Natural Killer (NK) cells and innate lymphocytes (ILC)

Objectives

1. Know how NK cells and ILCs develop and know their functions
2. Know how the function of NK cells is controlled
3. Know what renders a cell susceptible to NK cell mediated attack

   (in comparison to T cells)
4. Know how NK cells can influence adaptive immune responses

Index

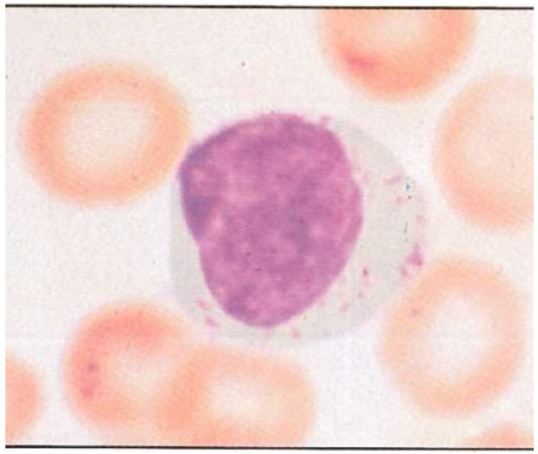
1. Introduction

1.1 NK cells: Phenotype

Morphology:

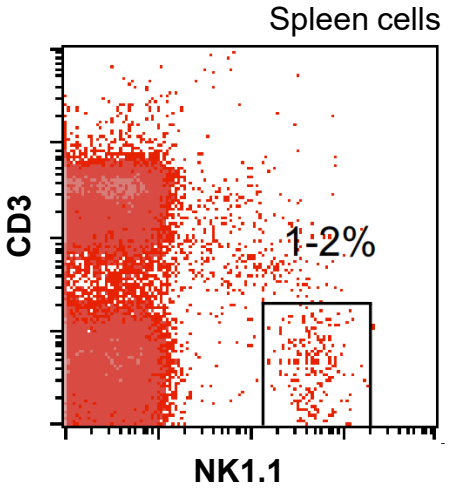
Large granular

lymphocytes



Phenotype:

Mouse	**Human**
TCR - / CD3 -; Ig -	TCR - / CD3 -; Ig -
IL2/15Rβ (CD122)	IL2/15Rβ (CD122)
NKp46	NKp46 (NCR1)
NK1.1, CD49b (integrin α2; DX5)	CD56

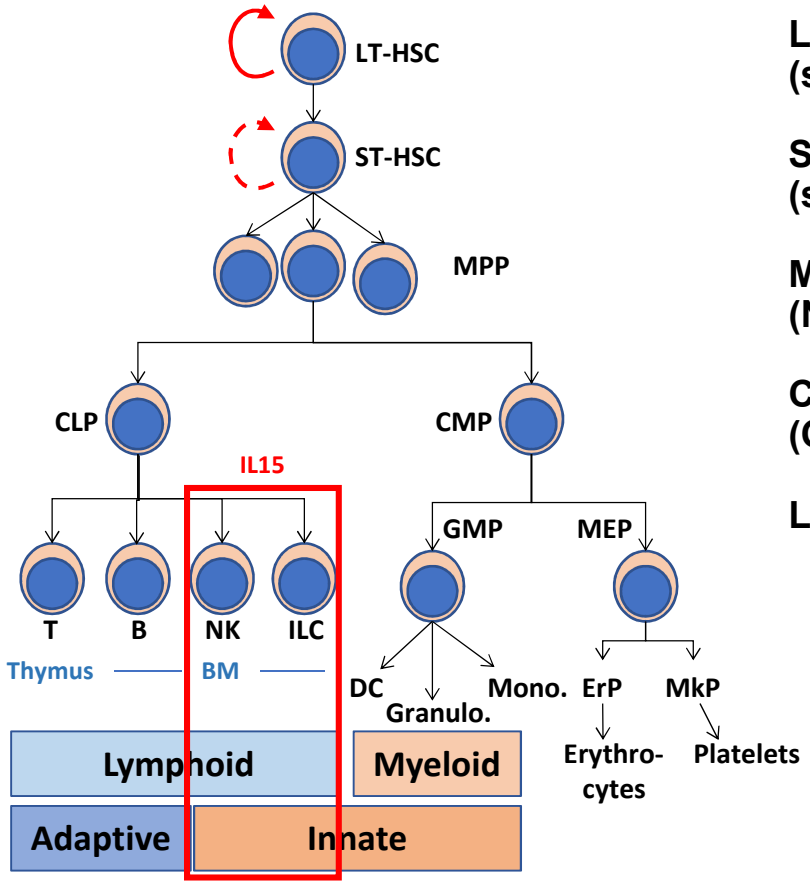


Blood up to 10% of lymphocytes

Founding member of a small family of Innate Lympoid cells (ILC)
Hallmark: Absence of antigen receptors (TCR or Ig)

1. Introduction

1.2 Development



Long term hematopoietic stem cell (LT-HSC) (self-renewal)

Short-term HSC (self-renewal, 4 weeks)

Multipotential progenitor (No self-renewal)

Common Lymphoid or Myeloid Progenitors (Oligopotent)

Lineage-restricted progenitors

NK cells derive from adult bone marrow HSC

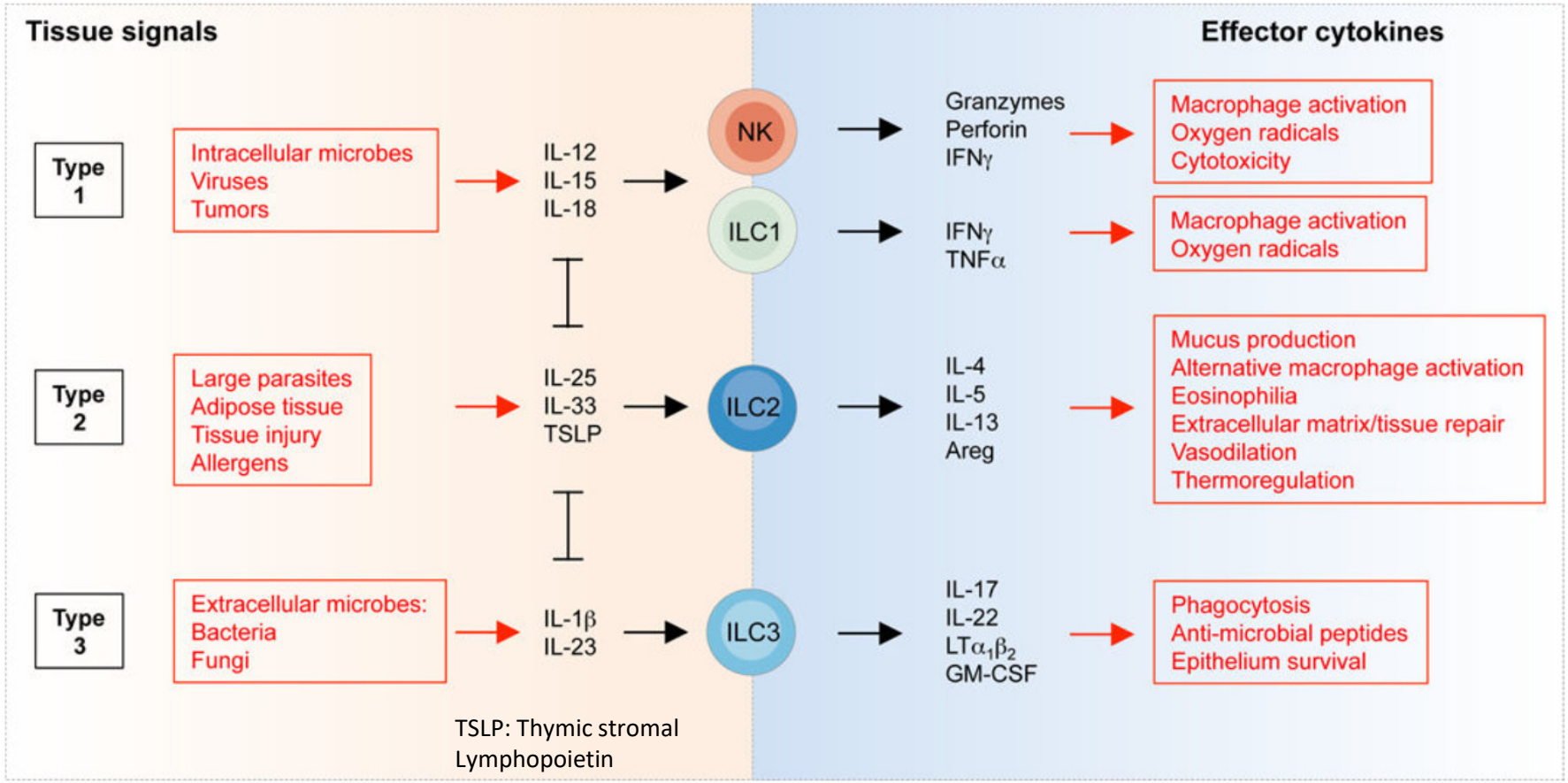
ILC derive from fetal liver HSC

innate lymphocytes:

- **lack of somatically re-arranged antigen receptors**
- **regulation (activation/inhibition) by pre-defined, invariant set of receptors and ligands**

1. Introduction

1.3 Function and regulation of ILCs



Adapted from Eberl et al 2015 Science 348: 6566

1. Introduction

1.4 Roles of NK cells

Innate immune protection

- against infection
  - direct recognition of pathogens
  - recognition of infected cells
- against cancer

Immunoregulation

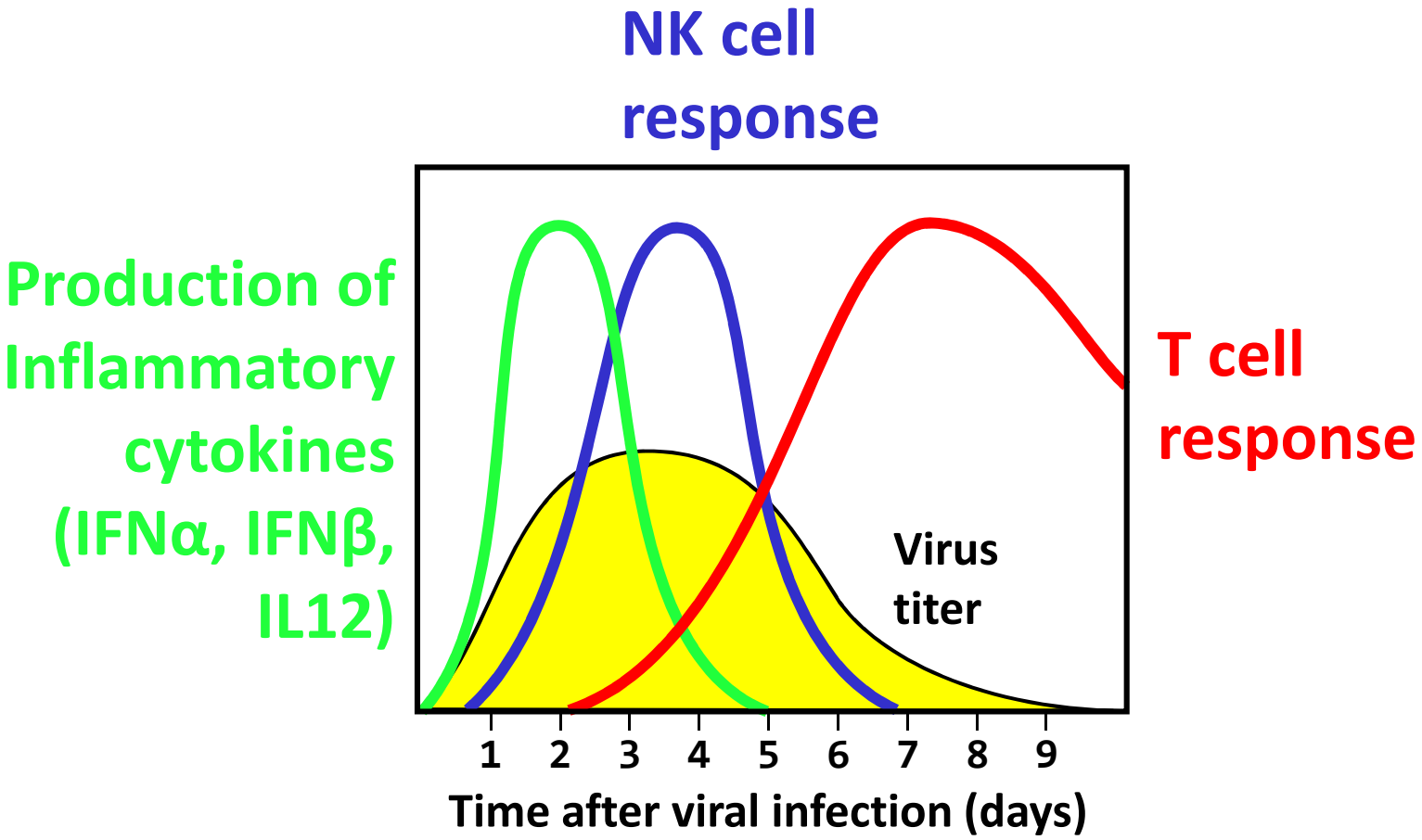
- Regulate innate immune responses
- Regulate adaptive immune responses

Clinical relevance

- Bone marrow graft rejection
- Graft versus leukemia reaction

1. Introduction

1.5 Time course



1. Introduction

1.6 Effector functions of NK cells

1. **Cell mediated cytotoxicity:**

   Granule exocytosis (Perforin, Granzymes)

   Natural killing

   NK cell-mediated lysis of infected or transformed cells without prior sensitization

2. **Production of cytokines**

   Pro-inflammatory: IFNγ, TNF-α, GM-CSF

   Anti-inflammatory: IL-10 (sustained activation)

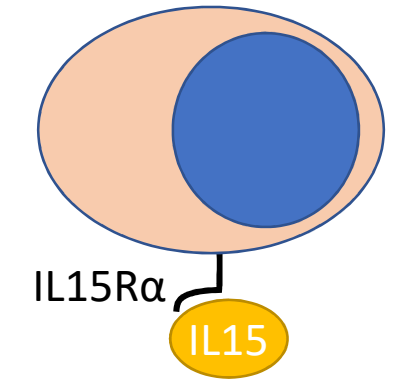
3. **Production of chemokines**

   Macrophage Inflammatory Proteins:

   - MIP-1a (CCL3); recruitment and activation of granulocytes
   - MIP-1b (CCL4): attraction of NK cells and monocytes
   - RANTES (CCL5): attraction of DC

2. NK cell function

2.1 NK cell homeostasis



Accessory cells:

Macrophage

Dendritic cell (DC)

Plasmacytoid DC (pDC)

IL15 required for formation and maintenance of NK cells

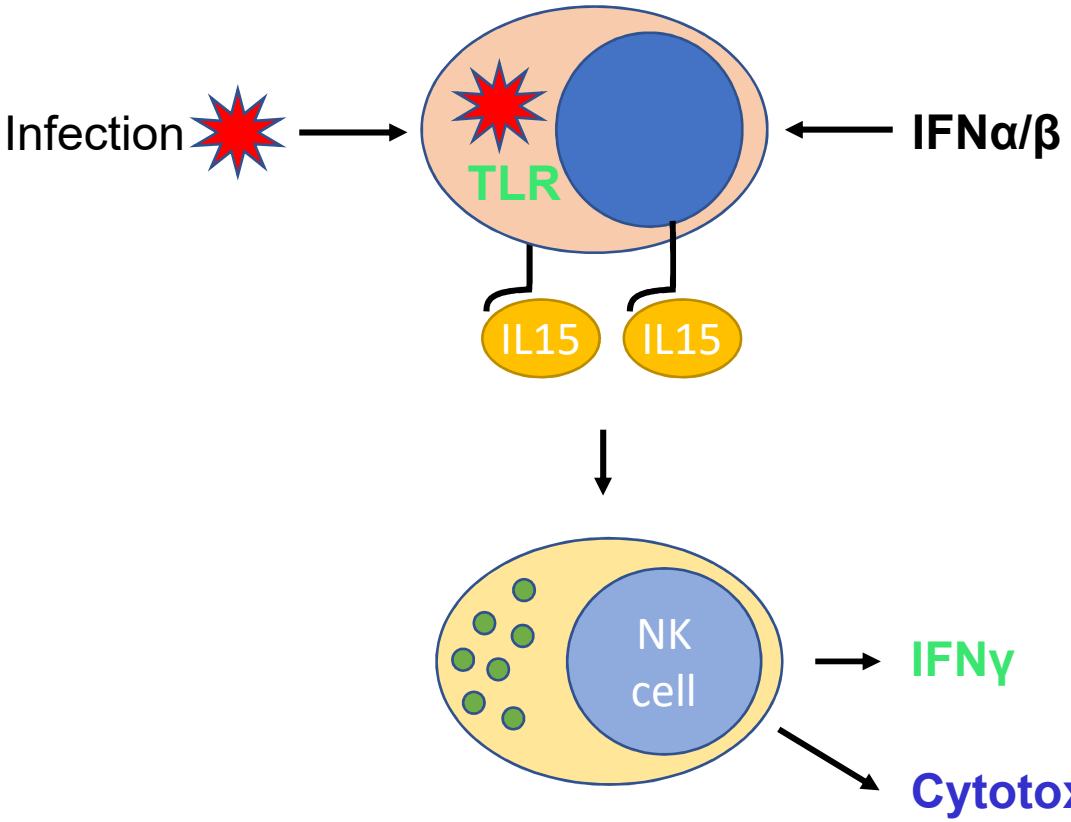
IL15 is presented via IL15Rα to NK cells by accessory cells

Bcl2, Mcl1 ⊣ Bim, Noxa

Survival

2. NK cell function

2.2 NK cell priming and activation



Accessory cells:

Macrophage

Dendritic cell (DC)

Plasmacytoid DC (pDC)

Effector responses depend on 2 signals

1.) NK cell priming (by IL15)

2.) Engagement of activating NK cell receptors

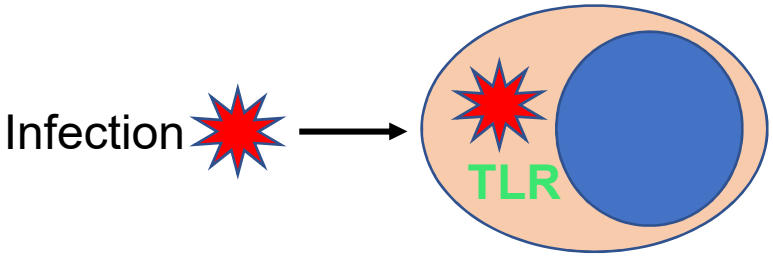
NK cell priming: Improves transcription/translation of IFNγ

Allows translation of perforin and Granzyme B mRNA

20-100 fold more active

2. NK cell function

2.3 Bystander activation



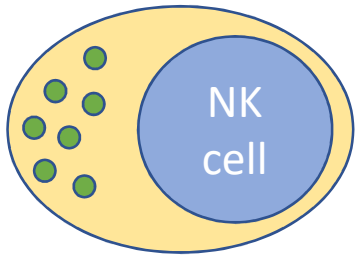
Macrophage

Dendritic cell (DC)

Neutrophils

IL12 or IL18 plus IL15

Two signals !!



IFNγ

Immunomodulatory function

-DC maturation

-increase MHC-I on APC

-increase APC cytokine production

-TH1 polarization

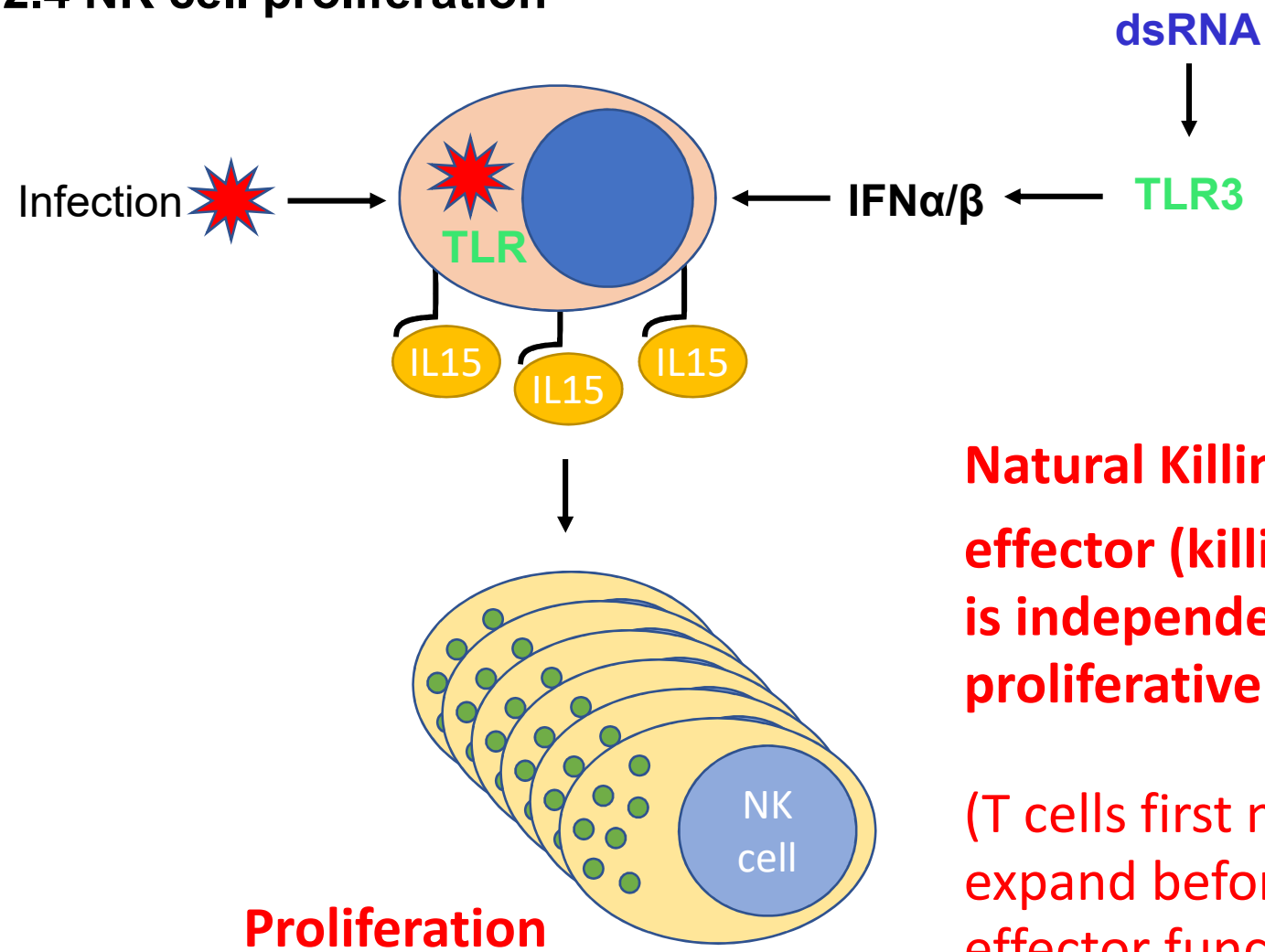
Pathogen restriction

-Activate MΩ killing of intracellular pathogen

-Antiproliferative effect on virus-infected and transformed cells

2. NK cell function

2.4 NK cell proliferation



Natural Killing:

effector (killing) function is independent of proliferative response

(T cells first need to expand before adapting effector function)

3. NK cell recognition

3.1 Recognition of <u>non-self</u>: Pathogen recognition

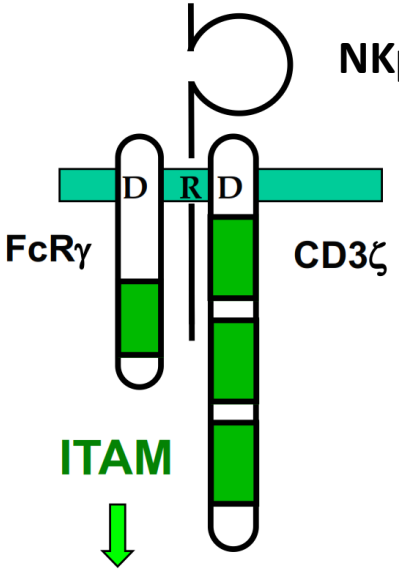
Fungal cells

Cryptococcus
Candida albicans

Cryptococcus
Candida galabrata

Cell wall:
β-1,3-glucan

Adhesins:
Epa1, Epa6 and Epa7

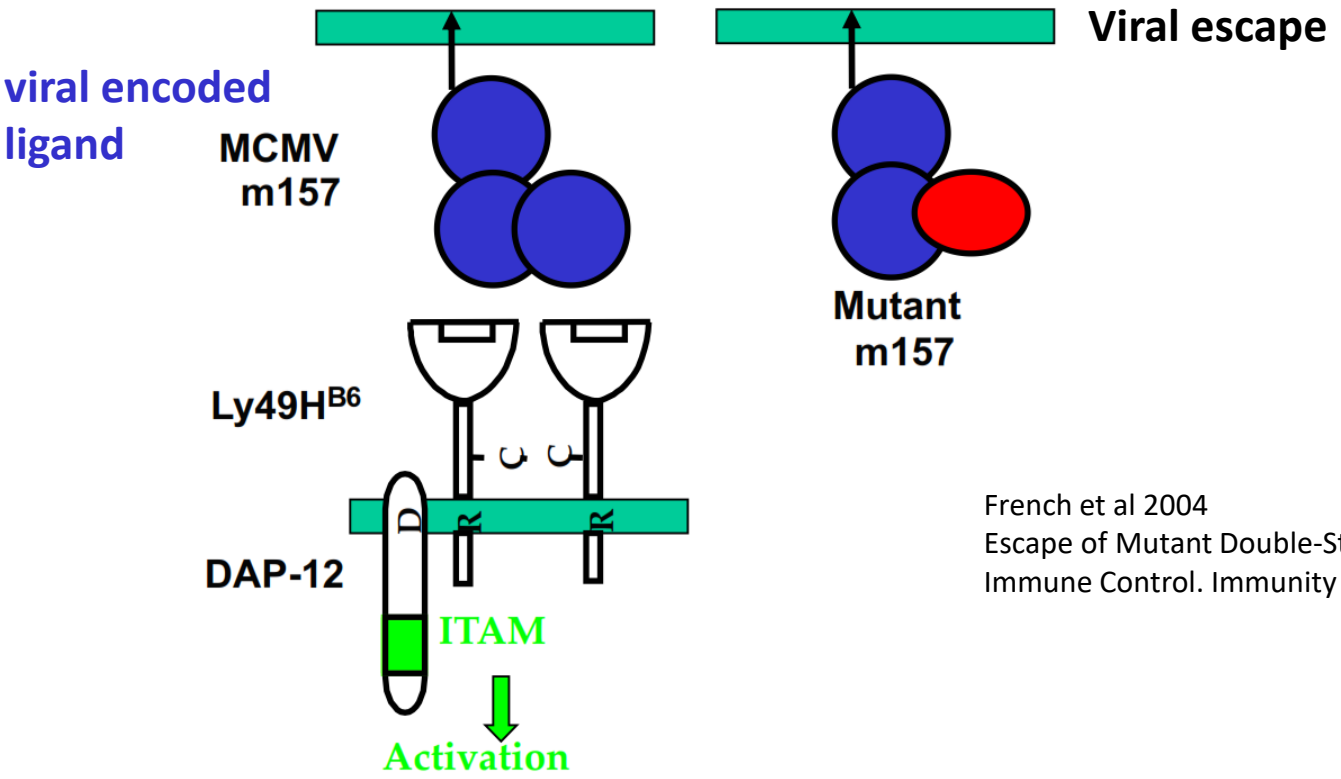


direct killing of fungal cells
by exocytosis of perforins ← **Activation**

Li 2013 Cell Host Microbes
Vitenshtein 2016 Cell Host Microbes

3. NK cell recognition

3.2 Recognition of non-self: Infected host cells

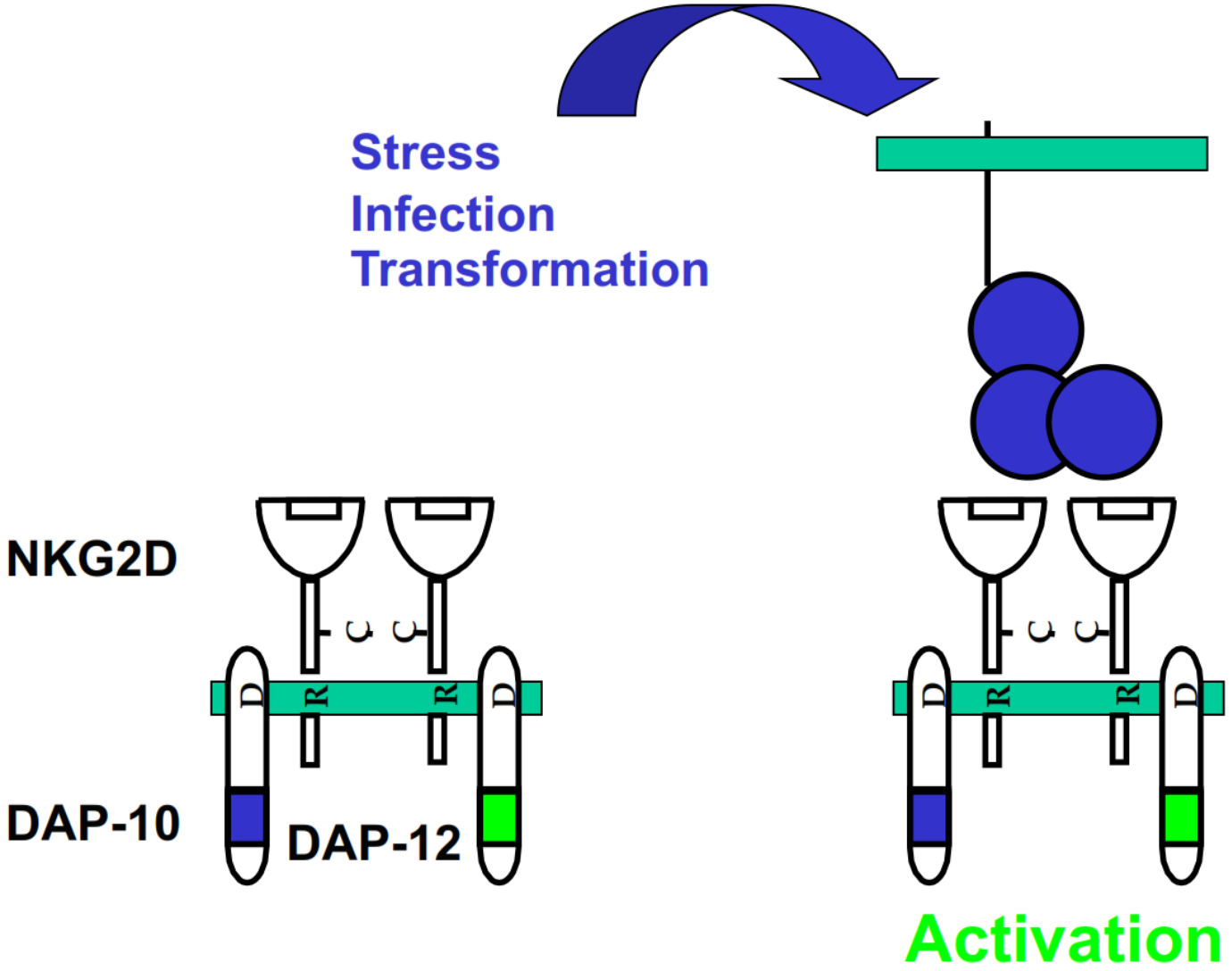


French et al 2004
Escape of Mutant Double-Stranded DNA Virus from Innate Immune Control. Immunity 20, 747–756

Pathogen	**Pathogen-derived ligand**	**NK cell receptor**
Mouse Cytomegalovirus	m157	Ly49H
	m12	NKR-P1A, NKR-P1C (NK1.1)
Influenza virus	Hemagglutinin	NKp46
Ectromelia virus (Mouse pox)	Virus-derived peptide (?) + host Qa-1b	CD94/NKG2C (Mouse pox)

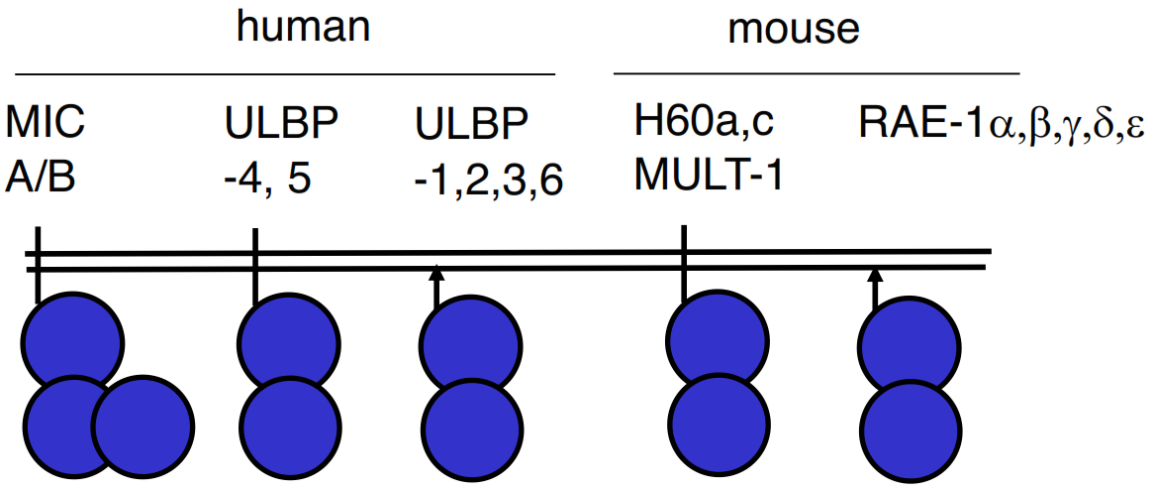
3. NK cell recognition

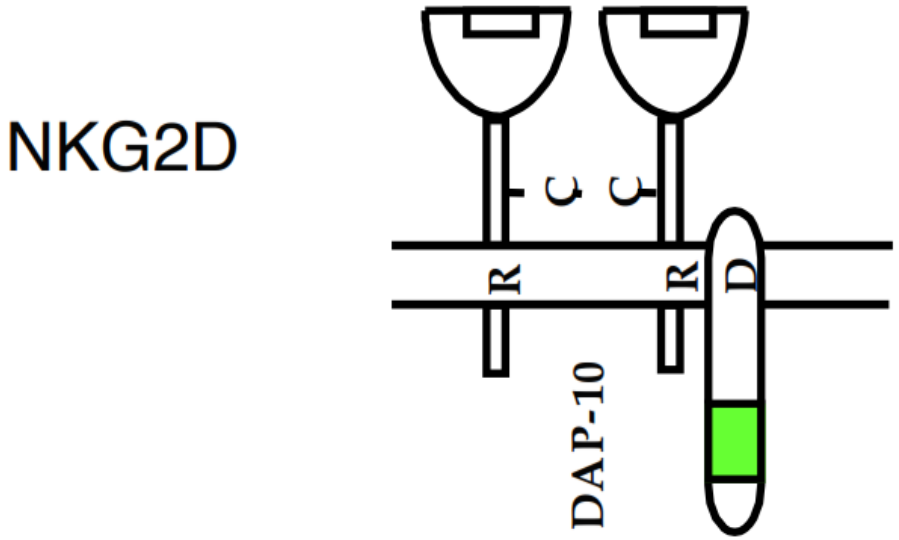
3.3 Recognition of induced-self: Stressed host cells



3. NK cell recognition

3.3 Recognition of induced-self: Stressed host cells

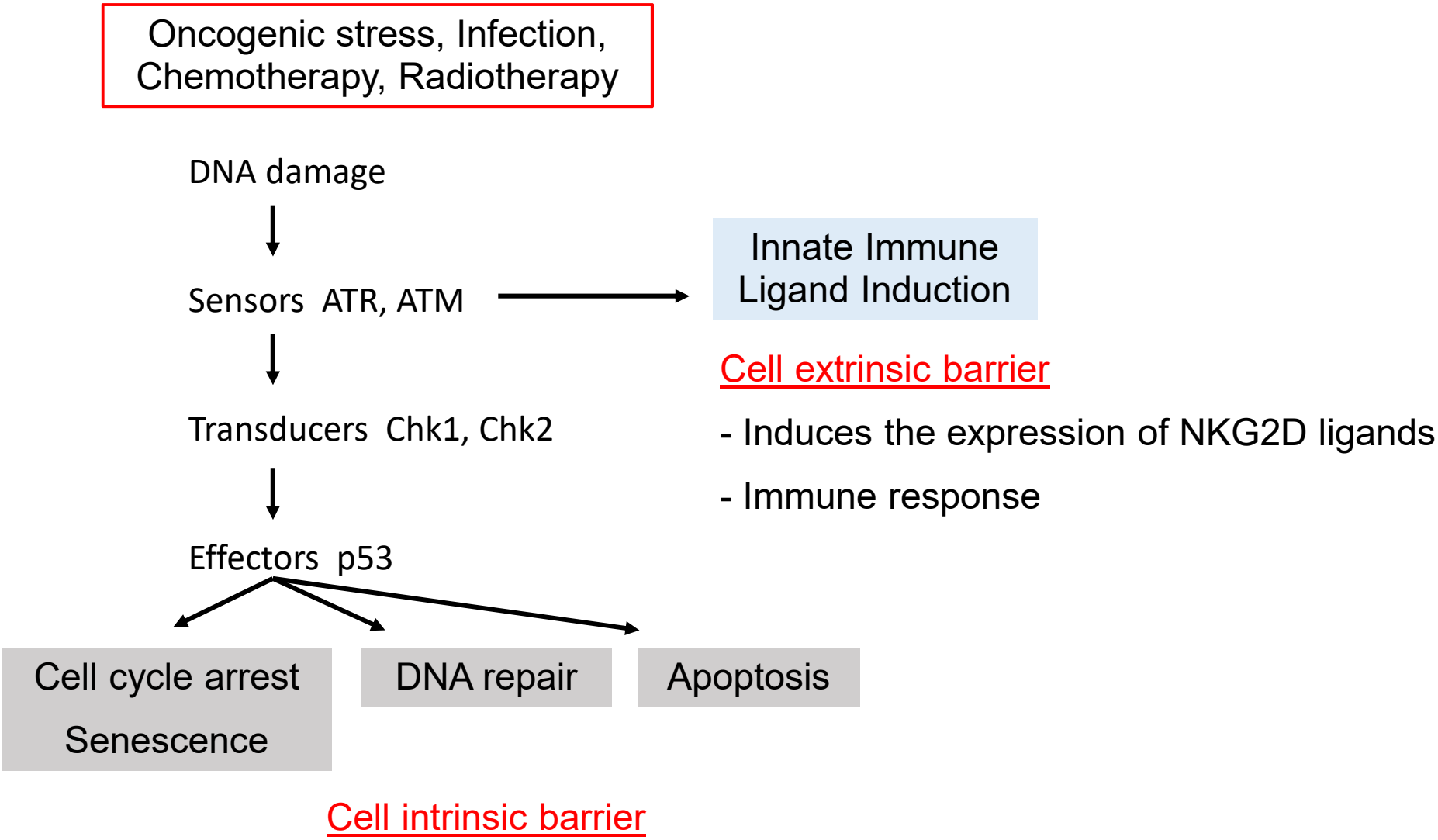




NKG2D LIGANDS:
- endogenous
- Ig-like molecules
- not expressed on normal cells
- single receptor / multiple ligands
- little sequence identity between MIC and ULBP
- high allelic variation in human population (MIC) due to viral inhibition mechanisms

3. NK cell recognition

3.3 Recognition of induced-self: Stressed host cells



3. NK cell recognition

3.3 Recognition of induced-self: Stressed host cells

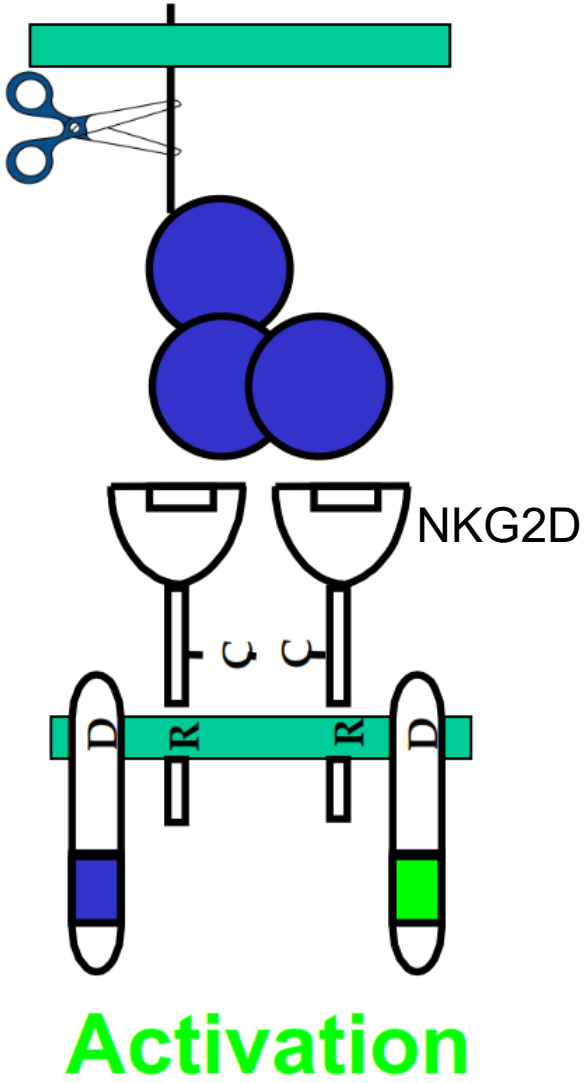
Induction of NKG2D ligands

pathway	level	ligands	regulatory component
DNA damage	mRNA	MIC, RAE, etc	ATM, ATR
Senescence	mRNA	RAE	ATM, ATR
Oncogenes	mRNA	MIC	E1A, BCR-ABL1
Infection	mRNA	RAE	TLR
Infection HIV			vpr
Proliferation	mRNA	RAE, ULBP	E2f
Wounding	mRNA	H60c	nd
Heat shock	mRNA	MIC	Hsf1
UV, heat shock	protein stability	MULT	E3 ubiquitin ligase

3. NK cell recognition

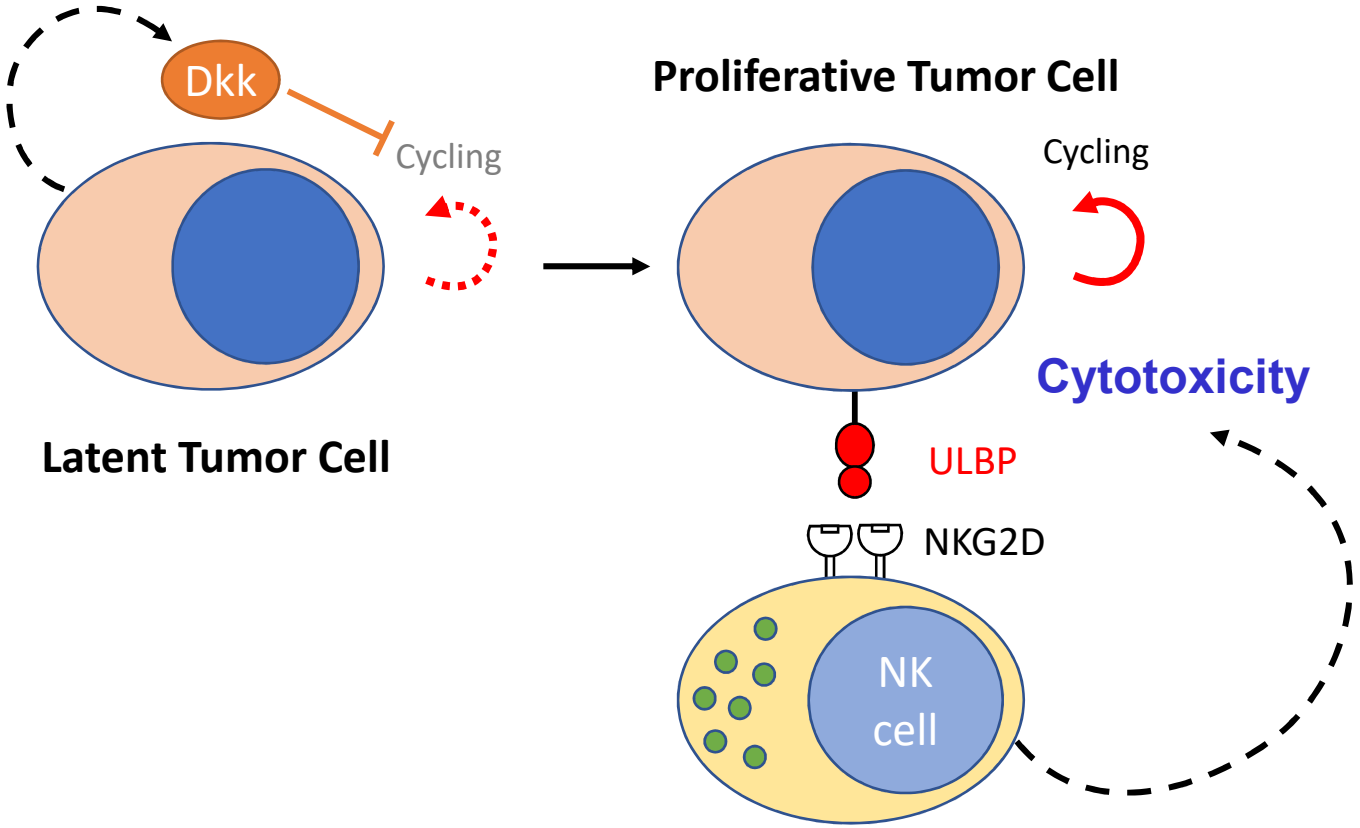
3.3 Escape from NKG2D recognition: Tumors

- Soluble NKG2D ligand in the sera of cancer patients
- Cleaved by matrix metalloproteases (MMPs)
- Bind (and block) NKG2D



3. NK cell recognition

3.3 Escape from NKG2D recognition: Metastasis



Malladi et al 2016 Cell 165, 45–60

3. NK cell recognition

3.3 Receptors for “induced-self”

Receptor	Ligand	Stimulus
NKG2D	mouse: MULT1, RAE, H60	Tumors, Infection, TLR3
	human: ULBP, RAET	Cellular activation and differentiation, DNA damage, heat shock
DNAM-1	CD155, CD112	Tumors, DNA damage
NKp30	BAT-3 (nuclear)	Tumors
	B7H6	Tumors
NKp44	truncated MLL5 (nuclear)	Tumors, HIV infected cells
	PCNA (nuclear)	Tumors
	Pdgf (growth factor)	Tumors
NKp46	?	Tumors
NKp65	CLEC2A	Keratinocytes, T cells
NKp80	AICL	Macrophages
KIR, Ly49 (EBV)	MHC class I up regulation	Infection
CD94/NKG2C	HLA-E + hsp70 peptide	Tumors

3. NK cell recognition

3.4 Normal-self recognition

Receptor	Ligand	Expression pattern
SLAM (Signaling Lymphocytic Activation Molecule) family receptors		
CD84	CD84	Haematopoietic cells -> explains preferential reactivity of NK cells to haematopoietic cells
2B4 (CD244)	CD48	
Ly9 (CD229)	Ly9	
CRACC (CD319)	CRACC	
NTB-A (Ly108)	NTB-A	
NKp46	?	
LFA-1 (CD11a/CD18)	ICAM1 (CD54)	

Summary: Activating NK cell receptors

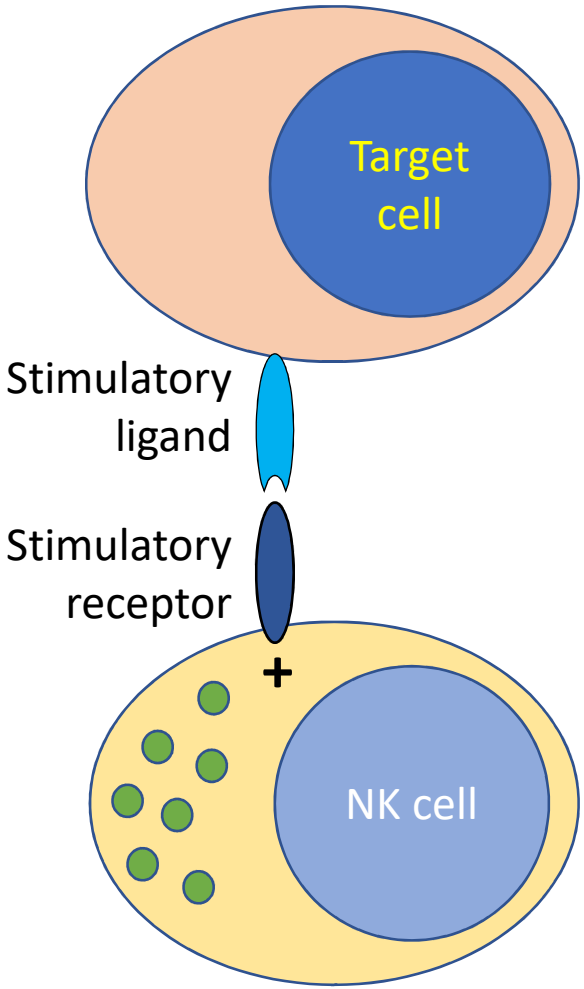
Non-self (Pathogen)
NKp30

Non-self
Ly49H
NKp46

Induced-self
NKG2D
DNAM-1
NKp30
CD94/NKG2C

Normal-self
SLAMs
NKp46

ADCC
CD16



Array of activating receptors

Specific for endogenous ligands, occasionally for foreign ligands

Inducible ligands

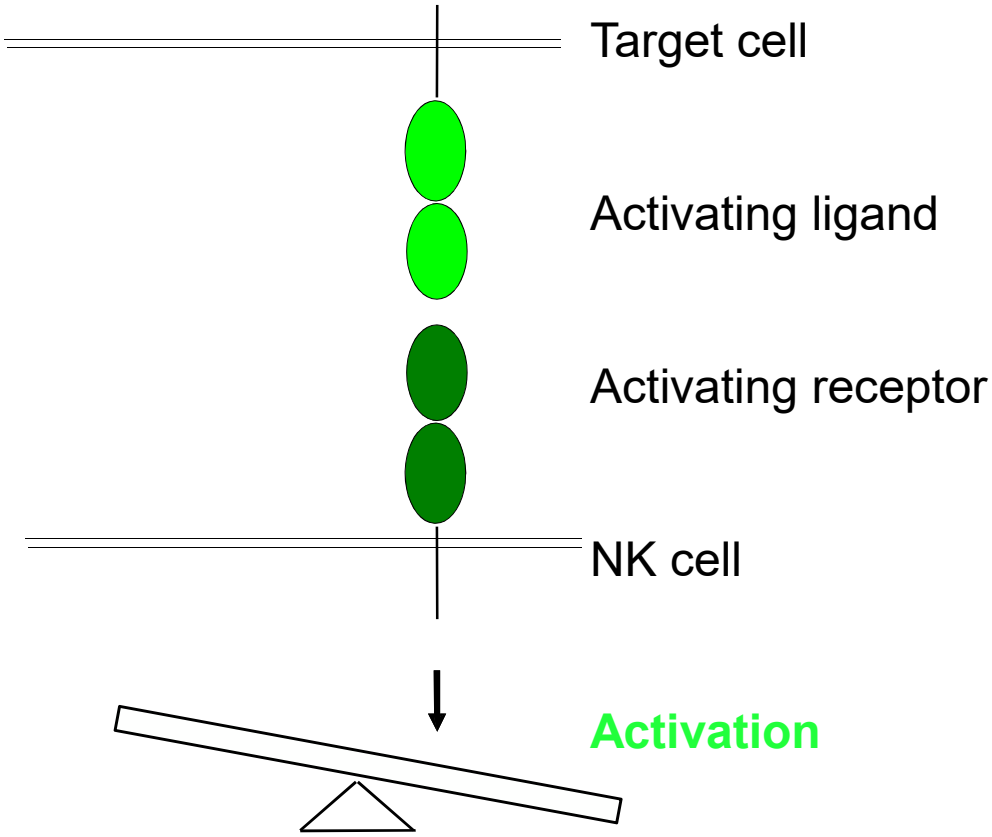
Inducible receptors

Endogenous ligands ?

Problem:
NK cells are activated by ligands, which are expressed by normal cells.

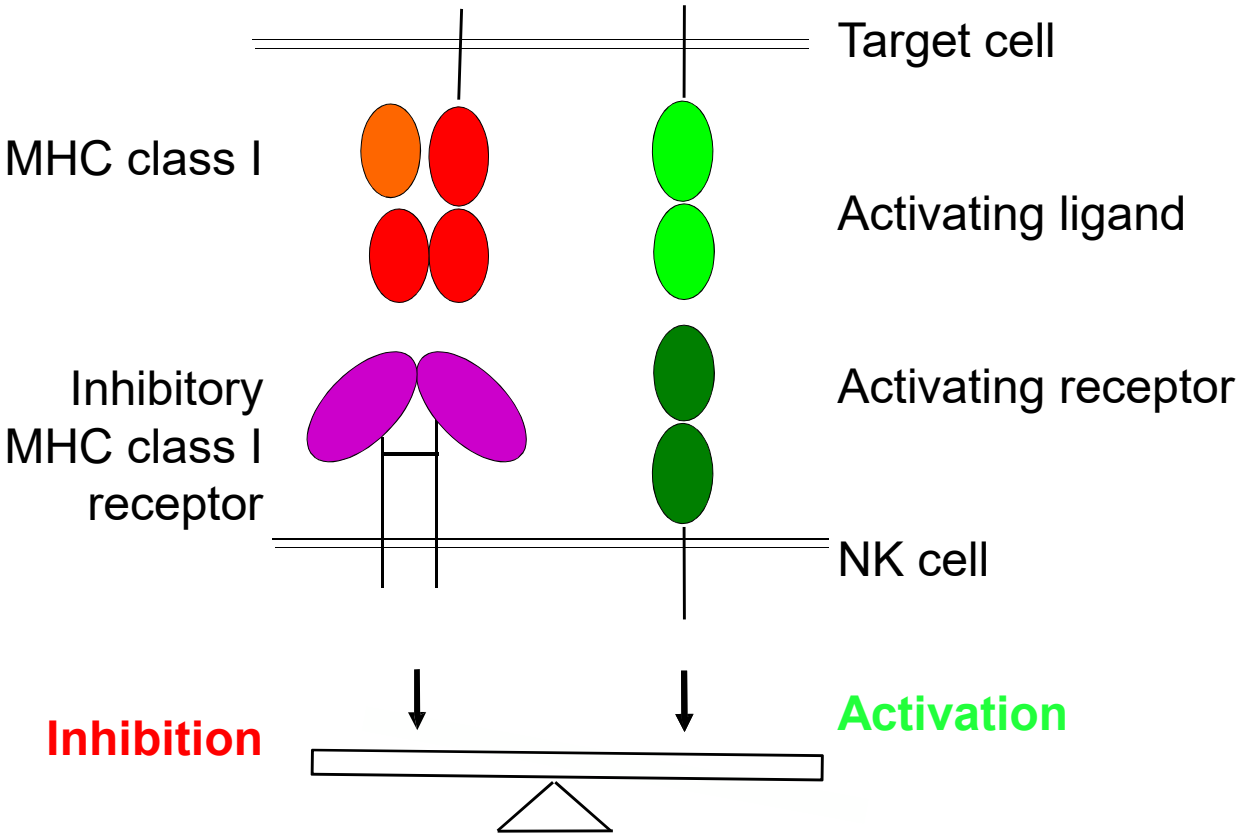
3. NK cell recognition

3.5 “Missing-self” recognition



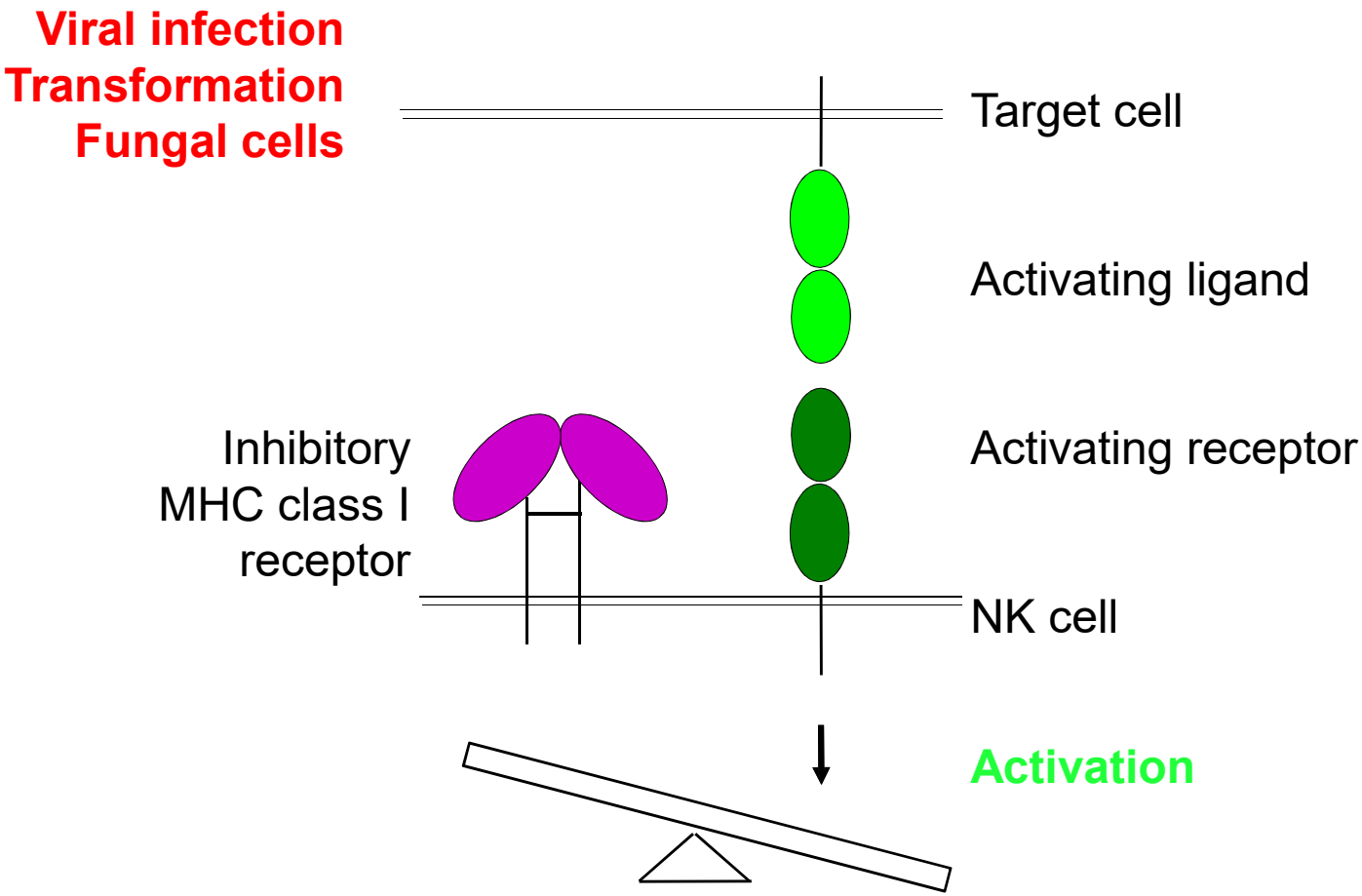
3. NK cell recognition

3.5 “Missing-self” recognition



3. NK cell recognition

3.5 “Missing-self” recognition

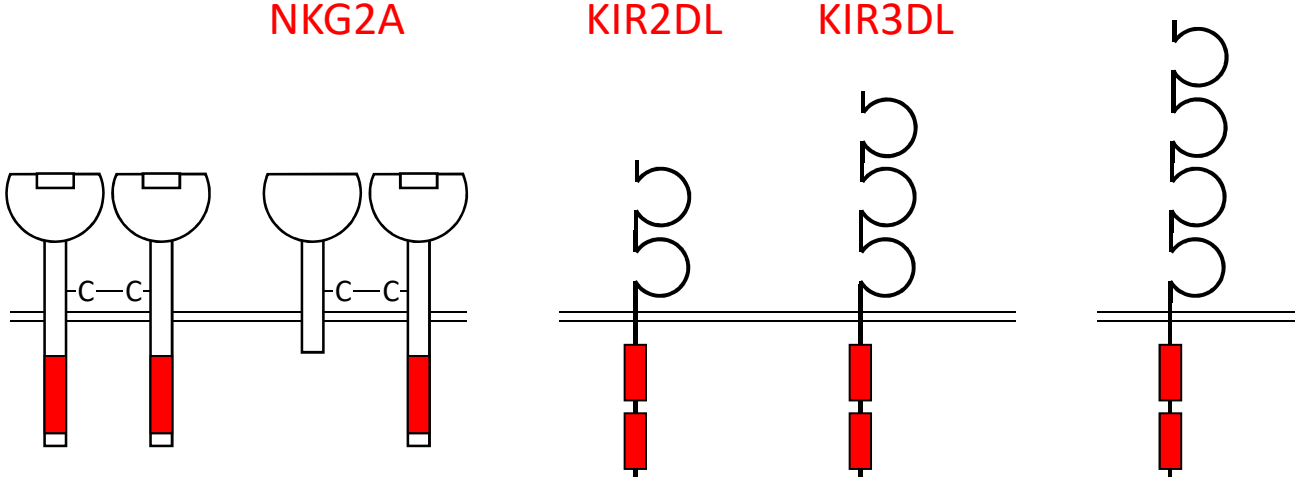


Klas Kärre (1981) « missing-self hypothesis »:
NK cells can kill cells when they do not express MHC class I molecules.

3. NK cell recognition

3.5 “Missing-self” recognition: Inhibitory receptors

Species	Mouse	Mouse / human	Human	Human	Human
Ligand	H-2K, H-2D and H-2L	Qa-1b / HLA-E	HLA-C	HLA-B, HLA-A	all MHC-I
Receptor	Ly49 family	CD94 / NKG2A	KIR family KIR2DL	KIR family KIR3DL	LILRB1



ITIM: I/VxpYxxL/V

Ig like domain

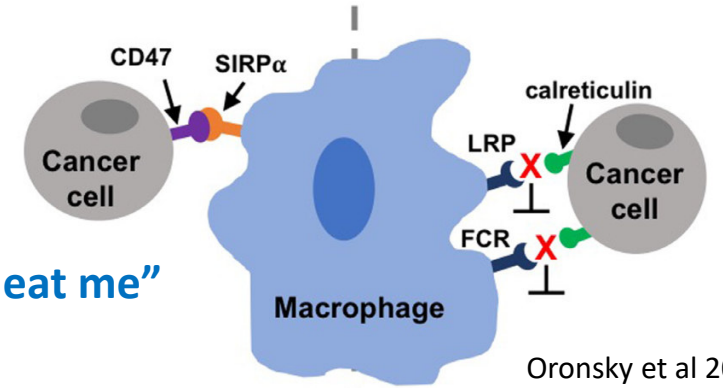
3. NK cell recognition

3.5 Missing-self recognition: Inhibitory receptors non-MHC specific

Receptor		Ligand	
NKR-P1B	mouse	Clr-b (Clec2d)	
p75/AIRM-1/Siglec-7	human	Sialic acid	
CEACAM1 (Carcino-embryonic cell adhesion molecule 1)	human	Homophilic	
2B4	mu/hu	CD48	e.g. SAP ko, XLP patients
KLRG1	mu/hu	E-, N-, R-Cadherin	

Similar mechanisms in other immune cells

PD-1	mu/hu	PD-L1, PD-L2	« checkpoint » for T cell activation and function
SIRP1α	mu/hu	CD47	« don't eat me » signal for Macrophages

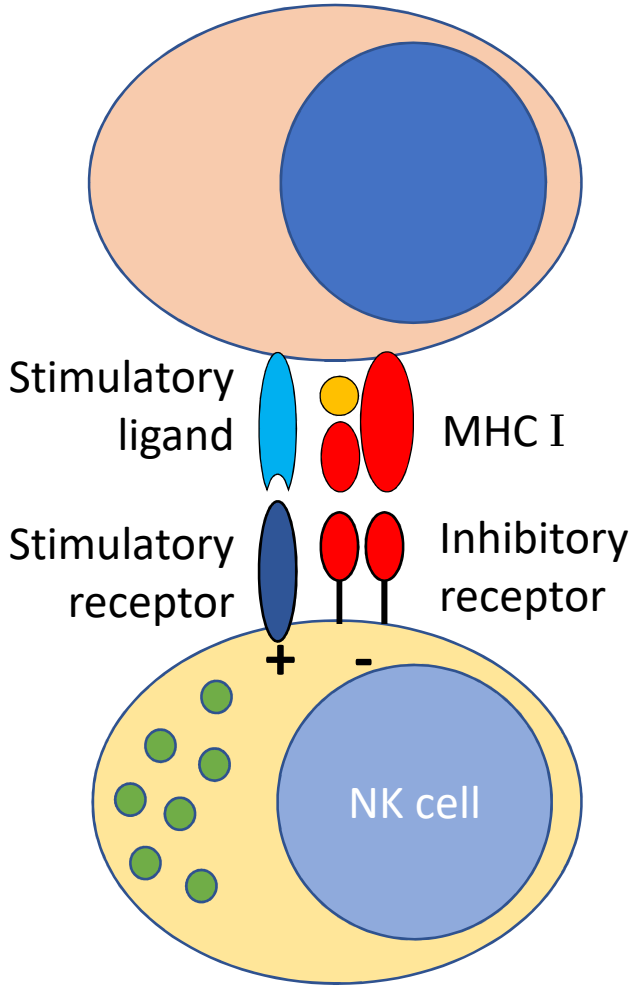


"Don't eat me"

"Eat me"

Oronsky et al 2020 Seminars in Oncology 47: 117

Summary: Inhibitory NK cell receptors



Missing-self

KIR / Ly49

CD94/NKG2A

LILRB1

KLRG1

Siglecs

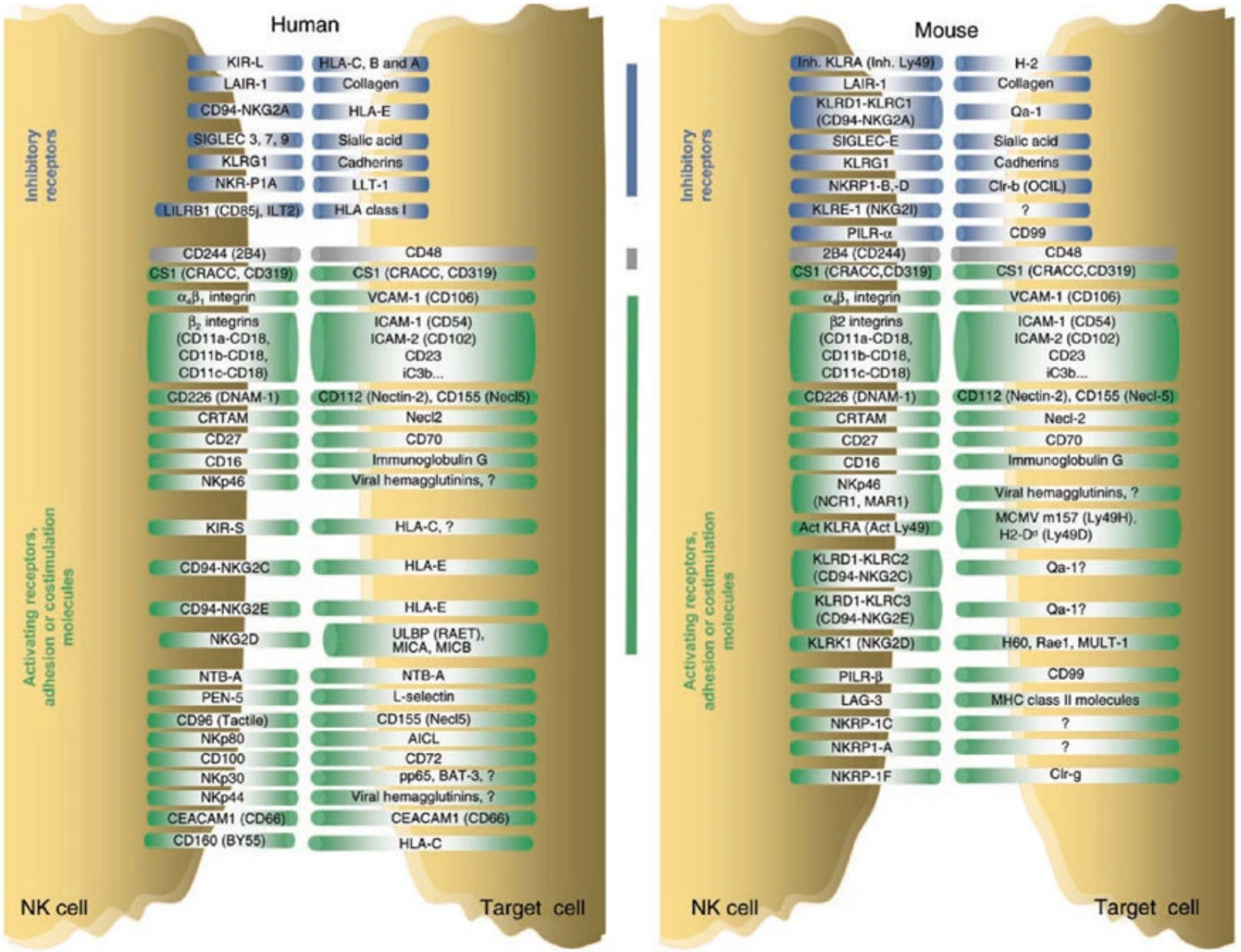
...

Non-self

Ly49C129

Specific for classical and non-classical MHC-I and for non-MHC molecules
Receptor engagement dampens NK cell effector function
The failure to engage inhibitory receptors explains « missing-self » reactivity

Summary: NK cell receptors

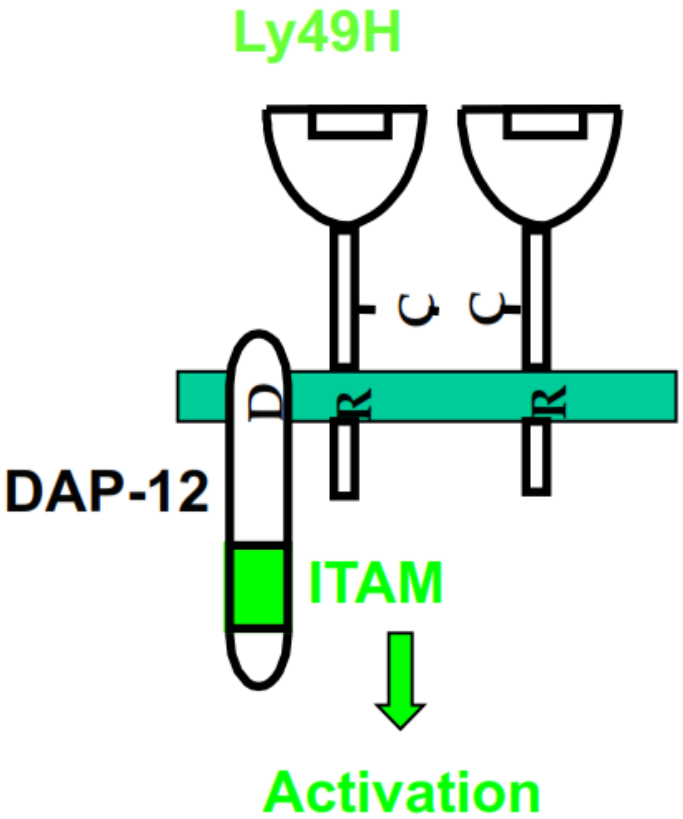


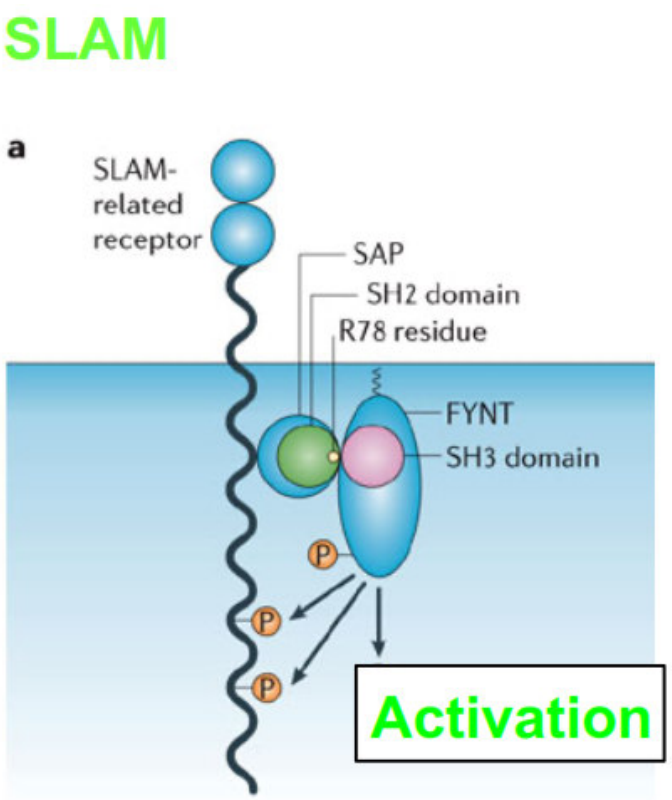
Vivier et al 2008 Nature Immunology 9 503

4. Signal transduction

Activation

Motifs		Adaptors	Recruits	Receptor
ITAM	pYxxL/Ixxxxxxxx**pY**xx(L/I)	DAP-12, FcRγ, CD3ζ	Syk, ZAP-70	NKG2D, NKp46
YINM	pYINM	DAP-10	PI-3K	NKG2D, Ly49H
ITSM	TIpYxxV/I	SAP	FynT	SLAM





Adapted from: Veillette 2006 NRI 6, 56-66

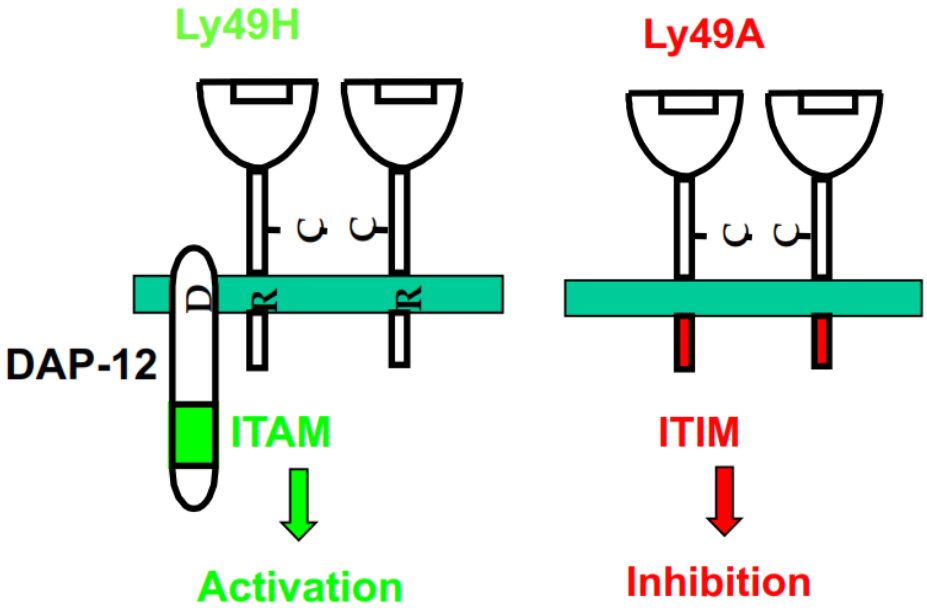
4. Signal transduction

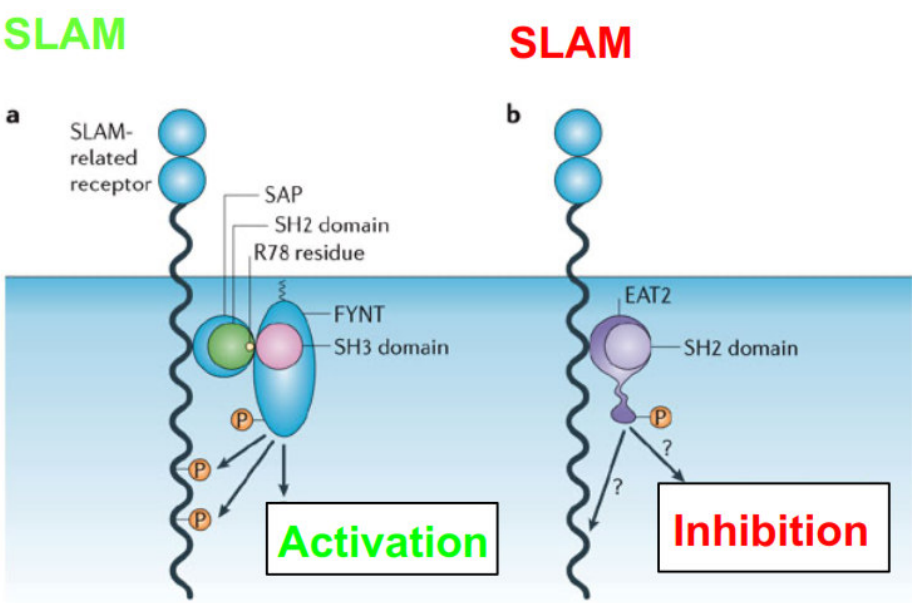
Activation

Motifs		Adaptors	Recruits	Receptor
ITAM	pYxxL/Ixxxxxxxpyxx(L/I)	DAP-12, FcRγ, CD3ζ	Syk, ZAP-70	NKG2D, NKp46
YINM	pYINM	DAP-10	PI-3K	NKG2D, Ly49H
ITSM	TIpYxxV/I	SAP	FynT	SLAM

Inhibition

Motifs		Adaptors	Recruits	Receptor
ITIM	L/IxpYxxL	-	SHP1, SHP2	KIR / Ly49
ITSM	TIpYxxV/I	EAT / no	SHIP	SLAM / 2B4

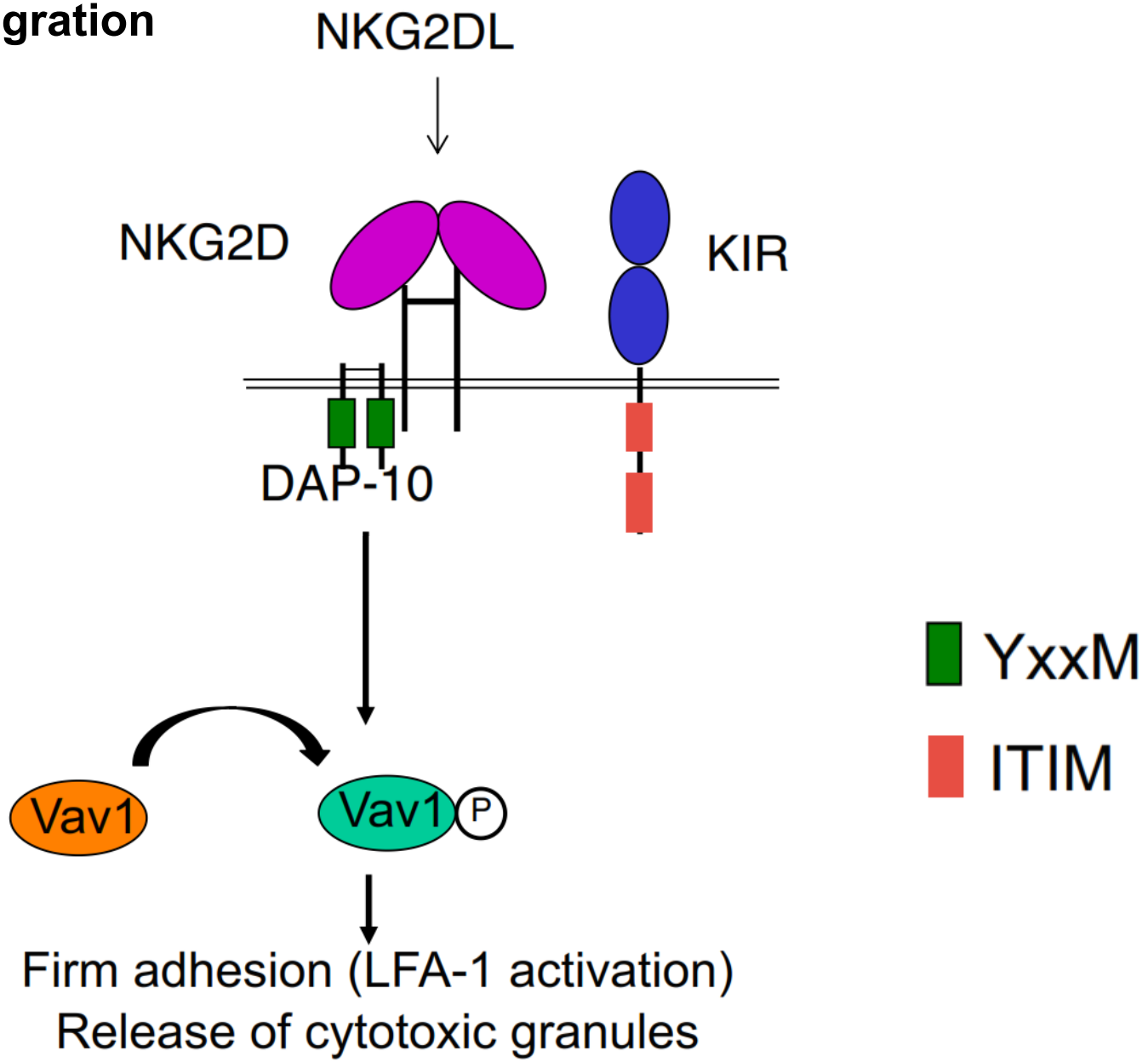




Adapted from: Veillette 2006 NRI 6, 56-66

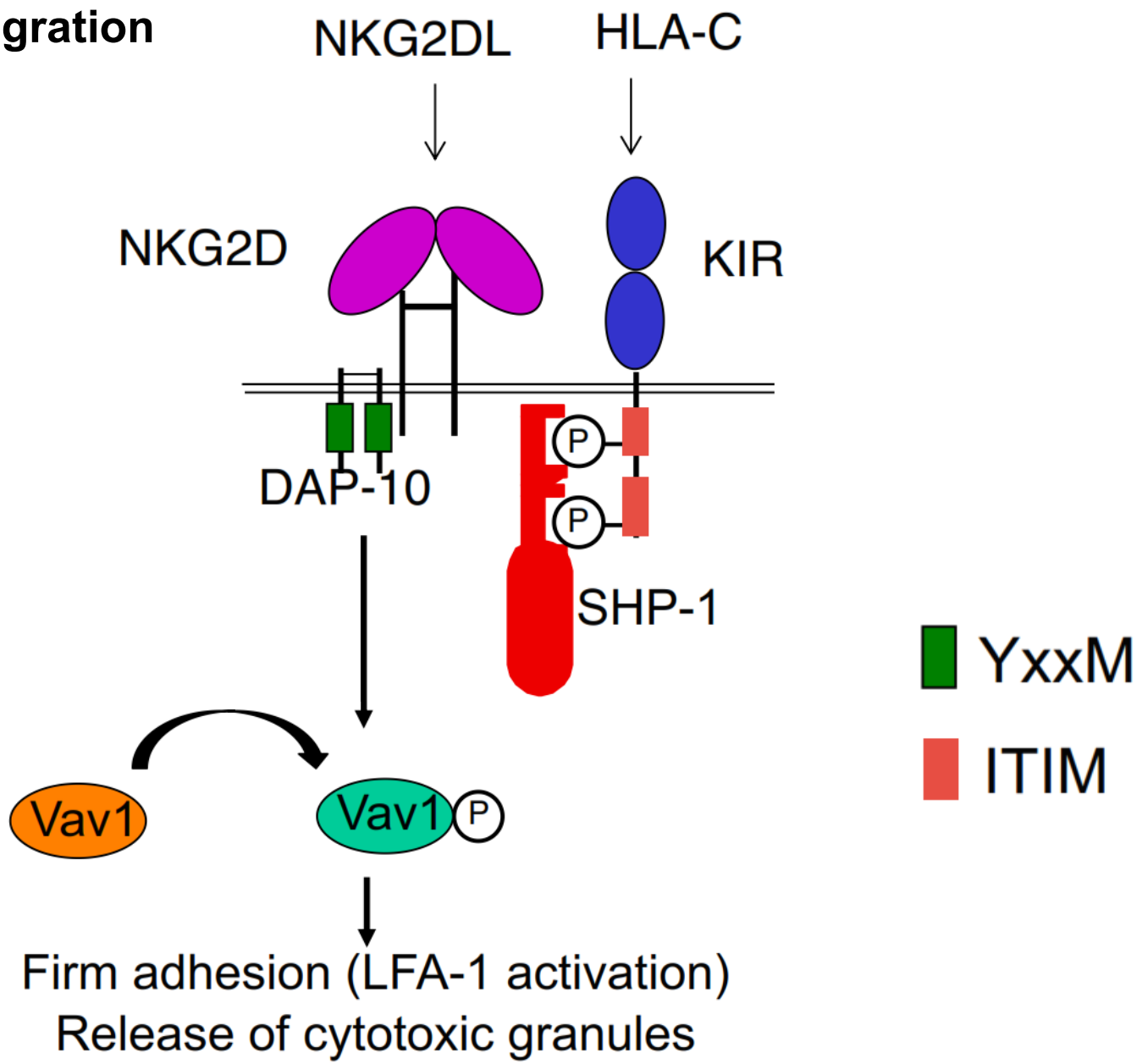
4. Signal transduction

Signal integration



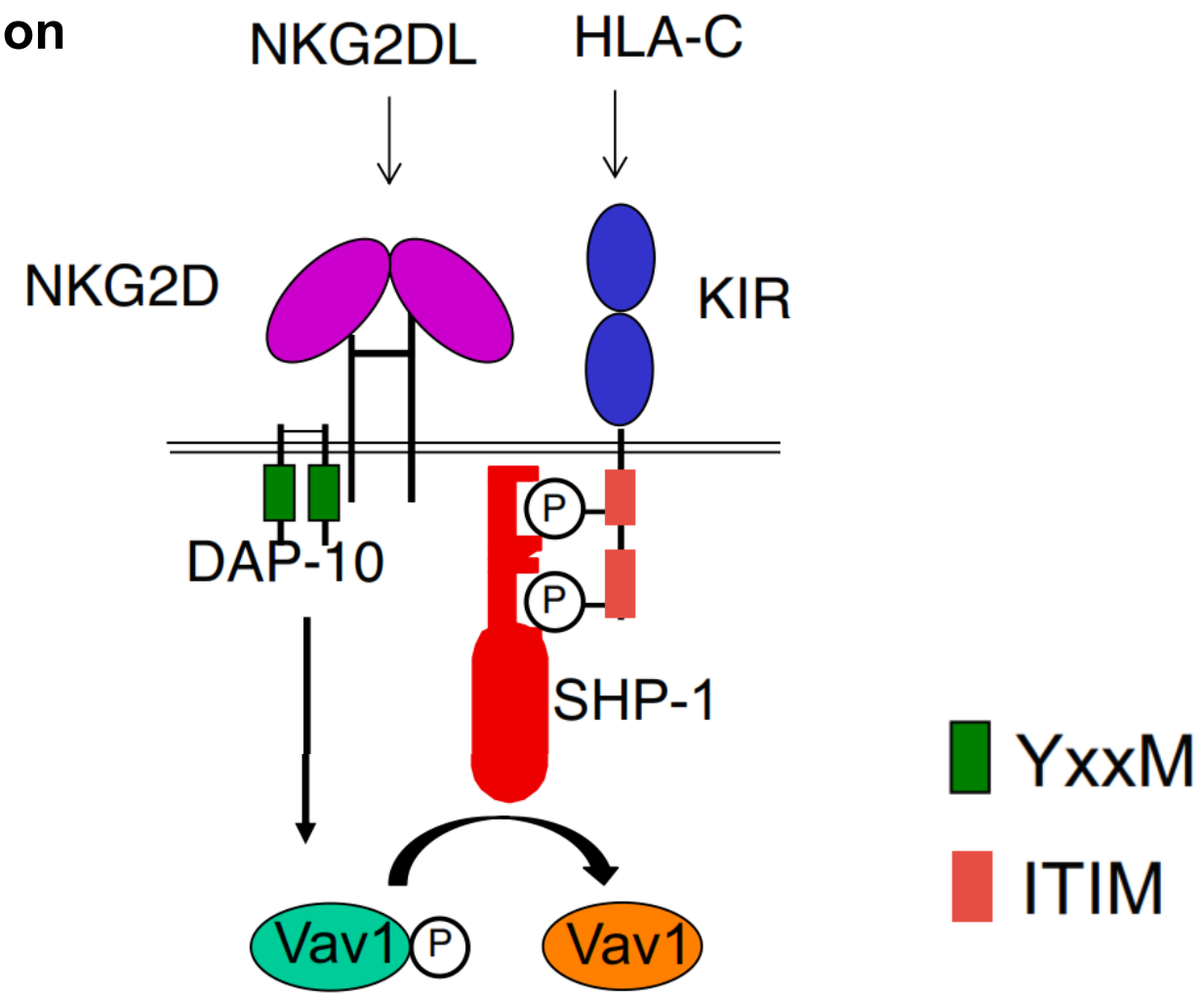
4. Signal transduction

Signal integration



4. Signal transduction

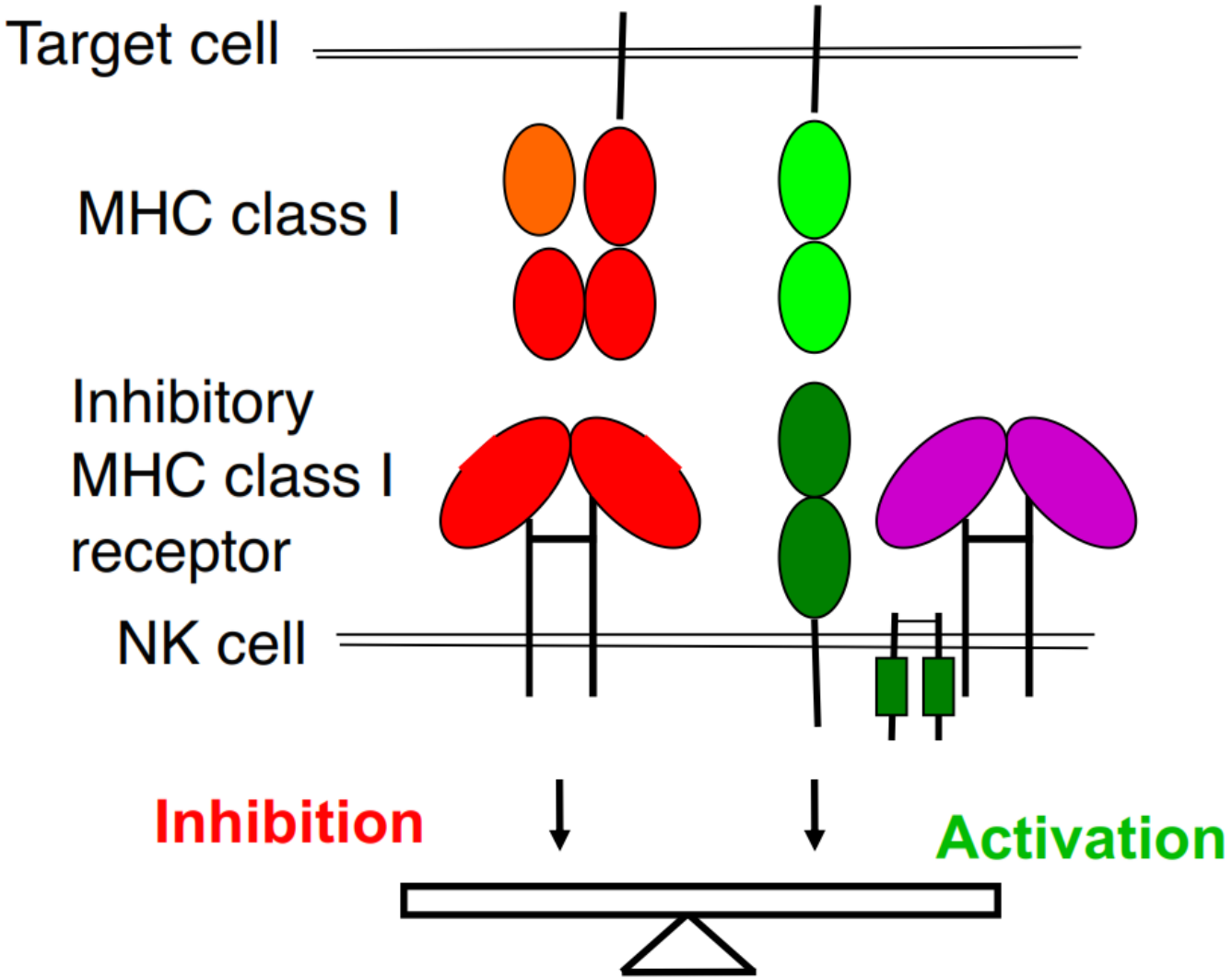
Signal integration



4. Signal transduction

Signal integration

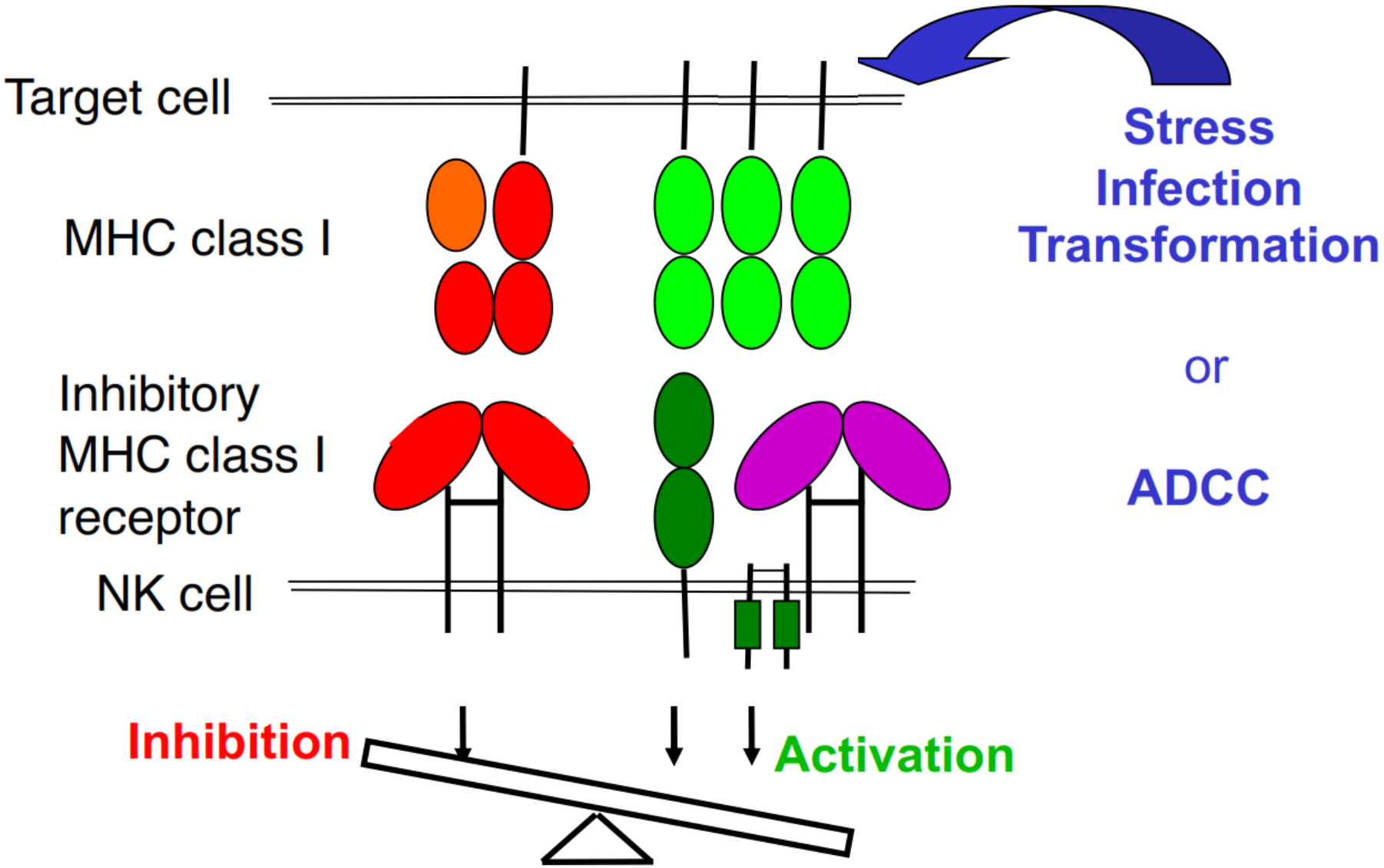
Induced self recognition



4. Signal transduction

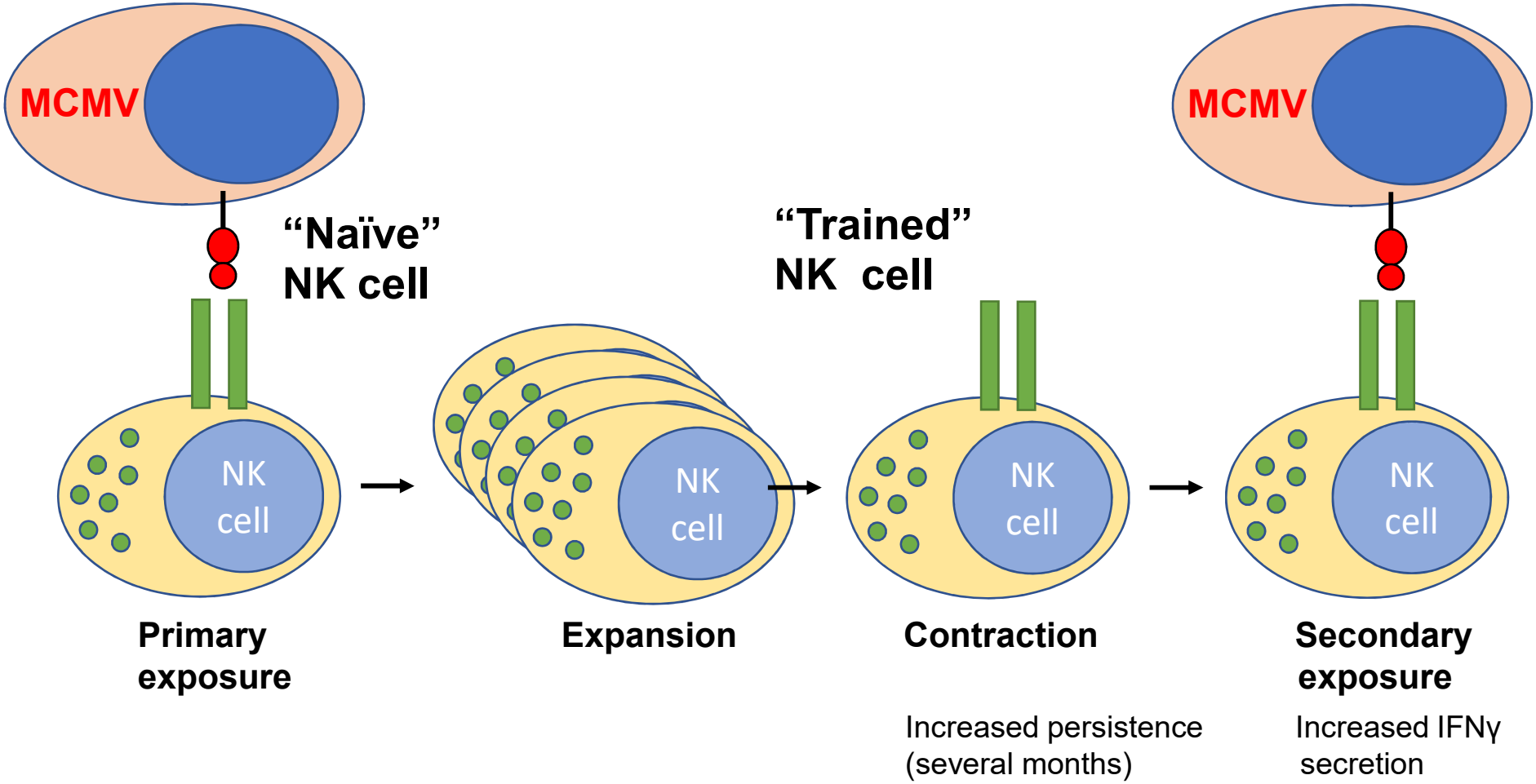
Signal integration

Induced self recognition



5. Adaptive features of NK cells

NK cell “memory” or “ trained immunity”



Can also be induced with cytokines (IL-12)

Sun et al 2009 Nature

6. NK cells and immunity to tumors

In vitro

Mouse and human NK cells kill many types of established tumor cells *in vitro*

In vivo: Tumor transplantation

- Rejection of transplanted tumors
- Mediate the anti-tumor effect of recombinant cytokines
  (IL-2, IL-12, IL-18 and IL-21)

  Guided by the presence/absence of NK cell receptor/ligands
  Promotes the development of tumor-specific T cell responses

Tumor immuno surveillance

-Protective role against chemically induced skin cancer
-Protect against spontaneous B cell lymphoma (arising in pfp/b2m-dko mice)
-NKG2D is protective against prostate cancer

Metastasis surveillance

- Recognize circulating tumor cells
- Prevent outgrowth of latent metastasis (Malladi 2016 Cell)

6. NK cells and immunity to tumors

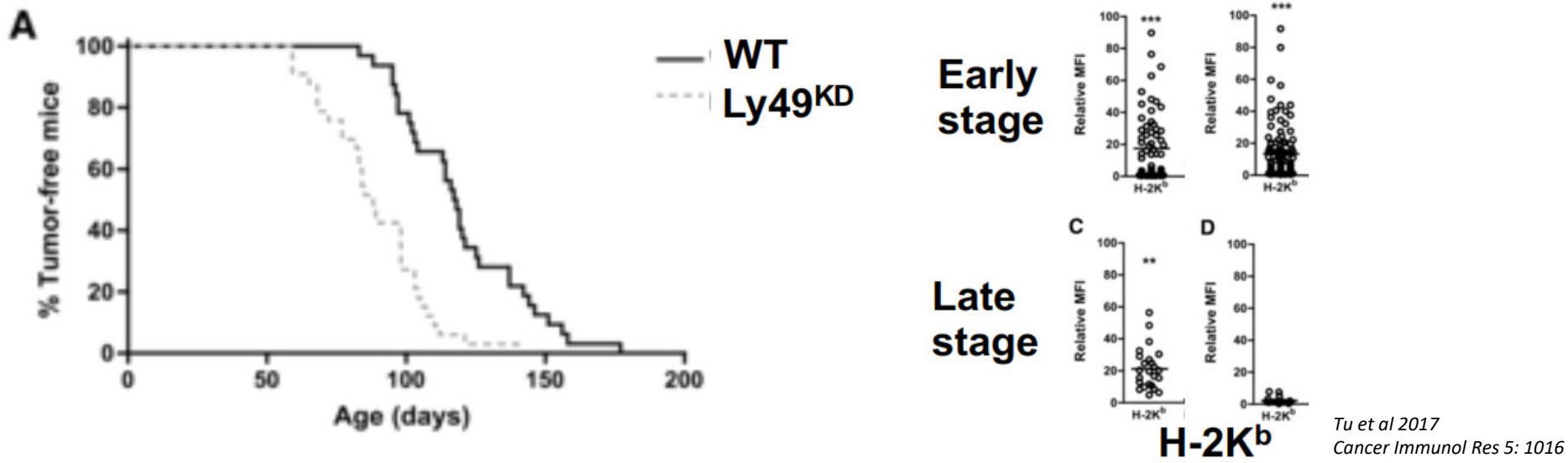
Loss or down regulation of MHC-I expression by tumor cells

Genome instability/DNA repair		-> mutations in *MHC-I* genes, chromosome breaks or loss, loss of the *b2m* gene or a lack of b2m expression. -> mutations in the machinery required for antigen processing and loading, including TAP1
Oncogenes	myc	-> Suppression of MHC-I expression
Tumor suppressors	PML	-> Suppression of MHC-I expression
Loss of TF	NLRC5	-> Required for MHC-I expression (hematop. cells)

By killing MHC-I^{+} cells, T cells enrich for MHC-I^{-} tumor variants

NK cell missing-self recognition can protect against MHC-I^{-} or MHC-I^{low} tumors

Ly49-deficient MMTV-PyMT mice



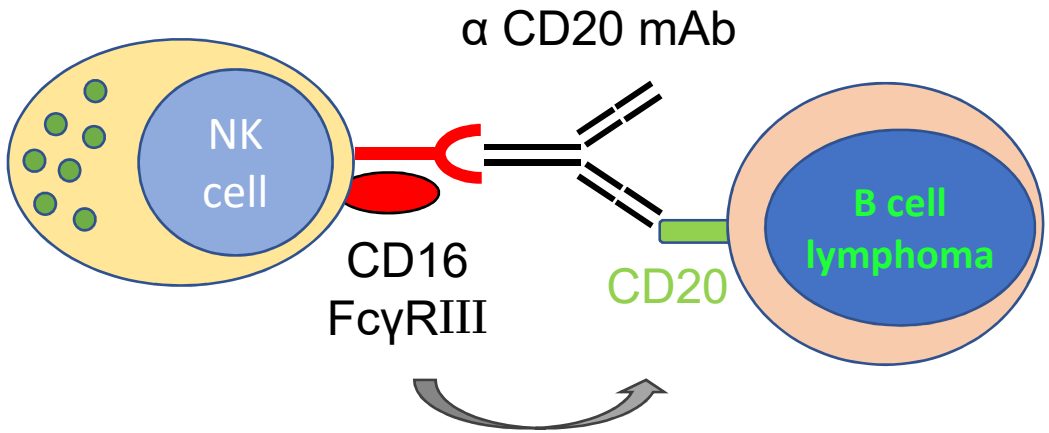
Tu et al 2017
Cancer Immunol Res 5: 1016

6. NK cells and immunity to tumors

Human Cancer Patients

- Low activity of blood NK cells is associated with increased cancer risk
- Patients with infiltration of NK cells into colon cancer have a better prognosis
- Tumor escape from NK cell control: soluble NKG2D ligand, NKp46 ligand low

- role of NK cell ADCC in the treatment of cancer
  e.g. anti-CD20 for treatment of non-Hodgkin B cell lymphoma

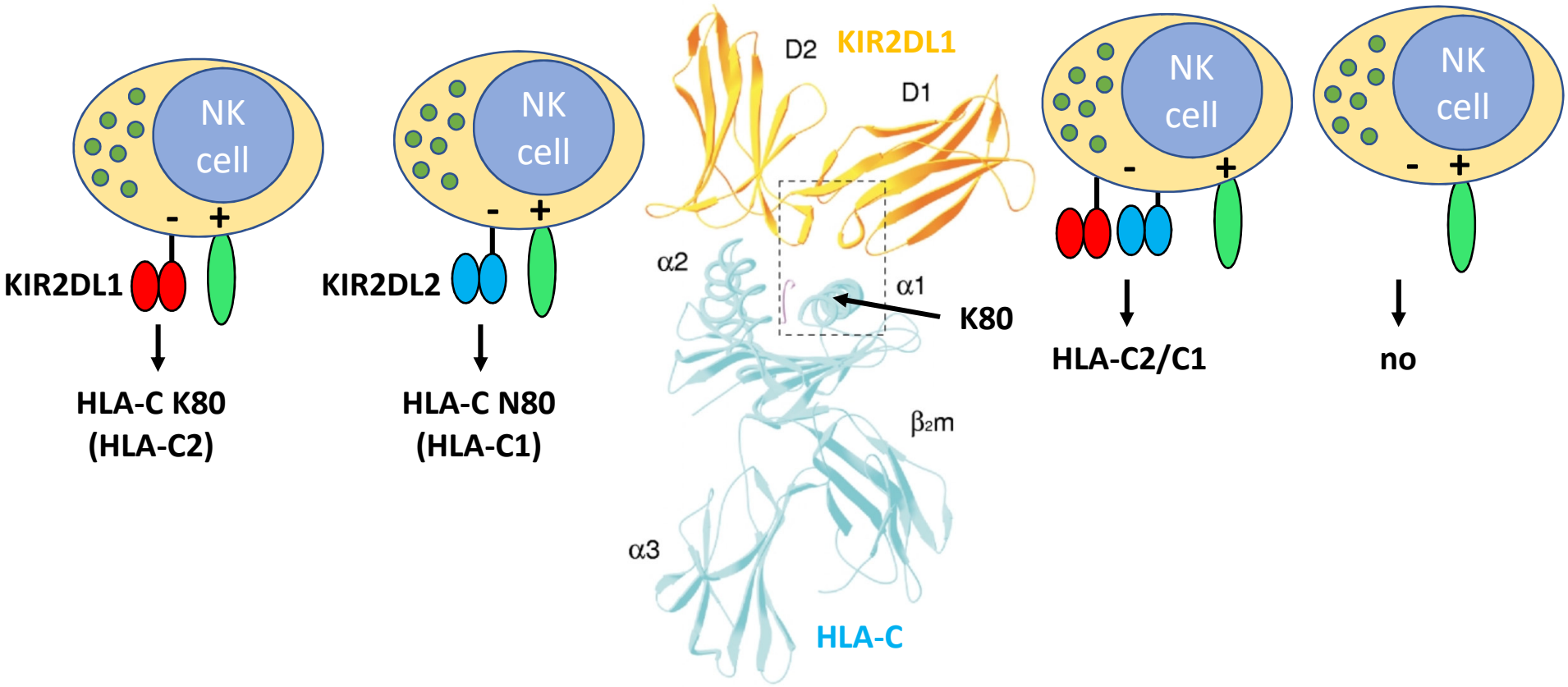


ADCC: Antibody dependent cell mediated cytotoxicity

6. NK cells and immunity to tumors

Graft versus leukemia effect

Diverse receptor distribution and ligand recognition



Fan et al 2001 Nature Immunology 2, 452 - 460

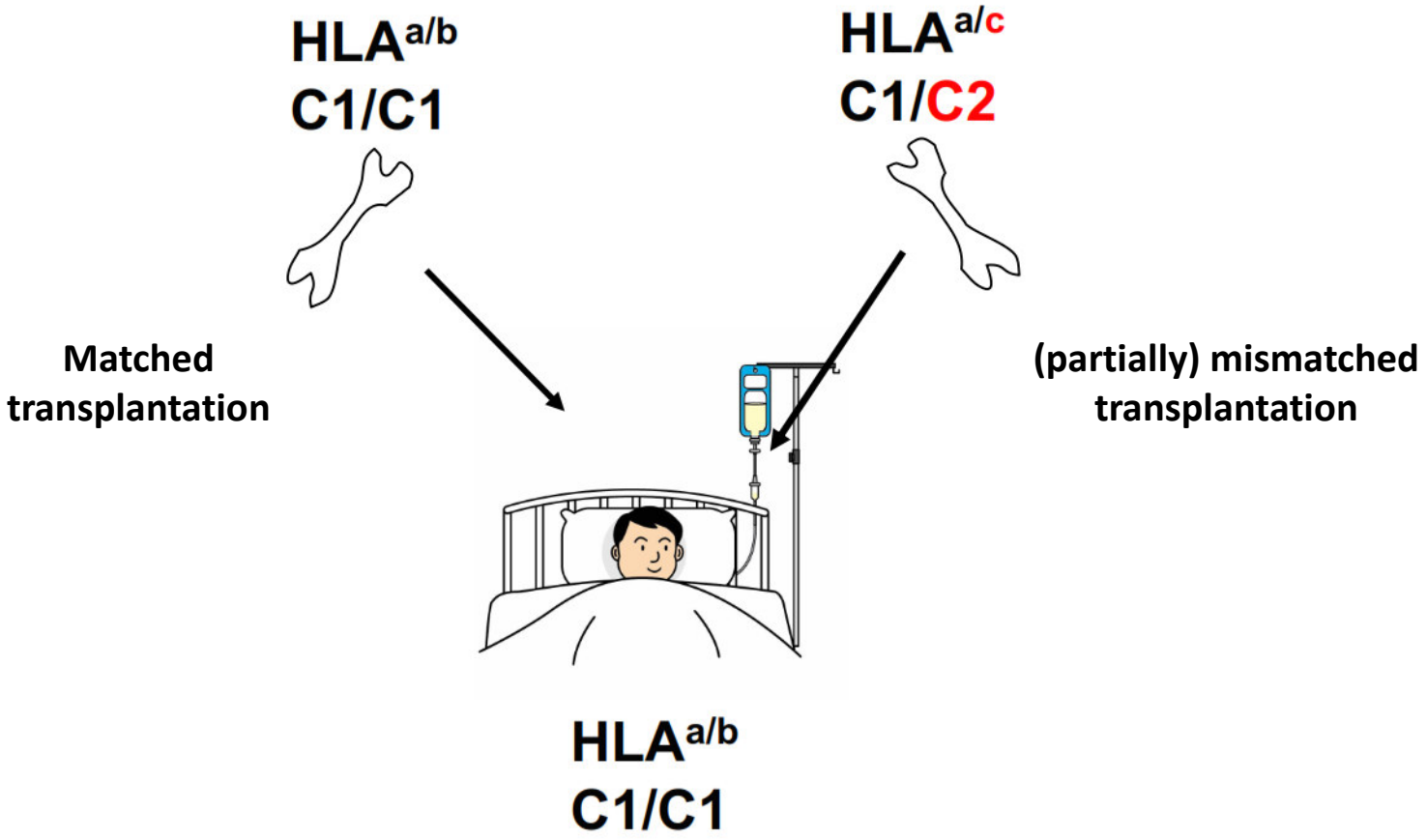
6. NK cells and immunity to tumors

Human bone marrow transplantation

Acute myeloid leukemia

-treated with allogeneic hematopoietic stem cell transplantations

-T cell and NK cell mediated **graft versus leukemia** (GvL) reactions



6. NK cells and immunity to tumors

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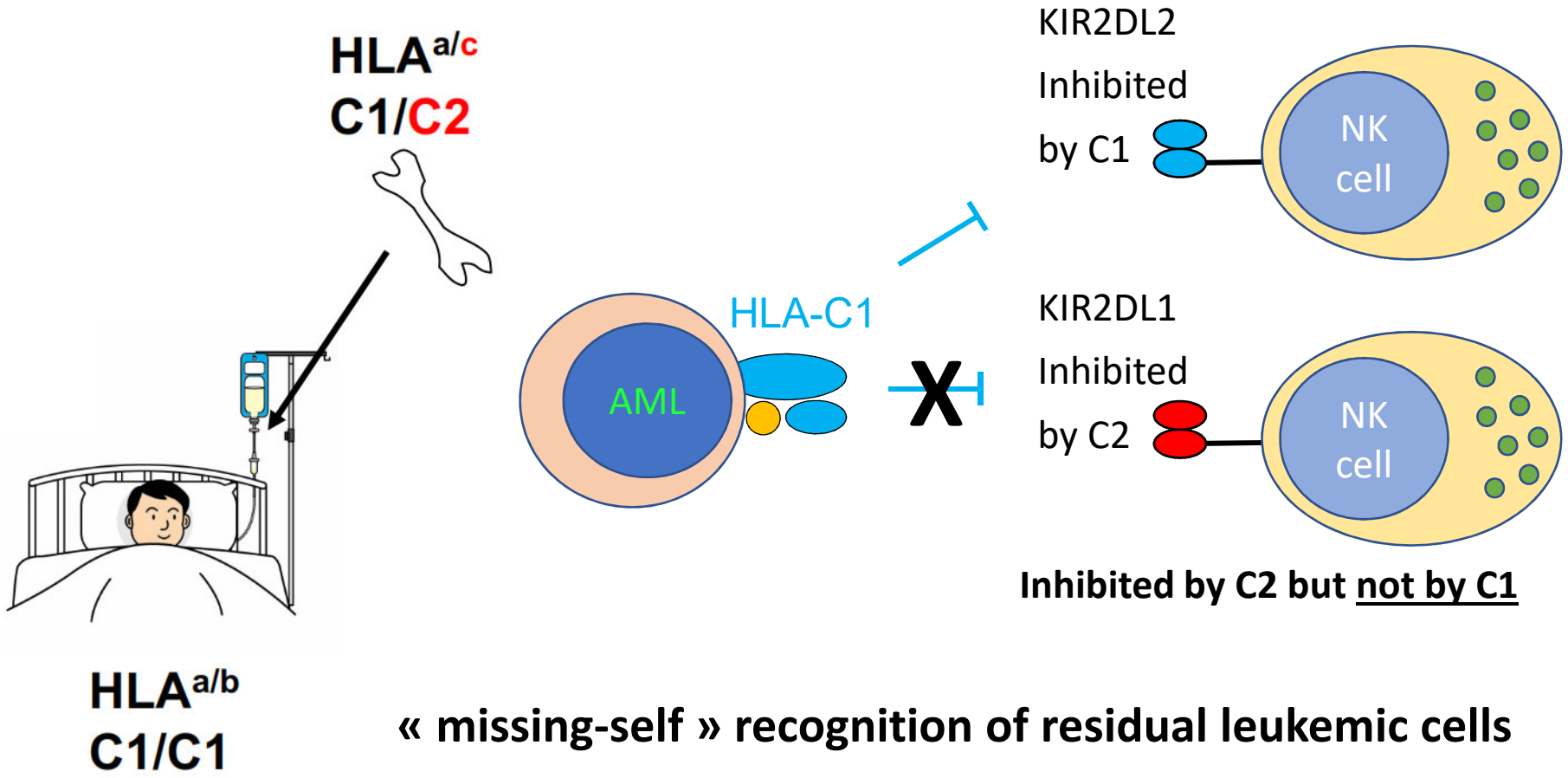
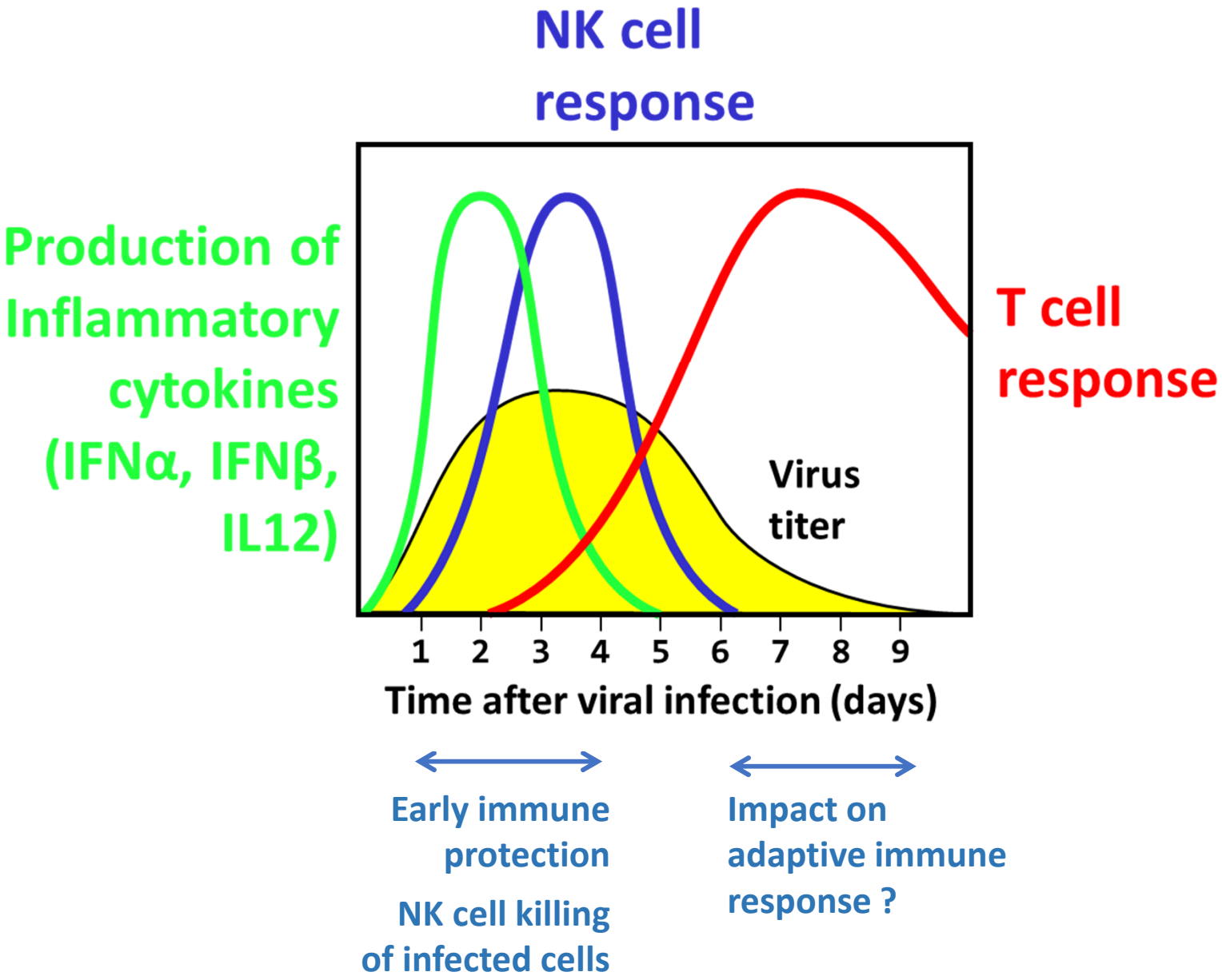


Inhibited by C2 but <u>not by C1</u>

« missing-self » recognition of residual leukemic cells

7. Immunoregulation



7. Immunoregulation

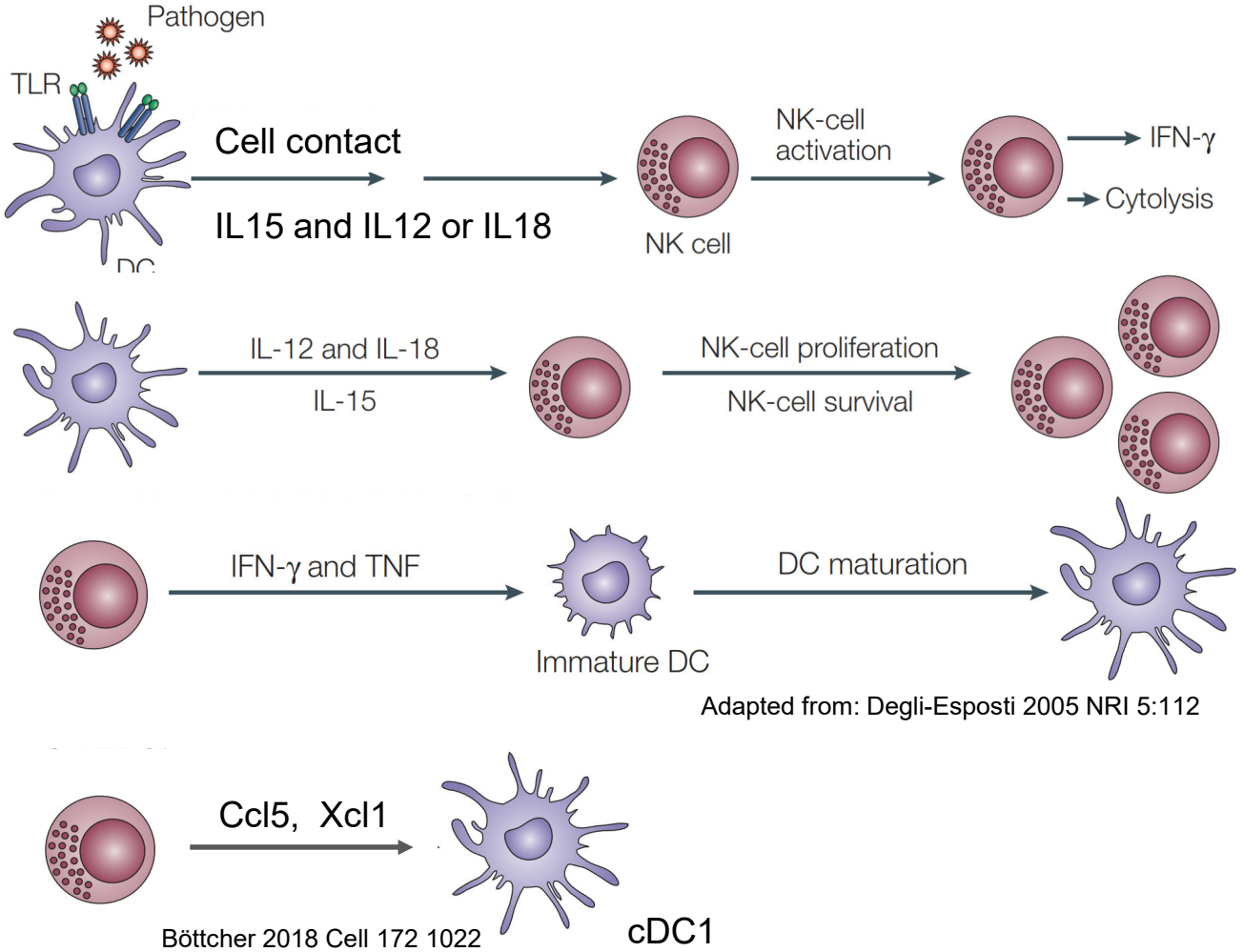
NK – DC crosstalk

DC-mediated NK cell activation

DC-mediated NK proliferation

NK cell-mediated DC activation

NK cell-mediated DC recruitment to tumors

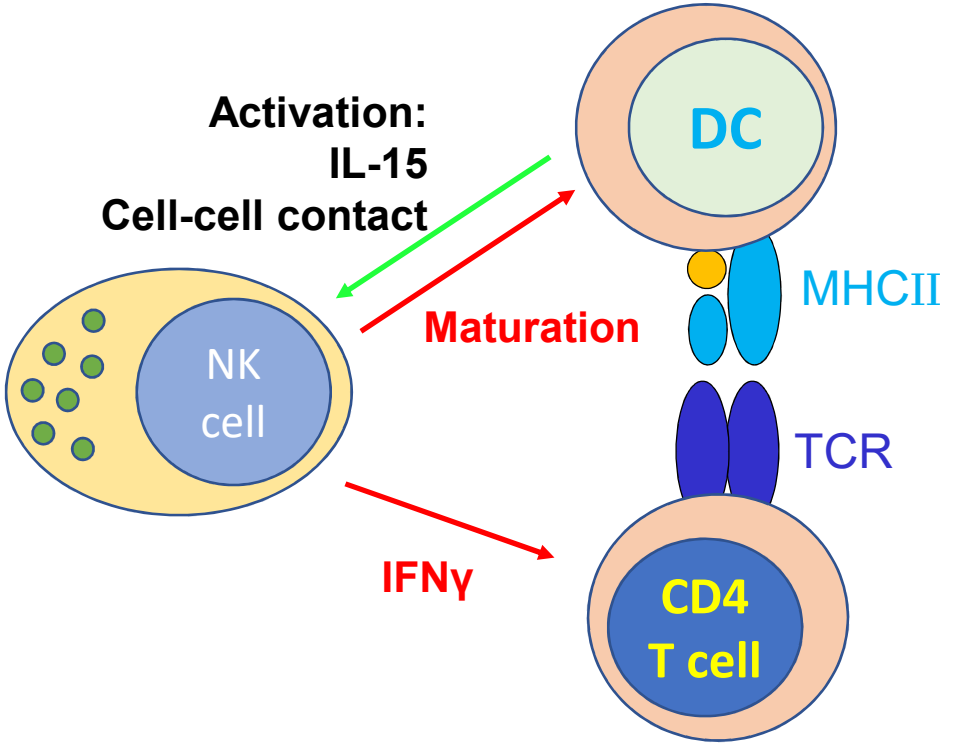


Adapted from: Degli-Esposti 2005 NRI 5:112

Böttcher 2018 Cell 172 1022

7. Immunoregulation

T cell priming

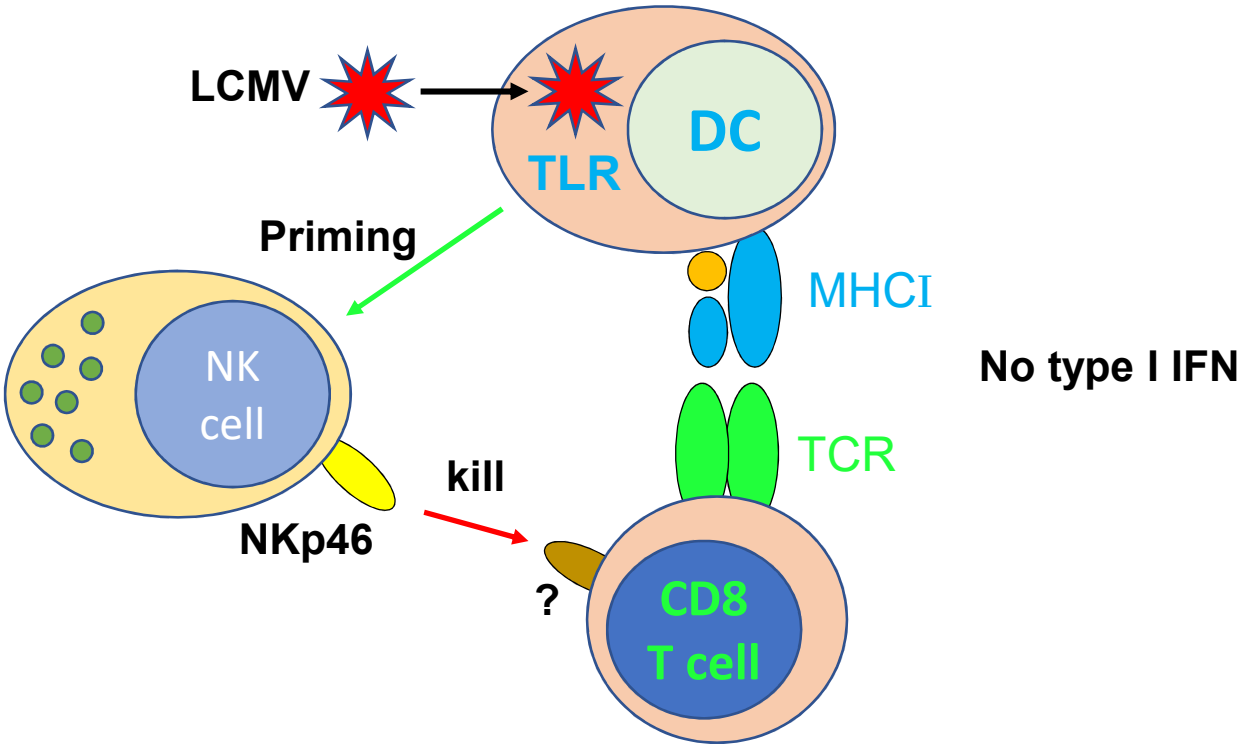


Promotes protective TH1 differentiation

Vaccination: Martin Fontecchia 2004 Nat.Imm 5 1260

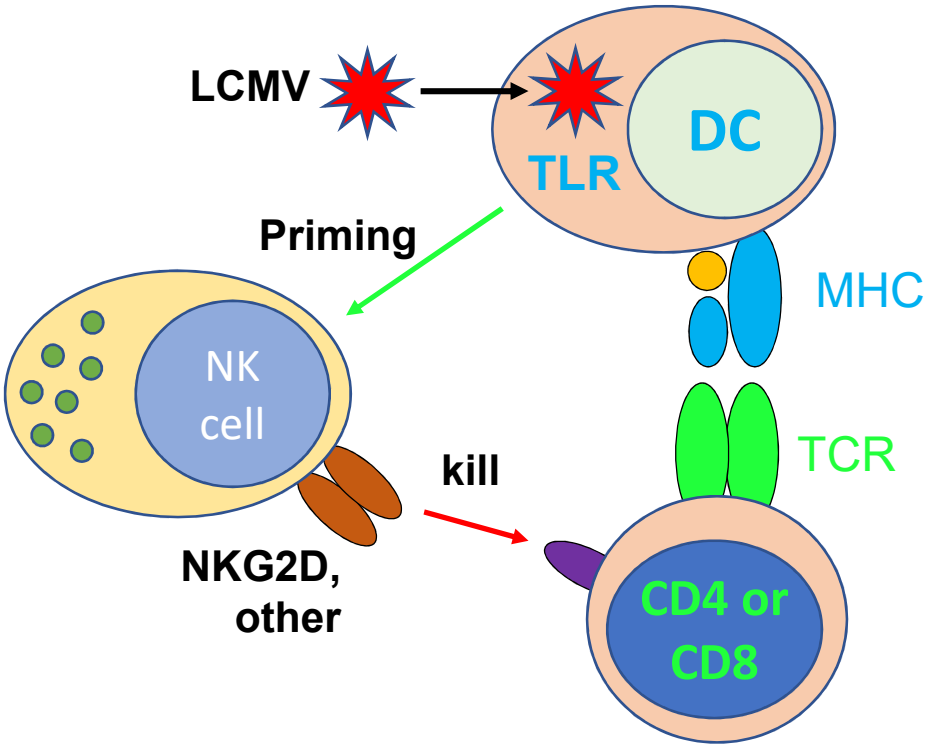
7. Immunoregulation

Elimination of aberrantly activated T cells



7. Immunoregulation

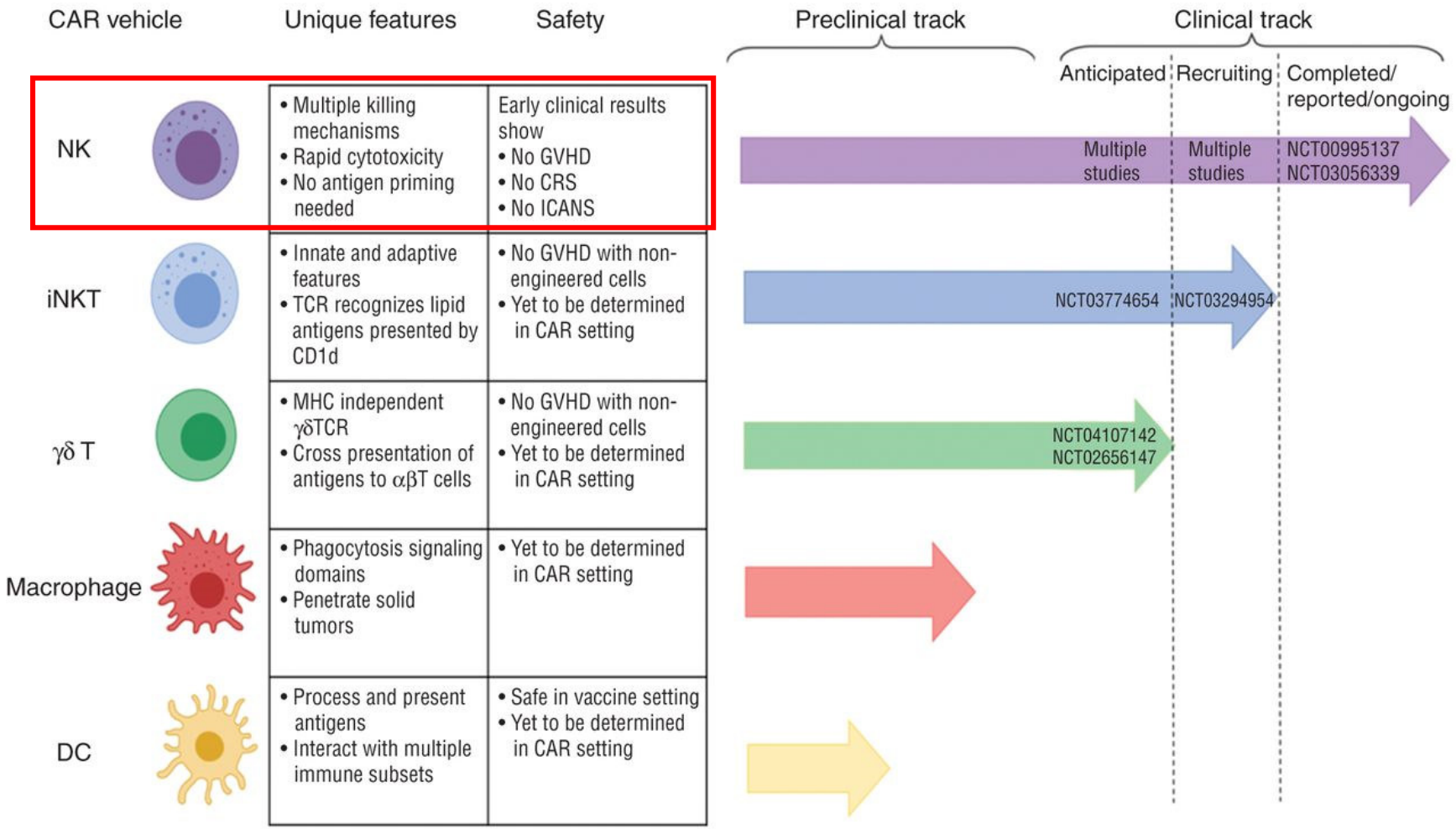
Restraining effector cells



- Dampens the immune response
- Reduces immunopathology during chronic infection

LCMV infection Waggoner 2011 Nature
Lang 2012 PNAS 109 1210
Vaccination Soderquest 2011 JI 186 3304

Natural Killer (NK) for cell based immunotherapies



Daher & Rezvani, Cancer Discovery (2021)

Frequent Problems with CAR T cell therapies:
Requirement for personal manufacturing

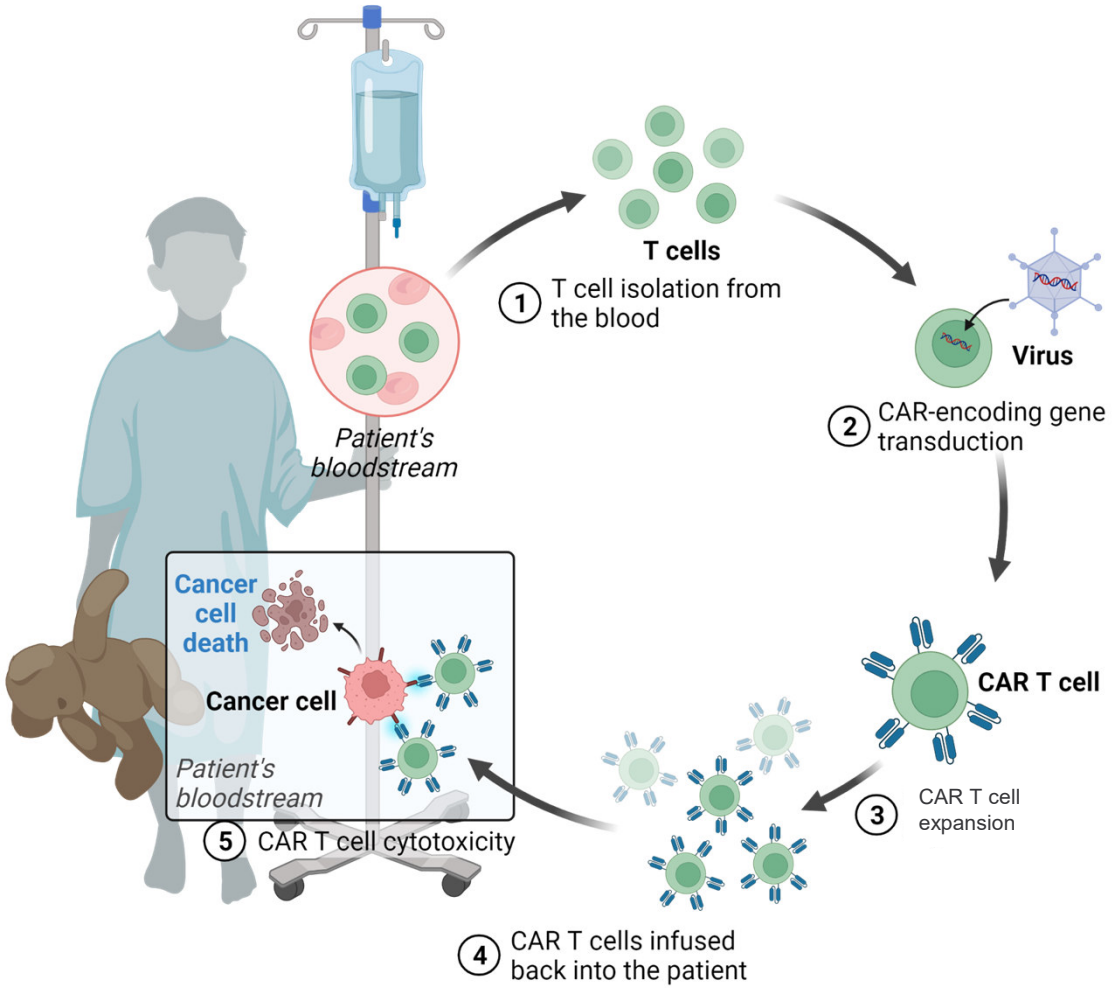
T cells need to be syngeneic, otherwise they can react to self-antigens

Manufacturing problems:

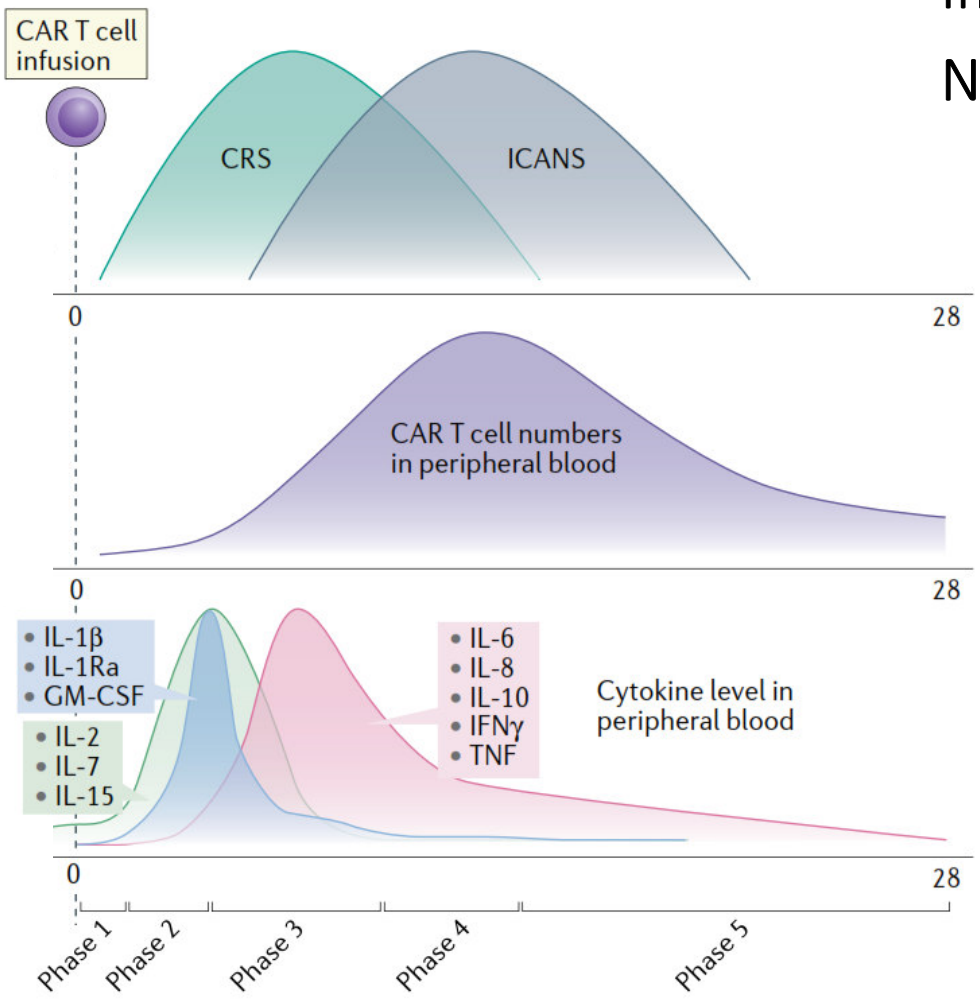
- Complex
- Costly
- Non-homogenous
- Failure to manufacture

NK cells can be allogeneic since many inhibitory receptors react to invariant MHCs

Manufacturing in large batches and even from cell lines possible



Frequent Problems with CAR T cell therapies:
Side effects



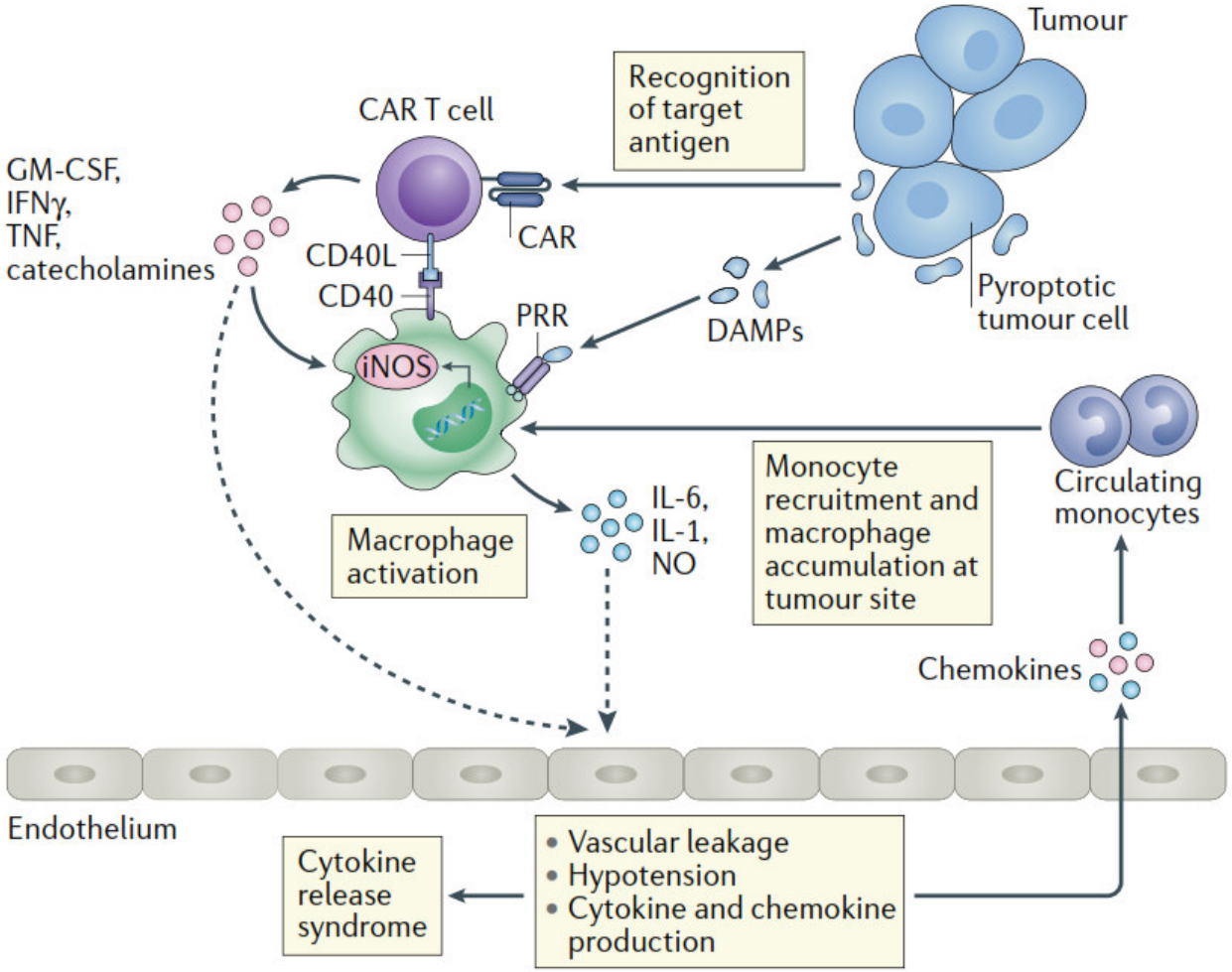
adapted from Morris et al., Nature Rev. Immunology 2022

Cytokine Release Syndrome (CRS)

Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS)

- CRS is observed in 60-90% of cancer patients treated with CAR T cells
  (≥ grade 3 CRS in 15-30%)
  grade3: fever, hypotension, hypoxia
- ICANS in 30-60% of treated patients
  (≥ grade 3 ICANS in 11-30%)
  grade3: epileptic seizures, cerebral oedema
- CRS and ICANS have been observed irrespective of the target antigen of the CAR, but only with CAR T cells, CAR NK cells don't show these side effects

Pathophysiology of CRS and ICANS



Morris et al., Nature Rev. Immunology 2022

in situ cytokine production by both activated CAR T cells and cells of the tumor micro-environment such as macrophages and recruited monocytes cause a systemic inflammatory response involving IL-6 and IL-1 which leads to endothelial injury and vascular leakage in multiple organs causing hypoxia, hypotension and/or organ damage

breakdown of the blood–brain barrier coincides with the onset of ICANS

Summary

Key functions of NK cells

Direct elimination of certain cells

- NK cells recognize « self » and attack cells that express « self » aberrantly (tumor and infected cells)
- NK cells can also react to virus infected cells by recognizing viral proteins (« non-self ») on infected cells (MCMV m157).
- NK cell recognition is exploited to mediate GvL (missing self)
- NK cells can kill Ab coated cells (ADCC)

Immunoregulatory roles

- Production of immunoregulatory cytokines
- «Talk» to DC and influence adaptive immune responses indirectly
- Recognize T cells and directly influence adaptive immune responses

Therapeutic functions

- CAR NK cells can be produced upfront and show better safety profiles than CAR T cells, but lack memory function