Antigen Recognition in the Adaptive Immune System and Lymphocytes Development

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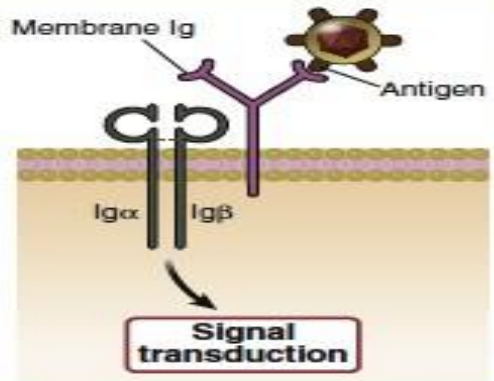
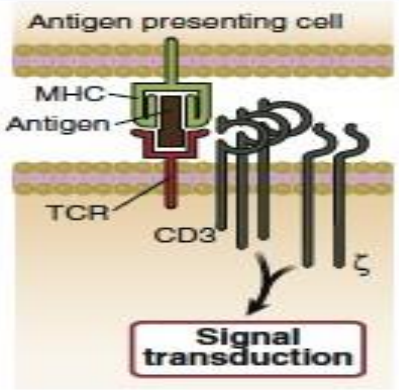
ANTIGEN RECEPTORS OF LYMPHOCYTES

Dr.’s Slides

	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

Additional Slide

ANTIGEN RECEPTORS OF LYMPHOCYTES

Lymphocytes are mainly divided into B lymphocytes and T lymphocytes, each defined by the type of antigen receptor they express.

- B lymphocytes express B-cell receptors (BCRs), which are also known as immunoglobulins (Ig) or antibodies.
- T lymphocytes express T-cell receptors (TCRs).

B-Cell Receptors (BCRs)
Structure and Forms

- BCRs are composed of two heavy chains and two light chains.
- In mature naïve B cells, the BCR is membrane-bound.
- After B-cell activation, the same receptor can be produced in a secreted (soluble) form, which is called an antibody.
- Therefore, BCRs exist in both membrane-associated and secreted forms.

Antigen Recognition
BCRs can recognize antigens:

- Directly (without processing)
- In their native conformational (3D) structure or linear structure

They can recognise a wide range of molecules, including: Proteins, Lipids, Polysaccharides, Nucleic acids or Small chemical moieties.

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ANTIGEN RECEPTORS OF LYMPHOCYTES

T-Cell Receptors (TCRs)
Structure and Binding

- TCRs are composed of α (alpha) and β (beta) chains in the majority of T cells (αβ T cells).
- TCRs are always membrane-bound and are never secreted.

Antigen Recognition and MHC Restriction

- TCRs cannot recognize free or native antigens.
- They recognize processed protein antigens (peptides) only.
- These peptides must be presented within the antigen-binding groove of an MHC molecule on a self antigen-presenting cell (APC).
- This phenomenon is called MHC restriction.

This rule applies to most T cells (αβ T cells).
Exception: γδ T cells can recognize certain ligands and non-classical antigens with less MHC restriction.

Feature	BCR	TCR
Antigen form	Native antigen	Processed peptide
Recognition	Direct	Indirect via MHC
Epitope type	Conformational or linear	Linear only
MHC restriction	No	Yes

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ANTIGEN RECEPTORS OF LYMPHOCYTES

Receptor Diversity and Clonal Nature

- Each B cell or T cell expresses ~10^4–10^5 identical receptors (BCR or TCR).
- All receptors on a single lymphocyte have the same antigen-binding specificity.
- A lymphocyte and all of its progeny form a **clone**, sharing the same idiotype.
- The total diversity of lymphocyte receptors reaches approximately **10^{16} specificities**.
- This enormous diversity **exists before any antigen exposure**, generated during lymphocyte development.

Antigen-Binding Sites (Paratope and Epitope)

- Antigen recognition occurs at the variable regions:
    - BCR: variable domains of heavy and light chains
    - TCR: variable domains of α and β chains
- Each variable region contains hypervariable regions called **Complementarity Determining Regions (CDRs)**.
    - BCR: 3 CDRs × 2 chains (heavy & light) = 6 CDRs
    - TCR: 3 CDRs × 2 chains (alpha & beta) = 6 CDRs
- The combined CDRs form the **paratope**, which binds the **epitope** or **determinant** on the antigen.

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ANTIGEN RECEPTORS OF LYMPHOCYTES

Constant Regions and Effector Functions

BCR (Immunoglobulin)

- The constant region of the heavy chain determines:
  - The isotype/class (IgM, IgG, IgA, IgE, IgD)
  - The effector function (complement activation, opsonization, placental transfer, etc.)
- The constant region of the light chain mainly provides:
  - Proper structural stability
  - Proper folding and secretion

TCR

- The constant regions of TCRs:
  - Do not mediate effector functions
  - Serve only in signal transduction

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ANTIGEN RECEPTORS OF LYMPHOCYTES

Receptor Complexes and Signaling Molecules

- B-cell receptor **complex**:
    - Immunoglobulin (BCR)
    - Associated signaling molecules: Igα and Igβ
- T-cell receptor **complex**:
    - TCR (αβ)
    - Signaling molecules: CD3 complex and ζ (zeta) chains
- These molecules are essential for transmitting activation signals into the cell.

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ANTIGEN RECEPTORS OF LYMPHOCYTES

Unique Processes in B Cells (Not in T Cells)

1. Receptor Editing

- Occurs in immature naïve B cells (haven't met the antigen yet).
- If a BCR is self-reactive, RAG enzymes can be reactivated.
- This leads to rearrangement of the light-chain variable region, altering specificity.
- Purpose: eliminate self-reactivity and allow survival (pass check point).
- This process does NOT occur in TCRs.

2. Affinity Maturation

- Occurs after antigen exposure in activated B cells.
- Involves somatic hypermutation and selection of higher-affinity clones.
- Results in antibodies with increased affinity.
- Affinity maturation does NOT occur in TCRs.

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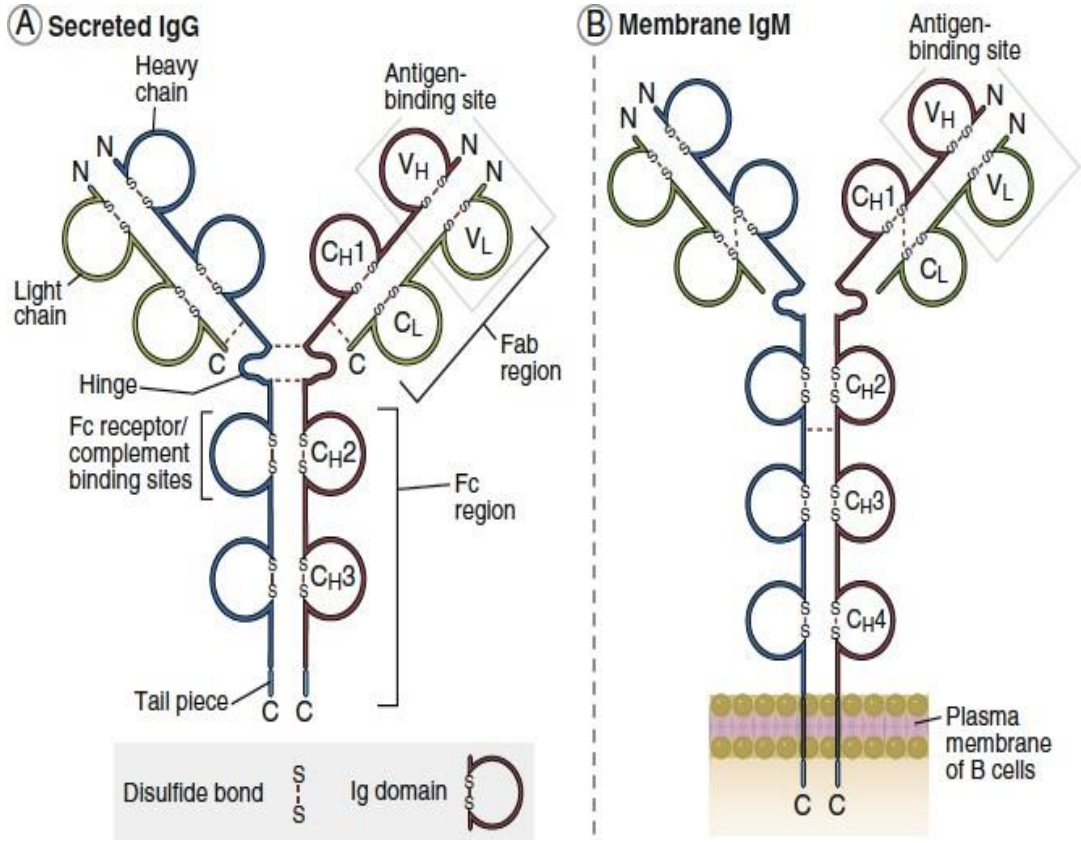
Antibodies

- 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 antigen-binding site of an antibody is composed of the V regions of both the heavy chain and the light chain, and the core antibody structure contains two identical antigen binding sites .
- In each Ig molecule, there are two identical Fab regions that bind antigen and one Fc region that is responsible for most of the biologic activity and effector functions of the antibodies.



Antibodies Structure

Dr.'s Slides

- 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.

- There are five types of heavy chains, called μ, δ, γ, ε, and α, which differ in their C 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 antigen, 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.

- The 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**

Additional Slide

Antibodies Structure

B-Cell Receptor (BCR) Structure

The B-cell receptor (BCR) is an immunoglobulin molecule composed of four polypeptide chains:

- Two identical heavy chains
- Two identical light chains

Light Chains

Each light chain consists of:

- One variable region (V^L) → involved in antigen binding
- One constant region (C^L)

Heavy Chains

Each heavy chain consists of:

- One variable region (V^H) → involved in antigen binding
- Three or four constant regions (C^H domains):
  - Three constant domains in the secreted (soluble) form
  - Four constant domains in the membrane-bound form
- A hinge region that provides flexibility

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Antibodies Structure

Immunoglobulin Classes (Isotypes)

The immunoglobulin class is determined by the type of heavy-chain constant region:

Heavy-chain constant region	Immunoglobulin class
μ (mu)	IgM
δ (delta)	IgD
γ (gamma)	IgG
ε (epsilon)	IgE
α (alpha)	IgA

Antibody Production During Immune Responses

Primary Immune Response

• During the first exposure to an antigen, the first antibody produced and secreted is IgM

Secondary (Memory) Immune Response

• Upon re-exposure to the same antigen: IgG (most commonly), IgA or IgE

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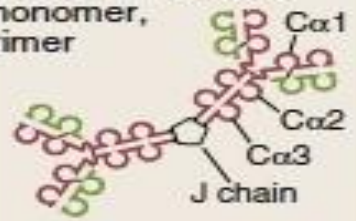
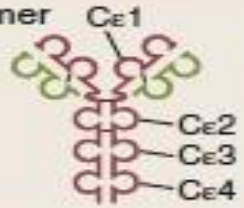
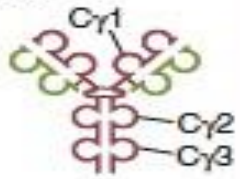
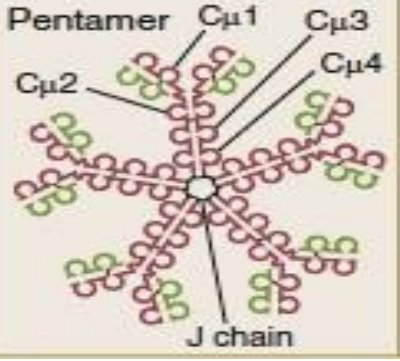
Isotype of antibody	Subtypes (H chain)	Serum concentration (mg/ml)	Serum half-life (days)	Secreted form	Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	Mainly dimer, also monomer, trimer 	Mucosal immunity
IgD	None (δ)	Trace	3	Monomer	Naive B cell antigen receptor
IgE	None (ε)	0.05	2	Monomer 	Defense against helminthic 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

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Antibodies

Immunoglobulin A (IgA) has two subclasses, **IgA1** and **IgA2**, and can exist in several structural forms, including a **monomer**, a **dimer**, and occasionally a **trimer**. Functionally, IgA is the principal immunoglobulin involved in **mucosal immunity** and predominates at major body **entry** sites.

Immunoglobulin M (IgM): exists in two main structural forms. The **monomeric** form is **membrane-bound** and is expressed on mature naïve B lymphocytes, where it functions as the B-cell receptor. The **pentameric** form is the **secreted** version of IgM. This pentameric structure is **stabilized** by a **J** (joining) chain, which links the individual monomers together. It functions in an **complement** activation.

A key distinguishing feature of IgA is the **secretory tail** that enables **trans-epithelial** transport. This feature differentiates IgA from IgM.

Immunoglobulin D (IgD) is primarily expressed on the surface of mature naïve B lymphocytes. Its expression results from alternative RNA splicing.

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Antibodies

Immunoglobulin E (IgE) is a monomeric immunoglobulin that plays a crucial role in defense against parasitic (helminthic) infections and in mediating type I (immediate) hypersensitivity reactions. These reactions underlie allergies and atopic diseases.

Immunoglobulin G (IgG) is the **most abundant** immunoglobulin in serum and is the dominant antibody produced upon **re-exposure** to a previously encountered antigen.

It has four subclasses—IgG1, IgG2, IgG3, and IgG4—with **IgG1** and **IgG3** being the most common and functionally active, while IgG4 has non-defined immune functions. IgG is a **monomer** and performs all the key roles of BCR, including **opsonization (tagging antigens),** fixation of the **complement system**, antibody-dependent cell-mediated cytotoxicity (**ADCC**), and negative feedback loops of **B-cell differentiation**. Importantly, IgG is the only immunoglobulin capable of crossing the placenta, thereby providing passive immunity to the newborn and protecting the infant up to 6 months of life.

Binding of Antigens by Antibodies

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

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Binding of Antigens by Antibodies

Antibody Structure and Enzymatic Cleavage

- Two proteolytic enzymes commonly used in immunology and molecular biology to study antibody structure are papain and pepsin.
- Papain cleaves the antibody produces three fragments:
- Two Fab (fragment antigen-binding) fragments (Each Fab contains one antigen-binding site)
- One Fc (fragment crystallizable) fragment
- Therefore, each antibody monomer has two antigen-binding sites.

Affinity and Avidity

- Affinity refers to the strength of binding between a single Fab site and a single epitope. It describes one-to-one interaction (molecule-to-molecule).
- Avidity refers to the overall strength of binding between an entire antibody (which has multiple binding sites) and an antigen.

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Binding of Antigens by Antibodies

Cross-Reactivity and Molecular Mimicry

- Sometimes antibodies generated against foreign antigens can bind to self antigens if they share structural similarity.
- This occurs because the antibody cannot distinguish between similar epitopes.

Streptococcus pyogenes Infection

- Group A β-hemolytic Streptococcus (Streptococcus pyogenes) commonly causes sore throat in children. The bacteria express **M protein** in their **cell wall.**
- After infection, the child clinically recovers, and antibodies are produced against M protein. Due to structural similarity, these antibodies **cross-react** with **Myosin** in **cardiac** muscle or structures in the **glomeruli**.

- This immune cross-reaction leads to Acute rheumatic fever or glomerulonephritis. These conditions are collectively called Post-streptococcal immune-mediated sequelae

- This phenomenon is known as **molecular mimicry**

Monoclonal Antibodies

- The realization that one clone of B cells makes an antibody of only one specificity has been exploited to produce **monoclonal antibodies.**
- To produce monoclonal antibodies, B cells, which have a short life span in vitro, are obtained from an animal immunized with an antigen and fused with myeloma cells (tumors of plasma cells), which can be propagated indefinitely in tissue culture
- by fusing the two cell populations and culturing them, it is possible to grow out fused cells derived from the B cells and the myeloma, which are called **hybridomas.**

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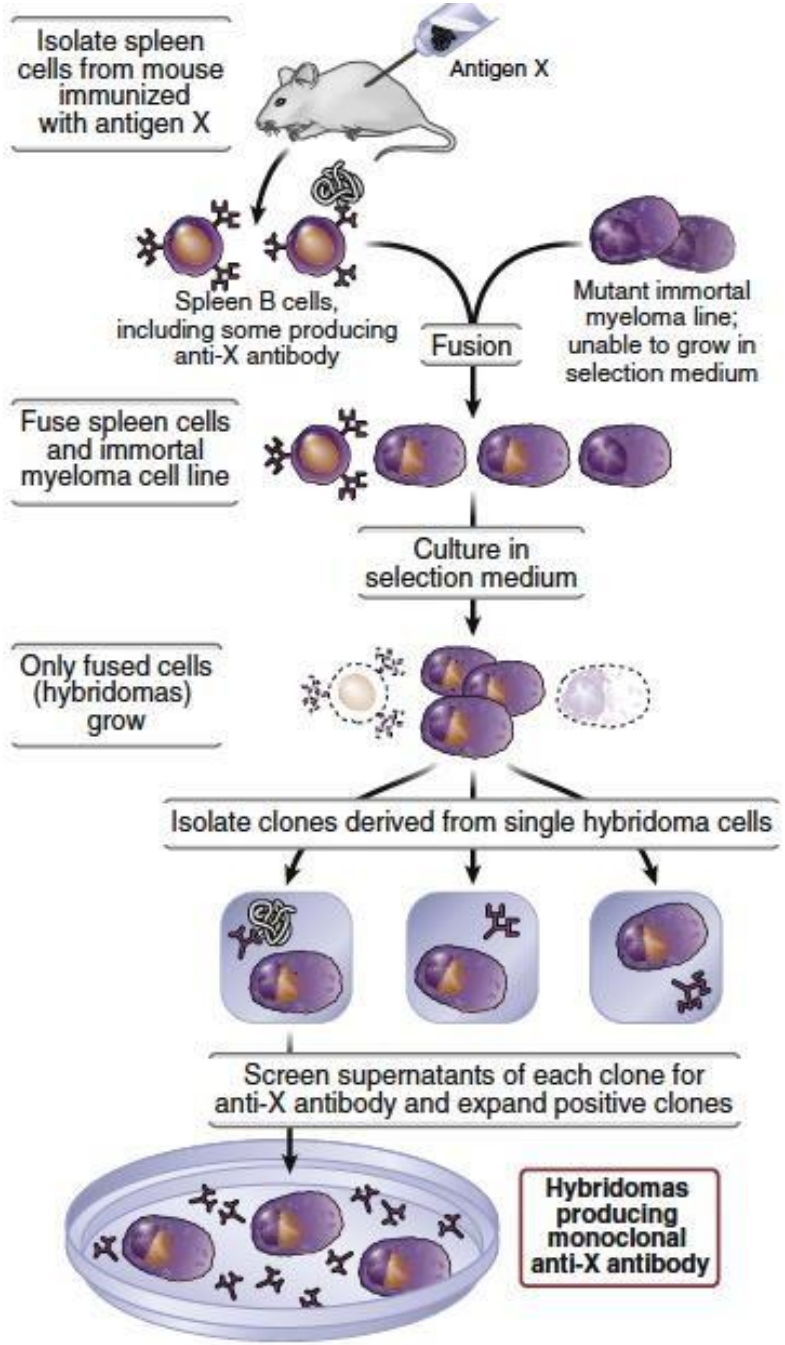
Isolate spleen cells from mouse immunized with antigen X
Antigen X
Spleen B cells, including some producing anti-X antibody
Fusion
Mutant immortal myeloma line; unable to grow in selection medium
Fuse spleen cells and immortal myeloma cell line
Culture in selection medium
Only fused cells (hybridomas) grow
Isolate clones derived from single hybridoma cells
Screen supernatants of each clone for anti-X antibody and expand positive clones
Hybridomas producing monoclonal anti-X antibody

Additional Slide

Monoclonal Antibodies

Monoclonal antibodies are a major breakthrough in immunology and modern medicine. They are widely used in **diagnostics** and in **therapeutics**, particularly for cancer treatment.

When a mouse is immunized with antigen X, multiple B-cell clones (parent B-cell with its progeny) are generated in peripheral lymphoid organs (like the spleen), specific for antigen X, and it produces antibodies specific to antigen X.
Knowing that mature naïve B cells and plasma cells are short-lived (days to weeks), whereas memory B cells are long-lived (years).

This process involves fusing normal splenic B cells (which produce antibodies but have a short lifespan) with myeloma cells (immortal B-cell malignancies that do not produce functional immunoglobulins). The fused cell, called a **hybridoma**, combines **immortality** from the myeloma cell with **antibody specificity** for antigen X from the B cell. Only hybridomas that survive and produce the desired antibody are selected.

Screening is performed using techniques such as ELISA, Western blot, antigen-antibody tests like precipitation, agglutination, or fixation. A single clone specific for antigen X is selected and expanded, producing identical, highly specific antibodies—this defines a monoclonal antibody.

Clinical Problem and Solution: Humanization
Mouse monoclonal antibodies can cause strong immune reactions in humans. This problem is addressed using recombinant DNA technology to produce humanized antibodies, reducing immunogenicity.

The T Cell Receptor Complex and T Cell Signaling

- **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.

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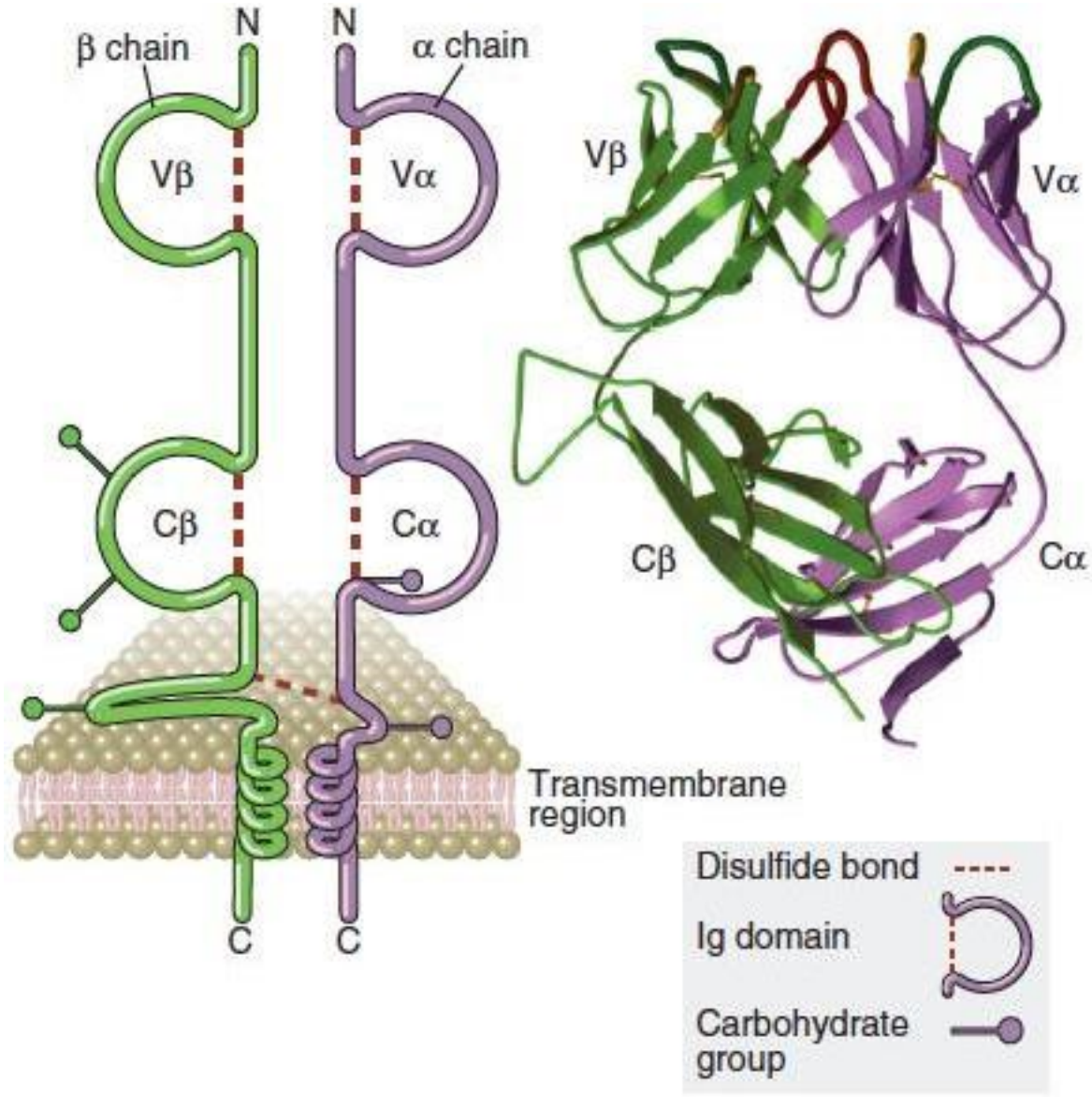
The Structure of the T Cell Receptor for Antigen

- The antigen receptor of MHC-restricted CD4+ helper T cells and CD8+ cytotoxic T lymphocytes (CTLs) 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.

Additional

Each chain contains a **variable** (V) domain, a **constant** (C) domain with a transmembrane region and a cytoplasmic tail (signal transduction).



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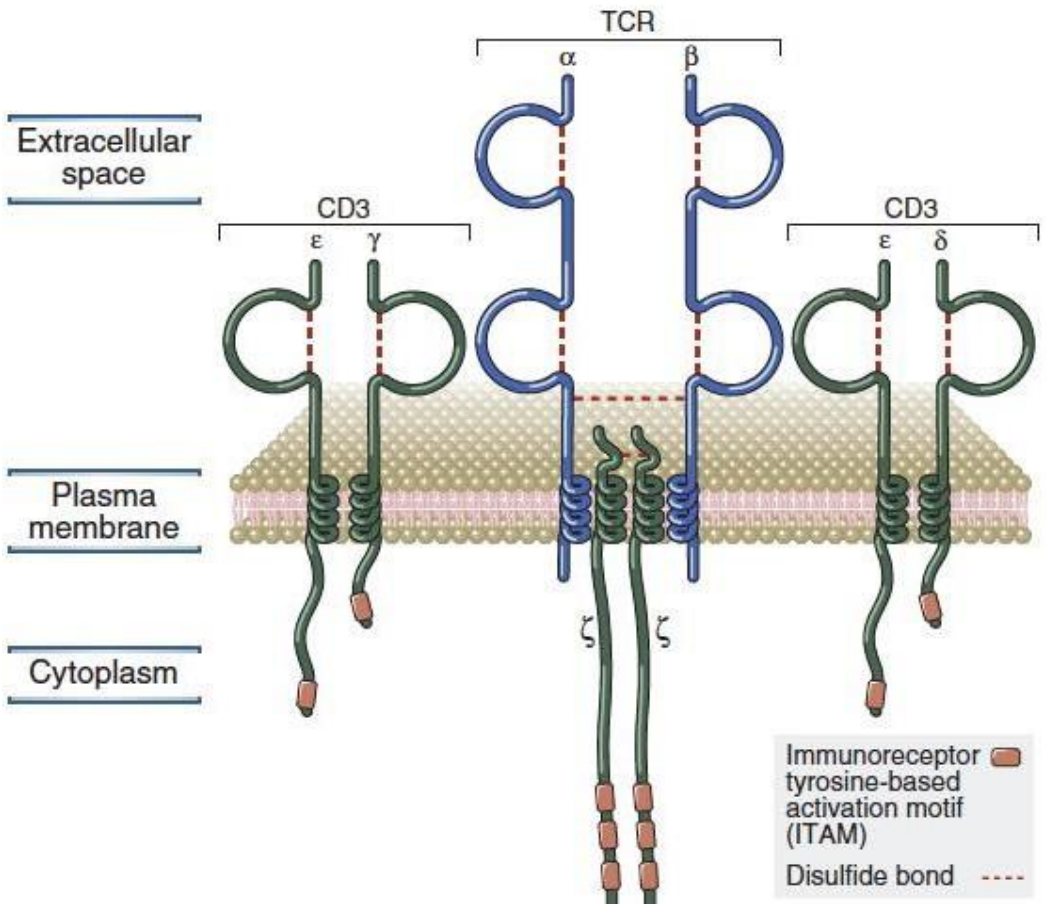
Components of the TCR complex.



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TCR Complex

The TCR complex consists of the TCR (αβ) associated with the CD3 complex and ζ (zeta) chains, which are essential for intracellular signaling.

Coreceptors

CD4 and CD8 are coreceptors and are not part of the TCR complex. CD4 interacts with MHC class II, while CD8 interacts with MHC class I.

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DEVELOPMENT OF IMMUNE REPERTOIRES

- As the clonal selection hypothesis predicted, there are many clones of lymphocytes with distinct specificities, perhaps as many as 109, and these clones arise before an encounter with antigen.
- **The process of lymphocyte maturation first generates a very large number of cells each with a different antigen receptor and then preserves the cells with useful receptors.**
- receptors are expressed on developing lymphocytes, selection processes come into play that promote the survival of cells with receptors that can recognize antigens, such as microbial antigens, and eliminate cells that cannot recognize antigens in the individual or that have the potential to cause harm.

Additional

The β chain of the TCR behaves similar to the heavy chain of the B-cell receptor (BCR), while the α chain corresponds to the light chain.

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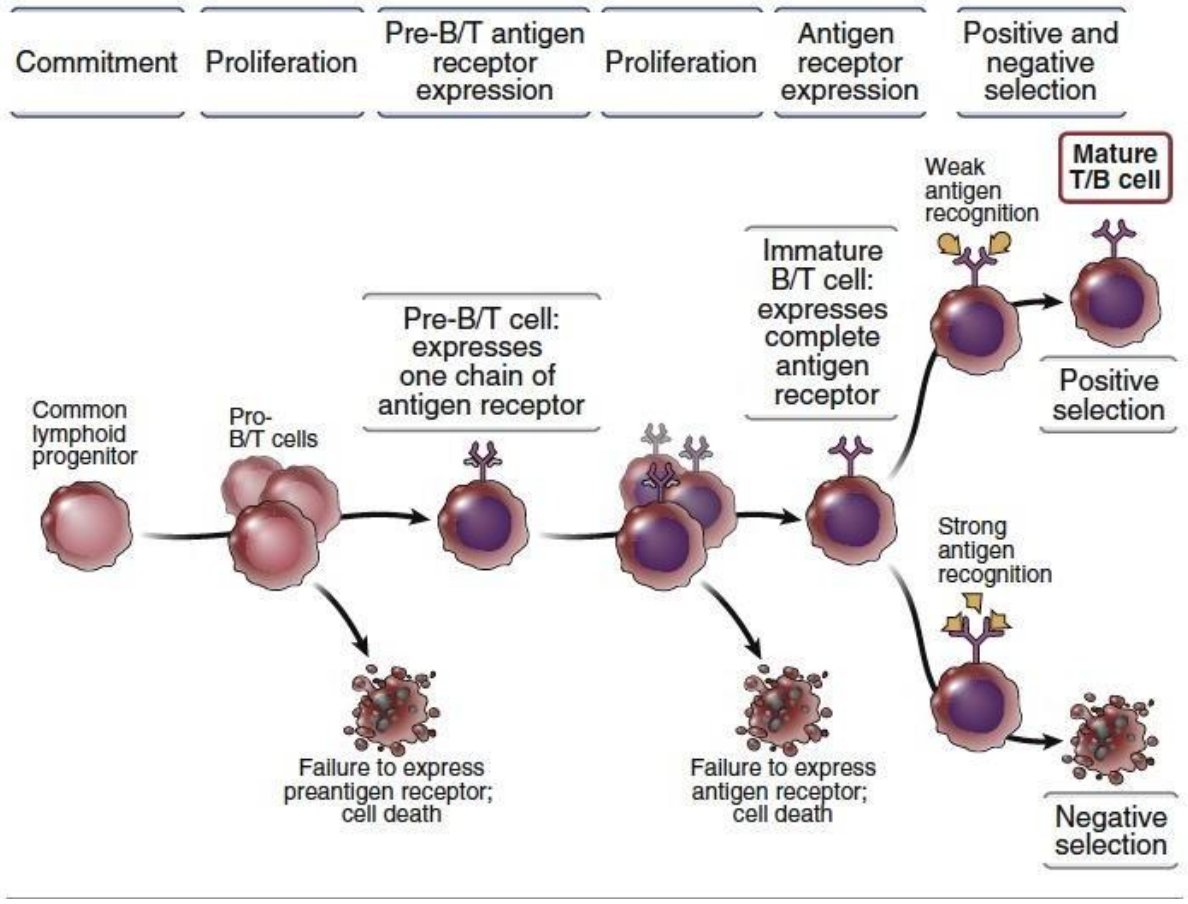
Lymphocyte Development



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Lymphocyte Development

As discussed earlier, each individual B cell and T cell expresses a single antigen specificity. However, the total number of specificities collectively present in an individual is called the **immune repertoire,** which can reach up to $\mathbf{10^{16}}$ different receptors before exposure to any antigen. This enormous diversity is generated through a major developmental event called **antigen receptor gene rearrangement**, which occurs **randomly** during lymphocyte development.

Common Lymphoid Progenitor (CLP)

Both **B cells and T cells** originate from the **common lymphoid progenitor** (CLP), which is distinct from the common myeloid progenitor.

The fate of the CLP (B cell vs T cell) is determined by up-regulation and down-regulation of **transcription factors** and **signaling** pathways:

- **B cell** lineage commitment: Driven by the transcription factor **PAX5**
- **T cell** lineage commitment: Driven by **NOTCH** signaling and **GATA-3**
- **IL-7** is required for the development of both B and T cells within their respective microenvironments

Additional Slide

Lymphocyte Development

Developmental Stages of B Cells

All the following stages occur before antigen encounter:

1. Pro-B cell
2. Pre-B cell
3. Immature B cell
4. Mature naïve B cell

Anatomical Location

- Common lymphoid progenitor: **Bone marrow**
- Pro-B, Pre-B, Immature B cells: **Bone marrow**
- Mature naïve B cells: **Peripheral (secondary) lymphoid organs** (Spleen or Lymph nodes)

Developmental Stages of T Cells

1. Pro-T cell
2. Pre-T cell
3. Immature T cell
4. Mature T cell

Anatomical Location

- CLP originates in the **bone marrow**
- Upon receiving T-cell commitment signals, the progenitor leaves the bone marrow and migrates to the **thymus**
- Maturation: From the thymic **cortex**, then to the thymic **medulla**
- Pro-T, Pre-T, and Immature T cells: **Thymus** (central lymphoid organ)
- Mature T cells: **Peripheral lymphoid organs**

Additional Slide

Lymphocyte Development

Both B and T cells possess **germline-encoded DNA segments** that code for antigen **receptors**. In these genes, recombination occurs.

Enzymes Involved

- RAG-1 and RAG-2 (Recombination Activating Genes) are active during the pro stage. They are responsible for cutting and rejoining receptor gene segments
- TdT (Terminal deoxynucleotidyl transferase) adds nucleotides without a template, increasing diversity

B Cell Receptor Rearrangement

- **Pro-B cell stage:**
  - Rearrangement begins here with the heavy chain (most diversity)
  - RAG enzymes mediate recombination
- **Pre-B cell stage:**
  - Light chain rearrangement begins
  - A pre-B cell receptor appears on the surface
  - **Pre-IgM, with a light chain called surrogate light chain (pre-ɣ5)**
- **Immature B cell stage:**
  - Expresses complete IgM (functional heavy + light chains)
  - Negative selection occurs
- **Mature naïve B cell stage:**
  - Alternative RNA splicing results in expression of both IgM and IgD

Additional Slide

Lymphocyte Development

T Cell Receptor Rearrangement

- **Pro-T cell stage:**
  - RAG enzymes and TdT initiate rearrangement
  - β-chain rearranges first
- **Pre-T cell stage:**
  - β-chain appears first in the cytoplasm, then on the surface
  - α-chain rearrangement follows
  - Formation of the pre-T cell receptor
- **Immature T cell stage:**
  - TCR is complete
  - Selection processes begin

CD4/CD8 Expression During T Cell Development

- Early thymocytes, Pre- & Pro- are **Double Negative** (DN): No CD4 & No CD8
- During the immature stage, cells become **Double Positive** (DP)
- After selection, **Single Positive (SP)** cells emerge:
  - $CD8^+$ cells recognize MHC I
  - $CD4^+$ cells recognize MHC II
- These SP cells are the **mature T** cells that exit to **peripheral** lymphoid organs

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Production of Diverse Antigen Receptors

- **The formation of functional genes that encode B and T lymphocyte antigen receptors is initiated by somatic recombination of gene segments that code for the variable regions of the receptors, and diversity is generated during this process.**
- 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 also contain D gene segments.

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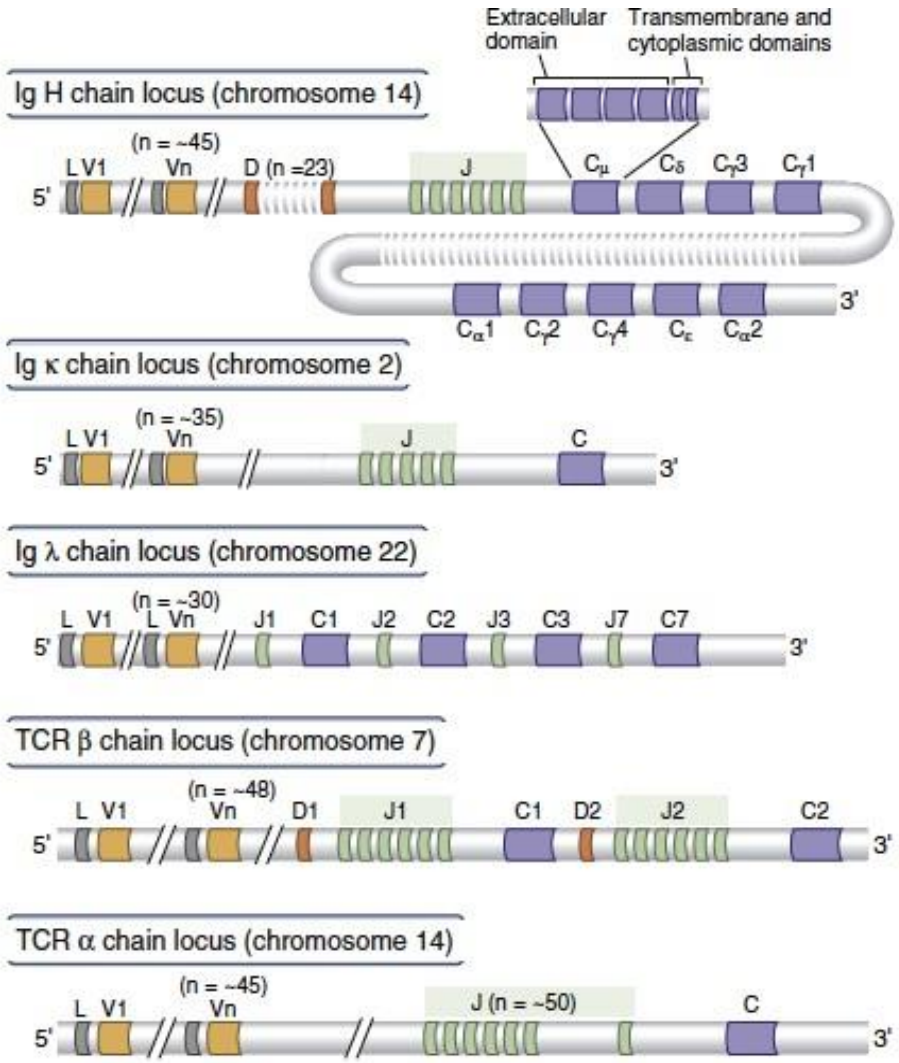
Germline organization of antigen receptor gene loci



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Germline organization of antigen receptor gene loci

Antigen receptor gene rearrangement is the **genetic** mechanism that enables lymphocytes to recognize a vast range of antigens. Humans have **46 chromosomes** arranged in **23 pairs**. However, because of **allelic exclusion**, rearrangement occurs on only one chromosome. Once a productive rearrangement is successfully completed on one chromosome, rearrangement on the other chromosome is halted. This ensures that each lymphocyte expresses receptors with a **single antigen specificity.**

The genes encoding B cell receptors (BCRs) and T cell receptors (TCRs) are located on specific chromosomes. In B cells, the immunoglobulin **heavy chain (IgH)** genes are found on **chromosome 14**. The light chain genes exist in two forms: **kappa (κ)**, located on **chromosome 2**, and **lambda (λ)**, located on **chromosome 22**. During B cell development, only one type of light chain is expressed. For T cells, the **β chain** genes are located on **chromosome 7**, while the **α chain** genes are found on **chromosome 14**, the same chromosome that carries the immunoglobulin **heavy** chain genes.

Antigen receptor genes are composed of different gene segments that participate in rearrangement. All receptor genes contain **V (variable)** and **J (joining)** segments. Only the immunoglobulin **heavy chain** and the **TCR β** chain also include **D (diversity)** segments. There are approximately 30 to 45 different V segments, and their large number contributes significantly to the **diversity** of antigen receptors.

Additional Slide

Germline organization of antigen receptor gene loci

Gene rearrangement occurs during lymphocyte development and is initiated by the enzymes **RAG-1** and **RAG-2**. The process begins with the joining of a **D segment** to a **J segment**, followed by the joining of a **V segment** to the newly formed **DJ unit**. The selection of these gene segments is **random**, meaning any V, D, and J combination can occur. This randomness is called **combinatorial diversity**, which is **limited only by the number of available gene segments** within the genetic loci.

To further increase diversity beyond these limits, the immune system uses junctional diversity. The enzyme **terminal deoxynucleotidyl transferase (TdT)** adds nucleotides at the **junctions** between gene segments **without** using a **template**. These random nucleotide additions greatly **increase variability**, making the potential diversity of antigen receptors effectively **unlimited**.

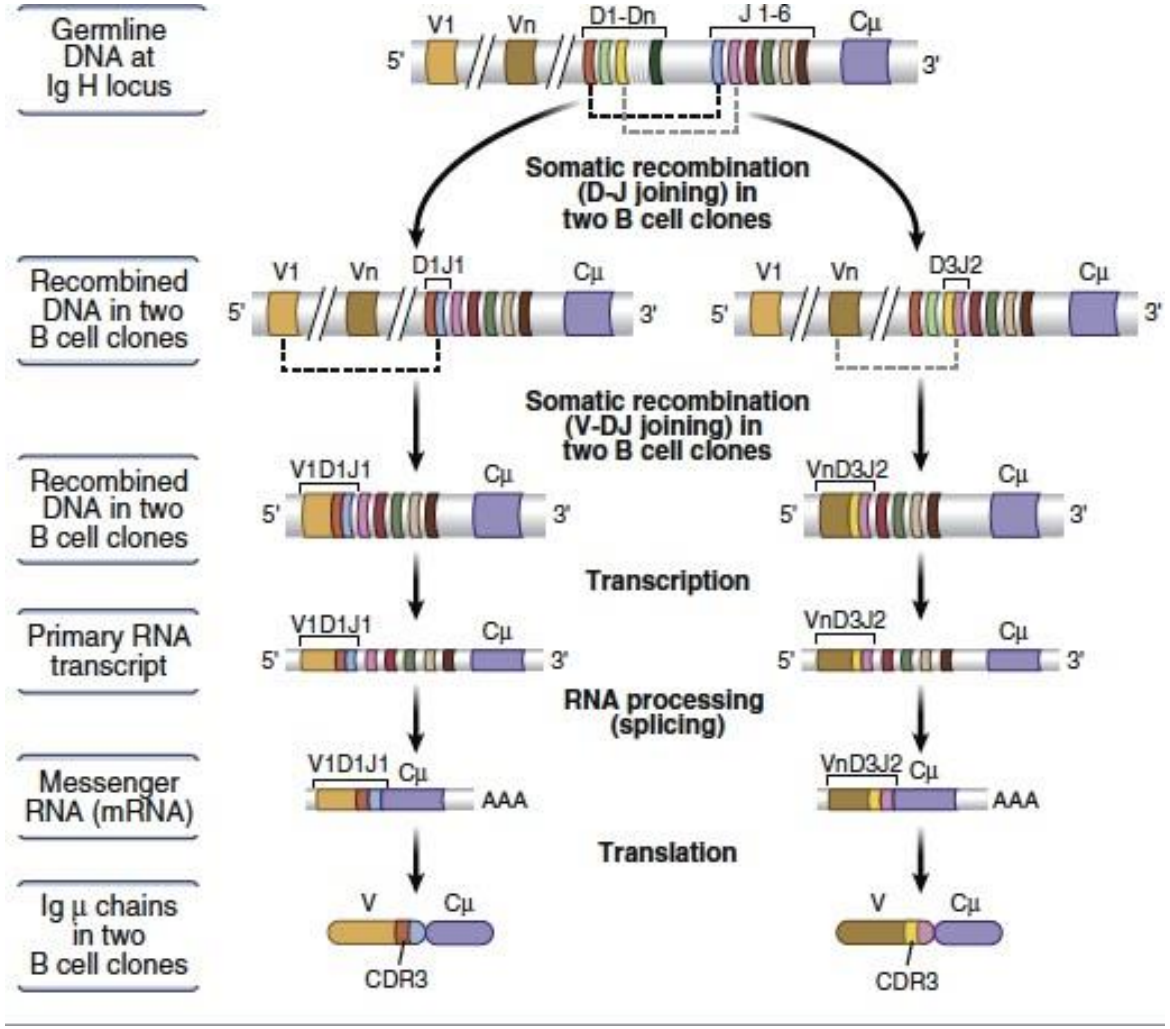
Once successful rearrangement of both the heavy and light chain **variable regions** has occurred in B cells, the cell begins to express the **constant regions**. In the heavy chain locus, the constant region genes are arranged **sequentially**, with the **μ (mu)** gene located first, followed by the **δ (delta)** gene.

After DNA transcription into pre-mRNA, **RNA** processing takes place. During this process, introns are removed and exons are joined together. Initially, **the μ constant region exon is included**, leading to the synthesis of **IgM**, which is **first** expressed in the cytoplasm and later on the cell surface. At a later stage, the μ exon is skipped and the **δ exon is included** instead. This regulated switching is achieved through **alternative RNA splicing**.

As a result of alternative RNA splicing, **mature naïve B lymphocytes** simultaneously express both **IgM** and **IgD** on their surface.

Dr.’s Slides

Recombination and expression of immunoglobulin (Ig) genes.



Additional ; Remember

The β chain of the TCR behaves similar to the heavy chain of the B-cell receptor (BCR), while the α chain corresponds to the light chain.

Mechanisms of V(D)J Recombination and Generation of Ig and TCR Diversity

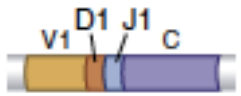
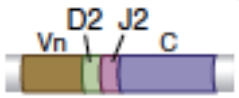
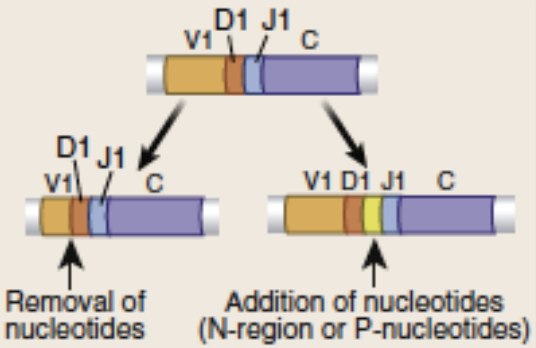
- **The somatic recombination of V and J, or of V, D, and J, gene segments is mediated by a lymphoid-specific enzyme, the VDJ recombinase** (RAG-1 and RAG-2) proteins**, and additional enzymes, most of which are not lymphocyte specific and are involved in repair of double-stranded DNA breaks introduced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

- **Diversity of antigen receptors is produced by the use of different combinations of V, D, and J gene segments in different clones of lymphocytes (called combinatorial diversity) and even more by changes in nucleotide sequences introduced at the junctions of the recombining V, D, and J gene segments (called junctional diversity)**

Dr.'s Slides

	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: TdT activity		
Total potential repertoire with junctional diversity	Ig: ~10^{11}	TCR: ~10^{16}

Additional Slide

Mechanisms of V(D)J Recombination and Generation of Ig and TCR Diversity

Antigen receptor gene rearrangement follows an ordered sequence during lymphocyte development. In B cells, the **heavy chain** is rearranged first, while in T cells the **β chain** is produced first. This occurs at the **pro-B** and **pro-T** stages, respectively, where **RAG-1 and RAG-2** enzymes are fully active and initiate V(D)J recombination. Successful formation of a functional heavy chain in B cells or β chain in T cells allows the cell to progress to **the pre stage.**

At the **pre-B stage**, rearrangement of the **light chains or α chain** begins. During this stage, **RAG activity is low to absent** once a productive light chain is formed. In the immature stage, RAG enzymes can be reactivated in a process known as **receptor editing**. This mechanism specifically alters only the **V and J segments of the light chain**. Importantly, receptor editing does **NOT** occur in T-cell receptors (TCRs). In contrast, in T cells, **RAG** enzymes and **TdT** remain active during both **β- and α-chain** rearrangements, which contributes to **greater** junctional diversity and explains why T lymphocytes possess a **broader immune repertoire.**

Dr.’s Slides

Maturation and Selection of B Lymphocytes

- 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.**

- **The Ig heavy-chain locus rearranges first, and only cells that are able to make an Ig μ heavy-chain protein are selected to survive and become pre-B cells.**

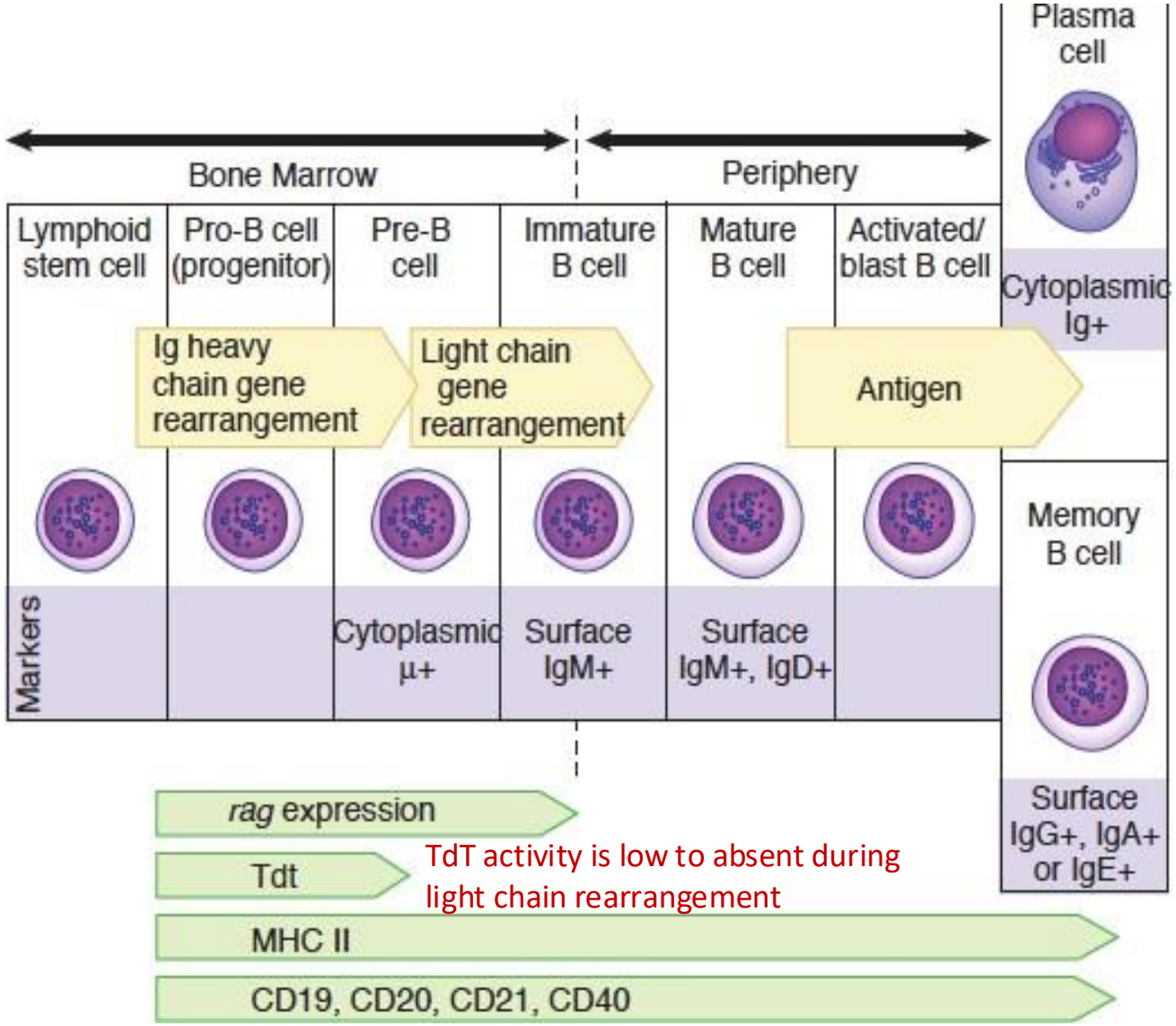
- **The assembled pre-BCR serves essential functions in the maturation of B cells.**

Dr.’s Slides

Completion of B Cell Maturation.

- The IgM-expressing B lymphocyte is the **immature B cell.**
- The IgM+ IgD+ cell is the **mature B cell,** able to respond to antigen in peripheral lymphoid tissues.
- Developing B cells are **positively selected** based mainly on **expression of complete antigen receptors**, and not on the recognition specificity of these cells.
- The B cell repertoire is further shaped by **negative selection against strong recognition of self antigens.**

Dr.’s Slides


Plasma cell
Bone Marrow
Periphery
Lymphoid stem cell
Pro-B cell (progenitor)
Pre-B cell
Immature B cell
Mature B cell
Activated/ blast B cell
Cytoplasmic Ig+
Ig heavy chain gene rearrangement
Light chain gene rearrangement
Antigen
Memory B cell
Markers
Cytoplasmic μ+
Surface IgM+
Surface IgM+, IgD+
Surface IgG+, IgA+ or IgE+
rag expression
Tdt
TdT activity is low to absent during light chain rearrangement
MHC II
CD19, CD20, CD21, CD40

Additional Slide

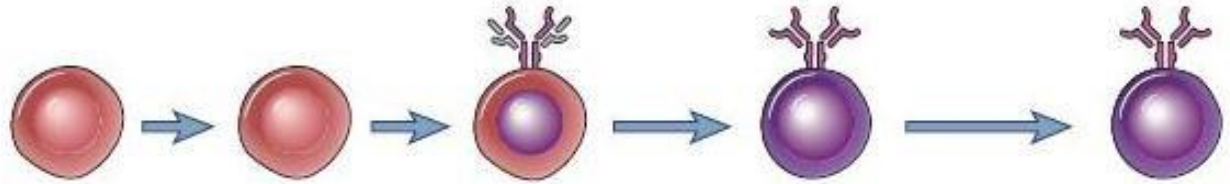
Completion of B & T Cell Maturation

Multiple developmental **checkpoints** ensure proper receptor formation and self-tolerance. In B-cell development, these checkpoints can be **summarized as HLS**. **The H (heavy chain)** checkpoint ensures that a (complete, intact, proceed); failure results in apoptosis. **The L (light chain)** checkpoint. The **S (selection) checkpoint** tests the newly formed BCR for self-reactivity. Because all these events occur in the bone marrow, there is no exposure to foreign antigens. Weak or absent binding to self-antigens allows survival, whereas strong binding indicates an **autoreactive** B cell, which undergoes **negative selection** through **deletion**.

B-cell surface markers also change during maturation. Early B cells express CD19 and CD20, which persist through most stages of development. As B cells mature further, they begin to express CD21 and CD23, which is called Fc receptors and are characteristic of mature naïve B cells.

In T-cell development, the main checkpoints can be summarized as **BAP**. The **B checkpoint** verifies successful **β-chain** rearrangement (complete, intact, proceed), allowing progression to the next stage. The **A checkpoint** confirms successful α-**chain** rearrangement. **The P checkpoint** refers to **positive selection.**

Dr.’s Slides



Stage of maturation	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Proliferation					
RAG expression					
TdT expression					
Ig DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor–associated μ	Membrane IgM (μ+ κ or λ light chain)	Membrane IgM and IgD
Surface markers	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	IgM^{lo} $CD43^-$	IgM^{hi}
Anatomic site	Bone marrow				Periphery
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

Dr.’s Slides

Maturation and Selection of T Lymphocytes

- **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 do not 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 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 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).

Dr.’s Slides

Selection of Mature T Cells.

- If the TCR of a T cell recognizes an MHC molecule in the thymus, which must be a self MHC molecule displaying a self peptide, and if the interaction is of low or moderate affinity, this T cell is selected to survive (**positive selection).**
- During this process, T cells 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. **single-positive T cells .**
- Immature, double-positive T cells whose receptors strongly recognize MHC-peptide complexes in the thymus undergo apoptosis. This is the process of **negative selection.**

Dr.’s Slides

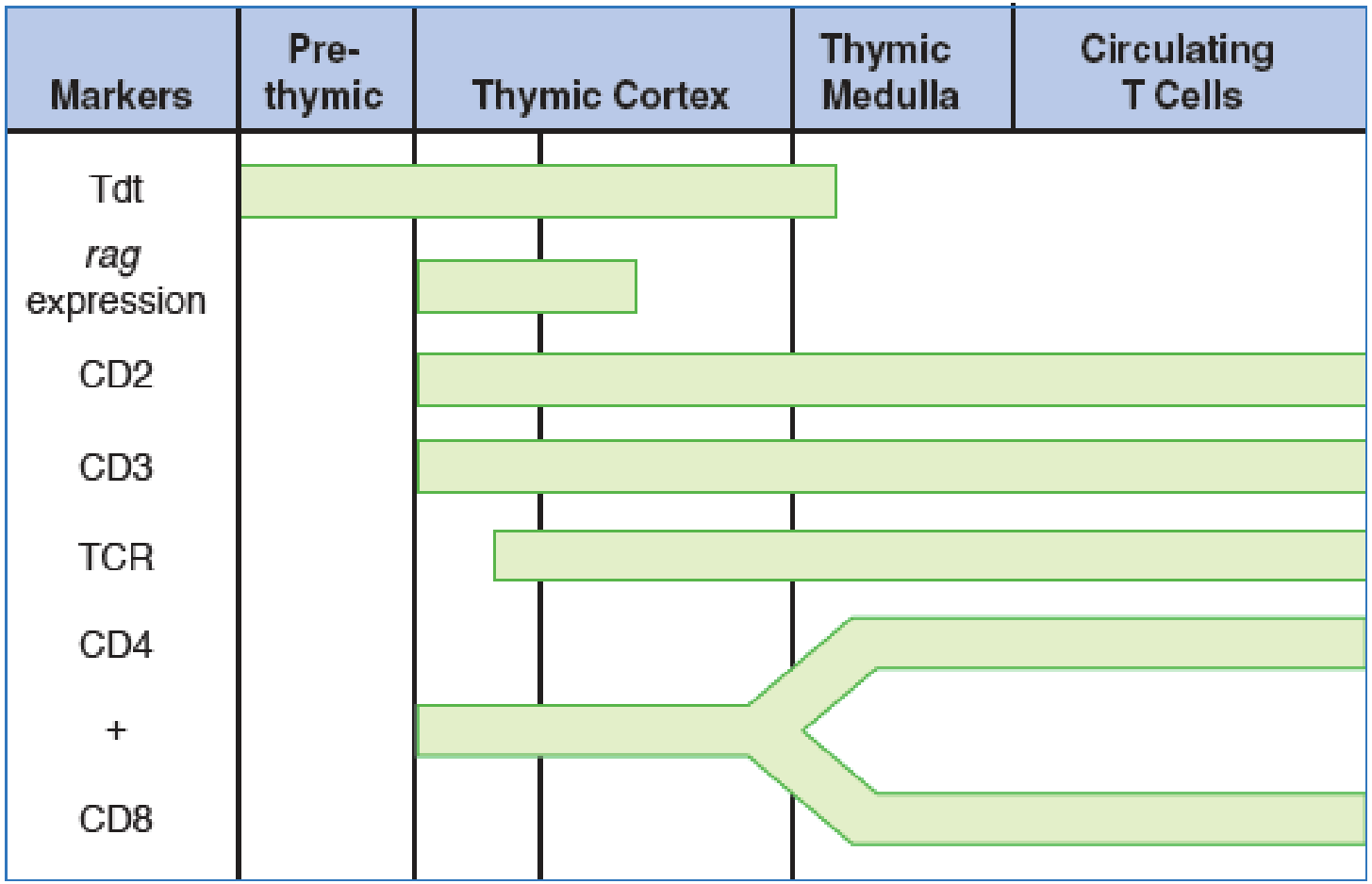
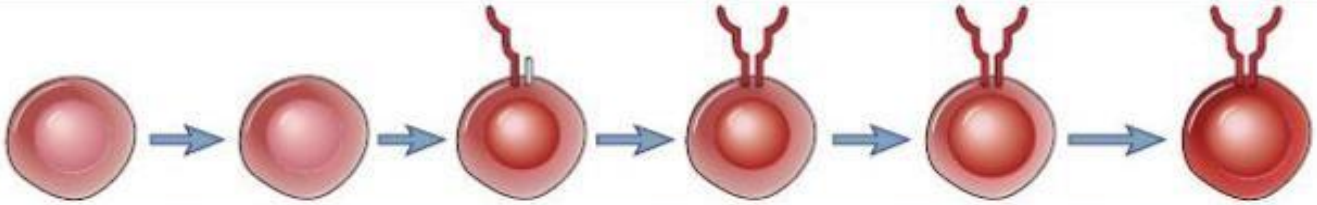


Figure I-3-13. Human T-Cell Differentiation

Dr.'s Slides



Stage of maturation	Stem cell	Pro-T	Pre-T	Double positive	Single positive (immature T cell)	Naive mature T cell
Proliferation						
RAG expression						
TdT expression						
TCR DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined β chain gene [V(D)J-C]; β chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA
TCR expression	None	None	Pre-T receptor (β chain/pre-T α)	Membrane αβ TCR	Membrane αβ TCR	Membrane αβ TCR
Surface markers	c-*kit* $^+$ $CD44^+$ $CD25^-$	c-*kit* $^+$ $CD44^+$ $CD25^+$	c-*kit* $^+$ $CD44^-$ $CD25^+$	$CD4^+CD8^+$ $TCR/CD3^{lo}$	$CD4^+CD8^-$ or $CD4^-CD8^+$ $TCR/CD3^{hi}$	$CD4^+CD8^-$ or $CD4^-CD8^+$ $TCR/CD3^{hi}$
Anatomic site	Bone marrow	Thymus				Periphery
Response to antigen	None	None	None	Positive and negative selection		Activation (proliferation and differentiation)

FIGURE 8–19 Stages of T cell maturation. Events corresponding to each stage of T cell maturation from a bone marrow stem cell to

The End