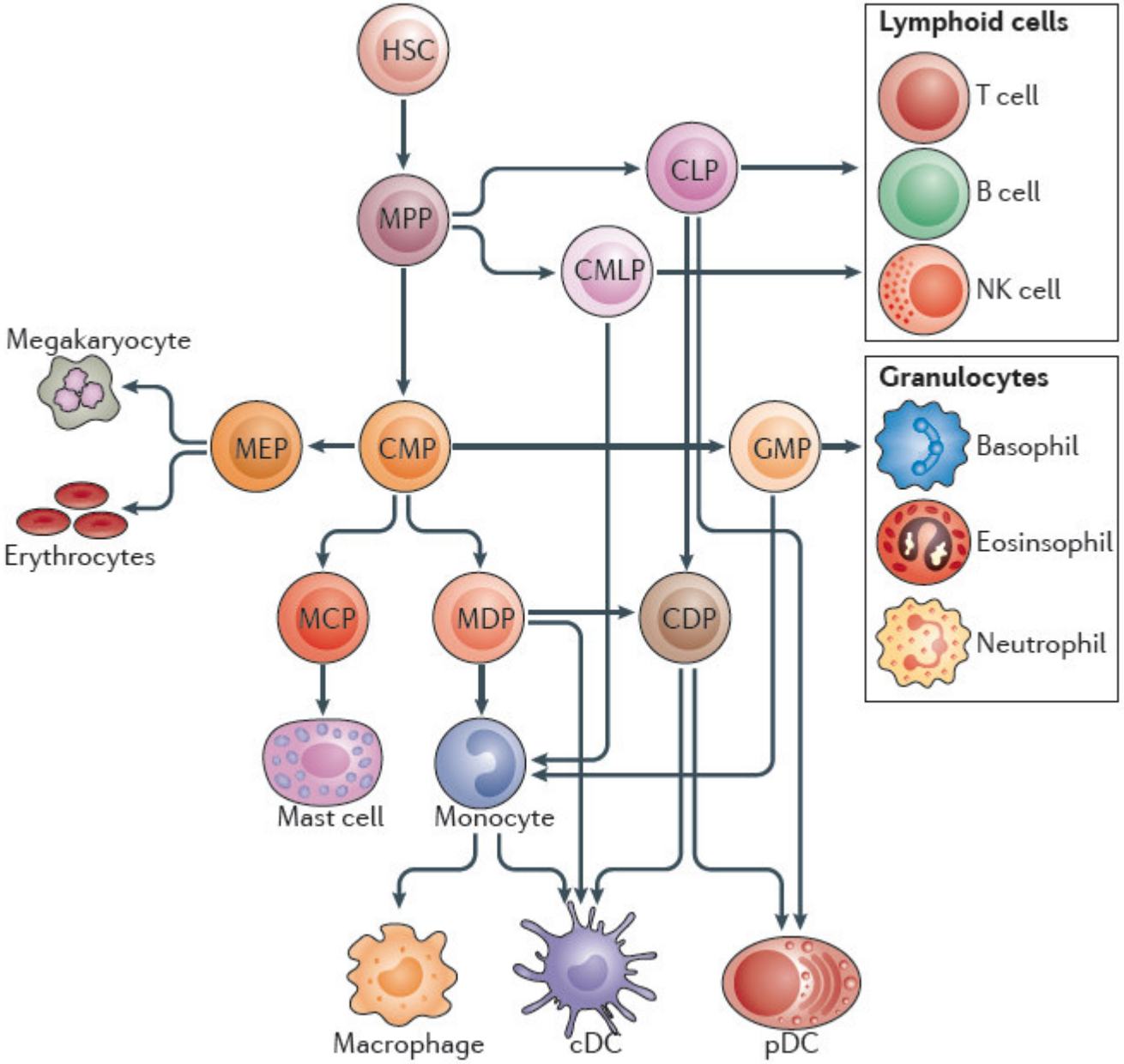
Innate Myeloid Immunity in Cancer

Learning objectives

- Major types of myeloid cells and their ontogeny
- Effector mechanisms: phagocytosis, radicals and immune regulation
- Chronic inflammation and myeloid cell polarization

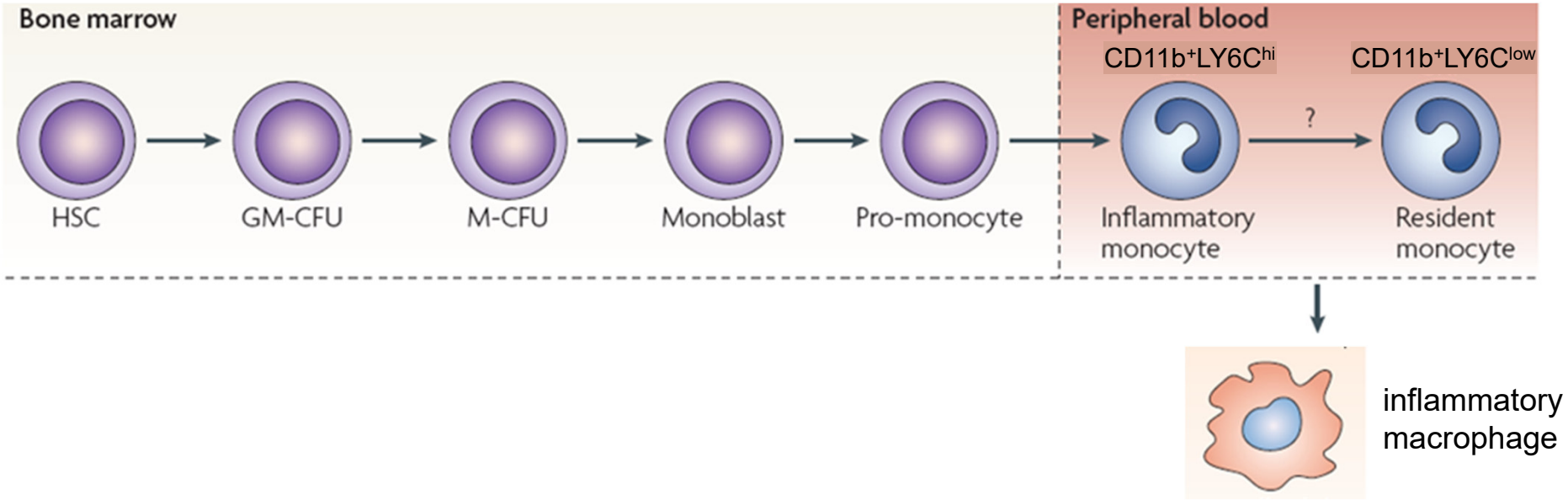
Adult hematopoiesis



cDC	conventional DC
CDP	common DC progenitor
CLP	common lymphoid progenitor
CMLP	common myelolymphoid progenitor
CMP	common myeloid progenitor
DC	dendritic cell
GMP	granulocyte and macrophage progenitor
MCP	mast cell progenitor
MDP	macrophage and DC progenitor
MEP	megakaryocyte and erythroid progenitor
NK	natural killer
pDC	plasmacytoid DC

Gabrilovich et al. Nat. Rev. Imm. 2012

Origin of monocytes and inflammatory macrophages

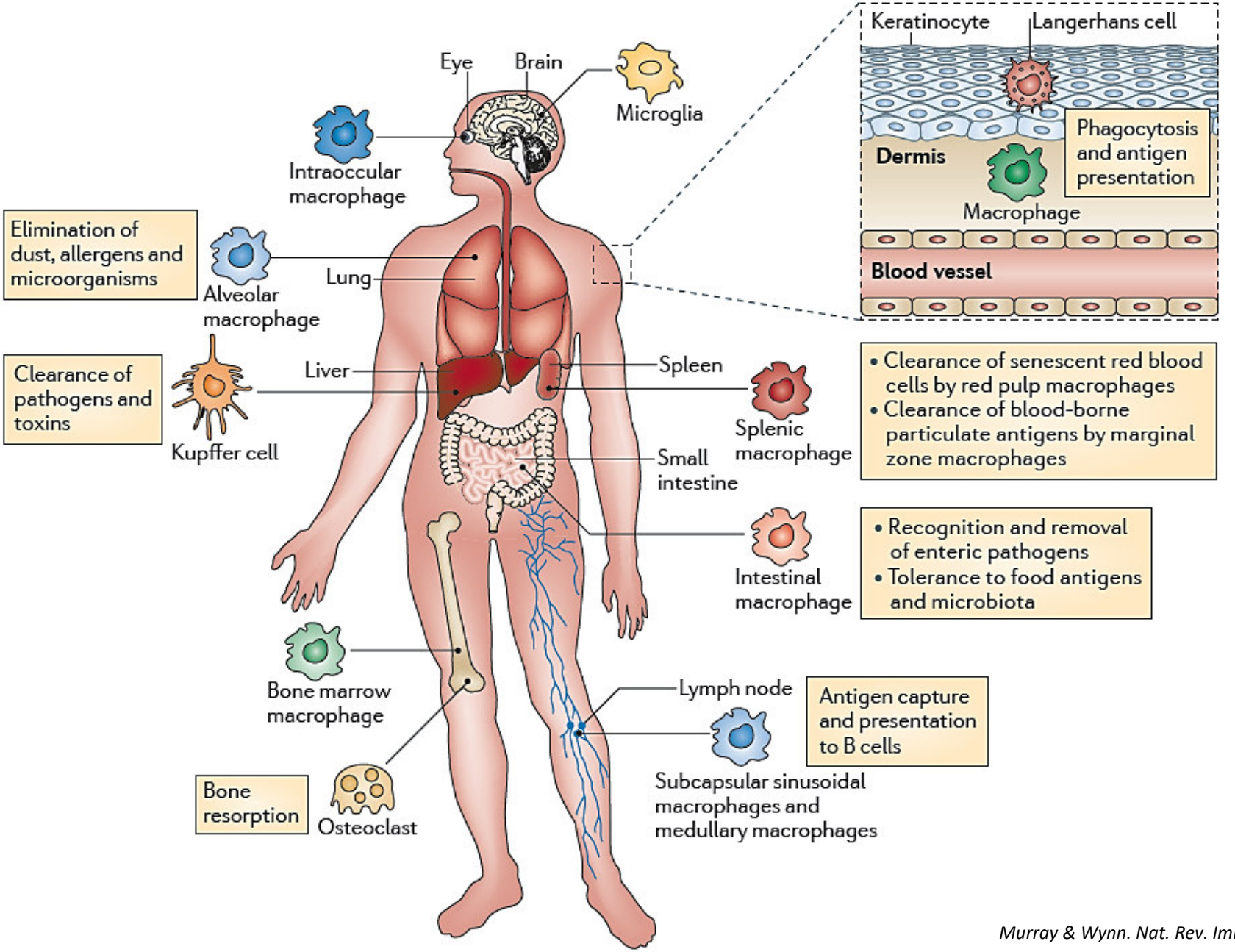


monocytes are constantly generated in the bone marrow and enter the bloodstream as two distinct populations:

- **inflammatory monocytes** ($CD11b^{+}LY6C^{hi}CCR2^{+}$) which rapidly exit the blood
- **blood-resident monocytes** ($CD11b^{+}LY6C^{-low}CX3CR1^{+}$) which circulate and patrol the luminal side of the endothelium of small blood vessels

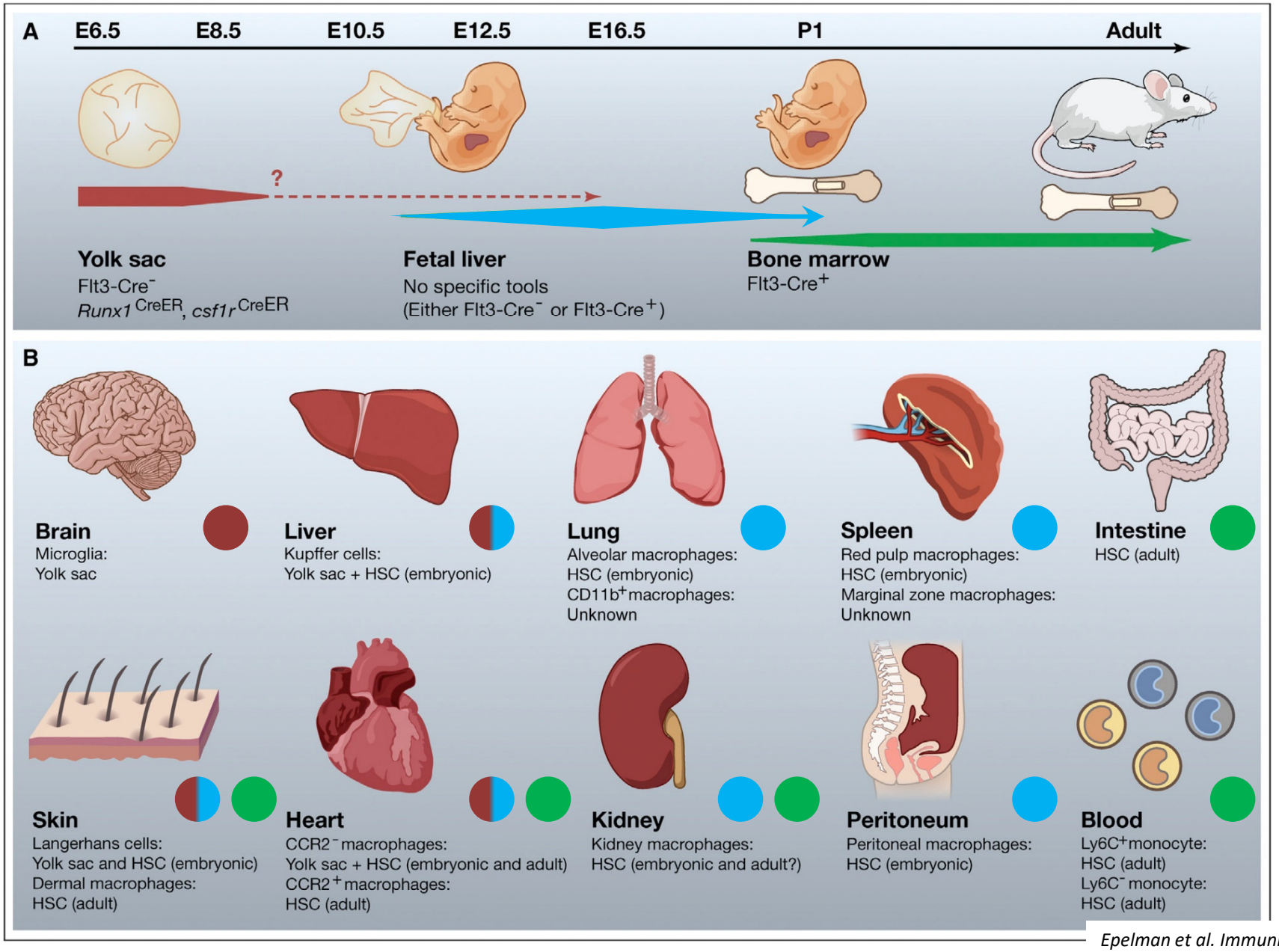
monocytes migrate to different tissues, where they differentiate into inflammatory macrophages (with a bias to M1-type macrophages for inflammatory monocytes and M2-type macrophages for resident monocytes)

Tissue macrophages perform very specific functions



Murray & Wynn. Nat. Rev. Imm. 2011

Ontogeny of tissue-resident macrophages



Epelman et al. Immunity 2014

Tissue-resident macrophages vs. monocyte-derived cells

- **tissue-resident macrophages** can self-maintain throughout life in most organs without necessary input from the bone marrow myeloid lineage (except alveolar macrophages)
- tissue-resident macrophage development is highly dependent on macrophage colony-stimulating factor 1 receptor (**CSF1R**; also known as M-CSFR), which is the receptor for the cytokines colony stimulating factor 1 (**CSF1**; also known as **M-CSF**) and **IL-34**. These cytokines are crucial for the differentiation and survival of most macrophages (but are also required for monocytes)
- each macrophage population is specifically adapted to its tissue of residence
- monocytes consist of two subtypes : LY6C^{hi} classical monocytes and LY6C^{low} non-classical monocytes. LY6C^{hi} classical monocytes derive from the recently identified cMoP
- undifferentiated **LY6C^{hi} classical monocytes** (inflammatory monocytes) are not only found in the blood but also in several tissues, including the spleen, lymph nodes, skin and lungs; LY6C^{hi} classical monocytes are the definitive **precursors of many mononuclear phagocytes** which influx into tissues upon inflammation and give rise to monocyte-derived DCs, monocyte-derived macrophages or myeloid-derived suppressor cells (MDSCs)
- **LY6C^{low} non-classical monocytes** (resident monocytes) remain mostly within the blood vessels where they patrol the vascular wall
- monocyte-derived cells can express DC markers such as CD11c and MHC class II, and they can present antigen to induce naive T cell activation; they can also express macrophage markers such as F4/80, the tyrosine protein kinase MER (MERTK) and CD64, and they are efficient at phagocytosis

Guilliams et al. Nat. Rev. Imm. 2014

Myeloid Markers

- **Gr1** (antibody recognizing both, Ly6G and Ly6C)
  - Ly6G
    on neutrophils
  - Ly6C
    on inflammatory monocytes
- **CD115** = CSF1R
  on monocytes, macrophages
- **CD11b** = Integrin αM (ITGAM) = Mac1 = CR3
  on monocytes, granulocytes, macrophages, NK
- **CD11c** = Integrin αX (ITGAX) = CR4
  on dendritic cells, monocytes, macrophages, neutrophils, and some B cells
- **F4/80** = EMR1
  on macrophages
- **MRC1** (Mannose receptor 1)
  on macrophages and dendritic cells
- **CCR2, CCR4** (receptors for CCL2 = MCP1)
  on inflammatory monocytes
- **CD80, CD86** = B7-1, B7-2
  on APC macrophages

Myeloid Markers

Table 1 | **Phenotypic definitions used for isolating different myeloid cell populations**

Tissue	Cell population	Subpopulations	Phenotype
Mouse lymphoid organs	DCs	ND	$CD11c^{+}F4/80^{-}GR1^{-}MHC\text{-}II^{+}$
		Conventional DCs*	$CD11c^{+}CD11b^{+}MHC\text{-}II^{+}CD205^{+}F4/80^{-}GR1^{-}CD115^{low}$
		Plasmacytoid DCs	$CD11c^{+}CD11b^{-}B220^{+}SIGLEC\text{-}H^{+}GR1^{+}F4/80^{-}$
	Monocytes	ND	$CD11b^{+}LY6C^{+}LY6G^{-}CD11c^{-}CD115^{+}$
		Resident monocytes	$CD11b^{+}LY6C^{low}LY6G^{-}CD115^{+}MHC\text{-}II^{-}F4/80^{hi}CD11c^{-}$
		Inflammatory monocytes	$CD11b^{+}LY6C^{hi}LY6G^{-}CD115^{+}MHC\text{-}II^{-}F4/80^{+}CD11c^{-}$
	Macrophages	ND	$F4/80^{+}CD11b^{+}GR1^{-}$
		M1 macrophages	$iNOS^{+}IL\text{-}12^{+}CD86^{+}MHC\text{-}II^{hi}$
		M2 macrophages	$CD206^{+}CD163^{+}CD36^{+}ARG1^{+}MHC\text{-}II^{low}IL\text{-}10^{+}IL\text{-}4R\alpha^{+}$ $FIZZ1^{+}YM1^{+}$
	Granulocytes	Various	$CD11b^{+}LY6G^{+}LY6C^{low}F4/80^{-}CD11c^{-}$
Mouse tumours	MDSCs	ND	$CD11b^{+}GR1^{+}CD11c^{-}F4/80^{+/-}CD124^{+}$
		Polymorphonuclear MDSCs	$CD11b^{+}GR1^{hi}LY6C^{low}LY6G^{+}CD49d^{-}$
		Monocytic MDSCs	$CD11b^{+}GR1^{mid}LY6C^{hi}LY6G^{-}CD49d^{+}$

Myeloid Cell Markers Humans

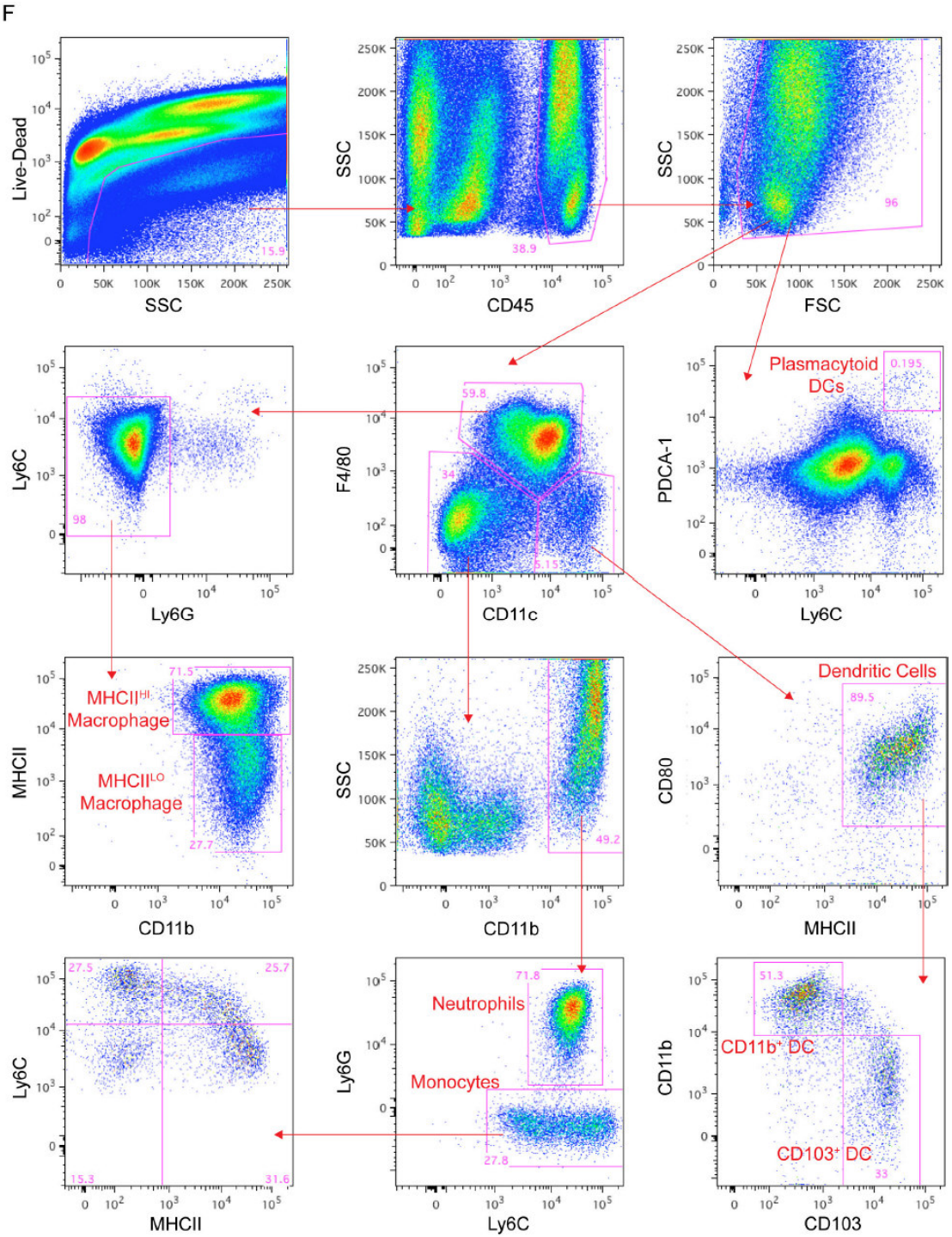
Cell Population	Subpopulations	Markers (those that I use)
All Immune cells	All	CD45
Monocytes	Inflammatory Monocytes	CD45+CD11b+Ly6C^{high}
	Resident Monocytes	CD45+CD11b+Ly6G^{Low}
Macrophages	M1 macrophages	CD45+CD11b+Ly6G-EMR1+MHCII+
	M2 macrophages	CD45+CD11b+Ly6G-EMR1+CD206+
Dendritic Cells	All	CD45+CD11b-CD11c+
Neutrophils	All	CD45+CD11b+Ly6G^{High}
Eosinophils	All	CD45+CD11b+Ly6G$^{neg\text{-}med}$Siglec8+
Myeloid Derived Suppressor Cells	*Monocytic Myeloid Derived Suppressor Cells*	*CD45+CD11b+Ly6G^{low}Ly6C^{high}*
	Granulocytic Myeloid Derived Suppressor Cells	*CD45+CD11b+Ly6G^{+}Ly6C^{Low}*

Note: EMR1 is also called CD68

Notes on Cell markers

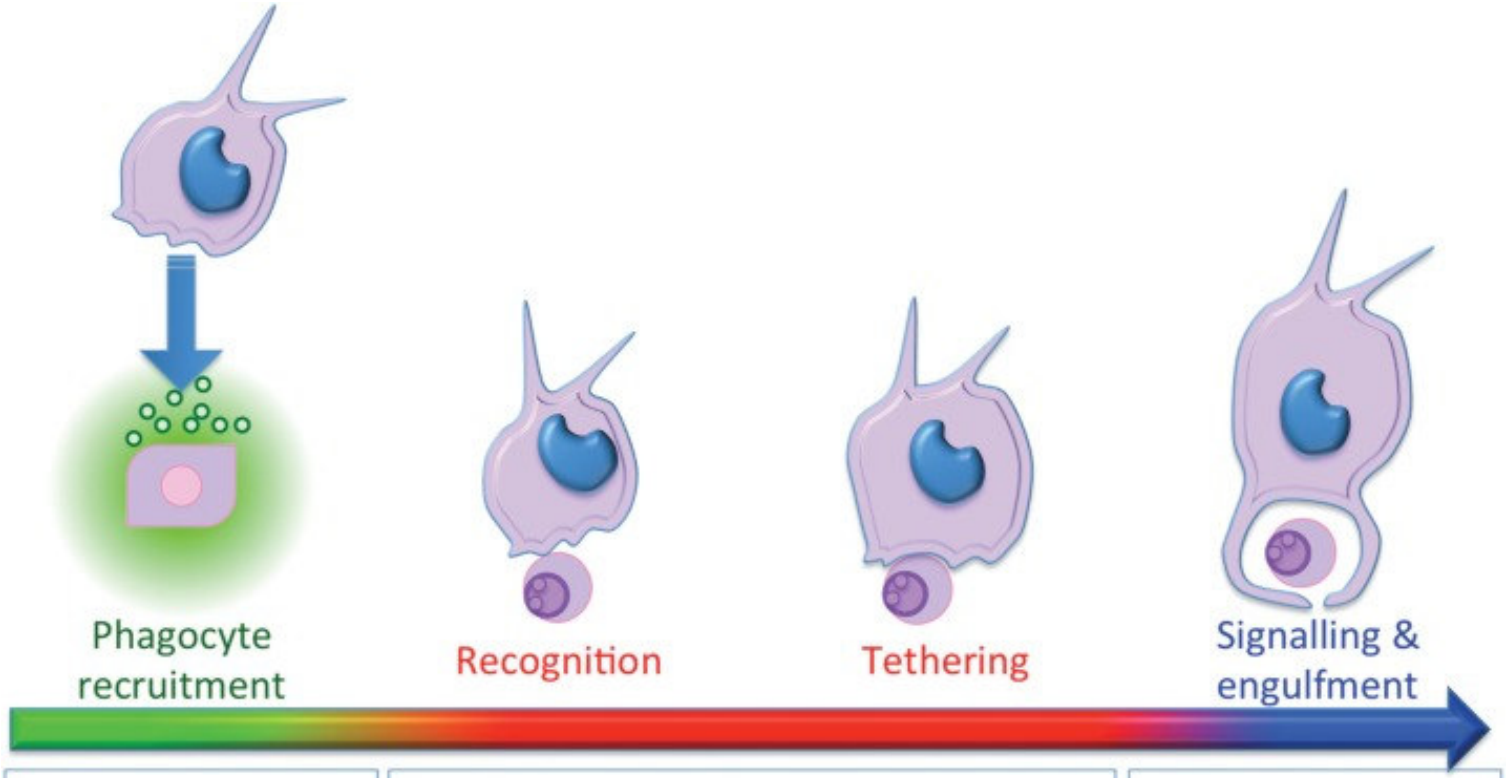
1. GR-1 = Ly6G+Ly6C, GR-1 is often used as a proxy for Ly6G
2. Depending on a cells' polarization and activation status their marker combinations can change
3. Some markers can be expressed on a low, medium or high level. This can change depending on their activation status, their differentiation, etc.
4. There are some cells that do not have one specific and fixed marker set
5. When analysing cell markers, it is crucial to have negative controls, positive controls, FMOs and even compensation beads if necessary.
6. FMO: Fluorescence Minus One, all markers are stained except one. FMOs are used to set gates.
7. Compensation beads: sometimes the fluorescence colors used overlap and this overlap has to be compensated.

Gating strategy for identification of myeloid-lineage populations

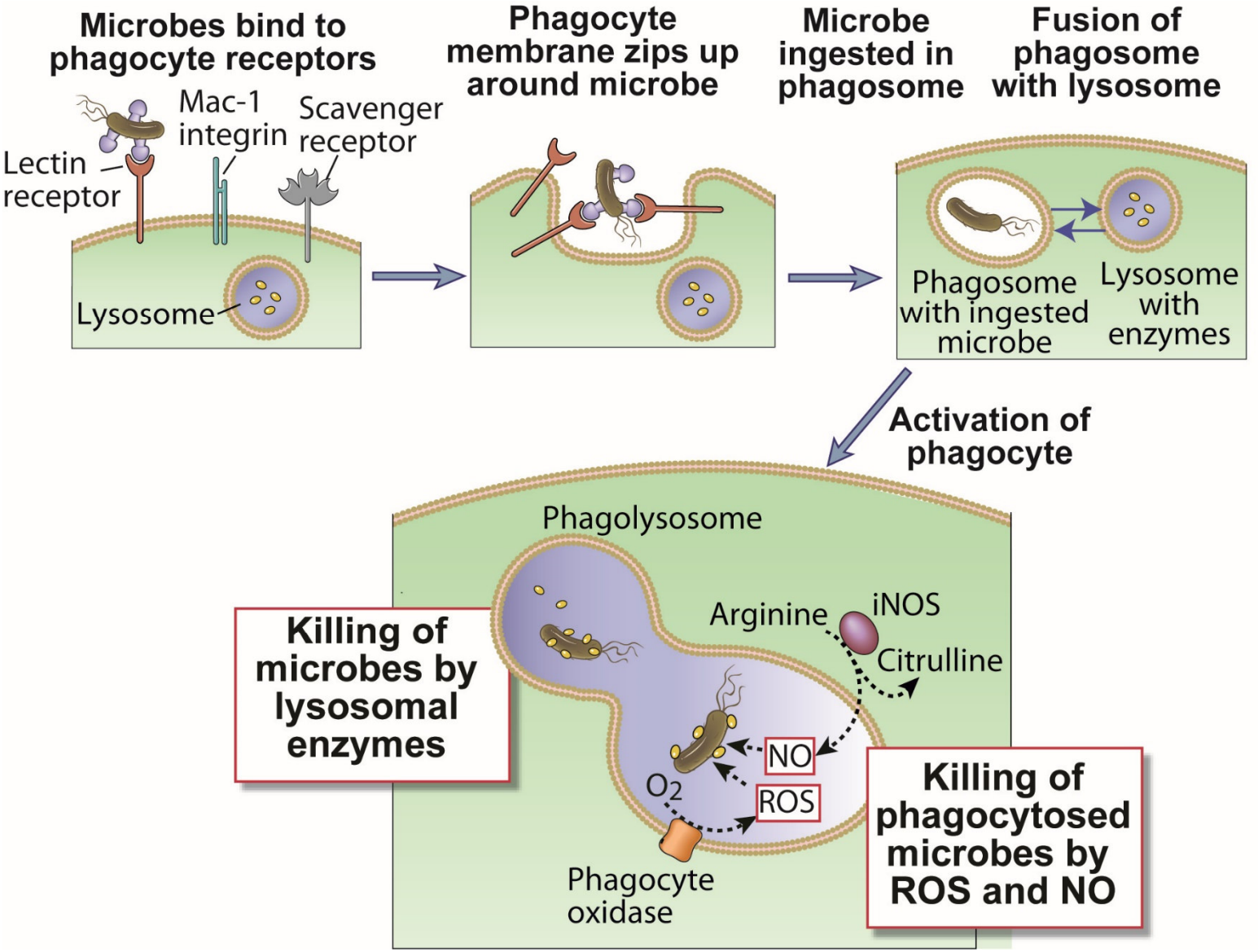


Ruffel et al. Cancer Cell 2014

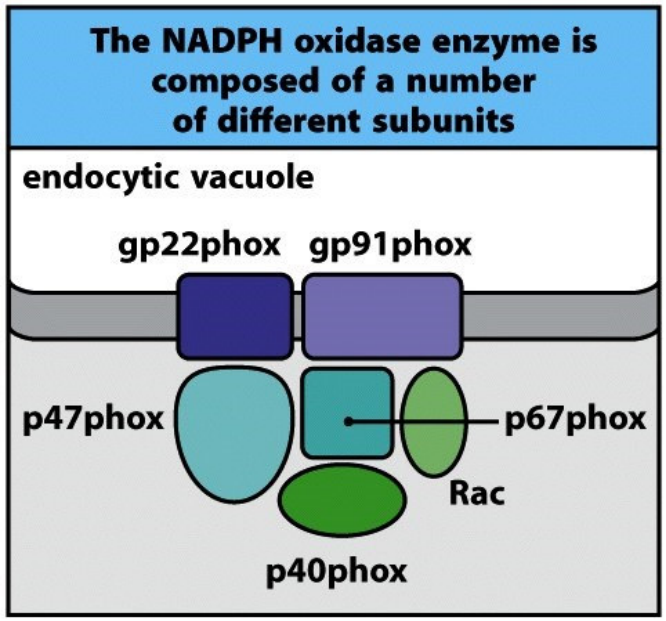
Macrophage phagocytosis



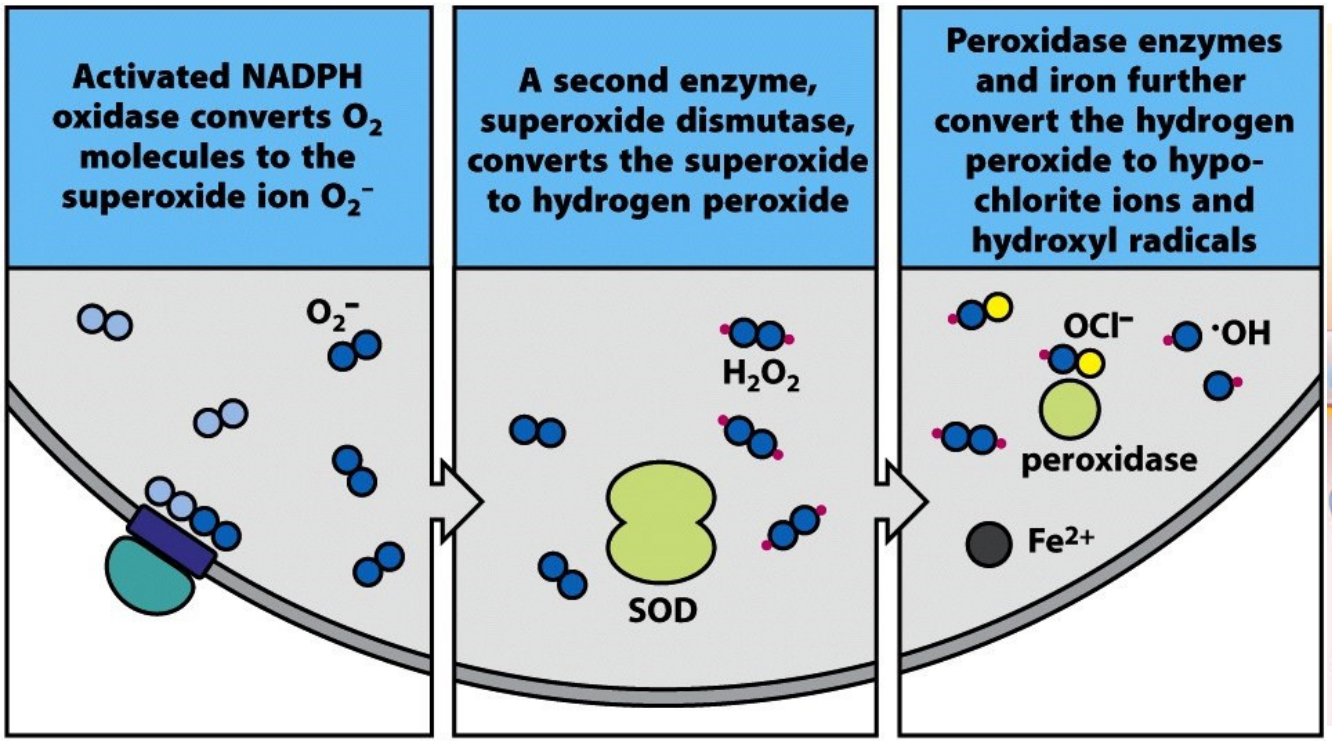
Macropahge phagocytosis of Microbes

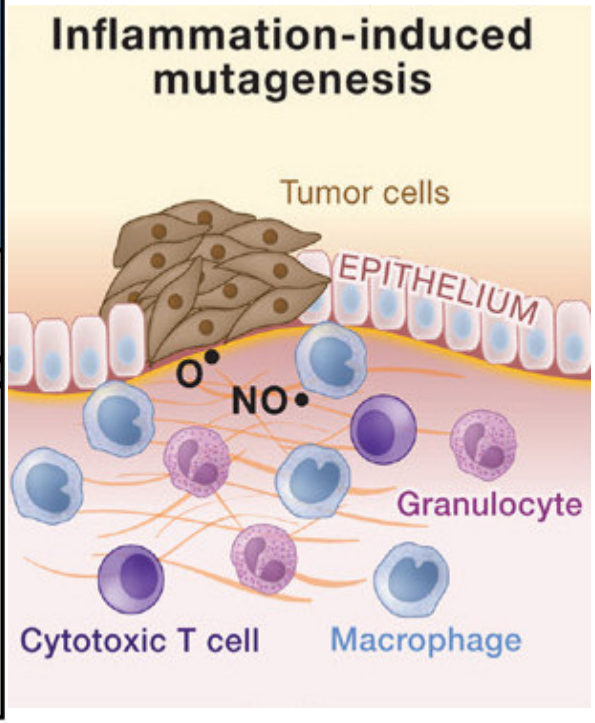


Oxidative burst (ROS generation)

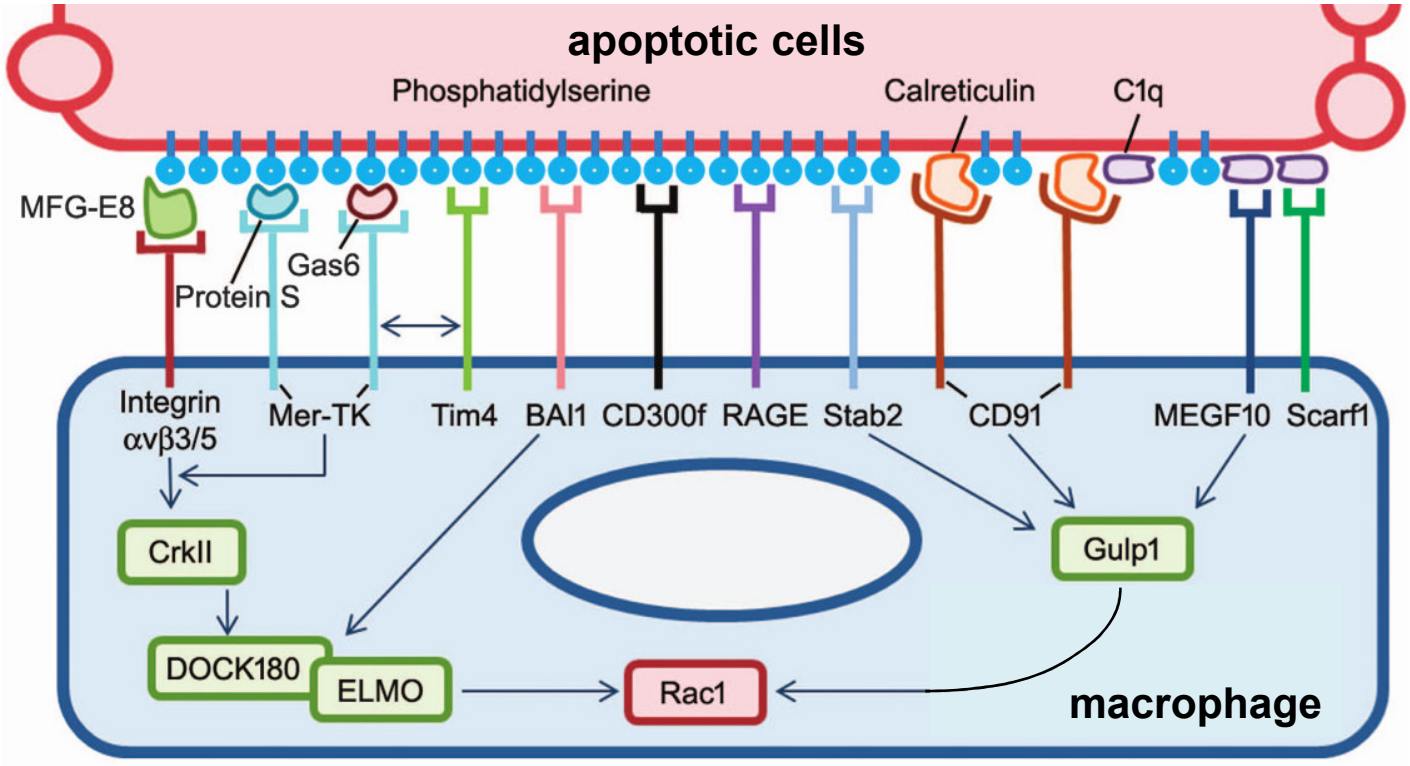


- macrophages and granulocytes can kill pathogens or infected cells by an => oxidative burst
  = release of short and long lived radicals
- NADPH oxidase generates O_2* from NADPH which is then converted to H_2O_2, which can be further processed to HO* and OCl- or react with NO_2 to peroxynitrite
- such radicals can damage DNA in nearby cells increasing the risk of cancer formation/progression





Phagocytosis of apoptotic cells



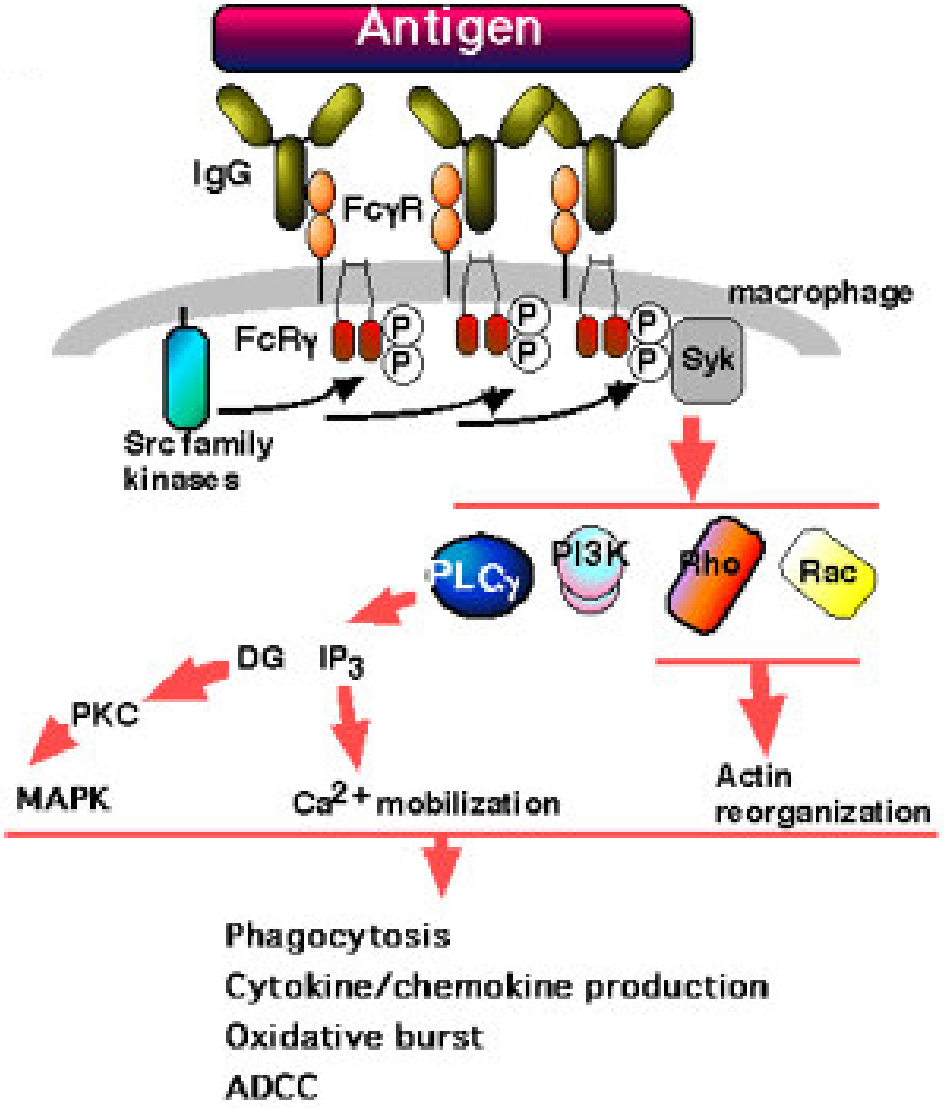
direct PtdSer bdg.

BAI1	functions upstream of the ELMO1–DOCK180–RAC module to mediate apoptotic cell recognition and engulfment; BAI1 interacts with PtdSer through its thrombospondin type 1 repeats
TIM4	no intracellular signaling function; a metal ion-dependent ligand binding site located in the immunoglobulin variable domain of TIMs mediates PtdSer binding
STAB2	Stabilin 2 functions upstream of GULP and thymosin-β4 to aid apoptotic cell clearance; binds PtdSer via its epidermal growth factor-like domain repeats

indirect PtdSer bdg.

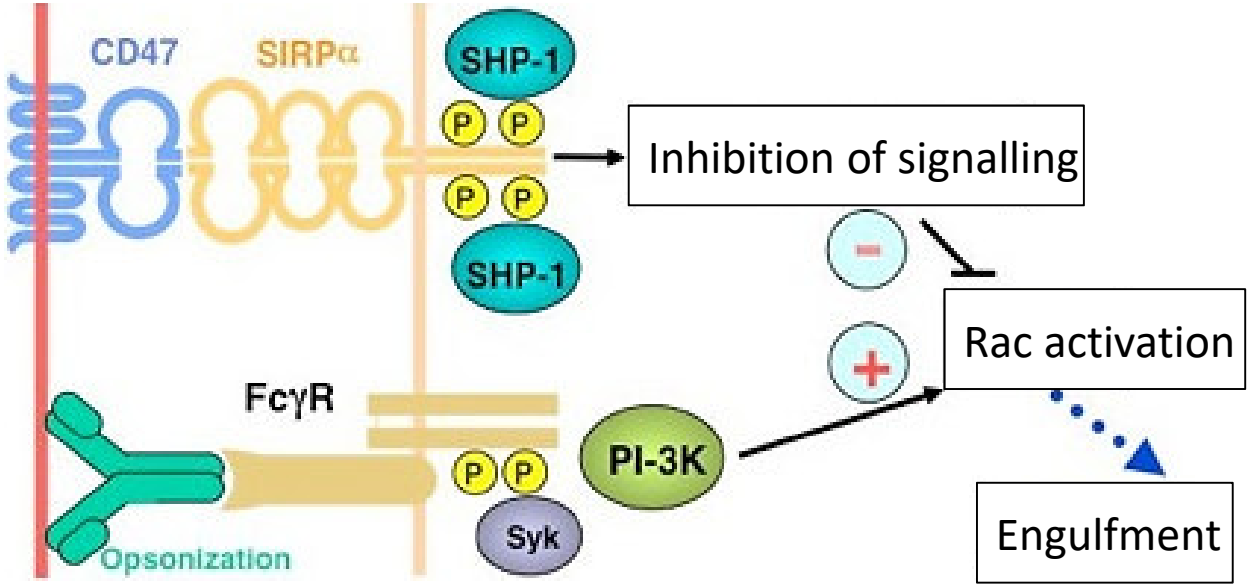
MFGE8	secreted by 'activated' macrophages and immature dendritic cells to promote apoptotic cell
calreticulin	engulfment, interact with PtdSer, uptake via integrin-αvβ3/5 and CD91=Lrp1
Protein S	interact both with PtdSer and Mer-TK receptors via their Gla domains
GAS6	and sex hormone-binding globulin domains, respectively

Phagocytosis of opsonized targets

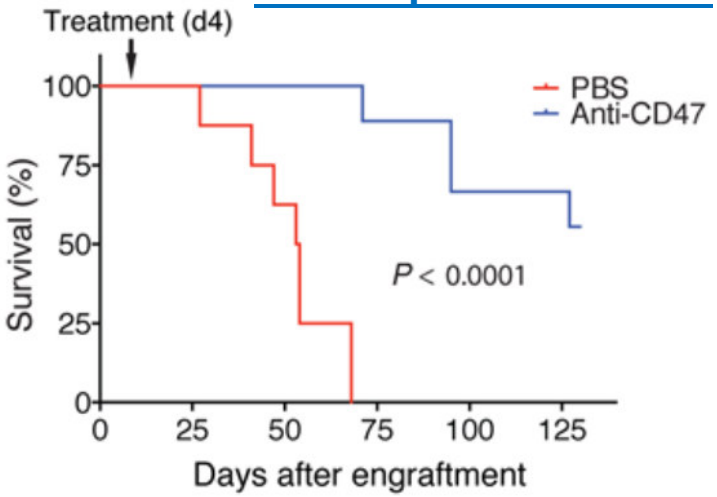


- activation of signaling relies on **ITAM**-bearing domains in the **FcγR** which binds to the constant part of IgG antibodies
- tyrosine residues in the ITAM domain become phosphorylated by **Src family kinases** such as Src, Fyn, Fgr, Hck and Lyn upon cross-linking of cell surface FcγRs by IgG-immune complexes
- recruitment and activation of **Syk** induces actin cytoskeleton reorganization

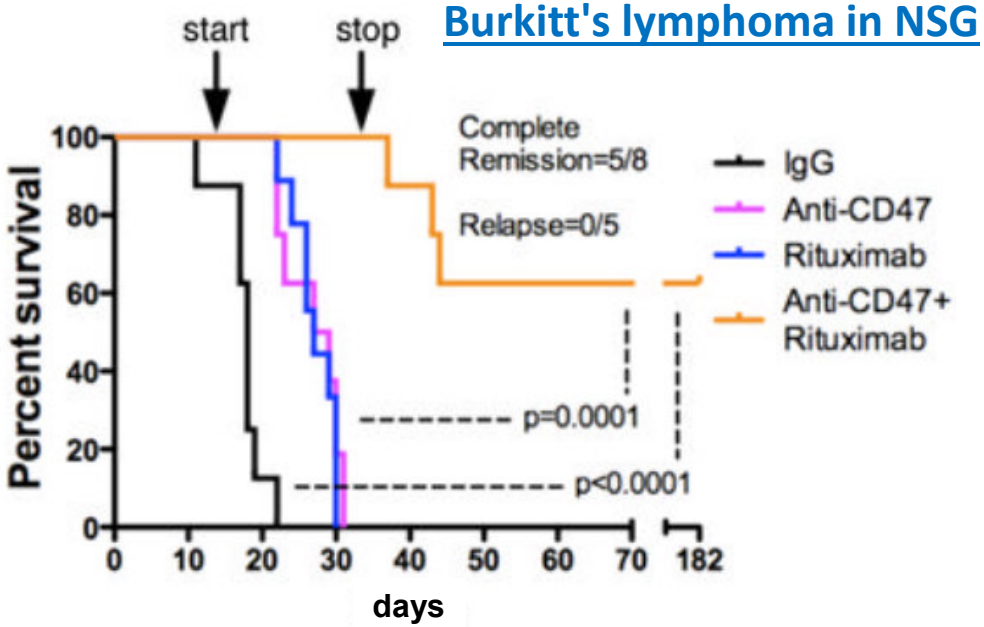
Blocking “Don’t eat me” signals targets various cancer types



orthotopic SCLC in NSG



Burkitt's lymphoma in NSG

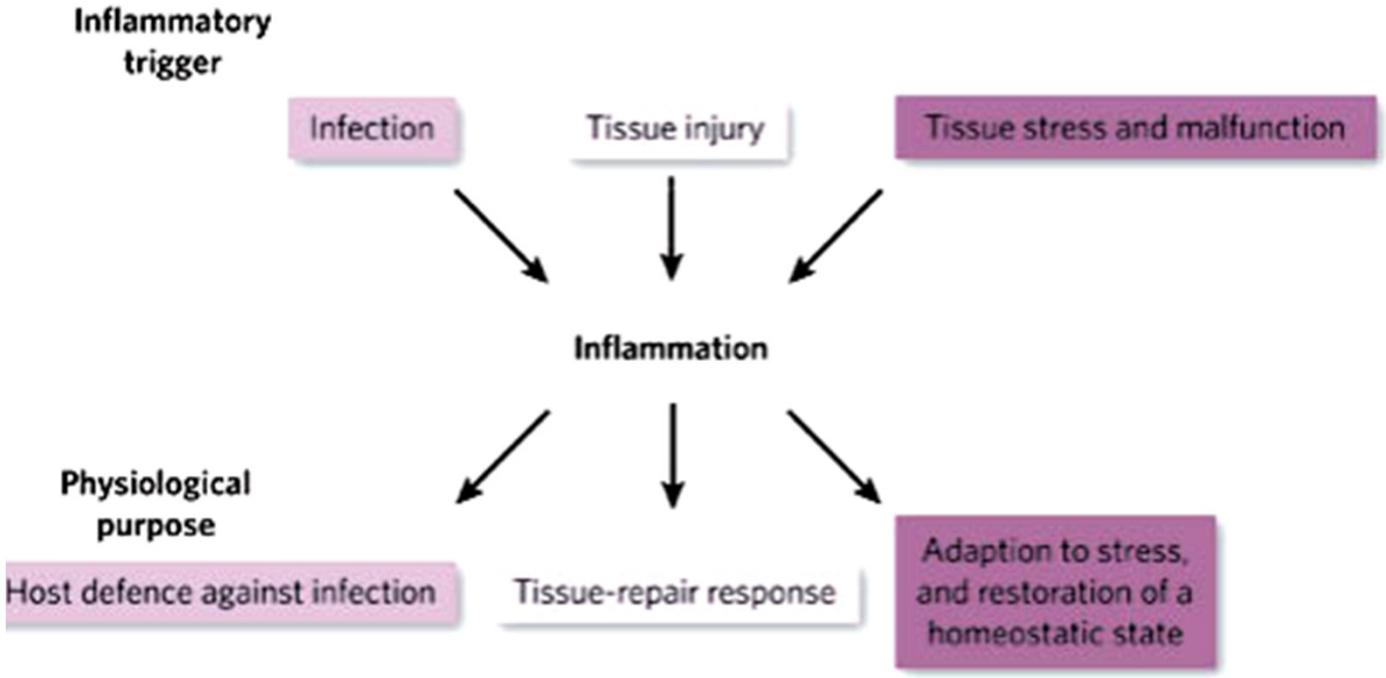


Key concepts about inflammation

inflammation: physiological process whereby tissues respond to **infectious & non-infectious insults** (also called **sterile inflammation**, including toxic, traumatic, or autoimmune insults)

four key signs:

- **Redness**
- **Swelling**
- **Heat**
- **Pain**



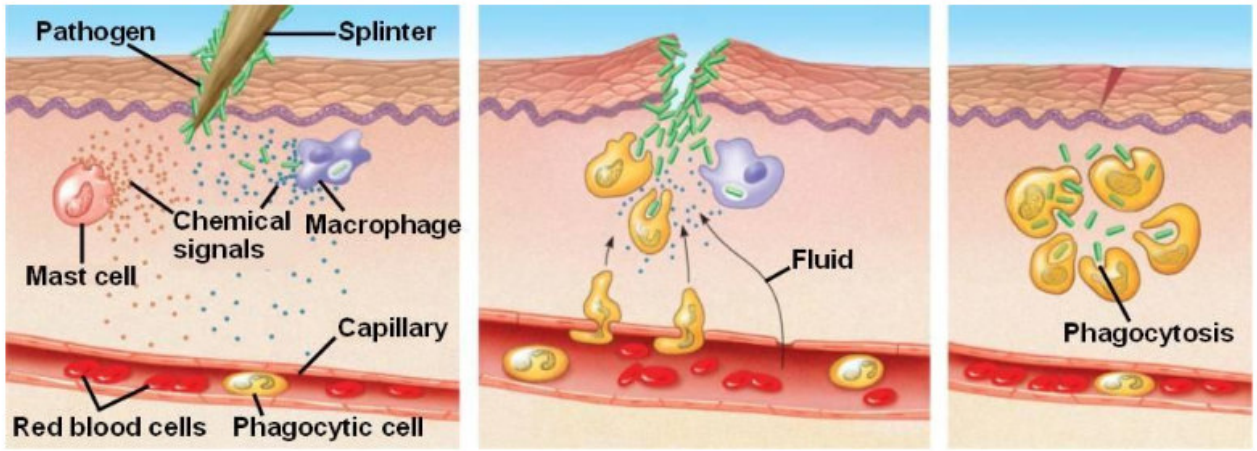
Key concepts about inflammation

inflammation includes several **phases**:

initial phase - changes in local blood flow & **accumulation of inflammatory cells** (neutrophiles, macrophages, DCs & lymphocytes)

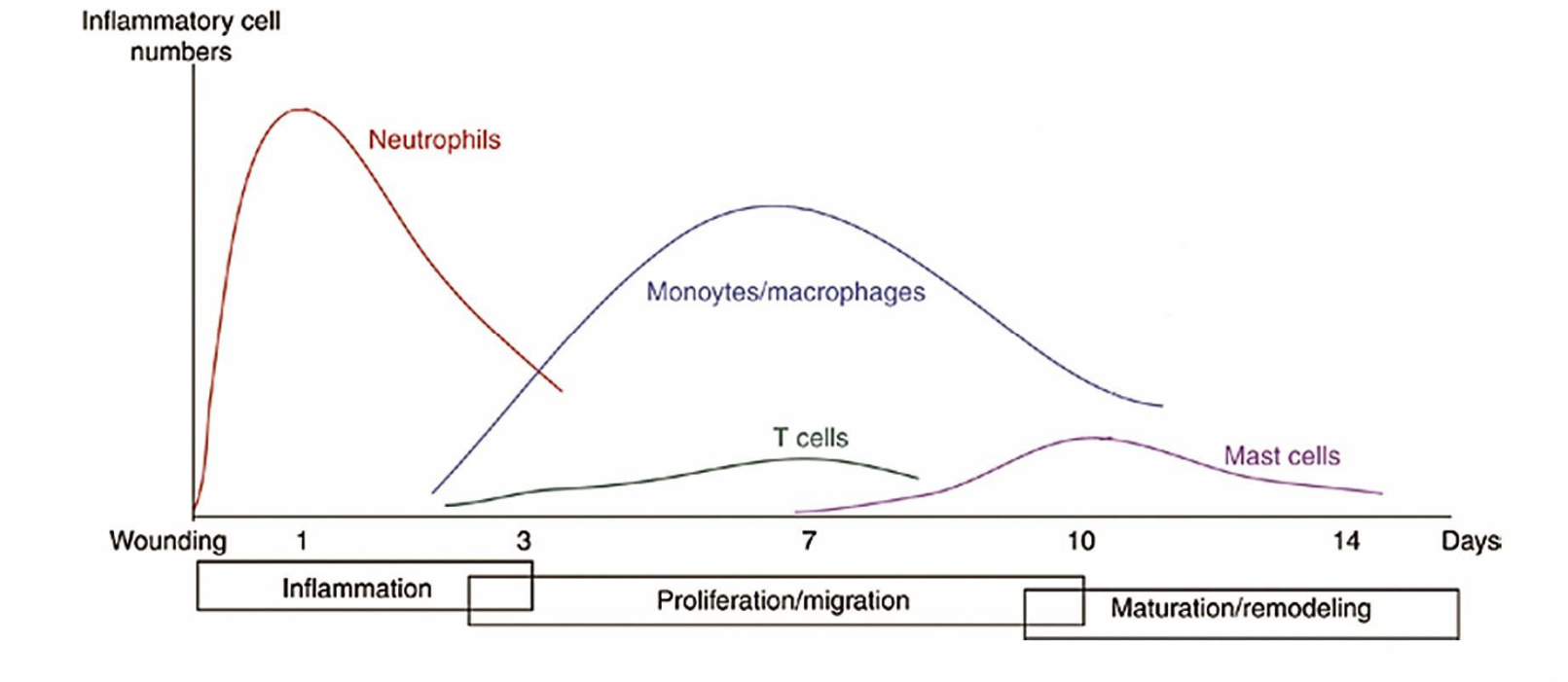
middle phase - **resolution** of initial insults

final phase - **termination** of inflammation & tissue repair



chronic inflammation fails to resolve the initial inflammatory response and continues to recruit bone marrow-derived inflammatory cells setting up a cascade of events in which growth-promoting effects of immune cells are progressively amplified

The inflammatory response during skin wound healing



In the adult organism, leukocyte influx is an inevitable and rapid response to skin injury:

- within 2-3 hours of wounding, neutrophils are attracted to the wound site
- these are followed by monocytes after about 48h, which mature into macrophages as they invade tissues, and finally lymphocytes (mostly T cells) and mast cells
- most of the recruited cells are drawn in from the blood, although there is likely to be some contribution from small numbers of resident cells (particularly Langerhans cells and mast cells)

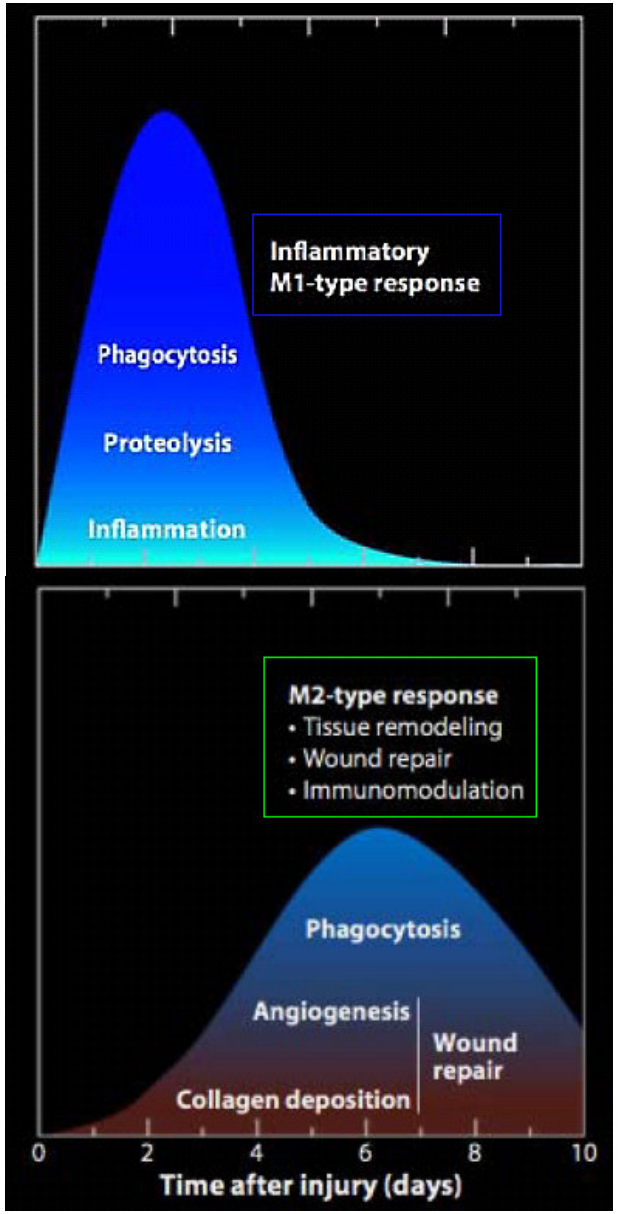
Macrophages in tissue injury and healing

"Inflammatory" macrophages (M1 phenotype)

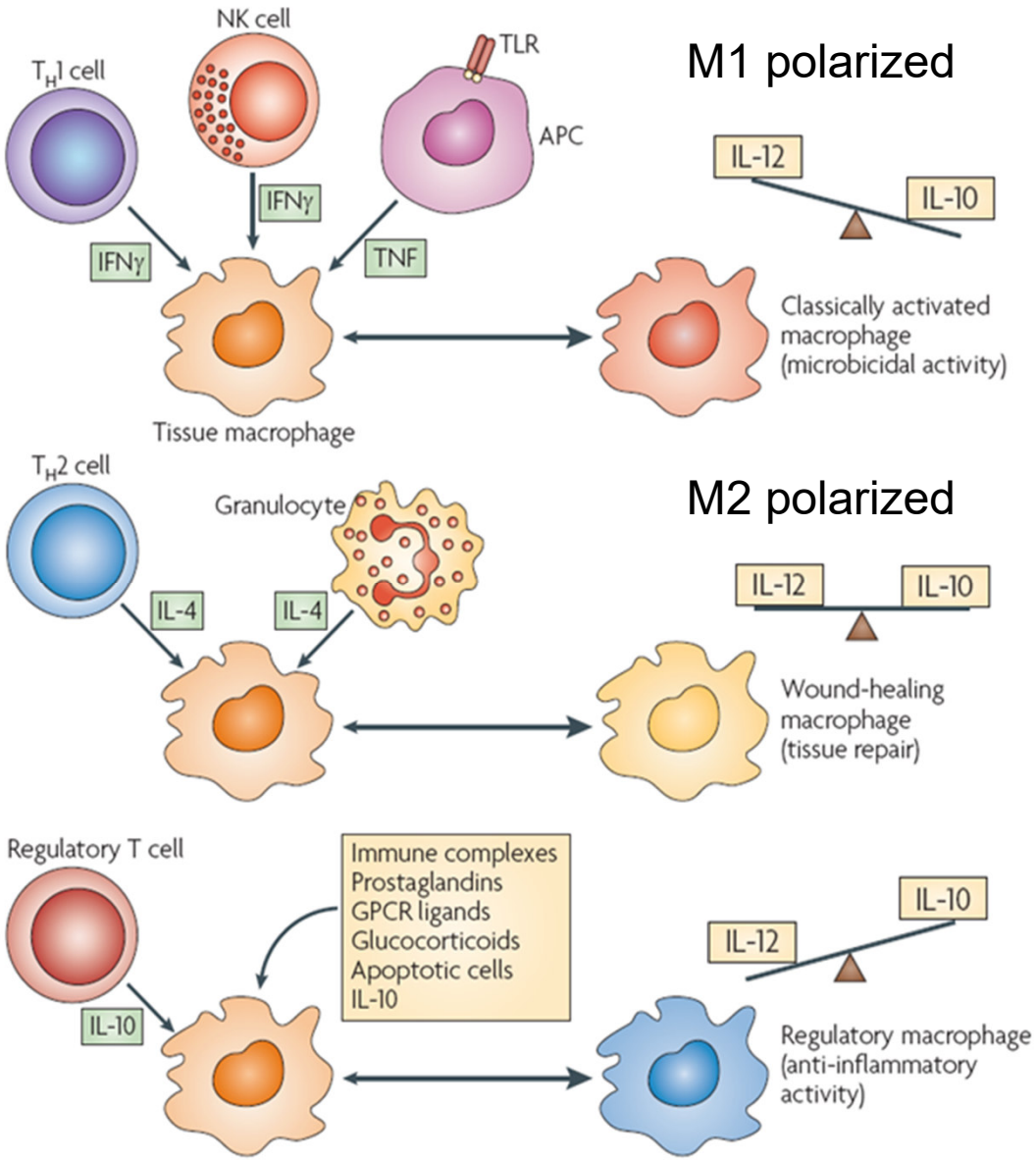
- clear pathogens and dying cells
- produce ROS and other inflammatory mediators
- cause cell and tissue damage
- stimulate immunity

"Healing" macrophages (M2 phenotype)

- promote tissue remodeling and angiogenesis
- tune down inflammation
- favor tissue regeneration by providing growth and survival factors and inducible signals leading to EMT
- stimulate immunological tolerance



Polarization of macrophage function



classically activated (M1) macrophages

- arise in response to interferon-γ (IFN γ) from T_H1, $CD8^+$ T or NK cells, and tumour-necrosis factor α (TNFα) produced by antigen-presenting cells (APCs)
  - cytotoxic activity against microorganisms and neoplastic cells (ROS production)
  - act as APCs, enhance CTL and NK by IL-12

wound-healing (alternatively act. M2)

- arise in response to interleukin-4 (IL-4) produced by T_H2 cells or granulocytes
- role in tissue repair
  - promote angiogenesis, remodeling, and repair of wounded/damaged tissues

regulatory (M2) macrophages

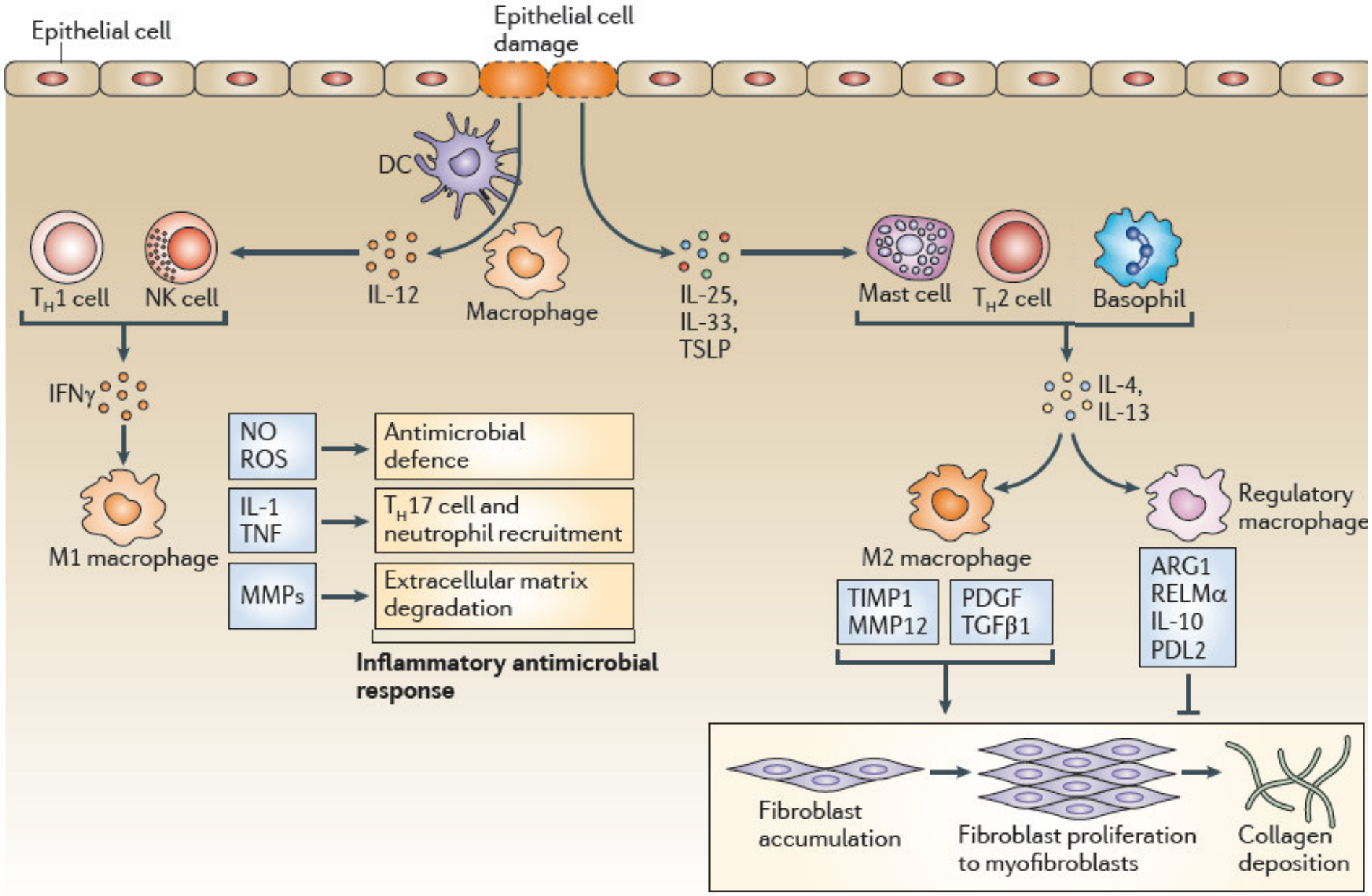
- are generated in response to various stimuli, including immune complexes, prostaglandins, G-protein coupled receptor ligands, glucocorticoids, apoptotic cells or IL-10
- suppress M1-mediated functions and adaptive immunity by IL-10

M1/M2 markers

Marker type	Associated signalling molecules	Gene (alternative names)	Comments
M2 markers	STAT6 phosphorylation *in vivo* and *ex vivo* without further perturbation (activated by IL-4/IL-13)	*Relma* (*Fizz1*, *Retnla*)	Highly induced by IL-4 and IL-13. Not expressed in humans
		Socs2	Highly induced by IL-4 and IL-13. Not macrophage-specific
		Irf4	Highly induced by IL-4 and IL-13. Not macrophage-specific
		Chia (*Amcase*)	Highly induced by IL-4 and IL-13. Not macrophage-specific
		Chi3l1 (*Gp39*, *Ykl40*)	Highly induced by IL-4 and IL-13. Not macrophage-specific
		Chi3l2 (*Ykl39*)	Not expressed in mice
		Chi3l3 (*Ym1*)	Not expressed in humans. Can be highly induced by IL-4 and IL-13 in some situations
		Cxcl13	Chemokine linked to T_H2 cell responses
		Ccl12	Chemokine linked to T_H2 cell responses
		Ccl24	Chemokine linked to T_H2 cell responses
		Klf4	Transcription factor induced by IL-4 in both mouse and human macrophages[171]
M1 markers	• STAT3 and/or STAT1 phosphorylation *in vivo* and *ex vivo* (linked to IL-6 and IL-10 in the microenvironment) • Evidence of an interferon-γ signature • Absence of STAT6 phosphorylation *in vivo* and *ex vivo*‡	*Marco*	Calmodulin-associated. Also found in other activation scenarios
		Socs3	Induced by IL-10, IL-6 and many other factors
		Nos2	Not readily expressed in human macrophages
		Il12b	Highly induced in M1 activation
		Ptgs2 (*Cox2*)	Highly induced in M1 activation
		Il23a (*Il23p19*)	Highly induced in M1 activation
		Ido1	Useful marker of human and mouse exposure to type 1 and 2 interferons
Context-dependent markers		*Arg1*	Can be induced by the STAT6 or STAT3 pathways[172,173]
		Il10	Differentially produced by most, if not all macrophages[174]
		Mrc1	Linked with M2 macrophages but widely expressed on many macrophage subsets

Arg1, arginase 1; *Ccl*, CC-chemokine ligand; *Chi3l*, chitinase 3-like; *Chia*, chitinase, acidic; *Cxcl13*, CXC-chemokine ligand 13; *Ido1*, indoleamine 2,3-dioxygenase 1; *Il*, interleukin; *Irf4*, interferon regulatory factor 4; *Klf4*, Krüppel-like factor 4; *Marco*, macrophage receptor with collagenous structure; *Mrc1*, mannose receptor, C type 1; *Nos2*, nitric oxide synthase 2, inducible; *Ptgs2*, prostaglandin-endoperoxide synthase 2; *Relma*, resistin-like molecule alpha; *Socs*, suppressor of cytokine signalling; STAT, signal transducer and activator of transcription; T_H2, T helper 2. *Shown are marker combinations that can be used to assign phenotypic characteristics to a mouse macrophage population. The use of multiple markers, especially when combined with assays for phosphorylated STATs, avoids the problems associated with markers, such as ARG1, that are widely expressed in either M1 or M2 polarized environments. ‡A notable exception is the infection of macrophages by *Fransicella* spp. — in this case, autocrine or paracrine IL-4 and IL-13 production is enforced by a myeloid differentiation primary response protein 88 (MYD88)-dependent pathway[175], and the subsequent activation of STAT6 favours bacterial survival.

Distinct macrophage subsets regulate inflammation and wound healing

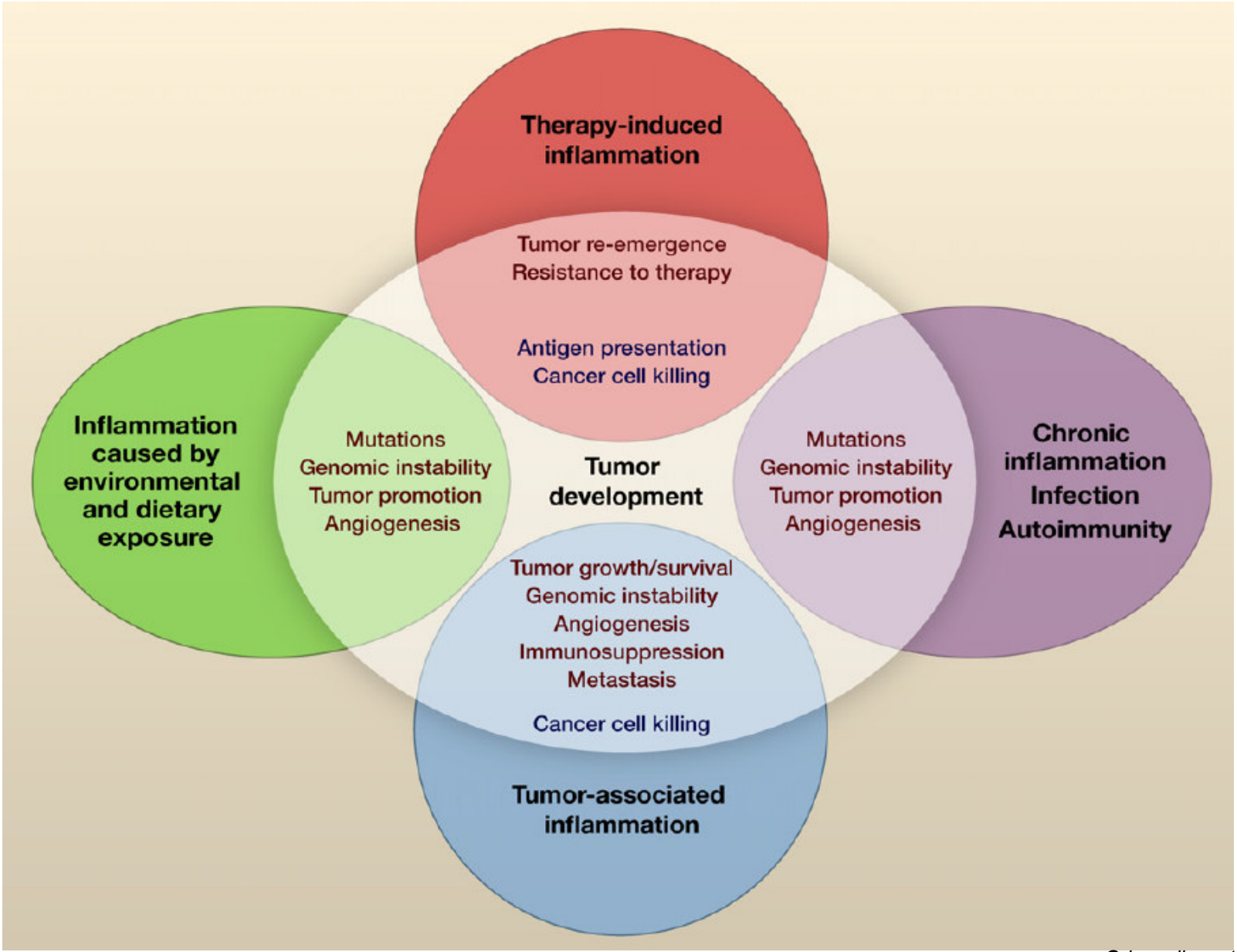


Murray & Wynn. Nat. Rev. Imm. 2011

Distinct macrophage subsets regulate inflammation and wound healing

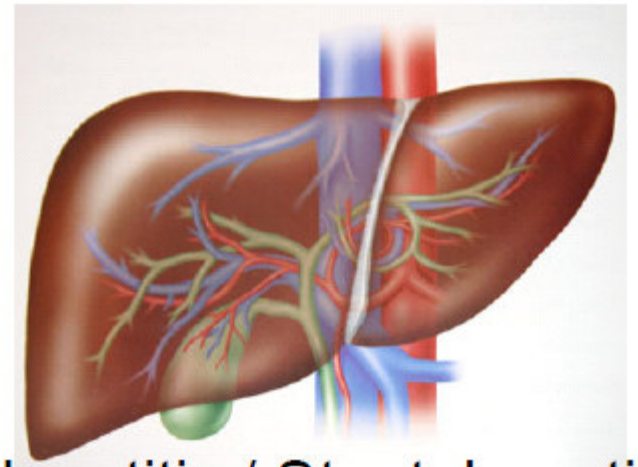
- tissue damage activates clotting, blood vessel dilation and increased vessel permeability, allowing recruitment of **inflammatory monocytes** via CCL2 which *in situ* **differentiate into macrophages** and can undergo further proliferation in the tissue
- cytokines such as **IFNγ** and pattern recognition receptor engagement lead to the differentiation of these macrophages into inflammatory macrophages with an **M1-like phenotype**, producing NO, ROS, IL-1 and TNF and secreting MMP2 and MMP9 for ECM remodeling
- persistent inflammatory stimulation recruits large numbers of TH17 cells and neutrophils, leading to substantial tissue damage
- damaged epithelial cells also release alarmins, including **IL-25**, **IL-33** and **TSLP**, which induce **IL-4** and **IL-13** secretion by a variety of innate and adaptive immune cells
- when the inflammatory stimulus diminishes, the alarmins and TH2-type cytokines drive the conversion of the immune response into a wound healing response, which is characterized by the accumulation of M2 macrophages that promote wound healing and fibrosis through the production of MMP12, TIMP1, PDGF and TGFβ1
- in the final stage, macrophages take on a regulatory/suppressive phenotype with ARG1, RELMα, PDL2 and IL-10, suppressing T cell proliferation and collagen synthesis by activated myofibroblasts

Roles of inflammation in cancer

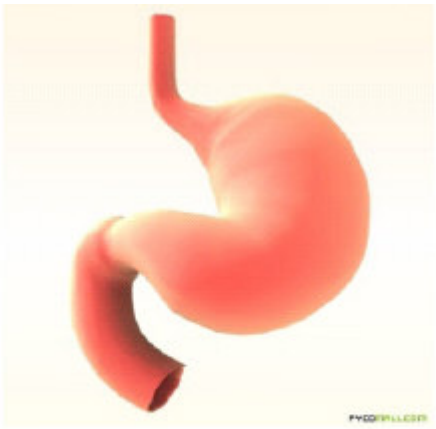


Grivennikov et al. Cell 2010

Chronic inflammation and cancer



Viral Hepatitis / Steatohepatitis



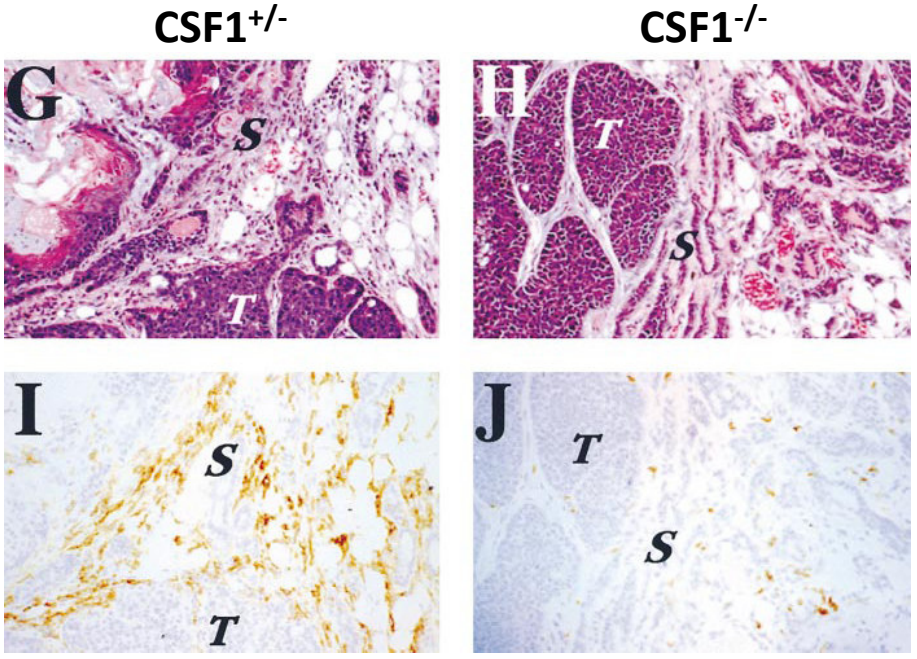
H. Pylori → Gastritis

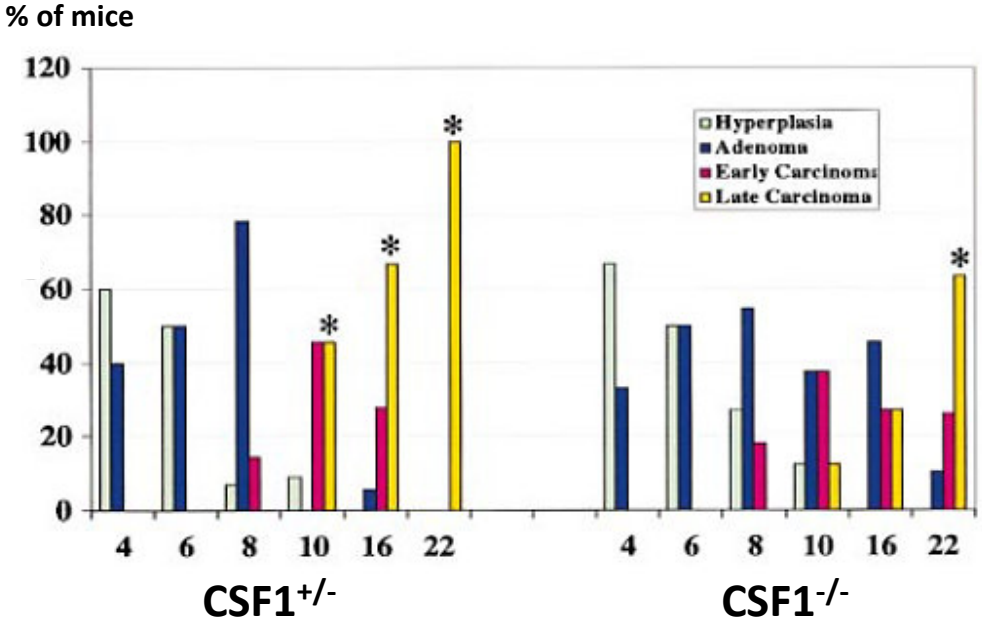
hepatocellular carcinomas are created by chronic hepatitis virus infections through
1) its ability to cause continuous cell proliferation since virus-induced killing of hepatocytes is compensated by proliferation of surviving cells
2) Viral antigens causes cells of the immune system to attempt to eliminate virus-infected cells, yielding a chronic inflammatory state in the liver

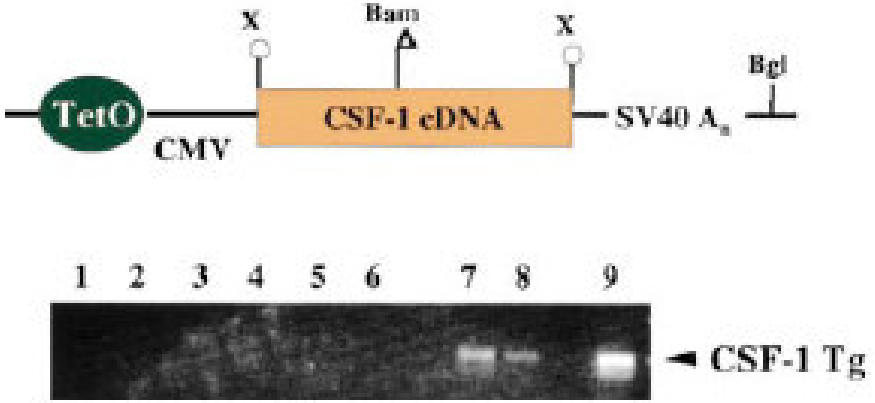
chronic *Helicobacter pylori* infections of the stomach can cause lymphomas arising in gastric mucosa-associated lymphoid tissue (MALT)

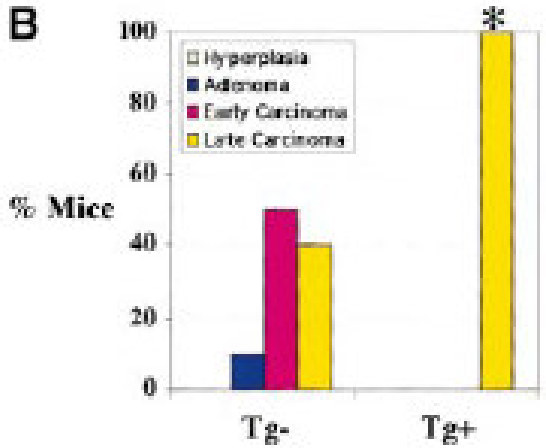
75% of MALT lymphomas can be cured if patients are treated with antibiotics that eradicate the *H. pylori* bacterial populations in the stomach

CSF1 Promotes Progression of MMTV-PyMT Mammary Tumors to Malignancy



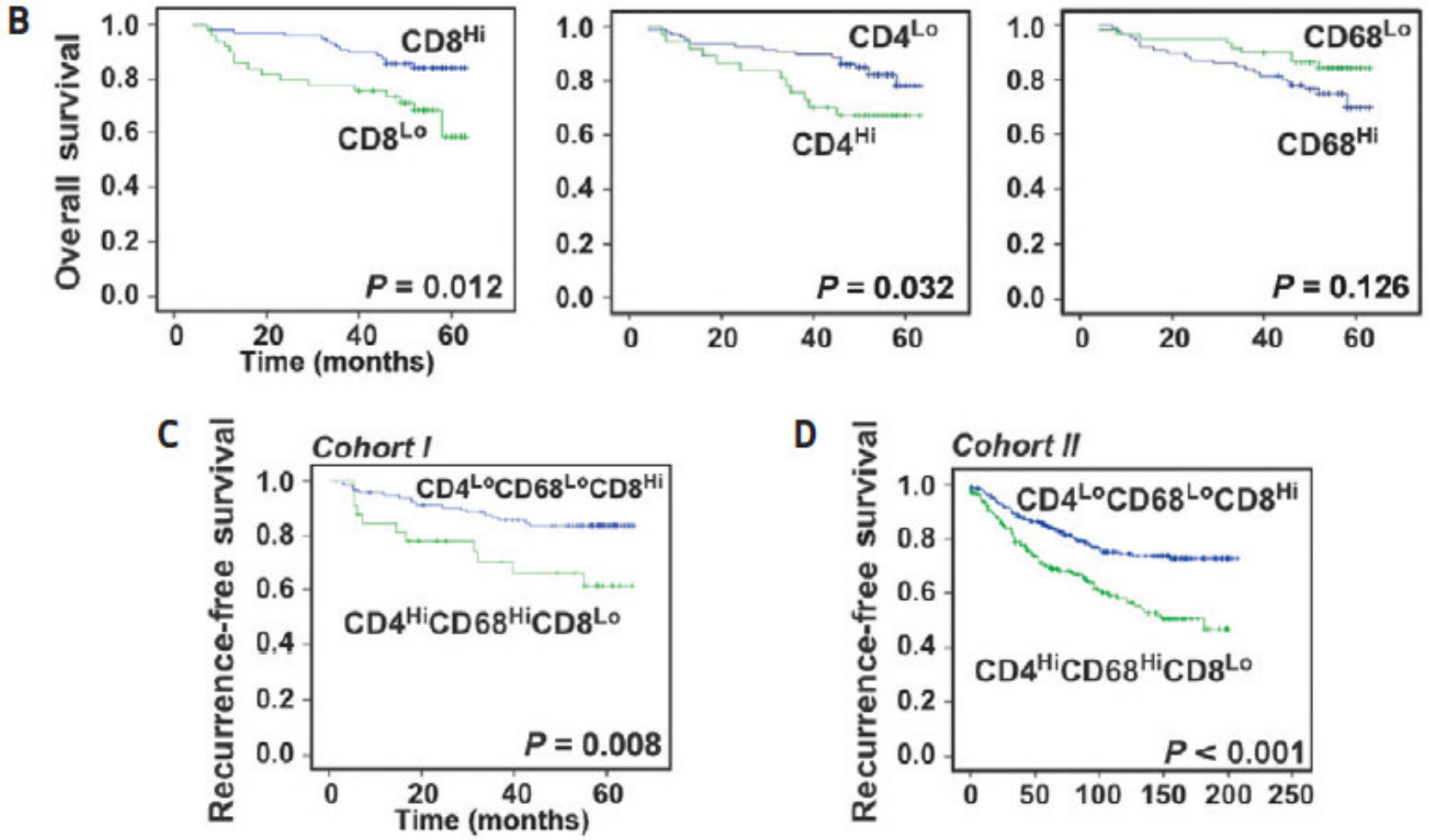






Lin et al., J. Exp Med. 2001

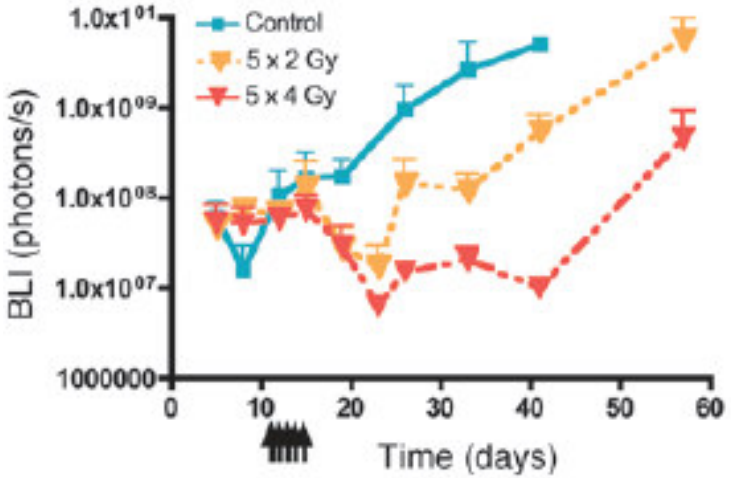
Macrophage and CD4 recruitment predicts worse outcome in breast cancer

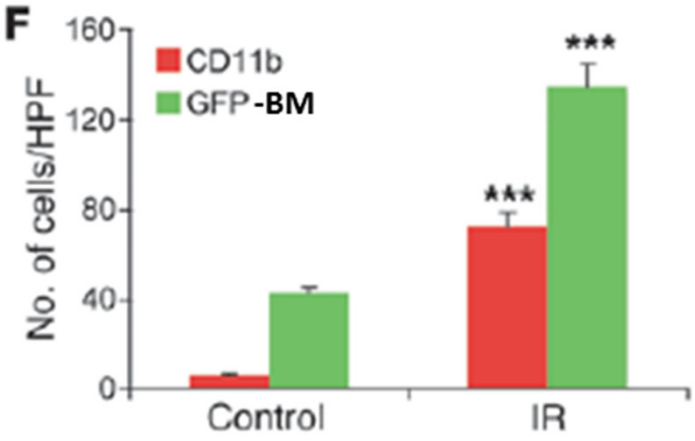


DeNardo et al., Canc. Discov. 2011

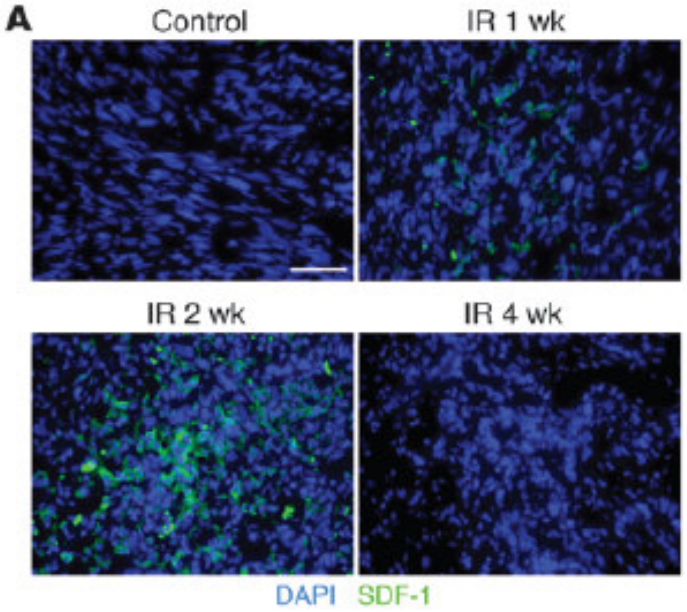
Inhibition of monocyte infiltration prevents recurrence of glioblastoma after irradiation

recurrence after irradiation

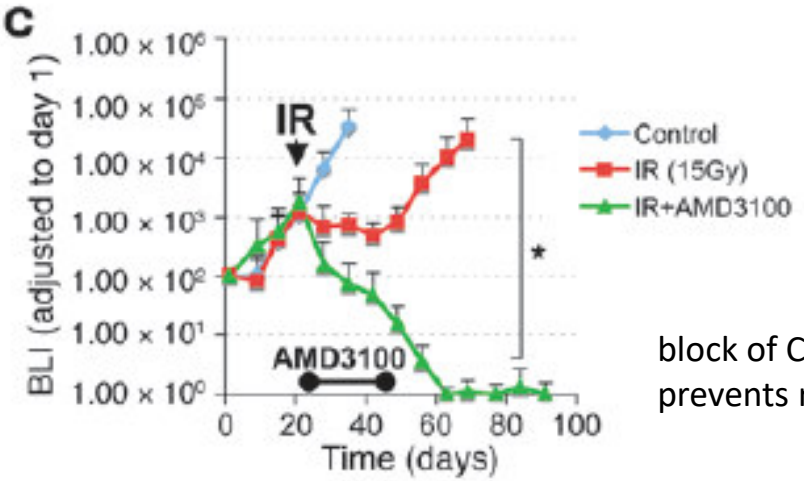




influx of GR1+ BMDC after irradiation



expression of SDF1(CXCL12) after irradiation

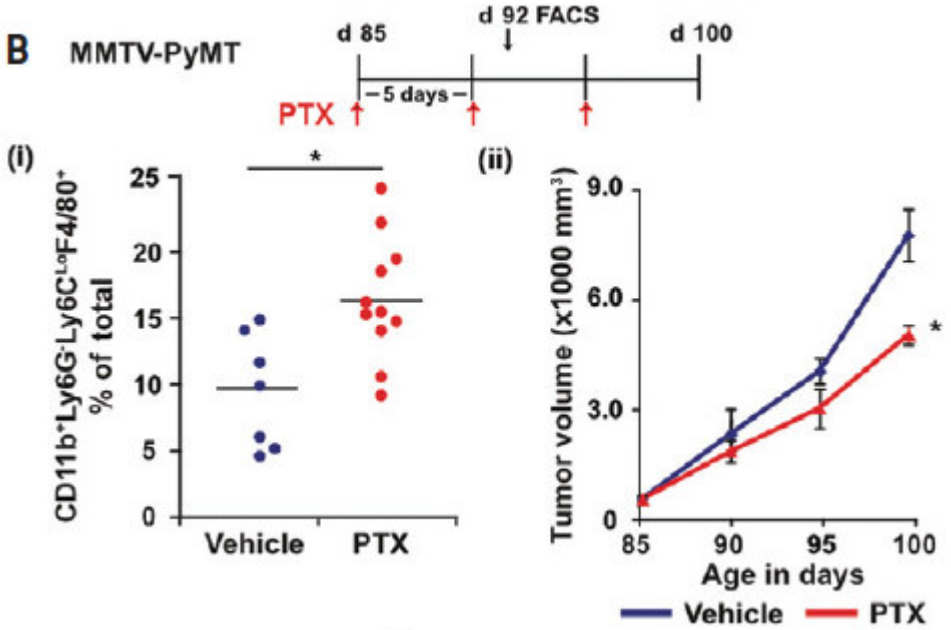


block of CXCR4 activity prevents recurrence

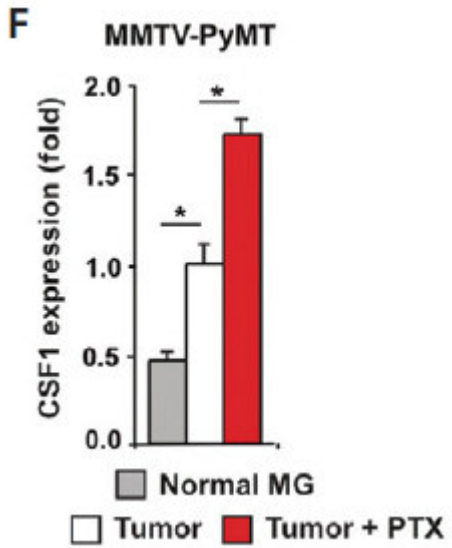
Kioi et a., J. Clin. Invest. 2010

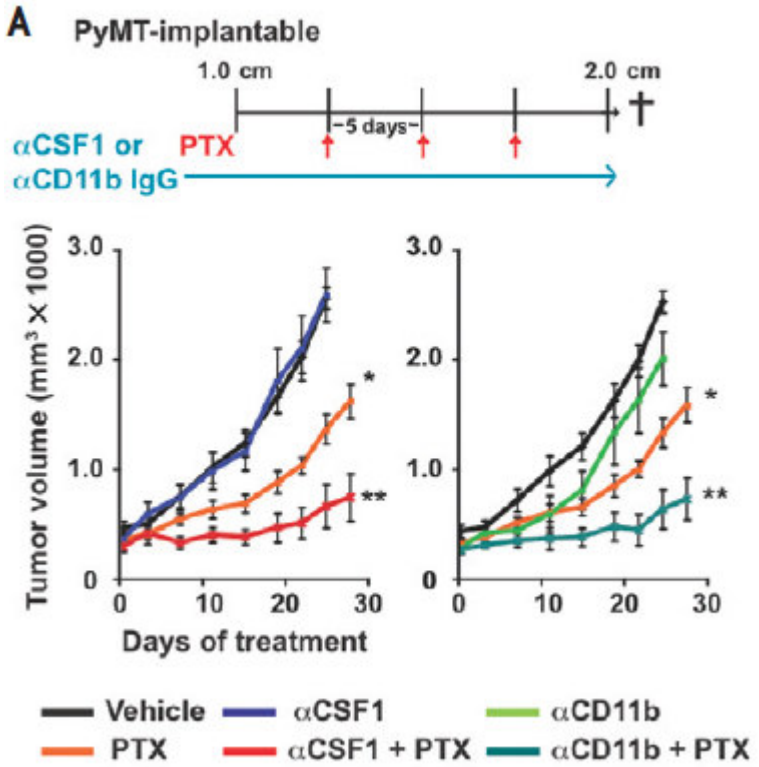
Macrophage recruitment counteracts chemotherapy

increased macrophage numbers after chemotherapy



CSF1 secretion supports macrophage expansion



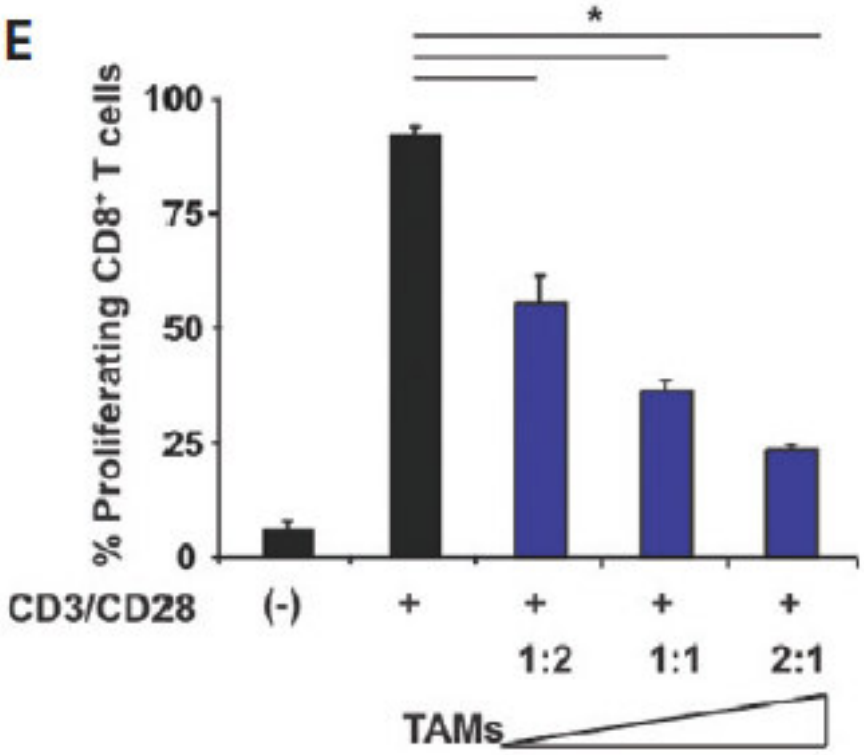


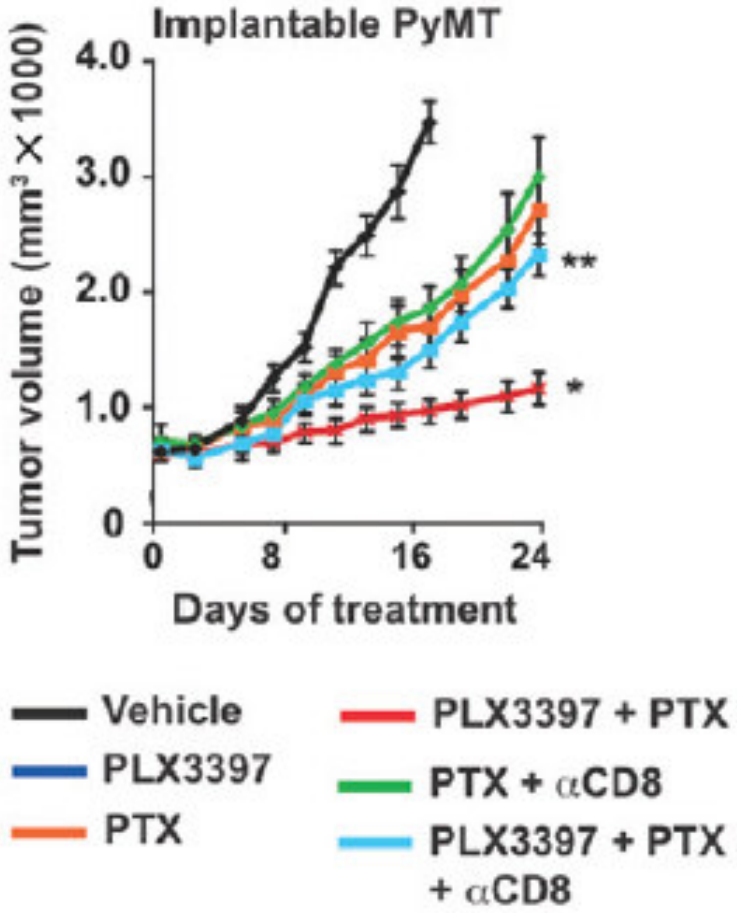
block of CD11b+ cells or CSF1 improves chemotherapy efficacy

DeNardo et al., Canc. Discov. 2011

Macrophages suppress CD8 CTL expansion which contributes to chemotherapy outcome

CSFE assay for T cell expansion (bead act.)

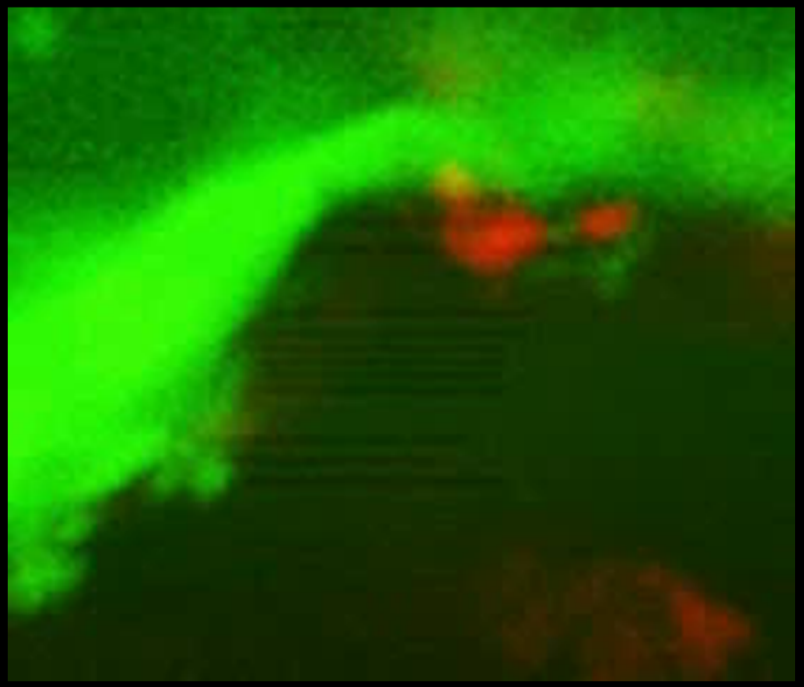




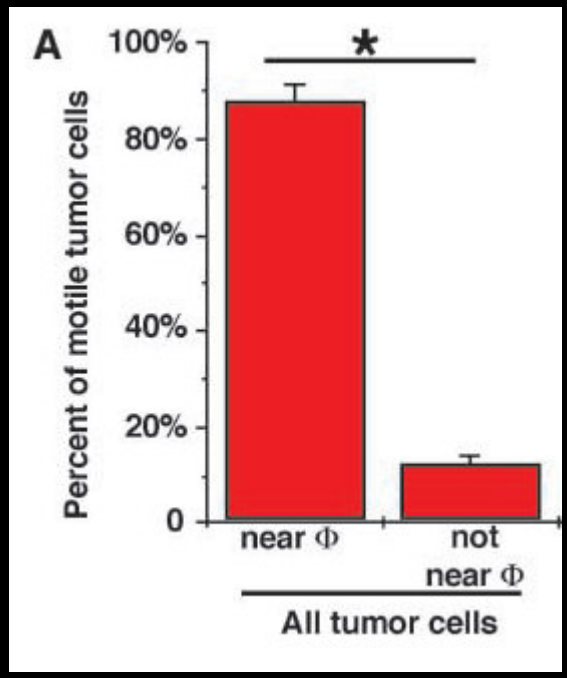
PLX3397: CSF1R inh.

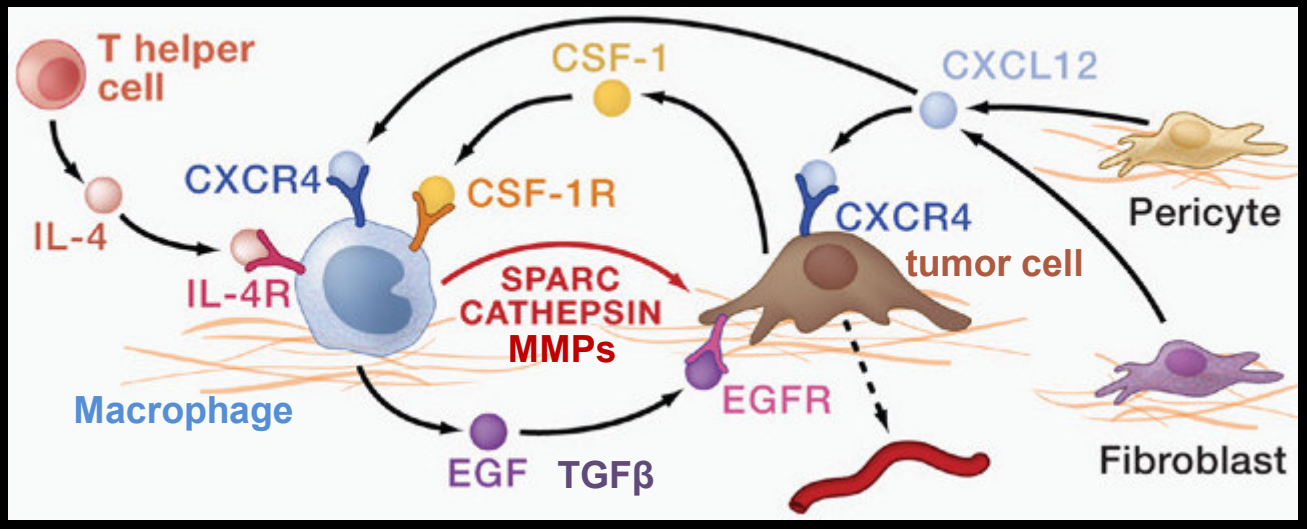
DeNardo et al., Canc. Discov. 2011

TAMs release ECM-remodeling and EMT-promoting factors that enhance tumor cell motility and invasion



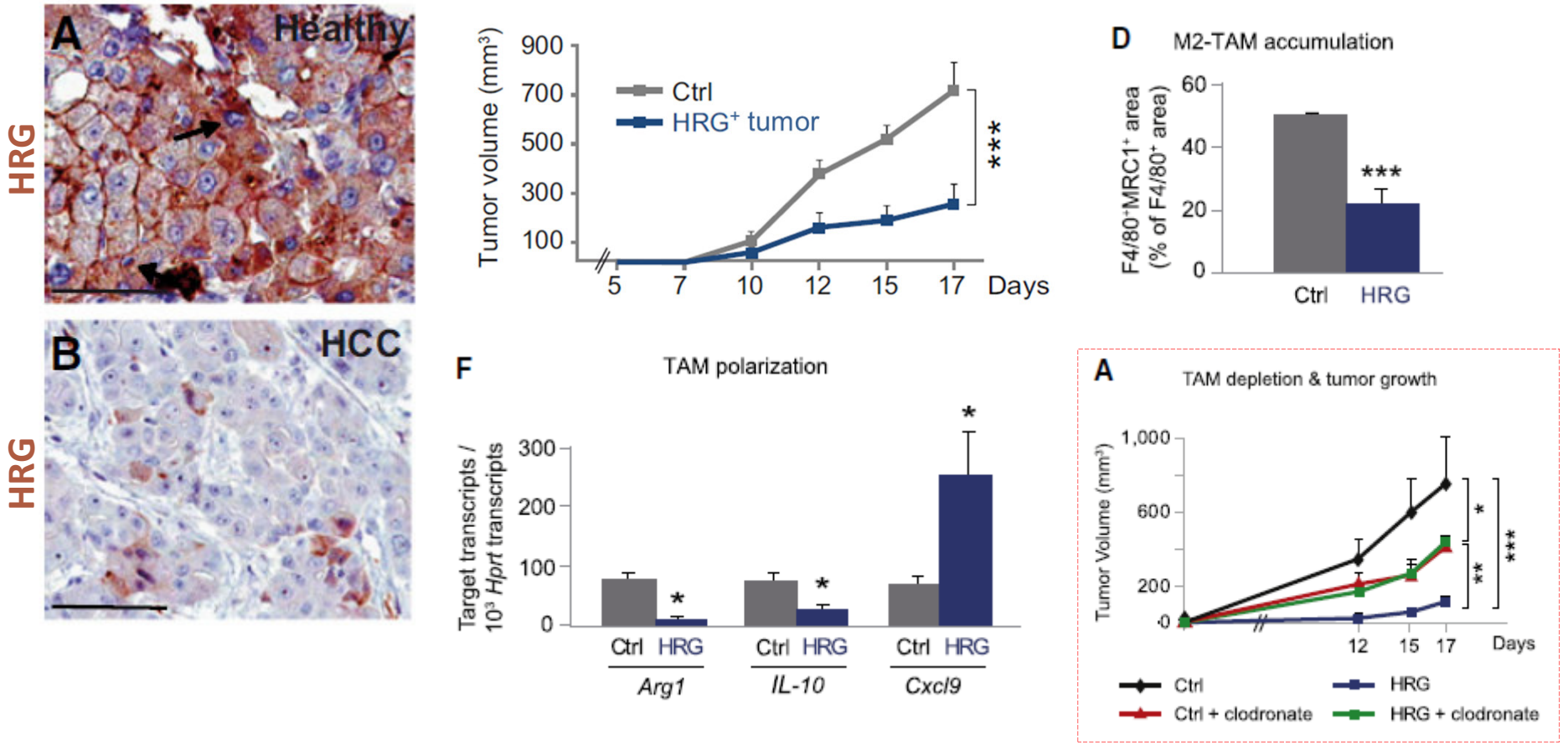
tumor cell
macrophage





Wyckoff et al., Cancer Res 2007
Qian & Pollard, Cell 2010

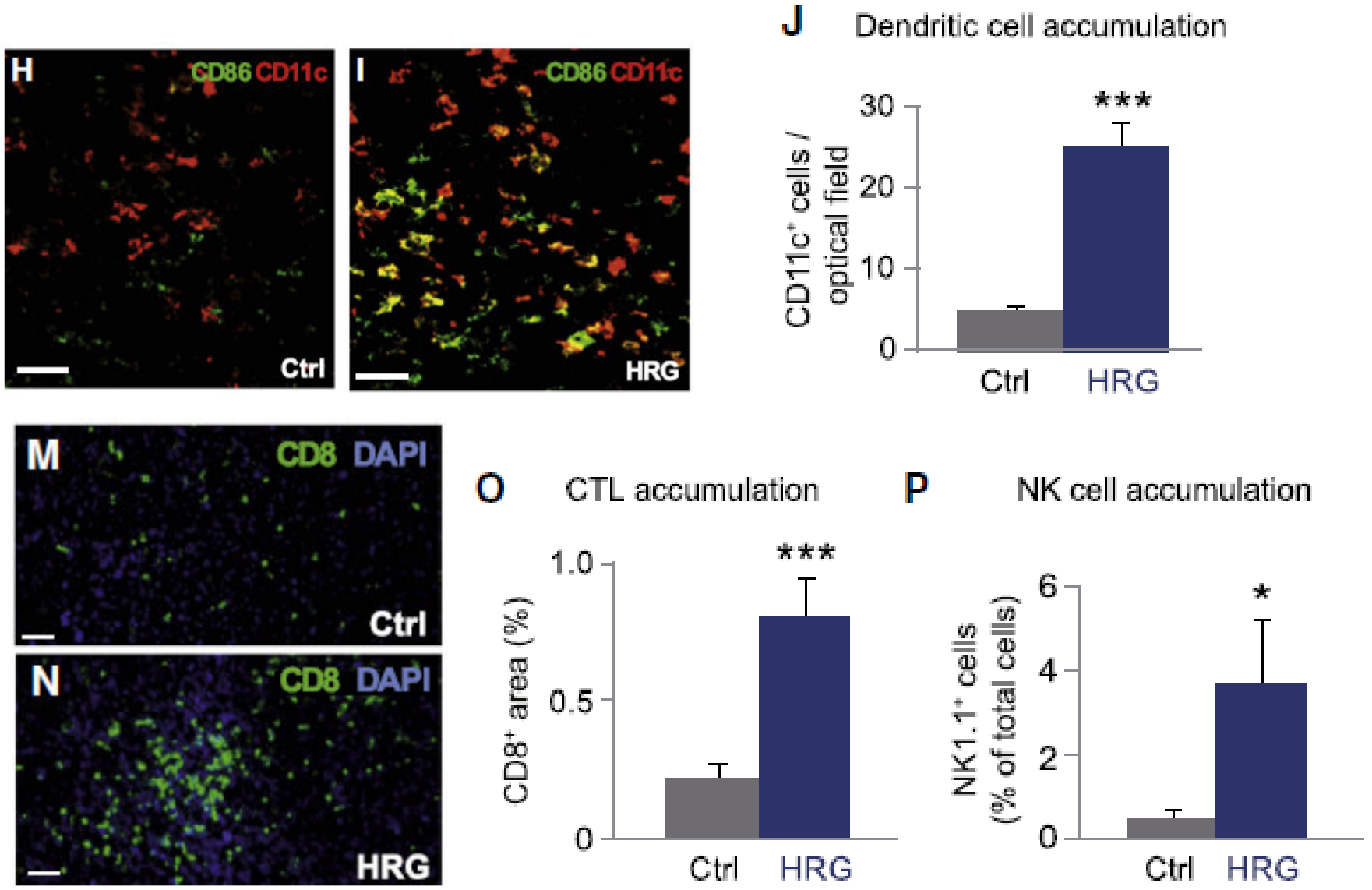
HRG slows tumor growth by altering macrophage polarization



- histidine-rich glycoprotein (HRG) is a multidomain protein that binds thrombospondins (TSPs), heparin, FcgR receptors and other molecules, implicated in tumorigenesis
- HRG is deposited in the tumor stroma from plasma or platelets
- HRG can trigger apoptosis of endothelial cells via its interaction with CD36
- HRG stimulates phagocytosis of dying cells via interaction with CD47

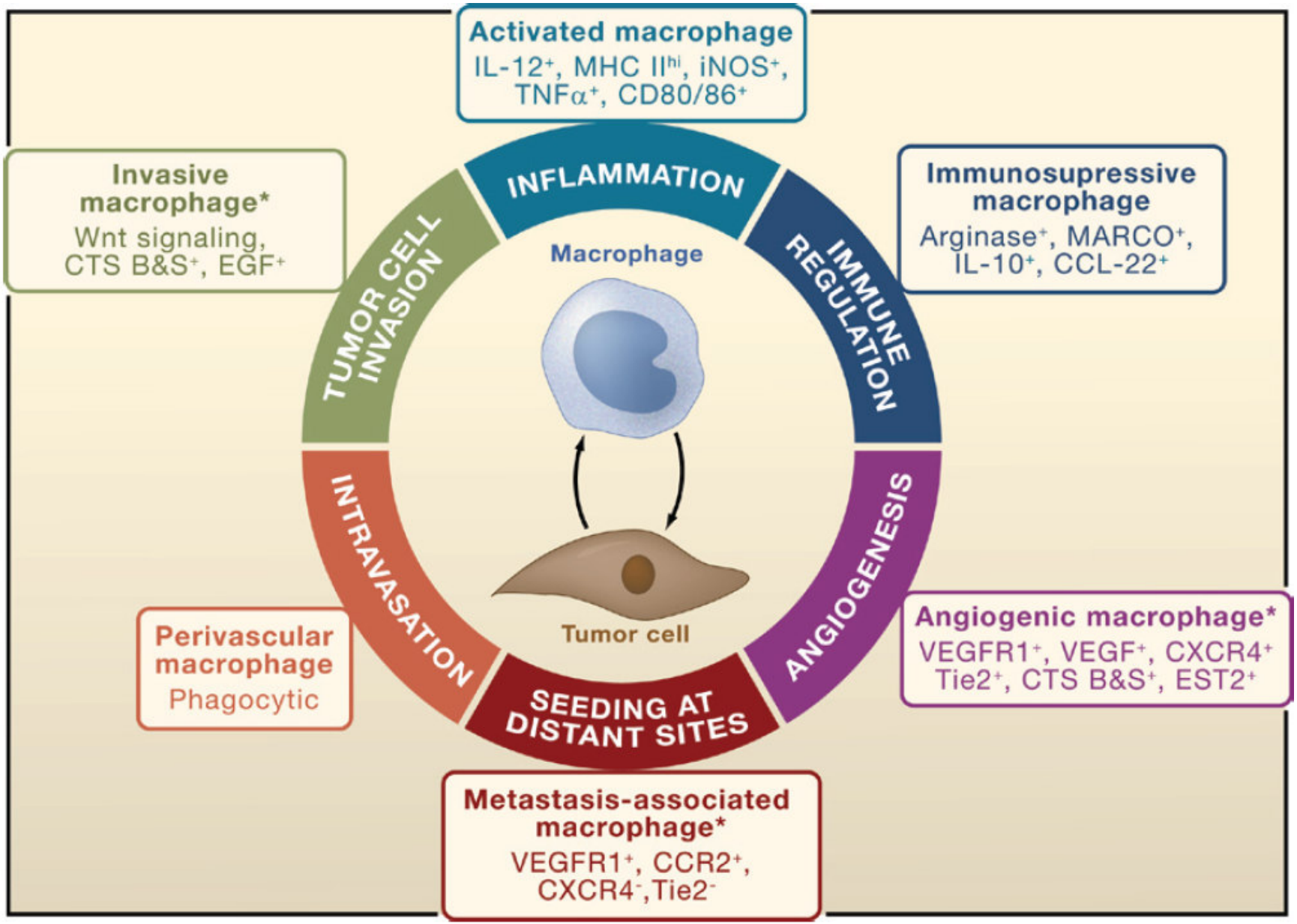
Rolny et al., Cancer Cell 2011

HRG slows tumor growth by supporting CTL responses



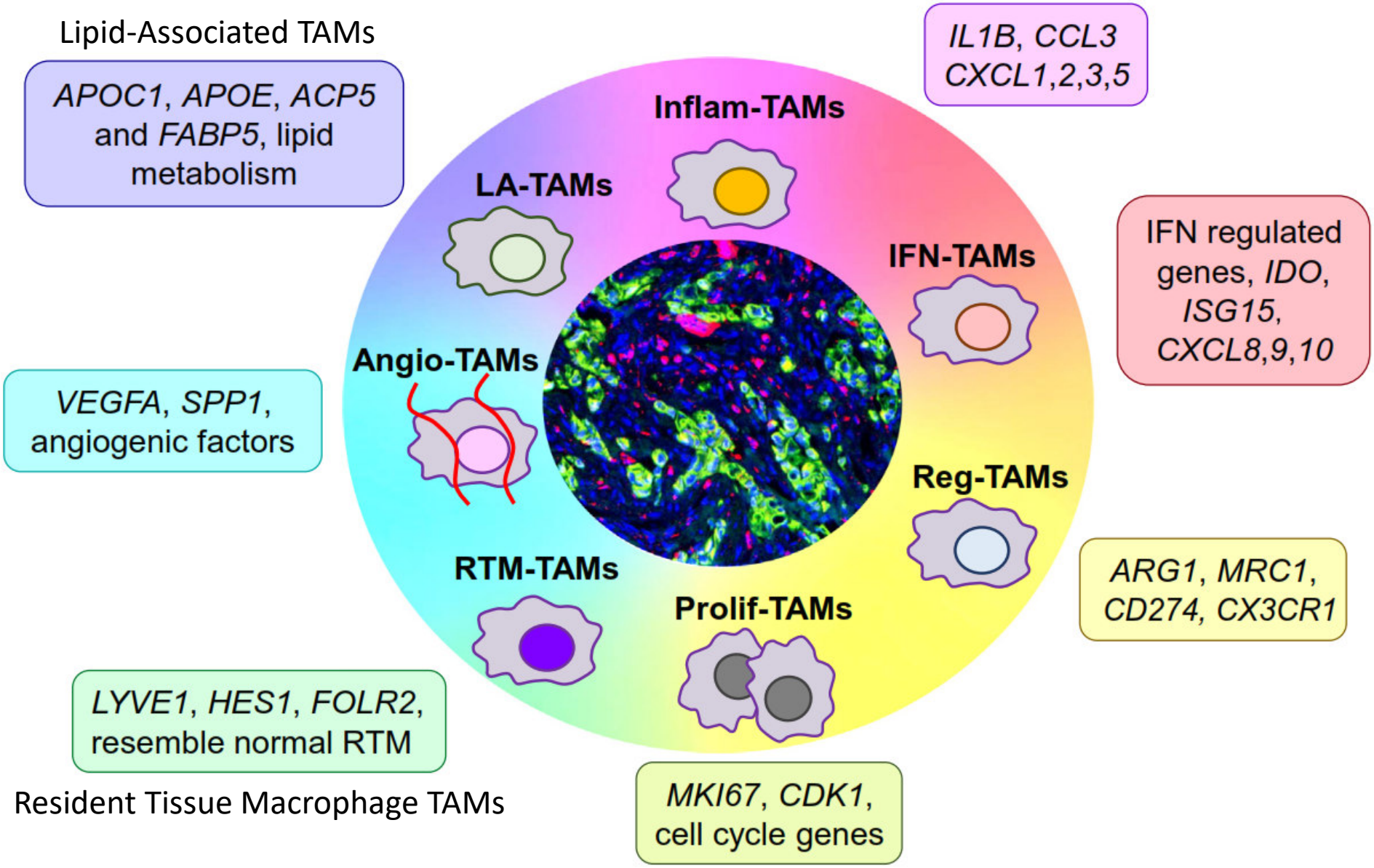
Rolny et al., Cancer Cell 2011

Different macrophage phenotypes in tumorigenesis



Qian & Pollard, Cell 2010

Molecular diversity of TAMs through single cell omics



Ma et al., Trends Imm. 2022

Neutrophils overview

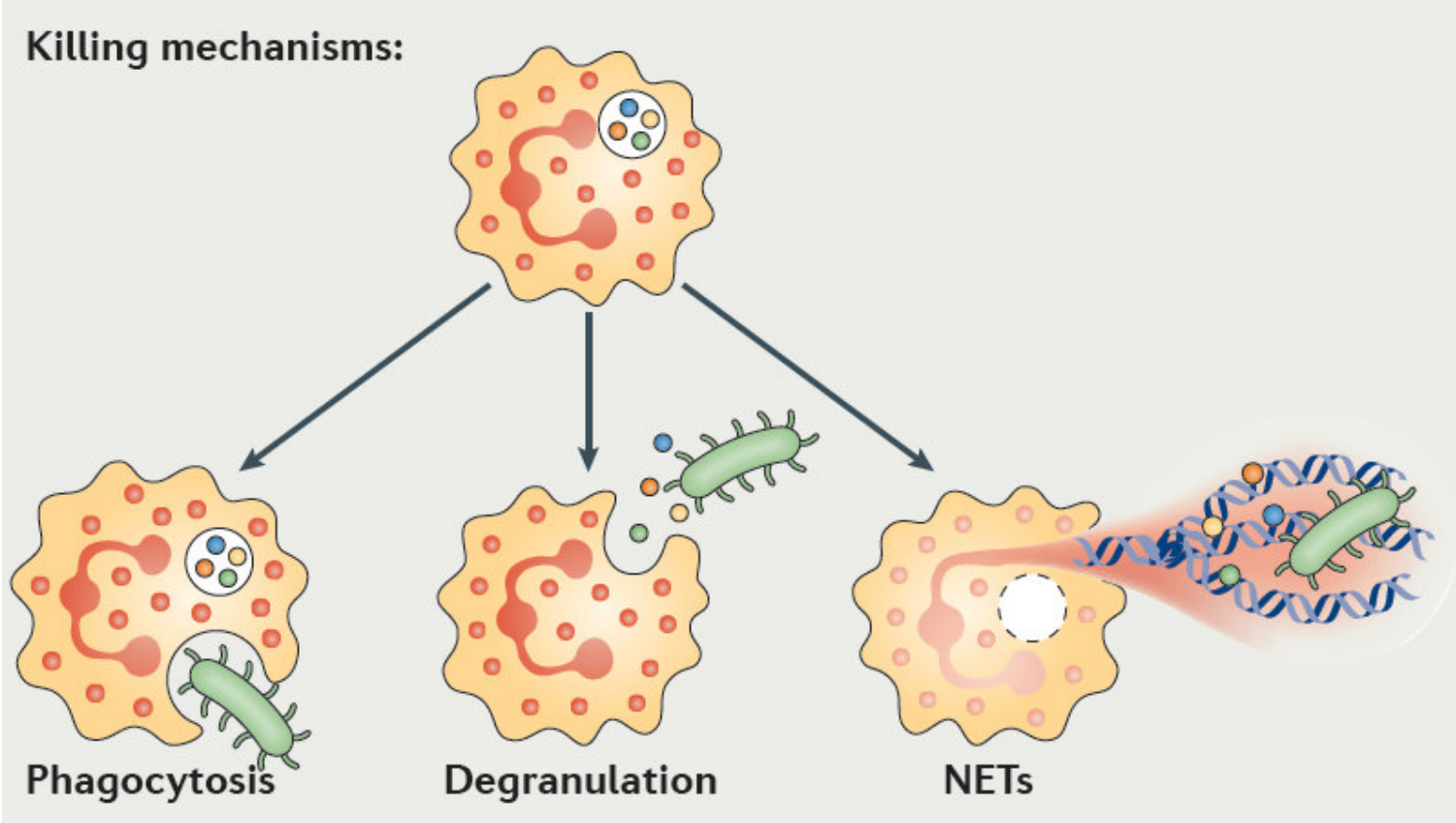
Origin and maturation:

- neutrophils are continuously generated in the bone marrow from myeloid precursors
- daily production can reach up to 2×10^{11} cells
- granulocyte colony stimulating factor (G-CSF) is the major cytokine driving production
- G-CSF is produced in response to IL-17A by γδ T cells, NKT and TH17 cells
- IL-17A is in turn under the control of IL-23 originating from tissue-resident macrophages and DCs

- short lifespan (12h) which is increased up to several days upon activation in tissues

- recruitment via CXCR2 chemokines (CXCL1, 2, 5, 8)
- activated via pattern recognition receptor (PRR) and TLRs
  e.g. FPR1 (receptor for N-formylmethionine found in bact. and mitochondrial proteins) => response to tissue damage or necrosis
- effectors:
  - **ROS production and phagocytosis**
  - **NETs formation and trapping and killing microbes**
  - lipid mediators: leukotriene B4 (potent chemoattractant)
  - LL-37, azurocidin, and chemokines (CCL3,4,20) to attract monocytes
  - primary granules: contain myeloperoxidase (MPO)
  - secondary granules: contain lactoferrin (antimicrobial, iron-removal) and gelatinase
  - tertiary granules: contain matrix metalloproteinase 9 (MMP9)

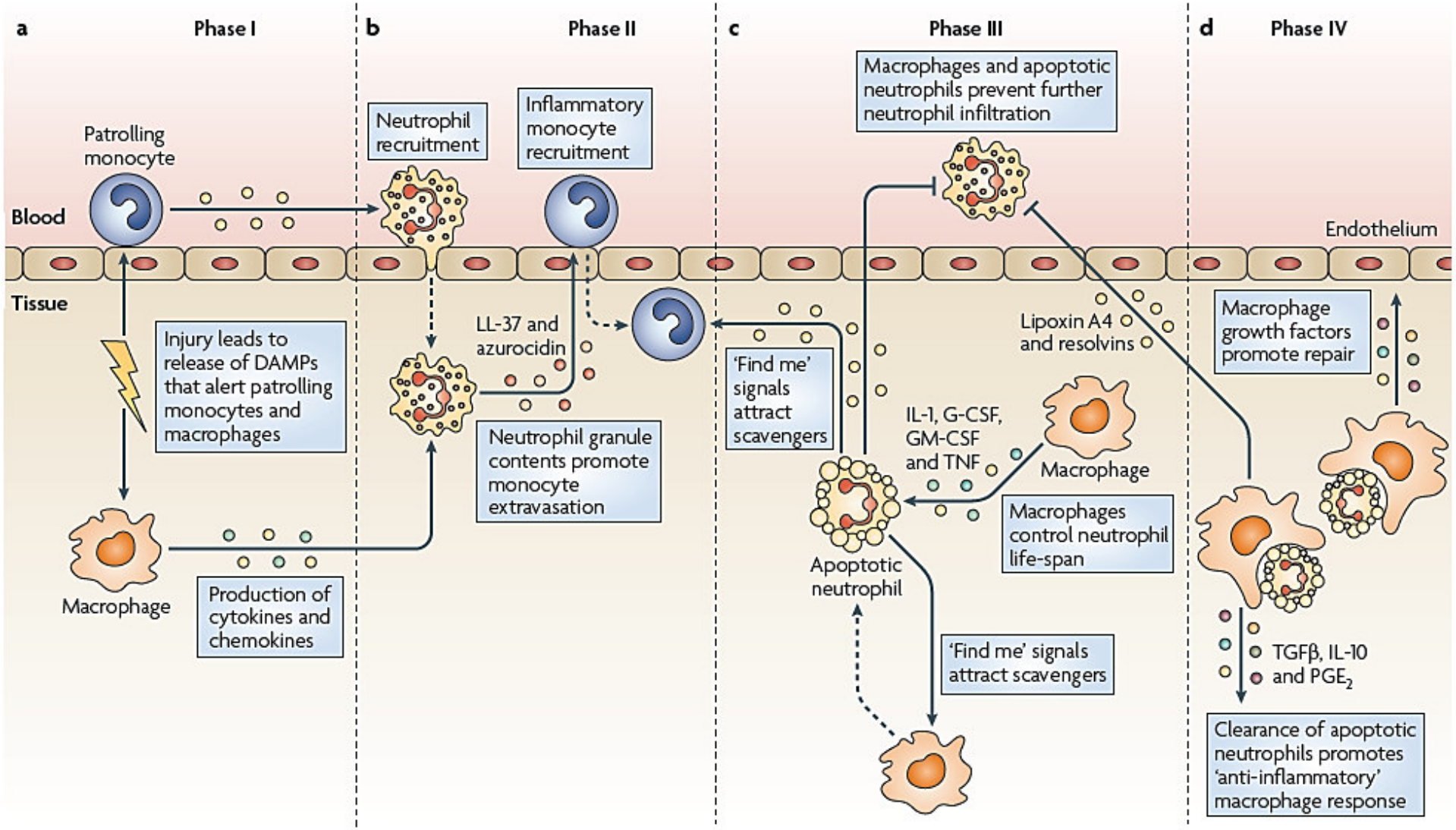
Neutrophil effector functions



- phagocytosis and NADPH oxygenase-dependent **ROS** production
- release of antimicrobial proteins (cathepsins, defensins, lactoferrin and lysozyme)
- release of neutrophil extracellular traps (**NETs**) composed of a core DNA element to which histones, proteins and enzymes (**MPO**, **elastase**) are attached; NETs immobilize target cells, thus preventing them from spreading but also facilitate subsequent phagocytosis

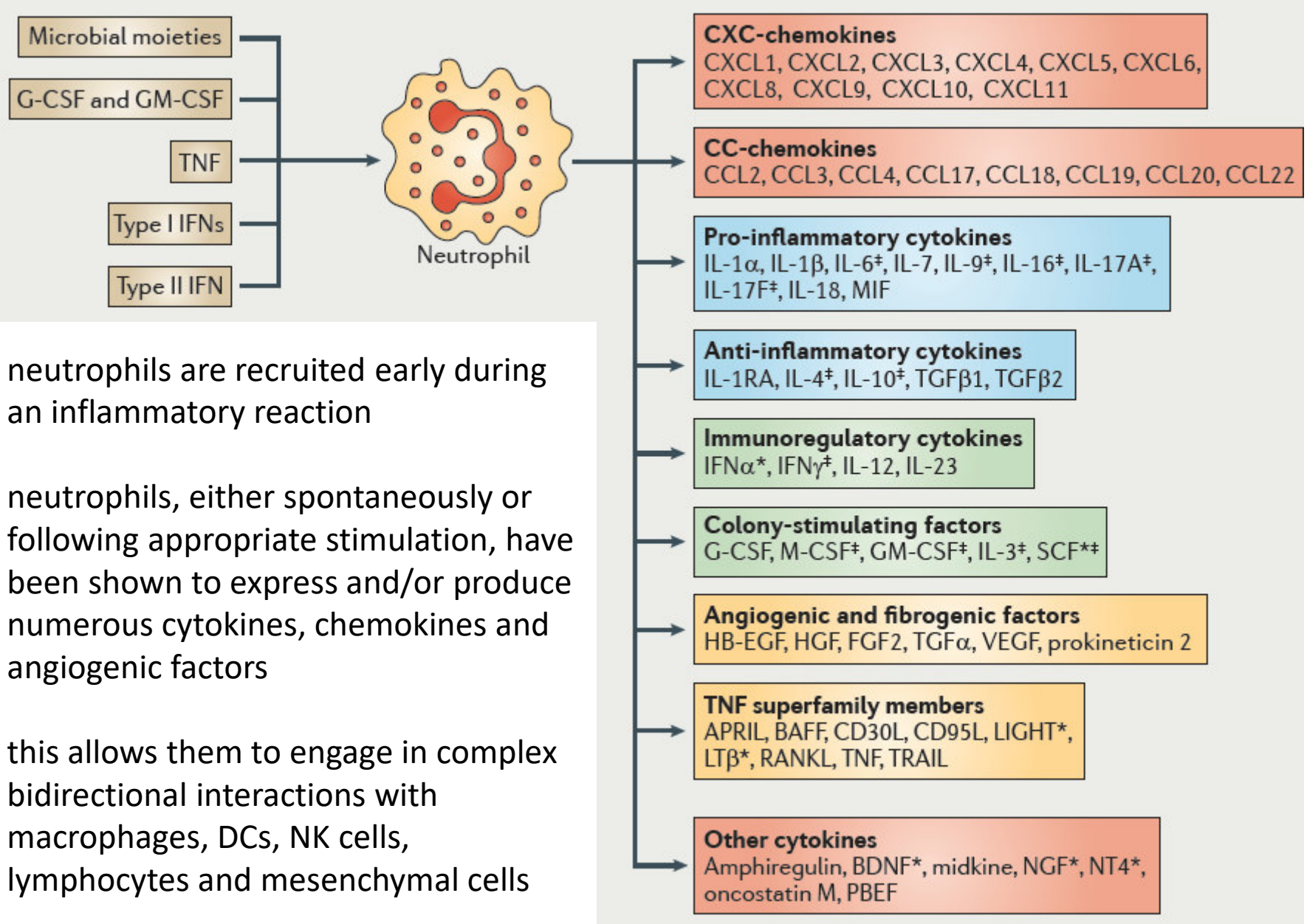
Kolaczkowska & Kubes Nat. Rev. Imm. 2013

Neutrophil recruitment, activation and resolution



Soehnlein & Lindbom, Nat. Rev. Imm. 2010

Neutrophil mediated immune regulation

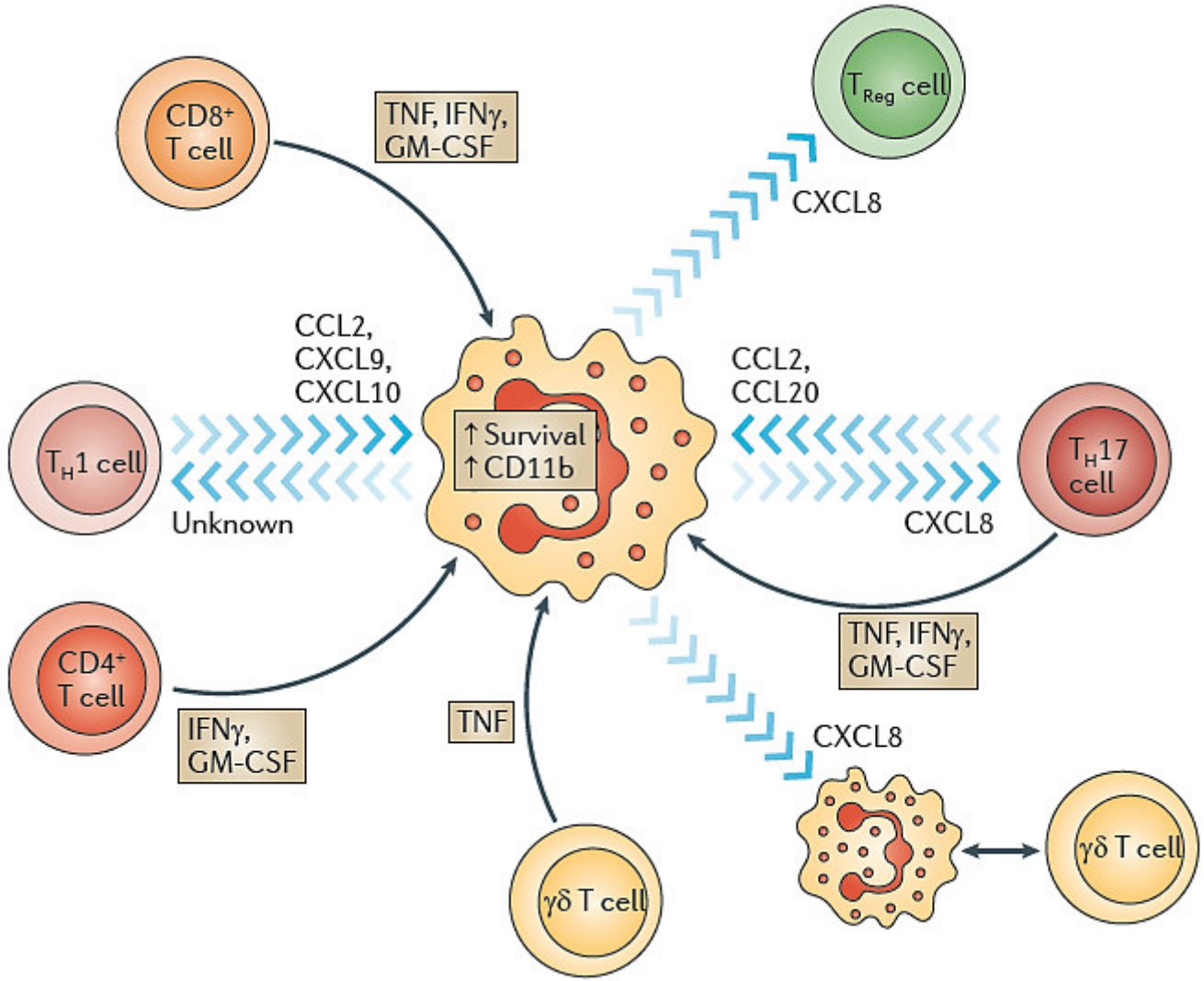


neutrophils are recruited early during an inflammatory reaction

neutrophils, either spontaneously or following appropriate stimulation, have been shown to express and/or produce numerous cytokines, chemokines and angiogenic factors

this allows them to engage in complex bidirectional interactions with macrophages, DCs, NK cells, lymphocytes and mesenchymal cells

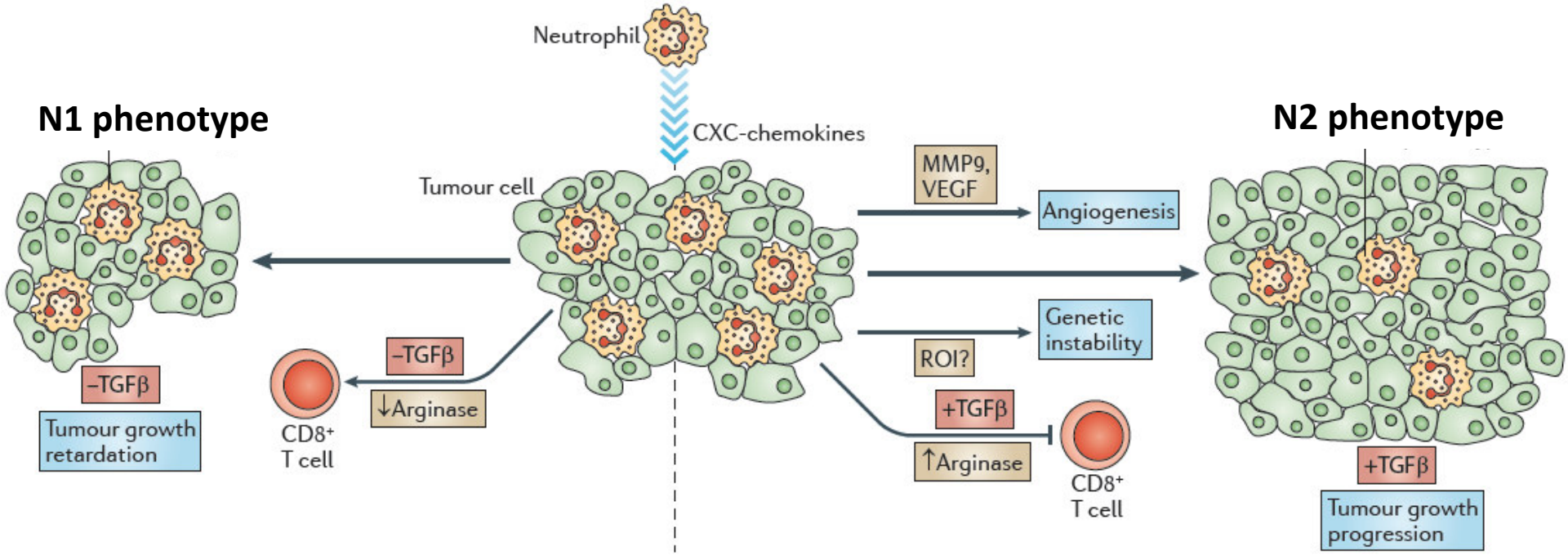
Neutrophils interact with a number of T cell populations



- recruitment of TH1 (CCL2, CXCL9,10) and TH17 cells (CCL2,20)
- TH1, TH17 and Treg attract neutrophils via CXCL8
- IFNγ, GM-CSF and TNF promote the survival of neutrophils

Mantovani et al. Nat. Rev. Imm. 2011

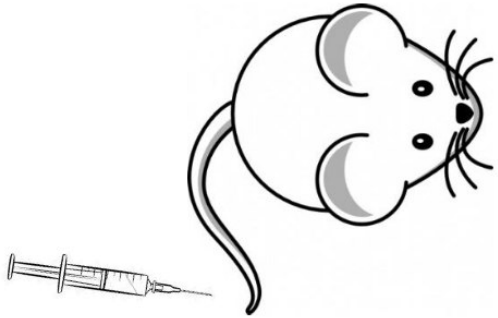
Neutrophils in cancer

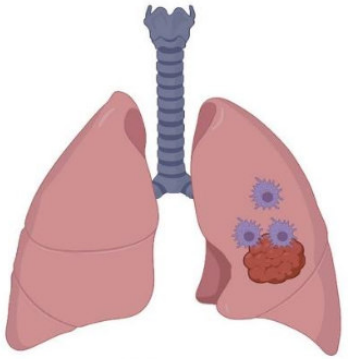


- neutrophils are driven by TGFβ to acquire a polarized, **pro-tumoral N2 phenotype** (characterized by high levels of arginase expression)
- **N1 phenotype** is associated with higher cytotoxic activity, higher expression of tumour necrosis factor (TNF) and lower expression of arginase leading to enhanced CD8+ T cell activation
- neutrophils **promote genetic instability** (possibly through ROS production) and **stimulate angiogenesis** (through MMP9 and VEGF)

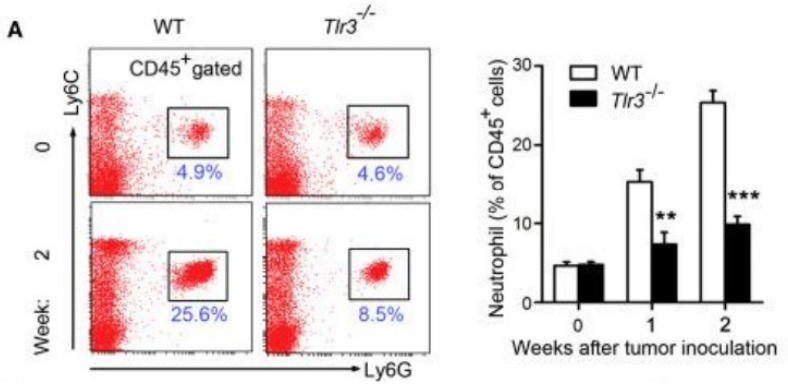
Neutrophils increase lung metastasis formation

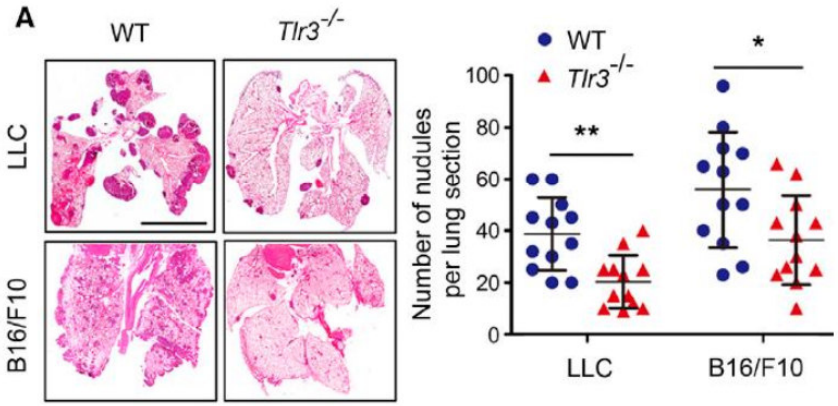
TLR3: activates epithelial cells in the lung to attract neutrophils





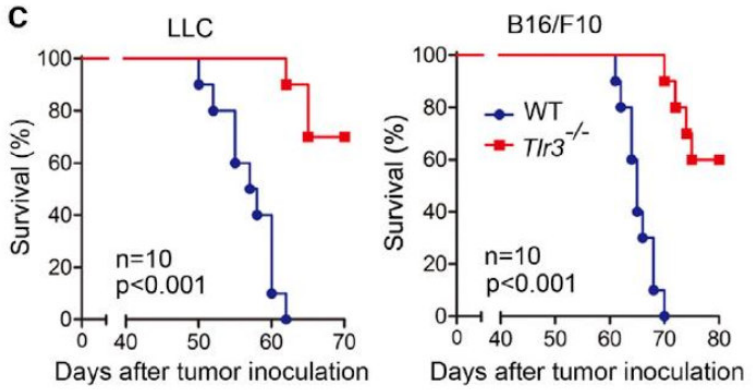
Lung Metastasis





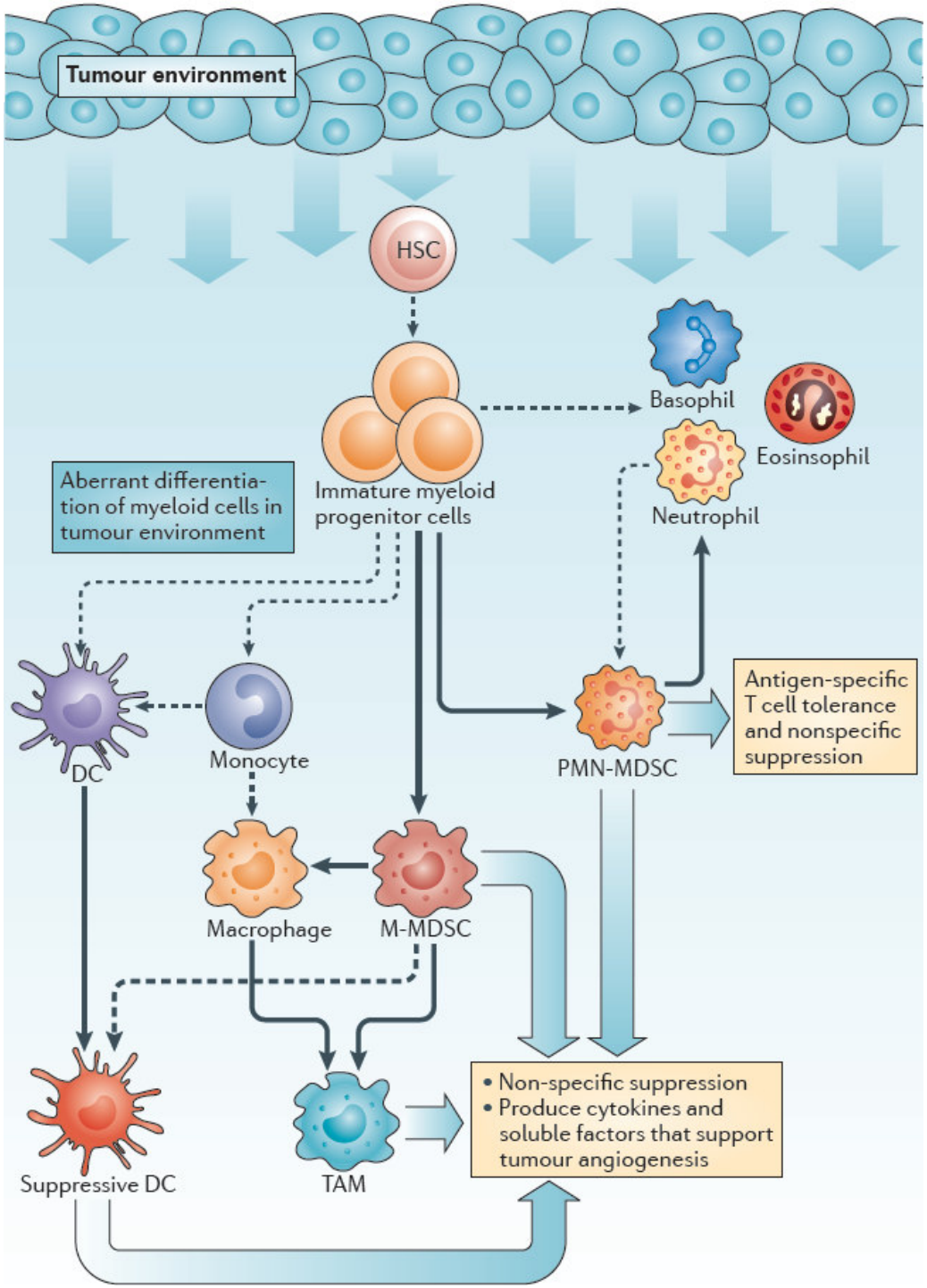
LLC: Lewis Lung Carcinoma, Lung cancer cell line

B16/F10: Melanoma (skin cancer) cell line



Tumor Exosomal RNAs Promote Lung Pre-metastatic Niche Formation by Activating Alveolar Epithelial TLR3 to Recruit Neutrophils
1.Liu, Yanfang et al.
2.Cancer Cell, Volume 30, Issue 2, 243 - 256

Aberrant differentiation of myeloid lineage cells



solid bold lines indicate the aberrant pathways of myeloid cell differentiation from **myeloid progenitors** that occur in cancer and chronic inflammation

these microenvironments promote the development of various immunosuppressive populations:

- monocytic myeloid-derived suppressor cells (**M-MDSCs**)
- polymorphonuclear myeloid-derived suppressor cells (**PMN-MDSCs = G-MDSCs**)
- **suppressive DCs**

the term MDSC has been questioned since these cells may represent normal intermediates of myeloid differentiation; **immature myeloid cells** (iMC) is used more and more to reflect this

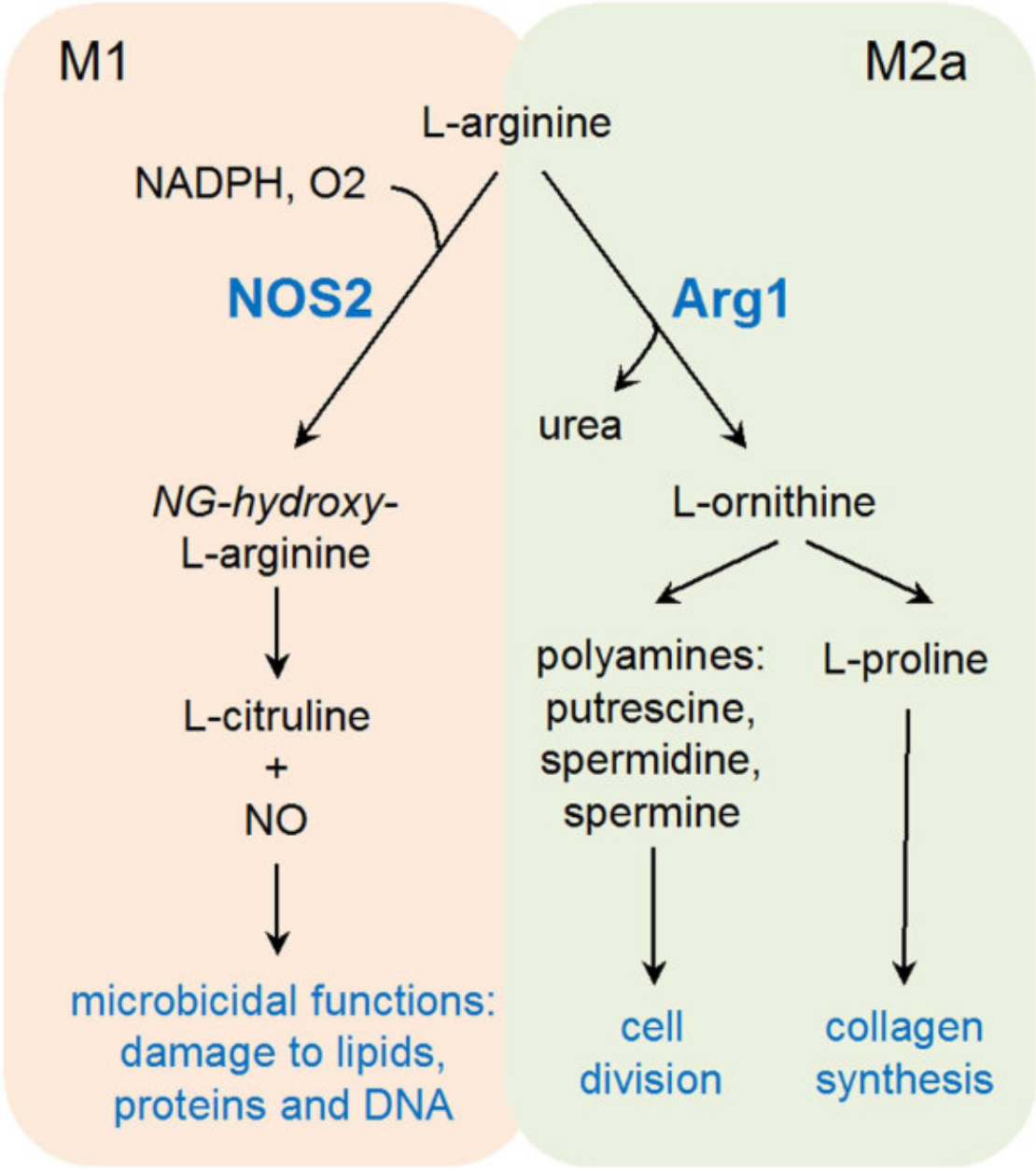
Gabrilovich et al. Nat. Rev. Imm. 2012

MDSCs / immature myeloid cells

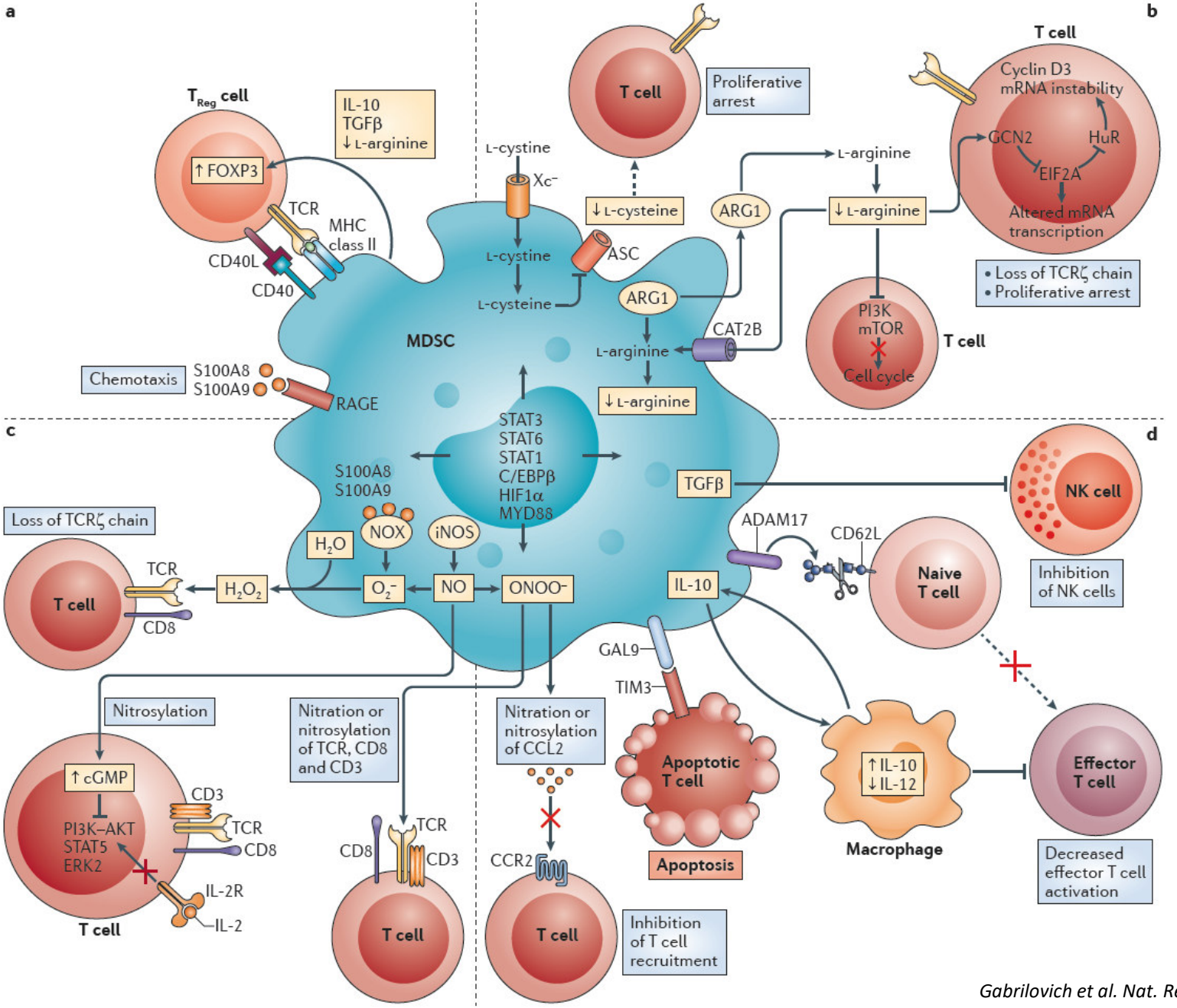
- immature myeloid cells (myeloid progenitor cells, immature macrophages, immature granulocytes, immature dendritic cells) characterized by the expression of **CD11b** and **GR1** (recognizes both Ly6C and Ly6G), and by the absence or reduced expression of mature myeloid cell markers
- MDSCs are a heterogeneous population of cells and are divided into two major subsets on the basis of their expression of the lymphocyte antigens LY6C and LY6G, and their morphology; these subsets are termed **monocytic MDSCs** ($CD11b^{+}LY6C^{hi}LY6G^{low}$ cells) and **granulocytic MDSCs** ($CD11b^{+}LY6C^{low}LY6G^{+}$ cells)
- monocytic MDSCs can differentiate into granulocytic MDSCs
- in humans, MDSCs are identified as $CD11b^{+}CD33^{+}HLA^{-}DR^{-}$, and are subdivided into $CD14^{hi}CD15^{-}$ monocytic MDSCs and $CD14^{low}CD15^{+}$ granulocytic MDSCs
- both MDSC subsets in mice express inducible nitric oxide synthase (iNOS) and arginase 1 (ARG1) at different levels, and suppress immune effector cell functions by producing reactive oxygen species
- MDSCs cultured *in vitro* differentiate into neutrophils, dendritic cells and macrophages
- adoptively transferred MDSCs migrate to the tumor site where they lose their GR1 expression and express the macrophage marker F4/80 within 48 hours. These results strongly suggest that MDSCs can be progenitor cells for tumour-associated macrophages and that the monocytic MDSCs are probably $LY6C^{hi}$ monocytes that have immunosuppressive characteristics

Arginine catablism as metabolic adaptation in myeloid function

- products of Arg1 vs iNOS appear to fulfill diametrically opposed functions:
- Arg1 enhances collagen synthesis and cell growth via L-ornithine production and has an important function in wound healing; furthermore ornithine can directly block worm infections
- NOS2 generated nitric oxide (NO) represents a major effector molecule in macrophage-mediated cytotoxicity controlling bacterial and parasitic infections



Effector functions of MDSCs

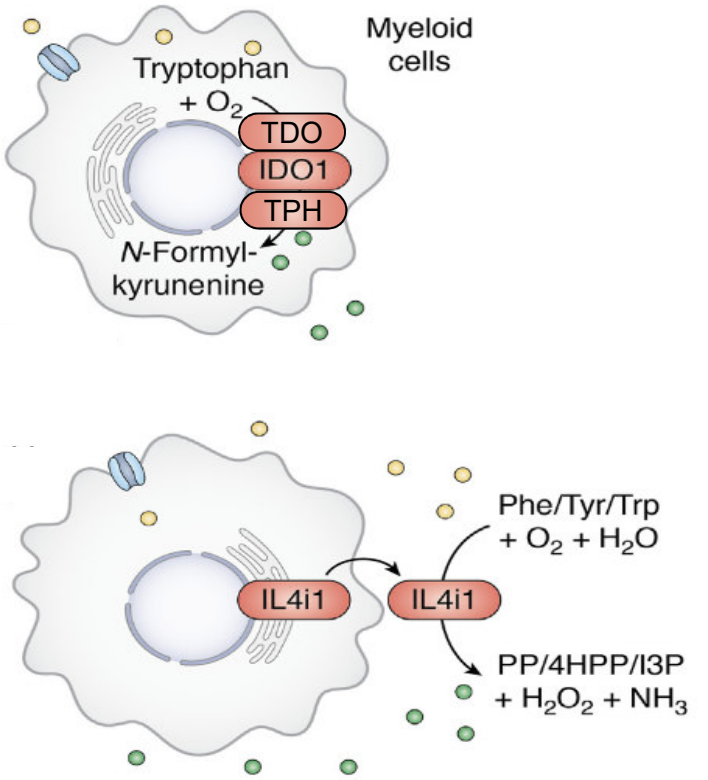


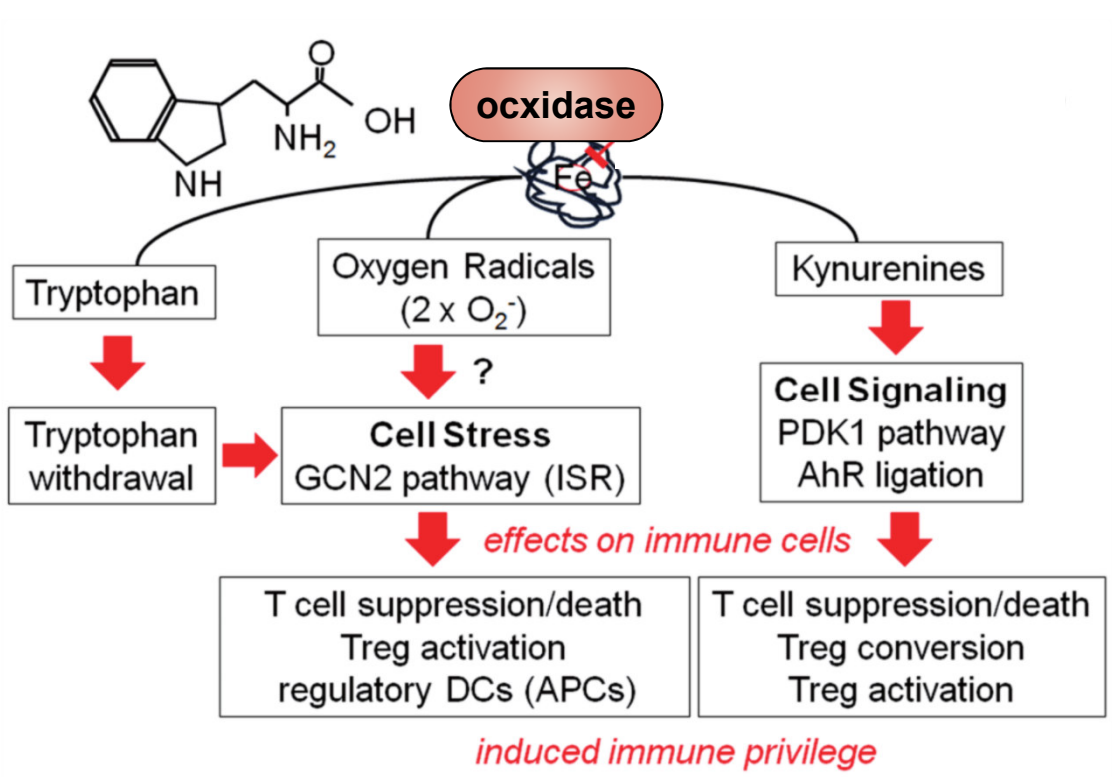
Gabrilovich et al. Nat. Rev. Imm. 2012

Effector functions of MDSCs

- produce **ROS** by NADPH oxidase (**NOX**) complexes and nitric oxide (NO) by **iNOS** resulting in release of oxidizing molecules, such as hydrogen peroxide (H_2O_2) and peroxynitrite (ONOO–). Peroxynitrite causes nitration and nitrosylation of components of the T cell receptor (TCR) signalling complex, and H_2O_2 causes the loss of the TCR ζ-chain, thereby inhibiting T cell activation through the TCR
- the metalloproteinase ADAM17 cleaves CD62L, which is necessary for T cell migration to draining lymph nodes
- induce apoptosis of T cells via galectin 9 (GAL9) that engage T cell immunoglobulin and mucin domain-containing protein 3 (TIM3) on T cells
- induce development of regulatory T (**Treg**) cells via IL10 and TGFβ, expand existing Treg cell populations (CD40) or contribute to the maintenance of Tregs (CD80)
- deprive T cells of amino acids that are essential for their growth and differentiation via **ARG1** (arginase), **Xc–cystine–glutamate transporter** and **IDO** (tryptohan depletion)

Tryptophan catabolism prevents T cell immune reaction





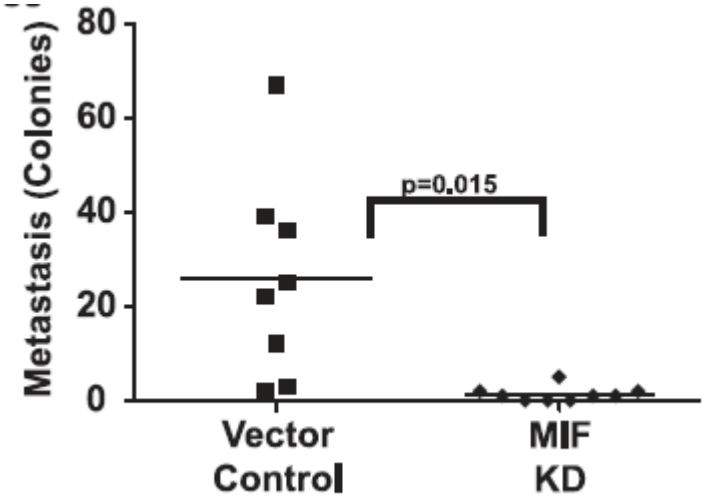
IL4i1: secreted aromatic amino acid oxidase
expressed in myeloid cells to suppress T cells

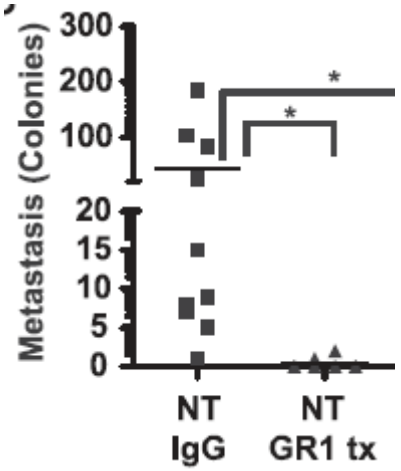
IDO: Indolamin-2,3-Dioxygenase
expressed in all cells, but upregulated in myeloid cells, essential for immune tolerance during pregnancy

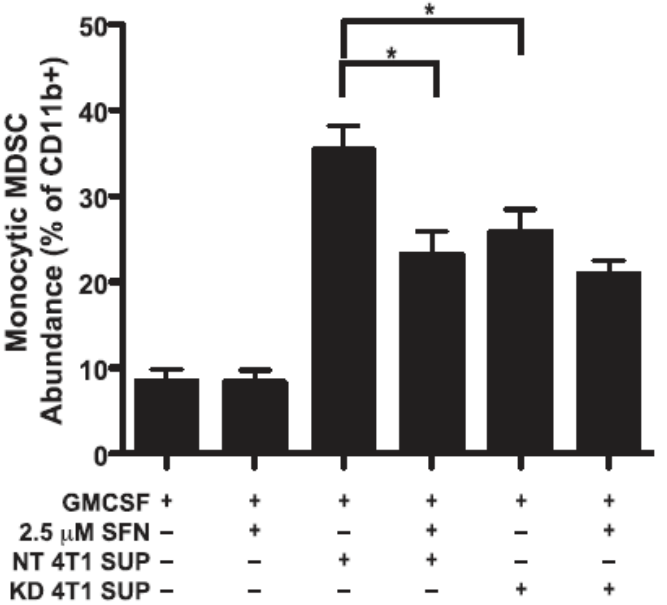
TDO: Tryptophan-2,3-Dioxygenase
expression restricted to liver but often upregulated in cancer cells

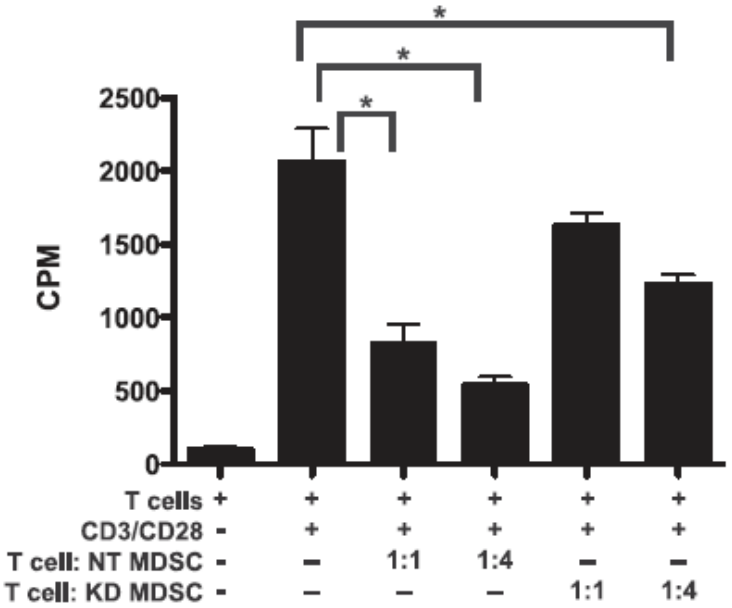
TPH: Tryptophan hydroxylase
upregulated e.g. in mast cells

MIF induces mo-MDSCs to facilitate metastasis







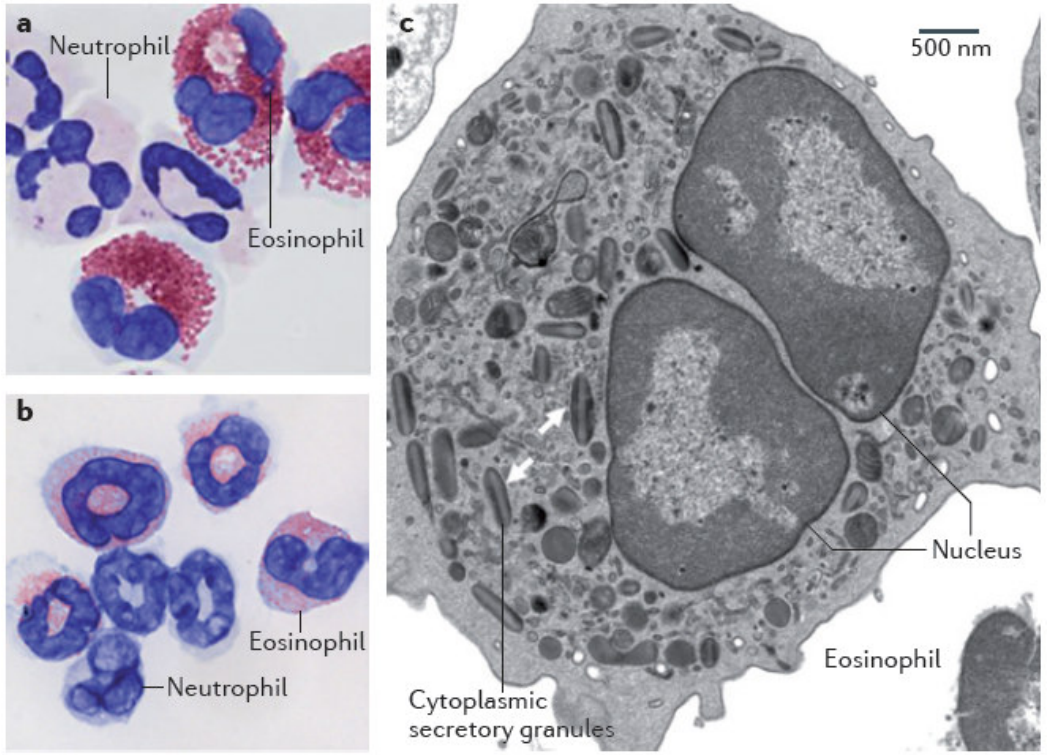


Simpson et al. J. Imm. 2012

Eosinophiles: inflammatory effector cells

human

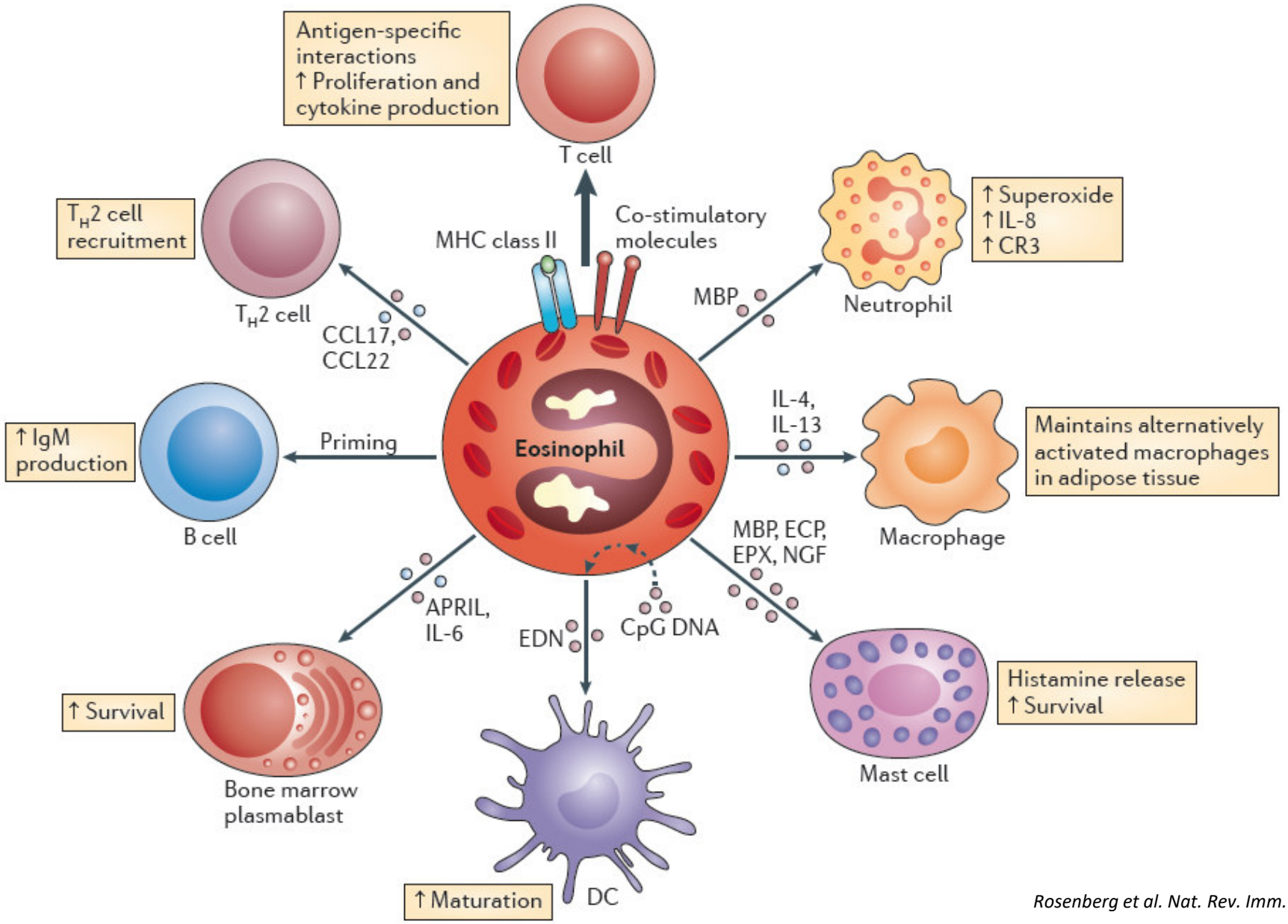
mouse



- markers: IL5R, CCR3, SIGLEC-F
- eosinophiles are released into the peripheral blood in a phenotypically mature state and are recruited into tissues in response to IL-5 (mostly T cell derived) and the eotaxin chemokines
- eosinophils have a half-life of ~18 hrs in the blood, but their survival is prolonged upon activation in tissues
- eosinophiles produce CCL17 and CCL22 which promote the recruitment of TH2 cells and IL-4/13 and IFNγ that promote TH1 and TH2 cells
- main function in anti-bacterial and anti-viral immunity, as well as in asthma

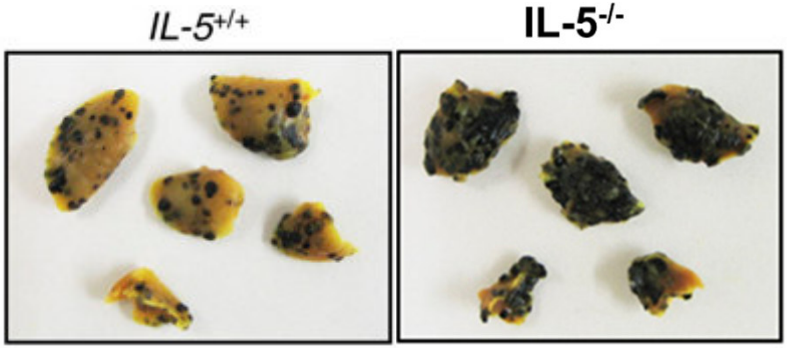
Rosenberg et al. Nat. Rev. Imm. 2013

Eosinophiles as modulators of immune reactions

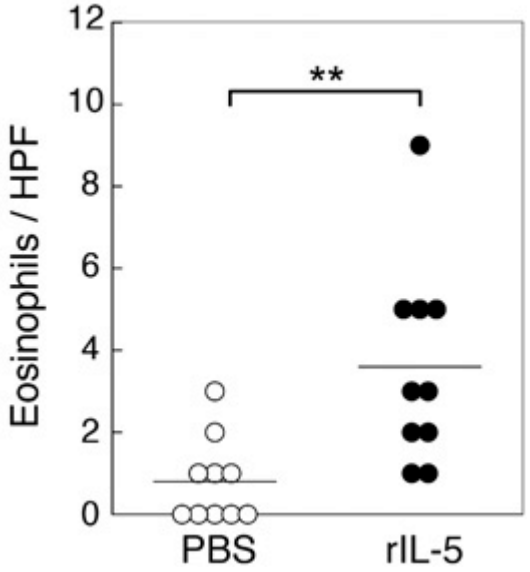


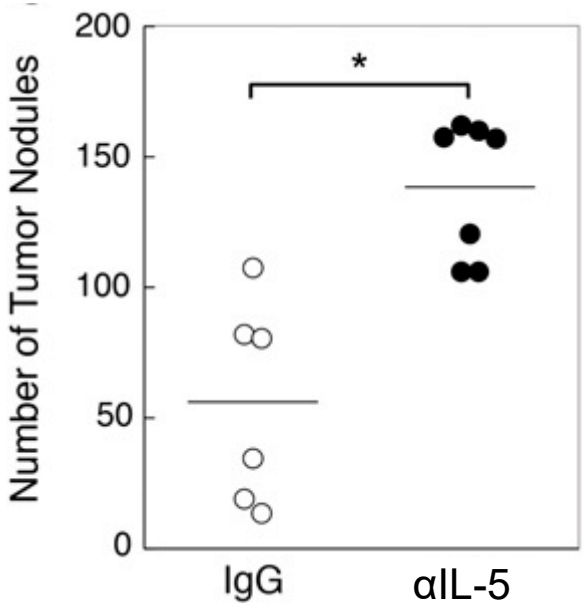
Rosenberg et al. Nat. Rev. Imm. 2013

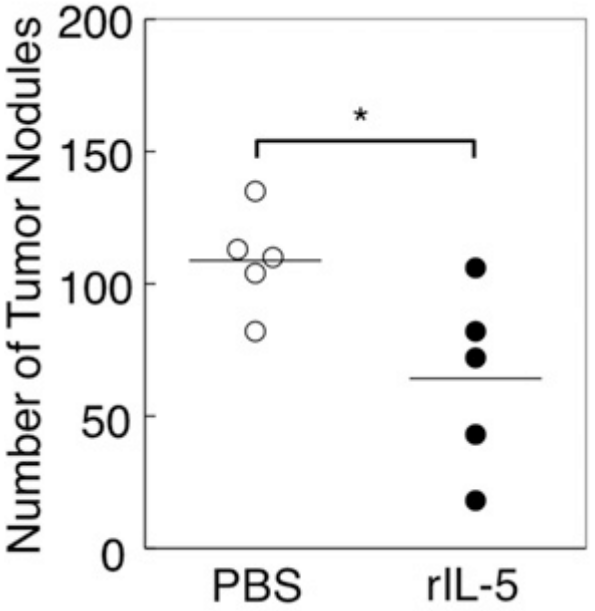
Eosinophil recruitment reduces lung metastasis



B16F10 model

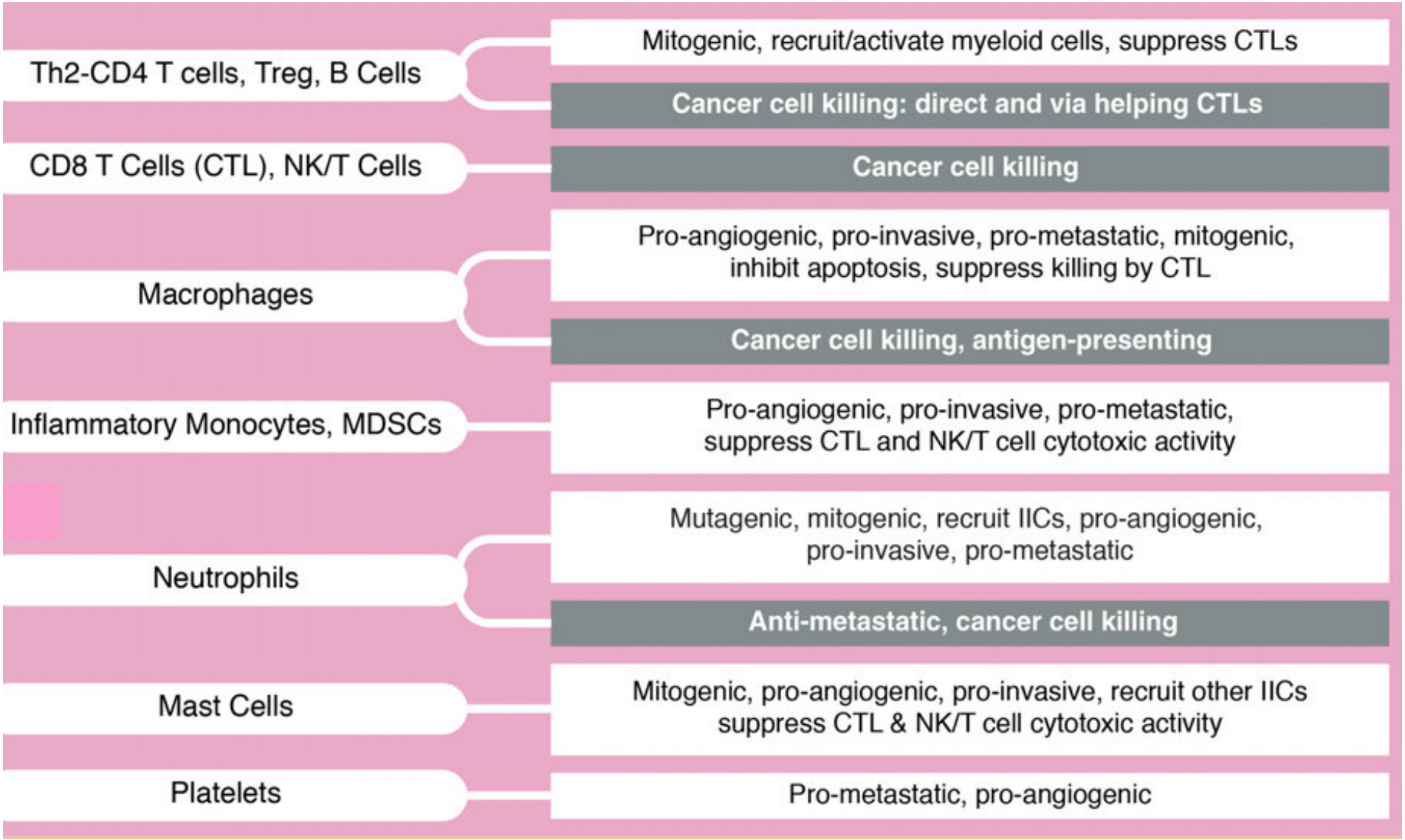






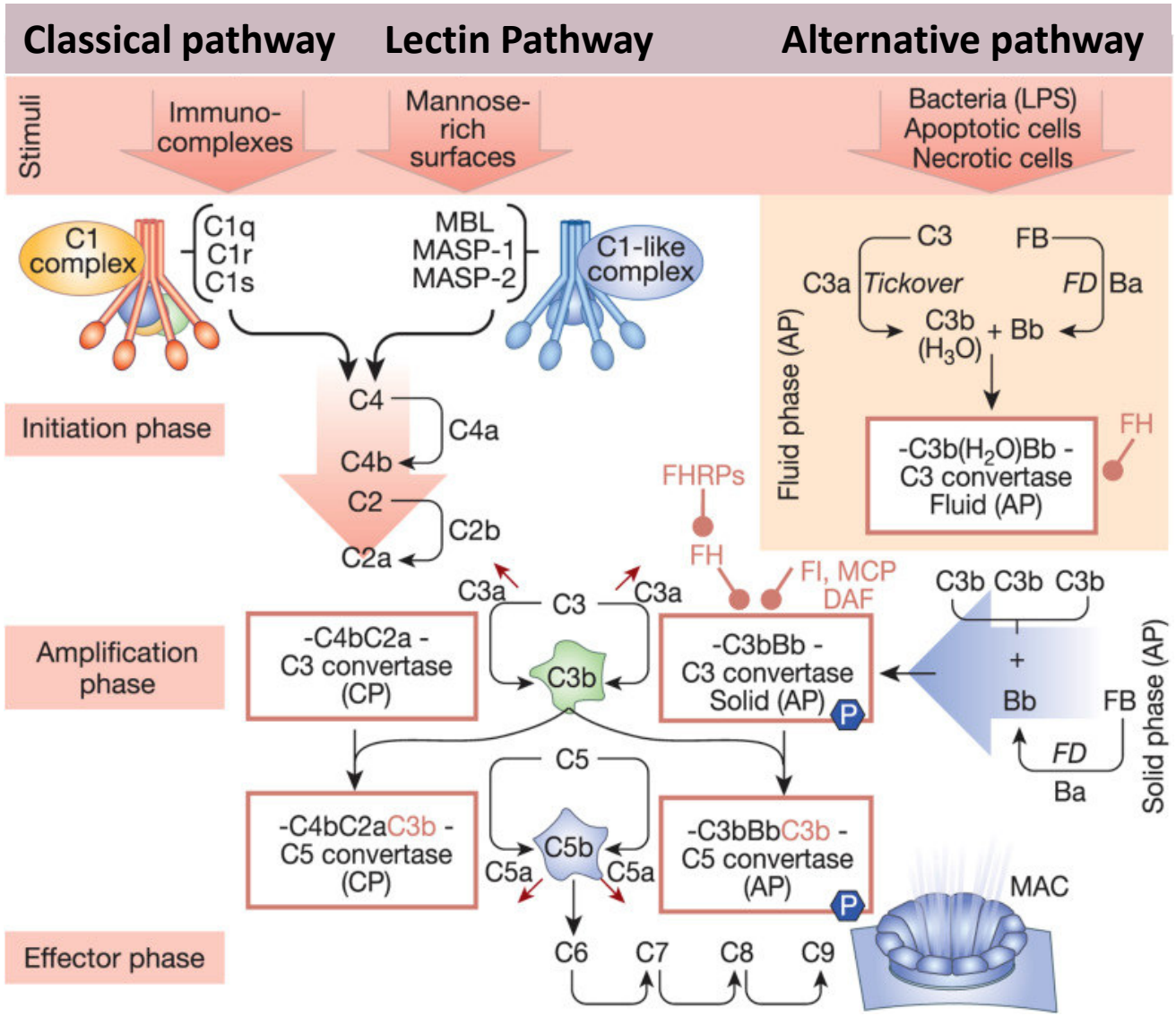
Ikutani et al. J. Imm. 2012

Immune cell functions in cancer



Hanahan & Coussens Cancer Cell 2012

Complement system



The three pathways of activation all generate variants of the protease C3-convertase

- C3-convertase cleaves and activates complement C3, creating C3a and C3b
- C5-convertase is formed when C3b complexes with C3 convertase and cleaves C5 into C5a and C5b

C3a, C5a: important chemoattractants for myeloid cells that can activate their degranulation, it further increases expression of adhesion molecules on endothel cells enhancing vascular permeability

C3b binds to the surface of pathogens (opsonization), leading to internalization by phagocytic cells

C5b initiates the membrane attack complex (MAC) leading to target cell permeabilization