Review Article

Signal transduction in the type I interferon system and viral countermeasures

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Type I interferons (IFN) including IFNα/β are cytokines of the immune system with critical functions in innate and adaptive immune response. Secreted IFN acts via JAK/STAT signaling pathways to direct a huge gene expression program, including antiviral, apoptotic, survival and immune genes. Only recently, the molecular patterns and their receptors as well as the connected signaling pathways leading to transcriptional activation of IFN genes have been elucidated. Ubiquitous cytosolic RNA helicases like RIG-I which sense intracellular triphosphate RNAs and activate the IFN-controlling transcription factors IRF3 and IRF7 seem to play a major role in antiviral defense and immunity. Recognition of extracellular nucleic acids by a subset of Toll-like receptors in addition contributes to a generalized host IFN response. During co-evolution with the host, viruses have learned to counteract every piece of the IFN network. Learning from viruses how to target the IFN system may lead us to novel strategies for therapeutic intervention.

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Introduction

The type I interferon (IFN) system, including a single IFNβ and a dozen IFNα variants, is an indispensable part of the immune system of all vertebrates, probably invented in response to viruses. Initially identified due to their immediate antiviral activity, IFNs are today recognized as multifunctional cytokines with pleiotropic activities in innate host cell defense and in coordinating adaptive immunity. IFN is involved in activating subsets of immune cells like NK, CTL and DC, which helps to eliminate pathogens or tumors, but which may also play a role in the establishment and maintenance of autoimmune disorders. IFN must therefore be under tight control of the host and expressed only in response to an immediate threat, like invading pathogens.

Innate immune recognition of pathogens is based on receptors for conserved, invariant structures, or molecular patterns [1]. Viruses are made in cells and lack an own metabolism. Nucleic acids are therefore the prime candidate patterns for recognition. Indeed, nucleic acids represent the main class of IFN inducers, as was appreciated...
early. Nevertheless, the first nucleic acid receptors able to trigger IFN induction were identified only during the past few years. These belong to two groups of protein families, the transmembrane Toll-like receptors (TLR), and the cytoplasmic RIG-I-like receptors (RLR). Members of the TLR9 subfamily, TLR3, -7, -8, and -9 sense nucleic acids approaching the cell from outside whereas the cytoplasmic RLRs identify dangerous RNA that has made it into the cytoplasm. Stimulation of these receptors leads to activation of the transcription factor kappa-B (NF-κB) and expression of inflammatory cytokines. In addition, they activate transcription factors of the interferon regulatory factor (IRF) family, IRF3 and IRF7, which control transcription of the early IFNβ and late IFNα genes, respectively. The activation of IRF3 and IRF7 by non-canonical members of the inhibitor of kappa B kinase (IKK) family of Ser/Thr kinases, TANK-binding kinase 1 (TBK1) and IKKε (IKKe), appears to be a central key event for IFN production in all body cells, with a remarkable exception in plasmacytoid dendritic cells (pDC). In a second round of receptor-mediated signal transduction, the secreted IFNs activate giant gene expression programs by the canonical Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling. This causes tremendous pleiotropic effects in all body cells, including a strong positive feedback to nucleic acid sensing.

Cytoplasmic receptors for RNA

Viral dsRNA, and synthetic dsRNA analogs like poly(I:C), are long known to play a major role both in the induction of IFN [2] and in stimulating antiviral responses [3, 4]. The Ser/Thr protein kinase R (PKR) is activated by dsRNA. In addition to its direct antiviral activity, PKR can activate NF-κB, but not IFR3 or IRF7, and is therefore synergistic in IFNβ induction, but it is not sufficient. The first cytoplasmic receptors able to activate IRF3 and IFN induction have been described only recently. Two related DExD/H box RNA helicases, retinoic acid inducible gene I protein (RIG-I) and the melanoma differentiation-associated gene product (MDA-5) were found essential for IFN production in response to RNA virus infection [5–7] (Fig. 1). RIG-I encodes a protein of 925 amino acids with an N-terminal region containing two caspase activation and recruitment domains (CARD) and a C-terminal region with ATP-dependent RNA helicase activity. MDA-5 has a similar structure, containing two CARD domains and a C-terminal RNA helicase domain. CARD domains may interact with CARDs from other proteins and are platforms for nucleating signaling events. Both the CARD domains and ATPase activity were required for IFN induc-

Figure 1. RIG-I mediated induction of type I IFN. The cytoplasmic RNA helicase RIG-I bound to viral triphosphate RNAs is recruited via CARD domain (C) interaction to the adaptor protein IPS-1, localized in the outer mitochondrial membrane. In a complex containing TRAF3 and chaperones Hsp90 and CypB, the kinase TBK1 phosphorylates the transcription factor IRF3 at C-terminal residues. Phospho-IRF3 forms homodimers that translocate to the nucleus and bind to the PRDIII-I enhancer region of the Ifnb1 gene, driving the mRNA transcription. The nuclear protein Pin1 targets IRF3 to proteosomal degradation. A20 and SIKE are cellular inhibitors of the IFN induction pathway. The helicase LGP-2 that lacks CARD domains probably interferes with RIG-I and MDA-5 function (see text for details).
MDA-5 did respond to poly(I:C) [11–15]. A study with IFN-inducing 21–27 nt long siRNA-like dsRNAs suggested that blunt dsRNAs are unwound efficiently by the RIG-I helicase activity, whereas those with a 3’-terminal 2nt overhang, as are found in Dicer-products, disturb unwinding and activation [16].

5’ triphosphate RNA is the molecular ligand for RIG-I activation

Besides dsRNA and poly(I:C), RNAs transcribed in vitro by the RNA polymerase of bacteriophages T7, T3, and Sp6 were recently found to trigger IFN expression [17]. Like T7 phage, many mammalian RNA viruses initiate viral RNA synthesis in a primer-independent manner, resulting in the presence of a triphosphate moiety at the 5’ end of RNAs. Notably, these include the paramyxoviruses and rhabdoviruses that are recognized by RIG-I, whereas picornaviruses like EMCV which are sensed by MDA-5, produce RNAs that lack a 5’ triphosphate and instead have a protein (Vpg) linked covalently to the 5’ terminus.

The identity of 5’ triphosphate RNA as the first, molecularly defined ligand for RIG-I was confirmed recently [18, 19]. IFN induction in rabies virus-infected cells was found to depend on RIG-I and on the presence of viral triphosphate RNA as a specific RIG-I ligand. RNA from rabies virus-infected cells and from purified rabies virus particles led to induction of IFN after transfection into cells. This activity was completely lost, when the 5’ triphosphate of the RNA was removed or replaced with a 5’-cap structure. Moreover, RIG-I directly bound not only dsRNA but also ssRNA oligonucleotides carrying a 5’-terminal triphosphate. IFN induction in response to triphosphate RNAs was independent of TLR recognition [20] and was abolished in RIG-I knock-out MEFs [18]. As confirmed in independent work, single-strand influenza virus triphosphate RNAs are also recognized by RIG-I [19]. These authors could also show that the viral NS1 protein co-localizes in complexes containing RIG-I and viral triphosphate RNAs, suggesting that NS1 does not only bind to dsRNA but also to viral triphosphate ssRNA, thereby acting as an antagonist of RIG-I. These findings fully support the previous observation that phage-transcribed ssRNA can induce IFN [17] and demonstrate that uncapped 5’ triphosphate RNA present in viruses serves as the molecular signature for the detection of viral infection via RIG-I. Triphosphate-containing RNAs are usually not present or accessible in healthy cells. Although cellular RNA polymerases do initiate transcription with triphosphate nucleotides, these are posttranslationally modified or removed in the nucleus. Triphosphate RNA in the cytoplasm of cells is therefore a pattern signature for “non-self” or dangerous [18].

An adapter for RIG-like-receptors

A direct and common downstream adapter to the CARD domains of active RIG-I and of MDA-5 required for activation of IFN and of NF-κB-controlled cytokines has recently been identified and was dubbed IFNβ promoter stimulator 1 (IPS-1) [21], mitochondrial antiviral signaling protein (MAVS) [22], virus-induced signaling adaptor (VISA) [23], and CARD adapter inducing IFNβ (Cardif) [24]. Intriguingly, IPS-1 is a transmembrane protein located to the outer mitochondrial membrane (Fig. 1). Its function in relaying signals to IFN induction is destroyed by targeting the protein to the plasma or ER membranes, suggesting that the mitochondrial localization is of functional relevance [22, 25]. IPS-1 contains a single N-terminal CARD domain, and a C-terminal region which interacts with TRAF6, FADD and RIP1 which are involved in NF-κB signaling [21, 23]. Details of how IPS-1-mediated downstream activation of the IRF kinases TBK1 and IKKi can occur, await clarification. However, TRAF3 is required [26] which is also involved in relaying signals of the TLR adapter TRIF to activation of IFN (see below), suggesting an important node function of this protein in IFN induction [27-29]. A negative regulator of the RIG-IPS-1-TBK1 activation cascade is A20, an NF-κB-inducible ubiquitin-editing protein [30, 31] whose C-terminal zinc-finger motifs are required for IRF3 and NF-κB inhibition.

Cytosolic receptors for DNA

Whereas cytoplasmic recognition of dsRNA has been appreciated for years, the existence of intracellular systems sensing DNA and responding with IFN production was corroborated only recently. TLR-independent induction of IFN was observed in response to transfected B-DNA [32], or to DNA escaped from apoptotic degradation [33] and required IRF3 [34]. Previously, TLR- and NOD-independent IFN induction by intracellular bacteria like Listeria monocytogenes has been observed [35]. The analysis of IPS-1−/− mice finally revealed that IPS-1 is not required for IFN induction in response to transfected DNA, infection with Listeria, or the dsDNA vaccinia virus [14, 15]. These observations strongly suggest a so far not appreciated distinct IFN inducing pathway which may have evolved to appropriately respond to DNA virus.
Transcriptional activation of IFN genes: Activation of IRFs by phosphorylation

Transcription of the IFNa/β genes is primarily controlled by proteins of the IFN regulatory factor (IRF) family, in particular the latent IRF3, and the IFN-inducible IRF7 (for a detailed review see [36]). In most body cells, activation of IRF3 triggers expression of a small subset of IFN genes, in particular IFNb. Transcription is promoted by an enhanceosome containing in addition the activated transcription factors, NF-κB and AP-1 (ATF-2-c-Jun)[37]. The secreted early IFN stimulates the synthesis of IRF7, which controls transcription of many additional members of the IFNb gene family.

Activation of the transcription factors IRF3 and IRF7 involves phosphorylation [38] by Ser/Thr kinases of the IKK family. Two non-canonical IKKs, TBK1 and IKKi which is expressed in hematopoietic cells after IFN stimulation, phosphorylate C-terminal Ser clusters of IRF3 and IRF7 [39, 40]. This is followed by IRF dimerization, import into the nucleus, and formation of the enhanceosome to initiate IFN mRNA synthesis. The major role in the initial activation of IRF3 is attributed to the ubiquitous and constitutively expressed kinase TBK1. Transcriptional activation of IRF3-controlled genes, including IFNb, is abrogated in TBK1 deficient mice in response to Sendai virus infection [41].

In cells not activated by TLR or RLR ligands, TBK1 and IKKi are bound to a protein called suppressor of IKK (SIKE) that prevents their association with IRF3 [42]. In contrast, a member of the peptidyl-prolyl isomerase proteins, cyclophilin B (CypB), and its interaction with latent IRF3, was found required for phosphorylation and dimerization of IRF3 and IFNb production. [43]. Another molecular chaperone, Hsp90, is part of the signaling complex leading to IFN induction (Fig. 1). Only upon viral infection, IRF3 dissociated from an IFR3-Hsp90-TBK1 complex to drive transcription of IFN mRNA. It seems that Hsp90 stabilizes TBK1 preventing its proteasome mediated degradation [44]. Stability of IRF and TBK1 seems to play an important role for IFN expression from a regulatory point of view. The half-life of the IFN-induced IRF7 controlling expression of the IFNa genes, is only 0.5–1 hour [45]. A nuclear peptidyl-prolyl isomerase, PIN1, appears to be involved in limiting IFN transcription. Via a WW domain, PIN1 binds to phosphorylated IRFs and causes their proteasomal degradation [46]. PIN1 knock-out mice show delayed IRF3 turnover and increased IFN production in stimulated cells. A positive feedback on IFR stability by IFN signaling was suggested by the observation that IRF3 is stabilized by conjugation with ISG15 [47].

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Toll-like receptor pathways inducing IFN

TLRs are single-span transmembrane proteins located at the cell surface or endosomal membranes of cells and act as sensors for microbial patterns. Upon ligation of extracellular ligands, TLR signals are transmitted via adapters that associate with the intracellular Toll-Interleukin receptor (TIR) domains of TLRs (Fig. 2). This leads to activation of NF-κB and expression of inflammatory cytokines, a general feature of TLR signaling [48]. Of the twelve mammalian TLRs identified, five TLRs can in addition activate IRFs and IFN induction, and four of these recognize nucleic acids. TLR3 binds dsRNA and poly(I:C) [49], TLR7 and 8 recognize ssRNA [50], and TLR9 binds methylated CpG DNA [51]. These TLRs are located in endosomal membranes [52]. A remarkable exception is the cell surface TLR4, as it can mediate IFN induction in response to a non-nucleic acid ligand, namely bacterial lipopolysaccharide (LPS). Activation of TLR4 to induce IFN involves a co-receptor, CD14, and binding of a complex of bacterial LPS with the MD-2 protein (for review see [53]). It must be noted that, in contrast to the ubiquitous RIG-I, the expression of TLRs is cell-type dependent. While TLR3 and TLR4 are expressed on many cell types including epithelial cells, fibroblasts and monocytes, TLR7, 8 and 9 are primarily expressed on myeloid DC (mDCs) and pDC [54]. pDC are known as the major producers of IFNa in humans. In contrast to other cells, pDC express high levels of IRF7 which otherwise is induced only after IFN JAK/STAT signaling by “early” IFNs, in particular IFNb [55–57].

As for RIG-I-mediated IFN induction, stimulation of TLR3 and TLR4 leads to the “classical” phosphorylation and activation of IRF3 and IRF7 by TBK1 and IKKi. This critically involves the TIR adapter TRIF [58] also known as TICAM1 [59], and is independent of IPS-1. TRIF binds to the TLR3 TIR directly, and to the TLR4 TIR via another TIR adaptor, TRAM [60, 61]. TRIF associates with TBK1 and IRF3. Similar to RIG-I/IPS-1-signaling, TRAF3 is required, suggesting an important role of this molecule at the merge of different upstream pathways [27, 28].

Notably, a completely different mode of IFN activation is observed for TLR7/9 (Fig. 2). This pathway is based on other adapters, other kinases and results in activation of exclusively IRF7. It plays a major role in pDC which express latent IRF7 and which therefore can produce the entire IFNa repertoire immediately upon stimulation of TLR7 and TLR9. This pathway is dependent on the TLR adapter MyD88 which otherwise is engaged in NF-κB activation [62, 63]. IRF7 activation requires a complex including MyD88, IRF7, and TRAF6 [63]. Three kinases, IRAK4, IRAK1, and IKKα, appear to be crucially involved.
Although in the absence of both IRAK1 and IRAK4 MyD88-dependent IRF7 activation is severely impaired [64, 65] it is suggested that IKKα is the kinase responsible for the final phosphorylation of IRF7 [66].

**Figure 2.** Interferon induction by Toll-like receptors. (A) TLR4 is the only Toll-like receptor that leads to IFN production in response to a non-nucleic acid ligand, namely the bacterial membrane component lipopolysaccharide (LPS). LPS binding to TLR4 and the co-receptors CD14 and MD-2 leads to signal transmission via the TIR adaptors TRAM and TRIF. Recruitment of TBK1, IRF3 and TRAF3 results in IRF3 phosphorylation by TBK1. (B) TLR3 is expressed in many cell types and located in endosomes. TLR3 recognizes dsRNA, released from disrupted viral particles or virus-infected cells. Signaling involves the TIR adaptor TRIF. TBK1 and IKKi phosphorylate IRF3 and 7 leading to production of IFN. (C) The endosomal TLR7, -8 and -9 are expressed predominantly in specialized hematopoetic cells like pDC. IFN induction depends on the adaptor protein MyD88 and the ubiquitin ligase TRAF6. In a complex containing IRAK1, IRAK4 and IKKα, IRF7, but not IRF3, is phosphorylated. Homodimers of phospho-IRF7 are imported into the nucleus and switch on transcription of IFNα mRNAs.

**Intracellular recognition of virus RNA is crucial for antiviral defense**

Although TLR-mediated activation may contribute considerably to systemic IFN levels, the recent characterization of knock-out mice suggests an outstanding importance of cytoplasmic recognition of viruses for antiviral host defense. Intriguingly, the absence of TLRs or of TLR adaptors did not have severe effects on the susceptibility of mice to RNA virus infection, in spite of a reduction of systemic IFN levels in some cases (for review see [67]). This indicated that intracellular recognition of viruses is not only required but is sufficient for mounting a protective antiviral inflammatory. Indeed, the lack of RIG-I or MDA-5 correlated with increased susceptibility to viruses recognized by these receptors [11]. In IPS-1 knockout mice, IRF3 and NF-κB responses to viruses and poly (I:C) was severely impaired. IPS-1−/− mice were more susceptible to EMCV, which is sensed by MDA-5. In addition, IPS-1−/− mice and even heterozygous IPS-1+/− mice were highly susceptible to infection with vesicular stomatitis virus (sensed by RIG-I) in spite of high IFNα levels probably produced by pDC, which are equipped with the TLR-dependent MyD88/IKKα/IRF7 pathway [14, 15]. These observations emphasize the importance of cytosolic recognition of viruses. They suggest that the mere production of sys-
temic endogenous IFN, as well as therapeutic application of exogenous IFN, does not guarantee the critical triggers for initiating an appropriate and sufficient innate antiviral response. Further work towards integrating the expression profiles of IRF, STAT, NF-κB, AP-1, and MAPK target genes may lead to identification of the relevant components beyond IFN [68].

IFN JAK/STAT signaling

All type I IFN family members have a common single and ubiquitous receptor, the IFNα receptor (IFNAR) which mediates the pleiotropic effects of IFN. Nevertheless, different IFNs may cause somewhat differential responses in a cell, and the biological response of different cell types to IFN may vary considerably (for comprehensive reviews see [69–71]). The major pathway of IFNAR signaling involves the activation of STAT family members by Janus kinases [72, 73] (Fig. 3). The two chains of IFNAR1 and IFNAR2c (a splice variant) are associated with the Janus kinases TYK2 and JAK1, respectively. Binding of IFN results in the tyrosine phosphorylation of the Janus kinases and of the recruited STAT1 and STAT2. STAT1 and STAT2 form heterodimers through SH2-phosphotyrosine interactions which associate with p48 (IRF9). This complex, known as IFN-stimulated gene factor 3 (ISGF3), binds to characteristic DNA sequences known as IFN-stimulated response elements (ISRE) in the promoters of more than hundred genes. STAT1 homodimers which are minor products of IFNAR signaling, but abundant in IFNγ signaling, activate a partially overlapping set of genes specified by gamma-activated sequences (GAS). IFNAR signaling can lead to activation of other STATs as well, resulting in a variety of STAT homo- and heterodimers. In addition, not only Janus kinases, but other kinases can contribute to STAT activation. Moreover, transcription factors other than STATs may participate in the expression of ISGs. The relative abundance of STATs, kinases, and other transcription factors may contribute to the observed flexibility of biological responses to IFN [69, 70].

Negative regulation of the JAK-STAT pathways occurs primarily at two levels, the activation of other STATs, and transcription of ISG by activated STATs. Members of the suppressor of cytokine signaling proteins (SOCS), SOCS1 and SOCS3, can inhibit JAK activity by binding through their SH2 domains to JAKs and to the receptor, respectively [74]. Members of the protein inhibitor of activated STAT (PIAS) family act as small ubiquitin-like modifier (SUMO) E3 ligases that target phosphorylated STAT in the nucleus and interfere with their transcriptional activity [75].

Antiviral activities of IFN

An important consequence of paracrine IFN signaling is the establishment of an antiviral state in the surrounding of virus-infected cells [76–78]. If not able to limit virus replication in previously infected cells in which viral antagonists are active, IFN response should at least impede further spread of viruses. Among the hundreds of ISGs induced are several coding for potent antiviral proteins. Antiviral mechanisms well established in cell culture and animal models comprise the PKR system [79], the 2′-5′OAS/RNaseL system [80], and the Mx protein family [81]. Additional proteins with potential antiviral activities are ISG20, promyelocytic leukaemia protein (PML), guanylate-binding protein 1 (GBP-1), P56, and the RNA-specific adenosine deaminase 1 (ADAR1) (for review...
see [78]. An IFN-induced protein family with rather specific activity is the apolipoprotein B editing catalytic polypeptide 3 (APOBEC3) family of cytidine deaminases, which incorporate into virdeficient HIV-1 virions [82] and which can block HIV-infection in resting T4 cells [83].

A probably not less important outcome of IFN signaling is the feedback stimulation of the RNA sensing and IFN induction machinery by inducing critical components like RIG-I, MDA-5, and IRF7. This leads to an amplified capacity to sense intracellular danger signals and allows a more comprehensive set of IFNs to be expressed. In addition, many components of the JAK/STAT pathway itself are among ISGs, including, STAT1, STAT2, and p48 providing a strong positive feedback for IFN signals. The importance of IFNAR signaling is illustrated by experiments with animals lacking the IFNAR or functional STAT1s. These are highly susceptible to viruses and rapidly succumb even to attenuated viruses [84–86].

**Viral IFN antagonists**

Most probably, viruses do not go completely unrecognized by the elaborate cellular security system. Exogenous viruses approach the cell as inert complex chemical entities. Although binding of viruses to cell surface receptors may already have inhibitory effects on cell signaling, the time between docking and viral gene expression is critical for detection and triggering of an initial IFN response. As soon as viral gene expression ensues, the potential of viruses is unchained and viral functions are expressed that can take over control of the cellular signaling pathways. A plethora of viral gene products working as specific IFN antagonists have been described in the past years, and their function characterized. In the following selected examples are provided which illustrate that viral functions can strike every step and piece of the host IFN regulatory network. Those viral proteins that cause a general cell transcription shut down (including IFN genes) are not included.

**Virus proteins concealing RNA**

Recognition of viral RNA is a key in IFN induction and in activating the antiviral PKR, in case of dsRNA. Any exposure of viral RNA is therefore critical. In this respect, all viral proteins associated with viral RNA may be considered important IFN antagonists by shielding RNA from recognition by host cells. The first viral proteins described as IFN antagonists are dsRNA binding proteins, namely the NS1 protein from influenza A virus, and the E3L of vaccinia virus. NS1 is a multifunctional pleiotropic protein that binds dsRNA, ss triphosphate RNA, RIG-I, and PKR. NS1 also inhibits the 3’end processing of cellular pre-mRNAs, regulates the virus replication cycle, and enhances translation initiation of viral mRNAs [19, 87–89]. More recently, the VP35 protein of Ebola virus, another negative strand RNA virus, was identified as a dsRNA binding protein with multiple functions in IFN escape [90]. Also DNA viruses encode proteins that interfere with cellular dsRNA binding proteins PKR and 2’5’OAS, thereby inhibiting the activation of these key antiviral enzymes, such as the E3L protein from the vaccinia poxvirus [91]. Intriguingly, E3L protein also binds Z-DNA. Whether this relates to a potential protective role in DNA-mediated IFN response, awaits clarification [92]. In any case, large DNA viruses like vaccinia encode a multiplicity of other proteins that interfere with innate and acquired immune response (for a comprehensive review see [93]. A special way of blocking the RNA-triggered induction of IFN is probably enabled by the E\textsuperscript{vif} protein of pestiviruses like BVDV and CSFV. E\textsuperscript{vif} is a structural glycoprotein of the virus and exhibits both RNase and dsRNA binding activity. Both properties of the protein have been found to be involved in blocking IFN induction by extracellular dsRNA by a so far not further specified mechanism [94].

Another strategy to interfere with recognition of viral RNA is targeting the function of the pattern receptors. This is applied by (-)RNA viruses of the Paramyxovirus genus, including for example Sendai virus, Simian virus 5, human parainfluenza virus-2, mumps virus and Hendra virus. The V protein of those viruses binds MDA-5, but not to RIG-I, and interfere with IFN induction by transfected RNA [7, 95]. The specificity for MDA-5 is puzzling, since RIG-I, and not MDA-5, appears to be the sensor for paramyxovirus RNAs. The V proteins of most paramyxoviruses are further remarkable as they simultaneously abolish JAK/STAT signaling, mostly by targeting either STAT1 or STAT2 for proteasomal degradation (see below). The typical mode of infection of paramyxoviruses involves fusion at the cell membrane and thereby may further minimize recognition of paramyxoviruses by avoiding encounter with endosomal TLRs. TLR-independent IFN induction in human pDC has been confirmed for respiratory syncytial virus (RSV) [96].

Exciting examples for viral proteins targeting the adaptors of recognition receptors are provided by (+)RNA viruses. The hepatitis C virus (HCV) NS3/4A protease is an essential virus protein required for processing of the immature viral polyprotein, and in addition, is instrumental in preventing IFN induction. HCV NS3/4A cleaves not only the TLR3/4 adapter TRIF [97] but also the RIG-I...
CARD adaptor IPS-1 [24, 25]. Cleavage of TRIF probably disables its association with TRAF3 and/or TBK1. NS3/4A cleavage of IPS-1 removes the transmembrane anchor from IPS-1, and precludes its mitochondrial localization. Treatment of HCV-infected cells with an inhibitor of the NS3 protease (BILN2061) restores IFN induction only partially, suggesting the presence of additional HCV inhibitory activities. The NS3/4A protease of the HCV-related GB virus, which causes generally acute and occasionally chronic hepatitis in small primates, cleaves IPS-1 as well. This observation provides further support for the use of GBV-B infection in small primates as an accurate surrogate model for deciphering virus-host interactions in hepacivirus pathogenesis [98]. Intriguingly, hepatitis A virus (HAV), which belongs to a different virus family, the Picornaviridae, has recently been found to follow the same strategy of cleaving IPS-1. Proteolytic inactivation of membrane-bound IPS-1 by HAV requires a protein precursor of the 3C cysteine proteinase (3ABC) that contains a transmembrane domain (SM Lemon, pers. comm.).

The A46R protein of vaccinia is targeting adapter function in a different way. This protein resembles the cytoplasmic TIR domains of the TLR adapter molecules TRIF, TRAM, and MyD88 and competes with TRIF for TLR3-TIR binding, thereby interfering with TRIF-dependent activation of IRF3. In addition MyD88, Mal and TRAM-mediated signaling is affected by A46R [99].

**Proteins targeting the step of IRF phosphorylation**

Obviously, the IRF kinases TBK1 and IKKi as well as the IRFs themselves are major targets for interference with IFN production. The phosphoproteins (P) of several (+)RNA viruses, including Borna disease virus, Ebola virus, and rabies virus, are essential proteins involved in viral RNA synthesis, and interfere with the activation of IRF3 and IRF7 by TBK1, though the mechanisms are mostly poorly understood. For Borna disease virus P, a decoy function was suggested as it was found to be phosphorylated by TBK1 overexpression [100]. Ebola virus P (VP35), in addition to binding dsRNA, interferes with IRF3 activation through TRIF and RIG-I pathways [90]. Rabies virus P prevents phosphorylation of both IRF7 and IRF3, by TBK1 and by IKKi [101] (and unpublished results). Since phosphorylation of the critical IRF3 serine 386 [102] is not possible in the presence of P, IRF3 dimerization, nuclear import and transcriptional activity is precluded. The rabies virus P protein in addition is active in preventing IFN JAK/STAT signaling [103, 104]. This dual function of simultaneously targeting IFN induction and IFN signaling is reminiscent of the situation with paramyxovirus V proteins, but involves distinct mechanisms. For rabies virus (unpublished) and Ebola virus [105] the relevance of P/VP35 IFN antagonistic functions for survival and pathogenesis in the host have been illustrated in animal experiments using engineered viruses. In case of KSV, inhibition of IRF functions requires the coordinate function of two non-essential, non-structural (NS) proteins, NS1 and NS2 [106, 107]. These two proteins mediate also the resistance to exogenous IFN [108, 109]. Finally, (+)RNA viruses may utilize even glycoproteins to fight IFN. Comparison of pathogenic and non-pathogenic hantaviruses revealed a correlation with IFN production which could be linked to mutations in the cytoplasmic tail of the G1 protein. Expression of the G1 cytoplasmic tail of the pathogenic NY-1 strain inhibited RIG-I and TBK1-triggered IFN activation, but failed in inhibition of a phosphomimetic form of IRF3 [110]. Thus, like rabies virus P, G1 appears to target the step of IRF3 phosphorylation by TBK1. Notably, often several viral proteins are employed to counteract IFN induction, as exemplified by the coronavirus causing severe acute respiratory syndrome (SARS). The SARS virus ORF 3b, ORF 6, and N protein all inhibit phosphorylation of IRF3 [111].

**Targeting IRF function**

IRF3 is the target of a variety of RNA and DNA viruses. The ML protein from Thogoto virus, an influenza-like insect virus, prevents IRF3 dimerization and subsequent CBP interaction, without interfering with the phosphorylation and nuclear import of IRF3 [112]. The Npro protein, a protease of HCV-related pestiviruses like BVDV and CSFV cause a decrease in the cellular IRF3 levels [113, 114]. Both a lack of IFN promoter activation after nuclear translocation of phosphorylated IRF3 dimers and a decrease in cytoplasmic IRF3 levels have been observed in Npro containing cells. The latter effect was attributed to polyubiquitination of IRF3 and proteasomal degradation. Similarly, the NSP1 protein of rotavirus interacts with IRF3 and targets it to proteasomal degradation [115]. Whether the viral proteins leading to IRF degradation mimic or stimulate the functions of the cellular Pin1 is unclear so far. Also IRF7 is a target of virus proteins. An EBV encoded protein, BZLF-1, physically associates with cytoplasmic and nuclear IRF7 and inhibits IRF7 activity after stimulation by dsRNA, as well as the activity of a constitutively active form of IRF7 [116]. Herpes viruses have acquired additional weapons to interfere with the functions of cellular IRFs. Human herpes virus 8 (HHV-8), the causative agent of Kaposi sar-
Interference with IFN function and JAK/STAT signaling

A far-reaching approach to counteract IFN functions is used by the large poxviruses like vaccinia virus. They express soluble IFN-binding proteins (“viroceptors” B8, B18) which compete with the cellular IFNAR for IFN binding [122, 123]. By neutralization of secreted IFN they prevent the establishment of an antiviral state in the non-infected tissue and disable the autocrine IFN feed-back loop.

The function of the IFNAR-associated Janus kinases JAK1 and Tyk2 is targeted by several DNA and RNA viruses. The E6 protein of Human papilloma virus 18 (HPV18) interacts with TYK2 and impairs its function [124] whereas the large T of polyomaviruses has a preference for JAK1. Also some paramyxoviruses interfere with Janus kinase function, although constitutive targeting of STATs is a more common strategy of the paramyxovirus interference with the ORF3b appears to interfere with nuclear translocation although STAT phosphorylation is not reduced whereas the ORF3b appears to interfere with nuclear translocation factors necessary for the IFN response [111]. The adenovirus E1A protein binds STAT1, and in addition IFN-γ activates STAT1 and STAT2 by rabies virus P to interrupt IFN-JAK-STAT signaling is unique among viruses. In view of the multiple tasks of P protein, including engagement in viral RNA synthesis and in IRF inhibition and a limited coding capacity of rhabdoviruses, the observed activity on demand may reflect a specialization to preserve capacity for other duties in non-alerted cells. A less specific mechanism to prevent nuclear import of activated STATs and of other proteins is applied by the Ebola virus VP24, a minor structural protein. VP24 binds importin-α, the main nuclear import factor for STATs. Recruitment of importin-α by overexpressed VP24 led to reduced nuclear accumulation of STATs and ISG transcription [133].

STAT-targeting proteins are known from positive strand RNA viruses or DNA viruses as well. The ORF6 proteins of SARS coronavirus inhibit STAT1 translocation although STAT phosphorylation is not reduced whereas the ORF3b appears to interfere with nuclear translocation factors necessary for the IFN response [111]. The adenovirus E1A protein binds STAT1, and in addition IFN-γ activates STAT1 and STAT2 by rabies virus P to interrupt IFN-JAK-STAT signaling is unique among viruses. In view of the multiple tasks of P protein, including engagement in viral RNA synthesis and in IRF inhibition and a limited coding capacity of rhabdoviruses, the observed activity on demand may reflect a specialization to preserve capacity for other duties in non-alerted cells. A less specific mechanism to prevent nuclear import of activated STATs and of other proteins is applied by the Ebola virus VP24, a minor structural protein. VP24 binds importin-α, the main nuclear import factor for STATs. Recruitment of importin-α by overexpressed VP24 led to reduced nuclear accumulation of STATs and ISG transcription [133].

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In rhabdoviruses like rabies virus which lack a V protein, the essential P protein is responsible for JAK/STAT signal inhibition [103, 104]. Unlike the V proteins of paramyxoviruses, rabies virus P does not target non-activated STATs, but interacts with STAT1 and STAT2 only after tyrosine phosphorylation [104]. The interaction with P retains activated STATs in the cytoplasm, thereby preventing STAT-mediated transcription of ISGs. Such conditional, activation-dependent targeting of STAT1 and STAT2 by rabies virus P to interrupt IFN-JAK-STAT signaling is unique among viruses. In view of the multiple tasks of P protein, including engagement in viral RNA synthesis and in IRF inhibition and a limited coding capacity of rhabdoviruses, the observed activity on demand may reflect a specialization to preserve capacity for other duties in non-alerted cells. A less specific mechanism to prevent nuclear import of activated STATs and of other proteins is applied by the Ebola virus VP24, a minor structural protein. VP24 binds importin-α, the main nuclear import factor for STATs. Recruitment of importin-α by overexpressed VP24 led to reduced nuclear accumulation of STATs and ISG transcription [133].

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Apparently, the same strategy can be used by small RNA viruses like HCV [139].

**Interference with IFN-induced antiviral proteins**

In a recent excellent review a comprehensive description of interference of viruses and viral proteins with IFN-induced antiviral proteins can be found [78]. Of note, virtually all viruses, including RNA viruses, DNA viruses, and retroviruses, have evolved means to interfere with the PKR and 2'5'OAS/RNase L system illustrating the importance of these systems in general antiviral defense. Some of the viral proteins competing with PKR for dsRNA are described above as they might also function in preventing recognition by RLR and IFN induction. Rather puzzling are some specific small noncoding viral RNAs like EBV-encoded RNAs (EBERs) or the adenovirus VA RNAs. On the one hand, EBERs are recognized by RIG-I and trigger IFNβ production [140] and on the other hand bind PKR to inhibit its antiviral activity [141, 142]. An intriguing alternative strategy to counteract PKR antiviral activity, just to mention, is applied by HSV-1, whose g34.5 protein “cures” the PKR-mediated shut down by recruiting phosphatase1-alpha to dephosphorylate eIF2 and thereby restore translation [143].

**Viral IFN antagonists as factors of tropism and host range**

The IFN network as well as the links to other systems, including other innate defense systems, like apoptosis, and the adaptive immune system, is highly variable and it is clear that IFN has different outcome in different cell types, tissues and organs. The tools developed by viruses ideally allow them to grow in the host for some time, at some privileged sites, and without killing the host too early. Whereas some viruses are eliminated after an acute infection, some are controlled partially by the immune system, and persist at low level. The restriction to certain organs may reflect at least in part the incapability of viruses to withstand the innate immunity elsewhere. There is also increasing evidence that the ability of IFN antagonists from viruses to counteract IFN pathways in a certain host species is a critical determinant of its host range. For example, species specific differences have been shown crucial for a couple of paramyxoviruses, including simian virus 5 and Sendai virus [144, 145], RSV [109, 146], measles virus [120], Newcastle disease virus (NDV) [147] and myxoma virus [148]. Indeed, the principle of host-specific IFN antagonism is being utilized for approaches using non-human paramyxoviruses like Newcastle disease virus for oncolytic virotherapy approaches [149]. This is based on the observation that many tumors have defects in the IFN system, particularly in JAK/STAT signaling.

Other members of this virus group do have a broad host spectrum, or are able to cross species barriers. Notably, these include “emerging” and zoonotic viruses, such as rabies- and rabies related viruses, Nipah-, Hendra-, dis.temper-, and Ebola viruses. In vitro and in vivo many of these viruses are able to enter cells from different species, suggesting that a severe “entry” barrier does not apply. Although successful infection of non-natural hosts, including human, may be a dead end with respect to further virus transmission and spread, the apparent ability to infect a foreign host provides the opportunity to adapt to the new host in different respects. Adaptation to appropriately counteract the new host’s IFN system appears therefore to be a key for the emergence of a new human pathogen [150]. The number and degree of molecular changes needed for the viral IFN antagonists to adapt and the intensity of contact may determine the probability of a virus to spill over to human and to convert into a human pathogen.

**Outlook**

Of the pathogens approaching hosts, viruses are the most intimate ones, because they are produced by host cells. The most singular features of viruses may reside in their nucleic acids. The IFN system appears to have developed primarily in response to nucleic acids. Receptors for external nucleic acids, like TLRs, and for internal nucleic acids, like RIG-I, can activate different defense and emergency conditions, respectively. A major challenge in the future is to exactly define those conditions.

The study of viruses tells us not only how to manipulate different arms of the IFN network but also how to stimulate the IFN system appropriately. Viruses with modified IFN antagonists are promising candidates for attenuated and immunogenic vaccines. Immune stimulatory nucleic acids binding to TLRs already find application as potent immune-modulators, agonists of the recently identified RLRs should provide us with additional tools for distinguishing manipulation of immune responses.

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References

[1] Janeway, C.A., Jr. (1989) Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb. Symp. Quant. Biol. 54 Pt 1: 1–13.

[2] Field, A.K., Tytell, A.A., Lampson, G.P., Hilleman, M.R. (1967) Inducers of interferon and host resistance. II. Multistranded synthetic polynucleotide complexes. Proc. Natl. Acad. Sci. U. S. A. 58: 1004 – 1010.

[3] Kerr, I.M., Brown, R.E., Ball, L.A. (1974) Increased sensitivity of cell-free protein synthesis to double-stranded RNA after interferon treatment. Nature 250: 57 – 59.

[4] Lebleu, B., Ben, G.C., Shaila, S., Cabrер, B., Lengyel, P. (1976) Interferon, double-stranded RNA, and protein phosphorylation. Proc. Natl. Acad. Sci. U. S. A. 73: 3107 – 3111.

[5] Yoneyama, M., Kikuchi, M., Matsumoto, K., Imaizumi, T., et al. (2005) Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J. Immunol. 175: 2851 – 2858.

[6] Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., et al. (2004) The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat. Immunol. 5: 730 – 737.

[7]Andrejeva, J., Childs, K.S., Young, D.F., Carlos, T.S., et al. (2006) The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. Proc. Natl. Acad. Sci. U. S. A. 101: 17264 – 17269.

[8]Rothenfusser, S., Vial, A., DiAlessio, D., Ablashi, D.V., et al. (1984) RNA and Virus-Inde- pendent Inhibition of Antiviral Signaling by RNA Helicase Lgp2. J. Virol. 80: 12332 – 12342.

[9] Weber, F., Wagner, V., Rasmussen, S.B., Hartmann, R., Paludan, S.R. (2006) Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. J. Virol. 80: 5059 – 5064.

[10] Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., et al. (2005) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 431: 101 – 105.

[11] Kato, H., Sato, S., Yoneyama, M., Yamamoto, M., et al. (2005) Cell type-specific involvement of RIG-I in antiviral response. Immunity 23: 19 – 28.

[12] Gitlin, L., Barchet, W., Lifshitz, S., Cell, et al. (2006) Essential role of md2-5 in type I IFN responses to polyriboinosinic-polyribocytidylic acid and encephalomyocarditis picornavirus. Proc. Natl. Acad. Sci. U. S. A. 103: 8459 – 8464.

[13] Kumar, H., Kawai, T., Kato, H., Sato, S., et al. (2006) Essential role of IPS-1 in innate immune responses against RNA viruses. J. Exp. Med. 203: 1795 – 1803.

[14] Sun, Q., Sun, L., Liu, H.H., Chen, X., et al. (2006) The specific and essential role of MAVS in antiviral innate immune responses. Immunity 24: 633 – 642.

[15] Marques, J.T., Devosse, T., Wang, D., Zamanian-Daryoush, M., et al. (2006) A structural basis for discriminating between self and nonself double-stranded RNAs in mammalian cells. Nat. Biotechnol. 24: 559 – 565.

[16] Kim, D.H., Longo, M., Han, Y., Lundberg, P., et al. (2004) Interferon induction by siRNAs and ssRNAs synthesized by phage polymerase. Nat. Biotechnol. 22: 321 – 325.

[17] Hornung, V., Ellegast, J., Kim, S., Brzoza, K., et al. (2006) 5’-Triphosphate RNA is the ligand for RIG-I. Science 314: 994 – 997.

[18] Pichlmair, A., Schulz, O., Tan, C.P., Naslund, T.I., et al. (2006) RIG-I-mediated antiviral responses to single-stranded RNA bearing 5’-phosphates. Science 314: 997 – 1001.

[19] Seth, R.B., Sun, L., Ellegast, K., Chen, J.Z. (2005) Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell 122: 669 – 682.

[20] Xu, L.G., Wang, Y.Y., Han, K.J., Li, L.Y., et al. (2005) VISA is an adapter protein required for virus-triggered IFN-beta signaling. Mol. Cell 19: 727 – 740.

[21] Meylan, E., Curran, J., Hofmann, K., Moradpour, D., et al. (2005) Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 437: 1167 – 1172.

[22] Loo, Y.M., Owen, D.M., Li, K., Erickson, A.K., et al. (2006) Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. Proc. Natl. Acad. Sci. U. S. A. 103: 6001 – 6006.

[23] Saha, S.K., Pietras, E.M., He, J.Q., Kang, J.R., et al. (2006) Regulation of antiviral responses by a direct and specific interaction between TRAF3 and Cardif. EMBO J 25: 3257 – 3263.

[24] Oganesyan, G., Saha, S.K., Guo, B., He, J.Q., et al. (2006) Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. Nature 439: 208 – 211.

[25] Hacker, H., Redeczke, V., Blagoev, B., Kratchmarova, I., et al. (2006) Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. Nature 439: 204 – 207.

[26] Saha, S.K., Cheng, G. (2006) TRAF3: a new regulator of type I interferons. Cell Cycle 5: 804 – 807.
[30] Lin, R., Yang, L., Nakhaei, P., Sun, Q., et al. (2006) Negative regulation of the retinoic acid-inducible gene iκB-induced antiviral state by the ubiquitin-editing protein A20. J. Biol. Chem. 281: 2095 – 2103.

[31] Wang, Y.Y., Li, L., Han, K.J., Zhai, Z., Shu, H.B. (2004) A20 is a potent inhibitor of TRIF3- and Sendai virus-induced activation of NF-kappaB and ISRE and IFN-beta promoter. FEBS Lett. 576: 86 – 90.

[32] Ishii, K.J., Coban, C., Kato, H., Takahashi, K., et al. (2006) A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. Nat. Immunol. 7: 40 – 48.

[33] Okabe, Y., Kawane, K., Akira, S., Taniguchi, T., Nagata, S. (2005) Toll-like receptor-independent gene induction program activated by mammalian DNA escaped from apoptotic DNA degradation. J. Exp. Med. 202: 1333 – 1339.

[34] Stetson, D.B., Medzhitov, R. (2006) Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. Immunity 24: 93 – 103.

[35] O'Riordan, M., Yi, C.H., Gonzales, R., Lee, K.D., Portnoy, D.A. (2002) Innate recognition of bacteria by a macrophage cytosolic surveillance pathway. Proc. Natl. Acad. Sci. U.S.A. 99: 13861 – 13866.

[36] Honda, K., Taniguchi, T. (2006) IRFs: master regulators of the IRF3 signaling pathway. Nat. Immunol. 7: 598 – 605.

[37] Sharma, S., Tenoever, B.R., Grandvaux, N., Zhou, G.P., et al. (2006) Role of cyclophilin B in activation of interferon regulatory factor-3. J. Biol. Chem. 280: 18355 – 18360.

[38] Yang, K., Shi, H., Qi, R., Sun, S et al. (2006) Hsp90 regulates activation of interferon regulatory factor 3 and TBK-1 stabilization in Sendai virus-infected cells. Mol. Biol. Cell 17: 1461 – 1471.

[39] Sato, M., Suemori, H., Hata, N., Asagiri, M., et al. (2000) Distinct and essential roles of transcription factors IRF3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. Immunity 13: 539 – 548.

[40] Obata, Y., Yamamoto, K., et al. (2006) Negative regulation of interferon-regulatory factor 3-dependent innate antiviral response by the prolyl isomerase Pin1. Nat. Immunol. 7: 598 – 605.

[41] Lu, G., Reinert, J.T., Pitha-Rowe, I., Okumura, A., et al. (2006) ISG15 enhances the innate antiviral response by inhibition of IRF-3 degradation. Cell Mol. Biol. 52: 29 – 41.

[42] Akira, S., Takeda, K. (2004) Toll-like receptor signalling. Nat. Rev. Immunol. 4: 499 – 511.

[43] Alexopoulos, L., Holt, A.C., Medzhitov, R., Flavell, R.A. (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413: 732 – 738.

[44] Hemmi, H., Takeuchi, O., Kawai, T., Kaisho, T., et al. (2000) A Toll-like receptor recognizes bacterial DNA. Nature 408: 740 – 745.

[45] Wagner, H. (2004) The immunobiology of the TLR9 subfamily. Trends Immunol. 25: 381 – 386.

[46] Kawai, T., Akira, S. (2006) Innate immune recognition of viral infection. Nat. Immunol. 7: 131 – 137.

[47] Hornung, V., Rothenfusser, S., Britsch, S., Krug, A et al. (2002) Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J. Immunol. 168: 4531 – 4537.

[48] Colonna, M., Trinchieri, G., Liu, Y.J. (2004) Plasmacytoid dendritic cells in immunity. Nat. Immunol. 5: 1219 – 1226.

[49] Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., et al. (2003) Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 301: 640 – 643.

[50] Oshiumi, H., Matsumoto, M., Funami, K., Akazawa, T., Seya, T. (2003) TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. Nat. Immunol. 4: 161 – 167.

[51] Fitzgerald, K.A., Rowe, D.C., Barnes, B.J., Caffrey, D.R., et al. (2003) LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. J. Exp. Med. 198: 1043 – 1055.
[61] Yamamoto, M., Sato, S., Hemmi, H., Uematsu, S., et al. (2003) TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat. Immunol.* 4: 1144 –1150.

[62] Honda, K., Yanai, H., Mizutani, T., Negishi, H., et al. (2004) Role of a transductional-transcriptional processor complex involving MyD88 and IRF-7 in Toll-like receptor signaling. *Proc. Natl. Acad. Sci. U. S. A.* 101: 15416 –15421.

[63] Kawai, T., Sato, S., Ishii, K.J., Coban, C., et al. (2004) Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. *Nat. Immunol.* 5: 1061 –1068.

[64] Yang, K., Puel, A., Zhang, S., Eidenschenk, C., et al. (2004) Functions of toll-like receptors. *Nat. Rev. Immunol.* 4: 465 – 478.

[65] Uematsu, S., Sato, S., Yamamoto, M., Hirotani, T., et al. (2005) Interleukin-1 receptor-associated kinase-1 plays an essential role for Toll-like receptor (TLR)7 and TLR9-mediated interferon-[alpha] induction. *J. Exp. Med.* 201: 915 –923.

[66] Kawai, T., Sato, S., Ishii, K.J., Coban, C., et al. (2004) Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. *Nat. Immunol.* 5: 1061 –1068.

[67] Darnell, J.E., Jr., Kerr, I.M., Stark, G.R. (1994) Jak-STAT pathways and transcriptional activation in response to type I interferons. *Nat. Rev. Immunol.* 5: 375 –386.

[68] Stark, G.R., Kerr, I.M., Williams, B.R., Silverman, R.H., Schreiber, R.D. (1998) How cells respond to interferons. *Annu. Rev. Biochem.* 67: 227 –264.

[69] Darnell, J.E., Jr., Kerr, I.M., Stark, G.R. (1994) Jak-STAT pathways and transcriptional activation in response to IFN and other extracellular signaling proteins. *Science* 264: 1415 –1421.

[70] Velazquez, L., Feltous, M., Stark, G.R., Pellegrini, S. (1992) A protein tyrosine kinase in the interferon alpha/beta signaling pathway. *Cell* 70: 313 –322.

[71] Kubo, M., Hanada, T., Yoshimura, A. (2003) Suppressors of cytokine signaling and immunity. *Nat. Immunol.* 4: 1169 –1176.

[72] Shuai, K., Liu, B. (2005) Regulation of gene-activation pathways by PIAS proteins in the immune system. *Nat. Rev. Immunol.* 5: 593 –605.

[73] Goodbourn, S., Didcock, L., Randall, R.E. (2000) Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. *J. Gen. Virol.* 81: 2341 –2364.

[74] Samuel, C.E. (2001) Antiviral actions of interferons. *Clin. Microbiol. Rev.* 14: 778 –809.

[75] Weber, F., Kochs, G., Haller, O. (2004) Inverse interference: how viruses fight the interferon system. *Viral Immunol.* 17: 498 –515.

[76] Williams, B.R. (1999) PKR: a sentinel kinase for cellular stress. *Oncogene* 18: 6112 –6120.

[77] Silverman, R.H. (1994) Fascination with 2-5A-dependent RNase: a unique enzyme that functions in interferon action. *J Interferon Res.* 14: 101 –104.

[78] Haller, O., Kochs, G. (2002) Interferon-induced mx proteins: dynamin-like GTPases with antiviral activity. *Traffic* 3: 710 –717.

[79] Sheehy, A.M., Gaddis, N.C., Malim, M.H. (2003) The anti-retroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat. Med.* 9: 1404 –1407.

[80] Chiu, Y.L., Soros, V.B., Kreisberg, J.F., Stopak, K., et al. (2005) Cellular APOBEC3G restricts HIV-1 infection in resting CD4+ T cells. *Nature* 435: 108 –114.

[81] Durbin, J.E., Hackenmiller, R., Simon, M.C., Levy, D.E. (1996) Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. *Cell* 84: 443 –450.

[82] Park, C., Li, S., Cha, E., Schindler, C. (2000) Immune response in Stat2 knockout mice. *Immunity* 13: 795 –804.

[83] van den Broek, M.F., Muller, U., Huang, S., Zinkernagel, R.M., Aguet, M. (1995) Immune defence in mice lacking type I and/or type II interferon receptors. *Immunol. Rev.* 148: 5 –18.

[84] Li, S., Min, J.Y., Krug, R.M., Sen, G.C. (2006) Binding of the influenza A virus NS1 protein to PKR mediates the inhibition of its activation by either PACT or double-stranded RNA. *Virology* 349: 13 –21.

[85] Noah, D.L., Twu, K.Y., Krug, R.M. (2003) Cellular antiviral responses against influenza A virus are countered at the posttranscriptional level by the viral NS1A protein via its binding to a cellular protein required for the 3' end processing of cellular pre-mRNAs. *Virology* 307: 386 –395.

[86] Garcia-Sastre, A., Biron, C.A. (2006) Type 1 interferons and the virus-host relationship: a lesson in detente. *Science* 312: 879 –882.

[87] Cardenas, W.B., Loo, Y.M., Gale, M., Jr., Hartman, A.L., et al. (2006) Ebola virus VP35 protein binds double-stranded RNA and inhibits alpha/beta interferon production induced by RIG-I signaling. *J. Virol.* 80: 5168 –5178.

[88] Chang, H.W., Watson, J.C., Jacobs, B.L. (1992) The E3L protein of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. *Proc. Natl. Acad. Sci. U. S. A.* 89: 4825 –4829.

[89] Langland, J.O., Kash, J.C., Carter, V., Thomas, M.J., et al. (2006) Suppression of Proinflammatory Signal Transduction and Gene Expression by the Dual Nucleic Acid Binding Domain of the Vaccinia Virus E3L Proteins. *J. Virol.* 80: 10083 –10095.

[90] Haga, I.R., Bowie, A.G. (2005) Evasion of innate immunity by vaccinia virus. *Parasitolology* 130 Suppl: S11 –S25.
[94] Iqbal, M., Poole, E., Goodbourn, S., McCauley, J.W. (2004) Role for bovine viral diarrhea virus Erns glycoprotein in the control of activation of beta interferon by double-stranded RNA. J. Virol. 78: 136 – 145.

[95] Childs, K., Stock, N., Ross, C., Andrejeva, J., et al. (2006) mda-5, but not RIG-I, is a common target for paramyxovirus V proteins. Virolology. Oct 13; [Epub ahead of print].

[96] Hornung, V., Schleider, J., Guenther-Biller, M., Rothenfusser, S., et al. (2004) Replication-dependent potent IFN-alpha induction in human plasmacytoid dendritic cells by a single-stranded RNA virus. J. Immunol. 173: 5935 – 5943.

[97] Li, K., Foy, E., Ferrecon, J.C., Nakamura, M. et al. (2005) Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proc. Natl. Acad. Sci. U. S. A. 102: 2992 – 2997.

[98] Chen, Z., Benureau, Y., Rijnbrand, R., Yi, J., et al. (2006) Vaccinia virus protein A46R targets multiple Toll-like receptors. J. Biol. Chem. 281: 13640 – 13645.

[99] Stack, J., Haga, I.R., Schroder, M., Bartlett, N.W., et al. (2005) Vaccinia virus protein A46R targets multiple Toll-like-interleukin-1 receptor adaptors and contributes to virulence. J. Exp. Med. 201: 1007 – 1018.

[100] Unterstab, C., Ludwig, S., Anton, A., Planz, O., et al. (2005) Viral targeting of the interferon-{beta}-inducing Traf family member-associated NF-{kappa}B activator (TANK)-binding kinase-1. Proc. Natl. Acad. Sci. U. S. A. 102: 13640 – 13645.

[101] Brzózka, K., Finke, S., Conzelmann, K.K. (2005) Identification of the rabies virus alpha/beta interferon antagonist: phosphoprotein P interferes with phosphorylation of interferon regulatory factor 3. J. Virol. 79: 7673 – 7681.

[102] Mori, M., Yoneyama, M., Ito, T., Takahashi, K., et al. (2004) Identification of Ser-386 of interferon regulatory factor 3 as critical target for inducible phosphorylation that determines activation. J. Biol. Chem. 279: 9698 – 9702.

[103] Vidy, A., Chelbi-Alix, M., Blondel, D. (2005) Rabies virus P protein interacts with STAT1 and inhibits interferon signal transduction pathways. J. Virol. 79: 14411 – 14420.

[104] Brzózka, K., Finke, S., Conzelmann, K.K. (2006) Inhibition of interferon signaling by rabies virus phosphoprotein P: activation-dependent binding of STAT1 and STAT2. J. Virol. 80: 2675 – 2683.

[105] Hartman, A.L., Dover, J.E., Towner, J.S., Nichol, S.T. (2006) Reverse genetic generation of recombinant Zaire Ebola viruses containing disrupted IRF-3 inhibitory domains results in attenuated virus growth in vitro and higher levels of IRF-3 activation without inhibiting viral transcription or replication. J. Virol. 80: 6430 – 6440.

[106] Bossert, B., Marozin, S., Conzelmann, K.K. (2003) Nonstructural proteins NS1 and NS2 of bovine respiratory syncytial virus block activation of interferon regulatory factor 3. J. Virol. 77: 8661 – 8668.

[107] Spann, K.M., Tran, K.C., Chi, B., Rabin, R.L., Collins, P.L. (2004) Suppression of the induction of alpha, beta, and lambda interferons by the NS1 and NS2 proteins of human respiratory syncytial virus in human epithelial cells and macrophages [corrected]. J. Virol. 78: 4363 – 4369.

[108] Schleider, J., Bossert, B., Buchholz, U., Conzelmann, K.K. (2000) Bovine respiratory syncytial virus nonstructural proteins NS1 and NS2 cooperatively antagonize alpha/beta interferon-induced antiviral response. J. Virol. 74: 8234 – 8242.

[109] Bossert, B., Conzelmann, K.K. (2002) Respiratory syncytial virus (RSV) nonstructural (NS) proteins as host range determinants: a chimeric bovine RSV with NS genes from human RSV is attenuated in interferon-competent bovine cells. J. Virol. 76: 4287 – 4293.

[110] Alff, P.J., Gavriloynskaya, I.N., Gorbunova, E., Endriss, K., et al. (2006) The Pathogenic NY-1 Hantavirus G1 Cytoplasmic Tail Inhibits RIG-I and TBK-1-Directed Interferon Responses. J. Virol. 80: 9676 – 9686.

[111] Kopecy-Bromberg, S.A., Martinez-Sobrido, L., Frieman, M., Baric, R.A., Palese, P. (2006) SARS coronavirus proteins ORF 3B, ORF 6, and nucleoposid function as interferon antagonists. J. Virol. 81: 548 – 557.

[112] Jennings, S., Martinez-Sobrido, L., Garcia-Sastre, A., Weber, F., Kochs, G. (2005) Thogoto virus ML protein suppresses IF3 function. Virology 331: 63 – 72.

[113] La Rocca, S.A., Herbert, R.J., Crooke, H., Drew, T.W., et al. (2005) Loss of interferon regulatory factor 3 in cells infected with classical swine fever virus involves the N-terminal protease, Npro. J. Virol. 79: 7239 – 7247.

[114] Hilton, L., Moganeradj, K., Zhang, G., Chen, Y.H., et al. (2006) The Npro product of bovine viral diarrhea virus inhibits DNA binding by interferon regulatory factor 3 and targets it for proteasomal degradation. J. Virol. 80: 11723 – 11732.

[115] Barro, M., Patton, J.T. (2005) Rotavirus nonstructural protein 1 subverts innate immune response by inducing degradation of IFN regulatory factor 3. Proc. Natl. Acad. Sci. U. S. A. 102: 4114 – 4119.

[116] Hahn, A.M., Huye, L.E., Ning, S., Webster-Cyriaque, J., Pagano, J.S. (2005) Interferon regulatory factor 7 is negatively regulated by the Epstein-Barr virus immediate-early gene, BZLF-1. J. Virol. 79: 10040 – 10052.

[117] Lin, R., Genin, P., Mamane, Y., Sgarbanti, M., et al. (2001) HHV-8 encoded vIRF-1 represses the interferon antiviral response by blocking IRF-3 recruitment of the CBP/p300 coactivators. Oncogene 20: 800 – 811.

[118] Lubovy, B., Kellum, M.J., Frisancho, A.J., Pitha, P.M. (2004) Kaposi's sarcoma-associated herpesvirus-encoded vIRF-3 stimulates the transcriptional activity of cellular IRF-3 and IRF-7. J. Biol. Chem. 279: 7643 – 7654.

[119] Seo, T., Park, J., Lim, C., Choe, J. (2004) Inhibition of nuclear factor kappaB activity by viral interferon regulatory factor 3 of Kaposi's sarcoma-associated herpesvirus. Oncogene 23: 6146 – 6155.

[120] Schlever, J., Hornung, V., Finke, S., Guenther-Biller, M., et al. (2005) Inhibition of toll-like receptor 7- and 9-mediated alpha/beta interferon production in human plasmacytoid dendritic cells by respiratory syncytial virus and measles virus. J. Virol. 79: 5507 – 5515.

[121] Moss, W.J., Ota, M.O., Griffin, D.E. (2004) Measles: immune suppression and immune responses. Int. J Biochem. Cell Biol. 36: 1380 – 1385.
[122] Symons, J.A., Alcami, A., Smith, G.L. (1995) Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. Cell 81: 551 – 560.

[123] Alcami, A., Symons, J.A., Smith, G.L. (2000) The vaccinia virus soluble alpha/beta interferon (IFN) receptor binds to the cell surface and protects cells from the antiviral effects of IFN. J. Virol. 74: 11230 – 11239.

[124] Li, S., Labrecque, S., Gauzzi, M.C., Cuddihy, A.R., et al. (2007) Alcami, A., Symons, J.A., Smith, G.L. (1995) Vaccinia virus

[125] Didcock, L., Young, D.F., Goodbourn, S., Randall, R.E. (1999) The V protein of simian virus 5 inhibits interferon signalling by targeting STAT1 for proteasome-mediated degradation. J. Virol. 73: 9928 – 9933.

[126] Ulane, C.M., Kentsis, A., Cruz, C.D., Parisien, J.P., Schneider et al. (2005) Composition and assembly of STAT-targeting ubiquitin ligase complexes: paramyxovirus V protein carboxy terminus is an oligomerization domain. J. Virol. 79: 10180 – 10189.

[127] Garcin, D., Marq, J.B., Strahle, L., le Mercier, P., Kolakofsky, D. (2002) All four Sendai Virus C proteins bind Stat1, but only the larger forms also induce its mono-ubiquitination and degradation. Virology 295: 256 – 265.

[128] Gotoh, B., Komatsu, T., Takeuchi, K., Yokoo, J. (2002) Paramyxovirus strategies for evading the interferon response. Rev. Med. Virol 12: 337 – 357.

[129] Ramaswamy, M., Shi, L., Varga, S.M., Barik, S., et al. (2006) Respiratory syncytial virus nonstructural protein 2 specifically inhibits type I interferon signal transduction. Virology 344: 328 – 339.

[130] Lo, M.S., Brazas, R.M., Holtzman, M.J. (2005) Respiratory syncytial virus nonstructural proteins NS1 and NS2 mediate inhibition of Stat2 expression and alpha/beta interferon responsiveness. J. Virol. 79: 9315 – 9319.

[131] Horvath, C.M. (2004) Weapons of STAT destruction. Interferon evasion by paramyxovirus V protein. Eur. J Biochem. 271: 4621 – 4628.

[132] Nagai, Y., Kato, A. (2004) Accessory genes of the paramyxoviridae, a large family of nonsegmented negative-strand RNA viruses, as a focus of active investigation by reverse genetics. Curr. Top. Microbiol. Immunol. 283: 197 – 248.

[133] Reid, S.P., Leung, I.W., Hartman, A.L., Martinez, O., et al. (2006) Ebola virus VP24 binds karyopherin alpha1 and blocks STAT1 nuclear accumulation. J. Virol. 80: 5156 – 5167.

[134] Leonard, G.T., Sen, G.C. (1996) Effects of adenovirus E1A protein on interferon-signaling. Virology 224: 25 – 33.

[135] Look, D.C., Roswit, W.T., Frick, A.G., Gris-Alevy, Y., et al. (1998) Direct suppression of Stat1 function during adenoviral infection. Immunity 9: 871 – 880.

[136] Paulus, C., Krauss, S., Nevels, M. (2006) A human cytomegalovirus antagonist of type I IFN-dependent signal transducer and activator of transcription signaling. Proc. Natl. Acad. Sci. U. S. A. 103: 3840 – 3845.

[137] Zimmermann, A., Trilling, M., Wagner, M., Wilborn, M., et al. (2005) A cytomegaloviral protein reveals a dual role for STAT2 in IFN-(gamma) signaling and antiviral responses. J. Exp. Med. 201: 1543 – 1553.

[138] Yokota, S., Yokosawa, N., Okabayashi, T., Suzutani, T., Fuji, N. (2005) Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 confines efficient viral replication. Virology 338: 173 – 181.

[139] Bode, J.G., Ludwig, S., Ehrhardt, C., Albrecht, U., et al. (2003) IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J 17: 488 – 490.

[140] Samanta, M., Iwakiri, D., Kanda, T., Imai, T., Takada, K. (2006) EB virus-encoded RNAs are recognized by RIG-I and activate signaling to induce type I IFN. EMBO J 25: 4207 – 4214.

[141] Elia, A., Laing, K.G., Schofield, A., Tilleray, V.J., Clemens, M.J. (1996) Regulation of the double-stranded RNA-dependent protein kinase PKR by RNAs encoded by a repeated sequence in the Epstein-Barr virus genome. Nucleic Acids Res. 24: 4471 – 4478.

[142] Mathews, M.B., Shenk, T. (1991) Adenovirus virus-associated RNA and translation control. J. Virol. 65: 5657 – 5662.

[143] He, B., Gross, M., Roizman, B. (1997) The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. Proc. Natl. Acad. U. S. A. 94: 843 – 848.

[144] Parisien, J.P., Lau, J.F., Horvath, C.M. (2002) STAT2 acts as a host range determinant for species-specific paramyxovirus interferon antagonism and simian virus 5 replication. J. Virol. 76: 6435 – 6441.

[145] Chatziandreou, N., Stock, N., Young, D., Andrejeva, J., et al. (2004) Relationships and host range of human, canine, simian and porcine isolates of simian virus 5 (paramyxovirus 5). J. Gen. Virol 85: 3007 – 3016.

[146] Riffault, S., Dubuquoy, C., Castagne, N., Baranowski, E., et al. (2006) Replication of bovine respiratory syncytial virus in murine cells depends on type I interferon-receptor functionality. J. Virol. 80: 5657 – 5662.

[147] Wang, F., Ma, Y., Barrett, J.W., Gao, X., et al. (2004) Disruption of Erk-dependent type I interferon induction breaks the myxoma virus species barrier. Nat. Immunol. 5: 2145 – 2148.

[148] Park, M.S., Garcia-Sastre, A., Cros, J.F., Basler, C.F., Palese, P. (2003) Newcastle disease virus V protein is a determinant of host range restriction. J. Virol. 77: 9522 – 9532.

[149] Wang, F., Ma, Y., Barrett, J.W., Gao, X., et al. (2004) Disruption of Erk-dependent type I interferon induction breaks the myxoma virus species barrier. Nat. Immunol. 5: 2145 – 2148.

[150] Garcia-Sastre, A. (2006) Antiviral response in pandemic influenza viruses. Emerg. Infect. Dis. 12: 44 – 47.