The Human Immune System

Part II
Immunogenesis

• Non-specific Responses:
  – Biological obstacles
  – Chemical obstacles
  – General obstacles
  – Physical obstacles
Immunogenesis

• Specific Responses – Acquired Immunity

1. Naturally Acquired Immunity
   1. Actively acquired – stimulates Ab synthesis from B cells; stimulates T cells; both destroy Ag
   2. Passively acquired – feto-placental transfer of IgG types; infant suckling for IgA sub-type transfer

2. Artificially Acquired Immunity
   1. Actively acquired – received an injection of an immunogenic substrate
   2. Passively acquired – injecting Ab’s produced in another animal or in vitro
# Different Kinds of Immunity -- 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Active/Natural Acquired</th>
<th>Immunizing Agent</th>
<th>Active/Artificial Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigens</td>
<td></td>
<td></td>
<td>Antigens</td>
</tr>
<tr>
<td>Lifetime</td>
<td></td>
<td>Duration of Immunity</td>
<td>Months to years</td>
</tr>
<tr>
<td>Endogenous</td>
<td></td>
<td>Ab Source</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td>Effectiveness in newborn</td>
<td>Low</td>
</tr>
<tr>
<td>Hi</td>
<td></td>
<td>Effectiveness in Adult</td>
<td>Hi</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td>Origin</td>
<td>Toxoid/vaccine</td>
</tr>
</tbody>
</table>
## Different Kinds of Immunity -- 2

<table>
<thead>
<tr>
<th>Passive/Natural Acquired</th>
<th>Characteristics</th>
<th>Passive/Artificial Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibodies</td>
<td>Immunizing Agent</td>
<td>Antibodies</td>
</tr>
<tr>
<td>4-6 months</td>
<td>Duration of Immunity</td>
<td>To 6 weeks</td>
</tr>
<tr>
<td>Exogenous</td>
<td>Ab Source</td>
<td>Exogenous</td>
</tr>
<tr>
<td>Hi</td>
<td>Effectiveness in newborn</td>
<td>Hi</td>
</tr>
<tr>
<td>Low</td>
<td>Effectiveness in Adult</td>
<td>Moderate</td>
</tr>
<tr>
<td>Transplacental Ab Passage</td>
<td>Origin</td>
<td>Ab-containing serum</td>
</tr>
</tbody>
</table>
Hypersensitivity: Allergy

- An immune response which results in exaggerated or inappropriate responses that are harmful to the host.

- Types I, II, III are antibody-mediated

- Type IV is cell-mediated
Type I – Immediate (Anaphylactic) Hypersensitivity

• Occurs within minutes
• Original “dose” is very small
• NOTE: if a reaction occurs in ANY member of a species = ANAPHYLAXIS
• NOTE: if a reaction occurs only in SOME members of a species = ATOPY
• Either way: regulated by IgE and basophils and mast cells – both of which degranulate to create the problem
Type I – Immediate (Anaphylactic) Hypersensitivity: Mediators

- **Histamine**: vasodilation with increased permeability; smooth muscle contraction
- **SRS-A**: Slow Reactive Substance of Anaphylaxis – a mixture of leukotrienes; primarily involved in bronchoconstriction of asthma; NOT a histamine!
- **PG’s**: bronchial roles
- **TX’s**: platelet aggregation
- **ECF-A**: Eosinophilic Chemotactic Factor of Anaphylaxis – tetrapeptide in mast cell granules; with degranulation, causes eosinophils to migrate to site of insult
- **Serotonin**: in mast cells; of minor cardiovascular importance in humans; makes you tired
Type I – Immediate (Anaphylactic) Hypersensitivity
The Allergic Problem

• Many people have allergies – literature varies with location
• Examples: hay fever, bronchial asthma, eczema, contact dermatitis, food allergies, drug eruptions
• ALL are associated with histamine release
Normal Distribution of Histamine in Tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Concentration (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>35</td>
</tr>
<tr>
<td>Nasal Membranes</td>
<td>15</td>
</tr>
<tr>
<td>Stomach</td>
<td>14</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.4</td>
</tr>
<tr>
<td>Heart</td>
<td>1.6</td>
</tr>
<tr>
<td>Abdominal Skin</td>
<td>6.6</td>
</tr>
<tr>
<td>Facial Skin</td>
<td>30.4</td>
</tr>
<tr>
<td>Basophils</td>
<td>1080 µg/10^9 cells</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.009 µg/10^9 platelets</td>
</tr>
</tbody>
</table>
## Histamine Receptors

<table>
<thead>
<tr>
<th></th>
<th>H₁</th>
<th>H₂</th>
<th>H₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP₃/Ca²⁺</td>
<td>cAMP</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Gₚ protein?</td>
<td>Gₛ protein</td>
<td>??</td>
<td>??</td>
</tr>
<tr>
<td>2-methylhistamine</td>
<td>4-methylhistamine</td>
<td>R-α-methyl-histamine</td>
<td></td>
</tr>
<tr>
<td>Pyrilamine</td>
<td>Cimetidine</td>
<td>Thioperamide</td>
<td></td>
</tr>
<tr>
<td>Allergies</td>
<td>Stomach acid</td>
<td>Pre-synaptic inhibition of histamine release</td>
<td></td>
</tr>
</tbody>
</table>

**H₁ dominates when H₁ and H₂ are in the same tissue**

Agonist; Antagonist
• In general, receptors are identified by the way they react with agonists (cause the same effect as the substance that's supposed to bind to it) or antagonists (block the effect of the substance that's supposed to bind to it). They are also identified by their second messengers. The table, above, summarizes some of that information regarding histamine receptors. Of interest is that H₂ receptors are inhibited by cimetidine (Tagamet).

• Of further interest is the fact that as men age (above the age of about 35), when they take Dimetapp, they develop a reversible impotence. It is thought that this occurs through the H₃ receptor. This was when Dimetapp contained PPO – no data in on the new version of Dimetapp
# Primary Histamine Actions

<table>
<thead>
<tr>
<th>System</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular System</td>
<td>Hypotension with tachycardia; facial erythema due to vasodilation of cutaneous vessels; throbbing headache due to dilatation of brain arterioles</td>
</tr>
<tr>
<td>Respiratory System</td>
<td>Bronchiolar smooth muscle contraction with increased secretions</td>
</tr>
<tr>
<td>Glandular Tissue</td>
<td>Increased catecholamine release from adrenals; hyperacidity and increased pepsin release in stomach</td>
</tr>
<tr>
<td>Intra-dermal Tissue: Lewis Triple Response</td>
<td>1) Dilation of capillaries in the immediate vicinity of injection leads to local red to blue color (FLUSH)</td>
</tr>
<tr>
<td></td>
<td>2) Dilation of arterioles in a wider area leads to redness (FLARE)</td>
</tr>
<tr>
<td></td>
<td>3) Appearance of swelling in the FLUSH area (= WHEAL) due to increased capillary permeability</td>
</tr>
</tbody>
</table>
# Histamine Receptor Locations

<table>
<thead>
<tr>
<th>Organ</th>
<th>Receptor Type</th>
<th>Histamine Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus</td>
<td>$H_2$ receptors</td>
<td>Relaxation</td>
</tr>
<tr>
<td>Stomach</td>
<td>$H_2$ receptors</td>
<td>Increased HCl production</td>
</tr>
<tr>
<td>Bronchi</td>
<td>$H_1$ receptors</td>
<td>Bronchoconstriction</td>
</tr>
<tr>
<td></td>
<td>$H_2$ receptors – small amount</td>
<td>Bronchoconstriction</td>
</tr>
<tr>
<td>CNS</td>
<td>$H_1$ receptors</td>
<td>Sedation</td>
</tr>
<tr>
<td></td>
<td>$H_1$ and $H_2$ receptors</td>
<td>Antiemetic effect</td>
</tr>
<tr>
<td>SNS</td>
<td>$H_2$ receptors</td>
<td>Inhibition</td>
</tr>
<tr>
<td>Heart</td>
<td>$H_1$ and $H_2$ receptors</td>
<td>Increased Atrial/Ventricular contraction force</td>
</tr>
<tr>
<td></td>
<td>$H_2$ receptors</td>
<td>Increased heart rate</td>
</tr>
<tr>
<td></td>
<td>$H_1$ and $H_2$ receptors</td>
<td>Increased coronary flow</td>
</tr>
</tbody>
</table>
Block Degranulation with Isoproterenol, Theophylline, Epinephrine, Cromolyn Sodium

Ag-bound Mast Cells

Degranulation

Releases:
Histamine
Serotonin
Heparin
Prostaglandins

That Cause

Block with antihistamines

Allergic Effects
Histamine Antagonism

- Histamine antagonism is a very profitable business in this country.
# Histamine Antagonism

<table>
<thead>
<tr>
<th>Histamine Release</th>
<th>Blocked by cromolyn sodium at mast cell and basophil level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H&lt;sub&gt;1&lt;/sub&gt; Receptor Blockade</strong></td>
<td>Competitive inhibitors such as Diphenhydramine HCl, Dimenhydrinate, Chlorpheniramine maleate, Promethazine HCl (Benadryl, Dramamine, Chlortrimetron and Phenergan)</td>
</tr>
<tr>
<td></td>
<td>Great use in allergic rhinitis, ACUTE phase due to tolerance, some childhood asthma, sedation</td>
</tr>
<tr>
<td><strong>H&lt;sub&gt;2&lt;/sub&gt; Receptor Blockade</strong></td>
<td>Competitive inhibitors such as Cimetidine, Famotidine and Ranitidine HCl (Tagamet, Pepcid and Zantac)</td>
</tr>
<tr>
<td></td>
<td>Great use in duodenal ulcers, benign gastric ulcer, ulcerogenic tumors of the pancreas, esophageal reflux</td>
</tr>
</tbody>
</table>
I've Been Stung!

Dizziness, seizure, loss of consciousness, death; 2º & BP

Labial + lingual swelling

Laryngeal swelling

Bronchoconstriction; asphyxia or similar

N/V

Dilation; ↑ BP → Brain + heart failure

Diarrhea

Cramps

Most Common

Rash; e.g., hives

Dilation = permeability

Diarrhea → shock (2º small vessel involvement)
Type II – Cytotoxic Hypersensitivity

• Occurs via Complement activation thru IgG or IgM

Examples:
• Hemolysis – HDN (Rh); ABO transfusion reactions
• Hemolysis – PCN, phenacetin, quinidine bind to surface of RBC
• ITP– quinine or ASA plus platelets cause bleeding and/or bruising
Hemolytic Disorder of the Newborn – Erythroblastosis Fetalis
Tx and Dx HDN

- Treatment
- RhoGAM
- Is anti-anti-Rh = anti-idiotype
- Give to woman who is Rh negative
  - Perigestationally or after Rh+ baby born
  - To any Rh negative woman after [spontaneous] abortion (fetal blood group unknown)

Diagnostic Testing: Coomb’s Test

Other Coomb’s uses: positive in patients with salmonellosis, brucellosis and who have autoimmune hemolytic anemias
\( X + \text{Drug} \rightarrow \text{anti drug} \text{Abs} \)

\( \text{PLT} \rightarrow \text{drug on its surface} \)

Complement activation

\( \text{Ab bound Ag on PLT} \)

Thrombocytolysis
Type III – Immune Complex Hypersensitivity

- Immune complexes deposited in tissues leads to dysfunction

- Activates complement system

- PMN’s attracted and cause inflammation and tissue injury
Type III – Immune Complex Hypersensitivity -- Examples

Arthus Reaction

- Immune complexes deposited in vessel walls
- A severe local inflammatory reaction with localized destruction of tissue, resulting from antigen-antibody (IgE) combination. [http://www.whonamedit.com/syndlist.cfm/6]
- Complexes form from giving high Ag dose which causes high [Ab], then give Ag SQ
- Causes edema and hemorrhage
- Not seen with much frequency these days
- Allergic Bronchopulmonary Aspergillosis (ABPA), Chronic obstructive pulmonary disease and Farmer's lung are examples of an arthus reaction.
- A severe, local, inflammatory, late-phase reaction accompanied by skin necrosis occurred after an infant was given an intramuscular injection of recombinant hepatitis B virus vaccine. The clinical course and appearance of the rash were typical of an Arthus reaction. Although not identical to this case, prior reported cases of complement-mediated reactions occurring after hepatitis B virus infection or vaccination provide theoretical support for this diagnosis. [http://www.vaccinationnews.com/DailyNews/August2001/ArthusReactionHepBVax.htm]
Type III – Immune Complex Hypersensitivity -- Examples

Serum Sickness

• First described in humans tx therapeutically with diphtheria/tetanus anti-toxin grown in horses
• Original dose is very large
• Immune complexes circulate or precipitate
• Causes fever, itching, arthralgia, lymphadenopathy, splenomegaly within 2 days to 2 weeks after injection
• Not seen with much frequency these days
Type III – Immune Complex Hypersensitivity -- Examples

**Immune Complex Diseases**

- Rheumatoid arthritis: autoimmune → inflammation
- Poststreptococcal glomerulonephritis: via Ag/Ab/complement → inflammation
- LATS (now known as Thyroid Stimulating Immunoglobulins – TSI) – hyperthyroidism
- SLE – from immune complex formation; sx: rash (butterfly), polyarthritis, nephrosis, hemolytic anemia, pleural effusion, CNS abnormalities – forms ANA – non-specific
Type III – Immune Complex Hypersensitivity -- Examples

Atopy

• Follows families; high IgE levels; require specific Ag’s
• Environmental: pollens, ragweed, dust, plants/toxins
• Foods: allergy to shellfish, nuts
• Any of which may lead to hay fever, asthma, eczema, urticaria
• Mechanisms: 1) ↓T₈ cells?? 2) ↑↑IgE levels??
Type III – Immune Complex Hypersensitivity -- Examples

• Drug Hypersensitivity
• Primary offender = antimicrobial agents
• Most common cause of hypersensitivity reactions
• Reaction occurs with [first or] second exposure to drug
• Results in rash, fever, anaphylaxis of varying severity (note “over-use” of “atopy” and “anaphylaxis” – follows more than 1 type of rxn)
Type IV – Cell Mediated (Delayed) Hypersensitivity

- A function of T cells NOT Ab!!
- Response is delayed – may take hours or DAYS after sensitization for response/reaction to occur and lasts for days
- Biggee: contact hypersensitivity
- Simple chemicals: Ni, formaldehyde
- Plants: poison ivy, poison oak, pumpkin vines, tomato bushes
- Topical drugs: sulfonamides, neomycin, cosmetics, soaps
- Within 24-48° develop erythema, itching, eczema or necrosis of skin
- AVOID offending substance
Contact Dermatitis

The image illustrates the sequence of events to cause contact dermatitis.
• In short an antigen is necessary to sensitize "a" T cell. After the second exposure to/of/by antigen, the sensitized T cell migrates to the site of the antigen and releases lymphotoxins that necroses the living tissues at the antigen-cell complex. At the same time, the sensitized T cell inhibits the migration of PMN's to the same site by releasing migration inhibition factor (MIF). The patient now has contact dermatitis.

• One of the most irritating aspects of contact dermatitis is the itch-scratch-itch cycle. This cycle is very difficult to break free from without proper medication. Once a person starts scratching the skin lesions, in all likelihood, he or she will develop infection in the lesions. Once treatment of the infection ensues and treatment of the inflammatory response ensues, the itch-scratch-itch cycle may be broken and the skin will heal.
Atopic Dermatitis

- It seems that atopic dermatitis is ALSO B cell mediated, making this disorder a mixed hypersensitivity (humoral and cell mediated). In addition, stress also increases levels of histamine, which makes the itch-scratch-itch cycle worse. Stress increases the activity of an enzyme called histidine decarboxylase which decarboxylates histidine to histamine that perpetuates the itching.
In the treatment of atopic dermatitis, trimming nails to reduce scratching and to reduce infections with children may prove useful. Mittens on small children, likewise, reduce the effects of scratching. Cotton clothing is better than other types as it does not snag on skin and further irritate it. Chapping due to cold dry air is most pathological -- remember it only takes a 0.2° C temperature change to trigger these receptors. In the wintertime, then, higher humidity and warmer temperatures tend to reduce the worsening effects of winter conditions, i.e., cold weather makes it worse. After bathing, pat dry: no rubbing. Reduce bathing time and maintain water in the cooler end of warm to reduce pruritis, i.e., too hot temperatures make the rash worse, too. Eucerin, steroids (topical, systemic) and non-defatting soaps work well. Remember, though, that topical steroids may also make the skin thinner at the site of usage and delay healing. Treat infections with anti-*Staphylococcal* agents, e.g., dicloxacillin.
## Selected Autoimmune Disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Hypersensitivity</th>
<th>Tissue Site</th>
<th>Ag</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>Cytotoxic (II)</td>
<td>Platelets</td>
<td>ASA, PCN’s; antihistaminies</td>
<td>Hemorrhage, bruising</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Cytotoxic (II)</td>
<td>Muscle fiber membrane</td>
<td>ACH receptor</td>
<td>Decreased muscle activity; eye muscle weakness</td>
</tr>
<tr>
<td>Graves’ disease</td>
<td>Cytotoxic (II)</td>
<td>Thyroid</td>
<td>??</td>
<td>Elevated $T_4$; elevated BMR</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>Cytotoxic (II)</td>
<td>Thyroid</td>
<td>??</td>
<td>Depressed $T_4$; depressed BMR</td>
</tr>
<tr>
<td>Arthus reaction</td>
<td>Immune Complex (III)</td>
<td>Blood vessels; Ag entry site</td>
<td>From person’s environment</td>
<td>Blood clots</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Immune Complex (III)</td>
<td>Joints</td>
<td>Self – connective tissues?</td>
<td>Arthritis, rheumatoid lung</td>
</tr>
</tbody>
</table>
The 5 Phases of [Superficial] Allergic Reactions
Allergic Response

- Normal Vascular Bed
Allergic Response

- Hyperemic Vascular bed
- Rubor and Calor (superficial)
Allergic Response

TUMOR

- Starling’s Law of the Capillaries says that the inner and outer fluid pressures are ± balanced between vessels and tissues
- More in/from Urinary lecture
- The increase in interstitial fluid leads to swelling due to:
  - Increase in BHP and EOP is > BOP
  - Increase in venular permeability
- Called swelling
Allergic Response

- With increased pressure, get increased flow
- If venule accepts this increased flow without dilating, SOMETHING has to give
- With the increased fluid in the tissues comes: DOLOR
- Dolor is due to
  - Change in pH – effects nerve ends
  - Increased fluid – increases pressure on nerve endings
  - Increased histamine – stimulates nerves
- ALL of which leads to Functio laesa to protect the site of inflammation
During Rubor and Tumor

- Chemotactic factors mediate interstitial movement of PMN’s to site of insult
• To make matters worse, during rubor and tumor, white blood cells, previous Figure on p. 99, are signaled to begin leaving the vasculature and migrate into the interstitium (diapedesis). Typically, the WBC marginate (they move to the edge of the vessel) and pavement (flatten out on the endothelium), then stick a piece of themselves through the endothelium at a region of bottlenecked flow (diapedesis, proper). They, then, snap the rest of their cell through the vessel to merge into the interstitium. Within several hours, more than 1,000,000 WBC are present in the tissues. Chemotactic factors mediate interstitial movement of PMN's, specifically, to the site of the insult.
Duration of Inflammatory Response

- **ACUTE**
- Active phase of exudation

- **SUBACUTE**
- Early repair phase with exudation

- **CHRONIC**
- Advanced repair phase with exudation
Exudation

• Accumulation of fluid in tissues due to inflammation

• 2 Kinds:
  – Non-cellular – zero to few WBC
  – Cellular – mostly PMN’s
Non-Cellular Exudates

1. Serous
   1. Lots of liquid with dissolved solutes
   2. Few WBC
   3. E.g., blister fluid

2. Fibrinous
   1. Contains fibrins
   2. Roughens surfaces of serosa (pleura, peritoneum, pericardium) and causes pain on movement
   3. Friction rubs heard by stethoscope

3. Mucinous
   1. Aka catarrhal
   2. ONLY on mucous membranes/surfaces
   3. For mucin release
   4. Runny nose
Cellular Exudate

1. Only one kind: Neutrophilic
   1. Primarily PMN’s
   2. So many PMN’s that fluid and dissolved particles are very minor
   3. Aka purulent
   4. Generally from infection by bacteria
   5. Prominent in areas of tissue necrosis
Cellular Exudate Formation

Diapedesis

PMN’s emigrate during rubor and tumor

PMN’s die, shatter and release hydrolytic enzymes INTO surrounding tissues

Enzymes digest and liquify tissue – known as suppuration (pus)

PMN’s (dead, live, shattered); lysed tissues; fluid; many times the bacteria causing the problem
Abscesses

Occur in solid tissues; “hole filled with pus”; difficult to treat with systemic antibiotics; generally treat with surgery to drain and collapse

Diffuse purulent inflammation beneath the skin
Complement Activation

3 “triggers”

1. Opsonization: direct binding of C3 fragment to bacterium

2. IL-6 stimulation: IL-6 secreted from macrophages due to bacterial identification; IL-6 causes liver to synthesize/secrete mannose binding protein (MBP); MBP binds to bacterial capsule and conjointly activates complement cascade

3. Ab-activated: Ab’s from B cells bind to bacteria; Fc fragment from 2 Ab’s bind C1q, C1r and C1s to activate complement cascade

1. NOTE: IgG4 will NOT bind complement, nor do IgA, IgD or IgE

2. NOTE: to bind complement, requires either 2 IgG (1, 2 or 3) or 1 IgM

4. NOTE: when complement “stripped” from blood, titer is measurable within 8 hours and at 18 hours is at normal concentrations
Classical Pathway

Complement Activation
• The following two figures illustrate the classical pathway of complement activation.
• C1q, C1r and C1s react together after C1q binds to the Fc fragment of either 2 IgG molecules (see above) or 1 IgM (see above) molecule after they have bound to a bacterium.
• The complex of complement 1 proteins is called activated C1.
• C1s cleaves C4 and C2 into two fragments, apiece: C4a and C4b; C2a and C2b.
• C4b and C2a bind together and react with C3.
• The product of this reaction is C2aC3bC4b and is called the "C5 convertase".
• NOTE: pay attention to your letter cases with these proteins as the case makes a difference in which proteins are being utilized.
Classical Pathway -- 1
• The "C5 convertase" reacts with C5 to form C2aC3bC4b + C5b. From this last reaction and the cleavage of C3 to C3b and C3a, there are two important products (C5a and C3a) that are involved in anaphylactic reactions and inflammatory reactions. These latter shall be discussed shortly.

• One C5b molecule reacts with sixteen C9 molecules, one C6, one C8 and one C7 molecule to form the "MAC": the Membrane Attack Complex. This MAC is a transmembrane channel, much like the escape pores we studied from Gram positive bacteria: the MAC is inserted into the cell like a spigot. This "spigot" permits the cell innards to rush out and other substance to rush into the complement and Ig-bound cell, causing its demise (cytolysis).
Classical Pathway

2
• As previously mentioned, C3a and C5a, both products from the classical pathway, are involved in anaphylaxis and inflammation.
• Both complement proteins, when bound in specific tissues (called complement fixation), cause degranulation of basophils and mast cells.
• Both cause increased vascular permeability so that not only may cells undergo diapedesis, but fluid may also leak into the surrounding tissues and cause swelling.
• By virtue of the fixation of, at least, C5a in various tissues, phagocytes are attracted their site of fixation (positive chemotaxis).
Anaphylotoxins/Inflammatoxins

Classical Pathway

Classical Pathway

Basophil

Blood Vessel

Mast Cell

Phagocytes

Platelet

Histamine

Degranulation

1. Degranulation

↑ Vascular Permeability

2. ↑ Vascular Permeability

Phagocyte Chemotaxis

Activated to site of Complement Fixation (C5a)

C3a

C5a
Complement Fixation Tests: CFT’s

• Since we've now mentioned complement fixation, it seems a good time to apply this concept, clinically. We do this through tests that are called, generically, complement fixation tests (Figure following). Examples of complement fixation tests (CFT's) include the Wassermann test for *T. pallidum* and tests for encephalitis viruses, histoplasmosis and Rocky Mountain Spotted Fever.
There are two reactions one may obtain when running this procedure: a positive test sample and a negative test sample.

In the first stage of the CFT (the complement fixation stage), the patients' serum is heated at 56° C for 30 minutes to inactivate the patient's own compliment. To the heat treated sample is added guinea pig complement in a fixed amount and the test antigen (from a bottle). The samples are then allowed to incubate for 30-90 minutes depending upon the CFT. In the positive test, note that the patient's own antibodies have fixated the guinea pig complement following binding of the bottled antigen. In the negative test, since there is no antibody in the patient's serum, there is no complement fixated.
CFT’s: Wasserman
• After the completion of this stage, sheep RBC and anti-sheep RBC (hemolysin) are added to the mixture and incubated for about 2 hours. This stage is called the indicator stage. After incubation, the mixtures are spun down in a centrifuge and examined for "RBC buttons". In the positive test sample, note that there is no hemolysis of the sheep RBC. This is because the hemolysin was unable to bind with the RBC and results in a "button" for the positive test. In the negative test, note that there is hemolysis of the sheep RBC. This is due the hemolysin fixing the guinea pig complement that turned on the hemolytic ability of the hemolysin and does not allow a button to form in the negative test.
CFT’s: Wasserman
Complement Activation

Alternative Pathway
The following figure illustrates the alternate pathway for complement activation. This pathway has been less studied than the classical pathway because it has not been known as long. Nevertheless, a microbial substance initiates this pathway, e.g., endotoxin, and causes C3 to react with a protein in the blood called "B" Note the case of the letter!

This forms the C3B complex. This complex then reacts on two substrates: another C3 molecule and with another protein in the blood called "D". The products of this reaction include C3C3bBD which is unstable, C3b (which may be used for opsonization -- coming up shortly) and C3a (for inflammation). The unstable C3C3bBD complex reacts with another protein in the blood called properdin (P) to form the stable C3C3bBDP intermediate. This intermediate reacts with another C3b molecule to form a different "C5 convertase". The latter C5 convertase then catalytically splits C5 into C5a and C5b and continues down the classical pathway.
Alternate Pathway
• The last topic regarding complement has to do with opsonization: this is process whereby microorganisms are immunologically covered (immune adherence) to enhance phagocytosis, Figure, following. It is very similar to hiding a pill in a piece of meat so that your pet will take it "without knowing about it".
Opsonization -- 1
There are 4 levels of opsonization:

1) No opsonin: the phagocyte has bonded with the bacterium via generic receptors. This is the lowest level of opsonization.

2) Antibody-bound bacterium: the phagocyte binds to the bacterium through its FC receptor.

3) C3b: the phagocyte binds to the C3b-coated bacterium through a specific C3b receptor.

4) The best level of opsonization is a bacterium that is bound to BOTH antibody and C3b. The phagocyte binds via each respective receptor and this level permits optimal phagocytosis, i.e., it "hides" the defenses of the bacterium from the phagocyte, e.g., capsule, so that the bacterium may be taken up for destruction.
Opsonization -- 2

<table>
<thead>
<tr>
<th>No Opsonin</th>
<th>Ab</th>
<th>C3b</th>
<th>Ab + C3b</th>
</tr>
</thead>
<tbody>
<tr>
<td>phagocyte</td>
<td>Fc receptor</td>
<td>C3b receptor</td>
<td>Best is both</td>
</tr>
<tr>
<td>B cell</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ELISA
• ELISA stands for Enzyme-Linked ImmunoSorbant Assay. Perhaps the best known ELISA is the screening ELISA for the presence of anti-HIV.

• In general, ELISA's work as illustrated in the Figure, following. A patient's serum is added to antigen bound beads. For our purposes of illustration, we'll expect that the patient's blood has the antibody we're interested in detecting. The mixture is incubated. Enzyme-bound anti-idiotype (an antibody to an antibody) is then added to the mixture. It is allowed to incubate. Substrate that will be chemically altered by the enzyme on the anti-idiotype -- assuming the anti-idiotype bound -- is added and the mixture is incubated, again. For discussion purposes, panel 4 is the positive response in the following Figure. If the antibody we're looking for is present, the substrate changes color and it's positive. If this were for anti-HIV, it would be repeated. If it were positive, again, a Western blot would be run to confirm the ELISA results and the patient notified of the results, either way. Panel 5, though, is negative, i.e., the substrate did not change color because the antibody we were looking for was not present, so the anti-idiotype was unable to bind to it to activate the enzyme bound to itself.
Immunological Applications

MAC-ELISA

IgM Antibody Capture - Enzyme Linked Immunosorbent Assay

Sample well

"trapper" Ab for Ag

Add patient's serum (with and without Ag*)

Add anti-idiotypic

Add anti-anti-idiotypic with horseradish peroxidase

Add chromogen

Positive = color change: Ab present

Negative = NO color change: no Ab present

color rxn = [+]

no color rxn = [-]
TITERS
• Titers are useful in determining if a person has a high enough level of antibodies to a specific disease or they may be used diagnostically to differentiate between, for example, rheumatoid arthritis and bursitis.

• Figure on p. 118 illustrates a simple titer determination. There are some conditions that need to be carefully maintained. These include adding an identical amount of antigen to all tubes and running a negative control so that you are able to identify a lack of clumping. Samples are run on undiluted samples and then on serially diluted samples. Each titer has its own set of specific dilutions. In Figure on p. 118, the undiluted sample has clumping. We want to find the highest dilution of that sample that exhibits NO clumping, i.e., there is only solution present, THEN go BACK to the nearest "solution" (the one that demonstrated clumping next to the one without clumping) and record the titer -- 1:20 in this example (Figure on p. 118). In Figure on p. 118, lack of clumping occurs at the 1:40 dilution, giving a 1:20 titer. Likewise, in Figure on p. 119, the titer is 1:20.
Titers

<table>
<thead>
<tr>
<th>Dilution:</th>
<th>1:5</th>
<th>1:10</th>
<th>1:20</th>
<th>1:40</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>RXN: Clump</td>
<td>less clump</td>
<td>less clump</td>
<td>less clump</td>
<td>solution/no clump</td>
<td>solution/no clump</td>
</tr>
<tr>
<td>Ab: pt’s</td>
<td>pt’s</td>
<td>pt’s</td>
<td>pt’s</td>
<td>pt’s</td>
<td>----</td>
</tr>
<tr>
<td>Ag: fixed amount</td>
<td>ID (same amount)</td>
<td>ID</td>
<td>ID</td>
<td>ID</td>
<td>ID</td>
</tr>
</tbody>
</table>

Titer for this patient = 1:20 (last dilution to clump)
## Titers

<table>
<thead>
<tr>
<th>Dilution:</th>
<th>1:10</th>
<th>1:20</th>
<th>1:40</th>
<th>1:80</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>RXN: Clump</td>
<td>clump</td>
<td>less clump</td>
<td>solution/no clump</td>
<td>solution/no clump</td>
<td>solution/no clump</td>
</tr>
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<td>pt’s</td>
<td>pt’s</td>
<td>pt’s</td>
<td>----</td>
</tr>
<tr>
<td>Ag: fixed amount</td>
<td>ID</td>
<td>ID</td>
<td>ID</td>
<td>ID</td>
<td>ID</td>
</tr>
</tbody>
</table>

Titer for this patient = 1:20 (last dilution to clump)
Monoclonal Antibody Production
Application of Monoclonal Ab’s

• Many laboratory applications

• BIGGEE: RadiolImmunoAssay (RIA) – already studied ELISA and Titers
THEORY

\[ Ag^o + Ab = Ag^o \cdot Ab \text{ complex} \]

\[ \therefore \]

\[ Ag^* + Ab = Ag^* \cdot Ab \text{ complex} \]

\[ Ag^* = \text{radioactive Ag} \]

it follows, then,

\[ Ag^o + Ag^* + Ab = Ag^o \cdot Ab + Ag^* \cdot Ab + Ag^o + Ag^* \]

if fixed amount of \( Ab \) added
THEORY

- $\text{Ag}^0\cdot\text{Ab}$ and $\text{Ag}^*\cdot\text{Ab}$ complexes are proteins, HENCE, they may be precipitated with Rx like PEG, SO:

![Diagram showing precipitation process with PEG](image-url)
THEORY

\[
\text{%Ag}^* \text{ bound to Ab} (\text{Ag}^* \bullet \text{Ab}) = \frac{\text{Ag}^* \bullet \text{Ab}}{(\text{Ag}^* + \text{Ag}^* \bullet \text{Ab})} \times 100 = \% \text{ Binding}
\]

• Requirements
  – Fixed amount of Ag* added to each tube  
  – Fixed amount of Ab added to each tube
• The < [Ag⁰], the > % Binding  
• The > [Ag⁰], the < % Binding
THEORY

Polyclonal Ab Binding Curve

Monoclonal Ab Binding Curve

%B

[Ag°]

From STANDARDS
Standard Curve –

Standards: known concentrations of the analyte under study; analyzed with UNKNOWN samples under identical conditions
Clinical Significance -- RIA

% binding is so ambiguous with polyclonal Ab’s that neither you nor your patient know what is the dx – MALPRACTICE!!!