Human cytomegalovirus evades IgG antibody-mediated immunity through endoplasmic reticulum-associated degradation of the neonatal Fc receptor (FcRn) for IgG

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Summary

Human Cytomegalovirus (HCMV) is known to evade host immunity, allowing it to persistently infect humans. Although the strategies of HCMV to evade cellular immunity are well studied, there is limited understanding on how HCMV antagonizes humoral immunity. The neonatal Fc receptor (FcRn), an MHC class I-related FcγR, The neonatal Fc receptor (FcRn), an MHC class I-related Fcγ receptor, plays a critical role in antibody immunity by controlling IgG transport from the mother to the fetus or across epithelial cell surfaces and prolonging the half-life of IgG. Through screening the HCMV proteome, we discovered that US11 specifically captured FcRn in both virally-infected and US11-expressing cells. US11 selectively inhibited the FcRn trafficking to the endosome by retention of FcRn in ER. Furthermore, US11 recruits Derlin-1, an E3 ligase mediator, and TMEM129, an ER-resident E3 ubiquitin ligase, together with the E2 enzyme, UbE2j2, to engage FcRn. These interactions initiate the dislocation of FcRn from the ER to the cytosol and facilitates its degradation in a ubiquitination and proteasome-dependent manner. Hence, our results reveal for the first time the mechanism by which HCMV infection exploits a Derlin-1/TMEM129/UbE2j2 pathway through US11 to disable FcRn. Because FcRn is vital to IgG transport and half-life of IgG in the cell populations supporting HCMV infection, these results have implications for vaccine development and antibody-mediated immune surveillance in the defense against HCMV.

Introduction

Human cytomegalovirus (HCMV) is a herpesvirus that infects humans throughout the world. Most infections with HCMV are asymptomatic, however, both initial and reactivated HCMV infections pose a life-threatening risk in immunocompromised patients, such as transplant recipients and HIV patients. In addition, due to its ability to infect the developing fetus via placental transmission, HCMV is the leading infectious cause of congenital abnormalities worldwide. HCMV has been extremely successful in infecting humans due to its ability to evade the immune system to establish lifelong latency. HCMV expresses US2, US3, US6, US10, US11, UL16, and UL18 proteins that inhibits MHC class I molecules functions to evade T cell-mediated immunity. However, little is known about how HCMV can circumvent antibody immunity.

The neonatal Fc receptor (FcRn) is composed of a membrane-bound heavy chain (HC) in non-covalent association with β_2-microglobulin (β_2m). Although FcRn shares structural characteristics with MHC class I molecules, it does not present antigenic peptides, instead, it binds IgG antibodies in a pH-dependent manner with FcRn binding IgG at a pH < 6.5 and releasing IgG at neutral or high pH. The FcRn is normally transported to early endosomes and has limited cell surface expression. Within acidic endosomes, FcRn binds endocytosed IgG. Depending on the cell type, FcRn either recycles IgG back to its original cell surface or transports IgG to the opposite cell surface where the extracellular neutral pH triggers the release of IgG from FcRn. Endocytosed IgG that does not bind FcRn moves to lysosomes where it is degraded . Therefore, FcRn prolongs the half-life of IgG and helps to establish passive immunity by transport IgG across the placental syncytiotrophoblast monolayer, as well as across mucosal epithelium monnolayer.

Although HCMV infects placental trophoblasts, epithelial cells, endothelial cells, where FcRn is expressed. Little is currently known about the interaction between HCMV and FcRn. Here, we have identified that the HCMV membrane glycoprotein US11 specifically captures human FcRn, inhibits its antibody trafficking functions, and causes its degradation. We therefore propose a novel mechanism through which HCMV escapes antibody-mediated immunity.

Results

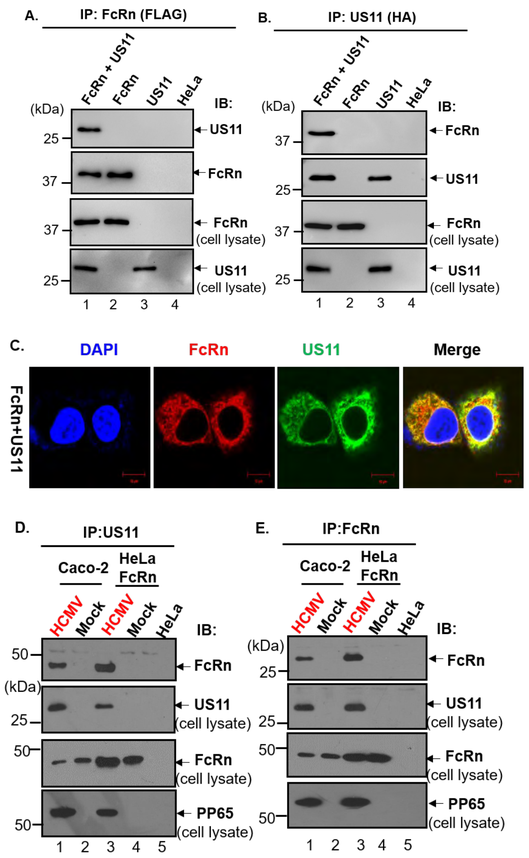


Figure 1. HCMV glycoprotein US11 interacts with FcRn. **A** and **B.** Co-immunoprecipitation of FcRn and US11 in cell lysates from HeLa$^{FcRn+US11}$. **C.** Colocalization of FcRn and US11 in HeLa$^{FcRn+US11}$ Cells. **D** and **E.** Co-immunoprecipitation of FcRn and US11 in cell lysates from virally infected cells.

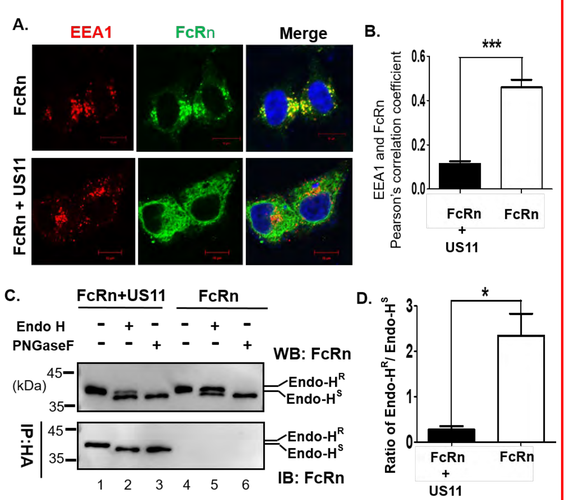


Figure 2. US11 inhibits FcRn trafficking to the endosome by retaining FcRn in the ER.
A. The expression pattern of FcRn with early endosome mark EEA1 in HeLa^{FcRn} and HeLa$^{FcRn+US11}$ cells. **B.** Averages of the EEA1 and FcRn colocalization coefficients. **C + D.** Sensitivity of US11-associated FcRn HC to Endo-H digestion. Endo H-sensitive (Endo-H^S) indicates FcRn retained in ER; Endo H-resistant (Endo-H^R) indicates FcRn matured from ER.

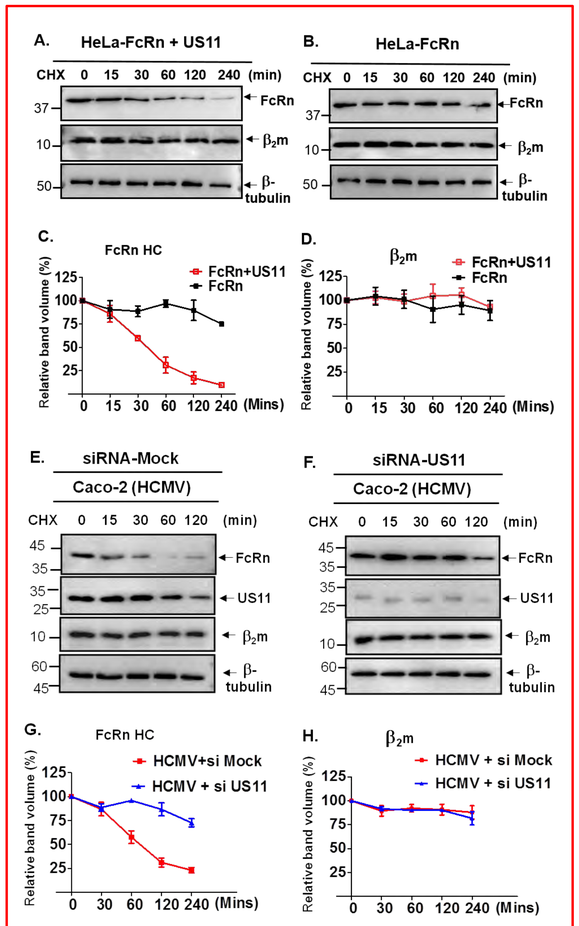
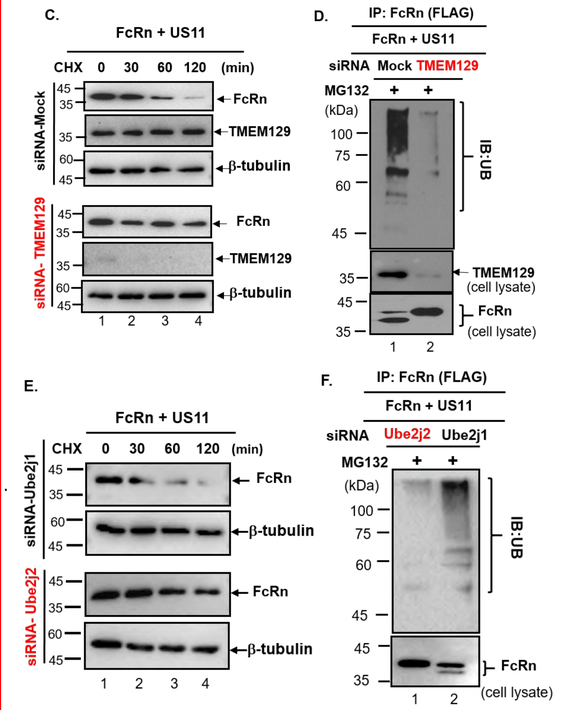


Figure 3. US11 induce FcRn protein degradation and is necessary for FcRn degradation during HCMV infection.
The FcRn protein expression level in HeLa^{FcRn} (**A**) and HeLa$^{FcRn+US11}$ (**B**) cells after cycloheximide treatment. The endogenous FcRn protein level in US11 siRNA-treated (**F**) or Mock siRNA-treated (**E**) Caco-2 cells after HCMV infection. The level of remaining FcRn (**C** or **G**) and β_2m (**D** or **H**) at different time points was quantified as the percentage of initial protein level.

Figure 4. US11 recruits FcRn to Derlin-1/TMEM129/Ube2j2 protein complex which induces FcRn ubiquitylation and degradation.
A and B. Co-immunoprecipitation of FcRn with Derlin-1 and TMEM129 in cell lysates from HeLa$^{FcRn+US11}$. The E3 ubiquitin ligase TMEM129 is required for US11-induced FcRn degradation (**C**) and ubiquitination (**D**). The E2 conjugating enzyme Ube2j2 is required for US11-induced FcRn degradation (**E**) and ubiquitination (**F**).



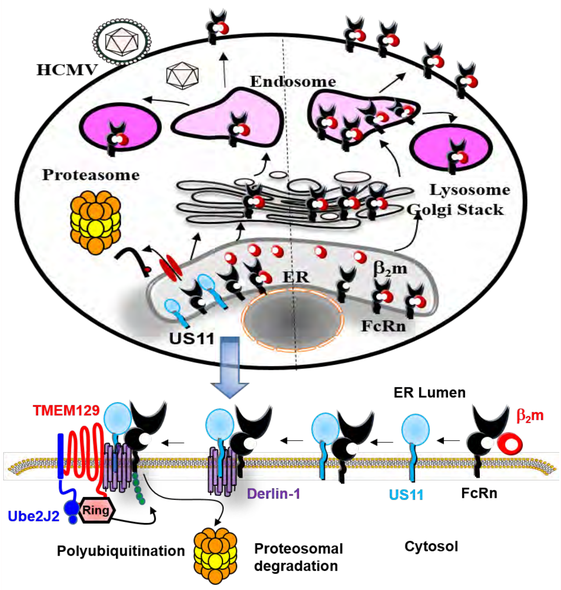


Figure5. Proposed model for US11 interacting with FcRn.

Conclusion

HCMV glycoprotein US11 interacts with FcRn.

US11 inhibits FcRn trafficking to the endosome by retaining FcRn in the ER.

US11 induce FcRn protein degradation and is necessary for FcRn degradation during HCMV infection.

US11 recruits FcRn to Derlin-1/TMEM129/Ube2j2 protein complex which induces FcRn ubiquitylation and degradation.