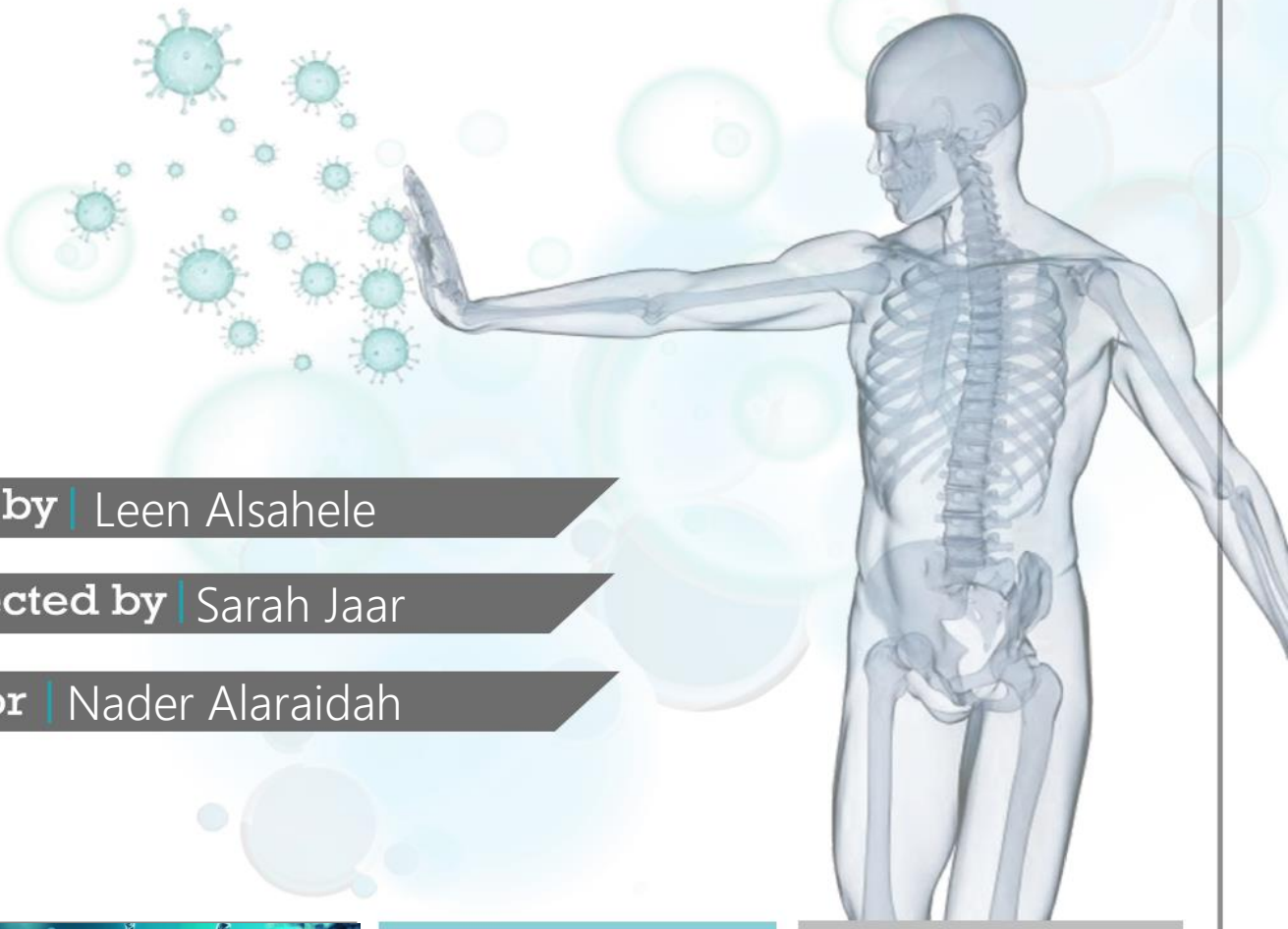
Sheet 12

# Immunology

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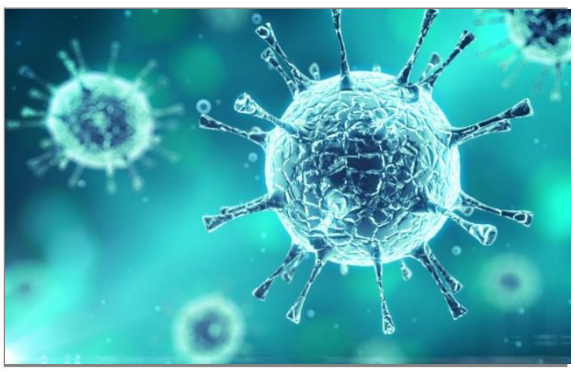


## Recap

It is better to see innate and adaptive immunity as a continuum rather than separate entities because they interplay with each other in several ways.

*The last lecture was a bridge between innate and adaptive immunity, and the 1st 1½ pages from this sheet are revision of the main topics of the previous lecture.

→What are the differences between antigens that are presented to T or B lymphocyte?

**T lymphocyte antigens** have to be linear peptides of a certain length & bound in self MHC molecule's cleft (these features are explained by the MHC restriction phenomenon)

**B lymphocyte antigens** can be diverse macromolecules (carbohydrates, lipids, protein antigens and metal ions) not only peptides.

Types of APCs:
1. Dendritic cells
2. Macrophages
3. B lymphocytes.
4. Vascular endothelial cells.
5. Thymic epithelial cells.

→There are many steps in the presentation of antigen to lymphocyte:

1- Antigen presenting cells **capture the antigen** at the site of entry.

The number of lymphocytes is limited in the body and they can't patrol all sites of entry of microbes. Microbes can enter the body by several ways: through skin, respiratory, GI, UT systems and through the blood.

Before APC captures the antigen, it's called a resting/immature dendritic cell.

2-After capturing, it will **become mature dendritic cell and it start to express co-stimulators as well as homing receptors** (CCR7). The ligands of CCR7 are *CCL19 and CCL21;* they are secreted from lymphocytes of T cell-rich areas. T lymphocytes will recruit dendritic cells to T cell rich zones. During that, maturation of dendritic cell is completed, and antigen processing starts.

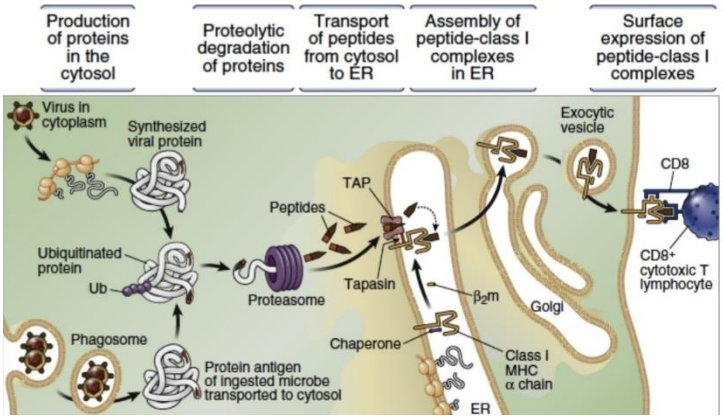
3- **Antigen presenting to lymphocytes**

What are the differences between the antigen processing of MHC II pathway and MHC I pathway?

### *1- MHC I pathway:*

If the foreign antigen is present in the ***cytosol*** (e.g. virus, mutated protein from a tumor cell) This defective protein gets tagged by ubiquitin, passing through the proteasome (cytosolic machinery for degradation of proteins) and gets degraded to peptides.

At the same time there is synthesis of MHC I molecule in ER.

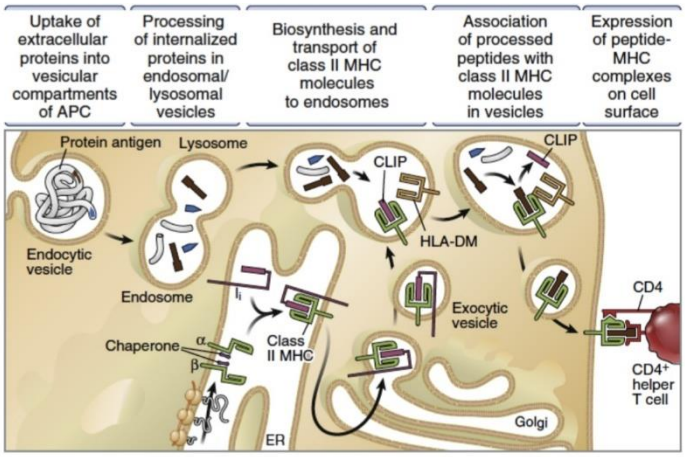



Then, TAP transporter (transporter associated with protein processing) attaches to the peptide that resulted from proteasomal degradation and translocates it into the ER. Why? Because TAP transporter has high affinity for an ER protein called Tapasin that allow entry of TAP transporter and associated peptide into the ER.

Peptide now is in ER, MHC I molecules were already produced, ***loading of peptide in MHC cleft occurs in the ER***. Then MHC I (with its peptide) comes out from ER and passes through the Golgi apparatus, then becomes an exocytic vesicle that moves towards the cell surface and finally activates Cytotoxic CD8 T-lymphocytes.

Cytosolic antigen→ tagged by ubiquitin→ passes through proteasome and gets degraded→TAP transporter attached to produced peptide→ go to ER→ loading of peptide into cleft of already-produced MHC I molecule→ go to Golgi apparatus→ exocytic vesicle→ to cell surface→ activate CTLs→ activate apoptosis of cell.

### 2- ***MHC II pathway:***




If we have extracellular antigens that get phagocytosed, they enter the cell in the form of membrane-bound vesicles (endosomes). The antigen now is in the phagosome, which fuses with lysosome and becomes phagolysosome (highly acidic pH). Proteolytic enzymes (like Cathepsins) start degrading proteins to peptides.

In the ER, there is MHC II synthesis: immediately after synthesis, a protein called the 'invariant chain' binds to the antigen-binding cleft to prevent peptides that come to the ER (MHC I antigens) from binding to the MHC II molecule.

MHC II leaves ER (with invariant chain inside it) and *goes to endosome, where loading takes place*. Then the peptide-MHC II complex continues the pathway through exocytic vesicle that travel to the cell surface and then activate Helper CD4+ T-cells.

Extracellular antigen→ phagocytosis →antigen is inside the phagosome→then phagosome fuses with lysosome → phagolysosome (acidic pH & contains proteolytic enzymes) → antigen degraded to peptide

MHC II is synthesized in ER →invariant chain binds to its cleft (to prevent binding of MHC I antigen peptides to MHC II) →leaves ER to endosome→ peptide loaded to MHC II molecule in endosome →exocytic vesicle→ cell surface→ activates CD4+ T helper cells.

## Adaptive Immunity

Topics of this lecture:

1-Structure of B cell receptors and T cell receptors

2-Lymphocyte development

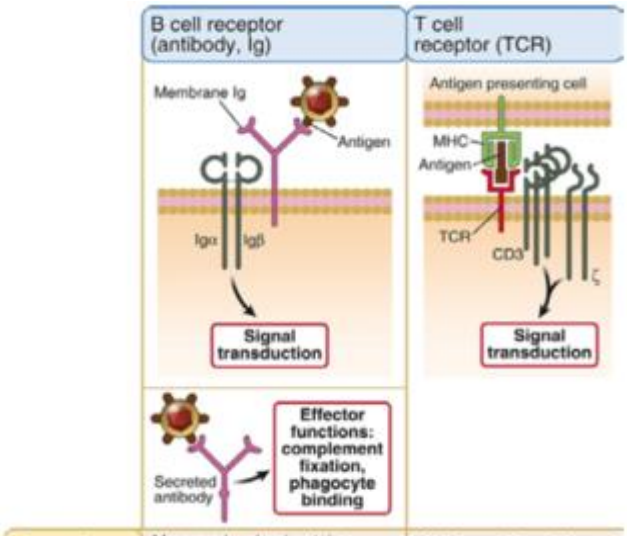
Differences between B cell receptors (BCRs) and T cell receptors (TCRs):

1-The antigen that is presented to the T cell has to be a linear peptide of a certain length & bound to self MHC molecule and presented on the surface of self APCs, whereas B cells receptor antigens can be of several types (carbohydrates, lipids, protein antigens and metal ions).

2-T cell receptors are only present as membrane-bound receptors, whereas B cell receptors are presents in two forms: membrane-bound and secreted/soluble form.

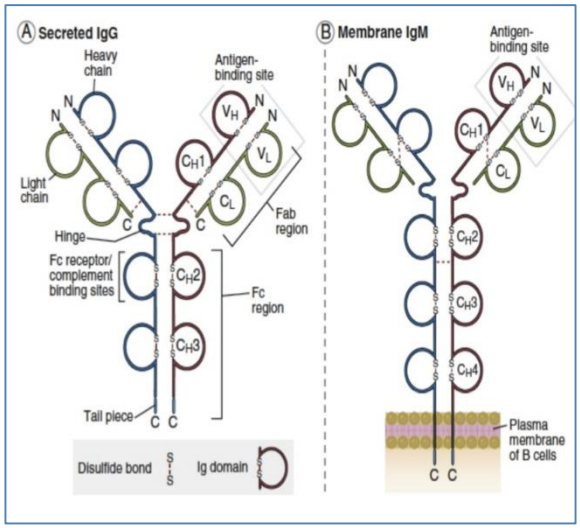
3-The antigen binding site in both of them is of the variable domain region.

4-T cell receptors don't have effector function. B cell receptors have effector functions via Fc portion of the antibody (phagocytosis, complement activation, ADCC…)




| | B cell receptor (antibody, Ig) | T cell receptor (TCR) |
|---|---|---|
| Forms of antigens recognized | Macromolecules (proteins, polysaccharides, lipids, nucleic acids), small chemicals<br>Conformational and linear epitopes | Mainly peptides displayed by MHC molecules on APCs<br>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 |

## B cell receptors BCRs (i.e. antibodies or immunoglobulins)




An antibody molecule is composed of four polypeptide chains: two identical heavy (H) chains and two identical light (L) chains. Each heavy chain binds to a light chain and the two heavy chains bind to each other by disulfide bonds

Light chain contains 1 variable domain and 1 constant domain

Heavy chain contains 1 variable domain and 3-4 constant domains (4 constant domains if it's membrane bound, and 3 if it's secreted form)

The antigen-binding site of an antibody is composed of the variable and constant domain of the light chain, plus the variable and 1st constant domain of the heavy chain.

If we fragment an antibody, we will find 2 different fragments: 2 identical Fab fragments (Fragment for Antigen Binding) and Fc region/portion (Fragment Crystallizable) that is responsible for most of the biologic activities and effector functions of the antibody.

As we said, antibody can come in 2 forms: either membrane-bound (anchored in the membrane) or soluble (not anchored).

In each variable domain, there are hypervariable segments/complementarity determining regions CDRs (3 short stretches in the V region of the heavy chain and 3 in the V region of the light chain) that give fine specificity of the receptor.

A monomer antibody can bind to 2 antigens (or 2 different epitopes/ determinants of the same antigen) because each Ig molecule has two identical Fab regions

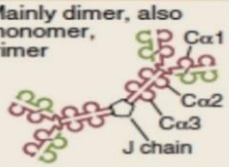
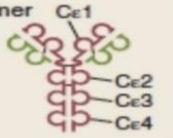
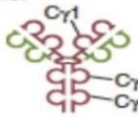
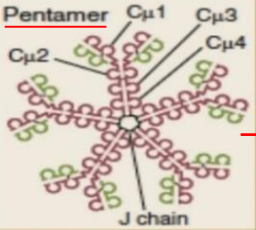
Between the Fab and Fc regions of most antibody molecules is a flexible portion called the **hinge region**. The hinge allows the two antigen-binding Fab regions of each antibody molecule to move independent of each other, so they can bind different antigens or epitopes.

Heavy chains determine antibody class or isotype, we have five classes of antibody: IgA, IgM, IgD, IgE as well as IgG

There is a feature of antibodies that is called class switching:

We said that the Fc portion determines the class of the antibody. So, if the same cell clones that produce IgM specific for antigen X are converted to produce IgG specific for antigen X, specificity did not change because it depends on the variable domain; what changed here is the constant Fc portion, so the class is changed. *This feature (class switching) is present in B cell receptors only and is not present in T cell receptors.*

Light chain has 2 types: kappa and lambda. Each B cell has either kappa or lambda -not both- and it won't change throughout its lifespan.

| Isotype of antibody | Subtypes (H chain) | Serum concentration (mg/ml) | Serum half-life (days) | Secreted form | Functions |
|---|---|---|---|---|---|
| IgA | IgA1,2 (α1 or α2) 2 subtypes | 3.5 | 6 | Mainly dimer, also monomer, trimer   | Mucosal immunity |
| IgD | None (δ) | Trace | 3 | Monomer | Naive B cell antigen receptor → Mature naïve B lymphocytes have 2 b cell receptors: IgM & IgD |
| IgE | None (ε) | 0.05 | 2 | Monomer   | Defense against helminthic parasites, immediate hypersensitivity |
| IgG | IgG1-4 (γ1, γ2, γ3 or γ4) 4 subtypes → 1+3 most found in humans | 13.5 | 23 | Monomer   | Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells → IgG is the only one to be transmitted vertically |
| IgM | None (μ) | 1.5 | 5 | Pentamer   | Naive B cell antigen receptor (monomeric form), complement activation → Can bind to 10 epitopes |

Information from the slides that we already know:

• The parts of antigens that are recognized by antibodies are called **epitopes** or **determinants**.
• The strength with which one antigen-binding surface of an antibody binds to one epitope of an antigen is called the **affinity** of the interaction. **affinity maturation can take place**
• 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 (avidity takes into account number of Fabs).

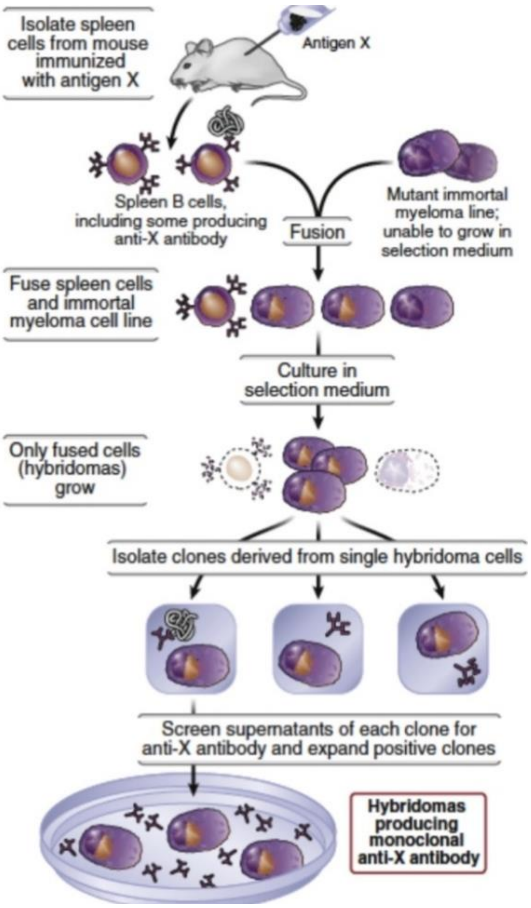
Antibodies produced against one antigen may *bind other, structurally similar antigens*. Such binding to similar epitopes is called **cross-reaction.**

We said that each B cell clone produces **monoclonal antibodies** specific for only single antigenic type. This feature has been exploited in medicine for therapeutic uses as well as research purposes.

How did this monoclonal antibody get extracted?

As you see in the right picture: 1-inject antigen X into a mouse 2-take a B cell population from the mouse's spleen 3-fuse them with plasma cells from an **immortal cell line** (myeloma that is unable of secreting its own immunoglobulins since we want only the immunoglobulins produced by the mouse) because B cells have short lifespan in vitro 4-immortal cells proliferate and produce new B cell clones 5-further selection & expansion of the clones that produce antibodies against antigen X. These fused cells are called **hybridomas.**

*The 1st problem in therapeutic purposes is that the origin of the antibody is from a mouse, so if we inject it into a human, their body will recognize it as foreign (use of recombinant DNA technology that produces human antibodies helps overcome the problem of mouse monoclonal antibodies).

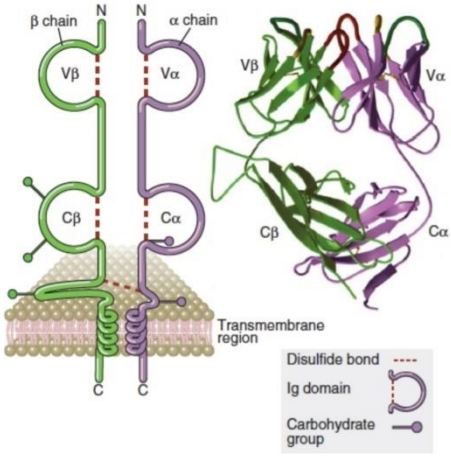



## T cells receptor complex and T cell signaling

The antigen receptor of CD4+ & CD8+ T-lymphocytes is a **heterodimer** consisting of two transmembrane polypeptide chains, designated TCR **α** and **β**, covalently linked to each other by a disulfide bridge between extracellular cysteine residues.

Each chain consists of a *variable domain* and a *constant domain.* The variable domains of **α** and **β** make the antigen binding site of T cell receptor.

T cell receptors, unlike B cell receptors, are always membrane-bound. But similar to B cell receptors, they have hypervariable regions that are called complementarity determining regions (CDR).

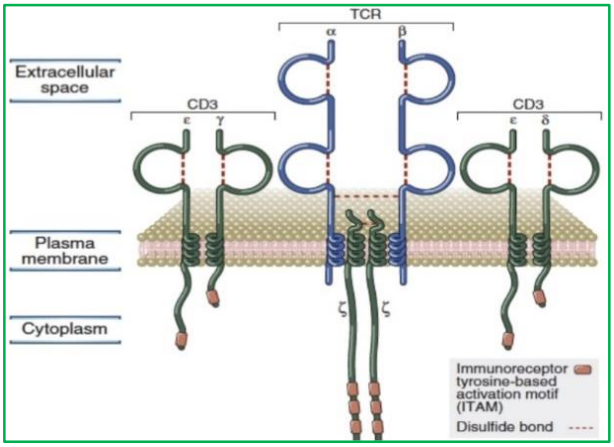


From slides (not mentioned by the doctor):

- T lymphocytes express different receptors that recognize antigens: T cell receptors (TCRs) on T lymphocytes.
- The antigen receptors of lymphocytes must be able to bind to and distinguish between many, often closely related, chemical structures.
- Each lymphocyte clone is specific for a distinct antigen and has a unique receptor, different from the receptors of all other clones.
- The total number of distinct lymphocyte clones is very large, and this entire collection makes up the immune repertoire.
- 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.

T receptor complex consists of the T-cell receptor (that binds the antigen) + signaling molecules:CD3 and zeta (ζ)

*Remember: B cell receptor complex consists of B-cell receptor (antibody) + signaling molecules: Igα and Igβ

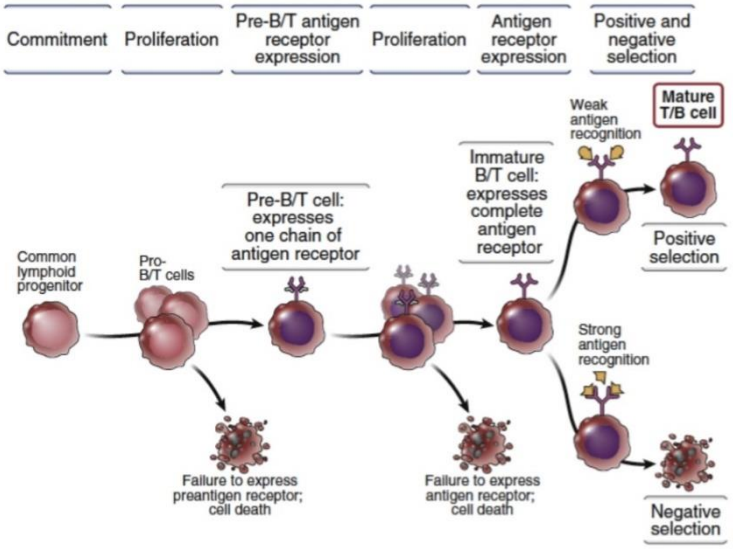



## *Lymphocyte development*

1st step in the development of lymphocytes (whether B or T) is the commitment of the common lymphoid progenitor cells to become either B cell lineages or T cell lineages.

Then, they go through cycles of proliferation and antigen receptor gene rearrangement (it is a very crucial step in the development of the lymphocyte).

Finally, there is a step of selection (positive and negative selection)




Production of Diverse Antigen Receptors:

Everyone has 2 copies of each chromosome (1 from the father and 1 from the mother).

In each chromosome there's a locus (group of genes) that encodes for the V domain and C domain of TCR, and V domain of light chain and heavy chain of BCR. All are inherited and gene-encoded. All antigen receptor gene loci contain V, J, and C genes, but only the Ig heavy-chain and TCR β chain loci also contain D gene segments.

What's happening is that throughout development, rearrangement of these segments occur.; whether it's change in their order between each other, or something else called junctional diversity. It is the same principle for T and B cell receptors.

Let's talk about B cell receptors: gene that encodes for heavy chain includes genes for the variable domain and genes for the constant domain. Between the genes for the V&C domains, there are short coding segments in the chromosome called ***joining*** (J) segments.

Also, there are coding segments called ***diversity segments***, found *only in the heavy chain in B receptors and in genes for the β chain in T cell receptors.*

What happens is that there's rearrangement of V, D, and J gene segments (one copy from father, other from mother, third from mother and so on); this leads to diversity in different clones of lymphocytes. Then there's expression in the form of T and B cell receptors because they encode for heavy chain or light chain in B cell receptors, alpha or beta chains in T cell receptors.

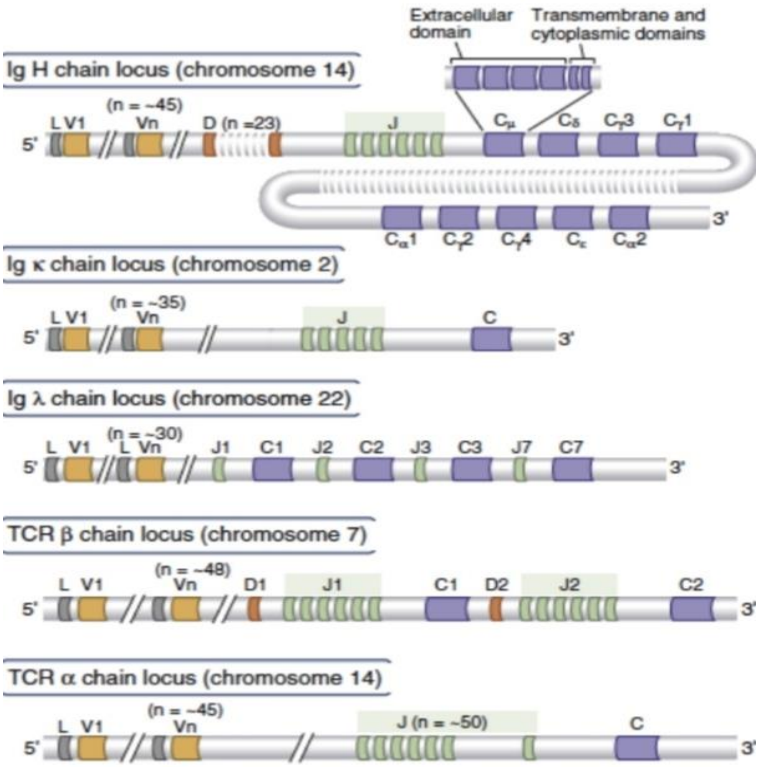
This rearrangement of the segments is called **combinatorial diversity.**

The other type is called **junctional diversity.** It results in an unlimited repertoire of B &T cell receptors.

Mechanism: The specific enzymes and exonucleases either insert or delete nucleotides (1 or 3 or 10 or any number of nucleotides) from the junction between those segments→ unlimited number of gene expression, that is called **antigen receptor gene rearrangement** (will be explained in lecture 14).

From slides: 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 the repair of double-stranded DNA breaks introduced by the recombinase.

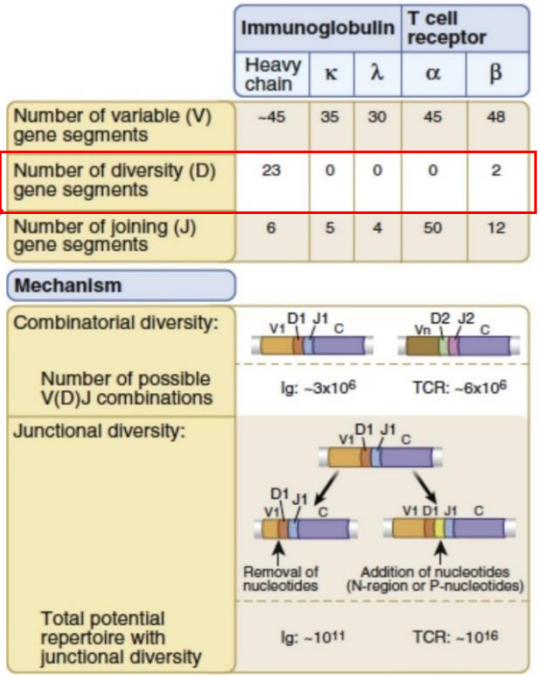
In this figure, you can see that the heavy chain of antibodies is encoded on chromosome 14. There are genes that encode for the variable domain and others encode for the constant domain. Between them, there are short coding signals (joining and diversity *present only on the heavy chain Ig or T cell receptor β chain*).



What happened is either rearrangement between these segments (combinatorial diversity) or change in the junction between them (junctional diversity).
So that way, the expression of them will be totally different → extremely diverse T & B cell receptors.

Genes that encodes for variable domain range between 30-45. This leads to lots of possibilities (but still less than junctional diversity).

Diversity region only present in the locus that encodes for heavy chain Ig as well as the locus that encodes for β chain of T cell receptor.




Let's go back to Maturation of lymphocytes (go back to the last picture on page 6)

Stem cells → common lymphoid progenitor cells→ commitment → B or T cell lineages → after commitment →pro-B/T cells (don't express any receptors) → pre-B/T cell

## Maturation and Selection of B Lymphocytes

→In bone marrow (some say in spleen)

→When common lymphoid progenitor cells in the bone marrow become committed to the B cell lineage, they proliferate, giving rise to a large number of precursors of B cells, called **pro-B cells** that don't express any receptor yet**.**

→Then, when V&C segments and segments between them (J&D) of the Ig heavy chain start to *rearrange*, coding for *heavy chain IgM* starts (that it is one of membrane antibodies on naïve B cell). And when expression of Ig heavy chain on B cell occurs, *many signals starts that either enhance survival or shut down the process*.

Only cells that are able to make an Ig μ 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.

→Then cells that express IgM are called **immature B cell**.

→The IgM+ and IgD+ cell is the **mature B cell**, able to respond to antigens in peripheral lymphoid tissues. Explanation: once the RNA that encodes for IgM heavy chain gets spliced, mRNA results; once the mRNA gets translated, it results in both IgM and IgD. Mature naïve B cell have to have 2 cell receptors on them (IgM and IgD).

→**Selection**: **positive** selection for B cells that express intact functioning B cell receptor (not based on the recognition specificity of these cells).
**negative** selection for B cells that react strongly with self-antigens.

## **Maturation and Selection of T Lymphocytes** (almost same as B lymphocytes)

T cell receptor rearrangement play a crucial role in maturation.

→T cell progenitors migrate from the bone marrow to the ***thymus***, where the entire process of maturation occurs.

→The least developed progenitors in the thymus are called **pro-T cells** or ***double-negative T cells*** (or double-negative thymocytes) because *they don't express CD4 or CD8.*

→ TCR β gene *recombination*, mediated by the VDJ recombinase, occurs in some of these double-negative cells.

If VDJ recombination is successful in one of the two inherited loci (maternal& paternal) 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.*

*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 and ***inhibit*** VDJ recombination at the second TCR β chain locus (**allelic exclusion**).

*Failure to express the α chain and the complete TCR again results in the *death of the cell*.

→The surviving cells *express* the complete αβ TCR and *both the CD4 and CD8 coreceptors*; these cells are called ***double-positive T cells*** (or double-positive thymocytes).

→**Selection:** **positive** selection: If the TCR of T cell recognizes **self MHC** (displaying a self peptide/not foreign antigen) molecule in the thymus, and interact with it in *low or moderate affinity*,
this T cell is selected to survive.
**negative** selection: If mature, double-positive T cells receptors *strongly recognize MHC-peptide* complexes in the thymus, they undergo apoptosis.
*In autoimmunity, negative selection isn't functioning properly.*

*During this process, selection from double positive to single positive cells occur. ***T cells that interact with MHC I*** (whose TCRs recognize class I MHC–peptide complexes**)** ***preserve the expression of CD8*** (the coreceptor that binds to class I MHC), ***and lose expression of CD4*** (the coreceptor specific for class II MHC molecules) and the other way around.

*The end*

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