# 7.016 Recitation 20 – Fall 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*



**Summary of Lectures 30 (11/28) & 31 (11/30):**

Important terms and cell types that you should know.

| Terms | Description |
|---|---|
| Antigen (Ag) | A molecule to which a T cell receptor (TCR) or antibody molecule binds |
| Antibody (Ab)/ Immunoglobulin (Ig) | A protein that is made of heavy (H) and light (L) polypeptide chains and has identical antigen binding sites; can be of different types (such as surface IgM or secreted IgG, IgA etc) |
| B cells | Produce antibodies responsible for adaptive immune response<br>-Memory B cells have surface IgM and plasma B cells have secreted IgG<br>-Memory B cells are responsible for the memory in antibody mediated immune response |
| $T_H$ | Helper T cells, express CD4 surface molecules and have unique T cell receptors (TcR) specific to an antigen |
| $T_C$ | Cytotoxic T cells, express CD8 surface molecules and have unique T cell receptors (TcR) specific to an antigen |
| APC | Antigen presenting cells (such as macrophages, dendritic cells, B cells) |
| MHC | Major histocompatibility complex; there are two types (Class I and Class II)<br>-MHC Class I located on surface of ALL nucleated cells, presents peptide antigens and is responsible for $T_C$ mediated killing of the infected cell<br>-MHC Class II located on the surface of APC, presents peptide antigens and is responsible for activation of $T_H$ cells and the antibody mediated immune response |
| Innate immune response | Inborn, is non-specific, does not have memory and is only against foreign antigen |
| Adaptive immune response | - This immune response is delayed, acquired, specific and has memory<br>- It forms the basis of vaccination<br>- Two types; $T_C$ mediated killing of infected somatic cells and antibody mediated humoral immune response |

The immune system protects the body from foreign entities that have invaded it, such as bacteria and viruses. The immune system provides **innate** and **adaptive** immunity.

**Mechanical and chemical barriers** such as the skin epithelium, saliva and mucus provide the first line of defense against approximately 99% of potential infections.

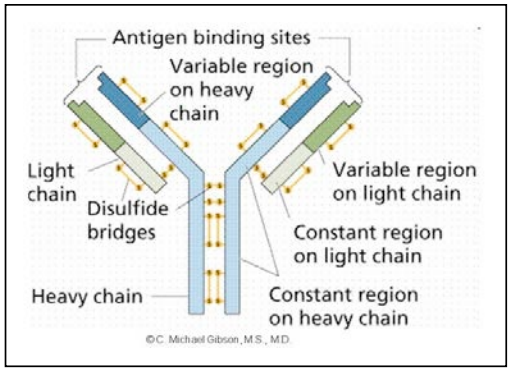
The second line of defense is the **Innate Immune response,** which commences immediately upon pathogen entry. This is an inborn defense mechanism; it is not antigen-specific and does not generate

memory. It involves cells such as macrophages, neutrophils and natural killer cells (NK cells). If these cells cannot rapidly eliminate pathogen, then the synthesis of cytokines and acute phase proteins induces inflammation.

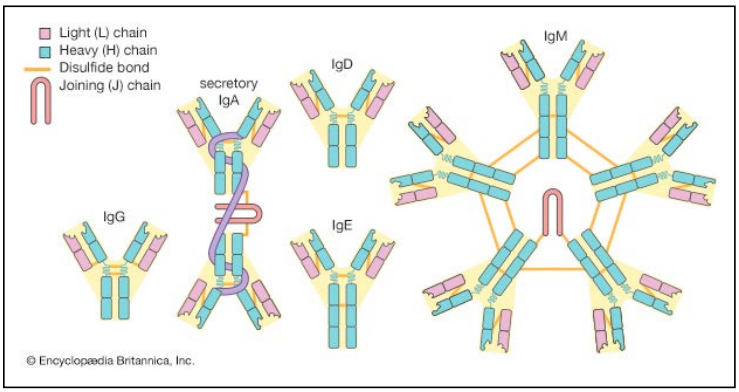
Activation of the **Adaptive immune response** is delayed with respect to the innate immune response. This response is stronger and more specific than the innate immune response, and has memory. It involves T- and B- lymphocytes. These lymphocytes originate from the hematopoietic stem cells (HSCs) in the bone marrow. The B-lymphocytes mature in the bone marrow and spleen whereas the T-lymphocytes mature in the thymus.

The antigen presenting cells (APC) reach the site of infection through signals that are provided by cytokines (small signaling proteins secreted by other cells or pathogens). The APC cells bind to specific antigen and engulf the foreign substance (i.e. everything the antigen is attached to), break it into small components and present these components on their surface through Major Histocompatibility Complex (MHC) molecules to activate T and B cells. The activated B cells then make and secrete antibodies, which can bind to and neutralize the antigens. In comparison, the activated $T_C$ cells secrete proteins (granzymes or perforins) that can poke holes in the membrane of the cell infected by the antigen thereby killing the infected cell.

**Antibody production by the B cells:** B cells make antibodies, which recognize and direct an attack against foreign entities. Antibodies are usually Y shaped proteins each composed of 2 heavy chains and 2 identical light chains. The heavy (H) and the light (L) chains are held together by the covalent disulfide bonds (S-S bonds).








There are several classes of antibodies that differ in structure and cellular location. For example, IgM class of antibodies forms a pentameric rossetta shaped structure with ten identical antigen-binding sites. In comparison, the IgG class of antibodies is monomeric and they have two identical antigen-binding sites. IgG antibodies are secreted by the plasma B cells; they bind to antigen so that it can be phagocytosed by phagocytic cells such as macrophages.

**Generation of antibody diversity:** Our bodies make billions of different antibodies. Antibodies are proteins and thus are encoded by genes. However, our genome contains fewer than 30,000 genes. So it is not possible to have a different gene for each antibody. Prof. Susumu Tonegawa was awarded the Nobel prize for discovering how a limited amount of genetic information can yield such a vastly diverse antibody repertoire.

Nobel lecture: https://www.nobelprize.org/prizes/medicine/1987/tonegawa/lecture

Each type of antibody light (L) chain (κ light chains and λ light chains) and heavy (H) chains has a separate pool of **gene segments** from which a single polypeptide chain is synthesized. The genes

encoding the H and L chains are on different chromosomes (H chain gene locus is on chromosome 14, κ light chains gene locus is on chromosome 2 and λ light chains gene locus is on chromosome 22 in humans).

There is a cluster of gene segments (V, D and J segments) in the section of the genome that encodes the variable (V) domain of Heavy (H) and Light (L) chains of antibodies. Each antibody- producing B cell rearranges these gene segments randomly to join one V gene segment to one D gene segment to one J gene segment thereby creating one rearranged antibody gene that makes the variable region of the Heavy (H) chain of the antibody molecule. Similarly, each antibody- producing B cell rearranges these gene segments randomly to join one V gene segment to one J gene segment thereby creating one gene that makes the variable region of the light (L) chain of the antibody molecule. Thus every antibody-producing B cells carries only one VDJ gene segment recombination to produce one unique antibody that is specific to one antigen.

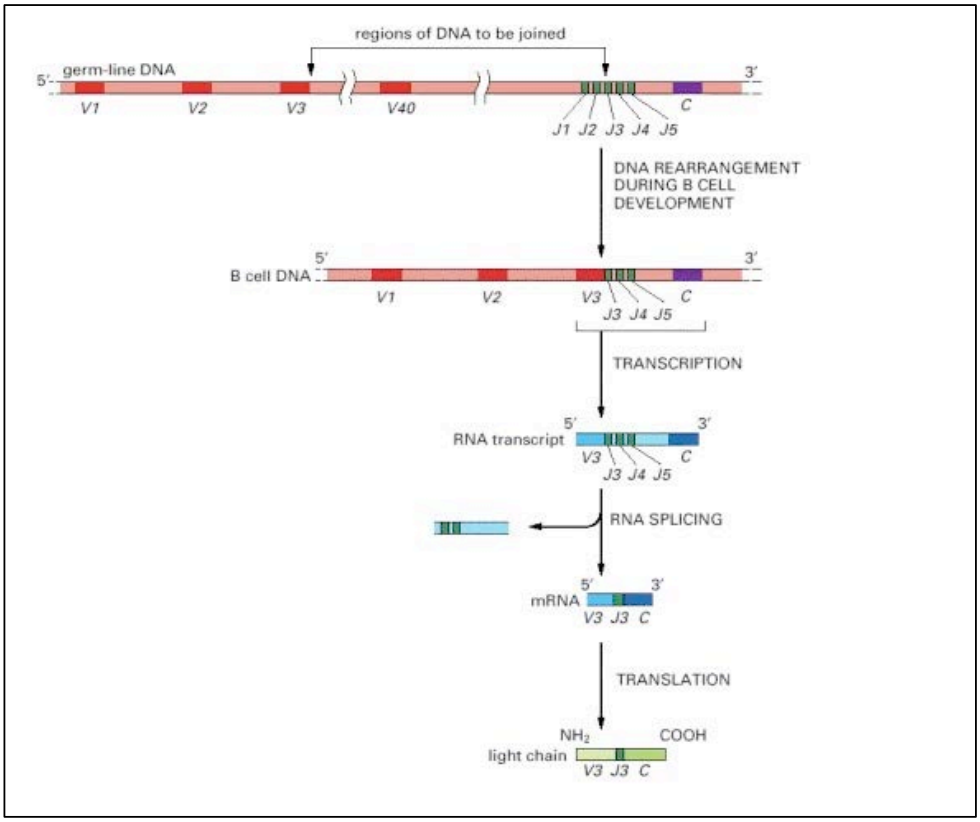
During development of a B cell, a complete coding sequence for each of the two antibody chains to be synthesized is assembled by site-specific **somatic gene recombination (i.e. DNA rearrangement)**. In addition to bringing together the separate gene segments and the C-region exons of the antibody gene, this VDJ DNA rearrangement also activates the transcription from the gene promoter (which is located prior to each V segment) through changes in the relative positions of the enhancers (and silencers) acting on the promoter. Thus, a complete antibody chain can be transcribed and then translated only after the DNA has been rearranged. This process of joining gene segments contributes to the diversity of antigen-binding sites in several ways.

**Junctional diversity:** When the selected V gene segment joins with a selected J and D gene segment in a B cell, nucleotide bases may be added or deleted at the point of stitching thus creating junctional diversity.

**Somatic hypermutation/ affinity maturation:** With the passage of time after immunization, there is an increase in the affinity of the antibodies produced against the antigen. This phenomenon is known as **affinity maturation**. This is due to the accumulation of point mutations specifically in the variable regions of both the heavy-chain and light-chain coding sequences. The mutations occur long after the coding regions have been assembled, when B cells are stimulated by antigen and helper T cells to generate memory cells in lymph nodes. Because this is about a million times greater than the spontaneous mutation rate in other genes, the process is called **somatic hypermutations**. The molecular mechanism is still uncertain, it is believed to involve some form of error-prone DNA repair process targeted to the rearranged V-region coding sequence by specific regions of DNA brought together by VDJ joining.

Only a fraction of the altered antibodies have an increased affinity for the antigen. The B cells expressing these higher-affinity receptors preferentially proliferate in response to the antigen, whereas the remaining B cells die by apoptosis. Thus, as a result of repeated cycles of somatic hypermutations, followed by antigen-driven proliferation of selected clones of memory B cells, antibodies of increasingly higher affinity become abundant during an immune response, providing progressively better protection against the pathogen.

A pictorial representation of the process by which of antibody diversity is generated is shown below.






**Clonal deletion and autoimmune diseases**: A person's body produces billions of different antibodies. Many of these randomly generated antibodies have the potential to recognize proteins that are made in one's own body. The immune system however has a way of distinguishing antibodies that act against "self" antigens from those that are against "non-self or foreign" antigens. It does so by destroying or preventing the proliferation of any antibody-producing cell or TCR producing cells that recognizes a self-made antigen. Furthermore, the coordination of adaptive and innate immune response also helps in distinguishing self from non-self (if an antigen generates an innate immune response it is a non-self antigen). This happens during the maturation of T and B cells in thymus and spleen.

**T cells:** There are multiple subtypes of T cells:

**-T-Helper ($T_H$)** express CD4 surface protein; are involved in activating B cells by producing cytokines.

**-T-cytotoxic cells ($T_C$)** express CD8 surface protein; can recognize and kill infected cells.

Each T cell has a unique T cell receptor (TcR), which is specific to an antigen and is produced by DNA rearrangement of the TcR gene as described earlier for the antibody gene.

T cells can recognize an antigen only if it is presented on the surface of antigen presenting cells (APC) or infected cells through MHC macromolecules. The $T_C$ cells recognize antigen presented on the surface of infected cells through MHC- Class I (specific for cytosolic antigen). In comparison, $T_H$ cells recognize antigens presented by the APC cells on their surface through MHC-Class II (specific for antigens derived from exoplasm). Non-compatibility between the different

polymorphic MHC- Class I molecules is the major cause of organ transplant rejection.

**Immunological memory** is a property of the adaptive immune response. We know this because the second time the immune system encounters the same antigen the response of B cells and T cells is faster, stronger and more specific due to the memory cells (such as the memory B cells as discussed in lecture) generated during the primary immune response. The principle behind vaccination is to expose an organism to some of the antigens of a harmful foreign particle that will create the immune system's memory in case the actual entire foreign particle ever invades that organism.

**Solution Key**

**1.** Some vaccines are injections of viral particles that have been inactivated in some way (such as extreme heat). Other vaccines are injections of a single viral protein that has been purified and produced using recombinant DNA techniques. How does a vaccine work? *Vaccination is the process of injecting either a weakened form of a virus or a single viral protein into an individual. Since both the weak virus and introduced viral protein are regarded as foreign particles by the host immune system, it results in cell mediated and /or humoral immune response and the production of memory B and T cells. If the individual encounters a viral infection later in life, these memory B and T cells can rapidly proliferate to produce more of their own kind and the antibody secreting plasma cells. This results in a rapid and strong secondary immune response that can combat viral infection.*

**2.** Each one of your gametes contains 3 X $10^9$ base pairs of DNA in its nucleus. How many base pairs of DNA are contained within:

**a)** Each nerve cell? *2 X (3 X $10^9$ base pairs) since it is diploid.*

**b)** Each antibody-producing white blood cell? *2 X (3 X $10^9$ base pairs) minus the base pairs that are lost after VDJ joining.*

**3.** The cellular arm of the immune system employs cytotoxic T lymphocytes (Tc) and natural killer cells. The $T_C$ cells can recognize body cells that have been infected by the virus. **Explain** why the Tc lymphocytes do not recognize an infected cell if the virus is dormant (latent) i.e. there is almost no or very little proliferation of the virus after infection.

*If the virus is latent, the virus protein will not be presented on the surface of infected cells through MHC-I molecules and will therefore not be recognized by $T_C$ cells.*

**4.** What is the cause of autoimmune disorders?

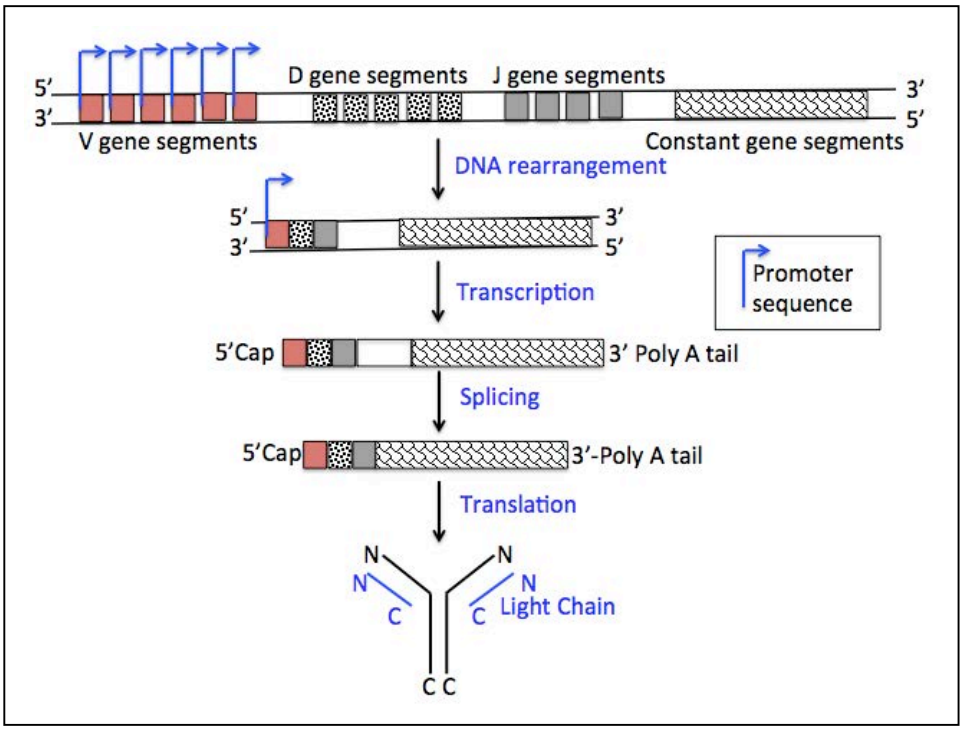
*The patient's self-reacting immune cells are not completed eliminated by negative selection during the course of development of the immune system.*

**5.** Shown below is a schematic of the production of a heavy chain polypeptide for an antibody. At the top is the chromosomal arrangement found in an immature B cell, at the bottom is shown the heavy chain polypeptide.

**a)** Label the process indicated by each arrow. By choosing from: each from: **transcription, translation, translocation, ligation, DNA rearrangement, splicing**

**b)** Indicate on the diagram the promoter region.

**c)** Draw the light chain of the antibody and label its N and C termini.



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