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MINIREVIEW

Inhibition of Interferon-Mediated Antiviral Responses by Influenza A Viruses and Other Negative-Strand RNA Viruses

Adolfo García-Sastre[1]

Department of Microbiology, Mount Sinai School of Medicine, New York, New York 10029

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The interferon (IFN) response represents an early host defense mechanism against viral infections, and it is known to be an important component of innate immunity (Vilcek and Sen, 1996). IFNs were discovered as acid-pH-resistant factors which were induced by treatment of cells with heat-inactivated influenza virus and which were able to inhibit (interfere with) virus replication (Isaacs and Lindenmann, 1957). Type I IFNs are typically secreted by eukaryotic cells in response to viral infection, leading to the stimulation of signal transduction pathways involved in the establishment of an antiviral state (for recent reviews, see Imada and Leonard, 2000; Stark *et al.,* 1998). Type II IFN or IFNγ is an important regulator of the cellular immune response and it is secreted by cells of lymphoid origin in response to various cytokine stimuli. The type I IFN genes consist of IFNβ and several IFNα subtypes. All type I IFN proteins recognize the same receptor, IFNAR, on the surface of eukaryotic cells within the same organism. Binding of type I IFN to its receptor results in the activation of two members of the Janus tyrosine kinase family, JAK1 and Tyk2, which in turn activate (phosphorylate) STAT1 and STAT2 transcription factors. As a consequence of phosphorylation, STAT1 and STAT2 heterodimerize, translocate to the nucleus, and associate with p48/IRF-9 to form the IFN-stimulated gene factor-3 (ISGF3). The ISGF3 complex binds specific DNA sequences containing IFN-stimulated regulatory elements (ISREs), promoting the transcription of downstream genes. Approximately 100 and 300 genes are known to be transcriptionally stimulated by IFNα and IFNβ, respectively (Der *et al.,* 1998). Among these genes, those encoding the dsRNA-activated protein kinase (PKR), the 2′,5′-oligoadenylate synthetases (2-5A synthetases), and the Mx proteins are known to interfere with viral replication by different mechanisms (Khabar *et al.,* 2000). Thus, secretion of type I IFN by virus-infected cells contributes to the induction of an antiviral state in neighboring uninfected cells.

Although the levels of PKR increase in cells in response to IFN, this antiviral enzyme is usually inactive unless it binds dsRNA. The same principle applies to the 2-5A synthetases. Binding of dsRNA to PKR causes a conformational change in this protein. As a result, PKR dimerizes, autophosphorylates, and becomes enzymatically active. A major target of the kinase activity of PKR is the translation initiation factor eIF-2α. PKR-mediated phosphorylation of eIF-2α leads to inactivation of this factor and to inhibition of protein synthesis (for recent reviews, see Gale *et al.,* 2000; Williams, 1999). On the other hand, dsRNA activation of 2-5A synthetases results in the synthesis of 2-5As, which in turn bind to and activate a latent RNase (RNase L). Activated RNase L induces the degradation of RNAs, including mRNAs and rRNAs, also contributing to inhibition of protein synthesis (Stark *et al.,* 1998). Finally, the IFN-induced Mx proteins are GTPases which inhibit replication of specific groups of viruses by mechanisms which are not well understood (Arnheiter *et al.,* 1996). In addition, it has become evident from studies using triple-knockout (PKR−/−, RNase L−/−, and Mx−/−) mice that additional IFN-regulated genes participate in the induction of the antiviral state by uncharacterized mechanisms (Zhou *et al.,* 1999). Some of these genes are known to interact with cellular components involved in translation, RNA synthesis, cell growth, and differentiation (Sen, 2000).

dsRNA appears to play a major role in the induction of the IFN response upon viral infection (Jacobs and Langland, 1996). dsRNAs are generated during virus infections as a result of overlapping bidirectional transcriptional units in DNA viruses, highly structured secondary structures of viral RNAs, and/or replicative intermediates

[1] Address correspondence and reprint requests to author at Department of Microbiology, Box 1124, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029. Fax: (212) 534-1684. E-mail: adolfo.garcia-sastre@mssm.edu.

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of different polarity in RNA viruses. dsRNA generated in infected cells can trigger synthesis of IFNβ and specific IFNαs by the activation of different transcription factors, including the interferon regulatory factors IRF-3 and IRF-7, NF-κB, and ATF2/c-Jun (Bragança and Civas, 1998; Marié *et al.,* 1998). Little is known about the mechanism by which dsRNA activates these transcription factors. At least in some systems, the dsRNA-mediated activation of constitutively expressed PKR is responsible for the stimulation of the NF-κB pathway (Chu *et al.,* 1999; Gil *et al.,* 2000; Kumar *et al.,* 1997; Yang *et al.,* 1995). Activated PKR in turn activates the IKK complex, which now phosphorylates IκB. Phosphorylation targets this NF-κB inhibitor to proteosome-mediated degradation (Ghosh *et al.,* 1998). NF-κB released from IκB translocates to the nucleus and cooperates with IRF-3/IRF-7 and ATF2/c-Jun in the induction of transcription from the IFN-B gene (Juang *et al.,* 1998; Wathelet *et al.,* 1998). Transcription factors activated by dsRNA also directly participate in the induction of a subset of IFN-stimulated genes in the absence of IFN (Daly and Reich, 1993). Once IFNβ (and IFNα4 in mouse cells) are secreted, interaction of these cytokines with its receptor results in activation of the STAT–JAK pathway and in the transcriptional stimulation of ISRE-containing promoters. Cells primed with IFN now express high levels of proteins encoded by IFN-stimulated genes, including the dsRNA-activated enzymes PKR and 2-5A synthetases. Viral infection of these cells results in the rapid stimulation of these enzymes by viral dsRNA, leading to protein synthesis inhibition and apoptosis/death of the infected cell (Stark *et al.,* 1998). In addition, some of the genes encoding transcription factors which participate in the activation of the IFN system, such as IRF-7, are also stimulated by IFN, and this is responsible for a positive feedback of the IFN response in cells primed with IFN (Marié *et al.,* 1998).

The type I IFN response is a powerful and immediate antiviral host response (Guidotti and Chisari, 2000). It is then not surprising that many viruses have evolved mechanisms to counteract this response (Alcami and Koszinowski, 2000; Cebulla *et al.,* 1999; Gale and Katze, 1998; Goodbourn *et al.,* 2000; Kalvakolanu, 1999; Ploegh, 1998). Subversion of the type I IFN system by viruses can occur at different levels and different viruses are known to prevent or inhibit IFN secretion, IFN-mediated signaling, and/or activity of IFN-stimulated genes. DNA viruses, such as poxviruses, herpesviruses, adenoviruses, and papovaviruses among others, encode inhibitors of IRFs (Juang *et al.,* 1998; Li *et al.,* 1998; Lubyova and Pitha, 2000; Moore *et al.,* 1996; Ronco *et al.,* 1998; Zimring *et al.,* 1998), inhibitors of NF-κB (Powell *et al.,* 1996; Revilla *et al.,* 1998), homologues of IFN receptors (Symons *et al.,* 1995), Jak inhibitors (Miller *et al.,* 1998; Weihua *et al.,* 1998), STAT inhibitors (Look *et al.,* 1998), p48 inhibitors (Barnard and McMillan, 1999; Miller *et al.,* 1999), and inhibitors of PKR and/or 2-5A synthetases (Cassady *et al.,* 1998; Chang *et al.,* 1992; Clarke *et al.,* 1991; Davies *et al.,* 1992; He *et al.,* 1997; Mathews and Shenk, 1991). Retroviruses, positive-strand RNA viruses, and dsRNA viruses are also known to inhibit the type I IFN system by several different mechanisms (Black *et al.,* 1989; Gale *et al.,* 1998; Gunnery *et al.,* 1990; Imani and Jacobs, 1988; Langland *et al.,* 1994; Roy *et al.,* 1990; Taylor *et al.,* 1999). By contrast, negative-strand RNA virus gene products involved in the inhibition of the type I IFN responses have only recently been identified. This article will focus on reviewing recent developments on our understanding of how negative-strand RNA viruses interfere with the IFN system, with special emphasis on the discussion of recent data generated in my laboratory in collaboration with Dr. Palese's and Dr. Muster's groups on the mechanism of inhibition of the type I IFN response by influenza viruses.

The negative-strand RNA viruses comprise a broad category of viruses belonging to the orthomyxovirus, paramyxovirus, rhabdovirus, filovirus, arenavirus, bunyavirus, and borna disease virus groups. Different negative-strand RNA viruses and even in some cases different strains within the same virus group have different abilities to induce IFN secretion in infected cells and/or different sensitivities to the antiviral action of IFN (Marcus *et al.,* 1992). The development of reverse genetics techniques allowing the genetic manipulation of negative-strand RNA viruses (Conzelmann, 1996; García-Sastre and Palese, 1993) made it possible to address experimentally the influence of specific viral genes on preventing the IFN-mediated antiviral responses of the host. These techniques as well as recent advances in the knowledge of the IFN system at the molecular level have greatly contributed to the reflourishing of the field of virus–host interactions involving the type I IFN system.

ORTHOMYXOVIRUSES: NS1 AND p58^{IPK} PROTEINS

Influenza A virus is considered the prototype virus of the orthomyxovirus group. Influenza viruses are important pathogens causing significant illness and death in humans and animals (Hayden and Palese, 1997). The negative-strand RNA genome of influenza A virus consists of eight segments which encode 10 proteins. Among these proteins, the only nonstructural protein of the virus, the NS1 protein, functions as an IFN antagonist and plays a key role in preventing the type I IFN-mediated antiviral response of the host during viral infection (Fig. 1) (García-Sastre *et al.,* 1998b). The NS1 protein of influenza A virus is an RNA-binding protein (Krug, 1998; Yoshida *et al.,* 1981). Interestingly, dsRNA is among the RNA molecules which have been shown to be able to bind *in vitro* to the NS1 protein (Hatada and Fukuda, 1992). Lu *et al.* were the first to show that NS1 protein inhibits *in vitro* the dsRNA-mediated activation of PKR and the subsequent inhibition of protein translation due

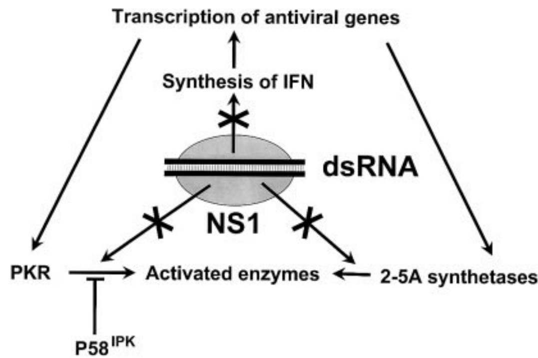

FIG. 1. Schematic representation of antiviral pathways activated by dsRNA and its inhibition by influenza A virus through the dsRNA-binding properties of the viral NS1 protein and through the activation of the cellular $p58^{IPK}$ protein. It should be noted that other cellular proteins that bind to dsRNA and that may participate in the host antiviral response, such as the dsRNA-dependent adenosine deaminase (ADAR), might also be inhibited by the NS1 protein of influenza virus.

to eIF-2α phosphorylation (Lu *et al.,* 1995). These properties are shared by the vaccinia virus E3L protein, a known virus-encoded IFN antagonist which binds to dsRNA (Watson *et al.,* 1991). The E3L protein was shown to prevent the dsRNA-mediated activation of PKR and 2-5A synthetases (Jacobs and Langland, 1996; Rivas *et al.,* 1998), contributing to the resistance of vaccinia virus to the antiviral effects of IFN (Chang *et al.,* 1995). However, the IFN antagonist activity of the NS1 protein of influenza A virus was not tested *in vivo* until 1998, when an NS1 knockout influenza A virus was generated (García-Sastre *et al.,* 1998b). This recombinant virus, delNS1, was compromised in its replication and pathogenicity in IFN-competent substrates and hosts, such as MDCK cells, laboratory strains of mice, and 10-day-old embryonated chicken eggs (García-Sastre *et al.,* 1998b; Talon *et al.,* 2000b). In contrast, delNS1 virus grows efficiently in substrates and hosts with deficiencies in the type I IFN system (García-Sastre *et al.,* 1998b; Talon *et al.,* 2000b), such as Vero cells, lacking the type I IFN genes (Diaz *et al.,* 1988), STAT1−/− mice, deficient in IFN signaling (Durbin *et al.,* 1996; Meraz *et al.,* 1996), and 7-day-old embryonated chicken eggs, with an immature type I IFN system (Sekellick *et al.,* 1990). Most significant is the fact that the delNS1 virus causes lethal disease in STAT1−/− mice (García-Sastre *et al.,* 1998b). The phenotypic rescue of delNS1 virus in IFN-deficient substrates clearly implicates the influenza virus NS1 protein in the inhibition of the type I IFN system.

What is the mechanism of the IFN antagonist activity of the influenza virus NS1 protein? It is possible that the NS1 protein, analogously to the E3L protein of vaccinia virus, binds to and sequesters dsRNA generated during virus replication, resulting in the inhibition of dsRNA-activated pathways (Fig. 1). Several lines of evidence support this hypothesis. First, the RNA-binding domain (RBD) of the NS1 is required for *in vitro* inhibition of PKR (Lu *et al.,* 1995). This inhibition might be exerted by a direct interaction of NS1 with PKR (Tan and Katze, 1998). Second, PKR is efficiently activated in cells infected with NS1 *ts* mutants of influenza A virus (Hatada *et al.,* 1999) or with delNS1 virus (Bergmann *et al.,* 2000), but not in cells infected with wild-type virus. Third, infection of cells with delNS1 virus, similarly to dsRNA treatment, results in the activation of IRF-3 (Talon *et al.,* 2000a) and NF-κB (Wang *et al.,* 2000), as well as in the subsequent activation of the IFN-B promoter (Wang *et al.,* 2000), promoting synthesis of type I IFN (Talon *et al.,* 2000a; Wang *et al.,* 2000). This in turn induces the stimulation of ISRE-containing promoters (García-Sastre *et al.,* 1998b), the expression of IFN-stimulated genes, and the induction of an antiviral state which prevent replication of heterologous viruses (Salvatore *et al.,* 2000). All these activities, including the stimulation of the IRF-3 and NF-κB transcription factors which are required for IFN-B gene transcription, are significantly reduced in wild-type virus-infected cells (Talon *et al.,* 2000a; Wang *et al.,* 2000). Fourth, a recombinant influenza A virus expressing only the RBD of the NS1 protein retains the ability to prevent NF-κB activation and IFNβ expression in infected cells (Wang *et al.,* 2000). Fifth, expression *in trans* of NS1 from a plasmid in the absence of any other influenza A virus protein prevents the dsRNA- and virus-mediated activation of IRF-3, NF-κB, and IFNβ synthesis (Talon *et al.,* 2000a; Wang *et al.,* 2000). The RBD of the NS1 is both required and sufficient for this inhibition. Sixth, the NS1 inhibits, by virtue of its dsRNA-binding properties, the dsRNA-mediated stimulation of the enzymatic activity of 2-5A synthetases *in vitro* (Donelan and García-Sastre, 2000). Seventh, the percentage of influenza A viruses which display higher resistance to IFN action in a given virus population correlates with the theoretical percentage of virions containing two or more copies of the NS segment (and expressing higher levels of NS1 protein) (Sekellick *et al.,* 2000). However, despite all this evidence, it is not yet clear whether the NS1 exerts its IFN antagonistic functions by merely sequestering dsRNA or by direct interactions with different host protein complexes which may or may not be mediated by dsRNA.

The importance of the interplay between PKR and NS1 during influenza A virus infections is further underscored by recent experiments showing that delNS1 virus regains its replication properties not only in STAT1−/− mice, but also in PKR−/− mice (Bergmann *et al.,* 2000). These observations also demonstrate a critical role of PKR in antiviral responses, as has also been recently shown by Leib *et al.* (2000) and Balachandran *et al.* (2000). The antiviral effects of PKR might be mediated by a combination of different activities, such as (i) its ability to stimulate the synthesis of type I IFN through the activation of IRF-1 (Kirchhoff *et al.,* 1995) and of the NF-κB pathway (Chu *et al.,* 1999; Gil *et al.,* 2000; Kumar *et al.,* 1997; Yang *et al.,* 1995), (ii) its role in Ser-phosphorylation

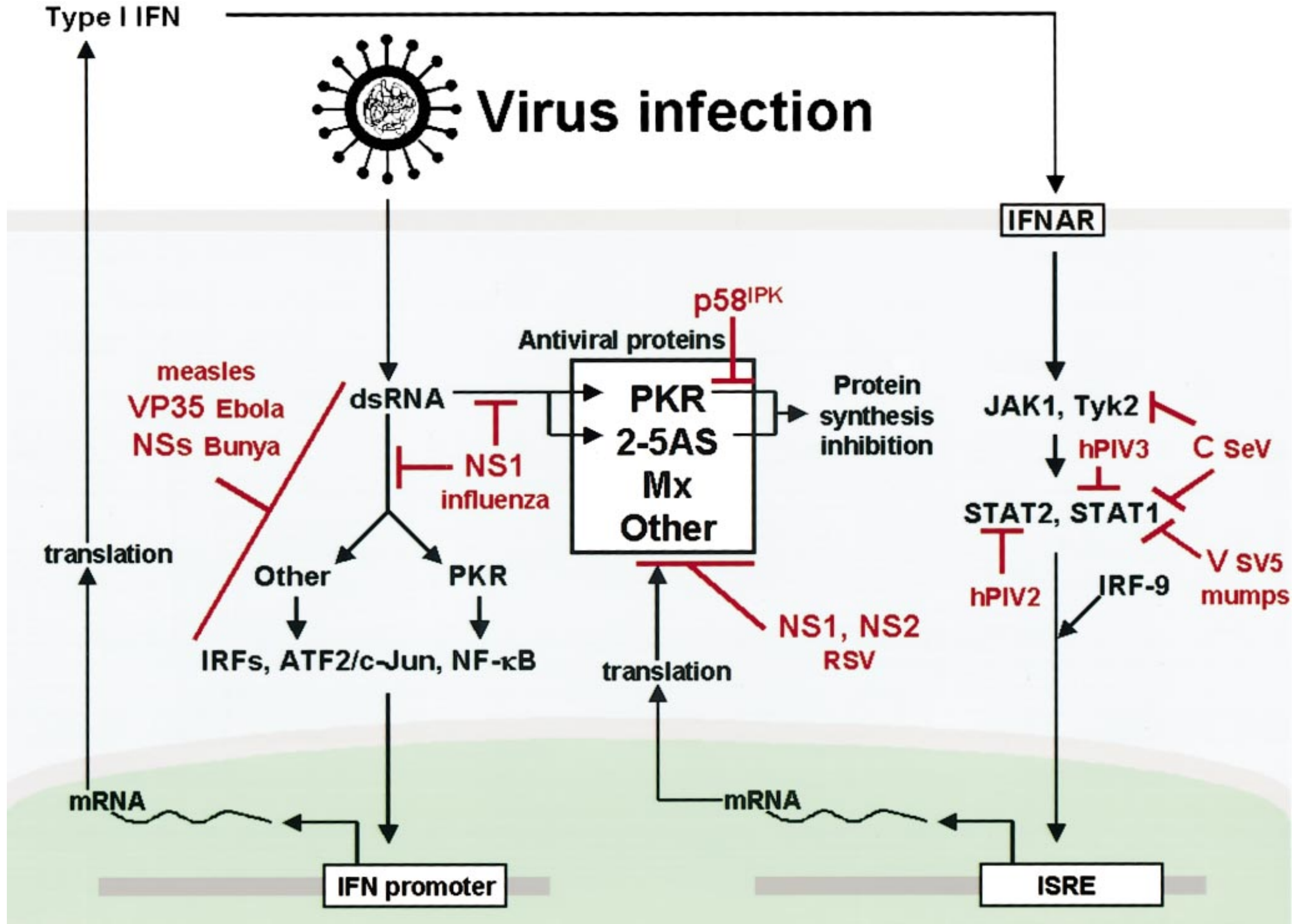

FIG. 2. Multiple mechanisms are used by negative-strand RNA viruses to subvert the type I IFN system. The figure represents the different levels at which different negative-strand RNA viruses and their encoded proteins have been shown to antagonize the type I IFN system. In addition, p58^{IPK} is a host cellular inhibitor of PKR activated during influenza A virus infection.

of STAT1 (Ramana *et al.,* 2000), (iii) its translational inhibitory properties as an eIF-2α kinase (Williams, 1999), and (iv) its proapoptotic properties (Balachandran *et al.,* 1998; Der *et al.,* 1997; Gil and Esteban, 2000; Takizawa *et al.,* 1996; Yeung *et al.,* 1999). Intriguingly, influenza A viruses appear to be able to inhibit the PKR-mediated phosphorylation of eIF-2α by yet a second mechanism, which have been extensively characterized by Dr. Katze's group (Gale and Katze, 1998; Gale *et al.,* 2000) (Figs. 1 and 2). Infection of cells by influenza A virus results in the activation of a host PKR inhibitory protein, P58^{IPK} (Lee *et al.,* 1990), which under normal circumstances is bound to the protein I-P58^{IPK} in an inactive complex (Lee *et al.,* 1992). This complex is disrupted in influenza A virus-infected cells, and the released P58^{IPK} physically interacts with PKR (Gale *et al.,* 1996; Polyak *et al.,* 1996), inhibiting its kinase activity by preventing PKR dimerization (Tan *et al.,* 1998) and/or by recruiting a cellular chaperone, Hsp70 (Melville *et al.,* 1999), to perform this inhibition. Thus, influenza A virus, like vaccinia virus (Chang *et al.,* 1992; Davies *et al.,* 1992) and hepatitis C virus (Gale *et al.,* 1998; Taylor *et al.,* 1999), appears to have evolved two different mechanisms to inhibit the antiviral enzyme PKR. It has now become clear that the expression of the NS1 protein in influenza A virus-infected cells is essential for overcoming the type I IFN responses (see above). Whether P58^{IPK} also plays an essential role in the inhibition of the type I IFN response by influenza A virus is still an open question which awaits the generation and characterization of p58^{IPK} knock-out animals. Finally, possible interconnections between the NS1- and P58^{IPK}-mediated inhibition of PKR in influenza A virus-infected cells remain to be elucidated.

Influenza B and C viruses, like influenza A viruses, also encode an NS1 protein from the shortest viral RNA segment. However, little or no homology is shared by the NS1 proteins of these viruses. Interestingly, the NS1 protein of influenza B virus is also able to bind to dsRNA and prevent the dsRNA-mediated activation of PKR *in vitro* (Wang and Krug, 1996). Moreover, two influenza B virus mutants expressing truncated NS1 proteins

showed a similar growth restriction in embryonated chicken eggs as that of influenza A virus NS1 deletion mutants (Talon *et al.,* 2000b). Further experimental support for the role of the NS1 proteins of influenza B and C viruses as IFN antagonists came from experiments demonstrating that these proteins are able to inhibit *in vitro* the dsRNA-mediated activation of 2-5A synthetases (Donelan and García-Sastre, 2000). Moreover, expression *in trans* of influenza B or C virus NS1 proteins complements the growth of influenza A delNS1 virus (Basler, Palese, and A.G.-S., unpublished). However, the precise mechanism by which the NS1 protein of influenza B and C viruses antagonize the type I IFN system in infected cells is not known.

PARAMYXOVIRUSES: V AND C PROTEINS

The first viral gene in the negative-strand RNA group of viruses which was clearly shown to be involved in antagonizing the type I IFN system was the NS1 of influenza A virus (García-Sastre *et al.,* 1998b). This observation was followed by the characterization of IFN antagonists encoded by several paramyxoviruses (Fig. 2). Paramyxoviruses are nonsegmented negative-strand RNA viruses and they include different human and animal viruses, such as Sendai virus, parainfluenza viruses, mumps virus, SV5, Newcastle disease virus, measles virus, canine distemper virus, Nipah virus, and respiratory syncytial viruses (RSV), among others. Fujii *et al.* (1988) showed that cells persistently infected with mumps or measles viruses have reduced levels of 2-5A synthetase activity. This effect correlated with an induction of STAT1 degradation in mumps virus-infected cells (Yokosawa *et al.,* 1998), providing a rational basis for the evasion of IFN-mediated antiviral responses by mumps viruses. In addition, Didcock *et al.* (1999a) showed that Sendai virus and SV5 inhibit the activation of IFN-responsive genes in infected cells. This is in agreement with results from Yokoo *et al.* (1999) showing that Sendai virus infection suppresses the antiviral action of type I IFN. It was subsequently found that SV5 infection resulted in targeting of STAT1 for proteosome-mediated degradation and that expression of the SV5 structural protein V was sufficient to induce STAT1 degradation in the absence of other virus proteins (Didcock *et al.,* 1999b). Strikingly, human parainfluenza virus 2 (hPIV2) infection leads to degradation of STAT2, and not of STAT1 (Young *et al.,* 2000). While elimination of STAT1 results in deficient type I and type II IFN signaling, elimination of STAT2 only interferes with type I IFN signaling, and this distinction might be responsible for different biological properties among different paramyxoviruses.

The V proteins of paramyxoviruses are expressed from the P gene, which in addition codes for the P protein, an important component of the viral RNA-dependent RNA polymerase. The V and P proteins have the same amino-terminal sequence, but differ in their carboxy-termini due to pseudo-template insertion of one or more nucleotides in the P-encoded mRNA (Lamb and Kolakofsky, 1996). Interestingly, inhibition of the type I IFN system by Sendai virus appears to be mediated by the C proteins, which are also encoded by the P gene. The C proteins of paramyxoviruses are expressed from an ORF overlapping the P ORF by translation from alternative initiation codons (Lamb and Kolakofsky, 1996). Depending on the use of one or more in-phase initiation codons, different paramyxoviruses encode different numbers of C proteins. In the case of Sendai virus, there are four C proteins (C′, C, Y1, and Y2) expressed in virus-infected cells. All these four proteins share the same carboxy-terminal sequences. The role of the C proteins of Sendai virus as IFN antagonists was established by experiments using recombinant Sendai viruses expressing mutant C proteins or containing mutations which prevented C protein expression (Garcin *et al.,* 1999; Gotoh *et al.,* 1999). Wild-type Sendai virus-infected cells demonstrated reduced levels of STAT1 activation after IFN treatment, resulting in a loss of IFN signaling (Garcin *et al.,* 1999; Gotoh *et al.,* 1999; Young *et al.,* 2000). By contrast, no IFN signaling deficiencies were found in cells infected with C mutant Sendai viruses (Garcin *et al.,* 1999; Gotoh *et al.,* 1999). Inhibition of type I IFN signaling during wild-type Sendai virus infection correlated with partial inhibition of IFN-mediated tyrosine phosphorylation of Tyk2 (Komatsu *et al.,* 2000), with a reduction in serine 727-phosphorylated forms of STAT1 (Young *et al.,* 2000) and with increased STAT1 instability (Garcin *et al.,* 2000). Individual expression of all four Sendai virus C proteins was sufficient to efficiently block IFN signaling, demonstrating that these viral proteins have IFN antagonistic activities (Garcin *et al.,* 2000). The individual role of the four Sendai virus C proteins, as well as of the C and V proteins of different paramyxoviruses, in inhibiting the IFN system during virus infections remains to be determined. Interestingly, it is clear that different paramyxoviruses antagonize the IFN system by inhibiting different cellular components (Fig. 2). For example, measles virus appears to inhibit type I IFN production (Naniche *et al.,* 2000); Sendai virus, SV5, and hPIV3 block type I and type II IFN signaling by targeting STAT1 activation; hPIV2 blocks only type I IFN signaling by targeting STAT2 activation; and RSV circumvents the antiviral IFN response without affecting IFN synthesis and/or signaling (Schlender *et al.,* 2000; Young *et al.,* 2000).

PNEUMOVIRUSES: NS1 AND NS2 PROTEINS

The C and/or V proteins of different paramyxoviruses, such as Sendai virus and SV5, respectively, appear to be involved in inhibiting the type I IFN system. However, pneumoviruses, among the paramyxoviruses, do not express C/V proteins. By contrast, pneumoviruses, includ-

ing RSV, encode two nonstructural proteins, NS1 and NS2, which are not present in the other paramyxovirus groups (Collins *et al.,* 1996). These proteins were found to perform a nonessential function during RSV replication, since it was possible to obtain infectious NS1 and/or NS2 knockout RSVs (Buchholz *et al.,* 1999; Jin *et al.,* 2000; Teng and Collins, 1999; Teng *et al.,* 2000). A recent report from Schlender *et al.* (2000) demonstrated that the NS1 and NS2 proteins of bovine RSV are involved in antagonizing the type I IFN system during viral infection by an unknown mechanism. Moreover, both NS1 and NS2 proteins are required for mediating resistance of bovine RSV or of a heterologous virus, such as rabies virus, to the antiviral action of IFNs (Schlender *et al.,* 2000). These two proteins of RSV are likely to be responsible for the ability of this virus to overcome the IFN-induced antiviral response (Atreya and Kulkarni, 1999; Young *et al.,* 2000).

FILOVIRUSES: VP35

Among the nonsegmented negative-strand RNA viruses, filoviruses include some of the most lethal viruses for humans, Marburg and Ebola viruses. Interestingly, Ebola virus infection results in a block on the ability to respond to dsRNA and IFNs by the infected cells (Harcourt *et al.,* 1998, 1999). Basler *et al.* (2000) found that the Ebola virus VP35 protein, which appears to be the equivalent of the P protein of paramyxo- and rhabdoviruses (Mühlberger *et al.,* 1999), antagonizes the type I IFN system. Expression of Ebola virus VP35 complemented growth of the IFN-sensitive influenza A delNS1 virus in IFN-competent cells. Expression of VP35 was also responsible for a block in dsRNA- and virus-mediated stimulation of the IFNβ promoter and of an ISRE-containing promoter in human cells (Basler *et al.,* 2000).

BUNYAVIRUSES: NSs PROTEINS

Bunyaviruses are three-segmented negative-strand RNA viruses. As with orthomyxoviruses, paramyxoviruses, and filoviruses, these viruses also seem to inhibit the type I IFN system. Haller *et al.* (2000) have recently found that attenuated isolates of Rift valley virus, a bunyavirus, differing from wild-type virus in their nonstructural NSs proteins, regain virulence and high levels of replication in IFNAR−/− mice. Moreover, high levels of type I IFN production were induced in mice infected with the attenuated viruses, but not in wild-type virus-infected mice (Haller *et al.,* 2000). Thus, the NSs protein of Rift valley fever virus most likely plays a role in inhibiting the type I IFN system during viral infection. Weber *et al.* generated a recombinant Bunyamwera virus, also a bunyavirus, which is deficient in NSs production (Weber *et al.,* 2000). The NSs knockout virus, and not the wild-type virus, induced the activation of IRF-3 and NF-κB transcription factors and the activation of the IFN-B promoter, demonstrating an IFN antagonistic function of the NSs protein of Bunyamwera virus (Weber *et al.,* 2000).

VIRUS-INDUCED SHUTOFF OF HOST CELL PROTEIN SYNTHESIS

In addition to the specific mechanisms discussed above, negative-strand RNA viruses, as well as other viruses, may inhibit the cellular antiviral responses through a generalized inhibition of host cell protein synthesis. Shutoff of cell protein synthesis is very efficiently induced by several negative-strand RNA viruses, such as VSV and influenza viruses, by mechanisms which are still poorly understood. Since the activation of the IFN-mediated antiviral response requires the induction of expression of different host factors, a general shutoff of cellular protein synthesis is likely to contribute to the evasion of the type I IFN system by viruses. In this respect, it has been shown that the VSV matrix protein inhibits host transcription (Ahmed and Lyles, 1998; Black and Lyles, 1992), including the transcription from the human IFN-B promoter (Ferran and Lucas-Lenard, 1997).

CONCLUDING REMARKS

Research studies conducted in the past 3 years and discussed above have shown that most negative-strand RNA viruses encode IFN antagonists. Remarkably, these viruses have evolved independent and diverse mechanisms to antagonize the IFN system (Fig. 2). These mechanisms are most likely the result of a coevolution between viruses and hosts and, in most cases, do not appear to completely abrogate the type I IFN antiviral response in the host. Recent advances in reverse genetics techniques (Conzelmann, 1996; Fodor *et al.,* 1999; Neumann *et al.,* 1999) and in the generation of knockout animals in different IFN pathways, as well as of transgenic animal models of infection (Rall *et al.,* 2000), will greatly facilitate future studies aimed at understanding the virus–host interactions leading to the inhibition of the IFN system. These interactions seem to play a critical role in viral pathogenicity, immunogenicity, persistence, and tissue/host tropism (Balachandran *et al.,* 2000; Didcock *et al.,* 1999a; Durbin *et al.,* 2000; García-Sastre *et al.,* 1998a). Most likely, IFN antagonist genes will also be identified in rhabdoviruses, hantaviruses, arenaviruses, and borna disease viruses. In addition, rationally engineered viruses with deletions/modification in their IFN antagonist genes might be suitable live attenuated vaccines (Talon *et al.,* 2000b). Finally, these recently identified viral encoded IFN antagonists among the negative-strand RNA viruses represent promising targets for novel antiviral therapies. Compounds inhibiting the activity of these IFN antagonists should restore innate host defense mechanisms resulting in inhibition of virus replication.

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