

Berlin, ZIBI lecture series
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Principles of innate immunity and Innate lymphoid cells (ILCs)

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Berlin

Principles of the Immune System

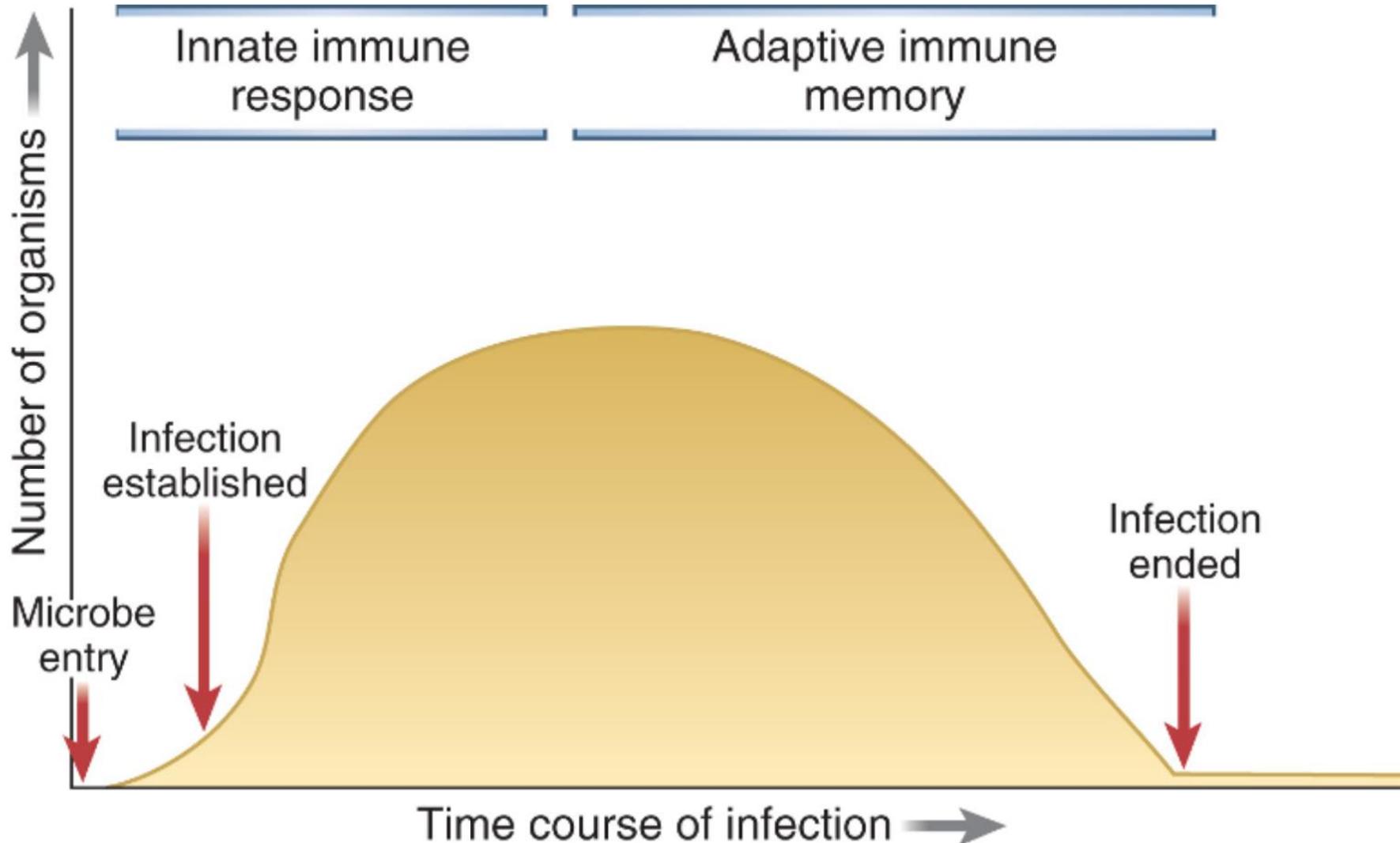
Evolutionary very old

Multicellular organism needs to keep „identity“

Necessity of discrimination between **SELF** and **NON SELF**

Evolution of effector functions for maintaining **SELF** and eliminating **NON SELF**

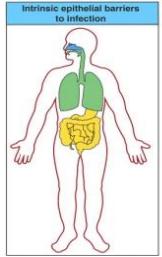
Innate and adaptive immunity



Innate immunity

Defenses against infections that are ready for immediate activation prior to attack by pathogen

Components

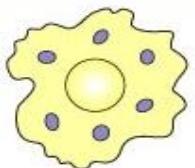


intrinsic barriers

epithelium

mucosa

cells



Immune cells:

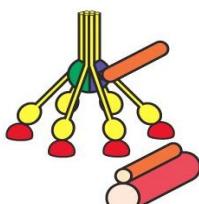
Myeloid cells:

macrophage, DC, mast cell, granulocytes

Lymphocytes:

Innate lymphoid cells (ILCs and NK cells)

MAIT, NKT, $\gamma\delta$ T cells



soluble factors

antimicrobial peptides

complement cascade

cytokines and chemokines

coagulation/fibrinolytic cascade

Functions

prevention of pathogen infection

alarm detection

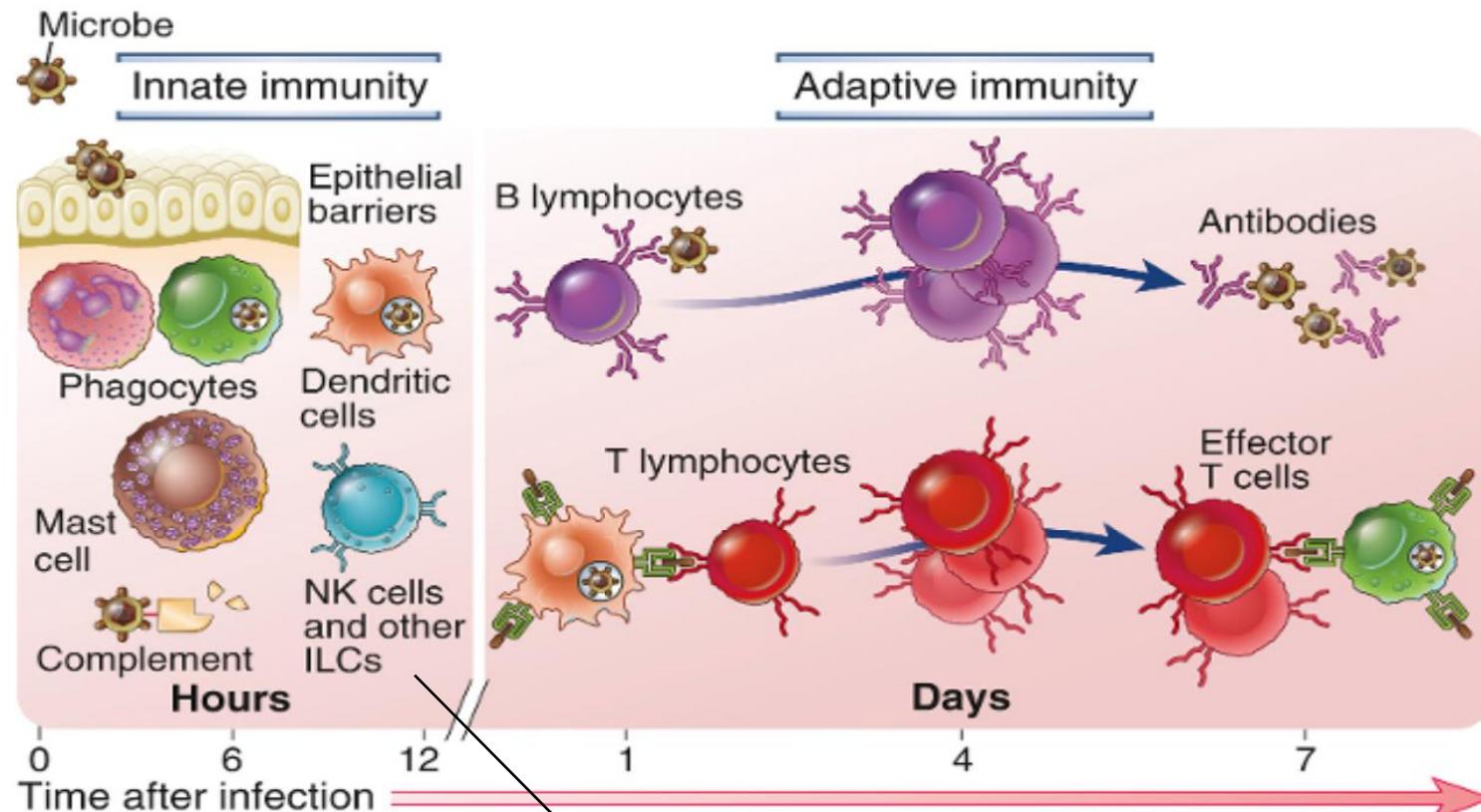
activation of local tissue response
immune cell recruitment

clearance of dead cells
pathogen elimination
wound healing

instructing adaptive immunity
(establishment of memory)

inflammation

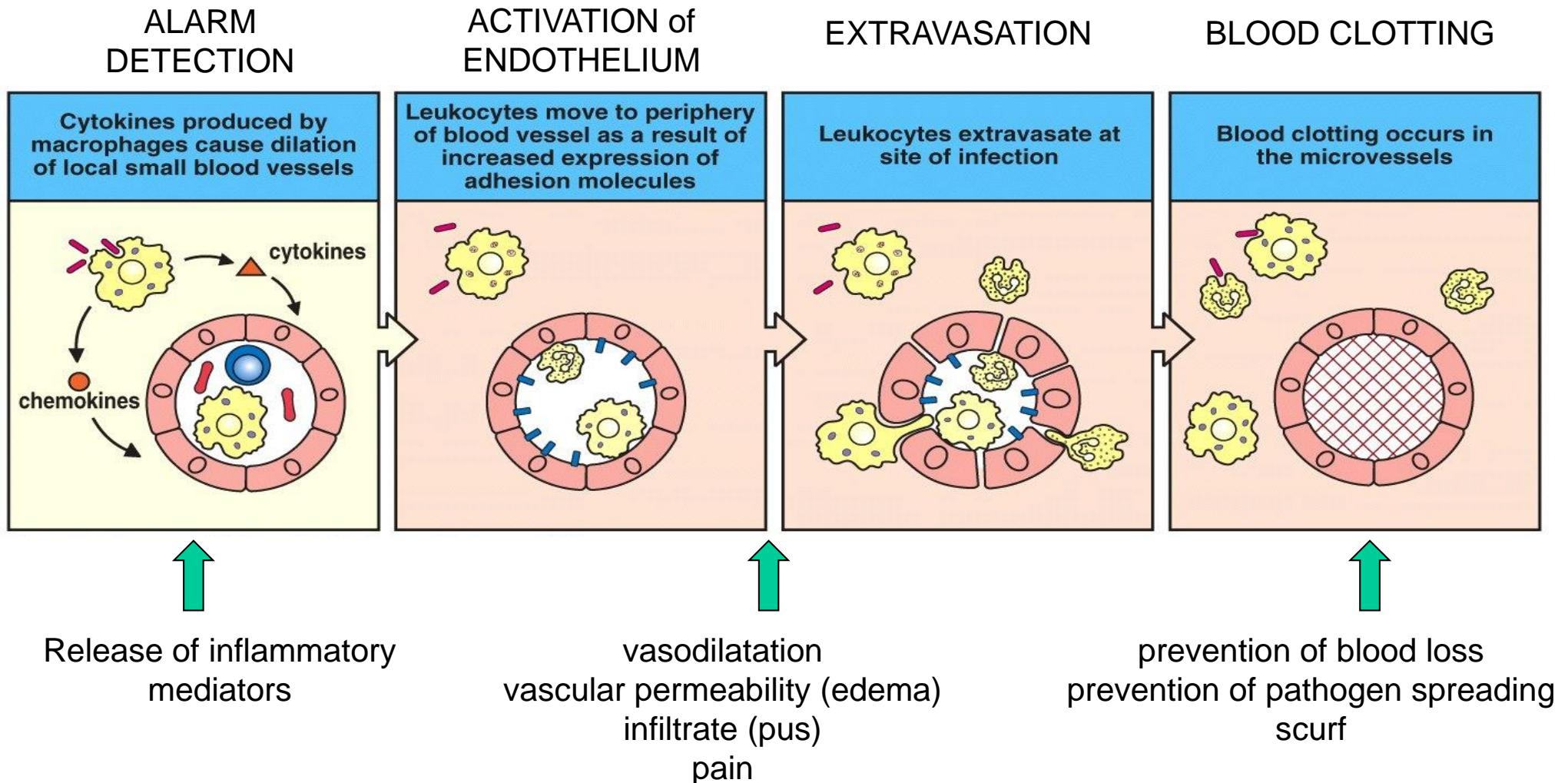
Innate and adaptive immunity



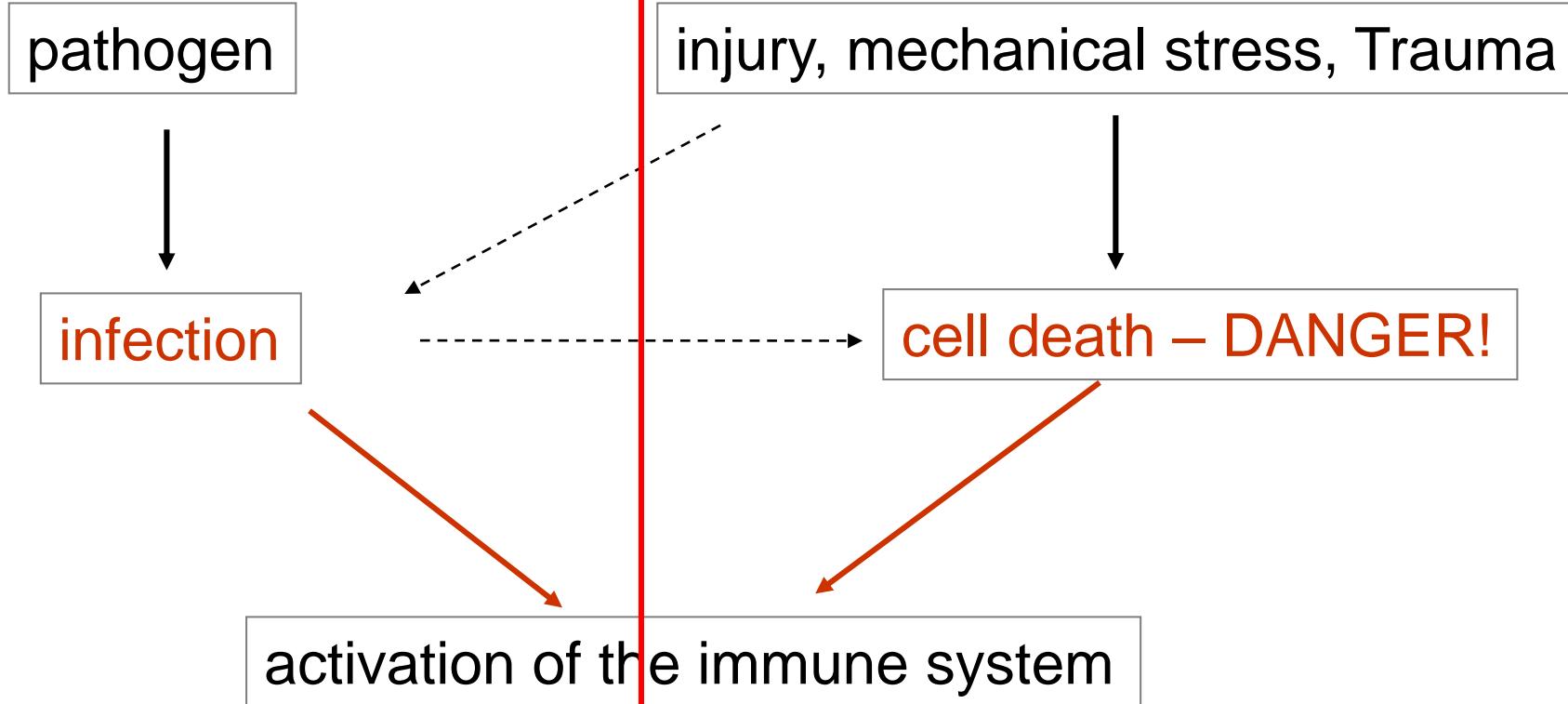
Natural Killer (NK) cells
Innate Lymphoid cells (ILCs)

Inflammation

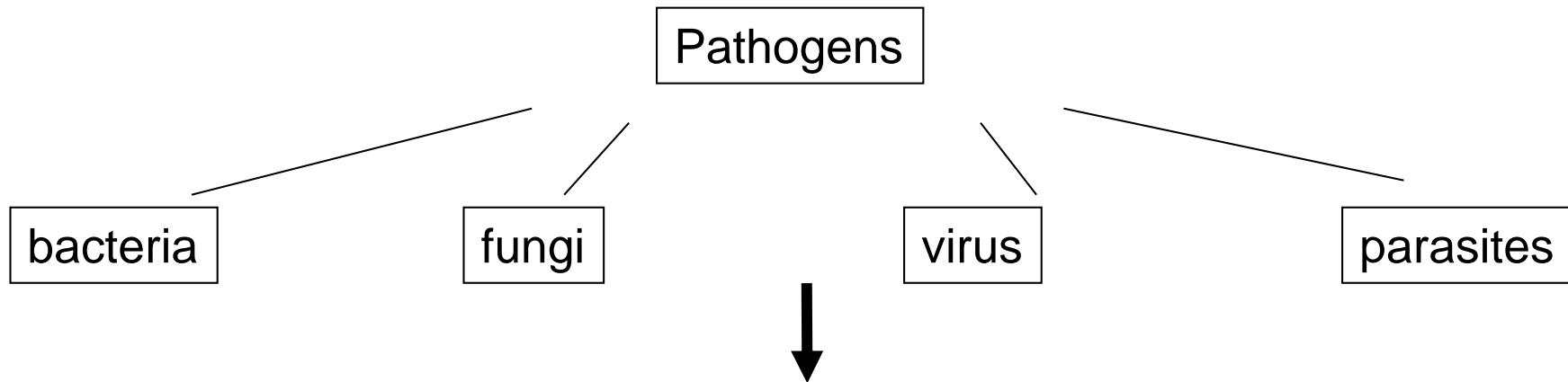
“local tissue reaction”



Which signals do trigger inflammation?



How do we recognize pathogens?

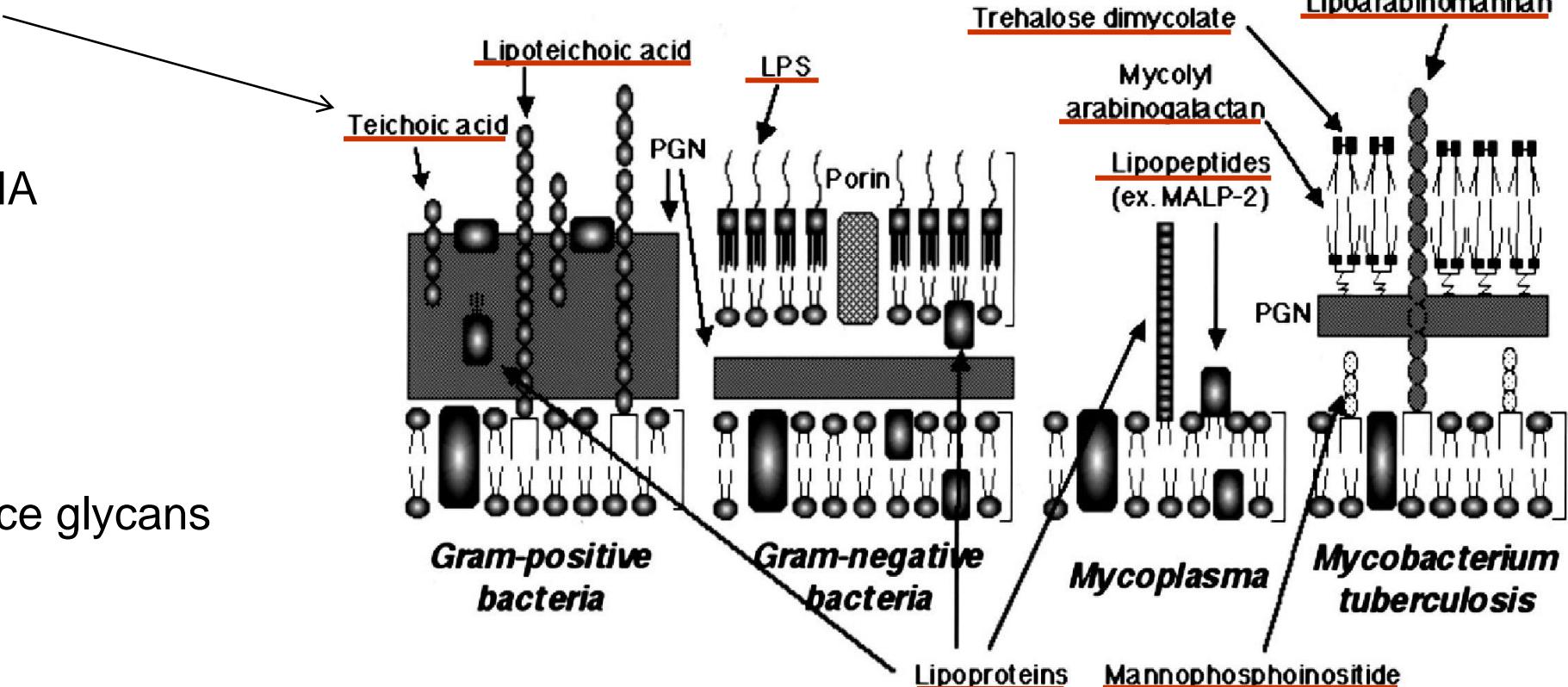


Pathogen associated molecular patterns
(PAMPs)

*typical biochemical structures characteristic
for a particular group of microorganisms*

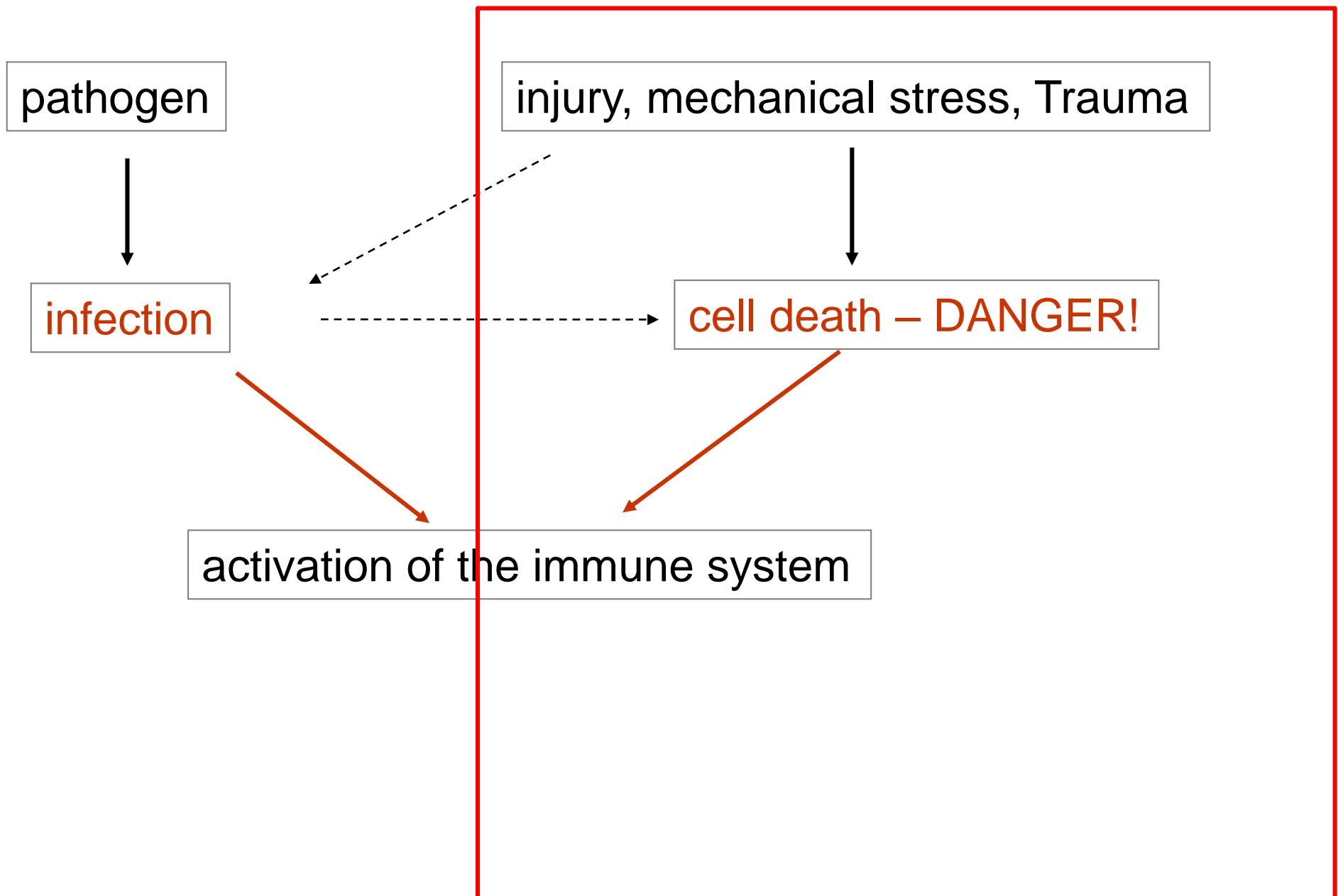
PAMPs

Bacteria:



Fungi:

Which signals do trigger inflammation?

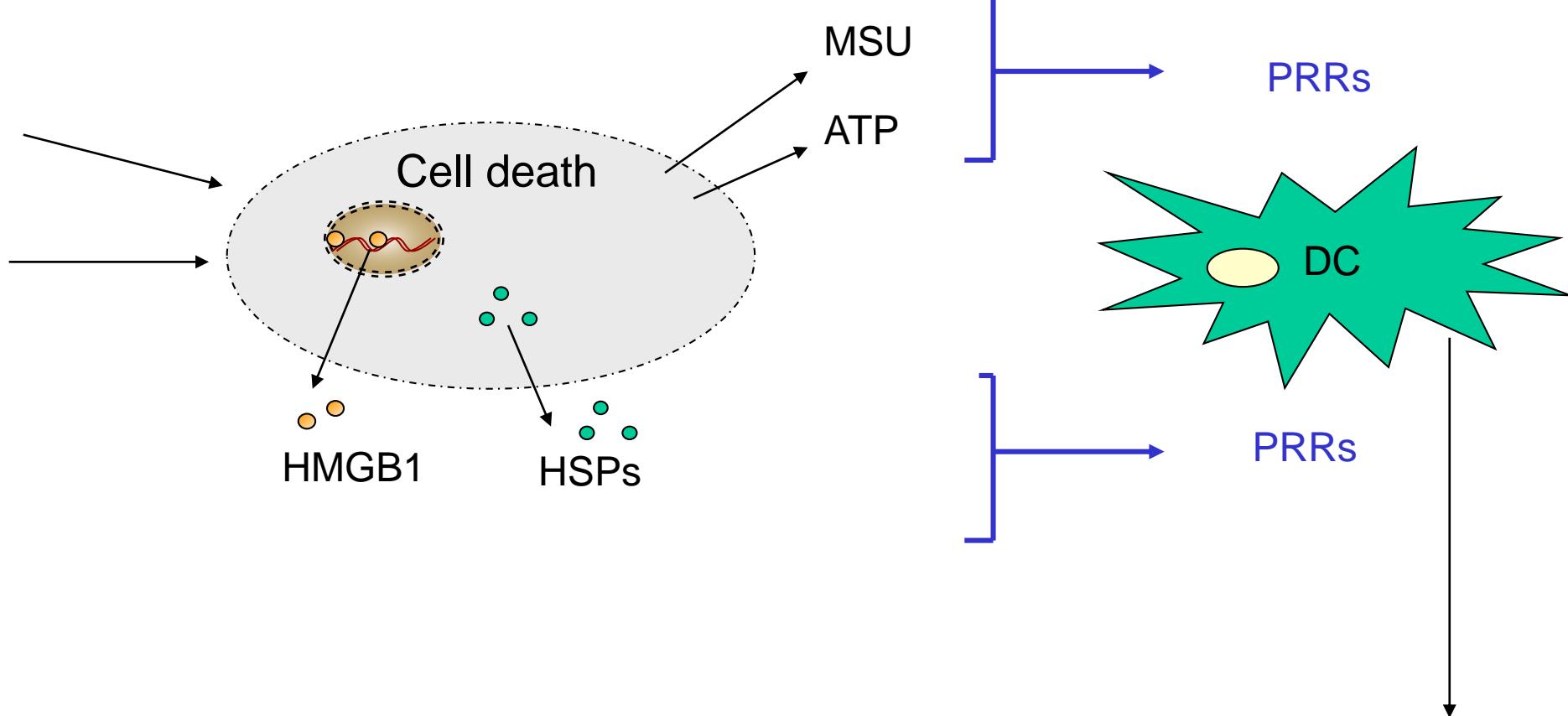


How do we recognize danger?

danger associated molecular patterns: DAMPs

necrotic dying cells release danger signals

infection
Trauma
Injury
burn



HMGB = high mobility group B

HSPs = heat shock proteins

MSU = monosodium urate; uric acid crystals

cytokines

Pattern Recognition Receptors (PRR)

Cell receptors that **recognize** conserved pathogen associated molecular patterns (PAMPs) and/or danger associated molecular patterns (DAMPs)

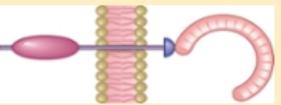
PRR belong to 5 main **families**:

Toll-like receptors (TLR), RIG-I-like (RLR), NOD-like (NLR), C-type lectin (CLR), cytosolic DNA sensors (CDC)

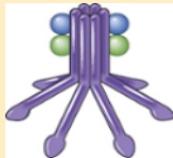
Cellular PRR are **expressed by**: professional phagocytes (macrophages, DC) but also cells target of infection

localization: cell surface, cytosol, endosomes

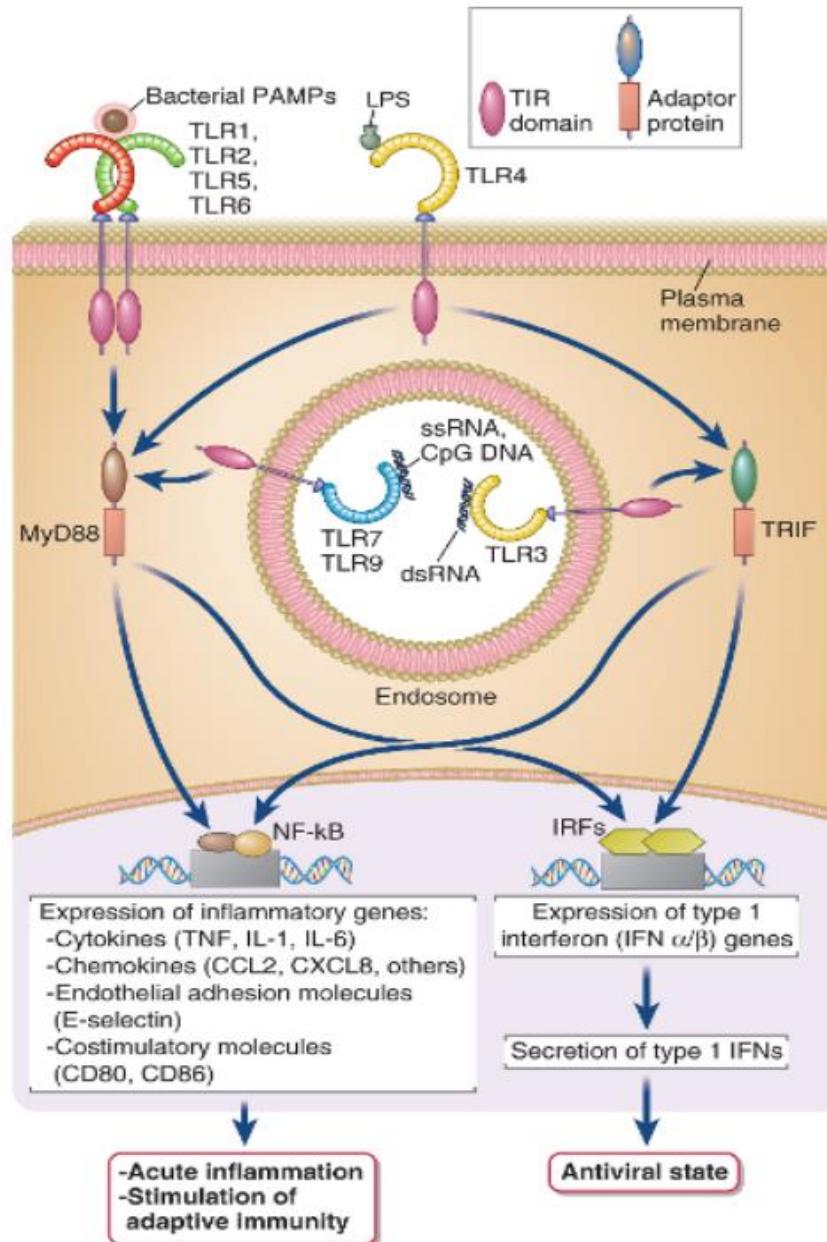
Cell-associated Pattern Recognition Receptors (PRR)

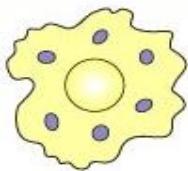
Pattern Recognition Receptors	Location	Specific Examples	Ligands (PAMPs or DAMPs)
Cell-Associated			
TLRs 	Plasma membrane and endosomal membranes of DCs, phagocytes, B cells, endothelial cells, and many other cell types	TLRs 1–9	Various microbial molecules including bacterial LPS and peptidoglycans; viral nucleic acids
NLRs 	Cytosol of phagocytes, epithelial cells, and other cells	NOD1/2	Bacterial cell wall peptidoglycans
		NLRP family (inflammasomes)	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	Microbial surface carbohydrates with terminal mannose and fructose
		DC-sign	
		Dectin-1, Dectin-2	Glucans present in fungal and bacterial cell walls

Soluble Pattern Recognition Receptors (PRR)

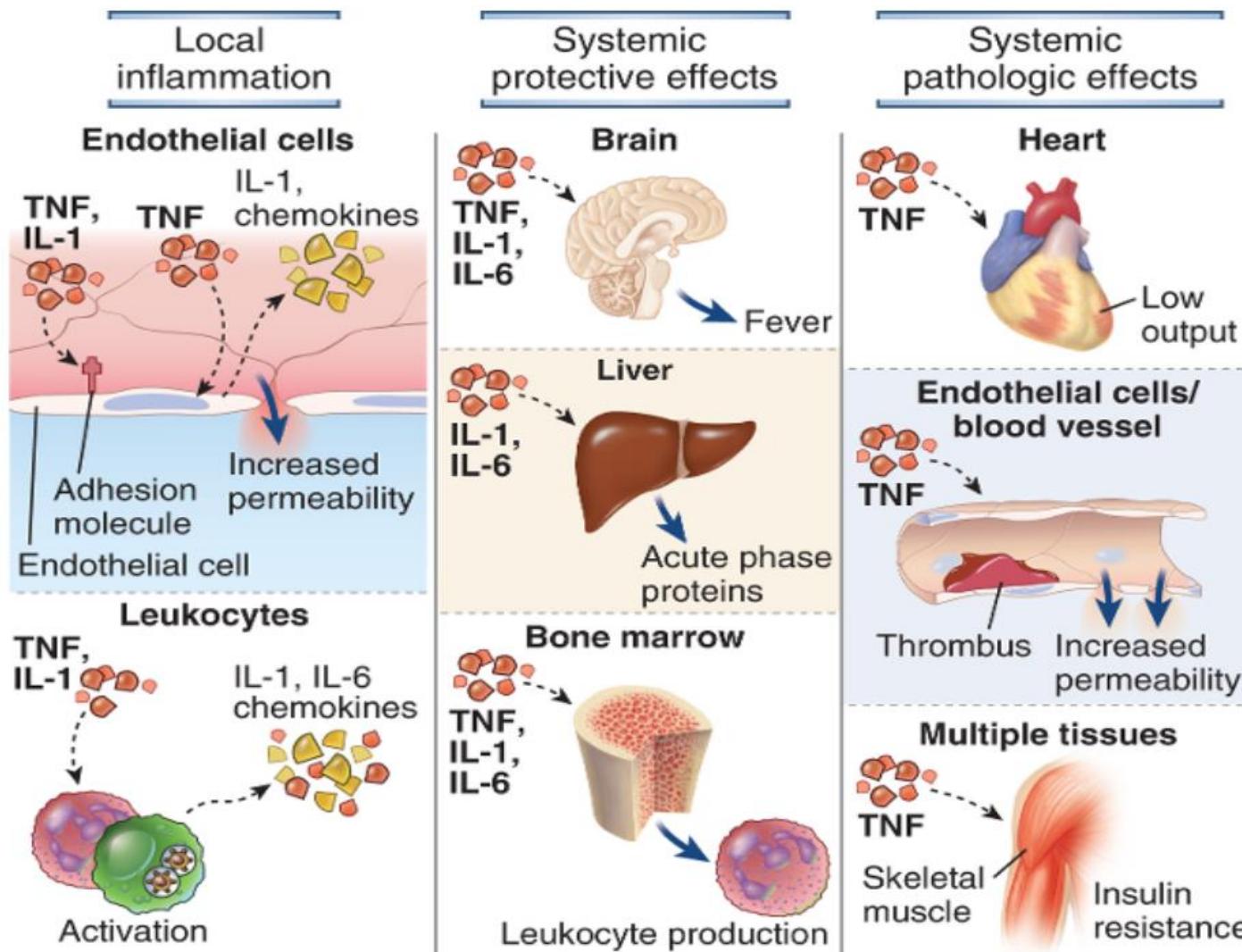
Pattern Recognition Receptors	Location	Specific Examples	Ligands (PAMPs or DAMPs)
Soluble			
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma	Mannose-binding lectin	Carbohydrates with terminal mannose and fructose
	Alveoli	Surfactant proteins SP-A and SP-D	Various microbial structures
Ficolins 	Plasma	Ficolin	<i>N</i> -acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	Various complement proteins	Microbial surfaces

Toll-like receptors (TLR) – Signalling and downstream targets





Inflammatory cytokines inducing endothelial cell activation



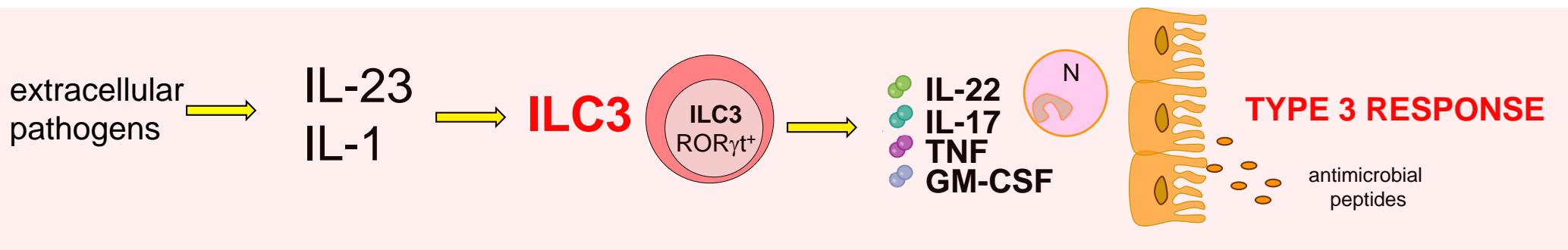
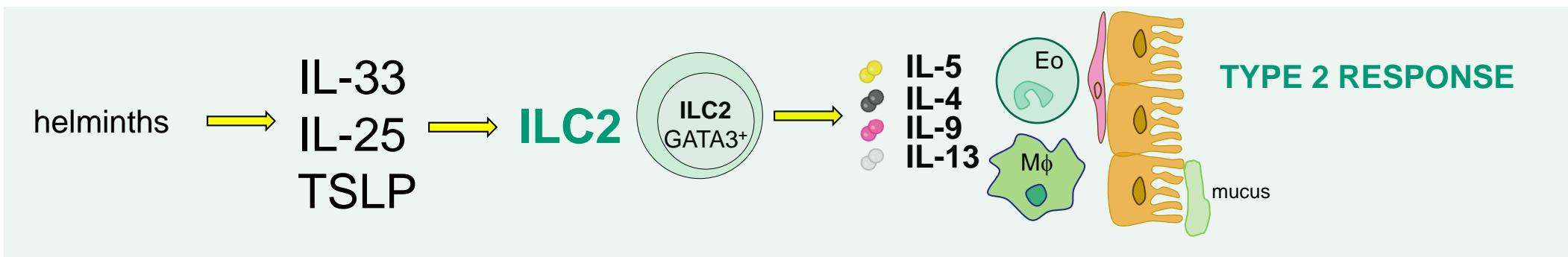
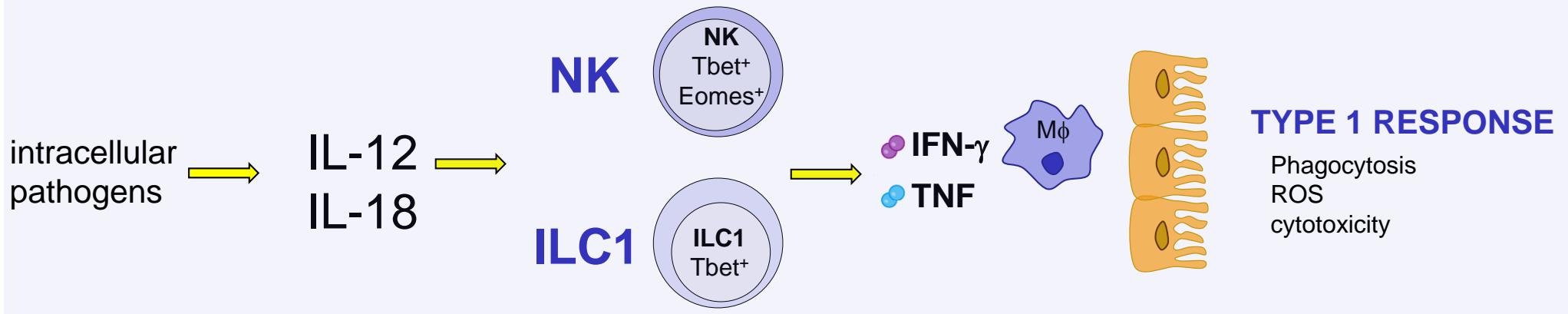
Inflammatory cytokines can instruct different types of tissue inflammatory responses

intracellular pathogens  IL-12
IL-18

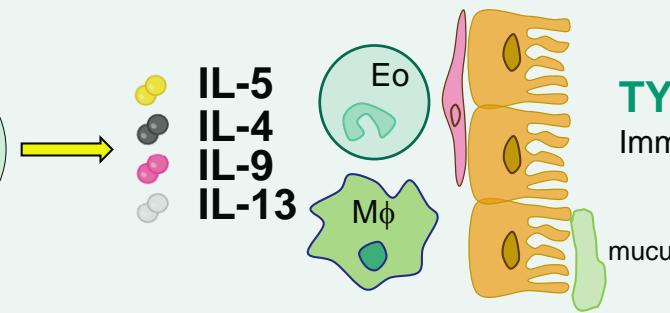
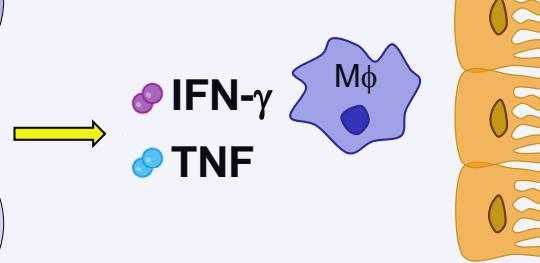
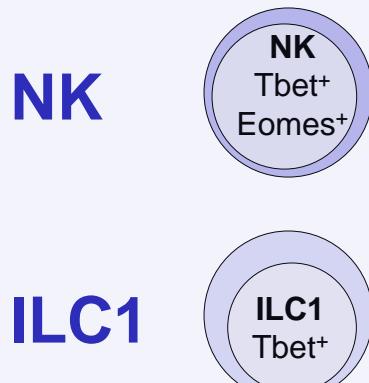
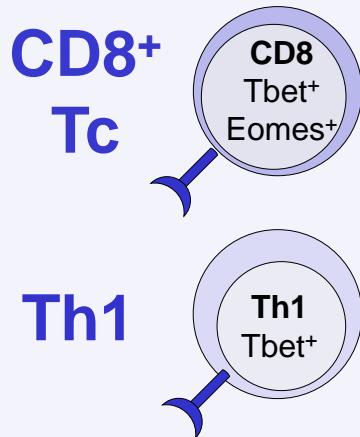
helminths  IL-33
IL-25
TSLP

extracellular pathogens  IL-23
IL-1

ILCs orchestrate the types of inflammatory responses depending on the infecting pathogen



ILC and T cells orchestrate the types of inflammatory responses depending on the infecting pathogen



What are Innate Lymphoid cells (ILC)?

Nomenclature introduced in Nat Rev Immunol 2013, updated in Cell 2018

GUIDELINES

Innate lymphoid cells — a proposal for uniform nomenclature

Hergen Spits, David Artis, Marco Colonna, Andreas Diefenbach, James P. Di Santo, Gerard Eberl, Shigeo Koyasu, Richard M. Locksley, Andrew N. J. McKenzie, Reina E. Mebius, Fiona Powrie and Eric Vivier

Abstract | Innate lymphoid cells (ILCs) are a family of developmentally related cells that are involved in immunity and in tissue development and remodelling. Recent research has identified several distinct members of this family. Confusingly, many different names have been used to characterize these newly identified ILC subsets. Here, we propose that ILCs should be categorized into three groups based on the cytokines that they can produce and the transcription factors that regulate their development and function.

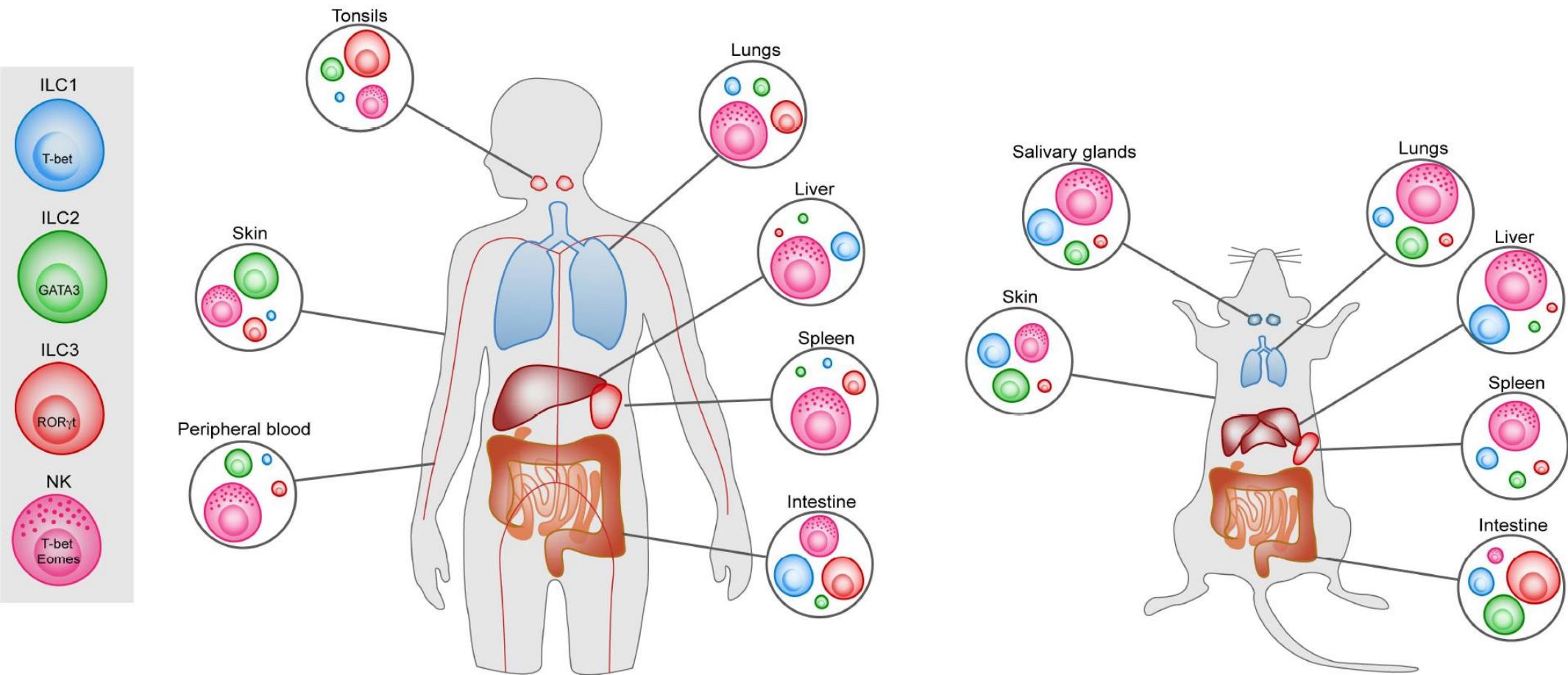
Here, we propose the classification of these ILC populations on the basis of their phenotypical and functional characteristics. Our proposed nomenclature is based on the T_h cell nomenclature and categorizes the ILC subsets into three groups (FIG. 1). Group 1 comprises ILCs that produce IFN γ . Group 2 comprises ILCs that produce type 2 cytokines (including IL-5 and IL-13) and are dependent on GATA-binding protein 3 (GATA3) and retinoic acid receptor-related orphan receptor- α (ROR α) for their development and function. Group 3 includes all ILC subtypes that produce IL-17 and/or IL-22 and depend on the transcription factor ROR γ t for their development and function. The proposed ILC nomenclature differs from that of the T_h cell nomenclature in that the ILC group names are not connected to the so-called 'signature' cytokines. This might help to avoid confusion, for example

A family of innate lymphocytes including **old** cells such as Natural Killer (NK) and lymphoid tissue inducer (LTi) cells as well as **new** cell types identified from 2008 on.

ILCs do not express rearranged receptors such as the **TCR**, but **respond to pro-inflammatory cytokines, neuromediators, nutrients** and express a largely **unknown receptor repertoire**.

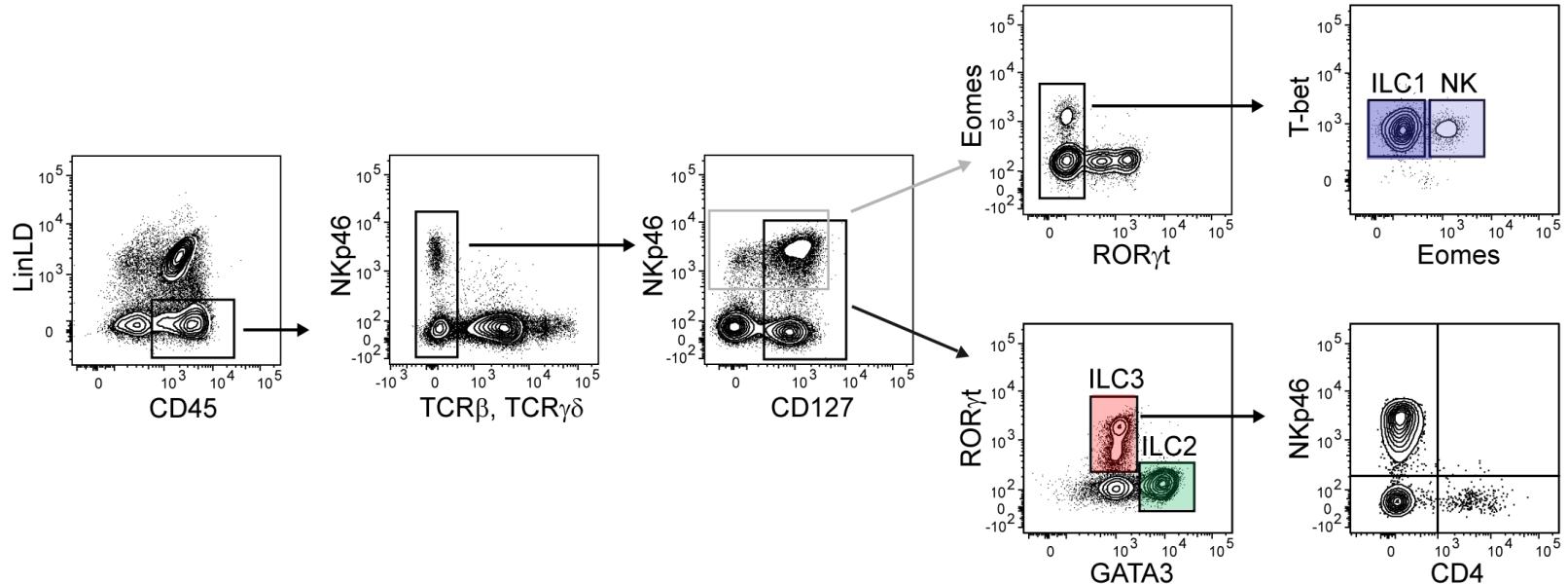
Where do we find ILCs in humans and mice?

ILC subsets reside in different tissues

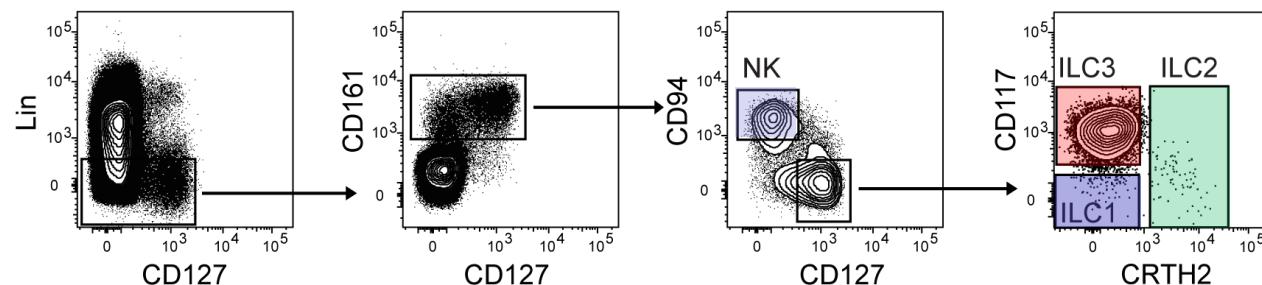


ILC subset identification in human and mouse tissues

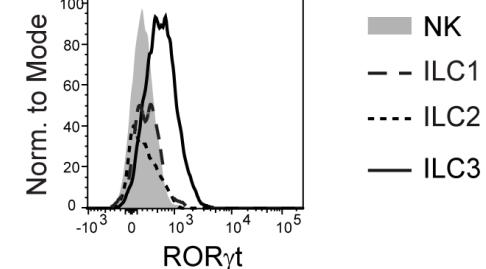
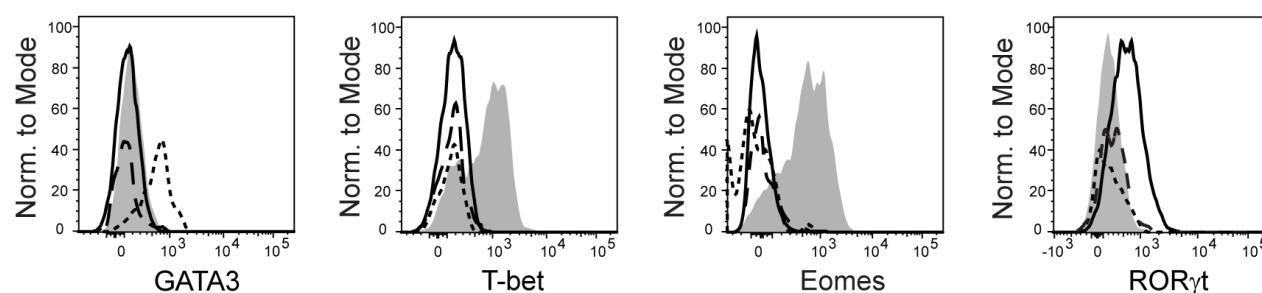
C57BL/6
small intestine LP



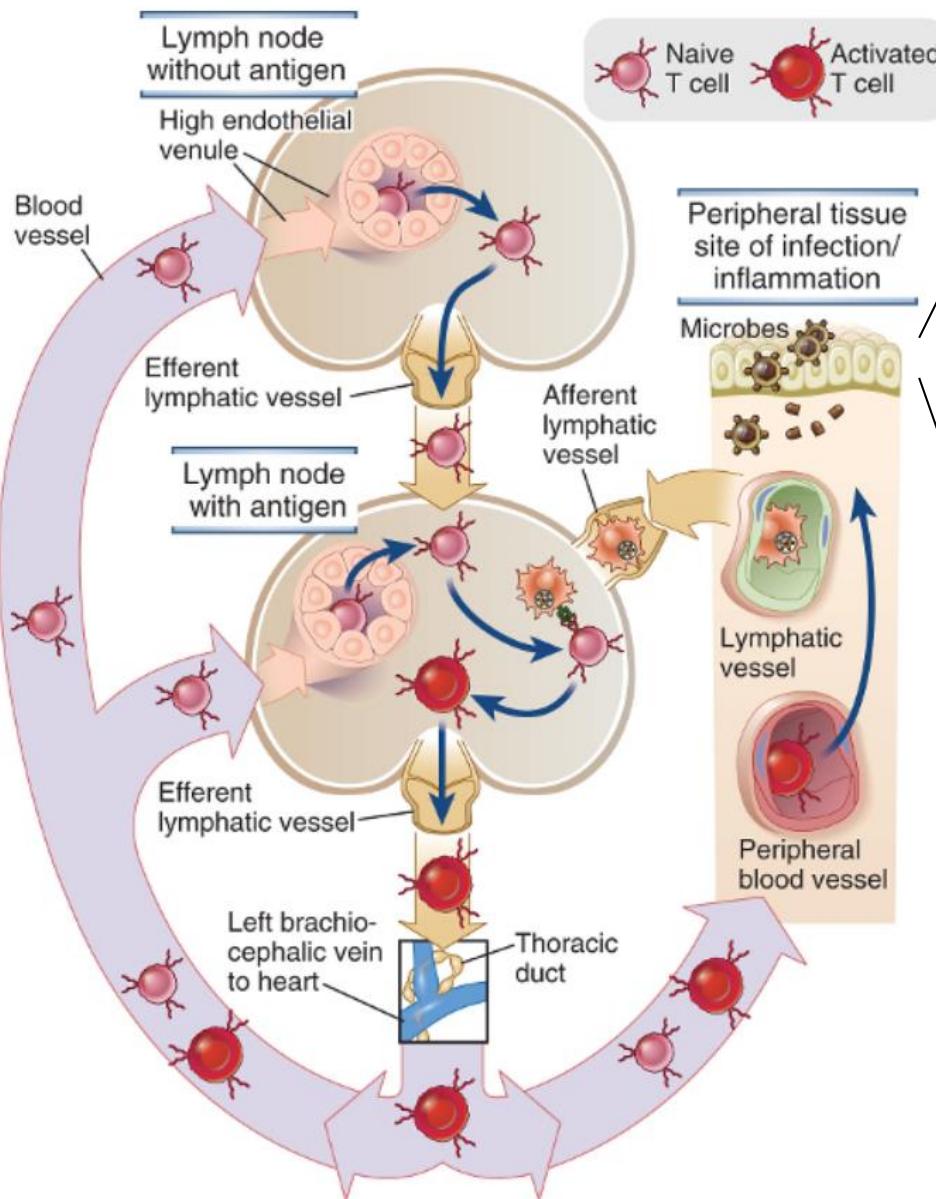
CD127 = IL7R



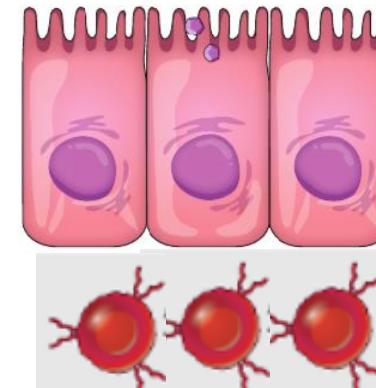
human tonsil



Circulating versus tissue resident lymphocytes

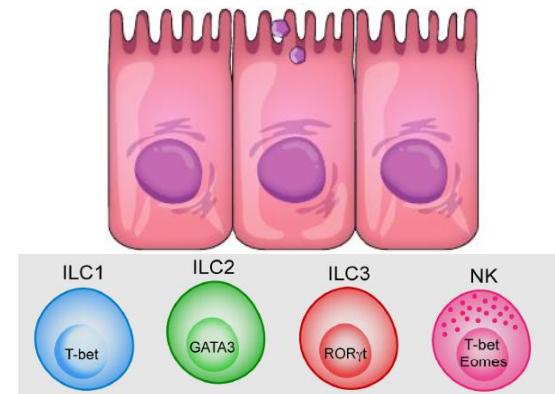


Naïve and memory T cells largely recirculate; only some memory T cells become tissue resident



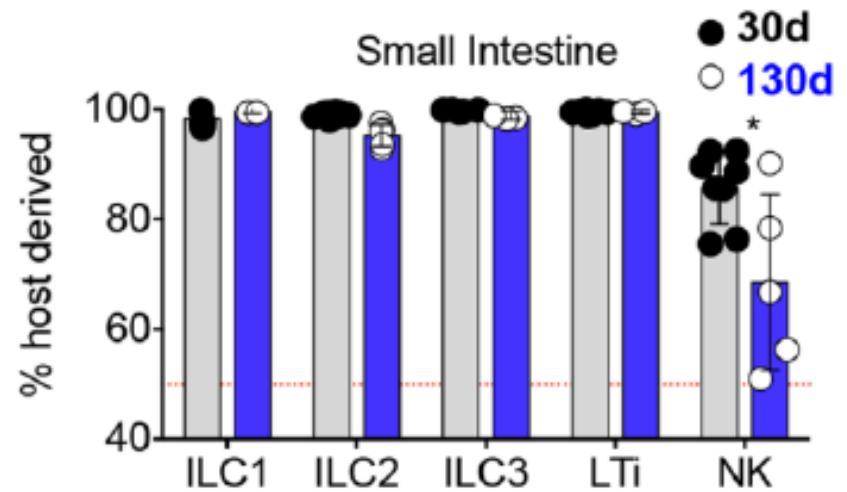
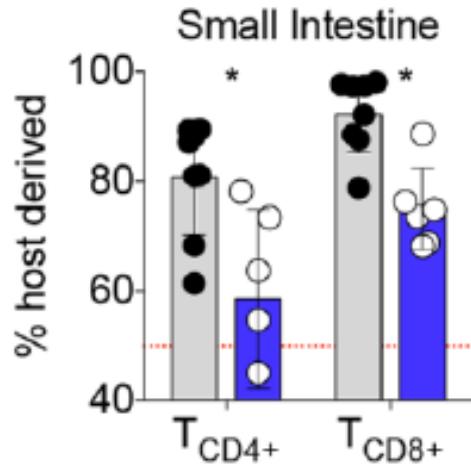
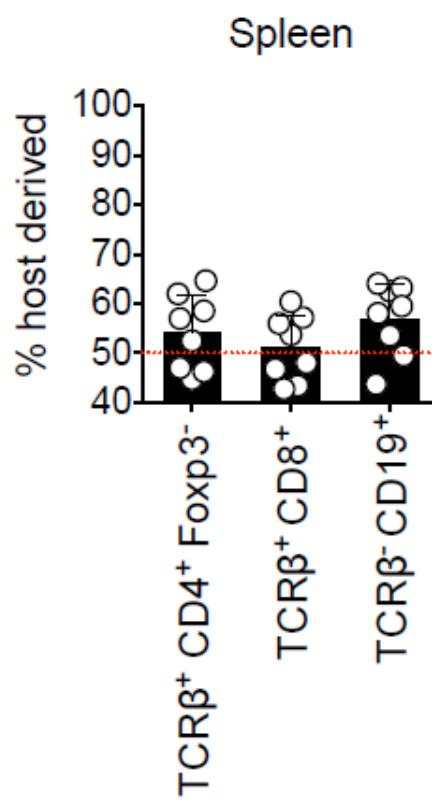
T cells

ILC preferentially reside in tissues



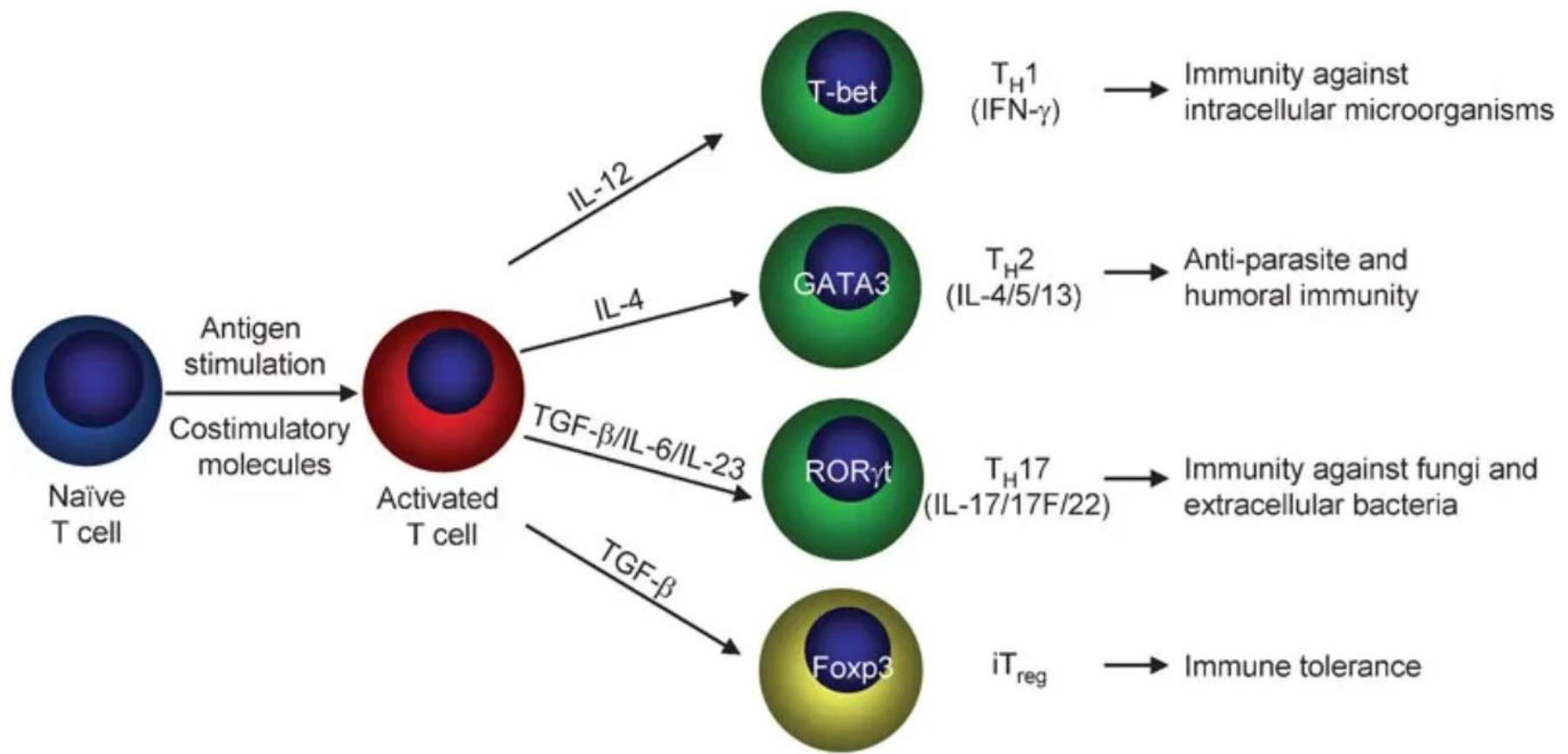
ILCs

Parabiosis experiments have shown that at steady state ILCs are largely tissue resident

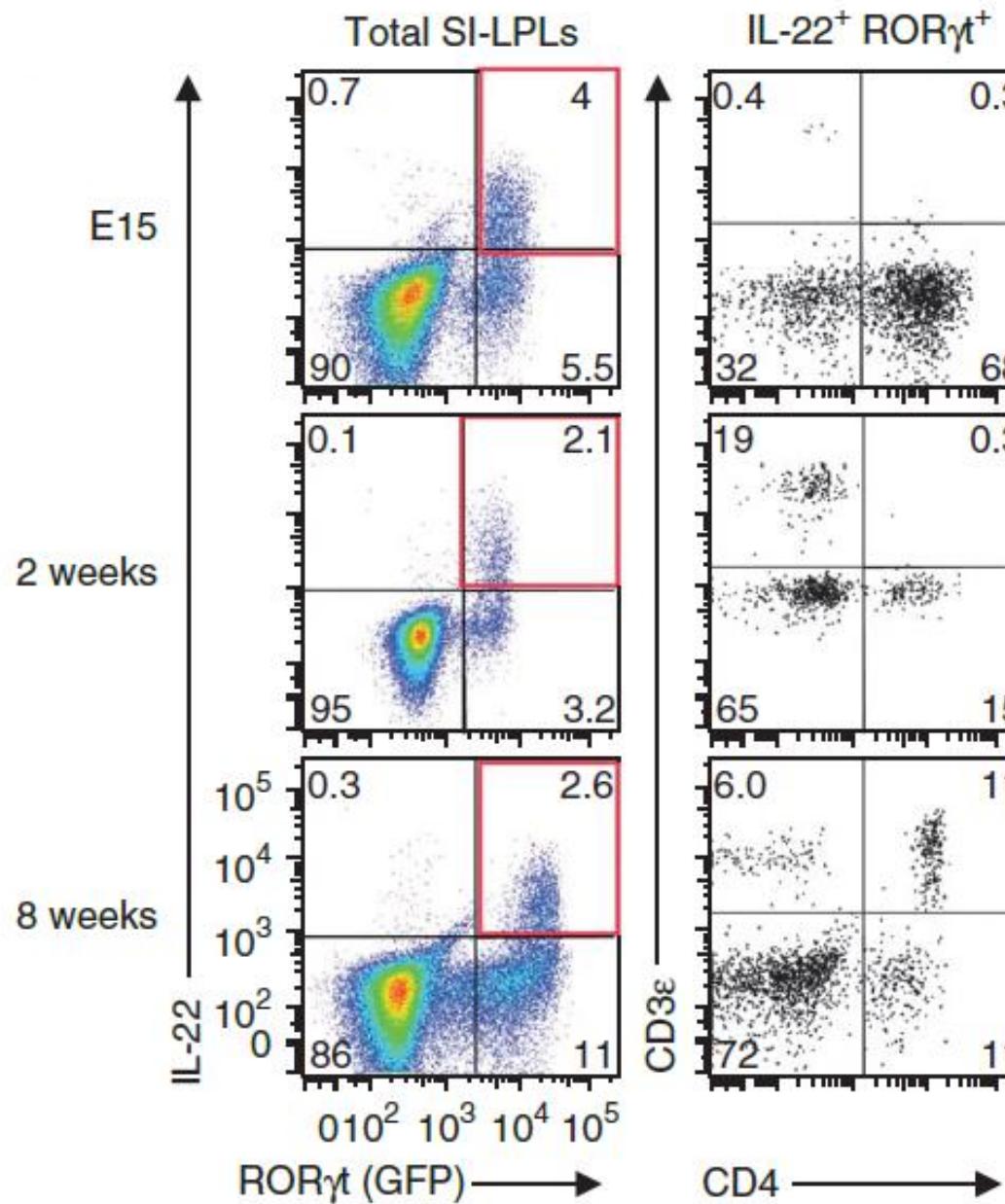


Where, when and how do ILCs acquire their effector programs?

Differentiation from naïve T cells towards different effector lineages relies on exposure to antigen and polarizing signals



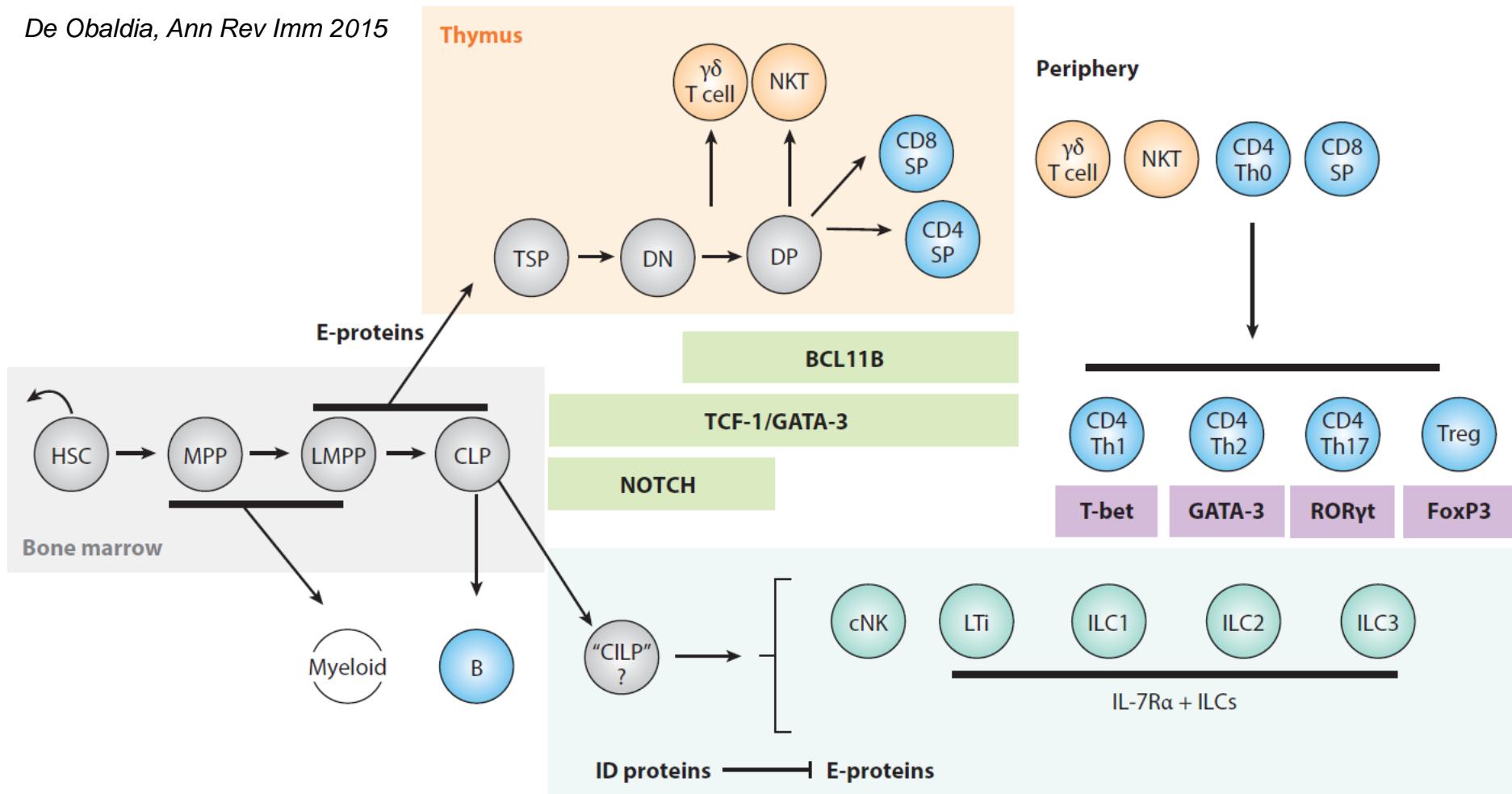
Tissue embryonic ILCs already express cytokines



From Sawa et al, Nature Immunol 2011

T cell-ILC differentiation

De Obaldia, Ann Rev Imm 2015



ILC progenitors have been identified in fetal liver, BM and blood

Progenitors

"Core" T cell and ILC TF network

Innate-like T lymphocytes

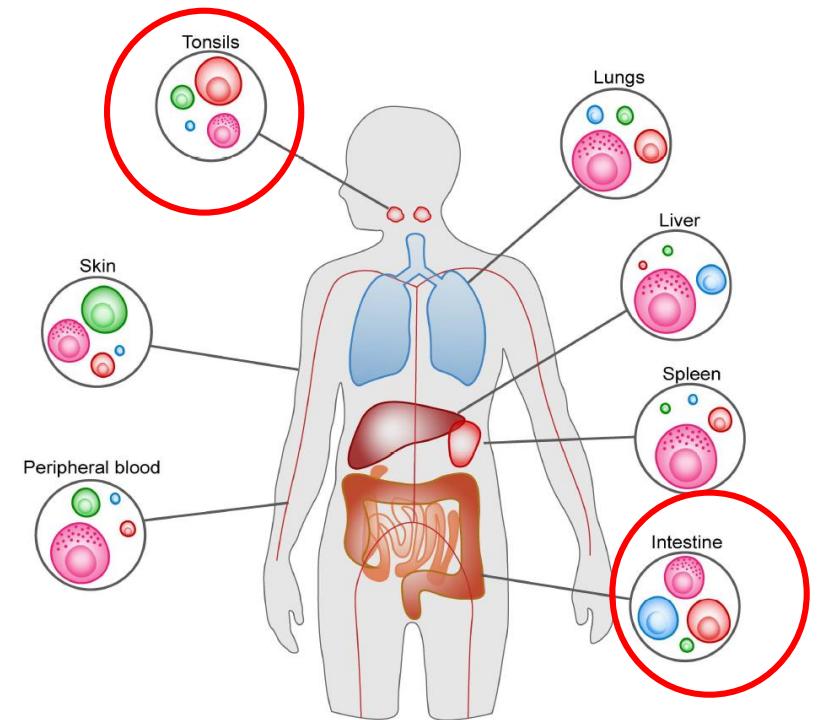
Innate lymphocytes

Adaptive lymphocytes

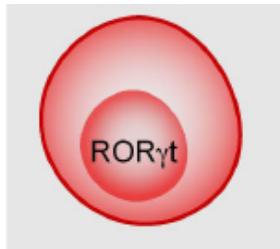
Master regulators of effector lymphocytes

Possot et al, *Nat immunol* 2011; Constantinides et al, *Nature* 2014; Klose et al, *Cell* 2014; Yang et al, *Nat immunol* 2016; Ishizuka IE et al, *Nat Immunol* 2016; Xu et al, *Immunity* 2019; Lim et al, *Cell* 2019

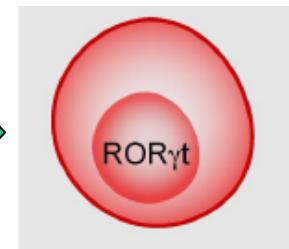
Committed precursors of ILCs are present in tissues



CD34⁺ HPC



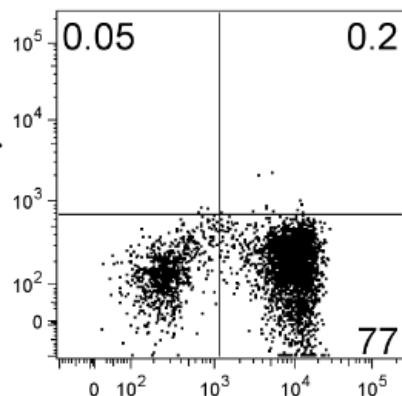
mature ILC3



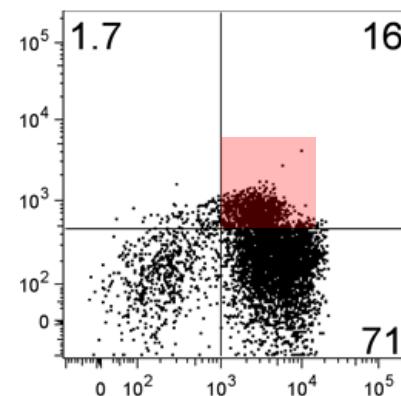
IL-22
IL-17

PB/BM/CB

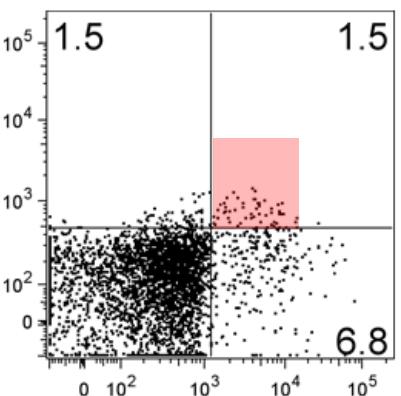
ROR γ t



tonsil



intestinal LP



Possot et al, *Nat Immunol* 2011
Montaldo et al, *Immunity* 2014
Bando et al, *Nat Immunol* 2015
Scoville et al, *Immunity* 2016

Montaldo et al. *Immunity*, 2014

Which signals induce activation of ILCs
beside inflammatory cytokines?

ILC sensing and specificity

cytokine receptors

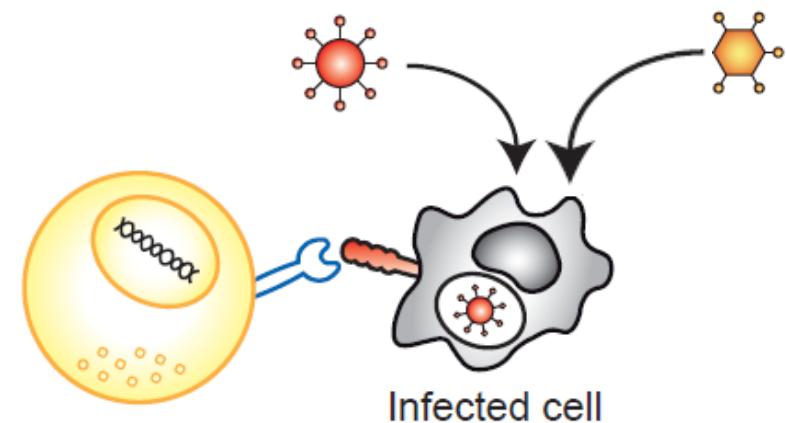
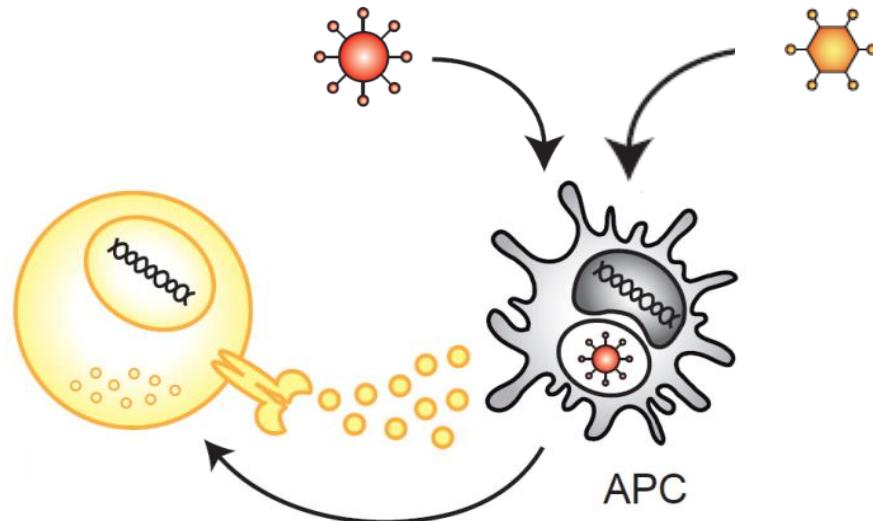


pro-inflammatory cytokines

activating receptors

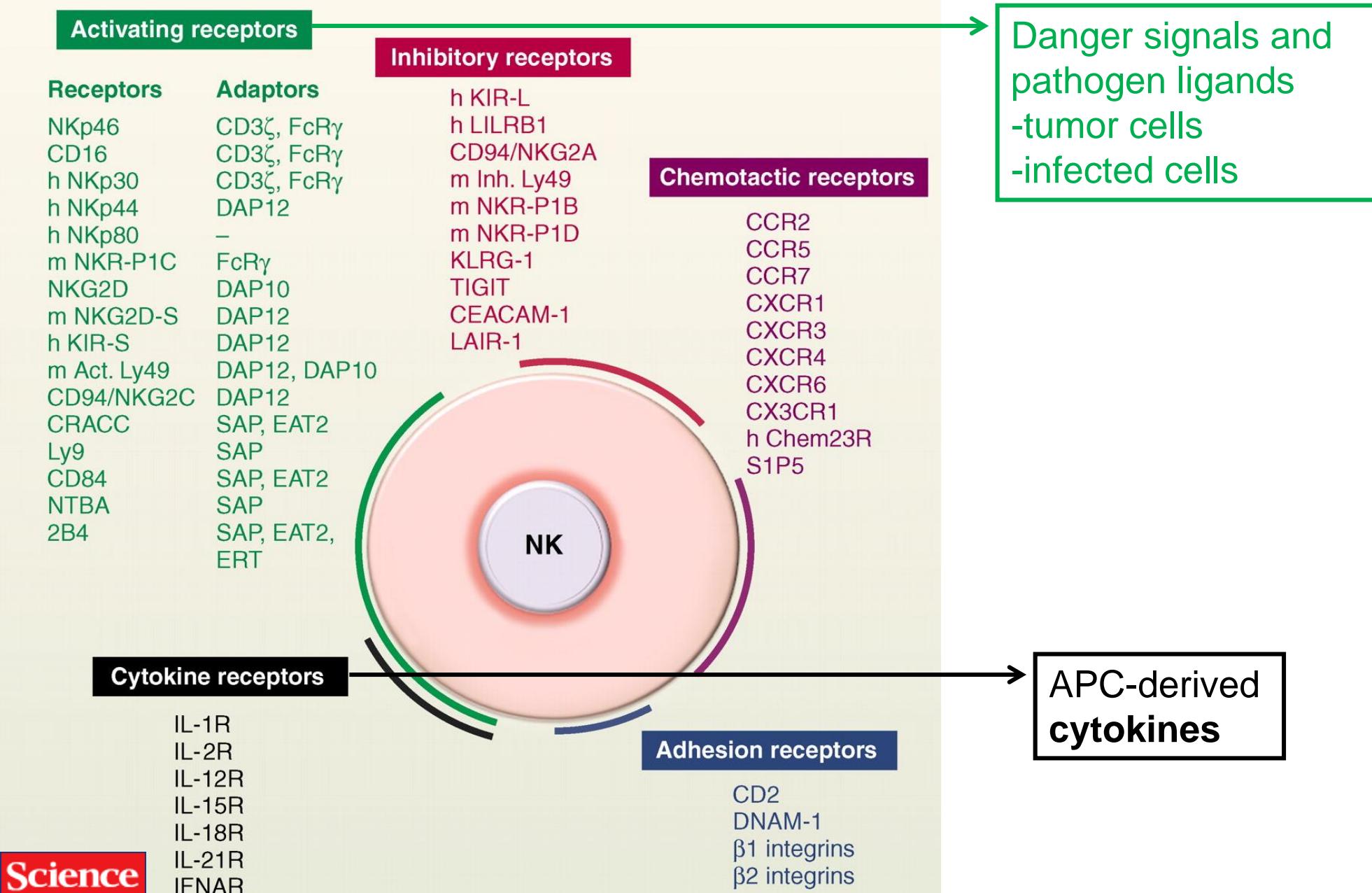


PAMPs or DAMPs



Dietary components: Retinoic acid, AhR ligands
Neuropeptides (VIP, Neuromedin)

NK cell/ILC1 activation



NK cell repertoire

Activating receptors

Receptors

NKp46

CD16

h NKp30

h NKp44

h NKp80

m NKR-P1C

NKG2D

m NKG2D-S

h KIR-S

m Act. Ly49

CD94/NKG2C

CRACC

Ly9

CD84

NTBA

2B4

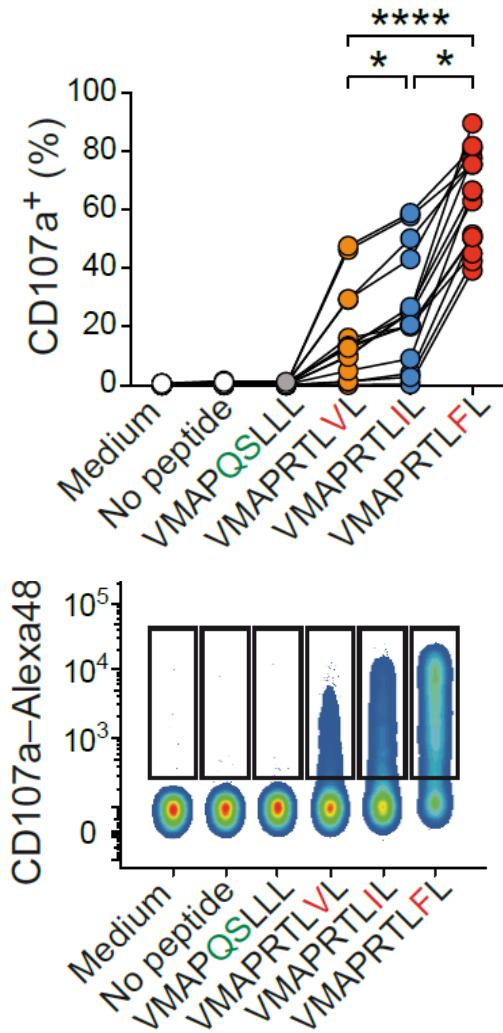
Receptors expressed on the large majority of NK cells

Receptors expressed on subsets of NK cells

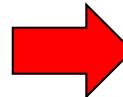
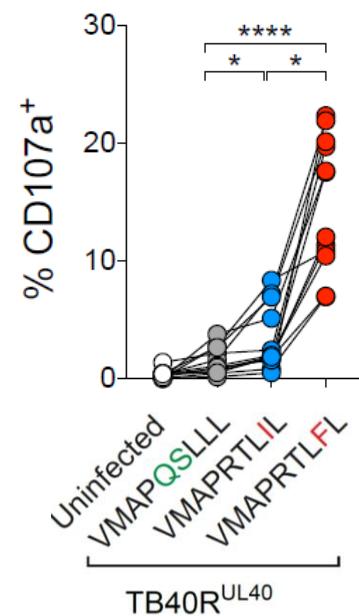
NKG2C⁺ NK cells differentially recognize viral peptides and have adaptive properties

NKG2C⁺ NK cells +

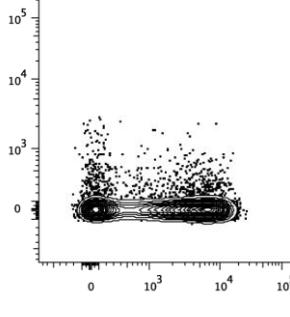
peptide pulse on RMA-S/HLA-E



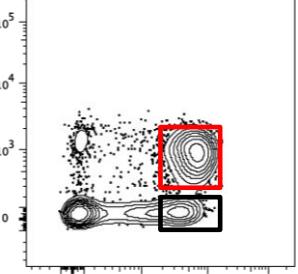
HUVEC infected with mutated HCMV strains



NKG2C

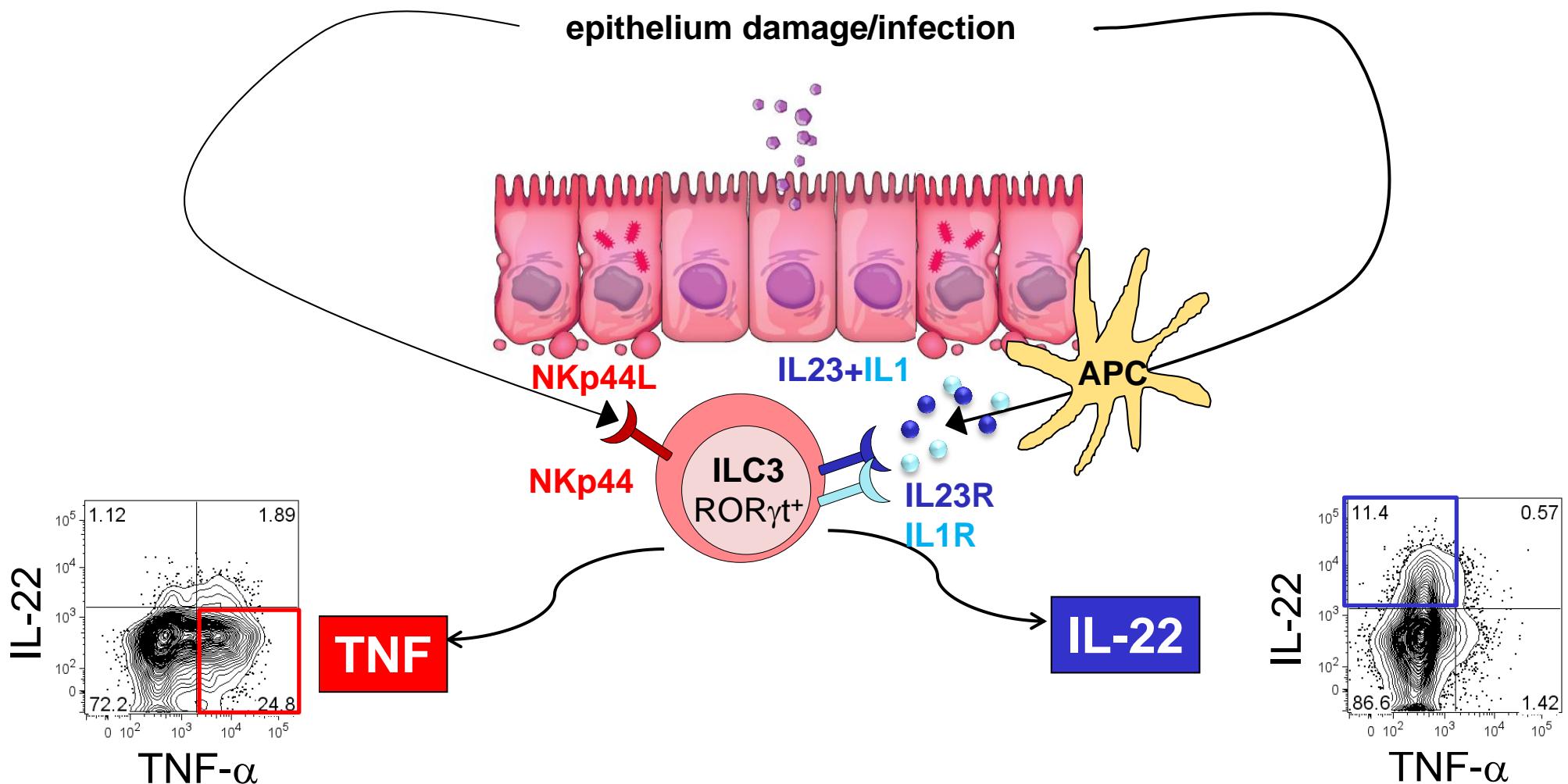


HCMV⁺



CD57

ILC3 can switch on distinct effector programs depending on the activating signals (NKp44 versus cytokines)



ILCs: open questions

Where and when do ILC differentiate?

Which recognition strategies do they employ? Neuro-immune synapse

What signals specifically drive ILC fate decision? Nutrients, microflora?

When during differentiation is the cytokine effector program imprinted?

Cross-talk with T cells and B cells

How do you switch off ILC responses?

Which are ILCs non redundant function? more specific depletion models..

How can we use and target ILCs to modulate inflammation?

Acknowledgements



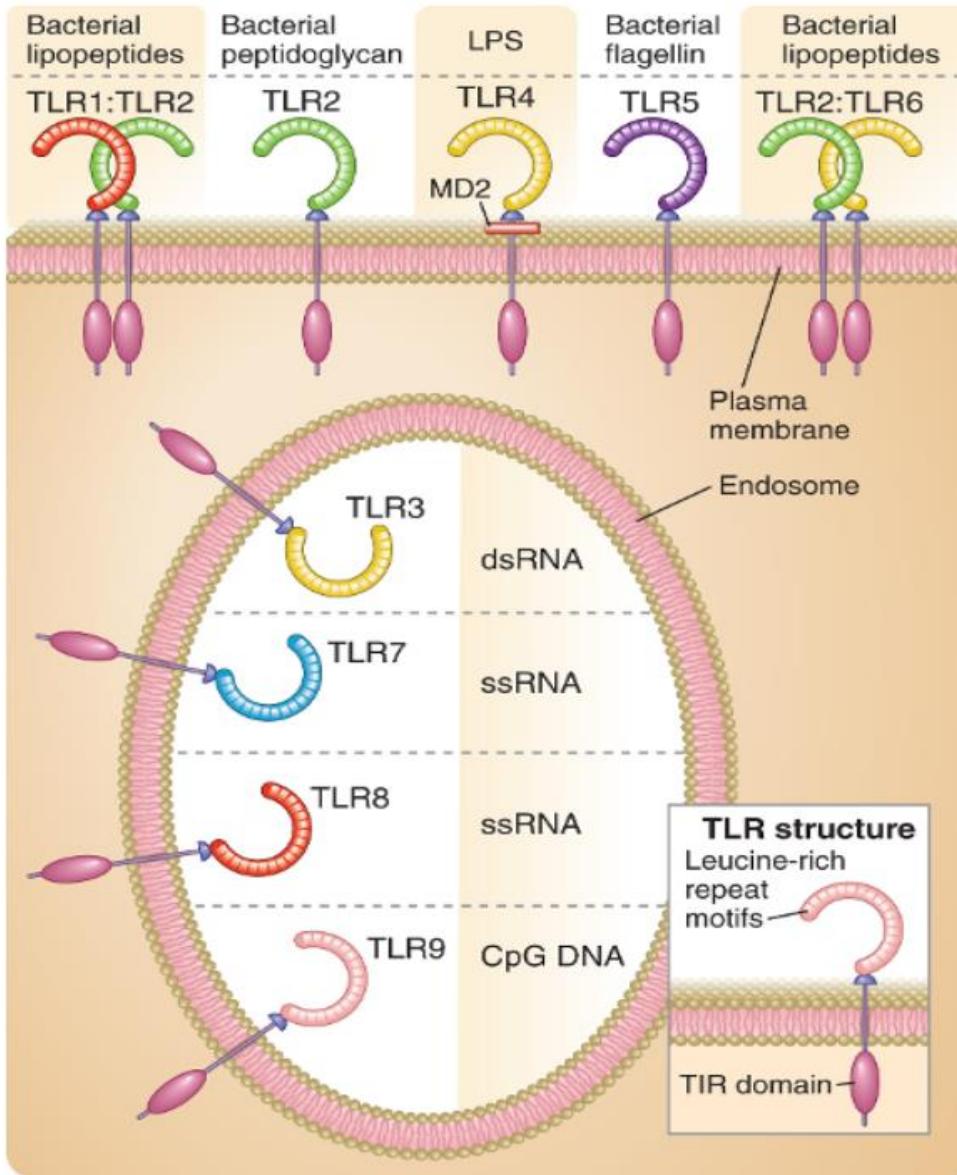
Innate Immunity Group

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Nils Müller
Christoforos Dimitropoulos
Quirin Hammer
Kerstin Juelke
Elisa Montaldo



pattern recognition receptors

- Toll-like receptors (TLR) –ligands



ILC2 activation

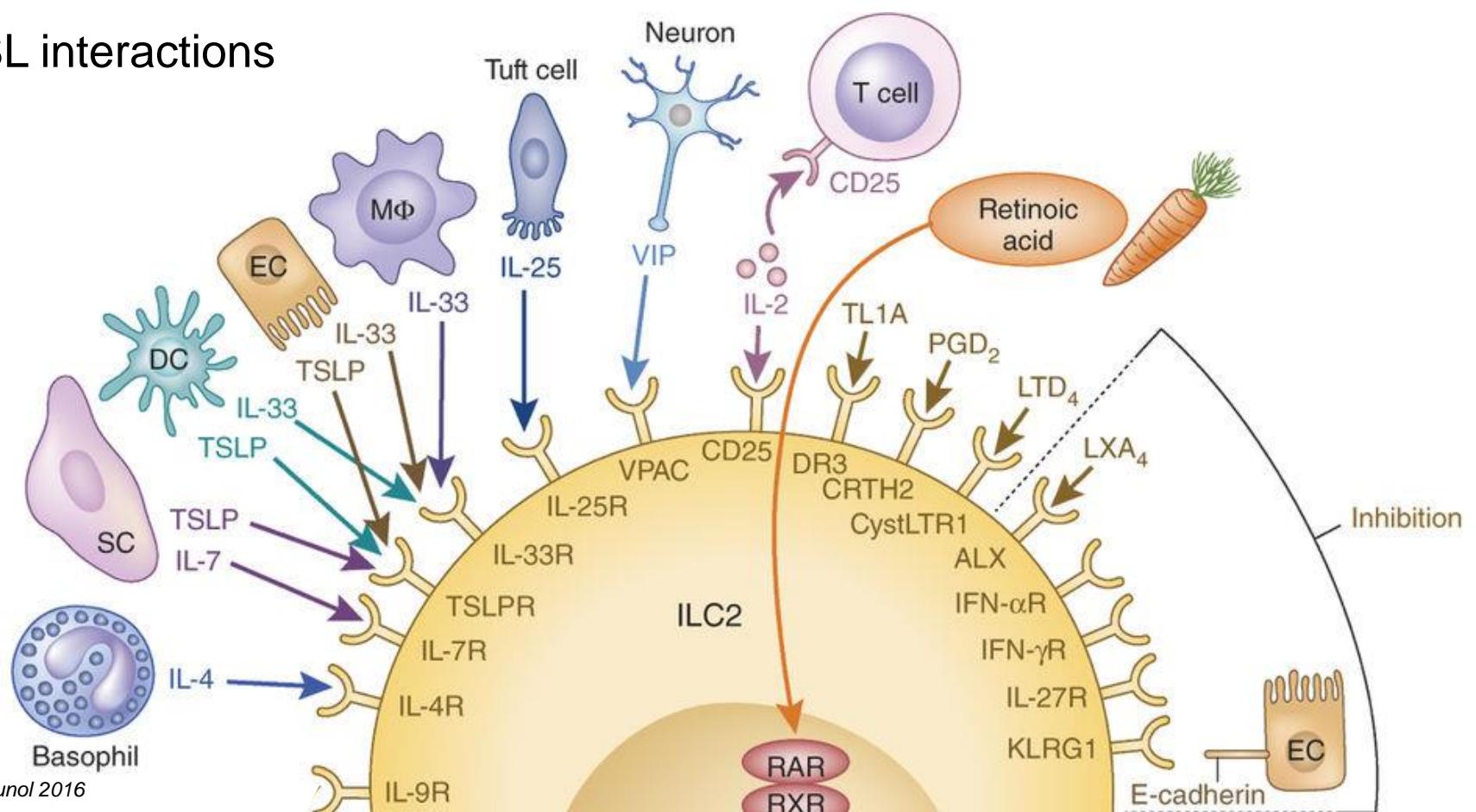
Activation:

Cytokines: IL-33, IL-25, TL1A, TSLP, IL-7, IL-2

PGD₂, LTD(E)₄

Neuropeptides: VIP, NeuromedinU

ICOS-ICOSL interactions



ILC3 activation

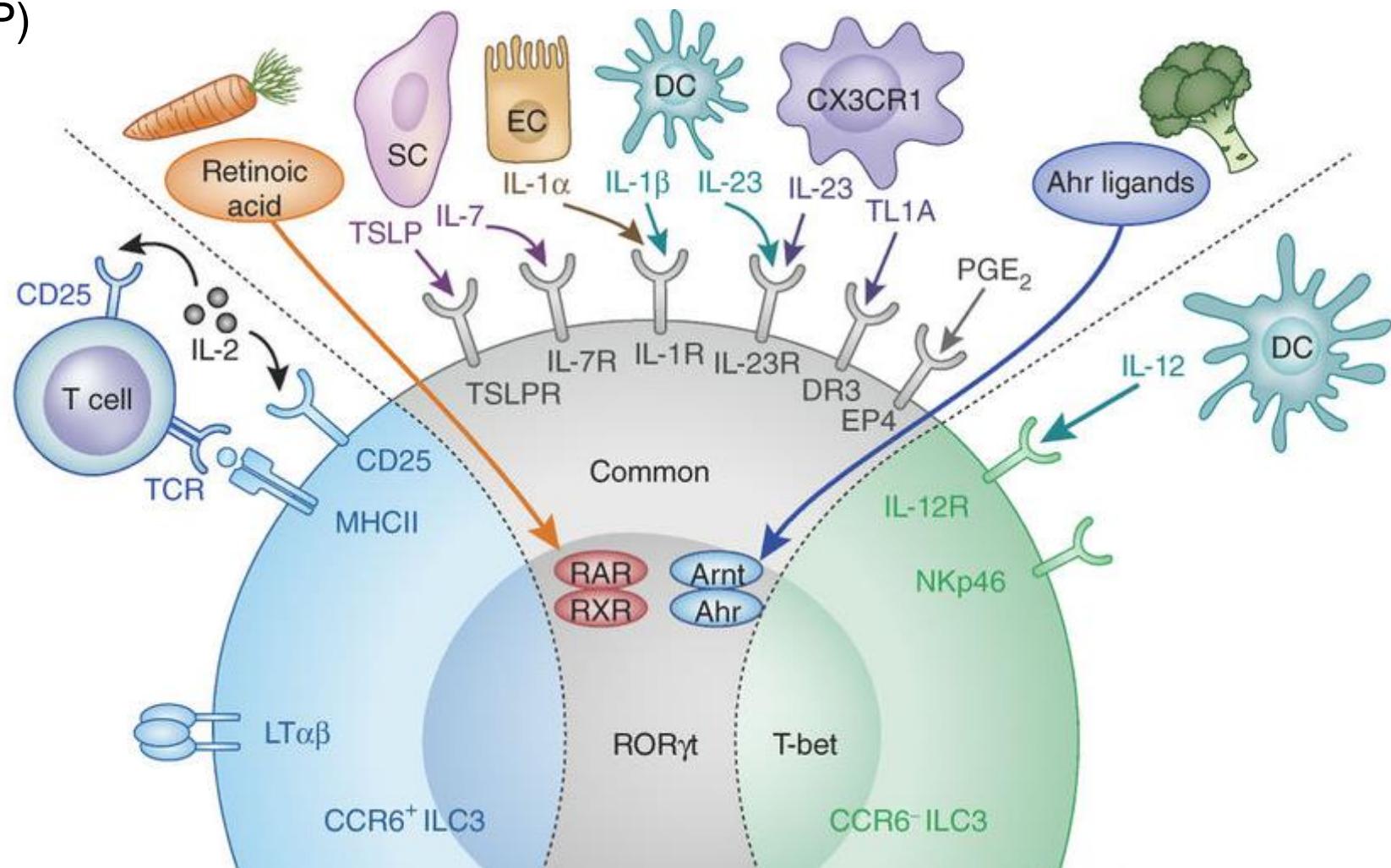
Activation:

Cytokines: IL-23, IL-1, IL-7, TL1A

Dietary components: Retinoic acid, Aryl hydrocarbon receptor (AhR) ligands

Neuropeptides (VIP)

NKp44L



pattern recognition receptors

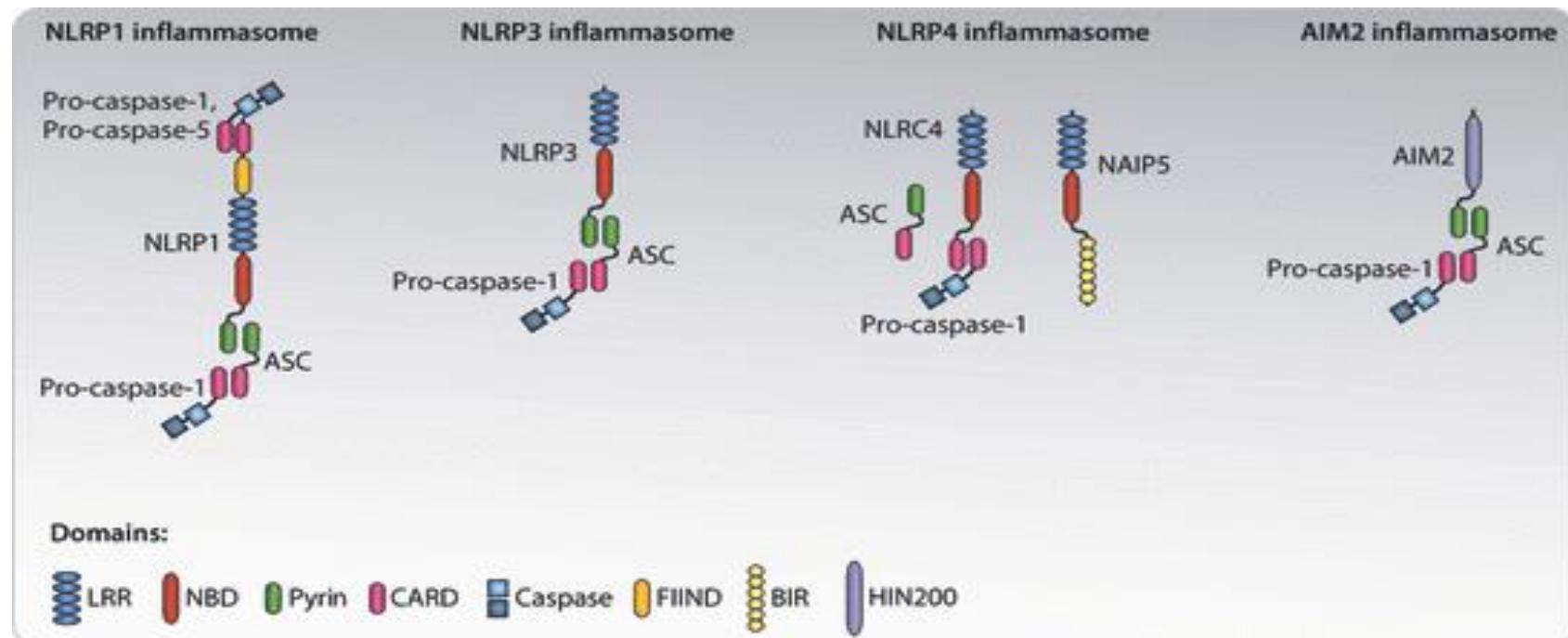
Inflammasome

Structure: receptor or sensor (NLRP3..)
ASC adapter
pro-caspase-1

Localization: cytosolic

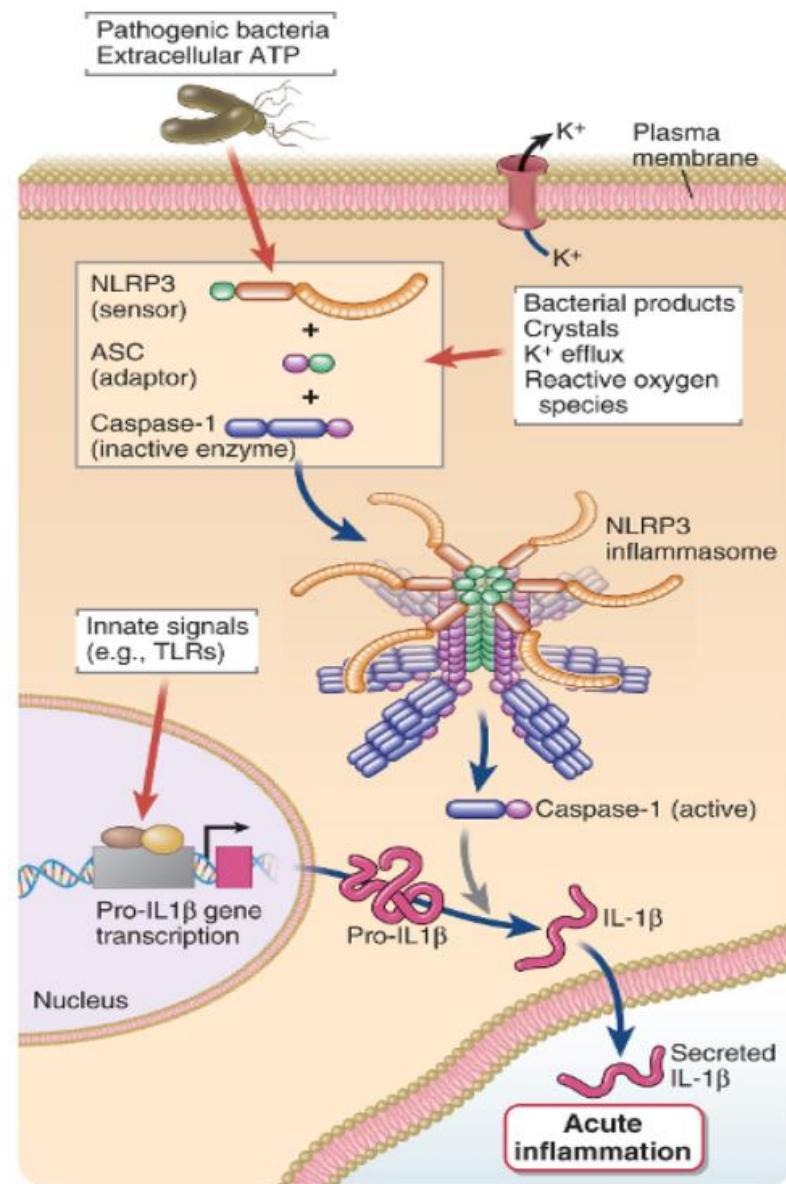
Signalling: activating caspase 1, a protease that converts proIL-1 β into IL-1 β

Expressed by: macrophage, DC, others (epithelial cells..)



pattern recognition receptors

Inflammasome and regulation of IL-1 production



TLRs induce transcription of pro-IL-1 β and other inflammatory cytokines, while the inflammasome controls IL-1 maturation

ILC functions

ARTICLES

nature
immunology

Evidence of innate lymphoid cell redundancy in humans

Frédéric Vély^{1,2,19}, Vincent Barlogis^{3,19}, Blandine Vallentin^{3,19}, Bénédicte Neven^{4–7,19}, Christelle Piperoglou^{1,2}, Thibaut Perchet^{8,9}, Maxime Petit^{8,9}, Nadia Yessaad¹⁰, Fabien Touzot^{5,11}, Julie Bruneau^{5,12}, Nizar Mahlaoui^{4,6,7}, Nicolas Zucchini¹³, Catherine Farnarier², Gérard Michel³, Despina Moshous^{4–7}, Stéphane Blanche^{4–7}, Arnaud Dujardin¹⁴, Hergen Spits¹⁵, Jörg H W Distler¹⁶, Andreas Ramming¹⁶, Capucine Picard^{4–7,17}, Rachel Golub^{8,9}, Alain Fischer^{4–7,18,20} & Eric Vivier^{1,2,20}

Follow-up of Jak3 deficient individuals who have undergone HSCT without myeloablation and characterized by poor ILC reconstitution in PB

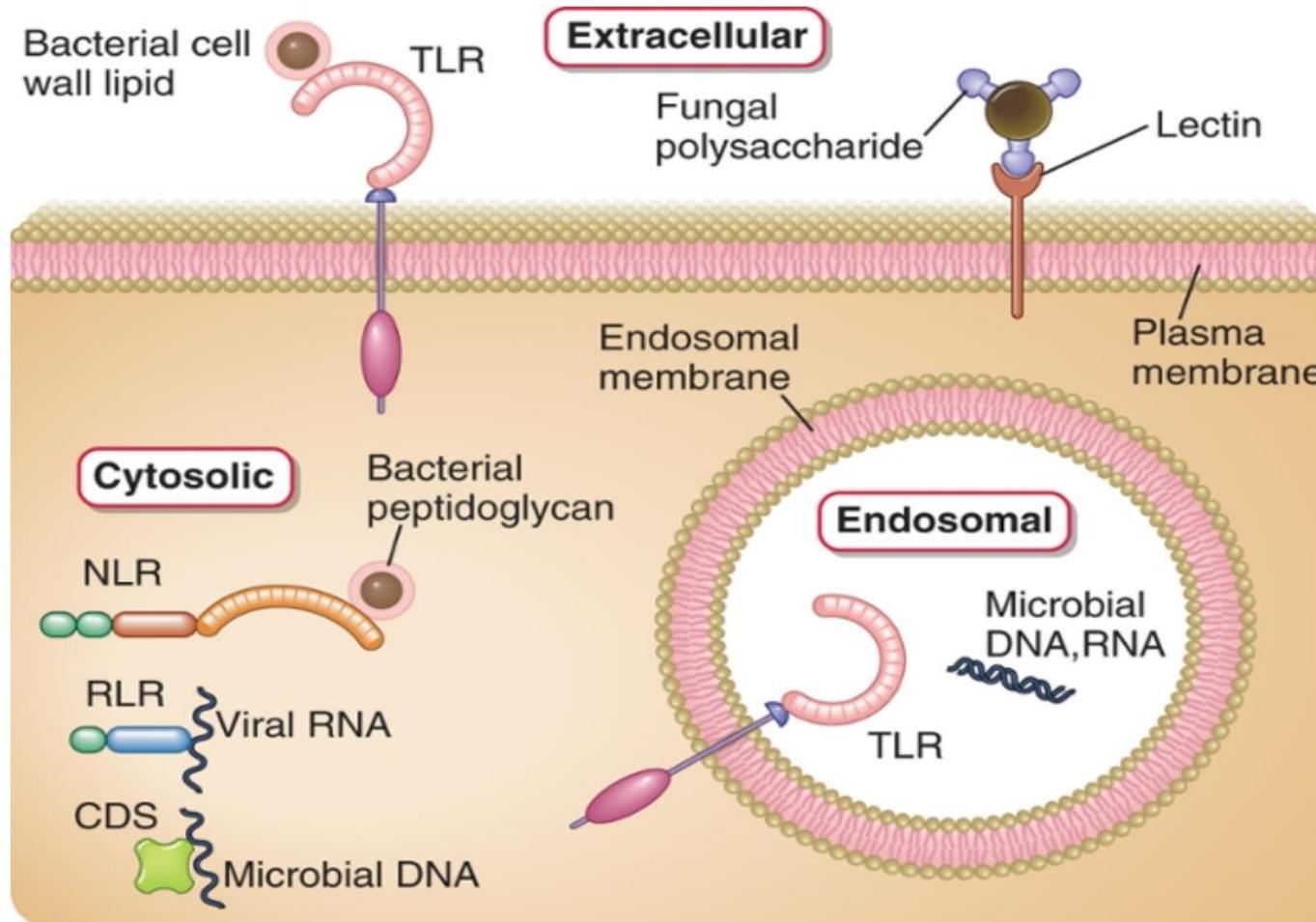
Embryonic and neonatal life

Containment of microbiota/pathobionts

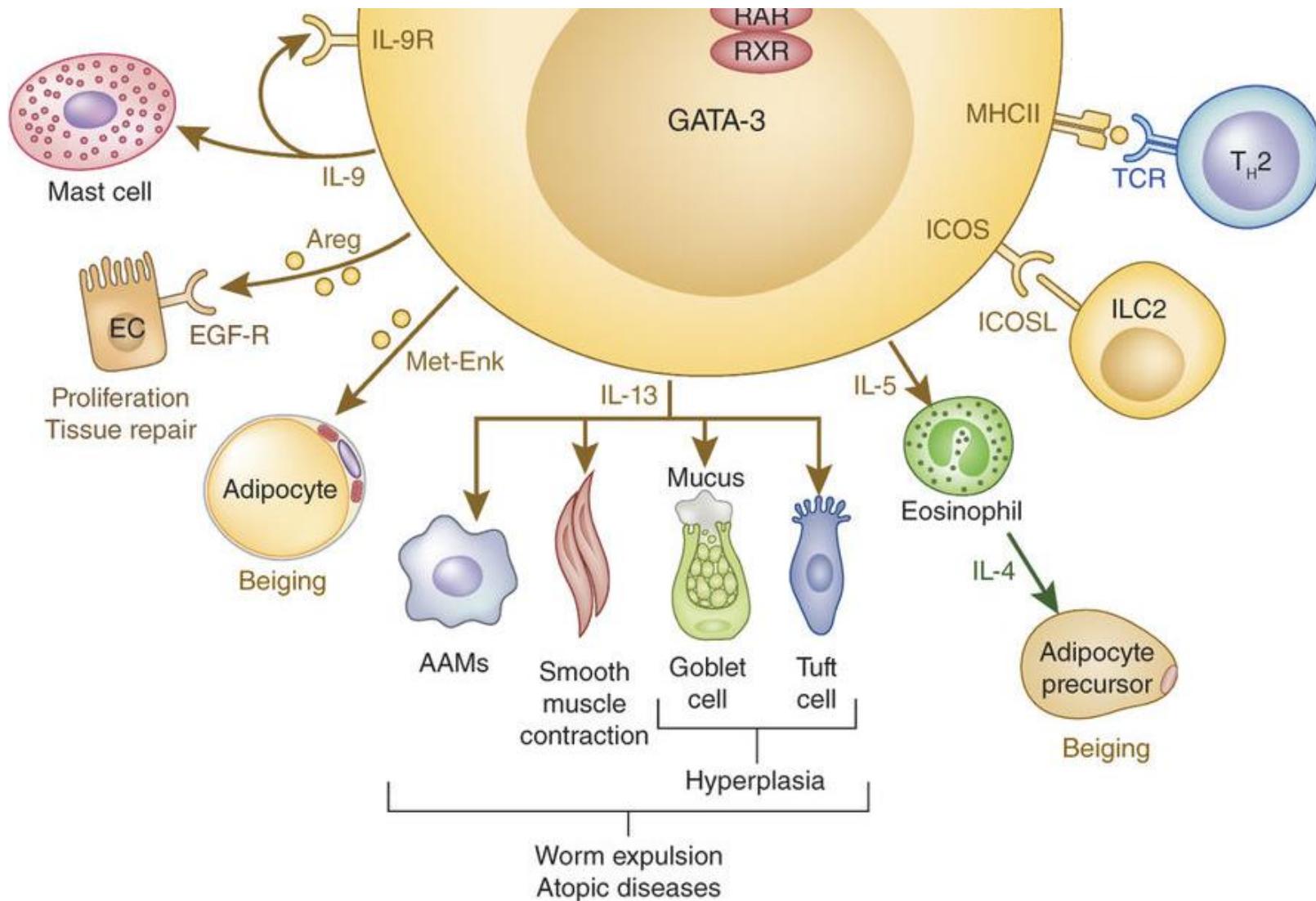
Tissue homeostasis

Inflammation

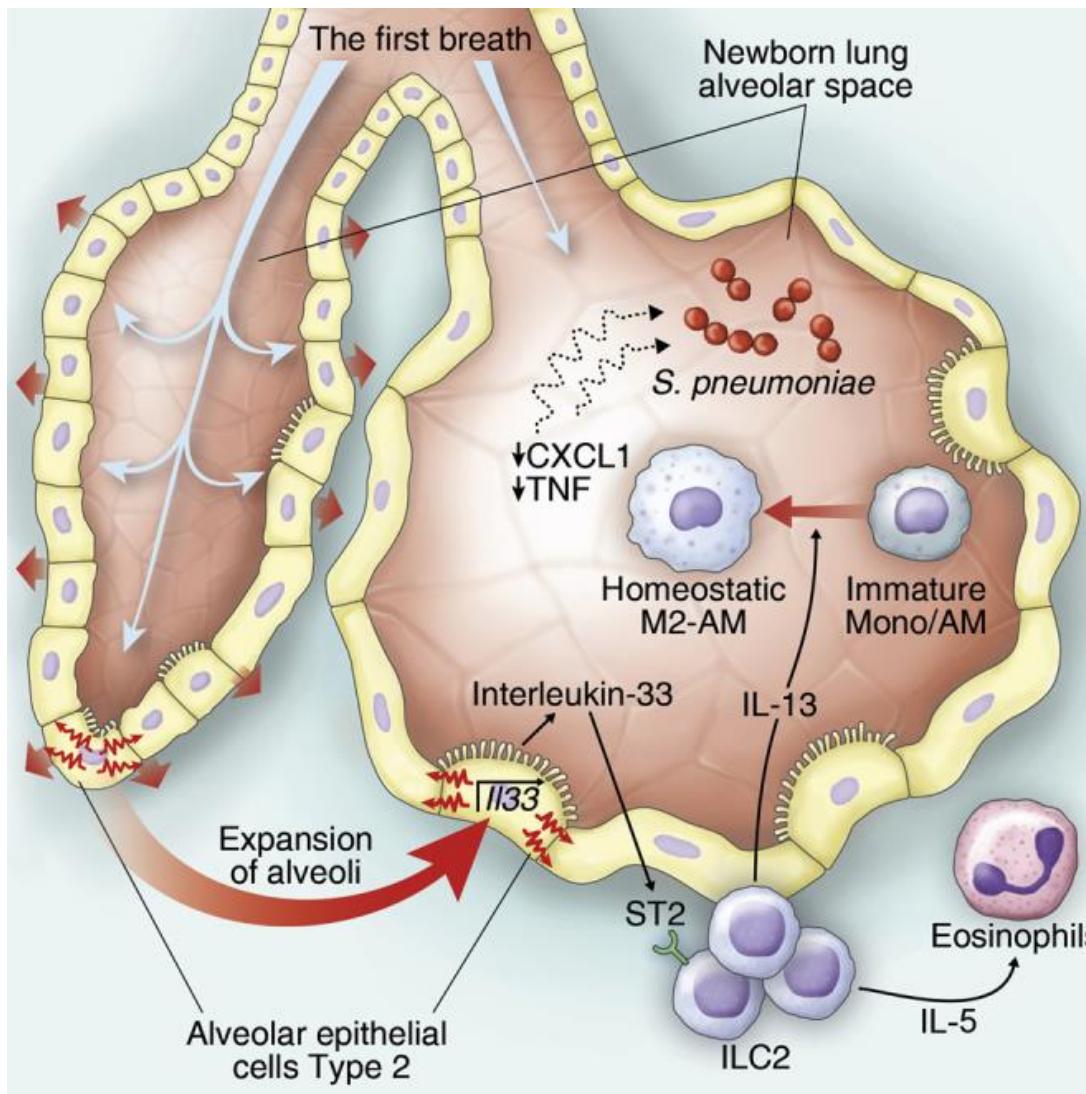
Pattern Recognition Receptors (PRR)-localization



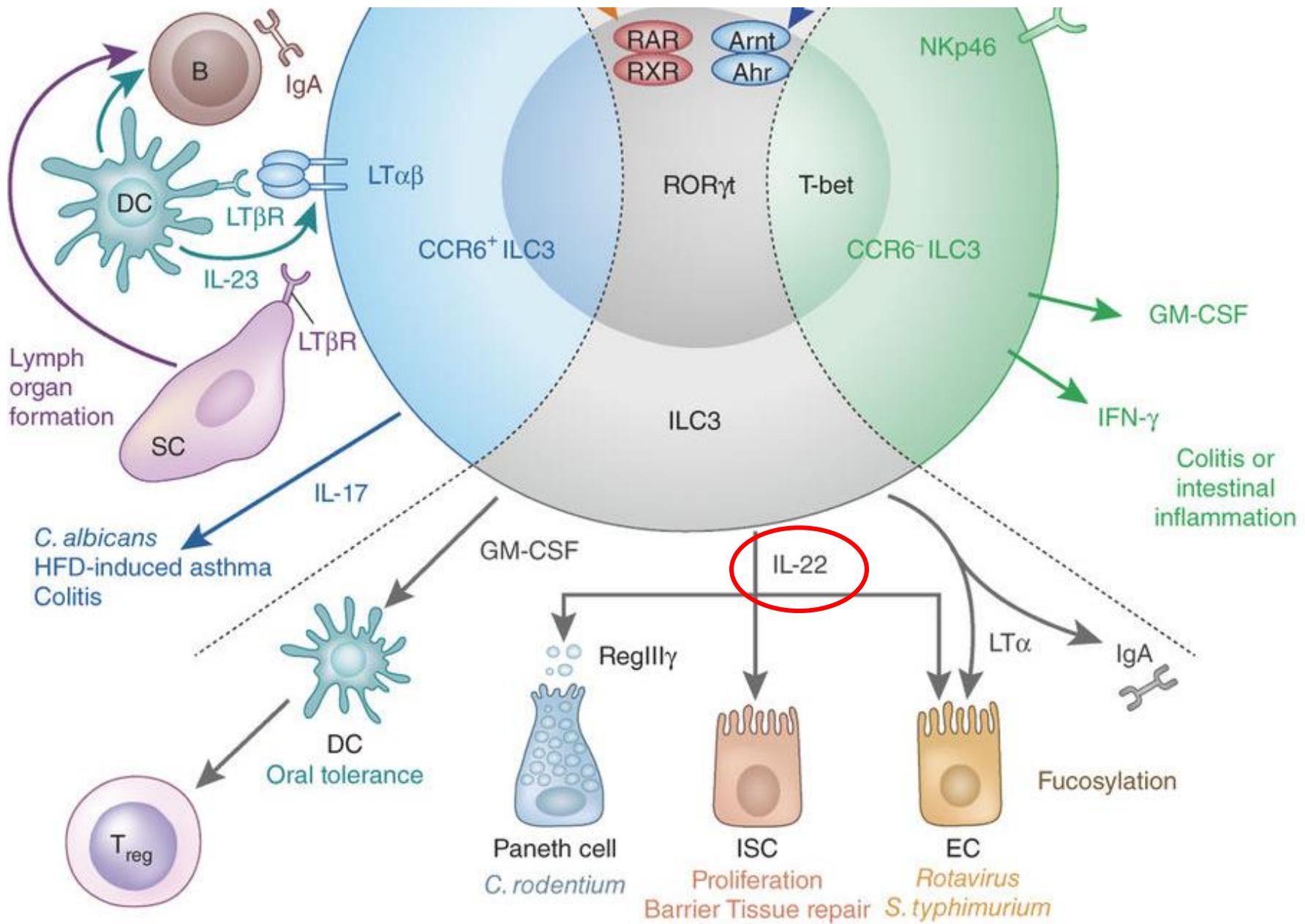
ILC2 functions



ILC2 in neonates shape lung environment



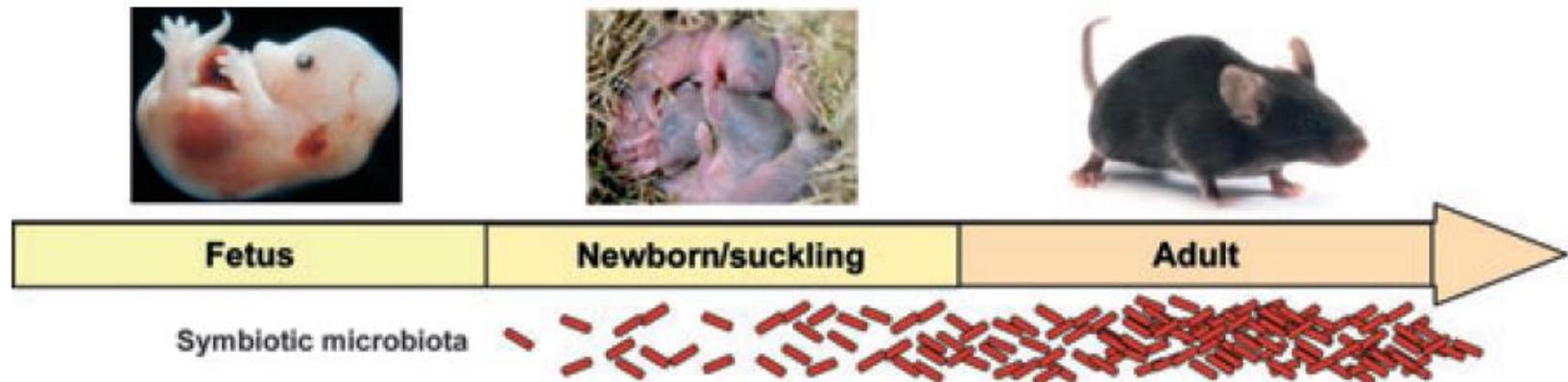
ILC3 functions



ILC classification – Vivier et al, Cell 2018

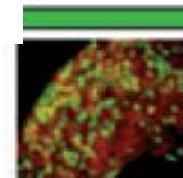
Stimuli	Mediators	Immune function
Tumors, intracellular microbes (Virus, bacteria, parasites)	NK IFN- γ Granzymes Perforin	ILC1 Type 1 immunity (Macrophage activation, cytotoxicity)
Large extracellular parasites and allergens	ILC2 IL-4 IL-5 IL-13 IL-9 AREG	Type 2 immunity (Alternative macrophage activation)
Mesenchymal organizer cells (Retinoic acid, CXCL13, RANK-L)	LTI RANK Lymphotoxin TNF IL-17 IL-22	fetus Formation of secondary lymphoid structures
Extracellular microbes (Bacteria, fungi)	ILC3 IL-22 IL-17 GM-CSF Lymphotoxin	Type 3 immunity (Phagocytosis, antimicrobial peptides)

Pre-natal and post-natal functions of ILC3 in homeostasis



I. "programmed" LT organization
largely $LT\alpha_1\beta_2$ -dependent

LTi cells



LNs, PPs



Cryptopatches \Rightarrow ILFs

IgA-producing B cells

II. Maintenance of barrier function
IL-22/LT-dependent

NKp46^{+/−} ILC3



IL-22 \Rightarrow Epithelial cells



Anti-microbial peptides

RegIII β , RegIII γ , lipocalin-2,
S100A8, S100A9

-induce production of anti-microbial peptides and mucus

-promote epithelial fucosylation (*Goto, Science 2015*)

-prevent overgrow and peripheral dissemination of commensal bacteria

(*Sonnenberg, Science 2012; Qiu, Immunity 2013*)

-induce EC proliferation and repair