# Biology of Innate Immunity: NK cells, Macrophages, PMN, PAMP/TLR

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# Innate vs. Adaptive Immunity

| Innate | Adaptive |
| --- | --- |
| Immediate response | Delayed response |
| Receptors -invariant, germline encoded | Receptors - require somatic genetic recombination |
| No 'memory'- constant number of precursors, constant response kinetics | Memory'- after primary exposure higher precursor frequency and faster response kinetics |

# Innate vs. Adaptive Immunity

| Innate | Adaptive |
| --- | --- |
| Epithelial cells | T cells |
| Granulocytes | B cells |
| Monocytes, dendritic cells & macrophages | |
| Mast cells | |
| NK cells | |

# Does innate immunity prevent or promoter tumor growth?

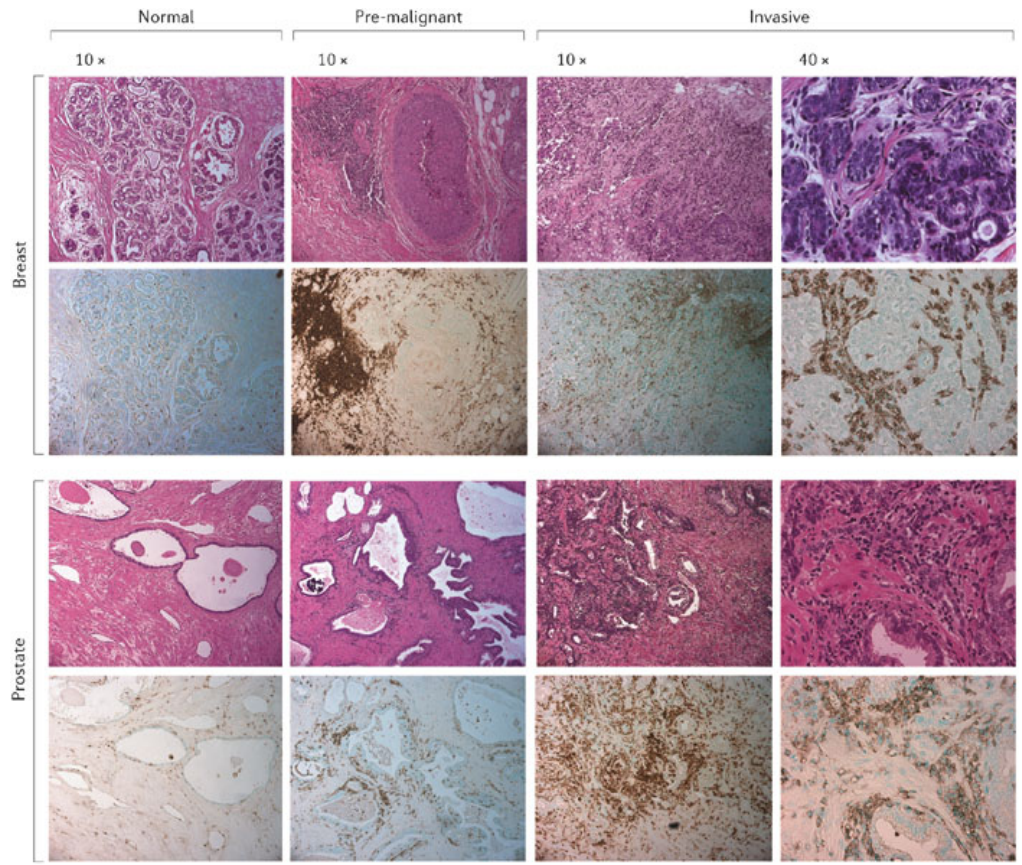
Innate immunity promotes tumor growth

Inflammation - activated macrophages & granulocytes
-provide angiogenic factors & growth factors and maxtrix metalloproteinases that promote tumor spread

Innate immunity prevents tumor growth

NK cells kill tumors and dendritic cells process tumor antigens and prime an adaptive (B & T cell) response

# Inflammation in human breast and prostate cancer






de Visser KE et al. (2006) Paradoxical roles of the immune system during cancer development Nat. Rev. Cancer. 6: 24–37 doi:10.1038/nrc1782

## Innate immunity promotes tumor growth

-Chronic inflammation predisposes to cancer (liver, colon)

-COX2 inhibitors diminish cancer risk

-TNF$\alpha$ activates NF$\kappa$b promotes tumor survival of hepatic and colon carcinomas in mouse models
(Pikarsky et al. Nature 431, 461, 2004 & Greten et al. Cell 118, 285, 2004)

## Innate immunity prevents tumor growth

-Direct cell-mediated cytotoxicity

-Cytokine-mediated anti-tumor effects

# Innate Cytokines

Epithelial cells ---- Type I interferon, pro-inflammatory cytokines

Granulocytes --- Pro-inflammatory cytokines, reactive oxygen species (ROS), IL-12

Macrophages -- Pro-inflammatory cytokines, ROS, VEGF

Conventional Dendritic Cells -- pro-inflammatory cytokines, IL-12, IL-15

Interferon-producing Dendritic Cells - Type I interferon, IL-12

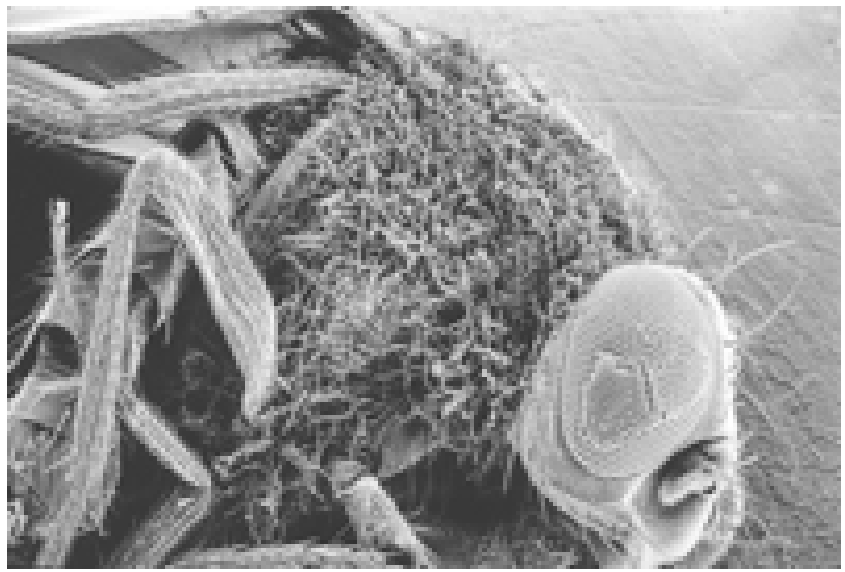
Mast cells - Pro-inflammatory cytokines, arachidonic acid, IL-4

NK cells - Interferon-$\gamma$, TNF, chemokines

What initiates cytokine production by innate immune cells?

# The story of the Toll-like receptors begins with insect immunity

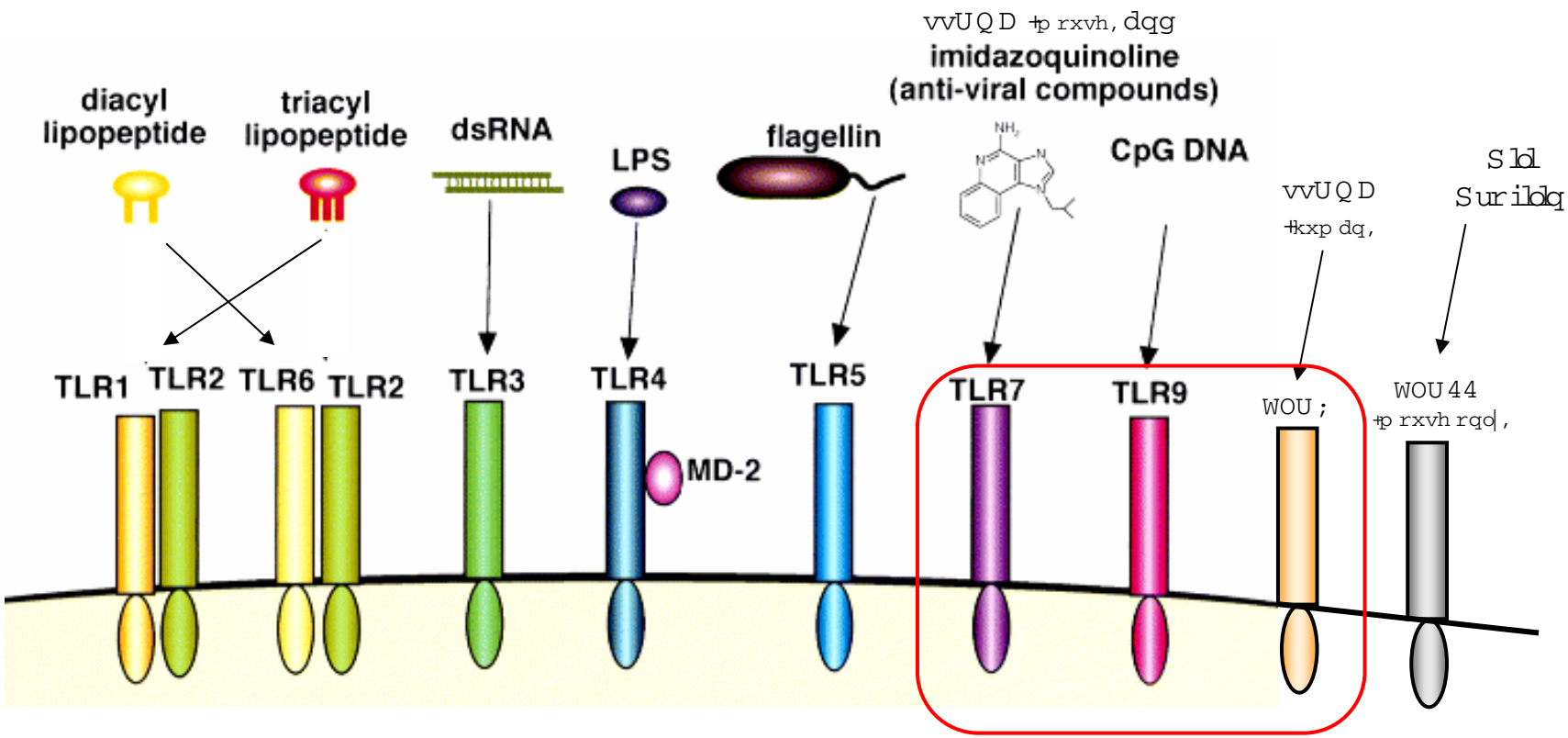
Toll-dependent innate immune responses in *Drosophila* to fungus and Gram+ bacteria



Lemaitre et al. 1996 Cell 86:973

Courtesy Mitch Kronenberg

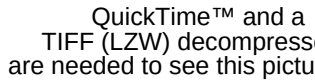
# Mammalian Toll-Like Receptors




## TLR recognize conserved structures in microbes

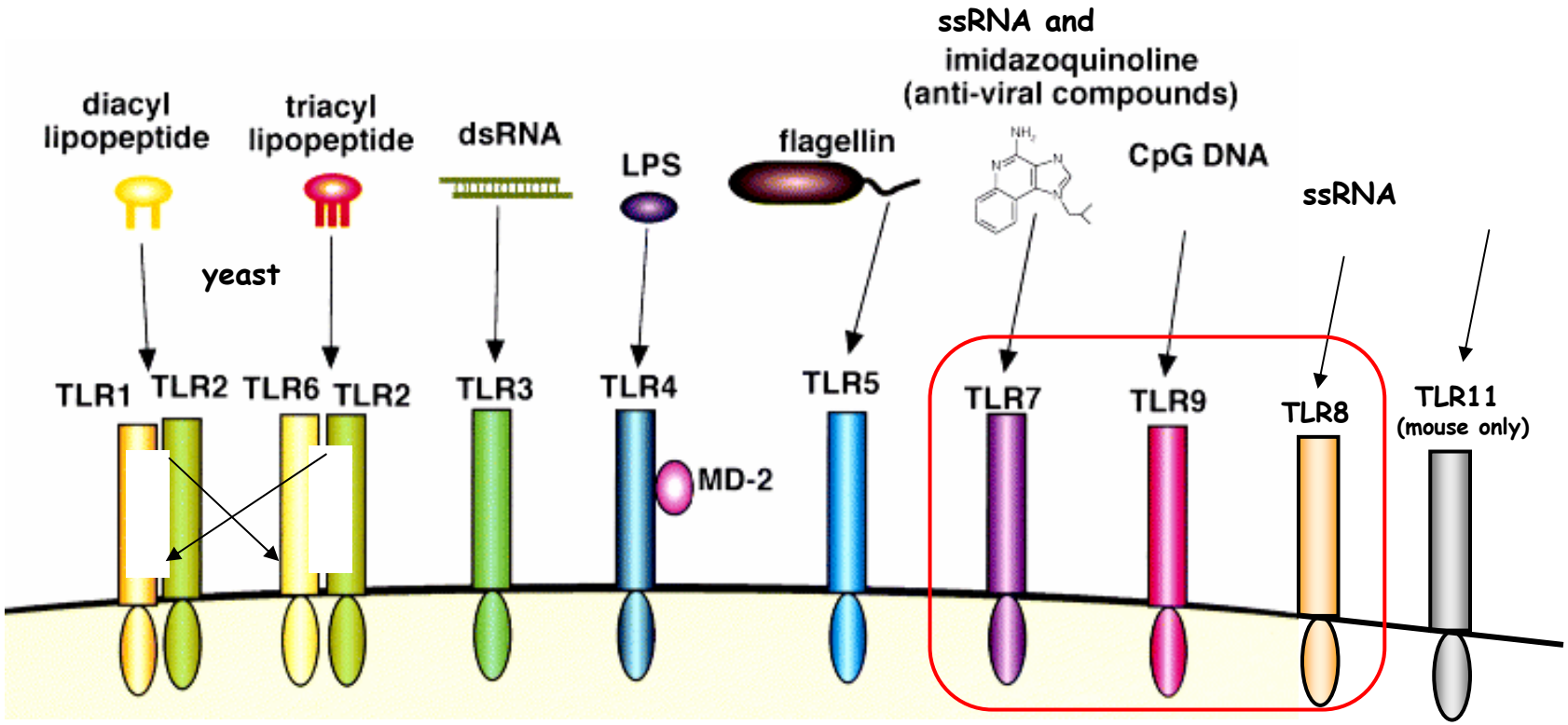
Courtesy Mitch Kronenberg

# TLR signaling pathways




From S. Akira Lab Website

# Mammalian Toll-Like Receptors




Interferon-producing dendritic cells -TLR 7, TLR9

Conventional dendritic cells - TLR1, 2, 4, 5, 6, 8

Resting NK cells - No functional TLR
Activated NK cells - TLR3, TLR9

# TLR-based cancer therapy
# 100 years ago!

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Bacterial infection post-surgery for cancer induces regression and prevention of metastasis

# Tumor Immunology and Immunotherapy

DR. WILLIAM B. COLEY

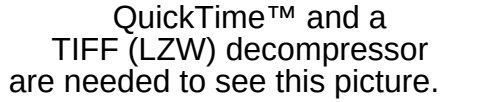
*First, a little history…*

**William B. Coley**

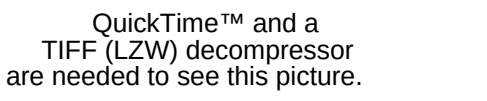
- Cancer Surgeon at Memorial Hospital (NYC) at the turn of the 20th Century
- Observed that a cancer patient who developed a severe bacterial infection (strep.pyogenes) had spontaneous regression of his tumors.
- Treated **over 900 solid tumor patients** with a crude bacterial extract ("Coley's Toxin") and reported a **40% response rate**, some leading to long term remissions.
- Approach largely abandoned after his death.
- His daughter, Helen Coley Nauts founded the Cancer Research Institute, which is one of the largest private foundations supporting basic and applied research in tumor immunology.

# TLR ligands as cancer therapies

Coley's Pharma TLR9 agonist CpG
effective in Non-small cell lung cancer




3M TLR7 agonist imiquimod
Approved for superficial basal carcinoma



# Role of macrophages and granulocytes in innate tumor immunity?

In vivo

- -primary tumorigenesis take weeks or months
- -no feasible way to deplete granulocytes and macrophages for extended periods

In vitro

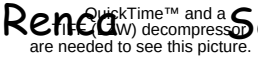
- -macrophages kill tumors *in vitro*
  but receptors (other than FcR)
  on macrophages & ligands on tumors
  not defined

# Eosinophil-mediated tumor immunity

Renca-IL4
wt

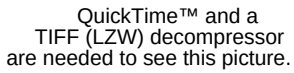
Renca-IL4
Scid or nude

Renca









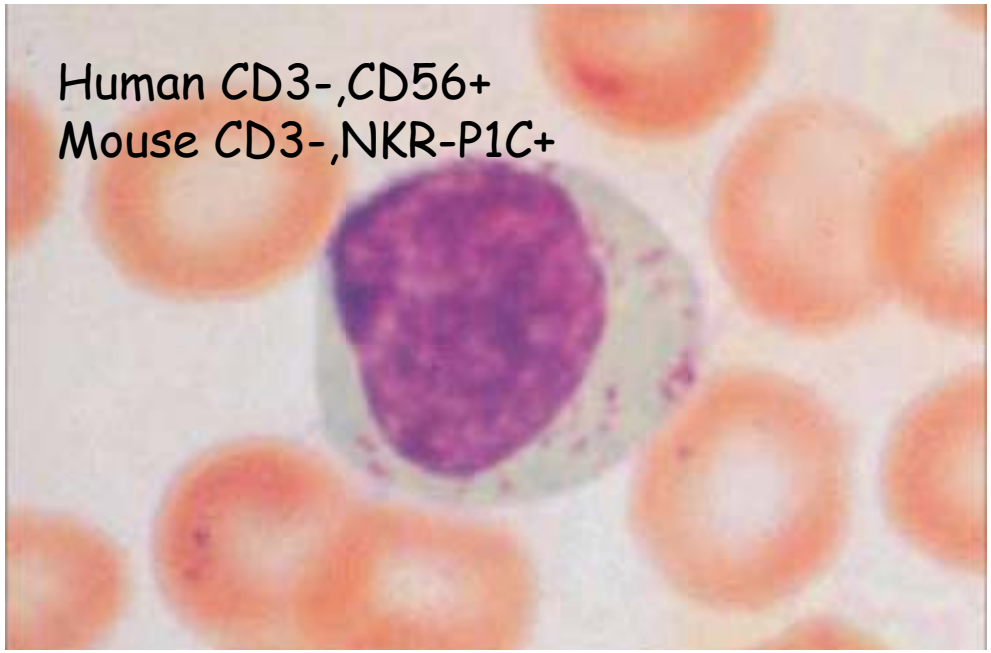
# Higher incidence of 3-MCA-induced fibrosarcomas in interferon-$\alpha/\beta$ receptor-/- mice

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Effect of INFa/b probably on host immune cells, rather than tumor

Dunn et al. Nat Immunol. 2005 6:722

# NK cells and tumor immunity




- Identified in '70s as lymphocytes from healthy humans and mice able to kill certain tumors in vitro
- Function in innate immunity to protect against viruses, bacteria, & tumors
- Produce cytokines & kill abnormal cells

# Immune surveillance against cancer by NK cells

QuickTime™ and a
TIFF (LZW) decompressor
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Mice depleted of NK cells with anti-AsialoGM1 or depleted of NK cells and NKT cells with anti-NK1.1 have higher incidence of 3-MCA-induced sarcomas

QuickTime™ and a
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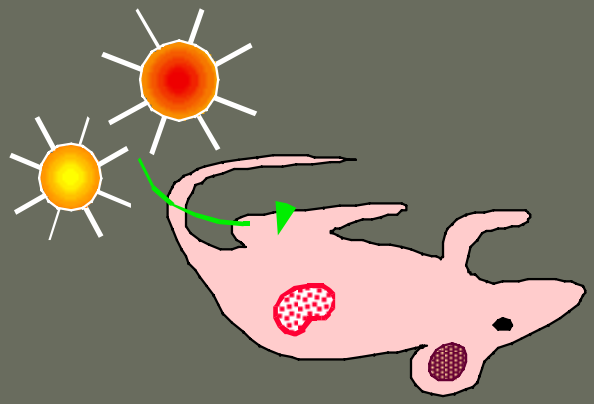
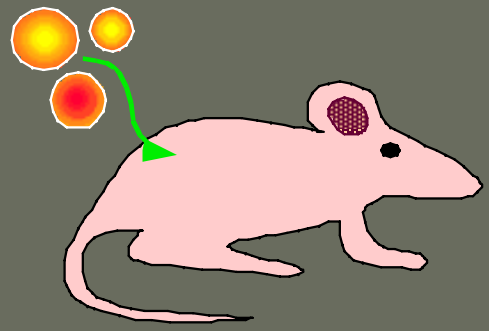
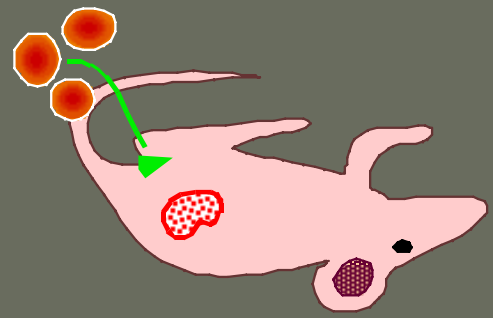
# NK Cells Reject Tumors Lacking MHC Class I



Class $I^+$ tumors grow *in vivo*

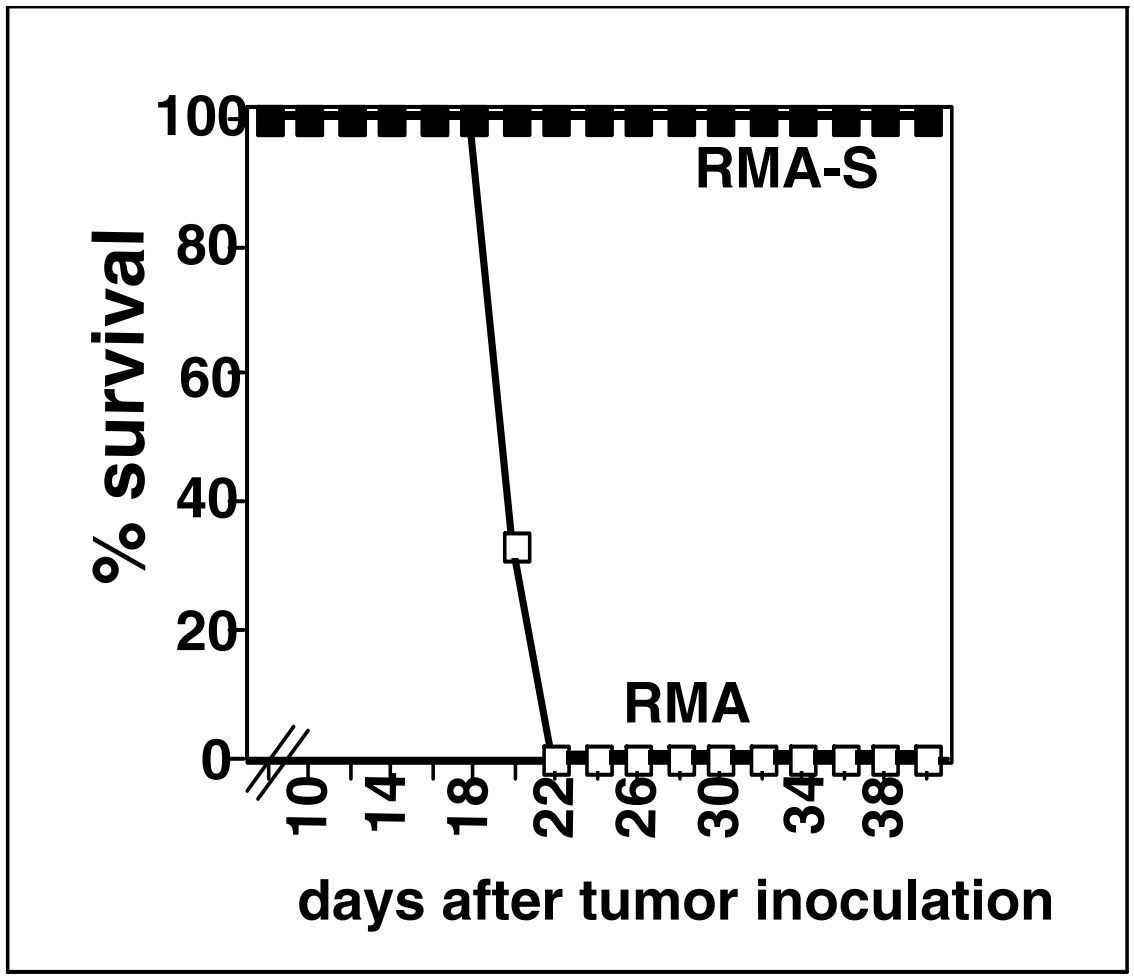


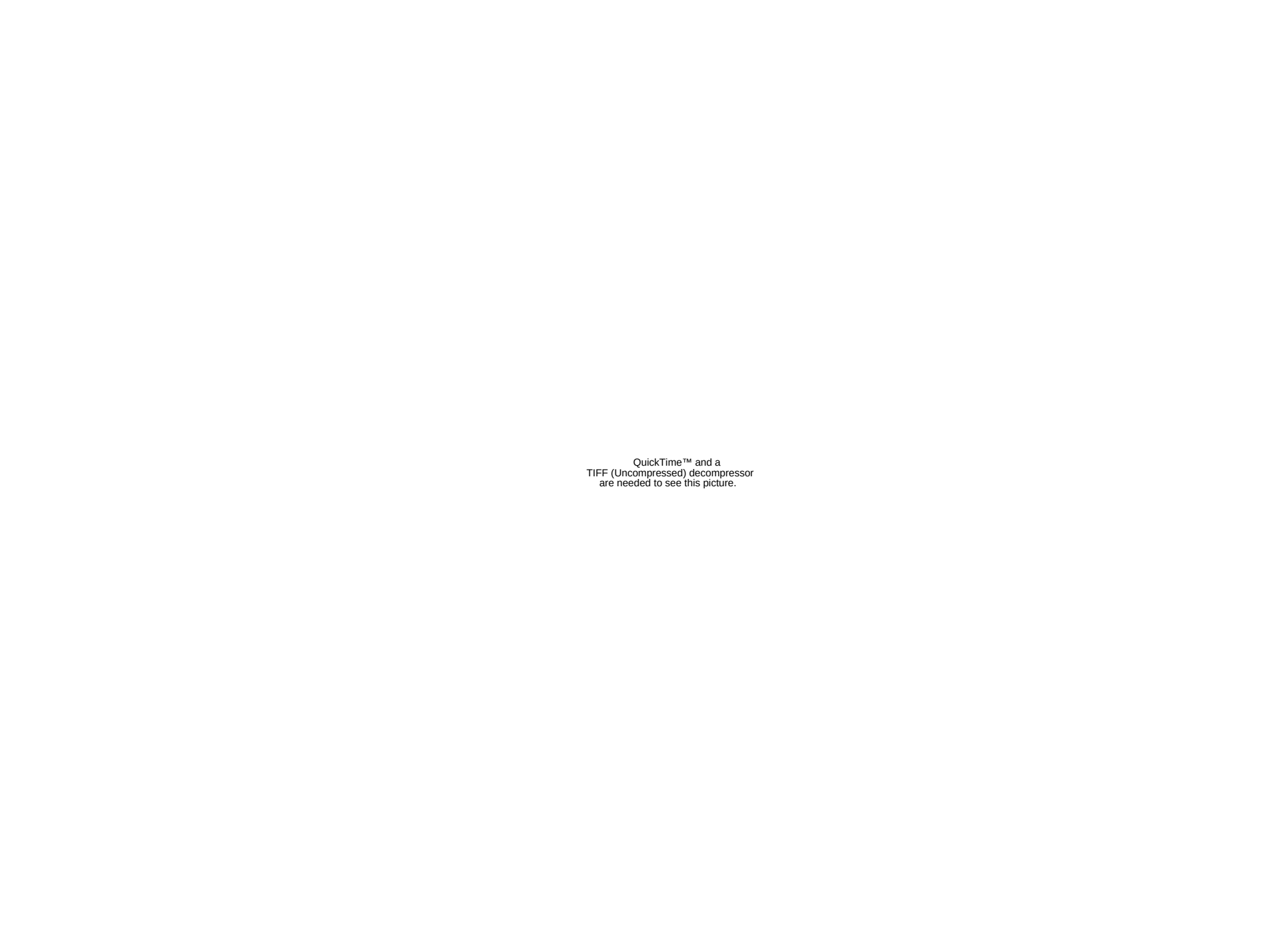
Class $I^-$ tumors are rejected



Class $I^-$ tumors in NK-depleted mice grow *in vivo*

# Mice reject MHC class I-negative RMA/S, but not class I-positive RMA lymphoma




QuickTime™ and a
TIFF (Uncompressed) decompressor
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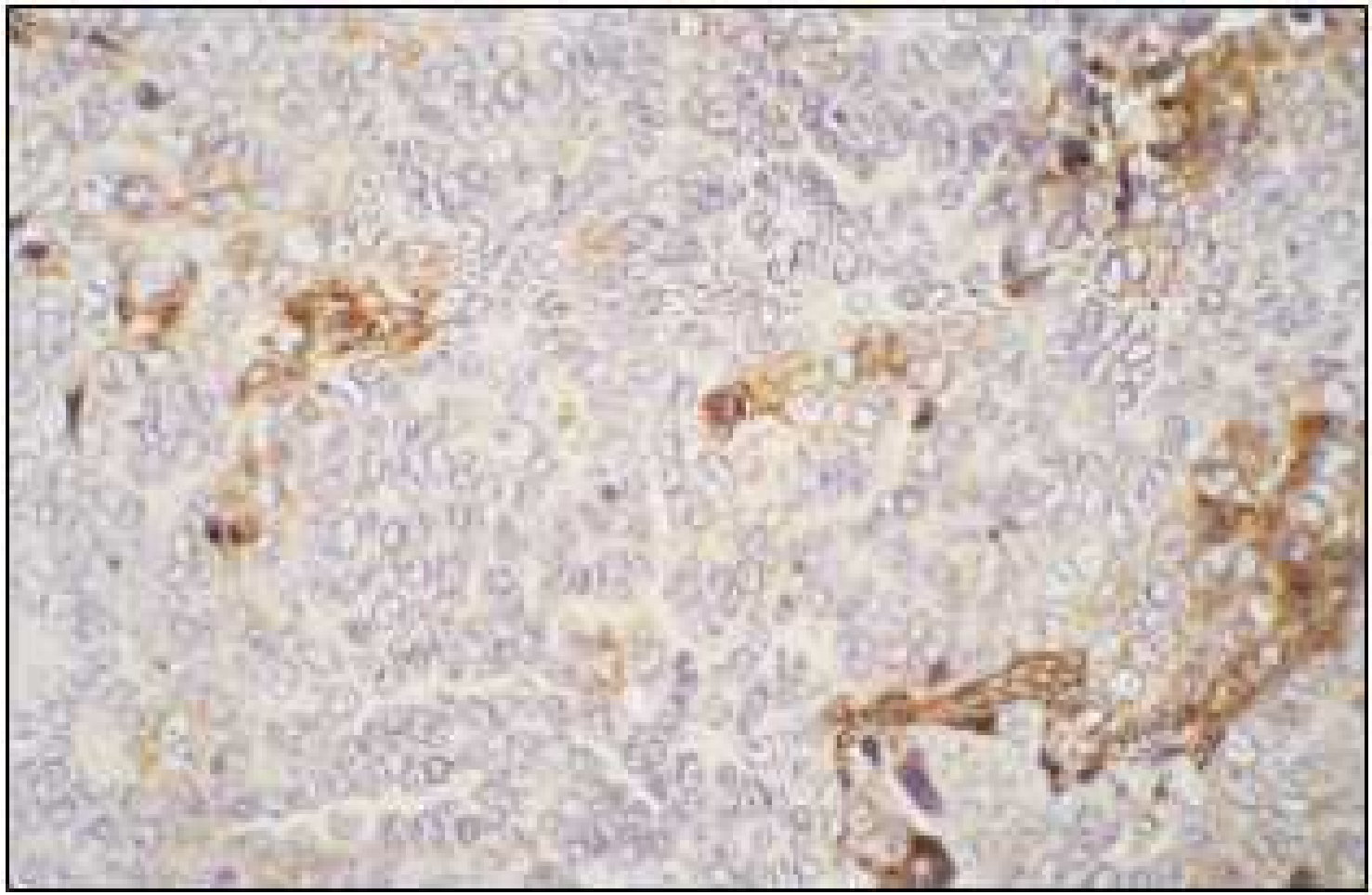
# Immune surveillance for ‘ Missing Self’

- NK cells preferentially kill cells that have lost MHC class I
- Provides protection against cells escaping T cell recognition
- Predicts existence of inhibitory receptors for MHC class I that spare normal cells from NK cell attack
  - *Karre et al Nature 319:675, 1986*

# Loss of Class I MHC Expression in a Prostate Carcinoma

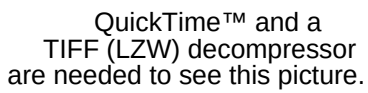


How are NK cells activated when they encounter tumors or virus-infected cells?

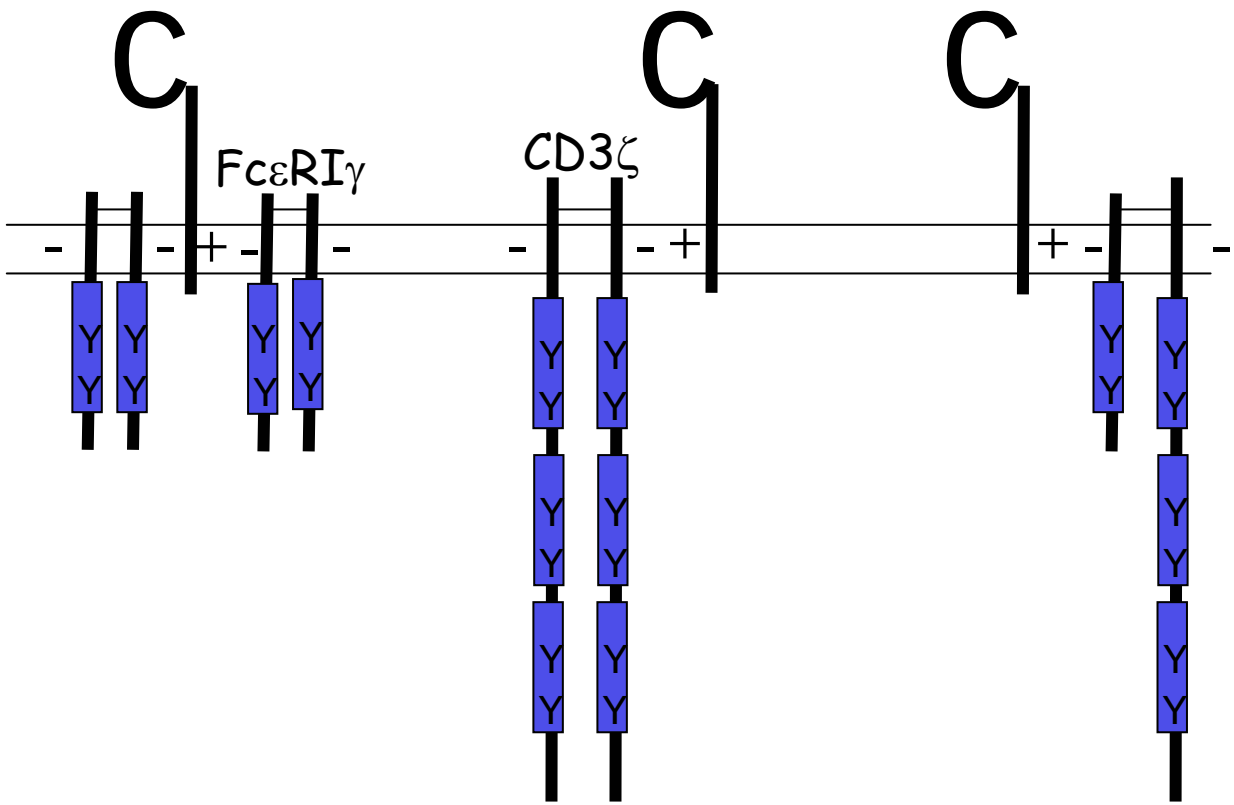
# Activating NK receptors - ligands

- Human/mouse CD16-FcεRIγ/ζ IgG
- Human CD2…… CD58
- Human 2B4 (CD244)-SAP… CD48
- Human DNAM-1 (CD226)… CD112, CD155
- Mouse PILRβ-DAP12…….. PILR-L
- Human NKG2D-DAP10……. MICA/B, ULBP
- Mouse NKG2D-DAP10/12… RAE-1,H60, MULT1
- Human/mouse NKp46-FcεRIγ/ζ…… ?
- Human NKp30-FcεRIγ/ζ…… ?
- Human NKp44-DAP12…… ?
- Mouse NKR-P1c-FcεRIγ…… ?

# Antibody-dependent cellular cytotoxicity

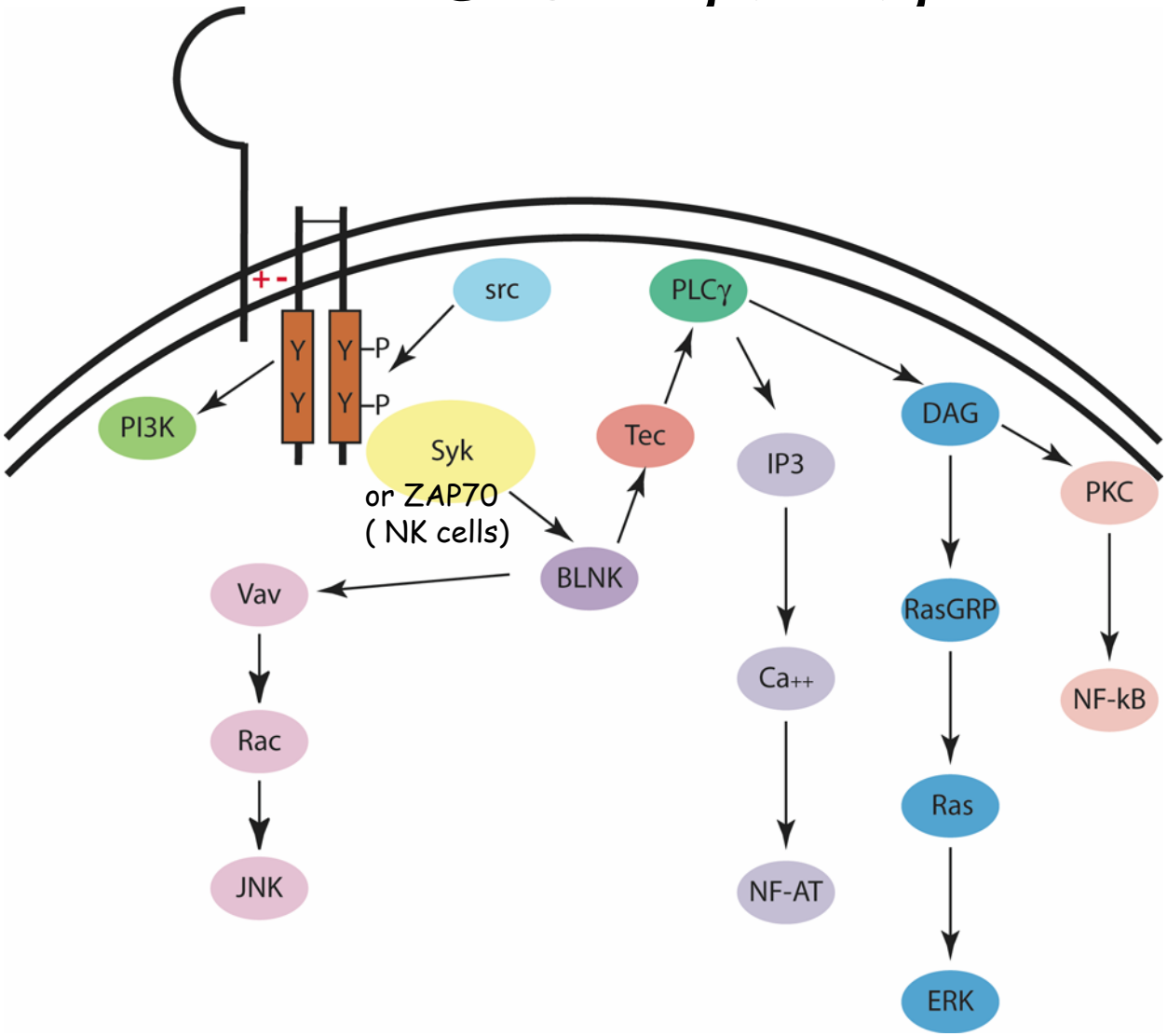


# CD16 (FcγRIIIA)




Expressed by NK cells and macrophages

# CD16 Signaling




Cytotoxicity, cytokine production

# FDA-approved therapeutic monoclonal antibodies

CD20

Her2

CD33

CD52

CD20

CD20

EGF-R

VEGF

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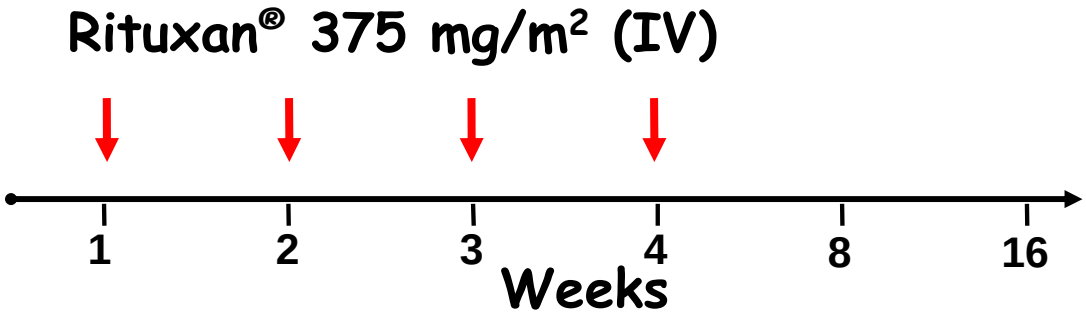
TIFF (LZW) decompressor
are needed to see this picture.

# Rituxan Pivotal Trial: Treatment of Patients With Relapsed Lymphoma

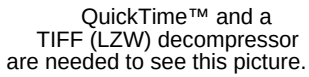


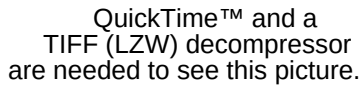

Monitoring every 3 months x2 years

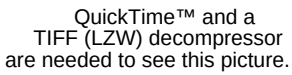
| | |
|---|---|
| Evaluable Patients | 166 |
| Overall Response | 80 (48%) |
| Complete Response | 10 (6%) |
| Partial Response | 70 (42%) |

McLaughlin et al. *J Clin Oncol*. (1998) 16:2825

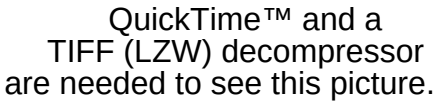
# Polymorphisms in CD16 correlate with therapeutic effects of anti-tumor monoclonal antibodies

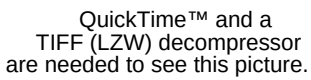


# CD16 (FcγRIII) mediates Herceptin and Rituxan mediate human tumor elimination in nude mice



# Activating NK receptors - ligands

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- Human NKp30-FcεRIγ/ζ…… ?
- Human NKp44-DAP12…… ?
- Mouse NKR-P1c-FcεRIγ…… ?

QuickTime™ and a
TIFF (LZW) decompressor
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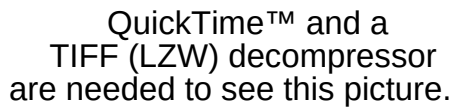
NKG2D

- 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

# NKG2D ligands in mice and humans




Many genes
Many alleles

# NKG2D ligands

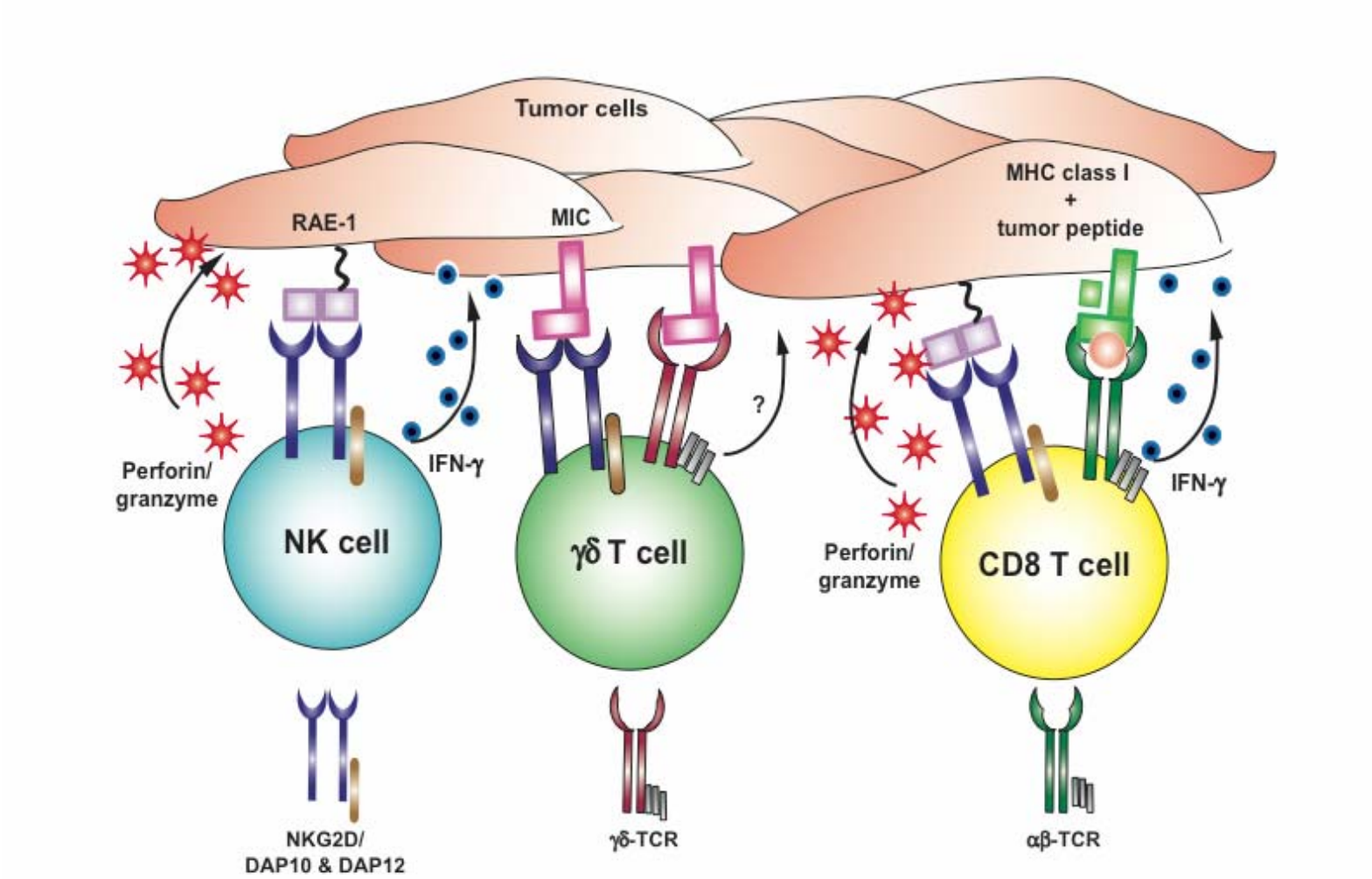
- MHC class I-like
  - don't require peptide or β2-microglobulin
- Bind with nM affinity to NKG2D

- Low levels expressed on healthy tissues
- Induced on virus-infected cells and tumor cells
- Induced by DNA damage
- Elevated in autoimmune diseases

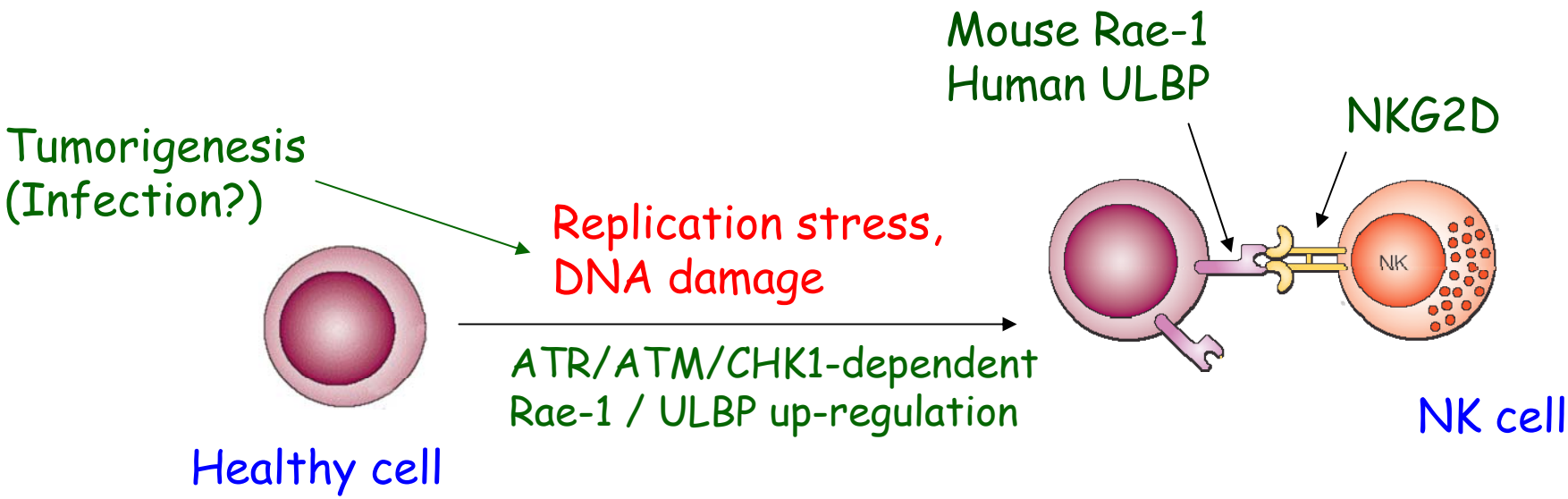
# What is the biological role of the NKG2D ligands?

"Danger signals" to alert the immune system to infection

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



# Induction of NKG2D ligands



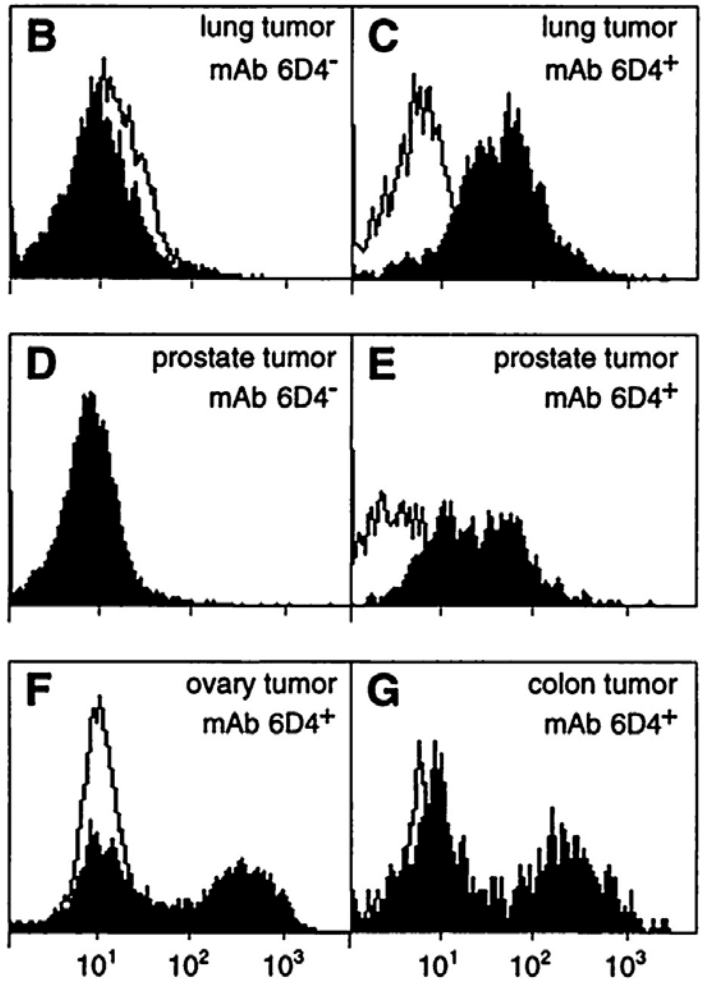

Thanks D. Raulet
Nature 2005

# NKG2D ligands (MICA/B) are expressed on many primary human tumors

Lung tumors

Prostate tumors

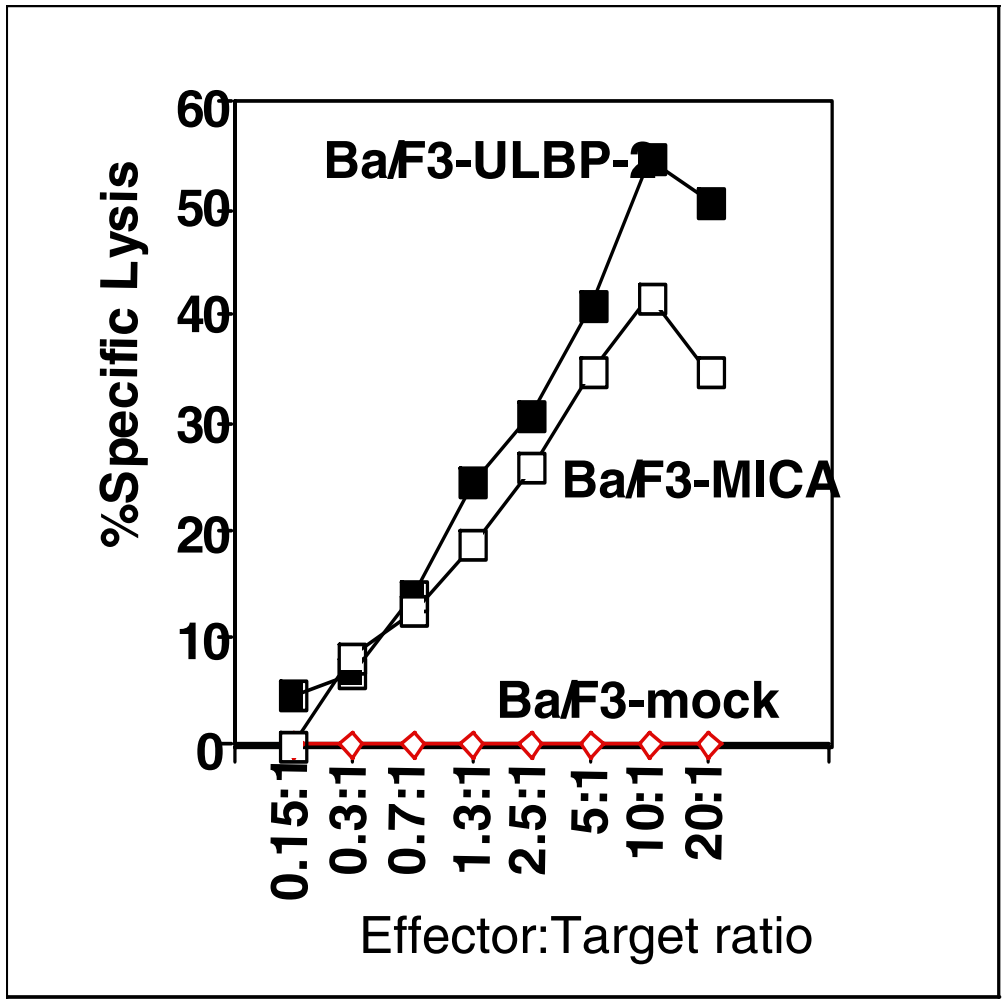
Ovarian tumors



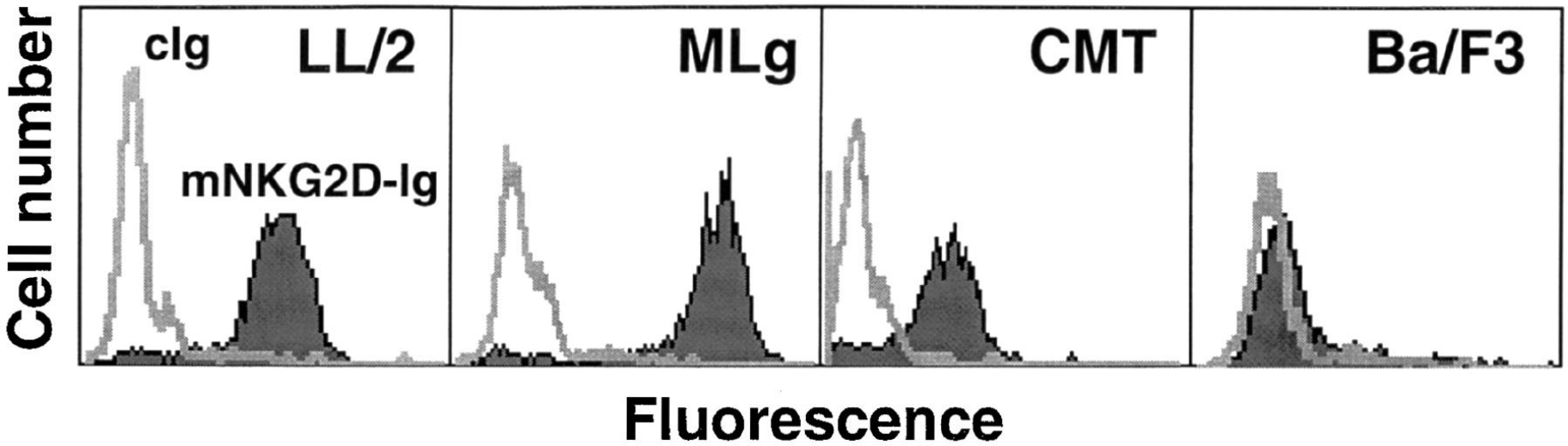

Colon tumors

Groh et al PNAS 96:6879, 1998

# Human NK cells kill NK-resistant mouse cells transfected with human NKG2D ligands



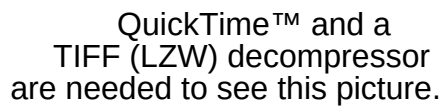
# NKG2D ligands are expressed on many mouse tumors



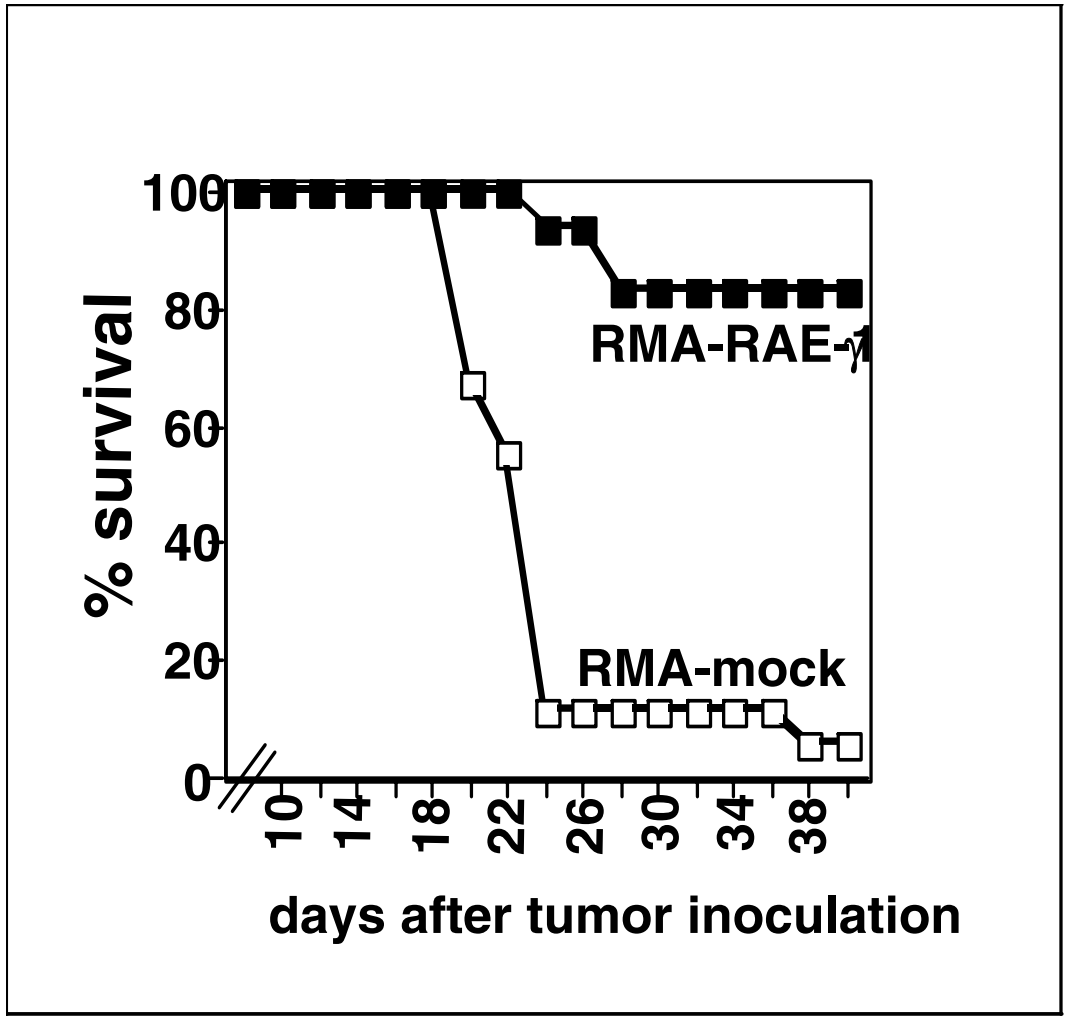

Lung tumors  Lung tumors  Colon tumors

Cerwenka, A. Immunity 12:721, 2000

# Mouse NK cells kill NK-resistant lymphomas transfected with NKG2D ligands



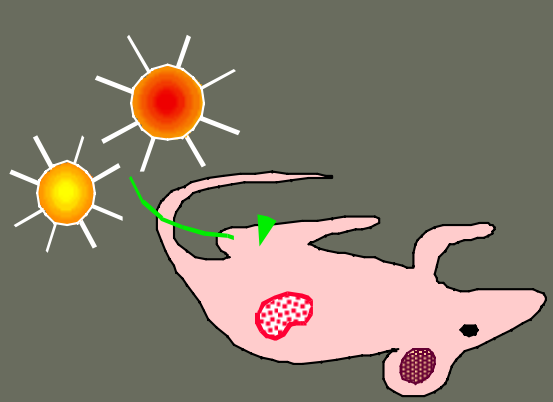
# Mice reject lymphomas transfected with NKG2D ligands



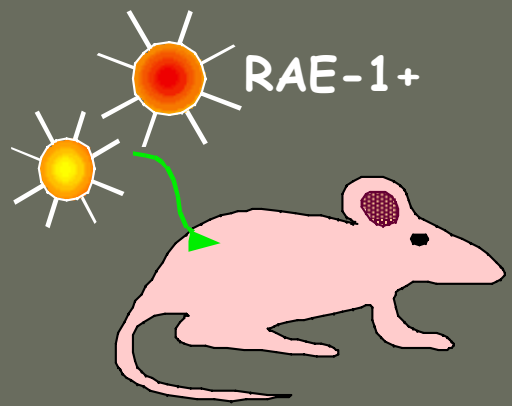

Rejection mediated by NK cells

# NKG2D-RAE-1 interaction overrides "self class I-inhibition" *in vivo*

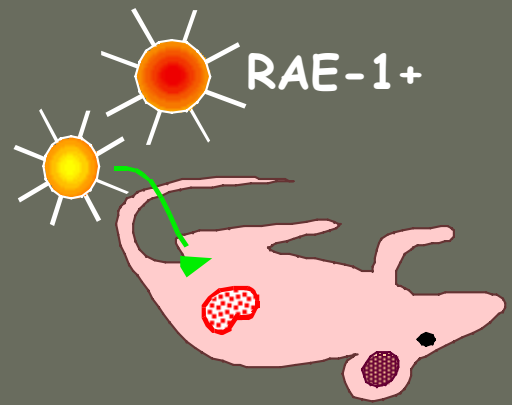
## NK Cells Reject RAE-1+ MHC class I+ Tumors!



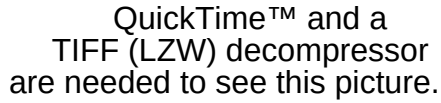
Class $I^+$ tumors grow *in vivo*

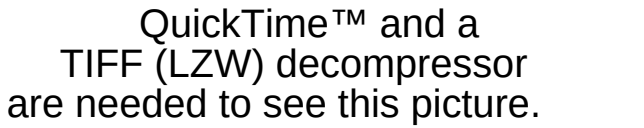



RAE-1+ Class $I^+$ tumors are rejected




RAE-1+ tumors in NK-depleted mice grow *in vivo*

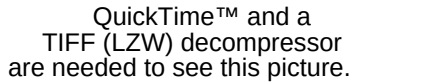


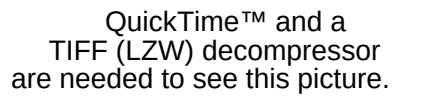




# Increased 3-MCA induced tumors in mice treated with anti-NKG2D mAb

If NK cells kill tumors expressing NKG2D ligands - how do the tumors survive?

# Shed or secreted NKG2D ligands in the sera of cancer patients



# NKG2D and Cancer

- Tumors frequently over-express NKG2D ligands
- DNA-damage induces expression of NKG2D ligands on tumors
- NK cells eliminate tumors expressing NKG2D ligands
- NKG2D ligands on tumors can (sometimes) augment tumor antigen-specific $CD8^+$ CTL
- Tumors shed or secrete soluble NKG2D ligands to act as decoys - immune evasion