Regulation of the NK Cell Alloreactivity to Bone Marrow Cells by the Combination of the Host NK Gene Complex and MHC Haplotypes

Koho Iizuka, Anthony A. Scalzo, Hong Xian and Wayne M. Yokoyama

*J Immunol* 2008; 180:3260-3267; doi: 10.4049/jimmunol.180.5.3260

http://www.jimmunol.org/content/180/5/3260

References

This article cites 59 articles, 24 of which you can access for free at:
http://www.jimmunol.org/content/180/5/3260.full#ref-list-1

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
Regulation of the NK Cell Alloreactivity to Bone Marrow Cells by the Combination of the Host NK Gene Complex and MHC Haplotypes

Koho Iizuka, Anthony A. Scalzo, Hong Xian, and Wayne M. Yokoyama

Host NK cells can reject MHC-incompatible (allogeneic) bone marrow cells (BMCs), suggesting their effective role for graft-vs-leukemia effects in the clinical setting of bone marrow transplantation. NK cell-mediated rejection of allogeneic BMCs is dependent on donor and recipient MHC alleles and other factors that are not yet fully characterized. Whereas the molecular mechanisms of allogeneic MHC recognition by NK receptors have been well studied in vitro, guidelines to understand NK cell allogeneic reactivity under the control of multiple genetic components in vivo remain less well understood. In this study, we use congenic mice to show that BMC rejection is regulated by haplotypes of the NK gene complex (NKC) that encodes multiple NK cell receptors. Most importantly, host MHC differences modulated the NKC effect. Moreover, the NKC allelic differences also affected the outcome of hybrid resistance whereby F1 hybrid mice reject parental BMCs. Therefore, these data indicate that NK cell alloreactivity in vivo is dependent on the combination of the host NKC and MHC haplotypes. These data suggest that the NK cell self-tolerance process dynamically modulates the NK cell alloreactivity in vivo.


Natural killer (NK) cells were initially described as having the capability to attack tumor cells without prior sensitization (1). The identification of MHC class I-specific NK cell inhibitory receptors in the early 1990s has revolutionized our conception of how NK cells selectively recognize and attack tumors while sparing normal cells (2–4). Upon ligand binding, NK inhibitory receptors transmit inhibitory signals to activating receptors. Through an ITIM in its cytoplasmic domain that becomes phosphorylated, inhibitory receptors recruit Src homology 2 domain-containing phosphatase 1, which then cancels out phosphorylation events involved in the NK activation pathways. Significant progress has been made since these initial findings with regard to NK cell inhibitory as well as activating receptors specific to MHC class I or class I-like molecules. This progress has led to the hypothesis that NK cells lacking cognate inhibitory receptors for tumor MHC will have greater activity against those tumor cells. Indeed, in the setting of haploidentical hematopoietic cell transplantation for acute myeloid leukemia, significantly fewer relapses and graft-versus-host disease episodes have been reported in NK receptor-ligand-mismatched donor/recipient pairs than in NK receptor-ligand-matched donor/recipient pairs (5). No correlation between NK receptor-ligand mismatches and relapses has been observed in patients undergoing allogeneic transplant for acute lymphoid leukemia. However, this observation has not been uniformly observed in other transplantation centers (6–8). Molecular mechanisms of allogeneic MHC recognition by NK receptors have been well characterized at the cellular and molecular levels (9, 10). However, guidelines to understand NK cell alloreactivity under the control of multiple genetic components in vivo remain undetermined and further study with animal models is required to deduce such guidelines.

In contrast to solid tissue transplant rejection that is mediated by components of specific immunity, i.e., T cells, the rejection of allogeneic bone marrow cells (BMCs)3 in lethally irradiated mice is primarily mediated by host NK cells. For example, β2-microglobulin (β2m)-deficient BMCs are readily rejected by wild-type, otherwise syngeneic hosts, indicating a role for MHC class I molecules on donor cells (11). NK cells are responsible for this process because rejection was abrogated by systemic administration of anti-NK cell receptor Abs that deplete NK cells. However, further definition of the NK cell effect is limited with the Ab approach due to the paucity of available mAbs, incomplete description of mAb specificities (12), and expression of multiple receptors by an individual NK cell (13). Furthermore, other host effects are difficult to dissect with this approach. Therefore, unlike the already detailed knowledge of the recognition processes involved in rejection of solid organs, the parameters affecting NK cell rejection of BMC grafts remain to be clearly determined.

Although MHC alleles clearly appear to play some role in bone marrow transplantation (BMT), the genetic transplantation laws governing rejection of BMCs by lethally irradiated mice also differ from those of solid organ transplantation. The most prominent example of these differences is hybrid resistance, whereby F1 hybrid

---

3 Abbreviations used in this paper; BMC, bone marrow cell; βm, β2-microglobulin; BMT, bone marrow transplantation; BM, bone marrow; NKC, NK gene complex; ASGM, asialo GM1 Ab.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00
offspring from two H2-disparate strains often reject parental BMCs (14, 15). This phenomenon has been explained by several hypotheses. One suggests that NK cells recognize recessively inherited histocompatibility Ags on parental (donor) BMCs, the Hh-1 (hemopoietic histocompatibility 1) theory (15). To account for the numerous rejection patterns observed in different F1 combinations, it was necessary to presuppose that there must be modifications of the Hh-1 gene expression, further complicating this hypothesis (15–17). Alternatively, the “missing self”-hypothesis is based on observations that there is an inverse correlation between target cell MHC class I expression and susceptibility to NK cells lysis (18).

However, the missing self-hypothesis does not explain all features of hybrid resistance. For instance, F1 hybrid mice sometimes reject one parental bone marrow (BM) graft but not the other, unless T cells are depleted from donor BMCs (19). Hence, there must be other considerations to explain the rules governing BMT with respect to MHC mismatches. “Licensing” is a recently described phenomenon to explain the NK cell tolerance process and functional competence (20). In the licensing process where the ligand-receptor interaction occurs, NK cells gain functional competence, such as killing and cytokine production, and these developmental effects are paradoxically conducted through the ITIM of inhibitory receptors. However, the mechanism of licensing is poorly understood in terms of modes of receptor and ligand interaction, signaling mechanisms and development stages (21). It is not known how the licensing process affects the hybrid resistance.

Allogeneic BM/T is a simpler experimental model with which to analyze these complex donor and host effects. The ability to reject allogeneic BMC is recipient strain dependent and donor determinant specific (22). For example, irradiated C57BL/6 (B6, H2b) mice can reject a large inoculum of BMC from H2d mice, whereas irradiated 129 (also H2d) mice do not (recipient strain dependent). By contrast, B6 mice fail to reject a large inoculum of BMCs from H2b haplotype mice (donor determinant specific). Thus, the rejection outcome is frequently unpredictable, when judged only from the perspective of the donor or recipient MHC haplotype (23).

It is formally possible that MHC haplotypes could influence the function of other loci that are involved in BM rejection. One candidate locus was identified using H2b recipients from a backcross panel derived from NK1.1+/B6 and NK1.1− (129) strains (23). NK1.1 expression was found to be genetically linked to the ability to reject H2d BMCs. However, in backcross panels of H2b recipients (B10.S-H2a and A.SW), NK1.1 expression did not segregate with rejection of H2ja. Other genetic loci, including host MHC, to reject H2s BMCs. However, in backcross panels of H2s recipient strain derived from NK1.1−/H11001 (B6) and NK1.1−/H11002 (129) strains (23).

The NK1.1 (Nkrp1c) locus resides in the NK gene complex (NKC) on distal mouse chromosome 6 (13). The NKC contains multiple clusters of genes that encode NK cell receptors belonging to the C-type lectin superfamily (24). Many of them recognize MHC class I- or class I-like molecules as ligand. Indeed, transgenic mice expressing inhibitory Ly49 receptors demonstrated their potential role in allogeneic BM rejection (25, 26), but it was not previously examined in detail how the interaction of a specific NK inhibitory receptor and its ligand regulates functional alloreactivity to third-party BMCs (meaning the allogeneic BMCs that are not recognized by the transgenic NK cell receptor). Moreover, the Ly49 receptors are inherited as haplotypes (27), i.e., clusters of genes that could influence allogeneic BM. In contrast to Ly49 receptors, Nkrp1 family members do not recognize MHC class I ligands but the C-type lectin-related (Clr) family, which colocalize within Nkrp1 loci (28, 29). With a rat CMV decoy ligand, poly-

![FIGURE 1](http://www.jimmunol.org/)  
**FIGURE 1.** Host MHC haplotypes differentially affect rejection of allogeneic BMCs in different genetic backgrounds. The indicated mouse strains were irradiated and received 10⁶ SJL BMCs i.v. Splenic ¹²⁵I-UdR uptake was measured as an index of bone marrow engraftment as described in Materials and Methods. All recipient groups contained at least five mice. In multiple comparisons (15 comparisons), the difference between B10 and B6 was not statistically significant (t test; p value two tail was 0.0048). This experiment was performed once. Similar results were obtained when 10⁶ T cell-depleted SJL BMCs were transplanted (data not shown).

Morphisms in the inhibitory Nkrp1b for ligand specificity was recently described (30). Hence, the NKC contains a large number of functional NK cell genes, including NK1.1, and these genes may have different alleles, thus possibly affecting allogeneic BM rejection in a MHC-dependent and -independent manner (31). Although it is theoretically possible to identify specific allotypes of the receptors in the NKC and the MHC class I alleles involved in BMT rejection, as yet, there are not even guidelines to explain the functional interplay of MHC and NKC haplotypes in BMT.

It has not been previously possible to isolate NKC effects or directly examine the influence of the MHC haplotype on the NK cell in vivo. In this study, we use NKC and H2-congenic mice in the BM/T system to directly assess the effects of NKC haplotypes and the influence of MHC on NK cell alloreactivity.

Materials and Methods

**Mice**

C57BL/6 (B6), BALB/c, and SJL/JCr were purchased from the National Cancer Institute (Frederick, MD). C57BL10JF1, B10.D2-H2b, B10.S-H2s, B10.BR-H2a, C57BL10-H2b, and SJL/J-B2m<sup>min</sup> were purchased from The Jackson Laboratory. BALB.B6-Cmv1r<sup>−</sup> is a congenic strain in which the murine CMV (MCMV) resistance allele, Cmv1r<sup>−</sup>, as well as other NK-cell-linked loci from B6 were transferred onto the BALB/c genetic background as described previously (32). Intra-NKC recombinant congenic strains were generated by backcrossing BALB.B6-Cmv1r<sup>−</sup> to BALB/c mice and identifying further intra-NKC recombinants. These strains (BALB.B6-C3T and BALB.B6-C7T6) have smaller segments of the B6-derived NKC region than BALB.B6-Cmv1<sup>−</sup> as described previously (Ref. 33 and see Fig. 5).

**BMT assay**

BMT and assessment of engraftment were performed by standard methods as previously described (35). Briefly, after gamma irradiation (9.5 Gy from a ¹³⁷Cs source) on day 0, recipient mice received the indicated number of BM cells from donor strain via i.v. tail vein injections. On day 5, recipient mice were injected i.v. with 3 µCi of ¹²⁵I-UdR and 1 × 10<sup>−11</sup> mol of FUDR. On day 6, the spleens were removed, rinsed with PBS, fixed in 70% ethanol for 3 h, and the radioactivity was counted with a gamma counter.
Incorporation of radioactivity into the spleens was used as an index of hemopoietic precursor cell proliferation. Where indicated, mice were treated with 50 μg H9262 l of the anti-asialo GM1 Ab (anti-ASGM; WAKO) via i.v. 2 days before BMT to remove endogenous NK cell activity. T cell depletion of BMCs was performed with anti-Thy1.2 Ab (HO-13-4; American Type Tissue Collection) and rabbit complement (Cedarlane Laboratories/Accurate Chemical & Scientific). Each group had at least four mice, unless otherwise noted. At least two experiments were performed for each figure unless mentioned in the figure legend.

**Statistics**

Multiple comparisons within each experiment were conducted. The experiment-wise error rate was held to the 0.05 level by performing a Sidak t test which held the comparison-wise error rate to be \( \frac{1}{n} \), where \( n \) is the number of comparisons (36, 37).

**Results**

**Multiple genetic factors affect allogeneic BMC rejection**

Genetic mechanisms governing allogeneic BM rejection are complicated and controlled by multiple genetic factors (Fig. 1). We transplanted \( 1 \times 10^6 \) BMCs from the SJL (H2s) strain into H2-congenic mice on the C57BL/10 (B10) and BALB genetic backgrounds. Five days after BMT, we measured the levels of 125I-UdR incorporation in proliferating donor cells. Whereas we observed a clear H2 effect of rejection of SJL BMCs on the B10 background (i.e., B10 (H2b) and B10.D2 (H2d)), we observed no significant H2 effect on the BALB/c background (i.e., C.B10-H2b (H2b) and BALB/c (H2d)). It is difficult to compare mice with the B10 and BALB backgrounds because we are not controlling for genetic factors other than the H2 loci. Because each strain has a different genetic background, the variability of proliferation could be due to genetic dissimilarities in sensitivity to irradiation or the splenic microenvironment required for donor cells to proliferate. Consistent with these notions, we observed that B10 and B6 mice displayed statistically different responses to allogeneic BMCs of DBA/1 mice in our preliminary studies (K. Iizuka and W. M. Yokoyama, unpublished data). To obtain insights into the genetic events regulating allogeneic BM rejection, we set out to analyze the effects of the NKC and H2 haplotypes by using H2 and NKC-congenic mice.

**Table I. Genetic background of mouse strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>H-2 Haplotype</th>
<th>NKC Haplotype</th>
<th>Genetic Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJL</td>
<td>s</td>
<td>SJL</td>
<td>SJL</td>
</tr>
<tr>
<td>SJL-B2m&lt;sup&gt;1/Cae&lt;/sup&gt;</td>
<td>Null</td>
<td>SJL</td>
<td>SJL</td>
</tr>
<tr>
<td>C57BL/6 (B6)</td>
<td>b</td>
<td>B6</td>
<td>B6</td>
</tr>
<tr>
<td>BALB/c</td>
<td>d</td>
<td>BALB</td>
<td>BALB</td>
</tr>
<tr>
<td>C.B10-H2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>b</td>
<td>BALB</td>
<td>BALB</td>
</tr>
<tr>
<td>BALB.B6-Cmv&lt;sup&gt;1/&lt;/sup&gt;</td>
<td>d</td>
<td>B6</td>
<td>BALB</td>
</tr>
<tr>
<td>(BALB × BALB.B6-Cmv&lt;sup&gt;1/&lt;/sup&gt;)F&lt;sub&gt;t&lt;/sub&gt;</td>
<td>b</td>
<td>B6/BALB</td>
<td>BALB</td>
</tr>
<tr>
<td>C.B10-H2&lt;sup&gt;b&lt;/sup&gt;-Cmv&lt;sup&gt;1/&lt;/sup&gt;</td>
<td>b</td>
<td>B6</td>
<td>BALB</td>
</tr>
<tr>
<td>(BALB × C.B10-H2&lt;sup&gt;b&lt;/sup&gt;)F&lt;sub&gt;i&lt;/sub&gt;</td>
<td>b/d</td>
<td>BALB</td>
<td>BALB</td>
</tr>
<tr>
<td>C57BL/10 (B10)</td>
<td>b</td>
<td>B10</td>
<td>B10</td>
</tr>
<tr>
<td>B10.S-H2&lt;sup&gt;s&lt;/sup&gt;</td>
<td>s</td>
<td>B10</td>
<td>B10</td>
</tr>
<tr>
<td>B10.D2-H2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>d</td>
<td>B10</td>
<td>B10</td>
</tr>
<tr>
<td>B10.WB-H2&lt;sup&gt;ae&lt;/sup&gt;</td>
<td>ja</td>
<td>B10</td>
<td>B10</td>
</tr>
</tbody>
</table>

**FIGURE 2.** The NKC regulates allogeneic BMC rejection. 

a. The indicated mouse strains were irradiated and received 10<sup>6</sup> SJL BMCs i.v. Splenic 125I-UdR uptake was measured. 
b. Irradiated mice of the indicated strains received 10<sup>6</sup> T cell-depleted SJL BMCs and bone marrow engraftment was assessed as in Fig. 1. Each mouse group, BALB/c, BALB.B6-Cmv<sup>1/</sup>, and (BALB/c × BALB.B6-Cmv<sup>1/</sup>)F<sub>t</sub>, was compared with each other for statistical analysis. An asterisk indicates a statistically significant difference between the groups designated with bars. Where asterisks and bars are not shown, there was no significant difference.

**FIGURE 3.** The NKC effect is \( \beta_{2m} \)-dependent. In brief, 10<sup>6</sup> \( \beta_{2m} \)-deficient SJL (SJL-B2m) BMCs were transplanted into recipient strains as described in Fig. 1. All recipient groups contained at least five mice, except for the SJL-B2m which contained two mice. Groups of BALB/c and BALB.B6-Cmv<sup>1/</sup> were statistically analyzed as in Fig. 2 and there was no significant difference. This experiment was performed once.

**FIGURE 4.** Rejection of BMCs from another H-2 haplotype. 

a. In brief, 10<sup>6</sup> B10.S-H2<sup>s</sup> BMCs were transplanted into indicated recipient strains as described in Fig. 1. The B10.S-H2<sup>s</sup> recipient group contained two mice. 
b. In brief, 10<sup>6</sup> T cell-depleted B10.S-H2<sup>s</sup> BMCs were transplanted into indicated recipient strains. Groups of BALB/c, BALB.B6-Cmv<sup>1/</sup> and (BALB/c × BALB.B6-Cmv<sup>1/</sup>)F<sub>t</sub> were statistically analyzed as in Fig. 2 and results are depicted as in Fig. 2.
To test whether allelic forms of the NKC affect allogeneic BMT rejection, we transplanted BMCs from SJL into irradiated B6, BALB/c, and BALB.B6-Cmv1r mice; the latter strain is a BALB/c strain congenic for the NKC derived from B6 (Table I). SJL BMCs, ranging from $4 \times 10^5$ to $2 \times 10^6$ were rejected by B6, but relatively poorly rejected by BALB/c recipients (Fig. 2 and data not shown). By contrast, BALB.B6-Cmv1r and (BALB/c × BALB.B6-Cmv1r)$^F_1$ mice rejected SJL BMCs well, to a level similar to that of B6 mice (Fig. 2a). Although splenic $^{125}$I-UdR uptake after BMT is an accepted assay of BM engraftment (11, 15), it is possible that the enhanced $^{125}$I-UdR incorporation was due to differences in capacity of mature T cells in the donor BM to proliferate in response to recipient allotypes. To eliminate this possibility, we transplanted T cell-depleted BMCs (Fig. 2b). T cell-depleted BMCs from SJL proliferated in BALB/c, but failed to do so in both BALB.B6-Cmv1r$^F_1$ and (BALB/c × BALB.B6-Cmv1r)$^F_1$ mice, an observation identical with that for mice without T cell depletion (Fig. 2a). This strongly suggests that the results were not due to alloreactive donor T cells. Finally, anti-NK cell depletion and transplantation into mice with selective NK cell deficiency (38) demonstrate that the rejection is NK cell dependent (data not shown), consistent with previous observations suggesting that NK cells are responsible for allogeneic BMT rejection (39, 40). Thus, these data indicate that the relevant donor epitope resides in $\beta_2$m-associated molecules, $\beta_2$m itself, or both.

Next, we transplanted BMCs from the B10.S-H2$^a$ strain, which has the same H2$^a$ haplotype as SJL. The rejection pattern of B10.S-H2$^a$ BMCs was comparable to that observed with SJL B10.S-H2$^a$ BMCs as well as BALB.B6-Cmv1r$^F_1$ and B6 mice did (Fig. 3), even though BALB/c were poor rejectors of SJL BMCs (Fig. 2a). Interestingly, $\beta_2$m-sufficient SJL recipient mice failed to reject donated $\beta_2$m-deficient SJL BMCs. This failure has not been previously reported but is consistent with the previously recognized abnormalities of SJL NK cells (41, 42), or NK cells in SJL may possess a dominant MHC-independent inhibitory receptors. More importantly, BALB/c mice retain both the signal transduction machinery for activation and effector mechanisms necessary to reject SJL BMCs, indicating that BALB/c has molecules to recognize and reject SJL BMCs depending on the recognition of transplanted BMCs. Furthermore, these data indicate that the relevant donor epitope resides in $\beta_2$m-associated molecules, $\beta_2$m itself, or both.

Next, we transplanted BMCs from the B10.S-H2$^a$ strain, which has the same H2$^a$ haplotype as SJL. The rejection pattern of B10.S-H2$^a$ BMCs was comparable to that observed with SJL

**FIGURE 5.** Mapping of the locus responsible for allogeneic BM rejection to the region between $Cd94$ and $D6Mit25$. a, Schematic representation of intra-NKC-congenic mice. A portion of mouse chromosome 6 with relevant markers is shown on top. For each strain, a thick bar indicates the genome derived from B6 mice, whereas a thin line indicates the BALB/c-derived genome. Schema shown below is an enlargement of the NKC region indicating the general location of the recombination break point for each strain. b, In brief, $10^6$ SJL BMCs were transplanted into indicated recipient strains as described in Fig. 1a. Groups of BALB/c, BALB.B6-CT3, BALB.B6-CT6, and BALB.B6-Cmv1r$^F_1$ were statistically analyzed as in Fig. 2 and results are depicted as in Fig. 2.
Allelic differences in the NKC affect hybrid resistance

In hybrid resistance, F1 mice often reject BMCs from either parental strain, but sometimes F1 mice reject only one parent’s BMCs and accept the other (15). When F1 mice are generated from non-H2-congenic mice, it is difficult to evaluate the influence of other genetic loci. Our data thus far suggest that allelic differences in the NKC have been overlooked previously and may be important in terms of affecting the outcome of hybrid resistance. To test this possibility, BALB.B6-Cmv1r mice were mated with C.B10-H2b to generate F1 mice that differed only with regard to NKC haplotype (see Table I). These F1 hybrid mice were transplanted with C.B10-H2b parental BMCs (Fig. 8). BALB/c × C.B10-H2b F1 mice treated with anti-ASGM (to eliminate host NK cells) accept parental BMCs more readily than nontreated F1 mice, indicating that hybrid resistance occurs in this combination although the resistance is relatively weak. In contrast, (BALB.B6-Cmv1r × C.B10-H2b)F1 mice rejected C.B10-H2b BMCs significantly more than (BALB/c × C.B10-H2b)F1 animals (7.6-fold vs 2.9-fold difference compared with anti-ASGM-treated BALB/c × C.B10-H2b)F1 mice). Thus, allelic differences of the NKC also directly affect the outcome of hybrid resistance.
Discussion
In the present study, we used NKC-congenic mice to show that NKC haplotypes control allogeneic rejection of SJL BMCs in vivo, formally demonstrating that this genetic region controls a complex outcome, successful engraftment or rejection of allogeneic BMCs. Most importantly, the MHC haplotype differentially influenced the effect of NKC haplotype, resulting in altered levels of rejection of allogeneic BMCs. This indicates that MHC does not always affect allogeneic BM rejection because the MHC influence depends on the NKC haplotype. Thus, these data lead to the conclusion that the combination of both NKC and H2 haplotypes determines the level of allogeneic BMT rejection, i.e., the alloreactivity of NK cells in vivo.

The allelic differences in the NKC also affected hybrid resistance. Although it is possible that different NKC loci regulate allogeneic rejection and hybrid resistance, we currently favor the reductionist interpretation that both rejection systems are regulated by the same NKC locus. Further evaluation of specific NKC loci will be needed to resolve this issue. In contrast, the influence of NKC alleles on hybrid resistance may explain, in part, the previous necessity to propose the Hh-1 theory to account for exceptions to rules otherwise determined by donor-derived MHC haplotypes in hybrid resistance. That the recipient MHC could control BM rejection has been evident for decades, especially in hybrid resistance (15, 43). However, previous studies have been focused on the MHC effect as being due to determinant molecules in MHC that are recognized by recipient. Although there is ample evidence that MHC molecules themselves are likely to be recognized directly on BMCs (44), the concept that the MHC effect is also due to its influence on the NKC control sheds important additional light on previous notions of the role of the MHC in BMT outcomes, such as in hybrid resistance. Furthermore, this study is the first to delineate the importance of NKC allotypes and their influence by MHC in NK cell activities in vivo.

In a previous genetic analysis of host factors that influence the outcome of allogeneic BMT, there was linkage to the NKL.1 locus (23). Due to the small number of backcrossed animals previously used and the close proximity of potentially relevant NKC loci, however, it was not possible to more precisely map the relevant locus or consider alternative hypotheses. Our studies made use of congenic and intra-NKC congenic mice that minimized any differences in non-NKC or non-MHC genes. This permitted more precise mapping to the region between C6d4 and D6Mit25, eliminating NK1.1 as being directly responsible for the “good responder status,” and allowed evaluation of other host factors, i.e., MHC, in regulating these effects. It is interesting that SJL mice have an apparent defect in rejecting β2m-deficient SJL BMCs, suggesting MHC-independent inhibition of NK cells (31). Nkrp1d was identified as an inhibitory NK cell receptor recognizing Clrb in a MHC-independent and ITIM-dependent manner (28). Although the Nkrp1 locus is not involved in the allogeneic rejection of SJL BMCs by mice with the B10 background, we examined the possibility that overexpression of Clrb on β2m-deficient BMCs can spare the lysis by engaging the Nkrp1d inhibitory receptor on B6 NK cells in a MHC-independent manner. We observed that β2m-deficient B6 progenitor BMCs transduced with Clrb failed to protect from lysis by host B6 NK cells in vivo (data not shown), suggesting that Clrb expression does not affect the rejection of β2m-deficient BMCs. Although Nkrp1b33H inhibitory receptor was shown to recognize Clrb33H (29), it is currently not known whether there is an allelic difference in Clrb between SJL and B6 mice. It will be interesting to examine whether Nkrp1b33H recognizes Clrb33H on SJL-β2m BMCs to inhibit rejection in vivo.

Several clues are available with respect to the nature of the allospecific NKC locus against H2. Since the BMT effect maps to the NKC, we postulate that the effect is due to the function of a NK cell receptor. Because the NKC encodes both activation and inhibitory receptors, the NKC effect may be due to a B6-derived activation receptor or a BALB/c-derived inhibitory receptor. In each case, the reciprocal allele for either hypothesized receptor would either be a null allele or encode a receptor that does not recognize the target ligand. The (BALB × BALB.B6-Cmv-1F)F1 hybrid results show a dominant B6 phenotype effect and is consistent with a B6 activation receptor. This receptor should be encoded in the C6d4 to D6Mit25 genomic region as shown by the BALB.B6-CT3 mouse which would be postulated to have gained the B6 activation receptor allele. In contrast, the data are also consistent with a BALB/c-derived inhibitory receptor encoded in the same interval. In this case, the BALB.B6-CT3 mouse would have lost the BALB/c inhibitory receptor allele. A BALB/c inhibitory receptor is consistent with the observation that BALB/c mice completely rejected BMCs from SJL-β2m-deficient mice, indicating that they already express an appropriate activation receptor to reject SJL-derived BMCs and that SJL-β2m-sufficient BMCs may deliver a MHC class I inhibitory signal that prevents rejection. Finally, it is possible that a cluster of highly related genes with overlapping functions and specificities may be involved inasmuch as the NKC contains numerous clusters of such genes (24). Many of those clusters contain genes for activating and inhibitory NK cell receptors and it is now generally believed that a balance of activating and inhibitory signals determines whether NK cells kill or do not kill at the single cell level. It therefore remains possible that the NKC effect on BMT is similarly dependent on both types of receptors.

It is obvious that allogeneic BMT has never exerted selective pressure on the evolution of the immune system in mice. This raises the question: what is the biological purpose or meaning of the combination of both NKC and H2 haplotypes in mice? We propose that the observed phenomenon reflects the NK cell self-tolerance process. B10 or B6 mice are fully capable of rejecting BMCs from B10.D2 mice (data not shown and Ref. 26), indicating that NKC derived from B10 mice encodes NK cell receptors capable of killing B10.D2 BMCs. However, when the H2d haplotype is introduced into B10 mice (i.e., B10.D2 mice), they no longer attack B10.D2 BMCs in BMT because they now are recognized as self. This is true for the combination of C.B10.H2b and BALB/c mice (data not shown). In other words, NK cells are educated to be self-tolerant by the H2d haplotype. Because NKC and MHC loci are located on different chromosomes, NK cell self-tolerance first needs to be established depending on the combination of NKC and H2. In an allogeneic BMT setting, this leads to an alteration in the alloreactivity of NK cells mediated also by the NKC haplotype. Thus, we believe that the mechanisms underlying NK cell tolerance are likely to be the same mechanisms at work in establishing the NK cell-differential alloreactivity by the combination of host H2 and NKC haplotypes.

The nature of the self-tolerance mechanism as it applies to allogeneic BMT rejection is not yet clear at the cellular and molecular levels. Is this the same as licensing (20)? It may be the case for the B10 background. Inasmuch as freshly isolated Ly49A-positive NK cells from B10.D2 mice (expressing H2d), the ligand for Ly49A) kill target cells better than the ones from B10 mice (20), Ly49A-positive cells may be licensed to kill SJL BMCs in B10.D2 mice but not in B10. In turn, licensing may be an inadequate explanation of observations from the Ly49C–H2d interaction point of view. B10 mice (expressing H2k), the ligand for Ly49C can license peripheral NK cells expressing Ly49C, but readily fail to
reject SJL BMCs. Similarly, NK cells licensed by Ly49A in the BALB background are evidently not competent to reject SJL BMCs, despite the fact that BALB/c and C.B10-H2b mice express Ly49A \(^{BALB}\) in the same way as Ly49A \(^{B10}\) in B10 and B10.D2 mice, including specificity, function, expression level, and its alteration by H2 haplotypes \((45)\). Whereas it is not known whether Ly49A \(^{BALB}\) or Ly49A \(^{B10}\) can license NK cells on the BALB/c background, several possibilities exist why the NK cell subset licensed by Ly49A \(^{BALB}\) does not kill SJL BMCs: 1) the NK cell subset licensed by Ly49A \(^{BALB}\) does not express a specific activating receptor for SJL or 2) the licensed subset does express a specific inhibitory receptor for SJL. The former possibility is strongly argued by the observation that BALB/c mice are competent to reject β\(_2\)-m-deficient SJL BMCs, inasmuch as the rejection is mediated by mature, thus “licensed” NK cells. Regarding the latter possibility, it is interesting that Ly49A \(^{B10}\) binds to Con A blasts from B10.S mice (H2\(^s\)) although the binding is weaker than to Con A blasts with the H2\(^d\) haplotype \((46)\). Alternatively, mechanisms in addition to licensing may exist to regulate the NK cell tolerance process and activity \((47)\). Elucidation of the cellular and molecular mechanism to explain differential effects of NK haplotypes by MHC allele requires further studies.

Several NKC loci that affect other NK cell functions in vivo were reported, such as \(Cmv1, Rmp1, Chok,\) and \(Nka\) \((48–51)\). Our studies demonstrate that the locus controlling allogenetic BM rejection in mice was also mapped to a region that includes the Ly49 cluster. Although the effector mechanisms underlying rejection of BM in vivo remain to be determined, attempts have been made to translate this in vitro killing of Con A blasts to hybrid resistance or allogenic rejection of BM in vivo \((52)\). However, differential effects on NK allelics by H2 have not been demonstrated in a genetically controlled manner. Regarding the differential NKC effects by H2, the control of MCMV by \(Cmv1\) is especially interesting. \(Cmv1\) was identified as Ly49H in the B6 NKC allele \((53–55)\) that recognizes m157, a MHC class I-like protein encoded by the MCMV genome \((56, 57)\). Further study of MCMV-resistant strains to self, nonself discrimination.


