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# Recognition of $\beta_2$-microglobulin-negative ($\beta_2m^-$) T-cell blasts by natural killer cells from normal but not from $\beta_2m^-$ mice: Nonresponsiveness controlled by $\beta_2m^-$ bone marrow in chimeric mice

**(major histocompatibility complex/$\beta_2$m-deficient mice)**

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**ABSTRACT** **The role of major histocompatibility complex (MHC) class I expression in control of the sensitivity of normal cells to natural killer (NK) cells was studied by the use of mutant mice made deficient for expression of $\beta_2$-microglobulin ($\beta_2$m) through homologous recombination in embryonal stem cells. T-cell blasts from $\beta_2$m-deficient ($\beta_2$m −/−) mice were killed by NK cells from normal mice *in vitro*, while $\beta_2$m +/− blasts were resistant. The $\beta_2$m defect also affected the NK effector cell repertoire: NK cells from $\beta_2$m −/− mice failed to kill $\beta_2$m −/− blasts, while they retained the ability to kill the prototype NK cell target lymphoma YAC-1, although at reduced levels. The inability to recognize $\beta_2$m −/− blasts could be transferred with $\beta_2$m −/− bone marrow to irradiated $\beta_2$m-expressing mice. In contrast, the development of CD8$^+$ T cells (deficient in $\beta_2$m −/− mice) was restored in such chimera. These results indicate that loss of MHC class I/$\beta_2$m expression is sufficient to render normal cells sensitive to NK cells, and that the same defect in the hemopoietic system of a mouse renders its NK cells tolerant to $\beta_2$m-deficient but otherwise normal cells. In the $\beta_2$m −/− mice, NK cells may be selected or educated by other bone marrow cells to tolerate the MHC class I deficiency. Alternatively, the specificity may be controlled directly by the class I molecules on the NK cells themselves.**

One function of innate immunity may be to detect "missing self"—i.e., incomplete expression of host major histocompatibility complex (MHC) class I products (1, 2). This is supported by natural killer (NK) cell rejection of tumor and bone marrow grafts lacking either an MHC class I transgene (3, 4) or $\beta_2$-microglobulin ($\beta_2$m; ref. 5), expressed by the recipients. Reduced MHC class I expression has been associated with high sensitivity to NK cells in a number of different murine and human tumor systems (for reviews, see refs. 2 and 6), but is loss of self MHC class I expression sufficient to render normal, nontransformed cells directly sensitive to NK cell lysis? Recognition of this phenotype would then be one important basis for the surveillance function of NK cells, regardless of antigens dependent on transformation or viral infection. If the MHC indeed influences the sensitivity of normal cells to NK cells, how is the NK cell repertoire affected by maturation in a class I-deficient environment? These questions were addressed in the present study by the use of $\beta_2$m-deficient mice obtained after homologous recombination in embryonal stem cells. The previous analysis of two such lines of $\beta_2$m-deficient mice has yielded important insights into the role of MHC class I molecules in immune functions (7–10). In particular, the mice were shown to be devoid of the CD8$^+$ T-cell subset in the periphery (8, 10) and were unable to mount a primary CTL response against allogeneic cells (10). As $\beta_2$m is required for the cell-surface expression of MHC class I heavy chains, these results show that MHC class I plays a critical role in the development of CD8$^+$ T cells.

Our experimental approach here was based on the use of concanavalin A (Con A)-activated blasts from $\beta_2$m −/− and $\beta_2$m +/− mice as targets for NK cells from different hosts. Since our results indicated that the $\beta_2$m deficiency affected not only the sensitivity of normal cells to NK cells but also the NK cell repertoire at the host–effector cell level, we used bone marrow chimeric mice to ask how MHC class I expression in the periphery influenced the development of NK cells from $\beta_2$m-deficient bone marrow. The development of CD8$^+$ cells under the same conditions was studied for comparison and control.

## MATERIALS AND METHODS

**Mice and Cell Lines.** Generation of the $\beta_2$m-defective mice has been described (7, 8). Briefly, one copy of the $\beta_2$m gene in the embryonal stem cell line E14TG2A [129(129/J)/Ola] was disrupted by homologous recombination. Cells carrying the mutated gene were injected into C57BL/6 (B6) blastocysts which, when allowed to continue development *in vivo*, gave rise to chimeric animals. When mated with B6 mice, a number of these chimeras transmitted the embryonal stem cell genome to their offspring. Half of these had also inherited the mutated $\beta_2$m gene. When these $F_1$ $\beta_2$m +/− animals were mated with one another, expected ratios of homozygous $\beta_2$m −/−, $\beta_2$m +/+, and heterozygous $\beta_2$m +/− animals were seen. Such $F_2$ animals were used in some of the experiments in this paper. Additional $\beta_2$m −/− and $\beta_2$m +/− mice were obtained by crossing $F_2$-generation animals. B6 and 129 mice were purchased from Alab (Sollentuna, Sweden) and The Jackson Laboratory, respectively. YAC-1 is a Moloney murine leukemia virus-induced T-cell lymphoma of the A/Sn strain, and P815 is a methylcholanthrene-induced mastocytoma from the DBA strain.

**Antibodies.** The following reagents were used in fluorescence-activated cell sorter (FACS) analysis and effector cell depletion; monoclonal antibodies (mAb) against H-2K$^b$ (AF6-88.5.3), H-2D$^b$ (28-14-8S) (American Type Culture Col-

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Abbreviations: $\beta_2$m, $\beta_2$-microglobulin; MHC, major histocompatibility complex; NK, natural killer; Con A, concanavalin A; CTL, cytotoxic T lymphocytes; mAb, monoclonal antibody; C, complement.

lection), CD8 [MAS 111; fluorescein isothiocyanate (FITC)-conjugated, Sera-Lab, Crawley Down, Sussex, U.K.], and NK1.1 (PK136; ref. 11); and antiserum against the ganglioside asialo-GM1 (Wako Biochemicals). FITC-conjugated anti-NK1.1 mAb was used for immunofluorescence.

**Generation of Con A-Activated T-Cell Blasts.** Spleen cells were depleted from erythrocytes by osmotic lysis and incubated for 48 hr at $5 \times 10^6$/ml in RPMI 1640 medium supplemented with 5% (vol/vol) fetal calf serum and 5 $\mu$g of Con A per ml. Before use as targets in a standard 4-hr $^{51}Cr$ cytotoxicity assay, dead cells were removed by centrifugation with Lymphoprep (Nycomed, Oslo).

***In Vitro* Cytotoxicity Assay and Effector-Cell Depletion.** NK effector cells were prepared from erythrocyte-depleted fresh spleen cells from mice treated with 2 mg of the interferon inducer tilorone (Sigma; T-8014) to augment NK cell activity 1–2 days before the assay. Cytotoxic T lymphocytes (CTL) were generated in a primary (Table 1, experiments 1 and 2) or secondary (Table 1, experiment 3; 1 week after immunization of responder mice with DBA spleen cells) mixed lymphocyte cultures. Chimeric responder spleen cells ($25 \times 10^6$) were mixed with 2000 R-irradiated DBA stimulator spleen cells ($10 \times 10^6$). The CTL assays were made on day 6 with Con A-activated blasts or P815 cells as target cells. To deplete effector cell subpopulations *in vitro*, spleen cells were incubated for 30 min at 4°C either alone or with the first antibody, washed, and further incubated with a 1:8 dilution of rabbit complement (C; Pel-Freez Biologicals) for 60 min at 37°C. Surviving cells were washed and used directly as effector cells. To deplete NK cells *in vivo*, 0.2 ml of ascites fluid containing anti-NK1.1 mAb or 25 $\mu$l of asialo-GM1 antiserum (diluted in phosphate-buffered saline to 0.2 ml) was inoculated respectively i.p. or i.v. 1 day before the assay.

**Immunofluorescence Analysis.** Expression of NK1.1 mAb was analyzed on nylon wool-passaged splenocytes and expression of H-2K$^b$, H-2D$^b$, and CD8 was analyzed on lymph node cells by using a fluorescence-activated cell sorter (FACS; Becton Dickinson). The cells were incubated for 30 min at 4°C with specific antibody, washed, and, in the case of H-2 antibodies, incubated with a second fluorescent antibody against mouse immunoglobulin (Dakopatts, Hägersten, Sweden).

**Bone Marrow Chimeric Mice.** To generate bone marrow chimeras, B6 recipient mice were irradiated with 900 R and inoculated i.v. with $20–30 \times 10^6$ bone marrow cells. Mice given $\beta_2$m −/− and $\beta_2$m +/− bone marrow cells were treated with anti-NK1.1 mAb before transplantation. Chimeric mice were tested after 2 months.

## RESULTS

**$\beta_2$m-Deficient T-Cell Blasts Are Killed by NK Cells *in Vitro*.** T-cell blasts were prepared by treatment of splenocytes isolated from $\beta_2$m −/−, $\beta_2$m +/−, and control 129 mice with the T-cell mitogen Con A. T-cell blasts from 129 and $\beta_2$m +/− mice were relatively resistant to lysis by fresh splenocytes from B6 or CBA, while blasts from $\beta_2$m −/− animals were sensitive (Fig. 1). Killing of the $\beta_2$m −/− cells was abrogated when splenocytes from mice treated with either anti-NK1.1 mAb (11) or antiserum against asialo-GM1 were used (Fig. 2 *A* and *B*). Both of these agents are known to deplete NK cells in mice after *in vivo* treatment. This indicated that, as is the case with a number of tumor cell lines (2), loss of MHC class I expression renders T-cell blasts sensitive to lysis by NK cells.

**NK Cells from $\beta_2$m-Deficient Mice Do Not Kill $\beta_2$m-Deficient Con A-Activated Blasts.** Previous results have led to the hypothesis that the NK cell repertoire is calibrated according to the host MHC class I products so that reactivity to cells expressing a complete set of self products is avoided (1, 2). For example, NK cells from H-2D$^d$ transgenic B6 mice (but not from normal B6 mice) rejected H-2$^b$ lymphoma and bone marrow cells (3, 4). H-2D$^d$ transfectant lymphoma cells (12) and H-2D$^d$ transgenic bone marrow cells (4) were accepted in the transgenic host. This hypothesis predicted that NK cells from $\beta_2$m −/− mice should tolerate MHC class I/$\beta_2$m-deficient cells. Spleen cells of $\beta_2$m −/− mice were indeed unable to kill $\beta_2$m −/− blasts *in vitro*, while those from $\beta_2$m +/− mice gave the same levels of killing as cells from B6 mice (Fig. 3). The phenotype of the killer cells in $\beta_2$m +/− mice against $\beta_2$m −/− Con A-activated blasts was CD8$^-$ and asialo-GM1$^+$—i.e., that of typical NK cells (Fig. 2*C*). The inability of $\beta_2$m −/− mice to kill and reject $\beta_2$m −/− cells was not due to total lack of NK activity; their

Table 1. NK cell activity, allospecific CTL response, and cell surface phenotype of lymphoid cells in bone marrow chimeric mice

| | | | | | | | CTL lysis | | |
|---|---|---|---|---|---|---|---|---|---|
| | NK cell lysis† | | Cell-surface phenotype‡ | | | | P815 (H-2$^d$) targets¶ | | |
| Mice* | $\beta_2$m −/− blasts | YAC-1 cells | H-2K$^b$ | H-2D$^b$ | CD8 | DBA blasts§ | C | C/anti-CD8 | C/anti-H-2D$^b$ |
| Exp. 1 and 2 | | | | | | | | | |
| $\beta_2$m −/− | 0, 0, 0 | NT | 0 | 0 | 2 | NT | | | |
| | 1, 2, 2 | NT | NT | NT | NT | 0, 0 | | | |
| $\beta_2$m −/− → B6 | 2, 0, 0 | 30, 20, 15 | 13 | 8 | 15 | NT | | | |
| | 4, 0, 0 | 23, 15, 11 | 9 | 7 | 17 | NT | | | |
| | 3, 0, 0 | 20, 16, 11 | NT | NT | NT | 24, 2 | | | |
| $\beta_2$m +/− → B6 | 27, 19, 15 | 42, 13, 18 | 97 | 88 | 16 | NT | | | |
| B6 → B6 | 33, 23, 15 | 58, 38, 25 | 95 | 98 | 35 | NT | | | |
| | 20, 12, 8 | 37, 24, 16 | NT | NT | NT | 17, 15 | | | |
| Exp. 3 | | | | | | | | | |
| B6 → B6 | | | | | | | 27, 15, 8 | 2, 2, 1 | 5, 0, 3 |
| 129 → B6 | | | | | | | 37, 11, 2 | 6, 5, 0 | 1, 1, 5 |
| $\beta_2$m −/− → B6 | | | | | | | 43, 26, 10 | 14, 0, 0 | 33, 18, 3 |

NT, not tested.

*Normal $\beta_2$m −/− mice were compared with chimeric B6 mice given either $\beta_2$m −/−, $\beta_2$m +/−, or B6 bone marrow. Individually tested mice from three experiments.

†Effector-to-target ratios were 200:1, 100:1, and 50:1.

‡Percent positive cells.

§Effector-to-target ratios were 50:1 and 10:1. The ability to generate anti P815 CTL was confirmed with additional $\beta_2$m −/− → B6 chimeras stimulated in mixed lymphocyte culture with B10.A(5R) spleen cells (data not shown).

¶Effector-to-target ratios were 50:1, 10:1, and 2:1.

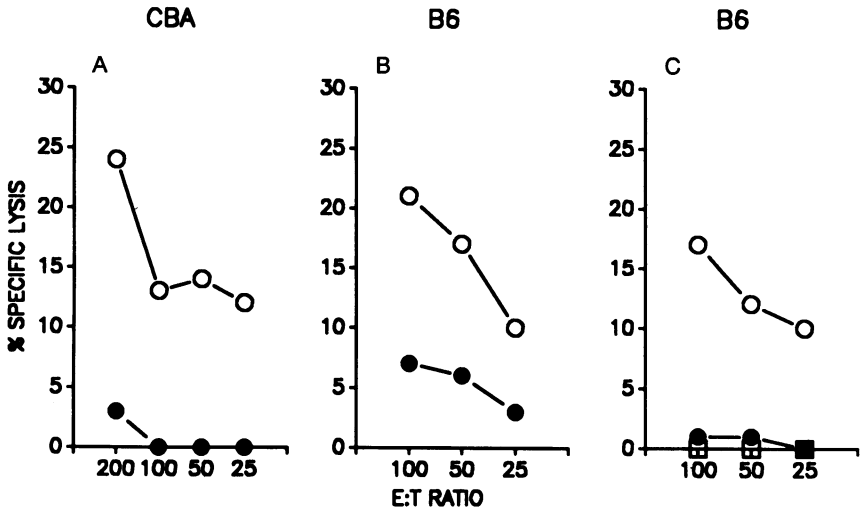


FIG. 1. Sensitivity of Con A-activated blasts to NK cells *in vitro*. (*A*) Lysis of blasts from $\beta_2$m −/− mice (○) and from 129 mice (●) by CBA spleen cells. (*B*) Same as in *A* but with spleen cells from B6 mice. (*C*) Lysis of blasts from $\beta_2$m −/− (○), 129 (●), and $\beta_2$m +/− heterozygotes (□) by spleen cells from B6 mice. E:T ratio, effector-to-target cell ratio.

spleen cells were able to kill the standard target YAC-1 (Fig. 3). This killing and the killing of YAC-1 by cells of $\beta_2$m +/− mice were mediated by asialo-GM1$^+$ cells (Fig. 2 *D* and *E*). In most experiments, the YAC-1 lysis by $\beta_2$m-deficient NK cells was lower when compared with +/− NK cells. In a comparison of data from four consecutive experiments (Table 2), the average lysis with effector cells from eight individually tested $\beta_2$m −/− mice was reduced 50% in terms of lysis and approximately 75% in terms of effector cell dilutions compared with effector cells from $\beta_2$m +/− mice. On the other hand, there was a considerable variation in lysis of YAC-1 cells with efficient killing also by effector cells from $\beta_2$m −/− mice in some experiments, whereas they always failed to kill $\beta_2$m −/− Con A-treated blasts. For example, in the second experiment in Fig. 3 (Fig. 3 *Right*), $\beta_2$m −/− effector cells killed YAC-1 more efficiently than $\beta_2$m +/− cells and yet failed to kill $\beta_2$m −/− Con A-treated blasts. The overall pattern suggests that the two activities are regulated in part by different mechanisms. Therefore, the inability to kill $\beta_2$m −/− Con A-activated blasts is not simply due to generally lower activity or fewer NK cells in $\beta_2$m −/− mice (see *Discussion*). This explanation is in line with the observation that the number of NK1.1$^+$ cells in the spleens of $\beta_2$m −/−, $\beta_2$m +/−, and $\beta_2$m +/+ mice was similar (10%, 13%, and 8% NK1.1$^+$ cells, respectively, in erythrocyte-depleted, nylon wool-passaged spleen cells stained with a FITC-conjugated anti-NK1.1 mAb; after correction for cell yields, each of the genotypes were found to have 2.5–4% NK1.1$^+$ cells in the spleen).

**Inability To Kill $\beta_2$m-Deficient Con A-Activated Blasts Is Determined by the Bone Marrow.** We used normal mice reconstituted with $\beta_2$m-deficient bone marrow (after anti-NK1.1 mAb conditioning of the host) to ask if a peripheral expression of MHC class I would break the "tolerance" of NK cells maturing from the $\beta_2$m-deficient bone marrow. We found that $\beta_2$m −/− to B6 chimeras showed the same selective defect in the NK repertoire as $\beta_2$m −/− mice (killing of YAC-1 cells but not $\beta_2$m −/− blasts; Table 1). In contrast, the deficiency in CD8$^+$ T-cells of $\beta_2$m −/− mice was restored when their marrow-derived precursors matured in a $\beta_2$m-expressing environment. The chimeras developed a normal peripheral pool of CD8$^+$ cells that are absent in $\beta_2$m

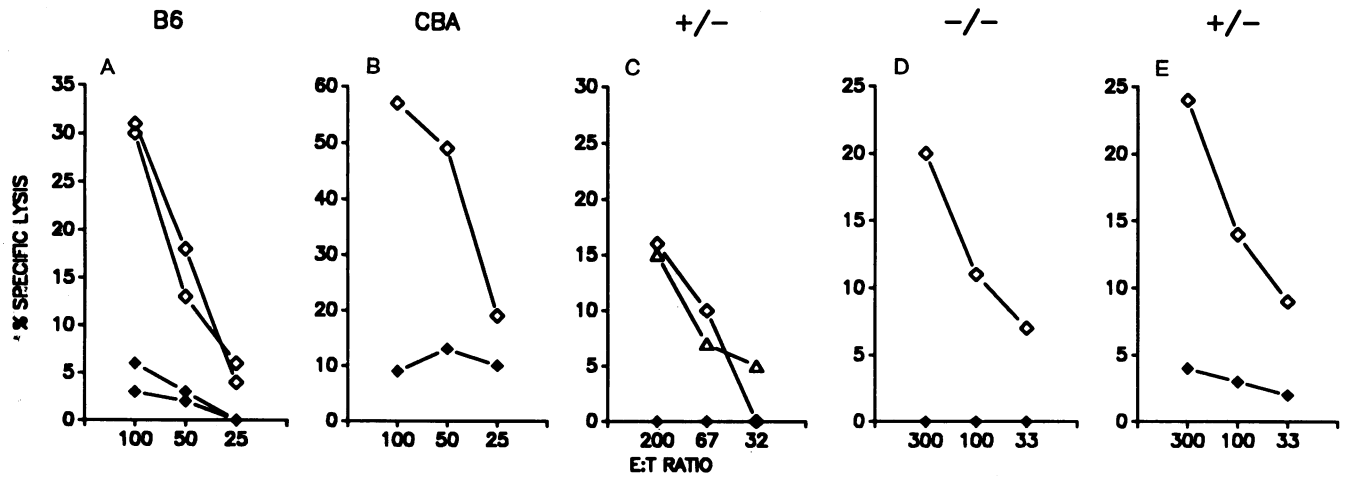


FIG. 2. Lysis of $\beta_2$m −/− blasts is mediated by NK cells. (*A*) Splenocytes from two individually tested nontreated (◇) and two individually tested anti-NK1.1-treated B6 mice (◆) as effector cells. (*B*) Same as in *A* but with splenocytes from two pooled nontreated (◇) and two pooled anti-asialo-GM1-treated (◆) CBA mice as effector cells. (*C*) Cell-surface phenotype of $\beta_2$m +/− effector cells against $\beta_2$m −/− Con A-treated blasts. Spleen cells were treated with anti CD8 + C (△), anti-asialo-Gm1+ C (◆), or with C alone (◇) before the test. Lysis of YAC-1 cells was mediated by asialo-GM1$^+$ cells in both $\beta_2$m −/− (*D*) and $\beta_2$m +/− (*E*) mice. Spleen cells were treated with complement alone (◇) or with anti-asialo-GM1 + C (◆). The donor of effector spleen cells is indicated above each figure. E:T ratio, effector-to-target cell ratio.

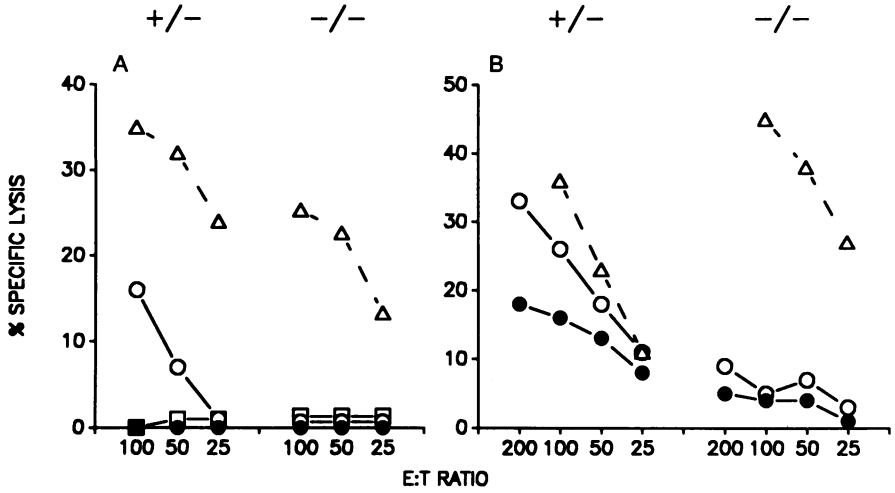


FIG. 3. $\beta_2$m −/− NK cells do not kill $\beta_2$m −/− blasts. (*A*) Lysis of blasts from $\beta_2$m −/− (○), 129 (●), and $\beta_2$m +/− (□) mice by spleen cells from $\beta_2$m +/− (*Left*) and $\beta_2$m −/− (*Right*) mice. Lysis of YAC-1 in the same experiment is indicated (△). (*B*) Same as in *A* but from another experiment. The values represent in each case individually tested mice from representative experiments. Killing of $\beta_2$m −/− blasts typically reached 15–25% lysis in a 4-hr cytotoxicity assay, although higher levels were observed occasionally with very efficient effector cells (*B*). Such effector cells were also able to kill 129 blasts to a certain extent, even when the relative difference between these and the more sensitive $\beta_2$m −/− blasts was maintained (*B*). E:T ratio, effector-to-target cell ratio.

−/− mice (8, 10) and they were able to mount a cytotoxic mixed lymphocyte culture response to allogeneic spleen cell and tumor targets (Table 1). This response was mediated by $CD8^+$, $H\text{-}2D^{b-}$ effector cells (Table 1), indicating that maturation in $\beta_2$m +/− mice allowed the differentiation of $CD8^+$ CTL from $\beta_2$m −/− bone marrow.

## DISCUSSION

Increased sensitivity to NK cells *in vitro* and rejection *in vivo* have been observed for several mutant tumor cell lines lacking MHC class I expression, but there are also examples of tumor systems where no such relation exists (for review, see refs. 2 and 6). It has not been clear whether the latter tumor lines are more representative of normal cells in this regard, implying that other changes besides (or without) loss of class I expression are required to make normal cells sensitive to NK cells. The present results answer this question, and we conclude (*i*) that homozygous loss of the $\beta_2$m gene, coding for the light chain of the class I molecule, is sufficient to make at least some normal cells susceptible to recognition by NK cells; (*ii*) that NK cells of $\beta_2$m −/− mice have lost the ability to kill normal $\beta_2$m-deficient targets, although NK cell recognition of the standard target YAC-1 persists; and (*iii*) that $\beta_2$m/MHC class I deficiency in the hematopoietic system ($\beta_2$m −/− to B6 chimeras) is sufficient to calibrate the NK cells to tolerate normal $\beta_2$m −/− cells, whereas selection of $CD8^+$ T cells, also deficient in $\beta_2$m −/− mice, can occur under conditions where class I expression is present only in nonhematopoietic cells. Similar to the findings reported by Bix *et al.* (5) during the progress of this study, we have found that bone marrow from our independently derived line of $\beta_2$m −/− mice was rejected by NK cells from normal mice (C.Ö., unpublished data). This finding and the observation that $\beta_2$m −/− mice were unable to reject $\beta_2$m-deficient bone marrow (ref. 5; C.Ö., unpublished data) can be explained on the basis of the *in vitro* results in this paper: $\beta_2$m −/− bone marrow cells are rejected because they are directly sensitive to NK cells in nonmutant mice. However, tolerance to autologous cells is maintained by NK cells in the $\beta_2$m-deficient mice, and the $\beta_2$m −/− marrow grafts are thus accepted.

Table 2. Killing of YAC-1 by effector cells from $\beta_2$m −/− and $\beta_2$m +/− mice

| Mice | NK cell lysis of YAC-1* | | |
|---|---|---|---|
| | 100:1 | 50:1 | 25:1 |
| $\beta_2$m −/− | 20 ± 14.8 | 17 ± 12.1 | 10 ± 11 |
| $\beta_2$m +/− | 37 ± 12 | 29 ± 9.1 | 21 ± 7.6 |

*Mean lysis values of YAC-1 from four consecutive experiments with effector cells from eight $\beta_2$m −/− and eight $\beta_2$m +/− mice tested individually. By using a two-tailed nonpaired *t* test, the differences between the two genotypes were found to be statistically significant in the two highest effector-to-target ratios ($P < 0.05$). Means ± SD are shown.

There are two possibilities for how NK cells may detect "missing self." The "effector inhibition" model postulates a MHC class I-dependent negative signal to NK cells (2). The alternative "target interference" model assumes a MHC class I-dependent masking or modulation of a triggering molecule at the target cell level, as originally proposed by Storkus and Dawson (for review, see ref. 6). In context of the latter model, the killing of $\beta_2$m −/− Con A-activated blasts may be triggered by molecules revealed only after phenotypic loss of dominant MHC class I products. If different class I alleles vary in their efficiency to interfere with target molecules, the latter will behave genetically as recessive MHC-linked antigens, whether or not they are encoded within the MHC proper. This pattern of inheritance is the one postulated for Hh antigens, proposed to be target molecules in NK cell-mediated rejection of allogeneic and parental bone marrow grafts (13, 14). Therefore, our present results, interpreted within a target interference model, may be reconciled with the previous genetic studies of bone marrow graft rejection in the mouse (13, 14) and more recent reports of allospecific NK cell responses in humans (15). These have shown that target sensitivity of T-cell blasts to certain NK cell clones may be under negative control of dominant, HLA-linked genes (16). It cannot be excluded that class I molecules are responsible for this down-regulation (16).

The allospecific reactivity by human NK clones is regulated independently of the tumor target killing (15). This pattern is reminiscent of the selective defect in natural killing of normal cells (as opposed to YAC-1 lymphoma cells) observed in the present study. There are at least two hypotheses to explain this "hole" in the NK repertoire against $\beta_2$m −/− cells: (*i*) deletion or nonresponsiveness of a specific

subset of NK1.1$^+$ cells in the $\beta_2$m $-/-$ mice and (*ii*) down-regulation of a specific receptor present on all NK1.1$^+$ cells. The equal number of NK1.1$^+$ cells in nonmutant and mutant mice speaks against deletion of a major subset of NK cells in $\beta_2$m $-/-$ mice, although the disappearance of a small fraction cannot be excluded. Further studies require methods to distinguish different NK1.1$^+$ cells, for example in the CD3$^+$ (17, 18), CD3$^-$/5E6$^+$ (19), and CD3$^-$/5E6$^-$ subsets. The CD3$^-$/5E6$^+$ population should be particularly interesting, since it contains all of the NK cell reactivity of B6 mice against mismatched bone marrow grafts, at least of the H-2$^d$ type (19). The idea of down-regulation of a specific receptor type (responsible for killing of Con A-activated blasts and bone marrow) on all NK cells must be kept open. It can also explain the reduced but not totally abolished killing of YAC-1 (Table 2). According to a "multiple choice" model for NK specificity (20), a single NK cell can operate with different receptor–target interactions in different situations. Thus, YAC-1 may be killed partly by an interaction dependent on its low class I expression (21) and partly by an interaction with tumor-associated structures. Only the former would be affected by the $\beta_2$m defect of the effector cell donor, thus explaining the remaining killer potential against YAC-1 targets. Whatever mechanism is responsible, it appears to be sufficient with a $\beta_2$m defect of bone marrow cells to prevent development of the ability to kill $\beta_2$m $-/-$ blasts in the NK repertoire. The defect may have consequences in the NK cells themselves (e.g., receptors may depend on MHC class I/$\beta_2$m for transport to the cell surface of effector cells) or in other bone marrow-derived cells whose class I molecules normally select certain NK subsets or NK receptors. To explore these possibilities further, $\beta_2$m must be reexpressed selectively in different types of bone marrow-derived cells. Previous work with chimeras has demonstrated that non-MHC genes in the bone marrow control the general levels of NK cell activity (22).

The present results with bone marrow chimeras also demonstrate that the defect in the NK cell repertoire in $\beta_2$m $-/-$ mice is not just secondary to the CD8$^+$ T-cell defect, since the latter but not the former was restored in $\beta_2$m $-/-$ to B6 chimeras. The development of CD8$^+$ T cells in these chimeras is hardly surprising, since thymic epithelium is considered as the crucial site of expression for MHC class I molecules in positive selection of CD8$^+$ T cells (23). Further studies of NK cells in the $\beta_2$m-deficient mice may lead to the molecular characterization of NK subsets and effector–target interactions as well as the process by which the NK cell repertoire is shaped by the class I phenotype of the bone marrow.

**Note Added in Proof.** After the submission of this report, similar data showing that $\beta_2$m $-/-$ Con A-activated blasts are recognized by NK cells from normal but not from $\beta_2$ $-/-$ mice have been reported by Liao *et al.*, using an independently derived $\beta_2$m-deficient mouse strain (24).

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