The Human Immune System

Part I
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
• Microorganisms are ubiquitous! They are in the air we breathe. They are on and in our bodies. They are on our clothes. They are in liquids and in food. They are on animals and in animals. They are in human waste and animal waste. They are on plants and on counter tops (these are called "fomites").

• As a general rule, wash your hands when you enter a patient's room, after touching anything in that room and lastly as you are exiting that room.

• Microorganisms may be spread in many ways! They may be spread in the air, in food and in water. They may be spread on food utensils, personal hygiene equipment and by direct contact. They may be spread by dressings, animals, dirty handkerchiefs, rusty nails and by trauma. Insects transmit many diseases. It is important to remember, though, that insects do NOT transmit HIV: they simply do not have the necessary machinery to be able to do that.
• The thymus, which overlies the heart, has long been misunderstood. Due to data obtained from soldiers in the Korean conflict upon autopsy, for many years it was believed that the thymus was large at birth, but by about 18-21 years of age, it "mysteriously" dissipated. We now know that the thymus slowly becomes smaller throughout our life span. It is the thymus that confers immunocompetence upon small lymphocytes destined to become T-cells (the "T" is for "thymus").

• The tonsils, both pharyngeal and palatine, and the lingual tonsil form a ring at the opening of the pharynx called Waldeyer's ring. We now know that Waldeyer's ring is protective against oropharyngeal cancer and that the tonsils need not be "prophylactically" removed unless they are inflamed to the point of threatening life.
• There are numerous lymph nodes throughout the body. They act as a filter and as a storage site for T cells and B cells (discussed later). Tonsils are the best known lymph nodes as they are the nodes most commonly enlarged due to throat infections by organisms such as *Streptococcus pneumoniae* and *Streptococcus viridans*.

• All nodes have the potential to enlarge based upon infectious processes presenting in close proximity to them. All nodes are connected to the lymphatic system via lymphatic vessels. Lymphatic vessels provide "pipelines" for the flow of immune support throughout the body. Additionally, the lymphatic vessels provide for the transport of long chain fatty acids during the absorption of fat from the diet during digestion.
To transport lipid, the lymph vessels interface between Peyer's patches (gut-associated lymphoid tissue) and the Cisterna chyli. The latter may be thought of as a "fat cesspool". The fatty chyle transported from the Cisterna chyli is "dumped" into the thoracic duct, transported to the right side of the heart, then dispersed as the body requires. The thoracic duct bifurcates, roughly at the level of the inferior heart border, into the left and right thoracic (lymphatic) ducts, which feed into the left and right subclavian veins.
• The chyle transported via the right (superior) thoracic duct is transported through a unique vessel: the superior thoracic duct drains the whole of the supero-lateral right side of the body (above the red and green line in Figure, right) into the heart. This is unique in that if there is some sort of damage to this duct, lymphatic fluid builds up in the tissues of the right arm and shoulder. This is very common in women who have undergone radical mastectomy on the right side. Because of this, these women are encouraged to immediately begin squeezing a rubber ball with their right hand or brush their hair with their right arm in order to "pump", "milk" or by gravity to remove this fluid from their extremity.
• The spleen is a useful organ, as well, in the immune system. In addition to storing the equivalent of an extra unit of blood to be used in the case of "fight or flight", the spleen also stores T cells and B cells. Although it stores both cells, it appears that this organ stores the two types of cells in two separate compartments, i.e., they do not come into contact with each other until they are dumped into common circulation as necessary.

• The appendix is now known to be useful as lymphatic tissue. It seems that if the appendix is removed "just 'cause we're there" the patient has a statistically higher chance of developing ileocecal cancer; those who have had their appendix removed due to inflammation/disease don't seem to have this same intriguing relationship.

• Lastly, the bone marrow. Remember that all blood cells develop from stem cells (hemocytoblasts) in the bone marrow. That includes all cells involved with the immune system, as well, e.g., lymphocytes, basophils, mast cells and eosinophils, to name a few.
Innate Defense Mechanisms

Nose: Filtering
Pharynx: Commensal organisms
Breast: Milk & IgA
Skin: 8x obstacle for commensal organisms
Colon: Commensal organisms
Prostate: Secretions

Eyes: Lysosome, saliva: Lysosome, Permease, Peroxidase
Lungs: Trachea & Mucus + Cilia
Stomach: Acid, Duodenum: Alkaline
Bladder: Flushing
Vagina: pH & 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. menengitidis -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.
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
3. Germicidal Activity

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
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
Outer Defenses -- 6

3. Germicidal activity
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
Around the end of 6 months gradually introduce the child to family meals. However, continue to breastfeed until the second birthday.
Outer Defenses -- 7

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 -- 8

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. menigitidis, 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 -- Example

Bactericidal Power of Blood in Humans
Inner Defenses

1. 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
Inner Defenses

2. 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

3. 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. Sickle 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)
One example of the effects of antibody-mediated immune responses is illustrated in the Figure, below. In a nutshell, a drug, like quinine or ASA is taken by the patient. Anti-drug antibodies are synthesized in response to its presence. The drug binds to platelets. The antibodies bind to the platelet-bound drug and activate complement that causes "thrombocytolysis", drug-induced thrombocytopenia. One effect of this response is easy bruisability.
• Other sorts of antibody mediated autoimmune disorders (where the body's immune system turns on itself) include the following disease states summarized, below. Note: more specifics on the exact types of hypersensitivities will be explained later in this section.
<table>
<thead>
<tr>
<th>Disease State</th>
<th>Hypersensitivity</th>
<th>Tissue Site</th>
<th>Antigen</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>Cytotoxic</td>
<td>Platelets</td>
<td>ASA, PCN's, anti-histamines</td>
<td>Hemorrhage; bruising</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Cytotoxic</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</td>
<td>Thyroid tissue</td>
<td>?</td>
<td>Elevated thyroxine; elevated BMR</td>
</tr>
<tr>
<td>Hashimoto's Thyroiditis</td>
<td>Cytotoxic</td>
<td>Thyroid tissue</td>
<td>?</td>
<td>Depressed thyroxine; depressed BMR</td>
</tr>
<tr>
<td>Arthus Reaction</td>
<td>Immune Complex</td>
<td>Blood vessels; antigen entry site</td>
<td>From the person's environment</td>
<td>Blood clots</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>Immune Complex</td>
<td>Joints</td>
<td>not certain; connective tissue</td>
<td>Arthritis</td>
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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

1. **Immunogenicity**: the ability to stimulate the formation of specific Ab’s.

2. **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*
Chemical Characteristics of Antigens

- Ag’s are generally foreign to the body
- Are generally large molecules of MW > 10,000

<table>
<thead>
<tr>
<th></th>
<th>Urea MW = 60</th>
<th>Glucose MW = 180</th>
<th>GH MW = 20,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structures</td>
<td><img src="image" alt="Urea Structure" /></td>
<td><img src="image" alt="Glucose Structure" /></td>
<td><img src="image" alt="GH Structure" /></td>
</tr>
</tbody>
</table>
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 $<< 10,000$
- 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

• Antigenic makeup of humans is very complex
• RBC antigens for blood groups and types have been extensively studied because of transfusions and rhesus disease
• 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
Grafts

- Allografts are also called “homografts”
- A co-isograft is a graft between inbred strains varying genetically by only a few genes – 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

- 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
Ab’s are Proteins

• 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
Ab’s are Proteins

| Fab | Fc | 2' Papain | \[ c = \text{constant} \]
|-----|----|-----------|----------------|
| L = light chain | H = heavy chain | \[ V = \text{variable} \] | T → Y

- s-s- = disulfide bonds
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

• 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
Ag-Ab Reaction in General

Diagram:

1. Ag
2. B cell differentiation
3. Plasma cell
4. Specific and attach to only 1 Ag
5. Harmless Ag
6. Ag-Ab Response

Ag-Ab Reaction
Specific Antibody Mechanisms of Action

Ag → Ag Ab complex → Ab

Inactivation mechanism → Fixation and Activation of Complement

Neutralization / Opsonization → Agglutination → Precipitation → All of which Magnifies Which Leads to

Phagocytosis → Inflammation → Cell Lysis

Ab C3b → Neutrophil → C5a → C5a

MAPPING → Degranulation → Basophil
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 helminth infections; anti-Rh; increases phagocytosis; fixes to skin

• There are 4 sub-types
IgG sub-types

• IgG$_1$ – seeks out protein Ag’s (such as you would find on *Yersinia pestis* that causes bubonic plague)

• IgG$_2$ – specific for polysaccharide Ag’s (e.g., pneumococcal; This is of particular significance for children who are born with Trisomy 21: many of these children do not make IgG$_2$ and are susceptible to *S. pneumoniae* infections which can be fatal unless dealt with appropriately.)

• IgG$_3$ – seeks out protein Ag’s (such as you would find on *Yersinia pestis* that causes bubonic plague)

• IgG$_4$ – blocks IgE binding to inhibit anaphylaxis
IgG₂ Comment

• 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
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
Brief Vaccination Note

• Immunization schedules go out of date about as often as a baby needs its diaper changed. Refer to your pediatrician for more up-to-date information on these immunizations.
• Td vaccinations should be watched closely.
• The MMR vaccine is now required to be given at least twice (and maybe even THREE times, now!) instead of only once as originally promised (much as Varivax vaccine, as well).
• School districts notify parents "religiously" about vaccination requirements. These schedules change more frequently than other protocols.
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 = IgA₁
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 helminthie 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
• How is it that 5 classes of antibodies regulate more than 10 billion different antigens without destroying the human body?
• Part of the answer came from Macfarlane Burnett in the 1950's when he proposed his Clonal Selection Model.
• This model suggests how the reticulo-endothelial system recognizes "self" from "non-self".
• In a nutshell, he proposed that germ line cells (endoderm, mesoderm and exo/ectoderm; you learned about these layers in the trilaminar disc in fetal development in A&P II) differentiate during embryological development into various embryological cells.
• The DNA of the antibody genes undergoes rearrangement.
• Those cells with the genes that recognize "self" die.
• Those cells with the genes to recognize "non-self" survive and begin our immune system.
• The bone marrow has the stem cells that will differentiate into B cells, which will, eventually, provide us with the machinations for humoral immunity through specific antibodies.
Heterophile Antibodies

- An antigen interacts with a B cell that differentiates into a plasma cell.
- This plasma cell releases many different (hetero) antibodies that are non-specific.
- Some of these non-specific antibodies are reactive to/with RBC's of some animals, e.g., horse.
- These heterophile antibodies may be used for the diagnosis of Epstein-Barr virus-caused mono (heterophile positive) vs. CMV-caused mono (heterophile negative).
Monosport Test is Based on Heterophile Antibody Production

- Patient's serum is mixed/incubated with guinea pig extract to which horse RBC's are added and mixed.
- Agglutination is a positive reaction and is positive for Epstein-Barr virus.
- Non-agglutination is a negative reaction and is indicative of the absence of Epstein-Barr virus.
- CMV still needs to be ruled out.
• This still doesn't answer the whole question.
• Some more of the answer occurs during the period of embryological development and growth: T cells receive information from the thymus in such a manner that if the T cell recognizes a "self" antigen, it is killed and the only T cells that survive are those T cells that have been "taught" to recognize foreign antigens.
• The foreign antigen recognition units in the case of the T cells, are of ANOTHER group of proteins in the immune system called the Major Histocompatibility Complex (MHC) or Human Leukocyte Antigens (HLA) – all of which follow in discussion after this section.
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

General Differentiation of Lymphocytes
- **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).
• If the thymus is removed from a newborn mouse, the animal completely or partially loses the ability to develop delayed hypersensitivity following appropriate sensitization, to reject skin grafts from foreign strains of mice and, in some cases, from rats, as well, and to produce antibodies against certain antigens, although Ig levels are usually not effected. There is great depletion in the number of small lymphocytes in the blood and the animal develops a fatal wasting disease 1-4 months later. These effects are greatly diminished if thymectomy is delayed for a few days and in adult mice the effects of thymectomy are negligible. Normal immunological functions are restored to neonatally thymectomized animals by transplanting normal thymus tissue or by injecting cells from the spleen or lymph nodes of normal mice. Thymus cells themselves are usually ineffective.
• Cell-mediated mechanisms are the basis of delayed hypersensitivity, contact dermatitis and the rejection of grafts. They are important in overcoming infections caused by viruses, bacteria that have a predilection for intracellular growth, fungi and protozoa. Many autoimmune diseases such as thyroiditis and uveitis (inflammation of the iris, ciliary body and choroid or the entire uvea [the second or vascular coat of the eye lying immediately beneath the sclera -- the pigmented layer]) depend in part on cell-mediated mechanisms.

• Cell mediated immunity is probably the mechanism by which the body rids itself of mutant cells (potential tumors) arising in the course of normal cell division. If such a mechanism for policing the body for malignant cells did not exist, the incidence of cancer would probably be vastly greater. The importance of the thymus is also illustrated by "nude mice", a breed of hairless mice which do not have a thymus or T-lymphocytes. The animals have defective cell-mediated immunity. They will readily accept grafts of human cancers and are used to test anti-cancer drugs.
Major Histocompatibility Complex (MHC or HLA) and T Cell Receptors
• To get through this section, it is important to grasp some new terms and definitions.

• Alloantigens are antigens that are present only on the cells of some people.

• HLA stands for human leukocyte antigen and determines the rate of success of organ transplants, e.g., host vs. graft disease.

• HLA-A1 is the number 1 allele at the HLA-A gene on 6p.
“Genetic Map” of MHC’s on 6p

• The centromere is on the left and the telomeric end is to the right. Note that the class III MHC's are "nestled" between the class II and Class I MHC's.
A haplotype is one of the two chromosomes containing the HLA loci, therefore, each child will inherit one HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DO, HLA-DR and HLA-DN from each parent, e.g., Punnett square, below:

<table>
<thead>
<tr>
<th>Female haplotypes ↓</th>
<th>Male haplotypes →</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A3, B7, Cw2</td>
</tr>
<tr>
<td>A32, B51, Cw2</td>
<td>A3, B7, Cw2</td>
</tr>
<tr>
<td></td>
<td>A32, B51, Cw2</td>
</tr>
<tr>
<td>A1, B27, Cw11</td>
<td>A3, B7, Cw2</td>
</tr>
<tr>
<td></td>
<td>A1, B27, Cw11</td>
</tr>
</tbody>
</table>
• Each of the gametes contains 1 haplotype; each zygote contains 2 haplotypes.
  – 2 siblings have a 25% chance of having the identical HLA;
  – 2 siblings have a 50% chance of having 1 identical HAL haplotype;
  – 2 siblings have a 25% chance of having NO identical haplotype.
• HLA-typing is the next step after blood grouping evidence if blood group data is incompatible.
• This works well since there is so little space (introns) between loci in the HLA region that there is virtually no crossing over during meiosis.
HLA typing is also used to predict BEST tissue matches
Example

- Based upon the above (p.69) genotypes which child would be the best kidney donor to child #3?
### Solution

<table>
<thead>
<tr>
<th></th>
<th>A antigens</th>
<th>B antigens</th>
<th>Cw antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  3  9  32</td>
<td>7  27  40  51</td>
<td>2  7  11</td>
</tr>
<tr>
<td><strong>Father</strong></td>
<td>+  +</td>
<td>+</td>
<td>+  +  +</td>
</tr>
<tr>
<td><strong>Mother</strong></td>
<td>+</td>
<td>+  +</td>
<td>+  +  +  +</td>
</tr>
<tr>
<td><strong>1st kid</strong></td>
<td>+</td>
<td>+  +</td>
<td>+  +  +</td>
</tr>
<tr>
<td><strong>2d kid</strong></td>
<td>+</td>
<td>+  +</td>
<td>+  +  +  +</td>
</tr>
<tr>
<td><strong>3d kid</strong></td>
<td>+  +</td>
<td>+  +</td>
<td>+  +  +</td>
</tr>
<tr>
<td><strong>4th kid</strong></td>
<td>+  +</td>
<td>+  +</td>
<td>+  +  +</td>
</tr>
</tbody>
</table>
• Child #1. Child #2 has 5 HLA's different from #3 (in green); child #4 has 3 HLA's different from #3 (blue); Child #1 has only 2 HLA's different from #3 (red).

• NOTE: "different from" means "foreign to"

• ASIDE: the mother would be an equal donor as she has only 2 HLA's different from #3, as well (violet).
The classes of HLA's are tabulated below. Data regarding each class is summarized, as well.

<table>
<thead>
<tr>
<th>Classes of HLA's</th>
<th>MHC-I</th>
<th>MHC-II</th>
<th>MHC-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>On almost every human cell</td>
<td></td>
<td></td>
<td>In plasma (complement)</td>
</tr>
<tr>
<td>1. presents foreign antigen (e.g., virus) to cytotoxic T lymphocytes (CTL's): $T_8$ cells</td>
<td></td>
<td>Recognized by/through $T_4$ cells; class II on DONOR cells initiate graft vs. host disease</td>
<td>Complement immune system</td>
</tr>
<tr>
<td>2. Class I's are the primary antigen recognized by the HOST'S CTL's during graft vs. host disease</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The structure of MHC-I proteins

- The H chain is a glycoprotein of molecular weight 44,000. It consists of 3 α chains.
- Associated with these three chains is the β2 microglobulin. This protein has a molecular weight of 12,000 and is coded for on chromosome #15, NOT on 6p. The whole structure is anchored in the cell membrane by the H chain. The α3 and β2 microglobulin are structurally similar to the C region of immunoglobulins.
- MHC class I's are members of the immunoglobulin supergene family. The α1 and α2 regions contain most of the alloantigenic determinants that are recognized by antibodies and T cells.
- These same regions (or domains) bind foreign antigens and then are acted upon by very specific T cells.
The structure of MHC-I proteins

- The most extra-cellular portion of this protein resembles a mushroom.
- The intra-cellular-most portion is phosphorylated and may be an intracellular switch.
- To code for this glycoprotein, 7 exons on the DNA are typically required.
- Both the extracellular and intracellular domains are hydrophilic, whereas the transmembrane domain is hydrophobic.
MHC-I

• The $\alpha_1$ and $\alpha_2$ regions when they are flipped. This region appears like a clamshell or a Venus fly trap. This shape is functional, as it "clamps" antigens for presentation to $T_\text{g}$ cells.
• Like B cells, T cells are also "educated" early on in embryogenesis to seek only 1 kind of antigen in association with Class I MHC proteins for presentation to CTL precursors.

• Once the CTL precursors meet with the antigen and MHC-I, the specific precursors divide and differentiate to mature CTL's (T₈).

• These CTL's, then, are said to be restricted in their activities to those cells in the body that have the SAME MHC-I and antigen as began the dividing and differentiating of the CTL precursors. More on this shortly

• CTL killing of the [target] human cell is at once 1) antigen-specific and 2) "Class I restricted".

• Remember that MHC-I glycoproteins are found on virtually every cell in the body.
• It seems that MHC I glycoproteins REQUIRE intracellular binding with antigen in order to be transported to/through/into the cell membrane, Figure, right.
• The antigen is internalized and binds to the ER-bound MHC-I.
• The MHC-I-Antigen complex is then partially ejected through the cell membrane.
• A T₈ cell recognizes the complex and the T₈ cell kills the cell.
The structure of MHC-II proteins

• This glycoprotein consists of an α chain and a β chain. The former has a molecular weight of 34,000 and the latter of 29,000. The lower domains on each chain (α2 chain and β2 chains) show C chain homology (as we saw with MHC-I's α3 and β2 microglobulin domains).

• The upper domains of each chain (α1 and β1 domains) interact with foreign antigens. The structure of the MHC-II also seems to be that of a clamshell or Venus fly trap.

• MHC-II glycoproteins on DONOR cells initiate the immune reaction with host T4 cells.
• Class II proteins work differently than Class I: **Class II glycoproteins are found only on immunocompetent cells.** It seems that when antigens come into contact with an immune cell, e.g., macrophage, the antigen is internalized and partially processed, Figure, right.

• The partially processed antigen and the newly synthesized MHC-II are enclosed in an acidic endosome and transported to the cell membrane where the MHC-II-antigenic particle are ejected part way through the cell membrane. The complex is recognized by/is the ligand for the CD4 receptor on T4 cells.

• This complex initiates T4-mediated immune responses. Like T8-MHC-I restriction, the **T4 cells are MHC-II restricted** in T4 recognition of antigens.
Major Histocompatibility Complex (MHC or HLA) and T Cell Receptors

T-Cell Receptors
• T cells are differentiated (identified) by various receptors on their surfaces: TCR’s
Figure, above, illustrates a generic T cell receptor. Note that it consists of two chains: an α chain and a β chain. Note the similarity of the T cell receptor to antibody and MHC-II (coming up) general structures.

Although the actual values vary by author and study, for the β chain, there are around 20-25 different genes for the V region; 1-2 for the D region; 6-12 for the J region; 2 different genes for the C region.
• The diversity of TCR's comes from VDJ recombination (like Ig and MHC's) and imprecise snipping. NOTE: the **diversity** of TCR's is much, much less than for the Ig genes.

• One T cell may have 1 of 2 kinds of proteins in their receptors: $\alpha\beta$ or $\gamma\delta$. In spite of the fewer genes for TCR's, T cells recognize just as many antigens as do B cells. This is due to the unusually high rate of mutation in TCR genes.

• Both Ig and TCR's undergo VDJ recombination. Numerous proteins are needed for this process. Two loci, RAG-1 and RAG-2 (recombination-activation gene) on chromosome #11, are next to each other. They are transcribed simultaneously and have NO introns. Typically, RAG-1 and RAG-2 are expressed in B and T cells -- ONLY during VDJ recombination.

• **This mechanism of action is not fully understood.**
MHC-I and TCR’s

- The bottom line is that with the action of MHC-I and TCR's, the body cell is killed by \( T_8 \) toxins.
MHC-II and TCR’s

- The combined action of MHC-II and TCR's is to turn on a macrophage to destroy the antigen in the cell and does NOT kill the cell (VERY different from MHC-I).