Natural killer (NK) cells express inhibitory receptors with varied binding affinities to specific major histocompatibility complex class I (MHC-I) haplotypes. NK cells can be classified as licensed or unlicensed based on their ability or inability to bind MHC-I, respectively. The role of donor vs host MHC on their development after allogeneic hematopoietic stem cell transplantation (allo-HSCT) is not known. Following reciprocal MHC-disparate allogeneic transplants and during de novo NK-cell recovery, depletion of the licensed and not unlicensed population of NK cells as determined by the licensing patterns of donor MHC-I haplotypes, resulted in significantly increased susceptibility to murine cytomegalovirus (MCMV) infection. A corresponding expansion of the licensed Ly49H+ NK cells occurred with greater interferon-γ production by these cells than unlicensed NK cells in the context of donor MHC-I. Thus, NK licensing behavior to MCMV corresponds to the donor, and not recipient, MHC haplotype after allo-HSCT in mice. (Blood. 2013;122(8):1518-1521)

Study design

Mice

Female B10.D2 (H-2b), B10 (H-2b), C57BL/6 (H-2b), and CB6F1 (H-2b) Jackson Laboratory mice were 6 to 8 weeks old and housed in Association for Assessment and Accreditation of Laboratory Animal Care–approved specific pathogen-free facilities under Institutional Animal Care and Use Committee–approved protocols.

HSCT

Bone marrow cells (BMCs) were extracted from donors depleted of NK cells (anti-NK1.1 [PK136] in vivo) and T cells (in vitro Thy1.2 [30H12] and rabbit complement13). Recipients were exposed to a lethal dose of 950 cGy γ-irradiation from a137Cs source and injected intravenously with syngeneic or allogeneic (full MHC-mismatch H2d vs H2b) BMCs (5 x 106 BMCs).

MCMV infection

MCMV Smith strain was obtained from American Type Culture Collection. MCMV (5 x 105 plaque-forming units) was administered intraperitoneally.
Results and discussion

We first compared the ability of NK subsets to mediate MCMV resistance by in vivo depletion of subsets following syngeneic or allo-HSCT. After syngeneic HSCT, B10 (H-2b) mice depleted of their licensed population of NK cells (Ly49Cl− cells which bind to H-2b) showed greater viral loads in the liver as compared with mice depleted of the unlicensed (Ly49G2+), which bind H-2d) cells along with expansion of licensed cells in nondepleted mice (supplemental Figure 1). However, when an allo-HSCT, devoid of NK and T cells to avoid graft-versus-host-disease, was performed using donor hematopoietic stem cells from B10.D2 mice into host B10 mice, a licensing pattern consistent with the donor H-2d MHC-I haplotype was observed with depletion of the Ly49G2+ population resulting in greater viral loads (Figure 1A). Depletions of the subsets are shown to be effective in B10 (supplemental Figure 2) and B10.D2 (supplemental Figure 3) mice.

The reciprocal transplant model using B10 donors into B10.D2 host mice showed an identical pattern of licensing based on donor MHC-I, suggesting the findings are intrinsic to licensing itself and not due to a specific strain of mouse or one of the NK subsets (Figure 1B). Additionally, utilization of CB6F1 mice that express both H-2b and H-2d as donors to C57BL/6 recipient mice (which were shown to have equivalent responses to MCMV as B10 mice10) in a HSCT resulted in equivalent viral loads upon depletion of either Ly49G2+ or Ly49Cl− subsets, suggesting equivalent licensing of both populations (Figure 1C).

The viral loads after allo-HSCT were greater than after syngeneic HSCT, implying potential differences in overall recovery and bone marrow reconstitution of the mice. However, a greater fold difference between licensed and unlicensed NK depletion in viral load between allo-HSCT and syngeneic HSCT is apparent (81.1 ± 6.7 vs 8.6 ± 2.3 in B10 host mice, respectively, and 12.9 ± 2.12 vs 4.5 ± 1.1 in B10.D2 host mice, P < .05 in both by t test), suggesting not only a licensing effect but a lack of inhibition of these cells by host MHC-I resulting in greater activity as suggested previously.15

A significant expansion of licensed NK cells, based on the donor MHC-I, was observed that were also Ly49H+ (activating receptor that binds to MCMV glycoprotein m15716,17). The NK cells observed were all donor-derived (Figure 2A) with expansion of the donor-licensed population post-MCMV infection in terms of percentage of cells (Figure 2B-D) and total numbers (supplemental Figure 4).

To determine whether there were differences in functional capabilities between the donor licensed and unlicensed cells beyond cell expansion differences, IFNγ production by the different subsets was examined. Based on donor MHC-I, the frequency of licensed, Ly49H+ NK cells producing IFNγ was significantly greater than the unlicensed population (Figure 2E-G). Potentially, increased exposure time to host MHC-I could result in licensing to host MHC-I, but infecting mice at a later time point post–allo-HSCT (day 17) resulted in MCMV-resistance patterns and activity consistent with licensing based on donor MHC-I (Figure 1D; supplemental Figure 5).

The data demonstrate that NK licensing post-HSCT does indeed occur based on donor MHC-I in terms of IFNγ production, expansion, and MCMV resistance in newly developing NK cells. Although the cytokine milieu and lymphodepletion post–HSCT and MCMV infection may alter the development and activation of NK cells, the pattern of licensing in anti-MCMV responses was still apparent in all mouse strains tested. These findings may appear to differ from previous work showing adoptive transfer of MHC-I–deficient NK cells into wild-type mice resulting in licensing of NK cells based on host MHC-I expression18 However, because our model used HSCT and we see a pattern of licensing consistent with donor MHC-I regardless of exposure time to host MHC-I, our
data indicate the cells involved with licensing seem to be of hematopoietic origin and donor-derived.

Our findings are in concordance with human studies regarding NK licensing being based on donor KIR ligands and show that mice also follow this pattern of licensing in a pathological model and reinforce the validity of preclinical mouse models. These data are of significant clinical relevance as recent studies have attempted to use NK cells after HSCT to reduce tumor relapse and viral reactivation. Having allogeneic NK cells that are licensed in the donor could provide significant antiviral protection in these patients.

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Authorship

Contribution: C.M.S. designed and performed experiments, analyzed data, and wrote the paper; Y.J.T.-F. maintained MCMV and helped perform experiments; A.E.Z. helped perform experiments and reviewed the paper; M.A. helped in experiment design and review of the paper; C.P. provided insight into MCMV experiments, designed experiments, and reviewed the paper; and W.J.M. designed experiments and wrote the paper.

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References


Murine NK-cell licensing is reflective of donor MHC-I following allogeneic hematopoietic stem cell transplantation in murine cytomegalovirus responses

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