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Host genetics of severe influenza: from mouse Mx1 to human IRF7

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Abstract

Influenza viruses cause mild to moderate respiratory illness in most people, and only rarely devastating or fatal infections. The virulence factors encoded by viral genes can explain seasonal or geographic differences at the population level but are unlikely to account for inter-individual clinical variability. Inherited or acquired immunodeficiencies may thus underlie severe cases of influenza. The critical role of host genes was first demonstrated by forward genetics in inbred mice, with the identification of interferon (IFN)- α/β -inducible *Mx* as a canonical influenza susceptibility gene. Reverse genetics has subsequently characterized the *in vivo* role of other mouse genes involved in IFN- α/β and $-\lambda$ immunity. A series of *in vitro* studies with mouse and human cells have also refined the cell-intrinsic mechanisms of protection against influenza viruses. Population-based human genetic studies have not yet uncovered variants with a significant impact. Interestingly, human primary immunodeficiencies affecting T and B cells were also not found to predispose to severe influenza. Recently however, human IRF7 was shown to be essential for IFN- α/β - and IFN- λ -dependent protective immunity against primary influenza *in vivo*, as inferred patient with life-threatening influenza revealed to be IRF7-deficient by whole exome sequencing. Next generation sequencing of human exomes and genomes will facilitate the analysis of the human genetic determinism of severe influenza.

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Introduction

The enormous human inter-individual variability of severity of infectious diseases is a century old medical enigma [1-3]. For the vast majority of microorganisms, only a small minority of infected individuals develops life-threatening infections. The colossal burden of infectious diseases mostly results from their enormous diversity. A good example is influenza, which typically causes a self-limiting infection that resolves within one week. In contrast, a small proportion of individuals develop life-threatening and occasionally fatal infections. Global influenza pandemics have tantalized researchers for a century, from the pandemics of 1918 through 2009 to the threats of pandemic from highly pathogenic avian influenza in the past decades. The many fascinating examples of virulence factors encoded by influenza A viruses (IAVs) [4,5] have been tested by viral genetics. Likewise, the emergence of any new IAV by reshuffling of RNA segments from two or more viruses typically accounts for each new pandemic. These viral genetic variations provide a compelling explanation for the emergence of new, more virulent viruses and therefore for inter-population variability of clinical outcomes in the course of influenza. They however can hardly account for inter-individual variability in a given human population, especially of small size.

For any given IAV, whether responsible for a devastating pandemic or a banal seasonal epidemic, only a minority of infected individuals develops severe sickness, including pneumonia and acute respiratory distress syndrome. The proportion of severe disease varies from about 0.04-0.4% of symptomatic cases during seasonal influenza to about 1-10% symptomatic cases being fatal during the worst known influenza pandemic in 1918-1919 [6,7]. A fundamental problem in the field of influenza is therefore that of inter-individual variability in the course of infection by any given influenza virus. Well-known risk factors have been documented epidemiologically since the identification of the first human influenza virus in 1933 by Wilson Smith [8], shortly after Richard Shope identified the first influenza virus in swine [9]. They principally include pre-existing chronic pulmonary disease, in children and adults, but apparently exclude HIV-positivity as a risk factor [10]. The vast majority of cases remain unexplained, whether during primary infection in early childhood or during re-infection later in life. Importantly, this notion applies to both epidemic and pandemic influenza. Although the proportion of severe cases can be 30-fold higher in the course of an emerging pandemic than during seasonal influenza, these cases remain the minority and unexplained in both settings [11]. We herein review the mounting evidence attributing severe influenza to inborn errors of immunity in both mice and humans, with an emphasis on discoveries made by forward genetics of primary infection.

The influenza virus

IAVs possess a genome consisting of eight ssRNA segments that encode 11-12 proteins [12]. On the virion surface are the glycoproteins, hemagglutinin (HA) and neuraminidase (NA), which mediate cellular entry and release from the infected cell, respectively, and the ion channel, M2. The matrix protein, M1, creates the structure of the virion under the lipid membrane. The ribonucleoprotein complex consists of the viral RNA (vRNA) surrounded by the nucleoprotein, NP, that interacts with the viral RNA-dependent RNA polymerase

subunits: PA, PB1, and PB2. An additional open reading frame in the PB1 RNA encodes PB1-F2, a modulator of apoptosis, while the function of a truncated gene product of the PB1 gene, PB1 N40, is still unclear. NS1 is the antagonist of host antiviral immunity, capable of sequestering double-stranded RNA and thus preventing activation of multiple pathways, including RIG-I/MAVS signaling, that converge on the activation of the transcription factors, IRF3 and IRF7, and transcription of IFN- α , - β and - λ [13,14]. Lastly, NEP (also known as NS2) is the nuclear export protein. When considering the causes of severe influenza disease, most of the attention has been placed on the viral genes. For example, the 1918 pandemic strain was partially explained by contributions from the HA gene and the polymerase complex genes and the emergence of 2009 pandemic H1N1 was explained by the triple-reassortment of genes from human, swine, and avian-like swine viruses [15,16]. Hypercytokinemia induced by infiltrating macrophages and other innate immune cells in response to high viral load is a potential source of lung injury during viral clearance in humans, as suggested by mouse models [17,18]. However, depletion of murine alveolar macrophages or neutrophils in mice can exacerbate lung injury due to delayed viral clearance [19,20]. Nevertheless, work in recent decades has begun to demonstrate the role that host genes play in susceptibility to severe or fatal influenza [21].

***In vivo* mouse studies – Mx**

Shortly after his 1957 discovery of IFN with Alick Isaacs [22], Jean Lindenmann made a chance discovery of the selective *in vivo* resistance of A2G mice to experimental infection by influenza virus and other myxoviruses [23-25]. The connection to IFN was proven using antiserum against mouse IFN that could ablate the natural protection from influenza enjoyed by the A2G mouse [26,27]. *Mx*, located on chromosome 16 and encoding a 72kDa IFN-inducible protein, Mx1, is the first mouse gene discovered by forward genetics of infectious diseases, following an expression cloning approach [28-30]. Mx1 deficiency, common in most inbred laboratory mouse strains, due to a nonsense mutation in CBA/J mice and a large deletion causing a frameshift and a premature stop codon in BALB/c mice, leads to profound susceptibility to influenza virus, although mice are not natural hosts for any known influenza virus [31]. The formal demonstration of Mx1 being the critical missing factor in these strains came from the transgenic expression of murine Mx1 or human MxA, which rescued the susceptibility phenotype [32,33]. Mx1 is a dynamin-like GTPase that, in the mouse, localizes to the nucleus. It prevents transcription by the viral RNA polymerase, effectively blocking both transcription and replication of the orthomyxviruses, which use a nuclear replication strategy [34-37]. The GTPase domain is critical for Mx1 binding to the viral ribonucleoprotein complex and the inhibition of transcription [38]. The IAV nucleoprotein is the main determinant of Mx1 sensitivity and various viruses, including avian strains like H7N9, have acquired mutations in NP that evade Mx1-mediated defense in mice [39,40]. Interestingly, DBA/2J mice (an Mx1-deficient mouse strain) crossed with A2G mice to express the wild-type Mx1 allele remain highly susceptible to H1N1 IAV [41]. Thus, in some cases the presence of Mx1 alone is insufficient for protection from IAV infection, indicating that regulation conferred by the genetic background and/or the influence of other alleles are at play and should be sought.

***In vivo* mouse studies – genome-wide screens**

Large-scale genome-wide studies offer the ability to identify novel pathways, genes and genetic variants affecting host control of IAV replication. The Collaborative Cross is an effort to measure the impact of genotypic diversity in eight inbred and three outbred mouse strains on multiple disease phenotypes including immune responses to infectious disease. Quantitative trait locus (QTL) mapping in the 8 inbred founders infected with IAV found several new candidate genes (*Grap2*, *Nox4*, and *Il16*) and led to the identification of a novel *Mx1* allele in CAST/EiJ with a reduced ability to inhibit IAV replication [42]. Additionally, QTL mapping identified sex-dependent effects in 29 closely-related mouse strains and novel candidates – *Hc*, *Pla2g7* and *Tnfrsf21* [43]. Several QTLs on chromosome 6, including *Samd9l* and *Ica1*, were found by linkage analysis on cytokine production following infection with highly pathogenic H5N1 IAV [44]. Recently, a novel *in vivo* screen consisting of a library of siRNA-encoding IAVs that each target one of 100 IAV-induced genes identified a previously unappreciated role for *Ifih1* (encoding melanoma-differentiation-associated gene 5 or MDA5), previously known for its recognition of positive-strand RNA viruses [45,46], in the immune response against IAV. Those viruses that targeted a necessary component of the innate immune response became more fit in the context of an *in vivo* infection and were identified by RNA-sequencing of the infected mouse lung [47].

***In vivo* mouse studies of IFN-inducing genes - reverse genetics**

The type I and III IFN signaling pathways are central to the immune response against influenza virus (reviewed by [48]). For some constituent parts of these pathways, their roles have been demonstrated *in vivo* by reverse genetics, i.e. by knockout (KO) studies (see Table 1). *Ifnb1* $-/-$ knockout mice are substantially more sensitive to IAV even in an *Mx1*-positive mouse [49]. *In vitro* studies showed that IAV infection is primarily recognized in hematopoietic cells by TLR7-MyD88 signaling [50,51] and in non-hematopoietic cells by RIG-I-MAVS signaling [46,52,53]. Mice deficient for *Unc93b1*, the adapter for endosomal TLRs – TLR3, 7, 8, and 9, show increased tissue pathology due to delayed viral clearance of influenza virus A/PR/8/34 infection [54]. However, *Tlr3* $-/-$ mice display decreased pathology and prolonged survival despite higher IAV lung titers pointing to TLR3 signaling in the promotion of inflammatory and adaptive responses that are ultimately damaging to the lung [55]. The *Myd88/Mavs* double knockout that cripples both signaling arms causes these mice to succumb to a lethal dose of IAV with more than ten-fold higher lung titers [56]. *Mavs* $-/-$ mice cope with infection like wild-type mice [56], whereas studies have conflicted on the absolute requirement for TLR7/MyD88 signaling for control of viral replication and development of IAV-specific antibodies in KO mice [56-59]. It should be noted that the above studies are performed in *Mx1*-deficient mice. Another study showed the necessity of TLR7 and MyD88 for survival after H7N7 IAV infection in an *Mx1*-positive mouse model [60]. This reflects the critical role of *Mx1* as an influenza restriction factor and the potential need to revisit many *in vivo* KO studies performed in *Mx1*-deficient mouse models [5,31]. Lastly, a recent study identified *Irf7* among 25 differentially expressed genes (DEGs) between C57BL/6J and DBA/2J mice infected with IAV and that overlapped with DEGs from other published studies. *Irf7* $-/-$ C57BL/6 mice (*Mx1*-negative) lost significantly more body weight with poor survival compared to wild-type control mice [61].

***In vivo* mouse studies of IFN-inducible genes - reverse genetics**

Downstream of IFN production is the potential expression of >400 IFN-stimulated genes (ISGs) that are the ultimate effectors of the innate antiviral response [62]. *Ifnar1*^{−/−} and *Stat1*^{−/−} mice do not respond to type I and III IFNs and allow overwhelming replication of IAV resulting in systemic influenza infection that is no longer restricted to the respiratory epithelium [63]. Influenza virus infection of *Ikbke*^{−/−} mice causes the production of normal amounts of type I IFN but they ultimately lack the induction of a subset of ISGs, including *Adar1*, *Ifit3*, *Ifi203*, due to a lack of STAT1 serine phosphorylation at position 708 by IKK-ε [64]. This reinforces the individual impact that specific ISGs, beside *Mx1*, can have during IAV infection. Mice deficient for ISG15, a 15kDa diubiquitin-like protein that can be similarly conjugated to viral target proteins, are more susceptible to influenza and other viruses [65,66]. However, IAV lethality in these mice is not due to higher viral titers but instead, a lack of conjugation-dependent immunity resulting in lung epithelial cell damage [67]. Double-stranded RNA (dsRNA) activated protein kinase R (PKR) phosphorylates eukaryotic initiation factor 2 alpha in response to dsRNA and inhibits protein synthesis [68]. PKR also sustains the IFN response by promoting the integrity of newly synthesized IFN-α and IFN-β mRNAs [69]. *Pkr*^{−/−} mice show 2-3 log higher IAV lung titers by day 2 of infection and show higher mortality compared to wild-type mice [68,70]. *In vivo* studies highlight the central role of IFNs and especially their induced genes in primary infections, whereas the control of secondary infections is less dependent on innate immunity and the IFN system.

Human *MX1* in vitro

Human *MX1* encodes a 78kDa protein called MxA [71,72] that localizes to the cytoplasm of the cell, which differs from the nuclear murine Mx1. Human MxA thus can inhibit viruses that replicate in the cytoplasm or in the nucleus such as VSV [73], hPIV3 [74], HBV [75,76], LACV [77], ASFV [78]. MxA, along with other ISG products, binds to NP of IAV [79,80] and retains the viral genomic RNA in the cytoplasm near late endosome [79]. It has since been shown that human MxA is induced predominantly by type I and III IFNs and that its non-constitutive expression is tightly regulated by these IFNs [81]. This tight regulation is perhaps due to the link of its expression to sensitivity to apoptotic stimuli [82,83]. Several high-throughput screens in humans have identified MxA as a restriction factor for replication of IAV, VSV and Yellow Fever virus [84,85]. Interestingly, inherited MxA deficiency has not currently yet been described in humans. Human *MX1* does have many heterozygous single nucleotide polymorphisms (SNPs) in the Exome Aggregation Consortium (ExAC) database, with frequencies ranging from 0.0008-0.27%, resulting in changes to the coding region including 12 heterozygous nonsense alleles. Eight of the missense alleles are present as homozygotes with 4 predicted to be damaging. None have yet been shown to be deleterious *in vitro* and the clinical status for these individuals, influenza infections in particular, is not reported. These data may be interpreted as suggesting that haploinsufficiency does not occur at the *MX1* locus, but they certainly do not exclude potential negative dominance for some heterozygous variations. Thus, the search for humans carrying deleterious mutations in *MX1*, including mono- and bi-allelic variations, should be continued especially among those who suffer from severe influenza.

Other *in vitro* human studies

Novel antiviral pathways have been uncovered using large-scale *in vitro* studies of human cell lines. Shapira *et al* [86] combined a yeast-two-hybrid analysis of IAV genes and 12,000 human genes with a transcriptome-wide array in IAV-infected human broncheoepithelial cells (HBECs) to create a network of genes that are positively and negatively regulated during infection. Among the most prominent hits, validated by small interfering RNA (siRNA)-mediated knockdown and measurement of viral titers in HBECs, were a group of inflammasome-related sensors and RNA-binding proteins. König *et al* [87] and Karlas *et al* [88] each also used RNA-interference (RNAi) screens in the context of influenza replication but identified those genes critical for the replication of influenza virus and thus identified potential therapeutic targets. Pulloor *et al* [89] focused their RNAi screen of 18,120 genes on regulators of the RIG-I pathway, which recognizes viral genomic RNA of IAV [90], using a GFP reporter driven by the IFN- β promoter in HEK293 cells transfected with synthetic double-stranded RNA. This approach identified the cellular pathway synthesizing inositol pyrophosphates as a critical regulator of IRF3 regulation and revealed the power of a screen coupled with a careful biochemical dissection to identify a previously unknown level of regulation. Winterling *et al* demonstrated the role of virus-inducible large intergenic non-coding RNAs (lincRNAs) in influenza virus replication, thus revealing a novel set of host factors affecting virus replication [91]. Dittmann *et al* screened a library of 401 ISGs overexpressed from a lentiviral library for the ability to reduce the spread of influenza virus in A549 lung adenocarcinoma cells. Extensive *in vitro* and *in vivo* functional assays validated *SERPINE1*, which encodes PAI-1, as a novel inhibitor of the host proteases that leave and mature the IAV attachment glycoprotein, HA[84]. Meta-analyses of large-scale screens [92-94] show little overlap between the hits identified within the various approaches, highlighting the importance of subtle differences in the libraries, cell systems, and time points examined. Thus, this emphasizes the necessity of validation experiments to confirm the involvement of these newly identified genes and/or pathways.

Human and mouse IFITM3

Using an RNA interference (RNAi) screen targeting host factors affecting early stages of influenza virus growth in human osteosarcoma cells, Brass *et al* identified the IFITM proteins, IFITM1, 2 and 3, as restriction factors for influenza virus, West Nile virus, and dengue virus [95]. Independently, Yount *et al* employed a proteomic approach, which purified fatty-acylated proteins in mouse dendritic cells, and also identified IFITM3 for its antiviral activity against influenza virus [96]. *IFITM3* is an IFN stimulated gene and resides largely in the endoplasmic reticulum. Post-translational modifications, S-palmitoylation and ubiquitination [96,97], regulate its function by relocating IFITM3 into the endosome where it blocks the virus fusion pore and thus the delivery of viral RNA into the cytosol. IFITM3 expression is negatively regulated by the NEDD4 E3 ligase, which targets IFITM3 for lysosomal degradation and can thus impact influenza replication [98]. IAV lung titers were higher, as were mortality and morbidity, in *Ifitm3* KO mice than in wild-type littermates [99,100]. Based on these results, Everitt *et al* also tested the association of a SNP in *IFITM3*, rs12252, with a C allele causing a truncation in IFITM3 (21) that is relocated throughout the cytosol [100]. Subjects homozygous for the C allele were significantly more

frequent among 53 patients from the UK hospitalized with severe influenza and further, among 32 Chinese patients with severe pH1N1 2009 infection [101]. However, the frequency of the C allele among healthy populations is quite high, especially in Asian populations: 49.7% (with 25.4% CC homozygous) in Han Chinese and 63.5% (43.8% CC homozygous) in Japanese populations [102]. Moreover, the association of rs12252 failed to reach significance with larger sample sizes [103]. This indicates that this *IFITM3* SNP could not be a major causal factor, but perhaps a modifier, of the outcome of influenza infection. Additionally, recent functional studies showed that expression of IFITM3 21 in A549 cells restricted influenza virus comparably to wild-type IFITM3 [104]. Thus, there is convincing experimental evidence for the role of wild-type IFITM3 but further work is required to define the role of common SNPs of *IFITM3* as modifiers for severe influenza.

Human *in vivo* studies – testing a common variant hypothesis

Common variants or SNPs have been tested in population-based studies of influenza outcomes, in “genome-wide association study (GWAS)-like” studies (reviewed in detail [105]). A SNP in *CD55*, rs2564978, was chosen from 5,166 SNPs identified by a small-scale GWAS study in 25 Chinese patients with severe influenza and 26 controls [106]. This SNP caused reduced expression of CD55 (also known as decay accelerating factor, DAF), which can reduce epithelial cell damage during influenza infection [106]. A similar GWAS study of 42 Chinese patients with severe pH1N1 or H7N9 infection and 42 controls crossed with a quantitative cis-QTL study in the lung yielded 1,114 SNPs. From these, a SNP (rs383510) that may regulate *TMPRSS2* expression was chosen based on mouse studies showing *Tmprss2* expression levels modulate infection outcomes [107,108]. Targeted screens of several candidate genes have also investigated SNPs associated with severe influenza without confirmed associations [105,109,110]. A deletion in the chemokine receptor *CCR5* was reported to be enriched in a Canadian cohort suffering from severe influenza [111]. However, another study failed to reach significance in a similar cohort of European ancestry [112]. More interestingly, regulatory SNPs affecting expression of surfactant proteins, *SFPTA* (rs1965708 and rs1059046) in a Spanish cohort and in *SFPTB* (rs1130866) in a Chinese cohort [113,114], were associated with severe pH1N1 2009 influenza. Independent studies of Japanese and Chinese children who developed severe and/or fatal influenza-associated encephalopathy found significant association with exonic variants in *CPT2* that result in thermolabile proteins that are hypomorphic during fever [115,116]. However overall, population-based studies have been underpowered and unreplicated and have not yet revealed major genetic determinants of severe influenza [110]. The common variants detected by these means might play a role, at best that of modifiers given the rarity of the disease, which needs to be experimentally tested [117,118].

Human *in vivo* studies – primary immunodeficiencies

Surprisingly, previously defined human inborn errors of immunity, including severe primary immunodeficiencies, such as severe combined immunodeficiency (a lack of T cells) or agammaglobulinemia (a lack of B cells) do not predispose children to severe influenza [119,120]. Although T and B cells are needed for generating acquired immunity to influenza virus or vaccine, these cells do not seem to be critical for the control of primary or secondary infection. This contrasts with parainfluenza viruses, which often kill children with

inborn errors of T cells [119]. Remarkably, several patients with MonoMAC syndrome caused by GATA-2 deficiency died from influenza and these patients are diminished in several leukocyte subsets, including plasmacytoid dendritic cells (pDCs) [121,122]. Known inborn errors of type I and III IFNs, including STAT1, IL10RB, TLR3 and MyD88 deficiencies, have so far not been shown to have severe influenza [123,124]. Moreover, humans with ISG15 deficiency do not develop severe infections with influenza but instead demonstrate a susceptibility to mycobacterial disease and a type I interferonopathy [125,126]. Infection with influenza A and B viruses can also trigger acute necrotizing encephalitis (ANE) [127]. This is distinct from severe pulmonary influenza virus infection because cerebral spinal fluid and brain tissue of these individuals is negative for influenza and parainfluenza viruses [127]. ANE is thus not commonly seen as a PID in the traditional sense of the term. Neilson *et al* identified a heterozygous missense mutation, p.Thr585Met, in *RANBP2* in 11 patients suffering from influenza-induced ANE [128]. *RANBP2*, a SUMO1 E3 ligase, regulates protein transport across the nuclear pore [129]. *RANBP2* deficiency is only found in familial cases of ANE and is inherited as an autosomal dominant incompletely penetrant trait with 40% of heterozygous carriers developing ANE [128]. The connection between *RANBP2* mutations and ANE remains unclear but there is no indication that those who carry these mutations have remarkable pulmonary complications from influenza. A molecular characterization is needed to pin down this mechanism and to elucidate the effect *RANBP2* mutations have on virus replication and/or the immune response meant to control it.

Human monogenic lesions *in vivo* - inherited IRF7 deficiency

We hypothesized that severe influenza may be caused by single-gene inborn errors of immunity, as previously documented for an increasing variety of infections [1]. We enrolled 22 patients suffering from severe influenza and requiring hospitalization, among which were 3 children less than five years old. Using whole exome sequencing, we discovered compound heterozygous mutations in *IRF7*, encoding interferon regulatory factor 7 (IRF7), in a girl who was severely ill with influenza at 2.5 years old [130]. Both of her *IRF7* alleles fail to upregulate type I IFN resulting in elevated IAV replication in fibroblasts and abolished type I and III IFN production in pDCs, which constitutively express high levels of IRF7 and produce large amounts of type I and III IFNs [131,132]. Perhaps not coincidentally, pDCs can be diminished in patients with GATA-2 deficiency [121]. The generation of airway epithelial cells from patient-specific induced pluripotent stem cells (iPSCs) allowed the demonstration of increased influenza virus spread associated with diminished IFN production due to a lack of IRF7. This represents the first demonstration of a genetic cause for severe disease influenza in humans. It is intriguing that known patients with mutations in *STAT1* or *IL10RB*, which impair type I and III IFN signaling, have not developed severe influenza. Of note, the *Irf7* KO mouse is highly susceptible to many RNA and DNA viruses and displays higher mortality when infected by IAV, while the IRF7 patient exhibited a narrower susceptibility [61,133-138]. IFN-mediated control of influenza virus has been known since the initial discovery of IFN itself [22], and the myriad ways that IAV antagonizes the IFN response underscores the *in vivo* role IFN plays in controlling pathogenesis [13]. The critically important question of the relative contributions of intrinsic

immunity in pulmonary cells and of innate immunity from pDCs is still incompletely understood.

IRF7 in cell-intrinsic immunity against influenza virus

The essential role of mouse IRF7 in the initial induction and subsequent amplification of type I and III IFN immunity was well established by reverse genetics [133,139-143]. In a study previously discussed, *IRF7* was also among the genes identified by Dittmann *et al* in a lentivirus-driven gain-of-function screen that selected for genes that control the spread of influenza virus *in vitro* [84]. Other recent forward somatic genetic studies point to a key role of IRF7 in anti-influenza immunity. A genome-wide screen of canine kidney cells showed that IRF7 knockdown led to the highest replication of influenza vaccine strain [144], while a loss of the translational repressors of IRF7, 4E-BP1 and 4E-BP2, led to dramatically lower influenza virus replication in mouse embryonic fibroblasts [145]. Moreover, a SNP diminishing IRF7 expression was associated with impaired antiviral responses in influenza-infected human DCs [146]. Such findings are consistent with the *in vitro* phenotypes observed in pDCs and iPSC-derived airway epithelial cells from the IRF7-deficient patient described above [130]. Further, these data, which all converged independently on IRF7, suggest that other inborn errors underlying severe influenza may be IRF7-related. For other human infections, a core gene was found, such as IFN- γ for mycobacterial diseases, IL-17 for mucocutaneous candidiasis, TLR3 for herpes simplex encephalitis, and CARD9 for invasive fungal diseases [147].

Conclusions

There is theoretical, experimental, clinical, and epidemiological evidence to propose and test the hypothesis that severe influenza in humans can be determined by inborn errors of immunity. Preliminary findings suggest that single-gene errors of innate immunity, affecting IRF7-dependent IFN- α/β and IFN- λ immunity in particular, can cause severe and life-threatening influenza. The study of influenza adds weight to the idea that severe infectious disease, in the course of primary infection, can be caused by monogenic lesions not necessarily fully penetrant [1]. Mutations that are found in humans and mice to underlie severe influenza point out the non-redundant pathways and molecules involved in protective immunity during primary infection. The clearest example is that of a single-gene inborn error of immunity, while the search for a polygenic explanation for this devastating disease has lagged behind. Of particular interest, genetic susceptibility to severe influenza in humans is probably narrow as most patients have been healthy otherwise. An understanding of the diversity of human antiviral responses and the deficiencies that confer susceptibility to each severe and fatal viral disease will fundamentally change the way we approach viral disease from a medical standpoint, including influenza in particular. The findings that can be confirmed *in vivo*, especially in humans, offer a clear target for manipulation in the clinic. For example, it is tempting to speculate that recombinant IFN- α may be useful in children with life-threatening influenza, especially in children deficient in an IFN-inducing gene such as *IRF7*. The advent of patient-tailored therapeutics and targeted functional screening based on next-generation sequencing data may augment host immunity to more effectively manage the disease course of influenza.

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Highlights

1. Severe influenza can be caused by single-gene inborn errors of immunity in mice and humans
2. Mouse *Mx1* was the first influenza susceptibility gene identified by forward genetics
3. Inherited IRF7 deficiency caused life-threatening influenza in an otherwise healthy child
4. Susceptibility genes are presumably influenza-specific as most patients are healthy prior to and after severe influenza

Table 1

Reverse genetics in mice of IFN- γ -inducing and IFN- γ -inducible genes

	gene knockout	protein name	mouse strain	Mx-background	virus strain	virus subtype	viral phenotype	morbidity	mortality	reference
IFN- γ -inducing	Ifnb $^{-/-}$	IFN- β	B6.A2G.Mxl	+/+	SC35M and A/PR/8/34	H7N7; H1N1	elevated lung titer, decreased LD50	<i>no data</i>	increased	Koerner et al 2007 (49)
	Ifnar1 $^{-/-}$	IFNAR1	B6.A2G.Mxl	+/+	SC35M	H7N7	dramatically decreased LD50	<i>no data</i>	increased	
	Ifnar1 $^{-/-}$	IFNAR1	129	-/-	A/WSN/33 and A/PR/8/34	H1N1	elevated lung titers	disseminated infection with hepatic and brain pathology	<i>no data</i>	Garcia-Sastre et al 1998 (63)
	Stat1 $^{-/-}$	STAT1	C57BL/6	-/-					<i>no data</i>	
	Ikkb $^{-/-}$	IKK- ϵ	C57BL/6	-/-	A/WSN/33	H1N1	<i>no data</i>	increased	increased	tenOever et al 2007 (64)
	Tlr7 $^{-/-}$	TLR7	B6.A2G.Mxl	+/+	SC35M	H7N7	elevated lung titers	<i>no data</i>	increased	Kaminski et al 2012 (60)
	Tlr7 $^{-/-}$	TLR7	C57BL/6	-/-	A/PR/8/34	H1N1	equal to wt mice	decreased cytokine production	survive sublethal infection	Pang et al 2013 (59)
	Mavs $^{-/-}$	MAVS		-/-			equal to wt mice	decreased cytokine production	survive sublethal infection	
	Tlr7 $^{-/-}$ Mavs $^{-/-}$	TLR7 and MAVS		-/-			reduced virus titer	<i>no data</i>	<i>no data</i>	
	Tlr7 $^{-/-}$	TLR7	C57BL/6	-/-	A/PR/8/34	H1N1	low antibody titers	<i>no data</i>	decreased	Jeisy-Scott et al 2012 (57)
	Myd88 $^{-/-}$	MyD88	C57BL/6	-/-	A/PR/8/34	H1N1	low antibody titers	<i>no data</i>	decreased	
	Myd88 $^{-/-}$ Trif $^{-/-}$	MyD88 and TRIF	C57BL/6	-/-	A/PR/8/34	H1N1	elevated lung titers	no difference in weight loss	increased	Seo et al 2010 (58)
	Tlr3 $^{-/-}$ Tlr7 $^{-/-}$	TLR3 and TLR7	C57BL/6	-/-	A/PR/8/34	H1N1		<i>no data</i>	increased	
	Myd88 $^{-/-}$	MyD88	B6.A2G.Mxl	+/+	SC35M	H7N7	elevated lung and brain titers	neurotropic infection	increased	Kaminski et al 2012 (60)
	Myd88 $^{-/-}$	MyD88	C57BL/6	-/-	A/New Caledonia/20/99	H1N1	normal lung titers	<i>no data</i>	<i>no data</i>	Koyama et al 2007 (56)
	Ips1 $^{-/-}$	IPS-1	C57BL/6	-/-			normal lung titers	<i>no data</i>	<i>no data</i>	
	Myd88 $^{-/-}$ Ips1 $^{-/-}$	MYD88/IPS-1	C57BL/6	-/-			elevated lung titers	<i>no data</i>	<i>no data</i>	
	Unc93b1 $^{-/-}$	UNC93B	C57BL/6	-/-	A/PR/8/34	H1N1	elevated lung titers	increase lung pathology	slightly increased	Lafferty et al 2014 (54)
	Tlr3 $^{-/-}$	TLR3	C57BL/6	-/-	A/Scotland/20/74	H3N2	elevated lung titers	decreased lung pathology	decreased	Le Goffic et al 2006 (55)
IFN- γ -inducible	Irf7 $^{-/-}$	IRF7	C57BL/6	-/-	A/PR/8/34 serially passaged in Mx1 $^{-/-}$ mice	H1N1	<i>no data</i>	increased weight loss	increased	Wilk et al 2015 (61)
	Isg15 $^{-/-}$	ISG15	C57BL/6	-/-	A/WSN/33 and B/Lee/40	H1N1; -	elevated lung titers	increased inflammatory cytokine production	increased	Lenschow et al 2007 (65)
	Eif2ak2 $^{-/-}$	PKR	129/Sv	-/-	A/WSN/33	H1N1	elevated lung titers	increased weight loss	increased	Balachandran et al 2000 (68)

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	gene knockout	protein name	mouse strain	Mx-background	virus strain	virus subtype	viral phenotype	morbidity	mortality	reference
	Eif2ak2 ^{-/-}	PKR	129/Sv(ev)xC57BL/6J	-/-	A/PR/8/34	H1N1	elevated lung titers	increased weight loss	increased	Bergmann et al 2000 (70)
	Serpine1 ^{-/-}	PAII	C57BL/6	-/-	A/PR/8/34	H1N1	elevated lung titers	increased lung pathology and weight loss	increased	Dittmann et al 2015 (84)