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Ly49I NK Cell Receptor Transgene Inhibition of Rejection of H2b Mouse Bone Marrow Transplants1,2

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The Ly49 family of genes encode NK cell receptors that bind class I MHC Ags and transmit negative signals if the cytoplasmic domains have immunoregulatory tyrosine-based inhibitory motifs (ITIMs). SE6 mAbs recognize Ly49C and Ly49I receptors and depletion of SE6+ NK cells prevents rejection of allogeneic or parental-strain H2b bone marrow cell (BMC) grafts. To determine the function of the Ly49I gene in the rejection of BMC grafts, we transfected fertilized eggs of FVB mice with a vector containing DNA for B6 strain Ly49I (Ly49IB6). Ly49IB6 is ITIM* and is recognized by SE6 as well as Ly49I-specific 8H7 mAbs. Normal FVB H2a mice reject H2b but not H2d BMC allografts, and the rejection of H2b BMC was inhibited partially by anti-NK1.1 and completely by anti-asialo GM1, but not by anti-CD8, Abs. In FVB mice, NK1.1 is expressed on only 60% NK cells. FVB.Ly49I B6 mice were crossed and back-crossed with 129 mice—H2b, SE6+, poor responders to H2b BMC grafts. While transgene-negative H2b/q F1, or first-generation back-crossed mice rejected H2b marrow grafts (hybrid resistance), transgene-positive mice did not. Thus B6 strain Ly49I receptors transmit inhibitory signals from H2b MHC class I molecules. Moreover, Ly49IB6 has no positive influence on the rejection of H2d allografts.

Materials and Methods

Mice

FVB mice were purchased from The Jackson Laboratory (Bar Harbor, ME) or Harlan Sprague-Dawley (Indianapolis, IN). (CB-17 × B6)F1 SCID mice were obtained from the Carolina's Medical Center (Charlotte, NC). All other mice were bred and maintained in our Microbiology Department Colony. BMC chimeras were made by exposing FVB mice to 7.5 Gy 137Cs in a Gamma Cell 40 irradiator (Atomic Energy, Ottawa, ON, Canada) and being infused of inocula of 5 × 106 BMC from either FVB or FVB.Ly49IB6 mice. The mice were given antibiotics in their drinking water for 10 days to prevent infection. Chimeras were challenged with donor marrow grafts 4–8 wk after reconstitution. To produce F1 mice, FVB, Ly49IB6 (H2b, Ly49I+++) and 129/Sv EMS (129) mice (H2a, Ly49I−−) were mated. Back-cross (BC1) mice were then produced by mating F1 Ly49I−− mice with 129/Sv EMS mice.
with 129 mice. Experiments were performed with mice housed in conventional and in specific-pathogen-free facilities. The Institutional Animal Care and Research Advisory Committee of the University of Texas approved all procedures.

The development of transgenic mice

A two-step cloning strategy was used to insert B6 Ly49I cDNA into an expression vector that has XhoI as the only cloning site (13). Ly49I cDNA from B6 mice was obtained by RT-PCR. Messenger RNA preparation and reverse transcription were conducted as described (5). The following two primers were designed based on published B6 Ly49I sequences (5, 6): 5’-AGC CTC GAG CCG GTA GAG ACA CAG AGA ACA-3’ and 5’-AGC CTC GAG TAG ATA GGA GAG TAC AGT CCC-3’. XhoI sites (underlined) were added for the convenience of subcloning. The B6 Ly49I cDNA was amplified by PCR as follows: denaturation at 95°C for 2 min, followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 1 min, and extending at 72°C for 90 s. The final product was extended at 72°C for 5 min, then incubated at 4°C before running the product out on a 1% agarose gel. A 0.94-kb band of DNA was removed under UV light and electroeluted to obtain pure PCR products (14). The electroeluted PCR product was ligated into a TA cloning vector and transformed into competent cells (Invitrogen, San Diego, CA). Plasmid DNA was purified with a Plasmid Maxi Kit (Qiagen, Chatsworth, CA). The B6 Ly49I cDNA was sequenced with a DNA sequencing kit (U.S. Biochemical, Cleveland, OH) using T7 and Sp6 primers in the TA cloning vector and primers synthesized based on known sequences inside the cDNA. The one amino acid difference between this protein sequence and the reported sequence (7) was in the transmembrane region at position 59, an I (isoleucine) instead of a V (valine).

The B6 Ly49I cDNA was excised with XhoI from the TA cloning vector, purified, and subcloned into the XhoI cloning site in the Thy-1 gene expression cassette (13) (kindly provided by Dr. van der Putten, Ciba Geigy, Basel, Switzerland) as described (14) to obtain the transgenic construct pTS-Ly49I (see below).

This construct was linearized with NotI and PvuI, isolated using a gel extraction kit (Qiagen, Chatsworth, CA), and the fragments were used for the injection of fertilized FVB eggs by Dr. Kathy Graves (University of Texas). Four transgenic mice were identified by dot-blot hybridization and PCR of tail genomic DNA. These mice were used to generate four transgenic lines. The expression of the transgene was demonstrated by staining PBL with FITC-conjugated 5E6 mAbs. Most of the mice used for these studies were heterozygous for Ly49I.

Enrichment of NK cells from fresh splenocytes

Enrichment of NK cells from fresh splenocytes employed StemSep gravity feed columns (StemCell Technologies, Vancouver, BC, Canada) according to their instructions and as previously described (15). Essentially, all non-NK cells are biotinylated with biotin-labeled mAbs to B, T, and myeloid cells, then aggregated with an anti-biotin tetramer. Incubation with StemSep magnetic colloid allows the biotinylated cells to adhere to the magnetic column. NK cells not bound by the colloid and trapped by the magnet are enriched from 1% to 5% about 50% of splenocytes.

Growth of lymphokine activated killer cells

Splenocytes enriched for NK cells were cultured in DMEM medium (Life Technologies, Grand Island, NY) supplemented with 500-1000 U/ml human IL-2 (Cetus Corporation, Emeryville, CA) and cultured for 4–5 days as described (15).

Abs and flow cytometry

Staining of T and NK cells and typing of mice were done with PBL as described (16). For two-color staining of T and NK cells (Fig. 1), PBL were first incubated with Fc receptor blocking Ab, 2.4G2 (PharMingen, San Diego, CA) and then stained with anti-NK1.1 and anti-Ly49I/C (5E6), anti-CD3 and 5E6, anti-CD4 and 5E6, anti-CD8 and 5E6, or anti-B220 and 5E6 in a sequential manner (Fig. 1). FITC-conjugated DX5 was also used (see Fig. 4). The mAbs were obtained from PharMingen.

PBL of F1 mice stained with FITC-conjugated mAb 5E6 (PharMingen) distinguished transgene positive from negative (FVB x 129)F1 mice. Two samples of PBL from each BC, mouse were stained with either FITC-conjugated anti-Kb (PharMingen) or FITC-conjugated mAb 5E6 to detect H2h1, Ly49IB6+/− mice. Stained PBL were analyzed by a FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

The production of the Ly49IB6-specific 8H7 mAb is described elsewhere (17, 18). BW5147 cells transfected with the B6 strain Ly49I were strongly positive when stained with 5E6 or Ly49C-specific 4L0311 mAbs, had minimal shift when stained with 8H7. For in vivo studies, we grew 8H7 hybridoma cells in (C.B-17 x B6)F1, SCID mice to obtain ascitic fluids that have high titers of mAbs. The fluid
was filtered over glass wool filters to remove any particulate matter. The ascites fluid was used to "block" Ly49I receptors on host NK cells at a dose that did not deplete NK cells.

The hybridomas GK1.5 (anti-CD4 mAb) and 2.43 (anti-CD8 mAb) were obtained from American Type Culture Collection (Manassas, VA). Ammonium sulfate precipitated PK136, GK1.5, and 2.43 mAbs were prepared as described (19). The depletion efficiency of PK136 and anti-asialo GM1 (Wako Chemicals, Dallas, TX) was tested by reversal of NK cell-mediated BMC graft rejections. The depleting efficiency of GK1.5 and 2.43 was confirmed by elimination of CD4$^+$ and CD8$^+$ cells, respectively, of PBL. 2.43 has also reversed CD8$^+$ T cell-mediated H2 k bone marrow cell transplants (20).

BMC transplantation, isotope assay, and statistics

These procedures were performed as described (21). Briefly, irradiated mice (8 Gy) were infused with 2.5 or 3.5 x 10$^6$ donor BMC. PK136 (anti-NK1.1) mAbs were injected i.p. 2 and 1 days, and 2.43 (anti-CD8) mAbs were injected i.p. 6 and 4 days before transplantation to deplete NK cells and CD8$^+$ T cells, respectively.

Proliferation of transplanted BMC in recipients was judged in terms of splenic uptake (%) of [125I]-iododeoxyuridine (125 IUdR), a specific DNA precursor and thymidine analogue 5 days after cell transfer (21).

The statistics used are described in detail (21). The percentage of injected $^{125}$I-UDR incorporated into each spleen was calculated and converted to log$_2$ values. Geometric means (95% confidence limits) values of groups (four to six mice) are presented. The significance of differences between any two groups was calculated by parametric and nonparametric methods using the Vax computer UTSTAT NGROUP program provided by the Academic Computing Service at the University of Texas.

Results and Discussion

Ly49I transgene is expressed by CD4$^+$ and CD8$^+$ T cells and NK cells

Thy-1 molecules are expressed on about 50% of fresh NK cells and nearly 100% of IL-2-activated NK cells (22–26). We concluded that the Thy-1 promoter was a good candidate to be used in the development of Ly49IB6 transgenic mice because expression would be limited to NK and T cells. We placed the Ly49I B6 transgene into FVB H2 q mice because NK cells of FVB mice were negative by staining with mAb 5E6 in preliminary experiments, an advantage in screening for transgenic mice. Moreover, FVB mice reject H2b, but not H2k, BMC grafts.

Four Ly49IB6 transgenic mouse lines were derived with the transgene expressed at different levels and on different percentages of PBL as determined by staining with mAb 5E6. Staining of PBLs from either transgenic FVB.Ly49I B6 mice or wild-type FVB mice with 5E6 was equivalent.
and a panel of Abs against T and B cell-surface Ags were compared (Fig. 1). About 50% of the PBL in 2120 mice express Ly49H<sup>+</sup>, and the expression on T cells is brighter than on NK cells (Fig. 1a). Two-color staining of PBL with 5E6 and anti-CD3 mAbs demonstrated that most cells expressing high level of the B6 Ly49 transgene were T cells (Fig. 1c). CD4<sup>+</sup> and CD8<sup>+</sup> T cells are Ly49I positive (Figs. 1, e and g). B cells do not express Ly49I (Fig. 1f).

To detect Ly49I expression on NK cells, we enriched NK cells from either FVB or FVB.Ly49I mice by PE-conjugated NK1.1. They were also stained with FITC-conjugated 5E6, or FITC-conjugated 8H7. Compared with the staining by an isotype control Ab, most transgenic NK cells are 5E6 positive (Fig. 2a) and 8H7 positive (Fig. 2c), indicating that most activated NK cells express the Ly49I transgene. In contrast, there was no difference between the histograms of FVB cells stained with 5E6, 8H7, or their respective isotype control Abs (Fig. 2e, data not shown). Similar results were obtained using (FVB × 129)<sub>F<sub>1</sub></sub> and (FVB.Ly49I B6 × 129)<sub>F<sub>1</sub></sub> NK cells (Fig. 2, d and f), although the staining intensity of (FVB.Ly49I B6 × 129)<sub>F<sub>1</sub></sub> NK cells is somewhat lower than that seen in FVB.Ly49I B6 NK cells. The level of Ly49I staining is lower than that seen in both B6 or B6D2<sub>F<sub>1</sub></sub> mice, where this epitope is expressed endogenously (Ref. 27 and data not shown).

To determine whether the distribution of Ly49 molecules was affected by the presence of the transgene, we stained enriched NK cells from both FVB and FVB.Ly49I B6 mice. Other than expression of the transgene, we found no significant changes in the Ly49 distribution. Neither FVB nor FVB.Ly49I NK cells stain positively for Ly49A (YE1/32), C (4LO3311), or G2 (4D11). However, about 30% of the NK cells from both parental and transgenic mice stain positively with 4E5 anti-Ly49D (data not shown). Because we are unable to detect most Ly49 receptors of FVB mice using the available Abs, FVB mice probably express Ly49 receptors that will require new mAbs to allow full characterization of Ly49 receptors in this strain.

Expression of the B6 Ly49I transgene in FVB hosts affects rejection of H2<sup>b</sup> but not H2<sup>d</sup> BMC grafts

To determine the ability of NK cells of FVB mice to reject allo- genetic BMC, we transplanted B6 or 129 (both H2<sup>b</sup>) or BALB/c (H2<sup>d</sup>) BMC into lethally irradiated FVB hosts. FVB mice reject inocula of 3.5 × 10<sup>6</sup> B6 BMC (Fig. 3a). This rejection can be partially reversed by anti-NK1.1 but not by anti-CD8 mAbs, suggesting that NK cells are responsible for marrow graft rejection. Whereas FVB mice rejected B6 or 129 (both H2<sup>b</sup>) marrow grafts, the FVB.Ly49I B6 mice failed to reject H2<sup>d</sup> BMC (Fig. 3c). It may be noted that B6 BMC grafts grew much better in FVB.Ly49I B6 hosts than in FVB hosts treated with the anti-NK1.1 (PK136) mAbs. Recent reports indicate that anti-NK1.1 (PK136) mAbs bind to NKR-P1B in Swiss.NIH mice and NKR-P1C in B6 mice (28); NKR-P1C is expressed on all NK cells in B6 mice, whereas NKR-P1B is expressed on about 60% of NK cells (28). Because FVB mice are inbred Swiss mice, their NK cells might well express NKR-P1B rather than NKR-P1C. We stained FVB NK cells for both the pan NK marker, DX5, and PK136 (Fig. 4). Only 60% of DX5<sup>+</sup> cells coexpressed PK136 (NK1.1), while almost all NK1.1<sup>+</sup> NK cells expressed DX5. By comparison, all DX5<sup>+</sup> cells in B6 mice express NK1.1 (Fig. 4). Greater than 98% of the DX5<sup>+</sup> cells were CD<sup>+</sup>, indicating that only rare DX5<sup>+</sup> cells are NK/T cells (Fig. 4, a and b; Ref. 29). We have also stained for NK/T cells by three-color analysis of anti-CD3, anti-DX5, and anti-5E6 mAbs. From this stain, most NK/T cells also express 5E6, and, therefore, we cannot exclude NK/T cells as effectors in the bone marrow grafts. We tentatively conclude that FVB NK cells express NKR-P1B and not NKR-P1C. Because NKR-P1C is not expressed on all NK cells of FVB mice, PK136 should not deplete all NK cells in this strain; this explains the partial rather than full loss of ability to reject H2<sup>b</sup> BMC in irradiated FVB hosts injected with PK136. In contrast, because Ly49I is expressed on all NK cells in the FVB transgenic mice, they lose the ability to reject H2<sup>b</sup> BMC completely.

To confirm that rejection of B6 marrow grafts is a property of hemopoietic cells (1), we generated irradiation BMC chimeras (Fig. 3b). The chimeras were irradiated FVB mice infused with 5 × 10<sup>6</sup> FVB or FVB.Ly49I B6 BMC 4–8 wk before challenge. The chimeras were lethally irradiated and challenged with inocula of
3.5 × 10^6 B6 BMC. The chimeras containing FVB. Ly49I^{B6}, but not FVB, hemopoietic cells had significant impairment in the ability to reject B6 marrow grafts (Fig. 3b), supporting the idea that presence of the Ly49I^{B6} transgene in donor-derived NK cells of FVB. Ly49I^{B6} origin inhibited rejection.

Depletion of the 5E6^{+} NK cells in irradiated hosts reversed the rejection of BALB/c (H2^d), but not B6 (H2^b), marrow grafts (30, 31), which led to the hypothesis that molecules recognized by 5E6 mAbs on B6 NK cells could be receptors for H2^d. Because 5E6 mAb reacts with both Ly49C and Ly49I, one or both 5E6^{+} NK cell subsets could be responsible for the rejection. To test if Ly49I of B6 origin is a stimulatory NK receptor for H2^d, 3.5 × 10^6 BMC from BALB/c (H2^d) mice were transplanted into FVB.Ly49I^{B6} transgenic or FVB hosts. The expression of the transgene failed to confer upon FVB mice the ability to reject BALB/c H2^d BMC (Fig. 3c), refuting the hypothesis that Ly49I^{B6} functions as an activating NK cell receptor for H2^d.

Inhibition of hybrid resistance by the Ly49I transgene

FVB. Ly49I^{B6+/−} (H2^d) mice were crossed with 129 (H2^b) mice to produce F_1 (H2^{b/b}) mice. Because NK cells of both FVB and 129 mice are negative for 5E6 (Fig. 2 and Ref. 31), only Ly49I^{B6} transgenic F_1 mice should have 5E6^{+} NK cells. Ly49I^{B6} transgenic and control F_1 hosts were lethally irradiated and challenged with B6 BMC. Transgene-positive F_1 hosts accepted B6 BMC, while transgene-negative F_1 hosts strongly rejected B6 BMC (Fig. 5a), indicating that B6 Ly49I molecules inhibited hybrid resistance. To ensure complete depletion of NK cells in (FVB × 129)F_1 mice, rabbit anti-asialo GM1 serum was used. All NK cells and a small portion of CD8^{+} T cells express asialo GM1. Depletion of CD8^{+} T cells by themselves had only a slight effect on the rejection of B6 BMC by (FVB × 129)F_1 mice (data not shown). Depletion of asialo GM1^{+} cells reversed the rejection of B6 BMC by (FVB × 129)F_1 mice, suggesting that NK (and/or NK/T) cells are the major effector cells responsible for rejecting B6 BMC in (FVB × 129)F_1 hosts (data not shown). The inability to reject B6 BMC by transgene-positive (FVB × 129)F_1 mice was due to the expression of the transgene on NK cells (Fig. 2, b and d).

Transgene-positive Ly49I^{B6+/−} (FVB × 129)F_1 mice were back-crossed to 129 mice lacking a recognizable Ly49I epitope to produce BC_1 progeny, and BC_1 H2^{b/b} mice were identified by staining their PBL with FITC-conjugated anti-K^{b} mAb. When challenged with B6 BMC, BC_1 mice with H2^{b/b} haplotype rejected B6 marrow grafts if they were transgene negative, but accepted B6 marrow grafts if they were transgene positive (Fig. 5b). These results confirm that rejection of H2^{b/b} BMC by H2^{b/b} hosts are inhibited by expression of Ly49I on host NK cells. To ensure that transgenic mice can reject susceptible BMC grafts, we used class I-deficient TAP1^{−/−} mice as donors. Both control and transgenic mice rejected TAP1^{−/−} BMC (Fig. 5c), indicating that the transgenic mice can reject grafts and do not have global defects in NK cell function.

Blocking Ly49I leads to rejection of B6 BMC grafts in FVB. Ly49I^{B6} mice

To verify that the transgene is responsible for transmitting a negative signal to the NK cell, we used 8H7 mAbs to block interactions between Ly49I^{B6} and prospective class I molecules. To determine whether 8H7 anti-Ly49I^{B6} mAbs could bind to NK cells without depleting them, several doses were given to mice in preliminary experiments. We determined that 50 μl of 8H7 ascites fluid sufficiently coats NK cells without depleting them (Fig. 6a). FVB. Ly49I^{B6} mice were injected with 50 μl of 8H7. Two days later, splenocytes were removed, enriched, and stained with anti-NK1.1PE, mouse anti-rat IgG FITC, and 5E6 biotin plus Streptavidin Red 670. In untreated mice, as well as control FVB mice treated with 8H7, the mouse anti-rat IgG FITC does not stain the splenocytes (data not shown). However, in FVB.Ly49I^{B6} mice injected with 8H7, the mouse anti-rat IgG FITC detected 5E6 on the surface of the splenocytes (Fig. 6a). After gating on live NK cells, we confirmed that the cells stained with mouse anti-rat IgG FITC were Ly49I^{B6} by staining the same cells with
the 5E6 mAb. Because 5E6 mAbs detect a different epitope of Ly49I<sup>B6</sup>, all cells positive for mouse anti-rat IgG FITC should also be positive for 5E6 (Fig. 6b). The orthogonal distribution of stained cells results from the double stain of 5E6 and mouse anti-rat IgG. Thus, cells that bound 8H7 were still present, and 8H7 could have blocked Ly49I<sup>B6</sup> interactions with H<sub>2</sub><sup>b</sup> class I molecules. We used the 50-μL dose in bone marrow transplantation experiments to block Ly49I<sup>B6</sup> interactions with H<sub>2</sub><sup>b</sup> class I molecules. Injection of 8H7 mAbs i.p. on the day of BMC transfer allowed transgene-positive (FVB<sup>Ly49I<sup>B6</sup></sup> × 129)<sup>F<sub>1</sub></sup> hosts to assess hybrid resistance. A total of 2.5 × 10<sup>6</sup> donor B6 BMC were transplanted into syngeneic, [(FVB × 129)<sup>F<sub>1</sub></sup> × 129]<sup>BC<sub>1</sub></sup>, or [(FVB, Ly49I<sup>B6</sup> × 129)<sup>F<sub>1</sub></sup> × 129]<sup>BC<sub>1</sub></sup> hosts. c, Donor TAP<sup>1−/−</sup> BMC were transplanted into both non-transgenic and transgenic F<sub>1</sub> hybrid hosts. * Geometric mean significantly less than other groups, p < 0.05.

**FIGURE 5.** BMC transplants in (FVB<sup>Ly49I<sup>B6</sup></sup> × 129)<sup>F<sub>1</sub></sup> and (F<sub>1</sub> × 129)<sup>BC<sub>1</sub></sup> mice. a, Donor B6 BMC were transplanted into B10 (H<sub>2</sub>-identical), (FVB × 129)<sup>F<sub>1</sub></sup>, or (FVB,Ly49I<sup>B6</sup> × 129)<sup>F<sub>1</sub></sup> hosts to assess hybrid resistance. b, A total of 2.5 × 10<sup>6</sup> donor B6 BMC were transplanted into syngeneic, [(FVB × 129)<sup>F<sub>1</sub></sup> × 129]<sup>BC<sub>1</sub></sup>, or [(FVB, Ly49I<sup>B6</sup> × 129)<sup>F<sub>1</sub></sup> × 129]<sup>BC<sub>1</sub></sup> hosts. c, Donor TAP<sup>1−/−</sup> BMC were transplanted into both non-transgenic and transgenic F<sub>1</sub> hybrid hosts. * Geometric mean significantly less than other groups, p < 0.05.

**FIGURE 6.** Monoclonal Ab to Ly49I<sup>B6</sup> (8H7) blocks interactions between Ly49I and H<sub>2</sub><sup>b</sup> class I molecules. a, FVB,Ly49I<sup>B6</sup> mice treated with 8H7 mAb on day 0 were sacrificed on day 2. Their spleens were removed, splenocytes enriched for NK cells as described in Materials and Methods, and stained with FITC-conjugated mouse anti-rat IgG and PE-conjugated NK1.1. A gate for NK1.1-positive cells was determined. The mouse anti-rat IgG stains only cells treated with 8H7 (a), and not those that are untreated (data not shown). b, NK cells from a stained with biotin-conjugated 5E6 followed by Streptavidin Red 670. Dot plot shows 5E6 (y-axis) vs mouse anti-rat IgG (x-axis) expression. In the transgenic mice, all NK cells that are positive for the mouse anti-rat IgG are also 5E6<sup>+</sup>, indicating that 8H7 mAbs block, rather than deplete, transgenic NK cells at this dose. c, FVB or FVB,Ly49I<sup>B6</sup> mice were transplanted with B6 BMC as in Fig. 3. One group of FVB,Ly49I<sup>B6</sup> mice was given the 50-μL dose. Bone marrow transplantation was assayed as described in Materials and Methods. # Geometric mean greater than other groups, p < 0.05, Geometric mean value less than (p < 0.05) FVB,Ly49I<sup>B6</sup> mice not injected with 8H7 mAbs.
there were minimal numbers of rat IgG\(^+\) or 5E6\(^+\) cells present (data not shown). Using transgenic mice, we have demonstrated that H2\(^b\) class I molecules are ligands for B6 Ly49 molecules in vivo. NK cells of H2\(^d\) FVB.Ly49\(^b\) transgenic mice are Ly49\(^d\) positive, unlike those of FVB mice. The ability to reject H2\(^b\) marrow grafts was impaired in transgenic mice. The expression of the transgene also abolished the ability of the (FVB \(\times 129\)F1) or (\(F_2\times 129\)) BC1 H2\(^b/d\) hosts to reject H2\(^b\) marrow grafts. In vitro studies either failed to demonstrate or demonstrated only weak binding of Ly49\(^b/d\) to H2\(^b\) class I molecules (3, 8). Our data refute the hypothesis that Ly49\(^b\) functions as an activating NK cell receptor for H2\(^d\) because the presence of the Ly49\(^b\) transgene does not endow FVB mice with the ability to reject BALB/c H2\(^b\) BMC. We cannot entirely rule out the possibility that 5E6 mAbs bind to yet uncharacterized ITIM-Ly49 receptors of mice of B6 strain NK gene complex, which recognize H2\(^d\) class I molecules on target cells.

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