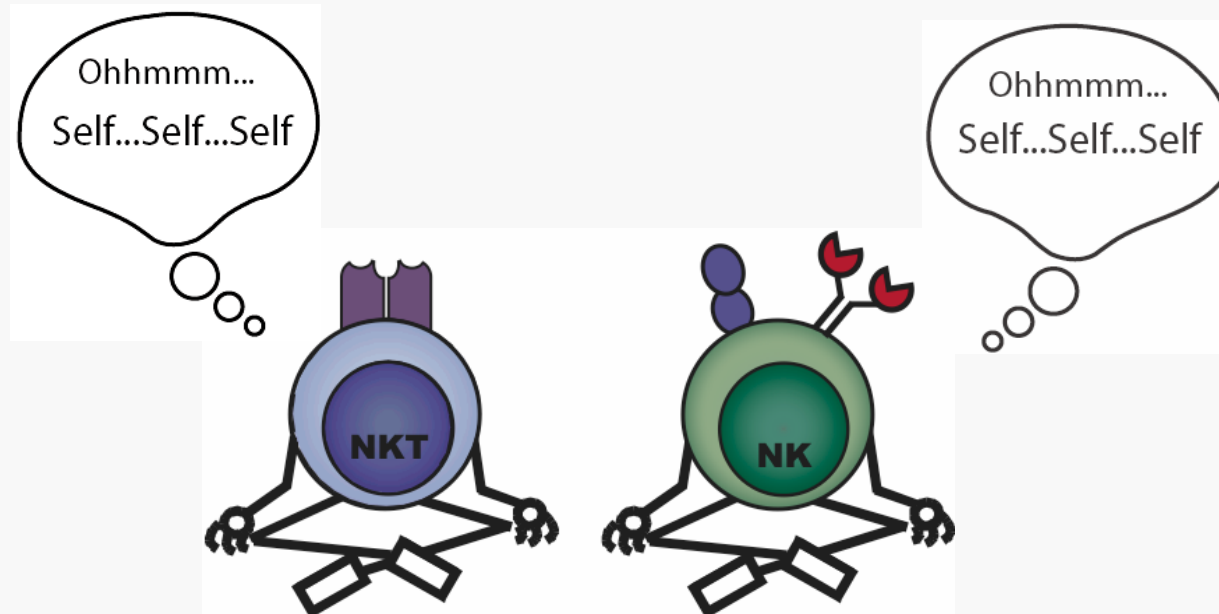


# NK cells and NKT cells: a brief overview of recognition mechanisms

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# Presentation Overview

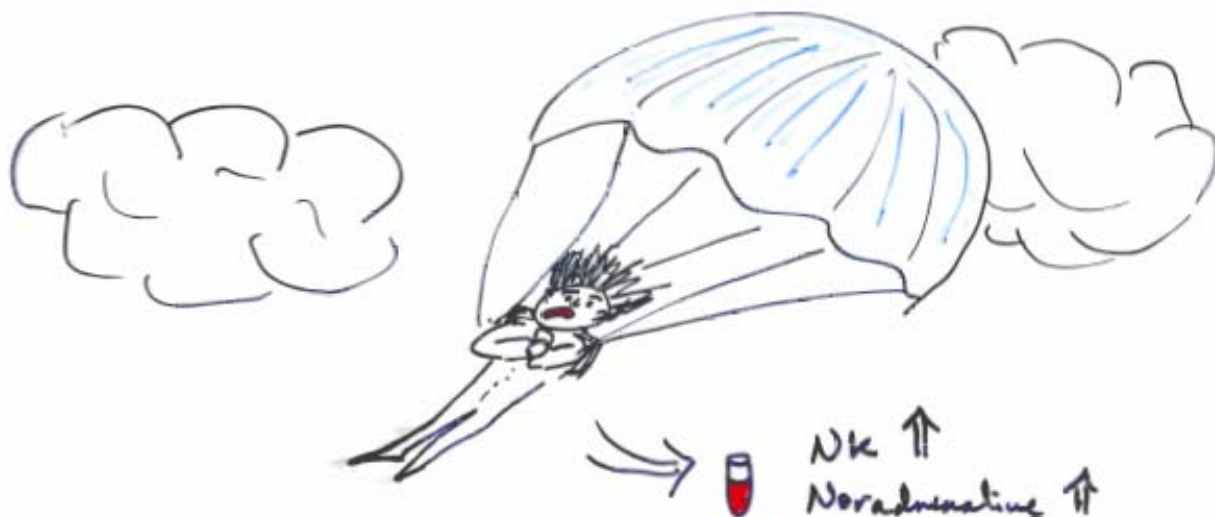
- I.)
- What are NK cells?
  - What makes NK cells tick?
  - NK receptors - structure and function

- II.)
- What are NKT cells?
  - How do NKT cells become activated?
  - NKT cells in anti-cancer responses

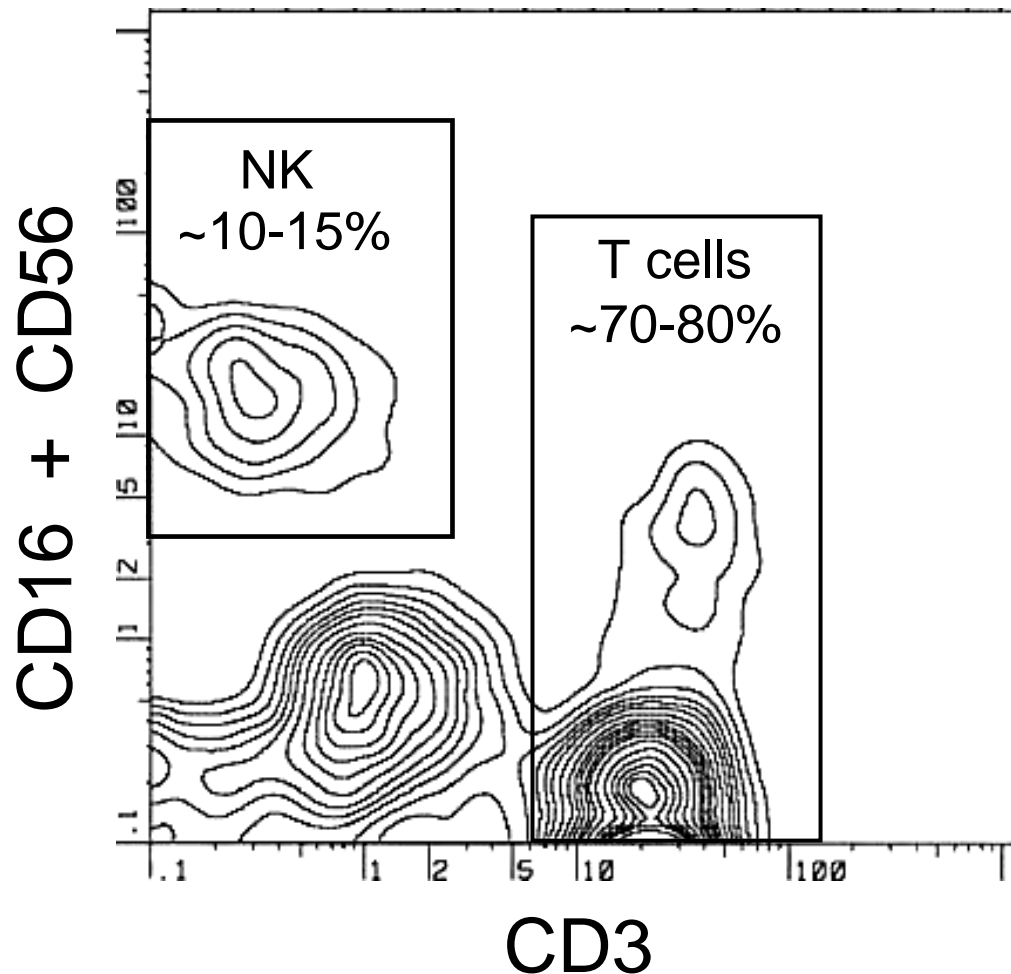
# I.) What are NK cells?

- Initially: “large granular lymphocytes”
- Kill tumor cells in vitro without prior exposure
- Important producers of  $T_{H1}$  cytokines, esp. IFN
- Rapid migration to sites of inflammation
- No memory - short lifespan

➤ “fight or flight” lymphocytes



# Human peripheral blood lymphocyte staining for NK cells and T cells

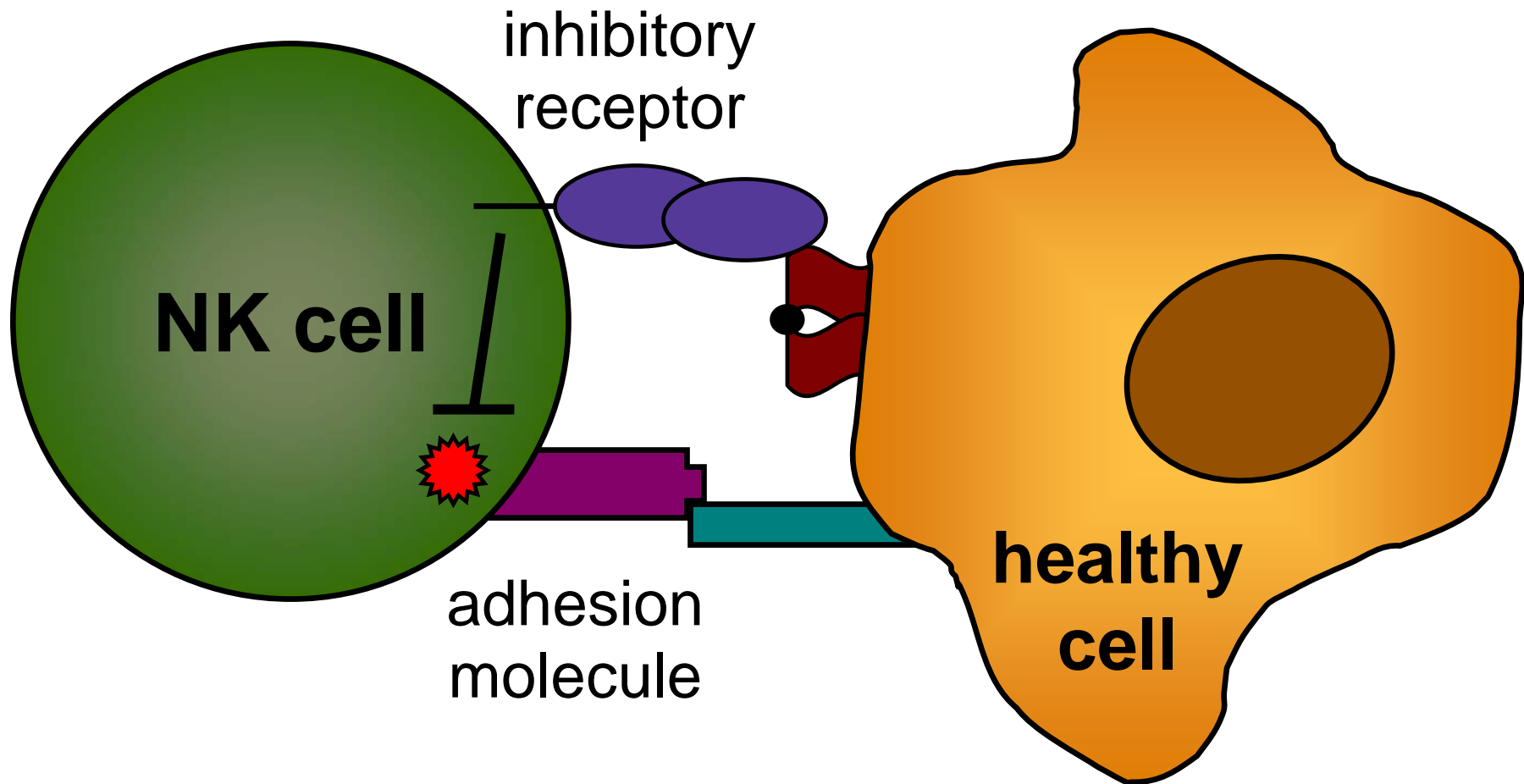


# What makes NK cells tick?

(how are the functions of NK cells regulated?)

- “priming” by exposure to cytokines
  - IL-12, IL-2, IFN  $\gamma$  , IFN  $\alpha$
  - Altered functions from exposure to T<sub>H</sub>2 cytokines?
- Positive and negative signals from cell surface receptors

# NK cell regulation by activating and inhibitory receptors

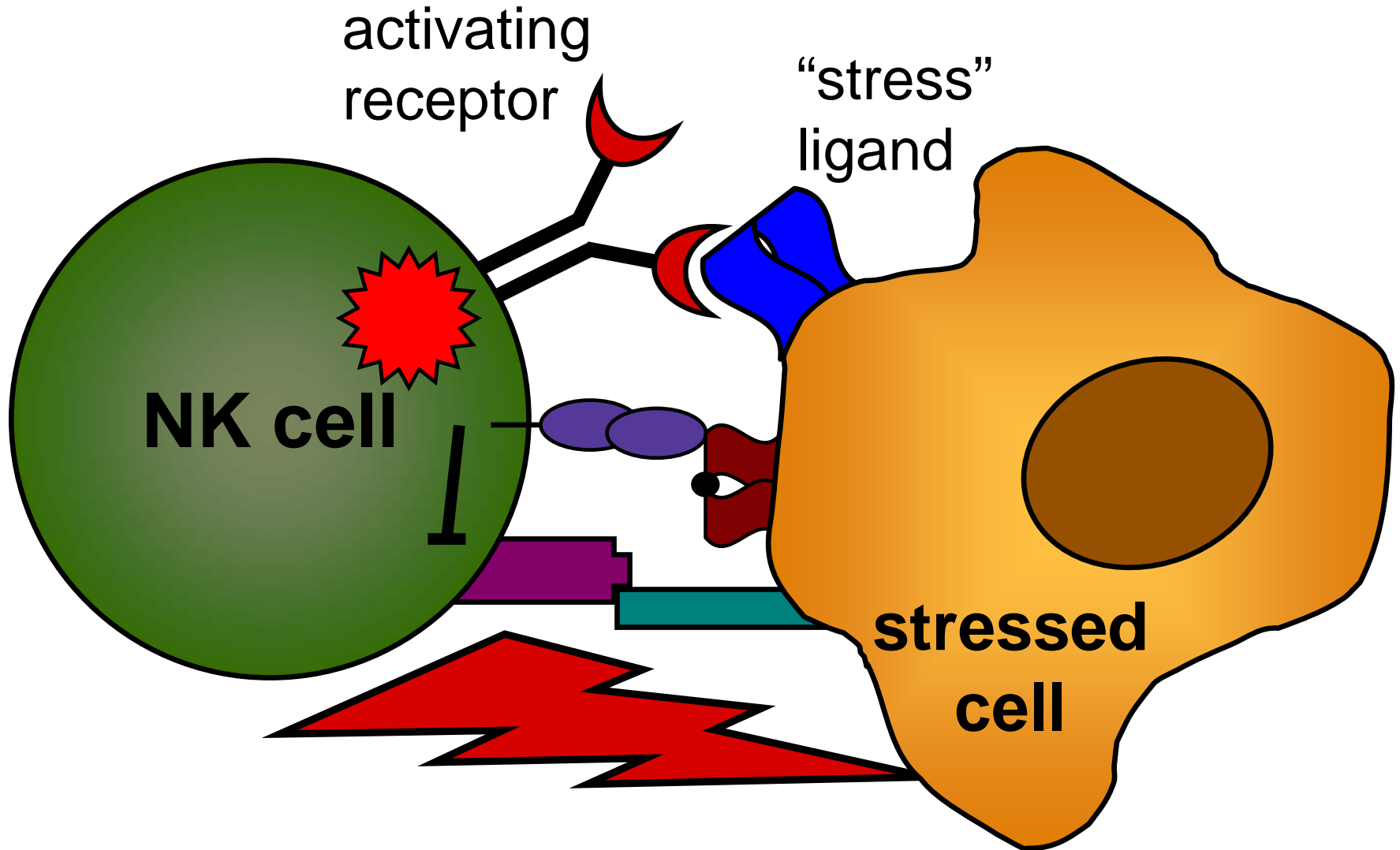


# NK cell inhibitory receptors bind:

- HLA class I molecules
  - Self
  - Allogeneic
- Other “normal” self molecules
  - CD48
  - $\alpha_3$  integrin
  - Sialic acid



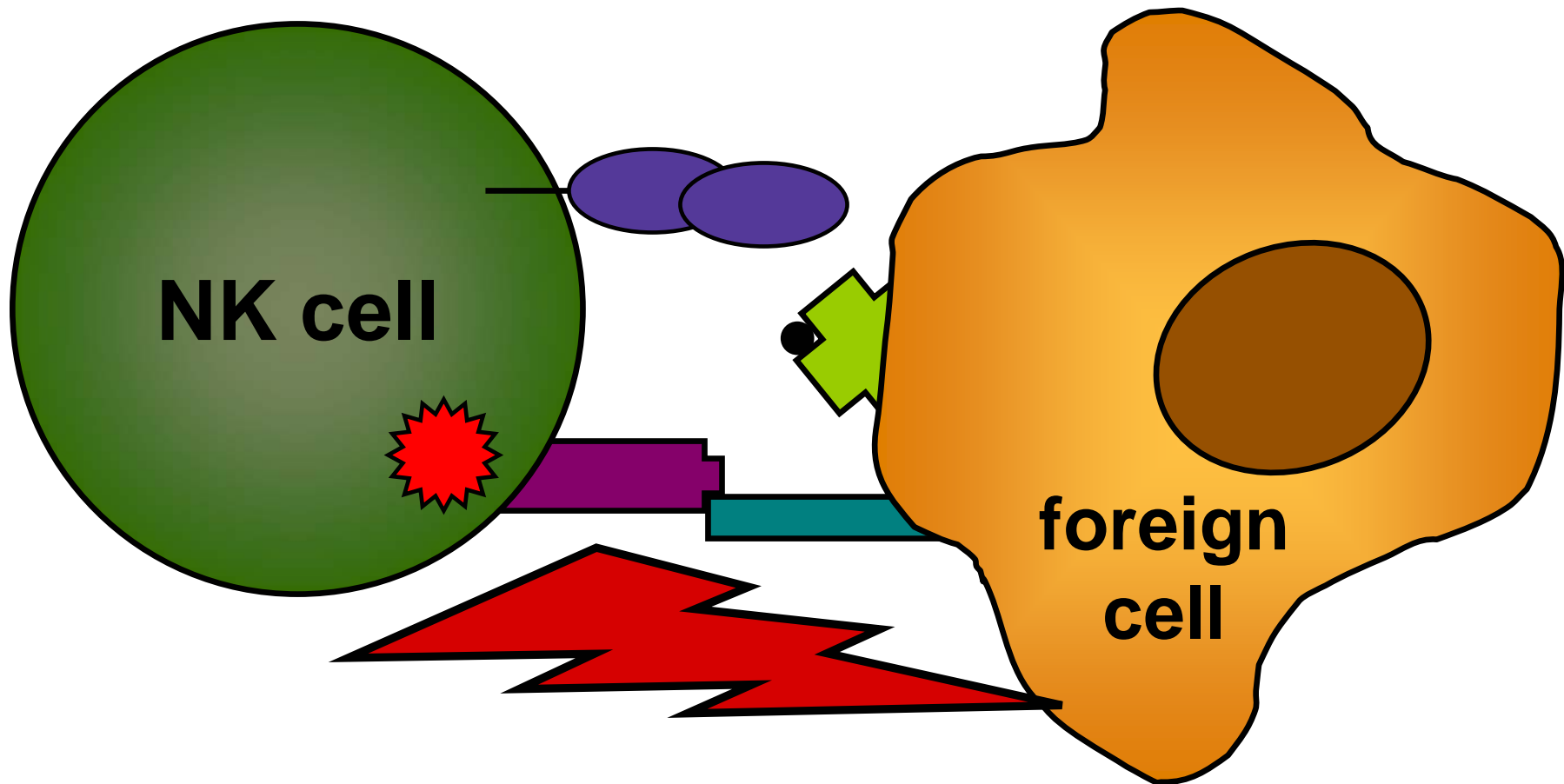
# NK cell activation by “dangerous” self



# NK cell activating receptors bind:

- Self molecules that are up-regulated due to cellular stress
  - Neoplastic transformation (MIC A/B)
  - Viral infection
  - Antibody coating (Fc RIII → ADCC)
- Normal HLA class I molecules
  - Foreign peptides?
- Foreign molecules
  - Virally encoded proteins

# NK cell regulation by “missing self”



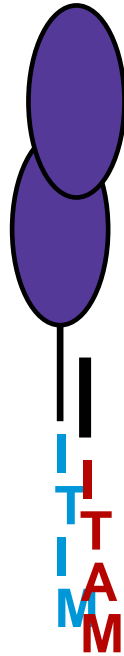
NK cell functions are regulated  
by a finely tuned, and complex,  
balance of power between  
diverse activating and  
inhibitory receptors

# NK receptor signaling

- Inhibitory receptors contain “ITIM” motifs
  - Activate SHP-1 and -2 tyrosine phosphatases that kill signals from other receptors
- Activating receptors use “ITAM” motifs
  - Activate tyrosine kinases to generate a signaling cascade
  - Often provided by association with partner chains

# Two molecular families of NK receptors

immunoglobulin  
superfamily

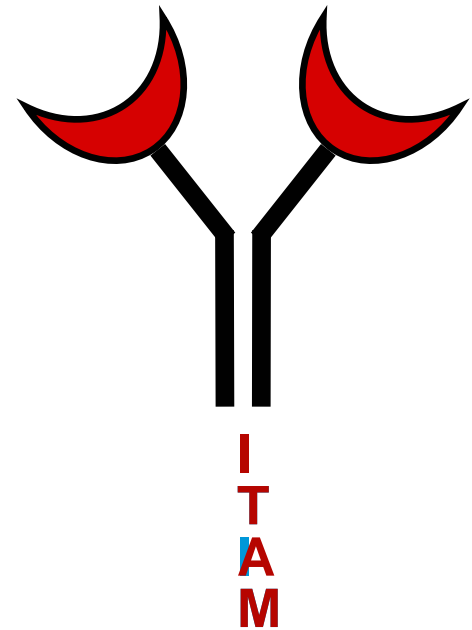


MHC class I

KIR

-

C-type  
lectin



CD94

Ly-49, CD94

Human

Mouse

# Human MHC class I receptors

Receptor	Ligand	Function
CD94/NKG2A	HLA-E	inhibitory
CD94/NKG2C	HLA-E (+ viral peptide?)	activating
KIR2DL	HLA-C (group 1 or 2)	inhibitory
KIR2DS	HLA-C?	activating
KIR3DL	HLA-B (Bw4), HLA-A3 or A11 + peptide	inhibitory
KIR3DS	HLA-Bw4?	activating

# NK cell HLA receptor “principles”

- Multiple HLA receptor types per NK cell
- Recognize polymorphic HLA epitopes
- Activating and inhibitory receptors for same ligands
- Inhibitory are “stronger” than activating
- Each NK cell has at least one inhibitory receptor that will bind a self HLA allotype
- Each NK cell usually has additional receptors that will bind allogeneic HLA



# Practical implications...

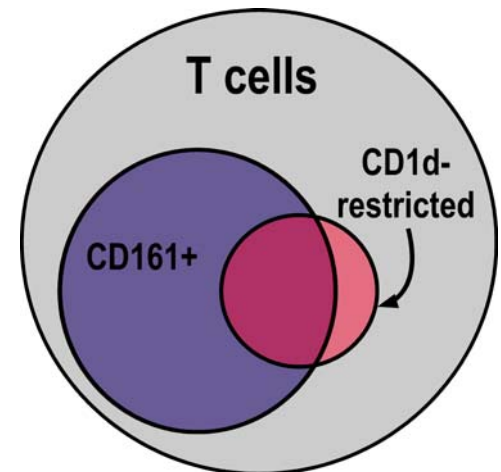
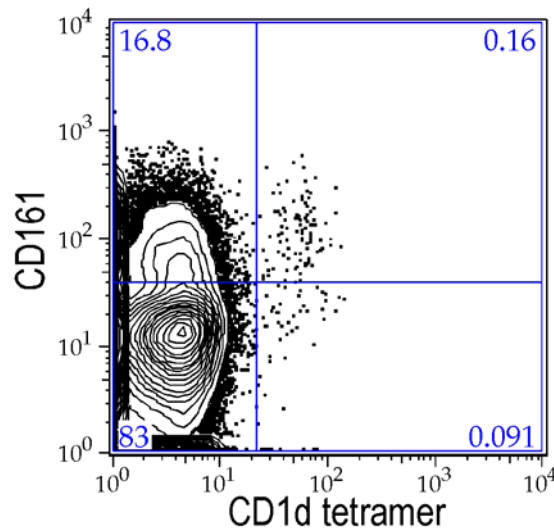
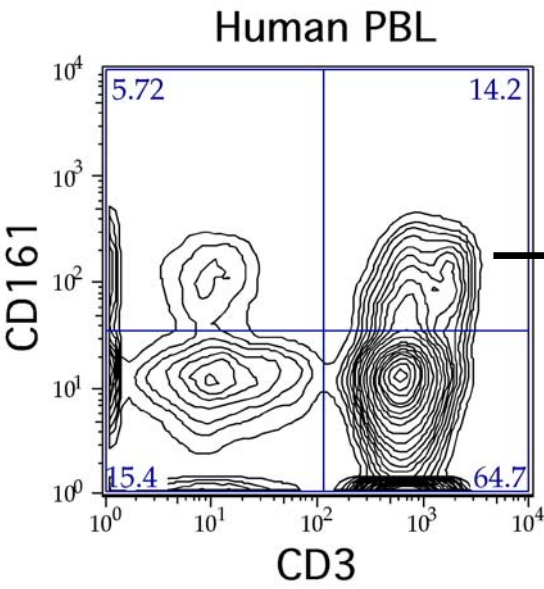
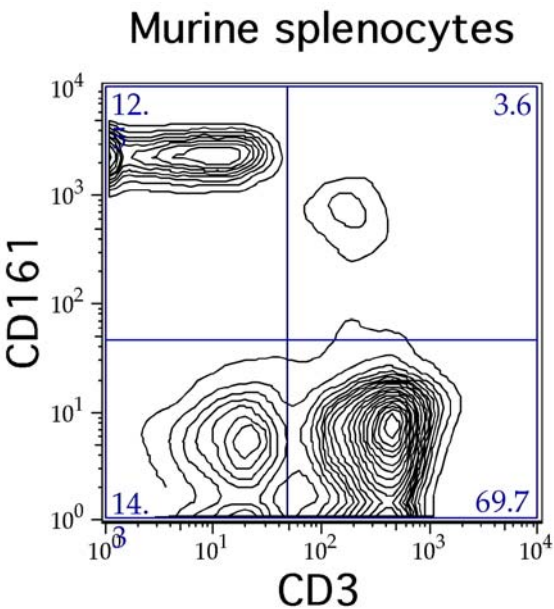
- Different KIR/HLA haplotype combinations have different potentials for activating NK cells
  - disease associations with KIR/HLA type
- Allogeneic BMT will create a new KIR/HLA combination
  - typing and “matching” for both KIR and HLA may improve outcome

# End of part I

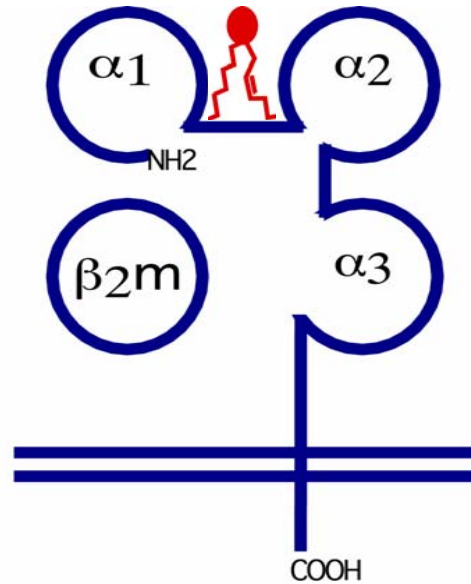
- Questions?

## II.) What are “NKT” cells?

- T cells with NK markers
- Semi-invariant TCR
- Innate functions?  
 (“Germ” independent, rapid response, no memory?)
- **CD1d-restricted T cells**

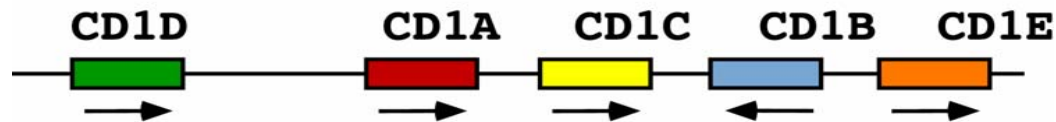


CD1 molecules:  
MHC class I-like  
antigen  
presenting  
molecules

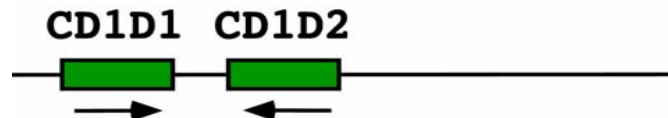


...that  
present  
lipids as  
antigens

### Human Chromosome 1

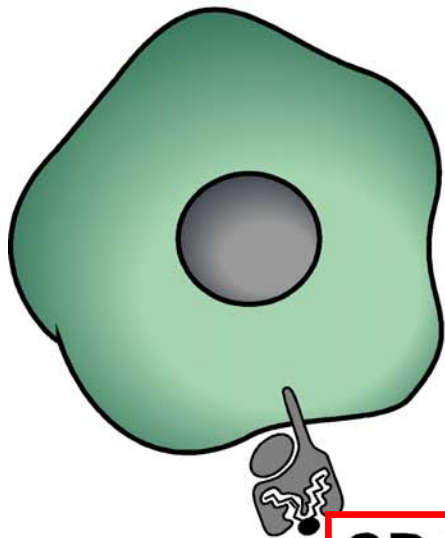


### Mouse Chromosome 3



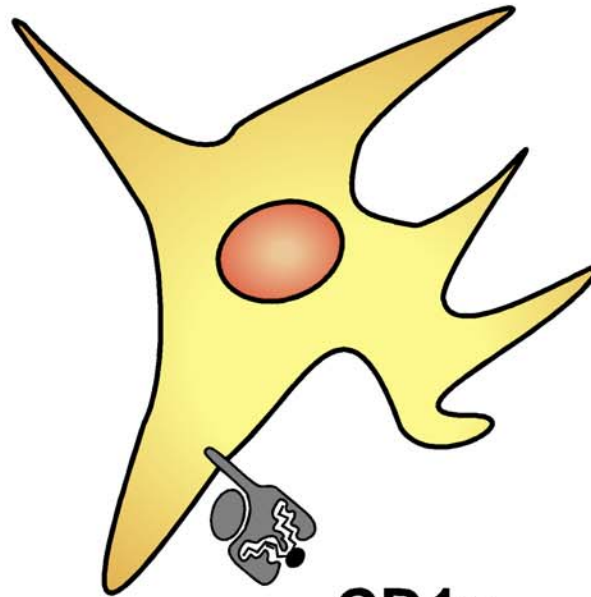
# CD1<sup>+</sup> Antigen Presenting Cells

Monocyte



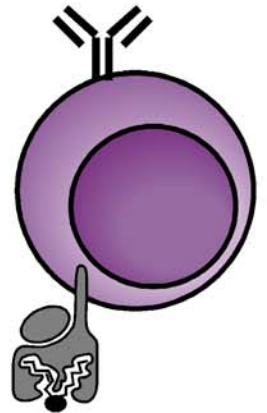
**CD1d**

Dendritic Cell



CD1a  
CD1b  
CD1c  
**CD1d**

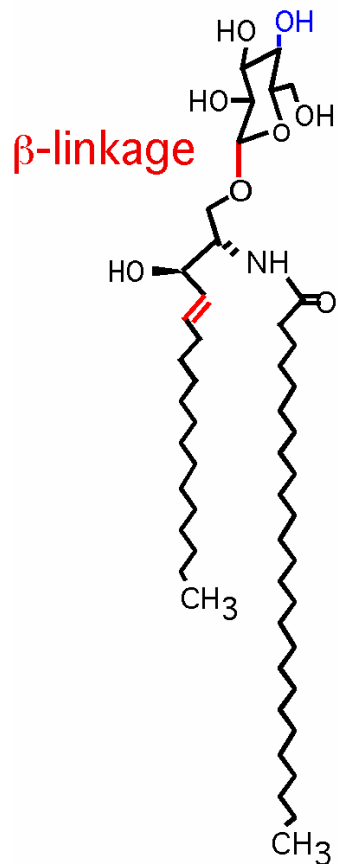
B cell



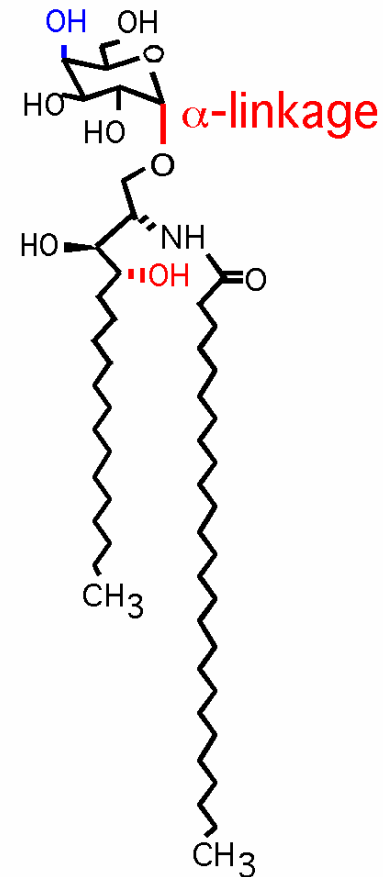
CD1c  
**CD1d**

# An unusual glycolipid is recognized by most NKT cells

$\beta$ -glucosylceramide  
(glucocerebroside)

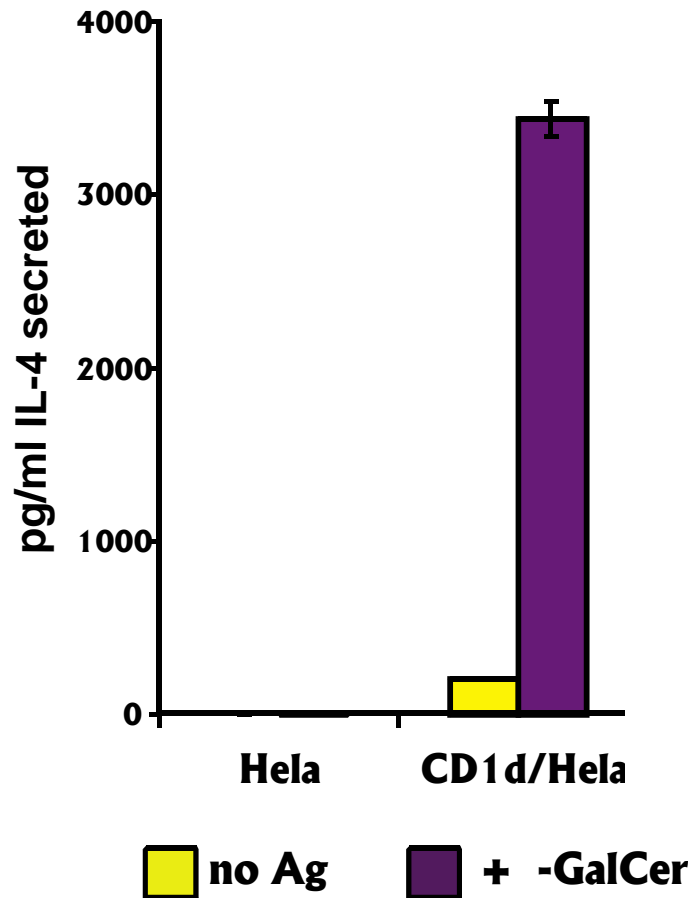


$\alpha$ -galactosylceramide

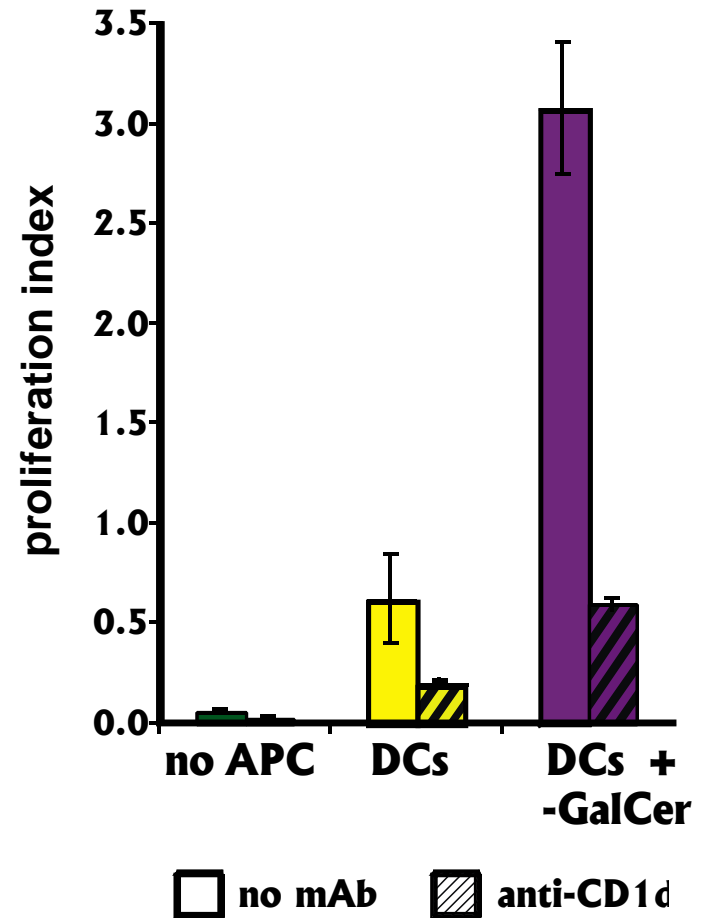


NK T cells respond strongly to  $\alpha$ -GalCer,  
but also (more weakly) to CD1d<sup>+</sup> APCs without added Ags

**CD1d transfected cell line**



**Myeloid Dendritic Cells**

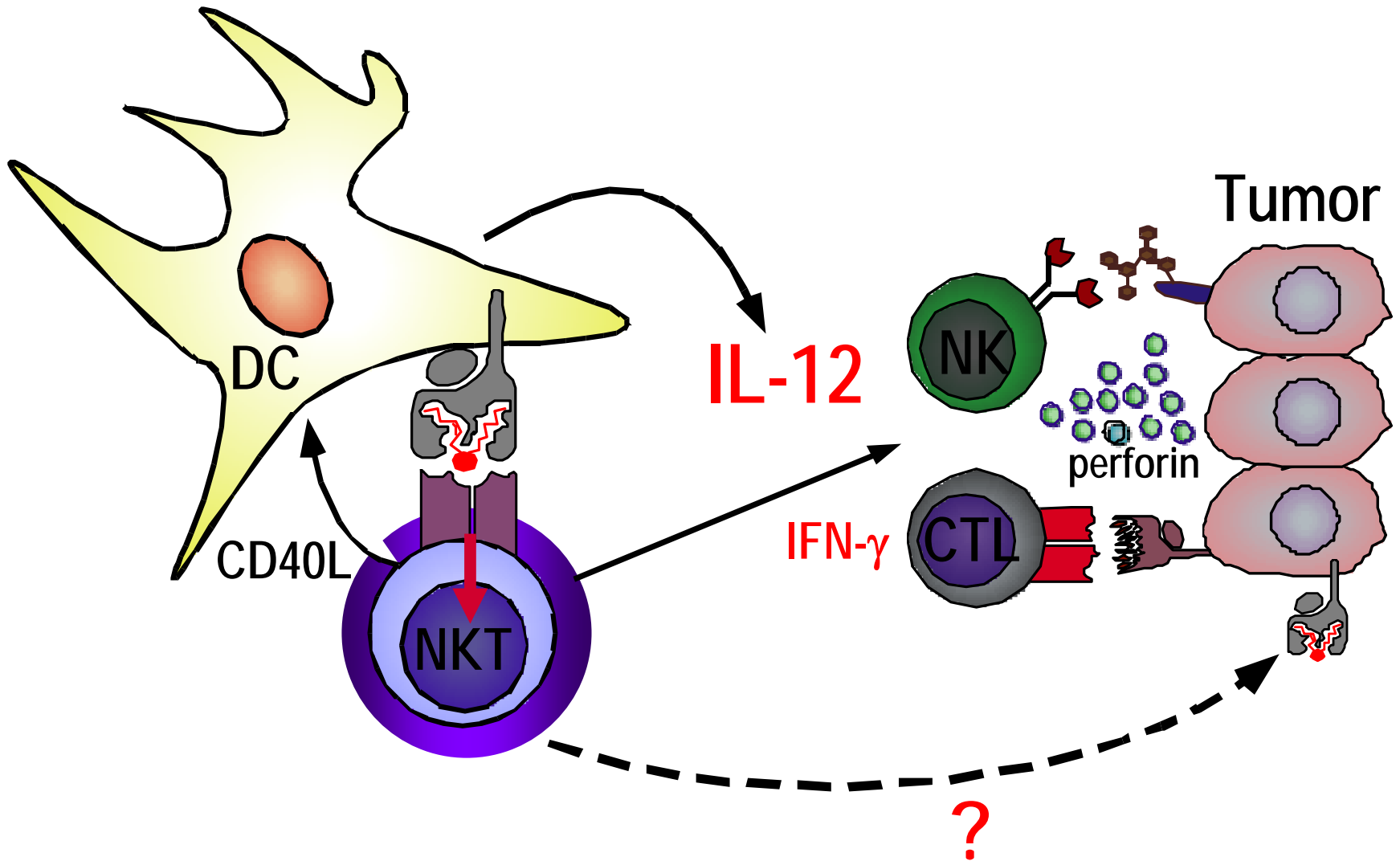


# Tumor rejection/immunosurveillance

- Activation by  $\alpha$ -GalCer leads to potent tumor rejection, (pulsed DCs even better)
- Tumor rejection due to administration of exogenous IL-12 is CD1d dependent
- NKT cells contribute to tumor immuno-surveillance via endogenous IL-12 pathway, early IFN
- NKT cells promote effective responses in anti-tumor “vaccine” systems
- CD1d down-regulated on tumor cells from human patients



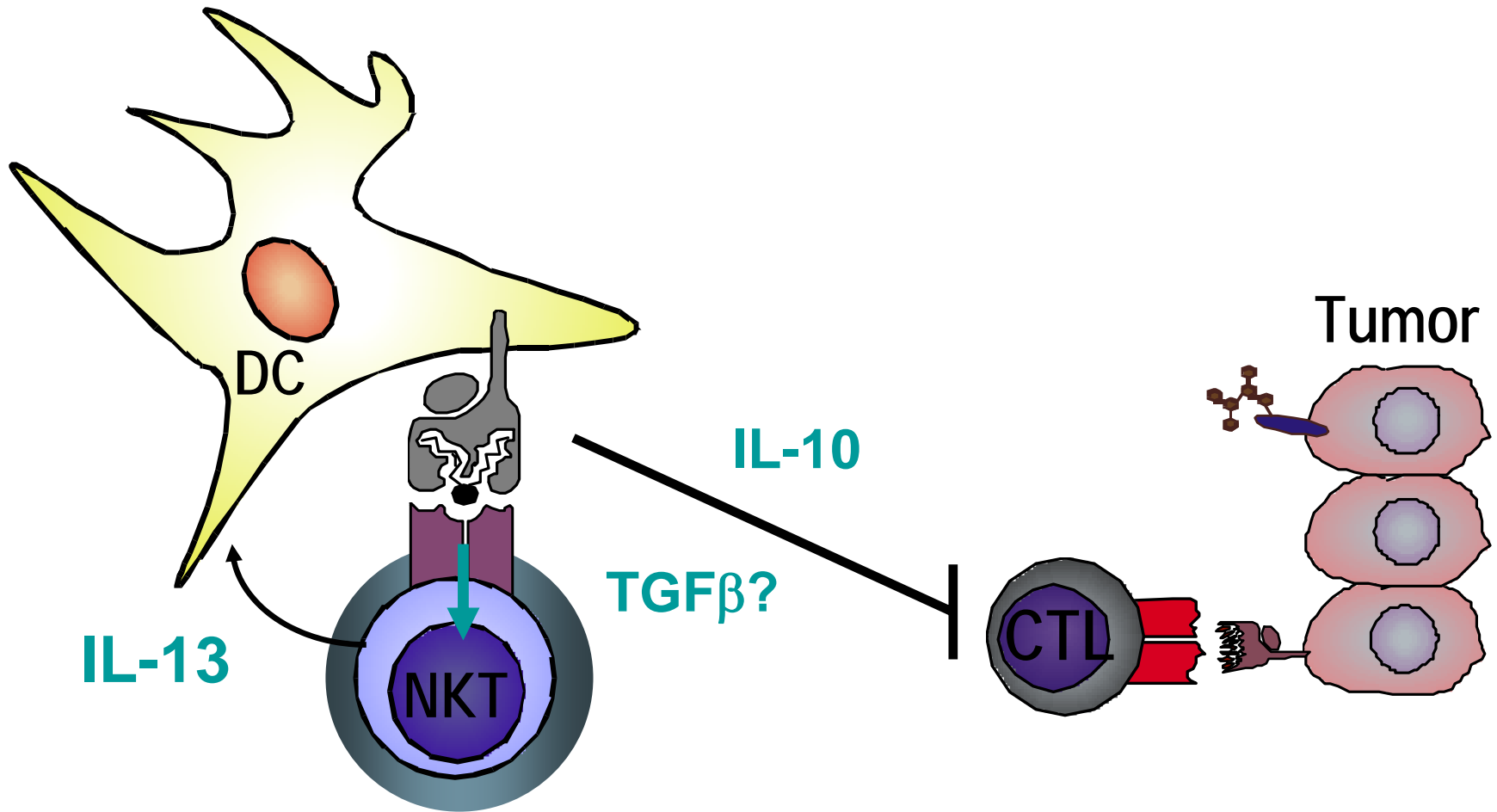
# NKT cell anti-tumor effect



# But, paradoxically, in other models...

- NKT cells suppressed anti-tumor CTL responses
  - IL-13 secretion important
- NKT cells suppressed anti-tumor responses to UV-irradiation induced sarcomas
- CpG oligo-dinucleotides induced anti-tumor responses better in CD1d KO mice

# NKT cell suppressive effect



# Summary

## NK cells

- Rapid cytolytic effectors
- Regulated mainly by cytokines and self
- Complex positive and negative signaling receptors
- Major ligand MHC I

## NKT cells

- CD1d-restricted T cells
- Stimulated by self and foreign glycolipid antigens
- Promote and inhibit subsequent immune responses
- Influence functions of DCs?