Week 5

Week 5

Questions from last week

- What's difference between immunity that wanes and life-long immunity?
  - Chris Brooke, Virology Professor at U of I: "That's the Holy Grail; no one knows"
  - Katherine Wu, Atlantic: https://www.theatlantic.com/science/archive/2021/09/waning-immunity-not-crisis-right-now/619965/
    - antibody levels vs serious disease (memory B cells)

Learning Objectives for Week 5

- **Review of clonal selection in adaptive immunity**
- Structure of antibodies and T cell receptors
- Variable and constant regions; constant regions and effector function
- Activation of T cells and B cells with Two-Factor Authentication
- Types of T cells, Helper and Cytotoxic
  - How Helper T cells help macrophages, CTLs and B cells
  - How pathogen-appropriate responses are generated

Adaptive Immunity: B cells and T cells

- B Cell (antigen) Receptor (BCR)
  - When secreted called an antibody
- T Cell (antigen) Receptor (TCR)
  - $\alpha\beta$ subunits for most TCR on "$\alpha\beta$ T cells"

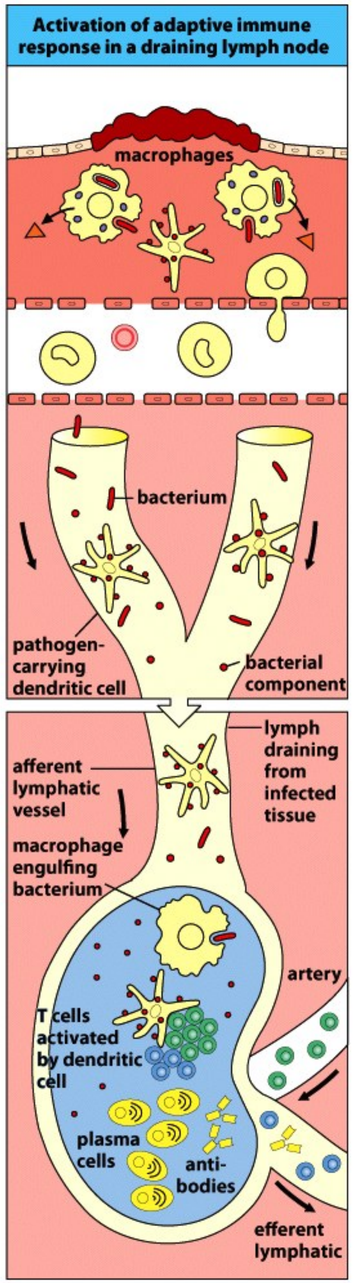


Figure 1.22 The Immune System, 3ed. (© Garland Science 2009)

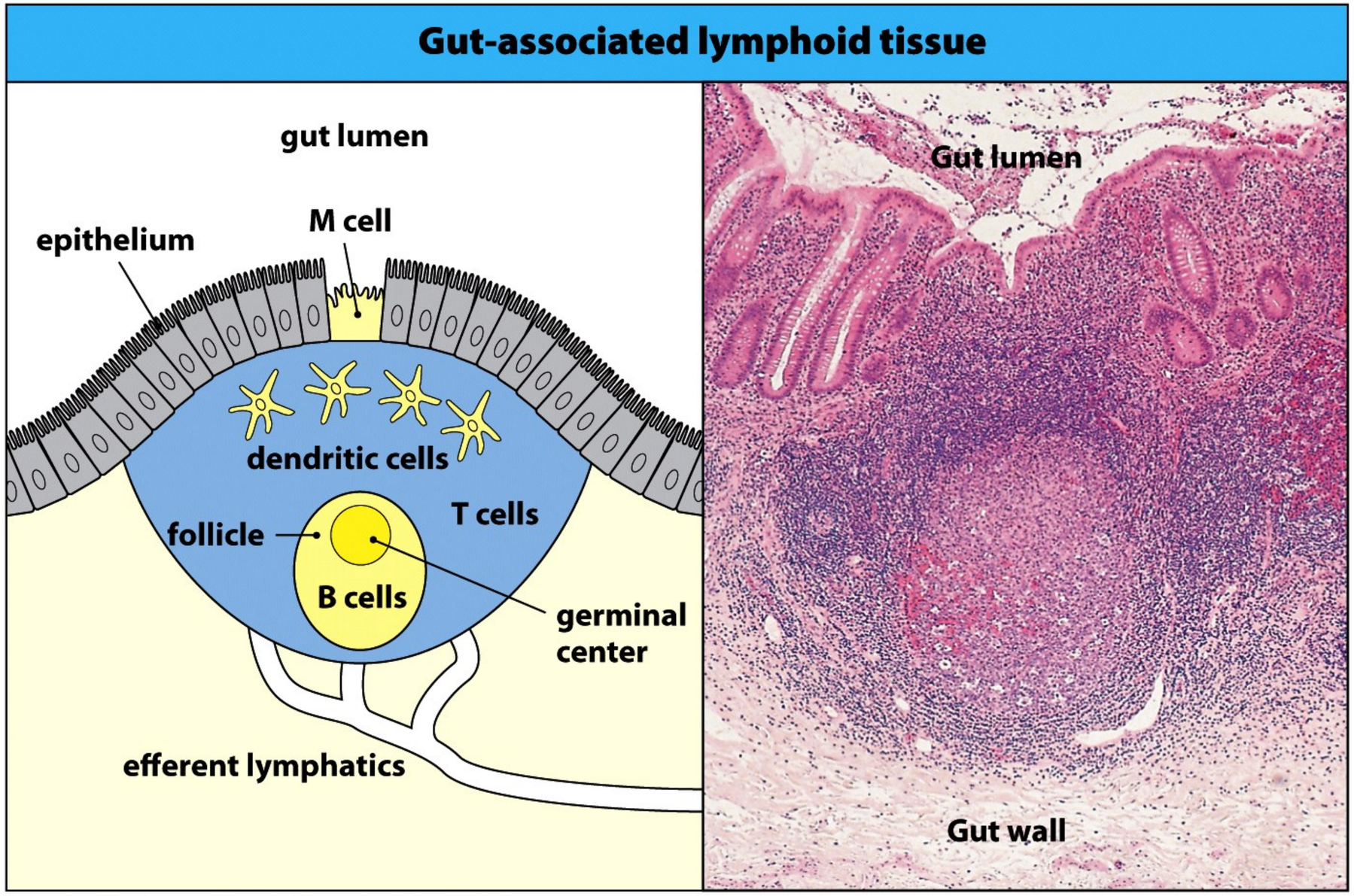


Figure 1.25 The Immune System, 3ed. (© Garland Science 2009)

clonal deletion

tolerance

clonal selection

memory

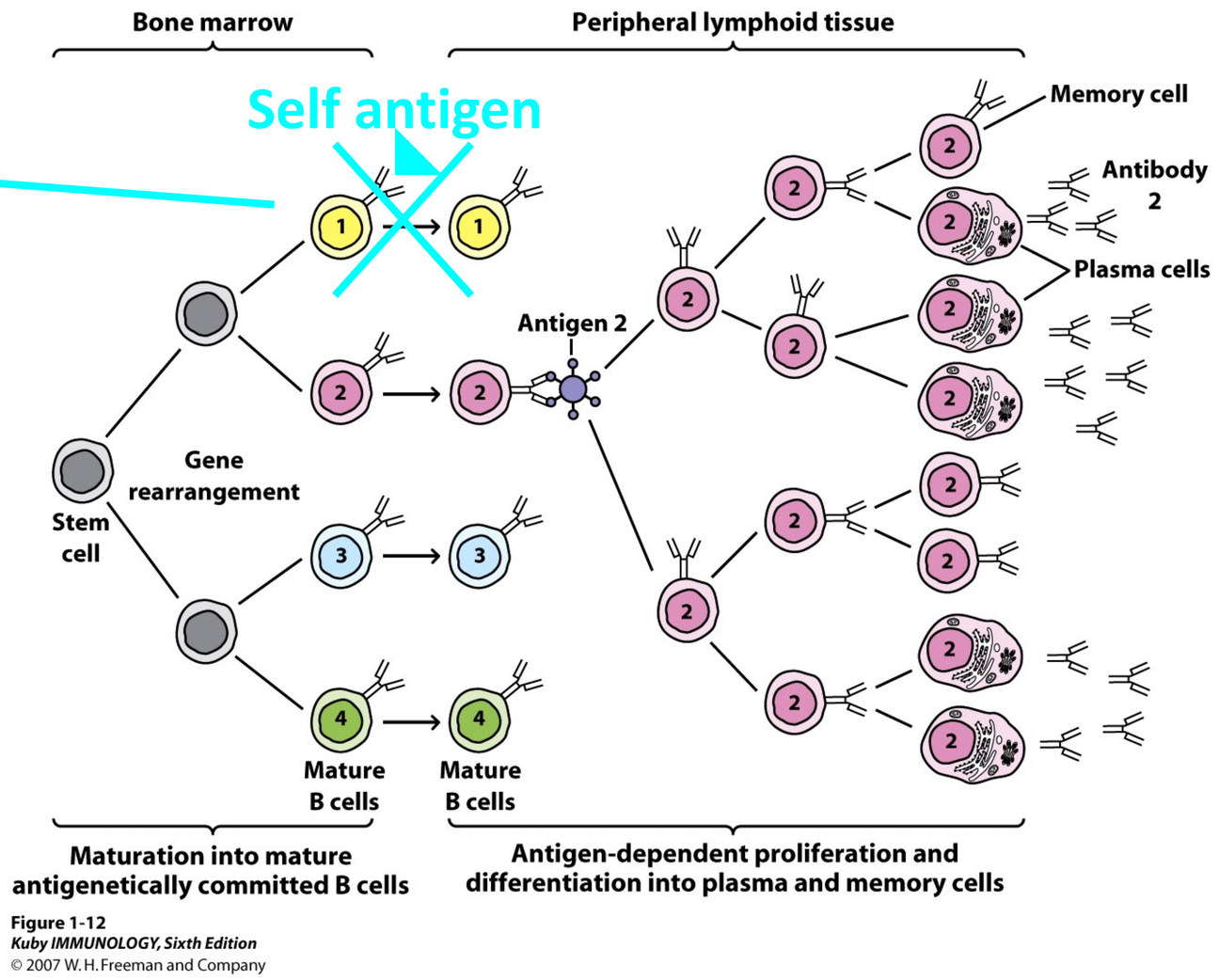


Figure 1-12
Kuby IMMUNOLOGY, Sixth Edition

Example showing B cells

Principle of Clonal Selection

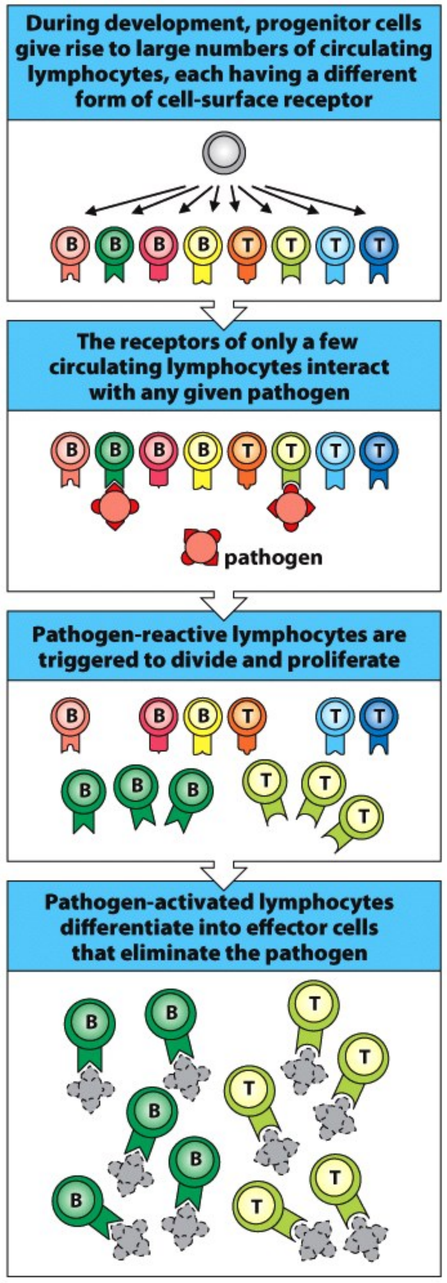


Figure 3.5 The Immune System, 3ed. (© Garland Science 2009)

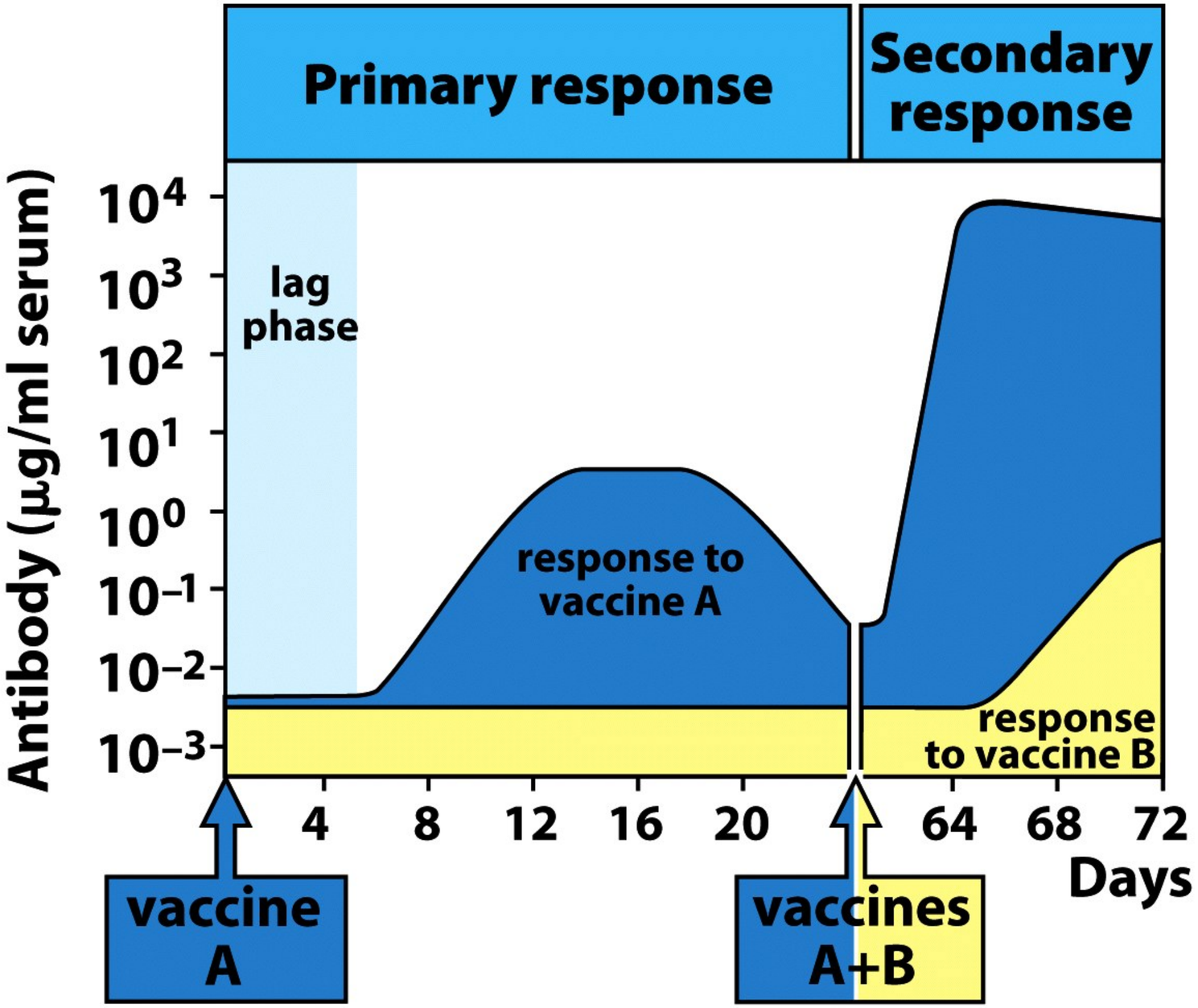


Figure 1.26 The Immune System, 3ed. (© Garland Science 2009)

- After first exposure and successful response, “immunity” is conferred
- Immunity is highly specific

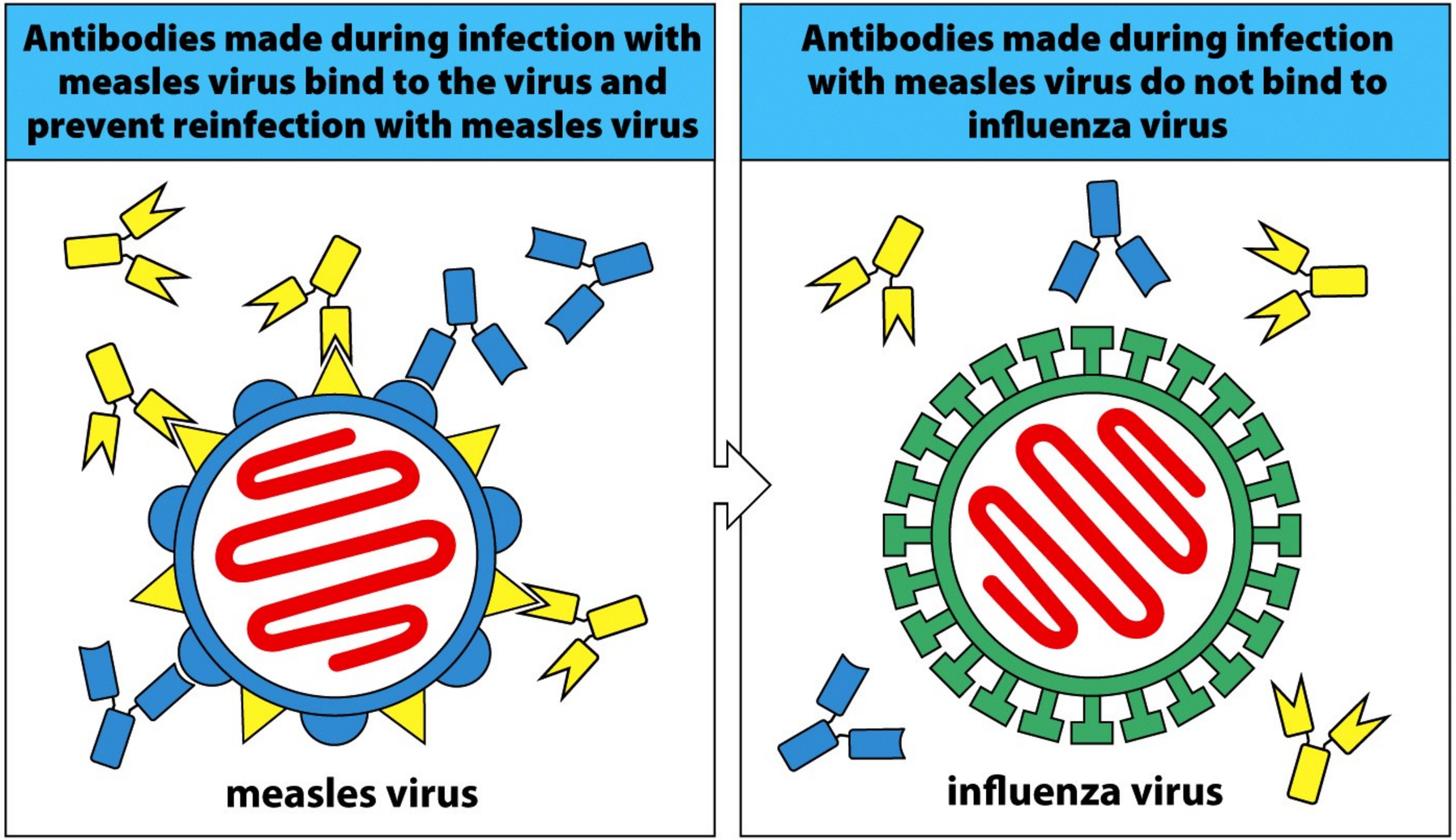
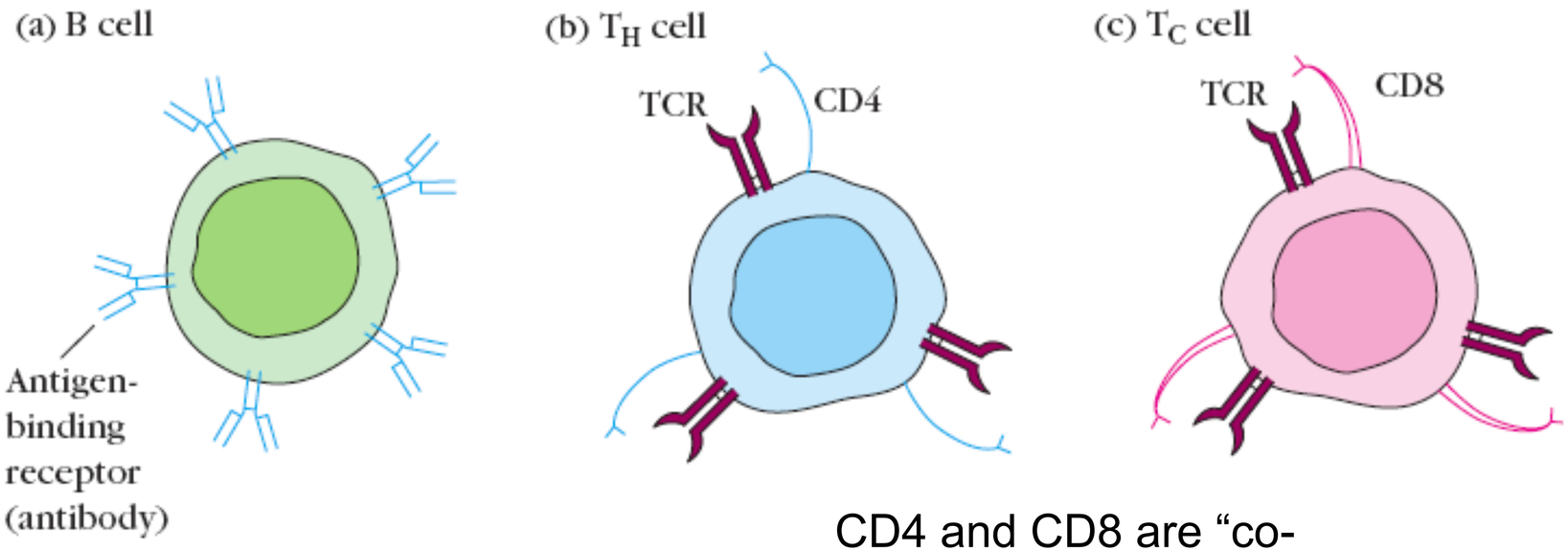


Figure 3.2 The Immune System, 3ed. (© Garland Science 2009)

B and T lymphocytes express distinctive receptors and coreceptors on their surface to recognize antigens



BCR

CD4 and CD8 are “co-receptors” that contribute to binding to MHC

Y-shaped protein

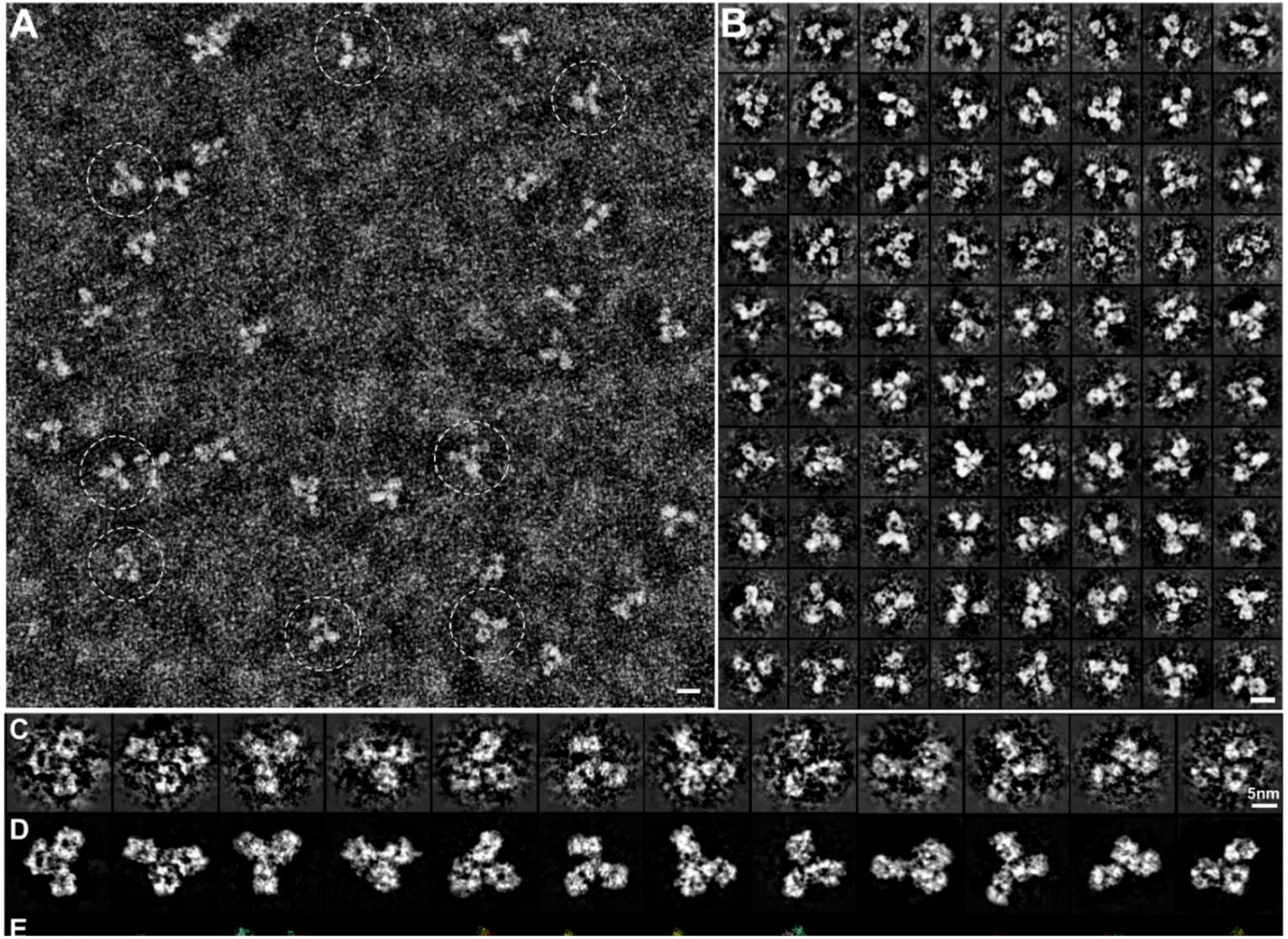


Figure 2. The Optimized Negative Staining (OpNS) Electron Microscopy (EM) images of IgG2 antibody particles. (**A**) Survey view of the IgG antibody particles imaged by OpNS EM. (**B**) Representative images of individual particles of antibody. (**C**) Zoomed-in views of selected individual particle images and (**D**) their corresponding denoised images are compared to (**E**) the crystal structure at a specific orientation. Copyright© 2015 the Authors, managed by Nature Publishing Group.

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IgG Antibody 3D Structures and Dynamics

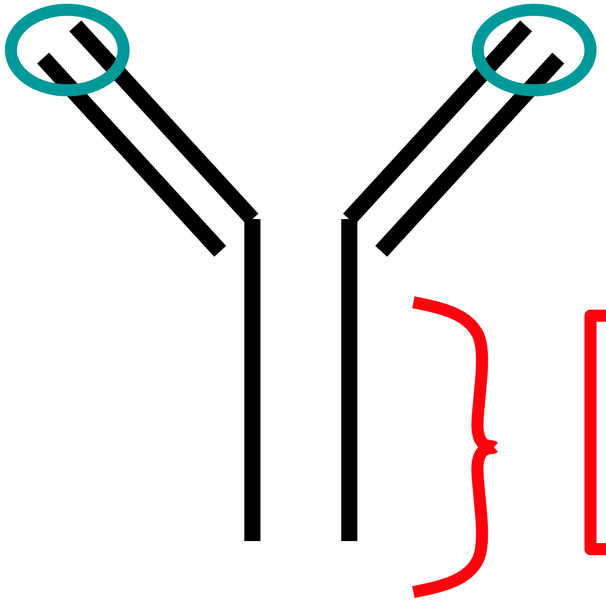
by Jacob White Jay [1,†], Brinkley Bray [1,†], Yaozhi Qi [1,2], Eseo
Hao Wu [3], Jinping Li [1,*] and Gang Ren [4,*]

Antibody Structure

Fab Fragment:

Antigen

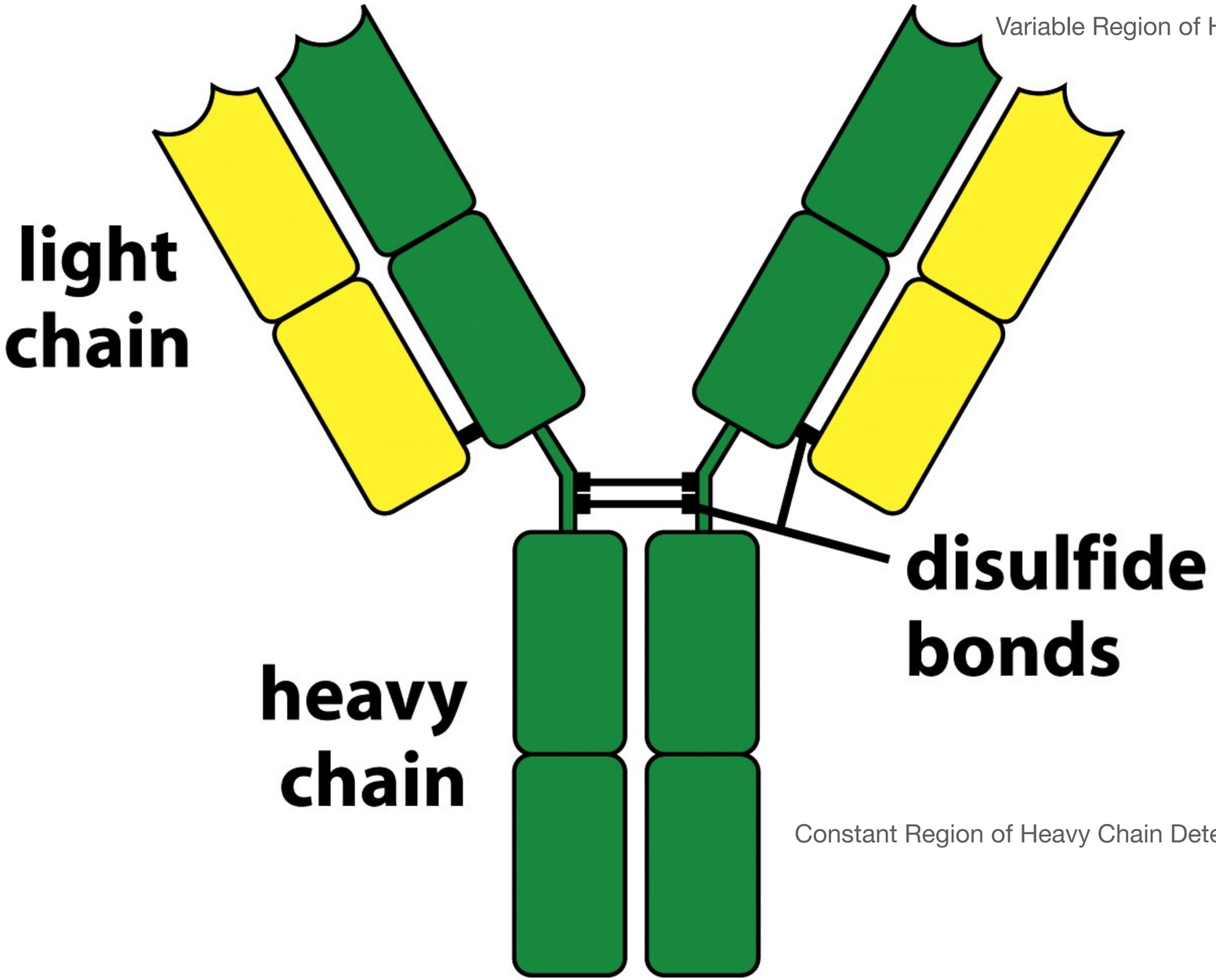
Binding



Fc Region

Biological Activity (Effector Function)

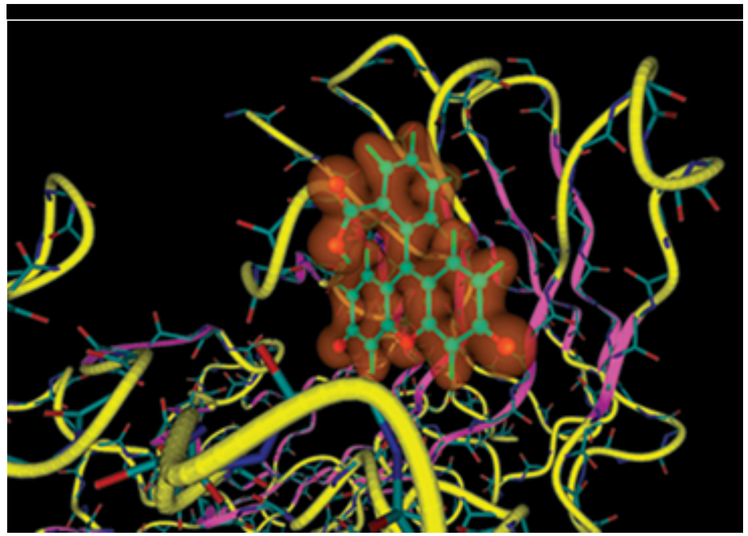
Variable Region of Heavy and Light Chains Determine Antigen Specificity

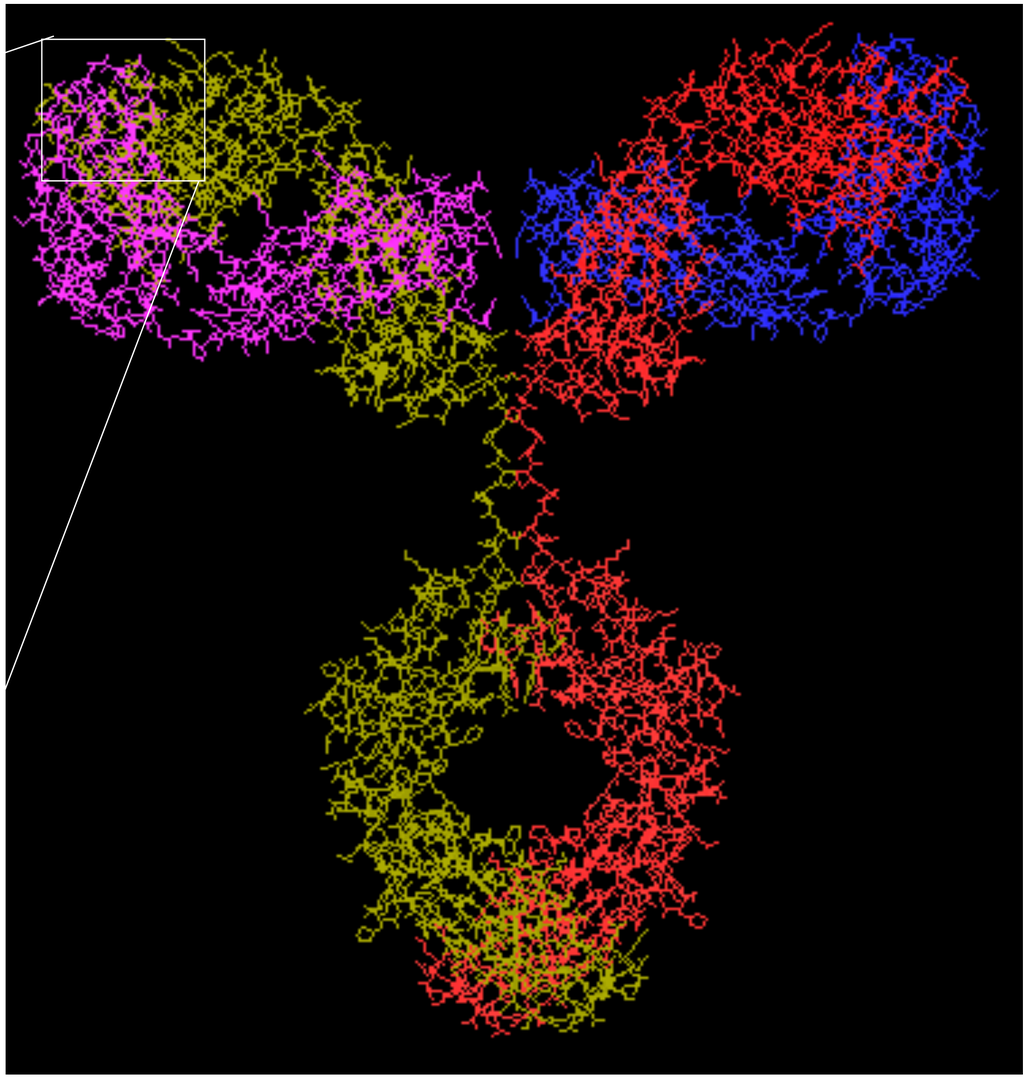


Constant Region of Heavy Chain Determines Effector Function and Is Species Specific

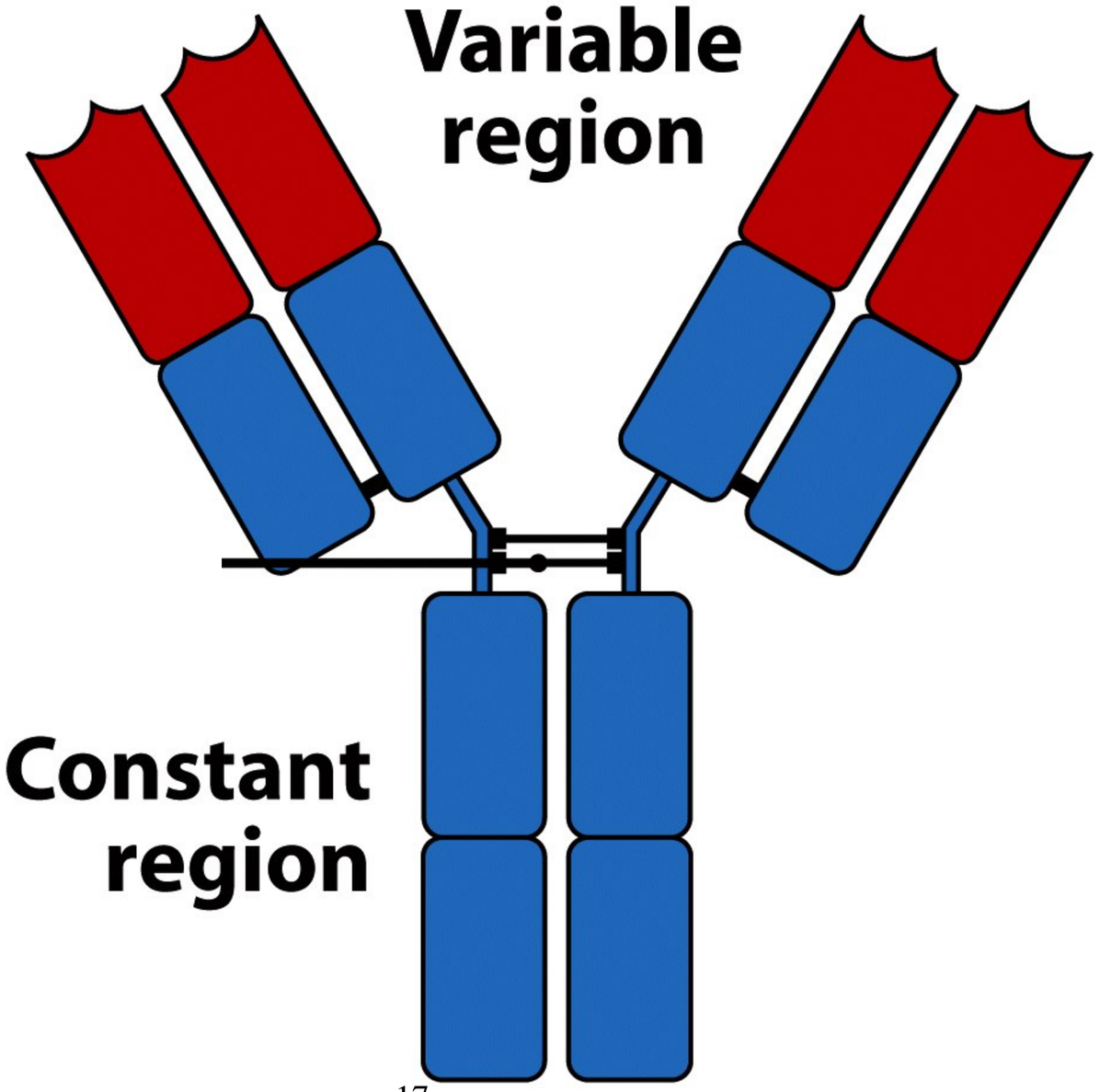
Figure 3-2 Immunobiology, 7ed. (© Garland Science 2008)

Antibody Heavy and Light Chains together form site of Antigen Recognition





Antibody Structure (Immunoglobulin)



Constant Region

- Constant from antibody to antibody, but there are several different “classes” or “isotypes”
- IgM, IgG, IgA, IgE
- As B cell is activated, the secreted antibody may change class
- The class determines the Effector Mechanism, and is specific to a given species
- If you inject a mouse IgG antibody into a donkey, the donkey will make antibodies that bind to all mouse IgG antibodies; useful for research. Humans will also make antibodies against mouse IgG, neutralizing the therapeutic antibody, problematic for therapies
- Therapeutic antibodies are now “humanized” before being injected into people

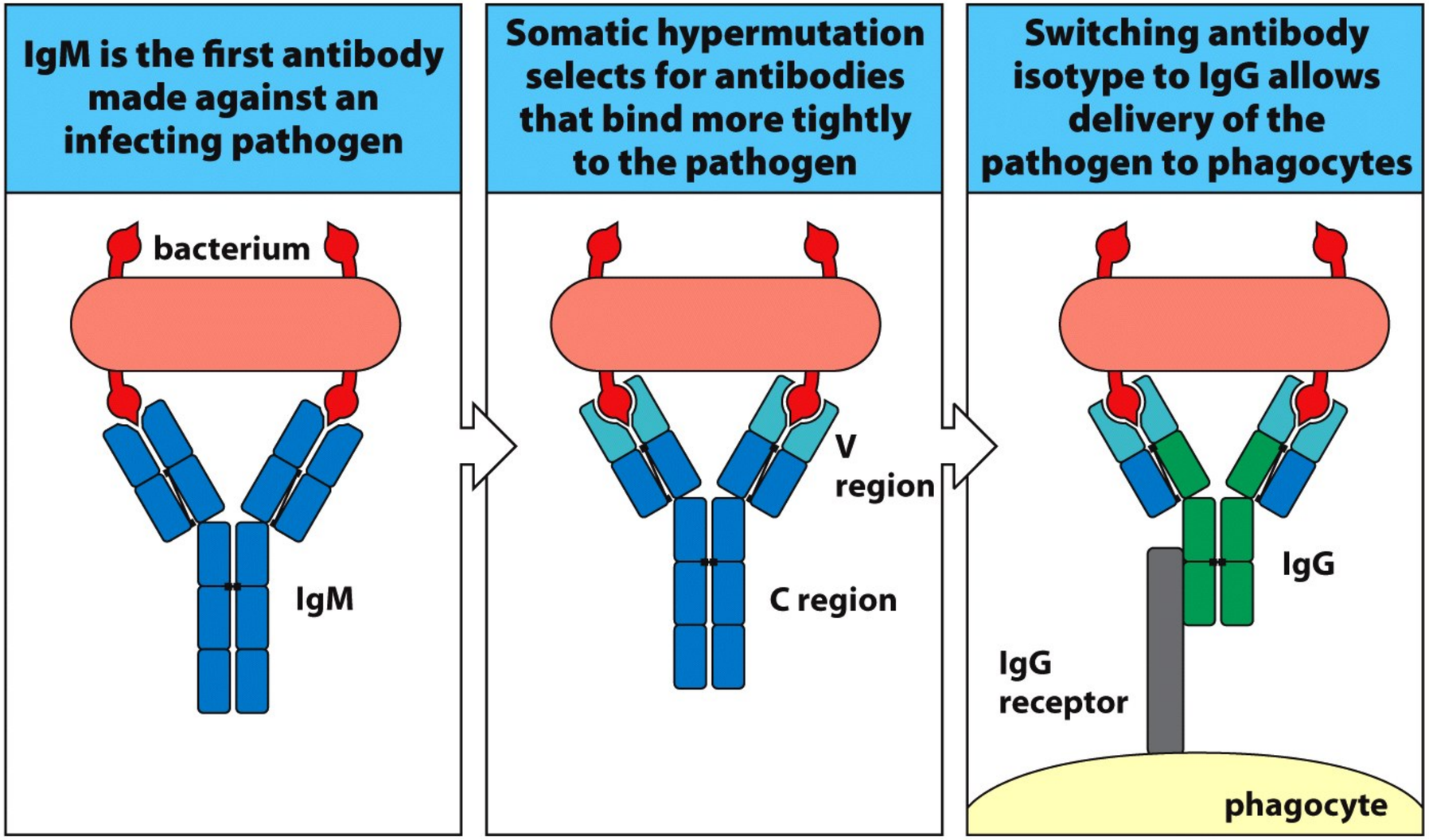


Figure 3.15 The Immune System, 3ed. (© Garland Science 2009)

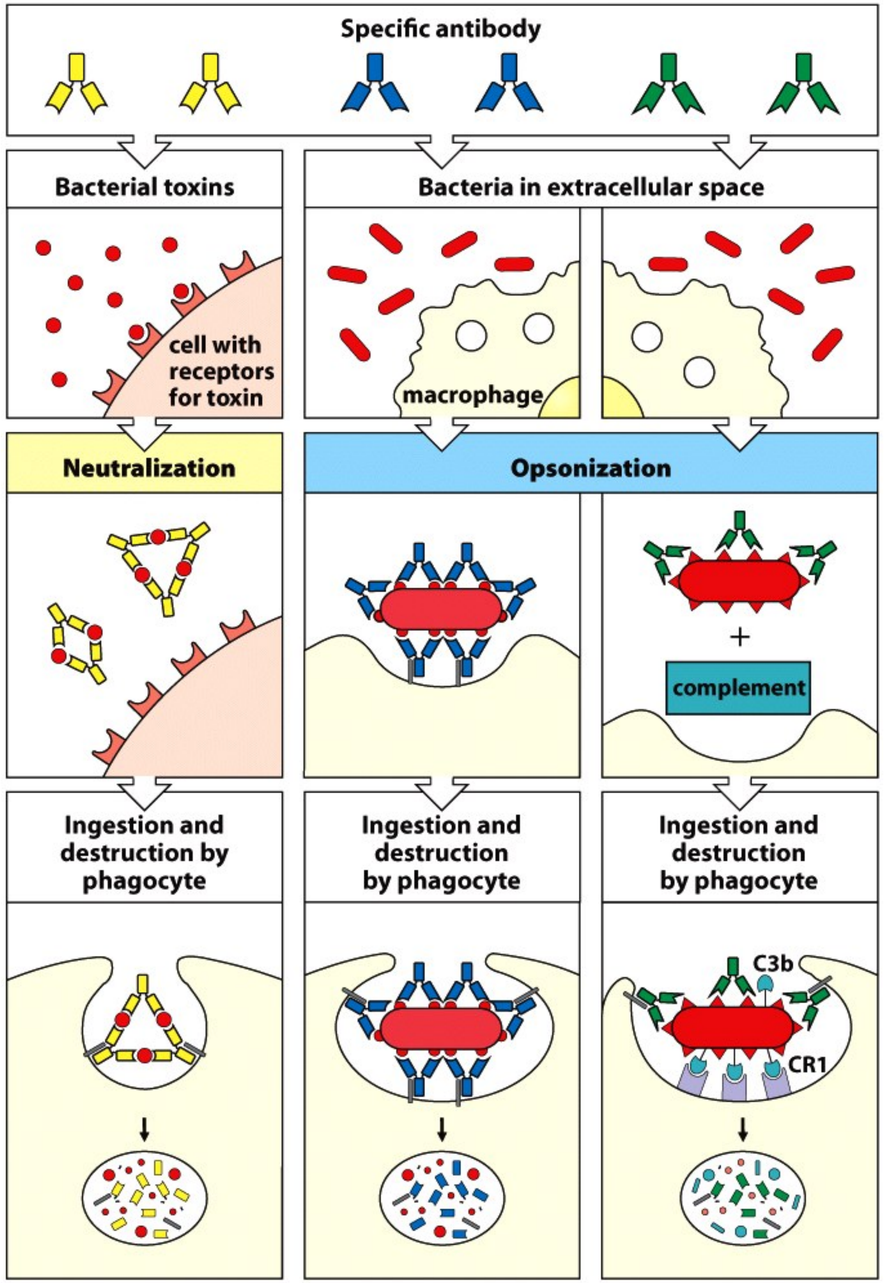


Figure 3.14 The Immune System, 3ed. (© Garland Science 2009)

Antibody and TCR Diversity

- Is generated by gene rearrangement (somatic recombination)
- No other somatic cells of the body recombine genes
  - Lots of other cells change transcription by epigenetic means, but B cells and T cells actually change their gene sequence

Learning Objectives for Week 5

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- Structure of antibodies and T cell receptors
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  - How Helper T cells help macrophages, CTLs and B cells
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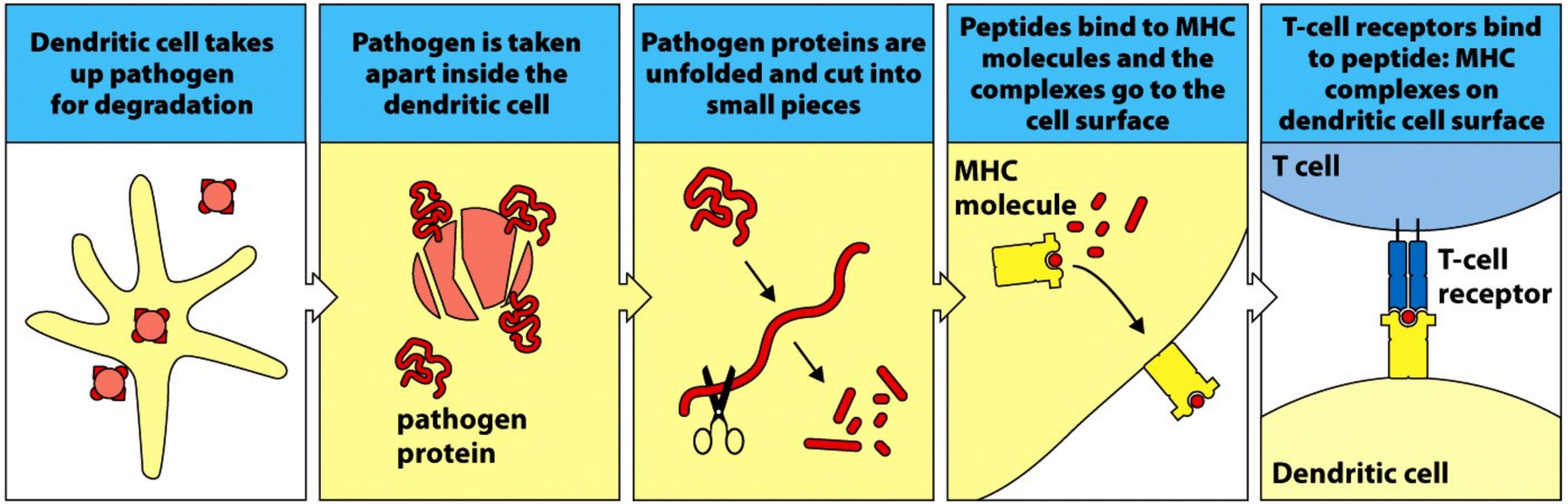


Figure 3.7 The Immune System, 3ed. (© Garland Science 2009)

Types of T cells

- Helper T cells
  - CD4+
  - Help macrophages, help B cells, help activation of Cytotoxic T cells
- Cytotoxic T cells
  - CD8+
  - Focused killing with perforin and Granzyme B cell suicide inducer

Helper T cell activates macrophage to continue phagocytosis

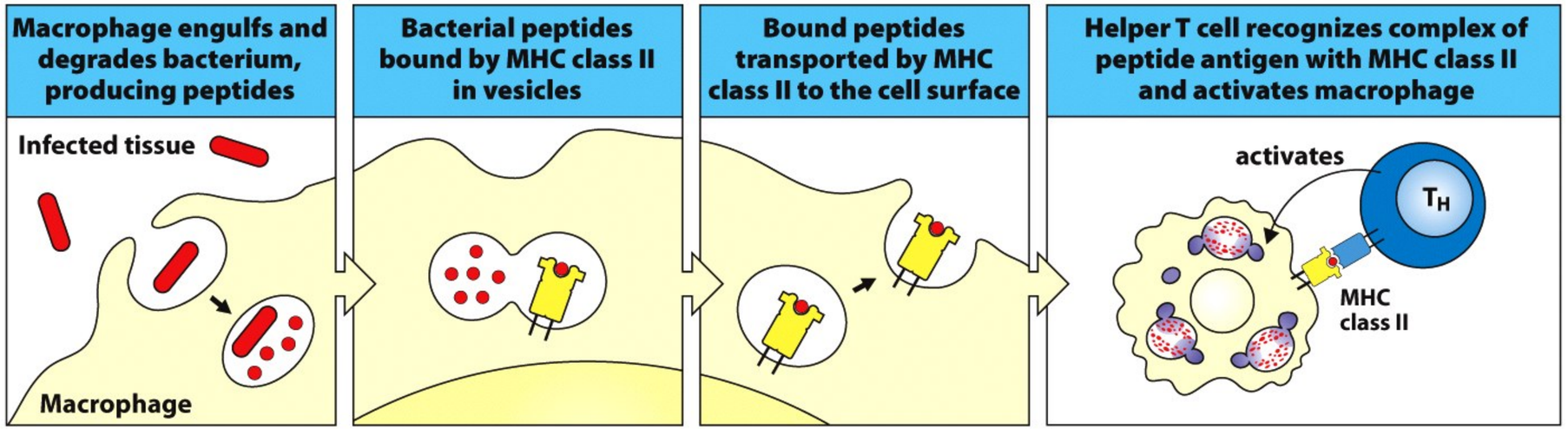


Figure 3.11 The Immune System, 3ed. (© Garland Science 2009)

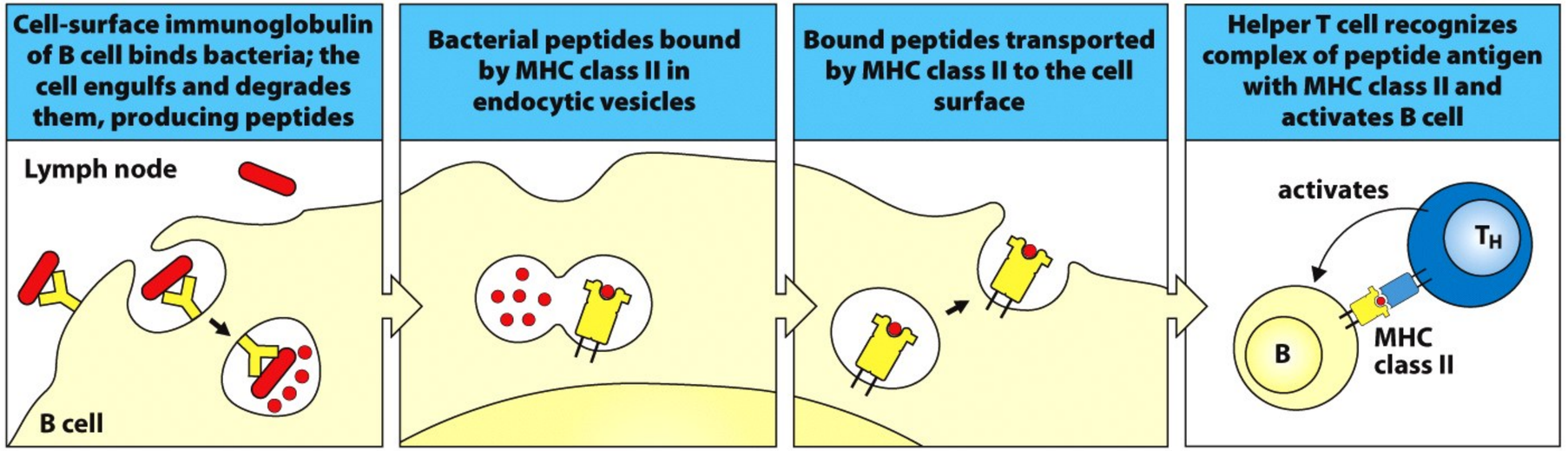


Figure 3.13 The Immune System, 3ed. (© Garland Science 2009)

Antigen-Specific Molecules: Antibodies

The Future of Monoclonal Antibodies Therapeutics: Innovation In Antibody Engineering, Key Growth Strategies And Forecasts To 2011
Business Insights
July 1, 2006, 180 Pages -$2,875 online download

The monoclonal antibody (mAb) market has grown rapidly in recent years, reaching sales of $14bn in 2005, an increase of 36.5% from 2004 sales of $10.3bn. Köhler and Milstein developed the hybridoma method of murine antibody production in 1975, which allowed the production of the first mAb to market; Johnson & Johnson's Orthoclone OKT3 (muromonab) in 1986. The mAb market is highly innovative and a key trend has been the move from murine to humanized and fully human antibodies. As technology has progressed these humanized mAbs have prevented immune responses (HAMA), thus having a larger market potential. The traditional therapy areas in the mAb market are oncology and autoimmune and inflammatory disorders (AIID), however this is forecast to change with the emergence of other therapy areas including infectious disease and ophthalmology. The clear leader in the mAb market is Genentech with 5 marketed drugs, with sales totaling $4,116.4m in 2005. A key theme of this report is the high level of innovation, as demonstrated by advancements in antibody engineering with the introduction of chimeric, humanized and fully human mAbs. Other innovation in antibody technology include advancements in non-invasive drug delivery technology, which is predicted to lead to a huge boost in sales in the long-term once drugs that utilize this technology come to market.

Antigen-Specific Molecules: Antibodies

Key findings of the report:

*** The antibody market is set to more than double in value over the next 5 years to $29.7bn, continuing the rapid growth witnessed in the past 2 years**. (actually 2009 figure was $36 billion, so that estimate was conservative)

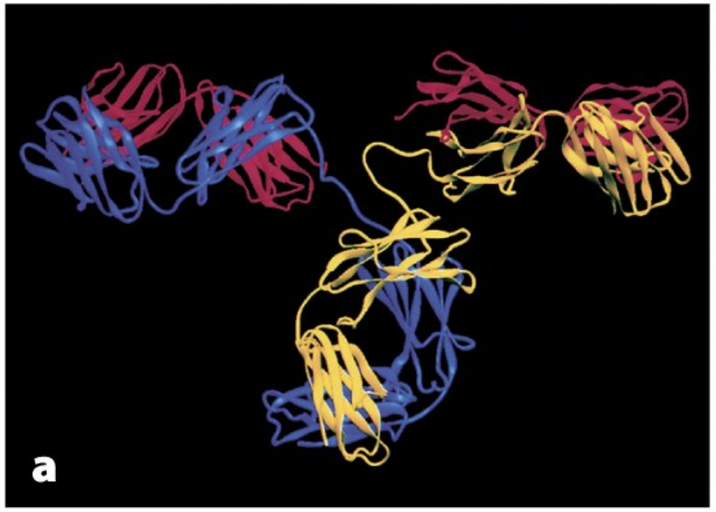
*** During the next 5 years antibodies for oncology and arthritis, immune and inflammatory disorders (AIID) are forecast to continue to lead the market, with sales of $14bn and $11bn, respectively in 2011.**

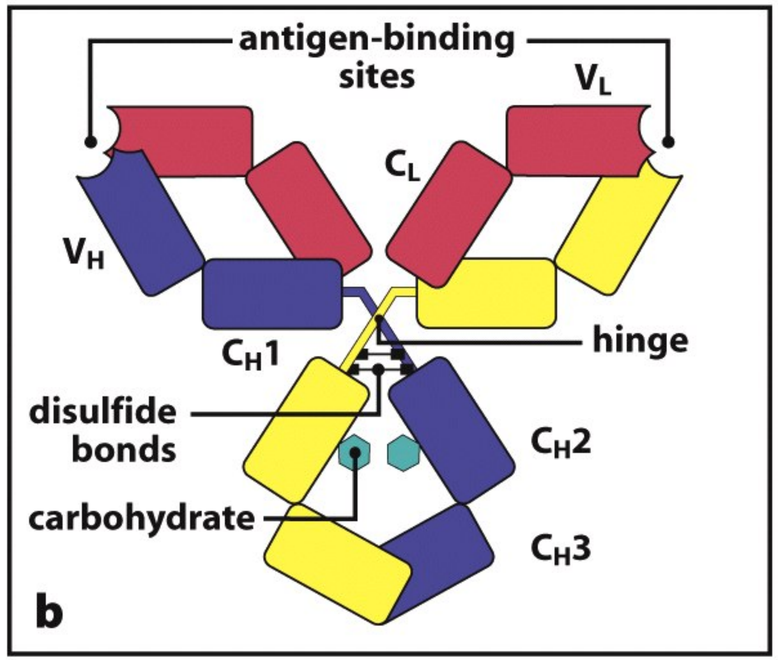
*** With the predicted launch of 2 pipeline antibodies in the next 5 years, the infectious disease area is set to grow in importance. Antibodies are also being developed in respiratory, cardiovascular and ophthalmology indications.**

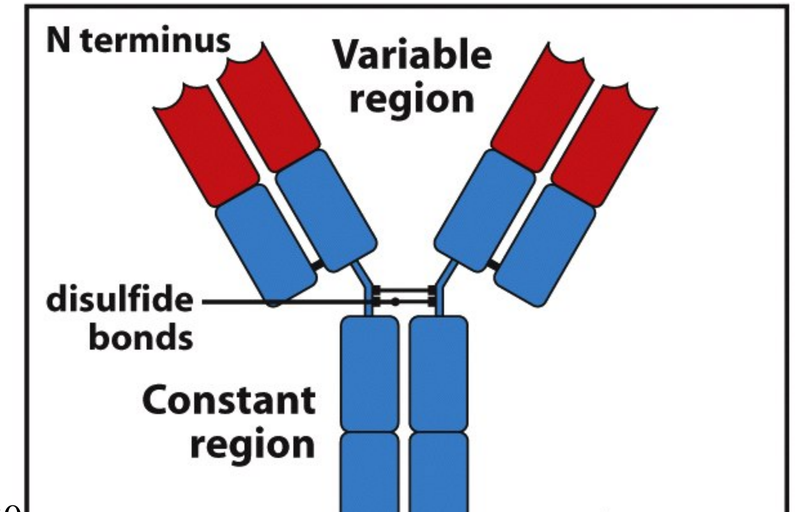
*** Analysis of early and late stage pipelines show development focus is shifting away from murine and chimeric mAbs to humanized and fully human antibodies. Abbott/CAT's Humira, launched in the US in 2003, was the first fully human antibody on the market.**

*** Genentech and Roche held a combined 48.7% share of the antibodies market in 2005, primarily due to the success of their collaborative oncology drugs, Rituxan, Herceptin and Avastin.**

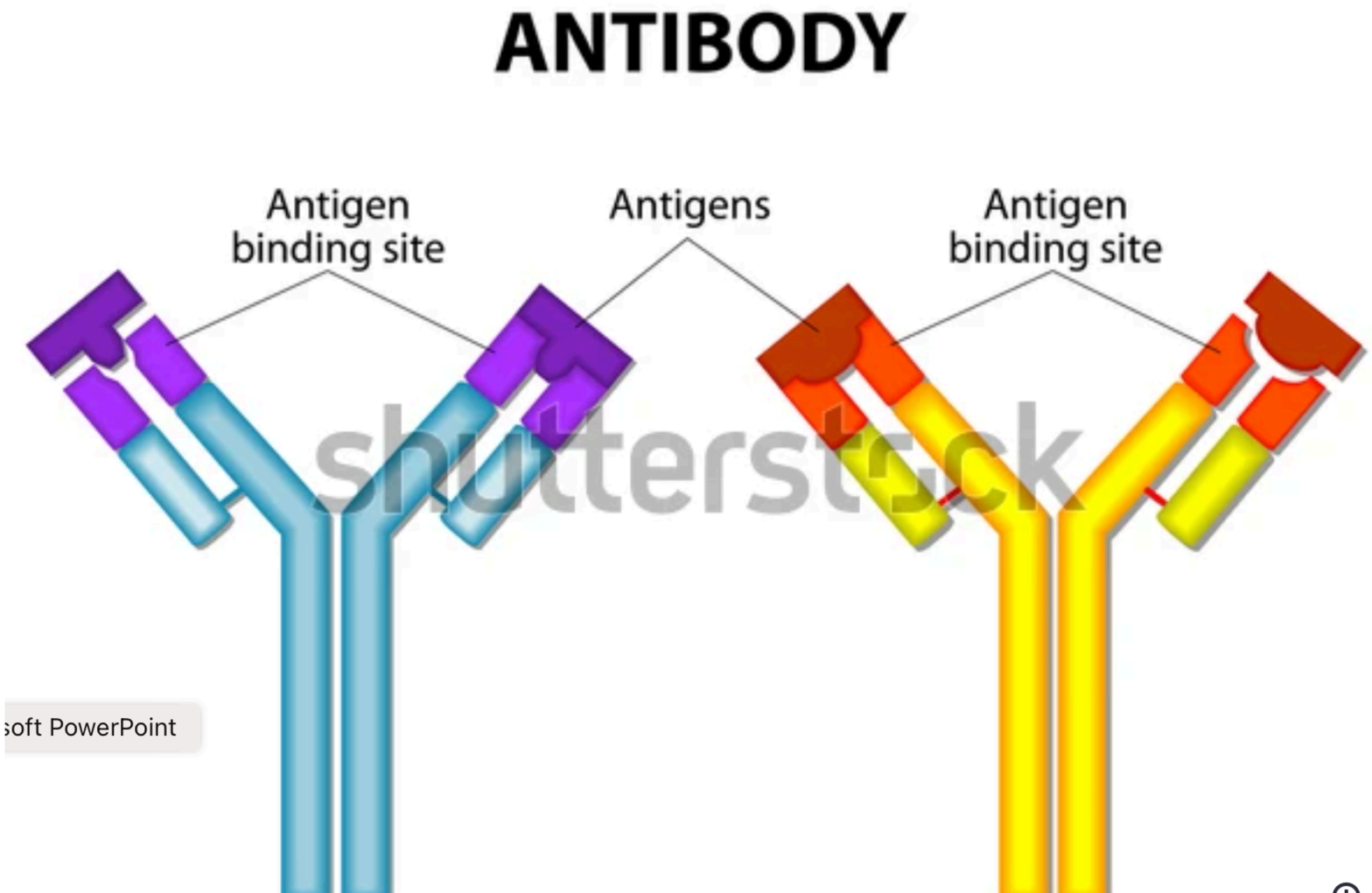
Antibodies have Variable Regions and Constant Regions







Antibody and Antigen are complementary



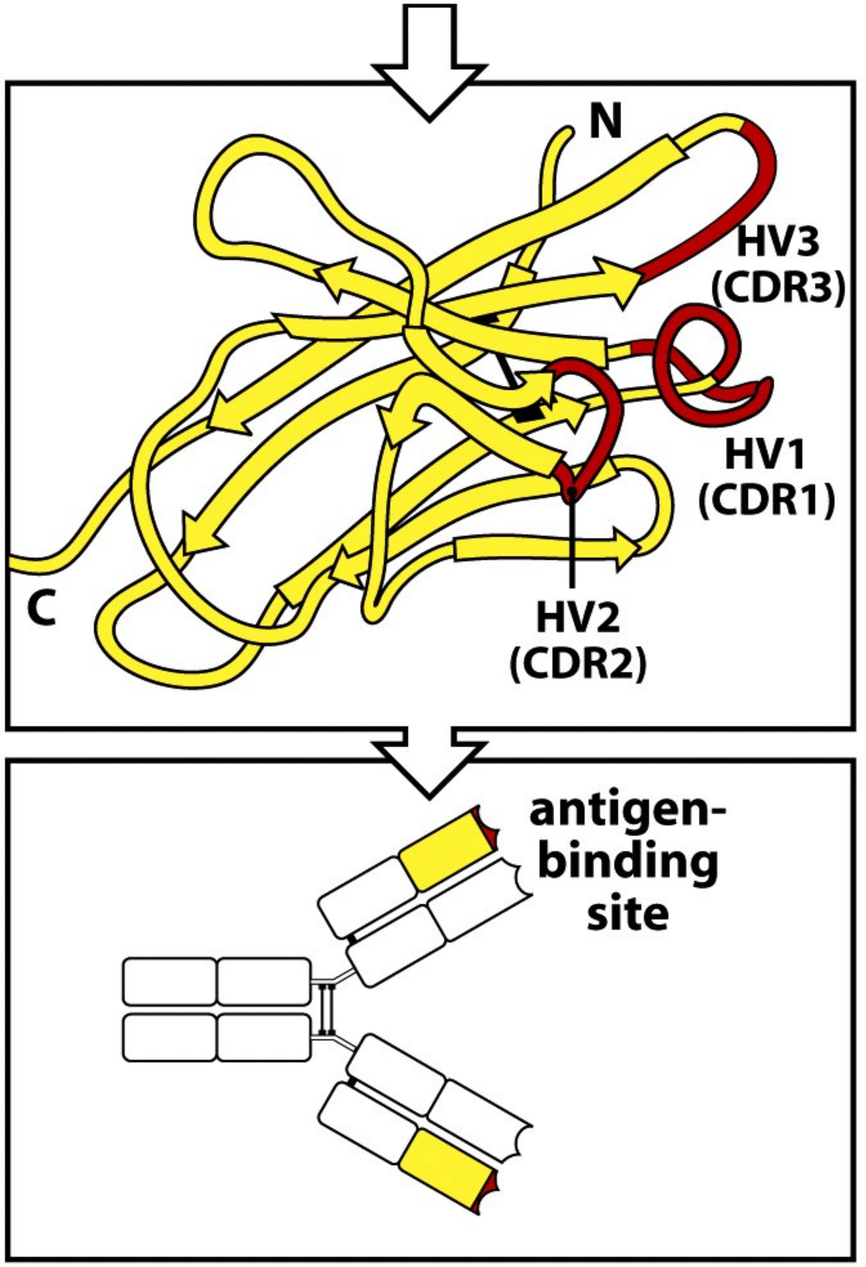


Figure 3-7 part 3 of 3 Immunobiology, 7ed. (© Garland Science 2008)

Antibody Diversity

DNA diversity

As B cells develop in bone marrow, diversity is created in the DNA sequences encoding the Light Chain Hypervariable domains

Some variability is created by combining already present sequences (e.g., there are about 40 V region segments to choose one from)

Some variability is created by random mutations in sequences at the junctions of the already present segments

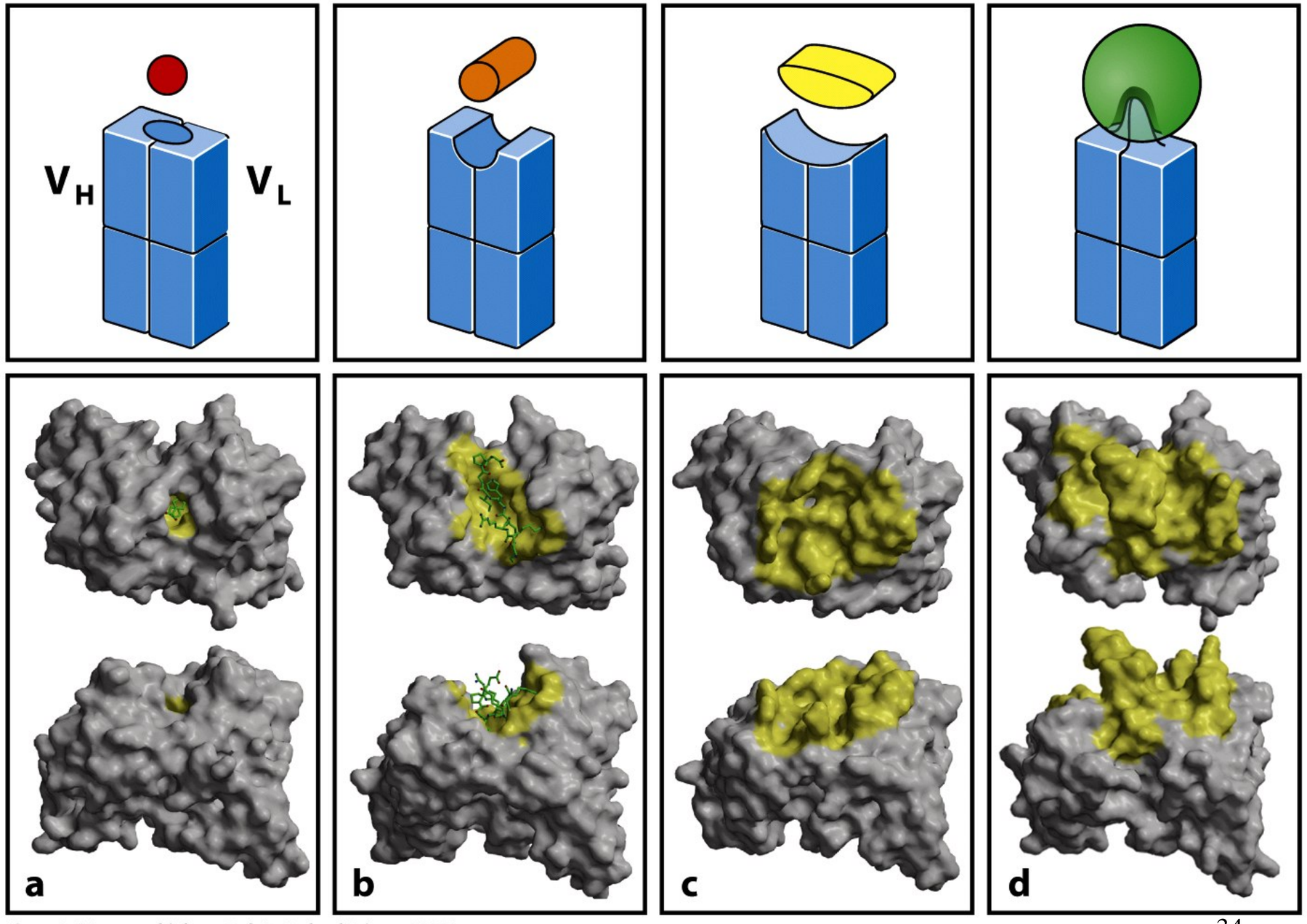
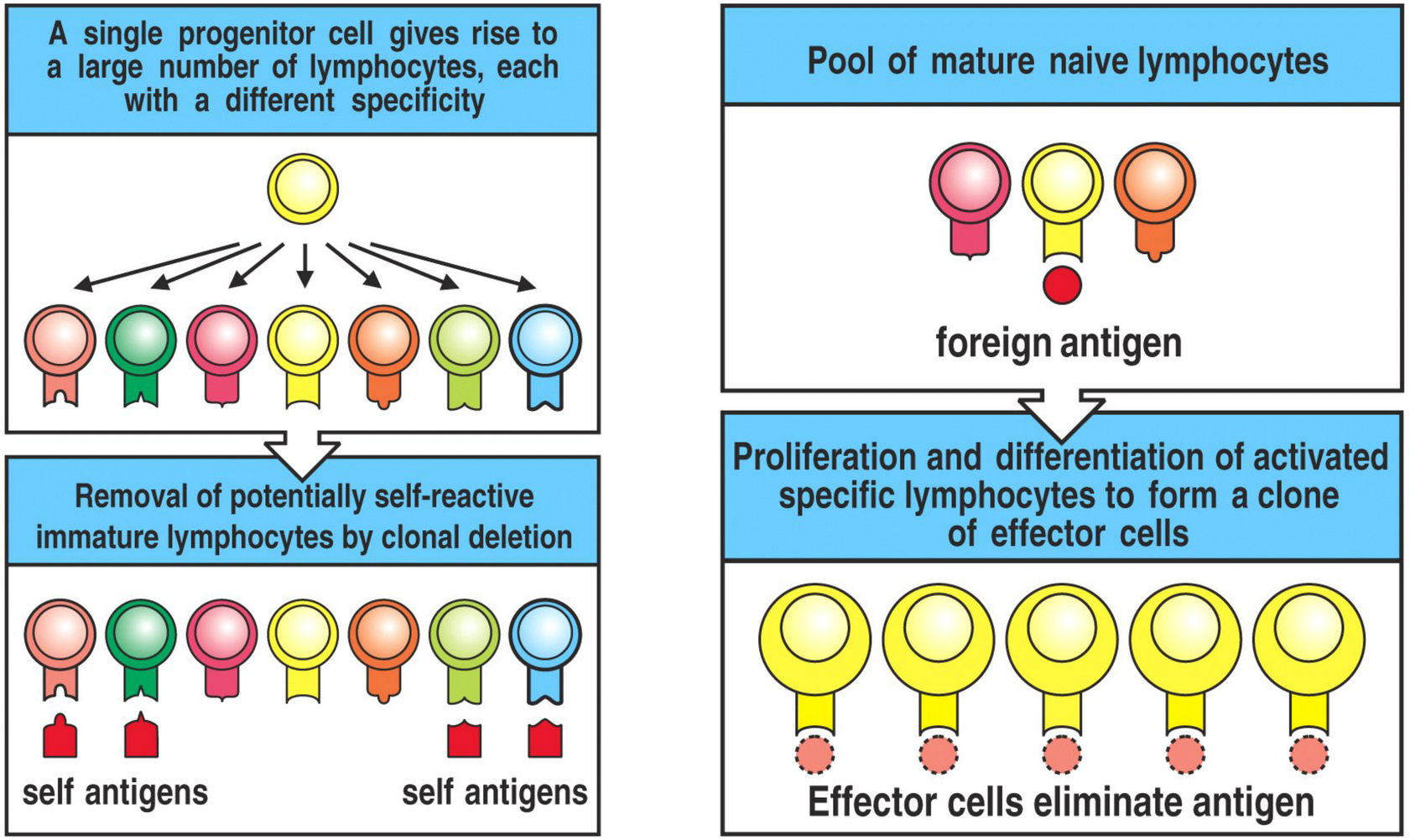


Figure 3-8 Immunobiology, 7ed. (© Garland Science 2008)

Clonal selection of lymphocytes is the central principle of adaptive immunity



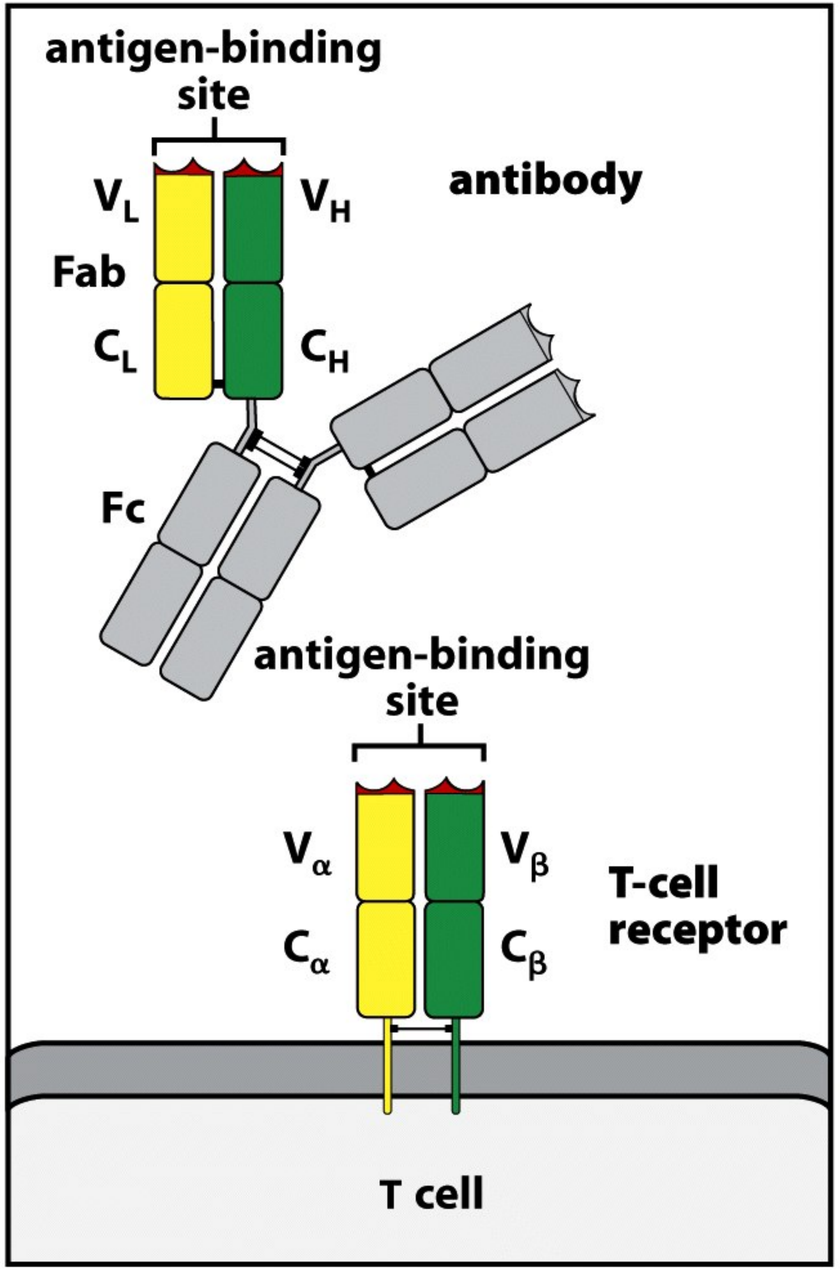


Figure 3-11 Immunobiology, 7ed. (© Garland Science 2008)

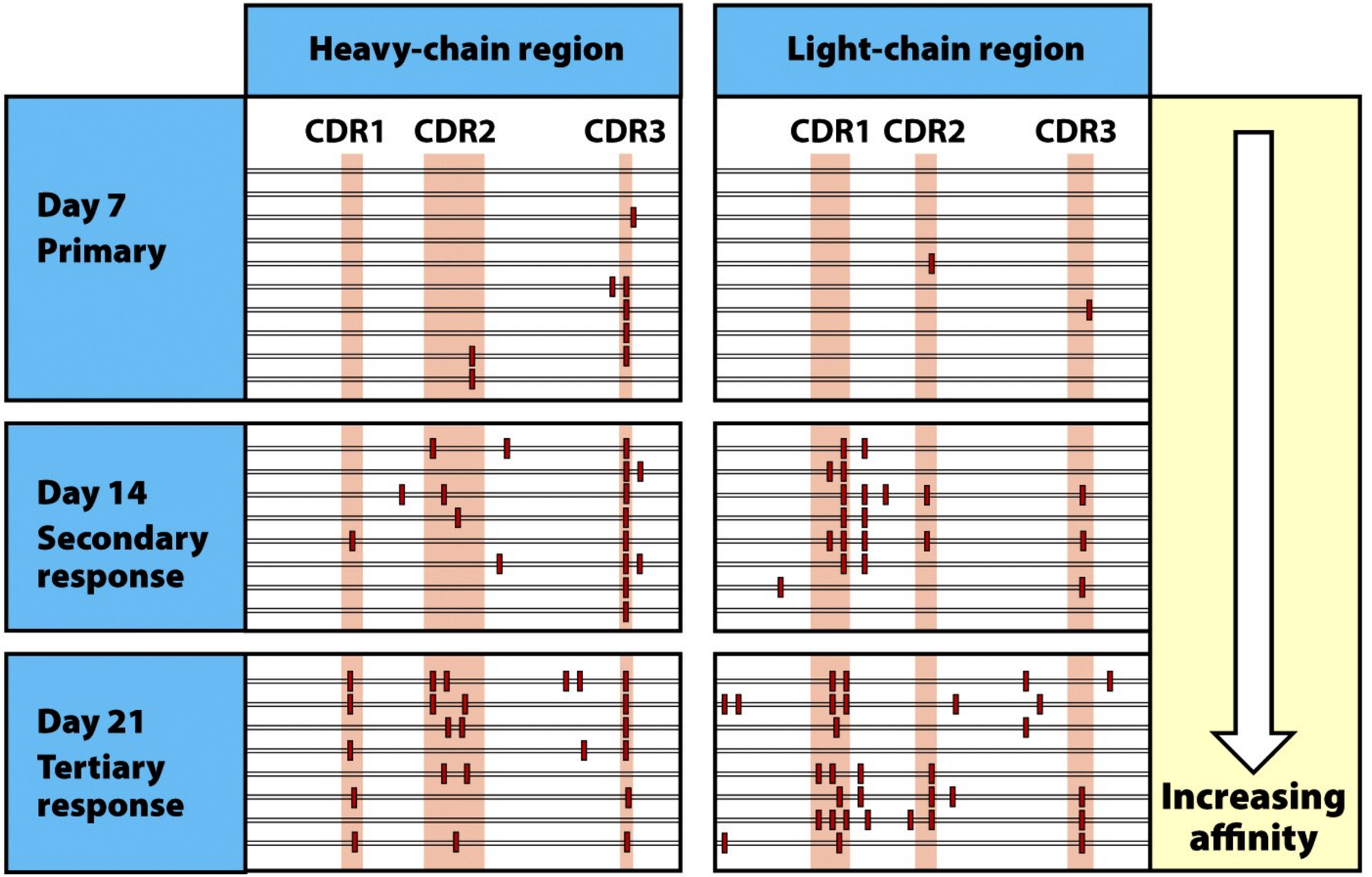


Figure 4-25 Immunobiology, 7ed. (© Garland Science 2008)

Element	Immunoglobulin		α:β T-cell receptors	
	H	κ+λ	β	α
Variable segments (V)	40	70	52	~70
Diversity segments (D)	25	0	2	0
D segments read in three frames	rarely	–	often	–
Joining segments (J)	6	5(κ) 4(λ)	13	61
Joints with N- and P-nucleotides	2	50% of joints	2	1
Number of V gene pairs	1.9×10^6		5.8×10^6	
Junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{11}$	
Total diversity	$\sim 5 \times 10^{13}$		$\sim 10^{18}$	

Figure 4-12 Immunobiology, 7ed. (© Garland Science 2008)

37 x 10E12 cells in whole body

Different Constant Regions (Isotypes)

These are called Isotypes, IgM, IgD, IgE, IgG, IgA, have different functions

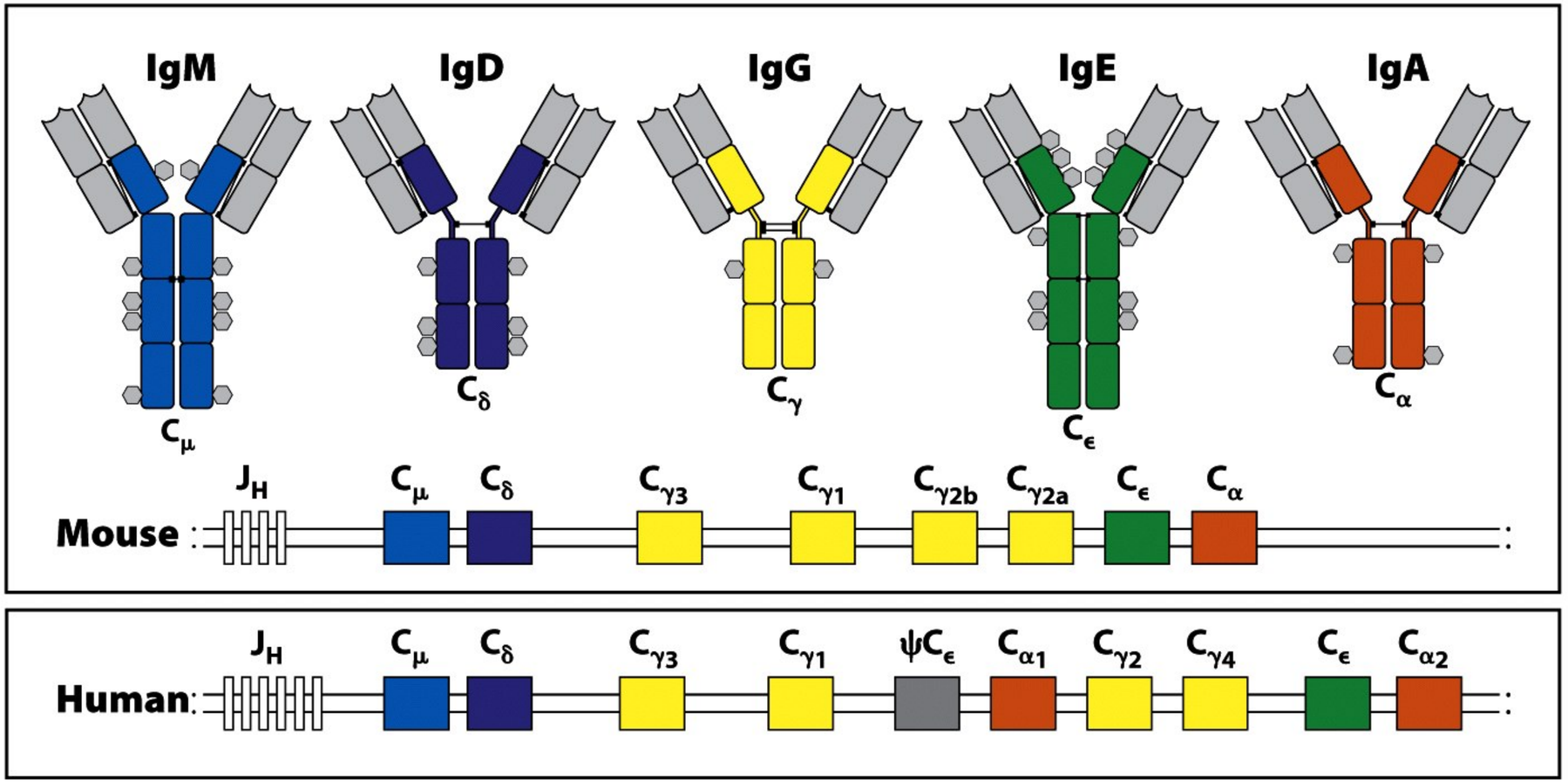
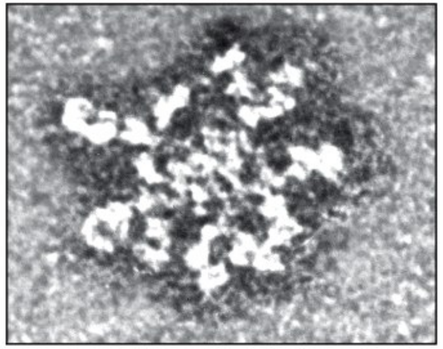
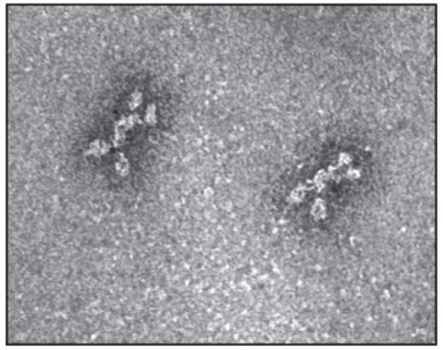
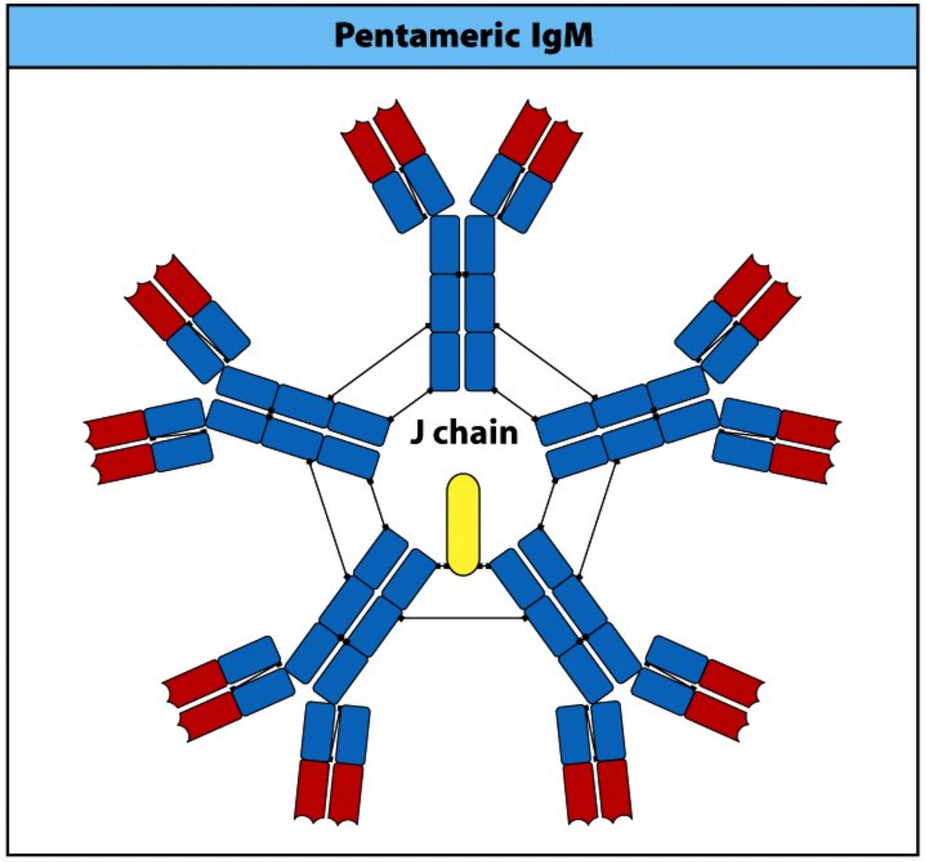


Figure 4-17 Immunobiology, 7ed. (© Garland Science 2008)







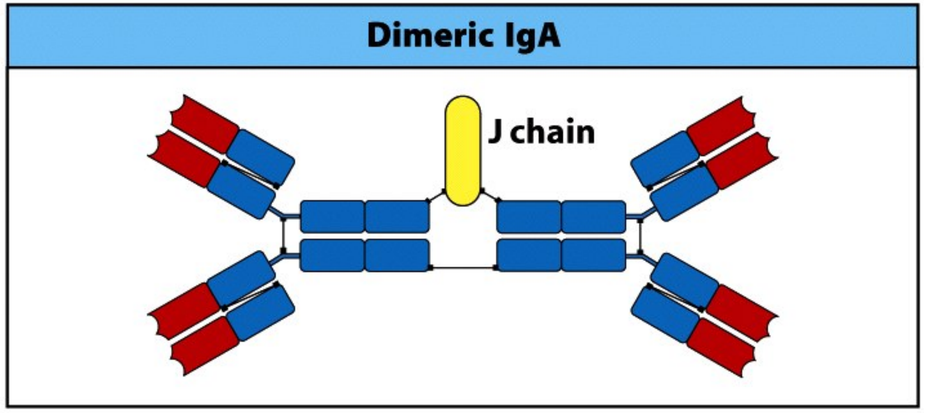


Figure 4-20 Immunobiology, 7ed. (© Garland Science 2008)

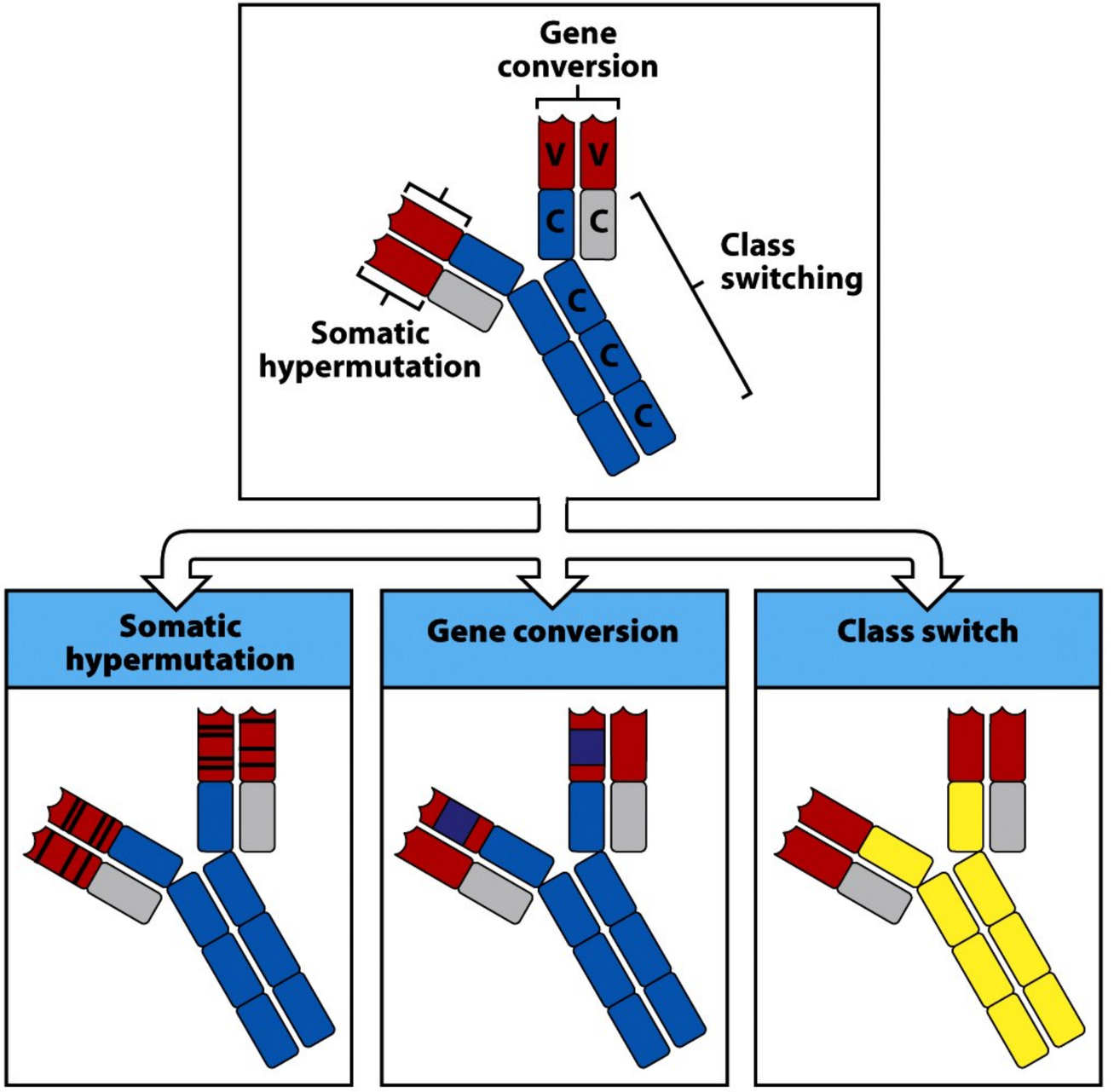


Figure 4-21 Immunobiology, 7ed. (© Garland Science 2008)

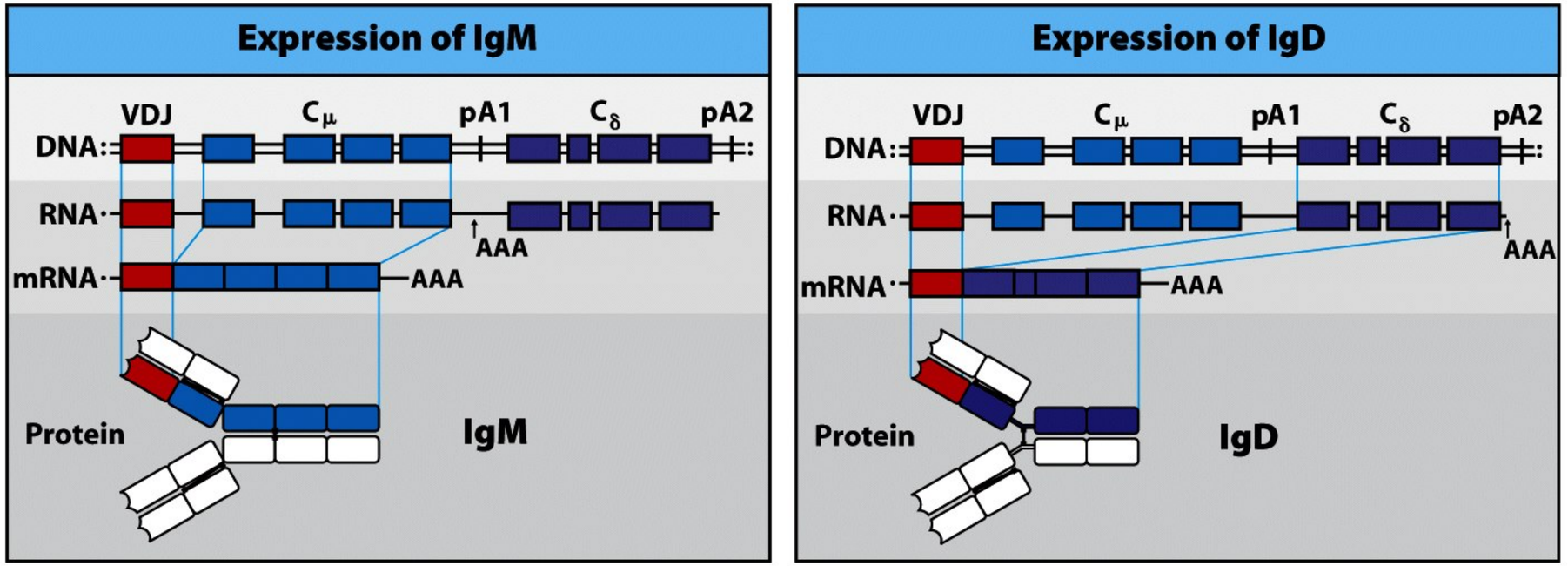


Figure 4-18 Immunobiology, 7ed. (© Garland Science 2008)

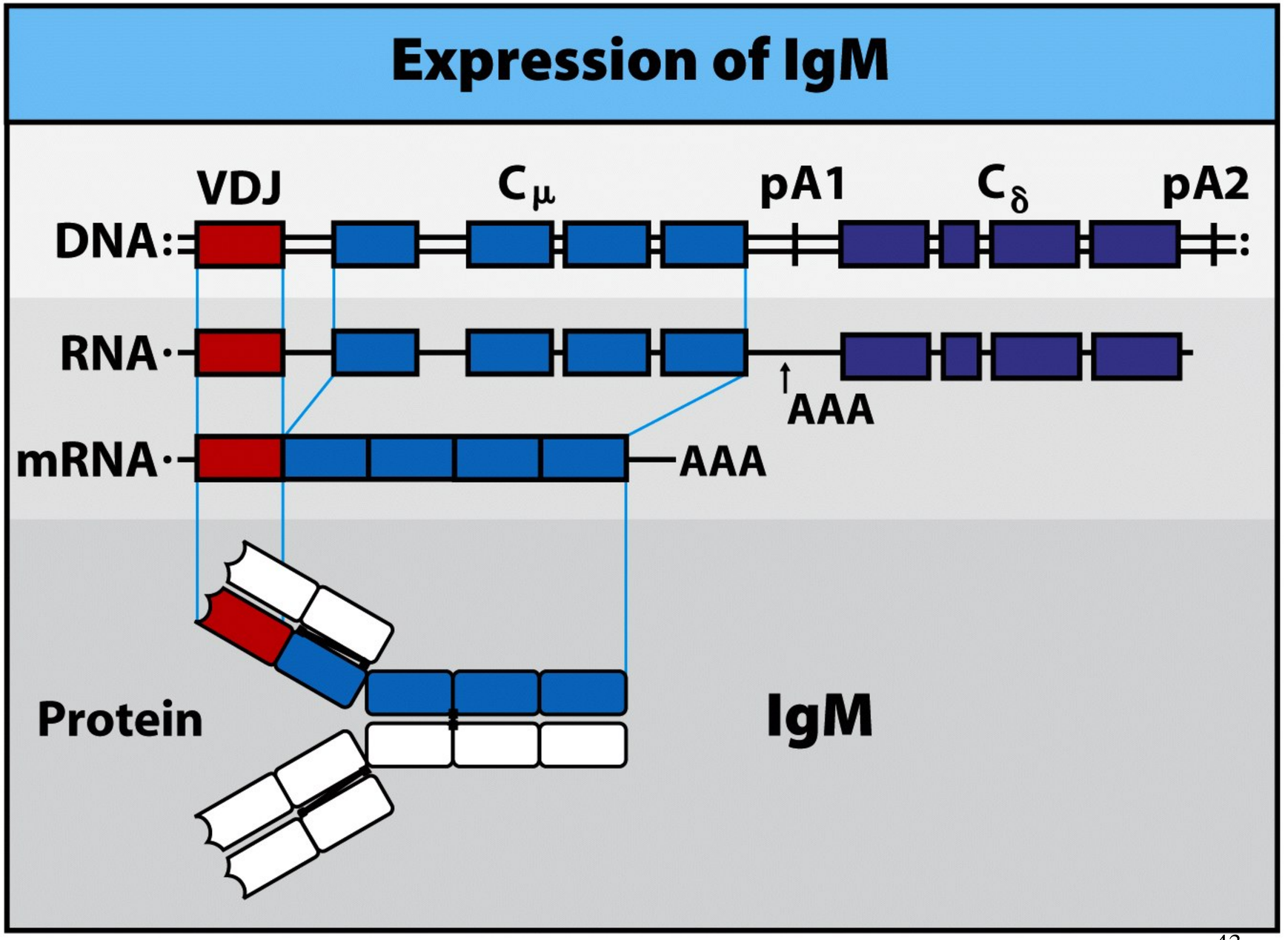


Figure 4-18 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

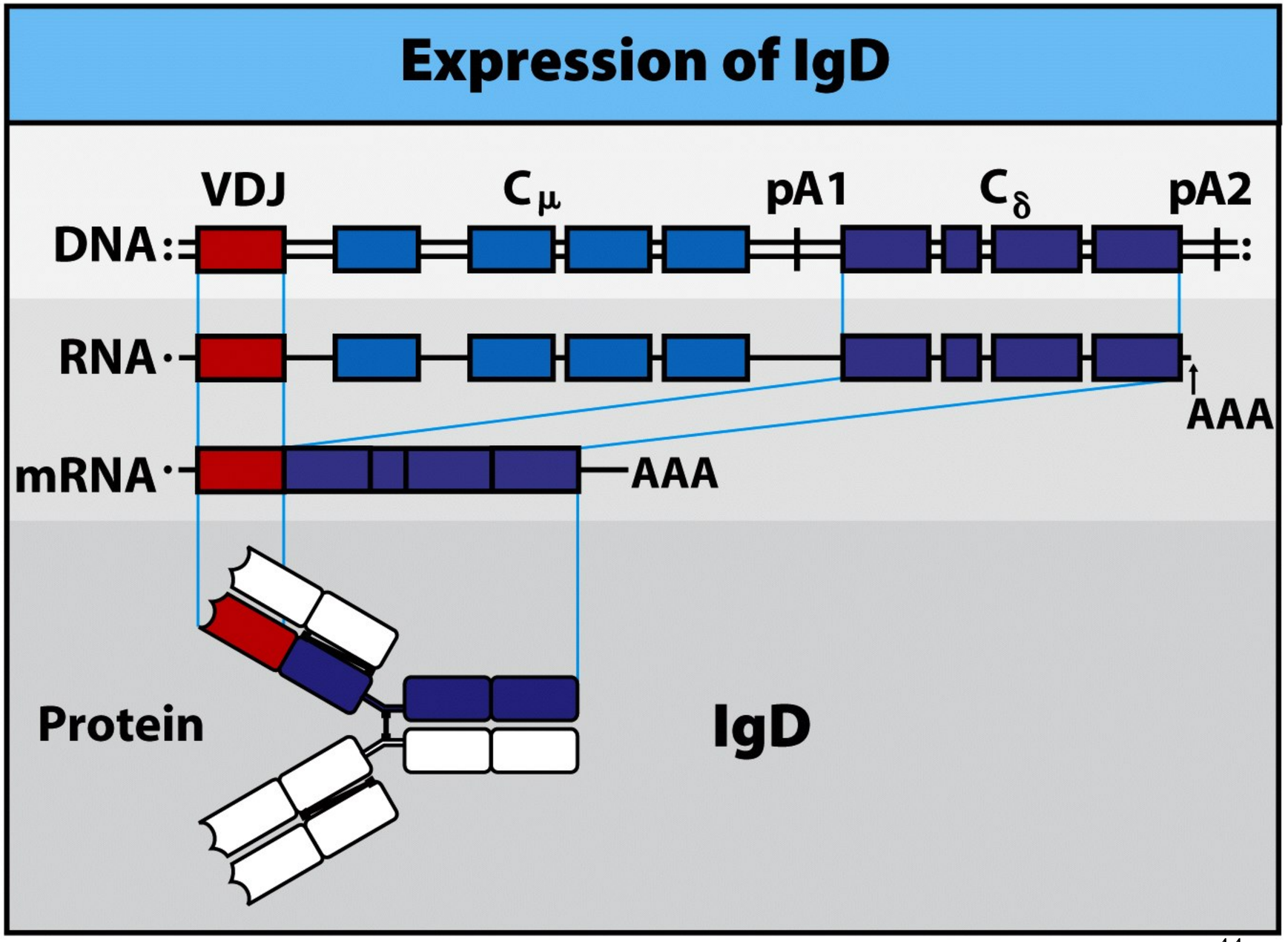


Figure 4-18 part 2 of 2 Immunobiology, 7ed. (© Garland Science 2008)

Learning Objectives for Week 5

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- **Activation of T cells and B cells with Two-Factor Authentication**
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  - How Helper T cells help macrophages, CTLs and B cells
  - How pathogen-appropriate responses are generated

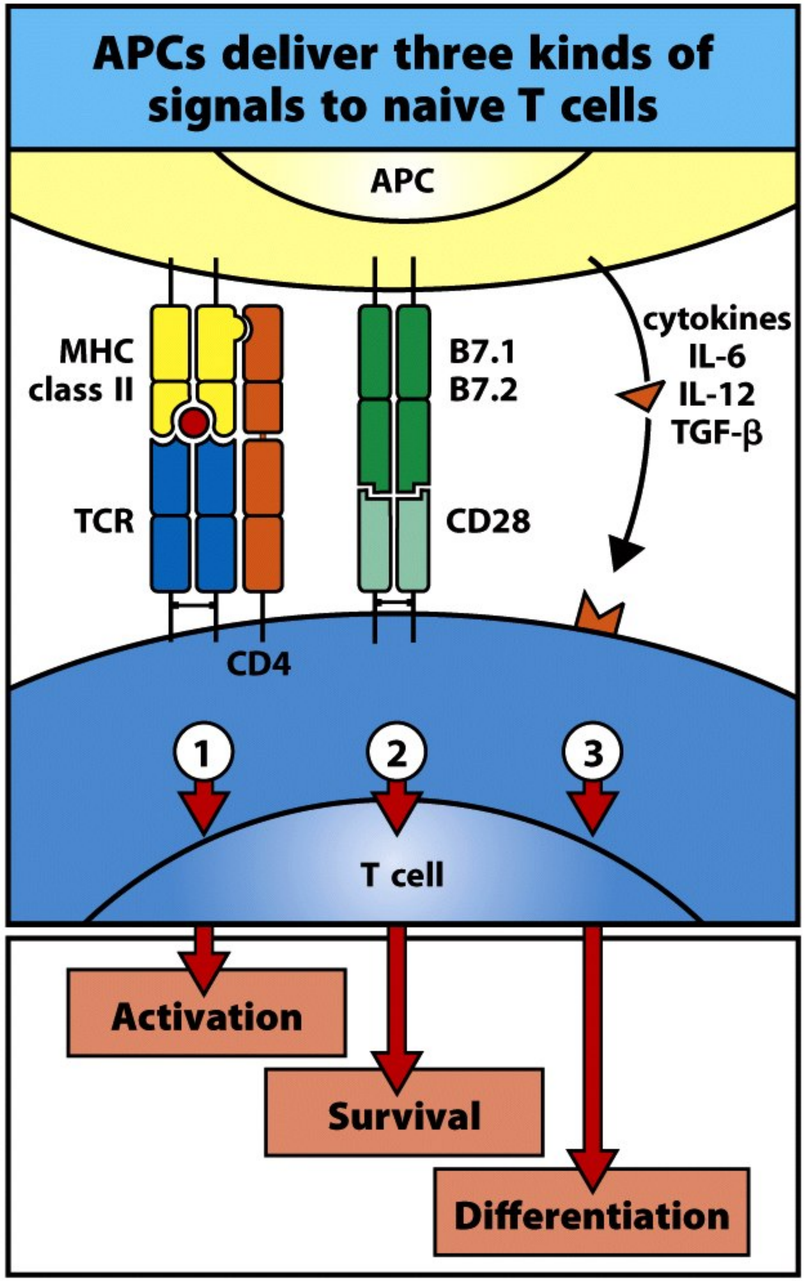


Figure 8-19 Immunobiology, 7ed. (© Garland Science 2008)

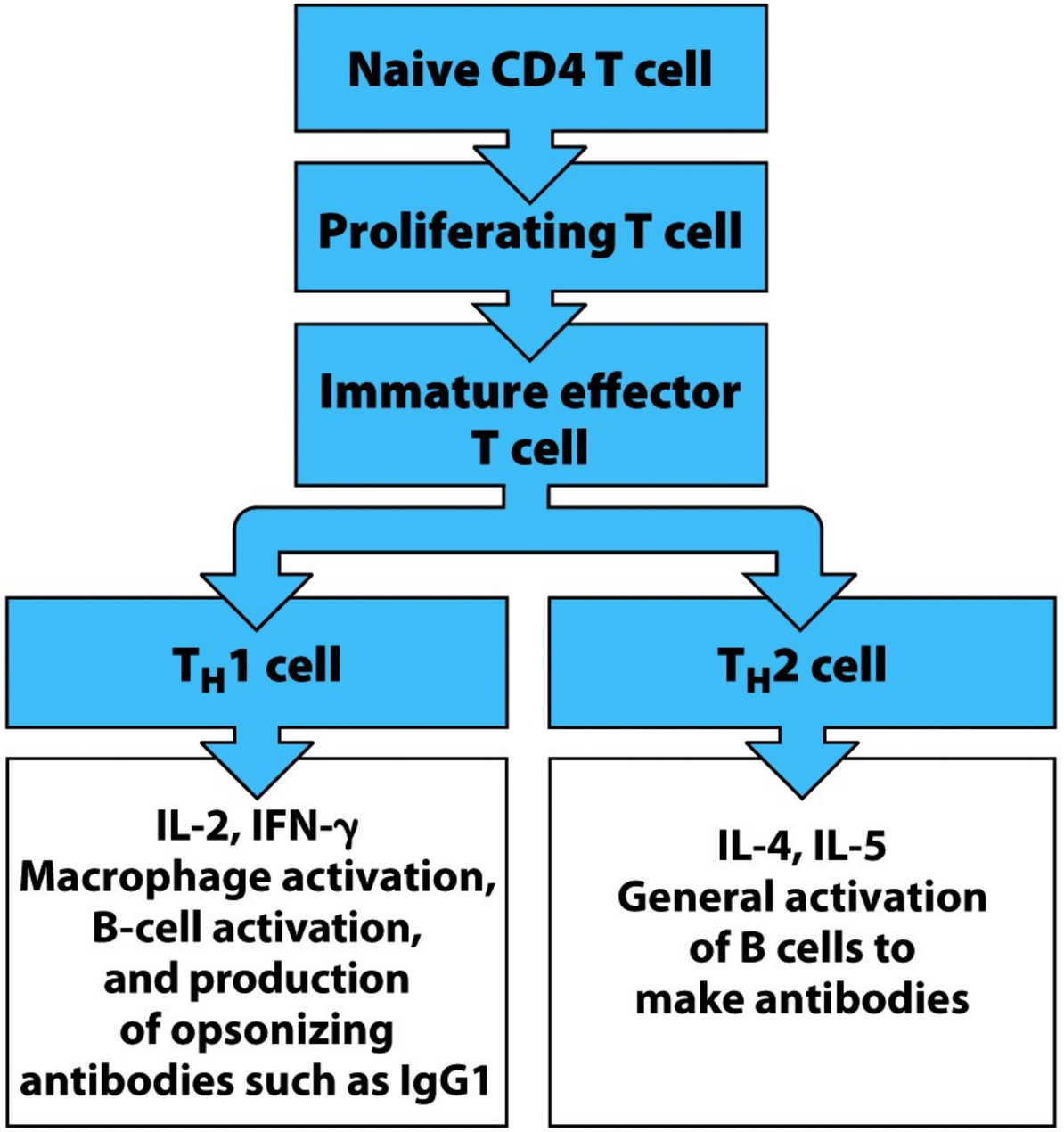


Figure 8.19 The Immune System, 3ed. (© Garland Science 2009)

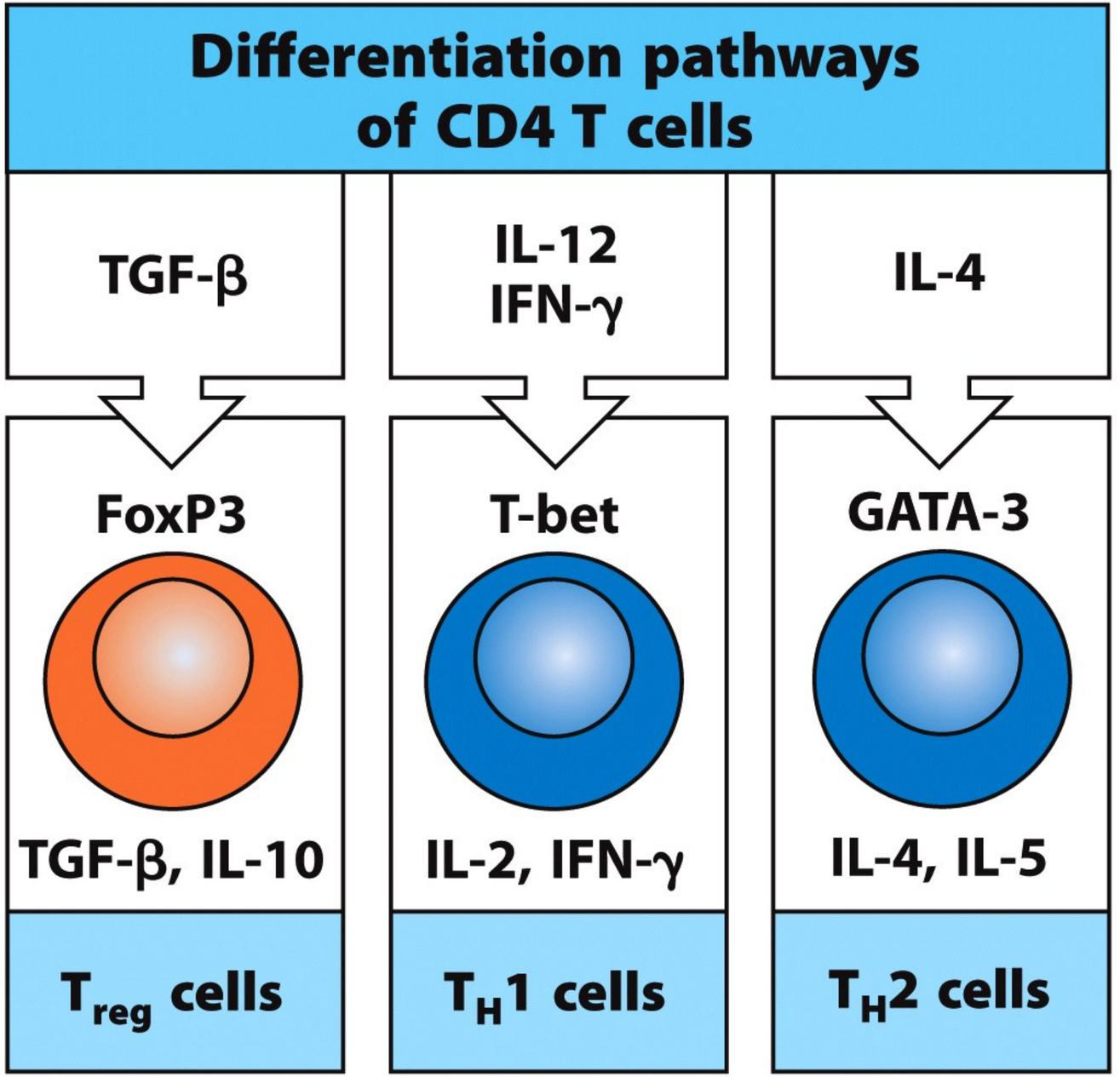


Figure 8.20 The Immune System, 3ed. (© Garland Science 2009)

What sets in motion Functions of the Immune System?

- Frank Macfarlane Burnet (1950's):
  - First distinguish "self" from "non-self" and protect the self from non-self, such as bacteria and viruses
  - Barbara Ehrenreich points out that "self" from Western individualism, and that "se
  - More recent problem: why does the imm microbiome, billions of non-self bacteria
- Polly Matzinger:
  - To protect 94
  - Danger is r at

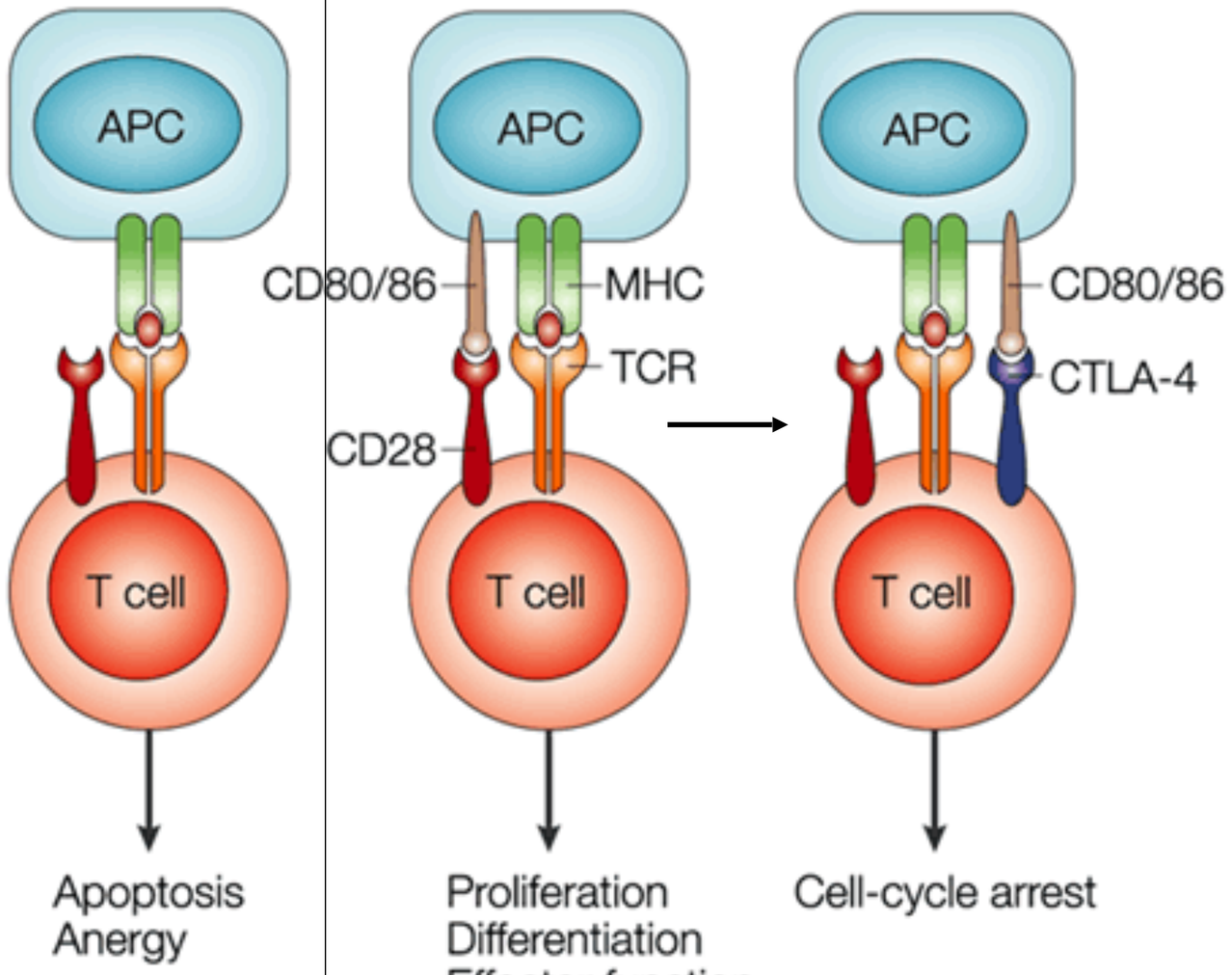
In one of her first publications, a paper for the *Journal of Experimental Medicine*, she listed a dog as a coauthor.[13] Ted Anton described the decision in his book *Bold Science*: "Refusing to write in the usual scientific passive voice ('steps were taken') and too insecure to write in the first person ('I took the steps'), she instead invented [a] coauthor": her Afghan Hound, Galadriel Mirkwood.[14] Once the deception was discovered, papers on which she was a major author were barred from the journal until the editor died and was replaced by another.[15]

•

Two-Factor Authentication of activation of adaptive immunity

- Both B cells and T cells require two signals to be fully activated
- Signal one is engagement of antigen-specific receptor (BCR or TCR)
- Signal two is “costimulatory signal” from APC for T cells, or helper T cell or “complement” for B cells
- Innate cells generate Signal Two in response to “danger signals” from pathogens or tissue damage

T-cell fate is dependent upon the conditions of TCR engagement.



Peripheral Tolerance

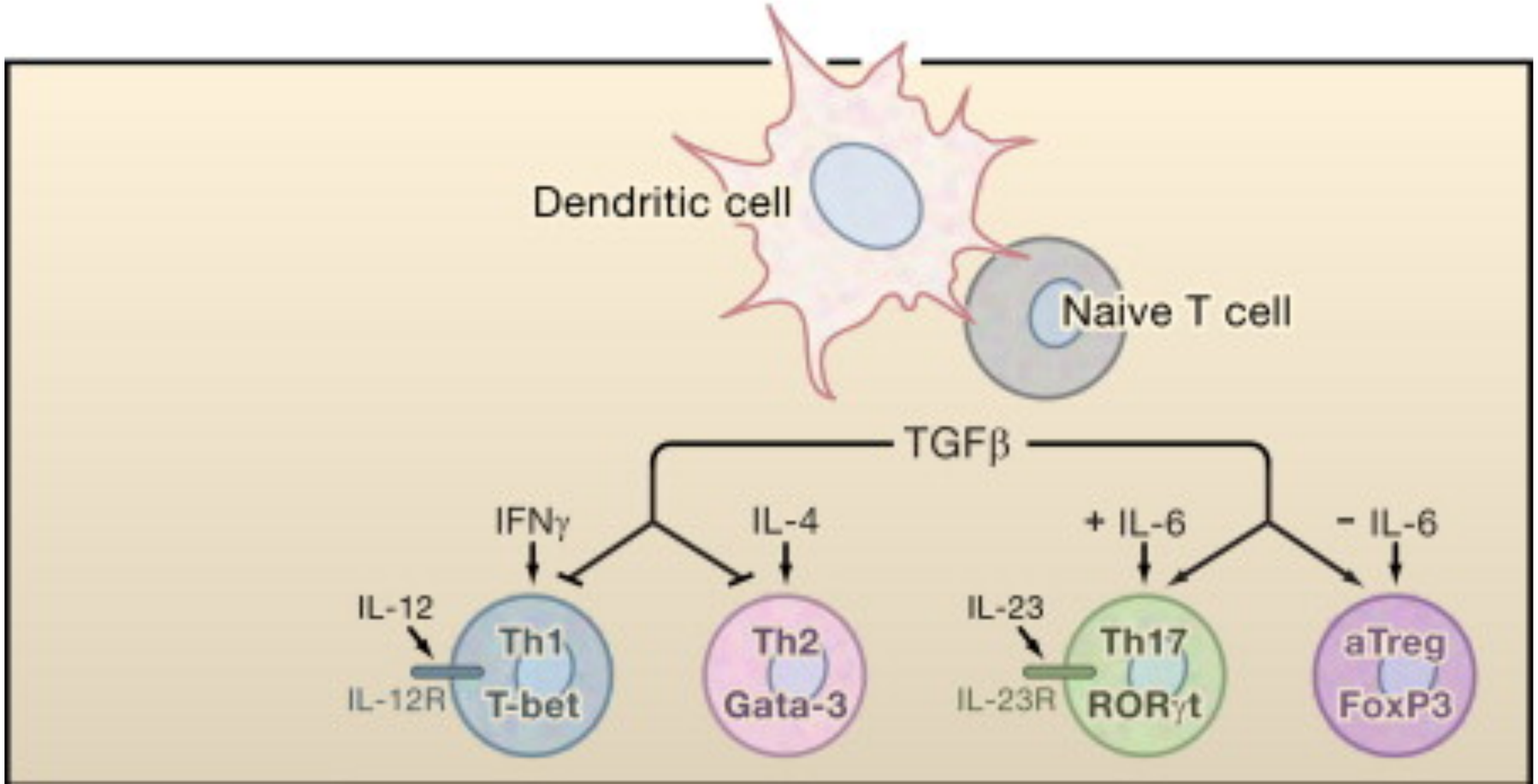
Nature Reviews | Immunology

Stronger Costimulation required to induce effective memory response

- For vaccines a replicating virus is more effective at eliciting memory, correlated with stronger signaling by OX40 and CD27 (Salek-Ardakani 2011)

- Depending on the cytokine milieu, CD4+ cells take on different phenotypes when activated

Effector helper T cells come in different flavors



PROTECTIVE ROLE	Intracellular pathogens	Parasitic worms	Extracellular bacteria	Counter-regulation
HARMFUL ROLE	Systemic pathology	Allergy and asthma	Autoimmunity Inflammation Cancer	

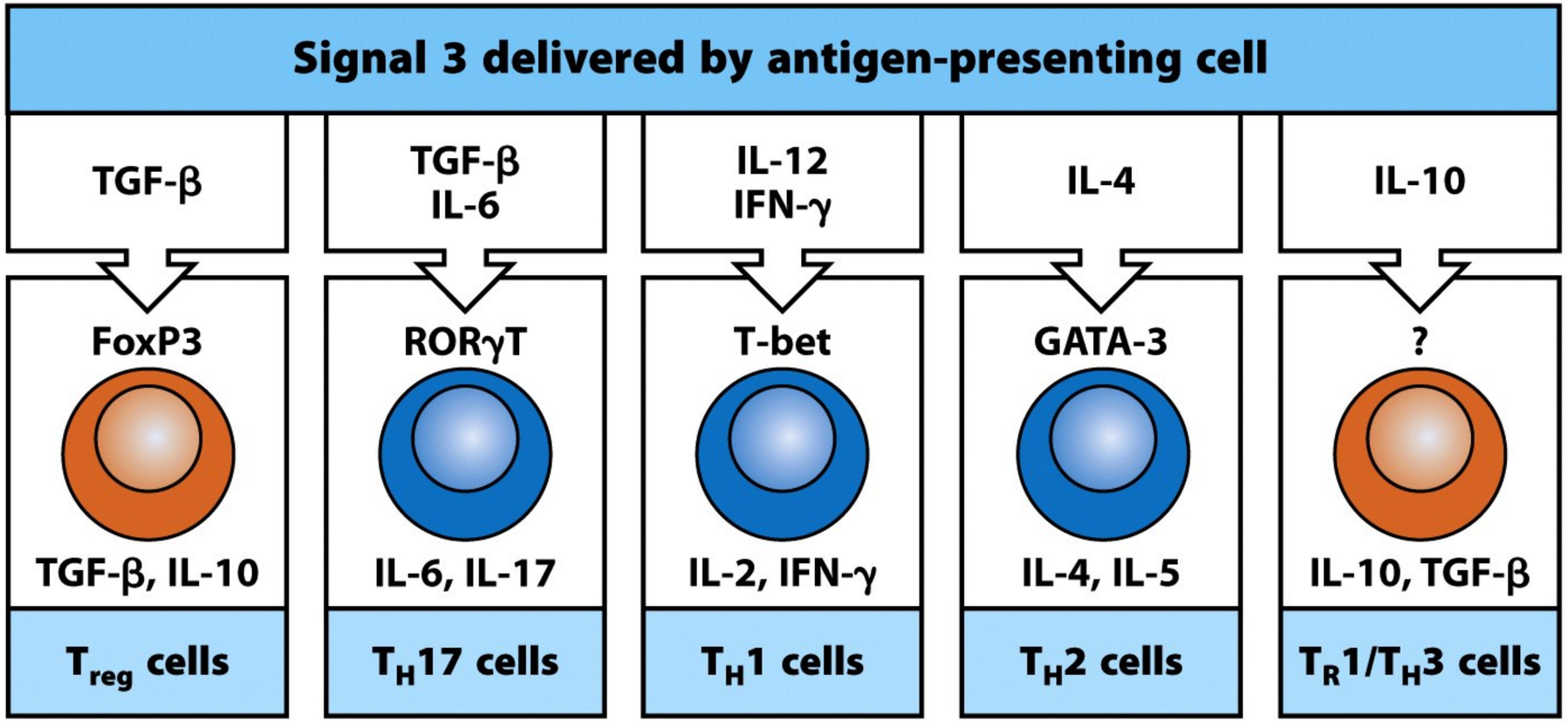


Figure 8-29 Immunobiology, 7ed. (© Garland Science 2008)

CD4+ T cells

- TH1 help macrophages and CTLs
- TH2 help B cells, especially production of IgE
- TH17 proinflammatory (activates neutrophils)
- Treg suppress CTLs and helper T cells
- Tfh follicular helper T cells help B cells

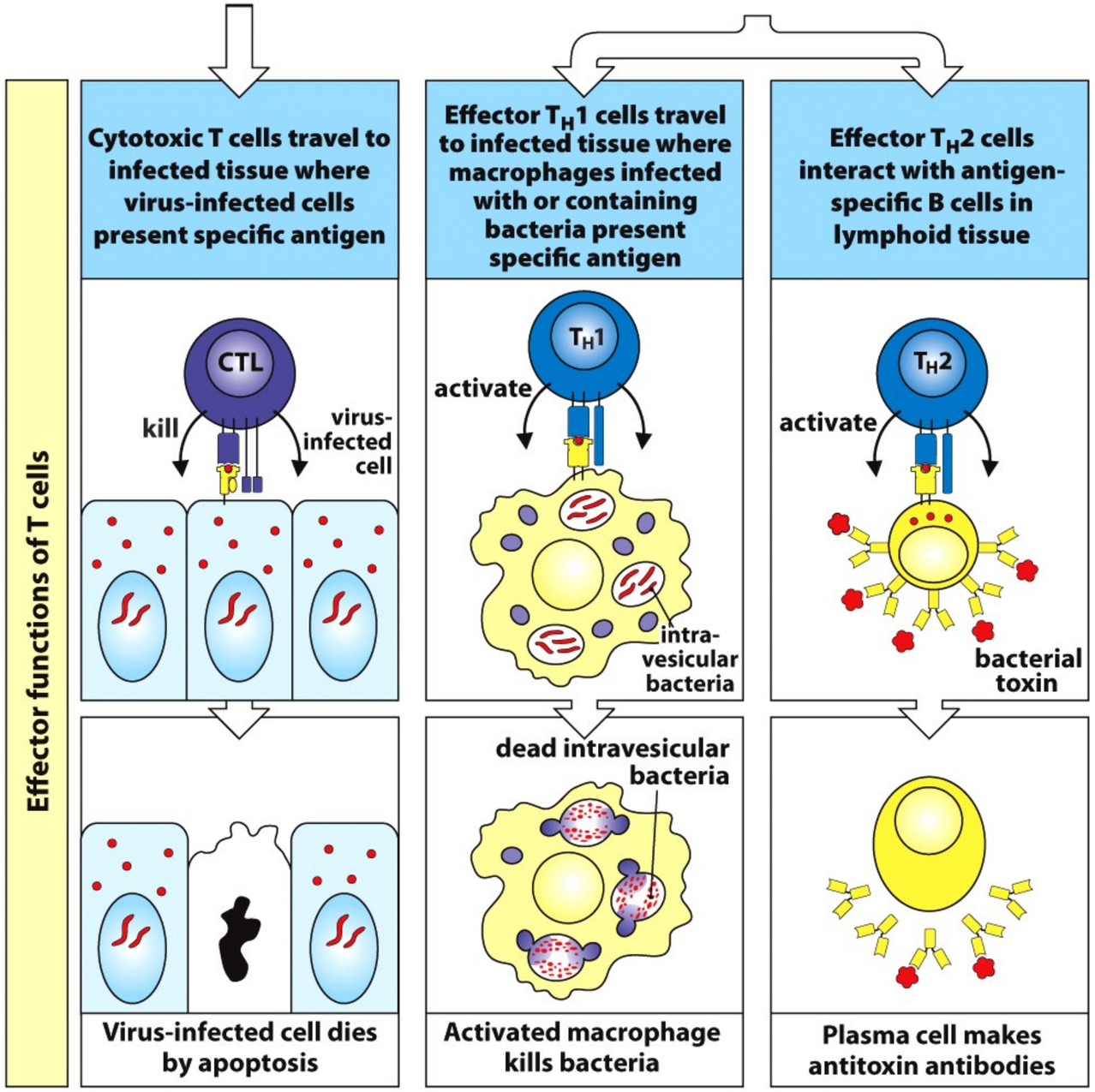


Figure 8.39 part 2 of 2 The Immune System, 3ed. (© Garland Science 2009)

Effector Function does not require costimulation (esp important for CD8+ CTLs)

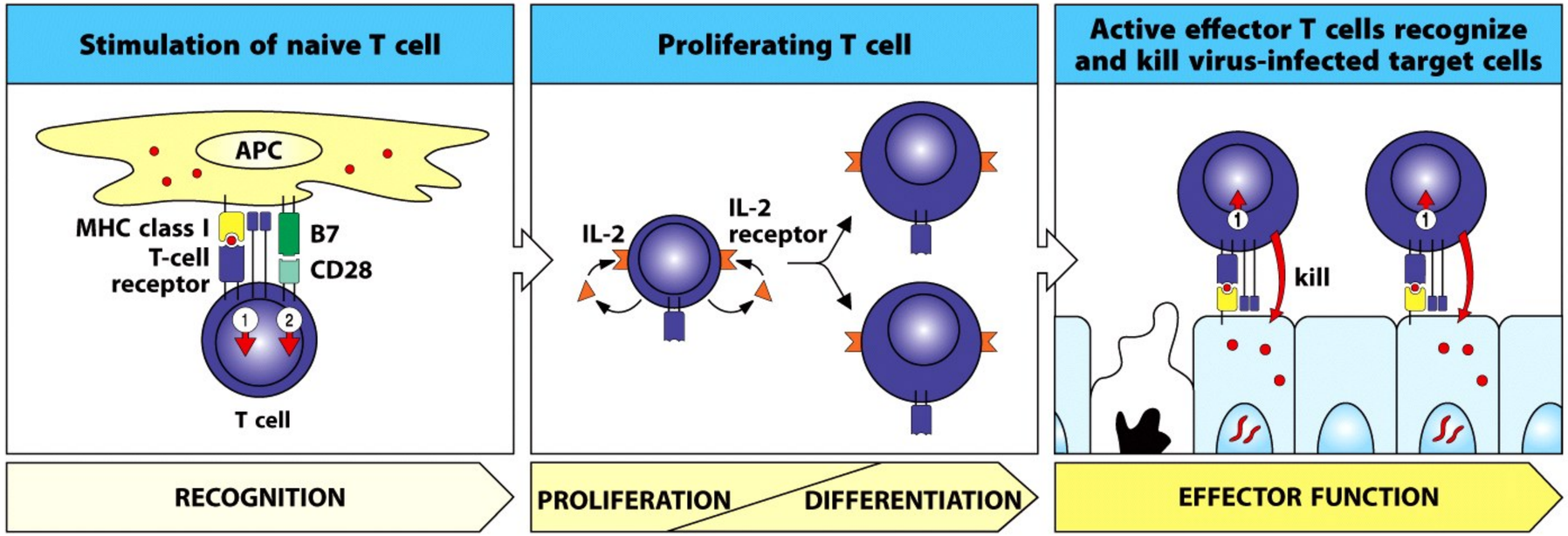
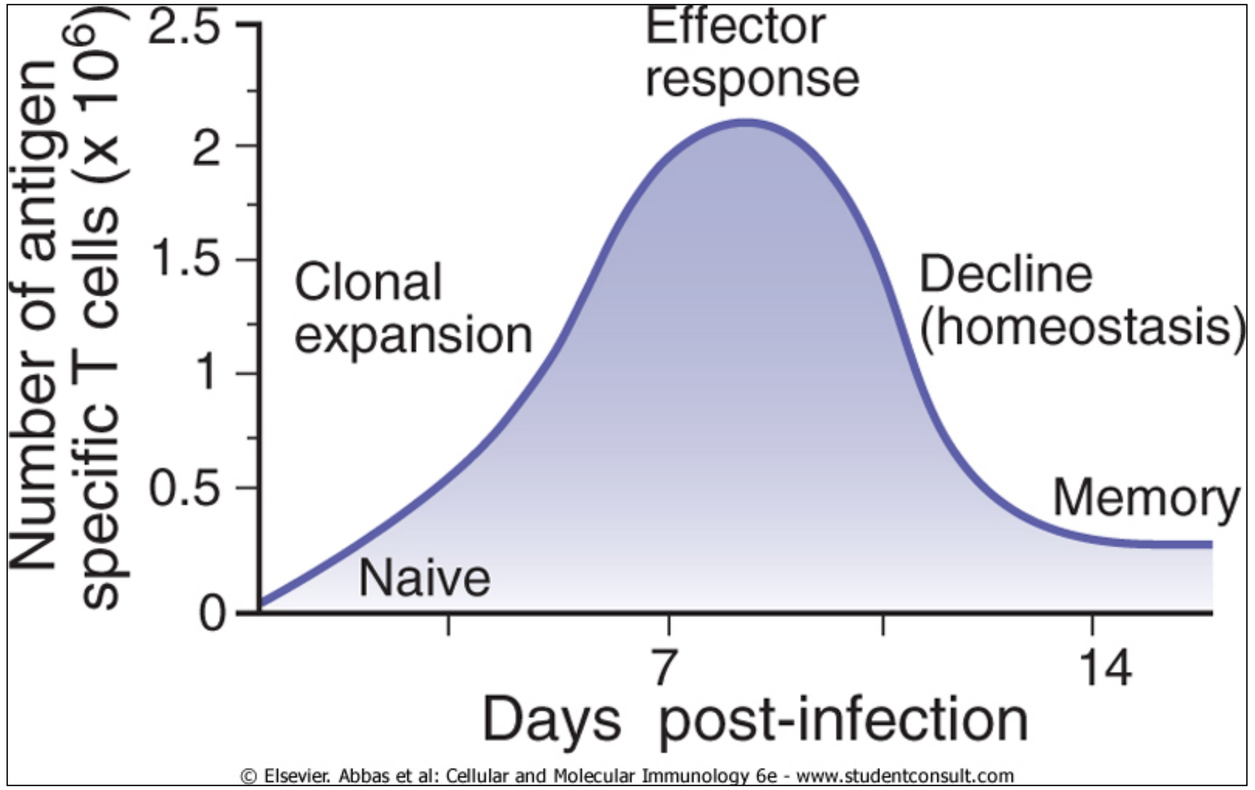


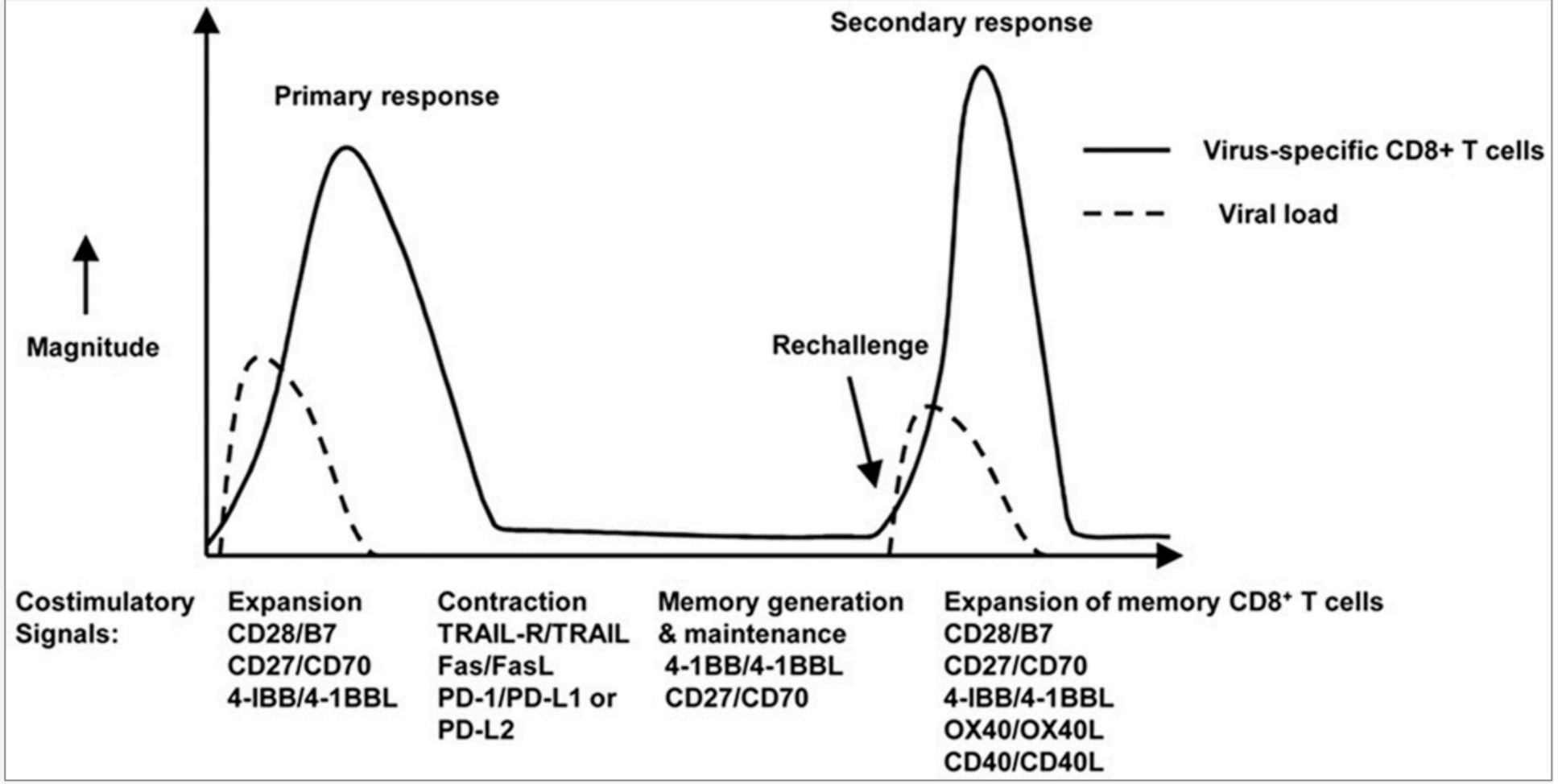
Figure 8.23 The Immune System, 3ed. (© Garland Science 2009)

Clonal expansion of T cells. T cells expand in response to infection, some of the progeny differentiate into effector cells, the majority die, and memory cells persist.



Memory T cells
Long lived clonally derived cells
Remain long after pathogen is gone
More easily activated than naïve T cells
Enable a more robust secondary response

Click on image to enlarge



From: Crit Rev Immunol. Author manuscript; available in PMC 2010 September 10.
Published in final edited form as:
Crit Rev Immunol. 2009; 29(6): 469–486.

- Activation sets in motion mechanisms to end the response: CTLA-4

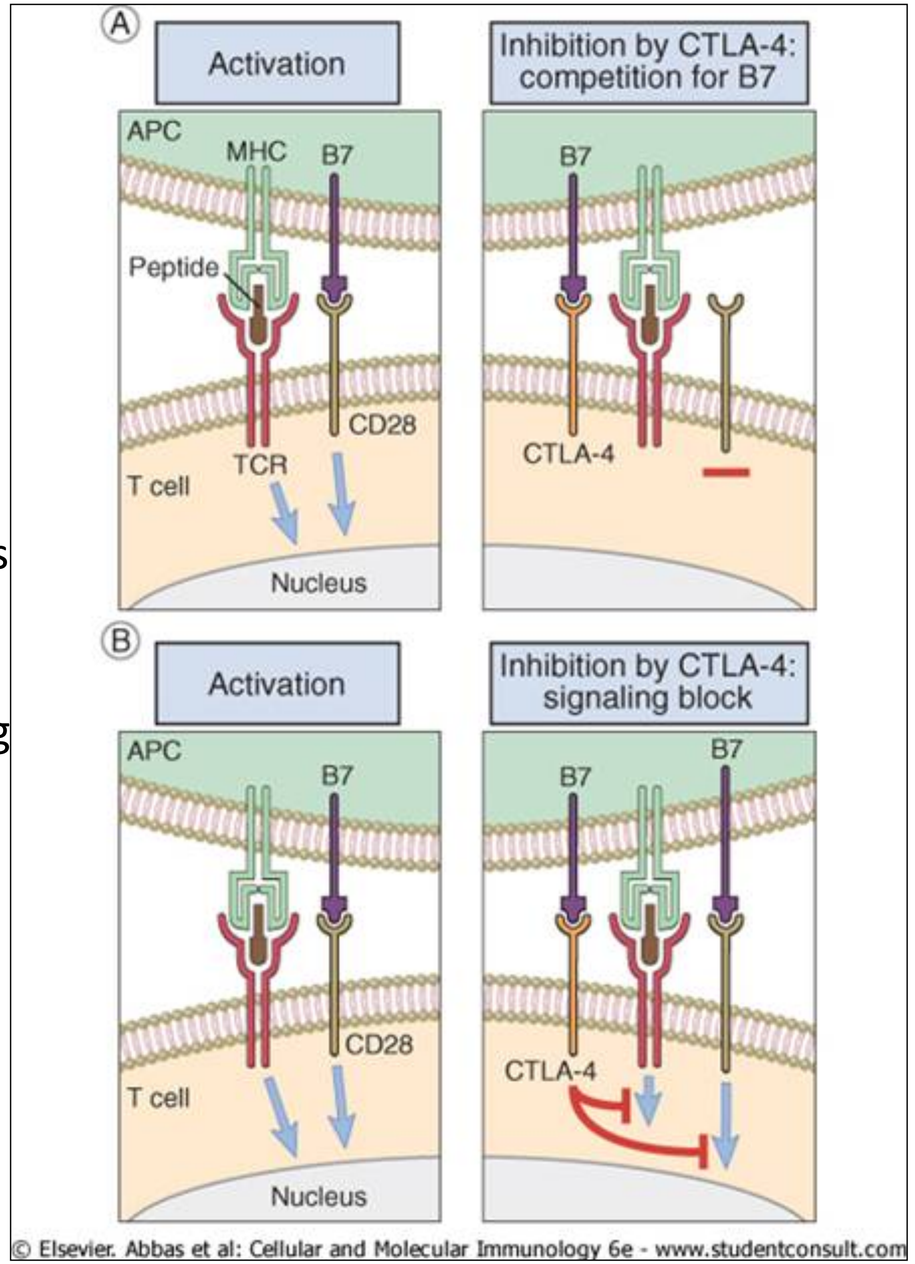
Engagement of CTLA-4 shuts down T cell activation

A) Once activated T cells begin to express CTLA-4 which competitively prevents CD28 binding to B7.

B) Following binding to B7, the **ITIM** motif of CTLA-4 associates with phosphatases resulting in **dephosphorylation** of the CD3 complex and subsequent inactivation of TCR-dependent signaling pathways.

This reduces T-cell receptor (TCR)-dependent activation of transcription factors NF-kB, AP-1 and NF-AT.

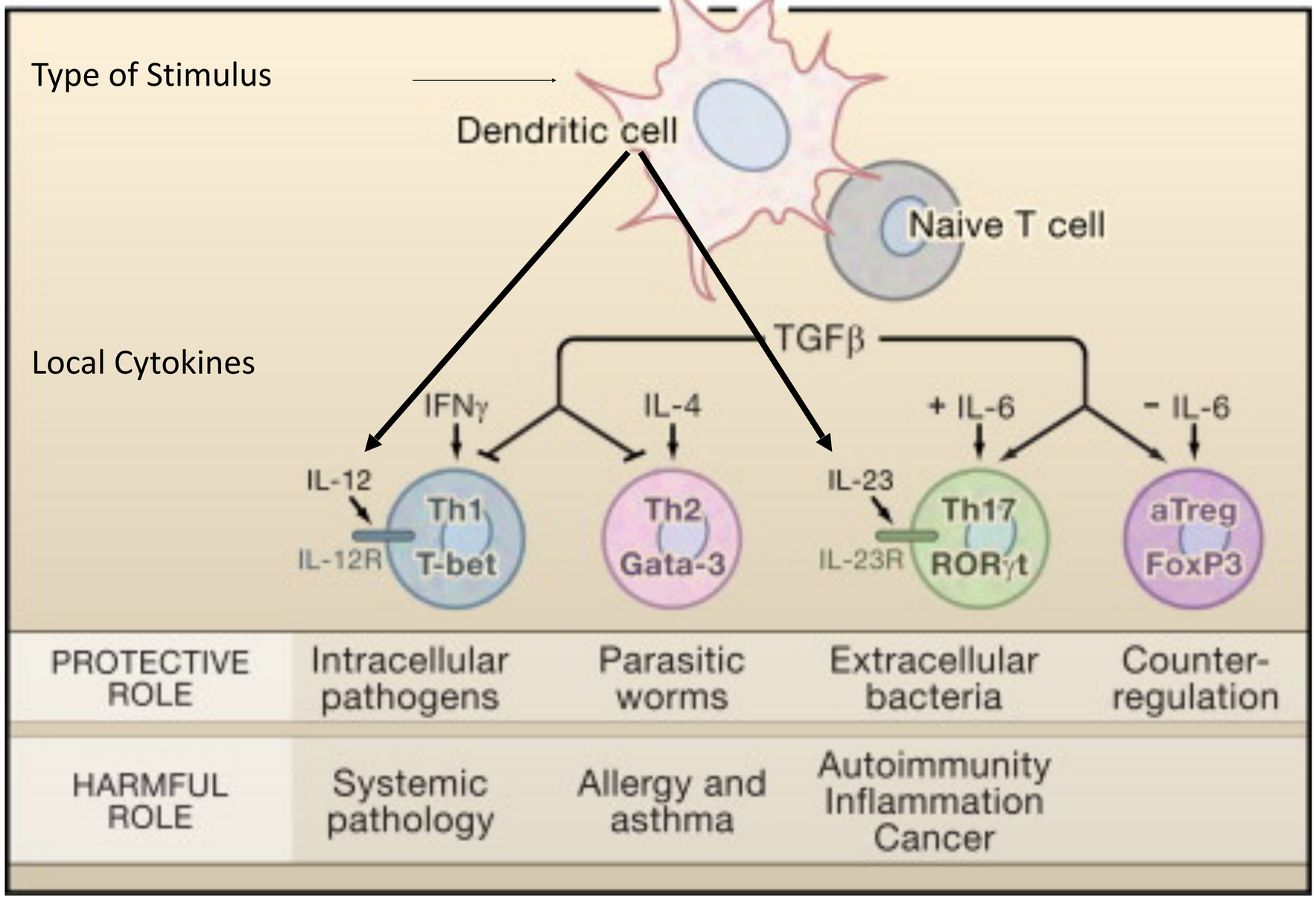
This results in decreased cytokine production by T cells and cell-cycle arrest.



Signal Three

Cytokines provide a Signal Three that prolongs survival (IL-12 and Type 1 interferons for CD8+ T cells, and IL-1 for CD4+ T cells) and determines differentiation

Differentiation to a particular T_H cell type is influenced by the DC type, innate immune sensing/response, and local cytokines (tissue type infected).



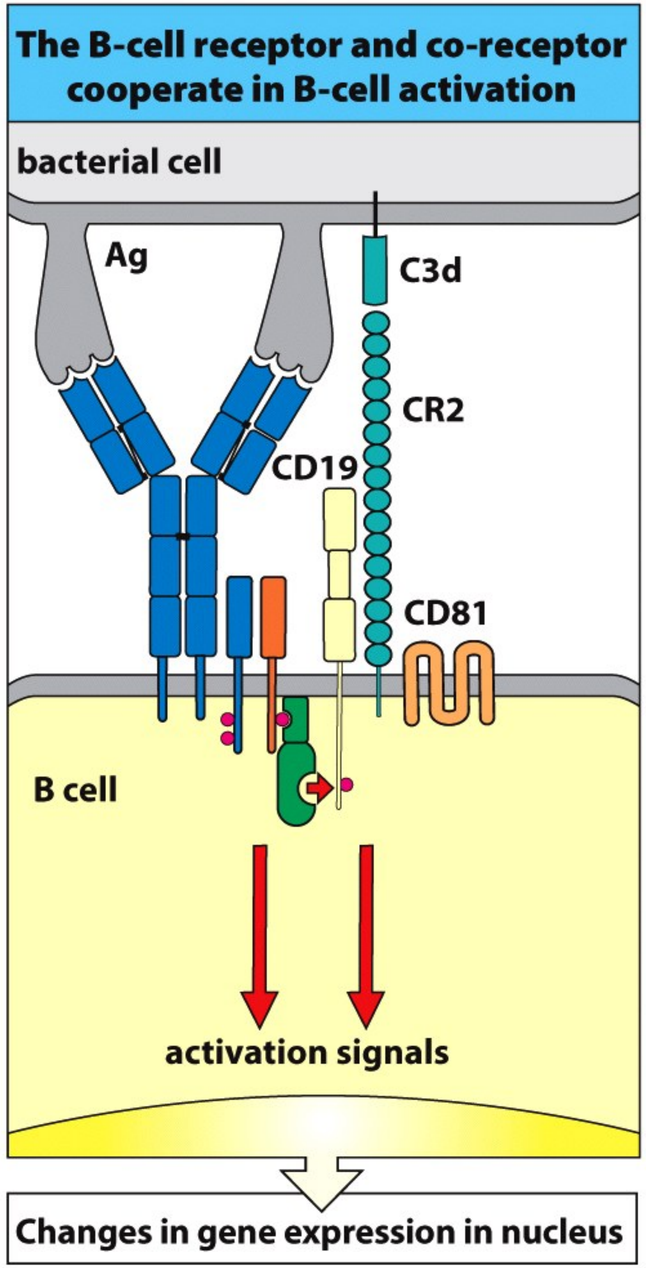


Figure 9.5 The Immune System, 3ed. (© Garland Science 2009)

- Simultaneous ligation of BCR and co-receptor increases activation from 1,000 to 10,000 fold over BCR alone
- So complement deposition of C3b onto pathogen surface greatly facilitates adaptive immune response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