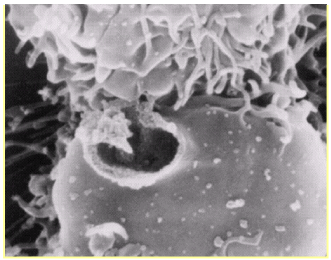
UCDAVIS

Natural Killer Cell Subsets: Unique Roles and Regulation during Viral and Tumor Immunity

William J. Murphy, Ph.D.

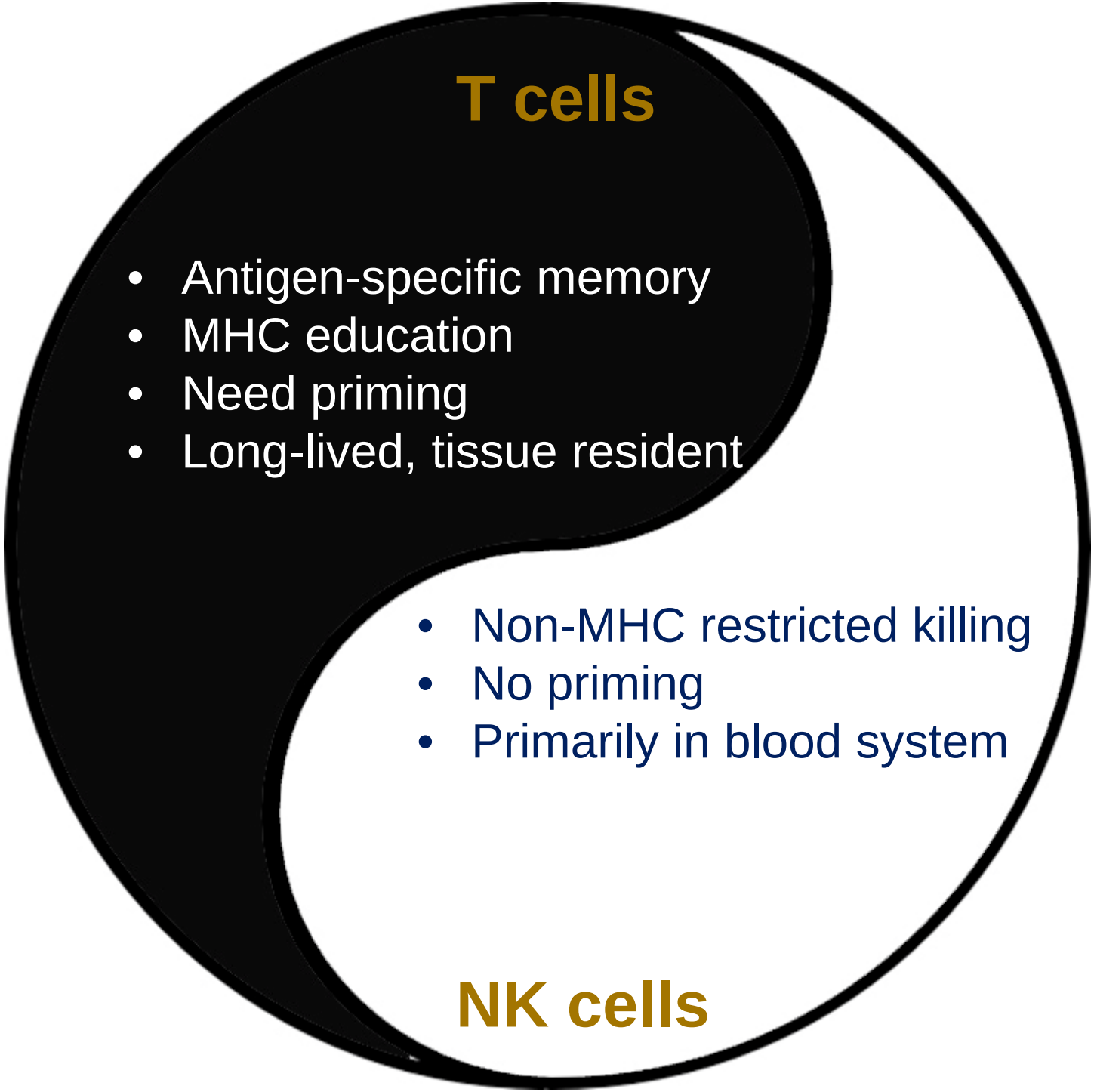
Departments of Dermatology and Internal Medicine

U.C. Davis School of Medicine



Natural Killer Cells

- Innate large granular lymphoid cell with anti-tumor and anti-viral activity
- Represents ~5-10% of peripheral blood lymphocytes
- Cytokine production (IFN-γ, IL-1-β, IL-3, IL-6, TGF-β, TNF-α, TNF-β, GM-CSF and M-CSF)
- Target lysis without prior immunization or pre-activation (granule exocytosis, ADCC, Fas/FasL and TRAIL/TRAIL-R pathways, TNF-α)
- Cytotoxic function based on:
  - "Missing self" recognition (Ljunggren and Karre, 1985)
  - Presence of stress ligands (MICA/B, Rae-1)
- MHC class I molecules recognition by inhibitory and activating NK cell receptors


T cells
• Antigen-specific memory
• MHC education
• Need priming
• Long-lived, tissue resident
• Non-MHC restricted killing
• No priming
• Primarily in blood system
NK cells

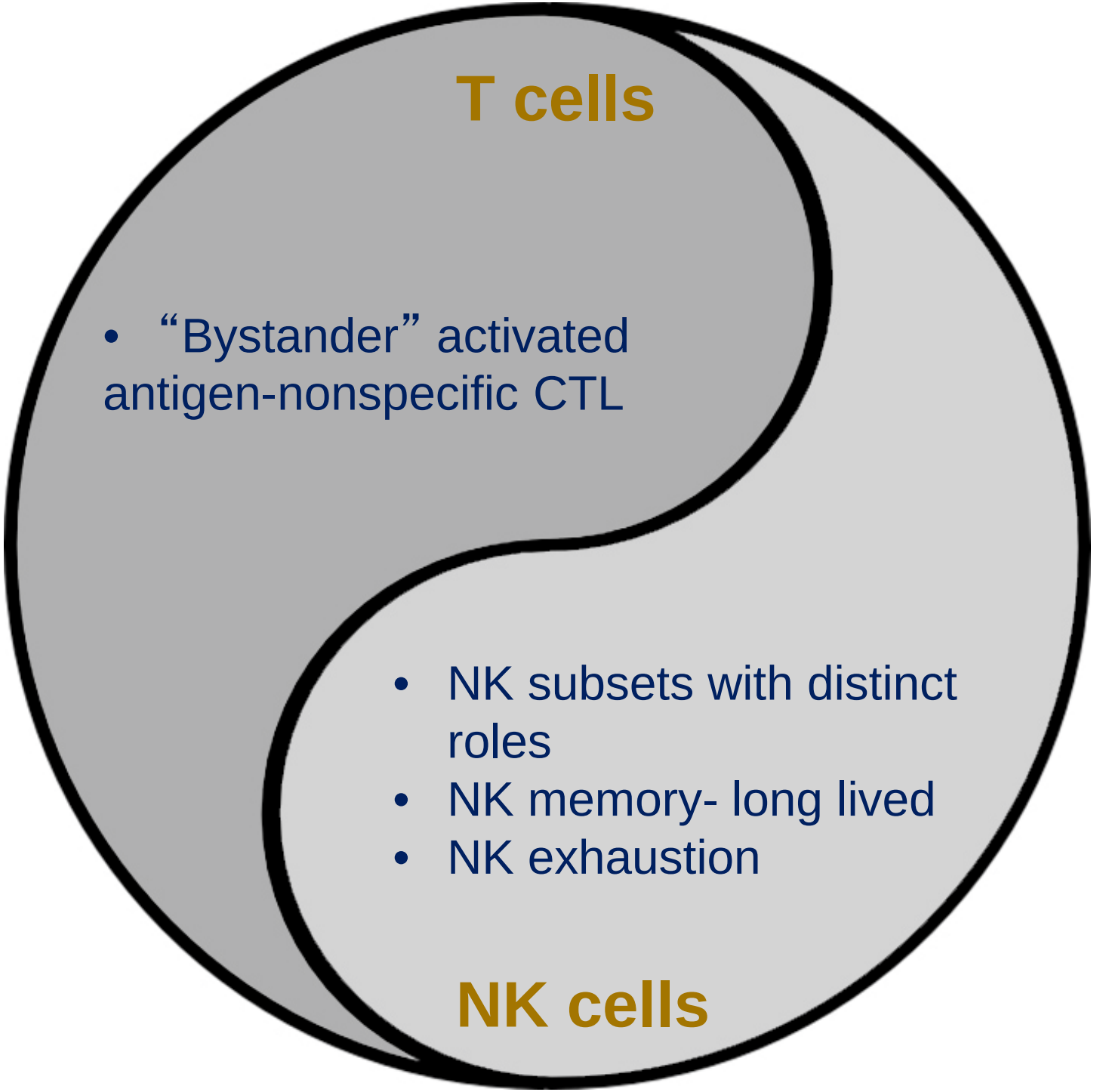
Human vs mouse

Human

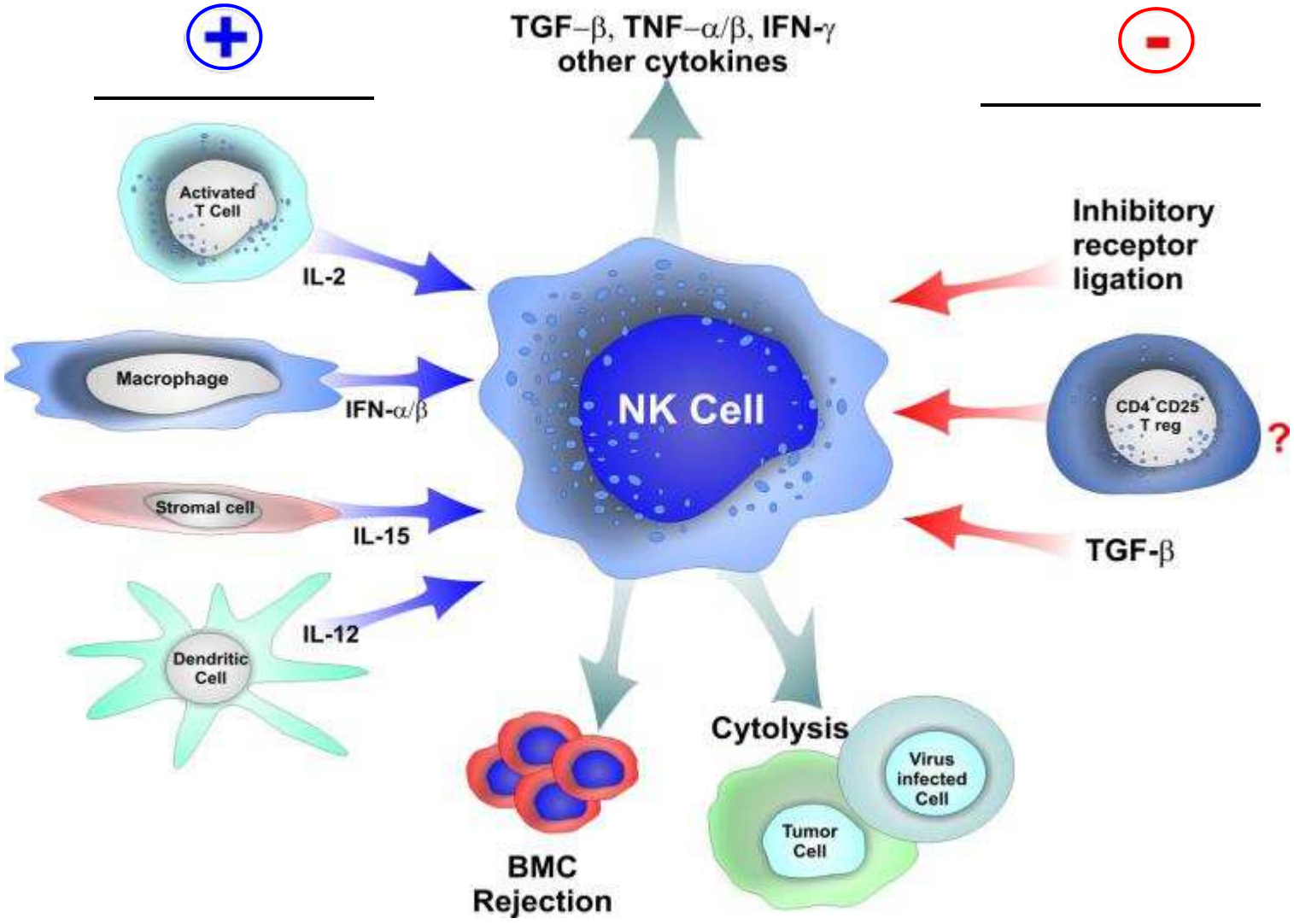
- CD56 – neuronal cell adhesion
  - dim – cytotoxic with perforin and granzyme. In periphery
  - bright – cytokine production (IFN-γ, GM-CSF, G-CSF, TNF, IL-6). In lymph nodes
- KIRs – immunoglobulin family structure
- Freshly isolated NK cells from peripheral blood exhibit cytolytic activity
- Can survive in vitro for long periods of time

Mouse

- No CD56 or similar molecule found
  - DX5 or NK1.1
  - Not found in lymph nodes
- Ly49 receptors (inhibitory or activating)
- Resting NK have poor cytotoxic function
  - low levels of perforin and granzyme
- Survive in vitro for shorter period of time (~2weeks)


T cells
• “Bystander” activated antigen-nonspecific CTL
• NK subsets with distinct roles
• NK memory- long lived
• NK exhaustion
NK cells

Positive and negative regulation of NK cell function



NK cell subset licensing

- Licensing of natural killer cells by host histocompatibility complex class I molecules in which only those NK cells bearing receptors for “self” MHC exhibit greater activity. Kim *et al*. *Nature*. 2005. 436(7051):709-13

- Mouse NK cells bearing Ly49 receptors for “self” MHC become “licensed” and primed for function. Primarily observed via in vitro activities.

Cytomegalovirus

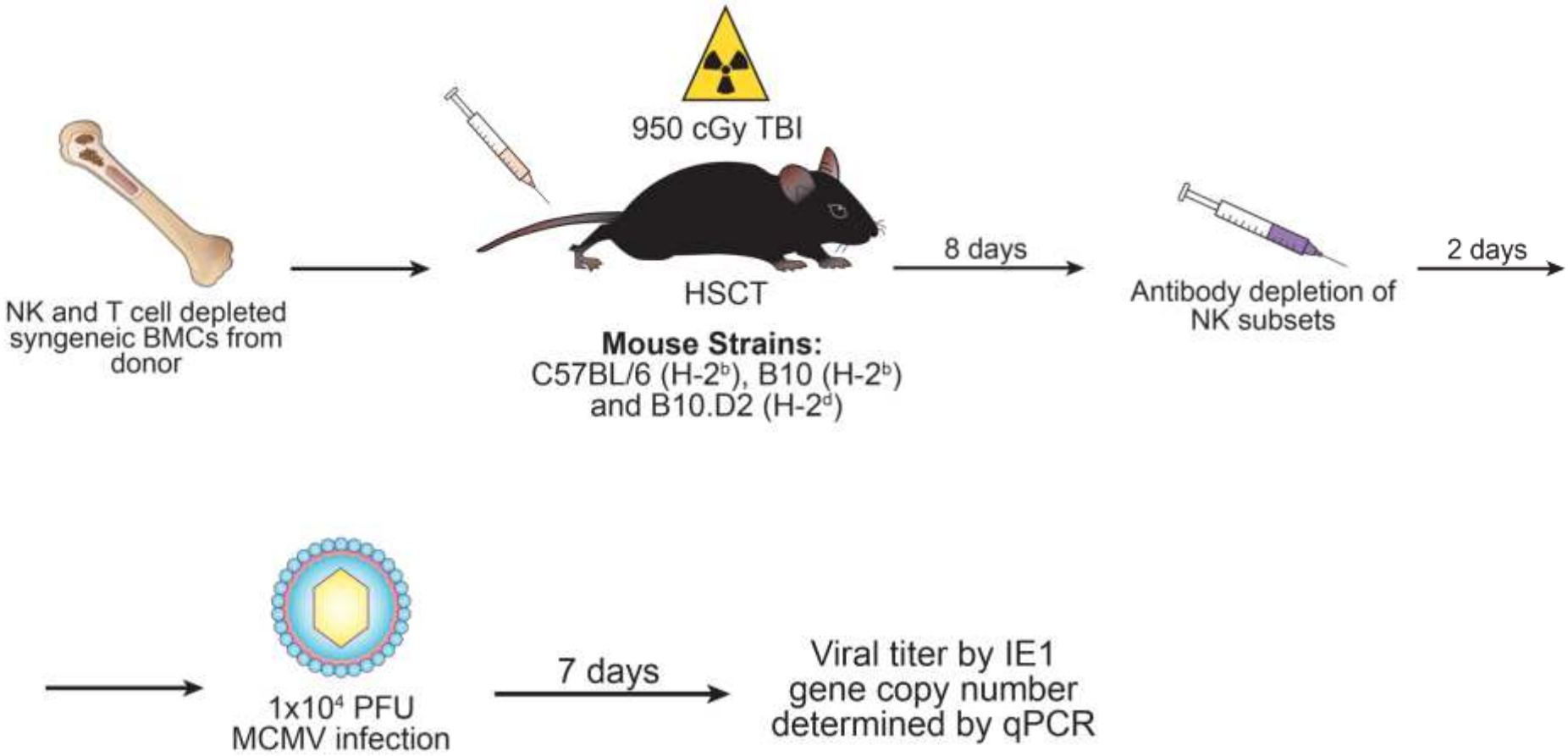
NK cells play significant role in CMV protection as shown by CMV pirating of MHC-like molecules in its genome

- 60-80% of people infected with CMV in U.S.
- Similar pathology, immune responses, and disease progression in human and murine CMV
- NK cells express activating Ly49H receptor that binds MCMV molecule m157 (Daniels *et al.*, J. Exp Med, 2001; Lee *et al.*, Nat Genetics, 2001; Dokun *et al.*, Nature Immunology, 2001)

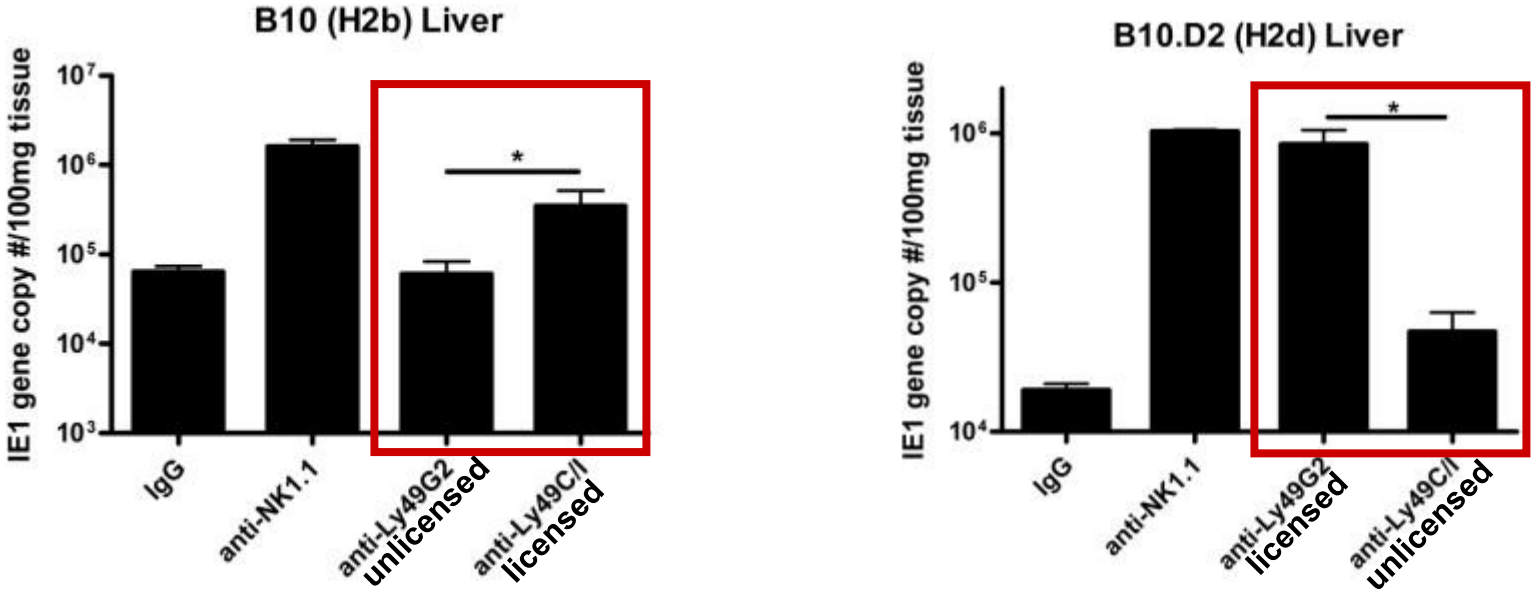
Hematopoietic stem cell transplantation

- Used for treatment of hematological malignancies (leukemia and lymphoma)
- Utilization of total body irradiation results in immunosuppression that can reactivate latent viruses and result in tumor relapse
- NK cells first lymphocytes to repopulation (7-10 days in mice, 14 days in humans) while T and B cells repopulate 30 days later in mice, variable in humans (greater than 2 years)
- What roles do NK cell subsets exert post-HSCT on anti-MCMV or leukemia responses?

Effects of NK subset depletion on MCMV resistance post-HSCT

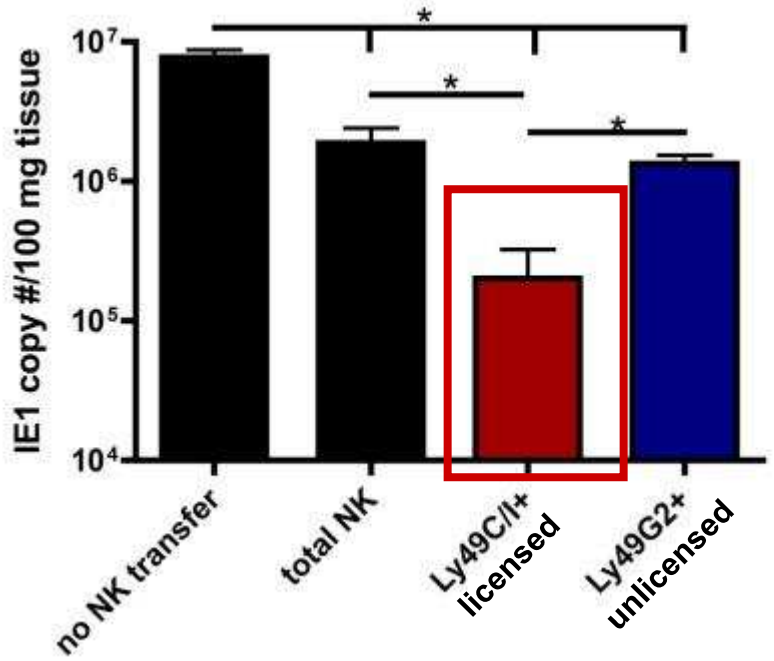


Licensed Ly49 NK cell subsets provide greater MCMV protection after syngeneic HSCT



Sungur *et al*., *PNAS* 2013. 110(18):7401-6

Adoptive transfer of licensed NK cell subsets offers greater anti-MCMV effects in immunodeficient mice



Sungur *et al*., *PNAS* 2013. 110(18):7401-6

Conclusions

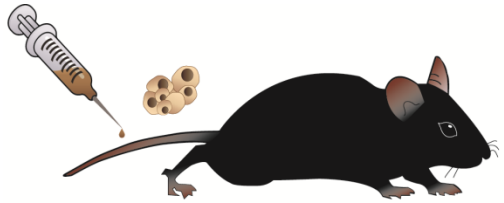
- Depletion of licensed NK cell subsets results in impaired MCMV control post-HSCT and Treg depletion
- Suggests licensed NK cells more active early during infection prior to suppression and regulation
- Tregs involved in suppressing and regulating licensed NK cell response

Aims/Questions

- Differential role of NK cell subsets when comparing HSCT vs non-HSCT setting, can this be seen in leukemia model?

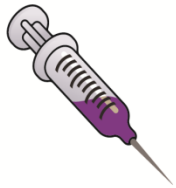
- Are there licensed and unlicensed NK cells functional parallels between viral and leukemia challenge models?

Experimental schema for leukemia non-HSCT experiments



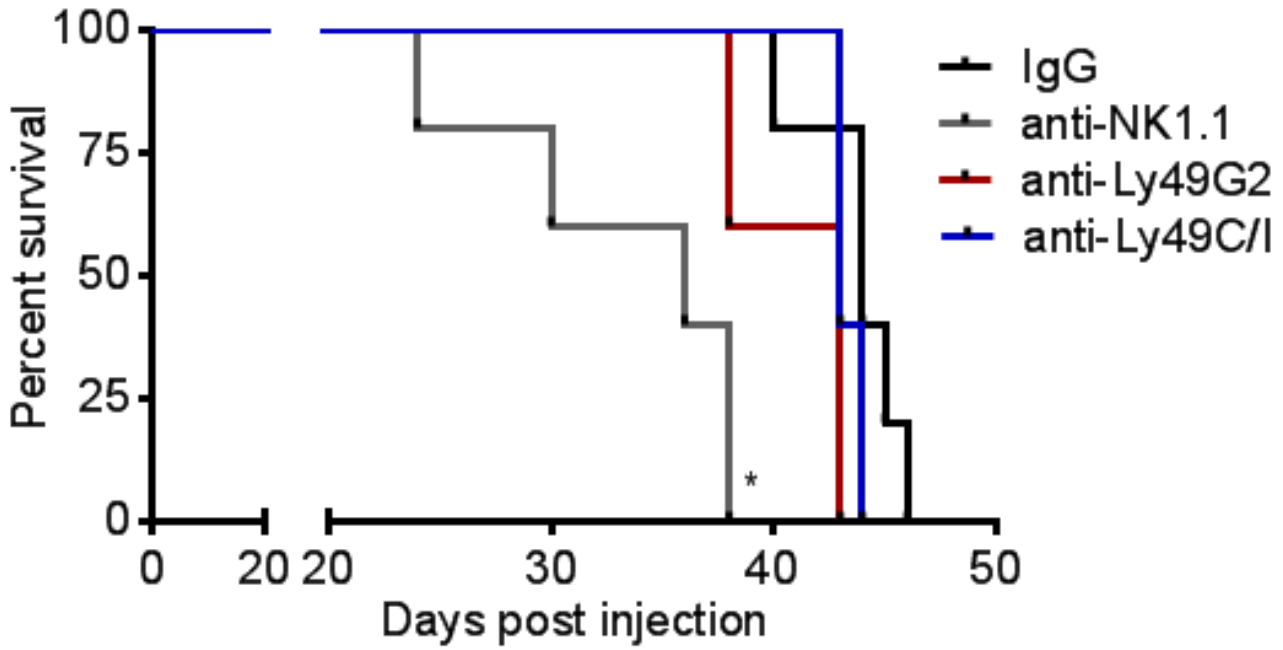
Recipient: C57BL/6
leukemia cells (C1498) I.V.

+

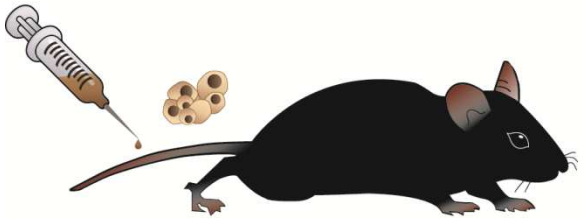


mAb depletion
of recipient mouse:
anti-NK1.1
anti-Ly49G2
anti-Ly49C/I

No difference in survival between subset depleted mice following leukemia challenge (non-HSCT)

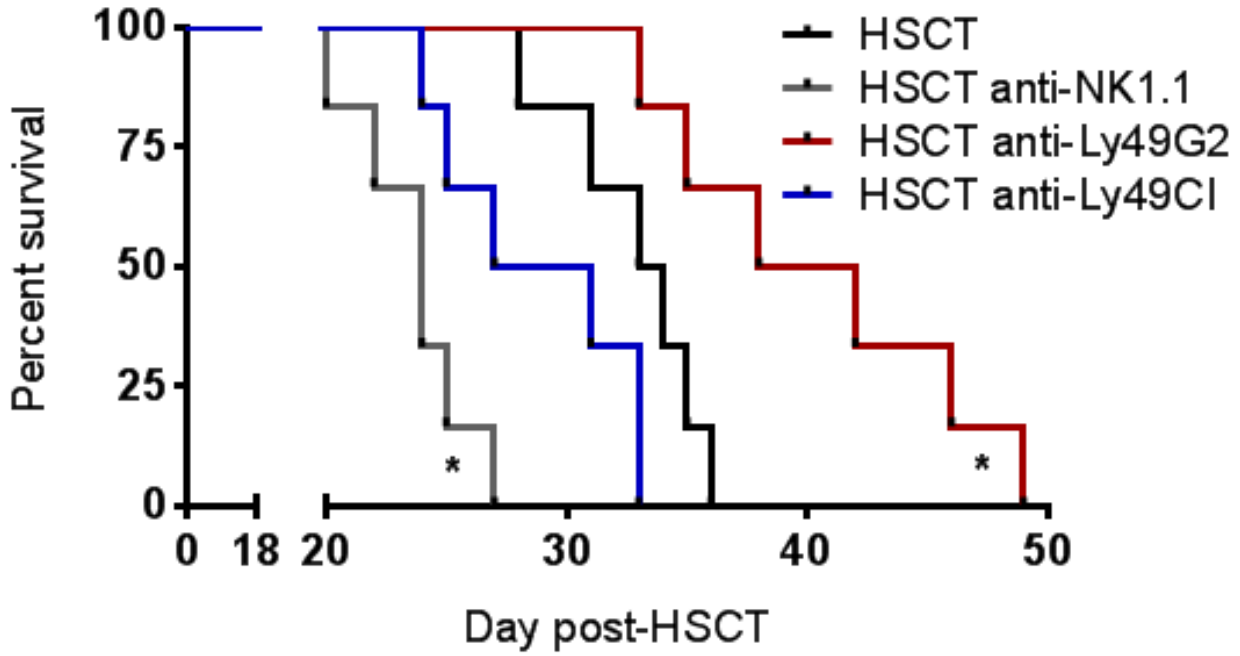


Experimental schema for leukemia HSCT experiments

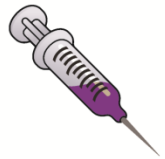


Recipient: C57BL/6
200,000 C1498 I.V.

Depletion of the unlicensed population following HSCT during leukemia challenge results in increased survival

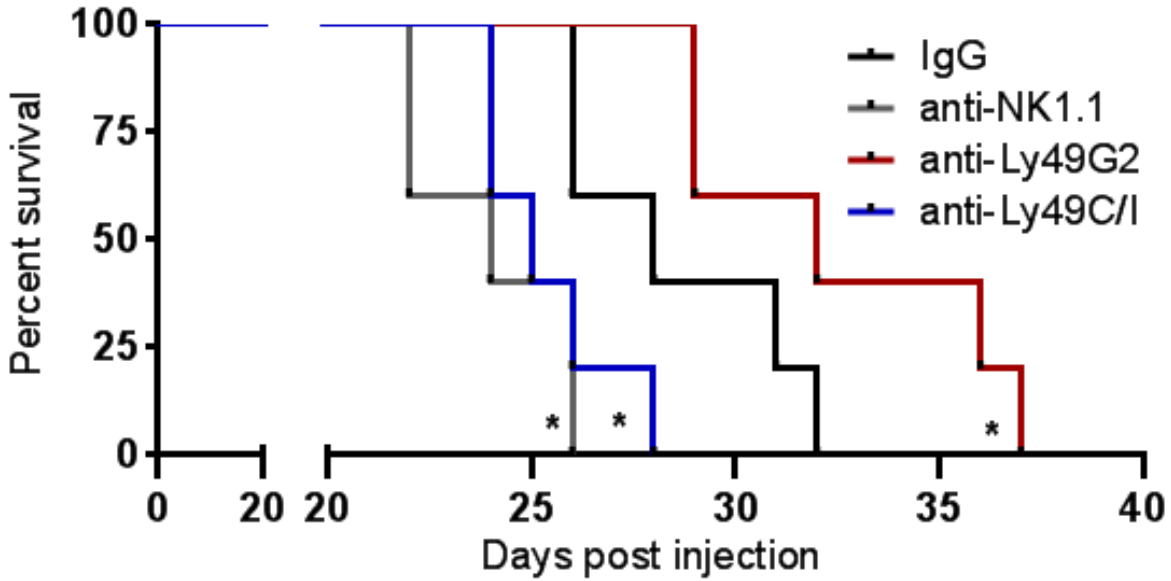


Experimental schema for leukemia + Treg depletion experiments

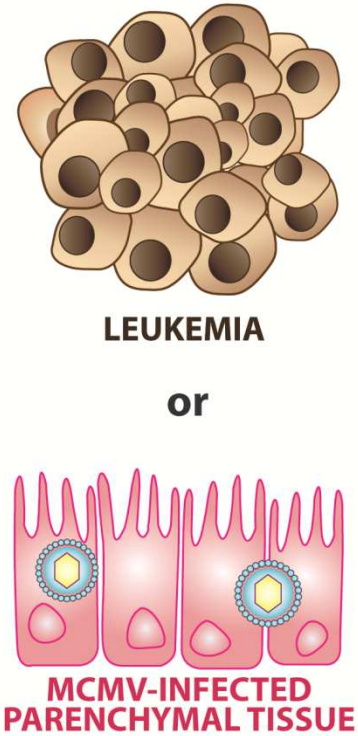


mAb depletion of Tregs

Depletion of Tregs results in survival patterns of HSCT mice following leukemia challenge



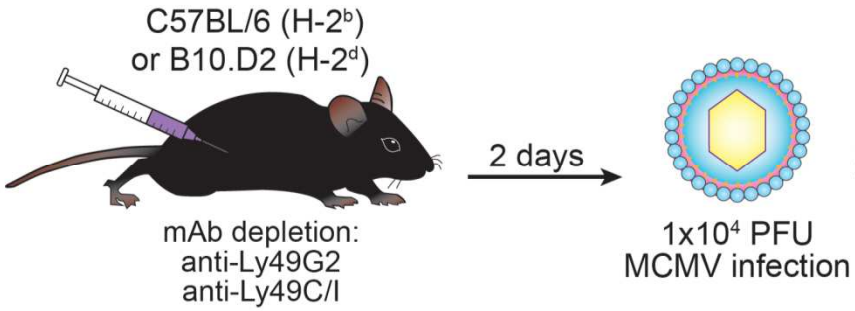
Treg-NK cell interactions during different inflammatory settings



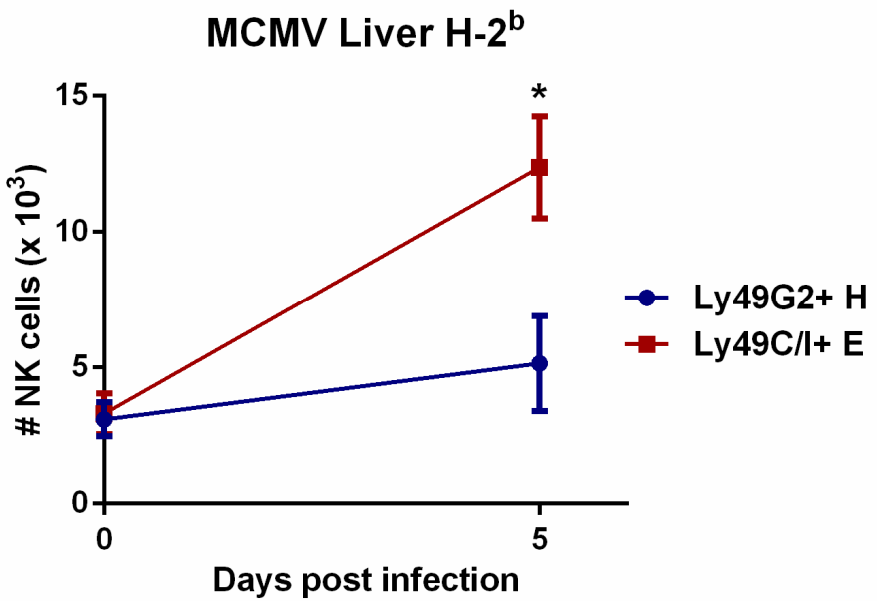
Aims/Questions

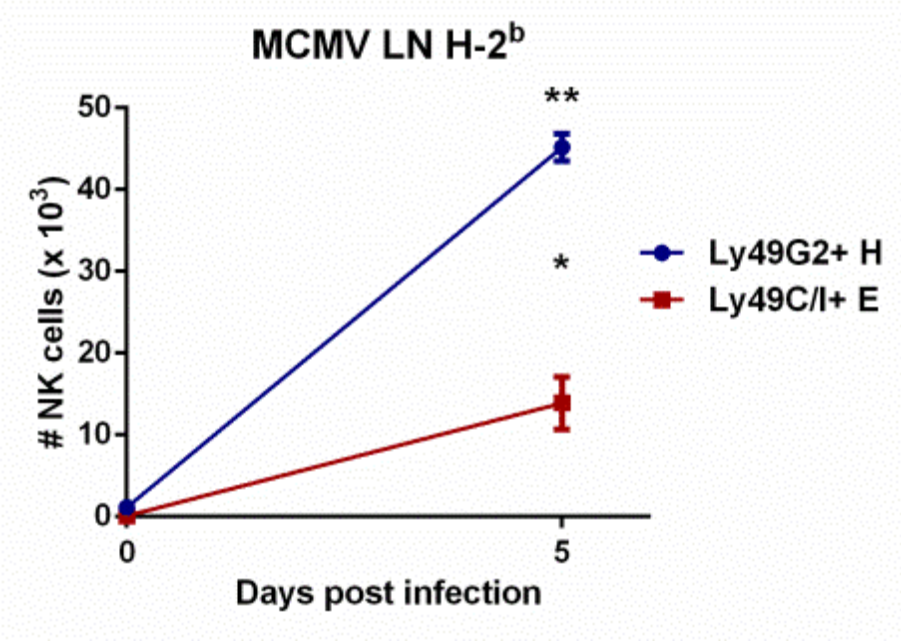
- In HSCT, NK cells are the predominant lymphoid cell to repopulate and therefore mediate resistance to MCMV. Thus, licensed NK effector (E) cells dominate.
- In non-HSCT situations, T cells may play a dominate role in MCMV resistance - what do NK cell subsets do to affect total MCMV resistance?
- Can effector (E) or helper (H) NK cells be delineated based on licensing?
- Are these functions carried out by distinct NK cell subsets? What do unlicensed NK cells do?

Effects of NK cell subsets on T cell responses

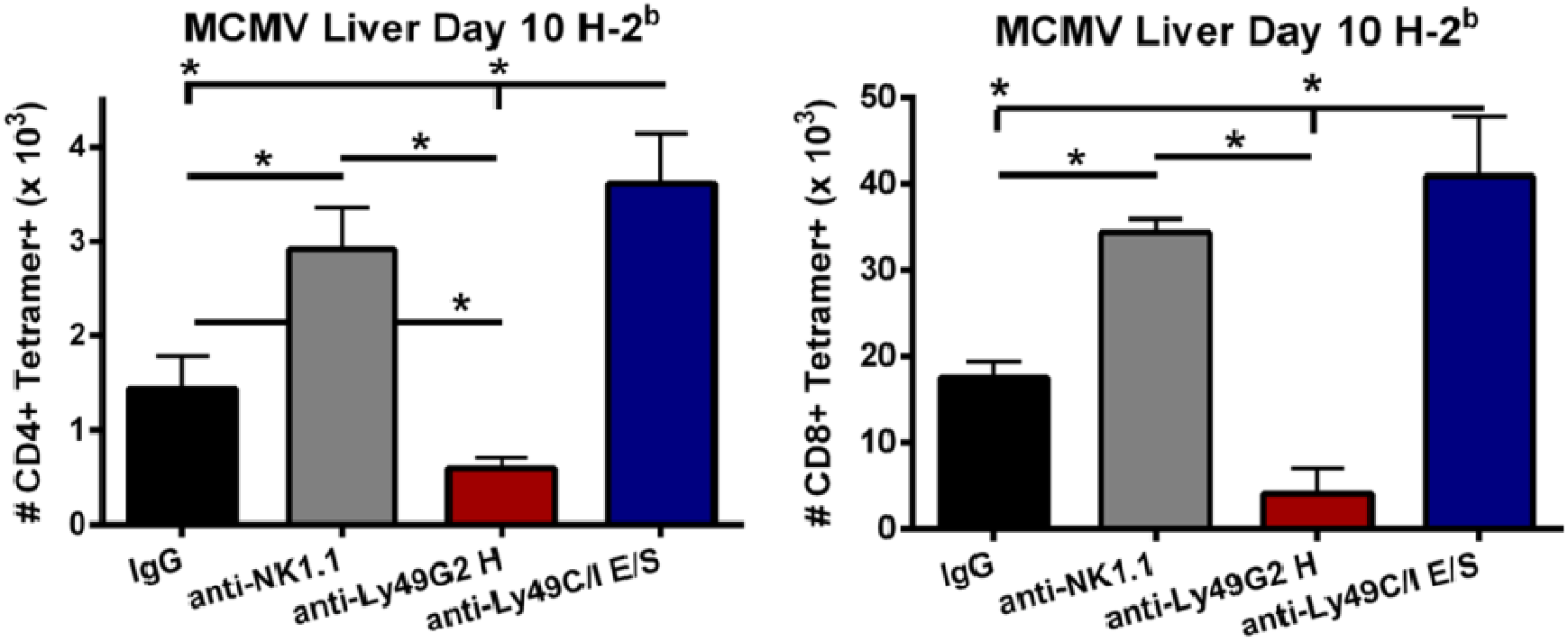


Licensed (Effector) NK cells at sites of infection while unlicensed (Helper) NK cells traffic to LN during early infection

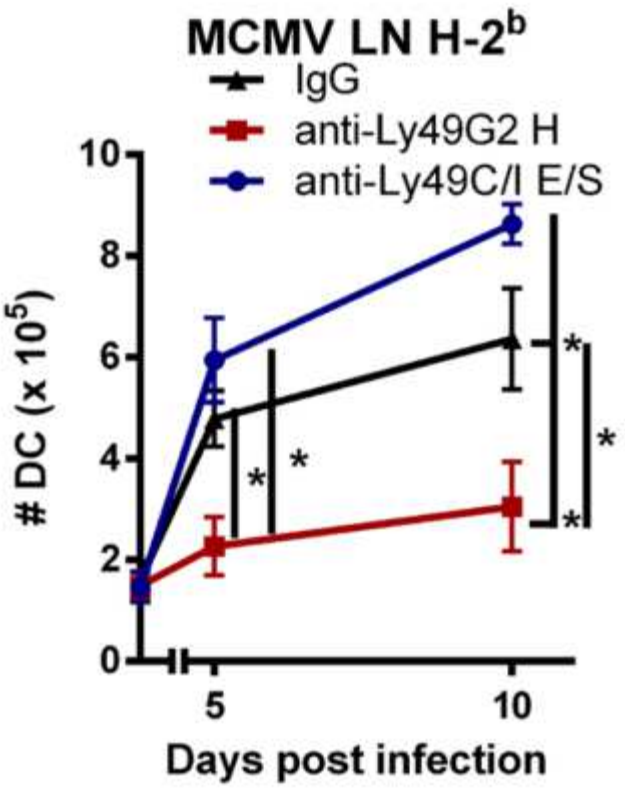


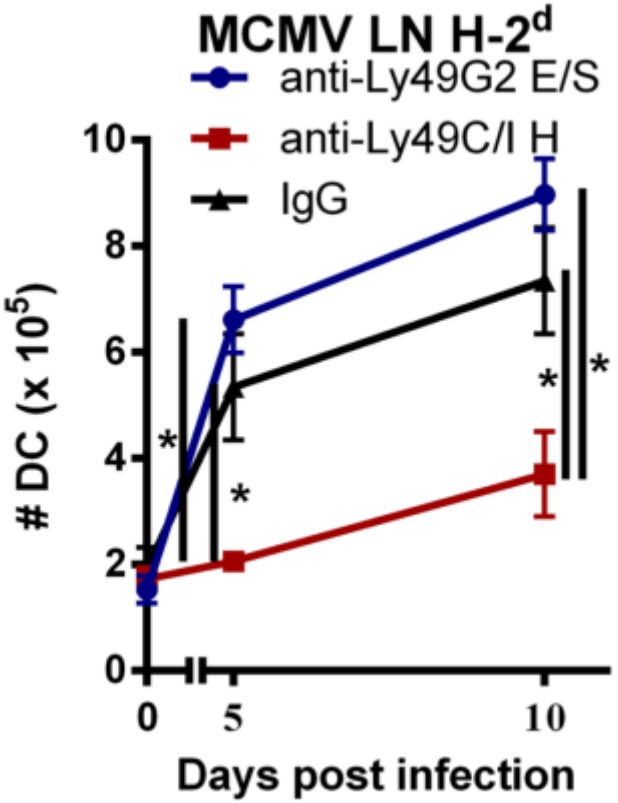


Depletion of unlicensed NK cells results in decreased numbers of Ag-specific T cells

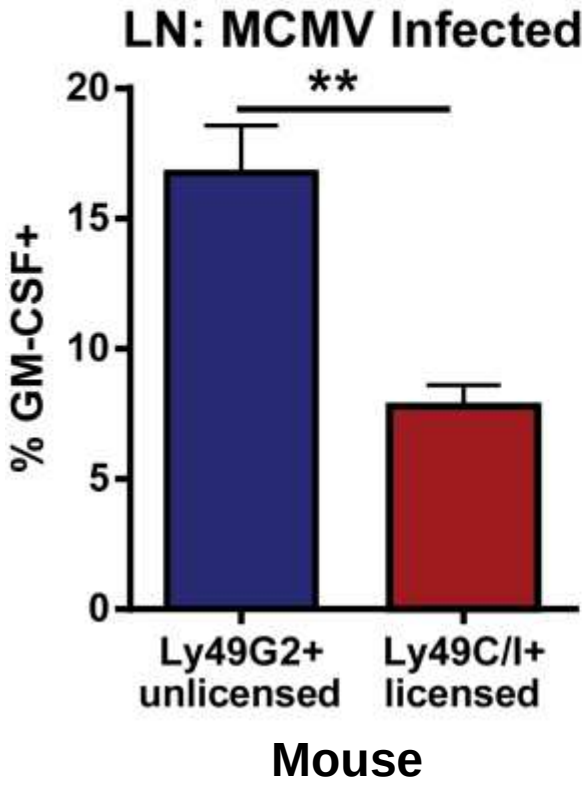


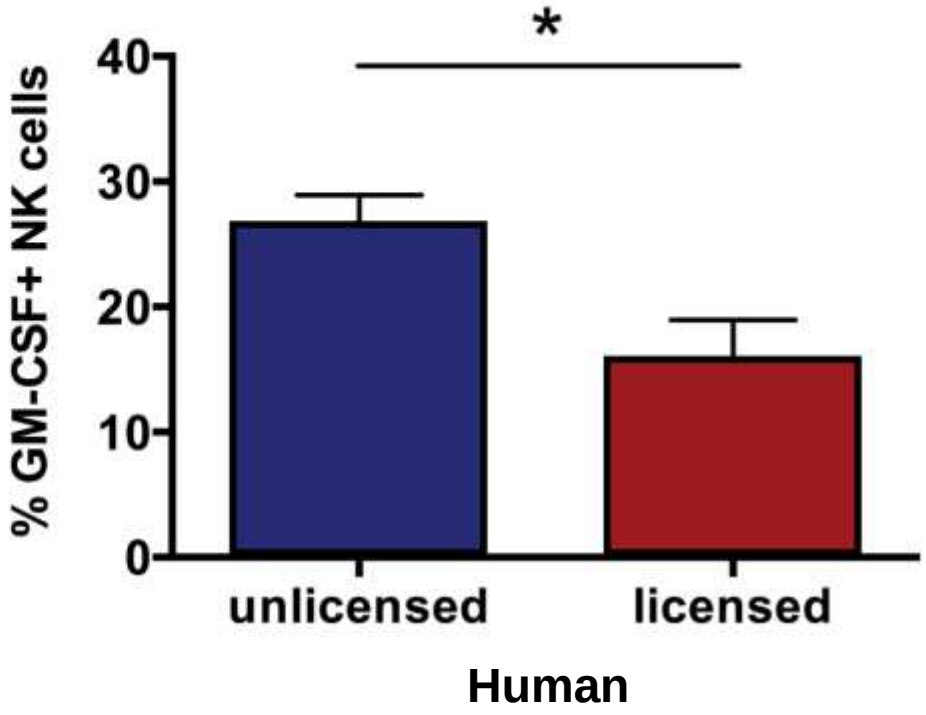
Helper (H) NK cells help expand DCs in LN





Cytokine profile parallels between mouse and human NK subsets

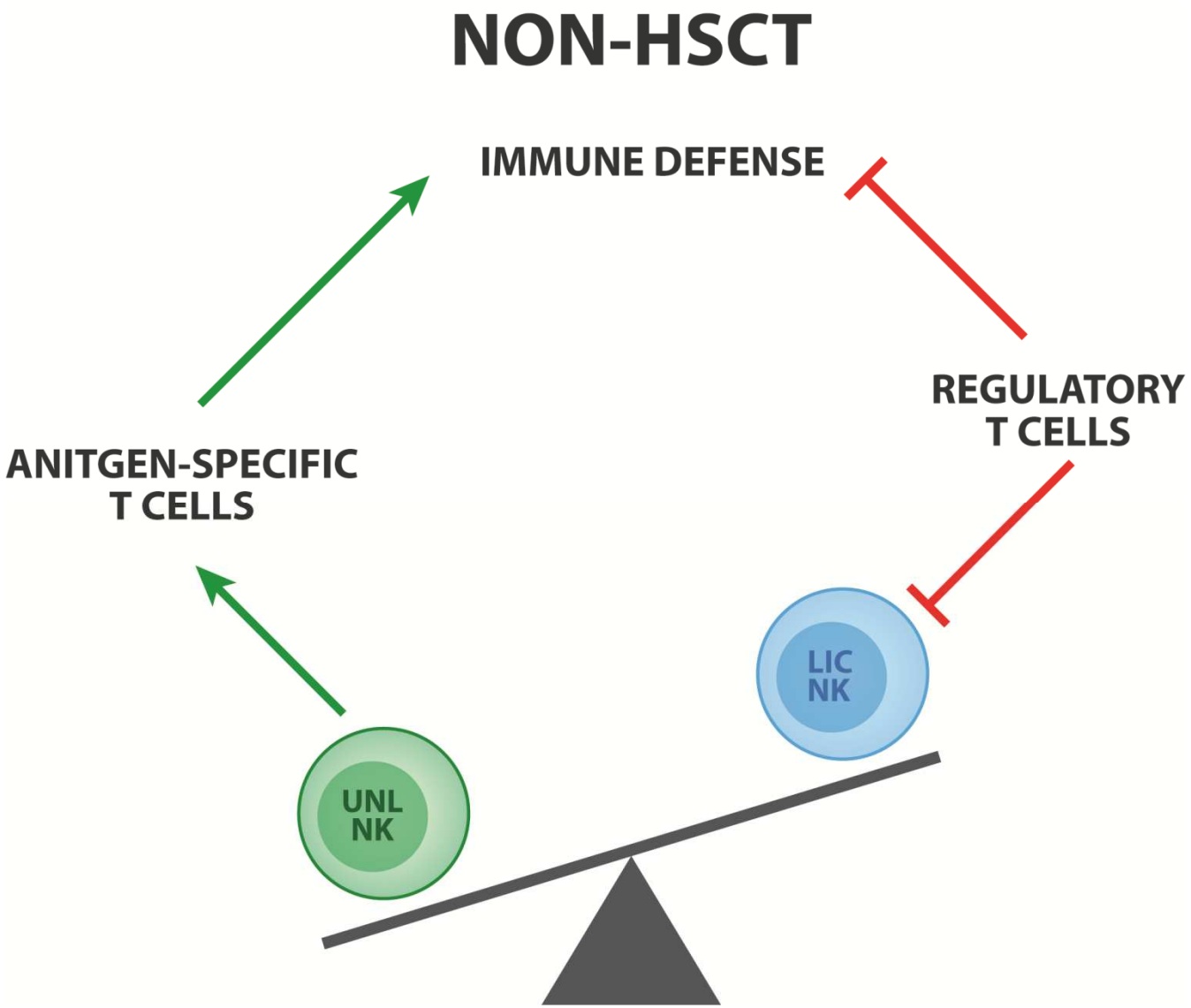




Advancements in NK Cell Biology and Function

- Licensing allows us to delineate NK cell populations into subsets with differential functional roles on pathogen resistance

NK Subsets and Host Response



Immunology Laboratory

University of California, Davis

Murphy Lab

William J. Murphy
Anthony Zamora
Ethan Aguilar
Gail Sckisel
Erik Ames
Steven Pai
Steven Grossenbacher
Annie Mirsoian
Christine Mall
Stephanie Mac
Jessica Stolfi
Ragheb Masoud
Janell Rivera
Robert Canter
Arta Monjazeb
Monja Metcalf
Weihong Ma

Pomeroy Lab

Claire Pomeroy
Yajarayma Tang-Feldman
Raymond Lochhead
Stephanie Lochhead

Baumgarth Lab

Nicole Baumgarth
Zheng Luo

University of California, San Francisco

Venstrom Lab

Jeffrey Venstrom
Juan Du

University of Minnesota

Blazar Lab

Bruce Blazar