Requirement for CD8-Major Histocompatibility Complex Class I Interaction in Positive and Negative Selection of Developing T Cells

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Summary

The interaction of the T cell surface glycoprotein CD8 with major histocompatibility complex (MHC) class I molecules on target cells is required for effective T cell activation. Mutations in the $\alpha 3$ domain of the MHC class I molecule can disrupt binding to CD8, yet leave antigen presentation unaffected. Here we show that such a mutation can interfere with positive and negative selection of T cells bearing T cell receptors (TCRs) that interact specifically with the mutant class I molecule. Autoreactive T cells in male mice expressing a transgenic TCR specific for the male antigen H-Y and H-2D^b were not deleted in the context of a transgenic D^b molecule bearing a mutation at residue 227. Similarly, CD8$^+$ cells were not positively selected in female mice expressing both the TCR and mutant class I transgenes. Endogenous MHC class I molecules were competent to bind CD8, but were unable to rescue the defect, indicating a requirement for coordinate recognition of antigen/MHC by a complex of the TCR and CD8 coreceptor for both positive and negative selection of thymocytes.

The repertoire of T lymphocytes is determined by interactions of immature thymocytes with MHC molecules in the thymus. Maturation involves positive selection of cells with $\alpha\beta$ TCRs specific for host MHC molecules and elimination of cells that react with self-antigen complexed to the same MHC molecules (1). The mechanisms permitting thymocytes to discriminate between self and non-self, and to transduce signals tailored to the nature of the antigen, are not understood. The signals clearly involve the TCR complex, which consists of the clonotypic α and β chains and several associated polypeptides (2). In addition, other thymocyte surface molecules, most notably the CD4 and CD8 glycoproteins, have been implicated as having important roles in the signal transduction (3, 4). CD4 and CD8 are coexpressed on most immature thymocytes, but mature T cells express one to the exclusion of the other. The expression of CD4 and CD8 correlates with the function and the MHC specificity of T cells. Cells with TCRs specific for antigen bound to class II MHC molecules are primarily helper cells and express CD4, while cells with receptors specific for antigen and class I MHC molecules have cytotoxic function and express CD8 (5, 6).

The extracellular domains of CD4 and CD8 bind to nonpolymorphic domains of class II and class I MHC molecules, respectively (7-9), while their cytoplasmic domains bind to a cytoplasmic tyrosine kinase, p56^{lck} (10-12). Effective activation of T cells requires that CD4 and CD8 bind both the MHC on the antigen-presenting cells and the tyrosine kinase on the cytoplasmic side of the plasma membrane (13, 14). Several studies have suggested that T cell activation results from simultaneous engagement of the same MHC molecule by the TCR and CD4/CD8, with consequent bridging of p56^{lck} and TCR-associated polypeptides (15, 16). It has thus been proposed that CD4 and CD8 function as coreceptors, contributing to signal transduction through the TCR (17).

Several studies have provided evidence that the CD4 and CD8 molecules have important functions during thymocyte development. Injection of anti-CD4 or -CD8 mAbs block the development of class II or class I-specific T cells, suggesting that these molecules have an important role in positive selection (18, 19). This conclusion is supported by the finding that mice bearing null mutations in the CD8α or CD4 gene have significant impairment in the development of T cells with the appropriate MHC restriction and with cytotoxic or helper function, respectively (20, 21). Treatment with anti-CD4 antibody also prevents the deletion of double positive T cells bearing self-superantigen–reactive TCRs, allowing the development of CD8$^+$ thymocytes that otherwise would not be observed (22, 23). CD4 has therefore also been implicated as having a signalling function in clonal deletion.

In earlier studies, we showed that the CD8α molecule binds

to the α3 domain of class I MHC molecules (9, 15). Mutations in the α3 domain that prevented binding of CD8 abrogated the ability of $CD8^+$ cytotoxic T cells to lyse target cells. A naturally occurring human class I MHC molecule, HLA-Aw68, was found to have a rare polymorphism in the α3 domain that resulted in decreased binding of CD8 (9). T cells restricted to interact with Aw68 were difficult to detect in some studies, raising the possibility that lack of interaction of CD8 and this class I molecule results in inefficient thymic positive selection of T cells having receptors specific for Aw68 (24). To determine whether the interaction of CD8 and class I MHC molecules is required, not only in activation of mature T cells, but also in developmental decisions during thymocyte maturation, we prepared transgenic mice expressing either wild-type class I molecules or mutant class I molecules deficient in CD8 binding. Analysis of T cell development in these animals indicates that the CD8–class I MHC interaction is essential both for positive selection and clonal deletion, and supports a model whereby CD8 serves as a coreceptor in these developmental processes.

Materials and Methods

Transgenic Mice. C57BL/6, C57BL/6×DBA/2 F_1 and DBA/2 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in micro-isolator cages in the University of California, San Francisco transgenic mouse facility. The H-Y-specific TCR transgenic mouse (25) was from the breeding colony at the University of British Columbia. D^b transgenic mice were created and analyzed according to standard procedures (26). The 10-kb genomic fragment including the D^b gene (27) was obtained from Dr. Robert Goodenow, University of California, Berkeley, and a 4.5-Kb XhoI/HindIII subclone containing the α3 domain was mutagenized (28) using the following oligo: 5'-acaagctccatcttctgggtca-3', which substitutes lysine for aspartic acid at residue 227. Purified 10-kb fragments containing either wild-type or mutant D^b genes were microinjected into C57BL/6 × DBA/2 F_2 zygotes. Founder mice were identified by Southern blot analysis of DNA from tail biopsies. F_1 mice, produced by back-crossing founders to DBA/2 animals, were mated to the H-Y/D^b-specific TCR transgenic mice which had also been similarly back-crossed. Double transgenic mice were identified by a combination of Southern blots on tail DNA and FACS® analysis of PBNC using antibodies against the TCR-β chain, D^b, and K^b (for the MHC background).

Antibodies and Flow Cytometry. H141-31 anti-D^b was purchased from Bioproducts for Science, Inc. (Indianapolis, IN). AF6-88-5.3 anti-K^b, and SF1-1.1.1 anti-K^d tissue culture supernatants were obtained from American Type Culture Collection (Rockville, MD) cell lines. Biotinylated antibodies against the transgenic TCR-α- (T3.70) and β (F23.1) chains have been previously described (29–31). PE-conjugated GK1.5 (PE-anti-L3T4), fluorescein-conjugated 53-6.7 (FITC anti-Lyt-2) and fluorescein goat anti–mouse IgG (FITC-GAMIg) were obtained from Becton Dickinson & Co. (Mountain View, CA). Fluorescein-conjugated goat antiserum specific for the Fc of mouse IgG was purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). A streptavidin tandem conjugate of PE and Texas Red was obtained from Southern Biotechnology (Birmingham, AL) or Gibco BRL (Grand Island, NY). 1–2 × 10^6 cells were analyzed on a FACScan® flow cytometer (Becton Dickinson & Co.) using FACScan® or LYSYS II® software (Becton Dickinson & Co.).

Cytotoxic T Cell Assays. Secondary CTLs were generated by culturing dendritic cells from male or female C57BL/6 mice with lymph node cells from female C57BL/6 mice that had been primed with a single intraperitoneal injection of 2 × 10^7 male C57BL/6 spleen cells for more than 14 d. Primed cells (5 × 10^6) were transferred to 24-well plates containing dendritic cells (5 × 10^5), in volumes of 2 ml of IMDM supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), and 5 × 10^{-5} M β-ME. After 5 d of culture, six wells from each group were pooled and analyzed for cytolytic activity against the indicated target cells in a 3-h ^{51}Cr-release assay. Target cells were prepared by culturing spleen cells in IMDM supplemented with Con A (2 μg/ml).

For preparation of dendritic cells from two spleens, tissue was minced with scissors in 4 ml of PBS and pushed through a steel screen mesh. The screen was rinsed twice in 4 ml of PBS and the debris retained by the screen was saved. Cells passing through the screen were further disaggregated by pipetting and pooled into a 12-ml vol. Spleen cells were centrifuged, the supernatant discarded, and the pellet resuspended by gentle mechanical agitation. Red blood cells were lysed by addition of 0.5 ml of sterile water at 4°C for 3 s followed by 12 ml of PBS. Large aggregates of cell debris were removed by sedimentation and the cell suspension centrifuged. The pellet was resuspended in IMDM, combined with debris retained by the screen described above, and plated in a single 100 × 15 mm petri plate (Cat. no. 8-757-12; Fisher Scientific Co., Pittsburgh, PA) in a total volume of 5 ml. Cells were incubated for 1–2 h at 37°C. The medium and large debris fragments were gently decanted from the plates or withdrawn by pipette with minimal disturbance of the adherent cell lawn. 2 ml of IMDM at room temperature were added, and the plate was agitated vigorously by hand in a generally horizontal plane for 10–20 s. Care was taken to expose the perimeter of the plate to sufficient turbulence to remove loosely adherent B cells that had accumulated. This washing process was repeated three more times with a final rinse of 5 ml. Adherent cells were then cultured overnight at 37°C to permit detachment of dendritic cells which were harvested by pipetting. This procedure typically yielded 4–6 × 10^5 cells per spleen equivalent, of which 60–80% were dendritic cells as defined by expression of the 33D1 surface marker (32). The vast majority of contaminating cells were Ig^+ B cells as determined by surface staining with anti–mouse Ig FITC and FACS® analysis.

Results

Experimental Strategy. Mice expressing transgenic TCR genes provide a facile means of observing the processes of positive and negative selection. One of the best characterized systems uses a transgenic TCR specific for the male antigen, H-Y, and the class I molecule H-2D^b (33). In female mice that express the D^b allele, thymocytes expressing the transgenic α and β chains are positively selected to become $CD8^+$ cells (29). In male animals that express D^b, there is massive clonal deletion that prevents the accumulation of double positive cells and results in thymuses containing fewer than 10% of the normal number of thymocytes (25).

All T cells in these mice express the transgenic TCR-β chain and lack endogenous β chains, since the functionally rearranged transgenic TCR-β gene prevents expression of endogenous β genes (34). In contrast, expression of the TCR-α chain does not prevent the expression of endogenous α genes, and the pairing of endogenous α chains with transgenic β chain can generate novel specificities. The transgenic α and

β chains are detected by the T3.70 and F23.1 mAbs, respectively (29, 31, 35). In mice that do not express the D^b allele, development of thymocytes bearing the transgenic TCR-α and β chains is arrested at the double positive stage, and progression to single positive mature cells requires surface expression of endogenous TCR-α chain. Hence, mature cells in these mice have preferential pairing of transgenic β chain with endogenous α chain and, consequently, little surface expression of the transgenic TCR-α chain.

To determine whether mutant class I molecules that do not bind CD8 can serve as substrates for positive and negative selection in this system, we prepared transgenic mice expressing wild-type or mutant D^b in a nonselecting H-2^d background. These mice were mated with the TCR transgenic animals, and male and female offspring were analyzed for expression of the transgenes and for the phenotype of the developing thymocytes.

Generation of Transgenic Mice. A mutant form of the H-2D^b gene was produced by site-directed mutagenesis at codon 227, substituting lysine for aspartic acid. Previous studies have shown that this mutation results in loss of CD8 binding and of the ability of this class I molecule to serve as a target for primary cytotoxic T cells (15, 36). Transgenic wild-type and mutant D^b were expressed at approximately the same relative level on both thymocytes and peripheral lymphocytes, as judged by flow cytometry (Fig. 1). The level of expression on thymocytes was slightly higher than that of endogenous D^b in C57BL/6 mice, but was roughly equivalent to that of endogenous D^d in DBA/2 mice (data not shown). Expression on peripheral lymphocytes was equivalent for the transgenes and the endogenous class I MHC molecules (Fig. 1, *bottom*).

Lack of Efficient Negative Selection in D^b-227/TCR Transgenic Mice. CD4$^+$CD8$^+$ thymocytes are deleted in male H-2^b mice that express the H-Y-specific transgenic TCR

(25). This situation is recreated in H-2^d mice transgenic for both the TCR and the wild-type D^b molecule (D^b-wt/TCR mice) (Fig. 2). The thymuses of these mice were diminished in size, lacked the usually predominant CD4$^+$CD8$^+$ population, and contained mostly CD4$^-$CD8$^-$ immature cells. Deletion was dependent on the transgenic TCR, as it was not observed in transgenic mice only expressing the D^b-wt gene (data not shown). In contrast, a relatively normal pattern of thymocyte development was observed in H-2^d male mice expressing the transgenic TCR and the D^b molecule with a mutation at residue 227 (D^b-227/TCR mice) (Fig. 2). The thymuses of these mice contained a prominent CD4$^+$CD8$^+$ population and also the more mature single positive CD8$^+$ or CD4$^+$ cells. These latter cells expressed low levels of the transgenic TCR-α chain, probably because of its displacement by endogenous α chains which would allow selection to occur through interaction with MHC molecules other than D^b. The capacity of D^b to signal deletion does not appear to be completely abolished by the mutation at 227, as there is an increase in the number of CD4$^-$/CD8$^-$ thymocytes in some of these mice relative to H-2^d/TCR mice. This result may be due to some residual binding between D^b-227 and CD8.

The peripheral lymphoid organs of male H-2^b mice that express the transgenic TCR were previously shown to contain a reduced complement of CD4$^+$ cells, a CD8^{lo} population, and a significant number of CD4$^-$CD8$^-$ cells expressing the transgenic TCR. The CD8^{lo} and double negative cells are thought to escape deletion because they do not express high levels of CD8, but their natural physiological counterparts are not readily identifiable. The D^b-wt/TCR mice have this characteristic pattern of T cells in their lymph nodes (Fig. 2, *bottom*). Some of the novel CD8^{lo} and CD8$^-$CD4$^-$ cells are also observed to varying extents in the periphery of male D^b-227/TCR mice, probably be-
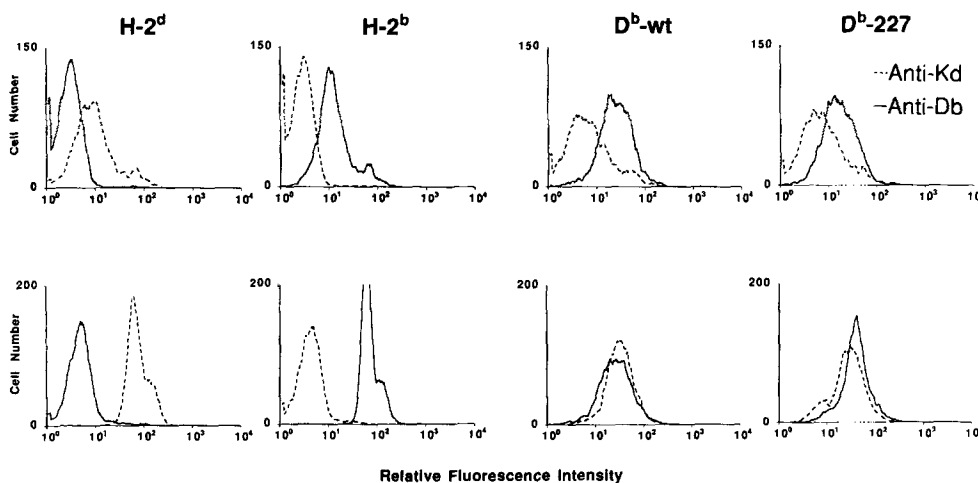

Figure 1. Expression of the transgenic MHC class I molecules on thymic and lymph node cells. Thymocytes (*top*) and lymph node cells (*bottom*) were taken from DBA/2, C57BL/6, and from the two H-2^d transgenic lines expressing D^b-wt and D^b-227. Cells were stained with H141-31 (Anti-D^b) and SF1-1.1.1 (Anti-K^d), and detected with fluorescein goat anti–mouse IgG Fc. Each histogram represents 10,000 gated events analyzed on a FACScan® flow cytometer (Becton Dickinson & Co.).
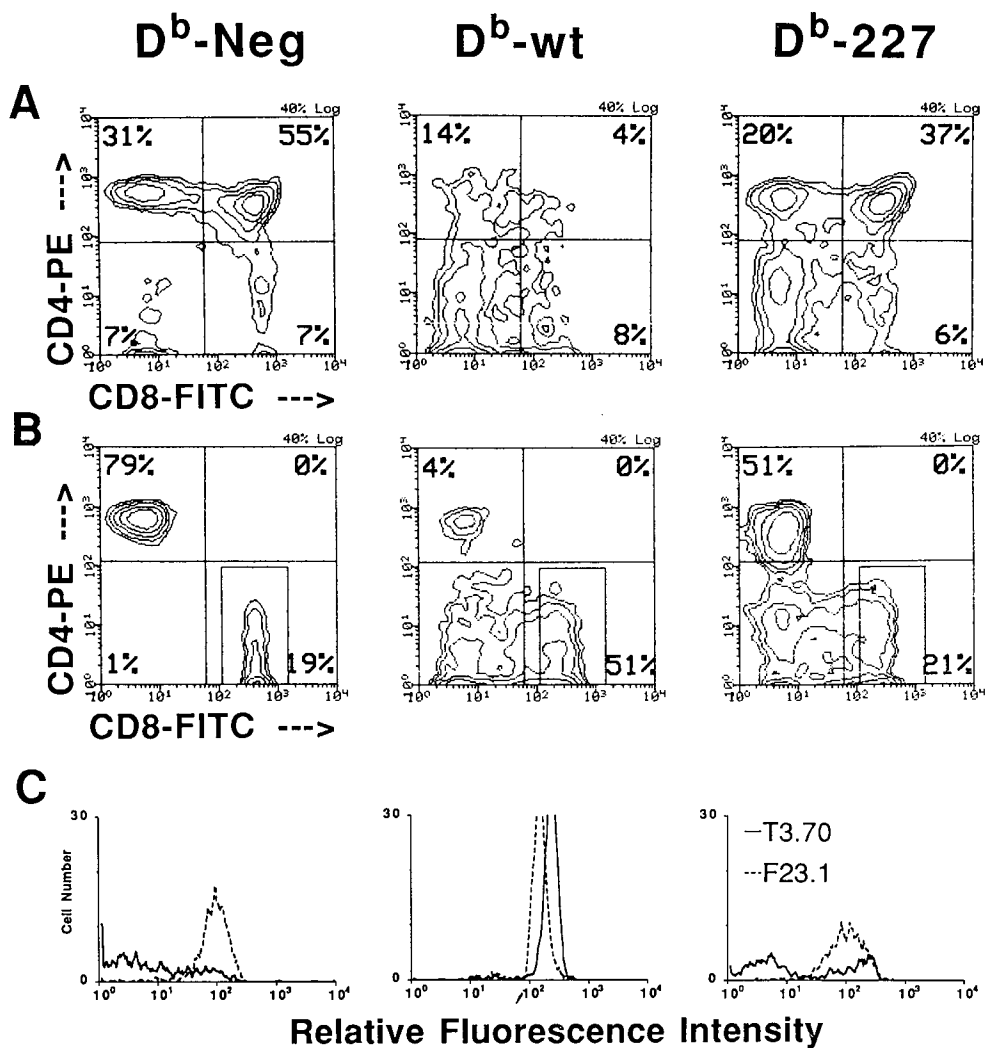
Figure 2. FACS® analysis of thymic and lymph node cells from male transgenic mice. Thymocytes (*A*) and lymph node cells (*B* and *C*) were taken from H-2^d TCR transgenic mice expressing either D^b-wt, D^b-227, or no D^b (D^b-Neg). Cells were stained with fluorescein anti-CD8, PE anti-CD4, and either F23.1-biotin or T3.70-biotin followed by streptavidin-tandem conjugate. Ten thousand events were analyzed on a FACScan® flow cytometer (Becton Dickinson & Co.). (*A*) Contour plot showing expression of CD4 and CD8 on thymocytes. (*B*) Contour plot showing expression of CD4 and CD8 on lymph node T cells gated for expression of the transgenic β chain (F23.1$^+$ T cells). (*C*) Histogram showing expression of the transgenic α (T3.70) and β (F23.1) chains on CD8$^+$ lymphocytes (gated within the rectangle in *B*).

cause the mutation in class I MHC does not completely abolish deletion. However, there is substantial rescue of single positive cells, which appear to express endogenous TCR-α chains in association with the transgenic β chain, as shown by the lack of significant staining with the α chain-specific antibody, T3.70. Thus, the mutation in the CD8-binding site of the D^b molecule significantly prevents clonal deletion, allowing the accumulation of double positive thymocytes that are then positively selected through their interaction with MHC molecules other than D^b expressed in the H-2^d mice.

Lack of Efficient Positive Selection in D^b-227/TCR Transgenic Mice. To analyze the effect of loss of CD8 binding on positive selection of D^b-restricted T cells, female animals expressing the transgenic TCR and the wild-type or mutant class I transgenes were analyzed. As had been previously shown

in female H-2^b mice (29), animals expressing the wild-type D^b transgene and the TCR transgene have a significant increase in the number of CD8$^+$ thymocytes. All of these cells stain positively with the T3.70 antibody specific for the transgenic α chain, thus indicating that positive selection of T cells expressing the complete αβ heterodimer had occurred (Fig. 3). This phenotype is not observed in female D^b-227/TCR transgenic mice. The CD8/CD4 ratio in mature thymocytes in these animals is not significantly changed relative to that in nonselecting H-2^d mice, and the CD8$^+$ cells do not stain brightly with the T3.70 antibody as is seen in D^b-wt/TCR mice. The D^b-227 molecule, therefore, fails to serve as an appropriate substrate for positive selection.

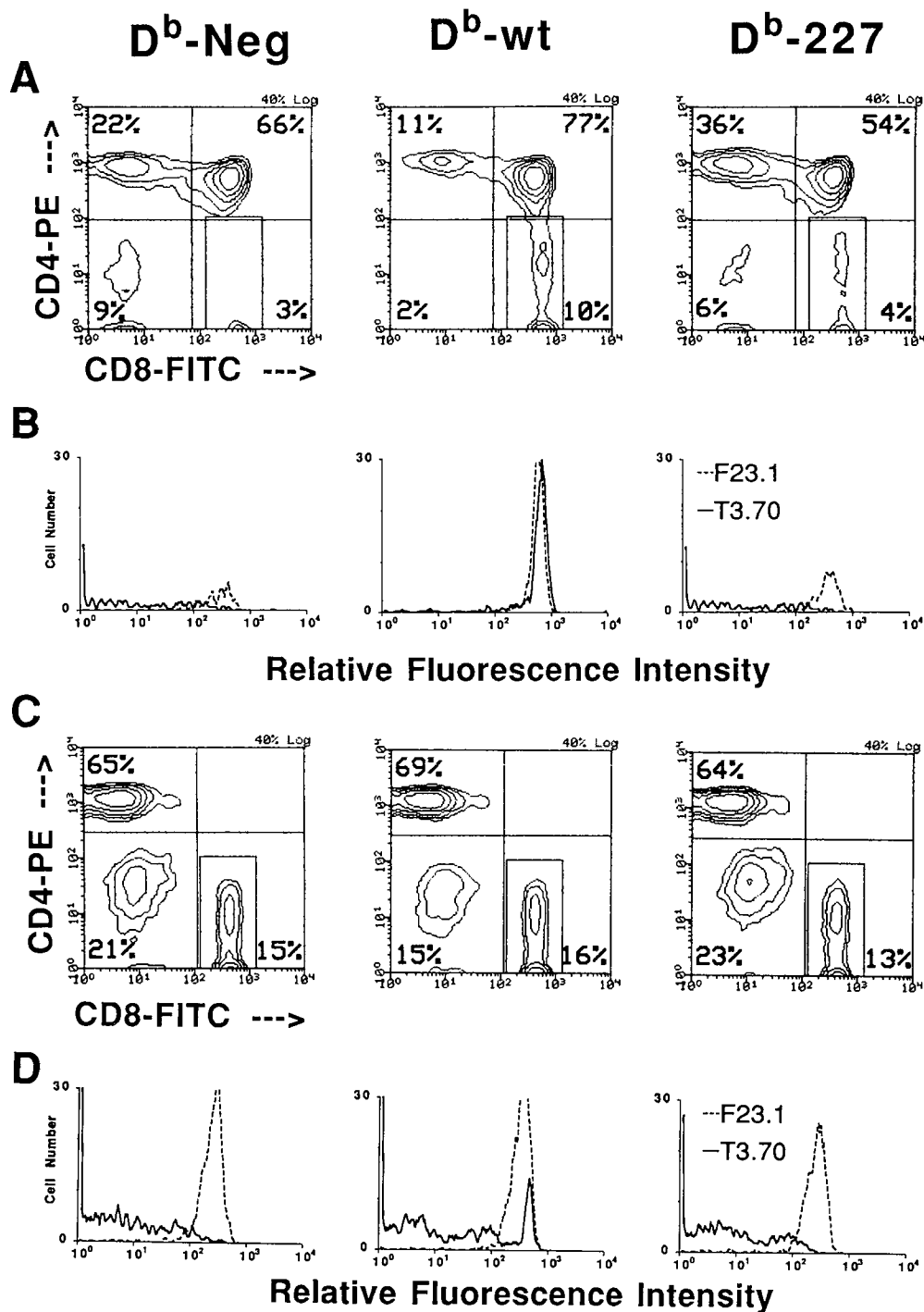The phenotype of T cells in peripheral lymphoid organs is consistent with that observed in the thymus. Many CD8$^+$

Figure 3. FACS® analysis of thymic and lymph node cells from female transgenic mice. Thymocytes (*A* and *B*) and lymph node cells (*C* and *D*) were stained as in Fig. 2. (*A*) Contour plot showing expression of CD4 and CD8 on thymocytes. (*B*) Histogram showing expression of the transgenic α (T3.70) and β (F23.1) chains on CD8⁺ thymocytes (*rectangle* in *A*). *C* and *D* are the same as *A* and *B*, but represent staining of total lymph node cells.

cells in the Dᵇ-wt/TCR mice express the transgenic TCR, although, unlike thymocytes, a significant proportion are TCR-α negative, possibly reflecting selective influences in the periphery. Few, if any, CD8⁺ T cells express significant levels of the transgenic TCR-α chain in Dᵇ-227/TCR mice.

Antigen Presentation by the Dᵇ-227 Molecule. Both positive and negative selection are adversely affected by the substitution of aspartic acid for lysine at residue 227. Based on previous studies which have shown that this residue is involved in binding of class I MHC to CD8, it is likely that the observed

developmental defects are due to loss of this interaction. However, these results could also be explained by a direct effect of the point mutation on the ability of D^b to present the H-Y antigen. To address this issue, the ability of H-Y–specific cytotoxic T cells to lyse cells expressing wild-type and mutant D^b was determined. Lymph node cells from C57BL/6 female mice primed in vivo with syngeneic male cells and restimulated in vitro with male or female C57BL/6 dendritic cells were tested for their ability to lyse Con A blasts from D^b-wt and D^b-227 mice. As shown in Fig. 4 (top), cells from male D^b-227 mice were lysed by the secondary CTLs, but lysis was less efficient than that of D^b-wt cells. Specificity for H-Y was apparent because female cells neither provided stimulation for CTLs nor served as their targets.

The antigen presentation function of D^b-227 was further analyzed by a cold target competition experiment in which unlabeled cells were used to inhibit the lysis of labeled male D^b-wt or male D^b-227 target cells. Lysis of D^b-wt targets was most effectively inhibited by male D^b-wt Con A blasts, but inhibition was also obtained with blasts from D^b-227 males, which were more efficient than the female D^b-wt or D^b-227 cells (Fig. 4, bottom left). In contrast, lysis of D^b-227 labeled targets was readily inhibited by blasts from either D^b-wt or D^b-227 male mice (Fig. 4, bottom right). These results are consistent with the interpretation that two populations comprised the secondary CTLs, one CD8-dependent, and the other CD8-independent. Lysis of D^b-227 targets presumably involved only the latter population, whereas the D^b-wt blasts could be lysed by either type of CTL. Overall, the results indicate that the D^b-227 class I molecule can present H-Y antigen and is recognized by antigen-specific T cells.

Discussion

The importance of the interaction of CD8 with class I MHC molecules in T cell activation and development was predicted a decade ago after it was recognized that CD8$^+$ cells are restricted to interact with class I MHC molecules (37). Subsequent studies have demonstrated a direct physical interaction between CD8 and class I MHC molecules (8, 38), and a requirement for CD8 expression in the function of class I-specific T cells (39). Results of studies with mice lacking expression of either CD8 (20) or of class I MHC (40, 41), and the demonstration that injection of anti-CD8 antibodies into mice blocks development of CD8$^+$ class I–restricted cells, but not of CD4$^+$ class II–restricted cells (18, 19), strongly suggested that the interaction of CD8 and class I molecules is required in development. However, these experiments did not conclusively establish that the observed developmental defects were solely due to abrogation of this interaction.

In this study, we have analyzed the consequences of disrupting the CD8-class I interaction on positive and negative selection of thymocytes expressing a well-characterized TCR. The disruption was achieved by introducing a mutation at residue 227 in the $\alpha3$ domain of the class I molecule D^b. This mutation has previously been shown to prevent binding to CD8α and to result in loss of recognition by primary, but not secondary, cytotoxic cells (15, 36). Residue 227 is located in a hydrophilic, solvent-exposed loop of the $\alpha3$ domain, and its substitution is unlikely to result in significant changes in the conformation of the class I molecule (15). Moreover, cells from male mice that express the mutant molecule are effectively lysed by secondary CTLs specific for H-Y/D^b, indicating that the antigen-presenting function of the molecule is not significantly impaired. Subtle effects on peptide presentation may still exist, but in the absence of structural data, this issue is difficult to resolve.

Although the mutant D^b molecule can be recognized by the specific TCR, its inability to bind CD8 was found to result in significant impairment in both positive and negative selection of thymocytes expressing the receptor. Deletion of autoreactive cells in male mice that express the transgenic H-Y/D^b-specific receptor is dramatically reduced in the context of the mutant D^b molecule, and the pattern of thymocyte development resembles that seen in the absence of a deleting MHC structure. However, some of the D^b-227 mice showed a variable increase in the number of CD4$^-$8$^-$ thymocytes, perhaps reflecting reductions in the other subsets
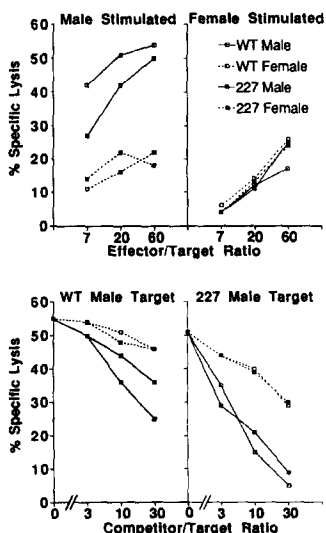

Figure 4. Recognition of D^b-wt and D^b-227 by cytotoxic T cells. (Top) Lysis of ^{51}Cr-labeled Con A blasts from male or female D^b-wt or D^b-227 transgenic mice by secondary CTLs. (Bottom) Inhibition of lysis of ^{51}Cr-labeled male D^b-wt or D^b-227 Con A blasts by unlabeled male or female Con A blasts.

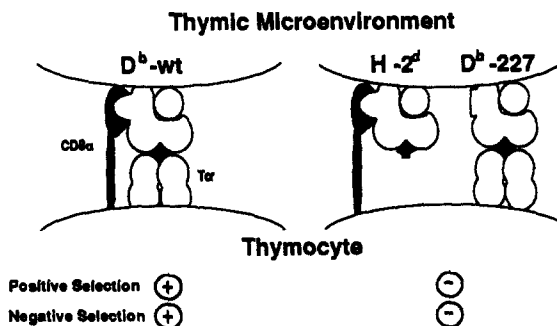
Figure 5. Model for the coreceptor function of CD8 in the development of thymocytes. The diagram represents thymocyte development in the presence of D^b-wt or D^b-227.

due to deletion. The mouse shown in Fig. 2 is an extreme example of this, and has 35% CD4⁻CD8⁻ thymocytes compared with 7% in the mouse that does not express D^b. Other D^b-227 mice were indistinguishable from the D^b-negative mice in their CD4/CD8 staining profiles. The peripheral T cells of male D^b-227/TCR mice include many more single positive cells than those of D^b-wt/TCR mice. In particular, the CD4$^+$8$^-$ subset is largely rescued by the mutation. However, there were also a substantial number of CD8^{lo} and CD4⁻CD8⁻ cells in some male mice bearing the mutant class I molecule. These cells dominate the periphery of male H-2^b/TCR (35) and D^b-wt/TCR transgenic mice. Recent data suggest that exit of these cells from the thymus does not depend on positive selection (42). The appearance of these cells in D^b-227/TCR mice may be a consequence of partial deletion due to residual binding between the mutant class I molecule and CD8. This possibility is difficult to evaluate since the physiologically active threshold for binding has not been adequately determined using in vitro assays. Alternatively, these cells may represent a distinct lineage that is independent of CD8–class I interaction and that may have a selective advantage in a male D^b environment. Nonetheless, it is clear that the capacity of wild-type D^b to mediate clonal deletion of self-reactive thymocytes in male mice is largely abrogated by a mutation in the CD8 binding region.

In female D^b-227 mice, positive selection of CD8$^+$ cells bearing both chains of the transgenic TCR is not detectable. Thus, the progression of thymocytes to CD8$^+$ T cells appears to require the binding of CD8 to the class I MHC molecule. This result is consistent with the findings of Ziljstra et al. (40) and Fung-Leung et al. (20), showing that development of the CD8 lineage does not occur in the absence of expression of class I MHC or CD8. In addition, it provides a likely explanation for the relative paucity of HLA-Aw68–reactive T cells in humans. A polymorphism at residue 245 in the α3 domain of this molecule results in decreased binding to CD8 and, hence, is likely to prevent positive selection of thymocytes reactive with Aw68.

The results presented here are consistent with the conclusions of two recent reports describing transgenic MHC class I molecules bearing analogous α3 domain mutations. These were shown to be inefficient in deleting CD8$^+$ cells specific for allo-determinants expressed on the same molecules (43, 44). Positive selection of L^d-restricted T cells specific for a murine cytomegalovirus peptide was also absent in one of the transgenic lines (43).

The D^b transgenic mice used in this analysis express normal H-2^d MHC class I molecules with unmodified α3 domains. As depicted in Fig. 5, these molecules could theoretically participate in thymocyte development by providing unimpeded CD8 interactions to accompany TCR recognition of H-Y/D^b-227. The fact that this type of complementation is unable to rescue either positive or negative selection indicates a requirement for coordinate recognition of a single MHC class I molecule by a complex of the TCR and CD8. A similar conclusion has been reached from the lack of target cell lysis by CTLs recognizing class I molecules that do not bind CD8 (15, 16, 45). These experiments showed that CD8 binding sites on other class I molecules could not substitute for the defective site on the molecule presenting antigen to the TCR. The lack of positive and negative selection in D^b-227 mice now provides in vivo evidence for the model of CD8 as a coreceptor in T cell recognition of antigen.

The initial description of TCR transgenic mice established that TCR specificity determines the CD4/CD8 phenotype of the developing T cell. For example, TCR transgenes isolated from a class I-restricted T cell clone cause preferential development of CD8$^+$ T cells in mice that express the appropriate MHC class I allele (29, 46, 47). The same phenomenon has been observed for class II-restricted TCRs and CD4$^+$ T cells (48, 49). The analysis of D^b-227/TCR mice has demonstrated the active participation of the CD8 coreceptor in the determination of the CD8 lineage. While the function of CD8 appears to require binding to MHC class I, its signaling role in thymocytes remains poorly understood. Both CD8α and CD4 bind to the cytoplasmic tyrosine kinase p56^{lck} through cysteine residues in their cytoplasmic tails (11, 12). This interaction is required for efficient in vitro T cell activation in coreceptor-dependent systems (13, 14). It remains to be determined whether the association of CD8α with p56^{lck} has a significant role in thymic differentiation and whether distinct signals are mediated by the CD4 and CD8 coreceptors.

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