Inhibition of Type I Interferon Response by Human Cytomegalovirus

Eui Tae Kim, Young Eui Kim, Yong Ho Huh, and Jin-Hyun Ahn*

Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon 440-746

Human cytomegalovirus (HCMV) has several strategies to evade host immune responses. HCMV contains several genes that inhibit antigen presentation to cytotoxic T cells. Recent studies also suggested that HCMV modulates host intrinsic and innate immune responses. The 72-kDa IE1 protein of HCMV was capable of antagonizing PML and PML nuclear body-mediated intrinsic antiviral activities of host cells [1, 2]. Recently, IE1 was reported to bind to STAT2 and, to a lesser extent, to STAT1, suggesting that these interactions may modulate the type I interferon signaling pathway during infection [3]. Here, we evaluated whether the IE1-STAT2 interaction inhibits the type I interferon response using recombinant viruses. We found that the Glu-rich acidic domain of IE1 is required for binding to STAT2. A mutant HCMV encoding the acidic domain-deleted IE1(Δ421-475) was generated using the HCMV-bacterial artificial chromosome (BAC) clone. The growth of mutant virus was only slightly delayed at high multiplicity of infection (MOI), but was severely impaired at low MOI with low level accumulation of viral proteins. When cells were pretreated with interferon β, the mutant virus showed an additional 1,000-fold reduction in viral growth even at high MOI, compared to wild-type. The inhibition of STAT2 loading on the target promoter upon infection was markedly reduced with mutant virus. These results provide genetic evidence that IE1 binding to STAT2 requires the acidic domain and promotes viral growth by interfering with interferon signaling .

Covalent modification of proteins by small ubiquitin-like modifiers (SUMO) can regulate protein-protein interaction. Interestingly, IE1 has been found to be modified by SUMO at a lysine residue within the acidic domain. We found that the SUMO-1 conjugated form of IE1 fails to interact with STAT2 in both in vitro binding assays and virus-infected cells, suggesting that the activity of IE1 to interfere with type I interferon signaling is negatively regulated by the cellular SUMOylation pathway. This led us to further ask whether virus has a strategy to reduce the SUMOylation level of IE1. We found that IE1 physically interacts with PIAS1 and that PIAS1 acts as a SUMO E3 ligase for IE1. The 86-kDa IE2 protein was previously found as a SUMO substrate and interacted with PIAS1. Interestingly, transiently expressed IE2 inhibited IE1 SUMOylation in a dose-dependent manner. IE1 SUMOylation reversely correlated with the accumulation of IE2 during the progression of lytic infection. Furthermore, IE2(346-542), which lacked both the SUMOylation sites and a SUMO-interacting motif but was sufficient to bind to PIAS1, efficiently inhibited IE1 SUMOylation in vitro assays, suggesting that IE2 may downregulate IE1 SUMOylation by targeting for PIAS1. Overall, our results

suggest that PIAS1-induced SUMOylation of IE1 inhibits STAT2 binding with attenuating the IE1 activity to inhibit the type I IFN response, but virus may have a strategy to reduce IE1 SUMOylation.

References

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