Characterization of the Elements and Proteins Responsible for Interferon-Stimulated Gene Induction by Human Cytomegalovirus

Shaojun Yang, James Netterwald, Weijia Wang, and Hua Zhu*

Department of Microbiology and Molecular Genetics, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey

Received 17 August 2004/Accepted 2 December 2004

Human cytomegalovirus (HCMV) infection of human fibroblast cells activates a large number of interferon-stimulated genes (ISGs) in a viral envelope-cell membrane fusion-dependent mechanism. In this study, we identified two interferon response elements, the interferon-stimulated response element (ISRE) and the gamma interferon-activated site (GAS), which act as HCMV response sites (VRS). Gel mobility shift assays showed that cellular proteins form specific and identical complexes with ISRE and GAS elements, and the binding of these complexes to ISRE and GAS is stimulated by HCMV infection. Point mutations in the consensus sequences of ISRE and GAS completely abolished their activities in response to HCMV-mediated transactivation, as well as their abilities to interact with HCMV-activated VRS-binding proteins. Interferon regulatory factor 3 does not appear to be present in the VRS-binding complexes or to be involved directly in HCMV-mediated ISG activation. Using ProteinChip technology, four potential proteins were identified, ranging from 20 to 40 kDa, in the VRS-binding complexes. The data suggest that HCMV infection activates VRS-binding proteins, which then bind to the VRS and stimulate ISG expression.

* Corresponding author. Mailing address: Department of Microbiology and Molecular Genetics, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07101-1709. Phone: (973) 972-6488, ext. 2-6488. Fax: (973) 972-8981. E-mail: zhuhu@umdnj.edu.

Human cytomegalovirus (HCMV), a member of the herpesvirus family, is a ubiquitous human pathogen (4). After primary infection, HCMV establishes lifelong latency in the host. It was found that the virus is latent in myeloid lineage cells in peripheral blood (26, 27). Latent HCMV periodically is reactivated in infected people and becomes particularly problematic in pregnant women and immunocompromised individuals. HCMV rarely causes symptomatic disease in an immunocompetent host. However, HCMV is a major cause of infectious morbidity and mortality in immunocompromised individuals, especially in AIDS patients and transplant patients. HCMV infection also is a leading cause of birth defects (5).

Like most viruses, HCMV infection affects host cell gene expression. Our previous work using differential display and GeneChip technology has allowed us to begin elucidating some of these changes (35, 36). In previous studies, it was found that among the more than 6,000 human genes examined, the expression of about 260 cellular genes is changed over fourfold in the first 24 h after HCMV infection. Interestingly and unexpectedly, a large number of interferon (IFN)-stimulated genes (ISGs) are strongly activated. UV-irradiated HCMV, which retains viral entry capability but lacks viral gene expression, is reduced. This leads to inhibition of expression of IFN-γ-stimulated major histocompatibility complex class I and II antigens, and associated antigen-processing genes, in endothelial cells and fibroblasts (16, 17). Interestingly, although HCMV specifically inhibits the Jak-Stat signal transduction pathway, the interplay between IFN-mediated responses and CMV replication is complex. It has been shown that in HCMV-infected human endothelial cells and fibroblasts, the Jak-Stat signal transduction pathway is disrupted and the expression of Jak 1 kinase and p48 (IRF-9) is reduced. This leads to inhibition of expression of IFN-γ-stimulated major histocompatibility complex class I and II antigens, and associated antigen-processing genes, in endothelial cells and fibroblasts (16, 17). Interestingly, although HCMV specifically inhibits the Jak-Stat pathway, the viral infection itself activates another signaling pathway, resulting in the strong induction of ISGs (35, 36).

In this report, we explore the components involved in this HCMV-mediated signal transduction pathway, using the isg54K gene promoter as the model. We demonstrate that both ISRE and GAS elements act as HCMV response sites (VRS). In addition, several cellular proteins, with molecular masses...
range from 20 to 42 kDa, are activated after HCMV infection and specifically interact with the VRS.

MATERIALS AND METHODS

Cells and viruses. Human foreskin fibroblast (HFF) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. HFF cells used in this study were between passages 8 and 20. The HCMV AD169 strain (American Type Culture Collection) was used to infect HFF cells at a multiplicity of infection (MOI) of 5 for another 3 days. The cells were either infected with live HCMV or UV-inactivated HCMV for 8 h before the luciferase assays, which were performed as described elsewhere (33). Each data point represents an average of triplicate points.

Nuclear extract and EMSA. To generate nuclear extracts, HFF cells were infected with HCMV AD169 at a multiplicity of infection (MOI) of 5 for 12 h. The nuclei were isolated and washed with phosphate-buffered saline (PBS). The nuclei pellets were resuspended by lysis on ice for 5 min in three volumes of lysis buffer A (10 mM HEPES buffer [pH 7.6], 60 mM KCl, 1 mM EDTA, 0.3% NP-40, 1 mM dithiothreitol [DTT], and 1 mM phenylmethylsulfonyl fluoride) followed by centrifugation at 450 × g for 10 min. Protein concentrations were measured using the Bio-Rad protein assay reagent. Aliquots of nuclear extracts were stored at -80°C. For electrophoretic mobility shift assays (EMSA), 120-bp isg54K promoter fragments containing an ISRE, two GAS, or a nonrelated NRS were amplified from the respective pElu plasmids by PCR, labeled with [γ-32P]ATP, and used as probes. Each reaction mixture (10 μl) contained about 15 μg of nuclear extract, 20 mM HEPES (pH 7.5), 0.1 mM EDTA, 1 mM MgCl₂, 2.5 mM DTT, 4% Ficoll, 1 ng of labeled probe, 1 μg of poly(dI-dC), 0.5 μg of herring sperm DNA, and 1 μg of bovine serum albumin (BSA). The reactions were carried out at room temperature for 30 min and loaded onto a 5% non-denaturing polyacrylamide gel containing 0.5× Tris-borate-EDTA buffer.

Immunodetection. The anti-IRF-3 monoclonal antibody, SL-12 (32), was used in these experiments. For immunofluorescence staining, the cells on the tissue culture plates were washed twice with PBS and fixed with 4% paraformaldehyde in PBS for 15 min at room temperature. The cells were then washed three times with PBS (PBS with 0.1% Triton X-100 and 0.05% Tween 20) for a total of 30 min, the samples were then stained with the secondary antibody, Alexa 568-labeled goat anti-mouse immunoglobulin G (Molecular Probes, Eugene, Ore.) for 30 min followed by three washes with PBS for a total of 1 h. The samples were examined under a fluorescence microscope. In vivo phosphorylation and immunoprecipitation were performed as described previously (32). Immunoblot analysis was performed as described previously (37).

SELDI analysis. DNA probes for surface-enhanced laser desorption ionization (SELDI) analysis were generated as for EMSA except that primers containing a 5'-biotin label were used in the PCR. A PS1 chip (preactivated surface ProteinChip; Ciphergen Biosystems, Fremont, Calif.) was washed for 2 min with 50% acetonitrile and then coated with streptavidin (1 μl of a 0.01-mg/ml solution in PBS; Sigma, St. Louis, Mo.). After incubation for 2 h at room temperature, the residual binding sites were blocked using 1 M ethanolamine for 30 min. Each spot was washed five times with PBS containing 0.5% Triton X-100. Biotinylated DNA was bound to each spot for 1 h at room temperature. The spots were washed twice with DNA binding buffer (20 mM HEPES [pH 7.5], 0.1 mM EDTA, 1 mM MgCl₂, 4% Ficoll). The binding reaction was carried out under the same conditions as for the EMSA described above. The nuclear extracts were added onto the ProteinChip and incubated for 1 h at room temperature. The chips were washed four times with the binding buffer and rinsed twice with PBS. At this point, 0.5 μl of a saturated solution of sinnipinic acid was added to the dried spots and allowed to dry. Chips were analyzed in the ProteinChip reader (PSI) at a laser intensity of 200 U and maximum sensitivity. Data were averaged over 65 data points using the SELDI ProteinChip software. Mass determinations were calculated using the external calibration method.

RESULTS

The ISRE is responsible for HCMV-induced ISG activation. To identify the elements on ISG promoters responsible for HCMV infection, a promoter analysis system was created in HFF cells. A 560-bp isg54K promoter region of the isg54K gene was cloned upstream of a luciferase reporter gene. This luciferase reporter plasmid was electroporated into HFF cells, and the expression of luciferase, after HCMV infection or IFN treatment, was measured. The basal level of luciferase activity was very high after transfection, because the transfection procedure itself induced this signaling pathway and activated ISG expression.
In order to solve this problem, a new reporter plasmid, pElu-ISRE (Fig. 1A), was constructed. This vector included the luciferase reporter under the control of the isg54 promoter, the Epstein-Barr virus (EBV) origin of replication (oriP), EBV nuclear antigen (EBNA-1), and a hygromycin resistance gene (Fig. 1). In this way, the reporter plasmid replicates and is maintained as an episome in transfected cells; successfully transfected cells can be enriched by hygromycin selection. HFF cells were transfected with pElu-ISRE and cultured for approximately 1 week to allow the cells to become quiescent. Then, the cells were either left untreated (mock), were infected with HCMV or UV-irradiated HCMV (UV-HCMV), or were treated with IFN-α (H9251). Luciferase assays were carried out 8 h after infection or 4 h after IFN treatment. As shown in Fig. 2A and B, the mock-infected cells had very low luciferase activity, while both HCMV and UV-HCMV infection strongly induced luciferase activity, resulting in 20- to 70-fold activation. IFN-α treatment also was able to stimulate reporter gene expression (Fig. 2B).

A major concern with this promoter assay was the possibility that activation of the reporter could be mediated by the interaction between virus and cell (i.e., binding and fusion) or that it could be mediated by viral major immediate-early (IE) proteins. It has been reported that the HCMV major IE protein IE2 alone, or in cooperation with IE1, can activate many mammalian and viral promoters in transient-transfection assays, including c-fos, c-myc, hsp70, adenovirus, SV40, and human T-cell leukemia virus promoters (1, 9, 11, 31). This activation is at least partially due to an interaction between IE2 and the TATA-binding protein (8, 10, 13). However, the activation of pElu-ISRE by HCMV infection was mainly independent of the HCMV major IE proteins, because UV-inactivated virus (lacking viral gene expression) (36) still was capable of activating the reporter (Fig. 2A and B). Thus, this experiment indicated that the HCMV-mediated activation of ISGs can be reproduced using this luciferase reporter assay and, further, an HCMV response element is located in this 560-bp fragment.

The GAS element is also a response to HCMV infection. GAS is another IFN response site present in some ISG promoters. Two GAS-like elements (VRS1) have been identified in the HCMV major IE promoter-enhancer regions, which play an important role in IE expression (20). To determine whether GAS elements are responsive to HCMV infection, the ISRE in pElu-ISRE was replaced with two GAS elements (identical sequences as in the major IE promoter), as indicated in Fig. 1B. Results from the luciferase assay demonstrated that, similar to IRSE, the GAS elements responded to UV-HCMV infection (Fig. 2C). Interestingly, both ISRE and GAS elements were able to support UV-HCMV-mediated transactivation; therefore, we named the elements containing this activity HCMV response sites (VRS).

In these luciferase assays, the fold activation could be quite different from experiment to experiment. This mainly was associated with differences in the basal levels of luciferase expression and was dependent on the VRS. When the VRS was mutated, the basal luciferase activity was significantly reduced, such that the fold activation was increased (Fig. 2A).
Infected cell proteins specifically interact with ISRE and GAS elements. To identify the proteins interacting with the VRS, we performed EMSA. The 120-bp fragments from pElu-ISRE, pElu-NRS, and pElu-GAS (Fig. 1B) containing ISRE, NRS, or GAS were amplified by PCR, labeled, and used as probes in EMSA. These probes are identical except for the middle 23 bp (Fig. 1B). Nuclear extracts were prepared from either mock-infected or 8-h UV-HCMV-infected HFF cells. The EMSA revealed no specific proteins interacting with the NRS probe; however, a few complexes did interact specifically with both the ISRE and the GAS probes. The bands were weak in using the mock-infected nuclear extract and became stronger after infection (Fig. 3A). In addition, a couple of bands were not observed in the mock-infected extract but were present in the HCMV-infected extracts. We also did not observe a difference in the VRS-binding activities between live HCMV and UV-HCMV nuclear extracts. Since the complexes with the VRS are similar between the mock-infected and virus-infected nuclear extracts, most, if not all, of these VRS-binding proteins are cellular proteins, not viral proteins, and either their nuclear abundance is increased or their VRS-binding activities are stimulated after HCMV infection. Interestingly, identical gel shift patterns were observed using either ISRE or GAS probes. To confirm whether the same protein complexes interacted with these two elements and to demonstrate specificity, competition experiments were performed with a 500-fold excess of unlabeled heterologous or homologous probe (Fig. 3B). Results from competition experiments showed that both the protein-ISRE and protein-GAS complexes competed with excess ISRE or GAS fragments, but not with the NRS fragment. These data indicated that ISRE and GAS sequences interact with either the same DNA-binding proteins or different proteins within the same protein complexes. Furthermore, these data suggest that HCMV activates ISGs via both ISRE and GAS elements.

Characterization of the VRS. To determine whether the consensus sequences of ISRE and GAS are crucial in the cellular response to HCMV infection, two point mutations were introduced into each ISRE and GAS element, as indicated in Fig. 4A. These mutated reporter plasmids, pElu-ISREm and pElu-GASm, along with two positive control plasmids, pElu-ISRE and pElu-GAS, and a negative control plasmid, pElu-NRS, were transfected into HFF cells. Each group of transfected cells was divided into two sets; one was infected with HCMV, and the other was infected with UV-HCMV and tested for luciferase activity. As a control, and consistent with an earlier experiment (Fig. 2), UV-HCMV activated the luciferase reporter with wild-type ISRE and GAS elements but did not activate the NRS promoter (Fig. 4B). However, UV-HCMV was no longer able to activate the promoters with the point mutations in ISRE or GAS (Fig. 4B). As a control, live HCMV (producing IE1 and IE2 proteins) was able to nonspecifically activate all reporters, indicating all transfection and luciferase assays were working. The above experiments showed that the consensus sequences of ISRE and GAS are important for activation of reporter genes by UV-HCMV. The DNA-binding activities of these elements also were tested by EMSA. Mock-infected and UV-HCMV-infected HFF nuclear extracts were reacted with wild-type and mutated ISRE and GAS probes (Fig. 4C). Binding was not detected when the NRS, ISREm, and GASm probes were used, which was consistent with the luciferase assays. Specific complexes were observed only when the wild-type ISRE and GAS probes were used in the reaction. Taken together, the luciferase assay and EMSA showed that the mutated VRS neither supported HCMV-mediated transactivation nor was involved in the formation of complexes with HCMV-activated proteins, indicating that the consensus sequences of ISRE and GAS are critical for activation of ISGs by HCMV.

IRF-3 is activated after HCMV infection but may not localize to the VRS-binding complex. IFN regulatory factors (IRFs) are a family of transcription factors that regulate both IFNs and ISGs. They share a conserved DNA-binding domain in their N-terminal portion. IRFs recognize DNA sequences that are similar to the ISREs (30). Recent studies have shown that some viruses stimulate a Jak-Stat-independent pathway in order to activate the genes with ISREs (2, 16, 18, 21–23, 32, 34). To determine whether IRF-3 is activated after HCMV infection and is responsible for induction of ISGs by interacting with the VRS, in vivo phosphorylation assays were performed to measure the phosphorylation state of IRF-3 after HCMV infection. As shown previously, IRF-3 was rapidly phosphorylated after HCMV infection at an MOI of 5 (2, 21) (Fig. 5B). More importantly, the phosphorylated IRF-3 reached a peak at 1 to 2 h postinfection (hpi) and was significantly reduced at 4 hpi (Fig. 5B and data not shown). The cellular localization of IRF-3 then was evaluated in immunofluorescence assays. HFF cells were infected with HCMV at an MOI of 5, and at the different time points postinfection the cells were fixed and
IRF-3 was determined using anti-IRF-3 monoclonal antibody. As illustrated in Fig. 5A, in mock-infected cells IRF-3 was localized in the cytoplasm, as shown by weak uniform staining (panel a). After infection, IRF-3 was translocated rapidly from the cytoplasm to the nucleus. The level of nuclear IRF-3 reached a peak at approximately 2 hpi (panel b). After 4 hpi (panels c to e), nuclear IRF-3 expression declined and cytoplasmic expression increased. By 8 hpi (panel e), very little nuclear IRF-3 was detected (Fig. 5B). These results suggest that IRF-3 is not directly involved in HCMV-initiated ISG induction, because the kinetics of IRF-3 translocation and ISG induction by HCMV do not overlap. It has been shown previously that after HCMV infection, ISGs are not induced to a detectable level until approximately 4 hpi, and expression typically peaks between 8 and 24 hpi (36). Furthermore, HCMV-induced ISG expression requires protein kinase C (PKC) activity: if PKC is blocked by inhibitor H7, ISG induction by HCMV is completely inhibited (20). When HCMV-infected cells were treated with H7, HCMV-induced IRF-3 translocation was not blocked (Fig. 5A, panel f). This result suggests that HCMV-induced IRF-3 translocation is not directly linked to ISG activation.

Two experiments were performed to determine whether IRF-3 is present in the VRS-binding complexes. In an EMSA, increasing amounts of anti-IRF-3 antibody, SL-12, were included in an EMSA reaction as described for Fig. 3. The VRS-specific binding complexes were not super-shifted or disrupted by the IRF-3 antibody. In previous studies, this same antibody was able to disrupt the interaction between IRF-3 and ISRE (32). In the second experiment, VRS-binding proteins were partially purified from HCMV-infected nuclear extracts by using a heparin column (Zhu, unpublished). The unbound proteins were removed by flushing the column with 0.1 M NaCl followed by 0.325 M NaCl, and the bound proteins were eluted with 1 M NaCl. The EMSA results indicated that VRS-binding activity was present in the 1 M NaCl fraction (Fig. 5D, left panel). However, immunoblotting results indicated that IRF-3 was not present in the 1 M NaCl fraction but in the unbound fraction (Fig. 5D, right panel). These results strongly suggest that HCMV-induced IRF-3 translocation is not directly linked to ISG activation.

Initial characterization of VRS-binding proteins. To further demonstrate that specific proteins are activated by HCMV infection and bound to the VRS, SELDI analysis was used. Specifically, HCMV-activated nuclear protein complexes were captured by PCR-generated, biotinylated VRS DNA fragments using ProteinChip technology, as described in Materials and Methods. The HCMV-infected (8 h) and mock-infected nuclear extracts were incubated with ISRE- or GAS-containing DNA fragments, we detected

FIG. 4. Mutagenesis of the VRS. (A) Two point mutations were introduced into the consensus sequences (bold) of ISRE and GAS as indicated to generate two mutant luciferase reporter plasmids, pElu-ISREm and pElu-GASm. (B) The mutant plasmids, along with the wild-type (pElu-ISRE and pElu-GAS) and ISRE deletion (pElu-NRS) plasmids, were transfected into HFF cells. Cells were mock infected or infected with HCMV or UV-HCMV for 8 h, and luciferase assays were performed. HCMV-induced luciferase activity (white bars) was set as 100% for each reporter plasmid. Luciferase activity in the mock-infected cells and UV-HCMV-infected cells is shown as black bars and gray bars, respectively. (C) The protein-binding activities of mutant ISRE and GAS were tested in EMSA as described in the legend for Fig. 3. The 120-bp fragments were generated from the plasmids by PCR and labeled for use as probes. The probes (PB, indicated on the top) were incubated with nuclear extracts (NE) from mock infected (M) or 8 h-HCMV-infected (c) HFF cells.
the same binding peptides that had been detected from the virus-infected cellular extract. However, the amounts of bound proteins were 5.4 to 6 times less for the 41.7-kDa protein (Fig. 6B and data not shown). These results are in agreement with observations from the EMSA (Fig. 3) and strongly suggest that HCMV infection activates several cellular proteins that specifically form complexes with the VRS.

**DISCUSSION**

The previous studies have shown that HCMV infection initiates a novel signal transduction pathway that leads to the induction of ISGs (35, 36). Later, it was discovered that the HCMV envelope glycoproteins, gB and gH, and subsequent virion fusion are required for this virus-induced activation (3, 19, 25). In this study, we demonstrated that ISRE and GAS elements are HCMV response sites (VRS). A number of cellular proteins are activated after HCMV infection that specifically interact with the VRS. These proteins have molecular masses between 19 and 41.7 kDa.

It was interesting and unexpected to determine that both ISRE and GAS elements, in fact, form identical complexes with HCMV-activated proteins. The ISRE normally interacts with IFN-α or -β-activated complexes or ISGF-3 containing Stat1, Stat2, and IRF-9 (p48). GAS normally interacts with the IFN-γ-activated Stat1 homodimer (6, 7). The fact that HCMV-activated complexes recognize both ISRE and GAS elements suggests that either a single protein in the complexes interacts with both ISRE and GAS or, alternatively, different proteins in the complexes interact with ISRE and GAS independently.

It is important to identify the VRS-binding proteins, because they are candidate proteins responsible for the induction of ISGs after HCMV-infection. The results from this study suggest that it is unlikely that the known ISRE- or GAS-binding proteins are present in the HCMV-activated complexes. First, HCMV does not appear to use known Stat proteins to activate ISGs (15–17). Stats are tyrosine phosphorylated transcription factors that are activated by Jak family kinases (6, 7). The members in this family include Stat1 to -6 (29). Stat1 and Stat2 are induced by IFN-α/β. However, in both an in vivo phosphorylation and translocation assays we were unable to detect activation of these proteins as a result of infection by HCMV (Zhu, unpublished). This result was consistent with the recent publications reporting that HCMV infection does not activate Stat1 and Stat2 and disrupts the Jak-Stat pathway (15–17).
Since other members in this family do not bind to ISRE or GAS elements (29), they are unlikely to be present in the HCMV-activated complexes. In addition, these Stat proteins contain 734 to 851 amino acids with molecular masses over 80 kDa, which is much larger than the potential VRS-binding proteins that were identified by SELDI (Fig. 6).

Secondly, HCMV does not appear to use most of the known IRFs to activate ISGs. IRFs are a family of transcription factors that regulate both IFNs and ISGs (30). They share a conserved DNA-binding domain in their N-terminal portion and recognize DNA sequences that are similar to the ISREs. Nine members of the IRF family have been reported in the human and mouse (30), with molecular masses ranging between 40 and 65 kDa. The expression of IRF-4 and IRF-8 is restricted to hematopoietic cells; these IRFs should not be in the HCMV-induced complexes generated in this study. Recent studies have suggested the existence of a Jak-Stat-independent pathway for induction of ISGs, and IRF-3 is a key factor in this pathway. This process requires the formation of a very large virus-activated factor complex that contains the cellular proteins IRF-3 and IRF-7 and the transcriptional coactivator proteins p300 and CBP (16, 18, 22, 23, 32, 34). However, our results indicate that IRF-3 is neither present in the VRS-binding complexes nor directly responsible for HCMV-mediated ISG induction, for the following reasons: (i) the IRFs/CBP/P300 complex observed by Wathelet et al. (32) is very large and migrates slowly in EMSA, which is markedly different from the complexes that we observed (Fig. 3, native gel conditions). (ii) A monoclonal antibody (SL-12) against IRF-3 had no effect on VRS-binding complexes in our EMSA (Fig. 5C). This same antibody was shown previously to completely abolish formation of an IRF-3/ISRE complex (32). (iii) The previous results showed that the induction of ISGs by HCMV was hardly detectable until 4 hpi and peaked after 8 hpi (36). Therefore, ISG induction is not detectable when IRF-3 peaks in the nucleus (1 to 2 hpi). However, by the time ISG is strongly activated, the nuclear IRF-3 level is greatly reduced (Fig. 5A). (iv) The PKC inhibitor H7 completely blocks HCMV-induced ISG activation (20) but does not block HCMV-induced IRF-3 translocation (Fig. 5A, panel f), indicating that IRF-3 translocation is not sufficient for ISG activation. (v) Chromatographic fractionation of VRS-binding proteins indicates that the fractions containing VRS-binding activity do not contain the IRF-3 protein (Fig. 5D). (vi) Lastly, IRF-3 is a 50-kDa protein, and the SELDI analysis indicated that the largest protein in these complexes was 41.7 kDa (Fig. 6). Taken together, these data suggest that either IRF-3 is not directly involved in the HCMV-mediated ISG induction or it is not sufficient for activation. If IRF-3 is involved indirectly, other factors, activated later, are required also.

It is a surprise that HCMV inactivates the IFN-induced Jak-Stat signal transduction pathway (15–17) but induces another signal transduction pathway to induce ISGs (35, 36). Why does HCMV induce ISGs that contain antiviral activities? What is the biological significance of this HCMV-initiated signal transduction pathway? Our recent studies have started to find the possible answers. We showed that this pathway regulates HCMV major IE expression through two GAS-like elements (VRS1) in the major IE promoter-enhancer region. Mutation of these two elements blocks the signal transduction pathway and dramatically reduces major IE gene expression and viral replication at low-multiplicity infection (20).

We speculate that HCMV infection activates the cytoplasmic VRS-binding proteins. These proteins translocate into the nucleus and form a complex with the VRS in the ISG promoter and then activate transcription. Identification of the VRS sequences provides a powerful tool to identify HCMV-activated proteins involved in ISG induction. Our current studies are
geared toward the purification and identification of VRS-bind-

REFERENCES

1. Biegalke, B. J., and A. P. Geballe. 1991. Sequence requirements for activa-


6. Darnell, J. E., Jr. 1998. Studies of IFN-induced transcriptional activation un-


tion: activation of a transcription factor complex containing IRF-3 and CBP/p300. EMBO J. 17:1087–1095.

