Immunology for the Rheumatologist

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AWIR 2019

Learning Objectives

We spend much of our time as rheumatologists 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
- 5) Discuss the immunopathogenesis of rheumatoid arthritis
- 6) Review the treatment of rheumatoid arthritis from an immunologic perspective

Disclosures

- Speakers' Bureau: Abbvie, Amgen, BMS, Janssen, Lilly, Merck, Novartis, Pfizer, Quest, Sanofi-Regeneron

Role of the immune system

- ***Defense against infection***
- Surveillance against tumors
- Recognizes and reacts against foreign proteins and tissues
- ***How do perturbations in the normal immune system result in disease?***
- ***How can we modulate the aberrant immune response to help our patients?***

Components of the Immune System

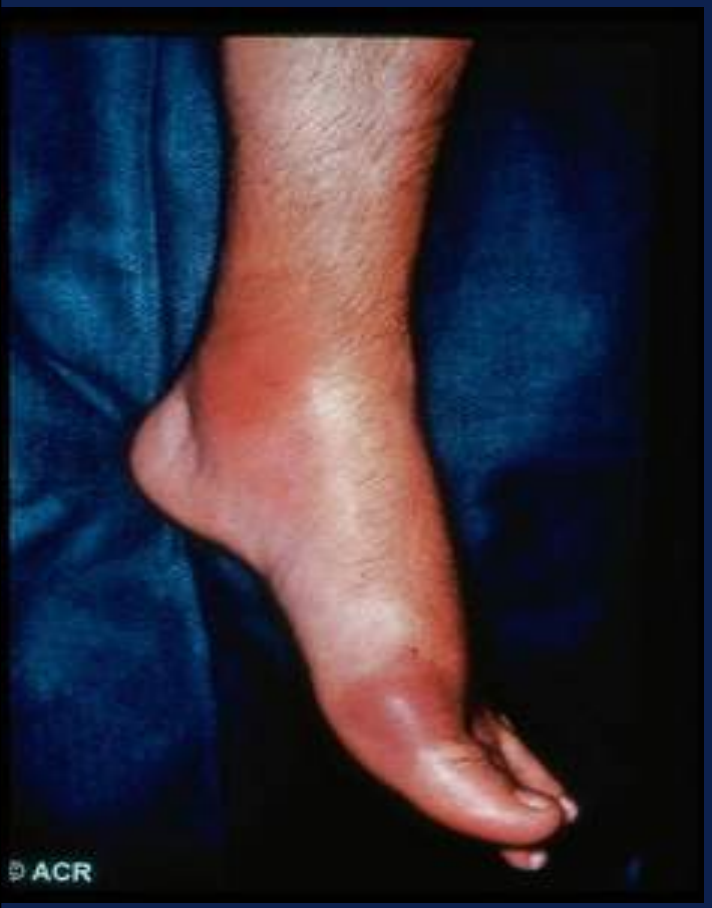
- **Physical Barriers-part of innate immune system**
  - Skin
  - Epithelial membranes

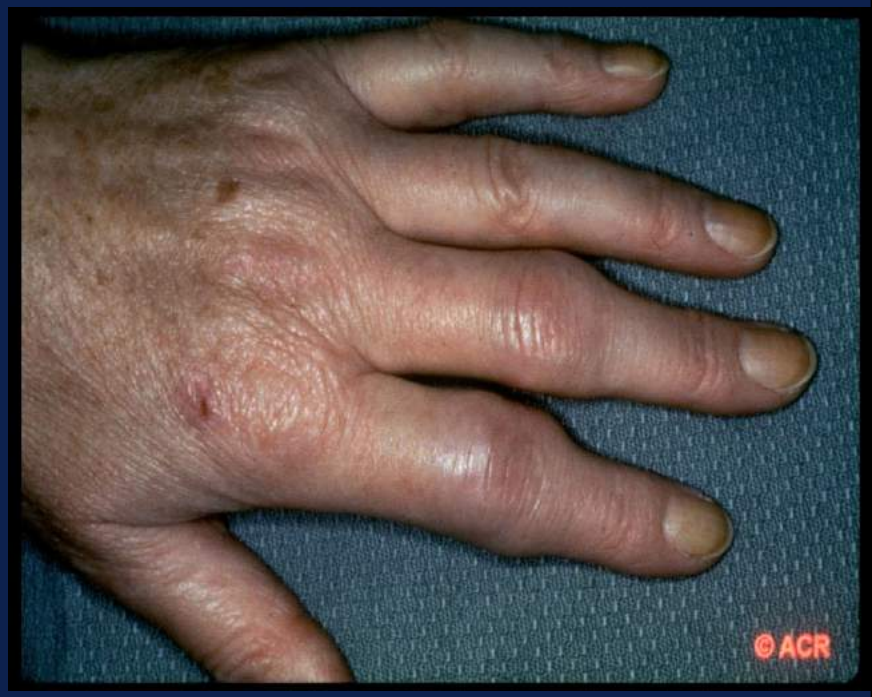
- **Innate Immune System**
  - "Non-specific" response
  - Involves both immune & non-immune cells
  - Immediate response
  - Response = **inflammation**
    cytokines/chemokines & co-stimulatory molecules

- **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 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)

Innate response

Adaptive response

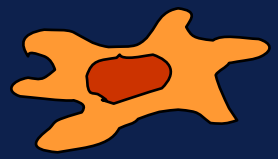
Neutrophil
(Phagocytosis,
Degranulation)

NK cell
(ADCC)

Eosinophil
(IL-5
producers)

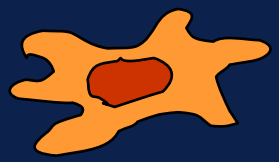
Basophil
(Histamine
Release)

Macrophage
(APC)

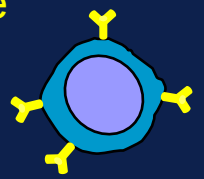


Dendritic cell
(APC)

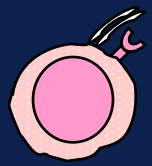
Dendritic cell
(APC)



Tc lymphocyte
(CD8+)



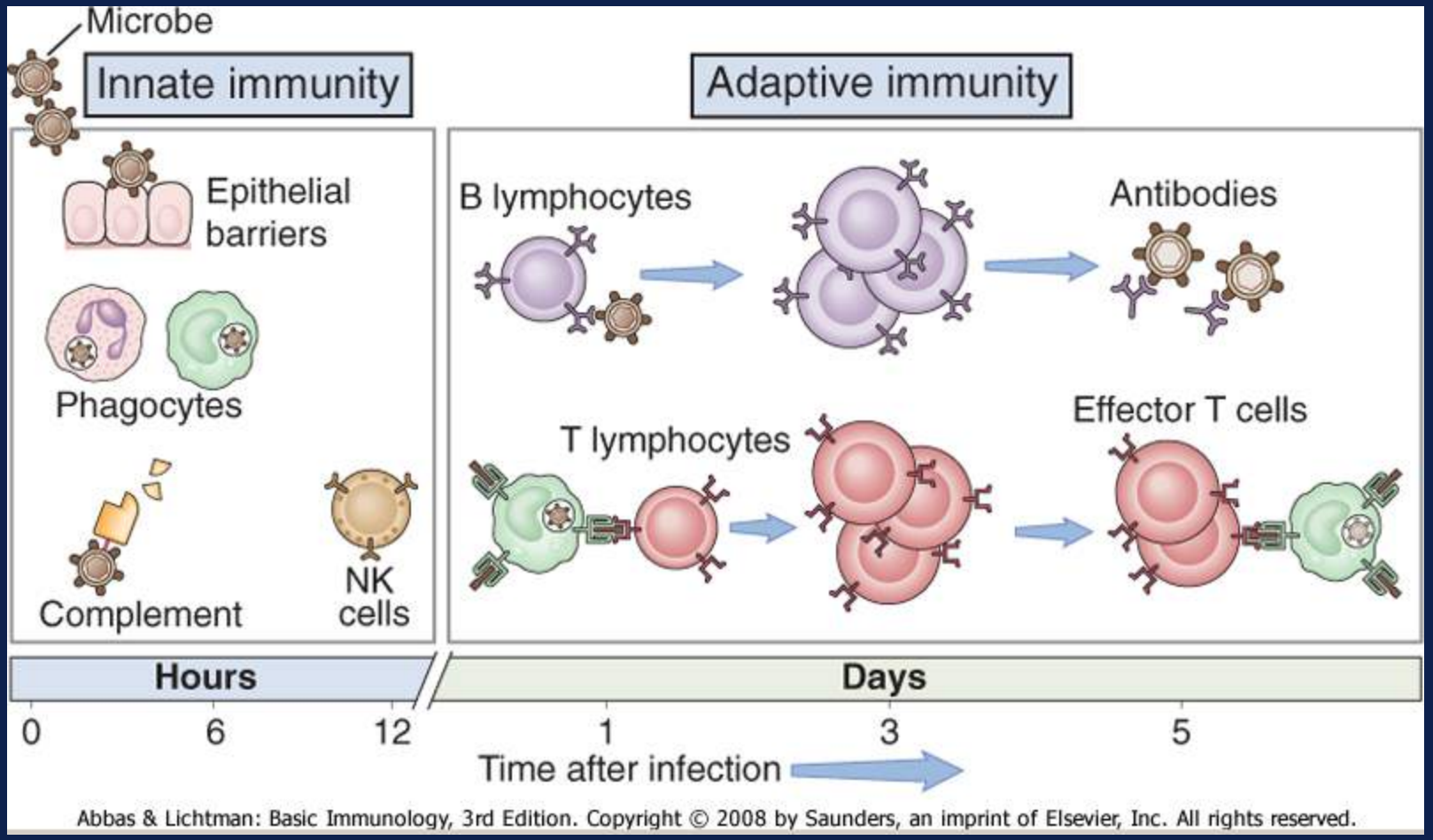
B lymphocyte



Th lymphocyte (CD4+)

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

Two Arms of the Immune System: Innate and Adaptive Immunity



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 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

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

Sensing Danger/Danger Signals
a.k.a. "pathogen-associated molecular ***patterns***" (PAMPS)
"danger-associated molecular ***patterns***" (DAMPS)

- Unique microbial structures
  - Bacterial cell wall components (LPS, PGN)
  - 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)

Present on cell surfaces

Present in blood and extracellular fluids

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

inflammation

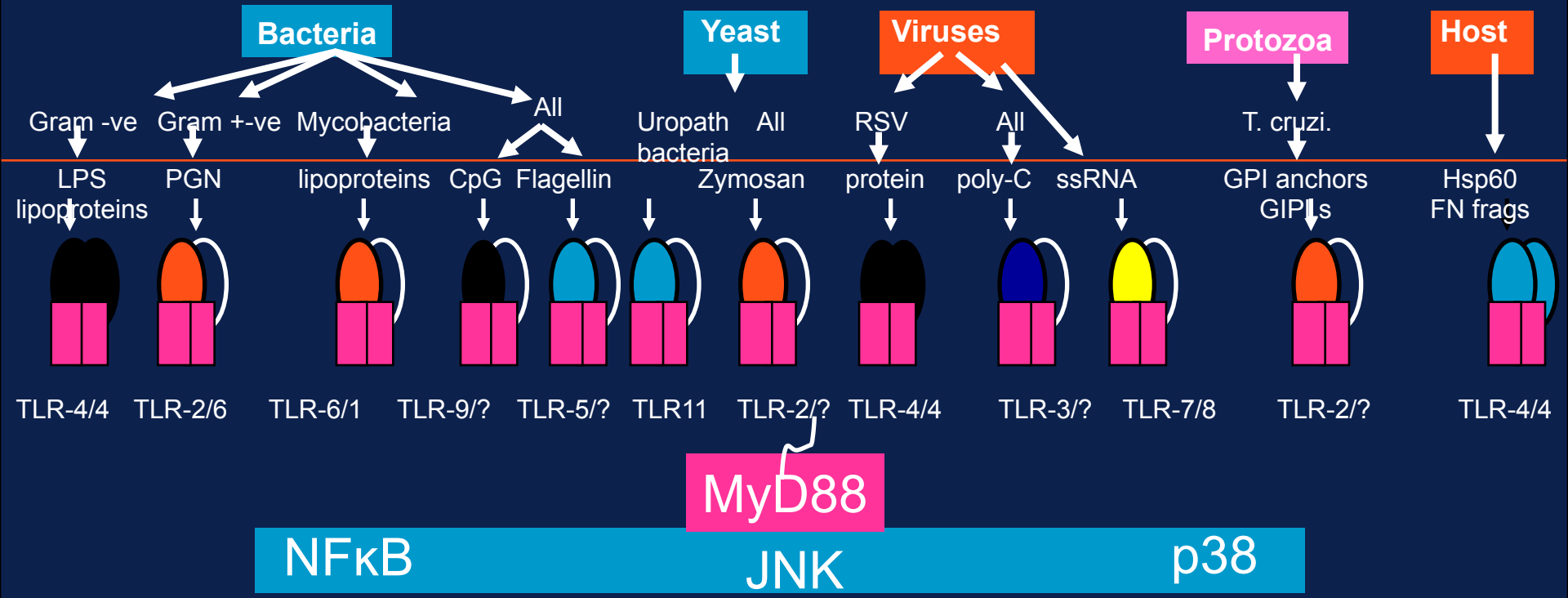
- Pentraxins
- ***Complement cascade***
- Collectins
- Ficollins

opsonization

- C-type lectins
- Scavenger receptors

phagocytosis

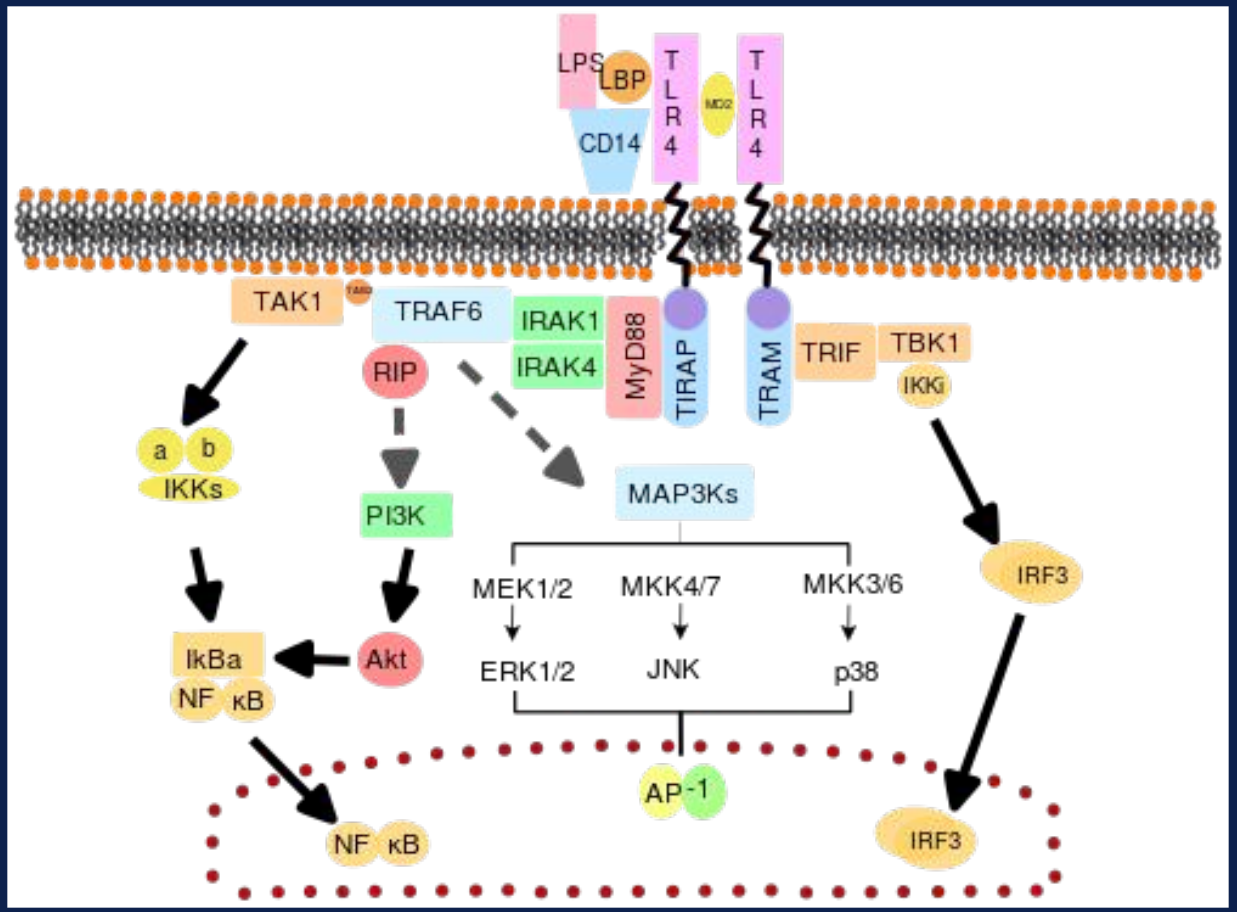
Recognition of pathogens by Toll-like receptors



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

TLR Signalling

Adapter proteins recruited
Signal transduction pathways activated
Drives gene transcription



Kawai et al. Nature Immunology. 11(5) 373-384. 2010

Macrophage Function

a) receptors for bacterial components

b) can bind and be activated by immune complexes

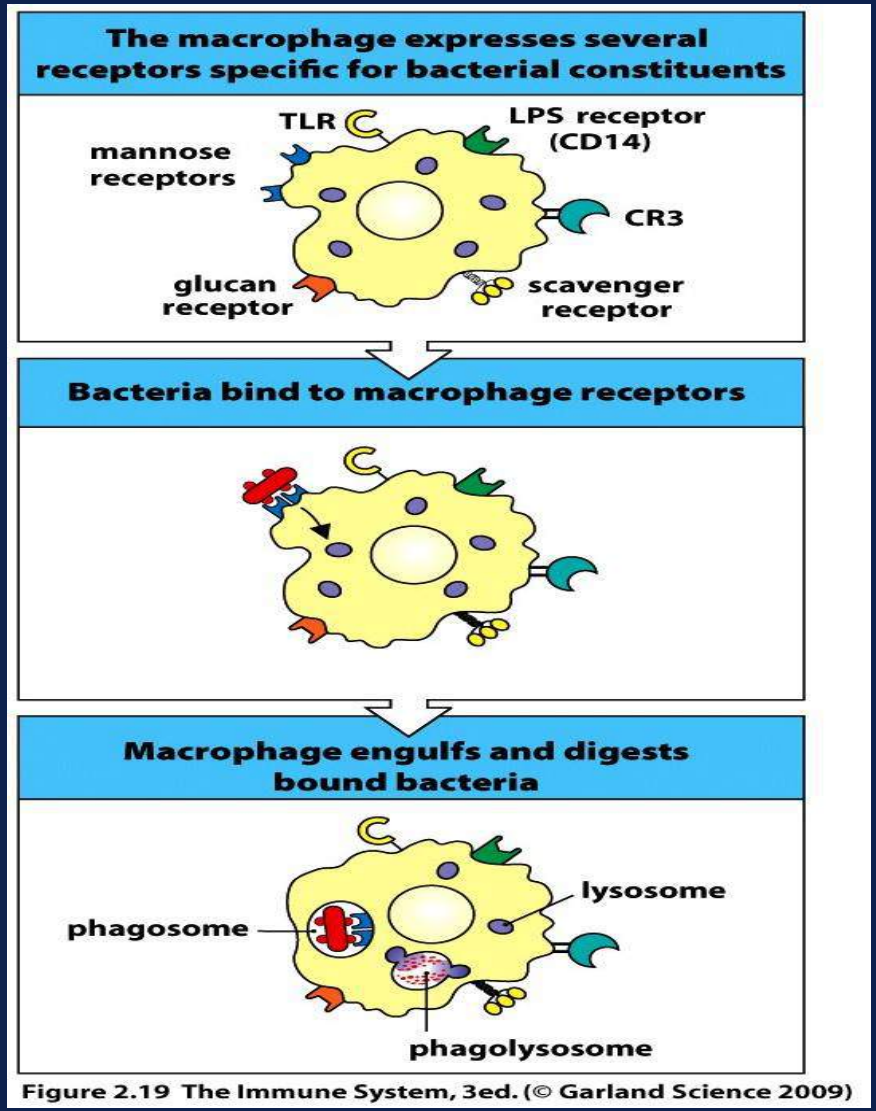


Figure 2.19 The Immune System, 3ed. (© Garland Science 2009)

Macrophage Function

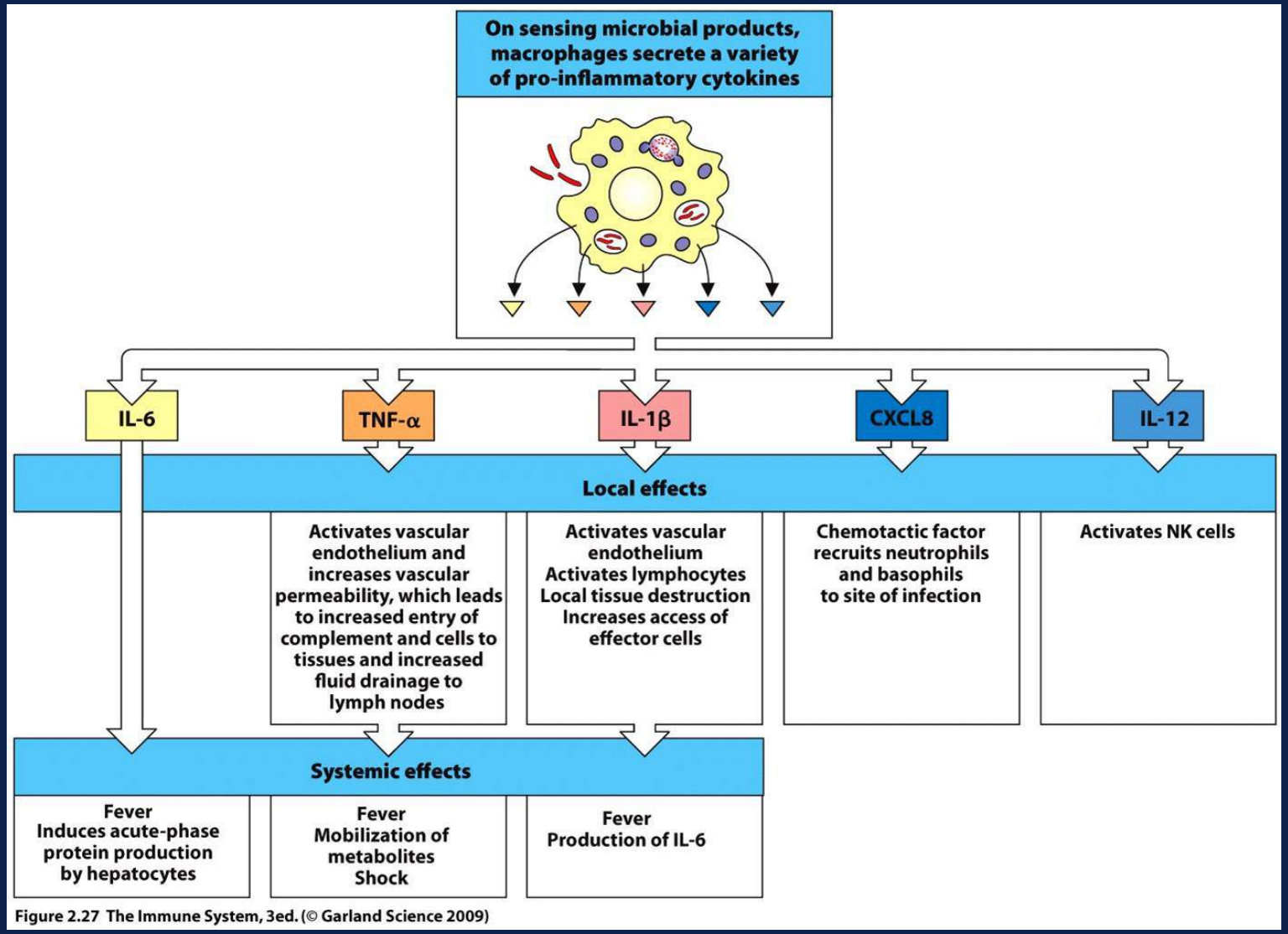
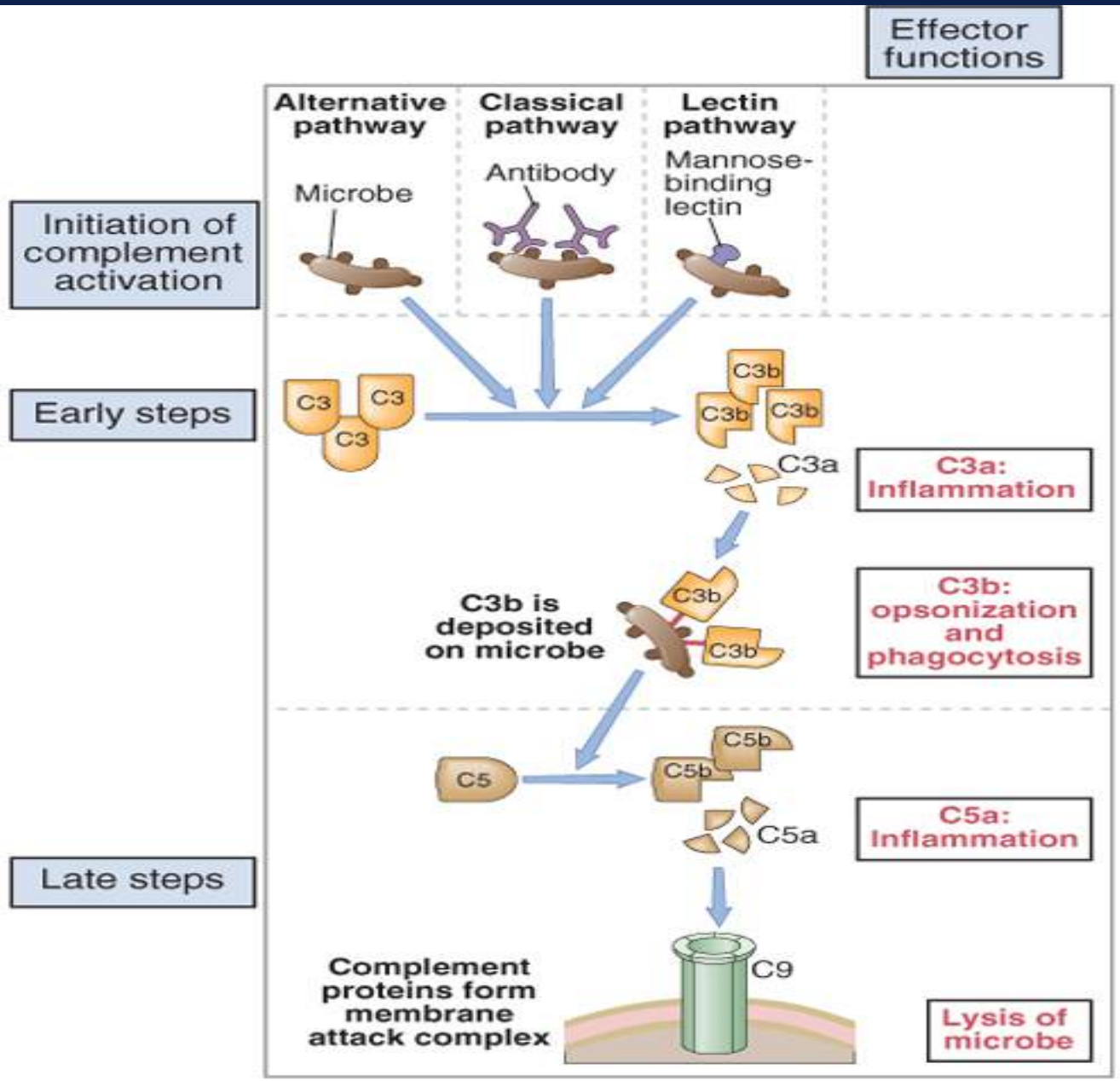
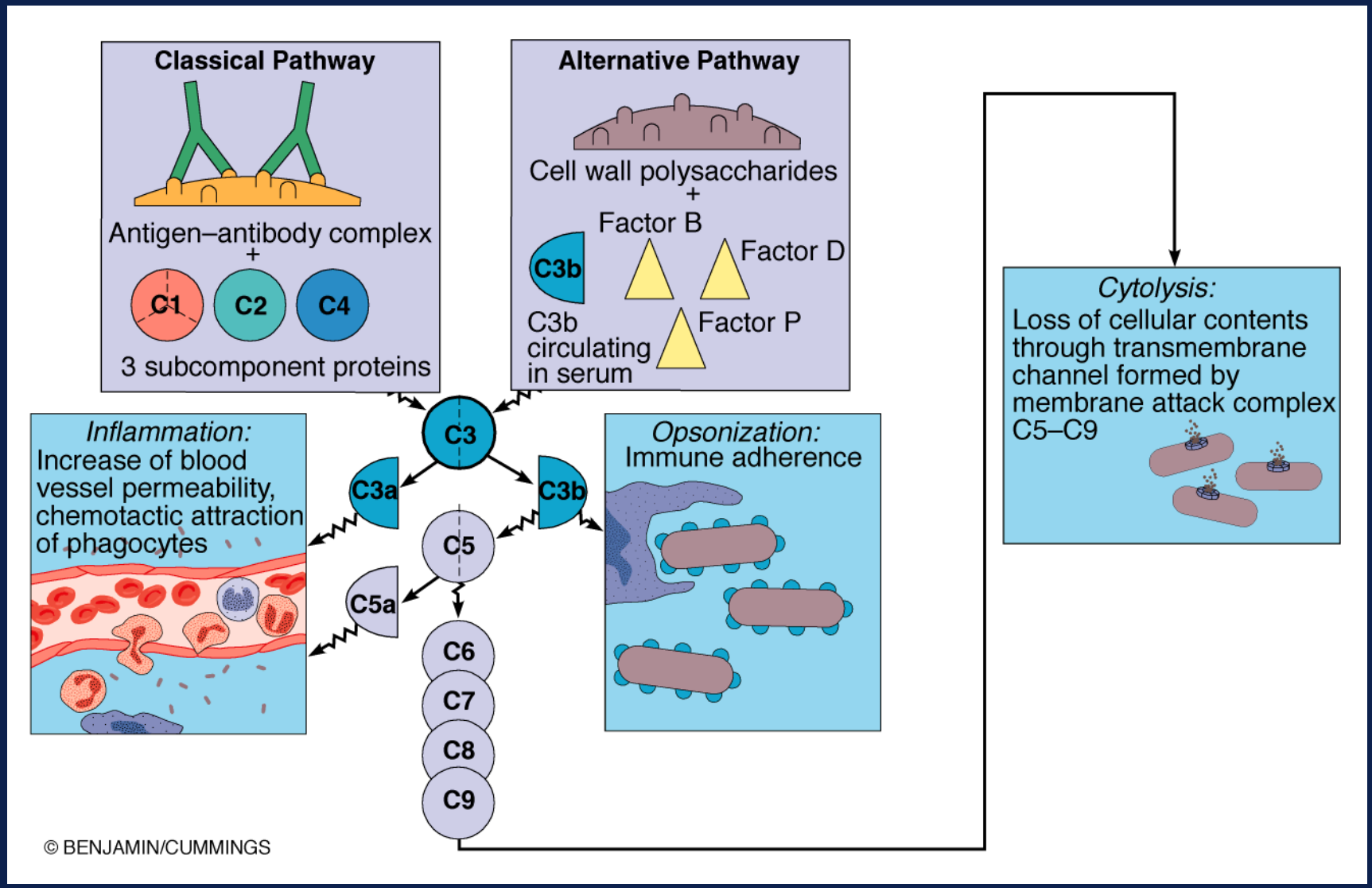


Figure 2.27 The Immune System, 3ed. (© Garland Science 2009)

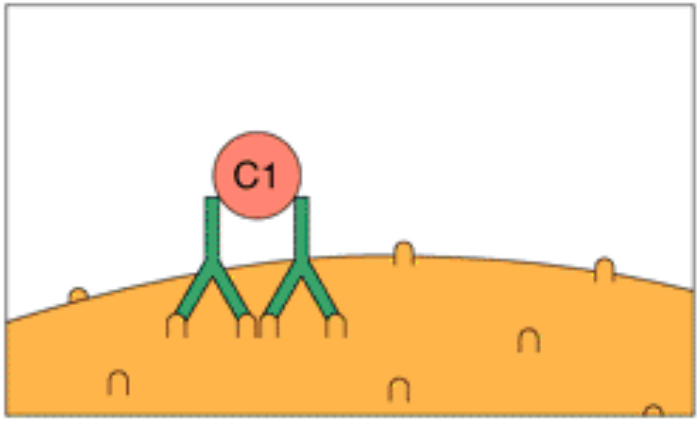
COMPLEMENT-3 distinct ways to activate all lead to C3b



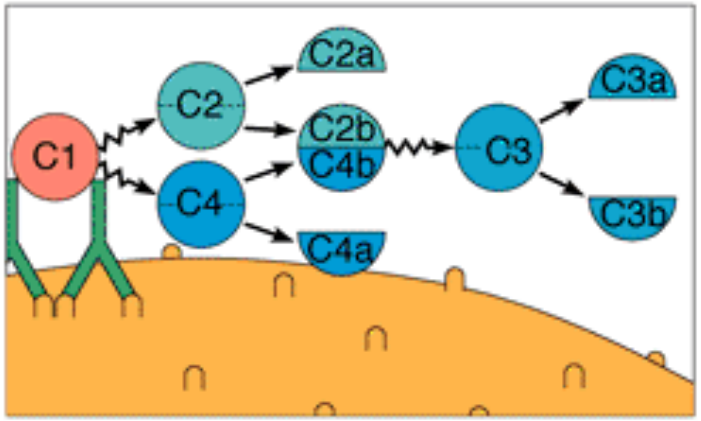
Both Classical and Alternative Complement Pathways Coat Microbe With C3b



Classical Complement Pathway is Triggered by Antibodies Binding to Foreign Cells



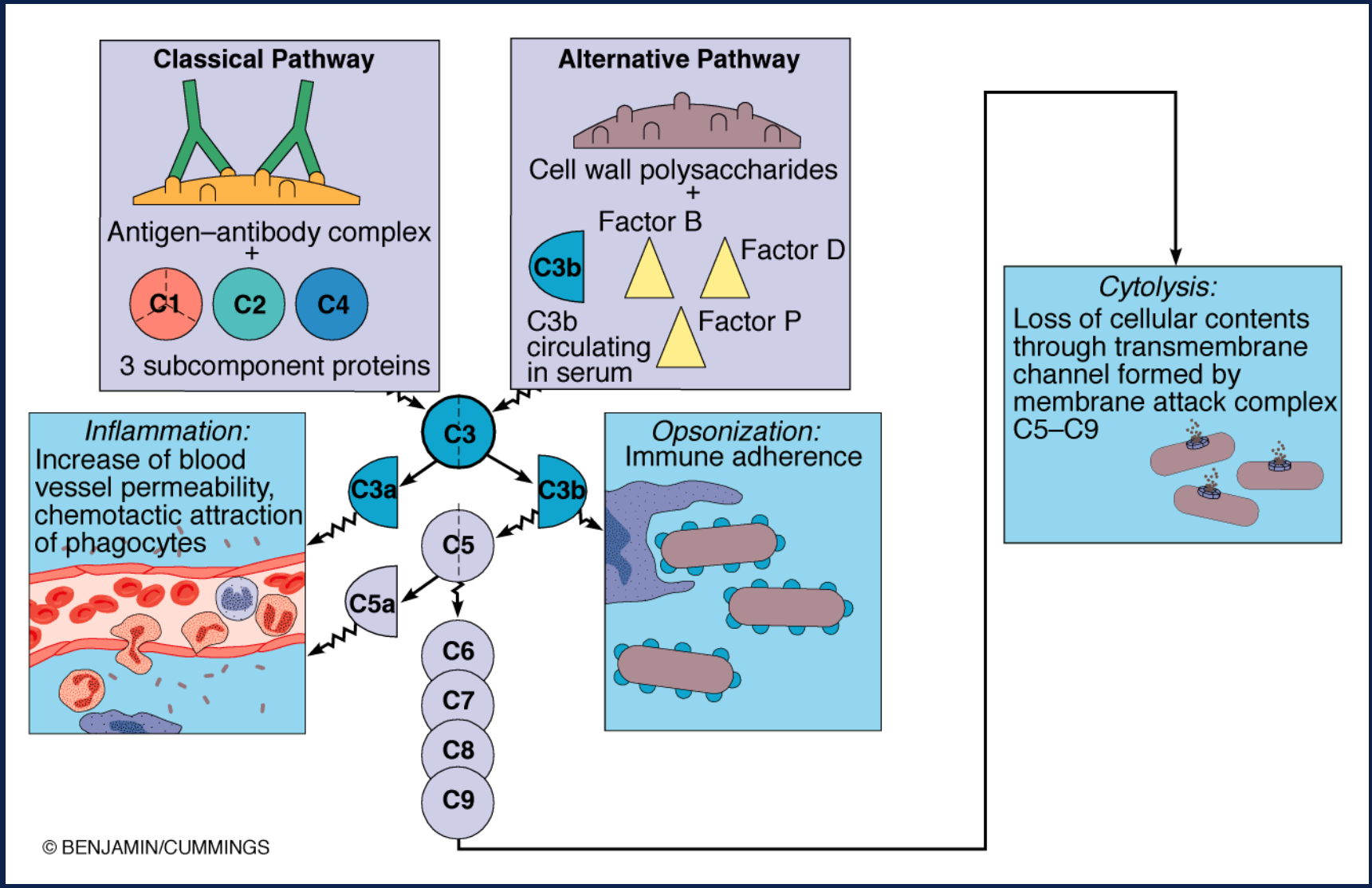
❶ Once antibodies recognize and attach to the antigen, complement protein C1 binds to two adjacent antibodies.



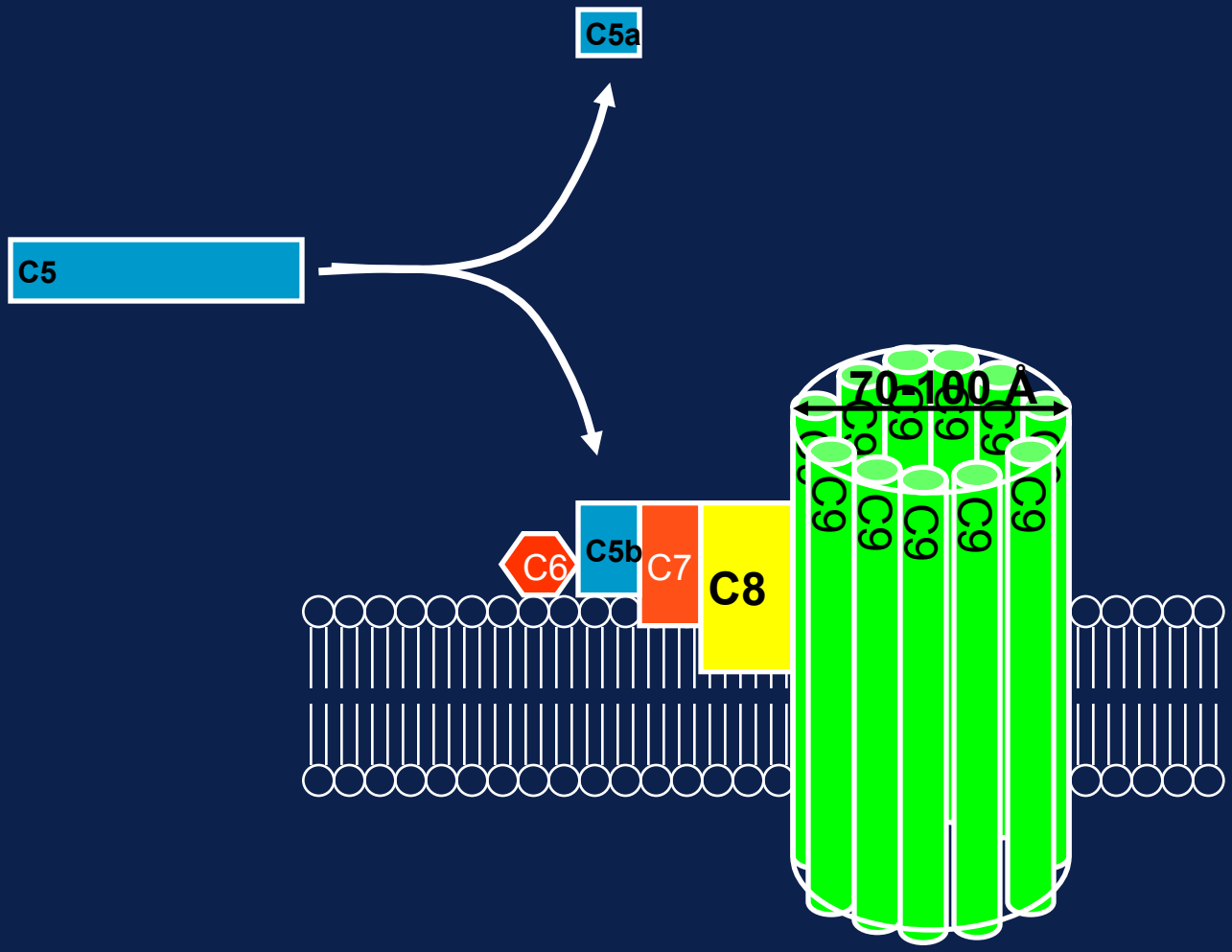
(a)

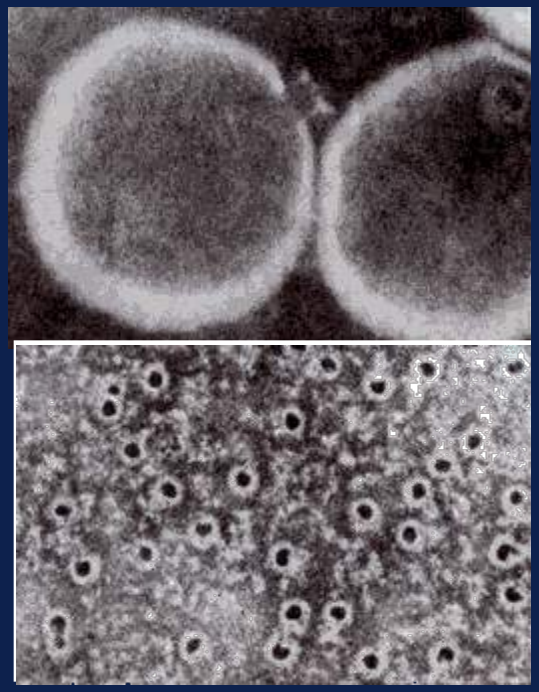
❷ C1 acts as an enzyme that splits the C2 and C4 proteins into fragments. Fragments C2b and C4b combine to form another enzyme, which splits C3 into two fragments. The active fragment is called C3b.

Classical and Alternative Complement Pathways Cause *Inflammation, Opsonization, and Cytolysis*

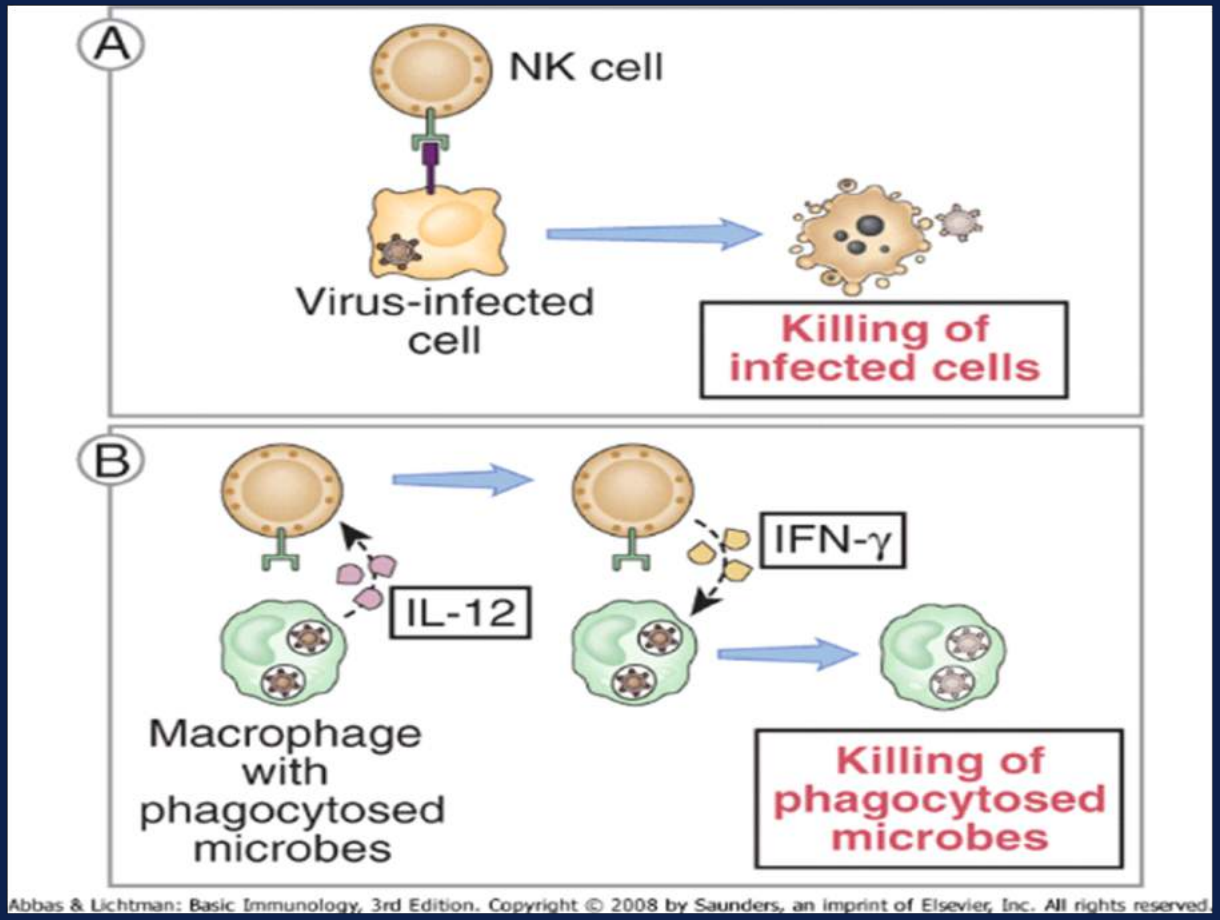


The Membrane Attack Complex





Functions of NK Cells



<ADCC

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

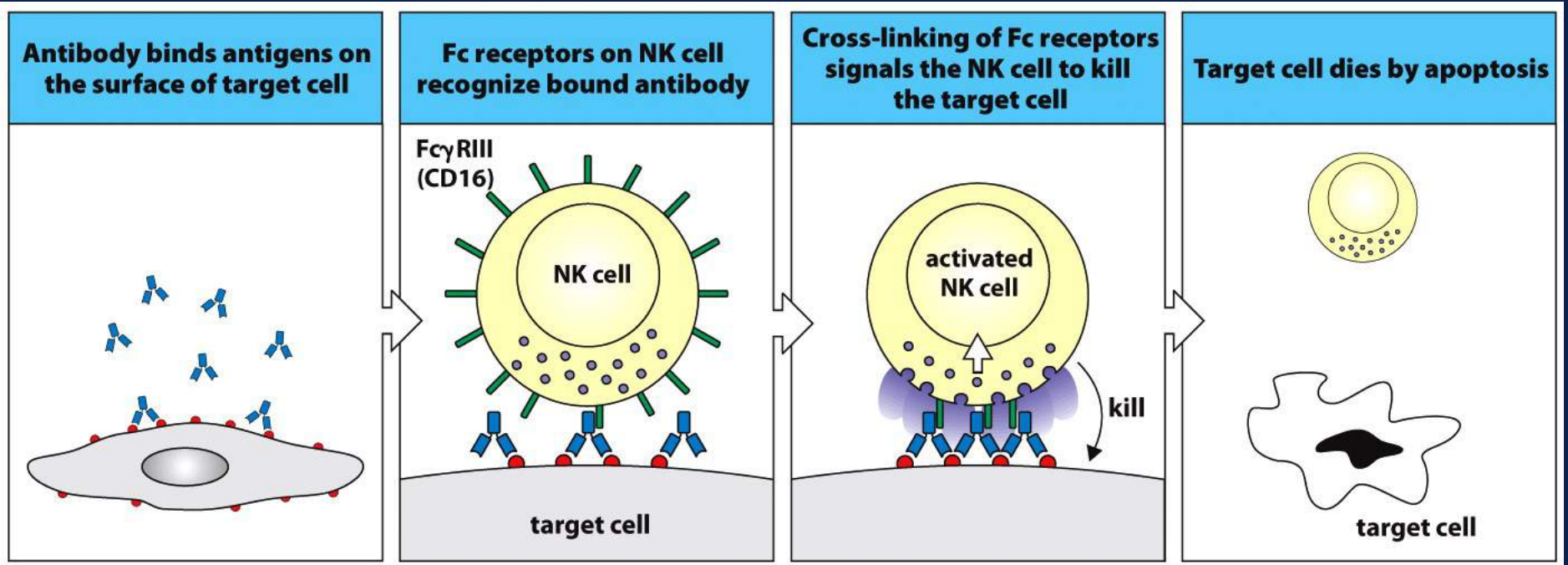


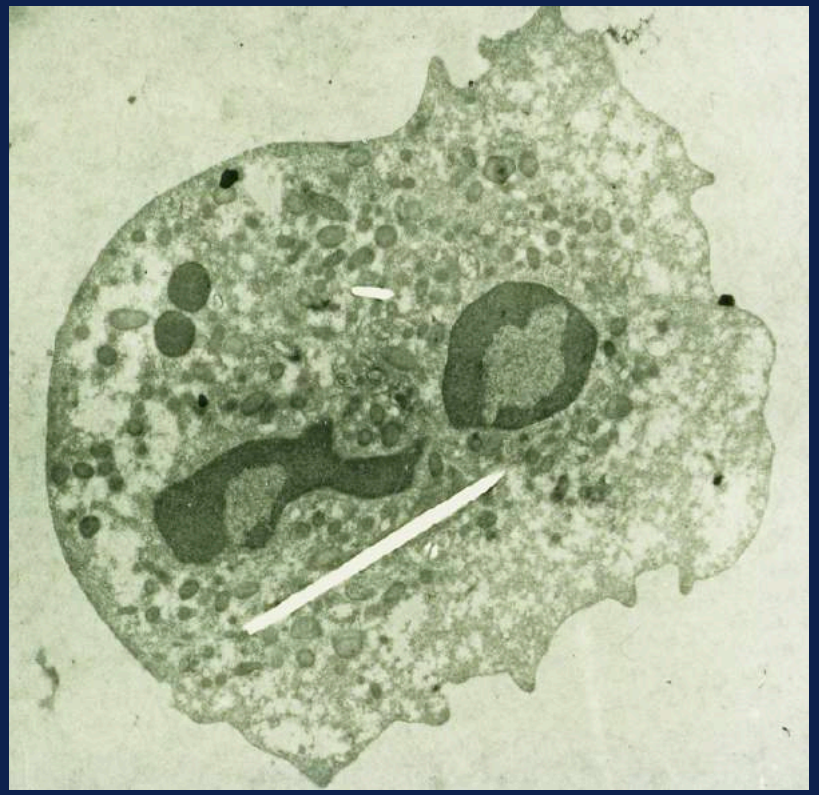
Figure 9.43 The Immune System, 3ed. (© Garland Science 2009)

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 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**

Toll-like receptors

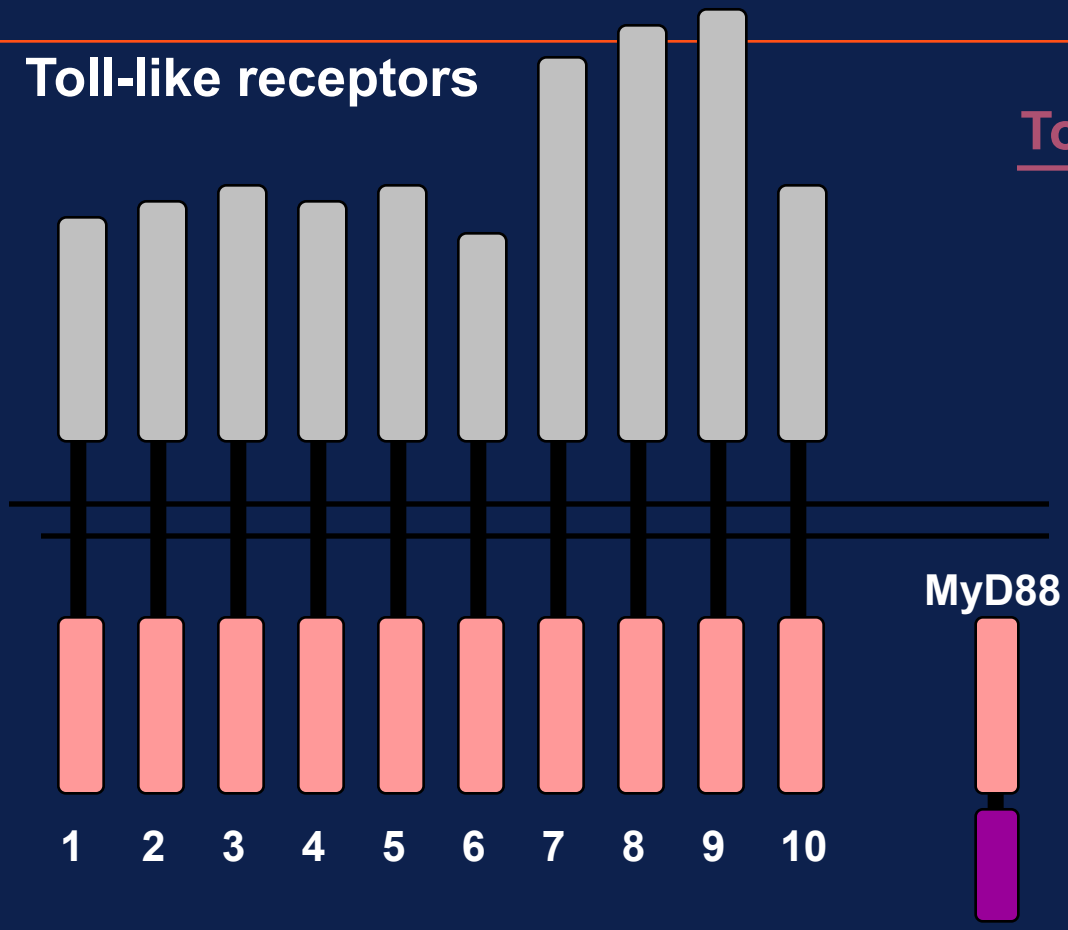
Lipoteichoic acid, endotoxin, flagellin, viral RNA, viral/bacterial DNA, **MSU/CPPD crystals**

Nod-like receptors

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

**DAMPS = Danger-Associate Molecular Patterns

Innate Immunity Sensors – Pattern Recognition Molecules (PRMs)

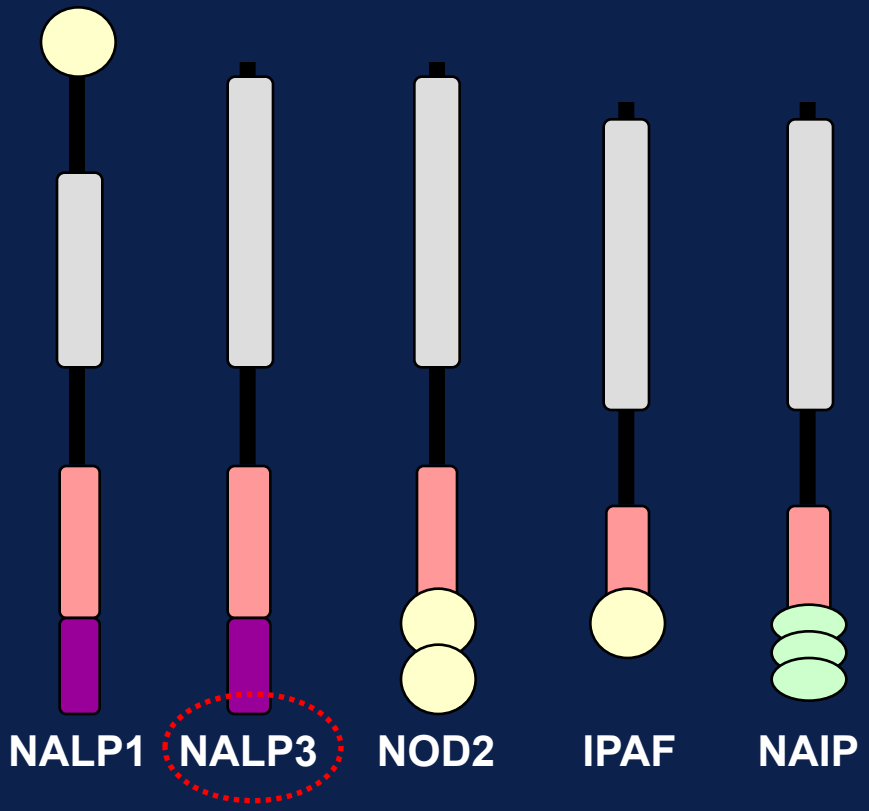


Toll-Like Receptors (TLRs)

14 members of TLR family

How TLR senses the presence of DAMPs is not clear

Innate Immunity Sensors – Pattern Recognition Molecules (PRMs)

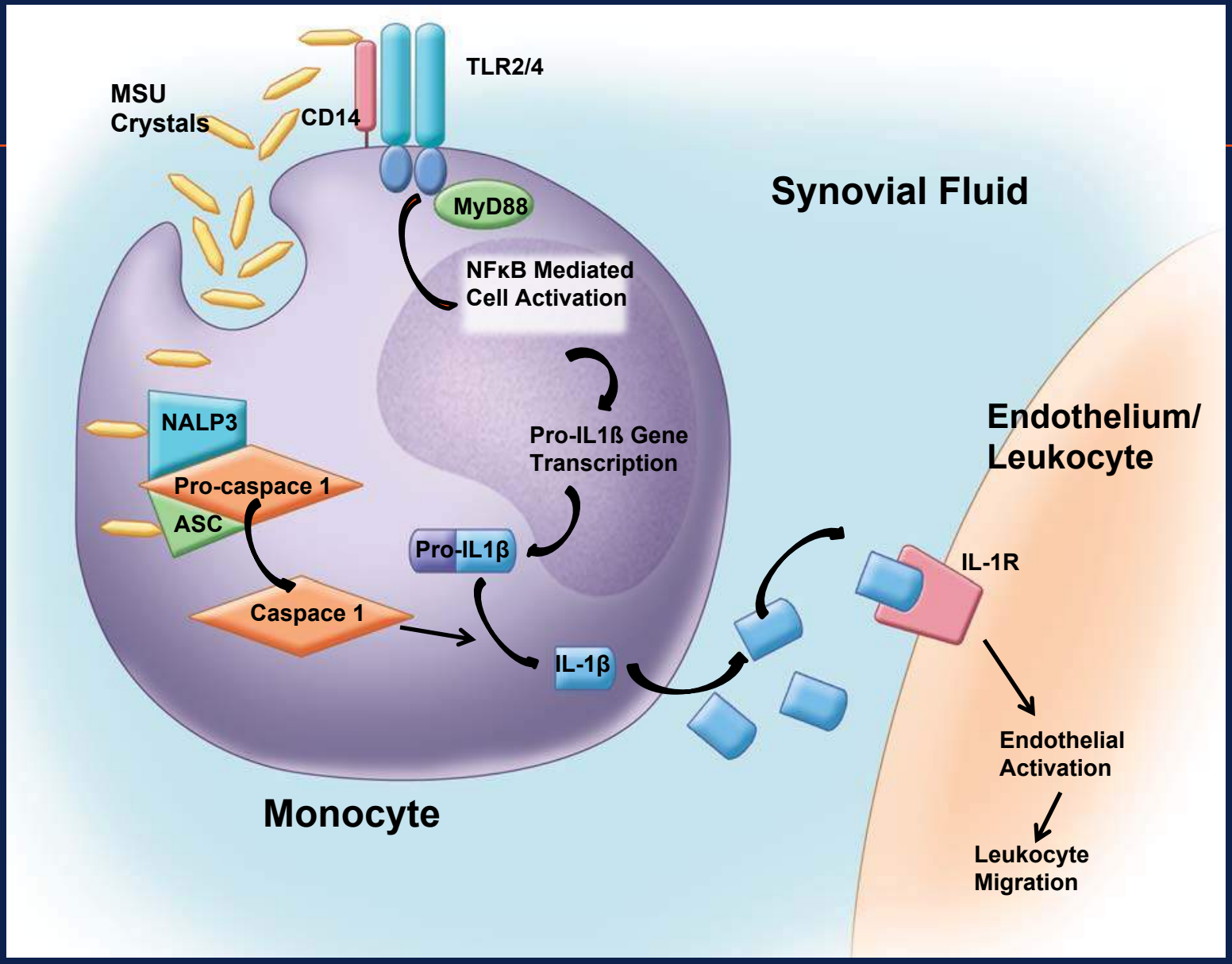


NOD-like receptors (NLR)

Cytoplasmic equivalent of TLR

22 members of the NLR family in humans- Ligands include:

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

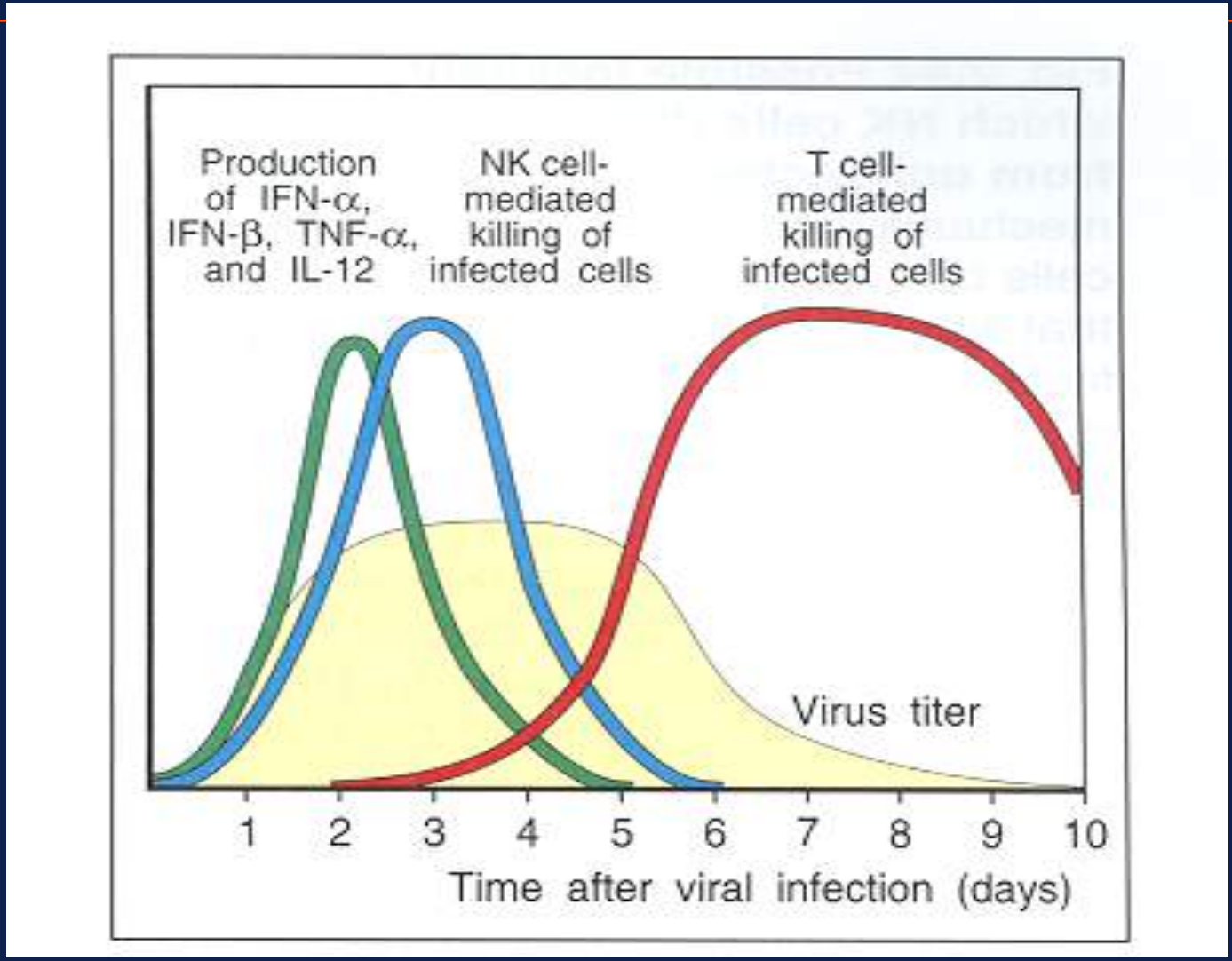


Edwards NL. Crystal-Induced Joint Disease, ACP Medicine Textbook, 2012

Adaptive Immunity

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

Time course of innate and adaptive immune responses



Mouse model of a viral infection

Classes of Lymphocytes-

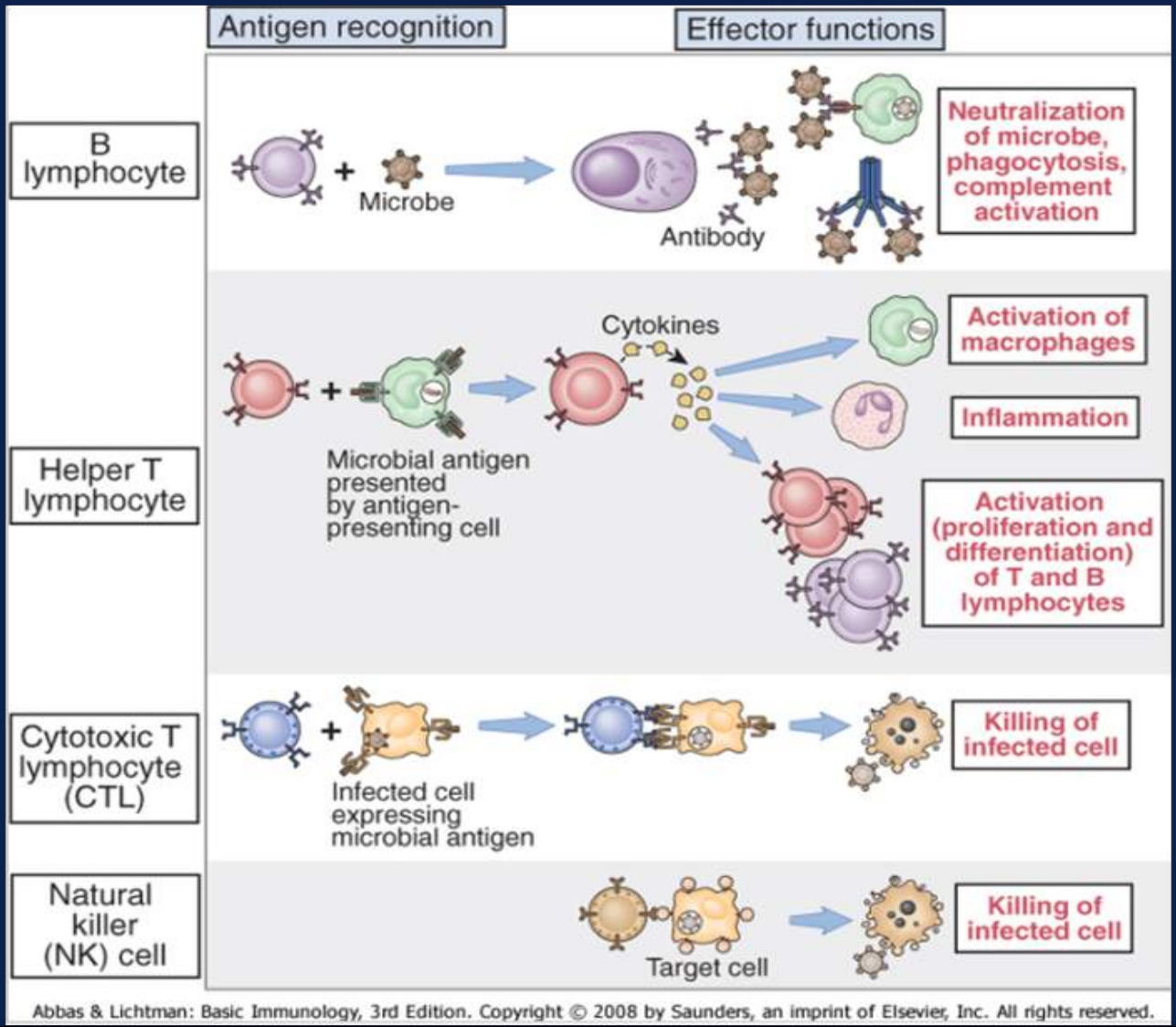
Recognize Different Types of Antigens

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

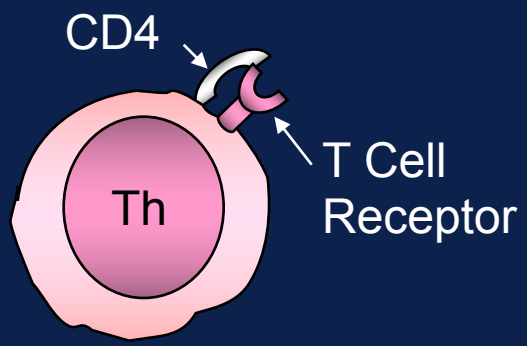


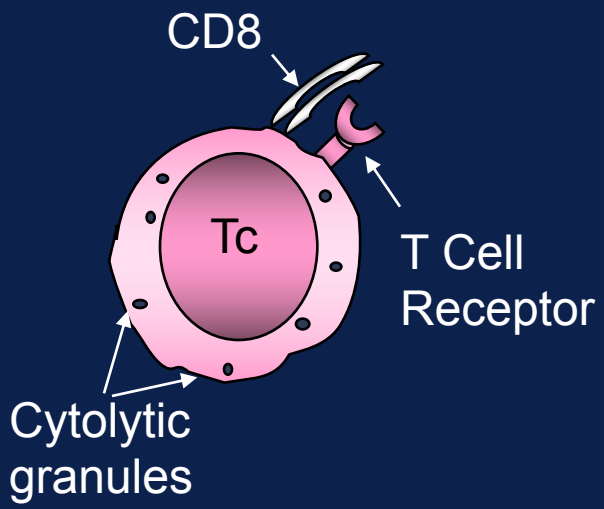
Three Strategies to Combat Microbes

- 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)

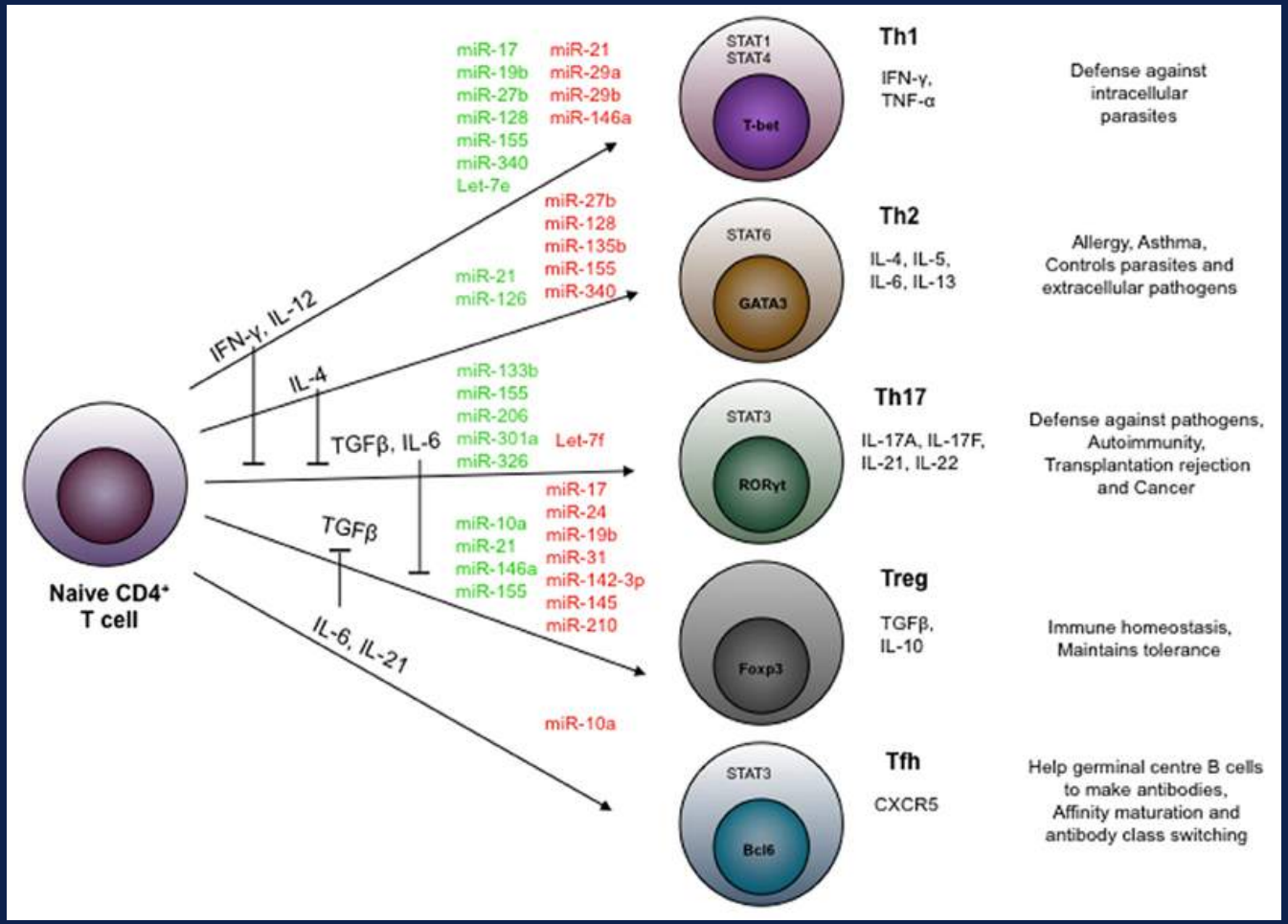
- T lymphocytes mature in the Thymus
- They express a ***specific*** receptor that binds antigen, called the T Cell Receptor (TCR)
- There are 2 main types:
  - CD8+ Cytotoxic T cells (Tc)
    Induce cell death in target cells via cytotoxic granule release
  - CD4+ Helper T cells (Th)
    Help B cells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





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

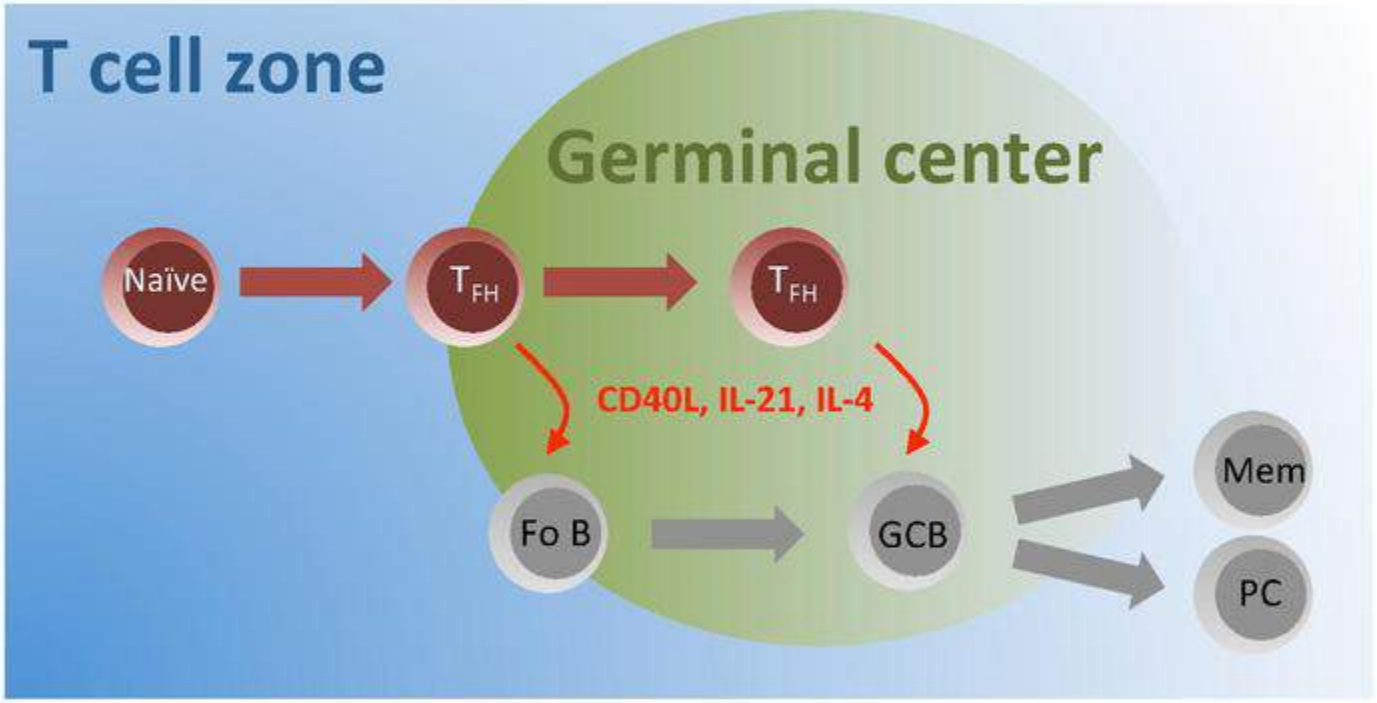
CD4 Subsets: Generation and Function



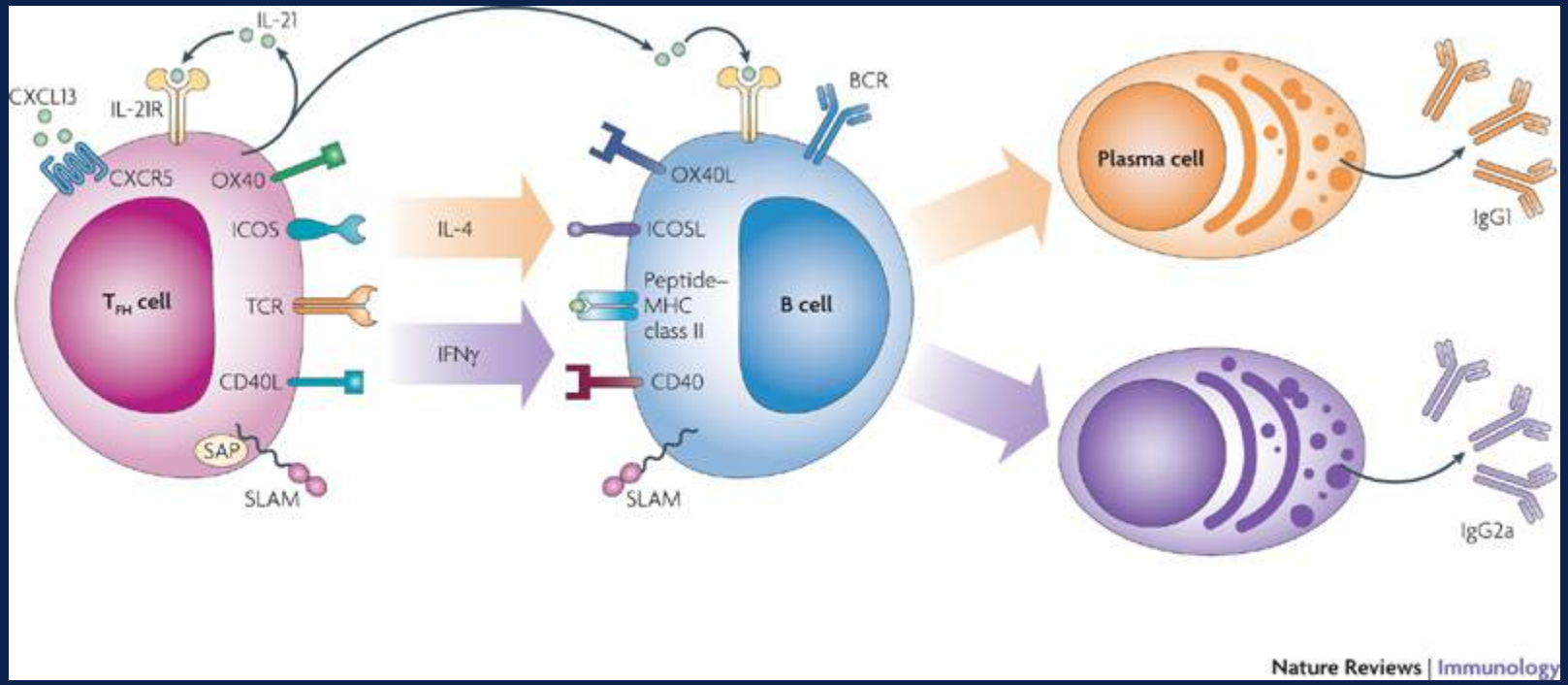
	CD8 cytotoxic T cells	CD4 T_H1 cells	CD4 T_H2 cells	CD4 T_H17 cells	CD4 regulatory T cells (various types)
Types of effector T cell					
Main functions in adaptive immune response	Kill virus-infected cells	Activate infected macrophages Provide help to B cells for antibody production	Provide help to B 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 cells

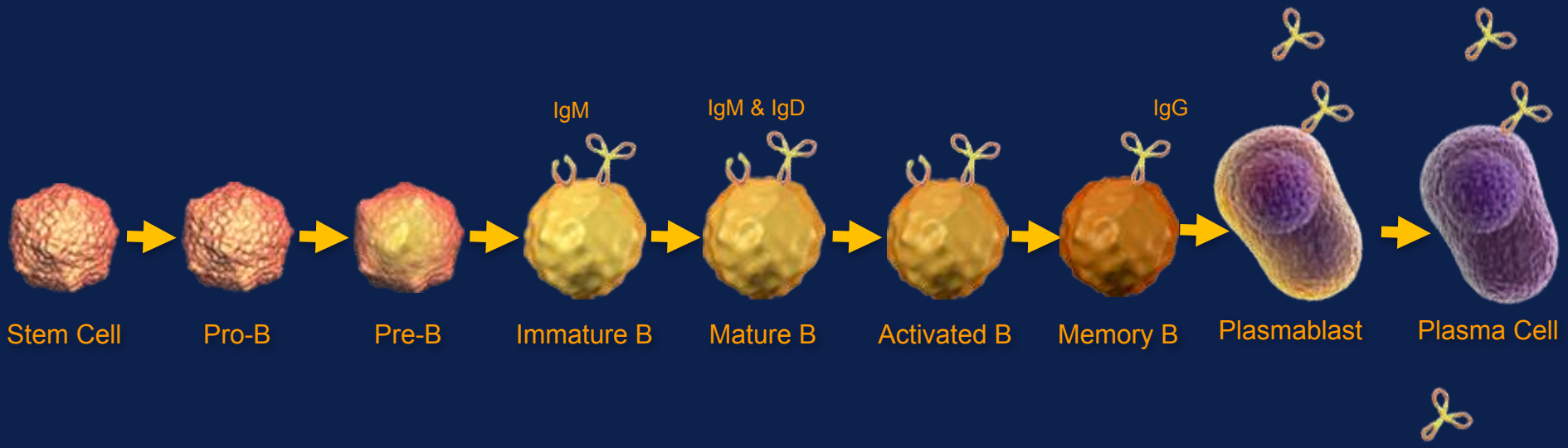


Trigger formation and maintenance of germinal centers
Stimulate plasma cell development
Stimulate development of memory B cells

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

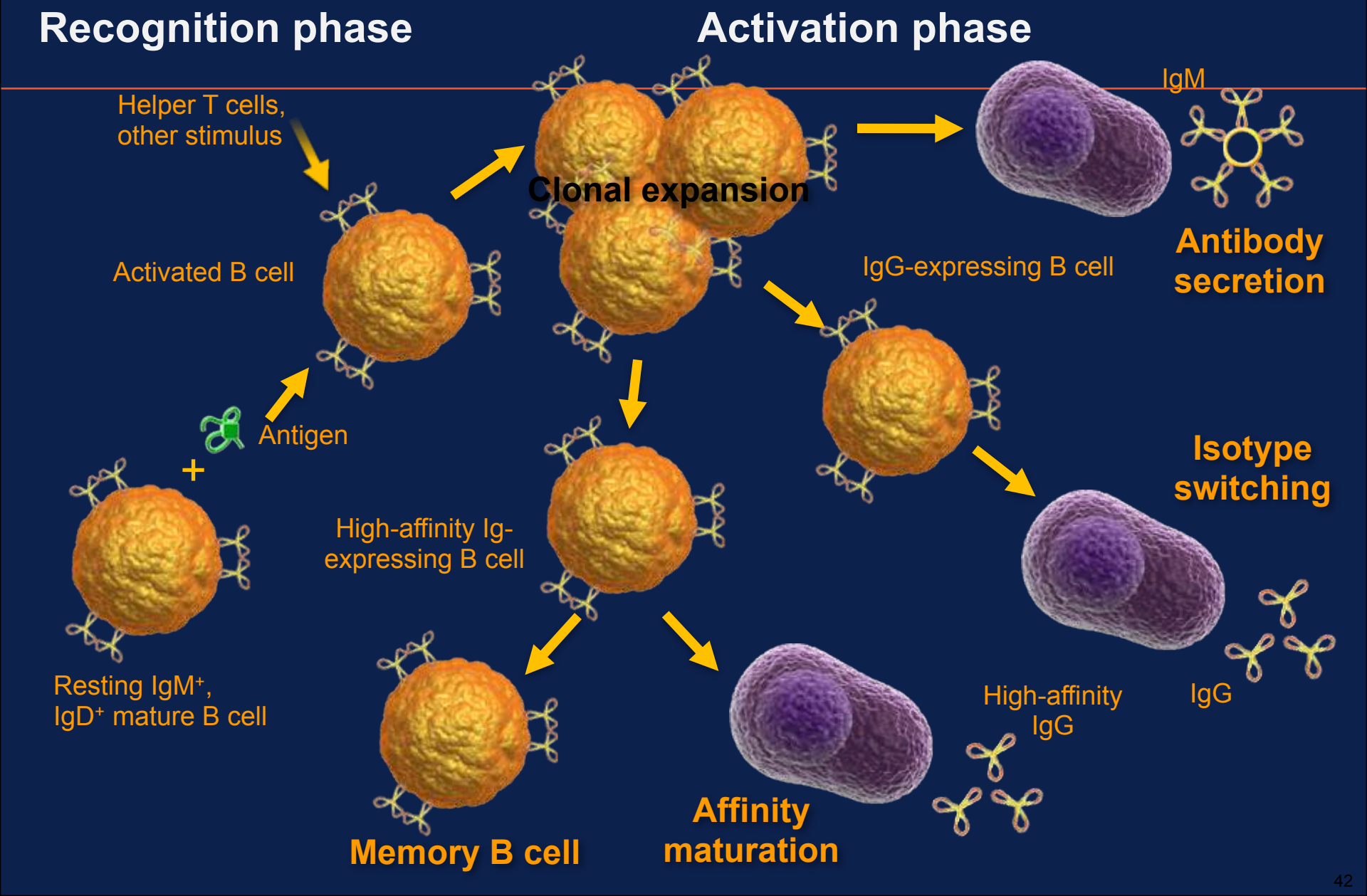
B-Cell Immunology: Lineage[1,2]



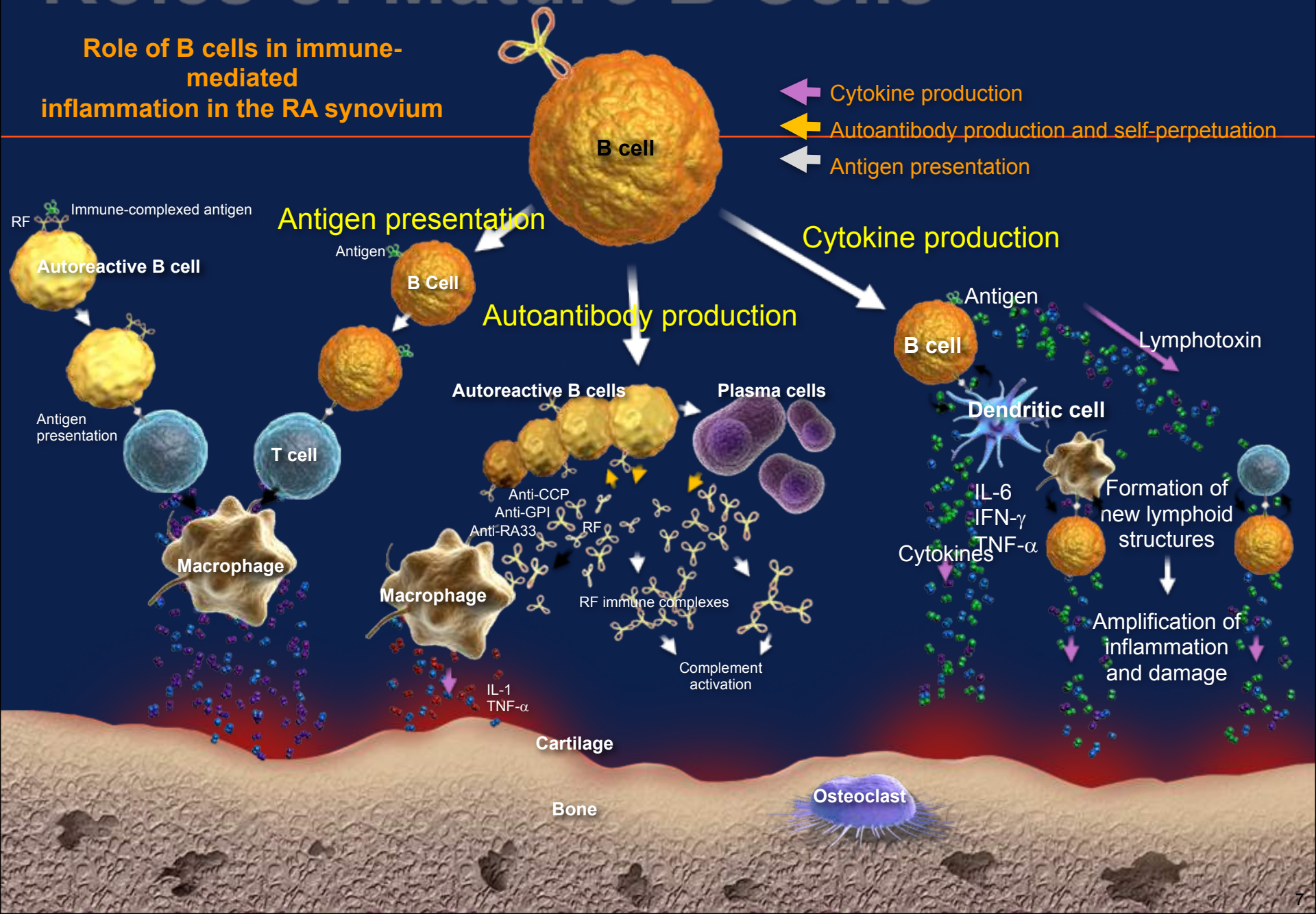
- B cells develop in the bone marrow and migrate to the peripheral lymphoid organs, where they can be activated by antigens[2]
- Activated B cells proliferate and differentiate into long-lived memory cells and antibody-secreting plasma cells[2]

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

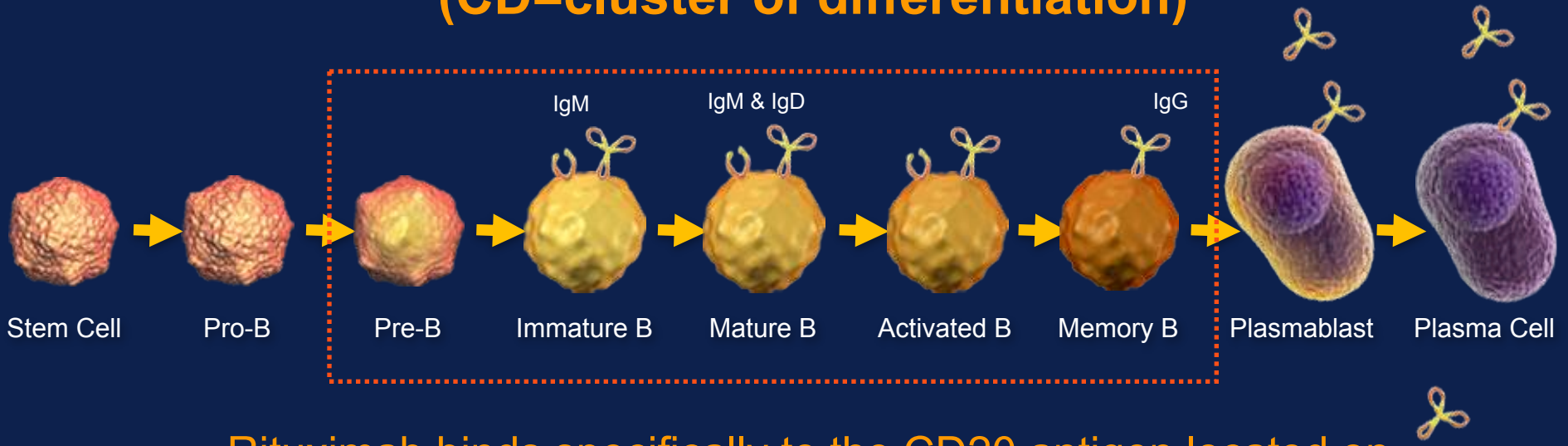


Roles of Mature B Cells



Targets of Rituximab[1,2]

Expression of CD20 During B-Cell Maturation[1] (CD=cluster of differentiation)



- Rituximab binds specifically to the CD20 antigen located on pre-B and mature B lymphocytes
- CD20 is not found on hematopoietic stem cells, pro-B cells, normal plasma cells, or other normal tissues
- Long-lived plasma cells do not express CD20 and are not directly targeted by Rituximab

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.
3. Rituxan® (rituximab) Full Prescribing Information. Genentech USA, Inc., and Biogen Idec Inc. October 2009.

Antibody Function

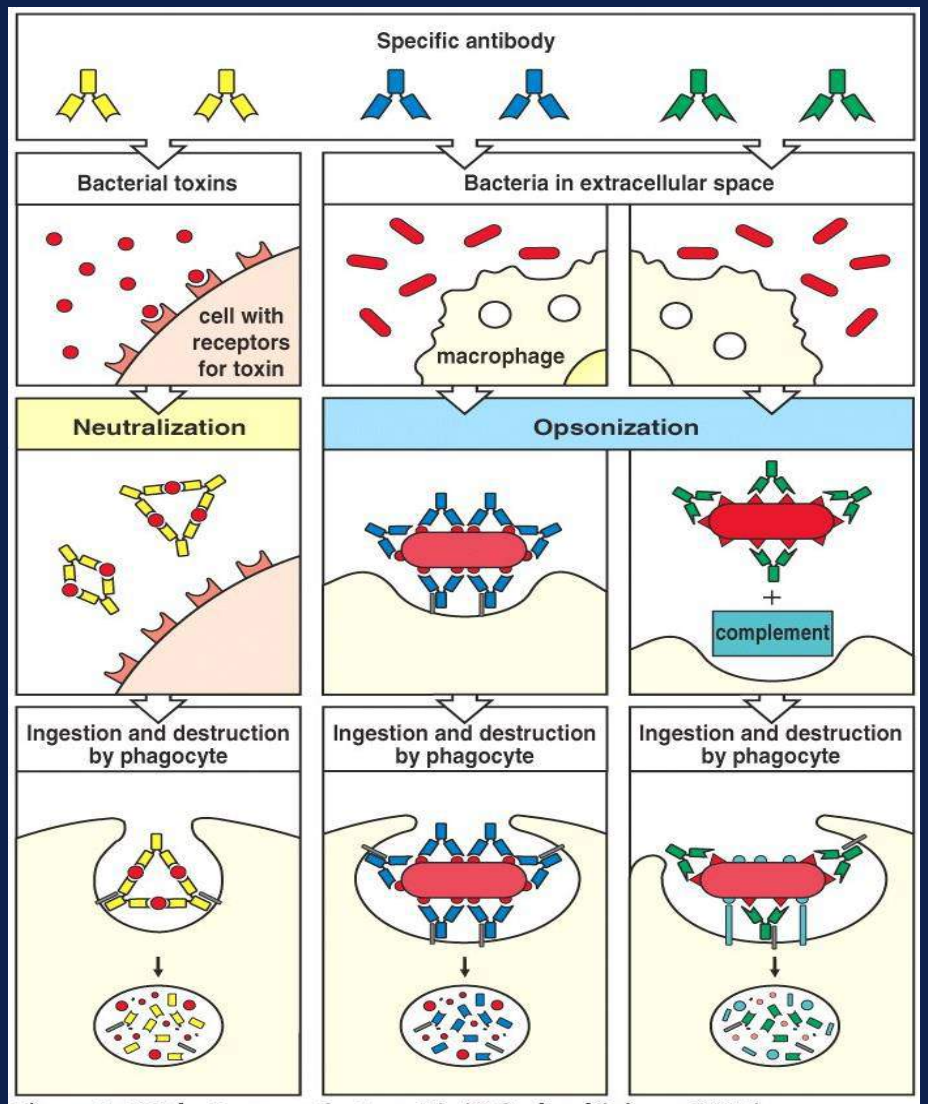


Figure 1-29 The Immune System, 2/e (© Garland Science 2005)

T Cell Function

MHC Based Antigen Presenting Cell-Lymphocyte Interactions

MHC II interacts with CD4+ lymphocyte

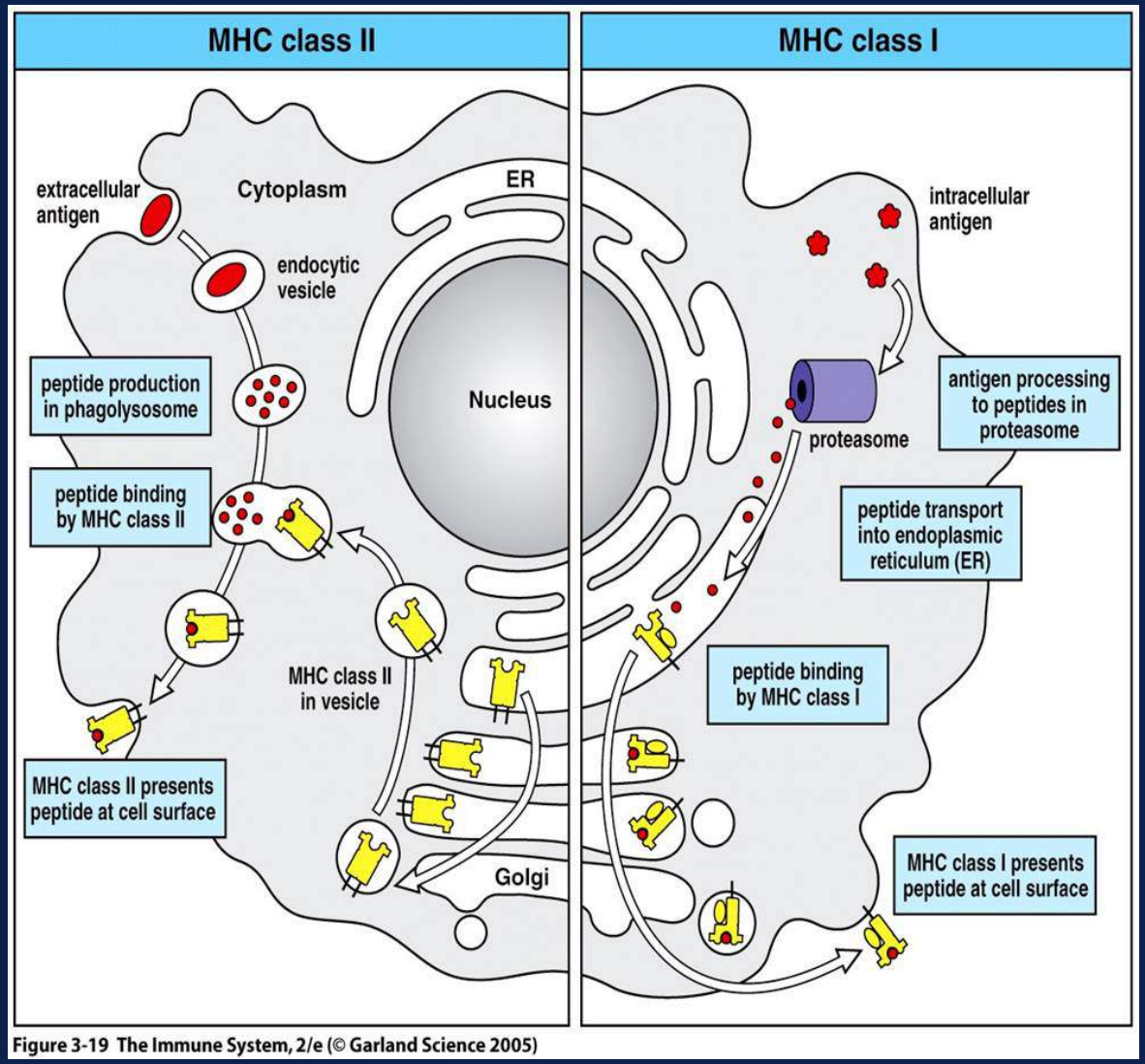


Figure 3-19 The Immune System, 2/e (© Garland Science 2005)

MHC I interacts with CD8+ lymphocyte

MHC Restriction

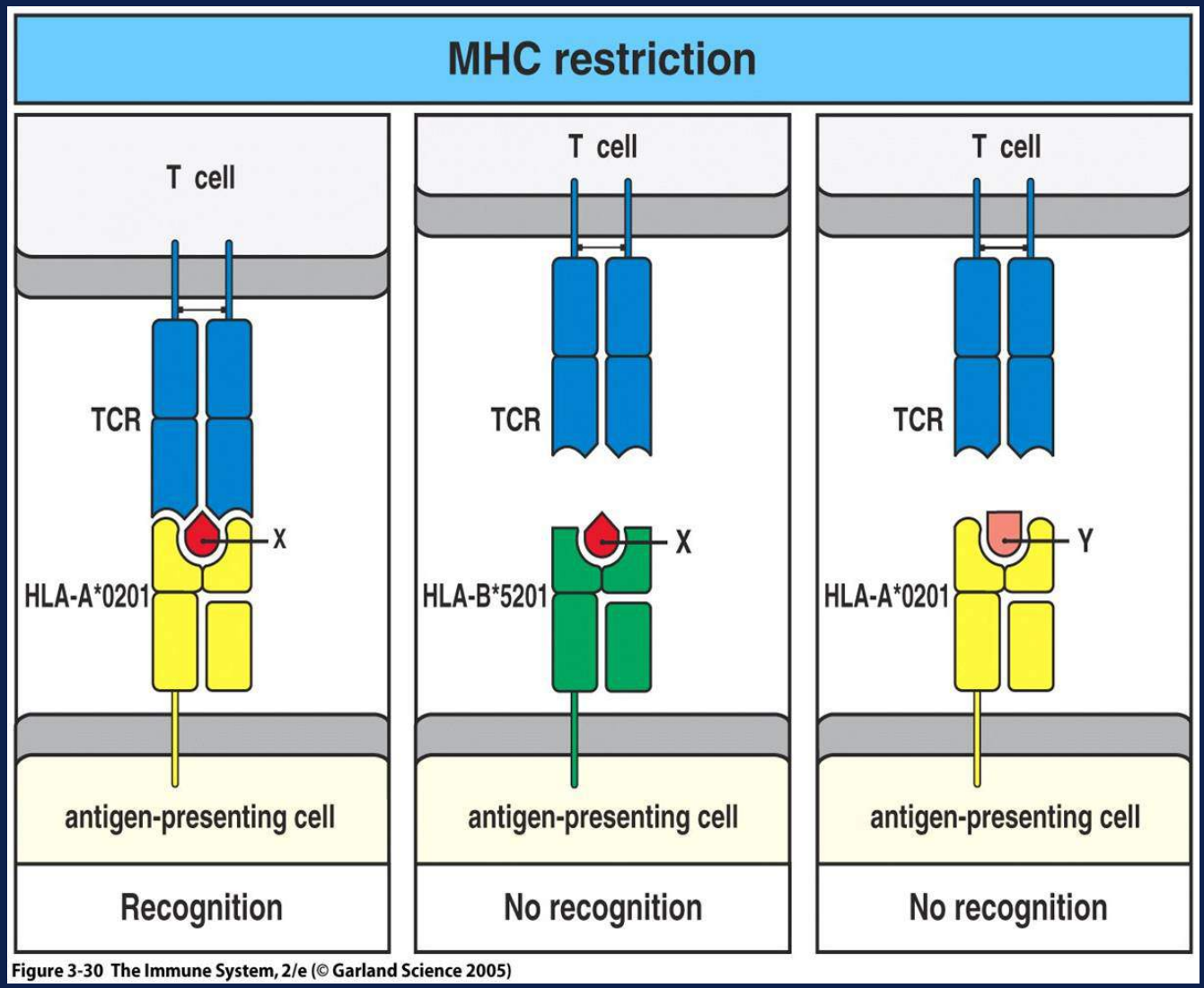


Figure 3-30 The Immune System, 2/e (© Garland Science 2005)

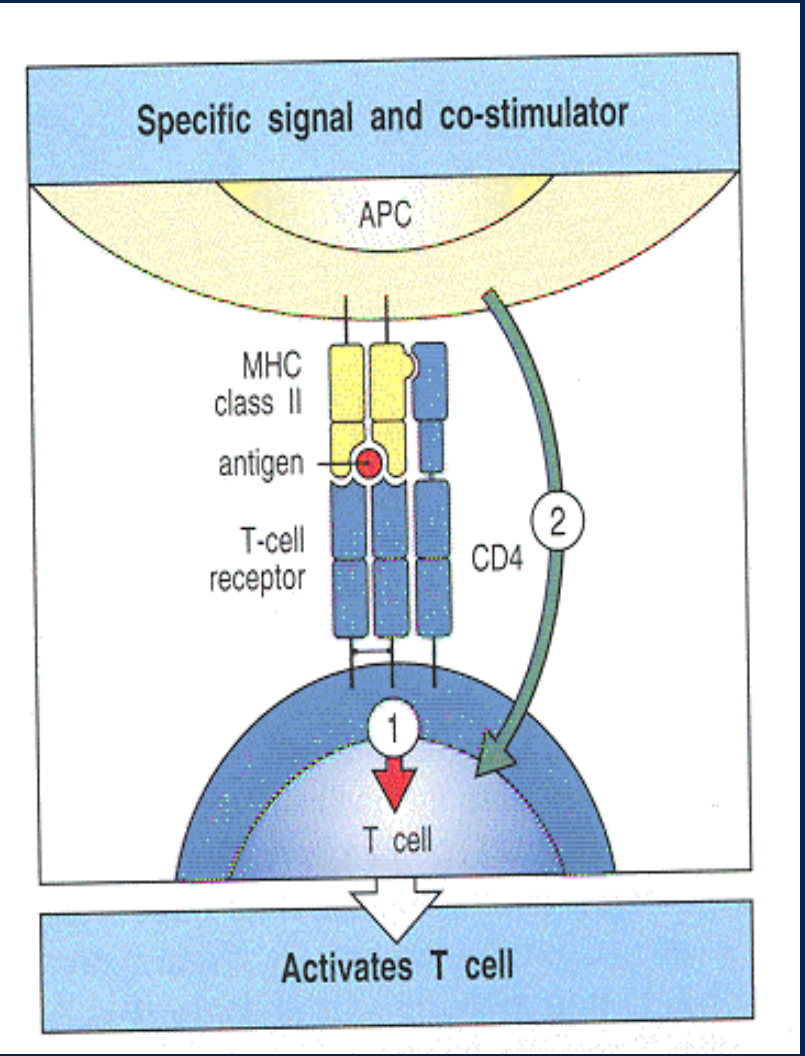
T cell activation

TCR ligation alone is insufficient to activate T cells

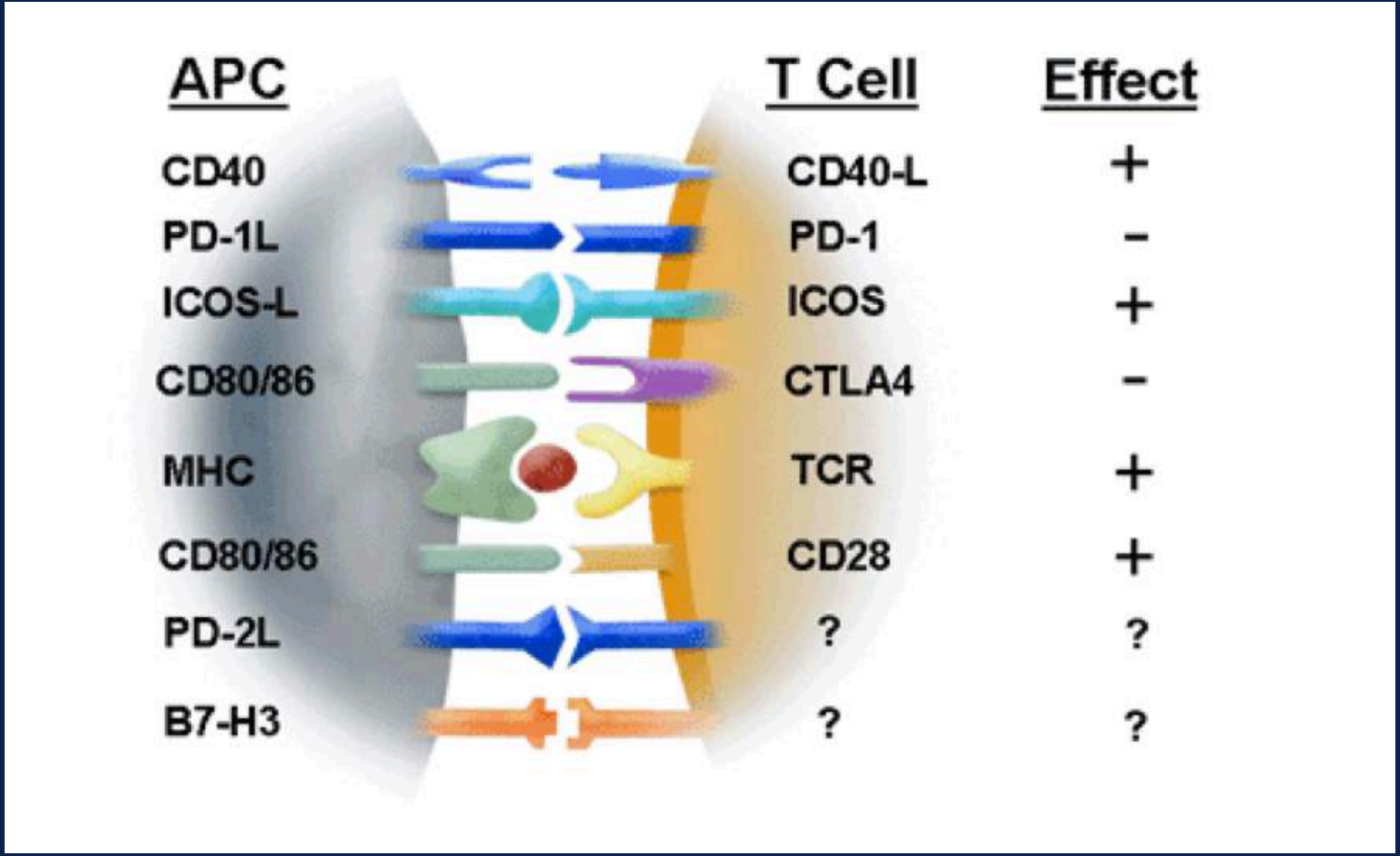
Safety control

Second signal is essential CO-STIMULATORY

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

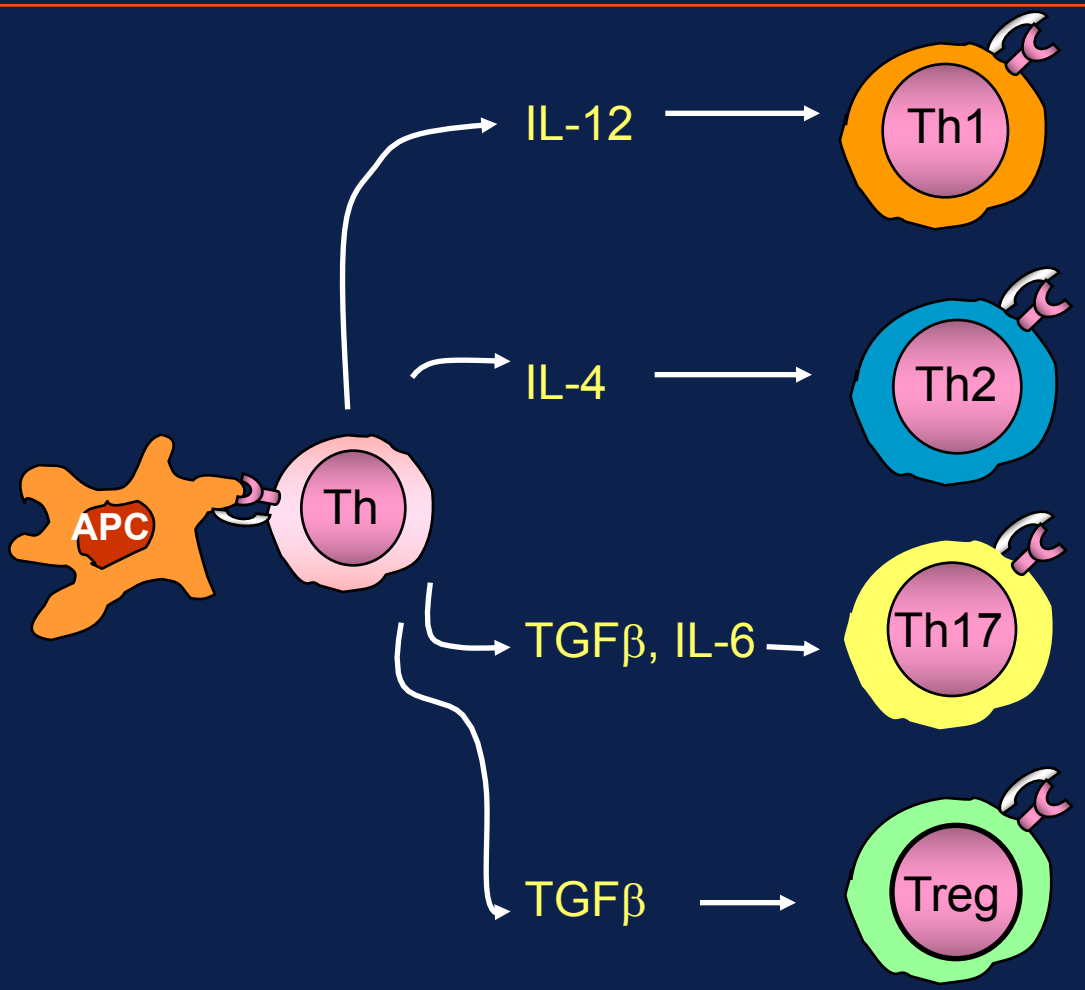


Co-stimulation



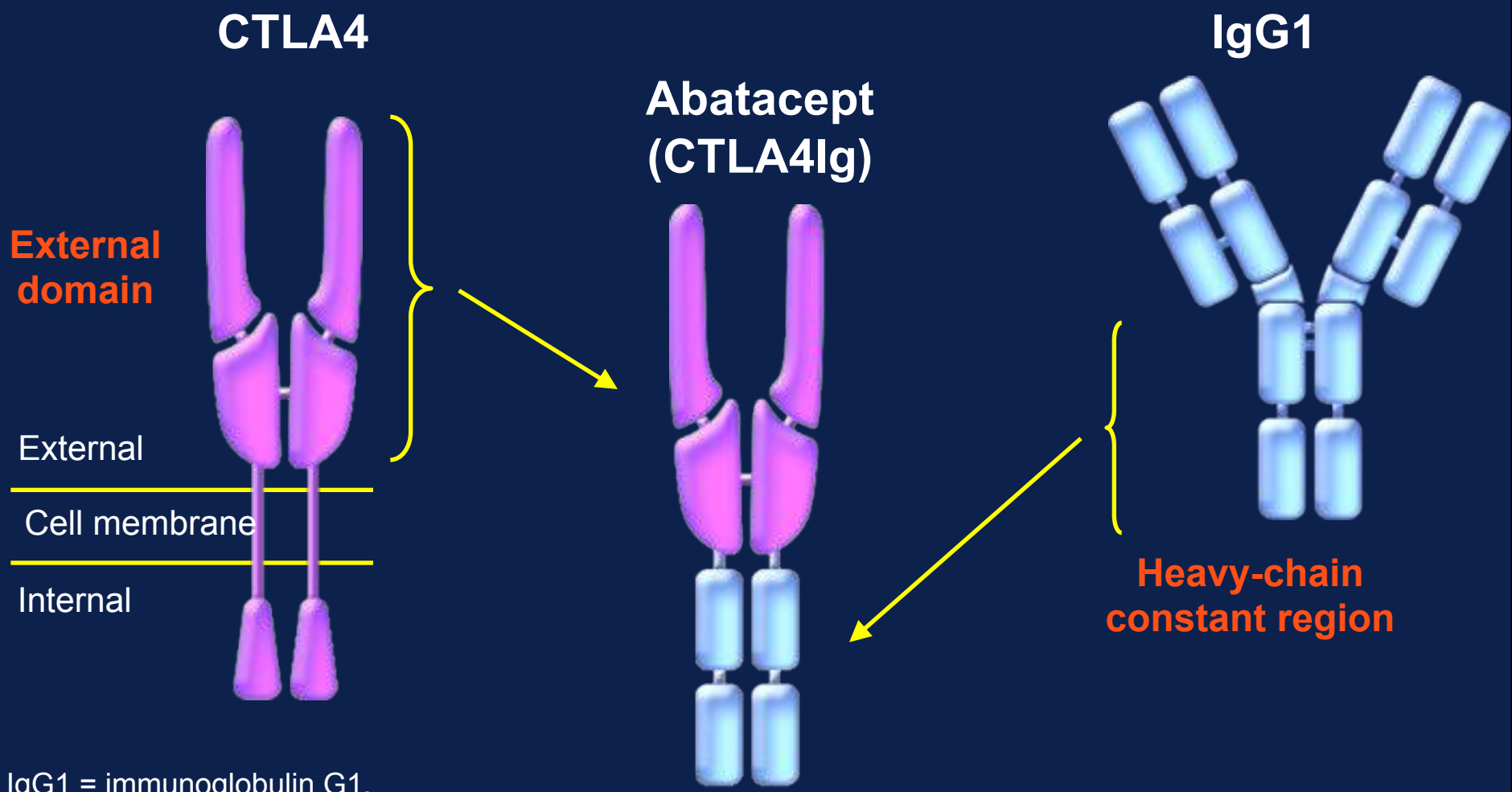
Carreno BM and Collins M. *Annu Rev Immunol*. 2002; 20:29-53.
Stuart RW and Racke MK. *Expert Opin Ther Targets*. 2002; 6(3):275-89.

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

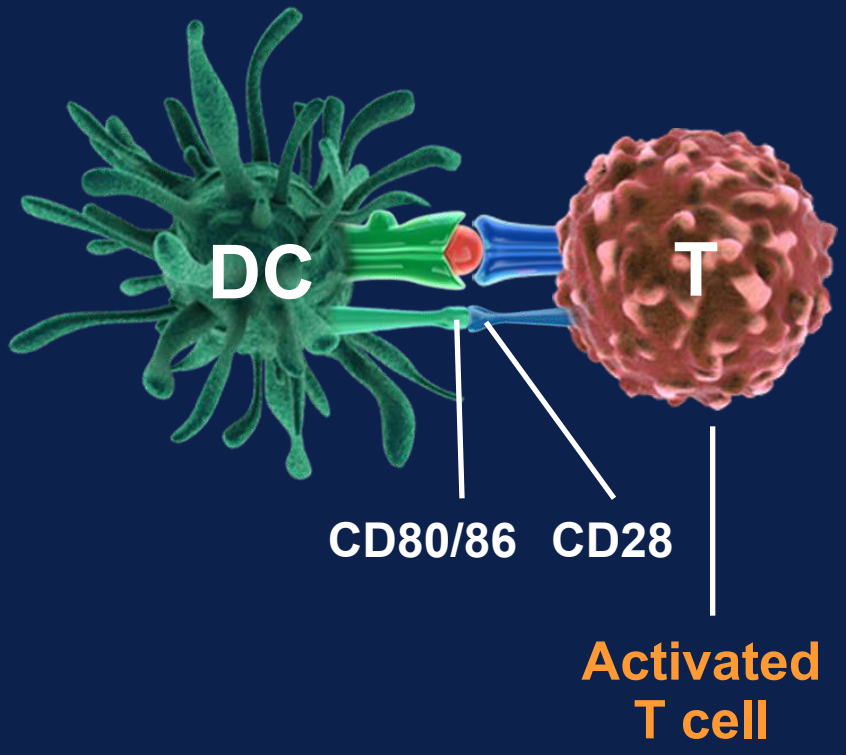
Abatacept: A Human Immunoglobulin Receptor Fusion Protein



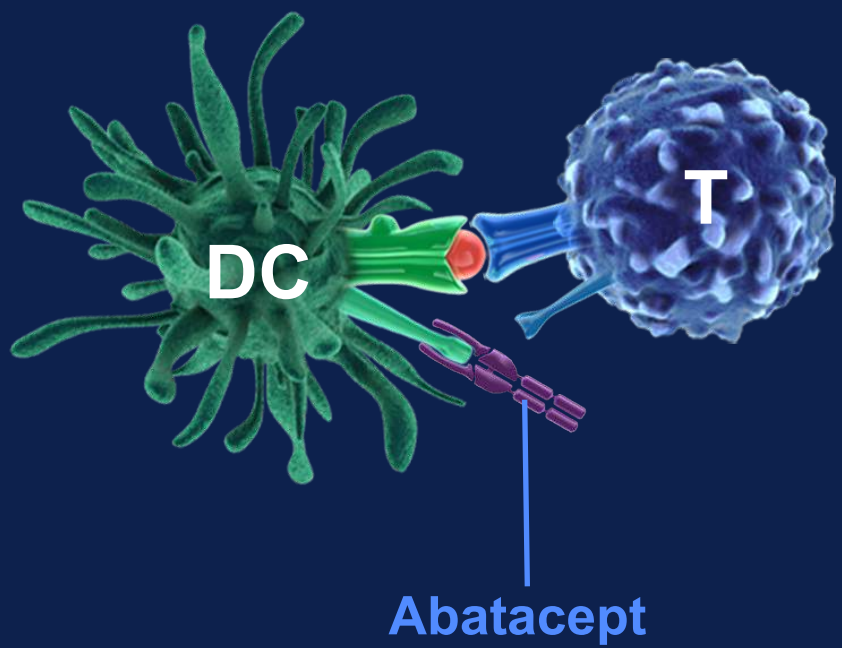
IgG1 = immunoglobulin G1.

Mechanism of Action of Abatacept

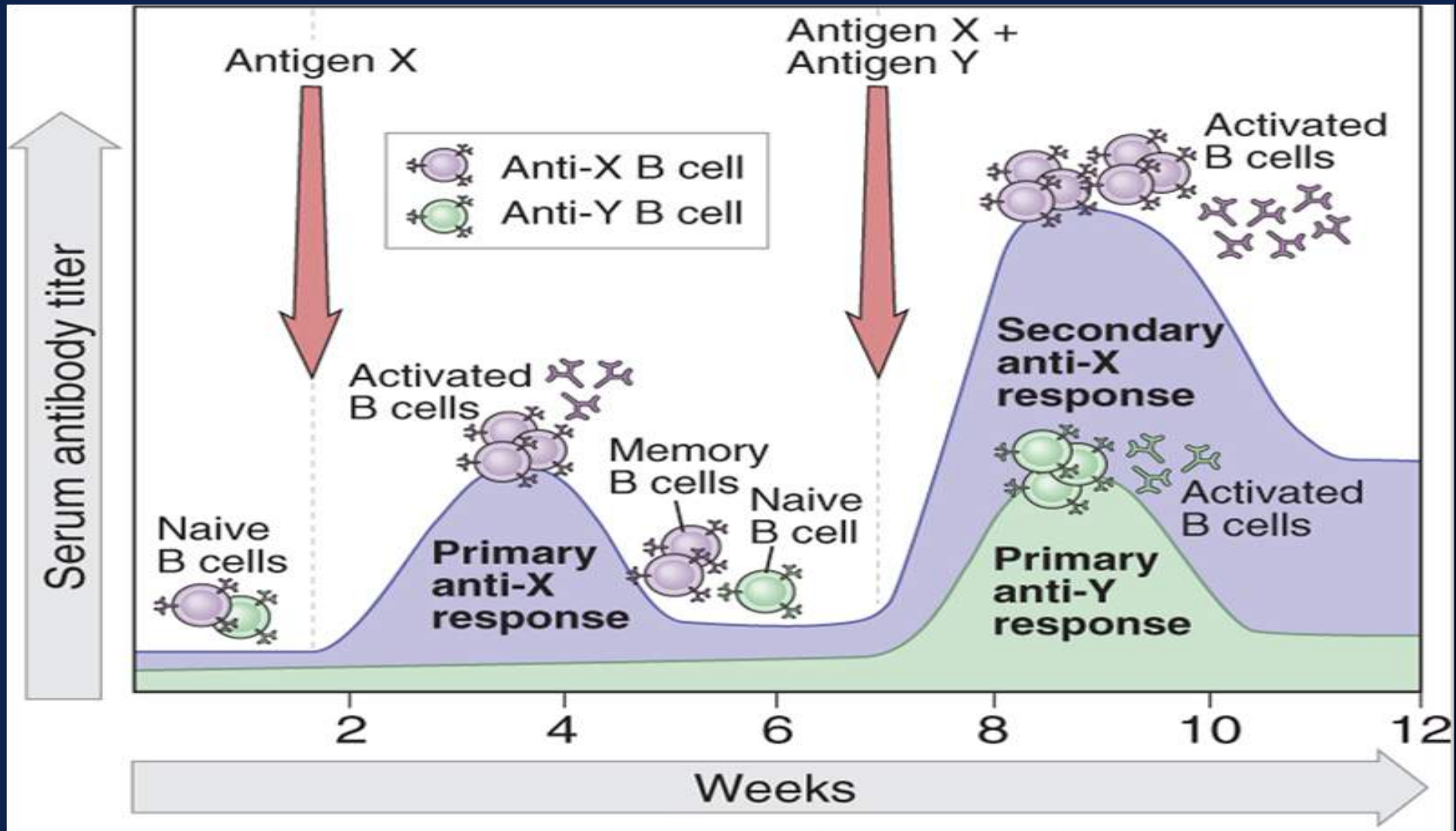
Without Abatacept



With Abatacept



Specificity of Immune Response



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 RA

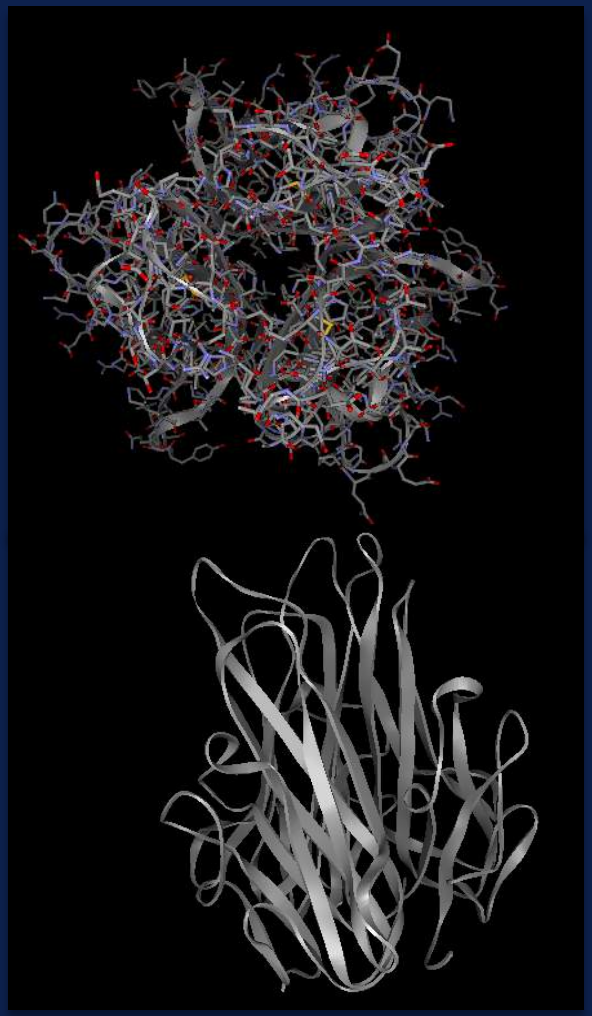
- Drive inflammation
- Drive joint damage
- Drive systemic manifestations

Targeted Therapies

- Treatments for rheumatoid arthritis (RA) prior to 1998 were discovered fortuitously.
- Now, due to a greatly improved understanding of the immunopathogenesis of the disease, we have developed targeted therapies.
- These include tumor necrosis factor α (TNFα), Interleukin-6, and Intracellular Signaling.

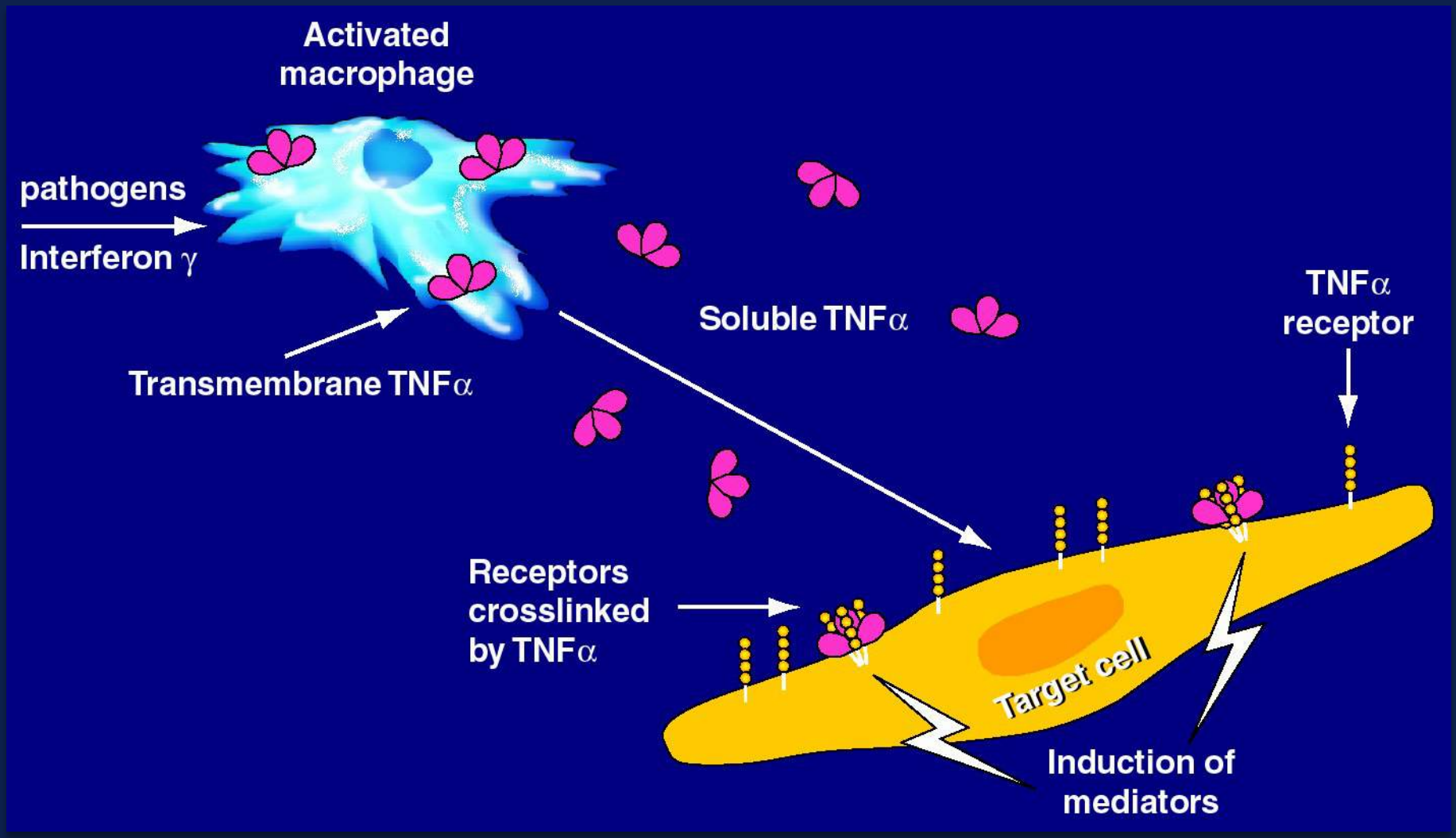
Tumor Necrosis Factor α (TNFα)

- 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.

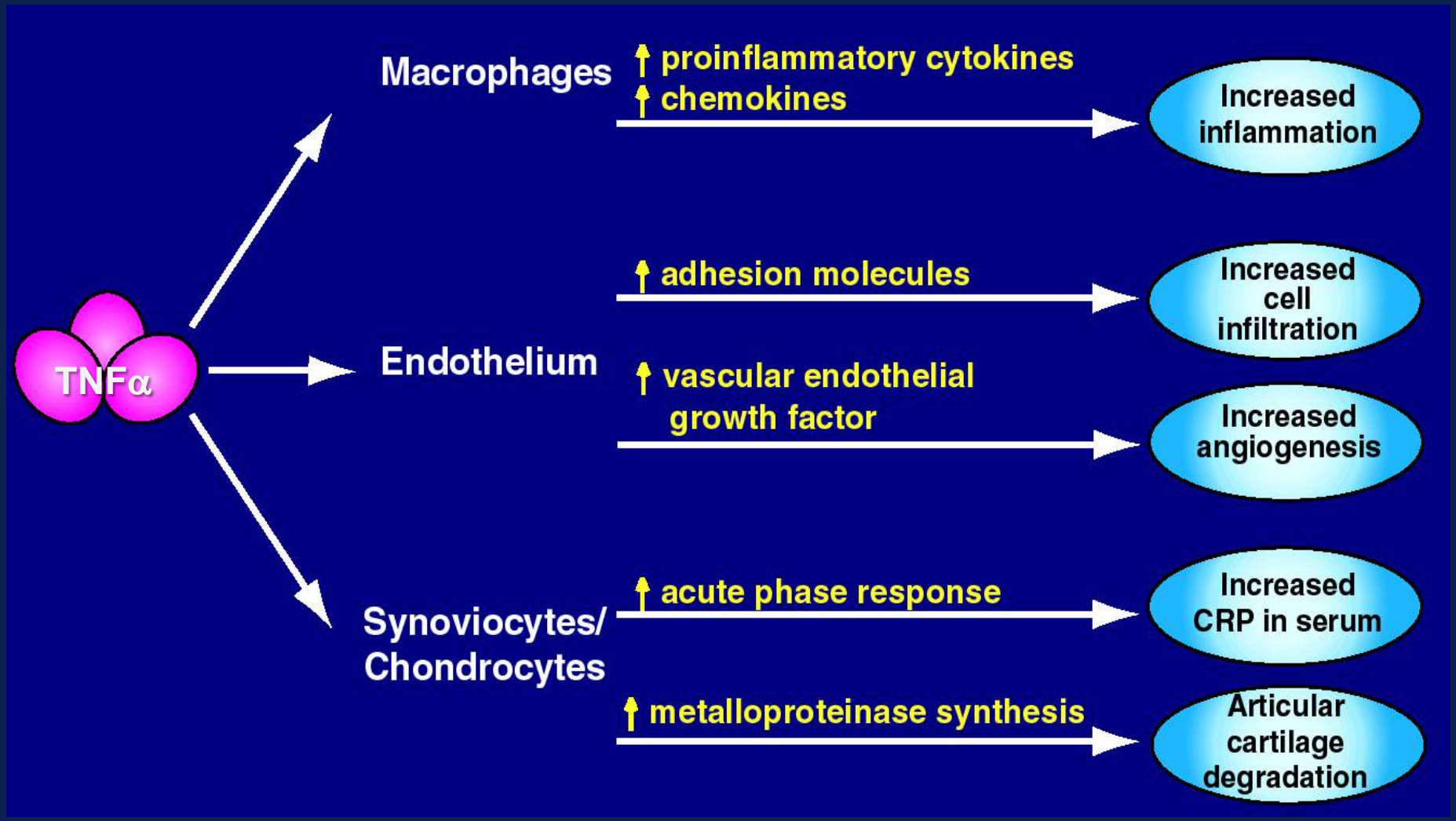
Synthesis and Function of TNFα



TNF receptors phosphorylate each other—induces signaling

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

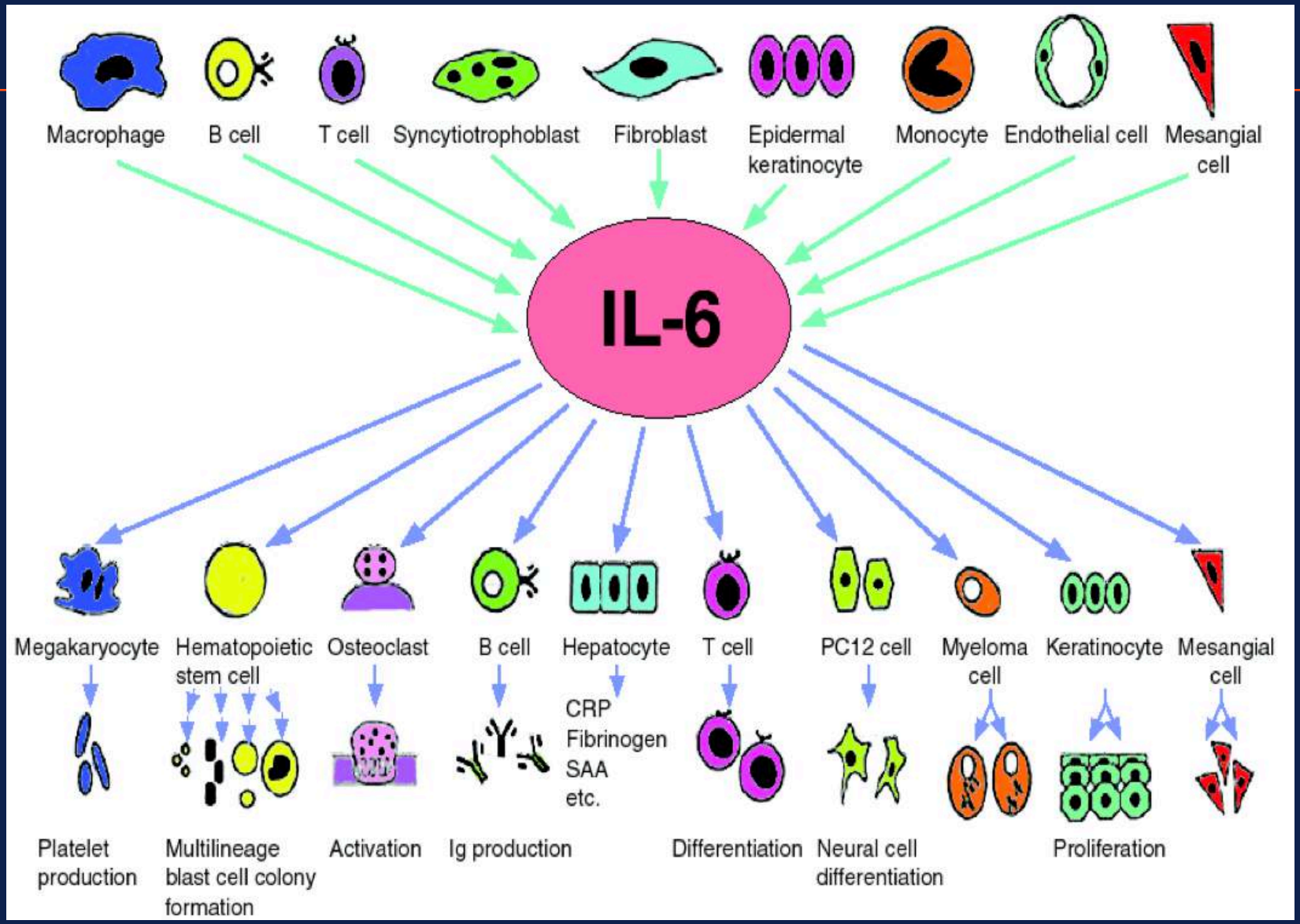
Key Actions of TNFα in RA



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

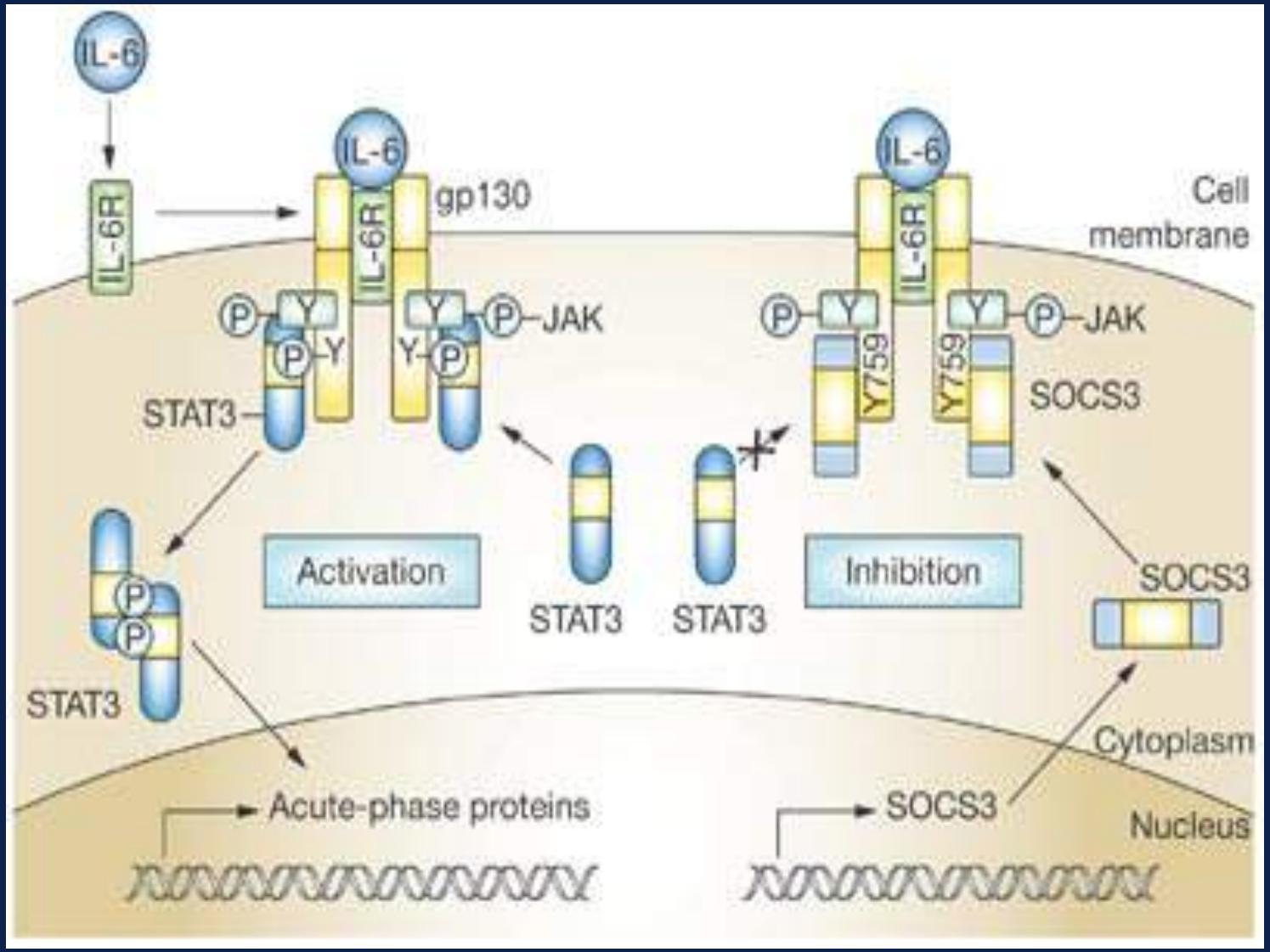
INTERLEUKIN 6 IS AN IMPORTANT CYTOKINE IN RA

Functions of IL-6



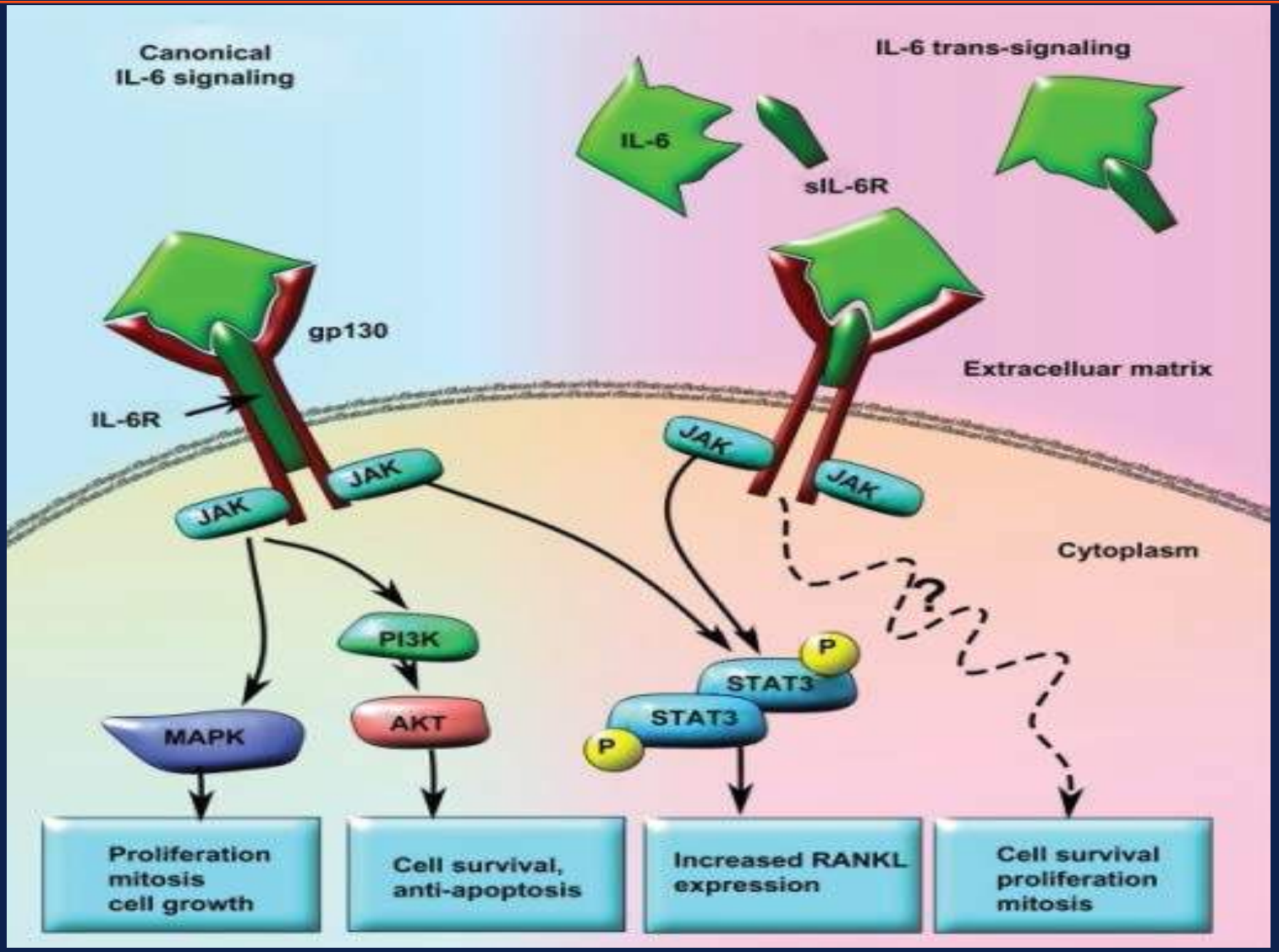
Source: Am J Health Syst Pham @2008 American Society of Health-System Pharmacists

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



Trans-signaling

Probably more important in chronic inflammatory diseases

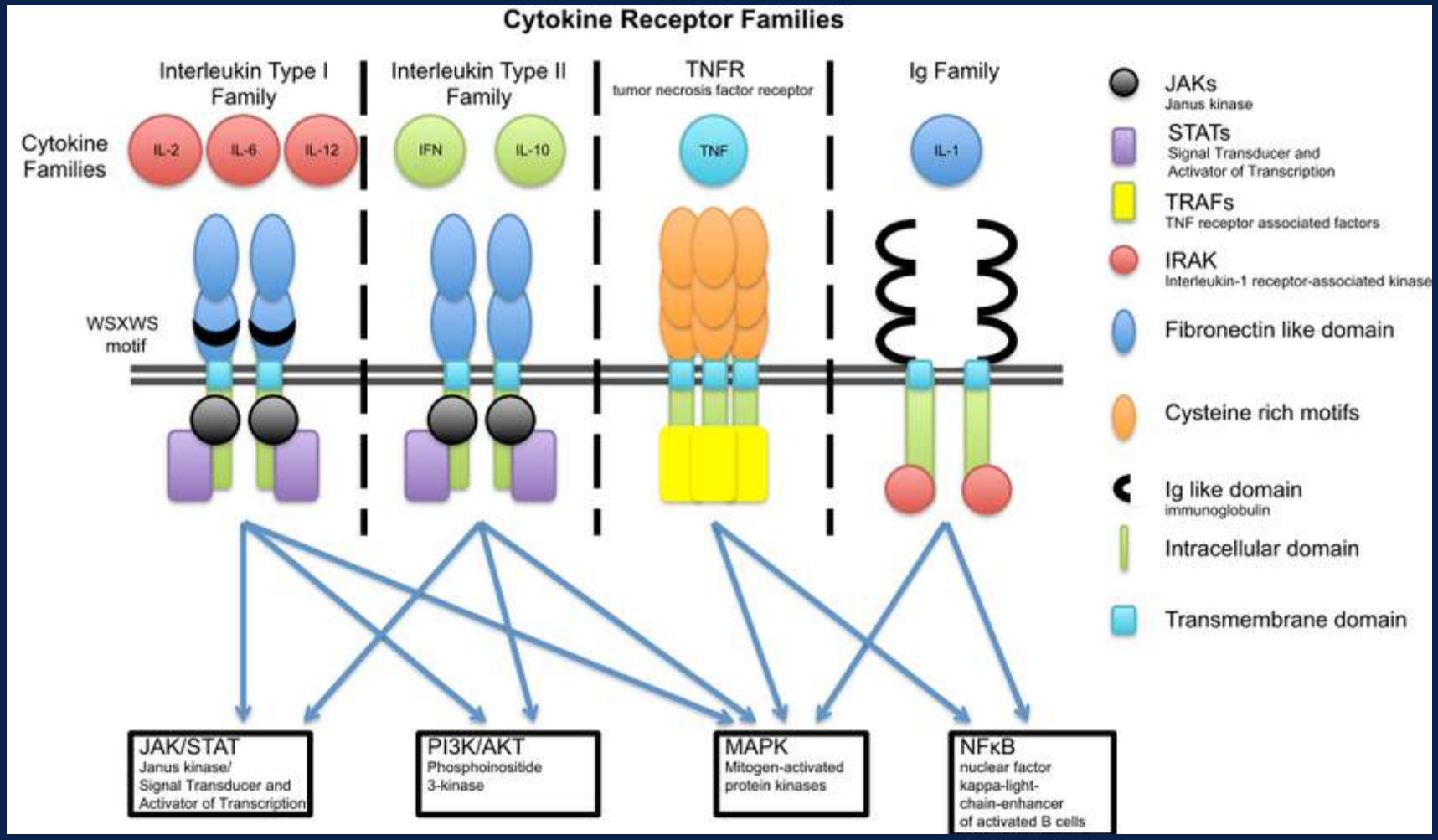


Intracellular Signaling

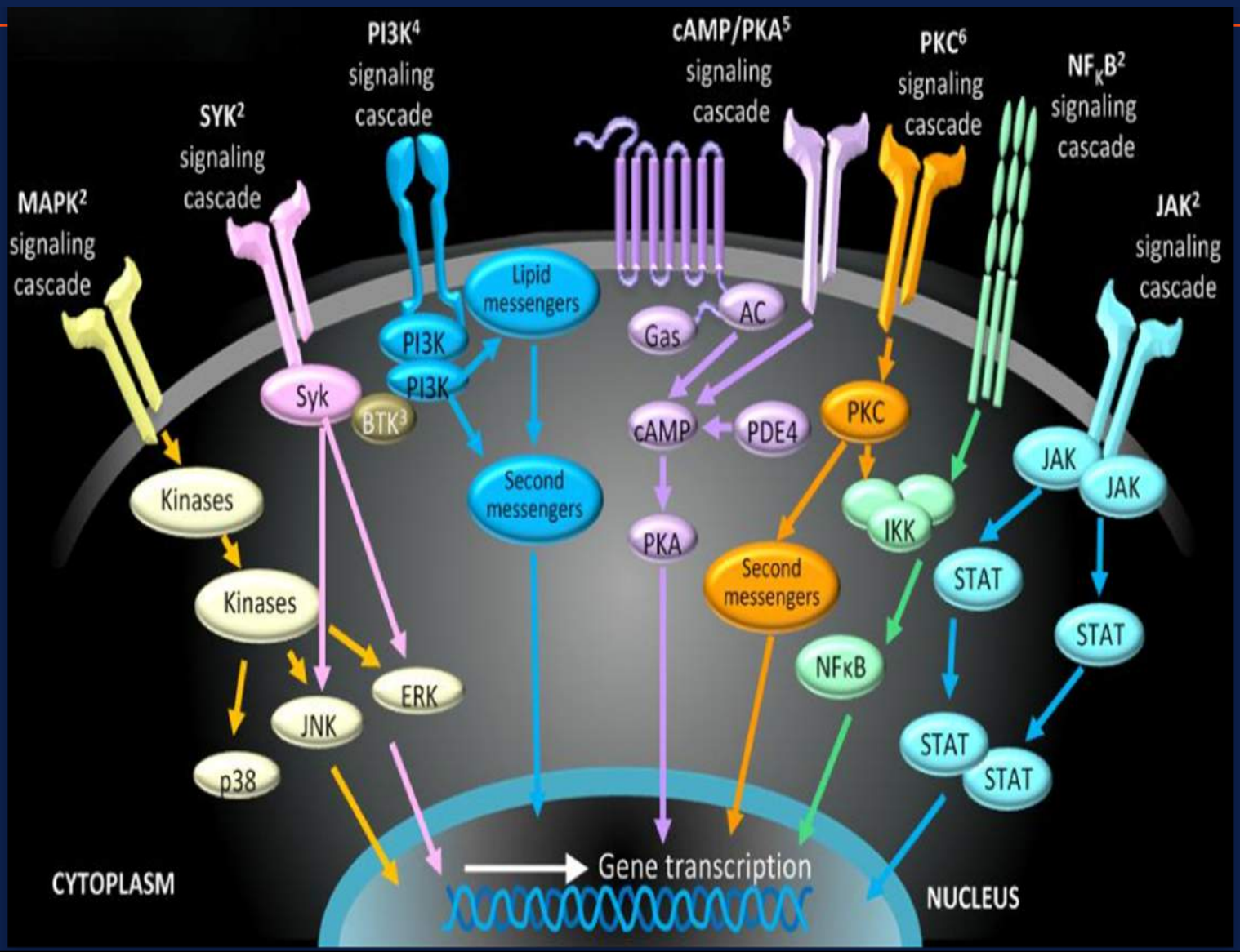
7 different cytokine receptors

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

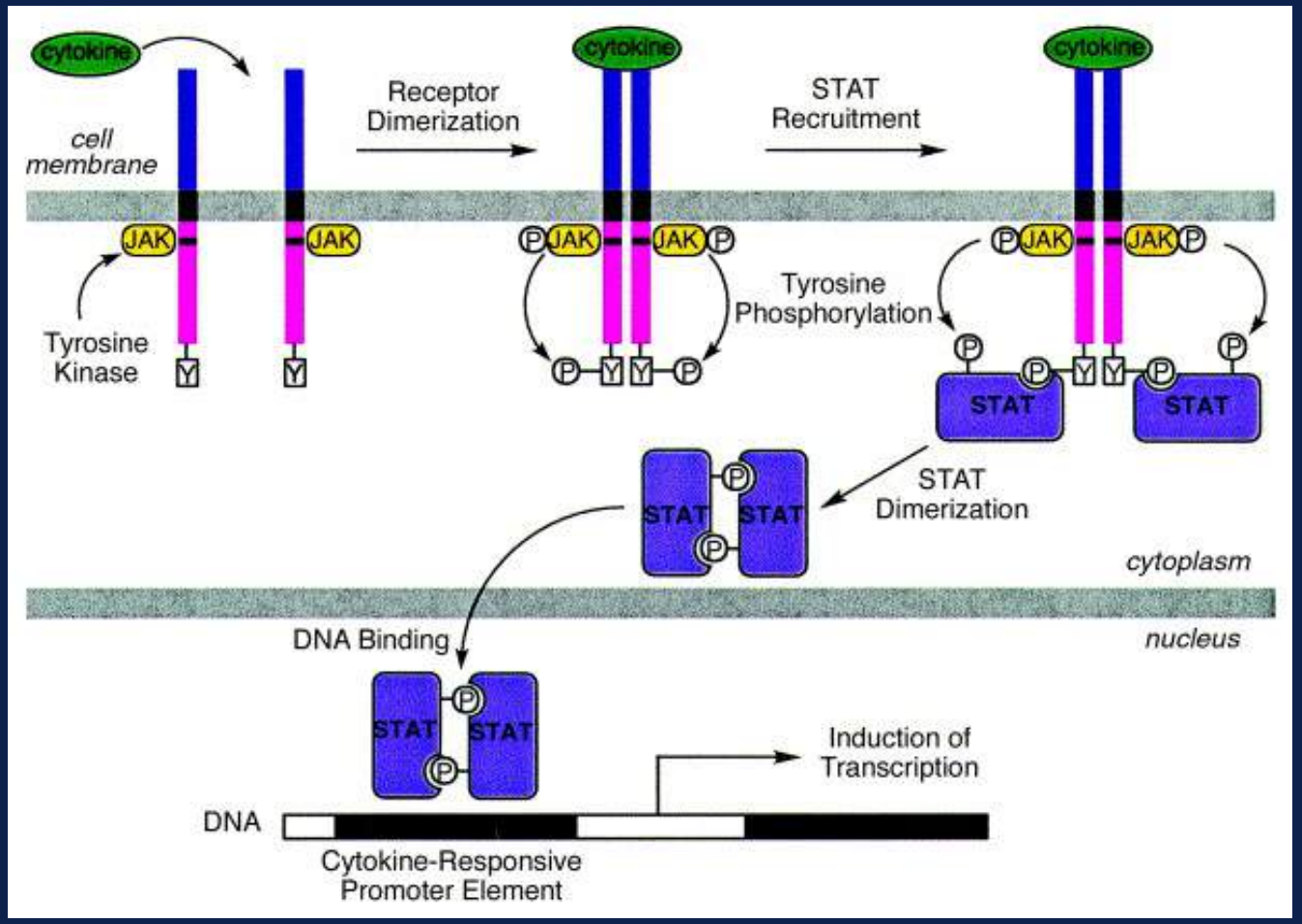
Cytokine Receptors Use Different Signaling Pathways



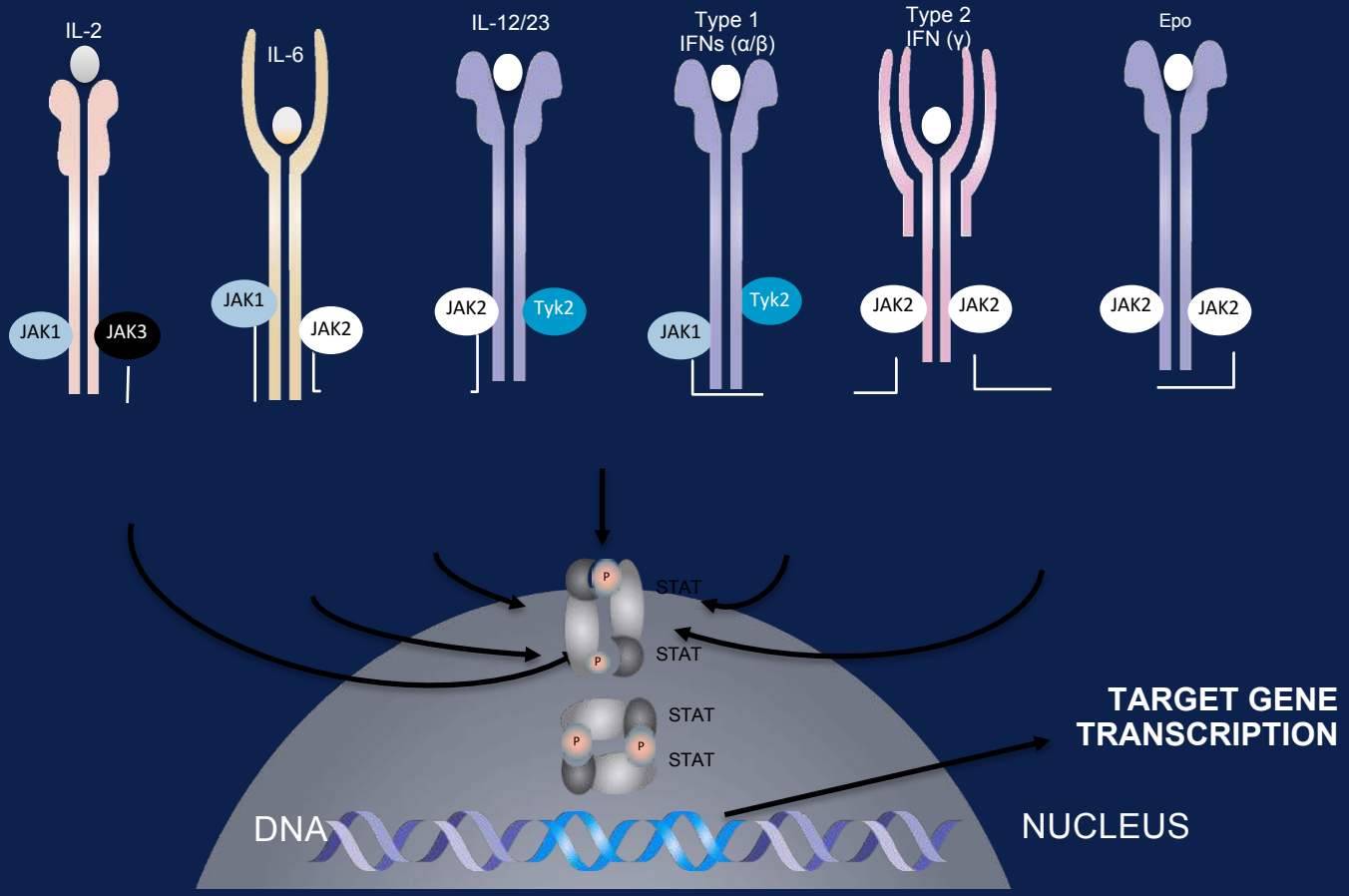
Cytokines Signal Through Different Pathways



JAK-STAT Signaling



Signaling by Different Cytokines Requires Unique JAK Pairings



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

Insights into the Initiation and Progression of RA

Etiopathogenesis of RA

- Genetic predisposition
  - *HLA-DRB1* [1]
  - *PTPN22* [2]
- Environment
  - Prior infections [3]
  - Hormones [4]
  - Smoking [5]
- Autoimmunity[3]
  - CD4+ helper and TH17 T cells [6]
  - B cells, plasma cells, and autoantibodies [7-9]
  - Proinflammatory cytokines: TNF-α, IL-1, IL-6, IL-17 [9]

Tayo BO, et al. *BMC Med Genet.* 2009;10:142.
Chen L, et al. *BMC Proc.* 2009;3 Suppl 7:S6.
Haier J. *Rheumatology.* 1999;38(6):504-9.
Karlson EW, et al. *Arthritis Res Ther*. 2009;11(3):R97.
Stolt P. *Ann Rheum Dis.* 2003;62(9):835-41.
Evans HG, et al. *Proc Natl Acad Sci U S A.* 2009;106(15):6232-7
Nanki T, et al. *Arthritis Res Ther.* 2009;11(5):R149.
Roll R, et al. *Arthritis Rheum*. 2006;54(8):2377-86.
Hueber W, et al. *Arthritis Res Ther. 2009;11(3):R76.*

Genetic Predisposition to RA

- **Strongest genetic factor associated with an increased risk of developing RA**:

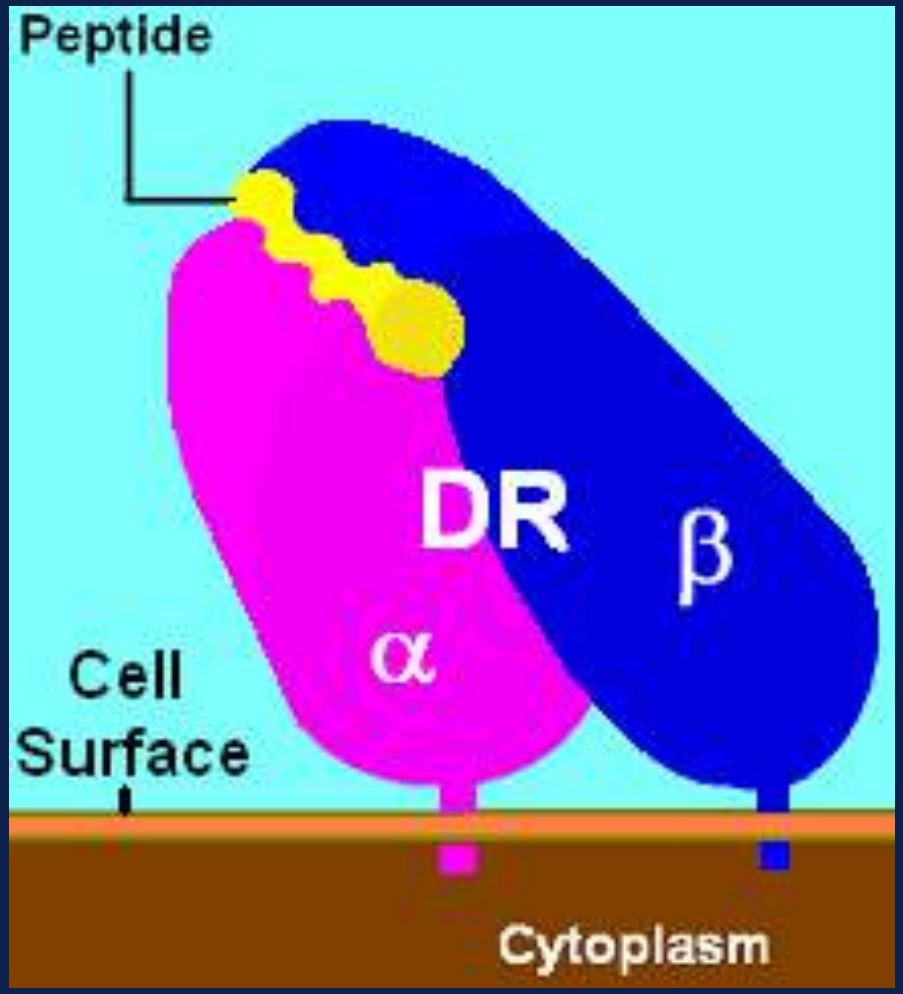
  - Polymorphisms at the ***HLA-DRB1*** locus (chr. 6p21.3) encoding HLA Class II β-chain molecules[1]

    - **Proteins (MHC molecules) involved in antigen presentation to T cells** [2]

- MHC molecules containing the shared epitope are able to accommodate citrullinated peptides in the binding site

Tayo BO, et al. *BMC Med Genet.* 2009; 10:142.
Weyand CM, et al. *J Clin Invest.* 1992; 89(6):2033-9.

Shared Epitope--part of the binding site of an MHC class II molecule



Immunologic Tolerance

- Definition:
  - **specific unresponsiveness to an antigen** that is induced by exposure of lymphocytes to that antigen

- Significance:
  - **All individuals are tolerant of their own antigens (self-tolerance); breakdown of self-tolerance results in autoimmunity**

HOW IS TOLERANCE BREACHED??

IE-WHY DOES THE RA PATIENT RECOGNIZE SELF AS FOREIGN??

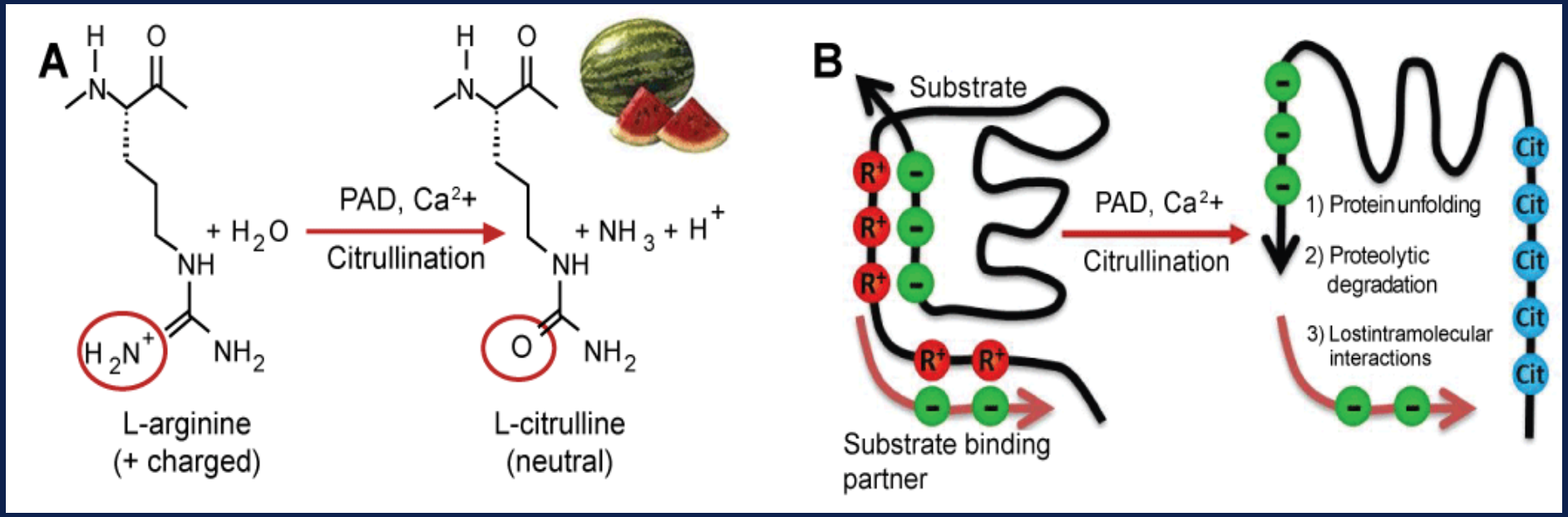
PADI Citrullination is the Target Resulting in Breaching Tolerance in RA

- Citrullination occurs at sites of inflammation[1]
- PADI post-translationally modifies proteins[1-3]
- Peptidyl **arginine** amino acid residues are modified to **citrulline** residues[1-3]
- This process occurs in multiple proteins[3]

RA in many pts characterized by autoantibodies that target citrulline-containing proteins

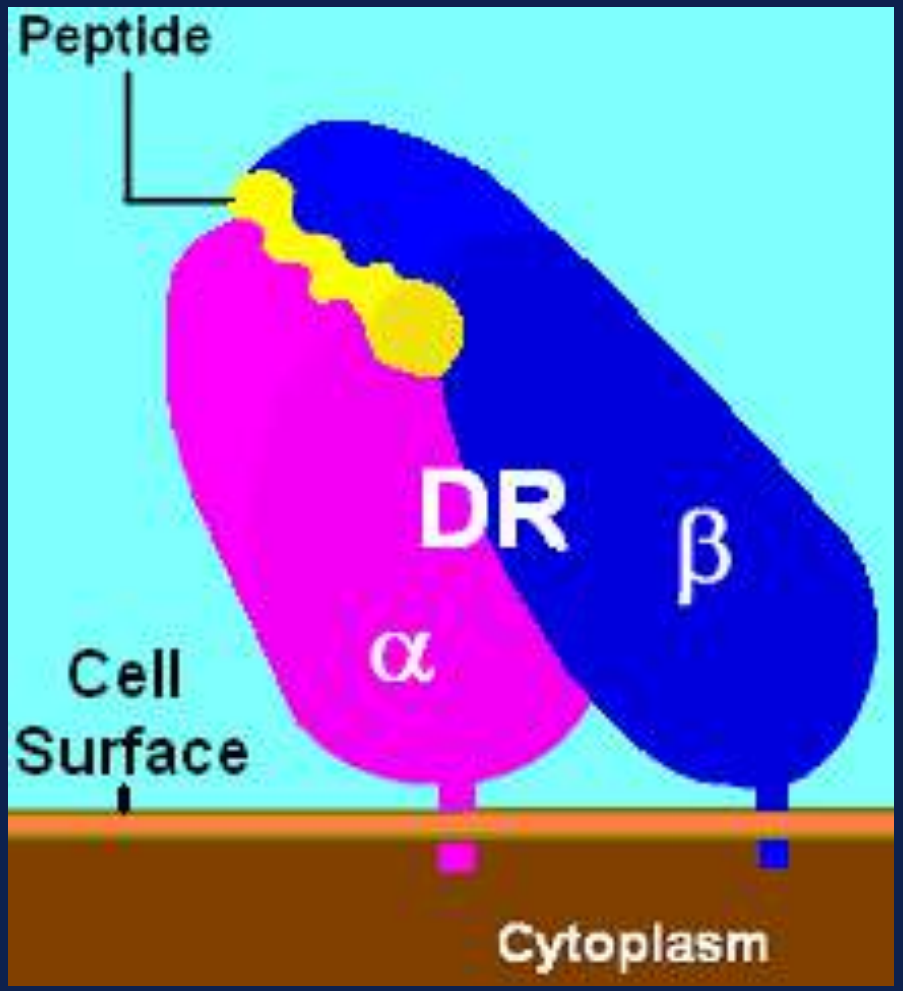
1. Sokolove J, et al. *Arthritis Rheum.* 2011;63(1):53-62.
2. Beltrami A et al. *J Bio Chem.* 2008;283(40):27189-27199.
3. Snir O et al. *Ann Rheum Dis.* 2009;68(5):736-743.

CITRULLINATION

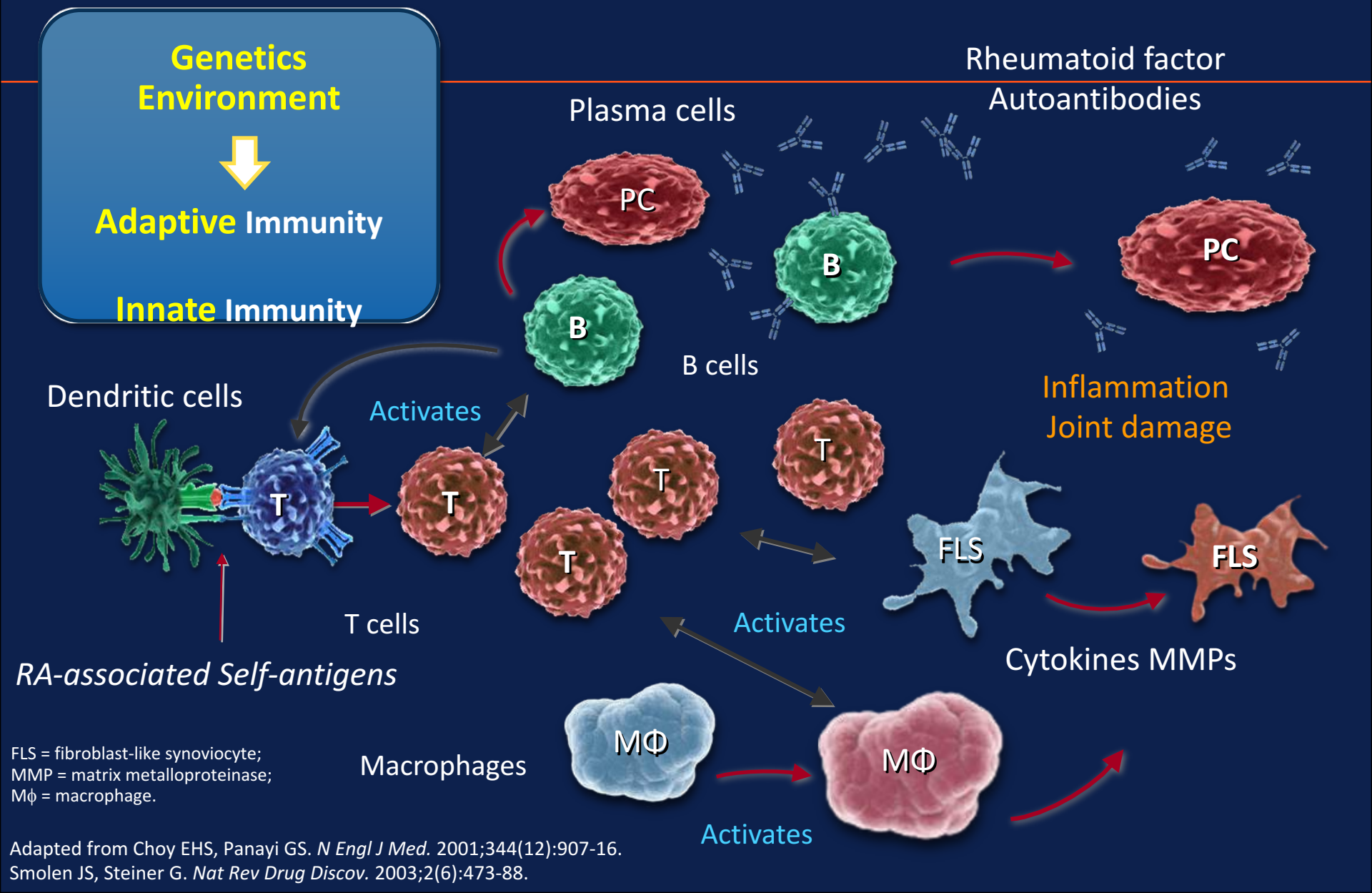


Shared Epitope

- Part of the MHC class II molecule
- Able to bind citrullinated self-antigens



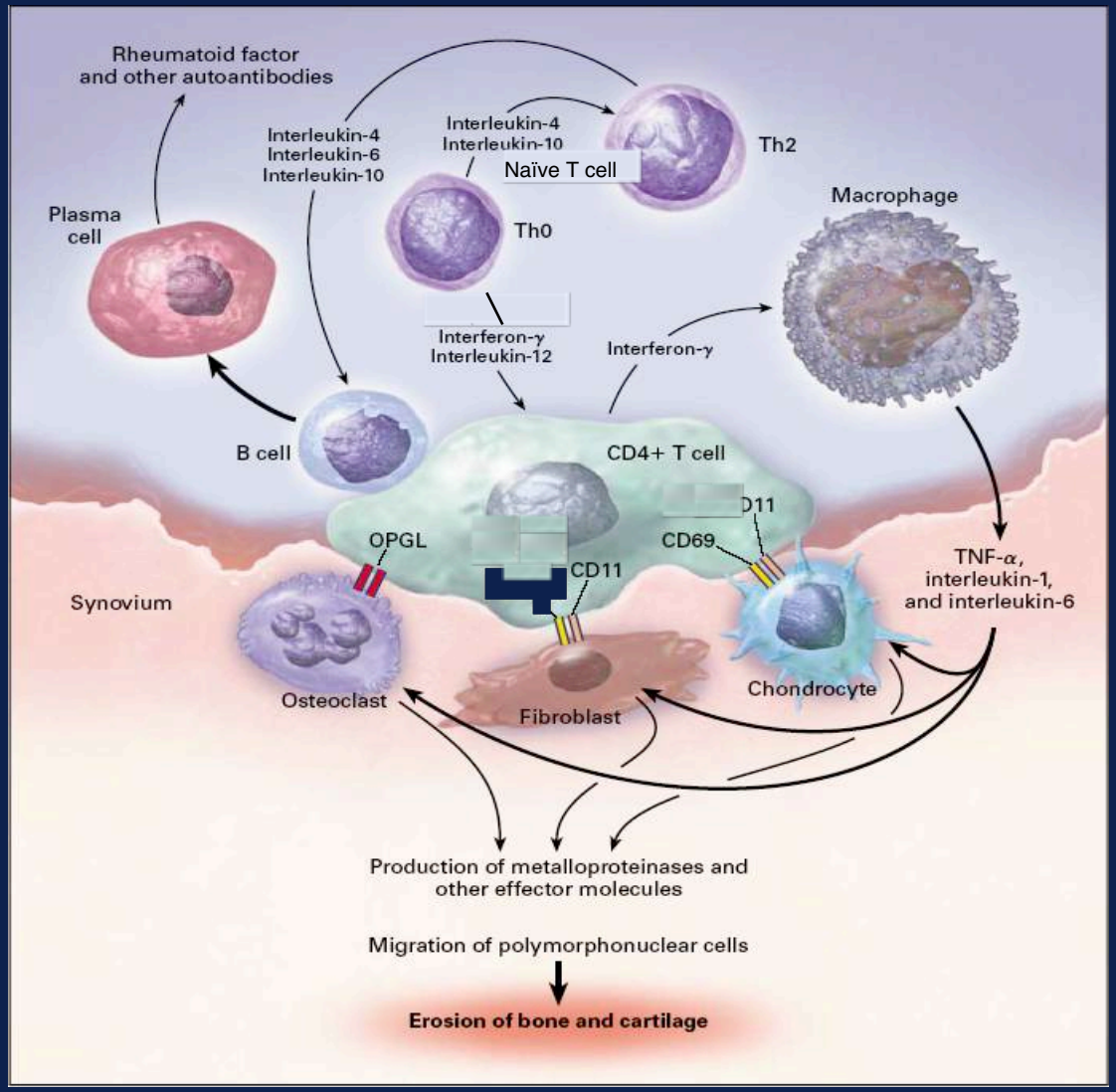
Etiopathogenesis of Rheumatoid Arthritis



FLS = fibroblast-like synoviocyte;
MMP = matrix metalloproteinase;
Mϕ = macrophage.

Adapted from Choy EHS, Panayi GS. *N Engl J Med.* 2001;344(12):907-16.
Smolen JS, Steiner G. *Nat Rev Drug Discov.* 2003;2(6):473-88.

There are multiple cell types and cytokines involved in RA. RA involves the entire integrated immune response-T, B, Innate. Understanding immunology allows a better understanding of the disease and its treatment.



Key cytokines in chronic inflammatory arthritis:

TNF-α
IL-1
IFN-γ
IL-6
RANK-ligand
IL-17

Modified from Choy, E. H.S. et al. N Engl J Med 2001;344:907-916

QUESTIONS ?