

Interferons and Viruses

Signaling for Supremacy

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Abstract

Interferon (IFN)- α and IFN- β are critical mediators of host defense against microbial challenges, directly interfering with viral infection and influencing both the innate and adaptive immune responses. IFNs exert their effects in target cells through the activation of a cell-surface receptor, leading to a cascade of signaling events that determine transcriptional and translation regulation. Understanding the circuitry associated with IFN-mediated signal transduction that leads to a specific biological outcome has been a major focus of our laboratory. Through the efforts of graduate students, postdoctoral fellows, a skilled research technologist, and important collaborations with investigators elsewhere, we have provided some insights into the complexity of the IFN system—and the elegance and simplicity of how protein–protein interactions define biological function.

Key Words

Interferon
Signal transduction
Antiviral

One Receptor–Multiple Type I IFN Subtypes

IFNs, originally identified for their ability to protect cells from viral infection (1), are now recognized as pleiotropic cytokines with additional roles in cell growth regulation and as modulators of the innate and adaptive immune responses (2). They are divided into

two classes, designated type I and type II, based on affinity for and activation of either the type I receptor, IFNAR, or the type II receptor, IFNGR. The type I IFNs are evolutionarily conserved and are comprised of α subtypes (14 human, 11 mouse) and single β , ϵ , κ , τ , ζ , and ω subtypes (3). Through a comprehensive analysis of how structural features in the IFN- α/β molecules, specifically

critical clusters of amino acids, affect the sensitivity of target cells to IFN-induced biological responses, we and others have identified two regions on the exposed surface of the IFN molecule that are associated with receptor recognition (4). The biological potency of a particular IFN- α/β subtype is determined at the level of receptor recognition, dictated by the nature of the interaction between the IFN subtype and the receptor complex. IFNAR is composed of two transmembrane subunits, IFNAR1 and IFNAR2c (5–7). Additionally, the presence of specific membrane glycosphingolipid species in cells correlates with sensitivity to IFN- α : association of IFNAR1 with membrane Gal α 1-4Gal containing glycosphingolipids facilitates receptor-mediated signaling. In the absence of these glycosphingolipids IFNAR is not functional (8). The enigma as to why multiple genes encoding distinct IFN- α species exhibiting considerable amino acid identity have been conserved, given that the different IFN- α s bind to the same cell surface receptor albeit with varying affinities, persists. The survival/selection of the “fittest” gene, i.e., the gene encoding the IFN- α with the highest affinity for the receptor, would presumably have ensured optimal activation of the receptor and subsequent optimal signal transduction. To address this conundrum, we examined the promoter regions of the different human and mouse IFN- α genes and identified different signature patterns for transcription factor binding sites, implying that different inducers may differentially activate the transcription of the different IFNs (3). Most recently, we have initiated studies directed at examining the virus-inducible IFN expression profiles in response to viruses that are tropic for different cell types, both in vitro and in vivo. Preliminary data suggest that viral pressure has determined the evolutionary conservation of multiple IFN- α subtypes. Specifically, different IFN gene signature pat-

terns are induced by different viruses, and the origin of the cell type infected with a specific virus also shapes this expression profile (unpublished). Our preliminary data suggest that pathogen-specific activation of discrete signaling effectors determines which IFN subtypes are inducibly activated, and tissue/cell origin defines the spectrum of IFN subtypes that are inducible.

IFN-Inducible STAT2 Activation

The binding of an IFN subtype with high affinity to IFNAR results in receptor activation and signaling cascades that coordinately effect both transcriptional and translational regulation in the target cell. The best characterized signaling cascade is the JAK-signal transducer and activator of transcription (STAT) pathway (reviewed in refs. 2 and 9). Immediately following the IFN–IFNAR binding event, the receptor-associated JAKs, JAK1 and TYK2, are phosphorylated-activated, then phosphorylate key tyrosine residues within the intracellular domains of the receptor subunits, which serve as recruitment sites for STAT proteins. Notably, recruited STATs, which are in turn phosphorylated-activated by the JAKs, include all the STAT family members: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6, dependent on the cell type. Phosphorylated-activated STATs form homodimeric and heterodimeric complexes that translocate into the nucleus to bind DNA sequences within the promoters of IFN-sensitive genes (ISG) (Fig. 1). Activated STAT1 and STAT2 heterodimers interact with IRF9 forming the ISG factor 3 (ISGF3) complex, which binds exclusively to IFN-stimulated response elements (ISRE). Multiple STAT homo- and heterodimers, independent of IRF9, recognize and bind to distinct palindromic DNA elements, gamma-activated sequences (GAS) (Fig. 1). ISGs have either

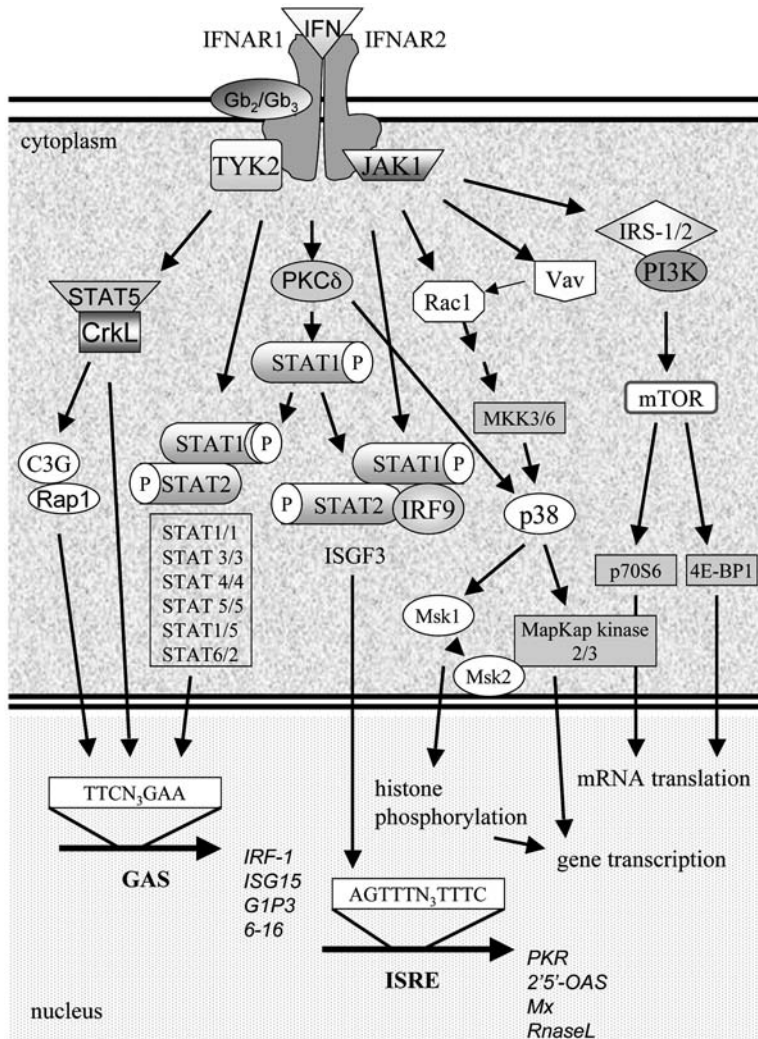


Fig. 1. Schematic representation of IFN–IFNAR activated signaling. IFN–IFNAR high-affinity interactions result in activation of multiple signaling effectors—kinases, adaptor proteins, transcription factors—that regulate transcription and translation. Adapted from ref. 2.

GAS or ISRE or a combination of both elements in their promoters. Various combinations of STAT dimers or complexes are required for optimal transcriptional activation of specific ISGs. Indeed, the nature of the specific STAT proteins expressed in distinct cell types contributes to cell-specific IFN-inducible gene expression (reviewed in ref. 10). Additionally, key residues in the DNA-

binding domains of the different STAT proteins contribute to their specificity for their cognate GAS-binding partners.

The inducible activation of STAT2 is distinctive for the IFN system, in contrast to the other STAT proteins that are activated by many and different cytokine–receptor interactions. Notably, STAT2 activation is critical for IFN- α/β inducible responses. In ISGF3, STAT1 and

IRF9 bind the ISRE, whereas STAT2 provides a potent transcriptional activation domain. Cognizant that, consistent with the other STAT proteins, STAT2 has a putative DNA-binding domain, a number of years ago we and others investigated the possibility that additional IFN-inducible STAT2-containing complexes may form that contribute to transcriptional activation. Indeed, IFN induces the formation of STAT2:STAT1 heterodimers (11,12) which preferentially bind to a GAS-like sequence (13). Using site-directed mutagenesis to substitute isoleucine residues for two critical valines at residue positions 453 and 454 within the putative DNA-binding domain of STAT2, we provided evidence for reduced chromatin binding of the IFN-inducible mutant STAT2:STAT1 heterodimer, with no effect on ISGF3 activation or ISRE binding (14). However, GAS-driven transcriptional activation is reduced in cells expressing the DNA-binding mutant STAT2, reflected in reduced IFN-inducible antiviral and growth inhibitory responses (14). The data infer that for IFN-inducible ISGF3-independent STAT2-containing complexes STAT2 binds DNA to invoke transcriptional activation of a subset of ISGs. In continuing studies, we have identified several IFN-inducible STAT2:STAT1 regulated genes, in further support of a functional role for ISGF3-independent STAT2-containing complexes in IFN-inducible biological responses.

Diverse IFN Signaling Pathways

Collectively, studies from many laboratories have enumerated the signaling effectors downstream of activated IFNAR (Fig. 1). In collaborative studies with Dr. Leonidas Platanias (Northwestern University School of Medicine, Chicago), we have identified and determined the functional relevance of a number of discrete signaling pathways.

CrkL, a cellular homolog of the *v-crk* proto-oncogene, serves as an SH2/SH3-containing

adaptor protein linking tyrosine phosphorylated receptors to downstream signaling effectors. IFNs- α , β , and ω induce the rapid and transient tyrosine phosphorylation of CrkL, mediated by IFNAR1-associated TYK2-SH2 interactions (15). CrkL interacts via its N terminus SH3 domain with the guanine exchange factor C3G that regulates activation of Rap-1, a small G protein that exhibits tumor suppressor activity. Thus, tyrosine phosphorylation of CrkL links IFNAR to the C3G-Rap-1 signaling cascade that mediates growth inhibitory responses. STAT5 interacts constitutively with IFNAR1-associated TYK2 and during IFN- α stimulation its tyrosine-phosphorylated form acts as a docking site for the SH2 domain of CrkL. CrkL and Stat5 then form a complex that translocates to the nucleus (16). This IFN-inducible CrkL-Stat5 complex binds in vitro to the TTCTAGGAA palindromic GAS element found in the promoters of a subset of ISGs. Thus, during activation of IFNAR, CrkL functions as a nuclear adaptor protein and, in association with STAT5, regulates gene transcription through DNA binding (17).

The p38 mitogen-activated protein kinases (MAP) are serine-threonine protein kinases activated in response to cellular stress, proinflammatory cytokines, and growth factors. p38- α MAPK is rapidly phosphorylated and activated following treatment of cells with IFN- α/β (18). The small GTPase Rac1 is activated in a type I IFN-dependent manner and its function is required for downstream engagement of the p38 MAPK pathway (19). In hematopoietic cells, type I IFN activation of IFNAR1 results in the tyrosine phosphorylation of Vav (20), a guanine-exchange factor, followed by Vav-mediated GDP/GTP exchange of Rac1, leading to the activation of p38 MAPK. In other non-hematopoietic cells, IFN- α activates both MAP kinase kinase 3 (MKK3) and MAP kinase kinase 6 (MKK6) (21). Such IFN-inducible activation of MKK3

and MKK6 is essential for downstream phosphorylation and activation of p38 MAPK. Subsequently, p38 MAPK regulates induction of the catalytic domains of MapKap kinase-2 and MapKap kinase-3 (22) and activation of Msk1 (22). Engagement of Msk1 may provide a potential mechanism by which gene transcription is regulated by IFNAR activation, as Msk1, and the related Msk2, have been implicated in regulation of histone phosphorylation, chromatin remodeling, and induction of transcription of early response genes in response to stress (23). The engagement of this p38 MAPK pathway, therefore, also plays a critical role in IFN-dependent transcriptional regulation. Interestingly, inhibition of the kinase activity of p38 MAPK blocks IFN- α -induced gene transcription (18,19) without inhibiting DNA binding or tyrosine phosphorylation of STAT proteins, implying that the p38 pathway acts in cooperation with the STAT pathway.

In other studies we have shown that the insulin-receptor-substrate (IRS) pathway also operates independently of the JAK-STAT pathway. Certainly, IFN-inducible JAK1 activation results in the tyrosine phosphorylation of IRS1 (24) and IRS2 (25), yet at this point the JAK-STAT and IRS pathways diverge. The phosphorylated IRS proteins provide docking sites for the SH2 domain of the p85 regulatory subunit of the phosphatidylinositol 3 kinase (PI3K). This p85-IRS interaction activates both the phosphatidylinositol and serine kinase activities of the p110 catalytic subunit of PI3K (26,27). Subsequently, the serine-threonine protein kinase mammalian target of rapamycin (mTOR) is activated, and in turn mediates both the activation of the p70S6 kinase and the inactivation of 4E-BP1 (28). Activated p70S6 kinase phosphorylates the 40S ribosomal S6 protein on serine residues, enabling mRNA translation. Inactivation of the repressor 4E-BP1 effects its dis-

sociation from eukaryotic translation initiation factor (eIF)-4E, thereby enabling translation initiation. Thus, IFN-inducible activation of the IRS-PI3K signaling cascade serves to complement JAK-STAT and p38 mediated transcriptional activation, effecting translation of ISGs.

As described, IFN-dependent tyrosine phosphorylated-activated STATs translocate to the nucleus to regulate gene transcription. In addition to tyrosine phosphorylation, phosphorylation of STAT1 on serine 727 is essential for induction of its transcriptional activity. Distinct from the serine-threonine protein kinases PI3K and mTOR is the protein kinase C (PKC) family of proteins. Several years ago we provided evidence that PKC- δ is activated during engagement of IFNAR and associates with STAT1 (29). Such an activation of PKC- δ appears to be critical for phosphorylation of STAT1 on serine 727, as inhibition of PKC- δ activation diminishes the IFN- α/β -dependent serine phosphorylation of STAT1. In addition, treatment of cells with the PKC- δ inhibitor rottlerin or the expression of a dominant-negative PKC- δ mutant results in inhibition of IFN- α/β -dependent gene transcription via ISRE or GAS elements. Interestingly, PKC- δ inhibition also blocks activation of p38 α MAPK, suggesting a dual mechanism by which PKC- δ participates in the generation of IFN- α/β responses.

IFN- α/β -IFNAR activation results in the activation of multiple signaling effectors and pathways that coordinately invoke gene and protein regulation in target cells to create an antiviral response. Activation of the JAK-STAT pathways is critical for control of viral replication. Notably, IFN-induced PI3K activation that mediates protection from cell death brought about by encephalomyocarditis virus (EMCV) or herpes simplex virus (HSV-1) infection does not involve activation of the STAT pathway (30). Additionally, the p38 MAPK pathway further

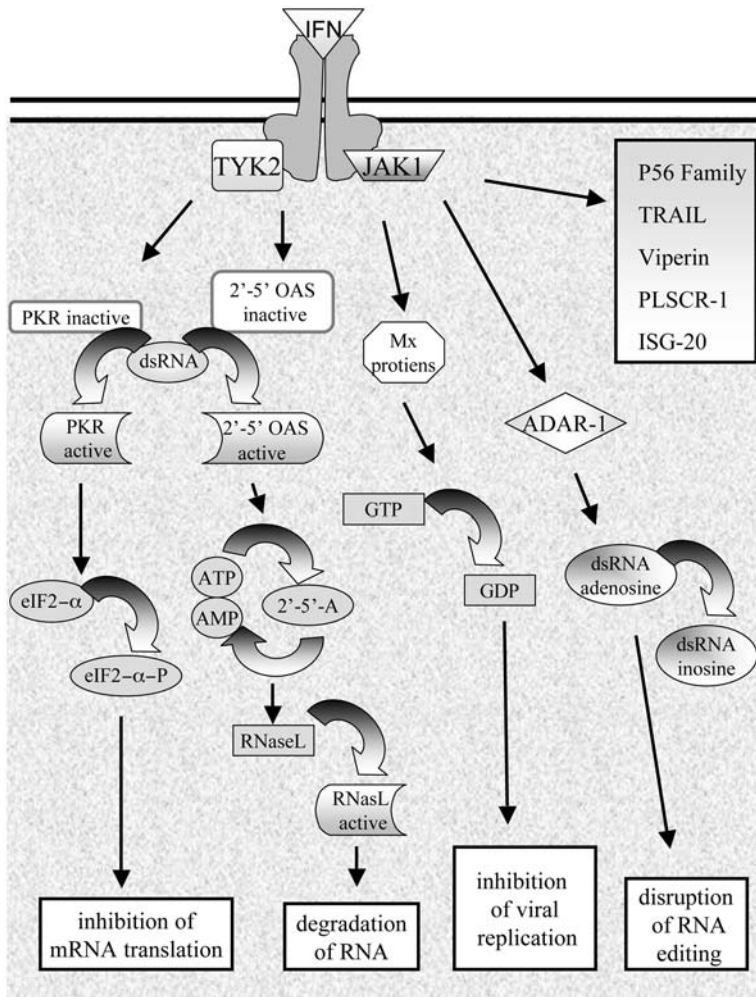


Fig. 2. Schematic representation of IFN-inducible effectors and targets involved in an antiviral response. IFN-induced signaling results in the transcriptional activation of ISGs whose protein products mediate antiviral activities: PKR inhibits the initiation of translation through phosphorylation of eIF2- α . 2'-5' OAS and RNaseL mediate RNA degradation. Mx GTPases inhibit viral assembly and ADAR-1 disrupts RNA editing. Members of the P56 family, including TRAIL, viperin, PLSCR-1, and ISG-20 contribute to the antiviral effects of IFN. Adapted from ref. 2.

contributes to IFN-inducible antiviral protection. Figure 2 provides a summary of many of the IFN-inducible effectors of an antiviral response, reviewed in ref. 2. Clearly, transcriptional activation of specific ISGs and translational regulation of targeted proteins defines the antiviral response to a particular virus.

Dependent on the virus and the target cell, the IFN- α/β -mediated inhibition of viral infection will be directed at viral entry, viral uncoating, DNA/RNA replication and editing, RNA translation, protein assembly, and/or viral egress. Additionally, IFNs mediate protection from virus infection either by limiting

virus-induced cell death or, conversely, invoking apoptosis of infected cells to prevent viral replication.

IFNs as Critical Effectors of an Innate Immune Response

The importance of IFN- α/β activation of IFNAR and subsequent signaling events to antiviral protection is underscored by viral challenge experiments in mice with targeted disruption of IFN- β (31,32), IFNAR1 (33–36), STAT1 (34,37–40), STAT2 (41), and TYK2 (42). Quite distinct from their direct antiviral effects, IFN- α/β are critical components of both the innate and adaptive immune responses to viral infection.

In the context of immune cells, type I IFNs influence T cell activation, by upregulating cytokines that influence T cell responses, by enhancing MHC class I expression, by influencing the expression levels of chemokines that are chemoattractant for T cells, and by complementing TCR-mediated signaling through IFNAR-mediated signaling effectors such as IRS proteins. IFN- α/β regulate NK cell functions, and influence the maturation and/or survival of dendritic cells, TH1 cells and B cells (reviewed in ref. 2).

Studies with IFNAR1^{-/-} mice revealed that these mice were highly susceptible to cardiotropic coxsackie virus B3 (CVB3) infection (36), suggestive of a protective role for type I IFNs against CVB3 pathogenesis and viral spread. Notably, results from in vitro studies identified IFN- β as exhibiting superior antiviral activity against CVB3 compared with IFN- α (43). Although studies with the IFNAR1 null mice have clearly identified the importance of the type I IFN system to protection from microbial pathogens, the contribution of individual subtypes, or even the IFN- α s vs IFN- β , cannot be addressed in a mouse that is non-responsive for all type I

IFNs. Accordingly, we have undertaken studies to address the specific in vivo functions of IFN- β , using an IFN- β null mouse generated by a former postdoctoral fellow in our group, Dr. Raj Deonarain. To distinguish the role of IFN- β from IFN- α , we examined the course of CVB3 infection in IFN- β ^{-/-} mice. We observed that CVB3 infection is more aggressive in IFN- β ^{-/-} compared with mice expressing IFN- β , with exacerbated cardiomyopathies and an incomplete IFN response to virus infection, as measured by reduced ISG expression in heart tissues (32). In other studies evidence was provided that mice null for IFN- β exhibit increased susceptibility to intranasal infection with vaccinia virus, succumbing to infective doses that are sublethal for mice expressing IFN- β (31). The data suggest that failure to invoke virus-inducible transcriptional activation of IFN- β results in a blunted IFN- α response.

A specific focus of our studies with the IFN- β null mice has been immune cell development and activation. IFN- β ^{-/-} mice exhibit defects in lymphoid development and myelopoiesis (32). Despite no abnormalities in the proportional balance of CD4 and CD8 T cell populations in the peripheral blood, thymus, and spleen of IFN- β null mice, activated lymph nodes and splenic T lymphocytes exhibit enhanced T cell proliferation and decreased TNF- α production relative to mice expressing IFN- β . Constitutive and induced expression of TNF- α are also reduced in the spleen and bone marrow macrophages, respectively, of IFN- β null mice. We observe an altered splenic architecture in IFN- β null mice and a reduction in resident macrophages. We identified a potential defect in B cell maturation in IFN- β null mice. Circulating IgM-, Mac-1-, and Gr-1-positive cells are also substantially decreased in IFN- β null mice. The decrease in the numbers of circulating macrophages and granulocytes likely reflects

defective maturation of primitive bone marrow hematopoiesis, specifically myelopoiesis that we detect.

Using an experimental autoimmune encephalitis (EAE) mouse model of human multiple sclerosis, we observe earlier onset and more severe disease in the IFN- β null mice, consistent with published data (44). Notably, in agreement with our findings of an enhanced proliferative response of T cells from IFN- $\beta^{-/-}$ mice to non-specific stimulation, we observe that memory T cells from peptide immunized IFN- $\beta^{-/-}$ mice re-challenged ex vivo with disease-inducing MOG peptide exhibit a greater proliferative response than those from the immunized IFN- $\beta^{+/+}$ mice (unpublished).

IFNs and Emerging Infectious Diseases

Viewed altogether, the implications from our in vitro and in vivo studies are that type I IFNs influence at least two facets of an antiviral response: (1) IFN- α/β inhibit virus infection directly, through IFNAR-activated signaling events that invoke targeted inhibition at multiple stages in the replicative cycle of different viruses, and (2) IFN- α/β influence immune cell development as well as activation, thereby contributing to shaping an immune response involved in viral clearance.

Severe acute respiratory syndrome (SARS) emerged as an international health crisis in 2003, with the SARS Urbani coronaviruses (CoV) identified as the etiologic agent. In response to the outbreak in Toronto, we undertook a clinical evaluation of IFN- α therapy in SARS patients. IFN alfacon-1 is a synthetic protein designed as a consensus of the more than 14 IFN- α subtypes, and exhibits superior bioactivity both in vitro and in vivo (45,46). To provide information on the potential therapeutic benefit and tolerability of IFN alfacon-1 for SARS, we conducted an open-

label study of 22 SARS patients in Toronto. IFN alfacon-1 treatment was associated with reduced disease-associated impairment of oxygen saturation, more rapid resolution of radiographic lung abnormalities, and lower levels of creatine kinase, a correlate of disease severity (47). To understand the mechanism of action of IFN alfacon-1 in limiting SARS-CoV infection in humans, we have undertaken a series of in vitro studies using a murine hepatitis coronavirus, MHV-1. We have evidence that MHV-1, when injected intranasally into mice, will induce a lung infection indistinguishable from that of the SARS-CoV infection in humans (Levy et al., unpublished). Specifically, A/J mice are highly susceptible to intranasal infection with MHV-1, develop pulmonary disease characterized by marked pulmonary congestion, hyaline membranes, interstitial thickening, and inflammation and alveolar exudates similar to SARS lung disease in humans. C3H murine L2 lung fibroblast cells are permissive for MHV-1 infection and IFN- α treatment protects from virus-inducible cytopathic effects in a dose-dependent manner (48). Using pharmacological inhibitors that specifically target IFN-inducible signaling effectors, we have evidence that JAK1, PKC δ , and p38 MAPK play important roles in IFN- α -mediated anti-MHV-1 activity. At the lower doses of IFN- α , pretreating the cells with JAK 1 inhibitor completely abrogated the protective effects of IFN- α . Because JAK1 activation is an early post-receptor activation event, it is not surprising that abrogating this upstream effector in the IFN-receptor signaling cascade has a profound effect on subsequent downstream signaling events and biological responses. By contrast, at the higher doses of IFN- α , inhibition of PKC δ inhibited IFN-induced antiviral activity to a greater extent, implying that STAT1-sensitive ISG activation is necessary for IFN-inducible anti-MHV-1 activity. Further-

more, we demonstrated that L2 cells treated with IFN- α induced the transcriptional activation of two ISGs associated with an antiviral response, namely *PKR* and 2'5'-*OAS*. Inhibition of IFN-induced activation of JAK1, PKC δ and p38 MAPK were shown to inhibit the transcriptional activation of *PKR* and 2'5'-*OAS* and abrogate the protective effects of IFN- α against MHV-1 infection (48).

A Signaling Paradox

The objectives of all viruses are to infect target cells, replicate large numbers of progeny virus, and spread these progeny to initiate new rounds of infection. Over time, viruses have evolved strategies to evade the host response to infection. An obvious target has been the type I IFN system, with many viruses encoding some form of IFN antagonist (49,50). Indeed, in order to escape activation of the IFN system, the SARS-CoV appears to block a step after the early nuclear transport of IRF-3, the transcription factor essential for IFN- β and IFN- α 4 promoter activity (51).

Chemokines and their receptors are critical for the clearance of infectious pathogens. Specifically, chemokines are implicated in directing lymphocyte trafficking to sites of infection and in activating the effector functions of these immune cells to eliminate infectious pathogens (52). Chemokines exert their activities through the engagement of specific seven-transmembrane G protein-coupled receptors. Viruses, in turn, have evolved various defences against chemokines. These defences range from the production of antagonists to either the chemokine or the chemokine receptor, to the co-opting of chemokine receptors for viral entry. In recent years, our laboratory has become interested in how poxviruses, specifically myxoma virus and vaccinia virus (VACV), have hijacked the

chemokine receptor CCR5 to invoke a permissive environment for viral replication.

Myxoma and VACV are members of the Chordopoxvirinae subfamily and Poxviridae family (reviewed in ref. 53). A comprehensive analysis of poxvirus genomic sequences has identified a number of virus encoded immunomodulatory genes, including soluble cytokine binding proteins, serpins, chemokine binding proteins, a complement control protein and members of the semaphorin and Toll/IL-1 receptor families (reviewed in ref. 54).

It has become apparent that chemokine receptors play a critical role in the transmission and pathogenesis of specific viral infections (55–57): CCR5 and CXCR4 serve as entry cofactors for HIV. Putative seven transmembrane proteins resembling chemokine receptors have been found in a number of viral genomes. CMV encodes several candidate chemokine receptors—US28, US27, and UL33. The herpes saimiri virus open reading frame (ORF) 74, ECRF3, binds CXCL8. Human herpesviruses 6 and 7 encode two chemokine receptors, U51 and U12. The Kaposi's sarcoma-associated human herpesvirus 8 ORF 74 encodes a constitutively active G protein-coupled receptor that binds chemokines including CXCL8. The poxviruses capripox, swinepox, and myxoma virus encode chemokine receptor homologs. Presumably, these virally encoded chemokine receptor homologs function to subvert chemokine binding to cell surface receptors, thereby precluding chemokine-mediated clearance of infectious virus.

A number of years ago, in collaboration with Dr. Grant McFadden's group at the University of Western Ontario, we followed up on their observations that exposure of cultured rabbit cells to CCL5 (RANTES) substantially reduced cellular infection by myxoma virus, and that ectopic expression of human chemokine receptors CCR1, CCR5, and

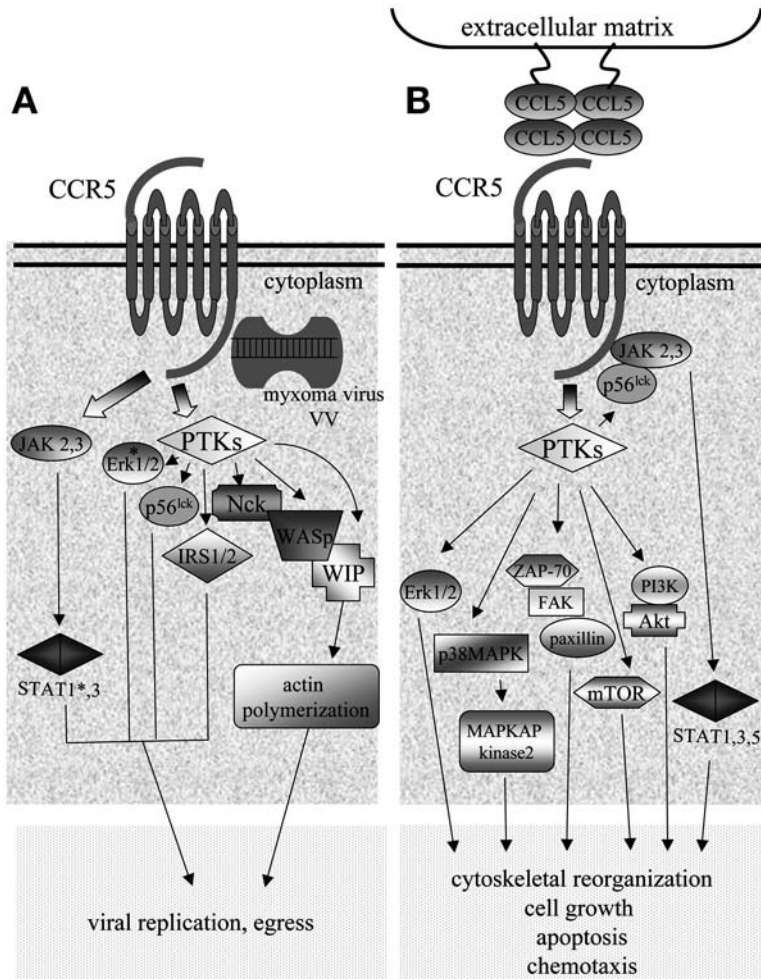


Fig. 3. Schematic representation of CCR5 mediated signaling. Activation of CCR5 by myxoma virus/VACV (A) or CCL5 (B) leads to various tyrosine phosphorylation events which culminate in either a permissive environment for viral replication, or an activated cell. (*) Erk1/2/STAT1 signaling induced by myxoma virus leads to a restrictive infection in mouse embryonic fibroblast cells (62), whereas vaccinia virus activation of Erk1/2 leads to permissive infection.

PTK, protein tyrosine kinase; Nck, non-catalytic region of tyrosine kinase; WASp, Wiskott–Aldrich syndrome protein; WIP, WASp interacting protein; ZAP-70, zeta chain associated protein kinase-70; FAK, focal adhesion kinase.

CXCR4 rendered non-permissive mouse cells susceptible to myxoma virus infection (58). Because CCL3 (MIP-1 α) and CCL4 (MIP-1 β) did not affect myxoma virus infectivity, the implications were that myxoma virus may preferentially “engage” CCR5 epitopes that are distinguished by CCL5–CCR5 interac-

tions. At the outset, we investigated whether myxoma virus infection of cells expressing CCR5 resulted in activated signaling effectors. Myxoma virus induces rapid tyrosine phosphorylation of CCR5, the association of CCR5 with JAKs and p56^{lck} and their phosphorylation-activation within minutes of virus adsorp-

tion. Additionally, we provided evidence for myxoma virus-inducible STAT and IRS activation (Fig. 3A) (59). Notably, CCL5 activation of CCR5 results in the rapid tyrosine phosphorylation-activation of CCR5, JAK2, and JAK3, the phosphorylation-activation of STAT1 and STAT3, and the formation of STAT1:STAT1 and STAT1:STAT3 dimers that exhibited DNA-binding activity (60,61). Phosphorylated JAK2 associates with phosphorylated CCR5 (Fig. 3B). CCL5-inducible JAK phosphorylation is insensitive to pertussis toxin inhibition, targeted at G_{ci} protein signaling, indicating that CCL5–CCR5 mediated tyrosine phosphorylation events are not coupled directly to G_{ci} protein mediated signaling events. Whereas CCR5 activation effected by HIV Env protein *is* inhibitable by pertussis toxin, myxoma virus-inducible phosphorylation events, as for CCL5–CCR5 stimulated signaling events, are not affected by pertussis toxin treatment. Given that myxoma virus infection of CCR5-expressing cells is blocked by herbamycin A and the JAK2 inhibitor, tyrophostin AG490, viral infectivity may be dependent on non-G protein–coupled signal transduction pathways triggered by the infecting myxoma virus particle.

We have extended these studies to examine VACV activation of CCR5. As for myxoma virus, the exact mechanism of VV binding and entry into cells is not known. Importantly, myxoma virus and VACV enter both permissive and restrictive cells, yet a post-entry event determines permissiveness for viral replication. We have evidence for VACV-inducible phosphorylation-activation of the signaling effectors JAK2, IRS-1, and Erk1/2 in permissive cells expressing CCR5 (62). Knockdown of CCR5 expression abrogates viral replication and these signaling events. As for myxoma virus, VACV infection is blocked by herbimycin A and tyrophostin AG490, but not by pertussis toxin, indicating

that VACV infectivity is also dependent on tyrosine phosphorylation and not G protein–coupled signaling events.

Our data highlight an interesting inconsistency, that IFN-induced activation of the JAK/STAT and IRS/PI3K pathways confers a protective phenotype to virus infections, while myxoma and VACV-induced activation of the same pathways, albeit mediated by a different receptor, lead to a permissive environment for viral infectivity. This apparent contradiction is confounded further when one considers that activation of an IFN response determines a restrictive environment for poxvirus infection (63,64), and poxviruses encode IFN antagonists, specifically to disrupt an IFN response (65–67). In ongoing studies we are investigating the downstream targets of these signaling pathways in poxvirus-infected cells, to discern which additional signaling effectors and networks distinguish the eventual biological response—viral replication or inhibition.

Conclusion

This review highlights our multifaceted approach to understanding how cell surface receptor activation results in a network of signal transduction pathways that converge to invoke specific biological outcomes, for the IFN–IFNAR interaction, an antiviral response. It is becoming clear that viral pressures have influenced the evolutionary preservation of multiple IFN- α subtypes, and that ongoing IFN evasion strategies by viruses include commandeering aspects of IFN-inducible signaling networks through activation of distinct cell surface receptors for viral advantage. Our future objectives are directed at understanding how signaling pathways and their effectors determine the equilibrium between a restrictive environment for viral replication and a permissive one.

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