Review Article

The Impact of Ly49-NK Cell-Dependent Recognition of MCMV Infection on Innate and Adaptive Immune Responses

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Clinical and experimental data indicate that a subset of innate lymphocytes, natural killer (NK) cells, plays a crucial role in the response against herpesviruses, especially cytomegaloviruses (CMV). Indeed, in mice, NK cells, due to the expression of germline encoded Ly49 receptors, possess multiple mechanisms to recognize CMV infection. Classically, this results in NK cell activation and the destruction of the infected cells. More recently, however, this unique host-pathogen interaction has permitted the discovery of novel aspects of NK cell biology, implicating them in the regulation of adaptive immune responses as well as in the development of immunological memory. Here, we will concisely review the newly acquired evidence pertaining to NK cell Ly49-dependent recognition of MCMV-infected cell and the ensuing NK cell regulatory responses.

If one way be better than another, that you may be sure is nature's way.

Aristotle

1. Introduction

Natural killer (NK) cells constitute a unique effector lymphocyte lineage, classically defined as part of the innate immune system. Their importance is well characterized in the context of health, under normal physiological conditions. For instance, NK cells are involved in tumor surveillance, given their natural cytotoxicity against neoplasms. In pregnancy, NK cells account for 70% of decidual leukocytes; they are thought to mediate trophoblast invasion into the uterine lining and the modification of maternal spiral arteries [1]. In addition to their implication in these crucial components of homeostasis and normal development, NK cells are well known for their considerable role in defense against infection.

Infection with a wide variety of pathogens can be limited by the action of NK cells, specifically intracellular microorganisms. Indeed, NK cells play a substantial role in the initial control of both bacterial (e.g., *Listeria monocytogenes*) and parasitic (e.g., *Plasmodium* species) spread [2]. Yet, in the context of infection, the hallmark of NK cells remains their potent antiviral activity. NK cells participate in the clearance of many different viruses (human immunodeficiency virus, coxsackievirus, influenza or poxviruses, etc.), but their contribution is indispensable with regards to infection with members of the *Herpesviridae* family (herpes simplex virus (HHV-1/2), varicella zoster virus (HHV-3), and cytomegalovirus (HHV-5)) [2]. Plentiful evidence supports this, namely, the cases of two young patients suffering from numerous and recurrent herpesviral infections due to their nonfunctional NK cells [3, 4]. In mice, NK cell depletion and adoptive transfer have long been known to increase, respectively, susceptibility and resistance to mouse CMV (MCMV).

The antiviral activity of NK cells relies on the various effector functions induced following their activation. On the

one hand, NK cells secrete several cytokines, such as IFN-γ, MIP1-α/β, RANTES, IL-10, activation-induced T-cell-derived chemokine-related cytokine (ATAC, lymphotactin), and others. On the other hand, NK cells display strong cytotoxicity. Indeed, they can kill virus-infected cells without prior activation, and they do so via a number of mechanisms, namely exocytosis of perforin-granzymes granules and signaling through members of the TNF death receptor family [5]. Yet, cytokine production and cytotoxicity must necessarily be preceded by the recognition of infected cells by NK lymphocytes, a phenomenon that has mostly been studied using a mouse model of infection.

CMVs are prototypical β-subgroup members of the *Herpesviridae* family. Given the nonredundant role played by NK cells in resolving infections with these viruses, it is not surprising that CMVs would be perfect candidates for the study of NK cell responses. Unfortunately, the strict species specificity of CMVs precludes experimental infection of mice with human CMV (HCMV). Yet, MCMV shares many features with its human counterpart, including genome structure and disease manifestations [6]. Both are natural pathogens of their respective host and have coevolved with them for eons. Moreover, both viruses have developed varied and analogous immune-evasion mechanisms that heavily implicate NK cells [7]. Therefore, early MCMV infection has become an established model to study NK cells and, more specifically, their impressive ability to distinguish self from nonself through their germ-line encoded receptors. In addition, at later times post-infection, this model reveled the unforeseen involvement of NK cells in the adaptive immune responses.

NK cells discriminate between infected and healthy cells using an extensive panel of cell surface receptors, both activating and inhibitory. Among the various receptor families involved in this process, Ly49 receptors have proven themselves to be particularly important for MCMV detection by murine NK cells. These polygenic and polymorphic receptors are clustered at the Natural Killer Cell Complex (NKC) on mouse chromosome 6 [8]. They are stochastically expressed as disulfide-linked homodimers primarily on the surface of NK cells, but also on subsets of monocytes, macrophages, dendritic cells (DCs), and T cells [9]. In terms of ligand specificity, inhibitory Ly49 receptors recognize self-MHC class I molecules (MHCI, also called H-2 in mice), whereas their activating counterparts can bind to various protein determinants of infection.

Ly49 receptors are structurally classified as type II trans-membrane, C-type lectin-like proteins. Their extracellular domain is comprised of a flexible stalk and a Natural Killer Domain (NKD), which provides ligand binding specificity and is structurally conserved among all members of the Ly49 family. Yet, activating and inhibitory receptors differ with regards to their intracellular domains. Indeed, inhibitory Ly49 receptors possess an immunoreceptor tyrosine-based inhibition motif (ITIM) within their intracellular domain. Conversely, activating Ly49 receptors lack this ITIM motif; instead, a positively charged arginine residue in their transmembrane domain interacts with the DAP12/DAP10 adaptor proteins, which bears an immunoreceptor tyrosine-based activation motif (ITAM).

During infection, Ly49 receptor triggering leads to the initiation of a signaling cascade, the result of which is either inhibition or activation of the NK cell. In the case of both activating and inhibitory receptors, the first step of this cascade is the phosphorylation of the tyrosine residue contained in their respective ITAM or ITIM, most likely by a Src family kinase [5]. This phosphorylation recruits either SHIP-1, SHP-1, or SHP-2 in the case of inhibitory receptors, or Syk, ZAP-70 and PI(3)K or Grb2 for activating receptors. In both cases, numerous downstream effectors are involved. The end result of inhibitory receptor triggering is the dephosphorylation of ITAMs linked to activating NK receptors and the prevention of Ca^{2+} influx, degranulation, cytokine production, and NK cell proliferation. In opposition, activating Ly49 receptor engagement induces the reorganization of the actin cytoskeleton to enable cell polarization and the release of cytolytic granules, as well as the transcription of many cytokine and chemokine genes.

Of note, inhibition is by far the most common signal received by NK cells over the course of their lifetime. Moreover, during NK cell development, binding of inhibitory Ly49 receptors to self-MHCI molecules renders them functional, that is to say capable of cytokine secretion (e.g., IFN-γ) and cytotoxic killing [10]. This model, called NK cell education or licensing, postulates that the stronger the inhibitory Ly49-self MHCI interactions are, the more competent the NK cell will emerge. The immune response which follows MCMV infection relies on the interplay of soluble factors and numerous cell types, among them NK cells. In the early innate response, Ly49 receptors are critical for NK cell activation, which in turn controls viral proliferation, due to their ability to recognize various viral and self-ligands. Nevertheless, initial NK cell proliferation is Ly49-independent and nonselective, while the subsequent preferential NK cell proliferation is driven by activating Ly49 receptors. At later stages of the infection, NK cell secreted cytokines and apt virus control allows them to regulate the adaptive response. Most surprisingly, Ly49-mediated activation of NK cells also allows the generation of NK cells with several characteristics seen in memory T cells (Figure 2(a)).

All of these aspects of MCMV-Ly49-driven NK cell responses will be examined in this paper. Most importantly, evidence will be presented to highlight the previously unanticipated sophistication of the NK cell response against infection, redefining the established concepts of innate and adaptive immunity.

2. Immediate Nonspecific NK Cells Response to MCMV Infection

From its initial peripheral sites of entry, MCMV spreads to the lymph nodes and via the bloodstream to the spleen, and the liver, infecting a broad spectrum of cell types. Macrophages, hepatocytes, and reticular fibroblasts are some of its primary targets [11, 12]. At 6–8 hours after infection (p.i.), lymphotoxin α/β expressed on the surface of B cells

interacts with its cognate receptor on splenic stromal cells, triggering type I interferon (IFN-α/β) secretion by the latter [13, 14]. Within 24 hours after the initial infection, before the end of the first round of viral division, this first wave of type I IFN secretion wanes. Subsequently, a second round of viral infection occurs, this time in many more organs, making the infection systemic.

Over the course of MCMV infection, several molecular viral byproducts are generated due to the lytic nature of the pathogen. Plasmacytoid DCs (pDCs) engulf infected cell debris and recognize them through their endosomal pattern recognition receptors (PRRs), that is, TLR3, TLR7 and TLR9; this recognition induces a second, much stronger wave of type I IFN secretion ($\sim$36 hours p.i) as well as the production of other proinflammatory cytokines (IL-12, IL-6, IL-1β, TNF-α, etc.) [15–17]. In parallel, the presence of MCMV particles in the cytoplasm of pDCs can lead to viral recognition by the NOD-like and RIG-I-like helicase sensors resulting in secretion of more pro-inflammatory cytokines; these molecules probably also contribute to pDC-independent secretion of type I IFN [18–20].

Conventional DCs (cDCs) are one of the many cell types that are initially infected by MCMV. In response to infection, they secrete IL-12, IL-18, IL-15 (trans-presented), and type I IFN all of which are required for NK cell activation. Indeed, different NK cell effector functions require different cytokine inputs for their initiation: while the IFN-$\alpha\beta$/STAT1 pathway is necessary for cytotoxicity and initial proliferation, NK cell IFN-γ production during the first 2 days following infection necessitates IL-18 or IL-12/STAT4 (Figure 2(a)i) [21–23]. Moreover, the IFN-γ secreted by infected cDCs, along with macrophage-secreted MIP1-α, are critical for the control of viral loads in the liver and lungs [24]. IFN-γ is also important for perforin-dependent control of viral replication in the spleen [25]. Once activated, NK cells start to eliminate infected cDCs and TNF-α-secreting macrophages, thus reducing the immunopathology caused by this cytokine [26]. In short, the initial NK cell response against MCMV is mediated by a cytokine storm; it is rapid, nonspecific, and global, involving NK cells with diverse Ly49 repertoires. Yet, it does not effectively control viral spread.

Indeed, despite the high levels of antiviral cytokines present in the serum, MCMV replication proceeds unhindered. This is true for all inbred strains at day 1.5 after infection. Yet, by day 3 p.i., mouse strains can be segregated according to their NK cell-dependent control of MCMV [27]. Most strains are susceptible to the virus, while a select few are resistant. These strains include C57BL/6 (B6) and MA/MyJ mice, whose resistance depends on their strain-specific activating Ly49 receptor, as well as PWK/Pas and NZW/LacJ mice, whose resistance is not yet characterized [27–29].

3. Specific Recognition of MCMV-Infected Cells

3.1. Ly49H/m157 Axis.

MCMV resistance in B6 mice depends on the presence of the activating Ly49H receptor on the surface of their NK cells [30–32]. In this mouse strain,

approximately 50% of NK cells express the receptor. Ly49H binds directly to m157, a MCMV-encoded glycoprotein with structural homology to host MHCI molecules; unlike the latter, however, m157 is a GPI-anchored protein, does not associate with β_2-microglobulin, or present peptides at the surface of the cell [33–35]. During the early phase of the infection, Ly49H-m157 ligation initiates an activating signaling cascade, resulting in clonal proliferation of Ly49H$^+$ NK cells, killing of infected cells, and secretion of cytokines (IFN-γ, MIP1-α, TNF-α, etc.), all of which result in the reduction of viral loads (Figure 1(a)).

The importance of this receptor-ligand pair in the control of MCMV infection was demonstrated by numerous studies. In the case of the virus, resistant mice became susceptible upon infection with a *m157*-deletant MCMV ($\Delta m157$) (Figure 1(b)) [36]. As for the host, deletion of Ly49H gene or deletion or loss of function mutation of the DAP12 gene were shown to render mice susceptible to MCMV infection, as revealed by high viral titers [37–39]. Conversely, transgenesis of Ly49H into initially susceptible strains was found to make them resistant to MCMV [40].

Once the interaction between Ly49H and m157 was established as an essential component of MCMV resistance in B6 mice, the nature of this interaction was investigated. Structural analysis, molecular modeling, and targeted mutations of both m157 and Ly49H have shown the importance of the receptor homodimerization domain. Indeed, Ly49H receptors carrying mutations in this region display reduced m157 recognition, while mutations in the putative ligand-binding regions had limited impact on this same interaction [41]. In the same vein, mutation of buried residues required for correct m157 folding ($\alpha0:\alpha2$ helix interactions) altered the ability of the viral molecule to bind Ly49H, while mutations in the exposed and scattered residues across the entire m157 structure did not [42]. Furthermore, posttranslational modifications can also affect Ly49H-m157 binding. Indeed, given the four putative N-glycosylation motifs (NXT/S) of m157, a variety of glycosylated isoforms were found to be expressed on the surface of MCMV-infected cells. Although these isoforms are also recognized by Ly49H, their binding stability and half-life are increased as compared to the unglycosylated m157 [43]. These results reveal a quite tolerant recognition of m157 by Ly49H where several amino acid substitutions are necessary in order to disrupt m157-Ly49H interactions. Such a molecular arrangement could allow Ly49H to recognize variable m157 molecules, emerging as the MCMV attempts to escape immune recognition.

From an evolutionary standpoint, capture of the *H-2* gene by MCMV is thought to have initiated a cascade of events leading to the emergence of the Ly49H-m157 interaction. Originally, this capture could have allowed the virus to exploit a MHCI homologue capable of triggering inhibitory Ly49 receptors. Since the Ly49 locus evolves rapidly, any inhibitory receptor could have been converted to an activating one, including those with binding specificity for m157 [44, 45]. In support of this, m157 can be bound by the inhibitory Ly49I receptor from 129/J mice; unfortunately, the involvement of Ly49I in susceptibility to MCMV infection could not be assessed due to a defect in
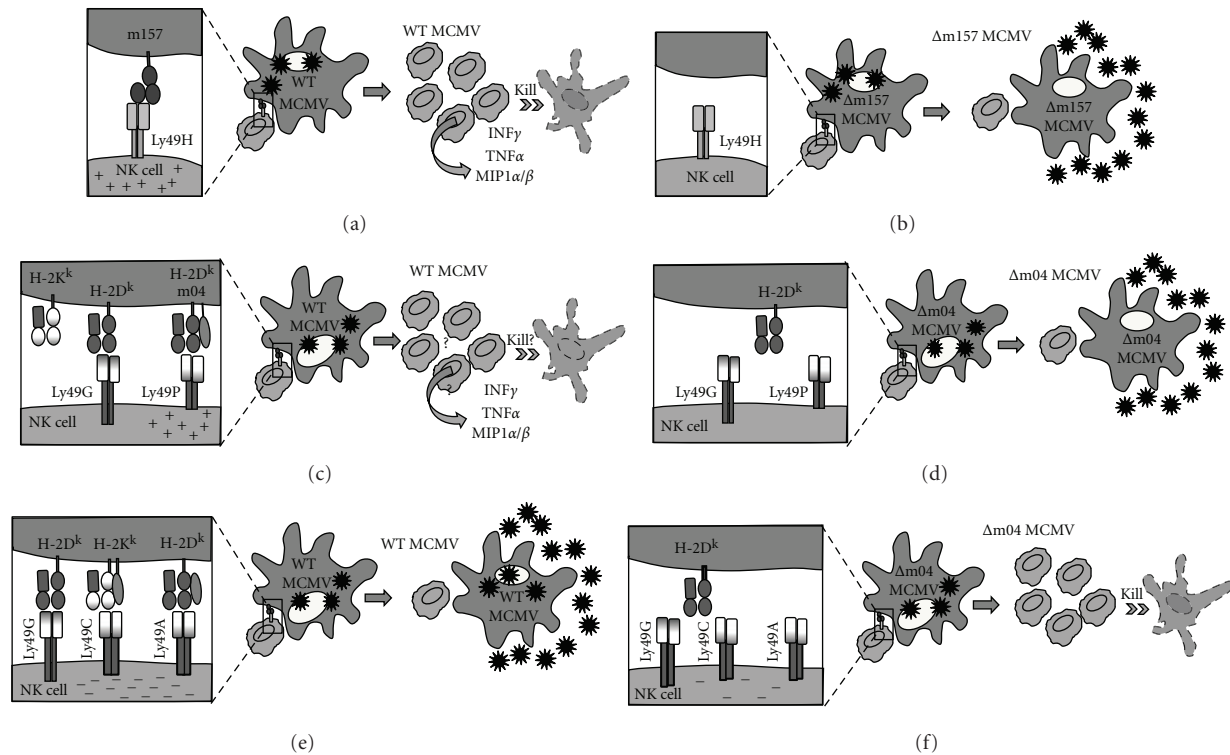
FIGURE 1: Multiple modes of Ly49-NK cell-dependent recognition of MCMV-infected cell. NK cell-dependent detection of MCMV infection occurs through different Ly49 receptors and viral proteins. (a, b) Ly49H-m157, operating in C57BL/6 mice; (c, d) Ly49P-*m04*:H-2D^k or Ly49G-H-2D^k, operating in MA/MyJ mice; (e, f) Inhibitory Ly49-H-2:*m04*, operating in BALB mice. (a, c) Triggering of the activating Ly49 receptors by their viral ligand, m157, or by their virally modified self-ligands, *m04*:H-2D^k, leads to NK cell cytotoxicity, proliferation, and secretion of cytokines. (b, d) The absence of these ligands prevents NK cells from recognizing and responding to infected cells. (e) However, triggering of inhibitory Ly49 receptors by virally modified self-ligands results in NK cell unresponsiveness during the infection. (f) NK cell responsiveness is restored upon infection with a deletant virus lacking the protein required for modifying self-ligands. WT MCMV: Wild-type MCMV, Δm157: MCMV lacking m157, Δm04: MCMV lacking m04, (+): activation, (-): inhibition.

NK cell signaling within this strain [33]. Recently, additional inhibitory Ly49 receptors, namely, Ly49C from the B6, BALB/c, and NZB strains, have been shown to interact with the m157 obtained from wild-derived MCMV isolates [46]. Interestingly, Ly49C-specific recognition of nonlaboratory strain-MCMV-infected cells did not seem to interfere with Ly49H-mediated NK cell activation and the ensuing antiviral response in B6 mice. The dominant Ly49H recognition of m157 is supported by the fact that sequential passage of wild-type (WT) MCMV in B6 mice leads to the emergence of "NK cell-escape" m157 variants, which evade Ly49H-mediated NK cell recognition [47]. Nevertheless, the m157 proteins of MCMV strains isolated from wild mice are highly variable, indicating that outside of laboratory settings, both inhibitory and activating Ly49 receptors could be targeted by the virus. In any case, the fact that m157, one of many MCMV-encoded proteins, can be recognized by different Ly49 receptors, both activating and inhibitory, suggested that other mechanisms of MCMV recognition might exist in other mouse strains.

3.2. Ly49P/m04/H-2D^k Axis. In this second mechanism of MCMV-infected cell recognition, an activating Ly49 receptor recognizes "altered" self-MHCI molecules rather than a viral MHCI homologue; this alteration of self-MHCI molecules

stems from their association with viral protein. This type of recognition could be used by NK cells from MA/MyJ mice, which are resistant to MCMV even though they lack Ly49H. Indeed, MA/MyJ resistance has been associated with a genetic interaction between the NKC and the *H-2^k* loci or the *H-2^k* locus alone [48–50]. At the cellular level, MA/MyJ resistance is correlated with the expression of the activating Ly49P receptor. This receptor can recognize infected cells given the surface expression of two specific elements: the viral *m04*/gp34 protein in association with H-2D^k molecules (Figure 1(c)). In order to dissect the contribution of *H-2* to this recognition, chimeric *H-2* molecules were produced, in which domains were swapped between H-2D^b and H-2D^k; these chimeric molecules were used to show that the H-2D^k-binding platform in particular is required for this recognition. Furthermore, the ectopic expression of *m04*/gp34 alone cannot stimulate Ly49P-bearing reporter cells in the absence of infection. This stimulation, however, is restored when target cells are infected with a viral deletion mutant lacking *m04* (Δ*m04* MCMV), suggesting that another host or viral factor is necessary for Ly49P/*m04*/H-2D^k-mediated recognition of infected cells. *m04*/gp34 is crucial for the *in vivo* NK cell response in MA/MyJ mice, since
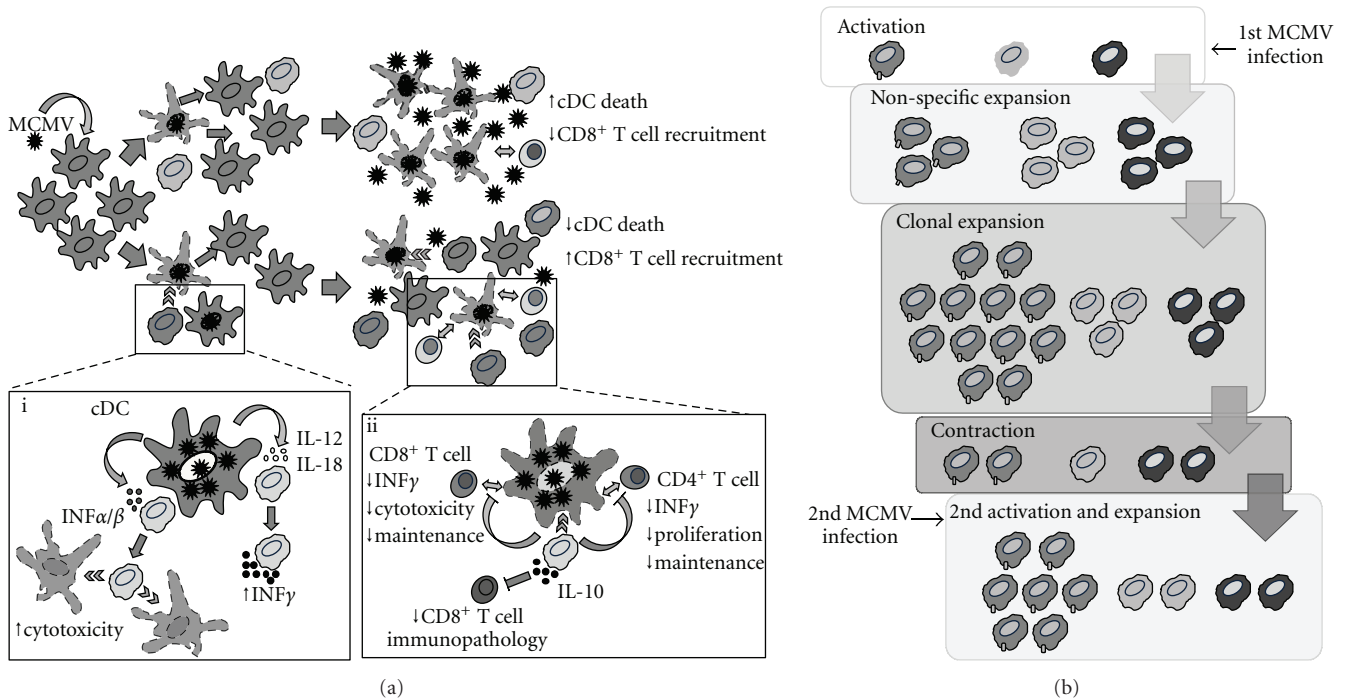
(a)

(b)

FIGURE 2: Different regulatory roles of Ly49H^{+} NK cells in adaptive immune responses. (a) Ly49H^{+} NK cells rapidly control viral replication and protect cDCs from MCMV infection. This allows for rapid recruitment and priming of CD8^{+} T cells and reduces viral and self-immunopathology. (i) Interaction of NK cells with MCMV-infected conventional dendritic cells (cDCs) induces the former's proliferation and cytotoxicity via IFN-α/β or secretion of IFN-γ via IL-12/IL-18. (ii) Ly49H^{+} NK killing of infected cDCs will also reduce the priming of CD4^{+}/CD8^{+} T cells and dampen the ensuing adaptive immune responses; NK cells will produce IL-10, which will limit excessive CD8^{+} T cell responses, thus reducing immunopathology. (b) Schematic representation of NK cell proliferative responses upon primary and secondary MCMV infections. ↑: increase, ↓: decrease.

normally resistant MA/MyJ mice are unable to control viral replication following infection with $\Delta m04$ MCMV [51] (Figure 1(d)).

The importance of H-2D^k in the resistance of MA/MyJ mice was also demonstrated *in vivo*: (1) by the creation of an H-2^k congenic mouse panel; (2) by the transgenesis of H-2D^k into susceptible H-2^b mice. In both cases, the mice became capable of controlling the viral infection, as opposed to H-2^b animals [50, 52, 53]. Xie et al. tested their H-2^k congenic mouse panel further and showed that Ly49G$^+$ NK cells expanded preferentially in these mice at 90 hours p.i. Their resistance to MCMV infection was abrogated following depletion of Ly49G$^+$ NK cells, which suggests that Ly49G is somehow involved [52, 53]. Since an inhibitory receptor cannot directly mediate resistance to MCMV, the authors hypothesized that appropriate licensing via Ly49G:H-2D^k interaction renders NK cells functional, capable of subsequently recognizing and eliminating MCMV through Ly49P/$m04$/H-2D^k (Figure 1(c)). Nevertheless, it remains unclear how or if licensing contributes to the ability of NK cells to resolve MCMV infection. Indeed, it has recently been shown that unlicensed NK cells respond better to MCMV infection [54]. Regardless of the role of licensing, the available evidence suggests that several Ly49 receptors might be involved in the control of MCMV infection in MA/MyJ mice.

3.3. Inhibitory Ly49/m04/MHCI Axis.

The contribution of inhibitory Ly49 receptors to the NK cell-mediated control of MCMV infection has recently been evaluated. Indeed, studies using BALB mice show that the presence of Ly49 inhibitory receptors negatively impacts early MCMV resistance, that is, it makes mice susceptible to the virus [55]. BALB mice are considered susceptible, that is to say that they are unable to control the replication of WT MCMV in the spleen at day 3 p.i. However, infecting these mice with $\Delta m04$ MCMV results in a significant reduction of viral loads in this organ and increased NK cell proliferation in a *H-2*-dependent manner. Indeed, BALB mice of the H-2^k (BALB.K) background showed the greatest reduction in viral load upon infection with $\Delta m04$ MCMV, whereas none was seen in H-2^b mice (BALB.By); the viral load reduction in H-2^d mice (BALB/c) was an intermediate value between that of H-2^k and H-2^b mice. This reduction in viral load is due to the absence of $m04$/gp34, which maintains surface expression of MHCI molecules, and the action of activating receptors such as NKG2D. As such, in WT MCMV-infected cells, sufficient levels of MHCI molecules are displayed on the cell surface; this allows inhibitory Ly49 receptors to be triggered and to deliver their inhibitory signal, the result of which is the unresponsiveness of NK cells (Figure 1(e)). In the case of $\Delta m04$ MCMV infection, the expression of MHCI molecules is severely downregulated, allowing NK

cell activation through the "missing-self" mechanism and improved control of viral spread (Figure 1(f)). Furthermore, viral loads were shown to return to high levels following depletion of certain inhibitory Ly49$^+$ NK cell fractions during $\Delta m04$ MCMV infection of H-2^d BALB mice. This effect was more pronounced following Ly49G$^+$ NK cell depletion than Ly49C$^+$ or Ly49A$^+$ NK cell depletion. In summary altered self-recognition by activating Ly49 receptors, requiring the presence of self-MHCI molecules associated with viral products, implicates involvement of inhibitory Ly49 receptors, which depending on mice strain can have opposite effects. Thus, when the predominant signal is $m04$-dependent activation (e.g., MA/MyJ via Ly49P), $m04$ ablation allows enhanced viral replication; however, when the predominant signal is $m04$-dependent inhibition (e.g., BALB via Ly49G/A), absence of $m04$ allows enhanced control of virus replication.

In short, these findings suggest that the early, NK cell-dependent control of viral replication is not solely due to activating Ly49 receptors, but rather that it involves a delicate interplay between signals generated by both activating and inhibitory receptors.

4. Beyond Natural Killing: Regulation of Inflammatory and Adaptive Responses by NK Cells

NK cells have classically been thought of as effectors of the antiviral response through their ability to directly eliminate infected cells. However, recent evidence demonstrates that their role extends beyond that of effector lymphocytes into regulators of the inflammatory and adaptive immune response against viral infection. More specifically, Ly49H$^+$ NK cells are essential for the protection and preservation of cDCs, which can themselves stimulate NK cells and potentiate their response [56, 57]. Nevertheless, infection does occur; NK cell-dependent elimination of cDCs and, in particular, macrophages, reduces the secretion of TNF-α, a major contributor to hemophagocytic lymphohistiocytosis syndrome during MCMV infection [26]. In addition, Ly49H-m157-dependent reduction of viral burden decreases the duration and intensity of pDC activation, which limits very high systemic levels of type I IFN and other cytokines that can be detrimental to the host [32, 58–60]. In doing so, NK cells also accelerate the recruitment and expansion of anti-MCMV specific CD8$^+$ T cells, as it was shown that in mice devoid of Ly49H, the appearance of CD8$^+$ T cells is delayed by ∼1 day (Figure 2) [57].

Triggering by m157 is essential for Ly49H$^+$ NK cell amplification and maintenance. In the context of MCMV infection, signaling through Ly49H/DAP12 induces NK cell clonal proliferation, ultimately leading to an increase in the Ly49H$^+$ NK cell frequency from 50% to 80% and clearance of the virus in the spleen and liver by day 6 p.i. [32]. Conversely, in Ly49H$^{-/-}$ mice, the overall amount of NK cells declines past uninfected levels, leading to high viral titers in the spleen at the same time point [59]. Another study, by Lee et al., attempted to dissect the role of Ly49H

in various aspect of the NK cell response using $Ly49h$ or $perforin$ 1 ($Prf1$) knockout mice. For instance, Ly49H-expressing $Prf1^{-/-}$ mice were susceptible to MCMV infection, even though Ly49H$^+$ NK cells proliferated substantially. Although in these mice the NK cytotoxic function was absent, they survived a dose that killed $Ly49h^{-/-}$ $Prf1^{-/-}$ double-knockout mice. Therefore, Ly49H expression was protective. This was explained by the fact that Ly49H$^+$ NK cells secreted IL-10, a potent immunosuppressive cytokine capable of limiting CD8$^+$ T cell cytotoxicity as well as IFN-γ and TNF-α secretion (Figure 2(a)ii) [59]. Thus, Ly49H is required for the maintenance of NK cells until late timepoints after infection, allowing them to exert a regulatory function over CD8$^+$ T cells and the immunopathology they can cause.

Paradoxically, the presence of activating Ly49 receptors can also be detrimental to the subsequent adaptive immune response by promoting viral persistence. For example, while Ly49H$^+$ mice could clear infected cDCs better than their Ly49H$^-$ counterparts, killing of these antigen-presenting cells (APCs) prevented them from priming naïve, virus-specific T cells [61]. This lack of appropriate T-cell priming in Ly49H$^+$ mice resulted in the reduced activation, cytotoxicity, IFN-γ secretion, and maintenance of CD8$^+$ T cells, as well as the reduced IFN-γ secretion and maintenance of CD4$^+$ T cell, contributing to MCMV persistence in the salivary glands (Figure 2(a)ii). However, MCMV-infected, immature cDCs have also been shown to induce anergy, poor effector functions, and inferior recall responses in CD4$^+$ and CD8$^+$ T cells [62]. Further research is needed to determine the precise effect of cDC clearance in the overall response to the virus. Nevertheless, Ly49H$^+$ NK cell recognition and removal of MCMV-infected cells can unquestionably shape the course of the adaptive immune response.

5. Memory-Like NK Cells

Another unforeseen NK cell role was evidenced by the discovery that, following activation, NK cells acquire features seen in memory T-cell populations. The existence of memory-like NK cells was first hinted at in a study of chemically-induced contact hypersensitivity (CHS). In this model, mice lacking T and B lymphocytes, the classical mediators of CHS, remained able to elicit an inflammatory response through hepatic NK cells, as measured by dermatitis [63]. Sun et al. [64] followed up on this observation using an MCMV model of infection and adoptive transfer into DAP12$^{-/-}$ mice. The investigators noticed that only Ly49H$^+$ NK cells underwent specific clonal expansion before contraction and persisted for up to 70 days p.i. This was rather astonishing as the generally accepted half-life of mature NK cells is 7 to 17 days [65]. Importantly, irrespective of initial transfer numbers the frequency of the MCMV-specific NK-cell response was similar to primary T-cells responses. They also observed that these long-lived, memory-like NK cells were m157-specific and had superior effector responses upon rechallenge with MCMV as compared to naïve NK cells [64].

The existence of memory-like NK cells was also confirmed by transferring cytokine-activated NK cells into naïve hosts and observing that they could still be detected as far as 22 days after transfer [66]. These memory NK cells were found to have similar cytotoxic activity to that of their naïve counterparts. Moreover, they were unable to constitutively produce IFN-γ yet upon restimulation produced significantly more of this cytokine than naïve NK cells. More recently, again in the CHS model, virus-specific memory-like NK cells were generated in response to influenza virus, VSV or HSV-1 infection. In this case, the adoptive transfer of specific, virus-sensitized hepatic NK cells into naive mice improved their survival following infection with a lethal dose of virus; this was dependent on the CXCR6 chemokine receptor expressed on NK cells [67].

All of these experiments have shown that innate immune cells can retain an intrinsic memory of prior activation, a function until now restricted to antigen-specific, adaptive immune lymphocytes. However, most of the aforementioned studies were performed with immunodeficient donor and recipient mice. Given the adaptability of the immune system, it is possible that donor NK cells, once transferred into these recipients, may have taken up more responsibility (i.e., memory status) than they normally would in WT animals in order to compensate for the absence of T and B cells. As for the work performed by Sun et al., even though their donor NK cells came from WT mice, they were transferred into neonate animals, whose adaptive immune responses are arguably as ineffective as that of immunodeficient mice.

6. Conclusions

Since their discovery over 30 years ago, it has become clear that NK cells possess numerous functions, going beyond their original "natural killers of tumors" role. Among these, they act as a first line of defense in the context of infection with a variety of pathogens, in particular viruses. Humans have been coevolving for millions of years with some of these viruses, namely CMVs; therefore, they "know us better than we know ourselves", having shaped our immune system and been shaped in return.

Mouse models of MCMV infection revealed that NK cells are not merely "killers" of infected targets, but very complex lymphocytes endowed with the molecular machinery necessary to perform a broad spectrum of functions. As part of the innate system, NK cells can immediately react to an infectious insult via fixed, germ-line encoded receptors that recognize pathogen-associated motifs. As the infection progresses, NK cells adapt, modulating the level of inflammatory mediators produced during the initial steps of infection. Finally, as adaptive responders, NK cells show clonal expansion, maintenance, and memory of previous insults in the case of reinfection.

Nevertheless, several questions remain: do NK cells recognize infections other than MCMV with their Ly49 repertoire of receptors? Can triggering of other NK receptors by MCMV-infected cells induce the same NK cell outcomes as in the Ly49H-m157 model (e.g., clonal expansion, memory, etc.)? What of other pathogens or different host species? Why does the host express so many different NK receptors targeting MCVM, several with opposite effects on NK cell activity (i.e., inhibitory versus activating)? Does the host benefit from the infection? If so, how? The possible paths of investigation are plentiful. As more inquiries are resolved, which is currently the case in the thriving field of NK cells in cancer, we will be able to harness the power of these lymphocytes in the treatment of infectious diseases [68, 69].

Acknowledgments

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