7.013 Recitation 17 – Spring 2018

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

Summary of Lectures 29 (4/27) and 30 (5/2)

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

Immunogen: Any foreign substance that elicits an immune response.

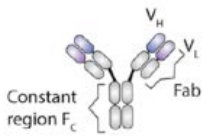
Innate Immune response: The **mechanical and the chemical barriers** such as the skin epithelium, saliva, mucous, etc provide the first line of defense and take care of approximately 99% of the infection. If however, these barriers are breached the **2nd line of defense, the innate immune response** kicks in.

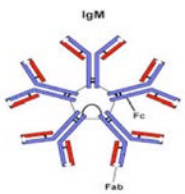
The innate immune response is not antigen-specific and does not generate immune memory; it is the **2nd line of defense**. However, it provides defense every time there is an infection. This commences immediately upon pathogen entry and involves cells such as macrophages, neutrophils, Natural killer (NK) cells etc. If the phagocytes cannot rapidly eliminate pathogen, inflammation is induced with the synthesis of cytokines and acute phase proteins and activation of the complement cascade. For the remaining infections we need the stronger, delayed and more specific adaptive immune responses, which form the **3rd line of defense**.

The lymphocytes originate from the hematopoetic stem cells (HSCs) in the bone marrow. The B lymphocytes mature in the bone marrow but differentiate in spleen and the T lymphocytes mature in the Thymus. The Thymus and bone marrow make the primary lymphoid organs. Both T and B cells get activated once they encounter the antigens either in spleen or lymph nodes (the secondary lymphoid organs).

Anything that is foreign is collected by the trash collecting cells or dendritic cells (or the antigen presenting cells, APC) of the immune system. They reach the site of infection through chemotactic signals that are provided by the chemokines. These dendritic cells then engulf the foreign substance (or antigen) and present it on their surface through Major histocompatibility complex (MHC) molecules to activate T_H or B cells of the adaptive immune response. The activated B cells then make and secrete antibodies, which can bind to and neutralize the antigens (neutralization). In contrast, the activated T_C cells (cytotoxic T cells) secrete proteins / enzymes (granzymes or perforins) that can poke holes in the membrane of cell infected by the antigen and thereby kill the infected cell. The two interactions are diagramed above.

Antibody production by the B cells: B cells make antibodies, which recognize and direct an attack against foreign entities. Antibodies are usually Y shaped protein molecules each composed of 2 heavy and 2 light chains, each Y shaped structure has two identical antigen binding sites (F_{ab}) and two heavy chains have a constant region (F_C region), the heavy and the light chains are held together by covalent disulfide bonds (S-S bonds). There are different types/ classes of antibodies; two radically different ones are IgG and IgM. IgM forms a rossetta shaped structure that binds avidly to the antigen. This activates the complement cascade that drills holes that forms the membrane attack unit to destroy the antigen.





Puzzle of antibody production: Immunoglobulins gene (Ig gene) has elements/ segments that make a stochastic choice, so that in each B cell one V segment joins with one J, or One V joins to form the light chain and One V joins with one D and one J to form Heavy chain of the antibody molecule. This makes lymphocytes the only cell type whose genetic composition differs between identical twins.

If you analyze the Heavy (H) and the light (L) chains of an IgG molecule, you will see that each H chain is approximately 500 amino acids long whereas each L chain is approximately 200 amino acids long.

- So coding region corresponding to 500 amino acids long H chain = 500 codons i.e. 1500 bp
- Coding region corresponding to 200 amino acids long L chain = 200 codons i.e. 600 bp
- Together this is (1500 + 600) = 2.1kb or approximately 2kb for each IgG antibody.
- We have 10^6 B cells each producing one unique antibody.
- If you assume that there is one gene for one antibody, then it comes to $2X\ 10^3\ X\ 10^6$ = **2×10^9bp**
- As per the above calculation, 66% coding capacity of our genome will be used just to create the B cell diversity!
- Now if we bring the T cells (10^6) into the picture each of which has its own unique TcR, then you need another 10^9 bp to account for this diversity.
- But our genome is only a 10^9 bp!
- So diversity of TcR and Ig CANNOT be created at the germline level. Instead, there are other mechanisms involved.

What are Bence Jones proteins? Myeloma is a type of hematological malignancy caused due to excessive proliferation of antibody producing neoplastic plasma B cells. If the urine samples of a myeloma patient are analyzed, one can detect monoclonal (only one kind) of immunoglobulin light chain. They can be κ (most of the time) or λ chains. The light chains can be immunoglobulin fragments or single homogeneous immunoglobulins.

If you analyze and compare the light chains from the urine samples of many myeloma patients, you see that they are unique for each patient but the difference is only in the first few amino acids (circled below) at the N terminus of the light chains. It is this part that forms the Fab (antigen-binding) portion of the antibody and reacts with the antigen.

Similarly, if you analyze the structure of antibody isolated from the serum of these patients, you will again see variation in the hypervariable regions (HV1, HV2 and HV3), which are close to the N terminus of the variable regions and in contact with the antigen/ immunogen. These hypervariable regions are the loops that connect different domains of the antibody molecule the H and the L chains.

Tonegawa's experiment: He isolated the mRNA corresponding to the L chains from the neoplastic plasma B cells of the myeloma patients. He radiolabeled it to use it as a probe in his experiments. He isolated the Ig gene from the somatic cells (muscles, liver, brain) and subjected it to digestion by a restriction enzyme. He also isolated the Ig gene DNA from the neoplastic plasma B cells of these patients and digested it with the same restriction enzyme. He ran these on the gel, transferred the electrophoresed DNA on a nitcrocellulose membrane, denatured them and hybridized them with radiolabeled mRNA. He found that it hybridized only with the DNA from the plasma B cells and not the rest. So clearly the Ig gene in germ-line or any other somatic cell was different from that in plasma B cells of the patient. The gene has undergone DNA rearrangement!

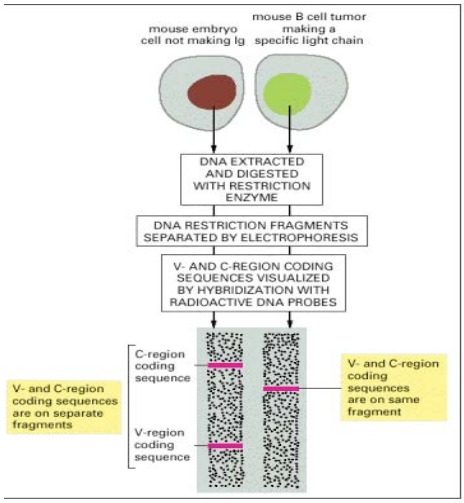


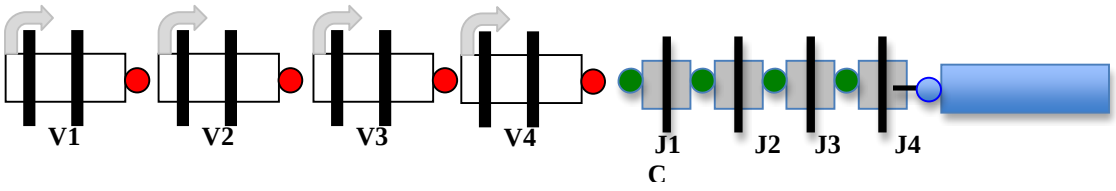
Figure 24-36
Drawing of an experiment that directly demonstrates that DNA is rearranged during B cell development. The B cell tumor arose from a single B cell and therefore makes a single species of antibody molecule. The two radioactive DNA probes used are specific (more...)

Nobel lecture:

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

This discovery ended up describing the process of generation of antibody diversity to show how limited amount of genetic information can yield such a vastly diverse antibody repertoire.

We now know that each type of antibody light chain (κ light chains, λ light chains), and heavy chains—has a separate pool of **gene segments** and exons from which a single polypeptide chain synthesized. Each pool is on a different chromosome and contains a large number of gene segments encoding the V region of an antibody chain and a smaller number of exons encoding the C region. 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**. In addition to bringing together the separate gene segments and the C-region exons of the antibody gene, these rearrangements also activate transcription from the gene promoter (which are 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 synthesized only after the DNA has been rearranged. This process of joining gene segments contributes to the diversity of antigen-binding sites in several ways.



V represents the variable gene segments, J the joining gene segments and C the constant region of the light chain of Antibody (Ab). The red and green circles represent the recombination recognition sequences, the blue circle shows enhancer sequence and the gray arrow shows the promoter. The V(D)J recombinase are encoded by two closely linked genes called *rag-1* and *rag-2 (rag* = *r*ecombination *a*ctivating *g*enes). The **RAG proteins** introduce double-strand breaks at the flanking DNA sequences, and this is followed by a rejoining process that is mediated by both the RAG proteins and the enzymes involved in general DNA double-strand repair.

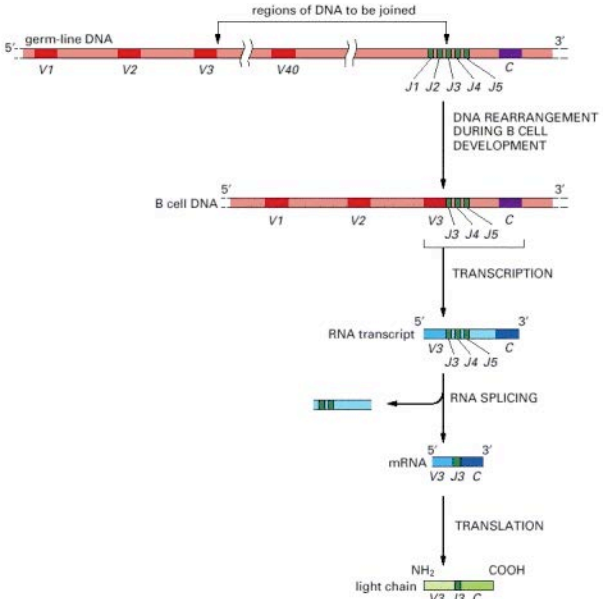
Junctional diversity: When the selected V segment joins with a selected J segment in a B cell, nucleotide bases can be added or deleted at the point of stitching thus creating junctional diversity.

Terminal deoxynucleotide transferase (TdT) enzyme further adds to the diversity by randomly adding bases at the point of joining of different segments. **Activation-induced deaminase** (AID) can also convert C to U thus creating further diversity. If however, the B cell produces a nonfunctional antibody it gets eliminated from the B cell repertoire.

Somatic hypermutation and affinity maturation: As mentioned earlier, with the passage of time after immunization, there is usually a progressive increase in the affinity of the antibodies produced against the immunizing antigen. This phenomenon is known as **affinity maturation**. This is due to the accumulation of point mutations specifically in both heavy-chain and light-chain V-region 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. They occur at the rate of about one per V-region coding sequence per cell generation. Because this is about a million times greater than the spontaneous mutation rate in other genes, the process is called **somatic hypermutation**. The molecular mechanism is still uncertain, but 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 V(D)J joining.

Only a small minority of the altered antigen receptors generated by hypermutation have an increased affinity for the antigen. The few B cells expressing these higher-affinity receptors, however, are preferentially stimulated by the antigen to survive and proliferate, whereas most other B cells die by apoptosis. Thus, as a result of repeated cycles of somatic hypermutation, 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 of antibody diversity is below.



There are 51 *V* segments, 27 *D* segments, 6 *J* segments, and an ordered cluster of *C*-region exons, each cluster encoding a different class of heavy chain. The *D* segment (and part of the *J* segment) encodes amino acids in the third hypervariable region, which is the most variable part of the V region.

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 that against

"non-self / foreign" antigens. It does so by destroying or preventing the proliferation of any antibody-producing cell that recognizes a self-made antigen.

So to summarize, our bodies make billions of different antibodies. These are proteins and thus are encoded by genes. However, our genomes contain less than 30,000 genes. So it is not possible that we would have a different gene to encode for each different antibody that we generate. The explanation for this is that there is a cluster of segments of genes (called V, D, and J segments) in the section of the genome that encodes antibodies. Every antibody-producing cell rearranges these DNA segments to join one V segment to one D segment to one J segment, thereby creating one gene that makes one antibody. Every different antibody-producing cell rearranges this cluster of DNA segments differently. Thus all antibody-producing cells contain only one gene that encodes an antibody, but every cell contains a different arrangement of that gene thus leading to antibody diversity. Other than cells of immune system, every cell in your body contains the exact same DNA as every other cell because no such rearrangement occurs.

The T cells: They can be of multiple subtypes: T-Helper (express CD4 surface protein) which are involved in activating B cells by producing cytokines, The T- cytotoxic cells (express CD8 surface protein) which can recognize and kill the infected cells.

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

The T cells can recognize an antigen only if it is presented on the surface of antigen presenting cells (APC) or infected cells through MHC. Tc recognize the antigen presented on the surface of infected cells through MHC-I (specific for cytosolic antigen). In comparison, the T_H recognize antigen presented by the dendritic cells (APC) on their surface through MHC-II (specific topology for the extracellular matrix antigen). Non-compatibility between the MHC-I is the major cause of organ transplant rejection.

The immune system displays memory; We know this because the second time the immune system encounters the same antigen, the response of B cells and T cells is faster and stronger. The principle behind vaccination is to expose an organism to some of the antigens of a harmful foreign particle that will spark the immune system's memory in case the actual entire foreign particle ever invades that organism.

A live virus will involve both TC and B cell mediated immune response since it infects a cell. In contrast, a foreign protein or a dead virus will elicit only a B cell response.

Questions

1. Viral infections can be treated using anti-viral drugs, or they can be prevented in the first place through the use of vaccines. Some vaccines are just 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?

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

a) Each nerve cell?

b) Each antibody-producing white blood cell?

c) Each red blood cell?

3. The cellular arm of the immune system employs cytotoxic T lymphocytes (T_c) and natural killer cells. The T_C cells can recognize the viral infected body cells.

a) What proteins are involved in the presentation of the antigen to the Tc lymphocytes?

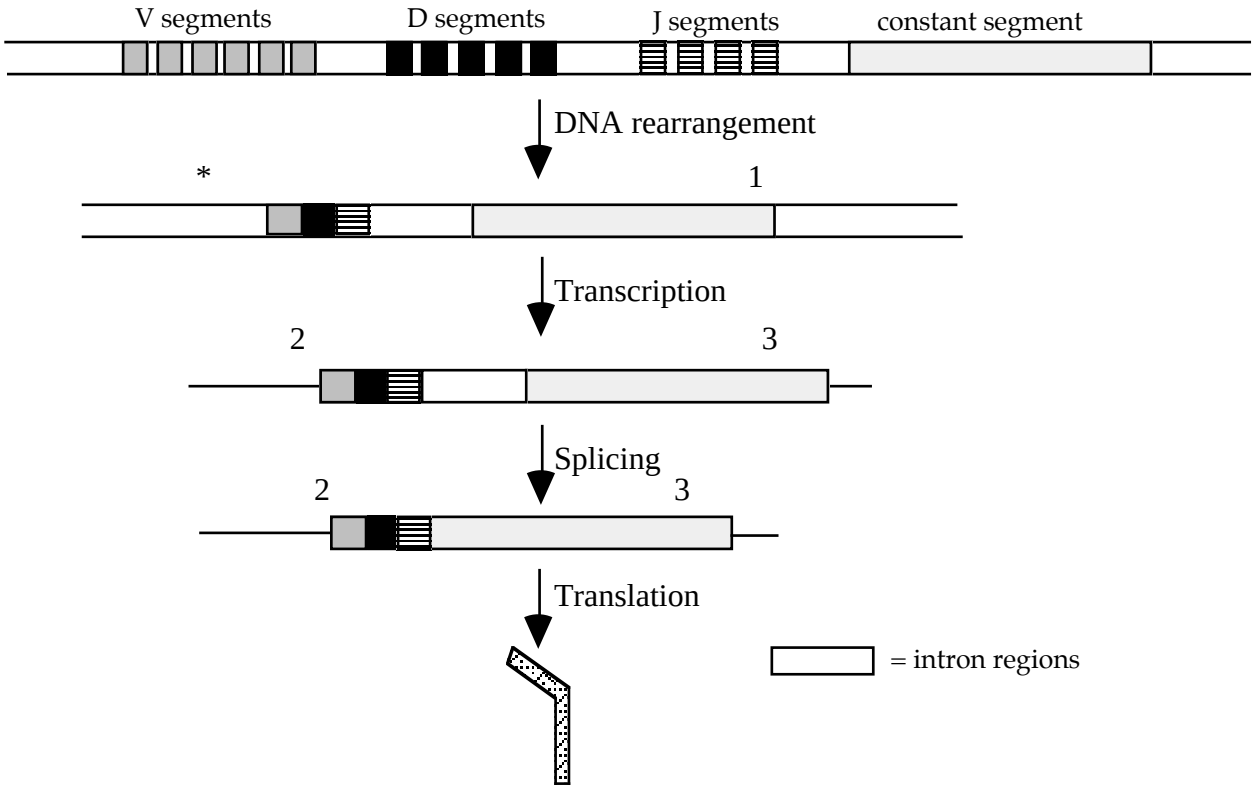
b) Explain why the Tc lymphocytes do not recognize an infected cell if the virus is latent i.e. viral capsid protein does not occur.

4. 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. Choose the one best option for each from: *homologous recombination, transcription, translation, translocation, ligation, DNA rearrangement, splicing*

b) Indicate on the diagram below where you would expect to find each of the following components: *Promoter (*), Transcriptional terminator (1), start codon (2), stop codon (3)*

c) Indicate on the diagram below the variable and the constant region of the heavy chain and the N and C terminus of this polypeptide.



5. What is cause of autoimmune disorders?

7.013 Introductory Biology

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