Immunology for the Rheumatology Provider

Alan L. Epstein, MD
Clinical Professor of Medicine
University of Pennsylvania School of Medicine

Learning Objectives

Immunology for the Rheumatologist

We spend much of our time as rheumatology providers dealing with the immune system gone awry. We rarely study the normal function of the immune system. Why is it important to have an understanding of immunology? Abnormal immune responses are the cause of many of our inflammatory diseases with serious morbidity and mortality. Antibodies are in widespread use to treat immunologic diseases. Understanding immunology helps us to better understand the diseases that we treat and their current therapies. It also prepares us for advances in understanding the immune mechanisms of inflammatory and autoimmune diseases and therapeutic options for these diseases in the future.

- 1) Review the innate immune system
- 2) Discuss acute gout as an example of a disease driven by aberrant innate immune function
- 3) Review the adaptive immune system
- 4) Discuss the details of T-cell function

Disclosures

- Speakers' Bureau and Consultant: Abbvie, Amgen, Astra-Zeneca, BMS, Chemocentryx, Fresenius Kabi, GSK, Janssen, Lilly, Novartis, Pfizer, Quest, Sanofi

Role of the Immune System

- ***Defense against infection***
- Surveillance against tumors
- Wound healing
- Recognizes and reacts to foreign proteins and tissues

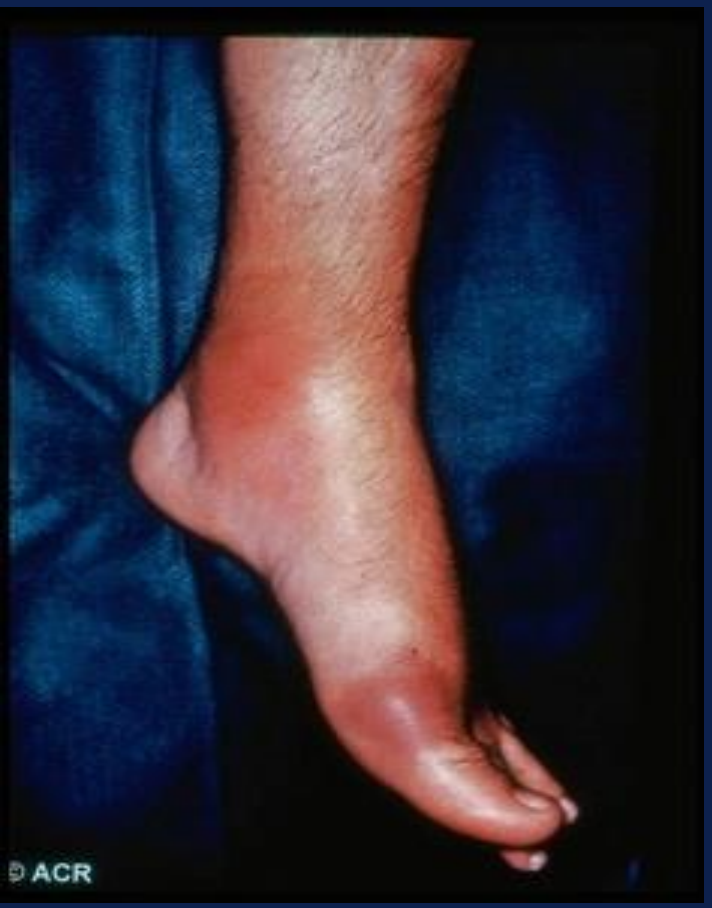
Components of the Integrated Immune System

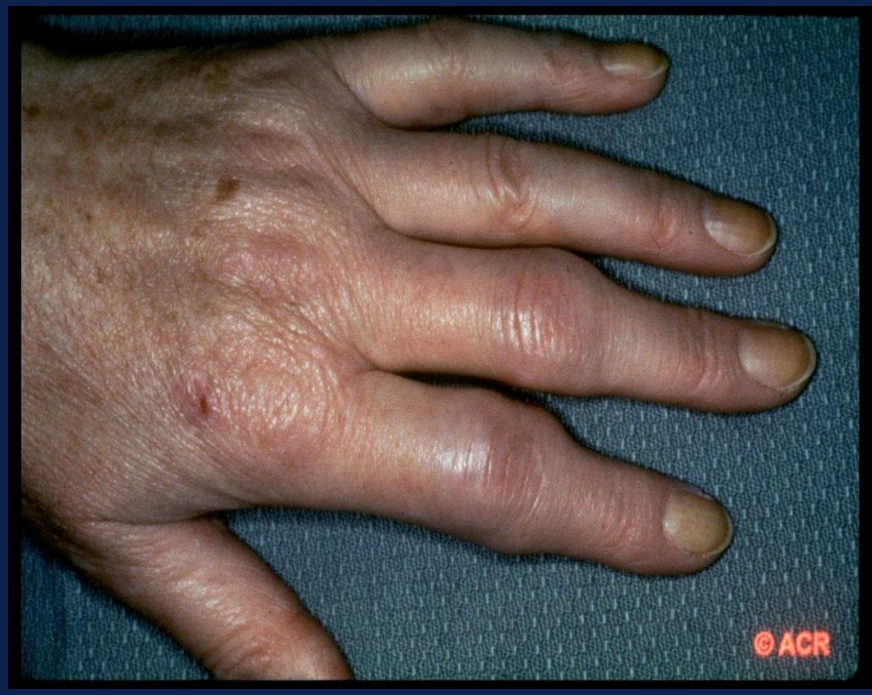
- **Innate Immune System**
  - -- Begins with physical barriers: skin and epithelial membranes
  - – "Non-specific" response
  - – Involves both immune & non-immune cells
  - – Immediate response
  - – Response = **inflammation**

    Cytokines and chemokines promote the ingress of neutrophils and macrophages
- **Adaptive Immune System**
  - – Specific recognition
  - – Immune cells only (T-, B-cells)
  - – Delayed response
  - – Response = clonal expansion & effector cytokine secretion
  - – Memory

Mechanisms of inflammation

Compare and contrast immunopathogenesis of gout and rheumatoid arthritis





Two arms of the immune system

Innate (acute) Immunity:

- First response—12+ hours
- Gout is an example of a disease driven by aberrant innate immune function

Adaptive (acquired) immunity

- Takes time to develop
- RA is an example of a disease driven (in large part) by aberrant adaptive immune function

Cells of the Immune System (Leukocytes)

BUT, don't forget cytokines

Innate response

Adaptive response

Neutrophil

(Cytotoxic-Phagocytosis,

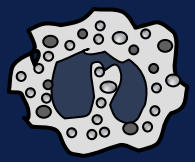
Degranulation)

NK cell

(Cytotoxic-ADCC)

Eosinophil

(IL-5 producers)

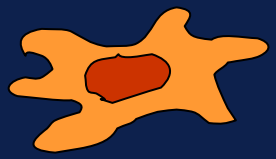


Basophil

(Histamine Release)

Macrophage

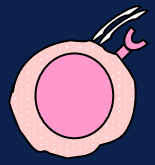
(APC)



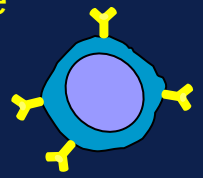
Dendritic cell

(APC)

Tc lymphocyte (CD8+)



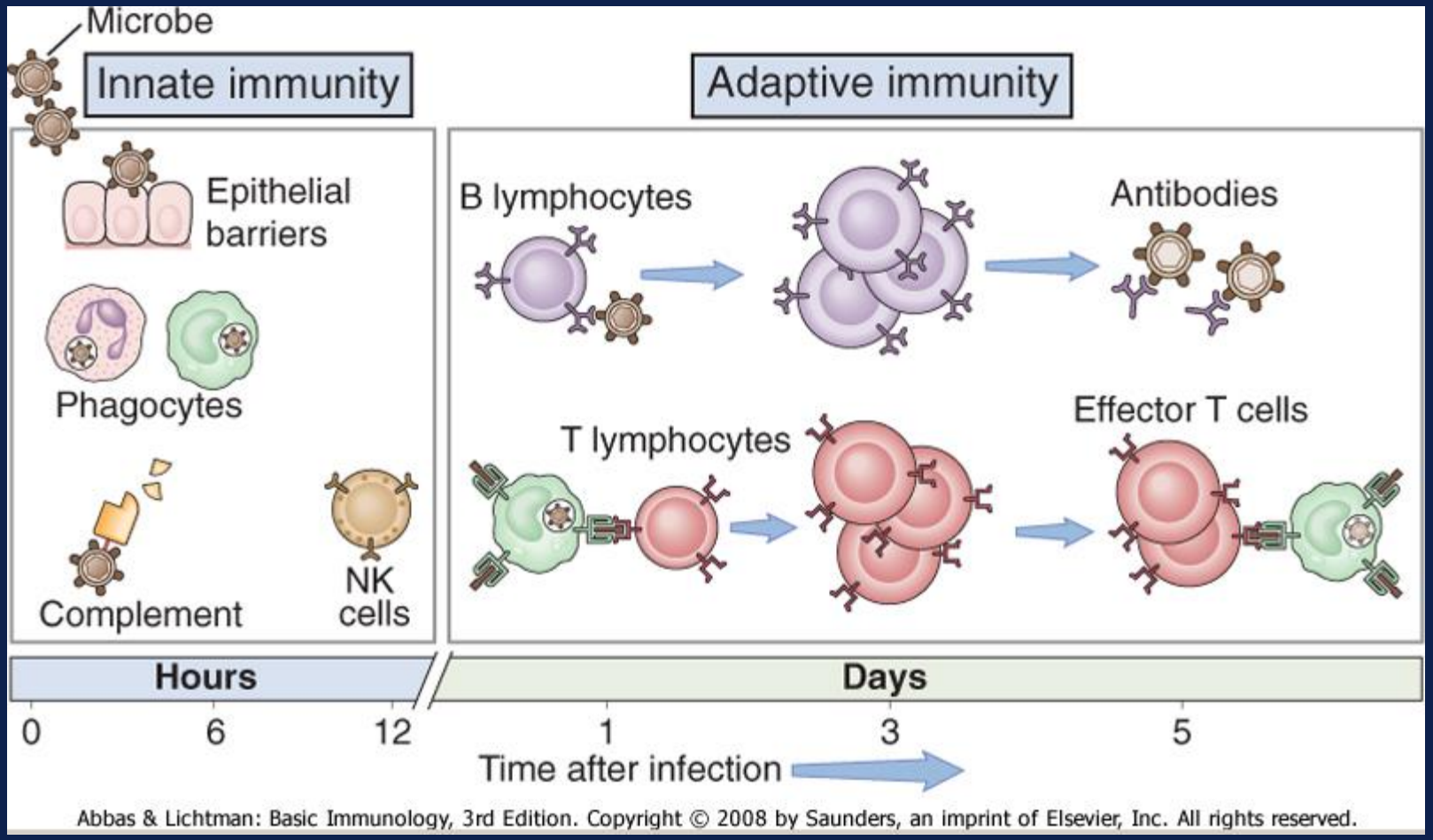
Th lymphocyte (CD4+)



B lymphocyte

Adapted from Goldsby, Kindt, Osborne and Kuby, Immunology 5th Ed. 2003 p25

Two Arms of the Immune System: Innate and Adaptive Immunity



Abbas & Lichtman: Basic Immunology, 3rd Edition. Copyright © 2008 by Saunders, an imprint of Elsevier, Inc. All rights reserved.

Prevent infections
Eliminate microbes

Antibodies block infections and eliminate microbes
T lymphocytes eradicate intracellular microbes

Innate Immunity: General features

- 1) Initial response to microbes
- Recognizes structures shared by classes of microbes
- Receptors for recognition encoded in germline, limited diversity
- Consists of epithelial barriers, phagocytes (neutrophils, monocytes and macrophages), NK cells, dendritic cells
- Complement system
- Cytokines + chemokines such as TNFα, IL-1, IL-6, IL-10, IFNγ
- ***All defenses without MEMORY***
- 2) Activates the adaptive immune response

Danger Is All Around Us

Remember, the main job of the immune system is protection

- Physical Danger
  - Tissue injury
  - Cell death
- Chemical Insults
  - Environmental toxins
- ***Infection***
  - Bacteria
  - Viruses
  - Parasites
  - Fungi

Sense danger by recognizing:
"pathogen-associated molecular ***patterns***" (PAMPs)
"damage-associated molecular ***patterns***" (DAMPs)

- Unique microbial structures
  - Bacterial cell wall components (lipopolysaccharide, peptidoglycan)
  - Microbial proteins (flagellin, zymosan, toxins)
- Nucleic acids
  - Double stranded RNA
  - CpG DNA
  - Viral and Microbial RNA
- Necrotic cell ATP
- Uric acid
- Hyaluronan fragments
- Cytochrome c

PAMPs=Molecular structures that are part of microbial pathogens

DAMPs=Endogenous molecules released from damaged cells

Pattern Recognition Molecules (PRMs)

Recognize PAMPs and DAMPs
Present on cell surfaces
Present in blood and extracellular fluids

- ***Toll-like Receptors (TLRs)***
- *NOD-like Receptors (NLRs)*
- RIG-I-like Receptors (RLRs)

inflammation

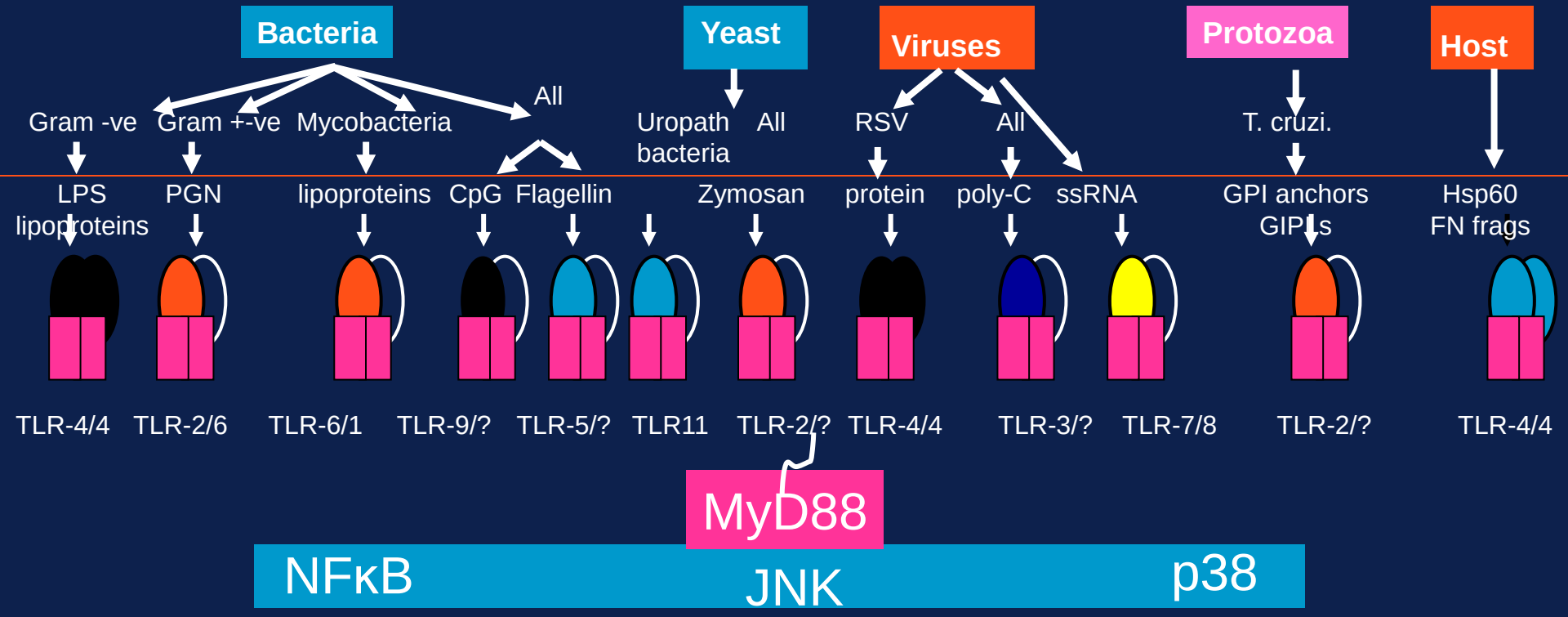
- Pentraxins
- ***Complement cascade***
- Collectins
- Ficollins

opsonization

- C-type lectins
- Scavenger receptors

phagocytosis

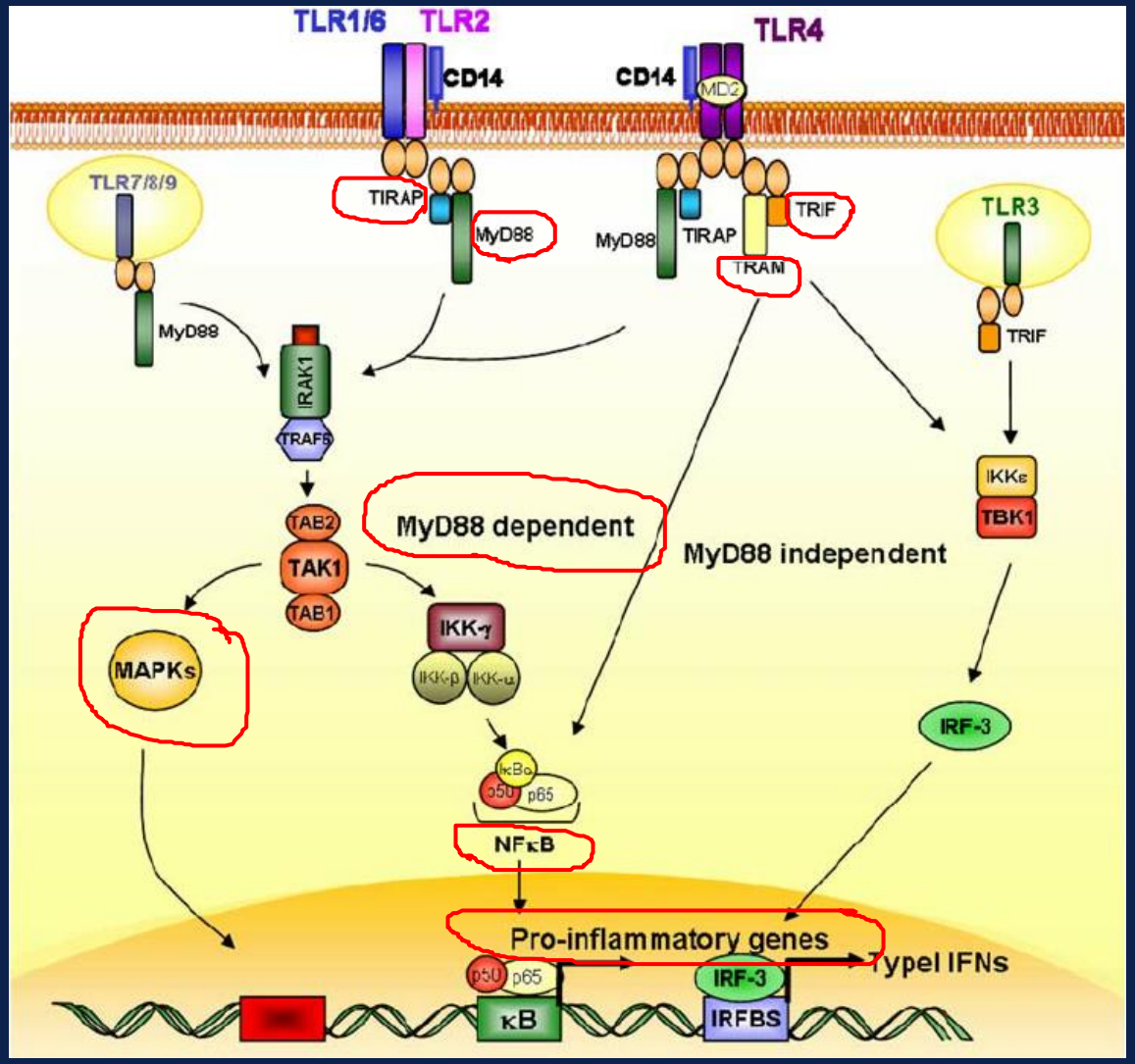
Recognition of pathogens by Toll-like receptors



- Found on macrophages, neutrophils, and dendritic cells
- Recognize distinct pathogen-associated molecular patterns conserved in microbes, eg, lipopolysacharides, lipoproteins,viral ds-RNA
- Activate the innate immune response

TLR Signalling

4 Adapter proteins recruited
2 Signal transduction pathways activated
Drives gene transcription



Macrophage Function

a) receptors for bacterial components(CHO, Lipids)
b) can bind and be activated by immune complexes

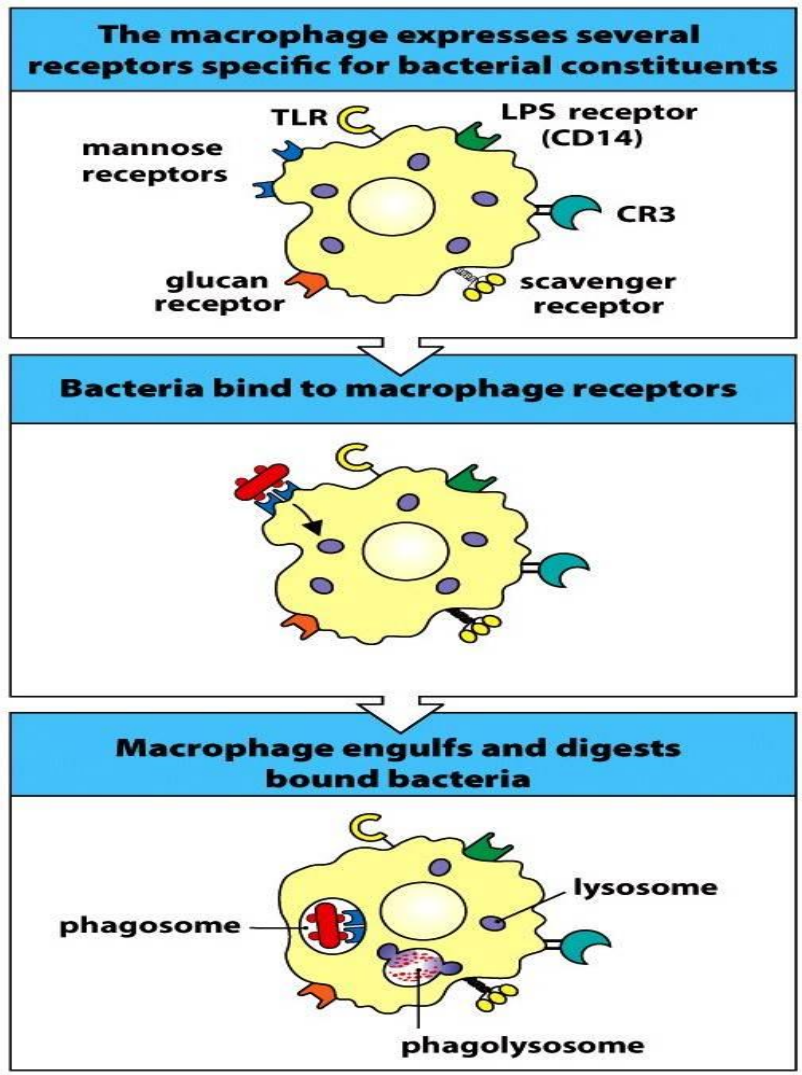


Figure 2.19 The Immune System, 3ed. (© Garland Science 2009)

Macrophage Function

Signaling through some receptors (such as TLRs) causes the release of pro-inflammatory cytokines

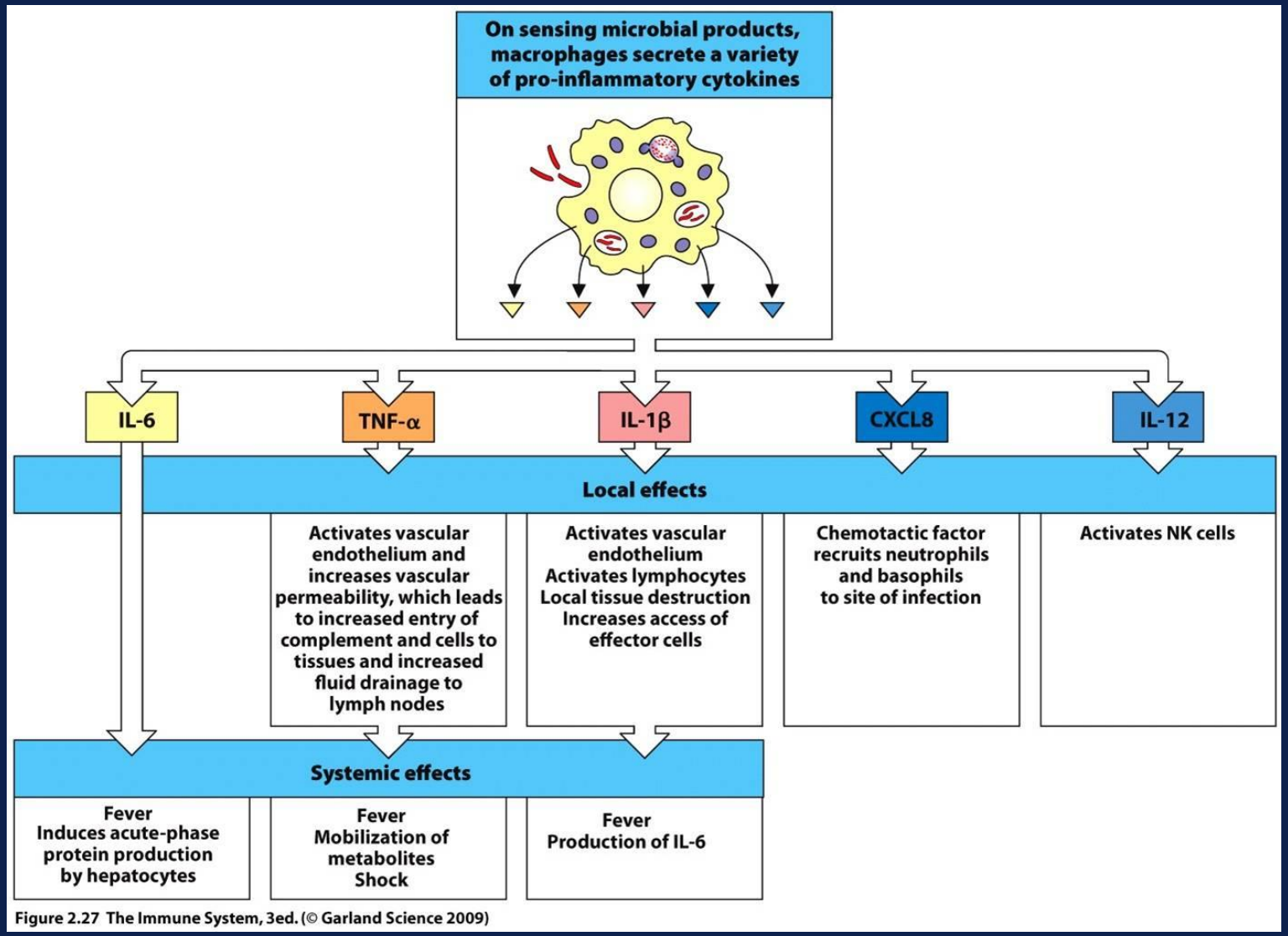
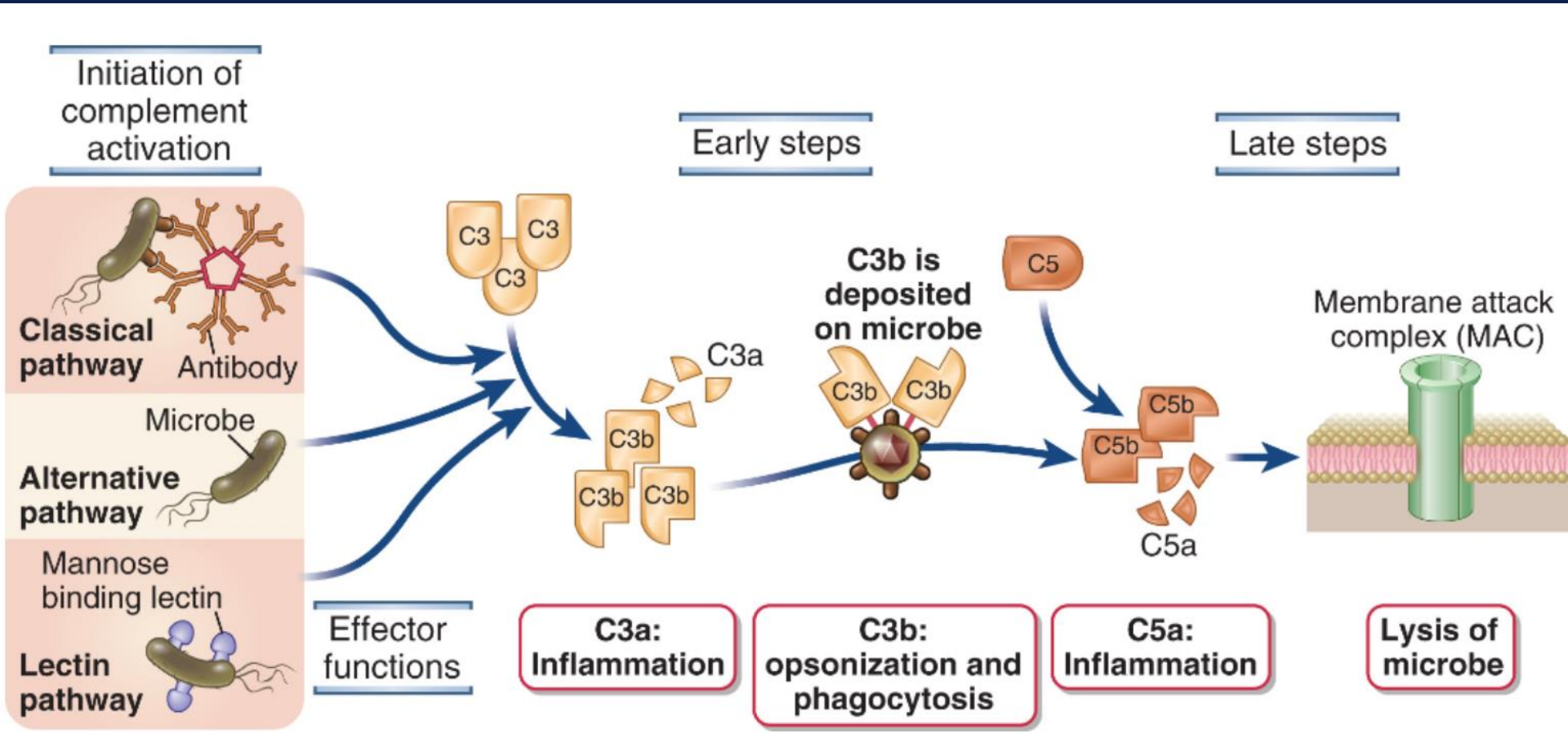


Figure 2.27 The Immune System, 3ed. (© Garland Science 2009)

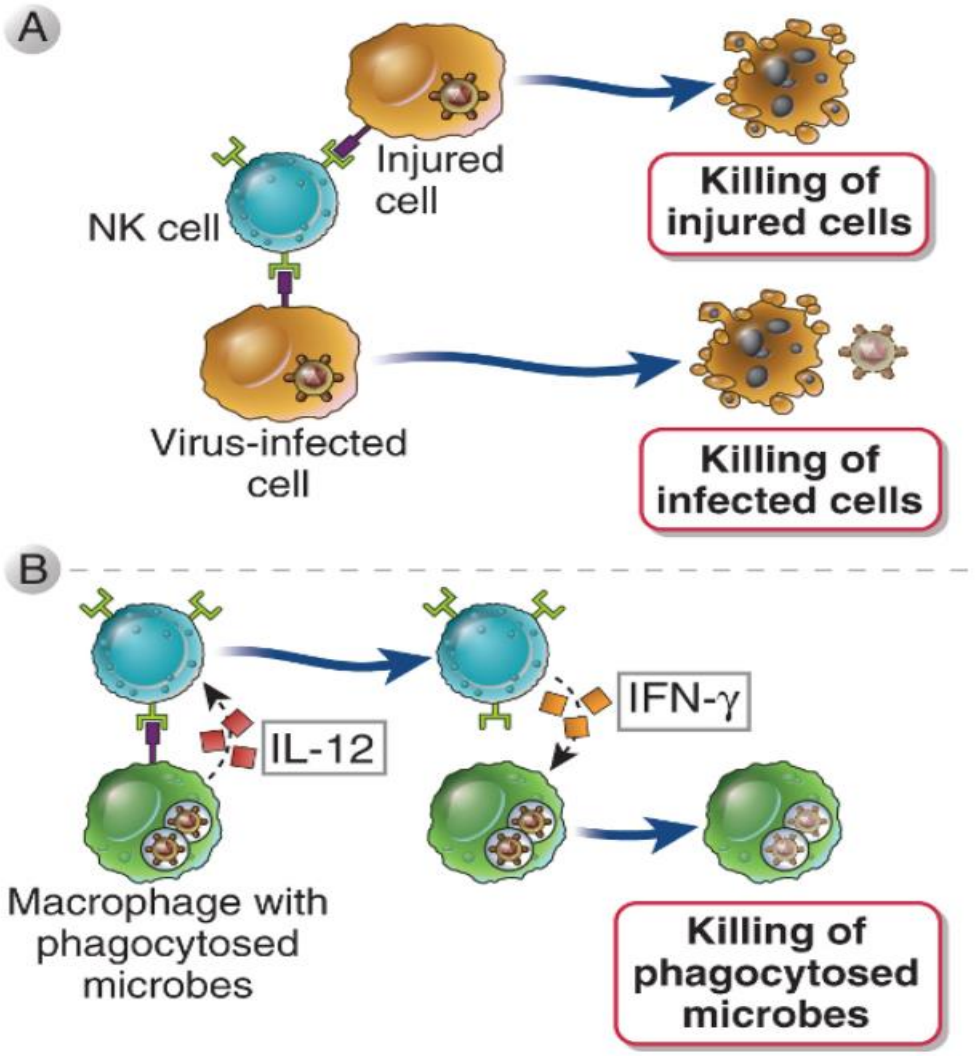
COMPLEMENT- 3 distinct ways to activate, all lead to C3B

Enhances (complements) ability of Abs + phagocytic cells to clear microbes, promote inflammation, and attack pathogen's cell membrane.



Abbas et al 2018

Functions of NK Cells



<ADCC

Abbas et al 2018

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

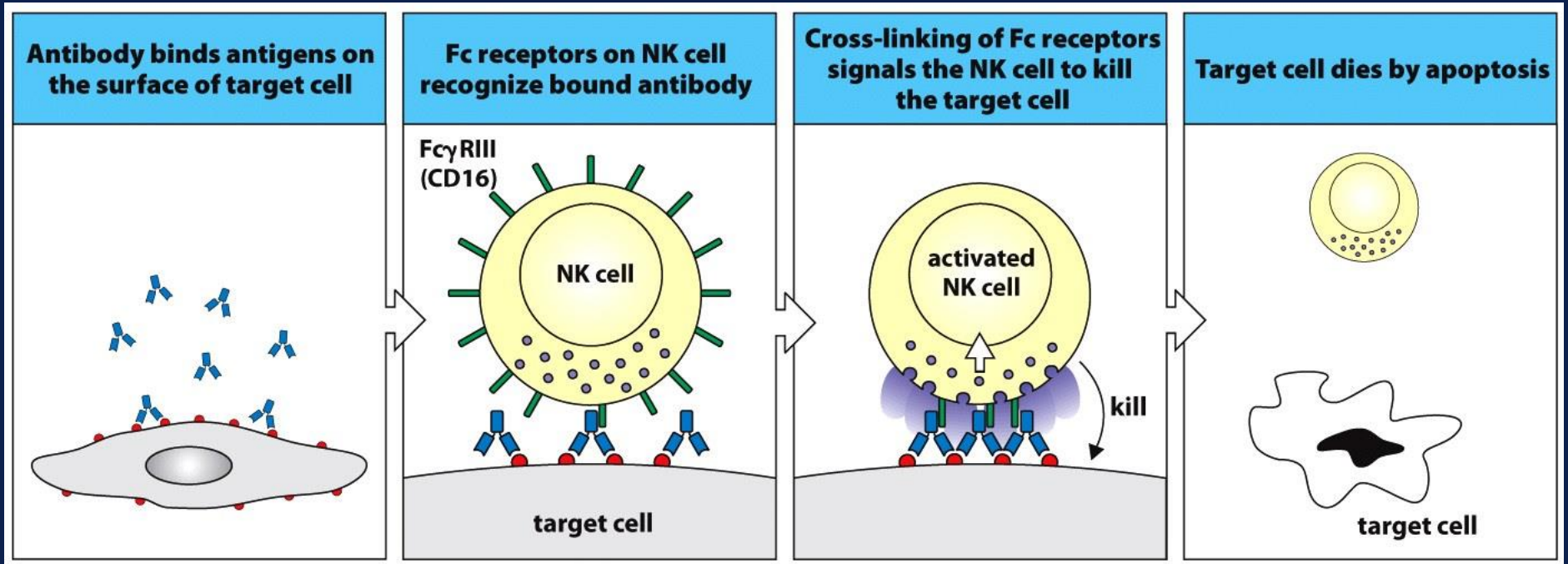


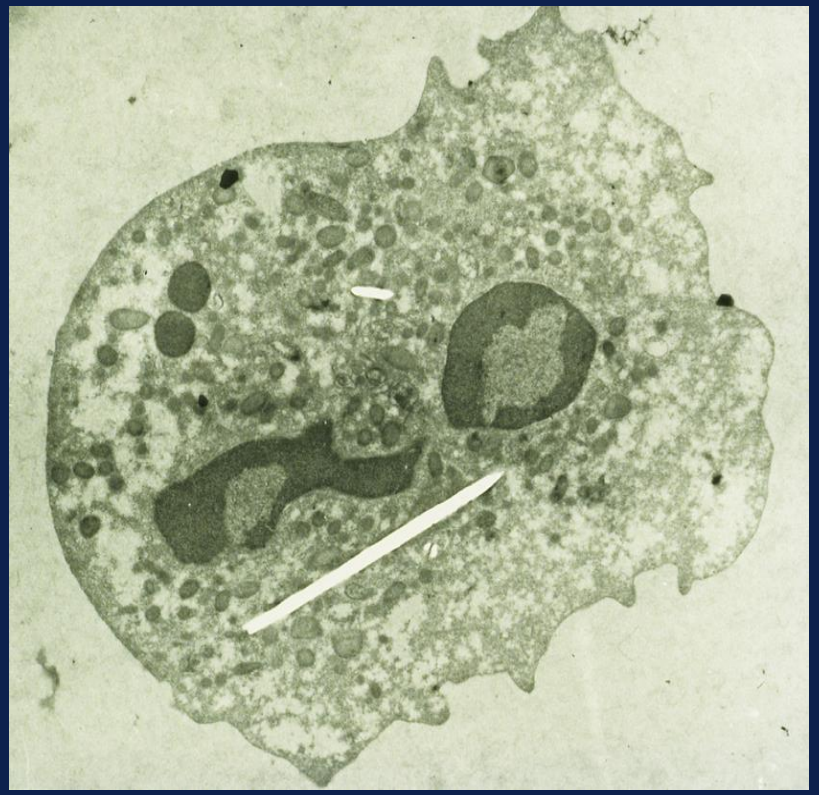
Figure 9.43 The Immune System, 3ed. (© Garland Science 2009)

Mechanisms of Acute Gouty Inflammation: Disorder of Innate Immunity

- Acute onset, self limited
- Urate is the inflammatory stimulus, resolves when urate is removed
- Predominant neutrophil response. No lymphocytic reaction (No T-cells or B-cells)
- No autoantibody formation

How Does a Crystal Incite Inflammation?

Interaction of crystals with synovial lining cells triggers neutrophil ingress.



Components of the Innate Immune System that Respond to DAMPs**

In this case uric acid

Toll-like receptors

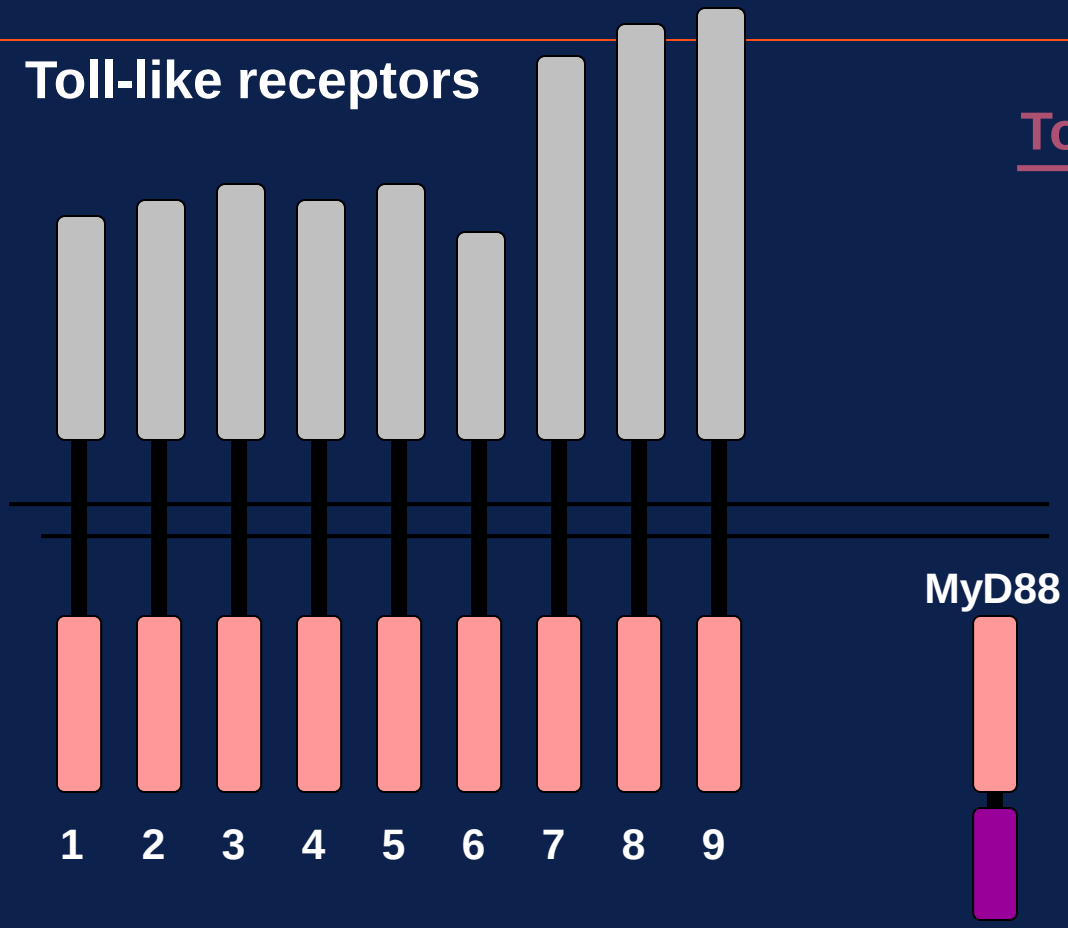
Lipoteichoic acid, endotoxin, flagellin, viral RNA, viral/bacterial DNA, **MSU/CPPD crystals**

Nod-like receptors

Bacterial products (*S. aureus*, Listeria, anthrax lethal toxin, flagellins, etc.), stress , K^+ efflux inducing agents, **MSU/CPPD crystals**

**DAMPS = Damage-Associated Molecular Patterns

Innate Immunity Sensors – Pattern Recognition Molecules (PRMs)

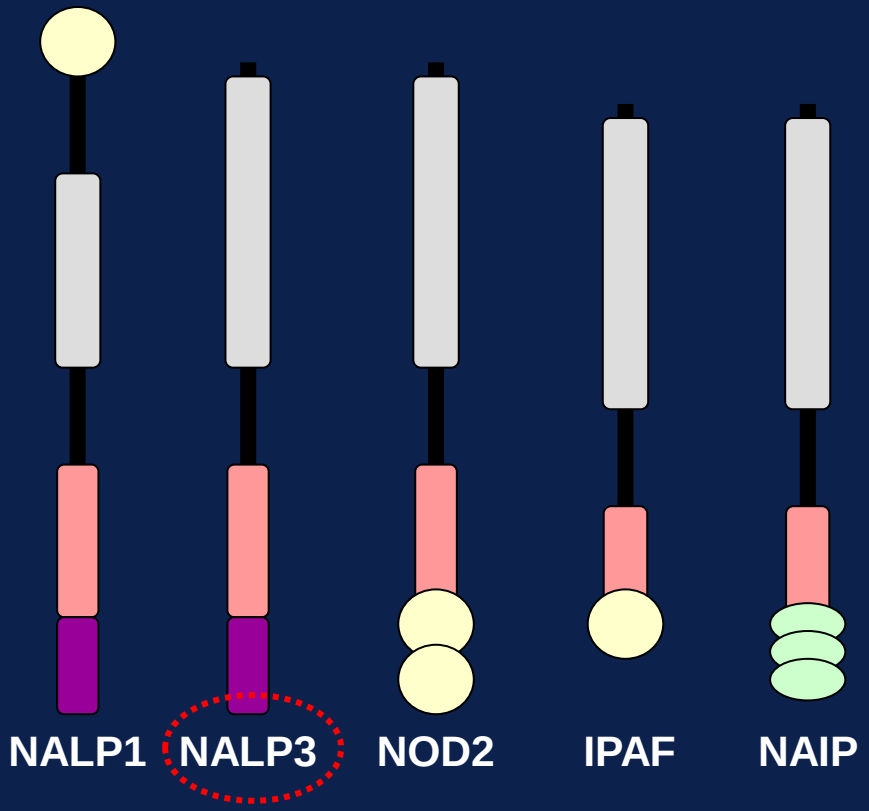


Toll-Like Receptors (TLRs)

11 members of TLR family-in humans

How TLR senses the presence of DAMPs is not clear

Innate Immunity Sensors – Pattern Recognition Molecules (PRMs)

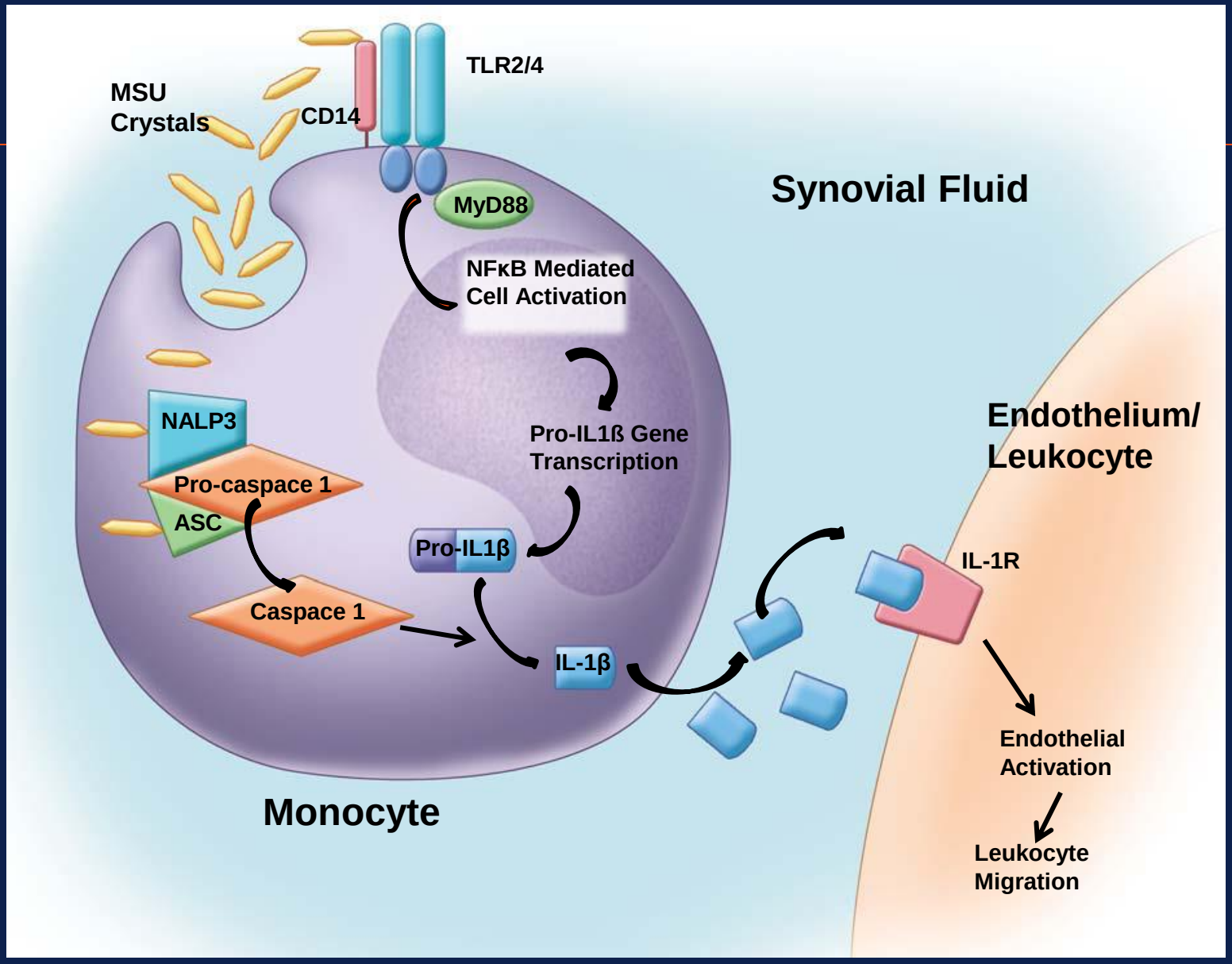


NOD-like receptors (NLR)

Cytoplasmic equivalent of TLR

22 members of the NLR family in humans- Recognize ligands including:

NALP1: anthrax lethal toxin
NALP3: *S. aureus*, Listeria, **uric acid crystals**, "stress"
NOD2: muramyl dipeptide
IPAF and NAIP5: *Legionella* flagellin

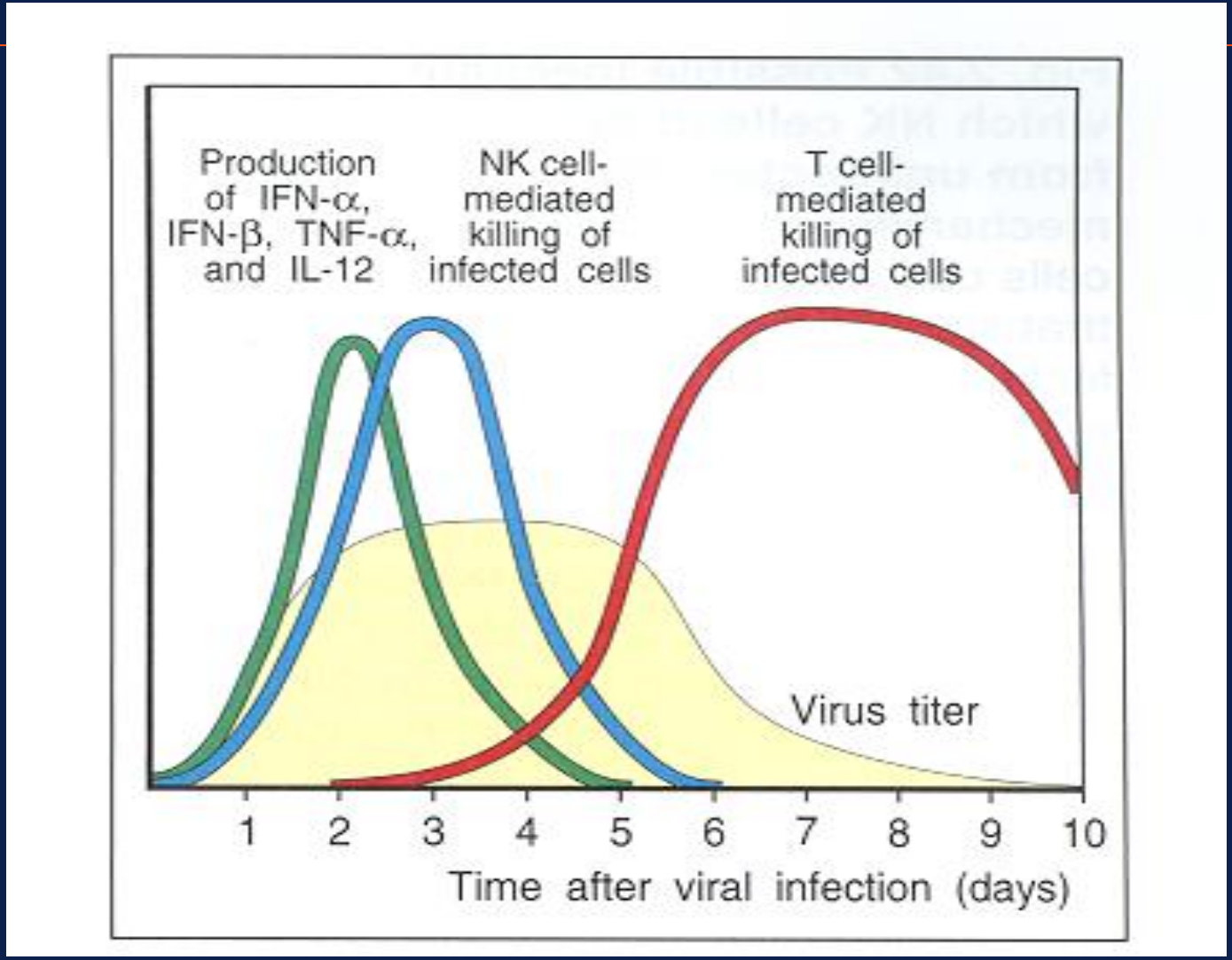


Edwards NL. Crystal-Induced Joint Disease, ACP Medicine Textbook, 2012

Adaptive Immunity

- Delayed response to an antigen demonstrating the features of SPECIFICITY and MEMORY
- Consists of lymphocytes and their products
- Utilizes specific receptors (T cell & B cell) ***generated by somatic mutation*** during development-ie system learns from what it sees
- Therefore, must be re-invented every generation!!

Time course of innate and adaptive immune responses



Mouse model of a viral infection

Classes of Lymphocytes-

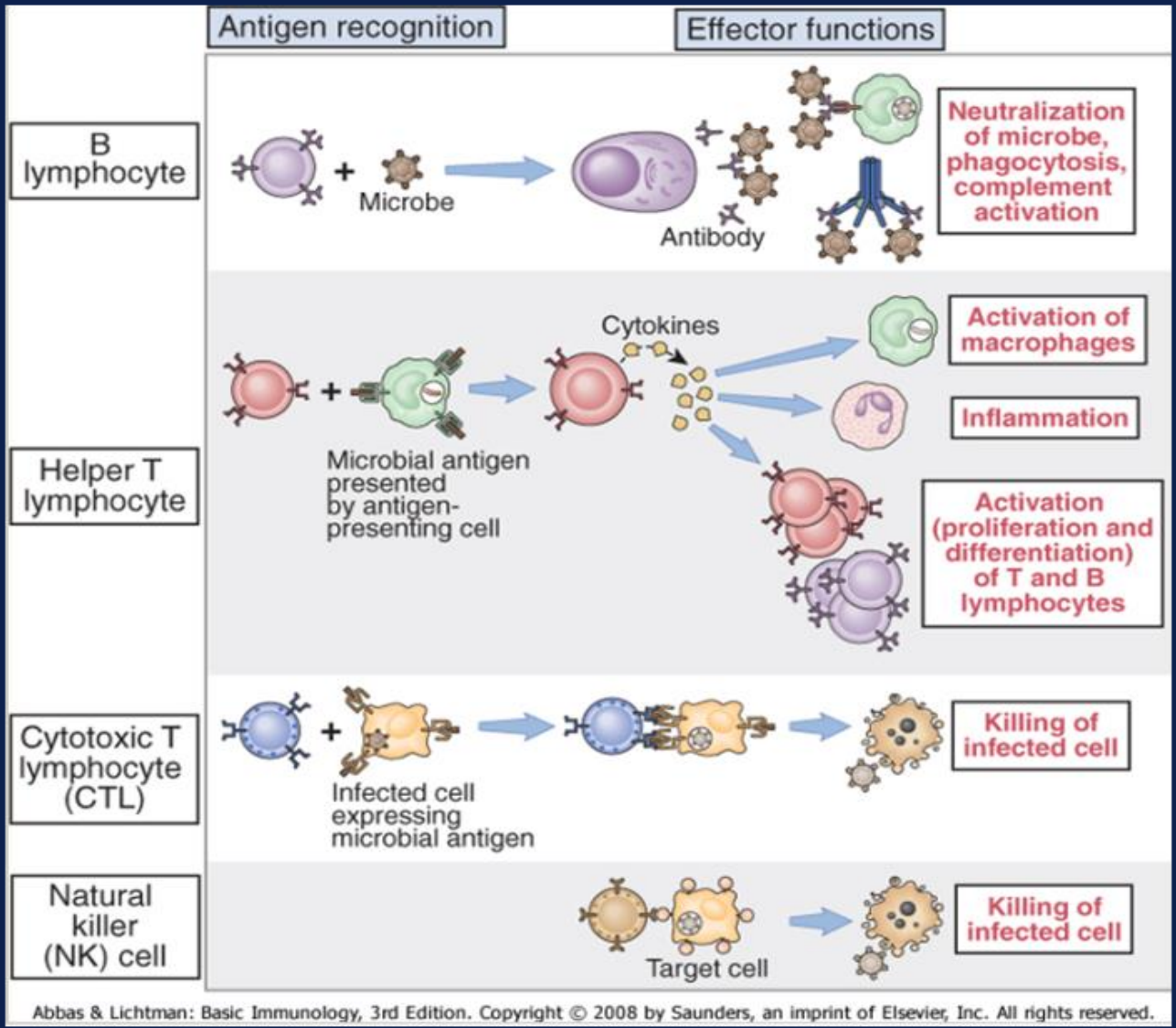
Recognize Different Types of Antigens

Antibodies recognize soluble or cell surface Ags

Recognize Ags on surface of APC's

Recognize Ags on infected cells

Recognize changes on surface of infected cells



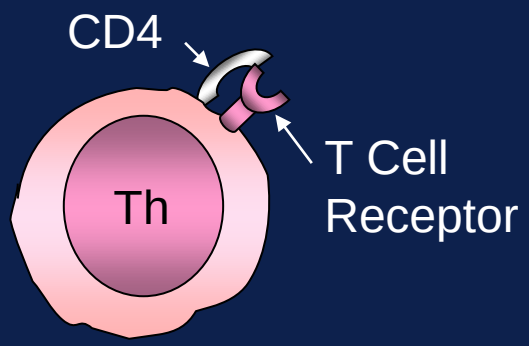
Abbas & Lichtman: Basic Immunology, 3rd Edition. Copyright © 2008 by Saunders, an imprint of Elsevier, Inc. All rights reserved.

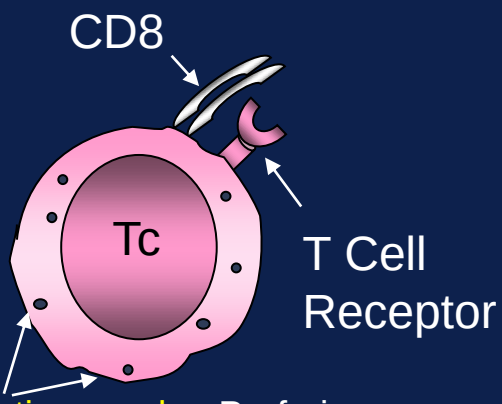
Three Strategies to Combat Microbes

- Secreted antibodies bind to extracellular microbes, block their ability to infect host cells, and promote their ingestion and subsequent destruction by phagocytes
- Phagocytes ingest and kill microbes—helper T cells enhance the killing by phagocytes
- Cytotoxic T cells destroy cells infected by microbes that are inaccessible to antibodies

T Cell Immunity (cell-mediated)

- T lymphocytes mature in the thymus
- They express a ***specific*** receptor that binds antigen, called the T Cell Receptor (TCR)
- There are 2 main types:
  - CD8+ Cytotoxic T cells (Tc)
    Induce cell death in target cells via cytotoxic granule release
  - CD4+ Helper T cells (Th)
    Help B cells to produce antibodies
    Help phagocytes to destroy ingested microbes.

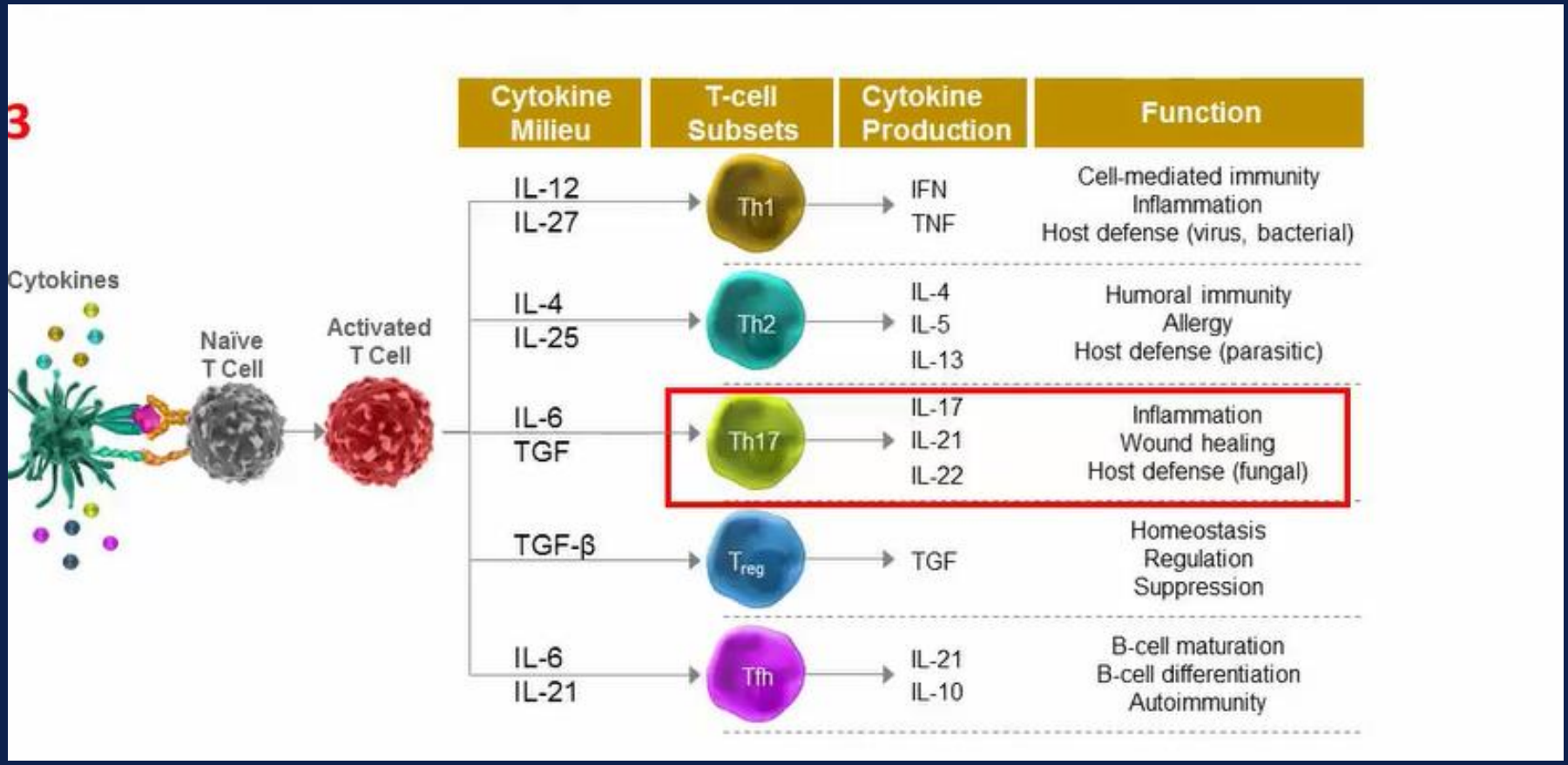




Cytolytic granules-Perforin>pore in membrane of target cell and Granzyme>activates apoptsis

Adapted from Goldsby, Kindt, Osborne and Kuby, Immunology 5th Ed. 2003

CD4 Subsets: Generation and Function



Functions of T Cell Subsets

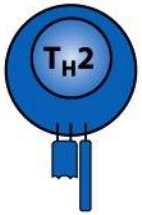
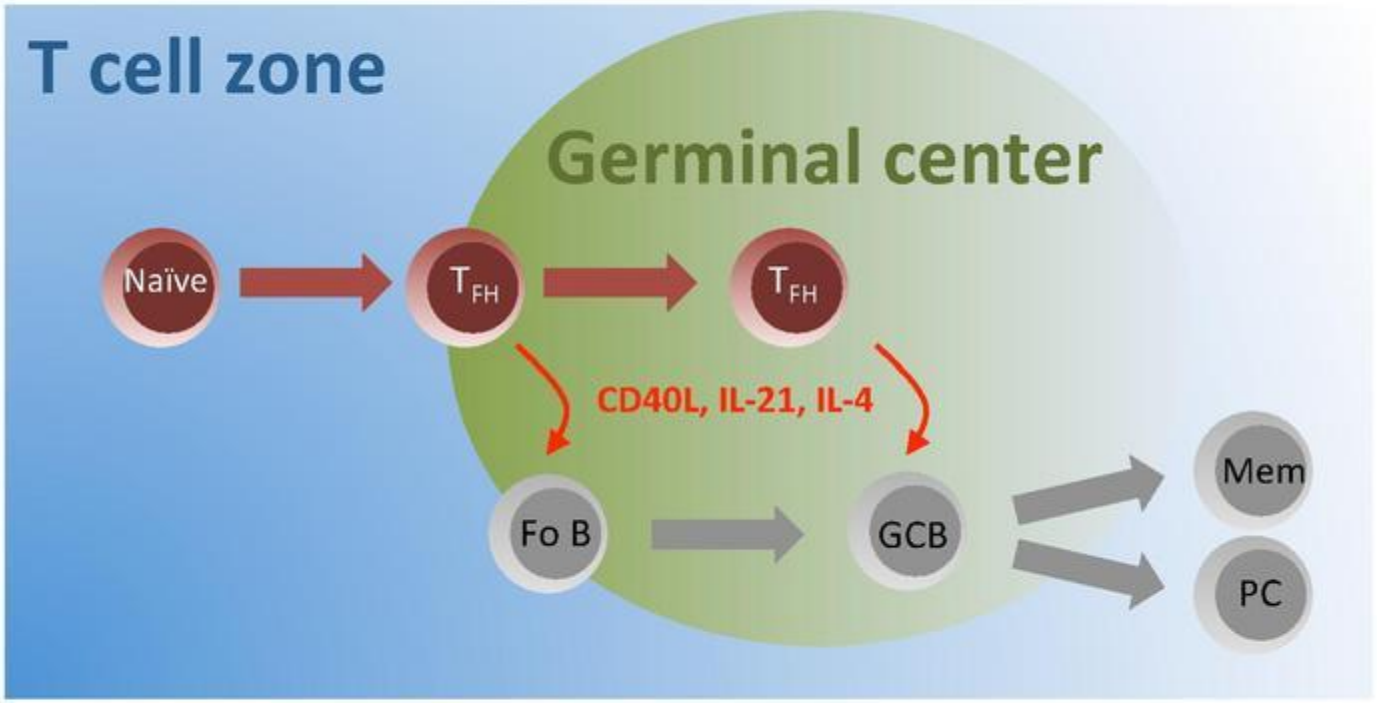
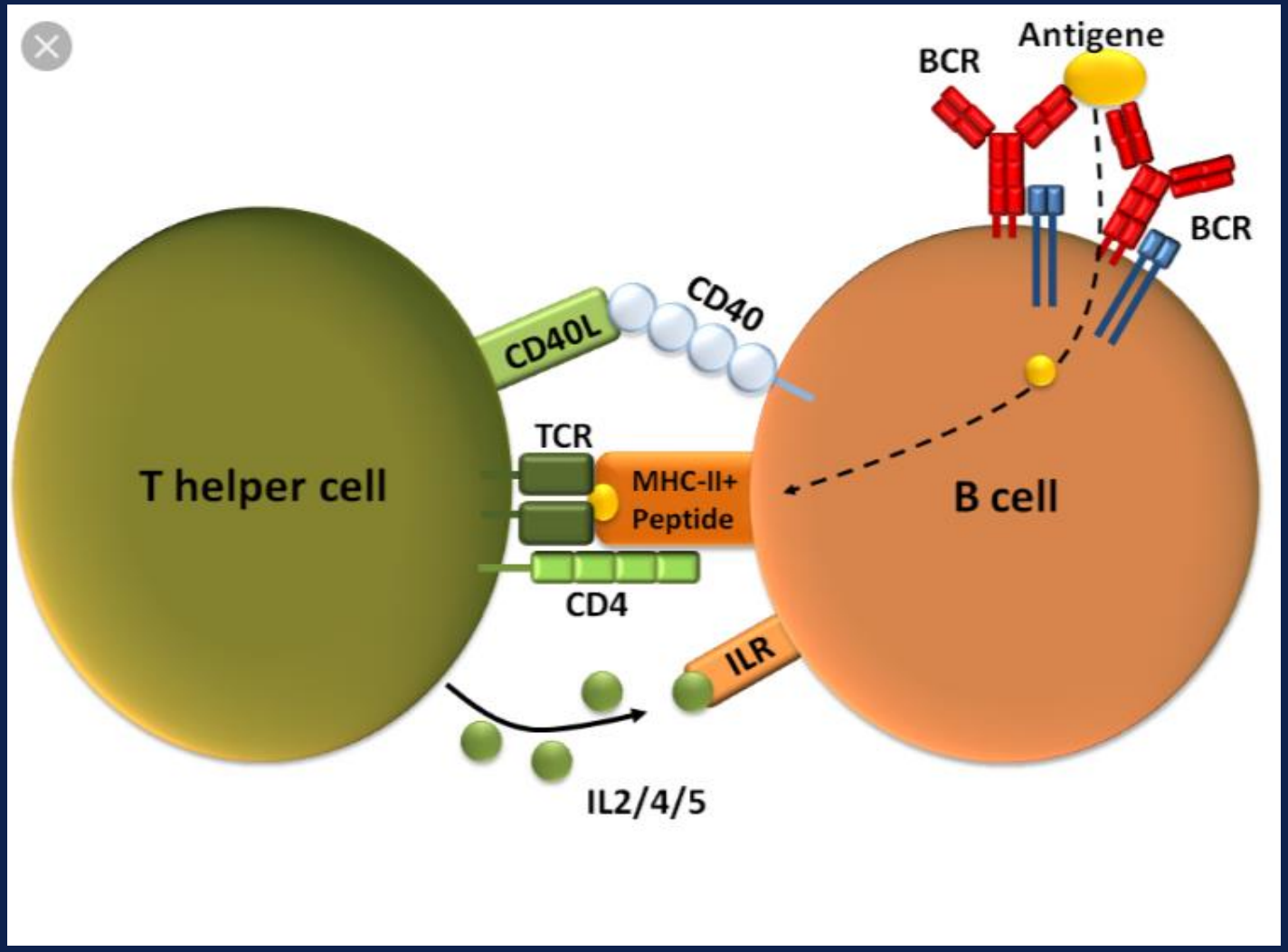
	CD8 cytotoxic T cells	CD4 T_H1 cells	CD4 T_H2 cells	CD4 T_H17 cells	CD4 regulatory T cells (various types)
Types of effector T cell					
Main functions in adaptive immune response	Kill virus-infected cells	Activate infected macrophages Provide help to B cells for antibody production	Provide help to B cells for antibody production, especially switching to IgE	Enhance neutrophil response	Suppress T-cell responses
Pathogens targeted	Viruses (e.g. influenza, rabies, vaccinia) Some intracellular bacteria	Microbes that persist in macrophage vesicles (e.g. mycobacteria, *Listeria*, *Leishmania donovani*, *Pneumocystis carinii*) Extracellular bacteria	Helminth parasites	Extracellular bacteria (e.g. *Salmonella enterica*)	

Figure 8-1 Immunobiology, 7ed. (© Garland Science 2008)

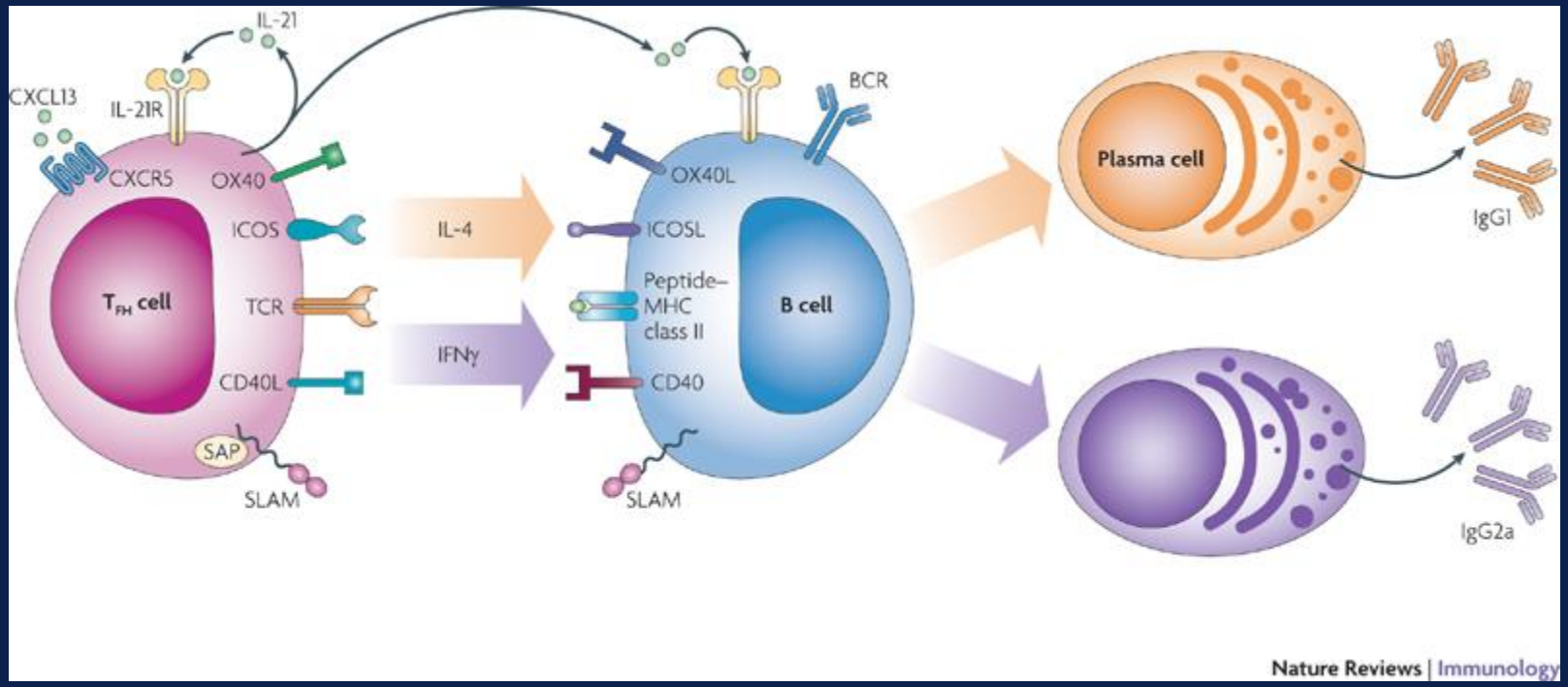
T follicular helper cells- migrate to follicles



T follicular helper cell



T follicular helper cells

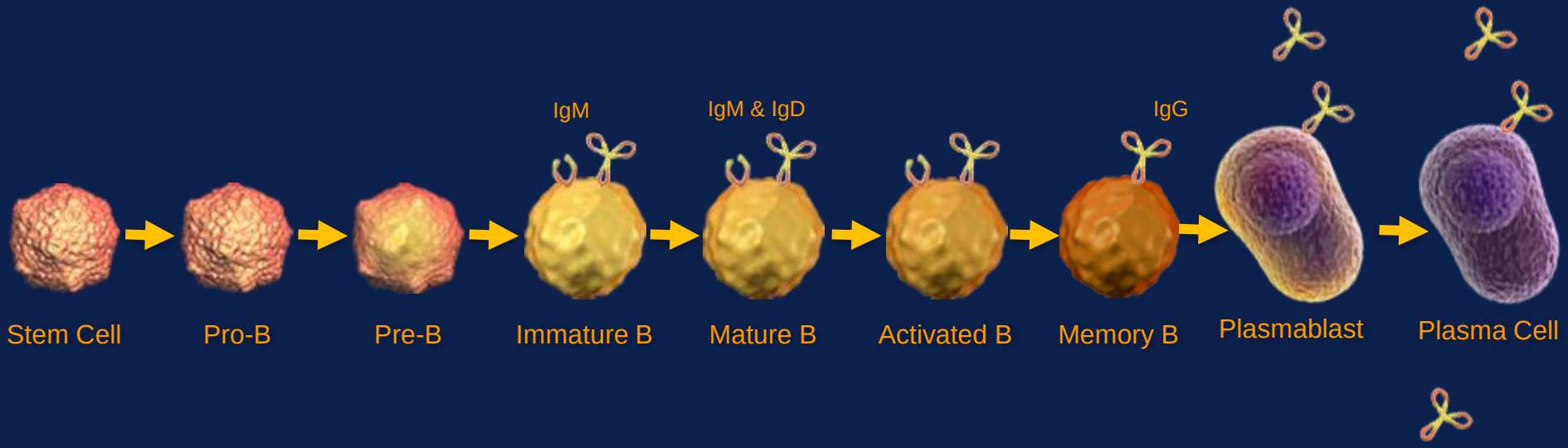


Trigger formation and maintenance of germinal centers
Stimulate plasma cell development
Stimulate development of memory B cells

B cells and Humoral Immunity

- Major limb of adaptive immunity
- Immunoglobulin is structurally homolgous to T cell receptor and also produced via somatic recombination
- Provides surveillance against blood born pathogens (bacteria, virus, parasites etc)
- Directly linked to innate immunity through complement activation

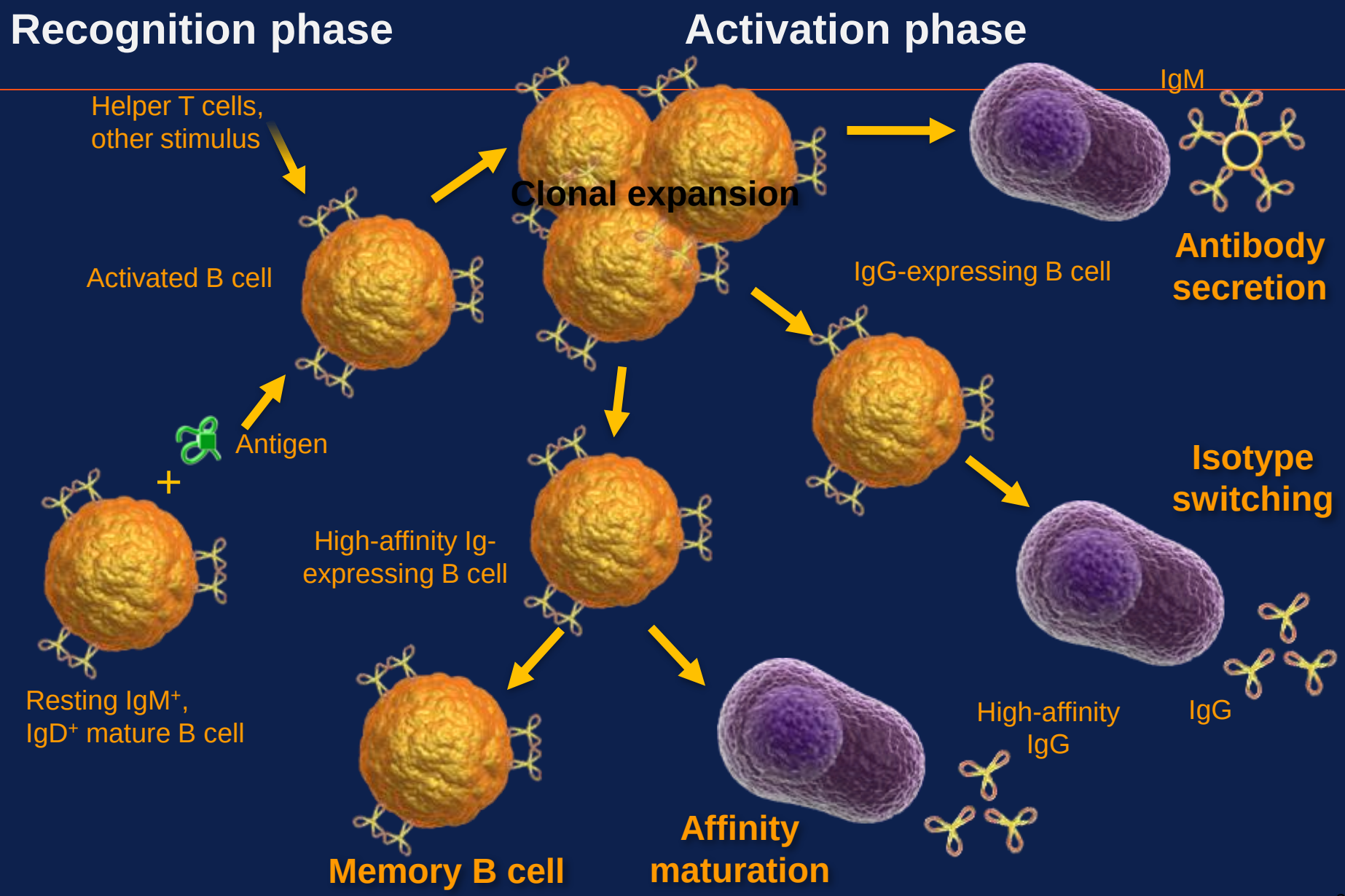
B-Cell Immunology: Lineage[1,2]



- B cells develop in the bone marrow and migrate to the peripheral lymphoid organs, where they can be activated by antigens[2]
- Activated B cells proliferate and differentiate into long-lived memory cells and antibody-secreting plasma cells[2]

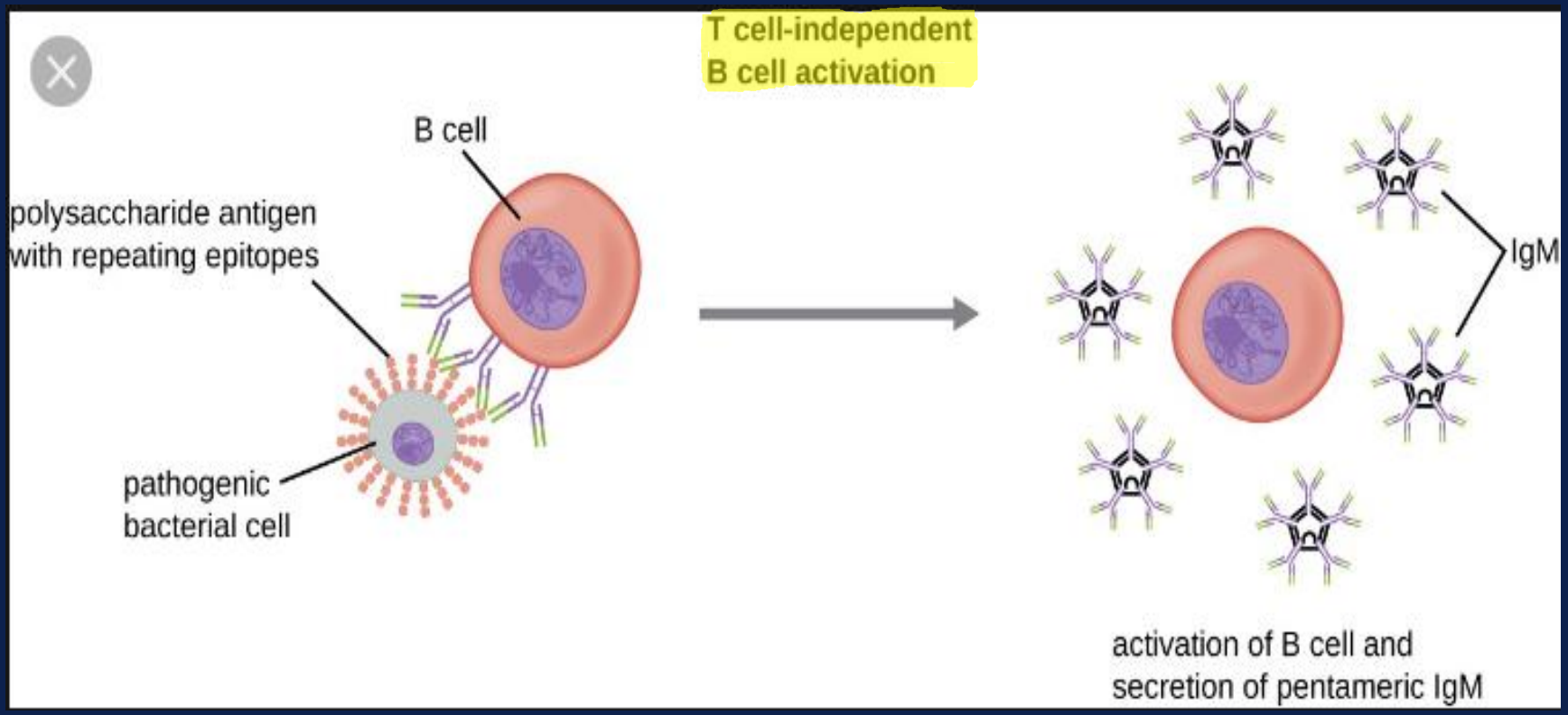
1. Roitt et al, eds. *Immunology*. 6th ed. 2001.
2. Murphy K et al. eds. *Janeway's Immunobiology.* 7th ed. New York, NY: Garland Science, Taylor & Francis Group, LLC; 2008:323-377.

B-Cell Activation-2 WAYS

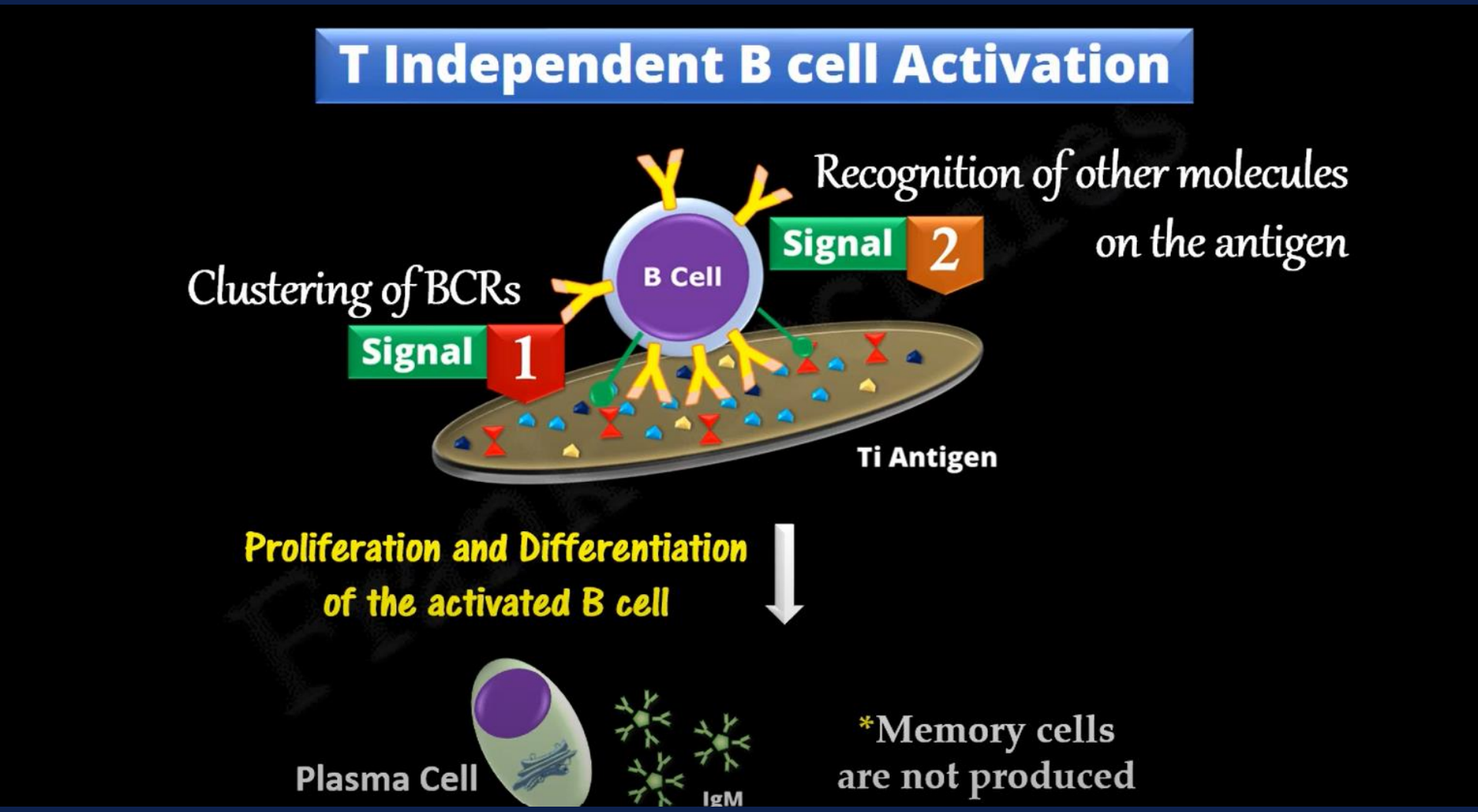


B Cell Activation

Non-Protein Antigens with Repeating Epitopes

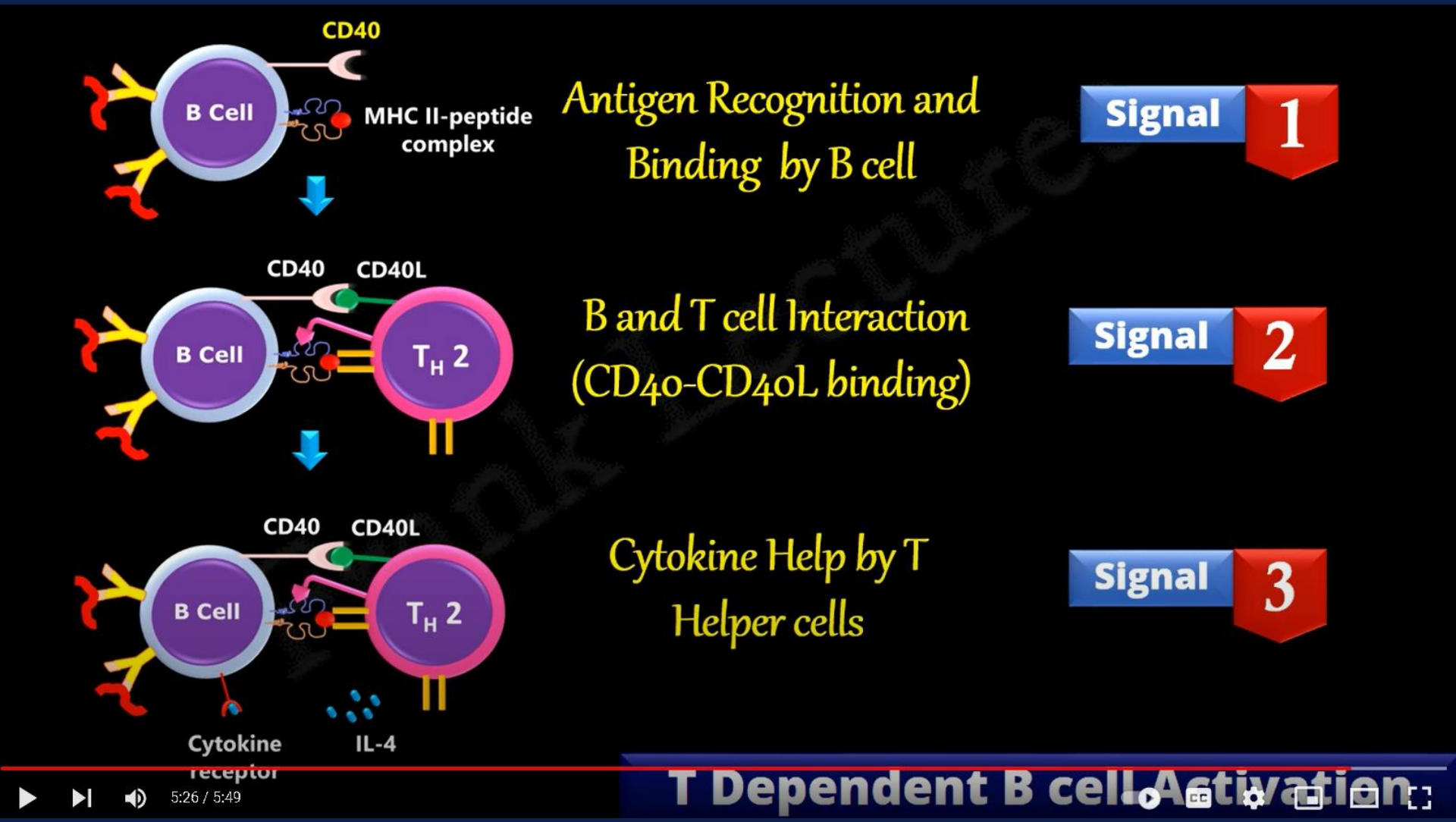


B Cell Activation



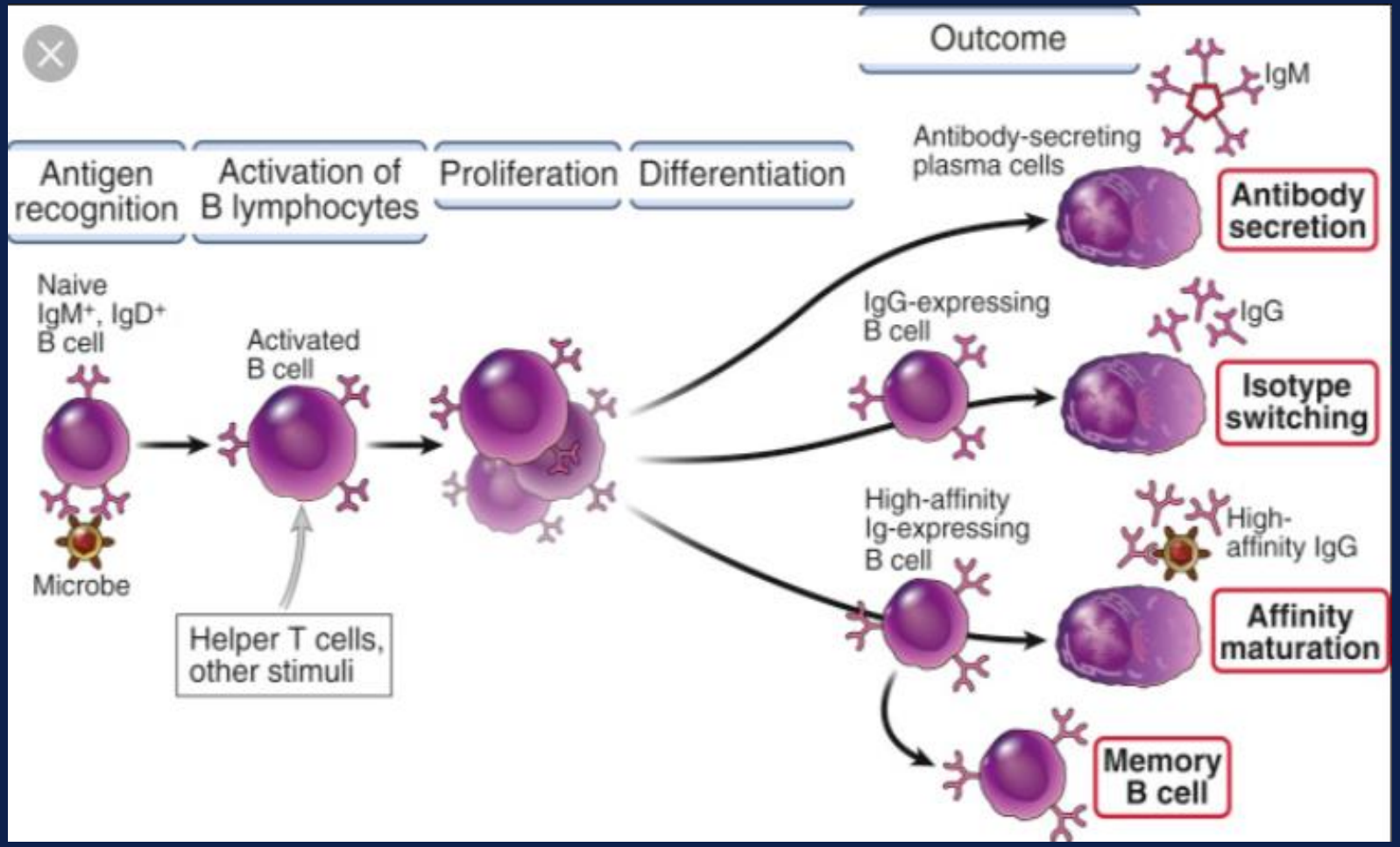
B Cell Activation

Protein Antigens-Requires T-Cell Help

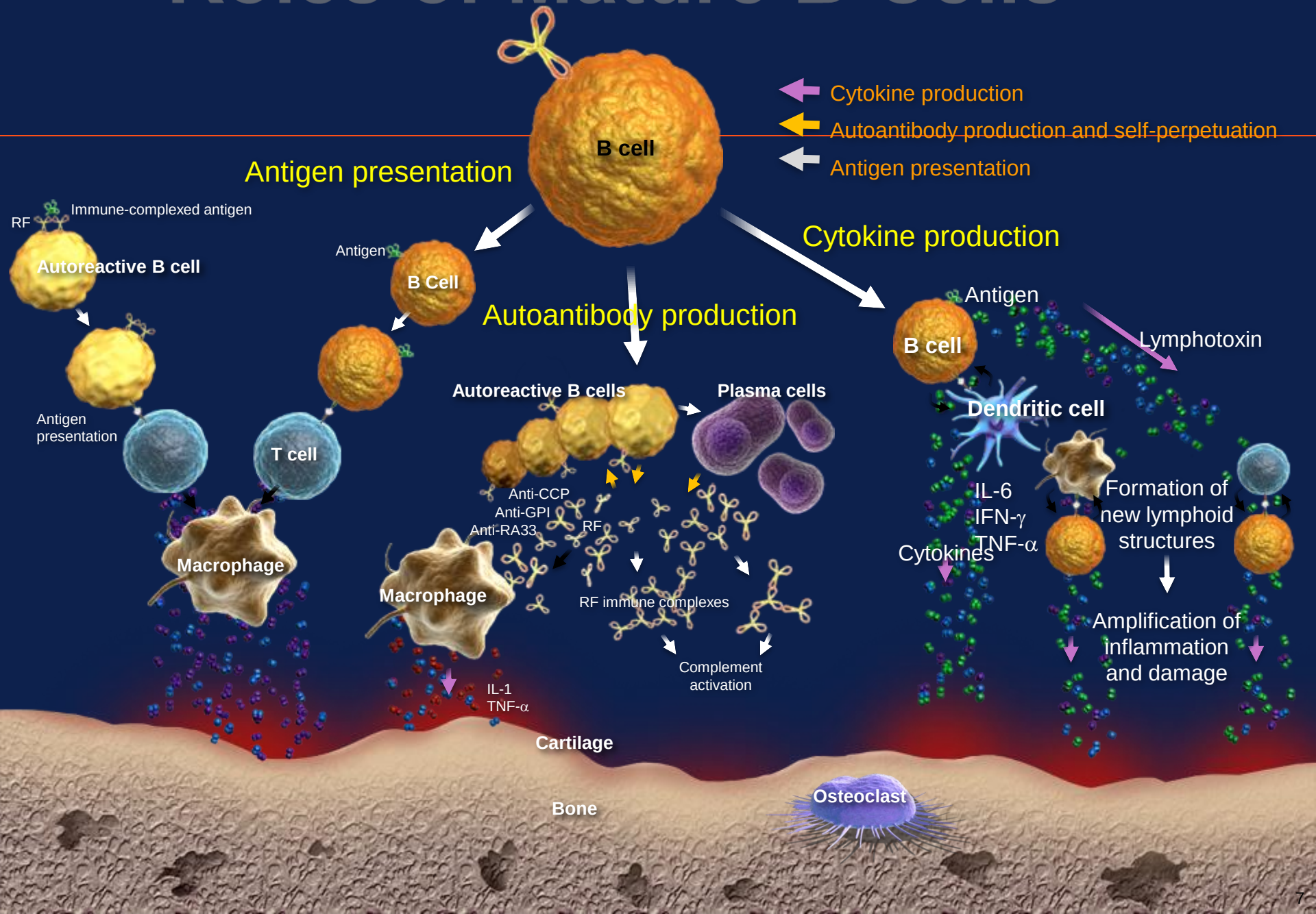


B Cell Activation

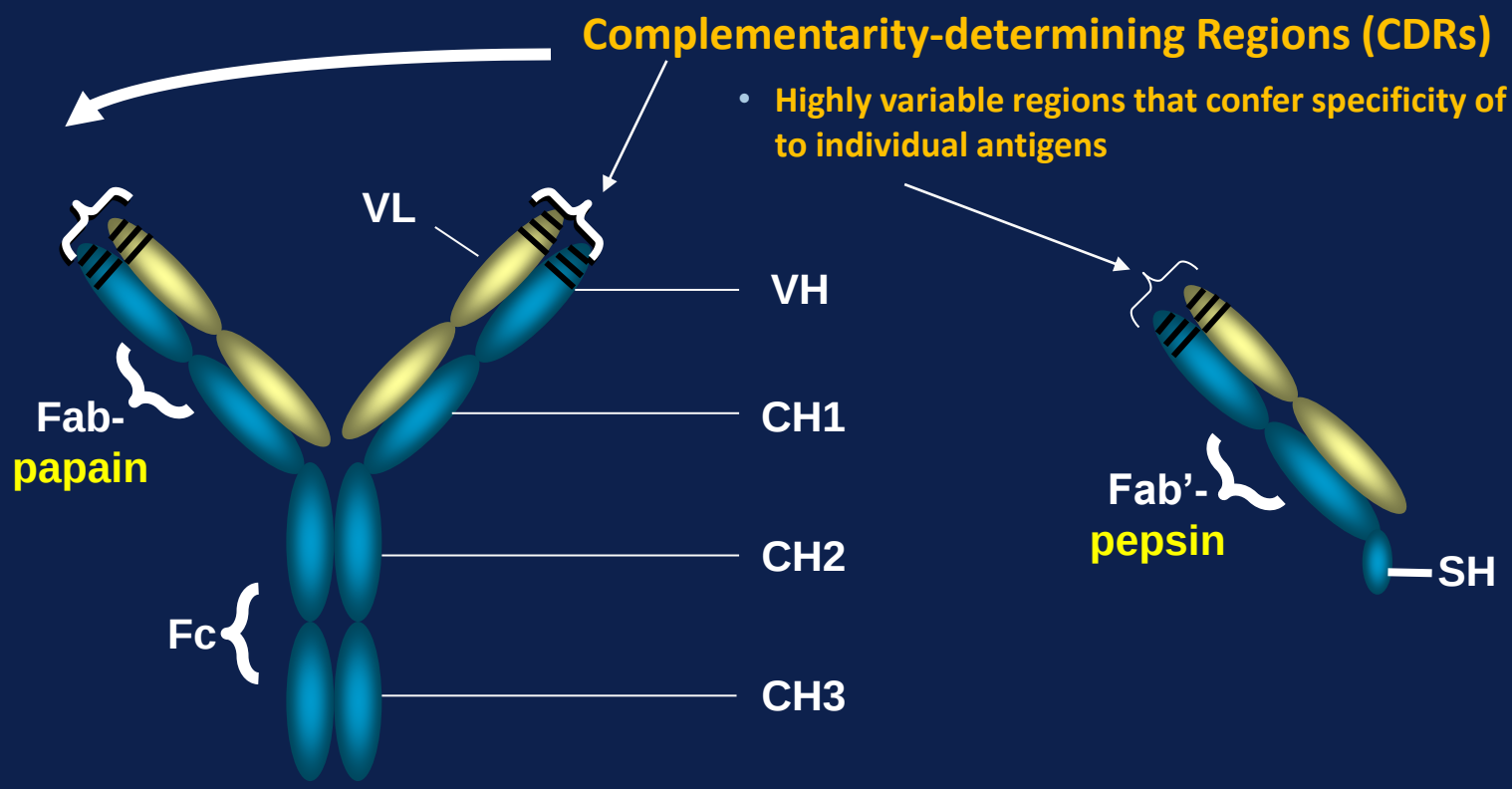
Protein antigen
T cell dependent



Roles of Mature B Cells



Antibody Structure



Janeway CA, et al. *Immunobiology*. 6th Edition, pp 104-115, Garland Science Publishing (2005).

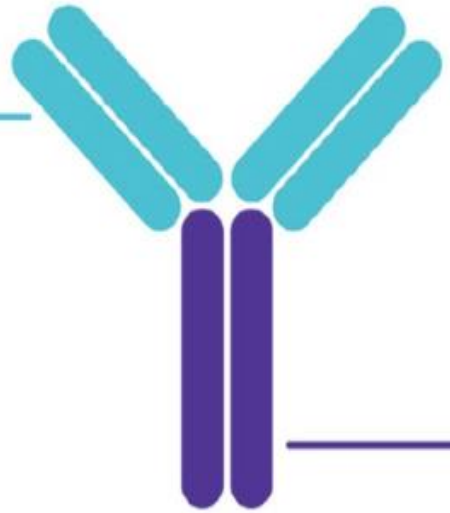
Antibody Functions

Fab Region

- Fab binding to antigenic drug target determines specificity of drug in vivo.
- Affinity of binding is a critical quality attribute that needs to be well characterized.

Biological Activity

- Binding to target triggers the desired biological effect.
- Fab-mediated mechanisms often may be supplemented with Fc-region–mediated effector functions following binding.



Fc Region

The Fc region can bind to

- Fcγ receptors on immune cells
- Neonatal Fcγ receptors (FcRn)
- The C1q component of complement.

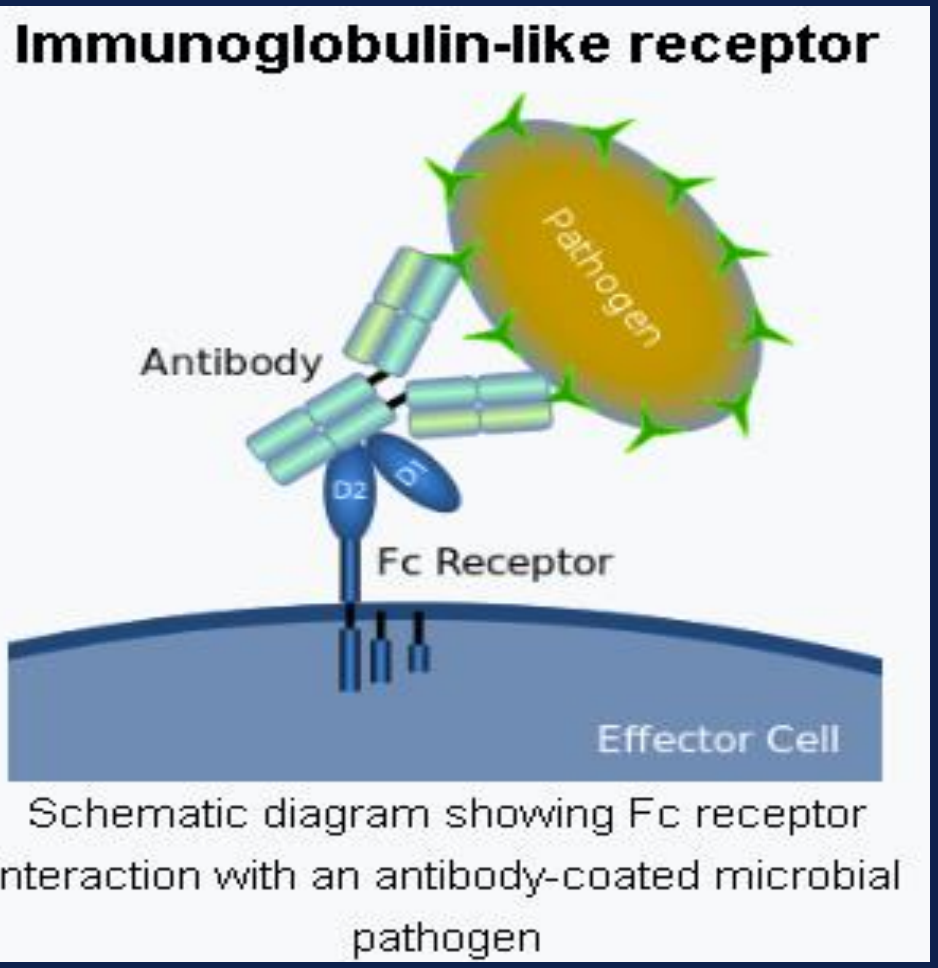
Biological Activity

- Antibody-dependent cell-mediated cytotoxity (ADCC)
- Complement-dependent cytotoxicity (CDC)
- Antibody-dependent cell-mediated phagocytosis (ADCP)

Selected Fc receptors and Their Importance

- Protein on surface of B cells, DCs, NK cells, macrophages, neutrophils, eos, basos, platelets, mast cells.
- Contribute to protective function of immune system.
- Fc receptors bind to Abs attached to infected cells or invading pathogens.
- Activity stimulates phagocytic or cytotoxic cells to destroy microbes or infected cells by phagocytosis or ADCC.
- Several types of Fc receptors based on type of antibody recognized. Fc gamma binds IgG, for example.
- All FcGamma receptors belong to immunoglobulin superfamily and are the most important Fc receptors for inducing phagocytosis of opsonized microbes.

Fc Receptor Activity



Schematic diagram showing Fc receptor interaction with an antibody-coated microbial pathogen

Antibody Function

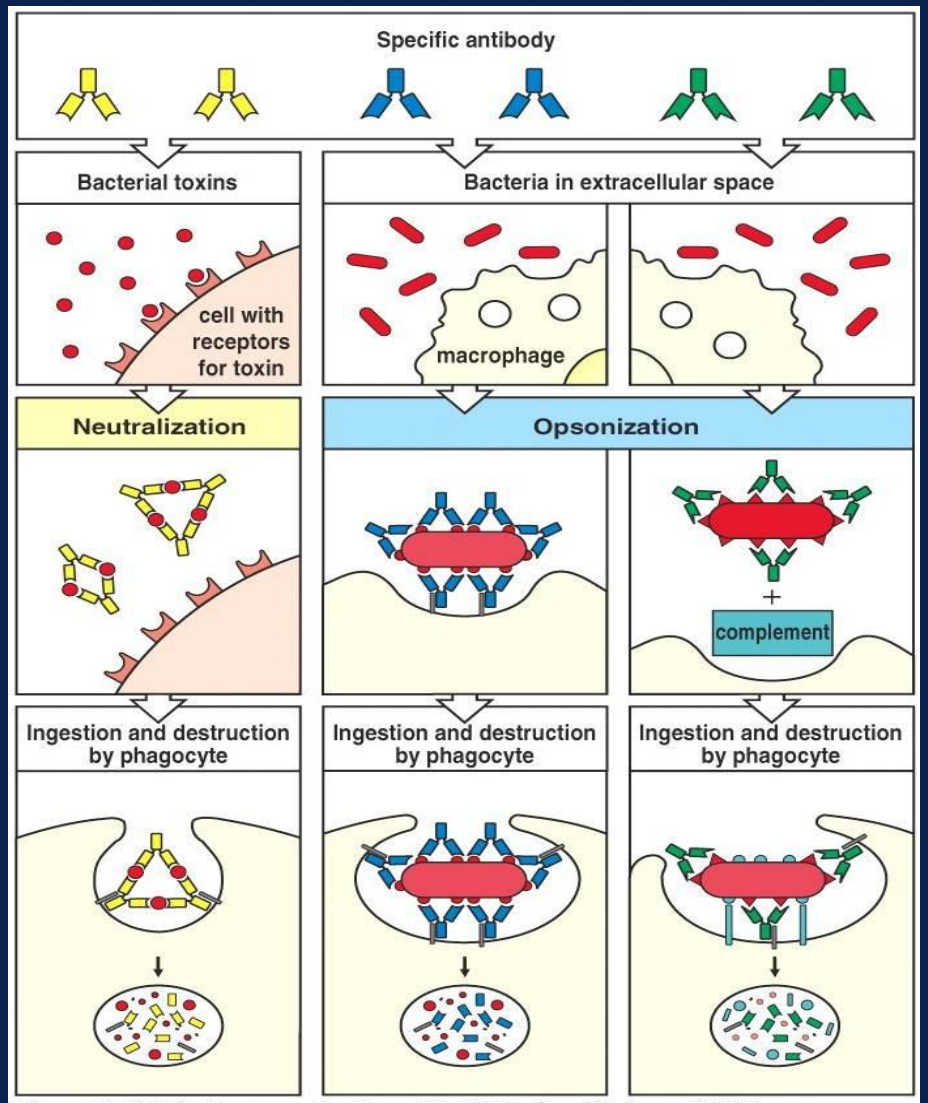


Figure 1-29 The Immune System, 2/e (© Garland Science 2005)

- Ab binds to pathogen and inhibits its toxic effect or infectivity=neutralization
- Abs coat pathogen enabling cells that recognize the Fc portion of the Ab to ingest and kill the organism=opsonization
- Abs can trigger complement activation=enhances opsonization or can directly kill

T Cell Function

MHC Based Antigen Presenting Cell-Lymphocyte Interactions

MHC II interacts with CD4+ lymphocyte

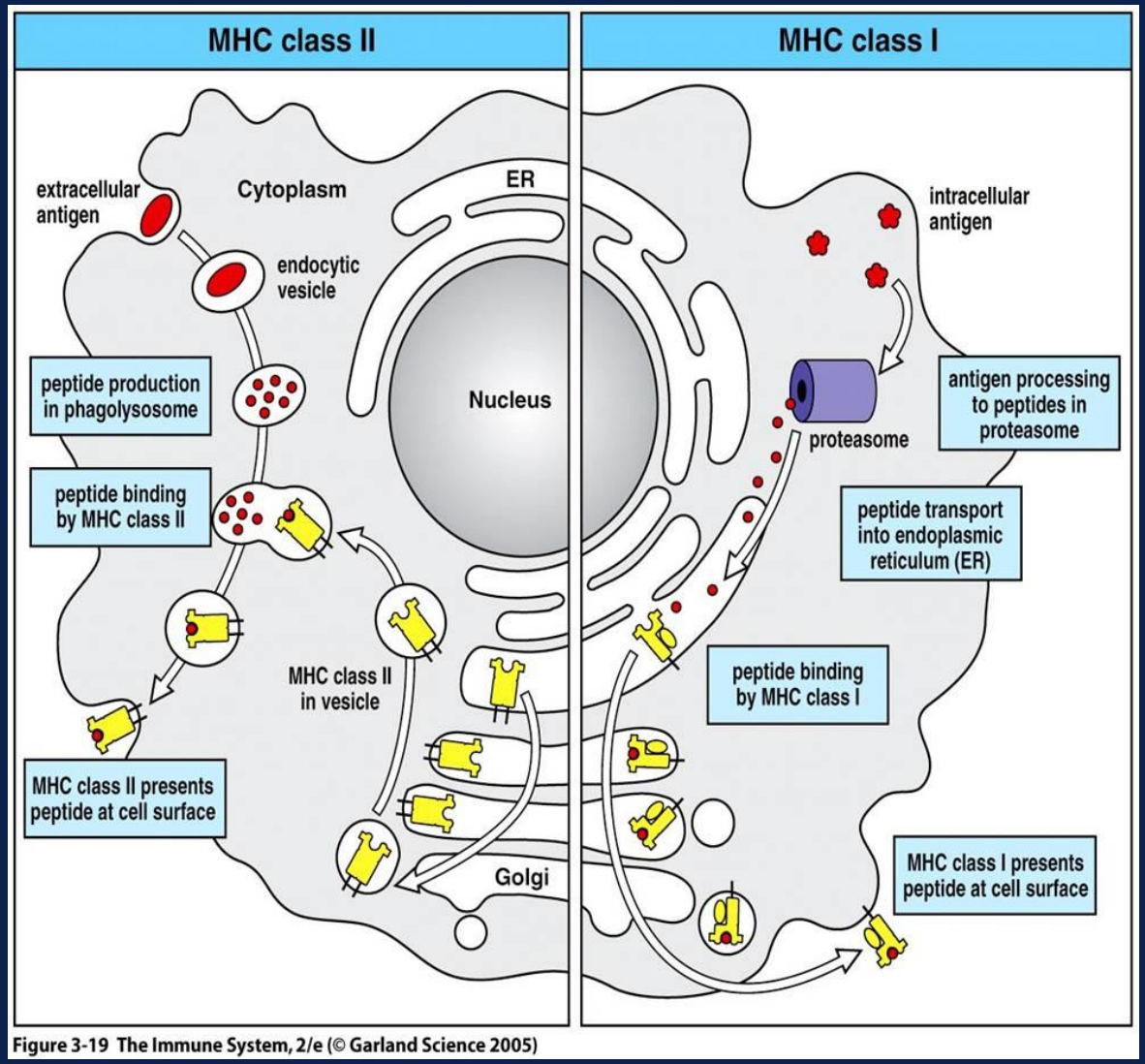


Figure 3-19 The Immune System, 2/e (© Garland Science 2005)

MHC I interacts with CD8+ lymphocyte

MHC Restriction

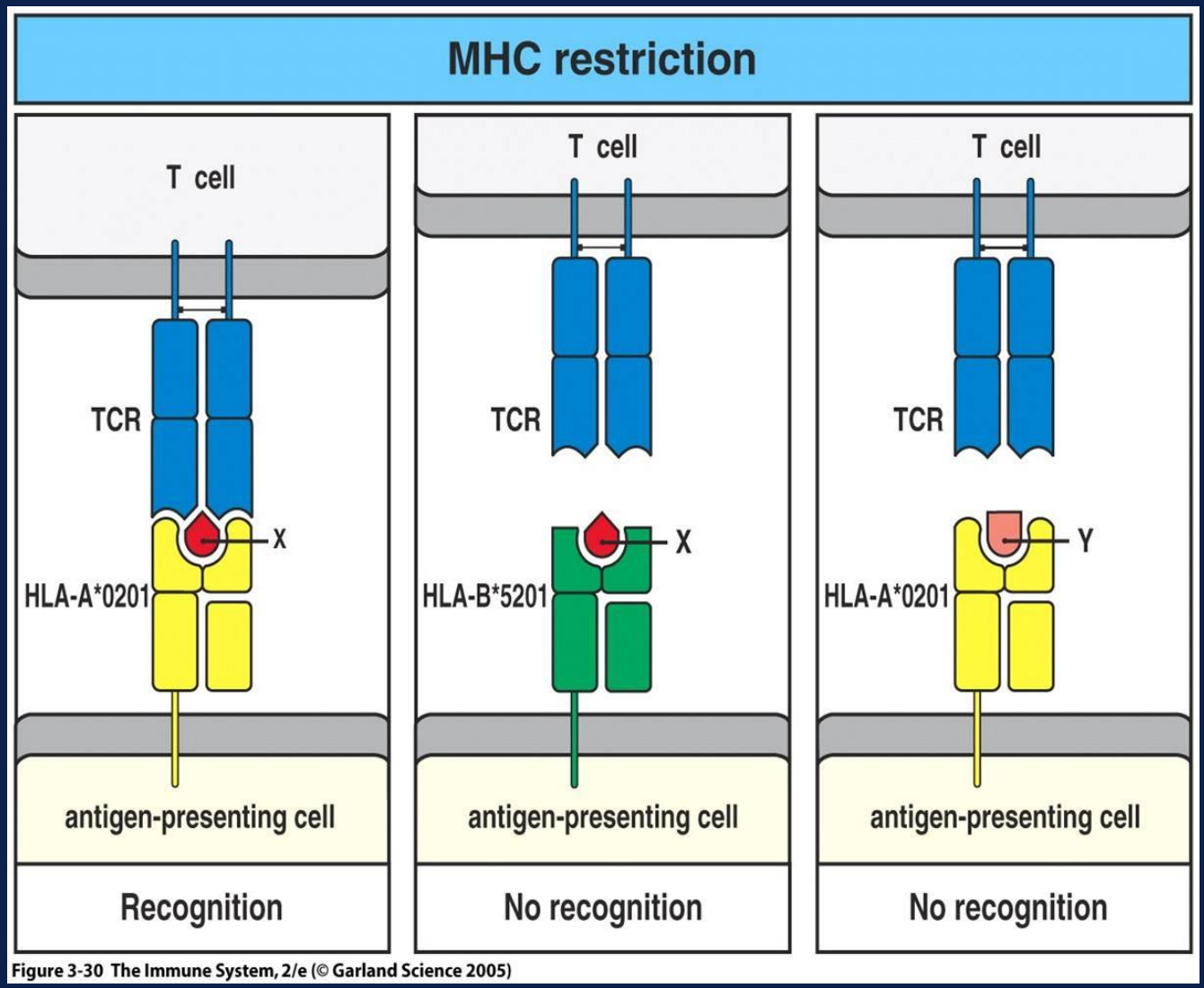


Figure 3-30 The Immune System, 2/e (© Garland Science 2005)

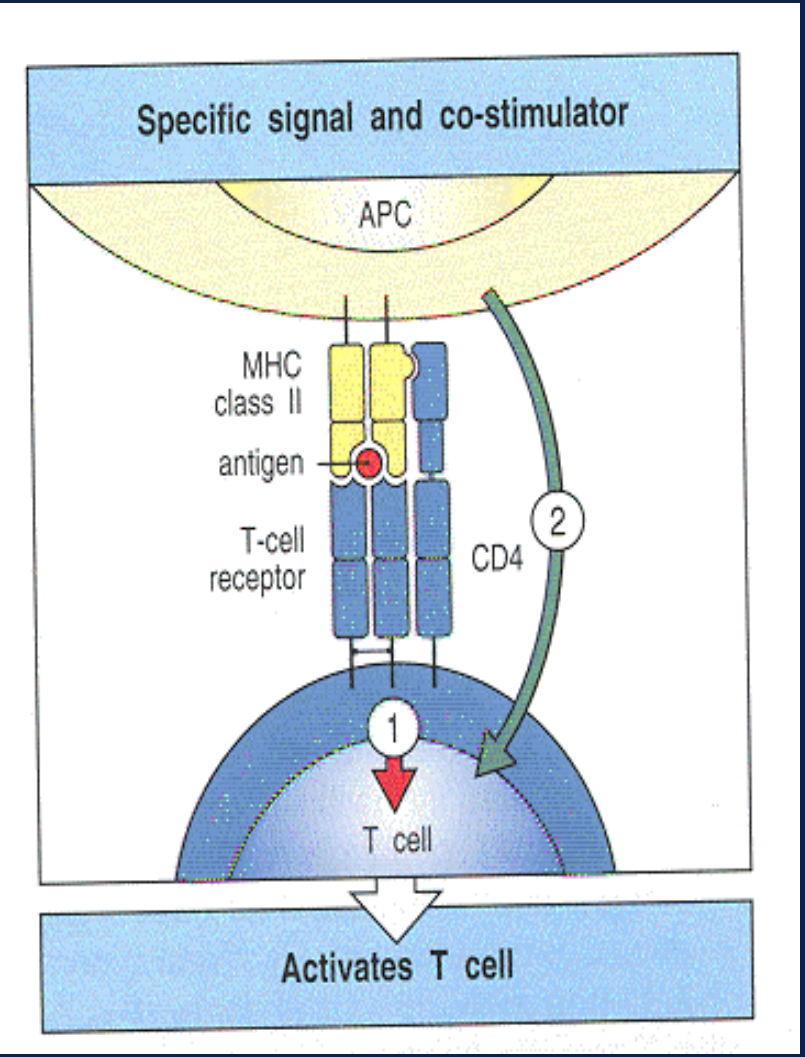
T cell activation

TCR ligation alone is insufficient to activate T cells

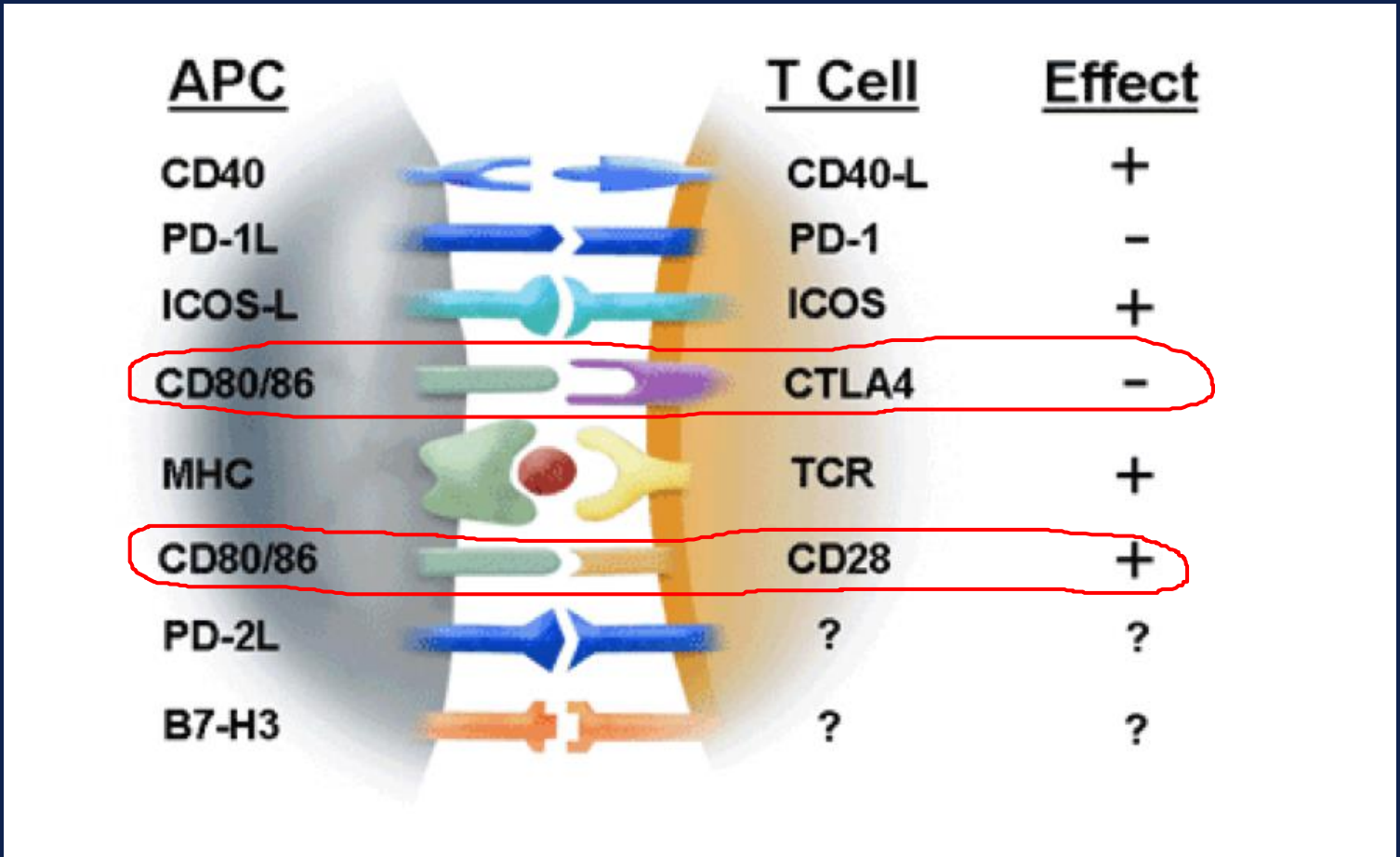
Safety control

Second signal is essential CO-STIMULATORY

Failure to co-stimulate results in ignorance, anergy or apoptosis

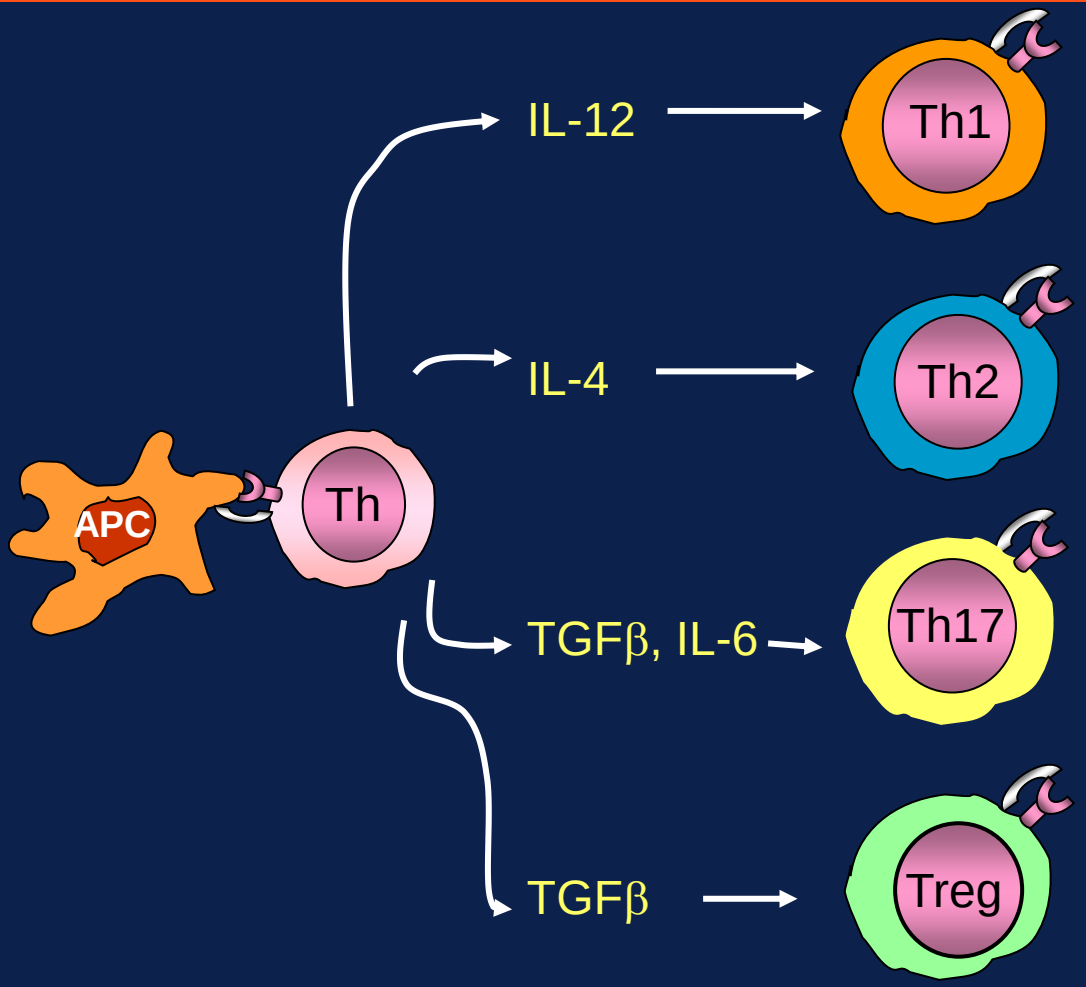


Co-stimulation-can have a positive or negative effect on the T cell



Carreno BM and Collins M. *Annu Rev Immunol*. 2002; 20:29-53.
Stuart RW and Racke MK. *Expert Opin Ther Targets*. 2002; 6(3):275-89.

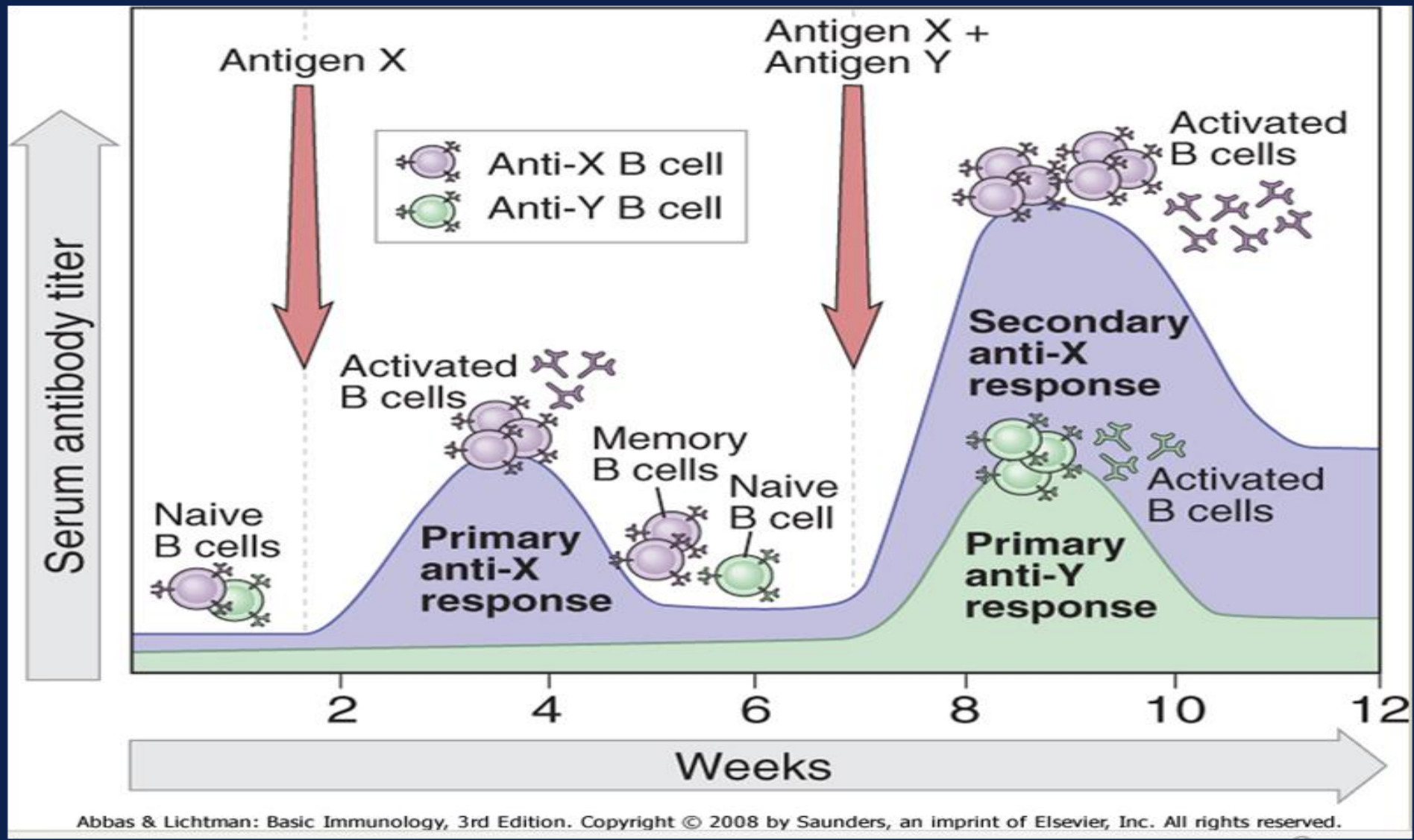
T Helper Cell Differentiation Driven by the Cytokine Milieu



Curtsinger *et al.*, 1999 Journal of Immunology, 162:3256–3262

Gutcher and Becher, 2007 Journal of Clinical Investigation 117(5):1119-1127

Specificity and Memory in the Immune Response



Abbas & Lichtman: Basic Immunology, 3rd Edition. Copyright © 2008 by Saunders, an imprint of Elsevier, Inc. All rights reserved.

Cytokine Biology

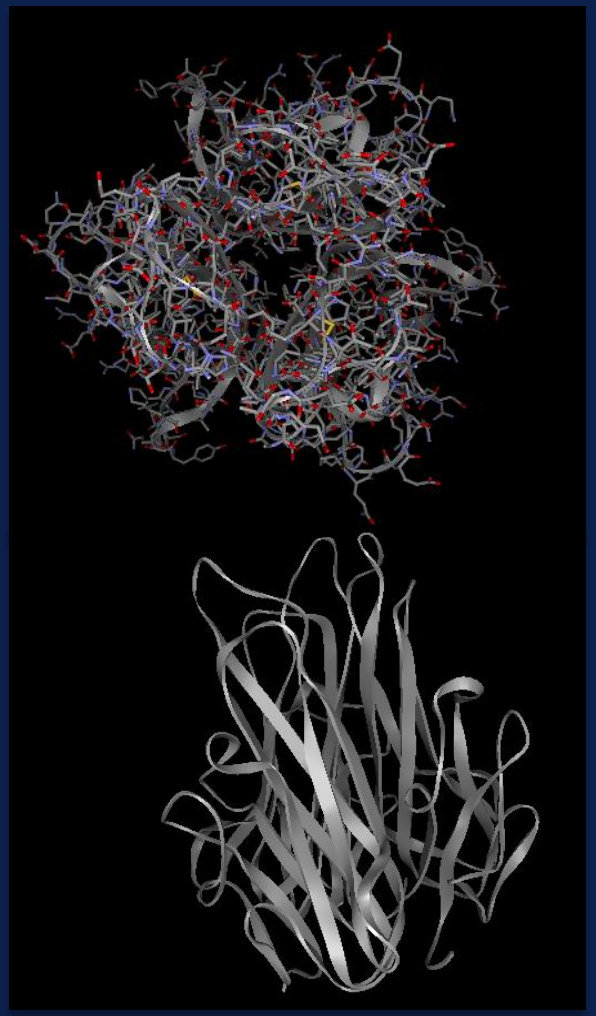
- Definition: Secreted proteins that function as mediators of immune and inflammatory reactions.
- Allow communication between immunocompetent cells.
- Innate immune response-produced mainly by macrophages and NK cells.
- Adaptive immune response-produced mainly by T cells.

Cytokines in Inflammatory Diseases

- Drive inflammation
- Drive joint damage
- Drive systemic manifestations

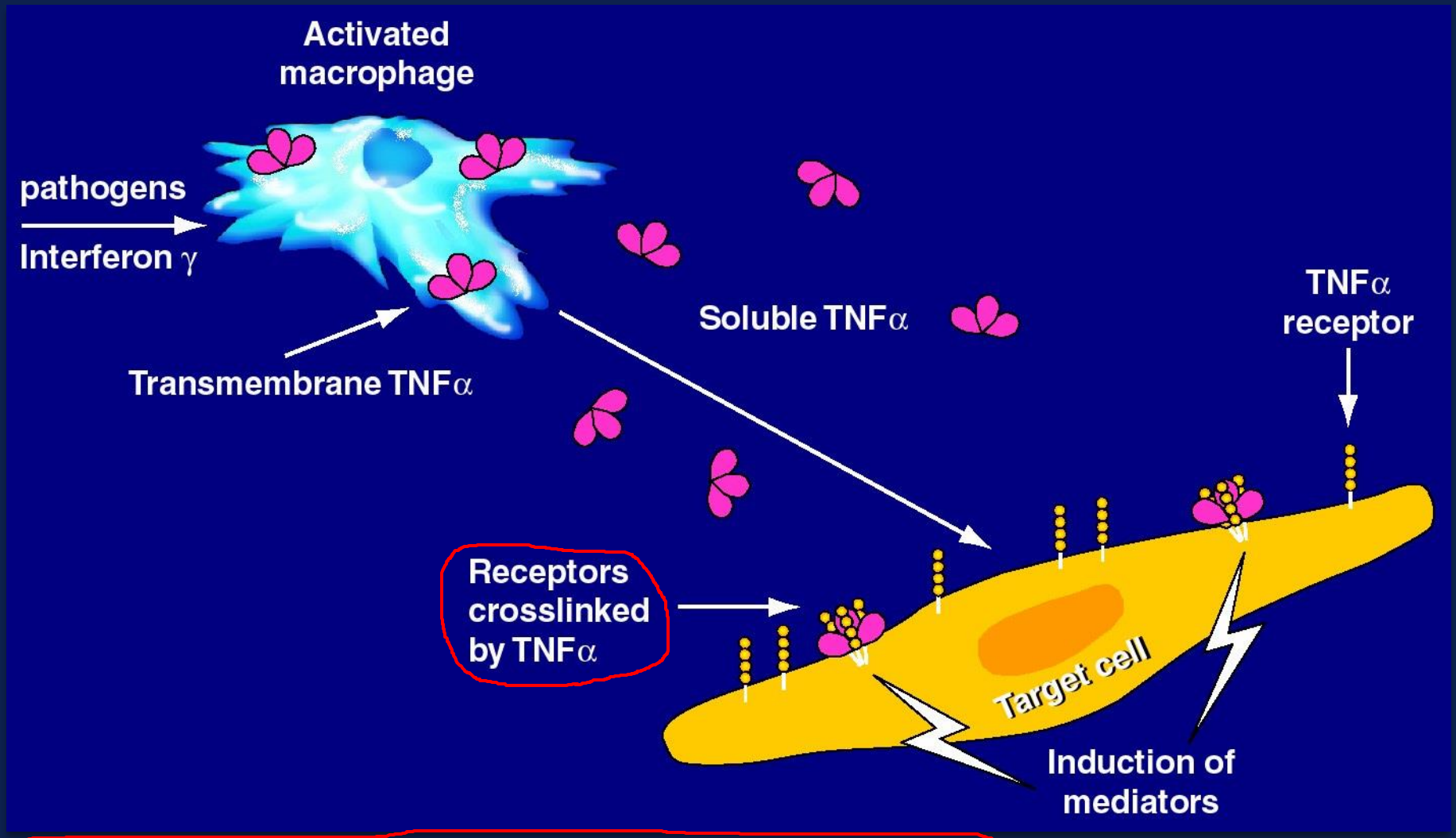
Tumor Necrosis Factor α (TNFα)

- Expressed as a transmembrane protein
  - Cleaved by TACE on cell surface
- Active protein is trimeric
  - 157 amino acids / monomer
  - Unglycosylated
  - One intrachain disulfide per monomer for stability
- Binds p55 (ubiquitous) and p75 receptors (hematopoietic cells)
  - Receptors present on virtually all cells (200 – 10,000!!)



Jones EY, et al. *Nature* 1989;338(2):225-228; Fiers W, et al. FEBS *Letters* 1991;285(5):119-212.

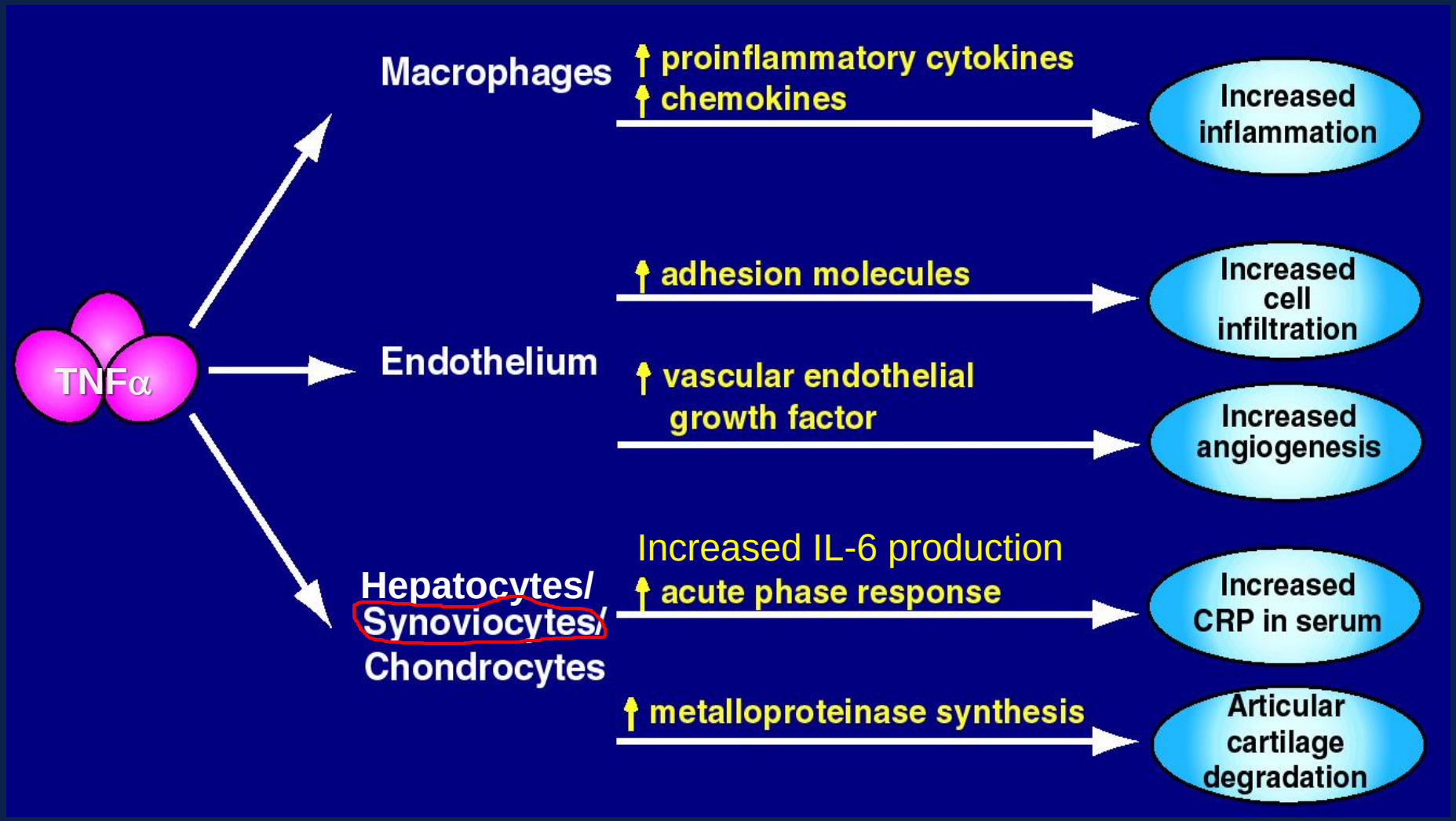
Synthesis and Function of TNFα



***TNF receptors phosphorylate each other—induces signaling

Fiers W, et al. FEBS *Letters* 1991;285(2):199-212.

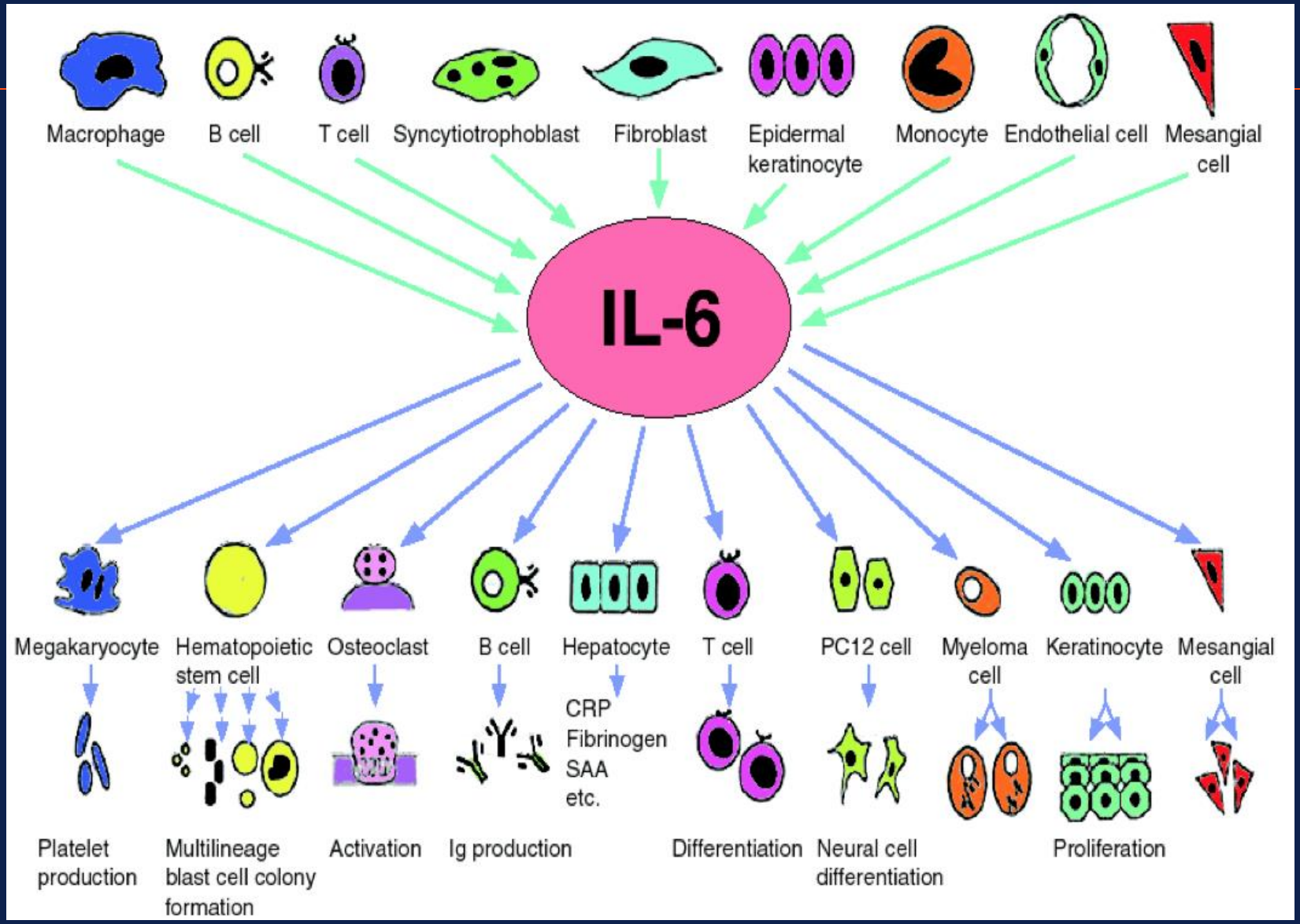
Key Actions of TNFα



Mcinnes IB, Schett G. *Nature Rev* 2007;7:429-442; Tracey D, et al. *Pharmacol Therapeutics* 2008;117:244-279.

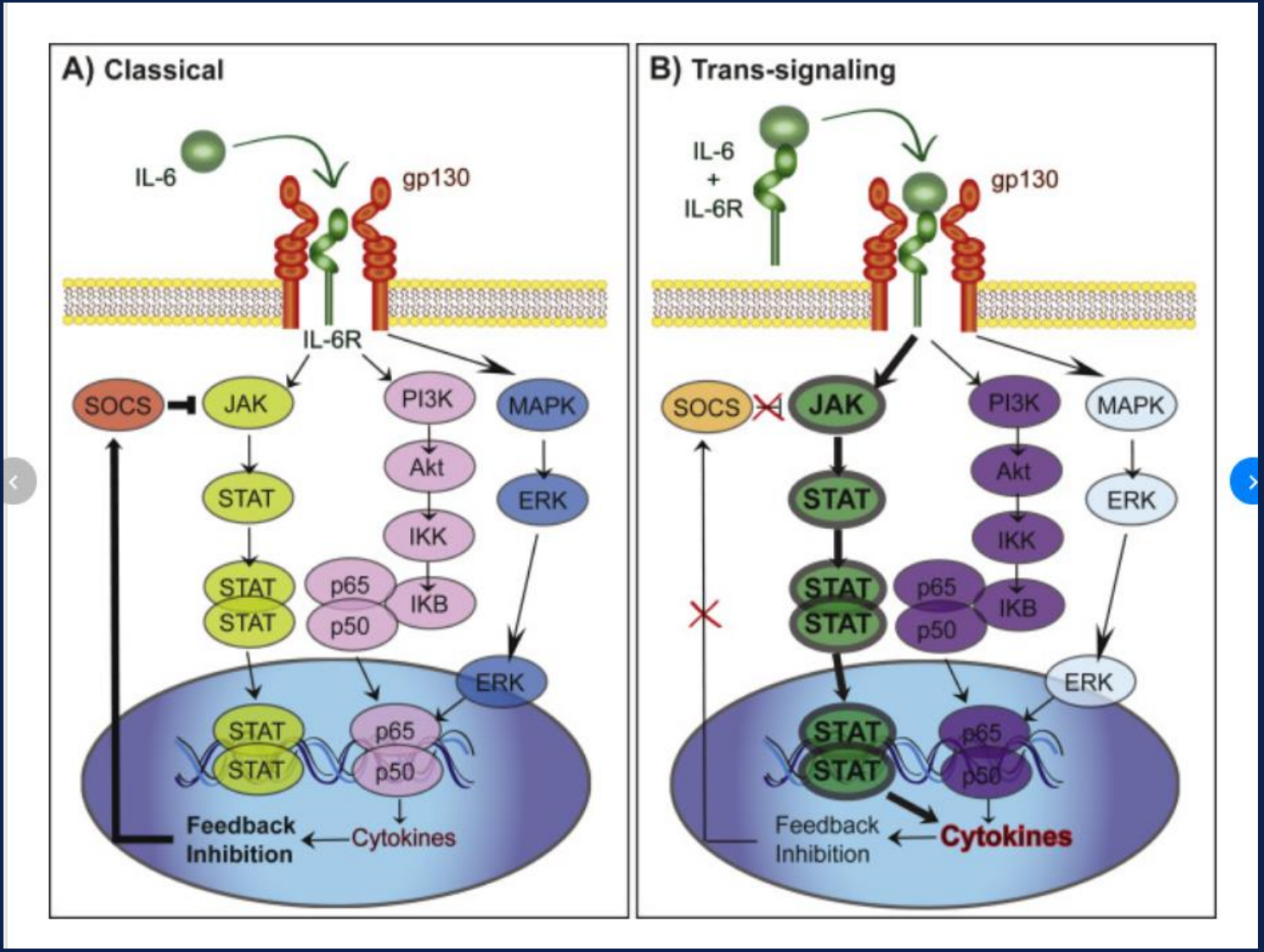
INTERLEUKIN 6

Functions of IL-6



Source: Am J Health Syst Pham @2008 American Society of Health-System Pharmacists

IL-6 affects a broad range of cells and tissues
It can do so because of its unique signaling mechanism



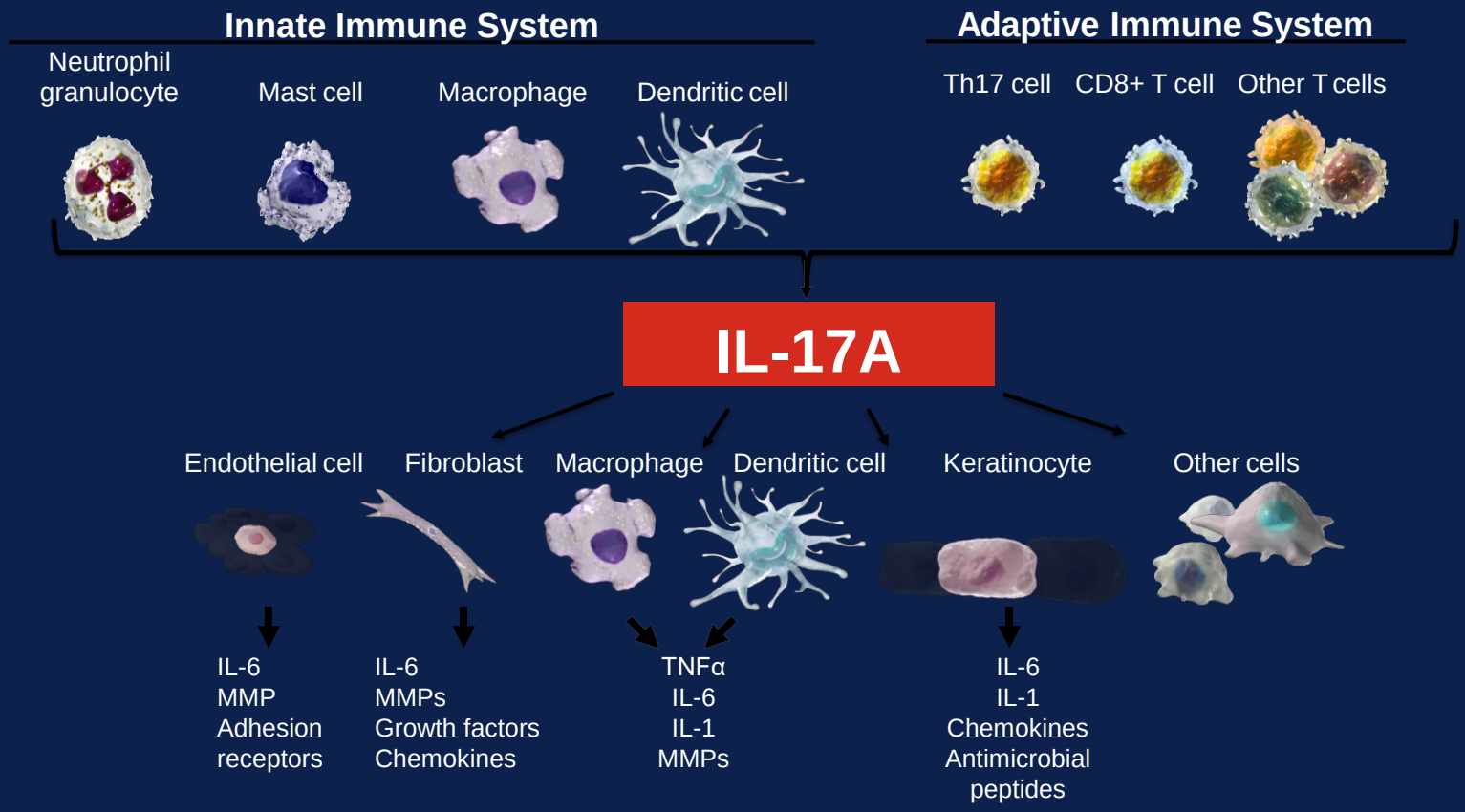
IL-17 Family of Cytokines

- A family of cytokine dimers formed from 6 different subunits[1]
- IL-17 cytokines exist as dimers[2,3]

IL=Interleukin.

1)Gaffen SL. *Nat Rev Immunol*. 2009;9:556-567.
2)Wright JF, et al. J Biol Chem. 2007;282(18):13447-55.
3)Chang SH, et al. Cell Res. 2007;17(5):435-40.

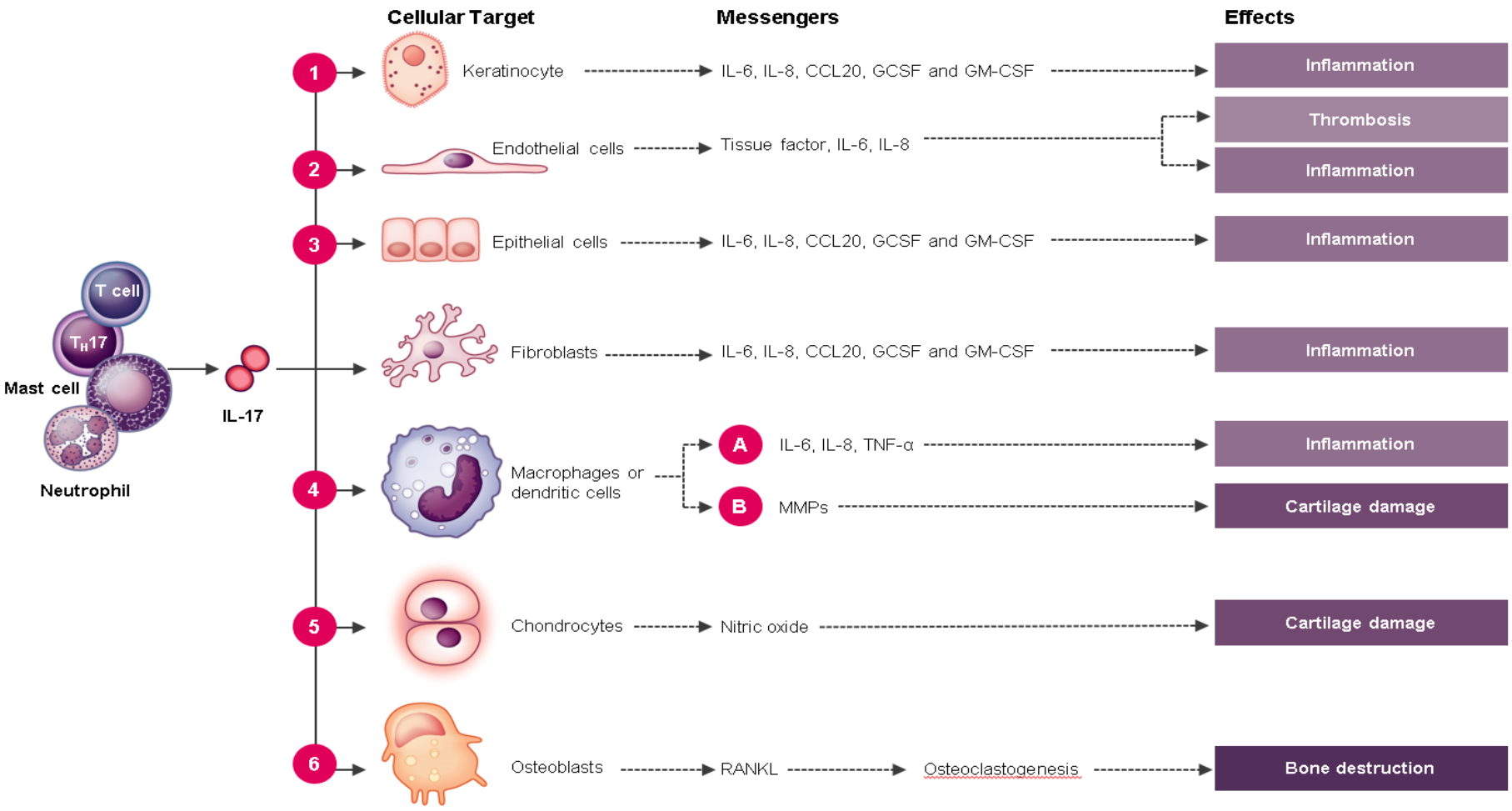
IL-17A at the Cross Roads: Producers and Responders



Adapted from Schön MP. *Exp Dermatol*. 2014;23:804–806.

Interleukin-17 Activities

Effects of IL-17 on Various Tissues



CCL = chemokine-ligand; GCSF = granulocyte-colony stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; TH = T helper; MMP = matrix metalloproteinases; TNF-α = tumor necrosis factor alpha; RANKL = receptor activator of nuclear factor kappa beta

PTI

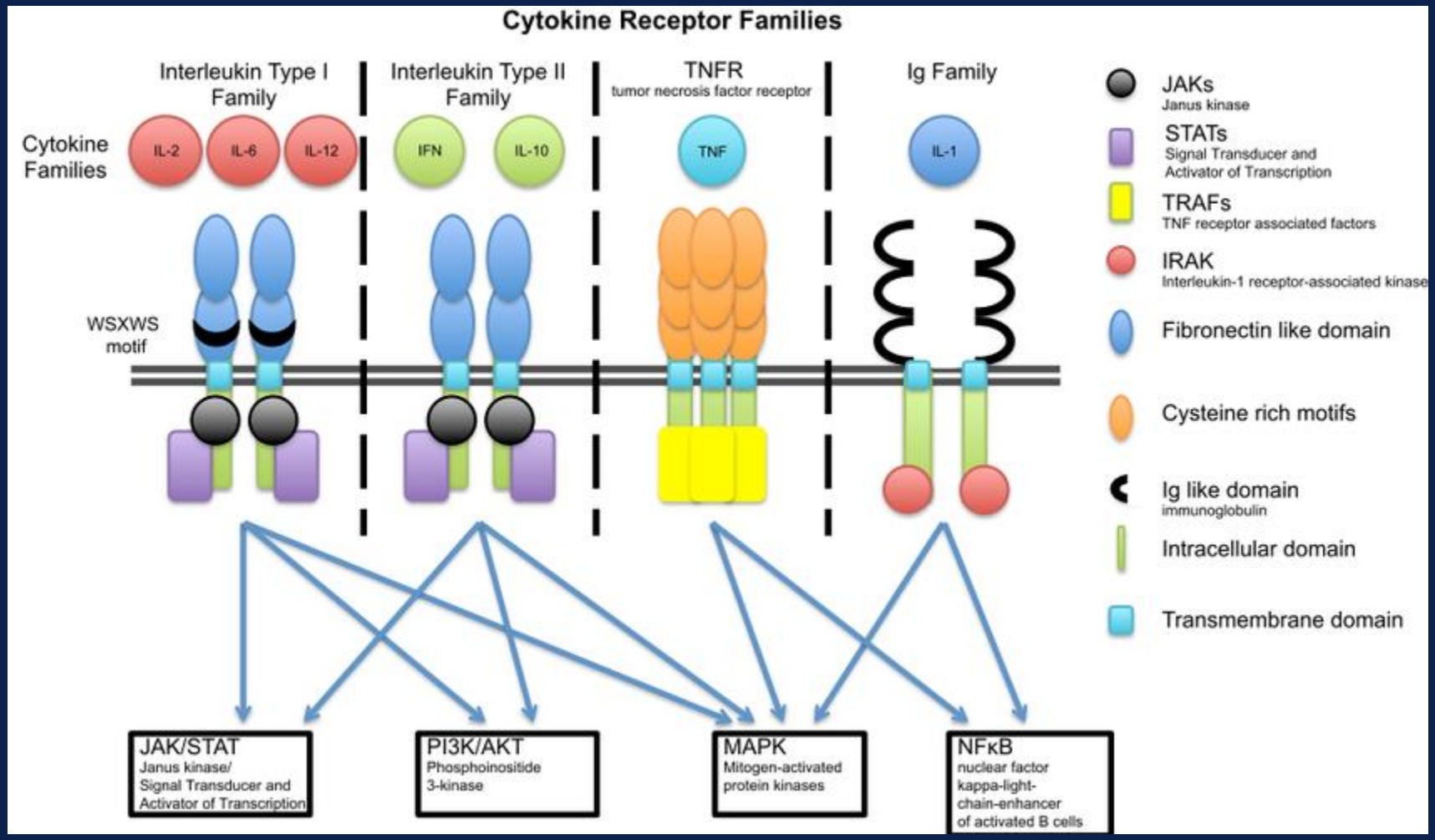
Intracellular Signaling

- When a cytokine/ligand binds to its cell surface receptor a message must be transmitted to the nucleus.
- In the case of inflammatory diseases this might generate the production of proinflammatory cytokines

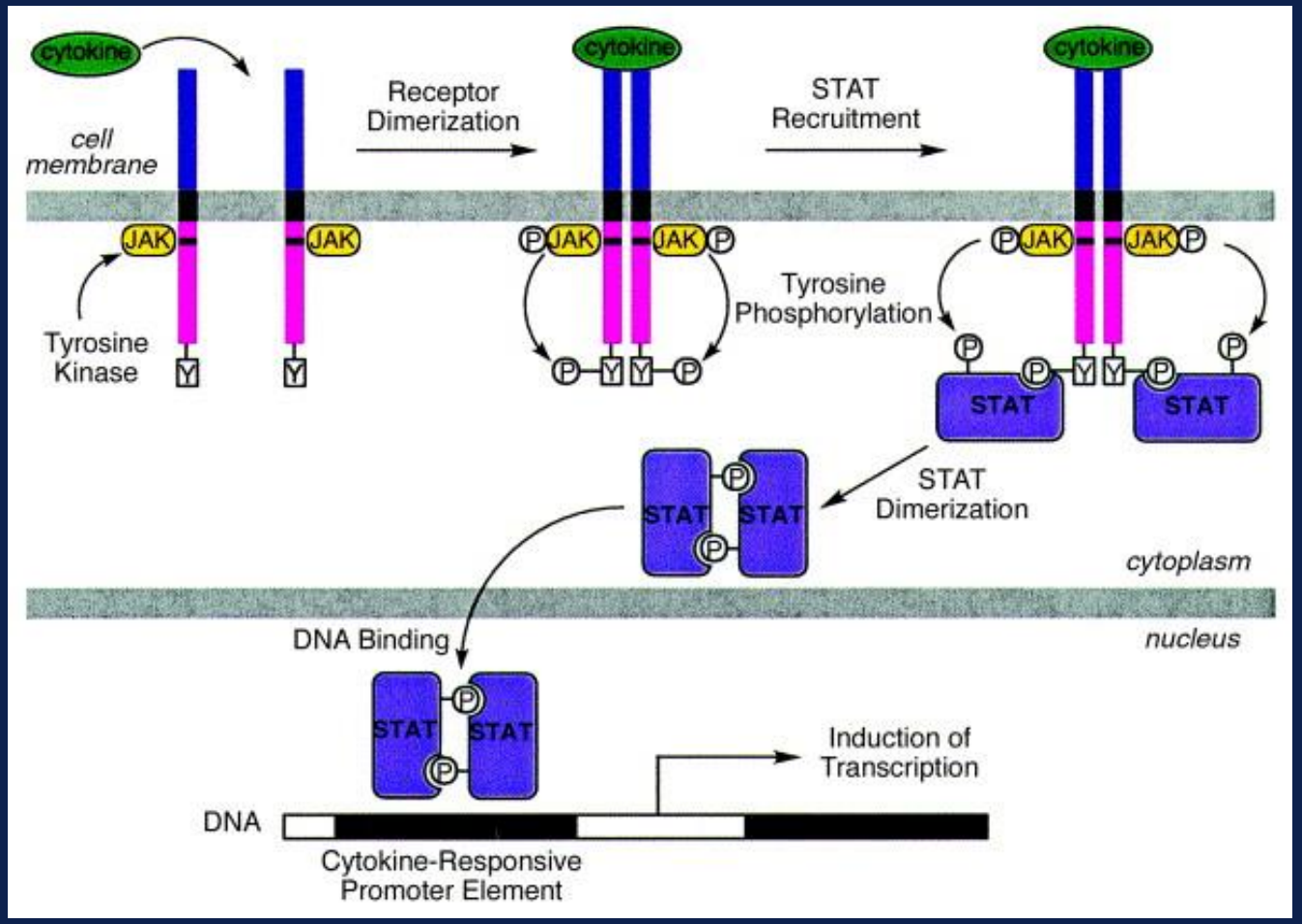
7 different cytokine receptors superfamilies

- IL-17 cytokine receptors
- Types I and II cytokine receptors
- TNF receptors
- Chemokine G protein coupled receptors
- Ig receptors
- TGF-B receptors

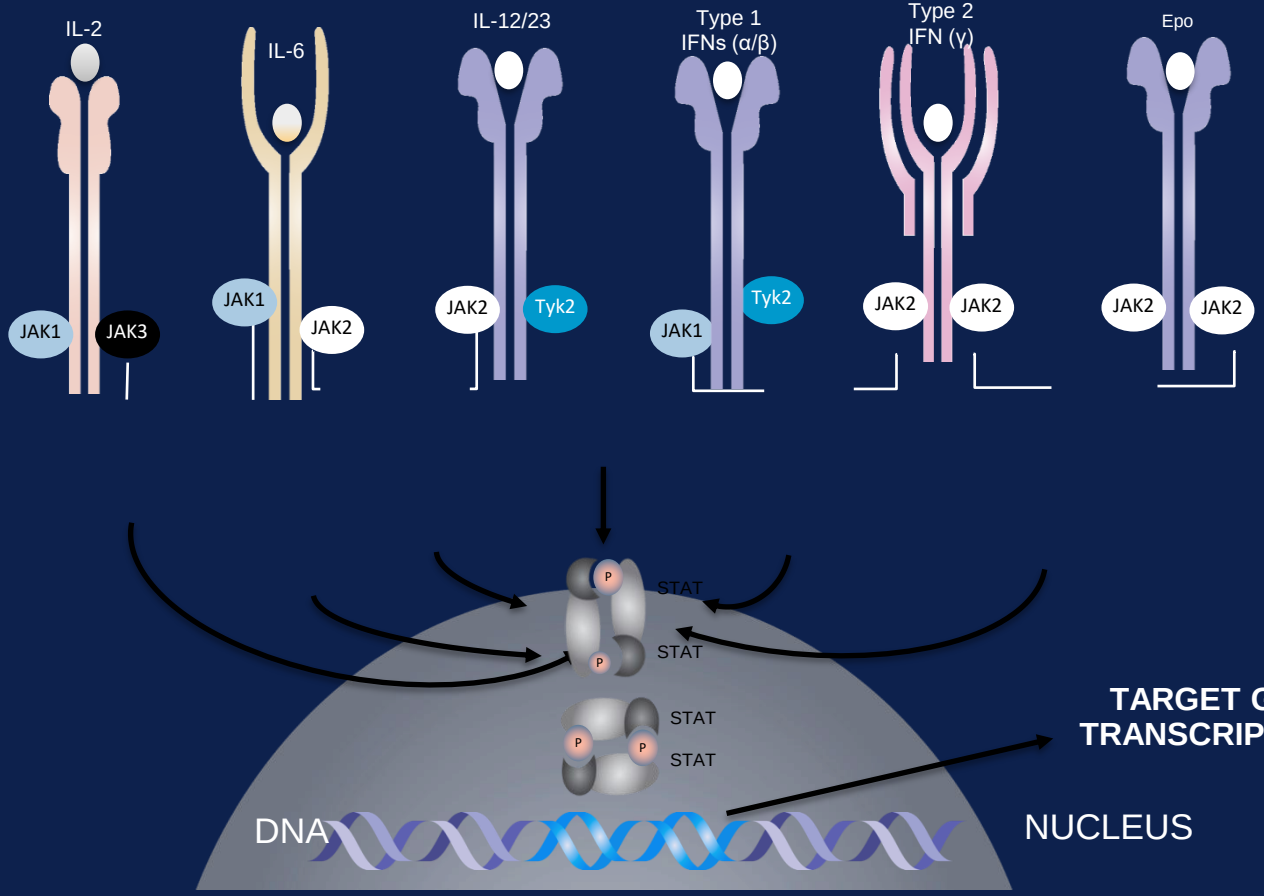
Cytokine Receptors Use Different Signaling Pathways



JAK-STAT Signaling



Signaling by Different Cytokines Requires Unique JAK Pairings and Unique STAT Pairings



Furumoto Y, et al. *Bio Drugs*. 2013; 27: 431-438.

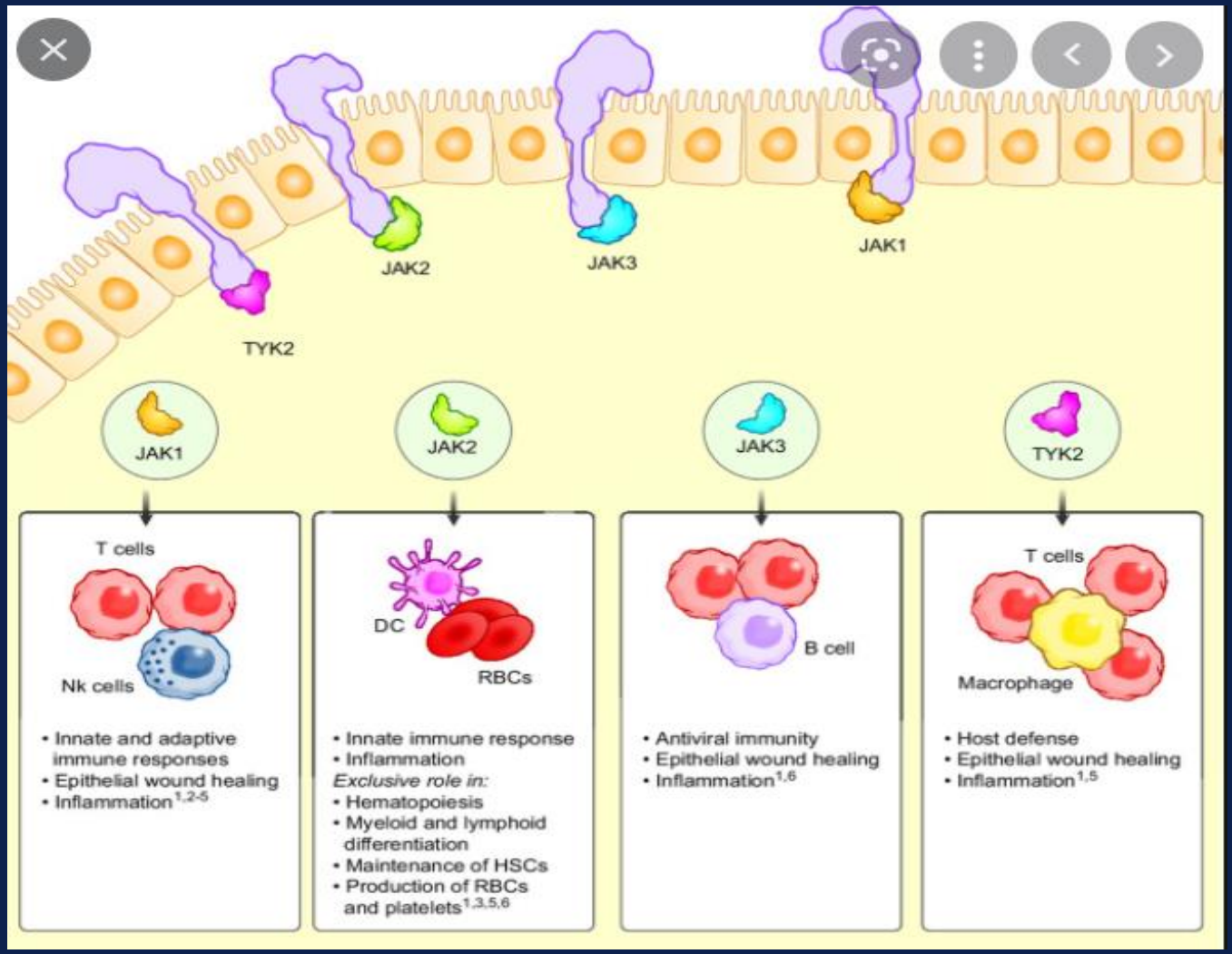
4 JAKs: JAK1, JAK2, JAK3,TYK2
Different Functions

JAK1
Plays major role in signaling of key proinflammatory cytokines[3]

JAK2
Mediates signals for hematopoietic growth factors[3]

JAK3=Innate>Adaptive Immunity/Antiviral immunity

TYK2=Innate>Adaptive Immunity/Antiviral immunity



Interleukin-17 Signaling

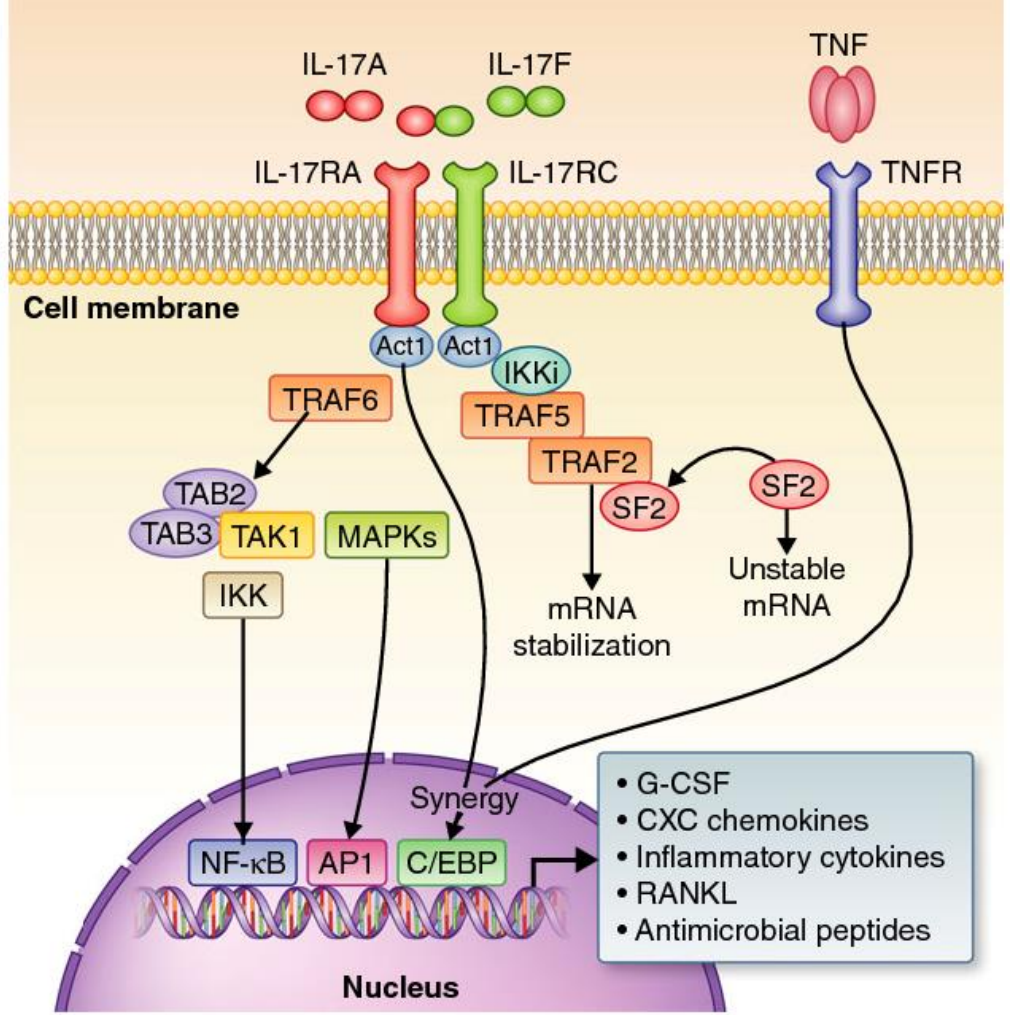


Fig. 1. Interleukin-17 receptor signaling. The interleukin-

Aging of the Immune System

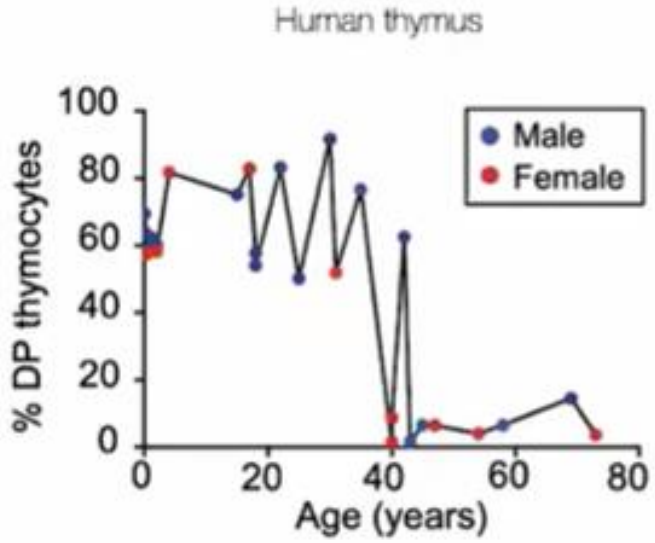
- Loss of adaptive immunity
  - Marked decline in the T-cell repertoire
  - Deterioration in Treg function
- Gain of nonspecific innate immunity

- Older individuals more susceptible to infection and cancer
- Older individuals unprotected from chronic tissue inflammation
- Inflammatory disease in the elderly promoted by loss of immunoinhibition = deterioration of Treg function

Weyand, Annals ATS, Volume 13, December 2016

Aging of the Immune System

Decline in naive T cell production with age



CD8>>CD4

Donna Farber and colleagues
Science Immunology 2016

Features of Immune System Aging

- Weakened antimicrobial immunity-more respiratory infections, shingles
- Impaired responses to vaccines
- Insufficient protection against malignancies
- Failing wound repair mechanisms
- Predisposed to unopposed tissue inflammation
    - Atherosclerotic disease
    - Osteoarthritis
    - Neurodegenerative disease

Weyand, Annals ATS, Volume 13, December 2016

Summary

Abnormal immune responses are the cause of many of our inflammatory diseases with serious morbidity and mortality.

Antibodies are in widespread use to treat immunologic diseases.

Understanding immunology helps us to better understand the diseases that we treat and their therapies.

QUESTIONS ?