FOCiS Advanced Course
in Basic & Clinical Immunology

Innate Lymphocytes

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Scientific Advisory Boards 2024

Innate Lymphoid Cells (ILC)

*”Lineage-negative” Id2-dependent cells arising from a common lymphoid precursor

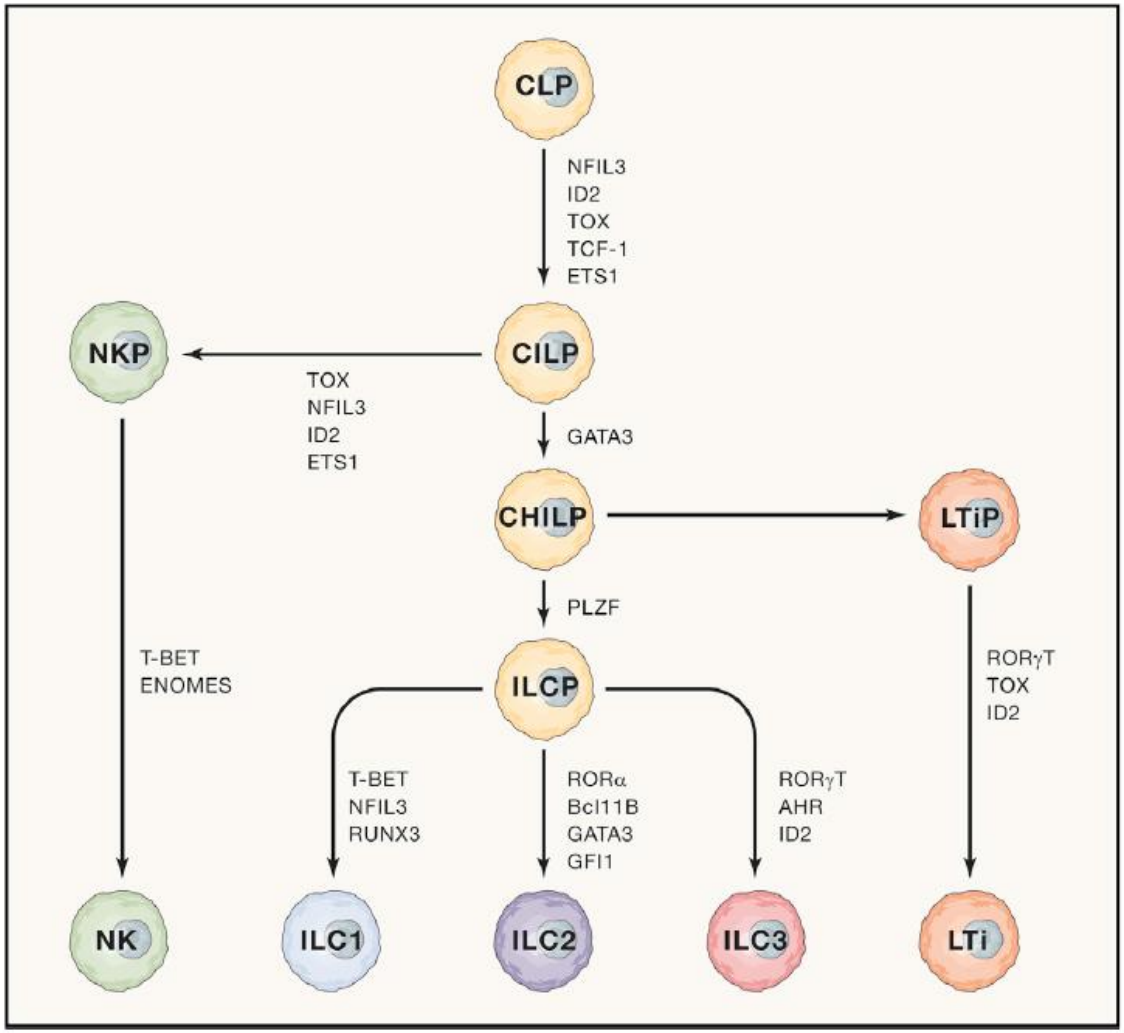
*Some mediate lymph node organogenesis during fetal development - “Lti” – lymphoid-tissue inducers

*Some become tissue-resident effector cells expressing cytokines

*Roles in homeostasis - establishing commensals, responding to dietary signals, responding to circadian cues, etc.

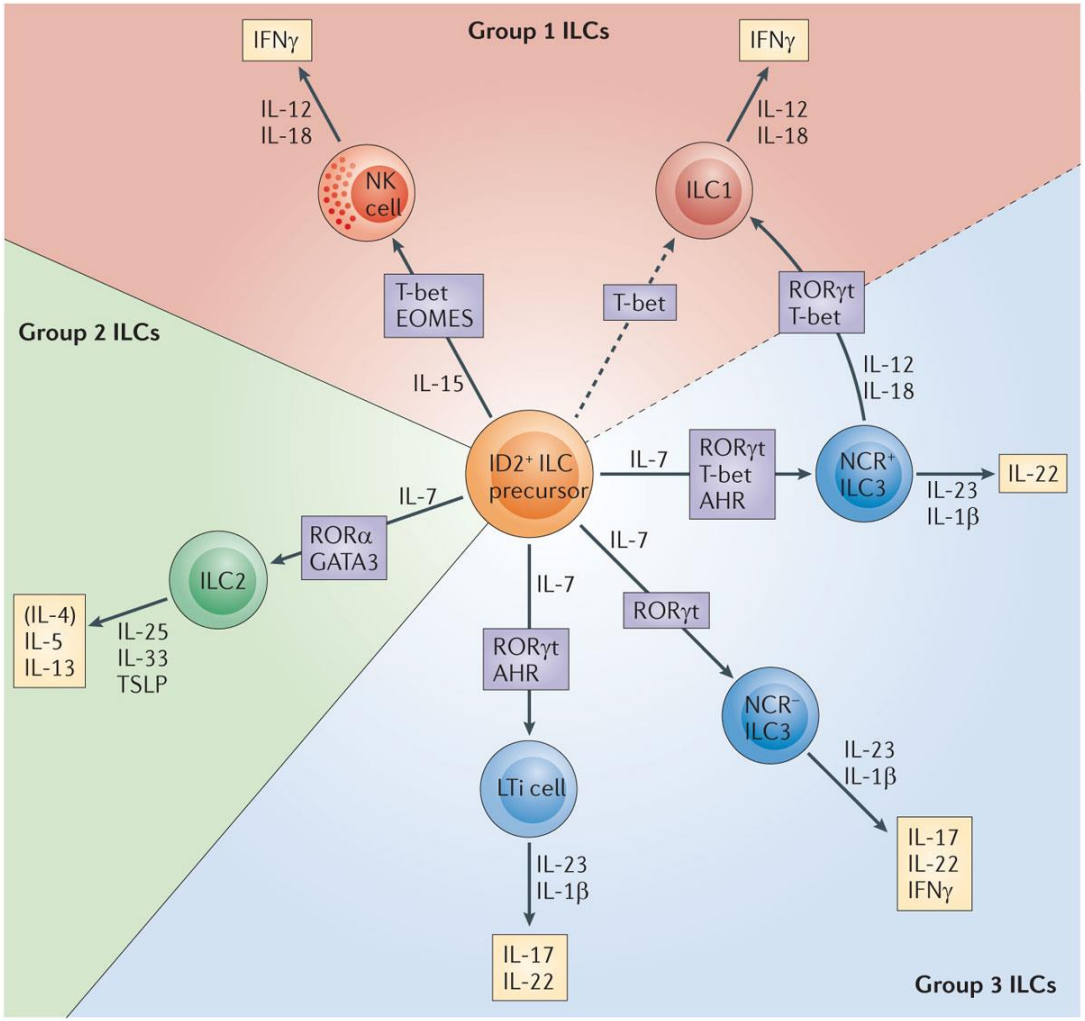
*Roles in inflammation and disease

Development of NK cells and ILC



Vivier et al. Cell 2018

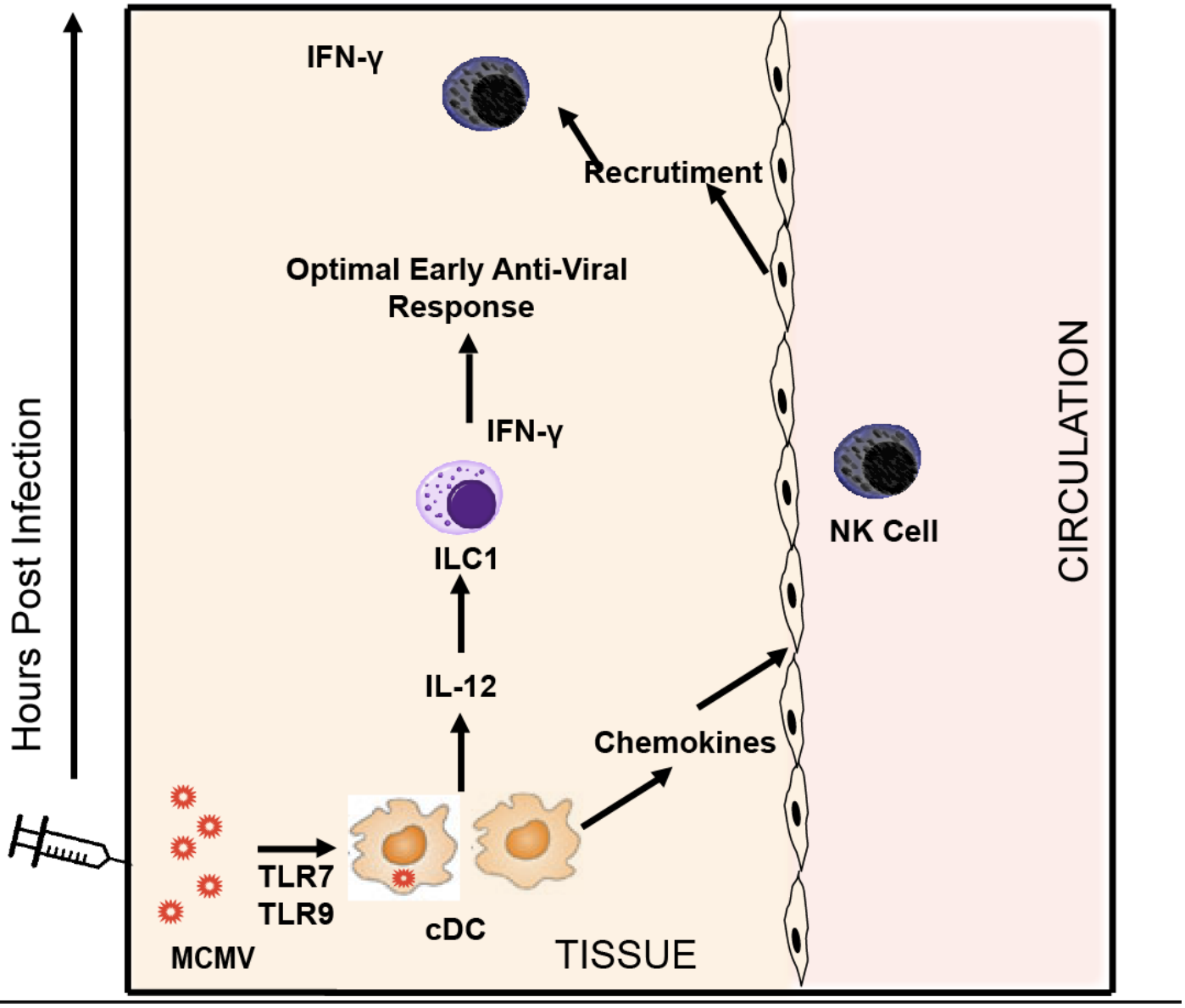
INNATE LYMPHOID CELLS



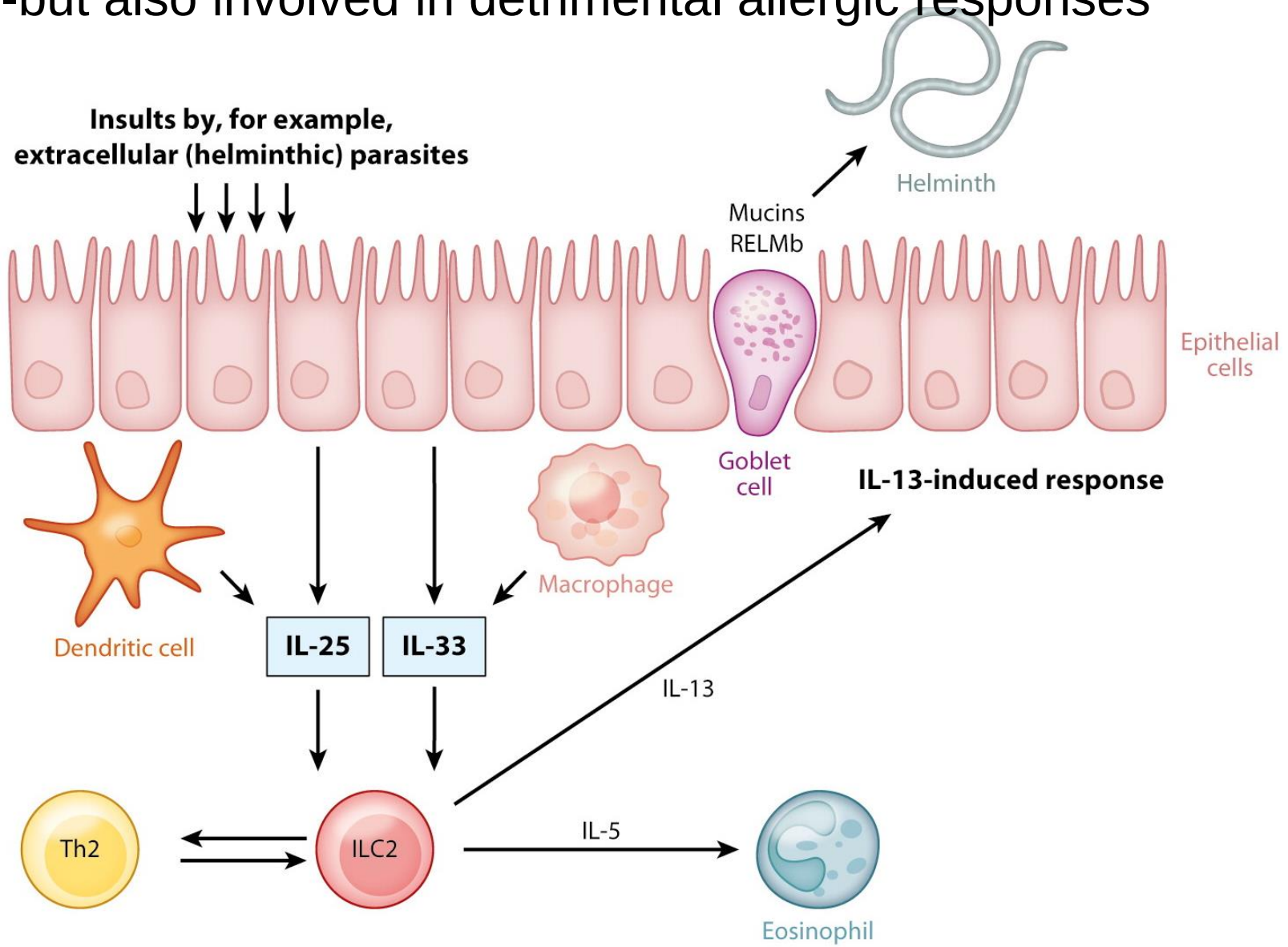
Nature Reviews | Immunology

Spits 2013

ILC1 restrict early viral replication in tissues



ILC2 –protect against parasites and help wound repair -but also involved in detrimental allergic responses

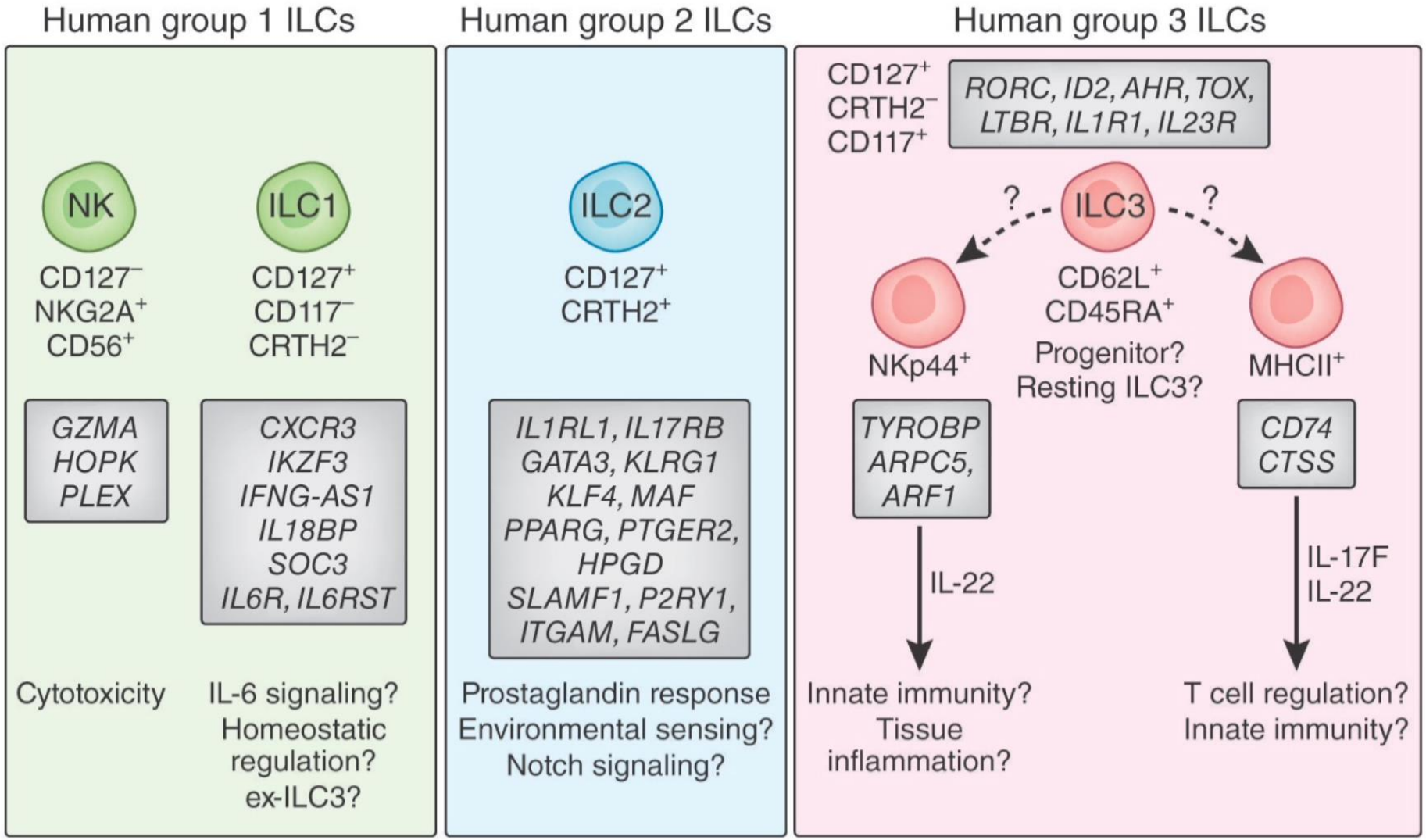


Spits & Cupedo Ann Rev Imm 2012

The heterogeneity of human CD127+ innate lymphoid cells revealed by single-cell RNA sequencing

nature immunology

Åsa K Björklund[1–3,6], Marianne Forkel[4,6], Simone Picelli[1], Viktoria Konya[4], Jakob Theorell[4], Danielle Friberg[5], Rickard Sandberg[1,2] & Jenny Mjösberg[4]



ILCs - the innate counterparts of T cells

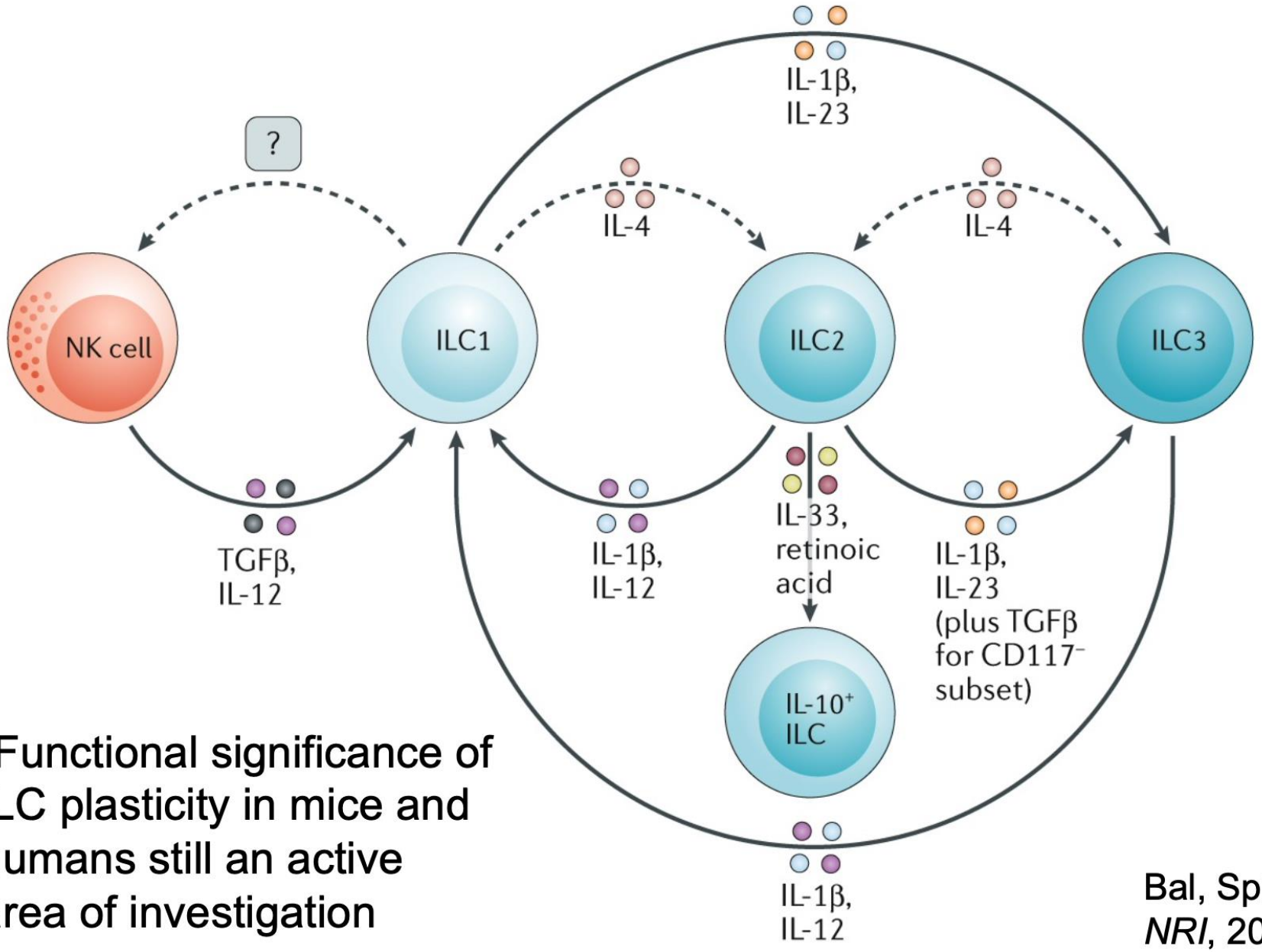
NK cells…..CD8+ T cells

ILC1…..Th1 cells

ILC2…..Th2 cells

ILC3…..Th17 cells

Human ILC subsets may exhibit substantial plasticity



-Functional significance of ILC plasticity in mice and humans still an active area of investigation

Bal, Spits *et al.* *NRI*, 2020

Class Discussion

What is the purpose of this redundancy?

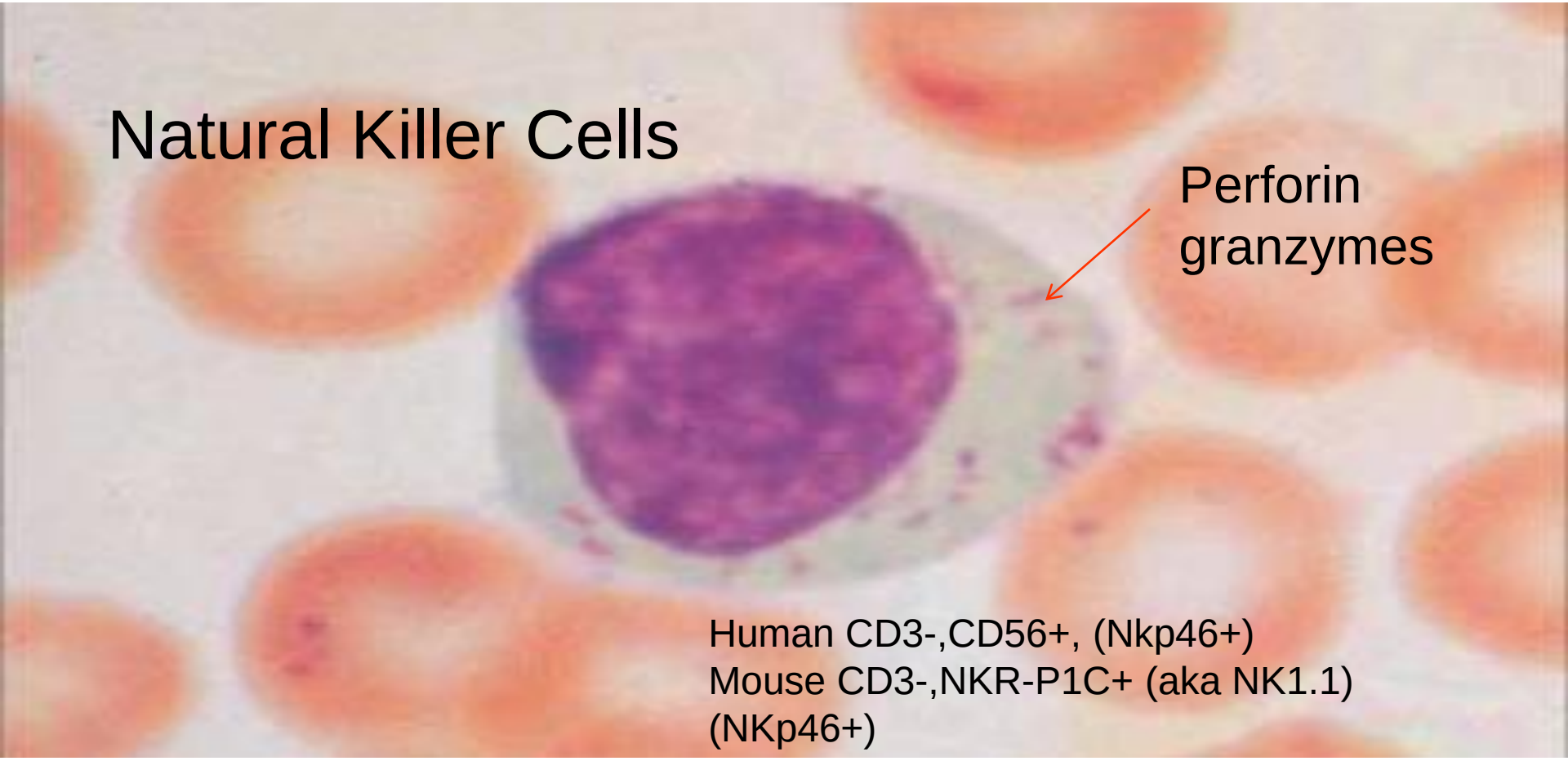
If Th cells exist, why also have ILC?

What is the purpose of this redundancy?

If Th cells exist, why also have ILC?

1. Speed
2. Location

Natural Killer Cells

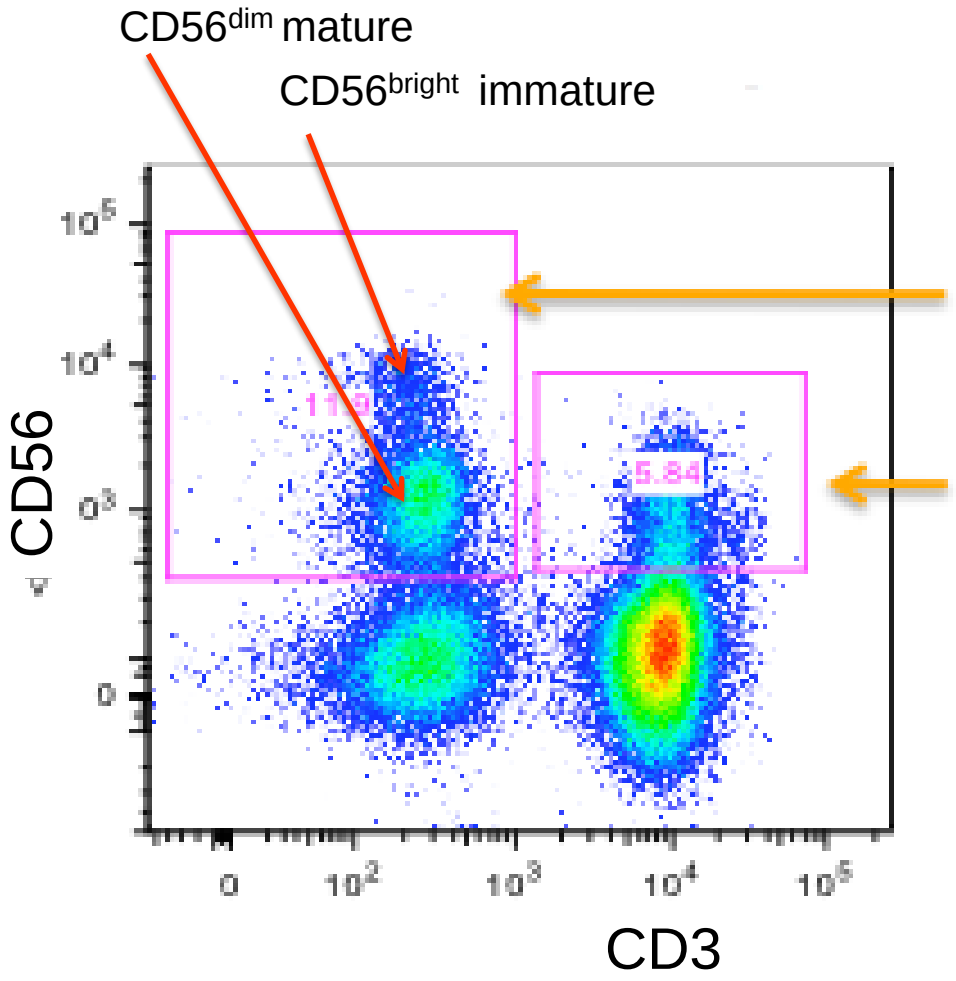


3rd lineage of lymphocytes

Function in innate immunity to protect against viruses, bacteria, parasites, fungi, & tumors

Produce cytokines & kill abnormal cells

Detecting NK cells in human peripheral blood



CD3-CD56+ NK cells

CD3+CD56+ T cells

(these are NOT “NKT” cells”)

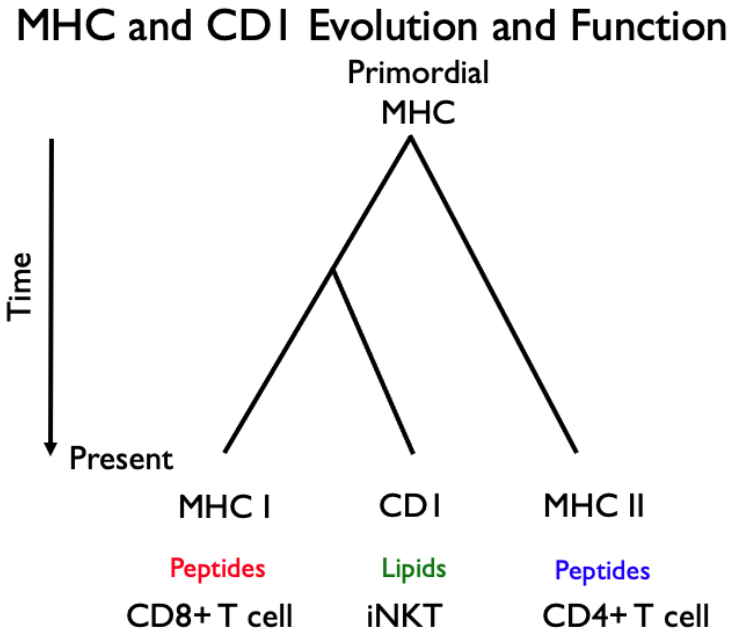
iNKT cells

T cells express an invariant TcRα chain

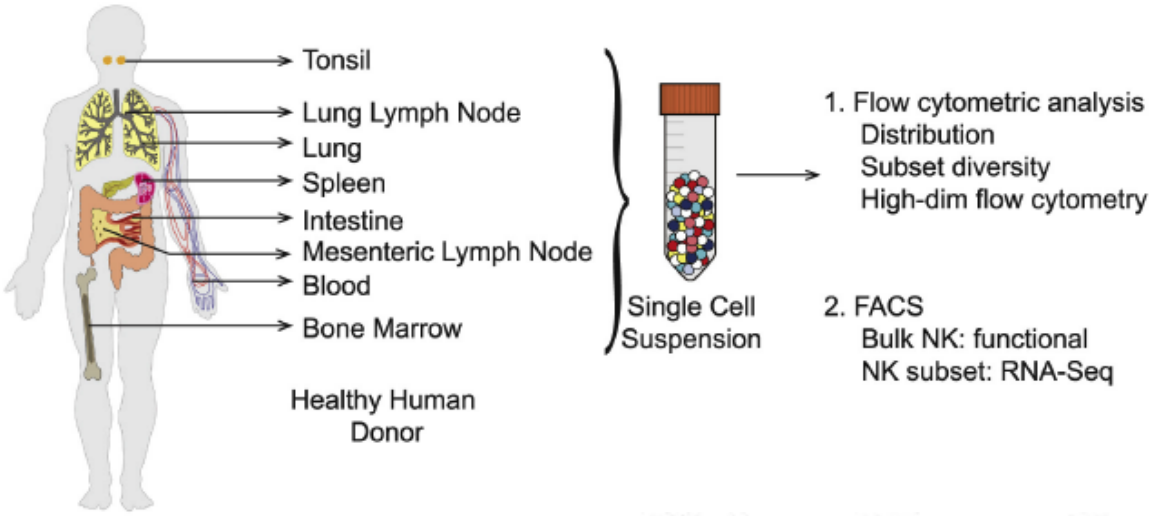
Recognize lipid antigens presented by CD1d

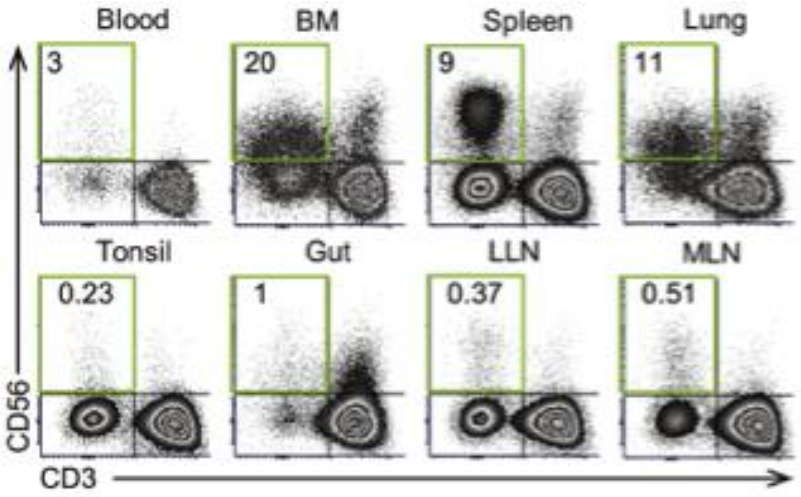
*In humans - invariant Va24 + Ja18 TcRα

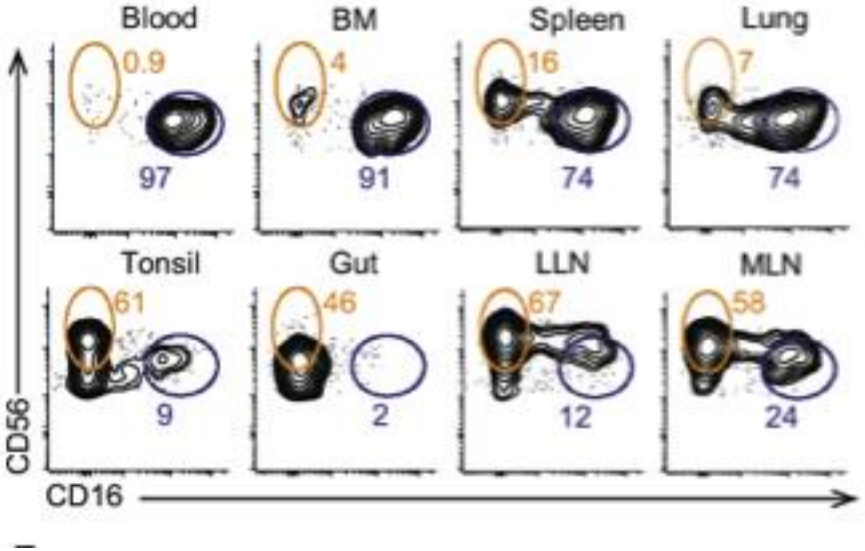
*In mice - invariant Va14 + Ja18 TcRα



Human NK Cells - Where do they live?







Dogra et al. Cell 2020

NK Cells - What do they do?

Cell mediated-cytotoxicity – “natural killing”

Antibody-dependent cellular cytotoxicity
(kill antibody-coated cells via activating Fc receptor CD16)

Early γ-interferon production

Secrete TNFα, LTα, GM-CSF, IL-3, M-CSF, IL-10, MIP-1α, MIP-1β, RANTES, etc.

(but <u>NOT</u> IL-2, IL-4, IL-17, or IL-22 – these are ILC not NK)

Immune regulatory role of NK cells

*Kill cells that are proliferating too much

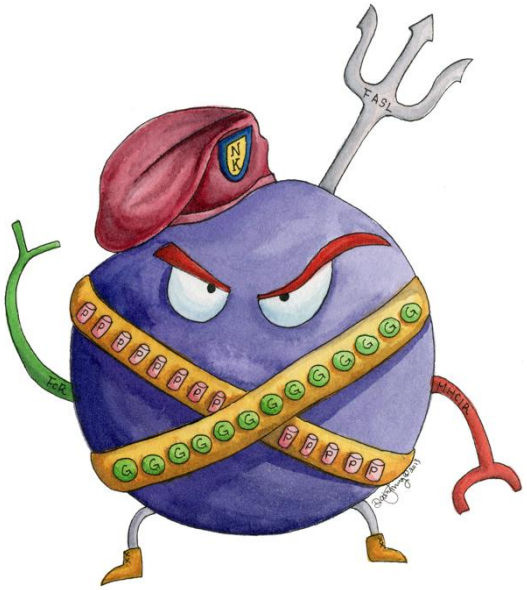
*Kill T cells causing autoimmunity

*Secrete pro-inflammatory cytokine – IFNγ

*Secrete suppressive cytokines – IL-10

Class Discussion

What do you think would happen if you had no NK cell?



New England Journal of Medicine 1989

Vol. 320 No. 26 MEDICAL INTELLIG

MEDICAL INTELLIGENCE

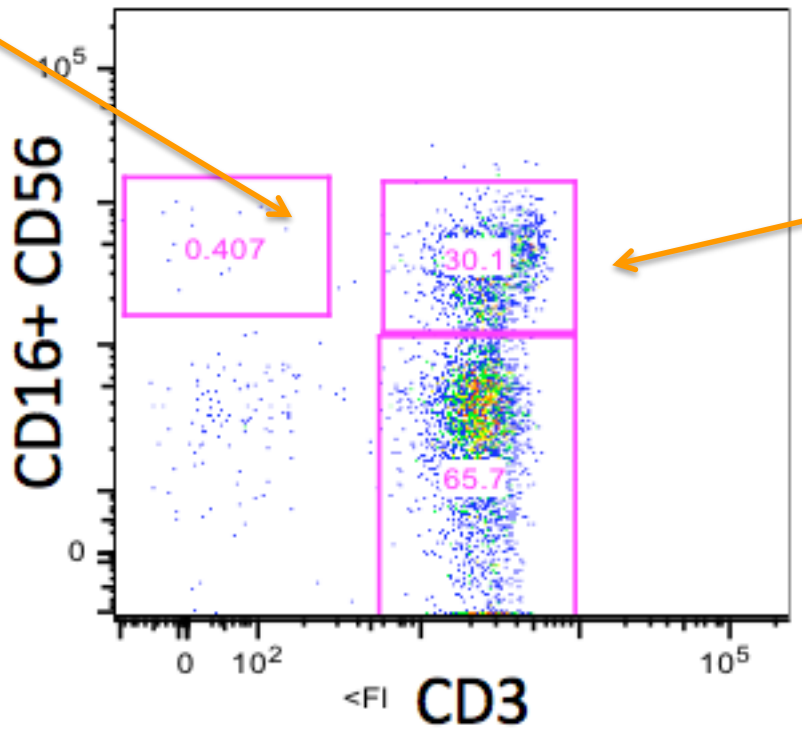
SEVERE HERPESVIRUS INFECTIONS IN AN ADOLESCENT WITHOUT NATURAL KILLER CELLS

CHRISTINE A. BIRON, PH.D., KEVIN S. BYRON, AND JOHN L. SULLIVAN, M.D.

Caused by loss-of-function mutation in one allele of *GATA2*

NK cell-deficient patient

$CD3^{-}CD56^{+}$ NK cells



$CD3^{+}CD56^{+}$ T cells

caused by heterozygous loss of *GATA2* – can't control warts (HPV)

Physiological role of NK cells is to protect against viral infections and cancer

Humans lacking NK cells are particularly susceptible to:

- Epstein-Barr Virus Fleisher, J. Pediatrics 1982

- Cytomegalovirus and other herpesviruses Biron, NEJM 1989

- Papillomavirus (cervical cancer) and Herpes Simplex Virus
  Ballas, J. Allergy Clinical Immuno 1991

- Varicella Zoster Virus Etzioni, J. Peditrics 2005

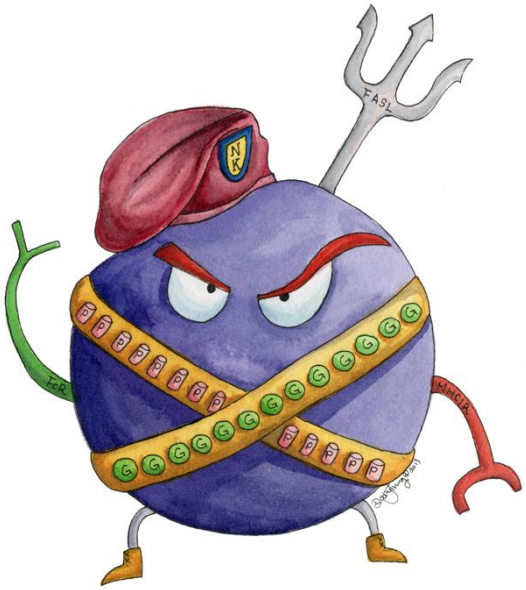
The NEW ENGLAND JOURNAL of MEDICINE 2021

BRIEF REPORT

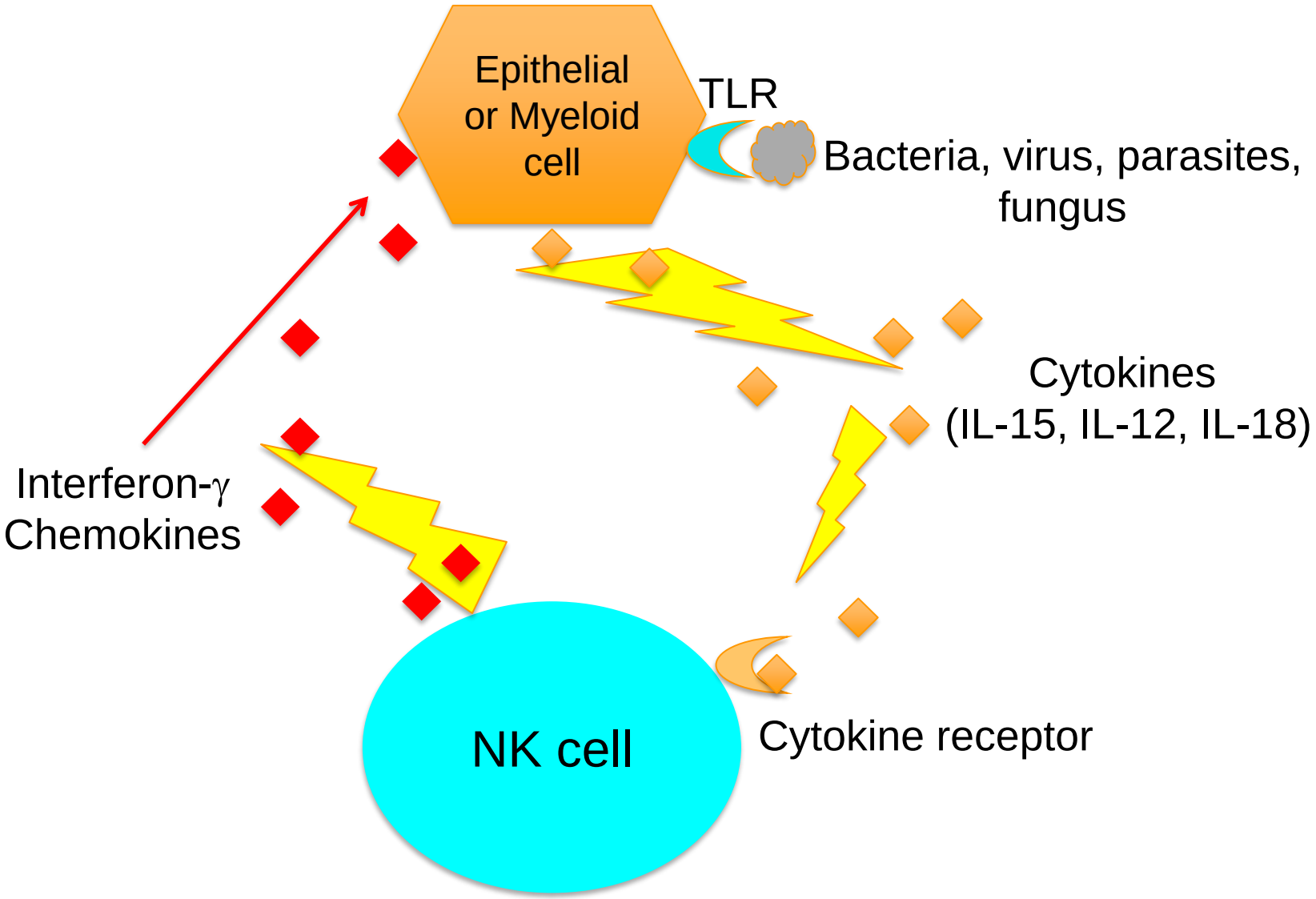
Treatment of Relapsing HPV Diseases by Restored Function of Natural Killer Cells

Andrea Lisco, M.D., Ph.D., Amy P. Hsu, B.S., Dimana Dimitrova, M.D., Diana M. Proctor, Ph.D., Emily M. Mace, Ph.D., Peiying Ye, Ph.D., Megan V. Anderson, R.N., B.A., Stephanie N. Hicks, R.N., B.S.N., Christopher Grivas, B.S., Dima A. Hammoud, M.D., Maura Manion, M.D., Gabriel J. Starrett, Ph.D., Alvin Farrel, Ph.D., Kerry Dobbs, M.S., Isaac Brownell, M.D., Ph.D., Christopher Buck, Ph.D., Luigi D. Notarangelo, M.D., Jordan S. Orange, M.D., Ph.D., Warren J. Leonard, M.D., Michael I. Orestes, M.D., Anju T. Peters, M.D., Jennifer A. Kanakry, M.D., Julia A. Segre, Ph.D., Heidi H. Kong, M.D., and Irini Sereti, M.D., M.H.S.

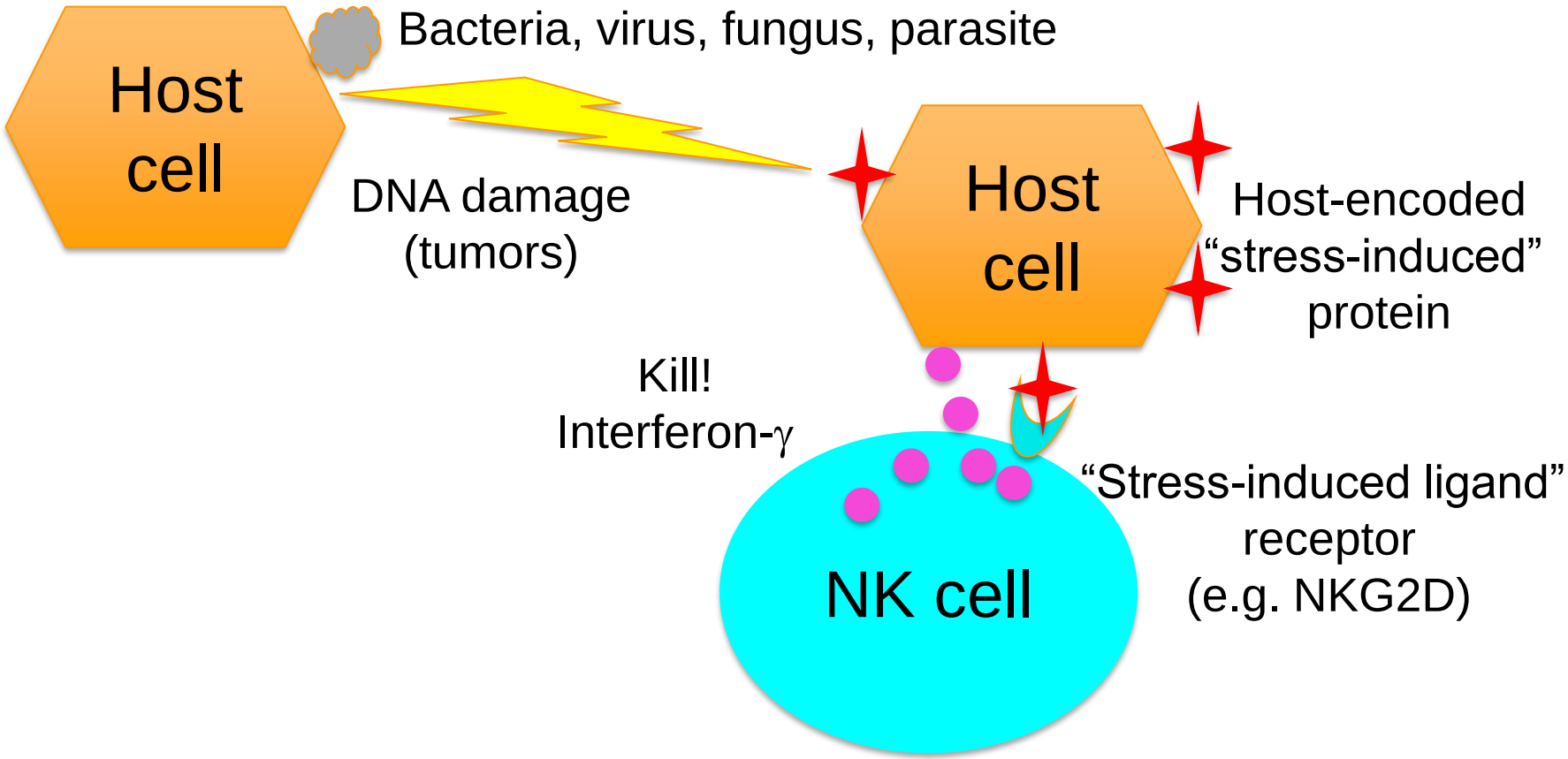
How do NK cells sense their environment?



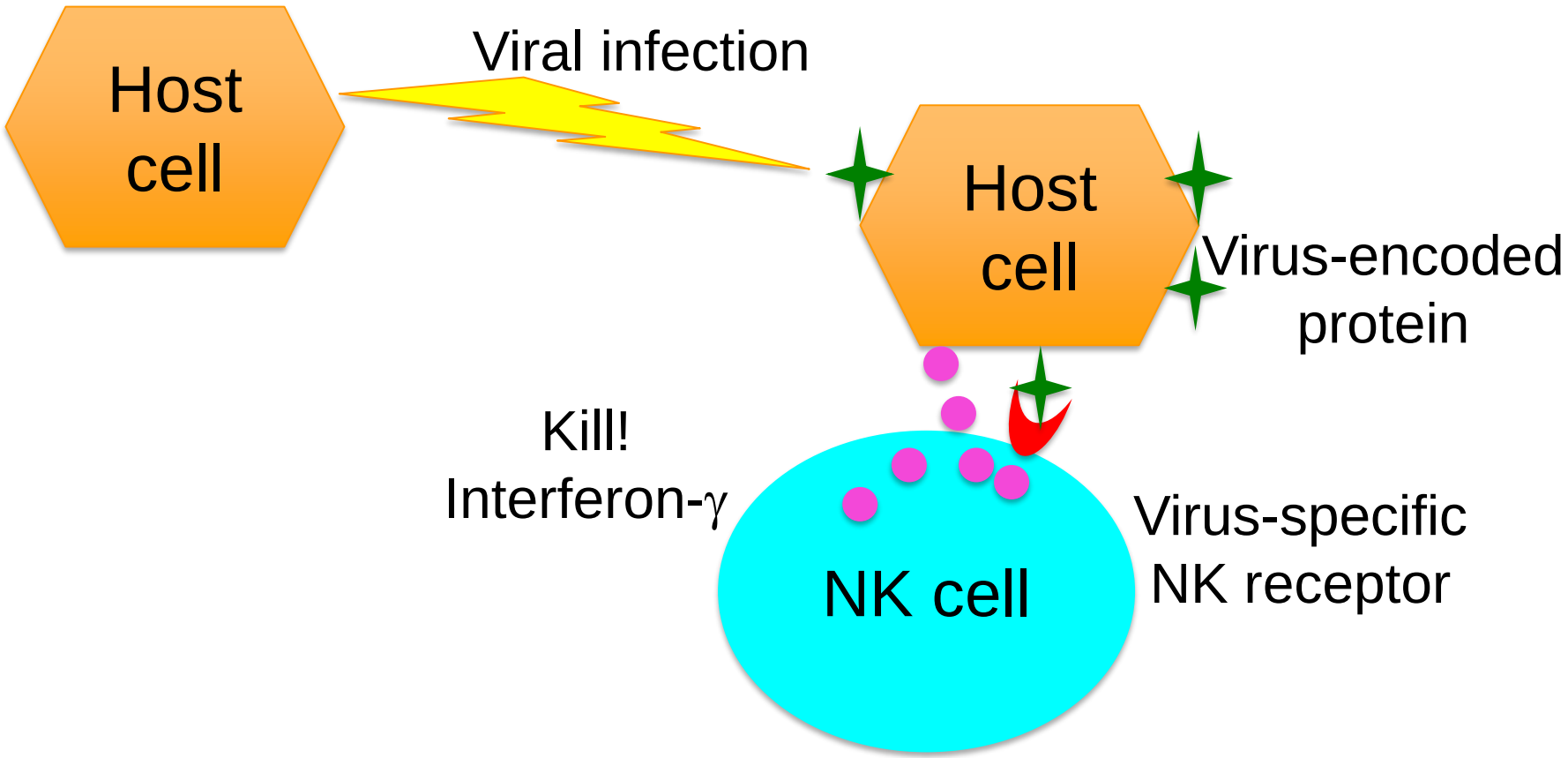
Cytokines produced by infected epithelial or myeloid cells



“Stressed” cells – upregulate host-encoded ligands for activating NK receptors

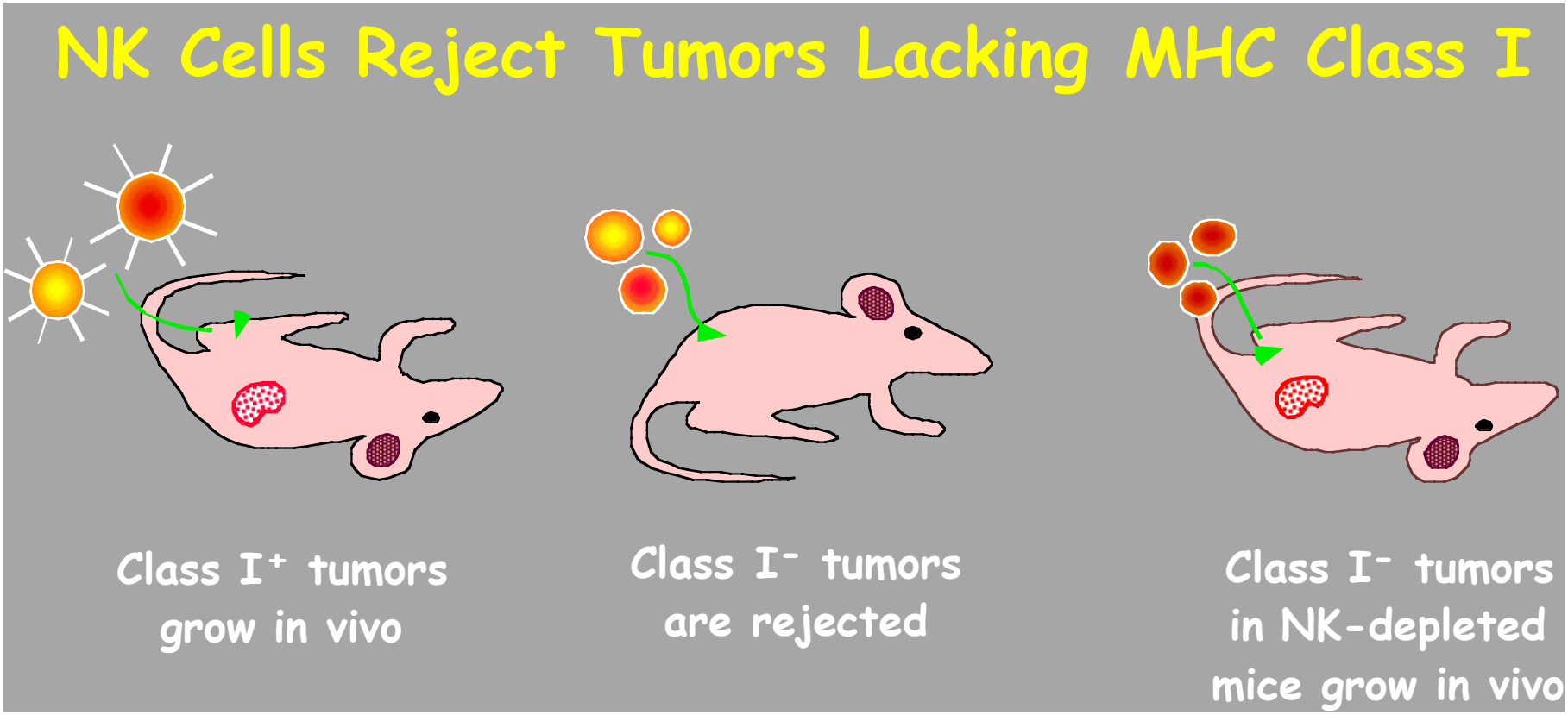


Infected cells express virus-encoded ligands for activating NK receptors



How are NK cell responses regulated?

NK cells like to kill cells lacking MHC class I – “missing-self”

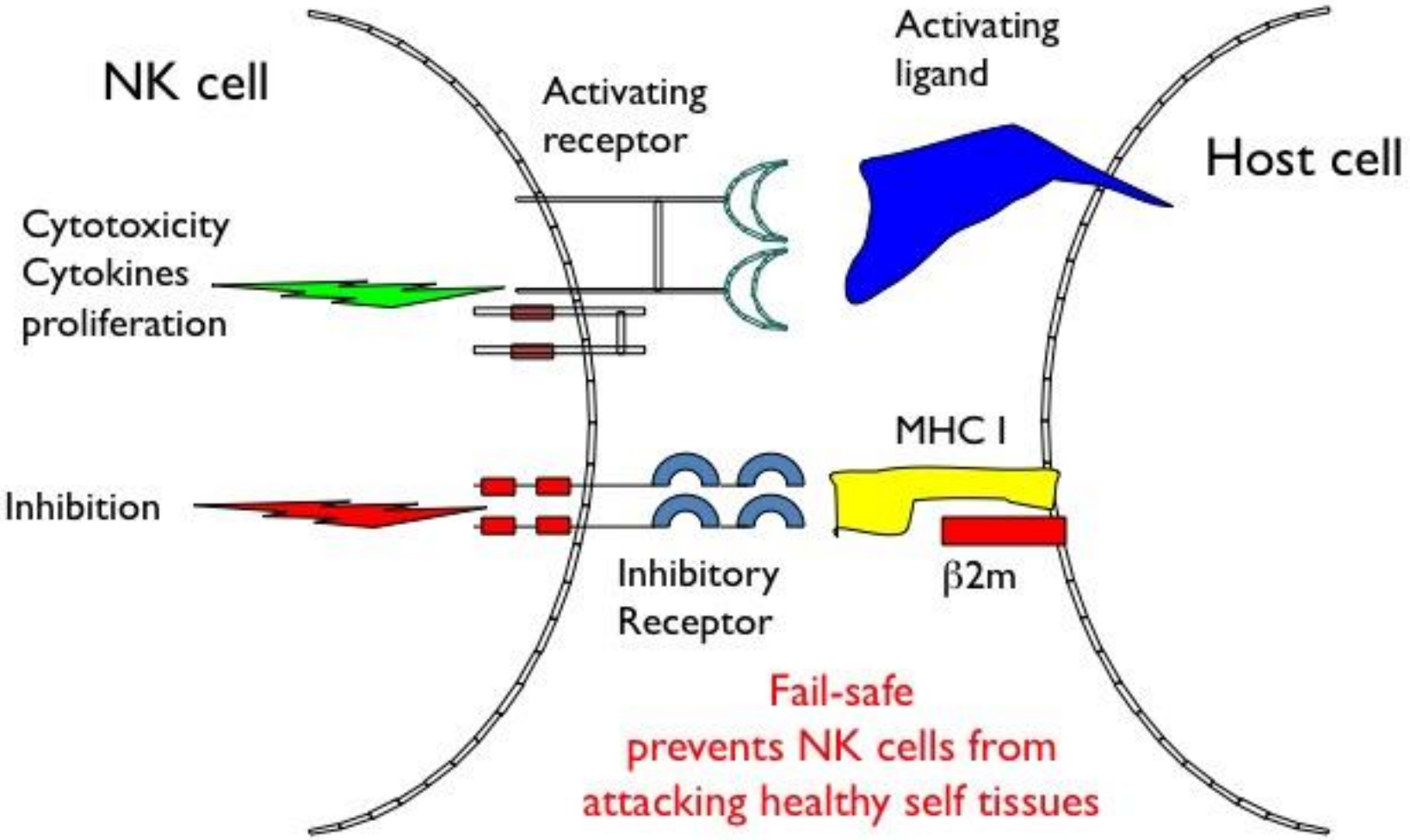


Karre *et al. 1986* Nature 319:675

“Missing-self” MHC on a cell is not sufficient for an NK cell to attack

NK cells require activating receptors to detect ligands on the target cell to initiate a response

NK cell functions are controlled by a balance of inhibitory and activating receptors



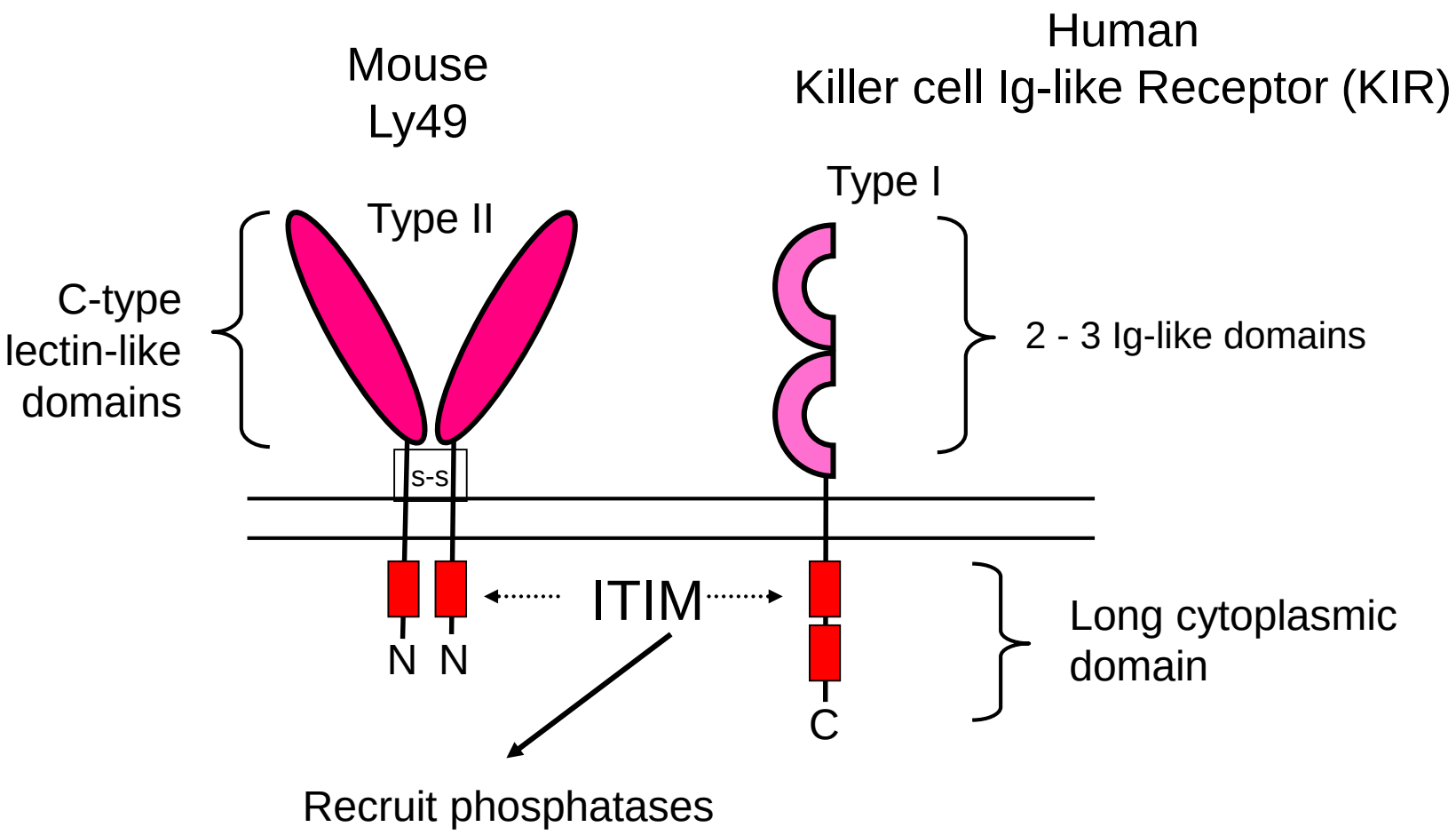
Physiological role for NK cell inhibitory receptors for MHC class I- detection of virus-infected or cancer cells?

Virus	Protein	Effect on class I
Adenovirus	E3-k19	Retain in ER
HSV-1,2	ICP47	Blocks TAP
EBV	EBNA1	Block peptide generation
HCMV	US2, US11	ER to cytosol
HCMV	US3	Retain in ER
HCMV	US6	Blocks TAP
HCMV	US10	Degrades HLA-G
MCMV	m152	Retain in ER
MCMV	m04	Associates with H-2
MCMV	m06	Lysosomal degradation
HHV8	K3, K5	Endocytosis
HIV-1	Nef	Endocytosis

37% of human melanomas have lost HLA class I

Rodig *Sci Trans Med* 2018 10:eaar3342

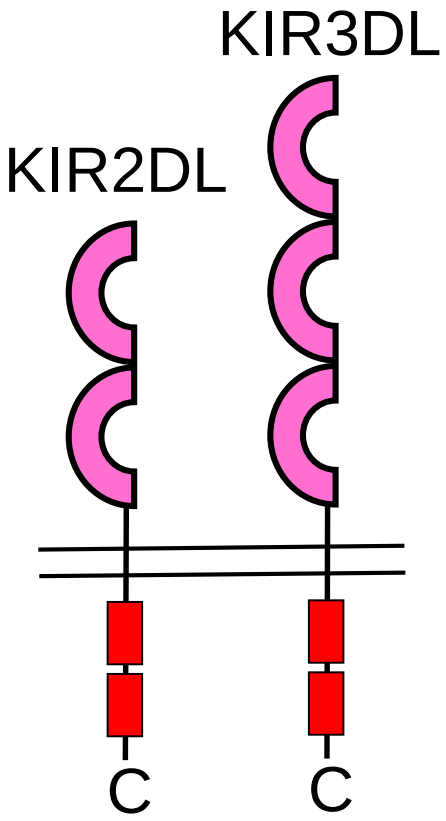
Structural differences between MHC class I-specific inhibitory receptors in mice and humans



Class Discussion

Why would primates need to evolve a new systemto recognize MHC?

HLA specificities of human KIRs



KIR2DL1 : HLA-C2 allotypes
(Cw2, 4, 5, 6=Lys80)

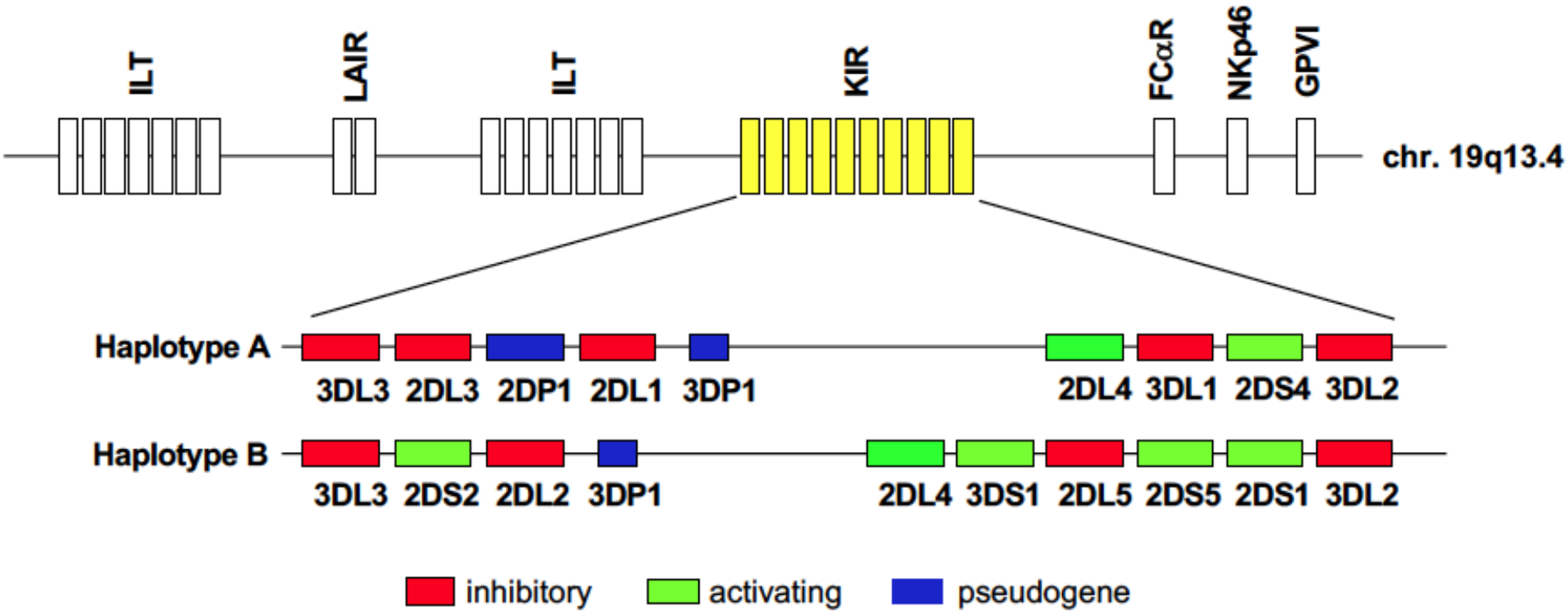
KIR2DL2 & KIR2DL3 : HLA-C1 allotypes
(Cw1, 3, 7, 8=Asn80)

KIR3DL1 : HLA-Bw4

KIR3DL2: HLA-A3

Lanier, *Ann Rev Imm* 2005

Different people have different KIR genes



KIR genes are highly polymorphic!

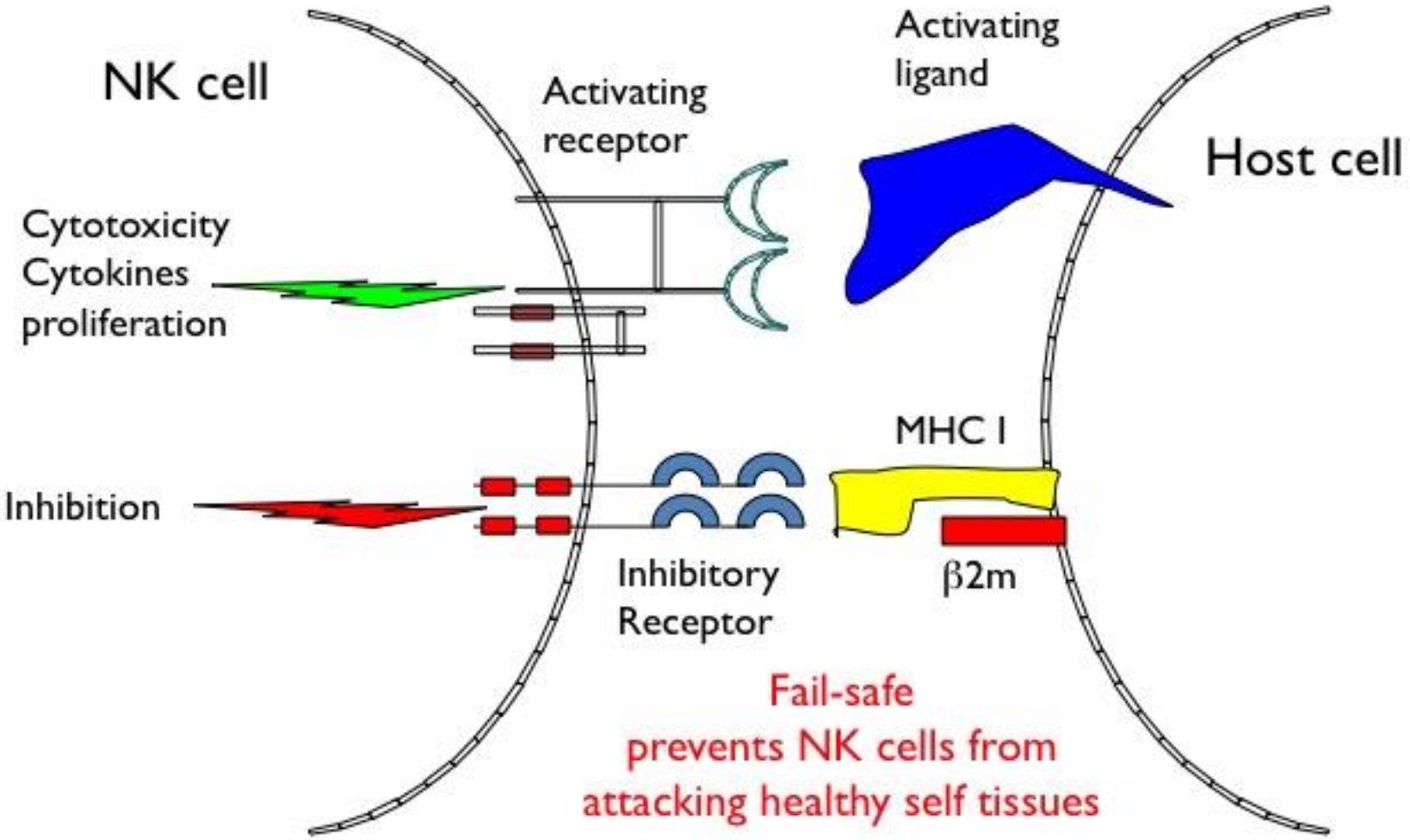
KIR Alleles									
Gene	***2DL1***	***2DL2***	***2DL3***	***2DL4***	***2DL5A***	***2DL5B***	***2DS1***	***2DS2***	***2DS3***
Alleles	428	35	68	114	45	47	33	65	71
Proteins	126	16	36	59	20	21	12	22	23
Nulls	9	0	1	0	1	0	0	0	2

Gene	***2DS4***	***2DS5***	***3DL1***	***3DL2***	***3DL3***	***3DS1***	***2DP1***	***3DP1***
Alleles	41	88	307	252	232	91	114	188
Proteins	22	38	138	141	116	37	0	0
Nulls	22	0	5	4	1	2	0	0

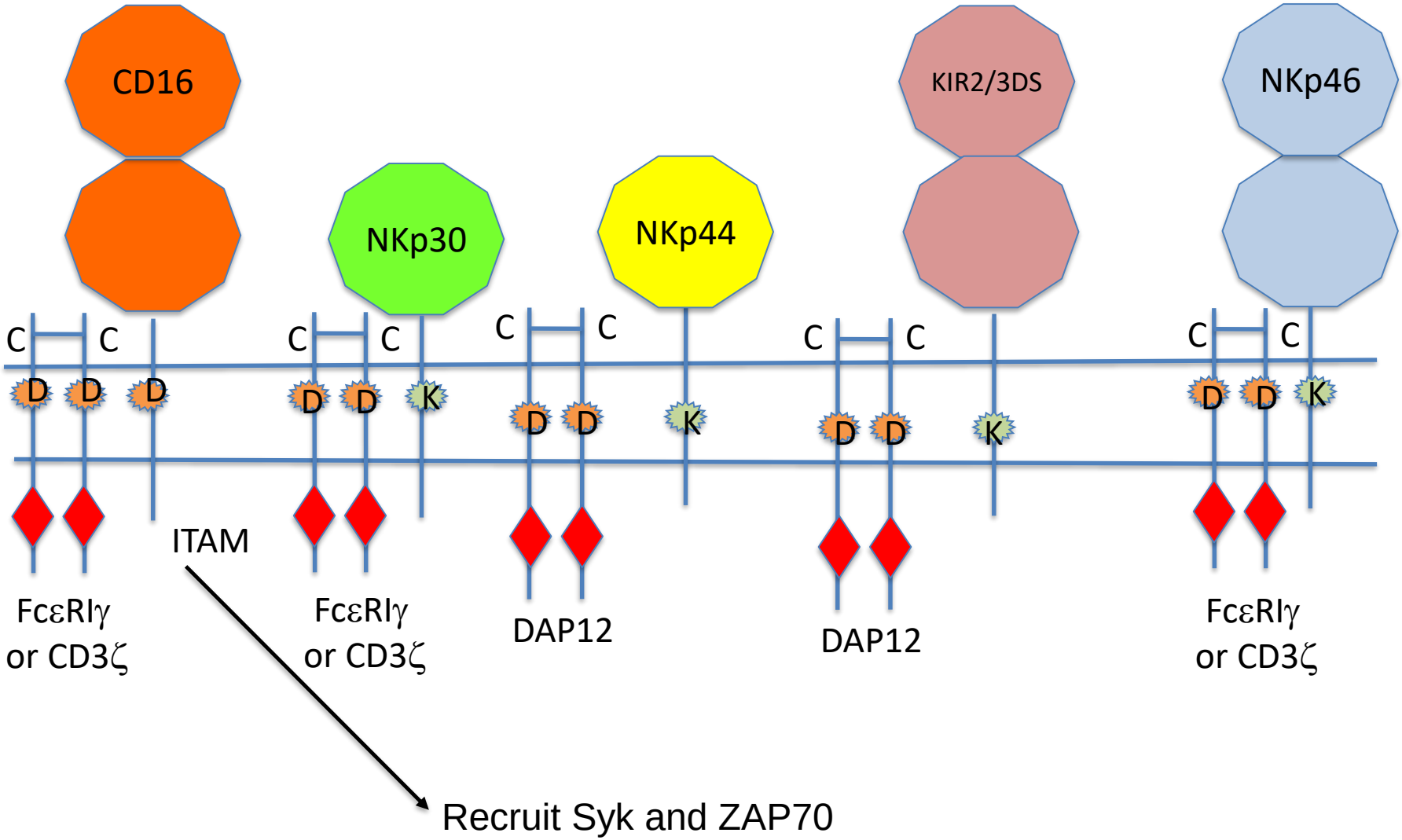
2219 alleles at 14 KIR loci

https://www.ebi.ac.uk/ipd/kir/

NK cell functions are controlled by a balance of inhibitory and activating receptors

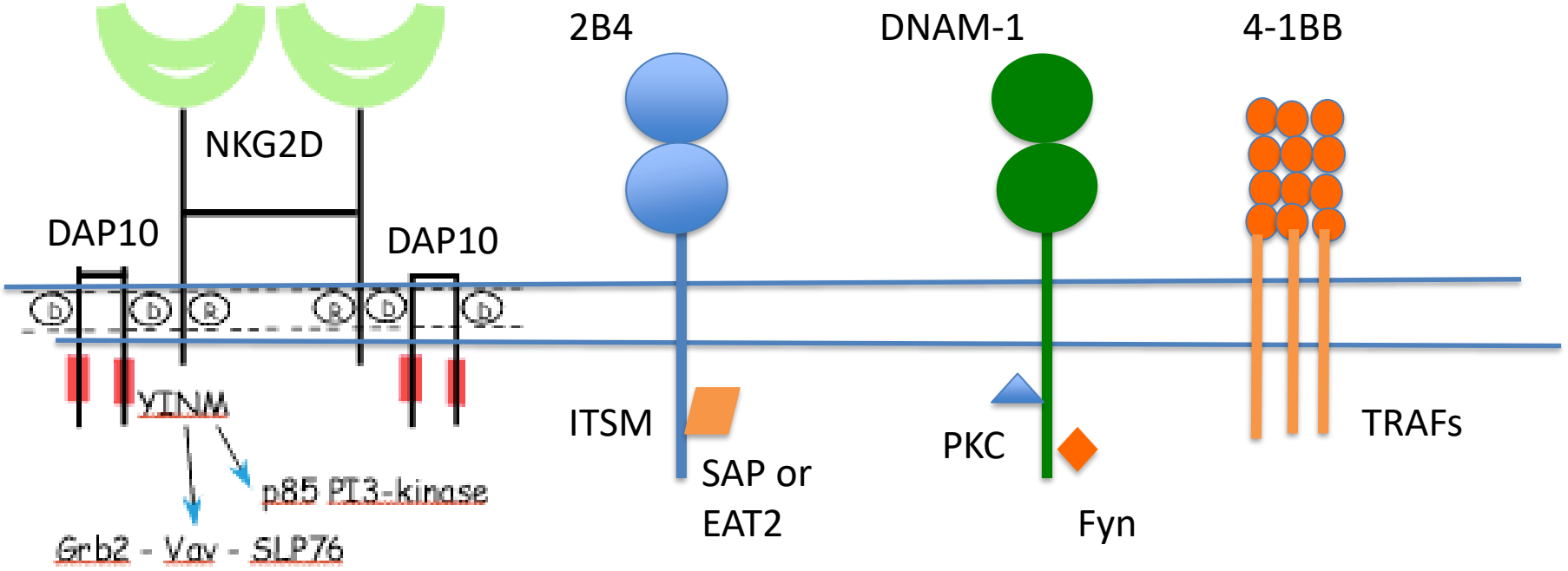


ITAM-based activating NK receptors

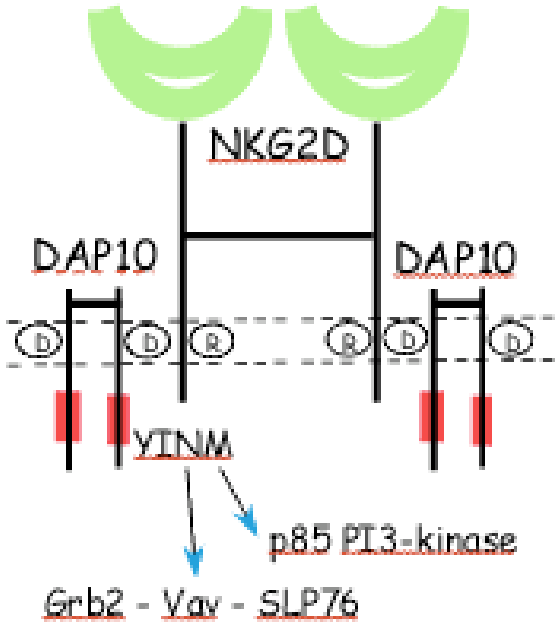


Miller & Lanier Ann Rev Cancer Biology 2019

Co-activating NK receptors



Miller & Lanier Ann Rev Cancer Biology 2019

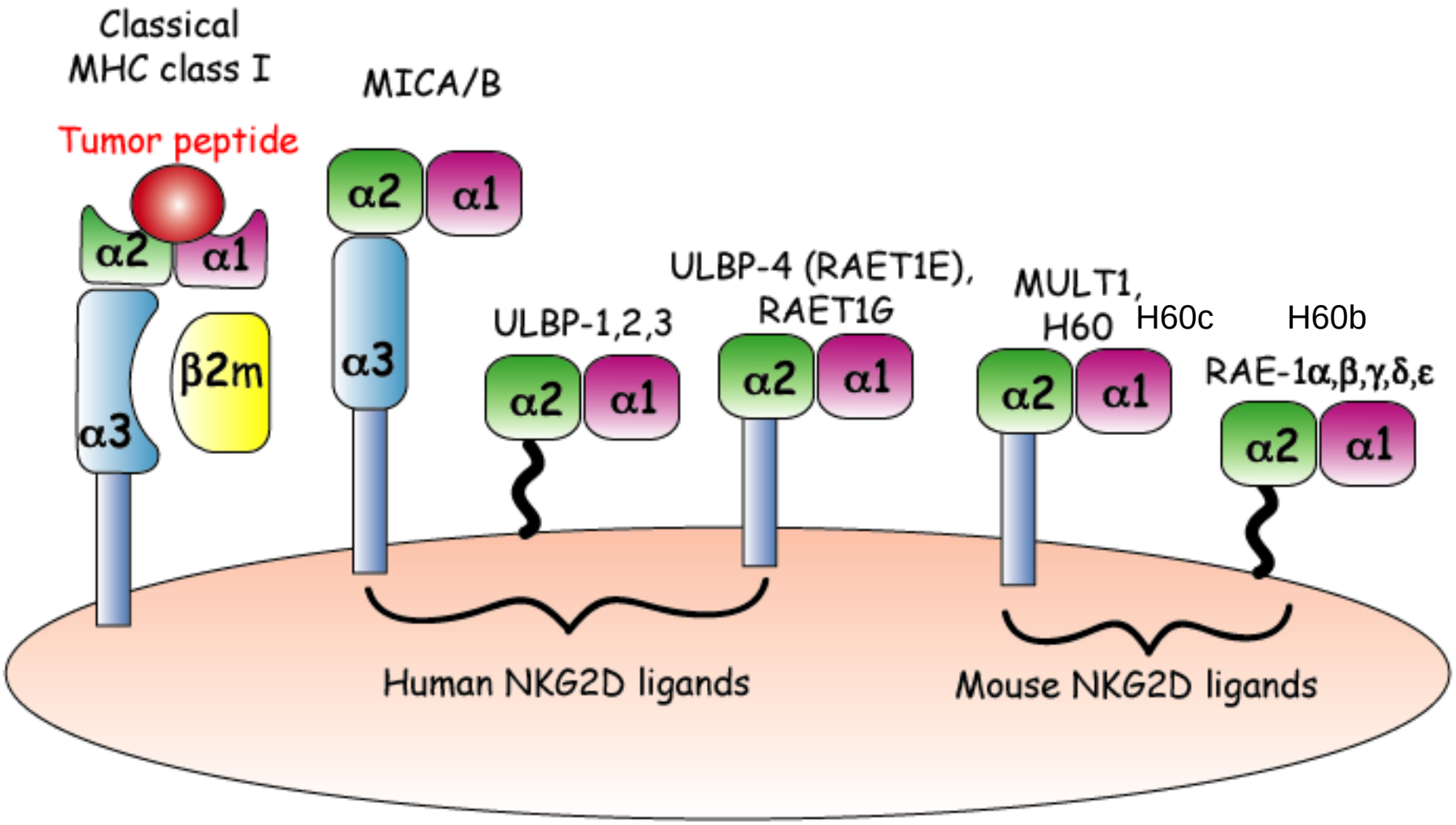


NKG2D

- C-type lectin-like superfamily
- 1 gene, non-polymorphic, conserved mice - humans
- Homodimer expressed on all NK cells, $\gamma\delta$ T cells, and $CD8^+$ T cells
- R in transmembrane associates with D in DAP10 transmembrane

DAP10

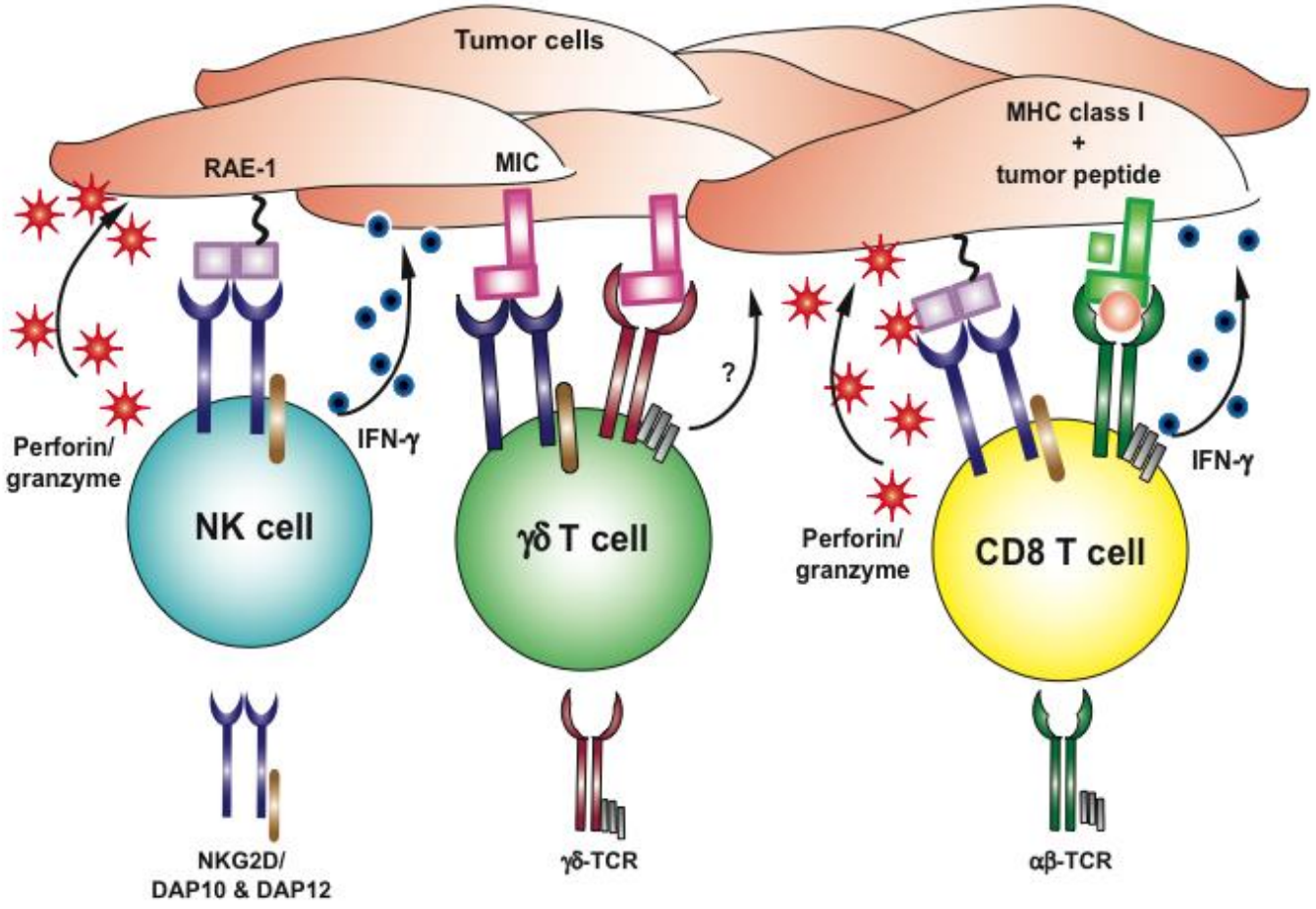
- 10 kd homodimer
- Cytoplasmic YINM recruits Grb2 & p85 PI3-kinase


Classical
MHC class I
Tumor peptide
α2
α1
α3
β2m
MICA/B
α2
α1
α3
ULBP-1,2,3
α2
α1
ULBP-4 (RAET1E),
RAET1G
α2
α1
MULT1,
H60
H60c
H60b
α2
α1
RAE-1α,β,γ,δ,ε
α2
α1
Human NKG2D ligands
Mouse NKG2D ligands

NKG2D ligands

- MHC class I-like
  - don't require peptide or β2-microglobulin
- Bind with nM affinity to NKG2D
- Low levels expressed on healthy tissues
- Induced on virus-infected cells and tumor cells
- Induced by DNA damage (ATM/ATR pathway)
- Elevated in autoimmune diseases
  (rheumatoid arthritis, celiac disease, diabetes, atherosclerosis)

NKG2D on NK cells, γδ T cells, and $CD8^+$ T cells detect NKG2D ligands on abnormal cells



NATURE BIOTECHNOLOGY | NEWS AND VIEWS

Of snowflakes and natural killer cell subsets

Lewis L Lanier

Nature Biotechnology **32**, 140–142 (2014) | doi:10.1038/nbt.2810
Published online 07 February 2014

You may have more than 30,000 NK cells subsets in your blood
- CyTOF analysis by Catherine Blish (Stanford)

Class Discussion

Why don’t NK cells kill HLA class I-negative tumors arising in cancer patients?

Why don't NK cells kill HLA class I-negative tumors arising in cancer patients?

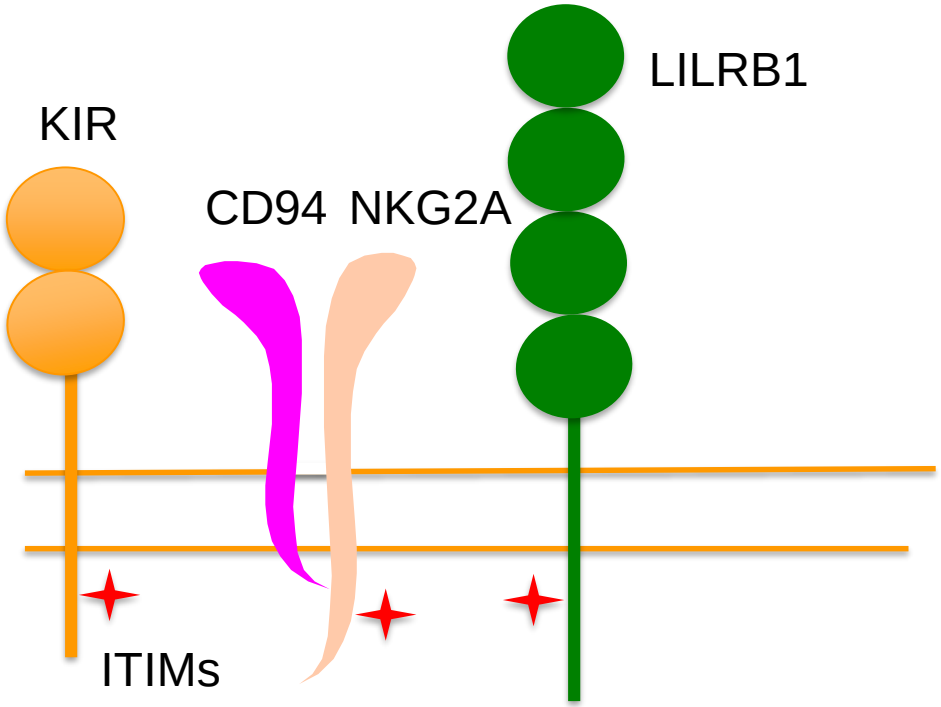
*Tumors lack ligands for activating receptors

*NK cells kill some tumors, but without cytokines don't expand – then become "de-sensitized"

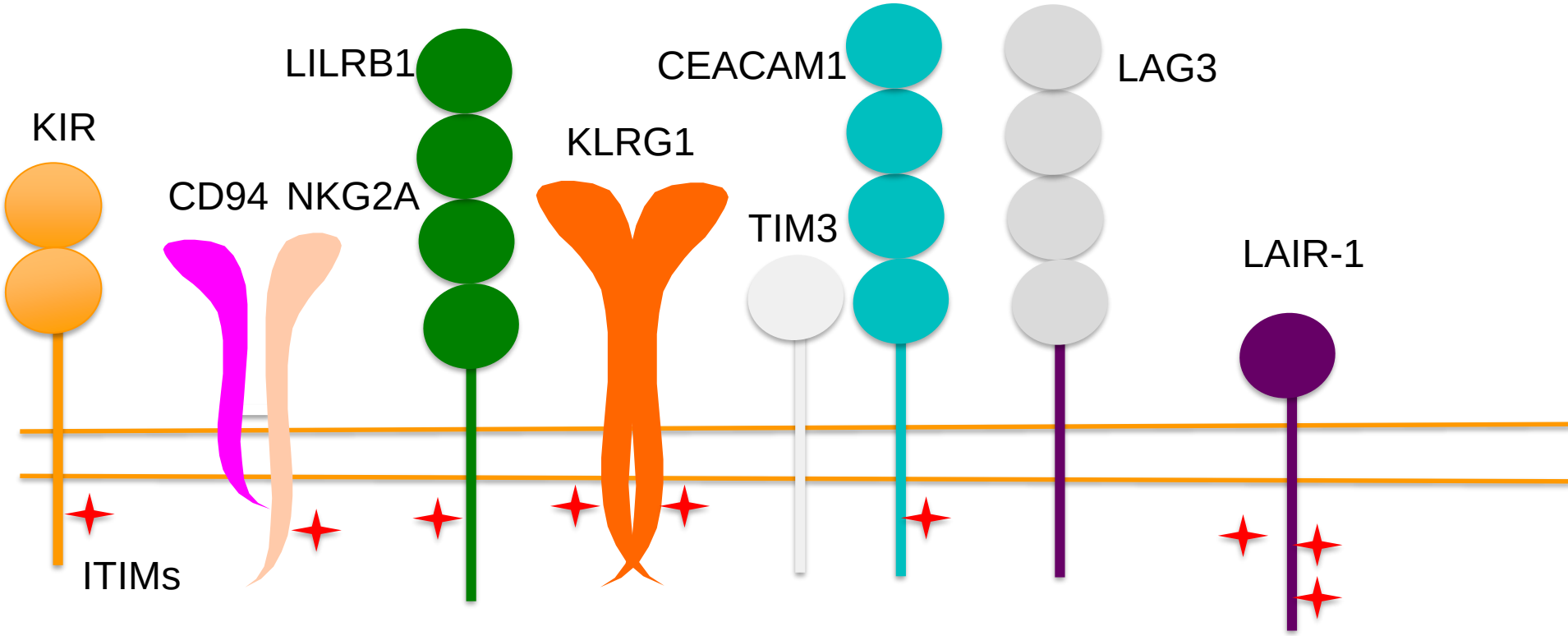
*Redundant inhibitory receptors other than for class I dampen NK cell responses

*Tumor microenvironment suppresses NK cell (e.g. NK cells hate Transforming Growth Factor β)

MHC class I Inhibitory Receptors on Human NK cells

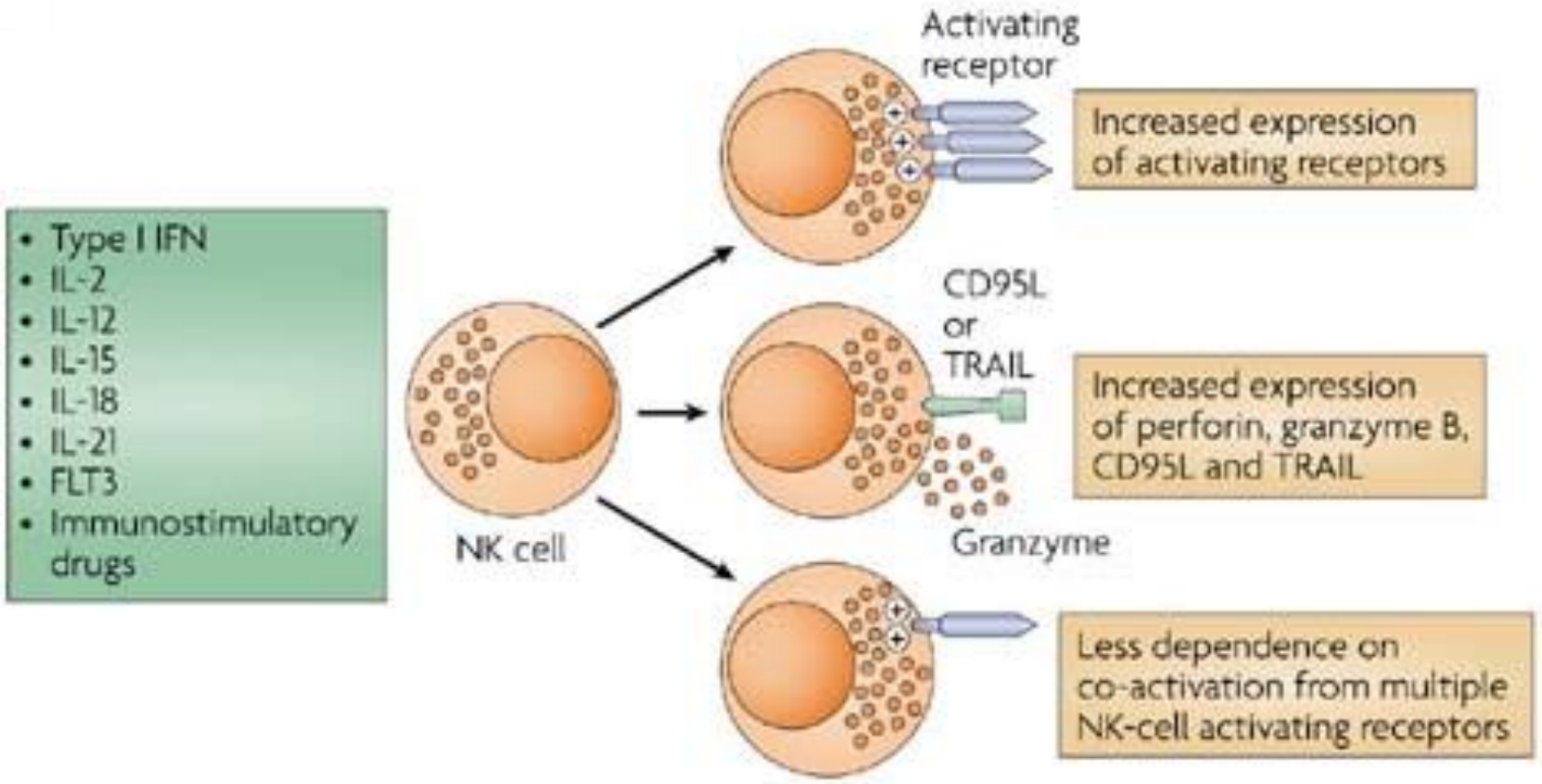


Inhibitory Receptors on Human NK cells



STRATEGIES FOR THERAPEUTICALLY MODULATING NK CELL FUNCTION

Factors boosting NK cell lytic activity



Ljunggren & Malmberg NRI 2007

Checkpoint blockade therapies

anti-KIR

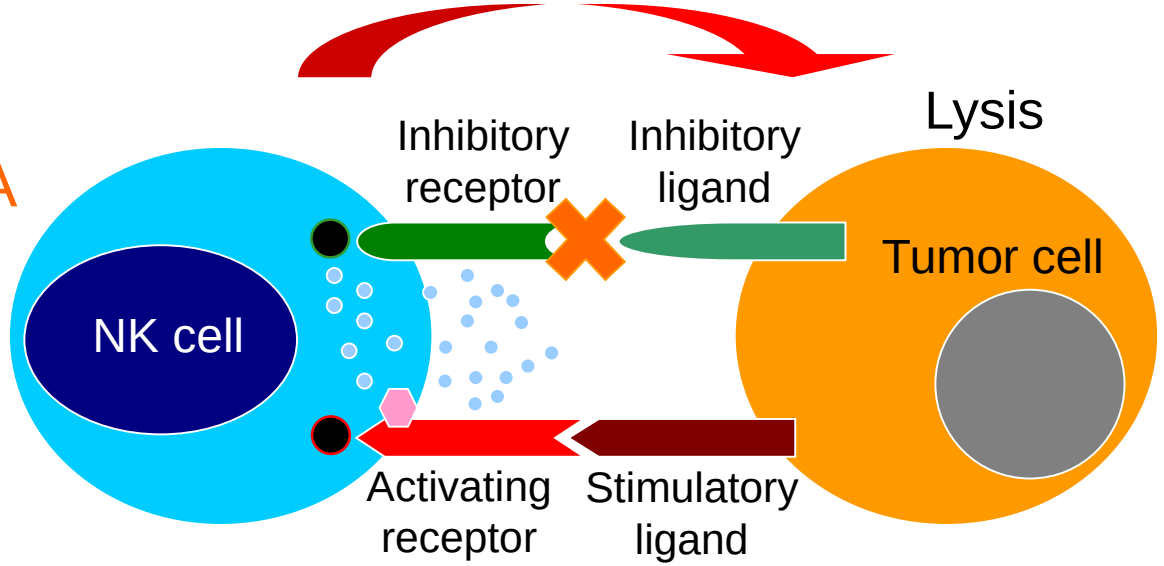
anti-NKG2A

anti-Tim3

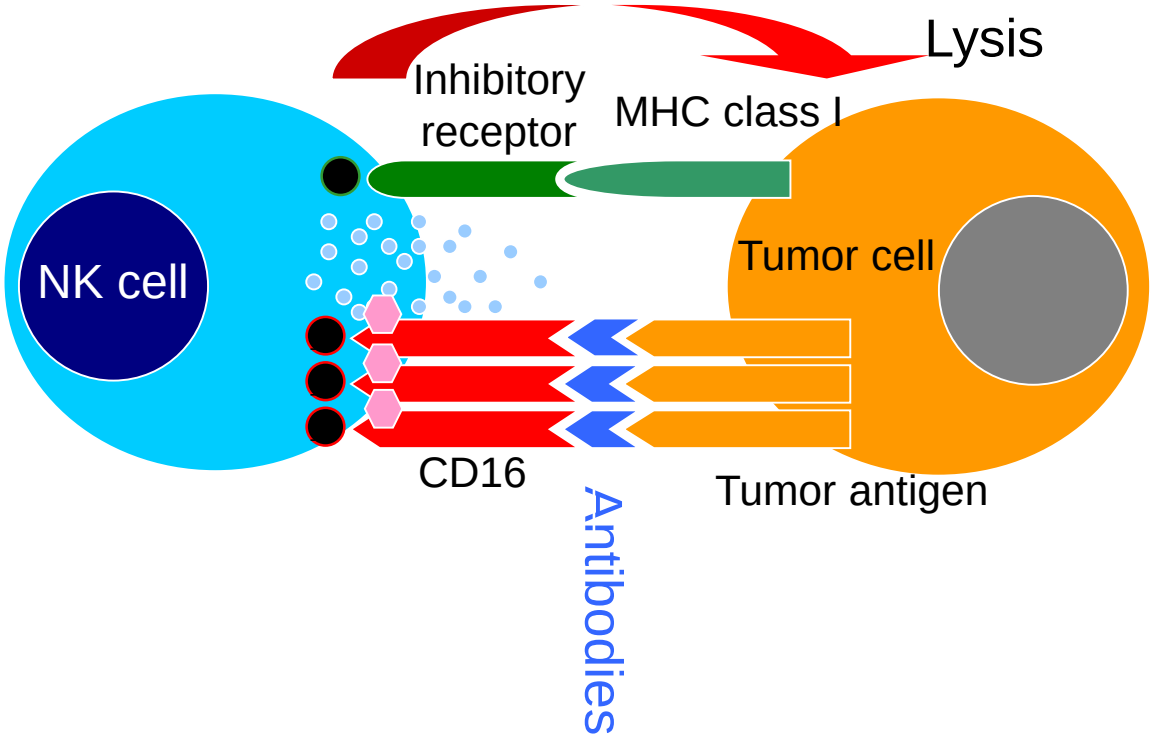
anti-LAG3

anti-TIGIT

anti-CD112R



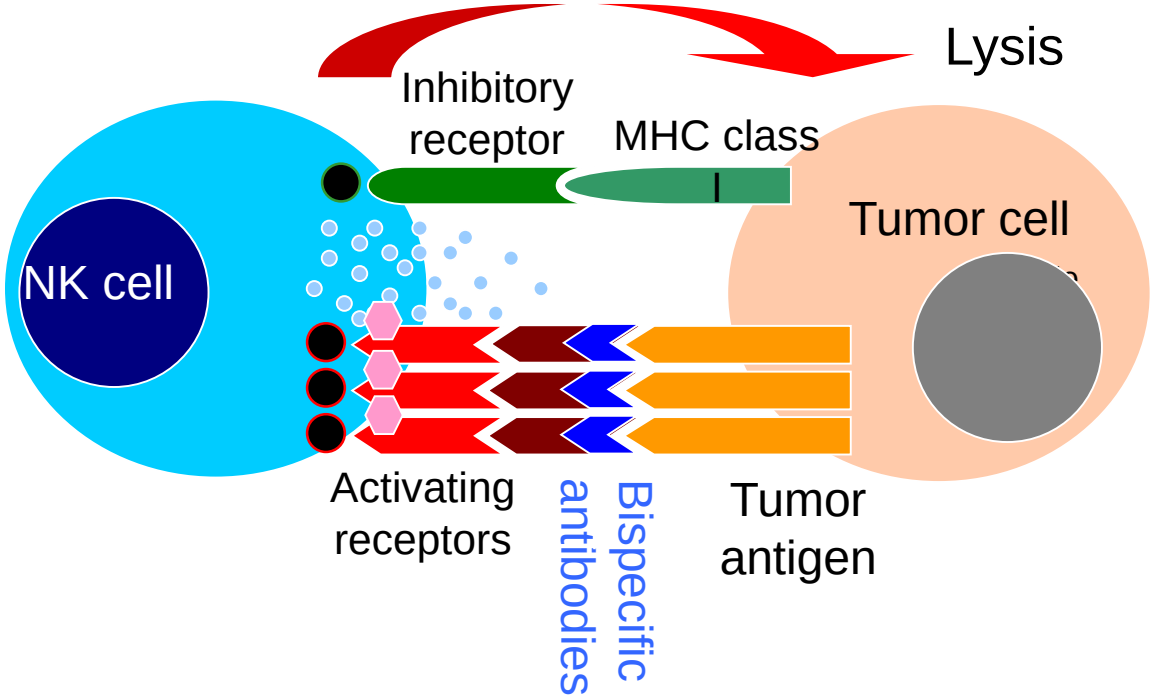
Antibody-dependent cellular cytotoxicity



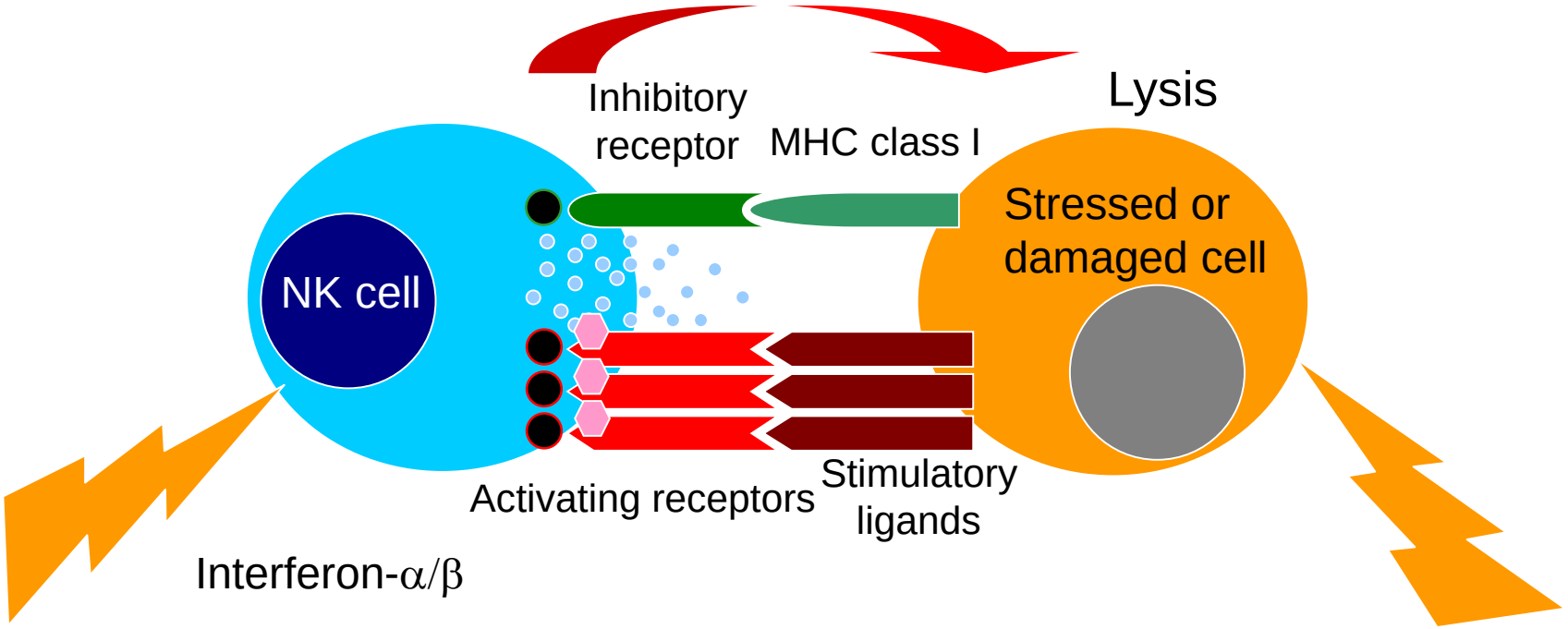
rituximab, trastuzumab, daratumumab

Bispecific antibodies

– anti-tumor x anti-NK activating receptor



Therapies that up-regulate stress-induced ligands on tumors or agents that activate NK cells



Interferon-α/β

IL-15

IL-12

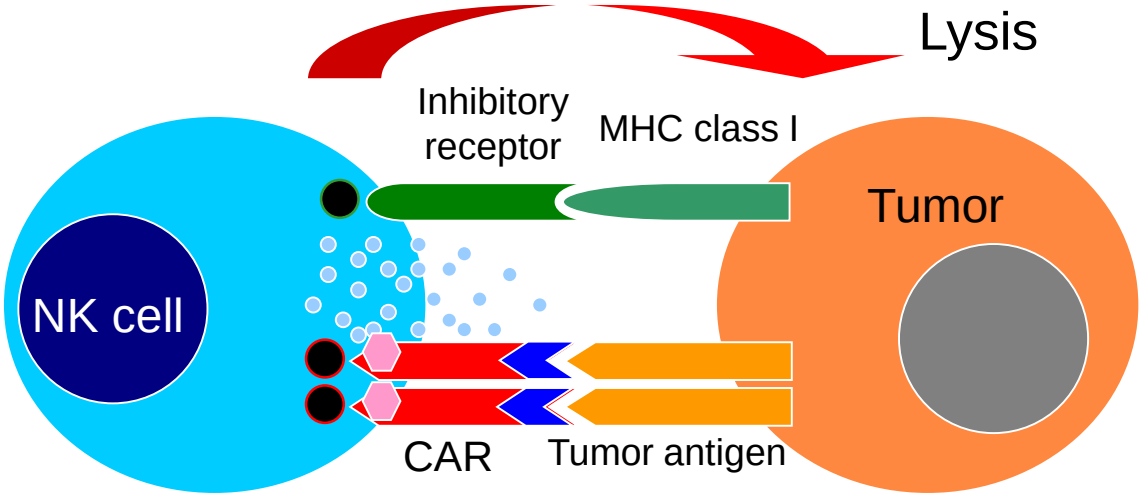
Agonist co-stimulatory antibodies (e.g. CD137, NKG2D)

Irradiation

Chemotherapy

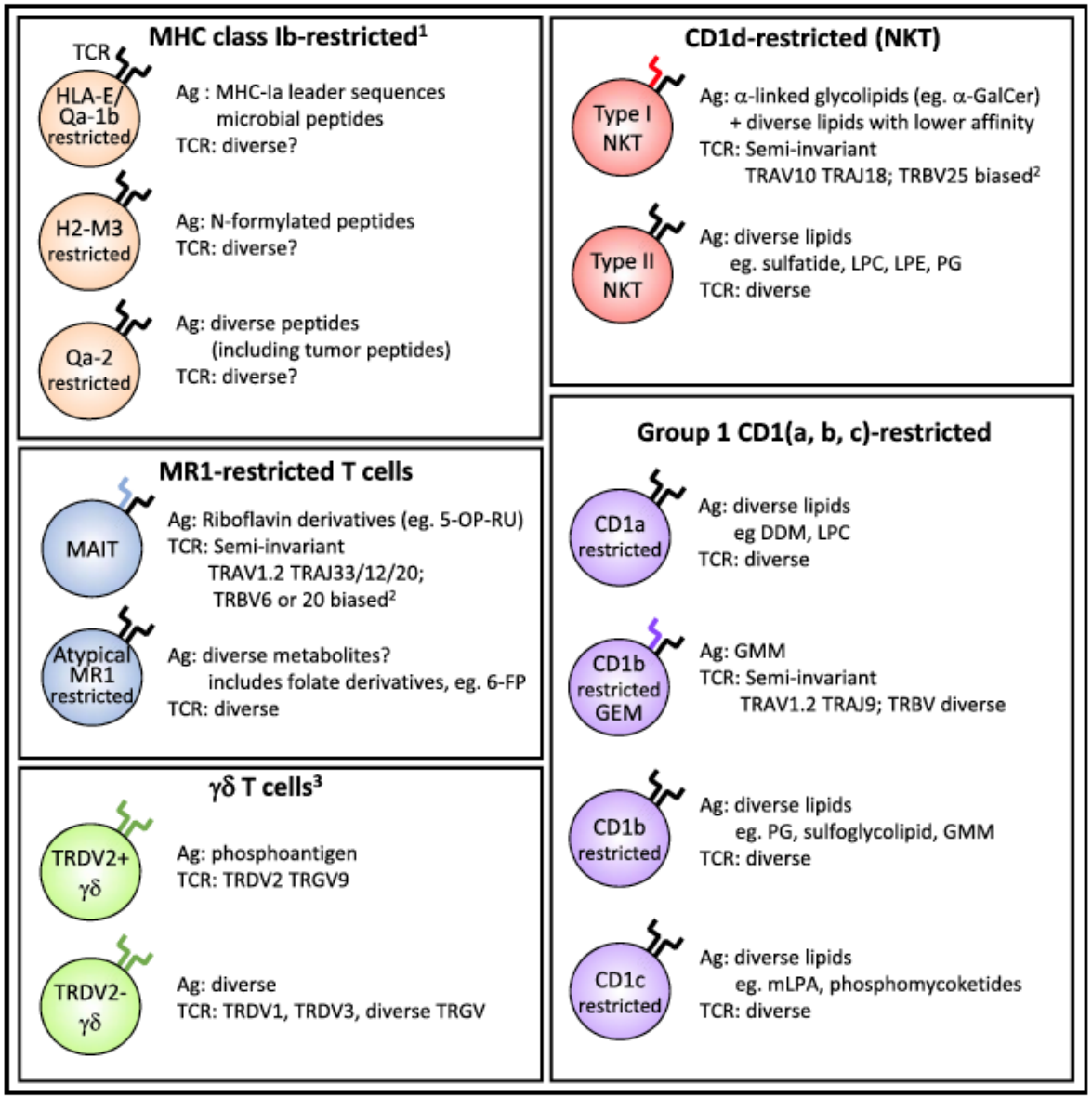
CAR NK cells

Chimeric antigen receptors



anti-CD19-cytoplamic CD137-CD3ζ

Innate T cells



Godfrey, Immunity, 2018

ILC and NK cells

- ILC – family of innate lymphocytes – rapid cytokine production
- NK cells keep you alive during certain viral infections
- NK cells regulated by inhibitory and activating receptors
- NK receptors are evolving rapidly
- NK cells possess immunological memory

Reviews

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