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Recent advances in understanding viral evasion of type I interferon

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Summary

The type I interferon (IFN) system mediates a wide variety of antiviral effects and represents an important first barrier to virus infection. Consequently, viruses have developed an impressive diversity of tactics to circumvent IFN responses. Evasion strategies can involve preventing initial virus detection, via the disruption of the Toll-like receptors or the retinoic acid inducible gene I (RIG-I) -like receptors, or by avoiding the initial production of the ligands recognized by these receptors. An alternative approach is to preclude IFN production by disarming or degrading the transcription factors involved in the expression of IFN, such as interferon regulatory factor 3 (IRF3)/IRF7, nuclear factor-κB (NF-κB), or ATF-2/c-jun, or by inducing a general block on host cell transcription. Viruses also oppose IFN signalling, both by disturbing the type I IFN receptor and by impeding JAK/STAT signal transduction upon IFN receptor engagement. In addition, the global expression of IFN-stimulated genes (ISGs) can be obstructed via interference with epigenetic signalling, and specific ISGs can also be selectively targeted for inhibition. Finally, some viruses disrupt IFN responses by co-opting negative regulatory systems, whereas others use antiviral mechanisms to their own advantage. Here, we review recent developments in this field.

Keywords: antiviral; evasion; inhibition; interferon; virus.

Introduction

Despite almost constant exposure to pathogens, mammals are only rarely infected to the point where disease becomes evident. The first line of defence consists of the interferon (IFN) family of soluble cytokines. The IFNs have anti-cancer, anti-proliferative, anti-viral and immunomodulatory functions[1] through the expression of more than 300 IFN-stimulated genes (ISGs).[2] There are three classes of IFNs which are produced by different cell types, bind unique receptors and have distinctive biological actions.[3] Here, we focus on the type I IFNs, which are produced by most cell types and have potent, inherent antiviral activity.[4] The type I IFN response is bimodal: first, detection of an invading virus leads to IFN production and secretion and second, IFN acts in an autocrine and paracrine manner to induce ISGs, the products of which work collectively to disrupt viral replication and spread.

To generate a productive infection, viruses must overcome antiviral responses, and accordingly, every aspect of these defences is targeted for inhibition. Here, we describe the IFN response and viral immune evasion strategies. As this topic has been extensively reviewed previously, we will focus on the most recent advances.

Virus recognition

In the first step of the biphasic type I IFN response, virus is detected through the recognition of pathogen-associated molecular patterns (PAMPs), highly conserved structural features found in broad classes of pathogens. PAMPs are sensed by pattern recognition receptors (PRRs), including the toll-like receptors (TLRs).[5] The TLRs recognize viral components including glycoproteins and nucleic acids such as dsRNA or CpG DNA. Via their cytoplasmic Toll/interleukin-1 receptor (TIR) domains, TLRs recruit TIR-containing adaptors such as MyD88, TIR-domain-containing adapter-inducing IFN-β (TRIF), Mal and TRIF-related adaptor molecule (TRAM), leading to the activation of nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3) (Fig. 1).

Recently, several viruses have been found to disrupt TLR signalling by interfering with the adaptor molecule
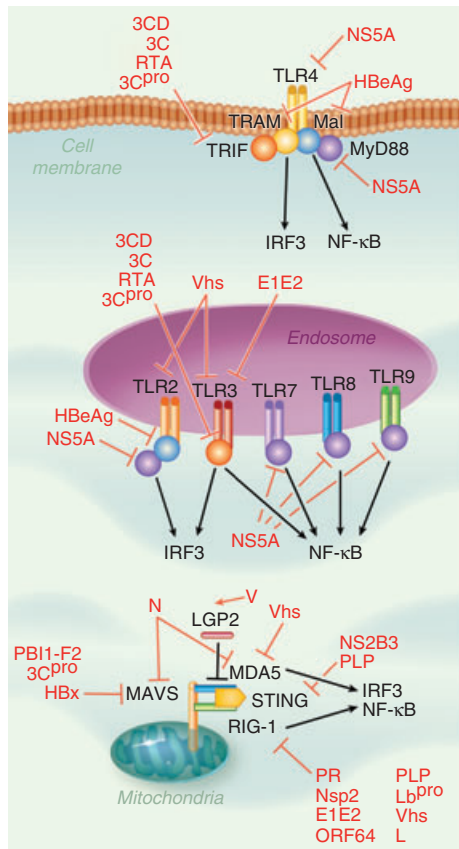
Figure 1. Pathways to virus detection. Viral pathogen-associated molecular patterns (PAMPs) are identified by various pattern recognition receptors (PRRs), such as the Toll-like receptors (TLRs) and retinoic acid inducible gene I (RIG-I)-like receptors (RLRs). TLRs are found both at the plasma membrane and in endosomes, detect a variety of virus-associated ligands, and signal through adaptors proteins Toll/interleukin-1 receptor-domain-containing adapter-inducing IFN-β (TRIF), TRIF-related adaptor molecule (TRAM), Mal and MyD88 to lead to the activation of transcription factors interferon regulatory factor 3 (IRF3) and nuclear factor-κB (NF-κB). RLRs retinoic acid inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), which are negatively regulated by LGP2, detect viral dsRNA, and signal through the adaptors mitochondrial antiviral signalling protein (MAVS) and STING to cause IRF3 and NF-κB activation. Virtually every step in this process can be impeded by viral proteins.

TRIF. For example, 3CD protease-polymerase, an intermediate in the polyprotein processing cascade of hepatitis A virus,[6] and 3C protein of enterovirus 71,[7] use protease activity to cleave TRIF. Replication and transcription activator (RTA) from Kaposi's sarcoma-associated herpesvirus (KSHV) also reduces TRIF levels, likely through a proteasome-mediated pathway.[8] Other TLR adaptor proteins are also affected – the hepatitis B virus HBeAg protein uses its precore specific sequence, which shows homology to the TIR motif, to compete with TIR-containing proteins Mal and TRAM to impede their interactions with downstream signalling molecules.[9]

A second class of PRRs is the retinoic acid inducible gene I (RIG-I)-like receptor (RLR) family, including RIG-I and melanoma differentiation-associated gene 5 (MDA5).[10] The RLRs detect cytoplasmic dsRNA, interact with the adaptor mitochondrial antiviral signalling protein (MAVS) and activate NF-κB and IRF3. Like TLRs, RLRs are hindered by viruses. For instance, the N protein from human respiratory syncytial virus (RSV) inhibits MDA5 and MAVS,[11] whereas the HIV protease decreases cytoplasmic RIG-I levels by targeting the sensor to the lysosome.[12] In contrast, the V proteins of several paramyxoviruses promote an interaction between RIG-I and LGP2,[13] an RLR that lacks signalling capacity.[14] Several viruses target RIG-I via viral de-ubiquitinating enzymes (DUBs), such as Arterivirus non-structural protein 2, Nairovirus L protein,[15] KSHV ORF64,[16] severe acute respiratory syndrome coronavirus (SARS-CoV) papain-like proteases,[17] and foot-and-mouth disease virus (FMDV) Lb^{pro}.[18] These DUBs remove K63-linked ubiquitin on RIG-I, preventing its interaction with MAVS.[19]

MAVS is also a popular focus of viral antagonists. The influenza A protein PB1-F2 binds the transmembrane domain of MAVS, causing a drop in the mitochondrial membrane potential,[20] which is required for MAVS function.[21] Coxsackievirus B3 encodes the cysteine protease 3C^{pro}, which directly cleaves both TRIF and MAVS, impeding both the TLR3 and RLR pathways, respectively.[22] Finally, the hepatitis B virus protein HBx associates with and blocks the action of MAVS.[23]

The adaptor protein STING, which interacts with RIG-I and MAVS and is involved in the detection of cytosolic DNA,[24] is also affected by viral proteins, such as the protease complex NS2B3 of Dengue virus, which cleaves STING into inactive fragments.[25] Interestingly, the papain-like proteases from human coronavirus NL63 and SARS-CoV, which possess protease and DUB enzyme activities, disrupt the dimerization of STING by decreasing its level of ubiquitination.[17]

Several viral proteins target both TLRs and RLRs at the expression level. The virion host shutoff (vhs) endoribonuclease from herpes simplex virus type 2 (HSV-2) specifically reduces TLR2, TLR3, RIG-I and MDA5 mRNA,[26] whereas hepatitis C virus (HCV) uses E1E2 to down-regulate the mRNA levels of TLR3 and RIG-I via E1E2,[27] and NS5A to decrease TLR4, MyD88, IRF3 and NF-κB2.[28]

The most straightforward mechanism of viral evasion of the IFN response is to avoid detection in the first place. Several viruses conceal or degrade dsRNA, a by-product of viral replication. For example, tick-borne encephalitis virus delays antiviral signalling by sequestering RNA molecules into cytoplasmic membrane-defined compartments, where they are inaccessible to PRR recognition.[29] Similarly, Japanese encephalitis virus (JEV) conceals its dsRNA among intracellular membranes.[30]

Amazingly, species-specific differences in the timing of the release of viral dsRNA into the cytosol account for the drastically different pathogenesis of JEV in humans compared with pigs.[30] Rather than hide it, Lassa fever virus uses the 3′–5′ exonuclease activity of its NP protein to degrade its dsRNA,[31] whereas the C protein from human parainfluenza virus type 1 is thought to regulate viral RNA production in such a way as to prevent dsRNA from accumulating at all.[32]

Transcription factor activation to IFN expression

Viral sensing by PRRs activates three main transcription factor complexes involved in IFN-β production: NF-κB, IRF3/IRF7 and ATF2/c-jun (Fig. 2).[33] In resting cells, NF-κB is held as an inactive complex in the cytoplasm by its inhibitor, IκBα.[34] PRR activation stimulates IκBα phosphorylation and degradation, releasing NF-κB to translocate to the nucleus and induce target genes. A recent example of viral disruption of NF-κB activation involves the V protein from measles virus, which binds to the nuclear location signal of the NF-κB subunit p65, impairing its nuclear translocation.[35] The NF-κB essential modulator (NEMO), a regulatory component involved in the phosphorylation of IκBα,[36] is also targeted, as it is cleaved into inactive fragments by the FMDV protease 3C$^{\text{pro}}$.[37]

Less is understood about ATF2/c-Jun. This complex is constitutively nuclear, even in its inactive form, and is stimulated by phosphorylation of its activation domains.[38] Virus infection triggers the stress-activated members of the mitogen-activated protein (MAP) kinase superfamily, which phosphorylate and activate ATF2/cJun. For the first time, a viral protein blocking this complex has been described; the Zaire ebola virus protein VP24 prevents the phosphorylation of p38 MAP kinase and the downstream activation of ATF2.[39]

Critical factors involved in IFN expression include IRF3 and IRF7.[40] IRF3, which is constitutively expressed in resting cells, is phosphorylated upon PRR signalling by the IκB kinase (IKK)-related kinases IKKε and TBK-1, causing IRF3 to homodimerize and translocate to the nucleus. There, IRF3 interacts with the histone acetyl transferases CBP and p300, and associates with the IFN-β promoter. IRF3 can also directly activate a subset of ISGs in the absence of IFN.[41,42] Accordingly, IRF3 is a popular target for viral inhibition. The V protein of Sendai virus directly binds IRF3, impairing its function.[43] Varicella zoster virus induces atypical, TBK1-independent phosphorylation of IRF3 that blocks downstream dimerization and activity, via the viral serine-threonine protein kinase ORF47.[44] Additionally, varicella zoster virus ORF61 interacts specifically with activated, phosphorylated IRF3, and uses its RING finger E3 ubiquitin ligase domain to ubiquitinate and degrade IRF3 via the proteasome pathway.[45] HIV immune evasion is complex and cell-type dependent;

in T cells, it has previously been shown that viral proteins Vpr and Vif disrupt the IFN response via the degradation of IRF3,[46,47] whereas in dendritic cells (DCs), IRF3 has recently been found to remain intact, but its activation and nuclear translocation are impeded by Vpr.[48] The HIV protein Vpu also degrades IRF3, by binding and directing it to the lysosome.[49] Instead of interfering with IRF3 activation, NS1 from RSV associates with both IRF3 and its co-activator CBP, impeding their interaction and impairing promoter binding.[50]

Several viral proteins indirectly disrupt IRF3 activation by interfering with the kinases TBK1 or IKKε. The papain-like protease domain 2 of NSp3 from mouse hepatitis virus (MHV) A59 has been found to de-ubiquitinate TBK1, decreasing its kinase activity and stabilizing it in an inactive conformation.[51] Although the mechanisms are currently unclear, the severe fever with thrombocytopenia syndrome virus NSs protein[52] and the HSV-1 γ34.5 protein associate with and inhibit TBK1,[53] while the Tula virus glycoprotein Gn disrupts IFN production at the level of the TBK1 complex.[54] Although they do not impede TBK1, the the NP proteins of several arenaviruses associate with the kinase domain of IKKε, impairing its binding to MAVS and preventing it from phosphorylating IRF3.[55] KSHV also inhibits IKKε signalling by encoding an miRNA known as miR-K12-11, which down-regulates IKKε mRNA translation.[56] Lastly, the C6 protein from vaccinia virus interferes with the activation of IRF3 and IRF7 at the level of TBK1/IKKε, via interaction with the kinase scaffold proteins TANK, NAP1 or SINTBAD.[57] As the exact contribution of these scaffold proteins to antiviral signalling is unclear, elucidation of C6 activity could provide valuable insight into IFN production.

Unlike IRF3, IRF7 is basally expressed at very low to undetectable levels in most cells. IFN-β production by IRF3, NF-κB and ATF2/c-jun induces the expression of IRF7. Like IRF3, IRF7 is phosphorylated by TBK1 and IKKε, causing it to heterodimerize with IRF3 and stimulate full type I IFN expression.[58] KSHV ORF45 impedes the phosphorylation and activation of IRF7 (but not IRF3) by competitive inhibition, as it is phosphorylated by IKKε and TBK1 more efficiently than IRF7.[59] ORF45 may also block IRF7 by associating with its inhibitory domain, stabilizing autoinhibitory intramolecular interactions to keep the protein in a closed, inactive conformation.[60] The V proteins of several paramyxoviruses associate with and disrupt IRF7,[61] as does N$^{\text{pro}}$ from classical swine fever virus.[62] Some strains of rotavirus use their NSP1 protein to cause IRF7 degradation via the proteasome, whereas other strains target IRF3, IRF5 or β-transducin repeat-containing protein (β-TrCP), a component of the E3 ubiquitin ligase complex that activates NF-κB.[63] Finally, the ebolavirus VP35 protein represents an interesting example of IRF7 inhibition: in macrophages and conventional DCs, VP35 interferes with
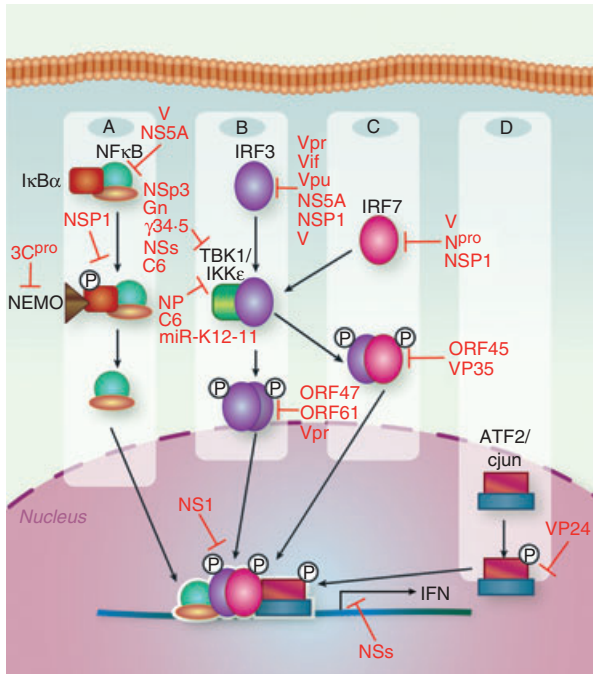
Figure 2. Transcription factor activation to interferon (IFN) production. Detection of viral components by pattern recognition receptors (PRRs) leads to the activation of a variety of transcription factors. (a) Nuclear factor-κB (NF-κB) is held in an inactive cytoplasmic complex via interaction with its inhibitor, IκBα. Upon virus recognition, IκBα is phosphorylated in a process involving the regulatory component NEMO, leading to degradation of the inhibitor, freeing NF-κB to translocate to the nucleus and bind the IFN promoter. (b) Upon virus detection, constitutively expressed interferon regulatory factor 3 (IRF3) is phosphorylated by the kinases TBK1 and IKKε, leading to its dimerization and nuclear translocation. (c) Although IRF7 is minimally expressed in most resting cell types, low level IFN production induces IRF7 expression, leading to its phosphorylation by TBK1/IKKε, heterodimerization with IRF3, nuclear translocation, and increased IFN expression. (d) The constitutively nuclear ATF2/cjun is phosphorylated upon virus detection by stress-activated members of the mitogen-activated protein (MAP) kinase superfamily, leading to the activation of the complex. Cooperative binding of NF-κB, IRF3/IRF7 and ATF2/cjun to the IFN promoter leads to full expression of type I IFN genes. Each of these pathways is subject to inhibition by viruses.

IRF7 activation via the RLR pathway, whereas in plasmacytoid DCs, VP35 does not block IFN production, because this cell type activates IRF7 through the TLR pathway.[64] Hence, non-redundant IFN induction pathways can help an organism to counteract specific virus evasion mechanisms.

Viruses can also impair IFN gene expression by inducing a general disruption of host cell transcription. The NSs protein from La Crosse encephalitis virus does just this, exploiting specific components of the DNA-damage response to cause the proteasomal degradation of the hyperphosphorylated form of RPB1, a component of cel-

lular RNA polymerase II (RNAP II), allowing it to selectively silence elongating RNAP II complexes. This does not impede the virus itself, as RNAP II is not required for the transcription or replication of the La Crosse encephalitis virus genome.[65]

IFN signalling

The second step of the biphasic IFN response, where secreted IFN binds its receptor (IFNAR) and activates ISG induction, is also actively disrupted by viruses. Although the exact mechanism is unknown, ORF54, a functional dUTPase from murine γ-herpesvirus-68, causes the degradation of the IFNAR1 protein, even in the absence of dUTPase enzymatic activity.[66] Several other viruses indirectly target IFNAR, by activating alternative signalling. For instance, HCV induces the Ras/Raf/MEK pathway, which increases the phosphorylation of a destruction motif in the cytoplasmic tail of IFNAR1, leading to its ubiquitin-dependent endocytosis.[67] The Kunjin strain of West Nile virus may employ a similar strategy, as the viral proteins NS4A and NS4B block IFN signalling by stimulating the unfolded protein response,[68] possibly via IFNAR degradation.[69]

Interferon binding to IFNAR activates the Janus family protein kinases (JAKs) Tyk2 and Jak1, inducing site-specific phosphorylation of tyrosine residues in signal transducers and activation of transcription 1 (STAT1) and STAT2, leading to their activation and formation of a heterotrimeric complex containing IRF9, known as IFN-stimulated gene factor-3 (ISGF3) (Fig. 3).[70] Each stage of the JAK/STAT signalling pathway is disrupted by viral proteins. Human metapneumovirus reduces Jak1 and Tyk2 mRNAs and proteins,[71] leading to decreased IFNAR cell surface expression by way of increased internalization but not degradation, possibly through the loss of Tyk2.[72] The E6 and E7 proteins from human papillomaviruses specifically interfere with the STAT1 promoter to block its transcription, in a mechanism that may involve histone deacetylation and DNA methylation.[73] The C protein of human parainfluenza virus type 1 impedes the nuclear translocation of STAT1 by physically retaining it in the cytoplasm in perinuclear aggregates associated with late endosomal markers.[74] RSV NS-1 and NS-2 prevent the phosphorylation and nuclear translocation of STAT1 and STAT2 after IFN-β treatment of bone-marrow-derived DCs,[75] whereas in the respiratory epithelium, NS2 causes the degradation of STAT2.[76,77] Viral interferon regulatory factor 2 (vIRF2) from KSHV decreases STAT1 and IRF9 levels to impair ISGF3 function.[78] HSV-2 causes the selective loss of STAT2 transcripts and proteins in some cell types, whereas in others, STAT2 levels remain constant but its phosphorylation and nuclear translocation are inhibited.[79] The papain-like protease from SARS-CoV has a complex mechanism of interference: it is a

de-ubiquitinating enzyme that up-regulates the expression of ubiquitin-conjugating enzyme E2-25k, leading to the ubiquitin-dependent proteasomal degradation of extracellular signal-regulated kinase (ERK) 1, which interferes with ERK1-mediated STAT1 phosphorylation.[80] Interestingly, adenovirus stabilizes tyrosine-phosphorylated, activated STAT1, sequestering it at viral replication centres, potentially through binding with viral DNA.[81] Adenovirus also impairs the dephosphorylation of STAT1 by obstructing its interaction with the protein tyrosine phosphatase TC45.[81]

ISG expression and function

Once activated, ISGF3 binds the promoters of ISGs, leading to their transcriptional activation.[70] While investigating how the human adenovirus protein E1A evades the type I IFN response, Fonseca et al.[82] furthered our understanding of this process, demonstrating how studying the virus leads to a better understanding of the host. They found that IFN-mediated antiviral activity requires the mono-ubiquitination of histone 2B (H2B) at lysine 120, a post-translational modification associated with transcriptionally active chromatin, in both the transcribed regions and the promoters of ISGs. This finding is a novel and unexpected aspect of antiviral signalling. Additionally, they found that E1A disrupts the hBre1 complex responsible for H2B mono-ubiquitination, preventing the expression of ISGs, and allowing viral escape of antiviral signalling.[82] In another elegant study, Marazzi et al.[83] demonstrated how viruses exploit epigenetic signalling to regulate antiviral gene expression. They found that the NS1 protein of influenza A strain H3N2 contains a short sequence that mimics the histone H3 tail. This permits histone-modifying enzymes to act on NS1; accordingly, NS1 is both acetylated and methylated in infected cells.[83] Modified NS1 associates with the human PAF1 transcription elongation complex, allowing the virus to hijack the host transcriptional elongation machinery. NS1 also disrupts transcriptional elongation at sites of active antiviral gene transcription, selectively impairing the expression of ISGs).[83]

Instead of globally obstructing ISG expression, some viruses target particular ISGs. For example, HCV infection up-regulates a microRNA that specifically decreases the expression of the ISG IFITM1.[84] The immediate-early 1 (IE1) protein of human cytomegalovirus (HCMV) down-regulates IFN-inducible Sp100 protein levels. While IE1 interacts with and causes proteasome-mediated degradation of Sp100A, it is unclear how IE1 affects additional Sp100 isoforms.[85]

Although the antiviral functions of many ISGs are not clearly understood,[86] those of 2'-5'-oligoadenylate synthetase (OAS) and protein kinase R (PKR) are well elucidated.[4] In response to dsRNA, OAS produces 2'-5'-linked
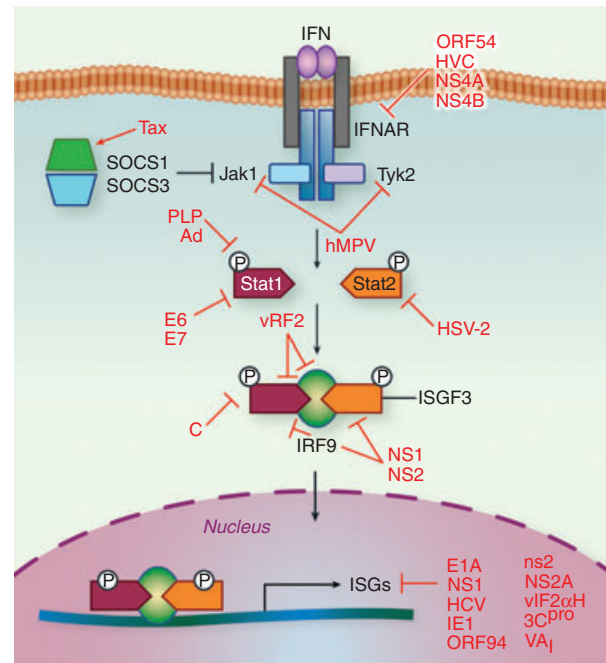

Figure 3. Interferon (IFN) signalling to ISG expression. Secreted IFN binds to the cell-surface receptor IFNAR, leading to the activation of kinases Tyk2 and Jak1, which phosphorylate and activate proteins signal transducer and activator of transcription 1 (STAT1) and STAT2. This results in the formation of a heterotrimeric complex containing interferon regulatory factor 9 (IRF9), known as IFN-stimulated gene factor-3 (ISGF3). Jak protein activation is negatively regulated by the IFN-inducible proteins SOCS1 and SOCS3. Binding of ISGF3 to the promoters of ISGs leads to their transcriptional activation, and the collective actions of the hundreds of ISGs induced by IFN inhibit both virus replication and spread. Each aspect of this process can be disrupted by viral processes.

oligoadenylates (2-5A) from ATP, which activate latent RNase L, leading to degradation of host and viral mRNAs, while PKR phosphorylates the eukaryotic protein synthesis initiation factor-2α subunit (eIF-2α), disrupting protein synthesis. HCMV ORF94 blocks the expression and therefore the activity of OAS.[87] Adenoviruses have an unusual mechanism for impeding OAS; they generate large amounts of virus-associated RNA (VA$_I$), which is processed by the host cell enzyme Dicer, producing small interfering RNAs.[88] VA$_I$ molecules act as pseudo-inhibitors, because they strongly bind, but poorly induce, OAS1.[89] Instead of interfering with OAS directly, MHV uses its ns2 protein, a phosphodiesterase, to cleave 2-5A molecules, preventing RNase L activation.[90] JEV NS2A physically interacts with PKR to impede its activation in response to various stimuli.[91] Poliovirus overcomes the PKR-mediated translational inhibition by cleaving an additional eukaryotic initiation factor, eIF5B, via the viral proteinase 3C^{pro}, creating a cleavage fragment that is able to rescue viral translation under conditions of eIF2α phosphorylation.[92] Interestingly, the *Ambystoma tigrinum*

virus, which infects ectotherms such as amphibians, reptiles and fish, was found to encode a protein homologous to eIF2α, called vIF2αH, which impairs eIF2α phosphorylation through the degradation of fish PKZ, a homologue of PKR. Although the exact mechanism for this process is not known, it is intriguing that the activity of PKZ was found to be required for vIF2αH to cause its degradation.[93]

In some cases, viruses turn the tables completely, using particular ISGs to their own advantage. For instance, MxA is a 76 000 molecular weight ISG, which interferes with the replication of HSV-1. Remarkably, HSV-1 stimulates the expression of a 56 000 molecular weight MxA isoform via alternative splicing, in the absence of type I IFN. This novel isoform of MxA, which associates with virion components and nuclear viral replication compartments, increases virus replication.[94] HCMV has long been known to directly induce the expression of the ISG viperin in the absence of IFN production.[95] Recently, it has been shown that via interaction with the viral protein vMIA, viperin is re-localized to the mitochondria, where it disrupts the actin cytoskeleton and enhances viral infection.[96] In fact, HCMV replication is decreased in cells lacking viperin. Rotavirus infection of intestinal epithelial cells leads to a strong induction of the type I IFN response, but instead of limiting virus growth, IFN signalling promotes rotavirus replication, particularly at the early stages.[97] The proposed mechanism is that type I IFN increases PKR levels, which the virus somehow exploits for its own replication.[97]

If a virus fails to completely block IFN production, a final subversion strategy is to modulate the negative regulation of the IFN response, which normally functions to turn off antiviral signalling upon viral clearance. The suppressor of cytokine signalling proteins SOCS1 and SOCS3 are induced by IFN, and directly interact with and inhibit JAK function in a negative feedback loop.[98] The human T-cell leukaemia virus type 1 takes advantage of this, using its Tax protein to both up-regulate SOCS1 expression through NF-κB activation and to stabilize the SOCS1 protein.[99] Surprisingly, SOCS was found to be required for Tax to impair IFN production, but was dispensable for Tax to block IFN signalling. Interleukin-6 up-regulates SOCS3; intriguingly, amino acid substitutions in the core region of HCV both produce interleukin-6 via activation of the unfolded protein response and render HCV more resistant to type I IFN.[100]

Conclusion

The number and diversity of viral targets for the disruption of the type I IFN response is staggering, as every step in this process can be inhibited in some way by viral proteins. Although developments in this field are rapidly accumulating, there is much still to learn. Each step taken

to characterize how viruses manipulate these pathways helps to further our understanding of antiviral signalling, truly exemplifying the saying: know thy enemy, know thyself.

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