

The Immune System

Immunogenesis

Non-specific Responses:

Biological obstacles

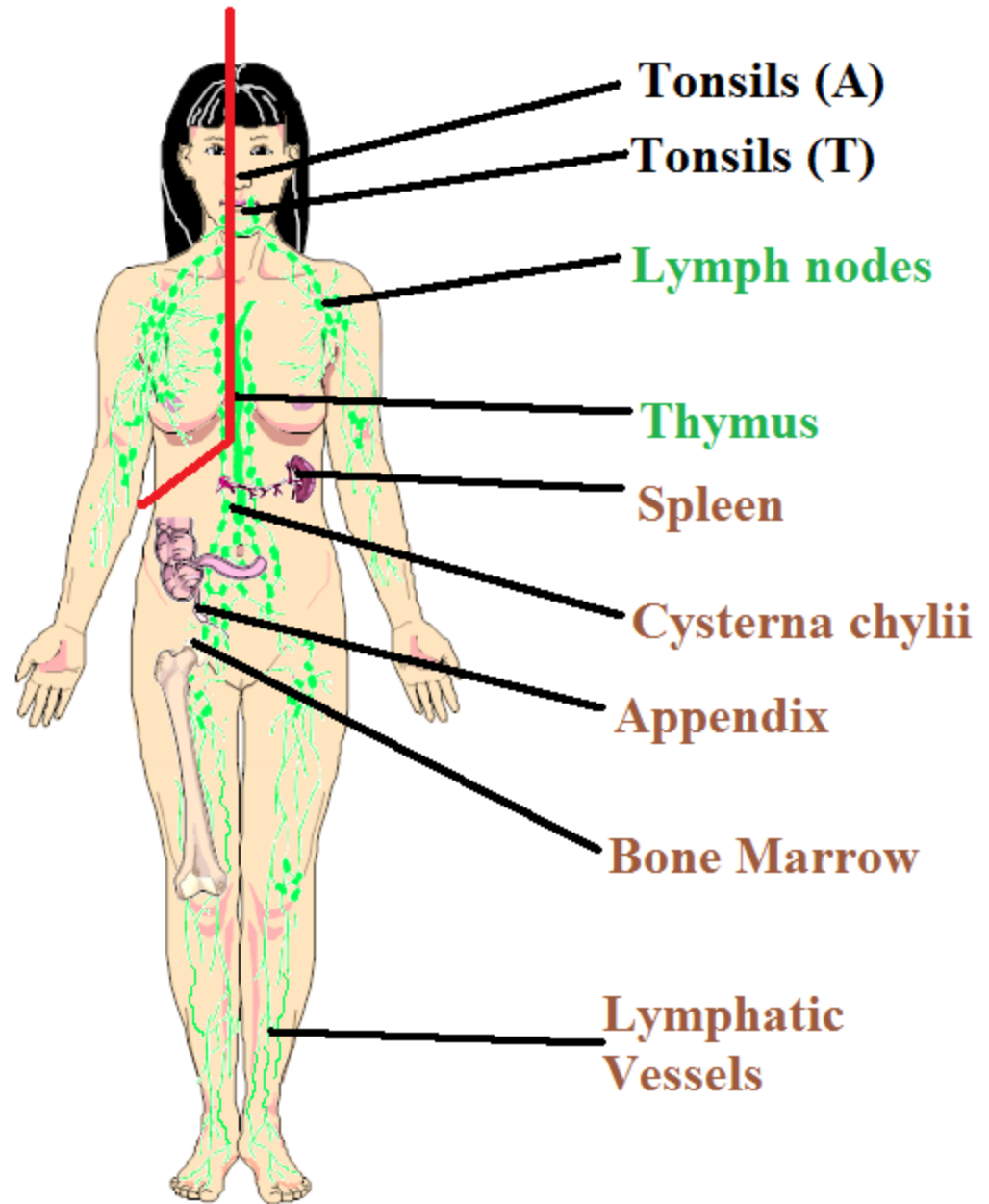
Chemical obstacles

General obstacles

Physical obstacles

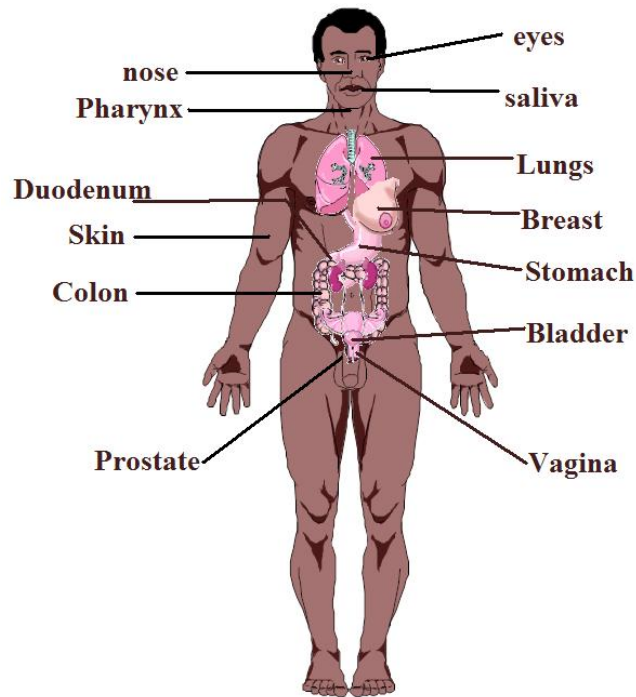
Definitions

1. Immunity: specific resistance to disease
2. Immunology: the branch of science that deals with the responses of the body to foreign object (antigen) challenge.
3. Acquired defense reactions: develop over time
4. Innate defense reactions: born with
5. Peyer's patches – GI immune tissue



Innate Defense Mechanisms

- Nose – filtering
- Pharynx – commensal organisms
- Breast – milk with IgA
- Skin – px obstacle, FA, commensal organisms
- Colon – commensal organisms
- Prostate -- secretions



- Eyes – lysozyme
- Saliva – lysozyme, permease, peroxidase
- Lungs – trachea with mucus and cilia
- Stomach – acid
- Duodenum – alkaline
- Bladder – flushing
- Vagina – pH and commensal organisms

Outer Defenses

1. Mechanical barriers:
 1. Intact skin highly effective
 2. Mucus membranes more permeable
 1. *N. gonorrhoeae* -genitals
 2. *T. pallidum* -genitals
 3. *N. meningitidis* -nasal
 4. *S. pneumoniae* -nasal
 5. *S. typhimurium* –intestinal mucosa
 3. Parasitization of mucus membranes is first step in many viral infections
 4. Mucus is also a mechanical barrier by discouraging viral penetration.

Outer Defenses -- 2

2. Mechanical Removal:

1. Respiratory tract has a “carpet of mucus” that is continuously conveying “stuff” towards the esophagus by ciliary action.
2. Coughing, sneezing, sniffing, blinking.
3. Flow of tears, sweat, saliva, urine and GI secretions, as well.

Outer Defenses -- 3

3. Germicidal Activity

1. Skin

1. Is bactericidal for *S. pyogenes* due to sebum secretion
2. Gram negative rods such as *E. coli* and *P. aeruginosa* are rapidly killed by skin
3. Experimentally, if large numbers of these organisms are placed on the skin, it's impossible to recover them an hour later
4. *Klebsiella* is one Gram negative organism that may survive for several hours
5. *S. aureus* (Gram positive) is highly resistant – MRSA and VRSA

2. Gastric juice

1. Quickly lethal due to strong acidity
2. *M. tuberculosis* is resistant
3. Ingested particles may escape destruction by being hidden in food particles
4. Ingested particles may escape destruction by diluting or buffering actions

Outer Defenses -- 4

3. Germicidal activity
3. Prostatic secretions
 1. Seminalplasmin seems to be antibacterial
 2. Enters bladder at end of complete micturition
 3. May explain the lower frequency of UTI's men experience compared to women (1:10, respectively) by both chemical and anatomical reasons
4. Breast milk
 1. Contains various antibacterial substances
 2. Contains an iron-binding protein which inhibits some E coli multiplication
 3. Contains an anti-viral agent (not of B cell origin) which protects infants against rotavirus (infantile gastroenteritis viruses) infection
 4. Contains an agent that kills Giardia
 5. Contains IgA, as well – protective against viruses (present in many secretions, as well as in breast milk)

NOTE: WEANING from breastfeeding

[<http://www.pediatriconcall.com/forpatients/CommonChild/breastfeeding.asp>]:

Around the end of 6 months gradually introduce the child to family meals. However, continue to breastfeed until the second birthday.

Outer Defenses -- 5

3. Germicidal activity

5. Lysozyme

1. A mucopolysaccharidase present in tissues and all body secretions except urine
2. Present in particularly high concentrations in tears
3. Has the ability to lyse and kill bacteria by breaking down their mucopeptides of their cell walls

Outer Defenses -- 6

4. Normal flora

1. Exceptionally well demonstrated in normal adult vagina
2. Flora are exclusively lactobacilli that hydrolyze the vaginal epithelial glycogen to lactate
3. This high acidity renders the vagina resistant to other organisms' invasion
4. When glycogen is not available (before puberty and after menopause), the flora is more variable, the secretions are more alkaline and invasion by pathogenic organisms is relatively common.

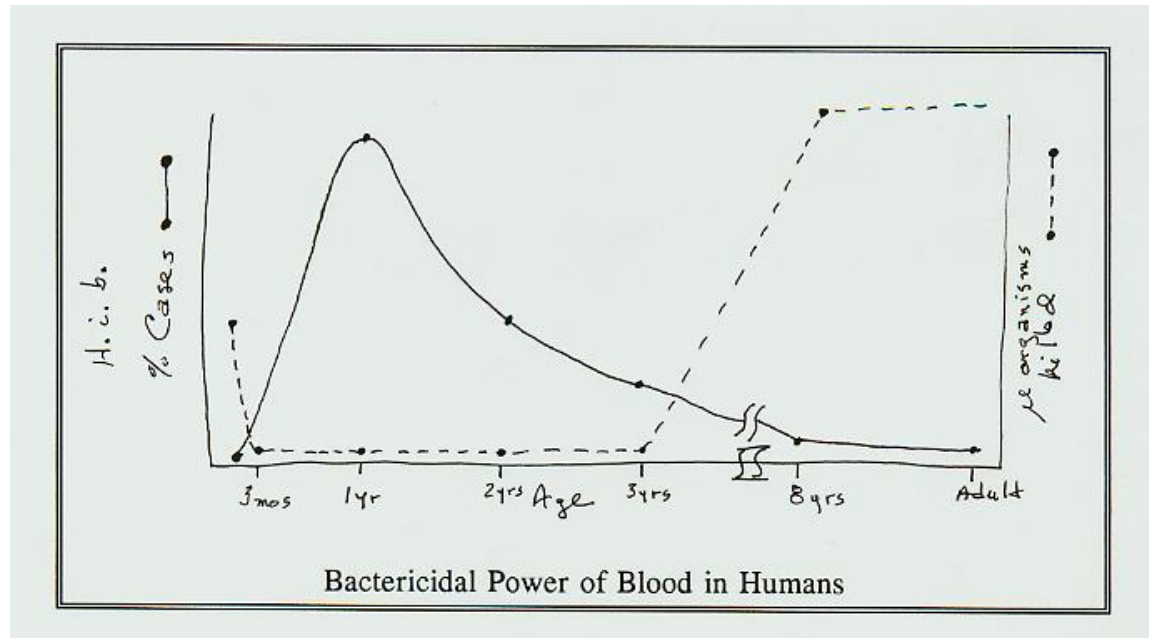
Complete Absence of Normal Flora

- This can be examined experimentally
- Developing fetus is already sterile
- With incredible aseptic techniques, sterile animal fetuses can be delivered by C-section
- These animals can live and breed in sterile environments
- Called **Germ Free Animals**
- With a proper diet, they are healthy and show no nutrient deficiencies
- They do not contract disease – nor do they produce Ab's against microbes
- Won't get caries unless have *S. mutans* introduced – can eat all the sugar they want as long as NO bacteria present
- If challenged by “non-pathogenic” bacteria, are unduly susceptible to generalized and lethal infections, e.g., *Lactobacillus*, *B. subtilis* and *S. faecalis*

Inner Defenses

1. Body Fluids

1. Serum exhibits bactericidal and bacteriolytic activities
2. These effects most clearly seen with Gram negative bacteria such as *E. coli* and avirulent strains of *V. cholerae*, *N. meningitidis*, *H. influenzae*, *Salmonellae* and *Shigellae*.
3. Virulent strains of the same species may be resistant to these actions
4. Gram positive organisms are less susceptible to bactericidal action and lysis simply does not occur.
5. Body fluids also have some power of neutralizing bacterial endotoxins and enzymes and possess weak viral neutralizing effects.



Inner Defenses

2. Viral Disposal

1. The body encounters viruses frequently – disposal is not obvious
2. Bactericidal and bacteriolytic mechanisms DON'T work against viruses and are ineffective
3. Clearance is primarily effected by fixed macrophages of the reticuloendothelial system
4. Wandering macrophages dispose of virally infected cells
5. Specific Ab's provide resistance against viruses

3. Viral Ab's – hodge-podge

1. Patients with agammaglobulinemia recover normally without Ab's
2. Ab's don't penetrate cells
3. Ab's don't inhibit intracellular multiplication

Inner Defenses

4. Viral defenses

1. T-lymphocytes play an important role
2. May have normal Ab production with defective lymphocyte production that will lead to viral susceptibility
3. Injections (experimentally) in animals and humans with antilymphocyte Ig increases susceptibility to viral infections
4. NKC's lyse virally infected cells before viral replication has occurred.
5. Interferon may be the most important defense mechanism against viruses
6. Interferon is detectable shortly after infection and long before Ab production
7. If mice are injected with anti-interferon and are then infected with various viruses, the illnesses they develop are much more severe than those in control animals
8. Many viruses will replicate only within narrow ranges of temperature and pH and an increasing body temperature and lowering of pH in areas of inflammation are important non-specific defense mechanisms
9. An elevated temperature (fever) and lower pH also increase interferon production and release

Factors Which Effect Innate Resistance

1. Genetic Factors

1. Responsible for variation in resistance by races, families and individuals
2. Selective breeding of mice permits strains of mice that are more susceptible (or less depending on the genes) than their parent stock to *S. typhimurium*
3. Not as easily “split out” in man, though, due to poor nutrition, poverty, over crowding
4. African Americans, Native Americans and Eskimos are more susceptible to *M. tuberculosis* than Caucasians. Seems to be due to exposure effects over several thousands of years.
5. Sick cell anemia, while protective to the 4 Plasmodia, is associated with increased susceptibility to *S. pneumoniae*.

Factors Which Effect Innate Resistance

2. Age

1. Infants are very susceptible to bacterial infections
2. Not very susceptible to chickenpox, measles or mumps' viruses
3. Poliomyelitis is more severe in adult life than in early childhood
4. Susceptibility to bronchitis and pneumonia is increased in old age

Factors Which Effect Innate Resistance

3. Gender

1. Women carry *S. typhi* in their gall bladders more frequently than do men
2. *Paracoccidioides brasiliensis* (causes mucus ulceration of mouth and nose with lymph spread) infects both genders equally – overt disease, though, is over ten times more common in men than women

Factors Which Effect Innate Resistance

4. Hormones

1. ACTH and corticosteroids inhibit the inflammatory reaction and lower the resistance to bacterial and viral infections.
2. Diabetes mellitus may be responsible for the susceptibility to staphylococcal infections found in diabetics
3. Estrogens are necessary for maintaining the resistance of the adult vagina against bacterial invasion

Factors Which Effect Innate Resistance

5. Fatigue

1. Has little effect on susceptibility to infections
2. Only rarely is there an occasional activation of a quiescent micro-organism in animals exercised to exhaustion on treadmills
3. In man, violent exercise in early polio predisposes to paralysis of the muscles used most actively

Factors Which Effect Innate Resistance

6. Temperature

1. There is no evidence that changes in temperatures and sitting in drafts cause minor human ills
2. Some animals seem to be a little different:
 1. If chickens are chilled, they develop susceptibility to *B. anthracis*
 2. If frogs are warmed, they develop susceptibility to *B. anthracis*

Factors Which Effect Innate Resistance

7. Nutrition

1. This is difficult to determine, at best, in man
2. Dental caries does go down when people are starved, e.g., POW camps
3. Ab response of starved individuals is virtually normal
4. Cell mediated immunity of starved people is depressed

Two Kinds of Acquired Defense Mechanisms

1. Antibody mediated immunity – aka humoral immunity – depends on the production of specific antibodies (Ig's)
2. Cell mediated immunity – aka cellular immunity – depends upon the development of specifically sensitized cells (T lymphocytes)

Acquired Defense Reactions

- Take time to develop because Ab's and sensitized lymphocytes do not appear for several days.
- Are more powerful because the Ab and lymphocytes react specifically against the invading substances [microbes] and their products.
- Leave varying degrees of acquired immunity because the body continues to produce Ab's or sensitized lymphocytes for long periods of time or may produce them at short notice if the body meets the same Ag on a subsequent occasion.
- (The latter is called immunological memory or secondary response.)




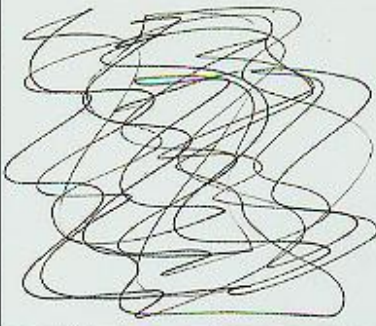
Antibody Mediated Immunity

- An antigen is any chemical substance that, upon entry into the body, causes the body to produce specific antibodies and/or specific cells (T-cells) which will react with the antigen, i.e., is capable of provoking an immune response.

Two Characteristics of Antigens

- Immunogenicity**: the ability to stimulate the formation of specific Ab's.
- Reactivity**: the ability of the antigen to react specifically with the produced antibody

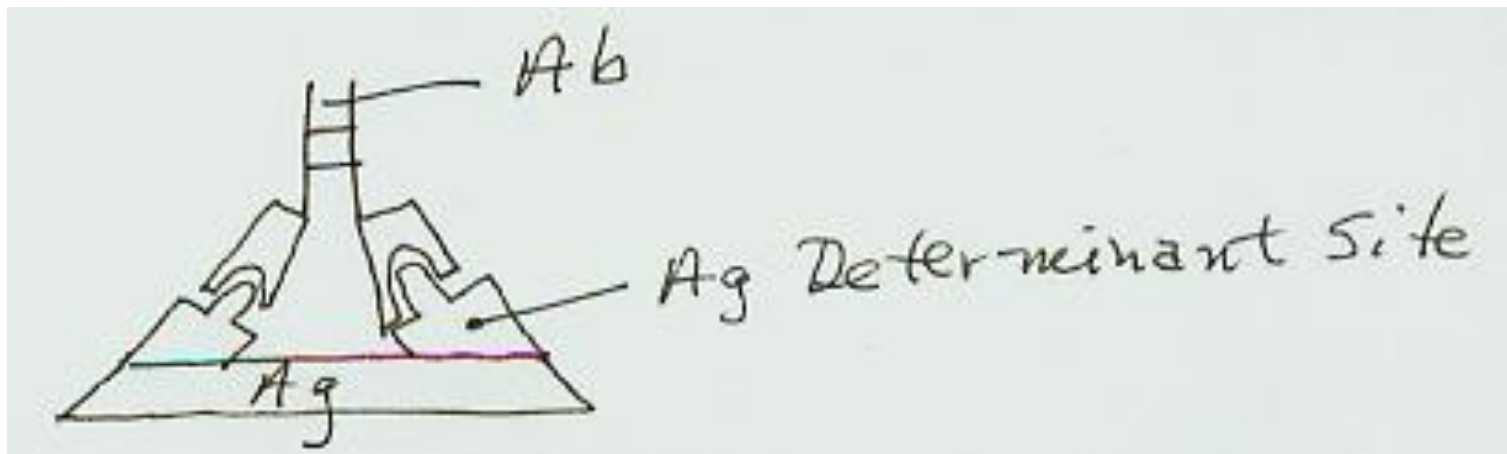
An antigen with both of these characteristics is called a **complete antigen**

 <chem>NC(=O)N</chem>	 	
Urea MW = 60	Glucose MW = 180	GH MW = 20,000

- Chemical Characteristics of Antigens
- Ag's are generally foreign to the body
- Are generally large molecules of MW > 10,000

Chemical Characteristics of Antigens

- Each Ag has a specific site which is recognized by the body for Ab synthesis
- These sites are called **antigenic determinant sites** or *epitopes*.
- The number of epitopes on the surface of the Ag is called the valence. Minimally, each Ag must be bivalent to induce Ab formation.
- Most Ag's are multivalent

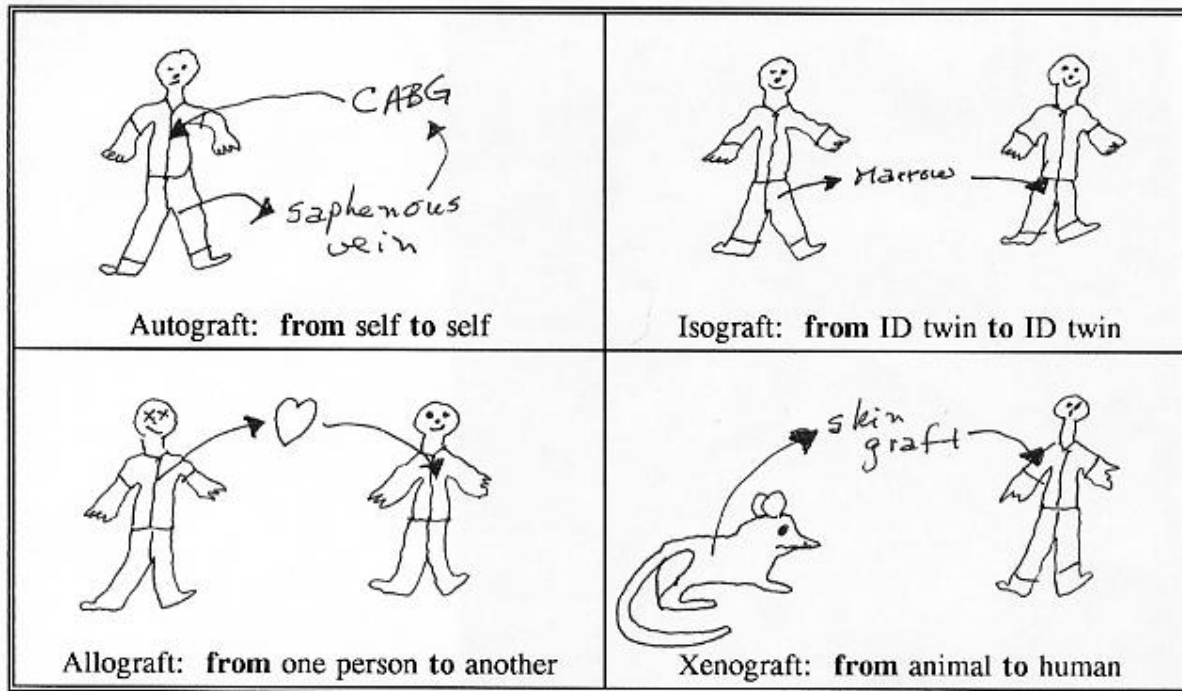


Antigenic Exception: Hapten

- A hapten is a low molecular weight substance that has a MW \ll 10,000
- To cause an immunogenic response, the hapten binds to a tissue or plasma protein which is of sufficient size to allow the epitopes of the hapten to become immunologically active
- T and B cells recognize this combination (hapten + protein) as an antigenic substance.
- The body does NOT react to the tissue or plasma protein

Human Antigens and Grafts

- The antigenic makeup of humans is very complex
- Because of tissue transplantation, tissue typing (using lymphocytes) is used to match donor and recipient as closely as possible



- The major histocompatibility complex (MHC – aka HLA) system consists of “strong” antigens which influence whether a graft will survive or not
- In autoimmune diseases, Ab’s are produced against various organ-specific antigens
- Allografts are also called “homografts”
- A coisograft is a graft between inbred strains varying genetically by only a few genes²⁶ – NOT of human importance

Human Antibodies (Ab's)

- An antibody is an Ig (immunoglobulin – a special group of proteins) which is produced as a result of the introduction of an Ag into the tissues of an animal and which reacts specifically with that Ag in some demonstrable way.
- **Specificity** is the most striking property of an Ab in its action, e.g., an Ab produced against tetanus toxin has **NO** action against Diphtheria toxin and vice versa

Ab's are Proteins

$\left. \begin{matrix} F_{ab} \\ F_c \end{matrix} \right\} 2^{\circ} \text{ Papain}$	$C = \text{constant}$ $V = \text{variable}$
<p>L = light chain H = heavy chain -S-S- = disulfide bonds</p>	

- Consist of polypeptide chains
- There are 4 of these chains:
 - 2 H chains – Heavy chains
 - 2 L chains – Light chains
- 1 H and 1 L chain make up a half of the Ab
- Each half is cemented together by –S-S– bonds
- Ab is in “T” shape when not complexed with Ag’s
- Ab is in “Y” shape when complexed with Ag’s

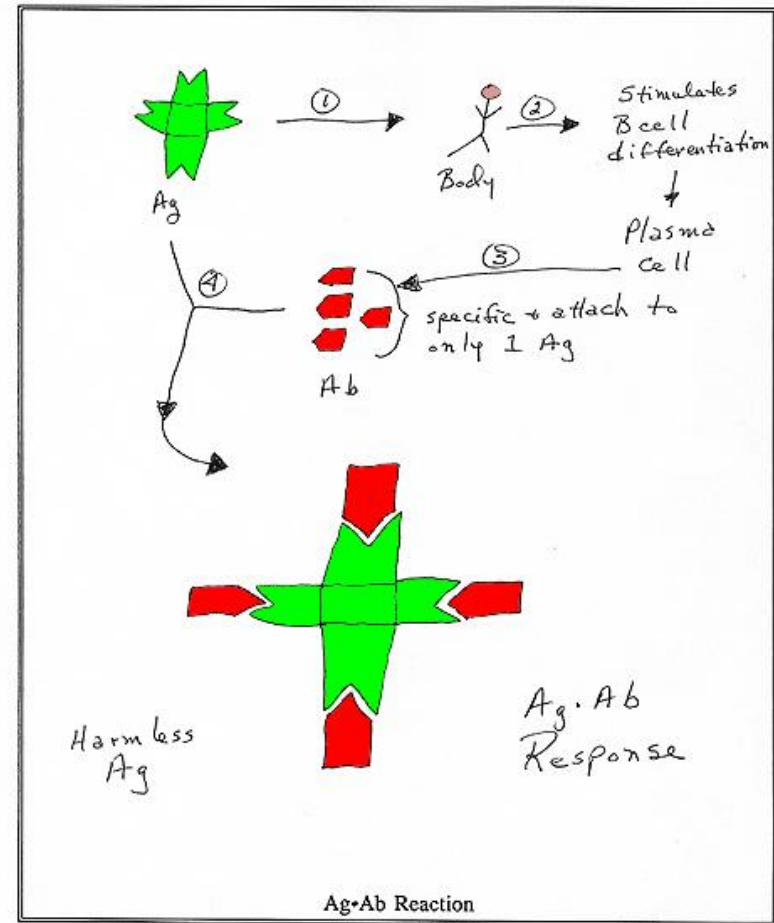
- Specific Ab activity depends on chemical structure of the individual Ig
- The L and H chains of Ig’s are peculiar in that they contain variable (V) and constant (C) regions
- Specificity is mediated by particular AA sequences in the V regions of the L and H chains
- These sequences are in a part of the Ab known as the Antigen Binding Fragment or F_{ab} fragment

Antibodies

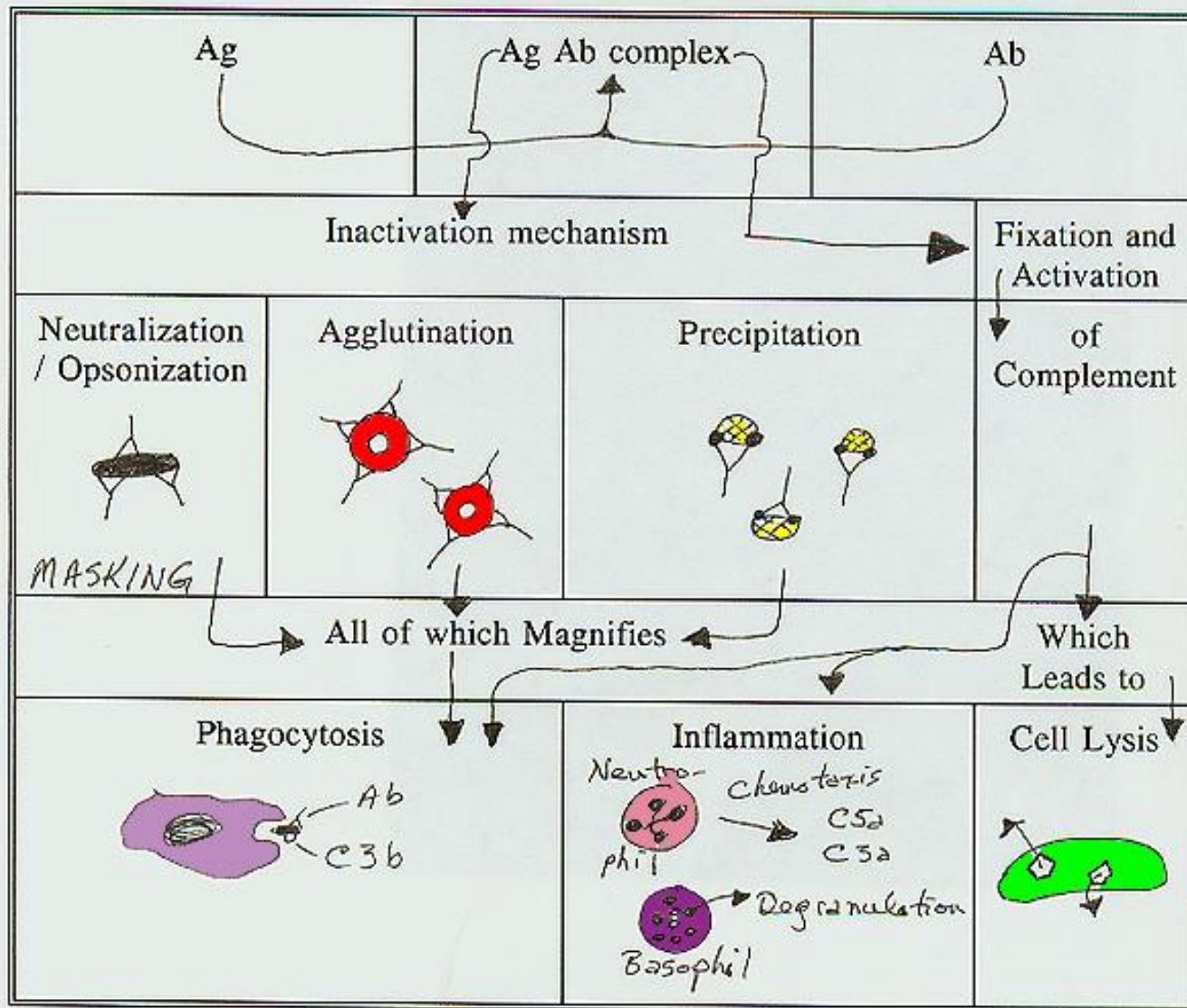
- Formed by cells of lymphoid tissues:
 - Spleen, lymph nodes, bone marrow, tonsils, Peyer's patches (in gut), solitary follicles
- When an animal is sufficiently antigenically stimulated, High [Ab's] can be detected in spleen and nodes
- Stimulated spleen and nodes continue to produce AB when grown in tissue culture or transplanted into other animals
- Ab formation is to some extent a local process
- Most actively producing Ab cells are those closest to Ag exposure
- Site first effected is site closest to challenge and receives first Ab's

Ag-Ab Reactions – In General

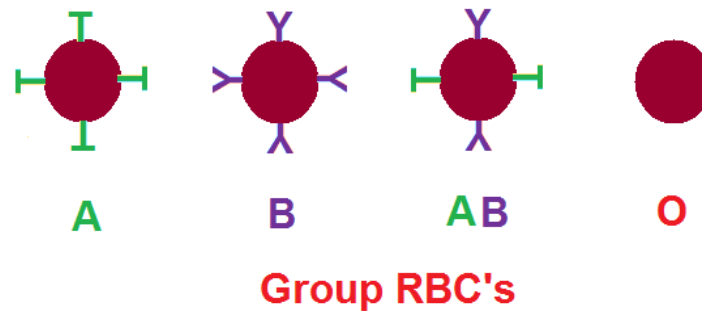
- Ag's and Ab's combine with each other
- The results of the combination are dependent upon
 - Ag nature
 - How Ag is presented to Ab
- If Ag is in solution, it is precipitated
- If Ag is part of cell surface, cell is agglutinated (clotted, more or less)
- Ag-Ab reactions form a major part of the body's acquired defenses against microbial invasion



Specific Antibody Mechanisms of Action



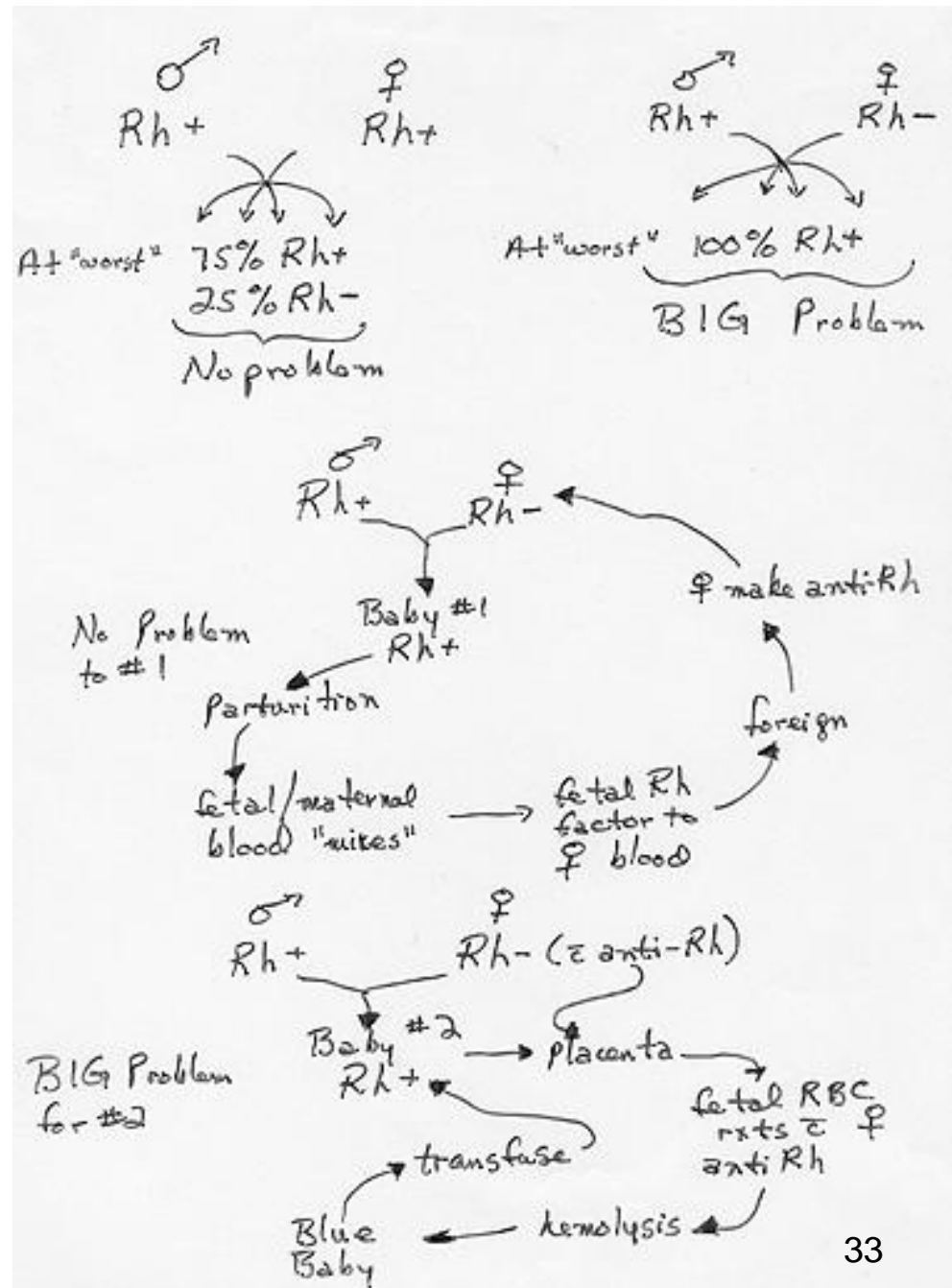
Human Antigens and Antibodies: Red Blood Cells



- RBC antigens for blood groups and types have been extensively studied because of transfusions and rhesus disease
- One use of glycoproteins by the body is as the antigens on the surfaces of red blood cells -- RBC.
- The illustration, above, shows that different glycoproteins provide RBC's with unique identifying markers.
 - RBC that are of the Group A persuasion have only that glycoprotein on their surfaces;
 - those with Group B have only the Group B glycoprotein;
 - those that are Group AB, have both glycoproteins;
 - those with Group O lack both glycoproteins.
- It is estimated that at least 85% of the population secretes soluble blood group substances in saliva, gastric juice, milk, seminal fluid, urine, ovarian cyst fluid and amniotic fluid.
- Indeed, before the invention of DNA testing, it was by these substances that people were determined to be at the scene of a crime.

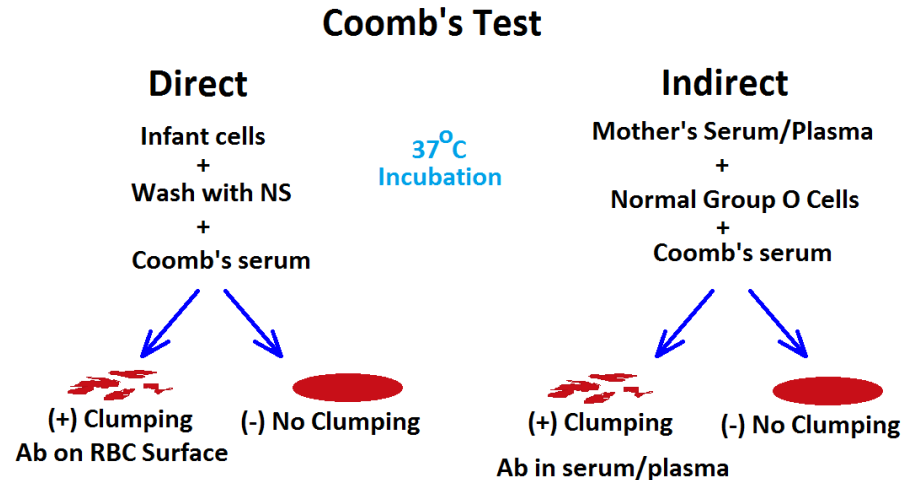
Hemolytic Disorder of the Newborn – Erythroblastosis Fetalis

- Treatment
- RhoGAM
- Is anti-anti-Rh = anti-idiotypic
- 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)

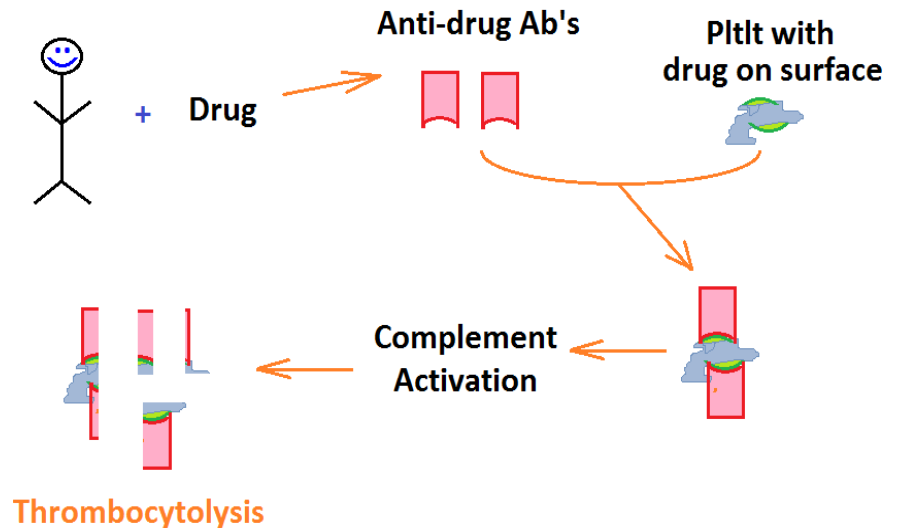


Dx HDN

Diagnostic Testing: Coomb's Test



Other Coomb's uses: positive in patients with salmonellosis, brucellosis and who have autoimmune hemolytic anemias



Specific Ig's: IgG

- Makes up approximately 75% of all Ab's

Characteristics

- Only Ig to cross the placenta; a later appearing Ig following natural infection (e.g., anti-HIV) or immunization; most effective in neutralizing soluble Ag's such as exotoxins and very small particulate Ag's such as viruses; monomeric (T or Y); protects fetus from diphtheria, polio, tetanus, measles, pneumococcal and streptococcal infections; F_c portion binds with eosinophils in helminthic infections; anti-Rh; increases phagocytosis; fixes to skin
- There are 4 sub-types

IgG sub-types and IgG₂ Comment

- IgG₁ – seeks out protein Ag's
- IgG₂ – specific for polysaccharide Ag's (e.g., pneumococcal)
 - If give 0.5 µg of pneumococcal capsule polysaccharide (SSS = specific soluble substance) to a human will cause an AgAb response
 - If give 0.5 mg (500 µg) of pneumococcal capsule polysaccharide (SSS = specific soluble substance) to a human WON'T cause an AgAb response
 - This is called immunological paralysis or immunological unresponsiveness
- IgG₃ – seeks out protein Ag's
- IgG₄ – blocks IgE binding to inhibit anaphylaxis

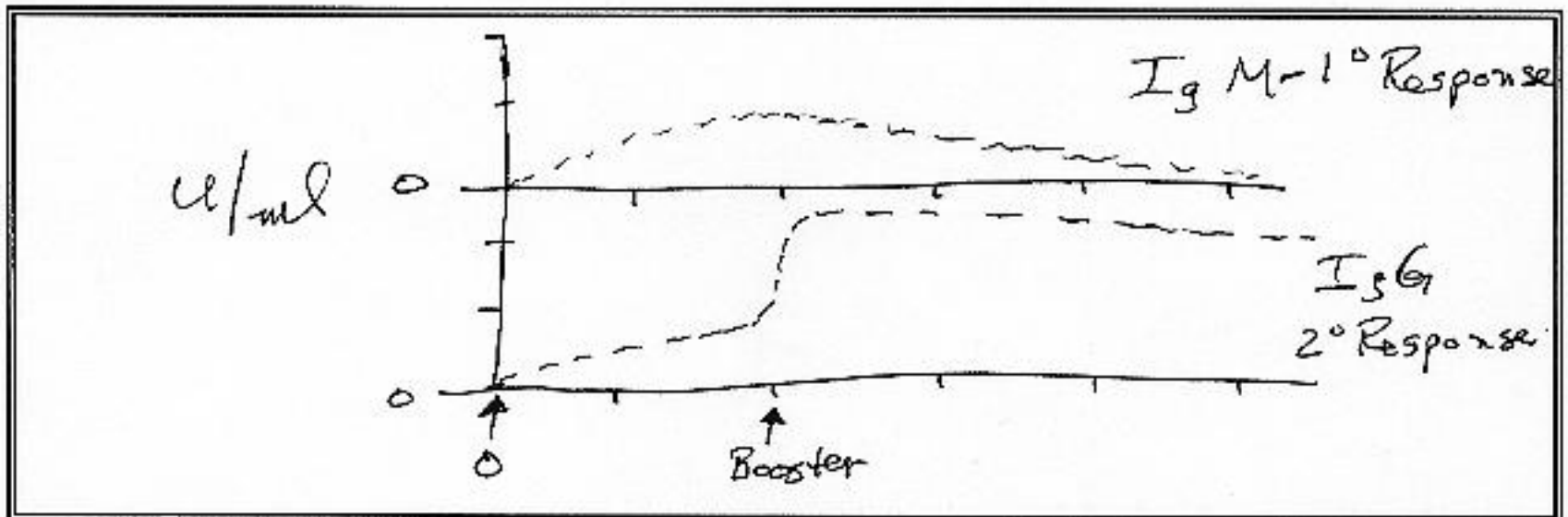
Specific Ig's: IgM

- Makes up approximately 5-10% of all Ab's
- Characteristics
- Is the first Ab to be made by young animals; “panic button” Ig; for primary response; responsible for ABO; kill gram negative bacteria (bind to LPS-O); monomeric on B cell surface (receptor); pentameric in plasma – J chain); primarily intravascular (*a priori* evidence of active infection); **IS** present in the neonate – is **ONLY Ab** to be made in utero by fetus

IgM Comment

- In primary atypical pneumonia (walking pneumonia), hemolytic anemia, IgM is produced
- The IgM produced is called “cold agglutinins”
- Called that because it agglutinates (clots) RBC between 0-4°C, but NOT at 37°C

IgM and IgG – Immunizations



Specific Ig's: IgA

- Makes up about 15% of all antibodies; same molecular size as IgG

Characteristics

- Nature's antiseptic ointment; **secretory Ig**; crosses cell barriers easily and appears in high concentrations in tears, saliva, nasal, bronchial or gut mucus, breast milk; repels microbial invasion across various mucus openings of the body; protects against enteric viruses (**breast fed babies**); decreases due to stress; dimeric (sIgA – IgA₂ with J chain – s = secretory); reaction of IgA at mucus membranes initiates inflammatory reaction for mobilization of other defenses; monomeric = IgA

Specific Ig's: IgE

- Makes up $< 0.1\%$ of all Ab's

Characteristics

- Prime Ig involved in allergies; true physiological role seems to be to induce anaphylaxis and appears during intestinal worm infestation; monomer on basophils and mast cells (receptor); mediates allergic reactions like hay fever; attracts eosinophils to sites of helminthic infections.

Specific Ig's: IgD

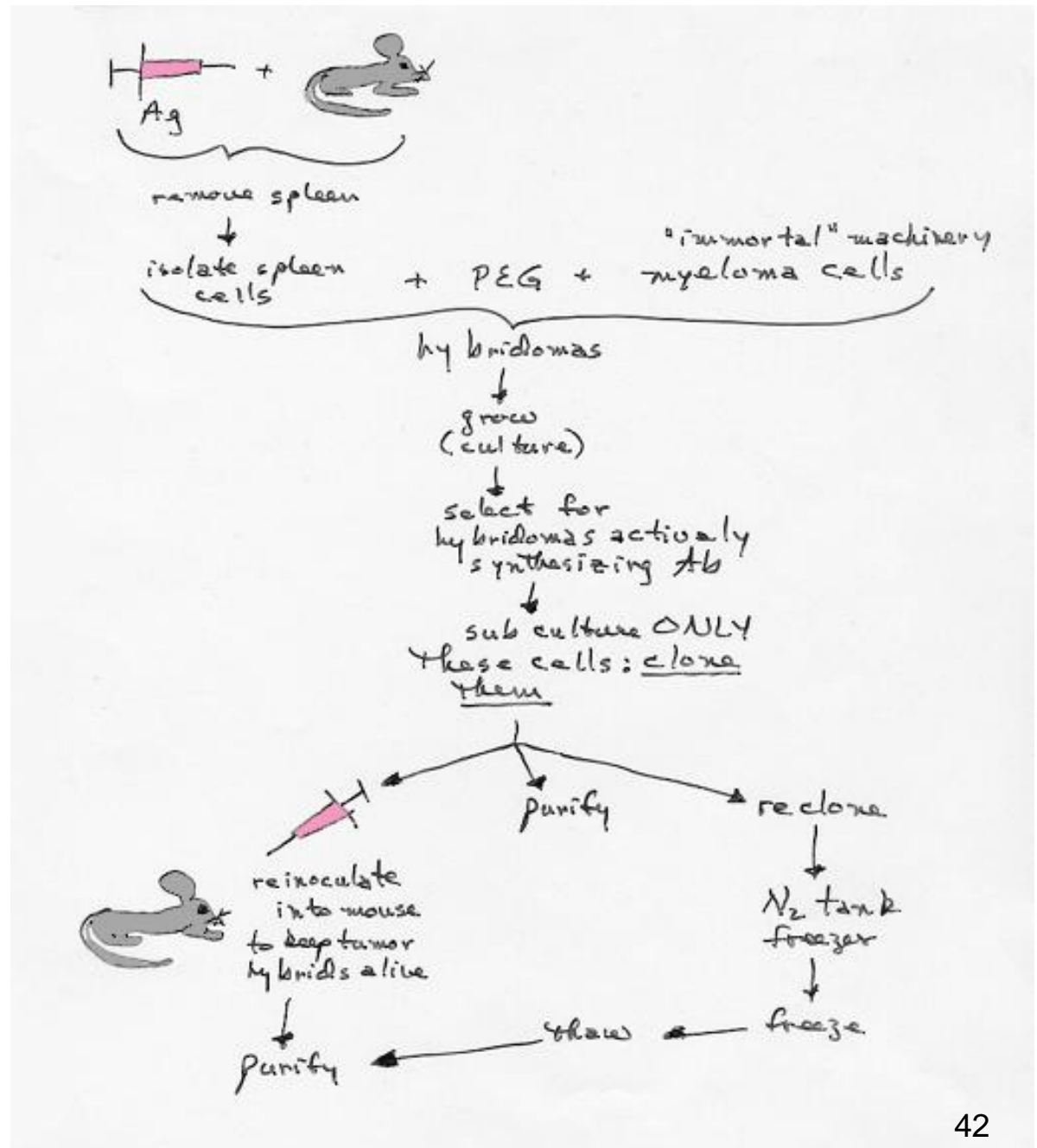
- Makes up less than 0.1% of all Ab's – present only in trace amounts.

Characteristics

- Similar to IgM; it is present in large amounts as a lymphocyte surface receptor; role not known for certain; but it is present in some kinds of leukemias; present on surface of B cells; monomeric; present in myelomas (a malignant tumor of the bone marrow)

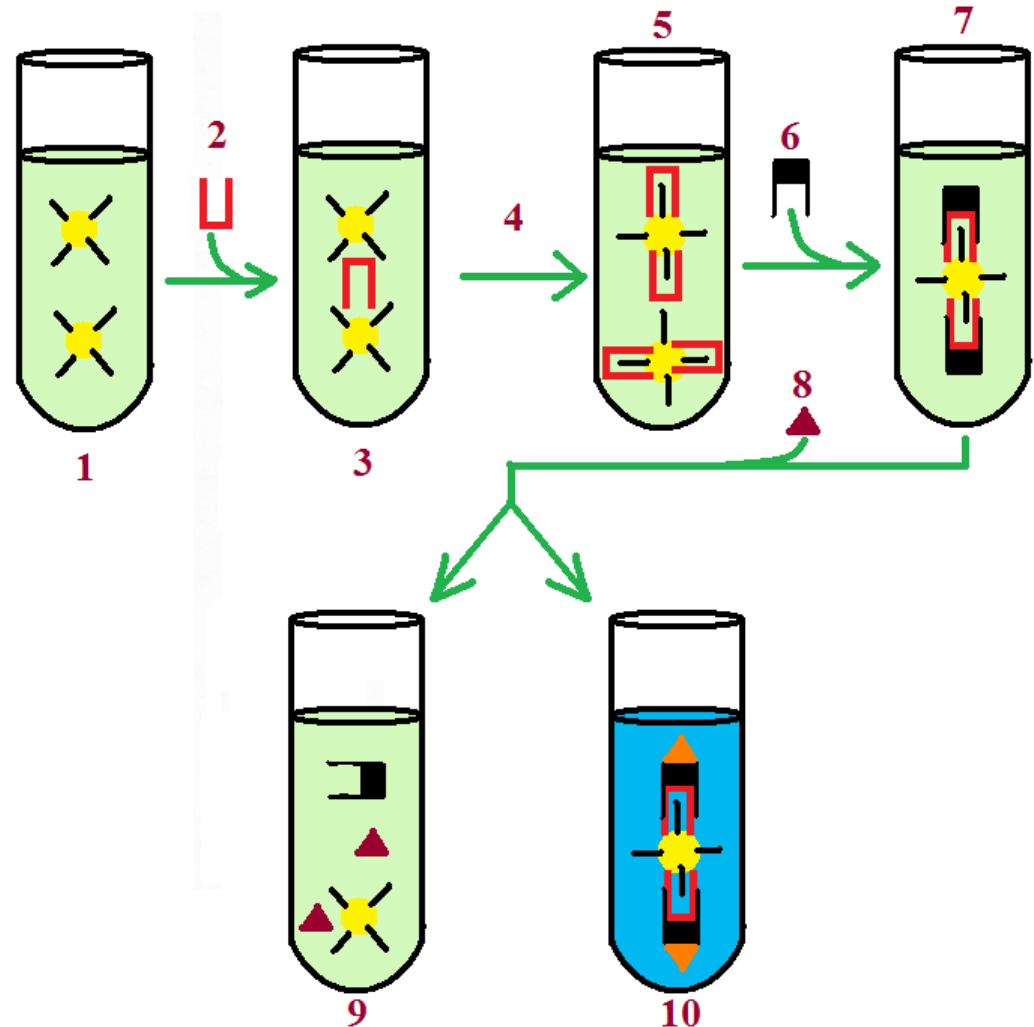
Monoclonal Antibody Production and Applications

- Many laboratory applications
- ELISA
- MAC-ELISA
- Titers
- RadioImmunoAssay (RIA)



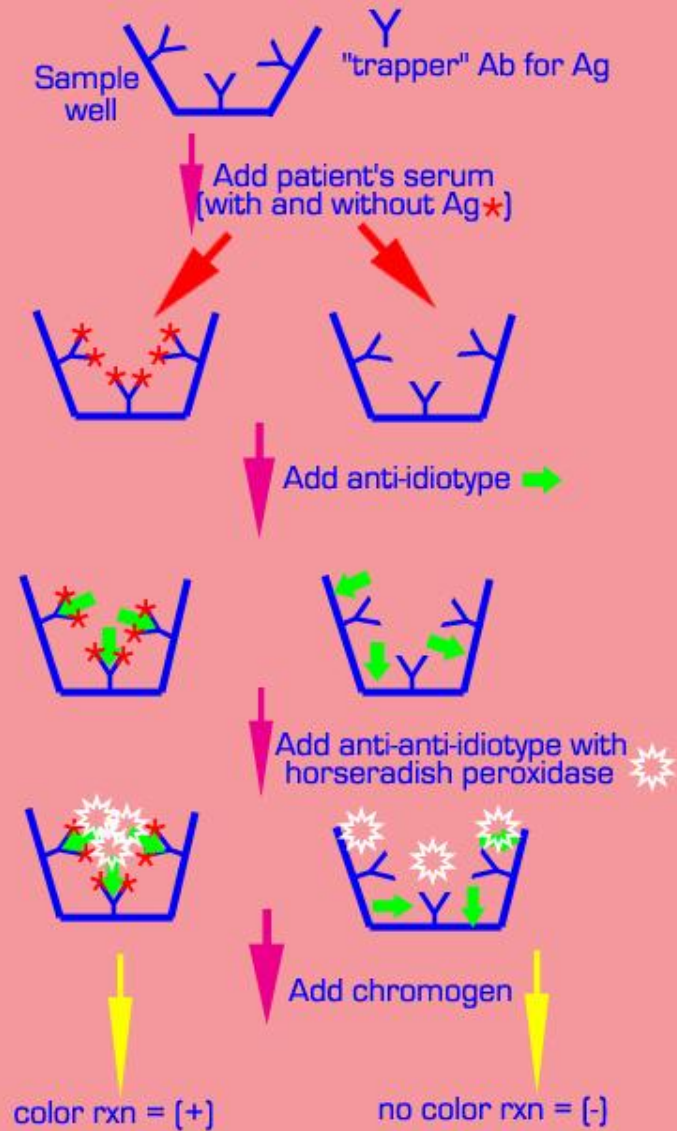
Immunological Applications -- ELISA

1. Buffer, Ag-bound beads in cuivet
2. Add pt's serum with[out] Ab
3. Mix
4. Incubate
5. Ab binds Ag on beads if present
6. Add enzyme-bound anti-idiotype
7. Incubate – Anti-idiotype binds pt's Ab (if present)
8. Add chromogen
9. Negative result – pt's Ab not present – chromogen isn't converted
10. Positive result – pt's Ab present – chromogen converted – color change to whole solution visualized



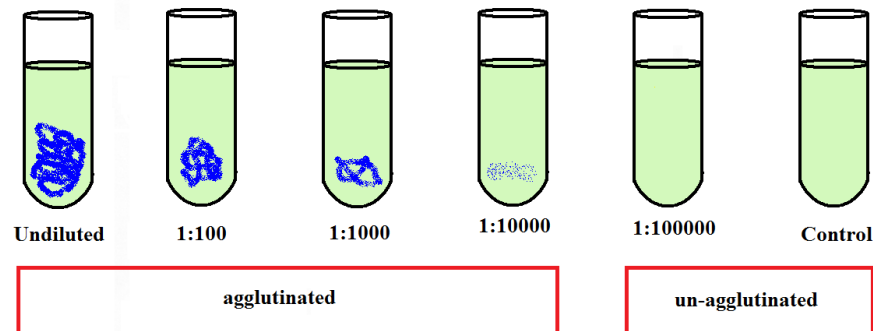
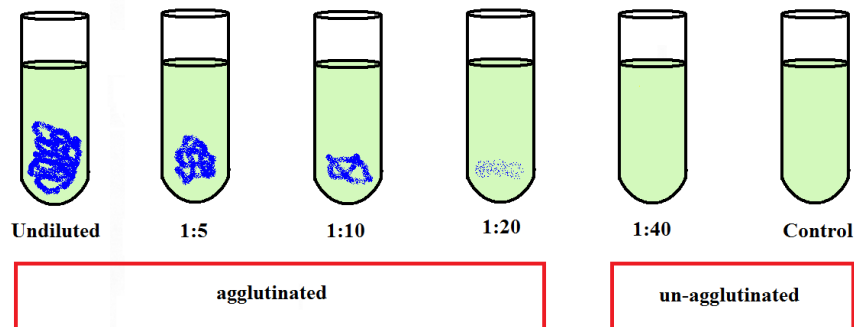
MAC-ELISA

IgM Antibody Capture - Enzyme Linked Immunosorbent Assay

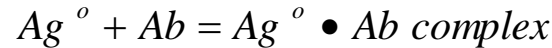


Titers

- Patient's Ab present in every tube except Control tube.
- Ag added (from bottle) in identical amounts to all tubes.
- TITER = 1:20 (last tube to agglutinate) – TOP Image
- TITER = 1:10000 (last tube to agglutinate) – Bottom Image
- ALWAYS run a control for comparison



RIA: THEORY and Application



\therefore



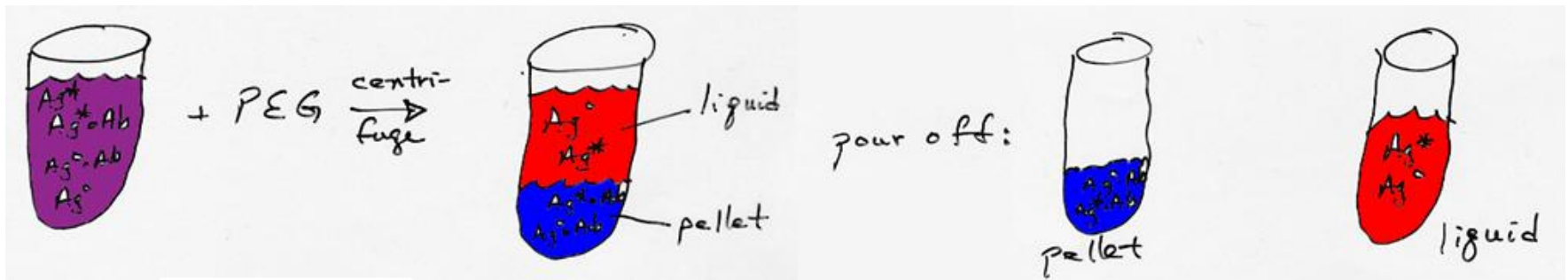
$Ag^* = \text{radioactive Ag}$

it follows, then,



if fixed amount of Ab added

- $Ag^o \bullet Ab$ and $Ag^* \bullet Ab$ complexes are proteins, HENCE, they may be precipitated with Rx like PEG, SO:



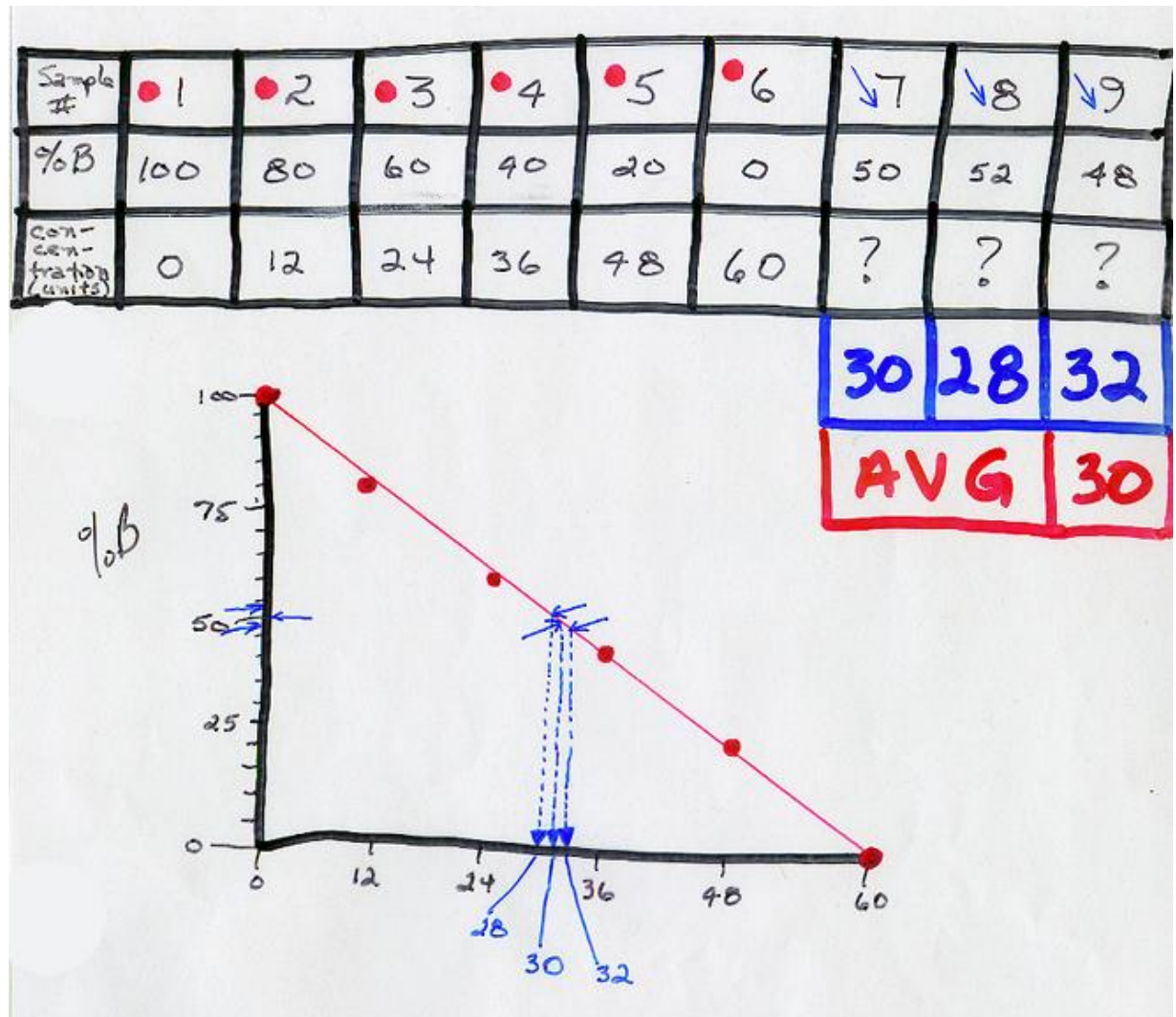
THEORY

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

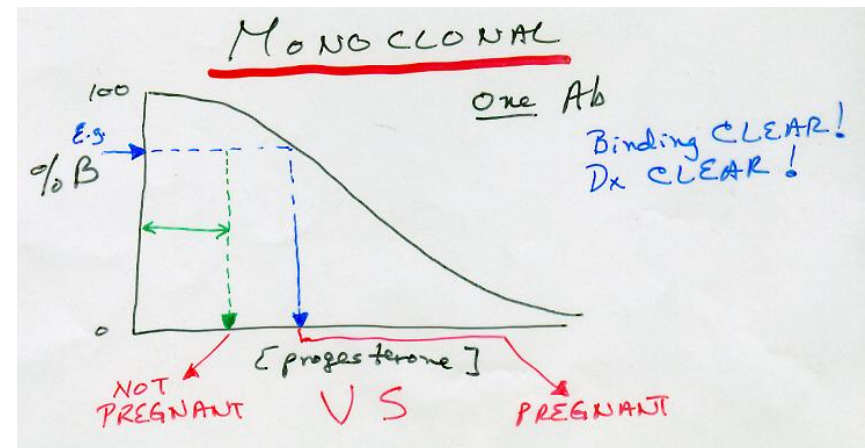
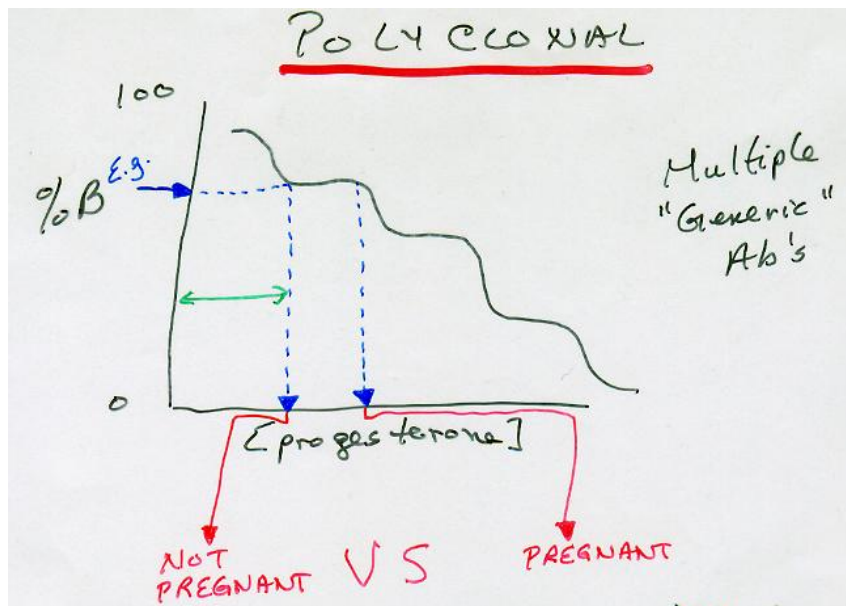
- Requirements
 - Fixed amount of Ag^* added to each tube
 - Fixed amount of Ab added to each tube
- The $< [Ag^0]$, the $> \% Ag^*$ Binding
- The $> [Ag^0]$, the $< \% Ag^*$ Binding

Standard Curve –

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



Importance of Monoclonal Antibodies & Clinical Significance -- RIA

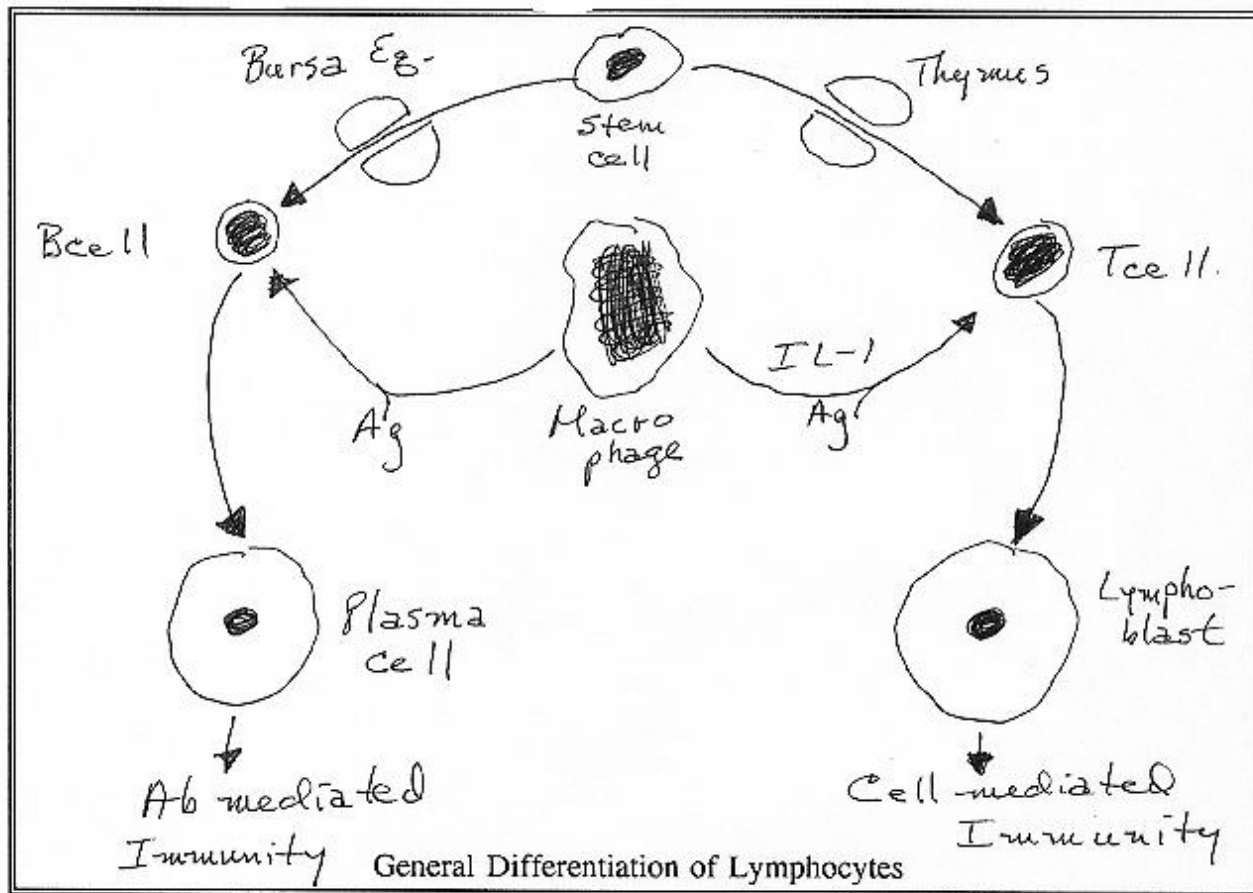


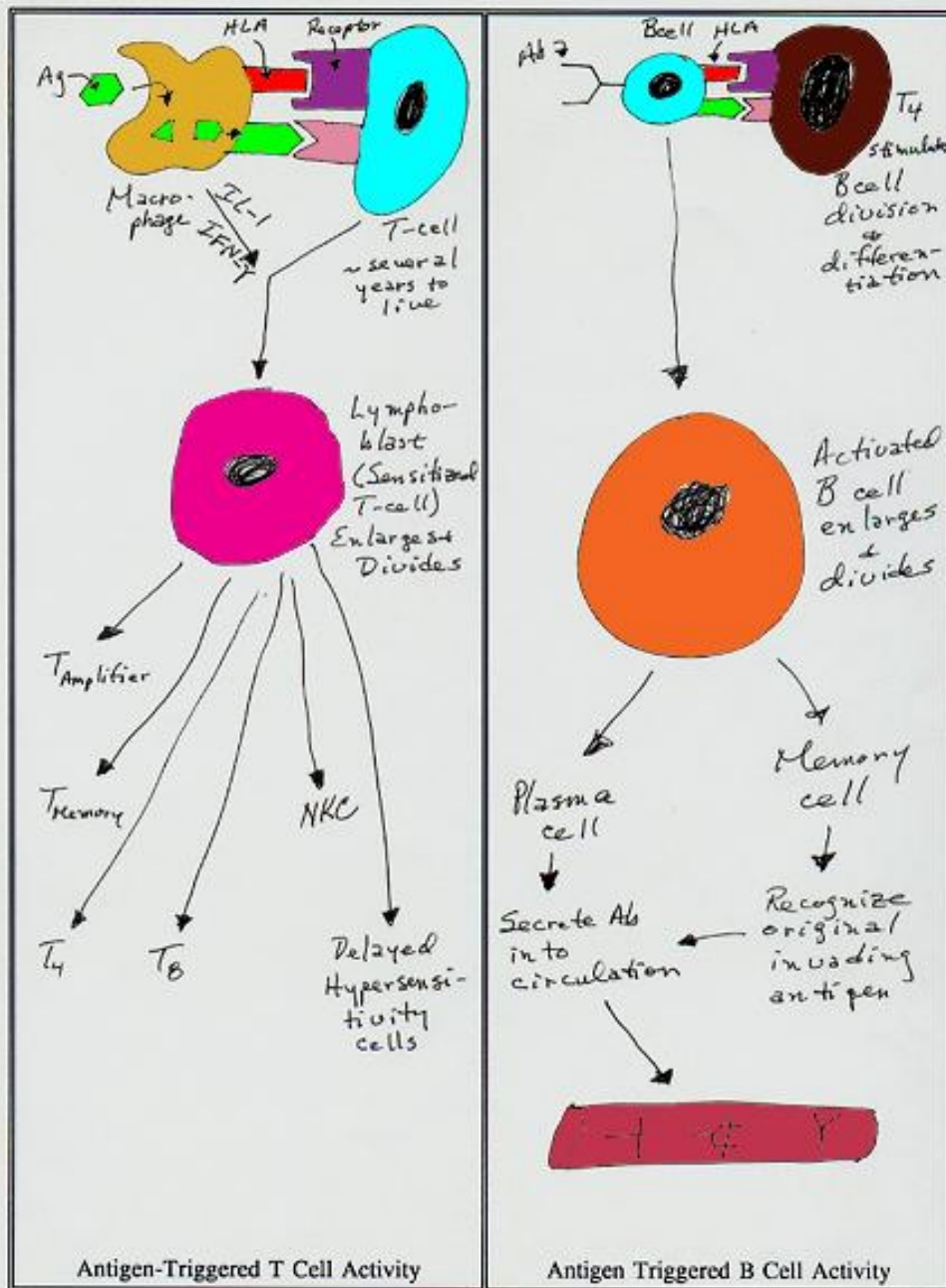
% binding is so ambiguous with polyclonal Ab's that neither you nor your patient know what is the dx – MALPRACTICE!!!

Cell Mediated Immunity

- In the old days, experts were aware that there were some phenomena that were clearly of an immune nature – just not of B cell derivation
- We now know that this was cell mediated immunity – mediated by T cells.
- The thymus elaborates a factor that induces immunocompetence in lymphocytes.
- The remainder of the stem cells destined to become lymphocytes – just not T cells – are processed, probably, in the bone marrow to become B cells.
- Although T and B cells occupy the same lymphoid tissues, they localize in separate areas of the tissues (compartmentalization)

B and T Cell Production





- **Amplifier T cells:** increase stimulation of T₄, T₈ and B cell descendants
- **Memory T cells:** Recognize original invading antigens
- **T₄ cells:** Induce Ab production by descendants of B cells and secrete IL-2 that stimulates proliferation of killer T cells
- **T₈ cells:** suppressor cells; suppress T₄ and some suppressive effects on B cells
- **NKC:** destroy antigen directly (lymphotoxins) or indirectly (other lymphokines [WBC stimulants])
- **Delayed Hypersensitivity cells:** secrete several lymphokines important in hypersensitivity (allergy)

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

Active/Natural Acquired	Characteristics	Active/Artificial Acquired
Antigens	Immunizing Agent	Antigens
Lifetime	Duration of Immunity	Months to years
Endogenous	Ab Source	Endogenous
Low	Effectiveness in newborn	Low
Hi	Effectiveness in Adult	Hi
Disease	Origin	Toxoid/vaccine

Different Kinds of Immunity -- 2

Passive/Natural Acquired	Characteristics	Passive/Artificial Acquired
Antibodies	Immunizing Agent	Antibodies
4-6 months	Duration of Immunity	To 6 weeks
Exogenous	Ab Source	Exogenous
Hi	Effectiveness in newborn	Hi
Low	Effectiveness in Adult	Moderate
Transplacental Ab Passage	Origin	Ab-containing serum

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

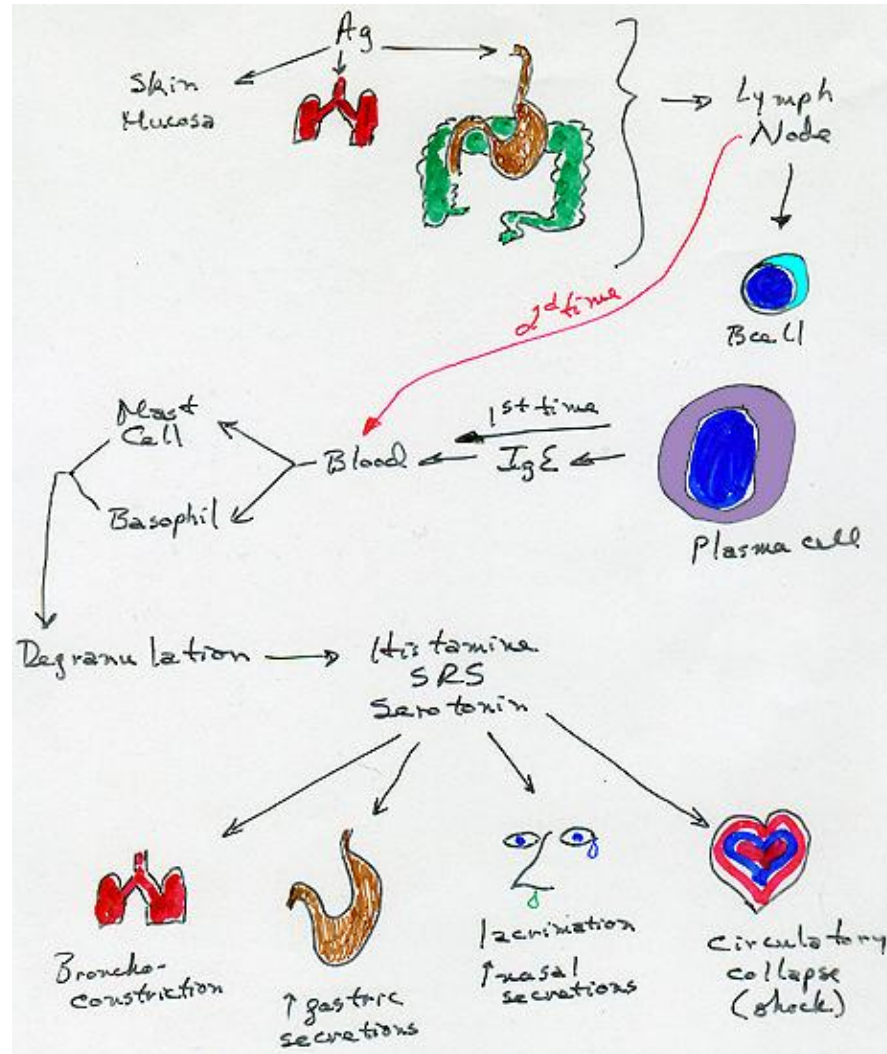
Type I – Immediate (Anaphylactic) Hypersensitivity

- 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

Tissues	Concentration ($\mu\text{g/g}$)
Lung	35
Nasal Membranes	15
Stomach	14
Spleen	3.4
Heart	1.6
Abdominal Skin	6.6
Facial Skin	30.4
Basophils	$1080 \mu\text{g}/10^9 \text{ cells}$
Platelets	$0.009 \mu\text{g}/10^9 \text{ platelets}$

Histamine Receptors

H ₁	H ₂	H ₃
IP ₃ /Ca ²⁺	cAMP	?
G _p protein?	G _s protein	??
2-methylhistamine	4-methylhistamine	R-α-methyl-histamine
Pyrilamine	Cimetidine	Thioperamide
Allergies	Stomach acid	Pre-synaptic inhibition of histamine release
Agonist; Antagonist		

Primary Histamine Actions

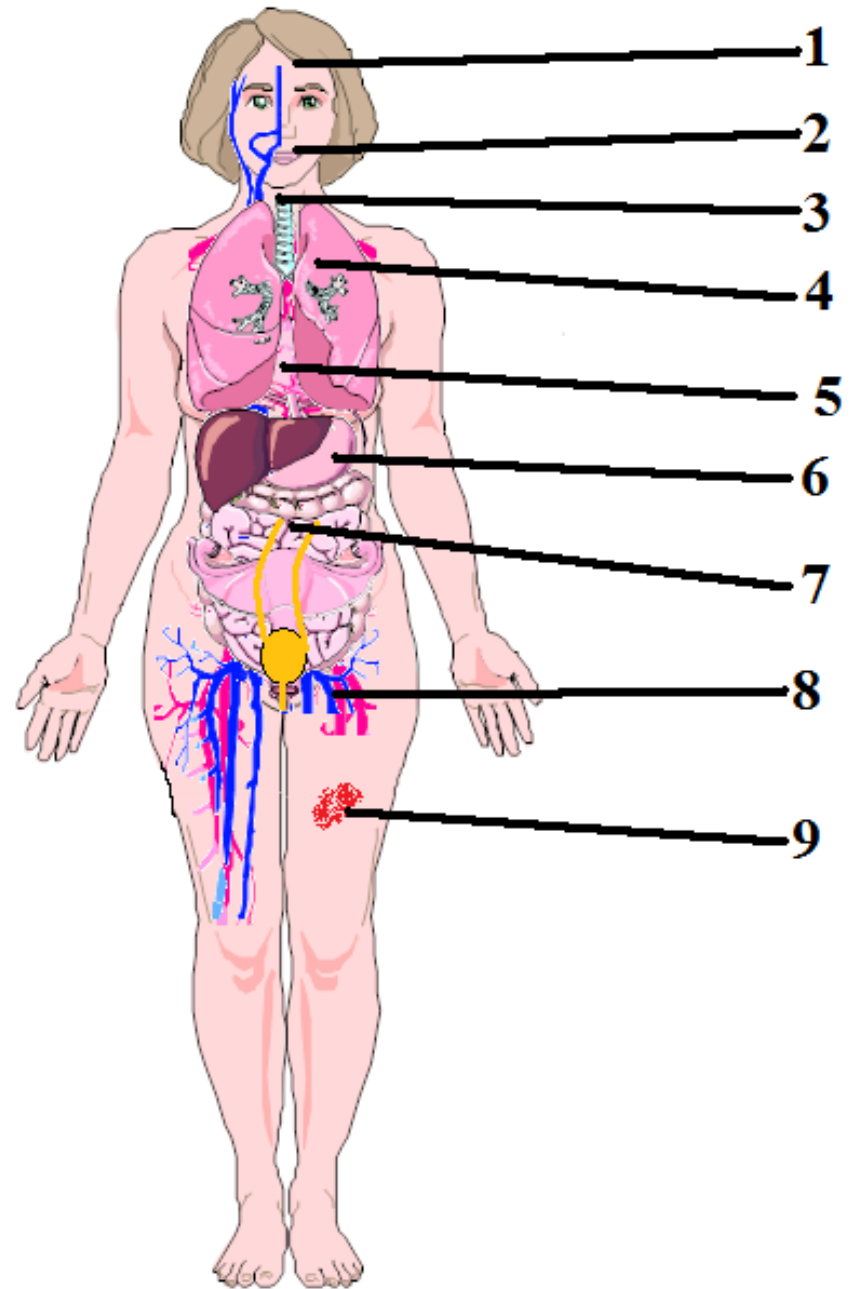
Cardiovascular System	HypOtension with tachycardia; facial erythema due to vasodilation of cutaneous vessels; throbbing headache due to dilatation of brain arterioles
Respiratory System	Bronchiolar smooth muscle contraction with increased secretions
Glandular Tissue	Increased catecholamine release from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

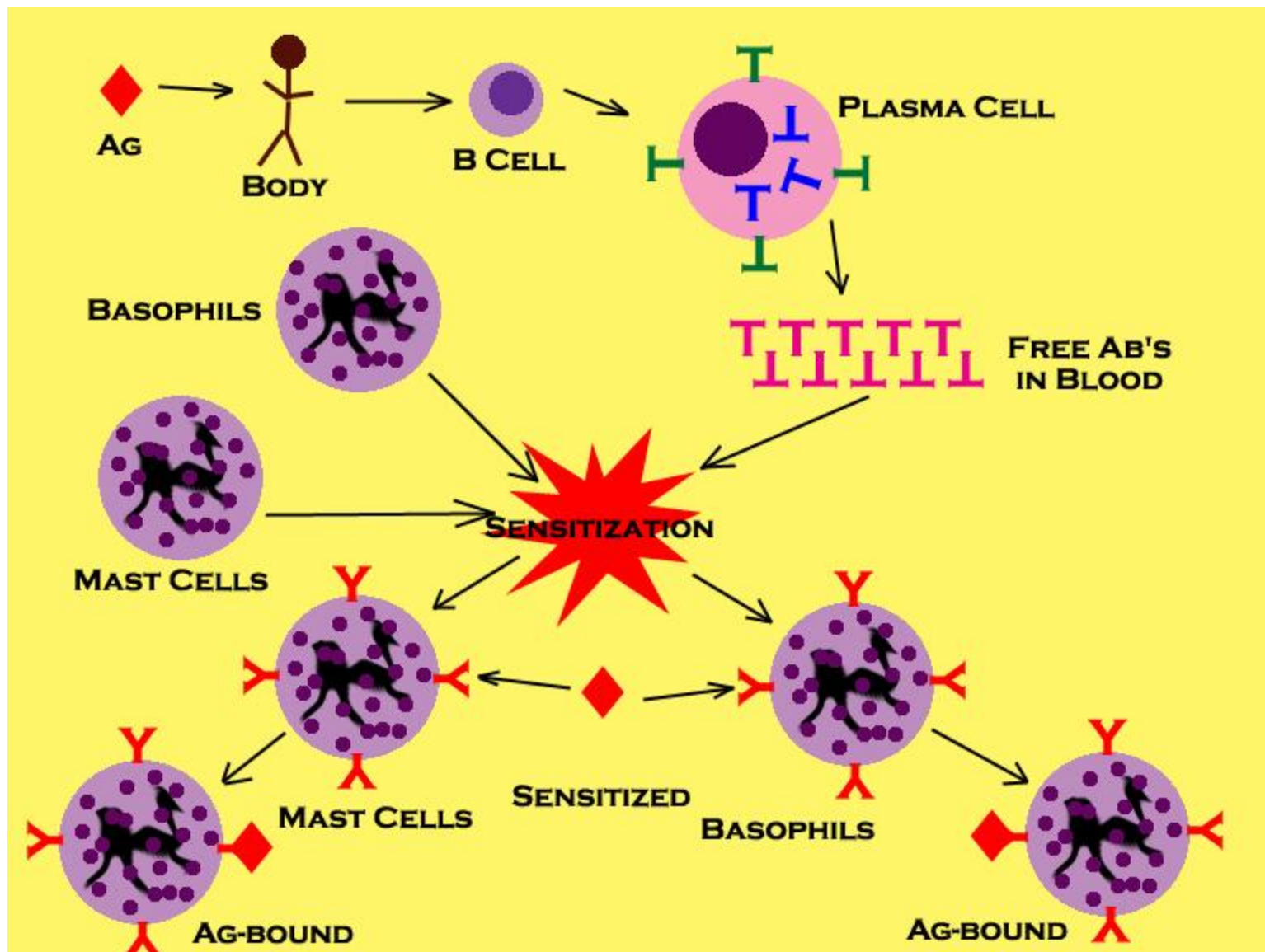
Histamine Receptor Locations

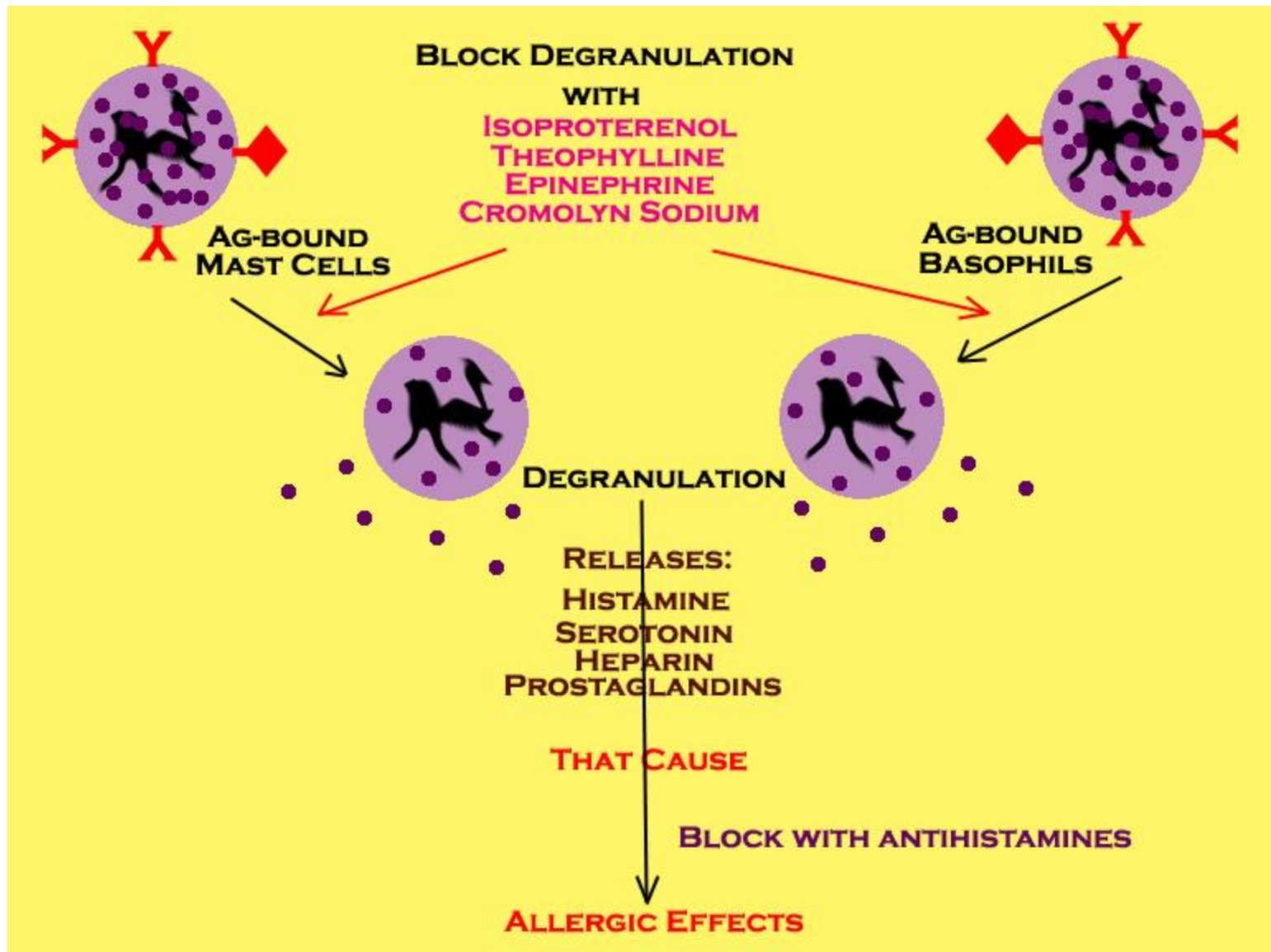
Organ	Receptor Type	Histamine Response
H ₁ dominates when both H ₁ and H ₂ receptors are present in tissue		
Uterus	H ₂ receptors	Relaxation
Stomach	H ₂ receptors	Increased HCl production
Bronchi	H ₁ receptors	Bronchoconstriction
	H ₂ receptors – small amount	Bronchoconstriction
CNS	H ₁ receptors	Sedation
	H ₁ and H ₂ receptors	Antiemetic effect
SNS	H ₂ receptors	Inhibition
Heart	H ₁ and H ₂ receptors	Increased Atrial/Ventricular contraction force
	H ₂ receptors	Increased heart rate
	H ₁ and H ₂ receptors	Increased coronary flow

I've Been Stung!

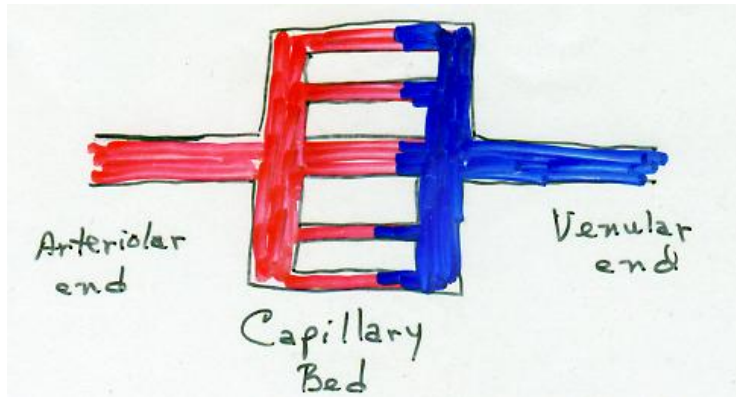
1. Dizziness, seizure, loss of consciousness, death 2° hypotension
2. Labial swelling
3. Laryngeal swelling
4. Bronchoconstriction: asthma or similar
5. Dilatation: hypotension leading to brain and heart malfunction (large vessel involvement)
6. N/V
7. Diarrhea, cramps
8. Dilation with increased permeability leading to shock (small vessel involvement)
9. Most common: rash, e.g., hives



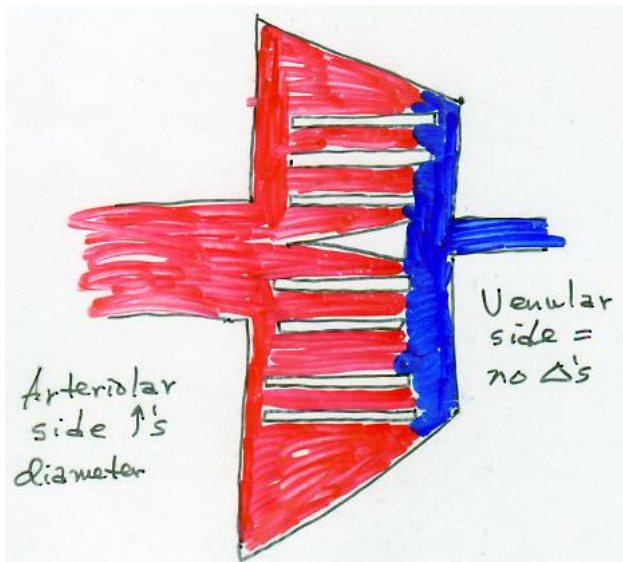




Allergic Response

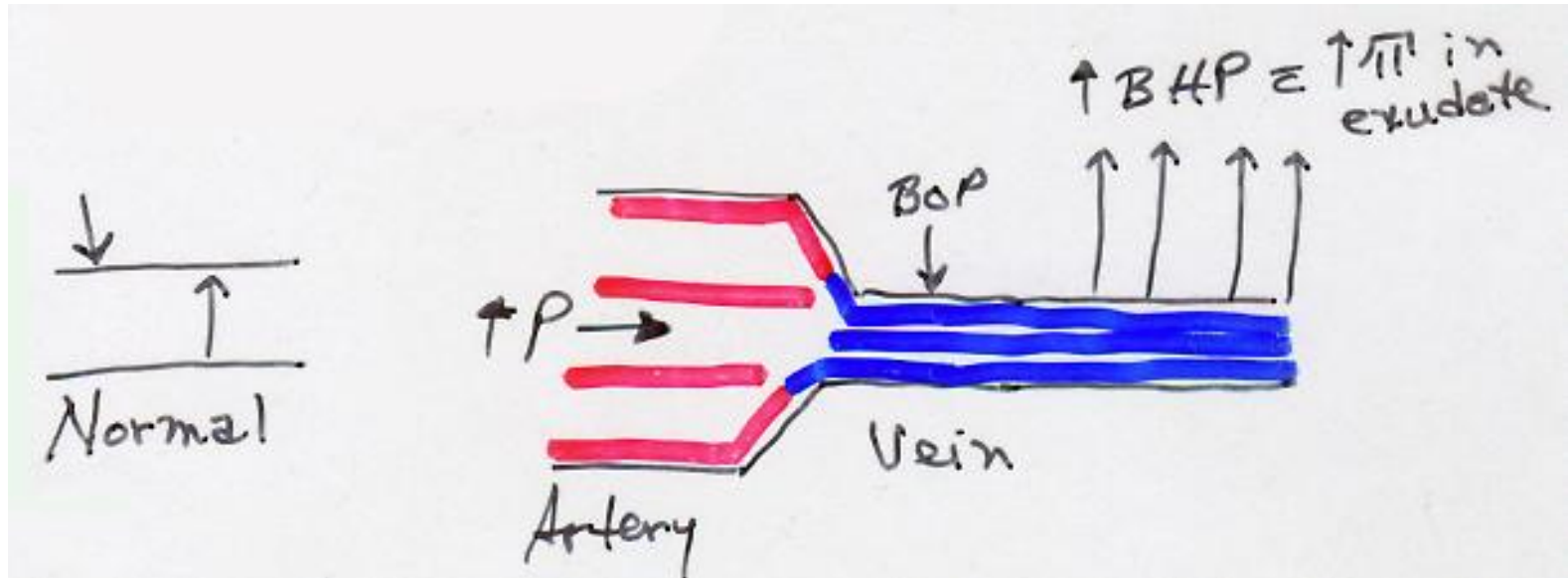


- Normal Vascular Bed



- Hyperemic Vascular Bed
- Rubor and Calor (superficial)

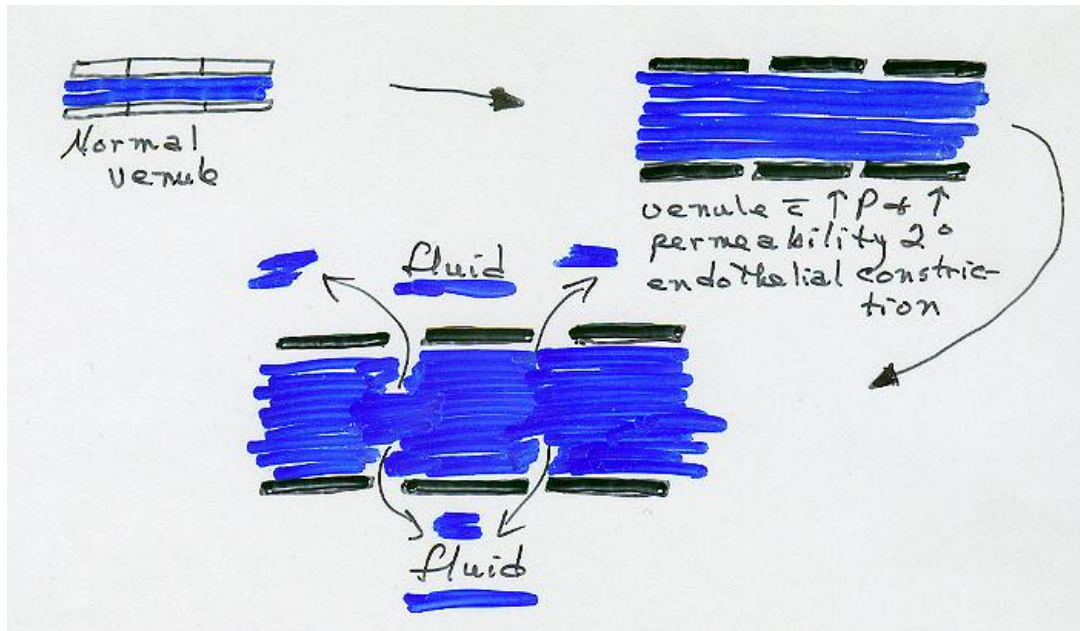
Allergic Response



TUMOR

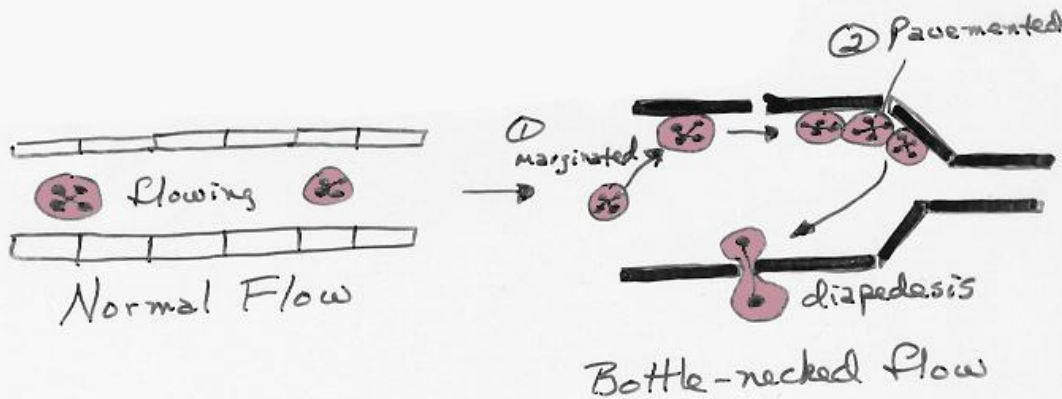
- Starling's Law of the Capillaries says that the inner and outer fluid pressures are \pm balanced between vessels and tissues
- More in Urinary lecture
- The increase in interstitial fluid leads to swelling due to:
 - Increase in BHP and EOP is $>$ BOP
 - Increase in venular permeability
- Called swelling

Allergic Response

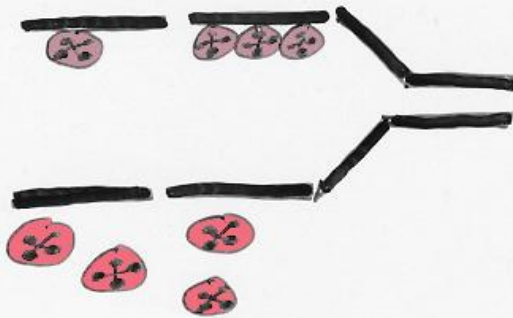


- With increased pressure, get increased flow
- If venule accepts this increased flow without dilating, SOMETHING has to give
- With the increased fluid in the tissues comes:
DOLOR
- Dolor is due to
 - Change in pH – effects nerve ends
 - Increased fluid – increases pressure on nerve endings
 - Increased 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



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
- 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** – mostly PMN's – next slide

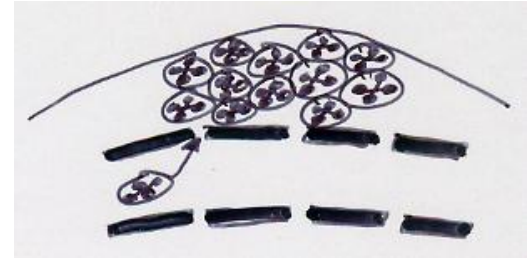
Cellular Exudate/Formation

1. Neutrophilic

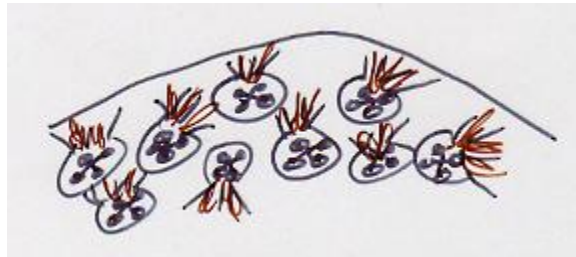
1. Primarily PMN's
2. So many PMN's that fluid and dissolved particles are very minor
3. Aka purulent
4. Generally from infection by bacteria
5. Prominent in areas of tissue necrosis



Diapedesis



PMN's emigrate during rubor and tumor



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



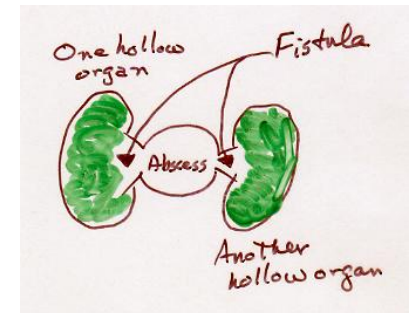
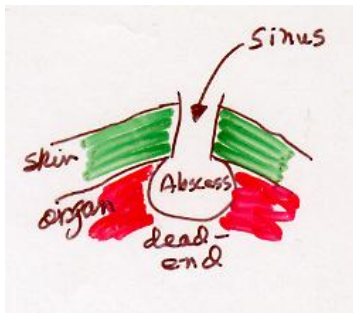
Enzymes digest and liquify tissue – known as supuration (pus)

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

Histamine Antagonism

Histamine Release	Blocked by cromolyn sodium at mast cell and basophil level
H ₁ Receptor Blockade	Competitive inhibitors such as Diphenhydramine HCl, Dimenhydrinate, Chlorpheniramine maleate, Promethazine HCL (Benadryl, Dramamine, Chlortrimetron and Phenergan)
	Great use in allergic rhinitis, ACUTE phase due to tolerance , some childhood asthma, sedation
H ₂ Receptor Blockade	Competitive inhibitors such as Cimetidine, Famotidine and Ranitidine HCl (Tagamet, Pepcid and Zantac)
	Great use in duodenal ulcers, benign gastric ulcer, ulcerogenic tumors of the pancreas, esophageal reflux

Type II –Cytotoxic Hypersensitivity

Examples:

- Occurs via Complement activation thru IgG or IgM
- Hemolysis – HDN (Rh; previously discussed); ABO transfusion reactions
- Hemolysis – PCN, phenacetin, quinidine bind to surface of RBC
- ITP– quinine or ASA plus platelets cause bleeding and/or bruising

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) $\downarrow T_8$ cells?? 2) $\uparrow\uparrow$ 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^o develop erythema, itching, eczema or necrosis of skin
- AVOID offending substance

Selected Autoimmune Disorders

Disease	Hypersensitivity	Tissue Site	Ag	Effect
Thrombocytopenia	Cytotoxic (II)	Platelets	ASA, PCN's; antihistamines	Hemorrhage, bruising
Myasthenia gravis	Cytotoxic (II)	Muscle fiber membrane	ACH receptor	Decreased muscle activity; eye muscle weakness
Graves' disease	Cytotoxic (II)	Thyroid	??	Elevated T ₄ ; elevated BMR
Hashimoto's thyroiditis	Cytotoxic (II)	Thyroid	??	Depressed T ₄ ; depressed BMR
Arthus reaction	Immune Complex (III)	Blood vessels; Ag entry site	From person's environment	Blood clots
Rheumatoid arthritis	Immune Complex (III)	Joints	Self – connective tissues?	Arthritis, rheumatoid lung

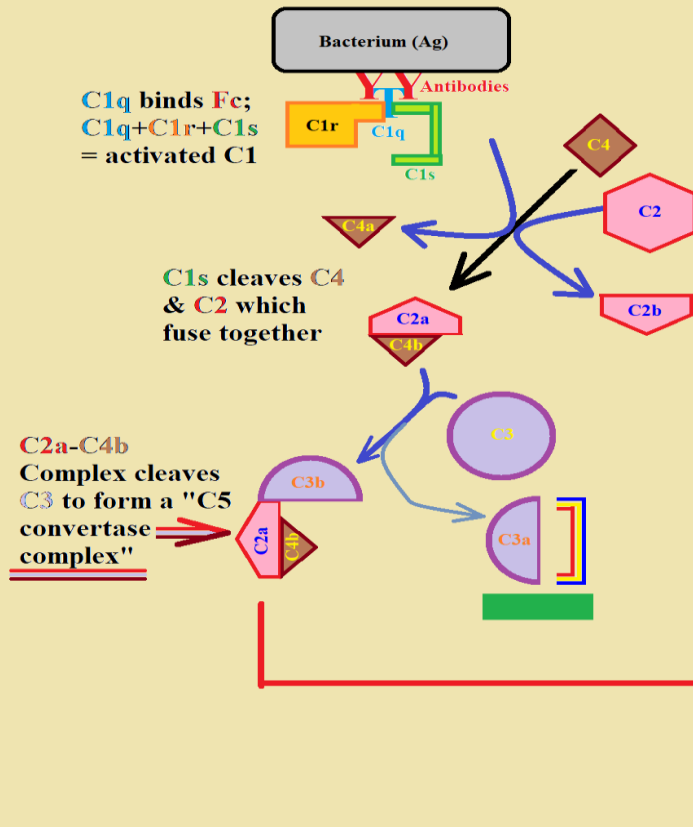
Complement Activation

3 “triggers”

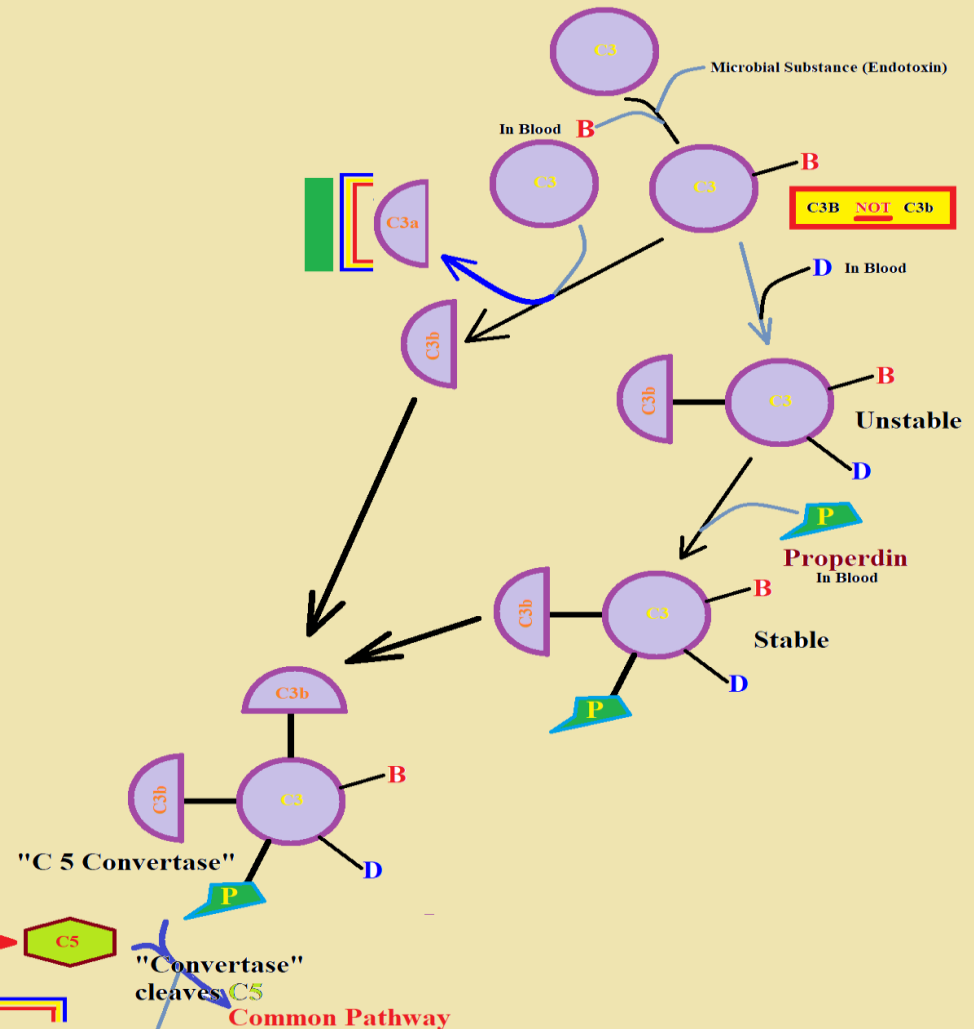
1. Opsonization: direct binding of C3 fragment to bacterium
2. IL-6 stimulation: IL-6 secreted from macrophages due to bacterial identification; IL-6 causes liver to synthesize/secrete mannose binding protein (MBP); MBP binds to bacterial capsule and conjointly activates complement cascade
3. Ab-activated: Ab's from B cells bind to bacteria; F_c 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

Complement System and Pathways

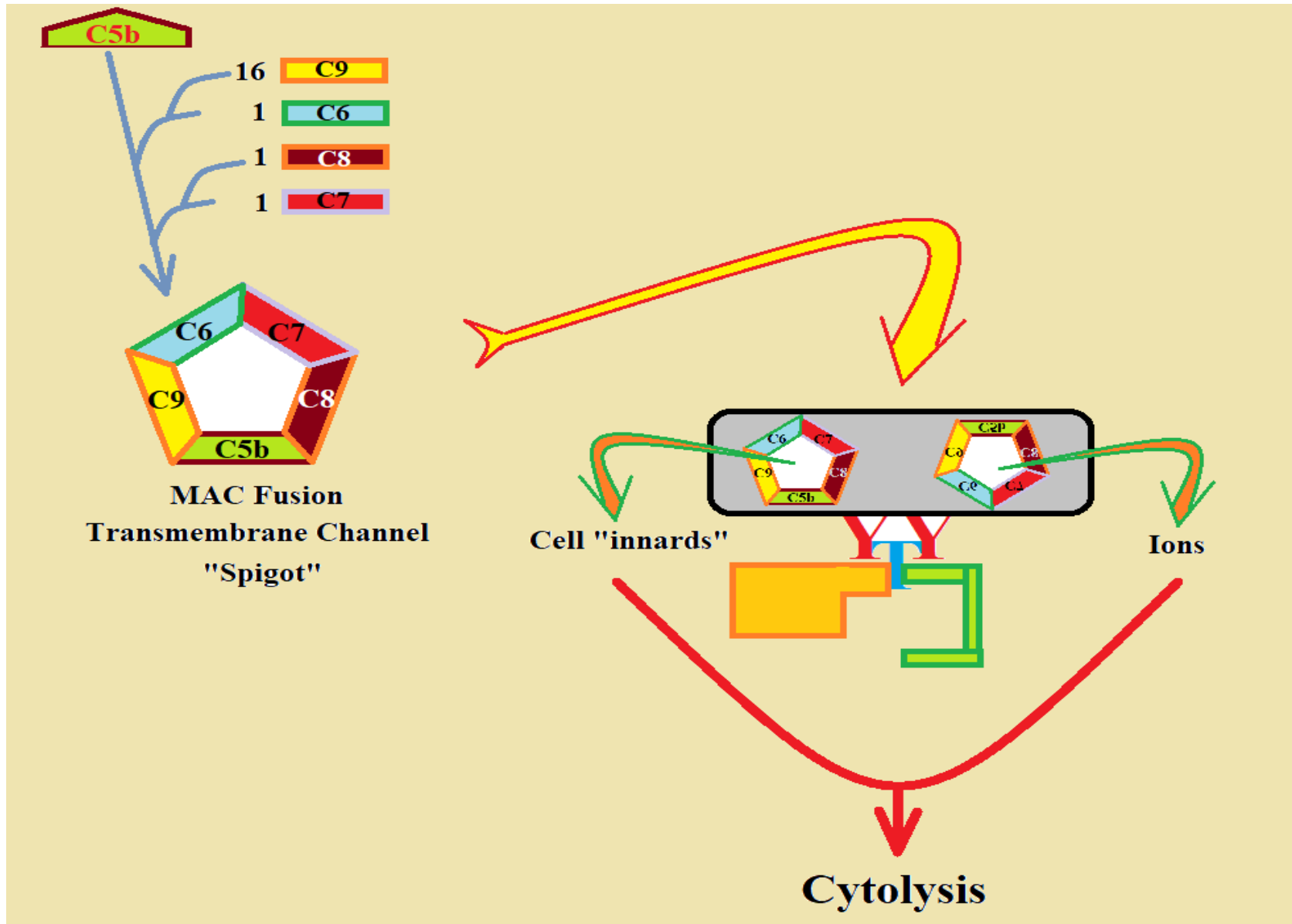
Classical Pathway Immune Triggered



Alternate Pathway



Complement: Common Pathway

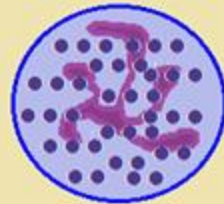


Anaphylotoxins/Inflammatoxins

Histamine, SRS-A,
ECF-A, Serotonin



Basophil degranulation



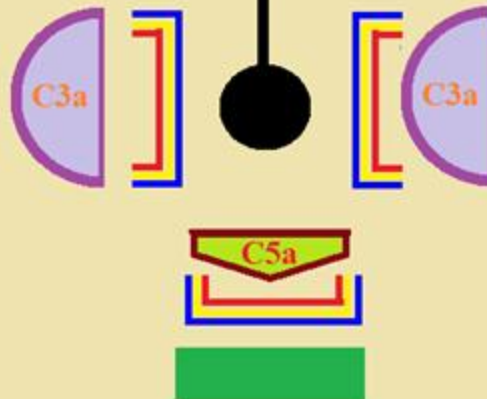
Positive chemotaxis



Increased vascular permeability;
Diapedesis to site of C5a fixation



Blood vessel with blood cells



Opsonization

