


Accelerating discovery, enabling scientists
Discover the benefits of using spectral flow cytometry for high-parameter, high-throughput cell analysis



SONY
Download Tech Note



NK Cells and Immune "Memory"

Joseph C. Sun, Sandra Lopez-Verges, Charles C. Kim, Joseph L. DeRisi and Lewis L. Lanier

This information is current as of August 4, 2022.

J Immunol 2011; 186:1891-1897; ;
doi: 10.4049/jimmunol.1003035
<http://www.jimmunol.org/content/186/4/1891>

References This article **cites 88 articles**, 33 of which you can access for free at:
<http://www.jimmunol.org/content/186/4/1891.full#ref-list-1>

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at:
<http://jimmunol.org/subscription>

Permissions Submit copyright permission requests at:
<http://www.aai.org/About/Publications/JI/copyright.html>

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
<http://jimmunol.org/alerts>



NK Cells and Immune “Memory”

Joseph C. Sun,^{*,1} Sandra Lopez-Verges,^{†,1} Charles C. Kim,[‡] Joseph L. DeRisi,[‡] and Lewis L. Lanier[†]

Immunological memory is a hallmark of the adaptive immune system. However, the ability to remember and respond more robustly against a second encounter with the same pathogen has been described in organisms lacking T and B cells. Recently, NK cells have been shown to mediate Ag-specific recall responses in several different model systems. Although NK cells do not rearrange the genes encoding their activating receptors, NK cells experience a selective education process during development, undergo a clonal-like expansion during virus infection, generate long-lived progeny (i.e., memory cells), and mediate more efficacious secondary responses against previously encountered pathogens—all characteristics previously ascribed only to T and B cells in mammals. This review describes past findings leading up to these new discoveries, summarizes the evidence for and characteristics of NK cell memory, and discusses the attempts and future challenges to identify these long-lived memory NK cell populations in humans. *The Journal of Immunology*, 2011, 186: 1891–1897.

The innate and adaptive immune systems have traditionally been segregated into well-defined compartments. Innate immunity features short-lived cells that respond rapidly and nonspecifically against pathogen exposure. Re-encounter with the same pathogen is thought to result in a qualitatively and quantitatively identical response as the first encounter. In contrast, adaptive immunity consists of T and B cells, which respond more slowly but with high specificity due to somatically rearranged genes that generate an infinitely diverse set of AgRs. The clonal expansion of a pool of Ag-specific effector T and B cells initiated by pathogen exposure results in a population of long-lived memory cells that are able to respond quicker and more robustly during subsequent encounters with the same pathogen. The lifespan of many innate immune cells are thought to be on the order of hours or days, relatively short compared with T and B cells, which

persist for months to years, making immune memory unimportant, or unnecessary, for short-lived cells comprising the innate immune system, such as granulocytes and dendritic cells (DCs). Although frequently discussed in isolation, these two sides of the immune system rarely act in isolation, and the interplay between cells of the innate and adaptive immune arms contributes to the most productive overall responses against pathogen invasion.

Evidence for innate immune memory

Although immunological memory has been one of the classical hallmarks that distinguishes adaptive immune T and B cells from all other cells of the hematopoietic lineage, evidence has been found in invertebrates, such as crustaceans, flies, beetles, and mosquitoes, and more primitive species, such as tunicates, sea urchins, and sea sponges, that immune memory exists independently of lymphocytes possessing rearranged AgRs (1–3). Exposure of the copepod *Macrocyclus albidus* (a minute crustacean) to its natural pathogen, the parasitic tapeworm *Schistocephalus solidus*, results in resistance to challenge with an antigenically similar tapeworm (4), providing evidence for the early existence of innate immune memory. *Drosophila melanogaster* injected with a sublethal dose of *Streptococcus pneumoniae* or *Beauveria bassiana* (a natural fruitfly pathogen) also demonstrated resistance against subsequent bacterial challenge compared with flies that were unprimed with bacteria (5). The underlying mechanisms for the observed protection following priming required activation of the Toll pathway in phagocytes, which was suggested to mediate the secondary responses and resistance. Specific priming of resistance against its natural bacterial pathogen *Bacillus thuringiensis* and the common bacterium *Escherichia coli* was observed in the red flour beetle, *Tribolium castaneum* (6). Demonstrating specificity in this innate immune response, beetles previously primed with heat-killed bacteria were more likely to survive a subsequent exposure to the same bacteria that was used in priming rather than exposure to a heterologous pathogen. Similarly, *Anopheles gambiae* mosquitoes (which are the major vector for malaria spread in Africa) pre-exposed

*Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065; [†]Department of Microbiology and Immunology, Cancer Research Institute, University of California, San Francisco, San Francisco, CA 94143; and [‡]Howard Hughes Medical Institute, Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94158

¹ J.C.S. and S.L.-V. contributed equally to this work.

Received for publication October 26, 2010. Accepted for publication December 6, 2010.

This work was supported by the Cancer Research Institute (to J.C.S. and S.L.V.), National Institutes of Health Grant AI085034 (to J.C.S.), and the Howard Hughes Medical Institute (to C.C.K. and J.L.D.). L.L.L. is an American Cancer Society Professor and is supported by National Institutes of Health Grants AI068129, CA095137, and AI066897.

Microarray data presented in this article have been submitted to the Gene Expression Omnibus under accession number GSE25672.

Address correspondence and reprint requests to Dr. Joseph C. Sun, Memorial Sloan-Kettering Cancer Center, 408 East 69th Street, ZRC-1402, New York, NY 10065. E-mail address: sunj@mskcc.org

Abbreviations used in this article: DC, dendritic cell; HCMV, human CMV; KIR, killer cell Ig-like receptor; KLRG1, killer cell lectin-like receptor G1; MCMV, mouse CMV.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/\$16.00

to *Plasmodium falciparum* demonstrate enhanced immunity upon parasite reinfection (7). The protective effect was attributed to circulating granulocytes, for which numbers are rapidly increased upon primary infection. Together, these reports suggest that innate immune cells in many simpler organisms (which lack adaptive immune cells) can be primed by previous infections and mount stronger secondary responses upon homologous pathogen challenge.

Evidence for NK cell memory

NK cells have long been categorized as a component of innate immunity. However, several lines of developmental, phenotypic, and functional evidence suggest that NK cells are closely related to T and B cells mediating adaptive immunity (8–10), with the exception of RAG-mediated rearrangement of AgR genes. First, similar to the generation of T and B lymphocytes, NK cells are derived from the common lymphoid progenitor (11). Second, like T and B cells, NK cells require cytokines of the IL-2R common- γ -chain family, particularly IL-15, for their development, homeostasis, and survival (12). Third, like thymocytes and pre/pro-B cells, immature NK cells undergo an education process whereby only appropriately selected cells (i.e., tolerant to self) are able to be functional effectors in the periphery (13–17). Fourth, activated NK cells express many of the same surface receptors (CD25, CD43, CD44, CD69, CD122, Ly6C, CD62L, and killer cell lectin-like receptor G1 [KLRG1]) as activated T cells (18, 19). Fifth, NK cells are functionally similar to T cells in their ability to produce IFN- γ and TNF- α following activating receptor-mediated or cytokine-induced stimulation (18, 19). Sixth, NK cells are functionally related to CTL through their shared ability to mediate cytotoxicity via perforin and granzymes (18, 19). With the exception of AgRs generated from somatically rearranged genes, the evidence above suggests that NK cells are evolutionarily similar to T and B cells of adaptive immunity. In fact, immature T cells that lack one specific transcription factor (Bcl11b) have been shown to de-differentiate into NK-like precursor cells (20–22). Taken together, these observations suggest that NK cells may be developmentally and functionally more closely related to adaptive immune lymphocytes than innate immune cells. In line with their lymphocytic nature, recent studies have further demonstrated that NK cells can undergo a clonal-like expansion following virus infection in both humans and mice and that previously primed NK cells can mediate secondary memory responses.

Early studies suggested the possibility that NK cell memory could exist. In a model of F1 hybrid resistance (in which the parental bone marrow graft is rejected by F1-recipient mice), B10 \times B10.D2 mice primed with parental B10 bone marrow cells rejected a second B10 graft more efficiently (23). When mice were primed with parental B10.D2 or a third-party allogeneic bone marrow prior to the parental B10 graft, the rapid rejection was abrogated. At the time, NK cells had not yet been identified as the cells mediating hybrid resistance; however, a retrospective interpretation of these early experiments suggests relevance for NK cell memory in graft rejection. Recent studies have indicated that NK cell priming can also occur nonspecifically during inflammation induced by TLR triggering on DCs or exposure to cytokines (24, 25). Thus, although NK cells were initially described by their

ability to kill certain tumor cells without previous sensitization (26–30), NK cells become even more potent effectors when stimulated. A study using the MHC class I-deficient RMA-S tumor model showed that NK cells primed by tumors that lacked MHC class I were also more activated and mediated greater effector responses (31). A significant increase in IFN- γ secretion and cytotoxic activity was measured in the activated NK cells only following inoculation with RMA-S tumor cells and not with RMA tumor cells expressing MHC class I, suggesting that in addition to inflammatory signals, NK-sensitive tumor cell targets can prime resting NK cells for greater effector potential, similar to T cell priming.

A more recent study demonstrated that NK cells mediate contact hypersensitivity responses to chemical haptens in RAG-deficient mice (32). In mice that lack T and B cells, contact hypersensitivity responses to 2,4-dinitrofluorobenzene and oxazolone persisted for >4 wk, and the responses were only elicited by the hapten to which mice were originally exposed and not by a different hapten. In the control mice lacking T, B, and NK cells due to a genetic deficiency in the RAG genes and the IL-2R common γ gene, no contact hypersensitivity was measured in previously sensitized mice. Interestingly, the only population of hapten-specific memory NK cells that could transfer sensitivity resided in the liver. Further studies are required to determine why only liver NK cells mediated the secondary response and the nature of the receptor-hapten interaction driving the responses. Following this report, our group used the well-characterized NK cell response to mouse CMV (MCMV) to determine whether immune memory could exist in virus-specific NK cells (33). NK cells in C57BL/6 mice bearing the Ly49H receptor have been shown to specifically recognize MCMV-infected cells expressing the viral glycoprotein m157 and undergo a clonal-like expansion (34–36). This proliferation was Ag-specific because infection of mice with a mutant MCMV lacking m157 could not drive Ly49H⁺ NK cell expansion (33). Using an adoptive transfer system, small numbers of Ly49H⁺ NK cells were observed to proliferate 100–1000-fold in lymphoid and nonlymphoid tissues in the recipient following MCMV infection, resulting in a long-lived pool of memory NK cells (Fig. 1A). This self-renewing population of NK cells was able to undergo secondary and even tertiary expansion following several rounds of adoptive transfer and virus infection (37). The memory NK cells recovered from previously infected mice several months later exhibited more robust effector functions *ex vivo* and were far more effective at protection against viral challenge compared with an equal number of resting NK cells from naive mice, demonstrating a qualitatively different secondary response in NK cells that had previously encountered viral Ag (33). More recently, another report showed that memory-like NK cells could be induced *in vitro* by exposure to inflammatory cytokines, such as IL-12 and IL-18 (38). Following adoptive transfer into recipient mice, these cytokine-activated NK cells were found to respond more robustly several weeks later (measured by IFN- γ production following activating receptor triggering) compared with resting NK cells. Altogether, these studies demonstrate that NK cells can persist for far longer than previously estimated (39–41) and have the potential to contribute alongside memory T and B cell responses during subsequent encounters with the same pathogen.

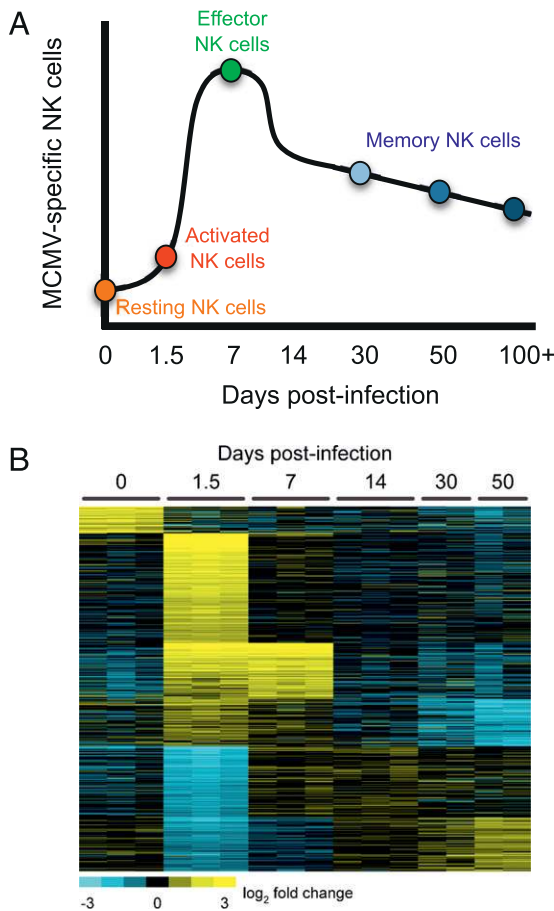


FIGURE 1. *A*, Virus-specific NK cell response to MCMV infection. During MCMV infection, resting Ly49H⁺ NK cells become activated and undergo an expansion phase resulting in the generation of more effector cells. The expansion phase is followed by the contraction of effectors resulting in long-lived memory NK cells months after initial infection. *B*, The gene array profile of different stages of the Ly49H⁺ NK cell response to MCMV infection. Congenic Ly49H⁺ NK cells were adoptively transferred prior to MCMV infection, as previously described (33). The transcriptional signature of naive (day 0), activated (day 1.5), effector (day 7), contracting (day 14), and memory (days 30 and 50) NK cells is unique at each time point following MCMV infection. Ly49H⁺ NK cells from three separate mice at each time point were individually sorted on a FACS Aria for RNA isolation (except for day 30 and day 50 time points done in duplicate). Samples were hybridized on the MEEBO microarray platform against reference mouse RNA, as previously described (88). All microarray data are available through the Gene Expression Omnibus under accession number GSE25672.

Characteristics of NK cell memory

How do memory NK cells differ qualitatively from resting NK cells? We know that CD8⁺ T cells undergo a programmed differentiation during virus infection that results in dramatic changes in their gene expression profile at each stage (42). Similarly, we have recently used gene array technology to profile transcription in Ly49H⁺ NK cells at different stages following MCMV infection: resting, acute activation, expansion, contraction, and memory maintenance phases (Fig. 1*B*). Although much work remains to determine the relative contributions of specific genes during each particular phase of the NK cell response, the global signature confirms that the mRNA transcriptional profile of resting NK cells is different from that of memory NK cells (Fig. 1*B*). Furthermore, the profiles of resting and memory NK cells differ greatly from

both early activated and clonally expanded Ly49H⁺ NK cells, demonstrating that NK cells at each phase of the response to MCMV exhibit a gene profile that is unique and stage-specific (Fig. 1*B*). The gene array road maps that immunologists studying memory T cell have generated (42–44), along with the identification of specific transcription factors for T cell differentiation, homeostasis, and survival (45), are useful tools that can guide the search for the factors that govern differentiation of NK cells following activation.

T cell studies have shown that during effector-to-memory cell differentiation different subsets of memory cells are generated, characterized by their anatomical location and expression of cytokine and homing receptors (46–48) (Fig. 2). Can the central memory versus effector memory T cell paradigm be found in memory NK cells as well? Memory NK cells generated during virus infection were all found to highly express KLRG1 (33), an inhibitory receptor recognizing E-cadherins, also predominantly expressed on effector memory CD8⁺ T cells (Fig. 2). Perhaps memory NK cells resemble this more terminally differentiated subset of memory T cells (Fig. 2). Unlike the overall maintenance of memory CD8⁺ T cell numbers, which plateau following contraction (49), the memory NK cell population contracts similarly to memory CD4⁺ T cell responses during lymphocytic choriomeningitis virus infection (50). Although memory NK cells have been shown to self-renew (measured by BrdU uptake over the course of several days) (37), absolute numbers steadily decline, and in the absence of secondary infection, memory NK cells in mice are difficult to detect 6 mo after MCMV infection; however, this represents a relative time span equivalent to decades in humans (J.C. Sun and L.L. Lanier, unpublished observations). Whether the overall long-lived NK cell population experiences preferential survival of the progeny of a subset of memory cells remains to be addressed.

Like memory T cells, memory NK cells have been shown to both self-renew and mount multiple rounds of expansion and contraction in numbers (37). Future studies will determine whether differences exist in the maintenance of memory NK cells compared with T cells and which cytokine signals (e.g., IL-7 and IL-15) and stromal cell interactions play an important role in the survival of long-lived NK cell populations. Many other outstanding tasks remain, the foremost of which is to identify a reliable and stable marker (or set of markers) to define memory NK cells, as has been identified for memory T cells (Fig. 2). This will allow direct analysis of long-lived NK cells within the endogenous NK cell population and circumvent the need for adoptive transfer to track Ag-experienced NK cells. Furthermore, we have observed that memory NK cells reside in both lymphoid and nonlymphoid tissue, but determining which soluble factors (cytokines and chemokines) modulate organ- and tissue-specific distribution and phenotype of memory NK cells is warranted. The contribution of stromal elements and other leukocytes, such as DCs and CD4⁺ Th cells, toward the generation of memory NK cells requires further elucidation. Lastly, the transcriptional control of NK cell differentiation and memory generation has not been well characterized, in contrast to the case with T cell differentiation. As more pathogen ligands recognized by NK cell receptors are characterized, additional infectious disease models will allow further analysis of the broader principles of NK cell activation, expansion, and memory.

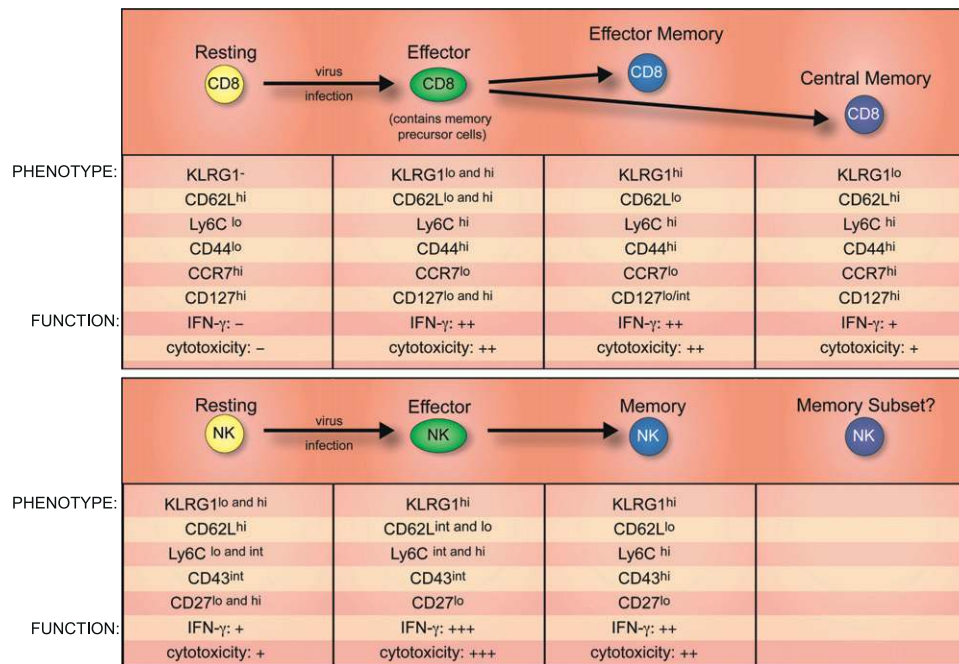


FIGURE 2. Comparison of CD8⁺ T cell and NK cell differentiation and memory generation following viral infection. Phenotypic and functional descriptions of resting, effector, and memory T and NK cells are shown. hi, high; int, intermediate; lo, low.

Do memory NK cells exist in humans?

In humans, NK cells also play a crucial role in the response against CMV. In an early study, a patient selectively lacking NK cells, but having normal B and T cells, was found to suffer a life-threatening illness postinfection with human CMV (HCMV) (51). Other reports of specific NK cell deficiencies (or NK cell functional deficiencies) in humans (52, 53) similarly describe overwhelming fatal infections during childhood or adolescence due to HCMV and other herpesviruses, such as varicella zoster virus and EBV, demonstrating the importance of NK cells in the immune response against certain viral infections. Functional Ly49 genes do not exist in humans, and thus far, there is no evidence that members of the killer cell Ig-like receptor (KIR) family, which encode human NK cell receptors analogous to activating and inhibitory Ly49 receptors in mice, can directly recognize HCMV-infected cells. Moreover, CMV is exquisitely species-specific; each CMV has coevolved and adapted within its own specific mammalian host. The CMV genes that are involved in immune-evasion mechanisms, such as genes responsible for downregulation of MHC class I and NKG2D ligands, and surface expression of decoy MHC ligands for NK inhibitory receptors have evolved independently in MCMV and HCMV and are tailored to counter the host response (54). Currently, there is no known direct counterpart of the Ly49H-MCMV m157 interaction described between a human NK cell receptor and an HCMV protein, although evidence suggests the possibility of a viral ligand for CD94-NKG2C. Healthy blood donors who have previously been exposed to HCMV have an increased proportion of NK cells bearing the lectin-like heterodimeric receptor CD94-NKG2C compared with HCMV-seronegative donors (55). HCMV has also been reported to induce the expansion of CD94-NKG2C⁺ NK cells in healthy adults and children, as well as in HIV-infected and leukemia patients (56–59). Moreover, HCMV shapes the

NK cell repertoire long after acute infection, as the percentage of CD94-NKG2C⁺ NK cells remains elevated even after therapeutic intervention and in asymptomatic HCMV⁺ donors that likely contracted the virus during childhood (55, 58). The expansion of CD94-NKG2C⁺ NK cells was observed in vitro when human NK cells were cocultured with HCMV-infected fibroblasts and was abrogated with a blocking CD94-specific mAb, supporting the involvement of a specific receptor–viral ligand interaction (60). Strikingly, a recent report showed a prolific expansion of CD94-NKG2C⁺ NK cells (>80% of all NK cells) in an immunodeficient infant in whom the NK cell response was carefully monitored during an acute infection with HCMV (61), comparable to the 100-fold expansion observed for mouse Ly49H⁺ NK cells during MCMV infection. Taken together, the evidence suggests that the CD94-NKG2C receptor on human NK cells might represent the functional counterpart of the MCMV-specific Ly49H receptor in mice. This potential Ag-specific recognition might represent an ideal system in which to initiate the search for human memory NK cells.

Similar to Ly49H, the activating CD94-NKG2C receptor complex associates with the adapter protein DAP12 (62, 63). Both CD94-NKG2C and the highly related inhibitory CD94-NKG2A receptor complex recognize HLA-E as their ligand (64, 65). However, the nature of the HCMV-induced ligand that drives CD94-NKG2C⁺ NK cell expansion remains elusive. Is HLA-E presenting a processed viral peptide recognized by CD94-NKG2C, or does HCMV encode a CD94-NKG2C ligand expressed on the cell surface? Alternatively, could HCMV be inducing a host protein that is subsequently being recognized by CD94-NKG2C? Recently, a report hinted that CD94-NKG2C, but not CD94-NKG2A, binds weakly to the HCMV UL18 glycoprotein (65). Our group has also previously shown that expression of UL18 resulted in increased killing of HCMV-infected cells by human NK

cell clones, but this appeared to not involve CD94 (66). Further investigation is warranted to conclusively determine whether a CD94–NKG2C–UL18 interaction is mediating expansion of this NK cell subset during HCMV infection.

The traditional cell-surface phenotype defining human NK cells is absence of CD3 and expression of CD56, the 140-kDa isoform of neural cell adhesion molecule (67, 68). Two NK cell subsets have been characterized according to the cell-surface density of CD56 and expression of CD16 (low-affinity Fc γ RIIIa), with CD56^{dim}CD16^{bright} cells comprising ~90% and CD56^{bright}CD16^{neg/dim} cells constituting ~10% of NK cells in the blood (69). Human NK cells, however, are a heterogeneous population with respect to the expression of KIR, NKG2A, and natural cytotoxic receptors. Recently, our group and others have examined expression of CD57, a carbohydrate Ag that is expressed on subsets of human NK cells and T cells (70). CD57 is expressed only on a minor fraction of NK cells in fetal tissues or cord blood (which represent the most naive NK cells in humans), and the percentage of NK cells and T cells expressing CD57 increases with age (71–73). A recent study showed that CD57 is a marker linked to cellular maturity of CD8⁺ T cells and NK cells and that it correlates with high cytolytic potential (74). CD56^{dim}CD57[–] NK cells can become CD56^{dim}CD57⁺ after stimulation *in vitro*, during the reconstitution of the immune system in a humanized mouse model, and in patients undergoing hematopoietic stem cell transplantation (75, 76). These studies suggest that as mature NK cells differentiate from CD56^{bright} to CD56^{dim}, they lose expression of NKG2A, natural cytotoxic receptors, CD27, and CD62L while acquiring CD16, LIR-1 (also named CD85J and LILRB1), Siglec-9, and KIRs, and the final stage of activation involves the acquisition of CD57 (75–77).

Is the CD57 activation marker on human NK cells the equivalent of KLRG1 on the mouse NK cells that have become activated and differentiated into effectors? Because CD8⁺ T cells expressing CD57 were reported to possess shorter telomeres than CD57[–] cells and express an effector/memory phenotype (78, 79), it is possible that NK cells expressing CD57 might also represent NK cells that have previously been driven into clonal expansion by encounters with pathogens. The frequency of NK cells expressing CD57 varies in different adult blood donors. Moreover, the frequency of NK cells expressing CD57 within a given NK cell subset in an individual is not uniform; the percentages of CD57⁺ and CD57[–] NK cells within the KIR2D, KIR3D, and NKG2A NK cell subsets in a single individual vary considerably, suggesting that NK cells within these subsets exist at different stages of activation or differentiation, likely as a consequence of different exposure to environmental pathogens (75, 76). Further studies are necessary to determine whether the CD94–NKG2C⁺ NK cells that specifically expand during HCMV infection will upregulate CD57 expression and whether CD57 will serve as a marker for NK cells that can form a long-lived memory cell population that responds against subsequent HCMV exposure.

As mentioned previously, memory NK cells in mice generated following MCMV infection produce higher amounts of cytokines and degranulate more robustly compared with resting NK cells (33). Surprisingly, the CD57⁺ NK cell population produced less IFN- γ than CD57[–] NK cells in re-

sponse to activation by cytokines (i.e., IL-12 and IL-18), but had higher amounts of granzymes (75). Although they degranulated similarly in response to stimulation via the majority of activating receptors, CD57⁺ NK cells responded better to stimulation through CD16 compared with CD57[–] cells (75). It remains to be determined how CD57⁺ NK cells bearing a virus-specific receptor, such as CD94–NKG2C, will behave *in vivo* during a secondary infection or HCMV reactivation. Thus, although CD57 is a good candidate as a marker of highly mature NK cells that may have been driven to expand in response to pathogens, further studies are necessary to determine if CD57⁺ NK cells represent a long-lived memory cell population in humans.

Challenges and barriers

The study of human NK cells poses many challenges and barriers, not least of which are the logistical difficulties. First, trafficking of NK cells may contribute to their maturation, function, and longevity, but there is a dearth of information about human NK cells residing in different organs or at the site of infection, as most human studies use NK cells from the peripheral blood. Second, there is high variability in the expression of NK cell receptors between individuals, in addition to the extensive polymorphism in the KIRs and their HLA ligands (80–82), thus making the number of donors or patients analyzed in any study crucial. Third, most of the functional data are obtained from *ex vivo* experiments or with cytokine-activated NK cells, which may alter surface receptor expression and cell function. Finally, assessing NK cell numbers and function in response to infection with pathogens is complicated by the fact that in most cases the dose, route, and time of pathogen exposure are unknown. In addition, treatment of infections with antibiotics and antiviral agents may adversely influence immune responses and NK cell responsiveness.

To demonstrate that a specific human NK cell population responds to a pathogen and that Ag-specific NK cells persist postinfection, longitudinal studies are required. For HCMV, like most herpesviruses, the majority of the infections are asymptomatic and occur during childhood or adolescence; therefore, determining when infection was initiated is difficult. HCMV persists in ~60% of the human population, and once infected, the virus is never eliminated but must constantly be restrained by the immune system. Although HCMV causes only subclinical disease in healthy humans, it can be life threatening in newborns and immunocompromised or immunosuppressed individuals (83–85). In solid-organ transplant patients, in whom immunosuppressive drugs are used to prevent graft rejection, more than half suffer from clinical manifestations of CMV infection if they are not treated prophylactically with antiviral drugs (83). Therefore, given that many (~30% for the bone marrow transplant patients) of these people will demonstrate reactivation of HCMV, transplantation patients could pose an interesting cohort for longitudinal study of NK cells in response to HCMV infection *in vivo*. Even so, caveats due to immunosuppressive therapeutics administered to the transplant patients and/or the immature differentiation state of the NK cells in the bone marrow transplant patients will likely influence the NK cell response to reactivation or infection with HCMV, potentially confounding interpretation of such studies. Moreover, these

patients are susceptible to opportunistic infections in addition to HCMV that may influence the NK cell response.

Conclusions

The study of NK cell memory in mice and humans is just beginning. The identification of these long-lived NK cells in mice opens up the possibility that similar populations exist in humans. As specific NK cell responses against many viruses (including herpesviruses, poxviruses, HIV, and influenza) have been described and more NK cell receptor–viral ligand interactions are being elucidated, NK cell subsets can be considered in the design of adoptive immunotherapy regimens against acute viral infection. Given the current interest in developing strategies to apply NK cells as therapeutic agents against a broad range of malignancies (86, 87) and approaches to augment NK cell function during chronic viral infections, such as HIV-1 and hepatitis C virus, NK cells have the potential to be exploited as another branch of immunity that can confer long-term protection through vaccination.

Acknowledgments

We thank Carrie Sun for generating the figures and Sue Kaech and members of the Sun and Lanier laboratories for helpful discussions and review of this manuscript.

Disclosures

The authors have no financial conflicts of interest.

References

- Kurtz, J. 2005. Specific memory within innate immune systems. *Trends Immunol.* 26: 186–192.
- Little, T. J., and A. R. Kraaijeveld. 2004. Ecological and evolutionary implications of immunological priming in invertebrates. *Trends Ecol. Evol. (Amst.)* 19: 58–60.
- Schmid-Hempel, P. 2005. Natural insect host-parasite systems show immune priming and specificity: puzzles to be solved. *Bioessays* 27: 1026–1034.
- Kurtz, J., and K. Franz. 2003. Innate defence: evidence for memory in invertebrate immunity. *Nature* 425: 37–38.
- Pham, L. N., M. S. Dionne, M. Shirasu-Hiza, and D. S. Schneider. 2007. A specific primed immune response in *Drosophila* is dependent on phagocytes. *PLoS Pathog.* 3: e26.
- Roth, O., B. M. Sadd, P. Schmid-Hempel, and J. Kurtz. 2009. Strain-specific priming of resistance in the red flour beetle, *Tribolium castaneum*. *Proc. Biol. Sci.* 276: 145–151.
- Rodrigues, J., F. A. Brayner, L. C. Alves, R. Dixit, and C. Barillas-Mury. 2010. Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science* 329: 1353–1355.
- Lanier, L. L. 2005. NK cell recognition. *Annu. Rev. Immunol.* 23: 225–274.
- Raulet, D. H. 2004. Interplay of natural killer cells and their receptors with the adaptive immune response. *Nat. Immunol.* 5: 996–1002.
- Sun, J. C., and L. L. Lanier. 2009. Natural killer cells remember: an evolutionary bridge between innate and adaptive immunity? *Eur. J. Immunol.* 39: 2059–2064.
- Kondo, M., I. L. Weissman, and K. Akashi. 1997. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 91: 661–672.
- Ma, A., R. Koka, and P. Burkett. 2006. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. *Annu. Rev. Immunol.* 24: 657–679.
- Sun, J. C., and L. L. Lanier. 2008. Tolerance of NK cells encountering their viral ligand during development. *J. Exp. Med.* 205: 1819–1828.
- Tripathy, S. K., P. A. Keyel, L. Yang, J. T. Pingel, T. P. Cheng, A. Schneberger, and W. M. Yokoyama. 2008. Continuous engagement of a self-specific activation receptor induces NK cell tolerance. *J. Exp. Med.* 205: 1829–1841.
- Johansson, S., M. Johansson, E. Rosmaraki, G. Vahlne, R. Mehr, M. Salmon-Divon, F. Lemonnier, K. Kärre, and P. Höglund. 2005. Natural killer cell education in mice with single or multiple major histocompatibility complex class I molecules. *J. Exp. Med.* 201: 1145–1155.
- Kim, S., J. Poursine-Laurent, S. M. Truscott, L. Lybarger, Y. J. Song, L. Yang, A. R. French, J. B. Sunwoo, S. Lemieux, T. H. Hansen, and W. M. Yokoyama. 2005. Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* 436: 709–713.
- Fernandez, N. C., E. Treiner, R. E. Vance, A. M. Jamieson, S. Lemieux, and D. H. Raulet. 2005. A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood* 105: 4416–4423.
- Williams, M. A., and M. J. Bevan. 2007. Effector and memory CTL differentiation. *Annu. Rev. Immunol.* 25: 171–192.
- Yokoyama, W. M., S. Kim, and A. R. French. 2004. The dynamic life of natural killer cells. *Annu. Rev. Immunol.* 22: 405–429.
- Li, P., S. Burke, J. Wang, X. Chen, M. Ortiz, S. C. Lee, D. Lu, L. Campos, D. Goulding, B. L. Ng, et al. 2010. Reprogramming of T cells to natural killer-like cells upon Bcl11b deletion. *Science* 329: 85–89.
- Li, L., M. Leid, and E. V. Rothenberg. 2010. An early T cell lineage commitment checkpoint dependent on the transcription factor Bcl11b. *Science* 329: 89–93.
- Ikawa, T., S. Hirose, K. Masuda, K. Kakugawa, R. Satoh, A. Shibano-Satoh, R. Kominami, Y. Katsura, and H. Kawamoto. 2010. An essential developmental checkpoint for production of the T cell lineage. *Science* 329: 93–96.
- Cudkowicz, G., and J. H. Stimpfling. 1964. Induction of Immunity and of Unresponsiveness to Parental Marrow Grafts in Adult F-1 Hybrid Mice. *Nature* 204: 450–453.
- Gidlund, M., A. Orn, H. Wigzell, A. Senik, and I. Gresser. 1978. Enhanced NK cell activity in mice injected with interferon and interferon inducers. *Nature* 273: 759–761.
- Carson, W. E., J. G. Giri, M. J. Lindemann, M. L. Linett, M. Ahdieh, R. Paxton, D. Anderson, J. Eisenmann, K. Grabstein, and M. A. Caligiuri. 1994. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J. Exp. Med.* 180: 1395–1403.
- Greenberg, A. H., L. Hudson, L. Shen, and I. M. Roitt. 1973. Antibody-dependent cell-mediated cytotoxicity due to a "null" lymphoid cell. *Nat. New Biol.* 242: 111–113.
- Herberman, R. B., M. E. Nunn, H. T. Holden, and D. H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int. J. Cancer* 16: 230–239.
- Kiessling, R., E. Klein, and H. Wigzell. 1975. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur. J. Immunol.* 5: 112–117.
- Sendo, F., T. Aoki, E. A. Boyse, and C. K. Buafu. 1975. Natural occurrence of lymphocytes showing cytotoxic activity to BALB/c radiation-induced leukemia RL male 1 cells. *J. Natl. Cancer Inst.* 55: 603–609.
- Zarling, J. M., R. C. Nowinski, and F. H. Bach. 1975. Lysis of leukemia cells by spleen cells of normal mice. *Proc. Natl. Acad. Sci. USA* 72: 2780–2784.
- Glas, R., L. Franksson, C. Une, M. L. Elooranta, C. Ohlén, A. Orn, and K. Kärre. 2000. Recruitment and activation of natural killer (NK) cells in vivo determined by the target cell phenotype. An adaptive component of NK cell-mediated responses. *J. Exp. Med.* 191: 129–138.
- O'Leary, J. G., M. Goodarzi, D. L. Drayton, and U. H. von Andrian. 2006. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat. Immunol.* 7: 507–516.
- Sun, J. C., J. N. Beilke, and L. L. Lanier. 2009. Adaptive immune features of natural killer cells. *Nature* 457: 557–561.
- Arase, H., E. S. Mocarski, A. E. Campbell, A. B. Hill, and L. L. Lanier. 2002. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* 296: 1323–1326.
- Dokun, A. O., S. Kim, H. R. Smith, H. S. Kang, D. T. Chu, and W. M. Yokoyama. 2001. Specific and nonspecific NK cell activation during virus infection. *Nat. Immunol.* 2: 951–956.
- Smith, H. R., J. W. Heusel, I. K. Mehta, S. Kim, B. G. Dorner, O. V. Naidenko, K. Iizuka, H. Furukawa, D. L. Beckman, J. T. Pingel, et al. 2002. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc. Natl. Acad. Sci. USA* 99: 8826–8831.
- Sun, J. C., J. N. Beilke, and L. L. Lanier. 2010. Immune memory redefined: characterizing the longevity of natural killer cells. *Immunol. Rev.* 236: 83–94.
- Cooper, M. A., J. M. Elliott, P. A. Keyel, L. Yang, J. A. Carrero, and W. M. Yokoyama. 2009. Cytokine-induced memory-like natural killer cells. *Proc. Natl. Acad. Sci. USA* 106: 1915–1919.
- Jamieson, A. M., P. Isnard, J. R. Dorfman, M. C. Coles, and D. H. Raulet. 2004. Turnover and proliferation of NK cells in steady state and lymphopenic conditions. *J. Immunol.* 172: 864–870.
- Prlic, M., B. R. Blazar, M. A. Farrar, and S. C. Jameson. 2003. In vivo survival and homeostatic proliferation of natural killer cells. *J. Exp. Med.* 197: 967–976.
- Koka, R., P. R. Burkett, M. Chien, S. Chai, F. Chan, J. P. Lodolce, D. L. Boone, and A. Ma. 2003. Interleukin (IL)-15R[alpha]-deficient natural killer cells survive in normal but not IL-15R[alpha]-deficient mice. *J. Exp. Med.* 197: 977–984.
- Kaech, S. M., S. Hemby, E. Kersh, and R. Ahmed. 2002. Molecular and functional profiling of memory CD8 T cell differentiation. *Cell* 111: 837–851.
- Sarkar, S., V. Kalia, W. N. Haining, B. T. Konieczny, S. Subramaniam, and R. Ahmed. 2008. Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. *J. Exp. Med.* 205: 625–640.
- Wherry, E. J., S. J. Ha, S. M. Kaech, W. N. Haining, S. Sarkar, V. Kalia, S. Subramaniam, J. N. Blattman, D. L. Barber, and R. Ahmed. 2007. Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity* 27: 670–684.
- Rutishauser, R. L., and S. M. Kaech. 2010. Generating diversity: transcriptional regulation of effector and memory CD8 T-cell differentiation. *Immunol. Rev.* 235: 219–233.
- Masopust, D., V. Vezys, A. L. Marzo, and L. Lefrançois. 2001. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 291: 2413–2417.
- Sallusto, F., J. Geginat, and A. Lanzavecchia. 2004. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu. Rev. Immunol.* 22: 745–763.
- Wherry, E. J., V. Teichgraber, T. C. Becker, D. Masopust, S. M. Kaech, R. Antia, U. H. von Andrian, and R. Ahmed. 2003. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat. Immunol.* 4: 225–234.
- Badovinac, V. P., B. B. Porter, and J. T. Harty. 2002. Programmed contraction of CD8(+) T cells after infection. *Nat. Immunol.* 3: 619–626.

50. Homann, D., L. Teyton, and M. B. Oldstone. 2001. Differential regulation of antiviral T-cell immunity results in stable CD8+ but declining CD4+ T-cell memory. *Nat. Med.* 7: 913–919.
51. Biron, C. A., K. S. Byron, and J. L. Sullivan. 1989. Severe herpesvirus infections in an adolescent without natural killer cells. *N. Engl. J. Med.* 320: 1731–1735.
52. Ezioni, A., C. Eidschchenk, R. Katz, R. Beck, J. L. Casanova, and S. Pollack. 2005. Fatal varicella associated with selective natural killer cell deficiency. *J. Pediatr.* 146: 423–425.
53. Orange, J. S. 2006. Human natural killer cell deficiencies. *Curr. Opin. Allergy Clin. Immunol.* 6: 399–409.
54. Sun, J. C., and L. L. Lanier. 2009. The Natural Selection of Herpesviruses and Virus-Specific NK Cell Receptors. *Viruses* 1: 362.
55. Gumá, M., A. Angulo, C. Vilches, N. Gómez-Lozano, N. Malats, and M. López-Botet. 2004. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. *Blood* 104: 3664–3671.
56. Gumá, M., C. Cabrera, I. Erkizia, M. Bofill, B. Clotet, L. Ruiz, and M. López-Botet. 2006. Human cytomegalovirus infection is associated with increased proportions of NK cells that express the CD94/NKG2C receptor in aviremic HIV-1-positive patients. *J. Infect. Dis.* 194: 38–41.
57. Mela, C. M., and M. R. Goodier. 2007. The contribution of cytomegalovirus to changes in NK cell receptor expression in HIV-1-infected individuals. *J. Infect. Dis.* 195: 158–159, author reply 159–160.
58. Petersen, L., A. S. Roug, A. Skovbo, A. H. Thysen, C. W. Eskelund, and M. E. Hokland. 2009. The CD94/NKG2C-expressing NK cell subset is augmented in chronic lymphocytic leukemia patients with positive human cytomegalovirus serostatus. *Viral Immunol.* 22: 333–337.
59. Monsiváis-Urenda, A., D. Noyola-Cherpiel, A. Hernández-Salinas, C. García-Sepúlveda, N. Romo, L. Baranda, M. López-Botet, and R. González-Amaro. 2010. Influence of human cytomegalovirus infection on the NK cell receptor repertoire in children. *Eur. J. Immunol.* 40: 1418–1427.
60. Gumá, M., M. Budt, A. Sáez, T. Brckalo, H. Hengel, A. Angulo, and M. López-Botet. 2006. Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. *Blood* 107: 3624–3631.
61. Kuijpers, T. W., P. A. Baars, C. Dantin, M. van den Burg, R. A. van Lier, and E. Roosnek. 2008. Human NK cells can control CMV infection in the absence of T cells. *Blood* 112: 914–915.
62. Smith, K. M., J. Wu, A. B. Bakker, J. H. Phillips, and L. L. Lanier. 1998. Ly-49D and Ly-49H associate with mouse DAP12 and form activating receptors. *J. Immunol.* 161: 7–10.
63. Lanier, L. L., B. Corliss, J. Wu, and J. H. Phillips. 1998. Association of DAP12 with activating CD94/NKG2C NK cell receptors. *Immunity* 8: 693–701.
64. Braud, V. M., D. S. Allan, C. A. O'Callaghan, K. Söderström, A. D'Andrea, G. S. Ogg, S. Lazetic, N. T. Young, J. I. Bell, J. H. Phillips, et al. 1998. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 391: 795–799.
65. Kaiser, B. K., J. C. Pizarro, J. Kerns, and R. K. Strong. 2008. Structural basis for NKG2A/CD94 recognition of HLA-E. *Proc. Natl. Acad. Sci. USA* 105: 6696–6701.
66. Leong, C. C., T. L. Chapman, P. J. Bjorkman, D. Formankova, E. S. Mocarski, J. H. Phillips, and L. L. Lanier. 1998. Modulation of natural killer cell cytotoxicity in human cytomegalovirus infection: the role of endogenous class I major histocompatibility complex and a viral class I homolog. *J. Exp. Med.* 187: 1681–1687.
67. Lanier, L. L., R. Testi, J. Bindl, and J. H. Phillips. 1989. Identity of Leu-19 (CD56) leukocyte differentiation antigen and neural cell adhesion molecule. *J. Exp. Med.* 169: 2233–2238.
68. Caligiuri, M. A., C. Murray, H. Levine, J. A. Longtine, and J. Ritz. 1989. Clonal evidence for the induction of NKH1 on activated human thymocytes. Functional changes associated with antigen expression. *Eur. J. Immunol.* 19: 1735–1739.
69. Lanier, L. L., A. M. Le, C. I. Civin, M. R. Loken, and J. H. Phillips. 1986. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J. Immunol.* 136: 4480–4486.
70. Lanier, L. L., A. M. Le, J. H. Phillips, N. L. Warner, and G. F. Babcock. 1983. Subpopulations of human natural killer cells defined by expression of the Leu-7 (HNK-1) and Leu-11 (NK-15) antigens. *J. Immunol.* 131: 1789–1796.
71. Abo, T., C. A. Miller, and C. M. Balch. 1984. Characterization of human granular lymphocyte subpopulations expressing HNK-1 (Leu-7) and Leu-11 antigens in the blood and lymphoid tissues from fetuses, neonates and adults. *Eur. J. Immunol.* 14: 616–623.
72. Tilden, A. B., C. E. Grossi, K. Itoh, G. A. Cloud, P. A. Dougherty, and C. M. Balch. 1986. Subpopulation analysis of human granular lymphocytes: associations with age, gender and cytotoxic activity. *Natural Immunity and Cell Growth Regulation* 5: 90–99.
73. Merino, J., M. A. Martínez-González, M. Rubio, S. Inogés, A. Sánchez-Ibarrola, and M. L. Subirá. 1998. Progressive decrease of CD8high+ CD28+ CD57- cells with ageing. *Clin. Exp. Immunol.* 112: 48–51.
74. Chattopadhyay, P. K., M. R. Betts, D. A. Price, E. Gostick, H. Horton, M. Roederer, and S. C. De Rosa. 2009. The cytolytic enzymes granzyme A, granzyme B, and perforin: expression patterns, cell distribution, and their relationship to cell maturity and bright CD57 expression. *J. Leukoc. Biol.* 85: 88–97.
75. Lopez-Verges, S., J. M. Milush, S. Pandey, V. A. York, J. Arakawa-Hoyt, H. Pircher, P. J. Norris, D. F. Nixon, and L. L. Lanier. 2010. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK cell subset. *Blood* 116: 3865–3874.
76. Björkstöm, N. K., P. Riese, F. Heuts, S. Andersson, C. Fauriat, M. A. Ivarsson, A. T. Björklund, M. Flodström-Tullberg, J. Michaelsson, M. E. Rottenberg, et al. 2010. Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK cell differentiation uncoupled from NK cell education. *Blood* 116: 3853–3864.
77. Béziat, V., B. Descours, C. Parizot, P. Debré, and V. Vieillard. 2010. NK cell terminal differentiation: correlated stepwise decrease of NKG2A and acquisition of KIRs. *PLoS ONE* 5: e11966.
78. Le Priol, Y., D. Puthier, C. Combadière, P. Debré, C. Nguyen, and B. Combadière. 2006. High cytotoxic and specific migratory potencies of senescent CD8+ CD57+ cells in HIV-infected and uninfected individuals. *J. Immunol.* 177: 5145–5154.
79. Monteiro, J., F. Batliwalla, H. Ostrer, and P. K. Gregersen. 1996. Shortened telomeres in clonally expanded CD28-CD8+ T cells imply a replicative history that is distinct from their CD28+CD8+ counterparts. *J. Immunol.* 156: 3587–3590.
80. Middleton, D., and F. Gonzalez. 2010. The extensive polymorphism of KIR genes. *Immunology* 129: 8–19.
81. McQueen, K. L., and P. Parham. 2002. Variable receptors controlling activation and inhibition of NK cells. *Curr. Opin. Immunol.* 14: 615–621.
82. Bashirova, A. A., M. P. Martin, D. W. McVicar, and M. Carrington. 2006. The killer immunoglobulin-like receptor gene cluster: tuning the genome for defense. *Annu. Rev. Genomics Hum. Genet.* 7: 277–300.
83. Fisher, R. A. 2009. Cytomegalovirus infection and disease in the new era of immunosuppression following solid organ transplantation. *Transpl. Infect. Dis.* 11: 195–202.
84. Reeves, M., and J. Sinclair. 2008. Aspects of human cytomegalovirus latency and reactivation. *Curr. Top. Microbiol. Immunol.* 325: 297–313.
85. Britt, W. 2008. Manifestations of human cytomegalovirus infection: proposed mechanisms of acute and chronic disease. *Curr. Top. Microbiol. Immunol.* 325: 417–470.
86. Moretta, A., F. Locatelli, and L. Moretta. 2008. Human NK cells: from HLA class I-specific killer Ig-like receptors to the therapy of acute leukemias. *Immunol. Rev.* 224: 58–69.
87. Vitale, M., M. Della Chiesa, S. Carlomagno, C. Romagnani, A. Thiel, L. Moretta, and A. Moretta. 2004. The small subset of CD56brightCD16- natural killer cells is selectively responsible for both cell proliferation and interferon-gamma production upon interaction with dendritic cells. *Eur. J. Immunol.* 34: 1715–1722.
88. Kim, C. C., S. Parikh, J. C. Sun, A. Myrick, L. L. Lanier, P. J. Rosenthal, and J. L. DeRisi. 2008. Experimental malaria infection triggers early expansion of natural killer cells. *Infect. Immun.* 76: 5873–5882.