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NK Cells Play a Critical Role in the Regulation of Class I-Deficient Hemopoietic Stem Cell Engraftment: Evidence for NK Tolerance Correlates with Receptor Editing

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The role that NK cells play in the rejection of hemopoietic stem cell (HSC) and tolerance induction has remained controversial. In this study, we examined whether NK cells play a direct role in the rejection of HSC. Purified HSC from MHC class II-deficient mice engrafted readily in congenic mice, while HSC from class I-deficient donors (β2-microglobulin−/− (β2m−/−)) failed to engraft. Recipient mice lacking CD8+, CD4+, or T cells also rejected HSC from class I-deficient donors, pointing directly to NK cells as the effector in rejection of HSC. Recipients, deficient in or depleted of NK cells, engrafted readily with β2m−/− HSC. Expression of the activating Ly-49D and inhibitory Ly-49G2 receptors on recipient NK cells was significantly decreased in these β2m−/− → B6 chimeras, and the proportion of donor NK cells expressing Ly-49D was also significantly decreased. Notably, β2m−/− chimeras accepted β2m−/− HSC in second transplants, demonstrating that NK cells in the chimeras had been tolerized to β2m−/−. Taken together, our data demonstrate that NK cells play a direct role in the regulation of HSC engraftment, and down-regulation and/or deletion of specific NK subsets in mixed chimeras can contribute to the induction of NK cell tolerance in vivo. Moreover, our data show that bone marrow-derived elements significantly contribute to NK cell development and tolerance. The Journal of Immunology, 2005, 175: 3753–3761.

Natural killer cells are primary cellular mediators of innate immunity. They can recognize and kill aberrant cells and produce chemokines and cytokines that mediate antimicrobial effects or prime other cells of the immune system. They are relatively radioresistant and kill MHC-disparate targets according to the missing self hypothesis (1, 2), and have also been demonstrated to play a role in regulation of hemopoiesis (3–5). Recent studies have suggested that NK cells do not play a direct role in rejection of pluripotent hemopoietic stem cells (HSC), and their mechanism of function on hemopoiesis remains controversial (5–8).

NK cells are regulated by multiple activating and inhibitory receptors. Recently, a number of NK subfamilies have been described that are characterized by the expression of inhibitory and/or activating receptors specific for MHC class I determinants (9). In mice, two families of cell surface receptors (Ly-49 and CD94/NKG2) have been identified on NK cells that bind MHC class I, resulting in either inhibition or activation of the cells (10). Most Ly-49 receptors (Ly-49A, C, G, I, and F) are inhibitory. Binding to their specific ligands transmits inhibitory intracellular signals through ITIM motifs in their cytoplasmic domain (11–13). Conversely, Ly-49D, Ly-49H, and Ly-49P are characterized as activating receptors. They differ from the inhibitory receptors in that they lack the ITIM domains and instead have the ITAM-bearing DAP12 adaptor protein (14–16). A second group of inhibitory receptors on NK cells is composed of disulfide-bonded heterodimers termed CD94/NKG2 (13). CD94 lacks signaling function and forms a heterodimer with the various NKG2 proteins A, B, C, D, and E (12, 13). The NKG2A receptor specifically recognizes the MHC-linked Qa-1 protein, which selectively binds peptides derived from the leader sequences of MHC class I H chains (9). Proper expression of Qu-1 is an indicator of normal expression of MHC class I molecules. NK cells can secrete Th1 cytokines (IFN-γ, TNF-α, and GM-CSF), Th2 cytokines (IL-4 and IL-13), and chemokines under specific circumstances (17). The balance of expression of the two types of receptors determines the final state of the cell and the cytokines and chemokines produced. The role of these receptors in regulating engraftment of HSC and the associated induction of tolerance has not been defined.

Recent studies have shown that NK cell cytotoxic function is regulated by a balance between the expression of specific activating and inhibitory receptors (13, 18). A role for inhibitory receptors (Ly-49A, C/I, and G2), as well as the activating receptor Ly-49D, has been described in the rejection of allogeneic bone marrow (BM) cells (15, 16, 19, 20). In mixed allogeneic chimeras, altered inhibitory receptor expression on NK cells has been reported (5, 21), suggesting that the expression of the various NK receptors may be regulated by the hemopoietic microenvironment. However, no change was noted in expression of the Ly-49D-activating receptor, leading to the conclusion that this was not the mechanism underlying NK cell tolerance (5). Strategies to influence the balance between expression of activating and inhibitory receptors on NK cells, or a possible removal of the Ly-49D NK subset may therefore be used to enhance allogeneic BM engraftment.
Materials and Methods

Animals

C57BL/6j (B6; H-2b); B10.BR SgSnJ (B10.BR; H-2d); C57BL/6J-Lyn tm1Wtr (beige; B6 background); C57BL/6J-TCR-β m1m1m1m1 (TCR-β KO); C57BL/6J-CD4tm1m1kow (CD4 KO); and C57BL/6-CD8tm1m1m1 (CD8 KO) mice were purchased from The Jackson Laboratory. MHC class I-deficient (β2m−/−; H-2b) and MHC class II-deficient (Abb−/−; Abh-2m−/−; H-2d) mice were purchased from Taconic Farms. Animals were housed in a barrier animal facility at the Institute for Cellular Therapeutics, University of Louisville, and cared for according to National Institutes of Health animal care guidelines.

Purification of HSC (c-Kit+/Sca1+/Lin−)

HSC were prepared, as previously described (22, 23). Briefly, BM cells (BMC) were resuspended at a concentration of 100 × 10^6 cells/ml in sterile cell sort medium, containing sterile 1× HBSS without phenol (Invitrogen Life Technologies), 2% heat-inactivated FCS (Invitrogen Life Technologies), 10 mM HEPES buffer (Invitrogen Life Technologies), and 30 μg/ml gentamicin (Invitrogen Life Technologies). Cells were incubated with directly labeled mAbs, purchased from BD Pharmingen: stem cell marker (Sca-1) PE (E13-161.7; rat IgG2a, c-Kit allophycocyanin (2B8; rat IgG2b), CD8α FITC (53-6-7; rat IgG2a), Mac-1 FITC (M1/70; rat IgG2b), B220 FITC (RA3-6B2; rat IgG2a), Gr-1 FITC (RB6-8C5; rat IgG2b), and TCR-β FITC (H57-597; armmenian hamster IgG) for 30 min and then washed. Cells were resuspended in cell sort medium at 2.5 × 10^6 cells/ml. HSC were obtained by live sterile cell sorting (FACSVantage SE; BD Biosciences).

Depletion of CD8+, CD4+, and NK1.1+ cells

B6 recipients were pretreated with anti-CD8 mAb (TIB 105; produced in our laboratory), anti-CD4 mAb (TIB 207; produced in our laboratory), or anti-NK1.1 mAb (PK136; 100 μl of ascites fluid diluted in 1 ml of HBSS), as previously described (24). A total of 400 μg of anti-CD8 or anti-CD4 mAb was injected i.p. at day −3 before HSC transplantation. The anti-NK1.1 mAb was administered on days −3 and −1 before HSC transplantation. To confirm depletion, peripheral blood was obtained on day 0 from recipients treated with anti-CD8, anti-CD4, or anti-NK1.1 mAb, and stained with anti-CD4 FITC, anti-CD8 FITC, anti-NK1.1 PE, and mouse anti-rat IgG2b FITC mAb (isotype control).

HSC transplantation

A total of 5000 HSC was transplanted by lateral tail vein injection into conditioned (950 cGy) beige-B6 recipients, or B6 at least 6 h after conditioning with 950 cGy total body irradiation (TBI), as previously described (23).

Analysis of MHC class I molecule expression on HSC

HSC were sorted, then stained, with one of the following mAb anti-class I A and D: H-2Kb FITC (AF6-88.5; BD Pharmingen) or H-2Dβ FITC (KH95; BD Pharmingen), with isotype staining serving as controls.

Expression of inhibitory or activating receptors on NK cells

Flow cytometric analysis was performed to determine the percentage of inhibitory (Ly-49A, Ly-49G2, Ly-49C/I, and CD49/NKG2) and activating (Ly-49D) receptors on NK1.1+ cells. Briefly, 250 μl of whole blood from recipients was collected in heparinized tubes, and aliquots of 50 μl were stained with anti-H-2Kb FITC vs specific mAbs for T cells (TCR-β, CD8α, CD4), B cells (CD45R/B220), NK cells (NK1.1), granulocytes (Gr-1), and macrophages (CD11b) for 30 min. RBC were lysed with ammonium chloride lysing buffer for 5 min at room temperature, then washed twice and fixed in 1% paraformaldehyde.

White blood cell (WBC) measurement

WBC counts (number × 10^9/ml) were determined using the MASCOT Multispecies Hematology Analyzer (CDC Technologies) on peripheral blood 3 mo after transplantation.

Statistical analysis

Experimental data were evaluated for significant differences using the Student t test; p < 0.05 was considered a significant difference. Graft survival was calculated according to the Kaplan-Meier method.

Results

Purified HSC from β2m−/− donors failed to engraft in wild-type B6 mice, but engrafted in beige-B6 mice

We first analyzed the level of expression of MHC class I K and D on purified HSC from wild-type B6, β2m−/−/−B6 (class I-deficient), and Abb−/−/−B6 (class II-deficient) mice. Low levels of expression of MHC class I K and D molecules were observed on HSC from β2m−/−/−B6 mice (Fig. 1B). As expected, Abb−/−/−B6 mice exhibited normal levels of expression of class I K and D compared with wild-type B6 mice (Fig. 1, A and C).

HSC were sorted from MHC class I- or class II-deficient mice, and 5000 purified HSC were transplanted into congenic recipient B6 mice conditioned with 950 cGy TBI 6 h after conditioning. Purified HSC from class II-deficient mice engrafted readily in B6 mice, with a median survival time of >120 days (Fig. 1D). In contrast, engraftment was significantly impaired in recipients of purified HSC from class I-deficient donors (p < 0.001), with failure of engraftment occurring rapidly (median survival time = 15 days). Recipients died between 11 and 20 days after HSC transplantation (Fig. 1D), coincident with radiation controls. Taken together, B6 mice reject class I-deficient HSC, but accept class II-deficient HSC, suggesting that the absence of MHC class I molecules on HSC may cause their rejection.
Although beige-B6 mice do not produce functional NK and CTLs (25), the level of expression of the various inhibitory and activating molecules on their NK cells was similar to those of wild-type B6 mice (Fig. 2A). When beige-B6 mice were reconstituted with purified HSC from /H92522m/H11002 mice, engraftment and long-term survival were significantly enhanced (Fig. 2B, p < 0.001). These data suggested that either NK1.1 cells or CD8 cells may mediate the rejection of purified HSC.

CD8 or CD4 T cells did not play a role in the rejection of /H92522m/H11002 HSC

To determine whether T cells are involved in the rejection of HSC, we depleted CD4 or CD8 T cells in vivo in B6 recipients using anti-CD4 or anti-CD8 mAb. Efficacy of depletion was confirmed by staining host peripheral blood (PB) cells with anti-CD4 or anti-CD8 mAbs (Fig. 3A). /H92522m/H11002 HSC were transplanted into these recipients as well as into untreated controls after conditioning with 950 cGy TBI. All recipient animals died between 10 and 17 days (Fig. 3C). When CD8 KO, CD4 KO, or TCR-β KO B6 mice were transplanted with HSC from B6 mice, all maintained long-term engraftment and survival (data not shown).

NK cells are the major effector cell in the rejection of purified HSC (/H92522m/H11002 model)

NK cells have been reported to be relatively resistant to radiation (26). To examine this, the percentage of NK cells in wild-type B6 mice was analyzed before and 6 h after irradiation, the time point when the HSC were transplanted in our model. Notably, our results show that the NK1.1 cells in peripheral blood persist after irradiation (Fig. 4A). However, /H92522m/H11002 recipients engrafted with the /H92522m/H11002 HSC, indicating that these HSC are functionally normal when placed in a syngeneic recipient.
To confirm that the B6-NK cell-depleted recipients of \( \beta_2 \text{m}^{-/-} \) donors, and transplanted into beige-B6 recipients conditioned with 950 cGy TBI. A, NK1.1\(^+\)/TCR-\( \beta \) cells were sorted from \( \beta_2 \text{m}^{-/-} \)-B6, beige-B6, or B6 mice. The cells were then restained and analyzed for inhibitory and activating receptors. The level of expression of Ly-49 A, C/I, D, G2, and NKG2 was evaluated by flow cytometric analysis. B, Survival of beige-B6 recipients of \( \beta_2 \text{m}^{-/-} \)-HSC (\( p < 0.001 \)). These results indicate that the normal development and function of NK cells depend on the interaction of NK cell receptors with their specific MHC class I ligands.

Expression of specific inhibitory or activating receptors is altered on donor NK cells in \( \beta_2 \text{m}^{-/-} \)-B6 chimeras

\( \beta_2 \text{m}^{-/-} \) HSC transplanted into NK-depleted B6 mice offer an excellent model to evaluate the influence of the presence of MHC class I on development of NK cells in vivo. To evaluate donor NK cell development in \( \beta_2 \text{m}^{-/-} \)-B6 chimeras, we analyzed the level of surface expression of inhibitory and activating NK cell receptors. Mixed chimeras were prepared by transplanting 5000 purified \( \beta_2 \text{m}^{-/-} \)-B6 HSC into B6 mice pretreated with anti-NK1.1 mAb and conditioned with 950 cGy TBI. The percentage of donor chimerism ranged from 73 to 85% at 1 mo after HSC transplantation. The NK distribution of inhibitory and activating cell receptors was analyzed on NK cells by flow cytometry 3 mo after transplantation. To analyze the levels of NK receptor expression, donor (\( \beta_2 \text{m}^{-/-} \)) cells were based on R2 (H-2\( ^{b} \)-negative cells) (Fig. 7A). Notably, the levels of expression of the activating receptor Ly-49D and the inhibitory receptor NKG2 were significantly decreased on newly produced donor-derived NK cells compared with syngeneic B6 controls and B6 NK-depleted B6 controls (\( p < 0.05 \); Fig. 7B). Additionally, there was a significant decrease in the Ly-49D/Ly-49G2\(^+\) population of NK receptors, but not the Ly-49D/Ly-49G2\(^+\) subset (Fig. 7C). These results suggest that the newly produced donor \( \beta_2 \text{m}^{-/-} \) NK cells were influenced by exposure to recipient MHC class I expression.

Altered expression of selected inhibitory or activating receptors on host NK cells occurs in \( \beta_2 \text{m}^{-/-} \)-B6 chimeras

We also evaluated the repertoire of host NK cells that developed in the \( \beta_2 \text{m}^{-/-} \)-B6 chimeras. The patterns of expression of activating and inhibitory receptors on host NK cells in the chimeras were
compared with the expression levels in B6→NK-depleted B6 mice and B6→B6 mice prepared as controls. We noted specific changes in receptor expression after chimerism was established. The level of expression of the activating receptor Ly-49D and inhibitory receptors Ly-49G2, as well as the coexpression of Ly-49D/Ly-49G2 on host NK cells, was significantly decreased compared with the controls (Fig. 7, D and E; p < 0.01). In contrast, the level of expression of other inhibitory receptors (Ly-49A, Ly-49C/I, NKG2) was similar to that for the controls. This further confirms the involvement of the Ly-49D and Ly-49G2 subsets in inducing tolerance in the H-2m/B6 congenic strain combination.

Evidence for specific tolerance to donor-type HSC in vivo
To test whether β₂m⁻⁻⁻→B6 chimeras had developed tolerance to β₂m⁻⁻⁻ HSC, primary chimeric mice that had been prepared by depleting B6 mice of NK cells, followed by transplantation of 5000 β₂m⁻⁻⁻ HSC, received a second transplant in which 5000 purified HSC cells from β₂m⁻⁻⁻ B6 mice were transplanted into the chimeras after 950 cGy TBI conditioning. The percentage of donor chimerism in the primary chimeras ranged from 89 to 95%. In contrast to naïve B6 or syngeneic chimeras (B6→B6) prepared as controls, all β₂m⁻⁻⁻→B6 chimeric recipients engrafted second β₂m⁻⁻⁻ HSC transplants and exhibited long-term survival (Fig. 7F). These data demonstrate that recipient NK cells are tolerized in vivo by prior engraftment of β₂m⁻⁻⁻ HSC and suggest that bone marrow-derived elements are important in determining the host NK cell repertoire and inducing tolerance in NK cells.

Discussion
We recently found that murine HSC express high levels of MHC class I molecules, especially class I K, and that MHC class I K plays a critical role in regulating long-term HSC engraftment via interaction with recipient NK cells (23). Notably, HSC must be matched to MHC class I K in the recipient for efficient and durable engraftment to occur (23). Interestingly, the pretreatment of wild-type recipients with anti-NK mAb overcomes this requirement for class I K matching between HSC and recipient, implicating a central role for NK cells in regulating HSC engraftment. However, it has remained controversial as to whether NK cells directly target HSC (5–8). Previous data demonstrating that NK cells do not act directly on HSC showed that purified c-kit⁺/Sca-1⁻/Lin⁻ HSC (KSL cells) incubated with activated allogeneic or semiallogeneic NK cells could rescue ablated syngeneic animals (28), suggesting that NK cell cytotoxicity is not due to a direct effect on HSC. Other
studies showed that NK cells have the ability to kill committed hemopoietic progenitor cells, but did not directly address their impact on HSC (6, 29). SCID mice, which are T and B cell deficient, but NK cell replete, reject allogeneic BMC, indicating that NK cells are critical in mediating the specificity of BM rejection (30). The ability of NK cells to recognize MHC class I molecules was first investigated in studies using MHC class I-deficient (disrupted β2m gene) mice (27, 31). NK cells from normal mice attack BMC or lymphoblasts from class I-deficient mice (27, 31, 32). NK cell-mediated alloreactivity has also been observed in allogeneic BM graft rejection (4, 5, 33, 34), indicating that NK cells can recognize the presence of allogeneic MHC molecules and may in fact regulate engraftment of HSC. Our present data demonstrate that recipient NK cells act directly on purified HSC, because recipients depleted of NK cells or NK-deficient B6-beige mice engraft readily with β2m−/− HSC, while untreated controls do not, and that tolerance can be induced in recipient NK cells by HSC transplantation.

In our present study, we transplanted purified HSC from β2m−/− donors to characterize the direct role of NK cells in HSC engraftment and tolerance. Our data indicate that wild-type B6 recipients readily engraft purified HSC from MHC class II-deficient mice, but not from MHC class I-deficient mice, confirming that loss of donor MHC class I expression results in direct rejection of purified HSC. B6 recipients of β2m−/− HSC expire at the time of irradiation controls, suggesting an early failure of engraftment or graft rejection. Purified HSC from β2m−/− donors engraft in B6-beige mice that are deficient in both NK cell and cytotoxic T cell function. We demonstrate that the rejection of purified HSC is not directly related to host CD8+ or CD4+ T cells, as MHC class I-deficient HSC failed to engraft in B6 recipients depleted of CD4+ or CD8+ cells as well as in CD8 KO, CD4 KO, or TCR-β KO recipients. However, depletion of NK cells dramatically prevented rejection of β2m−/− HSC, allowing all recipients to durably engraft. These data demonstrate that host NK cells play a critical and direct role in the rejection of class I-deficient HSC.

NK cell Ly-49 receptors include activating and inhibitory receptors that are able to distinguish individual MHC class I alleles and positively or negatively regulate NK cell cytolytic function in regulation of the innate immune response (13). Individual NK cells can express several (up to five) NK receptors and in different combinations (35). The Ly-49 receptors are also expressed on memory T or NK-T cells (36), and each member of the Ly-49 family receptor interacts with specific MHC class I molecules. For example, Ly-49A receptors recognize H-2Dd and H-2Db (37–40). Ly-49G2 receptors recognize H-2Dd and H-2Ld (41), and Ly-49C and/or...
Ly-49I recognize H-2Kb molecule (42). In BM transplantation (H-2d→H-2b), depletion of the inhibitory Ly-49C/I NK subset (which inhibits the rejection of allogeneic H-2b bone marrow grafts) significantly increased rejection of H-2b BMC (19). Similarly, removal of inhibitory Ly-49G2+ NK cells (which inhibit the rejection of allogeneic H-2d BM grafts) augmented the capability of the mice to reject allogeneic H-2b BMC (18). Ly-49D, an activator of NK cell cytotoxicity (43), has been shown to play a role in rejection of allogeneic BMC, as well as xenogeneic HSC (4, 44). Previous studies showed that Ly-49D NK cells in conditioned H-2b mice mediate rejection of H-2 homozygous allogeneic H-2d BMC. However, in successfully engrafted mice, self-tolerance of Ly-49D+ cells in H-2Dd mice was associated with increased coexpression of specific inhibitory Ly-49 receptors (Ly-49A or Ly-49G2) (3), while coexpression of H-2Kb-specific Ly-49C/I inhibitory receptor resulted in tolerance to Kb targets (3). We show in this study using the β2m−/−→B6 chimera model that recipient NK cells are modulated by a decrease in the subsets expressing Ly-49D and more precisely the Ly-49D receptor resulted in tolerance as was previously suggested (45, 46). Notably, receptor editing was also present on the donor NK cells developing in the chimeras, reflected by a decrease in the subset expressing Ly-49D. However, unlike for the newly produced recipient NK cells, this was not associated with a decrease in the Ly-49G2+ population. Taken together, these data suggest an educational process in donor NK cells to be tolerant to H-2a-expressing recipient MHC class I Ags. Moreover, these observations identified the Ly-49D-activating receptor as an important molecule for NK cell-mediated rejection of cells lacking class I expression, as well as the previously reported observation for H-2d and xenogeneic F344 and Lewis rat cells.

A role for NK cell tolerance remains controversial. Recent evidence suggests that NK cells are tolerated to donor alloantigen during engraftment. Our own studies in the β2m−/−→B6 chimeras strongly support a role for NK cell tolerance because second transplants of β2m−/− HSC into chimeras (β2m−/−→B6) were not rejected, while they were rapidly rejected by naive B6 recipients, as well as syngeneic B6→B6 chimeras. Therefore, the second TBI conditioning and BMT did not nonspecifically enhance engraftment of β2m−/− HSC, but rather an active process occurred and was associated with up- and down-regulation of specific receptors. It has been suggested that the balance between expression of specific inhibitory and activating receptors may contribute to regulation of the function of NK cytolysis (13), but a direct correlation remains to be defined. Altered expression of NK subsets has been reported in mixed allogeneic chimeras (21, 47). However, in mixed allogeneic chimeras (BALB/c→H-2b→B6 (H-2d)), the levels of expression of Ly-49A+, Ly-49C/I+, and Ly-49A+/Ly-49C/I+ inhibitory NK cell subsets were diminished after interaction with cognate ligands. Other studies indicated that NK cells in mixed chimeras exhibited specific tolerance to both donor and host Ags (5). However, down-regulation of the H-2d-specific activating receptor Ly-49D was not found, and the authors concluded that a change in the balance of inhibitory or activating receptors was not the underlying mechanism for NK tolerance (5). Our own results provide strong evidence for NK cell receptor editing in response to the establishment of mixed chimera (β2m−/−→B6→B6), because the expression of selected inhibitory and activating receptors Ly-49G2 and Ly-49D, as well as coexpression of Ly-49G2 and Ly-49D on newly produced donor and recipient NK cells in mixed chimeras was significantly decreased compared with syngeneic controls. Our findings parallel two recent reports of a role for Ly-49Q on precursor plasmacytoid dendritic cells in regulating the interface between innate and adaptive immune responses (48, 49). Our results therefore indicate that the environment of the mixed chimera shapes the NK cell receptor repertoire
of both donor and host NK cells, and that donor hematopoietic elements contribute significantly to the outcome during NK cell tolerance induction.

The mechanism of NK cell development and education is not clear. MHC class I expression contributes to NK cell development and/or activation because MHC class I-deficient mice have a normal number of NK cells and NK cell subfamilies present, although the function is significantly impaired (27). Our own data support this observation, because Ly-49 expression on NK cells from B6-beige mice resembled that for control B6 mice despite a significant difference in function. Previous studies have shown that NK cells from β₃m⁻/⁻ mice do not lyse MHC class I-deficient target cells, suggesting that the cytotoxic function of NK cells is impaired (27, 50). Other studies showed that NK cells can specifically acquire MHC class I molecules from surrounding cells in vivo or in vitro, and down-regulate certain inhibitory receptors (51, 52). Our data show that β₃m⁻/⁻ mice have normal levels of inhibitory and activating receptor expression on their NK cells compared with wild-type B6 mice. Importantly, the NK cells from β₃m⁻/⁻ mice failed to reject B6 or B10.BR HSC, further demonstrating that NK cell function is indeed impaired in class I-deficient mice.

In conclusion, we show that host NK cells play a critical role in the regulation of early engraftment of MHC class I-deficient HSC, and that NK cells can indeed be rendered tolerant to their MHC class I-deficient targets. The decrease in the Ly-49D subset in both donor- and recipient-derived NK cells reflects an educational process that leads to tolerance of cells that lack MHC class I. A better understanding of the role of inhibitory and activating NK cell receptors in early HSC engraftment and tolerance induction may allow a novel approach to condition a recipient for BMT through manipulation of the inhibitory and activating NK receptor families, and, as a result, significantly reduce the toxicity of the procedure.

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Disclosures

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