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Recovery from Friend Disease in Mice with Reduced Major Histocompatibility Complex Class I Expression

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Mice homozygous for the *b* allele of the MHC gene, *H-2D*, have a high incidence of recovery from Friend virus infections, while mice heterozygous for the *b* allele at *H-2D* have a very low incidence of recovery. Previous experiments indicated that the low recovery rates associated with heterozygosity at *H-2D* might be related to a gene dosage effect requiring the expression of two $H\text{-}2D^b$ alleles for high recovery. We investigated the effects of reduced $H\text{-}2D^b$ expression on recovery from Friend disease by using $H\text{-}2^b$ homozygous mice carrying a single β_2-microglobulin gene disruption. These mice had reductions in cell surface $H\text{-}2D^b$ expression comparable to those of $H\text{-}2D^{a/b}$ heterozygotes. Numerous cell types with various levels of $H\text{-}2D^b$ expression were examined, and in each case, the expression levels in the β_2-microglobulin mutants closely reflected those observed in the $H\text{-}2D^{a/b}$ heterozygotes. We found, however, that reduced expression did not affect recovery from Friend disease, indicating that heterozygous levels of $H\text{-}2D^b$ expression are sufficient for the high-recovery phenotype previously associated only with $H\text{-}2D^b$ homozygotes.

Friend virus (FV) is a retroviral complex composed of a replication-competent helper virus, Friend murine leukemia virus, and a replication-defective virus, spleen focus-forming virus (SFFV) (37). In susceptible adult animals, FV induces rapid polyclonal erythroblast proliferation caused by binding of the SFFV gp55 envelope glycoprotein to the erythropoietin receptor of erythroid progenitor cells (19, 27). This acute proliferation is followed by the immortalization of cells having SFFV proviral integrations at the *Spi-1* cellular oncogene locus (29, 31). Alterations in the p53 tumor suppressor gene have also been implicated in the immortalization of FV-induced erythroleukemias (24). Mice with Friend disease exhibit profound splenomegaly and abnormally high hematocrits of 85 to 95%. Left unchecked by host immunity, FV infections are usually lethal. Effective immune control of FV requires virus-specific humoral as well as cell-mediated immune responses (9, 15), and the in vivo depletion of either $CD4^+$ or $CD8^+$ T-cell subsets ablates recovery in normally resistant mouse strains (35).

Immune responses to FV are controlled by several host genes, including genes linked to the mouse major histocompatibility complex (MHC) and non-MHC genes (8). We have been particularly interested in the *H-2D* region of the MHC, as it has been shown to play a critical role in recovery from FV-induced erythroleukemia (10, 11). The effects of *H-2D* are most easily studied in F_1 mice that contain the proper background of non-MHC genes necessary to allow for MHC-mediated recovery. Using (C57BL × A)F_1 mice congenic for various MHC haplotypes, we demonstrated that after inoculation with high virus doses, all strains were equally susceptible to FV-induced erythroleukemia (11), but mice homozygous for the *b* allele at *H-2D* had a high-recovery phenotype while mice heterozygous for the *b* allele had a low-recovery phenotype. This effect has been mapped precisely to the *H-2D* gene in bm14 mutant mice. For example, (bm14 × A.BY)F_1 mice differ from (B6 × A.BY)F_1 mice by only a single nucleotide substitution in one of their *H-2D* alleles and yet have a dramatically reduced ability to recover from Friend disease (28).

The involvement of *H-2D* antigens in FV-specific immune responses is unusual in that the failure of *H-2D* heterozygous animals to recover from high virus doses is not due to the lack of appropriate peptide presentation molecules on infected cells. Rather, $H\text{-}2D^{b/b}$-associated recovery appears to act at the level of the T-cell repertoire. In previous experiments, immune T cells transferred from a high-recovery strain ($H\text{-}2^{b/b}$) into a low-recovery strain ($H\text{-}2^{a/b}$) could save the animals from a lethal dose of FV (4). Therefore the *H-2* haplotype of the T-cell donors appeared to be more critical than the haplotype of the FV-infected cells of the recipients.

Regardless of the mechanism of recovery, there are two possible explanations for the observation that $H\text{-}2D^{b/b}$-mediated recovery from FV is a recessive phenomenon. Either a non-*b* allele such as $H\text{-}2D^{bm14}$ or $H\text{-}2D^d$ can negatively affect the high recovery phenotype, or there is a gene dosage effect which requires the expression of two high-recovery alleles. To test the negative impact hypothesis, we previously examined the recovery status of high-recovery (A.BY × B6)F_1 mice ($H\text{-}2^b$) that expressed the $H\text{-}2D^d$ transgene from a low-recovery strain ($H\text{-}2^a$). Those mice displayed a high-recovery phenotype, suggesting that the $H\text{-}2D^d$ gene did not negatively affect recovery in $H\text{-}2D^{b/b}$ mice (28).

Therefore, we wished to investigate gene dosage effects by testing the recovery status of mice that expressed heterozygous levels of $H\text{-}2D^b$ without the possibility of negative effects potentially associated with coexpression of a second parental *H-2D* gene. A previous report indicated that MHC class I expression (*H-2K* and *H-2D*) was reduced in mutants heterozygous for a β_2-microglobulin (β_2-m) gene disruption (23). This finding was due to the fact that class I molecules generally require association with β_2-m light chains for proper folding, transport, and function (32), and the intracellular supply of β_2-m appeared limiting when only one allele was expressed. In

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the present study, we utilized this observation to produce a model for studying the impact of reduced *H-2D*^{b} expression on recovery from Friend disease.

MATERIALS AND METHODS

Mice. B6 β_2-m$^{+/-}$ mice were generously provided by the late Martin Zjilstra. These mice were at the fifth generation of backcrossing to C57BL/6 when used for the production of the (A.BY × B6 β_2-m$^{+/-}$)F$_1$ offspring used in experiment 3, the seventh generation of backcrossing for experiment 2, and the ninth generation for experiment 1. B6, A.BY, and A/WySn mice were obtained from the Jackson Laboratory, and the bm14 strain was provided by Roger Melvold, Northwestern University School of Medicine. All F$_1$ strains were produced in the animal care facilities of Rocky Mountain Laboratories.

Flow cytometry. Single-cell suspensions from mouse tissues were analyzed for antigen expression by using a FACStar I (Becton Dickinson, San Jose, Calif.) modified for five-parameter analysis. Thymic stromal cells were obtained by teasing the thymic lobes from two mice with forceps in 5 ml of phosphate-buffered balanced salt (PBBS) (9) with 2% fetal bovine serum (FBS) followed by vigorous pipetting. The stroma was transferred to 5 ml of fresh PBBS-FBS and vigorously pipetted again. The stroma was washed one additional time and digested with collagenase and DNase as described previously (38) to obtain a single-cell suspension. Histogram overlays were generated by using the Consort 30 program. Anti-*H-2D*^{b} monoclonal antibody (MAb) 95 (17) was purified by ion-exchange chromatography (DEAE) and directly conjugated with fluorescein isothiocyanate (FITC). Anti-FV gp70 MAb 720 (35) was purified by affinity chromatography and directly conjugated to biotin. The following tissue specific MAbs were purified by ion-exchange chromatography (DEAE) and conjugated to biotin: anti-CD4 (GK1.5) (14), anti-CD8 (53-6.72) (26), B220 (RA3-6B2) (13), MAC-1 (M1/70.15.11.5.HL) (36), and Gr-1 (RB6-8C5) (18). The biotin-conjugated MAbs were developed with phycoerythrin-conjugated streptavidin (Biomeda Corp., Foster City, Calif.) in all of the two-color analyses. In the three-color analyses of thymic stromal cells, anti-CD8 (53-6.72) was directly conjugated to phycoerythrin, and the biotin-conjugated anti-class II MAb (06062D; Pharmingen, San Diego, Calif.) was developed with streptavidin-Red613 (Immunoselect; Life Technologies Inc., Grand Island, N.Y.).

Virus. The B- and NB-tropic strains of FV were obtained from Frank Lilly and used as a 20% spleen homogenate prepared from infected BALB/c mice. Proper dilutions of virus stocks were determined by the spleen focus assay in (B10 × A)F$_1$ mice. Previous data showed that the number of spleen foci observed was not influenced by MHC genes (11).

Leukemia induction. Mice were infected by intravenous inoculation of 0.5 ml of virus diluted in PBBS with 2% FBS. The development and progression of leukemia was monitored by measuring splenomegaly by palpation of the mice under general anesthesia. Mice with obvious palpable splenomegaly had spleens greater than or equal to 0.5 g and were considered leukemic. This limit is consistent with our previous experience in monitoring many mice with periodic cross-checking of spleen size determined by palpation versus actual spleen weight. Previous experiments have shown that repeated palpations of individual mice over the course of the recovery period provide an accurate predictor of recovery as determined by a greater than 1-year survival (7, 11).

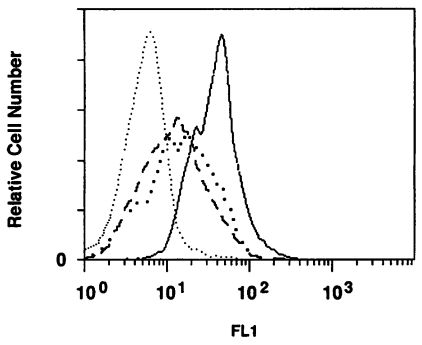

FIG. 1. Flow cytometric analysis of *H-2D*^{b} expression on nucleated spleen cells. Single-cell suspensions were stained with FITC-labeled anti-*H-2D*^{b} (MAb 95) and analyzed as described in Materials and Methods. Symbols for cell donors: —, *H-2*$^{b/b}$ β_2-m$^{+/+}$ mice; — — —, *H-2*$^{b/b}$ β_2-m$^{+/-}$ mice; · · ·, *H-2*$^{a/b}$ β_2-m$^{+/+}$ mice; ·····, *H-2*$^{b/b}$ β_2-m$^{-/-}$ mice.

RESULTS

***H-2D*^{b} expression in various hematopoietic lineages.** We compared the levels of *H-2D*^{b} surface expression on cells from mice that had one β_2-m gene disruption (β_2-m$^{+/-}$) with the levels of expression found on cells from normal littermate controls and *H-2* heterozygous (low-recovery) mice. Results from flow cytometric analyses using FITC-labeled MAbs specific for the *H-2D*^{b} molecule showed that nucleated spleen cells from (A.BY × B6 β_2-m$^{+/-}$)F$_1$ mice carrying a single β_2-m gene disruption expressed slightly lower levels of detectable surface *H-2D*^{b} than spleen cells from heterozygous *H-2D*$^{a/b}$ mice (Fig. 1). F$_1$ littermates that did not inherit the β_2-m mutation (β_2-m$^{+/+}$) expressed significantly higher levels of *H-2D*^{b} on spleen cells than either the *H-2* heterozygotes or the β_2-m$^{+/-}$ mice. We could detect no *H-2D*^{b} surface expression in F$_2$ progeny test animals that were homozygous for the β_2-m gene disruption (β_2-m$^{-/-}$; Fig. 1).

We further examined levels of *H-2D*^{b} surface expression on specific hematopoietic cell populations by using two-color flow cytometry. *H-2D*^{b} expression on CD4$^+$ T cells, CD8$^+$ T cells, B cells, macrophages, and granulocytes from the β_2-m$^{+/-}$ mice was significantly lower than in littermates that did not carry the gene disruption (Fig. 2). Different cell types expressed different quantities of surface *H-2D*^{b}. For instance, T-cell expression was much lower than B-cell expression. The expression levels in each cell type, however, reflected what was seen in the same cell types from *H-2D*$^{a/b}$ heterozygous mice. These results indicated that we had achieved the desired result of a widespread decrease in *H-2D*^{b} surface expression comparable to that seen in MHC heterozygous mice.

Recovery from FV-induced splenomegaly in β_2-m$^{+/-}$ mice. Three separate FV recovery experiments were done in which the dose and strain of FV were varied. In all three experiments, the (A.BY × B6 β_2-m$^{+/-}$)F$_1$ mice were typed for the presence or absence of the β_2-m gene disruption by flow cytometric analyses of *H-2D*^{b} surface expression on nucleated peripheral blood cells. The accuracy of this typing method was verified by progeny testing several F$_1$ offspring. F$_2$ progeny from two mice typed as β_2-m$^{+/-}$ always contained offspring with no detectable *H-2D*^{b} expression (β_2-m$^{-/-}$) such as the one seen in Fig. 1. Conversely, no β_2-m$^{-/-}$ offspring were detected in F$_2$ litters in which only one parent typed positive for the β_2-m mutation.

Disease incidence and progression in each of these experiments were monitored by spleen palpation of anesthetized

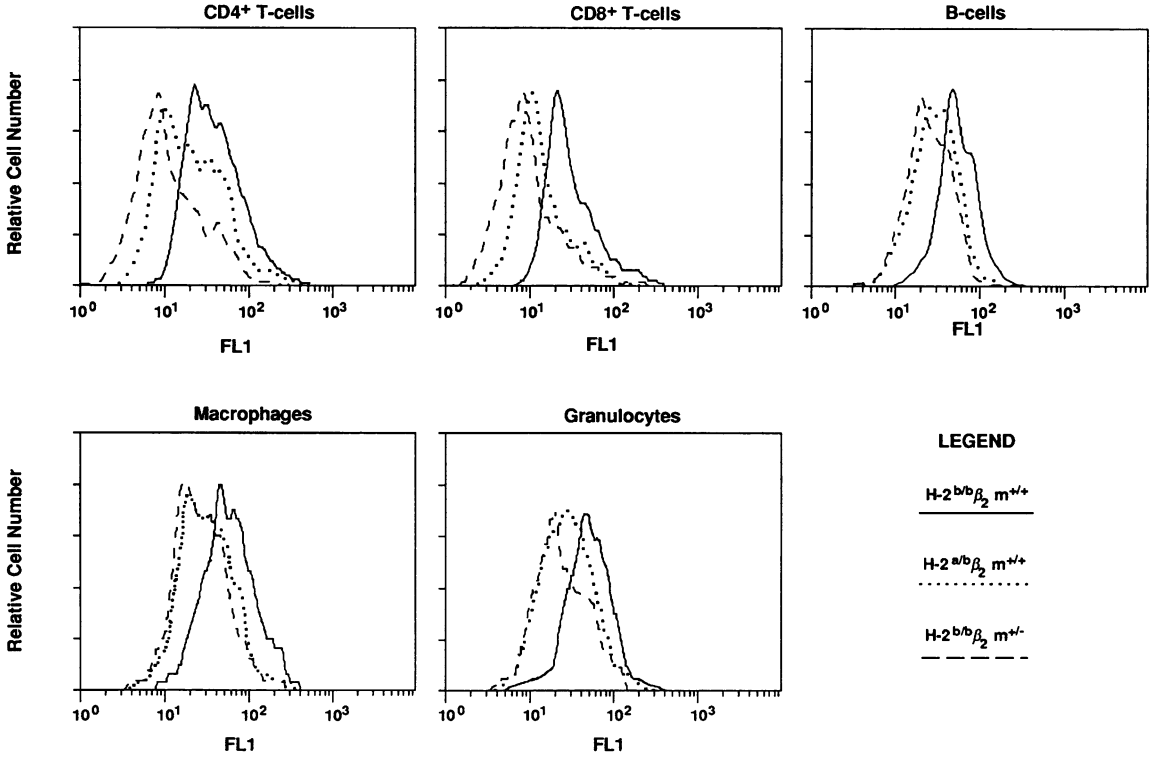

FIG. 2. Flow cytometric analysis of *H-2D*^{b} expression on lineage-specific spleen cells. Single-cell suspensions were gated for expression of the lineage-specific markers indicated in parentheses by staining with phycoerythrin-labeled antibodies as described in Materials and Methods. Cells analyzed were CD4 T cells (CD4); CD8 T cells (CD8); B cells (B220); macrophages (MAC-1); and granulocytes (Gr-1). Each gated population was then analyzed for *H-2D*^{b} expression (FL1).

mice. Figure 3 illustrates the kinetics of recovery in the mouse strains used in experiment 1. It should be noted that all of the strains were susceptible to acute FV infection, and at 21 days postinfection, more than 90% of the mice were still significantly splenomegalic. The eventual outcome of disease, therefore, was based on immune responses rather than initial resistance to infection. During the next 2 months, most of the *H-2*$^{b/b}$ homozygotes displayed a reduction in splenomegaly and eventually recovered while most of the *H-2*$^{a/b}$ and *H-2*$^{bm14/b}$ heterozygotes did not. Reductions in surface expression of *H-2D*^{b} in β_2-m$^{+/-}$ mice did not affect the kinetics or the efficacy of the immune responses during the recovery process, and these mice recovered at significantly higher rates than *H-2D* heterozygous mice.

A summary of three recovery experiments comparing β_2-m$^{+/+}$, and *H-2*$^{a/b}$ heterozygous mice is shown in Table 1. In each case, there was no difference in recovery rates between the two groups segregated for the β_2-m mutation, but both groups recovered at significantly higher rates than *H-2*$^{a/b}$ heterozygotes. In experiments 1 and 2, the mice were infected with two different doses of the B-tropic strain of FV. While recovery rates varied in a dose-dependent manner, it was evident that the decrease in surface *H-2D*^{b} expression associated with the β_2-m mutation did not affect the outcome of disease. In experiment 3, the mice were infected with a high dose of an NB-tropic strain of FV. This is a highly virulent strain of FV in our hands, and the recovery rates were correspondingly low. However, there was still no significant difference between mice carrying only one functional β_2-m gene (30% recovery) and normal littermates (31% recovery).

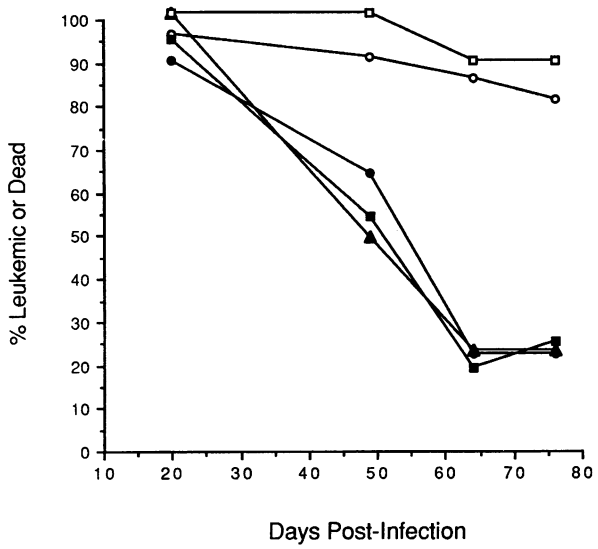

FIG. 3. Recovery from Friend disease. Mice were infected intravenously with 1,500 spleen focus-forming units of FV and were palpated for splenomegaly at the time points illustrated. The numbers of animals in this experiment were as follows: (B6 × A)F$_1$ (□), 9; (bm14 × A.BY)F$_1$ (○), 28; (B6 β_2-m × A.BY)$^{+/-}$F$_1$ (■), 27; (B6 β_2-m × A.BY)$^{+/+}$F$_1$ (●), 19; and (B6 × A.BY)F$_1$ (▲), 28. Differences between recovery rates in the low-recovery strains (open symbols) were not significant. Differences between the low-recovery strains and the high-recovery strains (closed symbols) were highly significant ($P < 0.001$ by Fisher's exact test).

TABLE 1. Recovery from Friend disease in β_2-m$^{+/-}$ mice

Expt no.	*H-2* type	β_2-m type	FV strain	FV dose (SFFU[a])	No. recovered total (%)[b]
1	*b/b*	+/+	B	1,500	15/19 (79)
	b/b	+/−	B	1,500	21/27 (78)
	a/b	+/+	B	1,500	1/9 (11)
2	*b/b*	+/+	B	500	10/11 (91)
	b/b	+/−	B	500	10/11 (91)
	a/b	+/+	B	500	1/6 (17)
3	*b/b*	+/+	NB	3,000	8/26 (31)
	b/b	+/−	NB	3,000	8/27 (30)
	a/b	+/+	NB	3,000	2/17 (12)

[a] SFFU, spleen focus-forming units.
[b] End point of recovery at 3 months postinfection.

***H-2D^b* expression in FV-infected cells.** Previous experiments indicated that cytotoxic T lymphocytes (CTL) recognized *H-2D$^{a/b}$* heterozygous target cells as well as homozygous cells (8). However, because *H-2D* expression on infected cells might influence their susceptibility to elimination by CTL, we wished to directly compare the levels of *H-2D^b* expression on FV-infected cells from *H-2* heterozygotes versus β_2-m$^{+/-}$ mice. Flow cytometric analyses of infected cells from these two types of animals revealed no differences in levels *H-2D^b* surface expression (Fig. 4). Interestingly, there was a small subpopulation of infected heterozygous cells that displayed very low levels of *H-2D^b* expression which could have allowed escape from CTL. However, forward-scatter analyses of this subpopulation revealed that the low-expression cells were of a uniformly small size characteristic of more highly differentiated erythroid cells. The same subpopulation was also seen in the high-recovery β_2-m$^{+/-}$ mice and even in uninfected controls and was therefore probably a differentiation state rather than a subpopulation of escape variants (data not shown).

***D^b* expression on thymic stromal cells.** Although previous adoptive transfer of T-cell experiments indicated that the MHC type of the T cells was important in recovery (5), there is no known mechanism by which the MHC molecules of the T cells themselves are directly involved in immune effector functions. Furthermore, the present data from the β_2-m$^{+/-}$ mice demonstrate that the expression of low levels of surface *H-2D^b* molecules on T cells is sufficient for high recovery (Fig. 2). It is likely that the MHC type of the animals in which the adoptively transferred T cells developed provided the critical elements for recovery in those experiments. At least two groups have demonstrated that the T-cell repertoire can be quantitatively affected by levels of MHC class I expression in the thymus (2, 22). For example, mice heterozygous for *H-2D^b* expression positively select only half as many *H-2D^b*-restricted T cells as *H-2D^b* homozygous mice (22). Therefore, we felt it was important to examine *H-2D^b* expression levels on thymus cells.

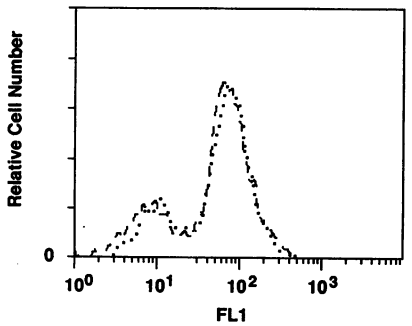

FIG. 4. Comparative analysis of *H-2D^b* expression on FV-infected cells by flow cytometry. Cells were obtained from spleens of mice infected 10 days earlier with a high dose of FV. The cells were gated for expression of viral envelope glycoproteins by staining with anti-gp70 MAb 720-phycoerythrin and analyzed for *H-2D^b* expression with MAb 95-FITC (FL1). Symbols for cell donors: — — —, *H-2$^{b/b}$* β_2-m$^{+/-}$ mice; · · ·, *H-2$^{a/b}$* β_2-m$^{+/+}$ mice.

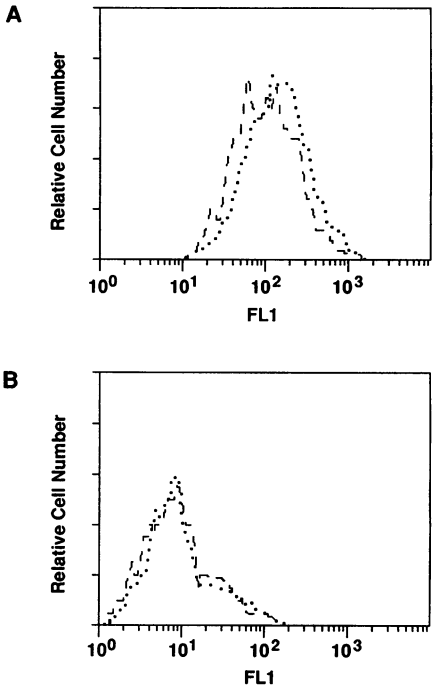

FIG. 5. Flow cytometric analysis of *H-2D^b* expression on thymic stromal cells. Thymic stromal cells were prepared and stained as described in Materials and Methods. The cells were gated for positive MHC class II expression and also for positive (A) or negative (B) expression of CD8. The gated populations were analyzed for *H-2D^b* expression with MAb 95-FITC (FL1). Symbols for cell donors: — — —, *H-2D$^{b/b}$* β_2-m$^{+/-}$ mice; · · ·, *H-2$^{a/b}$* β_2-m$^{+/+}$ mice.

The exact nature of the thymic cells involved in T-cell selection has not yet been determined, but evidence indicates the involvement of MHC class II-positive thymic stromal cells of two types: CD8$^+$ dendritic cells and CD8$^-$ epithelial cells (1, 16, 21, 38, 39). We found no significant difference in *H-2D^b* expression in comparisons of either cell type from H-2$^{a/b}$ heterozygous mice versus β_2-m$^{+/-}$ mice (Fig. 5), suggesting that a simple decrease in *H-2D^b* surface expression on thymic stromal cells could not account for the recovery differences between *H-2D^b* homozygotes and *H-2D^b* heterozygotes.

DISCUSSION

The recessive nature of the *H-2D^b* effect on recovery from Friend disease has been an intriguing phenomenon. MHC class I molecules are co-dominantly expressed and typically function as molecules which present viral peptides to CTL. Although the coexpression of two different MHC class I alleles results in the diminished expression of both, the vast majority of cells express far more class I molecules than are needed for CTL recognition (12), and heterozygosity is generally considered to be desirable in terms of the ability to present peptides

from the greatest number of different pathogens to the immune system (26). While at the population level heterozygosity is considered advantageous, for individuals infected with high doses of FV, it is generally fatal.

One possible explanation for the low recovery of *H-2D^b* heterozygotes is that heterozygous cells infected with FV are not good targets for MHC class I-restricted CTL. However, previous experiments demonstrated that heterozygous infected cells are well recognized by CTL in vitro (8). In addition, *H-2D^b* heterozygotes recover at high rates if infected with low rather than high doses of FV (8). Another explanation is that low recovery in heterozygotes is due to generalized FV-induced immunosuppression. This does not seem to be the case either, because we have found such immunosuppression only in animals homozygous for low-recovery *H-2D* alleles (30). We believe that the real defect in *H-2D^b* heterozygotes lies in the FV-specific T-cell repertoire. The best evidence supporting this theory is that T cells transferred from *H-2D^b* homozygous animals into heterozygous animals confer protection (4). One immune parameter controlled by *H-2D* that may be related to the T-cell repertoire is the kinetics of T-cell responses to FV. We previously found the kinetics of FV-specific T-cell responses to be significantly delayed in *H-2D^b* heterozygotes compared with homozygotes (6). The data are consistent with a reduction in FV-specific T cells from the repertoire during ontogeny, produced either through negative selection by an MHC molecule from a low-recovery haplotype or through a quantitative reduction in positively selected T cells due to the reduced levels of *H-2D^b* expression associated with heterozygosity. The data presented here indicate that the latter possibility is unlikely since we found high recovery rates in animals with only heterozygous levels of *H-2D^b* expression. Furthermore, we specifically examined the *H-2D^b* expression levels on the thymic epithelial cells thought to be involved in positive selection (21, 39) and found no unusual increases in expression that could account for the high recovery rates of β_2-m$^{+/-}$ mice.

Our finding that homozygous levels of *H-2D^b* expression are not required for high recovery suggests that an *H-2D* region gene (or genes) from the non-*b* haplotypes exerts negative influences on recovery in heterozygotes, probably through the negative selection of T cells required for an effective anti-FV immune response. In earlier experiments, we tested for negative effects on recovery by using *H-2D^b* homozygous mice expressing the *H-2D^d* transgene from a low-recovery strain but found that the transgene did not affect recovery (28). Using a different mouse strain, however, Polsky and Lilly found that *H-2D^d* transgene expression had a weak effect on the susceptibility of *H-2D^b* homozygotes to FV-induced splenomegaly, although they did not measure recovery (33). It is possible that stronger negative effects could be provided by a gene other than *H-2D^d*, such as *H-2L^d*. In fact, *H-2L^d* rather than *H-2D^d* is now considered to be the true allele to *H-2D^b* (40). Alternatively, negative effects might require the expression of both *H-2D^d* and *H-2L^d*. Also, our experiments do not exclude the possibility that negative effects might occur only in the context of reduced *H-2D^b* expression. It would be interesting to test the effects of the *H-2D^d* transgene in β_2-m$^{+/-}$ mice, but further breeding will be required to produce such a mouse strain.

It should be noted that we were observing only native cell surface *H-2D^b* molecules in the flow cytometry analyses. It has been reported that some *H-2D^b* molecules can be expressed on certain cell lines in the absence of β_2-m expression (3, 34), but the function of β_2-m-negative *H-2D^b* molecules is in considerable doubt. One of the functions of MHC class I molecules is the positive selection of CD8$^+$ T cells in the thymus. Using flow cytometry, we (data not shown) and others have not been able to detect CD8$^+$ T cells in β_2-m$^{-/-}$ mice (23, 41). At least by this criterion of function, then, the β_2-m-negative *H-2D^b* molecules in such mice are nonfunctional.

We have not completely ruled out the possibility that some obscure cell type in β_2-m$^{+/-}$ mice expresses high levels of *H-2D^b* expression that could alter recovery from FV infections. However, we have examined the cell types considered to be the most important in FV recovery as well as in T-cell development and found none with *H-2D^b* expression levels higher than those expressed in *H-2D$^{a/b}$* low-recovery mice. Therefore, reduced *H-2D^b* expression in the β_2-m$^{+/-}$ mice appears to be a general phenomenon, and given the low affinity of the *H-2D^b* molecule for β_2-m (20), it would be surprising to find high expression in the context of limited β_2-m expression. While we have not yet discovered the mechanism for the *H-2D^b* homozygosity requirement in recovery from Friend disease, the current data from β_2-m gene knockout mice indicate that it is not dependent on levels of *H-2D^b* surface expression.

REFERENCES

1. **Anderson, G., E. J. Jenkinson, N. C. Moore, and J. T. Owen.** 1993. MHC class II-positive epithelium and mesenchyme cells are both required for T-cell development in the thymus. Nature (London) **362:**70–73.
2. **Berg, L. J., G. D. Frank, and M. M. Davis.** 1990. The effects of MHC gene dosage and allelic variation on T cell receptor selection. Cell **60:**1043–1053.
3. **Bix, M., and D. Raulet.** 1992. Functionally conformed free class I heavy chains exist on the surface of beta 2 microglobulin negative cells. J. Exp. Med. **176:**829–834.
4. **Britt, W. J., and B. Chesebro.** 1980. H-2D (Rfv-1) gene influence on recovery from Friend virus leukemia is mediated by nonleukemic cells of the spleen and bone marrow. J. Exp. Med. **152:**1795–1804.
5. **Britt, W. J., and B. Chesebro.** 1980. Recovery from Friend virus leukemia is determined by the H-2 genotype of nonleukemic cells of the spleen and bone marrow, p. 329–335. *In* E. Skamene, P. A. L. Kongshavn, and M. Landy (ed.), Genetic control of natural resistance to infection and malignancy. Academic Press, New York.
6. **Britt, W. J., and B. Chesebro.** 1983. H-2D control of recovery from Friend virus leukemia: H-2D region influences the kinetics of the T lymphocyte response to Friend virus. J. Exp. Med. **157:**1736–1745.
7. **Chesebro, B., M. Bloom, K. Wehrly, and J. Nishio.** 1979. Persistence of infectious Friend virus in spleens of mice after spontaneous recovery from virus-induced erythroleukemia. J. Virol. **32:** 832–837.
8. **Chesebro, B., M. Miyazawa, and W. J. Britt.** 1990. Host genetic control of spontaneous and induced immunity to Friend murine retrovirus infection. Annu. Rev. Immunol. **8:**477–499.
9. **Chesebro, B., and K. Wehrly.** 1976. Studies on the role of the host immune response in recovery from Friend virus leukemia. II. Cell-mediated immunity. J. Exp. Med. **143:**85–99.
10. **Chesebro, B., and K. Wehrly.** 1978. Rfv-1 and Rfv-2, two H-2-associated genes that influence recovery from Friend leukemia virus-induced splenomegaly. J. Immunol. **120:**1081–1085.
11. **Chesebro, B., K. Wehrly, and J. Stimpfling.** 1974. Host genetic control of recovery from Friend leukemia virus-induced splenomegaly. Mapping of a gene within the major histocompatibility complex. J. Exp. Med. **140:**1457–1467.
12. **Christinck, E. R., M. A. Luscher, B. H. Barber, and D. B. Williams.** 1991. Peptide binding to class I MHC on living cells and quantitation of complexes required for CTL lysis. Nature (London) **352:**67–70.
13. **Coffman, R. L., and I. L. Weissman.** 1981. B220: a B cell-specific member of the T200 glycoprotein family. Nature (London) **289:** 681–683.
14. **Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M. Pierres, and F. W. Fitch.** 1983. Characterization

of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK 1.5: similarity of L3T4 to the human Leu-3/T4 molecule. J. Immunol. **131:**2445–2451.

15. **Doig, D., and B. Chesebro.** 1979. Anti-Friend virus antibody is associated with recovery from viremia and loss of viral leukemia cell surface antigens in leukemic mice. Identification of Rfv-3 as a gene locus influencing antibody production. J. Exp. Med. **150:**10–19.
16. **Fairchild, P. J., and J. M. Austyn.** 1993. Thymic dendritic cells: phenotype and function. Int. Rev. Immunol. **6:**187–196.
17. **Hasenkrug, K. J., J. M. Cory, and J. H. Stimpfling.** 1987. Monoclonal antibodies defining mouse tissue antigens encoded by the *H-2* region. Immunogenetics **25:**136–139.
18. **Hestdal, K., F. W. Ruscetti, J. N. Ihle, S. E. Jacobsen, C. M. Dubois, W. C. Kopp, D. L. Longo, and J. R. Keller.** 1991. Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. J. Immunol. **147:**22–28.
19. **Hoatlin, M. E., S. L. Kozak, F. Lilly, A. Chakraborti, C. A. Kozak, and D. Kabat.** 1990. Activation of erythropoietin receptors by Friend viral gp55 and by erythropoietin and down-modulation by the murine Fv-2^r resistance gene. Proc. Natl. Acad. Sci. USA **87:**9985–9989.
20. **Hochman, J. H., Y. Shimizu, R. DeMars, and M. Edidin.** 1988. Specific associations of fluorescent β-2-microglobulin with cell surfaces. J. Immunol. **140:**2322–2329.
21. **Hugo, P., J. W. Kappler, D. I. Godfrey, and P. C. Marrack.** 1992. A cell line that can induce thymocyte positive selection. Nature (London) **360:**679–682.
22. **Kisielow, P., H. S. Teh, H. Blüthmann, and H. von Boehmer.** 1988. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. Nature (London) **335:**730–733.
23. **Koller, B. H., P. Marrack, J. W. Kappler, and O. Smithies.** 1990. Normal development of mice deficient in β_2M, MHC class I proteins, and $CD8^+$ T cells. Science **248:**1227–1230.
24. **Lavigueur, A., and A. Bernstein.** 1991. p53 transgenic mice: accelerated erythroleukemia induction by Friend virus. Oncogene **6:**2197–2201.
25. **Lawlor, D. A., J. Zemmour, P. D. Ennis, and P. Parham.** 1990. Evolution of class-I MHC genes and proteins: from natural selection to thymic selection. Annu. Rev. Immunol. **8:**23–63.
26. **Ledbetter, J. A., and L. A. Herzenberg.** 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. **47:**63–90.
27. **Li, J.-P., A. D. D'Andrea, H. F. Lodish, and D. Baltimore.** 1990. Activation of cell growth by binding of Friend spleen focus-forming virus gp55 glycoprotein to the erythropoietin receptor. Nature (London) **343:**762–764.
28. **Miyazawa, M., J. Nishio, K. Wehrly, G. Jay, R. W. Melvoid, and B. Chesebro.** 1992. Detailed mapping of the *Rfv-1* gene that influences spontaneous recovery from Friend retrovirus-induced leukaemia. Eur. J. Immunogenet. **19:**159–164.
29. **Moreau-Gachelin, F., A. Tavitian, and P. Tambourin.** 1988. Spi-1 is a putative oncogene in virally induced murine erythroleukemia. Nature (London) **331:**277–280.
30. **Morrison, R. P., P. L. Earl, J. Nishio, D. L. Lodmell, B. Moss, and B. Chesebro.** 1987. Different H-2 subregions influence immunization against retrovirus and immunosuppression. Nature (London) **329:**729–732.
31. **Paul, R., S. Schuetze, S. L. Kozak, C. A. Kozak, and D. Kabat.** 1991. The *Sfpi-1* proviral integration site of Friend erythroleukemia encodes the *ets*-related transcription factor Pu.1. J. Virol. **65:**464–467.
32. **Ploegh, H. L., L. E. Cannon, and J. L. Strominger.** 1979. Cell-free translation of the mRNAs for the heavy and light chains of HLA-A and HLA-B antigens. Proc. Natl. Acad. Sci. USA **76:**2273–2277.
33. **Polsky, D., and F. Lilly.** 1991. Suppression of *H-2D*^{b}-associated resistance to Friend erythroleukemia virus by a class I gene from the *H-2*^{d} major histocompatibility complex haplotype. Proc. Natl. Acad. Sci. USA **88:**9243–9247.
34. **Potter, T. A., C. Boyer, A. S. Verhulst, P. Golstein, and T. V. Rajan.** 1984. Expression of H-2D^b on the cell surface in the absence of detectable β2 microglobulin. J. Exp. Med. **160:**317–322.
35. **Robertson, M. N., G. J. Spangrude, K. Hasenkrug, L. Perry, J. Nishio, K. Wehrly, and B. Chesebro.** 1992. Role and specificity of T-cell subsets in spontaneous recovery from Friend virus-induced leukemia in mice. J. Virol. **66:**3271–3277.
36. **Springer, T., G. Galfré, D. S. Sechler, and C. Milstein.** 1979. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. Eur. J. Immunol. **9:**301–306.
37. **Troxler, D. H., S. K. Ruscetti, and E. M. Scolnick.** 1980. The molecular biology of Friend virus. Biochim. Biophys. Acta **605:**305–324.
38. **Vremec, D., M. Zorbas, R. Scollay, D. J. Saunders, C. F. Ardavin, L. Wu, and K. Shortman.** 1992. The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. J. Exp. Med. **176:**47–58.
39. **Vukmanovic, S., A. G. Grandea III, S. J. Faas, B. B. Knowles, and M. J. Bevan.** 1992. Positive selection of T-lymphocytes induced by intrathymic injection of a thymic epithelial cell line. Nature (London) **359:**729–732.
40. **Watts, S. J., J. M. Vogel, W. D. Harriman, T. Itoh, H. J. Stauss, and R. S. Goodenow.** 1987. DNA sequence analysis of the C3H *H-2K*^{k} and *H-2D*^{k} loci: evolutionary relationship to H-2 genes from four other mouse strains. J. Immunol. **139:**3878–3885.
41. **Zijlstra, M., M. Bix, N. E. Simister, J. M. Loring, D. H. Raulet, and R. Jaenisch.** 1990. β2-Microglobulin deficient mice lack $CD4^-8^+$ cytolytic T cells. Nature (London) **344:**742–746.