MCB 150: Molecular Immunology

Spring 2007
Prof. Ellen Robey
Prof. Robert Beatty
Prof. Laurent Coscoy

Today’s game-plan

• Structure of the course and administrative issues
• Overview of the immune system
• Begin: Innate Immunity

MCB 150 Spring 2007

<table>
<thead>
<tr>
<th>Innate Immunity</th>
<th>Humoral Immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytes, Toll-like receptors</td>
<td>B cells, antibody, complement</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cellular Immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells, TCR, development, selection, tolerance, NK cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immune Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>inflammation, allergy, autoimmunity, infection</td>
</tr>
</tbody>
</table>

Course Instructors

Lecturers:
Ellen Robey
471 LSA 2-8669
Office hours: Tues 11-12

Laurent Coscoy
451 LSA 3-4128
Office hours: TBA

Robert Beatty
176 LSA 2-0671
Office hours: Tues 3-4

GSIs:

Dina Weilhammer
Dina@berkeley.edu
Office hours: TBA

Tim Nice
timnice@berkeley.edu
Office hours: TBA
Course Components

• Lecture series
• Discussion sections (not required, but highly recommended)
• Reading: Text (Kuby 6th addition), Journal Articles
• Problem sets (posted on web, these are not graded, but are to help reinforce what you learn in class)
• Exams-- two mid-terms, plus final exam

Grading

• Mid-terms: 25% each
• Final 50%

Half of the material final is a cumulative review and half focuses on the last third of the class.

Course Web Site

• www.mcb.berkeley.edu/courses/mcb150
• All lecture notes, problem sets, answers will be posted for viewing or download
• Powerpoint presentations shown in lecture will be posted for your review

Getting Help!!!

• Web site: www.mcb.berkeley.edu/courses/mcb150
• Discussion groups / GSIs
• Office Hours
• After lecture-- very brief questions only
• No Discussion sections this week.

• Sign up sheets available in class today

• You may attend any or all of the 4 discussion sections.

• Problem set/reading material for discussion will be posted on website

Overview of the Immune system

Microbes: why they are formidable foes.

Gross anatomy of the immune system

Cells of the immune system

Effector mechanisms: how the immune system protects

Immune recognition of pathogens: innate vs adaptive immunity

Cytokines and the inflammatory response

Microbes are ubiquitous in nature, extraordinarily diverse, rapidly evolve to exploit opportunities to infect hosts and to evade their immune systems.

Many pathogens can expand rapidly in the nutrient-rich environment of the host.

Exponential growth

8 hours = 280 trillion bacteria!!!!
Some microbes hijack cellular machinery to replicate and spread. Intracellular pathogens include viruses (influenza, HIV) and intracellular bacteria (listeria) and intracellular parasites (malaria, toxoplasma).

Listeria bacteria using the actin cytoskeleton of the host cell to spread from cell to cell. (Portnoy lab)  
Toxoplasma parasites (red) and dendritic cells (green) within a mouse lymph node. (Robey lab)

Pathogens rapidly evolve to avoid the host immune response

<table>
<thead>
<tr>
<th>Virus</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leporipapovirus (a myxoma virus)</td>
<td>Soluble IFN-γ receptor</td>
</tr>
<tr>
<td>Several poxviruses</td>
<td>Soluble IFN-γ receptor</td>
</tr>
<tr>
<td>Vaccinia, smallpox virus</td>
<td>Soluble IL-1 β receptor</td>
</tr>
<tr>
<td>Epstein-Barr</td>
<td>IL-10 homolog</td>
</tr>
<tr>
<td>Human herpesvirus-8</td>
<td>IL-6 homolog; also homologs of the chemokines MIP-1 and MIP-2</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Three different chemokine receptor homologs, one of which binds three different soluble chemokines (RANTES, MCP-1, and MIP-1α)</td>
</tr>
</tbody>
</table>

Overview of the Immune system

Microbes: why they are formidable foes.

Gross anatomy of the immune system

Cells of the immune system

Effector mechanisms: how the immune system protects

Immune recognition of pathogens: innate vs adaptive immunity

Cytokines and the inflammatory response

First some key definitions:

Pathogen: microbe that causes disease

Antigen: material (from a pathogen) that induces an immune response

Innate (natural) immunity: rapid, non specific immune response

Adaptive (acquired) immunity: slower, specific immune response

Leukocytes: blood cells

Lymphocytes: specialized blood cells that mediate adaptive immunity (e.g. T and B cells)
The cells of the immune system circulate through the body via lymph and blood. Pathogens and their antigens are transported from tissues via lymphatic vessels to the lymph nodes where they encounter immune cells.

The cells of the immune system spend much of their time in lymphoid organs. They develop (arise) in primary lymphoid organs, and they interact with antigens in secondary lymphoid organs.

**Thymus**: primary lymphoid organ for T cell development

**Bone marrow**: primary lymphoid organ for B cell development

**Lymph nodes**: collect antigens from tissues

**Spleen**: collects antigens from blood stream

---

**Overview of the Immune system**

Microbes: why they are formidable foes.

Gross anatomy of the immune system

**Cells of the immune system**

Effector mechanisms: how the immune system protects

Immune recognition of pathogens: innate vs adaptive immunity

Cytokines and the inflammatory response

---

Many different types of blood cells participate in the immune response to microbes:

**Innate immune cells**: “phagocytes”
- macrophage, neutrophils, dendritic cells

**Adaptive immune cells**: “lymphocytes”
- T cells, B cells
Most blood cells act to fight infection.

Overview of the Immune system

Microbes: why they are formidable foes.
Gross anatomy of the immune system
Cells of the immune system
Effector mechanisms: how the immune system protects
Immune recognition: innate and adaptive
Cytokines and inflammation

Immune Effector Mechanisms:

Cell-mediated immunity:
Phagocytosis (cellular eating)
cytotoxicity (cellular killing)

Humoral immunity:
complement: group of serum proteins that can directly kill pathogens.
antibodies: (also called immunoglobulin) proteins secreted by B cells that bind directly and specifically to pathogens. Antibodies target pathogens by marking them for destruction by other components of the immune system.
Macrophage fight microbes by engulfing and digesting them (phagocytosis or cellular eating).

A macrophage engulfing yeast

Bacteria fight back against phagocytosis

A macrophage attempting to engulf a bacterium *Streptococcus pneumoniae*, that is covered with a slimy coat.

Target cell killing by a cytotoxic (killer) lymphocyte

A cytotoxic T lymphocyte (CTL) killing a cell that has been infected by a virus. NK cells use a similar mechanism to eliminate tumor cells.

Overview of the Immune system

Microbes: why they are formidable foes.

Gross anatomy of the immune system

Cells of the immune system

Effector mechanisms: how the immune system protects

*innate and adaptive immunity*

Cytokines and inflammation
### TABLE 1-3: Comparison of adaptive and innate immunity

<table>
<thead>
<tr>
<th></th>
<th>Innate</th>
<th>Adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Response time</strong></td>
<td>Hours</td>
<td>Days</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>Limited and fixed</td>
<td>Highly diverse, improves during the course of immune response</td>
</tr>
<tr>
<td><strong>Response to repeat infection</strong></td>
<td>Identical to primary response</td>
<td>Much more rapidly than primary response</td>
</tr>
</tbody>
</table>

- **Found in:**
  - all multicellular organisms
  - Vertebrates only

- **Substances that trigger:**
  - A limited number of pathogen-associated molecular patterns (PAMPs)
  - Virtually any component of pathogens

- **Receptors:**
  - A limited number of Pattern Recognition Receptors (PRRs) expressed on many cell types
  - Highly variable receptors of 2 types: antibody made by B cells and TCR made by T cells

---

**Comparison of innate and adaptive immune recognition**

<table>
<thead>
<tr>
<th>Pathogen-associated molecular patterns (PAMPs)</th>
<th>Toll-like receptors (TLRs)</th>
<th>Pattern Recognition Receptors (PRRs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoarabinomannan</td>
<td>Tolllike (PAMP)</td>
<td>Flagellin</td>
</tr>
<tr>
<td>LPS (Gram-negative)</td>
<td>Tolllike (PAMP)</td>
<td>Flagellin</td>
</tr>
<tr>
<td>LPS (Gram-positive)</td>
<td>Tolllike (PAMP)</td>
<td>Flagellin</td>
</tr>
<tr>
<td>Flagellin</td>
<td>Tolllike (PAMP)</td>
<td>Flagellin</td>
</tr>
<tr>
<td>Virtually any component of pathogens</td>
<td>Tolllike (PAMP)</td>
<td>Flagellin</td>
</tr>
</tbody>
</table>

**Receptors that mediate**

- **Innate immune recognition: Toll-like receptors (TLRs)**
  - TLR2
  - TLR5
  - NOD-2
  - TLR9

- **Adaptive immune recognition**
  - Antibody and the T cell receptor (TCR)

---

**Overview of the Immune system**

- **Microbes:** why they are formidable foes.
- **Gross anatomy of the immune system**
- **Cells of the immune system**
- **Effector mechanisms:** how the immune system protects
- **Immune recognition:** innate and adaptive
- **Cytokines and inflammation**
Cytokines: (also called interleukins)

Small secreted peptides that used for intracellular communication between cells of the immune system

Can turn on or off immune responses

Mediate inflammation

Chemokines: subset of cytokines that specialize in regulating cell motility

Inflammation: a complex series of events induced by tissue damage (described in medical literature in 1500s)

- Redness (rubor)
- Pain (dolor)
- Swelling (tumor)
- Heat (calor)

Inflammation occurs when injured tissues release mediators that promote vasodilation (increased blood flow) and chemotaxis (directed migration) of leukocytes.

Infection can induce inflammation, but even sterile injuries can be sufficient to induce inflammation.
Blood cells (leukocytes) travel from the blood stream into tissues by a process known as **extravasation**

Blood cells can also be attracted to sites of infection by products produced by pathogens, as well as by chemoattractants made by host (chemokines, inflammatory mediators).

Neutrophils (a type of white blood cell) are attracted to bacterial products. Here they are moving toward a gradient of the bacterial peptide fMLP.

### Why study the immune system?

Importance of the immune system in human health

Provides model systems for studies of:
- gene regulation
- molecular recognition
- signal transduction
- etc, etc

Provides powerful techniques for use in medicine and science
Diseases associated with immune system dysfunction

- Autoimmunity (SLE, Arthritis, Myasthenia gravis, Graves disease)
- Immunodeficiency (inherited, acquired)
- Allergy (environment, drugs)
- Cancer (Leukemia, Lymphoma)

Myasthenia gravis

Asthma & Allergy

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Common allergens</th>
<th>Route of entry</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>Drugs, Soaps,</td>
<td>Intradermal</td>
<td>Systemic</td>
</tr>
<tr>
<td></td>
<td>Vitamin Pillars</td>
<td></td>
<td>response</td>
</tr>
<tr>
<td>Acute dealers</td>
<td>local shots</td>
<td>Subcutaneous</td>
<td>Local</td>
</tr>
<tr>
<td></td>
<td>Allergic rhinitis</td>
<td></td>
<td>rhinitis</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>Pollens (tree,</td>
<td>Intestinal</td>
<td>Intestinal</td>
</tr>
<tr>
<td></td>
<td>flower, grass)</td>
<td></td>
<td>rhinitis</td>
</tr>
<tr>
<td>Asthma</td>
<td>Pollens Dust,</td>
<td>Intestinal</td>
<td>Intestinal</td>
</tr>
<tr>
<td></td>
<td>food</td>
<td></td>
<td>rhinitis</td>
</tr>
<tr>
<td>Food allergy</td>
<td>Shells, Milk,</td>
<td>Oral</td>
<td>Oral</td>
</tr>
<tr>
<td></td>
<td>Eggs, Fruits</td>
<td></td>
<td>rhinitis</td>
</tr>
</tbody>
</table>

Therapeutics

- Vaccination
- Adoptive immunotherapy
- Transplantation
**Vaccination: THE major success**

Cases per 100,000 population:
- Diphtheria
- Polio
- Measles

**Tissue Transplantation**

<table>
<thead>
<tr>
<th>Tissue Transplanted</th>
<th>5 year graft survival</th>
<th>No. of grafts in USA (1992)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>50-80%</td>
<td>9736</td>
</tr>
<tr>
<td>Liver</td>
<td>40-50%</td>
<td>3064</td>
</tr>
<tr>
<td>Heart</td>
<td>70%</td>
<td>2172</td>
</tr>
<tr>
<td>Lung</td>
<td>80-40%</td>
<td>686</td>
</tr>
<tr>
<td>Cornea</td>
<td>~70%</td>
<td>40,000</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>80%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Treating cancer**

- Tumor-specific antibody
- Tumor-specific antibody conjugated to toxin
- Tumor-specific antibody conjugated to radionuclide

- Antibodies bind to the tumor cell
- Antibody-toxin conjugates bind to the tumor cell
- Radioactive antibody binds to the tumor cell

**Powerful methods for detecting and quantitating proteins and cells based on the highly specific binding of antibodies (immunoglobulins)**

- **ELISA**: enzyme-linked immunosorbant assay (measures small amounts of hormones, drugs, microbes in body fluids)
- **Western blot** (detects disease associated proteins)
- **Flow cytometry** (quantifies various cell types in mixed population-- HIV patients)
**Western Blot**

- Separate proteins by size using PAGE gel
- Transfer gel to blotting membrane
- Probe membrane with antibody specific for protein of interest
- Detect bound antibody by chemiluminescence

**Flow Cytometry: Quantitative Single-cell Immunofluorescence**

**Immunofluorescence**

**Innate (natural) immunity**
TABLE 1-3  Comparison of adaptive and innate immunity

<table>
<thead>
<tr>
<th></th>
<th>Innate</th>
<th>Adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response time</td>
<td>Hours</td>
<td>Days</td>
</tr>
<tr>
<td>Specificity</td>
<td>Limited and fixed</td>
<td>Highly diverse, improves during the course of immune response</td>
</tr>
<tr>
<td>Response to repeat infection</td>
<td>Identical to primary response</td>
<td>Much more rapid than primary response</td>
</tr>
</tbody>
</table>

Found in: all multicellular organisms, Vertebrates only

Substances that trigger: A limited number of pathogen-associated molecular patterns (PAMPs)

A limited number of Pattern Recognition Receptors (PRRs) expressed on many cell types

Vertebrates only

Virtually any component of pathogens

Highly variable receptors of 2 types: antibody made by B cells and TCR made by T cells

Innate Immunity

Innate immune effector mechanisms

Physical and biochemical barriers (defensins)

Phagocytosis and reactive oxygen

Cell autonomous defenses

Apoptosis

Interferons and PKR

Innate immune recognition

Discovery of the Toll-like receptors

Mammalian TLRs and their ligands

Non-TLR recognition of PAMPs

Connections between adaptive and innate immunity

Physical & Biochemical barriers

<table>
<thead>
<tr>
<th>Biochemical defense</th>
<th>Biochemical and physical defense</th>
</tr>
</thead>
<tbody>
<tr>
<td>lysozyme in most secretions</td>
<td>mucus</td>
</tr>
<tr>
<td>sebaceous gland secretions</td>
<td>cilia lining trachea</td>
</tr>
<tr>
<td>commensal organisms in gut and vagina</td>
<td>skin</td>
</tr>
<tr>
<td>sperm in semen</td>
<td>acid in stomach</td>
</tr>
</tbody>
</table>

TABLE 1-2  Summary of nonspecific host defenses

<table>
<thead>
<tr>
<th>Type</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomic barriers</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Mechanical barrier retards entry of microbes.</td>
</tr>
<tr>
<td></td>
<td>Acidic environment (pH 3-5) retards growth of microbes.</td>
</tr>
<tr>
<td>Mucous membranes</td>
<td>Normal flora compete with microbes for attachment sites and nutrients.</td>
</tr>
<tr>
<td></td>
<td>Mucus entraps foreign microorganisms.</td>
</tr>
<tr>
<td></td>
<td>Cilia propel microorganisms out of body.</td>
</tr>
<tr>
<td>Physiologic barriers</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>Normal body temperature inhibits growth of some pathogens.</td>
</tr>
<tr>
<td>Low pH</td>
<td>Acidity of stomach contents kills most ingested microorganisms.</td>
</tr>
<tr>
<td>Chemical mediators</td>
<td>Lysosome cleaves bacterial cell wall.</td>
</tr>
<tr>
<td></td>
<td>Interferon induces antiviral state in uninfected cells.</td>
</tr>
<tr>
<td></td>
<td>Complement types microorganisms or facilitates phagocytosis.</td>
</tr>
<tr>
<td></td>
<td>Tolllike receptors recognize microbial molecules, signal cell to secrete immunostimulatory cytokines.</td>
</tr>
<tr>
<td></td>
<td>Collects disrupt cell wall of pathogen.</td>
</tr>
<tr>
<td>Phagocytic/Inflammatory barriers</td>
<td>Various cells internalize (endocytose) and break down foreign macromolecules.</td>
</tr>
<tr>
<td></td>
<td>Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize phagocytosis, kill, and digest whole microorganisms.</td>
</tr>
<tr>
<td>Inflammatory barriers</td>
<td>Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity, and influx of phagocytic cells into the affected area.</td>
</tr>
</tbody>
</table>
Defensins

- Originally isolated from frog skin based on their ability to kill bacteria
- Small polypeptides (<10kDa) secreted at mucosal surfaces
- Direct bacteriocidal properties
- Insertion into biological membranes leading to target cell lysis
- Inhibited by cholesterol (specificity)

Phagocytosis was discovered by Ilya Mechnikov in 1882

In 1882, the Russian scientist Ilya Mechnikov was working in Messina, Italy, studying the larvae of the sea star. When he inserted a thorn into a larva, something weird happened. Mechnikov noticed strange cells gathering at the point of insertion. The cells surrounded the thorn, eating any foreign substances that entered through the ruptured skin. Mechnikov was thrilled. He decided to name these new cells phagocytes from the Greek words meaning "devouring cells."

Phagocytes use a variety of methods to destroy ingested microbes.

**Phagocytosis “cellular eating”**

1. Bacterium attaches to membrane
2. Bacterium is ingested, forming phagosome,
3. Phagosome fuses with lysosome.
4. Lysosomal enzymes digest the bacteria.
5. Digested material is released from cell.

**Phagocytes: macrophage, neutrophils, dendritic cells**

<table>
<thead>
<tr>
<th>Oxygen-dependent killing</th>
<th>Oxygen-independent killing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive oxygen intermediates</td>
<td>Defensins</td>
</tr>
<tr>
<td>O$_2^-$ (superoxide anion)</td>
<td>Tumor necrosis factor α</td>
</tr>
<tr>
<td>OH$^-$ (hydroxyl radicals)</td>
<td>(macrophage only)</td>
</tr>
<tr>
<td>H$_2$O$_2$ (hydrogen peroxide)</td>
<td>Lysozyme</td>
</tr>
<tr>
<td>ClO$^-$ (hypochlorite anion)</td>
<td>Hydrolytic enzymes</td>
</tr>
<tr>
<td>Reactive nitrogen intermediates</td>
<td>(household bleach)</td>
</tr>
<tr>
<td>NO (nitric oxide)</td>
<td>NH$_2$CL (monochloramine)</td>
</tr>
<tr>
<td>NO$_2$ (nitrogen dioxide)</td>
<td></td>
</tr>
</tbody>
</table>
Macrophage fight microbes by phagocytosis and production of toxic molecules

A macrophage produces reactive oxygen species to aid in destruction of the microbe

Reactive oxygen is revealed by the blue dye, NBT

Innate Immunity

Innate immune effector mechanisms
- Physical and biochemical barriers (defensins)
- Phagocytosis and reactive oxygen species

Cell autonomous defenses
- Apoptosis
- Interferons and PKR

Innate immune recognition
- discovery of the Toll-like receptors mammalian TLRs and their ligands
- non-TLR recognition of PAMPs

Connections between adaptive and innate immunity

Some microbes hijack cellular machinery to replicate and spread. Intracellular pathogens include viruses (influenza, HIV) and intracellular bacteria (listeria) and intracellular parasites (malaria, toxoplasma).

cell-autonomous defense:
cell produces an immune response that acts on itself

Apoptosis: Cellular Suicide

- Nuclear fragmentation
- Proteolysis
- Blebbing
- Death

Remnants undergo phagocytosis
Apoptosis versus Necrosis

- Tidy: contents of cells degraded from within, producing small cellular “blebs”
- Programmed from inside the cell
- Messy: contents of cell released.
- Induced by external insult

Cell death by necrosis is more likely to produce inflammation.

Interferons are cytokines that are produced in response to viral infection. Produce an “anti-viral state” in target cells. Acts on cell that produces it, as well as neighboring cells. Together with dsRNA, act to triggering the Protein Kinase R (PKR) pathway. Shuts down protein synthesis machinery of cells, thus preventing viral replication.

Protein Kinase R: Interfering with Infection
Innate Immunity

Innate immune effector mechanisms
Physical and biochemical barriers (defensins)
Phagocytosis and reactive oxygen
Cell autonomous defenses
Apoptosis
Interferons and PKR

Innate immune recognition
discovery of the Toll-like receptors
TLRs and their ligands
non-TLR recognition of PAMPs

Connections between adaptive and innate immunity

Comparison of the adaptive and innate immune responses

<table>
<thead>
<tr>
<th></th>
<th>innate</th>
<th>adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response time</td>
<td>hours</td>
<td>days</td>
</tr>
<tr>
<td>Response to repeat infection</td>
<td>identical to primary</td>
<td>stronger response upon second exposure</td>
</tr>
<tr>
<td>Receptors that Mediate pathogen Recognition</td>
<td>pattern recognition receptors (TLR)</td>
<td>antibodies and T cell antigen receptors (TCR)</td>
</tr>
<tr>
<td>Recognition</td>
<td>limited diversity, fixed in germline</td>
<td>unlimited diversity generated by V(D)J recombination</td>
</tr>
<tr>
<td>Ligands</td>
<td>Pathogen associated molecular patterns (PAMPs)</td>
<td>virtually any component of pathogen</td>
</tr>
</tbody>
</table>