



Massachusetts
Institute of
Technology



Under the Radar Screen: How Bugs Trick Our Immune Defenses

Session 5: MHC class I antigen presentation

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Human cytomegalovirus (HCMV)

- CMV is a member of the herpesvirus family, which includes the herpes simplex viruses and the viruses that cause chicken pox (varicella-zoster virus) and infectious mononucleosis (Epstein-Barr virus)
- Most common congenital (present at birth) infection in the United States.
- Once CMV is in a person's body, it stays there for life
- Most CMV infections are "silent" meaning they cause no signs or symptoms in an infected person.

Reagents

- Anti HC: antibody that reacts with the MHC class I heavy chain, regardless of its association with b2m and peptide
- W632: antibody that reacts with folded/assembled MHC class I
- LLnL, CbzLLL, lactacystin: proteasome inhibitors. In the presence of these inhibitors, proteins that are targeted for degradation accumulate inside the cell.
- BFA: brefeldin A, lactone antibiotic produced by fungi that blocks ER to Golgi transport.
- EndoH: endoglycosidase H, glycosidase that removes high mannose glycans from N-linked glycoproteins. High mannose glycans are found in the ER and are trimmed in the Golgi. EndoH is used to determine if proteins have moved from the ER to the Golgi.
- N-glycanase: cytosolic enzyme that removes glycans from proteins prior to their degradation

Techniques

- Pulse/Chase: Technique for following the movement of a substance through a biochemical or cellular pathway, by briefly adding the radioactively labeled substance (the pulse) followed by the unlabeled substance (the chase).
- Isoelectric focusing: separation of proteins according to their isoelectric point (PI)
- Cellular fractionation (microsomes): small vesicles generated by fractionation of the ER that are purified by differential centrifugation.
- RNA interference: RNA mediated downregulation of genes

Machinery involved in retro-translocation

- P97 ATPase (can be ER membrane associated but is cytosolic so it has to be through a receptor)
- Ufd1 and Npl4 (cofactors of P97)
- Sec61 (channel that transports nascent protein chains from the cytosol to the ER) ???

Substrate recognition machinery is still unknown

The papers

- Cell, vol 84 1996: HCMV infected cells have low class I levels at the cell surface and class I is degraded in these cells. How ? By what ?
- Nature, vol 429 2004: What cellular machinery is involved in the US11 mediated class I degradation ? What can be learned about normal cellular processes using viruses ?

Paper 1

“The HCMV US11 gene product dislocates MHC class I heavy chains from the ER to the cytosol”

E.J.H.J. Wiertz, T.R. Jones, L. Sun, M. Bogyo, H.J. Geuze and H. Ploegh

Questions

- fig1. Why do the pulse-chase at 26 degrees ?
- They use EM to determine the subcellular localization of US11. What other 2 techniques could have been used ?
- fig3. What could be causing the accumulation of a lower MW species in the presence of US11 ? See also fig 5. Hint: modifications to the protein ??
- fig5. How can they conclude that the 40KDa species is Endo H resistant when it runs at the same MW as the Endo H sensitive species ?
- fig6a. LLnI does not block completely MCH class I degradation. Why ? Would it be possible to block it completely ?

Paper 2

“A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol”

Y. Ye, C. Yun, D. Ron and T.A. Rapoport

Questions

- fig1c. How do they determine where the transmembrane domains are ? Is it reliable ?
- fig6. What do you think of the model ? Is it likely that Derlin-1 forms a channel in the ER membrane ?