Clinical Basis of the Immune Response and the Complement Cascade

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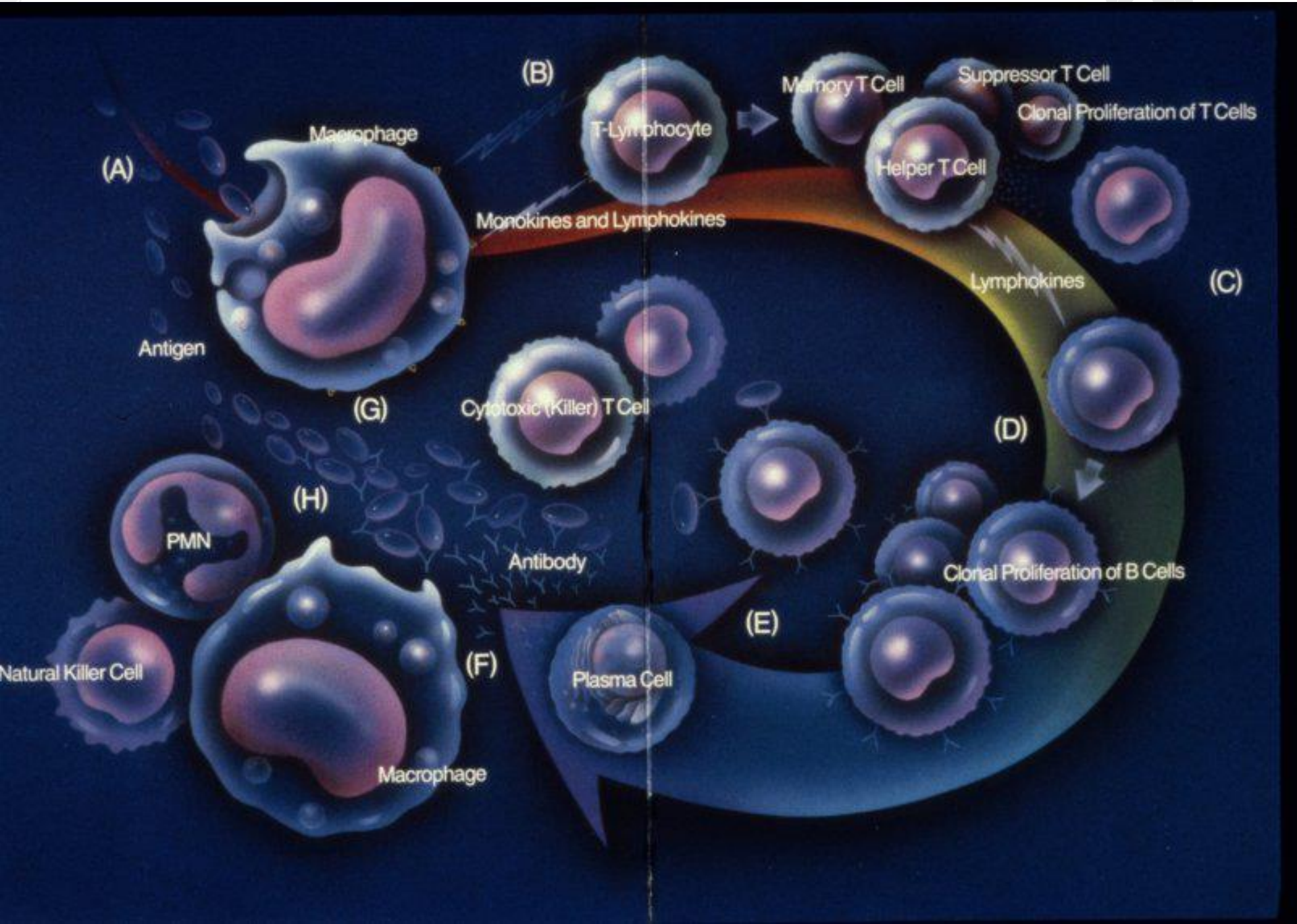
Disclosures

- None

Introduction

- Immunology is felt to be difficult to learn
  - There are too many details that you can get bogged down in
  - There's always an exception to every rule
  - We still don't really know that much: **but** what we know doubles every year
- The immune response: a system (or two) with a number of players working together as a team.

- All this means: A great source for questions


(A)
Macrophage
(B)
T-Lymphocyte
Memory T Cell
Suppressor T Cell
Clonal Proliferation of T Cells
Helper T Cell
Monokines and Lymphokines
Lymphokines
(C)
Antigen
(G)
Cytotoxic (Killer) T Cell
(D)
(H)
PMN
Antibody
Clonal Proliferation of B Cells
(E)
Natural Killer Cell
(F)
Plasma Cell
Macrophage

Immunity
Innate & Adaptive

- First line of defense
- Nonspecific
- Rapid onset
- No protective immunity
- No memory
- Phagocyte- mediated

- Activated
- Very specific
- Slower
- Protective immunity possible
- Memory possible
- Lymphocyte-mediated

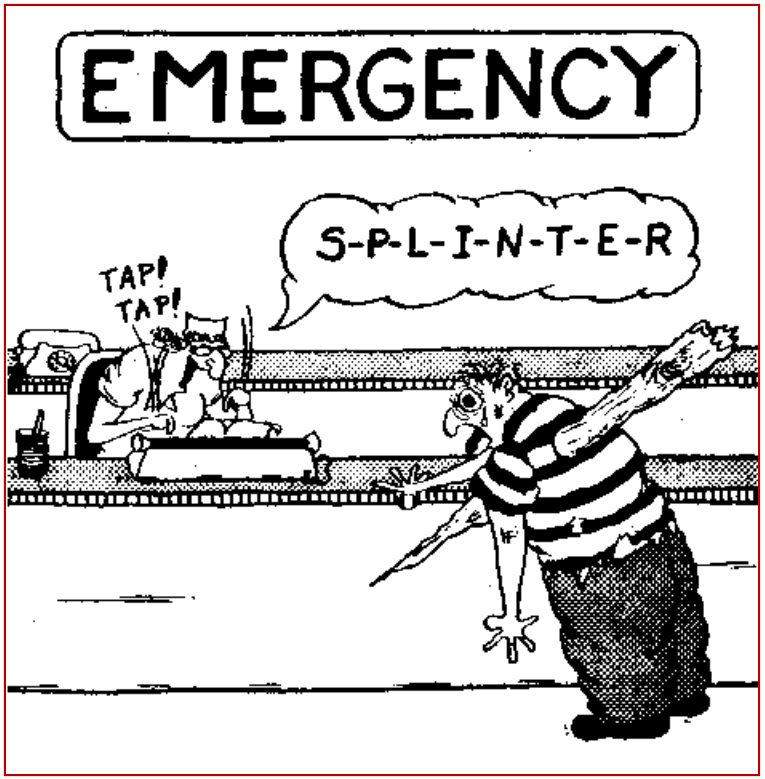
Innate immune system

- Immune system which all animals have “naturally” and always ready
- Components
  - Complement
    - Critical role against bacteria, fungi and virus
  - Phagocytes
    - Macrophages, neutrophils, NK cells

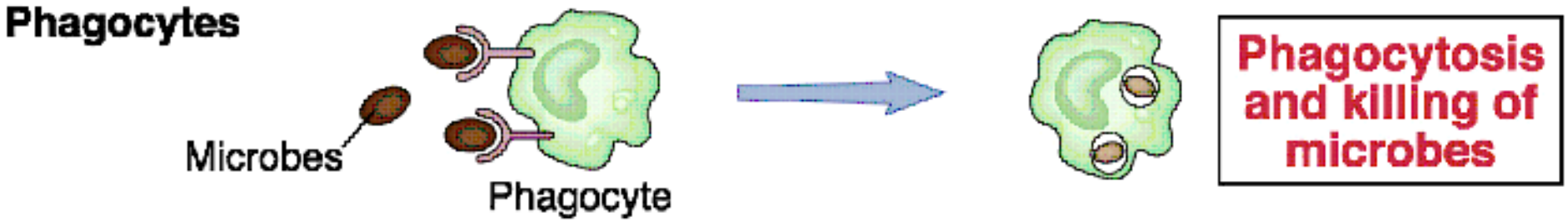
  (Physical barriers also are often thought of as part of the innate immune system)

So a you got a splinter?

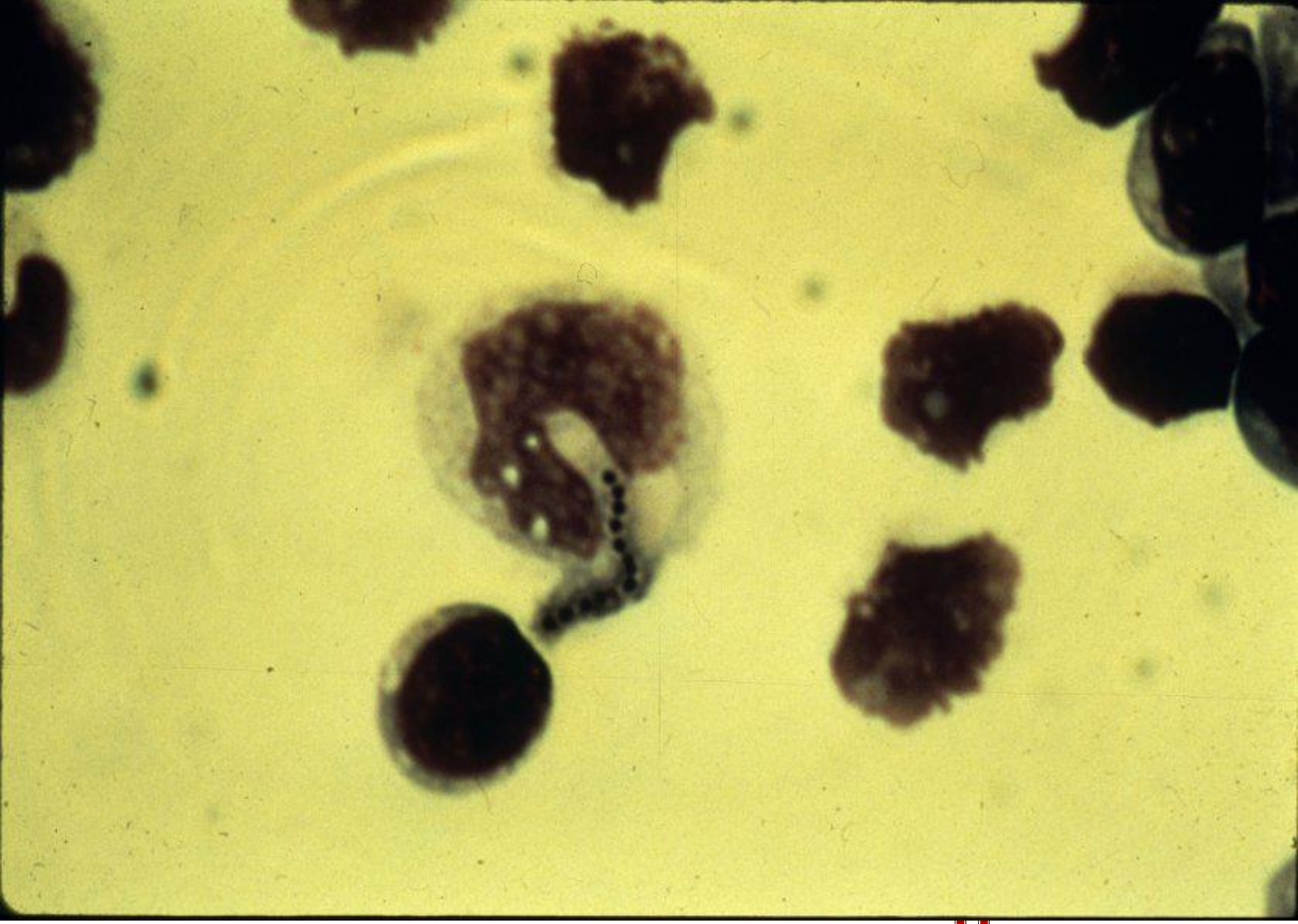
- Breaks the first line of defense
- Splinter is covered with bacteria
- What happens next?
  - Innate immune system kicks in fast, but is not specific
  - Bacteria give off signals that attract an important player in innate system – the **macrophage**



Macrophage = “big eater”



- Macrophage is waiting in tissue under the skin
- Macrophage engulfs invading bacterium and destroys it
- Process is called “phagocytosis”
- “Macro” means big and “phage” means eat – so it’s a “big eater”
- Macrophage is a “professional phagocyte”



Macrophage

- When macrophages activate, they make signaling proteins called **cytokines**
  - Cytokines signal to other immune cells that there's a problem at a particular location
  - Attract other "professional phagocytes" from blood to infected tissue to help out
    - Mainly **neutrophils**

Cytokine Communication: More than Alphabet Soup

- Cell communication via released peptides
- Low concentration
- Work through high affinity receptors
  - Can autostimulate
- Wide range of cellular effects
- Macrophages release cytokines to begin inflammatory response

Macrophage cytokine release

- IL-1: Activates vascular endothelium
- IL-8: Chemotatic factor for neutrophils
- TNF-alpha: Activates vascular endothelium & increases vascular permeability

- IL-6: Lymphocyte activation, increased antibody production
- IL-12: Activates NK cells: Induces differentiation of CD4+ T cells into TH1 cells

Cell Adhesion Molecules: Molecular Velcro

- Cell surface molecules with matching ligands on other cells
- Allow cell-to-cell binding for communication and homing
- Expression of CAMs variable and under complex control
- Example: Intercellular adhesion molecule-1 (ICAM-1) on APC's binding to lymphocyte function-associated antigen-1 (LFA-1) on T-cells

Selectin-mediated adhesion to leukocyte sialyl-Lewis^x is weak, and allows leukocytes to roll along the vascular endothelial surface

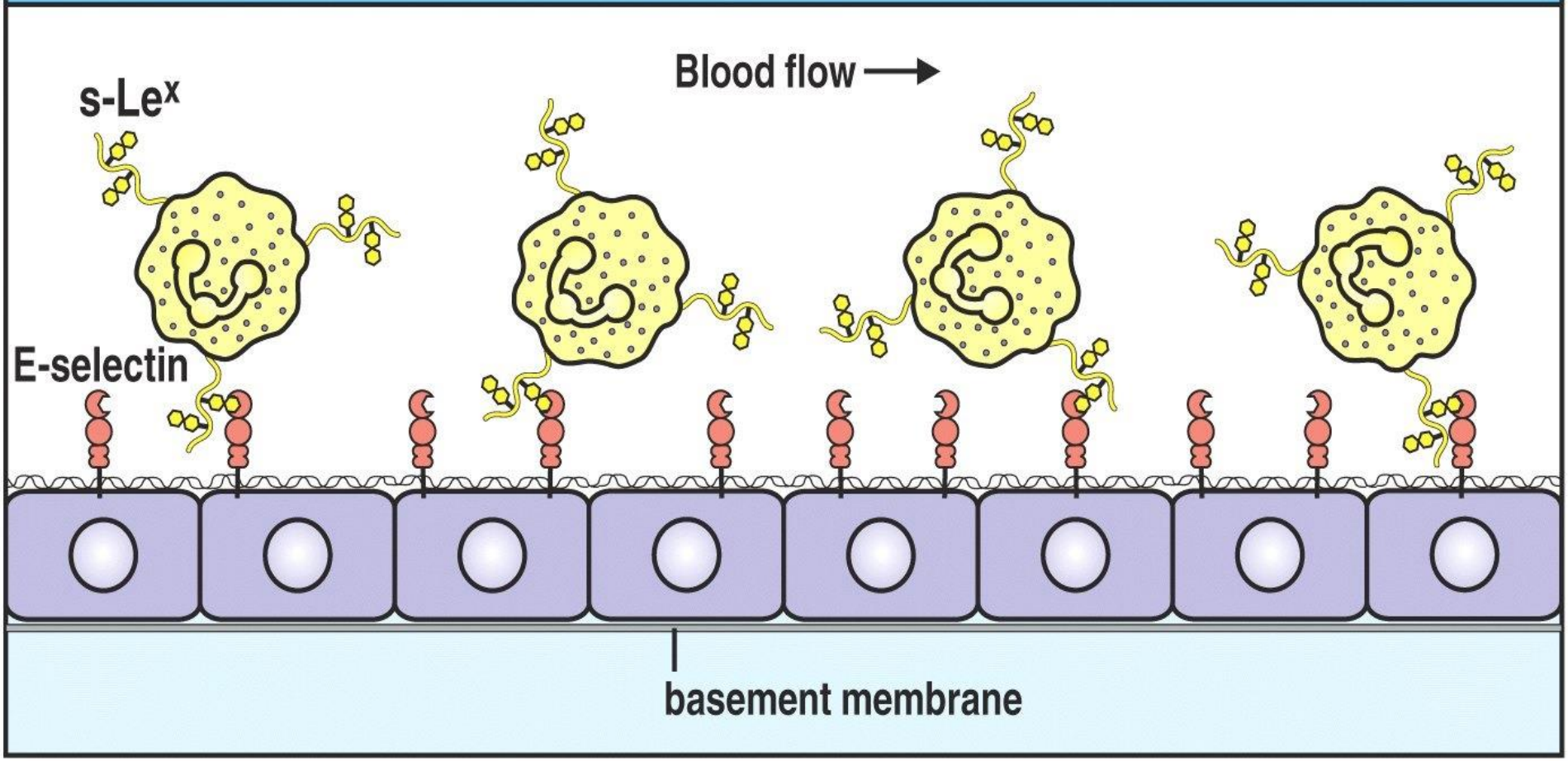
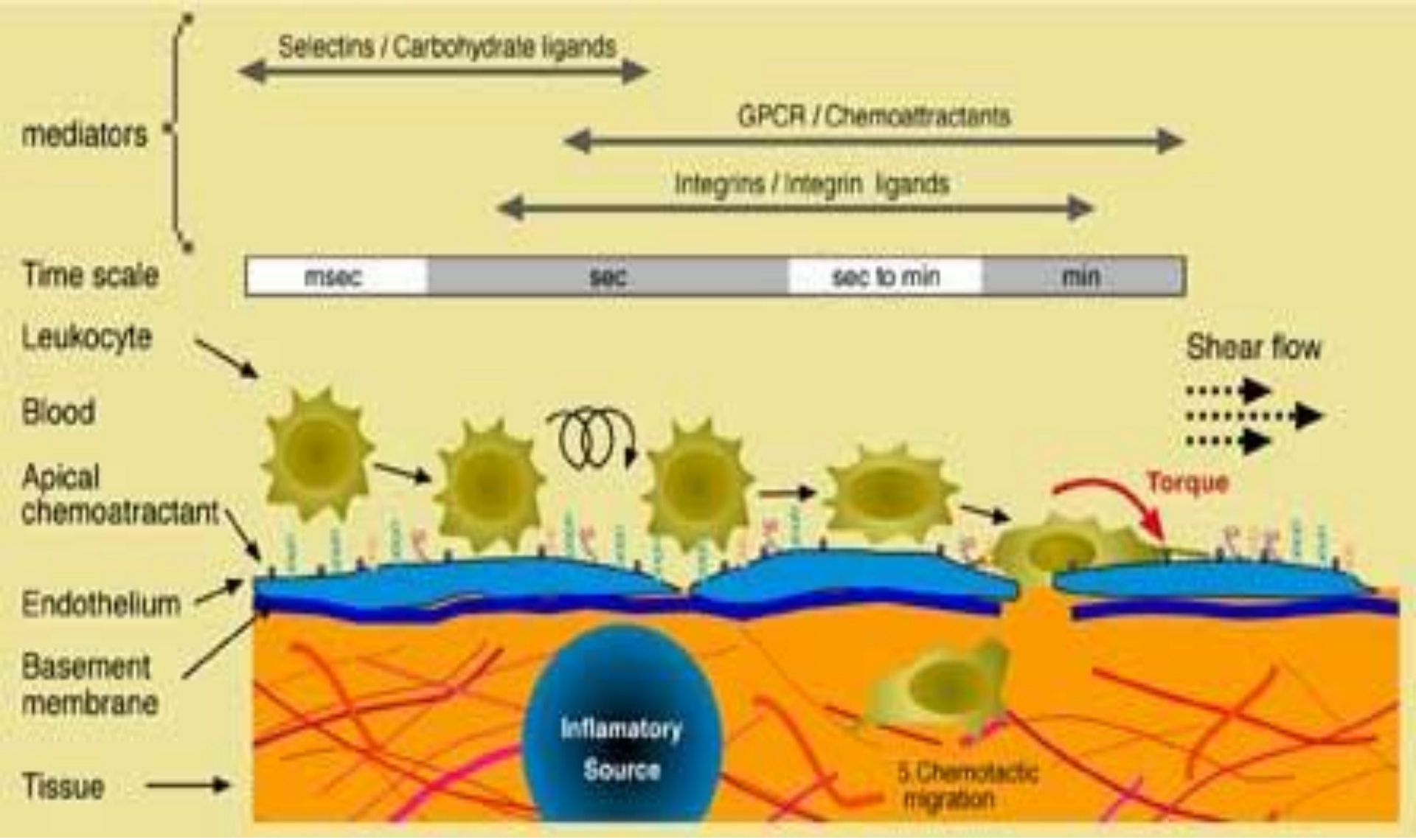


Figure 2-44 part 2 of 3 Immunobiology, 6/e. (© Garland Science 2005)


mediators
Selectins / Carbohydrate ligands
GPCR / Chemoattractants
Integrins / integrin ligands
Time scale
msec
sec
sec to min
min
Leukocyte
Blood
Apical chemoatractant
Endothelium
Basement membrane
Tissue
Shear flow
Torque
Inflamatory Source
5 Chemotactic migration

Macrophage – summary

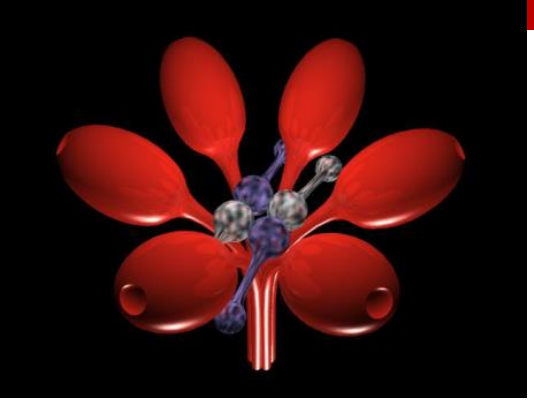
- Serve as sentinels to guard a large perimeter
- Recognizes standard invaders (bacteria) so response happens very fast
- When there's an infection, they begin by ingesting and destroying the organism and using cytokines to create an inflammatory response.
- IL-1, TNF-alpha and IL-8 magnify the innate immune response
- Antigen Presenting Cell (APC): the activated macrophage may become an APC and "home" to the lymph node (more on this later)

Other cells of innate immune system

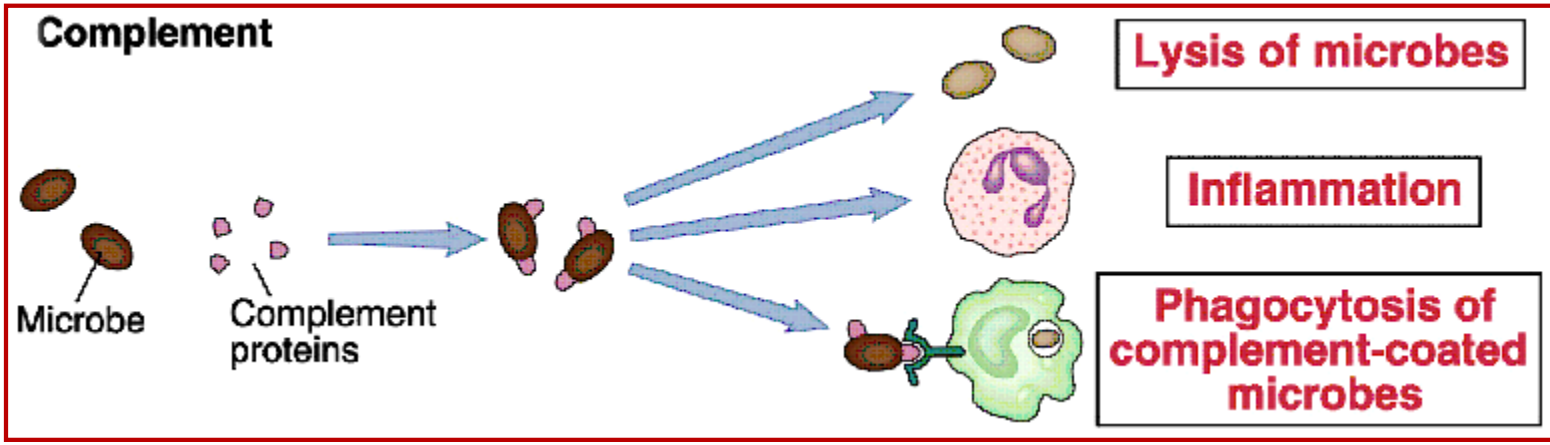
- Professional phagocytes
  - Macrophage
  - Neutrophil
- Eosinophil, Basophil, Mast Cell
  - Killers of parasites
- Natural Killer Cell
  - killers of host cells “gone bad”
  - Protects against cancers and viruses

What if bacteria escape into bloodstream?

- Another part of ***innate*** system kicks in
- **Complement** proteins are always present in blood
- "Naturally" recognize standard foreign invaders like bacteria
  - Punch holes in invaders
  - Attach "tags" onto invaders so can be easily phagocytosed – called **opsonization** (which means to "prepare for eating")



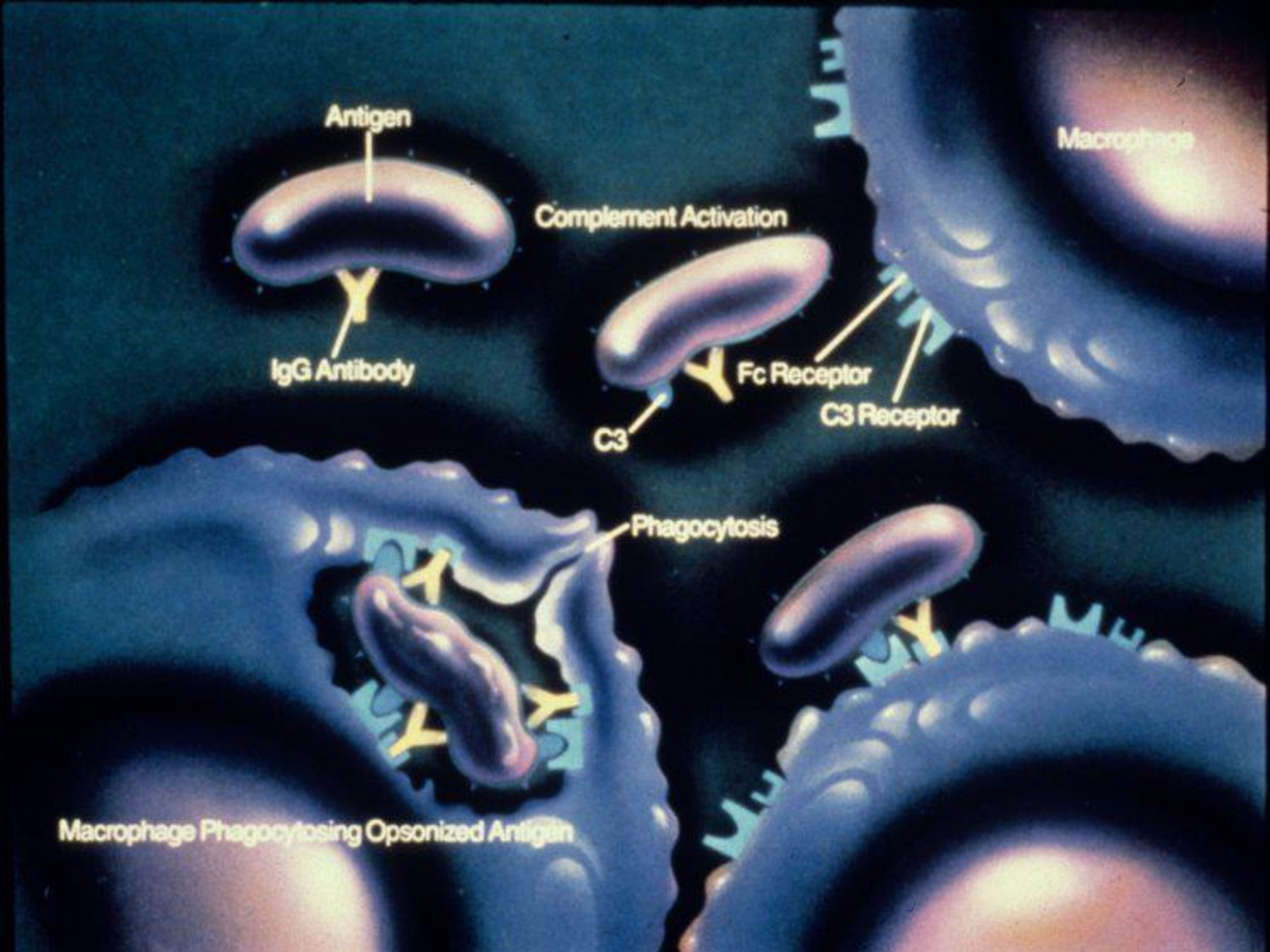
Complement Proteins



- Membrane Attack Complex: can cause lysis of microbes
- Allows more efficient phagocytosis

Three Complement Pathways

- **Classical Pathway**
  - C1, C4, C2, C3
  - Antigen-antibody complexes
  - IgM (most effective) and IgG bind complement
- **Mannan-binding Lectin Pathway**
  - Mannan-binding lectin binds mannose on pathogen surfaces
  - MBL, MASP, C4, C2, C3
    - MASP (mannan-binding lectin-associated serum protease)
- **Alternative pathway**
  - Binds to pathogen surface
  - Amplifies effects of the Classical Pathway
  - C3b, B, D, C3
- Although they initiate differently ALL pathways converge at C3 convertase


Antigen
IgG Antibody
Complement Activation
C3
Fc Receptor
C3 Receptor
Macrophage
Phagocytosis
Macrophage Phagocytosing Opsonized Antigen

ABC's of complement

- A is for anaphylatoxin (smaller cleavage fragment)
  - C3a, C4a and C5a are peptide mediators of local inflammation
  - C5a is the most active
  - C4a is relatively weak
- B is for binding (larger cleavage fragment)
  - C3b binds to complement receptors on phagocytes and allows for effective opsonization of pathogens
  - C5b associates with the bacterial membrane and forms membrane attack Complex
  - C4b is a weak opsonin

Adaptive Immune Response

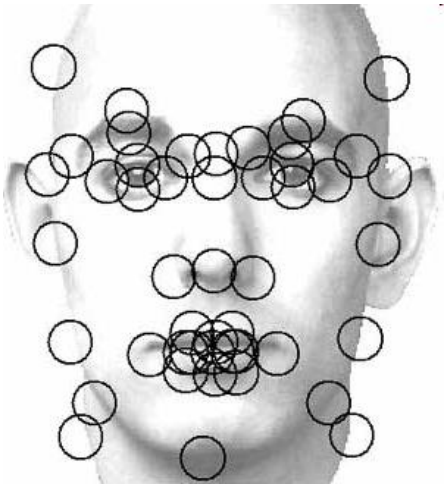
- Once immune system has been active for a little time, it learns from the battle and fine tunes its response: the adaptive immune response is "turned on"
- The fine tuned response is more specifically targeted against particular invaders and more effective
- Called **adaptive immune system** because it "adapts" to the specific invader
- Hallmark is specificity

Cells of the adaptive immune system

- Cell-mediated immunity
  - T cells
    - T helper cells
    - T killer cells
    - T regulatory cells
  - Viral, fungal, intracellular infections
- Humoral immunity
  - B cells
  - Antibodies
  - Extracellular bacterial infections

Immune system Components
Specific: Lymphocytes

- Recognition: Antigen Specific
  - B-cell
  - T-cell
- Each receptor on cell is identical
- Need 10-100 million different and unique lymphocytes



Lymphocytes

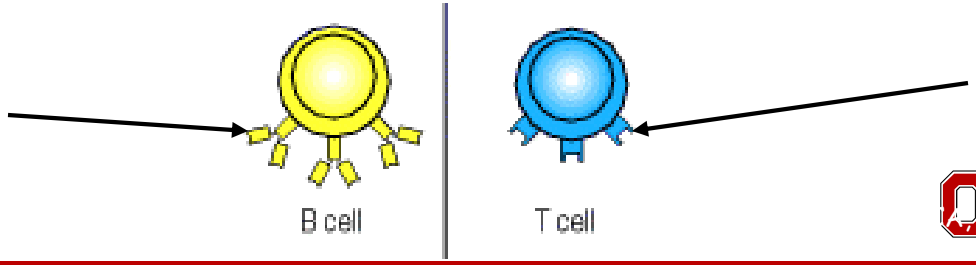
- Unique antigen receptor constructed early
- Selected and activated by non-self proteins
- Clones persist (memory cells)
- Lymphocytes with self-recognizing receptors are culled

B-cells

- Mature in bone marrow
- Lymphoid follicle
- Antigen receptor: Immunoglobulin molecule

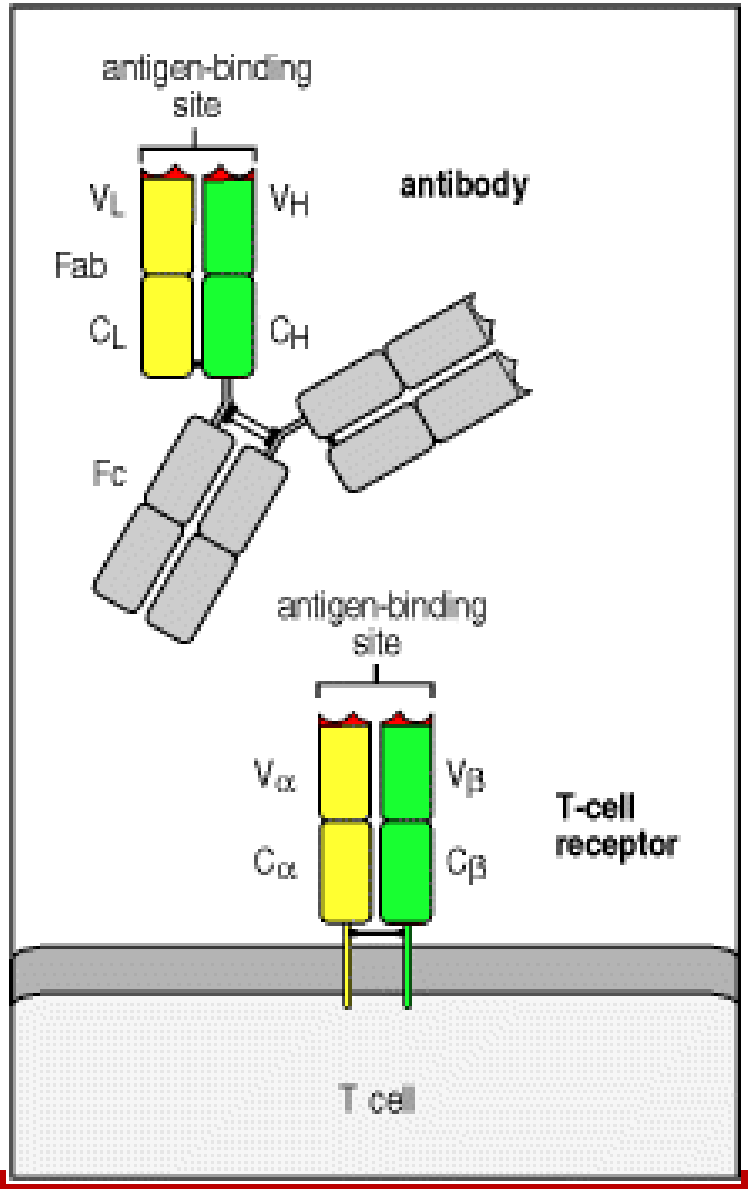
T-cells

- Mature in thymus
- Paracortical area
- Antigen receptor: T-cell receptor



Adaptive Immunity

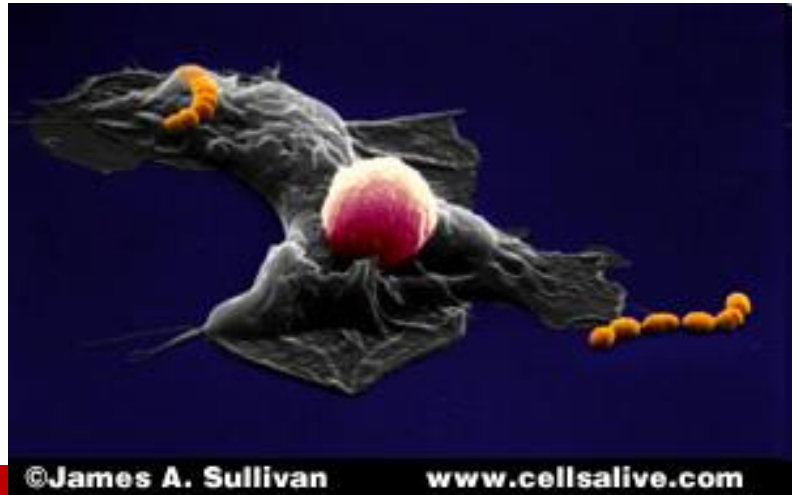
Antigen Receptors



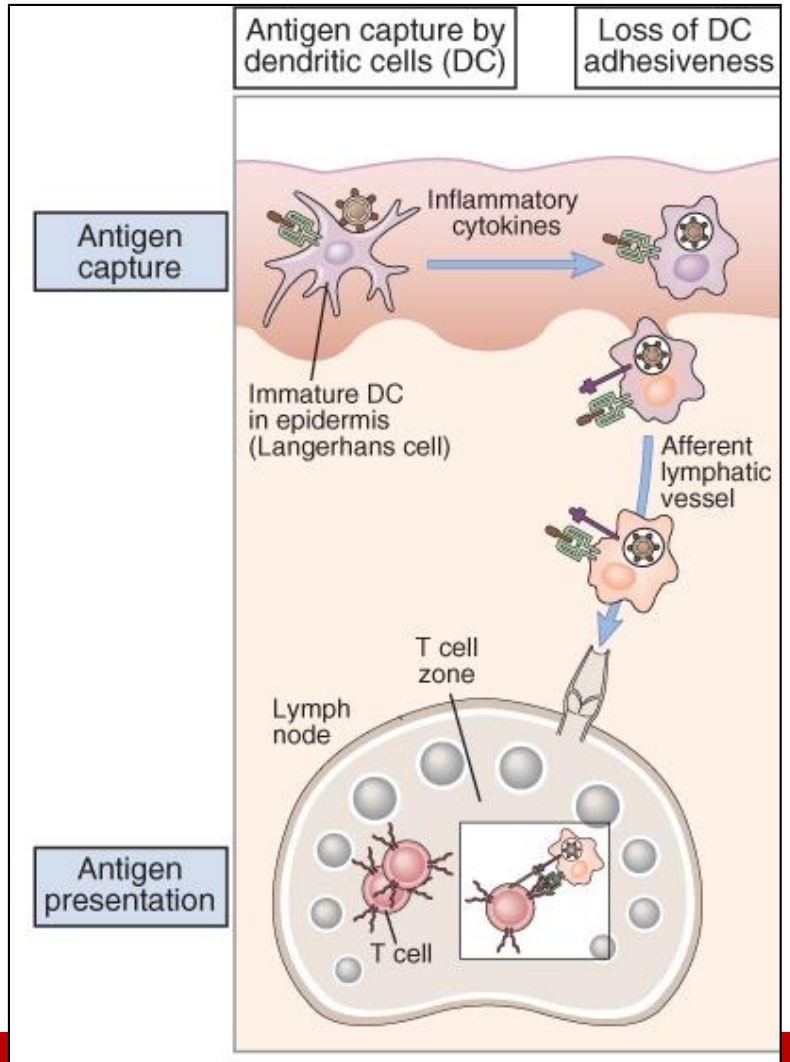
From Janeway, CA, *Immunobiology*, 5th ed.

Antigen stimulation of T cell

- Specific T cell proliferates in response to its matching antigen…but only when antigen is "presented" properly
- This is done by Antigen Presenting Cells (APCs)
  - Dendritic cells, Macrophages, B cells
- APCs phagocytose antigen, then "present" to T cell in lymphoid organs.



Antigen capture and presentation



Adaptive Immunity

"Professional" Antigen Presenting Cells

- Dendritic cells, macrophages, B-cells
- Efficiently process antigens
- Antigen peptides fit in MHC cleft
- MHC:peptide complex to cell surface
- Provide costimulatory 2nd signal

MHC Molecules

- Function: Bind processed antigen and transport to cell surface
- MHC I:
  - All nucleated cells
  - Process Ag from cytosolic compartment
  - Present to CD8+ cytotoxic T-cells
  - HLA-A, B, C
- MHC II:
  - Dendritic cells, macrophages, B-cells
  - Process Ag from vesicular compartment
  - Present to CD4+ helper T-cells
  - HLA-DR, DP, DQ

How are specific T cells produced?

- Like antibodies, human starts off with about 100 million different kinds of T cells
- Mature in thymus (thus T cell) where they undergo positive and negative selection
  - Don't recognize self: must be destroyed
  - High affinity for self (attack self): must be destroyed
- When pathogen stimulates one specific T cell, then just that one clone proliferates (clonal selection)
- For selection:
  - Approximately 1 trillion T cells in body.
  - 1 trillion T cells/100 million specificity = 10 thousand T cells/specificity

So now that specific T cells proliferate, what do they do?

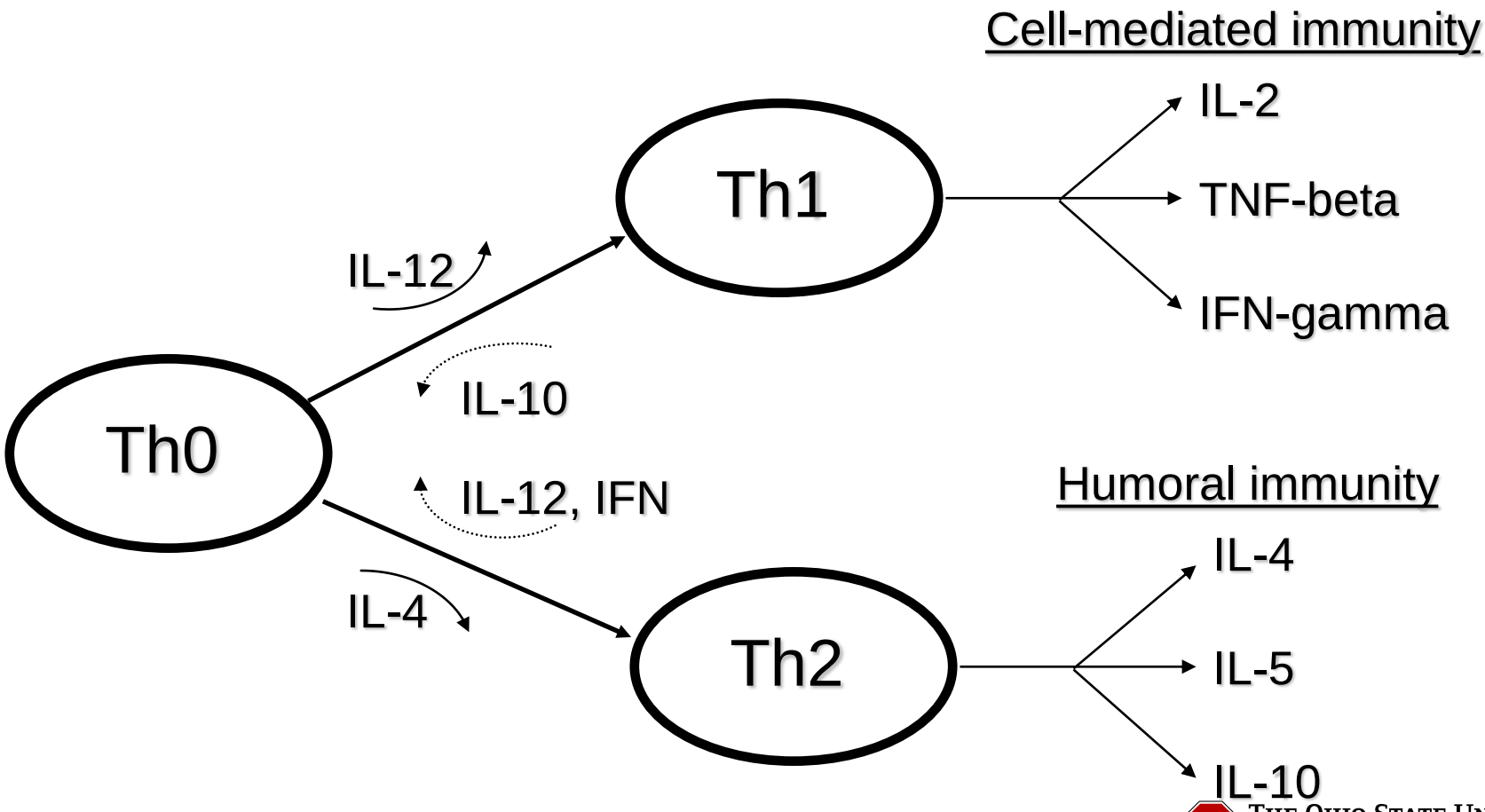
- CD8+ Killer (or cytotoxic) T cells
  - Kill virus-infected host cell
  - So invading virus can be destroyed even after inside cell!
- CD4+ Helper T cells
  - Direct immune response by sending out various signals (cytokines) – “Quarterback” of immune team
  - “Help” other cells do their thing
- Regulatory T cells
  - Involved in suppressing other immune cells

CD8+ Cytotoxic T-cell

- Directly cytotoxic to cells via binding to Ag:MHC I complex
- Cytosolic antigens (e.g., viruses)
- Induces apoptosis
- Cytotoxicity is specific and directional
- Cytotoxins include:
  - Perforin, granzymes
- Also produces cytokines
  - IFN-γ, TNF

CD4+ Th1/Th2 Paradigm

CD4+ T cell Binds to APCs via Ag:MHC II complex



CD4+ Helper T-Cells: Th1/Th2 Paradigm

- Th1 (type 1)
  - IL-2, TNF, IFN-γ
  - Activate macrophages and CTL's for intracellular pathogen killing and cytotoxicity
  - Facilitate cell-mediated immunity
  - Inhibit Th2 cell proliferation

CD4+ Helper T-Cells: Th1/Th2 Paradigm

- Th2 (type 2)
  - IL-4, 5, 10
  - Activate B cells and antibody production to neutralize extracellular pathogens & toxins
  - Facilitate humoral immunity
  - Inhibit Th1 cell proliferation

What Determines Th1 vs. Th2 Response?

- Type of pathogen
- Innate immune response to it
  - Macrophages, NK cells release IL-12, IFN-γ: TH1
  - Mast cells, basophils, $\gamma\delta$ T cells release IL-4: TH2
- Host's immune constitution
- Density of Ag presented on APC
  - High density Th1
  - Low density Th2

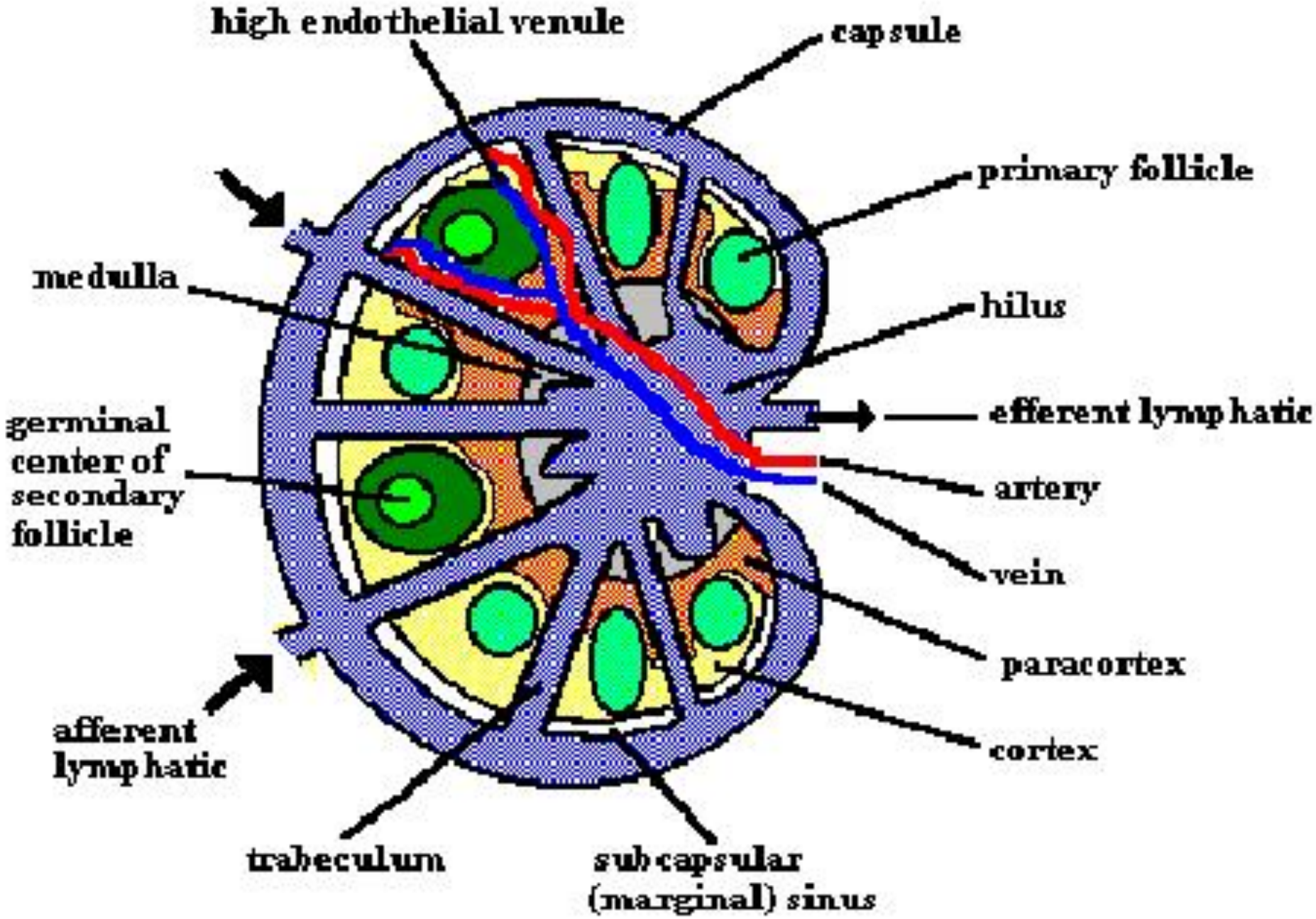
Where does adaptive immune response occur in the body?

- Only a few T cells and B cells specific for a particular antigen
- So how does antigen and matching antibody ever meet to stimulate specific response?
- Meet in secondary lymphoid organs
  - Lymph nodes, spleen, tonsils, appendix

("Primary" = bone marrow and thymus)

Lymph nodes are adaptive immunity meeting areas

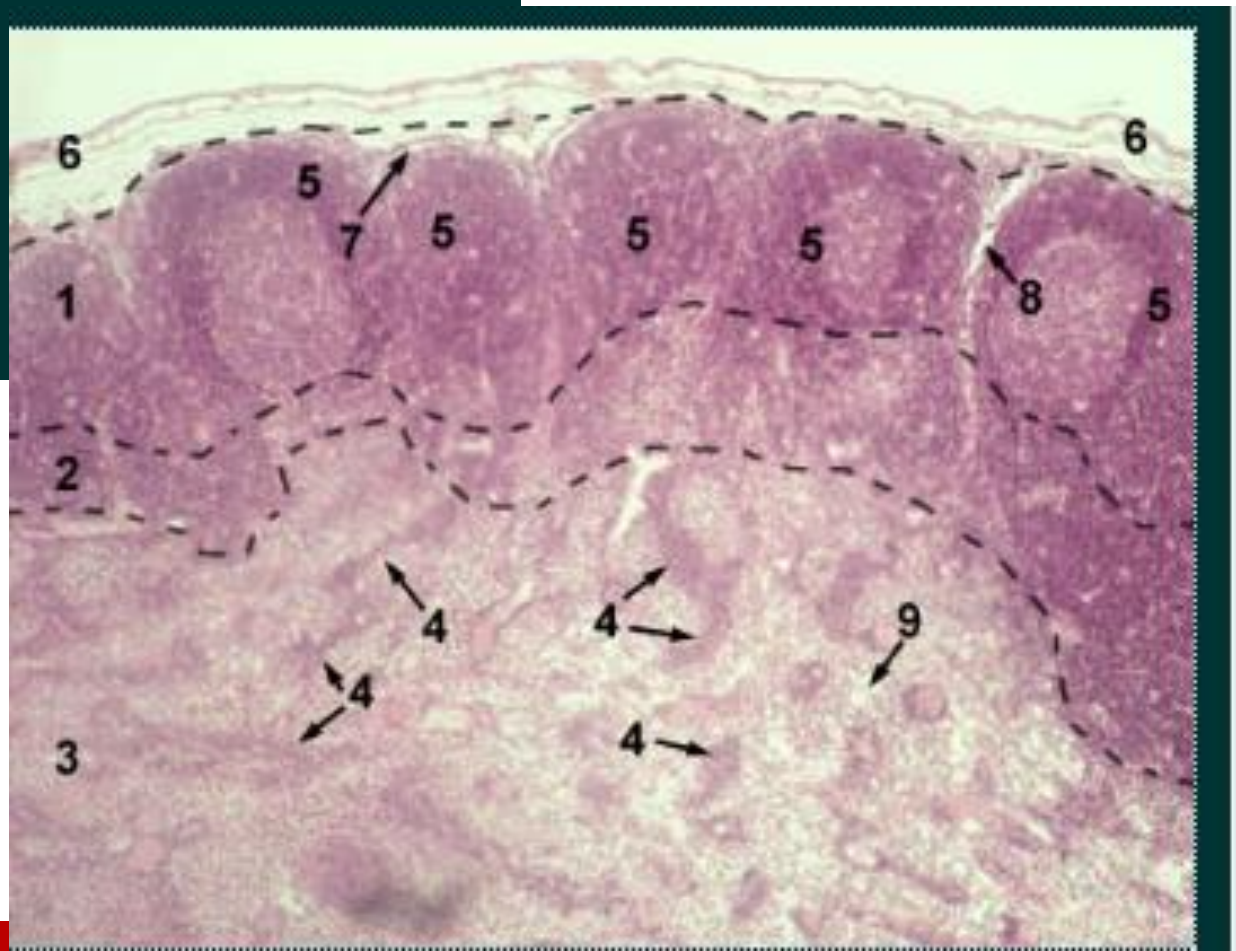
- pathogens in tissue drain with lymph fluid to lymph nodes
- Activated APCs “home” to lymph node
- Meanwhile, T cells and B cells circulate from node to node looking for match
- Coming together in lymph nodes makes matching much more likely
- This is why lymph nodes get swollen

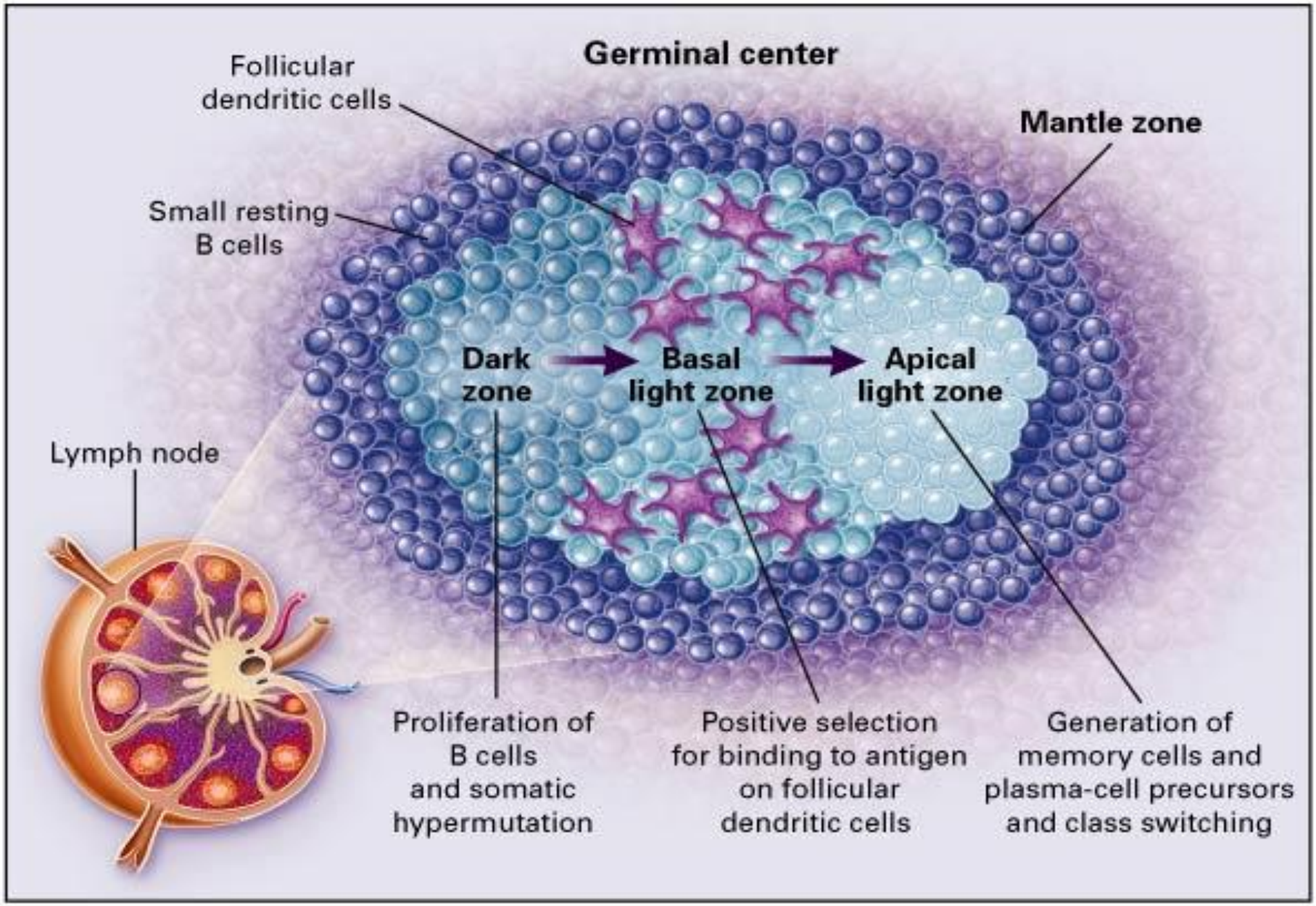

high endothelial venule
capsule
primary follicle
medulla
hilus
efferent lymphatic
germinal center of secondary follicle
artery
vein
paracortex
afferent lymphatic
cortex
trabeculum
subcapsular (marginal) sinus

LYMPH NODE

Stained with haematoxylin and eosin

1 - cortex
2 - paracortical zone
3 - medulla
4 - medullary cords
5 - lymphoid follicle of the cortex
6 - capsule
7 - subcapsular sinus
8 - cortical sinus
9 - medullary sinus



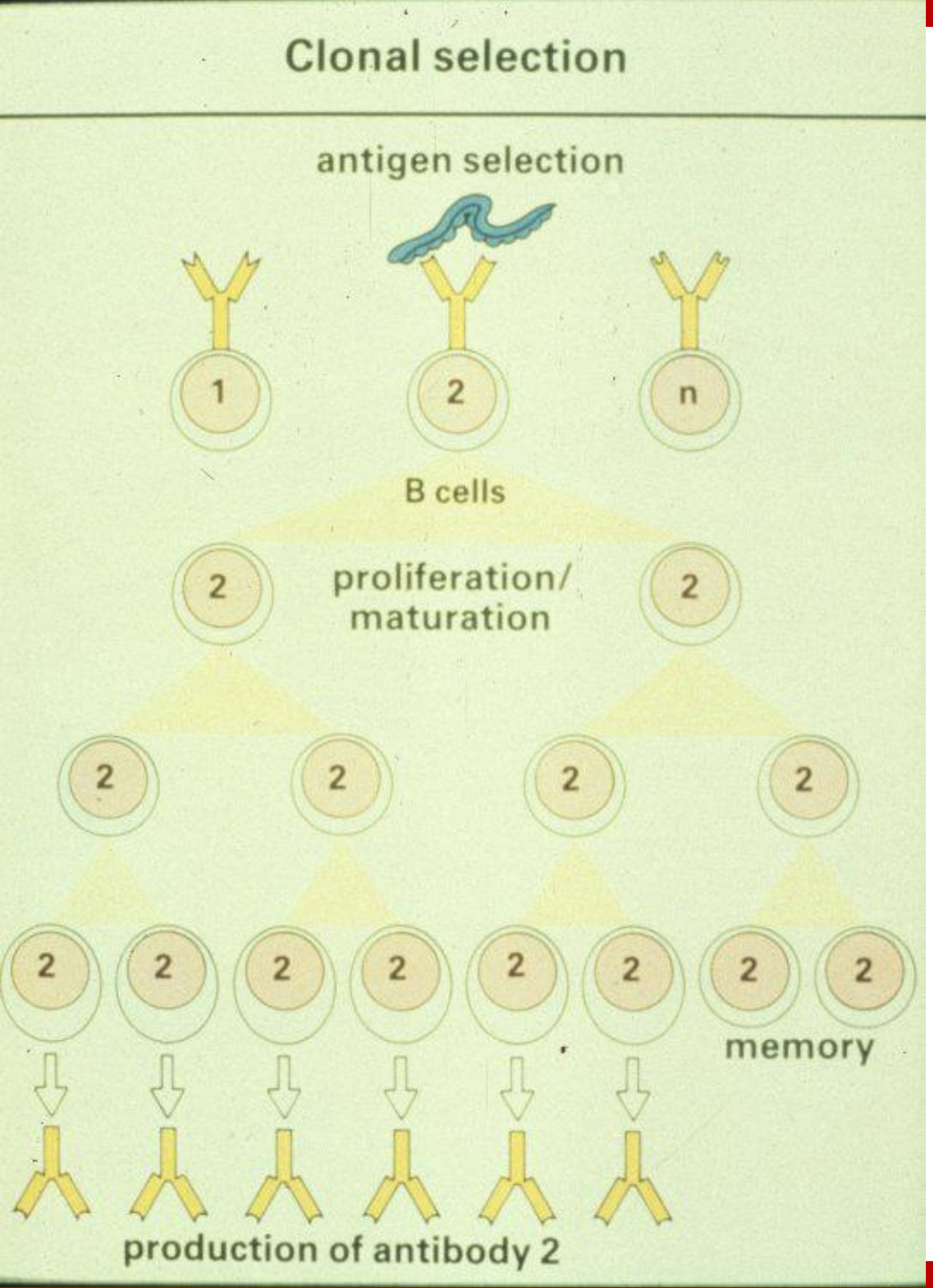

Germinal center
Follicular dendritic cells
Mantle zone
Small resting B cells
Dark zone
Basal light zone
Apical light zone
Lymph node
Proliferation of B cells and somatic hypermutation
Positive selection for binding to antigen on follicular dendritic cells
Generation of memory cells and plasma-cell precursors and class switching

Humoral Immune Response

- B cells, when stimulated, turn into antibody factories called plasma cells
- Takes about a week to start making antibody after first exposure to new antigen
- B cells come from bone marrow stem cells

How is specific antibody produced?

- Need about 100 million different kinds of antibodies to bind to every possible pathogen
- Your body actually starts off with that many different specificities of antibody!
- When pathogen (antigen) binds to one specific antibody, then only that antibody clone gets reproduced – clonal selection

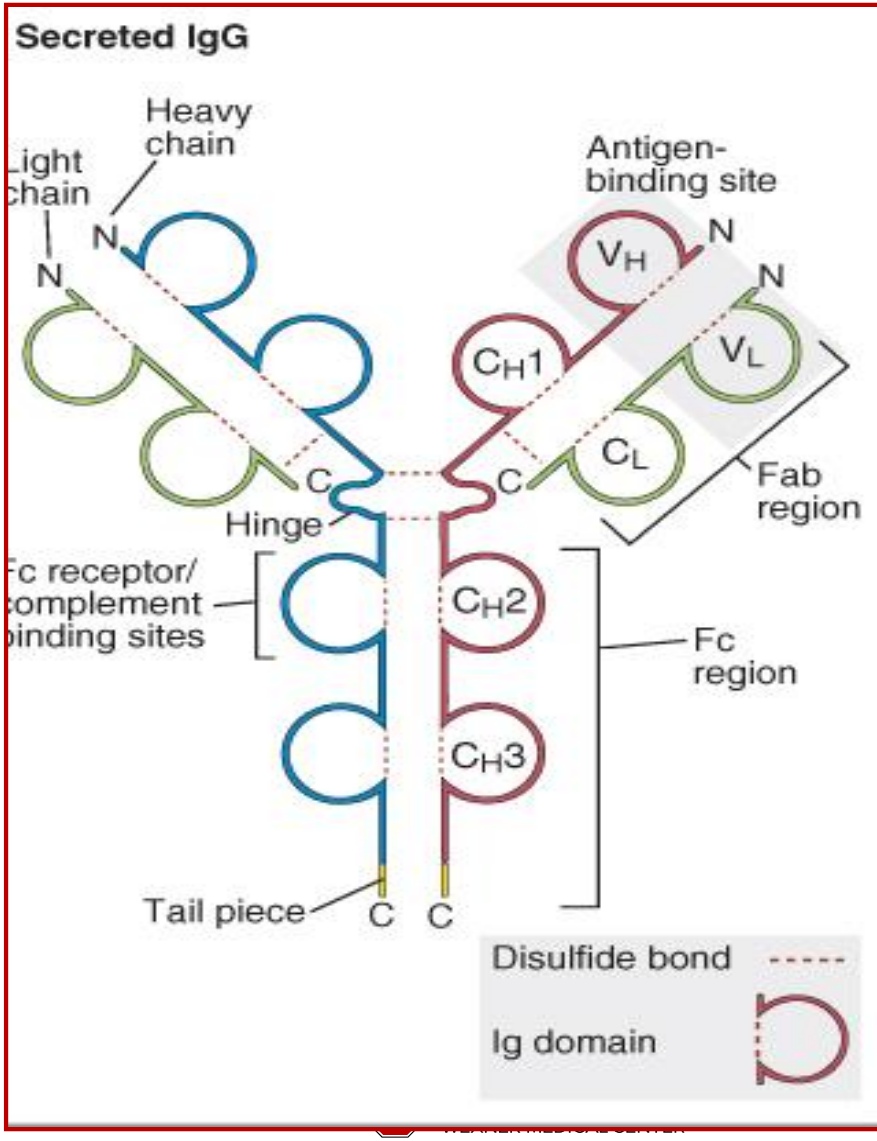


Clonal Selection

- 1950's theory
- Mount an overwhelming immune response
  - When needed
  - Can be "turned on and off"

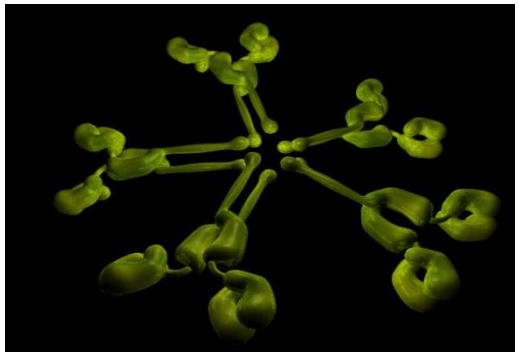
Antibodies

- Shaped like “Y”
- Two heads specific for particular antigen
- Base determines what class of antibody
- 5 classes (IgG, IgM, IgA, IgE, IgD)
- Class determines how antibody will function

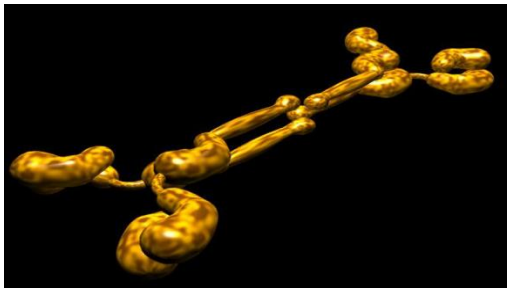


Immunoglobulins

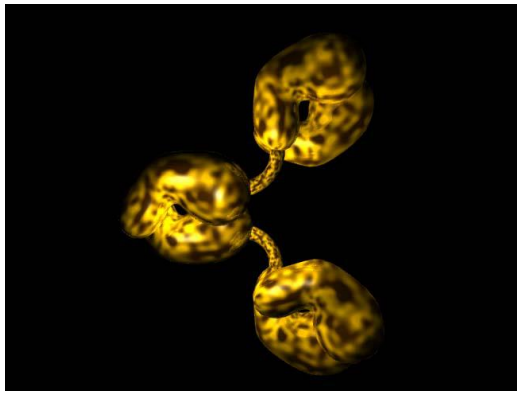
- Pentamer
  - IgM



- Dimer
  - IgA

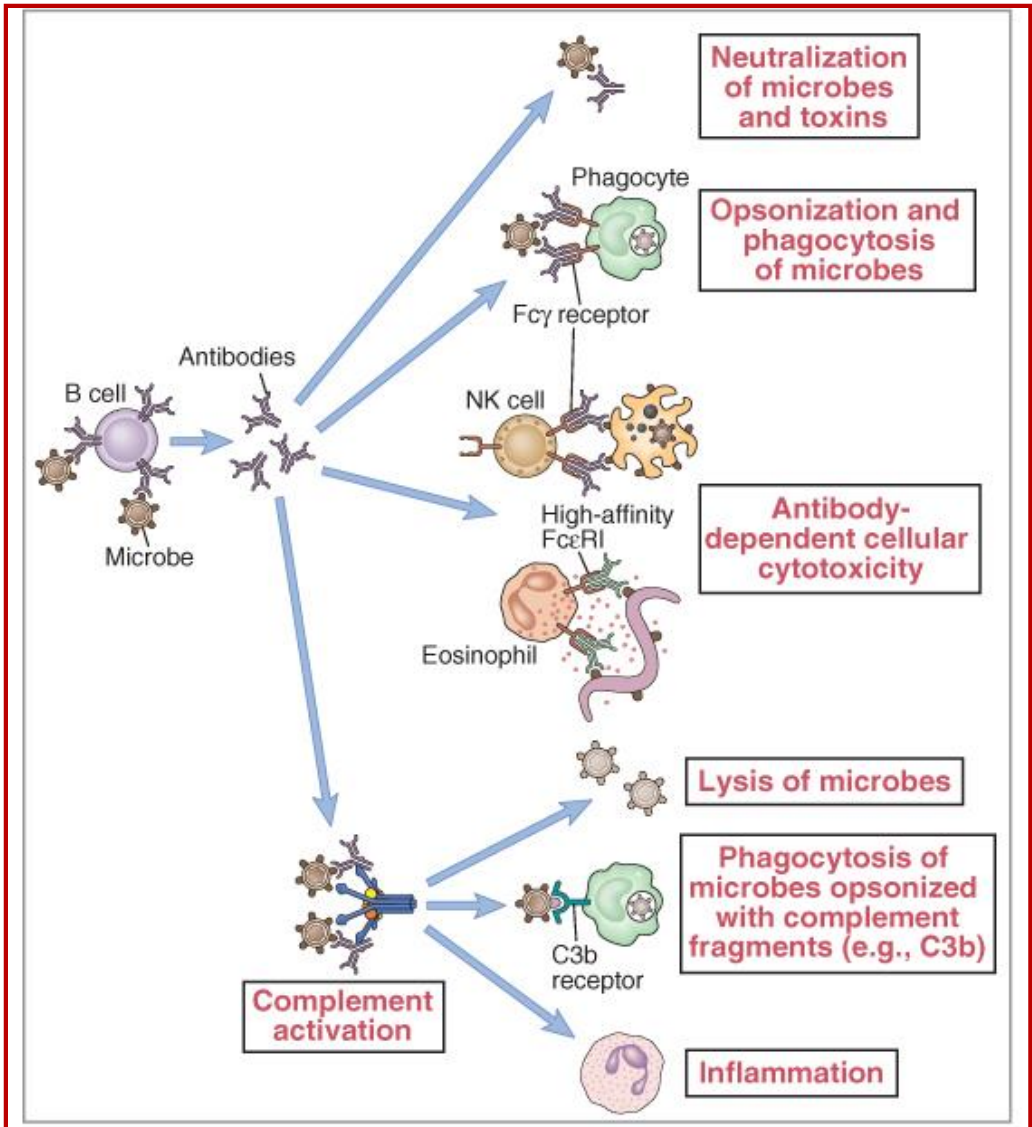


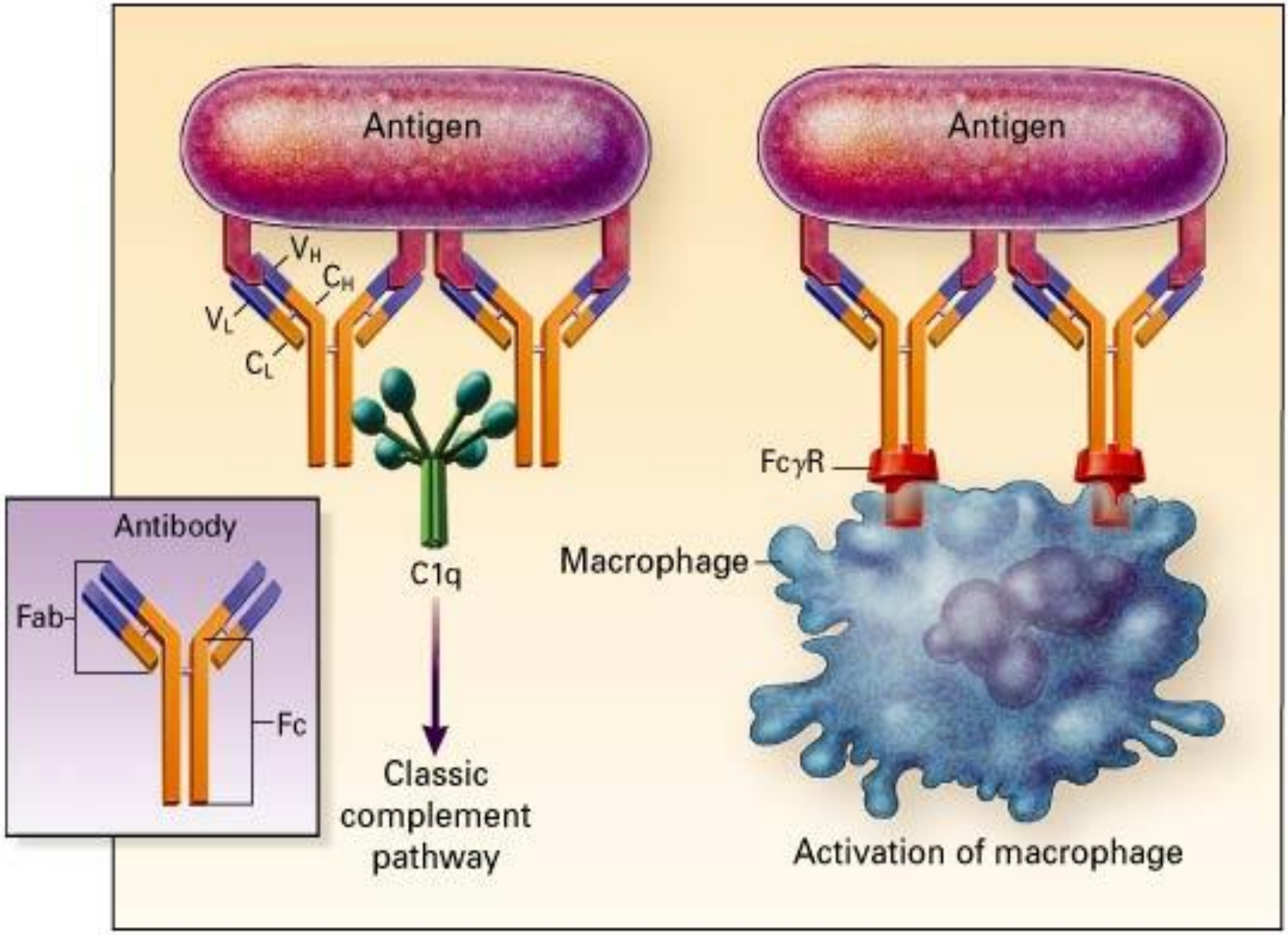
- Monomers
  - IgG, IgE, IgD



What do antibodies do?

- Don’t kill anything
- Attach to invader while it is outside a host cell and “tag” it for phagocytosis (opsonization)
- Can also “neutralize” antigen while it is outside a host cell so that it can’t function properly

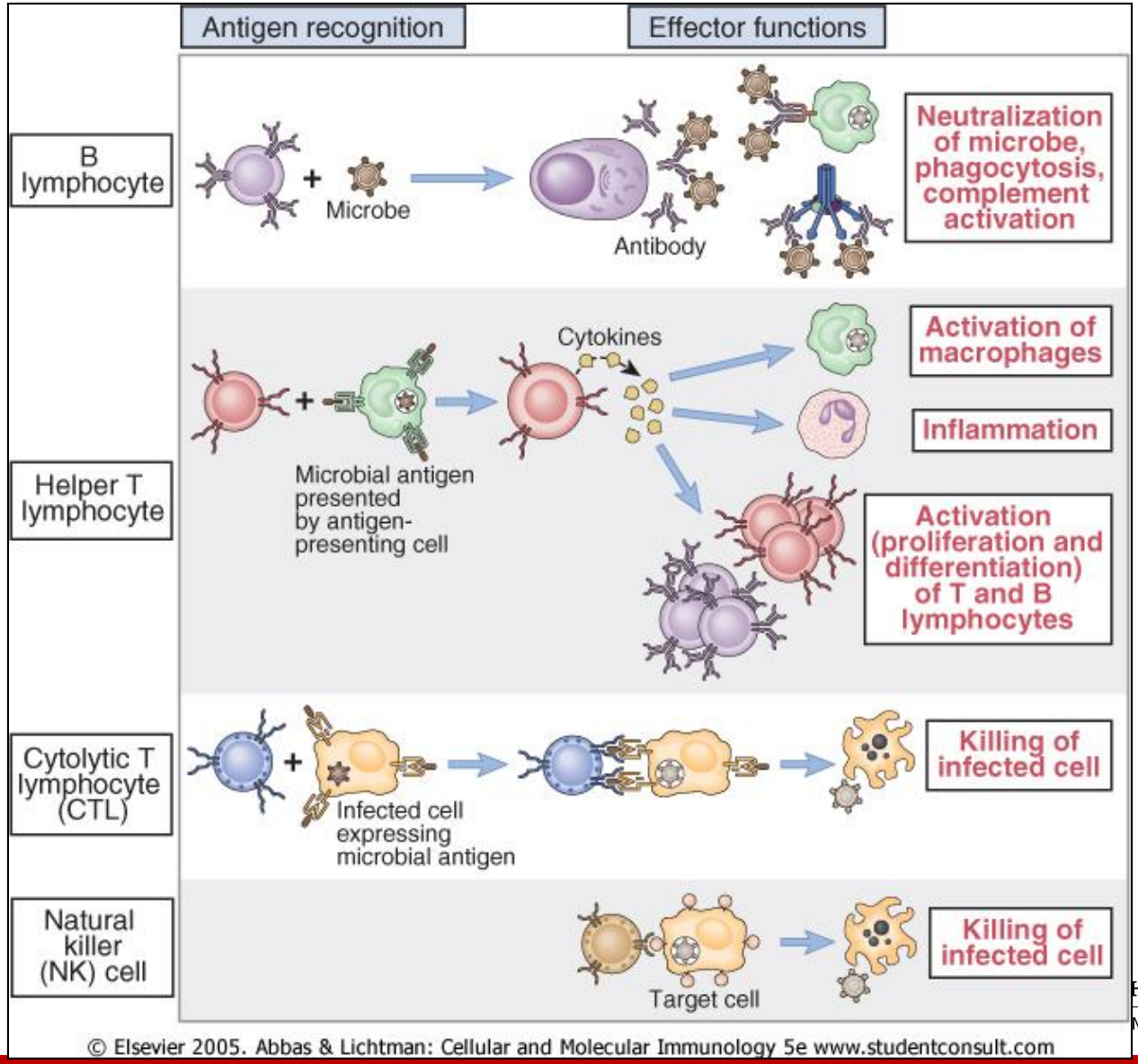



Antigen
V_H
C_H
V_L
C_L
C1q
Classic complement pathway
Antibody
Fab
Fc
Antigen
FcγR
Macrophage
Activation of macrophage

Cell-mediated immunity (T cells)

- There's a flaw in antibody defenses against viruses as opposed to most bacteria
- Once virus gets inside host cell, antibodies can't get to it
- T cells are the solution

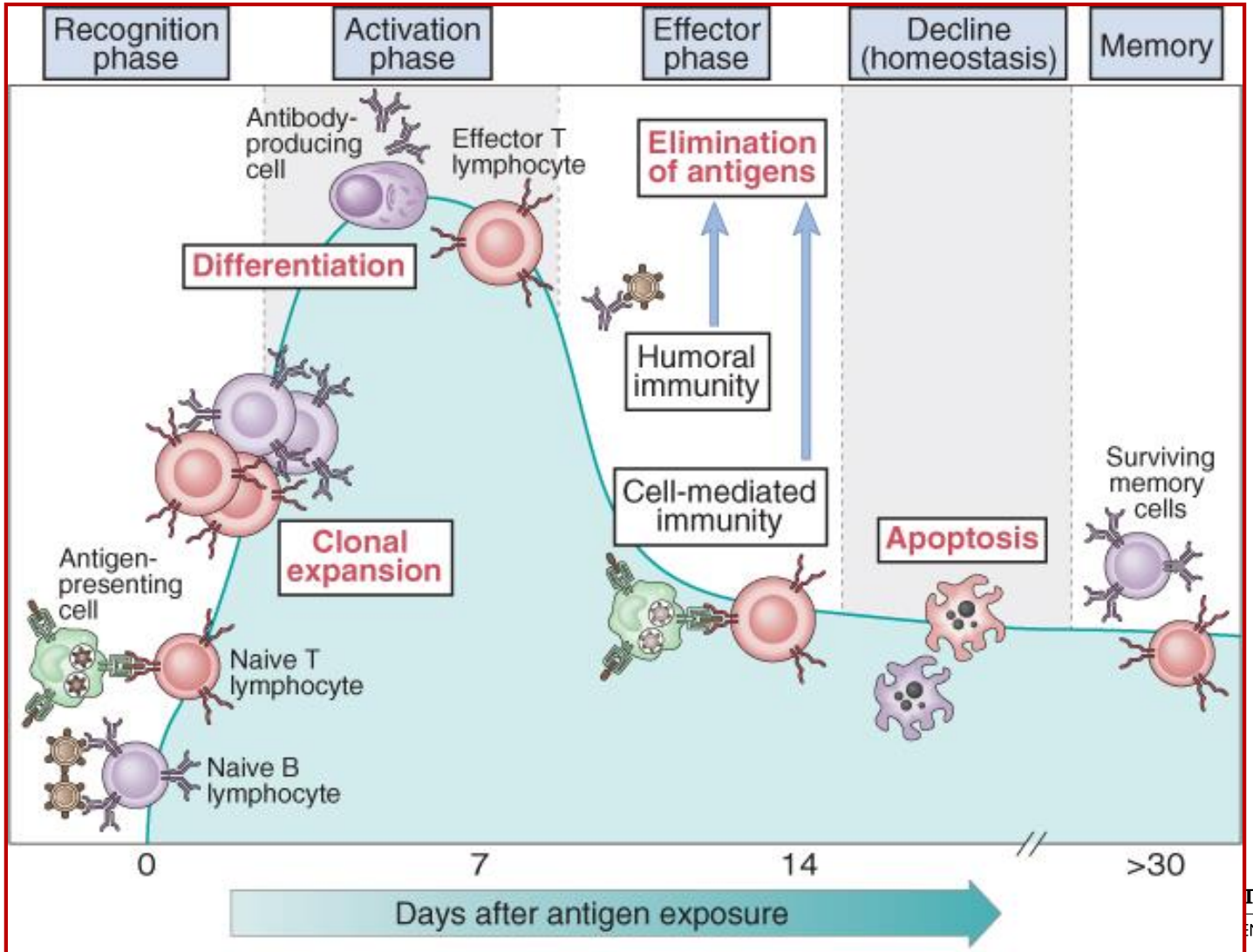
Cells of the immune system



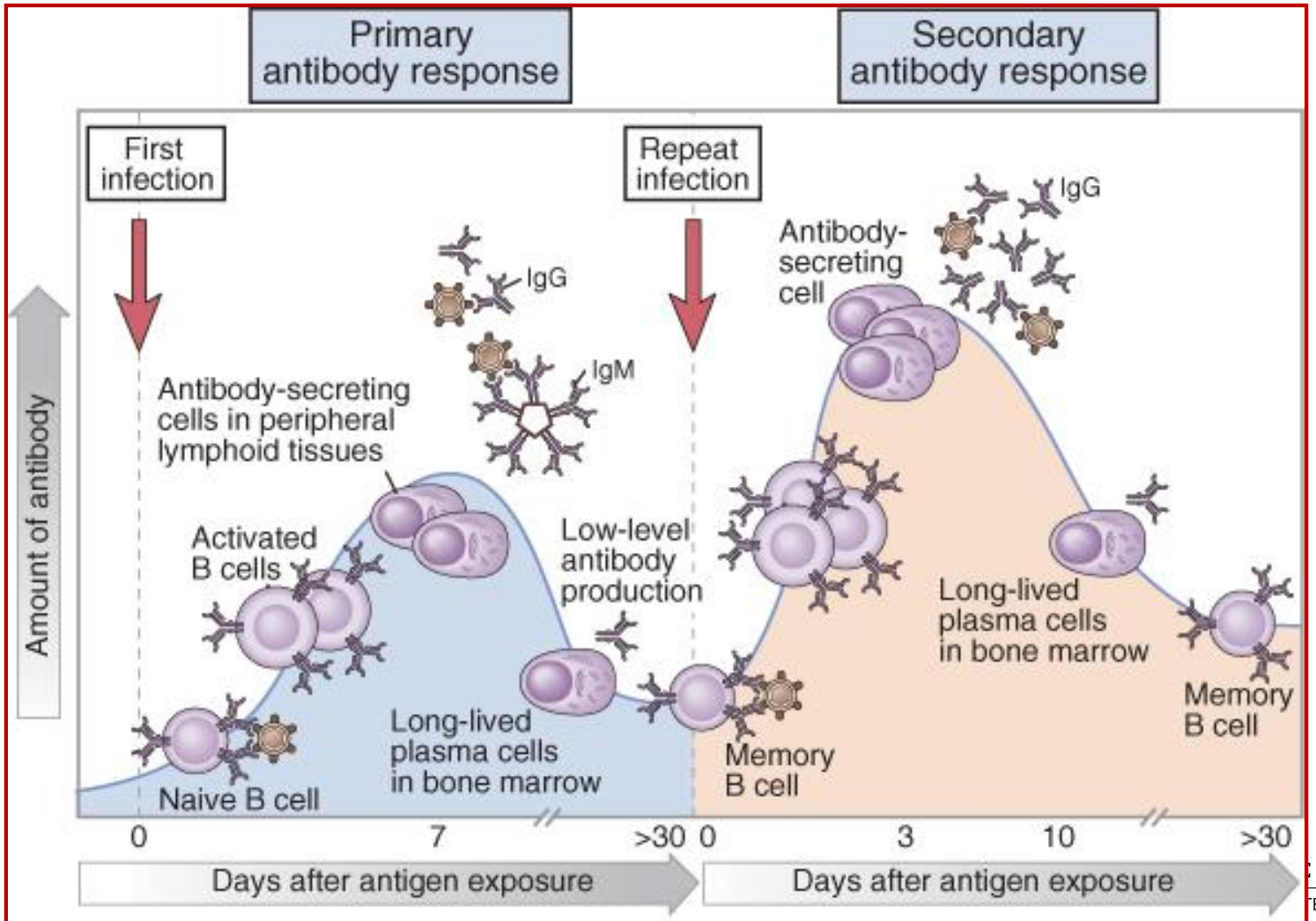
Adaptive immunity also has memory

- Once invader has been eliminated by adaptive immune response, then most of specific T cells and Plasma cells die
- A few stick around and serve as memory cells
- Next time the same invader comes, adaptive immune system kicks in much faster using memory cells – you're "immune!"

Distinct phases of immune response



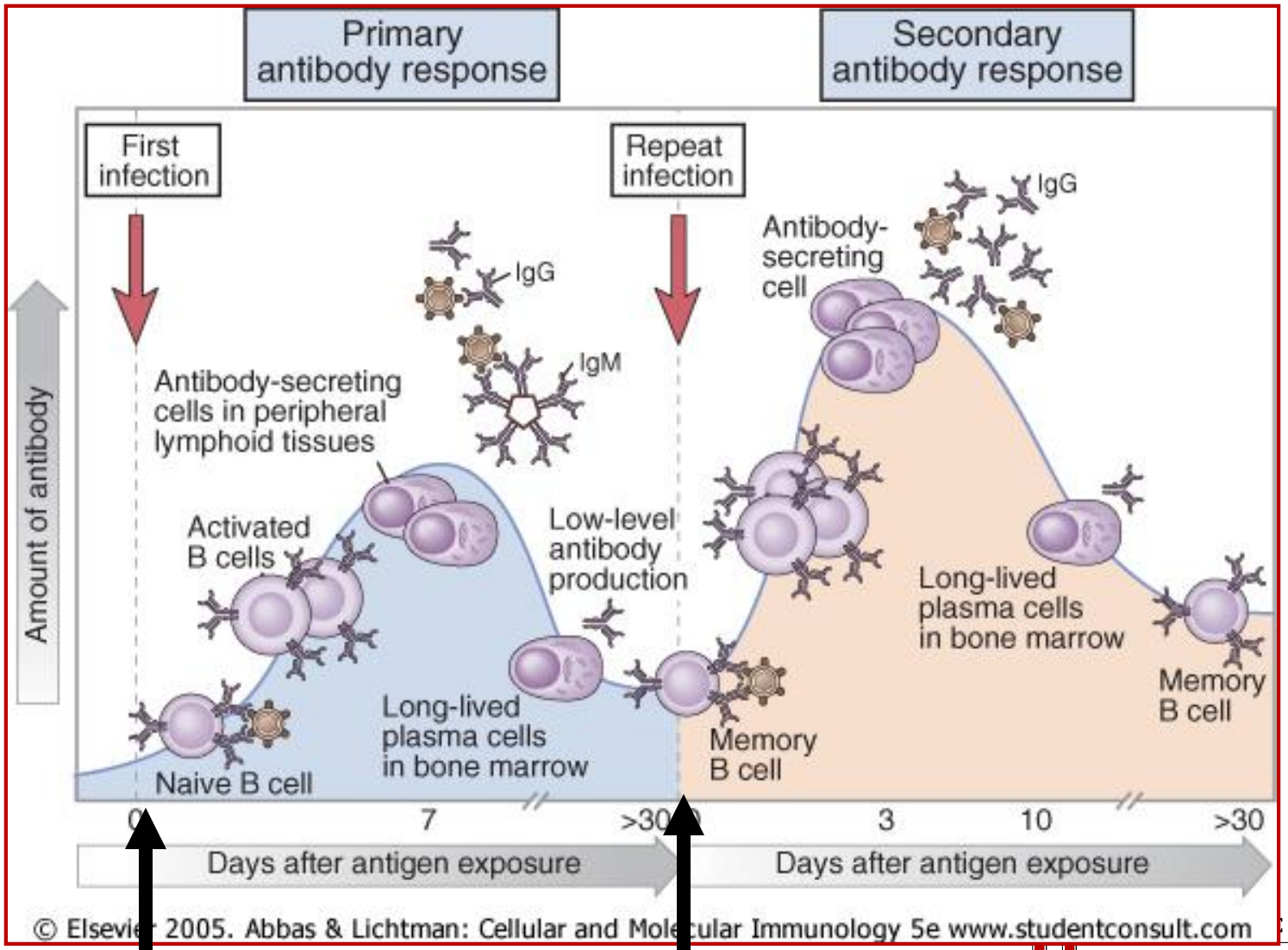
Primary and secondary response



What happens for example with a vaccination?

- Foreign “invader”
- Innate immunity
  - Macrophages eat, recruit neutrophils, cause inflammation, triggers adaptive immune system
- Adaptive immunity
  - Dendritic cells phagocytose, present antigen to specific T cells which proliferate and “help”
  - Antigen stimulates specific B cells to produce antibody
  - Since there’s nothing to fight, then become “memory cells” – you’re immune!

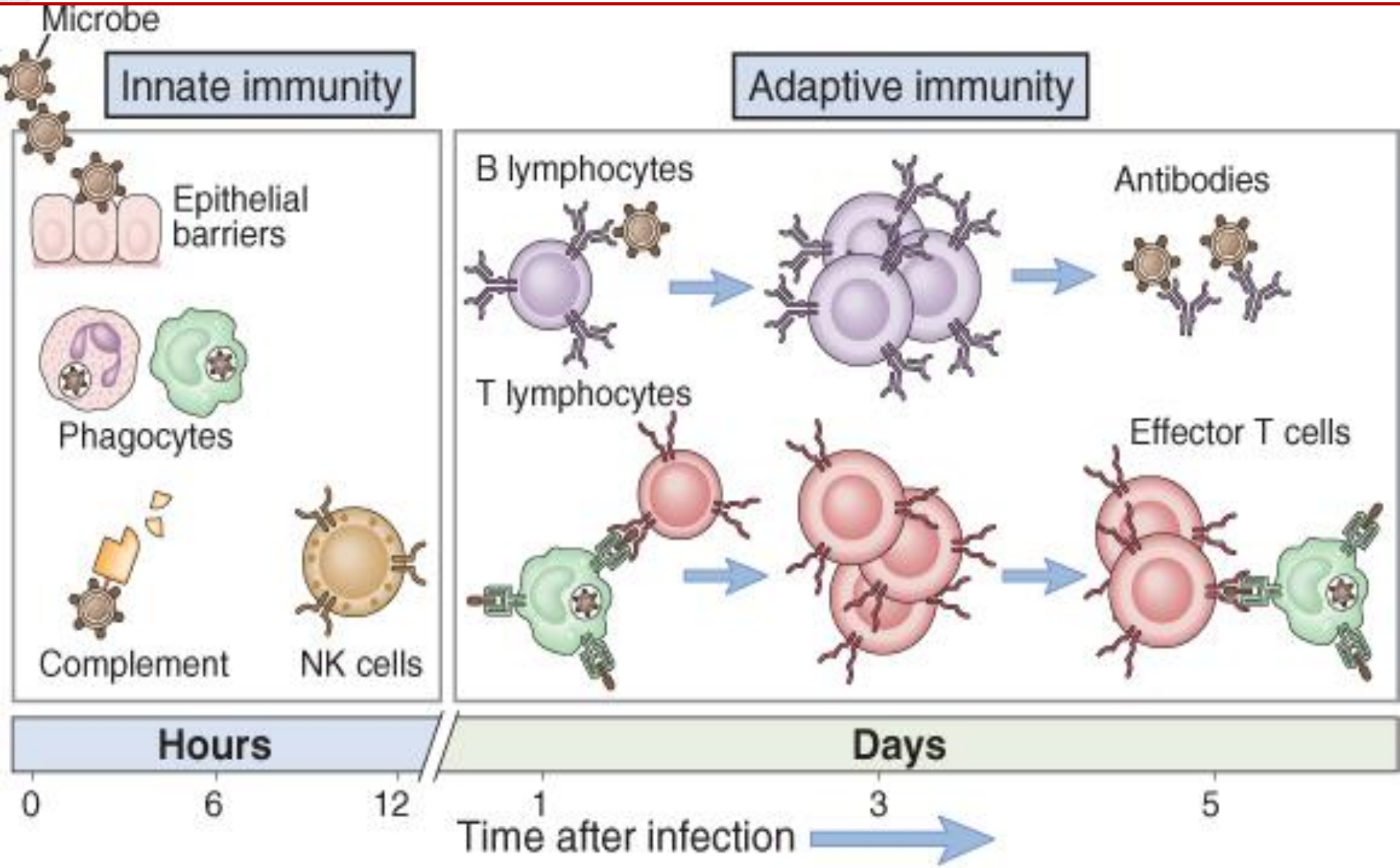
Vaccination Response

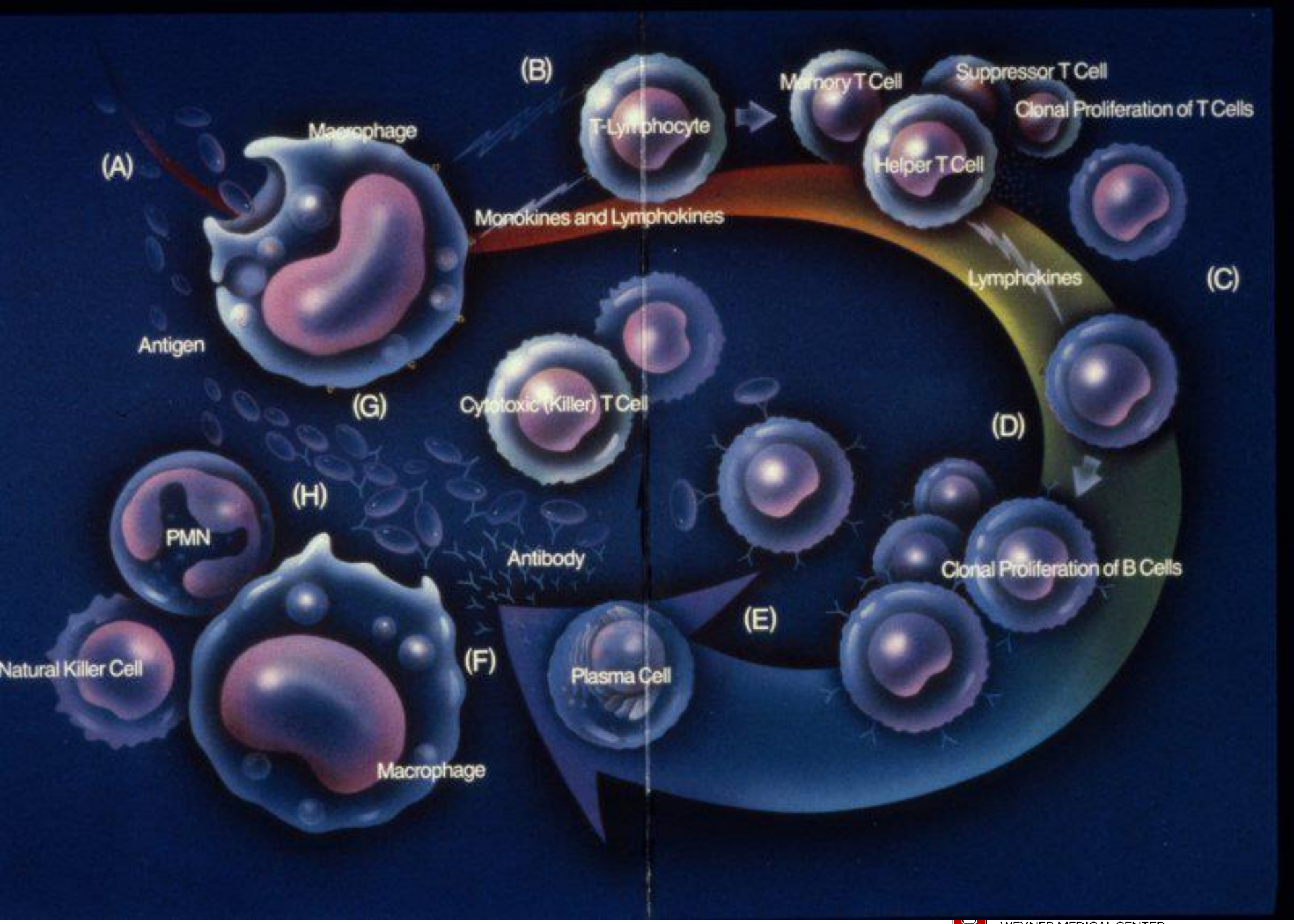


Innate system directs adaptive system

- Identifies real danger from an invader – activates adaptive immunity only when needed
- Directs the kind of response that adaptive immunity will make (e.g. humoral or cell-mediated)
- Targets response to particular site
- Provides a lot of the firepower for adaptive immune system (complement and phagocytes)

Immune Response




(A)
Macrophage
Antigen
(B)
T-Lymphocyte
Monokines and Lymphokines
Memory T Cell
Suppressor T Cell
Clonal Proliferation of T Cells
Helper T Cell
(C)
Lymphokines
(D)
Clonal Proliferation of B Cells
(E)
Plasma Cell
Antibody
(F)
Macrophage
(G)
Cytotoxic (Killer) T Cell
(H)
PMN
Natural Killer Cell

- Questions?