SITC Winter School

Innate Immunity: Cellular mechanisms and signaling

Lewis L. Lanier

UCSF

Useful Reviews – Innate Immunity in Cancer

Complement – Afshar-Kharghan J. Clin. Invest. 127:780, 2017

Interferons – Parker et al. Nat Reviews Cancer 16:131, 2016

Chemokines – Nagarsheth et al. Nat Reviews Immunology 17:559, 2017

Cytokines – Conlon et al. J. Interferon & Cytokine Research 39:6, 2019

Innate Immune Recognition of Cancer Woo et al. Ann Rev Imm 33:445, 2015

STING – Corrales et al. J. Clin. Invest. 126:2404, 2016

Neutrophils - Coffelt et al. Nature Reviews Cancer 16:431, 2016

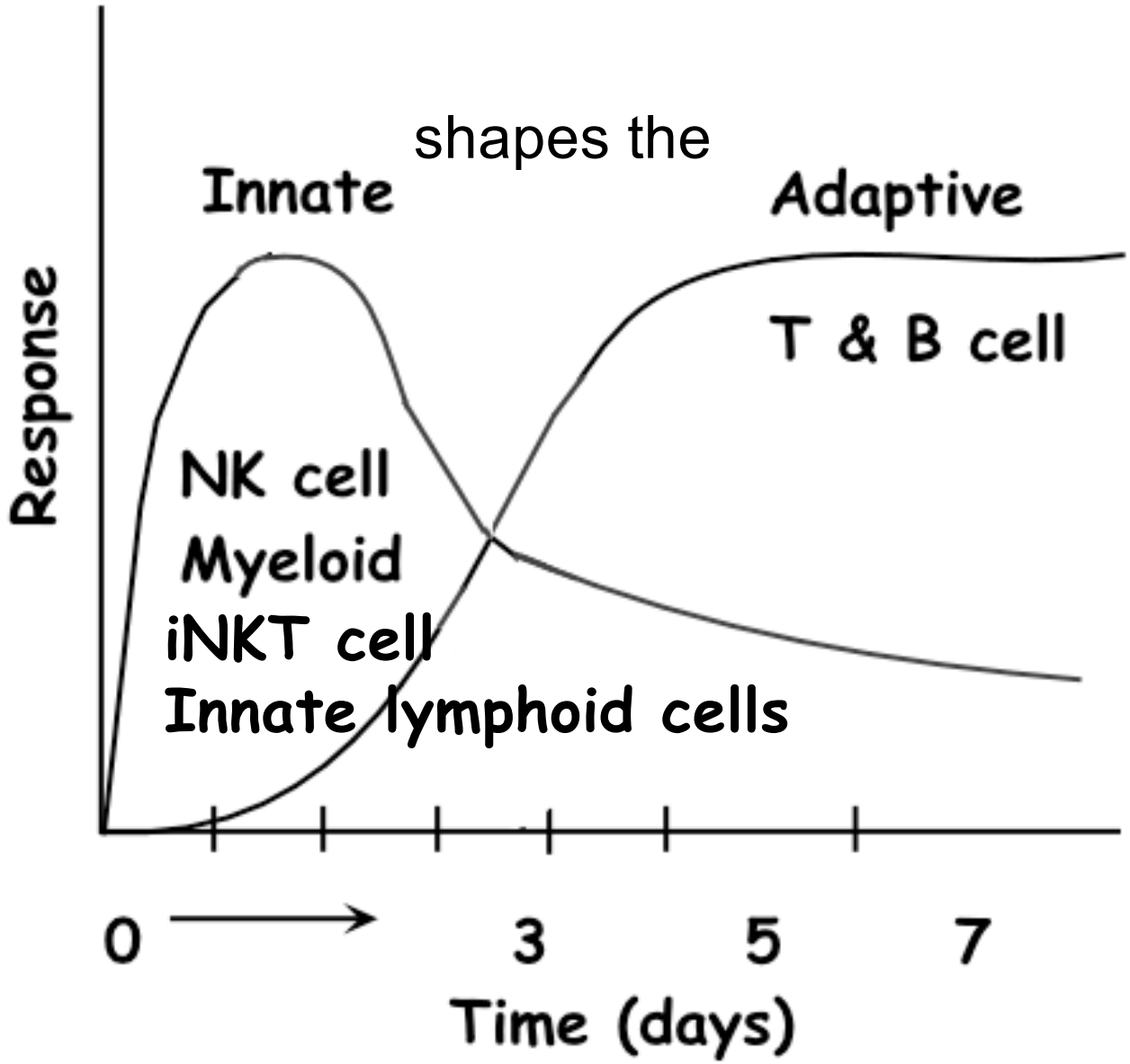
Mast cells - Varricchi et al. Frontiers Immunology 2017

Macrophages – Poh & Matthias Frontiers Oncology 2018

Dendritic cells – Veglia & Garilovich Curr Op Immunol 45:43, 2017

NK cells – Miller & Lanier Ann Rev Cancer Biology 2019

shapes the


Innate
Adaptive
T & B cell
Response
NK cell
Myeloid
iNKT cell
Innate lymphoid cells
0
3
5
7
Time (days)

What discriminates the innate and adaptive immune systems?

Innate immune cells use germline-encoded receptors

Adaptive immune cells somatically rearrange receptors (T cell receptors and Immunoglobulins)

Adaptive immune cells usually start from very low precursor frequencies and expand after encountering cognate antigens

Innate Immunity

- *Physical barriers and soluble factors in sera and tissues*
  - Some always present (protease inhibitors, complement)
  - Others induced by infection or inflammation (interferons, acute phase proteins, cytokines, & chemokines)
- *Cell intrinsic pathogen sensors*
  - In all cell types – Toll-like receptors, NODs, STING, RIG-I
- *Cells of the Innate Immune System*
  - Myeloid cells – phagocytes and antigen-presenting cells
  - Natural Killer cells - kill virus-infected cells and tumors
  - Innate Lymphoid Cells (ILC) – cytokine producers

Innate Immunity - Epithelial Cells

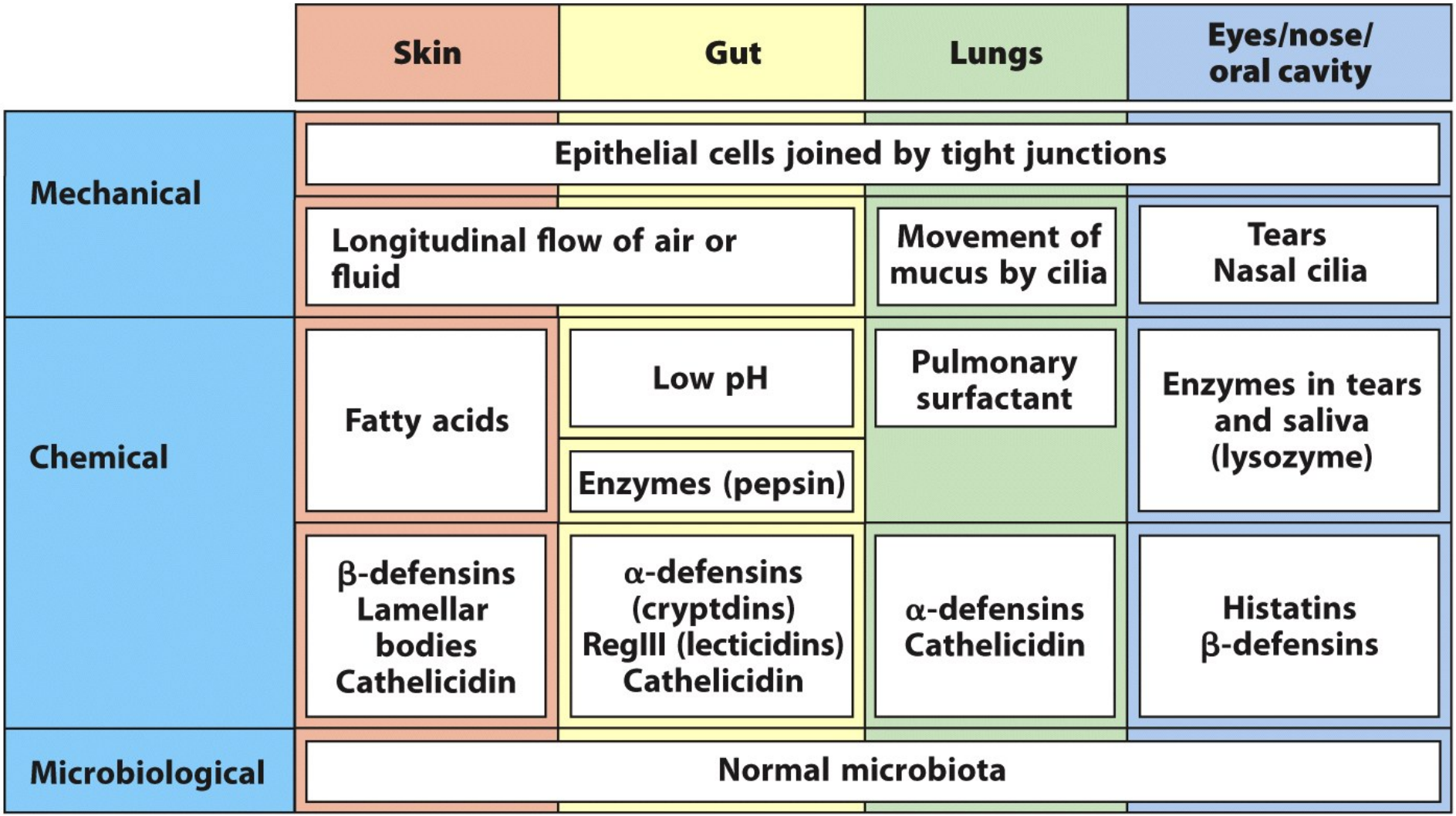


Figure 2.6 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Soluble factors

- Blood clotting system (physical barrier)

  Bradykinin (vasodilation – allow cell entry)
- Protease inhibitors
  - α2-macroglobulin
- Defensins
- Acute phase proteins
  - C-reactive protein, mannose-binding lectin
- Complement
- Cytokines
- Chemokines
- Interferons

Protease inhibitors

Bacteria frequently secrete proteases to invade host tissues
-these are neutralized by proteinase inhibitors present in sera

-10% of proteins in sera are protease inhibitors

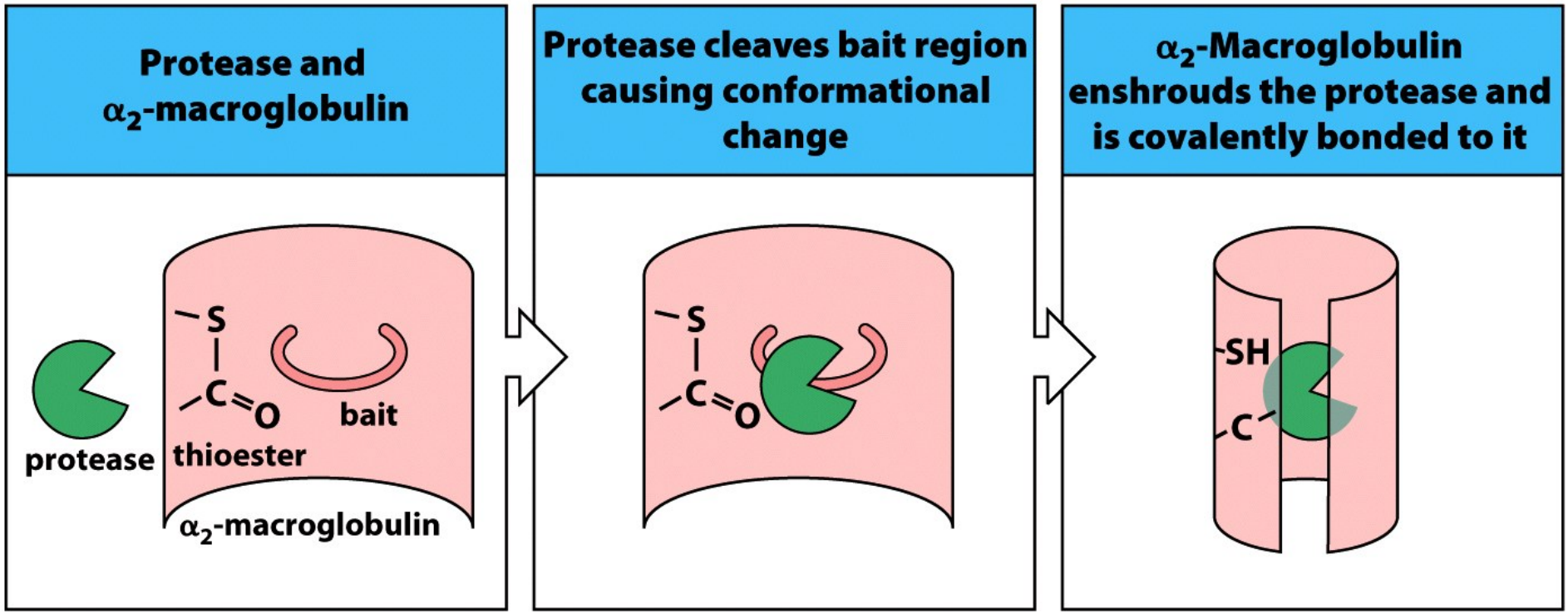


Figure 2.16 The Immune System, 3ed. (© Garland Science 2009)

Defensins produced
by epithelial cells in gut, lung, reproductive tract
by neutrophils recruited to site of infection

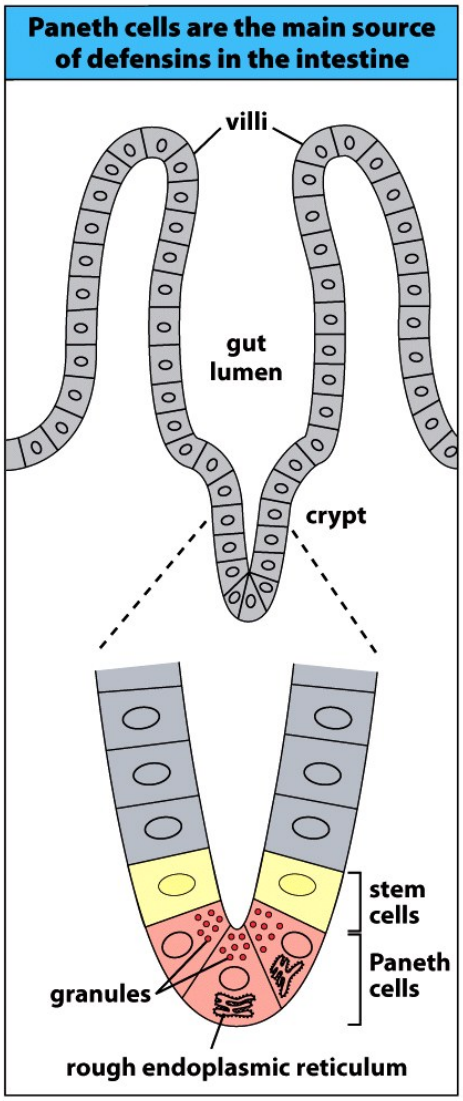


Figure 2.17 The Immune System, 3ed. (© Garland Science 2009)

Defensins

- α-defensins (cryptidins) and β-defensins – secreted by neutrophils and intestinal epithelial cells
- "mammalian antibiotics"
- Cysteine-rich 35-40 amino acid peptides – very stable!
- Rich in positively charged arginine residues
- Amphipathic (hydrophilic and hydrophobic) can insert in membranes

Humans Have Several Defensins

Defensin		Site of synthesis	Tissues defended	Regulation of synthesis
Class	Name			
α	HNP1	Neutrophils > monocytes, macrophages, NK cells, B cells, and some T cells	Intestinal epithelium, placenta, and cervical mucus plug	Constitutive
α	HNP2			
α	HNP3			
α	HNP4	Neutrophils	Not determined	Constitutive
α	HD5	Paneth cells > vaginal epithelial cells	Salivary glands, gastrointestinal tract, eyes, female genital tract, and breast milk	Constitutive and induced by sexually transmitted infection
α	HD6	Paneth cells	Salivary glands, gastrointestinal tract, eyes, and breast milk	
β	HBD1	Epithelial cells > monocytes, macrophages, dendritic cells, and keratinocytes	Gastrointestinal tract, respiratory tract, urogenital tract, skin, eyes, salivary glands, kidneys, and blood plasma	Constitutive and induced by infection
β	HBD2			
β	HBD3			
β	HBD4	Epithelial cells	Stomach (gastric antrum) and testes	

Figure 2.18 The Immune System, 3ed. (© Garland Science 2009)

Granulysin – unique to humans – expressed in NK and T cells

Cytokines – hormones of the immune system

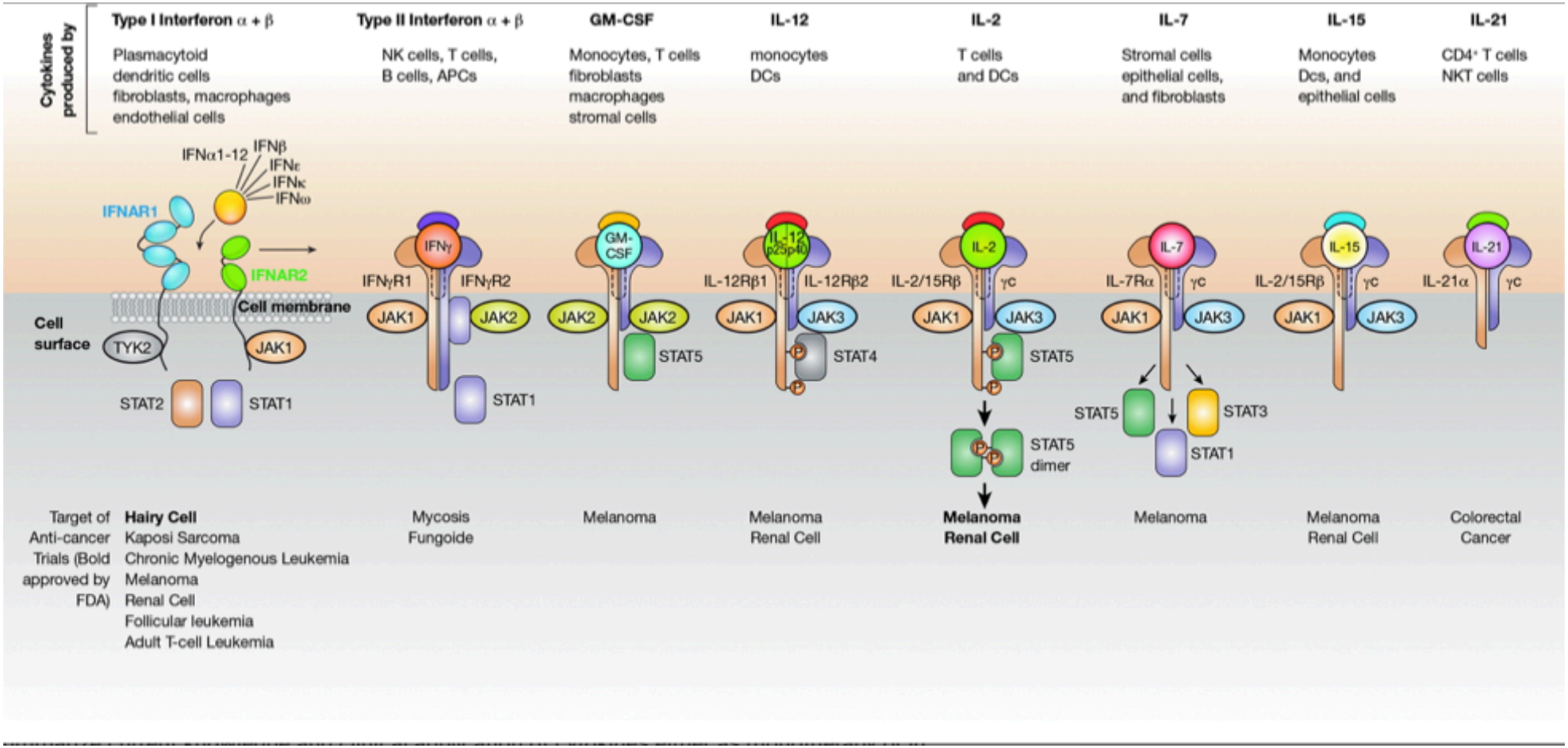
- Soluble proteins produced by immune cells and epithelial cells that can activate or suppress the immune system

- "Pro-inflammatory cytokines" – induced during infection or inflammation to activate immune system – e.g. Tumor Necrosis Factor-a (TNF), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-17 (IL-17), etc.

- "Regulatory cytokines" – suppress immune responses – e.g. Interleukin-10 or skew the adaptive immune system, e.g. IL-12 drives Th1 responses, IL-4 drives Th2 responses, TGF–β drives Treg cells, etc.

- Growth factors – e.g. IL-2, IL-7, IL-15 – for proliferation and survival

Cytokines and Cancer Immunotherapy



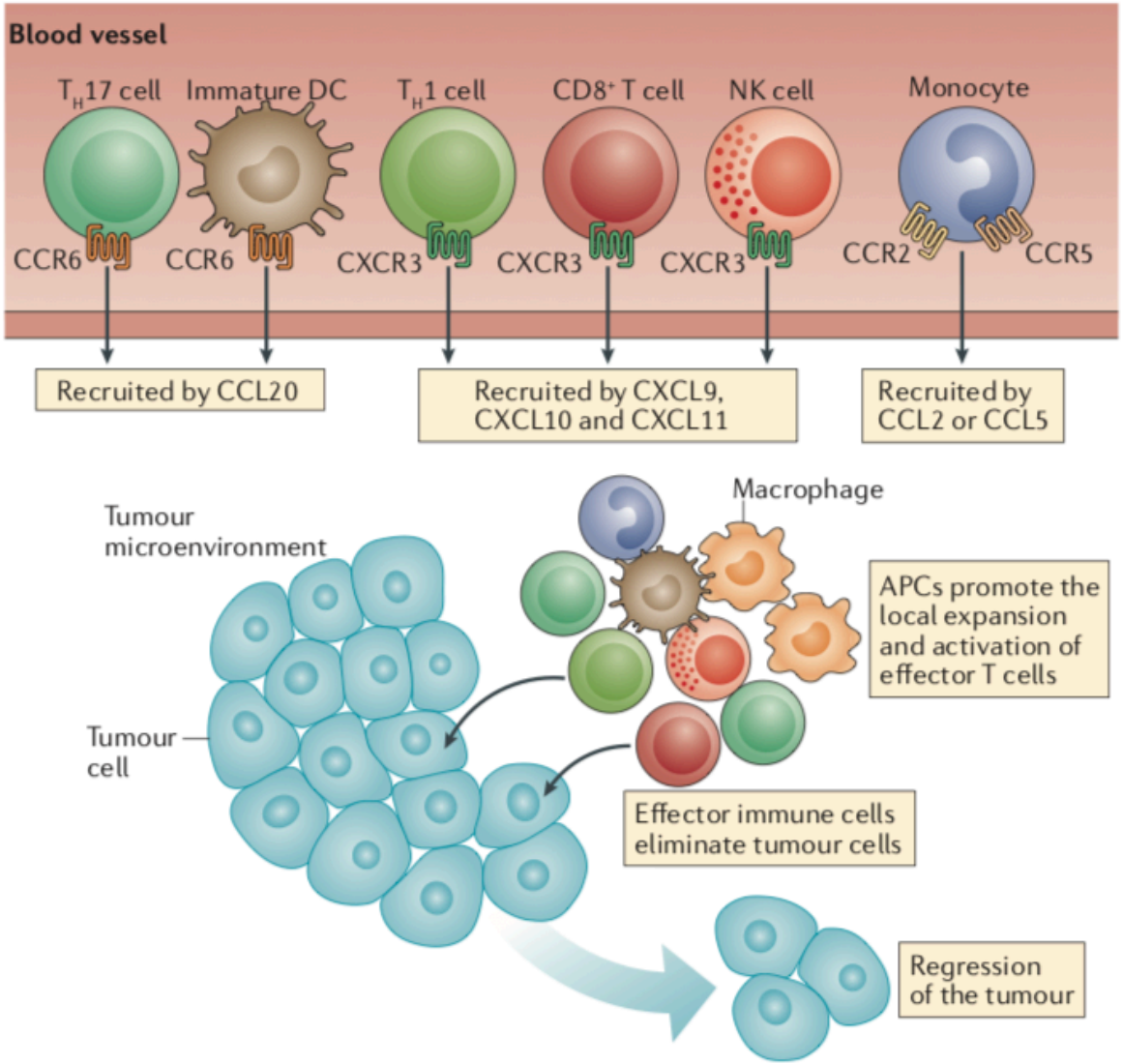
Issues – systemic toxicity IL-2, IL-12, IL-15, IFN
IL-2 expands Tregs

Conlon et al. J. Interferon & Cytokine Research 2019

Chemokines – traffic directors of the immune system

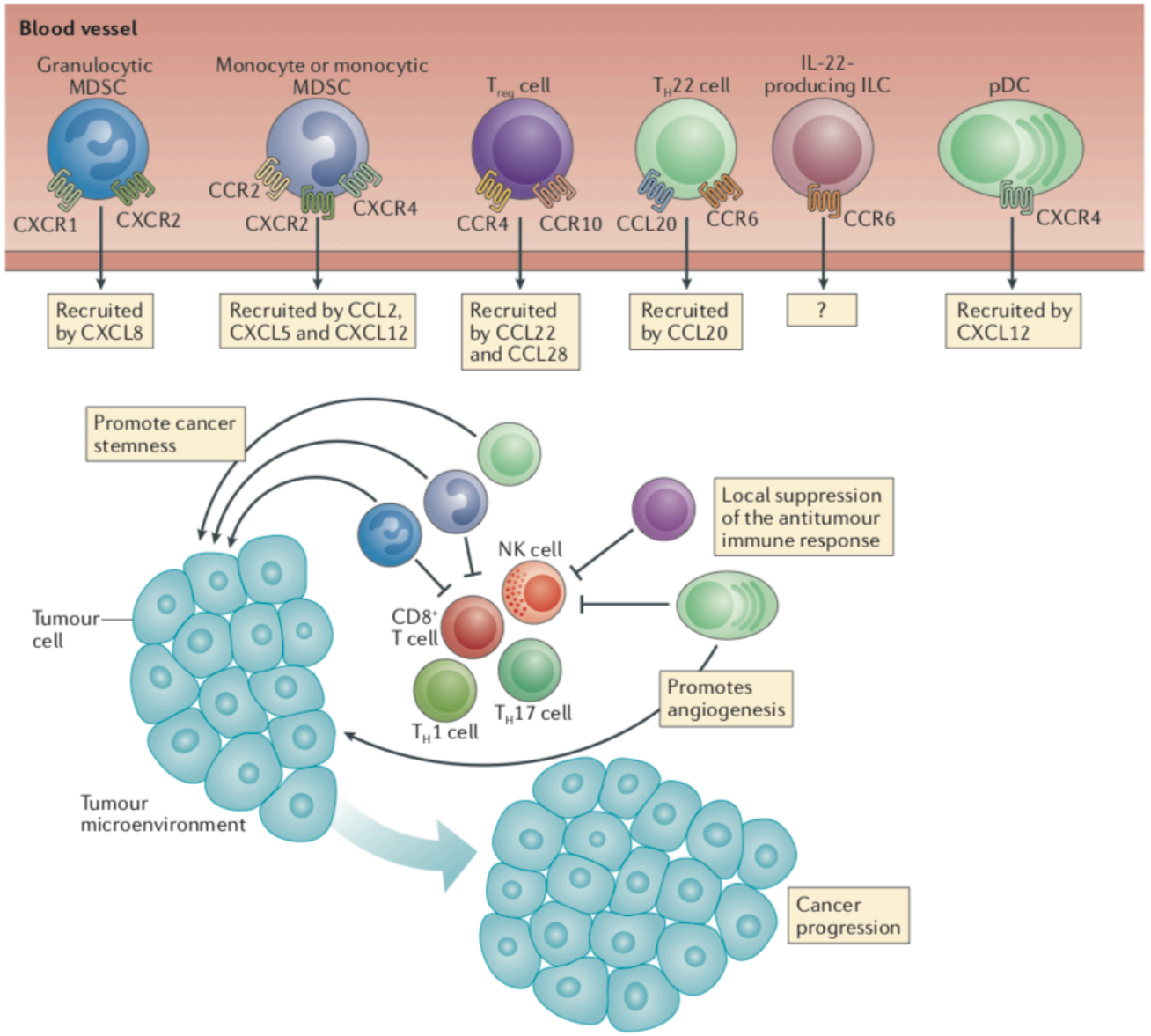
- Family of more than 50 soluble factors that induce the migration of cells, including CXC, CC, CX3C and XC chemokines
- Leukocytes expressing chemokine receptors (G-coupled protein receptors) migrate due to density gradients of chemokines
- Some chemokines are produced constitutively in tissues (e.g. lymph nodes) to organize cells within the tissue
- Chemokines are secreted by immune cells, epithelial cells, or endothelial cells in response to infection or inflammation to recruit granulocytes, myeloid cells, and lymphocytes to the site

How do effector cells get out of the blood or lymphatic vessels and into infected tissues or tumors?



Nagarsheth et al. Nat Reviews Immunology 2017

Chemokines in tumor microenvironment can also promote cancer



Nagarsheth et al. Nat Reviews Immunology 2017

Myeloid cells activated by pathogens secrete "inflammatory" cytokines

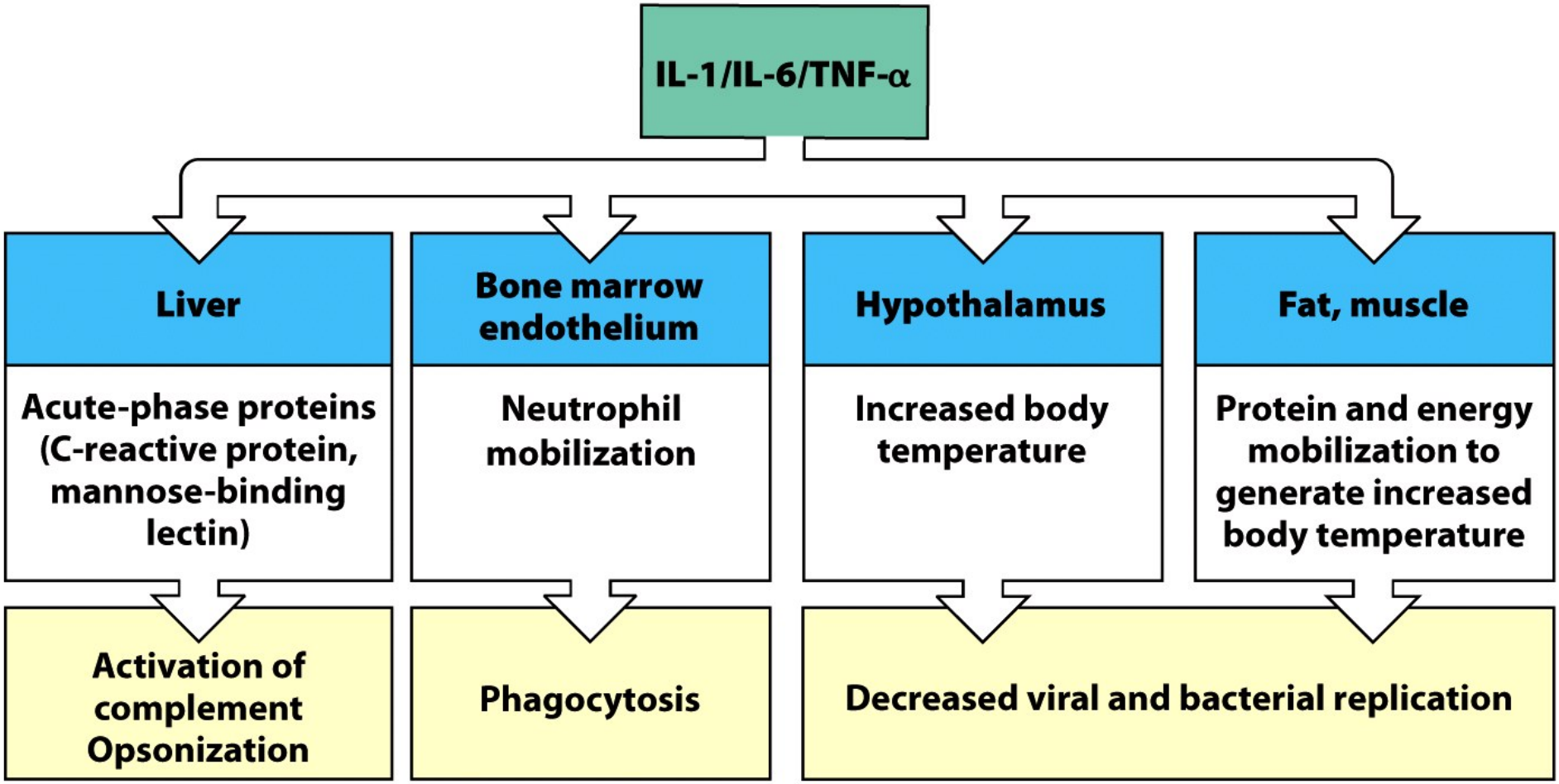


Figure 2.36 The Immune System, 3ed. (© Garland Science 2009)

"Pyrogens" causing Fever

Severe systemic inflammation

>>cytokines

nature medicine

Letter | Published: 28 May 2018

CAR T cell–induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade

Theodoros Giavridis, Sjoukje J. C. van der Stegen, Justin Eyquem, Mohamad Hamieh, Alessandra Piersigilli & Michel Sadelain

Nature Medicine **24**, 731–738 (2018) | Download Citation

ACUTE PHASE RESPONSE

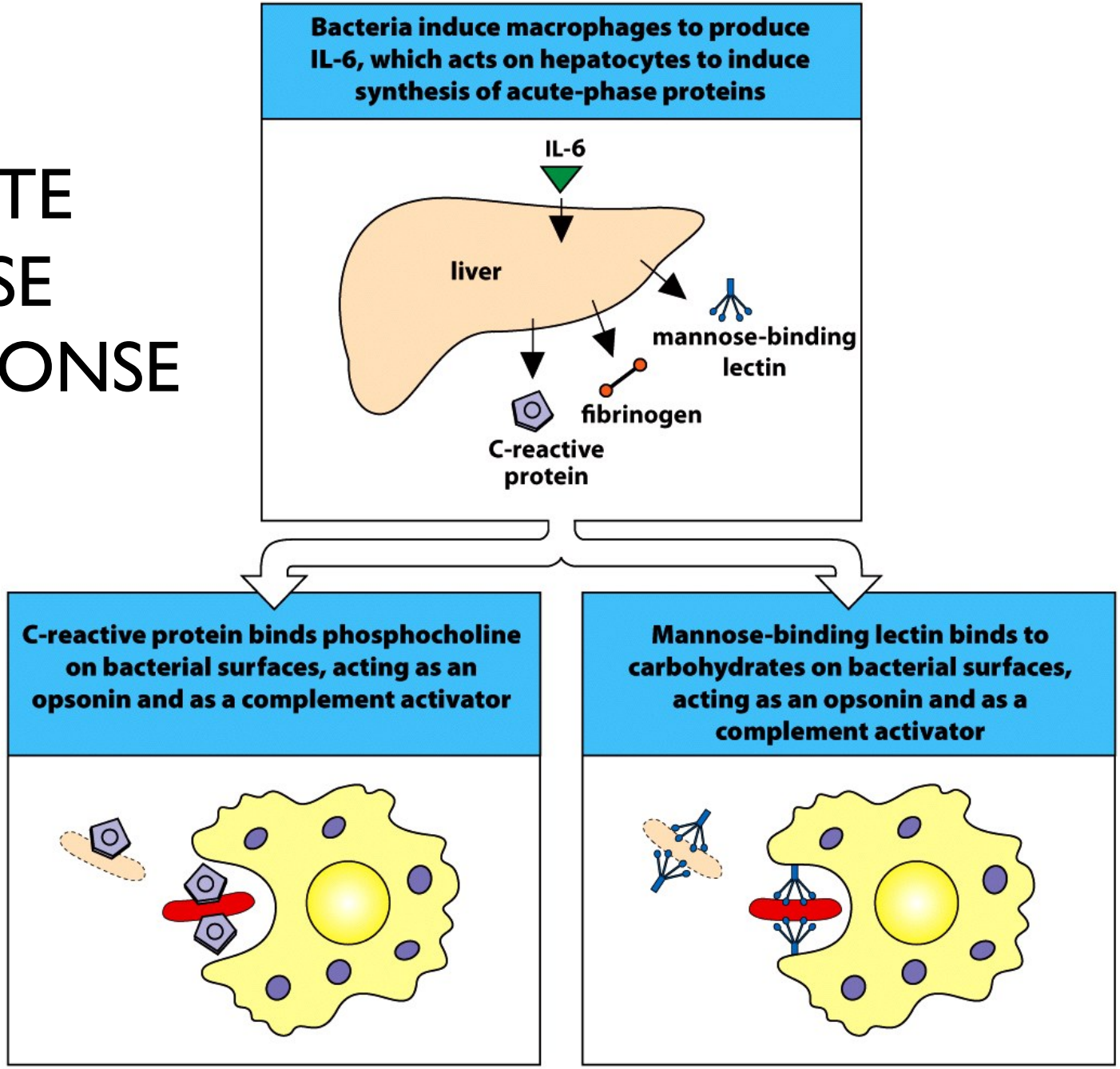
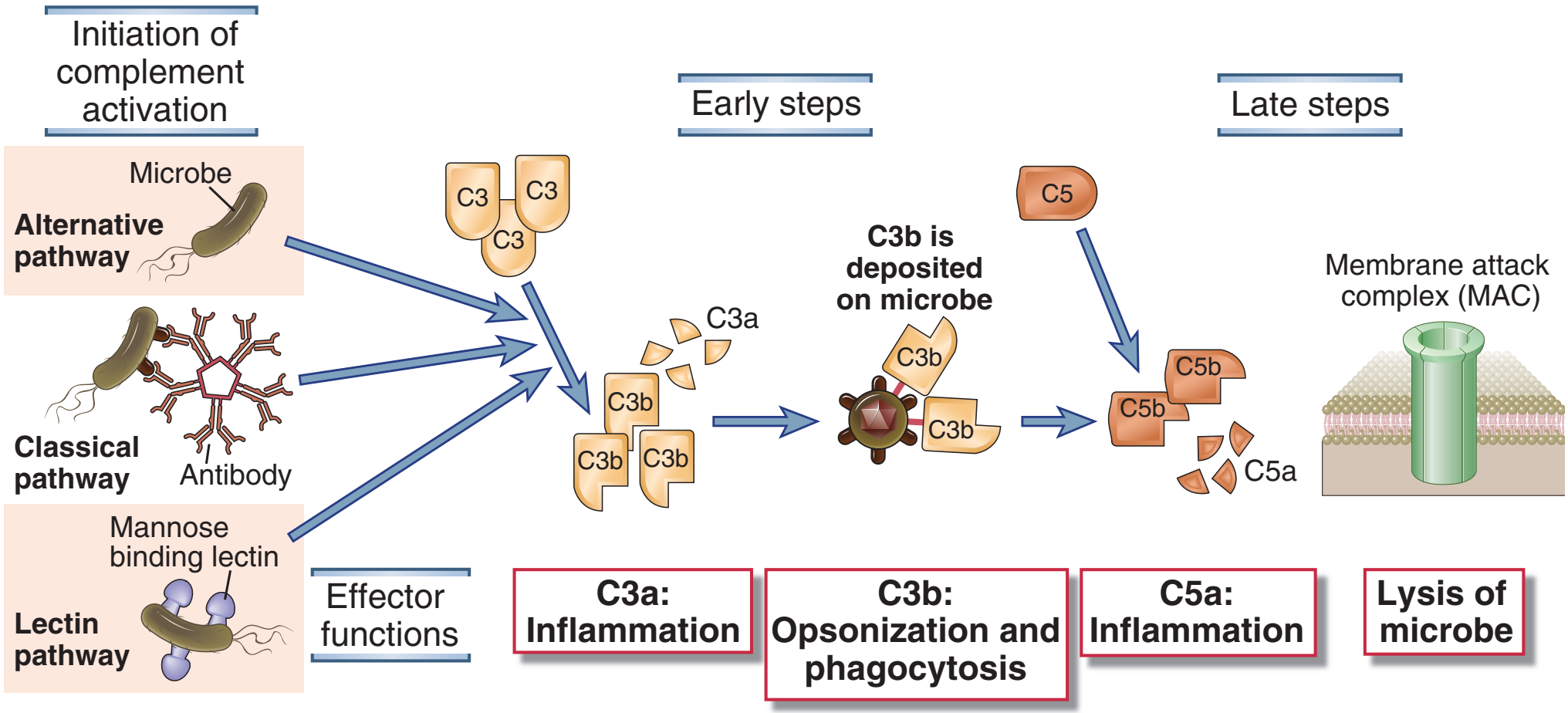


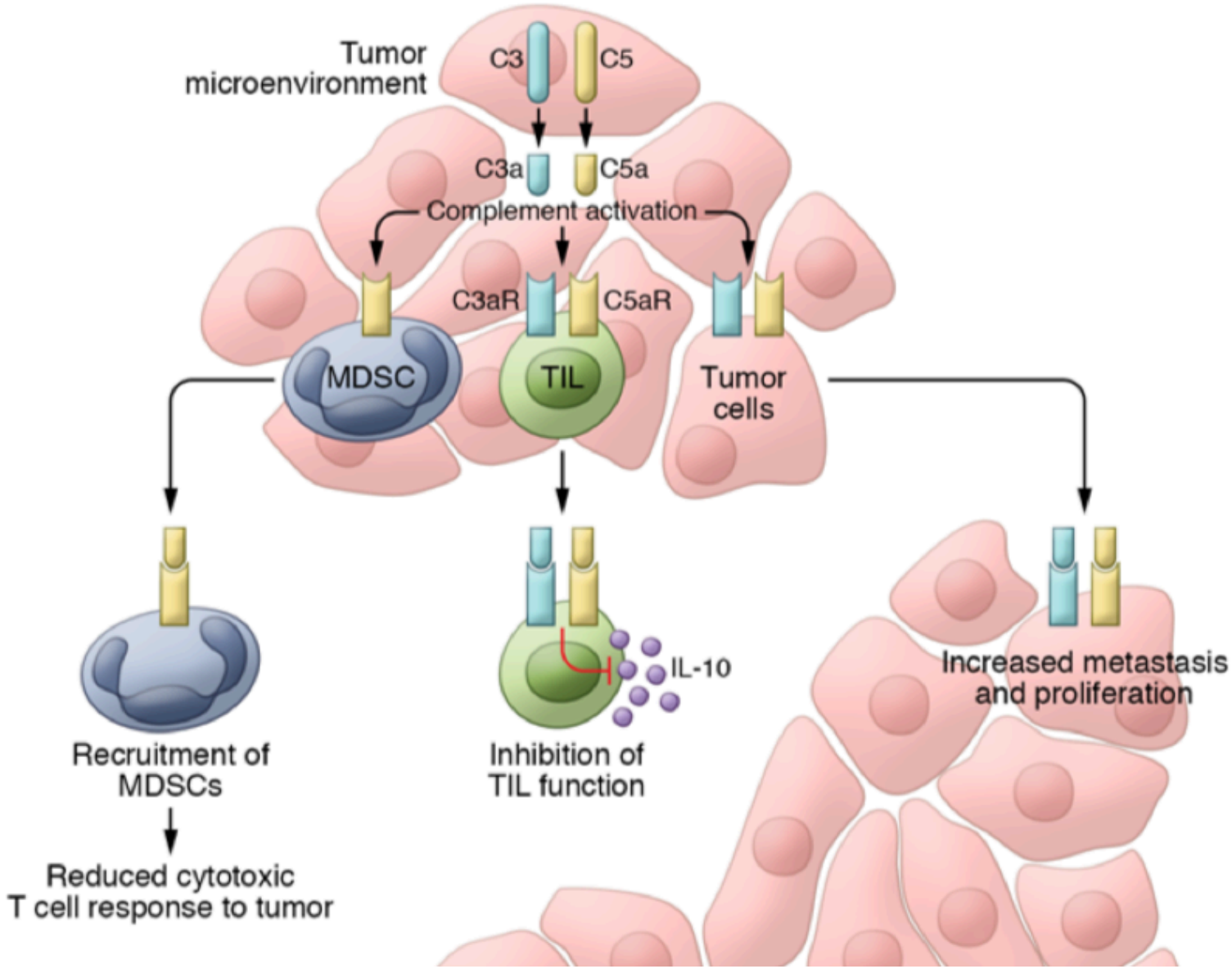
Figure 2.38 The Immune System, 3ed. (© Garland Science 2009)

Complement

- Circulating inactive serine protease enzymes
- Cascade of sequential activation



Role of Complement in Cancer – Complex



Afshar-Kharghan J. Clin. Invest.2017

Interferons

- Soluble proteins with anti-viral activity

  Type I interferons

  α….14 genes

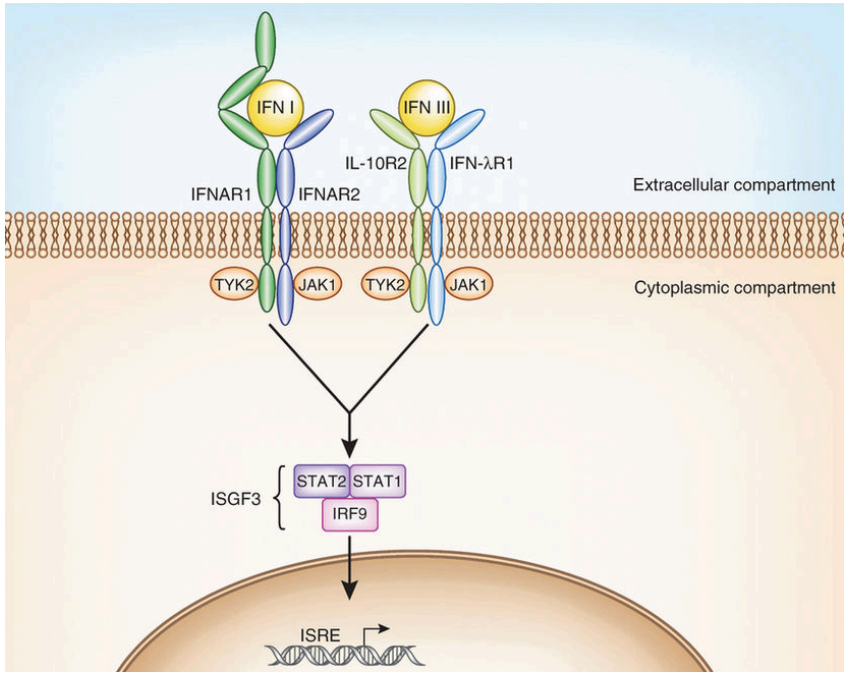
  β….1 gene

  Type II interferon

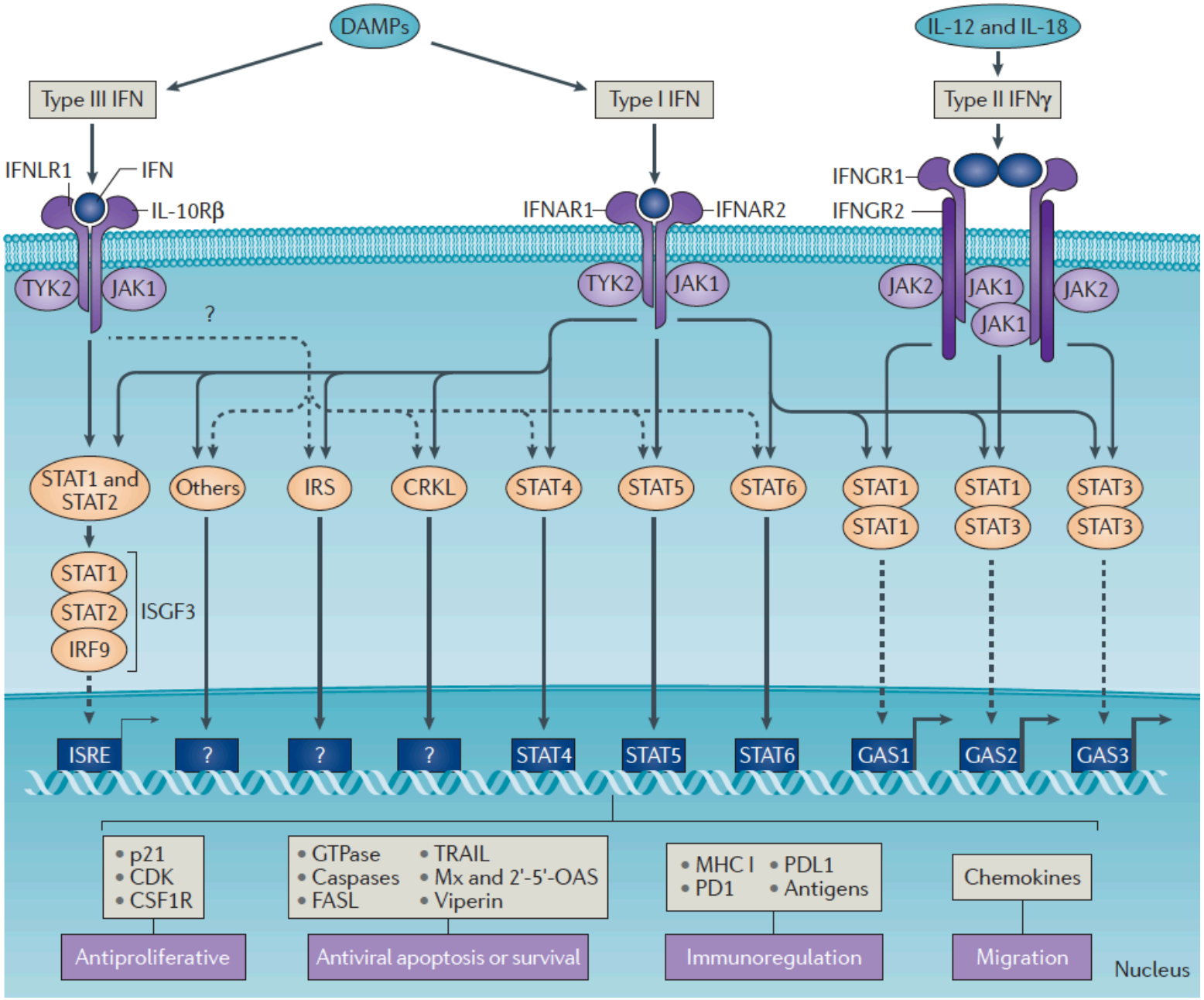
  γ….1 gene

  Type III interferons

  λ…..(IL-28A, IL-28B, IL-29, λ4)



- α and β interferons made by most virus-infected cells, high levels are made by specialized dendritic cells (plasmacytoid dendritic cells)
- γ interferon made by activated Natural Killer cells & T cells
- λ interferons made by myeloid and epithelial cells



Parker et al. Nature Review Cancer 2016

Pattern recognition receptors - cell intrinsic sensors

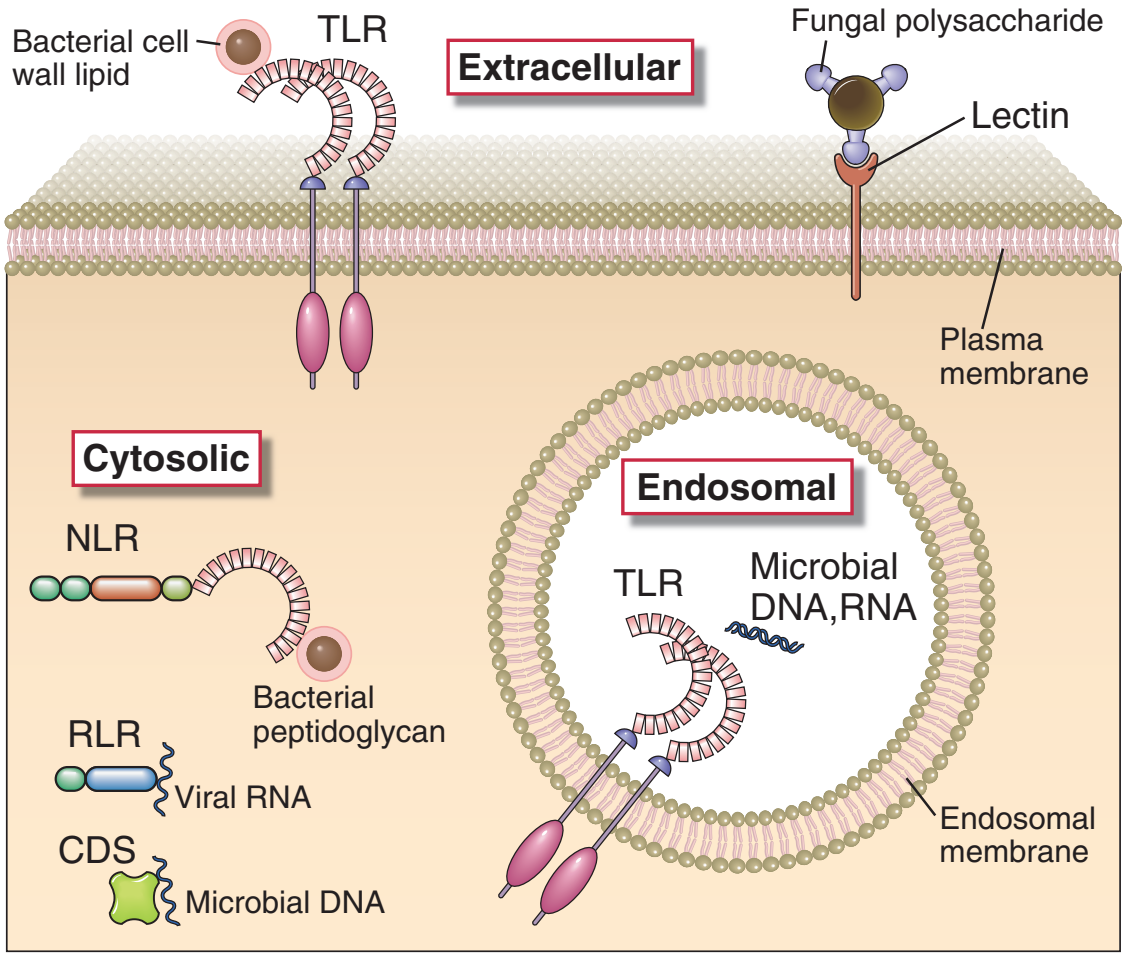
TLR (Toll-like Receptors) - PAMPs extracellular or phagocytosed ligands

CLR (C-type Lectins) – Carbohydrates

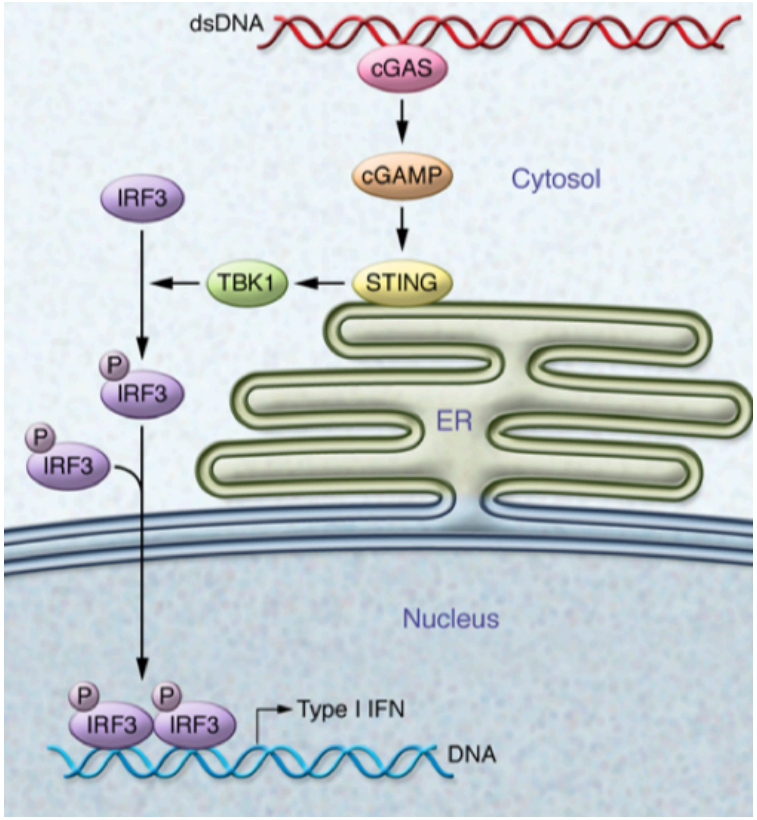
NLR (NOD-like Receptors) – Cytosolic PAMPs & DAMPs

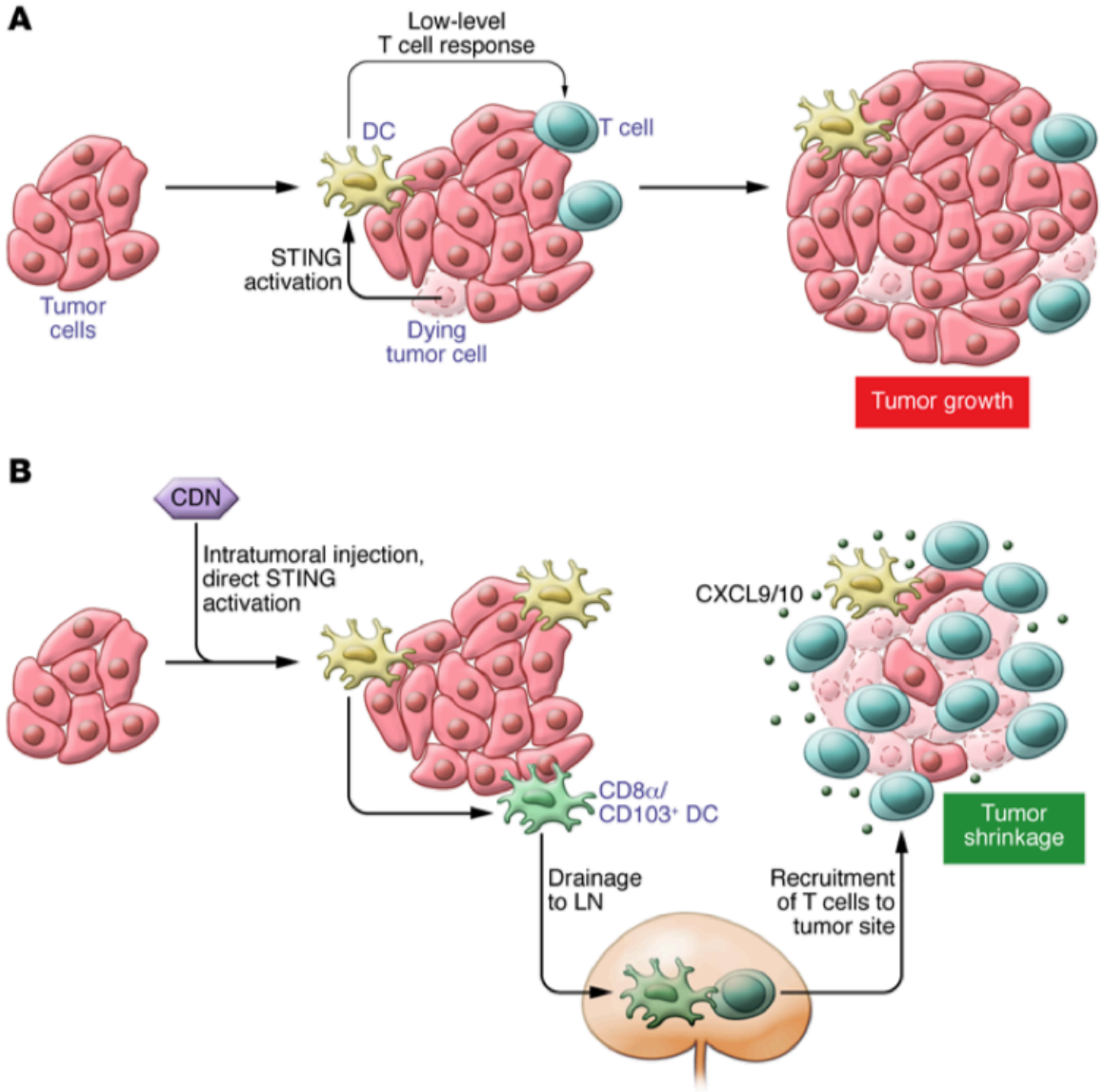
RLR (RIG-I-like Receptors) – Cytosolic Viral RNA

CDS (Cytosolic DNA Sensors) – Cytosolic nucleotides



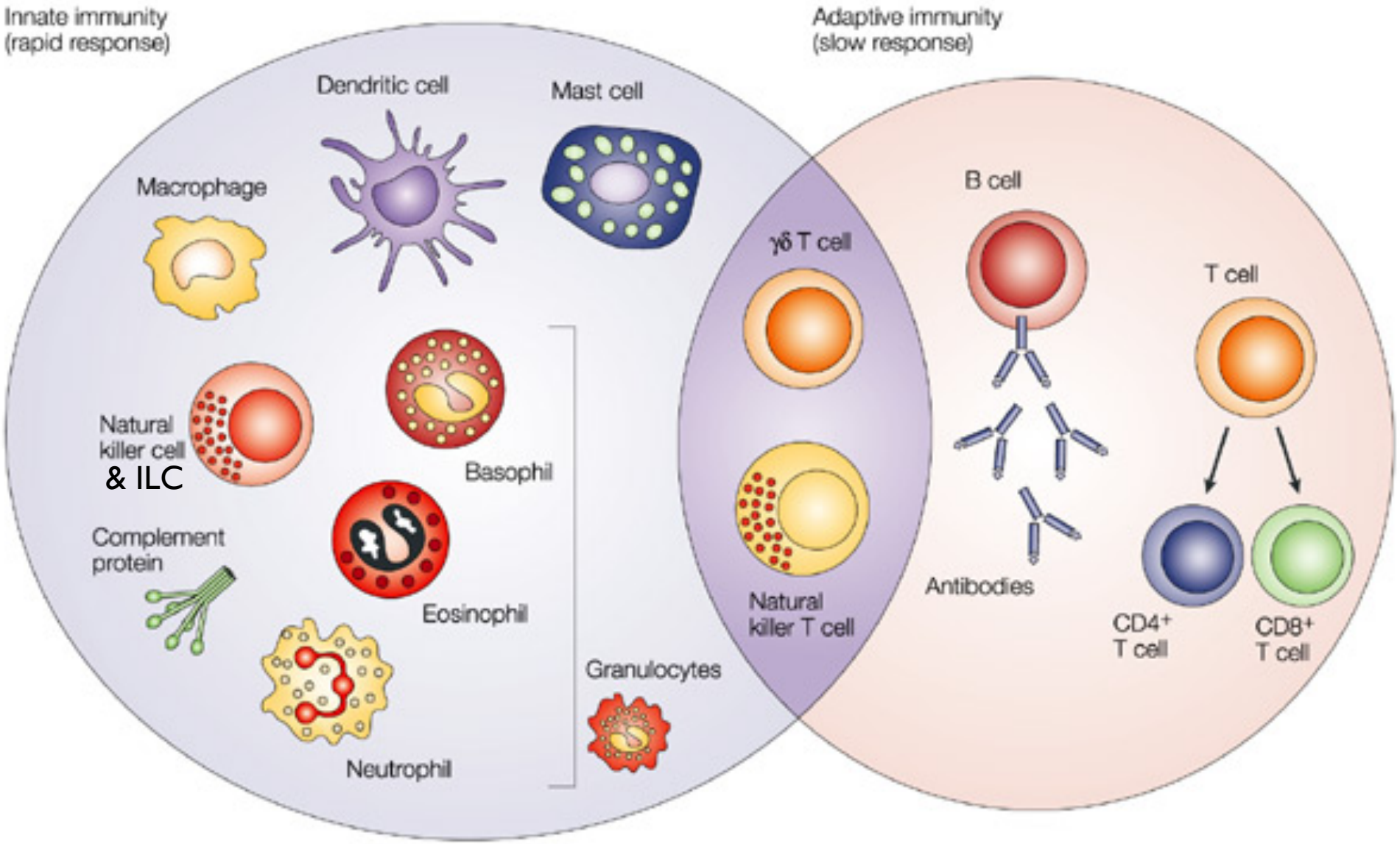
STING Agonist as Cancer Immunotherapeutics





Corrales et al. J. Clin. Invest. 2016

Cells of the Immune System

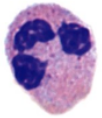


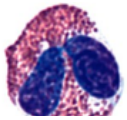
Nature Reviews Immunology April 2009

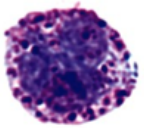
Nature Reviews | Cancer

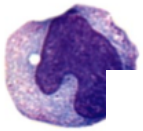
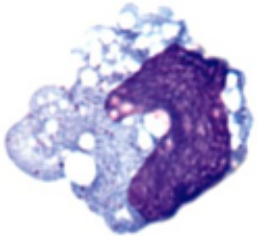
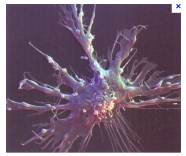
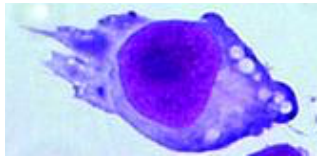
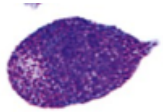
Myeloid Cells

- Granulocytes (Polymorphonuclear leukocytes)

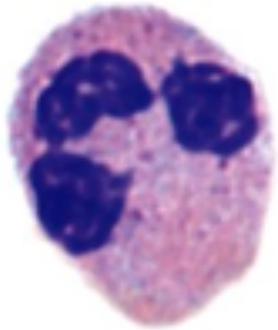
 – Neutrophils – most abundant in blood

 - Eosinophils – less abundant (helmites, bacteria, IL-5, IL-13)

 Basophils – rare (parasites, IgE-allergic responses)

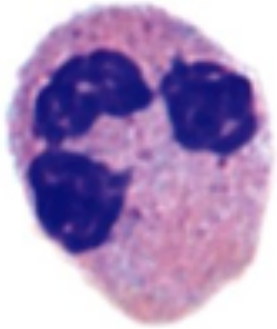
- Monocytes (in blood) 
- Macrophages (in tissues) 
- Dendritic Cells 
- Plasmacytoid dendritic cells (type I IFN) 
- Mast cells (in tissues) 

Innate Immunity - Neutrophils



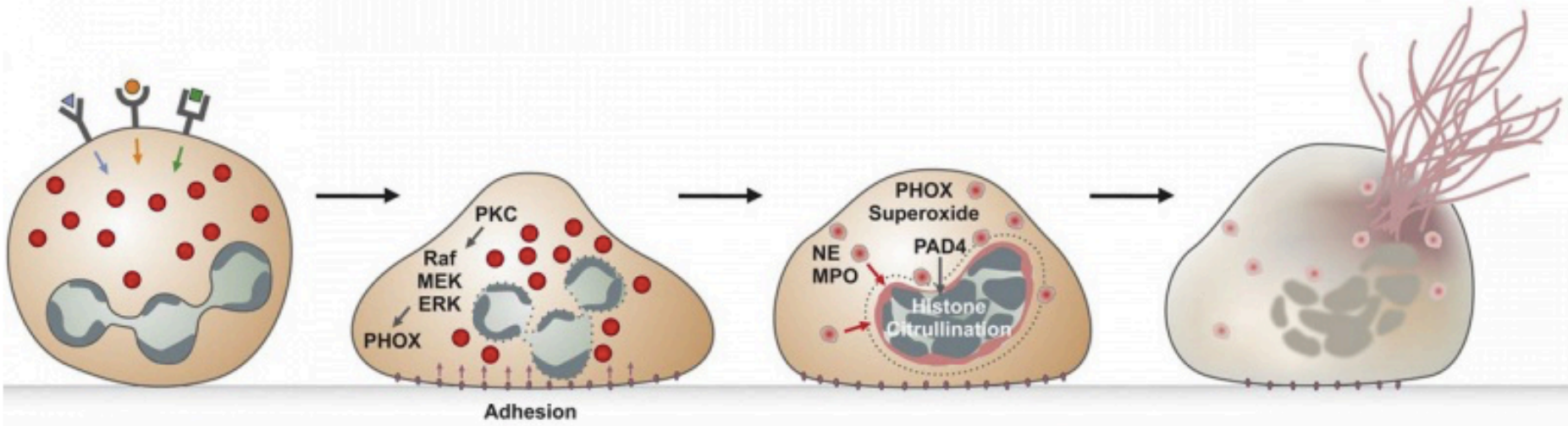
- Bone marrow-derived, short-lived (~ 5 days), rapid response - minutes!
- Increased production during infection - neutrophilia
- Cytoplasmic granules containing degradative enzymes, including lysozyme, collagenase, and elastase
- A type of MDSC (myeloid-derived suppressor cell)

Neutrophils



	Azurophilic granule	Specific granule	Small storage granule
Antimicrobial	Myeloperoxidase Lysozyme Defensins BPI	Lysozyme Lactoferrin Cathelicidin	
Neutral proteinases	Elastase Cathepsin G Proteinase 3	Collagenase Complement	Gelatinase Plasminogen activator
Acid hydrolases	Cathepsin B, D Glucuronidase Mannosidase Phosopholipase A2	Phospholipase A2	Cathepsin B, D Glucuronidase Mannosidase
Receptors		CR3, 4 FMLP Laminin	
Other	Chondroitin sulfate	Cytochrome b558	Cytochrome b558

Neutrophil Extracellular Traps



stimulation of receptors

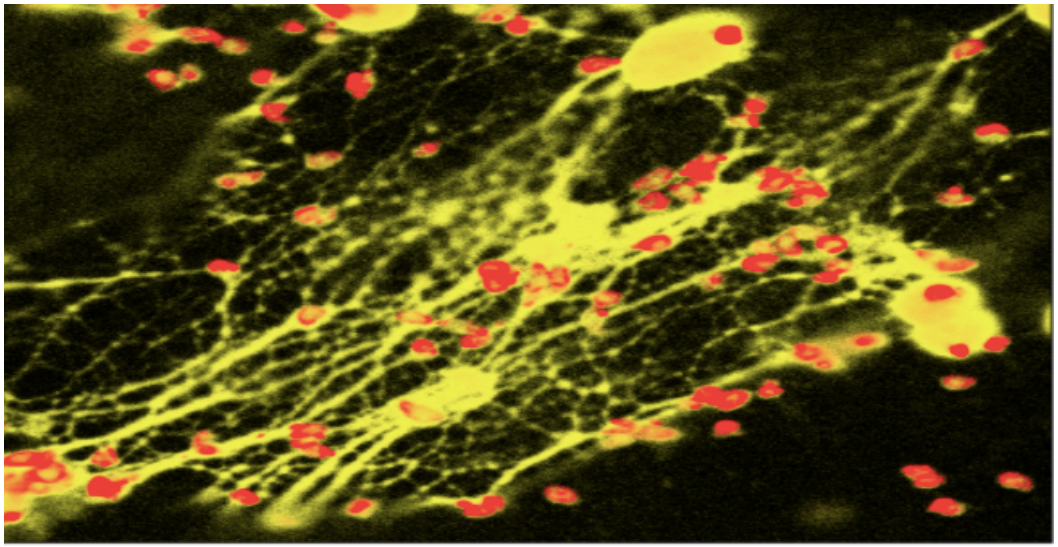
neutrophil adhesion to substrate

mobilize granule components

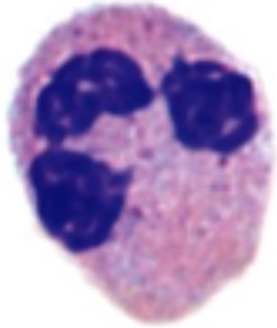
mixture of cytoplasm and nucleoplasm expelled to form NETs

Modified from: Brinkmann, V and Zychlinsky, A., J. Cell Biol.: 2012, 198(5);773-83

PMID: 22945932



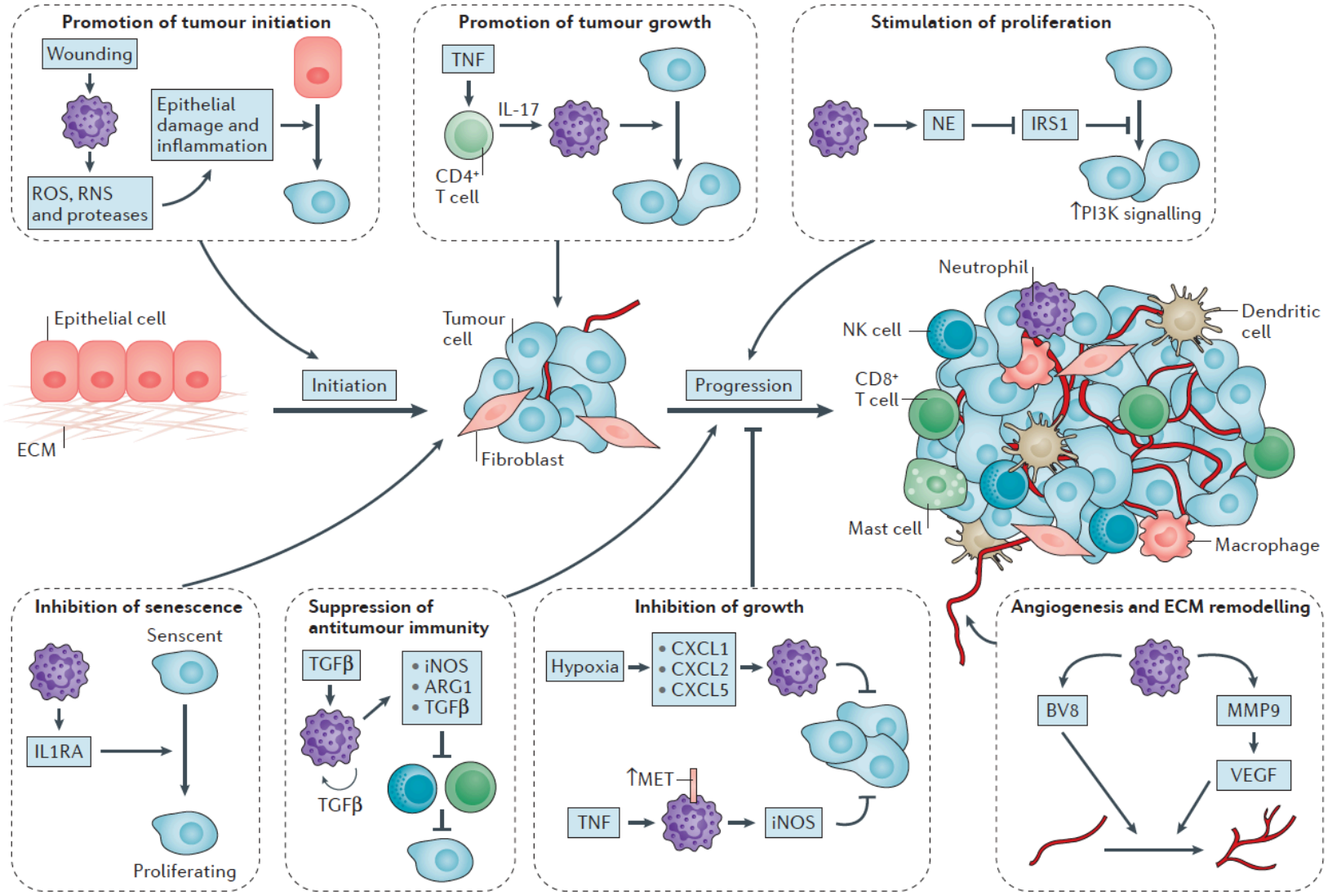
Activated neutrophils secrete soluble mediators



- CXCL8 (IL-8) - neutrophil adhesion and chemotaxis
- CCL3 (MIP1α)-chemotactic for many cells
- CXCL1 (GROα)- chemotactic for many cells
- CCL2 (MCP1)- chemotactic for many cells
- Defensins – directly kill bacteria

Neutrophils use chemokines to recruit monocytes, neutrophils, and initiate healing

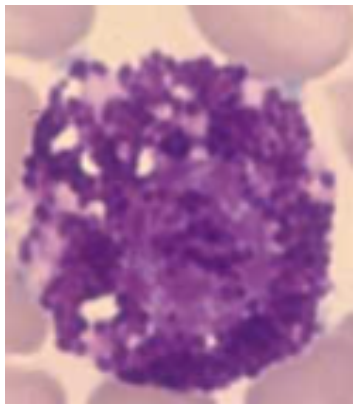
Role of Neutrophils in Cancer - Complex



Neutrophils = MDSC (myeloid-derived suppressor cells)

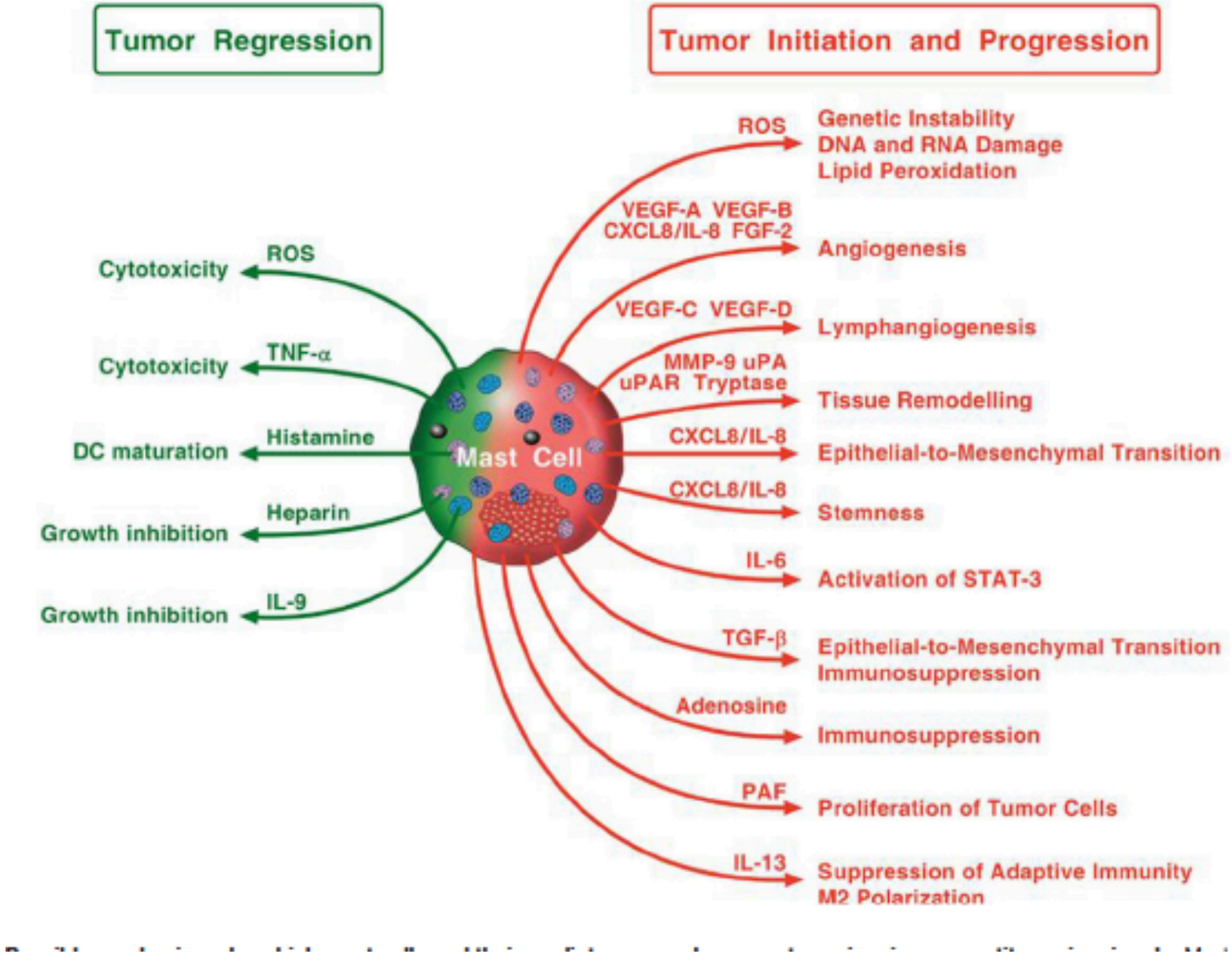
Coffelt et al. Nature Reviews Cancer 2016

Innate Immunity – Mast Cells



- Two types – connective tissue & mucosal
- Involved in defense against parasites and allergic reactions
- Connective tissue mast cell granules contain histamine, tryptase, chymase cathepsin G
- Mucosal mast cell granules contain chondroitin sulfate, tryptase, neutral protease
- Secrete IL-4, IL-13, leukotrienes
- Express high affinity receptors for IgE that can trigger release of histamine and other mediators

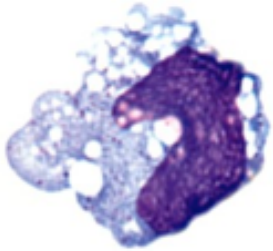
Role of Mast Cells in Cancer - Complex



Varricchi et al. Frontiers Immunology 2017

Innate Immunity - Monocytes and Macrophages

- Monocytes - bone-marrow derived, circulating mononuclear cells with kidney-shaped nucleus and abundant cytoplasm



- Tissue-resident macrophages
  - Kuppfer cells in liver, microglial cells in brain, alveolar macrophages in lung, Langerhans cells in skin (some seed organs during fetal life, others replenished in adults from bone marrow)

How do myeloid cells detect pathogens?

Toll-like receptors (TLR)
Wednesday Helen Goodridge
Innate Immunity: Introduction to Pattern Recognition and Intracellular Signaling

Glucan (lectin) receptors
(bind sugars)

Complement receptors
(if pathogen is coated with C')

Fc receptors
(if pathogen is coated with antibody)

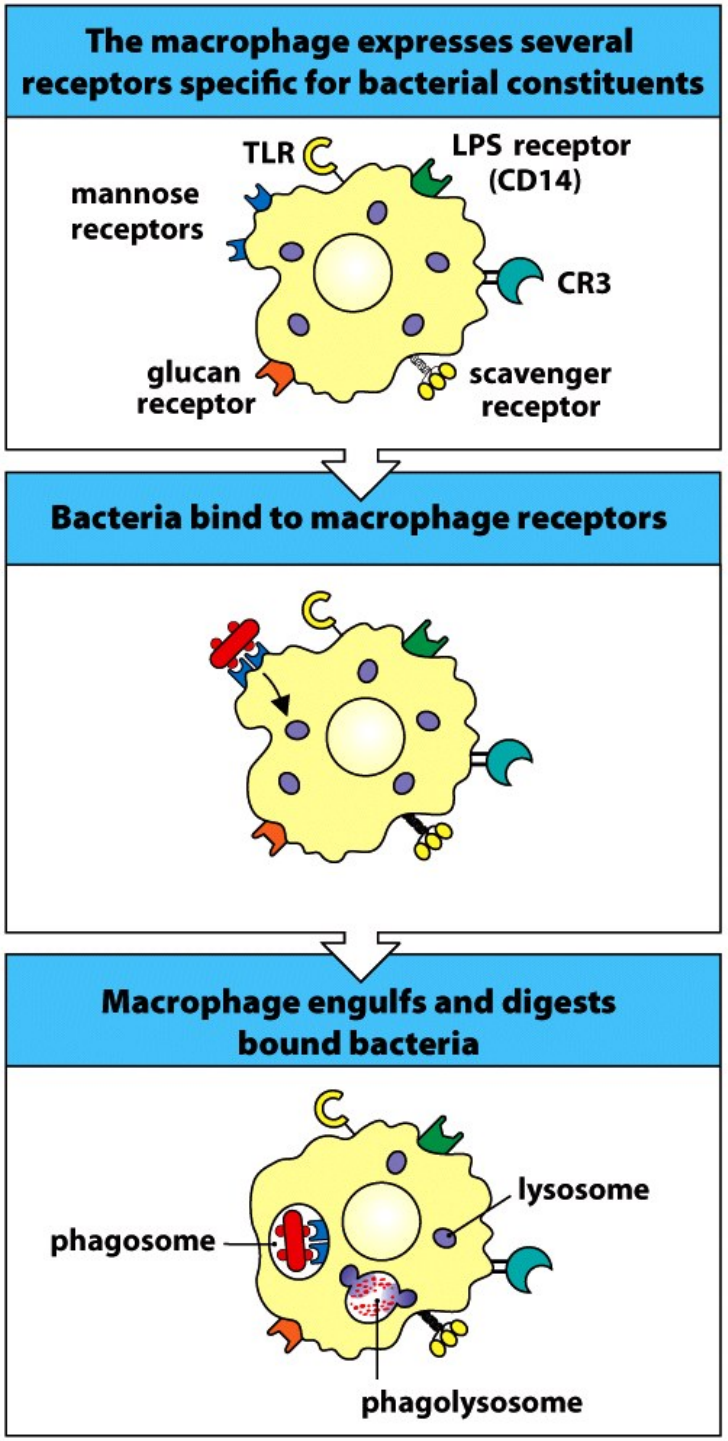
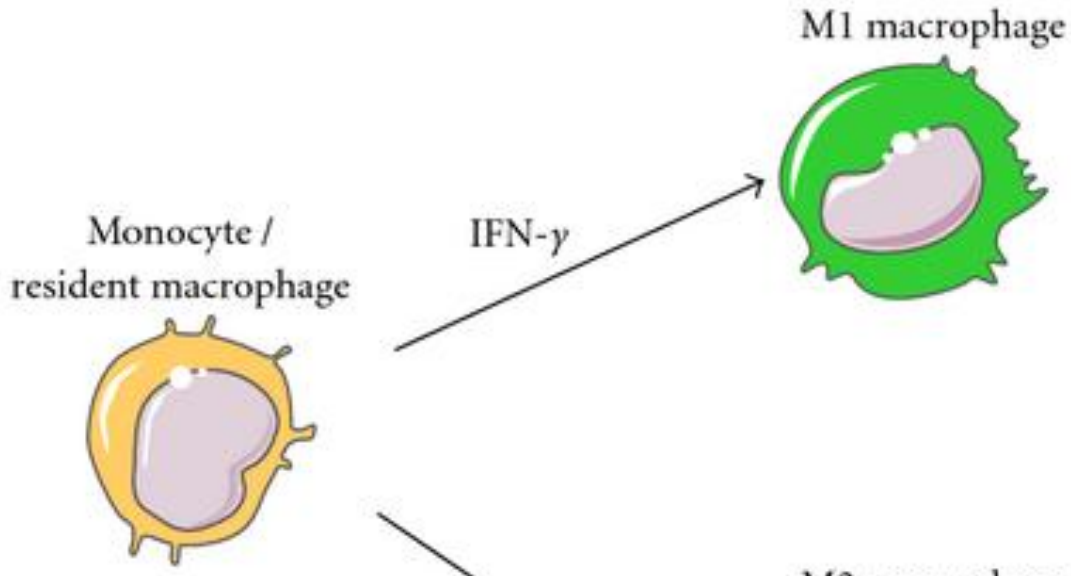


Figure 2.19 The Immune System, 3ed. (© Garland Science 2009)

Monocytes and Macrophages

- Recruited to sites of inflammation or cancer by complement and chemokines
- Ingest and degrade opsonized microbes
- Secrete 'pro-inflammatory' cytokines (including IL-12, TNF, IL-1, IL-6, and IL-15) and wound-repairing cytokines
- MDSC (Myeloid-derived suppressor cells)- inhibit T cell responses in tumors

Flavors of Macrophages
"M1" – "M2" – a vast oversimplification!



Pro-inflammatory
Resolve infection

TNF-α
IL-12
Reactive nitrogen
Oxygen intermediates

Promote Th1 response

Tissue Repair
Angiogenesis

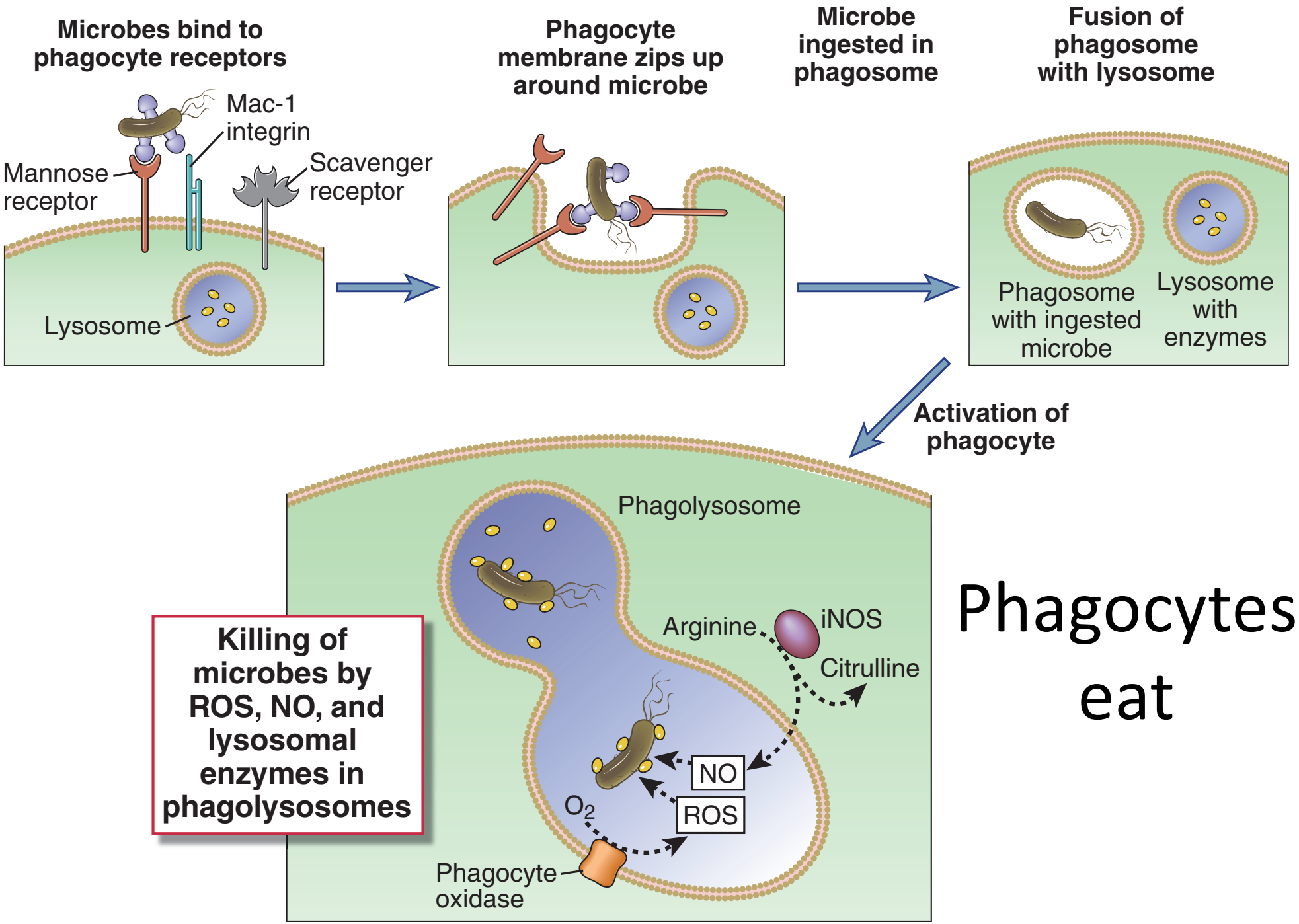
IL-1β
IL-6
IL-8
Growth factors (VEGF)
MMP

Arginase

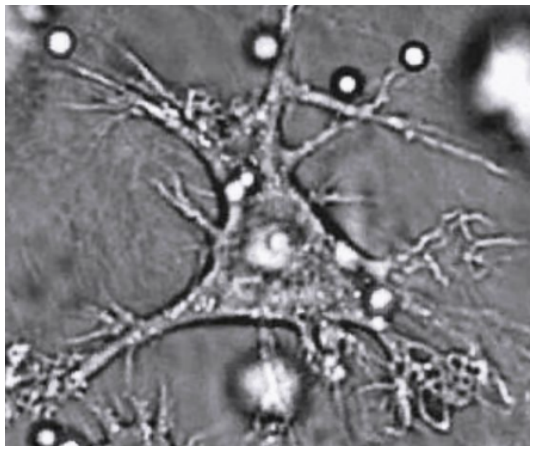
Promote Th2 response

Myeloid-derived Suppressor Cells (MDSC) suppress T cells responses – promote tumor growth?

Lavin et al. Nat Rev Immunol



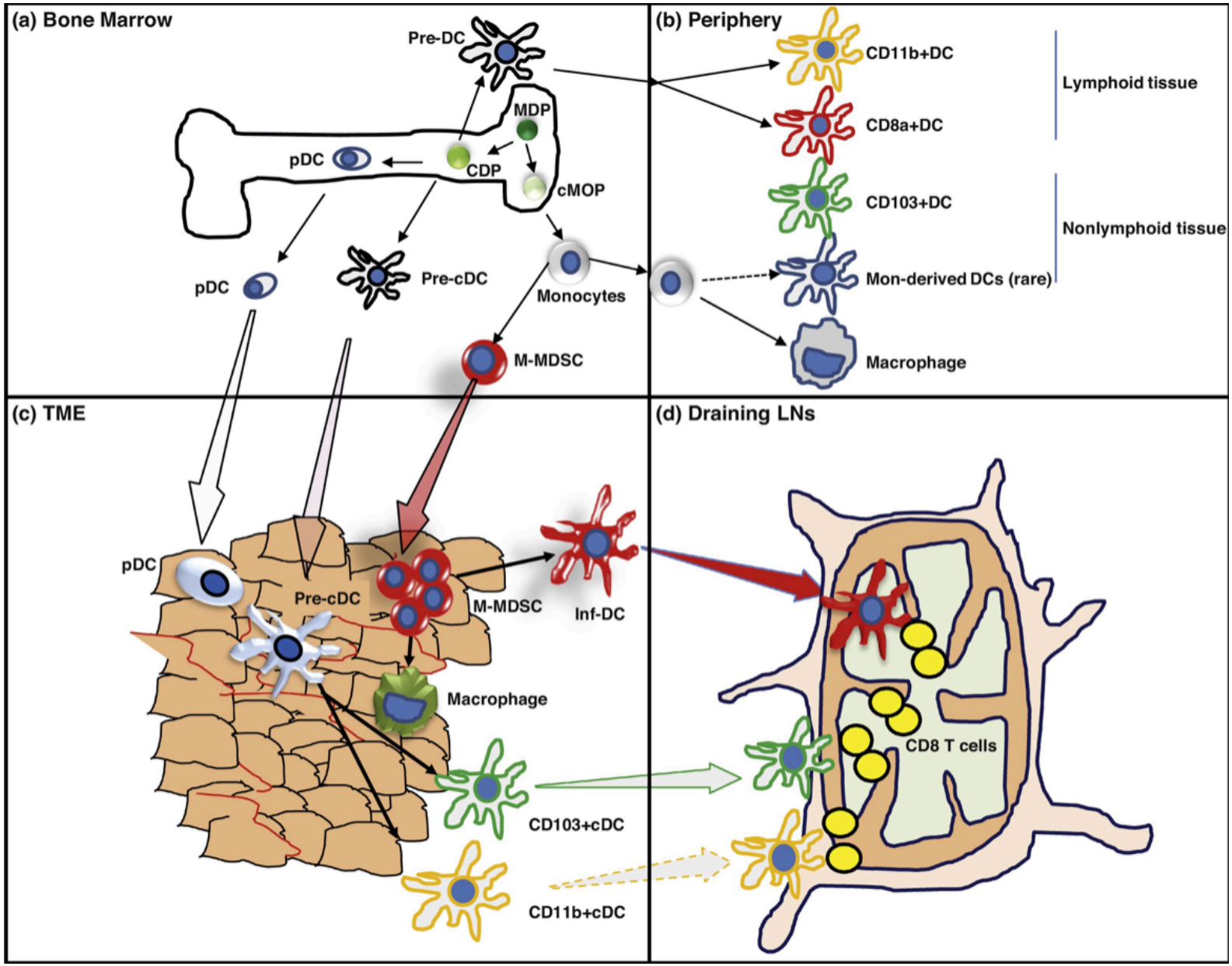
Phagocytes eat



Innate Immunity - Dendritic Cells

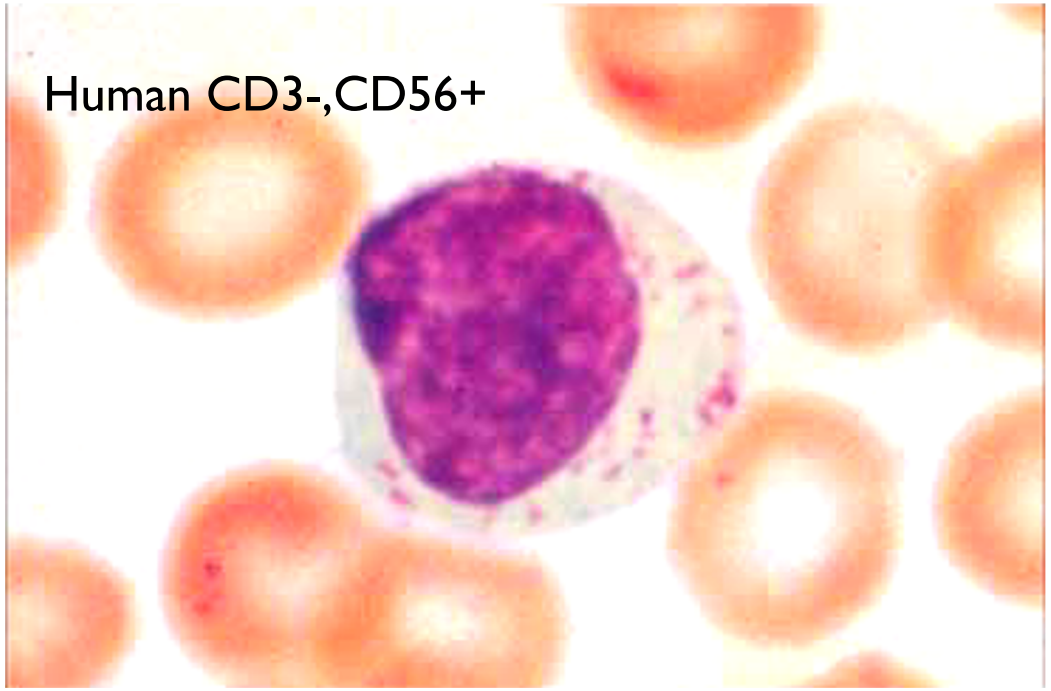
- In mucosal tissues and skin, immature DC efficiently capture antigens
- Pro-inflammatory cytokines induces their maturation, up-regulation of MHC class II, and co-stimulatory molecules, and migration to lymph nodes
- Considered the most potent 'antigen-presenting cells' in the body – activate T cells

Dendritic cell subsets in cancer immunity



Veglia & Gabrilovich Curr Op Immunol 2017

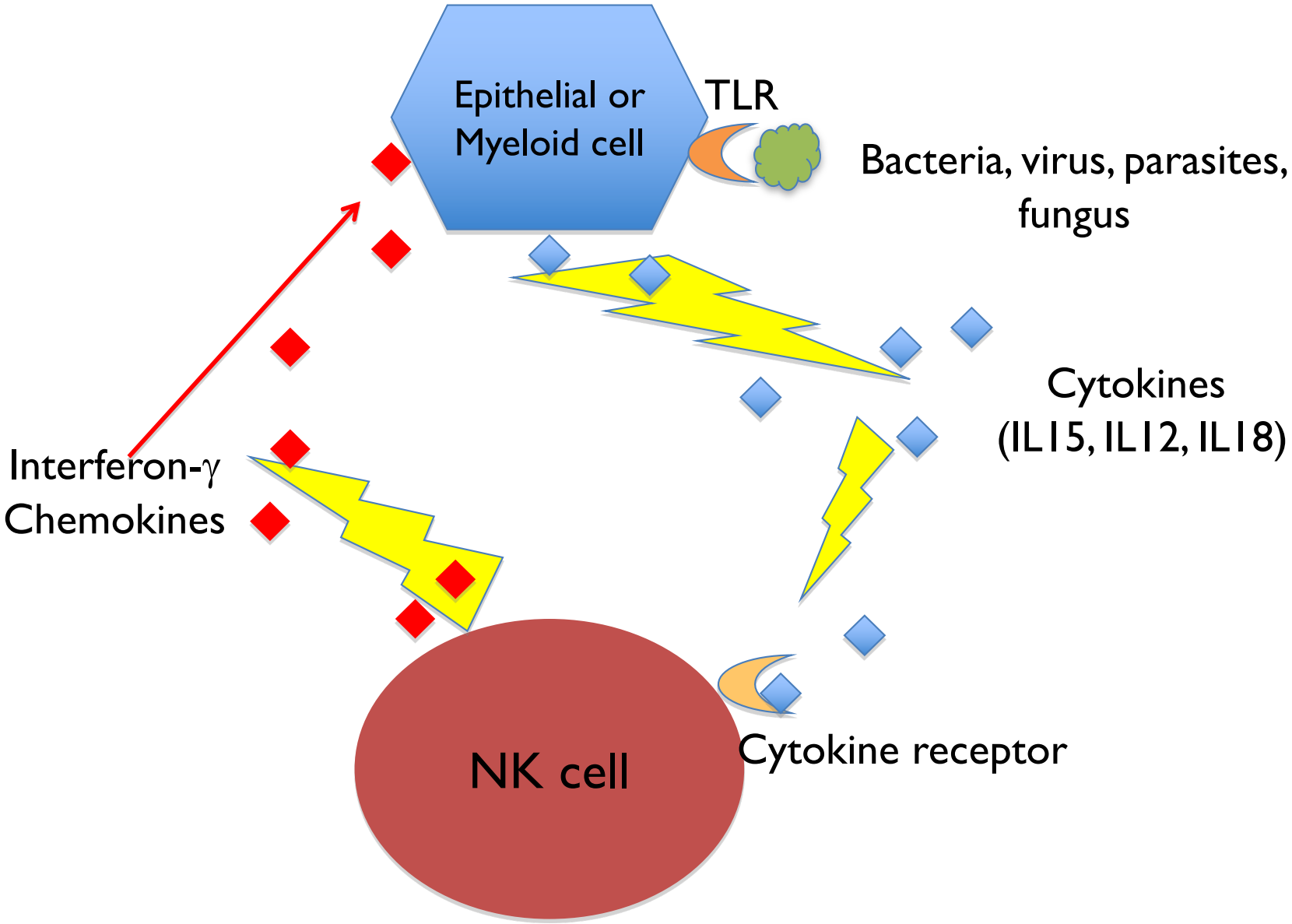
Natural Killer cells



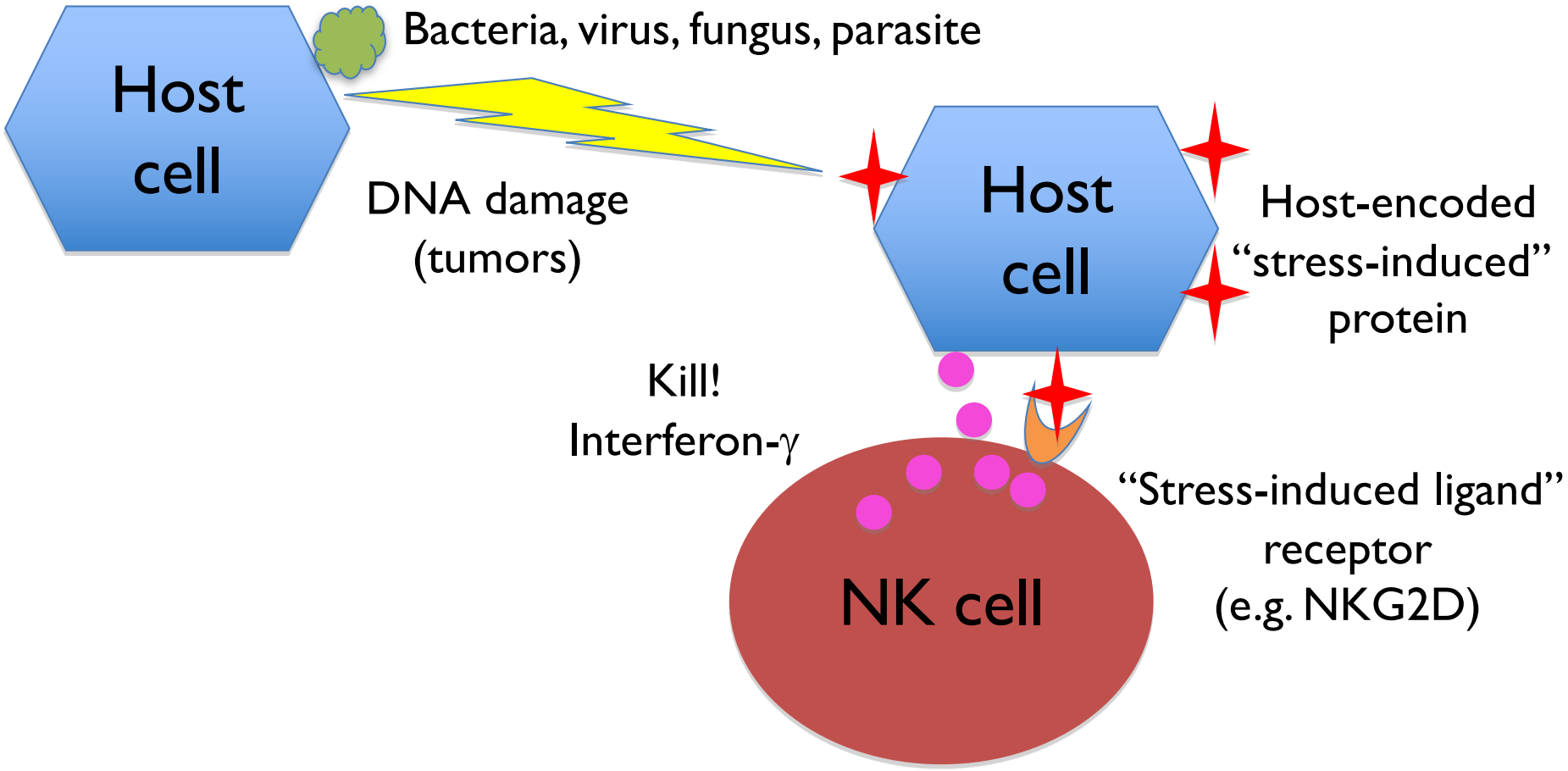
- Innate lymphocytes
- ~10-20% of blood lymphocytes
- Function in innate immunity to protect against viruses, bacteria, fungus, and tumors
- Produce cytokines & directly kill abnormal cells

How are NK cells activated by tumors and pathogens?

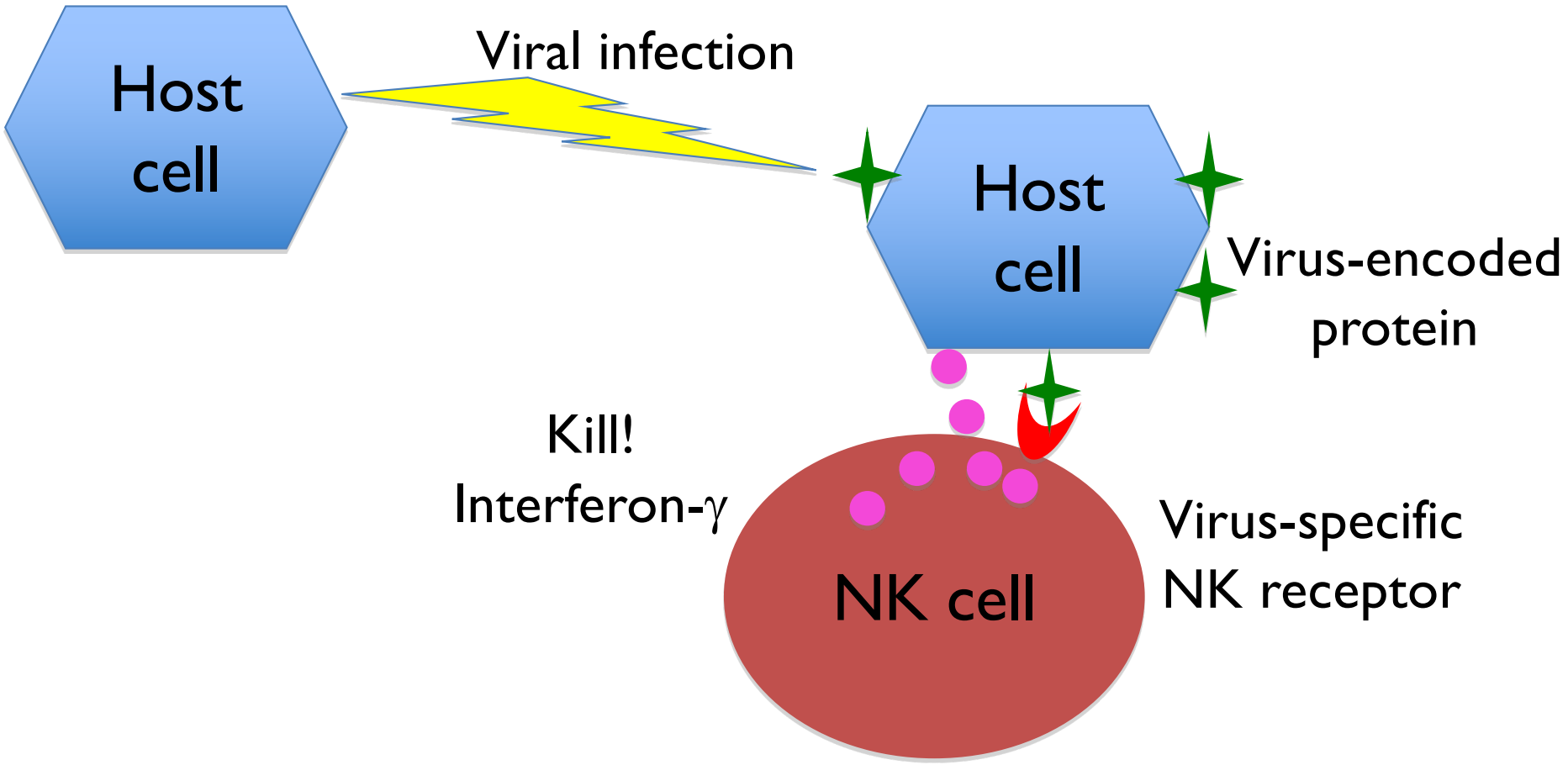
Cytokines produced by infected epithelial or myeloid cells activate NK cells to secrete Interferon-γ and chemokines



"Stressed" cells – upregulate host-encoded ligands for activating NK receptors



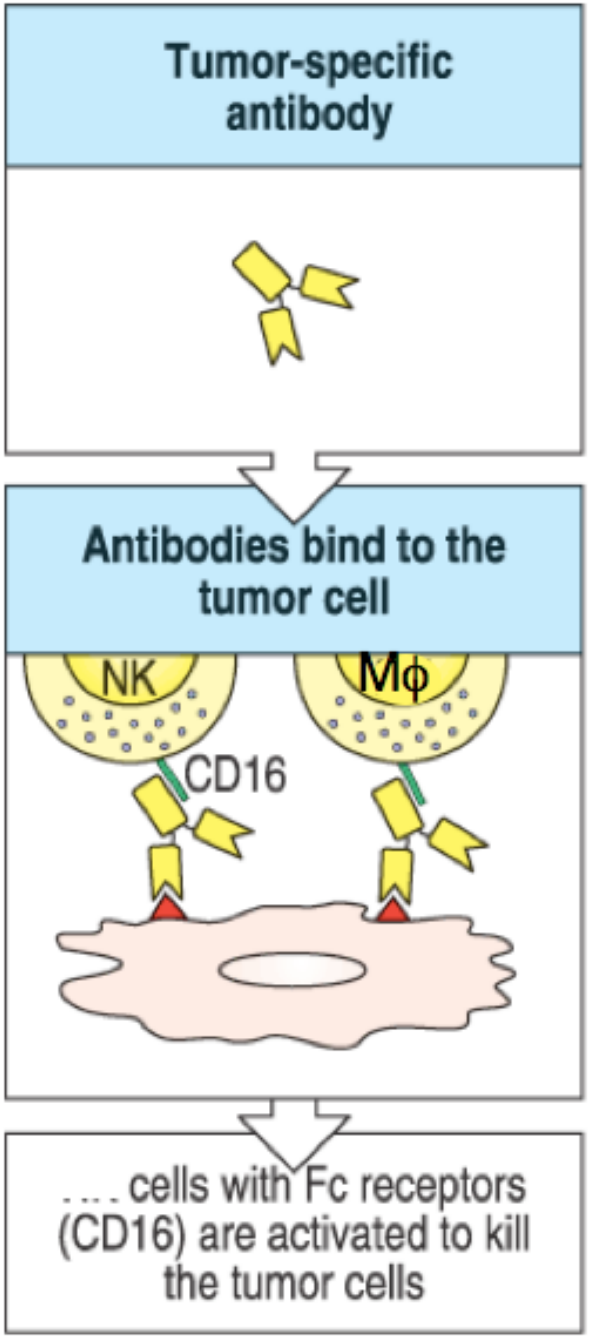
Infected cells express virus-encoded ligands for activating NK receptors



Antibody-dependent cellular cytotoxicity

NK cells and macrophages kill tumors or virus-infected cells coated with antibodies

Because they express activating receptors for the Fc region of antibodies



Immune regulatory role of NK cells

*Kill cells that are proliferating too much

*Kill T cells causing autoimmunity

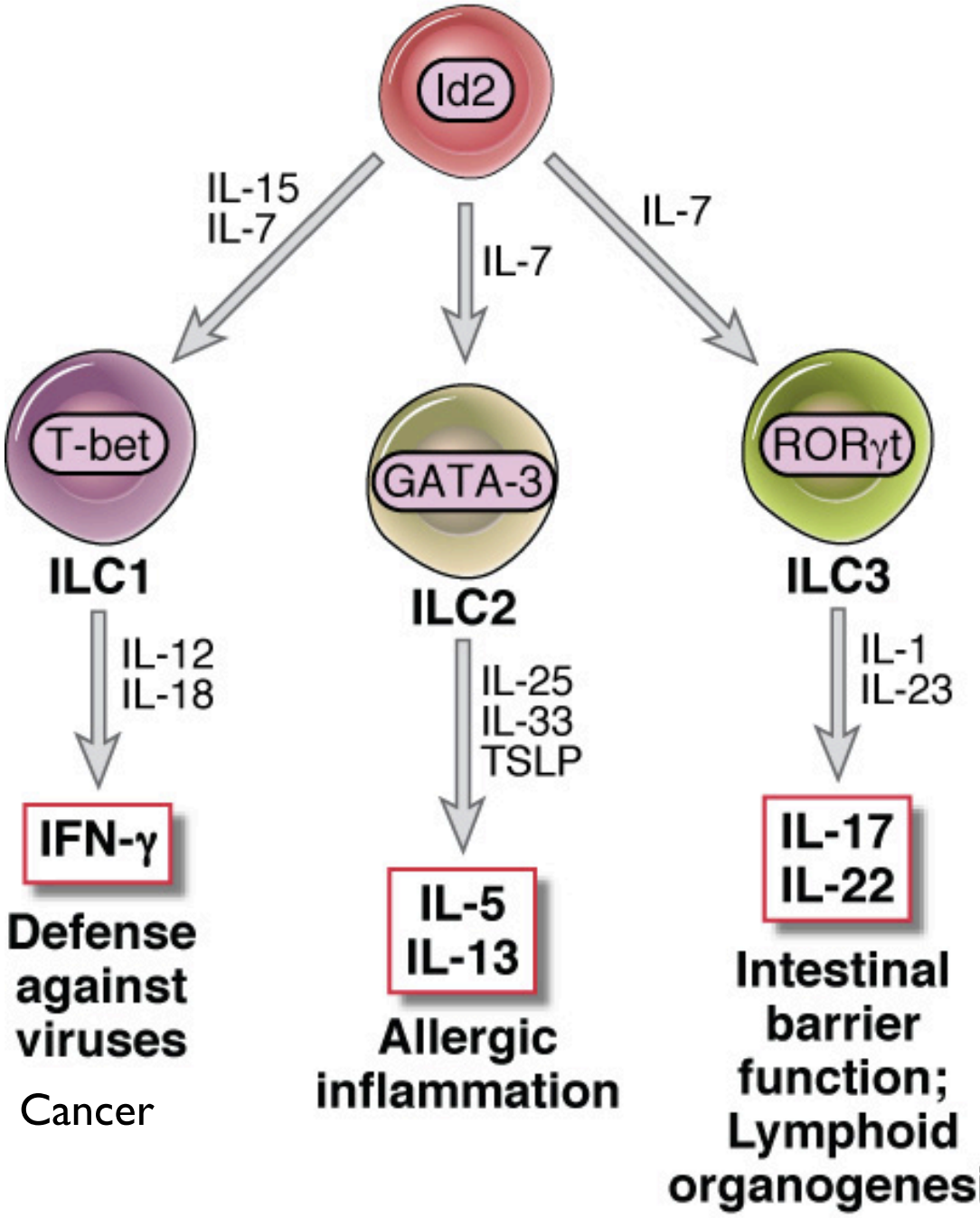
*Secrete pro-inflammatory cytokine – IFNγ

*Secrete suppressive cytokines – IL-10

Innate Lymphoid Cells (ILC)

present in mucosal tissues to regulate immune responses by secreting cytokines

Innate lymphoid cells

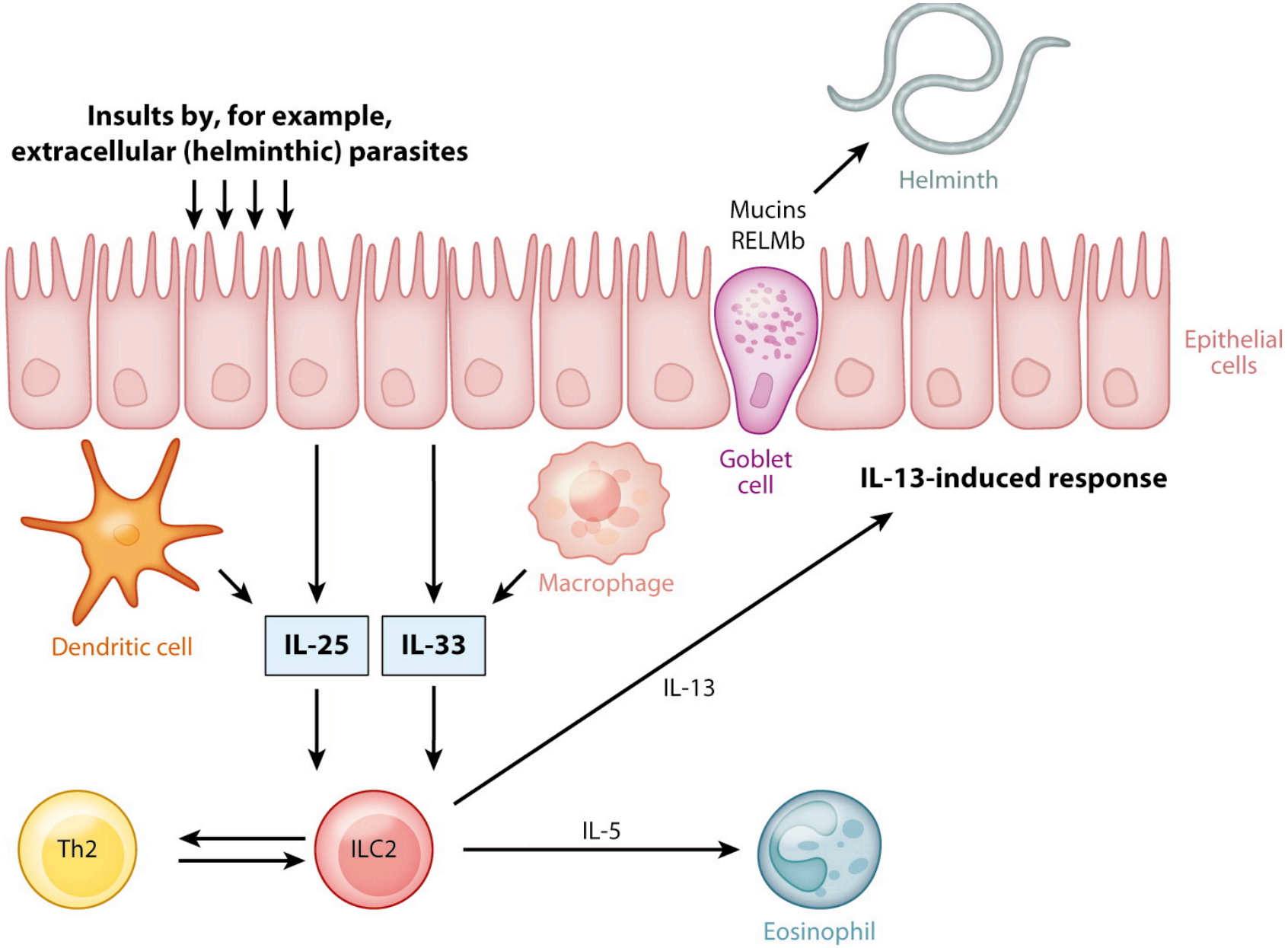


ILCs make many of the same cytokines as T cells but lack TCRs

May contribute to early cytokine responses in host defense and inflammatory diseases

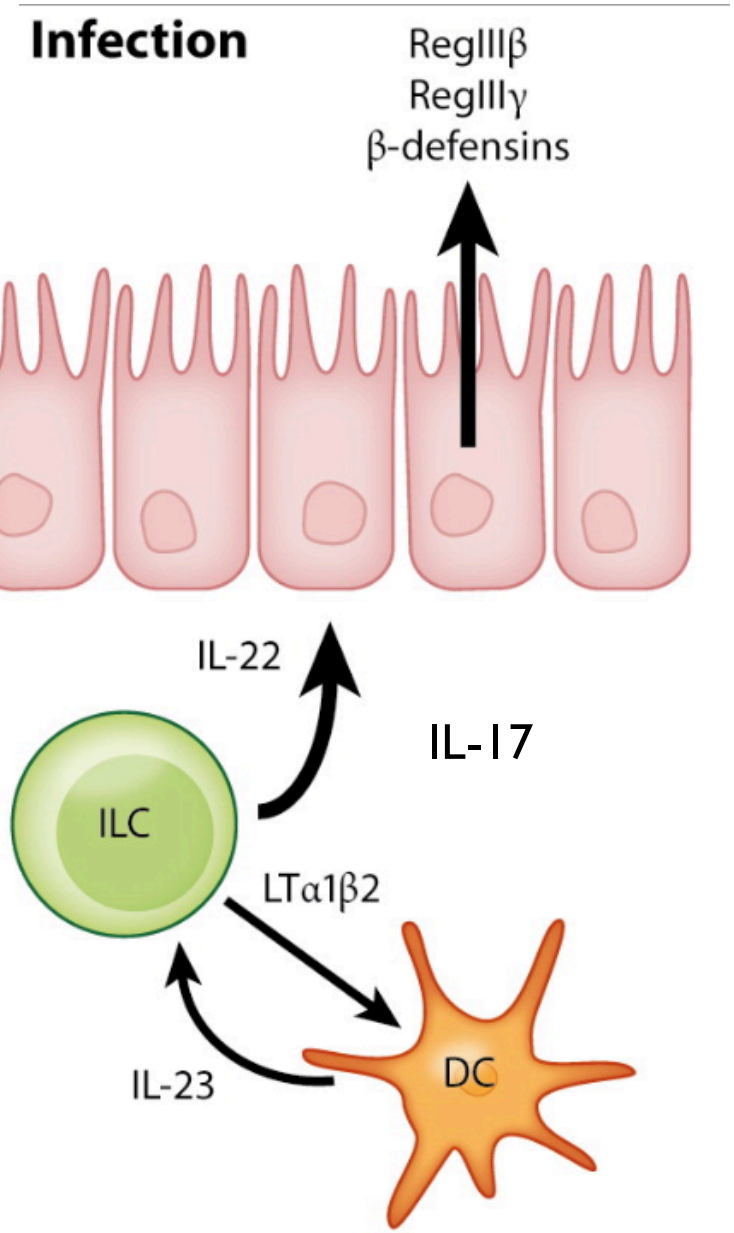
A. Abbas

ILC2 –protect against parasites and help wound repair



Spits & Cupedo Ann Rev Imm 2012

ILC3 –protect against extracellular bacteria and help wound repair



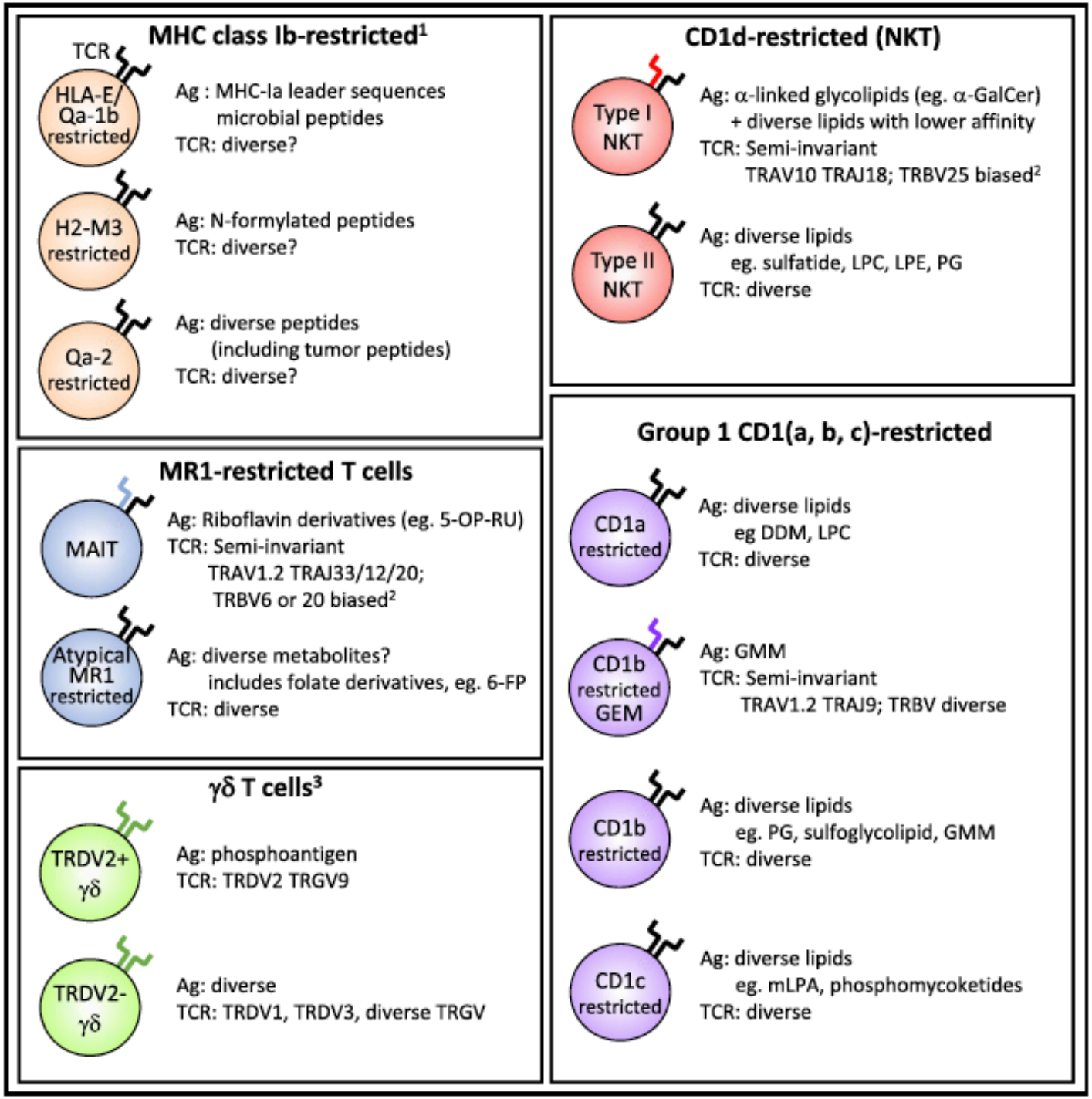
Spits & Cupedo Ann Rev Imm 2012

Some T cells behave like innate immune cells

*Express rather conserved T cell receptors

*Present in higher frequency that most "conventional" T cells prior to antigen encounters

*May possess germline-encoded "NK" receptors in common with innate lymphocytes



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