Assessing the binding of nectin-1 and CD96 in human cells to investigate how herpes simplex virus evades the innate immune system.

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1 *Introduction*

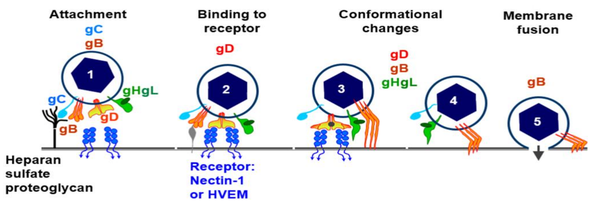
Nectin-1 is an adhesion protein that also serves as a receptor for herpes simplex virus (HSV) to enter cells. In epithelial tissues nectin-1 proteins naturally bind to other nectin-1 molecules on adjacent cells to organize intercellular junctions. Recently, our lab showed that human nectin-1 also binds to the natural killer (NK) cell receptor CD96 (Holmes et al, PloS One, 2019). CD96 modulates the immune response of NK cells, which are part of the innate immune system of mammals. NK cells play an important role in eliminating virally infected cells and cancer cells. When HSV infection occurs, nectin-1 located at the cell surface becomes internalized by endocytosis. We hypothesize that HSV down- regulates nectin-1 from the surface of infected cells to prevent their detection and destruction by NK cells. To study how nectin-1 and CD96 proteins bind to each other at cell contacts, cell lines expressing fluorescently tagged nectin-1 and CD96 are being generated. Previously, we used mouse melanoma B78H1 cells as a model which are normally deficient in both nectin-1 and CD96. B78H1 cells were transfected to stably express fluorescent human nectin-1 (hNectin-1GFP, green), however expression of fluorescent human CD96 (hCD96mCherry, red) appears to be unstable in these cells. Here, human cell lines 293 and K562 are being used. 293 cells are robust human embryonic kidney cells that are being used as a positive control for transfection. K562 cells are human myelogenous leukemia cells that have been shown previously in this lab to be deficient in nectin-1 and CD96. We are currently attempting selection of clonal cell lines for these constructs by fluorescent microscopy and flow cytometry. The cells that express fluorescent CD96 and nectin-1 will be mixed in co-cultures to observe co- localization of nectin-1 and CD96 at cell contacts. This approach will provide a unique setting to investigate and understand how HSV affects the NK cell innate immune response.

Holmes VM, Maluquer de Motes C, **Richards PT,** Roldan J, Bhargava AK, Orange JS, et al. (2019) Interaction between nectin-1 and the human natural killer cell receptor CD96. PLoS ONE 14(2): e0212443. https://doi.org/10.1371/journal.pone.0212443

2 *HSV entry*

HSV entry is a complex process with multiple virus-cell interactions.

- Encapsidated viral genome is delivered into the cell after fusion between the cell membrane and the viral envelope.
- Four essential viral glycoproteins (gD, gH, gL, gB) are needed to promote membrane fusion. The attachment glycoprotein C favors entry but is not required.



HSV gD binding to a specific cell surface receptor is critical to initiate entry.

Three cell surface molecules act as functional receptors for HSV:

Nectin-1 is cell-cell adhesion molecule which is used as the main receptor on epithelial cells and neurons.

HVEM is an immune modulator which act as HSV receptor on some epithelial cell, and may be used by HWSV to modulate the immune response.

3-O-Sulfated Heparan Sulfate also binds gD and allows HSV-1 entry in culture.

For review see:
Heldwein, E.E. and C. Krummenacher. 2008. Entry of herpesviruses into mammalian cells. Cell Mol. Life Sci. 65:1653-1668.
Krummenacher C., A. Carfi, R.J. Eisenberg and G.H. Cohen. 2013. Herpesvirus entry into cells: The Enigma Variations. Adv. Exp. Med. Biol. 790:178-195.

3 *How HSV may escape killing of NK cells by down regulation of nectin-1*

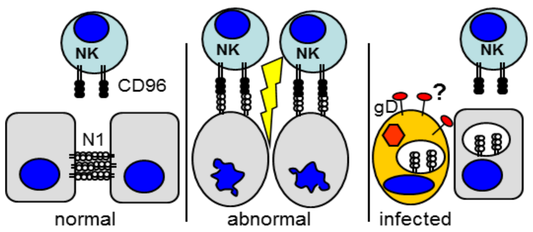
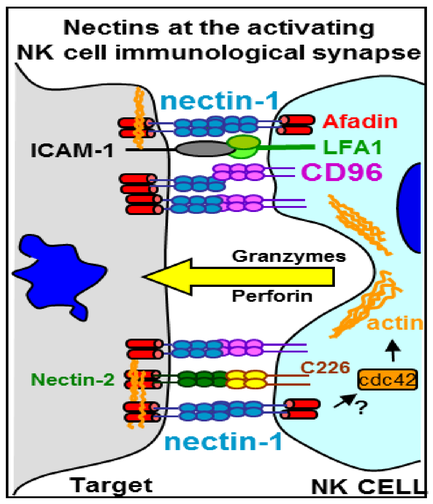


Figure 3: Role of nectin-1 in recruiting and activating NK cells. **A:** Nectin-1 (N1) in epithelial junctions is not accessible to NK cells. **B:** In pathologic cases, nectin-1 is exposed and recognized by CD96 at NKIS. NK cells are recruited and activated. **C:** gD is expressed in infected cells, nectin-1 is down-regulated from the infected cell surface and endocytosed in adjacent cells. No activation of NK cells.

Area's of Focus

- Is CD96 involved in binding to nectin-1 during the immune response of NK cells?
- Is nectin-1 involved in the activation of NK cells?
- How are these functions of nectin-1 possibly affected by HSV during infection?

4 *Role of nectin-1 in cell adhesion and NK cells*



Potential relationship between nectin-1 and surveilling NK cells.

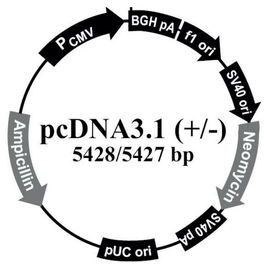
Schematic representation of various nectin-1 and CD96 and their ligands at the activating Natural Killer Cell Immunological Synapse.

5 *Hypothesis*

HSV binding of gD to nectin-1 down-regulates nectin-1 from infected cells which may protect HSV infected cells from the immune response of NK cells.

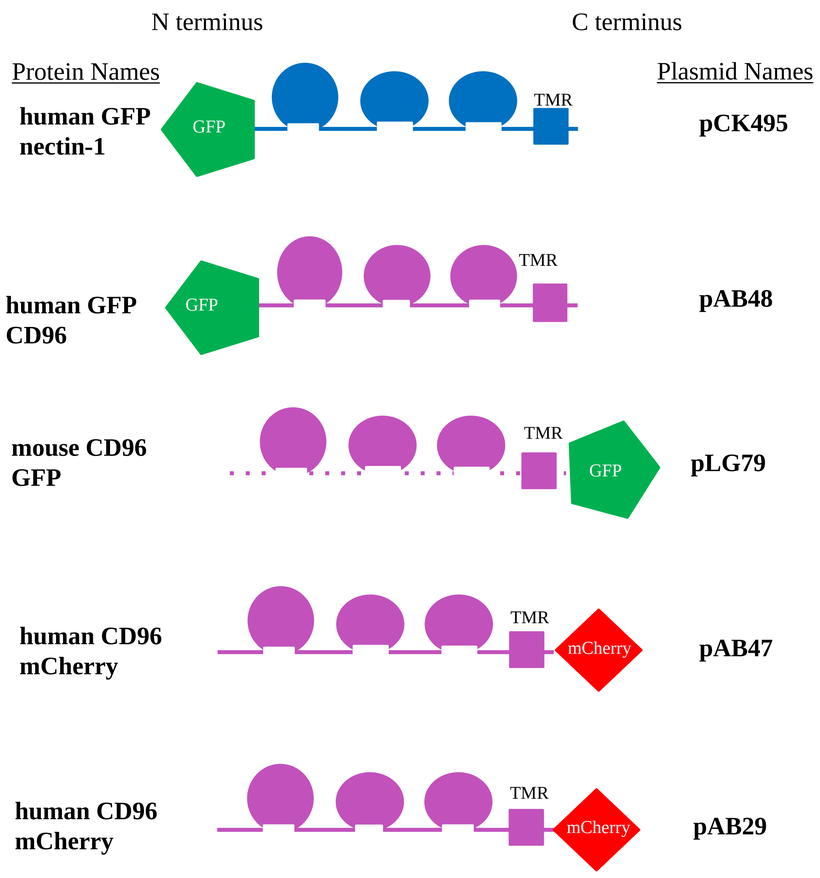
6 *DNA Constructs*

Plasmids for transfection are based on the vector pcDNA3.1+



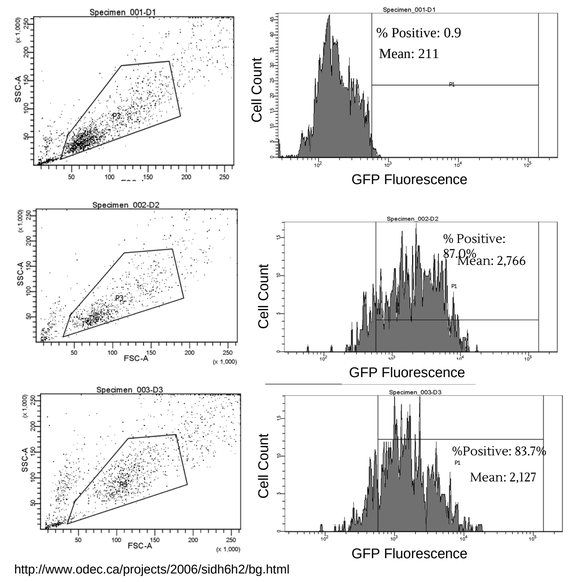
pUC origin: This is the origin of replication for the bacteria.
Ampicillin resistant gene: Plasmids are made in bacteria. The ampicillin will kill bacteria that do not have a plasmid in them.
P CMV: A promotor from the cytomegolovirus
BGH pA, f1 ori, SV40 ori: This is the multiple restriction site where specific genes can be put into the plasmid. These are specific for the pcDNA3.1 plasmid.
Neomycin: Antibiotic that is resistant to G418 cells. This will help select against cells that did not take in the plasmid.
SV40 pA: poly A site

http://www.biofeng.com/zaiti/buru/pcDNA3.1(-).html



7 *Flow Cytometry(FACS)*

Testing the fluorescence of GFP nectin-1 by measuring GFP fluorescence in transfected B78 cell lines.



B78 cell line- negative control

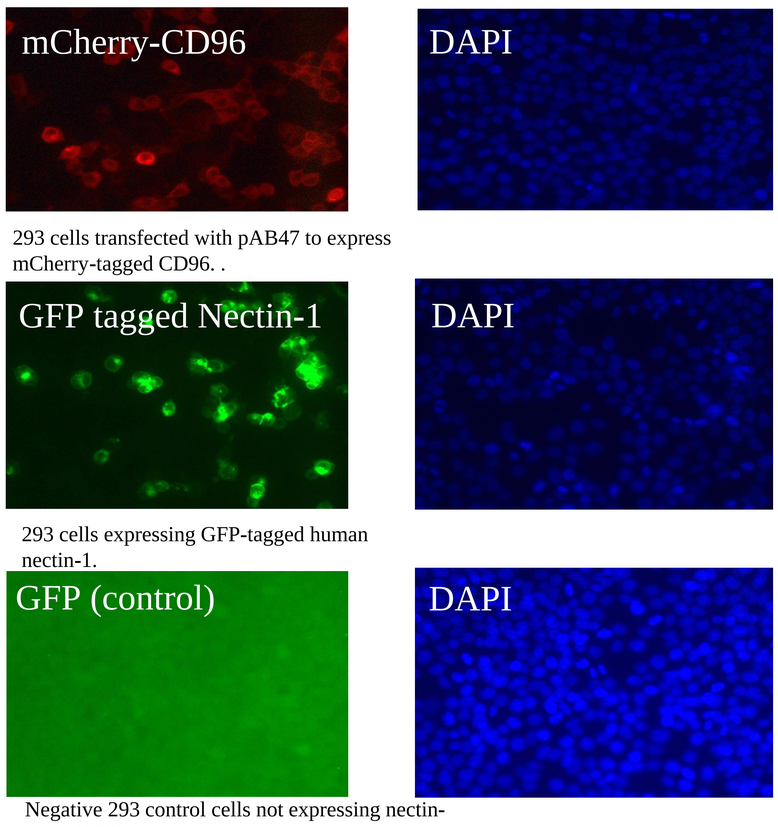
pCK495-1 cell line

pCK495-15 cell line

http://www.odec.ca/projects/2006/sidh6h2/bg.html

8 *Fluorescence microscopy*

To prepare for microscopy, cell lines grown in a 24 well plate were fixed and mounted on slides using Gold antifade reagent with DAPI; which stains cell nuclei for observation of individual cells. The figures on the left show fluorescing receptors through appropriate filters. Images on the right show DAPI nuclear staining of the same field.



293 cells transfected with pAB47 to express mCherry-tagged CD96. .

293 cells expressing GFP-tagged human nectin-1.

Negative 293 control cells not expressing nectin-1 nor CD96.

9 *Future directions*

- Detect co-localization of nectin-1 and CD96 through the use of fluorescent microscopy and confocal microscopy.
- Infect the transfected cells with HSV1 or add purified gD to see how this may affect the interaction between nectin-1 and CD96.

References and acknowledgments

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