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REVIEW

MCMV avoidance of recognition and control by NK cells

Ilija Brizić · Tihana Lenac Roviš · Astrid Krmpotić · Stipan Jonjić

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Abstract Natural killer (NK) cells play an important role in virus control during infection. Many viruses have developed mechanisms for subversion of NK cell responses. Murine cytomegalovirus (MCMV) is exceptionally successful in avoiding NK cell control. Here, we summarize the major MCMV evasion mechanisms targeting NK cell functions and their role in viral pathogenesis. The mechanisms by which NK cells regulate $CD8^+$ T cell response, particularly with respect to the role of NK cell receptors recognizing viral antigens, are discussed. In addition, we discuss the role of NK cell receptors in generation and maintenance of memory NK cells. Final part of this review illustrates how the NK cell response and its viral regulation can be exploited in designing recombinant viral vectors able to induce robust and protective $CD8^+$ T cell response.

Keywords MCMV · NK cell receptors · Immune evasion · Memory NK cells · CMV vaccine vector · NK cell - T cell crosstalk

Introduction

Natural killer (NK) cells are innate immune cells primarily known for their function in immune surveillance of viral infections and tumors. NK cells also have an important role in shaping the adaptive immune response through various mechanisms including crosstalk with other cells of innate and adaptive immunity. The importance of NK cells in control of herpes viruses, including cytomegalovirus (CMV), is best illustrated by the fact that individuals possessing NK cell deficiencies are more susceptible to these viruses [1]. Studies of NK cell control of CMV infection revealed some of the key features of NK cell biology. The mouse model of CMV infection with murine CMV (MCMV) has been particularly informative. Similar to NK cell deficient humans, NK cell-deficient mice are more susceptible to MCMV infection and show impaired control of virus replication [2]. MCMV uses various mechanisms to avoid NK cell recognition. The significance of MCMV evasion of NK cells for virus control in vivo is revealed by the fact that deletion of individual MCMV genes involved in NK cell evasion results in virus attenuation in NK cell dependent manner [3]. In addition, certain mouse strains possess NK cell receptors able to drive resistance to MCMV infection [4]. Here, we discuss the role of NK cell receptors in MCMV control and in regulation of adaptive immune response. In addition, we discuss various viral immunoevasion mechanisms aimed to compromise NK cell function particularly with respect to using engineered CMV mutants lacking NK cell immunoevasins as attenuated vaccine vectors.

NK cell receptors

Antiviral functions of NK cells include various mechanisms: (i) elimination of infected cells by release of cytotoxic granules, (ii) induction of apoptosis in target cells through Fas Ligand (FasL) or TNF-related apoptosis-inducing ligand (TRAIL), and (iii) secretion of cytokines. The activation of NK cells is regulated by a repertoire of receptors sensing cytokines induced by infection and those recognizing ligands expressed on target cells [5]. Receptors sensing cytokines potentiate NK cell function primarily by responding to type

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I IFNs, as well as IL-12, IL-15, and IL-18. NK cells also express a variety of germ-line encoded NK cell receptors (NKRs) that can be grouped based on either their chemical structure or role in NK cell response. Structurally, the majority of NKRs belong to the C-type lectin-like family or immunoglobulin-like family. Functionally, receptors may deliver either activating or inhibitory signal into the cell. Most of the ligands recognized by NK receptors are MHC-I or MHC-related molecules [4, 6]. Inhibitory receptors bind MHC-I molecules expressed on virtually all cells, maintaining NK cells in the state of inhibition. In mice, these include receptors such as CD94/NKG2A, inhibitory Ly49 receptors and KLRG1 [5]. Activating receptors are engaged by stress-induced molecules and nonself peptides or ligands. Examples of mouse-activating receptors are NKG2D, activating Ly49 receptors, NKp46 (NCR1), DNAM-1, CD94/NKG2E, CD94/NKG2C, and CD16 (FcγRIII). Engagement of some receptors, e.g., 2B4 or CD96, can result in NK cell inhibition or activation, depending on the engagement context [7, 8]. Distribution of NK cell receptors is stochastic, making individual NK cells heterogeneous and functionally diverse. Recent investigation that employed analysis of 28 receptors on human NK cells by cytometry coupled to mass spectrometry (CyTOF) has showed that there are 6 to 30,000 diverse NK cell subpopulations per individual and more than 100,000 NK cell subpopulations in analyzed group of individuals [9]. Interestingly, the expression of inhibitory receptors is genetically determined while the expression of activating receptors is strongly influenced by environmental stimuli.

Molecular mechanisms of MCMV evasion of NKG2D and NCR1 receptors

In order to avoid recognition by activating NK cell receptors, the virus needs to prevent the expression of their ligands on the plasma membrane of infected cells. The molecular mechanisms behind the subversion of various ligands interacting with the NKG2D receptor have been the subject of extensive studies [3]. NKG2D is a c-type lectin-like receptor expressed as a homodimer on majority of NK cells and functionally conserved in humans and mice. In mice, it is also expressed on some γδ T cell, CD8 T cell, and CD4 T cell subpopulations, where it functions as a costimulatory molecule [6, 10]. NKG2D receptor recognizes a broad set of ligands. In mice, these ligands include murine UL-16 binding protein-like transcript −1 (MULT-1), H60 family (H60a, H60b, and H60c), and retinoic acid early inducible cDNA clone-1 (RAE-1) family (α-ε). Protein structure of these ligands is MHC class I related and their expression is induced upon cellular stress, infection, and transformation [10]. To avoid NK cell activation via NKG2D, MCMV encodes several inhibitors that prevent the expression of NKG2D ligands on the surface of infected cells (Fig. 1).

Under physiological conditions, surface expression of NKG2D ligands is tightly controlled. The regulatory mechanism of the surface expression of MULT-1 includes ubiquitination and lysosomal degradation by cellular E3 ligases [11]. Once reaching the plasma membrane, MULT-1 recycles in clathrin-positive vesicles for a prolonged period of time [12]. Upon MCMV infection, virus induces MULT-1 transcription but manages to prevent its surface expression using two immunoevasins, m145 and m138 [12, 13] (Fig. 1). MCMV protein m138 induces endocytosis of MULT-1 molecules that have reached the plasma membrane and drive them to lysosomal degradation [12]. The second viral inhibitor of MULT-1, m145, targets mature MULT-1 protein in the secretory pathway, after the trans Golgi compartment, even though the complete mechanism is still not resolved [13]. The MCMV mutant that lacks MULT-1 inhibitor m145 is severely attenuated in vivo and this attenuation is NK cell dependent [13]. The m138 MCMV mutant is also attenuated in vivo, but m138 has been shown to have additional immunosubversive functions that could render a virus lacking this gene more susceptible to immune control [12, 14].

H60 family of NKG2D ligands comprises three members: H60a, H60b, and H60c [15, 16]. MCMV induces transcription of H60b, but not of H60a or H60c genes [15]. MCMV has devoted two inhibitors to prevent H60 surface expression [12, 17, 18]. MCMV proteins m155 and m138 target mature H60 molecules (Fig. 1). While m155 is ER resident protein involved in the downregulation of H60 by proteasomal degradation pathway, m138 downregulates H60 by lysosomal degradation pathway [12, 17, 19]. The m155 is another MCMV protein with dual immunosubversive function, since it inhibits the expression of CD40 in infected antigen-presenting cells [19].

MCMV infection induces transcription of all five *RAE-1* genes [20] by activating the phosphatidylinositol-3-kinase (PI3K) pathway [21]. However, the virus prevents RAE-1 surface expression using two immunoevasins, m152 and m138 (Fig. 1). The m152 viral protein is the first identified MCMV regulator with the ability to subvert NKG2D-mediated immune response [20, 22]. m152 is also known for its ability to retain MHC class I molecules in the ER [23] and the RAE-1 proteins are retained in the same compartment by m152 [24]. In contrast to m152, which retains all RAE-1 isoforms, m138 exclusively downregulates the surface resident RAE-1ε molecules. RAE-1δ has the longest half life due to the lack of specific PLWY motif that is present in other RAE-1 isoforms. As such, RAE-1δ remains present on the surface of infected cells for a prolonged period of time and increases the NKG2D-mediated resistance to MCMV infection. The PLWY motif has also been shown to influence the affinity of m152 to interact with the RAE-1β molecules [25].

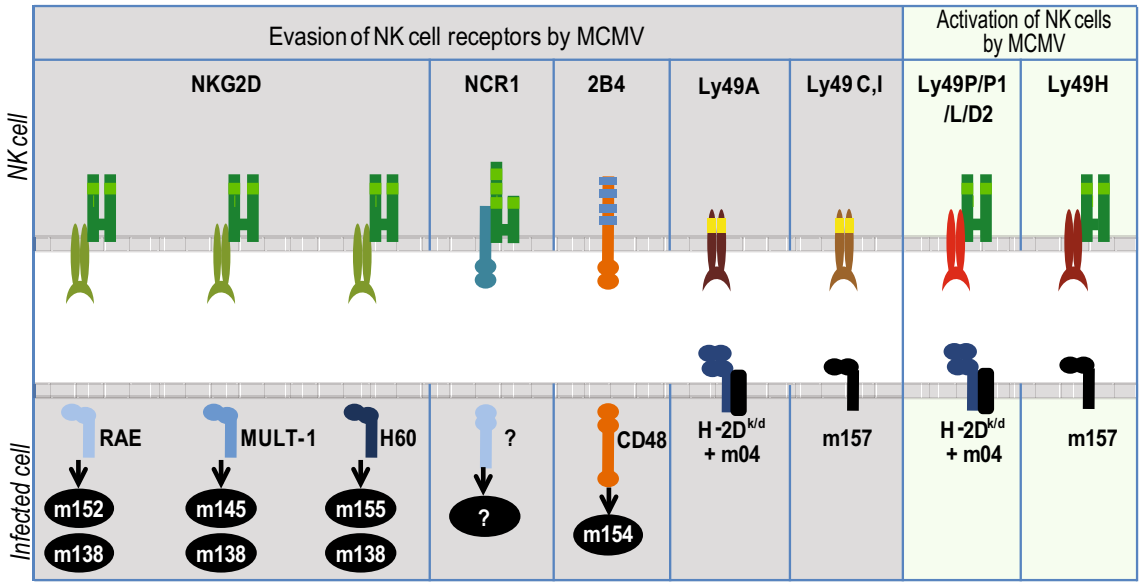

Fig. 1 MCMV subverts NK cell receptors by different molecular mechanisms. Most of MCMV immunoevasion mechanisms are aimed to prevent surface expression of ligands for activating NK cell receptors or include MHC I manipulation to avoid the 'missing self' recognition. Activating receptors NKG2D and NCR1 as well as 2B4 receptor are subverted by downregulation of ligands required for their engagement. In case of 2B4 and NKG2D, the downregulation is mediated by MCMV proteins that target either plasma membrane resident ligands (m138) or ligand maturation (m152), or induce their degradation (m154). Inhibitory Ly49 receptors are subverted either by expression of MHC homologues (m157), proteins mediating surface repopulation of MHC class I (m04), or by currently undefined mechanisms. However, certain mouse strains encode activating Ly49 receptors able to recognize viral products (m157, H-2D$^{k/d}$/m04)

Recently, the crystal structure of RAE-1γ in the complex with m152 was solved and proposed as a model for interaction of MHC-I-like ligands with MHC-I-like viral inhibitors [26].

Altogether, a range of viral immunoevasins is dedicated to preventing the expression of NKG2D ligands on the surface of infected cells. Moreover, a significant portion of NKG2D ligands is regulated by two MCMV immunoevasins targeting corresponding ligands at different cellular compartments. When either of the viral immunoevasins is missing, the respective NKG2D ligand is expressed on the surface of the infected cell, in spite of the presence of the second viral inhibitor. Thus, the redundancy of viral inhibitors of NKG2D is functionally relevant.

Natural cytotoxicity receptors (NCRs) are a family of activating NK cell receptors. Three NCRs are described on human NK cells: NKp46 (NCR1; CD335), NKp44, and NKp30 [27]. NKp46 is the only NCR with ortholog in mouse, named NCR1. While cellular ligand for NCR1 is still unknown, several pathogen-derived ligands have been reported (reviewed in [28]). The influenza hemagglutinin (HA) was shown to be a ligand for human NKp46 as well as mouse NCR1. Thus, mice missing the NCR1 receptor are more susceptible to lethal influenza infection [29]. Staining with NCR1 fusion protein revealed that MCMV is able to downregulate NCR-1 ligands from the surface of infected cells, and by doing so provides opportunity to avoid NK cell control (unpublished observation and [3]). Consequently, there is no difference in the early control of MCMV infection between NCR1$^{gfp/gfp}$ and wt mice (unpublished observation). However, the identity of the downregulated ligands or the molecular mechanism engaged is still not known. In contrast, NK cells from another mouse model of NCR1 deficiency, *Noé* mice, are hyperresponsive and these mice show enhanced resistance to MCMV and influenza infection [30]. The differences may be explained by the fact that *Noé* mice bear a point mutation in the gene encoding NCR1 receptor that leads to its absence from the cell surface, although the protein is present intracellularly. On the other hand, in NCR1$^{gfp/gfp}$ mice, part of the gene encoding for NCR1 has been replaced with gene encoding gfp, resulting in complete loss of NCR1 expression [29].

Evasion of paired receptors and receptors with dual function

Some activating and inhibitory receptors can be engaged by the same cellular ligand. The best example is PVR (CD155) which serves as a ligand for the activating receptor DNAM-1, inhibitory receptor TIGIT, and CD96, the receptor with dual function [7, 31]. The human counterparts of TIGIT and DNAM-1 receptors appear to be more promiscuous and bind other members of the nectin or nectin-like families, but in mice, their recognition seems to be restricted to PVR and to another, still unidentified ligand expressed on the mouse peripheral blood mononuclear cells (PBMCs) [32]. CMV

modulates the surface expression of PVR and its availability to ligate its cognate receptors. The UL141 protein of HCMV actively prevents expression of PVR on the cell surface [33]. However, recent data show the upregulation of PVR on MCMV-infected dendritic cells and macrophages in vivo, contributing to DNAM-1-mediated generation of MCMV-specific effector and memory NK cells [34]. It will be interesting to see how MCMV modulates surface expression of PVR on other permissive cell types in vitro and in vivo, as well as what the consequences of viral modulation on the NK cell activation following the recognition by DNAM-1, CD96, and TIGIT are.

The signaling lymphocyte activation molecule (SLAM) family of receptors is expressed on various immune cell types, including NK cells [8]. HCMV downregulates CD48, a SLAM family member, while the viral UL7 protein, which strongly resembles SLAM receptor's sequence, is able to attenuate proinflammatory cytokine production in myeloid cells [35, 36]. MCMV also downregulates SLAM family members including CD48, CD84, CD229, and Ly108 [37]. The MCMV m154 protein targets CD48 molecule, leading to its degradation by both lysosomal and proteasomal pathways (Fig. 1). CD48 is the interaction partner of the 2B4 receptor, also being a SLAM family member [8]. The 2B4 receptor is another example of NK cell receptor with dual function, since its engagement by CD48 can lead to activating or inhibitory signaling. It has been proposed that the dual function of 2B4 is regulated by CD48 and 2B4 expression levels, as well as by the availability of the signaling adaptor protein SAP in NK cells [38]. The MCMV *m154* deletion mutant virus has significantly reduced titers in all organs and depletion of NK cells has restored replication to the wt level, indicating that engagement of 2B4 receptor in this case leads to NK cell activation [37].

Ly49 receptors: viral subversion of 'missing self' mechanism and host counter responses

The Ly49 family comprises more than 20 members of activating or inhibitory receptors, some of them being paired receptors, expressed in different combinations and predominantly on NK cells [4]. The MHC class I molecules are common ligands of the inhibitory Ly49 NK cell receptors and their expression protects healthy cells from being lysed by NK cells, i.e., they are recognized as self. In order to avoid lysis by $CD8^+$ T cells, MCMV downregulates MHC I molecules from the surface of infected cells using two viral proteins, m06 and m152 [39]. Absence of MHC I renders infected cells susceptible to the 'missing self' recognition and lysis by NK cells. However, MCMV possesses a mechanism aimed at evading the 'missing self' recognition. The MCMV m04 protein antagonizes the function of m06 and m152 by binding to MHC I molecules and escorting them to the cell surface [40]. In addition, MCMV encodes MHC-I homologues aimed to engage inhibitory Ly49 receptors (Fig. 1) [41–43].

The inhibitory receptor Ly49I interacts with the MCMV-encoded MHC class I homologue m157 [41]. More recently, another inhibitory Ly49 receptor, Ly49C, has been shown to bind the m157 protein from wild type MCMV isolate G1F [42]. Interestingly, the inhibitory potential of Ly49C is *cis* regulated by the host's MHC I molecules [44]. The viral protein m157 is also recognized by the activating Ly49H receptor [45]. This might represent an evolutional host's response to the selective pressure imposed by the m157 evasive mechanism. The m157 gene sequence is highly variable among MCMV isolates and under selective pressure, in Ly49H positive mice, virus is able to mutate m157 [46, 47]. A crystal structure of the Ly49H receptor in complex with m157 identified the helical stalk of Ly49H molecule as a binding site of m157 [48]. In parallel, a binding model has shown that the final complex is a result of positive cooperative binding mechanism of two viral proteins to the Ly49 molecule [49]. The GPI anchor of m157 has been shown to be important for the m157 availability on the cell surface and corresponding efficacy of the Ly49-m157 complex formation [50].

In addition to mouse strains bearing Ly49H receptor, MA/My is another mouse strain that confers the MCMV resistance to the ability of Ly49 receptors to recognize viral products (reviewed in [4]). However, the activating Ly49P receptor specifically recognizes MCMV-infected cells in the context of $H\text{-}2D^k$ molecule [51]. This recognition involves the m04 protein of MCMV [52] and another still undefined viral component. Interestingly, another Ly49 family member, the inhibitory receptor Ly49G2, which also recognizes $H\text{-}2D^k$ molecules, further contributes to the MCMV resistance of MA/My mice [53]. Ly49G2 enables MCMV protection by licensing NK cells, shown to be essential for efficient detection and elimination of MCMV infected targets [54].

A huge coding potential of MCMV has enabled the virus to encode a variety of MHC class I homologues that could potentially attenuate NK cell response by engaging inhibitory NK cell receptors [55]. However, the alignment of putative MHC-I-like molecules of MCMV, mostly the members of the m145 family (m17, m145–m158), with classical MHC-I molecules have revealed varying and in general low level of sequence identity [56]. Nevertheless, currently available crystal structures of MCMV proteins m144, m152, m153, and m157 have confirmed their MHC-I-like fold [26, 56]. Direct recognition of MCMV product by NK cells, limited to a few mouse strains and best represented by m157, confirmed possible deleterious consequences for the virus in case when its MHC I homologue exposed on the cell surface is recognized by an activating counterpart of the immune system.

Memory-like NK cells in CMV infection

NK cells are traditionally considered to be a part of the innate immune system, as they lack gene-recombination machinery and are thought to be relatively short-lived. However, recent data show that NK cells can also mount an efficient long-term recall response [57–59]. Moreover, memory-like NK cells have been reported both in mice and humans [57–60]. The MCMV specific memory-like NK cells have been detected after MCMV infection of mouse strain bearing Ly49H receptor able to recognize viral m157 protein [59]. The interaction of Ly49H and m157 leads to IL-12 dependent expansion of $Ly49H^+$ NK cells [61]. The acute phase of infection is followed by the contraction and formation of a pool of long-lived $Ly49H^+$ NK cells, able to undergo secondary expansion and provide enhanced protection against MCMV infection [59, 61]. Although the mechanism behind formation of memory-like NK cells is still elusive, recent report shows that besides Ly49H, DNAM-1 signaling is required [34]. In addition, as for T cell memory, proapoptotic factor Bim is required for contraction and generation of mature memory $Ly49H^+$ NK cells [62]. In humans, such antigen-specific memory-like NK cells have not been characterized, even though the human NKG2C-activating NK cell receptor seems to play a role in the specific recognition of HCMV [63]. The $NKG2C^+$ NK cells are found in high frequencies in HCMV seropositive individuals and expand greatly during HCMV viremia or reactivation. Expansion of $NKG2C^+$ NK cells has also been observed during hantavirus infection or chikungunya virus, chronic hepatitis B or C virus and HIV infections, but only in HCMV seropositive individuals (reviewed in [64]). However, during EBV infection $NKG2C^+$ NK cells do not expand; instead, $CD56^{dim}NKG2A^+CD57^+$ NK cell population expands in CMV positive individuals and persists long-term [65]. In the majority of HCMV seropositive donors, $NKG2C^+$ NK cells are associated with lack of FcRγ expression [66, 67]. FcRγ-deficient NK cells respond more robustly through CD16, compared to FcRγ-expressing NK cells [66]. The analogous NK cells in mouse are unlikely to be found, since mouse FcRγ-deficiency abrogates CD16 expression. The classical T cell memory response after vaccination or exposure to antigen is characterized by decay over time. In case of CMV infection CD8 T cell memory inflation is observed, driven by continuous virus reactivations [68]. Could it be that the same reactivations, i.e., antigen and other accompanied forms of stimulation, also affect NK cells and sustain their numbers? The study of Foley et al. on hematopoietic cell transplants supports such hypothesis [63]. Transplanted $NKG2C^+$ NK cells declined in CMV seronegative recipients while they expanded in CMV seropositive recipients, even in the absence of viremia, indicating that expansion of $NKG2C^+$ NK cells requires the presence of CMV antigen [63].

Besides CMV specific, cytokine-induced and liver-restricted memory-like NK cells have also been described [57, 58]. Cytokine-induced memory-like NK cells derive upon stimulation with IL-12, IL-15, and IL-18 [58]. These cells show prolonged presence after adoptive transfer in naïve mice and produce more IFN-γ after restimulation compared to naïve NK cells [58]. Similarly, IL-12/15/18 prestimulated human NK cells show increased proliferation and effector function [69, 70] and express high levels of high affinity IL-2 receptor [69]. Interestingly, MCMV infection can also lead to induction of high affinity IL-2 receptor on NK cells [71].

Liver-restricted memory-like NK cells are induced upon sensitizing T and B cell deficient $Rag2^{-/-}$ mice with haptens [57]. In this model, mice could discriminate between haptens during secondary challenge. Therapeutic potential of these cells has been shown by vaccination of mice with viral antigens that induced hepatic memory-like NK cells, providing antigen-specific protection against several viruses [72]. Hepatic memory-like NK cells are dependent on the chemokine receptor CXCR6 [72], IFN-α, IFN-γ, and IL-12 [73] and are additionally defined by the expression of CD49a and by the absence of DX5 [74]. Interestingly, these cells seem to originate from hepatic stem cells, not from bone marrow [74]. It remains to be tested whether CMV infection also induces similar memory-like NK cells in liver.

NK cell memory is an exciting new research field with a possible huge therapeutic potential. However, many questions are still unanswered. CMV-specific memory-like NK cells could be explained by virus persistence and continuous stimulation. Cytokine-induced memory-like NK cells exert enhanced effector functions, for example IFN-γ production. This could be due to the epigenetic changes in *IFNG* locus, as it was shown for memory T cells [75], or to posttranscriptional IFN-γ mRNA regulation [58]. Such mechanism could also drive NK cell memory during CMV infection. Hepatic memory NK cells are even more complex as they break at least two dogmas in immunology. Not only that they obtain memory properties, but they also show specificity for many antigens [72]. Are memory-like NK cells beneficial for the host in control of viral infections and tumors? In CMV seropositive individuals increased frequency of $NKG2C^+$ NK cells is persistent for lifetime. Expansion of certain NK cell subpopulation could partially alter NK cell repertoire that might lead to reduction of immune surveillance capacities. However, $NKG2C^+$-associated FcRγ deficiency enhances NK cell response via CD16 [66]. Moreover, high percentage of $NKG2C^+$ NK cells is associated with reduced HIV progression [76]. In addition, cytokine-induced memory-like NK cells are protective against tumors in mouse model [69]. However, the study in patients following orthotopic liver transplantation indicated that while the presence of immature $NKG2C^-$ NK cells ($NKG2A^{high}CD62L^{high}CD57^{low}NKG2C^-TNF\text{-}\alpha^{low}IFN\text{-}\gamma^{high}$) is associated with the development of certain tumor types

(genitourinary tumors), expansion of NKG2C$^+$ NK cells (NKG2A^{low}CD62L^{low}CD57^{high}NKG2C$^+$TNF-α^{high}IFN-γ^{low} NK cells) is also associated with the development of specific tumors (head/neck or colorectal tumors) [77].

NK cell regulation of CD8$^+$ T cell responses during MCMV infection

The influence of NK cells on CD8$^+$ T cell response is still the matter of investigation, but currently available body of data indicates that the outcome is dependent on the infection context which can lead to the enhancement or reduction of CD8$^+$ T cell response magnitude [78–81]. Antiviral NK cell activities are shaping CD8$^+$ T cell response as they affect viral load, as well as amount of cytokines produced early during infection. Besides, recent reports indicate that regulation of CD8$^+$ T cell response by NK cells is much more complex as it was shown that NK cells can modulate T cell response also directly by killing T cells [82–84] or indirectly by interacting with APCs [85].

Most of the evidence describing regulation of CD8$^+$ T cell response by NK cells is generated on the MCMV model of infection. Su et al. have been first to report that depletion of NK cells in C57BL/6 mice leads to enhanced CD8$^+$ T cell response [81]. This enhancement is characterized by increased IFNγ production and CD8$^+$ T cell expansion. On the other hand, strong NK cell response induced by interaction of Ly49H and m157 negatively influences long-term virus-specific CD8$^+$ and CD4$^+$ T cell responses [79]. This is achieved by NK cell-mediated elimination of infected DCs and subsequent limitation of CD8$^+$ T cell priming. We have shown that lack of m157 and Ly49H interaction, upon infection of C57BL/6 mice with *Δm157* mutant virus, induces stronger CD8$^+$ T cell response when compared to infection with wt MCMV [78]. This effect is mediated by higher levels of IFNs and other proinflammatory cytokines, increased antigen load and preservation of early cDCs' function in the absence of m157. Further, upon MCMV infection of perforin knockout mice, Ly49H$^+$ NK cells suppress CD8$^+$ T response by secretion of anti-inflamatory cytokine IL-10 [86]. Additionally, it has been reported that IL-10 can also reduce CD4$^+$ T cell priming by interfering with NK-DC crosstalk [87]. Viral chemokine MCK-2 has also been shown to be implicated in NK cell-mediated shaping of CD8$^+$ T cell response [88]. MCK-2 recruits inflammatory monocytes to the site of infection, contributing to the enhancement of NK cell response, resulting in decreased CD8$^+$ T cell response. Even though the aforementioned studies indicate that stronger NK cell response will lead to weaker CD8$^+$ T cell response, some studies have demonstrated quite the opposite finding. Strong activation of NK cells mediated through Ly49H-m157 interaction has resulted in accelerated CD8$^+$ T cell responses due to limiting IFNα/β production by pDCs and preservation of splenic cDCs [80]. Additionally, Ly49G2$^+$ NK cells, able to recognize infected cells, mediate faster recovery of cDCs, leading to enhanced CD8$^+$ T cell response [54].

The role of NK cells in shaping T cell response has also been shown in lymphocytic choriomeningitis virus (LCMV) infection. Waggoner et al. have demonstrated that NK cells, otherwise unable to directly control LCMV infection, can regulate CD8$^+$ T cells response to this virus [82–84]. Regulation is achieved by elimination of activated helper CD4$^+$ T cells by NK cells, an effect strictly dependent on the infection conditions and virus dose. The results of this study have indicated that NK receptor 2B4 plays a role in specific CD4$^+$ T cell elimination [82]. Another study has shown that NK cell regulation of T cell response to LCMV is a consequence of perforin-dependent NK-cell functions; however, the authors claim that this effect is mediated through NKG2D [89]. In addition, recent studies showed that sensing of type I IFNs protects T cells from elimination by NK cells during LCMV infection [83, 84]. Type I IFN receptor deficient T cells are expressing lower levels of ligands for inhibitory receptors, MHC I and Qa-1b [84], and are recognized and eliminated by NK cells in NCR1 dependant manner [83]. Whether NK cells can shape T cell response by such mechanism during MCMV infection, is still not known.

Exploiting the ligands for activating NK cell receptor to potentiate efficacy of live attenuated viral vaccine and vaccine vectors

As CMV induces strong CD8$^+$ T response and has a large genome allowing the insertion of multiple foreign genes, it is increasingly being recognized as an excellent vaccine vector. A recent report by Hansen et al. has demonstrated how manipulation of CMV vector can lead to substantial improvement of vaccine properties [90]. For example, the absence of rhesus CMV (RhCMV) orthologs of HCMV pUL128-131A results in dominant MHC-II-restricted CD8$^+$ T cell response, while the expression of US11 ortholog suppresses the induction of canonical simian immunodeficiency virus (SIV) epitope specific CD8$^+$ T cell responses. Vaccination with such RhCMV vector bearing SIV antigens has been successful in protecting 50 % of the vaccinated macaques upon lethal SIV challenge [90].

Since NKG2D is expressed on NK and CD8$^+$ T cells, it represents an ideal candidate for targeted manipulation of vaccine properties. Diefenbach et al. have demonstrated that tumors ectopically expressing NKG2D ligands, RAE-1β and H60, induce potent T cell response and sensitize NK cells in vivo [91]. Vaccination with such tumor cells could protect mice from tumors that do not express RAE-1β and H60 indicating that NKG2D ligands potentiate the priming of

tumor specific CD8$^+$ T cells. We have recently tested the impact of expression of NKG2D ligand RAE-1 on the immunobiology of the recombinant virus possessing RAE-1γ in the place of its viral inhibitor [92]. In spite of dramatic attenuation, such virus demonstrates a robust capacity to induce CD8$^+$ T cell immune response and protect vaccinated mice against challenge infection (Fig. 2). Moreover, on the backbone of RAE-1γ expressing MCMV, we constructed several vectors expressing foreign CD8 epitopes and demonstrated enhanced CD8$^+$ T responses to vectored antigens [92, 93]. Surprisingly, the enhanced CD8$^+$ T cell response by MCMV vector expressing RAE-1γ was observed also in mice lacking NKG2D receptor or in mice treated with blocking anti-NKG2D mAbs. Therefore, the results indicate additional immune function of RAE-1γ protein. Further studies are needed to characterize this NKG2D independent function of RAE-1 including characterization of the so far unknown putative additional receptor.

Since recombinant MCMV expressing NKG2D ligand has shown such tremendous vaccine properties, translation of this knowledge to HCMV system is of great importance. UL16 binding protein 2 (ULBP2 protein), human RAE-1γ homologue, is the most promising candidate for the development of analogous HCMV vaccine vector. Recent progress in humanized mouse models could provide an opportunity for investigation of such vaccine model. Up to now several approaches have been applied to construct CMV vaccine/vaccine vectors, including spread deficient virus [94], cell culture-derived virus

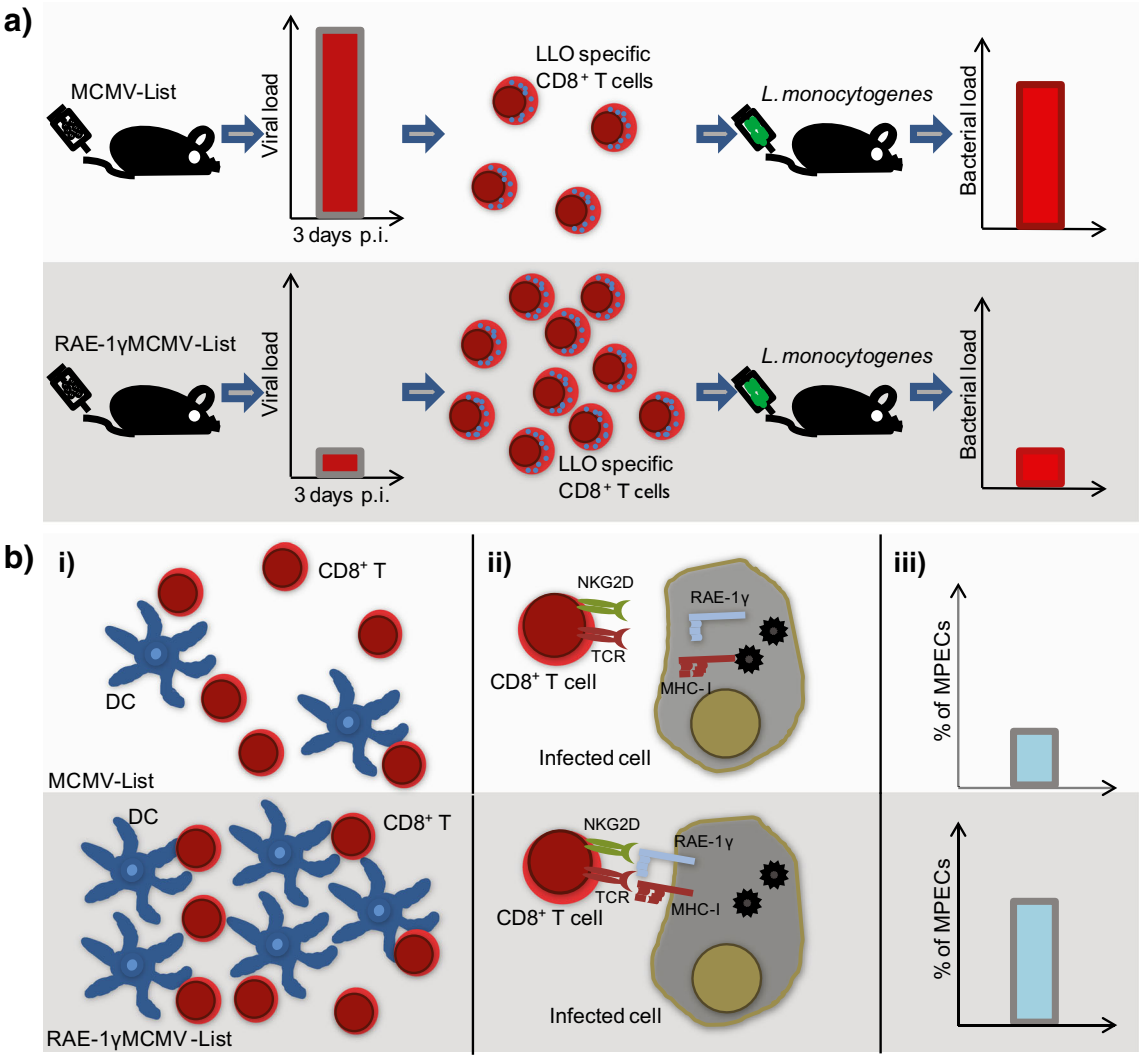

Fig. 2 Recombinant MCMV expressing RAE-1γ and vectored antigen is severely attenuated, elicits strong CD8$^+$ T cell response and provides protection against lethal challenge. **a** RAE-1γMCMV vector (RAE-1γMCMV-List) is attenuated following vaccination compared to wt MCMV vector (MCMV-List). Attenuation is due to NKG2D-mediated activation of NK cells and subsequent elimination of infected cells. RAE-1γ MCMV induces strong CD8$^+$ T cell response to vectored listeriolysin antigen (LLO) that provides protection against lethal *L. monocytogenes* challenge. **b** RAE-1γMCMV induces strong CD8$^+$ T cell response due to (*i*) preservation of DCs, (*ii*) NKG2D costimulation and high levels of MHC I expression, leading to better priming of CD8$^+$ T cells. In addition, CD8$^+$ T cell transition to memory precursors (MPECs) is enhanced (*iii*)

bearing mutations (e.g., in UL128-131A genes) [90], deletion of immunoevasion gene-rich regions [95], and others. An important question is, can CMV vector be engineered so that these beneficial manipulations act synergistically? For example, could combining NKG2D ligand insertion in vector with no gH/gL/pUL128-131A complex lead to an even better $CD8^+$ T cell response, i.e., protection?

Conclusions

The understanding of the NK cell role has evolved over the years: from plain innate eliminators of transformed and infected cells to complex immune cells able to strongly shape adaptive immunity and acquire adaptive functions. CMV is a complex DNA virus able to manipulate a plethora of immune response mechanisms including recognition by NK cell receptors. Yet, the host's evolutionary response seems to be capable of undermining some CMV evasion mechanisms, strongly influencing the immune response, and resulting in resistance to CMV. Recent studies have demonstrated that CMVs encode much more genes, transcripts and proteins than we originally thought [96, 97]. This complexity of virus and host immune response parameters and outcomes may explain some of the apparent discrepancies in the published literature. Better understanding of NK cells/CMV interactions will certainly help guide new strategies for the rational design of CMV vaccine and vaccine vectors. Indeed, several recent studies have demonstrated that recombinant CMVs are attractive candidates as vaccine vectors for a number of clinically relevant infections [90, 93, 98].

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