Mechanisms regulating NK cell activation during viral infection.

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Abstract:

Natural killer (NK) cells constitute a population of lymphocytes involved in innate immune functions, they play a critical role in anti-viral immune surveillance. Viruses have evolved with their host species for millions of years, each exerting a selective pressure upon the other. As a corollary, the pathways used by the immune system that are critical to control viral infection can be revealed by defining the role of viral gene products that are non-essential for virus replication. We relate here the battery of resources available to NK cells to recognize and eliminate viruses and reciprocally the immune evasion mechanisms developed by viruses to prevent NK cell activation.

Introduction:

Viruses and the hosts' immune system have exerted reciprocal selective pressure for eons in their mutual race to outsmart each other. Recurrent viral infections in individuals harboring genetic mutations resulting in Natural Killer (NK) cell function deficiencies proved the important role played by these cells in anti-viral immune surveillance[1, 2]. NK cells are lymphocytes of the innate immune system; unlike T and B lymphocytes, they do not express recombinant antigen receptors and do not require priming by antigen presenting cells (APC) for activation. Instead their activation is regulated by the integration of signals generated by germline encoded activating and inhibitory receptors, adhesion molecules and by sensors of inflammation (cytokine receptors, toll-like receptors (TLR)). Recent findings have provided a clearer understanding of NK cell biology and their role in anti viral immune response. This review summarizes the mechanisms of NK cell regulation, the activation pathways relevant to NK cell recognition of virus infected-cells and the multiple immune evasion mechanisms developed by viruses, including the most recent insights into the strategies used by cytomegalovirus.

NK cell functions

NK cells play a critical role under normal physiological conditions as they monitor for and remove transformed cells, thereby preventing tumour development and they are involved in pregnancy where they promote placentation[1]. In addition, their ability to recognize cells with altered MHC class I (MHC I) expression results in a potent activity in transplantation settings. In pathological conditions, their immune surveillance function is crucial against bacterial, parasitic and viral infections. Mice with impaired NK cells display an increased susceptibility to viruses; a key role of NK cells in viral immune surveillance has been seen against many human viruses (human immunodeficiency virus, influenza virus, hepatitis B and C viruses, coxsackievirus, poxvirus, flaviviridae viruses, herpesviridae family viruses). Once activated, NK cells secrete several cytokines and chemokines (such as IFN- γ , TNF- α , MIP1- α/β , GM-CSF, IL10) and kill target cells without requiring a preliminary step of selection and expansion. Cytotoxicity is mediated via various mechanisms such as release of cytolytic granules (perforin and granzymes) and signals transmitted through members of the TNF death receptor family (FasL, TRAIL).

NK cell receptors and regulation of NK cell functions

NK cells were for a long time only known for their ability to sense and eliminate cells with reduced MHC I expression, like tumor and virus-infected cells. This function is mediated by inhibitory receptors specific for MHC I; abnormal cells with reduced MHC I expression hardly engage these receptors, which results in weak inhibitory signals that fail to interfere with NK cell activation. This phenomenon known as "missing self recognition"[3] provided an explanation as to how NK cell functions are inhibited but not how they are activated. This regulation model implied that either NK cells are constitutively activated and will kill any cells they encounter that fail to display a satisfactory set of ligands for inhibitory receptors, or NK cells express alternate receptors inducing activating signals that are subject to regulation by inhibitory receptors. Studies undertaken during the last two decades provided a more precise understanding of NK cell biology. We now know that NK cells possess an array of receptors with a broad range of specificities and that are either activating or inhibitory; integration of their respective signals orchestrates NK cell functions.

NK cell receptors belong to two distinct structural families: the immunoglobulin (Ig)-like superfamily encoded on human chromosome 19 (7 in mouse) within the leukocyte receptor complex (LRC) and the C-type lectin superfamily clustered within the NK gene complex (NKC) on

chromosome 12 (6 in mouse)[4, 5]. Activating receptors possess a short intracellular domain; intracellular signals are mediated by immunoreceptor tyrosine based activation motifs (ITAM)-containing adaptor proteins that associate with the receptor's transmembrane domain charged residues. Engagement of inhibitory receptors results in the phosphorylation of one or more immuno-receptor tyrosine-based inhibition motifs (ITIM) displayed in the intracytoplasmic domain. ITIM phosphorylation recruits and activates Src homology 2 (SH2) domain-containing phosphatase-1 (SHP-1) or SHP-2; these phosphatases interrupt the activation signals by dephosphorylating essential signalling intermediates such as Vav1[6]. Inhibitory receptor signalling can also lead to adaptor protein Crk phosphorylation, which when associated with c-Abl is sequestered from activation signalling complexes[7].

MHC I specific NK cell receptors. KIRs are human NK cell receptors that belong to the Ig-like family, they recognize HLA A/B/C molecules while classical MHC I molecules in mouse are recognized by the C-type lectin Ly49 receptor family[8]. Although KIR and Ly49 sequences are strikingly divergent, they have each evolved through a process of convergent evolution to perform nearly identical functions by utilizing similar signalization and sharing comparable mechanisms that regulate their expression. In addition to KIRs and Ly49s, the CD94-NKG2A/C/or E heterodimeric receptors recognize non-classical HLA-E in human and Qa-1 in mouse, while inhibitory Leukocyte Ig-like receptor (LILR) 1 recognize HLA-G[8]. Members of the KIR, Ly49 and CD94-NKG2 families are composed of both activating and inhibitory receptors.

MHC I homolog-specific NK cell receptors. NKG2D is an activating C-type lectin-like receptor present on all NK cells; it binds various MHC I-homologous ligands (MICA/B and ULBP1-6 in human; RAE-1 α - γ , H60a-c and MULT1 in mouse) that are induced under various conditions of cellular stress and tumoral transformation[9]. Ly49H is an activating receptor that recognizes mouse cytomegalovirus (MCMV) m157 glycoprotein, which is structurally related to MHC I[10, 11].

Activating NK cell receptors specific for other types of ligands. Human natural cytotoxicity receptors (NCR) comprise NKp30, NKp44 and NKp46; they were named for their ability to trigger NK cell cytotoxic activity[12]. NKp30 is expressed on some NK cell subsets while all NK cells possess NKp46; both receptors are constitutively expressed. Conversely, NKp44 expression is induced on limited NK cell subsets upon activation. NCRs recognize ligands expressed on transformed cells and virus-infected cells[13]. The NCR repertoire is more restricted in mice with only one identified member, NKp46[13]. Another activating receptor, DNAM-1 binds CD155 and CD122, both ligands are expressed on tumor cells and virus-infected cells[14, 15]. 2B4 is a receptor implicated in anti-viral immunity[16]. Engagement of 2B4 by its ligand, CD48, mediates activating signals in human whereas it acts mostly as an inhibitory receptor in mouse; the nature of the signals engendered by 2B4 depends on the adaptor molecule it is associated with[16]. NK cells are also capable of antibody-dependent cell cytotoxicity (ADCC) upon interaction of their activating receptor CD16 (Fc γ RIIIA) with cells opsonized with IgG. Toll-like receptors (TLRs) constitute a family of receptors that recognize conserved pathogens-associated molecular patterns. TLR family members are constitutively expressed by NK cells and synergize with chemokines and cytokines receptors activating signals. TLRs were found to trigger and regulate human and mouse NK cell responses following bacterial and viral infection and to be responsible for the early production of type 1 IFN[17].

Although some of the receptors that constitute human and mouse NK cell repertoire are different between the two species, accumulating evidence demonstrate that these receptors provide similar specific functions through similar mechanisms, indicating that a convergent evolution has occurred. Selective pressure, at least partly due to infection by viruses, may have been responsible for the acquisition of distinct genes with a high degree of polymorphism. KIR family members display a strong degree of similarity; the same is true for Ly49s[18, 19]. Recombination between distinct

receptor member genes could be a key mechanism for the generation of novel receptors while some receptors appear to result from gene duplication and subsequent evolution.

Role of receptors during NK cell education

NK cell specificity and reactivity is acquired in developing cells in a process termed education. Every individual possesses a different set of receptor genes, and each NK cell expresses an array of different receptors with various functions and specificities; in addition, the frequency of NK cells expressing each of these receptors varies considerably between individuals. The diversity of KIR and Ly49 repertoire is further compounded by inheritance of different haplotypes and allelic polymorphism. It is recognized that distinct receptors are acquired sequentially and in a non-random order during NK cell development[20]. Comparative studies in WT mice and mice with reduced MHC I expression such as beta 2-microglobulin (β 2-m) knock out (KO) and TAP1 KO mice, showed that receptor expression on NK cells could be modulated by MHC I molecules in two ways: the frequency of NK cells expressing a given receptor and the amount of that receptor expressed[21, 22]. Thus, MHC I molecules affect the repertoire of NK cells both qualitatively and quantitatively. It remains unclear what mechanisms direct and calibrate repertoire acquisition in developing NK cells.

Different models have been proposed to explain the processes underlying NK cell education. Yokoyama's group proposed that NK cell education occurs through "licensing"[23]. In this model, the presence of MHC I (self)-specific inhibitory receptors provides NK cells with functional competence through an ITIM-dependent mechanism. On the other hand, Raulet's group proposed the "disarming" model; here, NK cells that lack self-specific inhibitory receptors are hyporeactive (disarmed) as a result of chronic activating signals; alternatively, "arming" occurs when NK cells exhibit self-specific inhibitory receptors that prevent disarming[24]. More recently, Hoglund's "rheostat" theory proposed that acquisition of NK cell responsiveness is quantitatively controlled by the extent of inhibitory signals transmitted by self-specific inhibitory receptors[25]. Interestingly, recombinant mice who constitutively express the MCMV protein m157 in the bone marrow possess hyporeactive NK cells[26]; it was proposed that chronic stimulation through Ly49H was responsible for this loss of function. This model evidenced that MHC I are not the only molecules that can control NK cell education and that education through activating receptors can also regulate NK cell functions; such education mechanisms fit the disarming and rheostat theories.

Members of the mouse Ly49 receptor family and human LIR-1 have the unusual ability to engage MHC I expressed not only on other cells (*trans* interactions) but also those in their direct vicinity on the NK cell membrane (*cis* interactions). Studies from Held's group showed that MHC I engagement in *cis* is necessary for NK cell education[27]. Masking of receptors due to *cis* interactions can restrict their detection by antibodies, resulting in false reported low expression levels of these receptors. This could also very possibly account for increased expression levels of some self-specific receptors reported in mouse strains with deficient MHC I expression. Thus, previous studies that concluded that MHC I molecules exert a negative effect on expression levels of self-specific Ly49 receptors may need to be re-addressed taking into consideration the impact of *cis*-interactions.

Peripheral regulation of NK cells

Even though a definite repertoire seems to be set once NK cells are fully developed, NK cell responsiveness can still be subject to regulation. Indeed, peripheral processes of tolerance induction have been documented. One may have predicted that NK cells from MHC I-sufficient mice adoptively transferred into MHC I-deficient host would be strongly activated due to constant

missing self recognition, instead such NK cells become hyporesponsive[28]. Chronic stimulation of mature NK cells through activating receptors by stress ligands[29, 30] or virus proteins[31] also induces NK cell exhaustion and hyporesponsiveness. This state is reversible when chronic stimulation is suspended in the presence of IL-2[29]. Central tolerance achieved during NK cell education can also be reverted in the periphery. β 2-m KO NK cells progressively elicit missing-self recognition function following infection by MCMV[32]. It is possible that reversion of tolerance in this scenario is due to inflammatory cytokines released during viral infection. This is supported by Yokoyama and Kim's findings that β 2-m KO NK cells cultured in IL-12 and IL-18 were capable of levels of IFN- γ production upon NK1.1 cross-linking similar to NK cells from β 2-m sufficient mice [33]. Thus, adjustments of NK cell reactivity are not restricted to a specific development stage as they also occur in mature cells, indicating that NK cells are able to adapt to their environment.

NK cell-mediated resistance to viral infection

Individuals with genetic abnormalities that cause selective NK cell deficiencies are highly susceptible to recurrent viral infections such as cytomegalovirus (HCMV), varicella zoster virus (VZV), herpes simplex virus (HSV) and human papilloma virus (HPV)[1, 2]. Associations between KIR repertoire and resistance to viruses have been described. Also, individuals who have been exposed to certain viruses harbor characteristic NK cell repertoire imprinting such as expansion of cells expressing activating NKG2C receptor, self-specific inhibitory KIR receptors, CD57 and LILRB1 and displaying low expression of CD56, NKp30 and NKp46. This feature is well documented following HCMV infection[34-38] and has also been reported with HIV-1[39], hantavirus[40] and chikungunya virus[41]. In HCMV⁺ individuals, NKG2C⁺ NK cells can be divided into two subsets: NKG2C^{dim} and NKG2C^{bright} cells. Coexpression of NKG2A is detected on NKG2C^{dim} NK cells that functionally and phenotypically resemble NKG2C⁺ NK cells from HCMV⁻ individuals, while NKG2A is absent on NKG2C^{bright} NK cells[42]. In population studies, homozygous deletion of the NKG2C gene (NKG2C^{-/-}) was reported in 4% of Japanese and Dutch cohorts while around 30% of the individuals were heterozygous (NKG2C^{+/-})[43]; similar results were observed in studies with Spanish[44] and Gambian donors[45]. In HCMV-infected individuals, greater numbers of NKG2C⁺ NK cells were detected in NKG2C homozygous than in heterozygous children[46] and adults[42]. Also, NKG2C was found to be present on NK cells at higher levels in NKG2C^{+/+} than in NKG2C^{+/-} individuals[42, 45]. Lower percentages of CD57⁺ NK cells were present in children with the homozygous deletion and were associated with higher anti-HCMV antibody titers; the boosted antibody response was possibly due to frequent viral reactivation[45]. A recent study analyzed the maturation of NK cells in three patients given cord blood transplants from NKG2C^{-/-} donors; the authors found that HCMV drove a rapid expansion of CD56^{dim}, KIR⁺, NKG2A⁻ NK cells that comprised a large proportion of cells expressing activating KIR able to trigger cytotoxicity and IFN γ release[47]. This study indicated that NKG2C is not necessary during HCMV-induced NK cell differentiation and that the absence of NKG2C can possibly be substituted by activating KIRs. Specific NK cell expansion has been reported in individuals infected by hepatitis virus (HCV)[48], however, coinfection by HCMV may have driven the repertoire re-shaping, so HCV role in altering NK cell repertoire remains unclear [49]. Alter et al reported that KIR3DS1⁺ and KIR3DL1⁺ NK cells expand in HIV-seropositive individuals that express HLA-Bw480I, the putative ligand for these receptors, but this study did not assess whether NKG2C was coexpressed with these receptors [50]. An important question yet to be answered is whether expression of self-specific KIRs provides the NKG2C⁺ NK cell subset with an enhanced ability to expand due to education processes or whether NKG2C directly recognizes a ligand present on infected cells, which drives the expansion.

Mouse studies where NK cells were depleted, or in NK cell-deficient mouse strains, evidenced the critical role played by NK cells in controlling infection by various viruses, including MCMV[51],

HSV-1[52], coxsackie virus[53], encephalomyocarditis virus (EMCV)[54], vaccinia virus (VV)[55], ectromelia virus (ECTV)[56], ebola virus[57] and influenza virus[58]. Activating NK cell receptors able to bind viral proteins were identified; they provide NK cells with means to recognize and eliminate infected cells. Table 1 summarizes the major receptors involved in regulating NK cell activity toward virus infected cells in human and mouse.

NCR-dependent NK cell response. The first ligands identified for NCRs were viral products. NCR engagement triggers NK cell activation. Human NKp46 binds influenza hemagglutinin and sendai virus (SV) hemagglutinin-neuraminidase[58] while sialylated NKp44 binds influenza and SV hemagglutinin[59]. NKp44 ligand (MLL5) is expressed on CD4 T cell during HIV-1 infection and its presence correlates with a decline of these lymphocytes during the course of infection[60, 61]. HCMV-infected dendritic cells (DC) activation of NK cells through NKp46 has also been reported[62]. In addition, human NCRs have been implicated in protective responses against HCV[63], west Nile (WNV)[64], dengue[64] and ebola[65] viruses. In mice, a role for NKp46 in controlling influenza infection was also proposed[66], but this remains unclear. Indeed, another study based on the *Noe* mouse strain, where the mutated *Ncr1* gene is non-functional, showed the presence of hyperactivated NK cells with elevated ability to control viruses such as MCMV and influenza[67]. Therefore the role played by mouse NCR in anti-viral NK cell response remains to be clarified.

Antibody dependent cellular cytotoxicity-dependent NK cell response. CD16 associates with FcR γ or CD3 ζ signaling adaptors in human NK cells. FcR γ -deficient NK cells can be detected in healthy individuals, they display low expression of NKp30 and NKp46[68]. HCMV⁺ individuals display an increased frequency of FcR γ ⁺ NK cells, which are mostly NKG2C⁺, CD57⁺ and NKG2A⁻, compared to samples from seronegative donors. *In vitro*, these cells produced low amounts of IFN γ in response to HCMV-infected cells, however, addition of plasma containing anti-HCMV antibodies triggered a strong cytokine production and degranulation of cytotoxic granules, which suggested an exacerbated ADCC function in this NK cell subset[69]. An ADCC-dependent activation of NK cells, characterized by production of IFN γ and degranulation of granzyme and perforin was also reported during HIV-1 infection[70-72]; these responses were found to be significantly stronger in patients with chronic infection than during early infection[71]. NK cells eliciting ADCC in the context of HIV-1, similarly to those in HCMV infection, displayed the same CD57⁺ and NKp46^{low} phenotype[71]. However, no correlation could be detected between the HLA and KIR haplotype in the ADCC-responsive and non-responsive NK cell populations, suggesting that the positive effect of NK cell licensing on ADCC effector function was lost during HIV-1 infection[73]. ADCC can also mediate NK cell activation, production of IFN γ and degranulation toward cells infected by influenza virus in both humans[74] and mice[75]. Killing of dengue-infected cells mediated through ADCC by NK cells has also been documented[76].

T cell dependent NK cell response. Several studies evidenced that NK cell activation by various viruses request virus-specific T cells, or is exacerbated in their presence. *In vitro* stimulation with HIV-1 derived peptides of HIV-1⁺ donor PBMC resulted in production of IFN- γ by NK cells while no response was detected with PBMC from HIV-1⁻ donors. Depletion of CD3⁺ cells from the PBMC populations abrogated the NK cell response, which indicated that activation was T cell dependent[77]. In the context of influenza A infection, IL-2 produced by virus-specific T cells was found to improve NK cell activation and to induce IFN- γ production[78]. A role for T cells in regulating anti-viral NK cell response was also reported after rabies virus vaccination. *In vitro* exposure of PBMC to rabies virus induced NK cells to degranulate in vaccinated but not in unvaccinated donor samples. This response relied on the production of IL-2 by CD4 T cells and on the presence of IL-12 and IL-18 released by myeloid accessory cells[79]. In the mouse, generation of memory NK cells in response to MCMV infection was also shown to require IL-12 dependent signals in NK cells[80]. Resting NK cells do not express, or very weakly express the IL-2 receptor α -chain CD25 while it is up-regulated in an IL-12 dependent manner during the first days that

follow infection by MCMV [81]. These results indicated an initial release of IL-2 by T cells in response to this virus that regulates NK cell activity. These studies suggest a regulatory role played by T cells on NK cell functions at late stage of infection once virus-specific T cells have expanded, in addition, pre-existing memory T cells generated by vaccination may play an important role in regulating the early NK cell response to primary infection. A better comprehension of the role played by T cells and of ADCC in controlling anti-viral NK cell responses, and defining which viruses trigger these mechanisms, may well pave the way to novel strategies aimed at promoting NK cell-mediated anti-viral response by vaccination.

MCMV infection: a model for anti-viral immune response. Studies on MCMV infection in mice have provided numerous insights into the mechanisms that NK cells have developed to mediate anti-viral surveillance. Early experiments observed that the beige mutant mouse strain, which has severe NK cell deficiencies, exhibits elevated morbidity and mortality rates following MCMV infection[82]. The role of NK cells was further evidenced by Bancroft *et al*, whose work demonstrated a positive correlation between NK cell activity and survival following MCMV infection in various strains of mice[83]. Later, NK cell depletion and reconstitution experiments provided the conclusive evidence that these cells are critical for survival in MCMV infected mice[51, 84].

a. The Ly49H-m157 axis. The fact that the early response to MCMV infection varies considerably between inbred mouse strains was revealed in ground-breaking studies conducted by Scalzo and Shellam[85]. They found that parameters associated with both MHC and non-MHC loci contribute to control the infection. The first MHC-independent resistance locus, termed *Cmv1*, was identified in C57BL/6 (B6) mice; it was found to be associated with limited MCMV replication in the spleen and lungs. Subsequent studies demonstrated that the *Cmv1* effect relies on NK cells; this locus was found to be encoded within the NKCC[86]. Genetic mapping studies identified *ly49h* as the gene responsible for the *Cmv1*-mediated control of acute MCMV infection[11, 87]; its product, Ly49H, is an activating receptor. Depletion of Ly49H⁺ NK cells in B6 mice resulted in increased viral replication in the visceral organs[11, 87]. Furthermore, insertion of *ly49h* as a transgene into mice normally susceptible to acute infection provides them with the ability to control MCMV replication[88]. Ly49H binds MCMV's gene product m157, which is an MHC I-like glycoprotein[10, 89]. Ly49H engagement results in activating signals triggering NK cell cytotoxicity and secretion of IFN- γ [89].

b. The m04-gp34/Dk axis. Another mechanism by which NK cells control MCMV infection relies on the detection of altered MHC I molecules. Scalzo *et al*. revealed that B6-related H-2^k MA/My mice exhibit resistance to MCMV in an NK cell-dependent manner[90]. Two independent experimental genetic approaches were used to identify a critical locus centered in the MHC I D^k coding gene in MA/My mice[91, 92]. At the cellular level, resistance is mediated by NK cells expressing the activating Ly49P receptor and requires the presence of the m04-encoded gp34 protein associated to D^k on infected cells [93]. Even though the set of data support well the conclusions, there is currently no Ly49P-specific antibody to block the receptor or to deplete Ly49P⁺ NK cells *in vivo*, which would confirm these findings. Therefore the role of Ly49P in this recognition axis is yet to be conclusively demonstrated.

c. Role of H-2. The importance of H-2^k in resistance to MCMV was also addressed using a panel of H-2^k congenic mouse strains and by introducing D^k into susceptible mice. A preferential expansion of Ly49G⁺ NK cells occurred with both strategies, and depletion of this NK cell subset abrogated the resistant phenotype[94, 95]. The authors suggested that Ly49G mediates NK cell "licensing" in H-2^k mice, thereby allowing the subset co-expressing Ly49P to recognize and eliminate MCMV infected cells expressing D^k/gp34. However, whether "licensing" improves NK cell ability to control MCMV is still a matter of debate[96]. Vidal and colleagues addressed the involvement of activating Ly49 receptors in MCMV resistance in various mouse strains. They found that Ly49P1, Ly49L and Ly49D2 in NOD/Ltj, BALB and PWK/Pas mice respectively,

provided NK cells with means to respond to MCMV infected cells in the context of D^k/gp34. Depending on the receptor concerned, recognition of gp34 could also be achieved in H-2^d and H-2^f, but not in H-2^b backgrounds[97]. This study demonstrated that MCMV infected cells can be recognized through various activating Ly49 receptor depending on the H-2 background. Babic *et al.* evaluated a role of self-specific inhibitory receptors in regulating NK cell response to MCMV[98]. BALB mice are devoid of activating Ly49 receptors that interact with MCMV gene products; these mice are considered susceptible to MCMV[85]. Still, H-2^k and H-2^f BALB mice infected with MCMVΔm04 (which m04 gene was deleted), displayed a significantly reduced viral replication compared to mice infected with WT virus in an NK cell-dependent manner. No resistance increase toward MCMVΔm04 was detected in H-2^b BALB mice while H-2^d BALB mice displayed an intermediate phenotype[98]. MHC I expression was strongly reduced in MCMVΔm04 infected cells. The increased anti-viral response against MCMVΔm04 was reverted to a weaker response, like against MCMV WT when specific inhibitory Ly49-expressing NK cell subsets were depleted[98]. A role for missing self recognition in NK cell anti-viral response was also suggested in [99]. These findings indicate that NK cell responses to viral infection are not only regulated by activating receptors targeting viral products but also by self-specific inhibitory receptors and support the importance of the H-2 background.

Viral immuno-evasion to NK cell response:

Viruses have developed several strategies that prevent NK cell activation; these include a direct targeting of NK cell receptors or interference with their ligands. The success of viruses such as cytomegalovirus, established based on its prevalence, is likely to rely on a large genome that contains multiple genes dedicated to non-essential functions. Table 2 summarizes the currently defined human and mouse cytomegalovirus genes with regulatory function on NK cell activity. Immune evasion genes (which encode immunoevasins) are best characterized in MCMV where they cluster in two defined loci, with the m02 family at one end and the m145 family at the other end of the virus genome[100].

Viral interference by altered MHC I recognition. It is well documented that various viruses target MHC I expression as a means to escape NK cell response. The widespread representation of viruses with genes responsible for this effect indicates that this constitutes a successful strategy of immune evasion. Decreased MHC I expression or expression of altered MHC I molecules both prevent CD8 T cell mediated immune response, however, these changes also promote “missing self recognition” by NK cells, which seems contradictory to the purpose of immune-evasion. Cytomegalovirus encodes several immunoevasion genes that target MHC I. In human, HCMV expresses US2 and US11 that bind to MHC I heavy chains within the endoplasmic reticulum (ER) and direct them to the cytosol where they are degraded by proteasome[101]. HCMV US6 glycoprotein binds the transporter-associated with antigen processing (TAP)/MHC I complex and inhibits translocation of peptide from the cytosol to the ER[102]. HCMV US3 blocks MHC I transit from the ER into the Golgi[103]. However, HCMV can also improve MHC I recognition by NK cells and promotes their inhibition. HCMV UL40 gene product mimics the signal peptides of various HLA-C haplotypes and binds to non-classical MHC I HLA-E, which favors its expression on infected cells and limits NK cell activation[104, 105] by engaging preferentially inhibitory NKG2A but not activating NKG2C receptors[106]. HCMV UL18 gene encodes an MHC I homolog that binds inhibitory LIR-1 receptor with an affinity 1000 times greater than its endogenous ligand HLA G[107, 108]. Similar strategies have been identified for MCMV: m04, m06 and m152 genes all alter MHC I expression. Both m06-gp48 and m152-gp40 interfere with MHC I expression on the cell surface by either redirecting MHC I molecules to the lysosome for degradation or retaining them in the ER/Golgi compartments[109, 110], respectively. A different strategy is used by the m04-gp34 protein that forms a stable complex with MHC I in the ER from where it will transit to the membrane[111]. It was proposed that m04-gp34 plays its immunoevasion role by preventing “missing self recognition”

and thus preventing NK cell activation. We now know that this immune evasion mechanism is context-dependent as it promotes a Ly49P dependent NK cell activation in H-2^k mice[93], as discussed above. MCMV m144 gene has a sequence that predicts a MHC I-like molecule; it was proposed that m144 contributes to immune evasion through interference with NK cell activation[112]. Besides cytomegaloviruses, it has also been documented that MHC I expression is altered following infection by HIV-1[113], human papilloma virus (HPV)[114], Epstein-Barr virus (EBV)[115], Kaposi's sarcoma associated γ -herpes virus (KSHV)[116]. Recognition of HLA/peptide complex by activating KIR2DS1 has been reported during EBV infection[117].

Down-regulation of NKG2D-dependent NK cell response. NKG2D recognizes a variety of MHC I-like stress-induced ligands and is a potent activating NK cell receptor. Both HCMV and MCMV have developed mechanisms to interfere with NKG2D-mediated recognition of infected cells by NK cells. HCMV-encoded UL16 glycoprotein blocks MIC-B, ULBP-1 and -2 expressions[118] while UL142 glycoprotein down-regulates MIC-A[119]. In mouse, MCMV m138, m145, m152 and m155 genes encode proteins that targets NKG2D ligands. The m138 gene product alters both MULT-1 and H60 expression[120], and MULT-1 is also affected by m145[121]. m152 down-regulates RAE-1 family members[122] and m155 affects H60[123]. In addition to viral genes encoding immunoevasins, viruses possess non-coding microRNA (miRNA) with immunoregulatory function. NKG2D ligand translation has been documented to be down regulated by HCMV miR-UL112, KSHV miR-K12-7 and EBV miR-BART2-5p[124, 125]. Repression of any of these ligands reduced killing of infected cells by NK cells. A recombinant MCMV with the m152 gene substituted with the NKG2D ligand RAE1- γ gene exerted a profoundly attenuated virulence *in vivo*[126]. Orthopoxviruses have developed a different mechanism to interference with NKG2D-dependent activation; these viruses produce an MHC I-like protein that binds NKG2D with higher affinity than its natural ligands and acts as a competitive antagonist[127]. The multiplicity of viral immunoevasion genes targeting NKG2D ligands attests to a strong immune pressure exerted on the virus by the host NK cells through this activation pathway.

Viral interference to DNAM-1 dependent response. The HCMV-encoded UL141 glycoprotein sequesters DNAM-1 ligand CD155 within the ER, thereby preventing DNAM-1 dependent NK cell activation[128]. CD96 receptor share the same ligands as DNAM-1; it was found to promote cell adhesion and to play a costimulatory role in human NK cells[129]. Therefore, it is very possible that HCMV UL141 also affects the CD96 costimulation pathway.

Viral interference to NCR-dependent NK cell response. Cellular ligands for NCRs have been only recently identified while a ligand for NKp46 is yet to be characterized. Also, little is currently known about viral regulation of NCR dependent responses. HCMV-encoded pp65 and VV hemagglutinin form a complex with NKp30 and interfere with its activating function[55, 130].

Down-regulation of ADCC. Viral sequence mutations that allow infected cells to escape NK cells' ADCC function have been reported for HIV-1[131]. However, another study did not detect alterations in ADCC function in HIV-1 viremic patients[132]. Thus, the existence of viral evasion mechanisms that target ADCC remains unclear and will require further investigation.

Viral interference to 2B4 dependent response. HCMV interferes with 2B4 dependent NK cell activation by down-regulating expression of its ligand CD48[133]. In mouse, MCMV m154 protein also targets this pathway, resulting in proteolytic degradation of CD48[134]. Although 2B4 is an activating NK cell receptor in human, it plays a dual role in murine cells. Mice infected with MCMV Δ m154 were shown to display reduced viral titers compared to mice infected with MCMV WT and depletion of NK cells abolished the protection[134]; these findings indicated that m154 encodes an immunoevasin that improves the virus ability to evade NK cell anti-viral response.

Viral interference to TLR and cytokine receptor function. Processes by which viruses compromise NK cell activation induced through cytokine receptors and TLRs have been reported[135]. TLRs

play a most important role in anti-viral defense; they are responsible for the early production of type I IFNs that follows viral infection. Viruses have developed various mechanisms to escape IFN response. Influenza[136] and HCV[137] were shown to antagonize type I IFN production, while poxviruses[138] and paramyxoviruses[139] were found to antagonize type I IFN receptor signaling. In addition, herpes simplex and influenza viruses were reported to block type I IFN-inducible genes and their products[140].

Regulation of NK cell response by MCMV m157. The MCMV infection model has provided valuable insights into how the immune system responds to viral infection and has helped to define numerous viral immuno-evasion mechanisms aimed at promoting unchecked viral replication and horizontal transmission to new hosts. The first MCMV gene to be identified with an NK cell regulatory function was m157[10, 89]; extensive research focusing on m157 generated a valuable knowledge and a better understanding of the mechanisms that regulate the antiviral NK cell response. NK cells lose their ability to control MCMV replication in B6 mice in the absence of m157, which demonstrates the critical role of m157 recognized by Ly49H⁺ NK cells[141]. However, Ly49H is not the only NK cell receptor capable of binding this immunoevasin. m157 is also recognized by inhibitory Ly49I receptor in 129/J mouse strain, whereas Ly49I in B6 mice cannot do so[10]. This observation highlights that immunoevasin functions are restricted to some mouse haplotypes due to receptor allelic variability. Based on the high level of sequence homology existing between Ly49H in B6 mice (Ly49H^{B6}) and Ly49I in 129/J mice (Ly49I^{129/J}), it has been speculated that natural selection initially retained m157 in MCMV's genome for its ability to inhibit NK cells[142]. This hypothesis was further supported by the fact that most MCMV strains isolated from wild mice possess m157 variants that do not bind Ly49H⁺ NK cells[143, 144]. In addition, m157 mutants found to dodge Ly49H dependent recognition rapidly emerged following repeated passages of MCMV in Ly49H⁺ B6 mice[144]. Therefore, NK cell activation and resistance to MCMV in B6 mice relies on Ly49H-dependent recognition of m157 while the regulatory function exerted by this viral gene varies in other mouse strains.

a. Differential binding of m157 variants. Various MCMV strains were isolated from mice caught in the wild and their m157 variants were tested for binding to NK cells from five mouse strains (BALB/c, B6, NZB, SJL/J and 129X1/SvJ) that are representative of the five major NKC haplotypes identified in inbred mice[11, 87]. MCMV m157 from the Smith, G1F and N5 strains m157 bound NK cells from B6 mice while all these three m157 variants and also the K17E strain m157 bound NK cells in NZB mice. Binding of the m157 variants to SJL/J and 129X1/SvJ NK cells was weak or nil. In BALB/c mice, only G1F and W8211 strains m157 were recognized by NK cells[143]. Therefore, m157 variants from various MCMV isolates show a range of binding patterns to NK cells in inbred mice. Interestingly, among the m157 variants tested, more were able to bind Ly49I^{129/J} than Ly49H^{B6}[143]; these findings strongly suggested that m157 was selected for its more frequent ability to inhibit than to activate NK cells in wild mouse populations. The fact that G1F m157 binds to NK cells from BALB/c mice despite the absence of Ly49H in this mouse strain revealed recognition of m157 by another receptor, which was identified as being Ly49C[143].

b. Integration of opposite signals induced by m157. The commonly used laboratory MCMV strain (Smith, K181) m157 binds either an activating receptor in B6 mice (Ly49H) or an inhibitory receptor in 129/J mice (Ly49I)[10]. The dual binding of G1F m157 to both inhibitory (Ly49C) and activating (Ly49H) in B6 mice constitutes a unique system to study how opposite signals induced by an immunoevasin are integrated in NK cells. Assays using reporter cells expressing these two receptors that were exposed to target cells expressing G1F m157 indicated that Ly49C-inhibitory signals can counteract Ly49H-dependent activation[143]. However, infection of B6 mice with a recombinant K181 MCMV virus expressing G1F m157 was controlled by NK cells similarly to a WT virus[143]. The persistent anti-viral response was most likely relying on Ly49H⁺ NK cells that did not co-express Ly49C, a subset that constitutes most of the Ly49H⁺ population in B6 mice. A precise analysis of Ly49H⁺ NK cells evidenced that those coexpressing Ly49C established less conjugates with m157-expressing targets, showed reduced F-actin polymerization and exhibited

reduced signs of activation than Ly49C–devoid NK cells[99]. In addition, transgenic B6 mice where all NK cells express Ly49C, infected with G1F m157-expressing MCMV, displayed reduced anti-viral Ly49H-dependent and –independent responses and increased viral replication[99]; possibly, this broad down-regulation of NK cell functions was because the immunoevasin targets a self-specific inhibitory receptor.

c. Ly49 receptor conformations and modes of binding to MHC I. Receptor structural studies provided a deeper understanding of the regulatory mechanisms at the molecular level. Analysis of the molecular interactions through which MHC I-specific Ly49s engage their ligands provided important information[145-148]. Ly49 receptors engage MHC I molecules via the lectin-like natural killer domains (NKD), which interact with a groove underneath the MHC I's peptide binding platform and establish contacts with $\alpha 1$, $\alpha 2$, $\alpha 3$ domains and with $\beta 2$ -m. Sequence alterations that exist within the NKD in the various Ly49 receptors allow distinct specificities for different MHC I molecules[148]. Held and Mariuzza evidenced that Ly49L adopts a backfolded (or opened) conformation with the proximal stalk passing between the two NKDs, thereby allowing a single Ly49 homodimer to engage two MHC I molecules on the surface of a neighboring cell (interaction in *trans*)[149, 150]. Some Ly49 receptors, including Ly49C, are also able to adopt an extended (or closed) alternate conformation whereby the NKDs are juxtaposed in such a way that they prohibit backfolding of the stalks[145, 146, 149]. The extended conformation reorients the NKDs toward the NK cell membrane, allowing binding to MHC I molecules expressed in the membrane vicinity (interaction in *cis*)[149]. The receptor flexibility required to switch from the back-folded to the extended conformation and allow *cis*-binding requires the presence of a 12 residue Ls loop that links $\alpha 3$ to the NKD[149].

d. Mechanisms of m157 binding to Ly49 receptors. Despite sharing little amino-acid sequence homology with MHC I, m157 adopts a similar structure including a set of α -helices lying above a β -sheet platform and supported by an $\alpha 3$ -like domain; however, m157 associates neither with a peptide nor with $\beta 2$ -m. Interestingly, a similar structure is also found in HCMV-encoded UL18[151]. The molecular interactions between m157 and Ly49H have recently been elucidated[150]. Unlike MHC I molecules that bind the NKD domain of Ly49 receptors, m157 docks Ly49H stalk region thereby allowing a single receptor to simultaneously engage two ligands[150]. The Ly49-m157 interactions involve an aromatic peg motif within the stalk distal $\alpha 3$ segment; this motif is present in all Ly49 receptors that recognize m157, suggesting that a common recognition strategy is employed by inhibitory and activating receptors[143, 150]. The Ly49 aromatic peg motif is buried between the NKDs in the backfolded conformation, required for binding in *trans*[149]. Therefore, the backfolded conformation appears to be incompatible with m157 binding. Kielczewska *et al.* analyzed the NKC from various mouse strains to identify residues critical for m157 binding. Mutation of amino-acids 146 and 151 in Ly49 receptors able to bind m157, such as Ly49H, resulted in loss of binding to the immunoevasin, whereas replacement of these residues in non-binding Ly49 receptors, such as Ly49I^{B6} and Ly49H^{129/J} enabled them to bind m157[152]. Based on these results the most logical interpretation was that m157 binding to Ly49 was mediated through interactions of the immunoevasin with these NKD residues. However, as the recently elucidated Ly49-m157 structure revealed that m157 does not interact with the receptor NKD but with its stalk domain[150] the initial conclusion drawn from Kielczewska *et al.* studies may need to be reconsidered. Instead, it is possible that mutation of the residues indicated above affected the stability of the backfolded conformation, thereby affecting the ability of the mutant Ly49s to recognize m157.

The stalk domain used to be considered to essentially play a role in linking the NKD to the membrane, and in translating an event of ligand binding into intracellular signalisation. We now know that viral products have the ability to bind the stalks of various immune receptors. Negative binding results previously obtained using chimeric fusion proteins composed of only the recognition domain of receptors linked to an antibody or another targeting partner should thus be

reconsidered. It will be interesting to investigate whether more immunoevasins encoded by viruses other than CMV can also bind NK cell receptors' stalk domains, which would indicate that this binding pattern represents a common strategy used by a range of viruses.

e. Cis-interactions regulate m157 binding to NK cells. Inhibitory Ly49 receptors engaging MHC I expressed on the surface of a juxtaposed cell (*in trans*) triggers inhibitory signals whereas no signals are induced upon interactions *in cis*[153]. Structural data indicated that m157 binding to Ly49C is only possible when the receptor adopts the extended conformation[150, 154], which is not compatible with *trans*-binding to MHC I[153], and we also know that Ly49C transmits inhibitory signals upon binding of m157[99, 150]. Therefore, MCMV immunoevasin binding to Ly49C in an extended conformation warrants that the receptor can transmit inhibitory signals and hinder NK cell activation even though the conformation adopted does not allow engagement of the endogenous MHC I ligands (illustrated in figure 1). The finding that m157 binds the Ly49 stalk and not the NKD represents a novel viral immuno-regulatory feature that supposedly does not compete with endogenous ligands for binding. Notwithstanding, m157 binding to Ly49C could not be detected when the receptor is already engaged in *cis* with MHC I molecules, indicating that both engagements do not occur concomitantly[99]. Possibly, the absence of a simultaneous dual-binding could be due to a Ly49-MHC I *cis*-bound complex sterically incompatible with m157 accessibility and/or to forces engendered under these conditions that alter the stalk conformation and make it improper to engage m157. The immuno-regulatory role of *cis* interactions in anti-viral immunity was demonstrated *in vivo*. In these experiments using B6 Ly49C Tg, β 2-m KO mice, all NK cells display Ly49C which is free of *cis*-interactions due to severely reduced MHC I expression; these NK cells cannot control infection by G1F m157-expressing MCMV [99]. A lethal dose (LD₅₀) was achieved upon inoculation of 5×10^4 PFU of MCMV[99], which is comparable to the LD₅₀ measured in Ly49H-devoid BALB/c mice[85]. On the other hand, β 2-m-sufficient B6 Ly49C Tg mice exhibited an NK cell-mediated protection, albeit diminished compared to WT mice in similar conditions of infection. This NK cell responsiveness was associated with a large fraction of Ly49C receptors that interacted in *cis* with MHC I[99]. A recent study evidenced the existence of similar regulatory mechanisms in human. HCMV impairs NK cell activation through the UL18 immunoevasin, which binds the inhibitory LIR-1 receptor; the authors showed that LIR-1 can engage HLA-G both in *trans* and in *cis*; the latter type of interaction interferes with UL18 binding and the subsequent inhibition[155]. Therefore in both murine and human, *cis*-interactions between inhibitory receptors and MHC I molecules constitute an NK cell-intrinsic regulatory mechanism that counters viral immunoevasin functions[99, 155]. An evolution-driven selection process of individuals that possess NK cell receptors able to interact in *cis* with their MHC I molecules might have resulted from pressure exerted by viral immunoevasins that target these receptors.

Coinfection and virus selection. Infection of the host with multiple strains of viruses has been reported for a number of viruses including HIV-1, dengue virus, HPV, HCV and Rotavirus[156]; this is a particularly common feature with herpes family viruses that display a high rate of mutations. In a multi-strain infection model, where competition between MCMV strains is dependent on Ly49H⁺ NK cells, virus strains encoding an m157 variant that are capable of binding Ly49H are excluded from the salivary glands; this process selects the shedding and transmission of only the most virulent viral strains to other hosts via the saliva[157]. Reciprocally, in humans as in wild mice populations, the NK cell repertoire is variable from one individual to another and is subject to polymorphism; as a result the virus strains selected by immune pressure may vary and therefore, the dominant virus strain may differ in host populations geographically isolated.

Role of NK cells in the adaptive immune response.

NK cells do not only control viral replication by directly eliminating infected cells and releasing cytokines, they also play a regulatory role on the adaptive immune response (reviewed in[158]).

Therefore, by regulating NK cell functions, viruses can affect broadly the anti-viral immune response. Accumulating evidence now support that NK cells possess properties previously attributed to the adaptive immune system. Long-lived NK cells differentiate following viral infection; they exhibit functions that recall those of memory lymphocytes (reviewed in [159]). DNAM-1 was recently shown to play a critical role in the generation of memory NK cells following MCMV infection [160]. MCMV-experienced NK cells strongly expand, rapidly degranulate and release cytokines upon reactivation; these cells confer a potent protection against infection when transferred into MCMV-susceptible newborn mice [161]. Similarly, NK cell differentiation has been documented in humans; a unique subset of NKG2C⁺ CD57⁺ NK cells persists at high frequency in HCMV-infected individuals and inflation of this subset was detected following virus reactivation [37, 162]. Differentiation of memory NK cells following MCMV infection requires the presence of inflammatory IL-12 [80]. It was reported that NK cells with memory-like functions develop after cytokine activation; this provides a ground to combine pre-activation with administration of cytokines into NK cell-based immunotherapies in the context of chronic virus infection [163, 164]. Von Adrian and coworkers reported liver-resident CXCR6⁺ NK cells that respond to vaccines containing antigens from influenza, vesicular stomatitis virus (VSV) and HIV-1 [165]. However, more recent findings tend to indicate that these cells do not derive from conventional NK cells but instead constitute a subset of innate lymphoid cells (ILC) that share NK cell markers but are functionally different [166]. An unsuspected role for RAG was recently identified in the mouse [167]. In addition to its requirement for V(D)J gene rearrangement during T and B lymphocytes development, RAG provides developing NK cells with a “cellular fitness”. WT NK cells expand more robustly than RAG KO NK cells following MCMV infection; in addition long-lived memory NK cells fail to differentiate in the absence of RAG. RAG proteins were found to play a role in promoting expression or activity of DNA damage repair enzymes, which endow NK cells with an increased ability to survive following cellular stress induced by intense proliferation and viral infection [167]. Interestingly, patients carrying inherited hypomorphic RAG1 mutations suffer from severe disseminated HCMV infection [168].

Conclusion:

The variety of mechanisms that viruses have developed to specifically regulate NK cells support strongly a critical role played by this cell population in anti-viral immunity. Our history recalls lethal viruses that had developed potent immune escape mechanisms such as the influenza A virus strain responsible for the pandemic in 1918 that caused 20 to 50 million deaths worldwide. However, a severe virulence, such as exhibited by Ebola virus, may not be a selective advantage as it results in rapid death of the host, which can be counterproductive to ensure a most efficient propagation of the pathogens. Instead, we propose that viral genes that trigger an NK cell response, which at first glance seems to go against the logic of natural selection for the virus point of view, actually balance against immunoevasins that strongly impair the antiviral response and that may lead to severe mortality rates.

Mouse studies with MCMV provided a wealth of information with regard to the variety of viral immunoevasins that modulate the immune response and reciprocally the pathways and mechanisms used by the immune system to counter the infection. However, we are yet to define an efficient strategy to regulate the immune response to our advantage in order to completely eradicate the virus and prevent life-long chronic infections. We need to define whether the regulatory mechanisms identified in the MCMV infection model could be transferred and applied to a large range of viruses.

Future perspective:

Considering how much knowledge about NK cell biology has been achieved during the last two decades, it is very plausible that much more of the mechanisms that regulate NK cell education and activation will have been unraveled in another ten years. This knowledge will be required to

properly interpret the results arising from the genetic studies ascribing certain combinations of KIR repertoire and HLA haplotype to susceptibility or resistance to viruses, and possibly will improve the donor/recipient pairing during hematopoietic cell transplantation. Clinically, immunodepressed individuals such as transplant recipients and cancer patients undergoing chemotherapy treatment are highly susceptible to viral infection and virus reactivation. CMV infection has been associated with reduced risks of leukemic relapse and improved survival in hematopoietic cell transplant recipients; studies aiming at defining whether in some cases the host can actually benefit from viral infection could identify more conditions where viral infection could be used to our advantage. Although the underlying mechanisms are yet to be understood, expansion of NKG2C⁺ NK cells appears to be a common trait induced by a large range of viruses in human. Exploitation of the mechanisms that govern NK cell activation and differentiation to our advantage, and *ex vivo* expansion of highly potent NK cell subsets for subsequent adoptive transfer also constitute immunotherapy strategies against viruses with a promising future. Also, considering that some viruses are largely control through ADCC and that virus-specific T cells play a critical role in regulating NK cell functions, it is likely that a better NK cell mediated cellular immunity can be achieved through vaccination.

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Disclosures

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Executive summary:

NK cell role in anti-viral immune surveillance

- NK cells play a critical role in anti-viral immune surveillance; individuals with NK cell deficiencies are affected by recurrent viral infections.
- Studies using mouse models demonstrated the implication of NK cells and identified multiple mechanisms involved in anti-viral immune surveillance.

Viruses target NK cell functions

- Expression of endogenous proteins can be induced, increased or repressed in virus-infected cells. Detection of cells that display an altered phenotype by NK cell receptors control NK cell activation and anti-viral response.
- Products of viral genes non essential for replication (immuno-evasins) are also expressed on infected cells; direct recognition of the immuno-evasins or recognition of altered endogenous proteins associated with immuno-evasins by NK cell receptors also regulate NK cell functions.

Lessons from MCMV

- MCMV provides an informative experimental model for human infection by HCMV due to strikingly similar pathogenesis and anti-viral immune response in both species.
- Studies about MCMV provided a wealth of information about the immune evasion strategies developed by viruses and the recognition mechanisms exhibited by NK cells.
- The MHC haplotype harbored by an individual constitutes another key component that controls NK cell reactivity, as it contributes to regulate NK cell function through interactions in *cis* with their receptors.
- Structural studies characterized how viral proteins interact with NK cell receptors and defined residues critical in this process.

Clinical implications and perspectives

- Understanding the factors that are critical at controlling NK cell anti-viral response provides novel parameters that can be used to predict the individuals' resistance or susceptibility to infection by a virus.
- Defining the receptors at play, the nature of the motifs recognized and understanding the mechanisms of regulation pave the way to new immune-therapy strategies, more particularly for immune compromised individuals.

Table 1**NK cell receptors involved during viral infection**

Receptor	Species	Ligand	Virus
<u>Inhibitory</u> KIRxDLx	H	HLA-C, some HLA-B	HIV-1, HPV, EBV, KSHV, HCMV
Ly49	M	classical MHC I, MHC I-like molecules	MCMV
CD94/NKG2A	H/M	HLA-E (H), Qa-1 (M)	HCMV
LIR-1 (LILRB1,CD85j)	H	HLA-G	HCMV
<u>Activating</u> KIRxDSx	H	HLA-C, some HLA-A	HCMV
Ly49	M	classical MHC I, MHC I-like molecules	MCMV
NKG2D	H/M	MICA/B, ULBP1-6 (H); RAE-1 α - γ , H60a-c, MULT1 (M)	HCMV, MCMV, KSHV, EBV, Orthopoxvirus, HIV-1, HBV
CD94/NKG2C,E	H/M	HLA-E (H), Qa-1 (M)	HCMV, MCMV
DNAM-1	H/M	CD155, CD112	HCMV, MCMV
NKp30	H	B7-H6, BAT3	ECTV, VV, Ebola virus
NKp44	H	PCNA, MLL5	Influenza virus, SV, WNV, Dengue virus
NKp46	H/M		Influenza virus, SV, NDV, HCMV, LCMV
CD16	H/M	Immunoglobulins	HIV-1, Dengue virus
TLRs	H/M	PAMPs	Influenza virus, HCV, Poxviruses, Paramyxoviruses, HSV-1

EBV: Epstein-Barr virus, ECTV: Ectromelia virus, HIV-1: human immunodeficiency virus-1, HBV: Hepatitis B virus, HCV: Hepatitis C virus, HCMV: human cytomegalovirus, HPH: human papilloma virus, HSV-1: Herplex simplex virus 1, KSHV: Kaposi's sarcoma associated γ -herpes virus, MCMV: mouse cytomegalovirus, NDV: New castle disease virus, SV: Sendai virus, VV: Vaccinia virus, WNV: West Nile virus

Table 2.**Cytomegalovirus genes that regulate NK cell response.**

Virus gene	NK cell receptor	Mechanism	NK cell regulation
<u>Human</u>			
US2, US11	self-specific inh. KIRs	HLA I degradation in proteasome	+
US6	self-specific inh. KIRs	Prevents TAP-dependent loading of peptides on HLA I	+
UL18	LIR-1 (ILT2, CD85j)	Produces HLA I surrogate that binds LIR-1	-
UL40	NKG2A/CD94	Promotes HLA-E interaction with NKG2A/CD94 while hinders interaction with NKG2C/CD94	-
UL16	NKG2D	Prevents MIC-B and ULPB-1, -2 expression	-
UL142	NKG2D	Prevents MIC-A expression	-
UL112	NKG2D	miR-UL112 inhibits MIC-B expression	-
pp65 gene	NKp30	Disrupt NKp30 association with signaling adaptor	-
UL141	DNAM-1	Prevents CD155 expression	-
<u>Mouse</u>			
m04	self-specific inh. Ly49s	Stabilizes MHC I expression	-
m04	Ly49P ^{MA/My}	Forms complex with H2-D ^k that binds Ly49P	+
m06, m152	self-specific inh. Ly49s	MHC I lysosomal degradation	+
m138, m145, m152, m155	NKG2D	Decreases NKG2D ligand expression	-
m144	self-specific inh. Ly49?	Sequence predicts MHC I-like molecule	-
m157	Ly49H ^{B6}	Directly binds Ly49H ^{B6}	+
m157	Ly49 (I ^{129/J} , C ^{B6, BALB})	Directly binds Ly49I ^{129/J} , Ly49C ^{B6, BALB}	-
m154	2B4	CD48 degradation	-

Figure 1.

Legend figure 1:

Regulation of NK cell inhibition by *cis*-interactions between MHC I molecules and inhibitory receptors. **A:** MCMV infects a host where the viral protein m157 is not recognized by NK cell receptors; NK cells are not activated and the virus replicates uncontrolled. **B:** MCMV infects an individual who possesses NK cells with activating receptors (Ly49H) that recognize m157; m157-expressing infected cells are recognized and killed by NK cells; viral replication is strongly controlled. **C:** MCMV infects a host who possesses NK cells expressing both Ly49H and Ly49C. Host's MHC haplotype does not allow *cis*-interaction between Ly49C and MHC I molecules. m157 from this viral strain binds Ly49C and induces inhibitory signals that prevent NK cell activation; the virus replicates uncontrolled. **D:** Like in C, MCMV infects a host who possesses NK cells expressing both Ly49H and Ly49C but MHC I binds Ly49C in *cis*, thereby preventing m157 engagement. Ly49H-dependent NK cell activation is mostly un-inhibited, allowing elimination of the infected cells; viral replication is controlled.