NK-Zellen & „innate lymphoid cells“ (ILC)

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IMMUNOLOGY
MAINZ

Vorlesungsinhalte

- Lymphopoese dient nicht nur der Bildung von T- u. B-Zellen:
  Natürliche Killer- (NK-) Zellen und „innate lymphoid cells“ (ILC)

- Vorkommen und Funktion von NK-Zellen

- Rezeptoren und Aktivierung von NK-Zellen: Toleranz
  missing self
  induced/altered self
  non-self

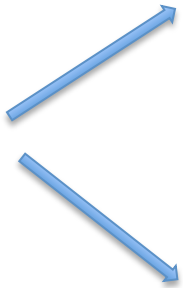
- Immunevasion: mCMV m157 und HIV nef

- Invariante NKT-Zellen

- Allgemeine Eigenschaften und spezielle Funktionen von ILC1, ILC2, ILC3

Die lymphoide Entwicklungsreihe ist komplexer als lange Zeit angenommen

Traditionelle Vorstellung



myeloische Reihe:
angeborene Immunität
first line of defence (Phagocytose)

lymphoide Reihe:
adaptive Immunität,
immunologisches Gedächtnis

☞ Es gibt Zellen, die ohne vorherigen Antigenkontakt cytotoxische Aktivität haben:
NK-Zellen sind entstammen der lymphoiden Reihe, sind aber Teil des angeborenen Immunsystems

NK-Zellen sind die dritte Entwicklungslinie der lymphoiden Zellen

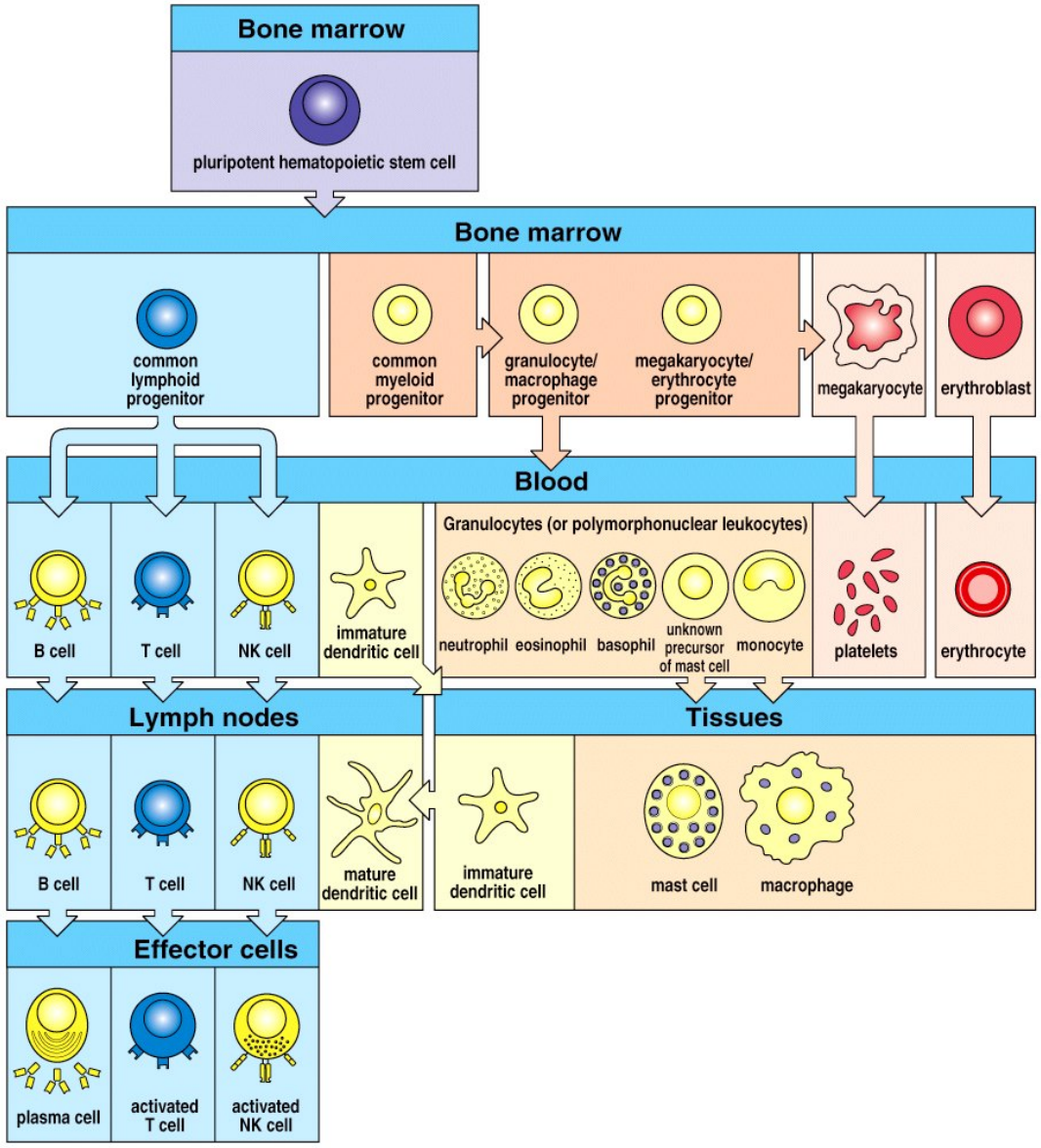
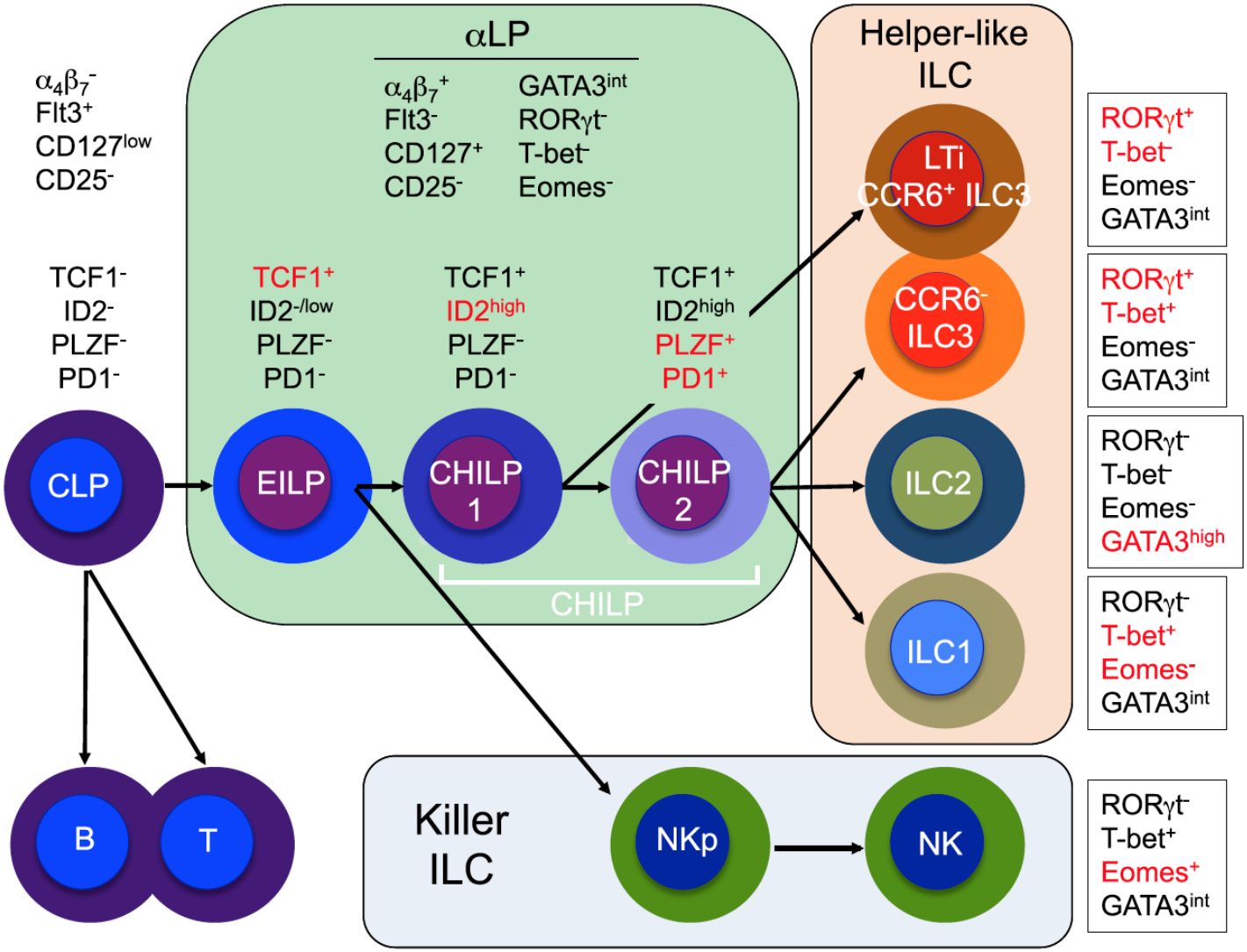


Figure 1-3 Immunobiology, 6/e. (© Garland Science 2005)

ILC bestehen aus Zellen mit cytotoxischen und T-Helfer-ähnlichen Eigenschaften

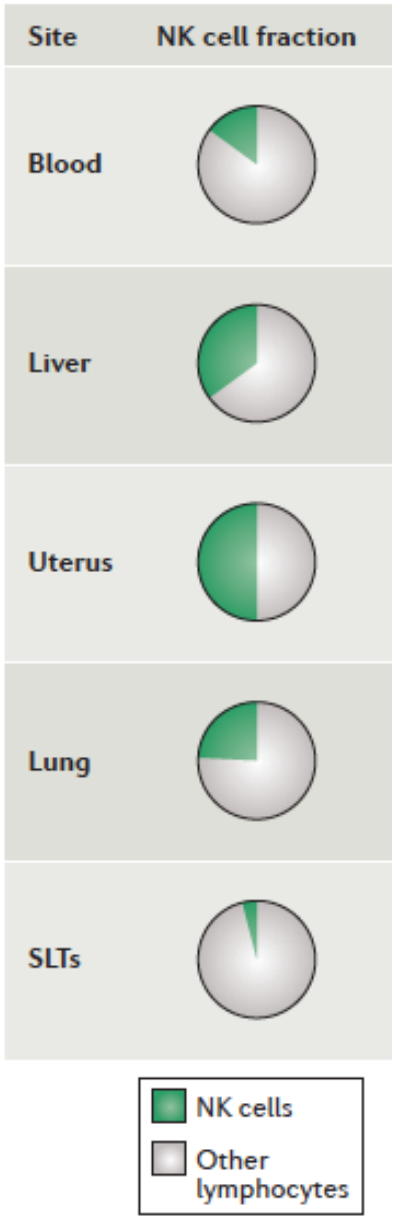


CLP: common lymphoid progenitor
EILP: early ILC progenitor

CHILP: common helper-like progenitor
LTi: lymphoid tissue inducer

Diefenbach et al. (2017) *Immunity*

Vorkommen von NK-Zellen



~5-15% peripheral blood lymphocytes (in humans)

~3-5% lymphocytes in spleen, abundant in liver

>70% of lymphocytes in decidual tissue

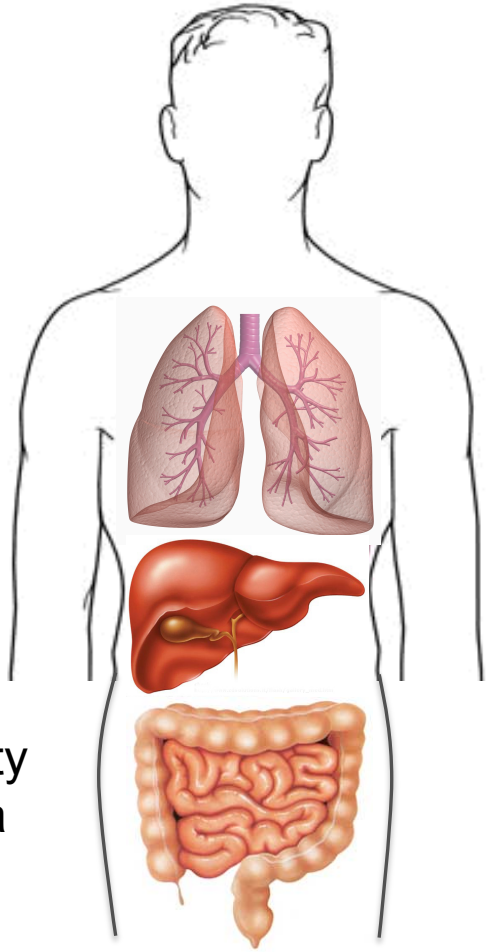
Skin

Lung

LN, Thymus
Spleen

Liver

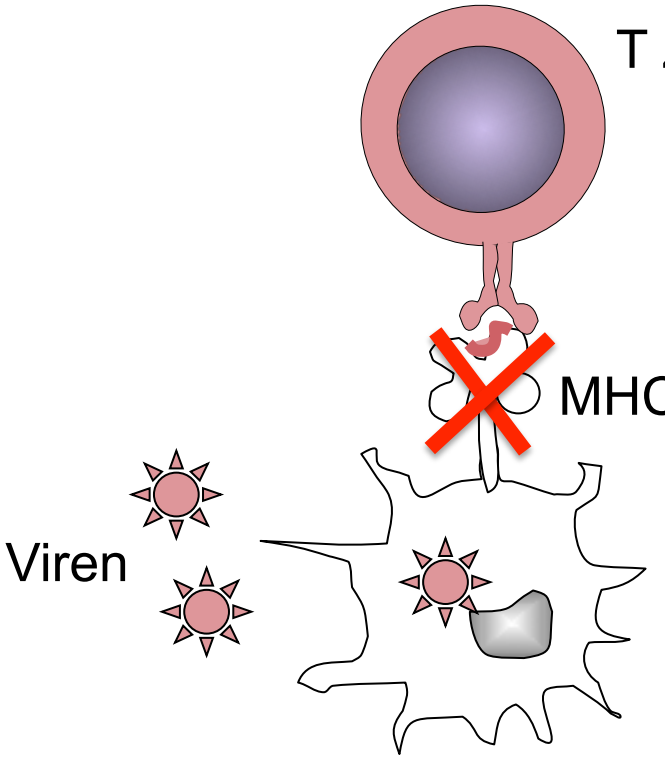
Gut
Peritoneal Cavity
Uterus/Placenta



T Zellen überwachen das “Innenleben” von Zellen

Schwachstellen der MHC-Abhängigkeit: Mutationen, Downregulation

MHC *Herpesviren (CMV)*
b2m *HIV*
TAP



„missing-self“

Infizierte Zelle: „fremd“ („non-self“)

Tumorzelle: mutiertes Genom („altered-self“)

T Zellen überwachen das “Innenleben” von Zellen

Schwachstelle MHC-Abhängigkeit: Mutation, Downregulation

MHC
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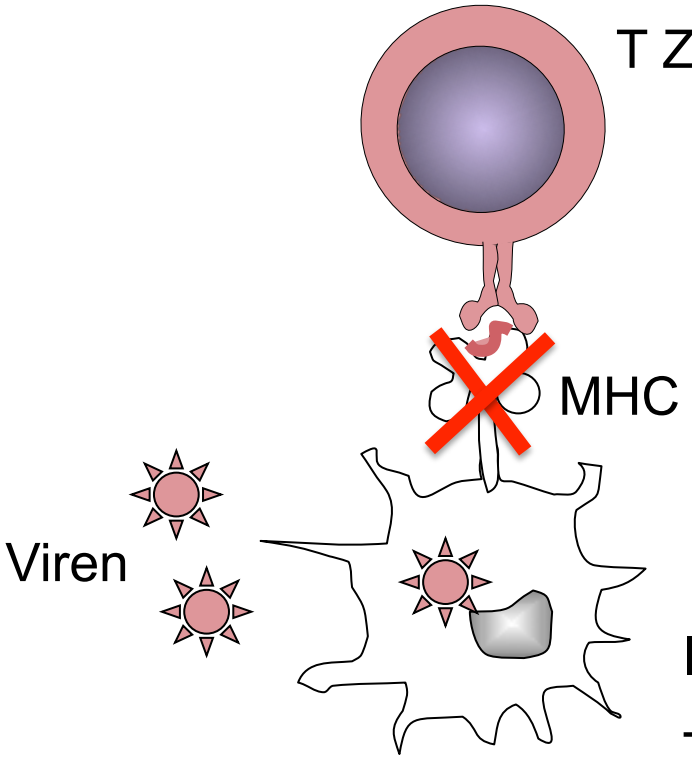
HIV



„missing-self“

NK-Zellen beheben die „Lücke im System“

Infizierte Zelle: „fremd“ („non-self“)

Tumorzelle: mutiertes Genom („altered-self“)

NK-Zell Rezeptoren können aktivierend oder hemmend auf die NK-Zelle wirken

- Aktivierende Rezeptoren erkennen Liganden auf gestressten oder infizierten Zellen
- Inhibitorische Rezeptoren binden an MHC-I oder MHC-I-ähnliche Moleküle

Table 1 NK cell receptors

Receptor family	*Species*	*Ligands*	*Activation/ inhibitory*
Ly49	M	MHC class I	ACT/INHIB
Ly49A		H-2D^{d,k,p}	Inhib
Ly49C		H-2K^{b,d}, H-2D^{b,d,k}	Inhib
Ly49D		H-2D^{d}	Act
Ly49H		m157	Act
Ly49I		H-2K/D^{b,d,s,q,v}	Inhib
Ly49P		H-2D^{d}	Inhib
KIR	H	HLA-A/-B/-C	ACT/INHIB
KIR2DL1		HLA-C2	Inhib
KIR2DL2/3		HLA-C1	Inhib
KIR2DL4		HLA-G	Act
KIR2DL5		?	Inhib
KIR3DL1		HLA-Bw4	Inhib
KIR3DL2		HLA-A3, -A11	Inhib
KIR2DS1		HLA-C2	Act
KIR2DS2		HLA-C1	Act
KIR2DS3		?	Act
KIR2DS4		?	Act
KIR2DS5		?	Act
KIR3DS1		HLA-Bw4	Act
CD94-NKG2	H/M	H: HLA-E M: Qa1b	ACT/INHIB
NKG2A			Inhib
NKG2C			Act
NKG2E			Act
NKG2D	H/M	H: MIC-A/-B, ULBP1/2/3/4 M: RAE-1, MULT-1, H60	ACT
NCRs	H/M	Viral HA	ACT
NKp30		BAT-3, HSPG, B7-H6	Act
NKp44		Viral HA	Act
NKp46		Viral HA, HSPG	Act
NKp80		AICL	Act
LILR	H/M	MHC class I, UL18	INHIB
2B4	H/M	CD48	ACT/INHIB
KLRG1	H/M	Cadherins	INHIB
NKR-P1	M	Ocil/Clr-b	ACT/INHIB
DNAM-1	H/M	PVR, CD122	ACT
PILR	M	CD99	ACT

Abbreviations: ACT, activation; BAT-3, HLA-B-associated transcript 3; H, human; HA, hemagglutinin; HLA, human leukocyte antigen; INHIB, inhibitory; KIR, killer immunoglobulin-like receptor; KLRG1, killer cell lectin-like receptor G1; LILR, leukocyte immunoglobulin-like receptor; M, mouse; MHC, major histocompatibility complex; MULT-1, mouse UL16-binding-like transcript-1; NCR, natural cytotoxicity receptor; NK, natural killer; PVR, polio virus receptor; RAE-1, retinoic acid early transcript-1.

Pegram et al. *Immunology & Cell Biology* (2011)

NK-Rezeptoren innerhalb einer Familie können aktivieren oder hemmen: Ly49 (Maus)

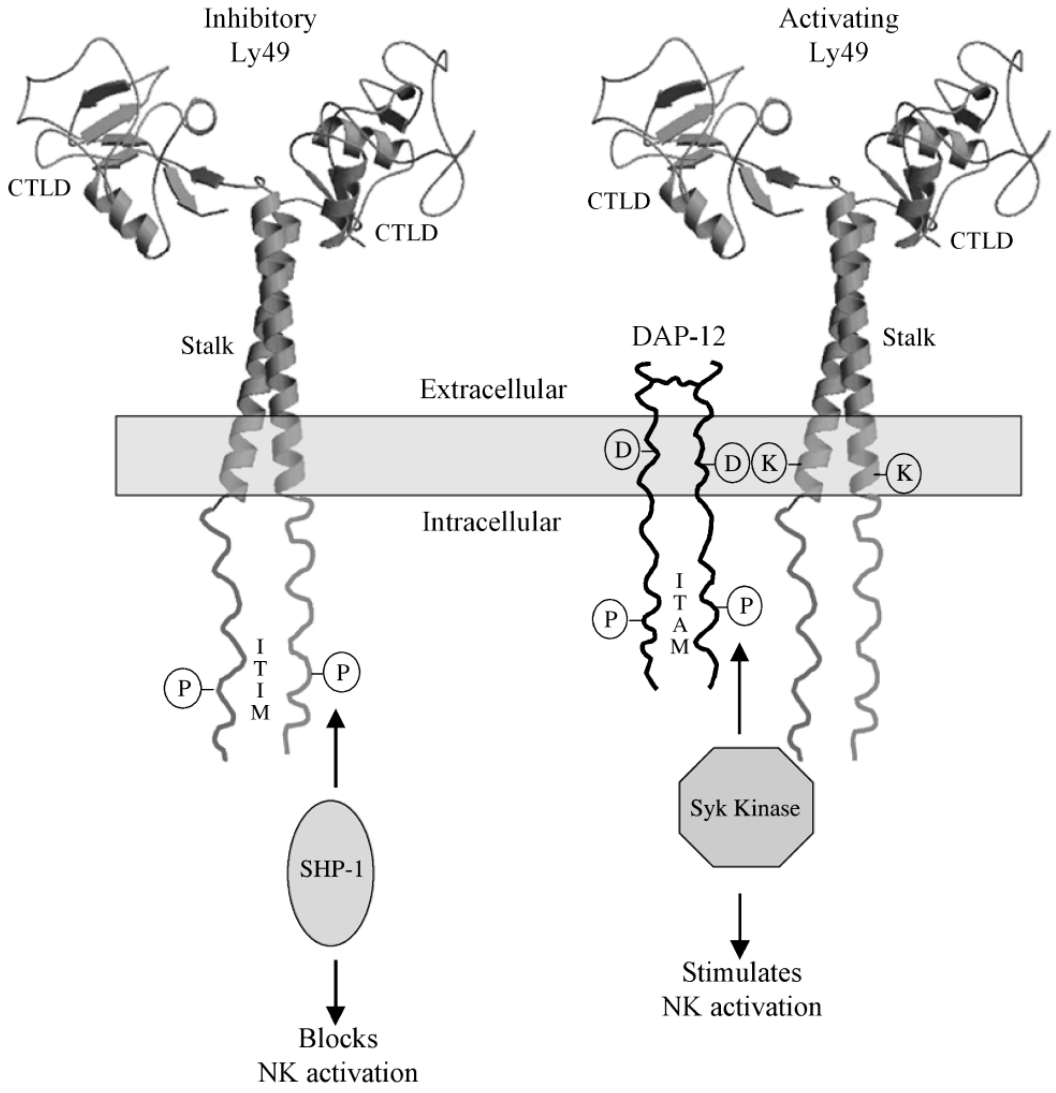


Figure 1 Inhibitory and activating mouse Ly49 NK cell receptors with their intracellular signalling counterparts. Ly49 NK cell receptors are 44 kDa type II homodimeric disulphide-linked transmembrane proteins consisting of a COOH terminal extracellular C-type lectin-like domain (CTLD), connected to the cell membrane by a stalk region of approximately 70 amino acids. Upon engagement, Ly49 inhibitory receptors recruit the tyrosine phosphatase SHP-1, which in turn inhibits NK cell activation. In contrast, engagement of Ly49 activating receptors results in DAP-12 immunoreceptor tyrosine-based activating motifs (ITAM) phosphorylation, with subsequent recruitment and activation of Syk kinase. This event initiates NK cell activation.

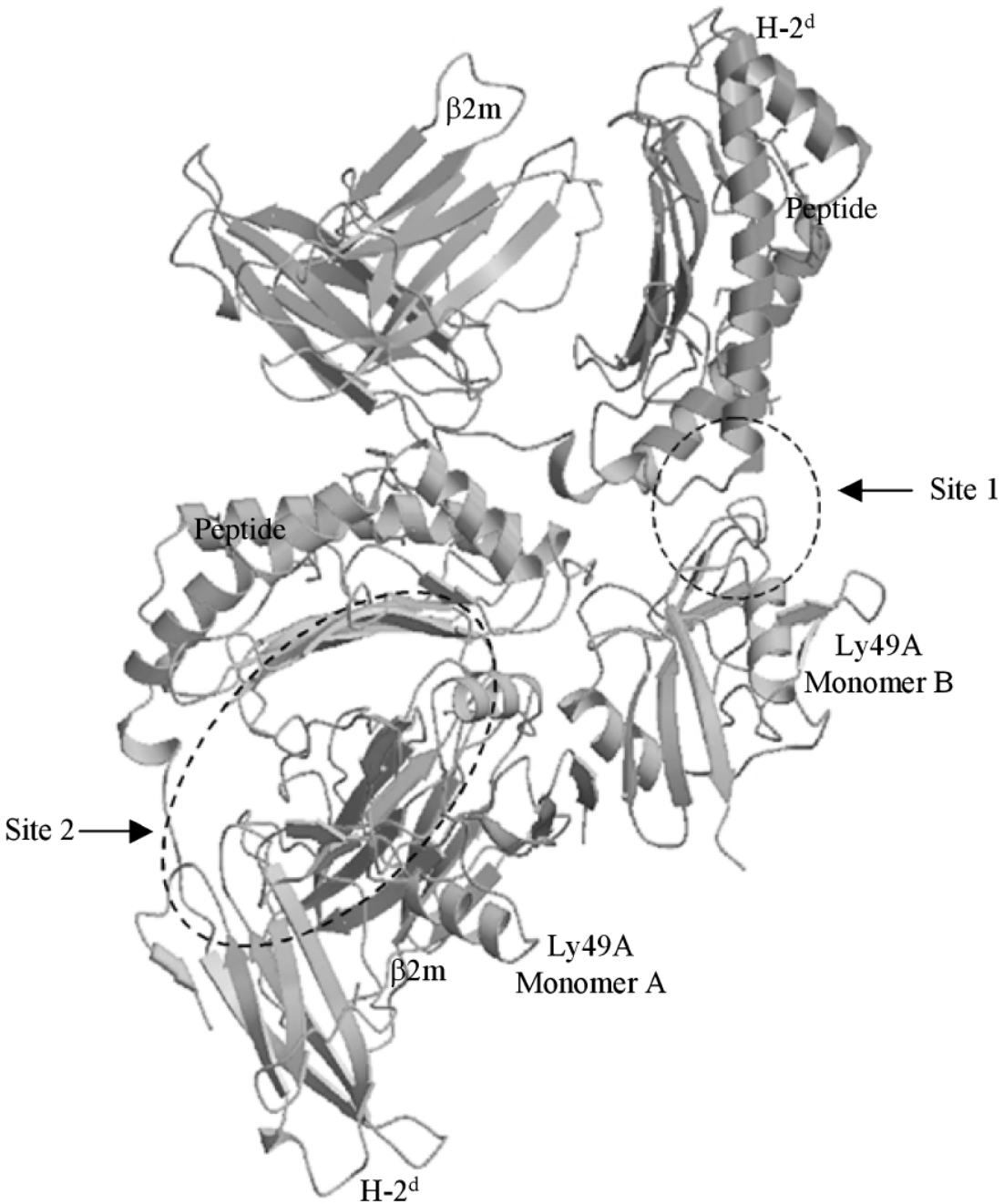
Table 1 Functions and ligands of Ly49 NK cell receptors

Receptor name	Function	Cellular ligand(s)[35–38]	Viral ligand
Ly49A	Inhibitory	D^b, D^d, D^p, D^k	
Ly49B	Inhibitory	?	
Ly49C	Inhibitory	K^b, K^d, K^k, D^d, D^b	
Ly49D	Activating	D^d	
Ly49E	Inhibitory	?	
Ly49F	Inhibitory	D^d	
Ly49G	Inhibitory	D^d, L^d	
Ly49H	Activating	D^b	MCMV-m157[32,33]
Ly49I	Inhibitory	K^d	MCMV-m157[32,33]
Ly49J	Inhibitory	K^b	
Ly49K*	Activating	?	
Ly49L	Activating	K^k	
Ly49M	Activating	?	
Ly49N*	Activating	?	
Ly49O	Inhibitory	D^b, D^d, D^k, L^d	
Ly49P	Activating	D^d	
Ly49Q	Inhibitory	?	
Ly49R	Activating	D^d, D^k, L^d	
Ly49S	Inhibitory	?	
Ly49T	Inhibitory	?	
Ly49U	Activating	?	
Ly49V	Inhibitory	D^b, D^d, K^k	
Ly49W	Activating	D^d, K^k	

Ly49 activating receptors are indicated in bold. To the best of our knowledge, no apparent full length transcripts are identified for Ly49K* and Ly49N*. Nevertheless, their translated nucleotide sequence suggests a possible activating function. Recently, a function in regulation of cytoskeletal architecture of macrophages through immunoreceptor tyrosine-based activating motifs (ITAM)-mediated signalling has been attributed to Ly49Q.[39]

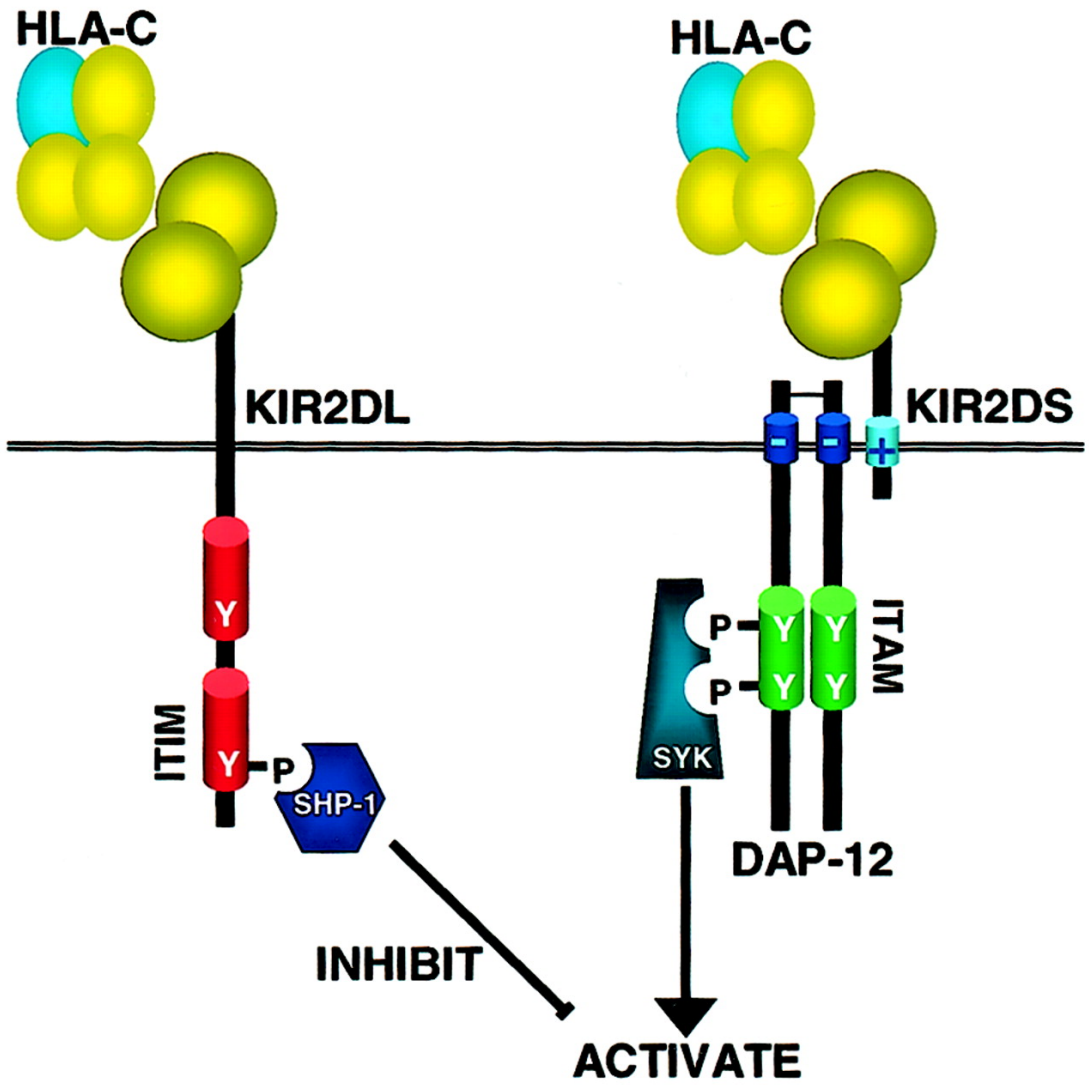
Dimasi & Biassoni *Immun. Cell Biol.* (2005)

Figure 4 X-ray crystal structure of Ly49A in complex with its MHC class I ligand H-2D^d (Protein Data Bank [PDB] entry code 1QO3)[19] highlighting the two sites of receptor–ligand interaction. These two different binding sites were identified by X-ray crystallography.[19] 'Site 1' buries a total surface area of 1000 Å^2 and is located at the N-terminal end of the α1-helix of the MHC class I molecule. This site is largely dominated by electrostatic interactions with a relatively high value for the shape correlation statistic, 0.78 (a shape complementarity value equal to 1 represents a perfect interface).[22] This site was suggested to be the functional binding site, based on the high shape complementarity value and on the perfect electrostatic interactions. Subsequently, functional binding assays and site-directed mutagenesis experiments revealed that 'site 2' is the functional binding site for the Ly49A NK inhibitory receptor.[23–27] Site 2 buries a total surface area of 3300 Å^2 and shows a moderately poor shape complementarity, 0.54.[22] This interface is primarily formed by hydrogen bond interactions. β_2m is the mouse β_2-microglobulin. This figure was prepared using the program Molscript.[34]



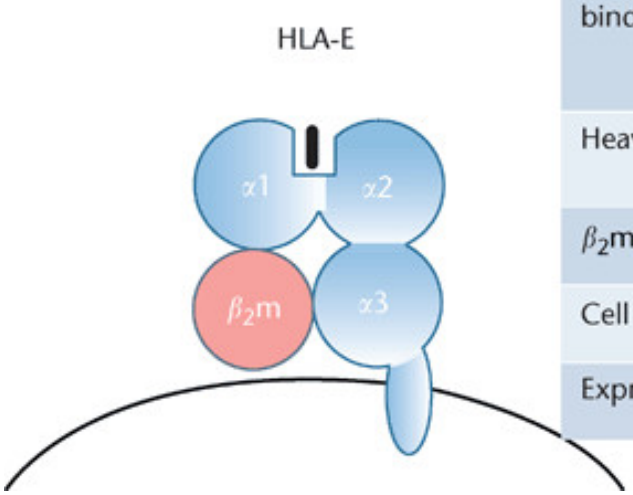
Dimasi & Biassoni *Immun. Cell Biol.* (2005)

Konvergente Evolution: *Killer immunoglobulin like receptors* (KIR) des Menschen haben andere Struktur aber gleiche Funktion wie die Ly49-Familie der Maus

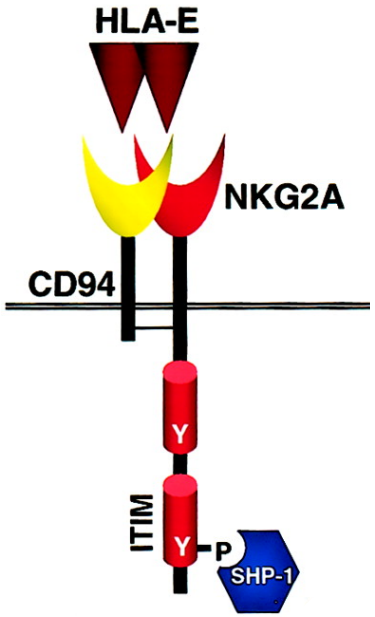


Farag et al. *Blood* (2002)

CD94/NKG2-Familie (Mensch & Maus): Indirektes Erfassen der MHC I - Expression



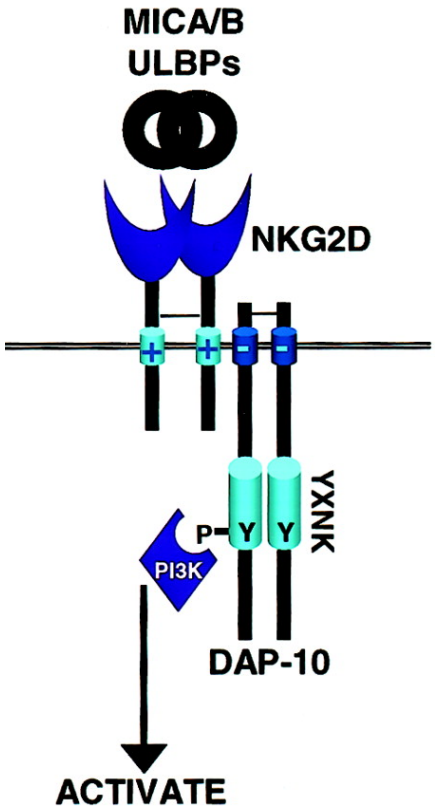
Receptor(s)	CD94/NKG2A-C and αβTCR
Contents of antigen binding groove	Short peptide fragment derived from leader sequence of MHC-Ia
Heavy chain	α1 α2 α3
β_2m associated?	Yes
Cell attachment	Transmembrane
Expression	Ubiquitous



- HLA-E (Mensch) und Qa-1 (Maus) sind MHC-ähnliche Moleküle und binden invariante Peptide aus den Leadersequenzen klassischer MHC I - Moleküle
- HLA-E + Peptid und Qa-1 + Peptid binden an CD94/NKG2 Heterodimere und NK-Zell-Aktivität wird gehemmt
- Ohne MHC-Peptide (MHC I ↓) erscheinen HLA-E/Qa-1 nicht an der Zelloberfläche, NK-Zelle wird aktiviert

Farag et al. *Blood* (2002)

NKD2D Homodimere binden stressinduzierte MHC-ähnliche Liganden und aktivieren NK-Zellen



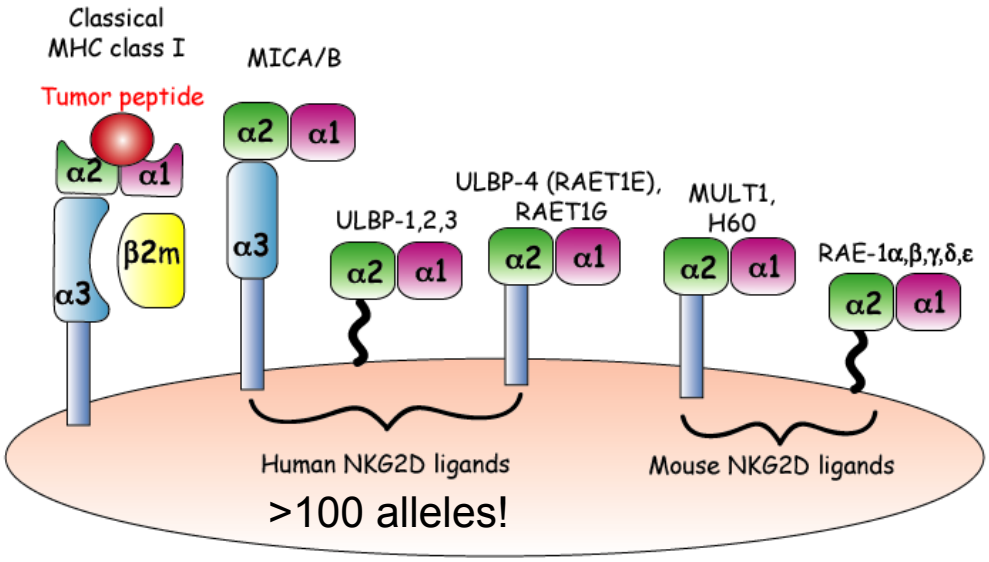
MICA/B: MHC class I chain-related A chain bzw. – B chain
ULBP: UL-16-binding proteins (human) bzw. H60/RAE-1/MULT-1 Proteine i. d. Maus

☞ „induced self recognition“ führt zur Aktivierung von NK-Zellen bei zellulärem Streß (heatshock, Transformation, Infektion, DNA-Schäden)

Farag et al. *Blood* (2002)

NKG2D Liganden – Marker für zellulären Stress

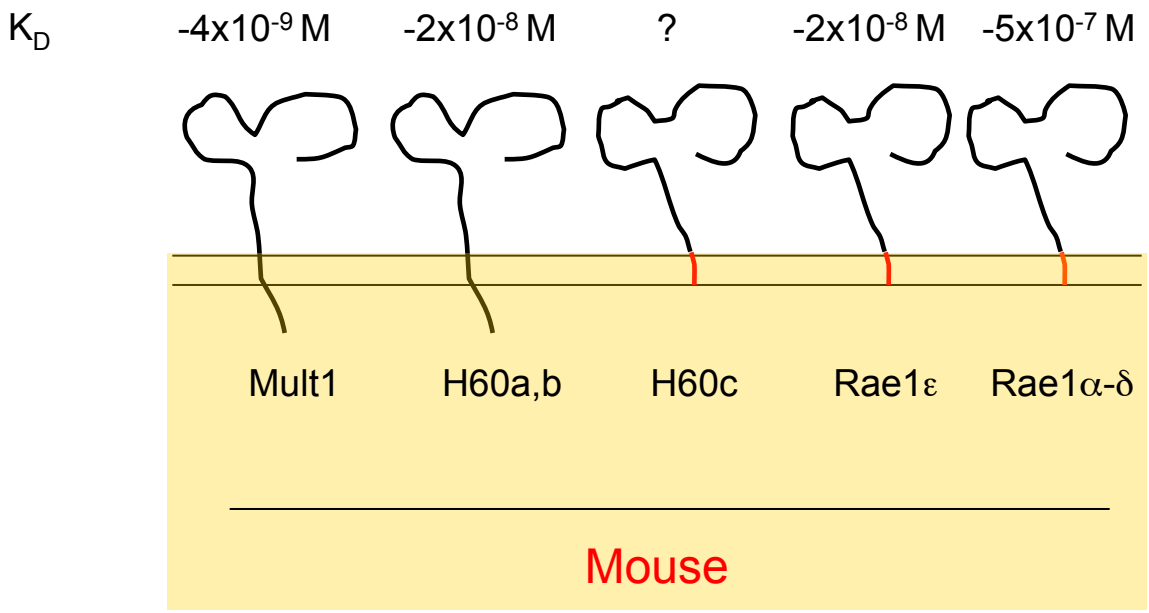
- MHC Klasse I-ähnlich, benötigen aber weder Peptid noch β-Mikroglobulin
- Binden mit nM Affinität NKG2D
- Niedrige Mengen werden auf gesunden Zellen exprimiert
- Induktion auf Virus-infizierten Zellen, Tumorzellen und nach DNA-Schäden.
- Erhöht bei autoimmun. Erkrankungen (rheumatoide Arthritis, Diabetes, Artherosklerose.)



Takaki & Lanier "Viral and Immunological Malignancies", 2005

Why are there so many ligands for one receptor?

- They might fulfill distinct biological functions



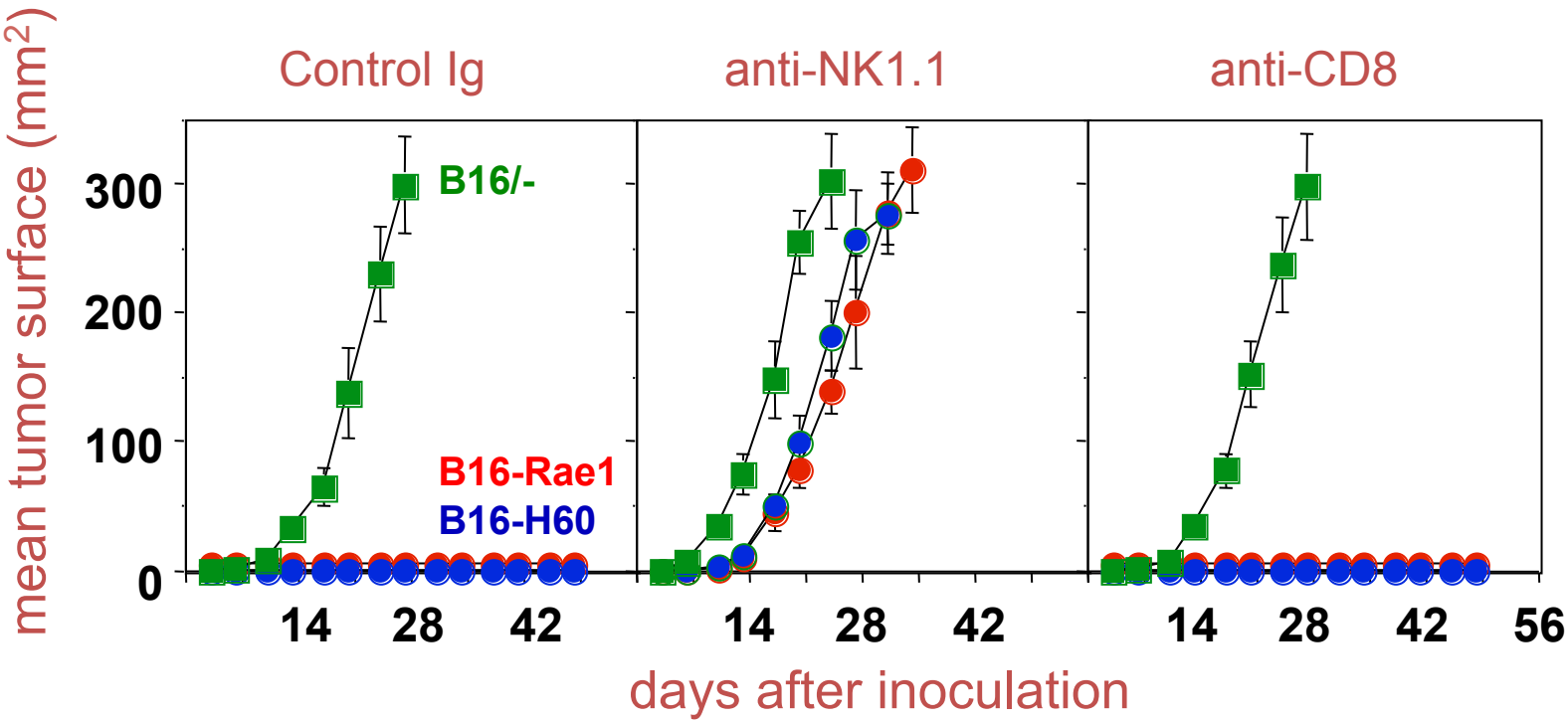
- They might be differentially upregulated in response to specific forms of cellular stress

- Organ-specific expression pattern

- Evolutionary race with viral evasion strategies

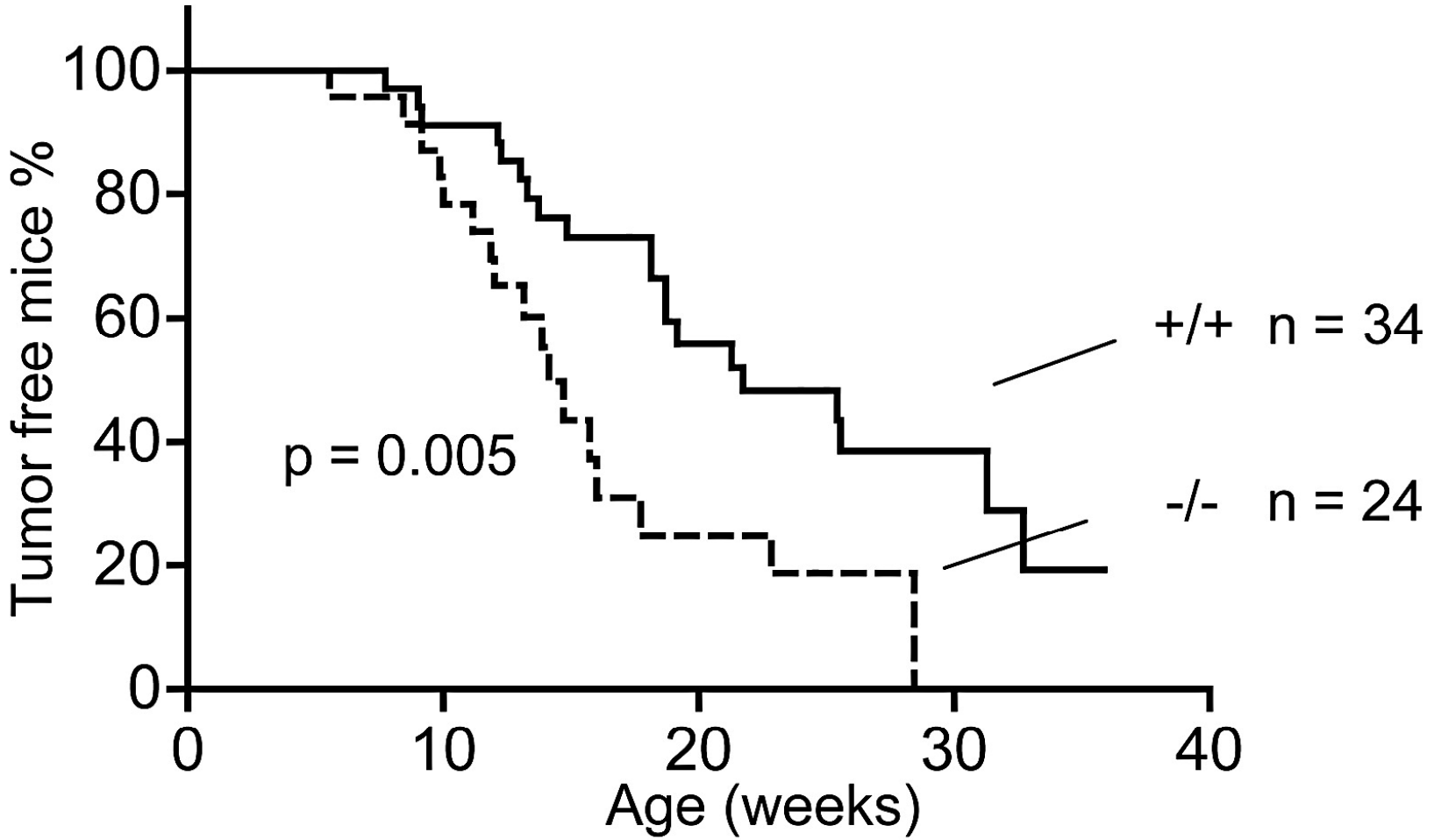
Slide: courtesy of A. Diefenbach

Altered-self recognition through the NKG2D receptor mediates tumor rejection



Diefenbach et al., *Nature* 2001

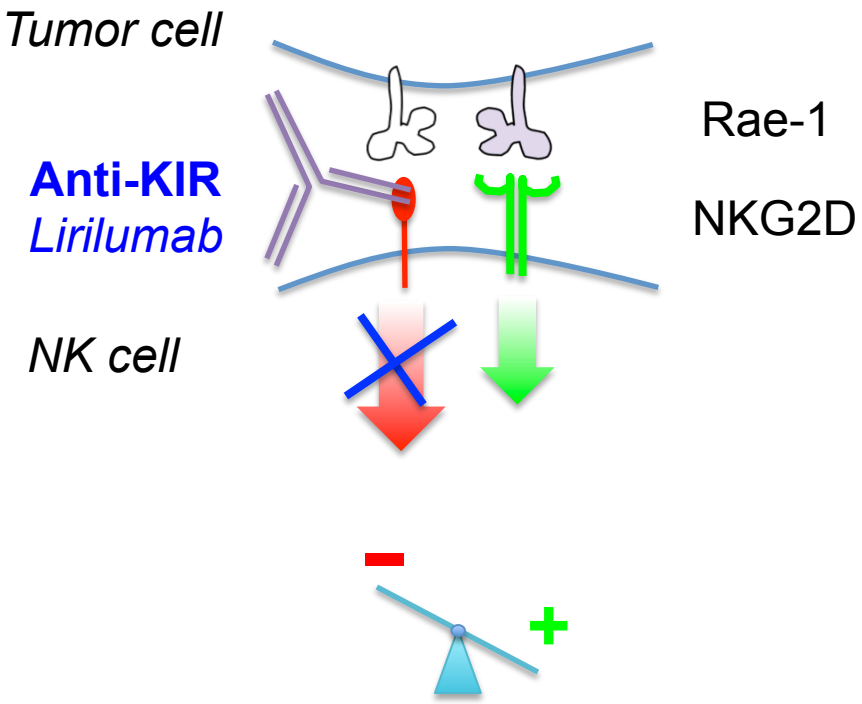
Mice genetically lacking the NKG2D receptor: increased incidence of spontaneously developing tumors



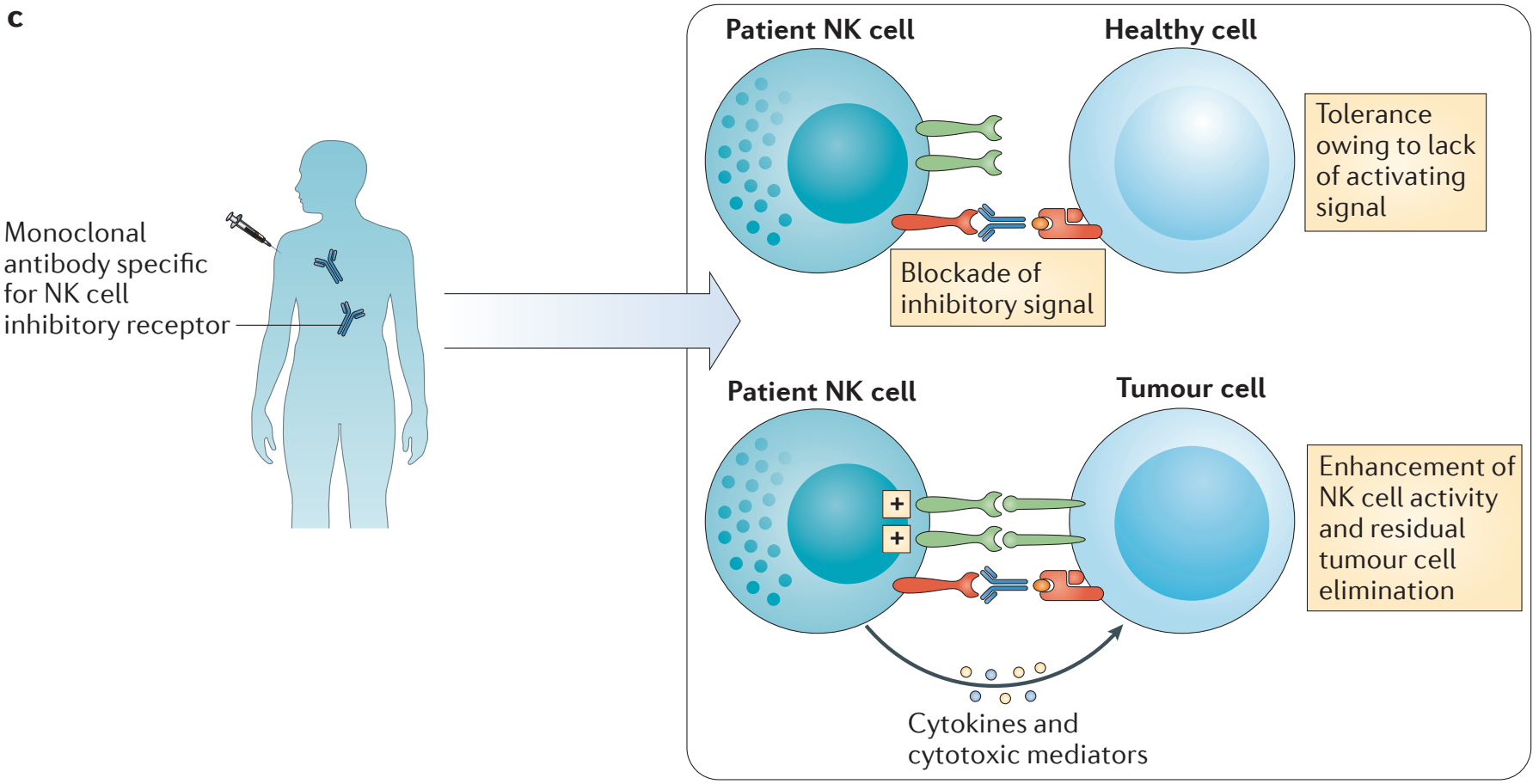
Guerra *et al*., Immunity 2008

„Missing-self“ in der Tumortherapie

Therapeutisch induziertes “missing-self”



Cancer therapies harnessing NK cell function



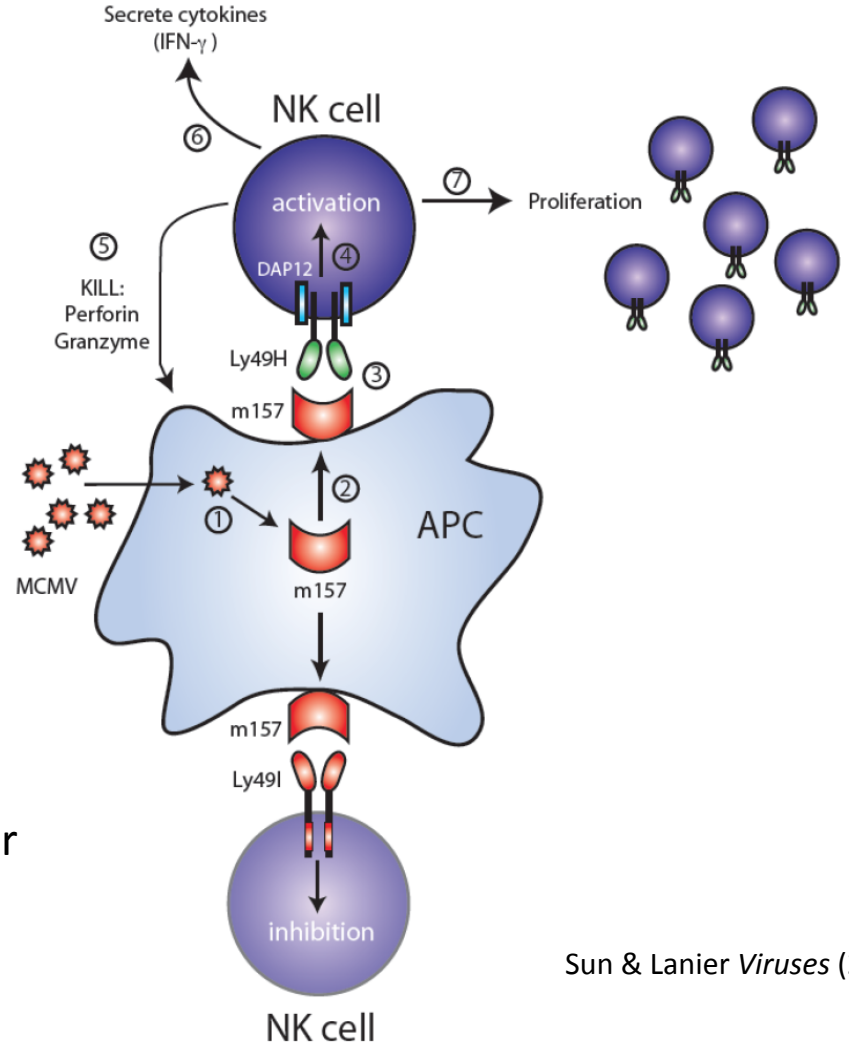
iKIR blockade = NK cell can't be inhibited.

(Vivier et al, Nat Rev Immunol, 2012)

„non-self recognition“: Murines Cytomegalievirus (mCMV)
Immunevasion: mCMV drosselt MHC I Expression und virales m157 imitiert MHC I Moleküle

C57BL/6: Aktivierender Rezeptor vermittelt Resistenz

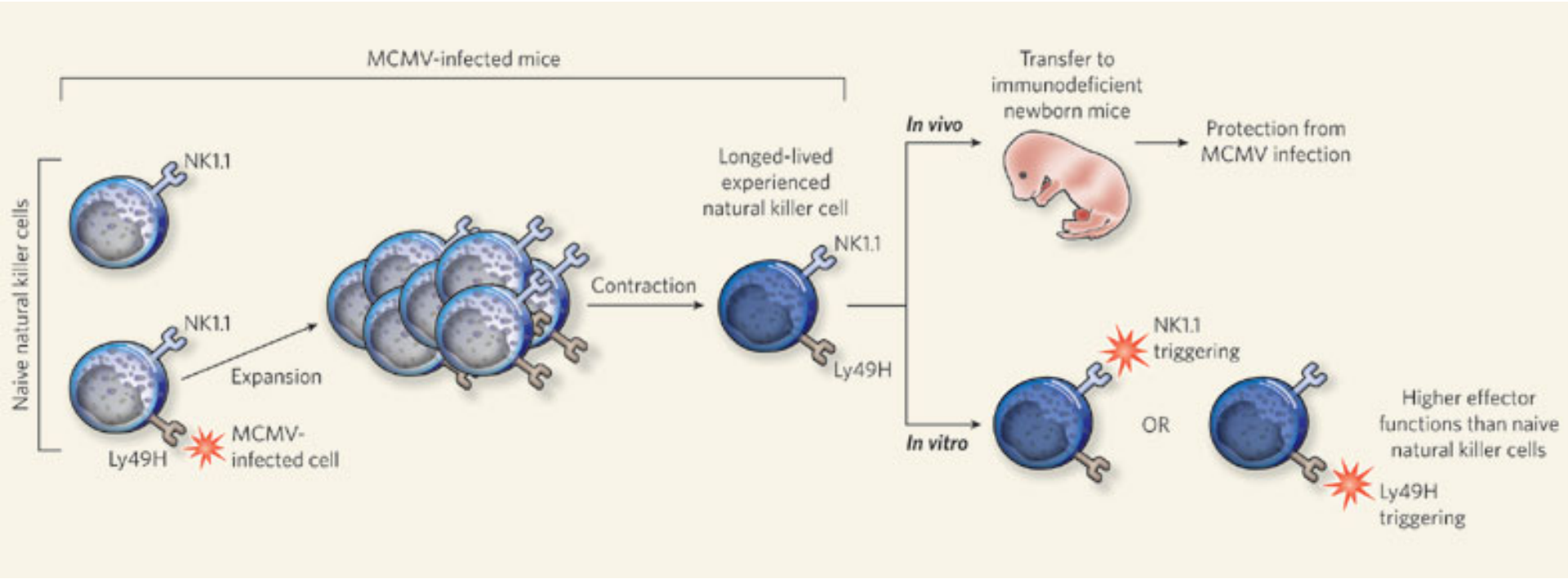
BALB/c: hemmender Rezeptor vermittelt Suszeptibilität



Sun & Lanier *Viruses* (2009)

Koevolution von Pathogen und Wirt:
Virus treibt die Evolution von Rezeptoren, Wirt selektiert auf virulentere Stämme

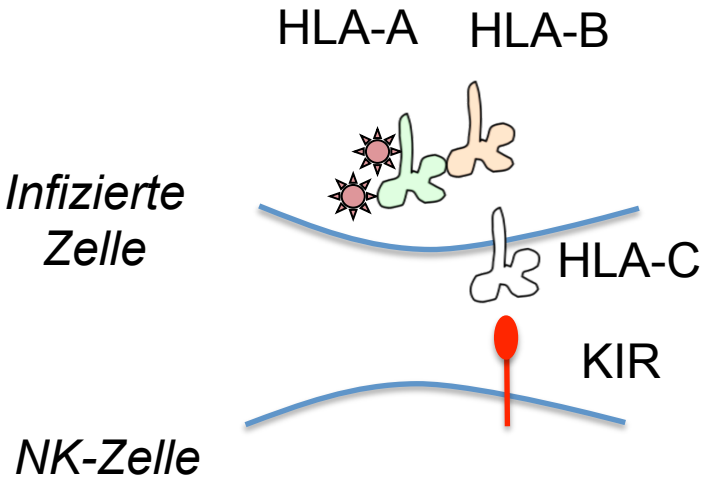
NK cells remember



Ugolini & Vivier, *Nature* 2009

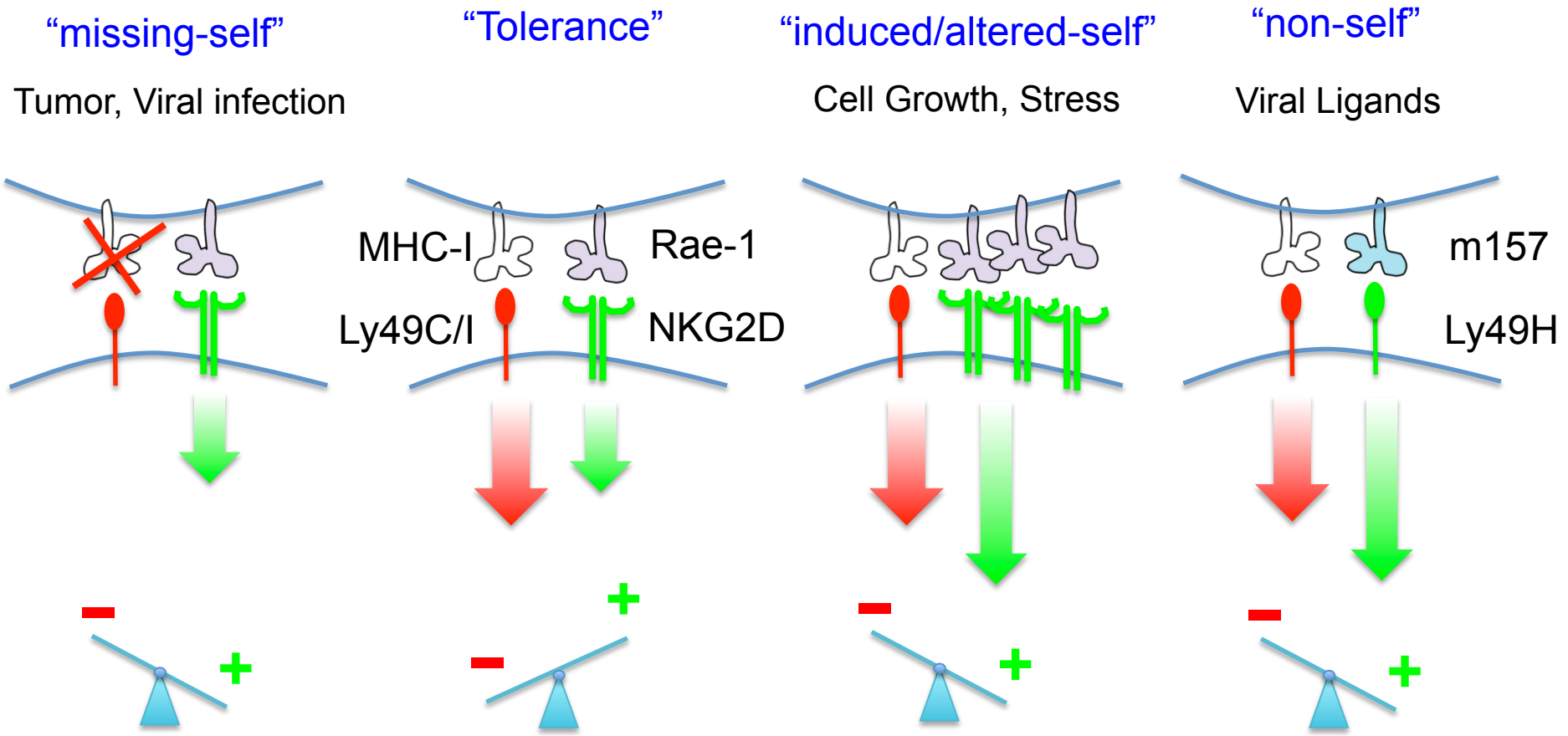
Weiteres Beispiel für Immunevasion von Viren: Das HIV Nef-Protein

HIV: Prävention der missing-self Erkennung durch NK-Zellen



HIV-nef supprimiert den Transport von HLA-A und -B (T-Zell-Evasion), weniger HLA–C (stark inhibitorisch für NK Zellen)

NK cell signal integration at the immunological synapse



Invariante NKT-Zellen

Gehören zu den T-Zellen und entwickeln sich aus DP Thymocyten im Thymus

Bilden TCR aus nur wenigen der möglichen Gensegmenten

Benötigen für ihre Entwicklung Interaktion des TCR mit CD1

Wandern aus Thymus in lymphat. Gewebe und Schleimhäute

CD1-Moleküle binden eine Vielzahl von Lipiden, Glycolipiden, Phospholipiden und Lipopeptide
in hydrophobem Kanal

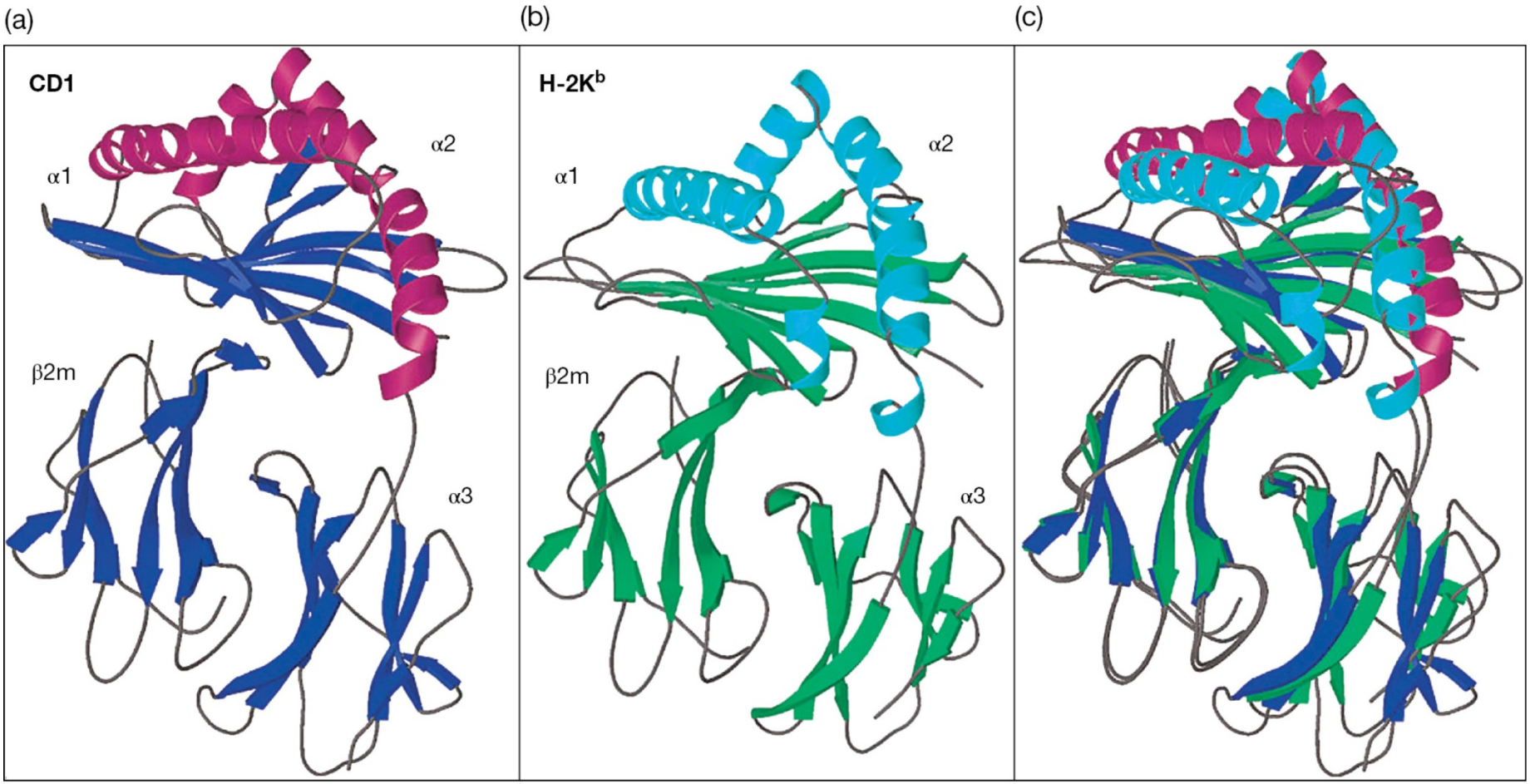


Figure 4.29 Comparison of the crystal structures of CD1 and MHC class I. (a) Backbone ribbon diagram of mouse CD1d1 (red, α-helices; blue, β-strands). (b) Ribbon diagram of the mouse MHC class I molecule H-2K^b (cyan, α-helices; green, β-strands). (c) Superposition using alignment of β_2-microglobulin highlights some of the differences between CD1d1 and H-2K^b. Note in particular the shifting of the α-helices. This produces a deeper and more voluminous groove in CD1d1, which is narrower at its entrance compared with H-2K^b. (Source: Porcelli S.A. *et al.* (1998) *Immunology Today* **19**, 362. Reproduced with permission of Elsevier.)

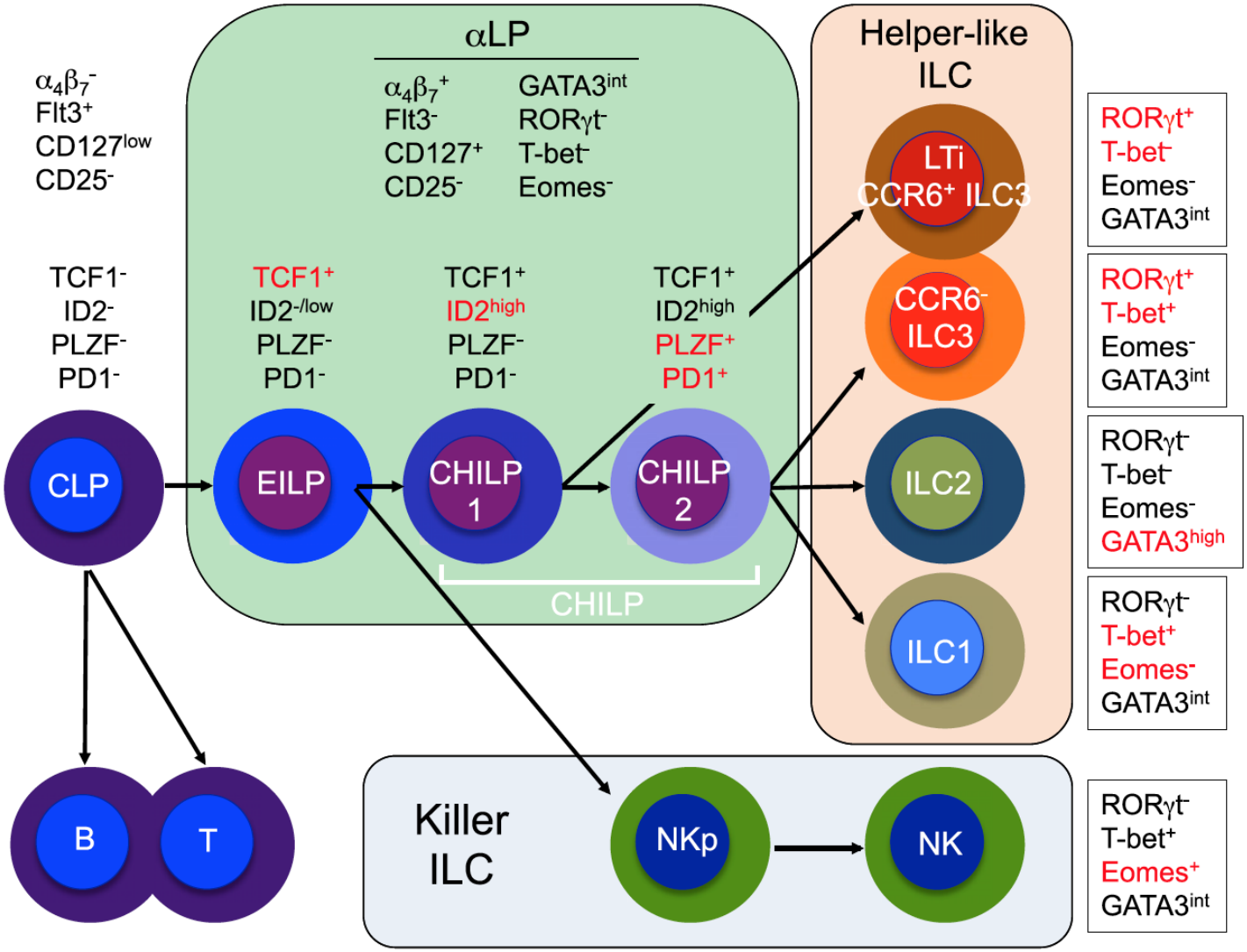
Roitt's Essential Immunology, Thirteenth Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, and Ivan M. Roitt.

Companion website: www.roitt.com

WILEY Blackwell

Innate Lymphoid Cells

ILC bestehen aus Zellen mit cytotoxischen und T-Helfer-ähnlichen Eigenschaften

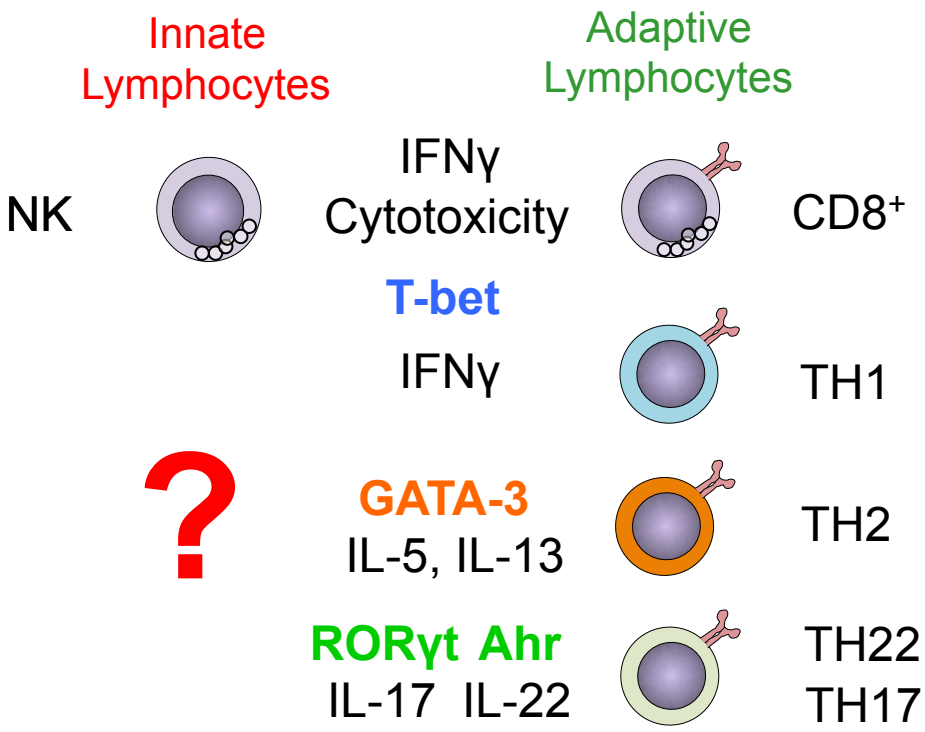


CLP: common lymphoid progenitor
EILP: early ILC progenitor

CHILP: common helper-like progenitor
LTi: lymphoid tissue inducer

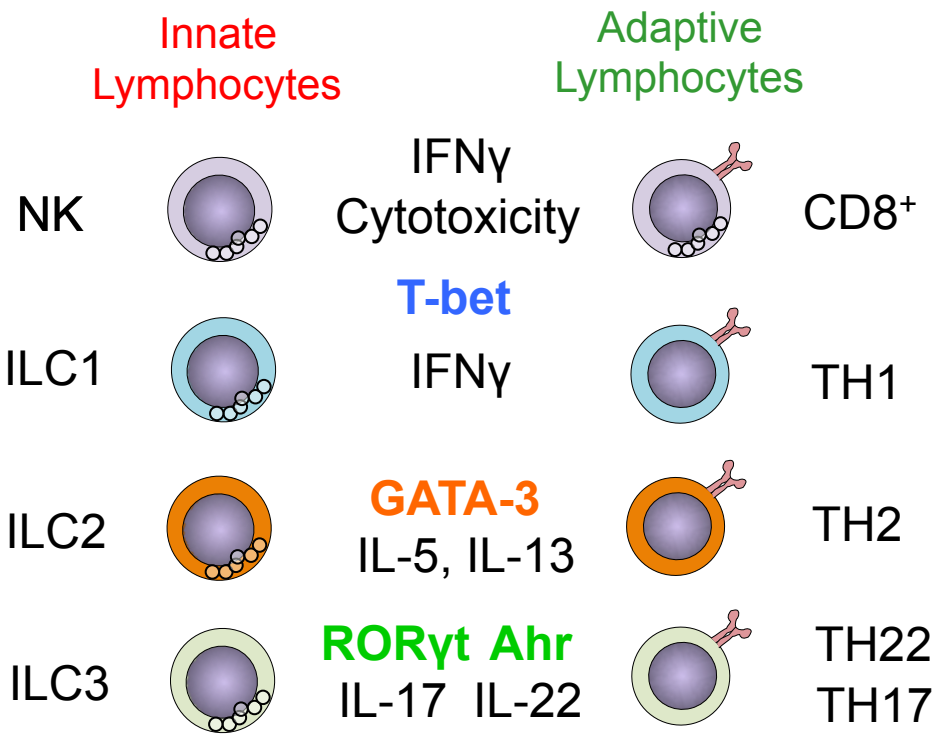
Diefenbach et al. (2017) *Immunity*

Spezialisierung nur bei adaptiven Lymphozyten ?



> Detektion von Helfer-Zytokinen in T-Zell-Defizienten RAG^{KO} Mäusen

Innate Lymphoid Cells (ILC)



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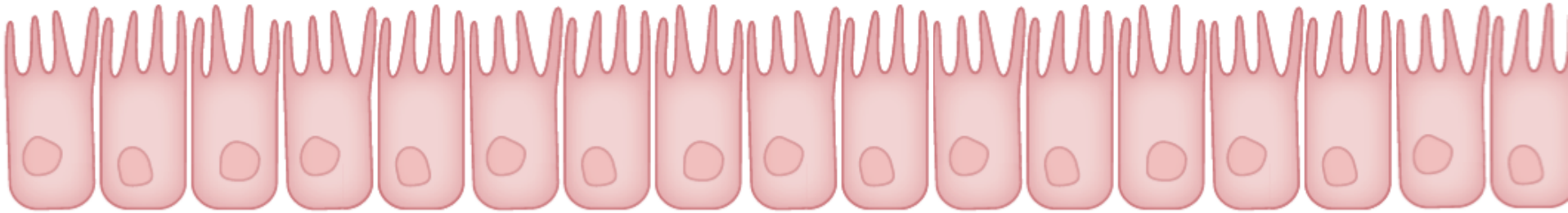
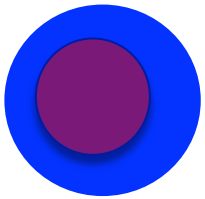


ILC1

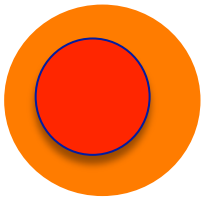


T-bet

IFN-γ, TNF

- Intracellular infections
- Inflammation?

ILC2

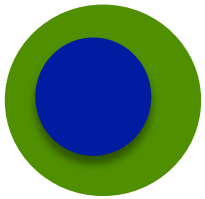


GATA-3
RORα

IL-5, IL-13
amphiregulin

- Worm infections
- Asthma, allergies

ILC3



RORγt

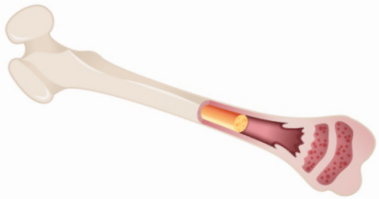
IL-22, IL-17A,
GM-CSF

- Intestinal infections
- Inflammatory bowel diseases

Type 1 immunity **Type 2 immunity** **Type 17 immunity**

Slide: courtesy of A. Diefenbach

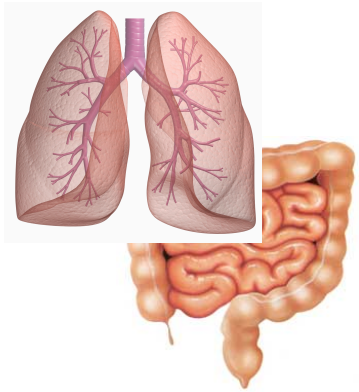
ILCs sind lokale, gewebe-residente Lymphozyten



ILC progenitor

peripheral blood

Embryonic and postnatal developmental window?



local ILC progenitor

immature ILC

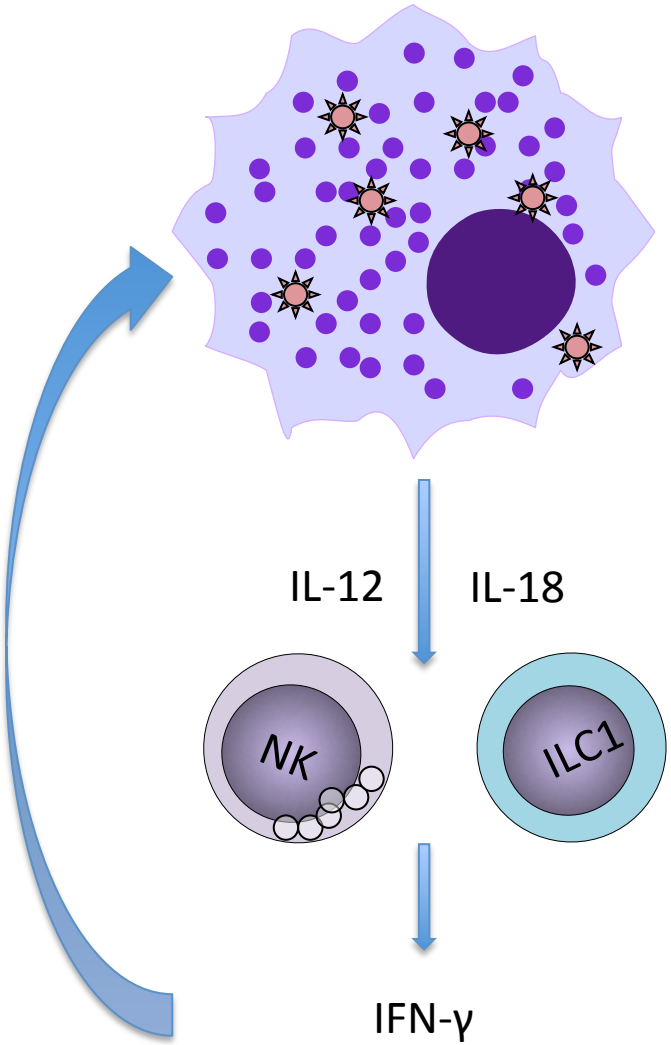
mature ILC

Gasteiger et al., Science 2015

Wichtige Eigenschaften der ILC

- Keine Antigen-spezifischen Rezeptoren
- Lokalisiert an Grenzen zur Außenwelt: Schleimhäute, Haut
- Werden durch Signale (Cytokine, Alarmine) von „Sensor-Zellen“ aktiviert
- Produzieren Cytokine in der Frühphase von Immunreaktionen
- Können lokal proliferieren

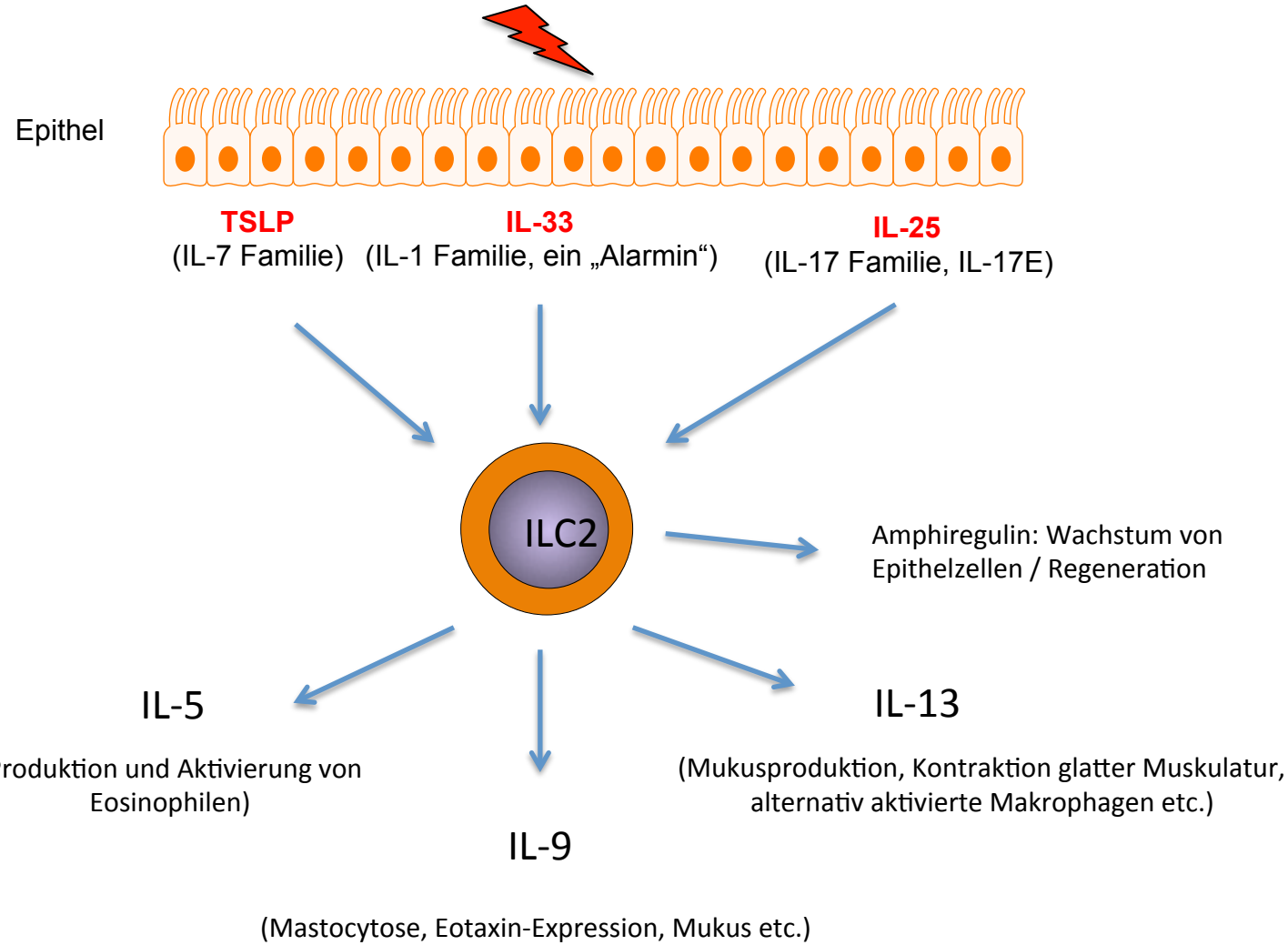
ILC1 (und NK-Zellen) produzieren IFN-γ nach Aktivierung durch infizierte DC und Makrophagen



Makrophagenaktivierung lange bevor Th1-Hilfe möglich ist.
Prinzipiell auch Einfluß auf Th1-Entwicklung

ILC2 werden durch Cytokine und Alarmine von Epithelzellen aktiviert

Helminthen, Pilze (Chitin !), Viren (Influenza), Allergene (Proteasen !)



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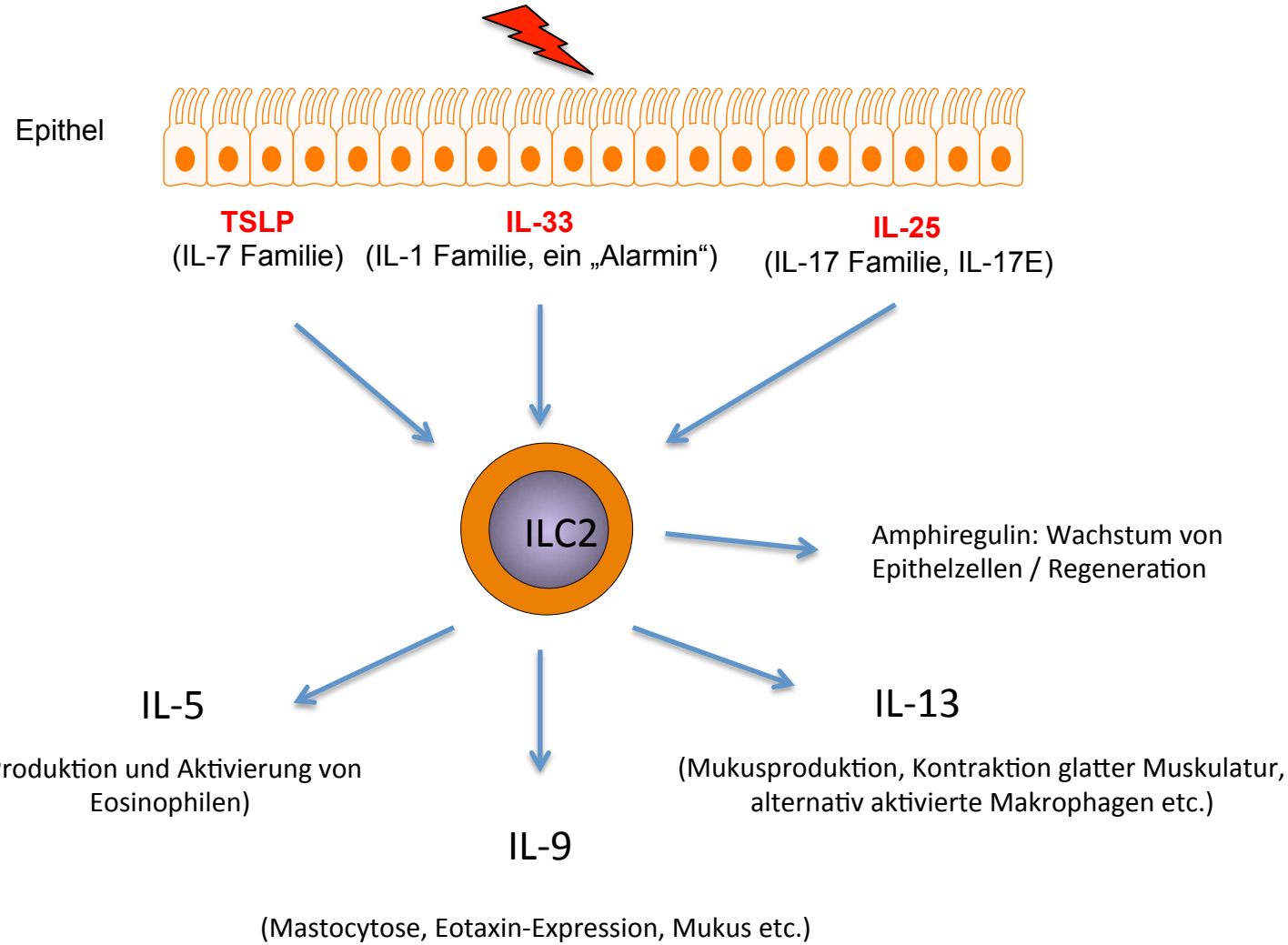
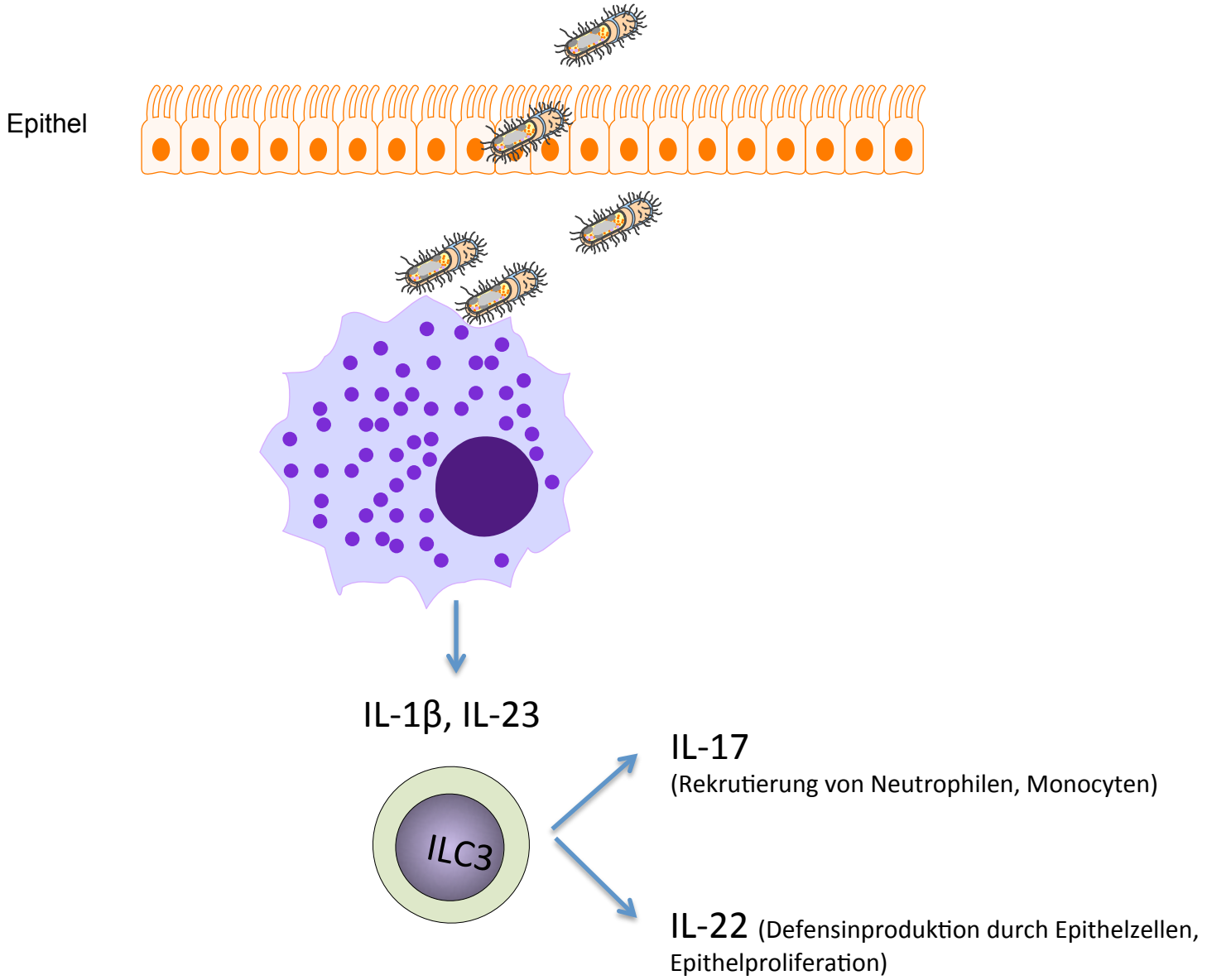


Infektionen mit extrazellulären Bakterien und Pilzen aktivieren ILC3



It appears that each of the three major ILC and effector CD4 T-cell subsets (ILC1/ILC2/ILC3 and Th1/Th2/Th17) evolved to enhance and coordinate the functions.....of different arms of the myelomonocytic pathway for optimal eradication of different classes of pathogens:

monocytes and macrophages are enhanced by Th1 cells;
eosinophils, basophils and mast cells by Th2 cells;
and neutrophils by Th17 cells

Janeway's Immunobiology, 9th Edition