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Human cytomegalovirus US3 modulates destruction of MHC class I molecules

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ABSTRACT

Human cytomegalovirus (HCMV), a member of the *Herpesviridae* family, is proficient at establishing lifelong persistence within the host in part due to immune modulating genes that limit immune recognition. HCMV encodes at least five glycoproteins within its unique short (US) genomic region that interfere with MHC class I antigen presentation, thus hindering viral clearance by cytotoxic T lymphocytes (CTL). Specifically, US3 retains class I within the endoplasmic reticulum (ER), while US2 and US11 induce class I heavy chain destruction. A cooperative effect on class I down-regulation during stable expression of HCMV US2 and US3 has been established. To address the impact of US3 on US11-mediated MHC class I down-regulation, the fate of class I molecules was examined in US3/US11-expressing cells and virus infection studies. Co-expression of US3 and US11 resulted in a decrease of surface expression of class I molecules. However, the class I molecules in US3/US11 cells were mostly retained in the ER with an attenuated rate of proteasome destruction. Analysis of class I levels from virus-infected cells using HCMV variants either expressing US3 or US11 revealed efficient surface class I down-regulation upon expression of both viral proteins. Cells infected with both US3 and US11 expressing viruses demonstrate enhanced retention of MHC class I complexes within the ER. Collectively, the data suggests a paradigm where HCMV-induced surface class I down-regulation occurs by diverse mechanisms dependent on the expression of specific US genes. These results validate the commitment of HCMV to limiting the surface expression of class I levels during infection.

1. Introduction

Viruses are organisms that subvert the cellular machinery of the host in order to replicate their genetic material and create infectious progeny. This dependence on living hosts for reproduction creates a unique interplay between these invaders and the components of the immune system. Indeed, the earliest steps of viral entry into the cell can be accompanied by the activation and initiation of a potent immune response (Liu et al., 2011). The ability of the host to recognize viral infection becomes paramount to halting the progression of disease and containing dissemination. The remarkable human immune defenses are highly coordinated and rely on the interaction of secreted proteins, receptor-mediated signaling, and intimate cell–cell communication. The competence of the host immune response determines how quickly and efficiently the pathogen is resolved as well as the severity of infection.

The interplay between virus and host is dynamic and evolution has seemingly provided viruses with mechanisms for bypassing, modulating, or disarming many facets of host immune defenses (Powers et al., 2008). Indeed, the genomes of many successful pathogens encode gene products that modify numerous steps of the immune response (Tortorella et al., 2000b). Human cytomegalovirus (HCMV) has the ability to initiate productive, or lytic, replication or to undergo a latent phase of infection with periodic bouts of reactivation. In order to persist indefinitely within the host, HCMV has evolved elaborate strategies to subvert cellular immune responses (Jackson et al., 2010). Viral functions targeting antigen presentation by major histocompatibility complex (MHC) class I molecules have been well documented (Tortorella et al., 2000a). By attenuating signaling by MHC class I molecules the virus circumvents clearance for a period of time by pathogen-specific cytotoxic T-lymphocytes (CTLs), thus allowing replication at a time when the virus is most vulnerable to host responses.

Abbreviations: CTL, cytotoxic T lymphocytes; ER, endoplasmic reticulum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HCMV, human cytomegalovirus; MHC, major histocompatibility complex; m.o.i., multiplicity of infection; US, unique short.

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The unique short (US) region of the HCMV genome encodes at least five endoplasmic reticulum (ER) resident glycoproteins (US2, US3, US6, US10, and US11) that modulate the cell surface expression of MHC class I molecules (Loenen et al., 2001). In addition, the major tegument protein pp71 has been suggested to modulate class I surface expression (Trgovcich et al., 2006). Each of these proteins is expressed at distinct phases of the viral life cycle and appear to target individual components of the MHC class I antigen presentation pathway. The immediate early US3 gene product retains class I molecules within the ER through inhibition of the tapasin/TAP interaction necessary for optimal peptide loading, in combination with modulating protein disulfide isomerase (PDI) stability (Park et al., 2004, 2006). The US2 and US11 genes encode early viral proteins that cause the rapid destabilization and destruction of class I heavy chains through a process mimicking endogenous ER quality control (Wiertz et al., 1996a,b). Infection with HCMV mutants expressing either US2 or US11 demonstrate an incomplete protection from CD8+ T-cell recognition (Besold et al., 2009), while co-expression of US2 and US3 resulted in almost complete down-regulation of MHC class I molecules from the cell surface (Noriega and Tortorella, 2009).

In this study, we examined the fate of class I molecules during the expression of HCMV US3 and US11. The co-expression of these viral proteins in cells led to enhanced ER-retention of class I and diminished proteasomal degradation properties, thus demonstrating a dominance of US3 function over US11. Furthermore, infection with viral mutants expressing both US3 and US11 corroborated these findings during the course of infection. This novel relationship between viral-encoded immune modulators demonstrates a more complex control of host defenses by HCMV than initially suspected.

2. Materials and methods

2.1. Cells

Human U373-MG astrocytoma cells, U373 transfectants that stably express HCMV US gene products, and MRC5 fibroblasts were maintained in Dulbecco's modified Eagle's medium supplemented with 8% fetal bovine serum (FBS), 1 mM HEPES, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere (95% air/5% CO_2). Gpg-293 cells (BD Biosciences) were utilized to generate retroviruses and were cultured in media similar to U373 cells. Primary human foreskin fibroblasts (HFF) were grown in minimal essential medium (MEM; PAA, Cölbe, Germany), supplemented with 10% FBS (Invitrogen, Karlsruhe, Germany), 2 mM L-glutamine, 50 mg/L gentamycin and 0.5 ng/mL basic fibroblast growth factor (bFGF; Invitrogen, Karlsruhe, Germany).

2.2. Antibodies and cDNA constructs

Rabbit polyclonal anti-US11 antibody was raised against the bacterially expressed GST-tagged lumenal domain of US11 (aa. 21–180). Rabbit polyclonal anti-US3 antibody was a gift from Dr. Hidde Ploegh (Whitehead Inst., Cambridge, MA). The monoclonal antibodies W6/32 (recognizes folded class I molecules) and HC10 (recognizes class I heavy chains) were purified from hybridoma cultured supernatant. Rabbit polyclonal antibody to class I heavy chain was raised against the bacterially expressed lumenal domain of HLA-A2 allele (aa. 25–308). Anti-glyceraldehyde-3-phosphate dehydrogenase was purchased from Upstate Biotechnology. US3 cDNA was cloned into the vector pLpCX (Clontech) and stably introduced into U373 astrocytoma cells by retroviral transduction. US3-expressing cells were clonally expanded in the presence of puromycin (0.375 μg/mL). U373–US3 cells were then transduced with a US11 retrovirus (retroviral vector pMIg) expressing GFP. The cells were sorted for GFP signal, thus isolating cells that only express US3 and US11. A similar cloning strategy was used to generate US2/US3 cells (Noriega and Tortorella, 2009).

2.3. BAC mutagenesis

The generation of RV-KB6 and RV-KB9 has been described elsewhere (Besold et al., 2007, 2009). Viral mutant RV-KB7 was generated analogously by BAC mutagenesis of the HCMV BAC pAD/Cre (Yu et al., 2002), using Red recombination in *Escherichia coli* strain EL250 as described by Lee et al. (2001). In this process, the open reading frames (ORF) US2, US6 and US11 were sequentially deleted from the HCMV genome. As a first step, ORF US2 was deleted by inserting a kanamycin resistance (Kan^R) gene. The Kan^R gene, flanked by FRT sites, was amplified from a derivative of vector pCP15 (Cherepanov and Wackernagel, 1995), using engineered primers. These primers contained, in addition to the priming sequence for the Kan^R FRT cassette amplification, at their very 5′-ends about 50 bp of homology to the nucleotide sequences directly adjacent to US2 (primer KB1: 5′-ATGGGTACTCGTGGCTAGATTTATTGAAATAAACCGCGATCCCGGGC-CTCTGGGATATAGTCTCGAGAAACGCAGCTTC-3′, primer KB2: 5′-CTCTGGGATATAAATTGGGAAAGAGCGTACAGTCCACACGCTGTTTCACCGGTACCCGGGGATCTTG-3′). The amplified Kan^R gene construct was inserted in the viral DNA by homologous recombination, thereby replacing ORF US2; individual colonies were selected by addition of kanamycin. To remove the Kan^R gene from the BAC, individual colonies were streaked out. Flp expression was induced by arabinose as originally described by Lee et al. (2001). The FLP recombinase removed the Kan^R gene from the viral DNA by site-specific Flp recombination at flanking FRT sites. The same strategy was then used in the second step to remove US11 (primer KB7: 5′-GGTGAGTCGTTTCCGAGCGACTCGAGATGCACTCCGCTTCAGTCTATATAGGTACCCGGGGATCTTG-3′, primer KB8: 5′-TTACAGCTTTTGAGTCTAGACAGGGTAACAGCCTTCCCTTGTAAGACAGATCGAGAAACGCAGCTTC-3′). Again, the Kan^R gene was removed by Flp recombination. Finally, for deletion of ORF US6 in the third step, insertion of an ampicillin resistance (Amp^R) gene was used, which was also amplified from the derivative vector of pCP15 (primer KB5: 5′-GAGAATGCCGTGTTGAAGGAACGCGCTTTTATTGAGACGATAAAACAGCAGCGGAACCCCTATTTGTT-3′, primer KB6: 5′-GAACATATATAATCGCCGTTTCGTAAGCACGTCGATATCACTCCTTCACTCTTGGTCTGACAGTTACC-3′). To avoid insertion of another FRT site into the HCMV genome, the Amp^R gene construct lacked FRT sites. Therefore, one copy of the Amp^R gene is contained in the final BAC pKB7.

2.4. Viral infection

Reconstitution of the wild type strain RV-BADwt and viral mutants as well as the generation of viral stocks were performed as described (Besold et al., 2009). Virus stock titration was performed by counting IE1 positive cells 48 h post infection, following staining with a monoclonal antibody (mAb) against IE1 (p63-27; (Andreoni et al., 1989)). Multiplicity of infection (m.o.i.) was defined as the number of IE1 positive cells. For cytofluorometric analyses HFF were infected at an m.o.i. of 5. For co-infection experiments, an m.o.i. of 5 was used for each virus.

2.5. Cell lysis, immunoprecipitation, and flow cytometry analysis

Cell lysis and immunoprecipitations were carried out as previously described (Noriega and Tortorella, 2009). Proteasome inhibitor (carboxylbenzyl-leucyl-leucyl-leucine vinyl sulfone [ZL_3VS]) was a kind gift from Dr. Matthew Bogyo (Stanford University, Stanford, CA) and Dr. Hidde Ploegh (Whitehead Institute,