The immune system and vaccines

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Two general types of defense

• Nonspecific defenses
  – Protection against any invader
  – Includes barriers, nonspecific cells and mechanisms
    • Phagocytes, inflammation, fever, antimicrobial substances

• Specific defenses
  – Specific responses to particular invader
  – Cellular and humoral immunity
Nonspecific defenses of host

• Barriers
• Phagocytosis
• Inflammation
• Fever
• Complement & interferon
First line of defense: skin and mucous membranes

- **Mechanical factors**
  - Epidermis: dead cells in upper layers, Langerhans and Granstein cells
  - Mucous membranes: mucus and other microbial secretions, flow of urine, normal microbial flora
  - Lacrimal apparatus: continual washing of eye
  - Saliva: dilutes microbes

- **Chemical factors**
  - Sebum: protects cracks from arising from drying
  - pH of skin: 3 & 5, keeps down bacteria
  - Normal microbial flora of skin
  - Sweat glands: flush microbes, eliminate wastes
    - Lysozyme breaks down cell walls of gram positive bacteria, damages walls of gram negative bacteria
  - Gastric juice
Leukocytes

- Two categories
  - Granulocyte
    - Neutrophils
    - Basophils
    - Eosinophils
  - Agranulocyte
    - Monocytes
    - Lymphocytes
# Leukocyte function

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Basophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
<th>Mast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-70% circulating WBC’s</td>
<td>1-3% circulating WBC’s</td>
<td>&lt;1% circulating WBC’s</td>
<td>5-8% WBC, become macrophage during extravasation</td>
<td>20-40% of circulating WBC’s in blood, 99% of cells in lymph</td>
<td>Undifferentiated precursor cells when released from bone marrow</td>
<td>Important in allergic response</td>
</tr>
<tr>
<td>Polymorphonuclear cells</td>
<td></td>
<td>Smallest WBC</td>
<td>Mononuclear cells</td>
<td>Specific immune response</td>
<td>Differentiate in tissues (stationary)</td>
<td>Produce and store TNF</td>
</tr>
<tr>
<td>1st at site of infection</td>
<td>Somewhat phagocytic</td>
<td>Allergic response</td>
<td>Phagocytosis, APC’s</td>
<td>B and T lymphocytes</td>
<td>Important in allergic response</td>
<td></td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>Involved in allergic reactions and against parasites (IgE dependent killing)</td>
<td>Histamine, heparin, serotonin, prostaglandins in granules</td>
<td>Antimicrobial activity</td>
<td>Secrete cytokines (IL-1, TNF-α)</td>
<td>Produce and store TNF</td>
<td></td>
</tr>
<tr>
<td>Produce lysozyme, myeloperoxidase, etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>APC’s (MHCII)</td>
<td></td>
</tr>
</tbody>
</table>
Mechanisms of phagocytosis

- Chemotaxis
  - Cytokines released from damaged cells in infection
- Adherence
  - Must adhere to cell to be phagocytosed
  - Opsonization = enhancement
- Ingestion
  - Adhesion followed by pseudopod formation enclosing microbe and forming phagosome
- Digestion
  - Phagosome fuses with lysosome
    - If digestion doesn’t occur
      - Microbe may destroy cell
      - Microbe may take up residence in cell
Phagocytosis

Organisms engulfing other cells
Inflammation

• Triggered by tissue damage
• Function
  – Destroy invader
  – Confine or wall off invader
  – Repair or replace damaged tissues
• Four cardinal signs
  – Rubor, dolor, tumor, calor
• Three stages
  – Vasodilation
  – Migration
  – Tissue repair
Vasodilation & increased capillary permeability

- Occurs in damaged area due to release of cytokines
  - Histamines and others from tissue mast cells and damaged cells
- Leads to arrival of phagocytic cells, proteins, plasma from blood vessels
- Results: redness, swelling, heat, pain
Migration of phagocytes

• Phagocytes move from blood vessels to effected area
  – Margination (phagocytes stick to endothelium)
  – Move out between cells
  – Neutrophils move out first, then granulocytes, then monocytes (become macrophage)
    • Macrophage = diapedesis
    • Others = extravasation

• “munch up” invaders
• Increased production of WBC’s in bone marrow
Inflammatory response summary

1. Bacteria and other pathogens enter wound.
2. Platelets from blood release blood-clotting proteins at wound site.
3. Mast cells secrete factors that mediate vasodilation and vascular constriction. Delivery of blood, plasma, and cells to injured area increases.
4. Neutrophils secrete factors that kill and degrade pathogens.
5. Neutrophils and macrophages remove pathogens by phagocytosis.
6. Macrophages secrete hormones called cytokines that attract immune system cells to the site and activate cells involved in tissue repair.
7. Inflammatory response continues until the foreign material is eliminated and the wound is repaired.
Tissue repair

- Begins during active phase of inflammation
- Completed when all injurious substances removed or neutralized
- All tissue does not repair
Fever

- Systemic response to bacterial or viral infection
- IL-1 induces release of prostaglandins from hypothalamus causes thermostat in hypothalamus to reset
- Body works to maintain new set point

Purpose
- Increases metabolic rate to facilitate healing
- High temperature make conditions “uncomfortable” for pathogens
- Increased WBC mobility
- Enhanced phagocytosis
- Decreased effect of endotoxins
- Increased T-cell proliferation
- Enhanced interferon activity
Complement

• Approximately 20-30 serum proteins circulate continuously in inactive state

• Two methods of activation
  – Classical pathway
    • Antigen-antibody complex causes cleavage of inactive complement proteins stimulating complement cascade
  – Alternate pathway
    • Activated directly by exposure to bacterial and fungal cell wall polysaccharides

• Four basic functions, all nonspecific
  – Opsonization
  – Chemotaxis
  – Increased vascular permeability
  – MAC
Complement cascade

The interlocking steps of the complement cascade end in cell death.
Interferons

• Chemicals produced by cells infected by virus
• Induce production of anti-viral proteins (AVP’s)
  – Interfere with viral replication in neighboring cells
  – Paracrine agents
Connecting specific & nonspecific immunity

• Antigen presenting cells (APC’s)
  – Macrophage, B-cells, Langerhan’s cells, others
  – Process phagocytized microbes and present on MHC
  – T-cells can only be triggered by presented antigen

• Cytokines
  – Chemicals produced by cells triggering responses in other cells, such as interleukins
Antigen Presenting Cells

(a) CD4+ T cells and CD8+ T cells

- Antigen-specific T-cell receptor
- Antigen
- MHC class II
- MHC class I
- Exogenous pathway
- Endogenous pathway

(b) CD4+ T cells and CD8+ T cells

- Cytokines
- IFN-γ
- Ag
- MHC class II
- T_H1 cells
- Cytolytic
- Perforin
- Granzymes
- Fas ligand
- Cytotoxic (killer) T cells
- Cytokines
- IFN-γ
- TNF-α
- Infected target cell (e.g., macrophage)
- Infected target cell
Definitions

• Innate resistance
  – Resistance to infectious diseases to which we have been exposed for generations (genetic)

• Immunity
  – Specific defense response of body, geared toward eliminating a particular invader or injurious substance

• Antigens
  – Cell surface receptors that can trigger an immune response.

• Antibodies
  – Special proteins (immunoglobulins) produced by plasma cells which target specific antigens
Types of immunity

• Naturally acquired
  – Active
    • get disease
  – Passive
    • antibodies pass from mom to fetus through placenta, breast milk

• Artificially acquired
  – Active
    • vaccination
  – Passive
    • preformed antibodies introduced via injection
    • Short term because antibodies degraded within two weeks
Humoral vs. cell-mediated immunity

• Humoral
  – Antibodies
  – Most effective against extracellular bacteria, toxin, viruses

• Cell-mediated
  – Cellular immunity
  – Most effective against intracellular pathogens including bacteria, viruses, fungi, protozoa, helminths
  – Involved in transplant rejection, cancer, hypersensitivity reactions
Antibody structure

• Most bivalent
• Two light chains and two heavy chains joined by disulfide bridges
• Variable region, constant region
• Fc = stem region = binding site for complement
  – Important factor in DHS
  – Binds IgE to eosinophils
    • Variable region binds to parasite = degranulation
Classes of antibodies

- IgM
- IgG
- IgA
- IgE
- IgD
**IgM**

- Initial exposure to antigen, short lasting
- Pentamer structure joined by J-chain
- Usually found in vasculature due to size
IgG

- 80% antibodies in serum, found in tissue fluids, crosses placenta
- Second and subsequent exposure, long lasting
IgA

- Most common form in mucous membranes, secretions
- Breast milk, colostrum
- Structure protects against degradation
IgE & IgD

• IgE
  – Fc to mast cells and eosinophils
  – Allergic reactions, parasites

• IgD
  – Marker on surface of B cells
B cells and humoral immunity

• Stem cells in bone marrow
• Migrate to lymphoid organs (lymph nodes, spleen)
• Activation = differentiation into plasma cells which produce antibodies
Clonal selection theory

• B cells in body in low numbers but of enormous variety
• In presence of antigen, clonal expansion of specific B cells = B cell army
• Differentiation into plasma cells = produce antibodies
  – Primary immune response = 30 days
  – Secondary immune response = few hours
  – Memory cells may result in life-long immunity
Gene rearrangement

Stem cell

Mature B cells

Antigen 2

Memory cell

Antibody 2

Plasma cells

4

4

3

3

2

2

2

2

1

1

2

2

2

2
Antigen-antibody complex

- Antibodies bind to antigen
- Do not directly kill or destroy injurious agent
- Increase ability of phagocytes and/or complement to destroy pathogen
- Agglutination = clumping of Ab-Ag
- Memory cells
  - Produced when B cells are activated
  - Quick response on second and subsequent antigen challenge
Antibody:antigen complex
T-cells and cell mediated immunity

• Stem cells in bone marrow to thymus for “education”
• Released to circulate in body
• TCR stimulated only by presented antigen
• Produce memory cells for quicker response to subsequent challenge
• Activation = differentiation into specific effector cells
Types of cells

- **Helper T cells**
  - $T_H$ cells, primarily CD4
    - Activate $T_C$ cells, other helper T’s
    - Enhance B cell response by producing cytokines that stimulate production of plasma cells
- **Cytotoxic T cells**
  - $T_C$ cells, usually CD8 = killer T’s
  - Kill infected cells on contact
  - Protect against intracellular parasites, transplants, cancer cells
  - Attaches to infected cell and “shoots”
- **Others**
  - $T_D$ cells = DTHS (poison ivy, transplant rejection, cancer)
    - Composed of both $T_C$ and $T_H$ populations
  - Suppressor T’s = turn off immune system, never isolated
NK cells

- Nonspecific type of cellular immunity
- Good protection against virus-infected cells and tumors
- Must contact cells for lysis to take place
Vaccines—Active immunization

• Goal
  – Control or eliminate disease by inducing long term protective immunity

• How do they work?
  – Activate the specific immune response
  – Stimulate antigen-specific immune effectors
  – And/or induce the production of memory cells that can be reactivated quickly when exposed to pathogen
Antigen-specific Immune Effectors

• Antibodies have efficacy against extracellular and intracellular pathogens
  – Bind to toxins and inactivate them or prevent them from diffusing
  – Keep viruses from binding and entering cells
  – Opsonize bacteria to enhance phagocytosis
  – Activate the complement cascade

• CD8\(^+\) T cells reduce, control, clear intracellular pathogens
  – Directly kill infected cells by releasing granzyme, perforin, etc.
  – Indirectly kill infected cells by releasing cytokines with antimicrobial properties

• CD4\(^+\) T cells help eliminate and reduce both intra and extracellular pathogens
  – Produce cytokines, such as IFN-\(\gamma\), TNF-\(\alpha/\beta\), IL-2, IL-3, etc.
  – Support activation and differentiation of B cells, CD8\(^+\) T cells, and macrophage through the production of various interleukins
## Immune effectors

- Type of immune effector elicited depends on nature of vaccine

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type</th>
<th>Serum IgG</th>
<th>Mucosal IgG</th>
<th>Mucosal IgA</th>
<th>T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diptheria toxoid</td>
<td>++</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza intranasal live atten.</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+(CD8+)</td>
<td></td>
</tr>
<tr>
<td>Meningococcal conjugate PS-protein</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pertussis, whole cell killed</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polio Sabin live attenuated</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Memory response

• Some vaccines elicit strong primary response, but no memory

• Importance of memory cells depends on incubation period
  (1) Ex. Influenza has 3-day incubation period
     – Pt exhibits symptoms by the time memory cells are activated
     – Protection depends on repeated immunizations = high levels of neutralizing antibody in circulation
  (2) Ex. Polio virus has long incubation period
     – Sufficient time for memory B cells to produce high levels of serum antibody
     – Polio vaccine induces high levels of immunologic memory
       • Peak serum antibody levels with Salk vaccine achieved in 2 weeks
       • Memory response reaches maximum at 6 months and persists for many years
Developing vaccines to produce humoral response

• Nature of the epitopes
  – Must be accessible to receptor on B cells
    • Hydrophilic
    • Native structure
      – Inactivated/attenuated proteins
      – Purified proteins, polysaccharides

• Location of potential pathogen
  – Oral vaccine vs. injection
    • Ex) *Neisseria gonorrhea* attaches to mucosal membranes in urethra
      – Vaccine must induce secretory IgA
    • Injectable vaccines induce IgG or IgM
Developing vaccines that elicit a CD8$^+$ T-cell response

- Intracellular pathogens such as viruses, some bacteria, protozoa, fungi
- Strong T-cell response required to activate “killer T cells”
  - Only recognize presented antigen
  - Antigen is not only presented, but processed by presenting cell
  - Characteristics of antigens
    - Internal
    - Hydrophobic
    - Linear peptides
      - Protein must be denatured inside cell to reveal these
T Cell Activation

Antigen presenting cell

Infected cell

MHC II

CD4+
(helper T)

TCR

interleukins

CD8+
(killer T)

TCR

CD8+
(killer T)

CD8+
(killer T)

CD8+
(killer T)
Steps in immune system activation by vaccines

• (1) Introduction of antigen
• (2) Stimulation of innate immune response
• (3) Antigen presentation
• (4) migration of APC’s to lymph nodes
• (5) production of immune effector cells
Summary of immune system activation

1. Macrophage captures, engulfs, and digests an antigen.

2. Macrophage presents a fragment of the antigen on its surface.

3. Interactions between proteins on the macrophage and helper T cell occur, activating the helper T cell.

4. Activated helper T cell proliferates into either TH1 or TH2 cells, which secrete different types of cytokines.

Humoral immune response

- Activated helper T cell
- TH2 cell
- CD40 ligand
- cytokine release
- Interaction between the TH2 cell and B cell causes the B cell to proliferate, differentiate, and produce antibodies.

Cell-mediated immune response

- Macrophage
- Helper T cell
- T-cell receptors
- CD28
- B7
- Cytokines secreted by the TH1 cell activate cytotoxic T cells to kill the infected target cell.

Activated cytotoxic T cell proliferates and differentiates into a mature cytotoxic T cell, which binds to an infected target cell and initiates the destruction of that cell.

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Types of vaccines

- General categories
  - Whole organism
  - Purified macromolecules
  - Recombinant antigen vaccines
  - Recombinant vector vaccines
  - Synthetic peptide vaccines
  - Multivalent subunit vaccines
### Whole Organism Vaccines: Comparison of attenuated and inactivated vaccines

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Attenuated vaccine</th>
<th>Inactivated vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacture</td>
<td>Virulent pathogen is weakened by growing under abnormal conditions</td>
<td>Virulent pathogen is inactivated (chemicals, irradiation, heat)</td>
</tr>
<tr>
<td>Booster requirements</td>
<td>Usually a single booster</td>
<td>Multiple boosters</td>
</tr>
<tr>
<td>Stability</td>
<td>Less stable</td>
<td>More stable, longer shelf-life, no need for cold chain</td>
</tr>
<tr>
<td>Type of immunity</td>
<td>Humoral and CMI</td>
<td>Mostly humoral</td>
</tr>
<tr>
<td>Reversion</td>
<td>May revert to virulent strain</td>
<td>Cannot revert</td>
</tr>
</tbody>
</table>
Purified Macromolecule Vaccines

• Eliminates some of risks of attenuated or killed vaccines
• Create vaccine from specific purified immunogenic macromolecules
• Examples:
  – Meningococcal meningitis vaccine
  – Pneumococcal pneumonia vaccine
• Limitations
  – Polysaccharide vaccines activate B cells in thymus-independent manner so that $T_H$ cells are not activated.
  – Produce IgM, little IgG, and limited if any immune memory
  – Solution: conjugate vaccines (PS-protein)
    • Ex: Hib vaccine
Recombinant Vaccines

• Antigen
  – DNA encoding antigenic determinants is isolated and cloned
  – Introduced into bacteria or yeast, induced expression of antigens
  – Allows for increased production of surface antigen
  – Ex: HBV vaccine
    • Genes for HBsAG (major surface antigen of HBV) cloned in yeast cells and purified

• Vector
  – Genetically engineered and attenuated viruses or bacteria that express surface antigens of pathogen
  – Replicate in the host
Herd immunity

- Vaccination not 100% effective
  - Inability to elicit strong enough immune response to provide long term protection
  - Medical conditions that contraindicate vaccination
- Goal: Vaccinate large number of individuals so that majority of the population is immune
- Decreased chance that a susceptible individual will encounter someone who is infected

Review the childhood and adult vaccine schedules you were provided by your instructor