



Massachusetts
Institute of
Technology



Under the Radar Screen: How Bugs Trick Our Immune Defenses

Session 8: Apoptosis

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Myxoma virus

- Poxvirus
- Infects rabbits and causes myxomatosis (skin lesions, swelling of the head, lumps on paws, ears and nose)
- Can be lethal but in time, rabbit populations can develop an immunity to the virus
- Was spread intentionally in Australia as an attempt to control the population of European rabbits that had grown out of proportion since their introduction in the 1950s

Epstein-Barr virus

- Herpes virus
- One of the most common human virus, it causes Mononucleosis or B cell lymphomas (has growth transforming capacity)

Apoptosis

- Deliberate death of a cell, aka programmed cell death. Opposed to Necrosis which is cell death resulting from an injury.
- Can be necessary during certain phases of development and can provide a source of antigens to APCs.
- Involves a cascade of biochemical events.
- Mitochondria: Key organelle in apoptosis. It controls the release of cytochrome C into the cytosol through the formation of pores.
- Role of BCL-2: BCL2 is the prototype for a family of genes. They govern mitochondrial outer membrane permeabilisation (MOMP) and can be either pro-apoptotic (including Bax, Bak and Bok) or anti-apoptotic (including Bcl-2, Bcl-xL, and Bcl-w). They can induce or block the release of cytochrome C into the cytosol, which activates caspase 9 and the following cascade.
- Caspases: Cysteine proteases that are “effectors” of apoptosis. They cleave other proteins after an aspartic acid residue. They often act in a cascade where they can activate each other.
cysteine-aspartic-acid-proteases.

Techniques

- TUNEL assay: DNA is fragmented during apoptosis. You can measure the level of apoptosis in a cell population by incubating cells with a dye that binds nicked DNA and performing flow cytometry.
- Mitochondria permeability: DiOC6 fluorescence correlates with mitochondrial membrane potential. + Control used protonophore CCCP causes dissipation of the proton gradient across the mitochondrial membrane and made sure that CCCP triggered change in fluo. Followed by flow cyt. TMRE and TMRM are similar to DiOC6 and give the same results.

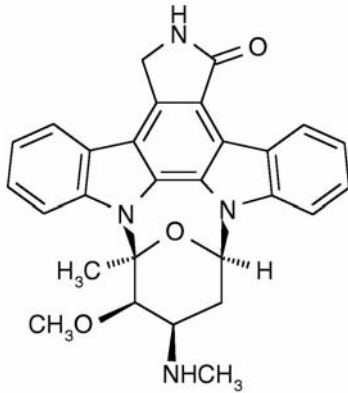
Relationship between EBV latent gene expression and cellular phenotypes in Burkitt's Lymphoma

Group I: - Low levels of activation antigens
- Low levels of latent genes
- High levels of apoptosis

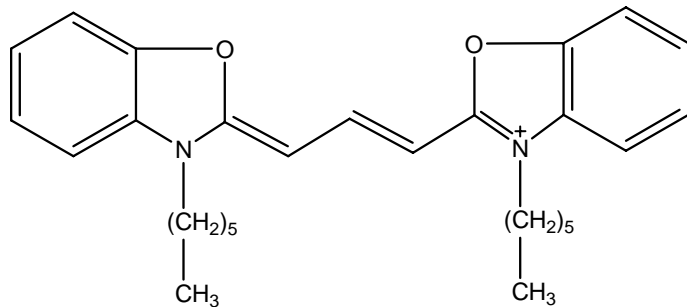
Group III: - High levels of latent genes
- Low levels of apoptosis

EBV latent genes provide some sort of survival signal !

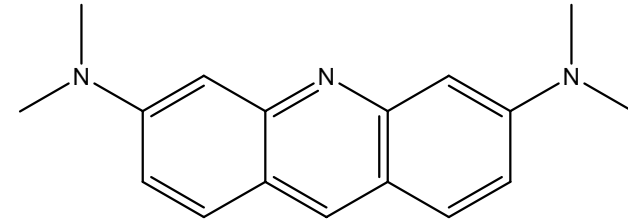
This weeks chemicals



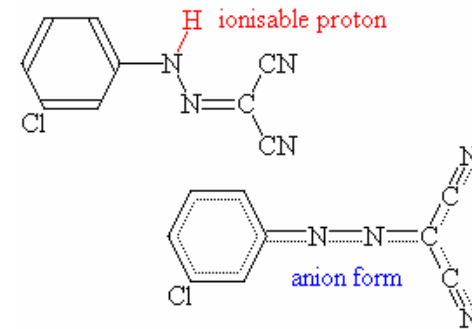
Staurosporine (*Streptomyces staurospores*)
is a relatively non-selective protein kinase inhibitor, which blocks many kinases to different degrees. Staurosporine is often used as a general method for inducing apoptosis



DiOC6(3) -(3,3'-dihexyloxacarboxyanine iodide)
is a fluorescent dye used for the staining of a cell's ER (endoplasmic reticulum), vesicle membranes and mitochondria



Acridine orange
Acridine orange is a nucleic acid selective fluorescent cationic dye useful for cell cycle determination. It is cell-permeable, and interacts with DNA and RNA by intercalation or electrostatic attractions.



CCCP: (carbonyl cyanide m-chloro phenyl hydrazone)
This is a lipid-soluble weak acid which is a very powerful mitochondrial uncoupling agent. The negative charge is extensively delocalized. This allows the anion to diffuse through non-polar media, such as phospholipid membranes. This behavior is very unusual: the vast majority of electrically charged ions are excluded from non-polar environments.

Paper 1

Everett, H., Barry, M., Lee, S. F., Sun, X. J., Graham, K., Stone, J., Bleackley, R. C., and McFadden, G. "M11L: A Novel Mitochondria-localized Protein of Myxoma Virus that Blocks Apoptosis of Infected Leukocytes." *Journal of Experimental Medicine* 191 (2000): 1487-1498.

Questions

- What is staurosporine and what does it do ?
- Does it make sense that a mitochondrial protein made by a cytoplasmic virus be also found in the cytoplasm ?
Hint: how are proteins targeted to the mitochondria ?
- Why does M11L not have a regular mitochondrial targeting sequence ?

Conclusions

- M11L on its own blocks apoptosis
- M11L is targeted to the mitochondria
- M11L reduces mitochondrial permeability transition (which is associated with apoptosis)
- M11L KO virus causes less apoptosis but more inflammation... why ? Target monocytes but not directly shown...

Paper 2

Henderson, S., Rowe, M., Gregory, C., Croomcarter, D., Wang, F., Longnecker, R., Kieff, E., and Rickinson, A. " Induction of Bcl-2 Expression by Epstein-Barr-Virus Latent Membrane Protein-1 Protects Infected B-cells from Programmed Cell-death." *Cell* 65 (1991): 1107-1115.

Prior evidence suggesting the existence of a chemokine related co-receptor for HIV-1 entry

- Human cells expressing CD4 are permissive for viral entry whereas mouse cells expressing the same human CD4 are not.
- The difference in tropisms (macrophages vs T cells and cell lines vs primary cells) seems to be linked to the sequence of gp120 (ENV subunit).
- Soluble factors such as Mip1 α and Mip1 β can block viral entry.
- HIV resistance in humans is linked to increased levels of chemokines.
- Fusin, a 7TM G coupled receptor functions as a co-receptor for entry of T tropic strains.

Questions asked in this paper:

- Are there other chemokine receptors used by HIV to enter the cells ?
- Could that explain some of the differences in tropisms ?
- What are these receptors ?

Questions

- They use acridine orange to measure cell viability as a way to assess apoptosis, is this appropriate ?
- In figure 2, are the results quantitative ? If not, what would be required to make them quantitative ?
- What should Figure 9 be in paper 2 ?