Human cytomegalovirus UL141 promotes efficient downregulation of the natural killer cell activating ligand CD112

Virginie Prod'homme,1,† Daniel M. Sugrue,1,† Richard J. Stanton,1 Akio Nomoto,2 James Davies,1 Carole R. Rickards,1 Daniel Cochrane,1 Melanie Moore,1 Gavin W. G. Wilkinson1 and Peter Tomasec1

1Department of Infection, Immunity and Biochemistry, Section of Medical Microbiology, School of Medicine, Cardiff University, Cardiff, UK
2Department of Microbiology, Graduate School of Medicine, University of Tokyo, Japan

Human cytomegalovirus (HCMV) UL141 induces protection against natural killer cell-mediated cytolysis by downregulating cell surface expression of CD155 (nectin-like molecule 5; poliovirus receptor), a ligand for the activating receptor DNAM-1 (CD226). However, DNAM-1 is also recognized to bind a second ligand, CD112 (nectin-2). We now show that HCMV targets CD112 for proteasome-mediated degradation by 48 h post-infection, thus removing both activating ligands for DNAM-1 from the cell surface during productive infection. Significantly, cell surface expression of both CD112 and CD155 was restored when UL141 was deleted from the HCMV genome. While gpUL141 alone is sufficient to mediate retention of CD155 in the endoplasmic reticulum, UL141 requires assistance from additional HCMV-encoded functions to suppress expression of CD112.

Human cytomegalovirus (HCMV), the prototype species of the subfamily Betaherpesvirinae, has a high prevalence in populations worldwide. Although HCMV is recognized to be an important human pathogen, particularly in immunocompromised individuals or following congenital infection, the vast majority of primary infections are subclinical and accompanied by asymptomatic lifelong carriage. HCMV encodes highly effective systems to provide for latency, persistent reactivation and transmission; as part of this process the virus acquired an impressive array of genes that act both to evade and redirect the host immune response (Wilkinson et al., 2008). The fact that individuals with genetic defects in their natural killer (NK) cell response are particularly susceptible to severe HCMV disease (Biron et al., 1989; Gazit et al., 2004) provided a rationale to focus attention on this arm of the immune response.

NK cells are composed of heterogeneous populations expressing a “mosaic” of different activating and inhibitory receptors, the function of each cell being regulated by integration of signals received from ligands presented on potential target cells (Lanier, 2008). Inhibitory signals received mainly from autologous MHC class-I molecules normally dominate, to maintain NK cells in a resting state. However, HCMV not only efficiently downregulates MHC-I (Ahn et al., 1997; Furman et al., 2002; Jones et al., 1996; Trgovcich et al., 2006; Wiertz et al., 1996a, b), but also stimulates the expression of recognized NK cell activating ligands, e.g. MHC-I-related chains (MIC) A and B, UL16-binding proteins (ULBP) 1–3, retinoic acid early transcripts (RAET)1E/ULBP4, RAET1G/ULBP5, RAET1L/ULBP6 and CD155 (Bacon et al., 2004; Bahram et al., 1994; Bauer et al., 1999; Chalupny et al., 2003; Cosman et al., 2001; Eagle et al., 2009; Groh et al., 2001; Tomasec et al., 2005). Despite this, HCMV-infected cells actually prove to be highly resistant to NK cells in functional assays (Cerboni et al., 2000; Tomasec et al., 2005). This resilience can be attributed to a substantial proportion of HCMV genome being directed towards evading the NK cell response.

Although HCMV downregulates endogenous MHC-I, the virus also encodes its own MHC-I homologue (gpUL18) that binds the inhibitory receptor LIR-1 (ILT-2) with high affinity (Beck & Barrell, 1988; Chapman et al., 1999; Prod’homme et al., 2007) and a peptide in the UL40 leader sequence that acts to promote cell surface expression of the non-classical MHC-I molecule HLA-E, the ligand for the inhibitory receptor CD94/NKG2A (Tomasec et al., 2000; Ulbrecht et al., 2000; Wang et al., 2002). The activating receptor NKG2D is remarkable in recognizing eight ligands. To combat their activation UL16 retains MICB, ULBP1 and ULBP2 in the endoplasmic reticulum (ER); miR-UL112 targets the MICB transcript, while UL142 downregulates MICA (Chalupny et al., 2006; Cosman et al.,...
The NK cell activating receptor DNAM-1 (CD226) recognizes both CD155 and CD112 (Bottino et al., 2003; Fuchs et al., 2004). We previously demonstrated that UL141 elicits efficient protection against NK cell-mediated cytolysis by sequestering CD155 in the ER yet, in isolation, had no effect on CD112 (Tomasec et al., 2005).

CD155 is the poliovirus receptor (PVR) or nectin-like molecule-5 (necl-5), while CD112 is also referred to as nectin-2, herpesvirus entry mediator B (HVEB) or poliovirus receptor-related protein 2 (PRR2). CD112 and CD155 are both structurally and functionally related. Nectins and necls are immunoglobulin-like molecules involved in cell adhesion, movement, proliferation, differentiation, polarization, virus entry and immune recognition (Takai et al., 2008). In view of its important role as an activating ligand for DNAM-1, we sought to analyse CD112 expression in the context of HCMV infection.

Initial flow cytometry studies revealed that CD112 was downregulated by the low passage HCMV strain Merlin, but not high passage strain AD169 (not shown). Strain AD169 has a 15 kb deletion encompassing UL132–UL150 that includes the NK cell evasion genes UL141 and UL142. Merlin was derived from a bacterial artificial chromosome (BAC) containing the entire strain Merlin genome (R. J. Stanton, unpublished data). MerlinΔUL141 was generated using technologies developed previously to facilitate manipulation of the adenovirus genome (Stanton et al., 2008). Briefly, a selectable cassette comprising ampicillin resistance, lacZ and SacB was PCR amplified and recombinereered into the Merlin BAC in place of nt 184597–185412 (relative to published Merlin sequence GenBank accession no. NC_006273) using primers SacBF-UL141 (5′-caggtagcatagaaacatacggtgaaaatactccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaatcccaaaa homology to pAL1111 underlined) and SacBR-UL141 (5′-ccagctttgagcggccgacacacggagcaggaacaggcgggcagcgtctctggca-aaaagggaagaaaagaatcatcctgaggttcttatggctcttg-3′, homology to pAL1111 underlined). In a second recombinereering step, the selectable cassette was removed using oligo delUL141 (5′-atatctccaataatccatcctcagttctgctcctctgtctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctc...
cells were compared at 96 h p.i. (Fig. 1d). Replicate samples from the flow cytometry study were analysed by immunoblot, in order to further assess the fate of the CD112 protein within the cell. Briefly, cells were extracted with Triton X-114 (Bordier, 1981), proteins were separated on NuPAGE gels (Invitrogen) and blots were analysed with two independent polyclonal anti-CD112 antibodies. In Merlin-infected cells, the loss of CD155 from the cell surface (Fig. 1) correlated with the emergence of elevated levels of an immature (endoglycosidase H-sensitive) form of CD155 complexed with gpUL141 in the ER (Cochrane, 2009; Tomasec et al., 2005) (Fig. 2a). In contrast to CD155, the CD112 signal gradually decreased in Merlin-infected cells and was not detected by 72 h p.i. (Fig. 2a).

Quantitative real time-PCR showed CD112 mRNA levels to be marginally increased throughout the infection (not shown), consistent with CD112 expression being regulated post-transcriptionally. To determine whether CD112 was targeted for proteolytic degradation, Merlin-infected cells were incubated in the presence of proteasome inhibitors. Treatment with either MG132 or Epoxomycin (Calbiochem) was able to restore CD112 expression, indicating that HCMV targeted CD112 for proteasome-mediated degradation (Fig. 2b).

UL141 was required for efficient downregulation of both CD112 and CD155 from the cell surface in HCMV-infected cells (Figs 1 and 3a), yet had no effect on CD112 in cells infected with recombinant adenovirus vector encoding UL141 [RAdUL141 (Tomasec et al., 2005); Fig. 3b]. We reasoned that UL141 acted in partnership with an additional HCMV-encoded function(s) to downregulate CD112. Indeed, the residual level of CD112 suppression mediated by the MerlinΔUL141 (Figs 1d, 2a and 3a) could potentially be mediated by this function operating sub-optimally. In cells co-infected with MerlinΔUL141 and RAdUL141, the HCMV deletion mutant was complemented; downregulation of both CD112 and CD155 was restored (Fig. 3c). Similarly, co-infection of strain AD169 with RAdUL141 also resulted in the downregulation of both CD112 and CD155 (Fig. 3d). These data are consistent with UL141 co-operating with additional HCMV-expressed function(s) to efficiently downregulate CD112, and that function also being intact within AD169 strain (thus excluding UL133–150). Through downregulation of CD112, HCMV eliminates from the cell surface an activating ligand for DNAM-1, which presumably contributes to the enhanced killing of HCMV-infected cells observed when UL141 is deleted from the virus (Fig. 3e, f), but not to the protection elicited when UL141 is expressed in isolation (Tomasec et al., 2005). HCMV thus targets both ligands for the NK cell activating receptor DNAM-1. GpUL141 alone is sufficient to sequester CD155 in the ER, while this study predicts that gpUL141 acts in concert with an additional viral function to induce proteasome-mediated degradation of CD112. This additional viral function could either directly co-operate with UL141, or act upon a cellular intermediate.

DNAM-1 is remarkable in being expressed on all NK cells and plays a major role in regulating their function. HCMV suppression of CD112 and CD155 may have ramifications that extend beyond the regulation of NK cell function. DNAM-1 is also expressed on activated T, NKT, myeloid and mast cells, megakaryocytes, platelets and a subset of B lymphocytes thereby impacting on a wide range of immunological responses and regulating platelet activation (Bachelet et al., 2006; Bottino et al., 2003; Burns et al., 1985; Kojima et al., 2003; Pende et al., 2006; Reymond et al., 2004; Scott et al., 1989; Shibuya et al., 1996, 1999, 2003; Xu & Jin, 2010). For example, the interaction between DNAM-1 and CD112/CD155 has been associated
with T-cell differentiation, proliferation, cytotoxicity and cytokine secretion (Tahara-Hanaoka et al., 2004). Furthermore, nectins and neclls regulate fundamental processes in cell biology including cell adhesion, movement, proliferation, differentiation, survival, polarization and signalling (Takai et al., 2008). HCMV infection is recognized to disrupt focal adhesions and intercellular connections, while inducing cell motility and transendothelial migration (Chan et al., 2009; Stanton et al., 2007). It will be important to determine how the modulation of CD112 and CD115 influences these processes.

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References


