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All is fair in virus-host interactions: NK cells and cytomegalovirus

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Abstract

The infection of mice with mouse cytomegalovirus (MCMV) as a model of human cytomegalovirus (HCMV) infection has been particularly informative in elucidating the role of innate and adaptive immune response mechanisms during infection. Millions of years of co-evolution between cytomegaloviruses (CMV) and their hosts has resulted in numerous attempts to overwhelm each other. CMVs devote many genes to modulating the host NK cell response and NK cells employ many strategies to cope with CMV infection. While focusing on these attack-counterattack measures, this review will discuss several novel mechanisms of immune evasion by MCMV, the role of Ly49 receptors in mediating resistance to MCMV, and the impact of the initial NK cell response on the shaping of adaptive immunity.

Natural killer cells and cytomegalovirus

Natural killer (NK) cells are essential in the control of various viral infections. They are among the first cells to sense the release of proinflammatory cytokines and decreased expression of MHC class I (MHC I) proteins on the surface of infected cells. NK cell activation depends on the integration of signals from activating and inhibitory NK cell receptors. MHC I molecules engage inhibitory NK receptors, including the KIR receptors in humans and the Ly49 receptors in rodents [1] (Box 1). Upon engagement, the target cell is recognized as self, thus preventing the lysis of an uninfected cell. Recognition of MHC I molecules is also required during the development of NK cells for them to achieve functional competence (for reviews see [2-6]). When surface expression of MHC I molecules is compromised, as it frequently is during a viral insult, inhibition signals are overridden by activating signals. As a result, the target cell is lysed via the ‘missing-self’ mechanism [7]. However, NK cells not only recognize infected cells that express a low level of MHC class I molecules but also cells that express cellular ligands for activating receptors, which are induced during infection [8]. Contrary to their inhibitory counterparts, only a small number of self-ligands have been described for activating Ly49 (KIR) receptors. In addition, NK cells can directly sense viral pathogens by recognizing and engaging specific viral proteins [9]. Curiously, a large proportion of activating Ly49 receptors recognize cells infected by mouse cytomegalovirus (MCMV), either directly or in an MHC class I-dependent manner [10]. Human cytomegalovirus (HCMV) is a clinically relevant pathogen

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[11]. An immunocompetent host is able to cope with HCMV infection, but the virus can cause severe damage to the host when immune control is impaired. Species specificity of HCMV precludes its study using animal models. This is the main reason why MCMV infection is the most intensively studied animal model for elucidating the NK cell response to viruses as well as the ongoing co-evolution of viruses and their hosts [12]. In that respect, it has been shown that the murine natural killer gene complex (NKC) encoding the Ly49 family of NK receptors controls the susceptibility of mice to MCMV infections [13]. In a similar manner to HCMV, MCMV is characterized by broad cell tropism and the ability to establish lifelong latency from which reactivation may occur. The hallmark of all cytomegaloviruses is the presence of a large group of immune evasion genes that target the host immune response. Various viral functions have evolved to counter the NK cell response, illustrating the evolutionary interplay between viruses and NK cells [14]. Despite the remarkable increase in our knowledge concerning the relationship between NK cells and CMVs, many questions remain to be answered.

Box 1

The Ly49 receptor family

Ly49 receptors belong to a family of polymorphic and polygenic receptors clustered in the natural killer complex (NKC) on mouse chromosome 6. Their structure classifies them as part of the type II transmembrane, C-type-lectin-like receptors. While their ectodomain is responsible for their specificity, their intracellular portion defines them as inhibitory or activating [66]. While inhibitory Ly49 receptors possess an immunoreceptor tyrosine-based inhibition motif (ITIM) in their cytoplasmic tail, activating receptors have a charged amino acid in their transmembrane domain, allowing them association with immunoreceptor tyrosine-based activation motif (ITAM) bearing adaptor protein DAP12. An activating signal leads to a signalling cascade that ends with cytoskeletal reorganization, release of cytolytic granules, enhanced expression of cytokines and chemokines, and NK cell proliferation [67]. However, during homeostasis this signal is usually inhibited by the dephosphorylation of ITAM residues that results from the triggering of the inhibitory receptor by its natural ligand, MHC I molecules. Expression of Ly49 receptors is stochastic and occurs primarily on NK cells, but is also observed on small portions of monocytes, macrophages, dendritic cells and T cells [68, 69]. In humans, functional homologs of Ly49 receptors belong to an immunoglobulin superfamily, named the killer-cell immunoglobulin-like receptors (KIR) [70]. The complete genomic sequence of four *Ly49* haplotypes has been determined to date. Although many inhibitory receptors are shared among different haplotypes (e.g. Ly49A, Ly49C, Ly49G2, and Ly49I), their contribution in inhibitory signaling is different with regards to MHC I haplotype-restricted education imposed during NK cell development. As opposed to their inhibitory counterparts, for the majority of activating Ly49 receptors no self-MHC I ligands have been described (Figure I to Box 1). Interestingly, most of them are directly or indirectly specific for MCMV proteins, suggesting an important evolutionary relationship with this virus. Information learned from studying the MCMV nicely supports the theory that activating Ly49 receptors evolved from inhibitory ones. Namely, it is suggested that the activating Ly49s were generated from the inhibitory receptors first by aquiring charged residue in the transmembrane region followed by a loss-of-function mutation in the ITIM motif which turns tyrosine into phenylalanine and possibly some recombination in the extracellular portion of the genes. This is supported in the model of evolution of KIR receptors where activating KIRs were generated first by STOP mutations in the cytoplasmic tail that truncated the ITIM motif, then by mutations ensuring the appearance of the charged amino acid in the transmembrane domain and finally, gene conversions and mutations resulting in the changed specificity of their extracellular domains [71]. The number of activating genes vary a great deal among

strains, from one (*Ly49l*) in BALB/c to seven (*Ly49d, u, p_3, p_1, w, m, h*) in NOD mice. The C57BL/6 (B6) haplotype includes two activating receptors (*Ly49d, h*), whereas 129/J mice encode three activating receptors (*Ly49r, u, p*) [72]. Our understanding of the interaction between Ly49 receptors with MCMV products gained from the use of reporter cells which on their surface expressed the ectodomain of the desired receptor which upon ligation initiated the signalling cascade leading to a final measurable product, i.e. GFP, IL-2, IFNγ, β-galactosidase [15, 22].

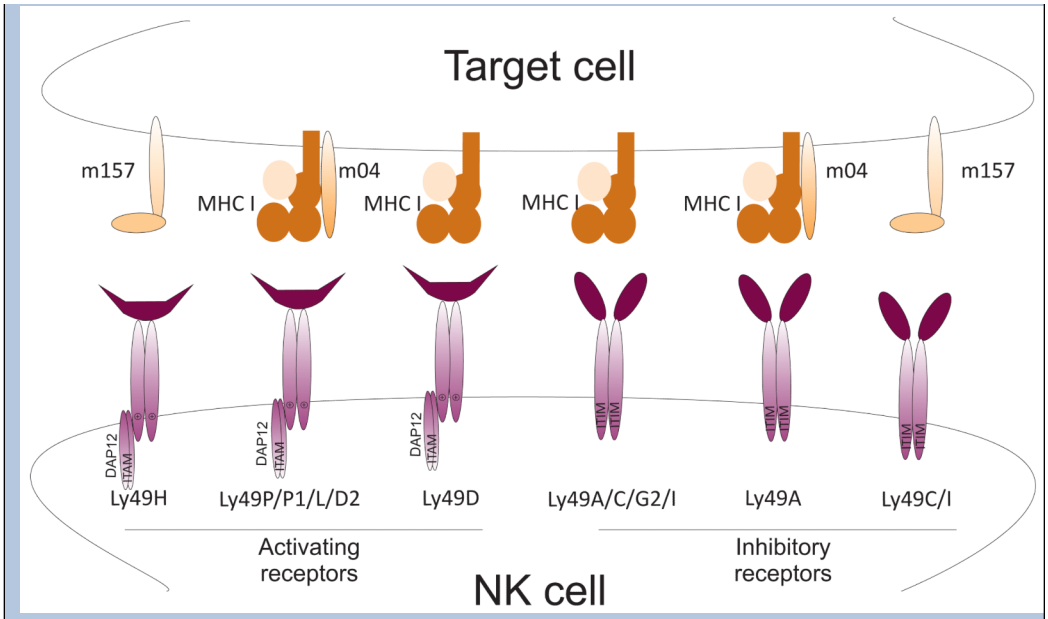

Figure I.

This review addresses various aspects of the relationship between NK cells and MCMV including: (i) the role of activating and inhibitory NK cell receptors in virus control, (ii) the interactions of MCMV immunoevasins with NK cell receptors and their ligands, and (iii) the impact of NK cells and viral immunoevasins on the adaptive immune response.

Recognition of MCMV infected cells by activating NK cell receptors

Despite a plethora of immune evasion strategies evolved in MCMV to avoid recognition by NK cells, a few mouse strains show a natural resistance to MCMV infection (reviewed in [10]). The most thoroughly studied example is the C57BL/6 mouse strain that expresses an activating Ly49H receptor on NK cells. This receptor directly interacts with MCMV-derived m157, an MHC class I-like protein expressed on the surface of infected cells during the early phase of infection. In turn, this leads to a direct recognition and elimination of infected cells via cytolytic mechanisms that include the secretion of perforin and granzyme B molecules [15-17]. The importance of the Ly49H-m157 axis was further supported by the insertion of the *Ly49h* gene into FVB mice and backcrossing them with a BALB/c background, rendering BALB/c mice which would otherwise be MCMV-sensitive, resistant to MCMV [18]. In addition, by backcrossing the BXD-8 mouse strain, which bears a natural mutation in the *Ly49h* gene, to a C57BL/6 background (B6.BXD8-Klra8$^{(Cmv1\text{-}del)}$/Wum or B6.BXD8TyJ-*Ly49h*$^{-/-}$) it was shown that the absence of Ly49H rendered the naturally resistant C57BL/6 mice susceptible to infection [19, 20]. Additionally, the importance of the Ly49H-m157 axis was demonstrated by infecting C57BL/6 mice with an MCMV mutant lacking *m157*, which prevented these mice from mounting a proper NK cell response [21].

Another principle of NK cell mediated resistance to MCMV has been described in MA/My mice. NK cells from these mice bear Ly49P, another activating NK cell receptor [22]. Ly49P-mediated recognition of infected cells requires the ligation of a molecular complex composed of an H-2D^{k} molecule together with an MCMV-derived m04 glycoprotein [23]. Unlike two other MCMV proteins that target MHC class I molecules, m04 does not downregulate cell surface expression of MHC class I. Instead it forms complexes with these molecules which travel to the cell surface to engage Ly49 receptors [24, 25]. Experiments

indicate that the m04/MHC class I complex is necessary but not sufficient for the recognition of infected cells by Ly49P and that additional viral factor(s) is/are required [23]. However, m04 also regulates the function of inhibitory Ly49 by rescuing cell surface MHC class I levels, which are able to engage these receptors (see below).

In addition to Ly49P, three other activating Ly49 receptors were linked to specific recognition of MCMV infected cells in an m04-dependent manner. These are Ly49L, Ly49P1 and Ly49D2, which were described in BALB/c (BALB.K and BALB.F), NOD/Ltj and PWK/Pas mice, respectively [26]. All these receptors have been shown to recognize a complex comprised of an MCMV m04 glycoprotein and an H-2 molecule (H-2D$^{d\ (k,\ f)}$, H-2D^{k} and H-2D^{k}, respectively). A recent report by Pyzik and colleagues specifically demonstrated that, compared with BALB/c and BALB.B mice, the resistance of BALB.K mice to MCMV and the faster viral clearance from organs is correlated with specific proliferation of Ly49L^{+} NK cells and their capacity to produce IFN-γ upon infection with WT MCMV [26]. Interestingly, unlike the Ly49H/m157 interaction in C57BL/6 mice, which leads to an immediate activation and proliferation of NK cells, in MCMV infected BALB.K mice inhibitory signals prevail during the first few days post-infection (Figure 1a). However, later during infection, Ly49L^{+} NK cells proliferate and increase the expression of these activating receptors, leading to virus control (Figure 1b).

What is the rationale behind having so many MCMV-specific NK cell receptors? Are they a product of a specific selective pressure imposed by MCMV as a relevant mouse pathogen? The most plausible explanation for the existence of several MCMV-specific activating Ly49 receptors is the host reaction to viral evasion of NK cells, as discussed below.

MCMV regulation of NK cell response by ligation of inhibitory receptors

In addition to Ly49H, MCMV m157 also serves as a ligand for the inhibitory Ly49I receptor in a strain specific manner. Although expressed in both C57BL/6 and 129/J mice, only the 129/J allele recognizes the m157 protein [27]. The meaning of this interaction has not been clarified until now, but it has been postulated that MCMV encodes proteins that can ligate inhibitory NK cell receptors to avoid recognition via the ‘missing self’ mechanism. To prevent antigen presentation and recognition by CD8^{+} T cells both MCMV and HCMV downmodulate the surface expression of MHC class I molecules [28]. This feature should render the cells sensitive to the ‘missing self’ mediated recognition by NK cells. However, in mice bearing NKC of BALB origin this does not occur, rendering them prone to infection. The explanation for this observation is that MCMV developed additional evasion strategies by encoding protein(s) which are able to restore the ‘self’ signature. Indeed, it has recently been reported on a novel mechanism employed by MCMV in avoiding the NK cell response [25]. It has been shown that despite a strong downregulation of MHC class I molecules, as a consequence of a WT MCMV infection, a sufficient amount of MHC class I molecules complexed with MCMV m04 glycoprotein reaches the cell surface and serves to ligate the inhibitory Ly49A NK cell receptor [25] (Figure 2a). It has also been demonstrated that m04 can antagonize the function of m152 and enable MHC I molecules to evade a complete downregulation from the surface of infected cells. By doing so, the m04 protein allows the engagement of inhibitory Ly49 receptors, thereby preventing NK cell activation via the ‘missing self’ mechanism. This interaction leads to an inhibited proliferation of NK cells and correlates with the inability of mice to control viral infection *in vivo* (Figure 2b). In the same report it was demonstrated that there is a potent role for an NK cell subset, bearing another inhibitory receptor, Ly49G2, in controlling the replication of an MCMV mutant lacking m04 (Δ*m04* MCMV). The role of inhibitory Ly49G2 receptor has also been outlined in a recent report by Xie and colleagues [29]. They have shown that in C57L.M-H2^{k} mice, the Ly49G2^{+} NK cell subset specifically expands in response to MCMV infection, acquires

cytolytic functions, and mediates the resistance to infection. Notably, it appears that the Ly49G2$^+$ NK cell subset specifically proliferates upon several other infections, as well, and is the first NK cell subset appearing upon hematopoietic stem cell transplantation [30-32]. Altogether, these findings outline the importance of inhibitory Ly49 receptors in controlling the early cytomegalovirus infection. It is likely that the expression of Ly49G2 on the NK cell subset is linked to another function important in virus control. These cells may be more permissive to cytokine signaling, leading to the induced expression of receptors involved in NK cell activation. Alternatively, inhibitory receptors may be important in forming stable immunological synapses and by doing so improve the signaling through the activating receptors.

Inhibitory Ly49 receptors can also be found on NKT and memory CD8$^+$ T cells [33]. There are no current studies emphasizing the interaction of MCMV with inhibitory receptors on CD8$^+$ T cells. MCMV expresses many MHC I-like molecules but their function is so far mostly unknown [34]. These viral proteins may bind inhibitory receptors expressed not only on NK cells but also on T cells and therefore, regulate their function. As mentioned above, the m157 protein also serves as a ligand for the inhibitory Ly49I receptor. A recent report by Corbett and colleagues reported on numerous variants of the *m157* gene isolated from wild type variants of MCMV [35]. This finding is important because these variants could have proteins which differ in their specificity for binding to the activating Ly49H and inhibitory Ly49I and Ly49C receptors compared to the m157 encoded by the Smith strain of MCMV. This is additionally supported by the fact that viral isolates bearing non-Smith *m157* replicate to high titers in otherwise resistant C57BL/6 mice. Another aspect of this study highlights the fact that, in the case of an m157 variant that binds to both activating Ly49H and inhibitory Ly49C on the very same NK cell, the outcome is the activation of NK cells. However, this contradicts the current paradigm that inhibitory signals mediated through Ly49 receptors override the activation signals and block NK cell functions. Additional efforts to study the strength of the interactions between NK receptors and m157 variants might explain the mechanism behind this observation.

MCMV evasion of NKG2D-dependent NK cell activation

NKG2D (natural killer group 2D) is a potent activating receptor expressed by cells of innate and adaptive immunity. It functions exclusively as an activating receptor that recognizes cell surface molecules structurally related to MHC class I proteins induced by infection or other type of cellular stress [36]. Ligation of NKG2D induces a strong signal that can override inhibitory signaling to promote NK cell-mediated cytolysis and cytokine release. NKG2D is also expressed on T cells. Because of the role the NKG2D receptor plays in controlling both NK- and T cell-mediated immunity, it is of tremendous importance to understand how and why NKG2D ligands are regulated upon viral infection. Four MCMV proteins are involved in the down-modulation of NKG2D ligand expression. The MCMV proteins m145, m152 and m155 interfere with the expression of all known NKG2D ligands, MULT-1, the RAE-1 family members and H60, respectively, whereas m138 affects the expression of MULT-1, H60 and RAE-1ε (reviewed in [12]). However, RAE-1 proteins differ in their susceptibility to down-regulation by MCMV. In contrast to RAE-1α, representing the sensitive isoform, surface resident RAE-1δ remains present on MCMV-infected cells. Although co-precipitation studies failed to demonstrate a physical interaction between m152 and RAE-1 protein, by using purified extracellular domains of RAE-1 and m152 in complex biochemical studies, which included measurement of binding by chromatography, analytical ultracentrifugation, and isothermal titration calorimetry, Zhi et al. confirmed a direct interaction of these proteins [37]. The binding affinity varies for different RAE-1 isoforms and corresponds to the differential susceptibility to downregulation by m152. Because the luminal domain of m152 is involved in the retention of MHC class I molecules in the

ERGIC (endoplasmic reticulum-golgi intermediate compartment) [38], it is not surprising that RAE-1 molecules and MHC I are similarly regulated by m152 [39]. The resistance of the mature form of RAE-1δ to m152 is associated with a lack of the PLWY sequence motif, which is expressed in the RAE-1αβγ proteins [39]. The deletion of the PLWY motif in RAE-1β caused a 10-fold decrease in its binding affinity to m152, suggesting that the PLWY motif has a key role in the interaction between m152 and RAE-1β [37]. Interestingly, deleting the PLWY motif from RAE-1γ or its insertion into the sequence of RAE-1δ does not influence their respective affinities for m152, suggesting that other differences contribute to the interaction between RAE-1 and m152. While m152 affects the maturation of newly synthesized RAE-1ε molecules, the surface-resident portion of RAE-1ε is downregulated by m138, which facilitates its endocytosis via a dynamin dependent mechanism [40].

The importance of viral regulation of the NKG2D signaling pathway is further illustrated by the fact that several herpesviruses, including HCMV, use microRNA (miRNA) to regulate the expression of NKG2D ligands [41, 42]. Compared to the effect of immunoevasion proteins, the effects of a single miRNA on the reduction of target protein production is 2- to 3-fold lower [43, 44]. Therefore, it has been hypothesized that viral miRNAs may play a role in fine tuning the interaction between the virus and the host, particularly during latency because preexisting target proteins must be degraded before any effect of viral miRNAs can be observed [45]. HCMV encodes at least 11 miRNAs. Among them, the best characterized is miR-UL112 which targets MICB [41]. Interestingly, the target site of this viral miRNA is conserved among different MICB alleles and a similar site exists in the MICA 3′ untranslated region. The same site is also targeted by cellular miRNAs, suggesting that HCMV uses a cellular mechanism to regulate the expression of this NKG2D ligand [46]. In mouse fibroblasts lytically infected with MCMV, 18 viral miRNAs were characterized [47, 48]. Moreover, at 72 h post-infection viral miRNAs contribute to more than 60% of the total miRNA pool. It has been shown that some MCMV miRNAs are post-transcriptionally regulated and that MCMV infection modulates cellular miRNAs profiles [49]. Although the levels of most cellular miRNAs are only modestly altered, miR-27, which exerts antiviral activity against MCMV, is downregulated in MCMV infected cells. Recently, it has been found that the same cellular miRNA, miR-27, is downregulated during infection with *Herpesvirus saimiri* by an interaction with viral small non-coding RNAs [50]. Dolken et al. reported the first functional *in vivo* phenotype of MCMV lacking two miRNAs, miR-M23-2 and miR-m21-1 [51]. The mutant virus was specifically attenuated in salivary glands during subacute infection, an organ essential for virus persistence and spread from host-to-host. Attenuation was abolished by a combined depletion of NK and $CD4^+$ T cells suggesting that miRNA-based immunoevasion strategy may be important for virus persistence. Although studies have thus far failed to reveal MCMV miRNA that are involved in the regulation of mouse NKG2D ligands, their existence is very likely.

The role of NK cells in shaping of adaptive immune response

Several recent studies demonstrated that NK cells strongly regulate viral infections through interactions with dendritic cells (DC). NK-DC ‘crosstalk’ can enhance NK cytotoxicity and proliferation, maintain splenic DC subsets after virus infection, and stimulate DC maturation and antigen presenting functions [52]. The impact of NK cells on the CD8 T cell response is an area of research that is being intensively studied (Figure 3). Robbins and colleagues have shown that strong NK cell activation during MCMV infection accelerates the CD8 T cell response *in vivo* by limiting the production of IFNα/β by plasmacytoid dendritic cells (pDC) to levels not immunosuppresive to the host (Figure 3a) [53]. According to this scenario, the activating axis of m157-Ly49H protects splenic conventional DCs (cDCs) and causes a prompt induction of the CD8 T cell response. These questions have been further assessed by

a selective depletion of pDCs which initially resulted in reduced IFNα production and enhanced viral replication followed by a specific proliferation of Ly49H$^+$ NK cells [54]. Although the authors saw no impact of pDC depletion on the subsequent MCMV specific adaptive immune response, the CD8 T cell response to VSV was severely impaired. Additional data has been obtained by Andrews and colleagues, who reported that a strong NK cell response limits the long-term efficacy of virus specific CD8 and CD4 T cell response and that this effect is mediated by the control of the frequency and duration of DCs infection (Figure 3b) [55]. Although the ultimate mechanism for this finding remained unclear, Lee and colleagues provided evidence suggesting that IL-10 secreted by Ly49H$^+$ NK cells can suppress CD8 T cell response during MCMV infection of perforin deficient C57BL/6 mice [56]. In another model of NK cell activation it has been shown that a strong NK cell response can improve, rather than diminish, the CD8$^+$ T cell response [57]. Despite the strong NK cell mediated attenuation, MCMV engineered to express the NKG2D ligand RAE-1γ (*RAE-1γ*MCMV) elicit a strong and long-lasting CD8 response in BALB/c mice, providing protection against a lethal MCMV challenge. *RAE-1γ*MCMV infection preserves the frequency of cDCs compared to the WT virus (Figure 3c). Notably, infection with *RAE-1γ*MCMV enhanced the CD8 T cell response even in C57BL/6 mice [57]. MHC I-specific inhibitory NK cell receptors may also influence the CD8$^+$ T cell response. We have recently shown that NK cell activation via the 'missing self' mechanism plays an important role in the early virus control (see above and ref. [25]). Indeed, it has been shown that MHC I H-2D^k, a cognate ligand for the Ly49G2 NK cell receptor, is essential for the NK control of MCMV infection [29]. This function is achieved through licensed Ly49G2$^+$ NK cells, which enable a faster recovery of cDC, after an initial drop caused by MCMV infection, eventually leading to an enhanced CD8$^+$ T cell response (Figure 3d) [58]. Altogether, the data collected thus far indicate that the impact of NK cells on the subsequent dynamics and overall quality of an adaptive immune response depends on the context of infection and NK cell activation, suggesting that there is no unique pattern in NK cell regulation of the adaptive immune response.

NK cells can memorize MCMV

Although originally described as part of the innate immune system due to the lack of receptor gene rearrangement, several reports have analyzed the ability of NK cells to acquire features of the adaptive immune system. These findings demonstrate that NK cells (i) experience a selective education process during development, (ii) undergo a clonal-like expansion during virus infection, (iii) generate a long-lived memory cells, and (iv) mediate a more efficient secondary response against previously encountered molecular structures [59, 60]. Although the ability of NK cells to memorize information and subsequently respond more robustly to the same antigen has been demonstrated in various models [61-63], MCMV infection proved to be extremely informative in the study of NK cell memory. Sun and colleagues demonstrated that, similar to T cells, NK cells sensitized by Ly49H interaction respond by dramatically increasing the proliferation capacity after encountering cells infected with MCMV [64]. Likewise, these cells were more protective after an adoptive cell transfer into syngeneic newborn recipients. Adoptively transferred immune NK cells appear to be a long-lived self-renewing population undergoing homeostatic expansion many months after the initial transfer and are able to respond robustly to viral challenge [65].

Despite the fact that the existence and function of memory NK cells has been well documented, their molecular signature is still unknown. Thus far, MCMV-specific memory NK cells have been described in mice expressing Ly49H, and their memory depends on the recognition of a specific viral protein, m157 [64]. MCMV is a potent inducer of various stress ligands including the ones for NKG2D. One can pose the question whether NK cell

activation via a different mechanism other than the Ly49H-m157 interaction would also lead to a generation of MCMV specific memory cells.

Concluding remarks

MCMV possesses different strategies aimed at preventing the activation of NK cells through the modulation of ligands for the activating and the engagement of inhibitory NK cell receptors. Consequently, the selective pressure imposed by the virus resulted in the generation of a plethora of host mechanisms aimed at counteracting the viral evasion of NK cells. In addition to Ly49H, several other activating Ly49 receptors are specific for MCMV infected cells. The interaction between NK cell receptors and their viral regulators are also important for the outcome of the adaptive immune response and are therefore, important for the pathogenesis of chronic virus infection. From our perspective, the precise role of NK cell signaling in the modulation of the adaptive immune response represents the single most important question that remains to be clarified experimentally. In this respect, we are still in the dark regarding the rationale for the functional redundancy of viral immunoevasins and the existence of viral proteins serving as ligands for the activating NK cell receptors. A more detailed understanding of the above mentioned viral and cellular mechanisms involved in the shaping of the NK cell response could result in new therapeutic targets and the development of novel vaccine protocols targeted against viral infections and tumors.

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Glossary

BALB/c	a commonly used inbred laboratory mouse strain, with albino appearance. These mice the bear H-2^d MHC I haplotype and are highly susceptible to MCMV infection
BALB.K	shares the same genetic background as BALB/c and differs only in MHC I haplotype, which is H-2^k in case of BALB.K mice
C57BL/6 (B6)	a commonly used inbred laboratory mouse strain that bears the H-2^b MHC I haplotype. These mice are resistant to MCMV infection due to recognition of the virus protein m157 by the Ly49H activating NK cell receptor
MA/My	a laboratory mouse strain resistant to MCMV infection. Resistance is dependent on Ly49P-mediated recognition of m04/MHC I complexes on infected cells

NOD/Ltj	mouse strain prone to spontaneous development of autoimmune (type 1) insulin dependent diabetes mellitus. This strain has a unique MHC I haplotype H-2^{g7}
PWK/Pas	wild-derived mouse strain resistant to MCMV infection independent of m157-Ly49H interaction. A *Cmv4* gene locus provides an additional NK cell dependent viral-resistance mechanism
129/J	mice of H-2^{b} MHC I haplotype. They do not express Ly49H but do express Ly49I, an inhibitory receptor that also recognizes MCMV m157
syngeneic or syngenic recipients	recipients that are genetically identical to donors, allowing for transplantation of cells or tissues
H-2 (Histocompatibility-2)	the mouse equivalent to the human HLA system
dendritic cells (DC)	antigen presenting cells (APC) which are important for linking innate immunity to adaptive immunity
conventional DCs (cDC)	common $CD11c^{+}$ APCs that directly activate both helper and cytotoxic T cells, mostly by secretion of IL-12 and through direct interaction via surface expressed CD40, CD70 and CD80/86
plasmacytoid dendritic cells (pDC)	In mice these cells express CD11c, B220, BST-2 (mPDCA) and Siglec-H but are negative for CD11b. Upon stimulation and subsequent activation, they produce large amounts of type I interferon (IFN-α/β)
$CD8^{+}$ T cells (cytotoxic T cells, CTL)	a lymphocyte subset capable of killing virus-infected cells or tumor cells. These cells express T-cell receptor (TCR) which specifically recognizes peptides presented by MHC I molecules and require co-stimulatory signal for activation
IFN-γ	a soluble dimerized cytokine that is secreted by activated helper and cytolytic T cells, as well as NK cells. It has antiviral, immunoregulatory and anti-tumor properties
granzyme B	a serine protease secreted by cytotoxic T cells and NK cells as part of cytoplasmic granules. In complex with granulysin and perforin it enters a virus-infected cell via mannose-6-phosphate receptor and induces caspase-dependent apoptosis
perforin	a cytolytic protein contained in cytolytic granules released by CTLs and NK cells. It forms pores in the cell membrane allowing entry of granzyme B into the cell
Smith strain MCMV	commonly used laboratory strain of MCMV, originally isolated by Margareth Smith in 1954
Immunoevasins	viral genes and their products devoted to subversion of the host immune system
m04/gp34	a MCMV gene (*m04*) and its product (gp34) that were initially described to complex with MHC I molecules

H60	an NKG2D receptor ligand that is primarily expressed on tumor cell lines and is negatively regulated by MCMV m155 and m138
MULT-1	an NKG2D receptor ligand that is primarily expressed on tumor cell lines and is negatively regulated by MCMV m145 and m138
RAE-1αβγδε	stress induced family of ligands for the NKG2D receptor that are primarily expressed on tumor cell lines. RAE-1αβγ isoforms are subject to downregulation by MCMV m152 while RAE-1ε is negatively regulated by m138

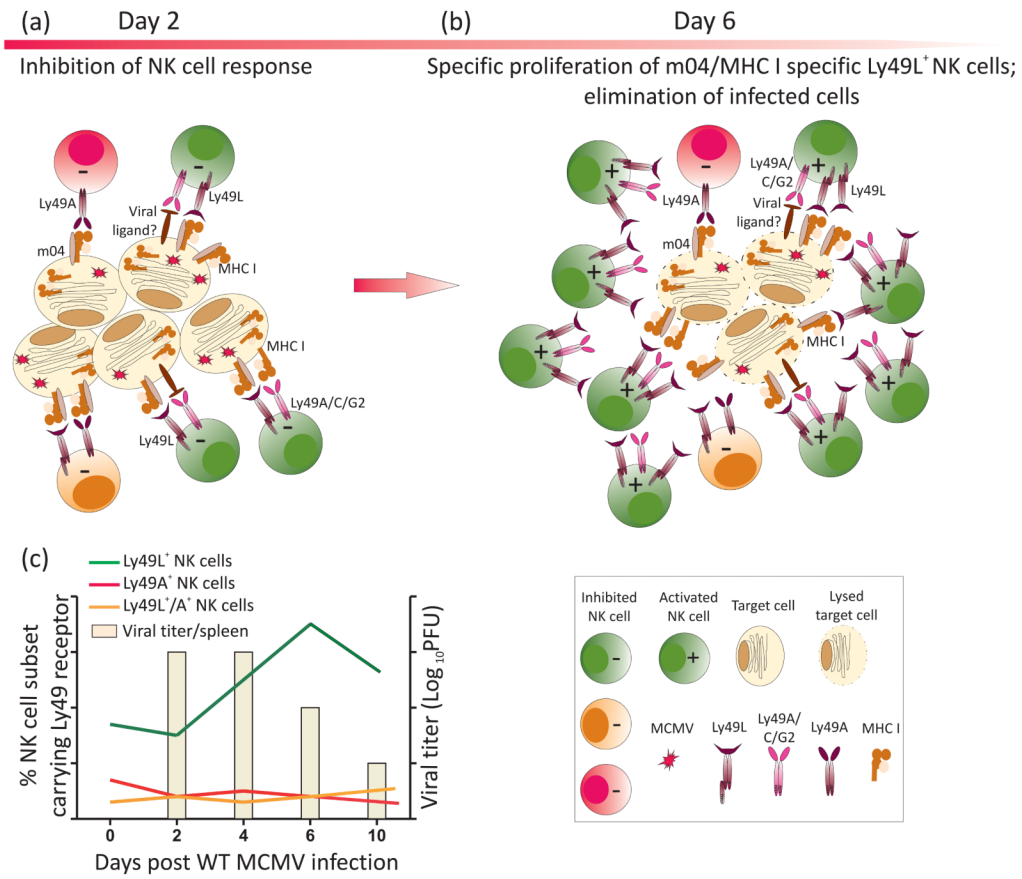

Figure 1. Specific proliferation of Ly49L$^+$ NK cells correlates with virus clearance in BALB.K mice

(a) At early time points post infection (day 2), natural killer (NK) cells failed to control mouse cytomegalovirus (MCMV) infection in spite of the fact that in addition to inhibitory Ly49A receptor activating Ly49L receptor can specifically recognize infected cells via the same ligand, the H-2D^k/m04 complex. In addition, inhibitory receptors might be engaged by viral ligands, therefore ensuring the dominance of inhibition (top, left). **(b)** During the course of infection (day 6) NK cells expressing Ly49L specifically proliferate and concurrently increase the density of Ly49L receptor on their surface. As a consequence, activation signals prevail, resulting in the containment of viral infection at later time points (top, right). **(c)** A schematic representation of these events is depicted in the left lower panel.

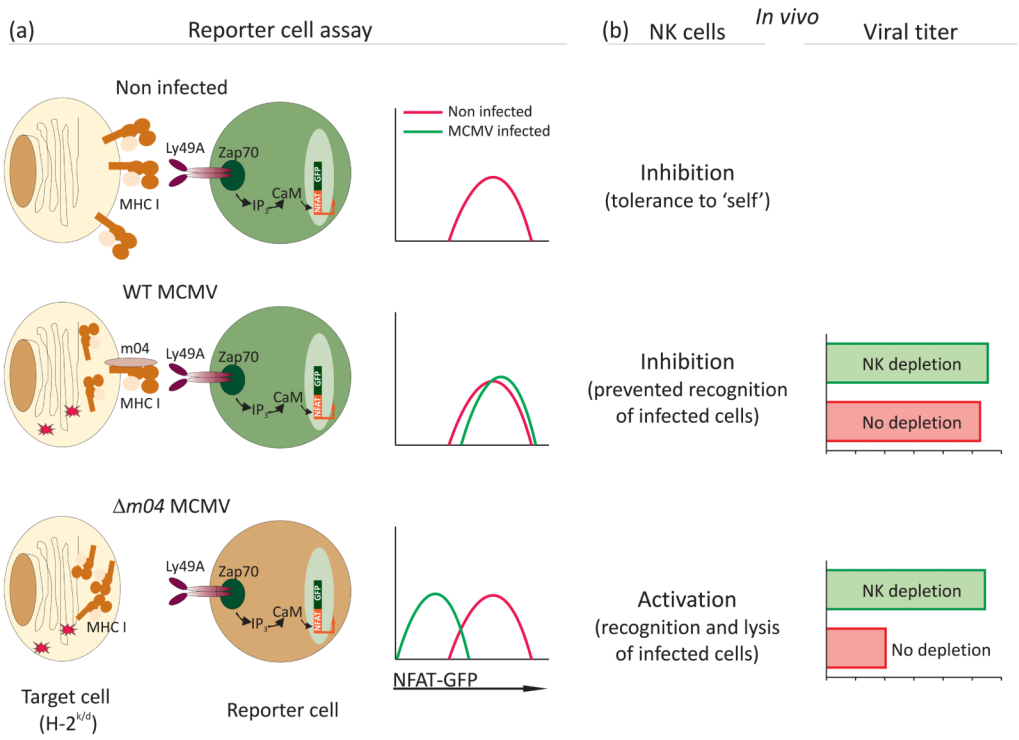

Figure 2. MCMV escapes 'missing self' dependent NK cell control

Schematic representation of the results obtained by a reporter cell assay **(a)** and natural killer (NK) cell dependent virus control *in vivo* **(b)**. MHC I expressed on target cells ligates inhibitory Ly49A receptor on reporter cells resulting in their activation as measured by GFP expression. This corresponds to *in vivo* tolerance of NK cells to normal cells (top). Although the infection with WT MCMV results in the downregulation of MHC I, this does not prevent the activation of Ly49A reporter cells due to the fact that *m04* encoded protein rescues enough surface MHC I to restore the engagement of the receptor. This correlates with the inhibition of NK cell response *in vivo* and the inability of mice to control the virus in a NK cell dependent manner (middle). In the absence of m04 (infection with Δ*m04* MCMV) the downmodulation of MHC I is more efficient, resulting in an inability of infected cells to engage the Ly49A receptor. As a consequence, NK cells are activated in a 'missing self' dependent manner and control the virus (bottom).

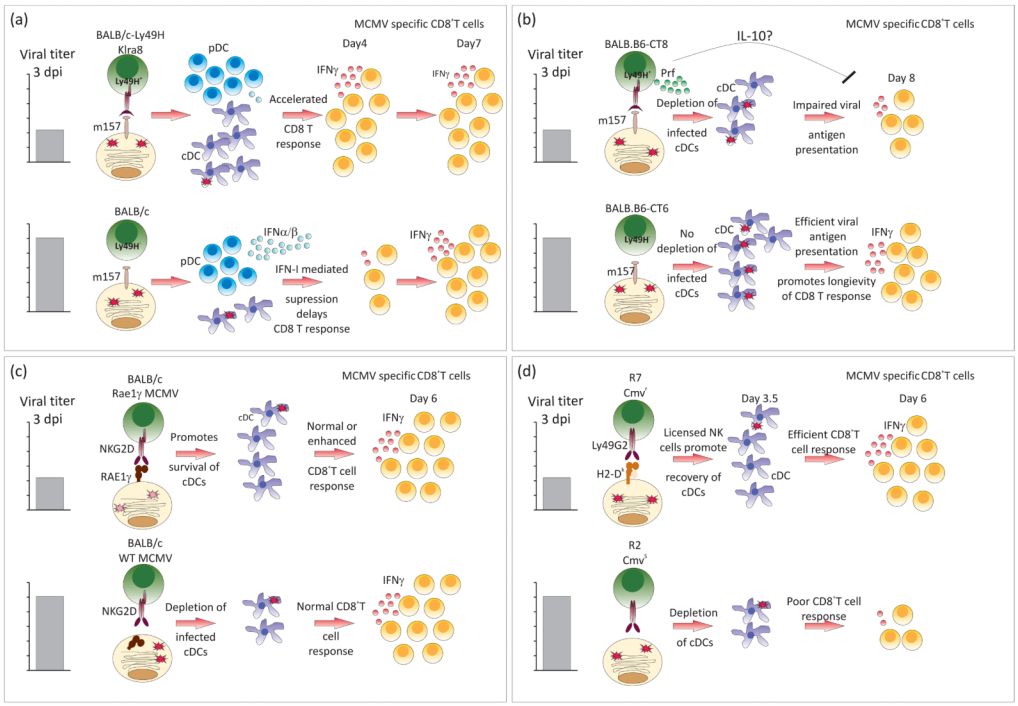

Figure 3. Various models used in studying the impact of NK cells on shaping the adaptive immune response to MCMV

(a) In Klra8 mice (C.B6-*Klra8*$^{Cmv1\text{-}r}$/UwaJ; these are BALB/c congenic mice carrying the C57BL/6 Natural killer cell gene complex, and are thus positive for NK1.1 and Ly49H and resistant to MCMV (mouse cytomegalovirus) [73]) Ly49H-m157 interactions not only result in an efficient virus control at day 3 post infection but also in an accelerated CD8 T cell response (top). By contrast, in normal BALB/c mice (which are Ly49H$^-$) a high virus load induces a surplus of type I IFN (interferon) which affects the kinetics of the CD8$^+$ T cell response (bottom) [53]. **(b)** In BALB.B6-CT8 mice (a BALB/c congenic mouse strain expressing NK1.1 and Ly49H and resistant to MCMV [73]) the Ly49H-m157 interaction results not only in efficient virus control but also in the depletion of infected cDCs, resulting in impaired antigen presentation and CD8$^+$ T cell response. The CD8$^+$ T cell response may be further suppressed by IL-10 derived from Ly49H$^+$ NK cells (top). In BALB.B6-CT6 mice (a BALB/c congenic mouse strain expressing NK1.1 but not Ly49H$^-$ that is sensitive to MCMV [73]) NK cells fail to control the virus but do preserve cDCs from depletion. This results in a more robust and prolonged CD8 T cell response (bottom) [55, 56]. **(c)** MCMV expressing the NKG2D ligand Rae1γ is efficiently controlled in BALB/c mice, which are otherwise sensitive to MCMV infection. This promotes the survival of cDCs and helps in establishing a level of CD8 T cell response comparable to the one in normal BALB/c mice, which cannot control the virus at early time points post infection [57]. **(d)** NK cells expressing Ly49G2 are efficiently licensed in R7 mice (C57L.M-*H2*^{k} – a recombinant congenic strain expressing H-2D from MA/My mice; resistant to MCMV [29]) and can mediate resistance to MCMV. This promotes a faster recovery of cDCs and an efficient CD8 response (top). In contrast, R2 mice (C57L.M-*H2*^{k} – a recombinant congenic strain expressing H-2D from C57L mice; sensitive to MCMV [29]), lacking the appropriate education ligand for Ly49G2, H-2D^k, fail to mount a proper NK cell response and thus are unable to control the virus, resulting in depletion of cDCs and an affected CD8 T cell response (bottom) [58].