

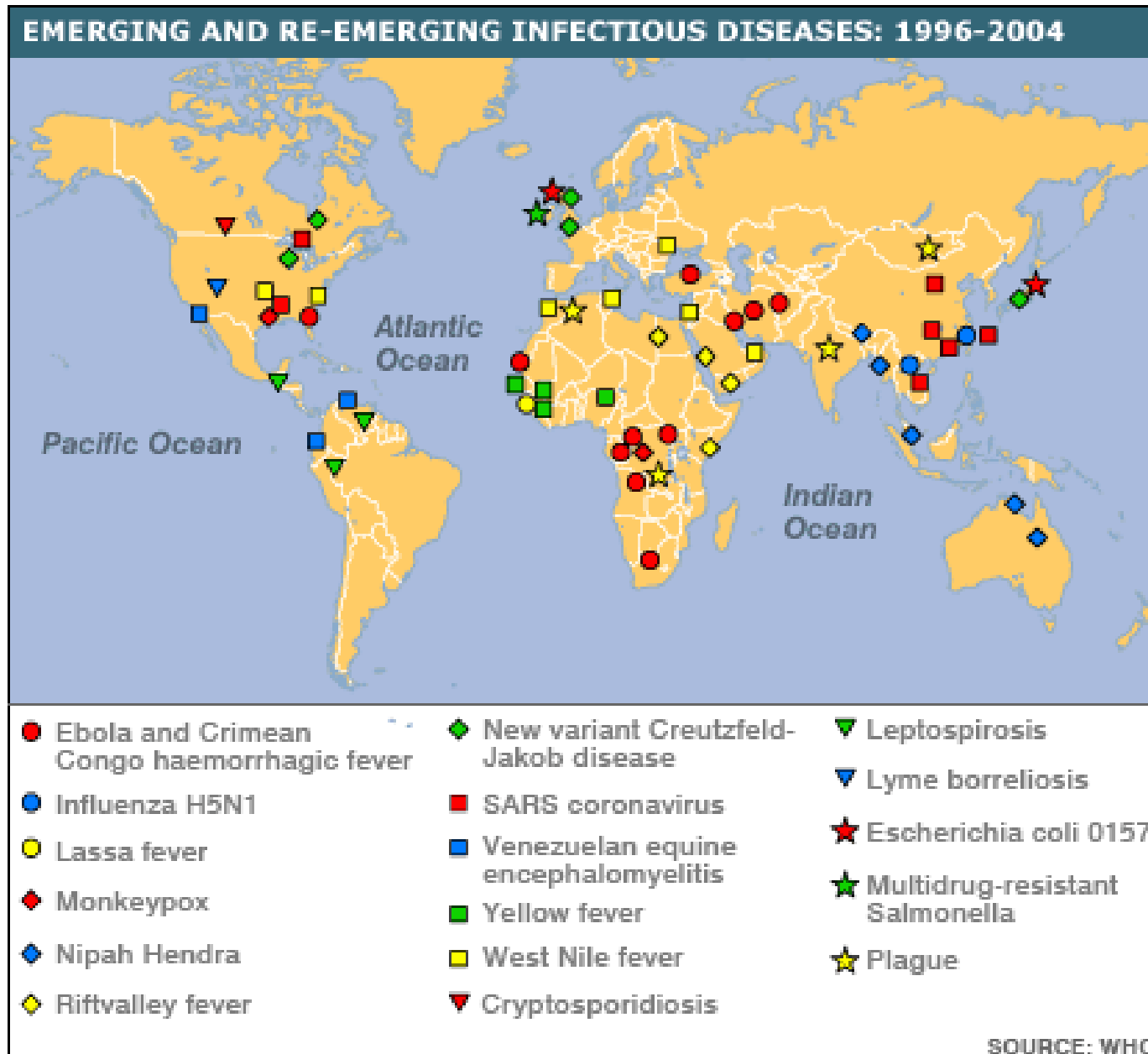


**INSTITUTE OF MICROBIOLOGY**  
CHINESE ACADEMY OF SCIENCES

# **Memory NK cells during mousepox infection**

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# Infectious Diseases are a Major Cause of Death Worldwide



May 14<sup>th</sup> 1796



# Prevalence of Smallpox in 2013



# Vaccine is based on immunologic memory

**TABLE 1** Antiviral vaccines licensed in the United States

<b>Vaccine</b>	<b>Type of antigen used</b>	<b>Route</b>
Adenovirus	Live virus	i.m.
Hepatitis A	Inactivated virus	i.m.
<b>Hepatitis B</b>	<b>Recombinant protein</b>	<b>i.m.</b>
Influenza	Inactivated virus, or viral antigens	i.m.
Japanese encephalitis	Inactivated virus	s.c.
<b>Measles</b>	<b>Live virus</b>	<b>s.c.</b>
<b>Mumps</b>	<b>Live virus</b>	<b>s.c.</b>
<b>Rubella</b>	<b>Live virus</b>	<b>s.c.</b>
<b>Polio</b>	<b>Inactivated Virus</b>	<b>s.c.</b>
Polio	Live virus	oral
Rabies	Inactivated virus	i.m.
<b>Varicella</b>	<b>Live virus</b>	<b>s.c.</b>
Yellow fever	Live virus	s.c.
Smallpox (Variola)	Live vaccinia virus	Intracutaneous

# Immunologic memory: hallmarks of adaptive immunity

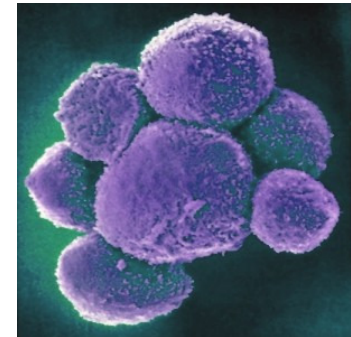
T cells & B cells : rearrangement of genes, antigen specific

- ✓ Developmental education
- ✓ Clonal expansion
- ✓ Longevity of memory cells
- ✓ Recall responses

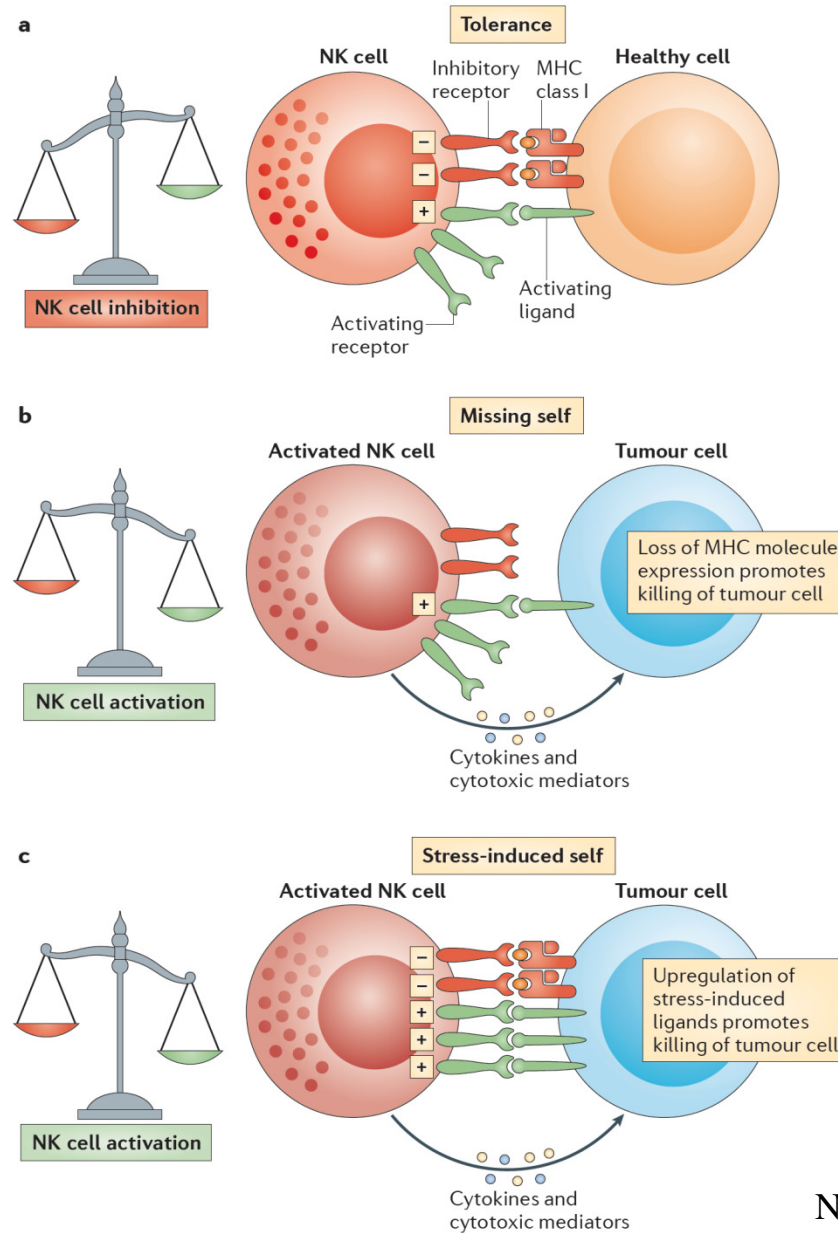
**NK cells memory?**

# Natural killer (NK) cells

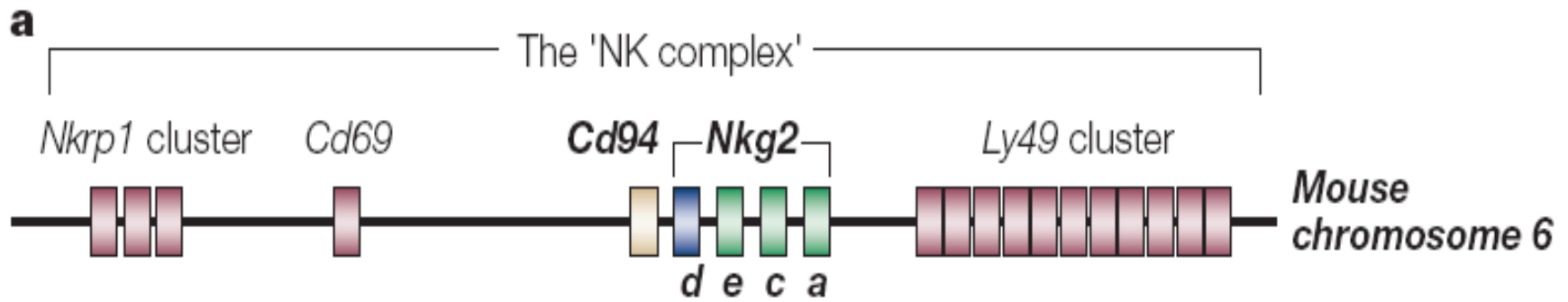
- Cytolytic lymphocytes
- Derived from bone marrow
- Innate immune system: NK cell receptors are encoded in the germline and do not undergo somatic recombination like B and T cell receptors
- NK cells are a key component of the innate immune system, providing early cellular defense against viruses and intracellular pathogens, and contributing to the early detection and destruction of transformed cells



# The rheostat concept of NK cell responsiveness

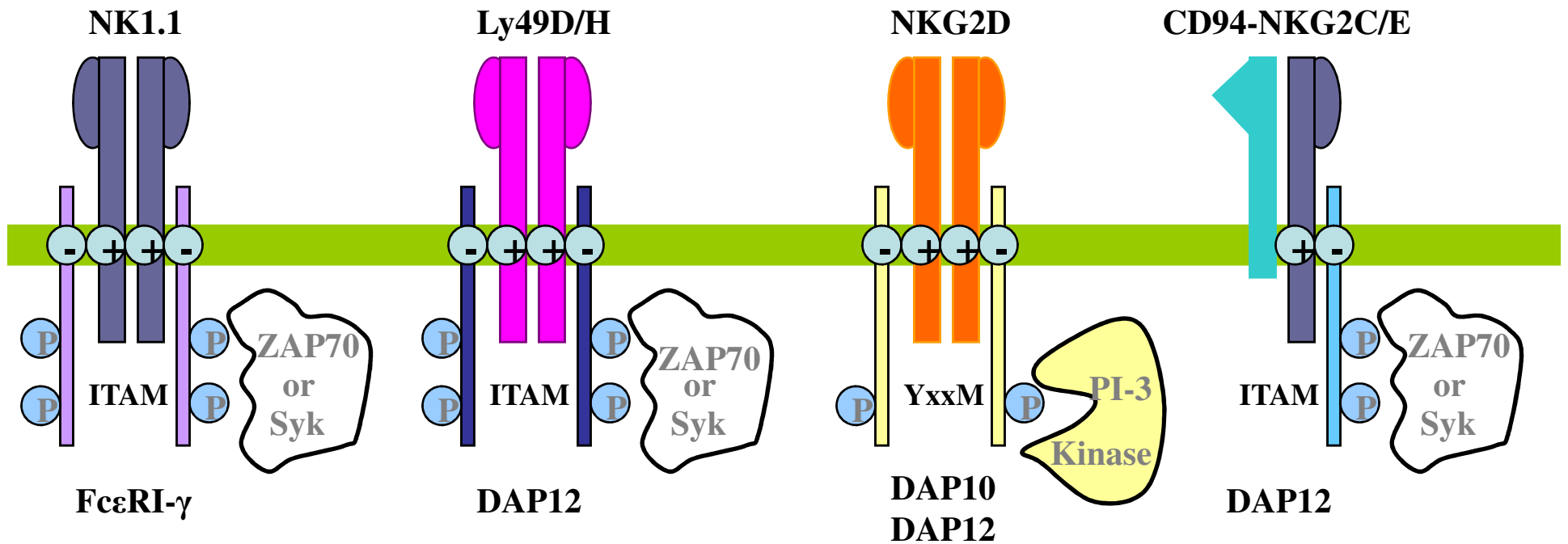






David H. Raulet *NATURE REVIEWS | IMMUNOLOGY* 2003

### Activating NK receptors in C57BL/6 (B6) mice



# Recent Studies of memory NK cells

## ➤ Specific NK cell subsets - Ly49H<sup>+</sup> NK cell memory to MCMV infection

Adaptive immune features of natural killer cells. Sun, J.C. et al. *Nature*, 2009.

## ➤ Non-specific NK cell subsets - hepatic NK cell memory to haptens and viruses

- T cell- and B cell-independent adaptive immunity mediated by natural killer cells. O'Leary, J.G., et al., *Nat Immunol*, 2006
- Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. Paust, S., et al., *Nat Immunol*, 2010
- Thy1 NK Cells from Vaccinia Virus-Primed Mice Confer Protection against Vaccinia Virus Challenge in the Absence of Adaptive Lymphocytes. Gillard, G.O., et al., *PloS Pathog*, 2011

## ➤ Human memory NK cells to CMV infection – CD57<sup>+</sup>NKG2Chi NK cells

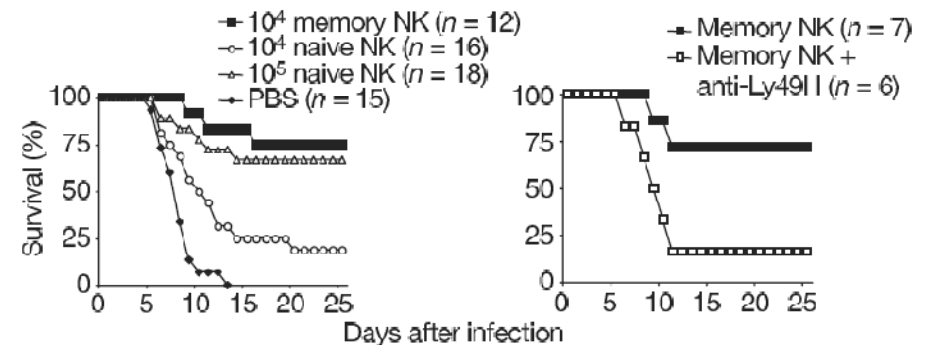
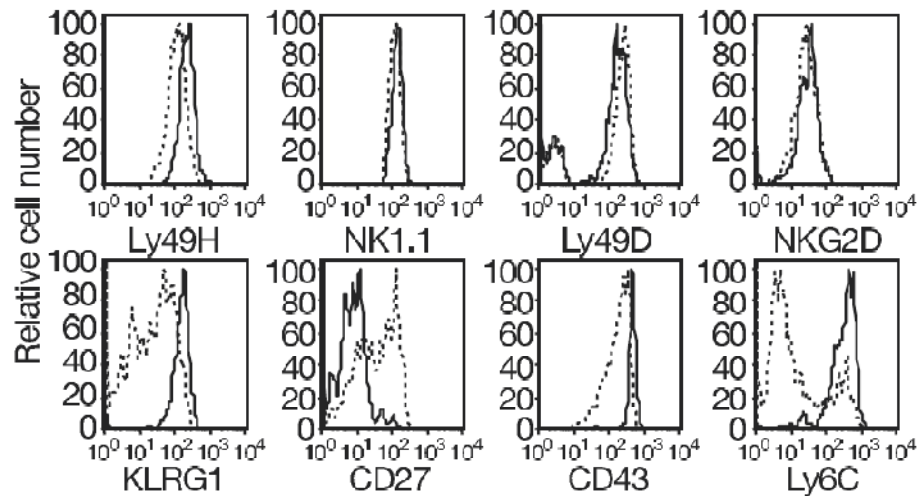
Expansion of a unique CD57<sup>+</sup>NKG2Chi natural killer cell subset during acute human cytomegalovirus infection. Lopez-Verges, S., et al., *Proc Natl Acad Sci U S A*, 2011.

**NK cells generate antigen-specific memory during viral infection or vaccination, and protect against subsequent pathogen infections**

# Adaptive immune features of natural killer cells

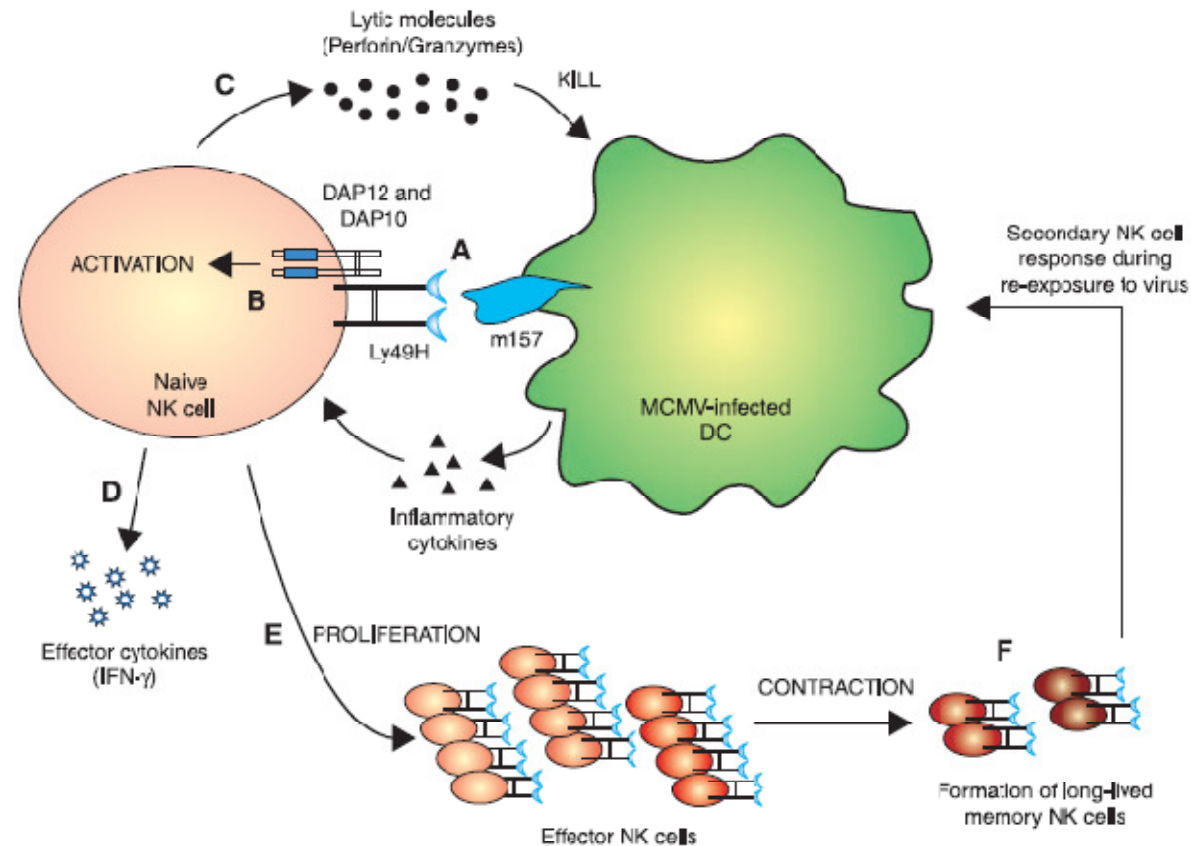
Joseph C. Sun<sup>1</sup>, Joshua N. Beilke<sup>1</sup> & Lewis L. Lanier<sup>1</sup>

## Ly49H-m157 (MCMV)



❖ Memory NK cells are more mature: high in Ly49H, KLRG1, CD43 and Ly6C, low in CD27.

❖ Memory NK cell are more protective than naïve NK cells



**Fig. 1. Naive and memory NK cells respond to MCMV.** Following MCMV infection of a dendritic cell, the m157 glycoprotein is expressed. NK cells from C57BL/6 mice express the activating Ly49H receptor, which associates with and signals through the ITAM-containing DAP12 and the DAP10 adapter molecules. Recognition of m157 by Ly49H-bearing NK cells (A) leads to activation via DAP12 and DAP10 (B). In addition to Ly49H ligation, IL-12 and pro-inflammatory cytokines produced by the infected dendritic cell act on NK cells allowing them to mediate cytotoxicity via perforin and granzymes (C), secrete cytokines such as IFN- $\gamma$  (D), and undergo clonal-like proliferation (E). Following NK cell expansion and viral clearance, effector Ly49H<sup>+</sup> NK cells undergo a contraction phase resulting in a long-lived memory pool (F). These memory NK cells can then respond again several months later when viral antigen is re-encountered (A). NK cell, natural killer cell; MCMV, murine cytomegalovirus; ITAM, immunoreceptor tyrosine-based activation motif; IL, interleukin; IFN- $\gamma$ , interferon-gamma.

# T cell– and B cell–independent adaptive immunity mediated by natural killer cells

Jacqueline G O’Leary<sup>1–3</sup>, Mahmoud Goodarzi<sup>1,3</sup>, Danielle L Drayton<sup>1,3</sup> & Ulrich H von Andrian<sup>1</sup>

It is commonly believed that only T lymphocytes and B lymphocytes expressing recombination-dependent antigen-specific receptors mediate contact hypersensitivity responses to haptens. Here we found that mice devoid of T cells and B cells demonstrated substantial contact hypersensitivity responses to 2,4-dinitrofluorobenzene and oxazolone. Those responses were adaptive in nature, as they persisted for at least 4 weeks and were elicited only by haptens to which mice were previously sensitized. No contact hypersensitivity was induced in mice lacking all lymphocytes, including natural killer cells. Contact hypersensitivity responses were acquired by such mice after adoptive transfer of natural killer cells from sensitized donors. Transferable hapten-specific memory resided in a Ly49C-I<sup>+</sup> natural killer subpopulation localized specifically in donor livers. These observations indicate that natural killer cells can mediate long-lived, antigen-specific adaptive recall responses independent of B cells and T cells.

- ❖ Hapten-specific memory NK cells
- ❖ Thy1<sup>+</sup>, Ly49C-I<sup>+</sup> NK cells
- ❖ Memory NK cells resided in the liver

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## Critical role for the chemokine receptor CXCR6 in NK cell–mediated antigen-specific memory of haptens and viruses

Silke Paust<sup>1,2</sup>, Harvinder S Gill<sup>3,4</sup>, Bao-Zhong Wang<sup>3</sup>, Michael P Flynn<sup>1</sup>, E Ashley Moseman<sup>1</sup>, Balimkiz Senman<sup>1</sup>, Marian Szczepanik<sup>5,7</sup>, Amalio Telenti<sup>2,6</sup>, Philip W Askenase<sup>7</sup>, Richard W Compans<sup>3</sup> & Ulrich H von Andrian<sup>1,8</sup>

A subset of hepatic NK cells that acquired antigen-specific memory of at least five structurally, chemically and biologically distinct antigens.

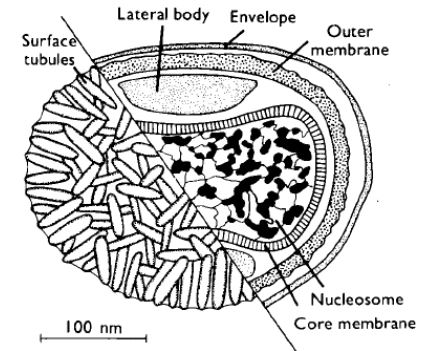
❖ Adaptive immune responses mediated by this population depended on CXCR6, which was expressed on about half of the hepatic NK cells

# Unanswered questions

- ❖ **Is NK cell memory a common phenomena during viral infections ?**
- ❖ **What is the properties of the memory NK cells ?**
- ❖ **Is the memory confined to a certain subset of NK cells ( for example: tissue specific, or receptor specific, ets) ?**
- ❖ **What epigenetic alterations account for the longevity and enhanced effector functions demonstrated by the memory NK cells ?**
- ❖ **Is it possible to vaccinate NK cells for enhanced host defense ?**

# Orthopoxvirus (OPV)

- DNA viruses,  $\approx 200$  Kb,  $\approx 200$  proteins
- The genome among all OPV are highly conserved (90% overall)  $\rightarrow$  strong antigenic cross reactivity
- Variola virus, humans  $\rightarrow$  smallpox
- **Ectromelia** virus (ECTV), laboratory mouse  $\rightarrow$  mousepox
- Vaccinia virus (VACV). Poorly or non pathogenic in humans and mice  $\rightarrow$  Vaccine against both, smallpox and mousepox
- Cowpox virus  $\rightarrow$  First vaccine



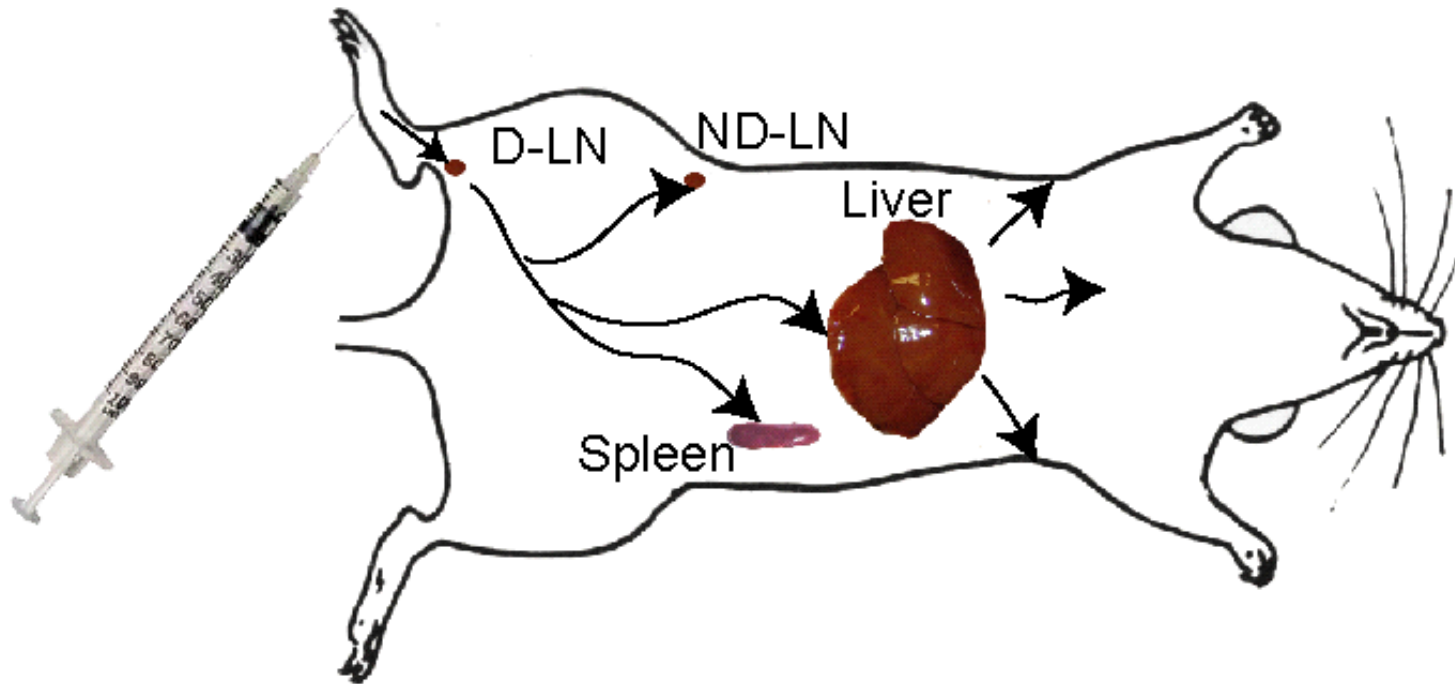


# Advantages of the ECTV model

- ECTV is a mouse pathogen
- Mousepox is very similar to smallpox
- Mousepox can be prevented by immunization with VACV
  
- *Resistant mouse strains*  
*C57BL/6 (B6), 129*  
Mechanism of natural resistance:  
Role of T cells, Abs, NK cells, aging etc.
  
- *Susceptible mouse strains*  
*BALB/C, DBA/2J*  
Mechanisms of acquired resistance  
Role of Immune Response Modifiers in virulence

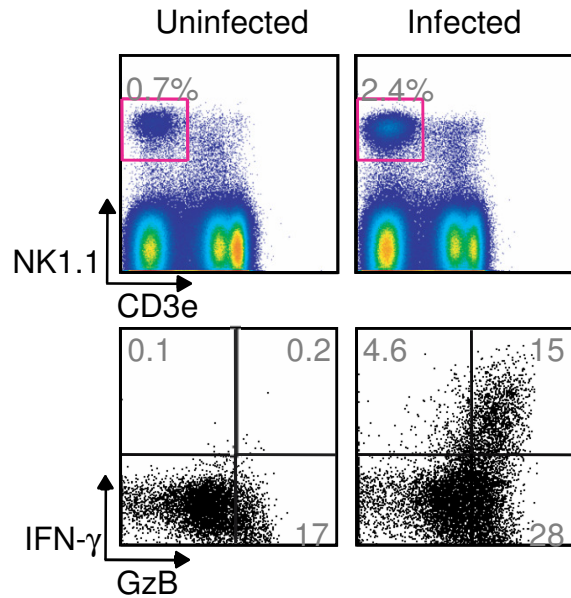
# ECTV lympho-hematogenous dissemination

## A peripheral → systemic infection



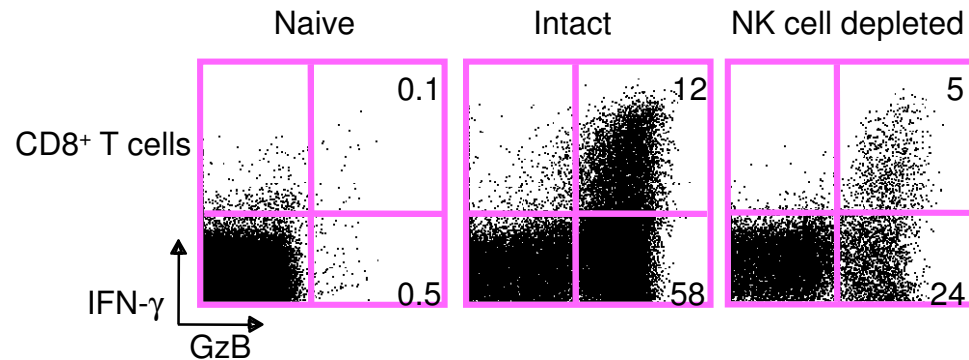
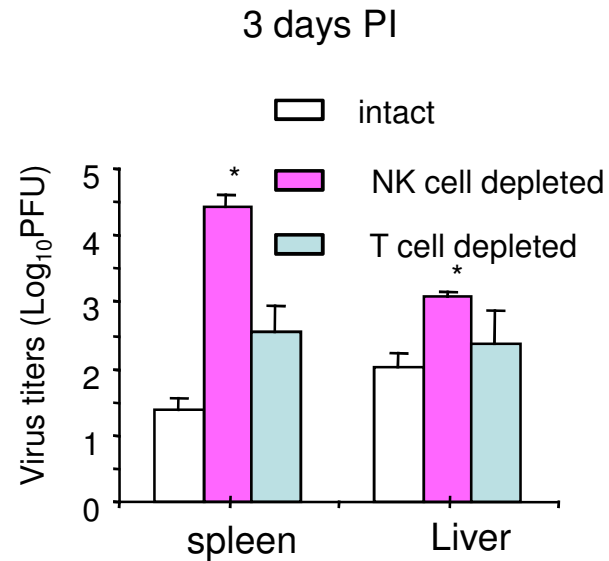
# NK cell directly kill viral-infected cells + support strong adaptive T cell responses

NK cells → Draining LN

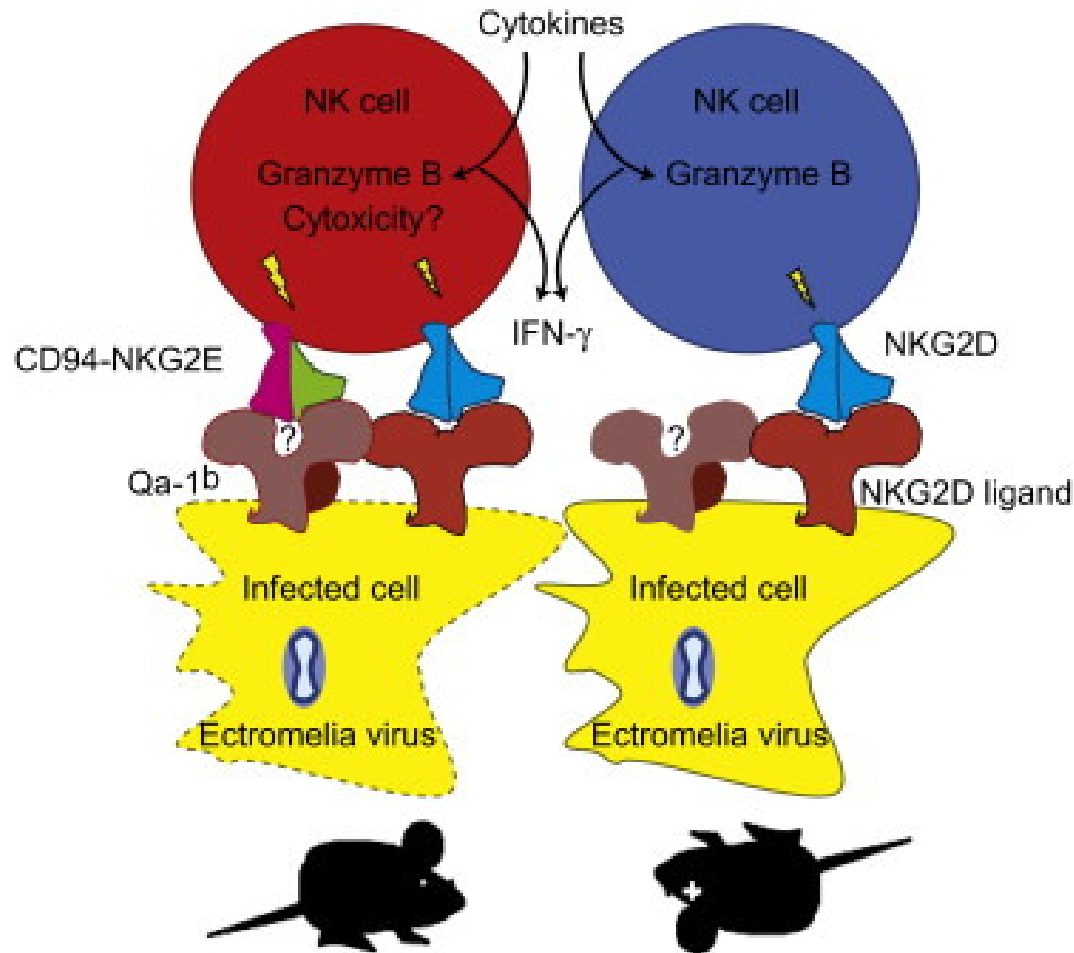


Supporting CD8<sup>+</sup> T responses

Control early viral dissemination



# CD94-NKG2E recognizes ECTV infected cells in the presence of NKG2D and in a Qa-1<sup>b</sup>-dependent manner



CD94-NKG2E



Qa-1<sup>b</sup>-Peptide?

NKG2D:

Co stimulating receptor

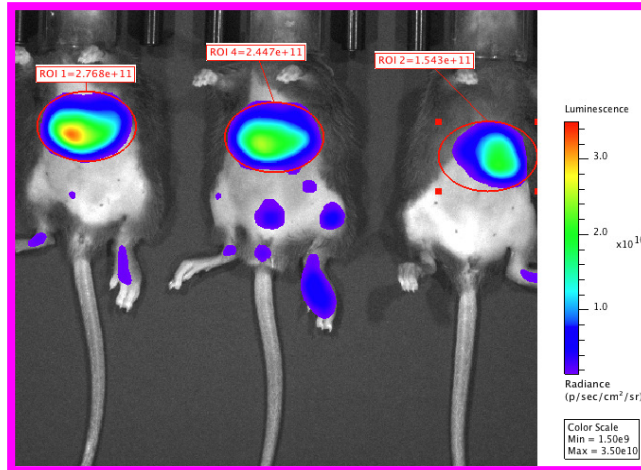
# **Anti-ECTV specific NK cell memory might be generated through expansion of CD94<sup>+</sup> NK cells during viral infection**

## **Unique system to investigate NK cell memory:**

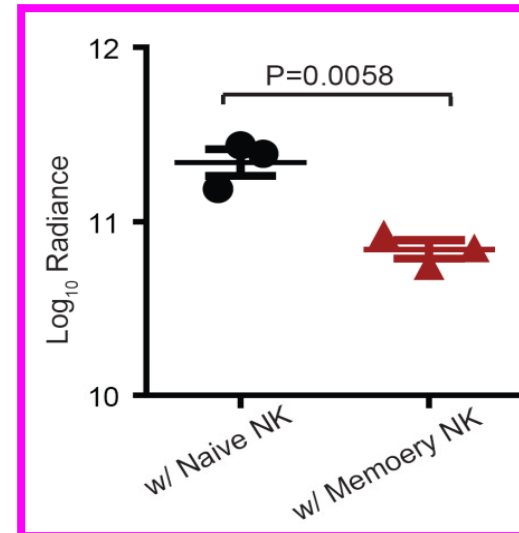
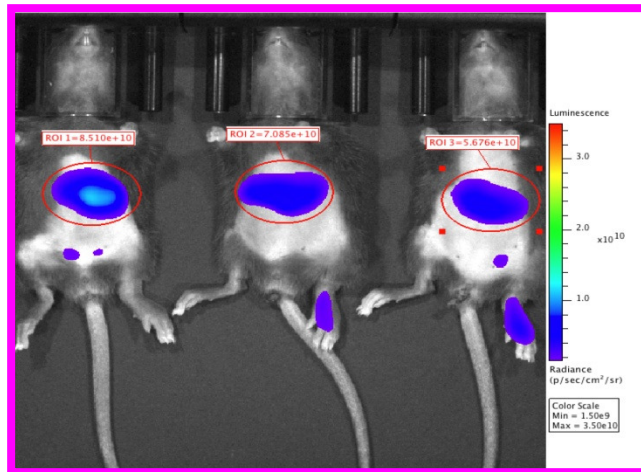
- Activating receptors involved are known**
- Vaccine design**
- Systemically study the mechanisms involved in the regulation and maintenance of memory NK cells**

# Memory NK cells protected susceptible neonatal B6 mice from ECTV infection

w/ naïve NK cells

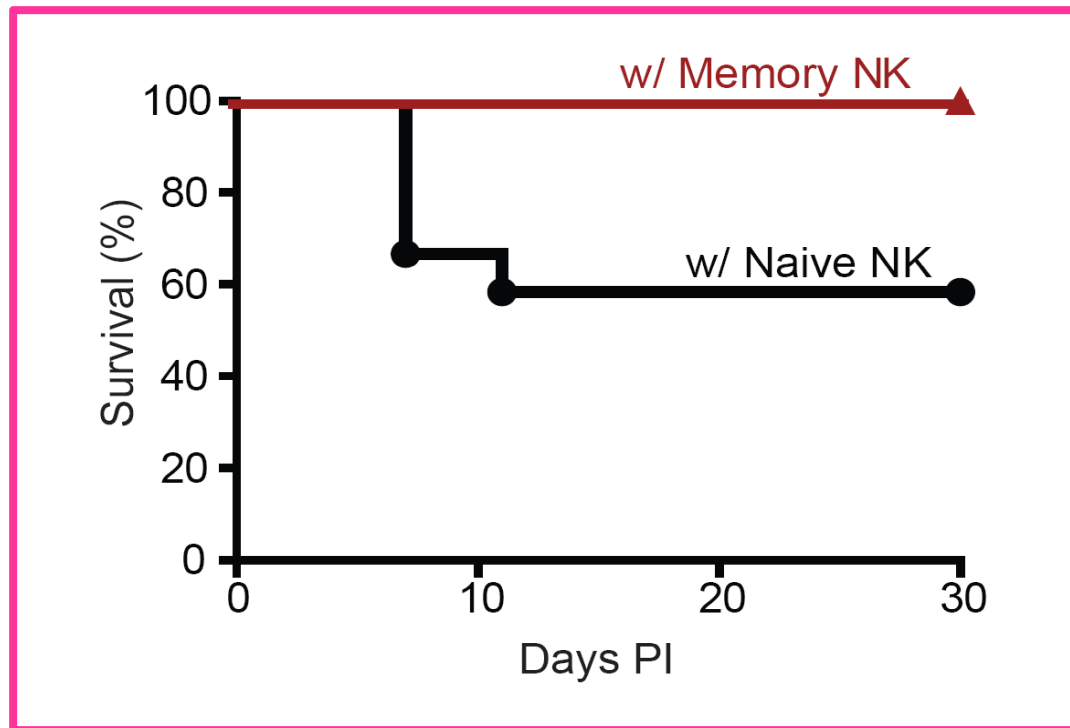


w/ memory NK cells



Neonatal B6 mice infected with 6 PFU of ECTV-Luc

# Memory NK cells significantly reduced mortality rate in neonatal B6 mice following ECTV infection



## **Future directions:**

- **Study the properties of the memory NK cells – receptor specific? Organ specific? Surface markers?**
- **Identify the peptide that bind to Qa-1<sup>b</sup> during ECTV infection – test for vaccine design**
- **Investigate the mechanisms involved in the regulation and maintenance of memory NK cells**



# Acknowledgements!

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