Introduction to Immunology

B-Cell Development and Antibody Maturation

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B-Cell Biology

Antigen-Independent B-Cell Development

Antigen-Dependent B-Cell Development

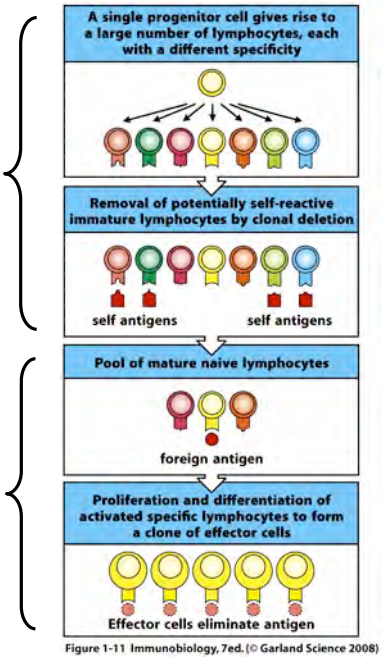


Figure 1-11 Immunobiology, 7ed. (© Garland Science 2008)

2 Phases of B-Cell Development at 2 Locations

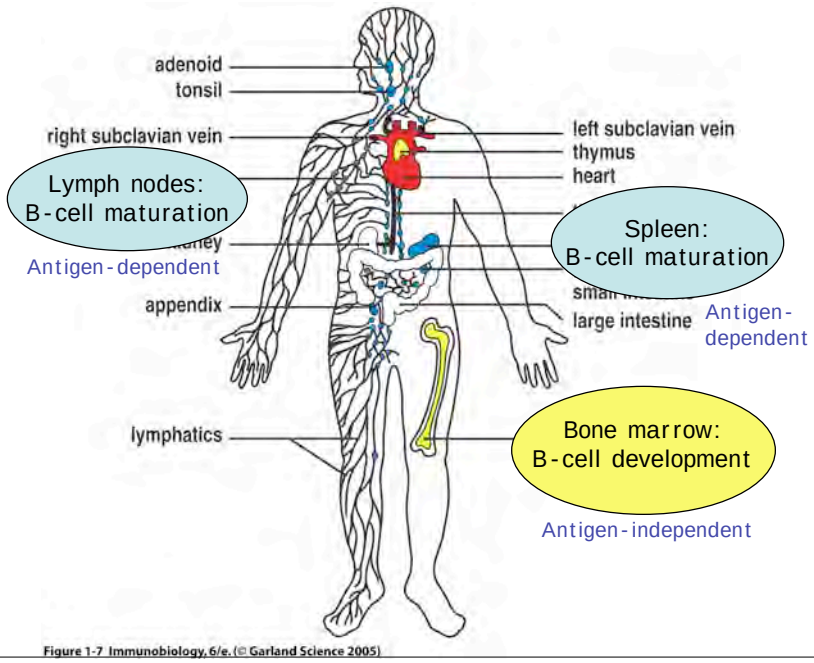


Figure 1-7 Immunobiology, 6/e. (© Garland Science 2005)

B-Cell Biology

Antigen-Independent B-Cell Development

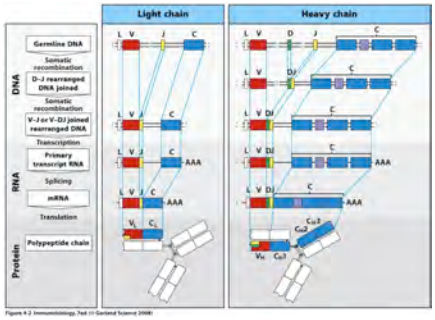
- Generation of B Cells in the Bone Marrow

Antigen-Dependent B-Cell Development

- Ig Class Switch, Somatic Hypermutation
- Germinal Center Reaction

3 Processes Establish Diversity of Pre-Immune Repertoire:

- Combinatorial diversity (V_H, D_H, J_H & V_L, J_L)
- Junctional diversity
- Combinatorial diversity through HC and LC combinations



- It is estimated that these processes could give rise to 10^{11} different antibody specificities that comprise the antigen receptor repertoire of naïve B-cells

Not All Rearrangements Lead to Suitable Antigen-Receptor:

- Some V_H and V_L gene segments are pseudogenes
- Junctional diversity can lead to reading frame shifts
- Junctional diversity can introduce translational stop codons

Additional Complexities:

- Each cells has 2 HC and 4 LC alleles
- Generation of autoreactive antibody receptors

⇩

It results a considerable cell wastage!

How is the generation of the pre-immune repertoire <u>regulated</u>?

Antigen-Independent B-Cell Development

Generation of B Cells in the Bone Marrow

1. DNA rearrangements establish the primary repertoire, creating *diversity*

2. Allelic exclusion ensures that each clone expresses a single antibody on the surface, establishing *specificity*

3. Deletion of self-reactive clones establishes immunological *tolerance*

Ordered Rearrangement of Ig Genes During B-Cell Development in the Bone Marrow

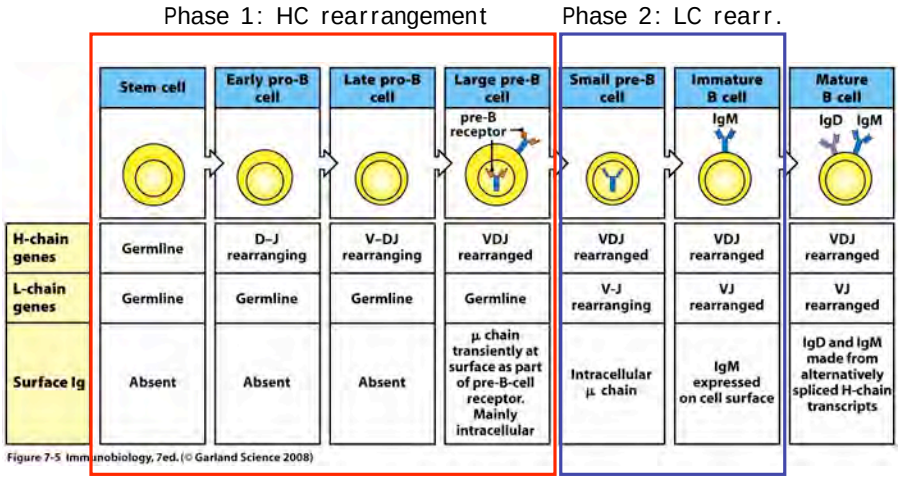


Figure 7-5 Immunobiology, 7ed. (© Garland Science 2008)

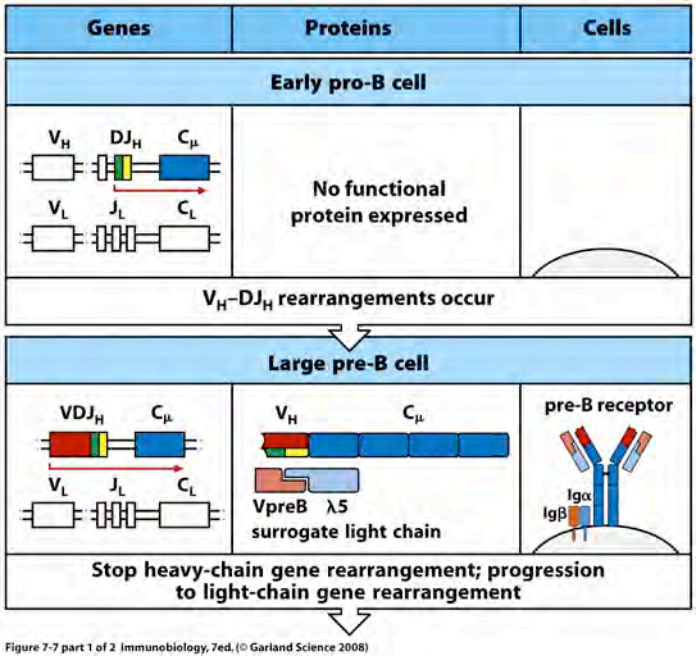


Figure 7-7 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

Pre-B Cell Receptor

- Membrane μHC
- λ5, VpreB (surrogate [or pseudo] LC)
- Igα and Igβ signaling components

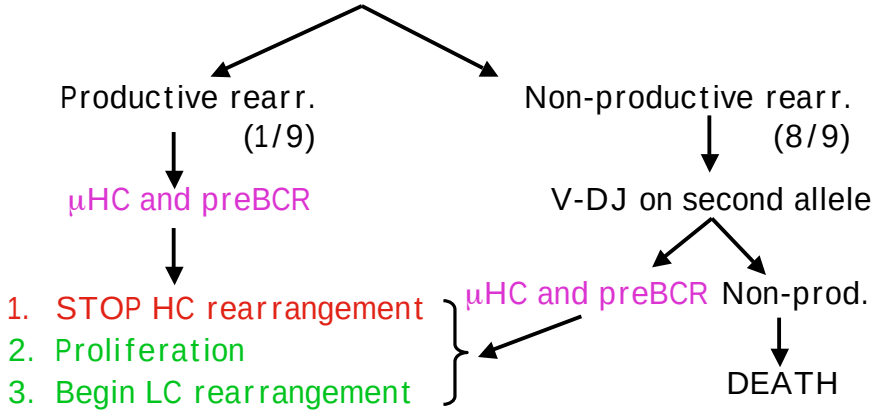
Tonic signaling - no known ligand

Ordered Rearrangement Gives Allelic Exclusion

Heavy chain rearrangement occurs first:

DJ on both alleles

V-DJ on one allele



(HC Alleles)	PR	NPR	NPR
	D-J	PR	NPR

Ordered Rearrangement of Ig Genes During B-Cell Development in the Bone Marrow

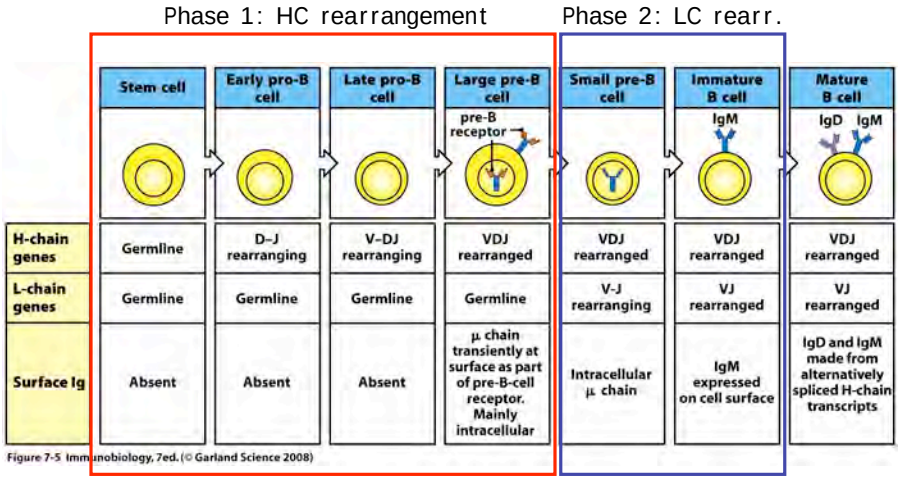


Figure 7-5 Immunobiology, 7ed. (© Garland Science 2008)

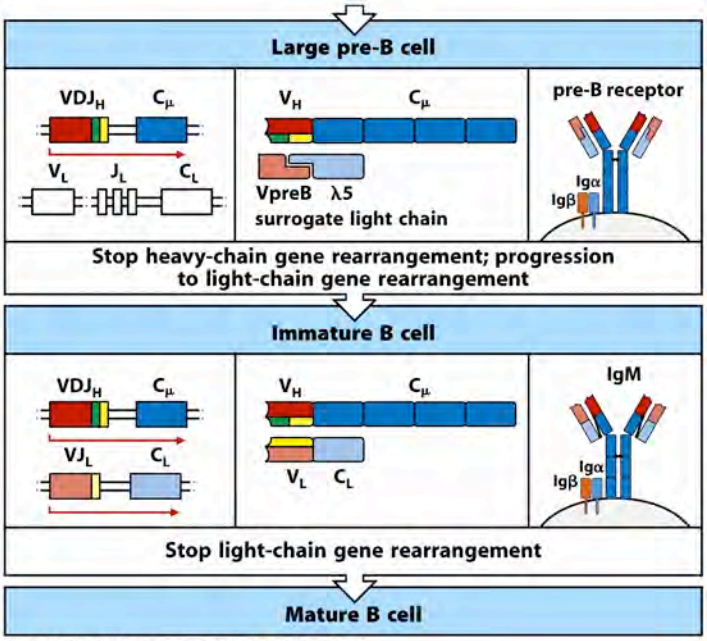


Figure 7-7 part 2 of 2 Immunobiology, 7ed. (© Garland Science 2008)

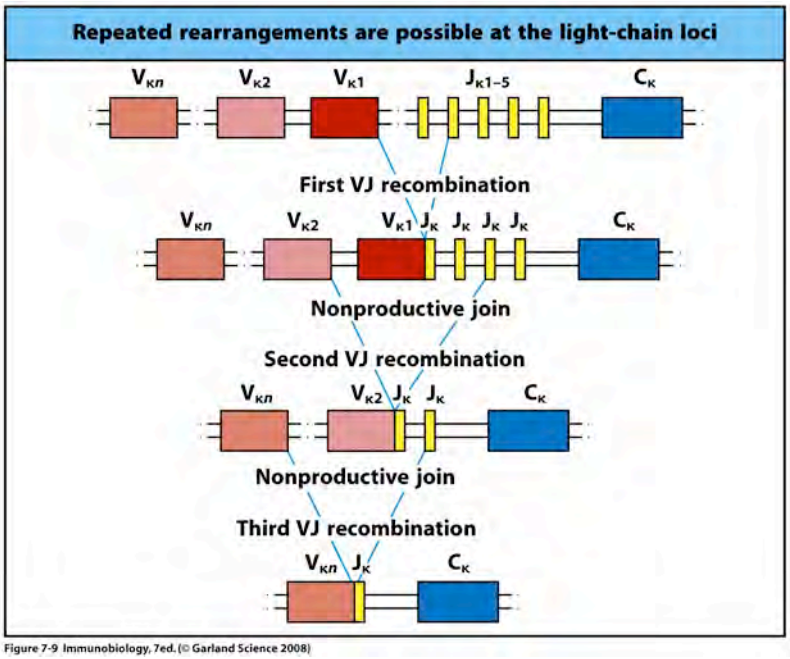


Figure 7-9 Immunobiology, 7ed. (© Garland Science 2008)

Rearrangement of Ig Alleles is Ordered and Regulated to Achieve Allelic Exclusion

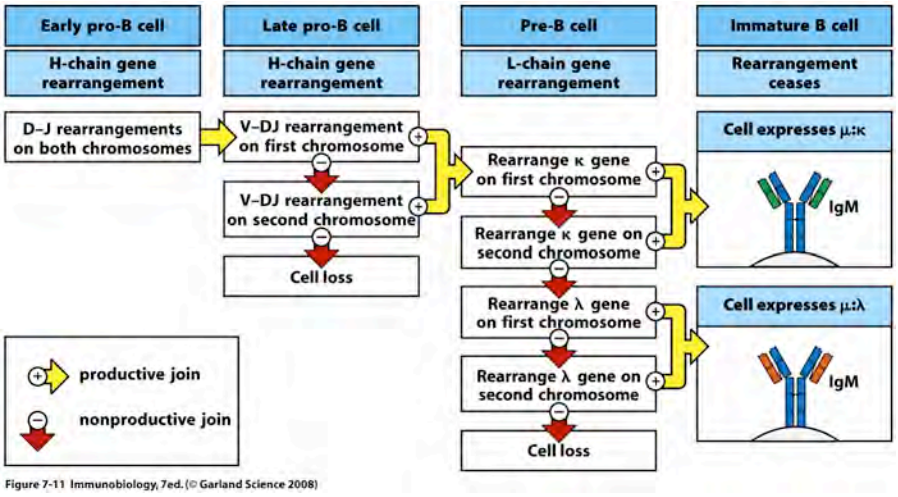


Figure 7-11 Immunobiology, 7ed. (© Garland Science 2008)

TWO checkpoints which confer allelic exclusion:

- pre-BCR
- and BCR

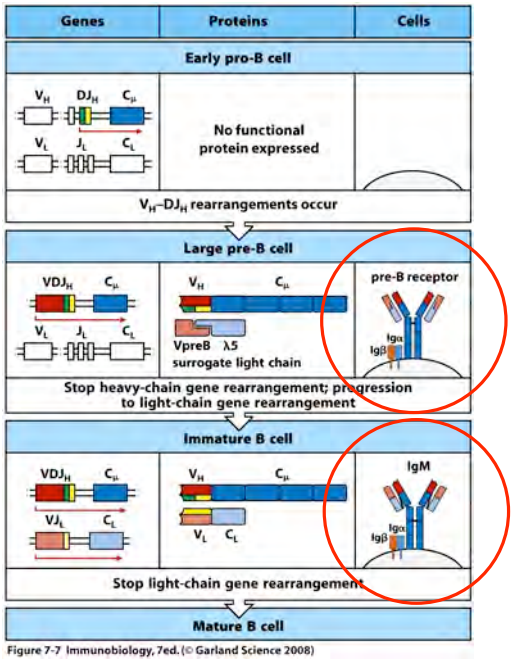


Figure 7-7 Immunobiology, 7ed. (© Garland Science 2008)

Establishment of Allelic Exclusion

Signaling through pre-BCR or BCR:

- reduces expression of RAG-1 and RAG-2
- targets RAG-2 for proteosomal degradation
- reduces access of the HC locus to the recombinase machinery (mechanism unclear)

BCR (B Cell Receptor)

{ Membrane μ HC
Kappa or lambda LC
Igα and Igβ signaling molecules

Tonic and ligand signaling

- Stop LC rearr.
- Survival

- Can sample self antigens

B Cell Tolerance

Central tolerance

- tolerance to self antigens that is established in lymphocytes developing in central lymphoid organs;
- main mechanism: clonal deletion

Peripheral tolerance

- tolerance to self antigens that is established in lymphocytes in the peripheral tissues
- clonal deletion, anergy, clonal ignorance

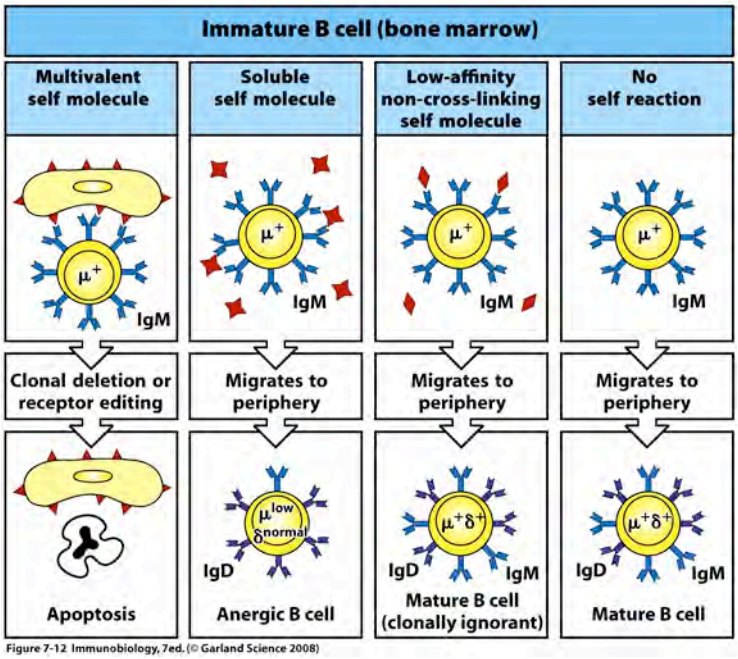


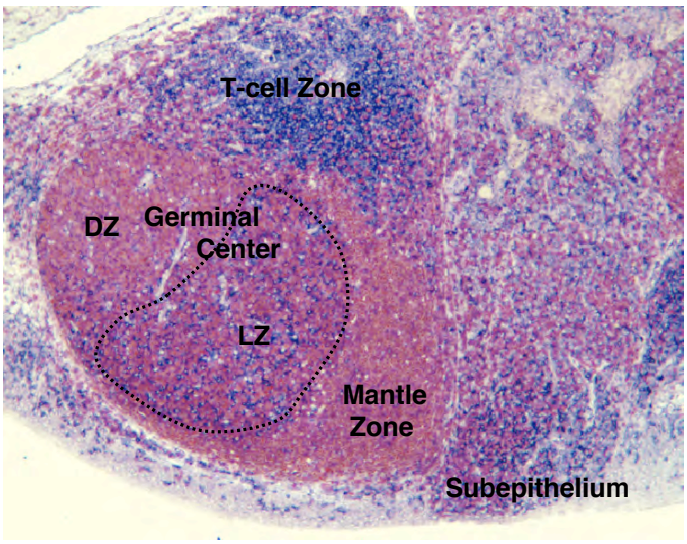
Figure 7-12 Immunobiology, 7ed. (© Garland Science 2008)

Antigen-Independent B-Cell Development

Generation of B Cells in the Bone Marrow

1. DNA rearrangements establish the primary repertoire, creating *diversity*
2. Allelic exclusion ensures that each clone expresses a single antibody on the surface, establishing *specificity*
3. Deletion of self-reactive clones establishes immunological *tolerance*

Antigen-Dependent B-Cell Development Occurs in the Germinal Center (GC) Reaction



Antigen-Dependent B-Cell Development

Generation of B cells with High-Affinity Antigen-Receptors in the Germinal Center (GC) Reaction

1. T-cell dependent activation of antigen-specific naïve B cells, the precursor cells of the GC-reaction

2. Somatic Hypermutation and Ig Class Switch during the GC-reaction generates high-affinity antigen-specific B cells with specialized effector functions

3. Differentiation of antigen-selected GC B cells into memory B cells and plasma cells, the carriers of antibody-dependent (humoral) immunity

Compartmentalization of Antigen-Dependent B-Cell Development

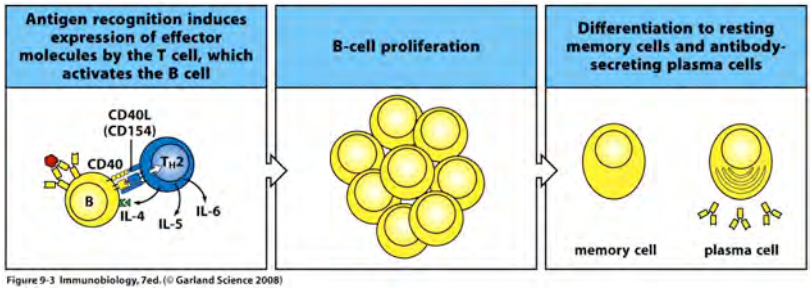


Figure 9-3 Immunobiology, 7ed. (© Garland Science 2008)

Location:	T-cell zone	GC dark zone	GC light zone
Process:	T-cell depend. activation	SHM	CSR; memory B & plasma cell differentiation

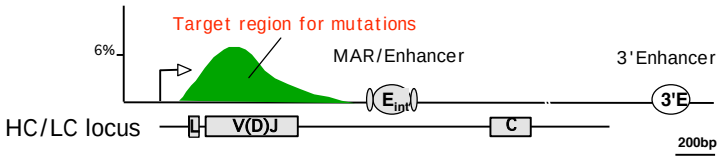
Somatic Hypermutation (SHM)

- Random mutagenesis, mostly single base changes
- Limited to V(D)J (does not extend to C regions)
- Hypermutation is 10^6 more than normal mutation rate (10^{-3}/bp/generation compared to 10^{-9}/bp/generation)
- Occurs only in mature, antigen-activated B-cells
- Combined with selection, results in clones making antibodies with increased affinity for antigen: Affinity Maturation

Features of SHM

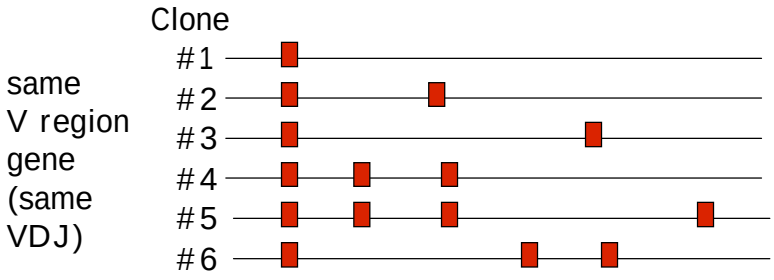
SHM Introduces Mutations into the Rearranged V(D)J Gene Segments

1. Distribution within ~2Kb from the promoter. Requires transcription



2. Requires cis-acting elements
3. Intrinsic characteristics
   - -Mostly single bp substitutions
   - -Predominance of Transitions over Transversions
   - -Specific hotspots motifs: AAGTT / CAGCT = RGYW
4. Associated with DNA strand breaks

Pattern of V Gene Mutations Provides Evidence of Cyclical Mutation and Selection Events



- Random mutation combined with selection

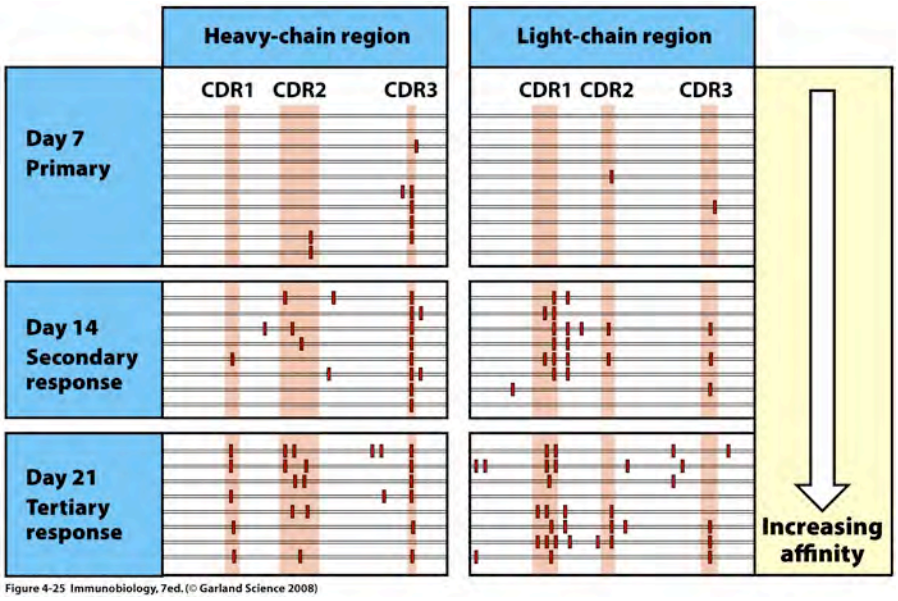
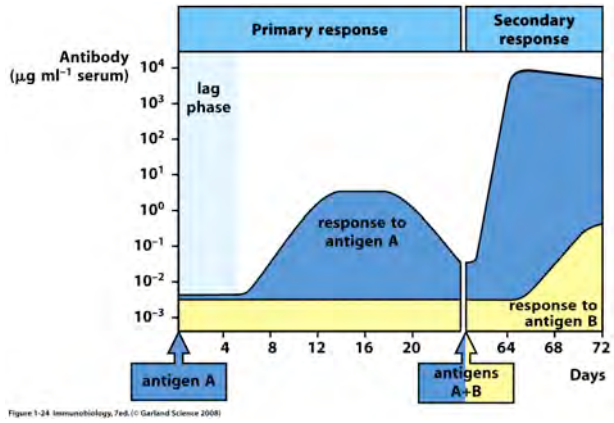


Figure 4-25 Immunobiology, 7ed. (© Garland Science 2008)

Affinity Maturation:

- Increase in the affinity for the specific antigen of the antibodies produced during a humoral immune response
- Particularly prominent in secondary (memory) immunizations



Class Switch Recombination (CSR)

- A DNA rearrangement that allows the same VDJ to be expressed with different heavy chain constant regions

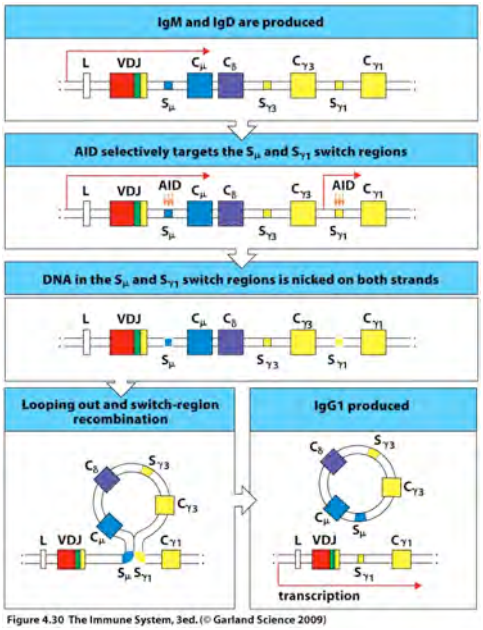


Figure 4.30 The Immune System, 3ed. (© Garland Science 2009)

Expression of Alternate Isotypes

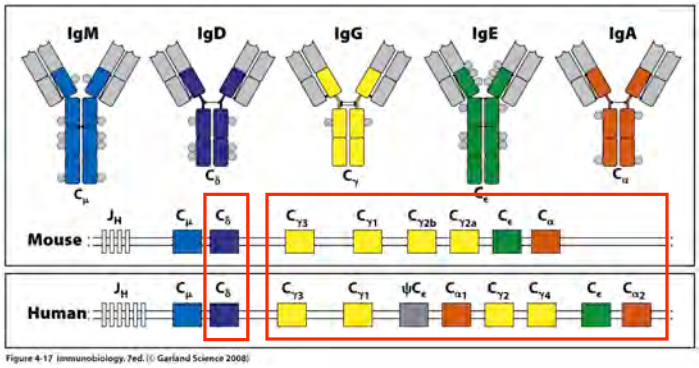


Figure 4-17 Immunobiology, 7ed. (© Garland Science 2008)

Two mechanisms for expression of alternate isotypes:

- IgM to IgD (and membrane to secreted) via differential RNA processing
- IgM to IgG, IgA or IgE by DNA rearrangement

Secreted Antibodies Function in Various Ways To Eliminate Foreign Invaders

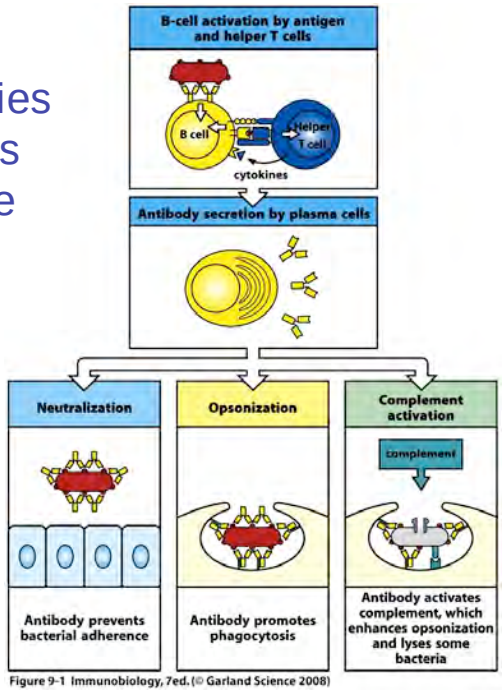
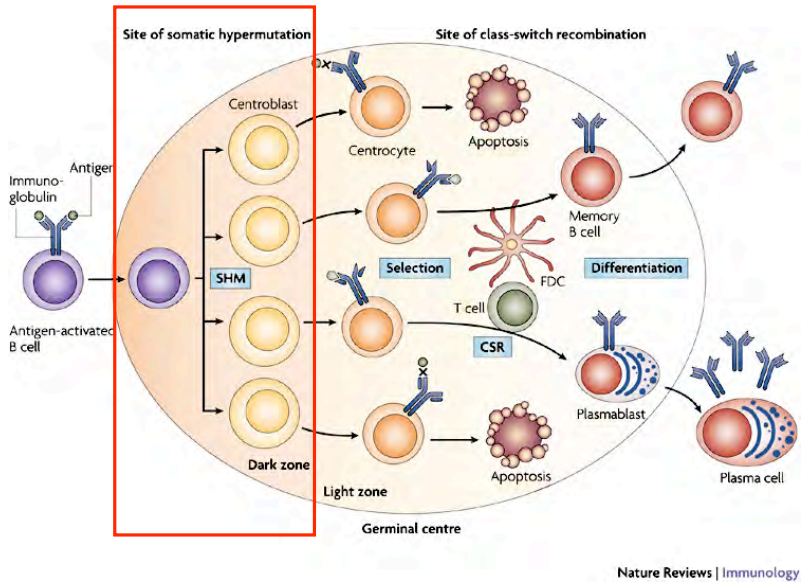


Figure 9-1 Immunobiology, 7ed. (© Garland Science 2008)

The Germinal Center Microenvironment

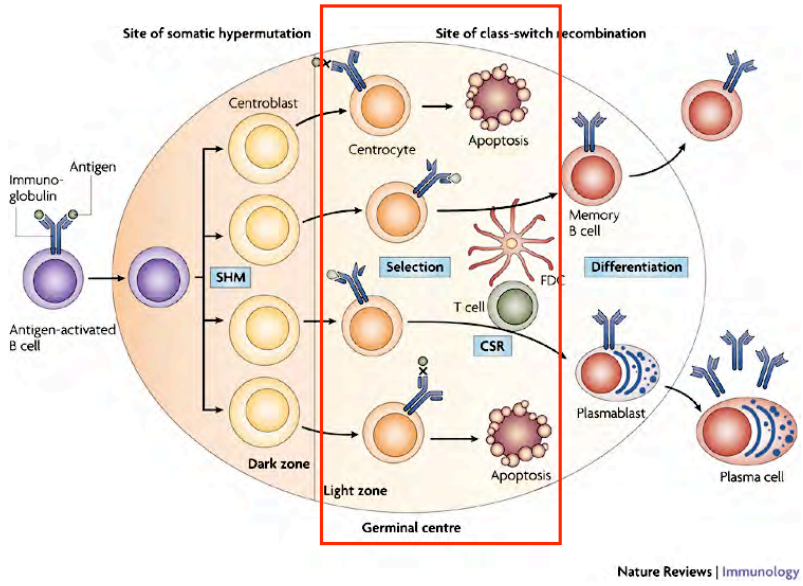


Dark Zone (Centroblasts)

1. Proliferation (one cell cycle completed in 12 h)

2. Generation of antibody-variants by SHM

The Germinal Center Microenvironment



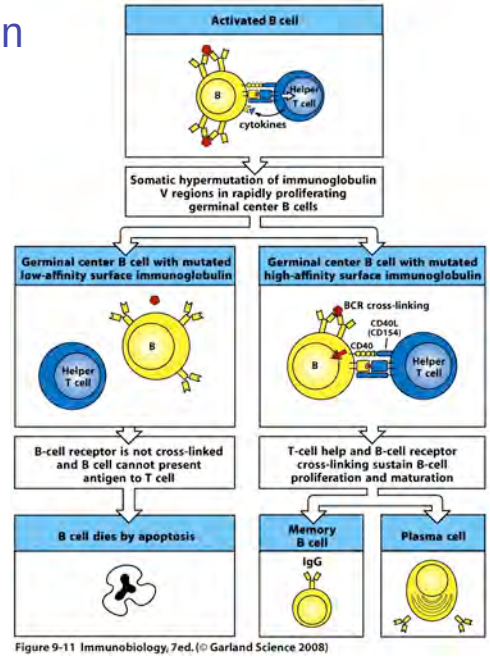
Light Zone (Centrocytes)

1. Selection
   - for high-affinity B cell clones
   - against newly generated self-reactive B cell clones

2. Generation of different antibody isotypes by CSR

3. Differentiation into memory B cells and plasma cells

Selection



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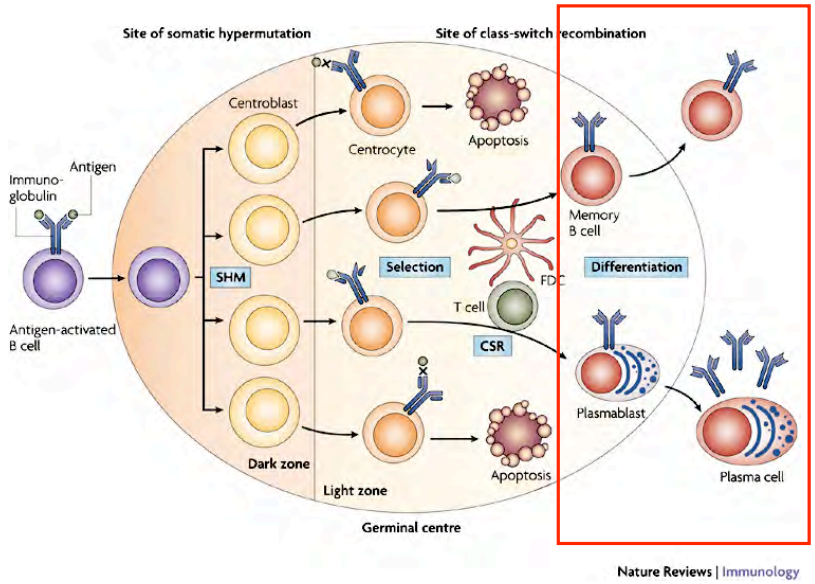
The Germinal Center Microenvironment



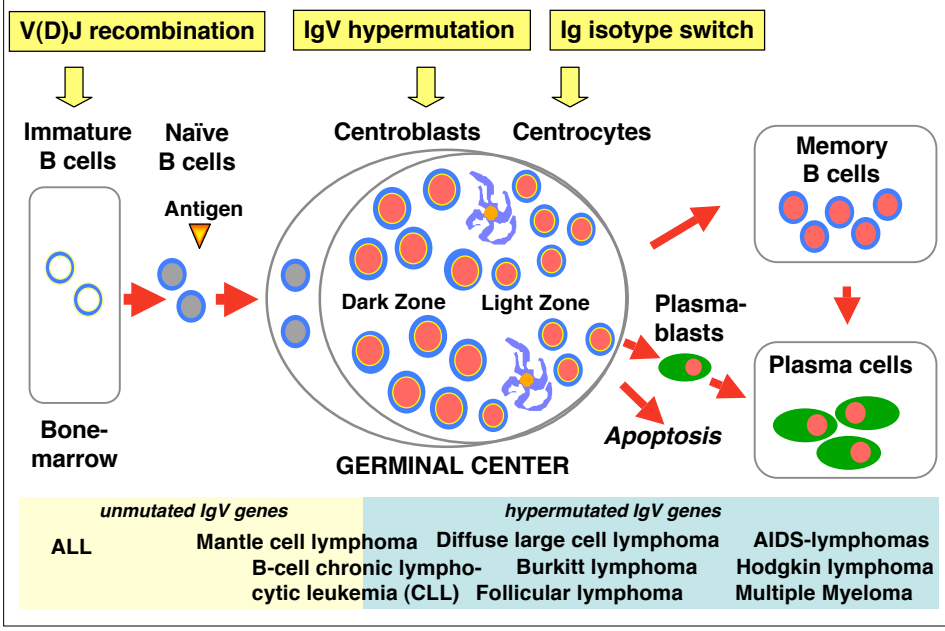
Nature Reviews | Immunology

Two Differentiated B Cell States Follow GC Reaction

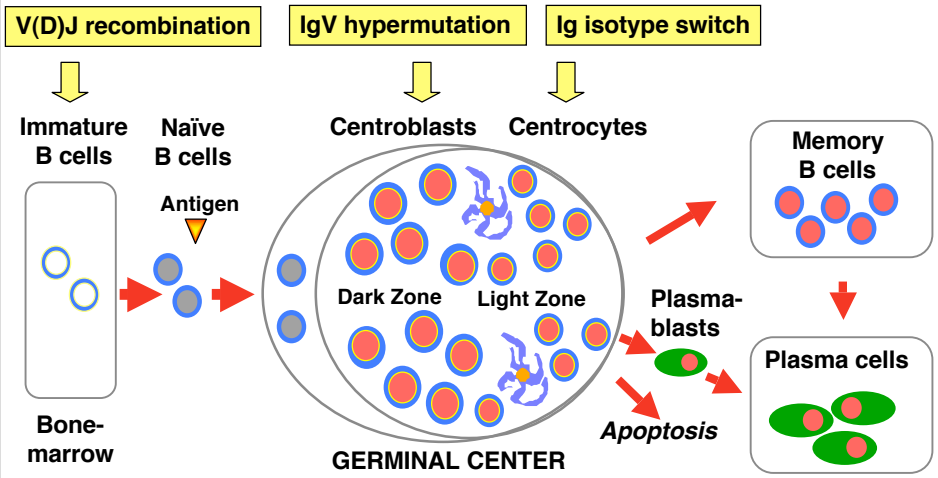
Memory B cell: No Ig secretion, but rapid response to renewed antigen-encounter with high affinity and switched isotypes; circulate b/w lymphoid tissues through the blood

Plasma cell: Ig secretion of high affinity and switched isotypes; home to the bone marrow

Most B-Cell Lymphomas Derive from the GC



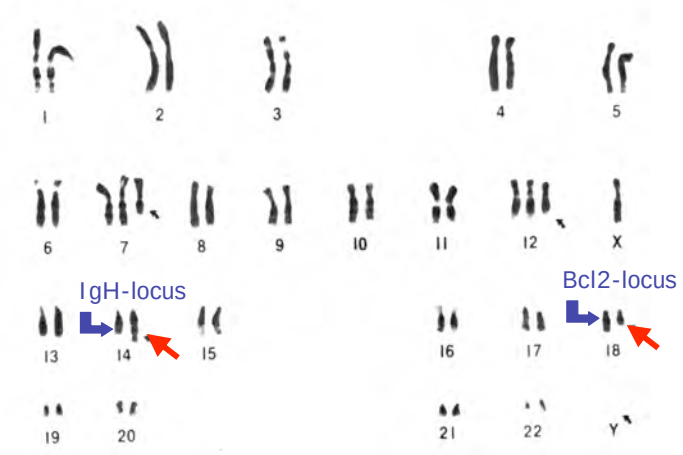
Most B-Cell Lymphomas Derive from the GC



- Many GC-derived lymphomas are characterized by reciprocal balanced chromosome translocations (*BCL2-IgH*, *c-MYC-IgH*, and *BCL6-IgH)*

t(14;18) Translocation in Follicular Lymphoma

(G-banded Karyotype)

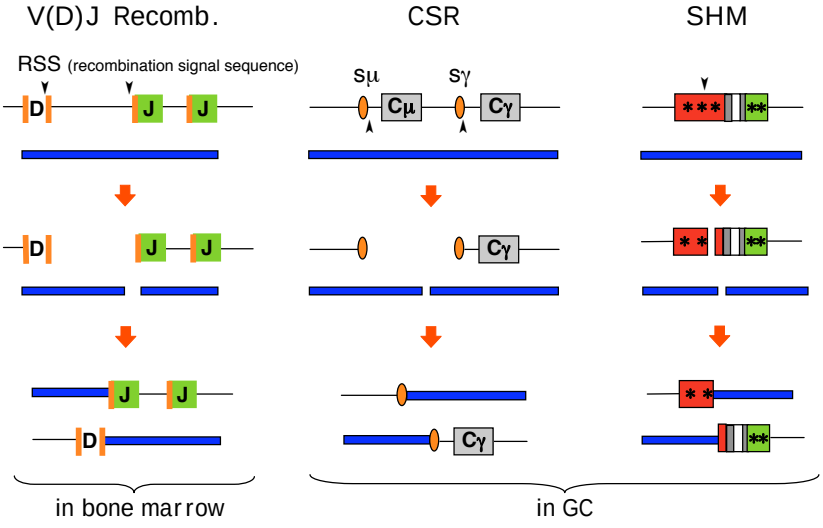


Chromosomal Translocations in B-Cell Lymphoma

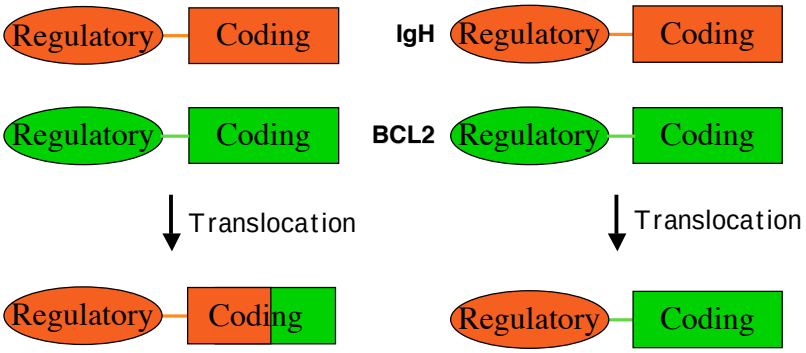
LYMPHOMA	TRANSLOCATION	GENE	PROTEIN
LYMPHOPLASMACYTIC	t(9;?)(p13;?)	PAX5	Transcription Factor
MANTLE CELL	t(11;14)(q13;q32)	BCL1	Cyclin D1
FOLLICULAR	t(14;18)(q32;q11)	BCL2	Anti-Apoptosis
MALT LYMPHOMA	t(11;18)(q21;q21)	API2/MLT	Anti-Apoptosis
DIFFUSE LARGE CELL	t(3;x)(q27;x)^	BCL6	Transcription Factor
BURKITT	t(8;14)(q24;q32)	cMYC	Transcription Factor

^x=various chromosomal partners

Reciprocal Translocations in B-Cell Lymphoma as Mistakes of the B-Cell Specific Ig-Locus Modifying Processes



Consequences of Chromosomal Translocations

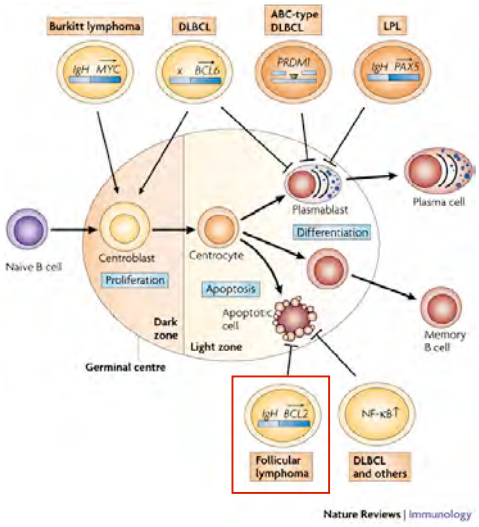


Fusion protein
(Sarcomas and leukemias)

Deregulated transcription
(Lymphomas)

- since IgH locus is expressed throughout B-cell development

Disruption of Normal Transcriptional Programs by Genetic Lesions Promotes Lymphomagenesis



Conclusions

- The GC-reaction generates memory B cells and plasma cells that produce high-affinity antibodies, which are necessary to protect against invading microorganisms

There is a caveat, however…

- The beneficial role of the GC in immunity is somewhat counterbalanced by its detrimental role in lymphoma-genesis, as the majority of B-cell lymphomas originate from GC B cells