We are all tired of immunology now

Week 4

Ed Roy, Marie Roy, Sue Ingels, Mary Kuetemeyer, Class Moderator

Questions from Last Week

- Where are the dogs?

Mabel’s Dream

- https://www.youtube.com/watch?v=6gor24MzXYs

Learning Objectives

Week 4

- Finish with resolution of innate immunity
- Brief overview of transcription and translation
- Intro to adaptive immunity

Innate and Adaptive

- Pattern recognition molecules of innate cells recognize conserved patterns
- A specific innate receptor has evolved over many generations
- Adaptive cells make no assumptions or predictions of what the pattern will be, but instead generate random diversity, and useful molecules will be selected by the pathogen within the lifetime of the individual
- You do not inherit a pattern-recognizing adaptive immune molecule, you inherit a mechanism for generating diversity in adapative immune cells

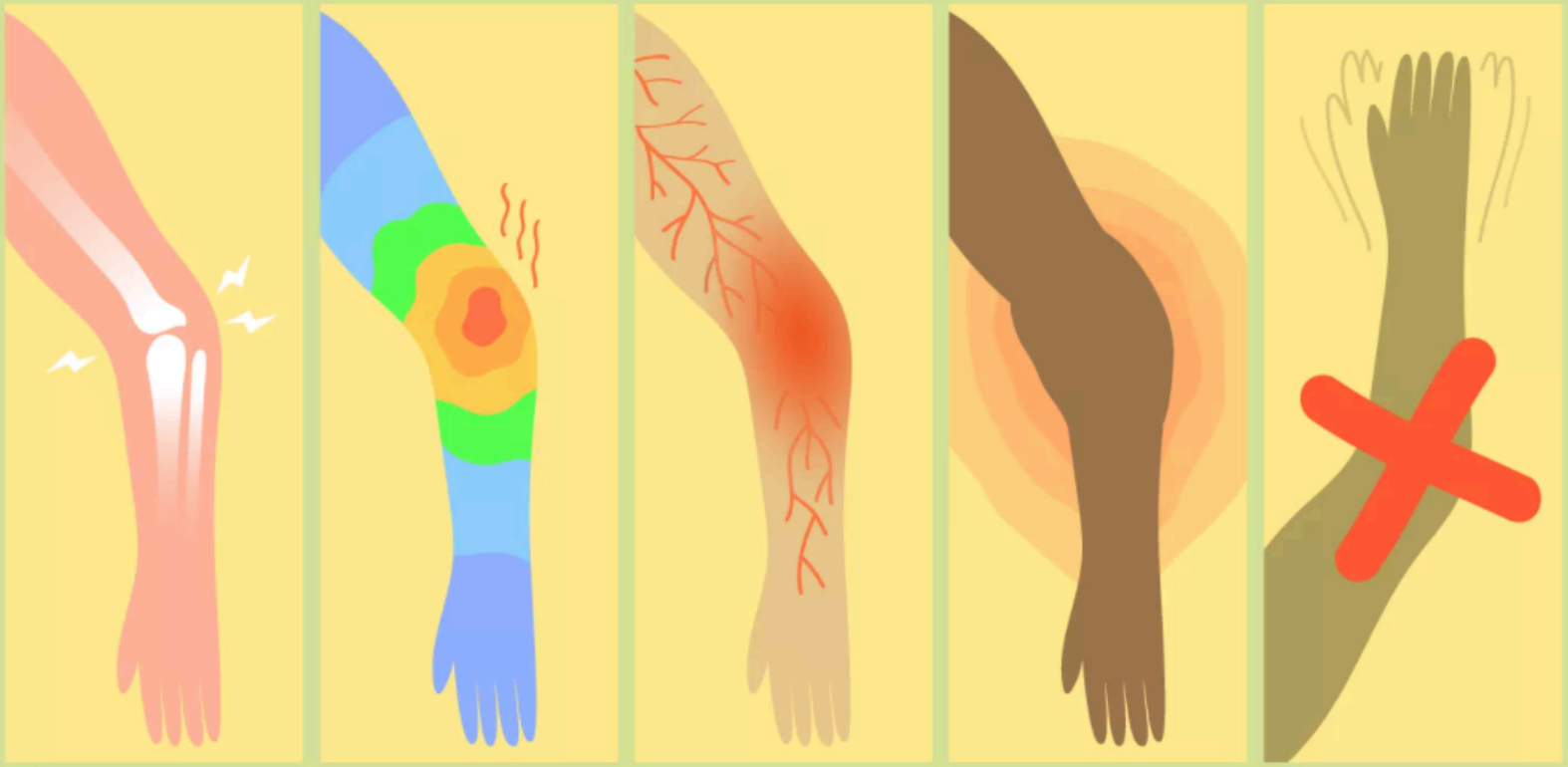
Two Components of Immune System, Innate and Adaptive

- Highly interdependent, common effector mechanisms for destroying pathogens
- Innate
  - Pathogen recognition and removal components that have evolved against conserved aspects of pathogens; germline encoded
  - Ready immediately; some cells in tissues
  - Doesn't change much with second exposure to pathogen

Inflammation key aspect of Innate System

- After recognition of pathogen by macrophages and dendritic cells, cytokines make vasculature leaky
- Cytokines call in more innate cells like neutrophils and macrophages, which phagocytose and destroy pathogens
- Five cardinal signs of inflammation

5 Cardinal Signs of Inflammation



Pain | Heat | Redness | Swelling | Loss of Function

verywell

Resolution of inflammation is active process

- Resolvins
- Negative feedback of stress responses
- Corticosteroids
- Parasympathetic feedback reflex

Anti-Inflammatory Reflex

- Parasympathetic nervous system
- Vagus nerve major pathway (afferent and efferent); senses cytokines
- Acetylcholine (ACh) is neurotransmitter
- Recent work showing the vagal ACh is anti-inflammatory

Cholinergic Receptors

- Nicotinic
  - Ion gated channel, fast response
  - Neuromuscular junction (somatic), vagal innervation of macrophages

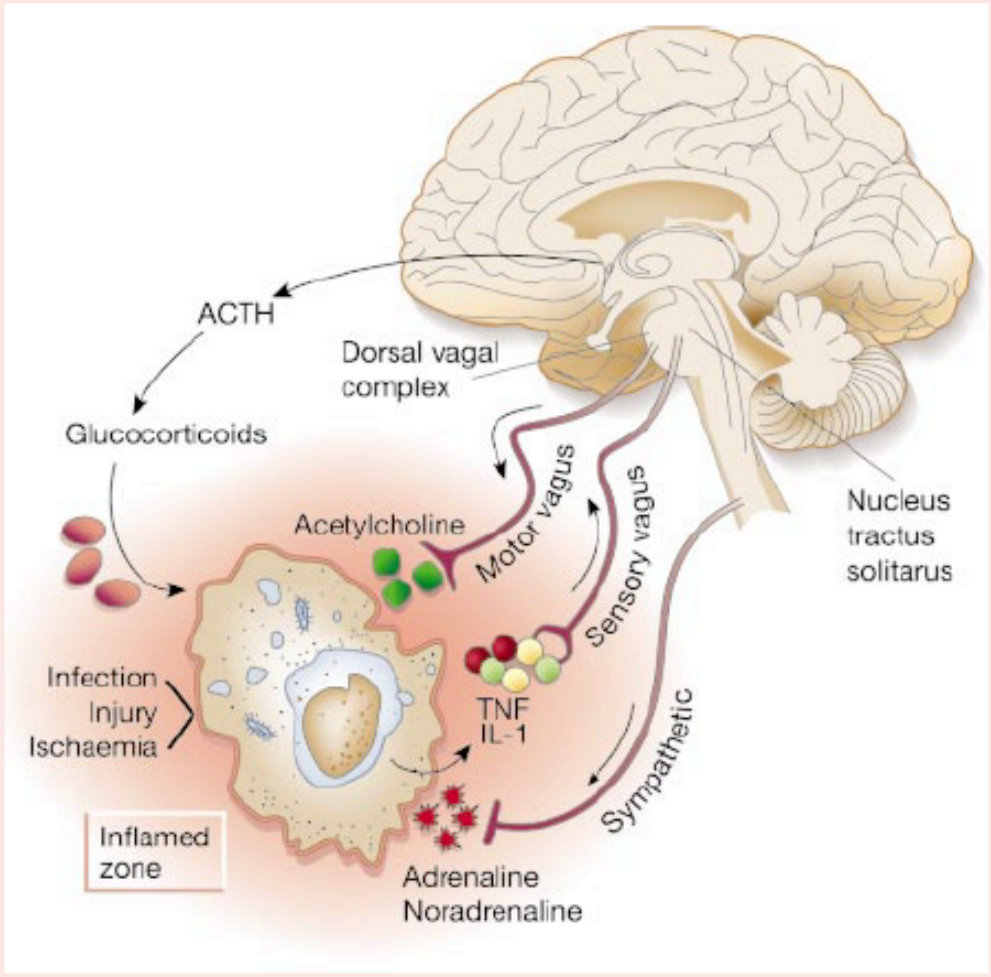


Figure 3 Wiring of the inflammatory reflex. Inflammatory products produced in damaged tissues activate afferent signals that are relayed to the nucleus tractus solitarius; subsequent activation of vagus efferent activity inhibits cytokine synthesis through the cholinergic anti-inflammatory pathway ('the inflammatory reflex').

Parasympathetic Nervous System

- Vagus nerve major pathway (afferent and efferent)
- Acetylcholine is postganglionic neurotransmitter
- Recent work showing the vagal ACh is anti-inflammatory
- Part of “anti-inflammatory reflex”

Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin

Lyudmila V. Borovikova*, Svetlana Ivanova*, Minghuang Zhang*, Huan Yang*, Galina I. Botchkina*, Linda R. Watkins†, Haichao Wang‡, Naji Abumrad§, John W. Eaton¶ & Kevin J. Tracey§

Nature 2000

Transcutaneous vagus nerve stimulation reduces serum high mobility group box 1 levels and improves survival in murine sepsis*

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Critical Care Med, 2007

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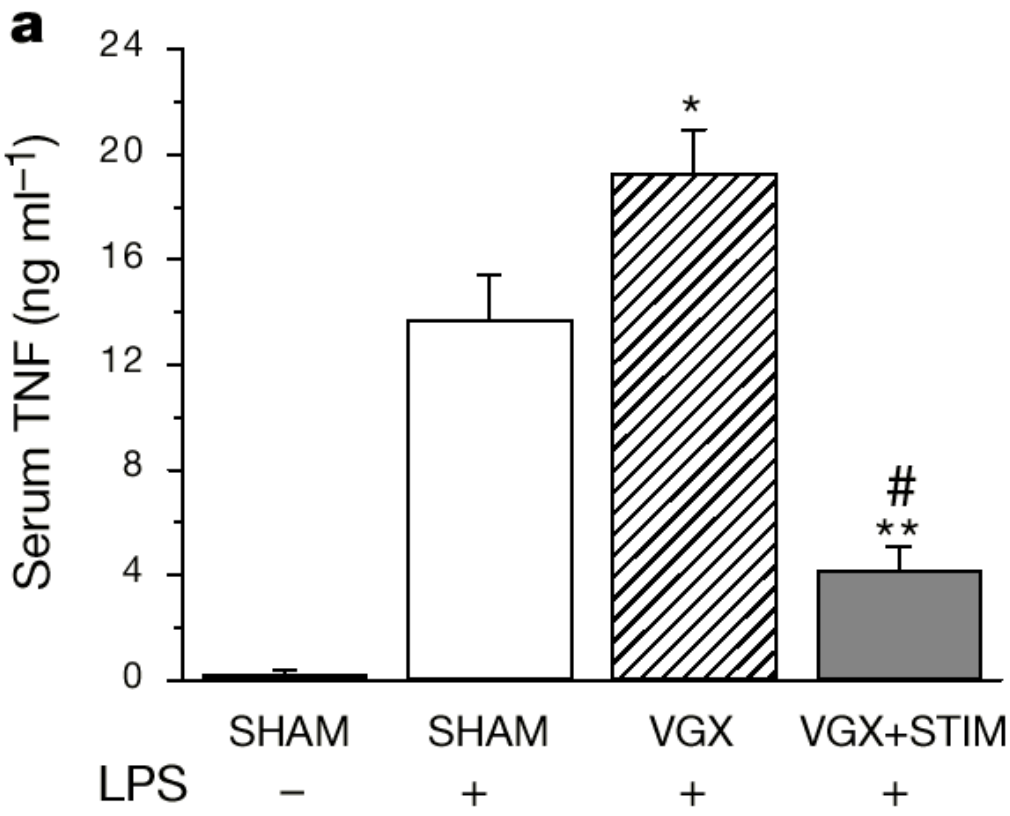

a
Serum TNF (ng ml−1)
24
20
16
12
8
4
0
*
#
**
SHAM
SHAM
VGX
VGX+STIM
LPS
–
+
+
+

Brain, Behavior, & Immunity - Health

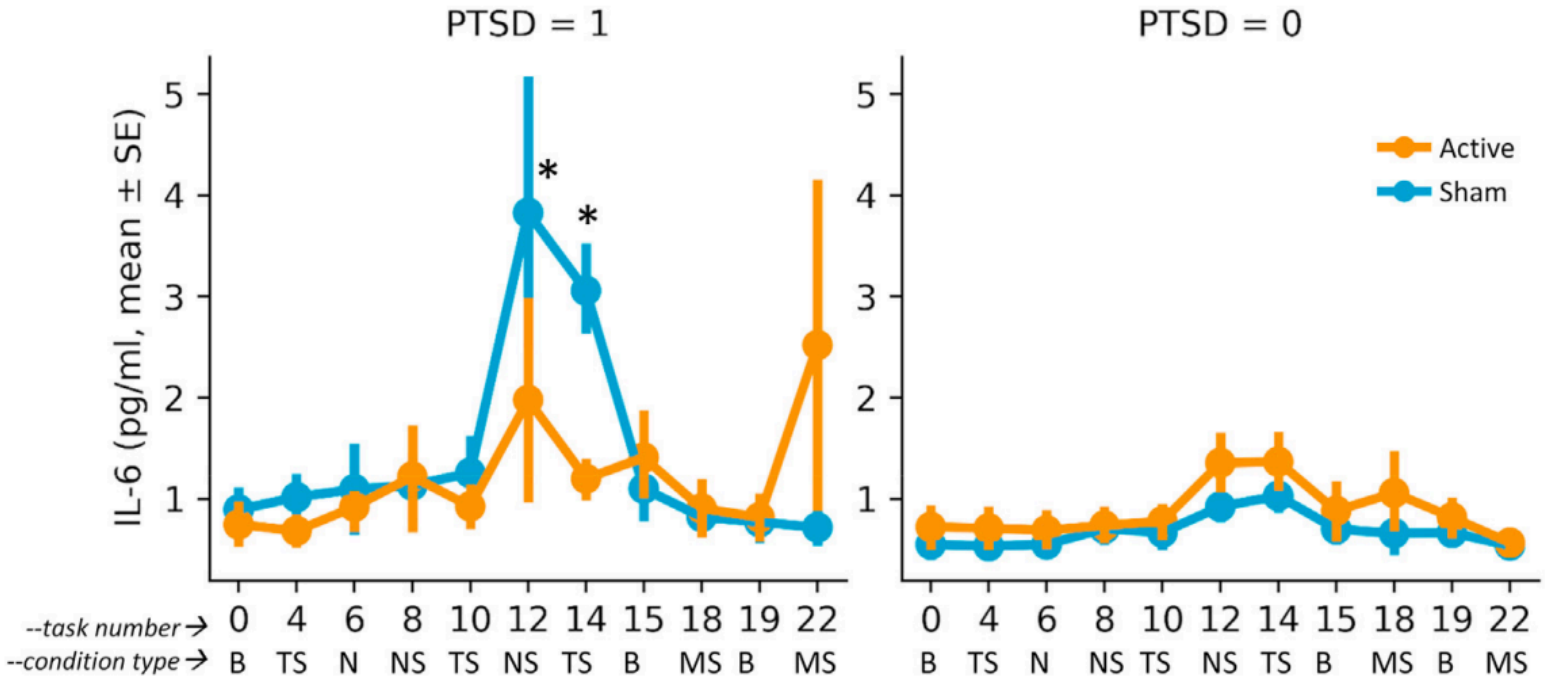
journal homepage: www.editorialmanager.com/bbih/default.aspx

Full Length Article

Transcutaneous vagal nerve stimulation blocks stress-induced activation of Interleukin-6 and interferon-γ in posttraumatic stress disorder: A double-blind, randomized, sham-controlled trial

J. Douglas Bremner [a,b,e,*], Nil Z. Gurel [f], Yunshen Jiao [h], Matthew T. Wittbrodt [a], Oleksiy M. Levantsevych [h], Minxuan Huang [h], Hewon Jung [f], MdMobashir H. Shandhi [f], Joy Beckwith [a], Isaias Herring [a], Mark H. Rapaport [a], Nancy Murrah [h], Emily Driggers [a,h], Yi-An Ko [i], MhmtJamil L. Alkhalaf [h], Majd Soudan [h], Jiawei Song [h], Benson S. Ku [a], Lucy Shallenberger [h], Allison N. Hankus [h], Jonathon A. Nye [b], Jeanie Park [d,e], Viola Vaccarino [c,h], Amit J. Shah [c,e,h], Omer T. Inan [f,g], Bradley D. Pearce [h]

[a] *Departments of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA*


PTSD = 1
PTSD = 0
IL-6 (pg/ml, mean ± SE)
1
2
3
4
5
*
*
Active
Sham
--task number →
--condition type →
0 4 6 8 10 12 14 15 18 19 22
B TS N NS TS NS TS B MS B MS

Adaptive Immune Response

- B cells and T cells (humoral and cellular)
- Requires time to become effective
- Constantly generating random diversity by gene rearrangements
- Effective cells proliferate in response to pathogens
- Retains memory of pathogen
- Second exposure response is faster and stronger

Lymphocytes: T cells and B cells

- Pieces of pathogen (antigens) are recognized by T cell Receptors (TCRs) and B cell receptors(BCRs)
  - BCRs are also called immunoglobulins, or antibodies when soluble (initially on the surface of B cells, later secreted into the blood and other fluids)

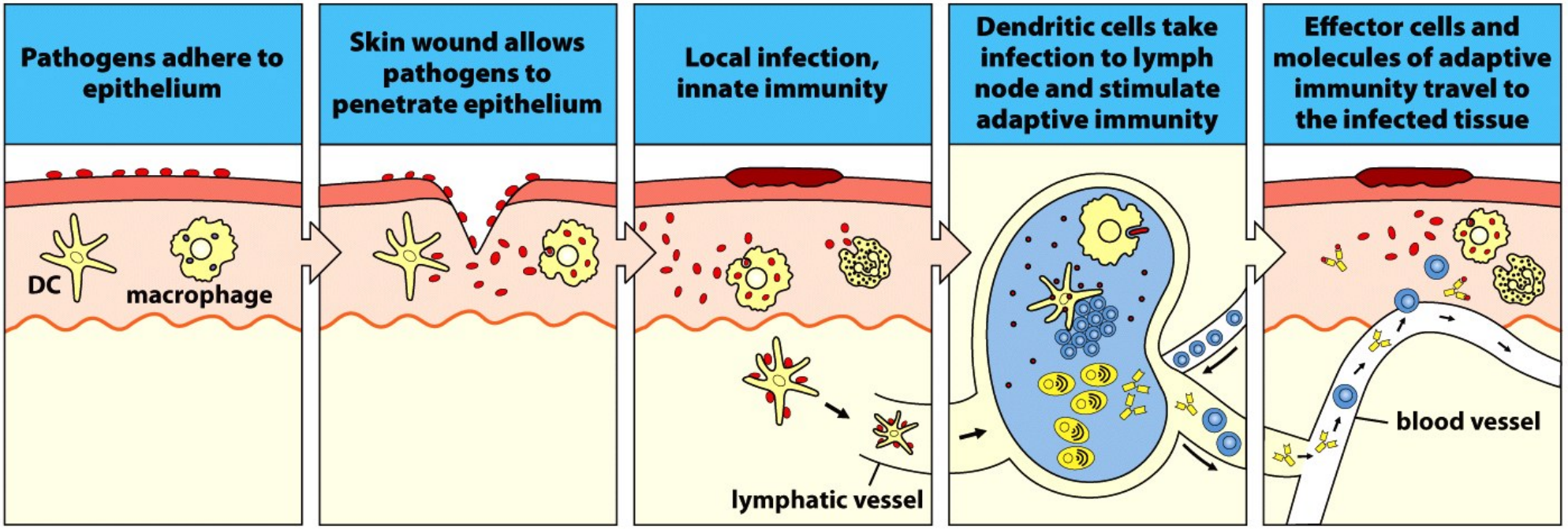


Figure 3.6 The Immune System, 3ed. (© Garland Science 2009)

Meanwhile back in the lymph node…..

Cells of the Innate System prompt selective expansion of particular lymphocytes of the adaptive system that recognize a piece of pathogen

This process is called Antigen Presentation

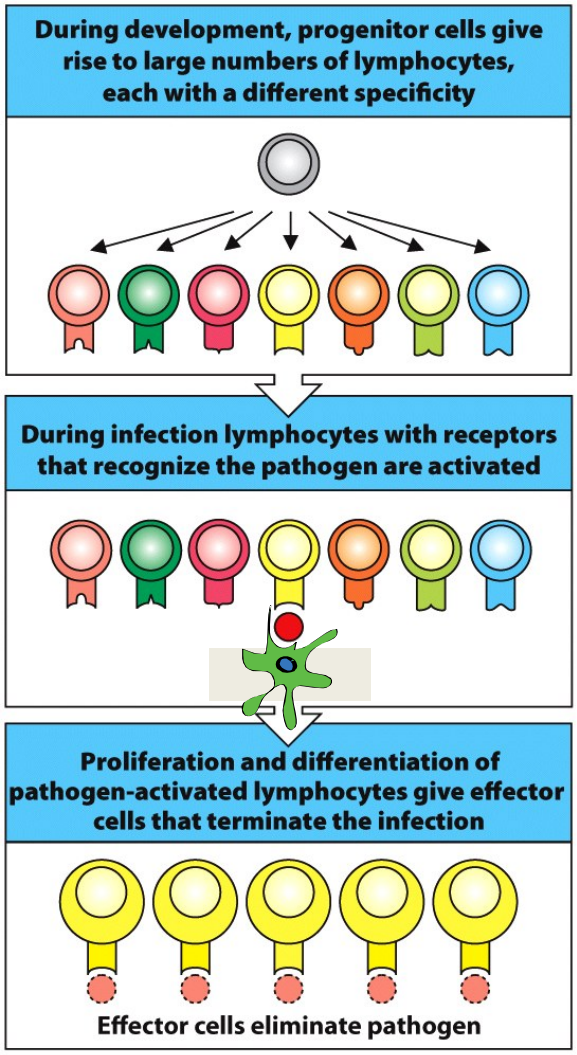
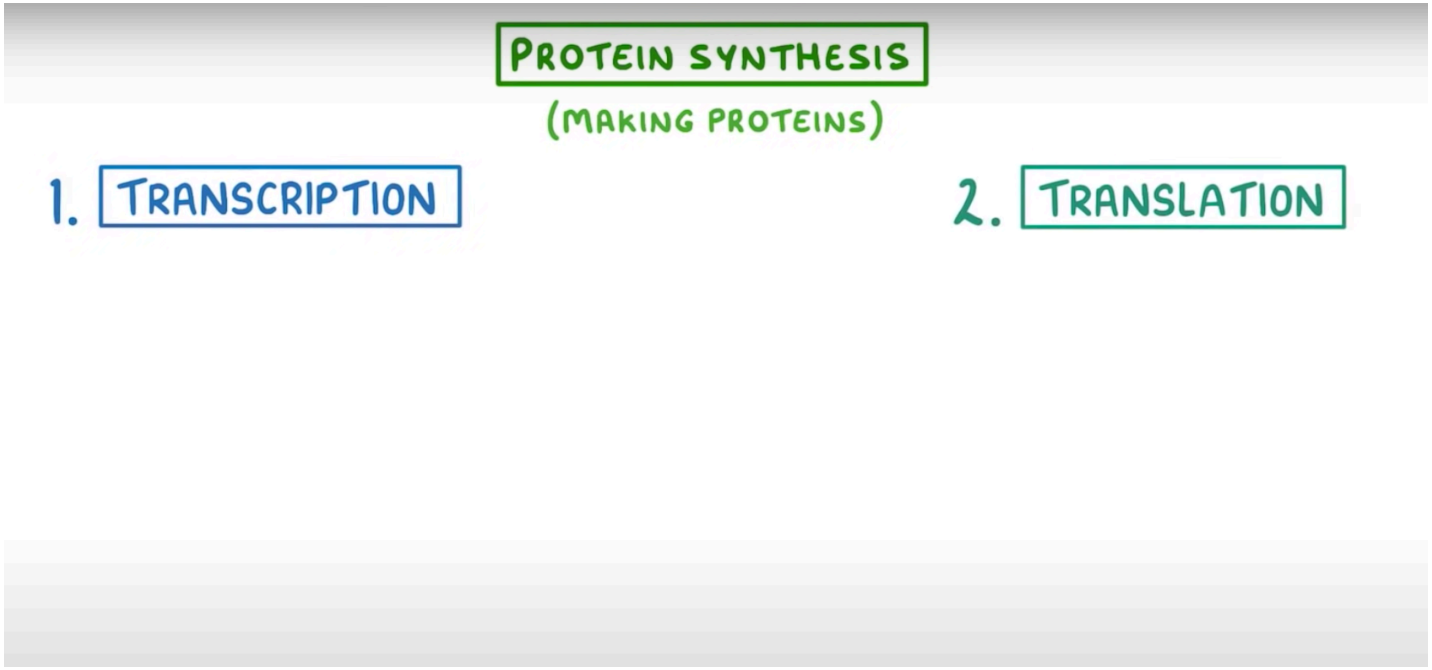


Figure 1.10 The Immune System, 3ed. (© Garland Science 2009)

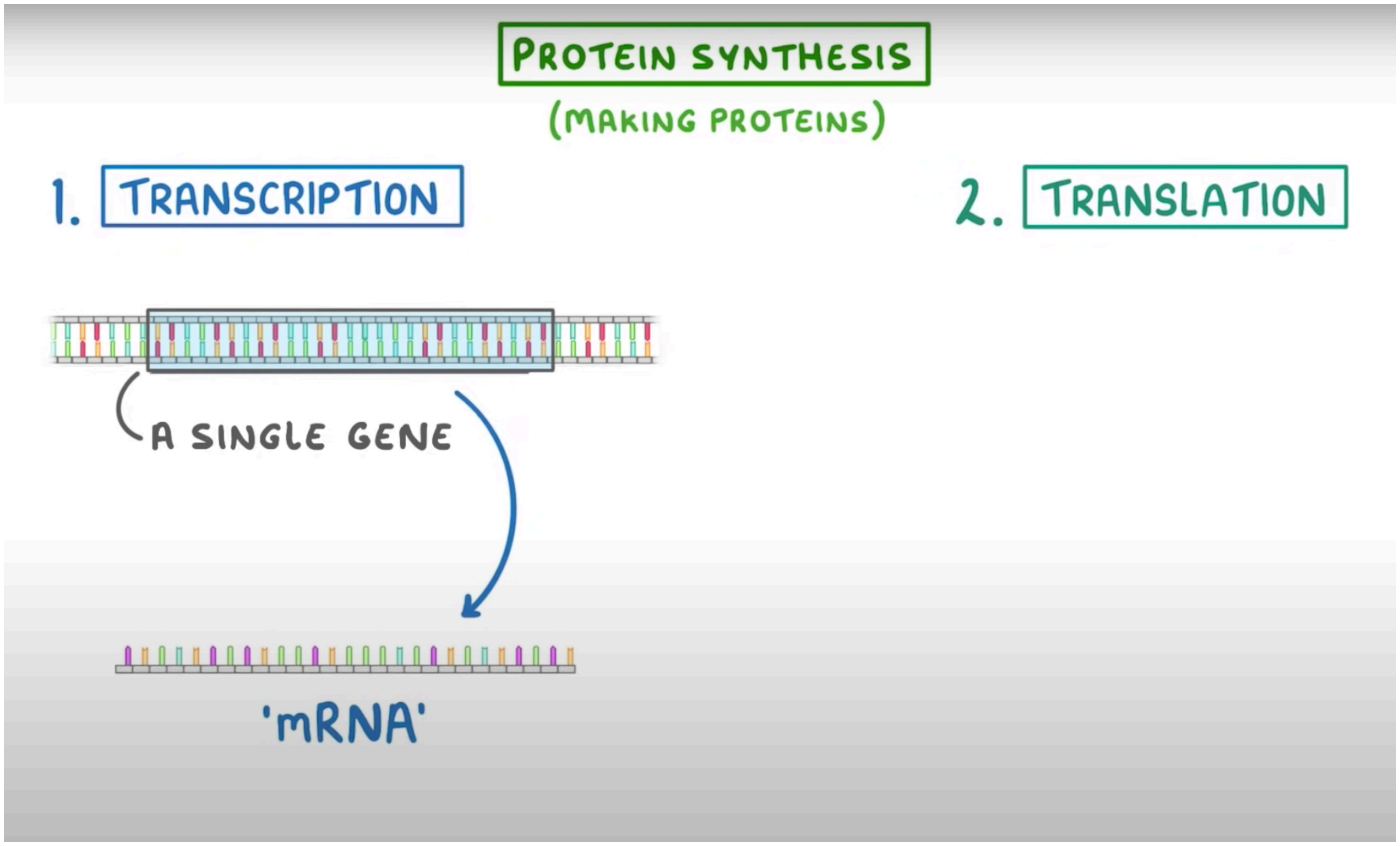
How is diversity of antibodies and T cell receptos generated? What does “translation” have to do with immunology?

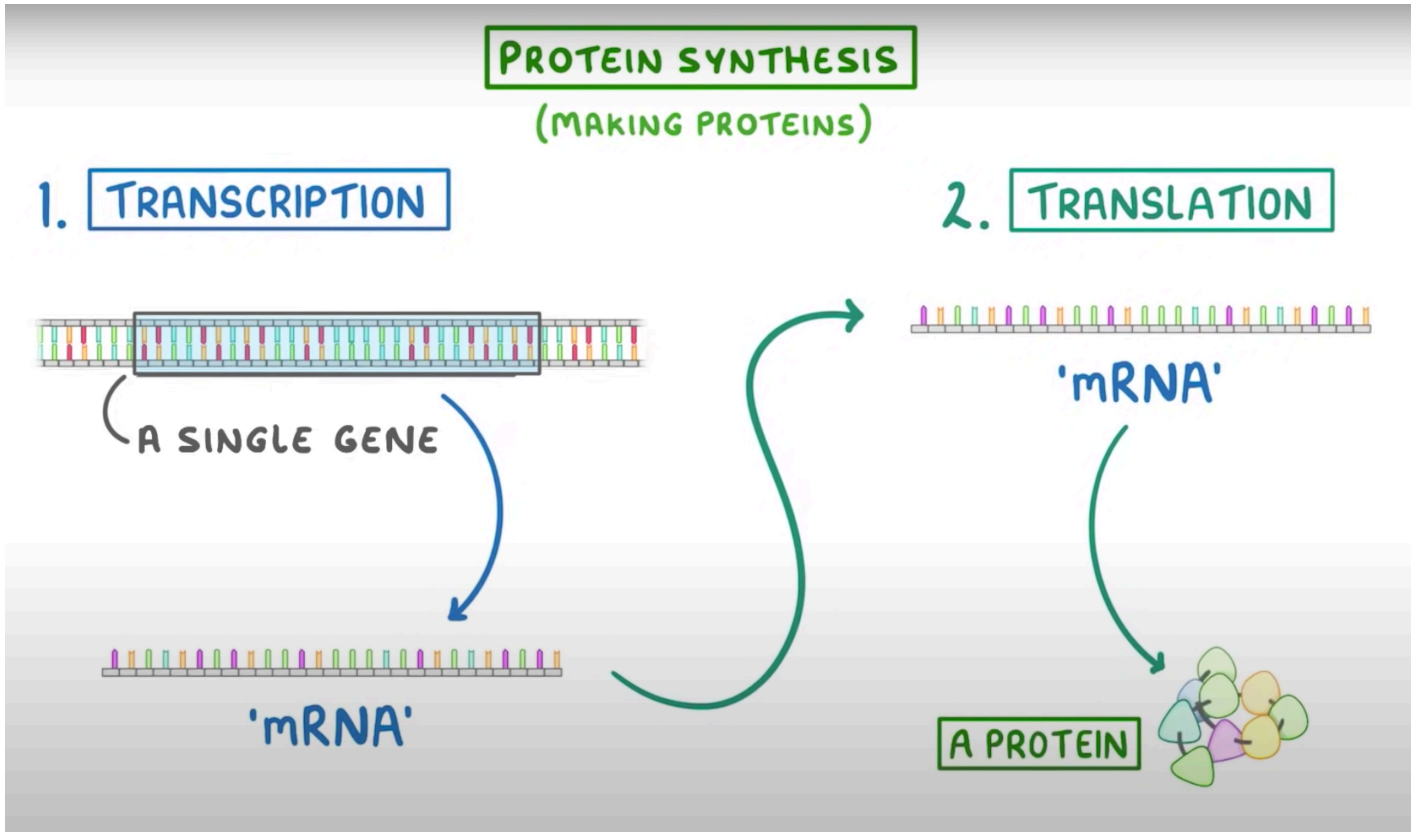
- Antibodies and T cell receptors have huge amount of diversity in amino acid sequences, to make sure they will recognize any novel pathogen
- B cells and T cells must create diversity in the DNA that encodes antibodies and T cell receptors
- This diversity in DNA sequence is created early in the development of B cells in the bone marrow and T cells in the Thymus
- The diversity is created only in the portion of the antibody that binds antigen
- Diversity in DNA is translated into diversity in protein (antibodies and T cell receptors)

Some Basic Biology of Protein Synthesis



- DNA
- mRNA
- Protein

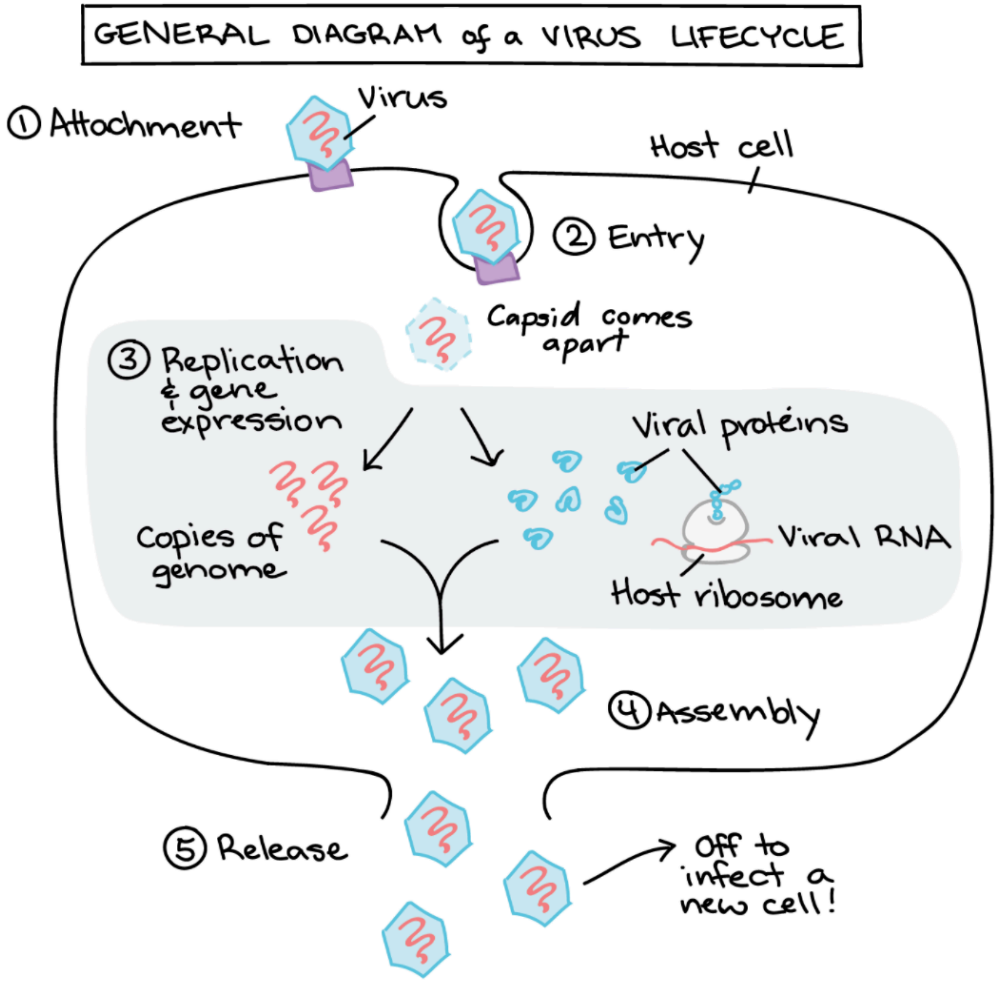



PROTEIN SYNTHESIS
(MAKING PROTEINS)
1. TRANSCRIPTION
2. TRANSLATION
A SINGLE GENE
'mRNA'
'mRNA'
A PROTEIN

Translation

mRNA to protein

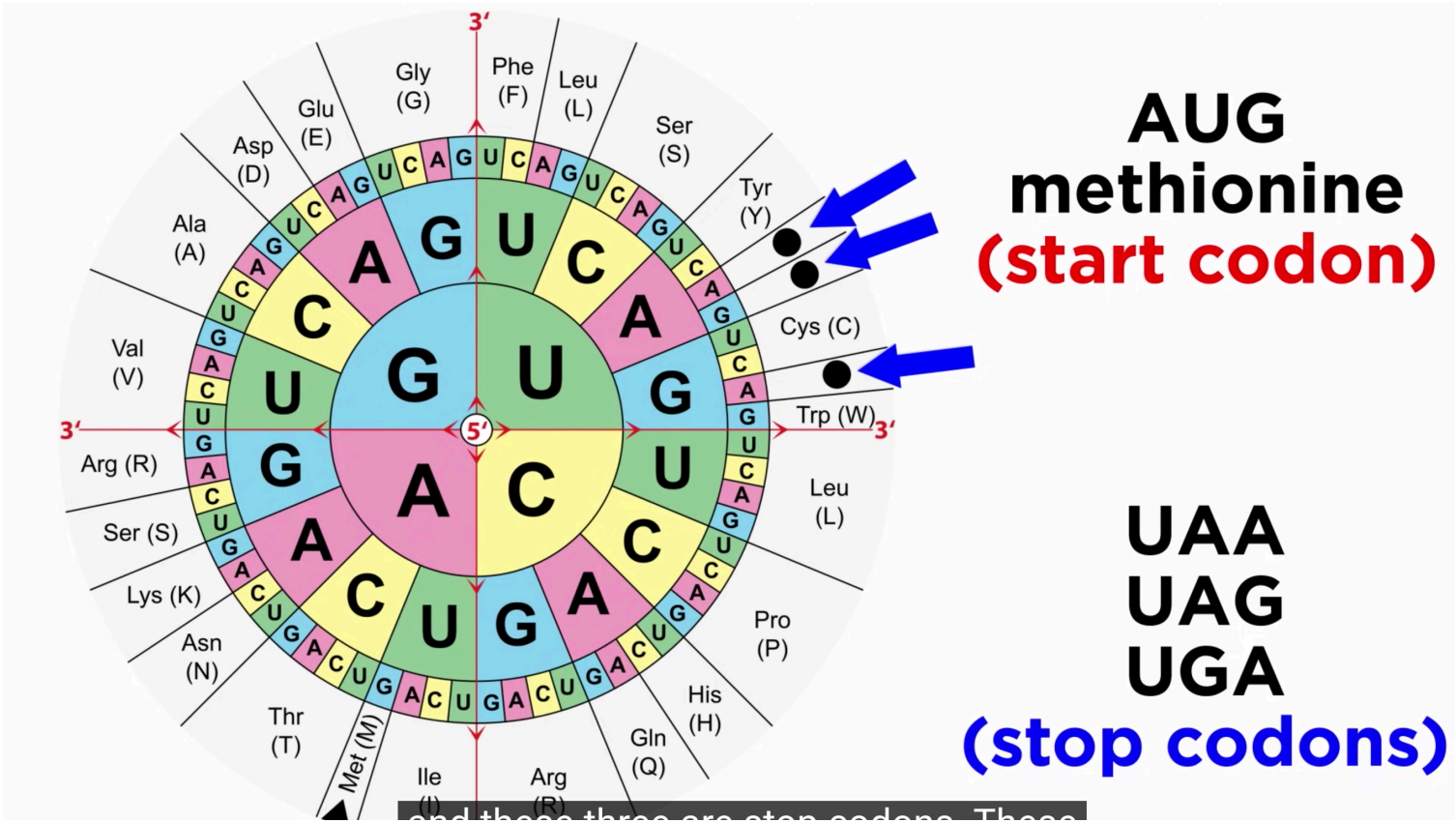
- https://www.youtube.com/watch?v=bKIpDtJdK8Q


GENERAL DIAGRAM of a VIRUS LIFECYCLE
① Attachment
Virus
Host cell
② Entry
Capsid comes apart
③ Replication & gene expression
Viral proteins
Copies of genome
Viral RNA
Host ribosome
④ Assembly
⑤ Release
Off to infect a new cell!

Anti-viral drugs from nucleoside analogs

Nucleoside is nucleotide without the phosphate

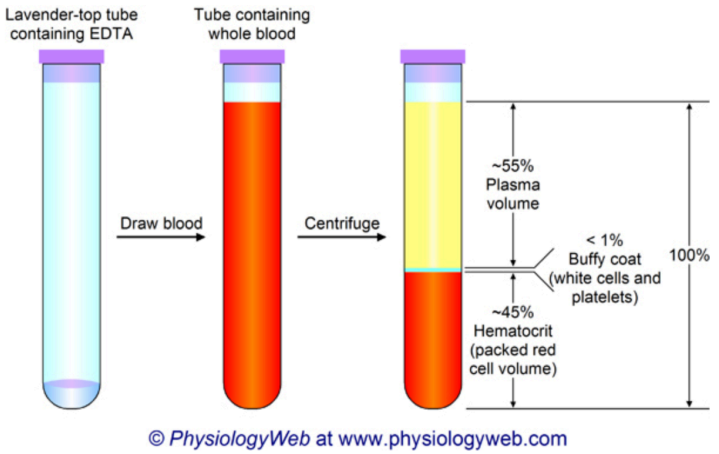
- Merck's molnupiravir, oral drug taken within 5 days of symptom occurence, prevented death and reduced hospitalization (8 deaths in control group)
- Monoclonal antibody treatment is also very effective, reducing hospitalization by 85%, but must be given i.v.
- Molnupiravir introduces modified nucleotides into viral RNA, creating mutations and disrupting replication of the virus; works with viral RNA polymerase but not human RNA polymerase


3′
Gly (G)
Phe (F)
Leu (L)
Ser (S)
Tyr (Y)
Cys (C)
Trp (W)
Leu (L)
Pro (P)
His (H)
Gln (Q)
Arg (R)
Ile (I)
Met (M)
Thr (T)
Asn (N)
Lys (K)
Ser (S)
Arg (R)
Val (V)
Ala (A)
Asp (D)
Glu (E)
5′
AUG
methionine
(start codon)
UAA
UAG
UGA
(stop codons)


CHEX
AGENT

Humoral Immunity and Cellular Immunity

- B cells secrete antibodies, which circulate in plasma of blood. It is called humoral immunity.
- T cells circulate as cells, therefore it is called cellular immunity or cell-mediated immunity.



ANTIGEN

Any molecule that binds to a T cell Receptor (TCR) or an antibody is an antigen. The specific region the antibody or TCR binds is an epitope.

Can be foreign proteins, altered self proteins, repetitive polymers of polysaccharides, glycolipids and nucleic acids.

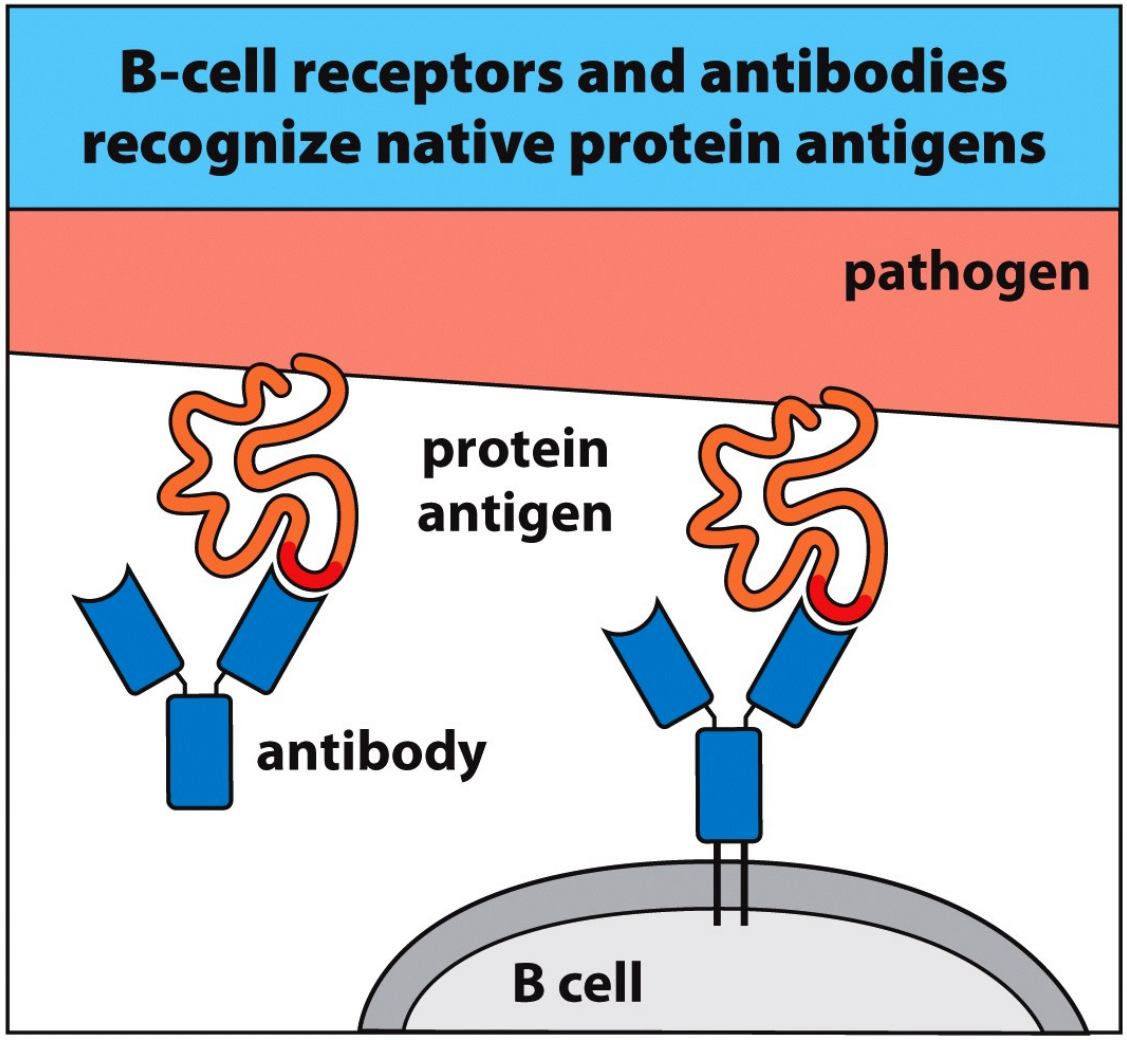
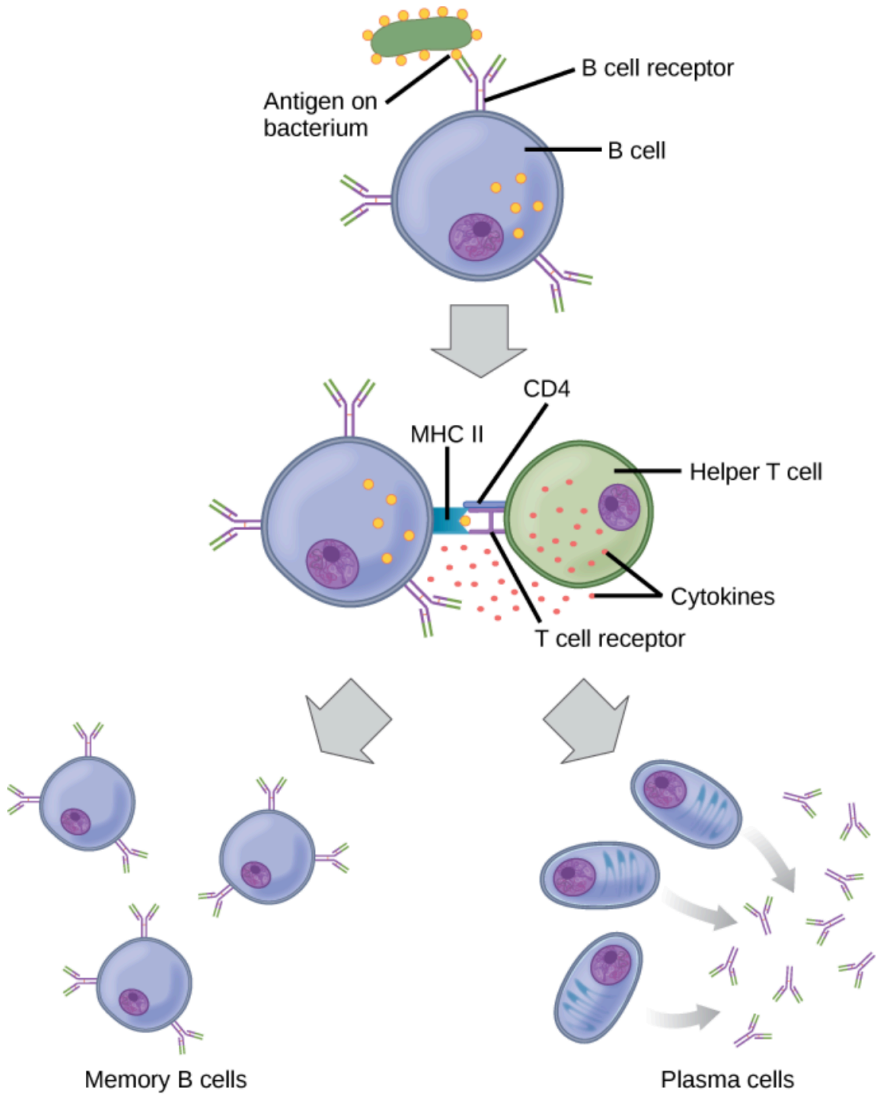


Figure 3.12 The Immune System, 3ed. (© Garland Science 2009)

- Each T cell or B cell has receptors that all recognize the same antigen
- Each TCR or antibody is slightly different than the TCR and antibodies of all other cells


Antigen on bacterium
B cell receptor
B cell
CD4
MHC II
Helper T cell
Cytokines
T cell receptor
Memory B cells
Plasma cells

Antibody (Ab) Diversity

- Extremely Specific
  - A single Ab binds a single epitope
- Antibodies can be targeted against anything
  - Protein, carbohydrate, nucleic acids, small molecules, drugs, etc
  - If protein, can be conformational or linear
- Antibody diversity is theoretically more than 10^{15} different specificities

TCR Diversity

- TCRs bind only peptides, 8 – 25 amino acids in length
- Recognize linear sequence, not conformation in native state of protein

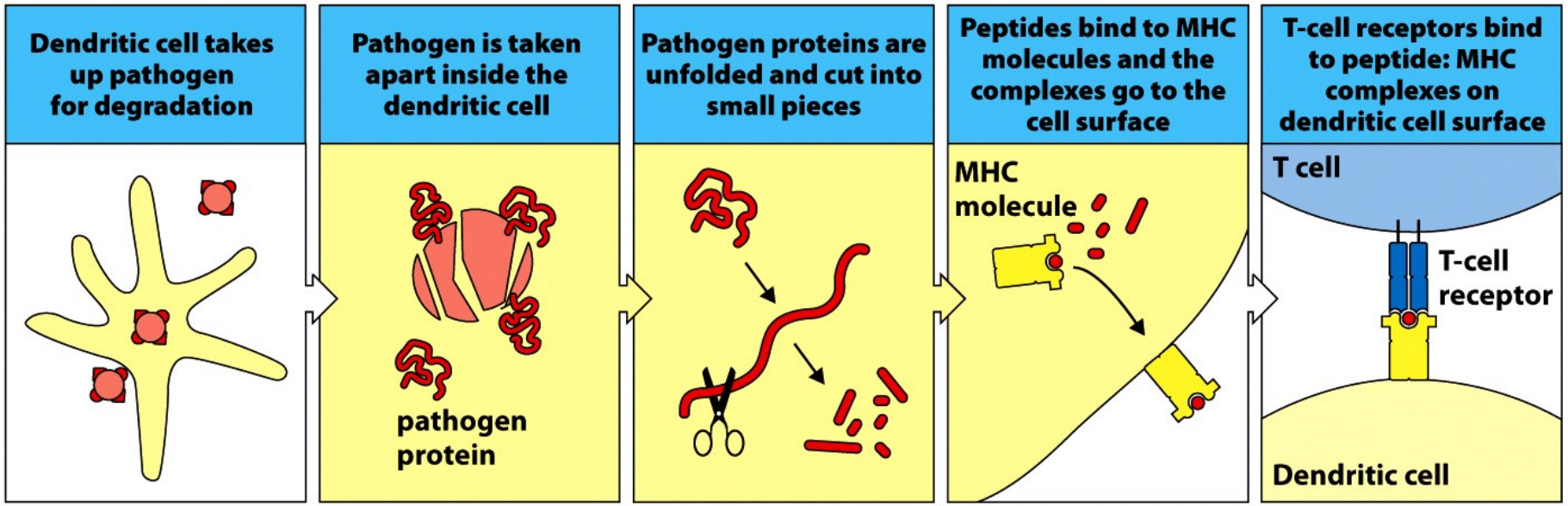


Figure 3.7 The Immune System, 3ed. (© Garland Science 2009)

Two Categories of T cells

- Helper T cells (CD4+)
  - Help macrophages
  - Help B cells become activated to secrete antibodies
  - Help cytotoxic T cells
- Cytotoxic T cells (CD8+)
  - Kill infected cells

Two major categories of molecules on antigen presenting cells that present antigen to TCRs

- MHC I to CD8+ T cells
- MHC II to CD4+ T cells
- MHCI is on all cells; MHCII is on professional antigen presenting cells like dendritic cells

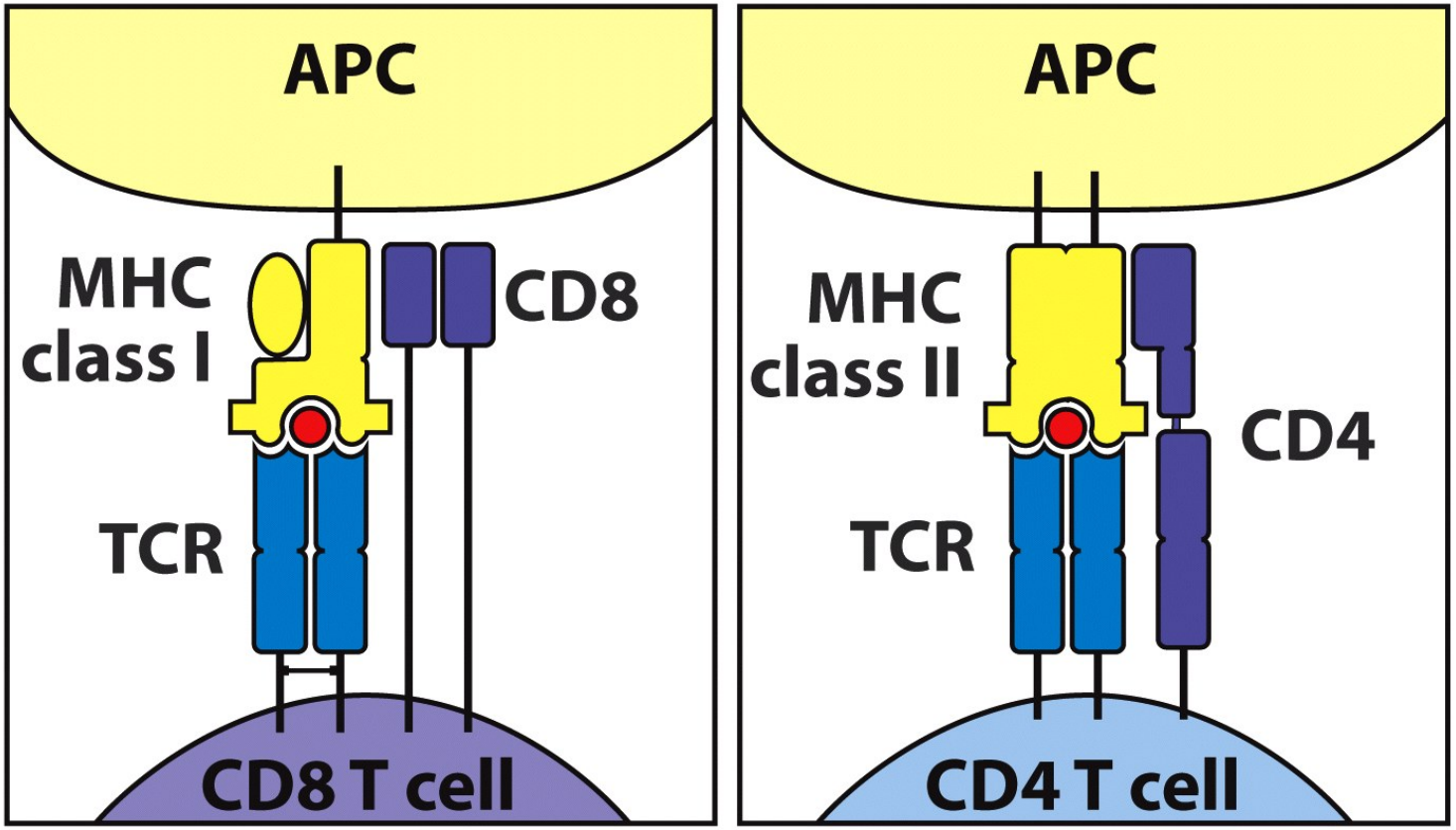


Figure 3.9 The Immune System, 3ed. (© Garland Science 2009)

A CD8+ Cytotoxic T cell Sees peptide/MHC three or four times

- During development in the Thymus
- When APC presents pathogen peptide to activate the T cell
- When Cytotoxic T cell encouters an infected cell and kills it
- Memory T cells proliferate rapidly upon re-exposure to pathogen

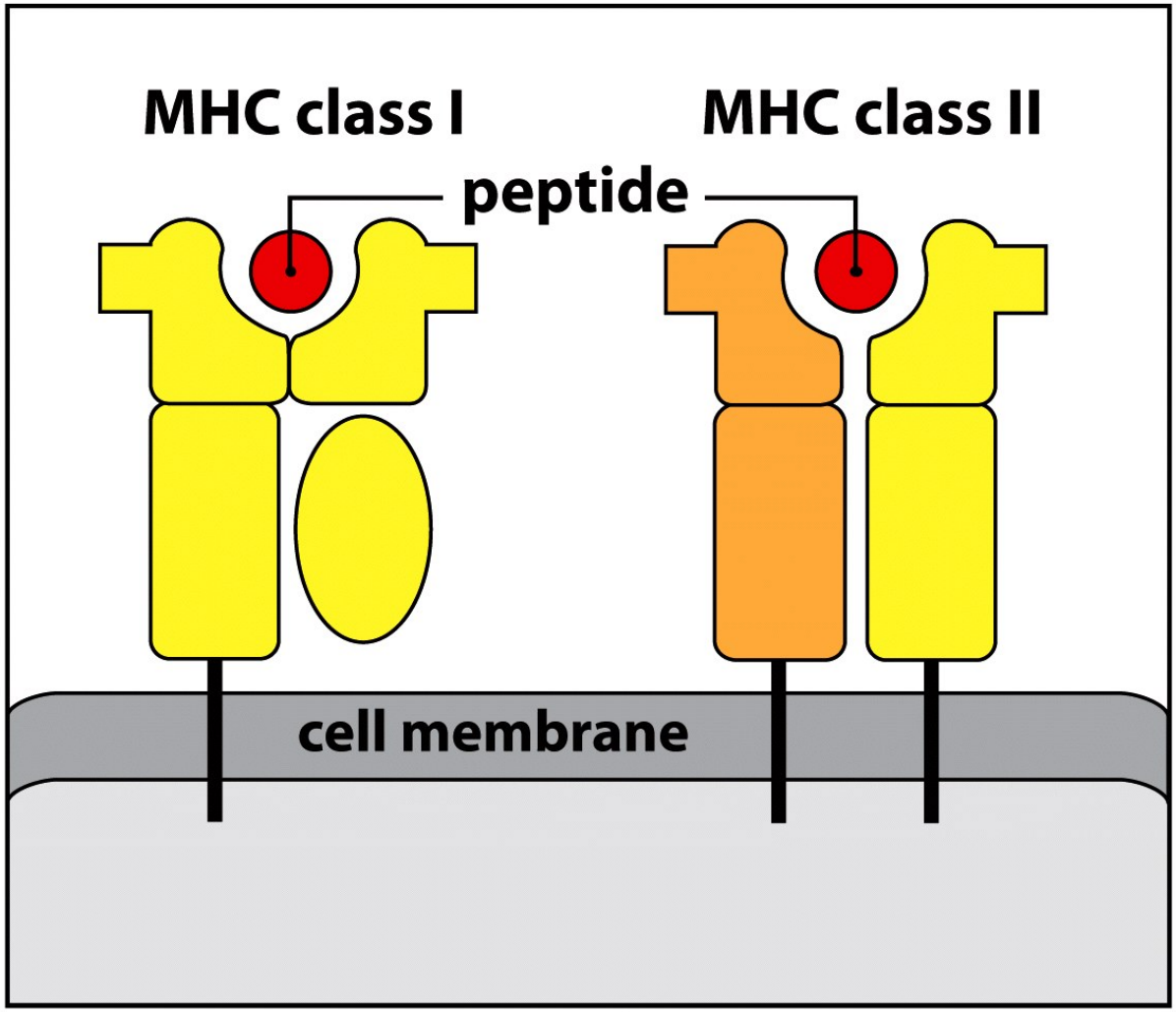


Figure 3.8 The Immune System, 3ed. (© Garland Science 2009)

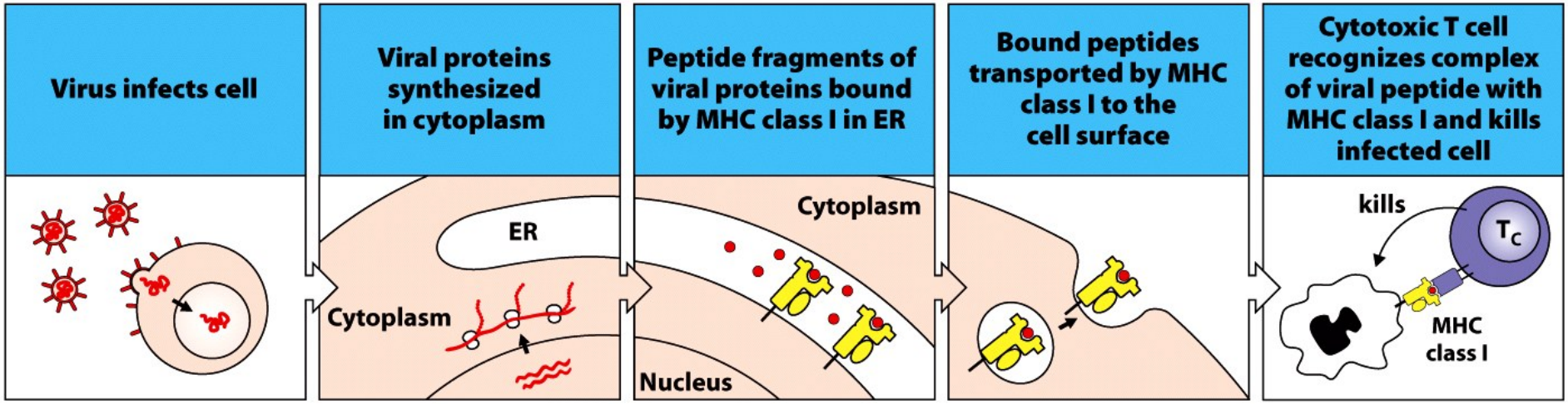
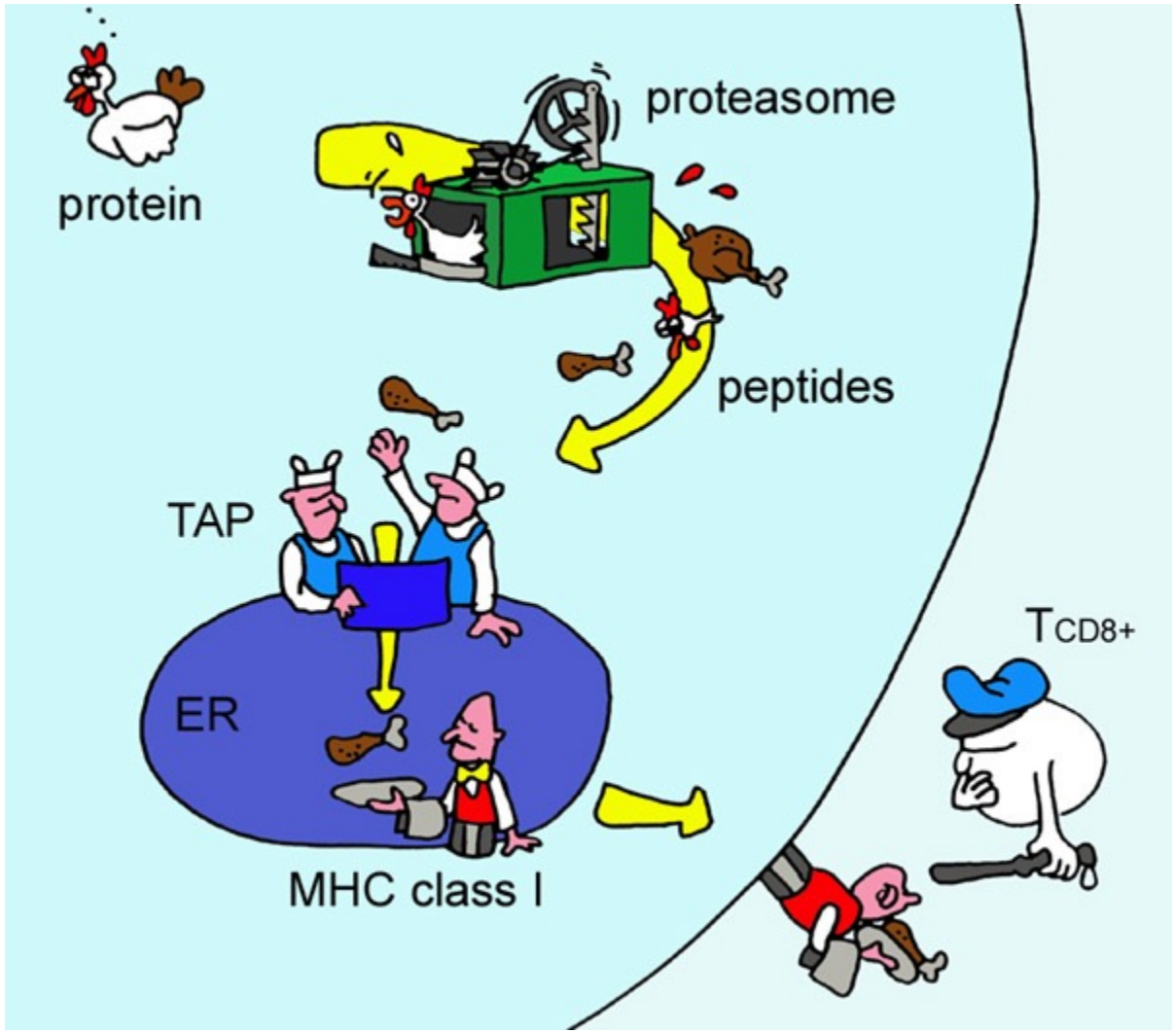


Figure 3.10 The Immune System, 3ed. (© Garland Science 2009)


protein
proteasome
peptides
TAP
ER
MHC class I
TCD8+

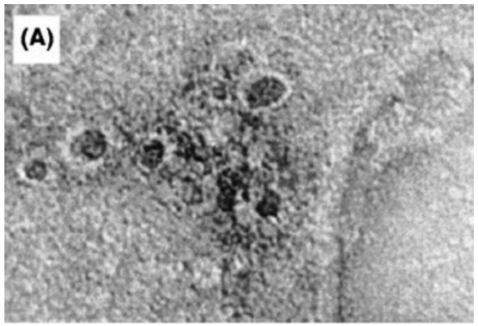

DUDE! FIND HIM YOURSELF

Cytotoxic T cells kill virus-infected cells and cancer cells

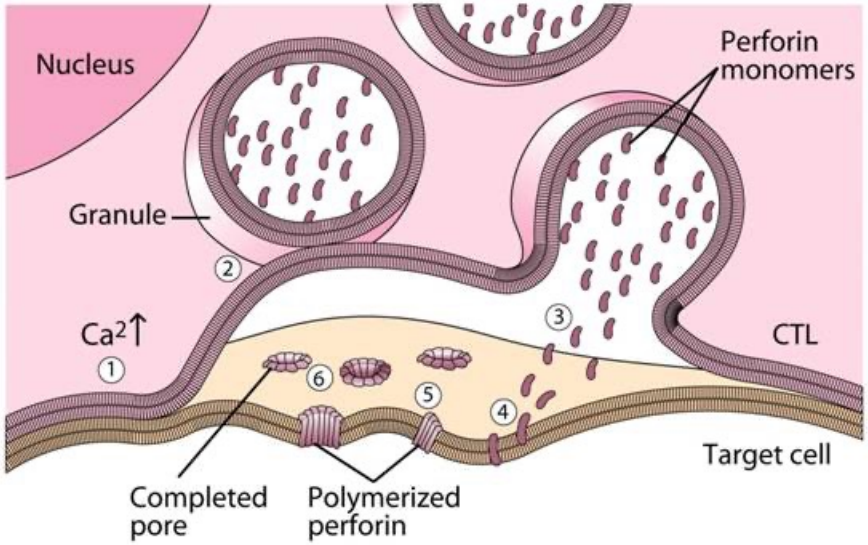
https://www.cbc.ca/news/health/serial-killers-attack-cancer-cell-in-video-1.3079500

Cytotoxic T cells' killing mechanism

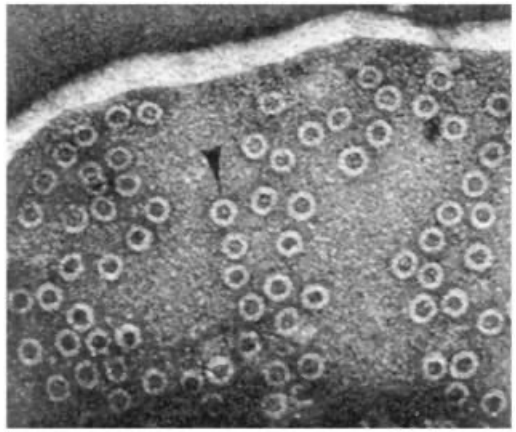
- Perforin pokes hole in membrane
- Granzyme B goes through holes
- and induces apoptosis (cell suicide)



CTL-mediated pore formation in the target cell membrane.



Delivery of Granzyme B which induces apoptosis



Helper T cell activates macrophage to continue phagocytosis

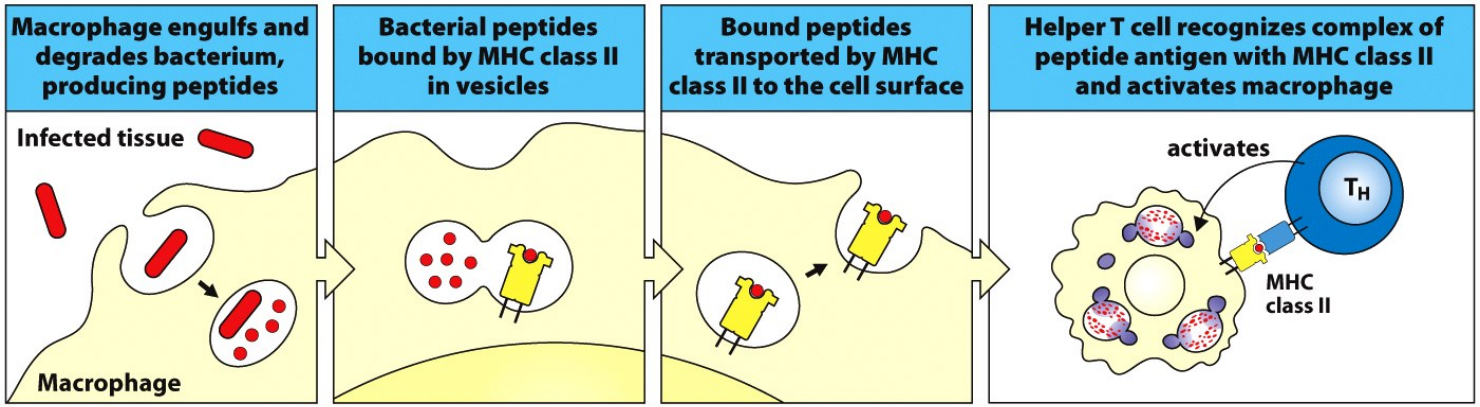
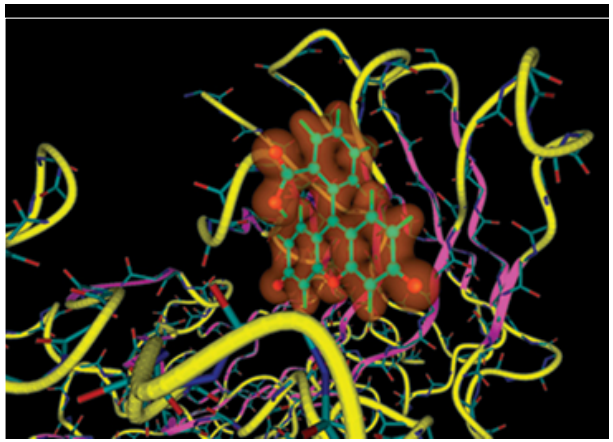
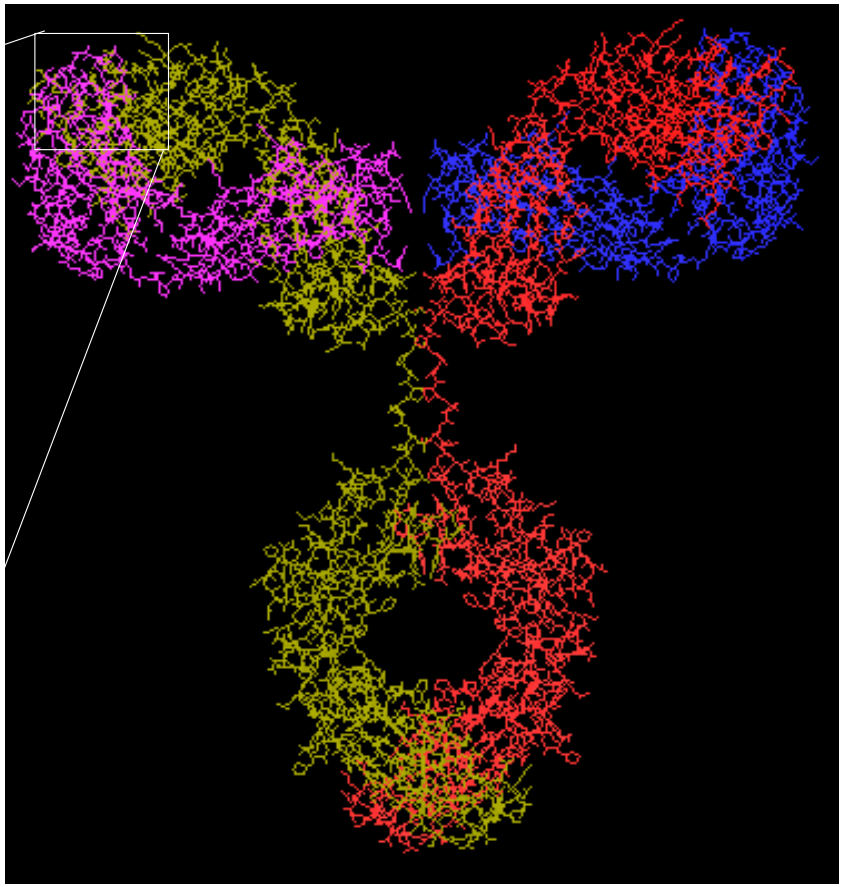


Figure 3.11 The Immune System, 3ed. (© Garland Science 2009)

Antibody Heavy and Light Chains together form site of Antigen Recognition





clonal deletion

tolerance

clonal selection

memory

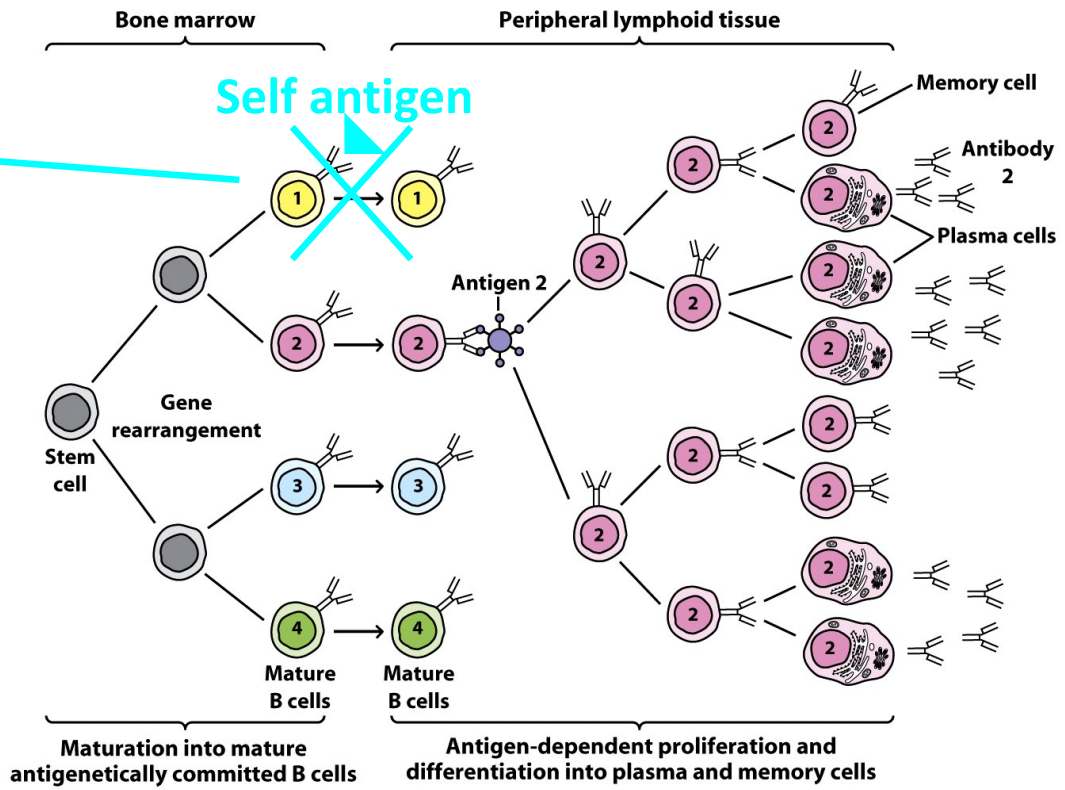


Figure 1-12
Kuby IMMUNOLOGY, Sixth Edition

Example showing B cells

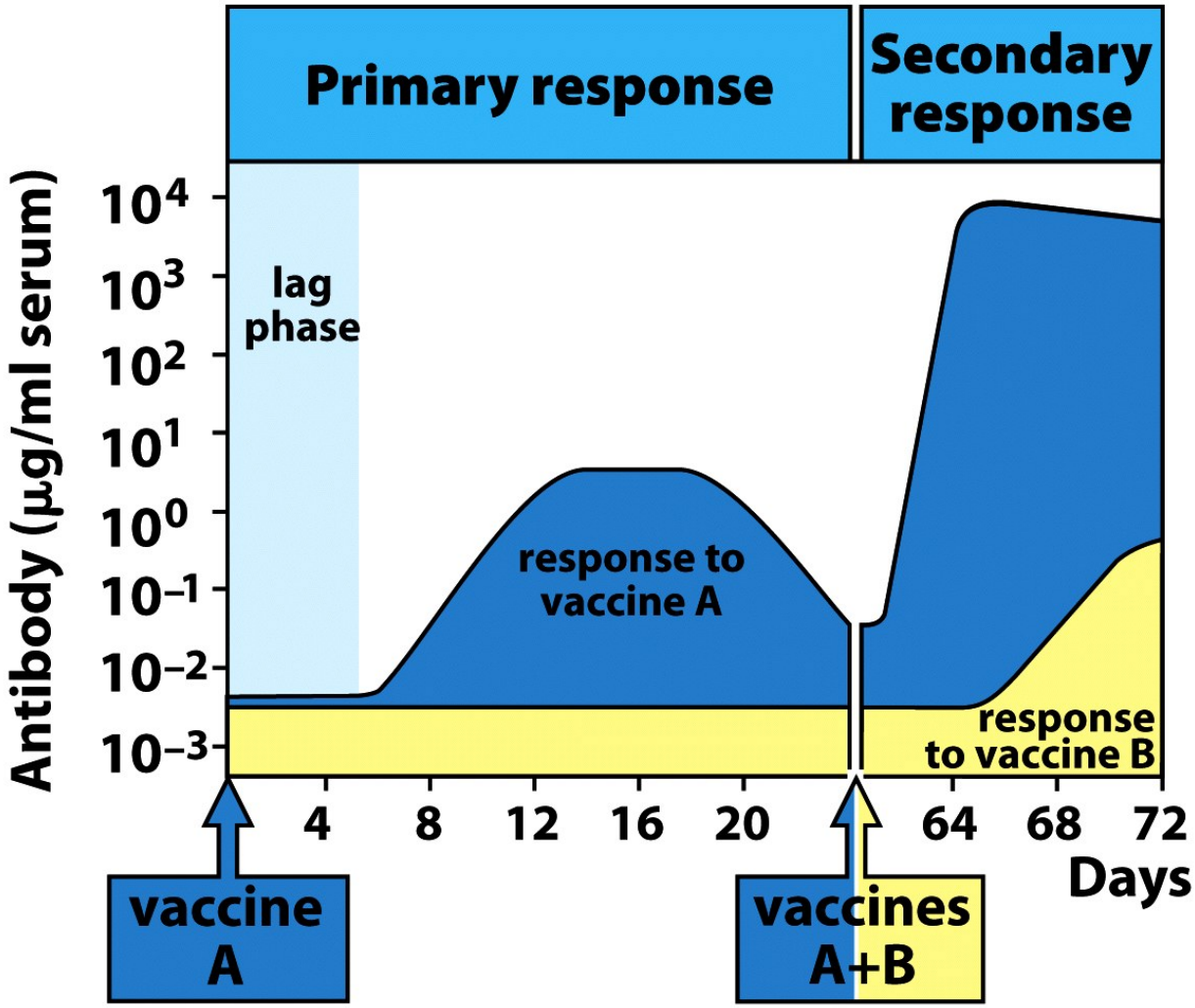


Figure 1.26 The Immune System, 3ed. (© Garland Science 2009)

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

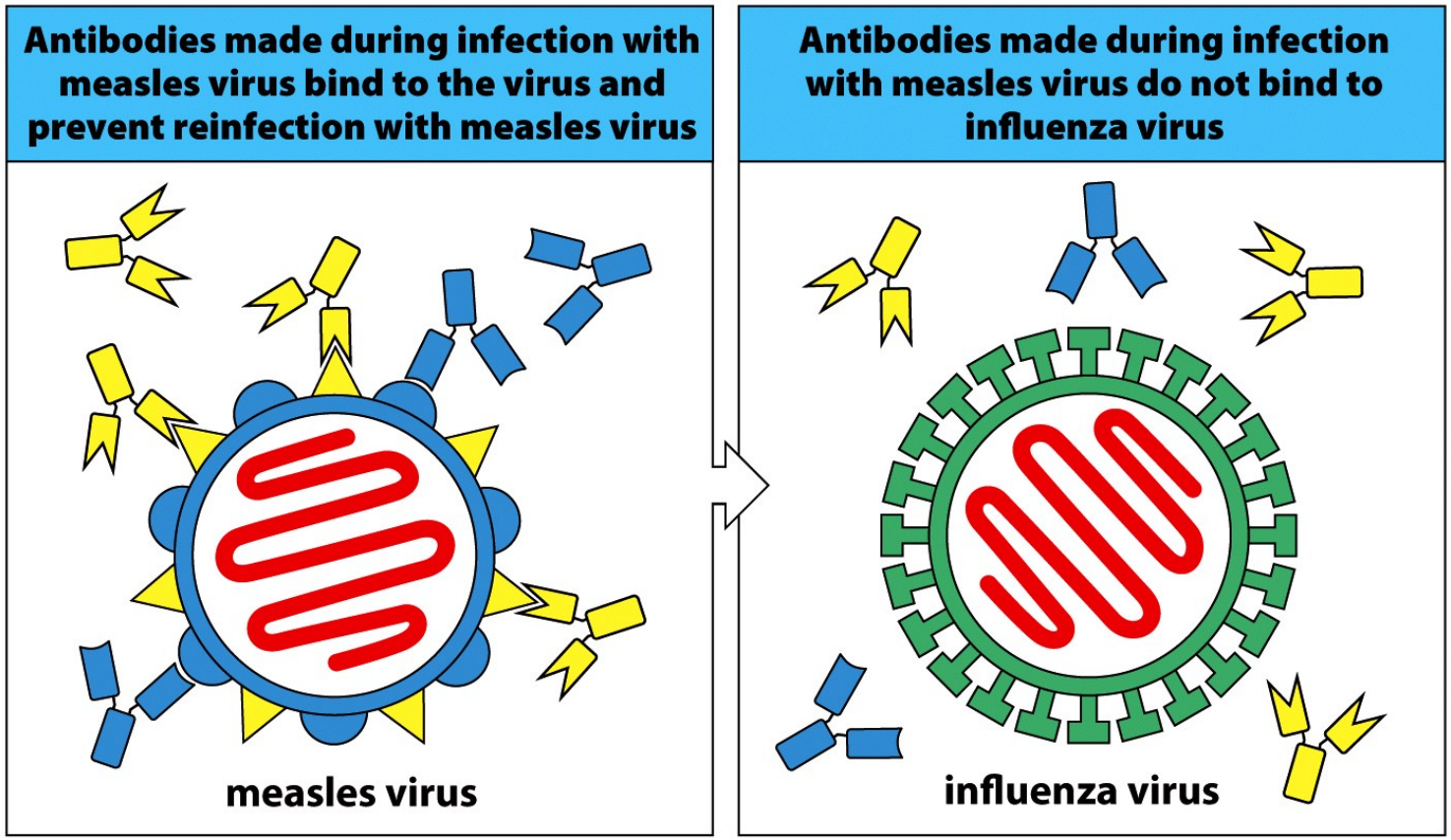


Figure 3.2 The Immune System, 3ed. (© Garland Science 2009)

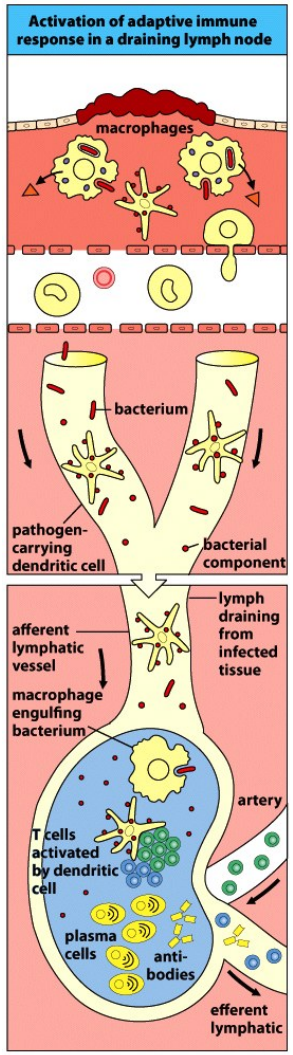


Figure 1.22 The Immune System, 3ed. (© Garland Science 2009)

Principle of Clonal Selection

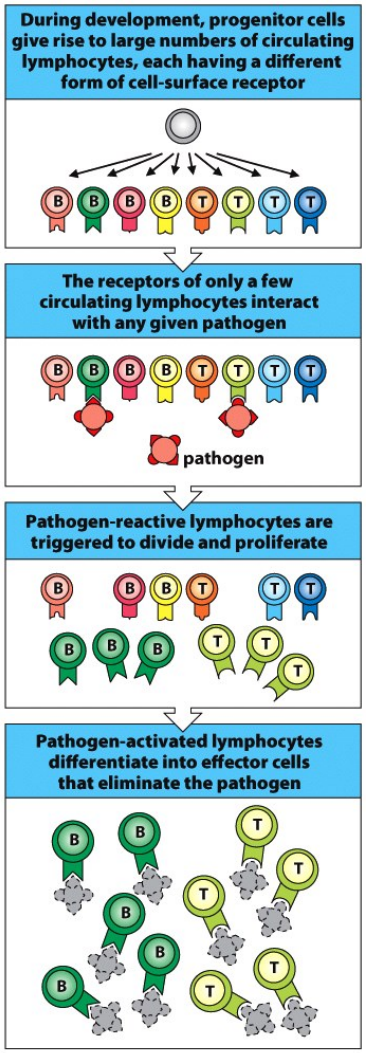


Figure 3.5 The Immune System, 3ed. (© Garland Science 2009)

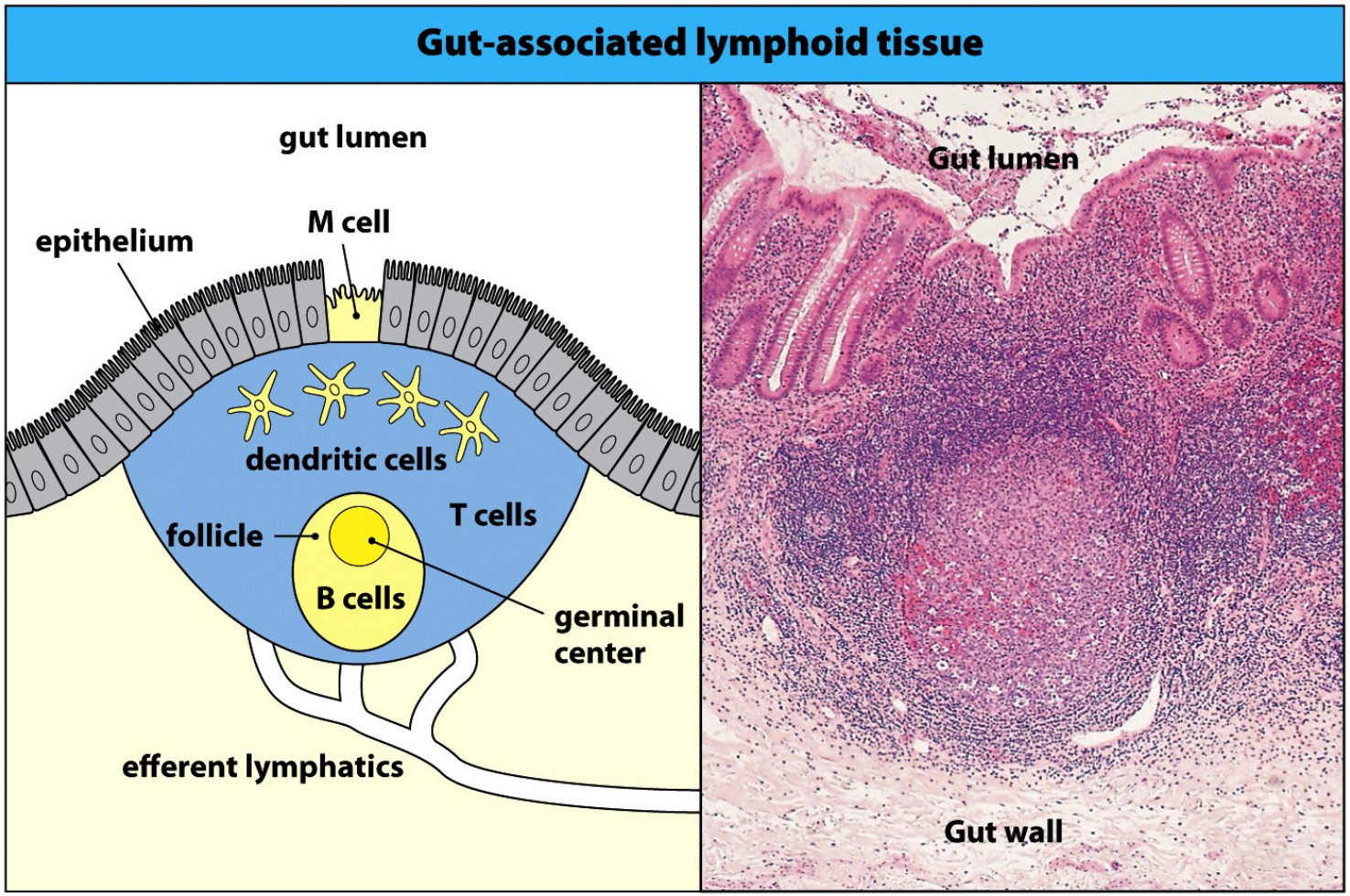


Figure 1.25 The Immune System, 3ed. (© Garland Science 2009)

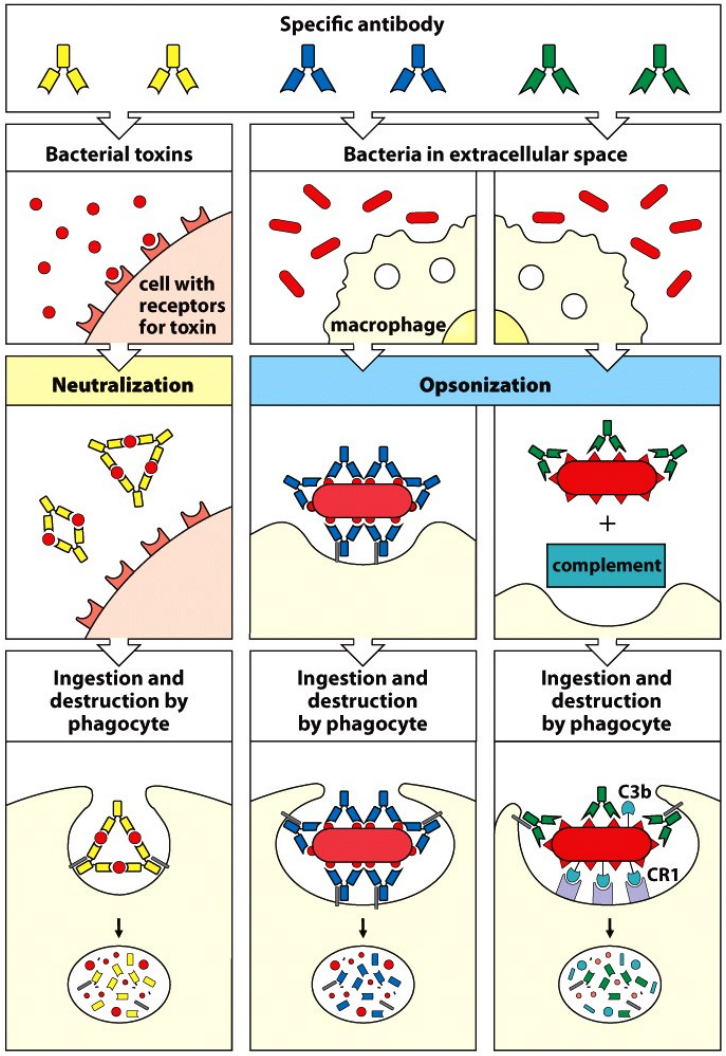
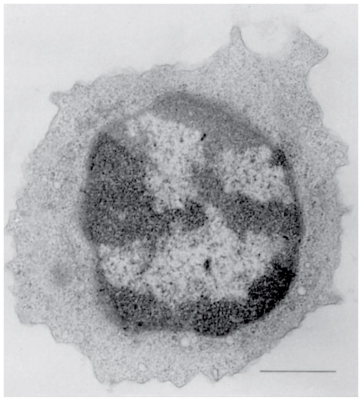


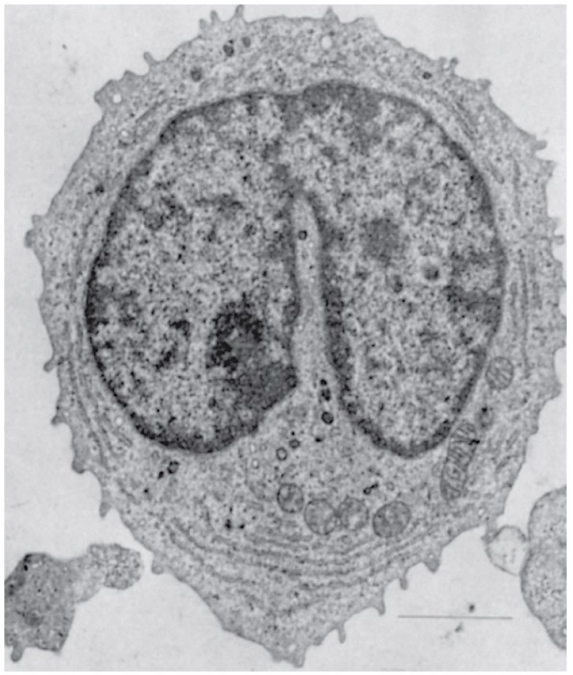
Figure 3.14 The Immune System, 3ed. (© Garland Science 2009)

Lymphocytes (B and T cells) are also white blood cells
The major cells of the adaptive immune system
Large nuclei; most are small and inactive (called “naïve”)



Small lymphocyte (T or B)
6 μm diameter

Figure 2-6b part 1
Kuby IMMUNOLOGY, Sixth Edition



Blast cell (T or B)
15 μm diameter

Figure 2-6b part 2
Kuby IMMUNOLOGY, Sixth Edition

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

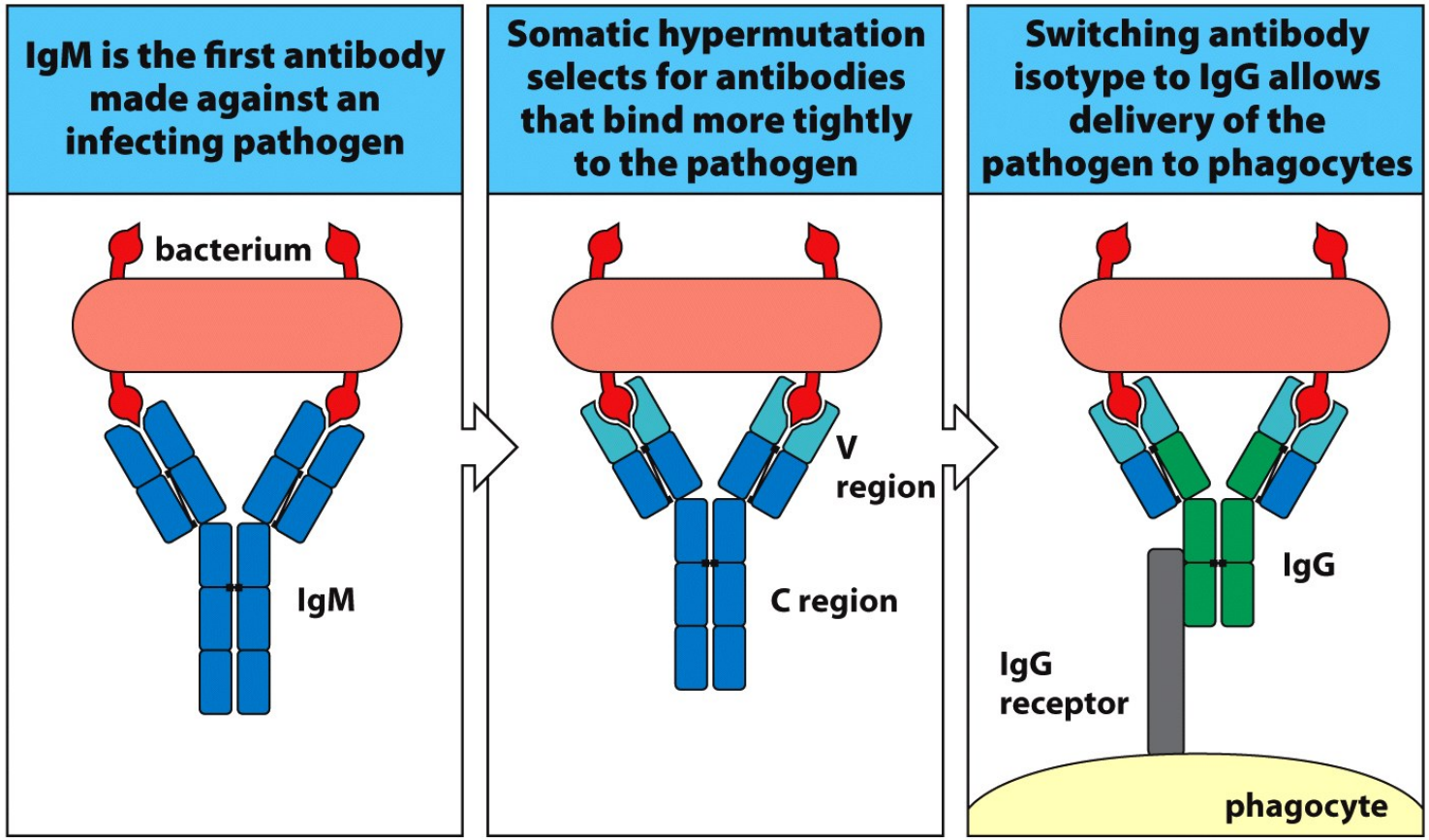


Figure 3.15 The Immune System, 3ed. (© Garland Science 2009)

Some self-reactive T cells can produce autoimmune disease after being activated by a pathogen

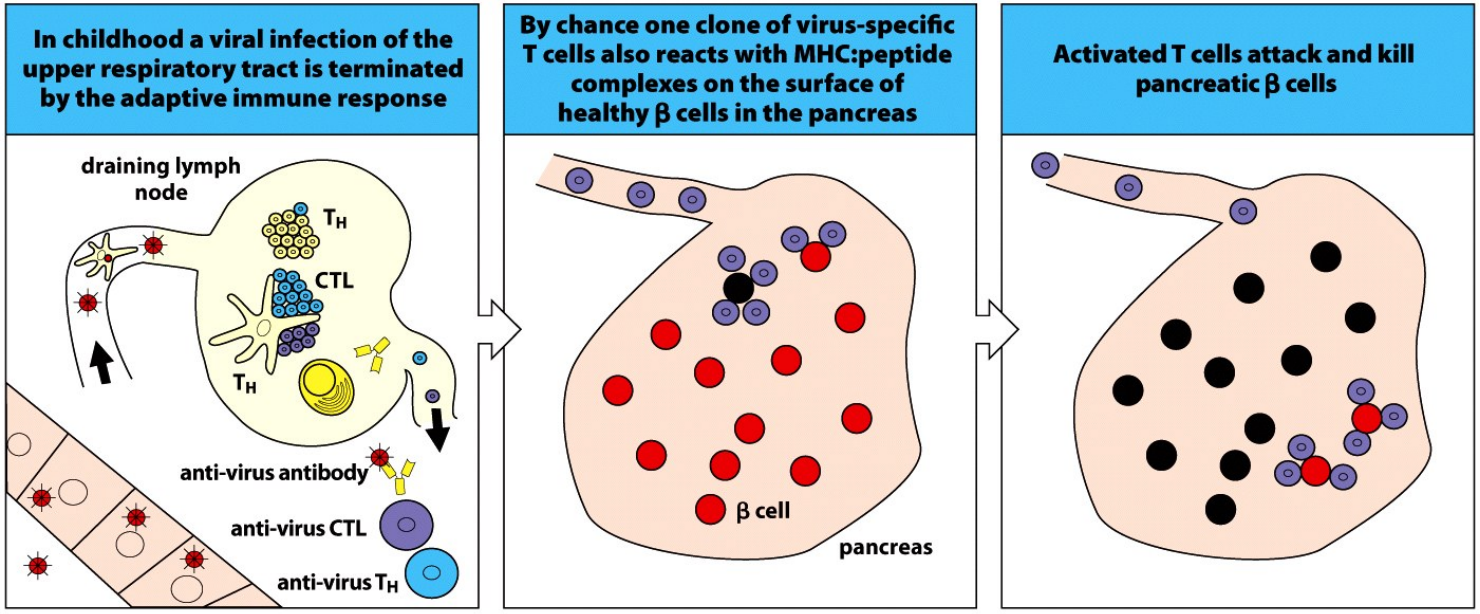


Figure 3.17 The Immune System, 3ed. (© Garland Science 2009)

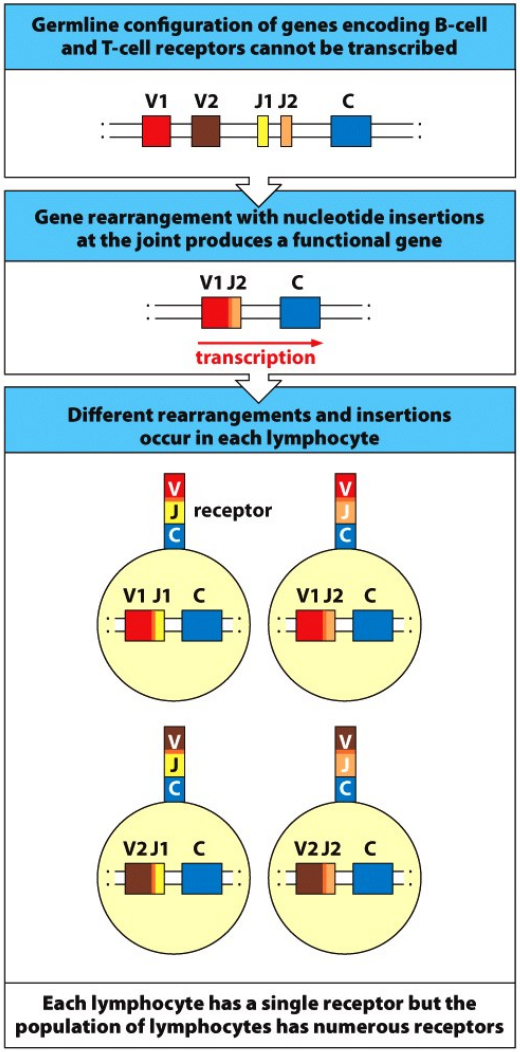


Figure 3.4 The Immune System, 3ed. (© Garland Science 2009)

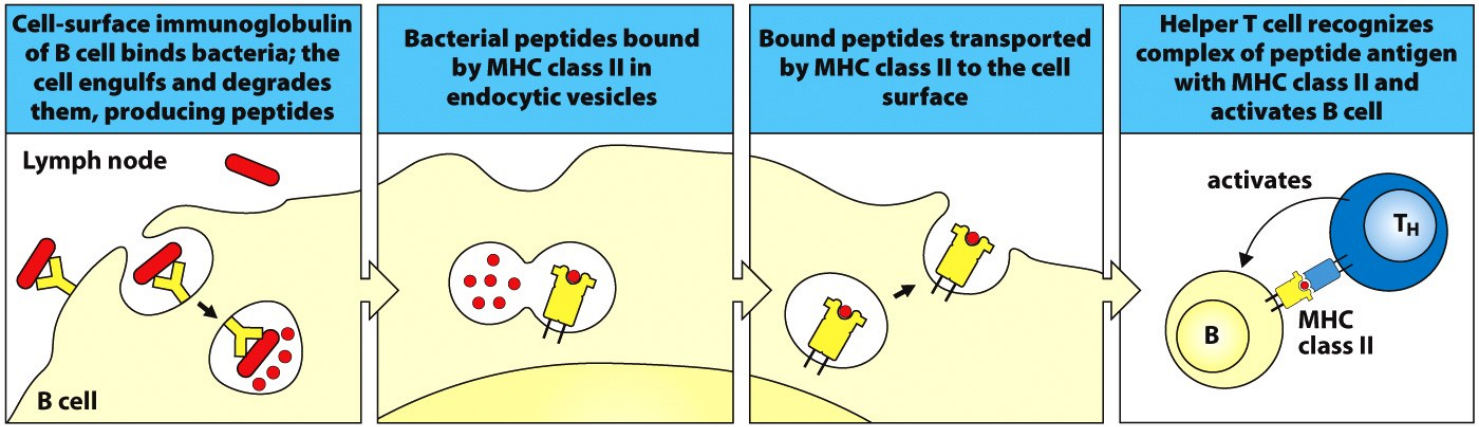


Figure 3.13 The Immune System, 3ed. (© Garland Science 2009)

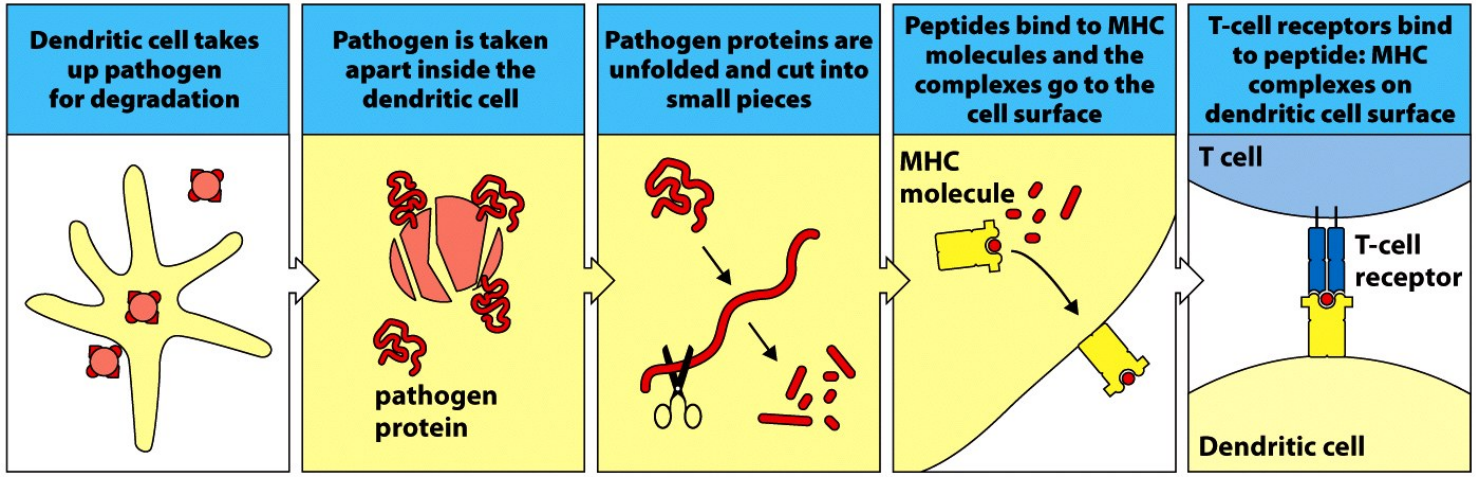


Figure 3.7 The Immune System, 3ed. (© Garland Science 2009)

Learning Objectives for Week 4

- **Review translation and transcription, how DNA codes for mRNA which codes for proteins**
- Start on adaptive immunity: structure of antibodies and T cell receptors, encoded by modifications of DNA sequences

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)

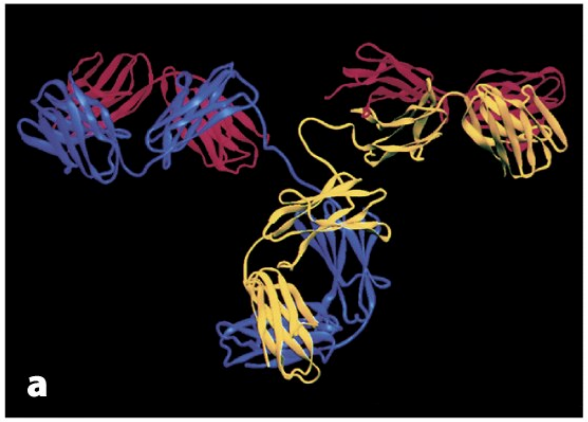
*** During the next 5 years antibodies for oncology and arthritis, immune and inflammatory disorders (AIID) are forecast to continue to lead the market, with sales of $14bn and $11bn, respectively in 2011.**

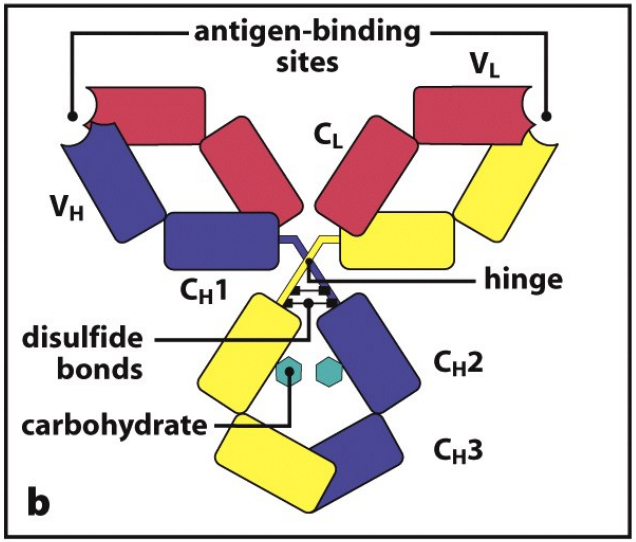
*** With the predicted launch of 2 pipeline antibodies in the next 5 years, the infectious disease area is set to grow in importance. Antibodies are also being developed in respiratory, cardiovascular and ophthalmology indications.**

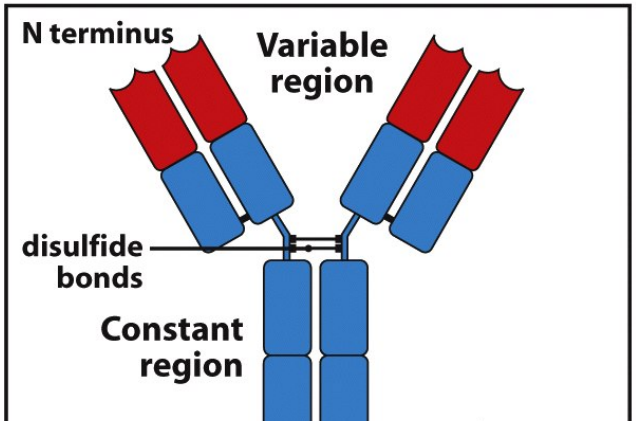
*** Analysis of early and late stage pipelines show development focus is shifting away from murine and chimeric mAbs to humanized and fully human antibodies. Abbott/CAT's Humira, launched in the US in 2003, was the first fully human antibody on the market.**

*** 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.**

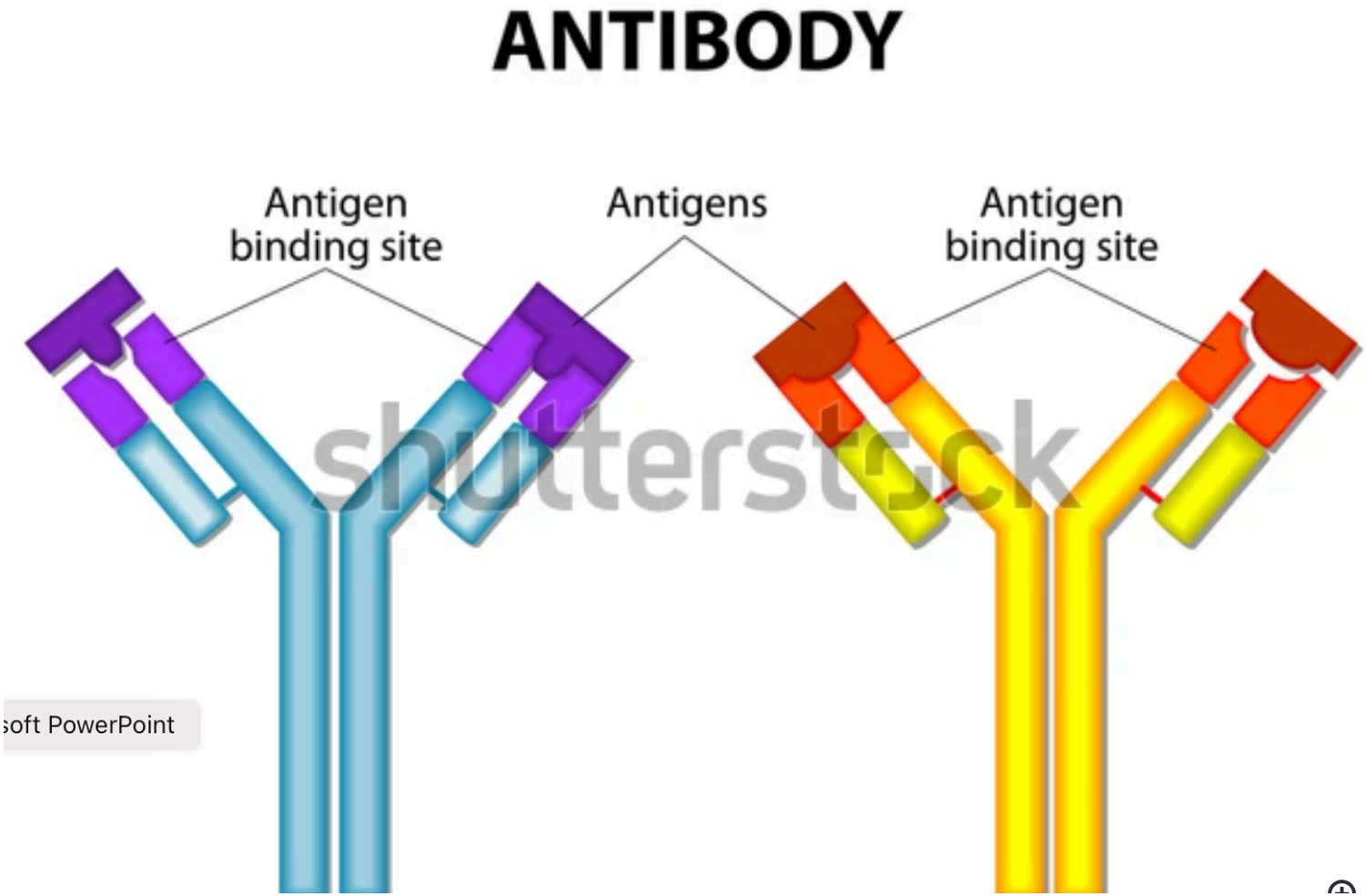
Antibodies have Variable Regions and Constant Regions



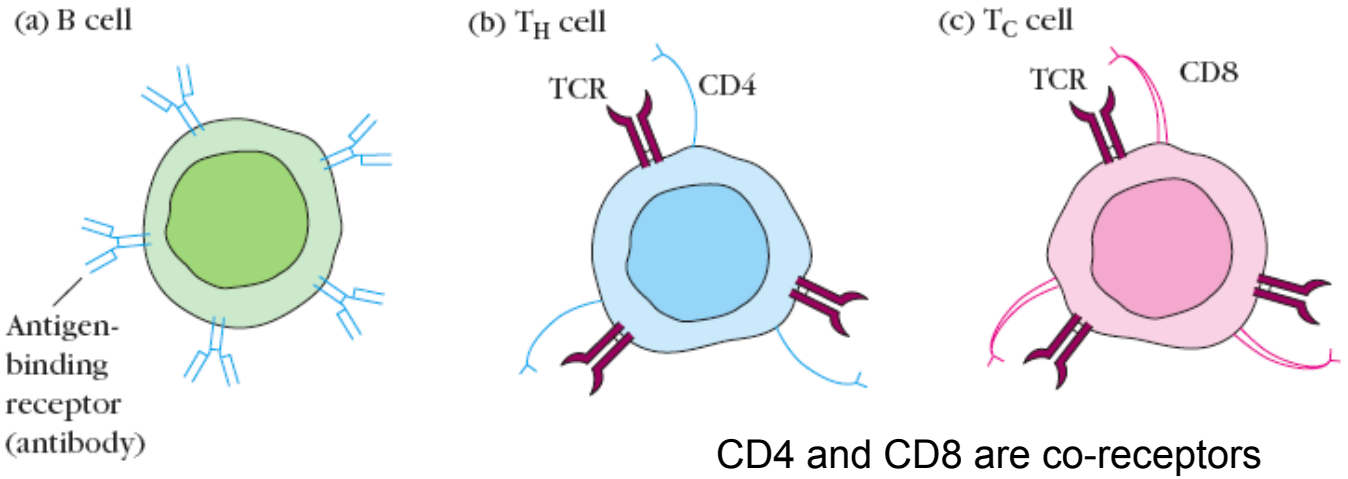




Antibody and Antigen are complementary



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



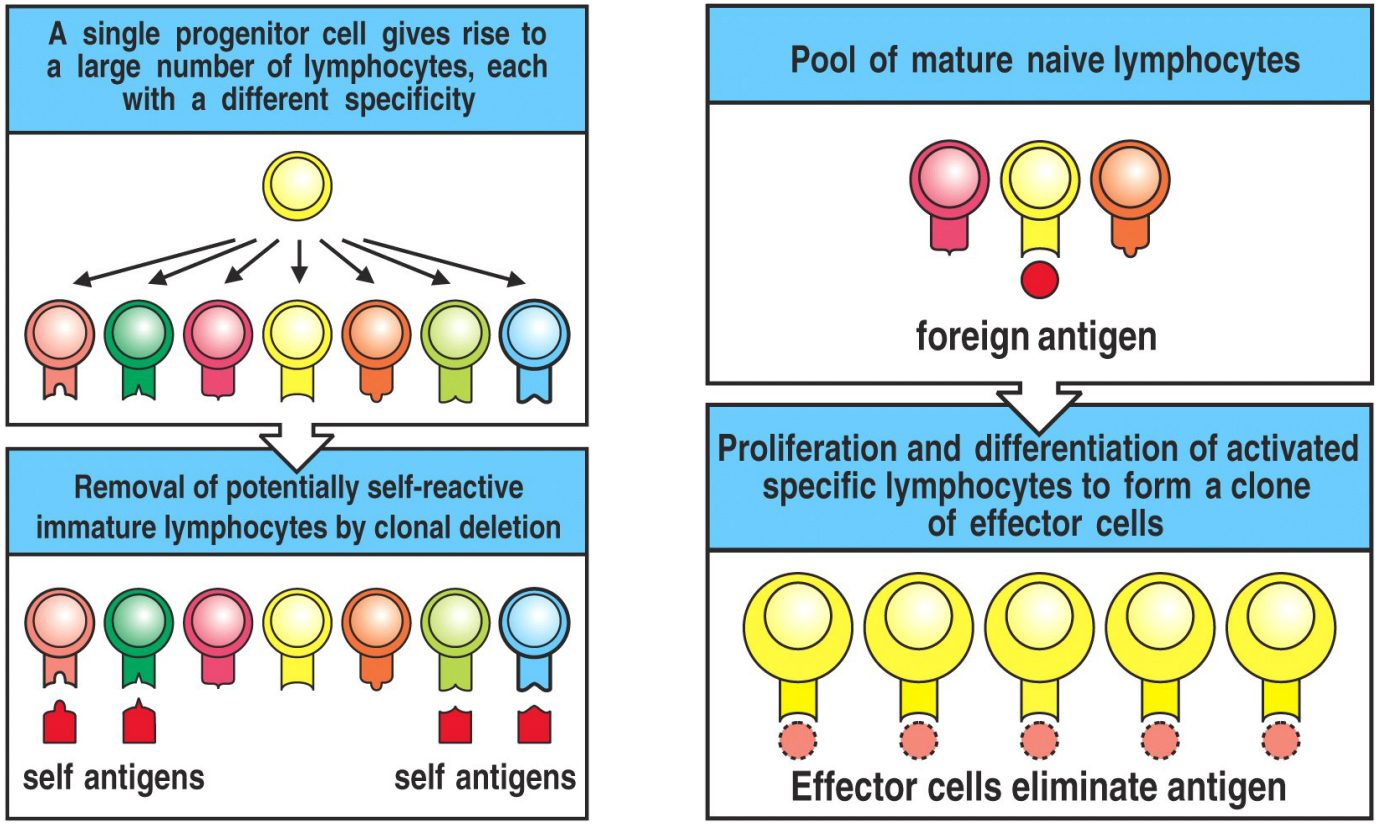
BCR

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

B cells and T cells

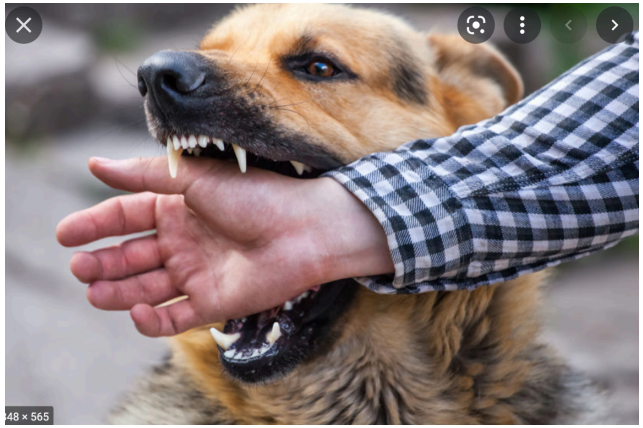
- B Cell (antigen) Receptor (BCR)
  - When secreted called an antibody
- T Cell (antigen) Receptor (TCR)
  - $\alpha\beta$ subunits for most TCR on "$\alpha\beta$ T cells"
  - $\gamma\delta$ subunits for "$\gamma\delta$ T cells"

Clonal selection of lymphocytes is the central principle of adaptive immunity

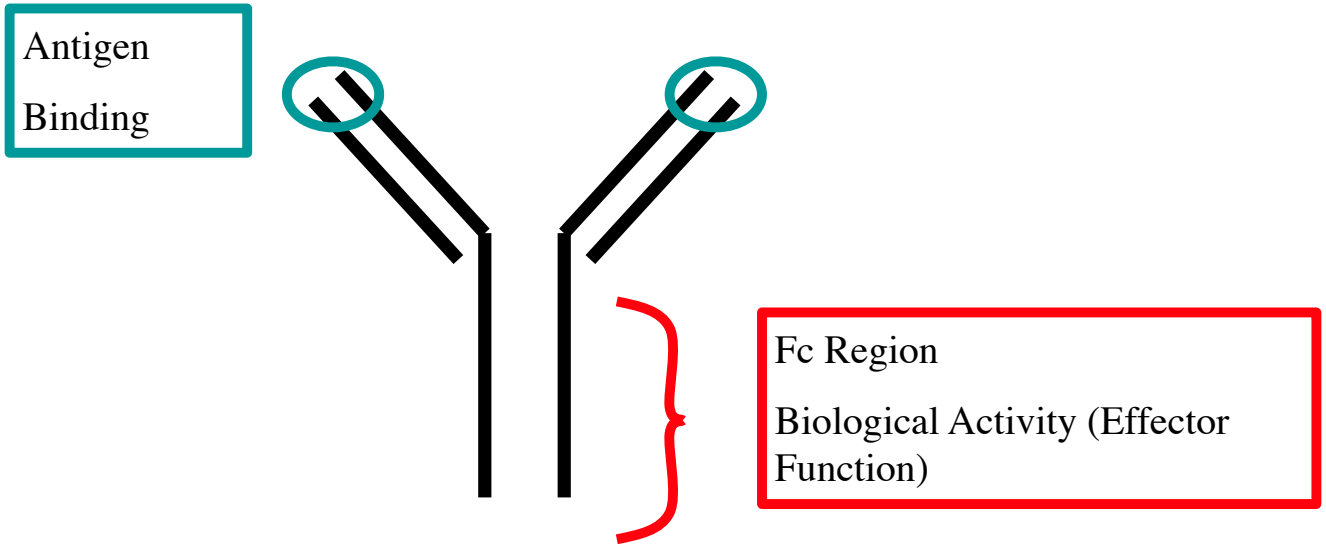


The Need for Structural Diversity

- Pathogens generate diversity on short time scale (rapid natural selection)
- Innate immune system recognizes conserved features, makes initial stop
- Adaptive immune system has huge diversity of antigen recognition receptors (antibodies and TCRs)
  - Life-long generation of diversity
  - Within-individual natural selection
  - Takes a few days to expand clones


48 × 565

Antibody Structure



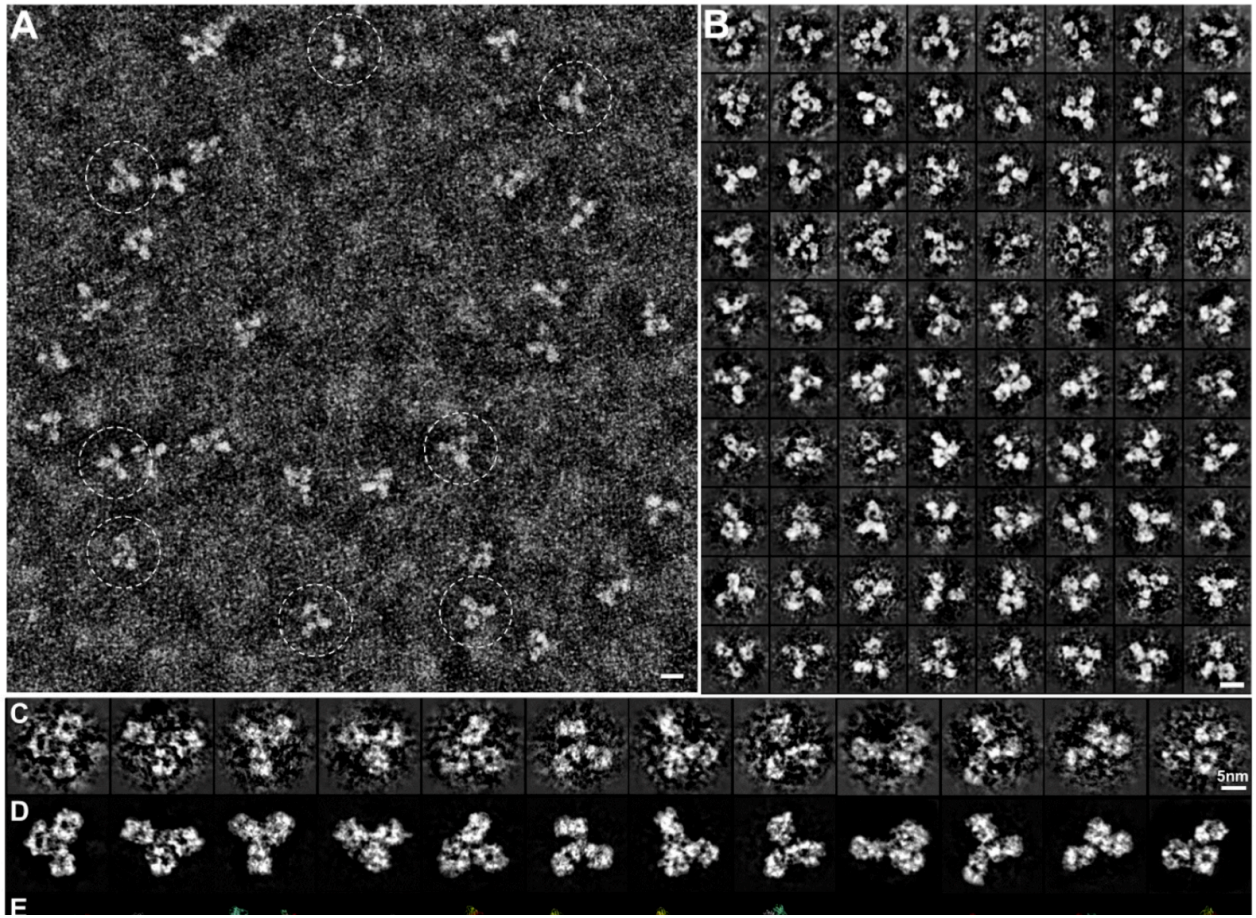


Figure 2. The Optimized Negative Staining (OpNS) Electron Microscopy (EM) images of IgG2 antibody particles. (**A**) Survey view of the IgG antibody particles imaged by OpNS EM. (**B**) Representative images of individual particles of antibody. (**C**) Zoomed-in views of selected individual particle 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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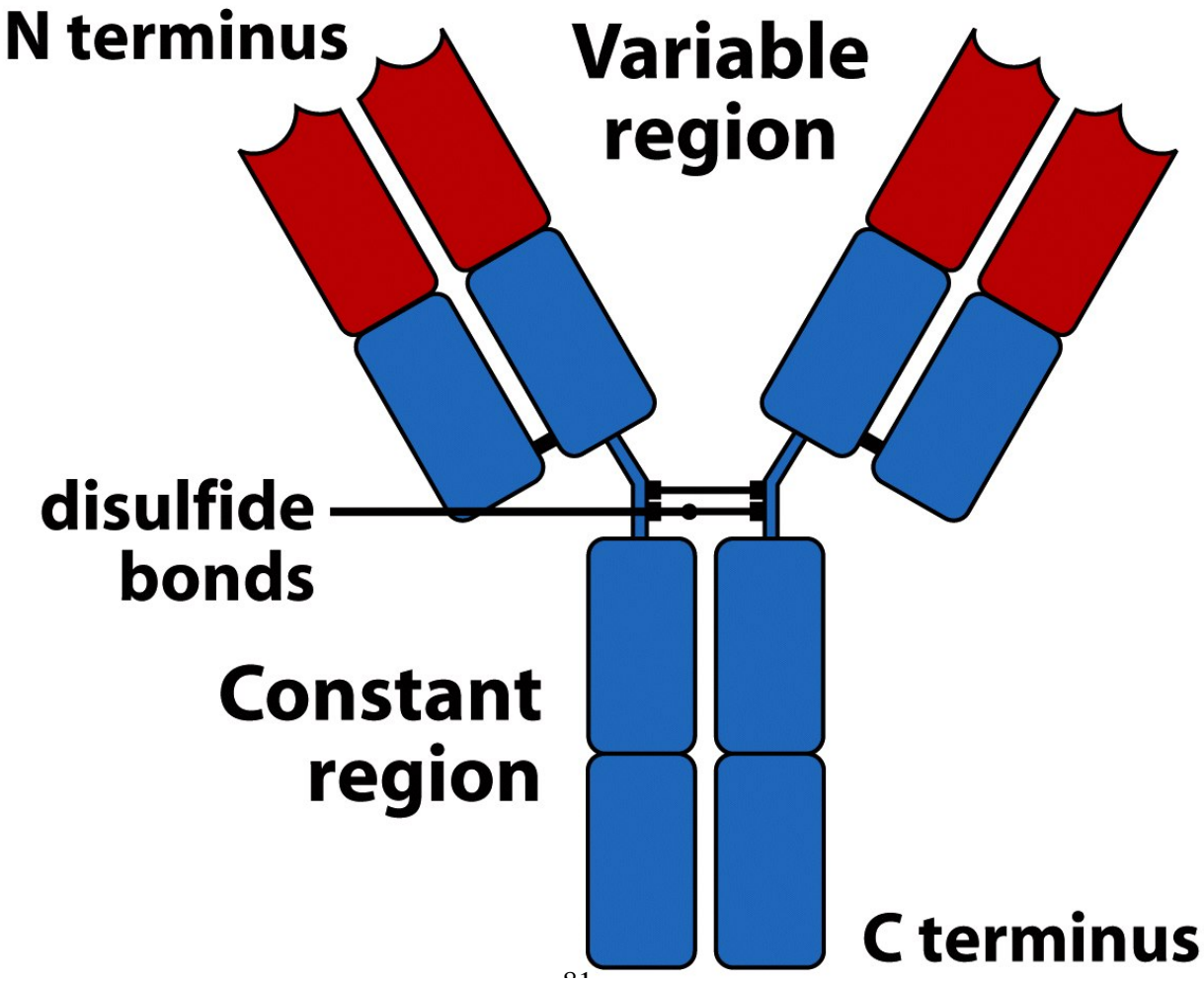
IgG Antibody 3D Structures and Dynamics

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

Structural Dimensions

- Based on various ways of dissecting protein:
  - Protease fragments
  - Sulfhydryl-bonded chains
  - Functional domains (homologous repetitive regions)

Antibody Structure (Immunoglobulin)



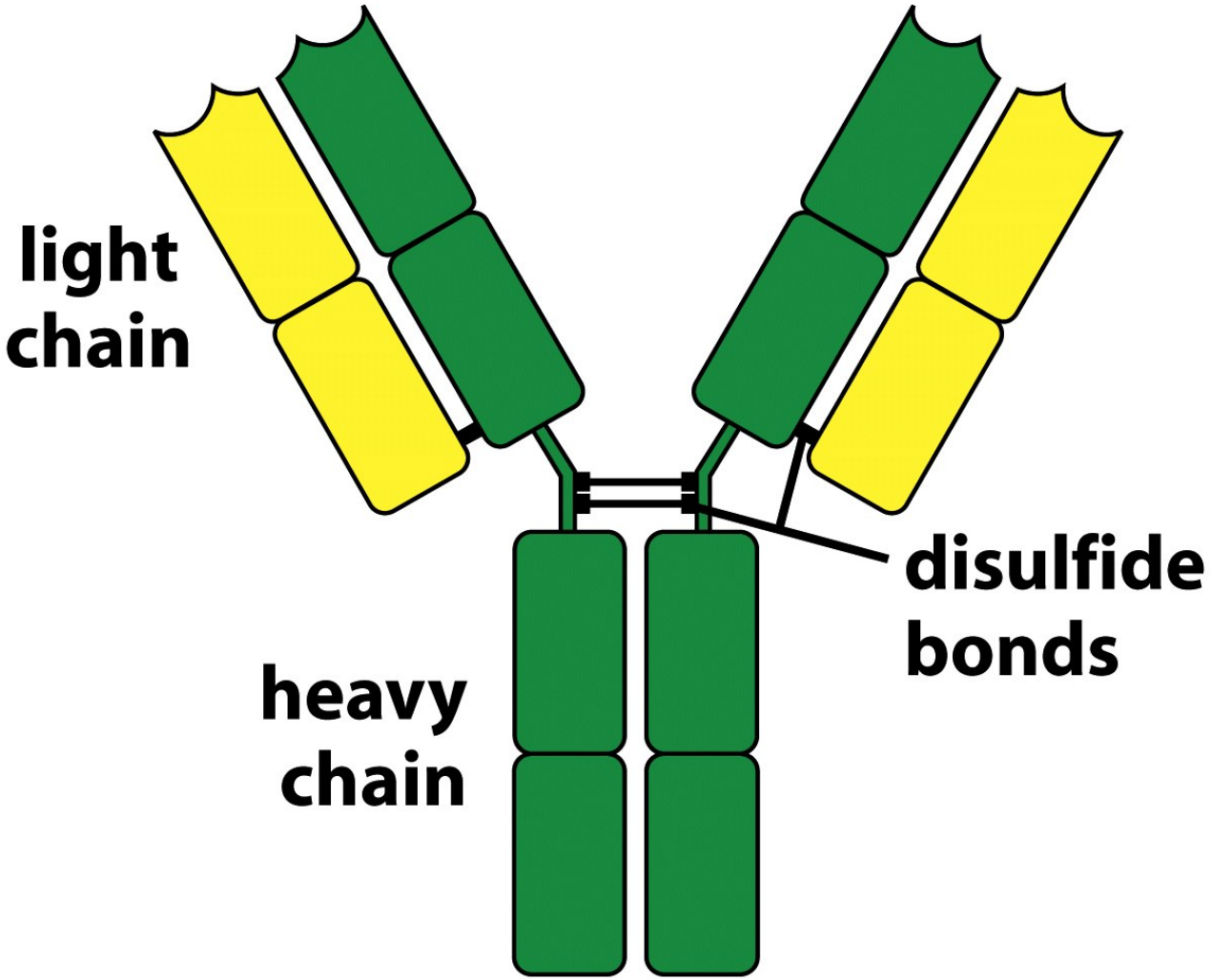
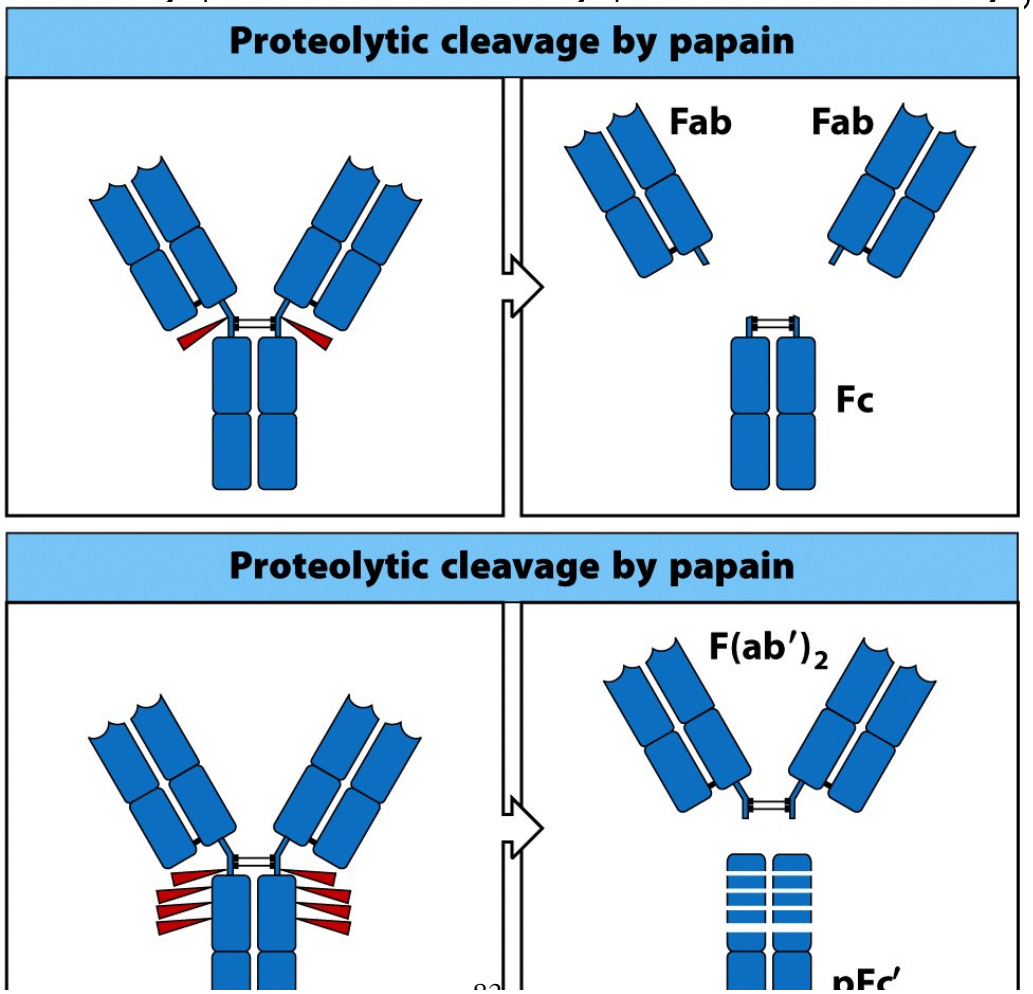


Figure 3-2 Immunobiology, 7ed. (© Garland Science 2008)

Antigen binding is on Fragment antigen binding

Fab



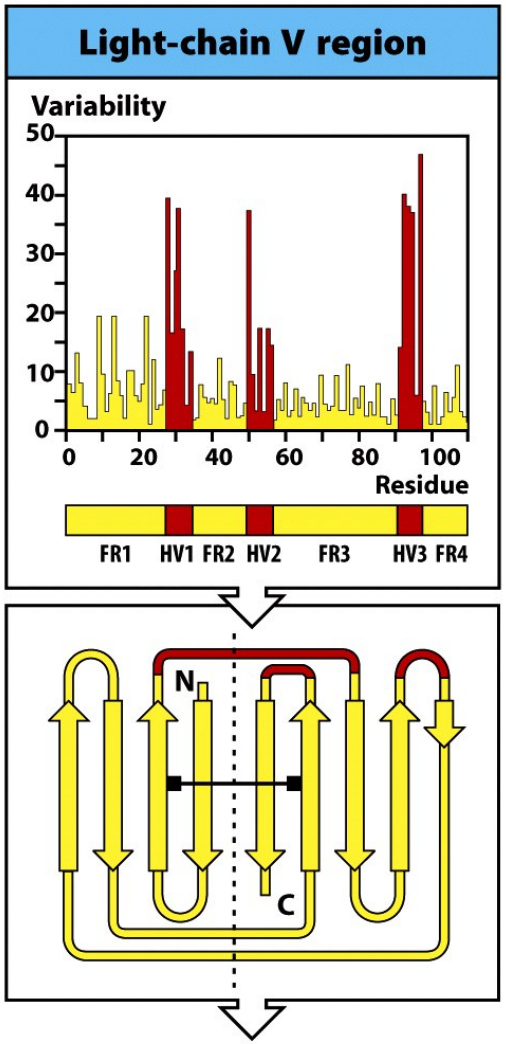


Figure 3-7 part 1 of 3 Immunobiology, 7ed. (© Garland Science 2008)

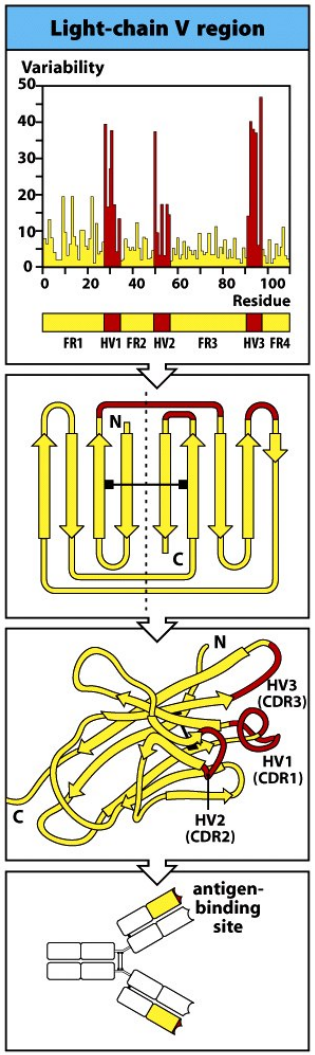
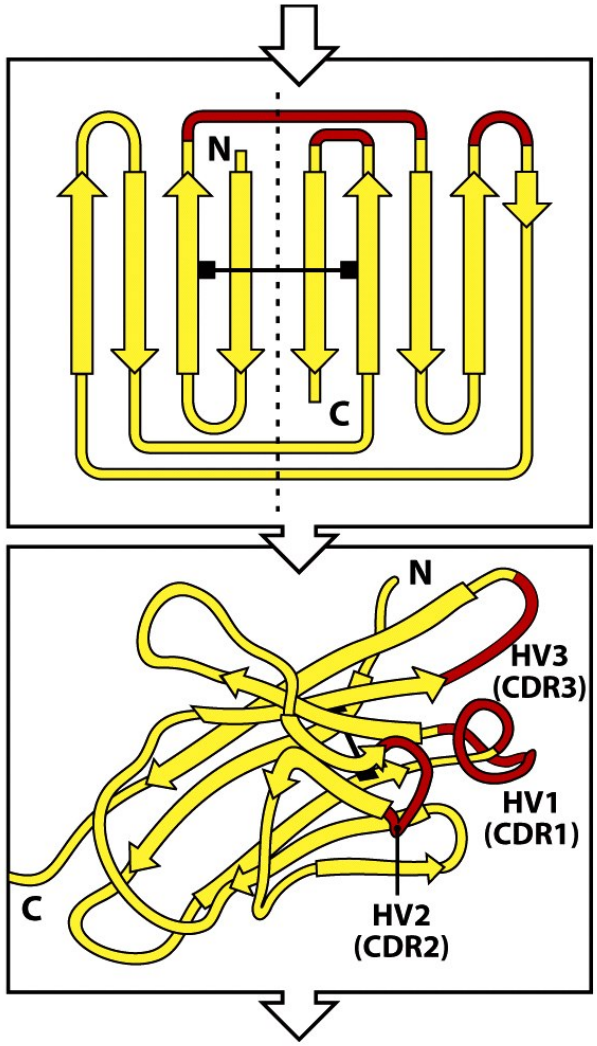


Figure 3-7 Immunobiology, 7ed. (© Garland Science 2008)



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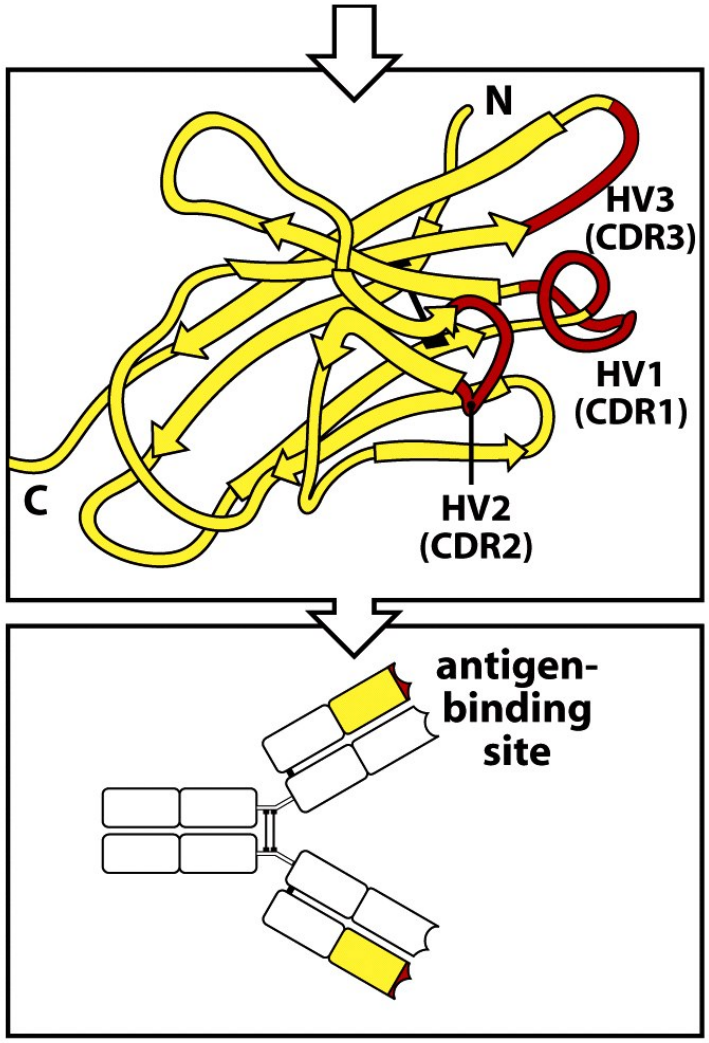


Figure 3-7 part 3 of 3 Immunobiology, 7ed. (© Garland Science 2008)

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

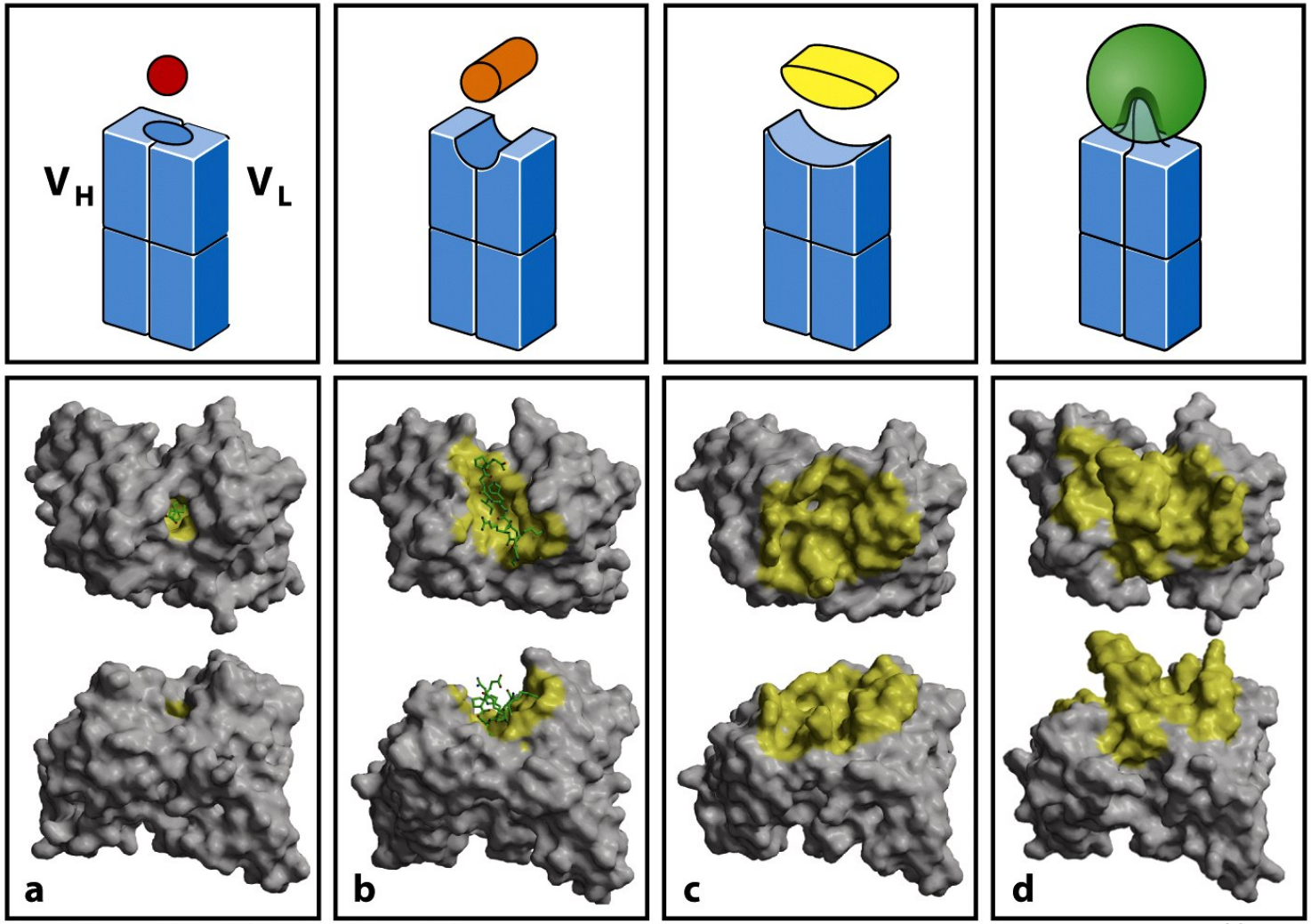


Figure 3-8 Immunobiology, 7ed. (© Garland Science 2008)

Number of functional gene segments in human immunoglobulin loci

Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	40
Diversity (D)	0	0	25
Joining (J)	5	4	6

Figure 4-3 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 create Different

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

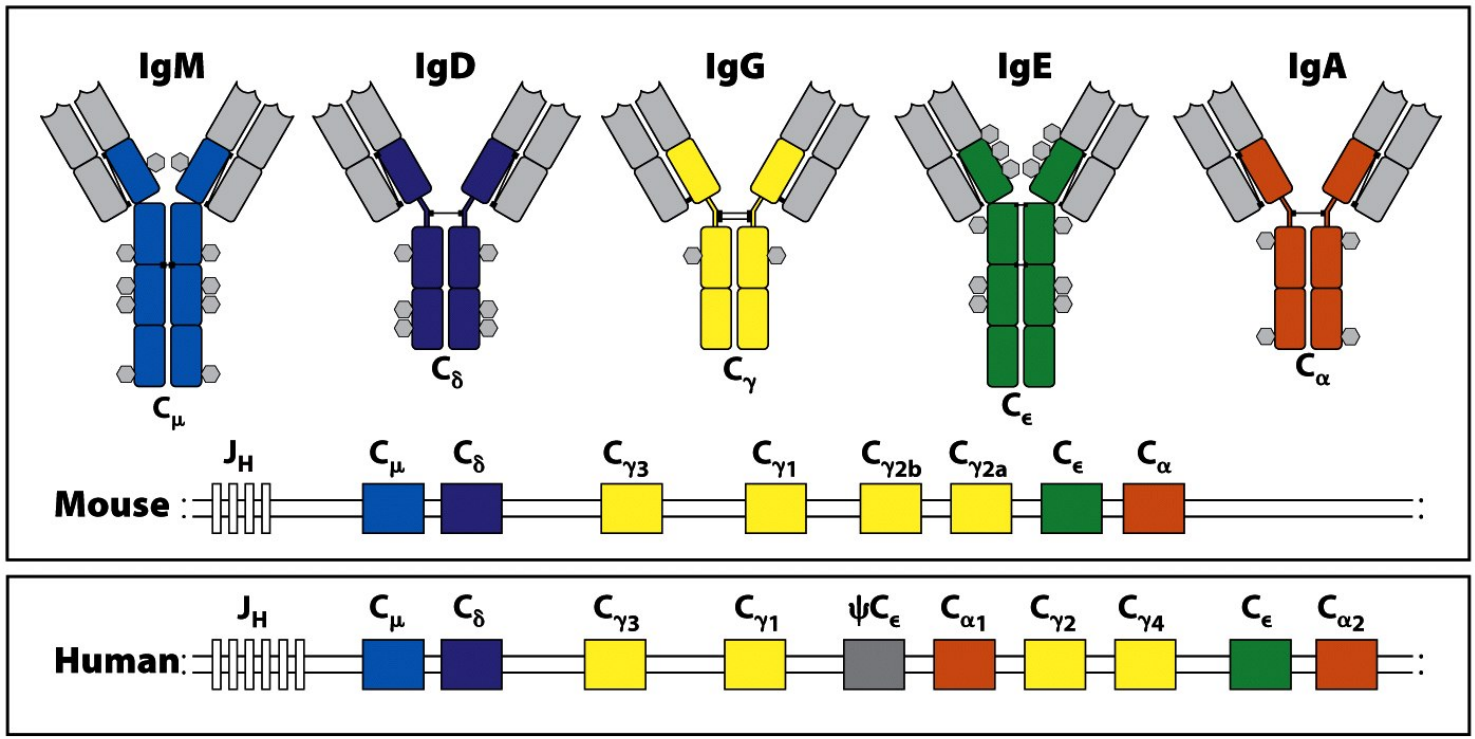


Figure 4-17 Immunobiology, 7ed. (© Garland Science 2008)

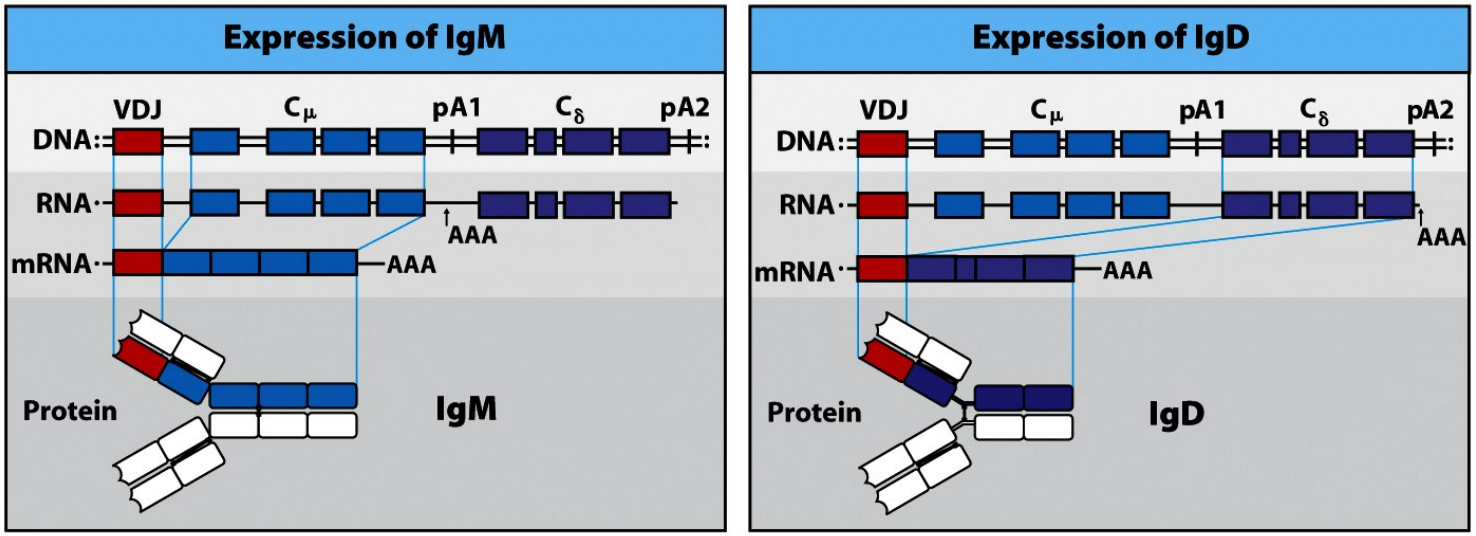


Figure 4-18 Immunobiology, 7ed. (© Garland Science 2008)

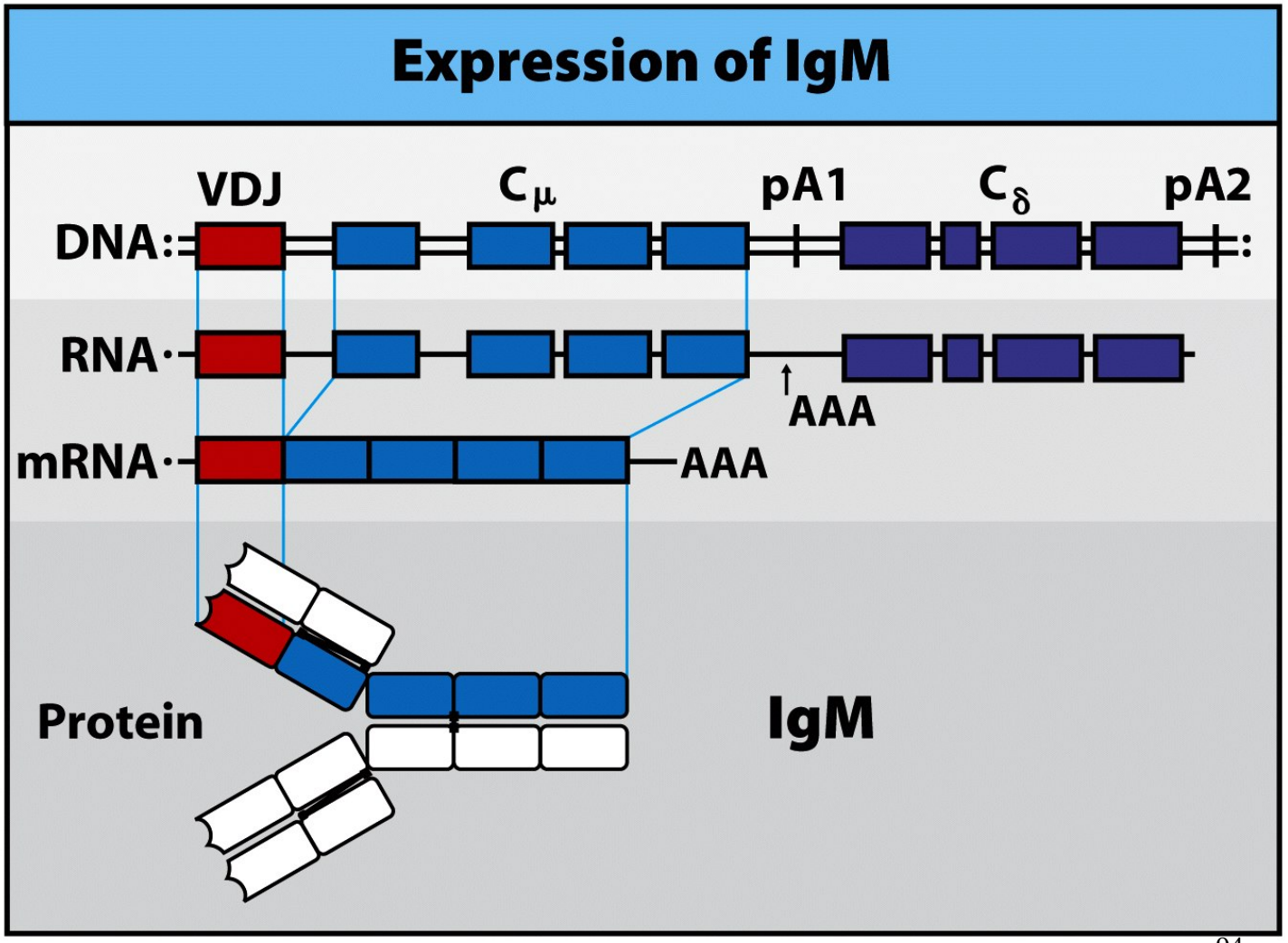
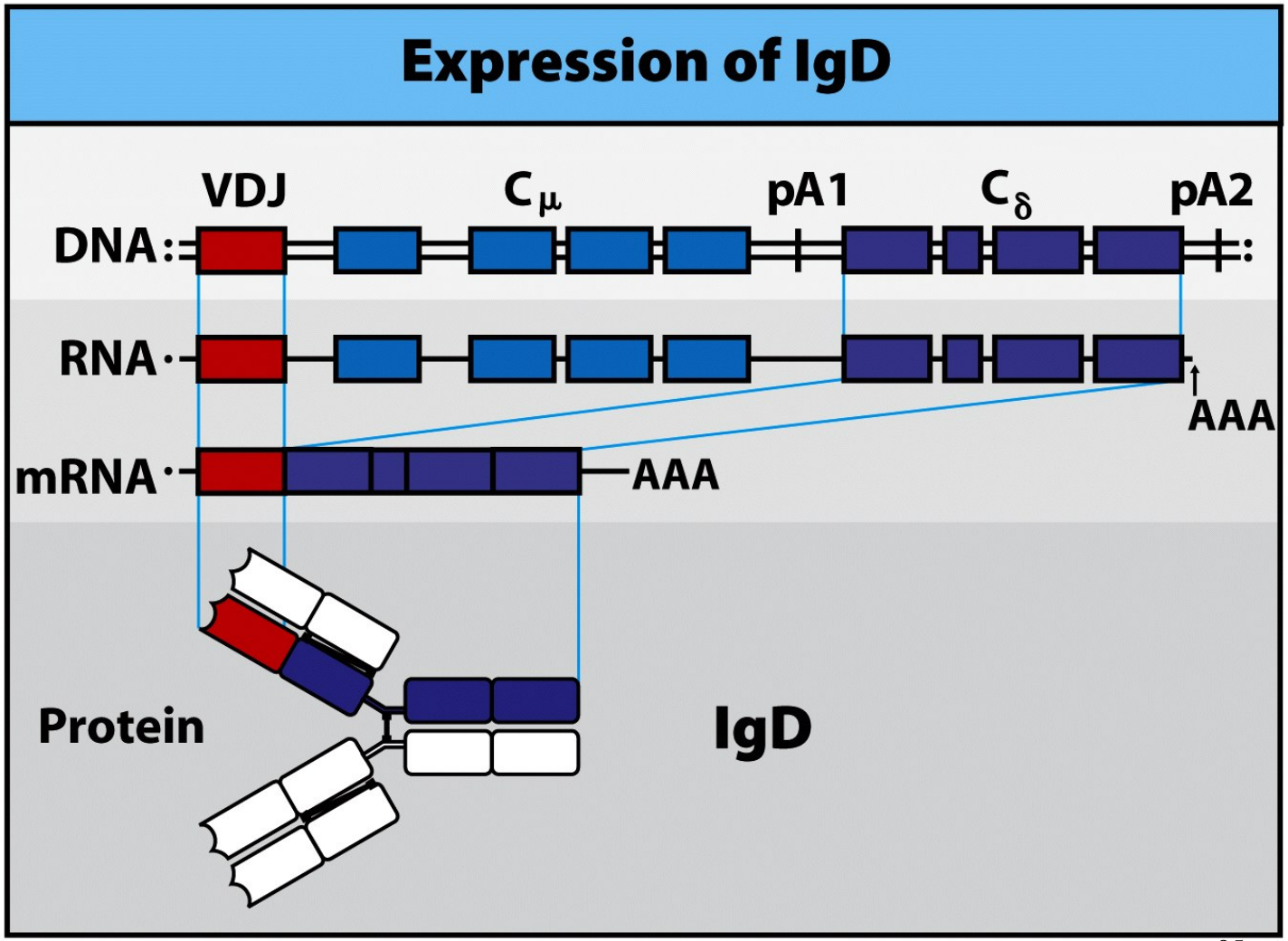


Figure 4-18 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)



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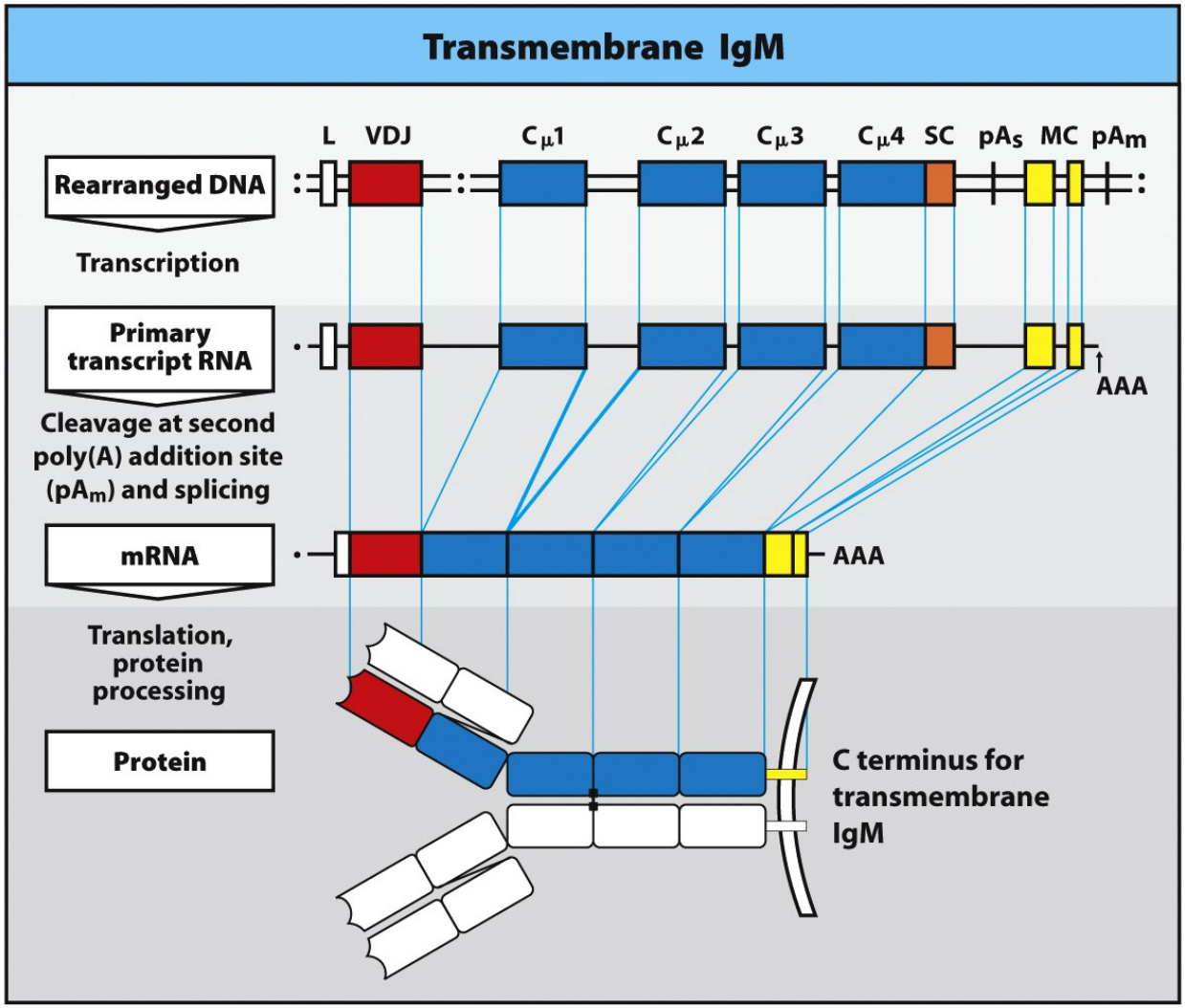

Figure 4-19 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

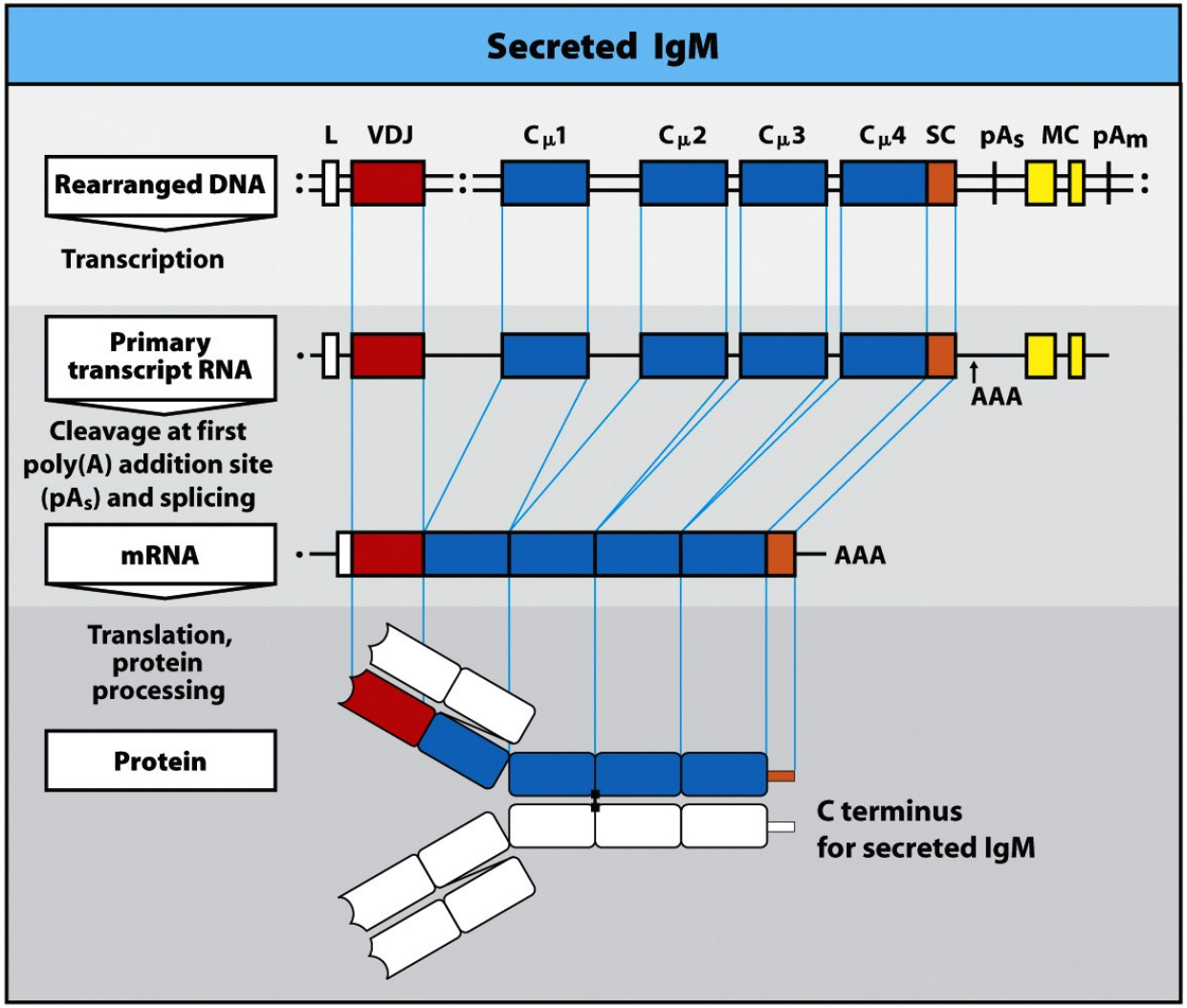

Figure 4-19 part 2 of 2 Immunobiology, 7ed. (© Garland Science 2008)

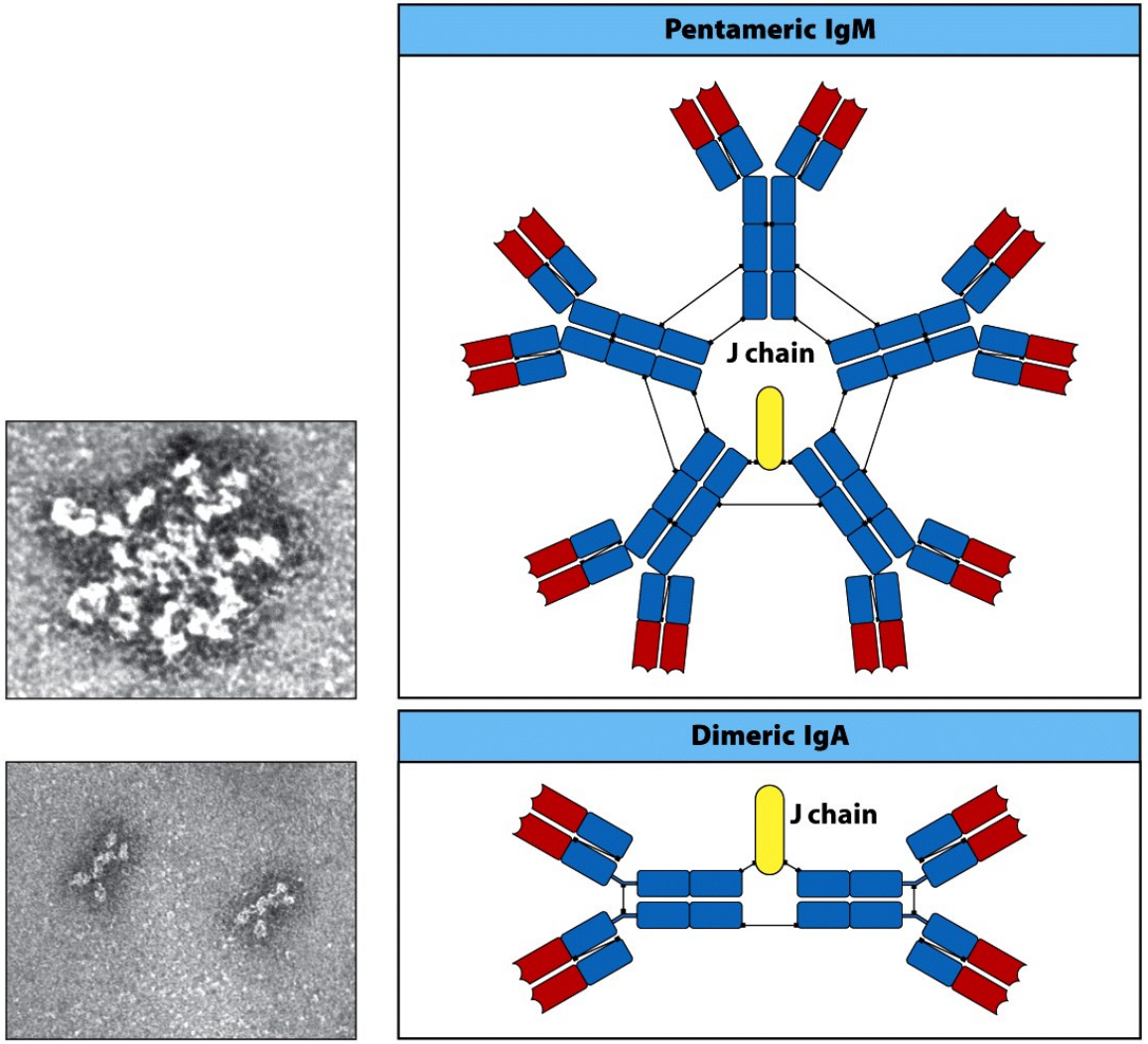

Figure 4-20 Immunobiology, 7ed. (© Garland Science 2008)

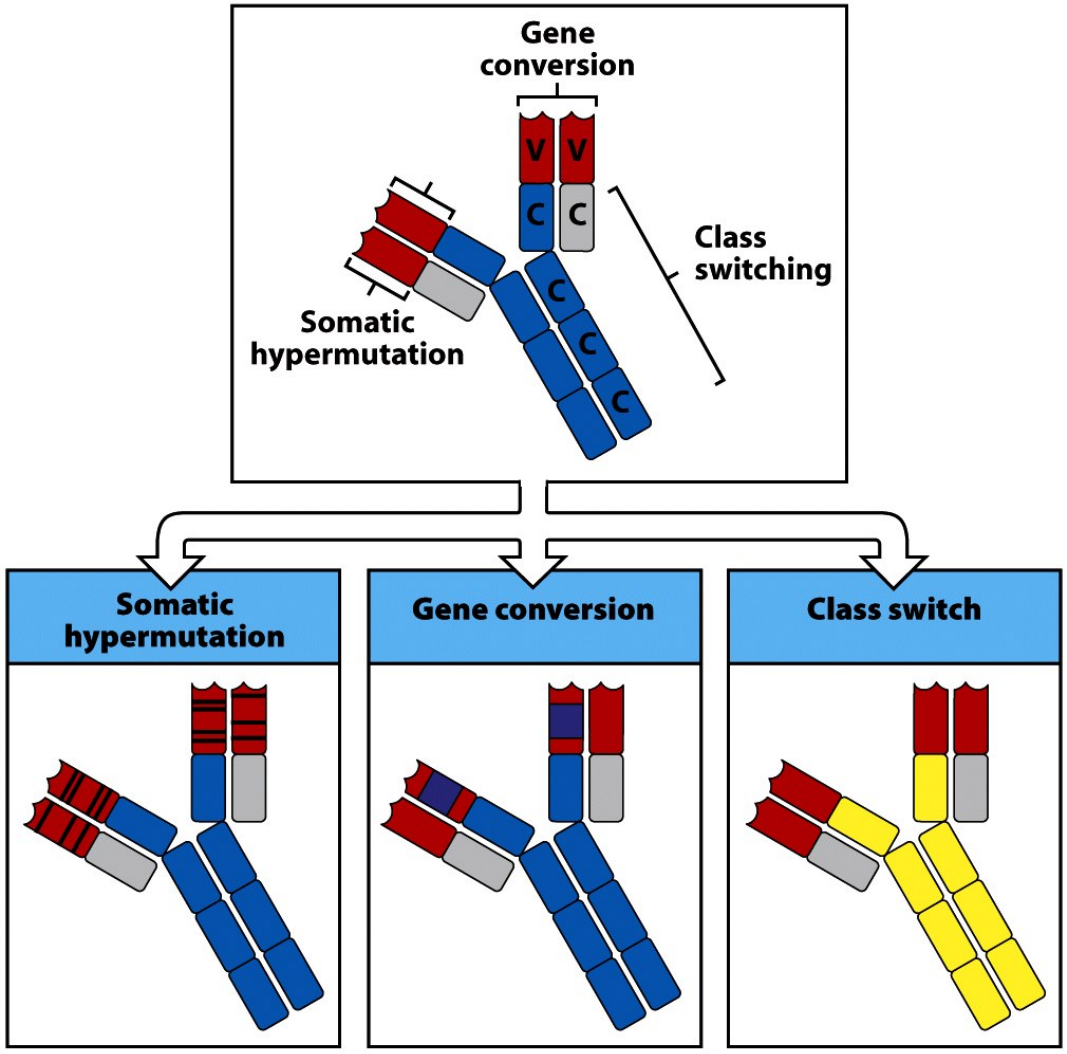

Figure 4-21 Immunobiology, 7ed. (© Garland Science 2008)

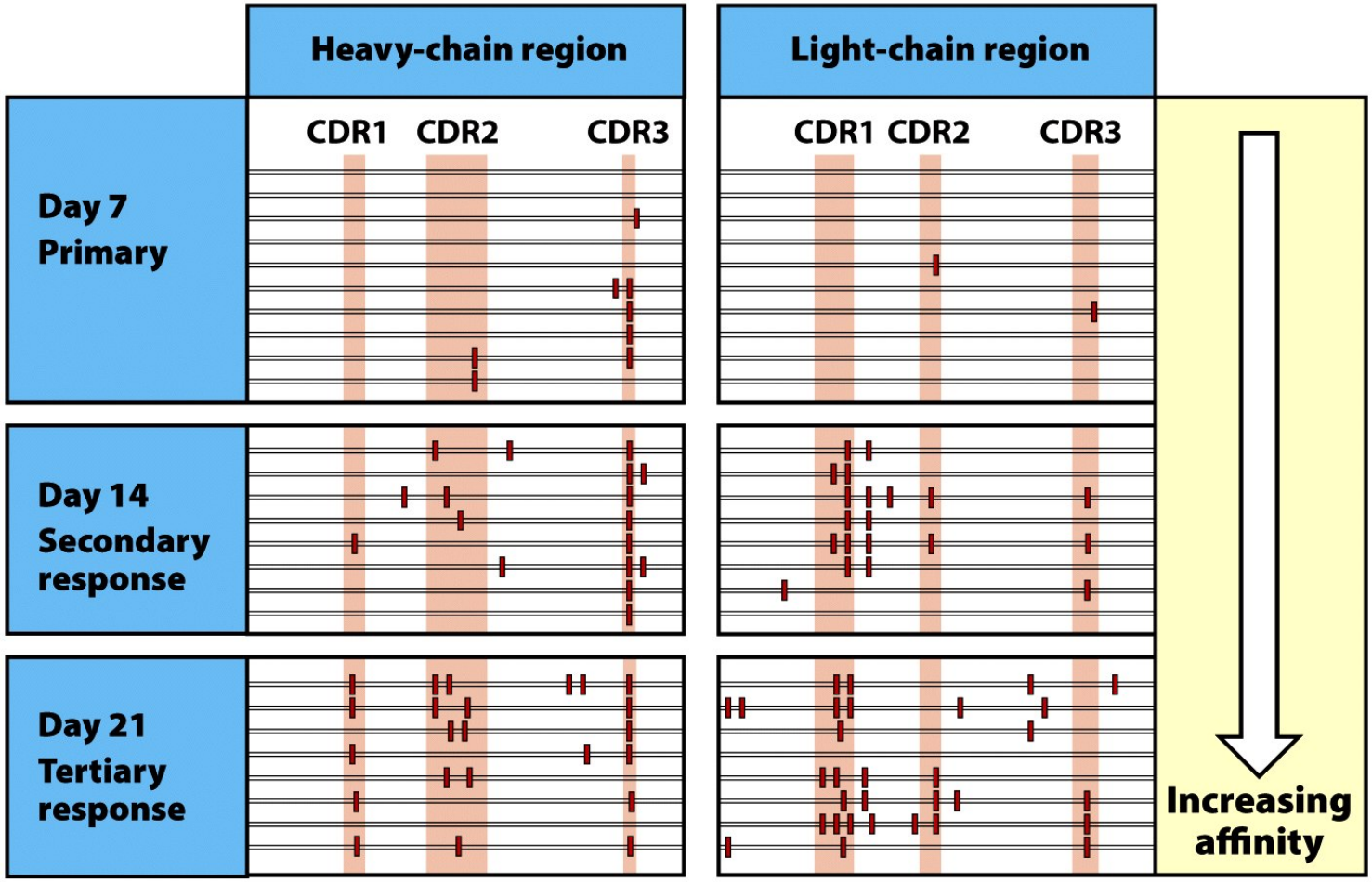

Figure 4-25 Immunobiology, 7ed. (© Garland Science 2008)

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 4-28 Immunobiology, 7ed. (© Garland Science 2008)

types of vaccines: https://www.gavi.org/vaccineswork/there-are-four-types-covid-19-vaccines-heres-how-they-work

https://www.cbc.ca/news/health/serial-killers-attack-cancer-cell-in-video-1.3079500