Interferons: cell signalling, immune modulation, antiviral responses and virus countermeasures

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Introduction

To establish infections in vivo, viruses must replicate in the face of powerful immune defence mechanisms including those induced by interferons (IFNs). The effectiveness of the IFN response has led to many viruses developing specific mechanisms that antagonize the production or actions of IFNs. Indeed, in order to replicate efficiently in vivo, it seems likely that all viruses must, at least to a degree, have some means of circumventing the IFN response either by limiting IFN production or by blocking IFN actions. However, virus countermeasures to the IFN response are rarely absolute and the IFN response, by limiting virus spread, buys time for the generation of an acquired immune response to the invading virus. Nevertheless, the speed and efficiency by which a given virus circumvents the IFN response may be critical determinants in its host range and pathogenicity. In part A of this article, we review how virus infections lead to the production of IFNs (section 1), how IFNs induce the transcription of their target genes (section 2) and how these target genes exert their antiviral effects (section 3). Part B of this article reviews the strategies used by viruses to inhibit IFN production (section 4), IFN signalling (section 5) and/or specific antiviral functions (section 6).

The IFNs are a large family of multifunctional secreted proteins involved in antiviral defence, cell growth regulation and immune activation. The IFNs may be classified into two distinct types. Type I IFNs are produced in direct response to virus infection and consist of the products of the IFN-α multigene family, which are predominantly synthesized by leukocytes, and the product of the IFN-β gene, which is synthesized by most cell types but particularly by fibroblasts. Type II IFN consists of the product of the IFN-γ gene and, rather than being induced directly by virus infection, is synthesized in response to the recognition of infected cells by activated T lymphocytes and natural killer (NK) cells (reviewed in Vilcek & Sen, 1996).

Type I IFN (IFN-α/β) and type II IFN (IFN-γ) share no obvious structural homology. However, functional similarities exist due to a broad overlap in the types of genes that they induce (reviewed in Stark et al., 1998; summarized in Fig. 1). It is clear that IFNs can induce transcription of a significant number of genes. In addition to the well-characterized gene products described below, large-scale screening using oligonucleotide arrays has identified several novel human IFN-inducible genes that are induced by either IFN-α/β or IFN-γ or both (Der et al., 1998). The importance of IFN in mediating responses to virus infections is established by the fact that mice lacking IFN-α/β (Muller et al., 1994; Fiette et al., 1995; Huang et al., 1995; Rousseau et al., 1995; Steinhoff et al., 1995; van den Broek et al., 1995a, b; Garcia-Sastre et al., 1998; Mrkic et al., 1998; Yeow et al., 1998; Cousens et al., 1999; Grieder & Vogel, 1999; Grob et al., 1999; Johnson & Roehrig, 1999; Nunez, 1999) or IFN-γ (Huang et al., 1993; Muller et al., 1994; Fiette et al., 1995; van den Broek et al., 1995a, b; Bovolenta et al., 1999; Cantin et al., 1999; Dorman et al., 1999; Grob et al., 1999; Nunez, 1999; Tay et al., 1999) receptors are unable to mount efficient responses to a large number of viruses. Importantly, there are often differences in the requirements for the two types of IFN in resolving specific virus infections, and the systems are non-redundant in many cases. Both types of IFN stimulate an ‘antiviral state’ in target cells, whereby the replication of virus is blocked or impaired due to the synthesis of a number of enzymes that interfere with cellular and virus processes. Both types of IFN can also slow the growth of target cells or make them more susceptible to apoptosis, thereby limiting the extent of virus spread. Finally, both types of IFN have profound immunomodulatory effects and stimulate the adaptive response. However, whilst both IFN-α/β and IFN-γ influence the properties of immune effector cells, they show significant differences, and it is these extended cytokine functions that probably account for the different spectrums of antiviral activities of the two types of IFN.

A. Production and actions of IFNs

1. Virus induction of IFN genes

The induction of IFN-β expression by virus infection of fibroblastoid cells has been the subject of intensive research. It
is generally assumed that the inducer is intracellular double-stranded RNA (dsRNA), provided by the viral genome itself or formed as a result of replication or convergent transcription of viral genomes (reviewed in Jacobs & Langland, 1996). The induction of IFN-β occurs primarily at the level of transcriptional initiation (see Fig. 2). The key induction event is the redistribution from the cytoplasm to the nucleus of the transcription factor NF-κB (Lenardo et al., 1989; Visvanathan & Goodbourn, 1989). NF-κB plays a role in the transcriptional induction of many immunomodulatory genes, including other cytokines, MHC class I and cell adhesion molecules (reviewed in Baldwin, 1996). NF-κB is normally held in a quiescent state in the cytoplasm by association with an inhibitor molecule called IκB. Upon receipt of a wide range of stress signals [for example lipopolysaccharide, tumour necrosis factor (TNF), interleukin (IL)-1 and viral dsRNA], IκB is destroyed, the associated NF-κB is freed from restraint and can enter the nucleus and activate transcription (reviewed in Israel, 2000). Exposure to dsRNA activates NF-κB via the dsRNA-dependent protein kinase R (PKR) (Maran et al., 1994; Yang et al., 1995; see section 3), which activates the IκKβ subunit of the multicomponent IκB kinase (Chu et al., 1999; Zamanian-Daryoush et al., 2000). PKR can also phosphorylate IκB directly (Kumar et al., 1994; Offermann et al., 1995), although the biological role for this is unclear.

NF-κB binds to the IFN-β promoter as part of a multiprotein transcription-promoting complex called the ‘enhanceosome’ (reviewed in Thanos, 1996), which also contains HMG-I/Y, ATF-2 homodimers or ATF-2/c-Jun heterodimers (Du et al., 1993) and a factor that binds to positive regulatory domain I (PRD I). Although the latter would appear to be a member of the interferon regulatory factor (IRF) family, its identity remains the subject of debate. Having been suggested to be IRF-1 (Miyamoto et al., 1988; Fujita et al., 1989a; Watanabe et al., 1991; Reis et al., 1992; Matsuyama et al., 1993), ISGF3 (Yoneyama et al., 1996), IRF-3 (Sato et al., 1998b; Schafer et al., 1998; Weaver et al., 1998; Yoneyama et al., 1998) or a combination of IRF-3 and IRF-7 (Wathelet et al., 1998). Since many of the IRF proteins bind both PRD I and the closely related IFN-stimulated response element (ISRE; an element that is found in genes that are transcriptionally responsive to type I IFN—see section 2), there may be some functional overlap in the properties of these proteins. One consequence of this overlap may be to ensure that virus infections cannot block IFN-β induction completely by inhibiting any single IRF (see section 4).

IFN-α can also be induced by virus infection in fibroblastoid cells, and the promoters of several IFN-α genes have been studied in detail (reviewed in Pitha & Au, 1995). Unlike IFN-β, the IFN-α promoters do not have an NF-κB site, but contain...
elements that are related to the PRD I- and ATF-2-binding sites, as well as distinct elements. IFN-α genes are not able to be induced in embryonic fibroblasts derived from mice lacking both copies of the IFN-β gene, implying that fibroblasts depend upon IFN receptor activation by IFN-β for IFN-α production (Erlandsson et al., 1998). It is thought that IFN-β works by inducing the synthesis of IRF-7, which, following activation by virus infection, leads to stimulation of IFN-α transcription (Au et al., 1998; Marie et al., 1998; Sato et al., 1998a; Yeow et al., 2000). IFN-α is also induced in leukocytes by virus infection. The induction mechanism is poorly characterized in the case of these cells, but is clearly distinct from induction in fibroblasts, since IFN-β production is not required (Erlandsson et al., 1998).

IFN-γ is produced by Th1 CD4^+ helper T cells and by nearly all CD8^+ cells, as a result of transcriptional activation induced by exposure to antigen-presenting cells (reviewed in Young, 1996). In naive and memory CD4^+ T cells, the IFN-γ promoter is under the control of two distinct regulatory elements (proximal and distal; Aune et al., 1997). In contrast, only the distal element is activated in CD8^+ cells, leading to a significantly weaker response than that seen in CD4^+ cells. The proximal element is activated by complexes containing c-Jun and ATF-2, whilst the distal element is activated by GATA-3 and ATF-1 (Penix et al., 1996; Zhang et al., 1998a). The signal transduction mechanisms involved in activating transcription of the IFN-γ gene are poorly characterized, but involve the p38 and JNK2 mitogen-activated protein kinase (MAP kinase) pathways (Rincon et al., 1998; Yang et al., 1998; Lu et al., 1999). IFN-γ production in response to antigen stimulation is enhanced markedly by IL-12 or IL-18, cytokines produced by activated antigen-presenting cells (reviewed in Okamura et al., 1998). Although neither IL-12 nor IL-18 alone can stimulate IFN-γ production significantly in unstimulated T cells, together these cytokines can stimulate IFN-γ production in an antigen-independent manner (Tominaga et al., 2000). The molecular

Fig. 2. Transcriptional induction of the IFN-β gene. Virus replication gives rise to dsRNA, which is able to activate PKR and perhaps additional cellular kinases. PKR in turn activates the IκB kinase and indirectly leads to the activation of the immunomodulatory transcription factor NF-κB. Together with ATF-2 and a member(s) of the IRF family, NF-κB assembles on the IFN-β promoter with the help of several copies of the accessory factor HMG-I/Y to form a multifactorial complex called the 'enhanceasome'. Components of the enhanceasome make contacts with factors that are part of the basal transcriptional machinery and, by stabilizing interactions with this machinery and causing a local 'remodelling' of the chromatin, recruit RNA polymerase II to the promoter to bring about transcription of the IFN-β gene. See text for details.
basis of this is unknown, but may involve activation of STAT4 by IL-12 and NF-κB by IL-18, and it may also involve an up-regulation of the IL-18 receptor by IL-12 (Yoshimoto et al., 1998). IFN-γ is also produced by activated NK cells in an antigen-independent manner and this is also dependent on IL-12 production by antigen-presenting cells and is stimulated by IL-18 (Singh et al., 2000).

2. Signal transduction in response to IFNs

The biological activities of IFNs are initiated by the binding of IFN-α/β and IFN-γ to their cognate receptors on the surface of cells, which results in the activation of distinct but related signalling pathways, known as the Jak/STAT pathways (reviewed in Stark et al., 1998; summarized in Fig. 3). The ultimate outcome of this signalling is the activation of transcription of target genes that are normally expressed at low levels or are quiescent. The upstream regulatory sequences of most IFN-α/β-inducible genes contain a variation of the consensus sequence [GAAAN(N)GAAA] called the ISRE, whilst the upstream regulatory regions of IFN-γ-inducible genes contain a unique element called the gamma activation sequence (GAS), which contains the consensus sequence TTNCNNNAA.

The IFN-α/β receptor is composed of two major subunits, IFNAR1 and IFNAR2 (reviewed in Mogensen et al., 1999). Prior to stimulation, the cytoplasmic domains of IFNAR1 and IFNAR2 are respectively associated with the ‘Janus’ tyrosine kinases Tyk2 (Colamonici et al., 1994) and Jak1 (Novick et al., 1994). IFNAR2 is also associated with the ‘signal transducer and activator of transcription’ (STAT) molecule STAT2 (Li et al., 1997). On IFN-α/β binding, IFNAR1 and IFNAR2 associate, facilitating the transphosphorylation and activation of Tyk2 and Jak1 (Novick et al., 1994). Tyk2 then phosphorylates the tyrosine at position 466 (Tyr466) on IFNAR1 (Colamonici et al., 1994), creating a new docking site for STAT2 through the latter's SH2 domain (Yan et al., 1996). STAT2 is then phosphorylated by Tyk2 at Tyr180 and serves as a platform (Leung et al., 1995; Qureshi et al., 1996) for the recruitment of STAT1 (also through its SH2 domain), which is subsequently phosphorylated on Tyr701 (Shuai et al., 1993). The phosphorylated STAT1/STAT2 heterodimers thus formed...
dissociate from the receptor and are translocated to the nucleus through an unknown mechanism, where they associate with the DNA-binding protein p48 (Veals et al., 1992) to form a heterotrimeric complex called ISGF3, which binds the ISRE of IFN-α/β-responsive genes. p48 is a member of the IRF family and is sometimes referred to as IRF-9; it should be stressed that, like the IFN-β promoter element PRD I, the ISRE sequence can also be bound by other members of the IRF family, notably IRF-1 and IRF-2, and this may have profound biological consequences (see below).

IFN-γ receptors are composed of at least two major polypeptides, IFNAR1 and IFNAR2 (reviewed in Bach et al., 1997). In unstimulated cells, IFNAR1 and IFNAR2 do not pre-associate strongly with one another (Bach et al., 1996), but their intracellular domains specifically associate with the Janus kinases Jak1 and Jak2, respectively (Kotenko et al., 1995; Sakatsume et al., 1995; Bach et al., 1996; Kaplan et al., 1996). Binding of the dimeric IFN-γ to the receptor triggers receptor dimerization, which brings Jak1 and Jak2 molecules on adjacent receptor molecules into close proximity (Greenlund et al., 1994, 1995; Igarashi et al., 1994; Bach et al., 1996); Jak2 is thus activated and in turn activates Jak1 by trans-phosphorylation (Briscoe et al., 1996). The activated Jak5 then phosphorylate a tyrosine-containing sequence near the C terminus of IFNAR1 (Tyr^410–Tyr^444), thereby forming paired binding sites for STAT1 that interact through their SH2 domains (Greenlund et al., 1994, 1995; Igarashi et al., 1994) and are phosphorylated at Tyr^701, near the C terminus (Shuai et al., 1993, 1994; Greenlund et al., 1994; Heim et al., 1995). The phosphorylated STAT1 proteins dissociate from the receptor and form a homodimer, through SH2 domain–tyrosine phosphate recognition, which translocates to the nucleus through a poorly characterized mechanism (Sekimoto et al., 1995). Active STAT1 homodimers, also called gamma-activated factor (GAF), bind to specific GAS elements of IFN-γ-inducible genes (reviewed by Stark et al., 1998) and stimulate transcription. IFN-α/β can also induce the formation of STAT1 homodimers, albeit less efficiently than IFN-γ (Haque & Williams, 1994), although the mechanism whereby STAT1 homodimers are activated by IFN-α/β remains obscure.

The transactivation function of STAT1 depends upon phosphorylation of Ser^727 (Wen et al., 1995) by a kinase with MAP-like specificity. The identity of this kinase remains controversial, although it may differ between cell types. Thus, p38 kinase has been shown to be important for Ser^727 phosphorylation in response to IFN-α/β and IFN-γ in mouse fibroblasts (Goh et al., 1999) but not in response to IFN-γ in macrophages (Kovarik et al., 1999). Furthermore, the protein tyrosine kinase Pyk2 has recently been shown to be a critical mediator of the Jak-dependent activation of Ser^727 phosphorylation of STAT1 in IFN-γ, but not IFN-α/β, signalling (Takaoka et al., 1999). It has also recently been shown that PKR plays a role in Ser^727 phosphorylation (Ramana et al., 2000), but this is unlikely to be direct. The role of Ser^727 phosphorylation is to facilitate interaction of STAT1 with the basal transcriptional machinery. Recent studies have revealed important connections between STAT1 and the CREB-binding protein (CBP)/p300 transcription factors. The CBP/p300 family of transcription factors potentiates the activity of several groups of transcription factors (reviewed in Janknecht & Hunter, 1996). Both the C- and N-terminal domains of STAT1 have been shown to bind CBP/p300 (Zhang et al., 1996). STAT1 also interacts with the chromatin-associated protein MCM5 in a Ser^727-dependent manner (Zhang et al., 1998) and with Nmi, a protein that acts to enhance the association between STAT1 and CBP/p300 (Zhu et al., 1999). Although STAT2 does not contain a MAP kinase consensus site and is not known to be serine-phosphorylated in response to IFN, it also binds CBP/p300 and facilitates interaction with the basal transcriptional machinery (Bhattacharya et al., 1996).

A second form of STAT1 (STAT1β) can be derived by differential splicing. STAT1β contains the tyrosine at position 701 and is recruited to the receptor complex, becomes tyrosine-phosphorylated, and binds DNA. However, STAT1β differs from the predominant form of STAT1 (STAT1α) by lacking the C-terminal 38 amino acids that include Ser^727 and, thus, it cannot activate transcription (Schindler et al., 1992; Shuai et al., 1993). The function of STAT1β is not clear. Although it can become incorporated into ISGF3 complexes that retain their transcriptional activation potential as a result of STAT2 function (Muller et al., 1993), the consequences of a potential STAT1α/STAT1β heterodimer have not been established, but these might well down-regulate transcription.

Recently, several other proteins have been identified that may also be required for IFN signalling. For example, the tyrosine phosphatase SHP-2, which pre-associates with IFNAR1, is phosphorylated in response to IFN-α/β and, in transfection experiments, a dominant-negative form of SHP-2 inhibits the IFN-α/β-induced expression of a reporter gene (David et al., 1996). IFN-α/β treatment also induces the phosphorylation and activation of cytosolic phospholipase A2 (CPLA2), an event that requires Jak1 and the p38 MAP kinase (Goh et al., 1999). The demonstration that CPLA2 inhibitors can block the expression of ISRE-containing genes induced by IFN-α/β implies that CPLA2 also plays a role in the transactivation of ISRE-containing genes (Hannigan & Williams, 1991; Flati et al., 1996).

As discussed above, other members of the IRF family can bind ISRE sequences and our understanding of IFN-mediated signal transduction is complicated by the fact that some of these IRF proteins are inducible by IFNs. Thus, both IFN-α/β and IFN-γ can induce IRF-1, which can then serve to sustain expression of genes that contain ISREs. Indeed, IRF-1-dependent gene expression in response to IFNs has been observed in a number of cases (see for example Kimura et al., 1994; Chatterjee-Kishore et al., 1998; Kano et al., 1999; Salkowski et al., 1999; Karlsen et al., 2000). This can give rise to complex patterns of gene expression whereby, for
example, IFN-γ can induce the synthesis of genes that lack GAS sites via the induction of IRF-1 (see for example Lechleitner et al., 1998; Foss & Prydz, 1999; Piskurich et al., 1999).

In contrast to the mechanism of IFN signal transduction, little is known about the mechanism of signal attenuation. Several IRF proteins, including IRF-2 (Harada et al., 1989) and the IFN-consensus sequence-binding protein (ICSBP, also called IRF-8; Nelson et al., 1993), are known to bind ISREs and negatively regulate expression, and may help to prevent expression in the absence of IFN or down-regulate the induced response. IFN-induced proteins play a major role in signal attenuation, since protein synthesis inhibitors prolong the transcription of IFN-induced genes (Friedman et al., 1984; Larner et al., 1986). One group of proteins with the potential to fulfill this role is the SOCS/JAB/SSI family, which are inducible by IFN-γ and several other cytokines and bind to and inhibit activated Jaks, leading to signal down-regulation (Endo et al., 1997; Naka et al., 1997; Starr et al., 1997; Starr & Hilton, 1999).

Activation by STAT1 is usually transient, as a result of dephosphorylation by a tyrosine phosphatase (Igarashi et al., 1993; Haque et al., 1995). However, it is not known whether the phosphatase acts on phosphorylated STATs in the nucleus or on phosphorylated Jaks or receptor subunits at the plasma membrane. The tyrosine phosphatase SHP-1 has been shown to be associated reversibly with IFNAR-1 after IFN-α stimulation (David et al., 1995) and Jak1 and STAT1 phosphorylation is increased significantly in macrophages isolated from mice that lack SHP-1 activity compared with normal control macrophages (Haque & Williams, 1998), suggesting that SHP-1 may play a role in signal attenuation. In addition to down-regulation by dephosphorylation, STAT1 is turned over by a mechanism involving proteasome-mediated degradation, but there is no evidence that this process is important in the regulation of STAT1 function (Kim & Maniatis, 1996).

3. The antiviral response

The best-characterized IFN-inducible components of the antiviral response are PKR and the 2′–5′ oligoadenylate synthetases, although it is clear that other factors may be involved, especially molecules that regulate the cell cycle or cell death and thereby limit the extent of virus replication. In many cases, IFN-inducible enzymes are inactive until exposed to virus infection, thus ensuring that uninfected cells do not suffer undue trauma. It is thought that the virus co-factor that activates these IFN-inducible enzymes is dsRNA (reviewed in Jacobs & Langland, 1996).

(i) dsRNA-dependent protein kinase R (PKR). The IFN-inducible PKR is a serine/threonine kinase with multiple functions in control of transcription and translation (reviewed in Clemens & Elia, 1997). The PKR protein has two well-characterized domains, an N-terminal regulatory domain that contains the dsRNA-binding site and a C-terminal catalytic domain that contains all of the conserved motifs for protein kinase activity (Meurs et al., 1990). PKR is normally inactive, but is activated by binding to dsRNA or other polyanions (Meurs et al., 1990; Katze et al., 1991; George et al., 1996), whereupon it undergoes a conformational change that leads to the unmasking of the catalytic domain. The active form of PKR is postulated to be a dimer, with two PKR molecules binding one molecule of dsRNA; the juxtaposed PKR molecules trans-phosphorylate each other on several serines and threonines. PKR activation is decreased when large amounts of dsRNA are present, due to saturation of dsRNA-binding sites and a shift in the equilibrium towards monomers. There are no sequence requirements for the dsRNA, although some RNAs are more potent activators than others. However, there are size requirements, with at least 50 base pairs of duplex being necessary for activation (reviewed in Robertson & Mathews, 1996).

Activated PKR has a number of important cell-regulatory activities. Firstly, it phosphorylates the α subunit of the eukaryotic translation initiation factor eIF2 and prevents the recycling of initiation factors (Meurs et al., 1992; reviewed in Clemens & Elia, 1997). In the initial step of translation, the initiator Met-tRNA is recruited to the 40S ribosomal subunit via an interaction with GTP-bound eIF2 (which consists of three subunits, α, β and γ). This complex then interacts with mRNA, other initiation factors and the large ribosomal subunit to form a pre-initiation complex, with subsequent hydrolysis of the GTP molecule bound to eIF2 and release of GDP-bound eIF2. In order to participate in another round of translational initiation, the GDP bound to eIF2 must be exchanged for GTP, a reaction that is catalysed by the guanine exchange factor, eIF2B. Phosphorylated eIF2α interacts strongly with eIF2B and traps it such that it cannot mediate the recycling of eIF2 (Ramaiyah et al., 1994; reviewed by Clemens & Elia, 1997). Since eIF2B is present in limiting amounts, translation is inhibited.

PKR also plays a role in mediating signal transduction in response to dsRNA and other ligands (reviewed in Williams, 1999). For example, the transcription factor NF-κB, which is essential for mediating induction of the IFN-β gene, is activated by PKR in response to dsRNA (see section 1). PKR has also been proposed to influence the activity of the transcription factors STAT1 (Wong et al., 1997; Ramana et al., 2000), IRF-1 (Kumar et al., 1997) and p53 (Cuddihy et al., 1999a, b), although the details of the activation events remain to be clarified. The elevated levels of PKR that would be found in a cell exposed to IFN would cause an enhancement of these signal transduction events, which may help to accelerate virus clearance. For example, enhanced activation of NF-κB activation would lead to increased cytokine, chemokine and MHC class I presentation.

PKR also aids in the clearance of virus infection by mediating apoptosis. It has been shown that dsRNA (and thus virus infection) can trigger apoptosis directly (Der et al., 1997; King & Goodbourn, 1998; Tanaka et al., 1998) and there
is considerable evidence that this effect works through PKR (Takizawa et al., 1996; Der et al., 1997; reviewed in Jagus et al., 1999; Tan & Katze, 1999), although PKR-independent mechanisms also operate for some viruses (Balachandran et al., 2000). The downstream targets for PKR-mediated apoptosis remain to be identified, but overexpression of PKR has been shown to induce apoptosis through a Bcl2- and caspase-dependent mechanism (Lee et al., 1997). Intriguingly, although mice with a targetted knockout of the dsRNA-binding domain of PKR are sensitive to virus-induced apoptosis (Yang et al., 1995), mice with a targetted knockout of the PKR catalytic domain are not (Abraham et al., 1999).

PKR also plays a role in mediating the apoptotic effects of dsRNA in an indirect manner. In this case, effects on protein synthesis are important (Srivastava et al., 1998; Gil et al., 1999), as are effects on the transcription factor NF-κB (Gil et al., 1999). Exposure of cells to dsRNA also enhances apoptosis by inducing the synthesis of Fas (Takizawa et al., 1995; Balachandran et al., 1998; Fujimoto et al., 1998) and Fas receptor (Fujimoto et al., 1998) in a manner that depends upon PKR (Balachandran et al., 1998). Finally, the apoptotic effects of TNF on promonocytic U937 cells require p53 to ensure a response to activated PKR (Yeung et al., 1996).

Although there is abundant evidence that PKR plays a major role in regulating virus infection, PKR is not sufficient to mediate the full antiviral response. Thus, mice with homozygous disruptions of the PKR gene (Yang et al., 1995; Abraham et al., 1999) still show resistance to virus infection, although the wild-type but not the PKR-deficient animals are protected to some extent by injection of dsRNA at virus doses that are normally lethal (Yang et al., 1995).

(ii) The 2′–5′ oligoadenylate synthetase system. 2′–5′ oligoadenylate synthetases are a group of enzymes that are induced by IFNs in mammalian cells and catalyse the synthesis from ATP of oligomers (three to five units) of adenosine linked by phosphodiester bonds in the unusual conformation of 2′ to 5′ (2′5′A; Kerr & Brown, 1978). The 2′5′A molecules bind with high affinity to endoribonuclease L (RNase L) and induce its activation via dimerization. Activated RNase L catalyses the cleavage of single-stranded RNA including mRNA, thereby leading to inhibition of protein synthesis (reviewed in Silverman, 1997). It has recently been demonstrated that RNase L also cleaves 28S ribosomal RNA in a site-specific manner, leading to ribosomal inactivation and thus translational inhibition (Iordanov et al., 2000). Since 2′5′A is highly labile, the activation of RNase L depends upon locally activated 2′–5′ oligoadenylate synthetase within the cell, thus ensuring that virus transcripts are destroyed preferentially over cellular mRNAs, since they are in the vicinity of the activator (viral dsRNA; Nilsen & Baglioni, 1979).

The 2′–5′ oligoadenylate synthetase/RNase L system has been suggested to play a role in the antiviral effects of IFN-α/β against vaccinia virus, reovirus and encephalomyocarditis virus (reviewed in Silverman & Cirino, 1997) and antiviral effects of IFN-γ are indeed impaired in RNase L−/− mice (Zhou et al., 1997). RNase L may also play a role in apoptosis, since RNase L−/− mice show defects in apoptosis in several tissues (Zhou et al., 1997) whilst activation of RNase L induces apoptosis (Diaz-Guerra et al., 1997). Although the exact role of RNase L in apoptosis is not clear, it seems likely that the 2′–5′ oligoadenylate synthetase/RNase L system may contribute to the antiviral activity of IFN by inducing apoptosis of infected cells (Zhou et al., 1997; Castelli et al., 1998a, b).

(iii) Alternative antiviral pathways. The IFN-inducible Mx proteins are highly conserved, large GTPases with homology to dynamin and have been found in all vertebrate species examined so far, including mammals, birds and fish (reviewed in Staehelei et al., 1993; Arnheiter et al., 1995). Mx proteins interfere with virus replication, probably by inhibiting the trafficking or activity of virus polymerases (Stranden et al., 1993), thereby impairing the growth of a wide range of RNA viruses at the level of virus transcription and at other steps in the virus life-cycle. The murine nuclear protein Mxi1 has been shown to suppress the growth of members of the Orthomyxoviridae (Staehelei et al., 1986, 1988; Haller et al., 1998). The human cytoplasmic protein MxA inhibits the growth of members of several RNA families, including the Orthomyxoviridae (Pavlovic et al., 1990, 1992; Fresé et al., 1995, 1997), Paramyxoviridae (Schneider-Schaulies et al., 1994; Zhao et al., 1996), Rhabdoviridae (Pavlovic et al., 1990), Bunyaviridae (Fresé et al., 1996; Kanerva et al., 1996) and Togaviridae (Landis et al., 1998). Mutant forms of Mx proteins lacking the ability to bind or hydrolyse GTP fail to suppress virus replication. Hefti et al. (1999) have analysed the behaviour of transgenic mice that constitutively express the human MxA gene in a mouse background lacking the IFN-α/β receptor and have shown that the MxA protein protects mice against Thogoto virus, La Crosse virus and Semliki Forest virus.

Recent studies involving the generation of mice that are triply deficient in RNase L, PKR and Mxi 1 indicate that there are additional antiviral effects of IFNs (Zhou et al., 1999). Other factors that clearly play a role in the IFN-induced antiviral response are caspases (see below) and the dsRNA-dependent adenosine deaminase (ADAR). The enzyme ADAR recognizes dsRNA as a substrate and unwinds it as a result of systematically replacing adenosines with inosine (Bass et al., 1989; Polson & Bass, 1994; O’Connell et al., 1995; Patterson et al., 1995). Since many viral RNAs go through a dsRNA-based replicative intermediate, this has the effect of being mutagenic, and there are several reports of genomic substitutions consistent with this activity (Bass et al., 1989; Cattaneo, 1994; Casey & Gerin, 1995; Hajjar & Linial, 1995; Horikami & Moyer, 1995; Polson et al., 1996). It has also been suggested that an inosine-specific ribonuclease could act in concert with ADAR to destroy modified viral RNAs (Scadden & Smith, 1997).
(iv) Antiproliferative activities of IFNs. IFNs can inhibit cell growth and thereby inhibit the replication of some viruses. However, the sensitivity of cells to the antiproliferative effects of IFNs is very cell-type dependent. For example, growth of the Daudi B cell line is arrested completely by as little as 1 unit/ml IFN-α/β, whereas many cell types are largely unresponsive at any dose tested. Because of the potential clinical importance of the cytostatic properties of IFN, the negative regulation of growth has been studied intensively and a number of aspects of this process have been described. There is evidence to support a role for PKR and RNase L in the antiproliferative functions of IFNs. The amount of PKR can vary according to the state of growth of mammalian cells in culture and this appears to correlate with the level of eIF2α phosphorylation (reviewed in Jaramillo et al., 1995), suggesting that, even in the absence of viral dsRNA, PKR can exhibit residual activity, presumably due to the presence of a cellular activator. Additionally, overexpression of PKR is growth suppressive and/or toxic in insect, mammalian and yeast cells (Koromilas et al., 1992; Chong et al., 1992; Dever et al., 1993), an effect which can also be shown to be due to eIF2α phosphorylation. Overexpression of the 40 kDa form of 2′–5′ oligoadenylate synthetase has been shown to reduce growth rates of transfected cells (Chebath et al., 1987; Rysiecki et al., 1989; Coccia et al., 1990) and expression of a dominant-negative mutant of RNase L in murine SVT2 cells inhibited the antiproliferative effect of IFN on these cells (Hassel et al., 1993).

IFNs can also exert negative regulation of the cell cycle at a more direct level. IFNs have been shown to up-regulate specifically the levels of the cyclin-dependent kinase inhibitor p21 (Chin et al., 1996; Subramaniam & Johnson, 1997; Subramaniam et al., 1998), which plays a crucial role in the progression from G1 into S phase (reviewed in Harper et al., 1993; Gartel et al., 1996). When p21 levels are elevated, cyclin-dependent kinase activity is turned off and consequently the phosphorylation of the retinoblastoma gene product (pRb) and the related pocket proteins is suppressed (Sangfelt et al., 1999). Since hypophosphorylated pRb and the related pocket proteins interact strongly with the E2F family of transcription factors, there is a consequent increase in the pRb–/pocket protein-bound E2F complexes (Iwase et al., 1997; Kirch et al., 1997; Furukawa et al., 1999). The significance of this is that free E2F is required for the transcription of many genes that are needed for transition from G1 to S phase and thus the elevation of pRb–/pocket protein-bound E2F complexes results in a block to the cell cycle.

Another major IFN-inducible activity that can act as a potent repressor of the cell cycle is the p202 gene product and related members of its ‘200 family’ (Kingsmore et al., 1989; Lembo et al., 1995; Gutterman & Choubey, 1999). The p202 product can bind both hypophosphorylated pRb (Choubey & Lengyel, 1995) and members of the E2F transcription family (Choubey et al., 1996; Choubey & Gutterman, 1997) as well as complexes containing both. The complex between E2F and p202 is unable to bind DNA and hence there is a loss of stimulation of transcription of genes important for the G1–S transition. Since the p202 protein also contains a transcriptional repression domain (Johnstone et al., 1998), any recruitment to DNA would also shut down gene expression. Finally, IFNs have been shown recently to downregulate directly the transcription of c-myc, an essential gene product that is required to drive cell cycle progression (Ramana et al., 2000).

(v) Control of apoptosis. IFNs, like other cytokines, can have either pro- or anti-apoptotic activities depending on various factors including the state of cell differentiation. For example, IFN-γ induces apoptosis of murine pre-B cells but inhibits apoptosis of chronic lymphocytic leukaemia cells (Buschle et al., 1993; Grawunder et al., 1993; Rojas et al., 1996). However, when a cell is infected with a virus, a major function of IFN is to ensure that the cell is triggered to undergo apoptosis (Tanaka et al., 1998). IFN appears to do this by inducing a pro-apoptotic state in uninfected cells (reviewed in Schindler, 1998). As discussed above, IFN-induction of PKR and the 2′5′A system plays a major role in the apoptosis response. However, IFN has also been demonstrated to induce caspase 1 (Chin et al., 1997), caspase 3 (Subramaniam et al., 1998) and caspase 8 (Balachandran et al., 2000) and thus to enhance the sensitivity of cells to virus-induced apoptosis. IFN-γ has also been shown to influence the sensitivity to apoptosis by inducing both Fas and Fas ligand (Xu et al., 1998).

(vi) Immunomodulatory functions of IFNs. Nearly all phases of innate and adaptive immune responses are affected profoundly by IFNs. All IFN family members share the ability to enhance the expression of MHC class I proteins and thereby to promote CD8+ T cell responses (reviewed in Boehm et al., 1997). In contrast, only IFN-γ is capable of inducing the expression of MHC class II proteins, thus promoting CD4+ T cell responses. IFNs play an important role in antigen processing by regulating the expression of many proteins involved in the generation of antigenic peptides to be displayed in association with MHC class I proteins. IFN-γ modifies the activity of proteasomes (reviewed in York & Rock, 1996) such that they enhance the generation of peptides that bind class I MHC proteins. In unstimulated cells, the proteasome contains the three enzymatic subunits x, y and z. However, following IFN-γ treatment of cells, the expression of the x, y and z genes is decreased and the transcription of three additional genes encoding enzymatic proteasome subunits LMP2, LMP7 and MECL1 is increased. This results in the formation of proteasomes with different substrate specificities, thereby altering the types of peptide produced and subsequently presented to the immune system. IFN-γ also increases the expression of TAP1 and TAP2, which are involved in the transfer of peptides (generated by the proteasome) from the cytoplasm into the endoplasmic reticulum to bind nascent MHC class I proteins (Trowsdale et al., 1990; Epperson et al., 1996).
Thus, IFNs enhance immunogenicity by increasing the repertoire and quantity of peptides displayed to CD8+ T cells. IFN-γ also plays an important role in regulating the balance between Th1 and Th2 cells. Firstly, it increases the synthesis of IL-12 in antigen-presenting cells (Dighe et al., 1995; Flesch et al., 1995; Murphy et al., 1995). IL-12 is the primary effector that drives developing CD4+ T cells to become Th1 cells (Hsieh et al., 1993; Trinchieri, 1995). Secondly, IFN-γ prevents the development of Th2 cells by inhibiting the production of IL-4, which is required for Th2 cell formation (Gajewski & Fitch, 1988; Szabo et al., 1995). IFN-γ also plays an important role in macrophage activation (Adams & Hamilton, 1984; Buchmeier & Schreiber, 1985; Dalton et al., 1993; Huang et al., 1993). Once activated, macrophages use a variety of IFN-γ-induced mechanisms to kill microbial targets. The most important of these mechanisms involve the production of reactive oxygen and reactive nitrogen intermediates. Reactive oxygen intermediates are generated as products of the enzyme NADPH oxidase, the assembly of which is induced by IFN-γ. Reactive nitrogen intermediates, especially nitric oxide (NO), are generated in murine macrophages as a result of the IFN-γ-dependent transcription of the gene encoding the inducible form of nitric oxide synthase (iNOS), which catalyses NO formation (MacMicking et al., 1997).

In addition to affecting humoral immunity indirectly by regulating the development of specific T helper cell subsets, IFNs can have direct effects on B cells by regulating development and proliferation, immunoglobulin (Ig) secretion and Ig heavy-chain switching. Since different Ig isotypes promote distinct effector functions in the host, IFNs can facilitate interactions between the humoral and cellular effector limbs of the immune response and increase the host defence against certain bacteria and viruses by selectively enhancing the production of certain Ig isotypes while inhibiting the production of others (Snapper & Paul, 1987; Snapper et al., 1988, 1992).

A major immunomodulatory function of IFN-α/β is to enhance the cytotoxicity of NK cells (reviewed in Reiter, 1993; Biron et al., 1999) by up-regulating the levels of perforins (Mori et al., 1998; Kaser et al., 1999). IFN-α/β also acts to stimulate the proliferation of NK cells to a limited degree, apparently via the induction of IL-15 from monocytes/macrophages (Ogasawara et al., 1998; Fawaz et al., 1999; Gosselin et al., 1999; Sprent et al., 1999). NK cells also synthesize and secrete IFN-γ in response to a combination of IL-12 and IL-15, which are released from infected monocytes/macrophages (Doherty et al., 1996; Fehninger et al., 1999). However, IFN-α/β blocks the production of IL-12 by infected monocytes (reviewed in Biron et al., 1999) and thus prevents NK cells from producing IFN-γ. The biological reasons, if any, behind this are unclear. Finally, IFN-α/β also play a role in stimulating the adaptive responses; IFN-induced IL-15 can stimulate the division of memory T cells (Tough et al., 1996; Zhang et al., 1998c; reviewed in Tough et al., 1999; Sprent et al., 1999), whilst IFN-α/β appears to be able to promote the survival of activated T cells directly (Marrack et al., 1999).

B. Virus countermeasures to the IFN response

4. Inhibition of IFN production

Viruses vary considerably in their ability to induce IFN. This may simply reflect the amounts of dsRNA produced during their replication cycles (in general, DNA viruses produce less dsRNA than RNA viruses and are therefore less potent inducers of IFN; reviewed in Jacobs & Langland, 1996) or it may reflect the fact that many viruses produce dsRNA-binding proteins as part of their life-cycle. The sequestration of dsRNA could inhibit the induction of IFN-α/β and might also act to minimize the dsRNA-dependent activation of antiviral gene products like PKR, 2′−5′ oligoadenylate synthetase and ADAR, as well as dsRNA-dependent apoptosis. For example, the reovirus major outer capsid protein σ3 is a dsRNA-binding protein (Lloyd & Shatkin, 1992). The multifunctional NS1 protein of influenza virus (Lu et al., 1995), the E3L protein of vaccinia virus (Chang et al., 1992) and products of the NSP3 gene of porcine rotaviruses (Langland et al., 1994) also bind dsRNA and a number of other viruses that have been reported to block IFN production at the transcriptional level may also do so by sequestering dsRNA [e.g. the core antigen of hepatitis B virus (HBV); Tuw & Scholemer, 1989; Whitten et al., 1991]. The sequestration of dsRNA by viral proteins might have a wider role in protecting the virus from antiviral mechanisms; dsRNA-activated PKR can activate NF-κB and induce the synthesis of immunomodulatory genes in addition to IFN-α/β.

Since the activation of NF-κB by infection is a key trigger to inducing IFN-α/β transcription and other immune responses, it would perhaps not be surprising to find that many viruses encoded inhibitors of NF-κB activation or function. Indeed, African swine fever virus (ASFV) encodes a homologue of IκB that inhibits the activity of NF-κB (Powell et al., 1996; Revilla et al., 1998). However, it is well established that NF-κB, as well as inducing proinflammatory cytokines, also induces anti-apoptotic genes (Liu et al., 1996; Wu et al., 1996; Wang et al., 1996; Van Antwerp et al., 1996; reviewed in Van Antwerp et al., 1998; Foo & Nolan, 1999) and any virus that blocks NF-κB activation may leave itself susceptible to enhanced induction of apoptosis. Interestingly, ASFV infections are indeed characterized by a significant degree of apoptosis (Oura et al., 1998). The increased risk of apoptosis associated with inhibition of...
NF-κB may be circumvented by viral gene products that act to block apoptosis; such gene products are widespread (reviewed in Cuff & Ruby, 1996; Gillet & Brun, 1996).

Another major strategy for blocking IFN-α/β production would be to target the activities of the IRF transcription factors that bind to the PRD I region of the IFN-β promoter. Intriguingly, the E6 protein of human papillomavirus type 16 (HPV-16) binds IRF-3 and can inhibit its virus-induced transcriptional activation function (Ronco et al., 1998). However, induction of IFN-β is not blocked completely by the E6 protein, suggesting that other cellular factors can substitute functionally for IRF-3, and indeed, as discussed above, there are several lines of evidence consistent with this hypothesis. The potential substitutes for IRF-3 include IRF-1 (Miyamoto et al., 1988; Fujita et al., 1989a; Watanabe et al., 1991; Reis et al., 1992; Matsuyama et al., 1993) and ISGF3 (Yoneyama et al., 1996), but these factors can themselves be targeted by virus functions. For example, IRF-1 is targeted by the K9 ORF gene product of human herpesvirus-8 (HHV-8) (Zimring et al., 1998), whilst the E7 protein of HPV-16 interacts with the p48 subunit of ISGF3 and prevents binding to DNA (Barnard & McMillan, 1999). Perhaps the plethora of factors that can bind to the PRD I region of the IFN-β promoter reflects a need of the cell to be able to circumvent virus blockades.

In addition to specific transcription factor blocks, viruses may inhibit the production of IFN by generally down-regulating host mRNA production or protein synthesis, and there is some evidence that these apparently non-specific effects can affect virus pathogenicity. For example, mutation in the gene encoding the matrix M protein of vesicular stomatitis virus (which in wild-type virus causes a general inhibition of host-cell transcription) leads to an attenuated virus with efficient IFN-β-inducing properties (Ferran & Lucas-Lenard, 1997). Similarly, the foot-and-mouth disease virus L proteinase gene encodes a protein that shuts off host-cell protein synthesis and mutation of this gene is sufficient to generate an attenuated strain that induces elevated levels of IFN-α/β (Chinsangaram et al., 1999).

Viruses may also have more subtle and indirect methods for reducing the level of IFN produced. For example, Epstein–Barr virus (EBV) produces a homologue of IL-10 (Hsu et al., 1990). Normally, IL-10 is produced by the Th2 subset of T helper cells and one of its biological functions is to inhibit the ability of monocytes and macrophages to activate Th1 cells by down-regulating the expression of class II MHC molecules. Activated Th1 cells produce a number of cytokines, including IFN-γ, that are critical for the induction of classical cell-mediated immune responses, including cytotoxic T lymphocytes. It has therefore been proposed that EBV produces the homologue of IL-10 in order to induce an inappropriate and less-effective immune response against the virus (Bejarano & Masucci, 1998). Similarly, human herpesvirus-6 may up-regulate IL-10, thereby causing immunodysregulation by causing a shift from a Th1 to a Th2 cytokine profile (Arena et al., 1999).

### 5. Inhibition of IFN signalling

There are clear advantages to viruses in having the ability to block IFN signalling. Since there are components in common between signalling pathways, it is possible for a virus to block IFN-α/β or IFN-γ signalling or both. Using such strategies, not only would the induction of cellular antiviral enzymes, such as PKR, 2′–5′ oligoadenylate synthetase and Mx, be inhibited but there would also be no up-regulation of class I MHC molecules within infected cells, making them poorer targets for cytotoxic T cells. Furthermore, virus-infected cells would be resistant to the actions of IFNs regardless of whether the IFNs were produced by infected cells or by activated leukocytes.

Blocking the IFN signalling pathways could occur at several levels and there is accumulating evidence that viruses can block at most, if not all, stages (Table 1). Several poxviruses have been shown to encode soluble IFN-receptor homologues that bind and sequester IFNs, thereby preventing their biological activity. For example, functional IFN-γ receptors are secreted by cells infected with rabbit myxoma virus, ectromelia virus, cowpox virus, camelpox virus and vaccinia virus (Upton et al., 1992; Mossman et al., 1995; Alcami & Smith, 1995). Vaccinia virus and most other orthopoxviruses also encode soluble IFN-α/β receptor homologues (Symons et al., 1995; Colamonici et al., 1995). It appears that the vIFN-α/β receptor of vaccinia virus can also bind to the surface of cells and inhibit IFN activity. Intriguingly, highly attenuated strains of vaccinia virus do not secrete the IFN-α/β receptor, consistent with its importance in virus pathogenesis. Interestingly, in terms of virus host range, both the IFN-α/β and IFN-γ receptor homologues secreted by poxviruses often have a broad species specificity, unlike their cellular counterparts.

Human cytomegalovirus (HCMV) has been shown to disrupt IFN signalling by decreasing the levels of Jak1 and p48 by a mechanism involving the proteasome (Miller et al., 1998, 1999), whereas the T antigen of murine polyomavirus (MPyV) binds to Jak1 thereby blocking the activation of the IFN-α/β and IFN-γ signalling pathways (Weihua et al., 1998). The STAT and p48 proteins that form part of IFN-inducible transcription complexes are targets for inhibition by several viruses. The V protein of the paramyxovirus simian virus 5 (SV5) targets STAT1 for proteasome-mediated degradation (Didcock et al., 1999b), thereby preventing the formation of ISGF3 and GAF complexes; indeed, at least part of the host range of SV5 appears to be determined by the ability to mediate STAT1 degradation (Didcock et al., 1999a). Surprisingly, whilst mumps virus also probably targets STAT1 for degradation (Yokosawa et al., 1998), human parainfluenza virus 2 (hPIV2) (a virus very closely related to SV5 and mumps) targets STAT2 (Young et al., 2000). As a consequence, whilst SV5 and mumps virus block both IFN-α/β and IFN-γ signalling, hPIV2 blocks only IFN-α/β signalling. Sendai virus (Didcock et al., 1999b; Yokoo et al., 1999) and hPIV3 also block IFN-α/β and IFN-γ signalling, although there was no evidence with these viruses that either
Table 1. Virus inhibition of IFN signalling and IFN-induced transcriptional responses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mechanism of action/inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Inhibition of IFN binding to cognate receptors</td>
<td></td>
</tr>
<tr>
<td>Poxviruses (many)</td>
<td>Soluble IFN-α/β receptor</td>
</tr>
<tr>
<td>Poxviruses (many)</td>
<td>Soluble IFN-γ receptor</td>
</tr>
<tr>
<td>ii. Inhibition of Jak/STAT pathway</td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>E1A decreases the levels of STAT1 and p48; sequesters the transcriptional co-activator, CBP/p300, which binds STAT1 and STAT2; interacts directly with STAT1</td>
</tr>
<tr>
<td>Ebola virus</td>
<td>Blocks IFN-α/β and IFN-γ signalling, mechanism unknown</td>
</tr>
<tr>
<td>Epstein–Barr virus</td>
<td>EBNA-2 blocks IFN signal transduction, mechanism unknown</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>Blocks IFN-α/β and IFN-γ signalling, mechanism unknown</td>
</tr>
<tr>
<td>Human cytomegalovirus</td>
<td>Reduces levels of Jak1 and p48</td>
</tr>
<tr>
<td>Human parainfluenza virus type 2</td>
<td>Blocks IFN-α/β signalling by targetting STAT2 for degradation</td>
</tr>
<tr>
<td>Human parainfluenza virus type 3 and Sendai virus</td>
<td>Block IFN-α/β and IFN-γ signalling by blocking STAT1 phosphorylation</td>
</tr>
<tr>
<td>Human papillomavirus type 16</td>
<td>E7 protein binds to p48 and blocks IFN-α/β signalling</td>
</tr>
<tr>
<td>Murine polyoma virus</td>
<td>T antigen binds to and inactivates Jak1</td>
</tr>
<tr>
<td>Simian virus and (mumps virus?)</td>
<td>V protein blocks IFN-α/β and IFN-γ signalling by targetting STAT1 for proteasome-mediated degradation</td>
</tr>
<tr>
<td>iii. Miscellaneous</td>
<td></td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Capsid protein specifically inhibits MxA gene expression, mechanism unknown</td>
</tr>
<tr>
<td>Human herpesvirus-8</td>
<td>Virus IRF homologue inhibits transcription responses to IFN-α/β and IFN-γ</td>
</tr>
</tbody>
</table>

STAT1 or STAT2 was specifically degraded. These viruses seem to prevent appropriate phosphorylation of STAT1 (Young et al., 2000; Komatsu et al., 2000). Interestingly, whereas SV5 utilizes the V protein to block IFN signalling, Sendai virus has been shown to use the C protein (Garca et al., 1999; Gotoh et al., 1999; Komatsu et al., 2000). In contrast, respiratory syncytial virus (another paramyxovirus) does not inhibit IFN signalling, although it clearly has some uncharacterized mechanism for circumventing the IFN response (Young et al., 2000). The adenovirus E1A protein can disrupt transcriptional responses to IFN-α/β and IFN-γ by decreasing the levels of STAT1 and p48 (Leonard & Sen, 1996), by sequestering the transcriptional co-activator CBP/p300, which binds STAT1 and STAT2 and is involved in transcription responses mediated by these proteins (Bhattacharya et al., 1996; Zhang et al., 1996), and by interacting directly with STAT1 (Look et al., 1998). Furthermore, the multifunctional E7 protein of HPV-16 interacts directly with p48, preventing the formation of ISGF3 and thus the activation of IFN-α/β-inducible genes (Barnard & McMillan, 1999).

HHV-8 encodes a homologue of the IRF family that represses transcriptional responses to IFN-α/β and IFN-γ; in this case, the inhibition does not appear to act at the level of IFN signalling, but rather inhibits the function of the IFN-inducible product IRF-1 (Zimring et al., 1998), thus transcriptional responses to IFN cannot be sustained. It has been reported that EBNA2 of EBV, which acts as a virus and cellular transcription factor, also inhibits IFN-α/β signalling, by an unknown mechanism that does not prevent the formation of ISGF3 complexes (Kanda et al., 1992). Ebola virus (Harcourt et al., 1998) and hepatitis C virus (HCV) (Heim et al., 1999) also block transcriptional responses to IFN-α/β and IFN-γ, although the cellular target(s) for inhibition and the viral proteins responsible have yet to be identified in these cases. It has also been reported recently that the capsid protein of HBV inhibits IFN-induction of the MxA gene (Rossmurduc et al., 1999).

Although blocking IFN signalling would seem to be of limited value to viruses in cells that had already been exposed to IFN before infection (such cells would have an established antiviral state), there is some evidence that it can still be advantageous to be able to down-regulate IFN responses. For example, although SV5 cannot initially replicate efficiently in cells that have entered an antiviral state, the ability of the virion-associated V protein to induce STAT1 degradation leads to an eventual decay of the antiviral state and subsequent virus replication (Didcock et al., 1999b). Viral proteins that require synthesis after infection might also be able eventually to inactivate an established antiviral state and permit replication, although it should be stressed that the delay in replication induced by IFN exposure would buy time for the host to mount an acquired immune response to help to resolve the infection.

Given that the immune response has co-evolved with viruses and that blocking IFN signalling seems an obvious
Table 2. Virus inhibition of IFN-induced antiviral enzymes

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mechanism of action/inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. PKR</td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Produces VA RNA that binds to but fails to activate PKR</td>
</tr>
<tr>
<td>Baculovirus</td>
<td>PK2 binds eIF2α kinases, including PKR, and blocks their activities</td>
</tr>
<tr>
<td>Epstein–Barr virus</td>
<td>Produces EBER RNA that binds to but fails to activate PKR</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>NS5A binds to and inhibits PKR; E2 also interacts with PKR and may inhibit its activity</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>ICP 34.5 redirects protein phosphatase 1 to dephosphorylate (re-activate) eIF2α; U11 blocks PKR activity</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>Down-regulates PKR by unknown mechanism; Tat and short Tat-responsive region RNA inhibit PKR</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>NS1 binds dsRNA and PKR to inhibit its activity. Influenza virus also induces cellular inhibitor of PKR (p58IPK)</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Induces the degradation of PKR</td>
</tr>
<tr>
<td>Poxviruses (many)</td>
<td>Example: vaccinia virus E3L binds dsRNA and PKR; K3L binds PKR</td>
</tr>
<tr>
<td>Reovirus</td>
<td>σ3 binds dsRNA and thus inhibits PKR (and 2′→5′ oligoadenylate synthetase)</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>NSP3 binds dsRNA and thus inhibits PKR (and 2′→5′ oligoadenylate synthetase)</td>
</tr>
<tr>
<td>ii. 2′→5′ Oligoadenylate synthetase/RNase L system</td>
<td></td>
</tr>
<tr>
<td>Various viruses</td>
<td>Produce proteins that sequester dsRNA (above)</td>
</tr>
<tr>
<td>Encephalomyocarditis virus</td>
<td>Induces RNase L inhibitor (RLI) that antagonizes 2′5′A binding to RNase L</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>2′5′A derivatives are synthesized that behave as 2′5′A antagonists</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>Induces RNase L inhibitor (RLI) that antagonizes 2′5′A binding to RNase L</td>
</tr>
</tbody>
</table>

strategy, it would be surprising if the immune system had not evolved a mechanism(s) for recognizing and eliminating cells in which IFN signalling has been blocked. Alternatively, the cell itself may have some compensatory strategy for inducing an antiviral response in cells in which the IFN signal-transduction pathway is blocked. Indeed, this may be an important function of IRF-1, which can bind to and activate many of the promoters normally activated by IFN-α/β (Pine, 1992; Henderson et al., 1997; Nguyen et al., 1997). IRF-1 levels can be raised by exposure of cells to a number of cytokines whose levels are up-regulated during infection, such as TNF-α, IL-1 and IL-6 (Fujita et al., 1989b; Harroch et al., 1994), and these potential alternative pathways to antiviral gene activation may be important survival mechanisms in the face of a blockade of IFN signalling.

6. Inhibition of IFN-induced antiviral enzymes

Many viruses encode factors that down-regulate the activity of IFN-induced antiviral enzymes such as PKR and 2′→5′ oligoadenylate synthetase; our current knowledge of these factors is summarized in Table 2 and is discussed below. (i) PKR. The importance of PKR in the induction of an antiviral state can be inferred from the wide variety of mechanisms that are employed by viruses to inhibit its activity (reviewed in Gale & Katze, 1998). As discussed above, a number of viruses encode dsRNA-binding proteins that act to minimize NF-κB activation, IFN induction and apoptosis and these proteins would also inhibit PKR. Interestingly, the dsRNA-binding proteins NS1 (Tan & Katze, 1998) and E3L (Sharp et al., 1998) also bind directly to PKR and inhibit its function, and this is also presumably true of the OV20.0L gene product of orf virus, which shares 33% homology with E3L (Haig et al., 1998). Although the NS1 protein of influenza virus is critical for its ability to overcome the IFN response (Garcia-Sastre et al., 1998; Hatada et al., 1999), influenza virus has also been reported to induce the activation of a cellular inhibitor of PKR termed p58IPK (Lee et al., 1990, 1992, 1994; Melville et al., 1997). NS1 probably also inhibits the IFN response indirectly (as discussed above) by being involved in the virus-induced shut-off of host-cell protein synthesis. Thus, NS1 regulates nuclear export of cellular mRNA (Fortes et al., 1994; Qiu & Krug, 1994) and affects pre-mRNA maturation by inhibiting splicing (Fortes et al., 1994; Lu et al., 1994) and poly-
adenylation-site cleavage (Chen et al., 1999; Shimizu et al., 1999).

In addition to binding dsRNA, viral gene products can inhibit PKR in other ways. Poliovirus induces the degradation of PKR (Black et al., 1989, 1993), HCV encodes the non-structural protein NS5A, which binds PKR directly, thus blocking its activity (Gale et al., 1997), whilst the baculovirus PK2 protein also binds PKR and inhibits its activity (Dever et al., 1998). Furthermore, the E2 protein of HCV contains sequences identical to the phosphorylation sites on PKR and eIF2α and its interaction with PKR may also contribute to the ability of HCV to circumvent the IFN response (Taylor et al., 1999). The K3L gene product of vaccinia virus has structural similarity to the N terminus of eIF2α and binds tightly to PKR, preventing autophosphorylation and hence activation of PKR and the subsequent phosphorylation of eIF2α (Davies et al., 1992, 1993; Carroll et al., 1993).

A more indirect method of overcoming the action of PKR is illustrated by the γ1, ICP34.5 protein encoded by herpes simplex virus (HSV). ICP34.5 interacts with cellular protein phosphatase 1α (PP1), redirecting it to dephosphorylate, and hence reactivate, eIF2α (He et al., 1997). A virus deleted in ICP34.5 is attenuated in normal mice but exhibits wild-type replication and virulence in PKR null mice, thereby demonstrating formally the importance of blocking the effects of PKR for HSV pathogenicity (Leib et al., 2000). The l14L protein of ASFV is a homologue of HSV ICP34.5 that contains the sequence thought to be important in its binding to PP1. However, l14L is found predominantly in the nuclei of infected cells and it is not yet clear whether it has a role in circumventing PKR activity (Goatley et al., 1999). Interestingly, HSV also encodes U8.11 (a γ2 protein), which, when expressed in mutants from an early promoter, can compensate for mutations in ICP34.5 by inhibiting PKR activity. Since U8.11 is an abundant tegument protein brought into the cells upon infection, it may act early to block phosphorylation of eIF2α. However, it appears not to be as important as ICP34.5 in preventing PKR-induced switch-off of HSV protein synthesis, and the exact role of U8.111 in the life-cycle of HSV has yet to be resolved (Mohr & Gluzman, 1996; Cassady et al., 1998).

Some viruses produce abundant short RNA molecules that inhibit PKR (reviewed in Robertson & Mathews, 1996). The adenovirus VAI transcript is an RNA molecule that can form a highly ordered secondary structure that binds avidly to the dsRNA-binding site on PKR and acts as a competitive inhibitor; the molecule is thought to be too short (160 nucleotides) to permit two molecules of PKR to juxtapose and transactivate (reviewed in Mathews, 1993, 1995). EBV also encodes two small RNAs, EBER-1 and EBER-2, that may be analogous to the VA RNAs of adenovirus. Thus, EBER-1 and possibly also EBER-2 can interfere with PKR activity (Sharp et al., 1993). Furthermore, EBER RNAs can partially complement VA-negative mutants of adenovirus (Bhat & Thimmappaya, 1985). Human immunodeficiency virus type 1 (HIV-1) also produces a short Tat-responsive region (HIV-TAR) RNA that inhibits PKR activity (Gunnery et al., 1990). However, HIV-1 also down-regulates PKR activity by an unknown mechanism (Roy et al., 1990) and the Tat protein, as well as being an activator of virus transcription, also interacts with and inhibits PKR (McMillan et al., 1995; Brand et al., 1997) by both RNA-dependent and RNA-independent mechanisms (Cai et al., 2000).

(ii) The 2′–5′ oligoadenylate synthetase/RNase L system. Since dsRNA is required to activate 2′–5′ oligoadenylate synthetase, virus proteins that sequester dsRNA, e.g. the E3L gene product of vaccinia virus (Rivas et al., 1998), inhibit both PKR and the 2′–5′ oligoadenylate synthetase/RNase L system. Several viruses also appear to have evolved strategies that specifically counteract the antiviral activity of the latter pathway. For example, during HSV type 1 and type 2 infection, 2′5′A derivatives are synthesized that behave as 2′5′A antagonists, thereby inhibiting the activation of RNase L (Cayley et al., 1984). Viruses such as HIV-1 (Martindale et al., 1999) and encephalomyocarditis virus (Cayley et al., 1982; Martindale et al., 1998) down-regulate RNase L activity by inducing the expression of the RNase L inhibitor (RLI), which antagonises 2′5′A binding to RNase L and hence prevents its activation.

Surprisingly, a number of the small RNAs produced by viruses that inhibit PKR, including HIV-TAR, adenovirus VAI and EBV EBER-1, appear to activate 2′–5′ oligoadenylate synthetase (Desai et al., 1995; Mordechai et al., 1995; Sharp et al., 1999), although the biological reasons for this are unclear.

Conclusion

The study of how viruses interact with the IFN system has told us much about virus pathogenesis and about the IFN system itself. Future studies on the molecular mechanisms that viruses have for circumventing the IFN response are likely to produce new and unsuspected insights into virus–host relationships. For example, given that viruses have co-evolved with the IFN system, it is possible that viruses have evolved subtle ways of exploiting the IFN response. In this context, it is intriguing to note that the IFN-α/β-inducible transcription factor IRF-7 may play a role in altering the pattern of latency in EBV infections (Zhang & Pagano, 2000), whilst HHV-8 can be induced from latency by IFN-γ (Chang et al., 2000).

The ability of viruses to block the IFN response may have consequences in terms of the chronic diseases caused by viruses and their treatments. Thus, IFN may be unsuccessful in the treatment of chronic virus infections because the viruses have mechanisms for circumventing the IFN response. For example, it has been suggested that IFN is ineffective as a treatment of some hepatitis C patients because the virus blocks PKR activity (Gale & Katze, 1998).

By understanding the molecular mechanisms by which viruses circumvent the IFN response, it may be possible to
identify novel antiviral drugs that work by preventing viruses from blocking specific cellular activities. Such drugs may be particularly useful in treating chronic virus-induced diseases such as persistent hepatitis B and C infections. In addition, it may be possible to generate attenuated vaccines by altering specifically the virus gene(s) that is responsible for virus inhibition of IFN function. We anticipate that research in the area of IFN–virus interactions will yield a wealth of information that has direct application to the control of virus infections.

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