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*J Immunol* 2006; 177:7923-7929; doi: 10.4049/jimmunol.177.11.7923

http://www.jimmunol.org/content/177/11/7923
Mapping of Quantitative Trait Loci Determining NK Cell-Mediated Resistance to MHC Class I-Deficient Bone Marrow Grafts in Perforin-Deficient Mice

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NK cells reject allogeneic and MHC class I-deficient bone marrow (BM) grafts in vivo. The mechanisms used by NK cells to mediate this rejection are not yet thoroughly characterized. Although perforin plays a major role, perforin-independent mechanisms are involved as well. C57BL/6 mice deficient in perforin (B6 perforin knockout (PKO)) reject class I-deficient TAP-1 KO BM cells as efficiently as normal B6 mice. In contrast, perforin-deficient 129S6/SvEvTac mice (129 PKO) cannot mediate this rejection while normal 129 mice efficiently reject. This suggests that in 129, but not in B6, mice, perforin is crucial for NK cell-mediated rejection of MHC class I-deficient BM grafts. To identify loci linked to BM rejection in perforin-deficient mice, we generated backcross 1 progeny by crossing (129 × B6)F1, PKO mice to 129 PKO mice. In transplantation experiments, >350 backcross 1 progeny were analyzed and displayed a great variation in ability to reject TAP-1 KO BM grafts. PCR-based microsatellite mapping identified four quantitative trait loci (QTL) on chromosomes 2, 4, and 8, with the QTL on chromosome 8 showing the highest significance, as well as a fifth epistatic QTL on chromosome 3. This study describes the first important step toward identifying BM graft resistance gene(s).

The Journal of Immunology, 2006, 177: 7923–7929.

Natural killer cells are the primary effector cells mediating acute rejection of bone marrow (BM) grafts (1). They also carry out cytotoxic and cytokine-mediated activity against tumor cells and cells infected with virus, intracellular bacteria, or parasites. These activities are strictly regulated by activating and inhibitory receptor-ligand interactions. Inhibitory receptors on NK cells prevent killing of healthy endogenous cells via recognition of self MHC molecules, but allow killing of MHC class I-deficient cells. In contrast, activating receptors on NK cells trigger lysis of target cells expressing ligands induced by tumor transformation and/or infection. Most activating receptors associate with small adapter molecules e.g., DAP10, KARAP/DAP12, CD3ξ, or FcɛRIγ (2). The adapter molecules contain ITAMs that become phosphorylated upon cross-linking of the receptor, allowing recruitment of downstream signaling molecules like SYK and Zap-70 (3). However, recognition of MHC class I-deficient tumor cells such as the T cell lymphoma RMA-S can occur in the absence of SYK and Zap-70 (4). The activating NK cell receptor NCR-1 (the mouse homolog of human NKp46) has recently been shown to participate in recognition of RMA-S cells (5). However, this receptor was not essential for rejection and it is thus still not clear which activating receptors and ligands mediate the recognition of these cells, as well as of MHC class I-deficient BM grafts in the mouse.

At least two mechanisms for NK cell-mediated lysis have been defined in recent years, calcium-dependent release of perforin and granzymes and interaction between death receptors and their ligands. The function of perforin in concert with granzymes appears to be a primary cytotoxic mechanism of NK cells. Conjugate formation occurs between the effector and the target cell resulting in degranulation and release of perforin and granzymes which cooperates in inducing DNA fragmentation and apoptosis of the target cell (6, 7). Perforin as a key mediator of NK cell-mediated cytolysis was first demonstrated by the lack of killing of Fas+ YAC-1 lymphoma cells, which are highly NK cell sensitive, by NK cells derived from perforin-deficient (perforin knockout (PKO)) mice (8). Thus, perforin deficiency results in effector cells with decreased ability to reject certain tumors and clear virus infections (9, 10). Alternatively, engagement of death receptors on the target cell leads to apoptosis mediated via death domain-containing proteins and caspase 8. Thus, NK cells trigger target cell apoptosis via death receptor ligands such as FasL and TRAIL (11–14). Interestingly, it has been demonstrated that NK cells have the ability to up-regulate cell surface levels of Fas on previously negative target cells before Fas-dependent lysis in vitro and in vivo (15). The mechanisms behind this regulation are unknown. It has also been shown that TRAIL is important for NK cell-mediated protection...
from metastasis of certain tumors and that this protection is dependent on IFN-γ-induced up-regulation of TRAIL on NK cells (16). In addition, TRAIL is responsible for NK cell-mediated elimination of immature dendritic cells in vivo showing that also non-transformed cells can be killed via TRAIL (17). NK cell-mediated cytolysis dependent on membrane-bound or secreted TNF has also been reported (18). Although these pathways represent important mechanisms of cytotoxicity, much remains to be studied when it comes to their role in NK cell-mediated BM rejection.

The role of perforin in NK cell-mediated BM rejection has been somewhat controversial. Previous reports have suggested that perforin is not necessary for rejection of BM grafts from allogeneic mice (19). However, using BM cells that were TAP-1 deficient, and thereby highly NK susceptible, we have shown that NK cells from perforin-deficient (PKO) mice of the 129:B6 strain have a decreased ability to reject these grafts. In contrast, B6 PKO mice rejected the grafts. Interestingly, this was only true when the mice were housed in a conventional facility: perforin-deficient mice kept under specific pathogen-free conditions were able to reject MHC class I-negative BM grafts regardless of genetic background (20). This result emphasized two factors that affect BM rejection in PKO mice: housing conditions and genetic background. Regarding the housing conditions, we recently demonstrated involvement of the Fas/FasL pathway in resistance to BM grafts and that diminished BM rejection capacity during conventional housing conditions may be explained by down-regulation of NK cell FasL expression (21). This down-regulation could be induced by pathogens infecting the animals (22), since sentinel mice in our conventional animal facility occasionally carry viral infections, e.g., mouse hepatitis virus, mouse parvovirus, and sendai virus. In addition, the inhibitor cellular FLIP, overexpressed in susceptible hemopoietic stem cells, was shown to convey partial engraftment in B6 PKO mice, suggesting involvement of death receptors in BM rejection (23). These results suggest a redundancy in the mechanisms involved in BM graft rejection. However, they do not explain the difference in rejection capacity between mouse strains. Besides the above-described strain differences in perforin-independent BM rejection under conventional conditions, the genetic background has also proven to be important in mice kept under specific pathogen-free conditions. Although 129:B6 PKO mice were unable to reject B6.lpr/TAP-1 KO BM grafts (negative for Fas), B6 PKO mice readily rejected such grafts (21). Furthermore, in another in vivo assay measuring rejection of large numbers of fluorescence-labeled β2-microglobulin (β2m)−/− spleen cells, wild-type (wt) 129 mice displayed impaired rejection compared with wt B6 mice (M. H. Johansson, unpublished data). These results emphasize the importance of genetic differences between the two mouse strains in regulation of rejection of hemopoietic cells, also under specific pathogen free conditions. The functional differences between B6 PKO and 129 PKO mice may be explained by genetic influence directly on killing mechanisms alternative to perforin. Alternatively, the explanation could lie in a differential regulation of NK cell recognition or activity in a more general way, but with changes subtle enough to result in functional differences only under suboptimal conditions like in the absence of perforin. Therefore, we set out to investigate how the genetic background of the mouse controls NK cell-mediated BM graft rejection in the absence of perforin and under conventional housing conditions.

We performed a whole genome scan of backcross mice displaying extreme BM graft rejection phenotypes and describe four quantitative trait loci (QTLs) mapping to chromosomes 8, 2, and 4. A fifth epistatic QTL, located on chromosome 3 and dependent on the genotype at the QTL on chromosome 4, is also described. By this study, we have taken the first important steps toward identifying genes involved in resistance to MHC class I-deficient BM grafts.
Materials and Methods

Mice

All mice were bred and maintained in a conventional mouse colony at the University of Texas Southwestern Medical Center as previously described (20). C57BL/6 (B6) and 129S6/SvEvTac (129) mice both carry the H-2b MHC haplotype. Perforin-deficient B6 PKO mice were originally purchased from The Jackson Laboratory. 129 PKO mice were derived from 129B6 PKO mice and were backcrossed to 129S6/SvEvTac mice seven generations. Perforin \( \alpha \) mice were then intercrossed, offspring tested for perforin, and PKO homozygous mice were bred and used for experiments. TAP-1 KO mice have a mixed background of 129 and B6 genes. Founder mice of the 129 background were crossed once to B6 and the mice have since then been inbred (\( \geq 10 \) generations). All work using animals was reviewed and approved by the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center.

BM transplantation

Transplantation was done as previously described (20, 24, 25). Experimental groups in general contained five mice with only a few exceptions where no less than three mice were included in a group. Briefly, recipient mice were lethally irradiated (850 cGy) and injected i.v. with 3.5 \( \times 10^8 \) TAP-1 KO donor BM cells. Five days after cell transfer, growth of donor-derived BM cells was assessed by spleen incorporation of \( ^{3}H \)-labeled Udr (Amersham Biosciences) which is a specific DNA precursor and thymidine analog. The results are expressed as the geometric mean (95% confidence level) percentage uptake of \( ^{3}H \)-labeled Udr. A high percentage of \( ^{3}H \)-labeled Udr uptake represents growth of the BM graft and a low percentage of \( ^{3}H \)-labeled Udr uptake denotes rejection. Using log\(_{10}\) values, parametric and nonparametric statistical analyses were performed to determine significance of differences between geometric mean values. The Student\’s t test was used to compare two groups and the Newman-Keuls multiple comparison test was used to determine significantly different groups at \( p = 0.05 \) level for experiments that consist of multiple recipient groups for a given donor. Values significantly (\( p < 0.05 \)) different from another group by nonparametric and parametric analysis are indicated in the figure legends.

Genetic mapping and statistical analysis

Genomic DNA was isolated from tail tips from backcross-1 (BC-1) progeny and control mice. Genotypes were determined by PCR amplification of loci polymorphic between 129 and B6 that contain simple sequence length polymorphisms, i.e., microsatellite markers. Primers were obtained from Invitrogen Life Technologies/ResGen. Standard PCR was performed at experimentally determined optimal annealing temperature for each primer pair. PCR products were visualized with ethidium bromide on 5% agarose gels to determine the genotype at each marker position (129 homozygous or 129/B6 heterozygous). Marker positions were obtained from The Jackson Laboratory Mouse Genome Database (www.informatics.jax.org) using positions from the Whitehead Institute Genetic Map. In total, 78 markers distributed throughout the 19 autosomes were analyzed (see Fig. 2). These markers allowed a genome coverage of \( \geq 75\% \), taking into account an area of 10 cM on each side of a marker.

All linkage analysis and permutation tests were performed using R/qtl software (26). BC-1 mice with the most extreme rejection phenotypes, 46 mice in each group, were selected for initial scan of the genome analyzing 61 markers distributed throughout the 19 autosomes. We analyzed BM rejection as a binary trait: rejecting mice were scored as 0 and accepting mice as 1. Loci of interest after the initial linkage analysis were more carefully investigated by PCR analysis of additional markers and including all 137 mice displaying the extreme phenotypes. We conducted linkage analysis with the aim to localize genomic regions linked to phenotype by analyzing coinheritance of genetic markers and phenotype. Linkage analysis was performed using a multiple imputation method that uses information in the marker genotype to simulate multiple versions of complete genotype information. This is a powerful tool to analyze sample material whose genome of the material is only analyzed for 2 markers in certain regions. Threshold levels for logarithm of odds (LOD) score significance of putative QTLs were determined by a permutation test procedure (27). In this procedure, the set of data is subjected to repeated random shuffling of phenotypes among the individual genotypes and the maximum LOD score obtained in each shuffle is recorded. These reflect maximum LOD scores that can occur by chance, because the relationship between the phenotype and genotype is broken in the shuffle (i.e., each animal has been assigned the wrong phenotype while retaining its genetic map). This test for significance is thus empirical and reflects the characteristics of the particular experiment to which it is applied (27). The 95th percentile of the distribution of maximum LOD scores serves as a genome-wide threshold for significance, while the 90th percentile is a threshold for suggestive QTLs.

Ninety-five percent confidence intervals, i.e., the most probable genomic location for each QTLs were determined using a bootstrap resampling method (28). This method uses repeated resampling with replacement (i.e., in each resampling random animals from the data set are duplicated and others are removed), each resample is then subjected to linkage analysis. The 95% confidence interval for each QTL is determined from the resulting distribution of maximum LOD score positions. Possible interactions between genes were tested by implementing a two-dimensional scan with a two-QTL model using a multiple imputation method that examines all pairs of genetic markers and intermarker positions for association with ability to reject BM grafts. To confirm the identified QTLs and interactions, we implemented a fit multiple-QTL model that is based on the creation of an initial model of phenotypic variance comprising all identified QTLs and their interactions. Each QTL or QTL combination is then excluded from the initial model in subsequent steps and the resulting influence on phenotypic variance is determined. A significant difference (\( p < 0.05 \)) between the influence on phenotypic variance caused by the whole set of QTLs and the influence when one has been excluded, confirms the relevance of the excluded component.

Results

A wide range of rejection capacities in BC-1 mice suggests polygenic control of BM graft rejection

We have shown that perforin can play a crucial role in BM graft rejection. Perforin-deficient mice on the 129:B6 background were
not able to mediate rejection of TAP-1 KO BM, while B6 mice with the same defect rejected such BM (Fig. 1A) (20). To elucidate genetic differences that may be responsible for the differential ability to reject class I-deficient BM, we set out to map genetic loci associated with BM rejection. First, 129:B6 PKO mice were crossed to B6 PKO to generate (129:B6 × B6)F1 PKO progeny. Transplantation of TAP-1 KO BM to perforin-deficient F1 progeny demonstrated that rejection was as efficient as in B6 PKO recipients (Fig. 1A). This suggests that rejection may be governed by dominant alleles of the B6 genotype.

129:B6 PKO mice were backcrossed to the 129 background for further studies (see Materials and Methods). The resulting 129 PKO were crossed to B6 PKO to generate (129 × B6)F1 PKO progeny. 129 PKO mice were unable to reject TAP-1 KO BM while (129 × B6)F1 PKO progeny rejected, confirming previous results with 129:B6 background mice (Fig. 1B). A larger variation in the rejection by F1 PKO on the 129 background (Fig. 1B) than F1 PKO on the 129:B6 background (Fig. 1A) is most likely due to the large mouse number in the latter case. However, genetic factors cannot be formally excluded. Importantly the F1 mice on both backgrounds rejected TAP-1 KO BM. Based on the dominant characteristic of BM graft rejection in this setting, we crossed F1 mice to 129 PKO to generate BC-1 progeny, which were subjected to TAP-1 KO BM transplantation. As shown in Fig. 1B, there was a wide variability in rejection capacity among the BC-1 progeny. Notably, the distribution was unimodal rather than bimodal, suggesting involvement of several independent loci in BM rejection (Fig. 1C).

**Microsatellite mapping reveals multiple loci that regulate BM graft rejection**

Of the 357 BC-1 progeny tested, we selected mice with extreme BM graft rejection phenotypes, 49 mice were defined as accepting the BM grafts and 89 mice were defined as rejecting (Fig. 1C). Forty-six mice from each extreme phenotype group were analyzed in an initial genome scan. Loci of interest (data not shown) were more carefully investigated by analyzing additional microsatellite markers and including all mice displaying the extreme phenotypes. Linkage analysis and permutation tests were performed implementing multiple imputation methods and analyzing BM rejection as a binary trait (rejecting vs accepting mice). Threshold levels for significant linkage were determined by a permutation test procedure, which is empirical and reflects the characteristics of the particular experiment to which it is applied. This whole-genome scan revealed multiple QTLs that displayed linkage to BM graft rejection in PKO mice (Fig. 2A). The QTL showing the strongest linkage to rejection was identified on chromosome 8. We designated it BM graft rejection (bmgr) 1 (Fig. 2A). The B6 allele at the peak marker (D8Mit249) conferred rejection (Table I). Furthermore, two significant QTLs, bmgr2 and bmgr3, mapped on chromosome 2 in vicinity of D2Mit237 and D2Mit291, respectively (Fig. 2A). Interestingly, whereas the B6 allele at bmgr2 conferred rejection, ability to reject BM grafts was linked to a 129 homozygous genotype at bmgr3 (Table I). This result shows that rejection alleles can also originate from the strain that allows acceptance of BM and highlights the fact that each mouse strain contains a mixture of rejection- and acceptance-predisposing alleles. The final outcome, rejection, or acceptance of BM, will be a net effect of multiple alleles. A suggestive QTL on chromosome 4, designated bmgr4, conferred rejection by the B6 allele (Fig. 2A; Table I). We used a bootstrap resampling method to determine chromosomal locations of the identified QTLs. The 95% confidence intervals for QTL locations are shown in Fig. 2B.

**Epistatic interactions affect BM graft rejection**

To test for possible gene-gene interactions regulating BM graft rejection, we performed an analysis using a two QTL model that examines all pairs of genetic markers and intermarker positions for association with ability to reject BM. The analysis enabled identification of a fifth QTL located close to D3Mit163 at the distal end of chromosome 3. This QTL did not show any effect on BM rejection when analyzed by itself (Figs. 2A and 3A). However, a 129 homozygous genotype at D3Mit163 seemed to enhance the effect of the genotype at the suggestive QTL bmgr4 (compare Fig. 3B and C), while a 129/B6 genotype at D3Mit163 eradicated or even reversed the effect of bmgr4 (Fig. 3D). This indicates epistatic interactions between QTLs on chromosome 3 and 4 affect BM graft rejection. The figure shows allele effects and interaction plots for QTLs detected on chromosomes 3 and 4. The relative I UdR uptake (on a scale from 0 to 10 where 0 is rejection and 10 is acceptance) is shown on the y-axis as mean ± SEM. A. Influence of genotype at the marker (D3Mit163) closest to the epistatic QTL (ebmgr1) on chromosome 3 alone. B. Influence of genotype at the marker (D4Mit145) closest to the epistatic QTL (bmgr4) on chromosome 4 alone. C. Influence of genotype at D4Mit145 in animals homozygous for the 129 genotype at D3Mit163. D. Influence of genotype at D4Mit145 in animals with a heterozygous genotype (129/B6) at D3Mit163.

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**Table I. Summary of QTLs linked to BM graft rejection**

<table>
<thead>
<tr>
<th>QTL</th>
<th>Chromosome</th>
<th>Peak Marker</th>
<th>LOD Score</th>
<th>Rejection Allele</th>
<th>Candidate Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>bmgr1</td>
<td>8</td>
<td>D8Mit249</td>
<td>3.87</td>
<td>B6</td>
<td>MMP2, -15, IL-12Rβ, IL-15, PLC-γ2, JAK3</td>
</tr>
<tr>
<td>bmgr2</td>
<td>2</td>
<td>D2Mit237</td>
<td>2.48</td>
<td>B6</td>
<td>IL-15Ra, Vav2, PKCδ, GATA-3</td>
</tr>
<tr>
<td>bmgr3</td>
<td>2</td>
<td>D2Mit291</td>
<td>2.55</td>
<td>129</td>
<td>MMP-9, -24, NFAT1, PLC-γ1</td>
</tr>
<tr>
<td>bmgr4</td>
<td>4</td>
<td>D4Mit145</td>
<td>2.01</td>
<td>B6</td>
<td>IFN-α family, IFN-β, JAK1</td>
</tr>
</tbody>
</table>

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**FIGURE 3.** Epistatic interactions between QTLs on chromosome 3 and 4 affect BM graft rejection. The figure shows allele effects and interaction plots for QTLs detected on chromosomes 3 and 4. The relative I UdR uptake (on a scale from 0 to 10 where 0 is rejection and 10 is acceptance) is shown on the y-axis as mean ± SEM. A. Influence of genotype at the marker (D3Mit163) closest to the epistatic QTL (ebmgr1) on chromosome 3 alone. B. Influence of genotype at the marker (D4Mit145) closest to the epistatic QTL (bmgr4) on chromosome 4 alone. C. Influence of genotype at D4Mit145 in animals homozygous for the 129 genotype at D3Mit163. D. Influence of genotype at D4Mit145 in animals with a heterozygous genotype (129/B6) at D3Mit163.
interactions between the two QTLs and the QTL at chromosome 3 was thus designated epistatic bmgr1 (ebmgr1; Table I). Analysis using a fit-multiple QTLs model confirmed that the phenotype is regulated by these four independent QTLs and the fifth ebmgr1 in concert with bmgr4 (data not shown). Thus, we demonstrate that multiple QTLs, including complex gene-gene interactions, regulate BM graft rejection in PKO mice.

Discussion

The recognition strategies and effector mechanisms involved in NK cell–mediated BM rejection have not been thoroughly elucidated. In this study, we have initiated a quest for genes affecting BM resistance in the absence of perforin. We used the fact that perforin-deficient 129 mice housed in a conventional animal facility are unable to reject TAP-1 KO BM grafts, while mice of the B6 genetic background under the same conditions readily reject such grafts in an NK cell–dependent manner.

A number of differences between 129 and B6 mouse strains have been reported with regards to NK cell function. 129 mice are not able to reject BM from BALB/c mice (H-2\(^d\)), while B6 mice readily reject such cells. This variance has been attributed to differences in the NK gene complex (NKC) on chromosome 6 (29). Indeed, the NKC is highly polymorphic between the two strains. For example, NKR1-C (NKR1-P1-C), a commonly used marker expressed exclusively on NK cells and a small subset of T cells, and encoded in the NKC, is present in B6, but not in 129 mice (30). The Ly49 gene family, encoding MHC class I-reactive activating and inhibitory receptors expressed on subpopulations of NK cells, is also located within the NKC. In three recent reports, the Ly49 gene family of the 129 strain was characterized and shown to be highly diverse from B6 (31–33). Importantly, we found no linkage in our study to the NKC. Moreover, 129/B6 PKO mice carrying the B6 NKC were as deficient as pure 129 PKO mice in rejection of TAP-1 KO BM graft rejection, further refuting influence of the NKC in this setting (data not shown).

A reported defect in 129/J NK cell function is aberrant DAP12 signaling. DAP12 is an adapter molecule used by activating Ly49 and some other receptors. McVicar et al. (34) showed that signaling downstream of DAP12 is defective in 129/J mice, a defect of unknown molecular nature. However, lysis of the prototypic NK target line YAC-1 was normal, showing that all activating pathways were not destroyed. The signaling defect may indeed explain the poor resistance to H-2\(^b\) grafts described above; this rejection is mediated via activating Ly49 receptors (Ly49D in B6 and Ly49R in 129) and dependent on DAP12 (34–37). However, these differences are unlikely explanations in our system. Activating Ly49 receptors are not involved in the rejection of MHC class I-deficient BM grafts and DAP12-loss-of-function mice are fully able to reject \(\beta_2\)-m\(^{-/-}\) spleen cells (37). Furthermore, rejection of H-2\(^b\) grafts was largely perforin dependent (21), while the present study investigates perforin-independent functions. However, it cannot be excluded that one of the putative receptors triggering reactivity against MHC class I-deficient BM grafts could be associated with DAP12 or use the same signaling pathway. Even so, none of the previously reported differences between B6 and 129 NK cell function can sufficiently explain the differential rejection patterns explored in this study.

A recent study has identified NKG2D ligands as recognition targets in BM graft rejection. BALB/c × C57BL/6\(\times\)F\(_1\) mice rejected BALB/c BM grafts in an NKG2D-dependent manner and expression of Rae-1 family members and H\(\delta\) was detected on BM cells from BALB/c but not C57BL/6 or F\(_1\) mice (38). It is not known whether NKG2D-mediated recognition is involved in the rejection of TAP-1 KO BM grafts. Although participation of NKG2D cannot be excluded, we find it unlikely that the strain difference in rejection capacity would be due to differential recognition of NKG2D ligands. First, NKG2D-induced BM rejection was MHC class I independent (38), second, an in vivo assay measuring rejection of large numbers of fluorescence-labeled B6\(\beta_2\)-m\(^{-/-}\) spleen cells revealed a similar difference where wt 129 mice displayed impaired rejection compared with wt B6 mice (M. H. Johansson, unpublished data). In the latter case, the B6 genetic background of the donor cells (lacking NKG2D ligand expression) argue against the importance of differences dependent on NKG2D.

Interestingly, mice with a nonfunctional NK cell receptor (ner1; mouse homolog of human \(NKp46\)) gene displayed a reduced rejection of the TAP-2-deficient tumor cells RMA-S compared with wild-type mice, but only in mice of the 129/Sv background. In contrast, rejection in B6 mice was independent of NCR1 function (5). This suggests that B6 NK cells may express additional activating receptor(s) recognizing RMA-S cells, receptors that may be lacking on 129/Sv NK cells. Such putative receptors are still unidentified, but could be important also for the recognition of TAP-1 knockout BM cells.

Our previous data suggested that death receptors may be involved in BM rejection (21, 23). It has also been shown that TRAIL is important for NK cell–mediated protection from metastasis of certain tumors and mediates resistance to certain virus infections. Furthermore, IFN-\(\alpha\)/IFN-\(\beta\) and IFN-\(\gamma\) can induce up-regulation of TRAIL on NK cells (16, 39, 40). Thus, it is not unlikely that TRAIL may be involved in rejection of BM grafts and may play a role in the rejection investigated in the present study. Regulatory T cells have been demonstrated to influence NK cell mediated rejection of allogeneic BM grafts and grafts lacking one MHC haplotype (hybrid resistance). Depletion of CD25\(^+\) T cells greatly enhanced rejection of these grafts (41). It may be possible that strain-dependent differences in this regulatory activity could be involved in the results described here.

As a first step toward identifying genetic factors in perforin-deficient mice, we performed a genome-wide linkage analysis for rejection of MHC class I-deficient BM grafts on progeny from a backcross of (B6 \(\times\) 129) PKO F\(_1\) mice to 129 PKO. This analysis revealed that the phenotype in 129 PKO mice is regulated by multiple QTLs. Although some of the QTLs are independent, others display complex gene-gene interactions. Three independent QTLs were significantly linked to BM graft rejection with the strongest linkage to a locus on chromosome 8 (bmgr1). There were two loci on chromosome 2. Interestingly, at bmgr2 BM graft rejection was linked to the B6 allele, while at bmgr3 a 129 homozygous genotype conferred rejection. In addition, bmgr4 on chromosome 4 was suggestively linked to BM graft rejection.

Because a whole genome scan, like the one performed, does not allow determination of the exact locations of QTLs, the chromosomal regions where the bmgr QTLs may be located are large and consist of vast numbers of genes. Nevertheless, we want to bring the attention to a number of candidate genes in these loci. The genes encoding matrix metalloproteinase (MMP)-2 and MMP-15, are located in bmgr1 on chromosome 8 and MMP-9 and MMP-24 are located in bmgr3. Expression of MMP-2, -9, and -15 have been demonstrated in mouse NK cells (42). MMPs have been implicated in regulation of NK cell function: first, they degrade extracellular matrix molecules and may promote extravasation of NK cells (42); second, MMP-9 cleaves the adhesion molecule ICAM-1 from the cell surface of tumor cells, protecting such cells from NK cell lysis (43); third, MMPs cleave FasL and TNF-\(\alpha\) from the cell surface and may be able to regulate lysis by NK cells (44, 45).
Genes encoding cytokines, cytokine receptors, or downstream signaling molecules affecting NK cell function are encoded within the bmgr loci. IL-12Rβ1 and IL-27Rα are encoded in bmgr1 and are both involved in NK cell responses involving IFN-γ production and proliferation (46, 47). However, both 129 and B6 mice seem to have intact responses to IL-12, arguing against polymorphisms in the IL-12Rβ1 gene between these strains (48). IL-15 (bmgr1) and its receptor (IL-15Rα; bmgr2) are essential for maturation, homeostasis, and proliferation of NK cells and can induce TRAIL expression. Deficiencies in any of these genes result in mice lacking mature NK cells (49, 50). JAK3 (bmgr3) associates with the common γ-chain of the IL-2, 4, 7, 9, 15, and 21 receptors. Deficiency in JAK3 in mice results in a lack of T, B, and NK cells. The NK cell deficiency is probably due to lack of IL-15 signaling (51, 52). Interestingly, one report on patients with JAK3 deficiency describes a phenotype with mature T cells and only mild immunodeficiency. However, the JAK3-deficient T cells did not up-regulate FasL in response to IL-2 (53). JAK1 (bmgr4) is involved in the signaling downstream of JAK3, as well as in the signaling in response to several other cytokines. The IFN-α gene family and IFN-β are located within bmgr4 on chromosome 4. These cytokines are known to stimulate NK cell cytotoxicity by stimulating expression of up-regulation of perforin and TRAIL. (40). Multiple genes in the IFN-α gene family have allelic differences between 129/Sv and B6 strains (54). However, NK cells in both mouse strains respond to type I IFN during virus infections (48) and it is not clear whether allelic differences in the IFN-α family may influence activation of NK cells. A number of signaling molecules important for NK cell activation may be involved in the BM graft rejection. Phosphopase C (PLC)-γ isoforms couple immune receptors to downstream calcium signaling. It was recently shown that mouse NK cells predominantly express PLCγ-2 (bmgr1) while PLCγ-1 (bmgr5) is expressed at minimal levels. Deficiency in PLCγ-2 resulted in impaired cytotoxicity in response to ITAM- and DAP10-dependent signaling (55, 56). In addition, the guanine nucleotide exchange factor vav2 (bmgr2) has been implicated in regulation of FcRγ and DAP12-dependent signaling in NK cells (57). Several intracellular signaling proteins are important in regulation of death receptor ligand expression. Protein kinase C (PKC)-θ (bmgr2) has been implicated in regulation of TCR/CD3-induced FasL expression in mouse CTL clones (58). NFAT (bmgr3) is probably important in the signaling pathway downstream of PKCθ and has been suggested to regulate CD16-induced FasL expression in human NK cells (59). The transcription factor GATA-3 (bmgr 2) is essential to T cell development, while GATA-3-deficient B and NK cells develop. GATA-3-deficient NK cells are fully cytotoxic but display an immature phenotype and are unable to produce IFN-γ (60).

We identified a locus in the distal end of chromosome 3 (ebmgr1) showing epistatic interaction with bmgr4 on chromosome 4. Interesting candidate genes in this locus may be the IFN-inducible guanylate-binding proteins (GBP)-1, -2, and -5. These proteins are induced by, e.g., LPS, Listeria monocytogenes infection, or hepatitis C core protein via IFN-γ and IFN-α/IFN-β. GBP s can inhibit viral replication and have antiproliferative activity, but have also been reported to promote fibroblast growth (61–63). Most of the described candidate genes have important functions in NK cells and other cells of the mouse. Thus, a complete deficiency in these proteins is highly unlikely. Nevertheless, polymorphisms slightly changing their function or expression level may result in the phenotype observed in 129 PKO mice. In conclusion, we have identified five loci responsible for BM graft rejection (bmgr1–4 and ebmgr1) in the absence of perforin. There are several possible candidate genes that may be involved in this rejection and may be regulating death receptor-mediated killing by NK cells, or other more general NK cell functions. QTL s defined in studies like the one presented here could provide information useful in studies of other complex traits, thus revealing links between these and NK cells or perforin-independent rejection mechanisms. Further studies will include generation and analysis of congenic mouse strains allowing血型 from loci and hopefully the identification of genes involved in NK cell-mediated BM resistance.

Acknowledgments

We thank Silvio and Maria Peña for excellent animal husbandry and Klas Kärre for critical reading of the manuscript.

Disclosures

The authors have no financial conflict of interest.

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