Increased bone marrow allograft rejection by depletion of NK cells expressing inhibitory Ly49 NK receptors for donor class I antigens

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Natural killer (NK) cells are the major effectors of acute rejection of incompatible bone marrow cell (BMC) grafts in lethally irradiated mice. The immunogenetics of BMC rejection are largely controlled by the coexpression (or not) of inhibitory and stimulatory Ly49 receptors whose ligands are class I major histocompatibility complex (MHC) molecules. The majority of the BMC rejection studies involved low numbers of BMCs that were resisted by host NK cells. In the present study, larger numbers of BMCs were given in which rejection was not detected and the role of different Ly49 NK subsets not presumably involved in the rejection of a particular BMC haplotype was examined. Surprisingly, the data show that the removal of NK cell subsets expressing Ly49 inhibitory receptors for donor class I antigens, which would be predicted to have no effect on the BMC rejection capability, resulted in the marked rejection of BMCs where no resistance was normally seen. These results extend the "missing self" hypothesis to suggest that NK Ly49 inhibitory receptors can both inhibit activation and killing by those cells, but also can in some way influence the function of NK cells that do not express that inhibitory receptor in a cell-cell interaction. This suggests that caution must be exercised before removal of host NK cell subset is applied clinically because enhanced BMC rejection may result. Altering the balance of Ly49 NK subsets may also affect other in vivo activities of these cells. (Blood. 2002;100:3026-3033)

Introduction

Natural killer (NK) cells are large granular lymphocytes capable of spontaneously killing various tumor cells and virally infected cells. Murine NK cells can also mediate the acute rejection of allogeneic bone marrow cells (BMCs) in lethally irradiated mice but not solid tissue allografts. They also exhibit a phenomenon called "hybrid resistance" in which H2 homozgyous parental BMC grafts are rejected by irradiated H2 heterozygous F1 hybrid mice. The recognition of major histocompatibility complex (MHC) class I molecules by murine NK cells is mediated by 2 families of cell surface receptors and has at least partially explained how NK cells can detect BMC allografts. Individual members of these Ly49 receptors have an affinity for particular MHC class I determinants. Several Ly49 receptors have been identified and cDNAs have been sequenced in mice with strain-specific allelic forms being reported. Most Ly49 molecules are "inhibitory receptors," because binding to their specific targets transmits inhibitory intracellular signals through an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domain. The ITIM recruits tyrosine phosphatase, which results in a potent inhibitory signal being sent to the NK cell. Three Ly49 receptors (Ly49D, Ly49H, and Ly49P) are characterized as "activating receptors," because they lack the inhibitory motif (ITIM) in their cytoplasmic domain and instead are dependent on a noncovalently associated molecule "DAP12," which contains an immunoreceptor tyrosine-based activating motif (ITAM). Recent studies have shown that NK cytotoxicity is regulated by a balance between expression of specific activating and inhibitory receptors. A second group of MHC class I-specific receptors on NK cells is composed of disulfide bonded heterodimers termed CD94/NKG2. CD94 has a short cytoplasmic tail with no signaling function and it forms a heterodimer with various NKG2 proteins A, B, C, D, and E.

We and others have shown the role of inhibitory Ly49A, C/I, and G2 NK subsets as well as the activating Ly49D NK subset in bone marrow allograft rejection in lethally irradiated mice. Although a small percentage of T cells also express Ly49 receptors, mice with severe combined immunodeficiency (SCID), deficient in T and B cells, also mediate class I-specific bone marrow allograft rejection, indicating that NK cells alone can mediate the specificity seen in BMC rejection.

Our previous studies were designed to delineate the role of various Ly49 NK subsets in mediating BMC rejection. These studies involved infusing relatively small numbers of allogeneic BMCs that were rejected by untreated irradiated mice unless specific depletion of a Ly49 NK subset directly mediating the rejection was performed, which instead resulted in BMC engraftment. During those studies using some Ly49 monoclonal antibodies (mAbs) as controls for examining specificity of function of other Ly49 subsets in mediating rejection, we made a novel and unexpected preliminary observation in mice. We observed that depletion of large subsets of NK cells expressing inhibitory receptors for a given H2 type of BMC had 2 effects: (1) increased resistance to the H2 type BMCs that these receptors recognized to

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receive negative signals and (2) weakening of rejection against BMC grafts that were not recognized by the particular NK receptor. For example, depletion of Ly49G2+ NK cells, which receive negative signals from H2d but not H2b class I antigens, led to decreased ability of H2b host mice to reject H2b BMCs.27 We describe here in more detail the in vivo effect of depletion of Ly49 NK subsets, which do not directly mediate the rejection of H2d or H2b haplotype BMCs, on the rejection of higher numbers of BMCs and discuss the potential relevance of these findings in clinical bone marrow transplantation (BMT).

Materials and methods

Mice

BALB/c (H2b), C.B-17 SCID (H2d), C57BL/6 (H2b), and BALB/c × C57BL/6 F1 (C66F1, H2fl), mice were obtained from the Animal Production Area, National Cancer Institute (Frederick, MD). All mice were kept under specific pathogen-free conditions until use at 8 to 12 weeks of age.

NK cell isolation and flow cytometric analysis

Groups of 5 to 10 C57BL/6 (H2b) mice were injected intraperitoneally with 500 µg mAb 5E6 (anti-Ly49C/I) or 200 µg mAb 4D11 (anti-Ly49G2) or 0.2 mL normal rat serum (NRS). Seventy-two hours later spleens were harvested and single-cell suspensions were prepared. Four-color flow cytometric analysis was performed to determine the percentage of Ly49C/I+, Ly49G2+, and Ly49D+ NK cells. Cells were incubated with phycoerythrin (PE)–conjugated mAbs 5E6 (anti-Ly49C/I) or 4E5 (anti-Ly49D), fluorescein isothiocyanate (FITC)–conjugated mAb 4D11 (anti-Ly49G2) or goat-antimouse/rat IgG, allophycocyanin (APC)–conjugated NK pan marker NK1.1 and peridinin chlorophyll protein (PCP)–conjugated anti-CD3 for 30 minutes at 4°C. After 2 washes, the cells were analyzed on an LSRII flow cytometer (Becton Dickinson, San Jose, CA). All secondary antibodies were titrated and tested for their ability to stain for primary antibodies.

Assays for BMC engraftment

Groups of 4 to 5 conventionally housed recipient mice were injected intraperitoneally with 500 µg mAb 5E6 (anti-Ly49C/I), 200 µg mAb 4D11 (anti-Ly49G2), 500 µg of mAb PK136 (anti-NK 1.1), 20 µL antisialo GM1 (anti-ASGM1; Wako Chemicals, Richmond, VA), 0.2 mL NRS, or 120 µg polyinosinic/polyycytidylic (poly I:C), 2 days before irradiation. The engraftment of donor BMCs was assessed by an in vitro colony assay for hematopoietic cell growth.26 Two days after antibody injection, mice were lethally irradiated with a 137Cs source (BALB/c at 850 cGy; C57BL/6 at 950 cGy; C.B-17 SCID at 350 cGy; and CB6F1 at 1000 cGy) and later injected intravenously with 0.5 × 106 to 25 × 106 BMCs in 0.5 mL phosphate-buffered saline (PBS). At day 8, spleens were gently crushed in medium and single-cell suspensions were prepared. Then, 106 spleen cells were plated in 0.3% sea plaque agar in 35-mm dishes containing optimal concentrations of recombinant murine cytokines (interleukin 3 [IL-3; 10 ng/mL] and granulocyte-macrophage colony-stimulating factor [GM-CSF; 10 ng/mL]). Cytokines were obtained from the Biological Resources Branch (Frederick, MD). Plates were incubated at 37°C for 7 days and the colonies with more than 50 cells were counted (colony-forming unit-culture [CFU-c]). The data are presented as mean ± SD total CFU-c per spleen, which was obtained by multiplying number of colonies by the cellularity. The Student t test was performed to determine if the mean values were significantly different (P < .05). Each experiment was performed 3 to 4 times with at least 4 mice per group.

Results

We have reported earlier that in BALB/c (H2b) mice, the Ly49G2+ NK subset can mediate the rejection of allogeneic H2b BMCs and conversely Ly49C/I+ NK cells are responsible for the rejection of H2b BMCs in C57BL/6 (H2b) mice as ascertained by the in vivo depletion of these subsets.25,26,29 While studying the in vivo role of Ly49G2+ NK subset in BALB/c (H2b) mice, we observed an interesting phenomenon by using antibodies directed against the Ly49C/I+ NK cell subset as control for the specificity of Ly49G2+ NK subset removal.26 Depletion of Ly49C/I+ NK cells in BALB/c (H2b) mice, which is the NK subset that receives negative signals from H2Kb and therefore cannot reject H2b BMCs, resulted in the ability of the recipient mice to reject higher numbers of allogeneic H2b BMCs.26 Therefore, depletion of a population of NK cells that were considered indifferent to H2b BMCs nevertheless resulted in enhanced rejection by H2b mice.

We carried out further transplantation studies to determine the effects of in vivo depletion of this and other Ly49 NK subsets that are not responsible for the rejection of a particular BMC allograft. To do this, we wanted to assess the effect of NK subset removal in instances where increasing BMC numbers could overcome resistance and result in engraftment. The number of allogeneic BMCs transplanted varied because mice differ in their BMC rejection capability.

Depletion of Ly49C/I+ NK subset in H2b mice enhances their ability to reject H2b BMCs

We examined the effect of in vivo depletion of Ly49C/I+ or G2+ NK subsets on allograft rejection in BALB/c (H2b) mice. Mice were injected with 200 µg mAb 4D11 (anti-Ly49G2) or 500 µg mAb 5E6 (anti-Ly49C/I) to deplete the respective Ly49 NK subset and 48 hours later mice were lethally irradiated and allogeneic BMCs were infused. After 8 days, the splenic CFU-c assay, which quantitates hematopoietic progenitors as reflected by growth in agar, was performed to assess BMC engraftment in mice. The CFU-c assay was chosen over the (125I) UdR radioisotope incorporation assay because though both assays detect progenitor cell function, the CFU-c assay, in addition, detects the production of CFU-c precursors/progenitor cells derived from the inoculum, whereas the other detects any proliferating marrow-derived cells in S-phase of the cycle. The results presented in Figure 1A demonstrate that irradiated BALB/c mice were capable of resisting 0.5 × 106 H2b BMCs, whereas the syngeneic control H2b C57BL/6 mice engrafted H2b BMCs. When the total NK cell population was removed by injecting ASGM1 antibodies, engraftment of allogeneic H2b BMCs was enhanced (P < .0001; Figure 1A). Removal of Ly49C/I+ NK subset, which does not mediate the rejection of allogeneic H2b BMCs as they receive negative signals from H2Kb BMCs, led to rejection of the BMCs but depletion of Ly49G2+ NK cells (which receive negative signals from H2Dd BMCs and therefore can mediate the rejection of H2d BMCs) significantly increased the engraftment of allogeneic H2b BMCs compared with the control group (NRS treated).36 However, when rejection was overridden by infusing larger numbers of (8 × 106) allogeneic H2b BMCs (Figure 1B–C), engraftment of the infused BMCs was noted in the control group (NRS treated), similar to syngeneic control (Figure 1B) and anti-ASGM1–treated group (Figure 1B–C), which had all of the NK cells depleted, suggesting that no resistance was occurring. Removal of Ly49G2+ NK cells, which mediates the rejection of H2b BMCs, did not increase the engraftment of allogeneic H2b BMCs compared with the control group (NRS treated) also indicating that no appreciable resistance was occurring (Figure 1B–C). In vivo activation of NK cells by poly I:C, which involves interferon α/β (IFN-α/β) production and increases BMC rejection ability of mice, prior to BM infusion resulted in inhibition of engraftment (P < .007) compared with the control group (NRS treated; Figure 1C). However, in vivo removal of the
supposedly irrelevant Ly49C/I⁺ NK subset (which does not mediate the rejection of allogeneic H2b BMCs because it receives negative signals from H2K b BMCs) resulted in significant rejection (P < .0001) of H2b BMCs compared with either of the control groups (NRS or ASGM1 treated; Figure 1B-C) or poly I:C-treated group (P < .0001). In lethally irradiated H2b mice, the Ly49C/I⁺ NK subset has been shown to mediate the rejection of allogeneic H2b BMCs when transplanted in limited (0.5 × 10⁶) numbers (Figure 2A).23,24 However, when allogeneic H2b BMCs were infused in larger numbers (25 × 10⁶ BMCs), the control group (NRS treated) could no longer reject the bone marrow allograft because the CFU-c content was similar to mice depleted of all NK cells by NK1.1 mAb and syngeneic control (Figure 2B-C). Under such circumstances,
depletion of the Ly49C/I+ NK subset (containing Ly49D+ cells) also did not affect the engraftment of H2b bone marrow allografts when compared with the control group (NRS-treated group; Figure 2C). However, the C57BL/6 mice treated with the anti-Ly49G2 mAb 4D11 demonstrated significant augmentation of H2b bone marrow allograft rejection (Figure 2B; P < .003) compared with the control recipients and this could be reversed if all the NK1.1+ cells were removed by injecting pan NK mAb, PK136 (αNK1.1) along with anti-Ly49G2 mAb 4D11, suggesting that BMC graft rejection was mediated by NK1.1+ cells (Figure 2C).

Thus, in H2b mice, the converse of the effect seen with NK subset removal in H2b mice (Figure 1B) occurs. Removal of the Ly49 NK subset that receives negative signals from H2b significantly enhances the ability of the remaining NK1.1+ cells to reject H2b BMCs.

In F1 (H2bxd) mice, rejection of higher numbers of either parental bone marrow allografts (H2b or H2d) is dependent on specific Ly49 NK subset depletion

NK cells in F1 (H2bxd) hybrid mice can reject both parental bone marrow allografts when transplanted in limited numbers, by a phenomenon called “hybrid resistance.” In F1 (H2bxd) mice, Ly49C/I and G2 receptors are also exposed to their “self-ligands” H2b and H2d, respectively, in vivo, which transmit inhibitory signals to NK cells. Therefore, it was of interest to examine the effect of depletion of Ly49 NK subsets in a hybrid resistance model, that is, depleting the NK subset that does not mediate the rejection of a particular haplotype BMC (Ly49C/I for H2b and Ly49G2 for H2d BMCs) on the rejection of higher numbers of those parental BMCs in F1 (H2bxd) mice.

We have reported earlier, and also show in Figure 3A, that in CB6F1 (H2bxd) hybrid mice NK cells expressing inhibitory Ly49G2 receptors mediate the rejection of parental H2b BMCs (0.5 × 106) because depletion of the Ly49G2+ NK subset in vivo results in the engraftment of the BMCs. However, when higher numbers of H2b BMCs (20 × 106) were transplanted to override the resistance, removal of Ly49G2+ NK cells (which plays a role in mediating the rejection of H2b BMCs in homozygous F1 [H2b/H2b] and homozygous H2b mice) did not augment allograft engraftment when compared to the control group (NRS-treated group; Figure 3B). Interestingly, similar to the results in homozygous H2b mice (Figure 1B), significant rejection of parental H2b bone marrow allografts occurred (P < .006) in Ly49C/I+ NK subset–depleted group when compared with anti-NK1.1–treated group, where all NK cells were depleted.

Weak resistance to parental BALB/c/H2b bone marrow allografts has been reported to occur by F1 (H2bxd) hybrid mice and this rejection can be enhanced by using BMCs that are depleted of T cells. We have used C.B-17 SCID (H2b) BMCs as donors because it has been shown that the rejection pattern of congenitally T cell-deficient C.B-17 SCID BMCs is identical to BALB/c BMCs that were depleted of T cells. The BMT results presented in Figure 4A demonstrate that as reported earlier, in CB6F1 (H2bxd) hybrid mice, NK cells expressing inhibitory Ly49C/I receptors mediate the rejection of parental H2b BMCs (0.5 × 106) as depletion of Ly49C/I+ subset in vivo results in the engraftment of the C.B-17 SCID (H2b) BMCs. Removal of the other Ly49 NK subset, that is, the Ly49G2 NK subset that does not mediate the rejection of H2b BMCs instead resulted in the rejection of the bone marrow allograft, similar to the control group, whereas syngeneic controls engrafted the BMCs (Figure 4A). Increasing the C.B-17 SCID (H2b) BMCs to 4 × 106 also resulted in rejection by the host (Figure 4B). However, when parental 4 × 106 BALB/c (H2d) BMCs were transplanted there was engraftment of H2b BMCs in the control group (NRS treated) and the reason is weak resistance to parental BALB/c (H2b) bone marrow allografts by F1 (H2bxd) hybrid mice. Removal of Ly49C/I+ NK cells (H2b rejecting) did not augment allograft engraftment when compared with the control group (NRS-treated group; Figure 4C). Interestingly, as observed in homozgyous H2b mice (Figure 2B-C), removal of Ly49G2+ NK cells, which does not mediate the rejection of H2b BMCs, resulted in enhanced rejection of H2d bone marrow allografts (P < .02) by the irradiated F1 recipients (Figure 4C).

Hence, the ability of F1 (H2bxd) hybrid mice to reject larger numbers of either parental BMCs (H2b or H2d) is enhanced on the depletion of a specific Ly49 NK subset, Ly49G2+ and Ly49C/I+, respectively.

Augmentation of the ability of C.B-17 SCID (H2b) mice to reject H2b BMCs following depletion of Ly49C/I+ NK subset

To ascertain whether enhanced rejection of allogeneic BMCs is indeed due to NK cells versus NK/T or T cells, we performed the BMT experiments in C.B-17 SCID (H2b) mice, which are deficient in T and B cells. Results presented in Figure 5 demonstrate that depletion of Ly49C/I+ NK subset in C.B-17 SCID (H2b) mice enhanced their ability to reject large numbers of allogeneic H2b BMCs when compared with the control group treated with NRS (P < .0001). This is consistent with results also observed in BALB/c (H2b) mice (Figure 1B). Removal of Ly49G2+ NK cells...
on the engraftment of H2b allografts.

0.5 NRS 2 days before irradiation. Irradiated mice (4 mice/group) received either

subset, capable of mediating rejection after depletion of the

– depleted mice might be due to an expansion of the Ly49 NK

subset and Ly49D/H11545/H11545. Depletion of Ly49G2 NK cells leads to enrichment of Ly49C/I

NK cells in H2bxd F1 mice

that mediate the rejection of H2b BMCs in C.B-17 SCID (H2b) mice did not augment the engraftment further compared with the control group because large numbers (7.5 \times 10^6) of allogeneic BMCs were transplanted. The results with C.B.-17 SCID (H2d) mice suggest that NK cells, and not T cells, are responsible for the increased marrow rejection as a result of Ly49 NK subset depletion.

Depletion of Ly49G2 NK cells leads to enrichment of Ly49CI+ and Ly49DI+ NK cells in H2b mice

We wondered whether the enhanced allograft resistance in the NK subset–depleted mice might be due to an expansion of the Ly49 NK subset, capable of mediating rejection after depletion of the nonrejecting subset. C57BL/6 (H2b) mice were injected with 200 μg anti-Ly49G2 (4D11 mAb) or 500 μg anti-Ly49CI (5E6 mAb) and 48 hours later half the mice were lethally irradiated. Seventy-two hours after mAb treatment (24 hours after irradiation) spleen cells were stained from nonirradiated and irradiated mice with various Ly49 fluorescent-conjugated mAbs. Staining with a goat–anti-mouse antibody (to detect mAb 5E6 bound to NK cells in mAb 5E6-treated mice) or goat–anti-rat antibody (to detect mAb 4D11 bound to NK cells in mAb 4D11–treated mice) was completely negative, suggesting that antibody treatment resulted in depletion of the subsets (Figure 6A). Results presented in Figure 6B and 6C, demonstrate that in vivo treatment of C57BL/6 mice with the mAb 4D11 (anti-Ly49G2) or mAb 5E6 (anti-Ly49CI) depleted 90% or more of splenic Ly49G2+ and Ly49CI+ NK cells, respectively.

Interestingly, in C57BL/6 (H2b) mice in which the Ly49G2 NK subset was removed by mAb 4D11, (Figure 6B,C) a significant increase in the percentage of Ly49CI+ (P < .0001) and Ly49D+ NK cells (P < .0001) was noted (control, 25.9% ± 2.4% Ly49CI+ and 19.8% ± 1.0% Ly49D+ cells; mAb 4D11 treated, 51.1% ± 2.3% Ly49CI+ and 47.8% ± 2.8% Ly49D+ cells). Therefore, enhanced rejection of H2d BMCs observed in Ly49G2 subset–depleted C57BL/6 (H2b) mice (Figure 2B–C) may be due at least in part to an increased likelihood of allogeneic BMCs coming in contact with NK cells responsible for rejection.

In agreement with these data, mice receiving the mAb 5E6 (anti-Ly49CI) also had a significant increase in the percentage of Ly49G2+ NK cells (Figure 6B–C), which correlated with the increased ability of H2d mice to reject H2b BMC allograft after Ly49CI– NK subset depletion (Figure 1B–C). The activating receptor for H2b BMC grafts has not been identified but Ly49H is a candidate because it is similar to Ly49C and Ly49I in the extracellular domain.32

Next, we did the flow cytometric analysis of Ly49 NK subsets 72 hours following depletion in the spleen cells of lethally irradiated mice. The results presented in Figure 7 indicate that the percentage of NK cells following irradiation increased in the untreated (nonirradiated, 3.3%; irradiated, 23.4%) as well as
Ly49C/I NK subset–depleted (nonirradiated, 1.4%; irradiated, 6.9%) or Ly49G2 NK subset–depleted group (nonirradiated, 2.6%; irradiated, 15.5%) because of killing of radiosensitive cells by irradiation and removal of NK subsets by Ly49 antibody treatments (Figure 7A). However, the total number of NK cells also decreased in the untreated control group following irradiation (nonirradiated, \(2.09 \times 10^6\); irradiated, \(0.86 \times 10^6\)) and even further in mAb-treated groups (Figure 7B).

Similar to the effects observed in nonirradiated C57BL/6 (H2b) mice (Figure 6), in lethally irradiated C57BL/6 (H2b) mice, Ly49G2 NK subset removal by mAb 4D1I led to a significant increase in the percentage of Ly49C/I+ cells, whereas the Ly49C/I NK subset removal by SE6 mAb treatment led to a significant increase in the percentage of Ly49G2+ NK cells (Figure 8A-B). These results indicate that antibody depletion of the subsets was not altered when irradiation was performed.

**Discussion**

We have previously described the roles of various Ly49 NK subsets (inhibitory and activating) in bone marrow allograft rejection in lethally irradiated mice.26,27,29 However, all previous studies involved transplanting limiting numbers of allogeneic BMCs, which were rejected by the control (untreated) irradiated mice. During those studies, we observed that in H2b mice, removal of the inhibitory Ly49C/I NK subset, which played no role in the rejection of allogeneic H2b grafts, augmented rejection of larger numbers of H2b BMCs.26 This suggested that such NK subsets are potentially "immunoregulatory" and may functionally regulate the NK cells that mediate marrow graft rejection.

Our previous studies examined the ability of Ly49 NK subset removal to abrogate bone marrow allograft rejection and the work...
subset but did result in enrichment of Ly49C/I shown) did not result in expansion of cell numbers of the other NK cells (Figures 6 and 8). Presumably, Ly49C/I/H11001 the rejection. Depletion of the Ly49G2 cell numbers or the enrichment of the NK subset directly mediating signals to NK cells, the NK cells were strongly capable of during development, which on interaction transmits inhibitory expressing inhibitory MHC.

It is possible that the enhanced rejection observed by removal of either Ly49G2+ or C/I+ NK cells is due to either the expansion in a particular haplotype if the appropriate inhibitory Ly49 NK cells are presented here suggests that NK subset modulation can also enhance allograft rejection. We have demonstrated that large numbers of allogeneic BMCs can be strongly rejected by mice of a particular haplotype if the appropriate inhibitory Ly49 NK cells are depleted in vivo. Thus, in homozygous H2b or heterozygous H2bd mice, removal of inhibitory Ly49C/I+ NK cells, which do not mediate the rejection of allogeneic H2b bone marrow grafts, significantly augmented the capability of the mice to reject H2b BMCs. The opposite effect was observed with homozygous H2b or heterozygous H2bd mice, where removal of inhibitory Ly49G2+ NK cells, which do not mediate the rejection of allogeneic H2d bone marrow grafts, significantly augmented the capability of the mice to reject allogeneic H2d BMCs. Although in F1 (H2bd) mice, both Ly49 receptors (Ly49C/I and G2) are exposed to their self-ligands (parental MHC class I, H2d and H2b, respectively) during development, which on interaction transmits inhibitory signals to NK cells, the NK cells were strongly capable of mediating allograft rejection despite living in an environment expressing inhibitory MHC.

It is well documented that BMC rejection in mice can be enhanced by treatment with poly I:C in vivo. Poly I:C treatment activates the macrophages, which produce IFN-γ/β, and that enhances NK cell cytotoxicity. Interestingly, removal of the appropriate Ly49 NK subset enhances the BMC rejection capability of the remaining NK1.1+ cells significantly greater than poly I:C-activated cells. NK cells also produce a variety of cytokines on activation, for example, GM-CSF, IFN-γ, tumor necrosis factor α, transforming growth factor β, and so forth. Therefore, one mechanism may be novel NK-NK cell immunoregulation whereby one Ly49 NK subset influences another Ly49 NK subset(s) mediating rejection possibly through the production of cytokines or other factors. Removal of that subset relieves the inhibition of NK cells mediating the rejection and results in enhanced rejection of allogeneic BMCs. These studies suggest that caution must be exercised when using other Ly49 mAbs because negative controls in in vivo assays examining the role or function of a particular NK subset because activity of the remaining subsets may be heightened.

We have previously observed that in H2b mice, Ly49G2+/- NK cells produce larger amounts of the growth-promoting cytokine (GM-CSF) than the growth inhibiting cytokine (IFN-γ). Similarly, in H2d mice, Ly49G2+/- NK cells produce larger amounts of the growth-promoting cytokine (GM-CSF) than growth inhibiting cytokine (IFN-γ; unpublished data, January 1994). Therefore, depletion of Ly49G2+/I- or Ly49G2+ NK cells in a particular...
haplotype strain of mice may create condition(s) unfavorable for the engraftment of the BMCs.

It is conceivable that following removal of either Ly49 NK subset (Ly49C/I or G2) more than one mechanism may be involved in augmenting the ability of the mice to reject larger numbers of BMCs. Our data suggest that NK subsets are complex in that a particular inhibitory Ly49 NK subset known to play no role in rejection of BMCs of a particular haplotype can apparently prevent rejection of allogeneic marrow. Human NK cells recognizing HLA-C may play a role in marrow graft rejection.57 This study has implications for human NK subset depletion for BMT. Removal of inappropriate NK subsets could have disastrous consequences by enhancing the recipient’s ability to reject bone marrow allografts. It is perhaps imperative to create reagents that deplete or inhibit activating but not inhibitory NK cell receptors.

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References

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