Modeling of HCMV Protein, US2, That Binds HLA-A2 and Down-Regulates its Expression on the Cell Surface

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Abstract

Viruses are parasitic microorganisms that invade cells to replicate. Once replicated, many viruses kill the host cell so their progeny can invade other cells, repeating the replication process and ensuring their survival. Recognition of viral-infected cells by the immune system is mediated by cell surface proteins called class I major histocompatibility (MHC-I) proteins. MHC-I proteins specifically bind viral peptides and present them to cytotoxic T lymphocytes (CTL), targeting the infected cell for destruction by the CTL (Fig. 1). Human cytomegalovirus (HCMV) produces a glycoprotein, called unique short 2 (US2), which binds to MHC-I inhibiting expression of MHC-I and preventing CTL recognition and destruction of HCMV-infected cells. When US2 binds to MHC-I in the rough endoplasmic reticulum (RER), the MHC-I molecules are sent to the cytoplasm for proteasomal degradation (Fig. 2), enabling HCMV to establish long-term, latent infections. X-ray crystallography of US2 revealed an immunoglobulin-fold (Ig-fold), which appears to play a role in evading the host's immune system; however, the exact mechanism is unknown.

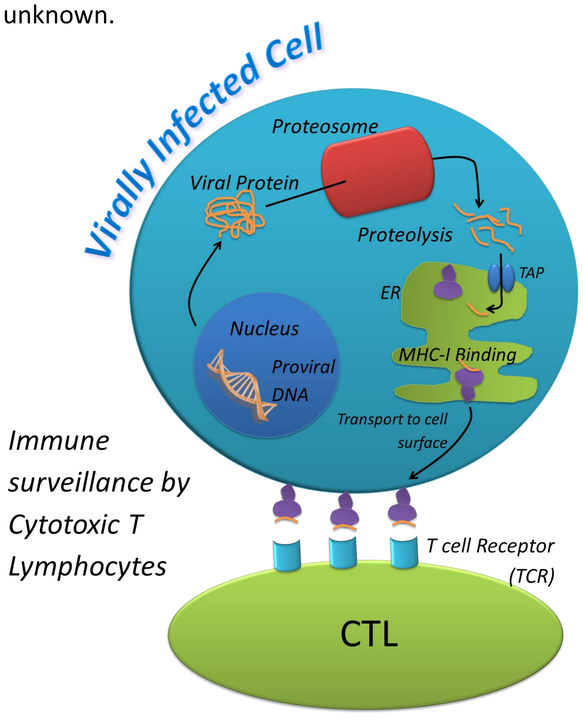

Figure 1: Proper viral antigen presentation on MHC-I. TAP: transporter associated with antigen presentation. Adapted from http://www.csa.com/discoveryguides/cancer/review.php

Introduction and Significance

•A significant number of organ transplants result in patients infected with human cytomegalovirus (HCMV). [1]
•Studies show HCMV latency requires the viral-encoded US2 protein to route MHC-I to the cytoplasm for destruction.

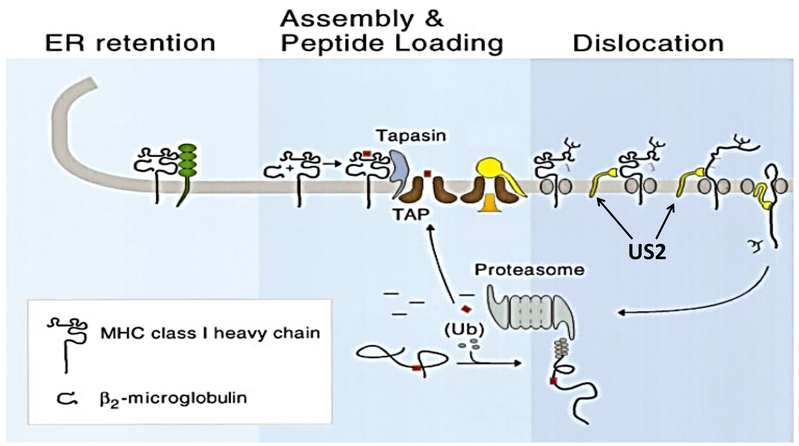

Figure 2: US2 binding to MHC-I induces dislocation into the cytoplasm where MHC-I is deglycosylated (scissors) prior to degradation in the proteasome.[2]

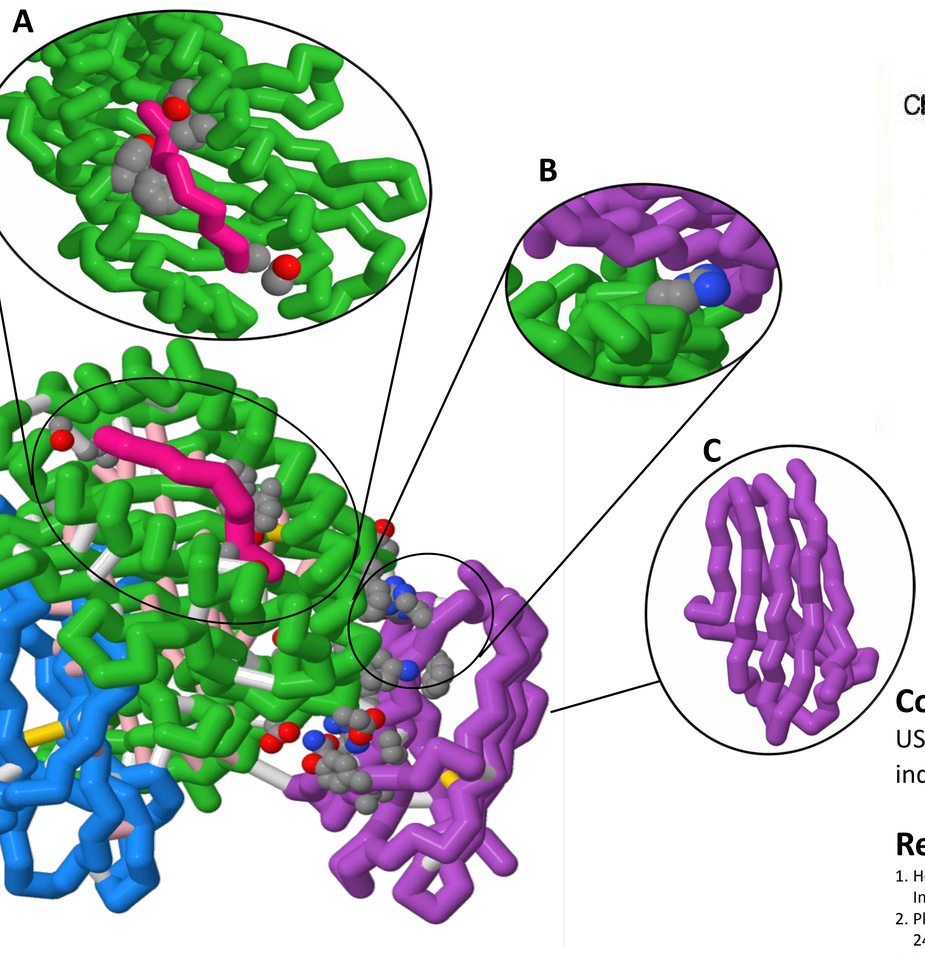

Figure 3: US2 complexed to HLA-A2. MHC-I alpha chain (green), beta-2-microglobulin (blue), viral peptide (pink), and US2 (orchid). **A:** Close up of HLA-viral peptide binding cleft. **B:** Mutant residue R181E on HLA. **C:** US2 has extensive binding sites with HLA along one of the beta sheets of its Ig-like fold. An Ig-fold consists of two anti-parallel beta sheets of 110 amino acid residues connected by a disulfide bond.[3]

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Story

•Viral-infected cells signal the immune system by MHC-I presentation of viral peptides on the cell surface.
•Cytotoxic T lymphocytes (CTL) bind the peptide-MHC complex and kill the infected cell, eliminating the virus.
•US2 disrupts the process of viral peptide presentation.
•In the RER, US2 binds to the alpha chain of MHC-I and redirects the MHC to the cytosol where it is destroyed in the proteasome (Figure 2).

Experimental Evidence

Structural studies by Gewurz et al., demonstrated that US2 contains an Ig-like fold (Fig. 3C) and binds to the HLA-A2/Tax peptide complex (Fig. 3A). Pulse-chase analysis of US2 mutants identified important interactions with HLA-A2, with which it forms a 1:1 complex (Fig. 3).

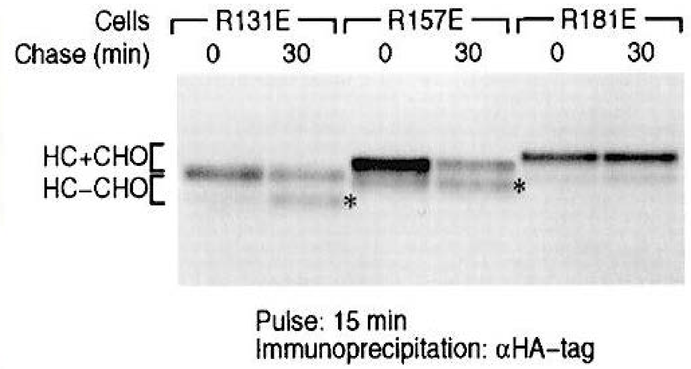

Figure 4: Pulse chase analysis of mutated HLA-A2 shows residue 181 (R181E) is essential for MHC-I dislocation. Dislocation of HLA-A2 to the cytoplasm results in deglycosylation and faster migration. Mutations at residues R131E, R157E, and R181E were tested for dislocation immediately following the pulse (0 minutes) and 30 minutes later. At time zero, single bands reflected newly synthesized HLA-A2 present in the RER. At 30 minutes, US2 dislocation and deglycosylation is seen with R131E and R157E by the presence of a second, smaller starred band, absent in R181E. This result indicates R181 is an important binding site for US2-mediated dislocation. [4]

Conclusion

US2 prevents CTL recognition of HCMV-infected cells by inducing the dislocation and degradation of MHC-I molecules.

References

1. Ho, M. (2008). The history of cytomegalovirus and its diseases. Medical Microbiology and Immunology, 197(2) 65-73.
2. Ploegh, H. L. (1998). Viral strategies of immune evasion. (Cover story). *Science*, *280*(5361), 248.
3. Berg J.M., Tymoczko J.L., Stryer L. (2002). The Immunoglobulin Fold Consists of a Beta-Sandwich Framework with Hypervariable Loops. Biochemistry.
4. Gewurz B, Gaudet R, Tortorella D, Wang E, Ploegh H, Wiley D. Antigen presentation subverted: Structure of the human cytomegalovirus protein US2 bound to the.... (Cover story). Proceedings Of The National Academy Of Sciences Of The United States Of America [serial online]. June 5, 2001;98(12):6794. Available from: Academic Search Complete, Ipswich, MA. Accessed April 17, 2012.