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Miletić, Antonija; Krmpotić, Astrid; Jonjić, Stipan

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Review

The evolutionary arms race between NK cells and viruses: Who gets the short end of the stick?

Antonija Miletić[1,2], Astrid Krmpotić[1] and Stipan Jonjić[1,2]

[1] Department of Histology and Embryology, Faculty of Medicine, Rijeka, Croatia
[2] Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

NK cells are innate lymphocytes that play a key role in the control of various viral infections. Recent studies indicate that NK cells may acquire some features of adaptive immune cells, including the formation of long-lived memory cells. A large and growing body of data indicates that NK cells regulate the adaptive immune response as well. The function and the activation status of NK cells are tightly regulated by signals induced by a broad range of inhibitory and activating cell surface receptors and cytokines released by other immune cells. Here, we review the function of mouse NK-cell receptors involved in virus control and in the regulation of the adaptive immune response. In addition, we discuss viral strategies used to evade NK-cell-mediated control during infection. Finally, the role of several activating Ly49 receptors specific for mouse cytomegalovirus (MCMV), as well as some controversial issues in the field, will be discussed.

Keywords: NK cells · NK-cell receptors · Viral immune evasion

NK cells in viral infections

NK cells are innate cytotoxic lymphocytes essential for the control of a broad range of viral infections. The activation and function of NK cells are based on a tightly regulated interplay between inhibitory and activating signals induced by the receptors expressed on their surface (reviewed in [1]) or by a variety of cytokines generated by the interaction between NK cells and other innate immune cells, such as macrophages and DCs [2, 3]. Type I IFNs, IL-12, IL-15, and IL-18 produced by macrophages and DCs are indispensable for NK-cell maturation and regulation of NK-cell function. More recently, it has been demonstrated that TGF-β signaling limits the maturation dynamics of NK cells during the neonatal period [4]. Mice lacking the TGF-β receptor on the surface of their NK cells and DCs possess more mature NK cells and can therefore control viral infection more efficiently compared with control mice [4], providing an explanation for the deficient NK-cell response early in life.

Correspondence: Prof. Stipan Jonjić
e-mail: stipan.jonjic@medri.uniri.hr

The impact of NK cells on virus control has been proven to play a key role in different infection models including those modeling infection with influenza virus, HIV, HCV, coxsackievirus, and poxviruses. Arguably, the role of NK cells in the control of herpesviruses, especially CMV, has been most widely studied up till now (reviewed in [5–7]). Upon activation and recruitment to the site of infection, NK cells use several mechanisms to limit virus replication: (i) a cytolytic response involving the secretion of cytolytic granules (granzymes and perforin) upon engagement of an activating receptor [8, 9], (ii) production of pro-inflammatory cytokines with antiviral activity such as IFN-γ and TNF-α [10], (iii) killing of target cells via FasL or TRAIL [11], and (iv) Ab-dependent, cell-mediated cytotoxicity-mediated lysis via CD16 [12].

Besides their well-recognized role in the control of virus replication, NK cells have been shown to possess additional functions that can be beneficial or detrimental to the host. For instance, decidual NK cells are considered to be important for placentation and maintenance of materno-fetal immune tolerance [13], but there is a very limited amount of data published on the role of NK cells in the control of infections during pregnancy. In mouse cytomegalovirus (MCMV)-infected mice, NK cells preserve the integrity and function of the salivary gland, an organ that is

important for horizontal virus spread [14, 15]. A study using the mouse model of salivary gland dysfunction after an acute MCMV infection showed the involvement of NK cells in the prevention of acinar atrophy and loss of secretions [16]. Conversely, the NK-cell response could play a negative role in virus infection, as demonstrated in the case of infection with RSV [17]. NK cells accumulate in the lungs of BALB/c mice at the early stage of RSV infection, where they gain an activated phenotype accompanied by high production of IFN-γ, and are responsible for the resulting acute lung injury [17], demonstrating a thin line between protective function and immunopathology. Likewise, although the role of NK cells in the control of influenza virus infection is well established [18], a recent study demonstrated that these cells can also contribute to the pathology by increasing pulmonary inflammation [19].

Studies in mice using the lymphocytic choriomeningitis virus (LCMV) showed that despite the induction of type I IFNs and the stimulation of NK-cell cytotoxic functions, NK cells do not play a role in the early clearance of this virus [20]. A recent study in LCMV-infected mice revealed a role for NK cells in the regulation of adaptive immunity [21]. A considerable number of studies using MCMV as an infection model confirm NK-cell involvement in shaping the adaptive immune response [22–27].

The main issues addressed in this review include the role of NK cells and their receptors in the control of virus infection and the strategies that viruses have evolved to evade and modulate immune response.

Mouse NK-cell receptors in response to viral infections

NK cells are regulated by a careful balance of signals from their inhibitory and activating receptors. Viruses, on the other hand, try to prevent the engagement of activating receptors or preserve a default inhibitory state at any cost (Fig. 1). The two most frequent approaches used by viruses to preserve the inhibitory state include direct targeting of NK-cell receptors and interfering with NK-cell receptor ligands. Unlike B and T cells, which use RAG enzymes for rearranging their receptor genes and consequently gain numerous combinations of Ag-specific receptors [28, 29], NK cells widen the diversity of Ag recognition by differential expression of a large set of germline-encoded inhibitory and activating NK-cell receptors on their cell surface.

NK-cell receptors are classified into two main families according to their extracellular domain: (i) the C-type lectin like family and (ii) the Ig-like receptor family [30]. In mice, many of the genes encoding members of the C-type lectin-like NK-cell receptors, as well as the genes that encode some of their ligands, are a part of the Natural Killer gene Complex (NKC) located on mouse chromosome 6 (reviewed in [31]). These include the killer lectin-like family a (Klra, or Ly49 receptors), representing the functional mouse homologues of killer Ig-like receptors (KIRs) found in humans. The Ly49 family comprises both inhibitory and activating members, many of them specific for MCMV (reviewed in [32,33]). Members of Klrc (or NKG2 receptors) and Klrd (or CD94) are expressed as heterodimers on overlapping NK-cell subsets, in which the NKG2A/CD94 complex encodes inhibitory receptors, while NKG2C/CD94 and NKG2E/CD94 exhibit activating properties [34]. Recent findings indicate that NKG2E/CD94 is essential for the resistance of C57BL/6 mice to mousepox, which is caused by the orthopoxvirus ectromelia virus (ECTV). ECTV-infected cells expressing the MHC-class-Ib molecule (Qa-1(b)) are specifically recognized by this activating receptor [35]. Klrb (or NKRP1) and its ligand Clr-b expressed within NKC were shown to be important in the control of poxvirus infection [36, 37]. Furthermore, Klrk (or NKG2D), one of the most important activating NK-cell receptors involved in the control of various infections and tumor transformation recognition (reviewed in [38]), also belongs in this large C-type lectin-like family, as do CD69 and Klrg1 (or MAFA1). Conversely, mouse Ig-like NK-cell receptors include members such as the paired-Ig receptor family (Pir, or ILT), the Natural cytotoxicity receptor 1 gene (Ncr1, or Nkp46) and the 2B4 receptor. The NCR1 receptor is the only member of the NCR receptor family expressed in mice. This activating receptor was found to be important in the control of several bacterial and viral infections including the influenza virus (reviewed in [39]). Furthermore, the CD96 receptor belonging to the same Ig-like family, shares a ligand with an activating (DNAM1) and an inhibitory (T-cell immunoreceptor with Ig and ITIM domains (TIGIT)) receptor expressed on NK cells. All three receptors competitively recognize the poliovirus receptor (PVR, or CD155), which is expressed on many cell types of epithelial origin as well as on monocytes and DCs (reviewed in [40]).

Finally, it is important to emphasize that the expression of some NK-cell receptors (e.g. Ly49s) is stochastic, so each NK cell is unique with regards to the repertoire of receptors it expresses, which can ultimately have a great influence on the immunological response and the resolution of the viral infection.

Viral evasion of NK cells by modulation of MHC class I molecules

Most of the known ligands for inhibitory NK-cell receptors are MHC class I (MHC-I) molecules with differential binding specificity and affinity for specific inhibitory receptors [41]. Since MHC-I molecules are expressed on practically all nucleated cells, it is not surprising that, in order to avoid the targeting of healthy cells, the default state of NK cells is inhibition. However, the downregulation of MHC-I expression from the surface of infected cells, which is a consequence of viral evasion mechanisms aimed at avoiding detection and lysis by $CD8^+$ T cells, should induce the activation of NK cells in a process termed "the missing self" recognition [42]. By this mechanism, the ligand-deprived inhibitory NK-cell receptors can no longer suppress NK-cell activity, thus leading to a prevalence of positive signaling coming from activating NK-cell receptors.

Inhibitory Ly49 receptors, which recognize MHC-I molecules as their cellular ligands, comprise the majority of inhibitory receptors found on mouse NK cells. MCMV encodes three proteins that modulate the expression of MHC-I molecules on infected

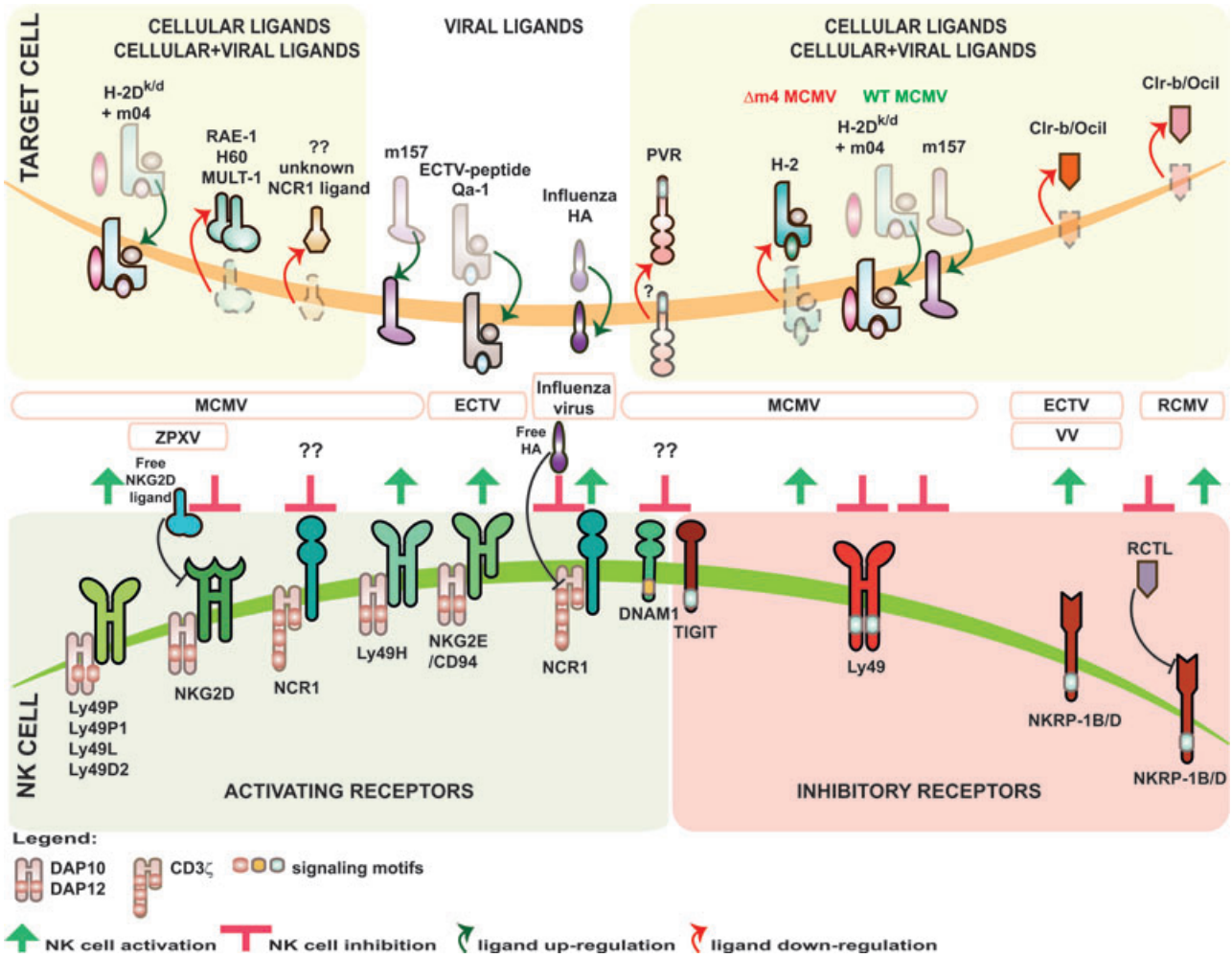

Figure 1. Modulation of ligand–receptor interactions as a result of viral immunoevasion. NK-cell activation is tightly regulated by the interplay between inhibitory and activating signals coming from receptors expressed on the cell surface of NK cells. In order to disrupt NK-cell activation, viruses employ a plethora of different immune evasion tactics. The schematic representation includes activating and inhibitory mouse NK-cell receptor-ligand pairs and the corresponding immune evasion mechanisms that viruses use in order to disrupt their interaction. MCMV evades NK cells by employing several immune subversive approaches including the downregulation of ligands for the activating (NKG2D, NCR1, DNAM-1) NK-cell receptors. The engagement of inhibitory receptors by viral ligand alone (m157) or in a complex with MHC-class-I molecules (m04/MHC-I) results in the avoidance of NK-cell activation by the missing self recognition. In some mouse strains the same viral proteins (m157, m04 in a complex with MHC-I) are recognized, in addition, by activating receptors resulting in NK-cell activation. Influenza virus HA expressed on infected cells is recognized by NCR1. To avoid this, influenza virus releases free HA which enters the NK cells and induces CD3-ζ degradation, thus limiting the signaling through NCR1. Zoonotic orthopox viruses (ZPXV) directly prevent NKG2D signaling by encoding competitive soluble ligands. Orthopoxvirus ectromelia virus (ECTV) upregulates the expression of the Qa-1 molecule on infected cells that leads to an increased proportion of NK cells expressing the activating CD94/NKG2E receptor at the expense of those expressing the inhibitory CD94/NKG2A receptor. NKRP-1 is important in the control of ECTV and vaccinia virus (VV) infection, since those viruses induce the downregulation of Clr-b, a ligand for this inhibitory receptor, which results in NK-cell activation. On the other hand, rat CMV (RCMV) encodes for the Clr-b homolog (RCTL) which, in addition to Clr-b downregulation, results in immune evasion.

cells [43]. The m152/gp40 [44] and m06/gp48 [45] proteins downregulate MHC-I molecules by retaining them at the level of the ERGIC/cis Golgi compartment or by redirecting them to lysosomes for degradation, respectively. However, the m04/gp34 protein does not downregulate MHC-I molecules but instead binds to these molecules and escorts them to the cell surface [46]. By doing so, the m04 protein not only antagonizes the function of two other MCMV inhibitors, but also provides sufficient surface MHC-I molecules to serve as a ligand for inhibitory Ly49 receptors [46, 47]. It has been proposed that the main role of m04 is viral avoidance of NK-cell killing by the missing self axis. The formal evidence for this hypothesis came from our studies showing that the deletion of m04 from the MCMV genome sensitizes the virus to NK-cell control in vivo [48]. Cowpox virus also encodes for proteins involved in the downregulation of mouse MHC-I molecules [49, 50], but it is not clear how this virus avoids missing self-dependent NK-cell activation.

Another viral mechanism used by MCMV to interfere with NK-cell dependent virus control is the generation of MHC-I homologues that serve as a decoys for inhibitory receptors. Most of

these viral proteins attain an MHC-I-like fold, but since these viral proteins do not present peptides they provide the virus with another evasion mechanism to avoid the activation of NK cells. MCMV encodes for several MHC-I-like molecules — m144, m145, m152, m153, m155, and m157 proteins within its m145 family [51]. The role of several of these molecules in immune evasion has been extensively studied. Likewise, the m152 protein has also been proved to be an important viral tool used in the downregulation of MHC-I molecules from the surface of the infected cell [44]. In addition, m152 has also been shown to play a part in the downregulation of NKG2D ligands along with two other members of the same family, the m145 protein and the m155 protein [52]. The m157 viral protein can be recognized by both inhibitory (Ly49I) and activating (Ly49H) NK-cell receptors, thus representing a very important factor in the regulation of host's immune response [53]. However, the exact function of some viral proteins, such as m144 and m153, and the corresponding mechanisms used in evading NK-cell-mediated viral control still remain to be elucidated [54, 55].

The engagement of inhibitory NK-cell receptors also plays a significant role in the functional NK-cell maturation. NK cells expressing inhibitory receptors that bind to self-MHC-I are considered "licensed" and are thus functionally more responsive to stimulation [56]. On the other hand, the so-called "unlicensed" cells lacking any known inhibitory receptor exhibit an impaired response upon ligation of activating receptors in vitro [57]. However, a study by Orr et al. [58] indicates that "unlicensed" NK cells are capable of mediating MCMV control in vivo, suggesting that NK cells lacking inhibitory receptors may be important for NK-cell control of certain infections. As an explanation the authors suggest that, despite the fact that MCMV downregulates the expression of surface MHC-I, residual amounts of MHC-I on the surface of infected cells are still sufficient to engage inhibitory receptors expressed by licensed NK cells and thus interfere with NK-cell activation via Ly49H recognition of m157 [58].

MCMV-specific activating Ly49 receptors

In response to viral immune subversion strategies, evolutionary pressure on the host has resulted in newly evolving activating NK-cell receptors [59]. For example, the activating receptor Ly49H, expressed on the surface of NK cells, confers resistance to MCMV in C57BL/6 mice [60–62]. Ly49H-exclusive and -direct ligation of m157 initiates strong NK-cell activation, involving a clonal expansion of Ly49H$^+$ NK cells and the secretion of proinflammatory cytokines, resulting in the killing of infected cells and a rapid control of viral replication by day 3 p.i. [51, 53, 63]. The m04 protein is another example of an MCMV protein that can influence the recognition by both inhibitory and activating Ly49 receptors. Contrary to the m157/Ly49 interaction, both the viral m04 protein and the host's H-2D$^{k/d}$ molecule are needed for the recognition of m04 by Ly49P [64]. Interestingly, another uncharacterized host or viral factor is required for successful Ly49P/m04/H-2D^k-mediated recognition of MCMV-infected cells since Ly49P reporter cells could not recognize H-2D^k-bearing target cells that artificially expressed m04. The infection of H-2D^k-bearing target cells expressing m04 with m04-deficient MCMV restored Ly49P recognition. In addition to Ly49P, several other activating Ly49 receptors can recognize MCMV-infected cells in an m04/H-2-specific fashion [65]. However, in contrast to the prompt activation and clonal expansion of Ly49H$^+$ NK cells upon Ly49H receptor engagement early after infection in C57BL/6 mice, Ly49 activating receptors recognizing the m04/MHC-I complex are hindered by inhibitory receptors competing for the same ligand. Therefore, the activating capacity of Ly49 receptors only becomes apparent at a later point in the infection. In addition, it has recently been shown that triggering of the activating Ly49P receptor by the m04/MHC-I H-2D^k complex can be abolished by the engagement of the Ly49 inhibitory receptor if the target cells simultaneously express MHC-I H-2^q molecules, which strongly inhibits NK cells [66].

According to these findings, numerous activating and inhibitory Ly49 receptors share ligands for the recognition of infected cells, which points to the possibility that activating receptors originated from inhibitory ones as a host response to selective pressure by the virus. This would most certainly explain why viral proteins such as m157 and m04, which originally evolved as ligands for inhibitory NK-cell receptors, can also be recognized by their activating counterparts.

Viral modulation of NKG2D receptor functions

NKG2D is a highly evolutionarily conserved receptor expressed on all NK cells, the majority of NKT cells, subsets of γδ T cells, all human CD8$^+$ T cells, activated mouse CD8$^+$ T cells, and a subset of CD4$^+$ T cells. NKG2D is considered one of the most important NK-cell receptors, responsible for triggering NK-cell cytolytic activity as a response to cellular stress due to tumor transformation or infection (reviewed in [67]). NKG2D recognizes a wide variety of MHC-like cellular ligands, characterized by a great diversity in structure and expression patterns but with one important feature in common – low expression in healthy cells that gets induced upon the first signs of cellular stress, ensuring that the immune system receives the necessary alert signal [68]. For example, infection with a neurotropic strain of mouse hepatitis virus (MHV) increased the transcription of NKG2D ligands in the brain of infected mice, thus enabling the signaling via this activating receptor. Signaling via NKG2D was abolished by the administration of an anti-NKG2D blocking Ab resulting in an increased mortality of MHV-infected mice [69], further confirming the role of NKG2D in the control of MHV.

The importance of NKG2D in immune surveillance is probably best illustrated by several viral approaches used to neutralize the activity of this NK-cell receptor. One such successful viral tactic for immune subversion is encoding for proteins that downregulate the expression of the ligands of this activating NK-cell receptor. MCMV protein m145 affects the surface expression of

MULT-1, m152 targets the RAE-1 family, whereas m155 targets H60 [52, 54, 70]. Furthermore, surface expression of H60, MULT-1, and RAE-1ε is additionally downregulated by m138/fcr-1 (Fig. 1). The deletion of any of the above-mentioned viral genes results in decreased viral titers in the organs of infected animals, emphasizing their importance in MCMV immune evasion. We have recently shown that the insertion of the NKG2D ligand RAE-1 in place of its viral inhibitor m152 dramatically increases the susceptibility of the virus to immune control in vivo, but does not affect its capacity to induce a protective $CD8^+$ T-cell response [71].

Zoonotic orthopox viruses employ a different approach to evading the NKG2D receptor. These viruses encode for a MHC-I-like competitive antagonist of NKG2D, focusing their evasion directly on the activating receptor rather than affecting the ligand expression [72].

Other MHC-I independent immune evasion strategies of NK cells

NKp46/NCR1 is another important NK-cell activating receptor implicated in the control of various viral and bacterial infections [18, 73–76]. Several groups have reported that undefined NKp46 ligand(s) are expressed on DCs and macrophages, thus emphasizing the importance of these receptor-ligand interactions in NK-cell activation during viral infections [75, 77]. While the cellular ligand for NKp46/NCR1 remains undefined, HA and HA-neuraminidase from several viruses, including the influenza virus, are known to ligate NKp46 (reviewed in [39]). It has been shown that human NKp46 recognizes viral HA in a sialic acid-dependent manner and that glycosylation is essential for NKp46 binding to viral HA. Recently, these findings were confirmed for the mouse ortholog, NCR1 as well [78]. In addition, free HA released with new virions from infected cells can inhibit NK-cell cytotoxicity by entering the cell and inducing CD3-ζ degradation, thus providing the virus with the mechanism to avoid efficient receptor activation [79]. Namely, as NCR1 employs the CD3-ζ adaptor molecule for signal transduction, signaling from this receptor is thus efficiently blocked (Fig. 1). The importance of NCR1 in the control of influenza virus has also been confirmed in vivo [18]. $NCR1^{gfp/gfp}$ mice, in which the gene encoding for NCR1 receptor is replaced with a green fluorescent protein reporter cassette, show increased susceptibility to lethal influenza infection [18]. In contrast, our own study addressing the role of NCR1 in MCMV infection revealed a lack of difference in the early control of the virus between $Ncr1^{gfp/gfp}$ mice and control mice (unpublished data). This was a surprising and unexpected finding that led us to question whether this is a consequence of viral evasion of NCR1, or whether signaling through this receptor plays no role in the control of this virus. Bearing in mind that we have previously shown that MCMV can completely block NKG2D-dependent virus control by downregulating its cellular ligands, we have decided to assess whether, similar to the situation for NKG2D ligands, MCMV downregulates the cellular ligands for the NCR1 receptor. Indeed, binding of NCR1 fusion protein was reduced in MCMV-infected mouse fibroblasts in comparison with that of uninfected cells, indicating that MCMV downregulates the cellular ligand for this receptor (unpublished data). This finding might explain the similar levels of virus control seen when comparing WT (control) and $Ncr1^{gfp/gfp}$ mice.

Interestingly, *Noé* mice, which have a mutation in Ncr1, show a different phenotype as compared to $Ncr1^{gfp/gfp}$ mice, with *Noé* mice displaying hyperactivation of NK cells [80]. The reason for this hyperactivated phenotype is unknown, but it results in better control of viruses such as MCMV and influenza virus, thus indicating an as-yet unrevealed regulatory role for this activating receptor in NK-cell viral control. These contradictory findings might be explained by differences between $Ncr1^{gfp/gfp}$ and *Noé* mice: although both mouse strains lack surface expression of NCR1, at the DNA level the $Ncr1^{gfp/gfp}$ mice retain only a portion of the Ncr1 extracellular domain, while *Noé* mice possess a single point mutation (W32R) in an otherwise completely preserved Ncr1 gene [18, 80]. Further investigations are needed to reveal the discrepancies concerning the role of this activating NK-cell receptor.

PVR/CD155 receptor, expressed on the surface of different hematopoetic cells including monocytes and DCs, was found to be a ligand for TIGIT [81–83], CD96, and DNAM1 receptors [84, 85], which further supports the necessity of a fine balance between inhibitory and activating signals in NK-cell function. Interestingly, TIGIT possesses the highest binding affinity for PVR among the three receptors, which would imply it has a binding advantage when the expression of PVR is limited [86]. HCMV exploits this fact and encodes UL141, a protein that partially downregulates PVR at the surface of infected cells, thus enabling the virus to evade DNAM1 and CD96-mediated killing [87]. Given its high affinity toward PVR, TIGIT can still bind PVR despite being partially downregulated by HCMV, thus assuring inhibitory signaling through this receptor. Thus, one could assume that MCMV also employs a similar mechanism to avoid NK-cell activation by PVR-specific activating receptors.

In contrast to other inhibitory NK-cell receptors, which mostly recognize MHC-I molecules as their ligands, NKRP1 uses Clr-b, a C-type lectin-like molecule as the ligand of choice to exert its inhibitory function [88]. A recent study by Williams et al. [36] investigated the impact of vaccinia virus (VV) and ECTV on the expression of Clr-b on infected bone marrow-derived macrophages. The authors found that Clr-b protein was successfully downregulated at the surface of infected cells, rendering poxvirus-infected cells sensitive to NK-cell-mediated lysis [36]. Rat CMV also downregulates the cellular ligand for the NKRP1 receptor, but at the same time prevents missing self-dependent NK-cell activation by encoding a viral homolog of Clr-b [89].

Immune regulatory role of NK cells during viral infection

A number of groups have been trying to elucidate the key pathways behind NK-cell-mediated immune regulation of T-cell responses (Fig. 2). Several factors including differential kinetics and the extent of viral replication, as well as different receptor–ligand

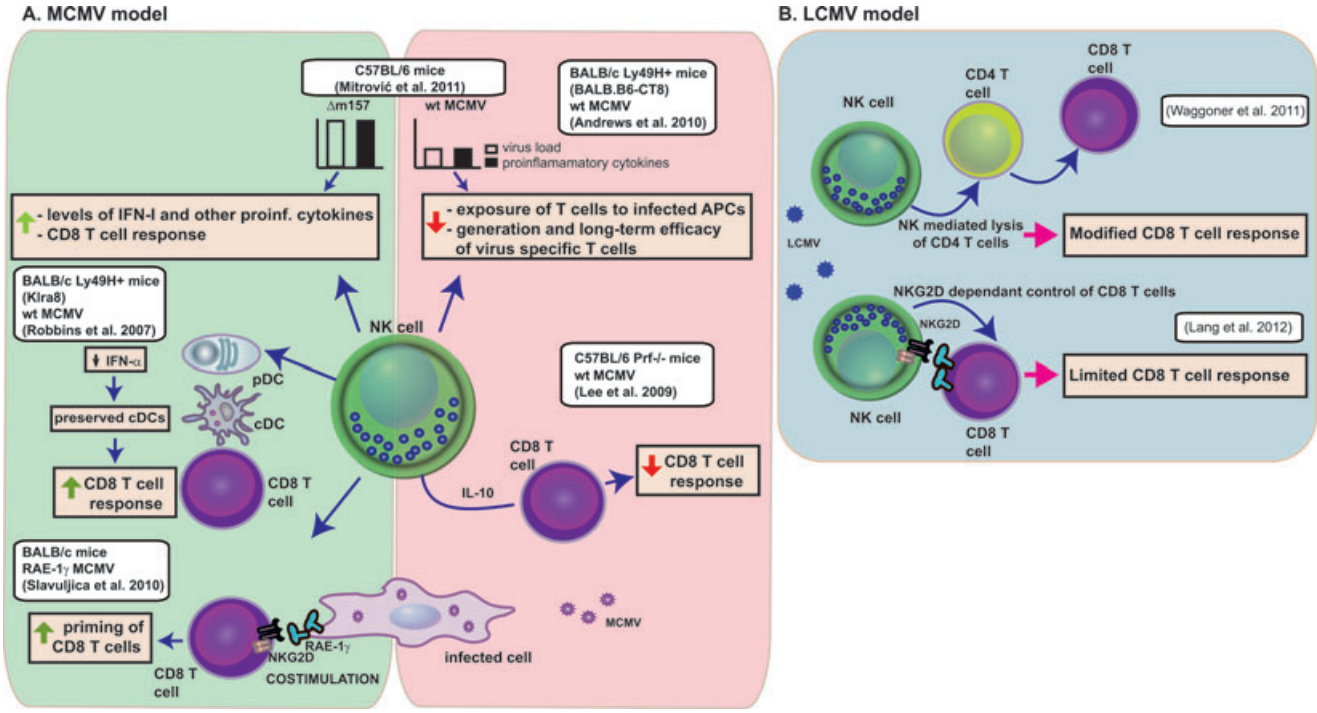

Figure 2. NK-cell-mediated regulation of CD8$^+$ T-cell responses to MCMV and LCMV infection. In addition to their already appreciated role in controlling virus replication, NK cells possess an immune regulatory potential during the formation of adaptive immunity. (A) The NK-cell response in Klra8 mice (BALB/c mice congenic for C57BL/6 NKC including the gene encoding Ly49H receptor) accelerates the CD8$^+$ T-cell response as a consequence of reduced activation of plasmacytoid DCs, decreased level of IFN-I and improved survival of cDCs (left panel middle section) [27]. An enhanced CD8$^+$ T-cell response was achieved in mice infected with MCMV expressing RAE-1γ, most likely as a consequence of costimulation of CD8$^+$ T cells by ligation of their NKG2D receptor and RAE-1γ on infected DCs (left panel bottom section) [71]. The results obtained in these two studies [27, 71], showing that improved NK-cell control positively correlates with an antiviral CD8$^+$ T-cell response, are in contrast with the results of two other studies [22, 23] using NK-cell-sensitive and NK-cell-resistant virus strains (left and right panels, upper section). Namely, in WT C57BL/6 mice and in BALB. B6-CT8 mice expressing Ly49H, Ly49H engagement with the viral protein m157 after infection with WT MCMV results in NK-cell-dependent virus control but impaired Ag presentation and reduced CD8$^+$ T-cell response (right panel, upper section) [22, 23]. In contrast, infection with MCMV lacking m157 (Δm157) results in a much higher virus load and elevated levels of proinflammatory cytokines accompanied by an improved CD8$^+$ T-cell response (left panel, upper section) [22, 23]. In MCMV-infected mice lacking perforin the CD8$^+$ T-cell response could be further suppressed by IL-10 derived from Ly49H$^+$ NK cells (right panel middle section) [24]. (B) In response to LCMV, activated NK cells regulate the CD8$^+$ T-cell response either by elimination of CD4$^+$ T cells [21] or by NKG2D-dependent limitation of the CD8$^+$ T-cell response [94].

pairs, may play a role in NK-cell regulation of specific immune responses determining the outcome of viral infection [90]. Robbins et al. [27] have shown that a strong NK-cell response to MCMV in Klra8 mice (mice on a BALB/c background congenic for the C57BL/6 NKC and thus positive for Ly49H) compared with that of control BALB/c mice, severely decreased the activation of plasmacytoid DCs (pDCs) and their production of IFN-α/β, aiding the survival of conventional DCs (cDCs) and eventually resulting in enhanced initiation of an antiviral CD8$^+$ T-cell response (Fig. 2A). In addition, the authors have shown that exogenous IFN-α administration in MCMV-resistant mice ablates cDCs and delays CD8$^+$ T-cell activation despite NK-cell control of viral replication [27]. In a different model, infection of BALB/c and C57BL/6 mice with MCMV engineered to express RAE-1γ also elicited a strong and long-lasting CD8$^+$ T-cell response despite efficient NK-cell-mediated virus control [71]. As the NKG2D receptor is also expressed on CD8$^+$ T cells, costimulation of CD8$^+$ T cells by ligation of RAE-1γ on infected DCs could explain the enhanced priming of CD8$^+$ T cells regardless of the restricted viral replication. Importantly, RAE-1γ MCMV infection preserves the frequency of cDCs and provides protection against a lethal MCMV challenge. Stadnisky et al. [25] have demonstrated the significance of the inhibitory NK-cell receptor Ly49G2 in the CD8$^+$ T-cell response to MCMV. Namely, they showed that licensed Ly49G2$^+$ NK cells support recovery of splenic cDCs and thus improve the CD8$^+$ T-cell response. NK cells were also shown to play a critical role in the initiation and shaping of the T-cell response after influenza A infection by supporting the recruitment of CD8$^+$ T cells and DCs to the posterior mediastinal lymph nodes, necessary for their priming [91]. In addition, it has been shown that NK cells influence the uptake and transport of influenza A virus by DCs in an IFN-γ and perforin-dependent manner.

In contrast to the results of Stadnisky et al. [25], Andrews et al. [23] reported that a strong NK-cell response results in a limited virus-specific CD4$^+$ and CD8$^+$ T-cell response upon MCMV infection. In addition, Lee et al. [24] suggested that IL-10 produced by Ly49H$^+$ NK cells in perforin-deficient mice is responsible for a reduced CD8$^+$ T-cell response. A more recent report reveals IL-10 involvement in CD4$^+$ T-cell priming as well, since it was shown

that, in the absence of IL-10, NK-DC crosstalk resulted in efficient priming of MCMV-specific $CD4^+$ T cells [92]. The proinflammatory cytokines IL-12, IFN-γ, and TNF-α, as well as the NK-cell activating receptors NKG2D and NCR-1, were shown to regulate this reciprocal NK/DC interplay [92]. Finally, a study performed using C57BL/6 mice showed that the $CD8^+$ T-cell response in early MCMV control inversely correlates with Ly49H-mediated virus control [22]. Mice infected with Δm157 showed elevated levels of type I IFN and several other proinflammatory cytokines as early as 1.5 days after infection, but at the same time early cDC function was preserved.

The immune regulatory role of NK cells can be particularly intriguing during infection with viruses that are otherwise resistant to NK-cell control. In the LCMV model, Waggoner et al. [21] showed that activated NK cells cytolytically eliminate helper $CD4^+$ T cells that eventually affects $CD8^+$ T-cell function [21]. Their finding of direct NK-cell-mediated lysis of $CD4^+$ T cells during LCMV infection introduces a novel mechanism by which NK cells can regulate T-cell functions, a mechanism that is in addition to contribution to the control of MCMV by lysis of virus-infected APCs, a process that in turn indirectly affects the activity of virus-specific T cells [93] (Fig. 2B). Moreover, a study by Lang et al. [94] showed that a limited T-cell response is a consequence of NKG2D and perforin-dependent regulatory NK-cell functions [94].

The big questions are not limited to how NK cells influence adaptive immunity, but also what are the conditions under which they do so, since NK cells can adjust to almost any given situation showing their great plasticity when responding to viral infection. Taking into account all the data published so far and the fact that different results are obtained by using different mouse or virus infection models, it appears that the impact of NK cells on the formation of an adaptive immune response greatly depends on the context of infection and the ability to activate NK cells through the engagement of certain activating receptors. This strongly suggests that there is no unique pathway in NK-cell regulation of the adaptive immune response.

Memory NK cells in viral infections

Even though NK cells were initially classified as exclusively part of the innate immune system, primarily due to their germline-encoded receptors and what was initially believed to be a short lifespan, recent studies have discovered some new adaptive-like NK-cell features such as the recognition of specific Ags, clonal expansion, and the formation of long-lived memory cells [95–97]. The clonal-like expansion upon recognition of specific viral Ags, a feature until now attributed only to the cells of adaptive immunity, has been reported during MCMV infection of C57BL/6 mice, in which the $Ly49H^+$ subset of NK cells plays a crucial role. The adoptive transfer of $Ly49H^+$ NK cells into DAP12–deficient hosts proved that $Ly49H^+$ NK-cell populations are fully capable of ensuring protective immunity against secondary infection following a ligand-specific expansion, which further confirms their memory-like features [96]. In addition, a recent study designated IL-12 as being important not only for achieving full NK-cell maturation and optimal expansion of virus-specific NK cells during infection, but also for the generation of a long-lived memory NK-cell population [98]. By the same token, can we expect that the ligation of other MCMV-specific Ly49 activating receptors will lead to a similar sort of virus-specific NK-cell memory? Although there is still no other evidence for MCMV-specific NK-cell memory, except the one via Ly49H receptor engagement, it is important to emphasize that even months after primary infection the majority of peripheral NK cells, including both $Ly49H^+$ and $Ly49H^-$ NK cells, display a higher proliferative capacity compared with that of NK cells in control (uninfected) mice; thus, primary antiviral immune responses induce long-term alterations in NK cells [99]. This is in line with a more recent study showing a higher expression of CD25, the high-affinity IL-2 receptor, on NK cells upon MCMV infection [100]. This finding suggests that innate cytokines released during early MCMV infection promote NK-cell proliferation even at low dose of IL-2. Further studies are required to determine whether this early induction of high affinity IL-2 receptor expression impacts subsequent memory NK-cell formation and NK-cell regulation of specific immune responses in general.

A study conducted after the Hantavirus outbreak in northern Sweden [101] demonstrated that human $NKG2C^+$ NK cells rapidly proliferate following Hanta-virus infection, and the kinetics and extent of this response can be compared with the clonal-like expansion of mouse $Ly49H^+$ NK cells during MCMV infection. Interestingly, this proliferation of $NKG2C^+$ NK cells in Hantavirus-infected individuals occurred in HCMV-seropositive, but not in HCMV-seronegative patients, suggesting that these $NKG2C^+$ NK cells may have been primed by prior recognition of HCMV [101]. Does this mean that NK cells can be primed by one pathogen and be able to have a secondary response upon exposure to a different pathogen?

It is important to mention that mouse NK-cell memory is not restricted to only MCMV. Namely, in the study conducted by Paust et al. [102] the results obtained by adoptive transfer of sensitized NK cells in mouse strains lacking T and B cells indicate that a discrete subset of liver $CXCR6^+$ NK cells acquired and retained Ag-specific memory to several distinct viral Ags. It is important to emphasize that the authors managed to show this Ag-specific response using a protocol that showed that adoptively transferred virus-sensitized NK cells enhanced the survival of recipient mice challenged with the sensitizing, but not with unrelated, virus. Another study, performed in the absence of adaptive T and B lymphocyte populations following VV infection, revealed liver $Thy1^+$ NK cells as a newly-identified NK-cell subset important for memory-like immune protection against lethal-challenge infection upon adoptive transfer of these $Thy1^+$ NK cells into VV-susceptible mice [103].

Although the discovery of NK-cell memory certainly represents a breakthrough in the field, closing the gap between innate and adaptive immunity, several questions still remained unanswered. For instance, it is still not clear if and how the specific T- and B-cell responses influence the generation of memory NK cells. In addition, we still need to identify the molecular mechanisms

behind the conversion of activated NK cells to memory cells, as well as the reason why only liver-derived NK cells can achieve a memory function.

Concluding remarks

The NK-cell response is one of the first lines of defense in fighting viral infections. Although NK cells have primarily been described as highly efficient effector cells that enable the early control of viral infection, they are now being shown to be an important link between innate and adaptive immunity, having an important immune regulatory role in both the formation of adaptive immunity and the overall outcome of infection; however, a large and increasing body of studies published in recent years in the NK-cell field leaves us with more questions than answers. NK-cell memory, the cross-talk of NK cells with other immune cells, NK-cell contribution to adaptive immunity, the constant addition of new members to the NK-cell receptor repertoire, together with as-yet unrevealed roles for the already discovered activation receptors are just some of the important issues that still need to be explored. It is likely that new approaches in our understanding of NK-cell biology in the context of viral infections will be based on genome-wide analysis of viral and NK-cell gene expression [104], as well as the use of information gained by other "omics" technologies.

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References

1 **Lanier, L. L.**, Up on the tightrope: natural killer cell activation and inhibition. *Nat. Immunol.* 2008. **9**: 495–502.

2 **Nguyen, K. B.**, **Salazar-Mather, T. P.**, **Dalod, M. Y.**, **Van Deusen, J. B.**, **Wei, X. Q.**, **Liew, F. Y.**, **Caligiuri, M. A. et al.**, Coordinated and distinct roles for IFN-alpha beta, IL-12, and IL-15 regulation of NK cell responses to viral infection. *J. Immunol.* 2002. **169**: 4279–4287.

3 **Akira, S.**, The role of IL-18 in innate immunity. *Curr. Opin. Immunol.* 2000. **12**: 59–63.

4 **Marcoe, J. P.**, **Lim, J. R.**, **Schaubert, K. L.**, **Fodil-Cornu, N.**, **Matka, M.**, **McCubbrey, A. L.**, **Farr, A. R. et al.**, TGF-beta is responsible for NK cell immaturity during ontogeny and increased susceptibility to infection during mouse infancy. *Nat. Immunol.* 2012. **13**: 843–850.

5 **Vidal, S. M.**, **Khakoo, S. I. and Biron, C. A.**, Natural killer cell responses during viral infections: flexibility and conditioning of innate immunity by experience. *Curr. Opin. Virol.* 2011. **1**: 497–512.

6 **Lodoen, M. B. and Lanier, L. L.**, Natural killer cells as an initial defense against pathogens. *Curr. Opin. Immunol.* 2006. **18**: 391–398.

7 **Scalzo, A. A.**, Successful control of viruses by NK cells—a balance of opposing forces? *Trends Microbiol.* 2002. **10**: 470–474.

8 **van Dommelen, S. L.**, **Sumaria, N.**, **Schreiber, R. D.**, **Scalzo, A. A.**, **Smyth, M. J. and Degli-Esposti, M. A.**, Perforin and granzymes have distinct roles in defensive immunity and immunopathology. *Immunity* 2006. **25**: 835–848.

9 **Sumaria, N.**, **van Dommelen, S. L.**, **Andoniou, C. E.**, **Smyth, M. J.**, **Scalzo, A. A.** and **Degli-Esposti, M. A.**, The roles of interferon-gamma and perforin in antiviral immunity in mice that differ in genetically determined NK-cell-mediated antiviral activity. *Immunol. Cell Biol.* 2009. **87**: 559–566.

10 **Orange, J. S.**, **Wang, B.**, **Terhorst, C.** and **Biron, C. A.**, Requirement for natural killer cell-produced interferon gamma in defense against murine cytomegalovirus infection and enhancement of this defense pathway by interleukin 12 administration. *J. Exp. Med.* 1995. **182**: 1045–1056.

11 **Sato, K.**, **Hida, S.**, **Takayanagi, H.**, **Yokochi, T.**, **Kayagaki, N.**, **Takeda, K.**, **Yagita, H. et al.**, Antiviral response by natural killer cells through TRAIL gene induction by IFN-alpha/beta. *Eur. J. Immunol.* 2001. **31**: 3138–3146.

12 **Trinchieri, G.**, Biology of natural killer cells. *Adv. Immunol.* 1989. **47**: 187–376.

13 **Santoni, A.**, **Carlino, C.** and **Gismondi, A.**, Uterine NK cell development, migration and function. *Reprod. Biomed. Online* 2008. **16**: 202–210.

14 **Jonjic, S.**, **Mutter, W.**, **Weiland, F.**, **Reddehase, M. J.** and **Koszinowski, U. H.**, Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes. *J. Exp. Med.* 1989. **169**: 1199–1212.

15 **McWhorter, A. R.**, **Smith, L. M.**, **Masters, L. L.**, **Chan, B.**, **Shellam, G. R.** and **Redwood, A. J.**, Natural Killer cell dependent within-host competition arises during multiple MCMV infection: consequences for viral transmission and evolution. *PLoS Pathog.* 2013. **9**: e1003111.

16 **Carroll, V. A.**, **Lundgren, A.**, **Wei, H.**, **Sainz, S.**, **Tung, K. S.** and **Brown, M. G.**, Natural killer cells regulate murine cytomegalovirus-induced sialadenitis and salivary gland disease. *J. Virol.* 2012. **86**: 2132–2142.

17 **Li, F.**, **Zhu, H.**, **Sun, R.**, **Wei, H.** and **Tian, Z.**, Natural killer cells are involved in acute lung immune injury caused by respiratory syncytial virus infection. *J. Virol.* 2012. **86**: 2251–2258.

18 **Gazit, R.**, **Gruda, R.**, **Elboim, M.**, **Arnon, T. I.**, **Katz, G.**, **Achdout, H.**, **Hanna, J. et al.**, Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1. *Nat. Immunol.* 2006. **7**: 517–523.

19 **Abdul-Careem, M. F.**, **Mian, M. F.**, **Yue, G.**, **Gillgrass, A.**, **Chenoweth, M. J.**, **Barra, N. G.**, **Chew, M. V. et al.**, Critical role of natural killer cells in lung immunopathology during influenza infection in mice. *J. Infect. Dis.* 2012. **206**: 167–177.

20 **Bukowski, J. F.**, **Woda, B. A.**, **Habu, S.**, **Okumura, K.** and **Welsh, R. M.**, Natural killer cell depletion enhances virus synthesis and virus-induced hepatitis in vivo. *J. Immunol.* 1983. **131**: 1531–1538.

21 **Waggoner, S. N.**, **Cornberg, M.**, **Selin, L. K.** and **Welsh, R. M.**, Natural killer cells act as rheostats modulating antiviral T cells. *Nature* 2011. **481**: 394–398.

22 **Mitrovic, M.**, **Arapovic, J.**, **Jordan, S.**, **Fodil-Cornu, N.**, **Ebert, S.**, **Vidal, S. M.**, **Krmpotic, A. et al.**, The NK cell response to mouse cytomegalovirus infection affects the level and kinetics of the early CD8(+) T-cell response. *J. Virol.* 2011. **86**: 2165–2175.

23 **Andrews, D. M.**, **Estcourt, M. J.**, **Andoniou, C. E.**, **Wikstrom, M. E.**, **Khong, A.**, **Voigt, V.**, **Fleming, P. et al.**, Innate immunity defines the capacity of antiviral T cells to limit persistent infection. *J. Exp. Med.* 2010. **207**: 1333–1343.

24 **Lee, S. H.**, **Kim, K. S.**, **Fodil-Cornu, N.**, **Vidal, S. M.** and **Biron, C. A.**, Activating receptors promote NK cell expansion for maintenance, IL-10 production, and CD8 T cell regulation during viral infection. *J. Exp. Med.* 2009. **206**: 2235–2251.

25 **Stadnisky, M. D.**, **Xie, X.**, **Coats, E. R.**, **Bullock, T. N.** and **Brown, M. G.**, Self MHC class I licensed NK cells enhance adaptive CD8 T cell viral immunity. *Blood* 2011. **117**: 5133–5141.

26 **Su, H. C.**, **Nguyen, K. B.**, **Salazar-Mather, T. P.**, **Ruzek, M. C.**, **Dalod, M. Y.** and **Biron, C. A.**, NK cell functions restrain T cell responses during viral infections. *Eur. J. Immunol.* 2001. **31**: 3048–3055.

27 **Robbins, S. H.**, **Bessou, G.**, **Cornillon, A.**, **Zucchini, N.**, **Rupp, B.**, **Ruzsics, Z.**, **Sacher, T. et al.**, Natural killer cells promote early CD8 T cell responses against cytomegalovirus. *PLoS Pathog.* 2007. **3**: e123.

28 **Igarashi, H.**, **Gregory, S. C.**, **Yokota, T.**, **Sakaguchi, N.** and **Kincade, P. W.**, Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity* 2002. **17**: 117–130.

29 **Borghesi, L.**, **Hsu, L. Y.**, **Miller, J. P.**, **Anderson, M.**, **Herzenberg, L.**, **Schlissel, M. S.**, **Allman, D. et al.**, B lineage-specific regulation of V(D)J recombinase activity is established in common lymphoid progenitors. *J. Exp. Med.* 2004. **199**: 491–502.

30 **Natarajan, K.**, **Dimasi, N.**, **Wang, J.**, **Mariuzza, R. A.** and **Margulies, D. H.**, Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination. *Annu. Rev. Immunol.* 2002. **20**: 853–885.

31 **Yokoyama, W. M.** and **Plougastel, B. F.**, Immune functions encoded by the natural killer gene complex. *Nat. Rev. Immunol.* 2003. **3**: 304–316.

32 **Pyzik, M.**, **Gendron-Pontbriand, E. M.** and **Vidal, S. M.**, The impact of Ly49-NK cell-dependent recognition of MCMV infection on innate and adaptive immune responses. *J. Biomed. Biotechnol.* 2011. **2011**: 641702.

33 **Ortaldo, J. R.** and **Young, H. A.**, Mouse Ly49 NK receptors: balancing activation and inhibition. *Mol. Immunol.* 2005. **42**: 445–450.

34 **Lanier, L. L.**, NK cell recognition. *Annu. Rev. Immunol.* 2005. **23**: 225–274.

35 **Fang, M.**, **Orr, M. T.**, **Spee, P.**, **Egebjerg, T.**, **Lanier, L. L.** and **Sigal, L. J.**, CD94 is essential for NK cell-mediated resistance to a lethal viral disease. *Immunity* 2011. **34**: 579–589.

36 **Williams, K. J.**, **Wilson, E.**, **Davidson, C. L.**, **Aguilar, O. A.**, **Fu, L.**, **Carlyle, J. R.** and **Burshtyn, D. N.**, Poxvirus infection-associated downregulation of C-type lectin-related-b prevents NK cell inhibition by NK receptor protein-1B. *J. Immunol.* 2012. **188**: 4980–4991.

37 **Carlyle, J. R.**, **Jamieson, A. M.**, **Gasser, S.**, **Clingan, C. S.**, **Arase, H.** and **Raulet, D. H.**, Missing self-recognition of Ocil/Clr-b by inhibitory NKR-P1 natural killer cell receptors. *Proc. Natl. Acad. Sci. USA* 2004. **101**: 3527–3532.

38 **Zafirova, B.**, **Wensveen, F. M.**, **Gulin, M.** and **Polic, B.**, Regulation of immune cell function and differentiation by the NKG2D receptor. *Cell. Mol. Life Sci.* 2011. **68**: 3519–3529.

39 **Seidel, E.**, **Glasner, A.** and **Mandelboim, O.**, Virus-mediated inhibition of natural cytotoxicity receptor recognition. *Cell. Mol. Life Sci.* 2012. **69**: 3911–3920.

40 **Stanietsky, N.** and **Mandelboim, O.**, Paired NK cell receptors controlling NK cytotoxicity. *FEBS Lett.* 2010. **584**: 4895–4900.

41 **Lanier, L. L.**, NK cell receptors. *Annu. Rev. Immunol.* 1998. **16**: 359–393.

42 **Karre, K.**, **Ljunggren, H. G.**, **Piontek, G.** and **Kiessling, R.**, Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* 1986. **319**: 675–678.

43 **Hengel, H.**, **Reusch, U.**, **Gutermann, A.**, **Ziegler, H.**, **Jonjic, S.**, **Lucin, P.** and **Koszinowski, U. H.**, Cytomegaloviral control of MHC class I function in the mouse. *Immunol. Rev.* 1999. **168**: 167–176.

44 **Ziegler, H.**, **Thäle, R.**, **Lucin, P.**, **Muranyi, W.**, **Flohr, T.**, **Hengel, H.**, **Farrell, H. et al.**, A mouse cytomegalovirus glycoprotein retains MHC class I complexes in the ERGIC/cis-Golgi compartments. *Immunity* 1997. **6**: 57–66.

45 **Reusch, U.**, **Muranyi, W.**, **Lucin, P.**, **Burgert, H. G.**, **Hengel, H.** and **Koszinowski, U. H.**, A cytomegalovirus glycoprotein re-routes MHC class I complexes to lysosomes for degradation. *EMBO J.* 1999. **18**: 1081–1091.

46 **Kleijnen, M. F.**, **Huppa, J. B.**, **Lucin, P.**, **Mukherjee, S.**, **Farrell, H.**, **Campbell, A. E.**, **Koszinowski, U. H. et al.**, A mouse cytomegalovirus glycoprotein, gp34, forms a complex with folded class I MHC molecules in the ER which is not retained but is transported to the cell surface. *EMBO J.* 1997. **16**: 685–694.

47 **Holtappels, R.**, **Gillert-Marien, D.**, **Thomas, D.**, **Podlech, J.**, **Deegen, P.**, **Herter, S.**, **Oehrlein-Karpi, S. A. et al.**, Cytomegalovirus encodes a positive regulator of antigen presentation. *J. Virol.* 2006. **80**: 7613–7624.

48 **Babic, M.**, **Pyzik, M.**, **Zafirova, B.**, **Mitrovic, M.**, **Butorac, V.**, **Lanier, L. L.**, **Krmpotic, A. et al.**, Cytomegalovirus immunoevasin reveals the physiological role of "missing self" recognition in natural killer cell dependent virus control in vivo. *J. Exp. Med.* 2010. **207**: 2663–2673.

49 **Byun, M.**, **Verweij, M. C.**, **Pickup, D. J.**, **Wiertz, E. J.**, **Hansen, T. H.** and **Yokoyama, W. M.**, Two mechanistically distinct immune evasion proteins of cowpox virus combine to avoid antiviral CD8 T cells. *Cell Host Microbe* 2009. **6**: 422–432.

50 **Byun, M.**, **Wang, X.**, **Pak, M.**, **Hansen, T. H.** and **Yokoyama, W. M.**, Cowpox virus exploits the endoplasmic reticulum retention pathway to inhibit MHC class I transport to the cell surface. *Cell Host Microbe* 2007. **2**: 306–315.

51 **Smith, H. R.**, **Heusel, J. W.**, **Mehta, I. K.**, **Kim, S.**, **Dorner, B. G.**, **Naidenko, O. V.**, **Iizuka, K. et al.**, Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc. Natl. Acad. Sci. USA* 2002. **99**: 8826–8831.

52 **Jonjic, S.**, **Babic, M.**, **Polic, B.** and **Krmpotic, A.**, Immune evasion of natural killer cells by viruses. *Curr. Opin. Immunol.* 2008. **20**: 30–38.

53 **Arase, H.**, **Mocarski, E. S.**, **Campbell, A. E.**, **Hill, A. B.** and **Lanier, L. L.**, Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* 2002. **296**: 1323–1326.

54 **Revilleza, M. J.**, **Wang, R.**, **Mans, J.**, **Hong, M.**, **Natarajan, K.** and **Margulies, D. H.**, How the virus outsmarts the host: function and structure of cytomegalovirus MHC-I-like molecules in the evasion of natural killer cell surveillance. *J. Biomed. Biotechnol.* 2011. **2011**: 724607.

55 **Vidal, S.**, **Krmpotic, A.**, **Pyzik, M.** and **Jonjic, S.**, Innate immunity to cytomegalovirus in the murine model. In Reddehase, M. J. (Ed.), *Cytomegaloviruses — From Molecular Pathogenesis to Intervention*. Caister Academic Press, Norwich 2013.

56 **Kim, S.**, **Poursine-Laurent, J.**, **Truscott, S. M.**, **Lybarger, L.**, **Song, Y. J.**, **Yang, L.**, **French, A. R. et al.**, Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* 2005. **436**: 709–713.

57 **Fernandez, N. C.**, **Treiner, E.**, **Vance, R. E.**, **Jamieson, A. M.**, **Lemieux, S.** and **Raulet, D. H.**, A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood* 2005. **105**: 4416–4423.

58 **Orr, M. T.**, **Murphy, W. J.** and **Lanier, L. L.**, 'Unlicensed' natural killer cells dominate the response to cytomegalovirus infection. *Nat. Immunol.* 2010. **11**: 321–327.

59 **Akkaya, M.** and **Barclay, A. N.**, How do pathogens drive the evolution of paired receptors? *Eur. J. Immunol.* 2013. **43**: 303–313.

60 **Daniels, K. A.**, **Devora, G.**, **Lai, W. C.**, **O'Donnell, C. L.**, **Bennett, M.** and **Welsh, R. M.**, Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. *J. Exp. Med.* 2001. **194**: 29–44.

61 **Brown, M. G.**, **Dokun, A. O.**, **Heusel, J. W.**, **Smith, H. R.**, **Beckman, D. L.**, **Blattenberger, E. A.**, **Dubbelde, C. E. et al.**, Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science* 2001. **292**: 934–937.

62 **Lee, S. H.**, **Girard, S.**, **Macina, D.**, **Busa, M.**, **Zafer, A.**, **Belouchi, A.**, **Gros, P. et al.**, Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily. *Nat. Genet.* 2001. **28**: 42–45.

63 **Adams, E. J.**, **Juo, Z. S.**, **Venook, R. T.**, **Boulanger, M. J.**, **Arase, H.**, **Lanier, L. L.** and **Garcia, K. C.**, Structural elucidation of the m157 mouse cytomegalovirus ligand for Ly49 natural killer cell receptors. *Proc. Natl. Acad. Sci. USA* 2007. **104**: 10128–10133.

64 **Kielczewska, A.**, **Pyzik, M.**, **Sun, T.**, **Krmpotic, A.**, **Lodoen, M. B.**, **Munks, M. W.**, **Babic, M. et al.**, Ly49P recognition of cytomegalovirus-infected cells expressing H2-Dk and CMV-encoded m04 correlates with the NK cell antiviral response. *J. Exp. Med.* 2009. **206**: 515–523.

65 **Pyzik, M.**, **Charbonneau, B.**, **Gendron-Pontbriand, E. M.**, **Babic, M.**, **Krmpotic, A.**, **Jonjic, S.** and **Vidal, S. M.**, Distinct MHC class I-dependent NK cell-activating receptors control cytomegalovirus infection in different mouse strains. *J. Exp. Med.* 2011. **208**: 1105–1117.

66 **Fodil-Cornu, N.**, **Loredo-Osti, J. C.** and **Vidal, S. M.**, NK cell receptor/H2-Dk-dependent host resistance to viral infection is quantitatively modulated by H2q inhibitory signals. *PLoS Genet.* 2011. **7**: e1001368.

67 **Champsaur, M.** and **Lanier, L. L.**, Effect of NKG2D ligand expression on host immune responses. *Immunol. Rev.* 2010. **235**: 267–285.

68 **Cerwenka, A.** and **Lanier, L. L.**, NKG2D ligands: unconventional MHC class I-like molecules exploited by viruses and cancer. *Tissue Antigens* 2003. **61**: 335–343.

69 **Walsh, K. B.**, **Lanier, L. L.** and **Lane, T. E.**, NKG2D receptor signaling enhances cytolytic activity by virus-specific CD8+ T cells: evidence for a protective role in virus-induced encephalitis. *J. Virol.* 2008. **82**: 3031–3044.

70 **Lisnic, V. J.**, **Krmpotic, A.** and **Jonjic, S.**, Modulation of natural killer cell activity by viruses. *Curr. Opin. Microbiol.* 2010. **13**: 530–539.

71 **Slavuljica, I.**, **Busche, A.**, **Babic, M.**, **Mitrovic, M.**, **Gasparovic, I.**, **Cekinovic, D.**, **Markova Car, E. et al.**, Recombinant mouse cytomegalovirus expressing a ligand for the NKG2D receptor is attenuated and has improved vaccine properties. *J. Clin. Invest.* 2010. **120**: 4532–4545.

72 **Campbell, J. A.**, **Trossman, D. S.**, **Yokoyama, W. M.** and **Carayannopoulos, L. N.**, Zoonotic orthopoxviruses encode a high-affinity antagonist of NKG2D. *J. Exp. Med.* 2007. **204**: 1311–1317.

73 **Mandelboim, O.**, **Lieberman, N.**, **Lev, M.**, **Paul, L.**, **Arnon, T. I.**, **Bushkin, Y.**, **Davis, D. M. et al.**, Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* 2001. **409**: 1055–1060.

74 **Vankayalapati, R.**, **Wizel, B.**, **Weis, S. E.**, **Safi, H.**, **Lakey, D. L.**, **Mandelboim, O.**, **Samten, B. et al.**, The NKp46 receptor contributes to NK cell lysis of mononuclear phagocytes infected with an intracellular bacterium. *J. Immunol.* 2002. **168**: 3451–3457.

75 **Magri, G.**, **Muntasell, A.**, **Romo, N.**, **Saez-Borderias, A.**, **Pende, D.**, **Geraghty, D. E.**, **Hengel, H. et al.**, NKp46 and DNAM-1 NK-cell receptors drive the response to human cytomegalovirus-infected myeloid dendritic cells overcoming viral immune evasion strategies. *Blood* 2010. **117**: 848–856.

76 **Moretta, A.**, **Biassoni, R.**, **Bottino, C.**, **Mingari, M. C.** and **Moretta, L.**, Natural cytotoxicity receptors that trigger human NK-cell-mediated cytolysis. *Immunol. Today* 2000. **21**: 228–234.

77 **Elhaik-Goldman, S.**, **Kafka, D.**, **Yossef, R.**, **Hadad, U.**, **Elkabets, M.**, **Vallon-Eberhard, A.**, **Hulihel, L. et al.**, The natural cytotoxicity receptor 1 contribution to early clearance of Streptococcus pneumoniae and to natural killer-macrophage cross talk. *PLoS One* 2011. **6**: e23472.

78 **Glasner, A.**, **Zurunic, A.**, **Meningher, T.**, **Lenac Rovis, T.**, **Tsukerman, P.**, **Bar-On, Y.**, **Yamin, R. et al.**, Elucidating the mechanisms of influenza virus recognition by Ncr1. *PLoS One* 2012. **7**: e36837.

79 **Mao, H.**, **Tu, W.**, **Liu, Y.**, **Qin, G.**, **Zheng, J.**, **Chan, P. L.**, **Lam, K. T. et al.**, Inhibition of human natural killer cell activity by influenza virions and hemagglutinin. *J. Virol.* 2010. **84**: 4148–4157.

80 **Narni-Mancinelli, E.**, **Jaeger, B. N.**, **Bernat, C.**, **Fenis, A.**, **Kung, S.**, **De Gassart, A.**, **Mahmood, S. et al.**, Tuning of natural killer cell reactivity by NKp46 and Helios calibrates T cell responses. *Science* 2012. **335**: 344–348.

81 **Yu, X.**, **Harden, K.**, **Gonzalez, L. C.**, **Francesco, M.**, **Chiang, E.**, **Irving, B.**, **Tom, I. et al.**, The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat. Immunol.* 2009. **10**: 48–57.

82 **Stanietsky, N.**, **Simic, H.**, **Arapovic, J.**, **Toporik, A.**, **Levy, O.**, **Novik, A.**, **Levine, Z. et al.**, The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc. Natl. Acad. Sci. USA* 2009. **106**: 17858–17863.

83 **Boles, K. S.**, **Vermi, W.**, **Facchetti, F.**, **Fuchs, A.**, **Wilson, T. J.**, **Diacovo, T. G.**, **Cella, M. et al.**, A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC. *Eur. J. Immunol.* 2009. **39**: 695–703.

84 **Bottino, C.**, **Castriconi, R.**, **Pende, D.**, **Rivera, P.**, **Nanni, M.**, **Carnemolla, B.**, **Cantoni, C. et al.**, Identification of PVR (CD155) and Nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. *J. Exp. Med.* 2003. **198**: 557–567.

85 **Fuchs, A.**, **Cella, M.**, **Giurisato, E.**, **Shaw, A. S.** and **Colonna, M.**, Cutting edge: CD96 (tactile) promotes NK cell-target cell adhesion by interacting with the poliovirus receptor (CD155). *J. Immunol.* 2004. **172**: 3994–3998.

86 **Stanietsky, N.** and **Mandelboim, O.**, Paired NK cell receptors controlling NK cytotoxicity. *FEBS Lett.* 2010. **584**: 4895–4900.

87 **Tomasec, P.**, **Wang, E. C.**, **Davison, A. J.**, **Vojtesek, B.**, **Armstrong, M.**, **Griffin, C.**, **McSharry, B. P. et al.**, Downregulation of natural killer cell-activating ligand CD155 by human cytomegalovirus UL141. *Nat. Immunol.* 2005. **6**: 181–188.

88 **Iizuka, K.**, **Naidenko, O. V.**, **Plougastel, B. F.**, **Fremont, D. H.** and **Yokoyama, W. M.**, Genetically linked C-type lectin-related ligands for the NKRP1 family of natural killer cell receptors. *Nat. Immunol.* 2003. **4**: 801–807.

89 **Voigt, S.**, **Mesci, A.**, **Ettinger, J.**, **Fine, J. H.**, **Chen, P.**, **Chou, W.** and **Carlyle, J. R.**, Cytomegalovirus evasion of innate immunity by subversion of the NKR-P1B:Clr-b missing-self axis. *Immunity* 2007. **26**: 617–627.

90 **Biron, C. A.**, Yet another role for natural killer cells: cytotoxicity in immune regulation and viral persistence. *Proc. Natl. Acad. Sci. USA* 2012. **109**: 1814–1815.

91 **Ge, M. Q.**, **Ho, A. W.**, **Tang, Y.**, **Wong, K. H.**, **Chua, B. Y.**, **Gasser, S.** and **Kemeny, D. M.**, NK cells regulate CD8+ T cell priming and dendritic

cell migration during influenza A infection by IFN-gamma and perforin-dependent mechanisms. *J. Immunol.* 2012. **189**: 2099–2109.

92 **Mandaric, S.**, **Walton, S. M.**, **Rulicke, T.**, **Richter, K.**, **Girard-Madoux, M. J.**, **Clausen, B. E.**, **Zurunic, A. et al.**, IL-10 suppression of NK/DC crosstalk leads to poor priming of MCMV-specific CD4 T cells and prolonged MCMV persistence. *PLoS Pathog.* 2012. **8**: e1002846.

93 **Andrews, D. M.**, **Andoniou, C. E.**, **Granucci, F.**, **Ricciardi-Castagnoli, P.** and **Degli-Esposti, M. A.**, Infection of dendritic cells by murine cytomegalovirus induces functional paralysis. *Nat. Immunol.* 2001. **2**: 1077–1084.

94 **Lang, P. A.**, **Lang, K. S.**, **Xu, H. C.**, **Grusdat, M.**, **Parish, I. A.**, **Recher, M.**, **Elford, A. R. et al.**, Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8+ T-cell immunity. *Proc. Natl. Acad. Sci. USA* 2012. **109**: 1210–1215.

95 **O'Leary, J. G.**, **Goodarzi, M.**, **Drayton, D. L.** and **von Andrian, U. H.**, T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat. Immunol.* 2006. **7**: 507–516.

96 **Sun, J. C.**, **Beilke, J. N.** and **Lanier, L. L.**, Adaptive immune features of natural killer cells. *Nature* 2009. **457**: 557–561.

97 **Cooper, M. A.**, **Elliott, J. M.**, **Keyel, P. A.**, **Yang, L.**, **Carrero, J. A.** and **Yokoyama, W. M.**, Cytokine-induced memory-like natural killer cells. *Proc. Natl. Acad. Sci. USA* 2009. **106**: 1915–1919.

98 **Sun, J. C.**, **Madera, S.**, **Bezman, N. A.**, **Beilke, J. N.**, **Kaplan, M. H.** and **Lanier, L. L.**, Proinflammatory cytokine signaling required for the generation of natural killer cell memory. *J. Exp. Med.* 2012. **209**: 947–954.

99 **Busche, A.**, **Schmitz, S.**, **Fleige, H.**, **Robbins, S. H.**, **Walzer, T.**, **Stewart, C. A.**, **Forster, R. et al.**, Genetic labeling reveals altered turnover and stability of innate lymphocytes in latent mouse cytomegalovirus infection. *J. Immunol.* 2011. **186**: 2918–2925.

100 **Lee, S. H.**, **Fragoso, M. F.** and **Biron, C. A.**, Cutting edge: a novel mechanism bridging innate and adaptive immunity: IL-12 induction of CD25 to form high-affinity IL-2 receptors on NK cells. *J. Immunol.* 2012. **189**: 2712–2716.

101 **Bjorkstrom, N. K.**, **Lindgren, T.**, **Stoltz, M.**, **Fauriat, C.**, **Braun, M.**, **Evander, M.**, **Michaelsson, J. et al.**, Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. *J. Exp. Med.* 2011. **208**: 13–21.

102 **Paust, S.**, **Gill, H. S.**, **Wang, B. Z.**, **Flynn, M. P.**, **Moseman, E. A.**, **Senman, B.**, **Szczepanik, M. et al.**, Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat. Immunol.* 2010. **11**: 1127–1135.

103 **Gillard, G. O.**, **Bivas-Benita, M.**, **Hovav, A. H.**, **Grandpre, L. E.**, **Panas, M. W.**, **Seaman, M. S.**, **Haynes, B. F. et al.**, Thy1+ NK [corrected] cells from vaccinia virus-primed mice confer protection against vaccinia virus challenge in the absence of adaptive lymphocytes. *PLoS Pathog.* 2011. **7**: e1002141.

104 **Bezman, N. A.**, **Kim, C. C.**, **Sun, J. C.**, **Min-Oo, G.**, **Hendricks, D. W.**, **Kamimura, Y.**, **Best, J. A. et al.**, Molecular definition of the identity and activation of natural killer cells. *Nat. Immunol.* 2012. **13**: 1000–1009.

Abbreviations: **cDC**: conventional DC · **ECTV**: orthopoxvirus ectromelia virus · **LCMV**: lymphocytic choriomeningitis virus · **MCMV**: mouse cytomegalovirus · **NKC**: Natural Killer gene Complex · **PVR**: poliovirus receptor · **VV**: vaccinia virus

Full correspondence: Prof. Stipan Jonjić, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, B. Branchetta 20, 51 000 Rijeka, Croatia
Fax: +385-51-651-176
e-mail: stipan.jonjic@medri.uniri.hr

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