Antigen Recognition in the Adaptive Immune System and Lymphocytes Development

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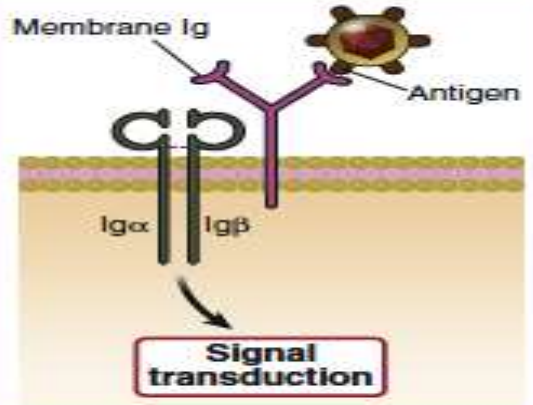
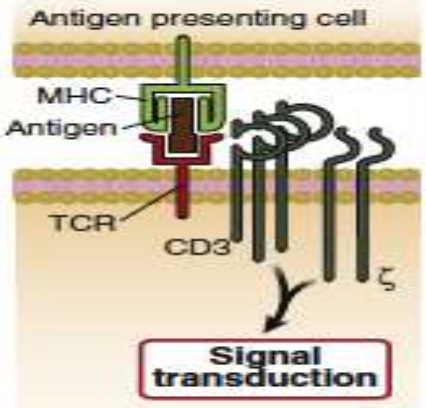
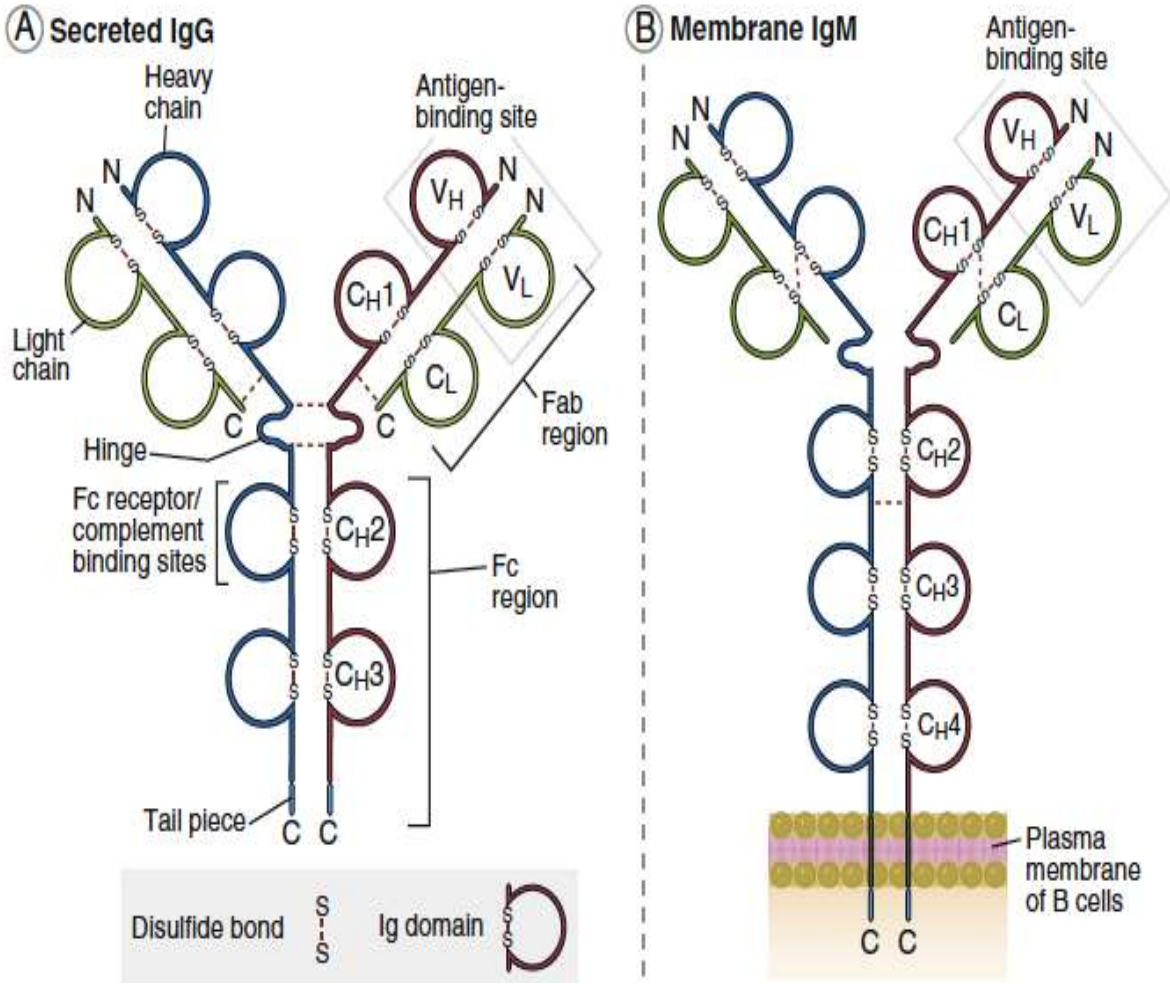
ANTIGEN RECEPTORS OF LYMPHOCYTES

	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

- 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

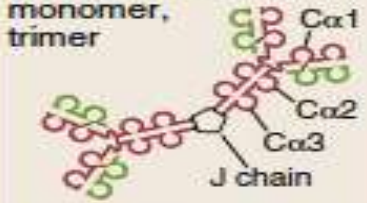
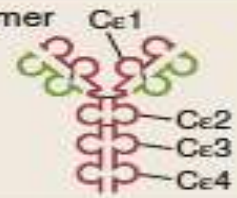
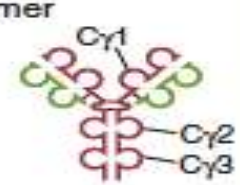
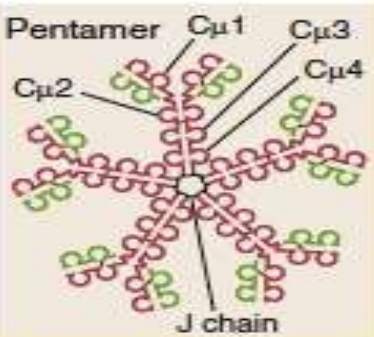
- Between the Fab and Fc regions of most antibody molecules is a flexible portion called the **hinge region**. The hinge allows the two antigen-binding Fab regions of each antibody molecule to move independent of each other.

- 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**

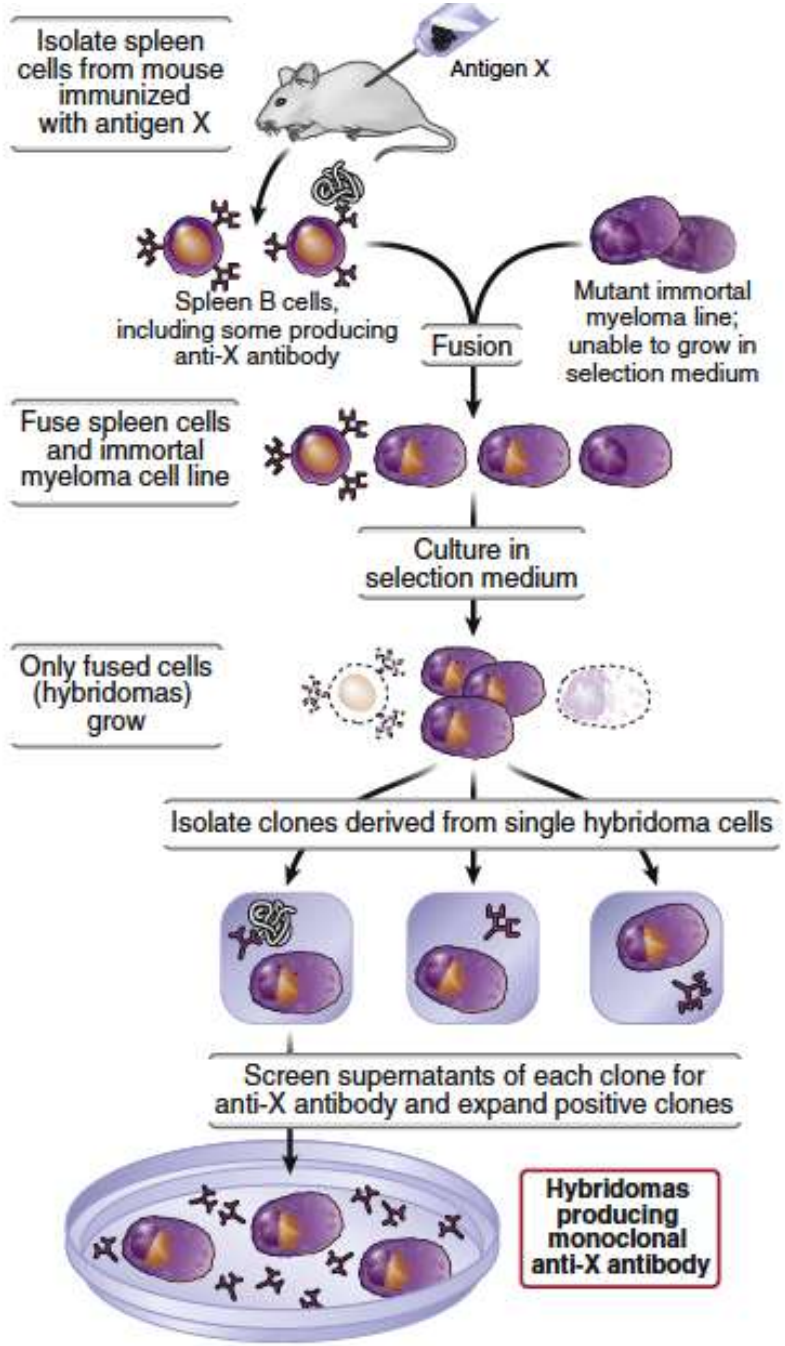
Isotype of antibody	Subtypes (H chain)	Serum concentration (mg/ml)	Serum half-life (days)	Secreted form	Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	Mainly dimer, also monomer, trimer 	Mucosal immunity
IgD	None (δ)	Trace	3	Monomer	Naive B cell antigen receptor
IgE	None (ε)	0.05	2	Monomer 	Defense against helminthic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

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.**

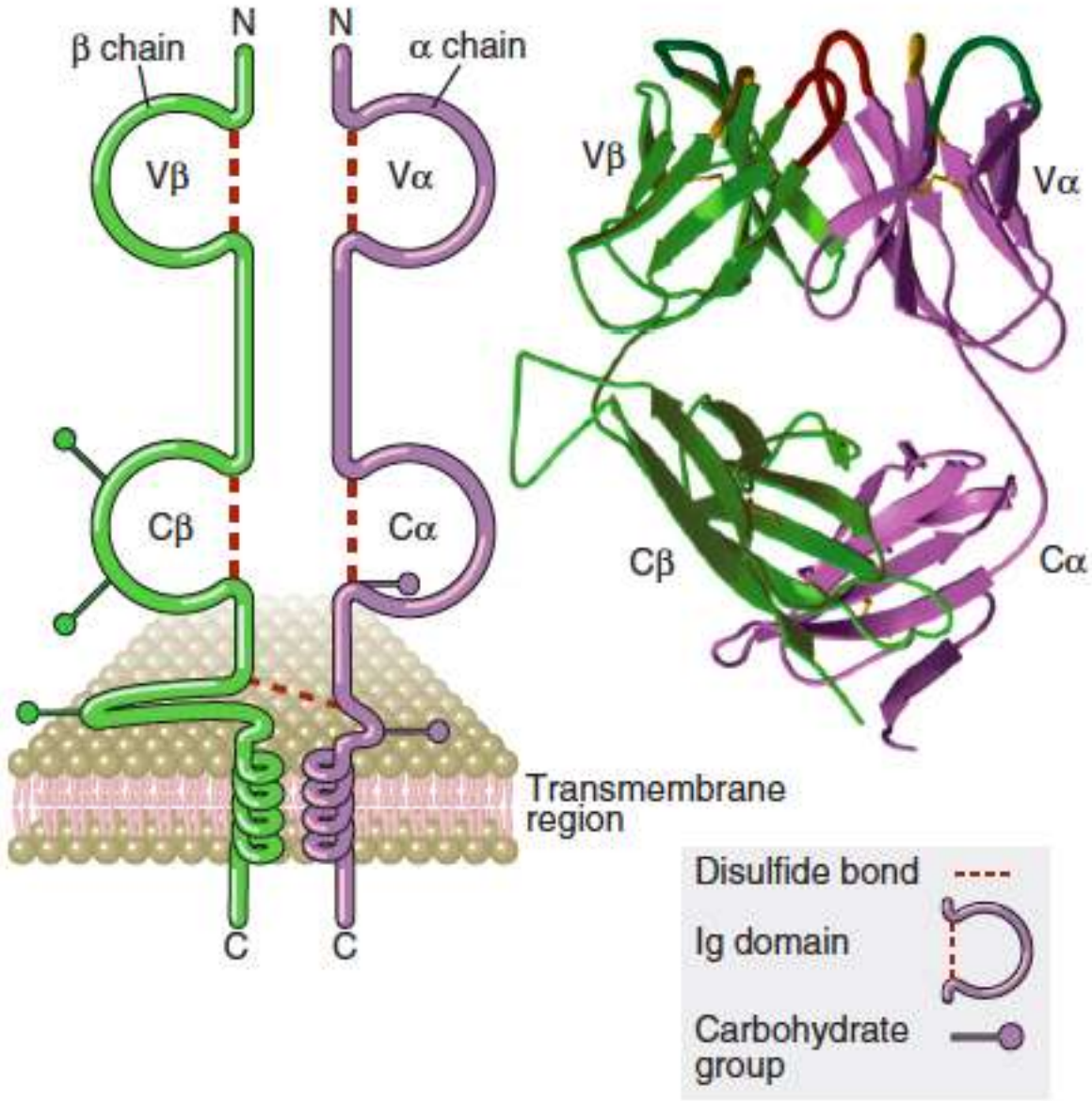

Isolate spleen cells from mouse immunized with antigen X
Antigen X
Spleen B cells, including some producing anti-X antibody
Fusion
Mutant immortal myeloma line; unable to grow in selection medium
Fuse spleen cells and immortal myeloma cell line
Culture in selection medium
Only fused cells (hybridomas) 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

The T Cell Receptor Complex and T Cell Signaling

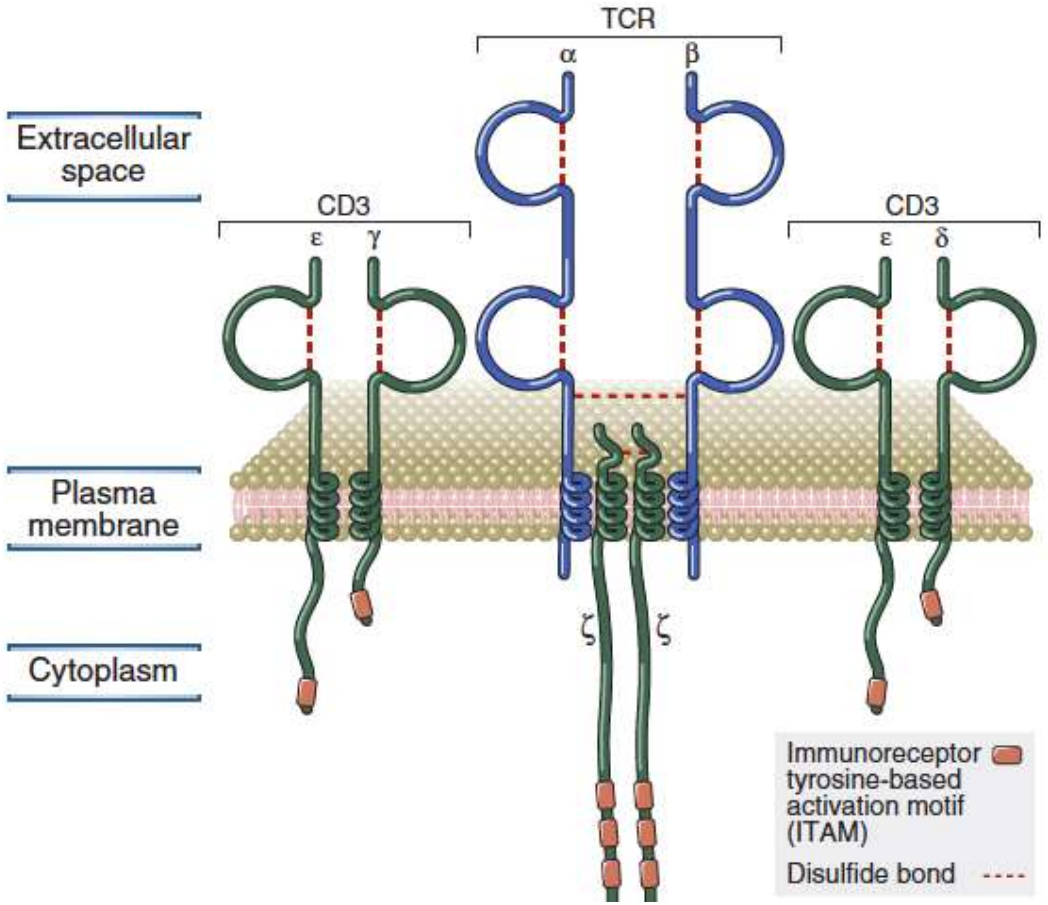
- **T lymphocytes express different receptors that recognize antigens: T cell receptors (TCRs) on T lymphocytes.**
- The antigen receptors of lymphocytes must be able to bind to and distinguish between many, often closely related, chemical structures.
- **each lymphocyte clone is specific for a distinct 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.

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.



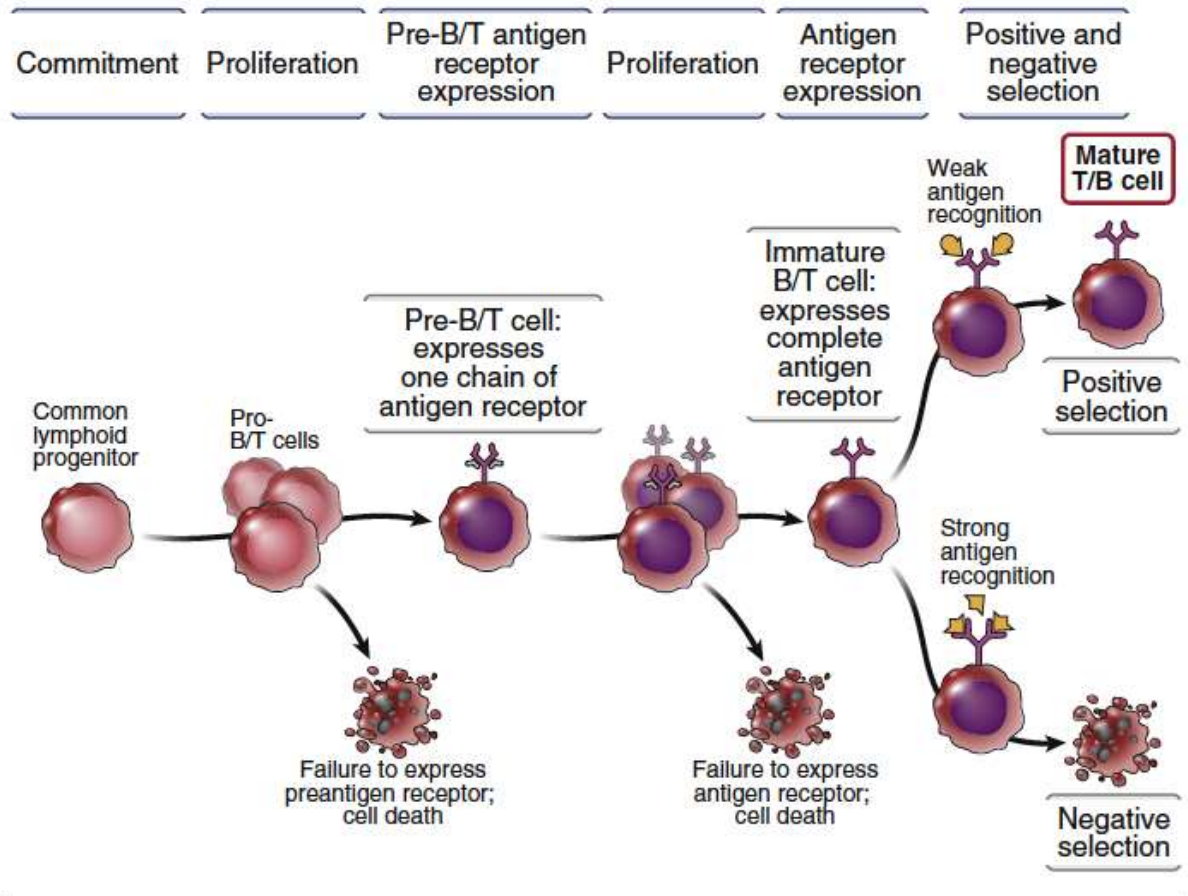
Components of the TCR complex.



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.

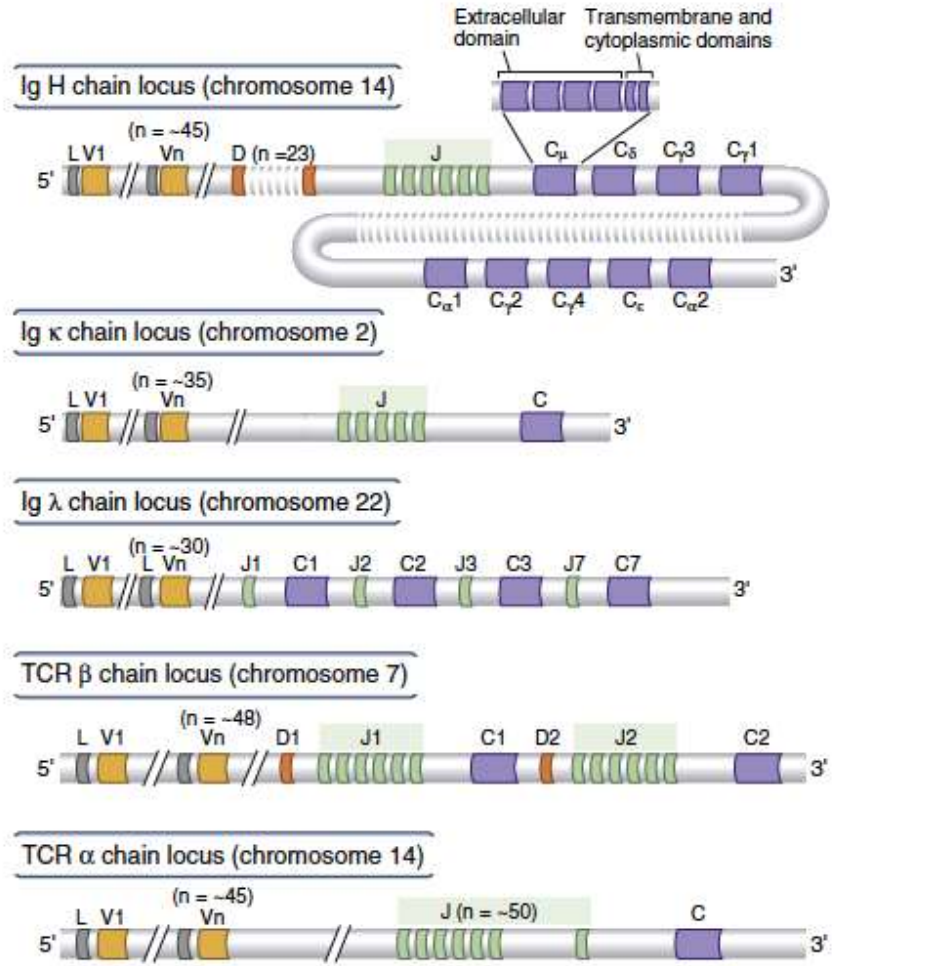
Lymphocyte Development



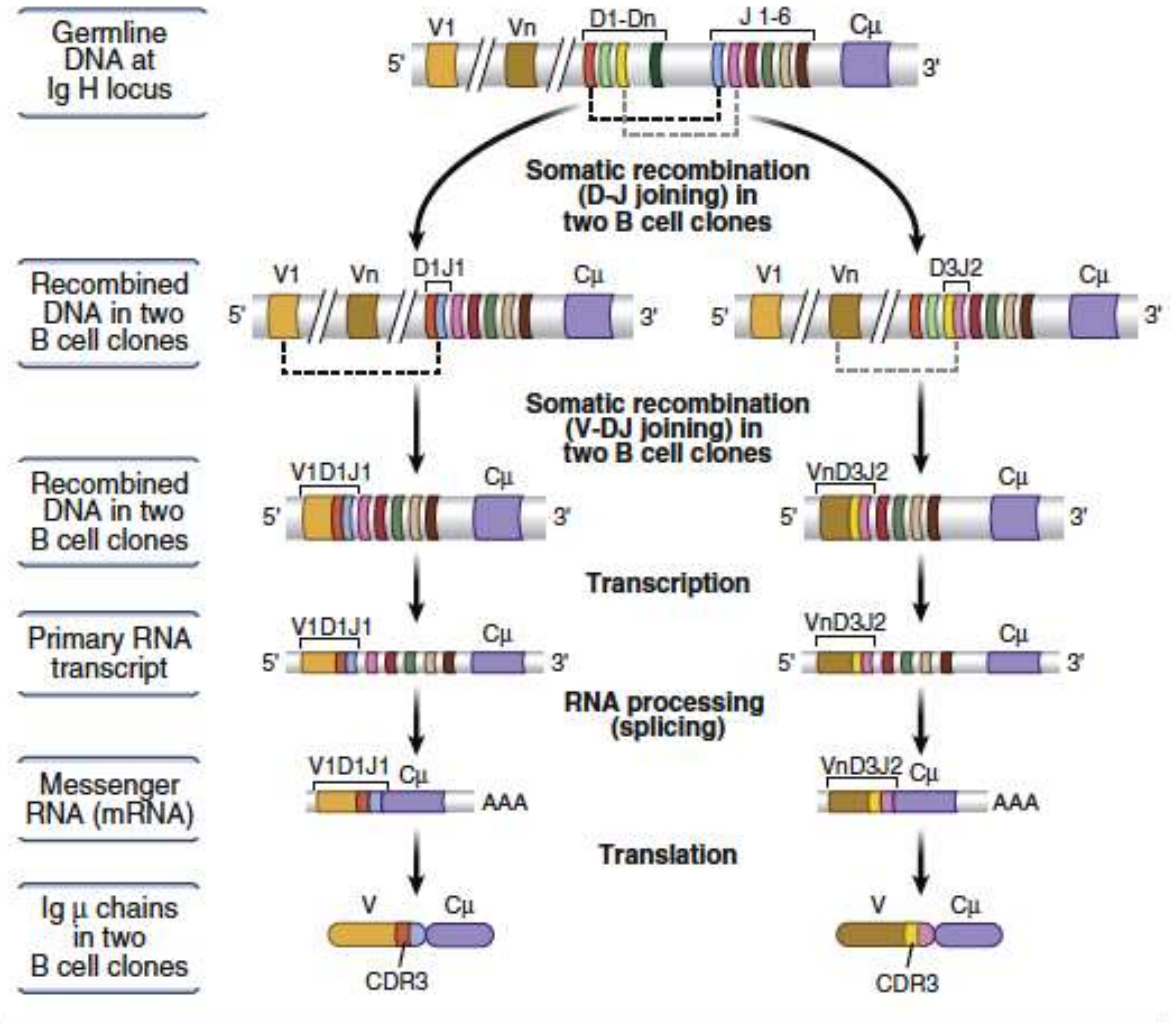
Production of Diverse Antigen Receptors

- **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.

Germline organization of antigen receptor gene loci



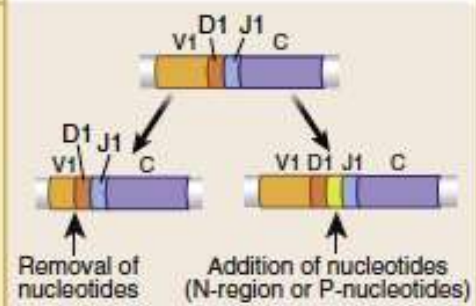
Recombination and expression of immunoglobulin (Ig) genes.



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)**

	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}	

Maturation and Selection of B Lymphocytes

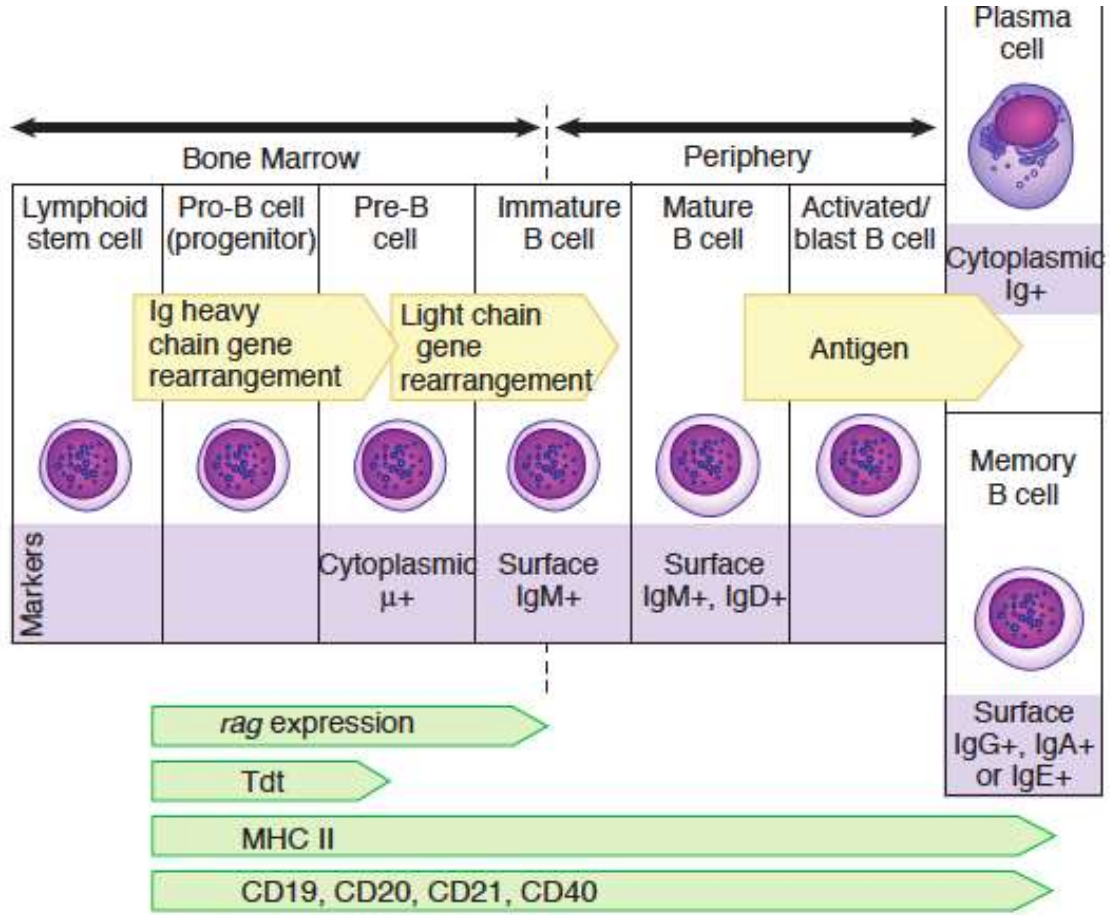
- Bone marrow progenitors committed to the B cell lineage proliferate, giving rise to a large number of precursors of B cells, called **pro-B cells.**

- **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.**

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.**


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
MHC II
CD19, CD20, CD21, CD40

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).

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.**

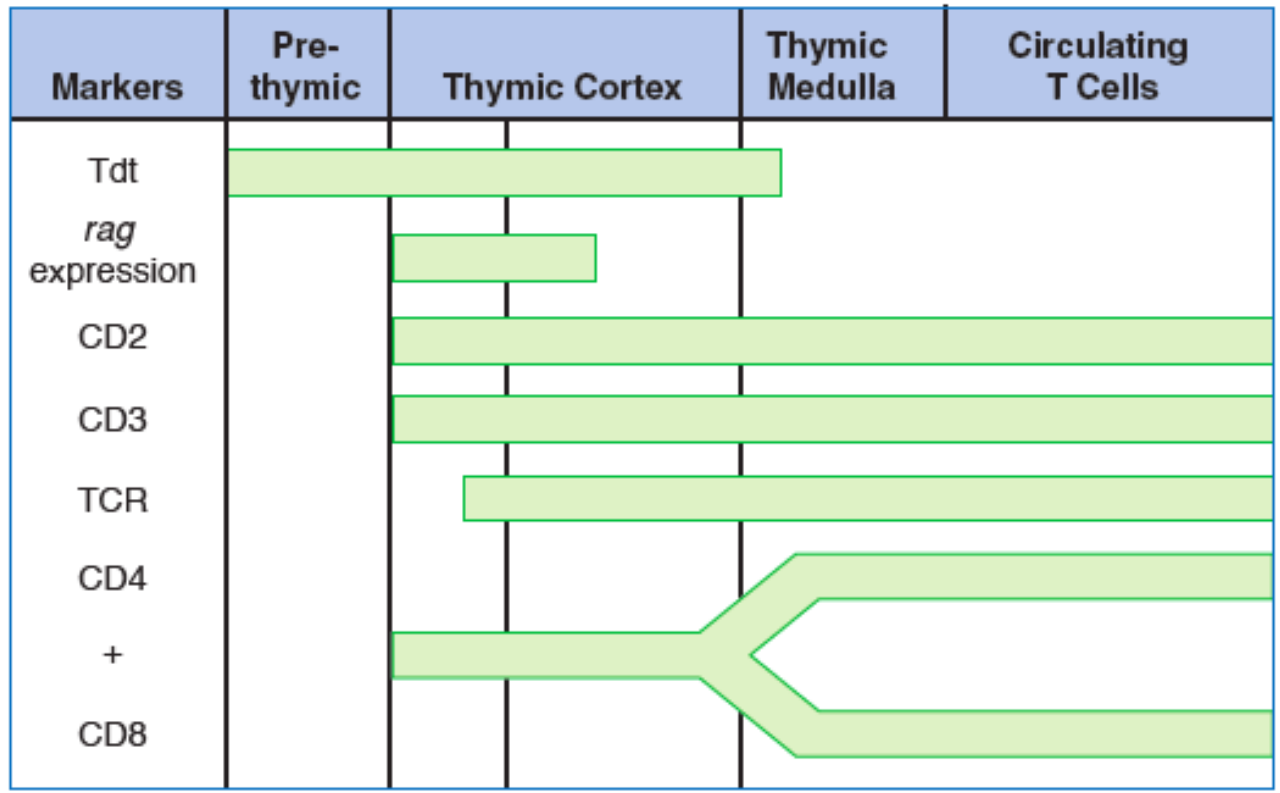


Figure I-3-13. Human T-Cell Differentiation

The End