IMMUNOLOGY STUDY NOTES

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CHAPTER 1

IMMUNOHISTOLOGY, INTRODUCTORY

- **ACQUIRED IMMUNITY:**
  - General Properties:
    - Ability to discriminate self from non-self
    - The Antibody is specific to the antigen
    - The system is diverse and can respond to a wide variety of antigens
    - The immunity is inherently transferable from donor to recipient
  - HUMORAL IMMUNITY: Acquired immunity mediated by antibodies and B-Cells, and transferable through blood serum.
  - CELL-MEDIATED IMMUNITY: Acquired immunity mediated by T-Cells, and not transferrable through blood serum.
- IMMUNIZATION:
  - **ACTIVE IMMUNIZATION:** Causing immunity by transferring an immunogenic (antigenic) substance into the host, and triggering his/her immune response to it.
    - Slow onset time: There is a lag-period before buildup of antibodies to the antigen.
    - Long-lasting
  - **PASSIVE IMMUNIZATION:** The transfer of immunity by transferring preformed antibodies from donor to recipient.
    - Fast onset time
    - Short lasting

    - **ADOPTIVE IMMUNIZATION:** Causing immunity by transferring immune cells from donor to recipient. The only time this is done clinically is bone marrow transplantations.
- **Primary Immune Response:** The initial response to an antigen, which requires antigen presentation and activation by T_H cells.
    - Slow onset period.
    - **IgM** is the antibody, secreted by B-cells.
    - **OLIGOCLONAL RESPONSE:** An initial antigen will usually trigger a handful of different B-Cells, each with varying levels of affinity. However, repeated exposures increase the affinity of the response, leading to the highest-affinity (**monoclonal**) response.
- **Secondary Immune Response:**
    - Fast onset period; stronger immune response; longer duration of response.
    - **IgG** is the antibody, secreted by Plasma cells.
- **CLONAL SELECTION THEORY:** Theory that explains how immunity can have both enormous diversity and specificity at the same time. The theory states that:
    - All antibody cells are precommitted to making a single antibody with a single specificity.
    - A single cell produces only one antibody, which interacts with only one antigen with the highest specificity.
    - When the right antigen interacts with that cell, it leads to *clonal expansion* and proliferation of that cell, so that many daughter-cells are made with the same specificity.
        - **Clone:** A group of cells in which all daughter cells are equal in their specificity.
    - The ability to recognize an antigen is dependent on a receptor (B-Cell receptor), and that receptor is a product of the same cell that secretes the antibodies. This ensures that made antibodies will fit with the antigen they are supposed to bind.

CHAPTER 2

HEMATOPOIETIC SERIES:

- **Lymphoid Series:** *Pre ------> Pro ------> Immature ------> Mature*
    - T-CELLS:
        - **T-Helper Cells**: T_H1 and T_H2
            - $CD4^+$, $CD3^+$
            - **T-Cell Receptors** recognize *altered-self*, an altered form of the Major Histocompatibility Complex (MHC), bound to antigen.
        - **Cytotoxic T-Cells**: T_C
            - $CD8^+$, $CD3^+$
    - B-CELLS: B-Cells ------> B-Memory Cells and Plasma Cells
        - **B-Cell Receptors (BCR)** recognize unprocessed antigen. The BCR are antibodies themselves: membrane bound IgM.
        - B-cells secrete IgM; Plasma cells secrete IgG.
- **Myeloid Series:**
    - Granulocytes:

        - Granulocyte ------> **Neutrophil**
            - **Opsonization:** Facilitating phagocytosis. Neutrophils have lots of opsonic receptors.
                - Neutrophils have **F_c-Receptors**, to recognize the F_c (common) portion of an IgG molecule bound to antigen. This extra affinity facilitates phagocytosis of the antigenic material.
                - Neutrophils have **CR1, CR2, CR3** receptors, all of which bind **C3b**, a **Complement** degradation product. Thus, the complement pathway facilitates opsonization of antigen.
        - Granulocyte ------> Eosinophil
        - Granulocyte ------> Basophil
    - Monocyte Series:
        - Monocyte ------> **Macrophage**
        - Monocyte ------> **Mast Cell**
            - Mast cells have allergic **IgE** receptors, which fit into the **F_c** portion of the IgE molecule.
            - IgE binding causes **degranulation** ------> release of histamine, vasodilation, increased vascular permeability, mucous secretion.
    - Megakaryocyte ------> **Platelets**
    - **Erythroid Series:** Erythrocyte, a subset of the Myeloid Series.

CHAPTER 3

IMMUNOHISTOLOGY:

- **THYMUS:** Each lobule is divided into cortex and medulla. The organ where T-Cells mature, and self-recognizing T-Cells undergo apoptosis.
    - **Cortex:** Densely packed with thymocytes.
    - **Medulla:** Sparsely packed with thymocytes.

- **SPLEEN:** Filters blood and traps systemic, blood-borne antigens.
    - **Red Pulp:** Isolated parts of spleen; contains old, destroyed erythrocytes, plus some macrophages.
    - **White Pulp:** Dispersed through rest of spleen, and surrounding interlobular arterioles. Contains T-cells
    - **Marginal Zone:**
- **LYMPH NODE:**
    - **Afferent Vessel:** Lymph vessels enter into the cortex from top of node. From here, antigen enters the lymph node from the blood.
    - **CORTEX:** Contains mostly B-cells and macrophages arranged into primary follicles -- a small cluster of dendritic cells and B-cells.
        - **Primary Follicles:** Rich in B-Cell and the site of initial B-Cell proliferation following activation.
        - **Secondary Follicles:** Activated, larger follicles, containing a **germinal center** in the middle which contains large B-memory cells and plasma cells.
    - **PARACORTEX:** Contains T-cells and dendritic (antigen-presenting) cells, which express the MHC-II complex. This region is sparsely populated in infants -- it is thymus-dependent.
        - *Initial antigenic interactions takes place here*, then activated T_H (and some B) cells migrate toward cortex where they convert the activate the primary follicles into secondary follicles.
    - **MEDULLA:** More sparsely populated. Contains formed antibodies.
    - **Efferent Vessel:** Activated B-Cells, plasma cells, T-Cells, and antibody exit through the efferent vessel via the intermedullary cords.
- **Mucosa-Associated Lymphoid Tissue (MALT):** In the submucosal spaces of gut (and respiratory tract). It contains a lot of plasma cells that secrete Secretory IgA.
    - **M-Cells** are specialized cells in MALT tissue that serve as antigen-presenting cells.
- **LYMPHOCYTE RECIRCULATION:** T-Cells are constantly circulated through the blood, which allows them to make continual contact with presented antigen. This greatly increases the likelihood that the right lymphocyte will come into contact with the right antigen.

- **CELL ADHESION MOLECULES:** They aid in recirculation of lymphocytes by getting them out of the blood (extravasation) and into the tissues or lymphoid organs.
    - **ICAM-1, ICAM-2, VCAM-1** are expressed on endothelial cells. They are members of the *Immunoglobulin Superfamily* and have lots of sequence homology with immunoglobulins.
    - **INTEGRIN FAMILY:** alpha-beta-Heterodimer. A separate family of adhesion molecules expressed by the lymphocytes. They serve as binding molecules for the ICAM's.
        - **LEUKOCYTE ADHESION DISEASE (LAD):** Abnormal beta-Chain of Integrin molecule, resulting in deficient immunity.
            - Susceptibility to bacterial infections.
            - Poor wound healing.
    - **SELECTIN FAMILY:** Another family of adhesion molecules.
        - Structure: **Lectin** Domain (Extracellular domain), EGF-like domain, and a domain of complement-regulatory proteins.
        - **HOMING RECEPTORS:** Many proteins in selectin family serve as homing receptors for B or T cells, allowing them to get to their intended destinations.
            - **VLA-4:** A homing receptor for B-Cells that allows it to home to MALT tissue.
            - **L-SELECTIN:** A homing receptor for T-cells, that allows it to get to peripheral lymph organs.

CHAPTER 4

IMMUNOGLOBULIN STRUCTURE and GENETICS

IMMUNOGENICITY: Capability of inducing an immune response (humoral or cell-mediated). Factors that increase immunogenicity:

- *Foreignness*
- *Higher molecular weight* (smaller molecules are less immunogenic)
- *Chemical complexity* (repeating subunits are less immunogenic)
- *Degradability:* Macromolecules that cannot be degraded are poor immunogens, because they cannot be processed by APC's.
- *Dosage:* Dosage must be within a prescribed range. If it's too high or too low, it won't promote immunity.
- **ADJUVANTS:** Substances that enhance immunogenicity when mixed with an antigen.

EPITOPES: Antigenic determinants. That portion of a molecule that specifically interacts with receptors to promote immunity.

- B-CELL EPITOPES: *B-Cells recognize soluble antigens, which bind to their membrane bound IgM immunoglobulins.*
    - Thus B-Cells tend to recognize large globular epitopes with hydrophilic edges (such as glycoprotein moieties) around the outside.
    - Because B-Cells can recognize tertiary conformation of molecules, the epitopic region can contain *sequential or non-sequential* amino acids.
    - Because of its tertiary structure, a complex globular protein contains multiple B-Cell epitopes.
    - Smaller antigens, such as Angiotensin II, interact within a deep and narrow cleft within the mIgM (membrane IgM).
- T-CELL EPITOPES: *T-Cells have **MHC-Restricted antigen recognition***. They can only recognize antigens that have been processed by APC's.

- **AGRETOPE:** The region of the antigen that binds to the MHC complex.
  - A given MHC molecule can bind a variety of different antigens.
- **EPITOPE:** The region of the antigen that binds to the T-Cell Receptor (TCR).
- The TCR binds much smaller epitopes, with linear sequence of amino acids -- because they have been processed by the APC.
  - Class I (processing of endogenous viral antigens) binds optimum length of 9 residues long.
  - Class II (processing of exogenous bacterial antigens, via the endocytic pathway) binds optimum length of 11-17 residues longs.
- T-Cell epitopes often contain *amphipathic* residues
  - Hydrophobic residues often act as agretopes and interact with MHC
  - Hydrophilic residues often act as the epitopes.

HAPTENS: Small molecules that are antigenic, but not immunogenic in themselves, because they are too small to elicit an immune response.

- They must first be bound to carrier proteins to form a Hapten-Carrier Conjugate. The hapten then becomes the epitope of the complex.
- Using haptens, researchers were able to demonstrate that the human immune system is highly specific.

VIRAL and BACTERIAL ANTIGENS:

- VIRAL ANTIGEN: Although B-Cells can recognize viral antigens when exposed to them, cell-mediated immunity is generally required to defend against viruses. T-Cells tend to recognize internal proteins.
  - CD8 T_C cells recognize MHC-I restricted viral antigens, that have infected a target cell.
  - CD4 T_H cells can also recognize viral antigens, if an APC takes up a naked piece of nucleocapsid and processes it, for example.
  - **ANTIGENIC SHIFT:** Making major variations to the viral protein coat, by means of Natural Selection and mutation. This makes it difficult to continually defend against the virus.

    - **ANTIGENIC DRIFT:** Minor variations to the viral protein coat.
- BACTERIAL ANTIGENS:
    - The **Peptidoglycan** component is the major epitope of the gram-positive bacteria.
    - The **Lipopolysaccharide (LPS)** component of the envelope is the major epitope of gram-negative bacteria.

MITOGEN: Substances that are non-specific polyclonal activators of lymphocytes. They induce polyclonal lymphocyte proliferation.

- **Lectins**: Polyclonal activators that bind to common sugar-residues found on many cells. They can induce agglutination and then proliferation of lymphocytes.
- CONCANAVALIN-A **(ConA):** A polyclonal T-Cell mitogen.
- PHYTOHEMAGGLUTININ **(PHA):** A polyclonal T-Cell mitogen.
- Pokeweed Mitogen: A polyclonal activator of both B and T cells.

CHAPTER 5

IMMUNOGLOBULIN STRUCTURE:

Two identical heavy chains and two identical light chains make up each immunoglobulin.

- Proteolytic Digestion:
    - **PAPAIN:** Protease cuts right above the joining region, generating three parts.
        - Two identical **Fab Fragments:** The antigen-binding fragment, that portion which changes for each immunoglobulin.
            - Contains the variable parts of both heavy and light chains.
        - One **Fc Fragment:** Common fragment, does not change for each immunoglobulin, but instead is dependent on which isotype (IgG, IgE, IgA, IgD, IgM) the immunoglobulin is.
            - Contains only heavy-chain components.

    - **PEPSIN:** Protease cuts right below the joining region, generating one main fragment:
        - **F(ab')$_2$**: This fragment had antigenic binding properties.
- **LIGHT CHAIN:** Contains two domains -- V_L and C_L
    - Contains two regions:
        - **VARIABLE REGION, V_L**
            - **HYPERVARIABLE REGION:** 15-20% of residues. Forms the epitope, antigen binding site of the molecule.
                - It contains three Complementarity-Determining Regions (**CDR's**), which make up three of the six CDR's of the epitope.
            - **Framework Region:** 80-85% of residues. beta-pleated sheet backbone.
        - **CONSTANT REGION, C_L**
    - Every immunoglobulin has one of two different classes of light chains:
        - **KAPPA, kappa:**
        - **LAMBDA, lambda:**
            - Divided into three subclasses: lambda1, lambda2, lambda3.
- **HEAVY CHAIN:** Contains four domains -- V_H, C_H1, C_H2, C_H3
    - Contains four regions:
        - **VARIABLE REGION, V_H**: The heavy-chain variable region usually contributes more to the antigen-binding site than the light-chain V-region.
            - **HYPERVARIABLE REGION:** 15-20% of residues. Forms the epitope, antigen binding site of the molecule.
                - It contains three Complementarity-Determining Regions (**CDR's**), which make up three of the six CDR's of the epitope.
            - **Framework Region:** 80-85% of residues. beta-pleated sheet backbone.
        - **CONSTANT REGIONS: C_H1, C_H2, C_H3**
    - Variability in the constant regions are divided into five different *classes*, which constitute the **isotypes** of the immunoglobulin. Each class, in turn, can be paired with either the kappa or lambda light chains. *The isotype is determined by the heavy-chain constant regions*.

        - **mu**: Heavy-chain structure of IgM.
        - **gamma**: Heavy-chain structure of IgG. This is further divided into gamma1, gamma2, gamma3
        - **alpha**: Heavy-chain structure of IgA. This is further divided into alpha1 and alpha2.
        - **delta**: Heavy-chain structure of IgD.
        - **epsilon**: Heavy-chain structure of IgE.
    - **HINGE REGION:** Only in alpha, gamma, and delta heavy chains (IgG, IgA, and IgD). It consists of oligosaccharide chains between C_H1 and C_H2 regions. IgE and IgM have no hinge region.
        - This region is rich in **Pro and Cys** and is flexible, giving the arms of IgG, IgA, and IgD flexibility.
        - The Pro and Cys make this region vulnerable to cleavage by proteolytic enzymes.
- FINE STRUCTURE:
    - **IMMUNOGLOBULIN FOLDS** constitutes the tertiary structure.
        - Two antiparallel **beta-Pleated Sheets**, hooked together by multiple disulfide bonds.
    - **DOMAIN** is a chain of about 60 aa, formed into a loop by a single disulfide bond.
    - TYPE OF ANTIGEN:
        - Tertiary structure tends to a form a flat beta-sheet structure with large globular antigens, where the CDR regions interact with a large surface.
        - Tertiary structure tends to form a deep groove with small antigens, and the antigen fits inside the groove.

ANTIGENIC DETERMINANTS: Immunoglobulins are themselves antigen determinants, as well as antigen-binding sites.

- **IDIOTOPE:** The individual antigenic determinant region of an immunoglobulin.
    - The idiotope may or may not be the same as the antigen-binding site, or it may overlap with it.

    - Each antibody has multiple idiotopes within it -- those regions capable of acting as antigen.
    - **IDIOTYPE:** The sum of the idiotopes of an immunoglobulin.
        - Monoclonal antibodies, all with same structure, all have the same idiotypes.
    - **IDIOTYPIC DETERMINANTS:** Common idiotopes that tend to show up in immunoglobulins even if they are polyclonal. This is due to the common germline structure of immunoglobulins.
- **ISOTYPE:** Constant-region antigenic determinants that are *species-specific*. They are contained with the constant region of an Ig and are thus endemic to the species.
- **ALLOTYPES:** Antigenic determinants that are inherited, and thus represent allelic variation within the constant region.
    - Only some of the constant region domains have allotypes (i.e. multiple alleles). Others do not, at least not known.

ISOTYPE CLASSES:

- **IMMUNOGLOBULIN G (IgG)**: ($gamma_2kappa_2$, $gamma_2lambda_2$); 80% of total serum immunoglobulin.
    - It is released by monoclonal Plasma cells in the secondary immune response.
    - It functions as an opsonin. Its **Fc region** can bind to **Fc-receptors** on neutrophils and macrophages.
    - It can activate complement.
    - It functions in fetal immunity. Some of its subclasses can cross the placenta to protect the fetus.
- **IMMUNOGLOBULIN M (IgM)**: (mu_2kappa_2, $mu_2lambda_2$)
    - IgM has a fourth heavy-chain constant region, C_H4
    - **MONOMERIC IgM** is expressed on B-cell membranes and constitutes the **B-CELL RECEPTOR (BCR)**, which B-cells respond to by proliferating and forming plasma cells and memory B-cells.
    - **PENTAMERIC IgM** is secreted by plasma cells.
        - Contains a **JOINING CHAIN (J-Chain)**, that links two of the five monomers together.

        - The monomers are further hooked together by two disulfide bonds which hook up monomers at their C_H3 and C_H4 regions.
    - Because of its large structure, IgM easily cross-links antigens, thus it takes less IgM to agglutinate an antigen than it takes IgG.
- **IMMUNOGLOBULIN A (IgA)**: ($alpha_2kappa_2$, $alpha_2lambda_2$). 10-15% of total serum immunoglobulin, plus the predominant type of secreted immunoglobulin.
    - **SECRETORY IgA:** A dimer or tetramer of IgA, with a J-Chain and a Secretory Component.
        - **POLY-IG RECEPTOR** is a homing receptor that homes polymeric IgA to secretory epithelial tissue.
        - **SECRETORY COMPONENT:** The receptor-IgA complex is then endocytosed into the epithelial tissue, and *the Poly-Ig Receptor then becomes the Secretory Component*.
        - The Secretory Component is thought to recognize the J-Chain of IgA and IgM, and it masks sites on the globulin that are susceptible to protease cleavage, thus making the IgA last longer in the harsh mucosal environment.
- **IMMUNOGLOBULIN E (IgE)**: ($epsilon_2kappa_2$, $epsilon_2lambda_2$). Extremely low serum concentration of 0.3g/mL or less.
    - IgE binds to Fc receptors on Mast Cells and basophils.
    - Once bound, it stays there until antigen comes along and **cross-links** with it, to cause Mast Cell degranulation and an anaphylactic response.
- **IMMUNOGLOBULIN D (IgD)**: ($delta_2kappa_2$, $delta_2lambda_2$). Very small total serum concentration. Unknown biological function.
    - It is expressed in immature B-Cells.

IMMUNOGLOBULIN SUPERFAMILY: Those proteins having structures similar to immunoglobulins.

- They all have the characteristic **immunoglobulin-fold.**
- They are believed to originate from the same primordial gene.
- Examples:
    - $beta_2$-Microglobulin

- TCR
- Cluster Designation proteins: CD2, CD4, CD8, CD28
- Cell adhesion molecules
- PDGF

MONOCLONAL ANTIBODIES:

- **HYBRIDOMA:** A cancerous myeloma (immortal B-Cell), that produces copious amounts of monoclonal (identical) antibody.
  - It is made by fusing a normal B-Cell with a cancerous myeloma.
- HETEROKARYONS: Fuse mouse and human cells, and the human cells will eventually lose all of their chromosomes. When you fuse the two, you must come up with a way to select for the desired hybrid, hence the HAT medium.
- HYPOXANTHINE-AMINOPTERIN THYMIDINE **(HAT)** MEDIUM: Use this medium to select for only the hybrid (hybridoma) cells.
  - **AMINOPTERIN:** It blocks the *de novo* pathway of nucleotide synthesis and forces the salvage pathway (recycling of old nucleotides).
  - **Hypoxanthine-Guanine Transferase (HGPRT):** This enzyme is required in salvage pathway.
  - You can then select for hybrids by giving them each a different mutation in the salvage pathway and then growing them on HAT medium. Only the cells that have hybridized will survive on the medium.

CHAPTER 6

ANTIGEN-ANTIBODY INTERACTIONS:

Highly specific, reversible, non-covalent interactions.

- **AFFINITY:** The sum of the interactions in a single binding site, between a single epitope and antigen-binding site.
- **AVIDITY:** The sum of the affinities between antibody and antigen, across multiple binding sites. This takes cross-linking into account and is a better measure of antibody interactions than affinity.
- **CROSS-REACTIVITY:** A single antibody reacting to more than one antigen.
    - This is the basis for **ABO-Blood Group** incompatibilities. The A or B antibody is actually formed against polysaccharides on intestinal bacteria. Incompatibility occurs because it then cross-reacts with the antigen on RBC-membranes.
    - *Streptococcal Pyogenes* possesses groups that mimic skeletal and cardiac muscles. Cardiac problems can result after getting over an infection of this bacterium.
- **PRECIPITIN REACTIONS:** Formation of an insoluble antigen-antibody complex from soluble components.
    - CRITERIA for formation: There must be the potential for cross-linking to occur.

        - The antibody must be bivalent.
        - The antigen must be at least bivalent -- have two copies of the same epitope, or different epitopes that each have corresponding antibodies to react with.
            - Myoglobin, which has repeating different subunits, will not precipitate with monoclonal antibodies because it can't cross link with close enough proximity. It will, however, precipitate with polyclonal antibodies.
    - **EQUIVALENCE ZONE:** The ideal ratio of antigen to antibody that results in maximal precipitation.
        - Having too much of antigen or antibody will result in suboptimal precipitation -- or none at all.
        - Plot a graph of antigen added -vs- amount precipitated, and it will form a bell-shaped curve, with the Equivalence zone at the maximum of the curve.
    - **PRECIPITIN GEL REACTIONS:**
        - **DOUBLE IMMUNODIFFUSION** method will allow you to test how similar two antigens are to each other. They each diffuse toward a common antibody, and form a line of precipitate wherever they interact.
            - **IDENTITY:** The two antigens share identical epitopes. They form a continuous line.
            - **NON-IDENTITY:** The two antigens share no epitopes. They cross each other and form an X.
            - **PARTIAL IDENTITY:** They share some epitopes in common but not others.
        - **RADIAL IMMUNODIFFUSION (Mancini Method):** Determine the relative concentrations of an antigen. Put antigen in center and let it diffuse outward through antiserum.
            - A precipitin ring will form. The area of that ring will be proportional to the concentration of antigen.
- **AGGLUTINATION REACTIONS:** Interaction between antibody and a particular antigen, to form a visible precipitate.
    - **PROZONE EFFECT:** An excess of antibody will offset the Ag:Ab ratio and thereby prevent agglutination.

    - **Incomplete Antibodies:** Antibodies that bind to antigen but do not induce agglutination. These are usually IgG instead of IgM.
        - **IgM** favors agglutination over IgG. If too much IgG is around compared to IgM, agglutination is less likely to occur.
    - **PASSIVE AGGLUTINATION:** Agglutination of a soluble antigen, by first mixing it with RBC's that have been treated with tannic acid or $CrCl_2$. This makes the antigen stick to (adsorb to) RBC membrane.
        - This technique is far more sensitive than precipitin reactions, for detecting concentration of soluble antigens.
    - **COOMBS TEST:** Test for the presence of maternal IgG to the Rh antigen, using the Coombs's reagent.
        - **DIRECT COOMBS'S TEST:** If the antibody is present in high enough concentration, then adding the antigen will directly agglutinate it to RBC's.
        - **INDIRECT COOMBS'S TEST:** You may have to add the Anti-Rh-Antibody (Anti-idiotype), in order to sufficiently crosslink the antigen and cause agglutination. A positive indirect Coombs's indicates that the antibody is present in very low concentration.
- **RADIO IMMUNO-ASSAY (RIA):** Tag radiolabeled antigen to its antibody, in order to quantify the amount of antigen present.
    - Add just enough radiolabeled antigen to saturate all the antibody you have.
    - Then add increasing amounts of unlabeled antigen, of unknown concentration. The unlabeled antigen will displace the radiolabeled antigen.
    - Then measure the amount of radiolabeled antigen in solution. The more you can measure, the higher the concentration of unlabeled antigen that you added.
    - **ELISA** is similar in principle to RIA, except that the reaction is linked to an enzyme, where reaction will yield a colored product.
- **WESTERN BLOTTING:** Use Electrophoresis, plus a radiolabeled antibody, to identify and isolate specific proteins.
- **IMMUNOFLUORESCENCE:** Tag antibodies to a fluorescent tag and add them to a medium. Then watch the fluorescence to see specifically where those antibodies bind.

CHAPTER 7

IMMUNOGLOBULIN GENES AND GENE-REARRANGEMENTS:

- LIGHT CHAIN GERM-LINE:

    - **L**: **Leader sequence.** Signal peptide guides the protein into the ER and is then cleaved.
    - **Variable Region**: Encoded by two genes
        - **V**: Variable-region gene. There are multiple V-segments for both the lambda and kappa light chains.
        - **J**: Joining segment. The J-segment is on a separate, adjacent exon, and it is joined to V-segment during splicing and rearrangement.
    - **C**: **Constant region** gene.
        - lambda-CHAIN: Three different C_{lambda} genes, which encode the constant region for the three lambda-subclasses: lambda1, lambda2, lambda3
            - Plus there is a fourth C-gene segment which is non-functional, called a **Pseudogene**.
        - kappa-CHAIN: Only one C_{kappa} gene, thus there is only one subclass of kappa-Chain.
- HEAVY CHAIN GERM-LINE:
    - **L**: Leader sequence. Signal peptide guides the protein into the ER and is then cleaved.
    - Variable Region: Encoded by three genes.
        - **V**: Variable region gene.
        - **D**: Additional **Diversity Region** gene.
            - It has about 13 different segments.
        - **J**: Joining gene
    - J-SEGMENT: A PSEUDO GENE separates the VDJ region from the C region. It is transcribed but is later spliced out.
    - **C**: Constant region. This gene determines the antibody isotype and is characteristic of the species.
- **HEAVY CHAIN REARRANGEMENTS:** Occurs in an ordered sequence during B-Cell Maturation. Note this is rearrangement of the DNA itself! This occurs before the light chain rearrangement.
    - Formation of VDJ Gene:
        - D and J segments join, and the intervening DNA is deleted.
            - This step may occur before the lymphocyte is committed to B-Cell lineage.

        - DJ Segment then joins to any of the V segments and the intervening DNA is deleted.
            - This step only occurs in cells committed to B-Cell lineage.
        - The VDJ gene is now transcribed.
    - Formation of Constant Gene: It is transcribed next.
    - mRNA Processing: VDJ transcript is joined to C transcript, and a Poly-A tail is added.
    - mRNA transcript is then secreted into ER lumen, and leader sequence is deleted. Then it goes through Golgi where glycosylation occurs.
- **LIGHT-CHAIN REARRANGEMENTS**: Occurs after heavy-chain rearrangements are done.
    - **kappa-CHAIN REARRANGEMENT:** It occurs first. If it successful, then subsequent rearrangement of the lambda-CHAIN is blocked.
        - The -heavy chain regulates this process by (1) promoting light chain rearrangement, and (2) blocking lambda-chain rearrangement (on a separate chromosome) while kappa-chain rearrangement is occurring.
            - This assures that any particular cell will only produce the kappa or lambda light chain, but not both.
            - This also implies that in kappa-producing B-Cells, the lambda-genes retain their germline configuration.
    - **lambda-CHAIN REARRANGEMENT:** It only occurs if both of the kappa rearrangements (on each of the homologous chromosomes) are unproductive.
    - **B-CELL APOPTOSIS:** If both kappa and lambda rearrangements, on both homologues, are unproductive, then the B-Cell dies. This is a common occurrence.
- **ALLELIC EXCLUSION:** The fact that Ig gene rearrangements only involve one of the two parental chromosome. The homologous chromosome remains in its germline configuration.
    - Rearrangement of one chromosome blocks rearrangement in the other.

MECHANISMS OF GENE REARRANGEMENT:

- **RECOMBINATION SIGNAL SEQUENCE (RSS):** These 7 or 9 bp sequences function to cause recombination in the region.
    - They are located in three discrete places along the germline:
        - Just distal (3') to the V-region.
        - On both sides of the D region.
        - Just proximal (5') to the J region.
    - Structure: Conserved structure
        - Palindromic Heptamer ------> **12 or 23 intervening bp** ----> AT-Rich Nonamer.
            - The intervening 12 or 23 bp correspond to one or two turns of a DNA helix.
            - V has 12bp -- one DNA turn
            - J has 23 bp -- two DNA turns
        - **ONE-TURN / TWO TURN JOINING RULE:** Segments having a 12-bp (one turn) RSS can join only to segments having a 23-bp (two-turn) RSS. This ensures that V will always join to J, and not to another V.
- **RECOMBINATION-ACTIVATING GENES (RAG):** Genes that mediate recombination.
    - These genes are only express in immature B and T-cells -- not mature cells. Thus rearrangements can only occur in the immature cells.
    - **RAG-1** and **RAG-2** act synergistically to promote recombination.
    - It is not known exactly how these genes promote the rearrangements.

- **CLASS-SWITCHING:** Changes in the constant-region germline, to change isotypes (from IgM to IgG, etc.)
    - Antigen binding to a BCR (IgM) causes switching from IgM or IgD ------> IgG, IgA or IgE.
        - *The V region remains the same*, such that the cell retains its same specificity, but the constant regions change to a different class.
        - **SWITCH SITES:** This occurs by a process called **switch recombination**, in which all constant-region genes (DNA) are deleted in progeny, except the ones being expressed.

        - Switch sites are located in the introns, proximal (5') to each C_H locus.
        - They contain tandem repeats that are highly conserved across species.
    - REVERSIBILITY: Switching is for the most part irreversible, because intervening C regions are deleted. However, a C-region that remains downstream remains intact and can thus still be switched to.
    - **Double Class-Switching** is possible with help of **IL-4**, which induces class two class switches in a row in the process of making IgE:
        - mu () ------> gamma-1 ($gamma_1$)
        - gamma-1 ($gamma_1$) ------> epsilon ()
- DIFFERENTIAL RNA PROCESSING:
    - IgM: Differential processing determines whether you get membrane bound monomeric IgM (mIgM) or secreted pentameric IgM.
        - IgM Gene Structure: The gene, C_H4 has two end-segments, M1 and M2.
            - **M1:** Encodes the transmembrane region of the membrane bound protein.
            - **M2:** Encodes the cytosolic region of the membrane bound protein.
        - M1 and M2 are transcribed, but *differential Poly-Adenylation* (where you put the Poly-A Tail) determines whether or not they are expressed.
            - If Poly-adenylation occurs at Site 1 (proximal to M1 and M2), then the protein is secreted as a pentamer.
            - If Poly-adenylation occurs at Site 2 (distal to M1 and M2), then the transmembrane parts are retained and it is put in the membrane.
    - In immature B-Cells, IgM and IgD can be secreted by the same cell.
        - The mRNA transcript includes both heavy-chain regions. Transcript Structure: **V, D, J, C, C_{delta}**
        - Alternative sites of Poly-adenylation again determine whether it is the C or C_{delta} that is expressed in the end.

    - TRANSCRIPTION: It does have an enhancer, a promoter, and a TATA-Box about 25 bp upstream of the Leading region.
        - **NF-kappaD**: A DNA binding protein that binds to the **NF-kappaD motif** of the gene, in order to enhance transcription of Ig genes.
- **GENERATION OF DIVERSITY (GOD)**: Several mechanisms contribute to the diversity of antibodies.
    - *V(D)J Rearrangement:* Multiple copies of each of these genes leads to a huge number of combinations possible in joining them.
    - *Junctional Diversity:* Due to imprecise DNA joining in the CDR3 region, in joining VD to J. It can actually join at one of several amino acids, generating more diversity as a result.
    - *N-Region Diversification:* In heavy chain, random addition of nucleotides to junctions between V(D)J rearrangements.
    - *Somatic Mutation:* Responsible for affinity maturation in stimulated Pre-B Cells. After antigen stimulation, B-Cells undergo somatic mutation in the variable region genes.
- **T-CELL RECEPTOR (TCR) GENES**: TCR is part of the Ig-superfamily, and has a germline very similar to the immunoglobulin genes.
    - They are subject to the same Generation of Diversity -- especially N-region additions and junctional diversity.
    - TCR genes are located on three separate chromosomes.
    - T-Cells express RAG genes, and TCR rearrangements are subject to RAG regulation.
    - T-Cells express the RSS, and they are subject to the 12/23 ONE-TURN / TWO-TURN RULE.

CHAPTER 8

MHC AND REGULATION OF IMMUNE SYSTEM

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC): The genes encoding the **Human Leukocyte (HLA) Antigen**, located on Chromosome #6.

- **MHC CLASS I:** Found on all cells.
    - **STRUCTURE**: A heterodimer consisting of the HLA plus a molecule of beta$_2$-Microglobulin.

        - PEPTIDE-BINDING DOMAIN: alpha1 and alpha2 domains form a groove of beta-pleated sheets, which are thought to form the peptide-binding domain.
            - **alpha1**: Distal to membrane, part of HLA.
                - It is the source of most diversity and thus usually the antigen-determining site. Most of polymorphism occurs in these residues.
            - **alpha2**: Distal to membrane, part of HLA
        - IMMUNOGLOBULIN FOLD: alpha3 and beta1-Microglobulin form an Immunoglobulin-fold like structure, and are thus considered part of the Ig-Superfamily.
            - **alpha3**: Proximal to membrane, part of HLA.
                - It binds to the **CD8** marker to facilitate signal transduction by that marker.
            - **beta2-MICROGLOBULIN**: Ig-superfamily protein.
    - Three genes encode it: **B, C, A** (in order)
    - FUNCTION: **CD8 Cytotoxic T-Cells** interact with MHC-I on a foreign cell. In response to this interaction, the cytotoxic T-Cell can then kill the target cell expressing the antigen.
- **MHC CLASS II:** Expressed only by **Antigen-Presenting Cells (APC's)**, such as macrophages, B-Cells, dendritic cells, Langerhans cells.
    - STRUCTURE: A heterodimer, consisting of an alpha and beta chain that associate by non-covalent interactions.
        - ANTIGEN-BINDING REGION: Forms a cleft, very similar to the HLA-I protein.
            - **alpha1**: Distal to membrane
            - **beta1:** Distal to membrane
        - PROXIMAL REGION: Once again is similar to an Immunoglobulin fold, and part of Ig-Superfamily.
            - **alpha2:** Proximal to membrane.
            - **beta2:** Proximal to membrane.
                - It binds to **CD4** in the T-Cell membrane, to facilitate signal-transduction by that marker,.
    - Three genes encode it: **DP, DC, DR**.

        - Each of those genes contains at least one region for the alpha-chain and one for the beta-chain.
    - FUNCTION: **CD4 Helper T-Cells** interact with MHC-II and foreign protein. In response, T-Helper Cell secretes cytokines and proliferates.
- **CLASS III GENES:** Located in-between the MHC I and MHC II. They express some complement factors and other members of the Ig superfamily.

GENERAL PROPERTIES OF MHC GENE-EXPRESSION:

- **HAPLOTYPE**: MHC genes are closely linked and are thus inherited usually as a group.
- **POLYMORPHISM:** Gene rearrangement does not occur in the MHC, but there are a large number of alleles at each locus. Thus there are a wide variety of different HLA's in the species.
- **CODOMINANT:** MHC genes are expressed codominantly.
    - 6 different MHC-I molecules are expressed in each cell (each version of A, B, and C, coupled with $beta_2$-Microglobulin)
    - 12 different MHC-II molecules are expressed in APC's (DP, DC, DR dimers). Plus, more molecules are displayed because there are multiple copies of some of the class-II genes.

Immune Responsiveness and the MHC:

- **IMMUNE-RESPONSE (IR) GENE:**
- Why are we susceptible to disease, in accordance with variability in our MHC?
    - **DETERMINANT SELECTION THEORY:** Says that MHC polymorphism within a species will generate different patterns of responsiveness and non-responsiveness. It's largely a function of which alleles you get, as to which things you can generate immunity to.
    - **HOLE-IN-REPERTOIRE THEORY:** Says that there is an absence of T-Cells capable of recognizing certain antigens, as they were eliminated during the thymic selection process (because those antigens happened to resemble self for that individual).
- **TYPHOID FEVER:** Reduced polymorphism of MHC tends to lead to increased disease. Dutch immigrants to South America had a different gene-arrangement of the HLA. It is hypothesized that they were therefore more susceptible to

disease than endemic South Americans -- they suffered from a Typhoid Fever epidemic shortly after arriving.

Antigen Processing and Presentation:

- **EXOGENOUS (ENDOCYTIC) PATHWAY: MHC-II** generally hooks up with exogenous antigens (bacterial antigens) that have been internalized, broken down, and processed by phagocytosis or receptor-mediated endocytosis.
    - Antigen is processed through three increasingly acidic compartments:
        - **Early Endosome**
        - **Late Endosome**
        - **Endolysosome**
        - **Lysosome**
    - **INVARIANT (Ii) CHAIN**: Added to Class-II proteins in the RER.
        - FNXNS:
            - It helps the HLA-II molecule get through the Rough-ER
            - It prevents other molecules from binding to it
            - It directs the HLA-II to the endocytic pathway.
        - Once the HLA-II molecule merges with a lysosome (it's not known when this occurs), the acidic environment causes the invariant chain to be cleaved.
- **ENDOGENOUS (CYTOSOLIC) PATHWAY: MHC-I** generally binds foreign peptides derived from within the cell, i.e. viral peptides that were transcribed within the cell.
    - The peptides most often are **nonamers**. Peptides of 8 or 9 residues are most efficient at stabilizing interactions between the alpha-chain and beta$_2$-Microglobulin.
    - **LOW MOLECULAR MASS POLYPEPTIDE (LMP):** Has high homology with **Proteosomes**, and thought to play a role in degrading antigen in the endogenous pathway.
        - Proteins targeted for proteolysis often have a small protein called **Ubiquitin** with it. The ubiquitin conjugate allows the antigen to be degraded by the LMP which is similar to a Proteosome.

- **TRANSPORTER of ANTIGEN PEPTIDES (TAP):** Once the antigen is degraded, the TAP transports it across the RER. Once in the RER, it associated with an alpha-chain and beta$_2$-Microglobulin, to form the final structure.
    - TAP1 and TAP2 genes are encoded within the MHC-II, near the LMP gene.

CHAPTER 9

B-CELL ONTOGENY AND RECEPTORS:

- **Antigen-Independent Phase:** B-Cell development in the bone marrow.
    - V-Gene Rearrangements: Progenitor B-Cell ------> Precursor B-Cell ------> Immature B-Cell ------> Mature B-Cell.
        - **RAG** genes are expressed during the Pro-B and Pre-B stages.
    - **SURROGATE LIGHT CHAIN:** Expressed in Pre-B cells, to assure that heavy-chain rearrangements have been productive. This is a form of Positive Selection.
        - If a B-cell is unable to associate with the surrogate light chain, then that means that the H-chain rearrangement was unproductive and the cell dies by apoptosis.
    - **Mature B-Cell** then leaves the bone-marrow expressing **Membrane immunoglobulin (mIgM and mIgD)**.
- **Antigen-Dependent Phase:** B-Cell class switching upon stimulation by an antigen.
    - Mature B-Cells go to spleen and lymph nodes, where they wait for antigen interaction. If they don't encounter antigen within a few days, then they die by apoptosis.
- **B-CELL RECEPTOR (BCR):** The mIgM have short cytoplasmic tails, thus they are hooked to a cytoplasmic complex, to allow signal transduction.
    - The cytoplasmic complex is a **Igalpha/Igbeta** heterodimer.

- The B-Cell receptor binds to antigen, endocytoses it, and presents it in association with MHC-II molecules to the T_H cell.

B-CELL ACTIVATION: Proliferation and maturation of B-Cells into Plasma Cells. Activated T_H Cells promote this process.

- General Process
  - B-Cell Receptor binds antigen.
  - B-Cell acts as an APC, and endocytoses, processes and presents that antigen to a T_H cell, thereby activating it.
  - T_H-B-Cell Conjugate forms, which induces directional release of cytokines by the T_H cell.
  - Proliferation and differentiation of B-Cells then occurs in response to the cytokines, forming plasma cells that secrete antibody for that antigen.
- **T_H-CELL <---> B-CELL CONJUGATE:** In peripheral lymphoid tissue, this conjugate is formed by means of at least two adhesion molecules.
  - **LFA-1** on T-Cell binds to **ICAM** on B-Cell.
  - **CD4** on T-Cell binds to **HLA-II** on B-Cell.
  - Both a membrane contact signal (as above) and cytokine signal are necessary to induce B-Cell proliferation.
- B-CELL CYTOKINES: T_H cells release cytokines to promote differentiation of B-Cells.
  - **IL-1** and **IL-4** are necessary competence signals, to drive B-Cell out of G_0 and into the growth cycle.
  - **IL-4** also promotes DNA replication.
- **AFFINITY MATURATION:** Increase in affinity of B-Memory cells for the antigen as a consequence of B-Cell activation. Occurs as a result of two processes:
  - **Somatic Hypermutation:** Random rearrangements in CDR regions of *mature* B-Cells can generate antibodies with higher or lower affinities for the stimulating antigen.
  - **Antigen Selection of High-Affinity Clones:** The mature B-cells that have *higher* affinity are selected for by binding interactions with antigen, thus the net result is mutation leading to higher affinity.

- **CLASS-SWITCHING:** Primary Immunity: IgM is secreted, followed by IgG later in the response. In Secondary Immunity, B-Memory cells switch to a different Ig, depending on cytokine signals.
    - **IL-4** promotes switching *first* to IgG1, and *then* to IgE.
    - **IL-5** and **TGF-beta** promotes switching to IgA.
- **B-Memory Cells:** Little is known about memory-cell formation. They differ from naive B-Cells in a few ways, and they have various lifespans depending on the antigen.

T-CELL INDEPENDENT (TI) ANTIBODY RESPONSES: B-Cells can also respond to certain types of antibodies directly, without the aid of T_H2 cells.

- Antigens that can elicit TI responses: Generally polysaccharides with repeating subunits.
    - Gram-Neg Bacterial LPS
    - Dextran
    - Pneumococcal capsular polysaccharide
- Type of Response Generated:
    - The response is weaker
    - No memory cells are formed
    - Only IgM is secreted, so affinity maturation does not occur.

T-CELL MATURATION: Most T-Cells die during maturation in the Thymus.

- **POSITIVE SELECTION:** The process of going from Double-Positive to Single-Positive. Only those T-Cells that recognize Self-MHC are allowed to develop. All others die by apoptosis.
    - This ensures *MHC-Restriction*.
    - **DOUBLE-POSITIVE ($CD4^+$, $CD8^+$)** cells undergo positive selection.
        - Some cells will bind to macrophage MHC-II in the thymus. This causes them to retain **CD4** and lose the CD8 molecule.
        - Other cells will bind to thymic epithelial MHC-I in the thymus. This causes them to retain **CD8** and lose the CD4 molecule.
        - SINGLE POSITIVE ($CD4^+$ or $CD8^+$) results in either case.
    - There may or may not be a surrogate *self-peptide* within the MHC groove during negative selection. That is unclear.

    - **DOUBLE-NEGATIVE** cells don't recognize either macrophages or epithelial cells, which makes them die by apoptosis. *Thus positive selection eliminates all (double-negative) cells that don't recognize self.*
- **NEGATIVE SELECTION:** Those T-Cells that recognize Self-MHC *alone* or with *high affinity* are killed off because they would be self-reactive.
    - This ensures *Self-Tolerance*.
    - **SINGLE-POSITIVE** ($CD4^+$ or $CD8^+$) undergo negative selection.

T-CELL RECEPTOR (TCR): Recognizes only antigen in association with an MHC complex.

- STRUCTURE: Heterodimer of alphabeta-chain or gammadelta-chain.
- CD MARKERS:
    - **CD3:** Pan-T-Cell marker. Signal-transducing molecule that is associated with the T-Cell receptor. When the TCR binds to MHC, CD3 transduces the signal.
        - **CD3-TCR COMPLEX:** All T-Cell Receptors form a complex with CD3, where CD3 is the primary signal transducing molecule.
    - **CD4:** T-Helper marker. Serves as a cell-adhesion molecule and a co-signaling molecule.
        - It binds to the $beta_2$ domain of the MHC-II complex.
    - **CD8:** T-Cytotoxic marker. Serves as both a cell-adhesion molecule and a co-signaling molecule.
        - It binds to the $alpha_3$-domain of the MHC-I molecule.
    - **CD28:** Essential co-stimulatory molecule on the T-Cell. It binds to B7 of an APC during T-Cell activation.
- TCR is member of Ig-superfamily. The alpha and beta chains are each thought to have heavy and light chains, similar to the BCR.
- **TRIMOLECULAR COMPLEX:** The (1) TCR, (2) antigenic peptide, and (3) HLA molecule form a trimolecular complex, which is the molecular basis for T-Cell activation.
    - **Agretope:** The interaction between antigen and HLA molecule.

T_H-CELL ACTIVATION: Initiated by interaction of the Trimolecular complex, along with the co-stimulatory signal.

- **CO-STIMULATION:** *Co-stimulatory signals are essential for T-Cell activation.*
  - **B7<-->CD28:** B7of the APC, and CD28 of the T-Cell.
    - Co-stimulatory signal up-regulates production of **IL-2 Receptors** by T-Cells, and causes secretion of **IL-2**.
  - **IL1**
  - **IL6**
- SIGNAL TRANSDUCTION: It is usually alpha-adrenergic (IP_3 / DAG ------> Ca^{+2}).
  - CD3 and CD4 or CD8 start the second messages.
- T_H CELL SUBCLASSES: Really a gradient from T_H1 to T_H2. *T_H-Cells are induced to secrete cytokines when activated by an APC.*
  - **T_H1**: Generally activates macrophages and **cell mediated immunity**.
    - Secretes **IL-2** and **IFN-gamma**
    - **c-fos** and **c-jun** are DNA-binding proteins that up-regulate the synthesis of IL-2 and the IL-2 Receptor.
  - **T_H2**: Generally activates monoclonal proliferation of B-cells ------> antibodies, to promote **humoral immunity**.
    - Secretes **IL-4**, **IL-5**, **IL-10**
      - **IL-4** is essential for B-Cell activation.
      - **IL-10** inhibits the activation of T_H1 by inhibiting macrophages, and thus serves a regulatory role.

MUCOSAL (MALT) IMMUNITY:

- Lymphocytes congregate in three regions of the mucosa:
  - Epithelial Regions: Mostly CD8 cells.
  - Lamina Propria: CD4 cells, and activated B and plasma cells.
  - Payer's Patches
- **PEYER'S PATCHES**: Similar to a tonsil. Rich in B-Cells with germinal centers.
  - **M-CELLS:** They are not APC's but serve a similar role. They overly the mucosal layer (in the epithelial layer) and they transcytose antigenic proteins to the peyer's patch.

    - Once exposed to antigen, activated T and B cells can migrate through the lamina propria ------> mesenteric lymph nodes ------> systemic circulation.
- **SECRETORY IgA:** A dimer or tetramer of IgA, with a J-Chain and a Secretory Component.
    - **POLY-IG RECEPTOR** is a homing receptor that homes polymeric IgA to secretory epithelial tissue.
    - **SECRETORY COMPONENT:** The receptor-IgA complex is then endocytosed into the epithelial tissue, and *the Poly-Ig Receptor then becomes the Secretory Component*.
        - The Secretory Component is thought to recognize the **J-Chain** of IgA and IgM, and it masks sites on the globulin that are susceptible to protease cleavage, thus making the IgA last longer in the harsh mucosal environment.

CELL-MEDIATED IMMUNITY: Antibody-independent immunity, mediated by T_H1 cells.

- Examples: Type-IV Hypersensitivity Responses, *mycobacterium tuberculosis* and other intracellular pathogens.
- **MACROPHAGE ACTIVATION: IFN-gamma**, secreted by T_H1 cells, activates macrophages. Effects are generally anaphylactic:
    - Increase expression of **MHC-II** molecules in macrophages, to enhance their roles as APC's.
    - Increase expression of **Fc-receptors** and **CR3-receptors**, to enhance opsonization.
    - Increase oxidative-burst ------> reactive O_2-intermediates and reactive N_2 intermediates (such as NO).
    - Increase synthesis of eicosanoids: PGD_2, PGI_2, TXA_2, LTB_4
- **CYTOTOXIC LYMPHOCYTE (CTL) ACTIVATION: IL-2** converts CTL-Precursors ------> Active CTL
    - IL-2 is secreted by T_H1 cells.
    - Antigen activation of a CD8 cell causes it to up-regulate its expression of IL-2 Receptors. Subsequent IL-2 activation (by T_H1 cells) causes CTL proliferation.

- After antigen activation, both CTL's and T_H-Cells are dependent on IL-2 for their proliferation.
- TERMINATION: After antigen clearance, *the levels of IL-2 decline and CTL's die by apoptosis.* This ensures that the immune response is limited and help to prevent excessive tissue damage.

- **CTL-MEDIATED CYTOTOXICITY:**
  - CONJUGATE FORMATION: CTL binds to MHC-I of the **target cell** (which is expressing antigen and is therefore targeted for destruction).
    - **TCR-CD3 complex** on CD8-Cell binds to **HLA-I** of target cell
    - Additional binding by **LFA-1** of CD8 cell with **ICAM-1** of target cell.
  - **PERFORIN MONOMERS:** After conjugate formation, the CD8 cell releases perforin monomers from storage granules.
    - These monomers polymerize within the membrane to form **perforin pores** ------> target-cell membrane lysis.
  - DEATH BY APOPTOSIS: Some evidence exists that the CTL cell sends a signal to target cell which causes the target-cell to die by apoptosis.
    - **TNF-beta** has been a signal that is implicated in this apoptosis signal.
- **NATURAL KILLER (NK) CELLS:** Non-specific (natural) killing of tumors and some virally infected cells.
  - They are believed to derive from **null cells**. Strange cell lineage.
  - KILLING METHOD: Similar to CTL's.
    - They degranulate perforin-like monomers to cause target-cell lysis.
    - They kill cells by apoptosis (mediated by TNF-alpha)
  - NK-CELL PROPERTIES:
    - They do not express CD8, CD3 or any other CD lymphocyte markers on their membranes.
    - They have no immunologic memory.
- **ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC):** Many cytotoxic cells express **Fc-receptors** on their membranes. They can then kill an **antibody(IgG)-coated cell** by a non-specific process.

- Cells involved: Most natural immunity cells have Fc-Receptors and can thus partake in ADCC. Neutrophils, Eosinophils, Macrophages, NK-Cells, Monocytes
- *Cells coated with IgM are not subject to ADCC because IgM has no available Fc region!* Primarily IgG is the antibody responsible for ADCC.

CYTOKINES: General Properties

- **Pleiotropy:** The same cytokine has different biological effects on different target cells.
- **Redundancy:** More than one cytokine accomplish the same action. This ensures that a lack of one will not lead to a deficient response.
- **Synergy:** Two cytokines acting in concert can often have a larger effect than the sum of either of them individually.
- **Antagonism:** Cytokines oppose each other's actions; they are self-regulating.
- SPECIFIC TYPES OF CYTOKINES:
  - **Lymphokines:** Cytokines released by B or T Cells.
  - **Monokines:** Cytokines released by macrophages and monocytes.
  - **Chemokines:** Cytokines that exhibit the property of chemotaxis.

Cytokine	Secreted by	Effects
IL-1	Activated Macrophages B-Cells	1) **Pyrogen** 2) Provides co-stimulatory signal to T-Cells. 3) Enhances activity of NK cells. 4) Provides a competence signal to allow B-Cell growth
IL-2	**T_H1**	*Generally induces CTL Proliferation* 1) Autocrine stimulation of T-Cells, to promote T-Cell growth and B-Cell growth. *IL-2 is essential for T_H proliferation and expansion.* 2) Promotes proliferation of macrophages in cell-mediated immunity. (Essential to Type-IV DTH Response) 3) Acts in synergy with IL-12 to promote growth of CTL's.

IL-3	T-Helper Cells	1) Stimulates degranulation of Mast cells (Type-I Anaphylaxis)
IL-4	**T_H2**	1) Promotes class-switching to IgG1, then to IgE in B-Cells. (Type-I Anaphylaxis) 2) Increases expression of MHC-II on resting B-Cells. 3) Increases expression of IL-2 receptors on T-Cells, in synergy with the CD28-B7 co-stimulatory signal. Also induces T-Cell proliferation. 4) Is essential for **B-Cell Activation**, hence this cytokine is released by T_H2 cells, which activate the humoral response.
IL-5	**T_H2**	1) Promotes B-Cell growth and induces class-switching to secretory IgA. 2) Promotes growth of **Eosinophils** in parasitic infections.
IL-6	Macrophages T_H2	1) Provides co-stimulatory signal to T-Cells during T-Cell activation. 2) Promotes B-Cell differentiation into **Plasma Cells**; stimulates antibody secretion of plasma cells.
IL-8	Macrophages Endothelial Cells	1) Chemotactic for Neutrophils -- a **chemokine** 2) Promotes vascular endothelium adherence and extravasation; inflammatory response
IL-10	**T_H2**	*Generally promotes the activity of T_H2 cells and inhibits T_H1 cells.* 1) **Inhibitory** Cytokine. It inhibits the activation of T_H1 indirectly, by suppressing cytokine production in macrophages. 2) Inhibits T_H1 and promotes growth of most cells. (Type-I Anaphylaxis)
IL-12	Macrophages B-Cells	1) Acts with IL-2 to promote growth of CTL's. 2) Stimulates proliferation of NK cells.
IL-13	T-Helper cells	1) Important **inhibitory** cytokine to inhibit the inflammatory response.

IFN-gamma	**T_H1** NK Cells	*Instrumental in cell-mediated immunity (CMI)* 1) Induces activation of macrophages in cell-mediated immunity and thus promotes the Type-IV hypersensitivity response. 2) Increases expression of MHC-I and II (where applicable) molecules on most cells. 3) Mediates important effects in inflammatory response. 4) Down-regulates IgE synthesis and thus helps to moderate the Type-I allergic response. It blocks IL-4.
CSF-G	Macrophages Endothelial Cells Fibroblasts	1) Promotes growth and differentiation of granulocytes in bone marrow.
CSF-M	Macrophages Endothelial Cells Fibroblasts	1) Promotes growth and differentiation of monocytes in bone marrow.
TGF-beta	Fibroblasts	1) Promotes B-Cell class-switching to IgA. 2) A **chemokine** -- chemotactic for macrophages and monocytes 3) *Inhibits proliferation of lymphoid cells*, to moderate the inflammatory response and promote wound-healing.
TNF-alpha	Macrophages (as part of Type-IV DTH)	1) **Pyrogen** 2) Increases expression of MHC-I molecules on most cells. 3) Long-term exposure causes fat and muscle wasting (cachexia) 4) Promotes growth of NK-Cells in Type-IV DTH response

BACTERIAL ENDOTOXIN: Lipopolysaccharide (LPS) coat causes anaphylaxis.

- **Macrophages** have receptors for released endotoxin. In response they release
    - **IL-1** and **TNF-alpha**: Released by macrophages in response to LPS.
        - Pyrogens
        - Anaphylaxis: They cause release of inflammatory mediators from endothelial cells: PAF, Up-regulation of Inducible NO Synthase ----> increased vascular permeability.
            - **PAF** then causes platelets to release histamine and serotonin ------> vascular permeability.
    - **IFN-gamma**: Enhances activity of macrophages.
- Gram-Negative bacteria that are likely in Urosepsis:
    - E. Coli.
    - Klebsiella
    - Proteus

Regulation of Immune Response: Ways in which we stop or moderate the immune response.

- **Catabolism of Antigen**: An important means by which the immune response has a limited duration.
- **Immune-Complex Suppression:** NEGATIVE FEEDBACK is exhibited on B-Cell secretion of antibodies. They have an **Fc-Receptor** which, when bound, turns off synthesis of further IgG.
- **CLONAL ANERGY:** Specific induction of unresponsiveness.
    - Lack of Co-stimulation (If the APC has no B7) will result in clonal anergy of the T-Cell, rather than activation of it.
    - Anergic T-Cells cannot proliferate in response to MHC.
    - Anergy is an *activate* state of unresponsiveness (active suppression), as opposed to passive lack of responsiveness.
- **Immunosuppressive Cytokines:**
    - **IFN-gamma** promotes cellular immunity: It is secreted by T_H1 cells and inhibits T_H2 cells.
    - **IL-10** promotes humoral immunity: It is secreted by T_H2 cells and inhibits T_H1 cells.
- **SUPPRESSOR T-CELLS:** They are $CD8^+$ cells that are known to exist, but little is known about them.

- **Anti-Idiotype Regulation:** Theory of self-regulating production of antibodies.
    - **Network Theory** says that antibody produced in response to an antigen in turn produces formation of antibodies to it. These anti-antibodies help to check the original antibody response.
    - As you create antibodies, anti-antibodies, and antibodies to those, the network becomes self-limiting, and the system is put into check.

CHAPTER 10

SERUM COMPLEMENT

CLASSICAL PATHWAY: Antibody-dependent activation of complement.

- INITIATION: **C1** has an **Fc-Receptor** that is activated when IgG and/or IgM *cross-link* the Fc-Receptors.
    - One molecule of IgM, because its pentameric is sufficient to activate complement.
    - ~1000 molecules of IgG are required in order to achieve high enough concentration to get the needed cross-linking.
- **C1q$r_2$$s_2$** then activates C4 and, separately, C2.
    - C4 ------> C4a + **C4b**
        - C4a is then released as anaphylatoxin.
    - C2 ------> **C2a**, which then catalyzes formation of C3-Convertase.
- **C3-CONVERTASE** is then formed by **C4b2a**. C1 is sloughed off.
- C3-Convertase then catalyzes C3 ------> C3a + C3b
    - **C3b** then participates in both classical and alternative C5-Convertases.
    - C3a is then released as anaphylatoxin.
- **C5-CONVERTASE** is then formed by **C4b2a3b**
    - **C5 ------> C5a + C5b**
        - C5a is released as anaphylatoxin and chemotactic factor.
        - C5b hooks to complement receptors and forms the initial part of the Membrane-Attack Complex (MAC).

ALTERNATIVE PATHWAY: Antibody-independent activation of complement.

- INITIATION: Initiation occurs whenever C3b and the necessary accessory enzymes are present. Alternative pathway is subject to a positive feedback loop.
    - C3 Slow Spontaneous Hydrolysis ------> **C3a + C3b**
    - **FACTOR-B ------> Ba + Bb**
        - Ba is sloughed off.
    - **C3-CONVERTASE: Bb** then binds to C3b to form **C3bBb**, which is C3-CONVERTASE.
        - Binding of Bb to C3b is catalyzed by **FACTOR-D**
        - The C3-Convertase is very unstable (5-min half life) unless it is stabilized by binding of **PROPERDIN**.

- AMPLIFICATION / POSITIVE FEEDBACK: C3-CONVERTASE can then generate more C3b by cleaving C3 ------> C3a + C3b
- **C5-CONVERTASE**: C3-Convertase (C3bBb) can bind to another C3b to form the C5-Convertase: **C3bBb3b**.
  - C5 ------> C5a + C5b
  - Again, C5a is given off as chemotactic factor
  - Again, C5b forms beginning of MAC.

MEMBRANE-ATTACK COMPLEX (MAC): C5b6789

- INITIATION: C5b binds to the target-cell by means of a complement-receptor. It provides the binding site for the subsequent addition of other factors.
- **C5b6** exposes hydrophobic regions of complex that nudge their way into the membrane.
- **C9** is a perforin-like molecule that can polymerize, and add 15 or more subunits to C5b678.
- The final MAC simply pierces the membrane.

Complement Regulation:

- **C1 INHIBITOR (C1Inh):** Glycoprotein that can block the Classical Pathway by preventing initial reaction of C1q, preventing activation of C2 and C4.
- C3-CONVERTASE REGULATORS: Family of proteins that inhibit C3-Convertase.
  - **CR1**: Classical and Alternative C3-Convertase Inhibition. Since this is complement receptor Type-I, this acts as a form of negative feedback.
    - It also acts to accelerate decay of alternative C3-Convertase.
  - **MEMBRANE-COFACTOR PROTEIN (MCP):** Classical and Alternative C3-Convertase inhibitor.
  - **C4b BINDING-PROTEIN (C4bp):** Classical C3-convertase inhibitor. Binds to C4b and prevents its association with C3a, preventing formation of Classical C3-Convertase.
    - It also acts to accelerate decay of alternative C3-Convertase.
  - **DECAY-ACCELERATING FACTOR (DAF):** Alternative C3-Convertase inhibitor. It accelerates the decay of C3-Convertase (which

has a short half-life without properdin), thereby effectively inhibiting the alternative pathway.
    - **FACTOR-H:** Prevents the association between C3b and Factor-B
        - It also acts to accelerate decay of alternative C3-Convertase.
        - **FACTOR-I:** *In all these cases*, factor cleaves the dissociated (decayed) C3-Convertase, to disable it and prevent it from reassembling.
- **CD-59**: Inhibits the MAC. It binds to C8, preventing the polymerized assembly of C9.
- **ANAPHYLATOXIN INACTIVATOR (AI):** Inactivates C3a, C4a, and C5a by cleaving an Arg residue from them.

COMPLEMENT RECEPTORS:

- **CR1:** Has a high affinity for C3b, to form the MAC.
    - Also plays an important role in regulating complement cascade (see above).
- **CR2:** Limited to B-Cells and some T-Cells. Function unknown.
- **CR3 / CR4:** Found on natural immunity cells (monocytes, PMN's, NK's)
- **C3a, C4a, C5a Anaphylatoxin Receptors:** Mast cells and Basophils have anaphylatoxin receptors which effect degranulation when bound.

Biological Consequences of Complement:

- Cell Lysis (via MAC):
    - Enveloped viruses are susceptible.
    - Most or all gram-negative bacteria
        - Resistant strains of *E. Coli* and *Salmonella* exist, associated with the smooth bacterial phenotype.
    - *Streptococcus Pneumoniae* is resistant to complement, due to its **capsule**.
- **Anaphylaxis**: **C3a, C4a, C5a** are the Anaphylatoxins
    - Smooth muscle contraction (bronchoconstriction)
    - Increased vascular permeability (endothelial cell constriction)
    - Adhesion of neutrophils to vascular endothelia (extravasation)
    - C5a is the most potent of all in mediating these effects.

- Chemotaxis: **C5a** is **Chemotactic Factor**. Very strongly chemotactic for neutrophils
- **Opsonophagocytosis**: **C3b** is an opsonin.
    - Phagocytes (Neutrophils, Macrophages, NK-Cells) express complement receptors (CR1, CR3, CR4) that bind C3b and thereby facilitate phagocytosis.
- Clearance and Solubilization of Immune Complexes:
    - **SLE:** In Lupus it was found that complement deficiencies can predispose you to Lupus, suggesting that complement plays a role in immune-complex clearance.
        - Specific deficiencies are the precursors of C3b -- C1, C2, and C4.
    - MECH: Coating of immune complexes with **C3b** is thought to facilitate its binding to CR1 on RBC's.

COMPLEMENT DEFICIENCIES:

- **HEREDITARY ANGIO-EDEMA (HAE):** Genetic inability to produce **C1Inh**
    - SYMPTOMS: Swelling of arteries.
    - PATHOPHYSIOLOGY: Defective C1Inh leads to a hyperactive classical pathway ------> C2-kinin split products ------> vascular permeability and angioedema.
- **PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH):** Defect in **CD59** and **DAF** inhibitors.
    - CD59 and DAF are alpha-adrenergic (IP_3) linked proteins.
    - SYMPTOM = **hemolytic anemia**.
- Early Component Deficiencies: Lead Type-III Immune Complex diseases such as Lupus.
- C3-DEFICIENCIES: Lead to disseminated ***Neisseria Gonorrhea***, as Neisseria proliferate in the absence of complement defense.
    - Neisseria = gram-neg bacteria.
- **LEUKOCYTE ADHESION DISEASE (LAD):** Abnormal beta-Chain of Integrin molecule, resulting in deficient immunity.
    - Susceptibility to bacterial infections.
    - Poor wound healing.

CHAPTER 11

IMMUNOPATHOLOGY

TYPE-I HYPERSENSITIVITY: IMMEDIATE. Mediated by IgE molecules binding to Fc-receptors on Mast Cells and Basophils.

- Examples:
    - Hay Fever, allergic rhinitis
    - Penicillin anaphylaxis.
    - Local anaphylaxis.
- **SENSITIZATION:** Initial formation of the IgE. Prior exposure to the allergen is required for an allergic reaction to happen later.
    - In atopic individuals, certain *allergens* promote the formation of IgE rather than IgG antibody.
        - **IL-4** promotes class switching to IgE.

        - IgE is normally only formed against parasites.
    - IgE binds to a **High-Affinity Fc-Receptor** on Tissue Mast Cells and Basophils.
        - Once bound, IgE sticks around for a long time. If it doesn't bind, it has a short half-life.
    - Once IgE is bound, the Mast Cells and Basophils are said to be *sensitized* and are subject to subsequent anaphylactic degranulation.
- **LOCALIZED ANAPHYLAXIS:** Allergic responses limited to a specific target organ.
    - **ATOPY**: The inherited tendency to manifest localized anaphylactic responses. Atopy is the property of being allergic.
    - EXAMPLES: Localized allergic reactions
        - **Asthma**: Allergic (IgE-mediated) Asthma differs from intrinsic Asthma. Allergic asthma is the anaphylactic response to airborne allergens in the lungs.
        - Hay Fever (Allergic Rhinitis)
    - PRIMARY SYMPTOMS OF ANAPHYLAXIS: See below for mediators
        - Bronchoconstriction
            - Mediated by TXA_2, PAF
        - Shock: Increased vascular permeability and vasodilation
            - Mediated by NO and Prostaglandins
        - Vomiting (GI, urinary smooth muscle contraction)
- **IgE CROSS-LINKING and DEGRANULATION:** In secondary allergic response, cross-linking of antigen with IgE on Mast cells causes degranulation.
    - CROSS-LINK: Proper ratio of 2:1 (IgE:antigen) must occur before cross-linking leads to degranulation.
        - Second messenger is an alpha-adrenergic (IP_3/DAG) pathway, leading to increased Ca^{+2} which causes degranulation.
    - DEGRANULATION occurs as a result of Ca^{+2} influx and causes release of the following mediators from Mast Cells and Basophils:
        - **Histamine**: Vasoactive amine.
            - Effects:
                - Intense bronchial smooth muscle contraction
                - Increased vascular permeability

        - Increased secretion by nasal, bronchial, and gastric glands.
      - **H1-Receptors:** Induces contraction of GI and bronchial smooth muscle
      - **H2-Receptors:** Found on exocrine glands and on vasculature -- increased permeability and secretion.
    - **Serotonin** has effects similar to Histamine.
    - **Eosinophil and Neutrophil Chemotactic Factors (ECF, NCF):** Attract Eosinophils and Neutrophils for the late phase response.
    - Proteases generate complement split-products and cause bronchial mucus secretion.
    - **EICOSANOIDS:** PGD2, LTB4, so called "secondary mediators" because they are not derived from granules.
      - **LEUKOTRIENES:** Slow-acting substances of anaphylaxis.
        - Extremely potent bronchoconstrictors.
      - **PROSTAGLANDINS**
      - Both are derived from **Arachidonic acid**, which is formed from **Phospholipase-A_2** in the membrane. The Phospholipase A_2 is activated by Ca^{+2} influx.
    - **PLATELET-ACTIVATING FACTOR (PAF):**
      - It is not derived from Arachidonic Acid.
      - Effects:
        - Platelet-aggregation and degranulation
        - Bronchoconstriction
    - Cytokines: **IL-1** and **TNF-alpha** are released by Mast Cells.
  - **EOSINOPHILIA** is a common sign of a Type-I allergic reaction. They normally accumulate late in a Type-I response.
    - Eosinophils normally mount attack against **parasites** and that's all.
    - Eosinophils will bind directly to antibody-coated allergen (ADCC) via their Fc receptors for IgG and IgE.
    - Eosinophils also release inflammatory mediators themselves, that aid in parasitic infections, but hurt in allergic responses:
      - **Leukotrienes** (slow-reacting substances of anaphylaxis).
      - **Major Basic Protein**

        - **Eosinophil-Derived Neurotoxin**
        - **Cationic Protein**
        - **PAF**
- **ALLERGENS:** Non-parasitic antigens that are capable of inducing the release of IgE. These substances all have the potential to cause anaphylaxis in *atopic* persons.
    - *Normally, IgE is only formed against parasitic infections.*
    - Examples:
        - DRUGS: Penicillin, Codeine, Vancomycin, Cephalosporin
        - Bee and Wasp venom
        - Ragweed
        - Various foods
- REGULATION OF TYPE-I RESPONSE: Generally promoted by **T_H2** cytokines.
    - T_H1 cells reduce the Type-I response.
    - T_H2 cells enhance the Type-I response, via IL-3, IL-4, IL-5, IL-10
    - **IL-4** is the most important promotor of anaphylaxis.
- **SKIN-TESTS**: Test for Type-I Hypersensitivity. Inject small amount of allergen intradermally, and look for a wheal and flare within 30 minutes.
    - The wheal and flare would result from local Mast Cell degranulation within the area
    - You run the very small risk of sensitizing someone to the allergen by doing a skin-test.
- THERAPIES for Type-I ALLERGIES:
    - Avoid the allergen.
    - **Desensitization:** Expose someone to increasing amounts of the allergen in the hopes of desensitizing Mast Cells to the allergen or exhausting the Mast Cells of their vasoactive components.
        - Repeated exposure appears to cause a shift toward IgG production in many people.
    - **Antihistamines** block the H_1 and H_2 receptors.
    - Induce **Anergy** by injecting them with T-Cell epitopes for the allergen. This would make future B-Cells unresponsive and prevent further production of IgE.

TYPE-II HYPERSENSITIVITY: ANTIBODY-DEPENDENT CYTOTOXIC HYPERSENSITIVITY. A reaction of soluble IgG, IgM antibody with membrane-bound antigen (usually autoantigen)

- Examples:
    - **ACUTE HEMOLYTIC ANEMIA** resulting from Blood-transfusion reactions: The ABO antigens are already present (if they are not self) because antibodies have been formed to normal gut flora, which happen to have polysaccharide antigens that mimic the RBC blood-groups.
        - PATHOPHYS: Transfusion reaction ------> Complement-mediated lysis of RBC's. Massive intravascular hemolysis. Reaction may be immediate or delayed.
        - SYMPTOMS: Fever, chills, nausea, hemoglobinuria, clotting.
    - **ERYTHROBLASTOSIS FETALIS** (Fetal reaction to maternal Rh antibody).
        - Pregnancy is at risk if Mother is **Rh^-** (and thus has Rh antibody) and has a baby with father who is **Rh^+**, thus the baby is Rh^+ and has Rh antigen.
            - First pregnancy is OK; the mother forms antibody against fetal blood during parturition, but it usually does not react with fetus.
            - Subsequent Pregnancies: The mother has *preformed antibodies*, and, if left untreated, they will react with fetal blood in-utero, resulting in fetal high immature RBC count (erythroblastosis).
        - TREATMENT: Give **Rhogam**, antibodies to the Rh-antigen. These antibodies bind to any fetal blood that enters mother's circulation and prevents antibody reaction.
        - **COOMBS'S TEST**: Test to see if maternal IgG is bound to fetal RBC's. Coombs's reagent will agglutinate if antibodies are present.
    - Autoimmune Hemolytic Anemia.
- **COMPLEMENT:** IgG and IgM and activate Complement via Fc receptors on endothelial cells. Complement is then activated via Classical (antibody-dependent) pathway. Complement effects:

    - Cell Lysis through **MAC**. This accounts for hemolysis in certain kinds of hemolytic anemias.
    - **OPSONIZATION** via **C3b** which acts as an opsonin: phagocytic cells express **C3b-receptors** and can thus bind to targets. This also occurs in certain autoimmune hemolytic anemias.
- **Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC): Natural Killer Cells,** as well as macrophages and some PMN's have Fc-receptors and can thus attack IgG-coated target cells and lyse them. This process occurs without phagocytosis.
    - This may play a role in Hashimoto's Thyroiditis.

TYPE-III HYPERSENSITIVITY: IMMUNE-COMPLEX HYPERSENSITIVITY. Accumulation of immune-complexes, formed by soluble antibody and soluble antigen.

- Examples:
    - Systemic Lupus Erythematosus (SLE)
    - Rheumatoid Arthritis
    - Glomerulonephritis
    - Goodpasture's Syndrome
- **SERUM SICKNESS:** Horse or bovine serum can be injected into human's as an antidote to bee venom or snake bites. The foreign serum will then induce formation of immune-complexes, which elicit symptoms 6 to 8 days later.
    - SYMPTOMS: Fever, arthralgia, vasculitis, acute glomerulonephritis.
- **ARTHUS REACTION:** Experimental **vasculitis**, in which a localized injury is produced by immune complexes. Immune-complexes accumulate on vessel walls which activated complement ------> vascular endothelial lesions.
- **PPD TB SKIN TEST** is also an example of a delayed hypersensitivity reaction.

TYPE-IV HYPERSENSITIVITY: DELAYED-TYPE HYPERSENSITIVITY (DTH). Antibody-independent, cell-mediated response of T_C cells against antigen. Reaction is generally 24 to 72 hours after allergen exposure.

- Examples:
    - Poison Ivy, Contact Dermatitis

    - Tuberculosis infections, and other intracellular parasites (where antibodies are inaccessible to them).
    - Graft rejection.
- SENSITIZATION is required, just like Type-I response.
- ACTIVATION: **T_H1** cells recognize the antigen directly and release lymphokines in response to it. Mature, specific T_H1 cells proliferate in response to antigen presented by macrophages or B-Cells. They then release cytokines:
    - **IL-2** stimulates growth of more T_H cells in an autocrine fashion.
    - **IFN-gamma** powerfully activates **MACROPHAGES**.
        - This stimulates them for phagocytosis (ADCC, opsonization).
        - Increases their oxidative burst ------> reactive oxidative intermediates.
        - This also makes them produce more MHC-II molecules, which makes them yet better APC's.
    - In **GRANULOMATOUS REACTION**, the macrophages can further turn into **Epithelioid Cells** and **Giant Cells**, in order to fight indigestible material or intracellular parasites such as *mycobacterium tuberculosis*.
    - Cytotoxic Cells recognize antigens directly, and proliferate in response to it.
    - **Natural Killer (NK)** cells can also proliferate in Type-IV responses. They have Fc receptors and respond primarily to membrane glycoproteins, virus-infected cells, or tumor cells.
- SKIN TEST: As compared to the Type-I skin test, a positive test here indicates that the individual has a specific population of T_{DTH} (sensitized T_H1) cells ready to fight this infection.
    - Inject antigen intradermally, and it will be processed by local Langerhans's cells (APC's) and then presented to local T-Cells for cell-mediated response.
    - Response is DELAYED rather than immediate -- 24 to 72 hrs after injection.

TOLERANCE: Active state of immunologic non-responsiveness to self.

- **TOLEROGEN:** A substance that induces tolerance. It shows specificity and memory, just like immunogens.

- Experimental Tolerance Induction:
    - Tolerance is induced more readily in immature cells than in mature cells.
    - EXPT: Take Strain-A mice at birth and inject them with Strain-B mice spleen cells.
        - As adults, the A-mice had tolerance to skin grafts B skin, because they were exposed to the B spleen cells during early development and thus perceived them as self.
    - Maintenance of tolerance depends on the persistence of antigen. *Tolerance does not last indefinitely!*
- Factors that promote tolerogenicity:
    - **Oral Tolerance:** Antigens introduced orally rend to promote tolerance.
        - Teleologically, this is probably because we ingest a lot of nutrients that are potentially antigenic. If it is nutritional food, then we should be tolerant of it immunologically.
    - Intravenous administration of antigen promotes tolerance more readily than subcutaneous administration.
    - High dosage of an antigen tend to induce tolerance. Also, extremely low doses may induce tolerance. Anything in between tends to induce immunity.
- B-Cells -vs- T-Cells: Both T and B cells are subject to clonal anergy and clonal deletion. But, T-Cells generally exhibit more tolerance than B-Cells.
    - Induction of T-Cell tolerance requires less antigen than that of B-Cells.
    - T-Cell tolerance is acquired sooner and lasts longer.
    - Loss of tolerance happens faster in B-Cells than in T-Cells.
- Theories of Tolerance Induction:
    - **CLONAL DELETION:** This occurs during negative selection in the thymus -- delete cells that respond with high affinity to self.
    - **CLONAL ANERGY:** Active state of unresponsiveness.
        - **Absence of Co-stimulatory Signal** promotes tolerance rather than immunity -- it leads to clonal anergy of B-Cell progeny.
        - Anergy can occur in mature, peripheral B-Cells.
        - Anergic cells express way less mIgM on their membranes, but about the same IgD.

THEORIES OF AUTOIMMUNITY: Autoimmune diseases have multiple etiologies.

- SEQUESTERED ANTIGENS: Freeing of sequestered antigens. Examples = anterior chamber of eye (lens uveitis), testis (autoimmune orchitis), myelin basic protein (MS).
- IMMUNE-COMPLEX INJURY: Autoimmunity induced by injury of immune-complexes.
- CO-STIMULATORY MOLECULES being inappropriately expressed can induce autoimmunity.
- INAPPROPRIATE EXPRESSION of HLA:
    - In IDDM, Pancreatic beta-Cells express too high levels of MHC-I and MHC-II.
    - Grave's Disease: similar finding
- CYTOKINE IMBALANCE: T_H1 activation over T_H2 can lead to autoimmune disease.
    - IL-2 is found in excess, plus too much IFN-gamma.
- ABNORMAL T-CELL FUNCTION: Lack of **suppressor T-Cells.** This theory has the most evidence supporting it.
- CROSS-REACTIVITY: Biological Mimicry with microbial antigens, as in **Rheumatic Heart Diseases**, in which antibodies against streptococcal antigens cross-react with myocardium.
- POLYCLONAL B-CELL ACTIVATION: Something stimulates anergic B-Cells to become active, usually non-specifically.
- FAILURE-TO-DELETE THEORY: Simple failure of thymic negative selection causes autoimmunity.

WITEBSKY'S POSTULATES: Experimental Autoimmune Encephalitis (EAE) is autoimmunity to Myelin Basic Protein in rats.

- Myelin Basic Protein is normally a sequestered antigen, protected by blood-brain barrier.
- If you experimentally expose rats to their own MBP, then they will show an immune response to it.
- This sequestered antigen could also be freed, however, by trauma, infection, etc.
- Once formed, the immunity can be transferred by a T-Cell clone to a recipient, and the same disease will be induced.
- **Multiple Sclerosis** = autoimmunity to Myelin Basic Protein

AUTOIMMUNE DISEASES: Most auto-immune diseases, for unknown reasons, occur predominantly in woman, sometimes by a margin of 10:1 or greater.

- **HASHIMOTO'S THYROIDITIS:** A combination of Type-II (organ-specific) and Type-IV (cell-mediated) auto-immune disease.
    - PATHOGENESIS: Type-II ADCC against thyroglobulin, and against thyroid peroxidase (microsomal bodies).
    - SYMPTOMS: **Goiter**, due to inflammatory infiltrates in the thyroid.
    - **HASHITOXICOSIS:** Severe hyperthyroidism found in this disease. This is often followed by destruction of thyroid tissue and hence severe hypothyroidism.
    - OTHER DISEASES: Many other autoimmune diseases are commonly associated with Thyroiditis: SLE, Rheumatoid Arthritis, Sjögren, Addison's, IDDM.
- **HEMOLYTIC ANEMIAS:** Complement mediated lysis or antibody mediated opsonization of red blood cells.
    - **WARM HEMOLYTIC ANEMIA:** IgG antibodies against Rh antigens.
    - **COLD HEMOLYTIC ANEMIA:** IgM antibodies specific for other RBC antigens (I and H).
        - Symptoms occur when blood is exposed to cold, such as extremities exposure to cold.
- **THROMBOCYTOPENIA:** Low platelet count. Autoimmunity against platelets is often found with autoimmune hemolytic anemias.
- **INSULIN-DEPENDENT DIABETES MELLITUS (IDDM):** Auto-immune attack against Insulin-secreting beta-Cells in pancreas.
    - The disease is a Type-IV cell mediated auto-immune disease.
- **PERNICIOUS ANEMIA:** Autoimmunity against **Intrinsic Factor (IF)** in Parietal Cells in the stomach.
    - No Intrinsic Factor ------> No absorption of B-12 in large intestine ------ > hemolytic anemia.
    - TREATMENT: Treat with supplement of B-12.
- **GRAVE'S DISEASE:**
    - PATHOPHYS: Type-II autoimmune attack against TSH-receptors in the thyroid gland, resulting in over activation of them ------> Hyperthyroidism.

    - This is a disease where an auto-antibody acts as an **Agonist**.
    - The antibodies are called "Long Acting Thyroid Stimulating" (LATS) antibodies.
- **MYASTHENIA GRAVIS:** Type-II autoimmune attack against Nicotinic Acetylcholine receptors ------> block Ach-receptors ------> Fatigable Weakness.
    - This is a disease where an auto-antibody acts as an **Antagonist**.
- **GOODPASTURE'S SYNDROME:** Type-II Autoimmune attack against Collagen-IV basement membrane components.
    - SYMPTOMS: In Goodpasture's, the auto-antibodies attack primarily the **Glomerular Basement Membrane** and **Pulmonary basement membrane** ------> Renal Failure (**Glomerulonephritis**) and Pulmonary dysfunction.
        - Classic dual symptoms are therefore **hemoptysis** and **renal failure**.
    - PATHOPHYS: Complement split products build up as a result of the inflammatory response against basement membranes.
    - DIAGNOSIS: Immunofluorescence shows a linear array of immunofluorescence, as antibodies bind to basement membrane.
- **MULTIPLE SCLEROSIS (MS):** Autoimmune (probably Type-II) attack against Myelin Basic Protein, resulting demyelination of nerves.
    - Activated T-Cells are found in cerebrospinal fluid.
- **SYSTEMIC LUPUS ERYTHEMATOSUS (SLE):** Type-III Hypersensitivity in which immune complexes are formed against nuclear components in any lysed cells. This is the most common systemic auto-immune disease.
    - Labs / Signs:
        - **Anti-Nuclear Antibodies, ANA, Anti-**dsDNA are the most common autoantibodies, but there are others.
        - Complement is overactive. High serum levels of complement split-products.
        - Hemolytic Anemia and Thrombocytopenia
        - Neutropenia results from chronic over activation.
    - **LE-TEST:** Old test of forming an LE-cell to test for Lupus.
        - Take blood and incubate it at 37C to get the cells to release their nuclei.

        - Anti-nuclear antibodies will then react with those nuclei.
        - Then, when those cells become phagocytosed by ADCC, they form a characteristic, identifiable cell called an **LE-Cell;**.
    - SYMPTOMS:
        - **Butterfly Malar Rash** is very common.
        - **Vasculitis**
        - **Glomerulonephritis**
        - **Polyarthralgia** is the most common complaint. **Synovitis** also occurs.
        - Heart-problems can occur but are less common: **Pericarditis**, and non-bacterial vegetations on valve leaflets called **Libman-Saks Endocarditis**.
        - **SUN EXPOSURE** makes the symptoms worse, as DNA is exposed to antibodies in the bloodstream.
    - DIAGNOSIS:
        - **BAND-TEST:** Look for immunofluorescence at the dermal-epidermal junction upon skin biopsy.
        - Immunofluorescence of **Anti-dsDNA Antibodies** is diagnostic of SLE.
        - Labs: Hemolytic **anemia, thrombocytopenia (low platelet count), leukopenia**.
- **SJÖGREN SYNDROME:** Also predominantly Type-III Autoimmune disorder characterized by **sicca** (dry eyes) and **xerostomia** (dry mouth). Second most common connective tissue disorder, after SLE.
    - **Rheumatoid Factor** is commonly found, whether or not they have Rheumatoid Arthritis.
    - Associated with a 4-fold increased risk for **malignant lymphoma**.
- **RHEUMATOID ARTHRITIS:** Autoimmune inflammation of joints.
    - **Rheumatoid Factor:** Antibody against the Fc portion of IgG, such that it forms an IgG complex which then deposits in joints.
        - **IgM-IgG** Complex is the most common to form.
- **CONTACT DERMATITIS:** Type-IV delayed hypersensitivity. Poison Ivy.

IMMUNE DEFICIENCY DISEASES: Most congenital immunodeficiency diseases are X-linked and thus occur only in males.

- PHAGOCYTIC IMMUNE DEFICIENCIES: As a group these diseases lead to recurrent bacterial and fungal infections.
    - **CONGENITAL NEUTROPENIA:**
        - Stem cells are present but don't mature.
        - Deficiency in **G-CSF**, such that granulocytes don't mature.
        - SYMPTOMS: Infantile bacterial infections.
    - **LEUKOCYTE ADHESION DEFECT (LAD):** Inability for neutrophils to extravasate due to inability to bind to endothelia.
        - PATHOPHYSIOLOGY:
            - **Complement Receptors, CR3 and CR4** deficient, or,
            - **LFA-1** could be deficient, resulting in no adhesion to ICAM-1 in endothelia.
    - **CHRONIC GRANULOMATOUS DISEASE (CGD):** X-Linked recessive
        - PATHOPHYS: Cytochrome-B deficiency resulting in **no NADPH Oxidase ------> No Oxidative Burst**, because of inability to recycle NADP.
            - Impairs the killing ability of neutrophils.
            - Catalase-negative bacteria can still be killed by the defective neutrophils, because they form their own H_2O_2 in bacterial metabolism, and they don't have the catalase to break it down.
        - PROGNOSIS: Children often die of septicemia by 7 years of age.
        - SYMPTOMS: Granulomas all over the place.
    - **MYELOPEROXIDASE DEFICIENCY:**
- HUMORAL (B-CELL) DEFICIENCIES: Usually subject to recurrent bacterial infections, but display normal immunity against viral and fungal infections.
    - **CONGENITAL X-LINKED HYPOGAMMAGLOBULINEMIA (XLA) (BRUTON'S DISEASE):** Deficient in all immunoglobulins.
        - PATHOPHYS: Caused by a defect in the early maturation B-Cells. Pre-B Cells are detected but cannot mature.
            - Appears to be a specific defect in the V-D-J gene-rearrangement machinery.
        - Age of Onset: Infantile about 6 months, after maternal passive immunity has worn off.

        - TREATMENT: Periodic gamma-globulin injections to passively protect them against common bacterial pathogens.
    - **COMMON VARIABLE IMMUNODEFICIENCY**: Late-onset Hypogammaglobulinemia (deficient IgG).
        - EPIDEMIOLOGY:
            - Age of Onset: Adult, 15-30 years.
            - Affects both men and women equally.
        - SYMPTOMS: Recurrent pyogenic bacterial infections.
        - PATHOPHYS: Appears to be in the activation of mature B-Cells to antibody-secreting Plasma Cells.
        - TREATMENT: Use IVIG (intravenous immunoglobulin?) to treat it.
    - **SELECTIVE IGA DEFICIENCY:** Most common of immunodeficiencies. B-Cell count is normal, but IgA is not synthesized or secreted. Could be a problem with class-switching or with the secretory pathway.
        - Symptoms:
            - Recurrent or opportunistic GI-tract and respiratory infections.
            - Increase incidence of allergic infections.
    - **TRANSIENT HYPOGAMMAGLOBULINEMIA OF INFANCY**
- T-CELL DEFICIENCIES: Increased susceptibility to viral and protozoal infections, and to intracellular pathogens such as *mycobacterium tuberculosis, candida albicans,* and *pneumocystis carinii*.
    - **DIGEORGE SYNDROME:** Congenital malformation of 3rd and 4th Brachial Pouches, resulting in no formation of Thymus.
        - SYMPTOMS:
            - Patient will present with severe **hypocalcemia** due to hypoparathyroidism (Parathyroid does not form normally).
            - Concurrent cardiac defects are common, and the most common cause of death.
            - TREATMENT: Grafting of fetal thymus tissue. Must use fetal tissue younger than 14 months to prevent Graft -vs- Host Disease.

- **ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS):** Infection of CD4 cells by HIV retrovirus.
    - Common Complications: Opportunistic infections. Of course this list is not complete.
        - **Persistent Generalized Lymphadenopathy**: Common early symptom. Persistent enlargement of lymph nodes with no apparent cause.
        - **Kaposi Sarcoma** is a common skin cancer that occurs all over the body in AIDS and rapidly metastasizes.
        - **Pneumocystis Carinii** is an opportunistic pathogen that frequently causes pneumonia in immunocompromised patients.
        - **Cytomegalovirus**
        - **Toxoplasmosis** occurs in the brain where it forms lesions that are evident on MRI. Toxoplasmosis occurs in normal people, too, but it doesn't form the lesion because our immunity can quickly wipe it out.
        - **Candida** infections.
    - **gp120** is the name of the viral-coat protein, which recognizes CD4 receptors on T_H cells in order to gain entry into the cells.
- COMBINED IMMUNODEFICIENCIES:
    - **BARE LYMPHOCYTE SYNDROME:** Deficiency in expression of MHC molecules resulting in impaired antigen presentation.
        - Types:
            - Type-I: Defective MHC-I
            - Type-II: Defective MHC-II.
            - Type-III: Both are defective.
    - **SEVERE COMBINED IMMUNODEFICIENCY (SCID):** Absence of both T and B-Cells. Onset at 6 months.
        - **Adenosine Deaminase Deficiency** is usually the cause in autosomal-recessive SCID (there is also an X-linked form). Deficiency of ADA leads to accumulation of deoxyadenosine and its derivatives (e.g., deoxy-ATP), which are toxic to immature lymphocytes.

        - TREATMENT: Bone-marrow transplant is a possibility. In the case of ADA deficiency, you can treat with the enzyme.
    - **WISKOTT-ALDRICH SYNDROME:** Rare X-linked, cause unknown.
        - PATHOPHYS: It is a complete failure to produce antibodies against polysaccharides. Absence of adhesio protein **CD43**.
        - FINDINGS: Normal IgG, low IgM, elevated IgA and IgE.
- COMPLEMENT DEFICIENCIES: See complement section.
- **CHRONIC MUCOCUTANEOUS CANDIDIASIS:** Specific immunodeficiency against Candida fungi and nothing else. Strange...

CHAPTER 12

TRANSPLANTATION

REJECTION:

- HYPERACUTE REJECTION: Type-II rejection of organ mediated by *pre-formed* antibodies to the graft.
    - RISK: This would be common in people who have had multiple transfusions, previous transplants, or multiparous women.
    - PROCESS: Activation of complement and immediate ischemia / inflammatory response in the grafted organ. The organ must be removed during surgery.

- ACUTE REJECTION: Cell-mediated (type-IV) reaction against alloantigenic HLA.
    - **CROSS-REACTIVITY** is shows against the alloantigenic HLA. No antigenic peptide is needed in the binding groove -- the T-Cells recognize and are activated by the foreign HLA itself.
    - T-Cells recognize alloantigenic HLA-I and HLA-II molecules of the graft, and are monoclonally activated by them.
- CHRONIC REJECTION: Just like acute rejection except longer time course.

WAYS TO PREVENT REJECTION:

- **MIXED LYMPHOCYTE REACTION (MLR)**: A way to detect a mixed population of cells.
    - Take the WBC's of prospective donor and recipient and mix them together.
    - Wait about a week for the delayed response to effect if one is going to occur.
    - **TWO-WAY MLR:** Then add radio tagged thymidine (^{3}H-Thy) and see if it is taken up by the cells (if it decreases in the medium), which indicates that cell proliferation has occurred. It doesn't tell you in which direction the immune reaction occurred.
    - **ONE-WAY MLR:** Irradiate the donor cell population to prevent it from proliferating, then add the ^{3}H-Thy and see if the recipient cells proliferate. If they proliferate then you have verified the directionality of the cell-mediated response.
- TISSUE-TYPING: Compare the haplotypes of prospective donors and the recipient. The closer the agreement, the better.
    - A homozygous donor can donate to a heterozygous recipient, because the recipient has the necessary HLA molecules to perceive the graft as self.
    - Class I and Class II incompatibilities combined are far worse than either one by itself.
    - Run serum cross-match tests to test for antibodies to the tissue (prevent hyperacute rejection) and to test for ABO BLOOD TYPES.

    - Kidney endothelial vascular cells express the ABO antigen, and blood-group incompatibilities can result in hyperacute rejection. Thus we must match blood-groups too.

GRAFT -VS- HOST DISEASE: In bone-marrow transplants, disease of grafted stem-cells reacting against donor's tissues.

- SYMPTOMS: Fever, exfoliative dermatitis, diarrhea, hepatitis with jaundice
    - Skin, liver, and gut are the primary targets for graft immune attack.
