RAISON D’ETRE OF THE IMMUNE SYSTEM:

To Distinguish Self from Non-Self Thereby Protecting Us From Our Hostile Environment.

Innate Immunity

Acquired Immunity
Innate immunity:

(Antigen nonspecific) defense mechanisms that are used by the host immediately or within several hours of encountering antigen.
lysozyme in tears and other secretions
commensals

skin
physical barrier
fatty acids
commensals

low pH and commensals of vagina

removal of particles by rapid passage of air over turbinate bones
mucus, cilia
acid
rapid pH change
commensals
flushing of urinary tract
Cellular Components of the Innate Immune Response

NK cells

Granulocytes

Antigen Presenting Cells:
Dendritic cells
Macrophages
Antigen Presenting Cells

These specialized cells internalize antigen by phagocytosis or endocytosis and then express parts of the antigen on the cell surface.

These cells are distinguished by two properties:

1. Express class II MHC molecules

2. Provide co-stimulatory signals necessary for activation of T-cells.
Acquired Immunity

Is adaptive and displays four characteristic attributes:

• Antigen specific
• Diversity
• Immunologic Memory
• Self/non-self recognition
Acquired Immunity

Involves two major types of cells:

Lymphocytes:

a. B-cells: Originate in the bone-marrow

b. T-cells: Originate in the thymus

• All lymphocytes have an antigen receptor, a surface protein that engages with a portion of an invading pathogen
B-cell Receptors

B-cells (CD19 and CD20):

• B cells secrete their antigen receptors: antibodies.
Antibodies cont.

• Antibodies can help elicit clearance of an antigen, or can prevent proper functioning of the antigen: neutralization.

• Antibodies are effective against extracellular pathogens, such as bacteria, or virus that has budded from the cell.

• Antibodies can work at distal sites. Are in interstitial fluids, blood and lymph fluids.

• Can bind soluble antigen
The T-cell Receptor

**T-cells (CD3):** Their antigen receptor is surface bound.

http://bioweb.wku.edu/courses/biol328/TcR.png
T-cell Subsets and Functions

1. Cytotoxic T cells (CTL) kill infected cells.
   - Are identified by the surface marker CD8 (CD8+ T-cells)
   - Control intracellular pathogens such as viruses and bacteria
   - Require cell to cell contact to bind antigen
   - Bind only antigen presented on the surface of cells
T-cell Subsets and Functions

2. Helper T cells (Th) provide “help” for cytotoxic T cells and B cells.

- Are identified by the surface marker CD4 (CD4+ T-cells).
- Also require cell to cell contact to bind antigen.
- Bind only processed antigen
- Secrete cytokines and chemokines.
Cell to Cell Communication

- **Cytokine**: Small molecules secreted during an immune response that help to signal and activate responding cells.

- **Chemokines**: Also small molecules secreted during an immune response, these often signal cells to migrate to areas of inflammation.
Antigen Processing

- Antigen presenting cells pick up, or endocytose, antigens and degrade them within endosomes via acid-dependent proteases
Antigen: ag

APC

CD4+ T-cell

CD8+ T-cell

Death signals: Perforin, Granzyme etc.

Cytokines and Chemokines

Lysis

B-cell

Clearance, Neutralization
Antigen Specificity:

Is determined by interactions between cellular receptors (T-cell receptor and B-cell receptor complex), antigen, and human leukocyte antigens (HLA).
Human Leukocyte Antigens (HLA):

Are a group of proteins encoded within the major histocompatibility complex (MHC) on chromosome 6 in humans.

Are the proteins that the body uses to identify self.

“Present” antigens for recognition by B- and T-cells.

Variation between individuals and between ethnic groups is extensive.
Human Leukocyte Antigens (HLA):

Class I antigens are found on all nucleated cells. = A,B,C

Present endogenous antigens.

CD8+ T cells recognize antigen when presented by HLA Class I molecules
Human Leukocyte Antigens (HLA):

Class II antigens are primarily on antigen presenting cells (macrophages, dendritic cells and B cells).
= DR, DP, DQ

Present exogenous antigens

CD4+ T cells recognize antigen plus MHC Class II
Human Leukocyte Antigens (HLA):

Each HLA allele encodes a surface protein that has molecule has its own distinct requirements for peptide binding.

For example, HLA-A*0201 prefers to bind to leucine and valine, while HLA-A*0301 prefers to bind to leucine and lysine.

Therefore, a person’s constellation of HLA molecules will determine which portions of a pathogen will be presented to the immune system.
Ribbon Structure of HLA Molecules

Class I

Class II

And David S. Goodsell for: Molecule of the Month

http://www.rcsb.org/pdb/molecules/pdb63_3.html
Processing and presentation of antigens
T-cell recognition of antigen

infected cell

MHC molecule presents peptide

antigen peptide bound to MHC molecule

T-cell receptor recognizes MHC and peptide
Diversity

Diversity of the adaptive immune response is due to the diversity of the T-cell and B-cell receptor complexes.

Comes at the level of the T-cell and B-cell population. The receptors expressed on each cell are specific for only one antigen, but vary from cell to cell.
Diversity is at the population level
B and T-cell receptors do not recognize the entire antigen

- CD8+ T-cells usually bind 9 to 10 amino acid sequences
- CD4+ T-cells usually bind larger amino acid sequences. Length is less clear ~12 to 14.
- B-cell receptors can interact with intact antigens, but only bind small stretches of either linear or continuous sequences.
MHC - peptide binding

T-cell recognition sequences

Anchor sequences bind to the MHC.
Peptide sequences affect MHC binding and TCR recognition:

Binds MHC And TCR:
- L
- V

Loss or decrease in MHC binding:
- G
- V

Loss or decrease in TCR binding:
- L
- V
Antibody – Antigen Recognition

Antibodies recognize either linear epitopes or epitopes in secondary structures. A change in the amino acid sequence or secondary structure can eliminate or diminish the antibody binding.
Memory

Is established through the clonal expansion of activated T or B cells:
Self/Non-self Recognition:

Is achieved through the interaction of antigen receptors, HLA, and antigen.

Responses to this complex are controlled through a process of “education”.
**Tolerance:**
The inability to react with self.

**Autoimmunity:**
The state in which tolerance to self is lost.
Immune responses are most efficient in tissue parenchyma.

Lymph nodes and the spleen provide architectural support for cell-to-cell interactions, and serve as “filters” for fluids draining other tissues.
Immune Response To HIV Infection
HOW THE IMMUNE SYSTEM RESPONDS TO HIV

1. Macrophages ingest viral particles and break them down into small peptides (epitopes).

2. Selected epitopes are displayed on the surface; helper T cells bind to them.

3. Helper T cells secrete proteins that activate other immune cells.

4. B cells release antibodies, which bind to the virus.

4a. Killer T cells attack infected cells bearing specific epitopes.

5. Macrophages ingest and destroy antibody-bound particles.

5a. Infected cell dies.
Infection Levels (Separate Scales)

CD4+ T-cell

HIV

CD8+ T-cell

Antibodies

Acute Asymptomatic AIDS and Death

4 – 8 weeks

Years
Immune Response to HIV

- CD4: Helper T-cell responses
- CTL: Cytotoxic T-cell responses
- B-cell: Antibody responses
- APC: Antigen Presenting Cells
CD4 Responses To HIV

CD4+ T-cell responses to antigens are usually indirectly measured by proliferation (cell division).

- $^3$H-Thy uptake
- CFSE

Cytokine production is another measure of activation

- Eliza
- ELISpot
CD4+ T-cell responses are predictive of disease progression.

In most individuals, the following pattern is observed:

CD4+ T-cell responses decline at various stages:
- response to HIV and recall antigens (early)
- response to alloantigens (mid)
- response to mitogens (late)
- expression of IL-2 receptor (CD25)

In addition, there is aberrant cytokine production:
- production of IFN-g, IL-2
- production of IL-4, IL-10
HIV SPECIFIC CTL

CTL responses are made to every HIV-1 protein:
Gag, RT, Env, Pol, Nef, Vif, Vpr are more frequently targeted during chronic infection

• Inverse correlation between viral load and levels of circulating HIV-specific CTL.

• Emergence of CTL escape mutants over time.

• Depletion of CD8+ T cells from macaques prior to infection with SIV, leads to higher viral loads and more profound immunosuppression.

• Absence of detectable HIV-specific CTL, or oligoclonal CTL responses are associated with poor clinical outcome.
CTL Responses To HIV

CTL responses are measured by
- $^{51}$Cr release assay (Killing)
- ELISpot (Cytokine release)

Antigen specific CD8+ T-cells can be quantified by tetramer staining. (Number of specific cells)
CTL fail to eliminate HIV-1

• Many chronically infected individuals have vigorous HIV-1-specific CTL responses yet they almost always fail to adequately suppress the virus. Why?

- Epitope escape?
- CTL Exhaustion?
- Suboptimal CTL?
Donor A: CD8 response to SL9

- **GAG tetramer**
- **Gamma-INF**
- **SLYNTVATL**
1. Original population of HIV replicates well.

2. Immune cells able to recognize original epitope reduce viral population.

3. Escape mutant carrying unrecognized version of epitope appears and replicates unopposed for a time.

4. Immune system eventually gains control over mutant, but another emerges.

5. Repeated production of mutant viruses leads to great viral diversity.
Antibody Responses
**General Properties of Anti-viral Antibodies**

- Can be generated to any accessible portion of the virus.

- Effective in blocking entry (neutralizing) if directed to viral receptors such as gp120 of HIV.

- Can block fusion (neutralizing) if antibody (Ab) binds to fusion protein such as gp41 of HIV.

- Can effect clearance of virus if it binds the virus and then binds Fc receptors on monocytes and macrophages.

- Can also bind complement and kill enveloped viruses.

- Most effective if they are present at the site of viral entry.
Gp120 and Gp41-mediated fusion
Neutralizing antibody responses to HIV are difficult to generate because:

Gp120 is presented as a trimer which protects some of the potential antibody binding sites.

Gp120 is highly glycosylated, meaning it has sugar molecules over much of its surface. Because many human proteins are glycosylated, humans rarely make antibody responses to glycosylated portions of proteins.

CD4 binding site is devoid of glycosylation and relatively conserved between isolates but is masked by V1V2 loops and is in a depression which is too small for good antibody binding.
CD4bs
V1V2
V3
V4
Gp41

CN
Inner
Outer

CD4bs
Bridging Sheet

Glycosylation:
Silent face
Non-neutralizing face
Trimerization

2G12
CD4bs
Bridging Sheet
Coreceptor bs
Neutralizing face

Glycosylation:
Silent face
Changes in gp120 glycosylation allow HIV escape from Nab responses

Fig. 2. Neutralization of autologous HIV. The neutralizing activity of plasmas obtained from patient 1 at months 0, 6, and 12 after presentation with primary infection is assayed against virus from months 0 and 12. The titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of virus replication (dashed lines). The error at each dilution reflects the standard error of duplicate wells.

Richman et al. PNAS 2003 vol. 100:4149
HIV and APC's

• APC’s may exhibit altered:
  chemotaxis
  IL-1 production
  antigen presentation
  oxidative burst response
  antimycobacterial activity

• Antigen presenting cells can act as trojan horses.
Dendritic Cells and DC-SIGN

DC-Specific, ICAM-3 Grabbing, Nonintegrin.

Interaction of DC-SIGN with ICAM-3 establishes the initial contact of the DC with a resting T-cell.

This is important because of the low number (100-1000 copies/cell) of MHC-peptide ligands on the DC. This enhanced binding allows the T-cell to scan the surface of the DC.

DC-SIGN also binds the glycan-rich HIV-1 envelope in the absence of CD4.
Proposed pathways for the transmission of HIV-1 to T-cells.
Why does the immune response fail to clear HIV?

• HIV integrates into the host genome. Therefore, to eliminate HIV, infected cells must be killed.

• Host factors can paradoxically enhance HIV replication. Therefore, by responding to HIV, CD4+ T-cells can be destroyed.
Why does the immune response fail to clear HIV?

• HIV can mutate and escape immune mediated opposition.

• Suboptimal CTL responses can be elicited.
Why does the immune response fail to clear HIV?

• Sugar coating (glycosylation) and folding of gp120 protects against Ab recognition.

• Critical binding sites on gp41 are revealed for only a short period of time.
Why does the immune response fail to clear HIV?

• APC’s may exhibit altered functions diminishing their ability to elicit immune responses.

• Antigen presenting cells can act as trojan horses, spreading HIV to CD4+ T-cells as they begin to respond to antigen.
Why does the immune response fail to clear HIV?

Role of viral genes:

Tat: Extracellular Tat stimulates CD4+ and CD8+ T-cells.

Nef: Intracellular Nef appears to activate cells to promote viral replication. Affect on cellular function?

Intracellular Nef downregulates CD4 and MHC class I molecules. In vivo significance?