# Innate Immunity #2

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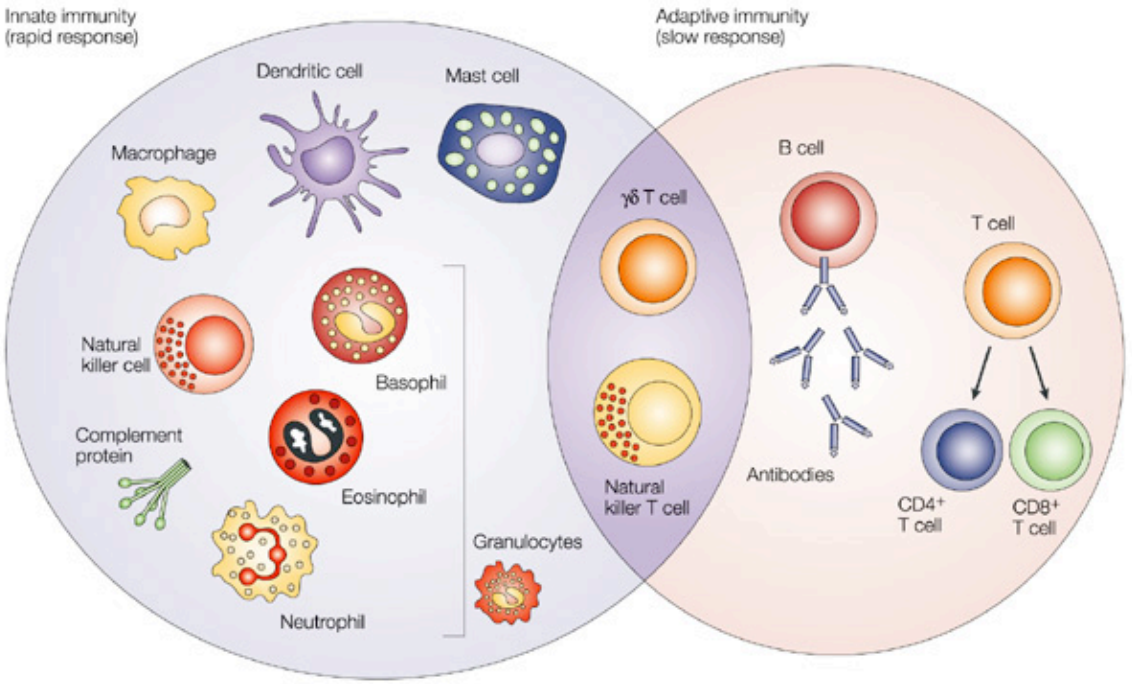
- Cells of the innate immune system
- Neutrophil functions
- Macrophage functions
- Dendritic cell functions
- NK cells/ILCs
- Basophils and eosinophils

# Cellular effectors of innate immunity

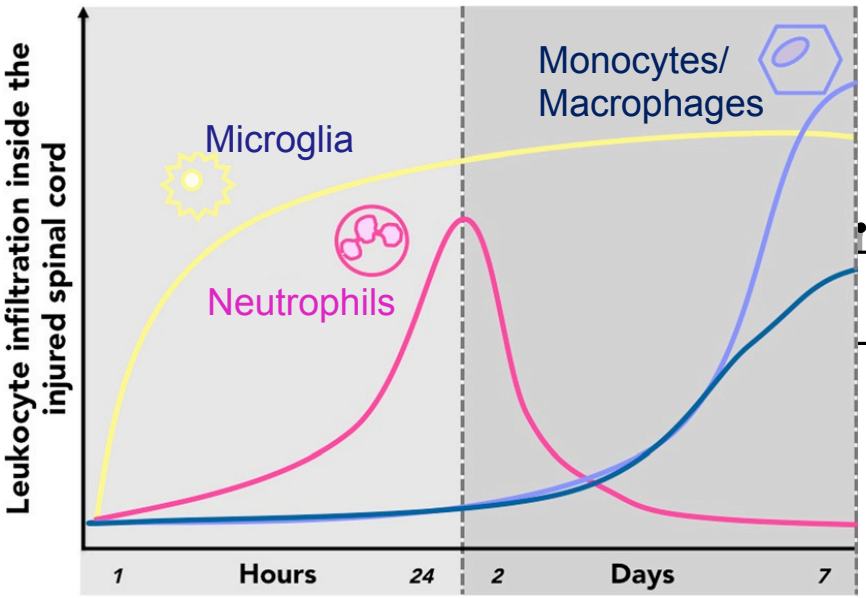


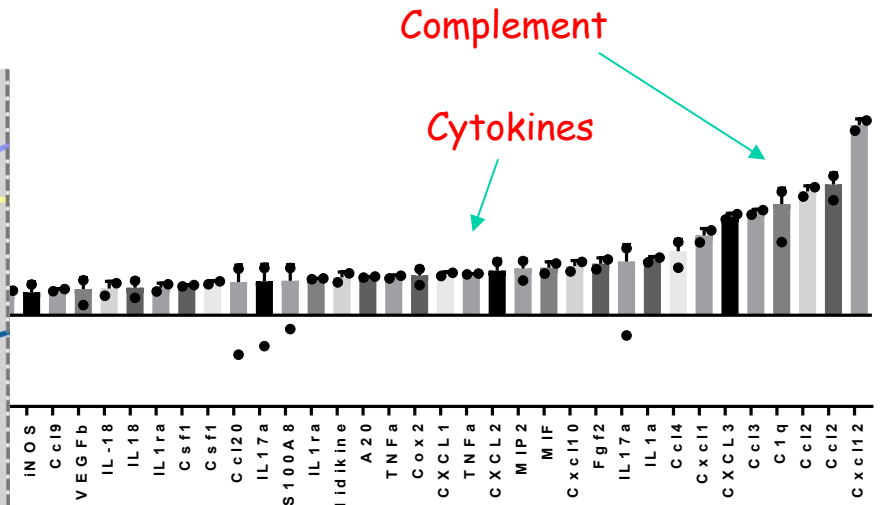

# Neutrophils

- Acute inflammation: first neutrophils; later monocytes.
- Controlled by pattern of chemokines are released by tissue macrophages and endothelial cells, in response to DAMPs.

# Waves of cells/cytokines during inflammation

## 24 hours post-Spinal Cord Injury

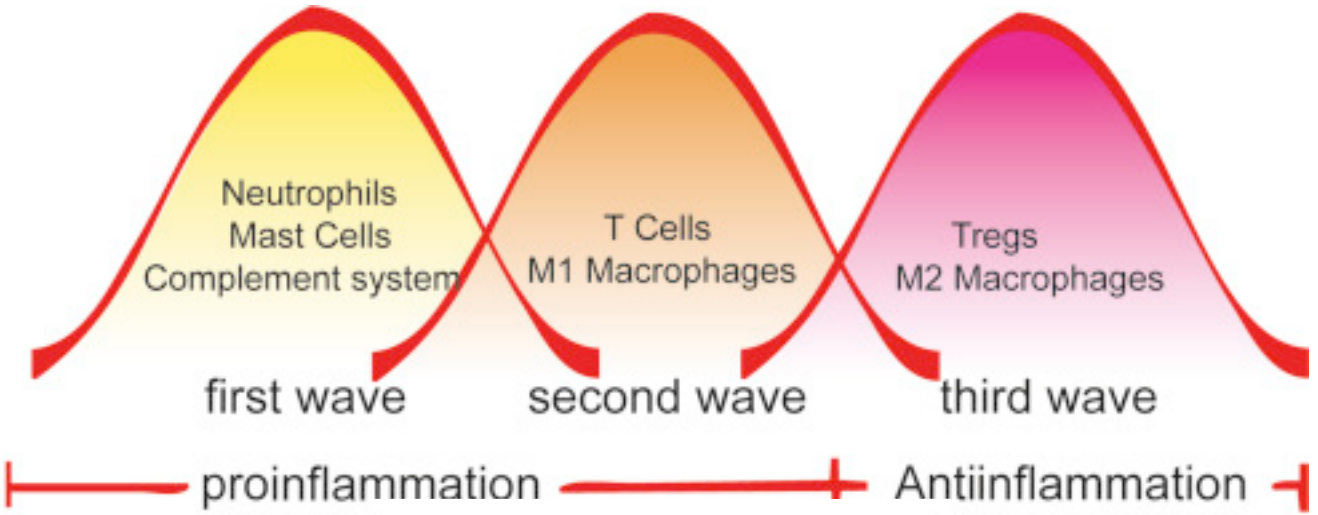


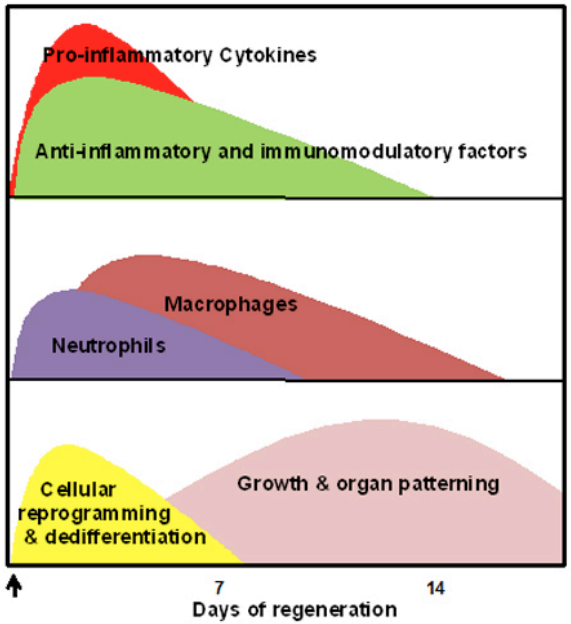

*Many monocyte and leukocyte chemokines are upregulated in neutrophils @ 24 hrs post-SCI

Dylan McCreedy (Lowell Lab), *unpublished*

# Waves of cells/cytokines during inflammation




- Neutrophils (other granulocyte types) first
- Monocytes/ macrophages follow
- Chemokine driven




Yang et al, J Ortho Trans 13:25 (2018) - muscle regeneration models….

# Neutrophils – lots of new functions in immunity

Classic roles

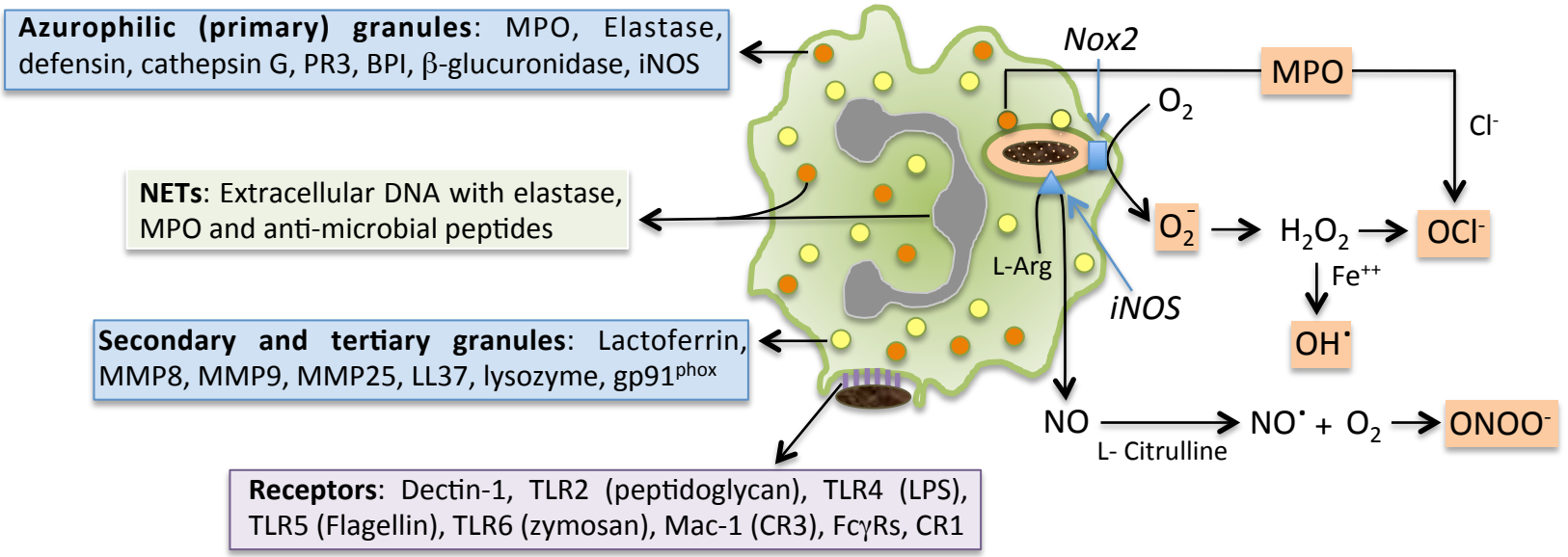
- Host defense in bacterial/fungal infections
- Mediator of tissue injury – only acute inflammation
- Short half-life, work like bombs, no interaction with other cells

New roles

- Producer of cytokines that influence adaptive cells
- Involved in chronic inflammation
- Involved in response to cancer
- Involved in auto-immunity (antigen source)
- Involved in allergic diseases

Kubes, Cell Tissue Res 371:399 (2018)

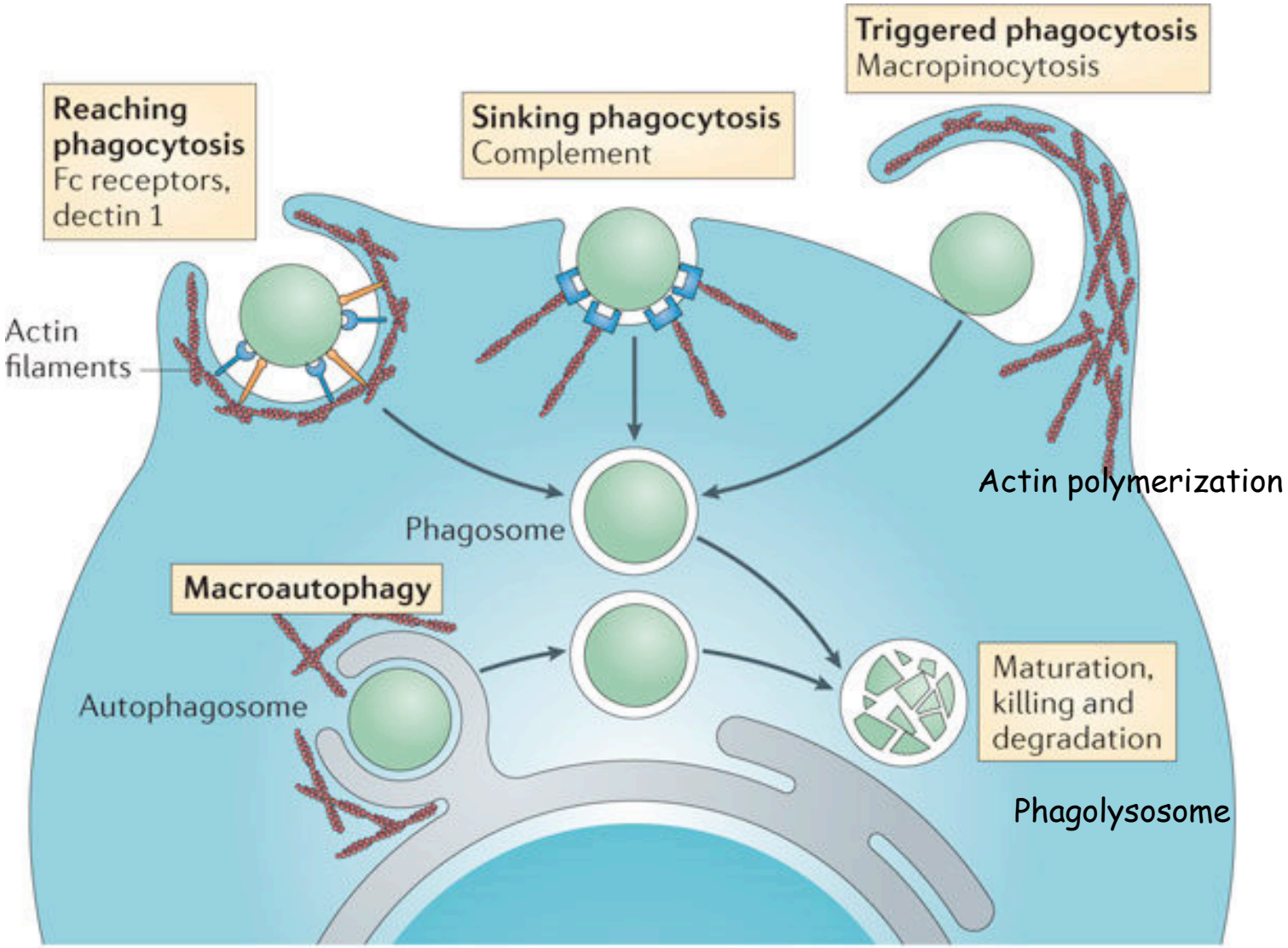
# Classical neutrophil effector functions: Phagocytosis and killing




Anti-microbial arsenal of neutrophils

Mayadas, Cullere and Lowell, Ann Rev Pathology, 9:181 (2014)

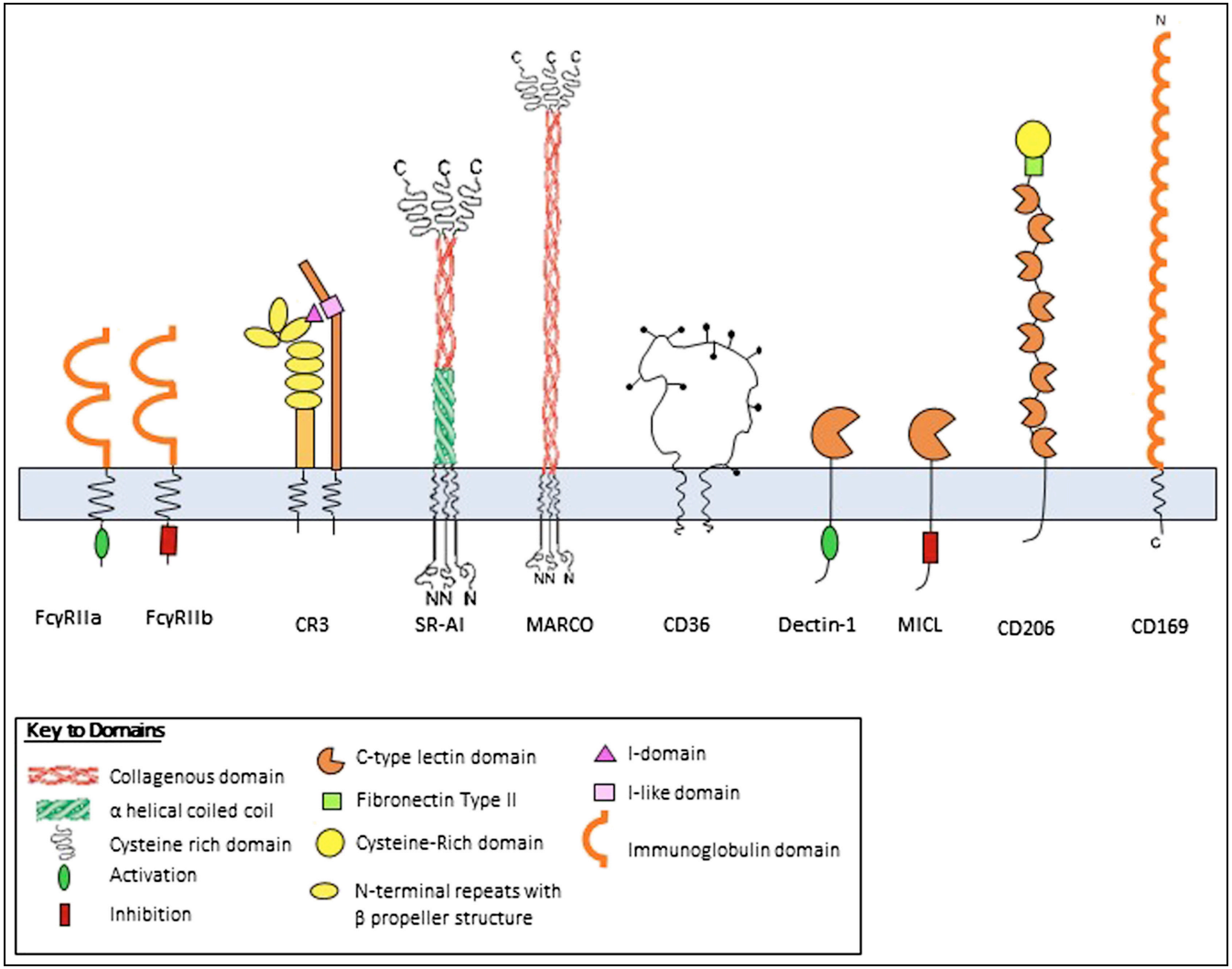
# Neutrophil phagocytic mechanisms




Underhill and Goodridge, Nat Rev Immunol, 12:492 (2012)

# Phagocytic receptors (neutrophils and macrophages)

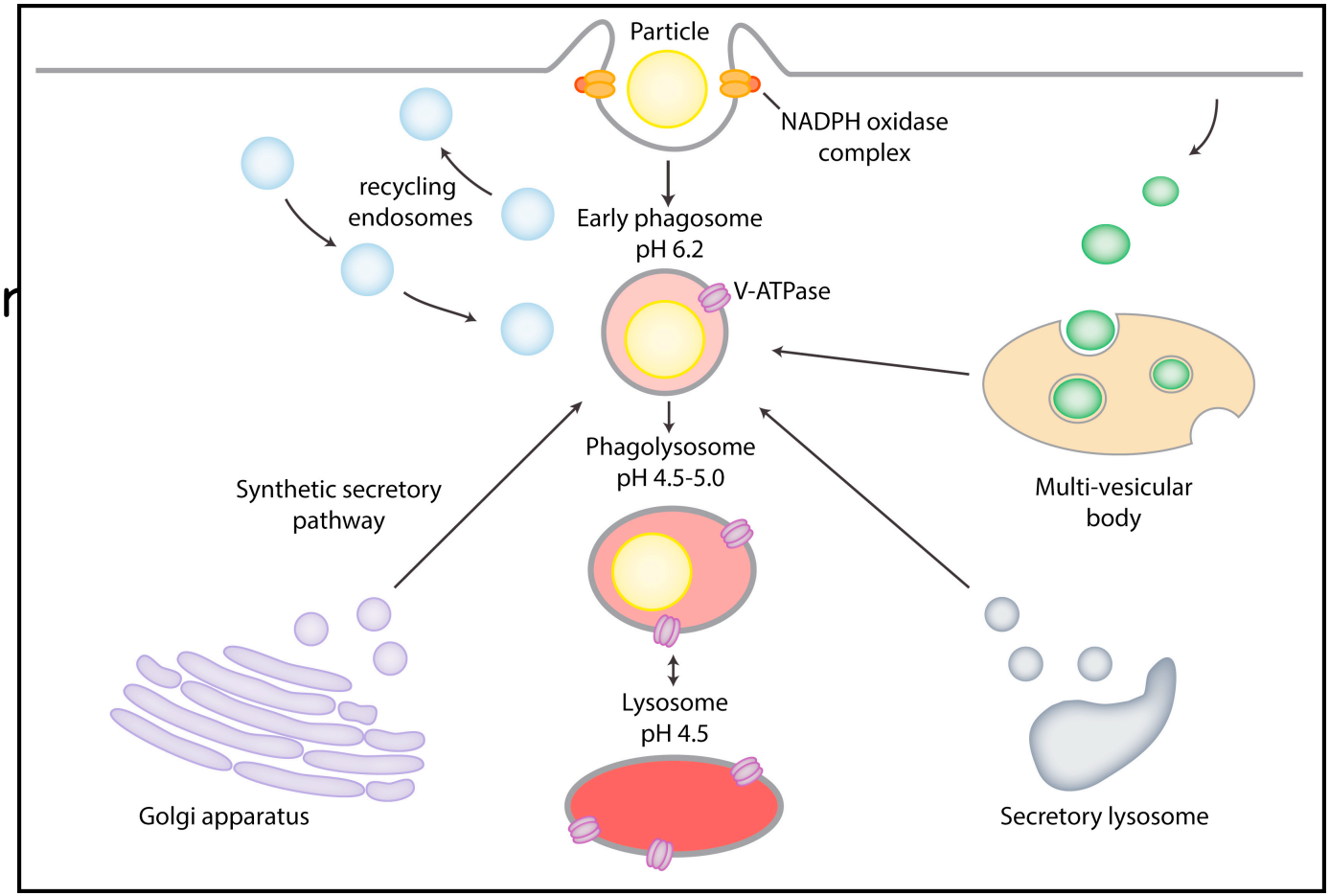
- Bind opsonized targets, pathogen molecules and apoptotic cells
- Signal differently (reaching vs sinking)
- Distributed differently amongst phagocytes




Gordon, Immunity, 44:463 (2016)

# Phagocytic receptors (neutrophils and macrophages)

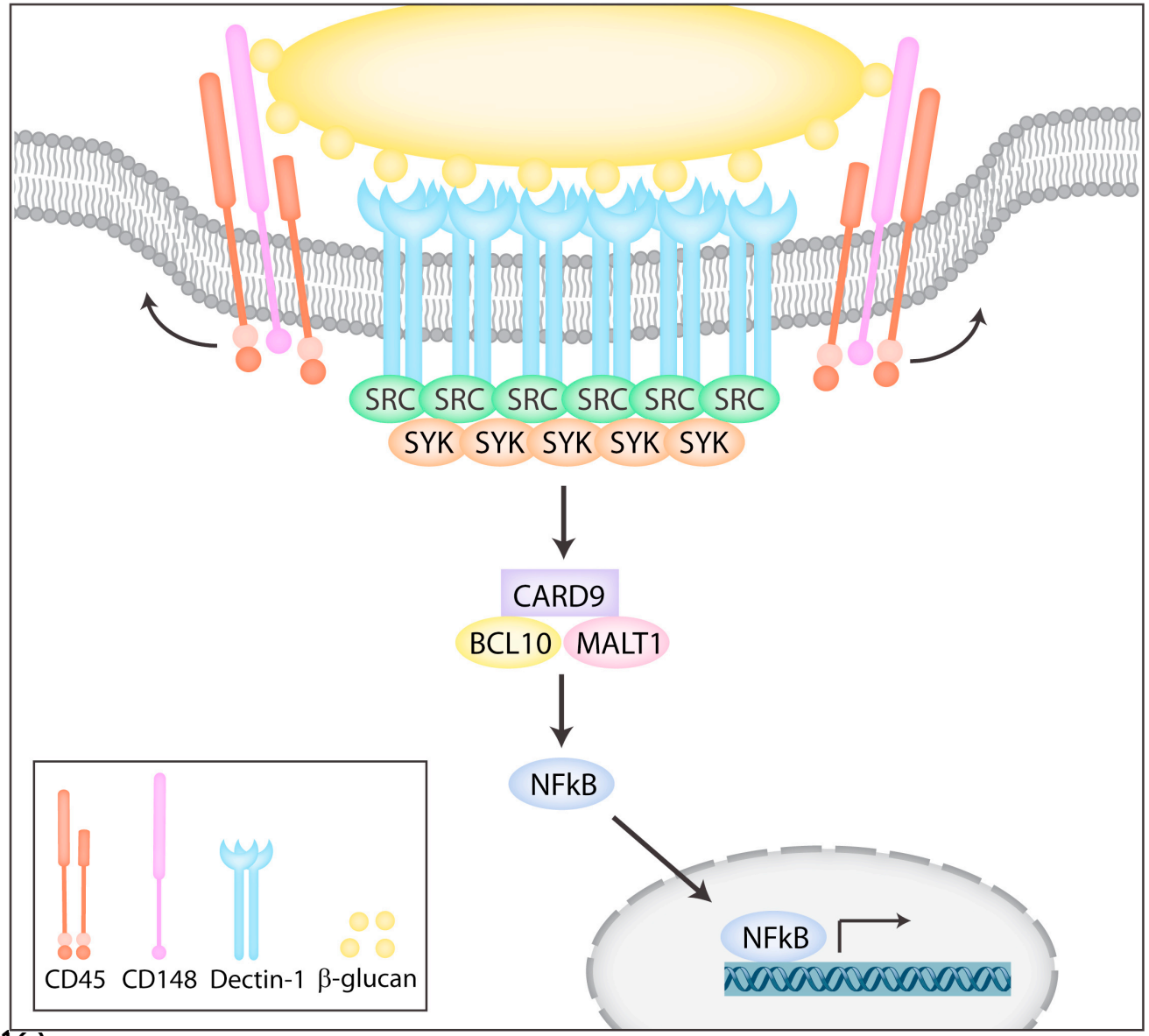
- Ultimate goal, delivery of cargo to phagolysome for destruction
- Also involved in recycling to pathways for antigen presentation




Gordon, Immunity, 44:463 (2016)

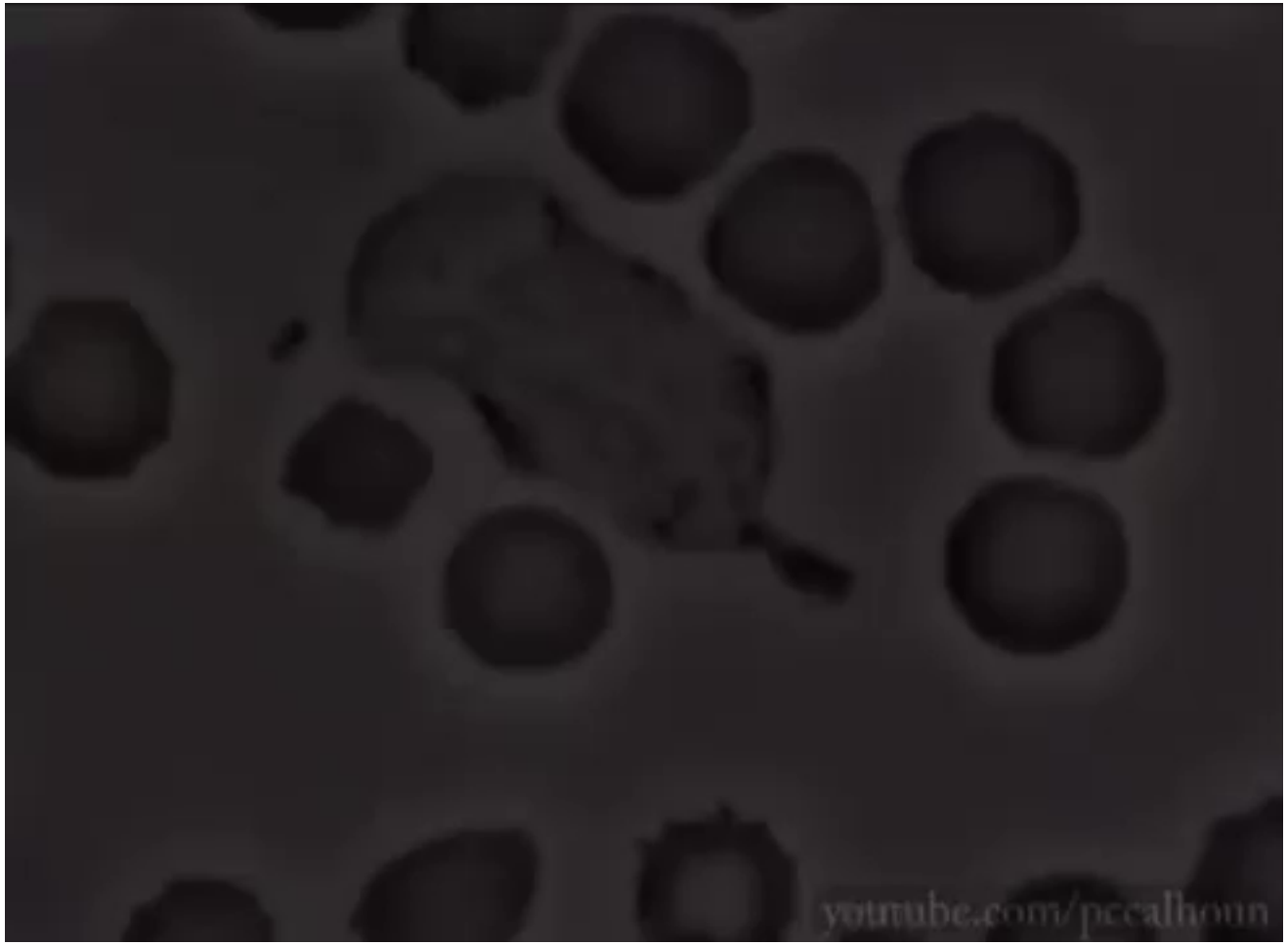
# Phagocytic receptors – the synapse

- Similar to the immunologic synapse in antigen presentation
- Concentration of signaling molecules at the PM



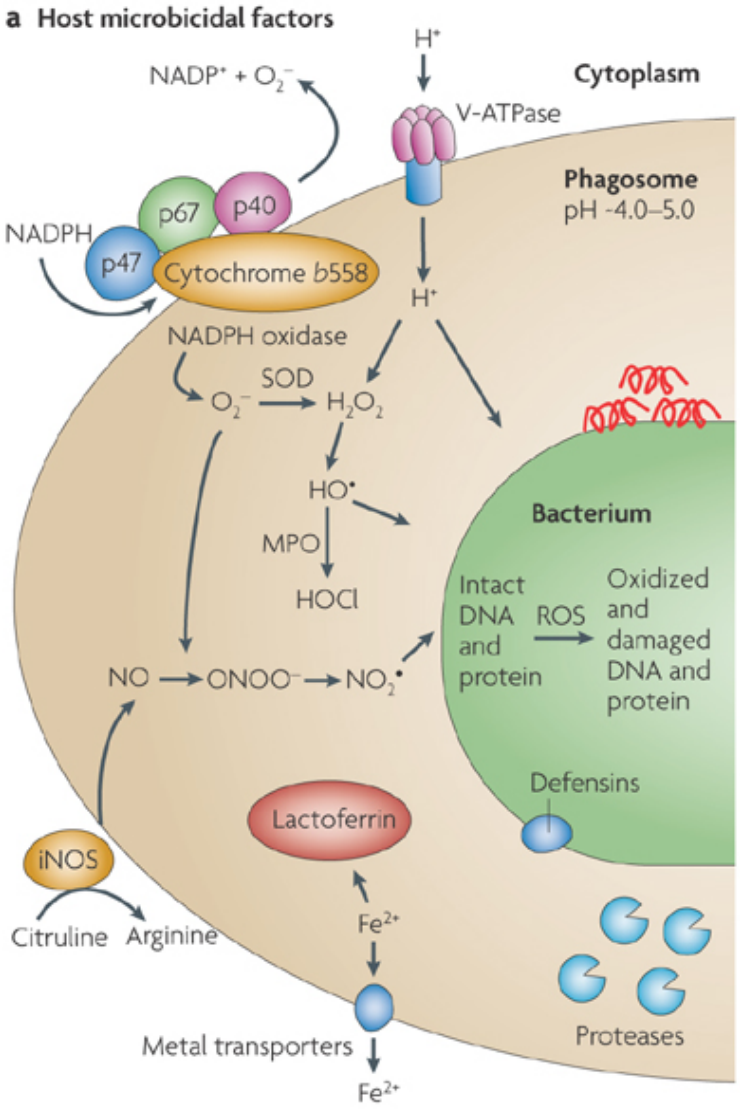

Gordon, Immunity, 44:463 (2016)

# Bacterial sensing and uptake



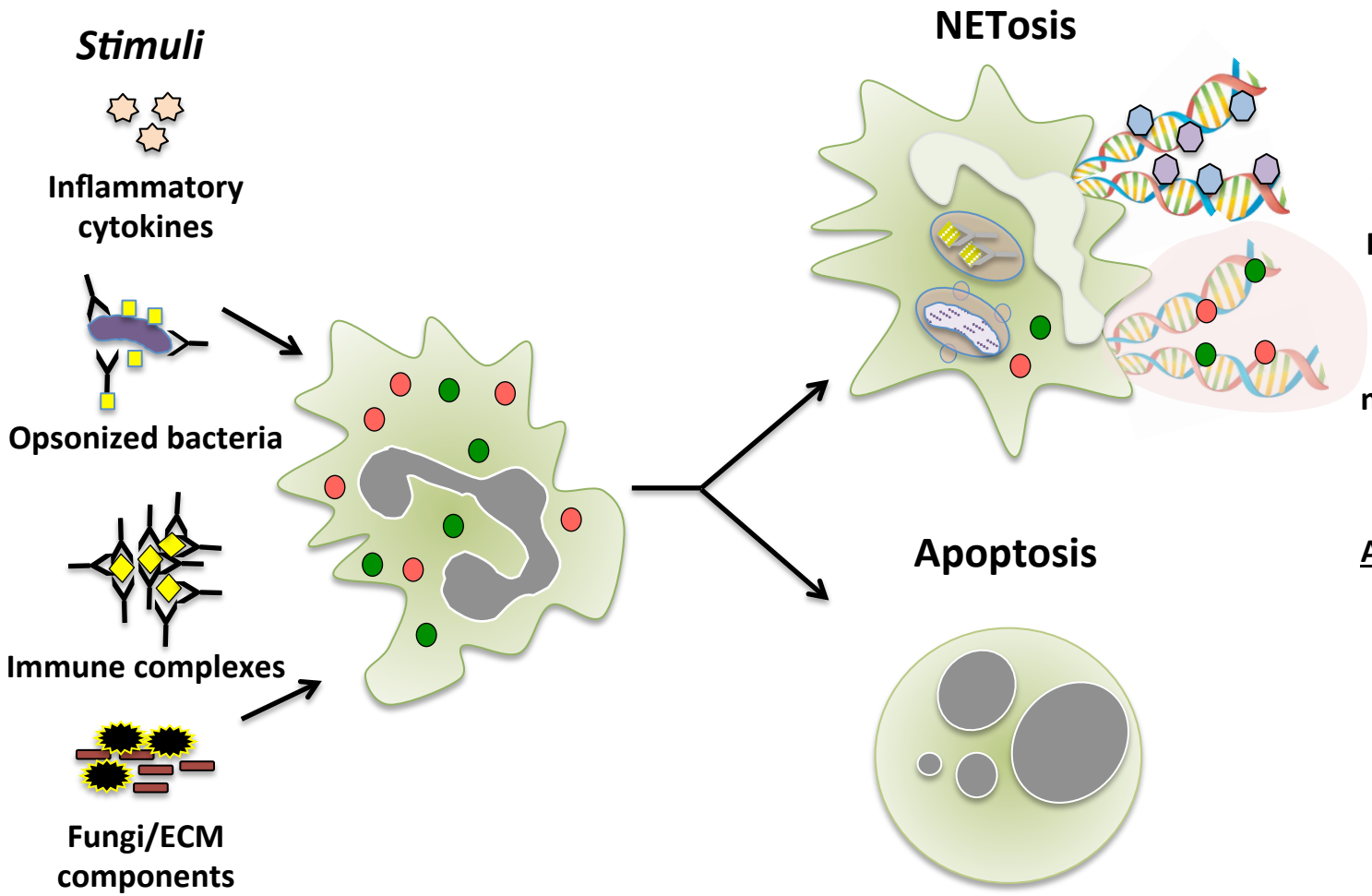
# Phagocyte oxidase

- Subunits in 2nd granules or plasma membrane
- Fuse with phagosome
- Superoxide causes DNA damage, protein oxidation
- Chronic granulomatous disease -- most common X-linked loss of NOX2
- Double deficiency (mice) iNOS and NADPH oxidase very severe




Flannagan et al, Nat Rev Micro Ann Rev Pathology, 7:355 (2009)

# Neutrophil Netosis




**Pro-inflammatory**

Release self Ags

Stimulate IFN$\alpha/\beta$

Form a chromatin lattice

Present anti-microbial peptides

**Anti-inflammatory**

Uptake by Macrophages

Stimulate anti-inflammatory cytokines

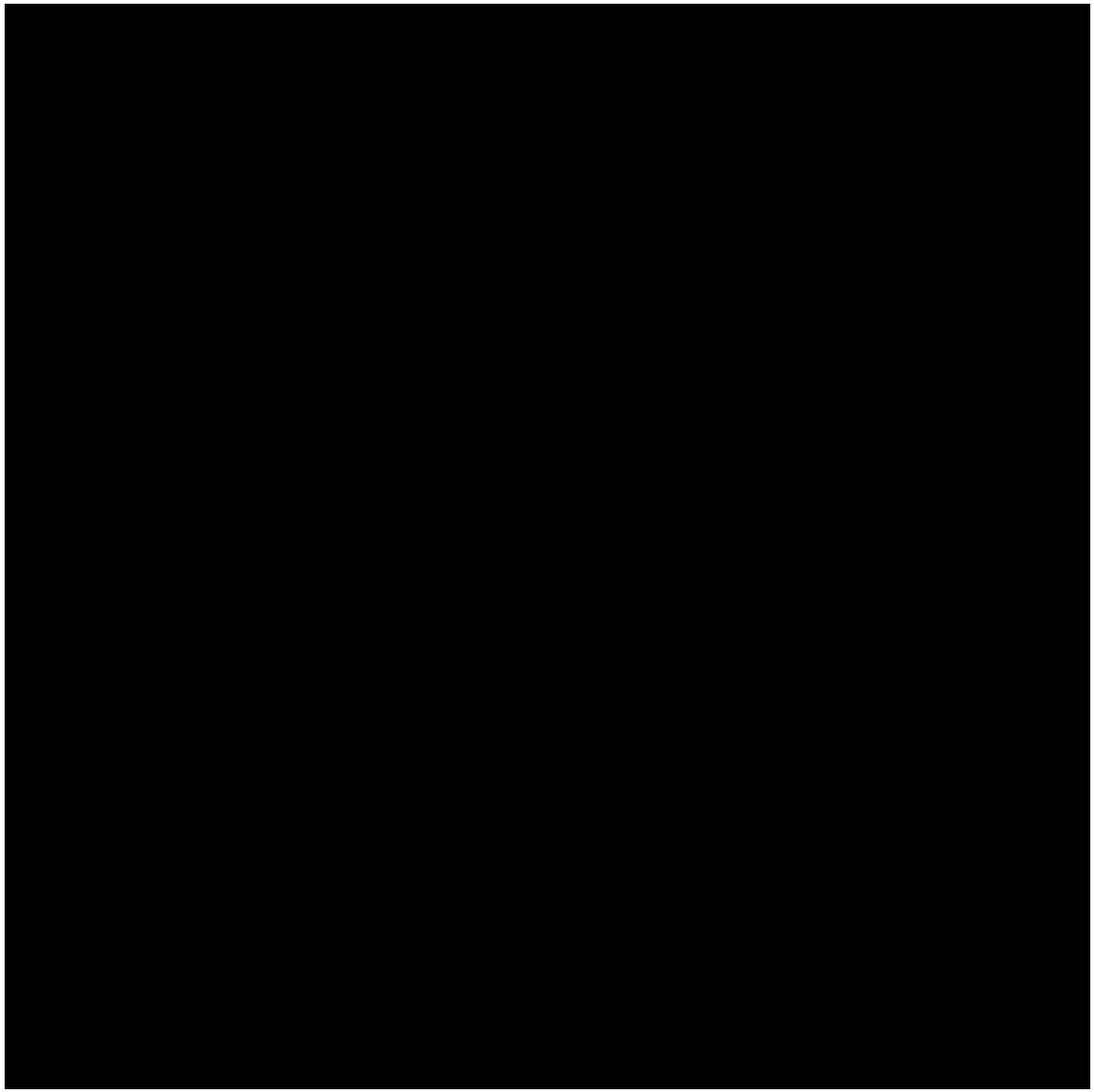
Removal of cellular debris

Mayadas, Cullere and Lowell, Ann Rev Pathology, 9:181 (2014)

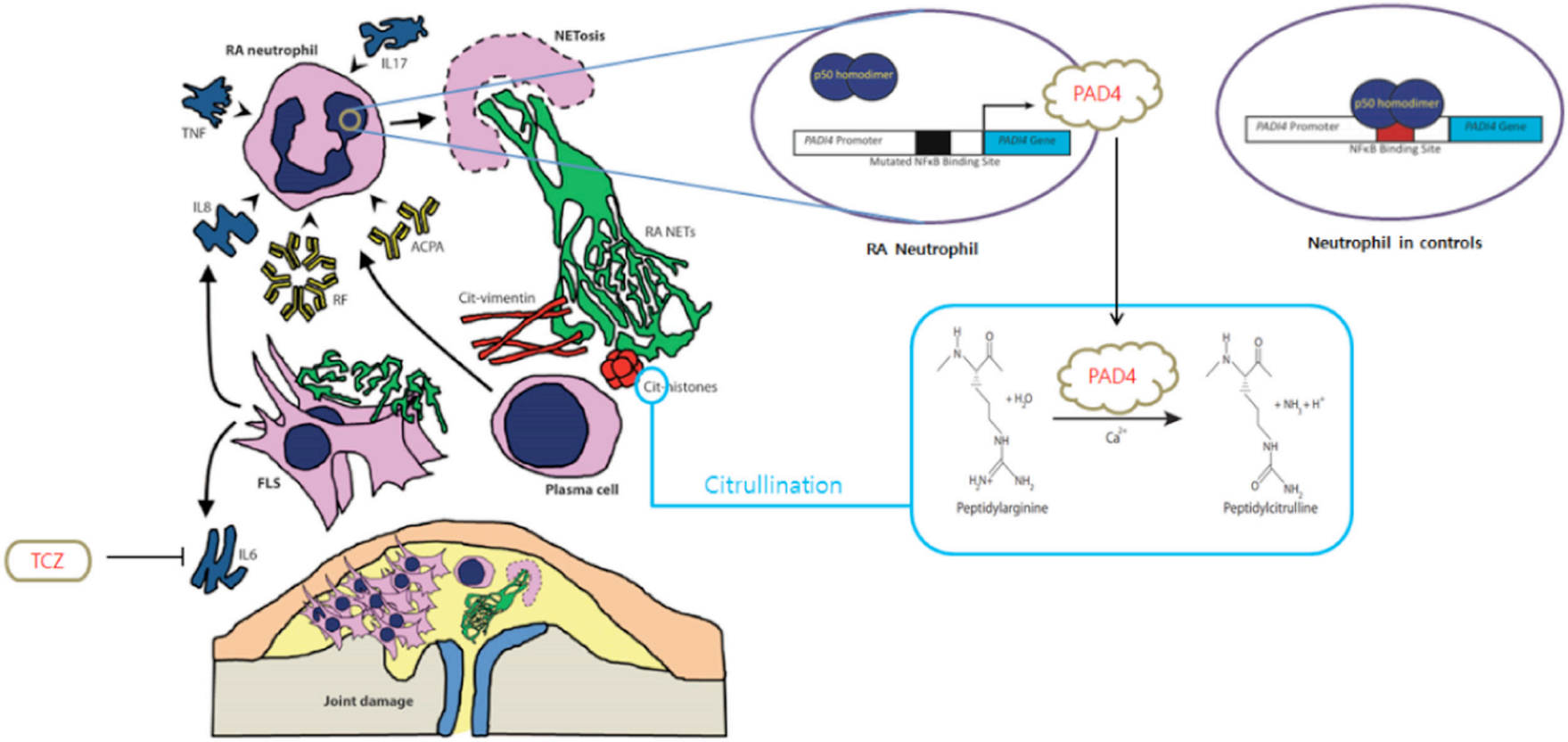
# Neutrophil NETosis

- Unique form of cell death
- Active process, results in release of DNA
- Requires activation of NADPH oxidase
- Sticky DNA forms "nets" which trap organisms
- Allows binding of anti-microbial peptides
- Very pro-inflammatory
- Assay by association of MPO with chromatin

# Neutrophil NETosis – in real time



# Neutrophil NETosis – major source of auto-antigens in rheumatoid arthritis




- Citrullinated proteins (histones, vimentin, collagen) Ags in RA
- Present in humans, PAD4 dependent in mice
- NETosis contributes to inflammatory diseases: gout, psoriasis

Lee et al, Autoimmunity Revs, 9.012 (2017)

# New roles for neutrophils

- Cytokine production:
  Clearly demonstrate *in vitro*, but *in vivo role* not clear
  Same true for chemokine production – CXCL12 production

- Roles in cancer
  Pro tumorgenic? Make growth factors, drive angiogenesis
  Anti tumorgenic? Drive inflammation for tumor immunity

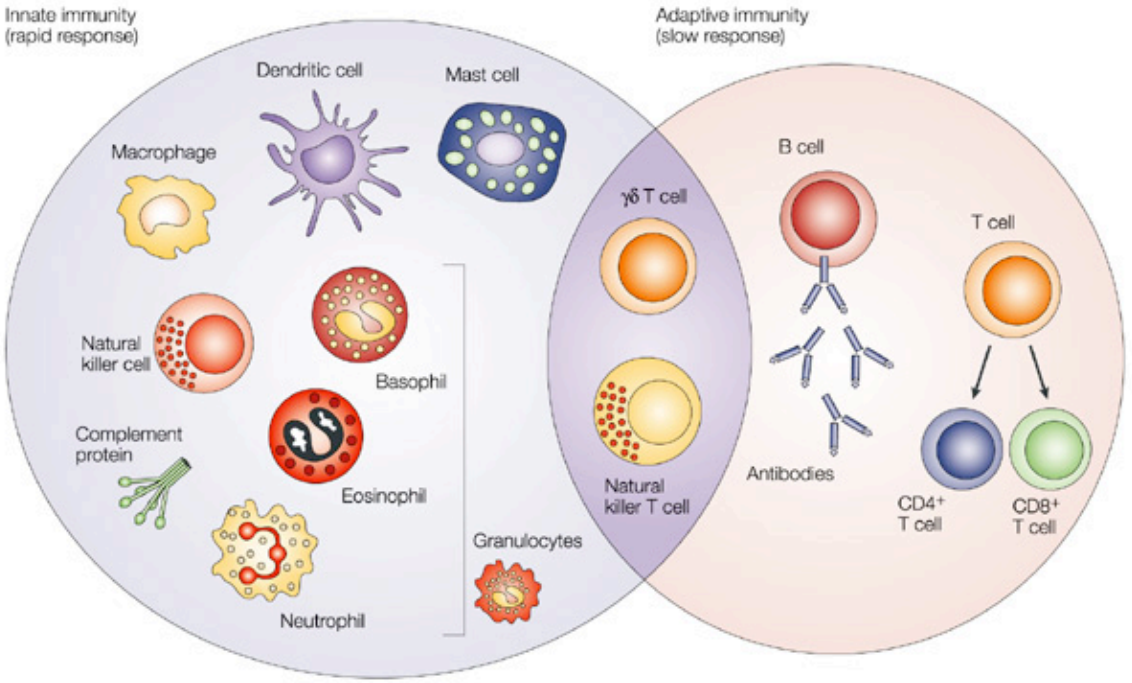
- Neutrophil subsets?
  N1 versus N2 in cancer; low density PMNs in lupus?
  Myeloid derived suppressor cells – immature PMNs

- Tissue resident neutrophils?
  Not just in the blood, spleen PMNs (Deniset JEM, 2017)

- Required for wound healing
  Neutrophil depletion often SLOWs healing (sterile injury)

Wang, Cell Tissue Res 371:531 (2018)

# Cellular effectors of innate immunity




# Monocytes -- Macrophages

- Enter inflammatory site, differentiate into M1 versus M2 types (IFNγ versus IL-4/IL-10)
- Major cytokine producers

# Monocytes and Macrophages – lots of new functions and lots of heterogeneity

Classic roles

- Monocytes circulate in blood, enter inflamed tissues
- Macrophages are differentiated monocytes, constantly replaced
- Cytokine producers, host defense and tissue repair

New roles

- Huge heterogeneity in macrophage subtypes
- Completely different lineages of tissue macrophages
- Different monocyte subsets, with different functions

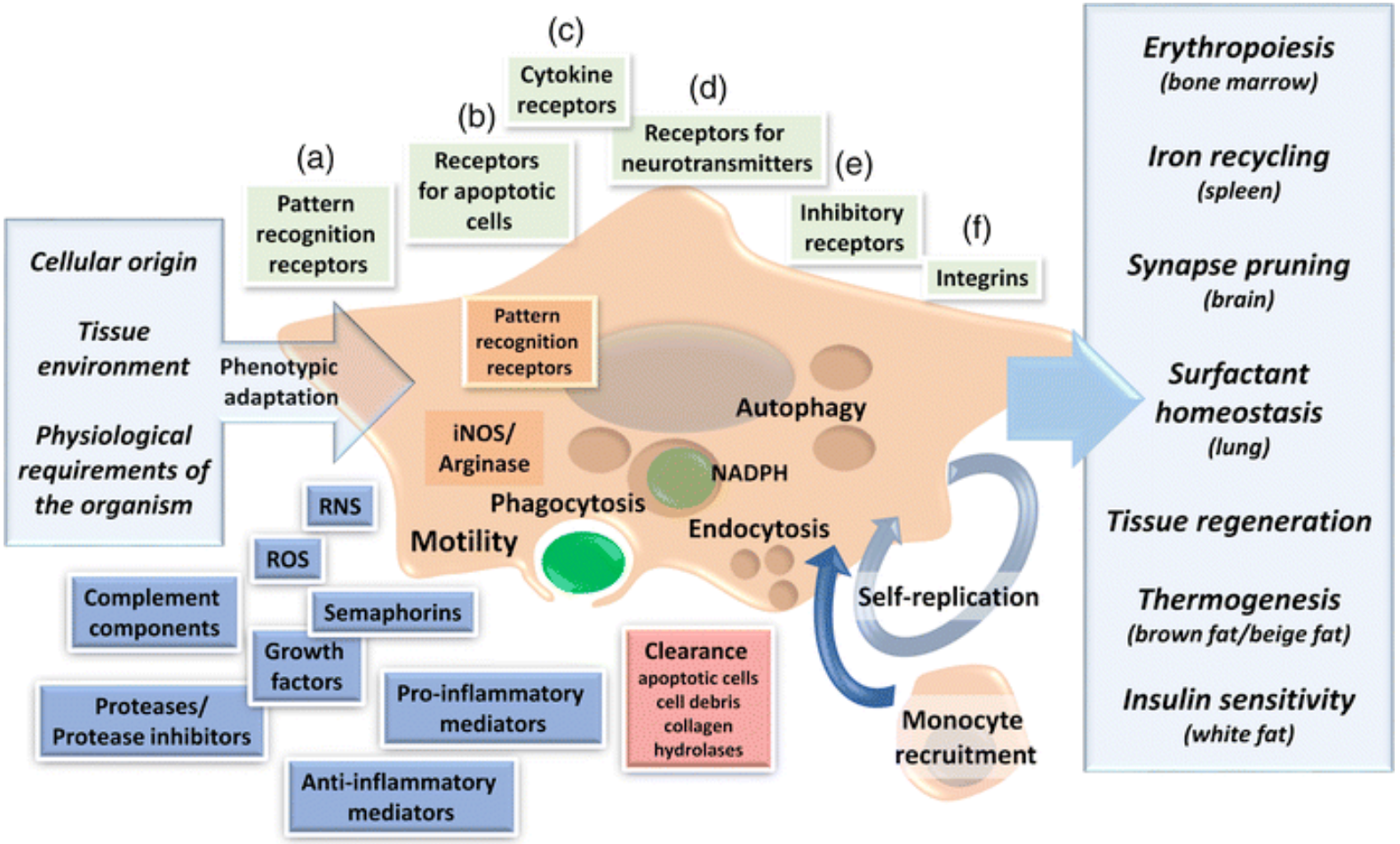
# Tissue Resident Macrophages "Reticuloendothelial System"

- Tissue resident macrophages as sentinels of immunity
  - Kupffer cells in the liver
  - Microglia in the brain
  - Osteoclasts in the bone
  - Alveolar macrophages in the lung
  - Splenic macrophage types (Red pulp vs marginal zone)
  - Foam cells in plaques
  - Bone marrow resident
  - Thymic resident
  - Intestinal (lamina propria and submucosal)
  - Serosal (peritoneal) resident

- Respond to stimuli in different ways
  - Different cytokine production
  - +/- iNOS upregulation and NOS production

Yona and Gordon, Front Immunol 6:328 (2015)

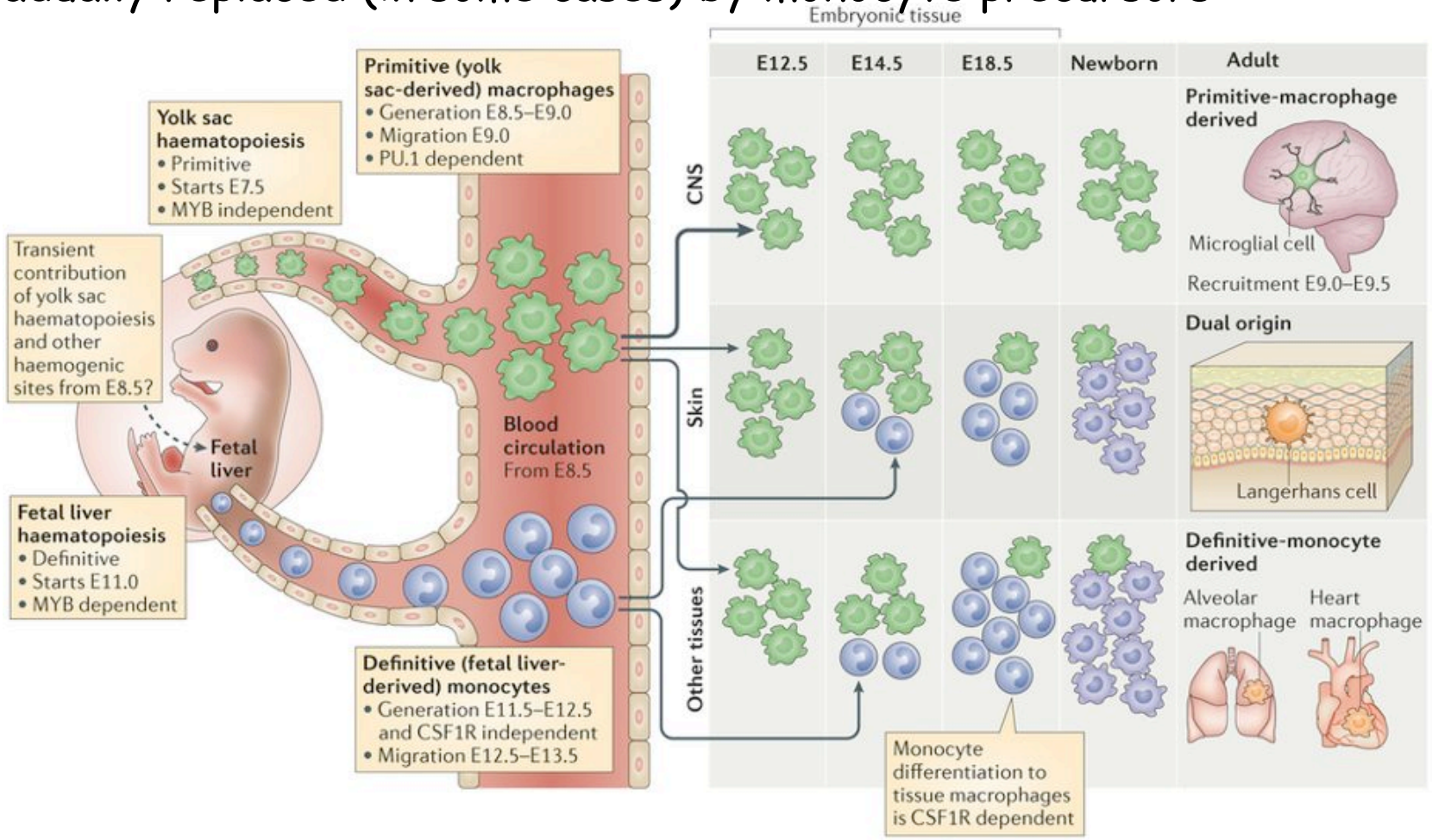
# Tissue Resident Macrophages modern view – more than host defense




Gordon and Martinez-Pomares, Pflügers Archiv 469:365 (2017)

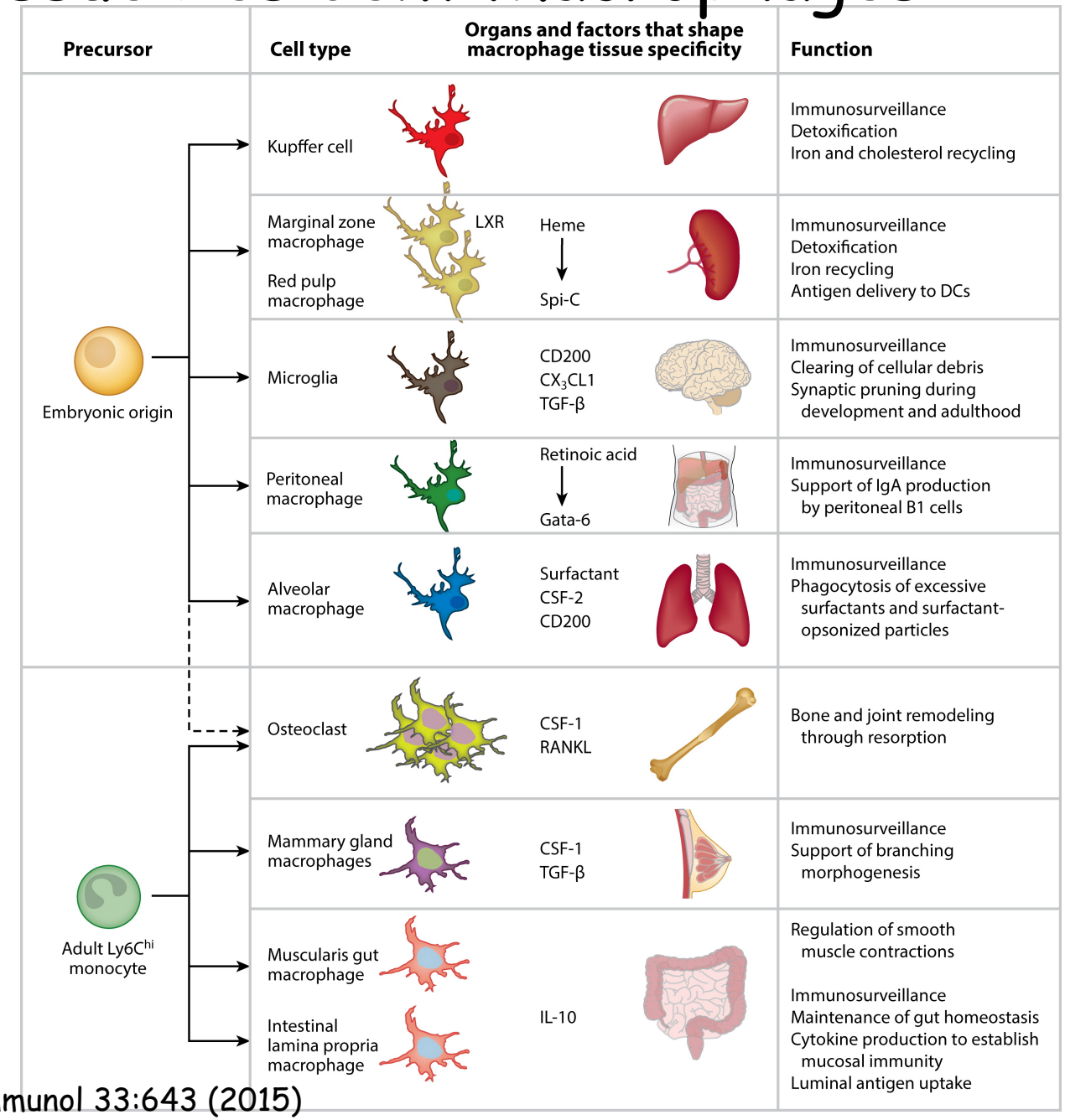
# Tissue Resident Macrophages
# Major new understanding

- many tissue macrophages seeded from yolk sac progenitors
- gradually replaced (in some cases) by monocyte precursors




Ginhoux and Jung, Nat Rev Immunol 14:392 (2014)

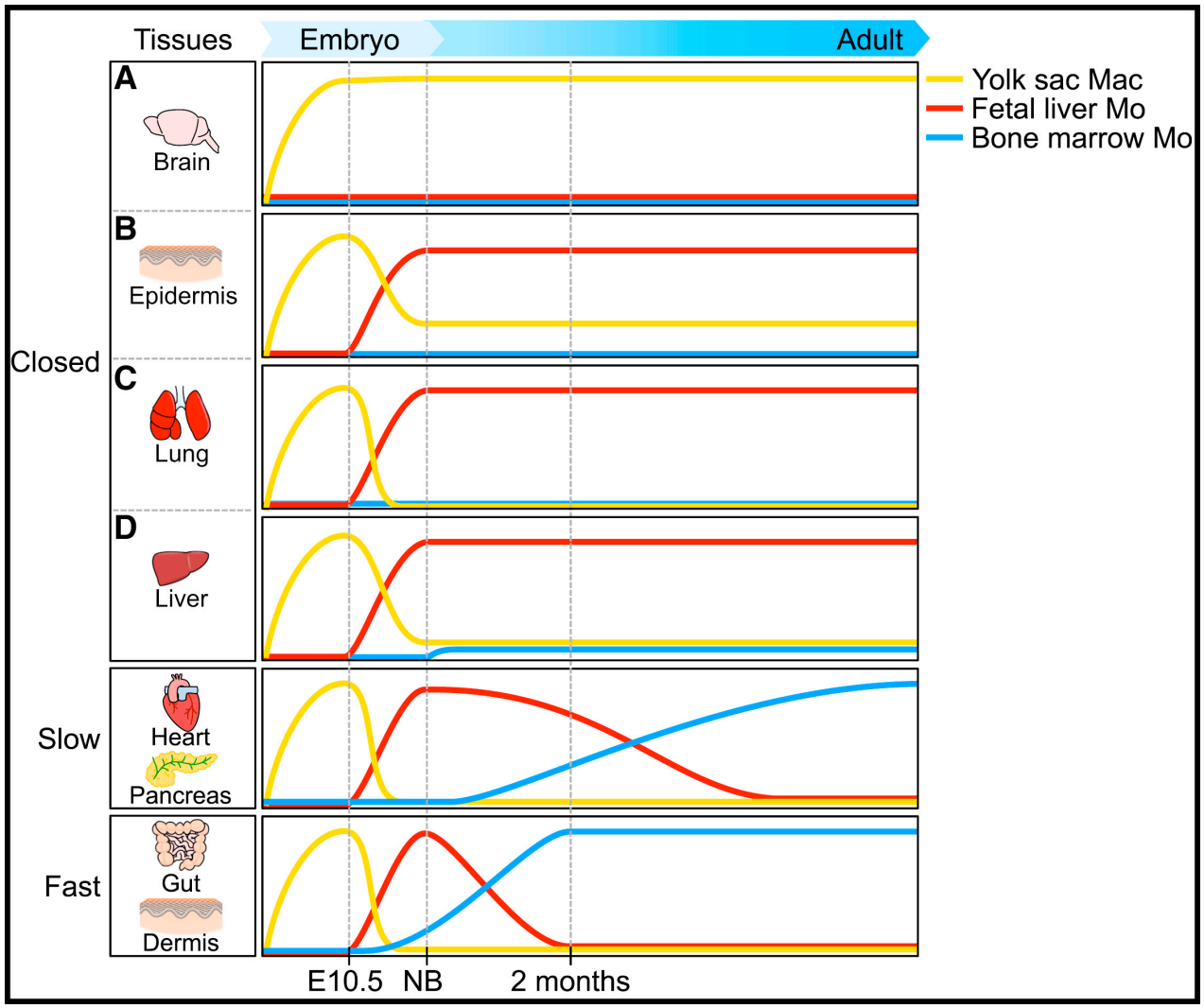
# Tissue Resident Macrophages




Varol et al, Annu Rev Immunol 33:643 (2015)

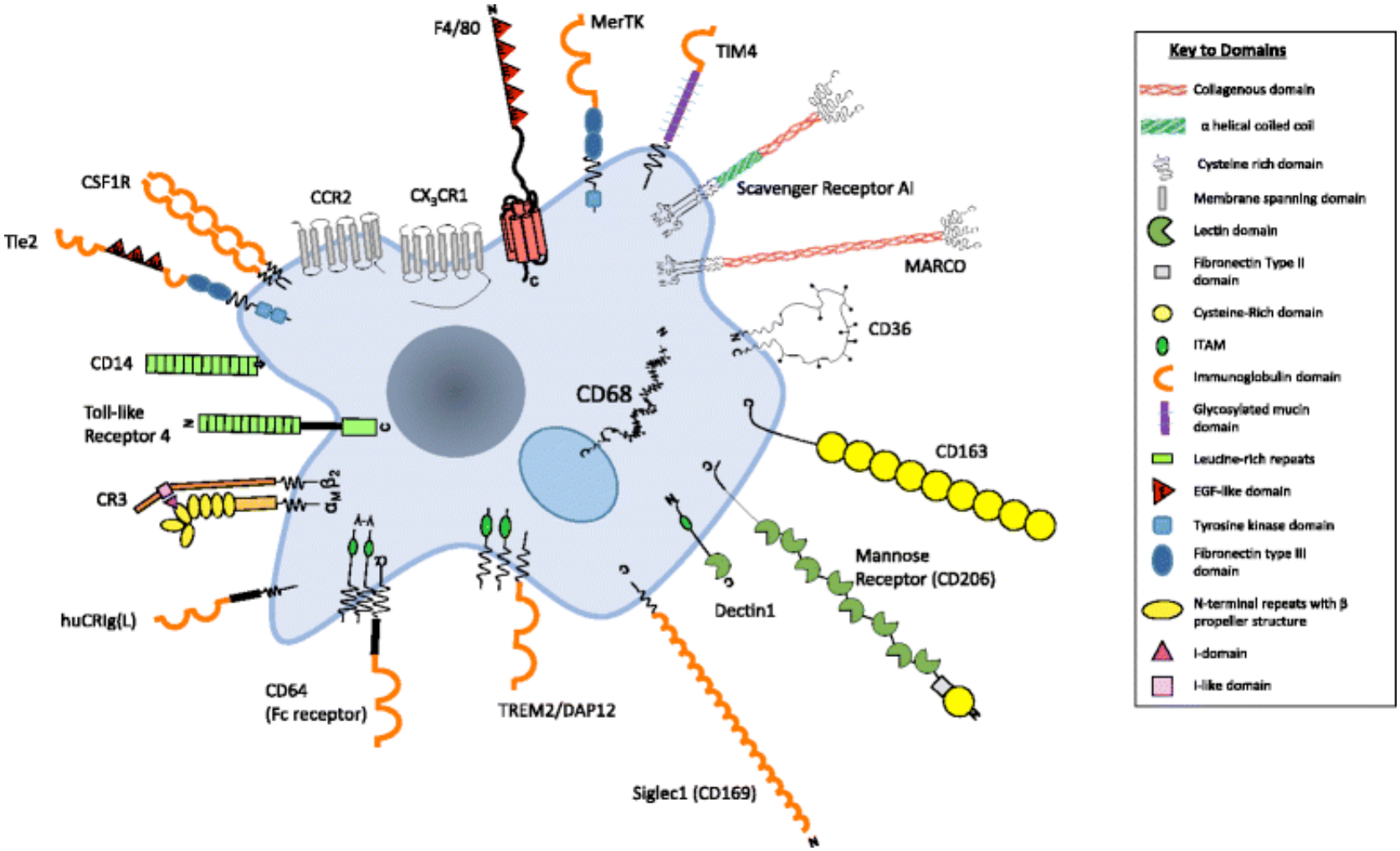
# Tissue Resident Macrophages –mix of both yolk sac and monocyte-derived

- Determined by lineage marking methods
- confirmed with parabiosis experiments
- Results: different mac origins in different tissues




Ginhoux and Guilliams Immunity 44:439 (2016)

# In the tissues, macrophages adapt to environment to carry out diverse functions




Gordon and Plüddermann, BMC Biology 15:53 (2017)

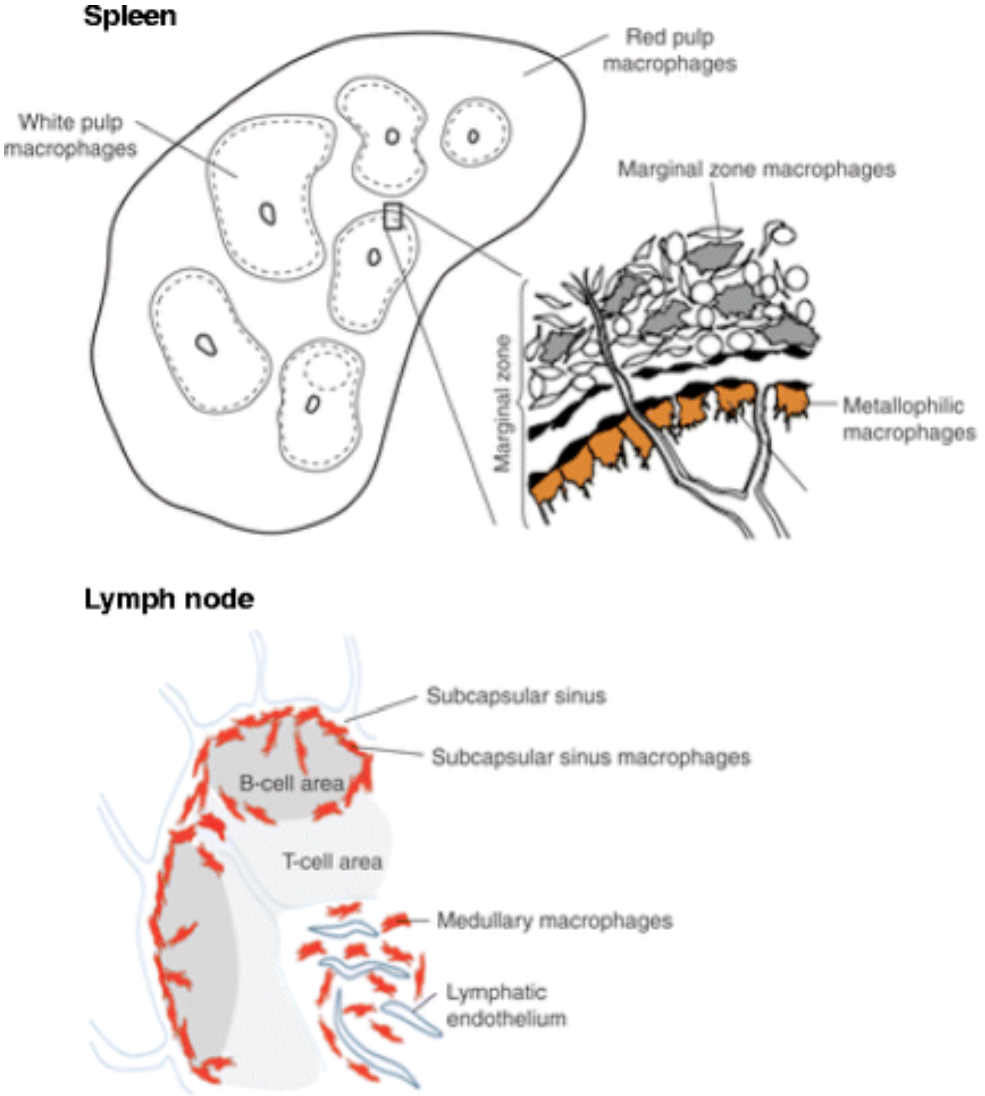
# Examples of tissue resident macrophage functions – not just host defense

- Bone marrow stromal macs
  Fe recycling, balancing hematopoiesis, phagocytosis RBCs
  osteoclasts, bone remodeling
- Spleen
  Red pulp macs, clearance of PMNs, RBCs
  Marginal zone macs, clearance of antigens/microbes
  White pulp macs, clearance apoptotic lymphocytes
- Gut
  interact with microbiome, often anti-inflammatory
- Lung
  Surfactant metabolism, immunosuppression, antigen capture
- Brain
  Microglia, clearance apoptotic cells, synaptic pruning, CSF secretion (choroid plexus macs)
- Adipose (fat) tissue
  remodeling of white to beige fat, metabolic regulation

- CAN REPROGRAM depending on tissue environment

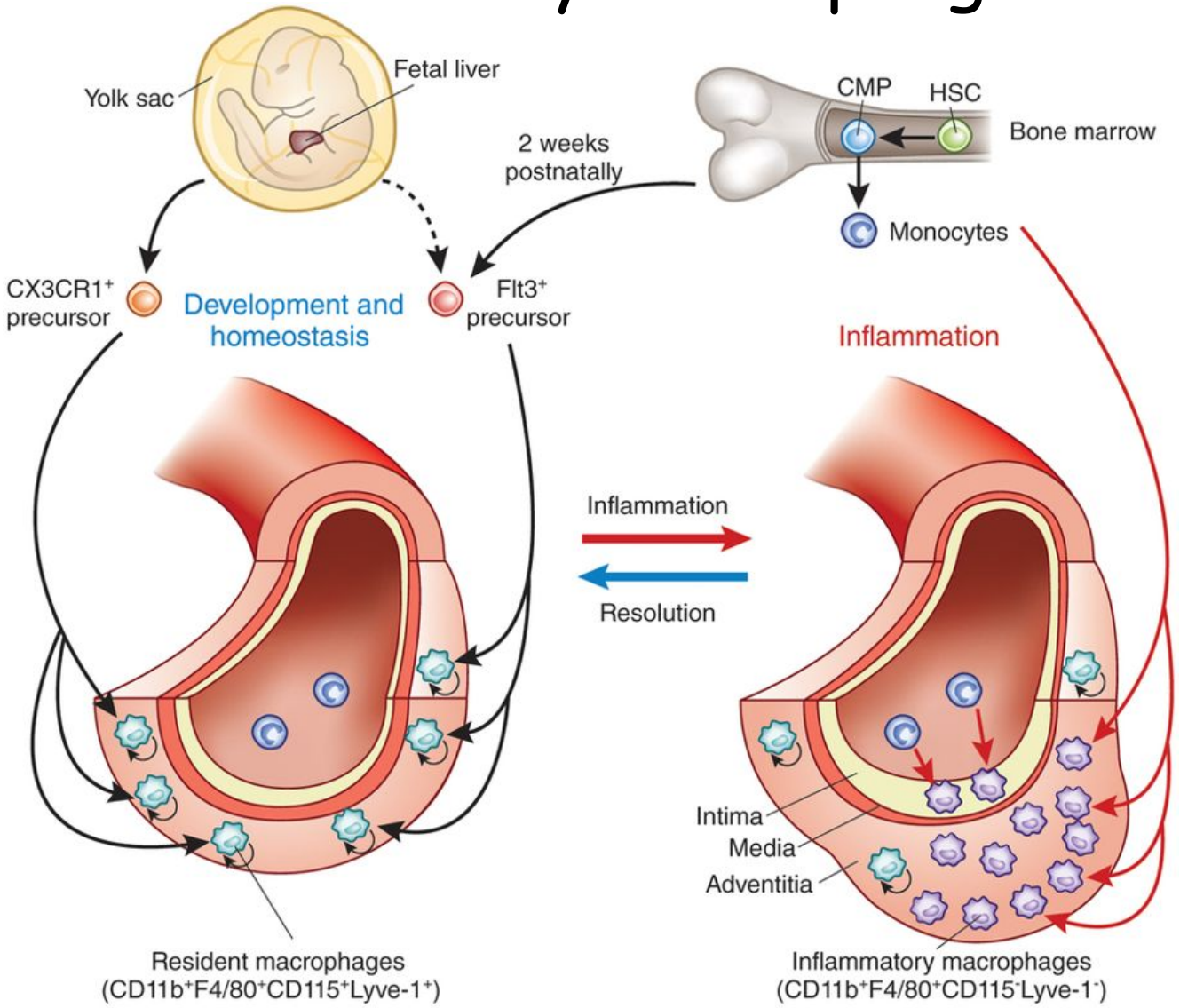
# Location of splenic/LN macs

- Distinguished by expression of different surface markers; CD68, CD169, F4/80, MARCO
- tissue resident macs often CX3CR1+




Gordon and Plüddermann, BMC Biology 15:53 (2017)

# Relationship between tissue resident and inflammatory macrophages




Klapproth et al, Nat Immunol 17:117 (2016)

# Inflammatory monocytes/macrophages

- Inflammatory monocytes (Ly6C$^{hi}$ or CD14$^{hi}$)
  - Steady state → enter/leave tissues, recirculate
  - Inflammatory state → enter tissue differentiate to macs
- Inflammatory states
  - Infection
  - Injury (ischemia)
  - Tumor
  - Obesity (major contributor to insulin resistance)
  - Atherosclerosis
  - Neurodegeneration (protein aggregation)
- Two major inflammatory macrophage subtypes
  - M1 versus M2

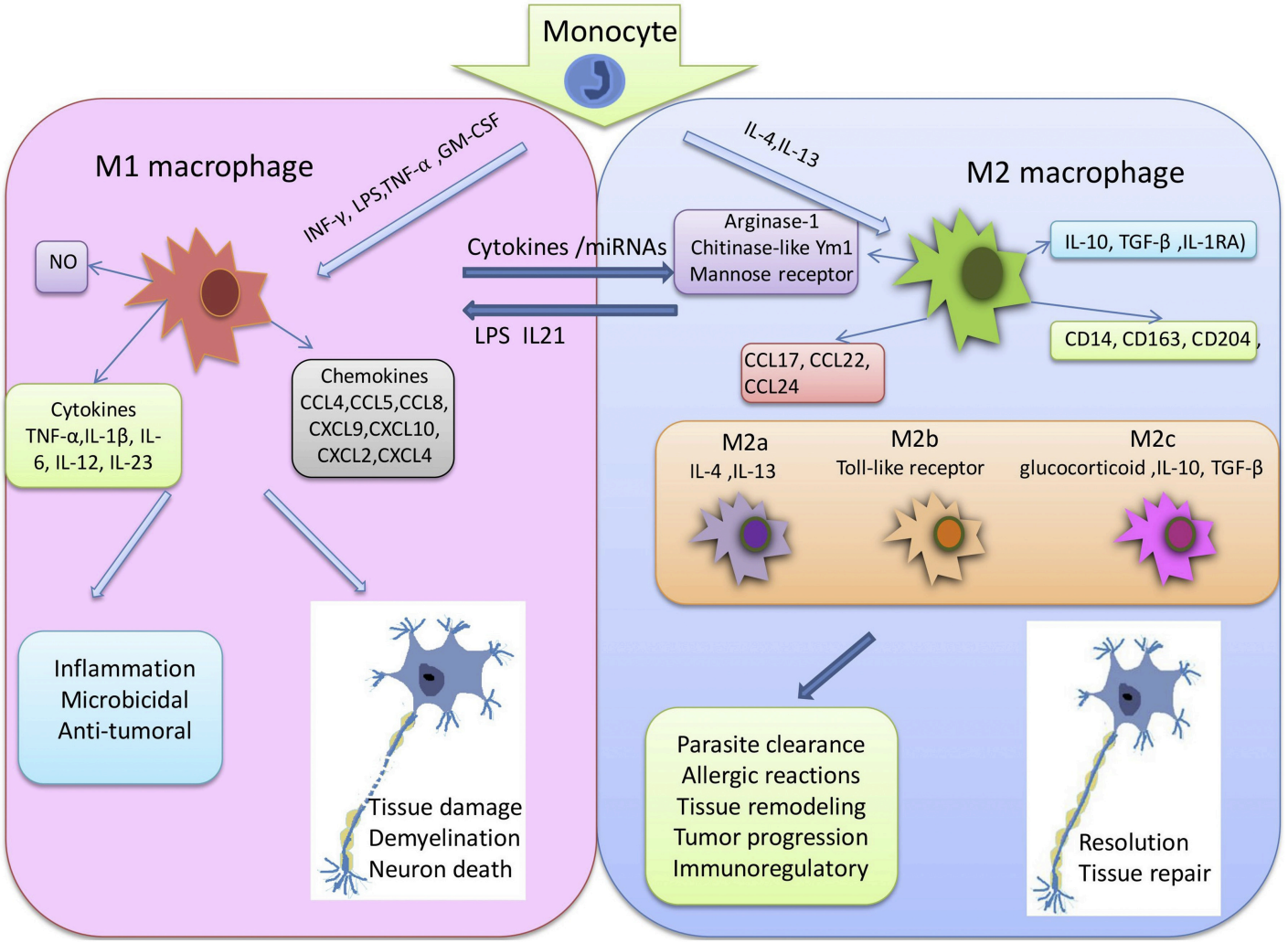
# M1 vs M2 macrophages

- M1 macrophages
  - Pro-inflammatory, associated with infection
  - Granuloma formation
  - Induced by inflammatory cytokines (IFNγ)
  - Highly phagocytic, high MHCII, lots of cytokines
  - High levels of iNOS, ROS production
  - Warburg metabolism

- M2 macrophages
  - Anti-inflammatory, associated with tissue repair
  - Often in tumors
  - Induced by IL-4, IL-13, apoptotic body phagocytosis
  - Express CD206, arginase and chitinase (YM1 in mouse)
  - Make more IL-10, TGFβ
  - Usually depend on PPARγ expression
  - Oxidative metabolism

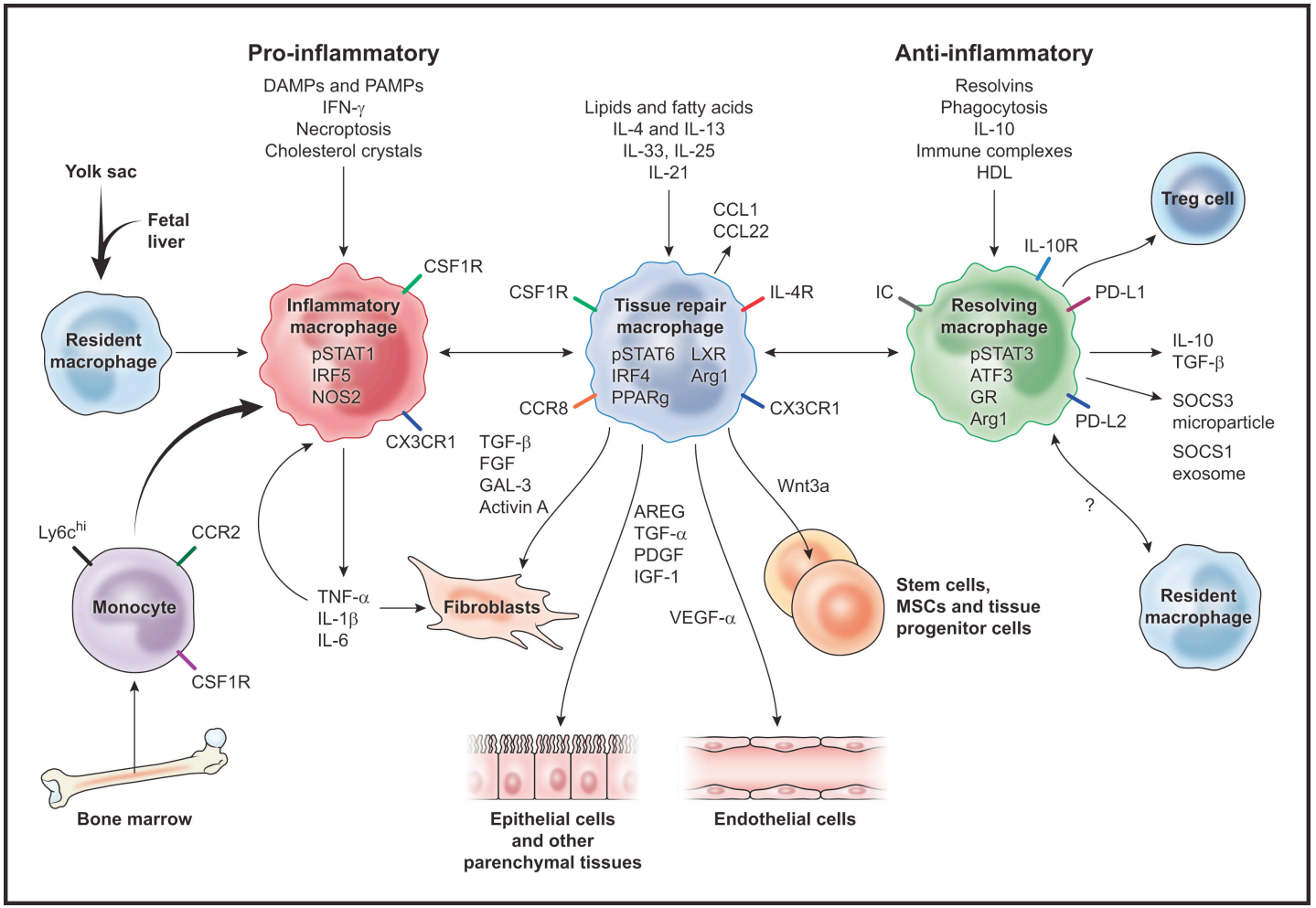
- LOTS OF OVERLAP → more of spectrum

# M1 / M2 ratio determines disease outcome – example multiple sclerosis




Chu et al, J Neuroimmunology 318:1 (2018)

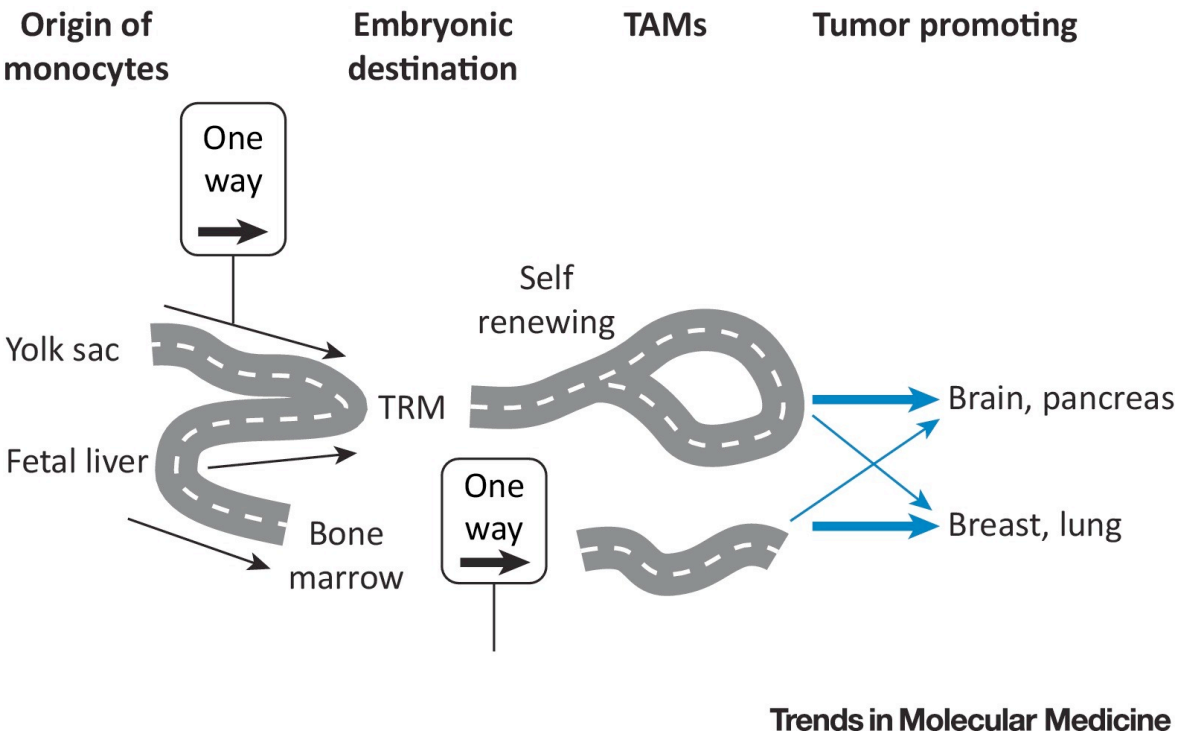
# M1 / M2 ratio determines disease outcome – example tissue repair vs fibrosis




Wynn and Vannella, Immunity 44:450 (2016)

# Tumor associated macrophages

- TAM → derived from both tissue resident and BM precursors
- Ratio of each varies depending on tumor type
- Can be both pro-tumor and anti-tumor → "M1 vs M2"
- New target in cancer therapy




Guerriero, Trends Mol Med 24:472 (2018)

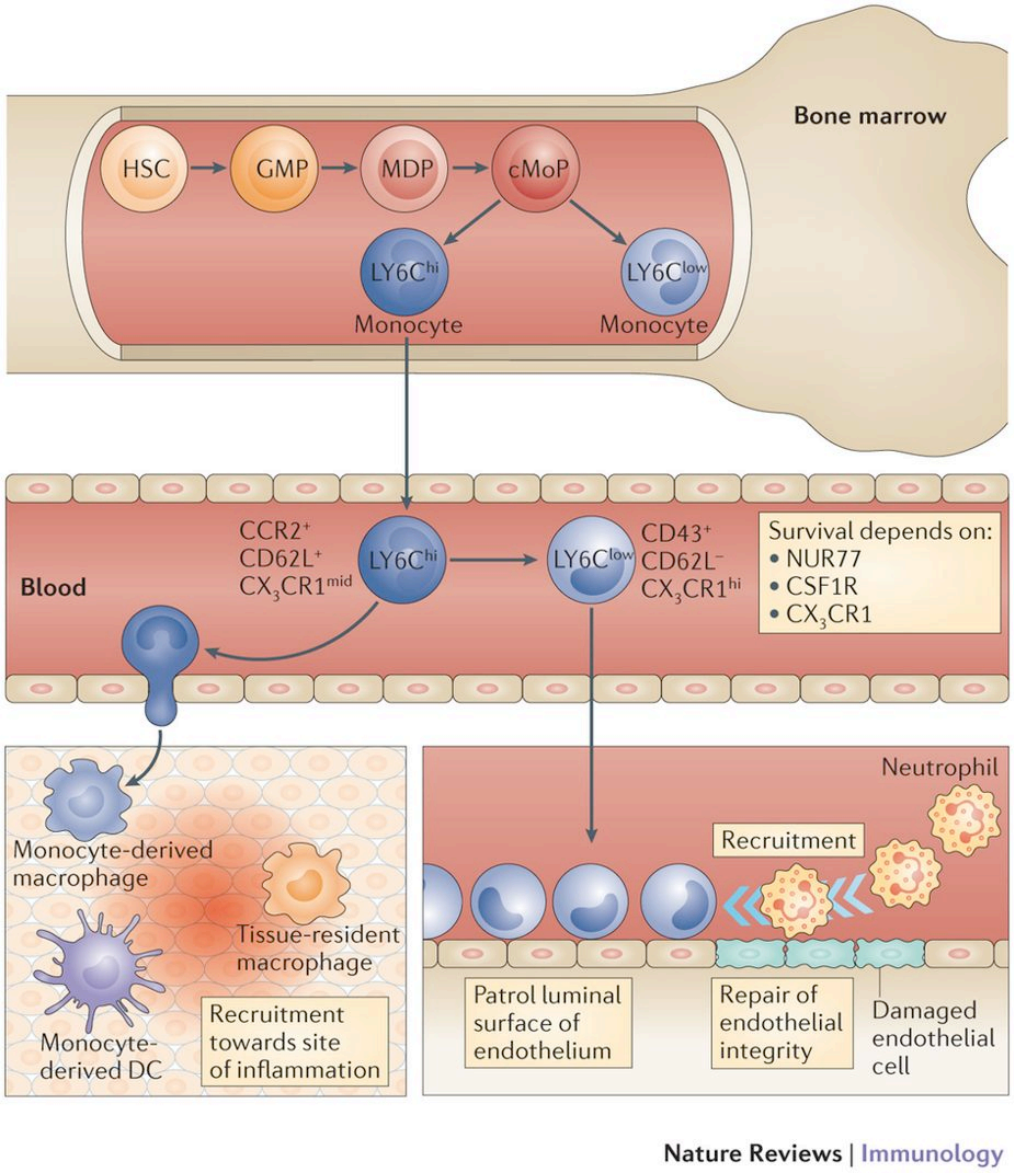
# Monocyte heterogeneity

- Monocytes → 5 – 10% of WBCs
  Previously thought to be just precursors of macrophages
  Two major types discovered with different functions

- Classical or inflammatory monocytes
  Mouse → $Ly6C^{hi}CCR2^{+}CD62L^{+}$
  Human → $CD14^{hi}$
  Derived from bone marrow precursors
  ~1 day (or less) half-life
  leave vasculature and differentiate into macrophages

- Patrolling or resident monocytes
  Mouse → $Ly6C^{lo}CD43^{+}CX3CR1^{hi}$
  Human → $CD14^{lo}CD16^{+}$
  Derived from $Ly6C^{hi}$ cells, depend on Nr4a1 transcription
  ~5 day half-life
  never leave vasculature and maintain vascular integrity

# Monocyte heterogeneity

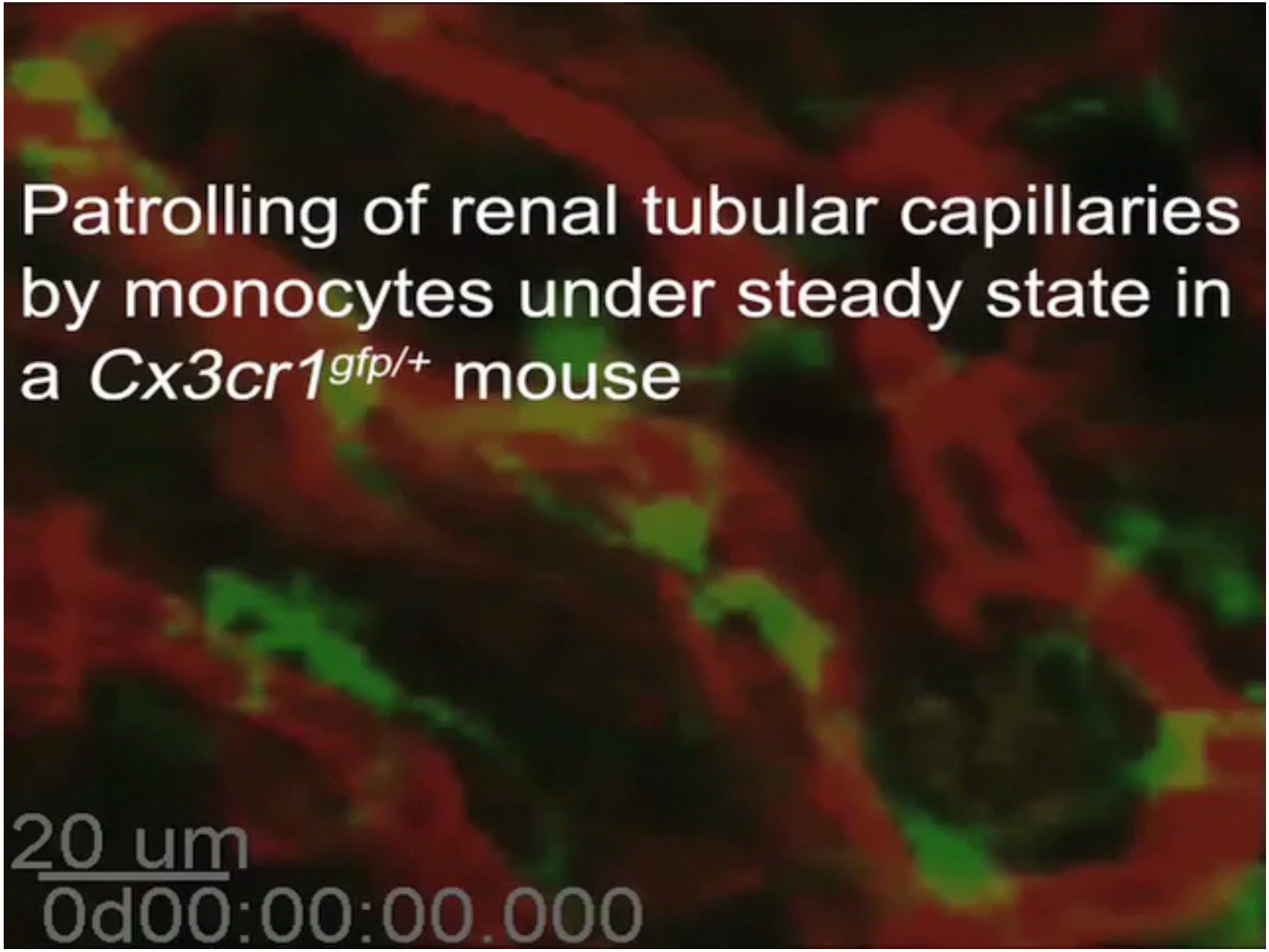



Ginhoux and Jung, Nat Rev Immunol 14:392 (2014)

# New functional roles for patrolling monocytes

- Roll along vascular beds (kidney and peritneum)
- Sense endothelial injury/damage (TLR7/9 dependent)
- Recruit neutrophils to mediate endothelial injury
- Thought to be the initiators of many inflammatory reactions
  - demonstrated in various kidney models (acute/chronic)
  - enriched in the lung – may control lung metastasis
  - major tool – Nr4a1$^{-/-}$ mouse

Buscher et al, Front Cardio Med 4:80 (2017)


Patrolling of renal tubular capillaries by monocytes under steady state in a *Cx3cr1*$^{gfp/+}$ mouse
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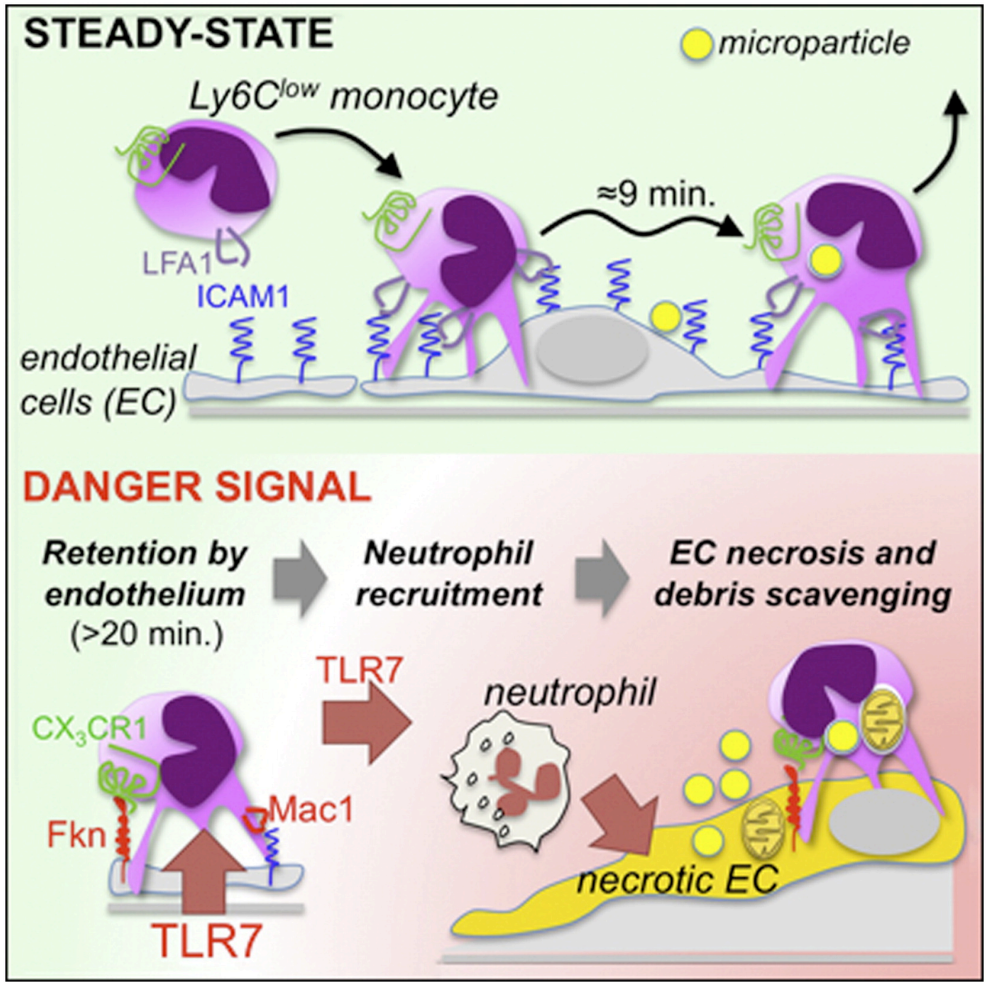
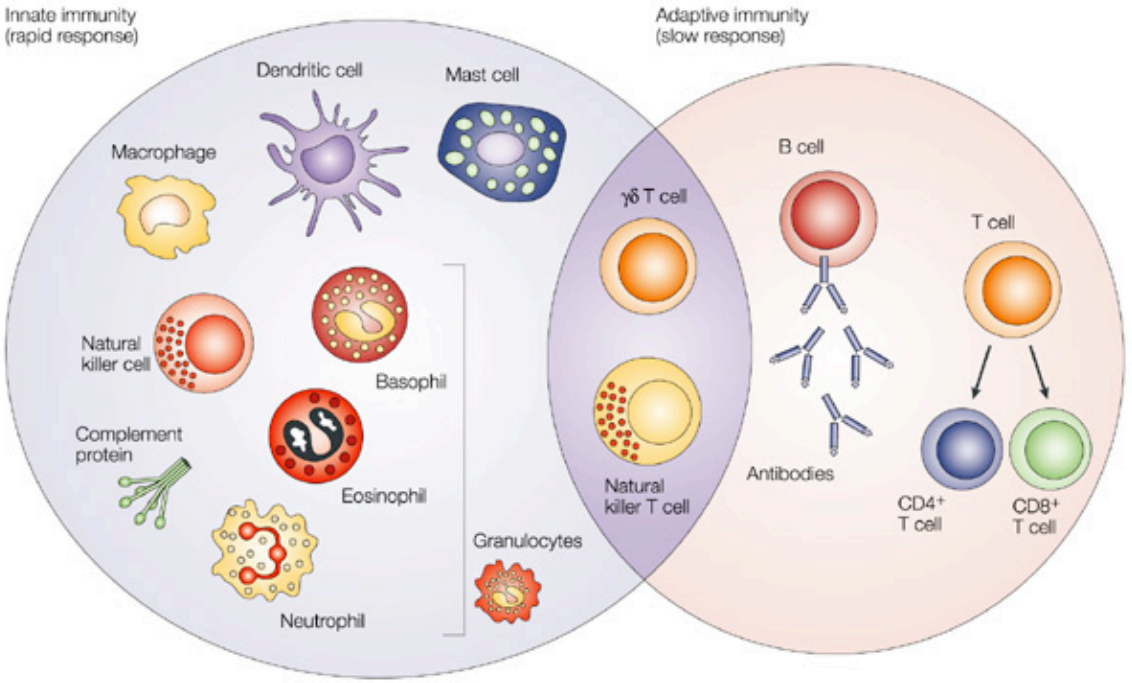
# Model for patrolling monocyte monitoring of vascular integrity




Carlin et al, Cell 153:362 (2013)

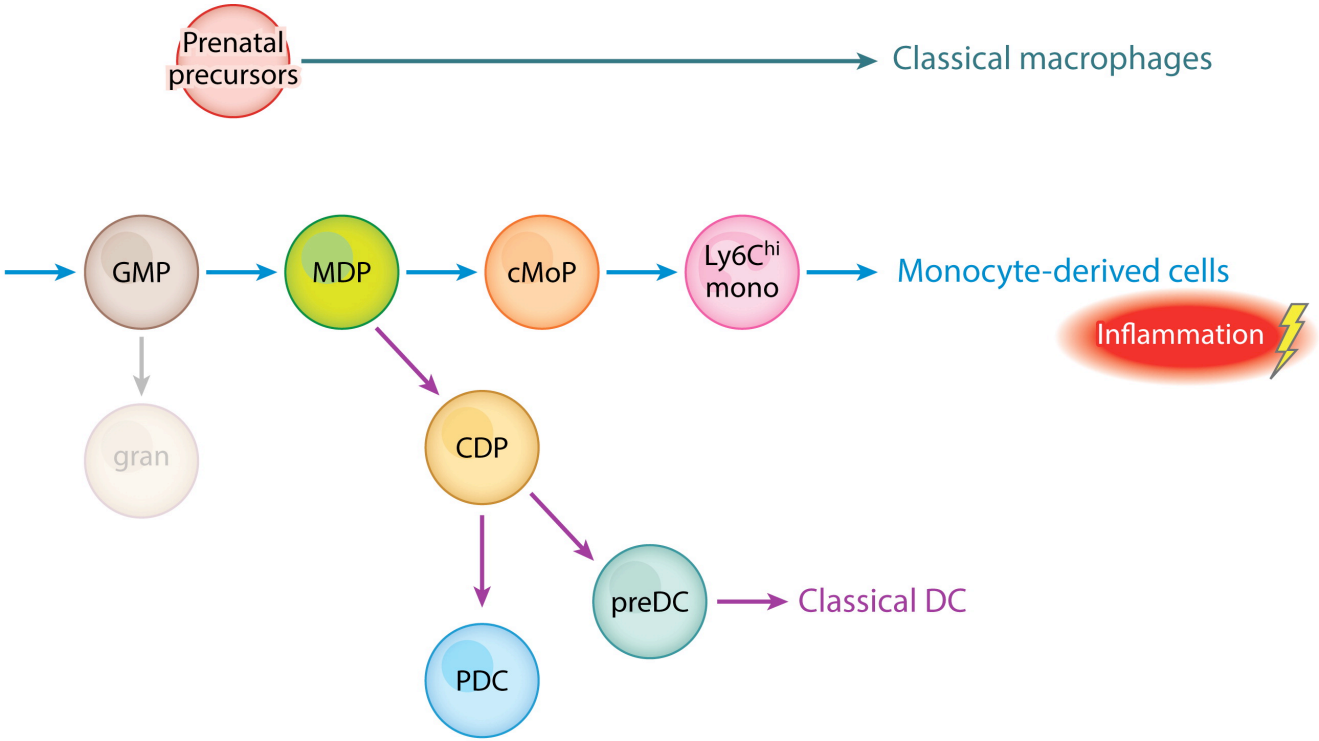
# Other major cellular effectors of innate immunity




## Dendritic Cells

- Conventional versus plasmacytoid
- Major roles in antigen presentation
- Many resident tissue types

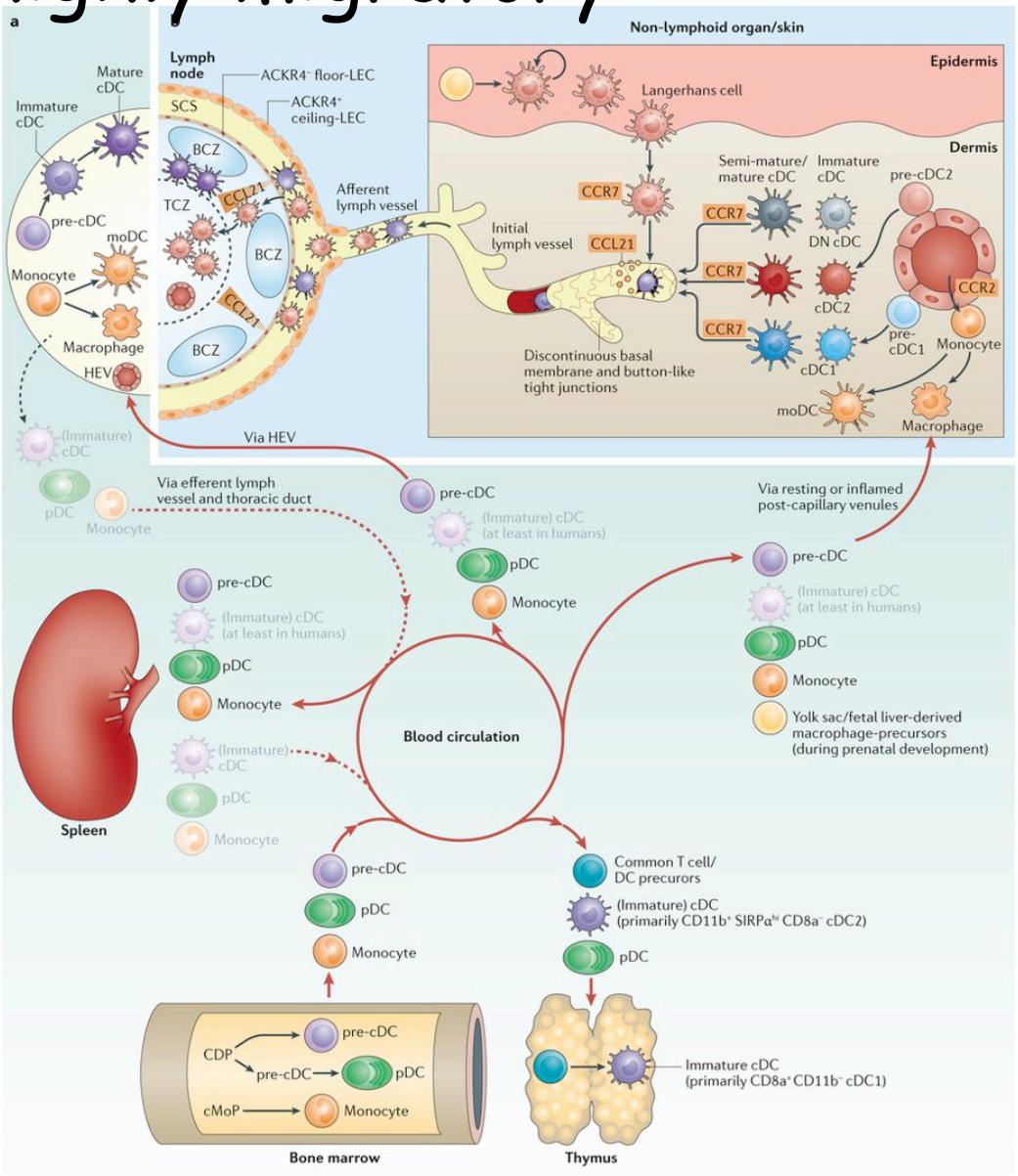
# Monocytes / DCs share common precursor




Varol C, et al. 2015.
Annu. Rev. Immunol. 33:643–75

# DCs are highly migratory

- immature DCs reside in tissues, process Ags (self and foreign)
- upregulate CCR7, respond to CCL21 in LN
- Ag presentation in LN (protective or tolerance inducing)




Worbs et al, Nat Rev Immunol 17:30 (2017)

# DCs subsets – MANY!

- prime different immune responses in different tissues
- different between mouse and man
- generally: cDCs, pDCs, monocyte-derived MoDCs
  - cDCs → classical DC types, subtypes
  - pDCs → plasmacytoid DCs, major interferon producers
  - moDCs → more tissue resident in nature

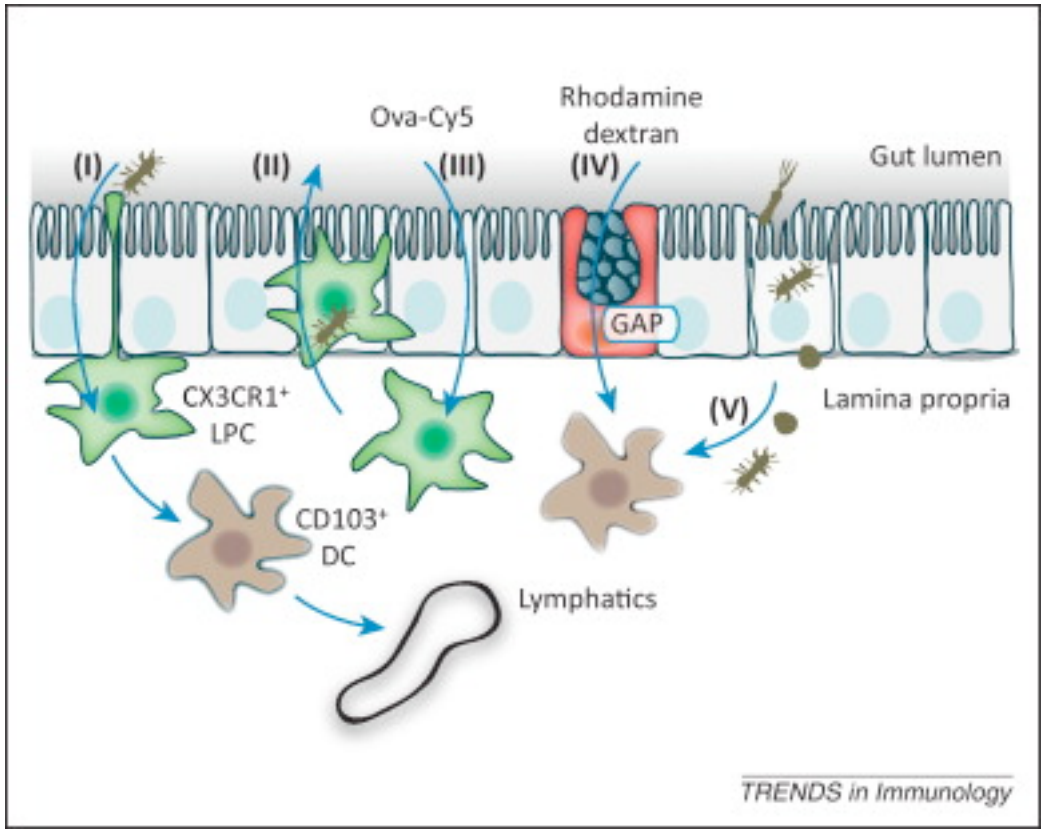
- Examples (all mouse):
  - Dermal cDC1 and cDC2, moDCs, Langerhans cell
  - Intestinal cDCs (CD103+ → involved in tumor immunity)
  - Lung cDCs → more tolerigenic
  - BatF3-dependent, IRF4 dependent

- Array of surface markers, many similar to macs

Worbs et al, Nat Rev Immunol 17:30 (2017); Segura, Dendritic Cell Protocol (Meth Mol Bio) 1423;3 (2016)

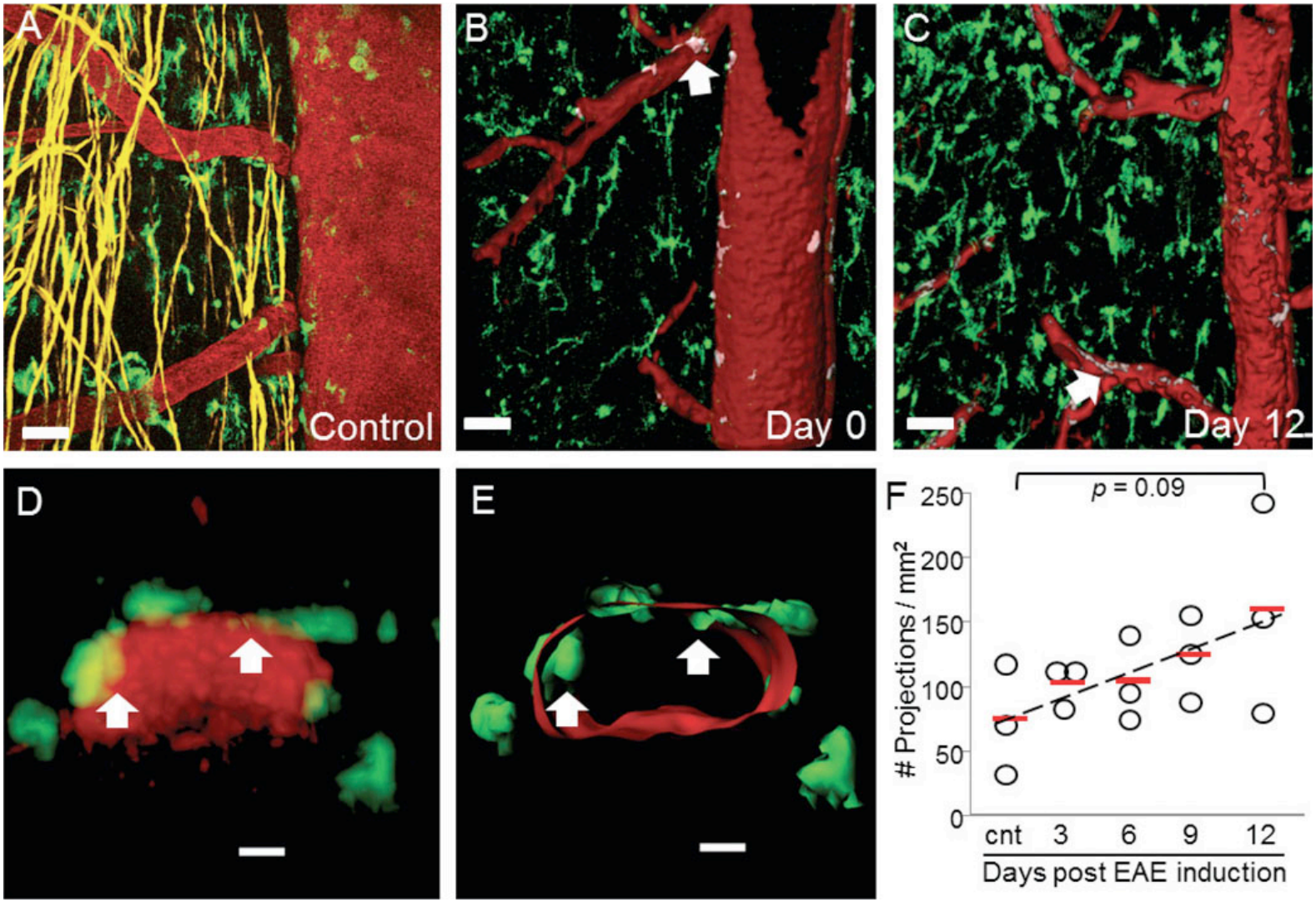
# Antigen sampling by DCs

- extend dendrites between epithelial cells
- pass Ags to migratory DCs
- Can cross the epithelium (less frequent)
- Detect Ags that traverse the endothelium



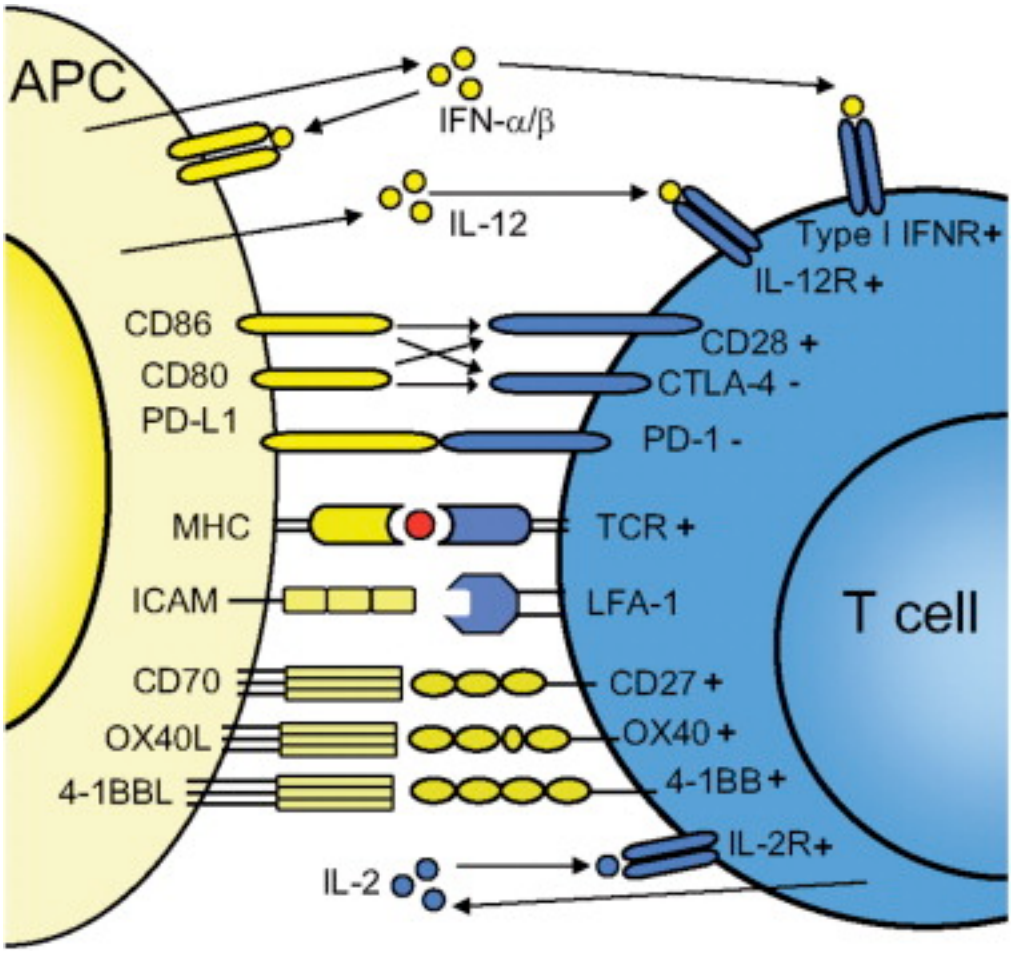

Schulz and Pabst, Trends Immunol 34:155 (2013)

# Antigen sampling by DCs - EAE model




Barkauskas et al, Miroscopy Microanaly 19:778 (2013)

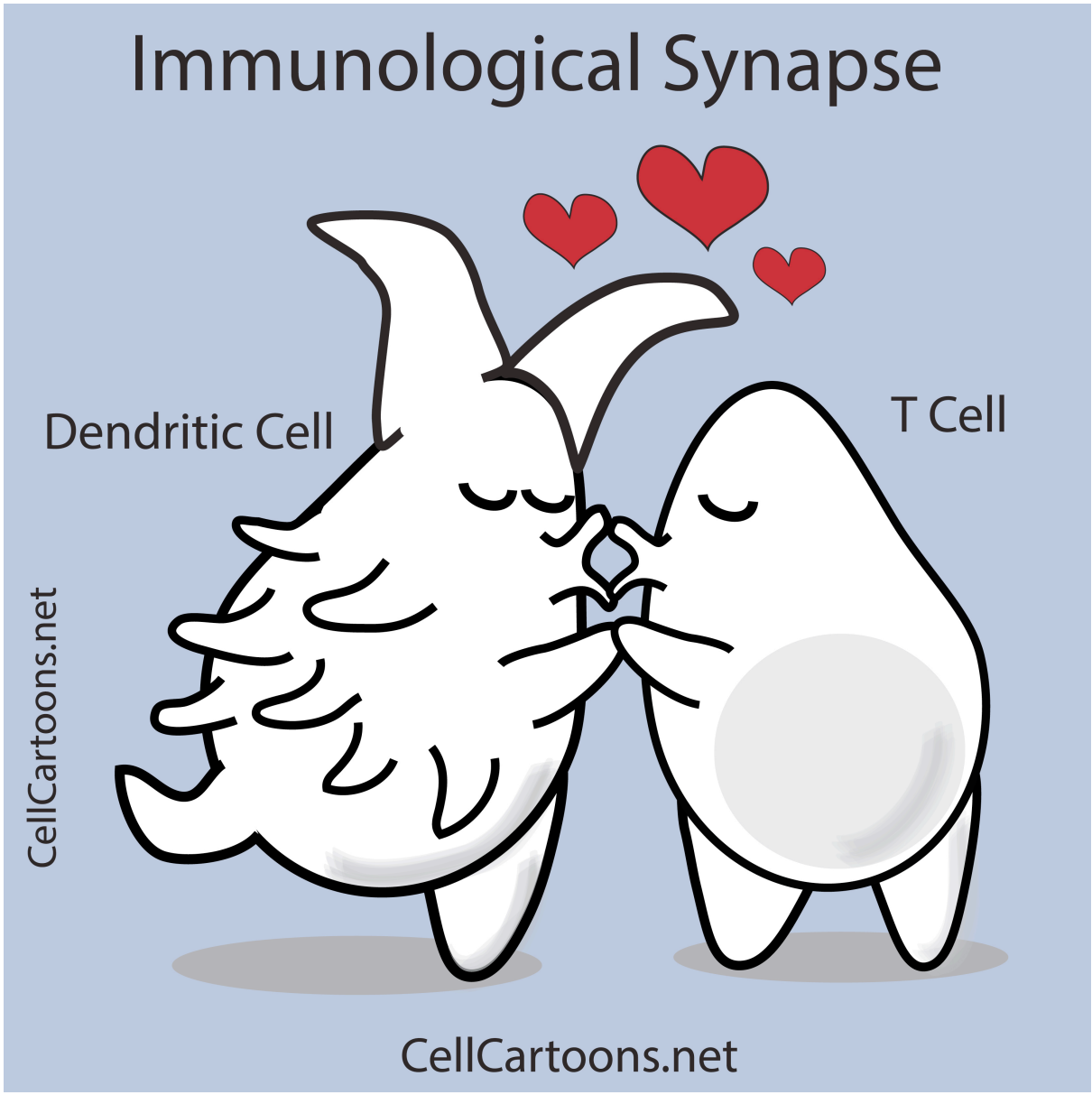
# Antigen presentation in the LN



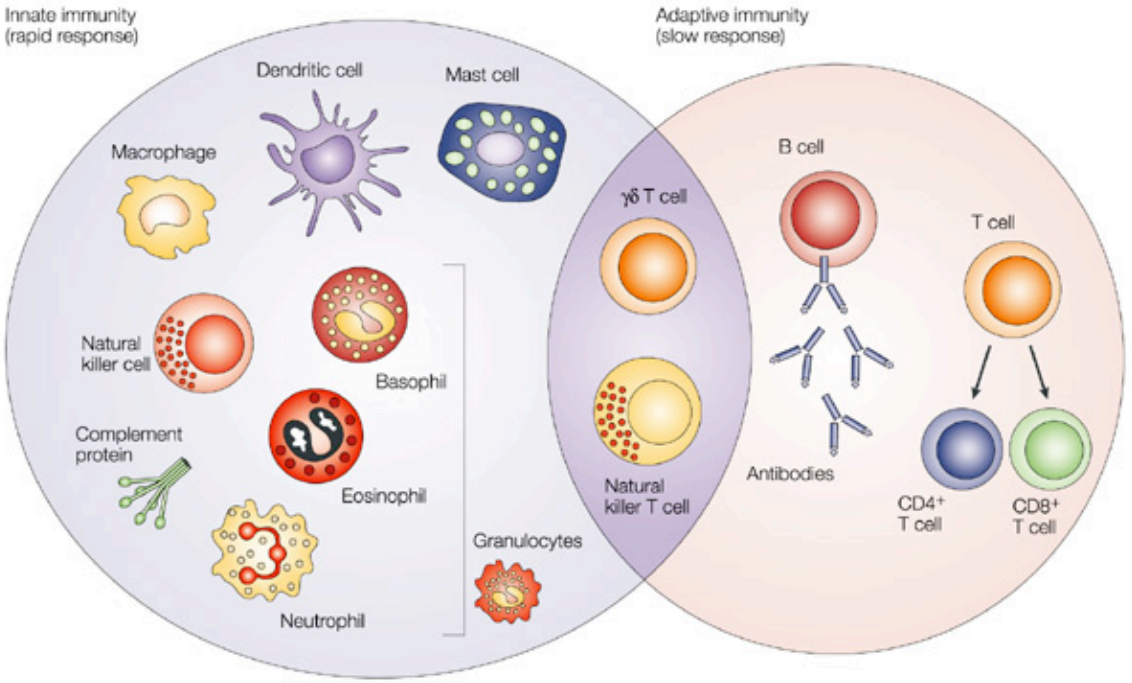

- whole lecture coming up

den Haan et al, Immunol Let 162:103 (2014)

# Immunological Synapse



# Other major cellular effectors of innate immunity



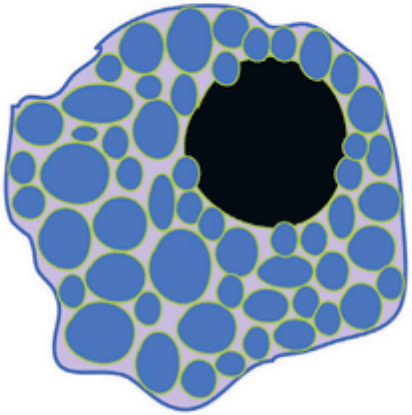

# Mast cells and Basophils

- Mediators of allergic inflammation

# Mast cells vs basophils

- Mast cells → tissue resident near exposed surfaces, long lived
- Basophil → circulating, short lived
- NO LINEAGE relationship

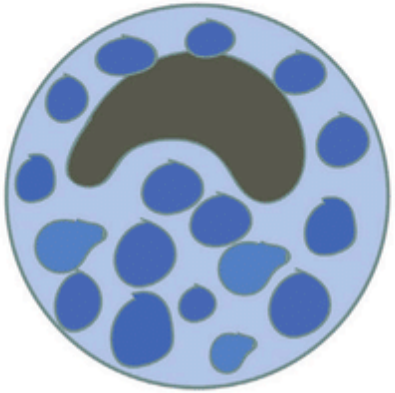
A **Mast cell**



**Granule content**

*Histamine*
*Heparin*
*Serine proteases*
*Carboxypeptidase A3*

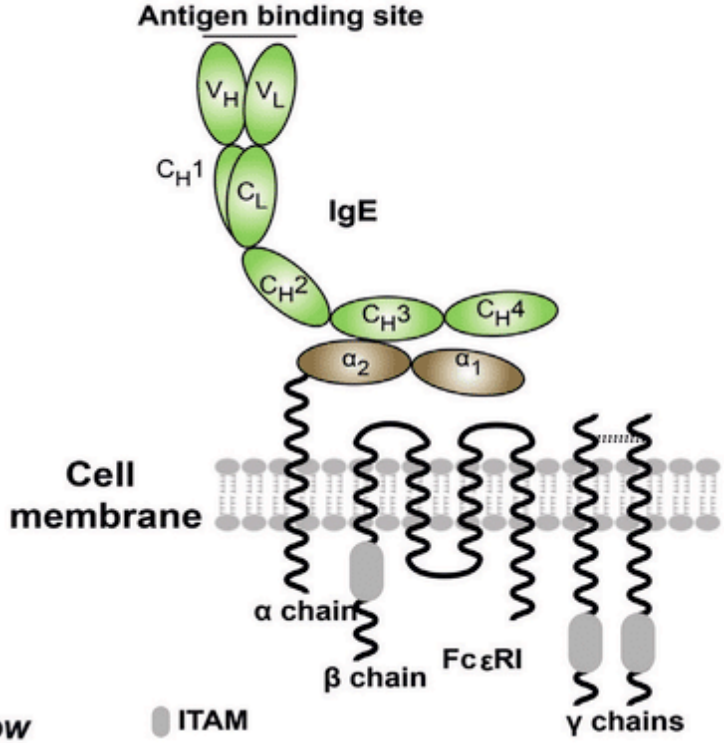
B **Basophil**



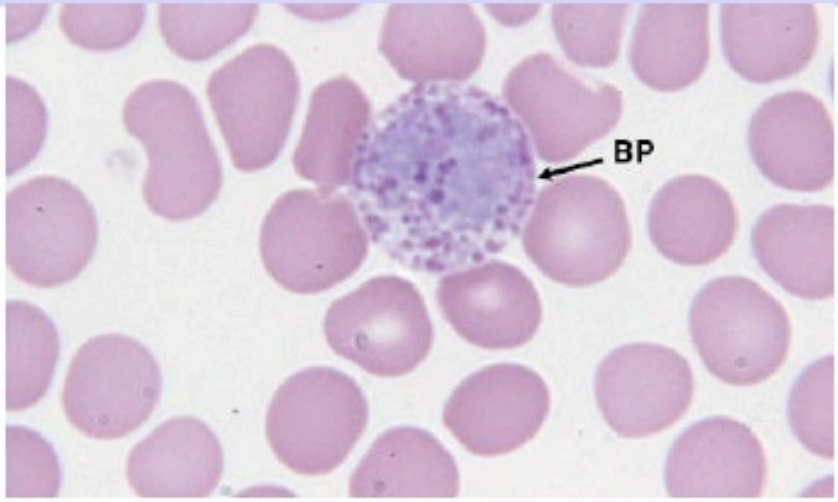
**Granule content**

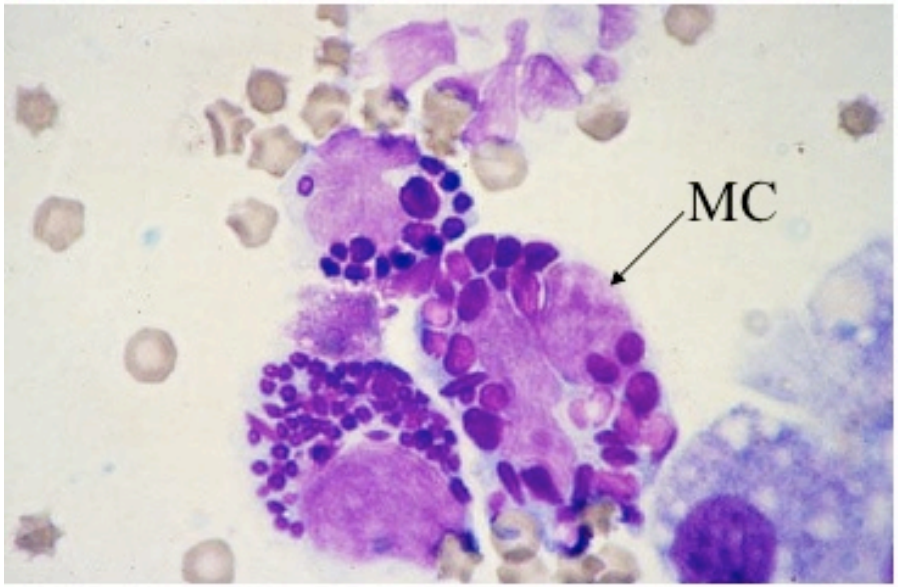
*Histamine*
*Chondroitin sulfate*
*Tryptase (mMCP-8)*
*Carboxypeptidase A3 low*

C **FcεRI with IgE**



# Basophils and mast cells



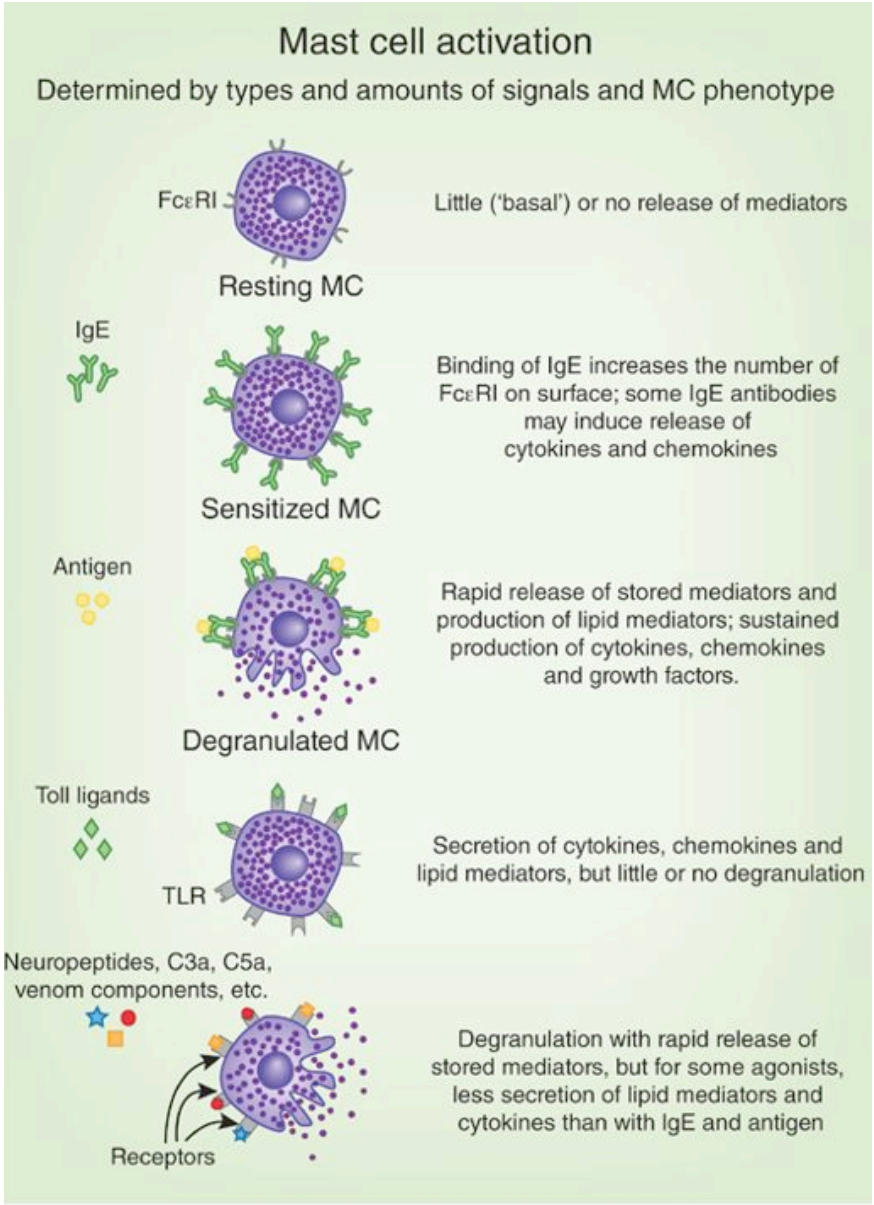

- Basophils and mast cells are the least prevalent of the leukocytes
- They possess high affinity Fc receptors for IgE
- They release the chemical mediators of immediate hypersensitivity, including:
  - Histamine
  - Prostaglandins
  - Thromboxanes
  - Leukotrienes
  - Heparin
- They also produce eosinophil chemotactic factor (ECF)
  - which causes eosinophils to enter the area of worm infestation or allergen localization

# Mast cells

- initiate allergic reactions – anaphylaxis
- also involved in chronic (allergic) type inflammation
  - contact dermatitis
  - forms of autoimmunity
  - forms of vasculitis
- recognize parasitic infections
- heterogeneous, like other tissue resident immune cells
  - mucosal mast cells – MCP4, 5, 6, 7, heparin
  - connective tissue mast cells – MCP 1, 2
- respond to FcεRI crosslinking
  - like macs, also respond to other stimuli (TLRs)
  - additive signaling for maximal response

# Mast cell activation

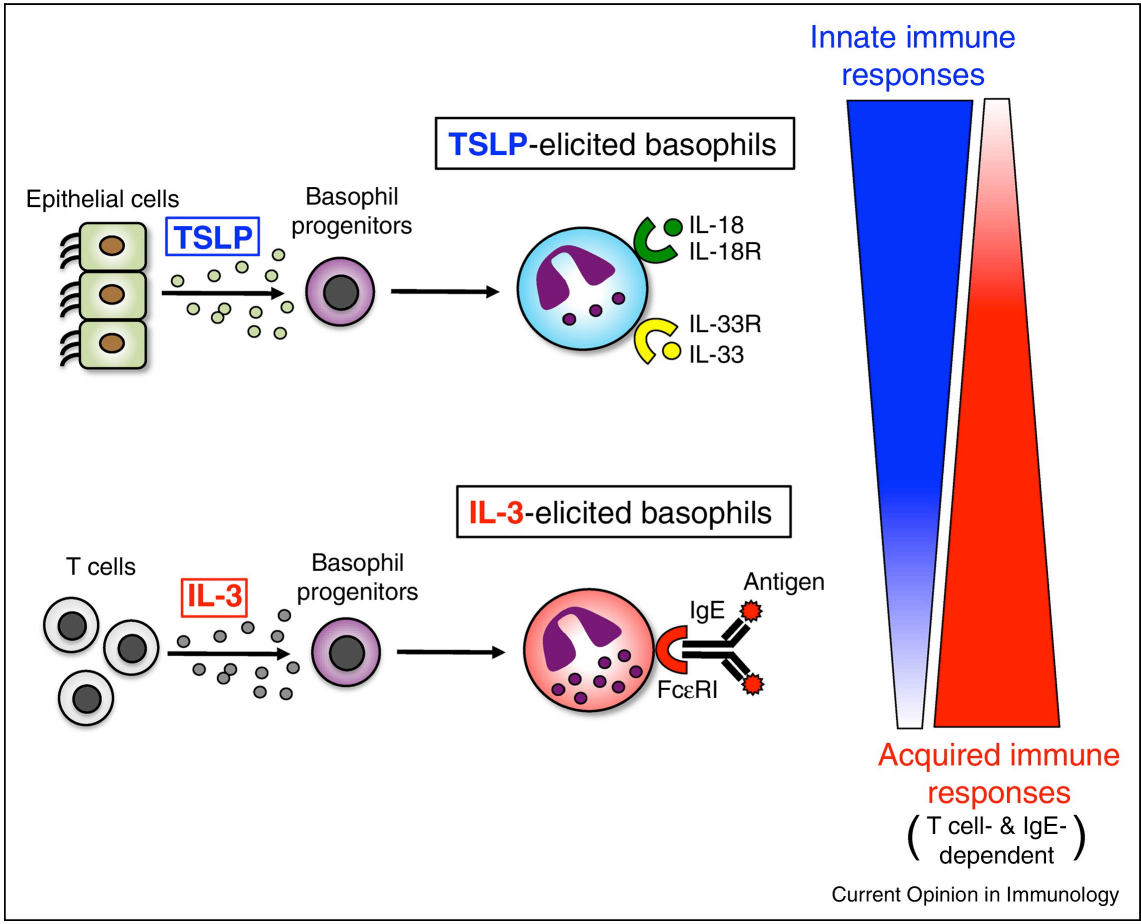
- preloaded with IgE
- allows response to tiny amounts of antigen (think peanut allergy)
- functionally → amplify inflammatory response
- cytokines drive adaptive response




Galli et al, Nat Immunol 12:1035 (2011)

# Basophil activation

- initiate allergic reactions – anaphylaxis (ALSO!!)
- expand and respond to parasite infections (ticks and worms)
- major source of IL4 to drive Th2 immunity
- like neutrophils → can form NETs
- TSLP vs IL-3 stimulation




Karasuyama, Curr Opin Immunol 31:1 (2014)

# Eosinophils

- host defense against helminths
- secondary role in allergic inflammation (asthma)
- pathologic role in eosinophilic tissue inflammation
  - dysregulated tissue repair?
  - intestinal, vascular, mucosal (nasal polyps)

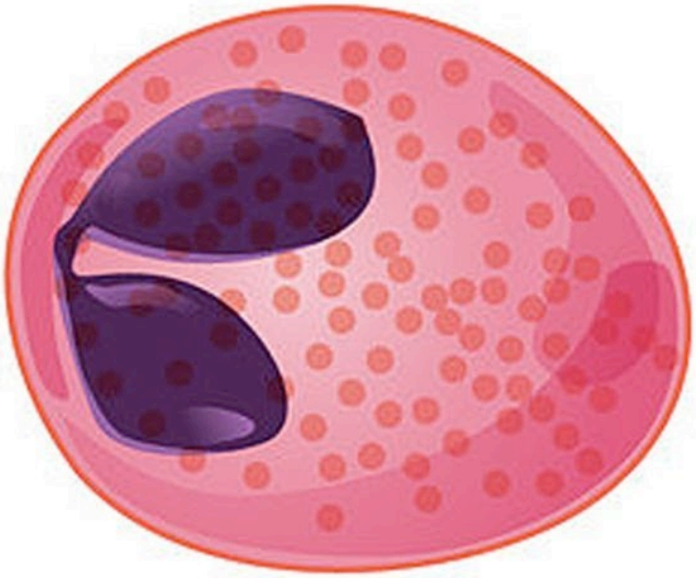
- New roles
  - major role in maintaining Th2 bias in adipose tissue
  - ?tumor surveillance – presence correlated with regression

- Still, experimental evidence for function in immunity is hazy
  - mouse deletion experiments → usually no effect

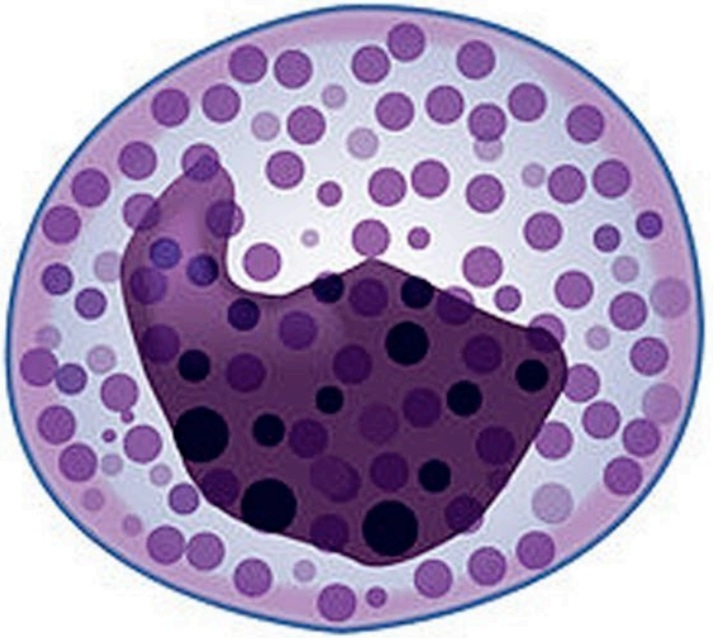
- 1-3% circulating WBCs, different lineage, IL-5 dependent
- Different repertoire of proteases/granule contents
- NETosis

Chusid, J Allergy Clin Immunol 6:1439 (2018)

# Eosinophils - name from their colors

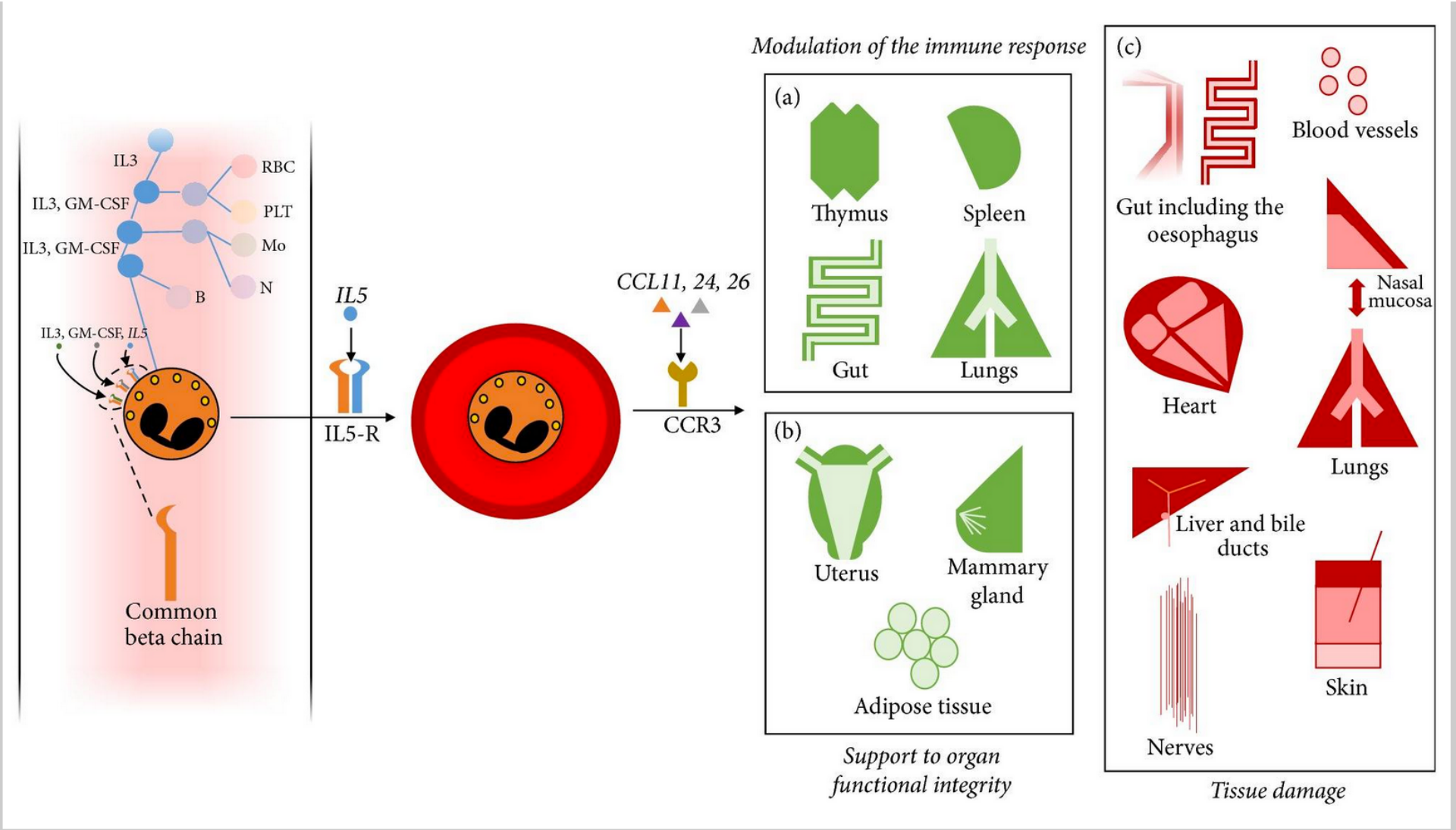


Eosinophil



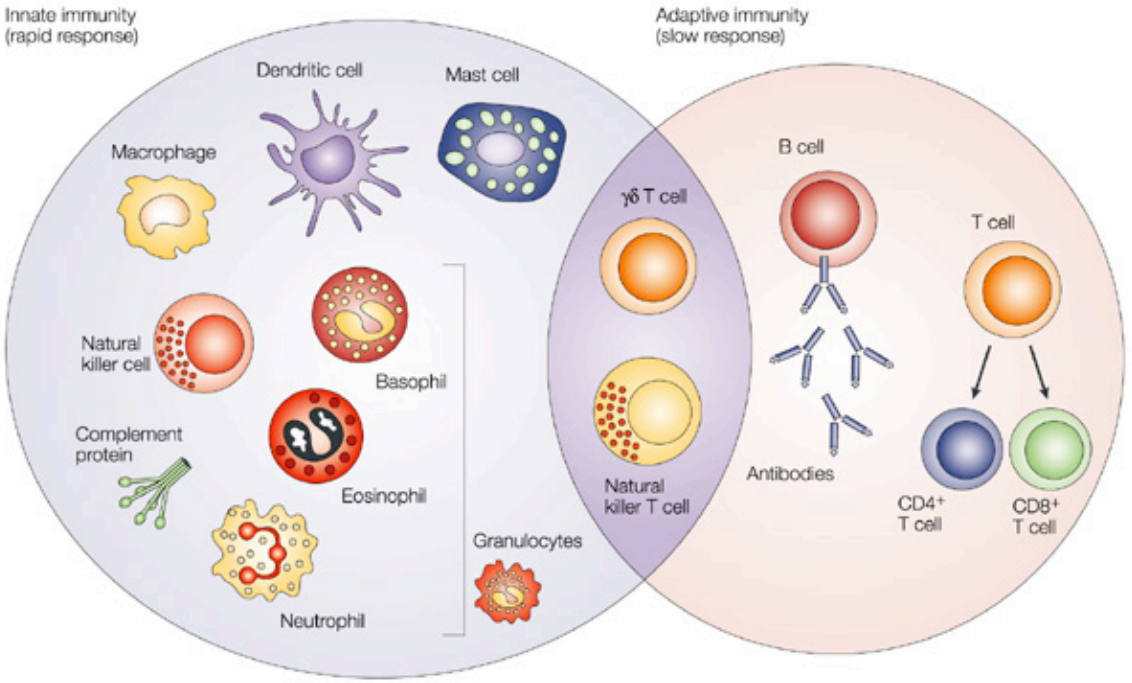
Basophil

# Eosinophils




Ramirez et al, Bio Med Res Jan28;9095275 (2018)

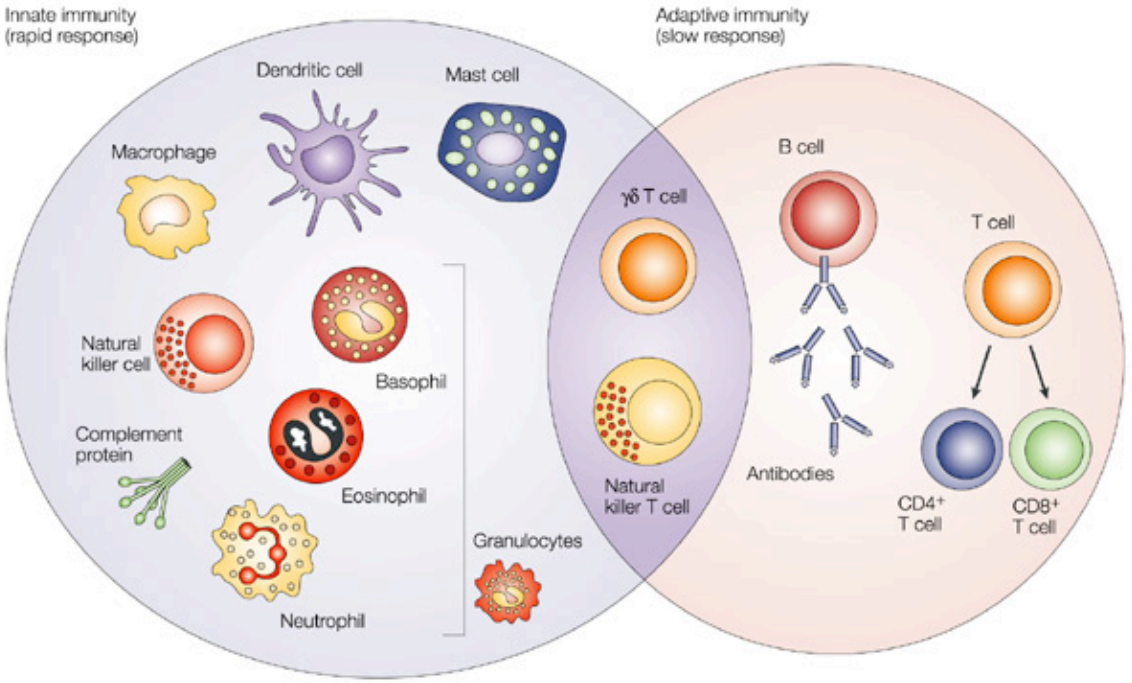
# Other major cellular effectors of innate immunity




## NK cells

- Recognition of stress induced ligands on targets
  --- covered in other lectures

# Other major cellular effectors of innate immunity




# Innate Lymphoid Cells (ILCs)

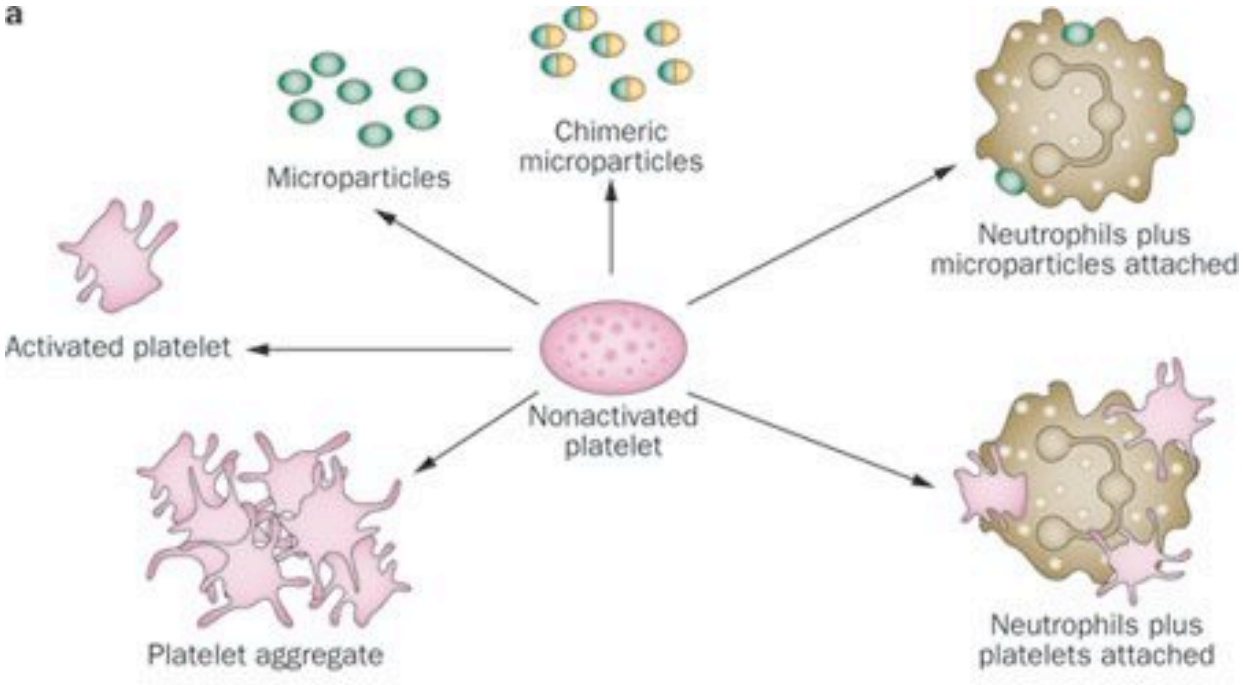
- Parallel T-cell types to drive type 1, type 2, type 17 inflammation
  --- also covered in coming lecture

# Other major cellular effectors of innate immunity

HEY! WHAT ABOUT ME???

THE PLATELET

I'm really important for immunology!

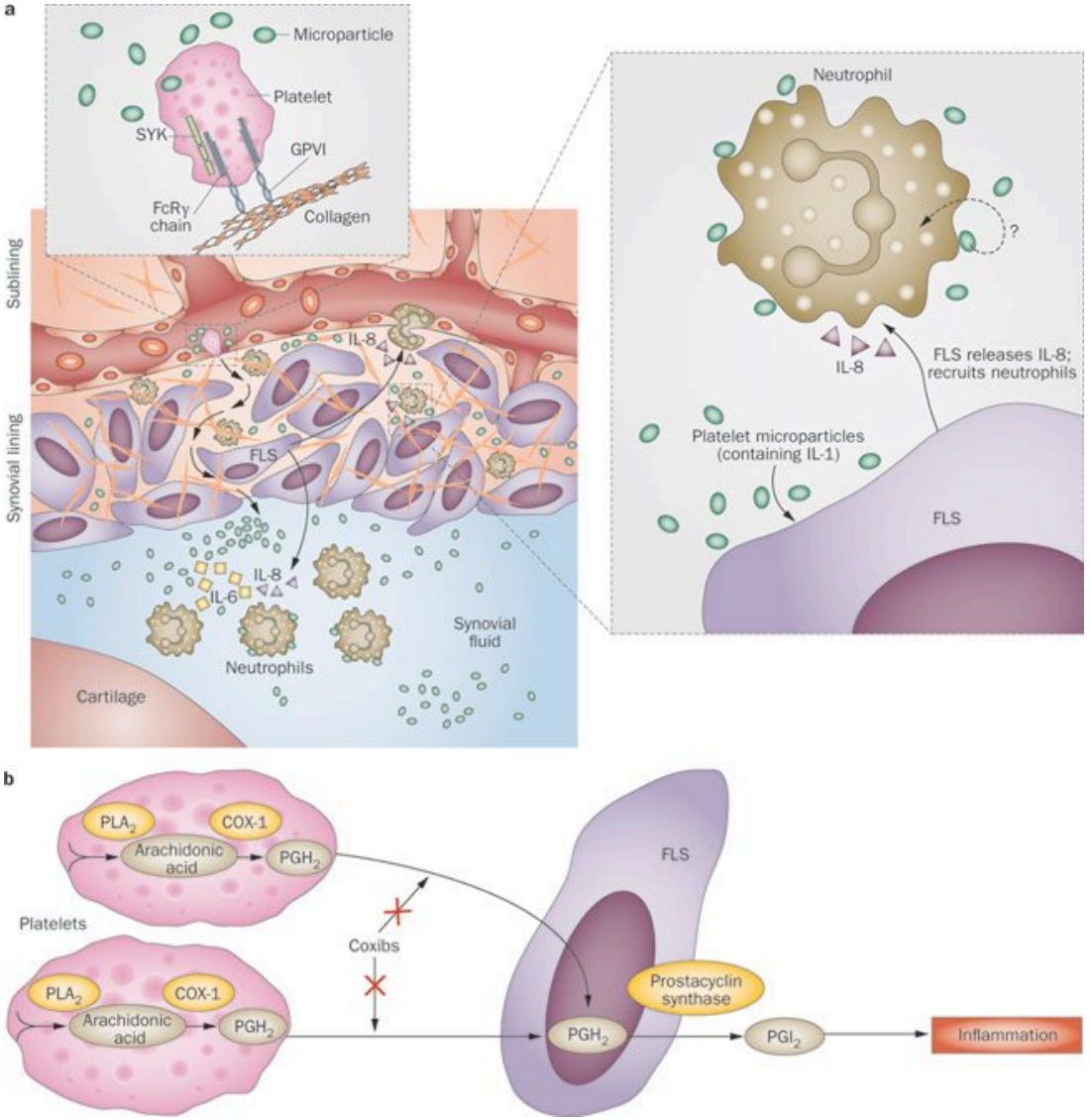



Boilard et al, Nat Rev Rheum 8:534 (2012)

# Platelet neutrophil interactions

- Direct cellular interactions
- major source of cytokines
- microparticles are major feature

- produce a ton of arachidonic acid metabolites




Boilard et al, Nat Rev Rheum 8:534 (2012)

Next lecture:

How immune cells get around