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_J Immunol_ 2006; 177:7094-7102; doi: 10.4049/jimmunol.177.10.7094

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Human Cytomegalovirus Envelope Glycoproteins B and H Are Necessary for TLR2 Activation in Permissive Cells

Karl W. Boehme,* Mario Guerrero,*† and Teresa Compton2*†

Human CMV (HCMV) is a ubiquitous member of the Herpesviridae family and an opportunistic pathogen that poses significant health risks for immunocompromised patients. HCMV pathogenesis is intimately tied to the immune status of the host, thus characterization of the innate immune response to HCMV infection is critical for understanding disease progression. Previously, we identified TLR2 as a host factor that detects and initiates inflammatory cytokine secretion in response to HCMV independent of viral replication. In this study, we show that two entry-mediating envelope gp, gp B (gB) and gp H (gH), display determinants recognized by TLR2. Neutralizing Abs against TLR2, gB and gH inhibit inflammatory cytokine responses to HCMV infection, suggesting that inflammatory cytokine stimulation by HCMV is mediated by interactions between these envelope gp and TLR2. Furthermore, both gB and gH coimmunoprecipitate with TLR2 and TLR1, indicating that these envelope gp directly interact with TLR2 and that a TLR2/TLR1 heterodimer is a functional sensor for HCMV. Because our previous studies were conducted in model cell lines, we also show that TLR2 is expressed by HCMV permissive human fibroblast cell strains, and that TLR2 is a functional sensor in these cells. This study further elucidates the importance and potency of envelope gp as a class of molecules displaying pathogen-associated molecular patterns that are recognized with immediate kinetics by TLRs in permissive cells. The Journal of Immunology, 2006, 177: 7094–7102.

We recently identified TLR2 as a host factor that activates inflammatory cytokine secretion in response to HCMV (17). The TLRs are a family of pathogen-recognition receptors that initiate innate immune responses to a myriad of invading microbes, including viruses (18, 19). Eleven mammalian TLRs have been identified, and they are predominantly expressed on phagocytic cells such as dendritic cells and macrophages; however, most cells express at least a subset of TLRs (19). The primary consequences of TLR activation include NF-κB activation, inflammatory cytokine secretion, dendritic cell maturation, up-regulation of immune co-stimulatory molecules, and for a subset of TLRs, the production of type I IFN (19–22). TLRs detect microorganisms on the basis of unique molecular structures termed pathogen-associated molecular patterns (PAMPs). Analysis of the innate response to bacterial PAMPs such as LPS, peptidoglycan, and unmethylated CpG DNA are a cornerstone of TLR research, and great strides have been made in our understanding of the relationship between bacteria and the innate immune system (23–28). In contrast, the mechanisms by which the TLR system recognizes and responds to viruses have only begun to be explored. Viral genomic nucleic acids are one major class of PAMP. TLR3 (dsRNA), TLR7 (ssRNA), TLR8 (ssRNA), and TLR9 (CpG DNA) (29–33) signal from the endosome (34–38) where degradation of virus particles exposes the viral genome for detection by this panel of TLRs (29, 31, 32). Although significantly less well studied, envelope gp that decorate the exterior of the virion are an emerging class of TLR activators (18). To date, three envelope gp have been identified as TLR agonists. The fusion protein from respiratory syncytial virus and the mouse mammary tumor virus envelope protein activate TLR4, while the hemagglutinin protein from measles virus activates TLR2 (39–42). Interestingly, a shared feature of these gp is that they play critical roles in the entry of their respective viruses, and this shared feature suggests that the molecular machinery used by viruses for entry is also targeted by the innate immune system (43, 44).

Although we demonstrated previously that TLR2 is activated by HCMV, the molecular trigger for TLR2 has not been determined.
In contrast with the RNA viruses listed above, HCMV displays as many as 12 envelope gp, four of which are required for entry. gp B and gp H works in concert with a tripartite complex comprised of gp H (gH), gp L (gL), and gp O (gO) to mediate the binding and entry of HCMV virions into host cells (45–48). In addition to their roles in entry, there is a growing body of evidence that gB and gH elicit responses from cells that are reminiscent of TLR activation. Abs against gB and gH block the induction of various innate markers, including NF-kB (49, 50), and cells expressed to soluble forms of gB activate NF-kB (50). Based on these observations, we hypothesized that gB and gH are the target of innate sensing by the host cell. In this study, we show that HCMV gB and gH activate TLR2 and associate with TLR1 and TLR2. Abs against gB and gH, but not gL, inhibit the inflammatory cytokine response to HCMV, and both gB and gH coimmunoprecipitate with TLR2 and TLR1, indicating that the functional sensor for HCMV is a TLR2/TLR1 heterodimer. We also extend our initial studies to HCMV permissive human fibroblast cells and show that TLR2 mediates NF-kB activation and inflammatory cytokine responses in cells that support productive HCMV infection.

Materials and Methods

Cell lines, reagents, and viruses

Human embryonic kidney (HEK) 293T cells (American Type Culture Collection) and normal human dermal fibroblast (NHDF) (Cambrex) cells were grown in 5% CO2 in DMEM (Invitrogen Life Technologies) supplemented with 10% FBS (HyClone) and 1% penicillin-streptomycin-ampicillin (PSF; BioWhittaker). Monomac-6 cells were maintained in Ham’s F12 medium supplemented with 10% FBS and 1% PSF in a 5% CO2 environment. LPS (from Escherichia coli 0111:B4) was obtained from Sigma-Aldrich and repurified by phenol extraction as described previously (54). Recombinant human IL-1β was obtained from R&D Systems, PamC3SK was obtained from EMC Microcollections, and soluble CD14 (sCD14) was from Biometec. The AD169 strain of HCMV was propagated in NHDF cells. Virion particles were purified from infected supernatants by density-gradient centrifugation (49–53). The supernatants by 10-column volumes of wash buffer (50 mM NaCl, 20 mM imidazole (pH 8.0)) containing Sepharoc S-200 substrate (Amersham Biosciences) in 1X PBS (Invitrogen Life Technologies) and run through by gravity flow at 4°C. Collected fractions were stored at −80°C.

Construction and generation of TLR2ΔC and TLR4ΔC-encoding retroviruses

The mutants were constructed using full-length FLAG epitope-tagged TLR2 and TLR4 provided by B. Williams (Cleveland Clinic Foundation, Cleveland, OH). The TLR2 and TLR4 cytoplasmic tails were deleted by PCR mutagenesis using a common upstream primer (5′-TAA TAT ACC GGT GCC ACC ATG TCT GTA CTT ATC C-3′) incorporating an AgeI restriction site and the TLR2-specific (5′-TTA AAT GCG GCC GCT TAT GTA TTT CAT ATA CCA CAG GCC-3′) and TLR4-specific (5′-TTA AAT GCG GCC GCC TAT GTG TTT CAT GTG TCA GCC AGC AAG ACC C-3′) downstream primers incorporating NcoI restriction sites. The fragments were digested and cloned into the retroviral transfer vector pCMMP.MCS.ires-GFP (a gift from B. Sugden, University of Wisconsin, Madison, WI). The constructs were confirmed by sequencing (University of Wisconsin Biotechnology Center) and recombinant retroviruses were generated as described previously (59). NHDF cells were transduced with retroviruses encoding TLR2ΔC, TLR4ΔC, or an empty vector control in a minimal volume for 1 h in the presence of 5 μg/ml polybrene. At 96 h posttransduction, GFP-positive cells were collected by FACS and used as indicated.

Cytokine ELISAs

Ninety-six-well plates were seeded with cells at a density of 5000 cells per well. At 24 h postgrowth, the growing medium was removed and replaced with serum-free DMEM. After 24 h serum starvation, the cells were challenged as indicated. At 18 h postchallenge, the supernatants were harvested and IL-6 or IL-8 levels were determined by ELISA. OptEIA IL-6 or IL-8 dual Ab detection assay (BD Pharmingen) was used according to the manufacturer’s instructions. For blocking Ab studies, virions were preincubated for 15 min with isotype control or anti-TLR2 Abs (eBioscience), anti-gB-27-78 or 9-3 (60), or anti-gH-14-4b (61) mouse mAbs at 100 μg/ml. For Gram, a rabbit polyclonal anti-gB and anti-gH (63) or rabbit IgG (Sigma-Aldrich) were used for blocking cell line. The gB and gH coding sequences from HCMV strain AD169 were cloned previously into the pCAGGS expression vector (63). The vector pCV55 encoding vesicular stomatitis virus G (VSV-G) was a gift from Y. Kawaoka (University of Wisconsin, Madison, WI), pFLAG-TLR1, pFLAG-TLR2, and pFLAG-TLR6 plasmids were donated by B. Williams (Cleveland Clinic Foundation). For coimmunoprecipitation experiments, 293T cells were cotransfected with plasmids encoding eGFP, gB, gH, or an empty vector control in a minimal volume for 1 h in the presence of 5 μg/ml polybrene. At 96 h posttransduction, GFP-positive cells were collected by FACS and used as indicated.

Coomassie blue staining and immunoblotting

The gB, gH, and gL coding sequences from HCMV strain AD169 were cloned previously into the pcDNA expression vector (63). The vector pCV55 encoding vesicular stomatitis virus G (VSV-G) was a gift from Y. Kawaoka (University of Wisconsin, Madison, WI), pFLAG-TLR1, pFLAG-TLR2, and pFLAG-TLR6 plasmids were donated by B. Williams (Cleveland Clinic Foundation). For coimmunoprecipitation experiments, 293T cells were cotransfected with plasmids encoding eGFP, gB, gH, or an empty vector control in a minimal volume for 1 h in the presence of 5 μg/ml polybrene. At 96 h posttransduction, GFP-positive cells were collected by FACS and used as indicated.
the following Abs: anti-gB 27-78 (60), anti-gH 6824 (62), anti-gL 6394 (62), anti-NS1G-15F9 (Sigma-Aldrich), or anti-FLAG M2 (Sigma-Aldrich). For the radiolabeled immunoprecipitation assay, the cells were transfected with gB and FLAG-TLR2 expression constructs individually or in combination. At 24 h posttransfection, the medium was replaced with DMEM supplemented 10% FBS, 1% PSF, and 150 μCi/mL 35-S-express label (NEN-DuPont). The cells were harvested at 48 h posttransfection and processed as described above. The immunoprecipitation products were resolved by 10% SDS-PAGE, the gel was dried to Whatman paper, and exposed to film. Images were collected using a Typhoon phosphor imager.

**IκBα degradation assay**

Cells were serum starved for 24 h before infection and pretreated with cycloheximide (100 μg/ml) for 1 h before infection. The cells were treated with IL-1β (100 pg/ml), Pam3CSK4 (20 μg/ml), LPS (1 μg/ml) plus 4 (1 μg/ml), or infected with UV-HCMV (multiplicity of infection (MOI) = 10) as measured before UV treatment. At 3 h posttreatment, the cells were harvested by scraping in Nonidet P-40 lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 30 mM NaF, 5 mM EDTA, 10% glycerol, 40 mM 2-glycerophosphate; Sigma-Aldrich), 1 mM Na3VO4 (Sigma-Aldrich), 0.1 mM of PMSF, protease inhibitor mixture (56), and 1% Nonidet P-40. The cells were subjected to two freeze-thaw cycles, and insoluble material was removed by microcentrifugation (13,000 rpm, 5 min, 4°C). The total protein content of each sample was quantitated using the Bio-Rad protein assay reagent. Equivalent amounts of total protein for each sample were separated by 10% SDS-PAGE, IκBα and actin levels were analyzed by immunoblotting as described previously (56) using anti-IκBα (sc-371; Santa Cruz Biotechnologies) and anti-actin Abs. Densitometry was performed using the ImageQuant software system (Amersham Biosciences).

**Statistical analysis**

The means of triplicate samples were compared using an unpaired Student’s t test with GraphPad Prism software (version 4.00; GraphPad).

**Results**

**gB and gH elicit inflammatory cytokine responses from cells**

To assess the ability of envelope gp to elicit inflammatory cytokine responses we used a panel of neutralizing Abs to block interactions between gB and gH and receptors on the surface of the cell (Fig. 1A). Transcriptionally inert UV-inactivated HCMV virions (UV-HCMV) were incubated with Abs for 15 min before infection, and IL-6 levels were measured by ELISA at 18 h postinfection as a marker of inflammatory cytokine activation. IL-6 levels were diminished by pretreatment of virions with gB (27-78 and 9-3) and gH-specific (14-4b) Abs, whereas the isotype control Ab had a modest effect on the IL-6 response. Furthermore, a rabbit polyclonal Ab against gL did not affect the IL-6 response (data not shown). These data suggest that gB and gH, but not gL, are interacting with cell surface receptors that elicit inflammatory cytokine secretion.

Additionally, the capacity of a soluble form of gB to activate TLR2 was assessed. The ectodomain of gB (HCMV strain AD169) was fused to the eGFP (gB-sGFP), the resulting protein purified and used to challenge HEK cells expressing CD14 alone (HEK/CD14), HEK cells expressing CD14 together with TLR2 or TLR4, respectively, and, consistent with our previous study, HCMV virions only activated cells expressing TLR2 (17). Similarly, gB-sGFP induced IL-8 secretion in a TLR2-dependent manner. A soluble eGFP control did not elicit cytokine responses. These data further support the hypothesis that HCMV envelope gp serve as agonists for TLR2.

**gB and gH physically associate with TLR2 and TLR1**

TLR ectodomains are composed of varying numbers of leucinerich repeats, a motif that is commonly involved in protein–protein interactions (64). Based on the preceding data, we hypothesized that gB and gH directly interact with TLR2. To test this hypothesis, we performed coimmunoprecipitation experiments from 293T cells cotransfected with gB and FLAG-TLR2 expression constructs. FLAG-TLR2 was immunoprecipitated with anti-FLAG Ab-conjugated agarose beads, the proteins resolved by SDS-PAGE, and products detected by immunoblotting for FLAG-TLR2 or gB (Fig. 2A). A dose-dependent pulldown of gB was observed from cells cotransfected with a constant amount of gB expression plasmid and increasing levels of FLAG-TLR2 expression plasmid. The amount of gB precipitated increased in proportion to the level of FLAG-TLR2 input. Immunoprecipitations from 35-S-labeled cells confirmed that coexpression of both gB and FLAG-TLR2 is required for this interaction (Fig. 2B). gH also coprecipitated with TLR2 indicating that both gB and gH physically interact with TLR2 (Fig. 2E). The envelope gp from VSV-G was included as a specificity control and did not coprecipitate with any of the TLRs tested (Fig. 2G).

In vivo, TLR2 functions as a heterodimer in combination with either TLR1 or TLR6 (65, 66). To determine the interacting partner for TLR2, gB and gH coimmunoprecipitation experiments were performed with TLR1 and TLR6. Both gB and gH coprecipitated with TLR1, but not TLR6, suggesting that the operon sensor for HCMV gB and gH is a TLR2/TLR1 heterodimer (Fig.
In contrast, gL did not coprecipitate with TLR2, TLR1, or TLR6 (Fig. 2F). These results are consistent with inability of anti-gL Abs to inhibit the inflammatory cytokine response to HCMV. Together, these results indicate that gB and gH directly interact with a TLR2/TLR1 heterodimer to activate inflammatory cytokine responses from cells.

To appreciate the relationship between TLR2 and HCMV in a context that is physiologically relevant to the life cycle of HCMV, it is critical to use cells that are fully permissive for HCMV infection. The initial studies that identified TLR2 as a host factor mediating innate responses to HCMV used cell types that do not support HCMV replication (17). Thus, we endeavored to translate our findings into HCMV permissive human fibroblast cells, the best-characterized cell culture system for the study of HCMV. The TLR repertoire of NHDF cells has not been reported, and it is not known whether these cells express TLR2. RT-PCR analysis of total RNA from NHDF cells revealed the presence of TLR2 (Fig. 3), as well as TLR1, TLR6, and TLR4 (data not shown). RNA harvested from Monomac-6 and 293T cells were included as positive and negative controls for TLR2 expression, respectively (Fig. 3). Efforts to detect TLR2 protein expression in NHDF cells have been unsuccessful; however, the presence of the TLR2 transcript, coupled with the ability of NHDF cells to respond to the synthetic TLR2 ligand Pam3CSK4 (Fig. 4), indicates that these cells express TLR2. Additionally, TLR1, TLR6, and TLR4 transcripts were detected in NHDF cells by RT-PCR (data not shown). NHDF cells secrete IL-6 in response to zymosan (data not shown).

To determine whether TLR2 mediates innate responses to HCMV in NHDF cells, we tested the effect of an anti-TLR2 Ab on the cytokine response to HCMV (Fig. 4). Compared with medium alone (No Ab), an isotype control Ab had no effect on the IL-6 response to UV-HCMV infection, the TLR-independent control
IL-1β, or the synthetic TLR2 ligand Pam3CSK4. In contrast, the levels of IL-6 secreted in response to UV-HCMV and the TLR2 control Pam3CSK4 were dramatically reduced by pretreatment with the TLR2 blocking Ab, but no effect was observed on the response to IL-1β. These results indicate that inflammatory cytokine responses to HCMV in permissive NHDF cells are mediated by TLR2.

To further address the role of TLR2 in NHDF cells, we constructed dominant-negative versions of TLR2 and TLR4 by removing their cytoplasmic tails (TLR2ΔC and TLR4ΔC, respectively) (Fig. 5A). These signaling-defective constructs lack the TLR1 domain common to all TLRs and cannot recruit the cytoplasmic adaptor molecules that propagate downstream signaling events (67). NHDF cells were transduced with recombinant retroviruses that coexpress FLAG epitope-tagged TLR2ΔC or TLR4ΔC in combination with eGFP (59). A control population expressing a GFP vector was also generated. GFP-positive cells were collected by FACS and expression of the dominant-negative constructs was confirmed by immunoprecipitation and immunoblotting (Fig. 5B).

IL-6 secretion was used as a marker of inflammatory cytokine activation after challenge of the dominant-negative cell panel with UV-HCMV (Fig. 6). The GFP vector control cells responded normally to all stimuli, including UV-HCMV. TLR2ΔC-expressing cells responded normally to IL-1β and LPS/sCD14, the TLR4 ligand. However, these cells displayed a reduced response to UV-HCMV and the TLR2 control ligand Pam3CSK4, confirming that TLR2 mediates inflammatory cytokine responses to HCMV in permissive NHDF cells. The IL-6 response from TLR4ΔC-expressing cells to IL-1β and Pam3CSK4 were unaffected, and as predicted, the response to LPS/sCD14 was completely eliminated. Interestingly, the response to UV-HCMV was partially diminished in these cells. No role for TLR4 was found in previous studies (17); however, these experiments were performed in nonpermissive
cells. It is possible that TLR4 is involved in the innate response to HCMV in permissive cells and this possibility is currently under consideration.

**TLR2 mediates NF-κB activation in response to HCMV infection**

Another signature TLR response is activation of the pleiotropic transcription factor NF-κB (19). Previous studies have shown that HCMV activates NF-κB within minutes after infection, kinetics that are suggestive of receptor-induced signaling (50, 68, 69). To determine whether TLR2 mediates NF-κB activation upon HCMV infection, we used our dominant-negative TLR cell panel to assess the degradation of IκBα as a marker of NF-κB activation (Fig. 7).

IκBα binds and sequesters NF-κB in the cytoplasm as part of a transcriptionally inactive complex (70). Many stimuli, including TLRs, induce IκBα degradation thereby releasing NF-κB to translocate to the nucleus where it complexes with numerous other factors to modulate transcription. Thus, the loss of IκBα though degradation correlates with the activation of NF-κB. In GFP control cells IL-1β caused complete IκBα degradation, whereas Pam3CSK4 and LPS + sCD14 induced a lesser degree of degradation. Furthermore, IκBα degradation is blocked in response to Pam3CSK4 and LPS + sCD14 in TLR2ΔC and TLR4ΔC-expressing cells, respectively. Similar results were observed in a second experiment. In response to UV-HCMV near-complete degradation of IκBα is observed in GFP vector control and TLR4ΔC-expressing cells. However, in cells expressing TLR2ΔC, the level of IκBα degradation is reduced. Densitometric analysis of the blots indicates that, although dominant-negative TLR2 completely prevents IκBα degradation in response to the TLR2 control ligand Pam3CSK4, IκBα degradation in response to HCMV infection is not completely blocked (Fig. 7, lower panel). This observation suggests that TLR2 is not the only mechanism by which HCMV can activate NF-κB. Together, these observations indicate that TLR2 mediates a portion of NF-κB activation in response to HCMV infection and further support the hypothesis that TLR2 is a key cellular factor for the innate immune response to HCMV.

**FIGURE 7.** HCMV activates NF-κB via TLR2 in permissive fibroblasts. NHDF cells expressing a GFP control vector (A), TLR2ΔC (B), or TLR4ΔC (C) were treated with cycloheximide for 30 min before challenge. The cells were mock infected, treated with IL-1β (1 pg/ml), Pam3CSK4 (40 μg/ml), LPS (1 μg/ml) + sCD14 (1 μg/ml), or infected with UV-inactivated HCMV at a MOI of 10. At 3 h postchallenge, whole-cell lysates were prepared. IκBα and actin levels were determined by SDS-PAGE analysis followed by immunoblotting (left panel). Right panel. The immunoblot shown in the upper panel was subjected to densitometric analysis using ImageQuant software. The intensity of the IκBα bands for each sample was normalized to the intensity of the corresponding actin band. The resulting IκBα intensity was plotted as a percentage of the IκBα signal in the mock-infected cells for each transductant. The results are indicative of two independent experiments.
Discussion

The goal of this study is to further elucidate the relationship between viruses and the host innate immune response. We previously identified TLR2 as a cellular factor that mediates innate immune responses to HCMV infection (17). However, many questions remain with respect to the mechanism by which HCMV activates TLR2, as well as the effect of TLR2 activation on the virus. In this study, we show that two HCMV envelope gp, gB and gH, activate TLR2. mAbs against both gB and gH inhibit cytokine responses to HCMV, and both gB and gH physically associate with TLR2 in coimmunoprecipitation experiments. gB and gH also coprecipitate with TLR1, but not TLR6, indicating that the functional sensor for HCMV is a TLR2/TLR1 heteromeric complex. Our previous studies used cell types that do not support HCMV infection, such as HEK and CHO indicator lines and murine fibroblasts. In this study, we extend our studies into HCMV permissive human fibroblasts, which allows for a greater appreciation for the role that TLR2 plays in HCMV biology. Although human fibroblasts have been shown previously to respond to TLR2 ligands, we demonstrate in this study that these cells express TLR2 mRNA (71, 72). Using TLR2 function-blocking Abs and dominant-negative TLR constructs, we show that TLR2 mediates NF-κB activation and inflammatory cytokine responses to HCMV in these cells. Together, these data add to the growing body of evidence suggesting that HCMV can activate innate immunity during binding and entry into host cells. We propose that envelope gp gB and gH, already well appreciated for their roles as mediators of virus entry, also interact directly with TLR2 and TLR1 during entry to initiate a signaling cascade that results in the activation of NF-κB and secretion of inflammatory cytokines.

HCMV gB and gH brings the number of viral envelope gp that are detected by TLRs to five (39–42). Notably, a shared feature of all of these gp is that they play critical roles in the binding and entry of their respective viruses (43–47, 60, 73–79). Viral envelope proteins are a compelling target for the TLR system, as they are the first component of the virus to come into contact with the cell. Consequently, detection of viral envelope gp would allow the cell to set the innate response in motion at the earliest stages of infection, perhaps even before the virus entering the cell. The rapid recognition and response could provide a temporal advantage for the host immune response, which would be extremely beneficial for combating a viral infection.

Activation of TLRs by envelope gp also suggests that the processes of virus entry and innate immune activation are coordinated. The viral envelope is studded with numerous copies of each gp, and each copy is able to interact with one or more cellular receptors. Multiple interactions between viral envelope proteins and different types of cellular receptors may induce the formation of an organized structure reminiscent of the immunological synapse (80). This type of receptor clustering would allow the cell to synchronize innate immune activation with the process of viral entry. Cellular integrins have been identified as receptors for HCMV gB and gH (46, 47) and have also been linked to TLRs (81, 82). It is possible that HCMV binding to integrins could facilitate interaction with TLR2/TLR1 heterodimers. Furthermore, receptor clustering may provide a mechanism by which with integrin and TLR signaling can be coordinated. Fig. 7 indicates that HCMV-mediated NF-κB activation is only partially attributable to TLR2. As NF-κB activation is also a downstream consequence of integrin usage, it is possible that TLR2 and integrins both contribute to the activation of NF-κB upon HCMV infection (83). However, it remains to be determined whether NF-κB activation by TLR2 and integrins is coordinated or coincidental.

In addition to HCMV, several other members of the Herpesviridae family activate innate immunity through TLRs. Herpes simplex virus type 1 (HSV-1), HSV-2 and mouse CMV harbor CpG-rich genomes that activate TLR9 (32, 33, 84, 85), and HSV-1 and varicella-zoster virus activate TLR2 (86, 87). An emerging possibility is that herpesviruses are subject to innate detection by multiple TLRs, with each TLR providing a distinct contribution to the overall response. For instance, TLR2 is associated with inflammatory cytokine responses, whereas TLR9 elicits the secretion of type I IFNs. Using multiple TLRs would allow the host to tailor its response to fit the pathogen through the combined actions of each TLR. In addition, HCMV infects a variety of cell types in vivo, including fibroblasts, endothelial cells, epithelial cells, monocytes/macrophages, smooth muscle cells, stromal cells, neuronal cells, and hepatocytes (4, 5), and each of these cell types may express a unique subset of TLRs and respond differently to HCMV infection. Thus, it is possible that the cell type infected and the different combinations of TLRs activated may have a profound influence on the outcome of infection. Experiments addressing the role of multiple TLRs simultaneously will provide valuable insights into how each TLR influences the global immune response to herpesviruses.

HCMV, like all herpesviruses, establishes a lifelong association with the host as a latent infection. To accomplish this goal, HCMV maintains a particularly close relationship with the host immune system and employs multiple immune modulation strategies that allow it to avoid detection by the host and persist in the face of a potent immune response (3). Because of this close relationship, it is tempting to speculate that HCMV may have adapted to use TLR responses to its advantage. HCMV disseminates in neutrophils and monocytes, and CD14-positive cells are hypothesized as a reservoir for latent virus (88). Each of these cell types are either activated by TLRs or are subject to recruitment by the mixture of cytokines and chemokines that result from TLR activation. Thus, HCMV may have evolved to use TLR responses as a means of recruit its dissemination and latency vehicles to the site of infection, where these cells could then become infected. Further examination of the role that TLRs play at both the cellular and organismal levels may provide further clues toward understanding the complex relationship between herpesviruses and their hosts.

Acknowledgments

We thank Bryan Williams (Cleveland Clinic Foundation) for the TLR1, TLR2, TLR4, and TLR6 plasmids; and Yoshi Kawaoka and Bill Sugden (both from the University of Wisconsin, Madison, WI) for the VSV-G construct and retroviral vectors, respectively. We also thank members of the Compton laboratory for critical review of the manuscript.

Disclosures

The authors have no financial conflict of interest.

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