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Cowpox virus employs a two-pronged strategy to outflank MHCI antigen presentation

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Abstract

Smallpox decimated humanity for thousands of years before being eradicated by vaccination, a success facilitated by the fact that humans are the only host of variola virus. In contrast, other orthopoxviruses such as cowpox virus can infect a variety of mammalian species, although its dominant reservoir appears to be rodents. This difference in host specificity suggests that cowpox may have developed promiscuous immune evasion strategies to facilitate zoonosis. Recent experiments have established that cowpox can disrupt MHCI antigen presentation during viral infection of both human and murine cells, a process enabled by two unique proteins, CPXV012 and CPXV203. While CPXV012 inhibits antigenic peptide transport from the cytosol to the ER, CPXV203 blocks MHCI trafficking to the cell surface by exploiting the KDEL-receptor recycling pathway. Our recent investigations of CPXV203 reveal that it binds a diverse array of classical and non-classical MHCI proteins with dramatically increased affinities at the lower pH of the Golgi relative to the ER, thereby providing mechanistic insight into how it works synergistically with KDEL receptors to block MHCI surface expression. The strategy used by cowpox to both limit peptide supply and disrupt trafficking of fully assembled MHCI acts as a dual-edged sword that effectively disables adaptive immune surveillance of infected cells.

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Keywords

antigen presentation; major histocompatibility complex; orthopoxvirus; viral immune evasion

INTRODUCTION

A recurrent theme in viral immune evasion is the disruption of MHC class I (MHCI) antigen processing and presentation pathways that otherwise lead to elimination of infected cells by cytotoxic T cells. Viral proteins that sabotage virtually every step of the process have been identified, including those that block peptide generation and transport, undermine peptide loading complex (PLC) assembly, and redirect MHCI intracellular trafficking (Hansen and Bouvier 2009). For example, herpesviruses have developed strategies to dislocate MHCI proteins from the ER for degradation in the cytosol as well as to internalize cell surface MHCI for degradation in the lysosome. The mechanistic study of these viral proteins has provided considerable insight into the physiologic pathways used in antigen presentation, and has, for example, led to a far better appreciation of the role played by ubiquitin conjugation in MHCI sorting and quality control.

In contrast to other large-DNA viruses, orthopoxviruses such as vaccinia, variola and ectromelia are not known to evade MHCI antigen presentation. However, work in the Fröh and Yokoyama labs independently discovered that cowpox does in fact block MHCI surface expression in a process facilitated by two immune evasion proteins. CPXV012 is a short-lived type II transmembrane protein that co-immunoprecipitates with the PLC and blocks transporter associated with antigen processing (TAP)-mediated transport of peptides into the ER (Byun et al. 2009; Alzhanova et al. 2009). Interestingly, CPXV012 appears to be a truncated C-type lectin domain with sequence similarity to a number of activating and inhibitory NK receptors. CPXV203, on the other hand, is a soluble protein that sequesters MHCI proteins within the ER by a mechanism that is independent of the PLC (Byun et al. 2007). However, the ER retention of MHCI by CPXV203 is dependent upon its C-terminal KTEL sequence, a motif known to bind to the KDEL receptor (KDELRL) whose cellular function is the retrieval of proteins from the Golgi to the ER. Of note, cowpox viruses with deletions of the CPXV012 and CPXV203 ORFs exhibit attenuated virulence in a manner dependent upon CD8⁺ T cell control (Byun et al. 2009), an observation that stands in stark contrast to similar manipulations of beta- and gamma-herpesviruses where it has been difficult to show an *in vivo* role for viral MHCI modulation during acute infection. More recent experiments indicate that MHCI targeting by cowpox affects virus-specific CD8⁺ effector responses but not T-cell priming (Gainey et al. 2012).

DISCUSSION

CPXV203 binds in a niche under the peptide-binding platform of MHCI

CPXV203 is capable of preventing the cell surface expression of wide array of MHCI proteins, and we recently have shown that this feat is accomplished by direct binding to the luminal domains of MHCI (McCoy et al, in press). Our work has also revealed that CPXV203 adopts a compact beta-sandwich structure surprisingly reminiscent of the vCCI and SECRET decoy receptors encoded by poxviruses, although the established determinants for chemokine binding are largely absent. CPXV203 binds underneath the MHCI peptide-binding platform, with its C-terminal KDEL motif located proximal to the transmembrane-spanning region of the class I heavy chain (Figure 1A). The structure of CPXV203 is quite distinct from that observed for two other viral proteins known to bind MHCI and disrupt antigen presentation, human cytomegalovirus encoded US2 (Gewurz et al. 2001) and adenovirus encoded E3-19K (Li, Muzahim, and Bouvier 2012) (Figure 1B). Interestingly,

both US2 and E3-19K are transmembrane proteins that mechanistically operate in the ER, with US2 targeting MHCI for ER-associated degradation (ERAD) and E3-19K retaining MHCI in a manner dependent on its cytoplasmic tail. CPXV203 binds an MHCI surface distinct from the overlapping binding surfaces of US2 and E3-19K that are located proximal to the A-pocket of the peptide-binding groove (Figure 1C).

CPXV203 binds to evolutionarily conserved regions of fully assembled MHCI

In agreement with the ability of CPXV203 to prevent surface expression of diverse MHCI in mouse and human cells, CPXV203 was found to bind different allelic and isotypic MHCI gene products of disparate mammalian species, including for example the non-classical protein TL that does not bind or present peptides. Based on the co-structure of a CPXV203/H2-K^b complex, CPXV203 contacts largely conformational determinants conserved among MHCI proteins in their heavy chain α 2- and α 3-domains, while also making significant contact with β 2m residues that are nearly invariant across species. There is also striking overlap of the footprint of CPXV203 on MHCI with the footprints of tapasin, CD8, and Ly49 proteins on MHCI (Figure 1D). Indeed, CPXV203 shares contact residues with Ly49 and tapasin in the MHCI α 2-domain, whereas CPXV203, CD8, and tapasin all interact with the highly conserved residues Asp227 and Glu229 in the acidic CD loop of the α 3-domain. Thus, the tri-domain interface engaged by CPXV203 is sequence conserved apparently by virtue of its critical role in chaperone and receptor binding functions, and is a feature primarily of fully assembled MHCI as these determinants are unlikely to be accessible or similarly organized in peptide-free or PLC-associated MHCI.

CPXV203 preferentially binds MHCI at the pH found in the Golgi

The binding of CPXV203 to MHCI was shown in a variety of biophysical assays to be enhanced significantly by decreasing pH below 7.4 (McCoy et al, in press). Based on the physiologic pH gradient between the ER and Golgi, it can be inferred that CPXV203 engages MHCI more avidly in the Golgi than the ER. Of note, the pH-dependent binding of CPXV203 with MHCI mimics that of the KDEL^R with its substrates, suggesting that the viral protein couples MHCI to KDEL^R in the early Golgi resulting in COPI-mediated retrograde transport of complexes back to the ER where they dissociate. Interestingly, mutagenesis data demonstrate that the pH regulation of CPXV203/MHCI complex stability is critically dependent on CPXV203 residues His75 and His80 that interacts with the acidic CD loop. The imidazole side-chain of histidine has a pK_a of ~6.0, and thus is frequently implicated in the pH regulation of Golgi and endosomal protein trafficking.

Interestingly, calreticulin and Erp57 also have C-terminal KDEL motifs conferring ER retrieval. Both of these ubiquitous ER chaperones assist folding and assembly of numerous oligomeric glycoproteins including MHCI. Indeed, Erp57 and calreticulin are members of the PLC that retains MHCI in the ER until it binds a peptide with suitable affinity. But non-PLC-associated calreticulin also accumulates in the cis-Golgi, and can return peptide-accessible MHCI to the ER. In this role, calreticulin functions to impose post-ER quality control to assure the presentation of peptides that can stabilize MHCI at the cell surface. Our findings suggest that CPXV203 has usurped a similar mechanism to retrieve MHCI but with the opposite goal. Calreticulin functions in host quality control by retrieving MHCI with suboptimal peptides, whereas CPXV203 blocks antigen presentation by retrieving quality peptide-loaded MHCI to prevent their surface display for CTL recognition.

The two-pronged strategy of CPXV012 and CPXV203 outflanks antigen presentation

The pH-dependent retrieval mechanism of fully assembled MHCI by cowpox helps define mechanistically how CPXV203 coordinates with CPXV012 to effectively block in vivo antigen presentation. CPXV012 blocks TAP-mediated peptide transport, thus CPXV012 is

targeting MHCI prior to loading with quality peptides. However, the ability of CPXV012 to restrict peptide supply is not absolute since the inhibition of peptide transport by TAP is leaky and certain MHCI-binding peptides in the ER are TAP-independent. Thus some MHCI with bound peptides can escape the CPXV012 block and exit the ER. This is the point at which CPXV203 functions, to retrieve fully assembled MHCI from the Golgi and return them to the ER. The fact that CPXV203 and MHCI interact more avidly in the Golgi than the ER effectively limits the pool of peptide-assembled MHCI that must be targeted by CPXV203. Thus CPXV012 and CPXV203 function synergistically before and after peptide loading using distinct mechanisms to effectively disable antigen presentation. A consequence of efficient MHCI downregulation, however, is NK cell susceptibility. In this regard, it is noteworthy that cowpox encodes OMCP, a secreted high-affinity ligand of the human and murine NKG2D-activating receptors that was recently shown to adopt an MHCI-like structure (Lazear et al. 2012).

Immune evasion proteins like CPXV012 and CPXV203 can be quite effective at sabotaging antigen presentation in cells that are infected with virus, yet it is commonly believed that non-infected DCs are required to cross-present viral antigens to prime CD8⁺ responses *in vivo*. Recent experiments by Gaaney et al. support this view, in that cowpox-specific T-cell development is compromised in Batf3-KO mice irrespective of the expression of the viral MHCI saboteurs (Gaaney et al. 2012). Thus, it will be of considerable interest to determine whether yet to be discovered immune evasion proteins might specifically target cross-presentation pathways of antigen presentation.

ABBREVIATIONS

β2m	beta-2 microglobulin
CPXV	cowpox virus
CTL	cytotoxic T lymphocyte
ER	endoplasmic reticulum
HCMV	human cytomegalovirus
KDEL	KDEL receptor
MHCI	major histocompatibility complex class I
NK	natural killer
PLC	peptide loading complex
TAP	transporter associated with antigen processing

REFERENCES

- Alzhanova, Dina; Edwards, David M.; Hammarlund, Erika; Scholz, Isabel G.; Horst, Daniëlle; Wagner, Mary J.; Upton, Chris; Wiertz, Emmanuel J.; Slifka, Mark K.; Früh, Klaus. Cowpox Virus Inhibits the Transporter Associated with Antigen Processing to Evade T Cell Recognition. *Cell Host & Microbe*. 2009 Nov 19; 6(5):433–445. [PubMed: 19917498]
- Byun, Minji; Verweij, Marieke C.; Pickup, David J.; Wiertz, Emmanuel JHJ.; Hansen, Ted H.; Yokoyama, Wayne M. Two Mechanistically Distinct Immune Evasion Proteins of Cowpox Virus Combine to Avoid Antiviral CD8 T Cells. *Cell Host & Microbe*. 2009 Nov 19; 6(5):422–432. [PubMed: 19917497]
- Byun, Minji; Wang, Xiaoli; Pak, Melissa; Hansen, Ted H.; Yokoyama, Wayne M. Cowpox Virus Exploits the Endoplasmic Reticulum Retention Pathway to Inhibit MHC Class I Transport to the Cell Surface. *Cell Host & Microbe*. 2007 Nov 15; 2(5):306–315. [PubMed: 18005752]

- Gainey, Maria D.; Rivenbark, Joshua G.; Cho, Hyelim; Yang, Liping; Yokoyama, Wayne M. Viral MHC Class I Inhibition Evades CD8+ T-cell Effector Responses in Vivo but Not CD8+ T-cell Priming. *Proceedings of the National Academy of Sciences*. 2012 Oct 29.
- BE, Gewurz; Gaudet, R.; Tortorella, D.; Wang, EW.; Ploegh, HL.; Wiley, DC. Antigen Presentation Subverted: Structure of the Human Cytomegalovirus Protein US2 Bound to the Class I Molecule HLA-A2. *Proceedings of the National Academy of Sciences of the United States of America*. 2001 Jun 5; 98(12):6794–6799. [PubMed: 11391001]
- Hansen, Ted H.; Bouvier, Marlene. MHC Class I Antigen Presentation: Learning from Viral Evasion Strategies. *Nature Reviews. Immunology*. 2009 Jul; 9(7):503–512.
- Lazear E, Peterson LJ, Nelson CA, Fremont DH. Crystal Structure of the Cowpox Virus Encoded NKG2D-ligand OMCP. *Journal of Virology*. 2012 Oct 31.
- Li, Lenong; Muzahim, Yasameen; Bouvier, Marlene. Crystal Structure of Adenovirus E3-19K Bound to HLA-A2 Reveals Mechanism for Immunomodulation. *Nature Structural & Molecular Biology*. 2012
- McCoy WH IV, Wang X, Yokoyama WM, Hansen TH, Fremont DH. Structural Mechanism of ER Retrieval of MHC Class I by Cowpox. *PLoS Biology*. 2012 In press.

Highlights

CPXV012 and CPXV203 work synergistically to block peptides from finding MHCI and targeting complexes that nevertheless form.

CPXV203 promiscuously binds MHCI using sequence-conserved determinants located under the peptide-binding platform.

CPXV203 preferentially associates with fully assembled MHCI in the Golgi, where it couples it to KDEL receptor mediated ER-retrieval.

Figure 1. Comparison of viral proteins that target MHCI antigen presentation

(A) Structure of CPXV203 in complex with murine H2-K^b. Depicted is a ribbon diagram of the complex with CPXV203 in magenta, H2-K^b in pale blue, β2m in grey, and the SIINFEKL peptide in yellow. (B) Structure of HCMV US2 (left, green) and E3-19K (right, blue) bound to HLA-A2. (C) Binding surface mapping of viral immune evasion proteins that target MHCI. (D) Overlap of CD8αα, CD8αβ, Ly49A, Ly49C, and tapasin binding sites on MHCI. Footprints are defined by <5Å contacts except for surface-exposed tapasin contacts identified by mutagenesis. CPXV203/MHCI interfaces (α2, α3, β2m) and proposed Tpn contact loops (α2 128–136, α3 222–229) are indicated on the second surface. Coordinates used in this figure: analysis (CD8αα/K^b, 1BQH; CD8αβ/D^d, 3DMM; Ly49C/K^b, 3C8K; Ly49A/D^d, 1QO3); graphical (CPXV203/K^b, 4HKJ; US2/A2, 1IM3; E3-19K/A2, 4E5X).