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MINIREVIEW

Human Cytomegalovirus Inhibition of Interferon Signal Transduction

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Cytomegalovirus (CMV), a beta-herpesvirus with worldwide distribution, exhibits host persistence, a distinguishing characteristic of all herpesviruses. This persistence is dependent upon restricted gene expression in infected cells as well as the ability of productively infected cells to escape from normal cell-mediated anti-viral immunosurveillance. Type I (IFN-α/β) and type II (IFN-γ) interferons are major components of the innate defense system against viral infection. They are potent inducers of MHC class I and II antigens and of antigen processing proteins. Additionally, IFNs mediate direct antiviral effects through induction of effector molecules that block viral infection and replication, such as 2', 5-oligoadenylate synthetase (2, 5-OAS). IFNs function through activation of well-defined signal transduction pathways that involve phosphorylation of constituent proteins and ultimate formation of active transcription factors. Recent studies have shown that a number of diverse viruses, including CMV, EBV, HPV, mumps and Ebola, are capable of inhibiting IFN-mediated signal transduction through a variety of mechanisms. As an example, CMV infection inhibits the ability of infected cells to transcribe HLA class I and II antigens as well as the antiviral effector molecules 2, 5-OAS and MxA I. EMSA studies have shown that IFN-α and IFN-γ are unable to induce complete signal transduction in the presence of CMV infection, phenomena that are associated with specific decreases in JAK1 and p48. Viral inhibition of IFN signal transduction represents a new mechanistic paradigm for increased viral survival, a paradigm predicting widespread consequences in the case of signal transduction factors common to multiple cytokine pathways.

***Key words*:** human cytomegalovirus, interferon signal transduction

Cytomegalovirus (CMV) is a ubiquitous beta-herpesvirus that has the distinguishing characteristic of all herpesviruses, the ability to persist in the host. Persistent CMV causes serious infections such as interstitial pneumonia, gastrointestinal mucosal ulceration, retinitis, and hepatitis in immunosuppressed patients. Elucidating mechanisms of CMV persistence is critical to a complete model of pathogenesis as CMV infection, morbidity, and mortality often are the result of dissemination of virus acquired prior to the onset of immunosuppression (6, 55).

The response to viral infection involves cellular and cytokine components of the host innate and adaptive immune system. However, viruses have developed protean means of subverting the host immune response (4, 56, 57, 61). This has been an explosive area of research that has revealed striking examples of the diverse strategies CMV has evolved to counteract host immunity.

The MHC is the ultimate interface between virus and adaptive immunity. $CD8^+$ and $CD4^+$ T lymphocytes recognize peptides derived from viral proteins presented in the context of MHC class I and class II molecules, respectively (11). This triggers the cell-mediated immune system armamentarium wherein virus-specific T lymphocyte clones proliferate and mediate cytolysis of virally infected cells and/or release cytokines that inhibit viral replication or recruit T cells and monocytes to amplify the antiviral response (11).

The interferons (IFNs) are potent stimulators of MHC expression and antigen presentation. Type I IFNs (IFN-α/β) induce MHC class I expression whereas Type II IFNs (IFN-γ) induce MHC class I and class II expression as well as components of the antigen processing machinery (3, 15, 52). Moreover, IFNs mediate direct antiviral effects through several distinct effector molecules that

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block viral infection or replication (27, 28).

It is increasingly evident that viruses have evolved mechanisms to inhibit MHC expression and IFN-mediated antiviral effects. Herein, we review CMV-mediated disruption of the IFN signal transduction pathway.

The role of IFNs in antiviral immunity and IFN-stimulated gene expression

In the early 1950s, Isaacs and Lindenmann discovered a family of proteins capable of interfering with viral replication, the interferons (IFNs) (23). Early investigations revealed IFNs rapidly induce the transcription of previously quiescent genes without the necessity of protein synthesis, i.e. immediate-early gene expression (8). IFNs mediate three major classes of biological activity; antiproliferative, antiviral, and immunoregulatory effects (8, 9, 29, 40, 48).

Type I (IFN-α/β) and type II (IFN-γ) interferons are major lines of defense against viral infection. IFNs mediate direct antiviral effector mechanisms that inhibit multiple steps of viral replication (47, 54). For example, 2,5-oligoadenylate synthetase (2',5'-OAS) activates ribonuclease L which degrades mRNA and limits the accumulation of viral transcripts. Protein kinase R blocks translation of viral transcripts by phosphorylating translation initiation factor eIF-2. Mx proteins block influenza, vesicular stomatitis virus, and herpes simplex virus replication by an unknown mechanism (47, 54).

IFNs are critical regulators of the antiviral immune response. IFNs are potent stimulators of antigen presentation thereby augmenting cell mediated antiviral immune responses. Type I IFNs up-regulate MHC class I protein expression, and type II IFNs up-regulate MHC class I and II (3, 8), Moreover, IFNs up-regulate components of the antigen processing machinery, such as proteasome expression and function (38).

There are *in vivo* and *in vitro* data supporting the critical role of the IFNs in anti-CMV immunity. Intramuscular IFN-α reduces the replication of MCMV in the spleen and liver of mice and IFN-α receptor knockout mice are 800-fold more susceptible to MCMV infection than their wild type litter mates (46, 58). Numerous *in vitro* studies demonstrate that pretreating cells with IFN-α inhibits human HCMV replication by decreasing transcription of the immediate-early (IE) HCMV gene products (16, 39). The antiviral effects of IFN also extend to clinical therapy, as IFN-α treatment significantly reduces the incidence of serious HCMV infections in seropositive renal transplant recipients (22).

Studies of IFN-γ in MCMV models demonstrate that IFN-γ is a critical cytokine in controlling acute and chronic CMV infection (2, 46). IFN-γ accounts for the majority of natural killer (NK) cell-mediated antiviral effects during acute infection (26, 44). Neutralization of IFN-γ prevents MCMV clearance from salivary glands and impairs control of MCMV infection and IFN-γ depletion increases MCMV titers in the liver and spleen (24, 37, 44). MCMV infection in IFN-γ receptor knockout mice leads to an uncontrolled persistent infection resulting in a severe vasculitis of large arterial vessels (46). Moreover, pre-treatment of diverse cell types with IFN-γ inhibits HCMV replication (10, 16, 19, 36, 50) and restores HCMV antigen processing and presentation to HCMV specific $CD8^+$ T lymphocyte clones (10, 16, 19-21, 36, 50).

Animal models demonstrate that the IFN stimulated JAK/STAT signal transduction pathway is critical for controlling CMV infections. STAT1 knockout mice and IFN-α receptor/IFN-γ receptor double knockout mice, which are deficient in IFN-stimulated signal transduction and biological responses, are exquisitely sensitive to viral infection (12, 46). In these mice, acute MCMV infection proceeds unchecked and rapidly leads to death.

Interferon Signal Transduction

IFN-γ binds to the heterodimeric interferon-γ receptor (IFN-γR), which is associated intracellularly with the Janus kinases (JAKs), JAK1 and JAK2 (8). Binding of IFN-γ to its receptor initiates JAK1- and JAK2-mediated tyrosine phosphorylation of the cytoplasmic tail of the IFN-γR (30, 43, 45). The phosphorylated IFN-γR serves as a docking site for latent cytoplasmic STAT-1 (8). After docking at the receptor, STAT-1 is phosphorylated by the JAKs and forms a homodimer through cognate phosphotyrosine-SH2 domain interactions (5, 7, 8). STAT-1 homodimers translocate to the nucleus where they bind IFN-γ activation sequence (GAS) elements present in the promoters of IFN-γ inducible genes (5, 8).

IFN-α signal transduction shares similar signaling molecules and basic signal transduction mechanisms as IFN-γ. IFN-α binds to its receptor, which stimulates the activation of kinases JAK1 and TYK2 (a JAK family kinase specific for IFN-α signaling) (33). STAT1 and STAT2 molecules are tyrosine phosphorylated by JAK1 and TYK2. Phosphorylated STAT1/STAT2 heterodimers unite with p48 to form the transcription factor complex ISGF3, which binds to the IFN stimulated response element (ISRE) sites in many IFN-α responsive promoters (14, 34, 49). Alternatively, phosphorylated STAT1 homodimers and STAT1/STAT2 heterodimers can translocate to the nucleus and bind to elements such as the inverted repeat (IR) element of the interferon regulatory factor-1 (IRF-1) gene to activate transcription in an ISGF3-*independent* manner (17, 35).

Cytomegalovirus inhibition of IFN stimulated gene expression and antiviral responses

HCMV infection blocks IFN-γ and IFN-α stimulated MHC expression. The defect in IFN induced MHC expression occurs proximal to IFN stimulated transcription factor activation and gene expression. Given that IFN-γ

and IFN-α stimulated responses are disrupted at a similar level, it is likely that they share a common defect in their signal transduction pathways.

Interferon-γ: Detailed investigation of the IFN-γ signal transduction revealed a lesion in this pathway that results in a complete block in IFN-γ signaling (41). EMSA experiments demonstrated that IFN-γ does not induce STAT-1 homodimer formation in HCMV infected cells suggesting that there is a disruption in either the phosphorylation of STAT-1 molecules or a decrease in STAT-1 levels or another component of JAK/STAT signal transduction. Immunoprecipitation experiments revealed that IFN-γ stimulates tyrosine phosphorylation of JAK1, JAK2, IFNγR, and STAT-1 is blocked in infected cells. Moreover, JAK1 protein was not detected in HCMV infected cells (41). JAK1 is a Janus family protein tyrosine kinase that is required for IFN-γ stimulated tyrosine phosphorylation, STAT-1 homodimer formation, and IFN-γ stimulated gene expression and biological responses (43). The HCMV mediated decrease of JAK1 protein levels disrupts the IFN-γ stimulated activation of the signal transduction pathway and the subsequent downstream responses such as STAT-1 homodimer formation and MHC induction. The decrease of JAK1 protein may be the lesion responsible for the lack of IFN-γ stimulated MHC class I, MHC class II, β_2-microglobulin, LMP2, LMP7, TAP1, and TAP2 expression in HCMV infected cells.

Further analyses of JAK1 expression in HCMV infected cells reveal that JAK1 levels are decreased posttranslationally by a subset of HCMV genes. Steady state JAK1 mRNA levels are not altered in the course of HCMV infection despite decreased JAK1 protein at 48 and 72 hours after infection (41). Moreover, treatment of HCMV infected cells with carboxybenzyl-leucyl-leucyl-leucine vinyl sulfone (Z-L_3VS), a specific inhibitor of the proteasome, reverses the decrease of JAK1 protein in HCMV infected cells, suggesting that JAK1 protein levels are reduced by a proteasome-dependent mechanism. In addition, treatment of infected cells with phosphonoacetic acid (PFA), which inhibits HCMV late gene expression, has no effect on the HCMV-mediated block in IFN-γ signal transduction suggesting that HCMV IE and/or E genes reduce JAK1 expression (41).

Interferon-α: JAK1 is an essential component of type I IFN signaling, therefore decreased JAK1 protein would inhibit IFN-α stimulated signal transduction, transcription factor activation, and gene expression and is likely to represent a common lesion in IFN signal transduction that blocks both IFN-γ and IFN-α stimulated responses in HCMV infected cells. However, differential display experiments in HCMV infected cells at 8 hours after infection have shown that a constituent of the viral particle, independent of the IFN signal transduction pathway, upregulates a subset of IFN responsive genes (60). Therefore, experiments were performed to directly test the integrity of IFN-α stimulated responses in HCMV infected cells.

Gene expression experiments revealed that HCMV blocks IFN-α stimulated ISGF3-dependent (MHC class I, 2',5'-OAS, and MxA) and ISGF3-independent (IRF-1) gene expression in infected fibroblasts and ECs (53). EMSA analyses, utilizing a DNA-binding element that binds STAT-1 homodimers and STAT-1/STAT-2 heterodimers, revealed that IFN-α stimulated transcription factor activation is blocked in HCMV infected cells (42). Immunoprecipitation experiments demonstrated that IFN-α stimulated tyrosine phosphorylation is blocked in HCMV infected cells, a cellular phenotype that correlates with decreased JAK1 protein. Thus, the HCMV mediated reduction of JAK1 protein may mediate disruption of IFN-α stimulated responses in an analogous manner to the defect in the IFN-γ pathway.

However, analyses of the individual components of the IFN-α signal transduction pathway uncovered an additional lesion in this signal transduction system. There is decreased expression of p48 protein (the DNA-binding component of ISGF3) in HCMV infected fibroblasts and ECs (42). Moreover, HCMV IE and/or E genes mediate the decrease in p48 protein expression.

These two defects in IFN-α signal transduction block distinct arms of the IFN-α signal transduction system. There is significant data demonstrating that IFN-α activates genes, such as IRF-1, independent of the p48 component of ISGF3 (17, 35). Thus, the decrease of JAK1 protein may inhibit IFN-α stimulated ISGF3-independent gene expression. In addition, p48 is the principal mediator of ISGF3 binding to ISRE DNA elements in IFN-α stimulated promoters (53). Studies in p48-negative cell lines and in p48 knockout mice show that IFN-α stimulated ISGF3-dependent gene expression is inhibited in cells lacking p48 (28). Moreover, p48 is critical to the establishment of IFN-α- and IFN-γ-induced antiviral states (28). Thus, the HCMV-mediated decrease in JAK1 and p48 may block IFN-α stimulated ISGF3-dependent gene expression.

Summary

These studies demonstrate that HCMV has evolved multiple mechanisms for globally blocking IFN stimulated responses. Specifically, HCMV appears to disrupt IFN-γ stimulated MHC class II expression through two distinct mechanisms; decreased JAK1 protein and disrupted IFN-γ signal transduction as well as a direct repression of CIITA expression. Moreover, IFN-γ stimulated MHC class I, β_2m, proteasome subunit, and TAP expression are inhibited by the decrease of JAK1 protein. IFN-α stimulated MHC class I expression and up-regulation of direct antiviral effector molecules such as 2',5'-OAS and MxA are blocked by disruption of ISGF3-dependent gene expression secondary to the HCMV decrease of JAK1 and p48 proteins. Finally, IFN-α stimulated ISGF3-independent gene expression is inhibited, perhaps as a result

of the decrease of JAK1.

Divergent viruses have evolved means of disrupting IFN stimulated JAK/STAT signal transduction and thus IFN stimulated antiviral and immunoregulatory responses. For example, adenovirus E1A gene products decrease p48, and overexpression of p48 can restore IFN-α signal transduction in E1A transfected cells (32). In addition, in HeLa cells E1A also decreases STAT-1 levels, further preventing IFN-α and IFN-γ induced signaling (31, 32, 51). The human papillomavirus (HPV) E7 protein inhibits the induction of IFN-α inducible genes through decreased ISGF3 formation and p48 nuclear translocation, without decreasing p48 levels (1). Mumps disrupts IFN-induced gene expression in infected cells through decreased STAT-1, mediated by a post-transcriptional mechanism (59). Ebola inhibits the induction of IFN induced genes including MHC class I, IRF-1, and 2',5'-OAS through decreased formation of STAT-1 homodimers and ISGF3 complexes (18). The Hepatitis B virus (HBV) terminal protein disrupts the signaling response to IFN-α and IFN-γ by interfering with the formation of active ISGF3 complexes (13). Moreover, the Epstein-Barr virus (EBV) nuclear antigen 2 (EBNA-2) gene similarly prevents activation of IFN-α by a mechanism downstream of the activation of ISGF3 (25).

In summary, there is increasing evidence that a relatively new paradigm of viral persistence exists, a paradigm in which interferons have exerted selection pressures resulting in the emergence of viruses with the ability to inhibit signal transduction. This is a fortuitous ability, for it interferes with multiple antiviral effects ranging from induction of antigen presentation, including MHC expression, to induction of antiviral enzymes.

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