Massachusetts Institute of Technology

Under the Radar Screen:
How Bugs Trick Our Immune Defenses

Session 06: Major Histocompatiblity (MHC) class II antigen presentation

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In this session

Paper 1. Attack on the T-cell:

Identifies the Nef-gene product of HIV to downregulate surface expression of CD4 (without transcriptional or translational defects) via a pathway different from normal downregulation after T-cell activation (which is Serine phosphorylation dependent)

Paper 2. Attack on the Antigen Presenting Cell (APC):

Identifies the Vpx-gene product of HIV to interact with the Invariant Chain (using cell-free, transfected and infected cells).

Human immunodeficiency virus (HIV) Biology

Viral Entry:

Macrophages and CD4+ T cells are targeted by HIV because of glycoproteins on the HIV surface (gp160 → gp120, gp41) which are capable of binding the CD4 co-receptor and chemokine receptors. Ultimately, this leads to membrane fusion and capsid entry

Replication and transcription:

Viral RNA transcription into DNA (error prone = drug resistance)
Viral DNA gets integrated into the host genome
Here, the virus can be dormant
Transcription factor dependent activation generates mRNA.
(T-cell activation (NFkB) leads to killing of T-cell !!)
Initially, mRNA is spliced
Regulatory and structural proteins are produced
Later, splicing is inhibited to give full-length viral RNA

Assembly and release:

Env-polyprotein (gp160) is cleaved by protease into gp120 and gp41 and are inserted in the plasma membrane of the host cell
Gag (p55), Gag-Pol (p160) and HIV genomic RNA associate with plasma membrane inner surface
Polyproteins are cleaved (protease) into functional proteins / enzymes.
The various structural components assemble to produce a mature HIV virion

Adapted from http://en.wikipedia.org/wiki/HIV

Human immunodeficiency virus (HIV-1) Biology

p24, p6, p7, p17 : proteins encoded by the gag gene that provide structural elements of the virus
Reverse transcriptase: transcribes the viral RNA into double-stranded DNA.
Integrase: integrates the DNA produced by reverse transcriptase into the host's genome.
Protease: HIV's gag and pol genes are produced as larger combination proteins and the specific protease used by HIV cleaves these into separate functional units.
Gp120: Exposed on the surface of the viral envelope and binds to the CD4 receptor on any target cell that has such a receptor, particularly the helper T-cell.
Gp41: glycoprotein is non-covalently bound to gp120, and provides assistance in fusion with the host cell
Tat: "Trans-Activator of Transcription" helps HIV reproduce by compensating for a defect in its genome: the HIV RNA initially has a hairpin-structured portion which prevents full transcription occurring. However, a small number of RNA transcripts will be made, which allow the Tat protein to be produced. Tat then binds to and phosphorylates cellular factors, eliminating the effect of the hairpin RNA structure and allowing transcription of the HIV DNA (Kim, 2001). This itself increases the rate of transcription, providing a positive feedback cycle. This in turn allows HIV to have an explosive response once a threshold amount of Tat is produced, a useful tool for defeating the body's response.
Rev : "Regulator of Virion" allows fragments of HIV mRNA that contain a Rev Response Unit (RRE) to be exported from the nucleus to the cytoplasm.

Nef: "Negative Regulatory Factor". The expression of Nef early in the viral life cycle ensures T cell activation and the establishment of a persistent state of infection. Nef also promotes the survival of infected cells by downmodulating the expression of several surface molecules important in host immune function. These include major histocompatibility complex-I (MHC I) and MHC II present on antigen presenting cells (APCs) and target cells,CD4 and CD28 present on CD4+ T cells.

Vif: "Viral infectivity factor" is essential for viral replication
Vpr: "Viral Protein R" plays an important role in regulating nuclear import of the HIV-1 pre-integration complex
Vpu: "Viral Protein U". Vpu is involved in viral budding, enhancing virion release from the cell.

Vpx: "Viral Protein X". Is absent in the HIV-1 viral strain but can be found in HIV-2 and SIV.

Adapted from http://en.wikipedia.org/wiki/HIV

Paper 1

Serine phosphorylation-independent down-regulation of cell-surface CD4

Nature, **350**, pp 508-511, (1991)

Garcia, J.V., and Miller, A.D.

Questions

- What is the function of sorting 2 populations in Fig. 1d ?
- What staining reagent was used in Fig. 1h-j and what does it show ?
- Why are there two different-sized NEO-transcripts in Fig. 2 ?
- Why is it important to make the point that Nef-downregulation is phosphorylation independent ?
- Can you recap all questions addressed by the individual figures ?
- Why would HIV downregulate surface expression of the receptor that is critical for entry of the host ?

Paper 2

Interaction of human immunodeficiency virus type 2 Vpx and invariant chain.

Journal of Virology, **74**, pp 6168-6172, (2000)

Pancio, H.A., Vander Heyden, N., Kosuri, K., Cresswell, P., and Ratner, L.

Questions

- For Fig. 3, would you consider it necessary to also do an IP with the other interaction partner (Vpx) ?
- Do the authors build a good argument for Vpx / Invariant chain interaction. Could you suggest additional experiments to show its involvement in antigen presentation ?