Massachusetts Institute of Technology

Under the Radar Screen:
How Bugs Trick Our Immune Defenses

Session 7: Cytokines

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HHV-8

- Discovered in the 1980's at the beginning of the AIDS epidemic
- Gamma herpes virus that only occurs in humans
- Causes Kaposi's sarcoma (proliferation of vasculature), mostly in HIV patients

HIV-1

- Retrovirus (RNA genome) that causes AIDS
- T tropic or Macrophage tropic strains
- Enters target cells through CD4 and possibly other co-receptors.

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.

ENV

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.

Chemokines/Cytokines

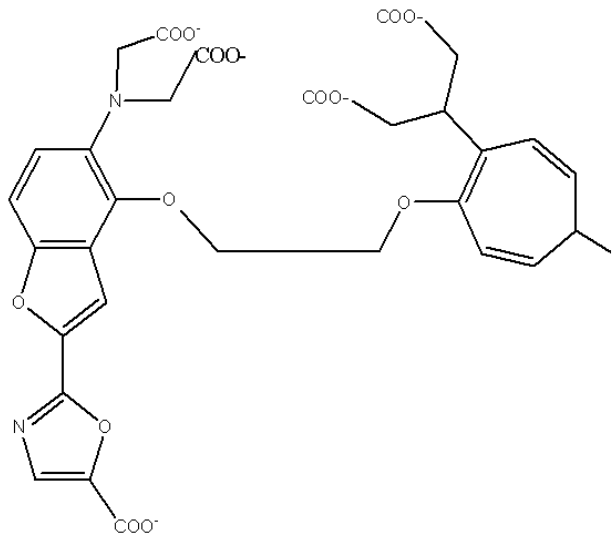
- Small molecules secreted by cells (app. 8-20 Kda)
- Can have chemotactic effects on neighboring cells
- Can be induced during an immune response and have a pro-inflammatory effect
- Bind to plasma membrane receptors on their target cells

cytokines

Name	Cells	Function
IL-1	Macrophage	T,Bcellactivation;fever;inflammation
IL-2	T cells	T cell proliferation
IL-3	T cells	Growth of many cell types
IL-4	T cells	B cell growth and differentiation
IL-5	T cells	B cell, eosinophil growth
IL-6	macrophages, T cells	B cell stimulation,inflammation
IL-7	stromal cells	Early B and T cell differentiation
IL-8	macrophages	Neutrophil (PMN)attraction
IL-9	T cells	mitogen
IL-10	T cells	Inhibits Th1 cytokine production
IL-11	Bone marrow stroma	Hematopoeisis
IL-12	APC	Stimulates T, NK cells
IL-13	T cells	Similar to IL-4
IL-14	dendritic cells,T cells	B cell memory
IL-15	T cells	same as IL-2
IL-16	- -	
IFNa	Most cells	Anti-viral
IFNb	Most cells	Anti-viral
IFNg	T, NK cells	inflammation,activates macrophages
TGFb	macrophages,lymphocytes	depends on target
TNFa	Macrophage	Inflammation; tumor killing
TNFb	T cells	Inflammation; tumor killing;enhance phagocytosis

Calcium release assay

Fura-2 is a widely used UV-excitable fluorescent calcium indicator. Its structure is derived from EGTA (Ethylen glycolbis(beta-amino-ethyl ether)) that has also 4 COOH-groups to bind calcium.



Upon calcium binding, the fluorescent excitation maximum of the indicator undergoes a blue shift from 363 nm (Ca2+-free) to 335 nm (Ca2+-saturated), while the fluorescence emission maximum is relatively unchanged at ~510 nm. The indicator is typically excited at 340 nm and 380 nm respectively and the ratio of the fluorescent intensities corresponding to the two excitations is used in calculating the intracellular concentrations.

Fura-2 is a calcium chelator which means that it is able to bind Ca2+ so that the ions are not able to perform a reaction with anything and easily can be transported.

Pertussis toxin (ptx) interferes with the regulation of the eukaryotic adenylate cyclase complex.

Normal regulation of adenylate cyclase activity in mammalian cells (Adenylate cyclase (AC) is activated normally by a stimulatory regulatory protein (Gs) and guanosine triphosphate (GTP); however the activation is normally brief because an inhibitory regulatory protein (Gi) hydrolyzes the GTP.

Adenylate cyclase activated by pertussis toxin (The pertussis A subunit transfers the ADP ribosyl moiety of NAD to the membrane-bound regulatory protein Gi that normally inhibits the eukaryotic adenylate cyclase. The Gi protein is inactivated and cannot perform its normal function to inhibit adenylate cyclase. The conversion of ATP to cyclic AMP cannot be stopped.)

Paper 1

“A Broad-Spectrum Chemokine Antagonist Encoded by Kaposi’s Sarcoma-associated Herpes Virus”

Viruses can produce chemokines that are similar to human cytokines and produce antagonistic effects.

Questions

- Fig 3. Why add EDTA in the calcium release assay and what does the EDTA do ?
- What does it mean if the calcium response induced by a cytokine is pertussis toxin dependant ?
- Why look at the effect of vMIP-II on HIV entry when vMIP-II is an HHV8 expressed chemokine ?

Paper 2

“Identification of a major co-receptor for primary isolates of HIV-1”

Viruses can also use the cytokine/chemokine receptor system as mode of entry into human cells.

Questions

- Fig 1. Is using a luciferase based assay adequate to measure infection/viral entry ?
- Why use RT-PCR to look at the expression of CC-CKR-5 mRNA rather than use Western blots and look at the protein levels.
- Is it advantageous to the virus to use a chemokine receptor to enter the target cell considering that cytokines secreted by our immune system can block it...?