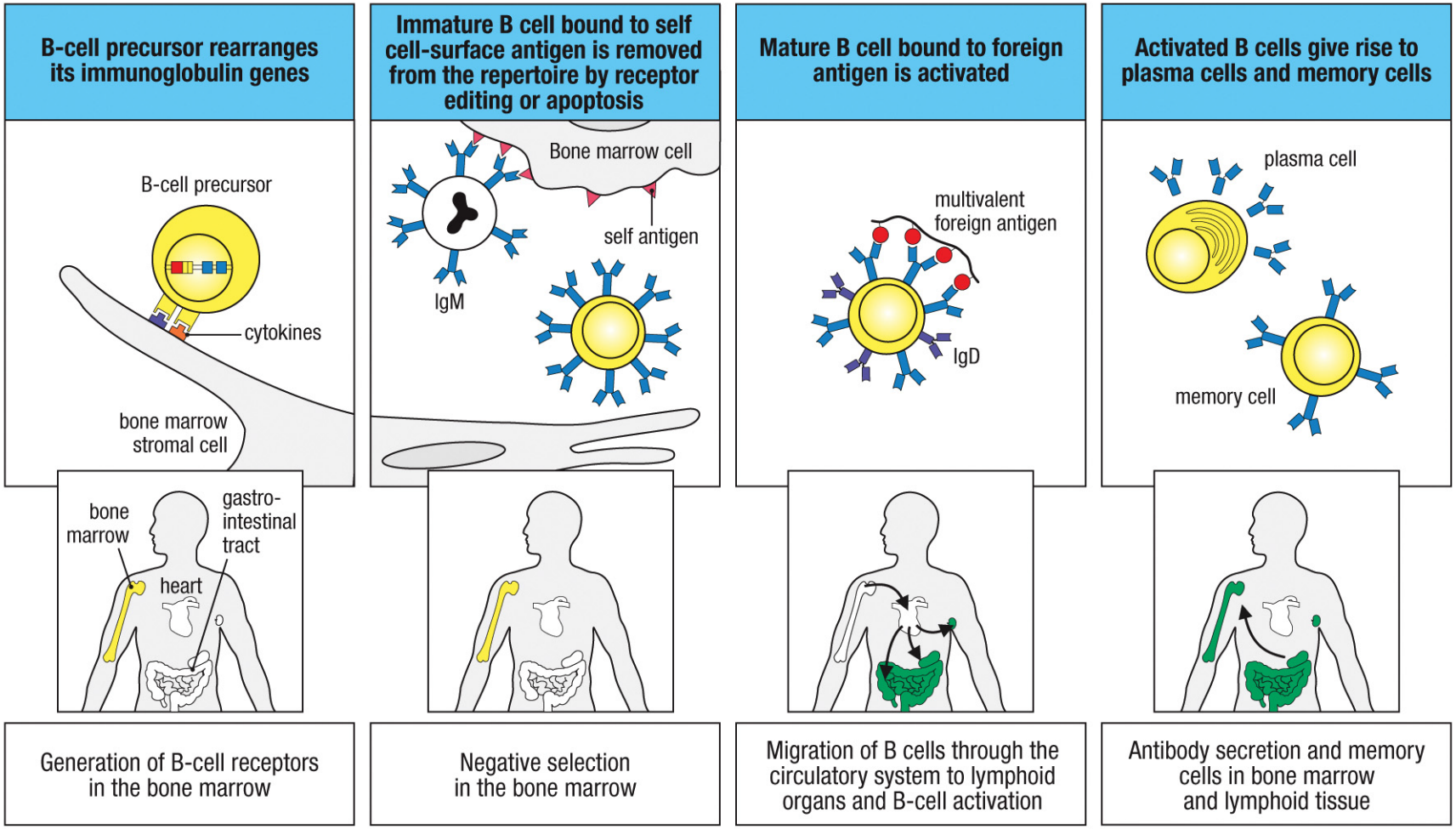
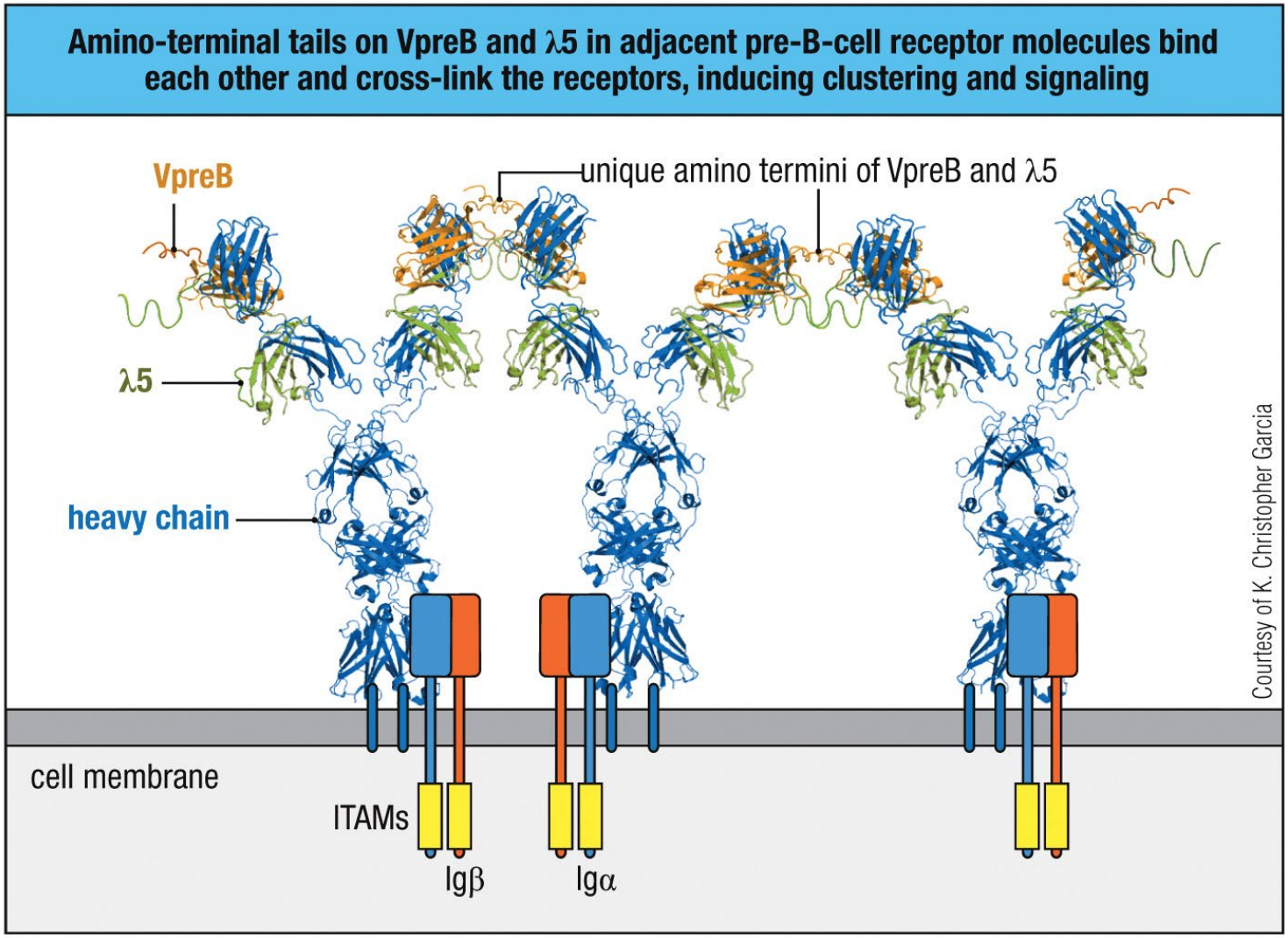
Life-Cycle of B Cells: Bone Marrow to Lymphoid Tissues



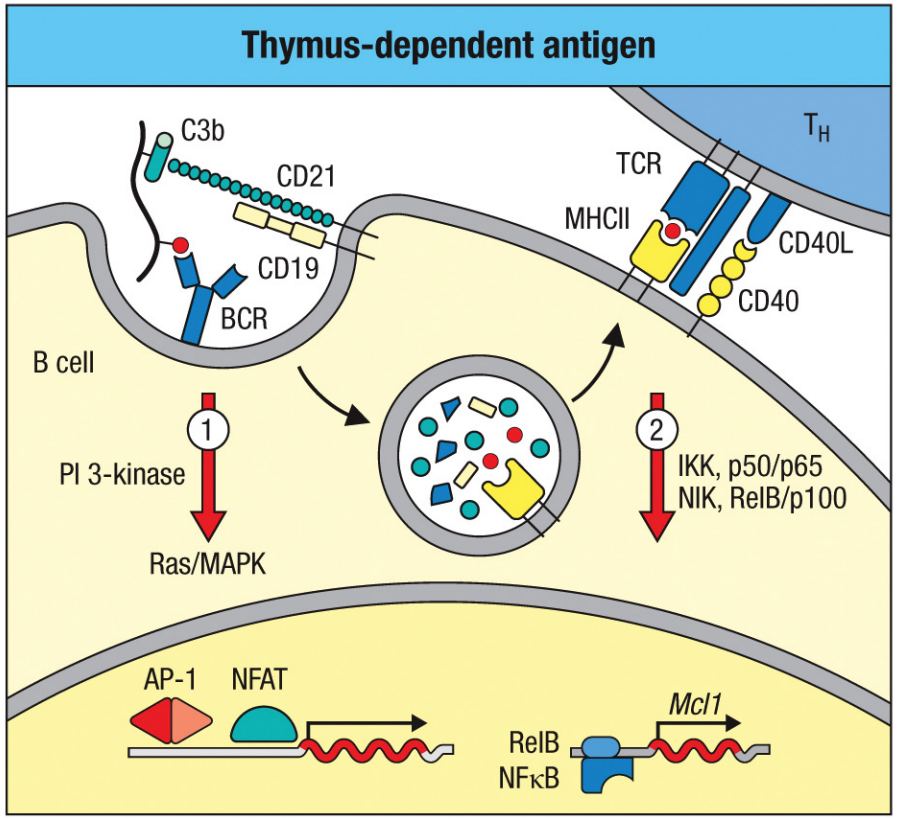
Pre-B-Cell Receptor

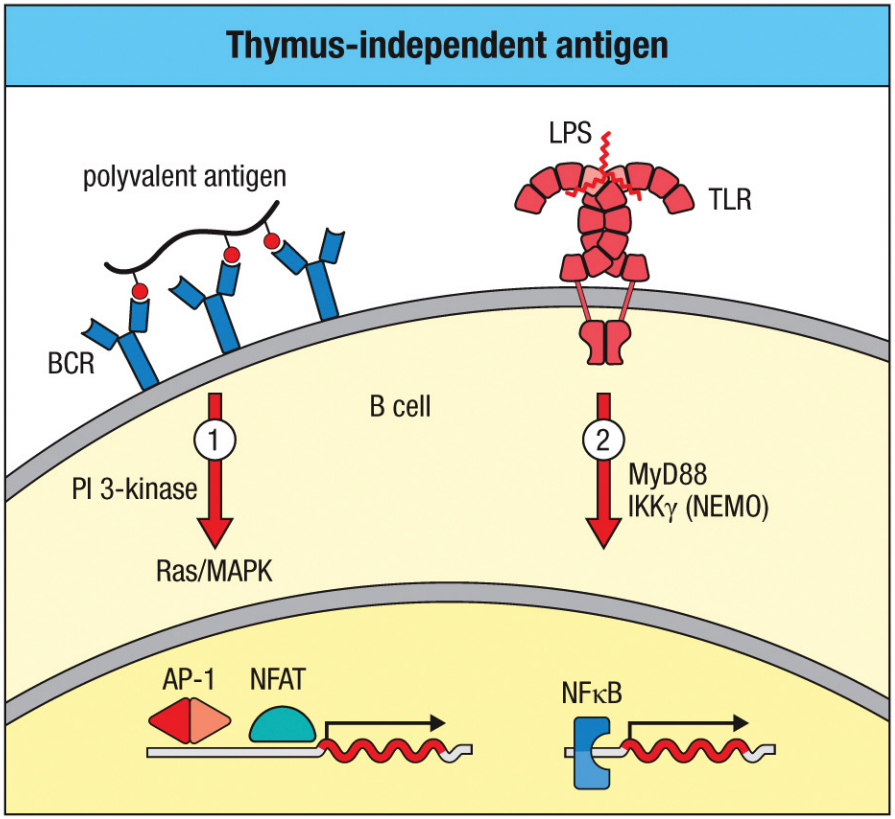


Outline

- Humoral Immune response
  - B cell activation (thymus dependent)
  - Affinity Maturation
  - Class Switching
  - Thymus-independent B-Cell activation

Two Signals Are Required for B Cell Activation

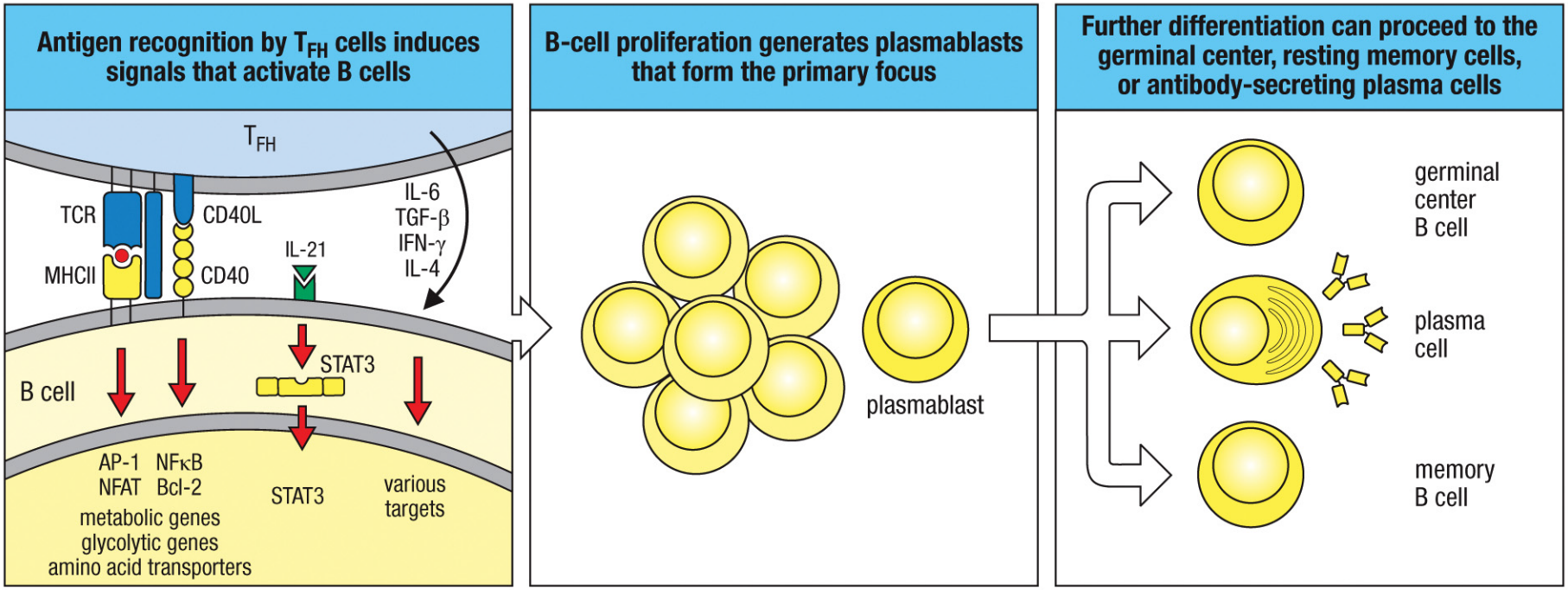




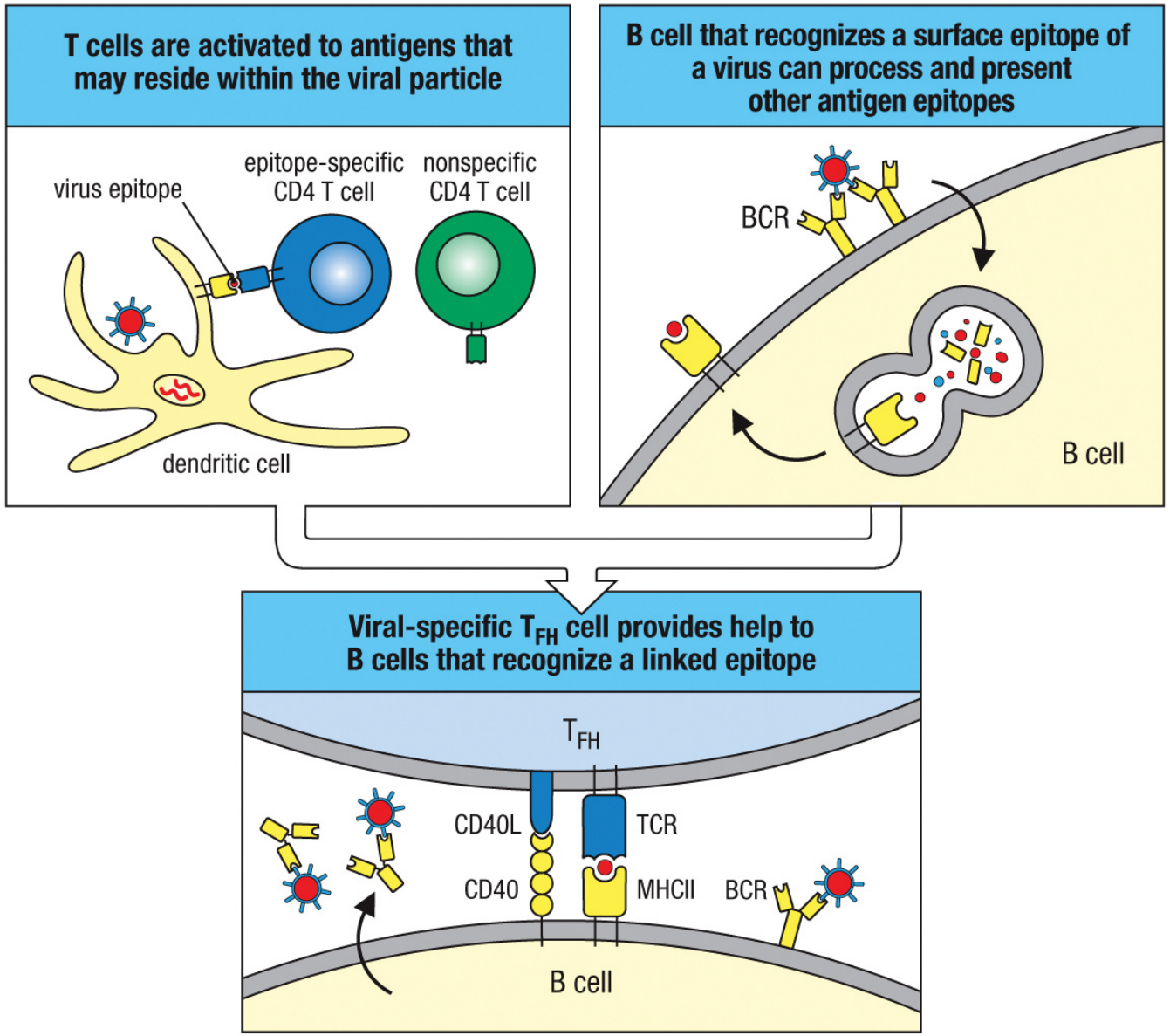
1. Crosslinking of the BCR
2. Signal from T helper Cells

1. Extensive cross linking of BCR (IgM)
2. Activation of TLR

T_H Cells Stimulate Proliferation Followed by Differentiation of B-Cells



T_H Cells and B-Cells Must Recognize Epitopes from the Same Pathogen to Interact



Non-Protein Antigens Are Linked with A Protein in Vaccines to Stimulate an Immune Response

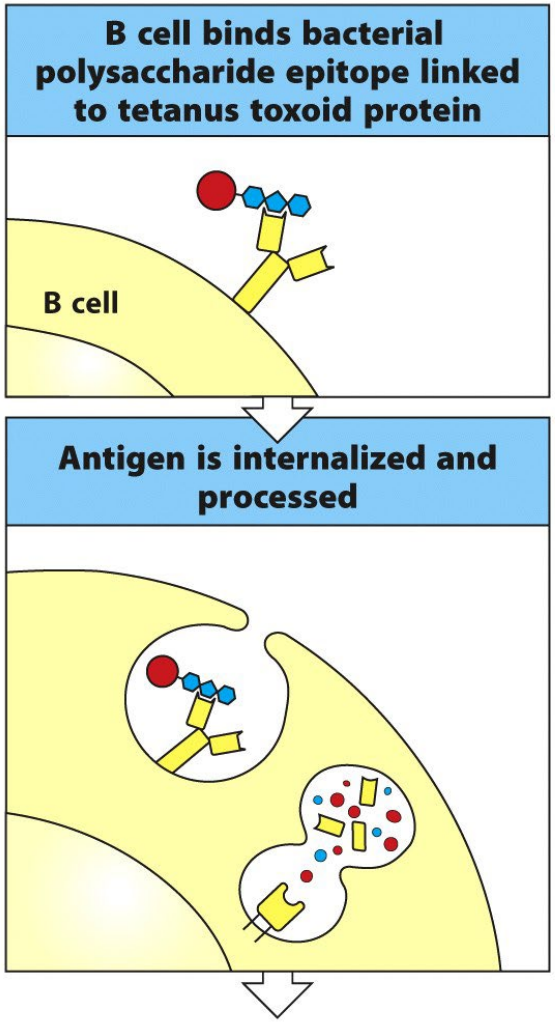


Figure 10.5 part 2 of 4 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

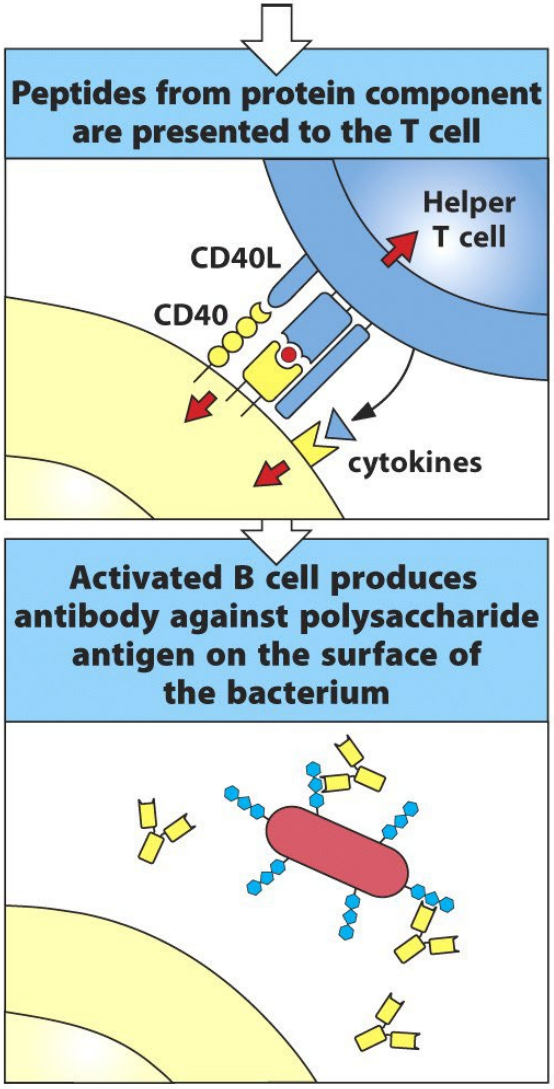
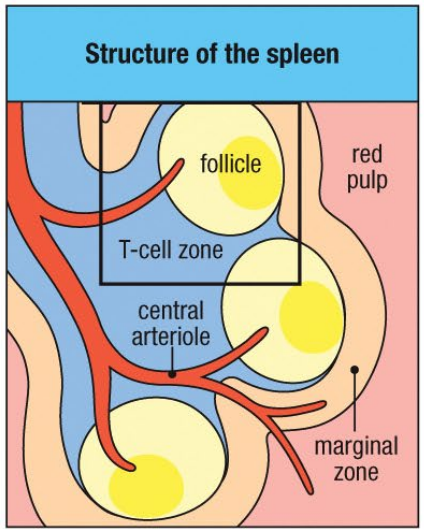
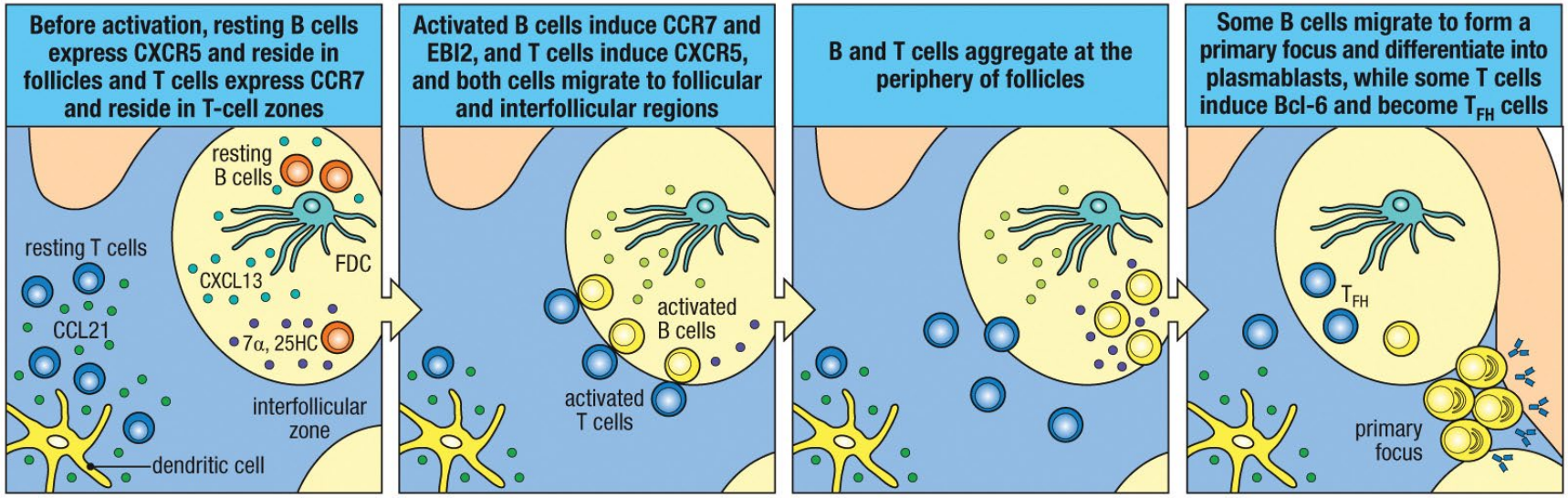


Figure 10.5 part 4 of 4 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

B Cells are Activated by T_H Cells at the Boundary of the Follicle

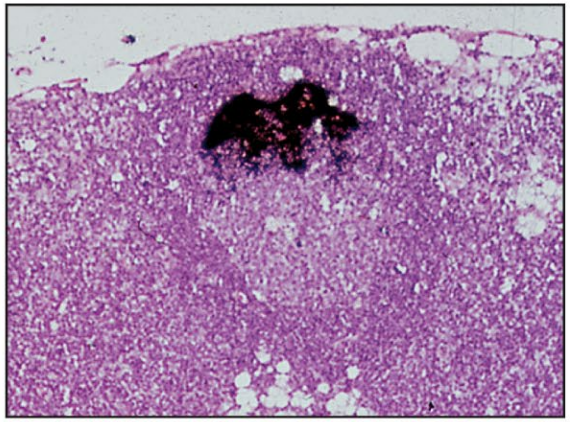


CXCL13—CXCR5 follicle homing
7alpha, 25-HC ----- EBI2, outer follicle homing
CCL21-----CCR7 T cell zone homing

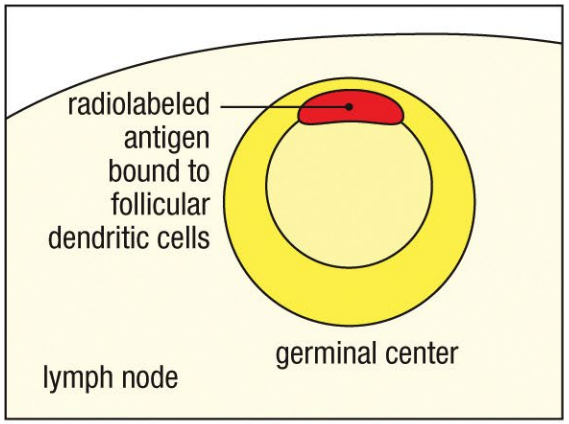


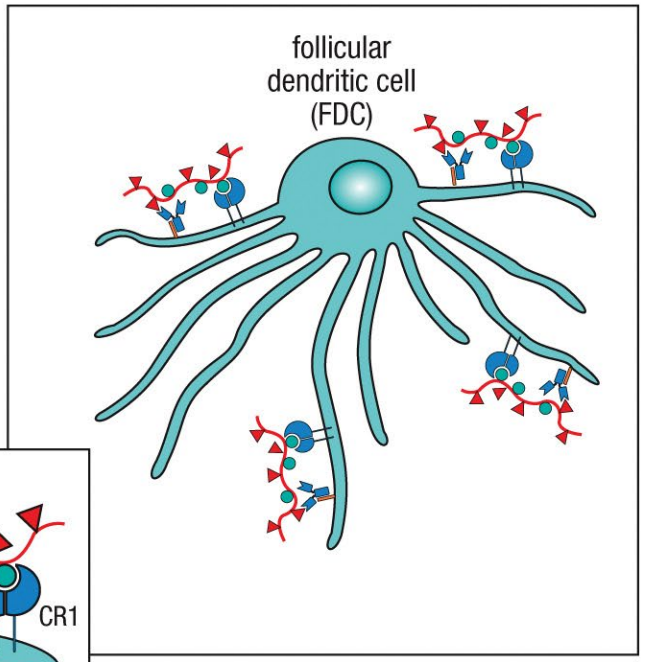
Follicular Dendritic Cells

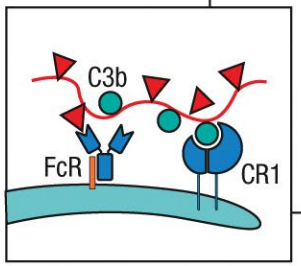
FDC display antigen-antibody-complement complexes on cell surface



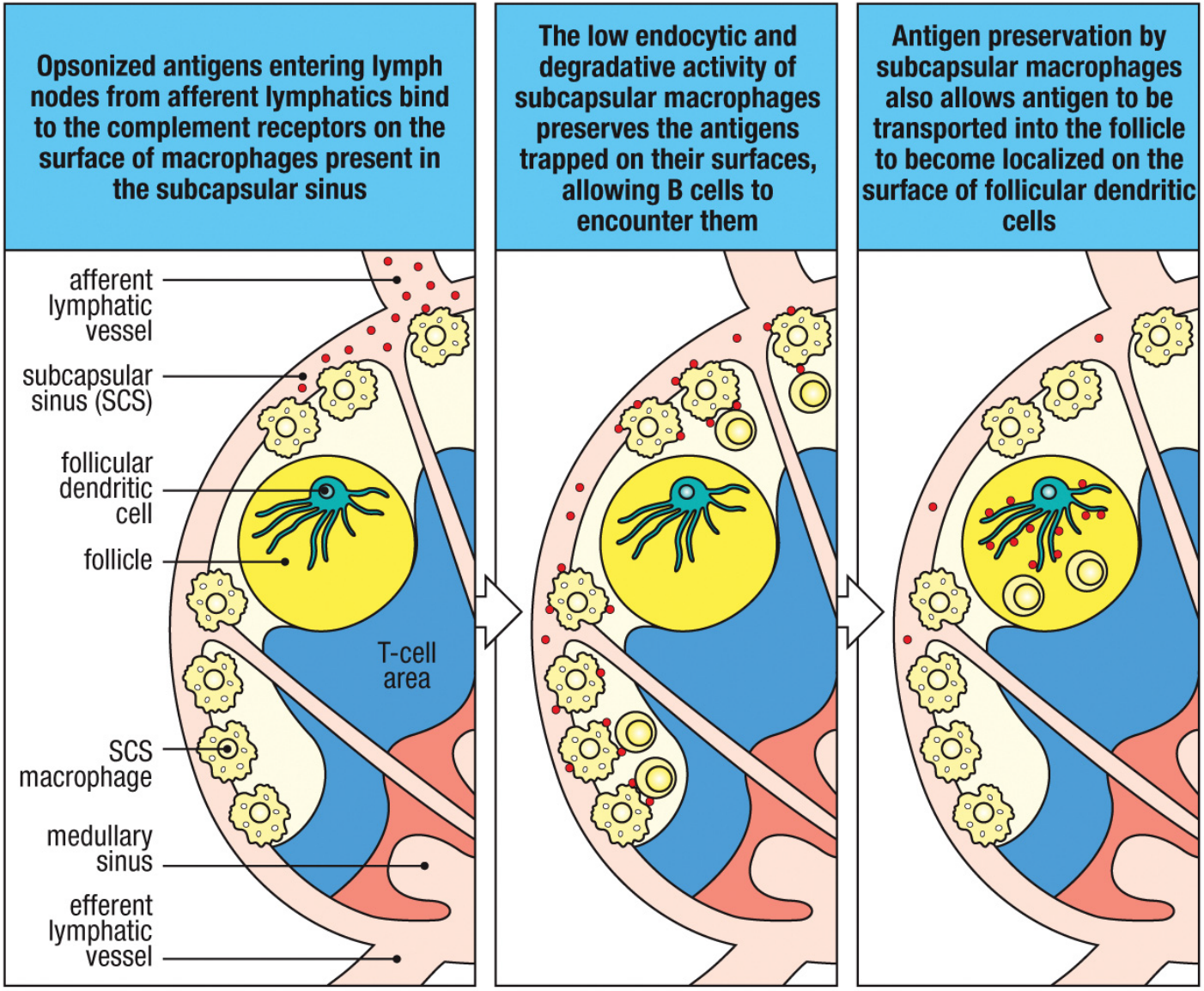
Photograph courtesy of Dr. John Tew



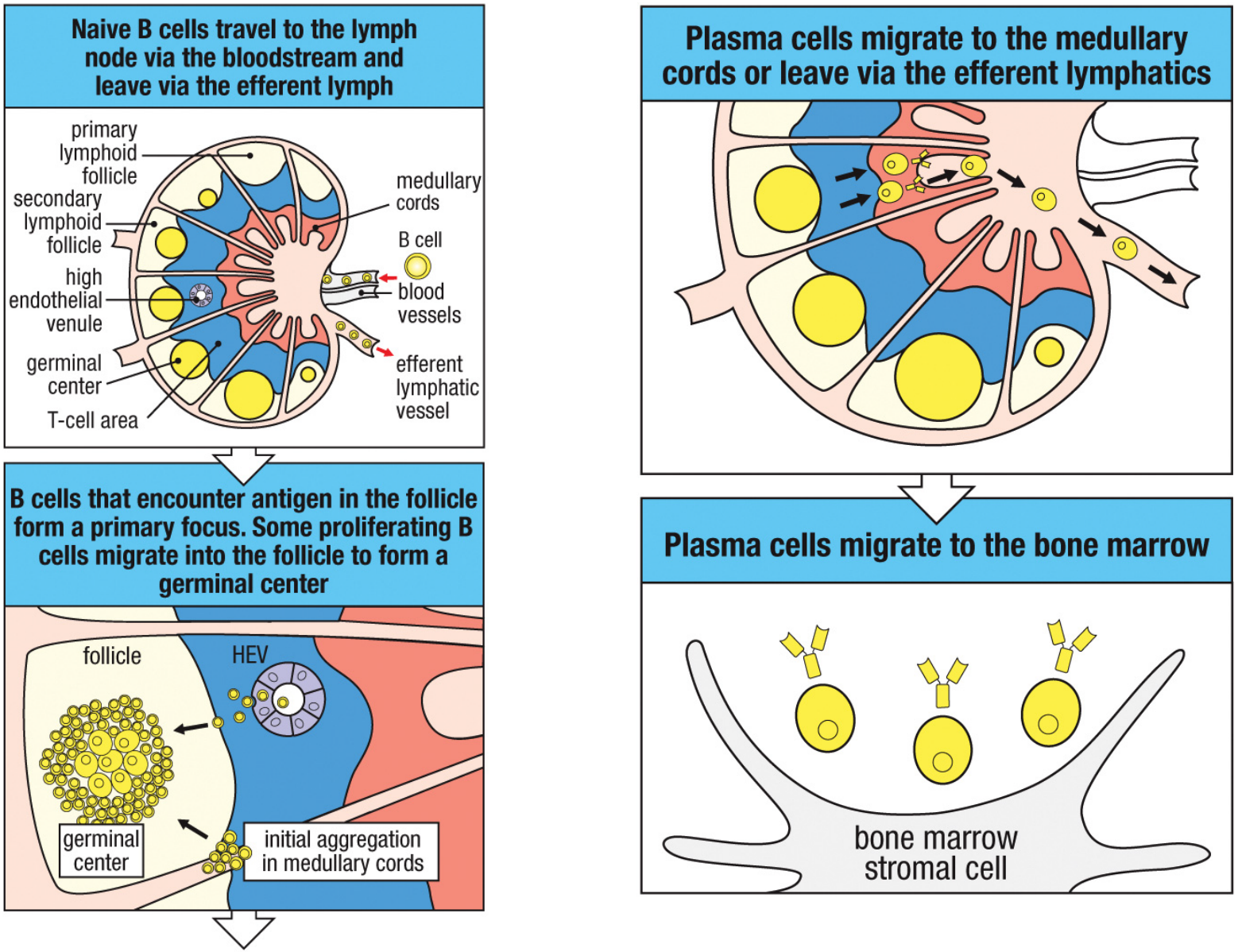




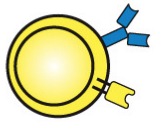
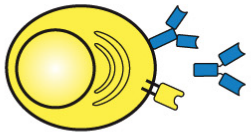
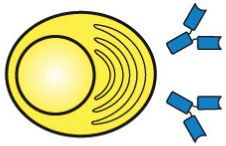
Opsonized antigens are captured and preserved by subcapsular sinus macrophages



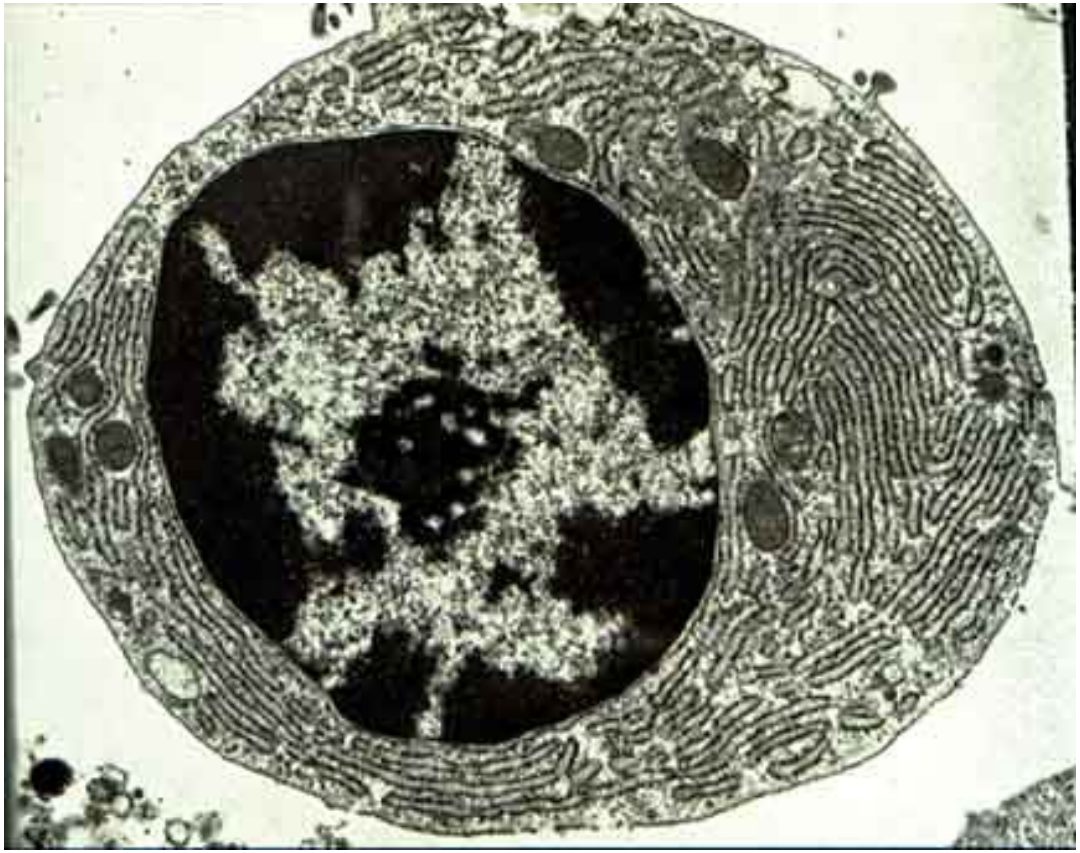
Activated B-Cells Form Germinal Centers in Lymph Node Follicles



Plasma Cell Differentiation

	Intrinsic properties			Inducible by antigen stimulation		
B-lineage cell	**Surface Ig**	**Surface MHC class II**	**High-rate Ig secretion**	**Growth**	**Somatic hyper-mutation**	**Class switch**
 Resting B cell	High	Yes	No	Yes	Yes	Yes
 Plasmablast	High	Yes	Yes	Yes	Unknown	Yes
 Plasma cell	IgA, IgE: High IgG: Low	Yes	Yes	No	No	No

Plasma Cells

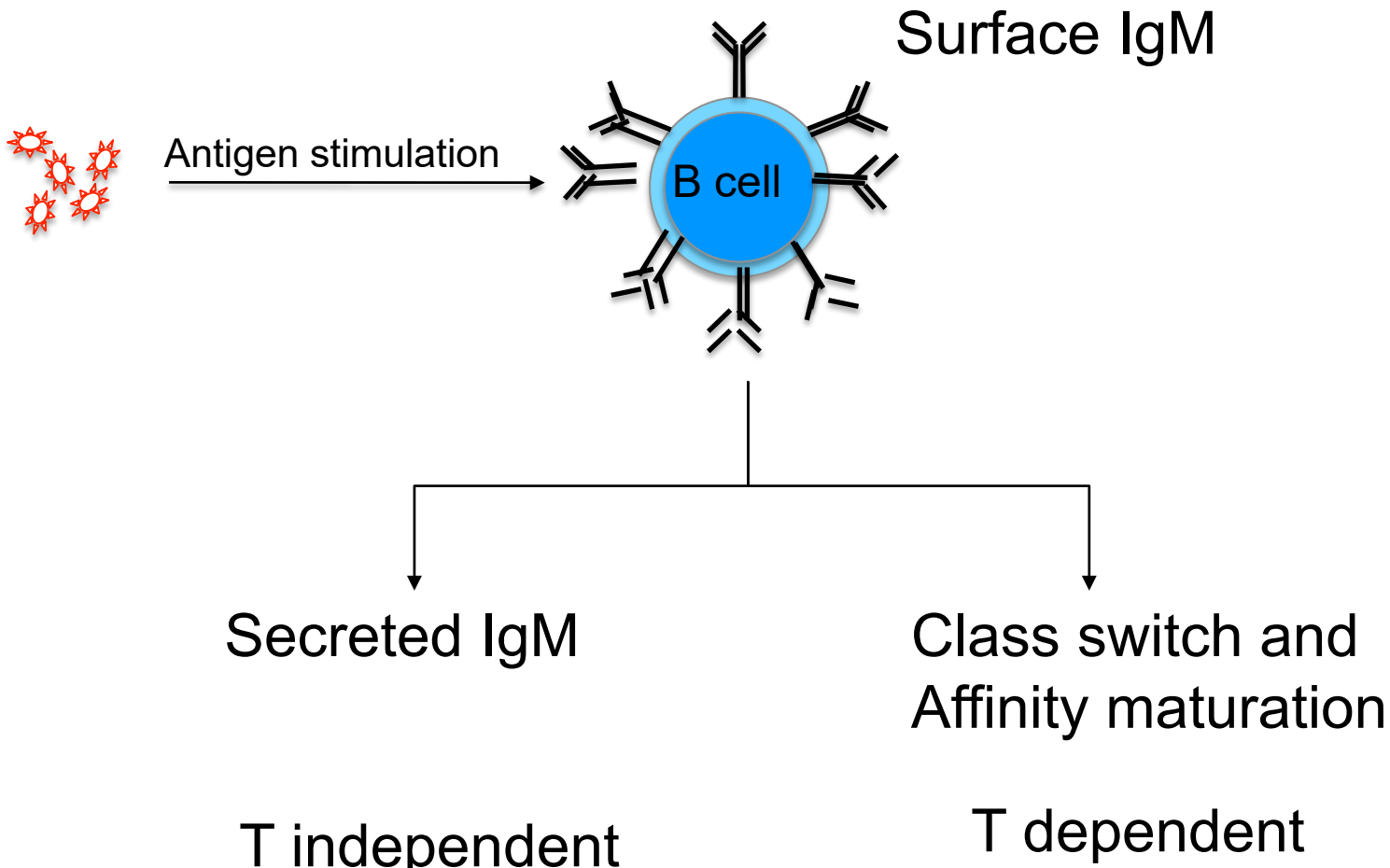


http://millette.med.sc.edu/Lab%206%20pages/C18%20image%20page.htm

Outline

- Humoral Immune response
  - B cell activation (thymus dependent)
  - Affinity Maturation
  - Class Switching
  - Thymus-independent B-Cell activation

Antigen Stimulation Induced Isotype Switch



Immunoglobulin Diversification

	Primary repertoire	***Secondary repertoire***
Stage of development	Naïve B cells	Activated B cells
Variable region	V(D)J recombination	Somatic hypermutation
Constant region	IgM or IgD	IgG, IgA, IgE, IgM, IgD

Immunoglobulin Diversification

Diversification mechanism	*Effect*
Somatic hypermutation	Antigen specificity
Class switching	Effector activity

Irreversible changes at the DNA level

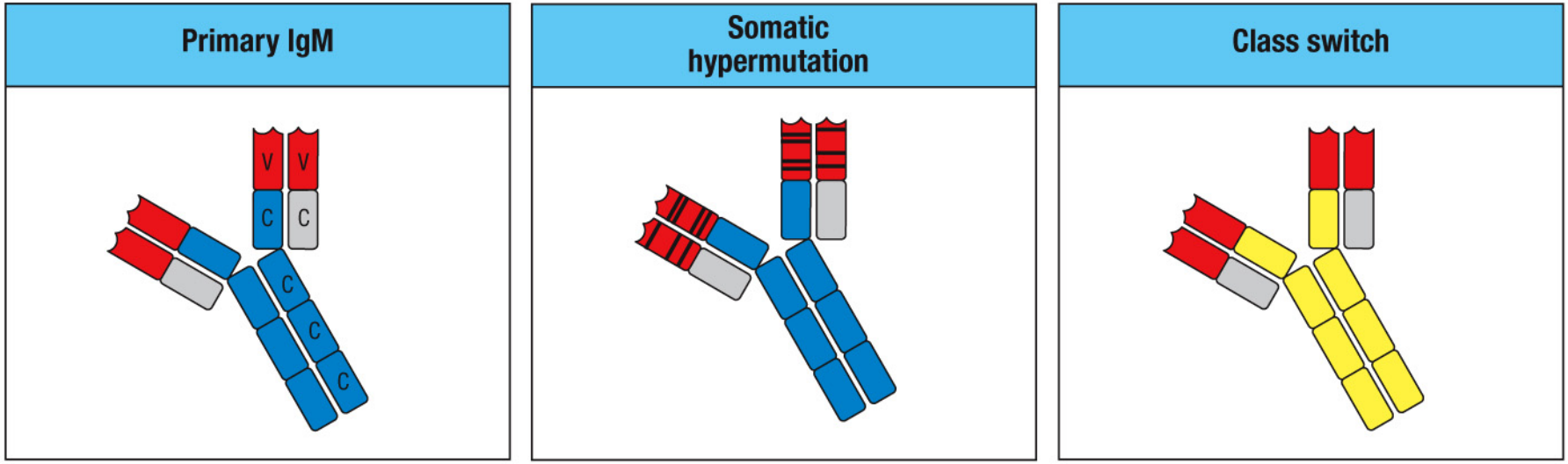


Figure 10.13 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

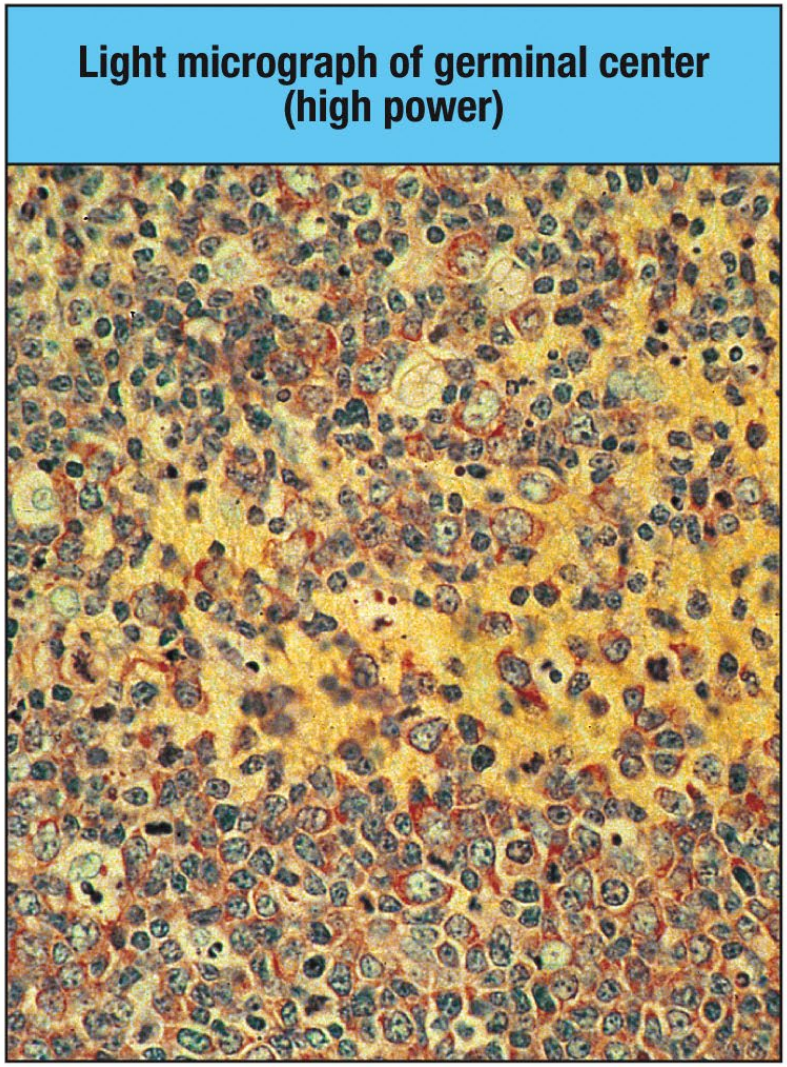
Somatic Hypermutation Is Required for Affinity Maturation of Antigen Specific Antibody

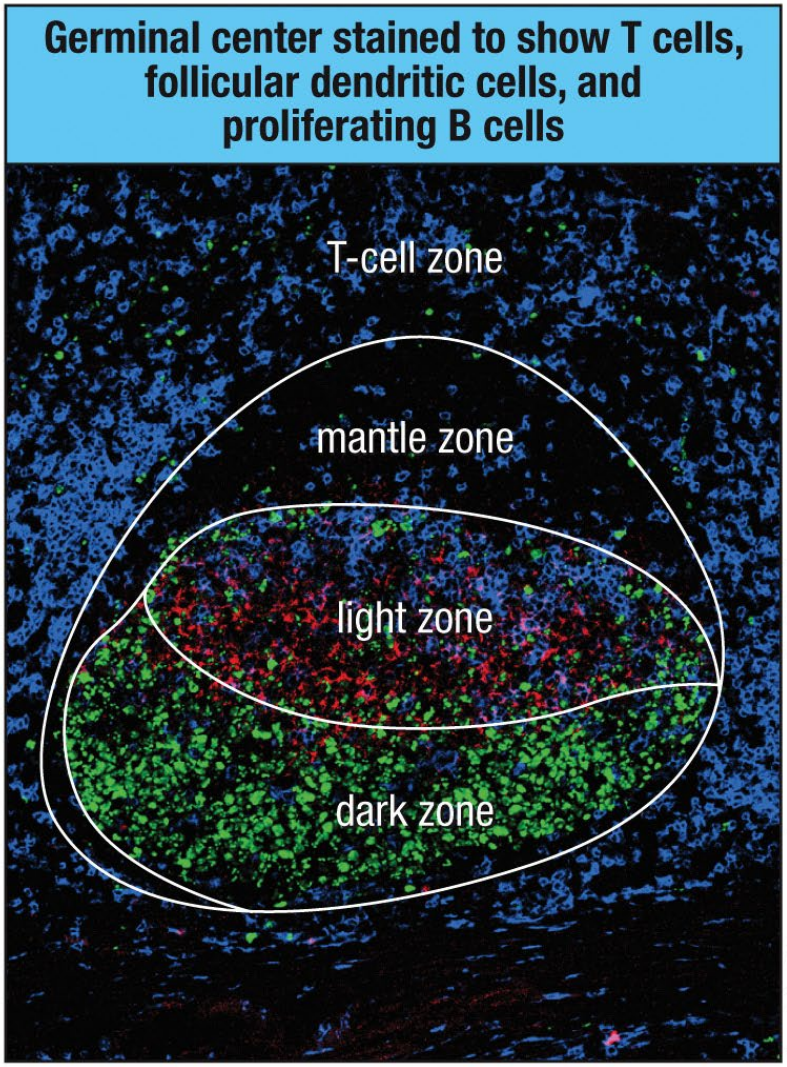
Affinity maturation

- Increase in the affinity of the antibody for the specific antigen
  - Many clones undergo independent mutations in the germinal center
  - Clones with the highest affinity are favored

- Prominent in secondary infections (memory)
- Immunization

Germinal Center

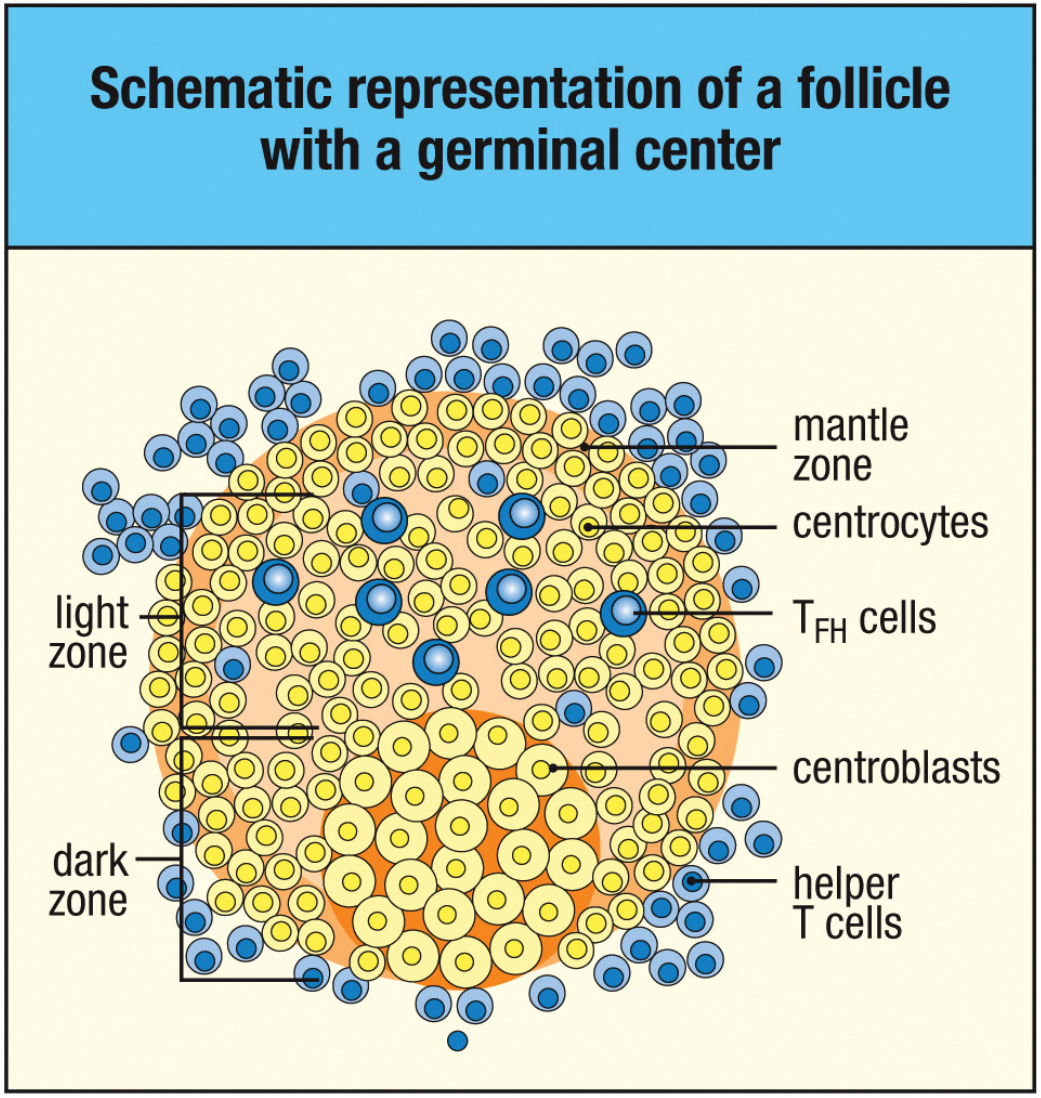




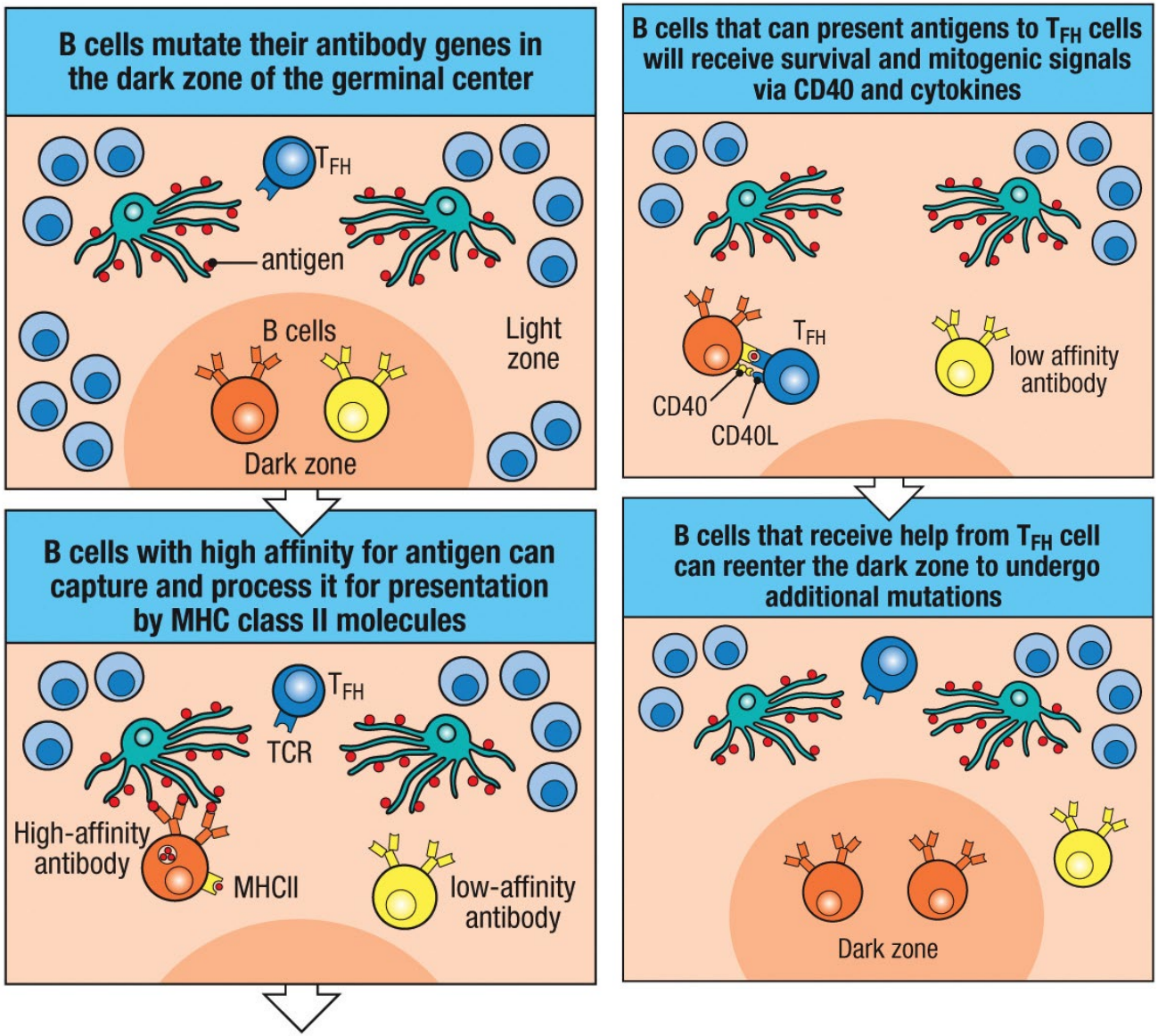
(both): Photographs courtesy of I. MacLennan

Green: Ki67, proliferating cells; Red: FDC; Blue: CD4 T cells

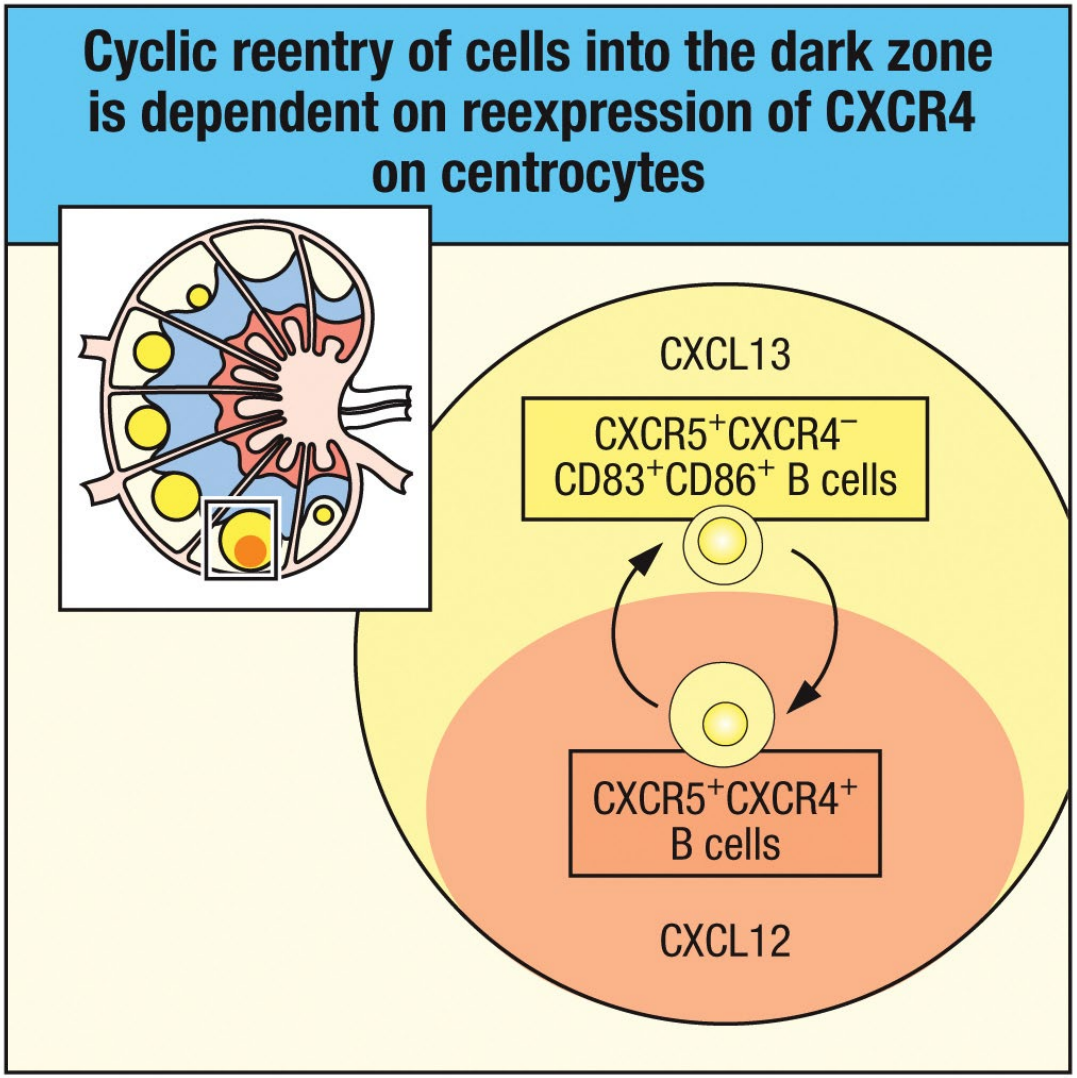
Germinal Center



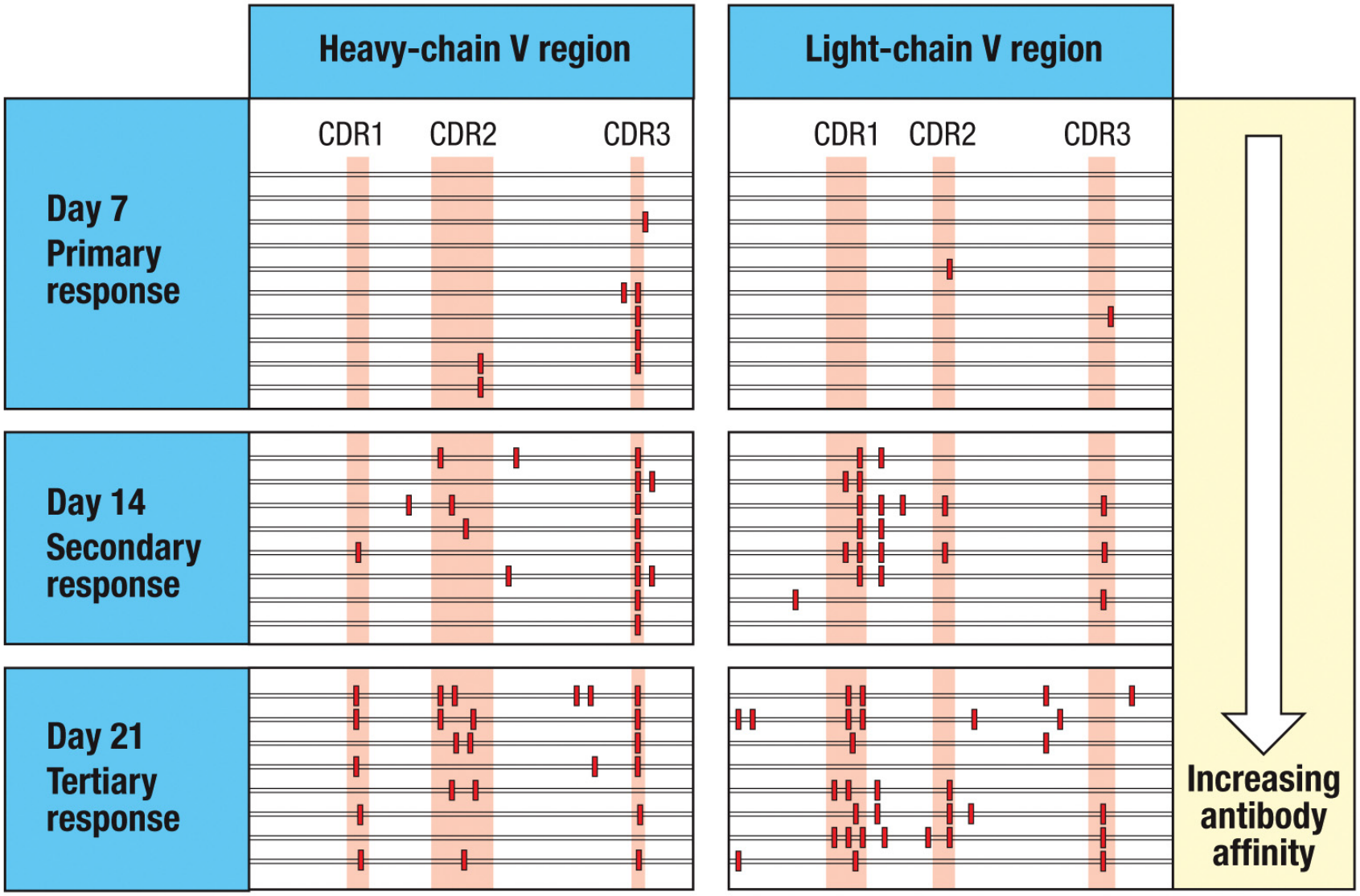
Affinity Maturation



Affinity Maturation



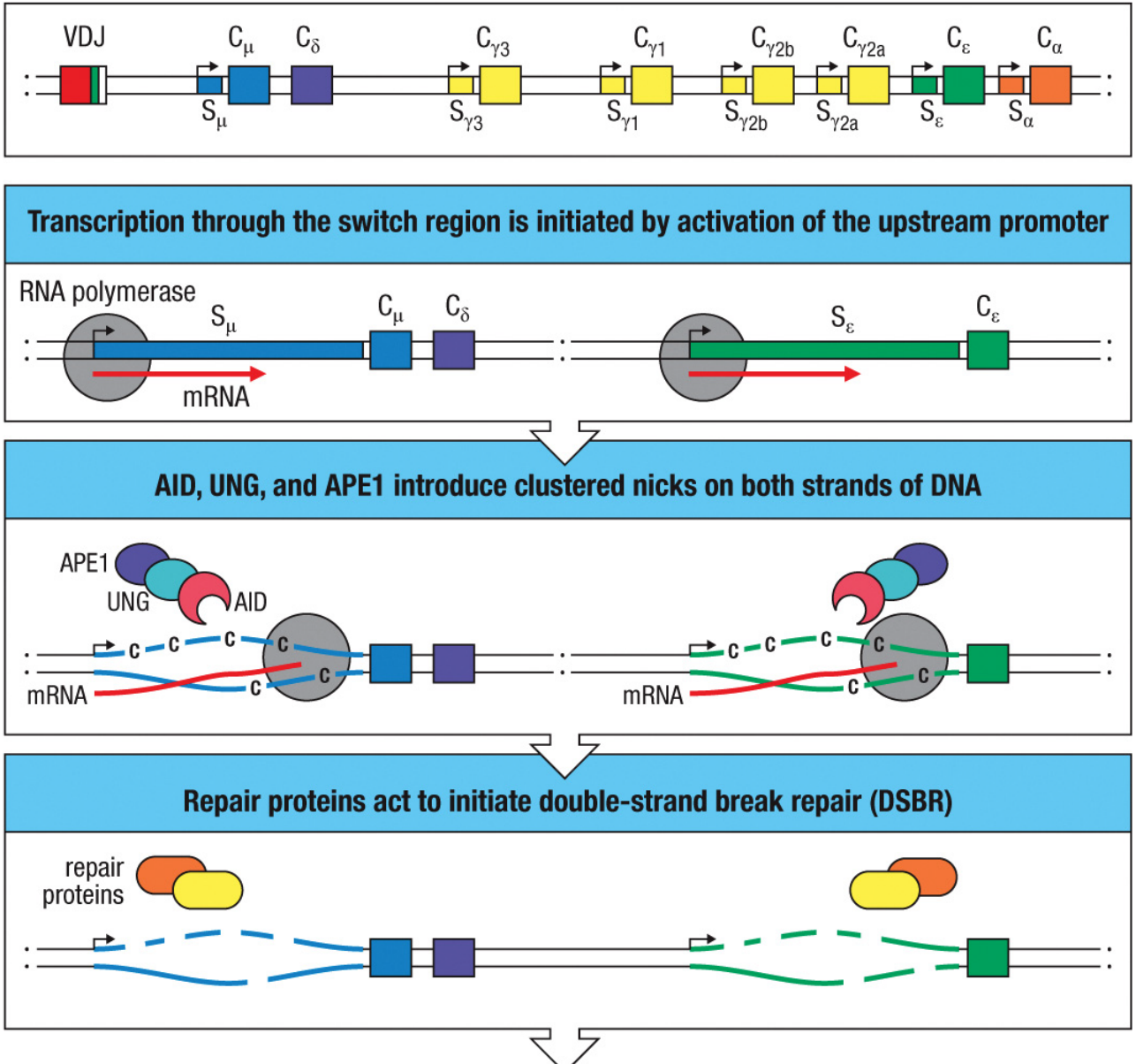
Increased Rate of Mutagenesis Generates Antibodies with Increased Affinity for Their Antigens



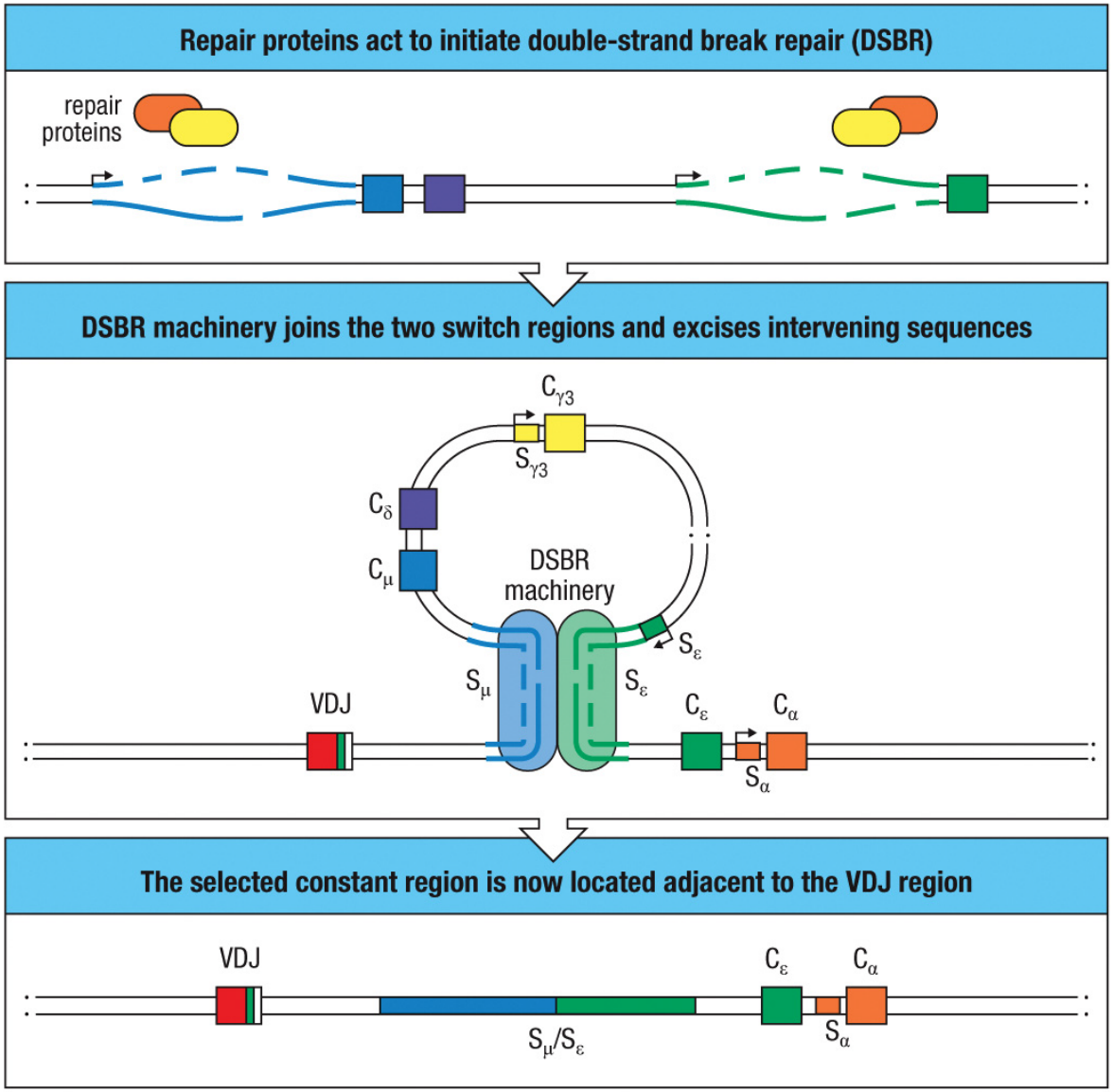
Class Switch

- Irreversible DNA recombination
  - non-homologous DNA recombination
- Always productive
- Initiated in response to:
  - antigen stimulation
  - cytokine stimulation
- Guided by switch regions
  - within intron between J and C segments
  - Dependent on AID, but not RAG

Class Switch Is Guided by Switch Signals



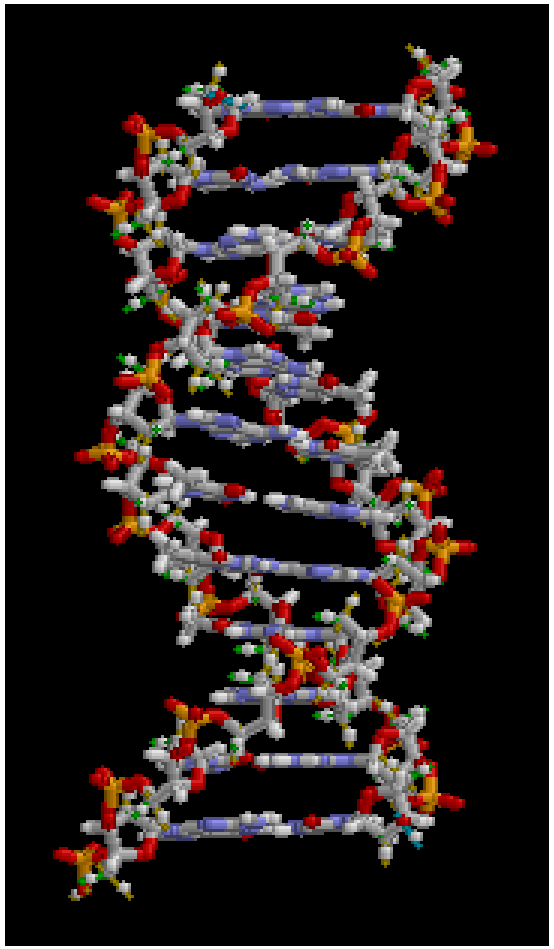
Class Switch Is Guided by Switch Signals

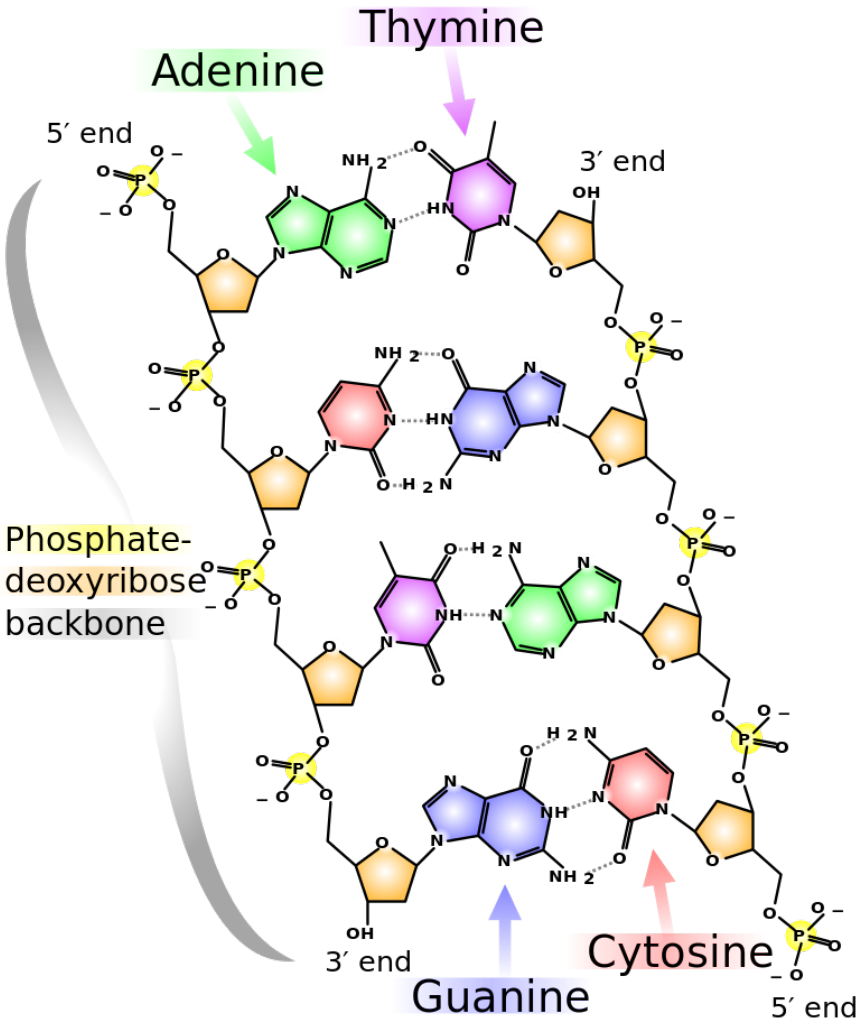


Activation-Induced Cytidine Deaminase (AID)

- Only expressed in B cells
- Only work on single strand DNA
  - Active transcription
- Initiates:
  - Mutations during somatic hypermutation
  - Class switching

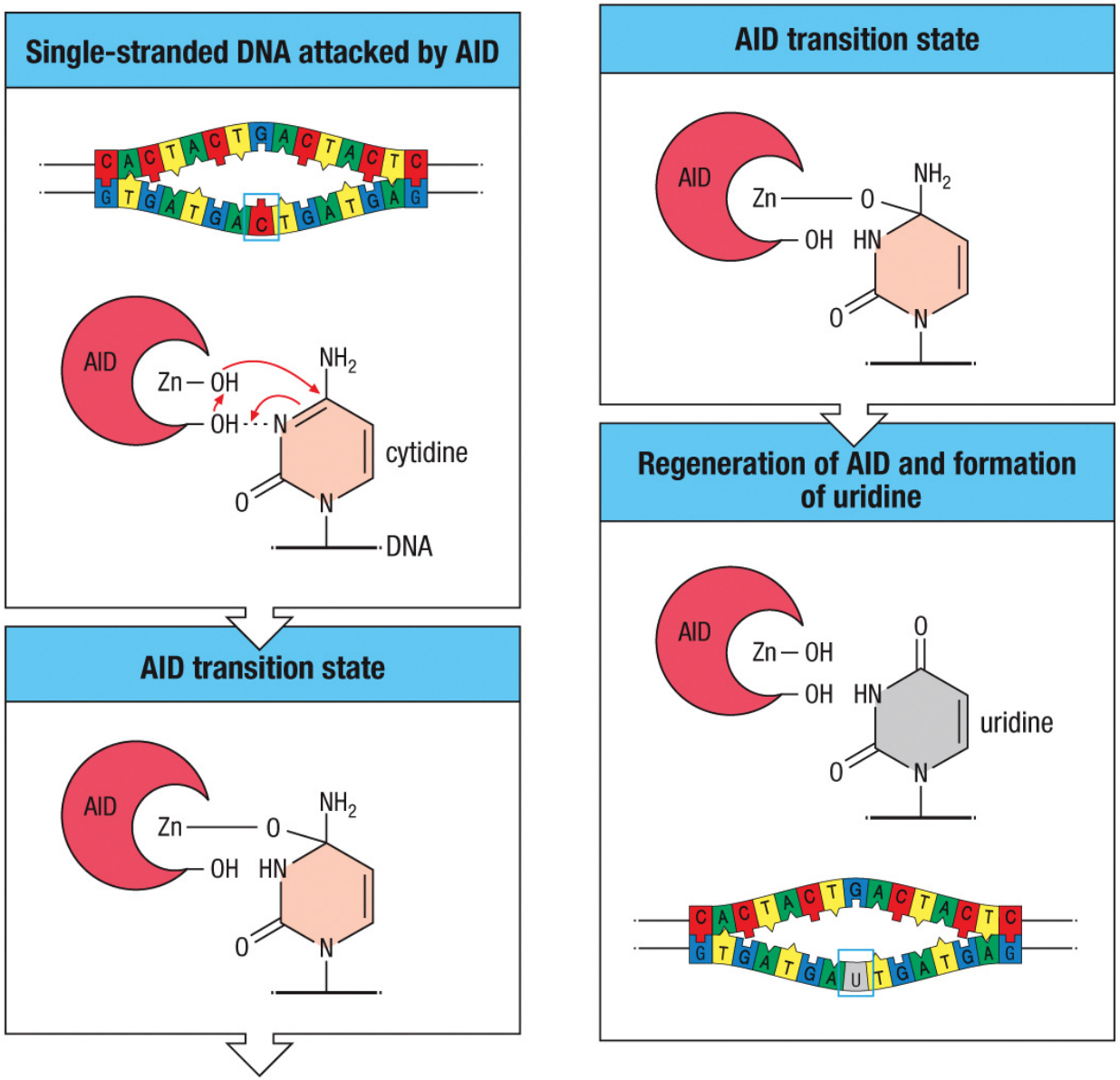
DNA



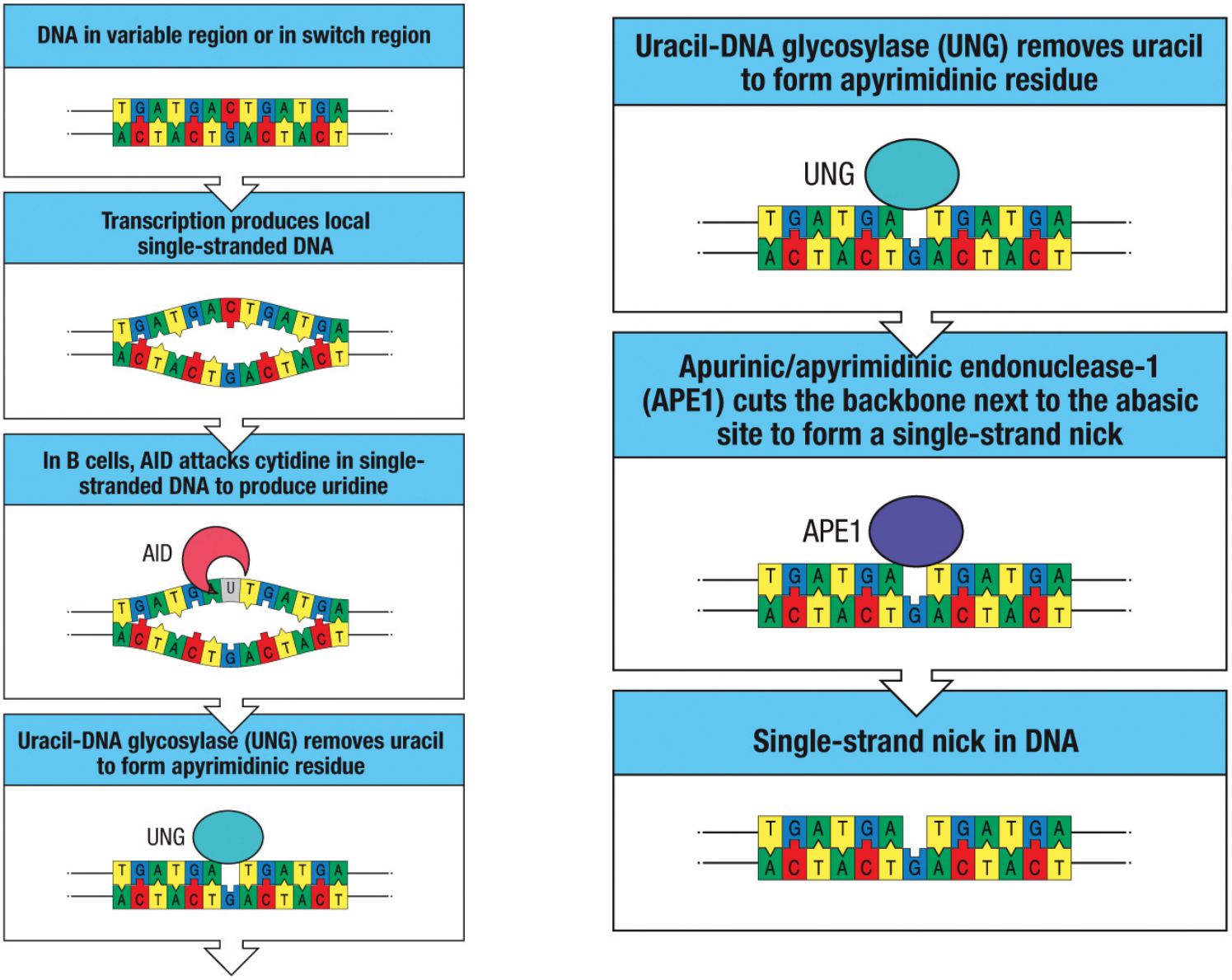


http://en.wikipedia.org/wiki/DNA

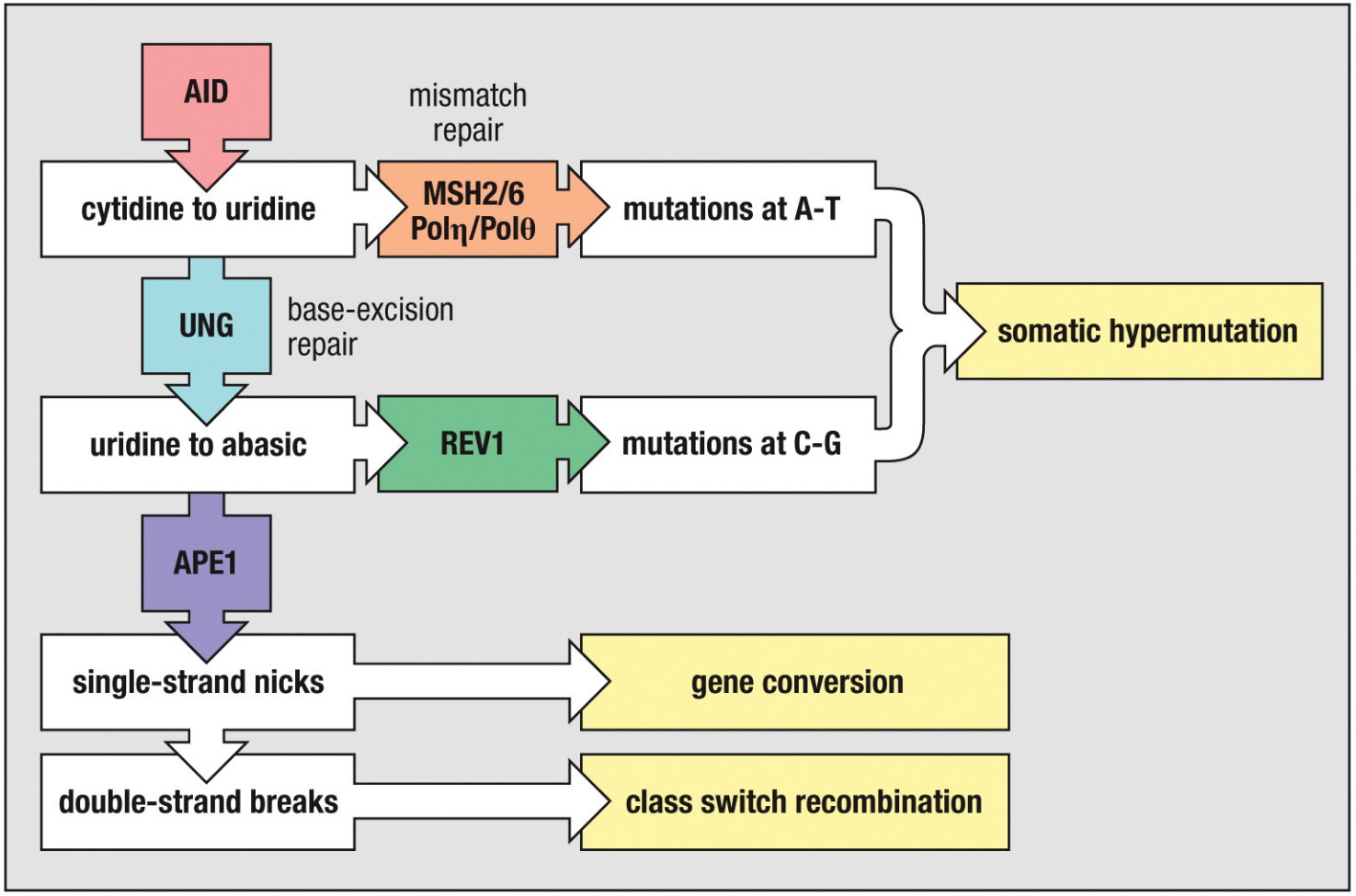
C-U Switch at Single-Stranded DNA



Generation of Single Stranded DNA Breaks



AID Initiates Ig Diversification



Class Switching is Mediated by Cytokines

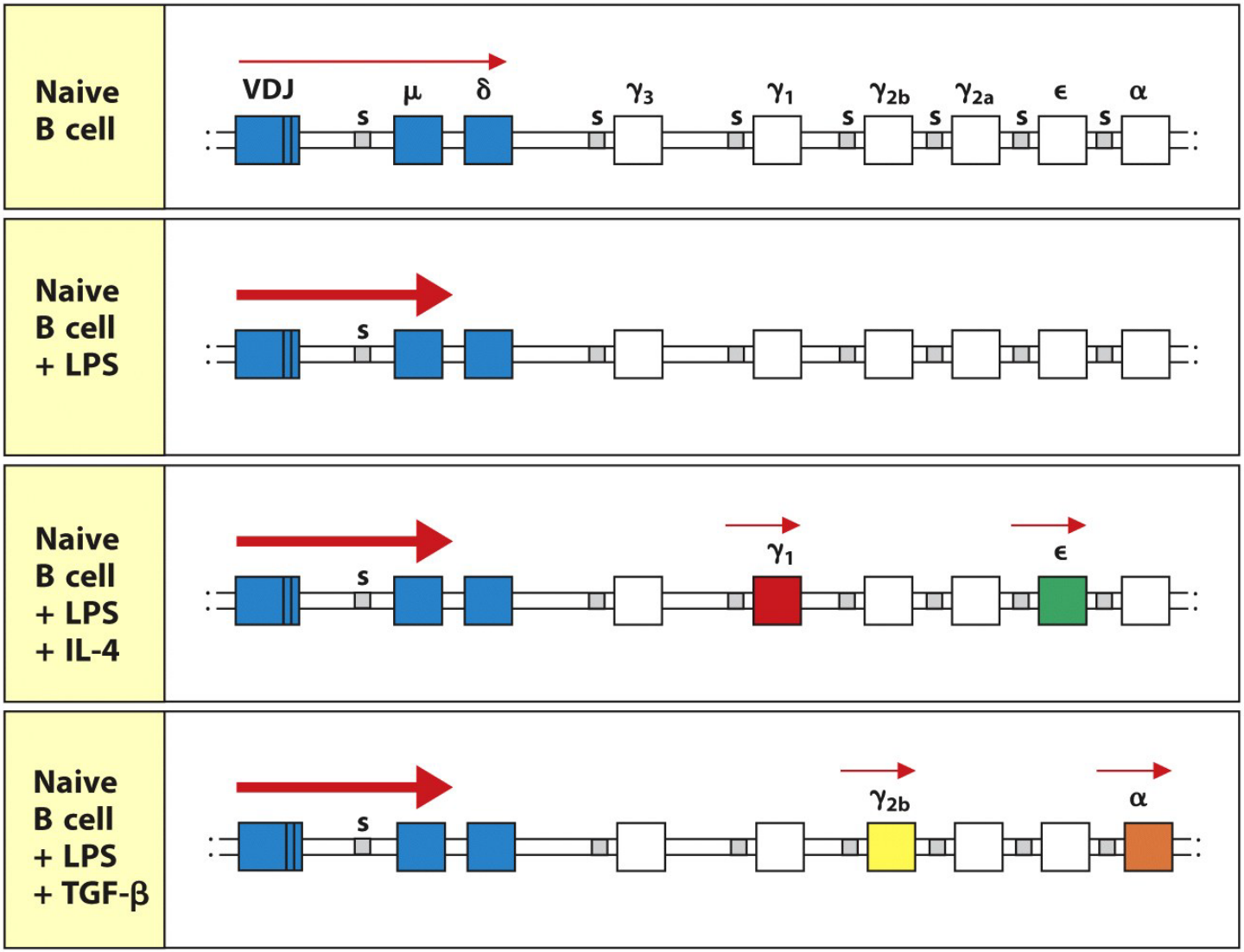


Figure 10.14 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Class Switching is Mediated by Cytokines

Cytokines	Isotype
IL-4	IgG1, IgE
IFN-γ	IgG3, IgG2a
TGF-β	IgG2b, IgA
IL-21	IgG3, IgG1, IgA
IL-5	IgG1, IgA

Class Switch

- Irreversible DNA recombination
  - non-homologous DNA recombination
- Always productive
- Initiated in response to:
  - antigen stimulation
  - cytokine stimulation
- Guided by switch regions
  - within intron between J and C segments
  - Dependent on AID, but not RAG

Changes in Ig and T-cell Genes During B- and T-cell Development

Event	Process	Nature of change	Process occurs in:	
			B cells	T cells
V-region assembly	Somatic recombination of DNA	Irreversible	Yes	Yes
Junctional diversity	Imprecise joining, N-sequence insertion in DNA	Irreversible	Yes	Yes
Transcriptional activation	Activation of promoter by proximity to the enhancer	Irreversible but regulated	Yes	Yes
Switch recombination	Somatic recombination of DNA	Irreversible	Yes	No
Somatic hypermutation	DNA point mutation	Irreversible	Yes	No
IgM, IgD expression on surface	Differential splicing of RNA	Reversible, regulated	Yes	No
Membrane vs secreted form	Differential splicing of RNA	Reversible, regulated	Yes	No

Figure 5.26 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Question

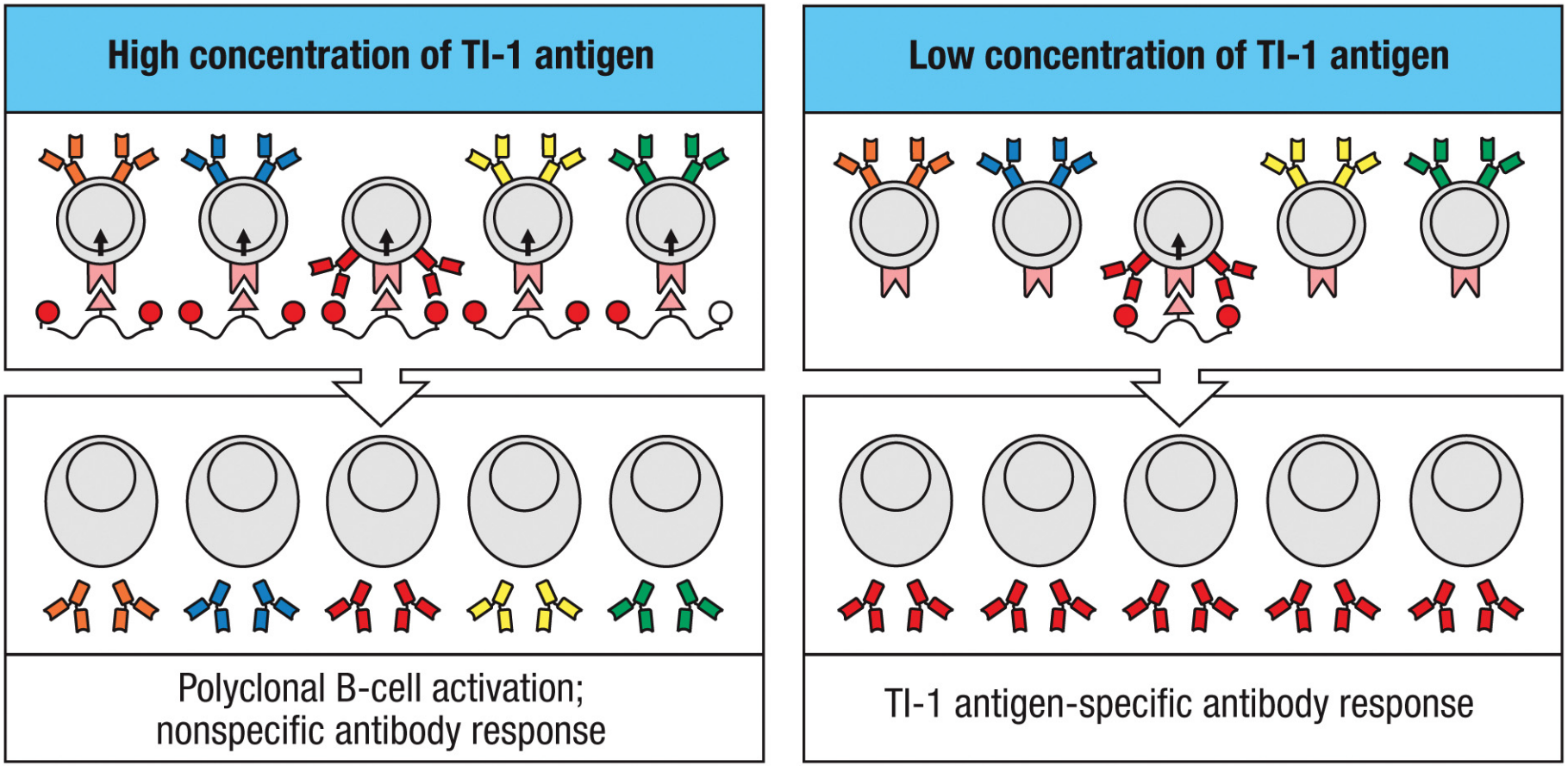
- How different is RAG and AID?

- Substrate
- Function

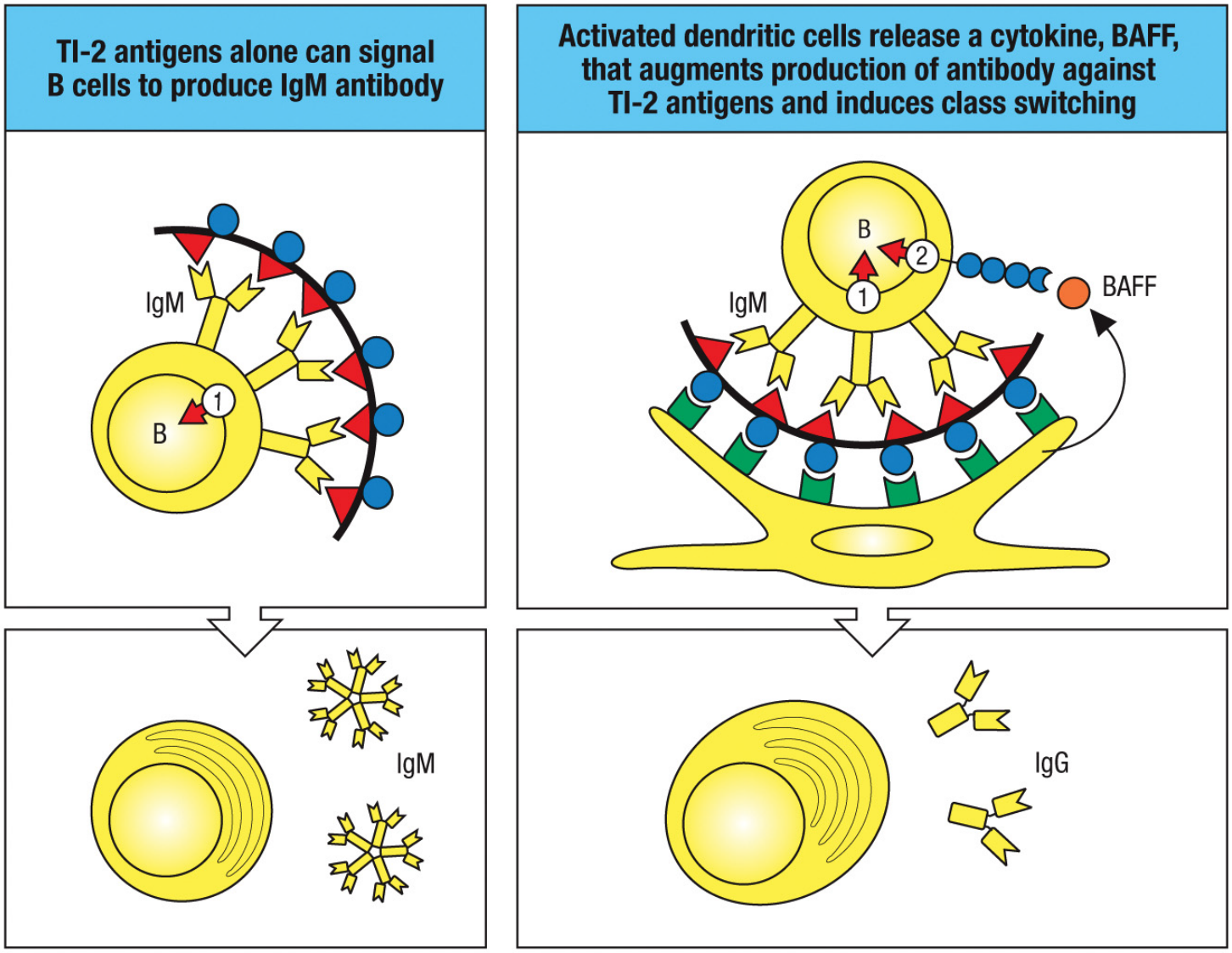
Outline

- Humoral Immune response
  - B cell activation (thymus dependent)
  - Affinity Maturation
  - Class Switching
  - Thymus-independent B-Cell activation

TI-1 Antigen: B Cell Mitogen-TLR



TI-2 Antigen: Extensive Crosslinking



Properties of Different Antigen Classes

	TD antigen	TI-1 antigen	TI-2 antigen
Antibody response in infants	Yes	Yes	No
Antibody production in congenitally athymic individual	No	Yes	Yes
Antibody response in absence of all T cells	No	Yes	Yes
Primes T cells	Yes	No	No
Polyclonal B-cell activation	No	Yes	No
Requires repeating epitopes	No	No	Yes
Examples of antigen	Diphtheria toxin Viral hemagglutinin Purified protein derivative (PPD) of *Mycobacterium tuberculosis*	Bacterial lipopoly-saccharide *Brucella abortus*	Pneumococcal polysaccharide *Salmonella* polymerized flagellin Dextran Hapten-conjugated Ficoll (polysucrose)

Figure 10.26 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Properties of Different Antigen Classes

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Question

- T cell help is not required for TD-B cells to:

- A) Proliferate
- B) Produce IgM
- C) Affinity maturation
- D) Class switching

Case Study: AID Defiency

- Patient:
  - 3 year old female
  - Recurrent infections
    - Pneumonia
    - Middle-ear infection
    - Enlarged lymph nodes
    - Blood culture of *Streptococcus Pneumoniae*
- Diagnosis:
  - High IgM, no IgG
- Treatment:
  - Antibiotics
  - Intravenous immunoglobulin therapy

Failure in Class Switch

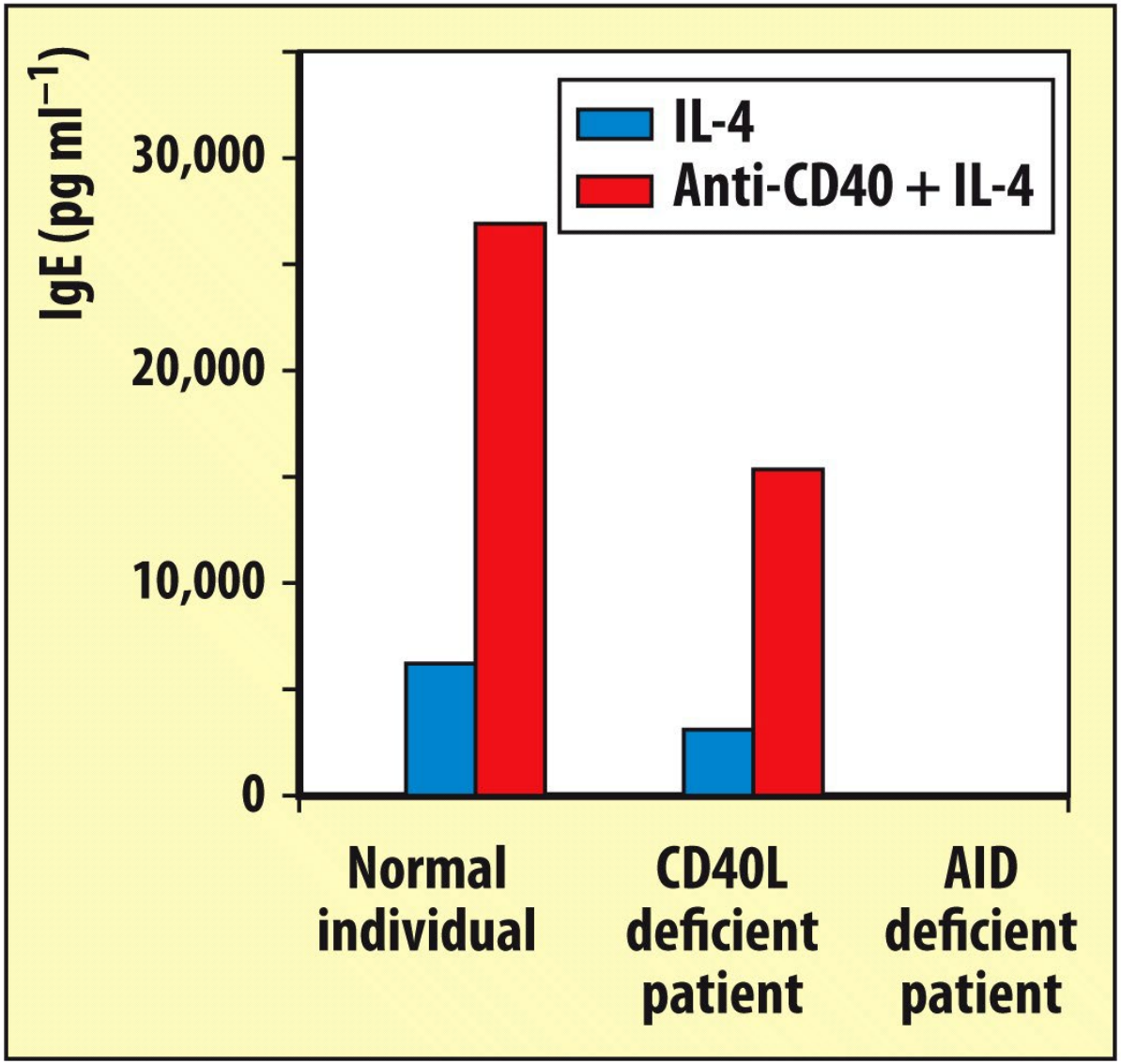

Figure 3.3 Case Studies in Immunology, 6ed. (© Garland Science 2012)

What's Wrong with the Patient?

Defect in AID

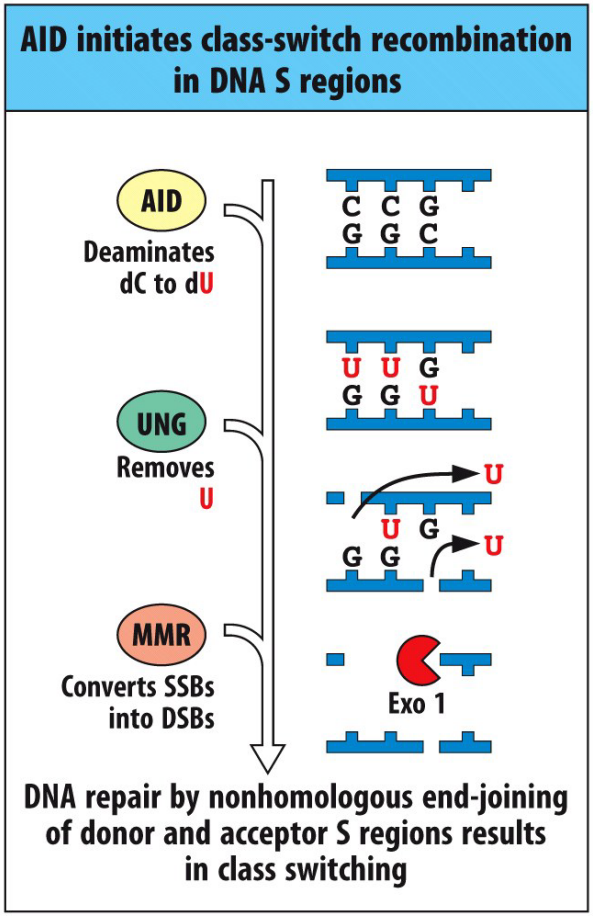

Figure 3.4 Case Studies in Immunology, 6ed. (© Garland Science 2012)

Enlarged Germinal Center

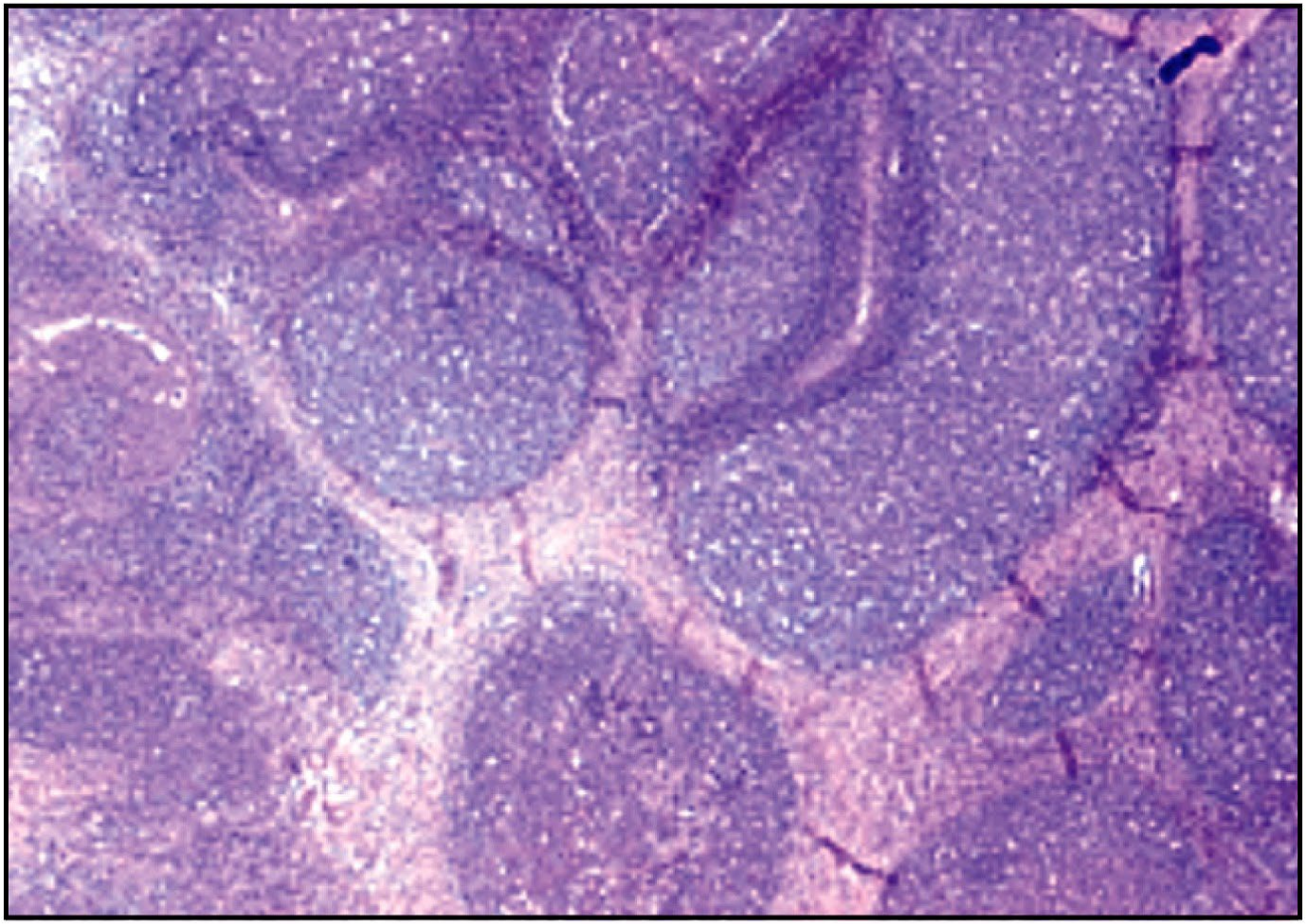

Figure 3.5 Case Studies in Immunology, 6ed. (© Garland Science 2012)

Proliferating B cells failed to class switch

What's Wrong with the Patient?

Failure in opsonization
Increased susceptibility of pygonenic extracellular bacteria

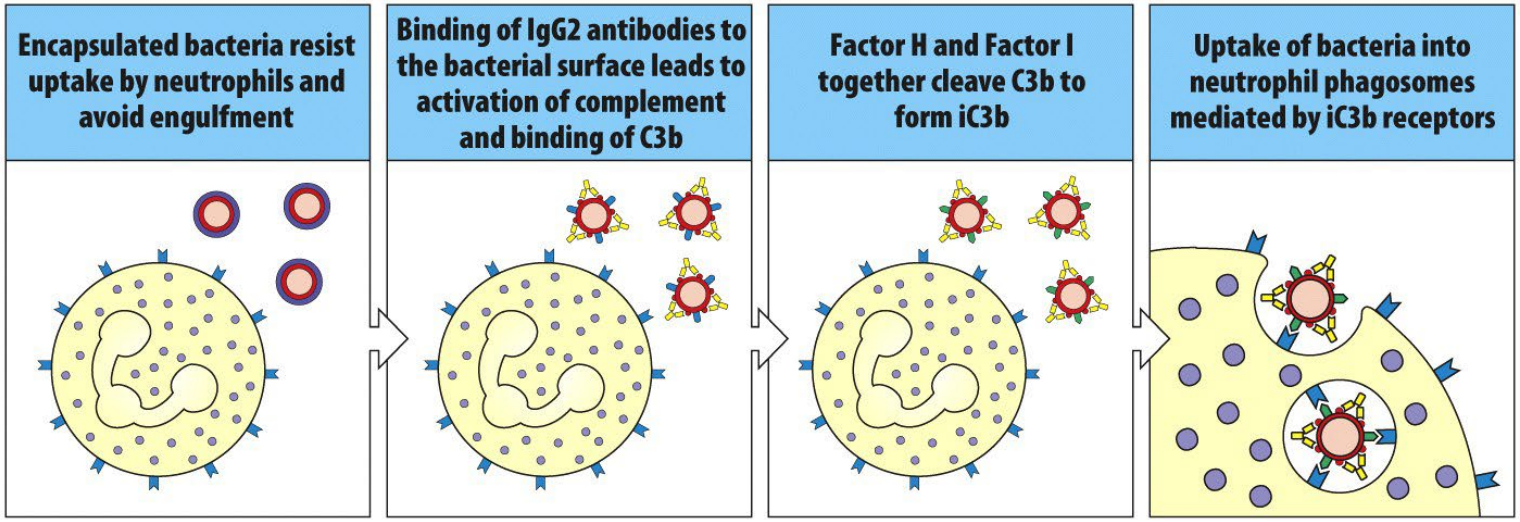

Figure 1.6 Case Studies in Immunology, 6ed. (© Garland Science 2012)

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	–	–	+++	*	++	+	+	–

Case Study: CD40 Ligand Deficiency

- Patient:
  - Male
  - Repeated infection since infancy
  - Antibody of IgM class only
- Diagnosis:
  - Absence of CD40 on activated T cells
- Treatment:
  - Weekly infusion with gamma globulin
  - Died of Liver failure at 21 years

No Surface CD40

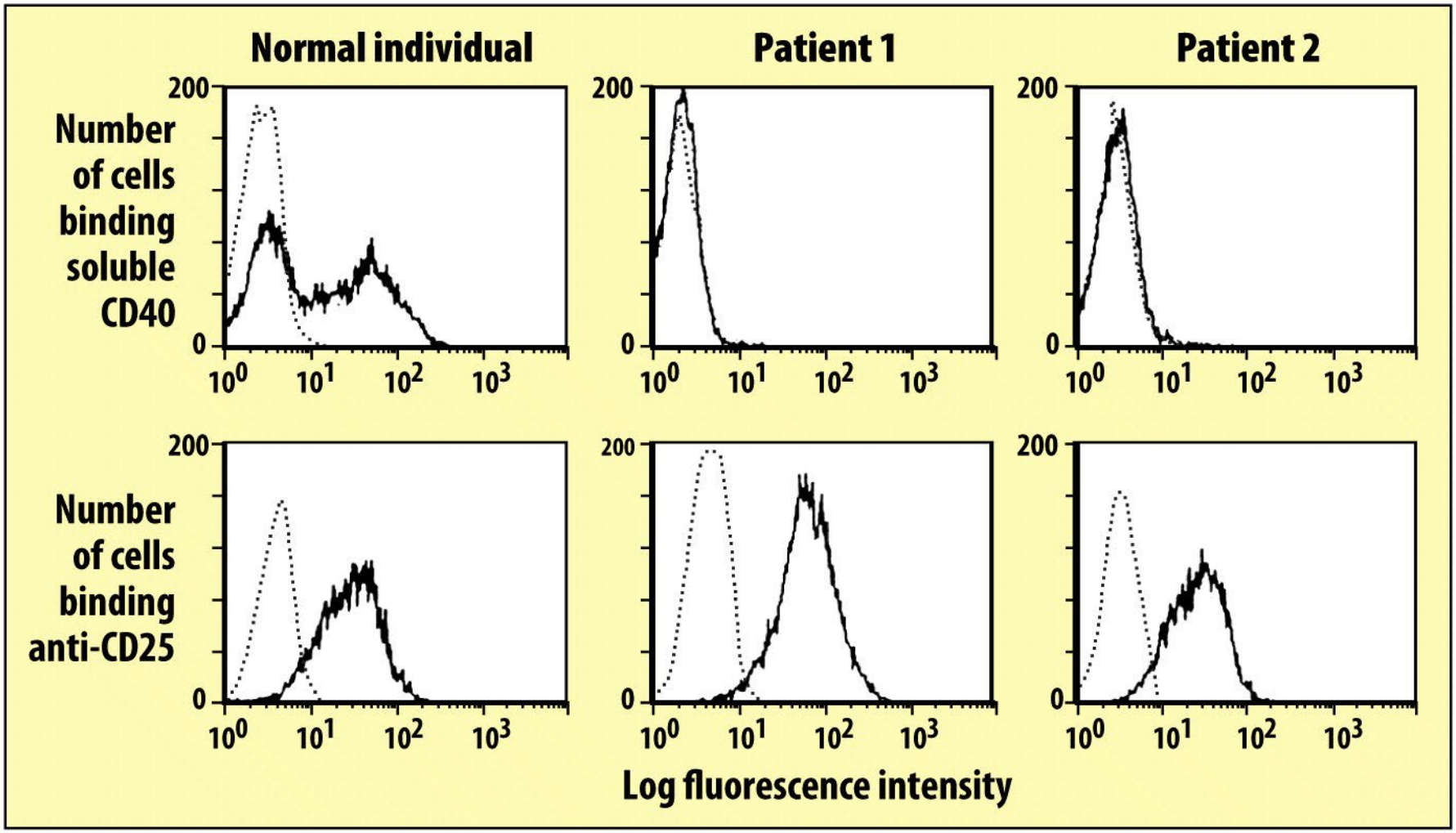

Figure 2.5 Case Studies in Immunology, 6ed. (© Garland Science 2012)

Absent of T Cell Help

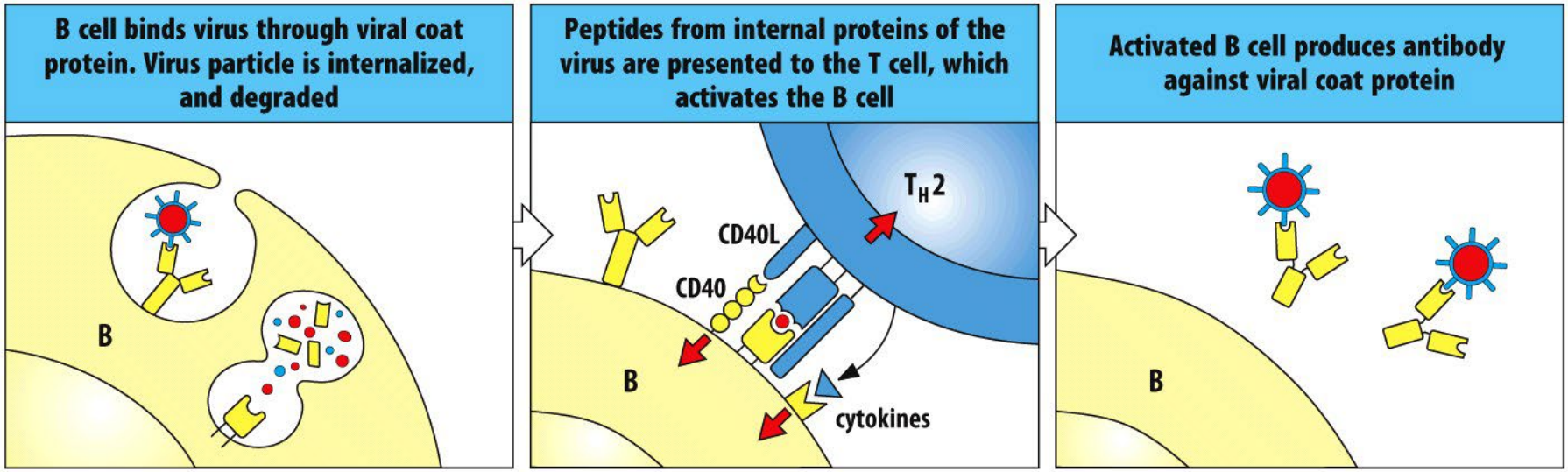

Figure 2.3 Case Studies in Immunology, 6ed. (© Garland Science 2012)

No Class Switching

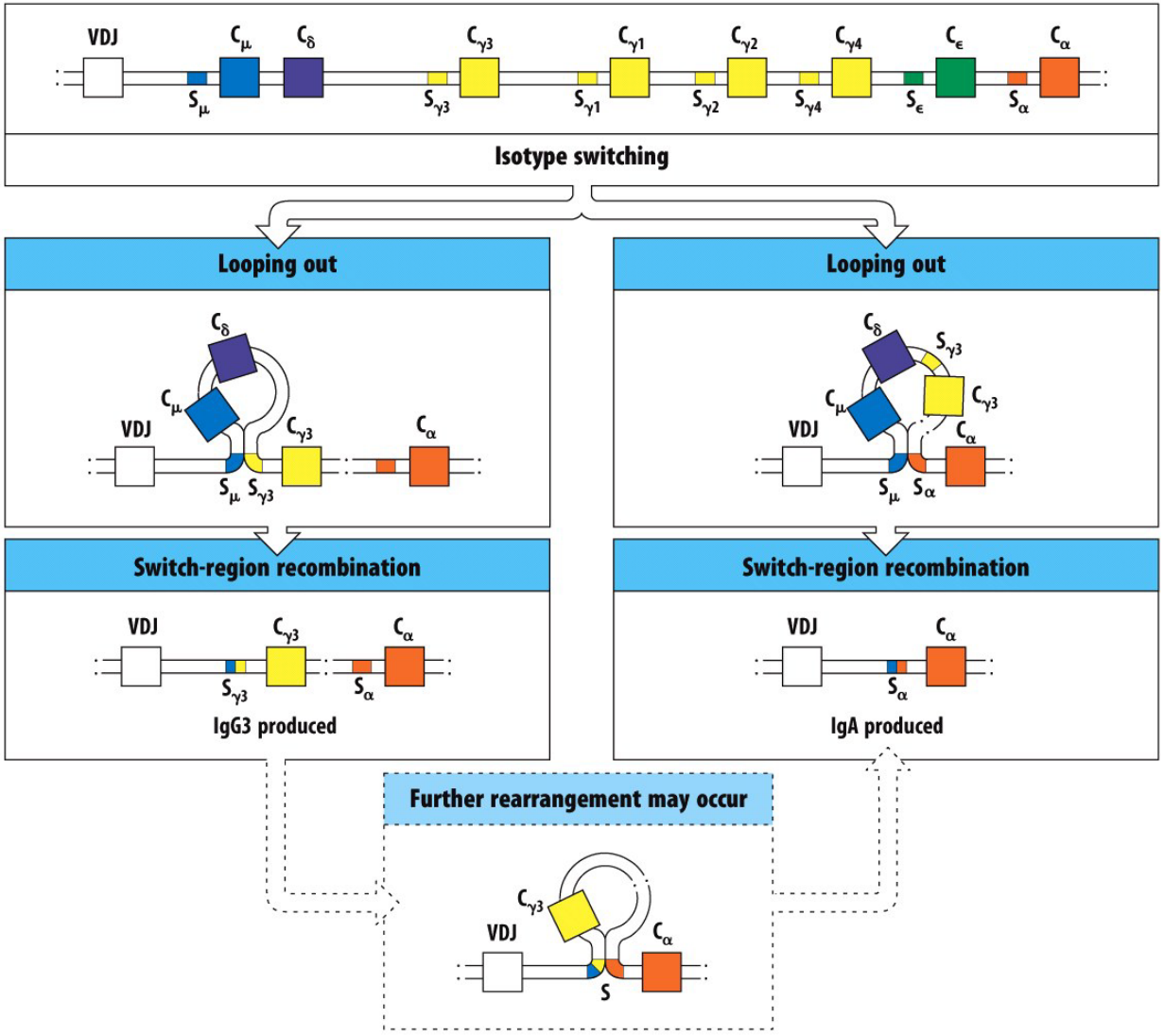

Figure 2.2 Case Studies in Immunology, 6ed. (© Garland Science 2012)

No Germinal Center

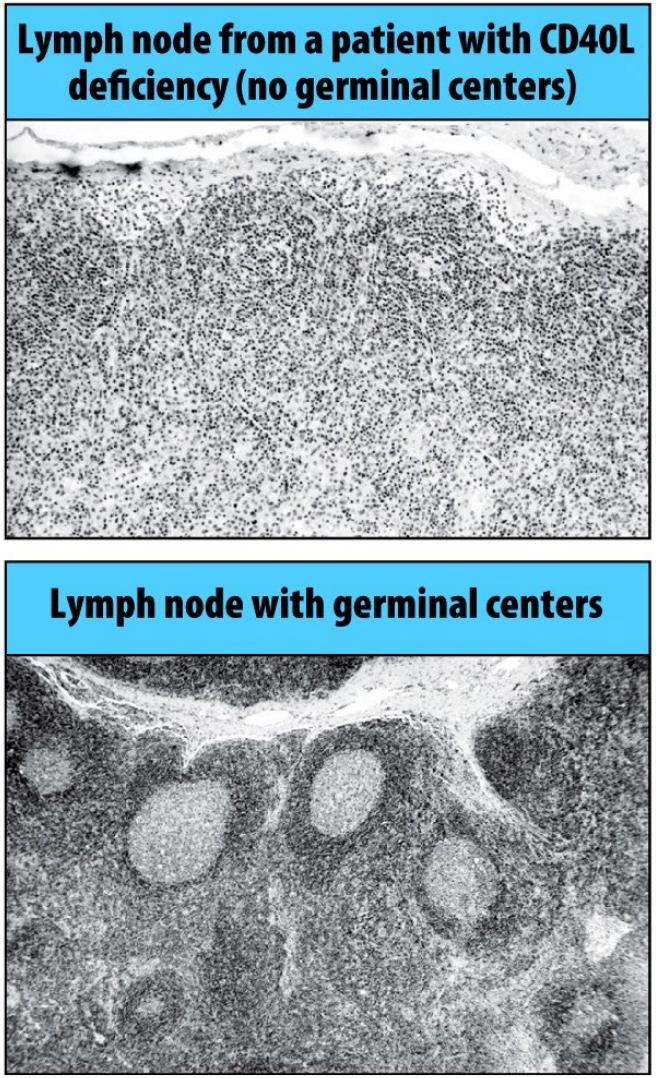

Figure 2.6 Case Studies in Immunology, 6ed. (© Garland Science 2012)

Failed Macrophage Activation

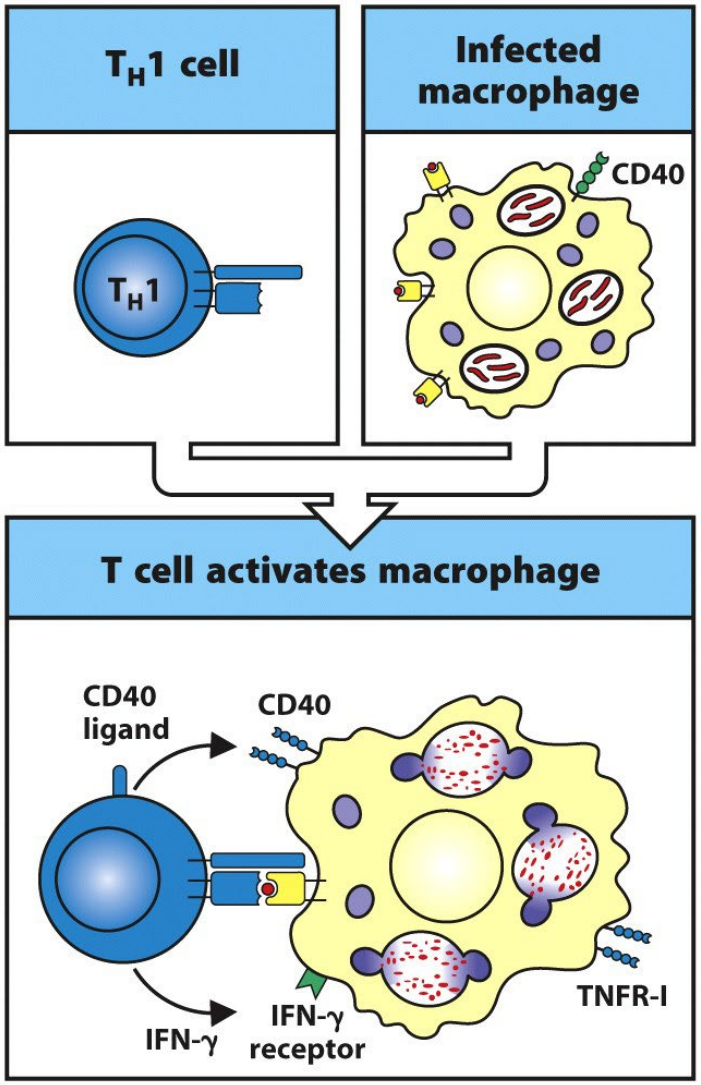

Figure 9.40 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

What's Wrong with the Patient?

Absent of CD40

Failed B cell affinity maturation and class switching

Failed to activate Macrophages

Severe Immune deficiency

Question

- What is different in AID and CD40 deficiency? Why?