

PREFERENTIAL DIFFERENTIATION OF T CELL RECEPTOR SPECIFICITIES BASED ON THE MHC GLYCOPROTEINS ENCOUNTERED DURING DEVELOPMENT

Evidence for Positive Selection

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One of the most fundamental questions remaining in cellular immunology is how the repertoire of antigen-specific T cell receptors is selected to recognize foreign antigens in association with the glycoproteins encoded by the MHC. The receptor on T cells is composed of two disulfide-linked variable chains, called α and β , and is associated with an invariant complex of proteins called CD3 (1-5). A single α/β heterodimer is known to confer both antigen and MHC recognition specificity to an individual T cell (6, 7), with most CD4⁺ CD8⁻ T cells using class II, and CD4⁻ CD8⁺ T cells using class I MHC molecules as recognition elements (8). T cells recognize antigens together with those MHC glycoproteins that they have encountered during their development rather than their own genetically encoded MHC products. This remarkable phenomenon, known as MHC-restricted recognition, is established in the thymus through a not yet fully defined selection process. One model used to describe this process, termed "positive selection," argues that only those T cells with sufficient avidity for the MHC glycoproteins expressed in the thymus are "positively selected," i.e., allowed to acquire functional maturity and to be exported to the periphery (9-12). More recent variants of this model propose that the selection process does not, in fact, involve "naked" MHC molecules, but rather thymic MHC complexed to a collection of self-peptides (13-16), possibly uniquely expressed on certain thymic stromal cells.

Much of the evidence supporting the positive selection model stems from studies with thymus-engrafted hemopoietic chimeras, which, given the complexities of such experimental systems, have generated much controversy with respect to this hypothesis (reviewed in references 17, 18). For this reason, a particular aim of our studies has been to examine the issue of thymic selection with experimental systems entirely different from chimeras. In the present paper, we examine the T cell repertoire specificities of CD4⁻ CD8⁺ cytotoxic T cells developing under conditions where one of the class I MHC-encoded molecules is blocked by treatment from birth with specific mAbs, while other class I MHC glycoproteins are still expressed. We show that in each experimental system used, antigen-specific T cells restricted to the blocked class

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I fail to develop, while generation of other class I-specific T cells proceeds undisturbed. These data provide strong support for the notion that the thymic MHC selection element and the T cells' MHC restriction element are one and the same, and are consistent with the recent evidence for positive selection in anti-class II mAb-treated mice (19) and TCR- α/β transgenic mice (20–22). Together, these results demonstrate that one of the crucial interactions required for the intrathymic selection of the repertoire of TCR specificities is that between the TCR and MHC or some form of MHC.

Materials and Methods

Mice and In Vivo mAb Treatments. All mouse strains were either bred at the Frederick Cancer Research Facility or obtained from the Jackson Laboratory, Bar Harbor, ME. Neonatal mice were treated within 24 h of birth with anti-H2-K^k mAb (hybridoma 11-4.1, reference 23, obtained from the American Type Culture Collection, Rockville, MD), purified from ascites as described (24). Mice were injected daily intraperitoneally with 500 μ g per gram body weight and analyzed when 2–3 wk old. Control mice were treated daily with saline, and other relevant controls were as previously described (25).

Flow Cytometry Analysis. Analysis was performed as described earlier (24), using a B-D Dual Laser FACS 440 interfaced to a PDP 11/24 computer. Data were collected on 50,000 viable cells (as determined by forward light scatter and propidium iodide gating) and displayed either as contour diagrams (Figs. 1 and 2), with a 3-decade log scale of increasing green fluorescence on the x -axis and red fluorescence on the y -axis, or as histograms with a 3-decade log scale of increasing green fluorescence on the x -axis, versus relative cell number (Fig. 3). In contour diagrams, the x - and y -axis coordinates that defined negative cells were selected as the intersection of positive and control negative profiles in each parameter. mAbs used for staining were: FITC-conjugated anti-CD8 or biotin-conjugated anti-CD8 (53.6 mAb; Becton Dickinson & Co., Mountain View, CA); biotin-conjugated anti-CD4 (GK1.5 mAb; reference 26); FITC-conjugated anti-CD3 (145-2C11, reference 27); FITC-conjugated anti-K^k (11-4.1, reference 23); FITC-conjugated anti-D^d (34-1-2, reference 28); and, as negative controls, FITC- or biotin-conjugated anti-human Leu-2 (Becton Dickinson & Co.). Binding of biotin-conjugated reagents was visualized with streptavidin-allophycocyanin.

Limiting Dilution Analysis of Cytotoxic T Cell Precursor Frequencies. Groups of 48 replicate microcultures of various numbers of control and treated responder cells were cultured in round-bottomed wells of 96-well microtiter plates in a final volume of 0.2 ml of Eagles Hanks' amino acid medium with 10% FCS, 2 mM L-glutamine, 5×10^{-5} M 2-ME, and 20% delectinated rat Con A supernatant (Collaborative Research Inc., Bedford, MA). Stimulator cells were 5×10^5 irradiated (2,000 rad) spleen cells from 2-6-mo-old mice, either allogeneic to the responder cells or autologous and modified with 0.1 mM trinitrobenzenesulfonate (TNBS)¹ according to earlier described procedures (29, 30). After 7 days of culture, 10^3 ⁵¹Cr-labeled target cells were added per well; plates were incubated for 6 hrs, and centrifuged; 0.15 ml of supernatant was removed from each well, and radioactivity of the samples was measured in a gamma counter. Target cells were either tumor cells, for allogeneic responses (i.e., RDM4, H-2^k; EL-4, H-2^b, P815, H-2^d), or autologous Con A blasts, for TNP-specific responses, modified with 10 mM TNBS. Positive wells were defined as those in which ⁵¹Cr-release exceeded the mean of control wells in which target cells were incubated with stimulator cells alone by more than 3 SD of the mean. Precursor frequencies were calculated as described (24, 31). When responder cells were thymocytes, mice had been pretreated at 24 and 48 h before sacrifice with 1 mg hydrocortisone acetate, to enrich for CTL precursors (31).

Results

K^k Expression Is Required for Generation of K^k-restricted CTL. In previous studies, we showed that mice chronically treated with anti-class I mAb specific for both K- and

¹ Abbreviations used in this paper: FCM, flow cytometry; LD, limiting dilution; TNBS, trinitrobenzenesulfonate.

D-encoded MHC gene products fail to develop the CD4⁻CD8⁺-subset of T cells that is responsible for class I-restricted cytotoxic T cell responses (25). We next examined CD4⁻CD8⁺ T cell development in mice in which only one of the class I molecules, those encoded for by the MHC K region gene, was blocked. C3H (H-2^k) mice were treated from birth with anti-K^k mAb (23) in daily doses sufficient to block most of the K^k molecules on spleen and thymus cells, as judged by flow cytometry (FCM; data not shown; see Fig. 3 for B10.A (H-2^a) mice). Two-parameter FCM analysis with anti-CD4 and anti-CD8 mAbs revealed no differences between the major T cell subsets of control and treated mice (Fig. 1). Thus, anti-K region mAb treatment does not interfere with the overall development of CD4⁻CD8⁺ T cells, or other T cell subsets.

We next investigated the ability of anti-class I K region antibody-treated mice to generate CTL responses to allogeneic and TNP-modified syngeneic stimulator cells. In this latter response, separate populations of CTL specific for TNP in association with either K or D region-encoded gene products are generated, and different mouse strains exhibit preference for using either K or D gene products as restriction elements (29, 30). To verify applicability of these rules in the limiting dilution (LD) system required for our CTL function analysis (25), we compared the CTL precursor frequency of TNP-specific CTL in different mouse strains (Table I). Consistent with earlier observations (29), H-2^k and H-2^a mice respond preferentially to K^k-TNP, H-2^d mice respond preferentially to D^d-TNP, while (H-2^k × H-2^d)F₁ mice exhibited preferential K^k-TNP responses when activated with K^k-expressing stimulator cells, and D^d TNP responses when activated with K^d D^d-stimulator cells (Table I). These

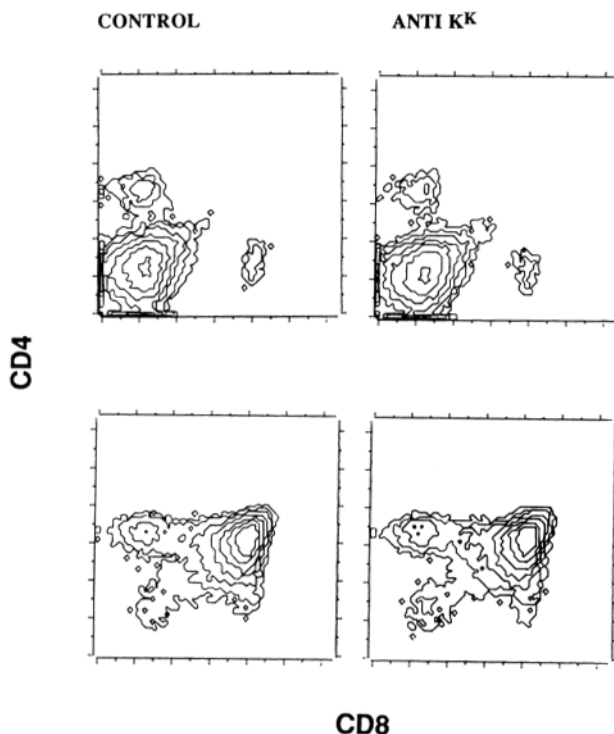


FIGURE 1. Effect of in vivo anti-class I (K^k) treatment on T cell subsets in spleen (*top*) and thymus (*bottom*). C3H (H-2^k) mice were treated from birth with saline (*left*) or anti-K^k mAb (11-4.1; *right*) and analyzed when 3 wk old. Spleen and thymocytes were reacted first with FITC-conjugated anti-CD8 mAb, followed by biotin-conjugated anti-CD4 mAb, followed by streptavidin-allophycocyanin. The proportions of CD4⁻CD8⁺ T cells are: for control spleen, 3.5; for treated spleen, 2.9; for control thymus, 2.4; for treated thymus, 2.2. The proportions of CD4⁺CD8⁻ T cells are: for control spleen, 9.3; for control thymus, 11.2; for treated spleen, 8.6; for treated thymus, 11.4. Data can be compared to those for C3H mice treated with anti-K^k-plus-D^k mAb that were previously published (25).

TABLE I
1/Frequency of CTL Precursors on Various TNP Targets

Response	B10.A K ^k D ^d	C3H K ^k D ^k	C3H.0L K ^d D ^k	B10.A(2R) K ^k D ^b	BALB/c K ^d D ^d	B6 K ^b D ^b
C3H-Anti-C3H (TNP)	NT	4,000*	>100,000	2,000	>200,000	>200,000
B10.A-Anti-B10.A (TNP)	12,000	10,000	>200,000	11,000	>200,000	NT
BALB/C-Anti-BALB/c (TNP)	10,000	>200,000	>200,000	>200,000	>11,000	NT
(C3H × BALB/c)						
F ₁ -Anti-F ₁ (TNP)	9,000	8,000	>200,000	9,000	>200,000	>200,000
Anti-C3H (TNP)	8,000	7,500	>200,000	7,500	>200,000	>200,000
Anti-BALB/c (TNP)	10,500	>200,000	>200,000	>200,000	12,000	>200,000

* Reciprocal of CTL precursor frequency.

differences were not related to non-MHC genes (data not shown), as congenic mouse strains with MHC-differences alone yielded identical results (29).

To determine how anti-K^k treatment affected the preferential K^k-TNP response in H-2^k mice, the TNP-specific CTL responses of spleen cells and thymocytes from control and treated mice were compared. The results clearly demonstrate a marked decrease in the precursor frequency of both splenic and thymic K^k-TNP-specific CTL (Table II). This decrease is not accompanied by a rise in the D^k-TNP-specific CTL response, as evidenced by the failure, under any circumstances, to lyse K^d D^k target cells (Table II). Additionally, the CD4⁻CD8⁺ T cells present in these mice (Fig. 1) were demonstrated to be functional, in that normal alloreactive CTL responses to H-2^b and H-2^d stimulator cells were generated (Table II). Also, examination of TCR expression by two-parameter FCM analysis with a mAb specific for the murine CD3-ε chain (27) versus anti-CD4 or anti-CD8 staining (Fig. 2) revealed no discernable differences between T cells from control and treated mice.

To address whether the selective inhibition of K^k-TNP-specific responses could be due to anti-K^k mAb carried over into the culture, we examined the TNP-specific

TABLE II
In H-2^k Mice Treated from Birth with Anti-K^k mAb, (TNP + K^k)-specific CTL Fail to Develop

Exp.	Responder cells	Stimulator: C3H-TNP			Stimulators: B6 C3H BALB/c		
		Targets: K ^k D ^k -TNP	K ^d D ^k -TNP	K ^k D ^b -TNP	Targets: B6	C3H	BALB/c
1*	Control*	5,500	>200,000	10,000			
	Treated (1)	50,000	>200,000	>100,000			
	Treated (2)	>200,000	>200,000	>200,000	NT		
	Treated (3)	>200,000	>200,000	>200,000			
2‡	Control	4,000	>100,000	3,500	2,500	>200,000	4,200 [§]
	Treated (1)	>100,000	>100,000	>100,000	3,600	>200,000	7,000
	Treated (2)	>100,000	>100,000	>100,000	4,800	>200,000	3,000
3‡	Control	1,500	>100,000	2,000	1,000		
	Treated (1)	>100,000	>100,000	>100,000	2,100		
	Treated (2)	>100,000	>100,000	>100,000	2,400		

* Exp. 1: Responder spleen cells.

‡ Exps. 2 and 3: Responder thymocytes (cortisone resistant).

§ Reciprocal of CTL-precursor frequency.

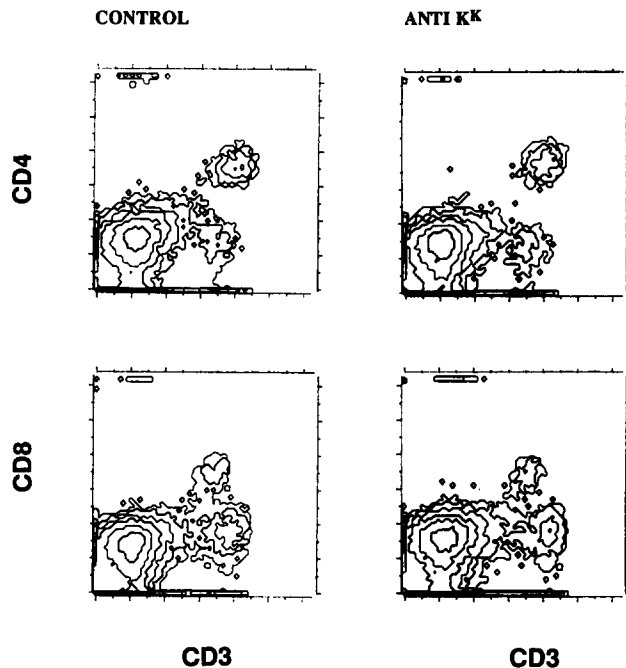


FIGURE 2. Effect of in vivo anti-class I (K^k) treatment on TCR expression in spleen cells. C3H ($H-2^k$) mice were treated from birth with saline (*left*) or anti- K^k mAb (11-4.1; *right*) and analyzed when 3 wk old. Spleen cells were reacted first with FITC-conjugated anti-CD3 mAb, followed by biotin-conjugated anti-CD4 mAb (*top*) or anti-CD8 mAb (*bottom*), followed by streptavidin-allphycocyanin. The proportions of $CD3^+CD4^-CD8^-$ T cells are: for control spleen, 3.4; for treated spleen, 3.0; $CD3^+CD4^+CD8^-$ T cells; for control spleen, 10.6; for treated spleen, 11.3.

CTL response of cells preincubated in anti- K^k mAb under saturating conditions. The results shown in Table III demonstrate that the K^k -TNP response of $H-2^k$ cells is not affected by anti- K^k mAb on the responder cells, making it unlikely that carry-over of the mAb could account for the defect observed in anti- K^k -treated mice. Taken together, these results therefore demonstrate that anti- K^k antibody treatment of $H-2^k$ mice selectively affected generation of K^k -TNP-specific $CD4^-CD8^+$ T cells, while development of other functional $CD4^-CD8^+$ T cells proceeded undisturbed.

Finally, we analyzed whether anti- K^k antibody-treated mice were still tolerant for K^k . Spleen cells (Table II) or thymocytes (data not shown) of anti- K^k -treated mice were stimulated with syngeneic or allogenic irradiated spleen cells in mixed lymphocyte cultures. Clearly, neither control nor treated cells responded with a CTL response to stimulation with syngeneic stimulator cells (Table II), while both responded

TABLE III
Does Blocking Antibody Remaining on Responder Cells
(from In Vivo Treatment) Affect CTL Responses?

Response against C3H-TNP	1/Frequency of CTL precursors on various TNP targets			
	C3H K^kD^k	C3H.0L K^dD^k	B10.A (2R) K^kD^b	B6 K^bD^b
C3H*	12,000	>100,000	18,000	>100,000
C3H coated with anti- K^k	15,000	>100,000	20,000	>100,000

* Responder spleen cells.

† Reciprocal of CTL-precursor frequency.

equally well to stimulation with allogeneic stimulator cells. Thus, mice treated with anti-K^k were found to be tolerant for K^k, while they could not “positively select” for K^k-restricted CTL. To explain the failure to positively select, and the success of tolerization in these mice, we invoke the following arguments: Since intrathymic clonal deletion of self-reactive cells (32-34) is thought to involve high-affinity interactions with predominantly bone marrow-derived cells (16, 35-37), it is possible that K^k molecules on these cells were not completely blocked by the injected mAb. Indeed, anti-K^k treatment did not saturate all K^k-molecules in thymus and spleen (see Fig. 3 for B10.A mice; same results for other mouse strains), hence there may have been sufficient K^k expression to allow for tolerization. Our data do not distinguish what cells are not blocked by antibody treatment. Because positive selection would involve interactions of a lower affinity (16), it should be easier to block positive selection than to block clonal deletion. Thus our data support these models of thymus function involving self-restriction and self-tolerance.

Blocking of K^k Class I Molecules During Development of the T Cell Repertoire Interferes with Generation of K^k-TNP-specific CTL, but not D^d-specific CTL. In the experiments reported above, generation of K^k-TNP-specific CTL was shown to require K^k-expression during development of the repertoire. These data would be consistent with the positive selection theory, provided that it can be proven that K^k-blocking alone does not affect development of all TNP-specific responses. To check whether any TNP-specific responses can be generated in anti-K^k-suppressed mice, we next examined the effects of K^k blocking in mice with a D region allele “permissive” for TNP responses, i.e., B10.A and (C3H × BALB/c)F₁ mice (see Table I).

As in the treated C3H (H-2^k) mice, B10.A (H-2^a) mice fail to generate K^k-TNP responses when treated from birth with anti-K^k-mAb (Table IV). However, although in BALB/c (H-2^d) mice CTL exhibit the ability to recognize TNP in association with D^d, no D^d-TNP responses are generated in the anti-K^k-suppressed B10.A mice. We therefore examined whether B10.A mice treated with anti-K^k still express D^d molecules. Since comodulation of class II (38) and class I glycoproteins

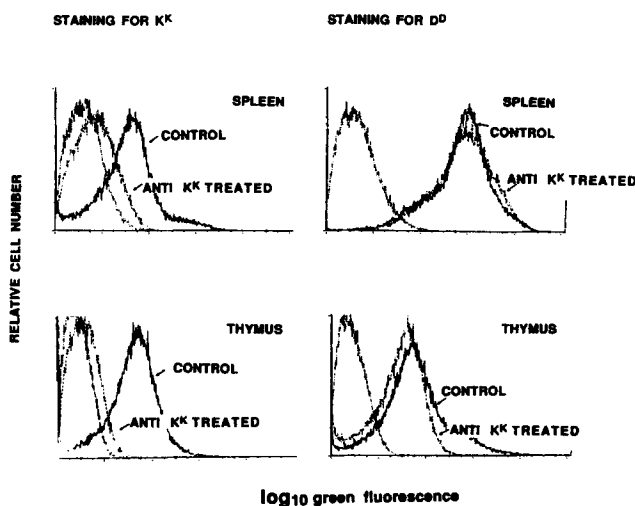


FIGURE 3. Effect of in vivo anti-class I (K^k) treatment on K^k and D^d expression in B10.A (K^kD^d) mice. B10.A (H-2^kD^d) mice were treated from birth with saline (solid line) or anti-K^k mAb (11-4.1; broken line), and analyzed when 3 wk old for expression of K^k (left) or D^d (right). Spleen cells or thymocytes were reacted either with FITC-conjugated 11-4.1 mAb (for K^k staining), or with FITC-conjugated 34-1-2 mAb (for D^d staining). Negative control staining (-----) is shown for only one of the samples in each diagram, since these curves essentially overlapped.

TABLE IV
*In B10.A Mice (H-2 K^kD^d) Treated from Birth with Anti-K^k mAb,
 (TNP + K^k)-specific CTL Fail to Develop*

Exp.	Responder cells	Stimulator: K ^k D ^d -TNP			Stimulator: B6
		Targets: K ^k D ^d -TNP	K ^d D ^d -TNP	K ^k D ^d	Target: B6
1	Control*	2,500 [†]	>100,000	>100,000	3,000
	Treated	>100,000	>100,000	>100,000	3,500
2	Control	2,400	>100,000	>100,000	1,000
	Treated	>100,000	>100,000	>100,000	2,100

* All responder cells: cortisone-resistant thymocytes.

[†] Reciprocal of CTL-precursor frequency.

(Ozato, K., personal communication) has been reported upon anti-MHC mAb treatment *in vivo*, the possibility that reduced D^d expression in anti-K^k-treated B10.A mice was responsible for the failure to positively select D^d-restricted CTL had to be considered. However, no evidence of comodulation K^k and D^d was found; D^d expression is, at best, only slightly reduced in anti-K^k-suppressed B10.A mice (Fig. 3), and identical results were obtained in (C3H × BALB/c)F₁ mice (data not shown). Thus, it appears that B10.A mice cannot generate D^d-TNP-CTL, despite presence of the D^d restriction element during development.

At first sight, these data are at variance with the predictions of the positive selection theory: if the inability to generate K^k-restricted responses in anti-K^k-treated mice is solely a reflection of a requirement for TCR-K^k interactions during devel-

TABLE V
*In C3H × BALB/c Mice (H-2 K^kD^k × K^dD^d) Treated from Birth with
 Anti-K^k mAb, (TNP + K^k)-specific CTL Fail to Develop,
 but (TNP + D^d)-specific CTL Do Develop*

Exp.	Responder cells	Stimulator: K ^k D ^k -TNP		
		Targets: K ^k D ^k -TNP	K ^d D ^k -TNP	K ^k D ^d -TNP
1	Control*	2,500 [†]	>100,000	3,000
	Treated (1)	>100,000	>100,000	>100,000
	Treated (2)	90,000	>100,000	>100,000
2	Control	3,300	>100,000	4,200
	Treated (1)	>100,000	>100,000	80,000
	Treated (2)	85,000	>100,000	90,000
		K ^d D ^d -TNP		
		K ^d D ^d -TNP	K ^d D ^k -TNP	K ^k D ^d -TNP
1	Control	6,000	>100,000	5,100
	Treated (1)	6,600	>100,000	6,300
	Treated (2)	11,000	>100,000	15,000
2	Control	19,000	>100,000	12,000
	Treated (1)	11,000	>100,000	15,000
	Treated (2)	12,000	>100,000	21,000

* All responder cells: cortisone-resistant thymocytes.

[†] Reciprocal of CTL-precursor frequency.

opment, remaining interactions between TCRs and D^d should still allow for development of D^d-restricted TNP responses. Another strategy applied to address this issue was the use of anti-K^k-suppressed (C3H × BALB/c)F₁ mice. In such mice (see Table I), D^d-TNP-CTL responses can be generated, provided H-2^d-TNP stimulator cells are used. Strikingly, anti-K^k treatment did not affect development of D^d-TNP-CTL in the (C3H × BALB/c)F₁ mice, as shown in Table V. The CTL precursor frequencies generated upon stimulation with H-2^d-TNP stimulator cells were very similar for treated and control mice, and responses mapped to D^d, as shown by the absence of lysis when K^dD^k-TNP target cells were used. At the same time, anti-K^k-treated (C3H × BALB/c)F₁ mice with unchanged D^d-TNP responses exhibited very strongly reduced K^k-TNP responses, as shown in the top half of Table V. Thus, anti-K^k treatment leads, in three different mouse strains (C3H, B10.A, and [C3H × BALB/c]F₁), to a failure to generate K^k-restricted CTL specific for TNP, while development of TNP-specific CTL using another restriction element, i.e., D^d, is not affected in the strain combination where such responses are "permissive." These results directly demonstrate that generation of CD4⁻CD8⁺ T cells with restriction specificity for a certain class I molecule requires interactions with that particular class I glycoprotein (or an unknown molecular complex containing that particular class I glycoprotein), and thus support the positive selection theory.

Discussion

In a search for a direct test of predictions derived from the "positive selection" theory, we analyzed the development of class I-restricted T cells under conditions where particular class I glycoproteins were blocked by mAb treatment from birth onward, while other class I molecules were unaffected. Clearly, the results show that, in mice with blocked K^k molecules, there is a selective failure to develop only K^k-restricted T cells, while other class I-restricted T cells are generated undisturbed. Thus, generation of T cells with a particular TCR specificity is dependent on recognition of a particular MHC glycoprotein during development, a result predicted by the positive selection theory. These findings with anti-class I MHC mAb have therefore confirmed the suggestions made by studies with hematopoietic and thymic chimeras (9-12) and are consistent with the recent demonstration of positive selection in other anti-MHC mAb treatment studies (19, 39) and TCR- α/β transgenic mice (20-22). Thus, a role for TCR-MHC interactions in the development of T cells in normal mice has now been firmly established.

Although our studies describe what we assume to be the general situation in which the thymic educating element (i.e., K^k) is identical to the T cells' restriction element, there appear to be certain exceptions to this rule. Our experiments reveal an example where the class I education and class I restriction element are discordant from each other. Under no circumstances could D^d-TNP CTL be generated from B10.A mice, i.e., a mouse strain expressing D^d glycoproteins. The explanation for this observation is unclear. It is possible that the development of D^d-TNP CTL is dependent on elements absent in B10.A, and present in BALB/c and (C3H × BALB/c)F₁ mice. Alternatively, cells with TCRs specific for D^d-TNP could be deleted in B10.A mice, but not in BALB/c and (C3H × BALB/c)F₁ mice. If the latter hypothesis were correct, the deletional elements would have to be non-MHC antigens, since all MHC glycoproteins present in B10.A are also present in (C3H × BALB/c)F₁. Because ear-

lier experiments (29, 30) demonstrated that the preferential D^d response was entirely MHC linked, we favor the first hypothesis, i.e., preferential differentiation of D^d-TNP CTL under the influence of non-D^d MHC molecules in BALB/c and in (C3H × BALB/c)F₁ mice. Experiments are currently underway to evaluate the mechanism of the generation of D^d-TNP responses. Another example of a T cell's reactivity and education element being disparate from each other can be found among T cells expressing a TCR with the Vβ17a-β chain. Such cells frequently recognize various allelic forms of the I-E class II MHC molecule (32, 33), or, as more recently proposed (40), different I-E-encoded glycoproteins complexed to a group of peptides selectively expressed in some but not other cells. Either way, such I-E-reactive T cells are generated in I-E⁻ mouse strains such as SWR (33). Assuming that most class II-specific T cells are dependent on class II expression during development (24), we predict that these I-E-reactive T cells are most likely positively selected through interactions with I-A (a testable hypothesis). Nonetheless, while these examples serve to illustrate that sometimes a dichotomy exists between the T cells' education and restriction element, we predict that, as a general rule, the MHC element used for positive selection predicts a T cell's restriction element. Without this rule, there would be no purpose for positive selection.

While the above data demonstrate that TCR-MHC interactions provide signals essential to the differentiation of T cells, it is likely that the selection of the TCR repertoire is additionally influenced by interactions other than those between TCRs and their ligands. It was postulated earlier that the CD4 and CD8 accessory molecules participate in the selection process (41), and more recent studies provide experimental support that this notion is correct, at least for negative selection (42, 43). Whether CD4 and CD8 molecules are also important participants in the process of positive selection remains to be resolved. Blocking of MHC glycoproteins during development may not only affect TCR-MHC interactions, as shown in the present study, but also interfere with significant accessory molecule-MHC interactions. In fact, it is conceivable that interactions between accessory molecules and MHC glycoproteins other than those bound by the TCR on developing T cells may sometimes "skew" the developing repertoire, i.e., generate T cells whose MHC specificity is disparate from the MHC molecules to which their receptors bound, or delete T cells whose TCRs had insufficient affinity for the MHC on which they were selected. Investigations of developing T cells under conditions where the accessory molecules are blocked are currently underway. Whatever the outcome of such experiments, the present results are not incompatible with an additional role for accessory molecule-MHC interactions in development. At the same time, however, our results demonstrate that accessory molecule-mediated signaling alone is not sufficient for generation of CD4⁻CD8⁺ T cells to occur. Instead, the findings suggest that TCR-mediated events represent the primary signals, i.e., when those signals are prevented, remaining CD8-mediated interaction are inconsequential. Clearly, therefore, a crucial interaction that determines T cells' MHC restriction specificity is between TCRs and MHC glycoproteins.

Summary

T cells recognize foreign antigens together with those MHC glycoproteins they have encountered during their development in the thymus. How the repertoire of

antigen-specific TCRs is selected has not yet been fully defined. We have investigated the T cell repertoire specificities of CD4⁻CD8⁺ cytotoxic T cells developing under conditions where one of the class I MHC-encoded molecules is blocked, while other class I-MHC glycoproteins are still expressed. We show that antigen-specific T cells restricted to the blocked class I fail to develop, while generation of other class I-specific T cells proceeds undisturbed. This highly selective perturbation of the T cell receptor repertoire demonstrates that development of CD4⁻CD8⁺ T cells with a certain TCR specificity requires expression of particular alleles of class I MHC. Thus, TCR-MHC interactions provide signals essential to the differentiation of precursor T cells.

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