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NK-Mediated Elimination of Mutant Lymphocytes that Have Lost Expression of MHC Class I Molecules

Yoichiro Kusunoki,¹ Seishi Kyoizumi,⁎ Masamitsu Honma, † Yoshiko Kubo,⁎ Hisashi Ohnishi,⁎ Tomonori Hayashi,⁎ and Toshio Seyama⁎

Mutant cells generated in vivo can be eliminated when mutated gene products are presented as altered MHC/peptide complexes and recognized by T cells. Diminished expression of MHC/peptide complexes enables mutant cells to escape recognition by T cells. In the present study, we tested the hypothesis that mutant lymphocytes lacking expression of MHC class I molecules are eliminated by autologous NK cells. In H-2b/k F₁ mice, the frequency of H-2Kb-negative T cells was higher than that of H-2Kk-negative T cells. The frequency of H-2K-deficient T cells increased transiently after total body irradiation. During recovery from irradiation, H-2K-negative T cells disappeared more rapidly than H-2K-positive T cells. The disappearance of H-2K-deficient T cells was inhibited by administration of Ab against asialo-GM1. H-2Kb-negative T cells showed higher sensitivity to autologous NK cells in vitro than H-2Kb/k heterozygous or H-2Kb-negative T cells. Adding syngeneic NK cells to in vitro cultures prevented emergence of mutant cells lacking H-2Kb expression but had little effect on the emergence of mutant cells lacking H-2Kk expression. Results in the H-2b/k F₁ strain correspond with the sensitivity of parental H-2-homozygous cells in models of marrow graft rejection. In H-2b/k F₁ mice, there was no significant difference between the frequencies of H-2Kb-negative and H-2Kb-negative T cells, although the frequencies of mutant cells were different after irradiation exposure among the strains examined. H-2b/k F₁ mice also showed rapid disappearance of the mutant T cells after irradiation, and administration of Ab against asialo-GM1 inhibited the disappearance of H-2K-deficient T cells in H-2b/k F₁ mice. Our results provide direct evidence that autologous NK cells eliminate mutant cell populations that have lost expression of self-MHC class I molecules.


Results of in vitro studies have shown that NK cells recognize and kill cells lacking expression of self-MHC class I molecules (1). NK cells may be involved in the elimination of cells infected with viruses that evade T cell recognition by interfering with expression of MHC class I molecules (2). NK-mediated elimination may also serve as a mechanism for protection against malignant cells that evade T cell recognition through loss of MHC class I molecules (3).

Increased frequencies of cells bearing genetic mutations have been demonstrated in atomic-bomb survivors up to five decades after the bombing (4–10). The relationship between the estimated dose of radiation and mutation frequency is shallow (7–9) and dose of radiation and mutation frequency is shallow (7–9). The relationship between the estimated dose of radiation and mutation frequency is shallow (7–9) and there is considerable variation in mutation frequencies among individuals (5, 6, 10); this could indicate that selection of mutant cells has occurred in vivo. Interestingly, there appears to have been no significant increase with dose in the frequencies of T cells that carry mutations that disrupt HLA-A expression among atomic-bomb survivors (11). We suspected that this may have been because cells lacking HLA-A expression have been eliminated by NK cells. This hypothesis is supported by previous results that indicate that NK cells can and do kill allogeneic cell lines that lack MHC class I molecules expressed by the NK cells (12, 13).

In the present study, we used murine models to evaluate the role of NK cells in eliminating T cells that lacked H-2 class I expression, and test the hypothesis that NK-mediated killing of autologous target cells can be triggered by a deficiency in the expression of self-MHC class I molecules. NK cells of certain hybrid F₁ strains can reject marrow from parental donors (reviewed in Refs. 14 and 15). For example, H-2b/k mice reject H-2b marrow but accept H-2k marrow (16). Therefore, we evaluated whether the frequency of H-2Kb-negative T cells was different from the frequency of H-2Kb-negative T cells in H-2b/k mice. We also used this model to investigate whether the survival of H-2K-deficient T cells would be affected by treating mice with an Ab that depletes NK cells in vivo (17, 18). In addition to these in vivo analyses, in vitro susceptibility to NK-mediated lysis was compared in wild-type T cells and H-2K-deficient mutant T cells. We also investigated whether NK cells eliminated H-2K-deficient mutant T cells during in vitro culture of T cells. Results of these experiments suggest that NK cells can eliminate autologous cells that have lost expression of self-MHC class I molecules.

Materials and Methods

Antibodies

Biotin-conjugated Abs against H-2Kb, H-2Kk, and H-2Kd; FITC-labeled Ab against NK1.1; and FITC-labeled and PE-labeled Ab against murine CD3 (145-2C11) were purchased from PharMingen (San Diego, CA). FITC-labeled Ab against murine CD4 was obtained from Caltag (South San Francisco, CA) and rabbit Ab against asialo-GM1 was obtained from Wako Pure Chemical (Osaka, Japan).

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Mice

C57BL/6j (B6), C3H/HeJ (C3H), BALB/c (B6 x C3H) F1 (B6C3F1), and (B6 x DBA2) F1 (BDF1) mice were purchased from Japan Clea (Tokyo, Japan), and (B6 x BALB/c) F1 (BCF1) and (B6 x NZB) F1 (B6NZB) mice were obtained from Japan SLC (Hamamatsu, Japan). All mice were housed in autoclaved cages and fed a regular mouse chow diet ad libitum. Mice were X-irradiated (220 kVp, 8 mA) at 8–12 wk of age. In some experiments, 200 μg of Ab against asialo-GM1 was injected via the tail vein every 4 days from 1 day before irradiation through 7 days after irradiation.

Enumeration of H-2K-deficient T cells

Lymphnodes (LN3) and spleens were harvested from euthanized mice. LN suspensions and nylon-wool-nonadherent spleen-cell suspensions were stained with biotin-conjugated anti-H-2Kk, anti-H-2Kb, or anti-H-2Kd Ab, and FITC-labeled anti-CD3 Ab followed by PE-labeled streptavidin. Cells were suspended in buffer containing 100 μg/ml propidium iodide and analyzed with a FACSscan (Becton Dickinson, San Jose, CA). Fluorescence data from a minimum of 5 x 10^6 lymphocyte-gated events were analyzed, and a window for normal T cells was set as a rectangle with the width extending from one-half of the modal FL-1 (CD3) value through twice the modal value. A variant window was set as follows: the upper limit of FL-2 was set at 1/25 the modal intensity of PE fluorescence for normal T cells and the lower limit was set at 10^6. The frequencies of T cells lacking H-2Kk expression were 2.6 x 10^{-5} (left) and 84.5 x 10^{-5} (right), respectively.

Enumeration of TCR-negative CD4 cells

Nylon-wool-nonadherent spleen cells (2 x 10^6) were stained with 10 μl PE-labeled anti-CD3 Ab and 10 μl FITC-labeled anti-CD4 Ab and then analyzed by flow cytometry as described previously (19). In brief, fluorescence data from a minimum of 2 x 10^6 lymphocyte-gated events were acquired, and a window for normal T cells was set as a rectangle with the width extending from one-half of the modal FL-1 value through twice the modal value. A variant window was set as follows: the upper limit of FL-2 was set at 1/25 the modal intensity of PE fluorescence for normal T cells, and the lower limit was set at 10^3. More than 90% of CD4 T cells appeared in the variant window when they were stained with FITC-labeled anti-CD4 Ab alone.

Purification and activation of NK cells

Nylon-wool-nonadherent spleen cells from B6C3F1 mice were stained with PE-labeled anti-CD3 Ab and FITC-labeled anti-NK1.1 Ab and then a NK1.1^+ CD3^- fraction was sorted with a FACStar (Becton Dickinson).

The NK-enriched fraction was cultured in a 1:1 mixture of RPMI 1640 (Nippon Laboratory, Kyoto, Japan) and Glick’s enhanced amino acid (Life Technologies, Rockville, MD) supplemented with 10% FBS, 10 mM l-glutamine, 200 U/ml penicillin G, 200 μg/ml streptomycin, and 5 x 10^{-5} M 2-ME (culture medium) in the presence of 200 ng/ml human recombinant IL-2 (h-IL-2) (kindly provided by Takeda, Osaka, Japan). After 5 days, the proportion of NK1.1^+ CD3^- cells exceeded 97%, and contamination with CD3-positive cells was <1%.

Allogeneic CTL and Con A blasts

CTL against H-2^d or H-2^k were generated by stimulating 2 x 10^6 cells/ml C3H or B6-derived LN cells with 5 x 10^6 cells/ml B6C3F1 splenocytes that had been irradiated with 30 Gy of x-rays. CTL against H-2^d were generated by stimulating 2 x 10^6 cells/ml B6-derived LN cells with 5 x 10^6 cells/ml similarly irradiated BALB/c splenocytes. Effector cells were tested for cytotoxic activity after culture for 5 days. B6, C3H, and BALB/c Con A blasts used as targets in cytotoxicity assays were prepared by stimulating LN cells with 5 μg/ml Con A for 2 days. YAC-1 leukemia cells were maintained in RPMI 1640 medium containing 10% FBS.

FIGURE 1. Enumeration of T cells lacking H-2Kk expression in splenocytes 2 wk after 3-Gy total body irradiation. Nylon-wool-nonadherent splenocytes were obtained from control (left) and irradiated (right) B6C3F1 males at 12 wk of age and stained with anti-CD3 and anti-H-2Kk Abs. A window for normal T cells (R2) was set as a rectangle with the width extending from one-half of the modal FL-1 value through twice the modal value. A variant window was set as follows: the upper limit of FL-2 was set at 1/25 the modal intensity of PE fluorescence for normal T cells and the lower limit was set at 10^6. The frequencies of T cells lacking H-2Kk expression were 2.6 x 10^{-5} (left) and 84.5 x 10^{-5} (right), respectively.

FIGURE 2. Frequencies of T cells lacking H-2Kk expression. LN cells (left) and nylon-wool-nonadherent spleen cells (right) from 8- to 12-wk-old B6C3F1 males (n = 9) were examined by flow cytometry. Mean frequencies of T cells lacking H-2Kk expression were 1.30 x 10^{-5} (SD = 0.69 x 10^{-5}) and 2.47 x 10^{-5} (SD = 1.38 x 10^{-5}) in LN and spleen, respectively. Mean frequencies of T cells lacking H-2Kd expression were 2.49 x 10^{-5} (SD = 1.53 x 10^{-5}) and 3.36 x 10^{-5} (SD = 1.38 x 10^{-5}) in LN and spleen, respectively. Wilcoxon signed rank sum tests showed significant differences in the frequency of T cells lacking H-2Kk as compared with H-2Kd expression (p < 0.005) and in the frequency of H-2K-deficient T cells in LN as compared with spleen (p < 0.01).
Isolation and in vitro culture of H-2K<sup>k</sup>- or H-2K<sup>b</sup>-deficient T cells

LN cells from B6C3F1 mice were irradiated with 3 Gy of x-rays and stimulated with 5 μg/ml Con A for 3 days. The cultured cells were washed twice with PBS and stained with biotin-conjugated anti-H-2K<sup>k</sup> or H-2K<sup>b</sup> Ab and FITC-labeled anti-CD3 Ab followed by PE-labeled streptavidin. H-2K<sup>k</sup>-CD3<sup>+</sup>, H-2K<sup>k</sup>-CD3<sup>+</sup>, H-2K<sup>b</sup>-CD3<sup>+</sup>, and H-2K<sup>b</sup>-CD3<sup>+</sup> fractions were sorted respectively with a FACStar, and the sorted cells were suspended in culture medium and distributed at a mean frequency of 5 × 10<sup>4</sup> cells/well in a 96-well round-bottom plate. The cells were cultured in the presence of 10 ng/ml h-rIL-2 for an additional 7 days and assessed for their sensitivity to NK- and CTL-mediated cytotoxicity in vitro. After the 7-day culture, the purity of each cell fraction exceeded 90%.

Cytotoxicity assays

Cytotoxic activity was measured with a <sup>51</sup>Cr release assay as described previously (20). Target cells (1 × 10<sup>3</sup>) labeled with <sup>51</sup>Cr were incubated with graded numbers of effectors (prepared as described above) in wells of 96-well U-bottom plates at 37°C for 4 h. After centrifugation at 450 × g for 3 min, radioactivity in the supernatant of each well was measured with an auto gamma counter (Aloka ARC-500, Aloka, Tokyo, Japan), and the percent of specific lysis at each effector/target cell ratio was calculated by standard methods (20). NK activity in splenocytes was evaluated for specific lysis of YAC-1 target cells at an effector/target cell ratio of 100:1.

In vitro elimination of cells that have lost expression of H-2K alleles during in vitro culture

LN suspensions and nylon-wool-nonadherent spleen-cell suspensions were stimulated with 5 μg/ml Con A and incubated in culture medium for 3 days. The resulting cells were washed twice with PBS containing 2% FBS and grown for 3 more days in 2 ml culture medium containing 200 ng/ml h-rIL-2 with or without graded numbers of NK cells purified and activated as described above. In some experiments, LN cells and nylon-wool-nonadherent splenocytes were irradiated with 2 Gy of x-rays before Con A

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**FIGURE 3.** Frequencies of T cells lacking H-2K<sup>k</sup> (■) or H-2K<sup>b</sup> (●) expression in B6C3F1 mice 2 wk after total body irradiation. Each point denotes the mean value of three to five mice, and each bar indicates the SD. Correlations between irradiation exposure and the frequencies of mutant cells were fit according to exponential models.

**FIGURE 4.** Frequencies of T cells lacking H-2K<sup>k</sup> (■) or H-2K<sup>b</sup> (●) expression in B6C3F1 mice 7 wk after total body irradiation. Each point denotes the mean value of three to five mice, and each bar indicates the SD. Correlations between irradiation exposure and the frequencies of mutant cells were fit according to exponential models.
stimulation. Cultures were then tested to determine the frequency of $H-2K$-allele-negative cells. The upper limit of $H-2K^k$-deficient variants was set at 1/25 of the modal intensity of PE fluorescence for normal T cells and the upper limit of $H-2K^b$-deficient variants was set at 1/12.5 the modal intensity of PE fluorescence for normal T cells. More than 90% of CD3-positive T cells from parental C3H or B6 Con A blasts appeared in the variant window when they were stained with anti-CD3 Ab and anti-H-2K$^k$ or -H-2K$^b$ Ab, respectively.

**Statistical analyses**

Statistical significance was assessed with the Wilcoxon rank sum test.

**Results**

**Frequencies of $H-2K$-deficient T cells in $H-2K^{b/k}$ F$_1$ mice**

We established a murine model to determine whether autologous NK cells could eliminate cells lacking expression of an MHC class I allele (Fig. 1). In B6C3F1 mice, splenic T cells lacking expression of $H-2K^b$ or $H-2K^b$ were observed at frequencies of $\sim 1–5 \times 10^{-5}$, and the frequencies of $H-2K^b$-negative cells were lower than the frequencies of $H-2K^b$-negative cells (Fig. 2). Differences in the frequencies of the two mutant populations were more apparent when mice were tested 2 wk after irradiation (Fig. 3). The difference in frequencies of mutant cells lacking $H-2K^k$ vs $H-2K^b$ corresponded with the sensitivity of parental H-2-homozygous cells in models of marrow graft rejection. $H-2K^{b/k}$ F$_1$ mice have been found to accept $H-2K^k$ parental marrow grafts, but reject $H-2K^b$ parental marrow grafts, by NK-mediated mechanisms (15, 16).

**Survival of irradiation-induced mutant T cells in $H-2K^{b/k}$ F$_1$ mice**

The frequencies of $H-2K$-deficient T cells were analyzed at 7 wk after irradiation (Fig. 4). Increased irradiation had only a small effect on the frequencies of $H-2K^b$-negative T cells and no significant effect on the frequencies of $H-2K^k$-negative T cells. As shown in Fig. 5, the frequencies of $H-2K$-deficient T cells peaked
frequencies of TCR-negative CD4 cells gradually decreased over time, possibly as a result of defective proliferation in vivo (19, 21, 22). The rapid decrease in frequencies of H-2K-deficient mutants suggested a mechanism involving active elimination in vivo.

Enhanced survival of H-2K-deficient T cells in H-2K<sup>k</sup>-<i>F</i> mice treated with anti-asialo GM1 Ab

To test whether NK cell activity is involved in the elimination of H-2K<sup>k</sup>-negative or H-2K<sup>b</sup>-negative T cells in vivo, mice were injected with 200 µg anti-asialo-GM1 every 4 days from 1 day before 2-Gy irradiation through 7 days after irradiation. Splenocytes from unirradiated mice treated with the Ab showed reduced NK activity as compared with splenocytes from untreated controls (Fig. 6). Splenocytes from irradiated mice showed higher NK activity than splenocytes from unirradiated controls, indicating that NK cells are relatively resistant to radiation (23). In unirradiated mice, the frequencies of H-2K-deficient T cells were not affected by the treatment with Ab against asialo-GM1 (Fig. 6).

Treatment with Ab against asialo-GM1 increased the frequencies of H-2K-deficient T cells on day 11 after irradiation (Fig. 6). The effect of Ab treatment was more pronounced for H-2K<sup>k</sup>-negative than for H-2K<sup>b</sup>-negative T cells, and on day 11, the frequency of H-2K<sup>k</sup>-negative T cells was almost as high as the frequency of H-2K<sup>b</sup>-negative T cells. Thirty-five days after irradiation, Ab-treated mice showed partial recovery of NK activity, and the frequencies of H-2K-deficient T cells decreased to levels observed in irradiated controls without the Ab treatment. Similar results were also observed with LN cells from the same mice (data not shown). By contrast, the frequency of TCR-negative CD4 cells in irradiated mice was not changed by treatment with Ab against asialo-GM1. These results suggest that NK cells are responsible for the elimination of radiation-induced H-2K-deficient T cells in vivo.

Cytotoxic sensitivity of cells after loss of H-2K expression

To test whether cells that have lost expression of self-MHC class I Ags become susceptible to lysis by NK cells, H-2K-deficient T cells were isolated from 3-Gy-irradiated Con A blasts. As shown in Fig. 7, H-2K-deficient T cells were more sensitive than wild-type T cells in cytotoxicity assays with an enriched population of NK cells.
activated syngeneic NK effectors. Sensitivity to NK cells was more than 3-fold higher in H-2K\(^d\)-deficient T cells than in H-2K\(^a\)-deficient T cells. In contrast, H-2K\(^a\)-deficient T cells were less sensitive than H-2K\(^b\)-deficient T cells in cytotoxicity assays with CTL against H-2\(^b\) but more sensitive to CTL against H-2\(^b\). Both H-2K\(^b\)-deficient and H-2K\(^a\)-deficient T cells were resistant to lysis by CTL against H-2\(^a\).

**NK cell-mediated elimination of cells that have lost H-2K allele expression during in vitro culture**

Con A blasts from B6C3F1 LN were tested to determine the frequency of H-2K\(^a\) or H-2K\(^b\)-negative cells after culture with or without activated NK cells in the presence of IL-2 for 3 days. As shown in Fig. 8, the frequency of H-2K\(^a\)-negative T cells decreased in proportion to the number of NK cells added to the culture, while the frequency of H-2K\(^b\)-negative T cells was not changed by the addition of NK cells. The frequency of H-2K-

deficient T cells increased when cells were irradiated with 2 Gy before stimulation with Con A. When NK cells were added to these cultures, the frequency of H-2K\(^a\)-negative T cells decreased in comparison to the frequency of H-2K\(^b\)-negative T cells. A similar decrease in the frequency of H-2K\(^a\)-negative T cells was observed when NK cells were added to Con A blasts from B6C3F1 splenocytes (Fig. 8). Interestingly, H-2K\(^a\) allele mutant frequencies were higher in LN cells that were irradiated in vitro with 2 Gy than in splenocytes that were similarly irradiated in vitro. The difference in mutant frequencies between LN cells and splenocytes irradiated in vitro may be due to the difference in NK activity between these cell populations. The LN cell population contained <10% NK1.1-positive cells and showed <10% NK activity against YAC-1 cells than the splenocyte population.

**Frequencies of H-2K-deficient T cells in H-2K\(^a/b\) F\(_1\) mice**

To test whether autologous NK cells could eliminate cells lacking expression of an MHC class I allele in vivo in other F\(_1\) hybrids, frequencies of H-2K-deficient T cells were analyzed in BDF1, BCF1, and B6NZB mice. Two weeks after irradiation, the mice showed dose-dependent increases in the frequencies of H-2K-deficient T cells, although there was no significant difference in radiation dose responses in the frequencies of H-2K\(^a\)-deficient T cells relative to the frequencies of H-2K\(^a/b\)-deficient T cells (Fig. 9). Radiation had a less striking effect on the frequencies of H-2K-deficient T cells in B6NZB mice, and the frequencies of H-2K-deficient T cells rapidly decreased after irradiation (Fig. 10). Treatment of 2-Gy-irradiated H-2K\(^a/b\) F\(_1\) mice with Ab against asialo-GM1 increased the frequencies of H-2K-deficient T cells on day 11 after irradiation (Fig. 11), but the effects of Ab treatment were less striking in B6NZB mice than in BCF1 mice.

**Discussion**

The identification of MHC class I Ag-specific inhibitory receptors has provided an understanding of molecular mechanisms that regulate the cytotoxic activity of NK cells (24–29). Our results provide direct evidence that autologous NK cells function in vivo to eliminate mutant cell populations that have lost expression of self-MHC class I molecules. A variety of mechanisms can cause down-regulation of MHC class I molecules. Defects in TAP (30), abnormalities in \(\beta\)-2-microgloblin (31), and structural alterations of MHC genes (32) have been reported in malignant and nonmalignant cells. Some viruses encode proteins that interfere with the formation of MHC/peptide complexes (2). HIV-1 Nef protein is known to induce endocytosis of MHC class I molecules (33), thereby allowing HIV-1-infected cells to escape lysis by HIV-1 Ag-specific CTL (34). Zheng et al. (35) reported that the proto-oncogene PML regulates MHC class I Ag presentation through induction of latent membrane protein-2, latent membrane protein-7, TAP-1, and TAP-2. Down-regulation of MHC class I molecules has been proposed as a mechanism that might allow malignant cells and virally infected cells to escape recognition by T cells. Results of our study suggest NK cells might prevent progression of certain malignancies and viral infections in vivo by recognizing and eliminating autologous cells that have lost expression of self-MHC class I molecules.

We found that the frequencies of T cells deficient in expression of H-2K alleles in B6C3F1, BCF1, and B6NZB mice were in the vicinity of 10\(^{-5}\). This value appears to be somewhat lower than that reported by Dempsey et al. (36) but higher than that reported by Klarmann et al. (37). Dempsey et al. (36) used (BALB/c × BALB/k)\(_{F1}\) mice and complement-mediated selection to detect mutant T cells, whereas Klarmann et al. (37) used (B10A × C57BL/6)\(_{F1}\) mice and an immunomagnetic bead selection method. The discrepancy in results among the three studies might
FIGURE 10. Frequencies of splenic T cells lacking H-2K\(^d\) (▲) or H-2K\(^b\) (●) expression and frequencies of TCR-deficient CD4 cells (○) from BCF1 (left) and B6NZB (right) mice after 2-Gy total body radiation. Open symbols denote frequencies in unirradiated, age-matched controls.

FIGURE 11. Effect of treatment with anti-asialo-GM1 Ab on the frequencies of mutant T cells after 2-Gy total body irradiation. BCF1 (left) and B6NZB (right) mice were injected with 200 µg anti-asialo-GM1 Ab at 4-day intervals from 1 day before irradiation to 7 days after irradiation. The frequencies of splenic T cells lacking expression of H-2K\(^d\) (top) or H-2K\(^b\) (middle) and splenic NK activity (bottom) were evaluated at 11 days after irradiation. Closed circles (●) denote values in irradiated mice treated with anti-asialo-GM1 Ab. Closed triangles (▲) indicate values in untreated irradiated controls. Open circles (○) indicate values in untreated controls.
reflect differences in the methods used for detecting H-2 class I-deficient T cells or differences between the strains that were selected for testing. It is known that the genetic background of the host can influence the repertoire of inhibitory NK cell receptors (38–40).

In B6C3F1 mice, H-2Kb-negative T cells were more frequent than H-2Kk-negative T cells. This finding is consistent with the observation that B6C3F1 recipients resist growth of C3H.H-2Kb (H-2KkDd) marrow cells (41). Although hybrid resistance effects have generally mapped to H-2D rather than H-2K, it is likely that B6C3F1 NK cells can respond to the absence of H-2Kb. Inhibitory NK receptors that recognize H-2Kk have not yet been identified. We would predict that H-2D-negative cells are more frequent than H-2D-negative cells in B6C3F1 mice. We were not able to test this prediction because H-2D allele-specific Abs could not distinguish adequately between H-2D-positive and -negative cells by the methods we used. Differences between the frequencies of H-2Kk-negative and H-2Kb-negative cells may reflect the expression of inhibitory NK cell receptors in H-2Kk-negative NK cells. Cells bearing H-2Kk-specific inhibitory receptors might be more numerous than those bearing H-2Kb-specific inhibitory receptors; alternatively, inhibitory signals delivered through H-2Kk-specific receptors might be weaker than those delivered through H-2Kb-specific inhibitory receptors.

Both BDF1 and BCF1 mice are known to accept parental H-2d marrow but reject parental H-2Kb marrow (41, 42). However, no differences in frequencies of H-2K-deficient H-2Kb and H-2Kk cells were observed in our study. Therefore, we were unable to determine whether differences in the frequency of H-2K-deficient T cells in F1 mice were relevant to the resistance of parental marrow cells in these particular F1 mice. In H-2Kd-negative F1 mice, hybrid resistance effects have mapped to H-2D rather than H-2Kk, and NK cells that respond in the absence of H-2D may primarily account for the resistance of these F1 mice to parental marrow. Drizlik et al. (41) reported that BDF1 mice do not reject marrow from C3H.H-2Kd (H-2KdDd) or B6.H-2Kd (H-2KdDd) donors. Rembecki et al. (42) also reported that BCF1 mice do not reject marrow from B10.A (5R) (H-2KdDd) and A.TL (H-2KdDd) donors. Our results are consistent with these earlier studies suggesting that the absence of H-2Kb or H-2Kk does not permit efficient NK responses in H-2k-negative F1 mice.

In the present study, radiation was less effective at inducing H-2K-deficient T cells in B6NZB mice than in the other strains examined. B6NZB mice are known to be able to reject both parental B6 and NZB marrow (42). Thus, NK-mediated elimination of H-2 class I-deficient cells might be more efficient in B6NZB mice than in other strains. Alternatively, B6NZB mice may be more resistant to radiation-induced cell damage and mutations. Such strain differences have been suggested in rejection of allogeneic marrow transplant (43) and in radiation-induced mutations (19). In humans, despite evidence for increased frequencies of cells bearing genetic mutations in atomic-bomb survivors (4–10), no significant radiation effect was observed in the frequency of T cells with mutations that lacked HLA-A expression in the survivors (11). We suspect that NK cells may have been able to eliminate any HLA-A mutant cells that were generated by exposure to radiation. This hypothesis is supported by evidence that NK cells can kill autologous cells that lack self MHC class I molecules (the present murine study and unpublished observations in human subjects). In HLA-A2/A24 heterozygous individuals, T cells lacking expression of HLA-A2 are significantly more frequent than T cells lacking expression of HLA-A24 (11). Although inhibitory NK cell receptors specific for HLA-A2 and -A24 have not been identified, the difference in frequencies of cells lacking expression of HLA-A2 and -A24 alleles might indicate variability in the expression or function of inhibitory receptors. NK susceptibility patterns among MHC class I-deficient cells might also influence the alterations in MHC phenotypes observed in cancers that escape T cell recognition. For example, metastatic melanoma cells that express only HLA-A24 have been detected in patients who received immunotherapy with melanoma Ag peptides (44). Further studies testing NK-cell susceptibility in MHC class I-deficient cells may help to establish improved therapeutic strategies for treatment of malignant diseases.

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