UCDAVIS

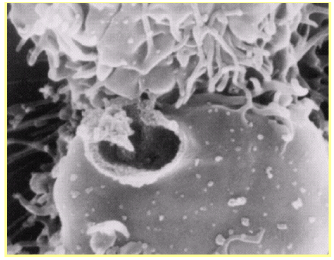
NK cell modulation of T cell responses during viral infection



William J. Murphy, Ph.D.

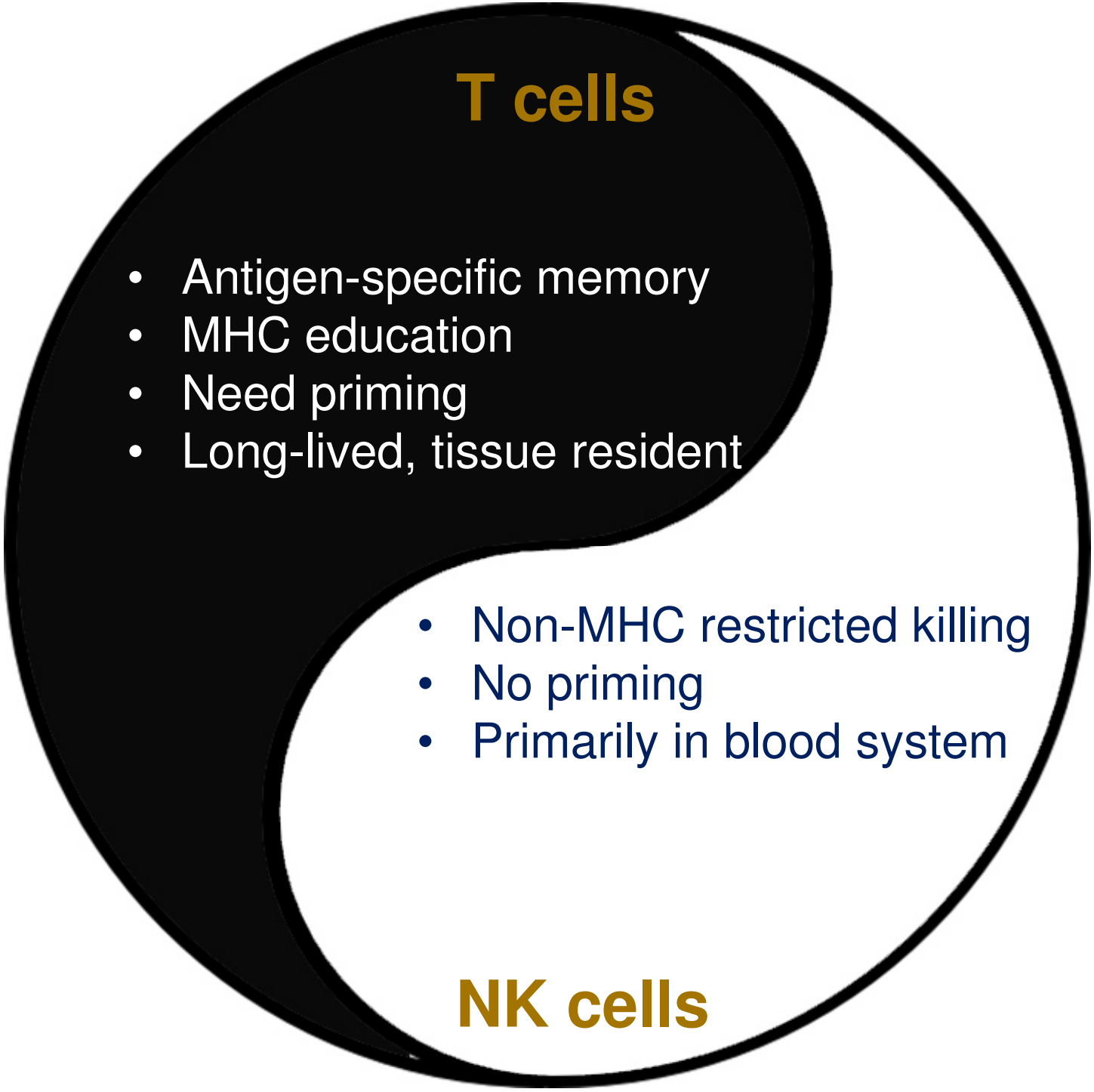
Departments of Dermatology and Internal Medicine

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

Natural killer cell subsets

- NK subsets exist with differing expression or isoforms of inhibitory (KIR/Ly49, NKG2A) and activating receptors (KIR/Ly49, NKG2D, NCR)

- NK cells regulate adaptive immune responses through lysis of DCs (Ferlazzo, JEM 2002), T cells (Waggoner et al., Nature 2011) and cytokines produced:
  - Suppressive: TGFβ, IL-10
  - Proinflammatory: IFNγ, TNFα, IL-6,

- Expression of unique isoforms of NKp30 activating receptor resulted in inflammatory/lytic versus suppressive (IL-10 producing) NK subpopulations (Delahaye, Nature Med 2011).

- $CD56^{bright}$ (cytokine producing) versus $CD56^{dim}$ (cytotoxic) subsets in humans

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.

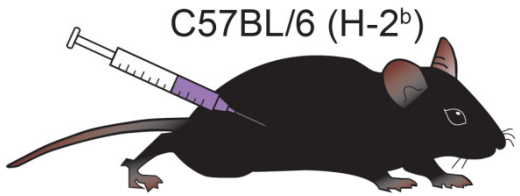
NK cell modulation of T cell responses

- NK cell function alters T cell responses to viral infection (Su et al. Eur J Immunol, 2001)
- NK cell lysis of target cells enhances T-cell responses by providing antigens for presentation, and promotes early CD8 T-cell responses to MCMV (Krebs et al. Blood, 2009)
- Presence of NK Cells can limit T-cell responses to either MCMV or LCMV infection and adaptive memory responses (Andrews et al. JEM, 2010)
- NK cell cytotoxicity limits CD8 T-cell responses, resulting in persistent viral infection (LCMV) and elevated infection-induced disease (Lang et al. PNAS, 2012)
- NK cells act as rheostats by modulating anti-viral T cells (Waggoner et al. Nature, 2012)

Hypothesis

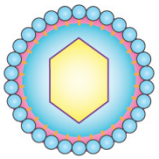
- NK cell populations exhibit unique functional roles during viral resistance based on licensing and kinetics
- The licensed population is the effector/suppressor population and involved in direct antiviral protection early and suppression of the adaptive immune response late in the infection
- The unlicensed population is the helper population, functioning to help promote the adaptive immune response during the early stages of viral infection

Experimental Schema



mAb depletion:
anti-Ly49G2
anti-Ly49C/I

2 days



1x10⁴ PFU
MCMV infection

3 days → 5 days → 7 days → 10 days

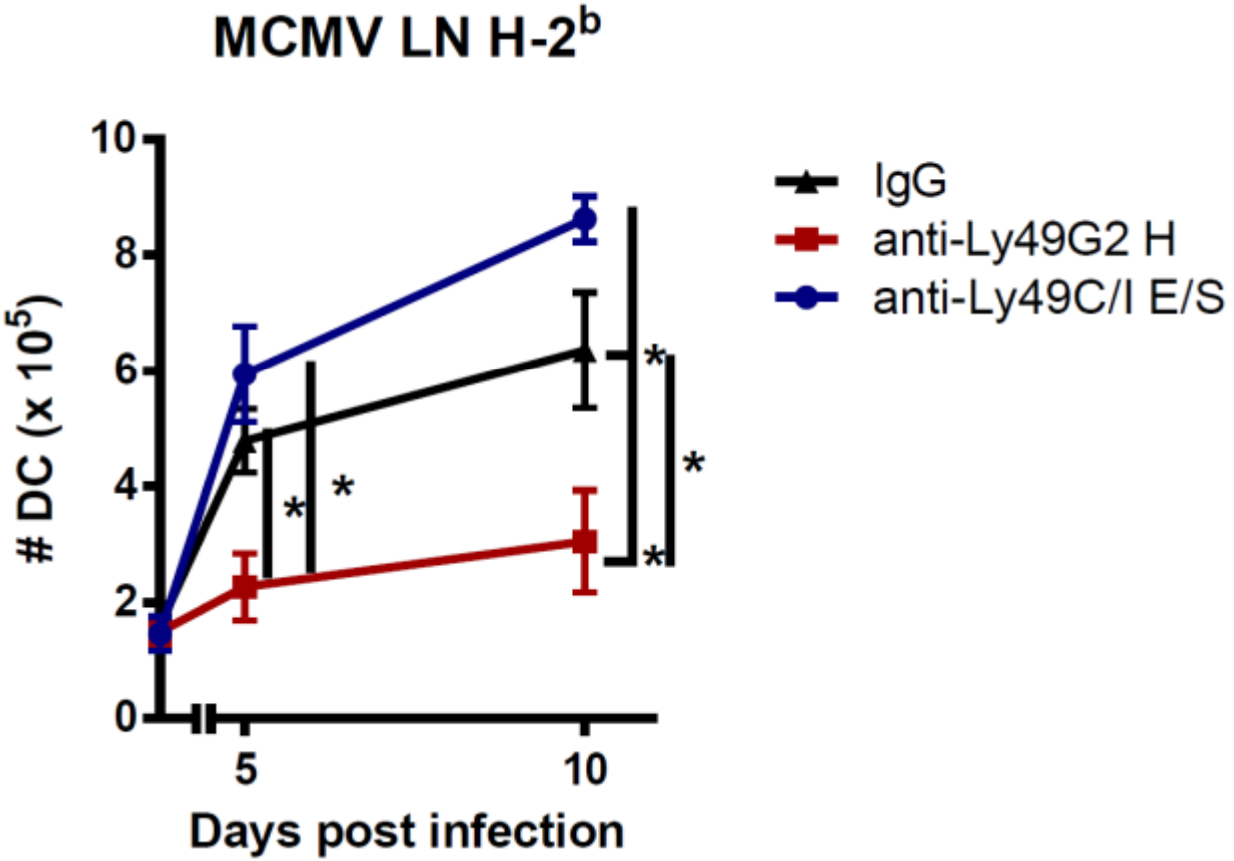
Analyzed:
Lymph Nodes
Liver
Spleen

Readouts:

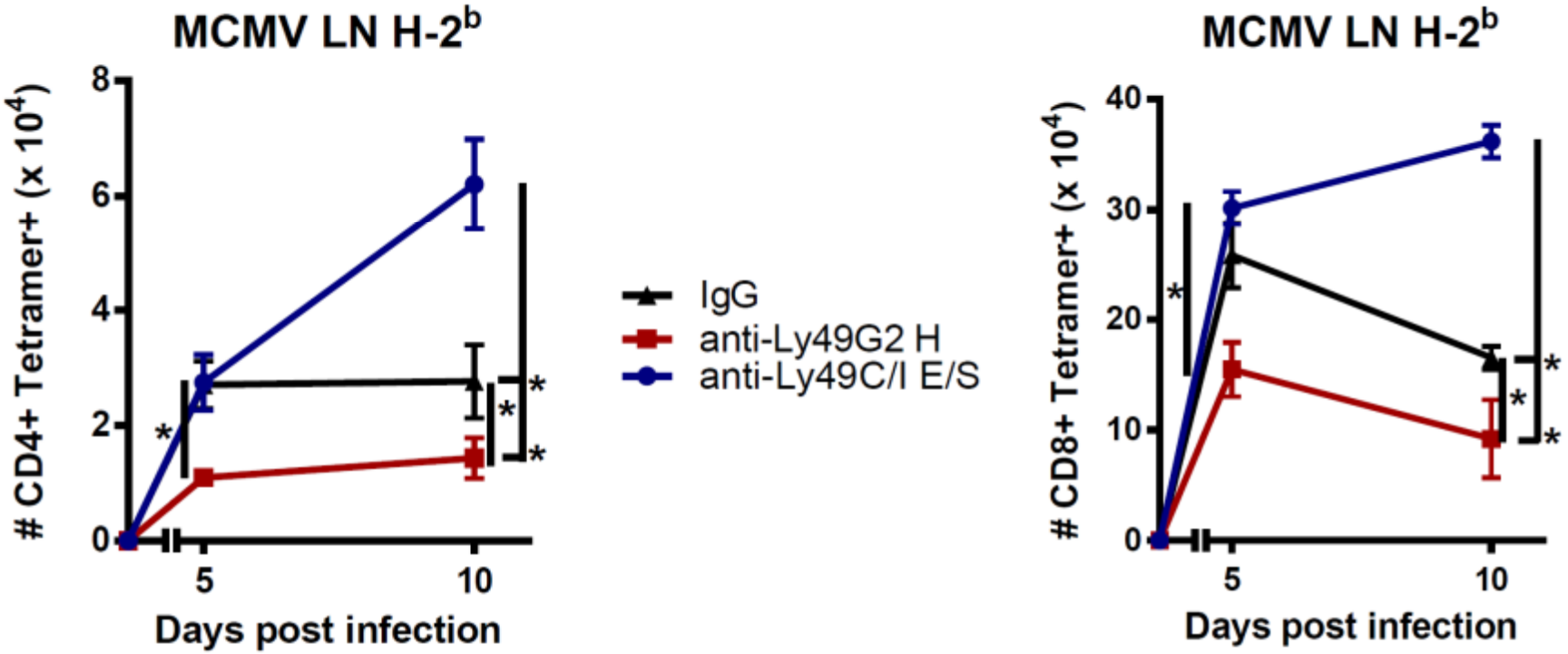
Flow cytometry:
- NK Cells
- DCs
- Antigen-specificT cells
- Intracellular staining for cytokines

Liver Pathology

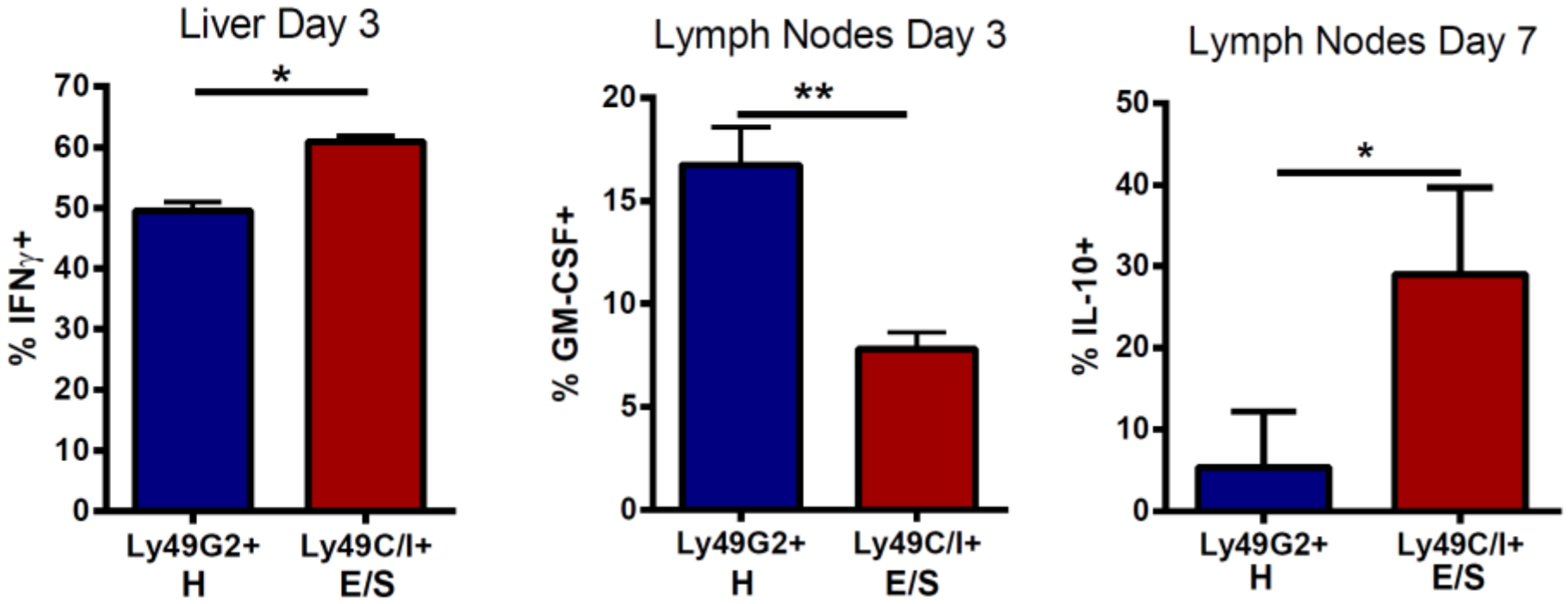
Unlicensed “Helper” NK cell Subsets help expand DCs in LN



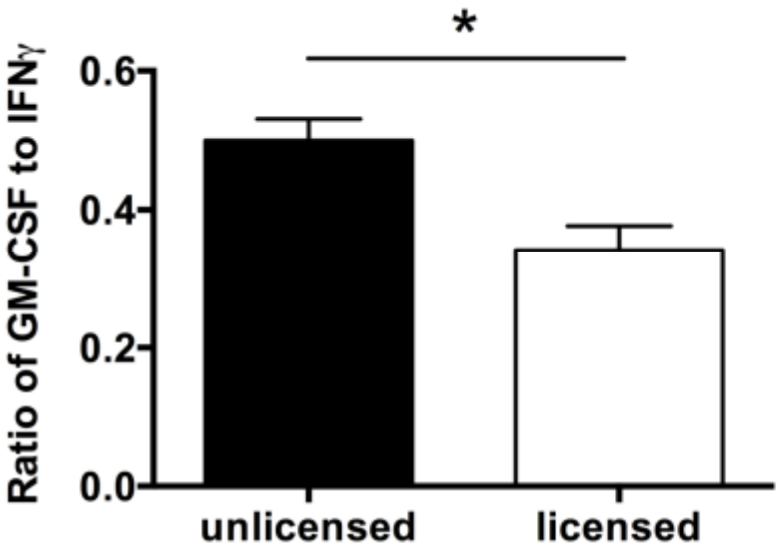
NK helper cells promote, while licensed NK effector/suppressor cells suppress adaptive T cell responses

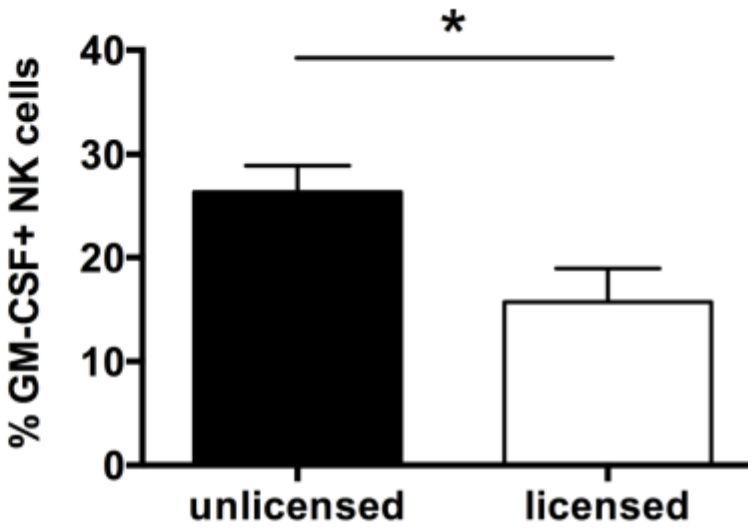


Cytokine profiles of Helper (H) and Effector (E/S) NK cell subsets reflect functionality

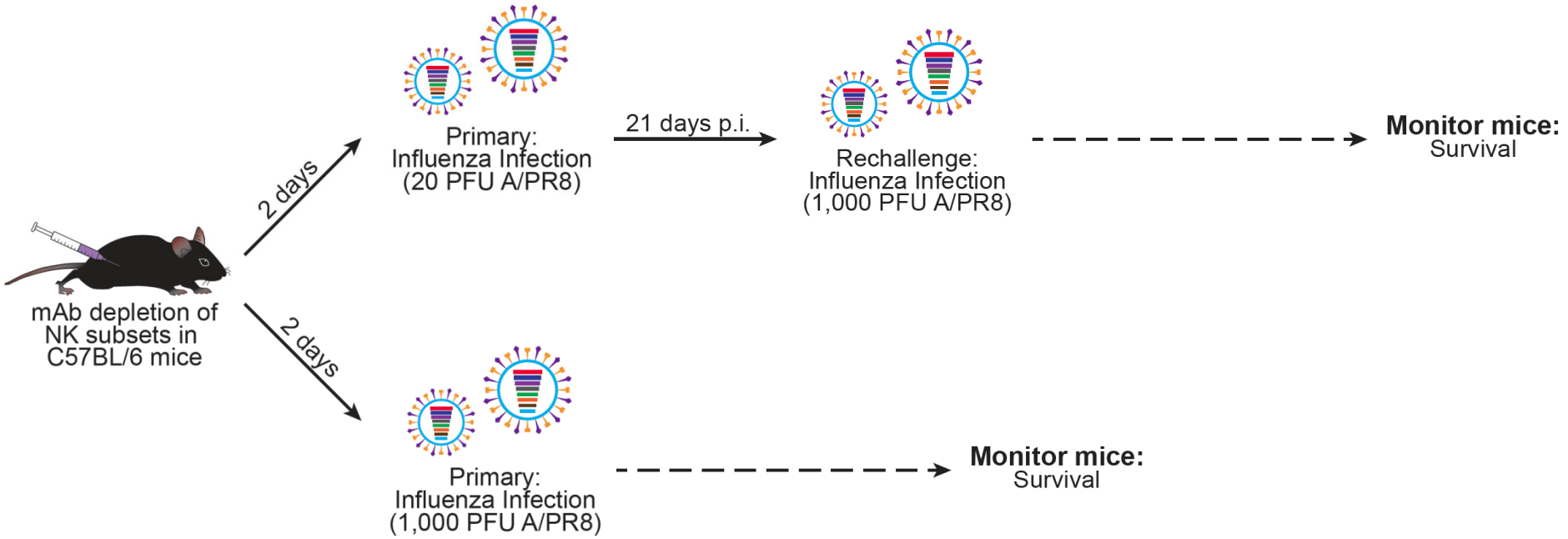


Similar Human NK cell subset cytokine production based on licensing

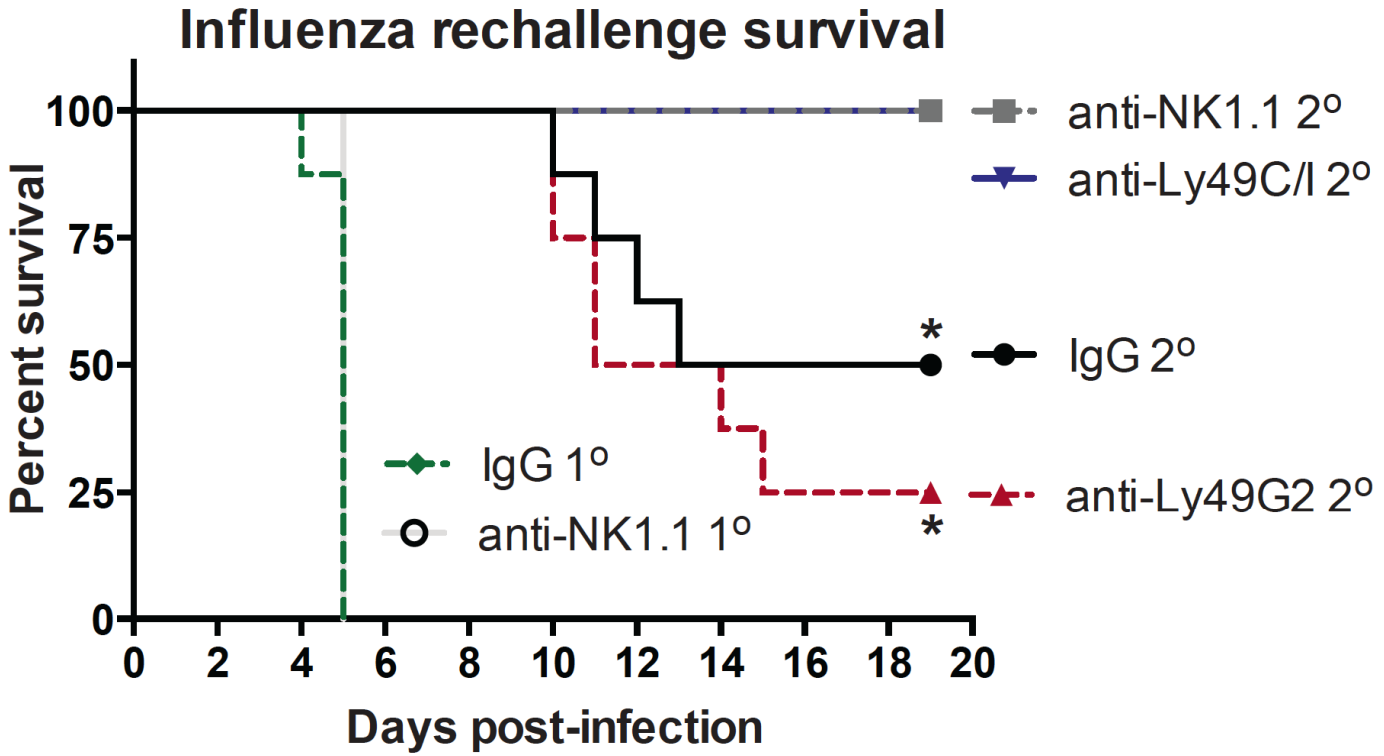




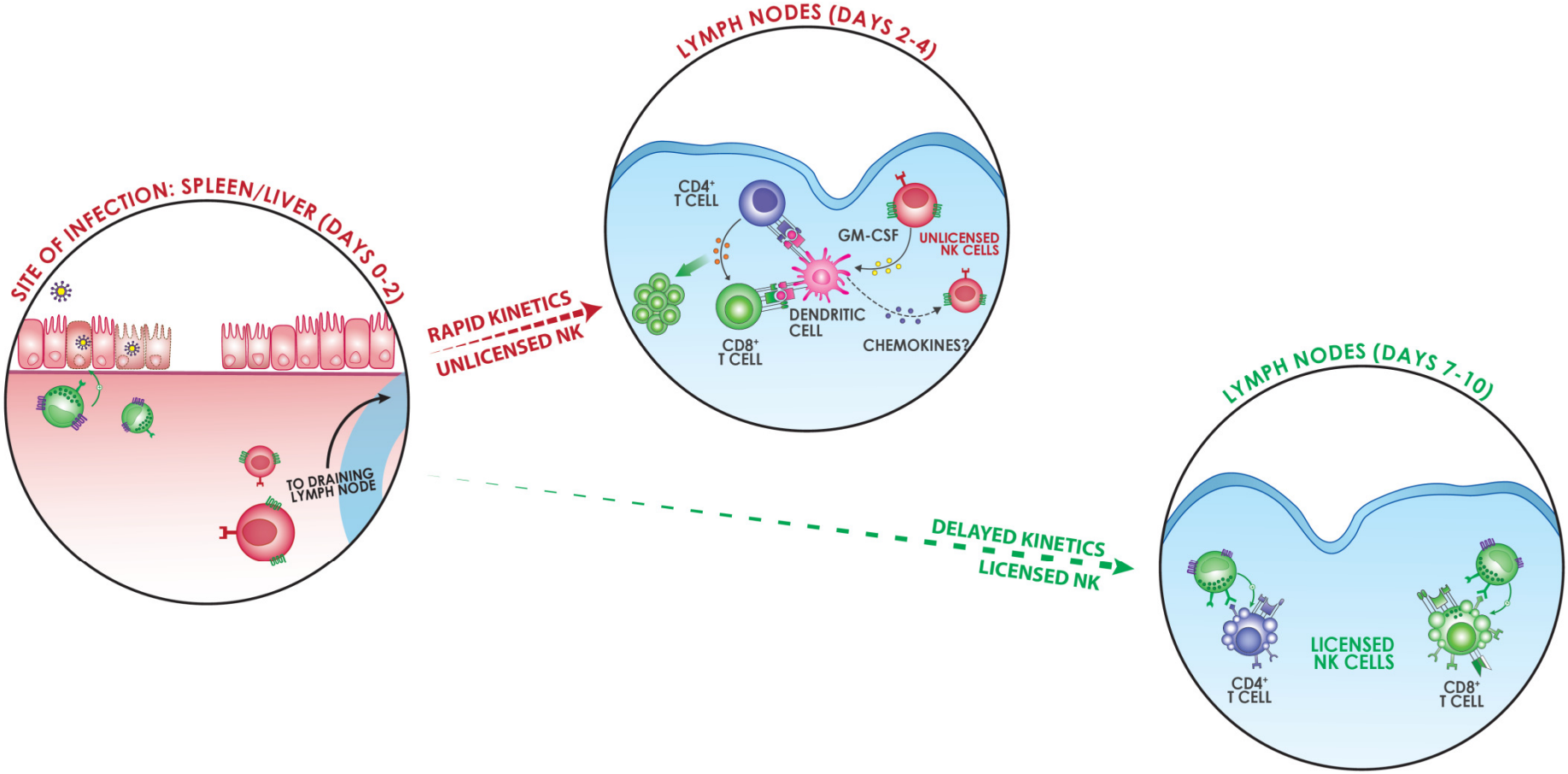
Rechallenge Experimental Schema



Removal of the NK helper subset impairs secondary anti-viral responses



Differential Roles of NK cell subsets on adaptive T cell responses



Conclusions

- Licensing serves as a marker for functional subsets of NK cells during viral responses
- Licensed NK cells serve as effector/suppressor cells by being at the sites of infection and aiding in antiviral responses early but inhibiting T cells later
- The unlicensed NK cells act as NK helper cells and aid in DC expansion in the LN early in the infectious course resulting in increased antigen-specific T cell responses
- Cytokine production differences observed in both human and mouse NK subsets correlates with their functional differences

NK cells as potential vaccine adjuvants

- NK cell subsets may differentially regulate adaptive immunity following vaccination
  - Depending on the viral vaccine, modulation of the effector/suppressor or helper NK subset may be desirable
  - NK subsets may alter the magnitude of additional innate responses – promoting or suppressing dendritic cell function
  - Cytokine production differences between the NK subsets may alter vaccine efficacy and skewing of adaptive immunity

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