New insight into strategies employed by HCMV in I M M U N O M O D U L A T I O N

Binding Mechanism of HCMV UL141 to CD155, TRAIL-R1 and TRAIL-R2

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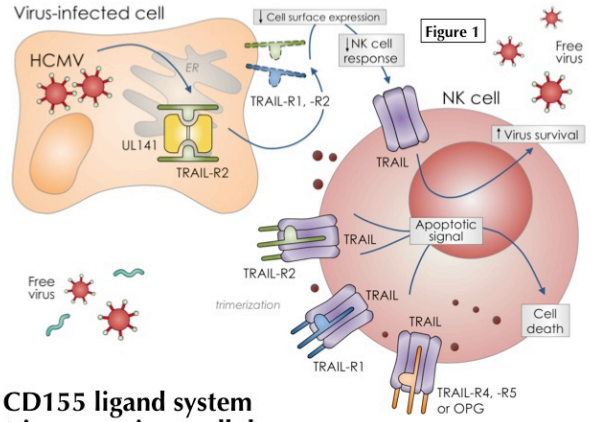
Introduction to human β-Herpesvirus

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that persistently infects the majority of the world's population (over 85% of the Slovak population are CMV positive). Following primary infection, HCMV persists for the lifetime of the host under the control of a healthy immune system (**1,2,3**). Reactivation from viral latency to productive infection in immunocompromised individuals, and acquisition of primary infection *in utero* or during transplantation can lead to serious disease (**4**). In addition, counting with the possibility of CMV being used as a vaccine vector (**5**), a complete understanding of its ability to modulate host immunity is paramount.

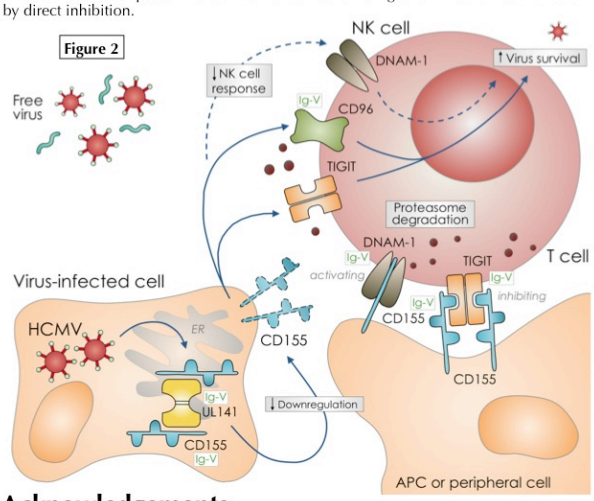
TRAIL-mediated apoptosis via Death Receptor signaling

Figure 1 – Death Receptors are cell surface receptors that transmit apoptotic signals initiated by specific ligands, e.g. TRAIL (TNF-related apoptosis-inducing ligand) . These receptors play a central role in instructive apoptosis and belong to the Tumor Necrosis Factor Receptor (TNFR) gene superfamily. They consist of cysteine rich extracellular subdomains and a homologous cytoplasmic sequence termed the "death domain". Adaptor molecules interact with the death receptors and transmit the apoptotic signal to the death-machinery.



CD155 ligand system triggers various cellular responses

Figure 2 – CD96, CD226 (DNAM-1) and TIGIT belong to an emerging family of receptors that interact with nectin and nectin-like proteins. CD226 activates natural killer (NK) cell-mediated cytotoxicity, whereas TIGIT reportedly counterbalances CD226. CD96 competes with CD226 for CD155 binding and limits NK cell function by direct inhibition.



Acknowledgements

IN gratefully acknowledges the contribution of the Slovak Research and Development Agency under the project APVV-14-0839. In addition, IN would like to appreciate the financial support from Marie Curie Fellowship SASPRO 003-01-02 and from Research Grant Agency VEGA 2/0103/15. In addition, we would like to thank the Institute of Neuroimmunology at the Slovak Academy of Sciences for allowing us to use SPR instrumentation.

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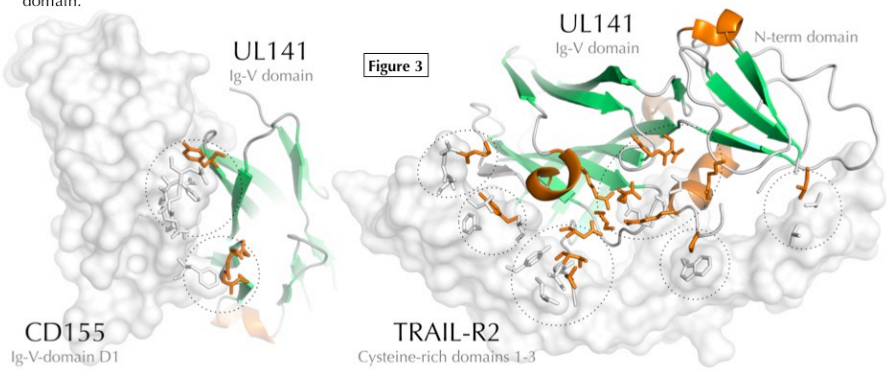
Immune modulatory function of HCMV UL141

Immune evasion genes help HCMV establish lifelong persistence. Without immune pressure, laboratory-adapted HCMV strains have undergone genetic alterations. Among these, the deletion of the UL/b' domain is associated with loss of virulence. In a screen of UL/b', there were identified several immune modulatory proteins that protect from natural killer (NK) and T cell attacks. Expression of the HCMV UL141 glycoprotein represents a unique mechanism to allow the virus to escape immune recognition as we show here that UL141 is capable of engaging diverse proteins containing disparate structural folds (**Figure 1, 2 and 3**).

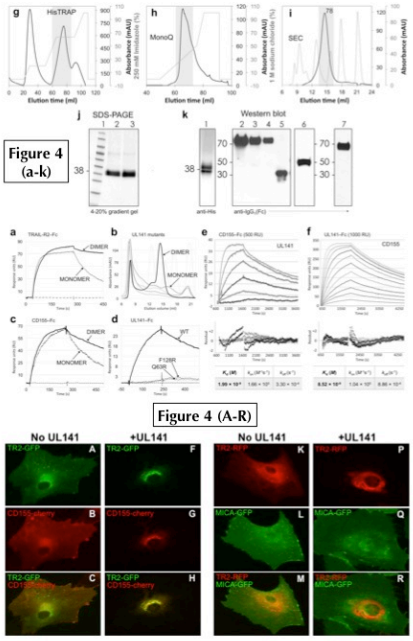
Binding interfaces to modulate NK-cell activating pathways

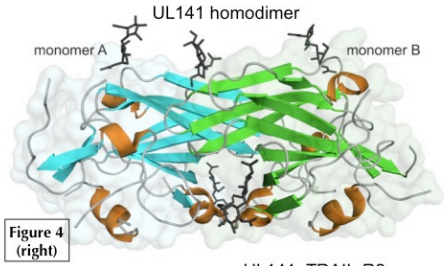
Figure 3 – HCMV UL141 is the first example of viral protein targeting two independent effector pathways. As of TRAIL-mediated killing (**Figure 1**), where UL141 retaining the TRAIL DRs in the ER and prevent their cell surface expression (**8**). We have found that UL141 binds TRAIL-R2 in a novel, non-canonical fashion using whole surface of the molecule (**7**). While at the same time UL141 is sufficient to downregulates CD155 (**Figure 2**), which is an NK cell activating ligand. We have revealed (**7**) that UL141 binds CD155 by lock-and-key mechanism using Ig-V-like domain.

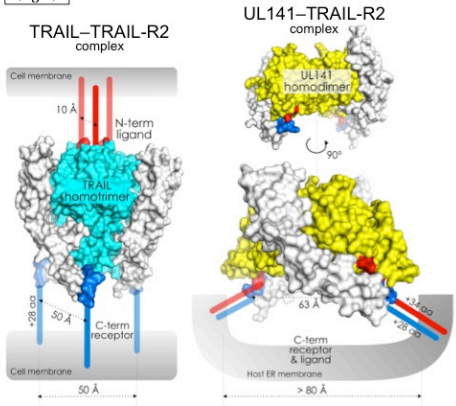


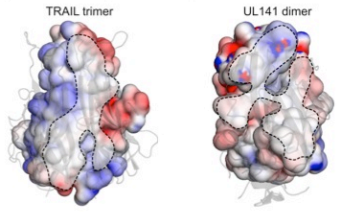
Experimental details for UL141 dimerization and cellular localization

Figure 4 (**a-k**) Dimeric and monomeric properties of UL141 analyzed by SPR, SEC, WB and SDS-PAGE; (**A-R**) Cellular co-localization of UL141, TRAIL-R and CD155; (**right-top**) Crystal structure of UL141 homodimer; (**right-center**) Membrane embedded model for studied complexes; (**right-bottom**) Electrostatic potential.









Summary

Our structural, biochemical and biological analysis has revealed that HCMV has evolved the pleiotropic **UL141 as a potent inhibitor of at least two different immune effector pathways**, the TRAIL DRs and nectin-like NK cell activating ligands (**6-8, Figure 1-4**). Our findings provide new insights into the structural basis of the evolutionary dynamic that exists between persistent viruses and host defenses, exemplified by the promiscuous targeting of immune effector pathways by UL141 which likely reveal new modalities that can be exploited for the design of therapeutics.