

CYTOTOXIC T-CELL RESPONSES SHOW MORE RESTRICTED SPECIFICITY FOR SELF THAN FOR NON-SELF *H-2D*-CODED ANTIGENS

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Responses of murine, thymus-derived, cytotoxic lymphocytes (Tc cells)¹ against foreign antigens displayed by various modified-self or H-2 compatible cells, exhibit H-2 restriction (1-3). Thus, Tc cells specifically recognize both foreign antigen and molecules coded in the *K* or *D* regions of the *H-2* complex (4, 5).

In the case of virus-infected self cells, it is apparent that various virus-specified proteins may carry the foreign component essential to the antigenic pattern recognized by Tc cells, thus conferring virus-specificity (6-10). The contribution of *H-2K* and *H-2D* regions has been clarified by the use of *H-2* mutants. Biological and biochemical studies of a series of mutants in the *H-2K* region of the *H-2^b* haplotype have demonstrated first that the classical, serologically-defined H-2 alloantigen molecule bearing private specificities is essential for Tc cell recognition of infected-self cells (11-15), and second, that the specificity of self H-2K antigen recognition by Tc cells is exquisite (11-13, 16). For example, mutant and wild-type H-2K molecules which are qualitatively indistinguishable by serological criteria (17, 18) and which crossreact markedly when recognized by alloreactive Tc cells (19-21), do not crossreact when recognized by Tc cells specific for virus-plus-self (11-13, 16).

Since high specificity of self recognition is an essential characteristic of the H-2 restriction phenomenon, it was investigated further with respect to the specificity of recognition of *H-2D*-coded molecules.

The *D* region of the *H-2^d* haplotype apparently codes for two different 45,000 dalton antigen molecules designated D and D' by Hansen et al. (22). D carries the serologically-defined private specificity H-2.4 and D' carries certain public specificities reactive to anti-H-2.28 sera (22, 23). The experiments reported here employed the BALB/c-*H-2^{db}* mutation, a loss mutation in the *H-2D^d* region which results in a lack of expression on cell surface membranes of D', while D is expressed normally (22, 23). Both D' and D are expressed on the cell membranes of wild-type BALB/c mice. The results show that Tc cells specific for *H-2D^d* coded antigens plus viral or minor histocompatibility (H) antigens on syngeneic

¹ Abbreviations used in this paper: D, *H-2D^d*-coded antigen molecule bearing private specificity H-2.4; D', *H-2D^d*-coded antigen molecule bearing public specificity H-2.28; MLR, mixed lymphocyte reaction; PFU, plaque-forming units; T cell, thymus-derived lymphocyte; T_c cell, cytotoxic T cell.

or H-2 compatible cells, respectively, recognize D but do not detectably recognize D'. On the other hand, BALB/c-*H-2^{db}* Tc cells respond in mixed lymphocyte reactions (MLR) to the D' present on BALB/c cells, and third party responder strains in MLR against BALB/c also produce Tc cells that recognize D'.

Materials and Methods

Animals. A/J, A.TH, A.TL, BALB/c, BALB/c-*H-2^b*, BALB/c-*H-2^{db}*, CBA/H, C3H.OH, C3H.OL, C57BL/6, DBA/1, and SJL/J mice were bred in conventional rooms and both sexes were used at 2-3 mo of age.

Immunization. Mice were immunized with ectromelia virus by intravenous (i.v.) injection of 10^4 plaque-forming units (PFU) of the attenuated Hampstead egg strain (24). Virus-specific memory T cells were obtained from the spleens 3 wk or more postimmunization (25).

Generation of Tc Cells in Vitro. The methods for secondary virus-specific responses, secondary responses to minor H antigens, and primary one-way MLR have been given in detail elsewhere (3, 25-27).

Briefly, secondary virus-specific T_c cells were generated by stimulating splenic memory T cells by coculturing them with syngeneic, ectromelia virus-infected splenic stimulator cells for 5 days at 39°C (nonpermissive for ectromelia replication) at a responder-stimulator ratio of 10:1 (25, 26).

Primary MLR utilized splenic responder cells and γ -irradiated, allogeneic, splenic stimulator cells cultured at 37°C for 5 days at a responder:stimulator ratio of 4:1 (27).

For the response to minor H antigens the method used was similar to that of Bevan (3). Responder mice were primed by i.v. injection of 10^7 spleen cells from appropriate H-2-compatible donor strains. After 2-4 wk spleen cells from the primed mice were stimulated a second time in vitro by coculturing for 5 days at 37°C with irradiated splenic stimulator cells, and Tc cell activity against minor H antigens was then assayed on macrophage targets.

Cytotoxicity Assay with Macrophage Target Cells. The ^{51}Cr release method using macrophages has been described fully elsewhere (13). Statistical significance was determined by Student's *t* test.

Results

Specificity of Tc Cell Recognition of Viral or Minor H Antigens in Association with Self H-2D-Coded Antigen. Previous results showed that ectromelia virus-infected target cells from BALB/c (*H-2^d*) and BALB/c-*H-2^{db}* mice were lysed to a similar extent in a 16-h assay by Tc cells generated during primary viral infection in vivo of various mouse strains which shared only the D region of the *H-2^d* haplotype with the target cells (15). Since BALB/c-*H-2^{db}* cells do not express the D' molecule on their surface membranes whereas BALB/c cells do (22) these original results implied that D' was not detectably recognized in association with virus-specific antigens by these Tc cells. The present studies have confirmed and extended this conclusion. Tc cells from A.TH mice (*K^s*, *D^d*) generated in a secondary response in vitro lysed infected BALB/c and BALB/c-*H-2^{db}* macrophage targets to the same extent and with similar kinetics in short-term assays (Table I).

Similar results (data not shown) have also been obtained with Sendai virus, a paramyxovirus quite different biologically and antigenically from ectromelia virus (a poxvirus).

Since the recognition by Tc cells of minor H antigens on H-2 compatible cells is H-2 restricted in a manner analogous to viral systems, the involvement or otherwise of D' was investigated in two examples of Tc cell responses to minor H antigens.

In the first example B10.A (*K^k*, *D^d*) mice were injected i.v. with A/J (*K^k*, *D^d*) cells and then their primed spleen cells were stimulated in vitro with A/J cells

TABLE I
Similar Lysis of Infected BALB/c-H-2^{db} and BALB/c Macrophage Targets by Virus-Immune A.TH T_c Cells Specific for H-2D-Region-Coded Antigens

Donors of virus-immune T _c cells*			Assay time h	Specific lysis of targets‡			
Strain	H-2K	H-2D		BALB/c-H-2 ^{db}		BALB/c	
				Infected	Uninfected	Infected	Uninfected
A.TH	s	d	4	9.1 ± 1.6	0	15.6 ± 1.7	1.7 ± 0.4
C3H.OL‡§	d	k	4	29.4 ± 4.8	1.9 ± 0.7	31.8 ± 4.3	3.4 ± 0.5
A.TH	s	d	6	25.0 ± 1.2	3.3 ± 1.3	30.5 ± 4.4	1.3 ± 0.9
C3H.OL	d	k	6	38.5 ± 3.9	2.7 ± 0.3	36.0 ± 4.7	3.0 ± 1.3
A.TH	s	d	9.5	40.9 ± 0.8	7.2 ± 1.3	39.4 ± 3.8	9.8 ± 1.8
C3H.OL	d	k	9.5	52.5 ± 6.4	7.1 ± 1.3	46.5 ± 5.9	7.2 ± 2.5

* From secondary responses in vitro. Killer:target ratio was 10:1.

‡ Data given are means of triplicates ± SE of mean with spontaneous release subtracted.

§ C3H.OL T_c cells were used as controls to demonstrate that the intrinsic susceptibility to lysis of infected BALB/c-H-2^{db} and BALB/c targets was similar in the case where recognition was via H-2K-coded antigen (unaltered by the BALB/c-H-2^{db} mutation).

TABLE II
T_c Cells Do Not Detectably Recognize D' in Association with Minor H Antigens on H-2 Compatible Cells

Exp	T _c cells*	Killer target ratio	Percent specific lysis of macrophage targets‡					
			A/J (k kkdd d)§	A.TH (s ssss d)	BALB/c (d dddd d)	BALB/c-H-2 ^{db} (d dddd "db")	BALB/c-H-2 ^b (b bbbb b)	C3H.OH (d dddd k)
1.	B10.A (k kkdd d)	2.5:1	65.3 ± 1.4	45.7 ± 2.3	49.0 ± 1.5	45.8 ± 4.2	2.4 ± 0.6	3.1 ± 0.1
	Anti-A/J (k kkdd d)	0.8:1	40.2 ± 2.0	24.5 ± 0.7	14.1 ± 0.9	11.5 ± 1.6	1.3 ± 0.4	0.8 ± 0.4
2.	DBA/2 (d dddd d)	7.5:1	33.5 ± 1.0	59.6 ± 2.3	9.8 ± 2.0	10.8 ± 1.0		
	Anti-BALB/c	2.5:1	12.6 ± 0.8	33.1 ± 1.2	4.5 ± 2.2	8.7 ± 1.9		
	DBA/2 (d dddd d)	7.5:1	32.4 ± 3.8	60.0 ± 2.0	11.7 ± 4.0	11.8 ± 1		
	Anti-BALB/c-H-2 ^{db}	2.5:1	13.1 ± 2.2	34.8 ± 2.3	2.5 ± 1.0	4.7 ± 2.5		

* As for Table I.

‡ As for Table I.

§ H-2 maps refer to K, I-A, I-B, I-C, S, and D regions.

|| Significantly more lysis than A.TH targets at the same killer target ratio (*P* < 0.001).

and the resulting T_c cells, directed against minor H antigens in association with K^k or D^d-coded molecules, were assayed against various targets. A/J (K^k, D^d) targets were lysed significantly more than A.TH (K^s, D^d) targets, presumably because only T_c cells recognizing minor H plus D^d coded determinants killed A.TH targets, whereas additional T_c cells recognizing minor H plus K^k-coded determinants would kill A/J targets (Table II, exp. 1). BALB/c and BALB/c-H-2^{db} targets were also lysed, presumably by T_c cells recognizing minor H antigens shared by BALB/c and A/J and associated with D^d-coded determinants. Lysis of BALB/c and BALB/c-H-2^{db} targets was similar; assuming the targets had the same intrinsic susceptibility, this result implies that D' (lacking in BALB/c-H-2^{db}) was not recognized in association with a significant number

TABLE III
BALB/c-*H-2^{db}* Anti-BALB/c T_c Cells Recognize Antigenic Patterns Dependent upon the *H-2D^d* Region

T _c cells	Killer: target ratio	Percent specific ⁵¹ Cr release from macrophage targets*				
		BALB/c- <i>H-2^{db}</i> (d dddd "db")‡	BALB/c (d add d)	A.TH (s ssss d)	C3H.OH (d dddd k)	SJL/J (s ssss s)
BALB/c- <i>H-2^{db}</i>	17:1	1.6 ± 1.9	40.6 ± 4.6	41.6 ± 0.8	5.9 ± 1.6	6.0 ± 2.1
Anti-BALB/c	5.6:1	1.0 ± 2.3	32.8 ± 5.7	28.5 ± 2.2	5.3 ± 2.0	7.6 ± 1.3

* See †, Table I.

‡ See §, Table II.

of minor H antigens by the B10.A T_c cells that responded to H-2-compatible A/J cells.

In a second approach to this question, spleen cells from DBA/2 (*H-2^d*) mice previously primed with either BALB/c or BALB/c-*H-2^{db}* cells, were stimulated a second time in vitro with irradiated BALB/c or BALB/c-*H-2^{db}* cells, respectively. T_c cell activity was then assayed against C3H.OL (*K^d*, *D^k*) and A.TL (*K^s*, *D^d*) macrophage targets that would display minor H antigens shared with BALB/c in association with either *K^d*-coded (C3H.OL) or *D^d*-coded (A.TL) determinants. At a given killer:target ratio, C3H.OL targets and nonspecific controls (CBA/H and SJL/J) were lysed to a similar extent by the two T_c cell populations resulting from stimulation by either BALB/c or BALB/c-*H-2^{db}* cells (Table II, exp. 2), thus indicating that the strength of the two responses was similar, and that valid comparison of lysis of A.TL targets was possible. A.TL targets were lysed to a similar extent, regardless of whether stimulation was from BALB/c or BALB/c-*H-2^{db}* cells (Table II, exp. 2). If DBA/2 anti-BALB/c T_c cells recognized significant numbers of minor H antigens in association with *D^d*, then they should have lysed A.TL targets to a greater extent than DBA/2-anti-BALB/c-*H-2^{db}* T_c cells, provided that the responses were approximately equal in other respects as indicated by the controls.

Taken together, these experiments suggest the general rule that *D^d* is not recognized in T_c cell responses against self *H-2D^d*-coded antigens plus foreign antigens. Instead, the *D* molecule is apparently recognized.

Recognition of D^d in T_c Cell Responses against Non-Self or H-2-Incompatible Cells. Primary MLR of BALB-c-*H-2^{db}* anti-BALB/c (*H-2^d*) gave strong T_c cell responses roughly comparable to other combinations with differences only in *K* or *D* regions (Tables III and IV). A.TH targets were lysed as much as BALB/c targets (Table III) though they share only the *D^d* region with BALB/c, whereas C3H.OH targets, which share all of the *H-2* gene complex with BALB/c, except for the *D* region, were lysed no more than SJL (*H-2^s*) nonspecific control targets (Table III). These data thus confirm other serological and cell-mediated results that map the BALB/c-*H-2^{db}* mutation in the *D* region (23).

Serologically, *D^d* does not bear private H-2 specificities, but reacts with antibodies present in antisera raised against the H-2.28 family of public H-2 specificities (22, 23). T_c cell responses against *D^d* also displayed marked crossreactivity; thus targets from other mouse strains bearing the H-2.28 family

TABLE IV
BALB/c-H-2^{db} Anti-BALB/c T_c Cells Recognize Mainly "Public" Rather Than "Private"
H-2 Determinants

Strain	Target cells		Titer with BALB/c-H- 2 ^{db} anti- BALB/c se- rum‡	Percent specific lysis* with BALB/c-H-2 ^{db} anti-BALB/c T _c cells at killer:target ratio	
	H-2	Serologically defined pub- lic specifici- ties of the "H-2.28 fam- ily"		10:1	3:1
BALB/c	d	27, 28, 29	32	62.8 ± 0.6	46.6 ± 2.7
DBA/1	q	27, 28, 29	64	62.4 ± 2.9	28.4 ± 1.5
C57BL/6	b	27, 28, 29	32	48.0 ± 4.1	25.1 ± 3.5
SJL/J	s	28	0	18.3 ± 3.6	3.6 ± 1.4
(DBA/1 × BALB/c-H- 2 ^{db})F ₁				42.5 ± 3.4	19.9 ± 2.9
(C57BL/6 × BALB/c-H- 2 ^{db}) ₁				26.0 ± 2.9	9.2 ± 3.6

* See ‡, Table I.

‡ Data from McKenzie et al. (23).

of specificities were also lysed significantly by BALB/c-H-2^{db} anti-BALB/c T_c cells (Table IV). The T_c cell crossreactivities were, however, not as prominent as in the serology, particularly in the case of C57BL/6 (H-2^b). This may mean that T_c cells recognize different determinants on D' from those defined serologically, as previously suggested by results using mutants in the K region of the H-2^b haplotype (18-20), or that T_c cell receptor affinity is lower than that of most immunoglobulins (16, 21).

The marked T_c cell crossreactivity seen in Table IV argues against the idea that the response to D' is H-2 restricted i.e., that determinant(s) on the D' molecule are recognized mainly or exclusively in association with determinant(s) unique to the H-2^d haplotype. This possibility was tested further by the use of targets from F₁ hybrids between DBA/1 or C57BL/6 and BALB/c-H-2^{db} (Table IV). The BALB/c-H-2^{db} genome codes for all other detectable H-2^d determinants other than those on D'. Thus, if the BALB/c-H-2^{db} anti-D' responses were H-2 restricted, the F₁ targets should be killed more efficiently than DBA/1 or C57BL targets. However, the converse was observed; they were lysed less (Table IV), thus confirming the lack of H-2 restriction.

Is there a T_c cell response to D' in MLR in which there are concurrent responses to other H-2K or H-2D antigen molecules? C57BL/6 (H-2^b) anti-BALB/c (H-2^d) T cells lysed A.TL (K^s, D^d) or A.TFR2 (K^l, D^d) targets significantly more than did C57BL/6 anti-BALB/c-H-2^{db} T_c cells, whereas C3H.OL (K^d, D^k) targets were lysed equally by both T_c cell populations (Table V, exp. 1). These data suggest that more C57BL/6 T_c cells reactive to H-2D-coded antigens were stimulated by BALB/c than by BALB/c-H-2^{db} cells. A response to determinants on D' (other than those shared by C57BL/6 and BALB/c) would account for this.

TABLE V
Evidence for a T_c Cell Response to D' in Allogenic MLR

Exp.	T_c cells	Percent specific lysis of macrophage targets*				
		A.TL (s kkkk d)‡	A.TFR2 (f fffs d)	C3H.OL (d dddk k)	CBA/H (k kkkk k)	SJL/J (s ssss s)
1	C57BL/6 Anti-BALB/c (b bbbb b) (d dddd d)	70.8 ± 0.9	68.3 ± 0.3	51.3 ± 1.4	18.3 ± 3.4	15.5 ± 0.6
	C57BL/6 anti-BALB/c- $H-2^d$ (b bbbb b) (d dddd "db")	56.9 ± 1.8§	56.1 ± 3.1§	51.8 ± 4.5	15.0 ± 3.4	15.2 ± 3.6
2.	CBA/H Anti-A.TFR5 (k kkkk k) (f fffk d)	CBA/H (k kkkk k)	A.TH (s ssss d)	DBA/1 (q qqqq q)		
		10.6 ± 0.9	86.9 ± 1.8	49.4 ± 3.2		

* See †, Table I; killer:target ratio was 3:1

‡ See §, Table II

§ Significantly less lysis than that caused by C57BL/6 anti-BALB/c T_c cells on the same target cells ($P < 0.02$).

Furthermore, CBA/H ($H-2^k$) anti-A.TFR5 (K^f , D^d), T_c cells which lysed A.TH (K^s , D^d) targets, crossreacted markedly on DBA/1 ($H-2^q$) targets that share determinants with D' (Table V, exp. 2). Thus a T_c cell response to determinants on D' probably occurred in both these MLR. Since other workers have reported similar crossreactivity involving the H-2.28 family of public determinants in MLR-generated T_c cells (28), these data suggest the general rule that D' is recognized in T_c cell responses against $H-2D^d$ -coded antigens where such antigens are non-self.

Discussion

Highly specific recognition of self H-2 antigens by T_c cells reactive to virus-infected self cells, or reactive to minor H antigens on uninfected H-2-compatible cells, is essential to the phenomenon of H-2 restriction. If such T_c cells were crossreactive or recognized determinants which are shared by different H-2K or H-2D antigens (i.e., public determinants), then H-2 restriction would of course, not be observed.

The present experiments illustrate this point with respect to recognition by T_c cells of the two antigens D and D' coded in the $H-2D^d$ region. D carries private, and D' carries public determinants (22, 23). Apparently, T_c cells reactive to virus-infected self cells, or T_c cells reactive to minor H antigens plus $H-2D^d$ -coded antigens on H-2-compatible cells recognize determinant(s) on the self D molecule together with physically associated non-self antigen(s). They do not recognize D' in detectable numbers. Since similar results were obtained with two very different viruses, ectromelia (a poxvirus) and Sendai (a paramyxovirus), and with two different minor H antigen systems, it seems reasonable to propose that these data reflect the general rule that recognition of self $H-2D^d$ -coded antigens by H-2 restricted T_c cells involves antigenic determinant(s) on the D molecule, not on D' .

One possible reason for this might be that D' does not associate physically with other antigens in cell membranes. Available data suggest that this is not the case. First, there is evidence that D and D' co-cap (29). Second, antisera specific for D or for D' can block virus-specific T_c cell-mediated lysis of virus-infected targets, thus indicating that both molecules are physically close to

virus-specific determinants in infected cell surfaces (15). Other reasons must, therefore, be considered. For example, the dictionary of Tc cell antigen-receptors employed in responses against infected self cells may not encompass D' determinant(s). This possibility is discussed further below.

In contrast to the case of recognition of infected self, D' is recognized by Tc cells which have developed in its absence. Thus, Tc cells from BALB/c-*H-2^{ab}* (D' negative) mice respond strongly in MLR to the D' antigen of BALB/c cells. D' is apparently recognized as a major alloantigen in its own right, rather than as a minor H antigen associated with D, i.e., as an example of "H-2 restriction". The evidence for this is threefold. First, BALB/c-*H-2^{ab}* anti-BALB/c Tc cells cross-react markedly on DBA/1 (*H-2^a*) and C57BL/6 (*H-2^b*) target cells, both of which display the "H-2.28 family" of public specificities but do not share the D molecule coded in the *H-2^d* haplotype of BALB/c which would be required for H-2 restricted recognition. Second, F₁ hybrid targets of the type (DBA/1 × BALB/c *H-2^{ab}*) or (C57BL/6 × BALB/c-*H-2^{ab}*) are killed less by such crossreactive Tc cells than DBA/1 or C57BL/6 targets. If the rules of H-2 restriction applied to this response, F₁ targets should be killed more than targets from DBA/1 or C57BL/6, since the D molecule is contributed by the BALB/c-*H-2^{ab}* haplotype of the F₁ hybrids. Third, Tc cells that recognize D' appear to comprise a significant part of the Tc cell populations that respond to *H-2D^d*-coded antigens in other MLR, e.g., C57BL/6 anti-BALB/c, and CBA/H anti-A.TFR5.

It would seem, therefore, that with respect to Tc cell recognition D' is in a category similar to I region-coded antigens. Such antigens can be recognized by some clones in allogeneic Tc cell populations, (30-32), albeit at a lower frequency than H-2K or H-2D antigens, but they are not directly involved in the recognition by Tc cells of virus-infected self cells, or H-2 compatible cells bearing non-self minor H antigens (3-5).

Another interesting aspect of the present results is their implication for mechanisms by which the antigen-receptor dictionary of the Tc cell pool is governed, presumably in the thymus during the differentiation of stem cells into mature, antigen-sensitive precursor Tc cells as illustrated by recent results of Bevan (33) and Zinkernagel et al. (34). This differentiation process presents an intriguing problem. On the one hand, Tc cell receptors must be diverse enough to recognize any potential viral pathogen that poses a threat to host survival. On the other hand, it seems that an H-2K or H-2D molecule is almost invariably recognized simultaneously with the foreign antigen, i.e., there is a restricted repertoire of determinants characteristic of *H-2K*- or *H-2D*-coded molecules that must also be recognized at the induction and effector phases of Tc cell operation.

Results from chimeric mice (33, 34) grafted with allogeneic thymic epithelium after removal of their own thymus (34), have fostered speculation that the thymic influence on Tc cell differentiation incorporates a selection or screening process in which progenitors are selected for further maturation by their capacity to recognize the *H-2K*- or *H-2D*-coded determinants displayed by self thymic epithelial cells (34, 35). If this is the case, why aren't Tc cells reactive to self D' selected? One trivial explanation might be that D' is not expressed on thymic epithelial cell surface membranes. This is testable with available antisera. Alternatively, the screening process in the thymus may be more

complex, and may permit only certain categories of self H-2 recognition to maintain self tolerance (36-39). Further investigation of factors controlling T-cell differentiation in the thymus should resolve this question.

Summary

The specificity of recognition of H-2 antigens by various subsets of Tc cells was investigated with respect to the two separate molecules known to be coded in the *H-2D^d* region (a) D which carries the private specificity H-2.4 and (b) D' which carries the public specificity H-2.28. BALB/c-*H-2^{ab}* mutant mice express D but not D' on their cell surfaces, whereas wild-type BALB/c mice express both D and D'. H-2 restricted Tc cells specific for viral-plus-*H-2D^d*-coded antigens on infected self cells, or minor H-plus-*H-2D^d*-coded antigens on H-2-compatible cells apparently recognize D, but do *not* detectably recognize D'. In contrast, BALB/c-*H-2^{ab}* anti-BALB/c Tc cell responses do recognize D' (the only known antigen which is not shared by mutant and wild-type); furthermore, D' is also detectably recognized by a significant proportion of the Tc cells that respond in MLR to *H-2D^d*-coded antigens. In these latter responses, D' was recognized separately from D, i.e., the response was not "H-2 restricted". These results indicate that H-2 restricted Tc cell responses to modified-self cells are more specific for self H-2D^d-coded antigens than are allogeneic Tc cell responses directed at the same antigens, in that haplotype-unique (private) specificity recognition (of the D molecule) exclusively occurs only in the former, not the latter case. The implications of this specificity of H-2 restricted responses for possible processes of somatic selection of anti-self recognition structures on progenitor Tc cells are briefly discussed.

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