Type I Interferon Activation of Natural Killer (NK) Cells by Cytomegalovirus (CMV) and Their Interaction with Dendritic (DC) and NKT Cells

Howaida A. Oulad Abdelati

Wright State University

Follow this and additional works at: https://corescholar.libraries.wright.edu/etd_all

Part of the Immunology and Infectious Disease Commons, and the Microbiology Commons

Repository Citation
Oulad Abdelati, Howaida A., "Type I Interferon Activation of Natural Killer (NK) Cells by Cytomegalovirus (CMV) and Their Interaction with Dendritic (DC) and NKT Cells" (2013). Browse all Theses and Dissertations. 1165.
https://corescholar.libraries.wright.edu/etd_all/1165

This Thesis is brought to you for free and open access by the Theses and Dissertations at CORE Scholar. It has been accepted for inclusion in Browse all Theses and Dissertations by an authorized administrator of CORE Scholar. For more information, please contact corescholar@www.libraries.wright.edu, library-corescholar@wright.edu.
TYPE I INTERFERON ACTIVATION OF NATURAL KILLER (NK) CELLS BY CYTOMEGALOVIRUS (CMV) AND THEIR INTERACTION WITH DENDRITIC (DC) AND NKT CELLS

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

By

HOWAIDA A. OULAD ABDELATI

M.D. Al-Fateh University 2005

2013

Wright State University
I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY HOWAIDA OULAD ABDELATI ENTITLED Type I Interferon Activation of Natural Killer (NK) Cells by Cytomegalovirus (CMV) and Their Interaction with Dendritic (DC) and NKT Cells BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

Committee on Final Examination

Nancy J. Bigley, Ph.D.
Professor of Microbiology and Immunology

Barbara E. Hull, Ph.D.
Professor of Biological Sciences

Cheryl Conley, Ph.D.
Professor, Department of Biology

R. William Ayres, Ph.D
Interim Dean, Graduate School
ABSTRACT

Oulad Abdelati, Howaida. M.S. Department of Microbiology and Immunology, Wright State University, 2013. Type I Interferon Activation of Natural Killer (NK) Cells by Cytomegalovirus (CMV) and Their Interaction with Dendritic (DC) and NKT Cells.

In our current study the roles of natural killer (NK) cells in regulation of the acute phase of murine cytomegalovirus (MCMV) infection were demonstrated. NK cells utilize perforin and gamma interferon to kill MCMV infected cells. Activation of NK cells is controlled by the balance between inhibitory and activation receptors and modulated by cytokines produced from the infected cells. Type I interferons are produced mainly by plasmacytoid dendritic cells (PDCs). Type I interferons have a substantial role in enhancing NK cells cytotoxic activity and NK cells proliferation and maintain cell survival through STAT mediated signaling pathway. IL-12 produced by dendritic cells activates NK cells and natural killer T (NKT) cells. Natural killer T cells activate NK cells through IFN-γ production at early time during MCMV infection. These immune cells and their produced cytokines are critical to regulate MCMV infection and any disturbance of this activation will lead to impaired host innate immunity.
# TABLE OF CONTENTS

I. Introduction ..................................................................................................................... 1

II. Literature review background ....................................................................................... 3

   NK cell development and education ............................................................................ 3

   Peripheral NK cell localization and homeostasis......................................................... 5

   Role of cytokines in regulation of NK cells ................................................................. 6

   NK cells receptors ........................................................................................................ 7

   NKT cells ...................................................................................................................... 8

   NK T cells development .............................................................................................. 10

   NK T cell ligand .......................................................................................................... 10

   Dendritic cells ............................................................................................................. 11

III. NK cells defense against CMV infection .................................................................... 12
IV. NK cell activation by type I interferon and IL-12 ..............................................16

Type I interferon activates NK cells through STAT mediated pathway ......20

V. NK cells activation by NK T cells stimulation during MCMV infection ..........23

VI. Plasmacytoid dendritic cells activate NK cells during CMV infection ..........31

VII. Conclusion and future studies .................................................................33

VIII. References ............................................................................................36
LIST OF FIGURES

FIGURE 1: Cross-talk between cells of innate immune system during MCMV infection. 2

FIGURE 2: NK cell development ........................................................................................................ 4

FIGURE 3: NK cell education ............................................................................................................ 5

FIGURE 4: TLR signaling pathway .................................................................................................... 12

FIGURE 5: Survival percentage of NK cells in NK cell deficient mice ............................. 13

FIGURE 6: Viral titer in the liver and spleen in different immune states ............................. 14

FIGURE 7: Perforin and IFN-γ requirements for the immune surveillance against MCMV infection ........................................................................................................... 15

FIGURE 8: Early production of innate cytokines in NK cell deficient infected mice  .... 17

FIGURE 9: Role of IL-12 in control of MCMV induced liver pathology ........................ 18

FIGURE 10: Role of IFN α/β on NK cell proliferation and cytotoxicity ........................ 19

FIGURE 11: MCMV induces Perforin, IFN-γ, and NK cell proliferation ........................ 21

FIGURE 12: STAT1 requirements for NK cell cytotoxicity ..................................................... 22
FIGURE 13: IFNα/β receptors and STAT1 requirements for NK cell proliferation ........22

FIGURE 14: IFNα/β receptors and STAT1 requirements for IL-15 messenger RNA expression ..........................................................................................................................................................................................................................................................23

FIGURE 15: α-GalCer induces rapid NK cell activation and IFN-γ production ..........24

FIGURE 16: MCMV replication in NK T cell deficient mice .........................................25

FIGURE 17: IFN-γ production by NK cells cocultured with MCMV infected dendritic cells..........................................................................................................................................................................................................................................................27

FIGURE 18: NK T cell activation at the early phase of MCMV infection .................28

FIGURE 19: Va14i NKT cells activated but not proliferate in response to MCMV infection ........................................................................................................................................................................................................................................................................29

FIGURE 20: NK cell and cytokine production in NK T cell deficient mice .............30

FIGURE 21: Activated NK cell numbers in DT- treated mice ................................32

FIGURE 22: NK cell cytotoxic activity in MCMV infected in PDC depleted mice ....33

FIGURE 23: IFN-γ production in MCMV infected PDCS depleted mice .................33
LIST OF TABLES

TABLE I: Major human and mice NK cell receptors ................................................................. 8

TABLE II: Human and mice NK T cell subtypes ......................................................................... 9

TABLE III: Role of IFNα/β and IL-12 in control of MCMV infection in E26 and C57BL/6 mice ........................................................................................................................................18
LIST OF ABBREVIATIONS

NK = Natural killer

CMV = Cytomegalovirus

HCMV = Human cytomegalovirus

MCMV = Murine cytomegalovirus

DCs = Dendritic cells

PDCs = Plasmacytoid dendritic cells

IPC = Interferon producing cell

RAG = Recombination activating gene

NKP = Natural killer cell precursor

HSC = Hematopoietic stem cell

KIR = Killer cell-immunoglobulin like receptor

NCR = Natural killer receptors

ITAM = Immunoreceptor tyrosine based activation motif

ITIM = Immunoreceptor tyrosine based inhibitory motifs sequence
TCR = T-cell receptor

α- GalCer = α-galactosylceramide

iGb3 = isogloboside 3

TLR = Toll like receptor

IFN-γ = Interferon gamma

IFNα/β = Interferon alpha/ beta

TNFα = Tumor necrosis factor alpha

MIP-1α = Macrophage inflammatory protein one alpha

MIP-1β = Macrophage inflammatory protein one beta

PFU = Plaque forming unites

NKD = NK cell-deficient mice

STAT = Signal transduction and transcription

I.P = Intraperitoneal

BrdU = Bromodeoxyuridine

DT = Diphtheria toxin.
ACKNOWLEDGEMENT

I would like to thank Dr. Nancy Bigley for all of her guidance and support that she has given to me throughout my graduate study. I would also like to thank Dr. Barbara Hull and Dr. Cheryl Conley for their suggestions and contributions in my work. I am also thankful to my parents for their love and encouragement.
DEDICATION

I would like to dedicate my thesis project to my husband Mohamed, my daughter Toyoob, and my son Alwatek who have always inspired and encouraged me.
I- Introduction

Cytomegalovirus (CMV) is a double stranded DNA virus belonging to the Betaherpesvirinae sub family. Causing mild to subclinical disease in about 50-90% of the population, most of patients are asymptomatic and the virus remains in their body in a latent state. It causes severe to lethal disease in immunocompromised hosts such as AIDS patients, and patients under immune suppressant drugs. Congenital infections may lead to fetal disease including deafness and severe neurological defects (Boppana et al., 2001). Immune surveillance against the CMV virus includes both arms of the immune system, the innate and the adaptive immunity. This study focuses on the innate immune defense including cells and effector cytokines.

The first line of the host defense in controlling CMV infection is activation of NK cells, early production of proinflammatory cytokines, and the activation of the innate immune cell effector function (Nguyen et al., 2002). Due to the similarity of the human cytomegalovirus (HCMV) and murine (MCMV) in disease pathogenesis and tissue tropism (Krmpotic et al., 2003), most of the studies used MCMV as the infective virus to study the mechanism and the immune regulatory pathway for CMV. Early studies demonstrated the role of type I interferon and NK cells in early detection and control of the MCMV replication and spread. During the course of the MCMV infection, biphasic type I interferon production was described where the IFNα/β was detected as early as six hours post infection, and the peak level at eight to thirteen hours, and declined by 36 to
48 hours post infection. In addition to CMV infected cells, other innate immune cells respond to CMV by producing type I interferon. These cells are referred to as interferon producing cells (IPC) and plasmacytoid dendritic cells are the primary population which release type I interferons in response to the CMV infection (Ito et al., 2006). In addition to the central role of the type I interferons in direct inhibition of the CMV replication, they orchestrate other immune cells, such as dendritic cells, macrophages and T cells and NK cells to mount their antiviral response. There is cooperation among the NK cells, IPC, and Natural killer T cells (NKT) cells to produce a powerful immune response. IPC and NKT cells promote the NK cells effector function through IFNα/β enhanced cytotoxicity and IL-12 inducing IFN-γ production. IFNα/β has a major role in NK cells activation, proliferation and migration of the cells to the infected compartment.

The hypothesis of this study is that Type-I interferon -producing dendritic cells as well as IFN-γ producing NKT cells activate the NK cell response against CMV.

**Figure 1**) Cross-talk between cells of innate immune system during MCMV infection.
II- Literature Review- Background

NK cells are bone marrow derived cells which have been described in the early 1970s as a lymphocyte subpopulation, and their cytotoxicity against tumors has been described (Vivier et al., 2011). Individual NK cells lack unique antigen recognition receptors and do not use recombination activating gene (RAG) enzymes for rearrangement of their receptor genes. The innate immune function of NK cells against different microorganisms has been studied. NK cells have the ability to distinguish self from non-self-antigens and affect the adaptive immunity through its production of cytokines and chemokines that affect other immune cells like dendritic cells and macrophages. The antiviral immune defense basically depends on two types of immunity, the innate and adaptive immunity NK cells are originally described as the effector cells of the innate immune response because of their rapid response to the challenge by direct cytolytic response and cytokine, and chemokine production. However, the adaptive immunity function of those cells has been recently described.

NK cell development and education

Early studies suggested that the bone marrow is the primary site for NK cell development and maturation (Huntington, et al., 2007). Although the thymus environment is critical for NKT cells maturation, NK cells develop independent of the thymus environment. In the bone marrow, NK cell generation begins with the development of the natural killer cell precursors (NKP), which express CD122 (B chain shared between the IL2 and IL 15) from the CD34+ hematopoietic stem cells (HSC).
NKP further development involve expression of CD161 (a family of C-type lectin receptors) cells which is expressed on all immature NK cells. CD161+ CD122+ cells differentiate to two major subsets, CD56<sup>bright</sup> CD16<sup>−</sup> NK cells, which are predominant NK cell phenotypes in the peripheral tissues, and CD56<sup>dim</sup> CD16<sup>+</sup> cells. CD56<sup>bright</sup> CD16<sup>−</sup> cells are potent IFN-γ producing cells which lack perforin granules and are unable to perform cytotoxic function. Ninety–five per cent of human blood NK cells are CD56<sup>dim</sup>CD16<sup>+</sup>. They possess perforin granules and have cytotoxic activity. Mouse CD161<sup>+</sup> cells are recognized as NK1.1, further development involves expression of CD49b (DX5) and Ly49 receptors. Human mature NK cells express CD56, natural cytolytic receptors (NCR), CD16 (FcγRIII) and KIR. Mature murine NK cells express CD16, Ly49 and CD49b (DX5) (Figure 2). Dim or bright, indicates the surface density expression of the cell marker. Bright indicates a strongly positive expression whereas dim indicates minimal or negative expression of the specific cell surface protein.

![Figure 2](image.png)

**Figure 2** Stages of human NK cell development and functional maturation. Adapted from Freud and Caligiuri 2006.

In addition to a distinct phenotype, NK cells undergo the education phase as a preparation toward fully responsive cells activated by activation receptors and tolerant to
self. Maturation of NK cells include the education of the immature cells to express cell surface molecules which enable them to interact with the environment such as the killer cell-immunoglobulin like receptor (KIR) in humans and LY49 in mice. During development, NK cell inhibitory receptors interact with self-ligands in the bone marrow stromal cells lead to mount their responsiveness (Figure 3), (Sun et al., 2011; Huntington et al., 2007).

![Figure 3](image)

**Figure 3**) NK cells education and increased responsiveness, a) increase the NK cells response with increase the interaction of inhibitory receptors with the MHC class I self-peptide in the bone marrow stromal cells, b) interaction of activation receptors with self MHC class I ligands causing cells anergy. Adapted from Sun et al., 2011.

**Peripheral NK cell localization and homeostasis**

Developing NK cells can leave the site of origin as they are maturing to reside in the spleen, liver, lung, gut, and other sites where they can be activated and initiate immune surveillance against tumors and cells infected with pathogens. Factors and mechanisms by which NK cells traffic between the lymphoid and non-lymphoid organs...
are still not fully understood. Some chemokines have been identified that regulate the migration of NK cells between organs. These include CC-chemokine ligands, CCL3, CCL4, CCL5, CCL19, CXC-chemokine ligands CXCL12, CXCL16 and CX3CL1, and some adhesion molecules such as αMβ2 integrin also called macrophage receptor 1; MAC1 plays a role in controlling of the NK cells trafficking between the different organs.

Role of cytokines in regulation of NK cells activity

A substantial role for cytokines is well studied in the development and homeostasis of the lymphoid NK cell lineage, development, maturation, and survival. Cytokines involved in this process are IL-2, IL-15, IL12 and IL18. NK cells activated by IL-2 and IL-12 augment tumorlysis function through enhanced perforin binding to the tumor cell surface (Lehmann et al., 2001). IL-2 is the best known growth factor for NK cells. It induces NK cells proliferation and augments their cytotoxic function in culture (Henney et al., 1981; Suzuki et al., 1983). IL-15 released by macrophages and monocytes maintains the NK cells survival through its effect on IL-2R, and by enhancing the expression of the anti- apoptotic factor BCL-2, (Carson et al 1997). Ranson et al. (2003) assessed the role of the IL-15 on the NK survival. In adoptive transfer assays, NK cells failed to proliferate in IL-15 deficient mice. In contrast, IL-4, and IL-10 have been shown to be negative regulators of NK cell responses. NK and T cell secretion of INF-γ is crucial for innate and adaptive immunity, IL-15 induces NK cells expression of INF-γ messenger RNA, and IL-18 and IL-21 augment the IL-15 effect on NK cells. IL-15 is the
main cytokine that activates NK cells to produce IFN-γ through its effect on the STAT pathway. IL-18 does not exert the same action though it causes a modest increase in the NK cell expression of IFN-γ upon stimulation; it acts through its effect on NF-κB activation of the IFN-γ gene promoter (Strengell et al., 2003). IL-18 is important in priming NK cells in a subsequent response to IL-12. IL-18, enhances the translation of IFN-γ mRNA and protein expression (Chaix et al., 2008)

NK cells receptors

NK cell activity is regulated mainly by two types of receptors. Activation receptors interact with cytokine transducing stimulatory signals to induce cell maturation and maintain survival. The inhibitory receptors suppress NK cells activity upon their interaction with the MHC class I, through the intracytoplasmic domain signals called immunoreceptor tyrosine based inhibitory motifs sequence (ITIM). Recognition of target cells with down regulation of MHC class I ligands is the function of the some types of the inhibitory receptors in a process called missing self-phenomenon (Höglund et al., 1997). Best examples of these receptors are the killer cell Ig-like receptors (KIR) in humans, Ly49 in the mouse, and CD94/NKG2 in both species. Cells with decreased expression of MHC class I are susceptible to NK cell killing since the inhibitory signals are suppressed by failure of MHC class I ligand engagement with the inhibitory receptor. NK cells deficient in KIR are less competent in target cells killing in contrast to cells expressing the KIR, (Anfossi, et al., 2006). The proper function of NK cells is controlled by the balancing signals between the inhibitory and the stimulatory receptor signaling pathways. Inhibitory receptors signals regulate the specific activation of the NK cells by the stimulatory receptors, Natural killer receptors (NCR) and CD16 are examples of
activation receptors. NKP46, NKP44, and NKP30 are NCRs linked to the type one intracytoplasmic anchored protein such as CD3-ζ, DAP12 and FcR-γ that contain an immunoreceptor tyrosine based activation motif (ITAM), (Table 1). NK cell activation is a result of the synergistic action resulting from a combination of a number of activating receptors (Bryceson et al., 2005). CD16 is a FcγRIII that binds to FC receptor of IgG antibodies to induce NK cells antibody-dependent cell-mediated cytotoxicity.

Table 1) Major mouse and human NK cell receptors. Adapted from Jost and Altfeld 2013

<table>
<thead>
<tr>
<th>Receptor family</th>
<th>Species</th>
<th>Receptor</th>
<th>Known ligand</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCR</td>
<td>M</td>
<td>NCR1</td>
<td>Viral HA</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>NKP46</td>
<td>Viral HA, HSPG, PEMP-1</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NKP30</td>
<td>BAT-3, HCMV pp65, B7-H6, HSPG, PEMP-1</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NKP44</td>
<td>Viral HA, HSPG</td>
<td>Activating</td>
</tr>
<tr>
<td>C-type lectin</td>
<td>M</td>
<td>Ly49A</td>
<td>H-2D^b,c,p</td>
<td>Inhibitory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ly49C</td>
<td>H-2K^b,d, H-2D^b,c,k</td>
<td>Inhibitory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ly49D</td>
<td>H-2D^b</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ly49H</td>
<td>m157</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ly49I</td>
<td>H-2K^b,d,q,v</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ly49P</td>
<td>H-2D^b</td>
<td>Activating</td>
</tr>
<tr>
<td>KIR</td>
<td>H</td>
<td>KIR2DL1</td>
<td>HLA-C2 (Lys 80)</td>
<td>Inhibitory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR2DL2/3</td>
<td>HLA-C1 (Asn 80)</td>
<td>Inhibitory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR2DL4</td>
<td>HLA-G</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR2DL5</td>
<td>Unknown</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR2DS1</td>
<td>HLA-C2 (Lys 80)</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR2DS2</td>
<td>HLA-C1 (Asn 80)</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR2DS3</td>
<td>Unknown</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR2DS4</td>
<td>Unknown</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR2DS5</td>
<td>Unknown</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR3DL1</td>
<td>HLA-Blw4</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR3DS1</td>
<td>HLA-Blw4</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR3DL2</td>
<td>HLA-A3/A11</td>
<td>Activating</td>
</tr>
<tr>
<td>C-type lectin</td>
<td>H, M</td>
<td>CD94/NKG2A</td>
<td>H: HLA-E, M: Qa1b</td>
<td>Inhibitory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD94/NKG2C</td>
<td>H: HLA-E, M: Qa1b</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD94/NKG2E</td>
<td>H: HLA-E, M: Qa1b</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NKG2D</td>
<td>H: HLA-E, M: Qa1b</td>
<td>Activating</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>NKR-P1</td>
<td>Ocil/Crd-b</td>
<td>Activating/Inhibitory</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>NKR-P1A</td>
<td>LL/T1</td>
<td>Inhibitory</td>
</tr>
</tbody>
</table>

Natural killer T cells (NKT)

NKT cells are T lymphocytes that express certain NK surface markers, for example, NK1.1 in the mouse, and CD161 in humans. Their role in immune surveillance
against different viral infection has been described (Tessmer et al 2009). NKT cells possess a distinctive T-cell receptor (TCR) and are restricted by CD1d presentation of antigen, CD1d restriction develops in the thymus (Bendelac et al., 1997). NK T cells express unique invariant β chain segments such as Vα14Jα18 TCR in mouse and Vα24Jα18 in human, this TCR is able to recognize self and foreign lipid molecules. Most NKT cells also express high levels of CD69 (very early activation marker) and CD44 (Pgp-1), and low levels of CD62L (L-selectin) a phenotype reminiscent of memory T cells. Different NKT cells subsets have been identified (Table 2), Type I NKT cells are defined as invariant NKT cells (iNKT), which are the most studied group and is the characterized with a highly restricted TCR repertoire in human and mouse (Table 1). The immune response of NKT cells is characterized by their cytotoxic function and their ability to produce INF-γ and/or IL-4 (Godfrey et al., 2000).

**Table 2:** Two types of NKT subsets identified in human and mouse and their different characteristics including the TCR, the CD1d restriction and their stimulatory ligands, Adapted from: Juno et al., 2012

<table>
<thead>
<tr>
<th>NKT Cell Subset</th>
<th>Mouse</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCR</td>
<td>Vα14-Jα18; Vβ8.2/7/2</td>
<td>Vα24-Jα18; Vβ11</td>
</tr>
<tr>
<td>Subsets</td>
<td>CD4+, DN</td>
<td>CD4+, CD8+, DN</td>
</tr>
<tr>
<td>Ligand</td>
<td>αGalCer</td>
<td>αGalCer</td>
</tr>
<tr>
<td>Restriction</td>
<td>CD1d</td>
<td>CD1d</td>
</tr>
<tr>
<td>NK receptors</td>
<td>NK1.1+/-</td>
<td>CD161+/-</td>
</tr>
<tr>
<td><strong>Type II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCR</td>
<td>Vα3.2-Jα9 or Vα8; Vβ8</td>
<td>Diverse</td>
</tr>
<tr>
<td>Subsets</td>
<td>CD4+, DN</td>
<td>CD4+, CD8+</td>
</tr>
<tr>
<td>Ligand</td>
<td>Sulfatide, lyosulfatide, lysophosphatidylcholine</td>
<td>Sulfatide, lyosulfatide, lysophosphatidylcholine</td>
</tr>
<tr>
<td>Restriction</td>
<td>CD1d</td>
<td>CD1d</td>
</tr>
<tr>
<td>NK receptors</td>
<td>NK1.1+/-</td>
<td>CD161+</td>
</tr>
</tbody>
</table>
NKT cell development

The NKT cells develop in the thymus from the same precursors as conventional CD4+ and CD8+ T cells, CD4+ CD8+ double-positive cells. In contrast to conventional T cells, which are selected by MHC–peptide complexes presented by thymic epithelial cells, iNKT are selected by lipid antigens presented by the non-polymorphic, MHC I-like molecule CD1d, present on the surface of thymocytes. NKT development in main four stages, stage 0 (CD24+CD44−NK1.1−) cells, stage 1 (CD24−CD44−NK1.1−), stage 2 (CD24−CD44+NK1.1−) and stage 3 (CD24−CD44+NK1.1+). NKT cells leave the thymus at stage 2 (Brennan et al., 2013).

NKT cells Ligands

Unlike MHC class I, CD1d presents lipid containing molecules to NKT cells TCR, rather than peptide fragments. Although natural ligands have yet to be identified, different studies have investigated the potential molecules that can stimulate NKT immune response and the initial work revealed the α-galactosylceramide (α-GalCer), as CDd1 represented NKT cell antigen, and has become the reagent of choice for pan-activation of the NKT cell compartment. Subsequent studies demonstrated other molecules such as the isogloboside 3 (iGb3), as an important endogenous ligand crucial for NKT cell development and self-recognition in mice and human, (Christiansen et al., 2008). Although the tissue distribution of NKT cells is well characterized in mouse, it is less defined in humans and wide range of NKT cell counts known in the human. In the
mouse, the highest number of NKT cells is present in the thymus, liver and spleen, and represent 0.5% of total thymus cells and 2.5% of T cells count in spleen and 30% of T cell count in the liver.

**Dendritic cells (DCs)**

DCs are a heterogeneous family of immune cells originating from hematopoietic progenitors in the bone marrow. Their function as antigen presenting cells is well known. They process endogenous and exogenous peptides and present it on their cell surface through the MHC class molecule. Immature cells are able to capture and process pathogens and are less efficient in presenting antigen to T cells (Blanco et al., 2008). When immature cells encounter pathogen, they recognize it through toll like receptors (TLR), (Figure 4), they mature and they migrate to the lymph node to present the peptide antigen to T lymphocytes. Upon pathogen encounter DCs upregulate some surface molecules such as CD80, CD86, and CD40 that enhance their interaction with the T lymphocyte. Disturbance of the DCs homeostasis has been linked to many autoimmune diseases such as diabetes mellitus, multiple sclerosis, systemic lupus erythematosus and others. DCs produce many cytokines and chemokines that regulate other immune cell activation and homeostasis; these include type I interferon, IL-12, IL-18, IL10 and IL-23. DCs are traditionally classified as myeloid DC and lymphoid DCs. Depending on the surface markers, DCs of uninfected mice showed three subsets of DCs: CD8α⁺, CD11b⁺ and LY6G/C⁺ CD8α⁺/⁻ (PDCs). PDCs are major cells known to produce IFN α/β in
response to CMV infection, and have a crucial role in NK cell activation in early CMV infection.

Figure 4) TLR domains and their adaptor molecules that send signals to the cell nucleus to produce cytokines, DCs sense MCMV through TLR 9. Adapted from Takeda and Akira, 2004.

III NK cells defense against CMV infection

In response to MCMV infection, NK cells are activated to produce different cytokines and chemokines such as IFN-γ, macrophage inflammatory proteins (MIP-1α and MIP-1β), and tumor necrosis factor α (TNFα) during in vivo and in vitro infection. Early NK cell activation and IFN-γ production during infection of C57BL/6 mice was due to proinflammatory cytokine nonspecific activation of NK cells during the first two days of infection. At the late phase of MCMV infection NK cells activated by direct interaction of the MCMV encoded m157 protein which mimics the MHC class I
molecule structure with the lectin-like type II protein (Ly49H) activation receptor (Dorner et al., 2004).

NK cells kill viral infected cells through pore forming cytolytic protein (perforin) and interferon γ production that directly inhibits viral replication. Loh et al. (2005) demonstrated that the primary mechanism of NK cells to control MCMV is to produce perforin and INF-γ in both the liver and spleen of the infected mice. Loh and his colleagues showed that NK cells, but not NKT cells, are responsible for the early protective response to the MCMV infection. In this study, C57BL/6 (B6) NK cell-deficient mice lacked NK cells and possessed normal NKT and T cells numbers and functions,. These mice were infected intraperitoneally with $10^5$ PFU of MCMV and monitored for survival. A few NK cell-deficient mice (NKD) died on the third day and 80% by the ninth day post infection, compared to wild type B6 mice infected with the same dose, which survived the infection (figure 5).

![Figure 5](image)

**Figure 5** Percentage of survival of NKD and B6 mice monitored for 21 days post MCMV infection. Adapted from Loh et al., 2005

Early control of MCMV infection does not require NKT or T cells, it requires NK cells. In other experiments, groups of B6 mice were infected with $5 \times 10^4$ PFU of MCMV.
These groups consisted of wild type B6 mice, NKD mice, Rag<sup>−/−</sup> (lacking mature T and B cells) and CD1d<sup>−/−</sup> (lacking NK T cells). Viral titers were measured at the 3rd day post infection in spleen and liver cells. An increase in viral titer occurred in NKD group of mice compared to the other groups. Rag<sup>−/−</sup>, CD1d<sup>−/−</sup> deficient mice had comparable viral titers to the B6 mice (figure 6).

![Figure 6](image)

**Figure 6** At the 3rd day post infection, NK cells deficient mice showed higher viral titer in the Liver and spleen comparing to the T, B, and NK T cells deficient mice. Adapted from Loh et al., 2005.

In this same study, perforin and IFN-γ were found to be crucial for protection from MCMV induced mortality, and to control the MCMV infection in both the spleen and the liver. They infected perforin deficient (pfp<sup>−/−</sup>), IFN-γ deficient (IFN-γ<sup>−/−</sup>), and B6 (control), mice with different doses of MCMV. Increased lethality occurred in pfp<sup>−/−</sup> and IFN-γ<sup>−/−</sup> mice in comparison to the B6 mice, indicating the importance of the perforin and IFN-γ in controlling the lethal MCMV infection (figure 7A). Perforin and IFN-γ were required in both the liver and the spleen to control the MCMV infection (figure 7B and C). These results contrasted with those of Tay and Welsh (1997) who found that perforin
was required for controlling MCMV infection of the spleen and IFN-γ was required for controlling liver MCMV infection.

Figure 7) Perforin and IFN-γ are required for the protection from lethal doses of MCMV. A) Increased lethality in pfp<sup>−/−</sup> and IFN-γ<sup>−/−</sup> mice and increased viral titer in liver and spleen of pfp<sup>−/−</sup> mice comparing with B6 mice. C) Increased viral titer in the liver and spleen of IFN-γ<sup>−/−</sup> mice compared with B6 mice. Adapted from Loh et al., 2005.
IV- NK cell activation by type I interferon and IL-12 during MCMV infection.

Type I interferons, discovered by Isaacs and Lindenmann in 1957, binds to their receptors and activates interferon stimulating gene factor3 which enhances the transcription of interferon inducible genes through STAT1, STAT2, and STAT4 (Malmgaard el al., 2002; van Boxel-Dezaire et al., 2006). The biologic effects of Type I interferons involve the synthesis of essential molecules for cell growth, maintenance of cell survival, and immune cells activation. In 1996, Orange and Brion, demonstrated that MCMV infection induces type I interferon and IL12 expression at early times of infection (Figure 8). These cytokines play a crucial role in NK cells activation and their NK cell-dependent and independent anti-CMV response is shown in (figure 9). Two groups of mice, C75B/6 and NK cell/ T cell-deficient (E26) mice, were infected with MCMV and monitored for the production of IFNα/β, IL12 and IFN-γ. An increase in the production of these cytokines were seen after 2 days post infection in vivo and in ex vivo using splenic leukocytes. IFNα/β and IL12 were produced in MCMV infected E26 mice and C57BL/6 mice. IFN-γ was not induced by infection of the E26 mice (Figure 8). This result indicated the dependence of IFN-γ production on the presence of NK cells. This study demonstrated that the early reaction against MCMV is caused by IFNα/β effect and not by NK cells. Serum levels of IFNα/β and IL12 were higher in the infected E26 mice than infected immune competent mice. An increase in viral titer was seen in the liver of E26 mice. Both mice groups were treated with blocking antibodies. About 1 log increase
in viral load was seen in C57BL/6 compared to untreated group. On the other hand, 1.3 log increased viral load and viral replication in E26 mice treated with the IFNα/β blocking antibodies. This observation indicated the early production of IFNα/β was independent of NK or T cells. In contrast, IL-12 neutralization significantly increased the viral load in E26 infected mice at 3\textsuperscript{rd} day post infection. About 2 log increase in viral burden was observed in NK cell deficient mice (Table III), this observation showed that IFNα/β, and IL-12 produced early during infection in the presence of NK cells have a significant antiviral effect.

Figure 8) Early production of IFNα/B, IL-12 p40, and IFN-γ at the day 2 after MCMV infection of C57BL/6 or E26 mice in serum and in splenic leukocytes. Adapted from. Orange and Biron. 1996
Figure 9) IL-12 role in control of MCMV induced hepatic necrosis is independent on NK cells. Adapted from Orange and Biron, 1996.

Table III) Role of IFNαβ and IL-12 in control of viral burden in E26 and C57BL/6 mice. Cytokine neutralizing anti bodies were used to detect NK independent response. Adapted from Orange and Biron 1996.

<table>
<thead>
<tr>
<th>Study</th>
<th>Mouse Strain</th>
<th>Number of Mice</th>
<th>Day After Infection</th>
<th>Antibody Treatment</th>
<th>Viral Titer Log PFU/g Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-αβ</td>
<td>C57BL/6</td>
<td>4</td>
<td>2</td>
<td>Control</td>
<td>4.08 ± 0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>2</td>
<td>Anti-IFN-αβ</td>
<td>4.92 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>E26</td>
<td>4</td>
<td>2</td>
<td>Control</td>
<td>4.63 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>2</td>
<td>Anti-IFN-αβ</td>
<td>5.29 ± 0.16</td>
</tr>
<tr>
<td>IL-12</td>
<td>C57BL/6</td>
<td>3</td>
<td>3</td>
<td>Control</td>
<td>4.08 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>Anti-IL-12</td>
<td>5.21 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>NK-depleted</td>
<td>5.24 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>NK-depleted + anti-IL-12</td>
<td>6.32 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>E26</td>
<td>3</td>
<td>3</td>
<td>Control</td>
<td>4.91 ± 0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>Anti-IL-12</td>
<td>5.61 ± 0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>Control</td>
<td>5.17 ± 0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>Anti-IL-12</td>
<td>5.46 ± 0.15</td>
</tr>
</tbody>
</table>

*Roles of early cytokines were evaluated using neutralizing Abs administered to animals before infection, as described in Materials and Methods.

*pValue significantly different from control, \( p < 0.05 \).

*Value significantly different from control, \( p < 0.1 \).

*Value significantly different from all other treatment groups, \( p < 0.01 \).
In the same study, Orange and Brion (1996) assessed the role of IFNα/β in regulating NK cell cytotoxicity and NK cell proliferation during MCMV infection. They evaluated splenic and bone marrow NK cell mediated cytotoxic function against $^{51}$Cr-labeled YAC-1 target cells in MCMV infected C57BL/6 mice treated with IFNα/β neutralizing antibodies. IFNα/β neutralization caused significant reduction in NK cell cytotoxicity of the spleen and bone marrow cells. There was about 75% less NK cell cytotoxic activity observed in mice treated with anti IFNα/β (Figure 10 A). The frequency and percentage of blast NK cells (NK1.1$^+$ CD3$^-$) were assessed by flow cytometry. Significant reduction in NK cell proliferation and blast formation was observed in C57BL/6 infected mice treated with IFNα/β neutralizing antibodies compared with untreated mice (Figure 10 B).

![Figure 10](image)

**Figure 10** The crucial role of IFNα/β in induction of NK cell proliferation and cytotoxic activity during MCMV infection. A) Splenic and bone marrow NK cell cytotoxic activity inhibited by IFNα/β neutralization. B) Percentage and frequency of blast NK1.1$^+$ CD3$^-$ cell significantly reduced by IFNα/β neutralization. Adapted from Orange and Biron. 1996.
Type I interferon activate NK cells during MCMV infection through STAT mediated pathway.

Nugynus el al. (2002) studied the mechanism of INFα/β and IL-12 regulation of NK cells function during MCMV infection and the mediators required for this regulation. They assessed the role of type I interferon mediated NK cell cytotoxicity and proliferation. This study showed for the first time that INFα/β mediates NK cell proliferation through the STAT mediated pathway in vivo. They demonstrated in MCMV infected C57BL/6 mice that the NK cell expansion is through the IL-15/ STAT mediated pathway and not through the direct effect of IFNα/β on NK cells. Within the first 2 days of CMV infection, NK cell proliferation and cytotoxic activity were enhanced by type I interferon and INF γ produced in response to IL-12. Nuguyen et al. (2002) demonstrated that NK cell proliferation starts as early as 1.5 day post MCMV infection where about 25% of NK cells were proliferated, 60% of them perform cytotoxic activity through perforin secretion, 30-60% secreted INF-γ, all IFN-γ secreted cells was able to produce perforin, and 20% of NK cells perform all of the three activities(Figure 11). This observation indicated that NK cells can exert more than one antiviral response during MCMV infection. Genetic mutation of INFα/β receptor blocked the NK cell response to INFα/β action and reduced cytotoxicity but the production of INF-γ was not affected. Cytotoxic function mediated by STAT1 activation. STAT1 knockout mice from C57BL/6 background showed reduced NK cell cytotoxic activity (Figure 12). STAT1- mice NK cells showed higher viral content than the STAT4- mice. On the other hand STAT4- mice showed decreased number of IFN-γ producing NK cells. IFN-γ serum level decreased in
STAT4- mice and surprisingly, serum IFN-γ levels were higher in STAT1- than STAT4- infected mice (Nguyen et al., 2002). This observation was attributed to the compensatory production of IL-12 in mice with impaired NK cell cytotoxic function. NK cell proliferation in response to the infection was detected by BrdU incorporation of the cells in vivo. Numbers of BrdU+ cells were markedly reduced in IFNα/βR- mice but not in IL-12 deficient ones (IL-12P35-) (figure 13).

IL-15 is important in NK cell survival and homeostasis (Ranson et al., 2003), IL-15 production is enhanced by IFNα/β, during CMV infection through the STAT1 mediated pathway (Nguyen et al., 2002). MCMV infected IFNα/βR- mice showed decrease level of IL-15 and STAT- mice showed impaired IL-15 production (figure 14).

Figure 11) MCMV induced perforin, IFN-γ, and NK cell proliferation at day 1.5 post i.p infection. Adapted from Nguyen et al., 2002
Figure 12) NK cell cytotoxicity requires STAT1 mediated signaling. Adapted from Nguyen et al., 2002.

Figure 13) NK cell proliferation indicated by BrdU+ NK cell numbers and frequency, were markedly decreased in IFN α/βR− and STAT1− MCMV infected mice. Adapted from Nguyen et al., 2002.
Figure 14) STAT1 and IFNα/β receptor are essential for IL-15 mRNA expression. IL-15 production in MCMV infected mice and mice treated with recombinant IFN-α require STAT1 signaling. Adapted from Nguyen et al., 2002.

V- NK cell activation by NK T cell stimulation during CMV infection.

Although the role of NK T cells in innate immune surveillance against tumors is well studied, the antiviral response and the immune regulation for NK T antiviral function is less well understood. Various studies used α galactocylceramis (α-GalCer) as NKT cell external stimulators to activate NK T cell immune response in vivo (Kitamura et al., 2000). α-GalCer is a glycolipid molecule presented by CD1d to the invariant TCR of the NK T cells. After NK T cell stimulation with the α-GalCer, a rapid release of IFN-γ occurred. Carnaud and his colleagues, (1999), used the α-GalCer to study the functional link between NK T and NK cells. This study showed that NK cells activated by IFN-γ that produced by α-GalCer stimulated NK T cells. IFN-γ is produced as early as 90 minutes after α GalCer injection in immunocompetent mice and continues up 6 hours post α-GalCer injection. This first peak of IFN-γ is resulted from activation of NK (CD3′
NK1.1$^+$) cells, (figure 15). In this study immunocompetent (C57BL/6), T, B, and NK T cell deficient (RAG$^{-/-}$), and selective NK T deficient (CD1d$^{-/-}$) mice were injected by $\alpha$-GalCer and monitored for IFN-\(\gamma\) production. IFN $\gamma$ was not produced by NK cells in RAG$^{-/-}$ and in CD1d$^{-/-}$ mice, indicating that activation of NK cells to produce IFN-\(\gamma\) is a secondary event to NK T cell activation (Figure 15).

**Figure 15** $\alpha$-GalCer induce rapid NK cells activation and IFN-\(\gamma\) production in C57BL/6 (B6) mice but not in RAG$^{-/-}$ (B), and CD1d$^{-/-}$ mice (C). NK cell activation require CD1d/NK T cells pathway (C). Adapted from Carnaud et al., 1999.
Dommelen et al. (2003) tested the effect of α-GalCer activated NKT cells on NK cell activation during MCMV infection in visceral organs. In this study, they detected an inhibition of the viral replication in MCMV infected and α-GalCer treated C57BL/6 mice at day two, and four post intraperitoneal (i.p) infection compared to infected untreated mice. They found viral replication in NKT cell deficient mice equal to the wild type mice treated with α-GalaCer, and concluded that the antiviral effect of NKT cells was not a result of direct NKT cell involvement in viral clearance, but rather a result from transactivation of NK cells by IFN-γ production. NKT deficient mice (B6.Jα281−/−), (Figure 16) showed equal viral titer to the control immune competent mice (C57BL/6) infected with MCMV and treated with a GalCer. The NKT cell antiviral effect in response to α GalaCer is a result of their production of IFN-γ which enhances more IFN-γ production from NK cells and promotes their perforin mediated toxicity. In mice deficient in perforin and IFN-γ, αGalaCer did not induce significant decrease in viral titer (Figure 16).

![Figure 16](image)

**Figure 16**) MCMV replication in C57BL/6 and NKT cell deficient (Jα281−/−) mice liver and spleen. At day 2,4, and 6, viral replication in NKT deficient mice is equivalent to that in control mice. Adapted from Dommelen et al., 2003.
NK T cell activation during MCMV infection and cytokines requirement for this activation still under continues study. Tyznik et al. (2008) hypothesized that iNKT cell activation during MCMV infection was induced by IL-12 secretion from DCs. The iNKT cell response to MCMV infection in mouse is independent to antigen presentation through CD1d / TCR interaction. The mechanism of activation of iNKT cells by DCs during MCMV was studied in cell culture and in vivo as well. Mouse bone marrow derived DCs were infected by MCMV and cocultured with splenic iNKT cells. iNKT cell IFN-γ production was induced but not IL-4 in contrast to iNKT cocultured with mock infected DCs where no IFN-γ was detected (Figure 17A). Furthermore, they postulated that the iNKT activation is dependent on TLR9 activated DCs, while DC derived from TLR9 mutated mice failed to stimulate iNKT cells IFN-γ production. In addition, iNKT stimulated by DC derived from mice lacking the IL-12p40 (IL-12p40\(^{-/-}\)), and CD1d (CD1d\(^{-/-}\)) exhibited decreased the iNKT cell induced IFN-γ production. CD1d\(^{-/-}\) DCs induced iNK T cells IFN-γ production was similar to that produced with wild type DCs coculture, and CD1d blocking antibodies showed no effect on the IFN-γ production in iNK T/ DCs culture. Collectively these results indicated that DCs recognize MCMV virus through TLR9 and produce IL-12. IL-12 activates IFN-γ production by iNKT cells independently on CD1d/TCR antigen presentation (Figure 17 B).
Figure 17) A) IFN-γ production by iNKT cells when cocultured with wild type MCMV infected and Mock non infected DCs, B) IFN-γ production by iNKT cells cocultured with MCMV infected and Mock non infected DCs generated from TLR9,IL-12, IL-18, and CD1d deficient mice, and wild type and CD1d deficient mice treated with CD1d blocking antibodies. Adapted from Tyznik et al., 2008

In vivo, iNKT cell activation has been detected in MCMV infected C75BL/6 mice by analyzing α-GalCer/CD1d tetramer+ iNKT. Early activation markers, CD25 and CD96 were up regulated to be detected at 12 to 36 h post infection in the liver and spleen (figure 18 A, B) and intracellular level of IFN-γ was detected ex vivo from liver and spleen at 36 hours post infection. The result is similar to those of the in vitro study. MCMV infection in vivo induces early iNKT cells activation and IFN-γ production but not IL4 or TNF production (Tyznik et al., 2008).
Figure 18 A) iNKT cell activation at the early phase of MCMV infection detected by expression of CD25 iNKT cells in the liver and spleen of the MCMV infected mice (open histogram) compared with the control uninfected mice injected with buffer saline (shaded histogram). B) Time course of CD25 expression in liver and spleen of MCMV infected mice. Adapted from Tyznik et al., 2008.

Wesley et al., 2008, studied in C57BL/6 mouse the Vα14iNKT cells response to MCMV infection. They demonstrated that Vα14iNKT cells were activated to produce IFN-γ cytokine. In contrast to NK cells, Vα14iNK T cells did not proliferate during MCMV infection. C57BL/6 mice were infected by MCMV virus for 20 to 40 hours and the cells were analyzed for signs of activation and INF-γ production. Vα14iNKT decreased in number over time, in MCMV-infected mice compared with uninfected mice (Figure 19A). Although the iNKT cells did not proliferate in response to MCMV infection, they were rapidly activated and the expression of CD25+ cells on the surface of the Vα14iNKT cells was detected at 20 hrs post MCMV infection (figure 19B,C) similar to the observation of Tyzanic and colleagues (2008).
Figure 19 A) Va14i NK T cells are activated but do not proliferate in response to MCMV infection in vivo, number of Va14iNKT are less at 40 hrs than at 20 hrs post infection. Adapted from Wesley et al., 2008

Figure 19 B, C) Increased iNKT cell activation indicated by increase in CD25 expression, the highest level at 20 hrs post infection of C57BL/6 mice. Adapted from Wesley et al., 2008

Figure 19D) percentage, and total iNKT and NK cell numbers detected in cells from C57BL/6 mice liver, decreasing the number and percentage of iNKT after 1.5 day post infection, and an increase of NK cells number and percentage first 4 days post infection. Adapted from Wesley et al., 2008.
Wesley et al. (2008) demonstrated that iNKT cells enhanced NK cell activation and IFN-γ production at 30 hours post infection of C57BL/6 mice. The percentages of IFN-γ⁺NK and IFN-γ⁺NKT cells was measured and the result showed comparable numbers of both IFN-γ⁺ producing cells in the infected liver, while NK cells are the predominant IFN-γ producer in the spleen. IFN-γ and other inflammatory cytokines were significantly reduced in NK T cells deficient mice (CD1d⁻/⁻) spleen compared with CD1d⁻/+ (figure 20), In conclusion, these observations suggests that Vα14iNKT cells are activated but did not proliferate in response to MCMV infection, and activated iNKT cells secrete IFN-γ that amplify the IFN-γ production by NK cells, and other innate cytokine production.

![Figure 20](image)

**Figure 20**) NK cell activation (A) and innate cytokines production (B) are significantly decreased in NKT cells deficient mice. Adapted from Wesley et al., 2008
VI- Plasmacytoid dendritic cells activates NK cells during CMV infection

Plasmacytoid dendritic cells (PDC) are a dendritic cell subtype that recognize DNA and RNA viral antigen through the Toll like receptor 7 (TLR7) and TLR9 which promotes Type I interferon secretion through MyD88-IRF7 signaling pathway. During MCMV infection, PDCs and their produced cytokines interact with NK cells leads to their activation and cytokine secretion. The only well-known antiviral response of the PDC is through the production of Type I interferons that regulate other immune cells such as NK cells and other DCs subtypes to shape the innate immune response (Dalod et al., 2003).

Swiecki et al. (2010) studied the crucial role of PDCs in NK cells antiviral activity against MCMV. They used a new approach by using transgenic mice on C57BL/6 background that express diphtheria toxin receptor, these mice selectively depleted of PDCs with the diphtheria toxin (DT) treatment. Such mice were infected with MCMV intraperitoneally. In contrast to control immune competent mice, DT-treated mice exhibited increased viral replication in the liver and the spleen during early infection and in the salivary gland at day eight post infection. NK cell immune response to MCMV includes two phases; the early phase develops by a nonspecific activation of NK cells by the innate cytokines and independent on LY49H receptor interaction. The late phase of NK cell immune surveillance involves expansion of LY49H+ NK cells that activated by the direct interaction of the LY49H receptor with the MCMV protein (m157). To assess the effect of PDC depletion on the nonspecific (early) phase of NK cell
activation and IFN-γ production and to exclude the NK cell activation through the direct interaction of LY49H with mcmv protein (m157), they used MCMV virus lacking m157 protein. The effect of PCDs depletion on NK cells activation was apparent since the early hours of infection. Reduced number of activated NK cells (CD96+) (figure 21), and impaired NK cell cytotoxic activity were observed in PDC depleted mice compared with wild type, (figure 22). The percentage of IFN-γ producing NK cells was increased at later times post infection of the DT treated mice. There was no effect observed on the NK cells selective activation, where the number of LY49H+ NK cells increased in later time during infection of PDC depleted mice and more IFN-γ was produced than in control mice (figure 23, A and B).

![Figure 21](image_url)  
**Figure 21**) Decrease in activated NK cells number of DT-treated mice at the early time post MCMV infection. Adapted from Swiecki et al., 2010
Figure 22) NK cells cytotoxicity during different time points of MCMV infection to the DT treated and control mice. Adapted from Swiecki et al., 2010

A

B

Figure 23 A) IFN γ production in DT-treated and control MCMV infected mice. B) Effect of PDC depletion on specific and nonspecific NK cell activation, indicated by NK 1.1+ and Ly49H+ NK cells respectively. Adapted from Swiecki et al., 2010.

VII- Conclusion and future studies

Collectively, the data presented in this study illustrate the innate immune response exerted by NK cells and other innate immune cells to destroy MCMV-infected cells. NK cells are activated either directly by interaction of activation receptors with viral encoded proteins, or indirectly through the effect of the proinflammatory cytokines. The m157 viral protein interacts with Ly49H receptor to initiate specific activation of NK cells in
C57BL/6 mice. Nonspecific activation of NK cells through the action of Type I interferon occurs at an early time during infection. The early response to MCMV infection is Type I interferon production by infected DCs subsets. PDCs recognize MCMV virus through TLR9 and produce IFNα/β, IL-12, and other cytokines. IFNα/β promoting cytotoxic activity by perforin release, inducing NK cell proliferation and maintaining NK cell survival through STAT1/IL-15 pathway. IL-12 is also produced by DCs during MCMV infection. IL-12 induces NK cells as well as NKT cell activation and IFN-γ production. Although NK cell proliferation is detected during MCMV infection, NKT cells do not expand in infected mice. One of the lethal sequelae of MCMV infection is the neurological complications such as, hearing loss and visual impairment. Most of the studies assessed the innate immune response in visceral organs, and the immune response against neuronal MCMV infection needs to be evaluated.

In future studies, factors involved in NK cell activation during MCMV infection of neurons need to be identified. NK cell activation by IFNα/β in MCMV neuronal infection can be studied by assessing the NK cell-mediated cytolysis of YAC-1 target cells in MCMV-infected mice. Compared to MCMV-infected mice infected intracerebrally, MCMV-infected mice treated with IFNα/β blocking antibodies should show increased neurological deficits, increased viral titers, and decreased NK cell cytolysis. The requirement of type I interferons in inducing NK cell proliferation can be assessed by detecting NK1.1⁺ CD3⁻ cells by flow cytometer in infected brain tissue. Future evaluation of the role of NKT cells in activating NK cells in MCMV-infected neuronal tissue will provide a better understanding of the innate antiviral immune surveillance. IFN-γ production in brain tissue (ex vivo) in C57BL/6 (control), and NKT
cell-deficient (CD1d−/−) C57BL/6 mice should indicate NKT cell involvement in immune surveillance against neuronal MCMV infection in the control but not CD1d-deficient C57BL/6 mice.
References


