] have been observed to date in IFNAR-deficient humans.

### IFN-III Compensation?

The apparent lack of vulnerability to naturally occurring viral pathogens initially encountered at mucosal surfaces in IFNAR-deficient patients is striking and at first glance appears in stark contrast to IFNAR-deficient mice, which are profoundly susceptible to multiple viruses. However, it is important to consider the route of viral exposure in judging these apparent phenotypic differences. *Ifnar1*<sup>-/-</sup> mice are indeed highly susceptible to viruses when challenged by intravenous or intraperitoneal inoculation [3]. However, when challenged with influenza or respiratory syncytial virus (RSV) via the respiratory mucosa they displayed no increase in viral susceptibility compared with WT controls [12]. This has been attributed to the capacity of IFN-III to compensate entirely for the loss of IFN-I antiviral activity at the respiratory mucosa [12,14,39]. Deletion of *Ifnar* and *Ifnlr* together renders mice extremely vulnerable to infection of the respiratory tract [12,14,39]. An obvious hypothesis for why IFNAR-deficient patients become severely ill following immunization with some live-attenuated vaccines is that intramuscular injection bypasses the IFN-III response, which would normally be encountered during natural infections, leading to widespread dissemination of the attenuated virus and disease. This has been (indirectly) tested in mice, where *Ifnar1*<sup>-/-</sup> mice are vulnerable to the dissemination of vaccine-strain YFV (YFV-17D) when it is injected by the intramuscular or intraperitoneal routes [40]. An interesting corollary is that patients with defective IFN-I immunity may be susceptible to viruses that naturally bypass the mucosa; for example, arthropod-borne pathogens such as dengue virus. As with any susceptibility state, pathogen exposure is a key factor governing disease expressivity [32], and IFNAR-deficient patients have not been knowingly exposed to arboviruses [33,35]. An open question is whether similar compensation occurs in exclusive defects of IFN-III (Box 2).

The implication of these experiments of nature is that there is redundancy between IFN-I and IFN-III in mediating the antiviral state at mucosal surfaces. One would predict a more significant phenotype to arise in defects that compromise both IFN-I and IFN-III responses simultaneously. For such an example, we turn to defects of the shared signal transduction pathway downstream of these receptors, beginning with defects of the **interferon-stimulated gene factor 3 (ISGF3)** complex [STAT1, STAT2, and interferon regulatory factor 9 (IRF9)].

### Glossary

**Antiviral state:** a cellular state of resistance to virus entry, replication, assembly and/or release, governed by the expression of multiple antiviral effector gene products (ISGs), many of them induced by interferons.

**Bacille Calmette–Guérin (BCG):** a live-attenuated form of *Mycobacterium bovis* developed in 1921 as a vaccine against *Mycobacterium tuberculosis*.

#### Haemophagocytic

**lymphohistiocytosis (HLH):** a life-threatening systemic immune disorder characterised by widespread immune cell activation (predominantly lymphocytes, NK cells, and macrophages) and dysregulated cytokine signalling, leading to end-organ damage.

**Inborn errors of immunity:** rare genetic disorders of human immune function, due to germline pathogenic variants, typically monogenic. These are usually inherited, although they can also arise *de novo* or result from somatic mutation.

**Interferons (IFNs):** the term applied to secreted cytokines mediating 'viral interference', defined as the ability of virus-infected cells to produce a substance that, when added to uninfected cells, 'interferes with' viral infection.

**Interferon-stimulated gene factor 3 (ISGF3):** a heterotrimeric complex of STAT1, STAT2, and IRF9 and the principal transcription factor responsible for ISG induction in response to IFN-I and IFN-III.

**Interferon-stimulated genes (ISGs):** a group of genes induced in response to IFNs, responsible for the functional activity of IFNs.

**Interferon-stimulated response element (ISRE):** ISRE in the promoters of ISGs is bound by ISGF3 to activate transcription in response to IFN-I and IFN-III. This is distinct from the IFN $\gamma$  activation site (GAS), which is bound by homodimers of tyrosine phosphorylated STAT1, also known as GAF.

**Systemic inflammatory response syndrome (SIRS):** a syndromic description for systemic inflammation arising from a broad range of noxious stimuli (including trauma, infection, malignancy, immune-mediated disease, etc) that, if unchecked, can lead to multiple organ dysfunction, cardiovascular collapse, and death.

### Homozygous STAT1 Deficiency

AR complete STAT1 deficiency leads to a profound defect of immunity with broad susceptibility to infectious pathogens, extending to herpesviruses as well as intracellular bacteria (including mycobacteria). This pattern reflects the participation of STAT1 in signalling by all IFNs [i.e., IFN-I, IFN-II (IFN $\gamma$ ), and IFN-III], thereby disrupting critical functions of IFN $\gamma$  among specialised cells of the immune system. It is the only disorder described in this review where haematopoietic stem cell transplantation (HSCT) is indicated and has documented life-saving potential in patients [41,42]. Heterozygosity for hypomorphic variants in human *STAT1* produces clinical vulnerability to mycobacteria but not viruses due to the selective impact on IFN $\gamma$  signalling and thus will not be considered further.

Complete AR STAT1 deficiency was first identified in unrelated infants presenting with disseminated **bacille Calmette–Guérin (BCG)** infection who succumbed to severe viral illness [caused by recurrent herpes simplex virus 1 (HSV1) encephalitis in one and an unknown viral pathogen in the other] [43]. Sequencing of the candidate disease gene *STAT1* revealed in one child a homozygous frameshift deletion (c.1757-1758delAG) while the other bore a homozygous missense variant (p.L600P) and preserved mRNA expression. Consistent with the complete absence of STAT1 protein in each child, resulting in loss of ISGF3 and IFN $\gamma$ -activated factor (GAF) DNA-binding activity, patient cells were completely refractory to IFN in the induction of an antiviral state *in vitro*. Subsequent cases of complete STAT1 deficiency broadly recapitulated this phenotype [44], although sometimes with additional inflammatory features such as severe hepatitis [45] or HLH [41] provoked, at least in the latter case, by viral infection. Homozygosity for a hypomorphic *STAT1* allele was shown to produce a less severe phenotype both *in vitro* and *in vivo* [46,47]. The profound clinical impact of STAT1 deficiency and the therapeutic benefit of HSCT reflect the broad importance of this transcription factor in immunity.

### Homozygous STAT2 Deficiency

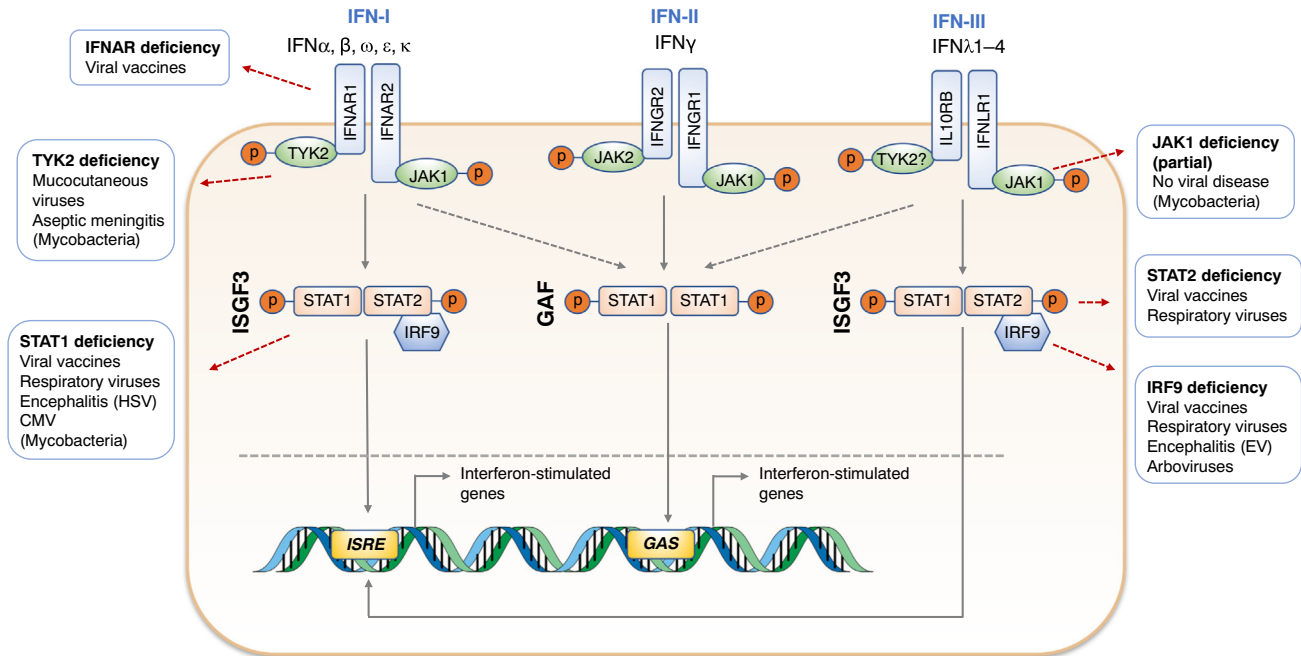
Biallelic loss-of-expression variants of the transcription factor *STAT2* produce a milder clinical phenotype than STAT1 deficiency comprising viral susceptibility without associated problems in handling bacteria, owing to STAT2's more restricted role in IFN-I and IFN-III signalling. Compared with defects of IFNAR, STAT2 deficiency has a broader clinical phenotype, encompassing viral disease caused by vaccine-strain viruses as well as common viral pathogens encountered at mucosal surfaces [31,48–50]. Presumably, this is due to the parallel disruption of IFN-I and IFN-III responses. As previously mentioned, the phenotype of viral susceptibility in STAT2 deficiency also has variable expressivity, for reasons that remain unclear.

STAT2 deficiency was originally described in a kindred of five individuals with a spectrum of disease ranging from death in infancy to survival into middle age with no apparent phenotype [31]. Homozygosity for a variant (c.381+5G>C) causing aberrant *STAT2* mRNA splicing and undetectable STAT2 protein expression was accompanied by a profound *in vitro* defect of the expression of **IFN-stimulated response element (ISRE)**-containing genes and failure to mount an IFN-I-mediated antiviral state [31], defects that were rescued by *STAT2* complementation. Patient cells expressed a small subset (10%) of **interferon-stimulated genes (ISGs)** induced in WT cells [31], most of which contained gamma activation site (GAS) elements within their promoters, presumably reflecting a residual amount of IFNAR-dependent signalling via GAF. Whilst STAT2 deficiency almost certainly compromises ISGF3 function in response to IFN-III, this prediction has yet to be formally tested owing to the lack of available patient cell types that reliably express a functional IFN lambda receptor (IFNLR). Treatment with IFN $\gamma$  protected STAT2-deficient cells against cytopathic effects of vesicular stomatitis virus [49], suggesting the compensatory induction of an antiviral state, as described in IFNAR-deficient cells.

**Type I interferons (IFN-I):** a group of cytokines comprising 13 subtypes of IFN $\alpha$ , IFN $\beta$ , IFN $\epsilon$ , IFN $\kappa$ , and IFN $\omega$ , with a range of antiviral, growth inhibitory, and immunoregulatory properties in vertebrate immune systems; increasingly recognised to have immunopathological potential, subject to stringent negative feedback control.

**Type II interferon (IFN-II):** a single cytokine, IFN $\gamma$ , produced mainly by lymphocytes, which in addition to its primary role in immunomodulation has antiviral properties through the induction of a transcriptional programme distinct from but overlapping that of IFN-I and IFN-III.

**Type III interferons (IFN-III):** comprises four cytokines – IFN $\lambda$ 1, IFN $\lambda$ 2, IFN $\lambda$ 3, and IFN $\lambda$ 4. IFN-III activates an intracellular signalling cascade similar to that of IFN-I. It is produced by most cells in response to pattern recognition receptor signalling but acts on a more restricted range of cell types.



Trends in Genetics

**Figure 1. Canonical Interferon (IFN) Signalling Pathways and Their Genetic Lesions.** Displayed are the three IFN pathways and a brief summary of the viral susceptibility phenotypes that accompany molecular defects of these pathways. IFN-I receptor (IFNAR) deficiency selectively impairs the IFN-I response, predisposing to disease secondary to inoculation with live-attenuated viral vaccines. Since IFN-I responses play a part in preventing the systemic dissemination of viruses, IFNAR-deficient individuals would hypothetically be vulnerable to arboviruses transmitted via the bloodstream. STAT2 and IRF9 are central to the response to both IFN-I and IFN-III, the latter mediating antiviral immunity at mucosal surfaces. Thus, these molecular defects are accompanied by problems in handling both live-attenuated viral vaccines and mucosally transmitted viruses such as influenza. In the case of STAT2 deficiency, variable expressivity of the phenotype is recognised. STAT1 deficiency is the most clinically serious defect since it compromises the response to all IFNs simultaneously. This is associated with susceptibility to a broad range of viruses, including herpesviruses. Because IFN-II is also critical to responses to mycobacteria, STAT1 deficiency is accompanied by life-threatening complications of mycobacterial infection. Tyrosine kinase 2 (TYK2) and (partial) Janus kinase 1 (JAK1) deficiency are also associated with mycobacterial susceptibility, alongside a much milder phenotype of viral disease. In the case of TYK2 deficiency, this may be due to the ability of IFN-III, and to a lesser extent IFN-I, to signal independently of TYK2. Complete JAK1 deficiency has not been reported. Abbreviations: CMV, cytomegalovirus; EV, enterovirus; GAF, IFN $\gamma$ -activated factor; HSV, herpes simplex virus; ISGF3, interferon-stimulated gene factor 3.

Complete deficiency of STAT2 was subsequently reported in a further five children in three kindreds [48–50], totalling ten individuals (additional patients have been identified but their details remain unpublished). Some of these individuals had more obvious problems with common childhood viruses, such as viral pneumonia and enteroviral meningitis, than in the original kindred [49]. In total, viral disease had a fatal outcome in two of ten cases. All six children known to have been exposed to MMR have experienced dissemination of vaccine-strain viruses to the lungs and/or brain (Table 1). The outcome of herpesvirus infections in these patients was seemingly unremarkable, except for one child with prolonged EBV replication following primary infection [49] and another with herpes gingivostomatitis [31]. There were no reports of CMV disease despite serological evidence of exposure. Measures of adaptive immune cell number and immunoglobulin production were normal, with one or two minor exceptions [49]. This suggests that adaptive immunity, and/or IFN-II signalling, provides compensatory immunity to CMV and possibly other herpesviruses in STAT2-deficient patients. In some cases, viral disease has also been associated with significant systemic inflammation, reaching the criteria for HLH [50], apparently responsive to immunoglobulin therapy [49,50]. This and related inflammatory phenotypes are observed in other disorders (IFNAR2, STAT1, IRF9), implying immunopathology caused by aberrant activity of compensatory immune pathways.

Table 1. Human Molecular Defects of IFN-I Signalling and Their Associated Phenotypes<sup>a,b</sup>

Gene	Protein expression	Severe/recurrent viral disease	Uncomplicated infection	Asymptomatic exposure	Other clinical manifestation	Refs
<i>IFNAR1</i>	Absent	P1: MMR encephalitis P2: disseminated 17D YFV (MMR without incident)	P1: none P2: none	P1: CMV P2: CMV, HSV1, HSV2	No	[35]
<i>IFNAR2</i>	Absent	P1: MMR encephalitis (fatal) P2: none (MMR withheld)	P1: HHV6 P2: none	P1: CMV, EBV P2: unknown (SCIG)	No	[33]
<i>STAT1</i>	Absent	P1: recurrent HSV encephalitis (fatal) P2: unknown viral pathogen (fatal)	Unknown	Unknown	Both: disseminated BCG	[43]
<i>STAT1</i>	Absent	Vaccine-strain polio virus shedding EBV-driven LPD post-HSCT	HRV, PIV2, polio vaccine	None	Disseminated BCG Severe hepatitis Multiorgan failure post-HSCT (fatal)	[45]
<i>STAT1</i>	Reduced expression of truncated nonfunctional protein ( Ex3)	Recurrent CMV pneumonitis Encephalitis (cause not identified)	Viral GI infections and RTIs Cutaneous HSV1	Unknown	Pulmonary NTM infection Sepsis Recurrent pneumonia (BCG naïve)	[44]
<i>STAT1</i>	Absent	HLH, possibly related to MMR and/or HHV6	Nil stated	Unknown	(BCG naïve)	[41]
<i>STAT1</i>	Splicing defect ( Ex23) with low-level expression of WT protein	P1: None noted P2: HSV1 gingivostomatitis, hospitalisation for CMV and VZV infection	Unknown	Unknown	P1: recurrent NTS infection (BCG naïve) P2: <i>Salmonella</i> meningitis (BCG naïve)	[46]
<i>STAT1</i>	Splicing defect ( Ex8) with low-level expression of WT protein	P1: severe varicella P2: none noted	Unknown	P1: CMV, EBV P2: unknown	P1: disseminated NTM infection (BCG naïve), <i>Candida</i> line infection P2: disseminated BCG Septic shock (fatal)	[47]
<i>STAT2</i>	Absent (multiple splicing defects)	P1: MMR pneumonitis/hepatitis HSV1 gingivostomatitis IAV pneumonia P2: fatal viral illness (10 weeks) P3: none noted (childhood history unknown) P4: bronchiolitis MMR encephalitis (SNHL) P5: hospitalisation with viral illness	P1: none P2: N/A P3: unknown P4: varicella P5: varicella	P1: EBV P2: N/A P3: MMR, VZV, CMV P4: Unknown P5: Unknown	None	[31]
<i>STAT2</i>	Absent	P1: opsoclonus–myoclonus syndrome post-MMR with late recurrence P2: MMR encephalitis (MuV)	P1: unknown P2: unknown	P1: Unknown P2: Unknown	Mild renal tubulopathy Defect of mitochondrial fission	[48]
<i>STAT2</i>	Absent (premature stop/splicing defect)	P1: MMR hepatitis Severe RSV, EV, AdV Fatal febrile illness (7 years) P2: severe varicella MMR pneumonitis/hepatitis EV meningitis Prolonged primary EBV	P1: unknown P2: RSV, IAV, EV, AdV	P1: Unknown P2: Unknown	'Inflammatory' responses to viral infection	[49]
<i>STAT2</i>	Absent	HLH post-MMR	Unknown	HHV6, CMV, VZV, RSV, AdV, PIV1–3	No	[50]

(continued on next page)

Table 1. (continued)

Gene	Protein expression	Severe/recurrent viral disease	Uncomplicated infection	Asymptomatic exposure	Other clinical manifestation	Refs
<i>IRF9</i>	Reduced expression of truncated product ( Ex7)	Severe IAV pneumonitis Biliary perforation post-MMR Recurrent bronchiolitis	HMPV, RSV, AdV, PIV1-4	HSV1, CMV, HRV, EV	(i) Periodic fever (ii) C1q autoantibody	[52]
<i>IRF9</i>	Absent	P1: disseminated VZV post vaccination Severe dengue fever and Zika virus disease EV encephalitis IAV pneumonia RSV bronchiolitis P2: none (on SCIG)	P1: dengue, IBV pneumonia P2: none	P1: Unknown P2: Unknown	P1: recurrent pneumonia and bronchitis, septic shock with purpura fulminans P2: none	[53]
<i>JAK1</i>	Unaffected	None	VZV (shingles), HPV	Unknown	Recurrent mycobacterial infection Metastatic bladder carcinoma (fatal)	[57]
<i>TYK2</i>	Absent	P1: PIV3 pneumonia, recurrent oropharyngeal HSV1 P2: none P3: none P4: none P5: none P6: unspecified viral infection of skin P7: none P8: recurrent herpes gingivostomatitis and aseptic meningitis	P1: molluscum contagiosum P2: shingles	Unknown	P1: eosinophilia, elevated IgE Disseminated BCG NTS infection Candidiasis Ocular sarcoidosis P2: disseminated BCG Recurrent <i>Brucella</i> meningitis P3: TB, bacterial meningitis (fatal) P4: meningitis, otitis media, UTI, asthma P5: disseminated BCG P6: disseminated BCG P7: TB P8: none	[60]
<i>TYK2</i>	Absent	None	Unknown	HHV6, IAV, parvo-B19 MMR vaccinated	Recurrent bronchopneumonia Duodenal perforation Anal/skin abscesses	[61]
<i>TYK2</i>	Absent	Recurrent herpes gingivostomatitis and aseptic meningitis	VZV	Unknown	Disseminated BCG	[62]

<sup>a</sup>All of the variants included above are pathogenic in homozygosity or compound heterozygosity.

<sup>b</sup>AdV, adenovirus; CNS, central nervous system; ECMO, extracorporeal membrane oxygenation; EV, enterovirus; HHV6, human herpesvirus 6; HPV, human papillomavirus; HRV, human rhinovirus; IAV/IBV, influenza A/B virus; LPD, lymphoproliferative disease; MuV, mumps virus; NTM, nontuberculous mycobacteria; NTS, non-typhoidal *Salmonella*; PIV, parainfluenza virus; RTI, respiratory tract infection; SCIG, subcutaneous immunoglobulin; SNHL, sensorineural hearing loss; TB, tuberculosis.

### Homozygous IRF9 Deficiency

The clinical and molecular impact of IRF9 deficiency echoes STAT2 deficiency, reflecting the intimate cooperation of these transcription factors in IFN-I and IFN-III signalling. IRF9 is the main DNA-binding component of ISGF3. Alone, IRF9 has minimal ISRE-binding capacity or transcriptional activity [51]. AR IRF9 deficiency has been described in three children in two kindreds

#### Box 2. IFN-I Compensation?

Compensatory interactions between IFN-I and IFN-III go both ways. The IFNLR is formed from IL10RB, the ubiquitously expressed subunit of the IL10 receptor, and IFNLR1, which displays tissue-restricted expression mainly in epithelial cells and hepatocytes. AR loss-of-expression mutations of *IL10RB* confer susceptibility to severe, early-onset inflammatory bowel disease, a phenotype indistinguishable from null mutations of *IL10RA* or *IL10* itself [100], presumably reflecting the dominant role of loss of IL10 signalling in this phenotype. Notably, there have been no reports of clinical susceptibility to mucosal viral disease [either respiratory or gastrointestinal (GI)] in over 20 patients reported to date with deficiency of IL10RB (reviewed in [101]). This implies, but does not prove, that defects in IFN-III signalling are compensated by IFN-I, in apparent contrast to the situation in mice, where IFN-III plays a nonredundant role in defence against rotavirus and norovirus in the intestine [13,15] and in restricting influenza virus to the upper airway [16]. An open question is whether IL10RB deficiency might confer a subtler defect of mucosal antiviral immunity in specific circumstances.



[52,53]. In the original report, the proband bore a homozygous *IRF9* missense variant producing a truncated IRF9 protein lacking the IRF association domain (IAD), a critical domain for STAT interaction. Full-length IRF9 lacks a nuclear export signal and is predominantly localised to the cytosol through its constitutive interaction with STAT2 [51,54]. Loss of the IRF9 IAD led to an inability to bind STAT2 and was associated with constitutive nuclear localisation of IRF9 Ex7 [52]. In the second kindred, homozygosity of an *IRF9* variant allele (c.577+1G>T) in two siblings was accompanied by undetectable IRF9 protein expression [53]. An elder sibling who died with pathological dissemination of yellow fever vaccine was probably similarly affected. In cells from IRF9-mutated patients, STAT1 and STAT2 were appropriately phosphorylated on IFN $\alpha$  treatment; however, binding to an *ISRE* probe was completely impaired, consistent with the failure to form a functional ISGF3 complex. Consistent with this defect of ISGF3 assembly in IRF9-deficient cells, the transcriptional induction of classical ISRE-containing ISGs was abolished [52,53]. RNA-seq studies of IRF9 Ex7 cells revealed the expression of a subset of ISGs in patient cells, some to a higher magnitude than controls, implying the activity of ISGF3-independent complexes such as GAF [52]. Responses to IFN $\gamma$  were preserved in cells bearing the truncated IRF9 protein (IFN $\gamma$  response was not reported in IRF9-null cells), in contrast to reports in *Irf9*<sup>-/-</sup> mouse embryonic fibroblasts [55] and in U2A cells, an IRF9-deficient fibrosarcoma cell line [56].

As in STAT2 deficiency, IRF9-deficient children experienced life-threatening problems in controlling vaccine-strain viruses and evidence of increased susceptibility to mucosal viral pathogens but not herpesviruses [52,53] (Table 1). Adaptive immune parameters were normal when monitored during health in the first child; the proband in the second kindred had mildly reduced IgG and was treated with intravenous immunoglobulin (IVIG) [53]. In another similarity with some STAT2-deficient patients, periodic fevers and severe **systemic inflammatory response syndrome (SIRS)** suggestive of autoinflammation were noted, the trigger for which was not identified [52,53].

### Receptor-Associated Kinases

The receptor associated kinases Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) play key roles in linking innate IFN receptor ligation to transcriptional activation. However, unlike most of the signalling molecules considered in this review, which have specific functions in IFN signalling, JAK1 and TYK2 participate in a wide range of additional cytokine and growth factor signalling pathways. Thus, from our IFN-centric perspective, these disorders are nonspecific and less informative. Nevertheless, they provide some interesting insights.

#### Partial JAK1 Deficiency

Biallelic hypomorphic mutations in *JAK1* have been reported in a single individual to date [57]. The impact of these variants on IFN-I signalling was modest, with minimal compromise of the transcriptional response. Consistent with this partial defect of IFN-I signal transduction, no evidence of a clinically relevant defect of antiviral immunity was observed in this patient, beyond a single episode of uncomplicated varicella zoster virus (VSV) reactivation (shingles). JAK1 participates in signalling through a large range of cytokines, including IFN $\gamma$ , and compromise of these cytokine pathways appears to dictate the clinical phenotype, which was dominated by susceptibility to mycobacterial infection and early-onset bladder carcinoma (Table 1). Complete JAK1 deficiency has not been described in humans. Based on observations in *Jak1*<sup>-/-</sup> mice, this might be expected to be incompatible with postnatal life [58].

#### AR TYK2 Deficiency

A different picture emerges for patients deficient in TYK2. In the canonical model of IFN-I signalling, based on studies in TYK2-deficient U1A fibrosarcoma cells [59], TYK2 is essential. However,

complete deficiency of TYK2 in humans has a relatively mild clinical impact on antiviral immunity (Table 1). Reported in 11 individuals, in all cases accompanied by absent TYK2 protein expression, the clinical phenotype is dominated by susceptibility to mycobacterial and/or fungal infections [60–63], again presumably due to the involvement of TYK2 in other cytokine pathways. Initially considered a cause of hyper-IgE syndrome [63], this was refuted by the subsequent identification of multiple TYK2-deficient cases lacking this phenotype [60]. No patients experienced problems handling live-viral vaccines. Naturally encountered viral infections reported in TYK2-deficient patients were in most cases mild, with herpes gingivostomatitis in three patients accompanied in one case by viral pneumonia; two individuals developed aseptic meningitis.

Studies in virally transformed fibroblasts, T cells, and B cells from TYK2-deficient patients demonstrated reductions in IFNAR1 and IL10RB expression [60,61], consistent with the loss of a scaffolding function of TYK2 towards these receptor subunits [64]. Interestingly, in response to IFN-I there was residual – albeit substantially reduced – STAT1 phosphorylation, ISGF3 and GAF DNA binding, and ISG expression [60,61]. Cell-type variation was apparent, since responses to IFN $\alpha$  in transformed T cells (as well as monocytes from a single case) were absent [60,61]. Unexpectedly, IFN-III signalling (assayed in patient EBV-transformed B cells and *TYK2* knockout HAP1 cells) was preserved, suggesting that it may be TYK2 independent [60,61]. While these findings are inconclusive, they imply that residual IFN-III (and possibly also IFN-I) responses in TYK2-deficient patients are of a sufficient magnitude to sustain antiviral immunity. These results are broadly compatible with *Tyk2*<sup>-/-</sup> mice, which are less susceptible than *Ifnar1*<sup>-/-</sup> mice to experimental VSV infection [65], and where IFN-I signal transduction is only partially impaired [65,66]. Thus, IFN-III and IFN-I responses appear to be at least partially TYK2 independent.

### Interspecies Differences in IFN-I Biology?

Since much of our current understanding of IFN-I biology is derived from mouse models, we briefly consider how the phenotypes of deficient mice and humans compare. Human and mouse genomes contain 17 *IFN-I* and one *IFNG* genes; humans have four *IFN-III* genes (*IFNL1–4*), whereas mice have two (*Ifnl2/3*) [8–10]. IFN-I signalling molecules show variable interspecies diversity. Human and mouse IFNAR1 and IFNAR2 proteins share only 50% homology [67]. While STAT1 is highly conserved, STAT2 has 70% homology, the lowest of all STATs [68]. Consequently, certain viral IFN-I antagonists (e.g., flaviviral NS5 proteins) do not function in the mouse because they fail to bind murine STAT2 [69,70]. Another difference is that human but not murine hepatocytes respond to IFN-III [39,71,72]. Accordingly, recombinant human IFN $\lambda$ 1 is being explored as a therapy for viral hepatitis [73].

Despite the inherent limitation of comparing results from experimental and natural infection, and the fact that most viral pathogens are human adapted, viral susceptibility phenotypes of deficient humans and mice broadly correspond, particularly in the case of TYK2, STAT1, and STAT2 [65,66,74–76]. Viral susceptibility has not been systematically analysed in *Irf9*<sup>-/-</sup> mice [55]. The apparent exception is IFNAR-deficient mice, which demonstrate broader susceptibility to herpesviruses, such as HSV [77] and (murine) CMV [78,79], than humans. In addition, a range of defects in haematopoietic stem cell (HSC) maintenance [80,81], immune cell number [e.g., reduced natural killer (NK) cells [82], expanded CD11c<sup>+</sup> myeloid cells [83]], increased bone turnover [84], and altered responses to alternative cytokines [colony stimulating factor 1(CSF-1), interleukin (IL)6, and IFN $\gamma$  [36,37,85]], attributed to the loss of constitutive IFN-I signalling in IFNAR-deficient mice [38], were not observed in human patients [33,35]. Despite several caveats (low patient numbers, the absence of deep immunophenotyping data), an obvious question is why human immunity is seemingly not impacted to the same degree by defective IFN-I signalling.

### Outstanding Questions

Why do disease outcomes vary significantly between patients with the same genetic defect? Is this explained by differences in exposure or do these disorders highlight variation in other immune compensatory mechanisms?

How relevant are IFN-I/IFN-III defects to antiviral immunity across an individual's lifespan? Does susceptibility diminish with age, as seen in other monogenic innate immune disorders (often attributed to the immunological education of the adaptive immune response)?

To what extent, if any, do defects in IFN-I signalling impact subtler aspects of adaptive immune maintenance and/or function in humans?

Does IFN-III protect IFNAR-deficient human epithelial surfaces from viral infection? Epithelial cells respond to both IFN-I and IFN-III, and IFNAR-deficient patients appear no more vulnerable to mucosally acquired viruses than the general population.

Is the route of live-viral vaccine delivery a determinant of pathogenicity? Does the process of attenuation facilitate mutations that confer enhanced replication capacity, pathogenicity, or tropism in IFN-I-incompetent hosts?

What pathomechanism underlies hyperinflammatory phenotypes in some patients deficient in STAT2 or IRF9? Inflammatory disease in this scenario may reflect failure to control viral replication, the exaggerated immunopathogenic activity of compensatory immune pathways, a defect of negative regulation, or a combination of these.

Can knowledge of the organisation of the IFN system be harnessed in antiviral therapy or human vaccine design? For example, IFN-III may provide an effective agent for prophylaxis or therapy of mucosal viral disease, free from the systemic toxicity associated with IFN-I, and is being explored in three Phase II clinical trials currently recruiting for novel coronavirus disease (COVID-19) (NCT04343976<sup>6</sup>, NCT04354259<sup>8</sup>, NCT04344600<sup>9</sup>). Rationally designed replication-competent viruses deleted

### Concluding Remarks

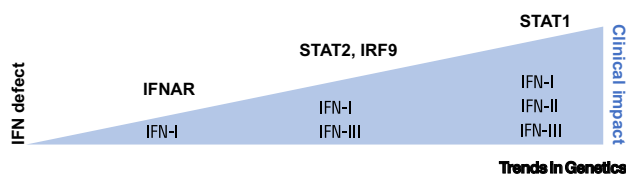
Human genetic disorders reveal that IFN-I is an important, and in certain circumstances essential, component of the concerted antiviral response, at least in early life. However, IFNAR-deficient patients appear significantly less susceptible to viruses that are restricted in their replication to mucosal surfaces than patients deficient in STAT2 or IRF9. This is likely to be because IFNAR-deficient patients will have preserved IFN-III responses in mucosal sites, whereas STAT2- and IRF9-deficient patients will not, although this has yet to be proved in patient material (see Outstanding Questions).

for key IFN-evasion proteins are showing promise as viral oncotherapy and as vectors in animal vaccines and may play an important role in next-generation vaccines or gene-therapy vectors.

A spectrum of disease severity is also apparent, whereby the impact of STAT1 deficiency is more serious than loss of STAT2 or IRF9, seeming to correlate with the extent of compromise to the IFN system as a whole (Figure 2). Another notable difference between complete STAT1 deficiency and other disorders is the vulnerability of patients with the former to herpesviruses, and in particular CMV – presumably due to defective IFN $\gamma$  responses in STAT1 deficiency. The apparent lack of CMV disease in patients with defects in IFN-I responses is striking (Table 1) and implies that CMV has evolved such sophisticated immune evasion strategies so as to render IFN-I effectively redundant, in contrast to the situation *in vitro*, where CMV has an early growth advantage in IFN-I/IFN-III-incompetent fibroblasts [86]. CMV susceptibility is a clinical feature of defects of IFN $\gamma$  signalling such as IFNGR- or STAT1- deficiency [87,88]. The only monogenic defect of IFN-I signalling in which CMV susceptibility was reported – involving a hypomorphic *IFNAR1* variant [89] – was probably explained by digenic inheritance of a null mutation in *IFNGR2*.

An intriguing phenotype of immune dysregulation accompanies monogenic defects in *IFNAR2*, *STAT2*, *STAT1*, and *IRF9*, which warrants further investigation. Hyperinflammatory manifestations range from periodic fever [52] and hepatitis [45] to HLH [33,41,50] and severe SIRS [53]. While temporally associated with viral infection, there is not always sustained viral replication, and in some instances no obvious infectious precipitant could be identified [53]. We speculate that a combination of distinct aetiological factors may contribute, including increased pathogen load and overactivity of compensatory immune pathways, such as IFN $\gamma$  and/or inflammasome-dependent IL1 signalling (which is negatively regulated by IFN-I [90,91]).

While providing insight, these disorders also raise several interesting avenues for further enquiry (see Outstanding Questions). Data from TYK2-deficient humans are intriguing, implying that responses to IFN-III and possibly also to IFN-I proceed independently of TYK2 in certain cell types and that the residual IFN-I/IFN-III signalling in TYK2 deficiency may be sufficient for antiviral defence. This would be consistent with the capacity of very low concentrations of IFN-I to induce an antiviral state [92]. From the clinical perspective, IFN $\gamma$  might offer a potential therapeutic option in some of the molecular disorders described. IFN $\gamma$  provides a degree of antiviral compensation in IFNAR-, STAT2-, and IRF9-deficient cells *in vitro*, although *in vivo* its use may come at a cost of immunopathology. Some of the findings in patient cells – for example, the preserved responses



**Figure 2. The Extent of the Defect in the Interferon (IFN) System Correlates with Its Clinical Impact.** IFN-I receptor (IFNAR) deficiency, selectively impairing IFN-I, results in susceptibility to a more limited range of viruses than signal transducer and activator of transcription (STAT)1 deficiency, which disables responses to all IFNs. STAT2 and interferon regulatory factor 9 (IRF9) deficiencies, impeding both IFN-I and IFN-III responses, are intermediate.

to IFN $\gamma$  – contrast with what has been observed in IFNAR- or IRF9-deficient murine or fibrosarcoma cell systems [36,37,55,56], calling into question the clinical relevance of a large body of literature derived from these models.

Viruses face a race between the speed of virus replication and the establishment of an antiviral state mediated by any of the three IFN types. Any viral defect has the potential to tip the balance in this race against the virus. Consequently, in a normal acute infection, the IFN system is likely to constitute a constant selective pressure that keeps viruses maximally fit in terms of replication speed and competence, as well as in the maintenance of mechanisms to circumvent the IFN response [93,94]. The outcome of this race is also likely to be extremely important in governing viral pathogenesis and host range. Collectively, the insights from human genetics summarised here reveal the essential nature of the human IFN system as a whole and how certain viral pathogens (e.g., CMV) subvert restriction by some, but not all, IFN types. These insights are helping to transform our understanding of the function and interconnectedness of the IFN system and its place in the concerted antiviral response in humans, which should inform the development of next-generation vaccines and antiviral therapies.

### Acknowledgments

Funding was provided by the Wellcome Trust [211153/Z/18/Z (C.J.A.D.), 207556/Z/17/Z (S.H.), 101788/Z/13/Z (R.E.R.)], Sir Jules Thorn Trust [12/JTA (S.H.)], and the British Medical Association (C.J.A.D.).

### Resources

<sup>i</sup>[www.clinicaltrials.gov/ct2/show/NCT04343976](http://www.clinicaltrials.gov/ct2/show/NCT04343976)

<sup>ii</sup>[www.clinicaltrials.gov/ct2/show/NCT04354259](http://www.clinicaltrials.gov/ct2/show/NCT04354259)

<sup>iii</sup>[www.clinicaltrials.gov/ct2/show/NCT04344600](http://www.clinicaltrials.gov/ct2/show/NCT04344600)

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