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

By Nigel Killeen,* Ann Moriarty,* Hung-Sia Teh,† and Dan R. Littman*

From the *Department of Microbiology and Immunology and Howard Hughes Medical Institute, University of California, San Francisco, California 94143-0414; and the †Department of Immunology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z3

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 α3 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-2Db were not deleted in the context of a transgenic Dα 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 αβ 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 non-polymorphic domains of class II and class I MHC molecules, respectively (7–9), while their cytoplasmic domains bind to a cytoplasmic tyrosine kinase, p56lck (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 p56lck 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 F1, 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. D8+ transgenic mice were created and analyzed according to standard procedures (26). The 10-kb genomic fragment including the D8 gene (27) was obtained from Dr. Robert Goodenow, University of California, Berkeley, and a 4.5-kb Xhol/HindIII subclone containing the α3 domain was tagged with the following oligo: 5′-acaagctccatcttctggg-3′, which substitutes lysine for aspartic acid at residue 227. Purified 10-kb fragments containing either wild-type or mutant D8+ genes were microinjected into C57BL/6 × DBA/2 F1 zygotes. Founder mice were identified by Southern blot analysis of DNA from tail biopsies. F1 mice, produced by back-crossing founders to DBA/2 animals, were mated to the HY/Db-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 using antibodies against the TCR-β chain, D8+, and K8 (for the MHC background).

Antibodies and Flow Cytometry. H141-31 anti-D8 was purchased from Bioproducts for Science, Inc. (Indianapolis, IN). AF6-88-5.3 anti-K8, and SFI-1.11 anti-K8 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 (FITCGAMiG) 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 × 106 cells were analyzed on a FACS® flow cytometer (Becton Dickinson & Co.) using FACS® 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 × 107 male C57BL/6 spleen cells for more than 14 d. Primed cells (5 × 107) were transferred to 24-well plates containing dendritic cells (5 × 106), 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 51Cr-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 × 10⁶ 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 IgB+ 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-2Db (33). In female mice that express the Db allele, thymocytes expressing the transgenic α and β chains are positively selected to become CD8+ cells (29). In male animals that express Db+, 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\textsuperscript{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\textsuperscript{b} in a nonselecting H-2\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{b} in C57BL/6 mice, but was roughly equivalent to that of endogenous D\textsuperscript{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\textsuperscript{b}-227/TCR Transgenic Mice.** CD4\textsuperscript{+}CD8\textsuperscript{+} thymocytes are deleted in male H-2\textsuperscript{b} mice that express the H-Y-specific transgenic TCR (25). This situation is recreated in H-2\textsuperscript{d} mice transgenic for both the TCR, and the wild-type D\textsuperscript{b} molecule (D\textsuperscript{b}-wt/TCR mice) (Fig. 2). The thymuses of these mice were diminished in size, lacked the usually predominant CD4\textsuperscript{+}CD8\textsuperscript{+} population, and contained mostly CD4\textsuperscript{+}CD8\textsuperscript{−} immature cells. Deletion was dependent on the transgenic TCR, as it was not observed in transgenic mice only expressing the D\textsuperscript{b}-wt gene (data not shown). In contrast, a relatively normal pattern of thymocyte development was observed in H-2\textsuperscript{d} male mice expressing the transgenic TCR and the D\textsuperscript{b} molecule with a mutation at residue 227 (D\textsuperscript{b}-227/TCR mice) (Fig. 2). The thymuses of these mice contained a prominent CD4\textsuperscript{+}CD8\textsuperscript{+} population and also the more mature single positive CD8\textsuperscript{+} or CD4\textsuperscript{+} 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\textsuperscript{b}. The capacity of D\textsuperscript{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\textsuperscript{−}/CD8\textsuperscript{+} thymocytes in some of these mice relative to H-2\textsuperscript{d}/TCR mice. This result may be due to some residual binding between D\textsuperscript{b}-227 and CD8.

The peripheral lymphoid organs of male H-2\textsuperscript{b} mice that express the transgenic TCR were previously shown to contain a reduced complement of CD4\textsuperscript{+} cells, a CD8\textsuperscript{+} population, and a significant number of CD4\textsuperscript{−}CD8\textsuperscript{−} thymocytes expressing the transgenic TCR. The CD8\textsuperscript{+} 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\textsuperscript{b}-wt/TCR mice have this characteristic pattern of T cells in their lymph nodes (Fig. 2, bottom). Some of the novel CD8\textsuperscript{+} and CD8\textsuperscript{−}CD4\textsuperscript{−} cells are also observed to varying extents in the periphery of male D\textsuperscript{b}-227/TCR mice, probably be-
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 Dβ 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 Dβ expressed in the H-2b mice.

Lack of Efficient Positive Selection in Dβ-227/TCR Transgenic Mice. To analyze the effect of loss of CD8 binding on positive selection of Dβ-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-2b mice (29), animals expressing the wild-type Dβ 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 Dβ-227/TCR transgenic mice. The CD8/CD4 ratio in mature thymocytes in these animals is not significantly changed relative to that in nonselecting H-2b mice, and the CD8+ cells do not stain brightly with the T3.70 antibody as is seen in Dβ-wt/TCR mice. The Dβ-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 (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 D227 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
molecules in T cell activation and development was predicted a decade ago after it was recognized that CD8+ cells are re-

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 re-

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

Figure 5. Model for the co-receptor function of CD8 in the development of thyocytes. The diagram represents thyocyte development in the presence of Db-wt or Db-227.

strained 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 thyocytes expressing a well-characterized TCR. The disruption was achieved by introducing a mutation at residue 227 in the α3 domain of the class I molecule Db. 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 α3 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/Db, 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 Db 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 thyocytes expressing the receptor. Deletion of autoreactive cells in male mice that express the transgenic H-Y/Db-specific receptor is dramatically reduced in the context of the mutant Db molecule, and the pattern of thyocyte development resembles that seen in the absence of a deleting MHC structure. However, some of the Db-227 mice showed a variable increase in the number of CD4+ 8- thyocytes, perhaps reflecting reductions in the other subsets...
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 Db. 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^b 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^a-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^b 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 p56lk 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 p56lk has a significant role in thymic differentiation and whether distinct signals are mediated by the CD4 and CD8 coreceptors.

We are grateful to Soo-Jeet Teh for expert technical assistance and to Kathy Smith for help in the initial phase of this work. We thank Thomas Jenuwein for providing instruction in transgenic techniques, and Craig Davis, Art Weiss, Peter Parham, and Anne Norment for their advice and helpful discussions.

This work was supported by a long-term fellowship from the European Molecular Biology Organization to N. Killeen, by a grant from the Canadian Medical Research Council to H.-S. Teh, and by a grant from the National Institutes of Health (RO1 AI23513) to D. R. Littman. D. R. Littman is an investigator of the Howard Hughes Medical Institute.

Address correspondence to Dr. Dan R. Littman, Department of Microbiology and Immunology, Howard Hughes Medical Institute, University of California, San Francisco, CA 94143-0414.

Received for publication 24 February 1992 and in revised form 9 April 1992.
References

1. von Boehmer, H., H.-S. Teh, and P. Kisielow. 1989. The thymus selects the useful, neglects the useless and destroys the harmful. *ImmunoL Today*. 10:57.

2. Ashwell, J.D., and R.D. Klausner. 1990. Genetic and mutational analysis of the T-cell antigen receptor. *Annu. Rev. Immunol.* 8:339.

3. Turner, J.M., and D.R. Littman. 1991. Function of CD4 and CD8 in T cell activation and differentiation. In Advances in Regulation of Cell Growth, Vol. 2. J. Mond, A. Weiss, and J. Cambier, editors. Raven Press, New York. 10:645.

4. Janeway, C.A., Jr. 1992. The T cell receptor as a multicomponent signalling machine. *Annu. Rev. Immunol.* 10:645.

5. Littman, D.R. 1987. The structure of the CD4 and CD8 genes. *Annu. Rev. Immunol.* 5:561.

6. Parnes, J.R. 1989. Molecular biology and function of CD4 and CD8. *Adv. Immunol.* 44:265.

7. Doyle, C., and J.L. Strominger. 1987. Interaction between CD4 and class II MHC molecules mediates cell adhesion. *Nature (Lond.)* 330:256.

8. Norment, A.M., R.D. Salter, P. Parkham, V.H. Engelhard, and D.R. Littman. 1988. Cell-cell adhesion mediated by CD8 and MHC class I molecules. *Nature (Lond.)* 336:79.

9. Salter, R.D., A.M. Norment, B.P. Chen, C. Clayberger, A.M. Krens, D.R. Littman, and P. Parkham. 1989. Polymorphism in the alpha 3 domain of HLA-A molecules affects binding to CD8. *Nature (Lond.)* 338:345.

10. Rudd, C.E., J.M. Trevillyan, J.D. Dasgupta, L.L. Wong, and S.F. Schlossman. 1988. The CD4 receptor is complexed in detergent lysates to a protein-tyrosine kinase (pp58) from human T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 85:5190.

11. Shaw, A.S., K.E. Amrein, C. Hammond, D.F. Stern, B.M. Sefton, and J.K. Rose. 1989. Characterization of a murine monoclonal anti-CD4 antibody treatment. *Nature (Lond.)* 335:174.

12. Parham, P. 1989. Getting into the groove. *Nature (Lond.)* 342:617.

13. Kisielow, P., H. Bluthmann, U.D. Staerz, M. Steinmetz, and H. von Boehmer. 1988. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD48+ thymocytes. *Nature (Lond.)* 333:742.

14. Hogan, B.L.M., F. Costantini, and E. Lacy. 1986. Manipulating the Mouse Embryo. A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

15. Watts, S., J.M. Vogel, W.D. Harriman, T. Itoh, H.J. Strauss, and R.S. Goodenow. 1987. DNA sequence analysis of the C3H H-2Kd and H-2Dd loci. Evolutionary relationships to H-2 genes from four other mouse strains. *J. Immunol.* 139:3878.

16. Kunkel, T.A. 1985. Rapid and efficient site-specific mutagenesis without phenotype selection. *Proc. Natl. Acad. Sci. USA.* 82:488.

17. Teh, H.-S., P. Kisielow, B. Scott, H. Kishi, Y. Uematsu, H. Bluthmann, and H. von Boehmer. 1988. Thymic major histocompatibility complex antigens and the alpha beta T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature (Lond.)* 335:229.

18. Teh, H.-S., H. Kishi, B. Scott, H. von Boehmer, and P. Kisielow. 1990. Early deletion and late positive selection of T cells expressing a male-specific receptor in T cell receptor transgenic mice. *Dev. Immunol.* 1:1.

19. Staerz, U.D., H.G. Rammensee, J.D. Benedetto, and M.J. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotype determinant on T cell antigen receptor. *J. Immunol.* 134:3994.

20. Nussenzweig, M.C., R.M. Steinman, M.D. Witmer, and B.A. Gutchnick. 1982. A monoclonal antibody specific for mouse dendritic cells. *Proc. Natl. Acad. Sci. USA.* 79:161.

21. von Boehmer, H. 1990. Developmental biology of T cells in T cell receptor transgenic mice. *Annu. Rev. Immunol.* 8:531.

22. Uematsu, Y., S. Ryser, Z. Dembic, P. Borgula, P. Krumpenfort, A. Berns, H. von Boehmer, and M. Steinmetz. 1988. In transgenic mice the introduced functional T cell receptor beta gene prevents expression of endogenous beta genes. *Cell.* 52:831.

23. Teh, H.-S., H. Kishi, B. Scott, and H. von Boehmer. 1989. Deletion of autospecific T cells in T cell receptor (TCR) transgenic mice spares cells with normal TCR levels and low levels of cytotoxic T cell activity. *J. Immunol.* 143:1467.

24. Zuniga-Pflucker, J.C., L.A. Jones, D.L. Longo, and A.M. Kruisbeek. 1990. CD8 is required during positive selection of CD4+/CD8+ T cells. *J. Exp. Med.* 171:427.
of CD8 molecules. J. Exp Med. 169:795.
36. Potter, T.A., TV. Rajan, R.F. Dick 2nd., and J.A. Bluestone. 1989. Substitution at residue 227 of H-2 class I molecules abrogates recognition by CD8-dependent, but not CD8-independent, cytotoxic T lymphocytes. Nature (Lond.). 337:73.
37. Swain, S.L. 1981. Significance of Lyt phenotypes: Lyt-2 antibodies block activities of T cells that recognize class I major histocompatibility complex antigens regardless of their function. Proc. Natl. Acad. Sci. USA. 78:7101.
38. Rosenstein, Y., S. Ratnofsky, S.J. Burakoff, and S.H. Herrmann. 1989. Direct evidence for binding of CD8 to HLA class I antigens. J. Exp Med. 169:149.
39. Gabert, J., C. Langlet, R. Zamoyska, J.R. Parnes, A.M. Schmitt-Verhulst, and B. Malissen. 1987. Reconstitution of MHC class I specificity by transfer of the T cell receptor and Lyt-2 genes. Cell. 50:545.
40. Zijlstra, M., M. Bix, N.E. Simister, J.M. Loring, D.H. Raulet, and R. Jaenisch. 1990. Beta-2-microglobulin deficient mice lack CD4 + T cells. Nature (Lond.). 344:742.
41. Koller, B.H., P. Marrack, J.W. Kappler, and O. Smithies. 1990. Normal development of mice deficient in beta 2m, MHC class I proteins, and CD8 + T cells. Science (Wash. DC). 248:1227.
42. von Boehmer, H., J. Kirberg, and B. Rocha. 1991. An unusual lineage of alpha/ beta T cells that contains autoreactive cells. J. Exp Med. 174:1001.
43. Aldrich, C.J., R.E. Hammer, S. Jones-Youngblood, U. Koszinowski, L. Hood, I. Stroynowski, and J. Forman. 1991. Negative and positive selection of antigen-specific cytotoxic T lymphocytes affected by the alpha 3 domain of MHC I molecules. Nature (Lond.). 352:718.
44. Ingold, A.L., C. Landel, G. Evans, and T.A. Potter. 1991. Co-engagement of CD8 with the T cell receptor is required for negative selection. Nature (Lond.). 352:721.
45. Kalinke, U., B. Arnold, and G.J. Hammerling. 1990. Strong xenogeneic HLA response in transgenic mice after introducing an alpha 3 domain into HLA B27. Nature (Lond.). 348:642.
46. Sha, W.C., C.A. Nelson, R.D. Newberry, D.M. Kranz, J.H. Russell, and D.Y. Loh. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. Nature (Lond.). 336:73.
47. Sha, W.C., C.A. Nelson, R.D. Newberry, D.M. Kranz, J.H. Russell, and D.Y. Loh. 1988. Selective expression of an antigen receptor on CD8-bearing T lymphocytes in transgenic mice. Nature (Lond.). 335:271.
48. Berg, L.J., A.M. Pullen, B. Fazekas de St. Groth, D. Mathis, C. Benoist, and M.M. Davis. 1989. Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. Cell. 58:1035.
49. Kaye, J., M.L. Hsu, M.E. Sauron, S.C. Jameson, N.R. Gascoigne, and S.M. Hedrick. 1989. Selective development of CD4 + T cells in transgenic mice expressing a class II MHC-restricted antigen receptor. Nature (Lond.). 341:746.