12

Immunology

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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Review (First 10 min of Video Lecture)

The topic of the last lecture was a bridge between innate and adaptive immunity. It is better to see innate and adaptive immunity as a continuum instead of separate entities. This is because these processes interplay with each other; cells of innate immunity interact with cells of adaptive immunity and vice versa.

- The word "lymphocytes" refers to B and T cells.
- B and T cells can recognize **different antigens**. T cell receptors are limited, they can only recognize linear peptides of a certain length that must be bound to the cleft of MHC molecules. This is why T cells exhibit **MHC restriction**. B cells, however, can recognize a wider range of antigens. They can recognize peptides, proteins, carbohydrates, lipids and metal ions.
- **MHC II** presents **extracellular antigens** to **CD4** T cells, while **MHC I** presents **intracellular** antigens to **CD8 cells**. There is a difference in the processing of these molecules and the antigens they present.
- **The antigen goes to the lymphocyte** - The number of lymphocytes is limited, therefore, it would not be efficient for lymphocytes to go and look for microbes that enter from the multiple portals of entry (skin, GI tract, respiratory tract) or are blood-borne. Instead, antigen presenting cells (APCs) can be found at these sites, and they capture the antigens (this is the first step of antigen presentation to lymphocytes). Then the antigen can be brought to the T cell
- **There are five APCs** – All express MHC II. The most efficient type is dendritic cells, which can activate naïve T lymphocytes. Macrophages and B cells activate effector T lymphocytes. There are also thymic epithelial cells (important for lymphocyte development) and vascular epithelial cells
- Once the **dendritic cell** captures the antigen, it is no longer a resting/immature dendritic cell. The cells mature and start expressing co-stimulators and the homing receptor CCR7 (chemokine receptor 7). In T cell rich zones, CCL19 and CCL21 are secreted and they are ligands for the homing receptor. This will recruit dendritic cells to the T cell rich zone. During this process, the dendritic cells will complete their maturation and process the antigen.
- **MHC I** – Intracellular antigens such as viruses and mutated proteins of tumor cells – They get tagged by ubiquitin, which sends them to the proteasome for degradation. At the same time, MHC synthesis occurs in the ER. The degraded peptide attaches to the TAP transporter, which will translocate the peptide into the ER. The TAP has high affinity for tapasin, which will allow the TAP to bring the peptide with it into the ER. Then, the peptide is loaded onto the MHC molecule, passed through the golgi apparatus, and finally sent through an exocytotic vesicle to the cell surface.

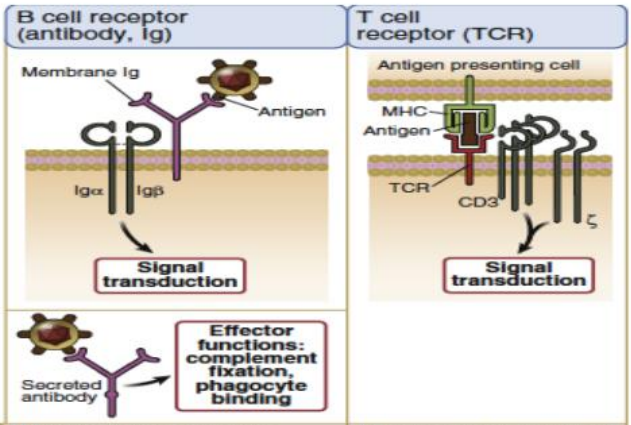
- **MHC II** – Extracellular Antigens – The antigen enters the cell in a membrane bound vesicle known as an endosome. This endosome becomes a phagosome, then it fuses with a lysosome to become a phagolysosome. This phagolysosome has a very low pH and enzymes such as cathepsins which cause degradation of proteins into peptides. MHC II synthesis occurs in the ER and immediately a protein, known as the invariant chain, binds to the cleft. This ensures that peptides that are meant to bind to MHC I won't accidentally bind to MHC II. The MHC II molecule is then sent to the phagolysosome and that's where loading will occur.

Antigen Receptors of Lymphocytes

This lecture discusses adaptive immunity, so the focus is on B and T lymphocytes. Lymphocytes must have receptors so that they can recognize antigens. Although B and T cell receptors differ in many ways, they both have constant and variable regions and antigens bind to the variable region.

B and T cell receptors differ in many ways. This includes:

1. **Form of Antigens Recognized** – As mentioned earlier, B cell receptors can recognize a larger variety of molecules, while T cells have MHC restriction.
2. **Location** – B cell receptors may be membrane bound or in a secreted soluble form. T cell receptors are always membrane bound.
3. **Effector Functions** – T cell receptors do not have any effector functions, while B cell receptors have effector functions done by the Fc portion of the antibody. Effector functions include complement fixation (activation) and phagocyte binding.



	B cell receptor (antibody, Ig)	T cell receptor (TCR)
Forms of antigens recognized	Macromolecules (proteins, polysaccharides, lipids, nucleic acids), small chemicals Conformational and linear epitopes	Mainly peptides displayed by MHC molecules on APCs Linear epitopes
Diversity	Each clone has a unique specificity; potential for $>10^9$ distinct specificities	Each clone has a unique specificity; potential for $>10^{11}$ distinct specificities
Antigen recognition is mediated by:	Variable (V) regions of heavy and light chains of membrane Ig	Variable (V) regions of α and β chains of the TCR
Signaling functions are mediated by:	Proteins (Igα and Igβ) associated with membrane Ig	Proteins (CD3 and ζ) associated with the TCR
Effector functions are mediated by:	Constant (C) regions of secreted Ig	TCR does not perform effector functions

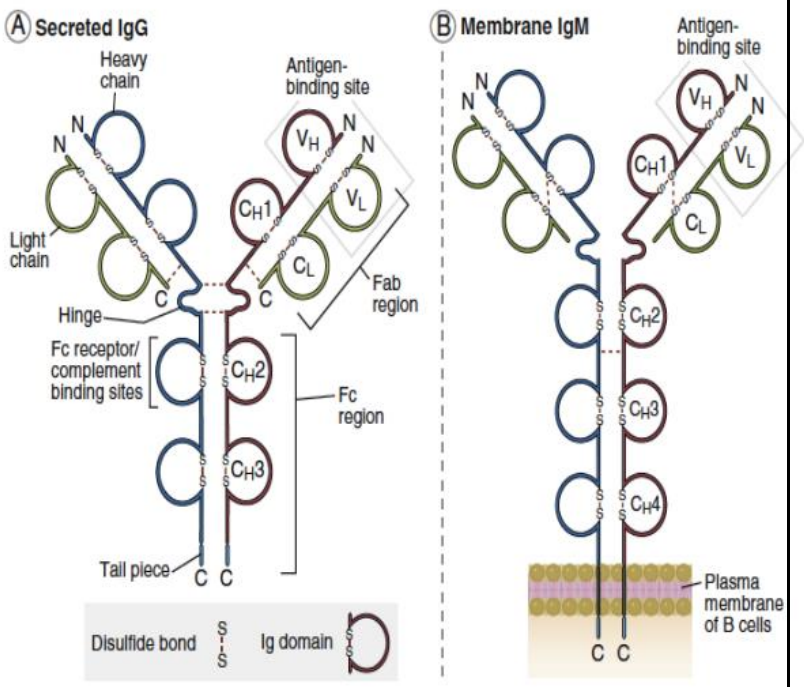
Antibodies

Antibodies = B Cell Receptors (BCRs) = Immunoglobulins (Ig)

These are all different names for the same molecule!

Structure

An antibody molecule is composed of four polypeptide chains—two identical heavy (H) chains and two identical light (L) chains—with each chain containing a variable region and a constant region.



- The light chain consists of 1 variable domain and 1 constant domain
- The heavy chain consists of 1 variable domain and 3 (when secreted) or 4 (when membrane bound) constant domains.

Each light chain is attached to a heavy chain and the two heavy chains are attached to each other by disulfide bonds.

Each variable domain also has **hypervariable regions** (also known as complementarity-determining regions {CDRs}). This is what gives the fine specificity of the **receptor**. The fine specificity of the **antigen** comes from the **epitope** (also known as the determinant). This basically means that these are the components that allow for the highly specific binding between an antibody and its antigen.

Parts of the Antibody:

1. Fab (fragment of antigen binding) region - The part of the antibody that binds to the antigen. Each antibody has two, identical Fab regions.
    a. The Fab region is composed of the variable and constant domain of the light chain and the variable and *first* constant domain of the heavy chain.
    b. As a monomer, an antibody can bind to 2 antigens for the two Fab regions (or two epitopes of the same antigen). However, antibodies can also exist as dimers, trimers, or pentamers. Therefore, they can be bind to up to 10

epitopes, for the 10 Fab regions present in an antibody that exists as a pentamer.

2. Fc (fragment that crystallizes) region - Responsible for most of the biologic activity and effector functions of the antibodies.
3. Hinge region – It is a flexible region between the Fab and Fc parts. It allows the two antigen-binding Fab regions of each antibody to move independent of each other. This allows them to bind to different epitopes separately.

Isotypes

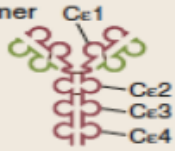
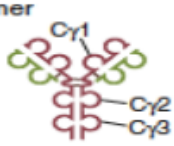
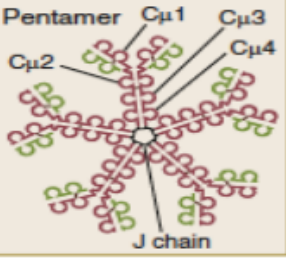
There are five types of heavy chains, called μ, δ, γ, ε, and α, which differ in their constant regions. Antibodies that contain different heavy chains belong to different classes, or isotypes, and are named according to their heavy chains (IgM, IgD, IgG, IgE, and IgA).

The antigen receptors of naive B lymphocytes, which are mature B cells that have not encountered any antigens, are membrane-bound IgM and IgD. After stimulation by antigen and helper T lymphocytes, the antigen-specific B lymphocyte clone may expand and differentiate into progeny that secrete antibodies.

These same B cells may produce antibodies of other heavy-chain classes. This change in Ig isotype production is called heavy-chain class (or isotype) switching. For example, a class switch may be from an IgM which recognizes antigen X to an IgG which also recognizes antigen X. **The heavy chains (more specifically, the Fc portion) can change during the B cell life span, but the light chain (which can be of κ-kappa or λ-lambda types) doesn't.**

The next table illustrates the different isotypes. While most of what the professor stated is in the table, there are a few additional points mentioned:

- Out of the four IgG subclasses, IgG1 and IgG3 are most common in humans. IgG antibodies have multiple functions, including complement fixation (activation), opsonization, and neonatal immunity (immunity transferred from the mother to the fetus since IgG antibodies are the only antibodies that can pass through the placenta)
- IgM in its **soluble form** is a pentamer. Therefore, it can bind to up to 10 different antigens or 10 different epitopes of the same antigen.

Isotype of antibody	Subtypes (H chain)	Serum concentration (mg/ml)	Serum half-life (days)	Secreted form	Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	Mainly dimer, also monomer, trimer 	Mucosal immunity
IgD	None (δ)	Trace	3	Monomer	Naive B cell antigen receptor
IgE	None (ε)	0.05	2	Monomer 	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3 or γ4)	13.5	23	Monomer 	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	Pentamer 	Naive B cell antigen receptor (monomeric form), complement activation

Binding

The parts of antigens that are recognized by antibodies are called epitopes (or determinants).

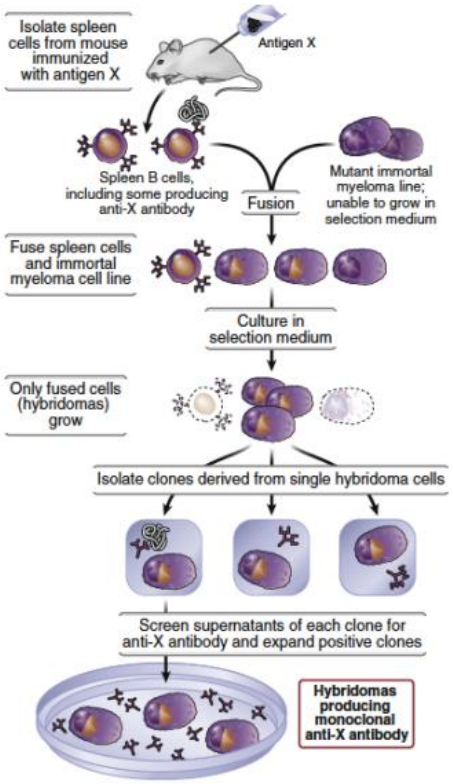
The strength with which **one** antibody binds to **one** epitope of an antigen is called the **affinity** of the interaction. Affinity can mature (affinity maturation).

The **total strength** of binding is much greater than the affinity of a single antigen-antibody bond and is called the **avidity** of the interaction. This considers the binding of all the units of the antibody (so it takes into account whether the antibody is monomeric, dimeric, etc.)

Antibodies produced against one antigen may bind to other, structurally similar antigens. Such binding to similar epitopes is called a **cross-reaction**.

Monoclonal Antibodies

The realization that one clone of B cells makes an antibody of only one specificity (recognizes one antigen) has been exploited to produce monoclonal antibodies. They have therapeutic and research uses.



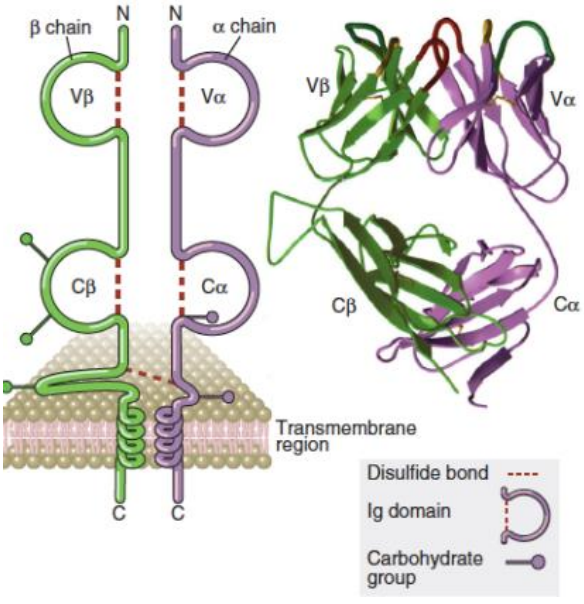
Making Monoclonal Antibodies

1. Antigen X is injected into a mouse. Then, the B cell population of the spleen is taken, as the spleen has clones of the B cells that produce antibodies that are specific to antigen X.
2. B cells have a short life span in vitro. Therefore, they are fused with plasma myeloma cells, which are immortal, tumor cells. This fusion results in a hybridoma cell.
    a. The plasma myeloma cell **must** be unable to produce its own antibodies. This is because we only want the antibodies produced by the B cell clones of the mouse.
3. Selection and expansion are done on the clones that produce antibodies against antigen X.

The issue with using these antibodies for medicinal purposes is that they originate from a mouse, which the immune system will recognize as foreign. Recently, monoclonal antibodies have been generated by using **recombinant DNA technology** to clone the DNA encoding **human** antibodies of desired specificity.

T Cell Receptors

The T cell receptor (TCR) is a heterodimer of two polypeptide chains, TCR α and β, covalently linked to each other by a disulfide bridge between extracellular cysteine residues.

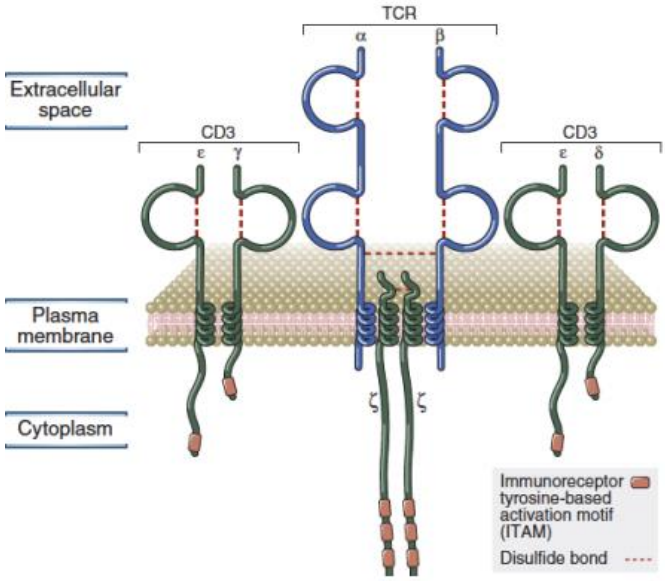


Each chain is made up of a variable and constant domain. The variable domains of the α and β chains form the antigen binding site of T cell receptors. Of course, there are also hypervariable regions within the variable region. The chain also has a transmembrane sequence.

TCRs do not exhibit class switching or affinity maturation.

While most T cells receptors are αβ, there is a small population of γδ (gamma delta).

The **T cell receptor complex** is a complex of the TCR and associated signaling molecules. These signaling molecules help in signal transduction after the antigen binds. For T cells these signaling molecules are CD3 and zeta (ζ).



Antibodies also have signaling molecules, Igα and Igβ. So, the B cell receptor along with these signaling molecules form the **B cell receptor complex**.

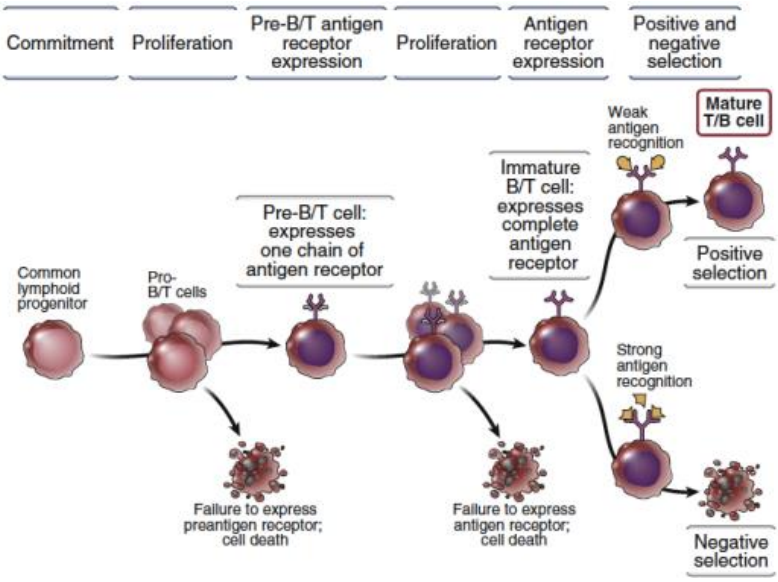
The Immune Repertoire

As mentioned earlier, the CDRs give the fine specificity of the receptor. But why do they need this high specificity?

As adaptive immunity is very specific, the antigen receptors of lymphocytes must be able to bind to and distinguish between many, often closely related, chemical structures. So each lymphocyte clone must have a unique receptor that can differentiate between distinct antigens. The process of lymphocyte maturation first generates a very large number of cells (perhaps as many as 10^9) before any encounter with antigens. This large collection of distinct lymphocytes makes up the immune repertoire. However, not all of these cells have useful receptors. The selection process now comes into play and promotes the survival of cells with receptors that can recognize antigens. Cells that cannot recognize antigens or have the potential to cause harm will be eliminated.

Remember that this specificity is for the sake of **antigen binding**. Therefore, although each clone of T lymphocytes recognizes a different antigen, the antigen receptors transmit biochemical signals that are fundamentally the same in all lymphocytes and are unrelated to specificity.

Lymphocyte Development



A Basic Idea:

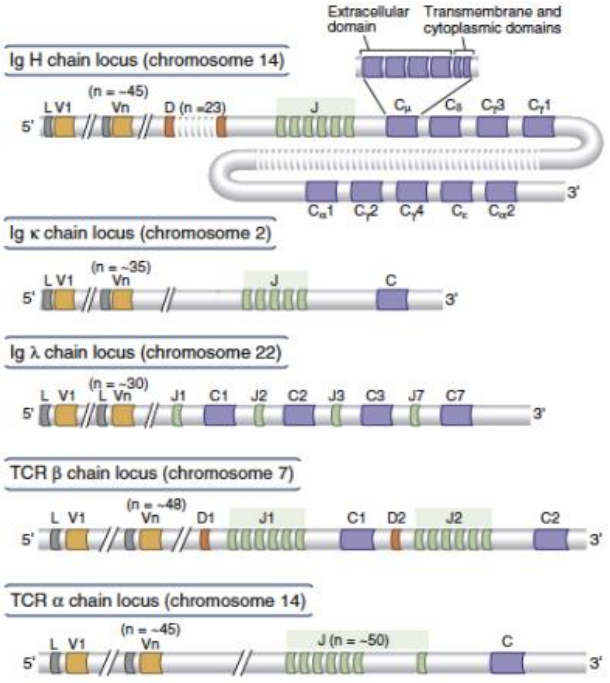
The first step in lymphocyte development is the commitment of common lymphoid progenitor cells to either B cell lineages or T cell lineages. Then, the cells go through cycles of proliferation and *antigen receptor gene rearrangement* (this is a very important step!). Finally, either positive or negative selection occurs.

Production of Diverse Antigen Receptors

All of the components of antigen receptors (i.e. heavy and light chains of B cells and alpha and beta chains of T cells) are inherited and gene encoded. The formation of functional antigen receptor genes is initiated by **somatic recombination**, or the **rearrangement** of gene segments that code for the **variable regions** of the receptors. This is what gives the large diversity of the immune repertoire.

Early lymphoid progenitors contain Ig and TCR genes in their inherited, or germline, configuration. In this configuration, Ig heavy-chain and light chain loci and the TCR α-chain and β-chain loci each contain multiple variable region (V) gene segments, numbering about 30-45, and one or a few constant region (C) genes.

Between the V and C genes are groups of several short coding sequences called diversity (D) and joining (J) gene segments. All antigen receptor gene loci contain V, J, and C genes, but **only** the Ig heavy-chain and TCR β chain loci contain D gene segments too.

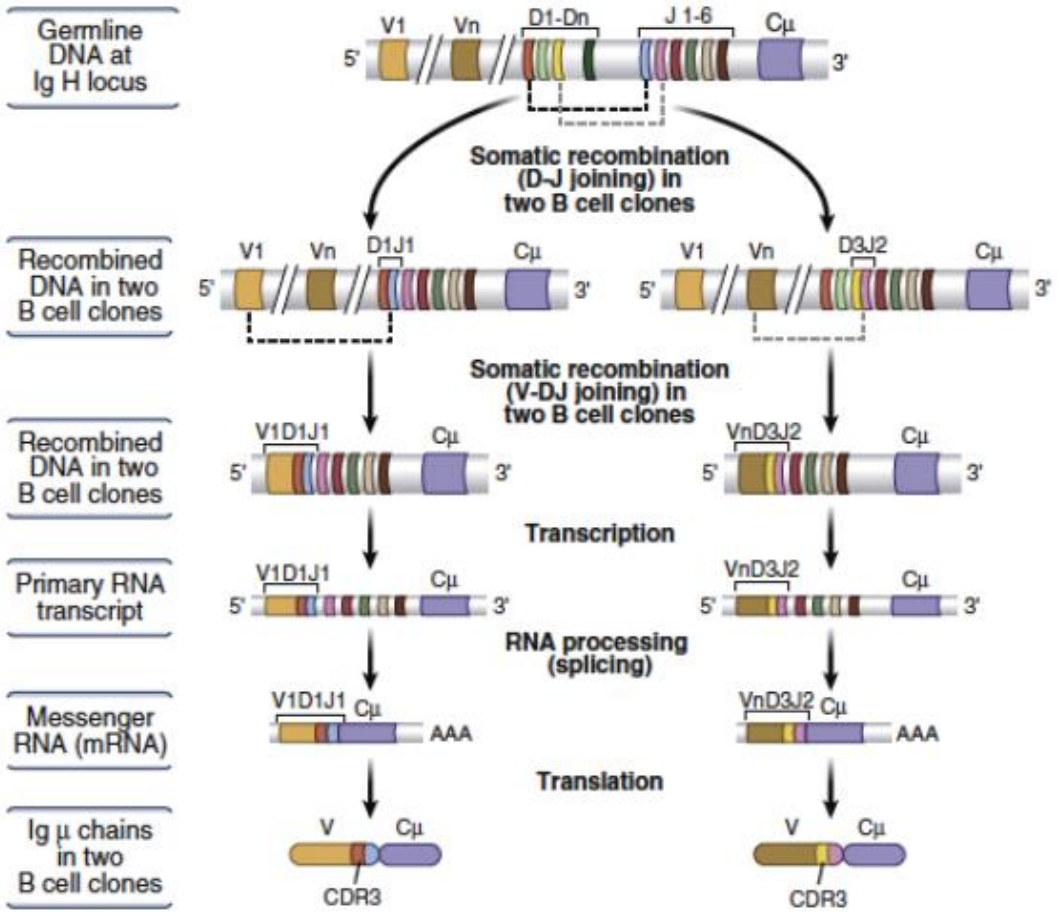


Antigen Receptor Gene Rearrangement

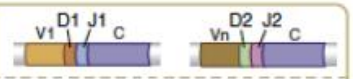
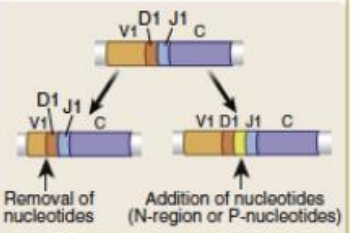
The somatic recombination of V and J, or of V, D, and J, gene segments is mediated by a lymphoid-specific enzyme, the VDJ recombinase (RAG-1 and RAG-2) proteins, and additional enzymes, most of which are not lymphocyte specific and are involved in repair of double-stranded DNA breaks introduced by the recombinase.

Types of Gene Rearrangement:

1. Combinatorial Diversity – The segments are rearranged to give different combinations of V, D, and J gene segments in different clones of lymphocytes. This gives a limited number of possible recombinations. Additionally, the number of nucleotides does NOT change.
2. Junctional Diversity – Endonucleases either add or remove nucleotides at the junctions of the recombining V, D, and J gene segments. A variable number can be added or removed, for example one segment may have 3 nucleotides removed and another segment may have 10 added. This is what gives unlimited outcomes of receptors.



The genes that encode for the variable domains range from 30-45 gene segments. This does give many different possibilities and diversity, because there are many different possible combinations of the V,D, and J segments. However, this diversity is still less than that given by junctional diversity (because you can add or remove as many nucleotides as possible).

	Immunoglobulin			T cell receptor	
	Heavy chain	κ	λ	α	β
Number of variable (V) gene segments	~45	35	30	45	48
Number of diversity (D) gene segments	23	0	0	0	2
Number of joining (J) gene segments	6	5	4	50	12
Mechanism					
Combinatorial diversity:					
Number of possible V(D)J combinations	Ig: ~3×10^6			TCR: ~6×10^6	
Junctional diversity:					
Total potential repertoire with junctional diversity	Ig: ~10^{11}			TCR: ~10^{16}	

Notice how junctional diversity gives a much larger potential repertoire

B Cell Maturation

B cell maturation occurs mostly in the bone marrow, but some maturation may also occur in the spleen.

1. Bone marrow progenitors committed to the B cell lineage proliferate, giving rise to a large number of precursors of B cells, called pro-B cells. (Commitment = pro-B cells). Pro-B cells do not express any receptors.
2. Then, it starts expression of genes for BCRs. The Ig heavy-chain locus rearranges first, and only cells that are able to make an Ig μ (IgM) heavy-chain protein are selected to survive and become pre-B cells. The assembled pre-BCR serves essential functions in the maturation of B cells. The IgM-expressing B lymphocyte is the immature B cell.
3. The mature B cell expresses both IgM and IgD. How does this occur? The mRNA for the heavy chain gets spliced. The result of this splicing is that the mRNA once transcribed gives IgM and IgD. This cell is now able to respond to antigens in peripheral lymphoid tissues.
4. Now selection must occur. **Positive selection** occurs for B cells that express intact, functional BCRs. So developing B cells are positively selected, based mainly on the expression of complete antigen receptors and not on the recognition specificity of these cells. **Negative selection** occurs for B cells that react strongly with self-antigens.

T Cell Maturation

1. T cell progenitors migrate from the bone marrow to the thymus, where the entire process of maturation occurs.
2. The least developed progenitors in the thymus are called pro-T cells or double negative T cells (or double-negative thymocytes) because they do not express CD4 or CD8.
3. The first thing expressed is the β chain of T cell receptors. Once it is expressed it is known as a pre-T cell
    a. TCR β gene recombination, mediated by the VDJ recombinase, occurs in some of these double-negative cells.
    b. If VDJ recombination is successful in one of the two inherited loci and a TCR β chain protein is synthesized, it is expressed on the cell surface in association with an invariant protein called pre-Tα, to form the pre-TCR complex of pre-T cells. If the recombination in one of the two inherited loci is not successful, recombination will take place on the other locus. If that too fails and a complete TCR β chain is not produced in a pro-T cell, the cell dies.
    c. The pre-TCR complex delivers intracellular signals once it is assembled, similar to the signals from the pre-BCR complex in developing B cells. These signals promote survival, proliferation and TCR α gene recombination. The signals inhibit VDJ recombination at the second TCR βchain locus (allelic exclusion). Failure to express the α chain and the complete TCR again results in death of the cell.
4. The alpha chain is expressed so that the cell expresses the complete αβ TCR and **both** the CD4 and CD8 coreceptors. These cells are called double-positive T cells (or double-positive thymocytes).
5. Selection of Mature T Cells from double positive to single positive.
    a. In all this maturation of B and T cells, and especially T cells, there are no foreign antigens. Therefore, in the selection process, recognition of self-peptides of self-antigens occurs.
        i. If the TCR of a T cell recognizes an MHC molecule in the thymus and interacts with low or moderate affinity, this T cell is selected to survive (positive selection).
        ii. Immature, double-positive T cells whose receptors strongly interact with the MHC molecule undergo apoptosis (negative selection). In abnormal cases of autoimmunity, the negative selection is not functional and the resulting T cells are very reactive to self-peptides.
    b. During this process, single positive selection occurs where the cell changes from having both CD8 and CD4 coreceptors to having only one.
        i. T cells whose TCRs recognize MHC I-peptide complexes become CD8 cells.
        ii. T cells whose TCRs recognize MHC II-peptide complexes become CD4 cells.