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Human Cytomegalovirus Inhibits Major Histocompatibility Complex Class II Expression By Disruption of the Jak/Stat Pathway

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Summary

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that is able to persist for decades in its host. HCMV has evolved protean countermeasures for anti-HCMV cellular immunity that facilitate establishment of persistence. Recently it has been shown that HCMV inhibits interferon γ (IFN-γ)–stimulated MHC class II expression, but the mechanism for this effect is unknown. IFN-γ signal transduction (Jak/Stat pathway) and class II transactivator (CIITA) are required components for IFN-γ–stimulated MHC class II expression. In this study, we demonstrate that both a clinical isolate and a laboratory strain of HCMV inhibit inducible MHC class II expression at the cell surface and at RNA level in human endothelial cells and fibroblasts. Moreover, reverse transcriptase polymerase chain reaction and Northern blot analyses demonstrate that neither CIITA nor interferon regulatory factor 1 are upregulated in infected cells. Electrophoretic mobility shift assays reveal a defect in IFN-γ signal transduction, which was shown by immunoprecipitation to be associated with a striking decrease in Janus kinase 1 (Jak1) levels. Proteasome inhibitor studies with carboxybenzyl-leucyl-leucyl-leucine vinyl sulfone suggest an HCMV-associated enhancement of Jak1 protein degradation. This is the first report of a mechanism for the HCMV-mediated disruption of inducible MHC class II expression and a direct virus-associated alteration in Janus kinase levels. These findings are yet another example of the diverse mechanisms by which HCMV avoids immunosurveillance and establishes persistence.

Human cytomegalovirus (HCMV),[1] a ubiquitous betaherpesvirus, causes extensive morbidity and mortality in neonatal and immunocompromised patients. In these individuals, the majority of HCMV-associated disease is the result of the spread of latent or persistent virus acquired before immunosuppression (1, 2). Therefore, understanding the means by which the virus avoids clearance by the immune system is critical for a complete model of pathogenesis.

The primacy of cell-mediated immunosurveillance in controlling HCMV infection is established by the prominence of HCMV disease in individuals with impaired cellular immunity (i.e., AIDS patients and transplant recipients) (1, 2). Although cell-mediated immunity can protect from disease, it rarely clears the virus from the host. Consistent with this ability to persist, HCMV has evolved multiple mechanisms for escape from cellular immune responses. HCMV-infected cells are resistant to NK cell lysis through surface expression of an MHC class I–like molecule (3, 4), and HCMV escapes $CD8^+$ T lymphocyte immunosurveillance by decreasing MHC class I expression through the action of three independent HCMV glycoproteins (5–10).

Although NK cells and $CD8^+$ T lymphocytes have been classically shown to be important in controlling HCMV infection, recent in vivo studies have demonstrated an expanded role for $CD4^+$ T lymphocytes in control of replication and clearance of the virus (11–13). Moreover, the profound decrease in $CD4^+$ T lymphocytes in AIDS patients frequently results in HCMV pneumonia and retinitis (1, 2).

$CD4^+$ T lymphocytes recognize antigens presented in

[1]*Abbreviations used in this paper:* CIITA, class II transactivator; E, early; EC, endothelial cell; EMSA, electrophoretic mobility shift assay; g, glycoprotein; GAF, IFN-γ activation factor; GAS, IFN-γ activation sequence; HCMV, human cytomegalovirus; IE, immediate-early; IP, immunoprecipitation; IRF, IFN regulatory factor; Jak, Janus kinase; L, late; PFA, phosphonoformic acid; RT, reverse transcribed; Stat, signal transducers and activators of transcription; Z-L_3VS, carboxybenzyl-leucyl-leucyl-leucine vinyl sulfone.

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the context of MHC class II molecules, highly polymorphic heterodimers consisting of an α and β chain. MHC class II molecules are expressed constitutively on B cells, monocytes, dendritic cells, and thymic epithelial cells, whereas IFN-γ is the most potent inducer of MHC class II expression in many other cell types, including endothelial cells (ECs) and fibroblasts (14).

MHC class II expression is controlled predominantly at the level of transcription (14). IFN-γ induces MHC class II expression by activating the Jak/Stat pathway and upregulating class II transactivator (CIITA). CIITA is believed to activate transcription by interacting with ubiquitous DNA binding proteins at MHC class II promoters (14–18). In the IFN-γ signal transduction (Jak/Stat) pathway, IFN-γ binds to extracellular heterodimeric receptor subunits IFN-γR1 and IFN-γR2, which are associated intracellularly with the Janus kinases (Jaks) Jak1 and Jak2, respectively (19, 20). The binding initiates phosphorylation of tyrosine residues in Jak1, Jak2, and the cytoplasmic tail of IFN-γR1 (21–24). Each phosphorylated IFN-γR1 chain becomes a docking site for a member of the family of signal transducers and activators of transcription (Stat), Stat1α (19, 20). After docking at the receptor, Stat1α is phosphorylated by the Jaks and forms a homodimer known as IFN-γ activation factor (GAF) (19, 25, 26). GAF migrates to the nucleus where it binds the IFN-γ activation sequence (GAS) elements present in the promoters of IFN-γ–inducible genes (19).

As with NK cell responses and $CD8^+$ T cell immunosurveillance, there is accumulating evidence that HCMV has evolved a means of escaping $CD4^+$ T cell immunosurveillance as well. HCMV-infected alveolar type II pneumocytes in patients with HCMV pneumonia do not express MHC class II molecules in vivo (27). In vitro studies demonstrate that IFN-γ induction of MHC class II expression is impaired in HCMV-infected ECs and fibroblasts (28–30). However, the mechanism by which HCMV inhibits IFN-γ–induced MHC class II expression is unknown.

In this study, we investigated IFN-γ–induced MHC class II expression in HCMV-infected human ECs and fibroblasts. We show that HCMV disrupts IFN-γ induction of MHC class II expression by inhibiting the Jak/Stat pathway, a phenomenon associated with decreased Jak1 protein.

Materials and Methods

Cells. Human umbilical vein endothelial cells were isolated from vessels and propagated as previously described (28). ECs were infected with HCMV strain VHL/E (31). HCMV-infected ECs were generated by a dispersion method which yields a culture of >95% infected ECs (28). Human embryonic lung fibroblasts (MRC-5), passages 22–35, were cultured in Eagle's minimal essential medium supplemented with 10% fetal bovine serum (GIBCO BRL, Gaithersburg, MD) at 37°C in a 5% CO_2 incubator. HCMV Towne strain was propagated in MRC-5 at low multiplicity of infection (MOI) with aliquots frozen at −80°C and titer determined as described elsewhere (32). In all experiments, Towne HCMV was incubated (MOI: 3) with fibroblasts for 2 h at 37°C and free virus was washed off (time 0). To inhibit HCMV late gene expression, cells were infected with Towne in the presence of 2 mM phosphonoacetic acid (PFA; Sigma Chemical Co., St. Louis, MO) and 0.6 mM Ganciclovir (GCV; Roche Labs, Nutley, NJ).

Flow Cytometry. Cells were harvested with 0.005% trypsin/0.01% EDTA, stained with FITC-labeled HLA-DR antibody (Genclone, Plymouth Meeting, PA) or an isotypic IgG1–FITC conjugate (Becton Dickinson), and analyzed by flow cytometry on an EPICS Profile II flow cytometer (Coulter Corp., Hialeah, FL) (27, 28).

Northern Blot Analysis. 10 μg of total cytoplasmic RNA, isolated by guanidium thiocyanate extraction and cesium chloride centrifugation, was separated on a 1.4% agarose/0.22 M formaldehyde gel and transferred to nylon membranes (Hybond-N; Amersham Corp., Arlington Heights, IL). For Jak1 detection only, mRNA from 30×10^6 fibroblasts was isolated (Invitrogen Corp., Carlsbad, CA) and fractionated as above. Random priming (DecaPrime II Kit; Ambion Inc., Austin, TX) of glyceraldehyde phosphate dehydrogenase (GAPDH) and HLA-DRα probes was performed (28). PCR labeling was used for interferon regulatory factor (IRF) 1, glycoprotein (g)B, immediate early 1 (IE1), and Jak1 probes. In brief, 50 ng of full-length human IRF-1, gB, IE1, or Jak1 was incubated in a 50-μl PCR reaction containing IRF-1 primers (IRF-1 sense: 5′ CTTCCCTCTTCCACTCGGAGTC 3′; IRF-1 antisense: 5′ CTGGTCTTTCACCTCCTCGATATCT 3′); gB primers (gB sense: 5′ CACCAAGTACCCCTATCGCGT 3′; gB antisense: 5′ TTGTACGAGTTGAATTCGCGC 3′); IE1 primers (33); Jak1 primers (Jak1 sense: 5′ GAAACTTTGACAAAACATTACGGTGC 3′; Jak1 antisense: 5′ TCCTTCTTGAGGATCCGATCG 3′); dNTP-dCTP; and 700 nM α-P32-dCTP (Amersham Corp.). Reaction products were purified from unincorporated isotope via a Mini Spin G-50 column (Worthington Biochemical Corp., Freehold, NJ), melted, hybridized, and detected as previously described (28). After hybridization overnight at 42°C, the final wash was carried out at 56°C with 0.2 × SSC and 0.1% SDS for 30 min. Autoradiography was performed with BioMAX MS film (Eastman Kodak Co., Rochester, NY) at −80°C for 4–8 h.

Reverse Transcriptase PCR. 10 μg of cytoplasmic RNA was treated with 10 U RNase-free DNase (Stratagene Inc., La Jolla, CA) for 30 min at 37°C followed by phenol/chloroform extraction and ethanol precipitation at −80°C. Samples were reverse transcribed (RT; GIBCO BRL), and one 5-μg aliquot for each sample served as a no-RT control to control for genomic contamination in subsequent PCR reactions. After heating at 94°C for 3 min the reaction mixture was cycled 30 times: 1 min at 94°C; 2 min at 60°C; 3 min at 72°C; and a final 10 min elongation step at 72°C. PCR reactions were performed with β-actin (540-bp PCR product), CIITA (680-bp PCR product), and HLA-DRα primers (273-bp PCR product) and products were analyzed on ethidium bromide–stained 2% agarose gels. All PCR products were cloned into pCRII vector (Invitrogen Corp.) and sequenced by the dideoxy chain termination method. Primer sets are as follows: CIITA primers: previously published primers CIITA-2 and CIITA-3 (34); β-actin sense primer: 5′ GTGGGGCGCCCCAGGCACCA 3′; β-Actin antisense: 5′ CTCCTTAATGTCACGCACGATTTC 3′; HLA-DRα sense: 5′ AAAGCGCTCCAACTATACTCCGA 3′; HLA-DRα antisense: 5′ ACCCTGCAGTCGTAAACGTCC 3′.

Electrophoretic Mobility Shift Assay. Nuclear extracts were prepared by a modification of Dignam et al. (35). 3 μg of nuclear extract was combined with 1 μl of 5× binding buffer, 0.8 μl of poly (dI-dC), and 1 μl of ^{32}P-labeled IRF-1 GAS element (5′ GATC-

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GATTTCCCCGAAATCATG 3′) probe (21). The reaction was incubated at room temperature for 20 min and resolved on a 6% nondissociating polyacrylamide gel. For controls, 1 μl (100 ng) of 100× cold GAS element probe, 1 μg of Stat1α mAb (Santa Cruz Biotechnology, Santa Cruz, CA), or 1 μg of IgG1 (DAKO Corp., Carpinteria, CA) was added to the binding reaction before the addition of radiolabeled probe for competition and supershift assays, respectively.

Immunoprecipitation and Western Blot Analysis. Immunoprecipitation (IP) was performed as previously described (21, 23). For Stat1α, 6×10^6 cells per treatment were lysed in IP lysis buffer consisting of 1% Triton X-100, 0.15 M NaCl, 50 mM Tris (pH 8.0), 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM PMSF, 1 mM orthovanadate, and 5 μg/ml each of pepstatin, leupeptin, and aprotinin. Stat1α Ab was added to postnuclear lysates and incubated at 4°C overnight. For Jak1, Jak2, and IFN-γR1 IP, 12×10^6 cells per treatment were solubilized in IP lysis buffer. For Jak1 and Jak2, 10 μg of rabbit Ig and protein A–Sepharose (Pharmacia Biotech, Piscataway, NJ) were added to postnuclear lysates and incubated at 4°C overnight. Fresh phosphotyrosine, protease inhibitors, and primary antibodies were added to the precleared lysate, followed by another overnight incubation at 4°C. Immune complexes were collected with an excess of protein A–Sepharose (Jak1, Jak2) or protein G–Sepharose (Stat1α, IFN-γR1) and fractionated under reducing conditions on 7.5% SDS-PAGE (Stat1α, Jak1, and Jak2) or 12% SDS-PAGE (IFN-γR1). Equal volumes of lysates from an equal number of cells were resolved by SDS-PAGE.

For proteasome inhibitor experiments, carboxybenzyl-leucyl-leucyl-leucine vinyl sulfone (Z-L_3VS; gift of Hidde Ploegh, MIT, Cambridge, MA) was used as previously described (36). Cells at 48 h after infection were incubated with 50 μM Z-L_3VS for 12 h and lysed for IP. Control cells were treated with an equivalent amount of solvent (DMSO) without Z-L_3VS.

Western blot analyses of IP experiments and standard Western analyses were as follows: Westerns were performed with primary antibodies (1:1,000) Jak1, Jak2, Stat1α, and IFN-γR1, followed by 1:3,000 anti–rabbit horseradish peroxidase–conjugated IgG (Santa Cruz Biotechnology) or horseradish peroxidase–conjugated protein (Bio-Rad Laboratories, Hercules, CA), and were developed using Ultrachemiluminescence (Pierce Chemical Co., Rockford, IL). For the standard Western lysates (see Fig. 5 *C*), ECs were lysed in 5% SDS, 0.5 M Tris HCl (pH 6.8), 0.5 mM EDTA, and protease inhibitors. After centrifugation at 15,000 rpm for 15 min, equal volumes of supernatant from an equivalent number of cells were fractionated by SDS-PAGE and Western blot analysis was performed as described above.

Results

HCMV Inhibits IFN-γ–induced MHC Class II Expression. We used human ECs and fibroblasts to investigate the effect of HCMV on IFN-γ–stimulated MHC class II expression. ECs and fibroblasts are infected by HCMV in vivo and require IFN-γ stimulation to upregulate MHC class II expression (14, 15, 37, 38). We infected ECs with an EC-tropic clinical isolate, VHL/E, and fibroblasts were infected with a common laboratory strain of HCMV (Towne).

Our previous studies showed that IFN-γ stimulation of HCMV-infected ECs did not induce MHC class II expression at the cell surface, in the cytoplasm, or at the RNA level (28). In this study, our analysis of HCMV-infected fibroblasts yielded similar results. Flow cytometry analysis of HLA-DR surface expression demonstrated that IFN-γ treatment induced MHC class II expression in noninfected but not in HCMV-infected fibroblasts (Fig. 1 *A*). UV-inactivated HCMV did not inhibit MHC class II surface expression, demonstrating that inhibition of IFN-γ–induced MHC class II expression was dependent upon virus replication (Fig. 1 *A*). Northern blot analyses of IFN-γ–stimulated MHC class II RNA expression revealed that noninfected cells treated with IFN-γ accumulated HLA-DRα mRNA, whereas HCMV-infected cells did not (Fig. 1 *B*). Therefore, our findings in fibroblasts paralleled our previous observations in ECs. That is, HCMV inhibits IFN-γ–stimulated MHC class II surface expression and the accumulation of HLA-DRα RNA.

HCMV Inhibits IFN-γ–induced CIITA Expression. We hypothesized that one of two known levels of transcriptional control of MHC class II expression, either the Jak/Stat pathway or CIITA expression, was nonfunctional given the lack of IFN-γ–stimulated MHC class II RNA upregulation in HCMV-infected cells. CIITA, an IFN-γ–induced transcription factor, is required for activation of MHC class II promoters and transcription of class II genes (16). We determined the expression of CIITA in HCMV-infected ECs and fibroblasts by RT-PCR. Noninfected cells treated with IFN-γ expressed CIITA and HLA-DRα mRNA, whereas HCMV-infected IFN-γ–treated cells did not (Fig. 2). We next investigated the expression of IRF-1, an IFN-γ–stimulated gene that plays a central role in regulating MHC class I and II expression in vivo, to determine if HCMV infection globally blocked IFN-γ–stimulated gene expression (39). IRF-1 RNA was upregulated by IFN-γ treatment in noninfected cells but not in HCMV-infected cells (Fig. 3). These data suggested that there was a

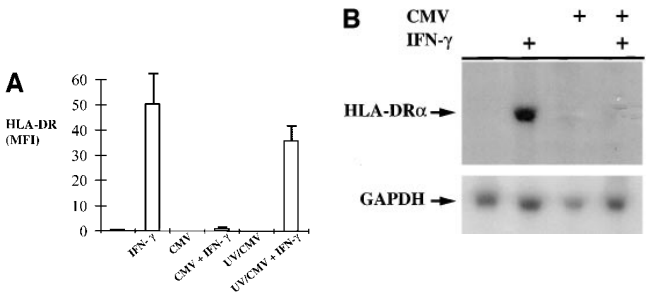

Figure 1. IFN-γ–stimulated MHC class II expression is inhibited in HCMV-infected cells. (*A*) IFN-γ does not induce HLA-DR surface expression in HCMV-infected fibroblasts. Shown is a bar graph displaying the mean fluorescent intensity (*MFI*) of HLA-DR surface expression in noninfected, sham-infected (inoculated with UV-inactivated HCMV), and HCMV-infected fibroblasts. Beginning at 12 h after infection, cells were treated with 200 U/ml IFN-γ for 72 h. The mean value of three independent experiments is displayed with error bars representing the SEM. (*B*) IFN-γ treatment does not upregulate HLA-DRα RNA in HCMV-infected cells. IFN-γ treatment was identical to that in *A*. A Northern analysis is displayed in which IFN-γ–stimulated HLA-DRα RNA expression was compared with glyceraldehyde phosphate dehydrogenase (GAPDH) expression in fibroblasts.

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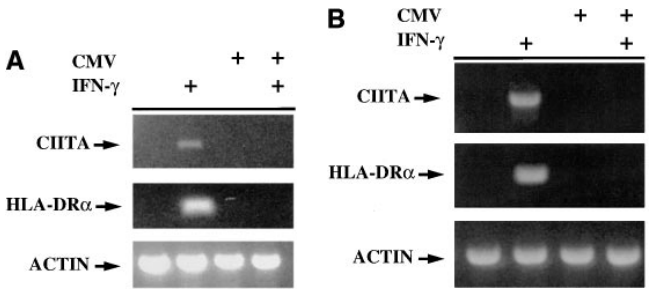

Figure 2. HCMV abrogates IFN-γ–stimulated CIITA expression. IFN-γ treatment was as described for Fig. 1 *A*. RT-PCR analysis of CIITA expression in noninfected and HCMV-infected fibroblasts (*A*) and ECs (*B*) reveals that IFN-γ–stimulated CIITA expression is inhibited in HCMV-infected cells.

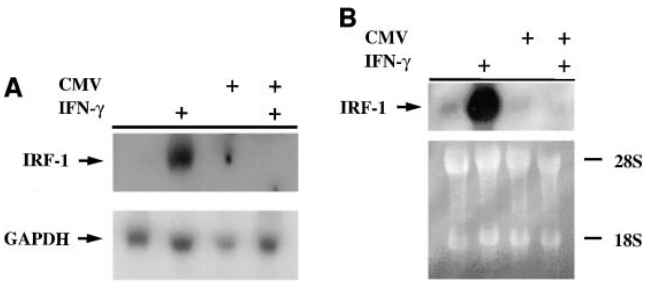

Figure 3. HCMV inhibits IFN-γ–stimulated IRF-1 expression. Northern analysis of IRF-1 RNA levels shows that IFN-γ–stimulated IRF-1 expression is blocked in infected fibroblasts (*A*) and ECs (*B*).

general disruption of IFN-γ–stimulated gene expression in HCMV-infected ECs and fibroblasts.

HCMV Inhibits IFN-γ–stimulated MHC Class II Expression by Inhibiting IFN-γ Signal Transduction (Jak/Stat Pathway). We analyzed IFN-γ–stimulated GAF induction to determine if HCMV disables inducible MHC class II expression at the level of the Jak/Stat pathway. IFN-γ induces GAF, a homodimer of phosphorylated Stat1α proteins, which binds GAS elements in the promoters of IFN-γ–stimulated genes and activates transcription (19). IFN-γ–stimulated GAF induction was assayed using electrophoretic mobility shift assay (EMSA) with the GAS element of the IRF-1 promoter as probe. IFN-γ induced GAF in noninfected cells, but not in HCMV-infected fibroblasts and ECs (Fig. 4). The specificity of our probe was verified by supershift analysis, in which Stat1α antibody, but not an isotypic IgG1 control, supershifted the GAF–GAS complex. Furthermore, GAF–GAS complex formation was inhibited by 100× GAS competitor (Fig. 4).

The formation of GAF is ultimately dependent on the upstream signaling events of the IFN-γ signal transduction system (Jak/Stat pathway). Stat1α, IFN-γR1, Jak1, and Jak2 are phosphorylated on tyrosine residues when IFN-γ binds its receptor. We investigated the integrity of this signal transduction pathway by immunoprecipitation. Noninfected and HCMV-infected fibroblasts were treated with IFN-γ for 30 min and Stat1α, IFN-γR1, Jak2, and Jak1 were immunoprecipitated from whole cell lysates. Each immunoprecipitate was split in half before Western analyses of phosphotyrosine (Fig. 5 *A*) or Stat1α, IFN-γR1, Jak2, or Jak1 (Fig. 5 *B*) immunoreactivities. IFN-γ stimulated tyrosine phosphorylation of Stat1α, IFN-γR1, Jak2, and Jak1 in noninfected cells, but none of these proteins were phosphorylated in infected cells (Fig. 5 *A*). Western analyses of the immunoprecipitated proteins revealed that Stat1α, IFN-γR1, and Jak2 were equivalently expressed in noninfected and HCMV-infected cells, whereas there was a dramatic decrease of Jak1 protein in infected cells (Fig. 5 *B*).

These IP experiments in fibroblasts demonstrated a decrease of Jak1 protein in HCMV-infected cells. To rule out the possibility that our antibody was cross-reacting with a protein immunoprecipitated from HCMV-infected cells, we analyzed Jak1 expression by standard Western analysis of whole cell lysates. No Jak1 was detected in infected fibroblasts (data not shown). These findings were also extended to HCMV-infected ECs, which had no detectable Jak1 protein in contrast to Stat1α (Fig. 5 *C*).

We performed a Northern blot analysis to determine if the decrease in Jak1 protein in infected cells correlated with a change in steady state mRNA. The levels of Jak1 mRNA were equivalent in noninfected and HCMV-infected fibroblasts (Fig. 6), which suggested that JAK-1 was decreased by a posttranscriptional mechanism.

Recent investigations have demonstrated that the posttranscriptional decrease in MHC class I heavy chains in infected cells was mediated by the proteasome, a multicatalytic proteolytic complex (5, 6, 36). We tested whether HCMV targeted Jak1 for degradation by a similar mechanism using the proteasome inhibitor Z-L_3VS, which covalently inhibits the trypsin-like, chymotrypsin-like, and peptidyl-glutamyl peptidase activities of the proteasome (36). Noninfected fibroblasts and HCMV-infected fibroblasts were treated either with solvent alone (DMSO) or Z-L_3VS for 12 h, and Jak1 was immunoprecipitated. By Western analysis, Z-L_3VS treatment increased the steady state levels of the Jak1 protein in HCMV-infected fibroblasts (Fig. 7). The specificity of this finding was confirmed by the absence of Jak1 immunoreactivity in the presence of a blocking peptide. These results suggest that the posttranscriptional decrease of Jak1 protein in infected cells was mediated by a degradative process involving the proteasome.

HCMV Immediate-early or Early Genes Inhibit Inducible MHC Class II Expression. HCMV has the second largest genome of the herpesvirus family, encoding >200 proteins that are expressed in a temporal fashion, e.g., immediate-early (IE), early (E), and late (L). We examined the role of late genes in inhibiting IFN-γ–stimulated MHC class II expression using phosphonoformic acid (PFA) and GCV, inhibitors of HCMV DNA polymerase. HCMV infection in the presence of PFA/GCV inhibited the L gene product gB, without inhibiting IE1 gene expression (Fig. 8 *A*). IFN-γ–stimulated GAF formation was inhibited in the presence of these inhibitors (Fig. 8 *B*). This finding was consistent with the hypothesis that HCMV IE and/or E

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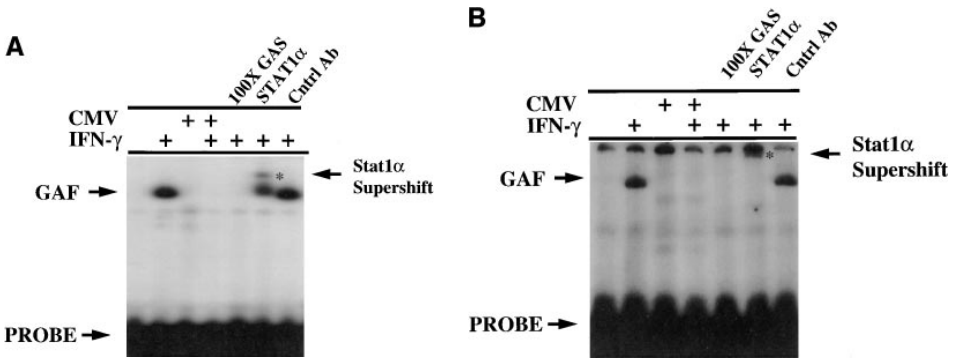

Figure 4. IFN-γ–stimulated GAF formation is inhibited in HCMV-infected cells. At 72 h after infection, noninfected and HCMV-infected (*A*) fibroblasts and (*B*) ECs were treated with 200 U/ml IFN-γ for 30 min and then nuclear extracts were recovered. EMSA binding reactions were performed as described in Materials and Methods. For controls, 100× GAS element competitor, Stat1α Ab, or IgG1 was added to the binding reaction before the addition of radiolabeled probe. The supershifted GAF–GAS band is denoted by *. IFN-γ–stimulated GAF formation is inhibited in infected fibroblasts (*A*, lane *4*) and ECs (*B*, lane *4*).

genes, but not L genes, inhibit IFN-γ–stimulated signal transduction and MHC class II expression.

Discussion

The studies presented here are the first to reveal a mechanism for HCMV inhibition of MHC class II expression. Specifically, we found that IFN-γ–stimulated signal transduction (Jak/Stat pathway) is disabled in infected cells. Jak/Stat signaling is the most proximal of the levels required for the induction of MHC class II expression, and its disruption prevents the upregulation of CIITA and activation of MHC class II transcription (Fig. 9).

Fibroblasts and ECs are major targets of CMV infection in vivo (37, 38, 40–42). ECs play a particularly important role in CMV pathobiology, not only as reservoirs of persistence but as critical components of the dissemination pathway involving circulating leukocytes (43, 44). However, ECs also play a vital role in inflammatory responses, and as such are poised to interact as inducible antigen-presenting cells with leukocytes. Therefore, it is important for HCMV-infected ECs to escape cell-mediated immunosurveillance and persist by inhibiting expression of MHC molecules. It has been shown that HCMV decreases MHC class I expression on ECs, for which several mechanisms have been recently uncovered (5–10). Similarly, we have previously demonstrated that HCMV inhibits IFN-γ–mediated MHC class II induction on ECs (27–29), and that this inhibition occurs at the same time after infection as the decrease in constitutive MHC class I (data not shown).

Inhibition of IFN-γ upregulation of MHC class II expression has coevolved in divergent viruses including mouse hepatitis virus, HIV-1, Kirsten murine sarcoma virus, and measles virus, suggesting that escape from $CD4^+$ T lymphocyte immunosurveillance provides a survival advantage to the pathogen (45, 46). $CD4^+$ T cells augment $CD8^+$ T lymphocyte and B lymphocyte responses to viral infection. There is significant evidence that $CD4^+$ T cells can control CMV infection independent of the $CD8^+$ T cell subset: mice depleted of $CD8^+$ T cells halt CMV dissemination with similar kinetics to immunocompetent mice (13), and clearance of CMV from select organs is

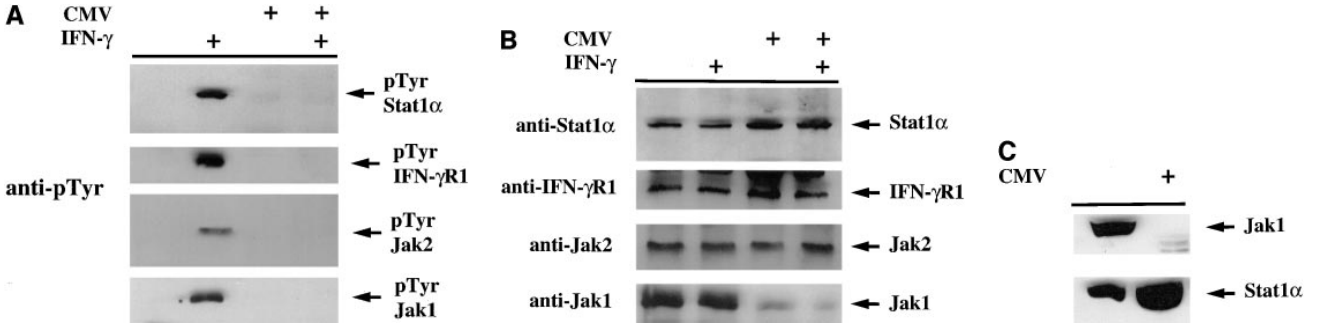

Figure 5. IFN-γ–stimulated Jak/Stat signal transduction is inhibited by HCMV. Fibroblasts at 72 h after infection were treated with IFN-γ, solubilized in a 1% Triton X-100 lysis buffer, and IPs were performed. Immunoprecipitates were divided in half for Western blot analysis of phosphotyrosine immunoreactivity (*A*) or Western blot detection of the immunoprecipitated protein (*B*). (*A*) IFN-γ stimulates phosphorylation of Stat1α, IFN-γR1, Jak2, and Jak1 in noninfected cells (lane *2*), but none of these proteins are phosphorylated in HCMV-infected cells (lane *4*). (*B*) There is an equivalent amount of Stat1α, IFN-γR1, and Jak2 protein in noninfected and HCMV-infected cells, but Jak1 protein is decreased in HCMV-infected cells. (*C*) Standard SDS lysates from ECs were fractionated by SDS-PAGE and analyzed by Western blot analysis. Jak1 protein is decreased in HCMV-infected cells in contrast to Stat1α.

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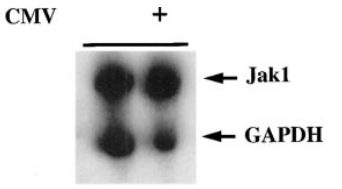

Figure 6. Jak1 protein is decreased by a posttranscriptional mechanism. mRNA was isolated from equal numbers of noninfected and HCMV-infected fibroblasts at 72 h after infection, a time when there is no detectable Jak1 protein. Jak1 mRNA is not decreased in HCMV-infected cells despite the decrease of Jak1 protein at this time point.

completely dependent upon the $CD4^+$ T cell subset (12, 47). A direct role for $CD4^+$ T cells in anti-CMV activity is supported by the findings of CMV-specific class II–restricted cytolysis and direct antiviral effects of the $CD4^+$ T lymphocyte cytokine mileu, specifically IFN-γ (11, 12, 48–50). The release of cytokines from CMV-specific $CD4^+$ T cells has significant direct and immunoregulatory anti-CMV effects in vivo and in vitro (11–13, 51, 52). Our results suggest that HCMV may inhibit these direct and indirect IFN-γ antiviral effects by knocking out IFN-γ responses at their most proximal point, the level of IFN-γ signal transduction.

IFN-γ signal transduction is dependent upon the function of Jak1 (53). In mutant cell lines lacking this protein, IFN-γ–stimulated tyrosine phosphorylation of IFN-γR1, Jak1, Jak2, and Stat1α is inhibited (53). This pattern of phosphorylation is analogous to what we found in HCMV-infected cells (Fig. 5), suggesting that the HCMV-associated posttranscriptional decrease in Jak1 protein results in inhibition of IFN-γ–stimulated MHC class II expression. Northern analysis of Jak1 mRNA in infected cells revealed steady state levels equivalent to those in noninfected cells. This data, in conjunction with experiments with the proteasome inhibitor Z-L_3VS, suggest that increased degradation by the proteasome complex is at least partly responsible for the decrease in Jak1 protein.

Lastly, we found that CMV IE and/or E genes inhibit IFN-γ–stimulated MHC class II expression by disrupting IFN-γ–mediated Jak/Stat signal transduction. CMV IE and E genes mediate the majority of known HCMV immunoregulatory effects. They downregulate MHC class I expression (5–10), inhibit the transporter associated with antigen

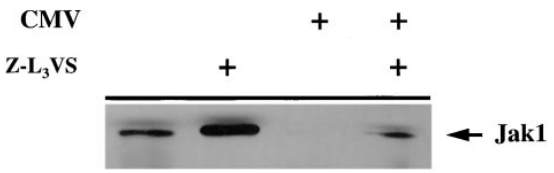

Figure 7. Jak1 is degraded in infected cells. Noninfected and HCMV-infected fibroblasts at 48 h after infection were treated with solvent (DMSO) or Z-L_3VS for 12 h. After Z-L_3VS treatment, equal numbers of cells were lysed and Jak1 was immunoprecipitated. There is an increase in steady state levels of Jak1 protein in HCMV-infected cells treated with Z-L_3VS (lane *4*).

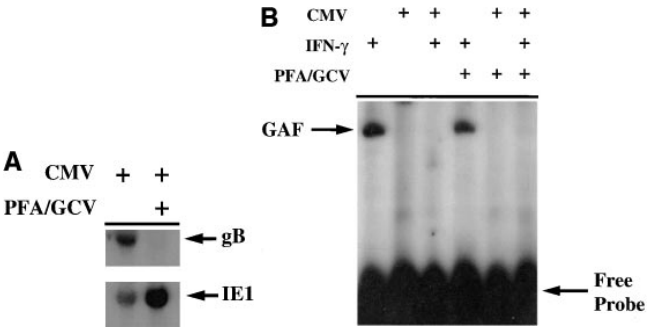

Figure 8. HCMV IE and/or E genes, but not L genes, inhibit IFN-γ signaling. (*A*) Northern analysis shows that PFA and GCV treatment inhibits the expression of an L gene, gB, but not IE gene expression in HCMV-infected fibroblasts. (*B*) At 72 h after infection, HCMV-infected fibroblasts, and HCMV-infected fibroblasts treated with PFA/GCV were stimulated with 200 U/ml IFN-γ for 30 min, nuclear extracts were recovered, and EMSA was performed. HCMV infection inhibits IFN-γ–stimulated GAF formation under conditions where only IE and E genes are expressed (lane *6*).

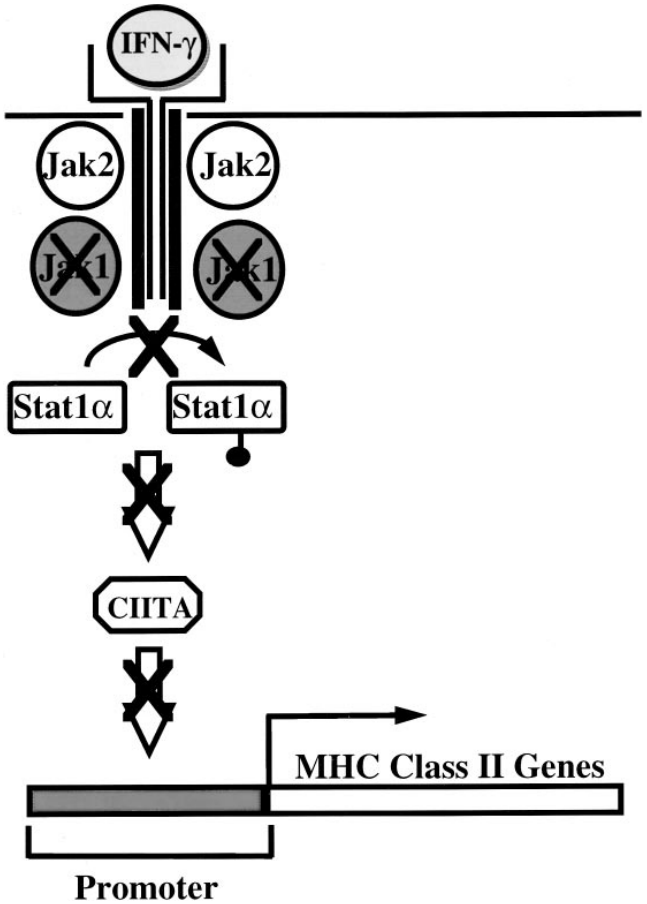

Figure 9. Model for HCMV inhibition of IFN-γ–stimulated MHC class II expression. HCMV IE and/or E genes disable Jak/Stat signal transduction by decreasing Jak1 expression, which inhibits IFN-γ–stimulated signal transduction. Inhibiting IFN-γ signaling prevents the upregulation of CIITA and protein–protein interactions between CIITA and class II promoter binding proteins that are believed to be required for MHC class II transcription.

processing (54), and encode an MHC class I homologue (3, 4).

In conclusion, we have demonstrated that HCMV inhibits inducible MHC class II expression in ECs and fibroblasts by disabling IFN-γ stimulated signal transduction. To our knowledge, this is the first report of a mechanism for the HCMV-mediated disruption of inducible MHC class II expression and the first report of a direct virus-associated alteration in Janus kinase levels. These findings are yet another example of the diverse mechanisms by which HCMV, and thus viruses in general, are capable of avoiding immunosurveillance and establishing persistence.

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