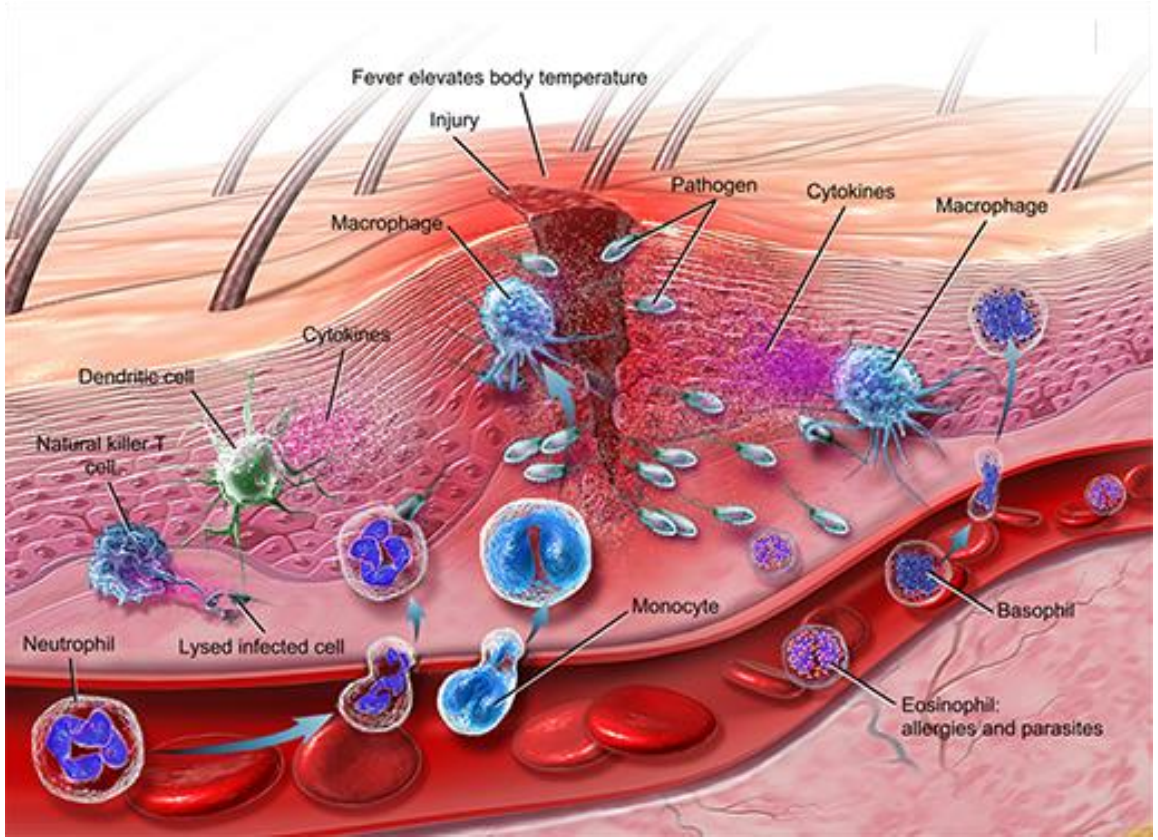
Innate Immunity



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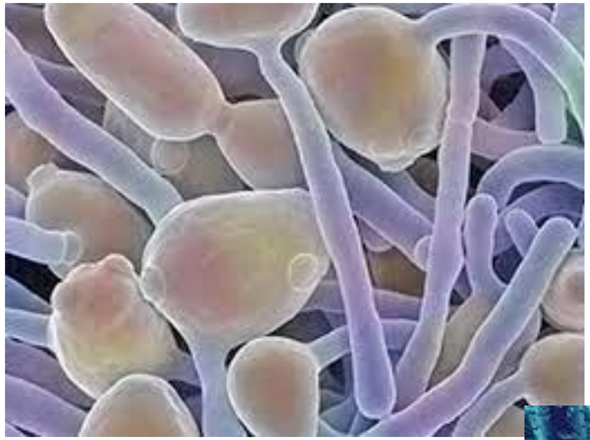
Laboratory of Rheumatology, Autoimmunity and Inflammation

4th Immunology Workshop for Clinicians
16-18 June 2023, Heraklion, Crete

What does the immune system do?



fungi

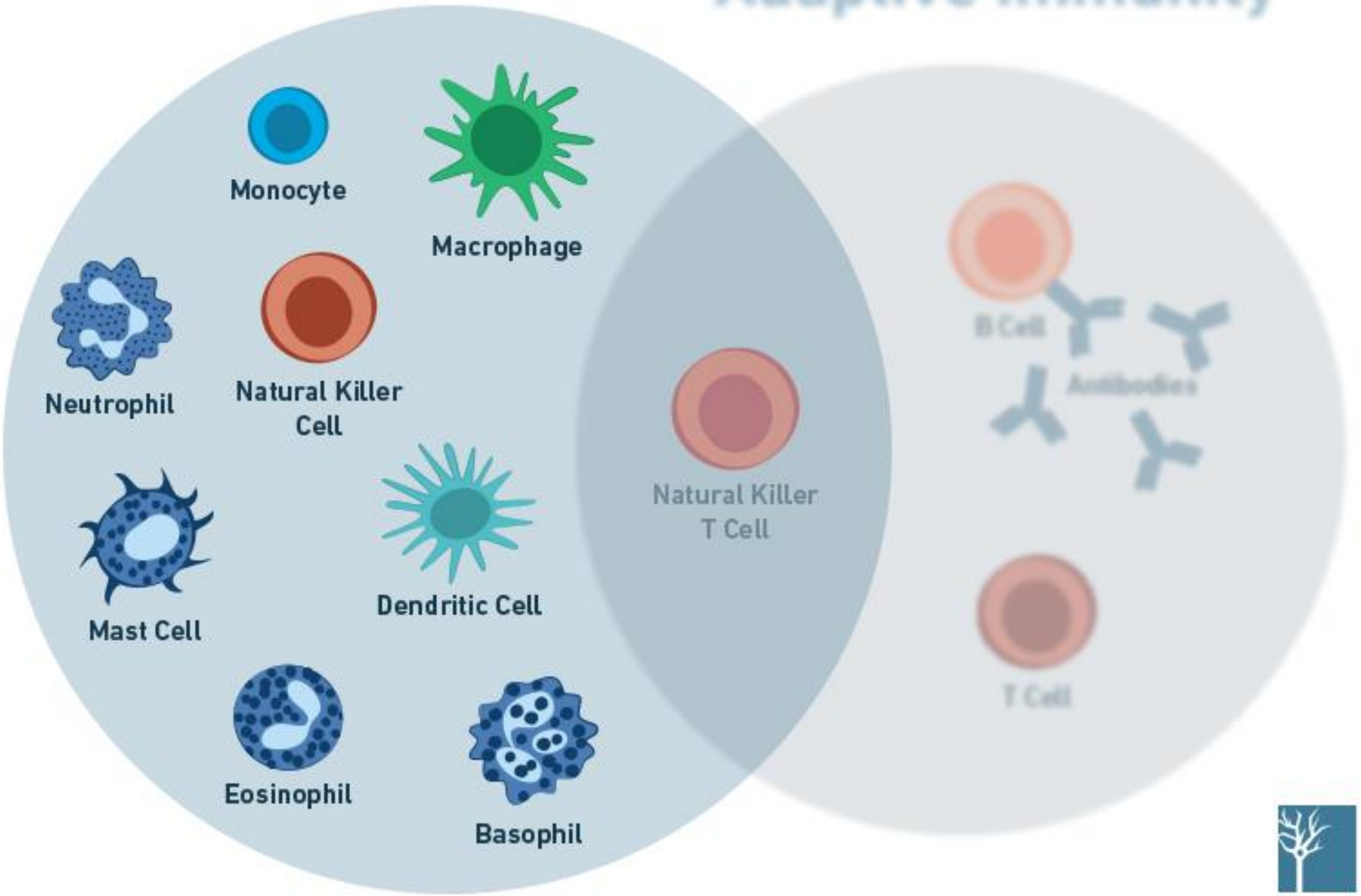
bacteria

parasitic protozoa

helminths

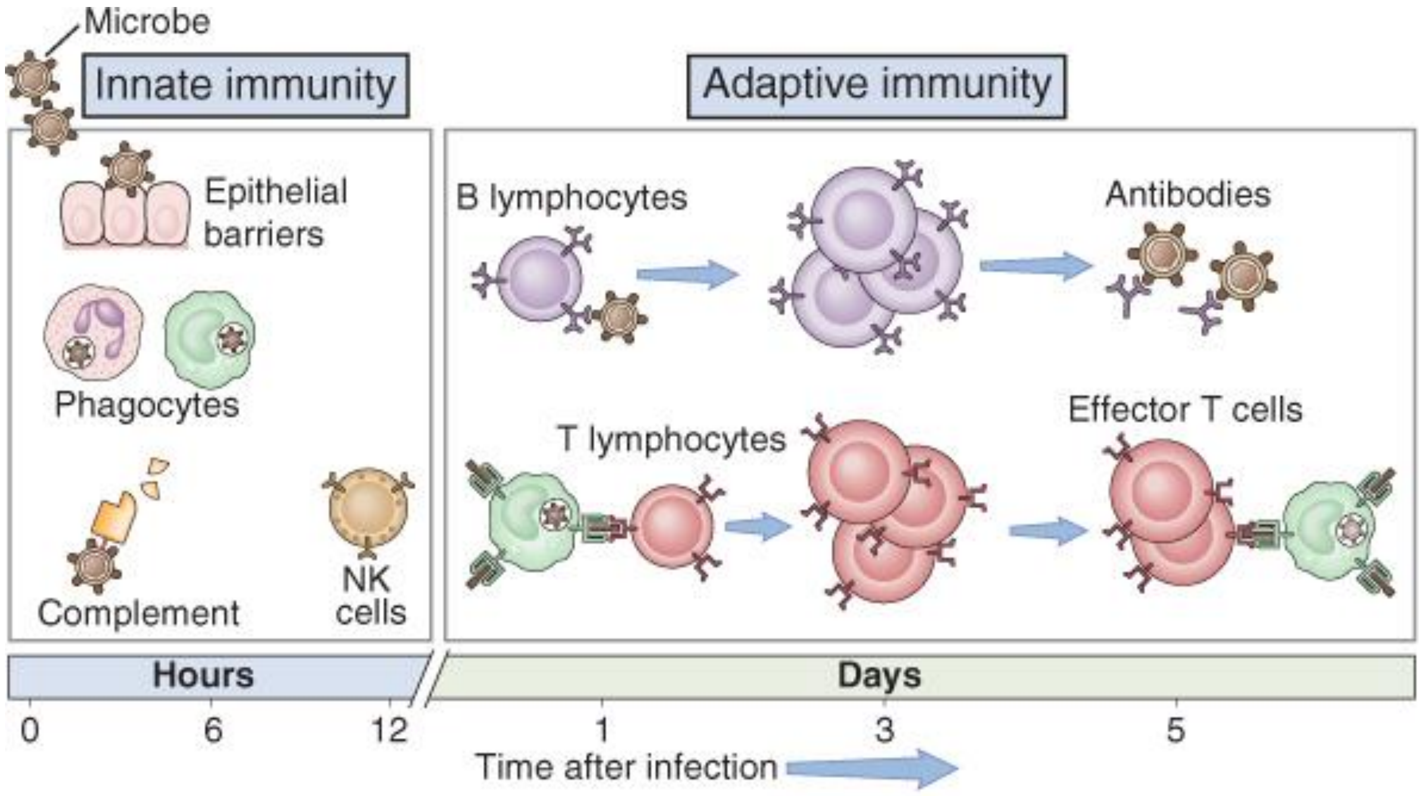
Innate Immunity

Adaptive Immunity



Properties of innate immunity

- Exists before the entrance of a pathogen
- First line of defense (skin barriers, mucosal surfaces)
- Initial response to microbes (within min.)
- Not specific for an antigen
- No immunological memory
- Stimulates and shapes adaptive immune responses

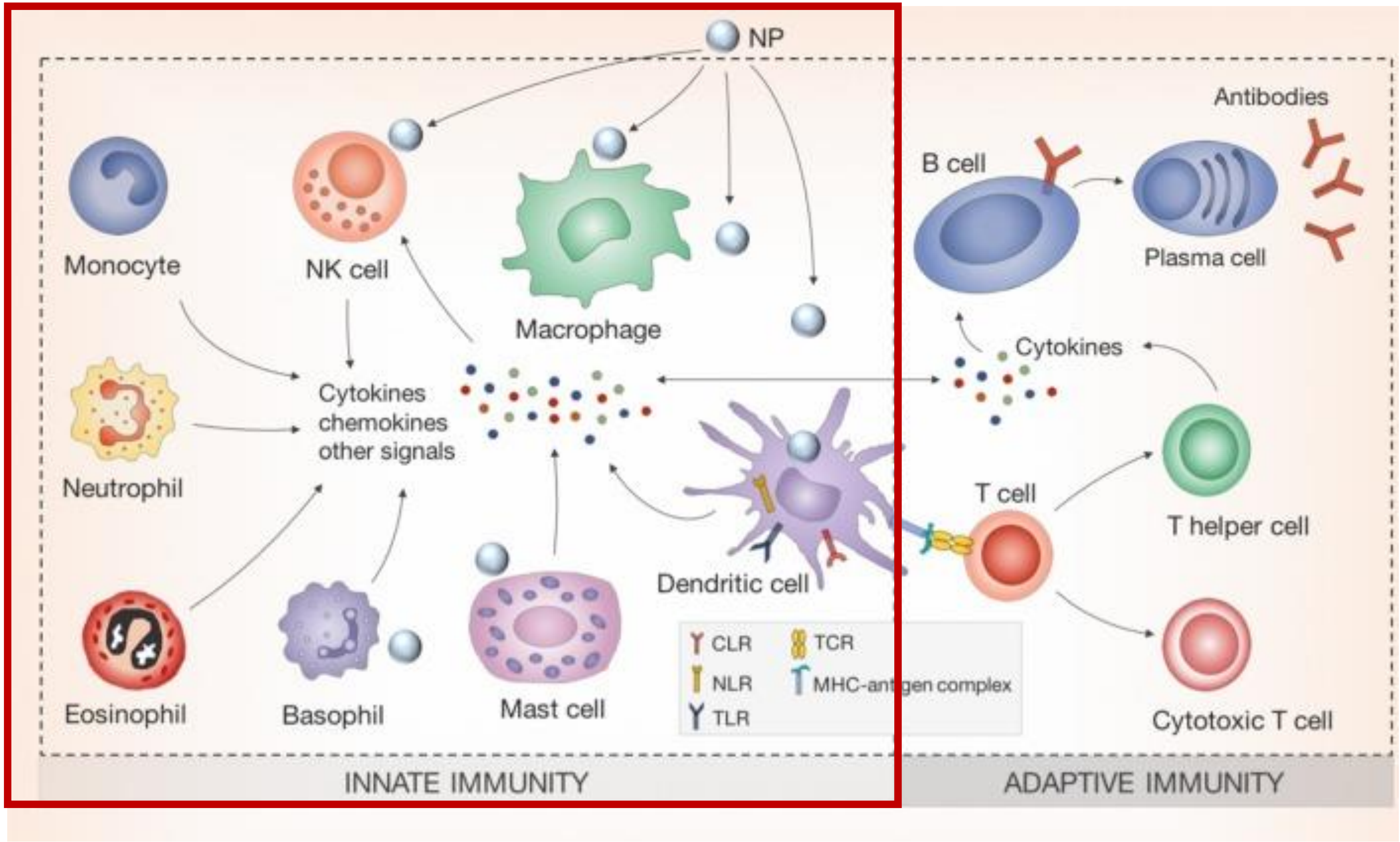


Innate immune system: first line of defense

	Skin	Gastrointestinal tract	Respiratory tract	Urogenital tract	Eyes
Mechanical	Epithelial cells joined by tight junctions				
	Flow of fluid, perspiration, sloughing off of skin	Flow of fluid, mucus, food, and saliva	Flow of fluid and mucus, e.g., by cilia Air flow	Flow of fluid, urine, mucus, sperm	Flow of fluid, tears
Chemical	Sebum (fatty acids, lactic acid, lysozyme)	Acidity, enzymes (proteases)	Lysozyme in nasal secretions	Acidity in vaginal secretions Spermine and zinc in semen	Lysozyme in tears
	Antimicrobial peptides (defensins)				
Microbiological	Normal flora of the skin	Normal flora of the gastrointestinal tract	Normal flora of the respiratory tract	Normal flora of the urogenital tract	Normal flora of the eyes

Figure 1.6 The Immune System, 3ed. (© Garland Science 2009)

Cells involved in innate immune responses



Phagocytosis of extracellular microbes: a cytoskeletal-dependent process

phagocytes

Neutrophil

Monocyte

Macrophage

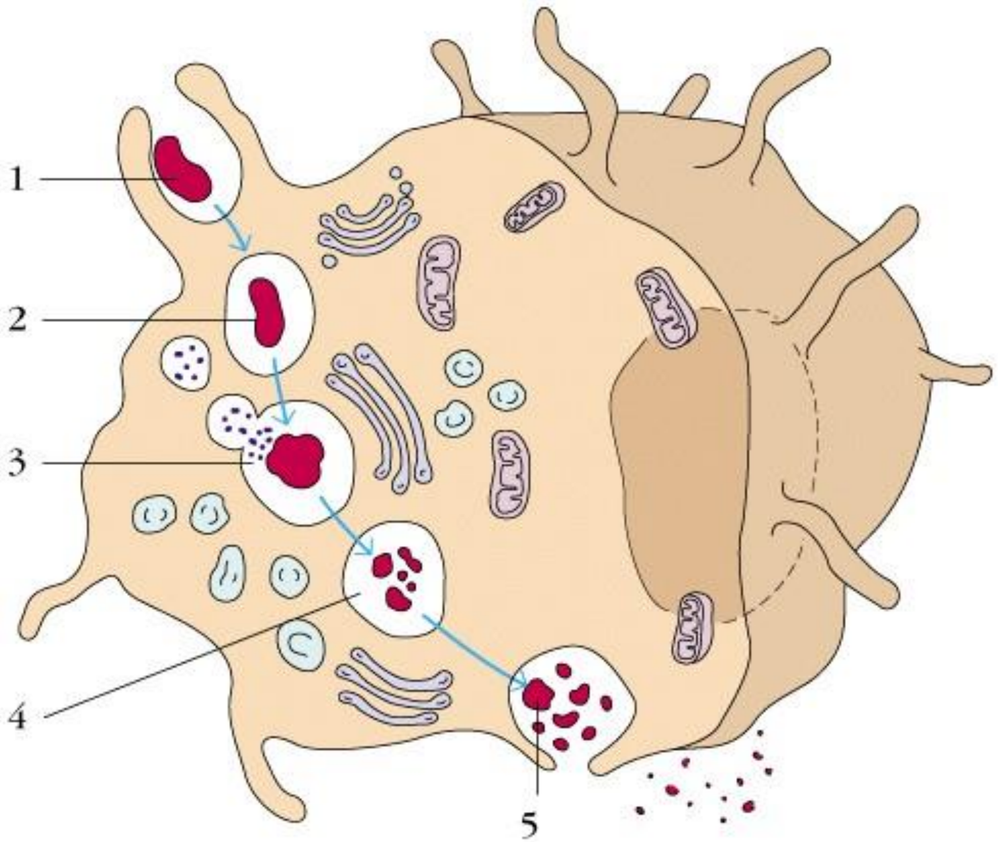
Dendritic cell

Engulfment of
> 0.5µm bacteria by
pseudopodia

phagosome

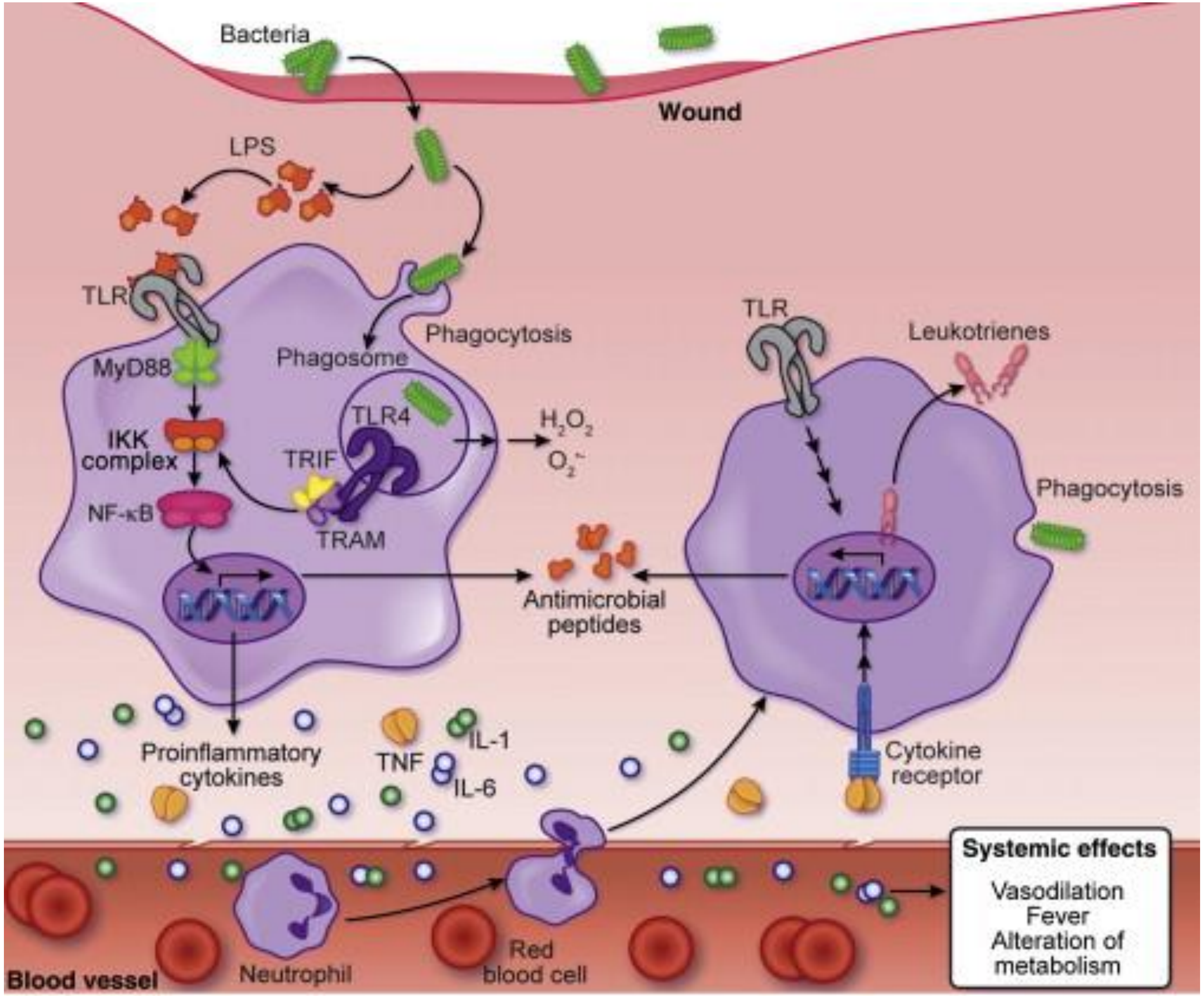
fusion to form
phagolysosome

killing, digestion



waste release

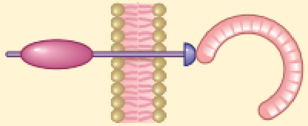
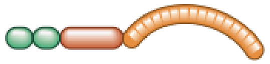
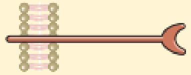
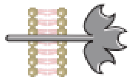
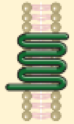
Receptor-mediated phagocytosis



PAMPs-DAMPs

		Microbe Type
Pathogen-Associated Molecular Patterns		
Nucleic acids	ssRNA dsRNA CpG	Virus Virus Virus, bacteria
Proteins	Pilin Flagellin	Bacteria Bacteria
Cell wall lipids	LPS Lipoteichoic acid	Gram-negative bacteria Gram-positive bacteria
Carbohydrates	Mannan Glucans	Fungi, bacteria Fungi
Damage-Associated Molecular Patterns		
Stress-induced proteins	HSPs	—
Crystals	Monosodium urate	—
Proteolytically cleaved extracellular matrix	Proteoglycan peptides	—
Mitochondria and mitochondrial components	Formylated peptides and ATP	—
Nuclear proteins	HMGB1, histones	—

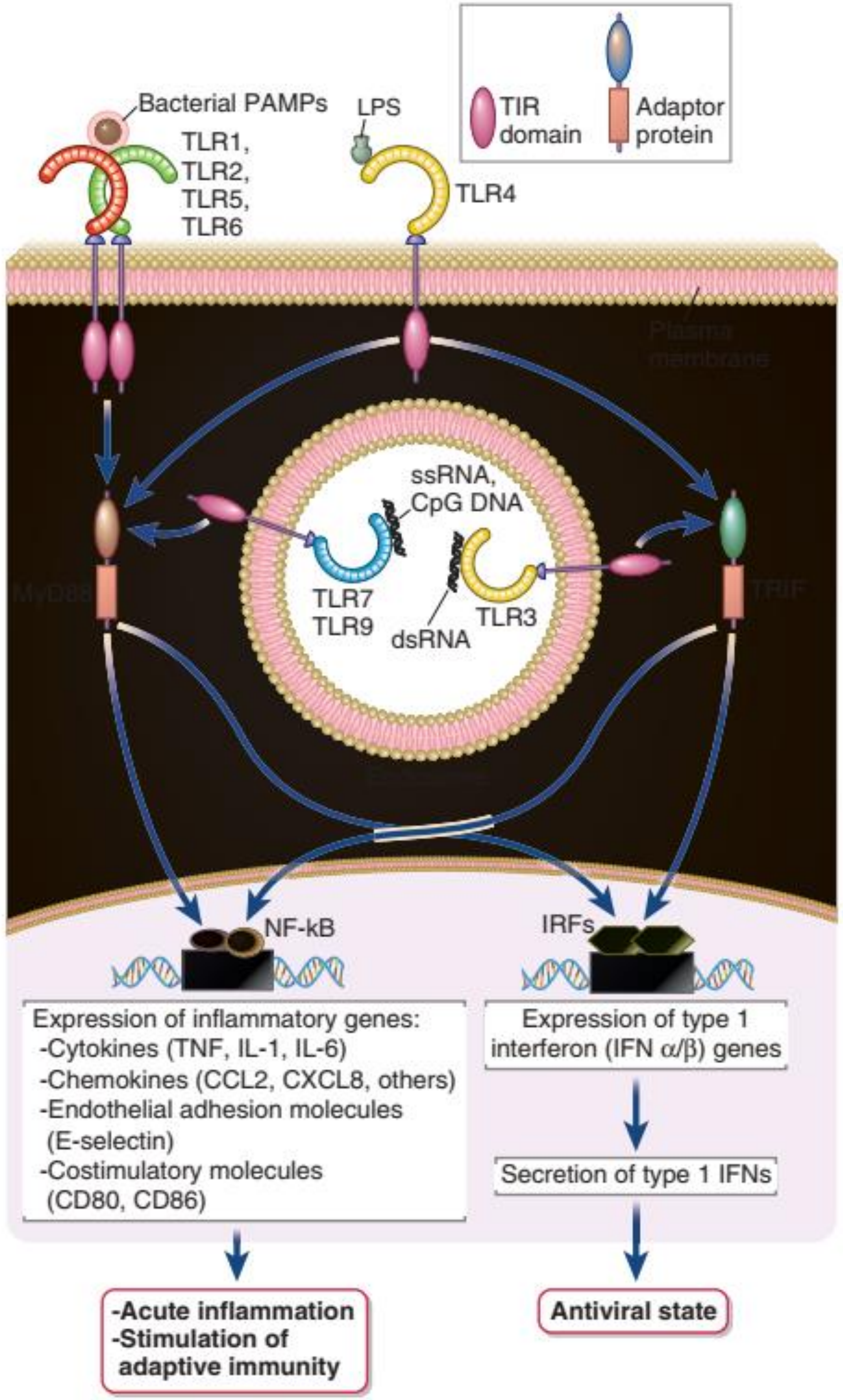
PRRs

Pattern Recognition Receptors	Location	Specific Examples	Ligands (PAMPs or DAMPs)
Cell-Associated			
TLRs 	Plasma membrane and endosomal membranes of DCs, phagocytes, B cells, endothelial cells, and many other cell types	TLRs 1–9	Various microbial molecules including bacterial LPS and peptidoglycans; viral nucleic acids
NLRs 	Cytosol of phagocytes, epithelial cells, and other cells	NOD1/2 NLRP family (inflammasomes)	Bacterial cell wall peptidoglycans Intracellular crystals (urate, silica); changes in cytosolic ATP and ion concentrations; lysosomal damage
RLRs 	Cytosol of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
CDSs 	Cytosol of many cell types	AIM2; STING-associated CDSs	Bacterial and viral DNA
CLRs 	Plasma membranes of phagocytes	Mannose receptor DC-sign Dectin-1, Dectin-2	Microbial surface carbohydrates with terminal mannose and fructose Glucans present in fungal and bacterial cell walls
Scavenger receptors 	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
N-Formyl met-leu-phe receptors 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues

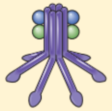
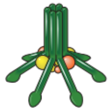
adapted from Cellular and Molecular Immunology 9th Edition

The example of TLRs

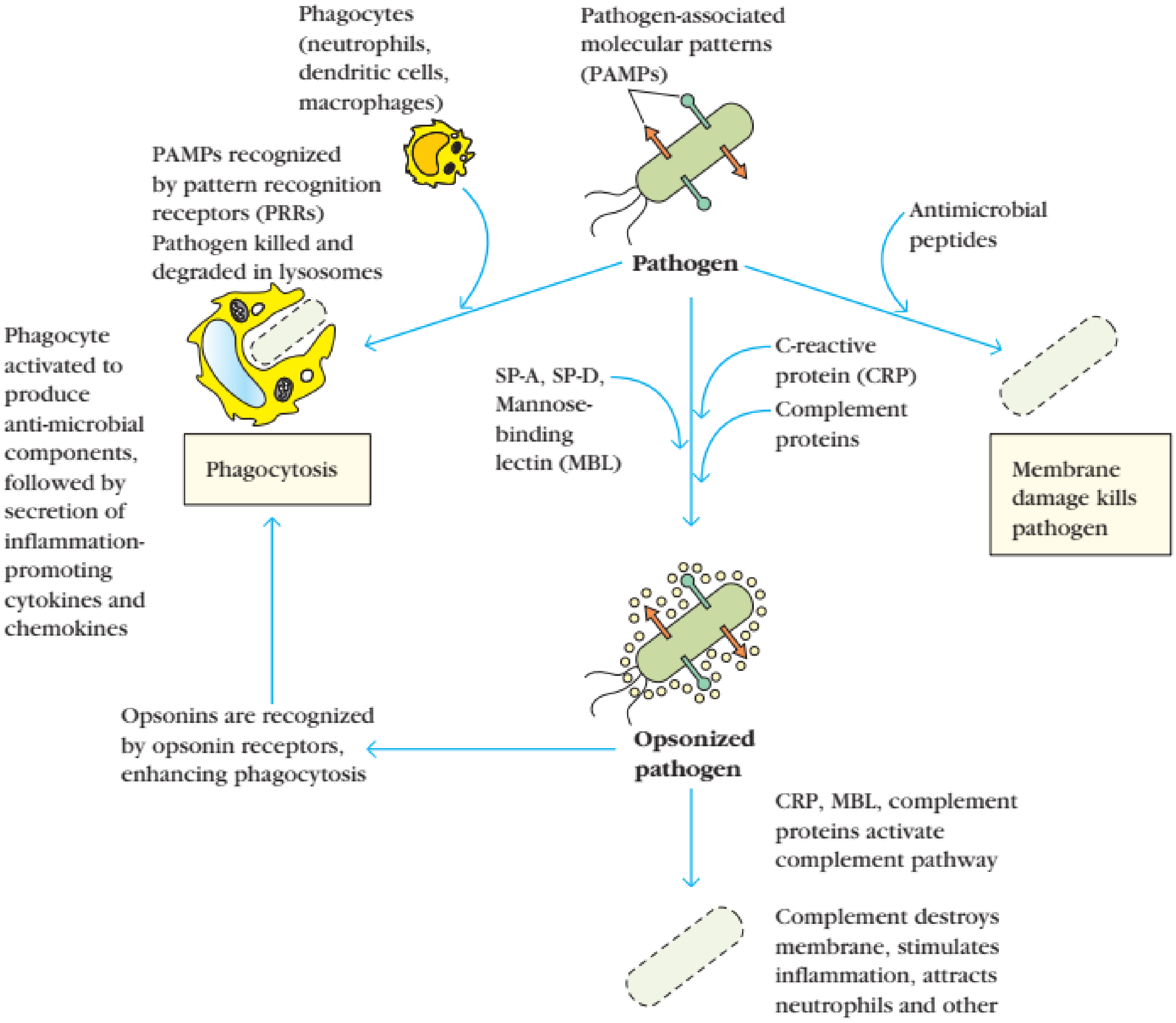
- *Toll* was discovered as a Drosophila gene involved in the dorsal- ventral axis during development of the fruit fly
- 11 TLRs have been identified in humans
- Some TLRs are expressed on the cell surface and others in endosomes



Soluble effector molecules of innate immunity

Soluble			
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma Alveoli	Mannose-binding lectin Surfactant proteins SP-A and SP-D	Carbohydrates with terminal mannose and fructose Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	Various complement proteins	Microbial surfaces

adapted from Cellular and Molecular Immunology 9th Edition



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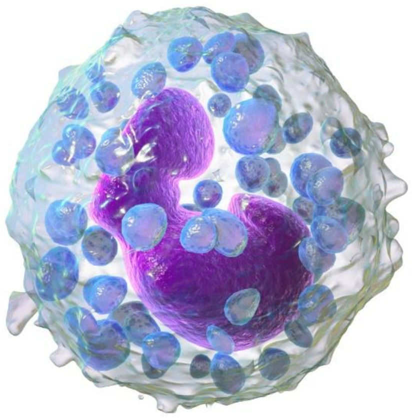
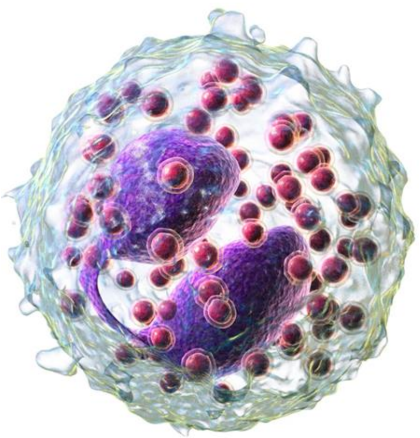
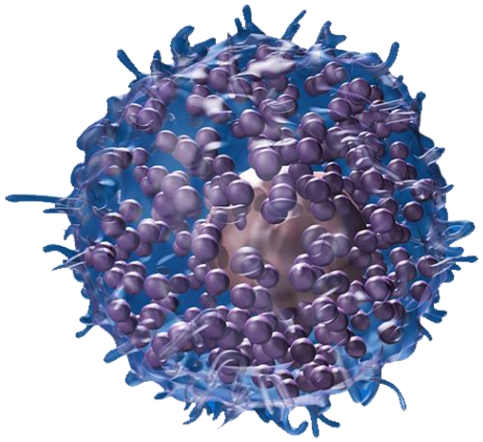
Granulocytes: critical players of the immune system



basophils



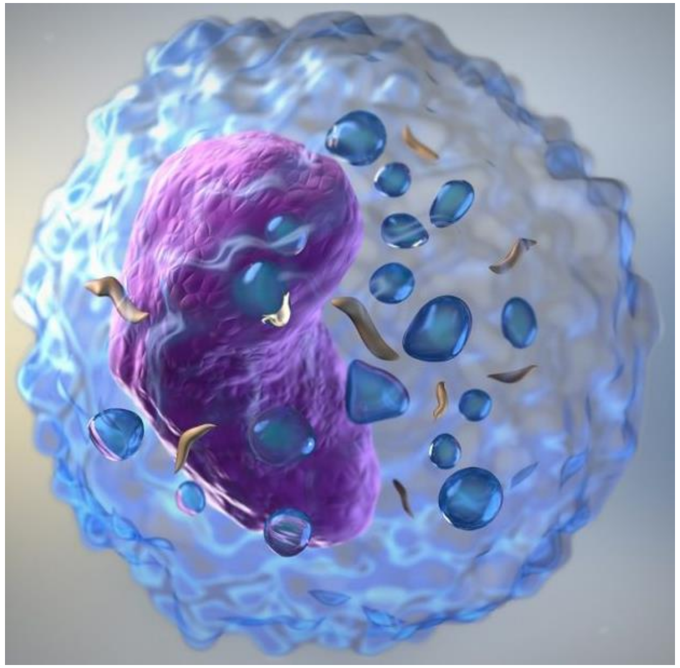
eosinophils



mast cells

- release compounds from pre-formed granules to destroy bacteria and other pathogens
- degranulation leads to secretion of lytic enzymes, inflammatory mediators
- molecules released include toxins, histamine, proteases, inflammatory molecules
- major role in allergic responses (e.g.IL-4 production by basophils)

Natural killer cells

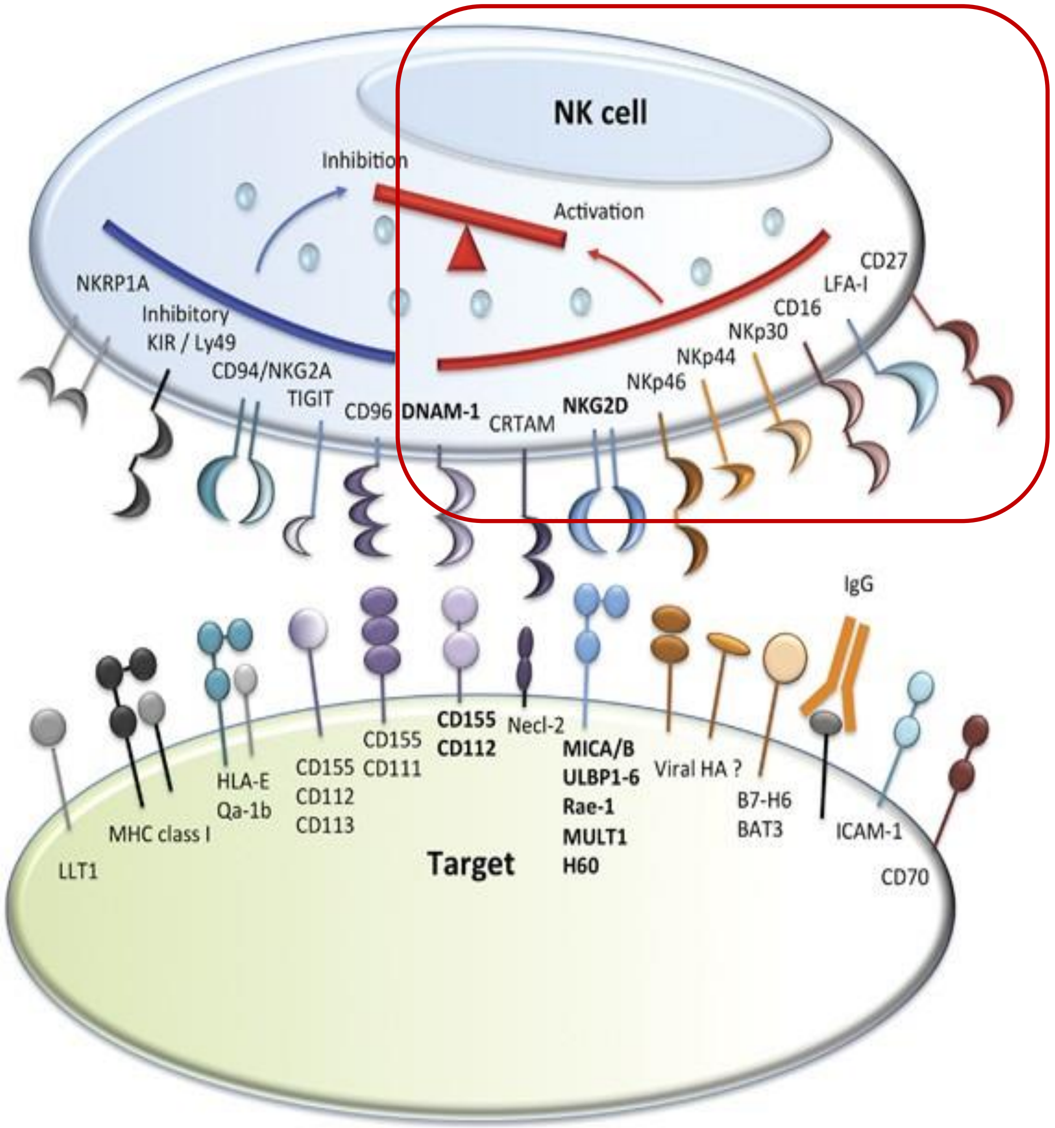


- large granulated lymphocytes, 5-10% blood lymphocytes
- also found in skin, gut, liver, lung
- important in host defence, cytotoxic to tumour cells and virally infected cells (i.e herpes)
- no antigen-specific receptor
- complex sets of activating and inhibitory receptors: balance of signals
- provide link between innate and adaptive immunity

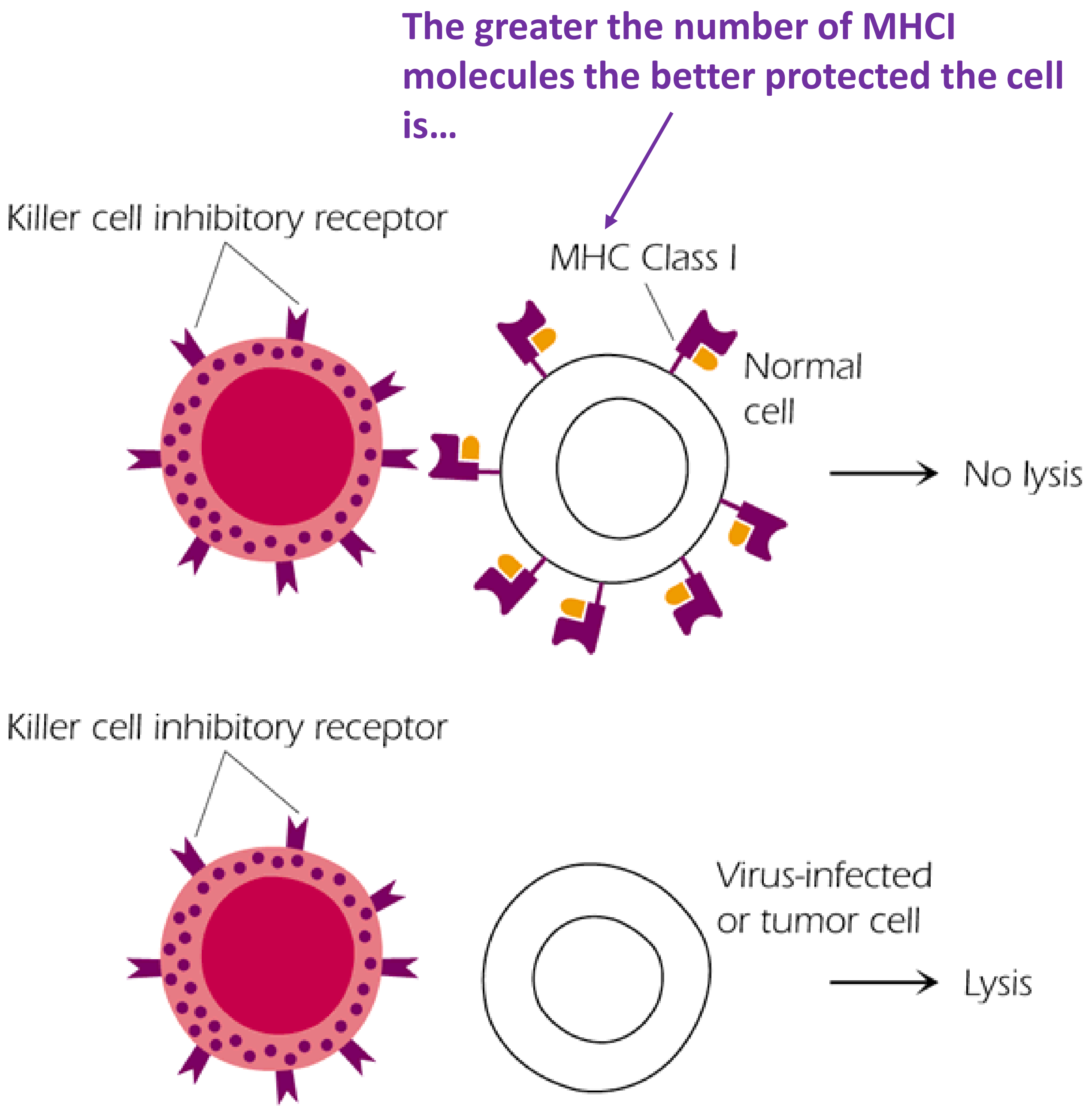
- NK cell express various combinations of **activating receptors** and **inhibitory receptors**

- **Activating receptors** recognize cell-surface proteins induced on target cells by metabolic stress, such as malignant transformation or microbial infection, DNA damage, heat-shock related stress etc.

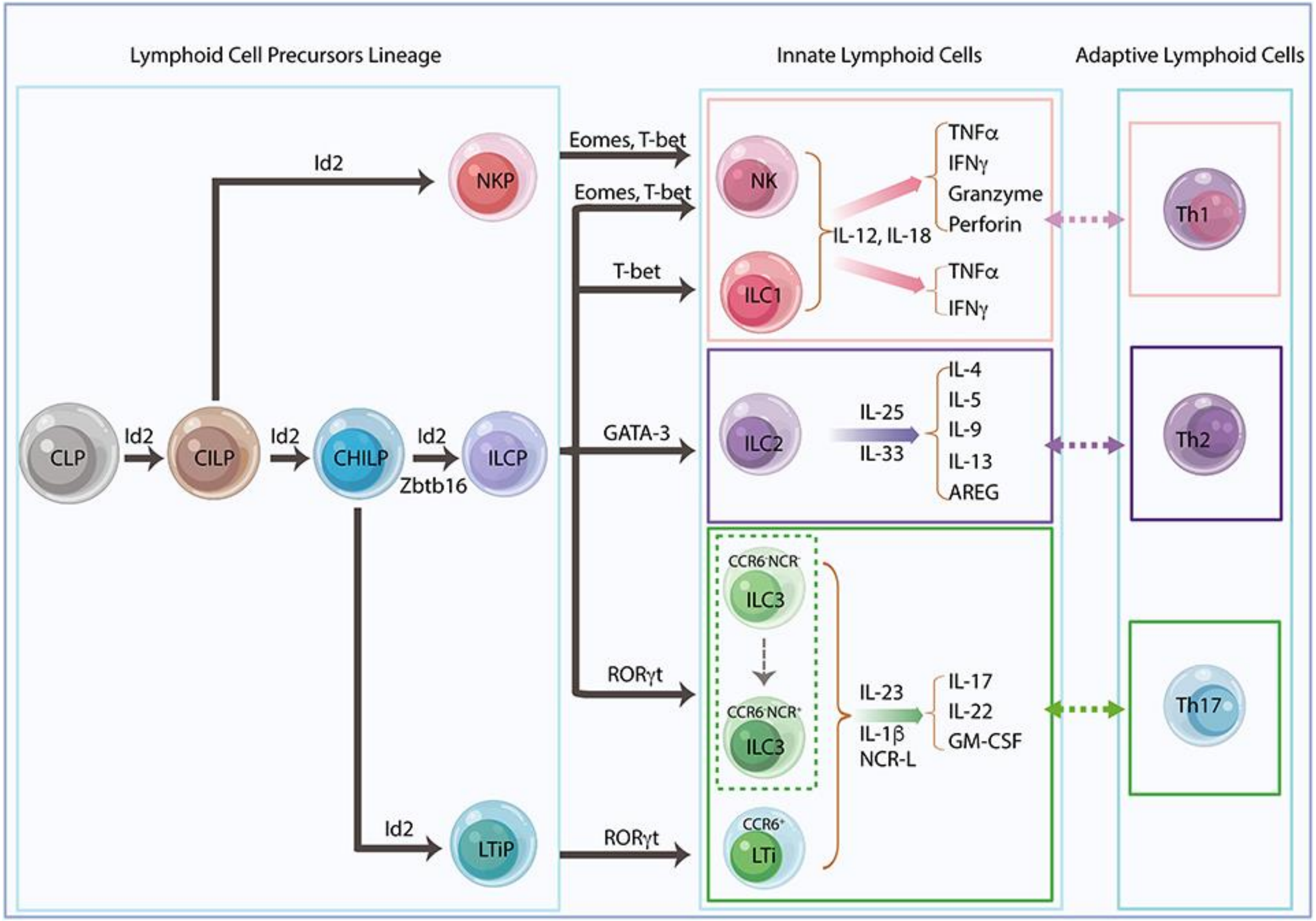
- Stimulation of activating receptors leads to the release of cytokines (IFN-γ, TNF-α, GM-CSF) and chemokines (CCL1-5, CXCL8) by NK cells that enhance their cytotoxic capacity



- Inhibitory receptors on NK cells recognize surface molecules such as MHC Class I molecules
- This recognition prevents NK cells from killing normal host cells
- If the MHC I molecules are missing or downregulated (tumor cells, virally-infected), the inhibitory receptors are activated and kill the target cells through caspase activation

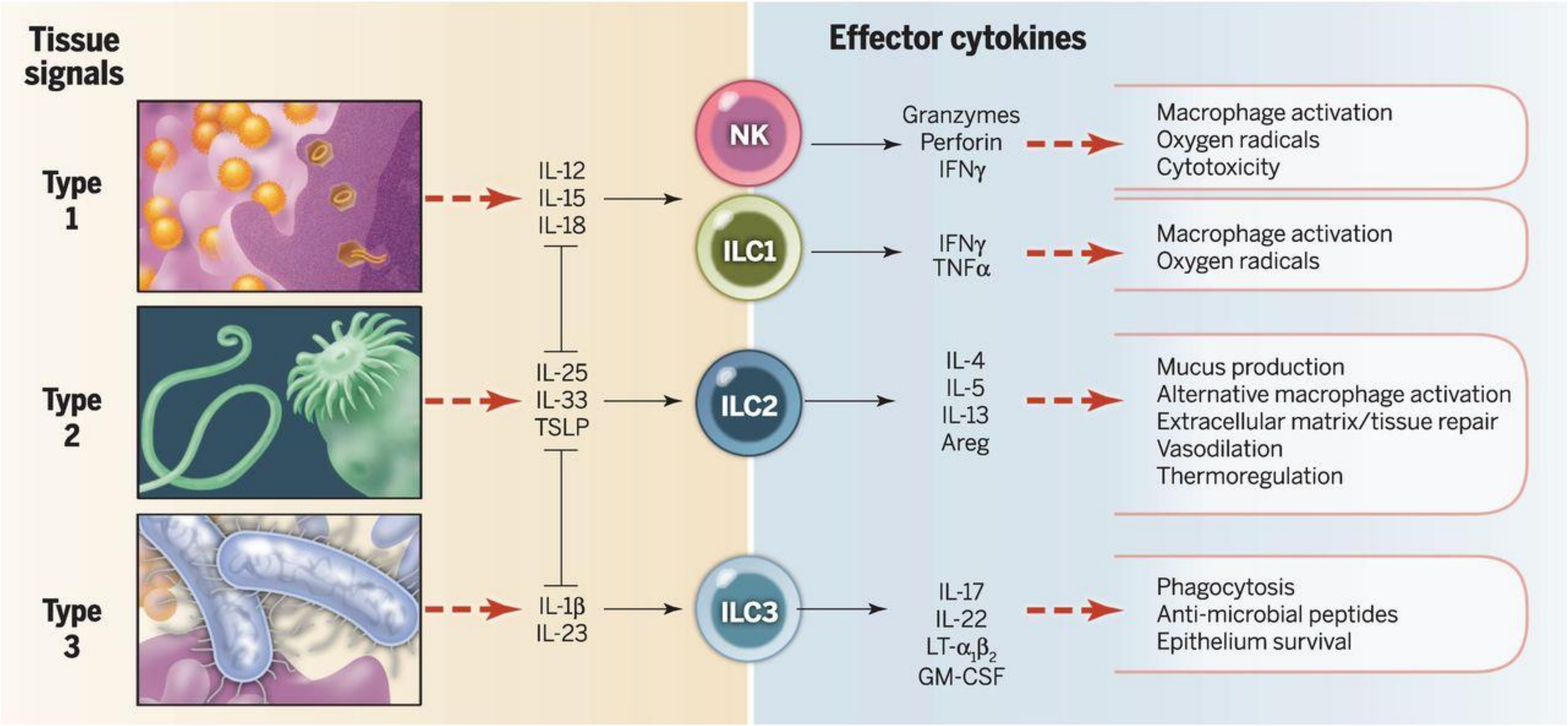


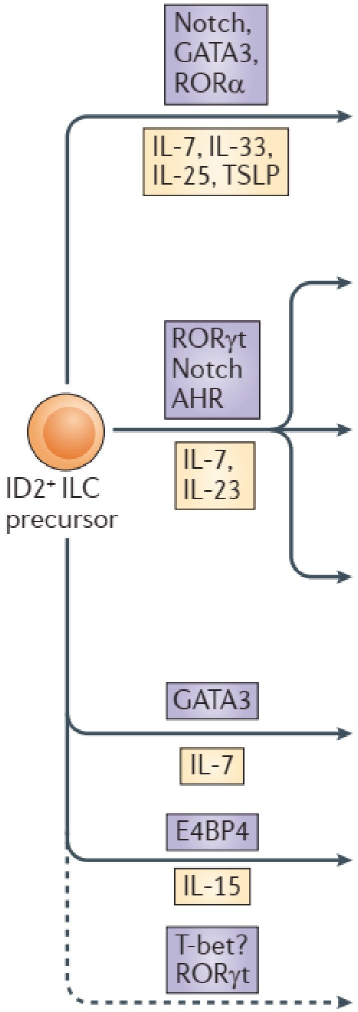
Innate lymphoid cells



- ILCs are primarily tissue resident cells, found in both lymphoid and non-lymphoid tissues and rarely in the blood

Effector functions of ILCs

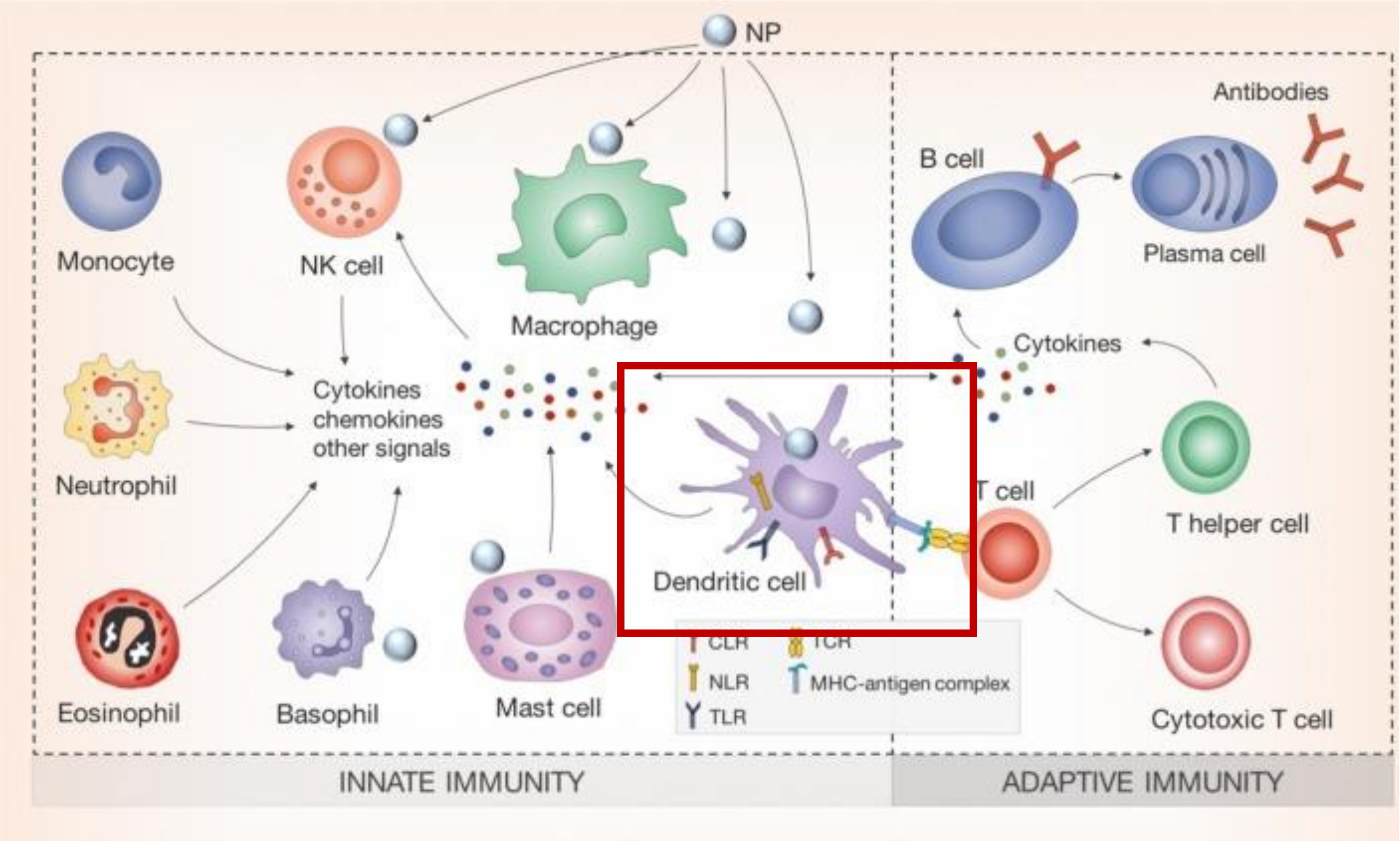




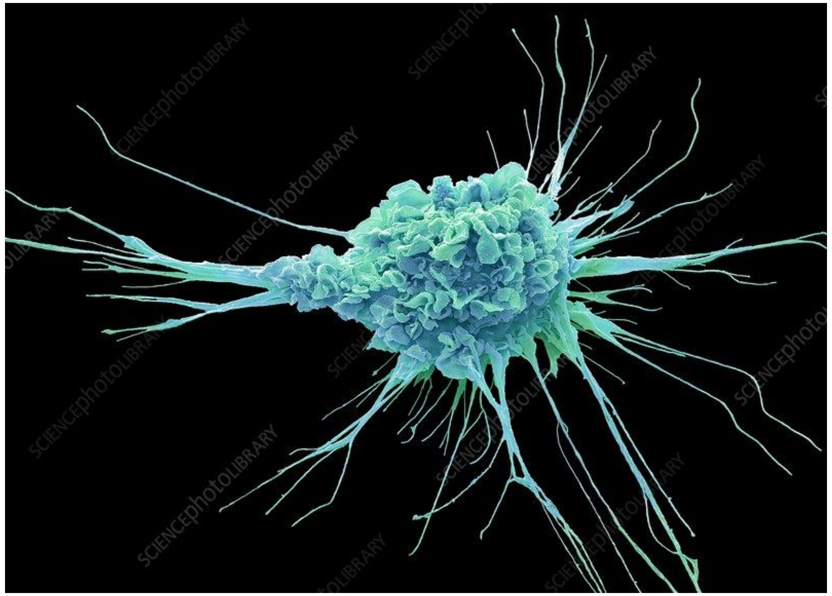
	New nomenclature	New group nomenclature	Mediators produced	Function	Disease association
Nuocyte, NHC, I_H2 cell	ILC2	Group 2 ILCs	IL-5, IL-9, IL-13, amphiregulin	• Immunity to helminths • Wound healing	Allergy and asthma
LTi cell	LTi cell	Group 3 ILCs	Lymphotoxin, IL-17, IL-22	• Lymphoid tissue development • Intestinal homeostasis • Immunity to extracellular bacteria	
NK22 cell, NCR22 cell, NKR-LTi cell, ILC22	NCR+ ILC3		IL-22	• Homeostasis of epithelia • Immunity to extracellular bacteria	IBD?
ILC17	NCR− ILC3		IL-17, IFNγ	• Immunity to extracellular bacteria?	IBD
Thymic NK cell	NK cell	Group 1 ILCs	IFNγ (high levels)	• Immunity to viruses and intracellular pathogens • Tumour surveillance	Inflammatory conditions, IBD
Conventional NK cell	NK cell		IFNγ (low levels), perforin, granzymes	• Immunity to viruses and intracellular pathogens • Tumour surveillance	Inflammatory conditions, IBD
	ILC1		IFNγ	• Inflammation?	IBD?

Aberrant ILC-related immune responses can lead to autoimmunity (IBD), allergic responses (asthma)

DCs: the bridge between innate and adaptive immunity



Dendritic cells



named after their 'tree-like' or dendritic shapes

- DCs are found in the skin, gastrointestinal tract, respiratory system, spleen, blood (immature)
- "sentinels" of the immune system, recognise microbial pathogens, initiate adaptive responses, secrete cytokines
- Immature state: capture and process pathogens
- Mature state: migrate to lymph nodes and present antigens to naïve T cells

Effector cytokines of innate immunity

- ✓ **IL-1, TNF-a, IL-6**: produced by phagocytes and infected cells ⟶ phagocyte recruitment and activation, chemokines and adhesion molecule expression, synthesis of acute phase proteins

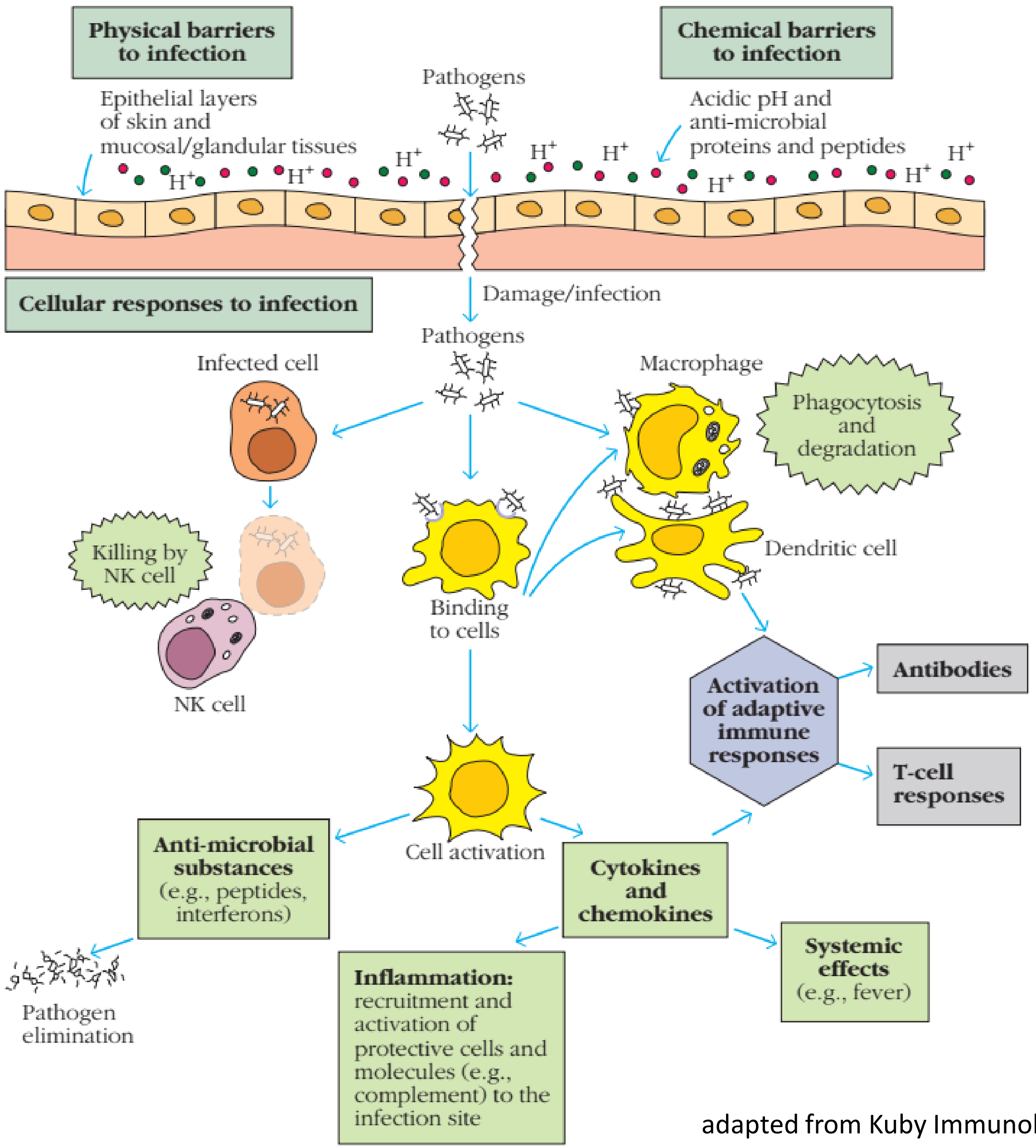
- ✓ **Type-I IFNs (IFN-α, IFN-β):** "interfere" with viral infections, produced by infected cells, growth factors for NK cells and DCs, antiviral immunity

- ✓ **IFN-γ**: produced by NK and T cells, macrophage activation, antiviral immunity

- ✓ **IL-12**: produced by APCs, Th1 cell differentiation factor, directs adaptive immunity, activates NK cells

- ✓ **IL-10, TGF-β**: immunosuppression, T-reg function and survival, inhibit effector cell proliferation, promote tissue remodelling

Effector mechanisms of innate immunity against pathogens



adapted from Kuby Immunology 7th Edition

Protective immunity vs hyperinflammatory response

- ✓ The immune system is expected to recognize foreign invaders, respond proportionally to the pathogen burden and then return to homeostasis

- ✓ A balance is required between sufficient cytokine/chemokine production to eliminate the pathogen and avoidance of a hyperinflammatory response which causes clinically significant collateral damage.

protective immunity

resolution of inflammatory responses

Mechanisms that limit innate immune responses

- The production of regulatory cytokines from macrophages and DCs ⟶ the example of IL-10

- The secretion of natural antagonists from mononuclear phagocytes ⟶ the example of IL-1RA

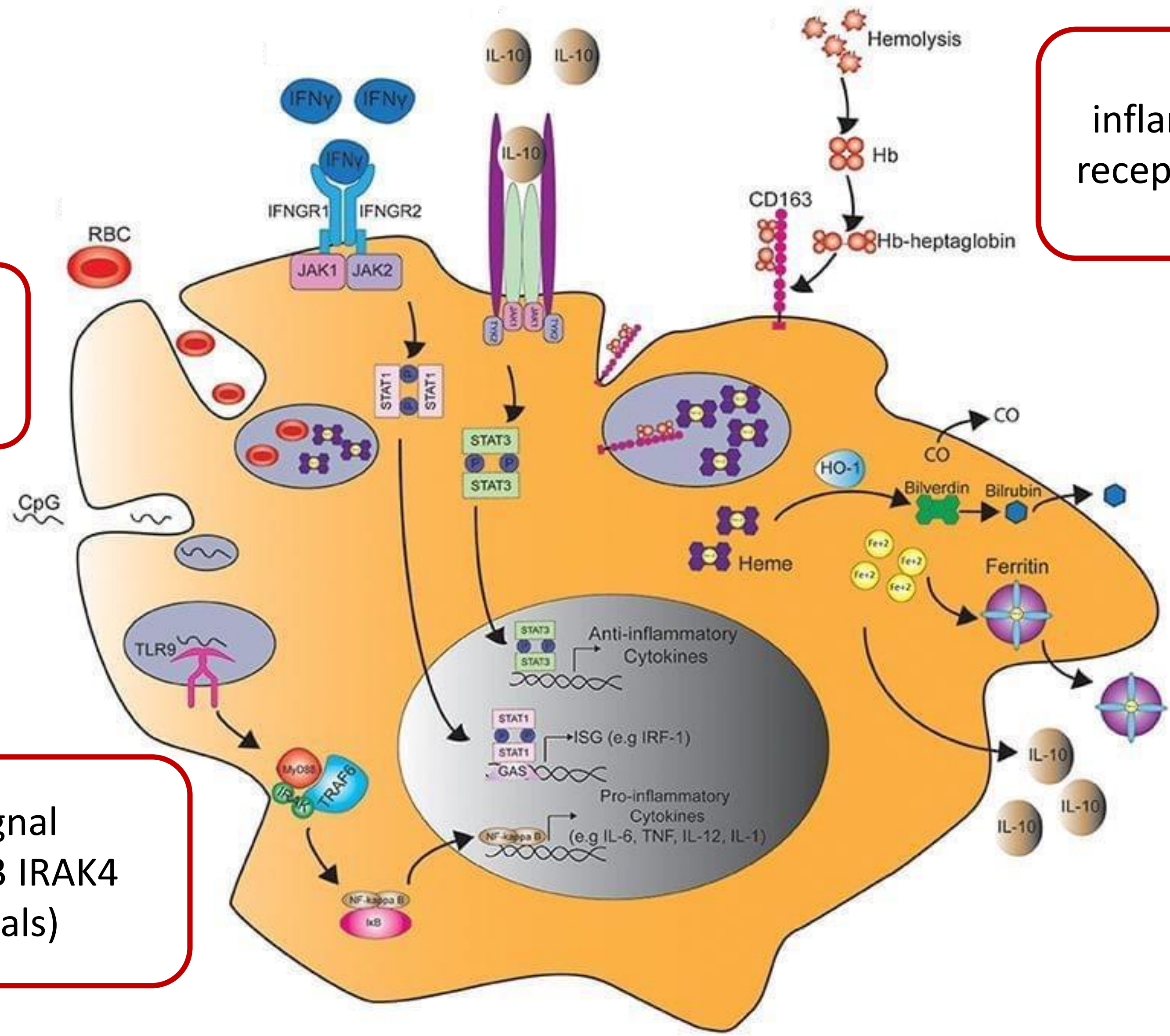
- Targeted mutations in autophagy genes ⟶ enhanced IL-1β and IL-18 secretion and development of hyperinflammation

- Negative regulatory signaling pathways that block the activating signals generated by PRRs and inflammatory cytokines ⟶ the paradigm of SOCS (suppressors of cytokine signaling), SHP-1 etc.

Targeting the innate immune system

TLR antagonists (Abs, drugs, small molecules), 8 in clinical trials

Inhibitors targeting signal transduction molecules (3 IRAK4 inhibitors in clinical trials)



Inhibitors targeting pro-inflammatory cytokines/cytokine receptors (9 for RA and 6 for SLE in clinical trials)

JAK/STAT inhibitors (tofacitinib, baricitinib)

Table 1 The characteristics and clinical applications of inhibitors targeting innate immune sensors in autoimmune diseases

Target	Inhibitor	Character	Application	Autoimmune disease	References
Inhibitors targeting innate receptors					
TLR4	NI-0101	Antibody	Phase II clinical trial	RA	[145]
TLR3/4	Baclofen	Small molecule	Phase III clinical trial	MS	[146, 147]
TLR7/9	IMO-3100	Oligonucleotides	Phase II clinical trial	Psoriasis	[209]
TLR7/9	Chloroquine	Small molecule	Clinical medicine	SLE and RA	[46, 141]
TLR7/9	Hydroxychloroquine	Small molecule	Clinical medicine	SLE and RA	[46, 141]
TLR7/9	Quinacrine	Small molecule	Clinical medicine	SLE and RA	[46, 141]
TLR7/8/9	CpG-52364	Small molecule	Phase I clinical trial	SLE	[143]
TLR7/8/9	IMO-8400	Oligonucleotides	Phase II clinical trial	Psoriasis	[144]
Inhibitors targeting signal transduction molecules					
IRAK4	PF-06650833	Small molecule	Phase II clinical trial	RA	[171]
IRAK4	BAY1834845	Small molecule	Phase I clinical trial	RA and psoriasis	[171]
Syk	R788	Small molecule	Phase I clinical trial	RA	[210]
Inhibitors targeting terminal proinflammatory cytokines					
TNF	Adalimumab	Monoclonal antibody	Clinical medicine	RA	[6, 202]
TNF	Certolizumab pegol	F(ab') fragment of a humanized monoclonal antibody	Clinical medicine	RA	[6]
TNF	Etanercept	p75 (of TNFRII)-Fc (of IgG1) fusion protein	Clinical medicine	RA	[6, 202]
TNF	Golimumab	Monoclonal antibody	Clinical medicine	RA	[6]
TNF	Infliximab	Monoclonal antibody	Clinical medicine	RA	[6, 202]
IL-6R	Tocilizumab	Monoclonal antibody	Clinical medicine	RA	[197]
IL-6	Sarilumab	Monoclonal antibody	Phase III clinical trial	RA	[203]
IL-6	ALX-0061	Small molecule	Phase II clinical trial	RA	[203]
IL-6	Sirukumab	Monoclonal antibody	Phase II clinical trial	RA	[211]
IL-6	MEDI5117	Monoclonal antibody	Phase I clinical trial	RA	[212]
IL-6	Clazakizumab	Monoclonal antibody	Phase II clinical trial	RA	[213]
IL-6	Olokizumad	Monoclonal antibody	Phase II clinical trial	RA	[214]
IL-1	Anakinra	Recombinant	Clinical medicine	RA	[198]
IL-1	Rilonacept	Soluble decoy receptor	Clinical medicine	RA	[198]
IL-1	Canakinumab	Monoclonal antibody	Clinical medicine	RA	[198]
IFN-α	Sifalimumab	Monoclonal antibody	Phase II clinical trial	SLE	[215]
IFNAR	Anifrolumab	Monoclonal antibody	Phase III clinical trial	SLE	[216]
IFN-α	Rontalizumab	Monoclonal antibody	Phase II clinical trial	SLE	[204]
IL-18	Tadekinig alfa	Recombinant	Phase III clinical trial	NLRC4 and XIAP deficiency	[205]


thank you