Brief Definitive Report

POSITIVE AND NEGATIVE SELECTION OF T CELL RECEPTOR Vβ DOMAINS CONTROLLED BY DISTINCT CELL POPULATIONS IN THE THYMUS

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The TCR repertoire is shaped by positive and negative selection events occurring within the thymus. Recent genetic analysis in transgenic and normal mice has implicated recognition of self MHC molecules by TCR in both positive (1–5) and negative (6–12) selection processes, thus raising the issue of how molecular discrimination between the two events may occur. Historically, this question has been addressed by functional assays of MHC-restricted immune responses in radiation bone marrow chimeras, but results in this complex system are frequently equivocal (reviewed in reference 13).

Recently (9), we reported that a TCR β chain V domain ($V_{\beta6}$) confers reactivity to the Mls-1^a antigen, and that $V_{\beta6}{}^+$ T cells are clonally deleted in Mls-1^a mice that express MHC class II gene products (especially I-E). Positive selection of the same TCR $V_{\beta6}$ domain was observed in mouse strains that express I-E but not Mls-1^a (3). This system thus provides a convenient and direct method to address the role of MHC class II–expressing cells in the thymic selection process by quantitating $V_{\beta6}{}^+$ cells in allogeneic radiation bone marrow chimeras where donor and host differ in expression of I-E and/or Mls-1^a. We show here that largely (if not entirely) nonoverlapping thymic cell populations are responsible for positive and negative selection of $V_{\beta6}{}^+$ T cells during development.

Materials and Methods

Animals. Inbred B10.BR, B10.D2, and DBA/2 mice were purchased from the Institute für Zuchthygiene, Tierspital, University of Zürich, Switzerland. B10.G and DBA/1 mice were obtained from Olac, Bicester, Oxon, UK. The characteristics of these strains relevant to the present study are summarized in Table I.

Chimeras. Recipient mice were lethally irradiated (950 rad, 117 rad/min, ^{137}Cs source) and reconstituted 1 d later with 5×10^7 T cell–depleted bone marrow cells obtained from the femora and tibia of donor mice (14). T cell depletion was performed using anti-Thy-1.2 mAb HO 13-3-9 and a polyclonal mouse serum plus rabbit complement. The transplanted

mice had a survival rate of >90%. Chimeras were analyzed not earlier than 10 wk after reconstitution.

mAbs and Flow Microfluorometry. Cytotoxic rat IgM mAb against CD8 (3.168.1) was used in the presence of rabbit complement to deplete thymocyte suspensions of CD8$^+$ cells (3). For immunofluorescence, aliquots of thymocytes or lymph node cells were stained at 4°C with rat mAbs 44-22-1 (anti-V$_{\beta6}$), KJ16-133 (anti-V$_{\beta8.1}$/V$_{\beta8.2}$), or KT11 (anti-V$_{\beta11}$) followed by a fluorescent goat anti-rat second reagent. For two-color immunofluorescence, additional incubations with phycoerythrin-conjugated mAb GK1.5 (anti-CD4) were done. To monitor the chimerism of transplanted mice, haplotype-specific IgG2a mAbs 100-27.55 (anti-K^{k}D^k), 34-1-2 (anti-K^{d}D^d, crossreactive with K^{q}D^q), 100-5.28 (anti-K^{k}D^k, crossreactive with K^{q}D^q), and 34-2-12 (anti-K^{d}D^d) were revealed by a fluorescent goat anti-mouse IgG2a reagent (Southern Biotechnology Associates Inc., Birmingham, AL). Single and two-color immunofluorescence were performed as described previously (9).

Results and Discussion

Positive selection was first analyzed in classical F$_1$ → parent chimeras. We showed previously that B10.BR mice (E^k) had about twice as many V$_{\beta6}^+$ cells in the CD4$^+$ subset as congenic B10.G (E$^-$) mice, and that the high expression of V$_{\beta6}$ was dominant in F$_1$ animals, as would be expected for positive selection (3). When (B10.BR × B10.G)F$_1$ bone marrow was used to reconstitute irradiated B10.BR or B10.G hosts, it was apparent that the increased levels of V$_{\beta6}^+$ CD4$^+$ cells correlated with the genotype of the irradiated thymus (Fig. 1 and Table II). Thus, F$_1$ → B10.BR chimeras had 7-8% V$_{\beta6}^+$ CD4$^+$ cells (comparable with control B10.BR → B10.BR), whereas F$_1$ → B10G mice expressed much lower levels (3-5%), equivalent to B10G → B10G controls. The proportion of V$_{\beta6}^+$ CD4$^+$ cells was identical in thymus and peripheral lymph nodes of chimeric animals (Table II), indicating that positive selection was most likely occurring within the thymus. Similar results were obtained in fully allogeneic chimeras in a situation where negative selection via Mls-1^a did not apply (see below); thus, V$_{\beta6}^+$ CD4$^+$ cells were twice as frequent in B10.G → DBA/2 (E^d) as compared with B10.G → DBA/1 (E$^-$) mice (Table III).

The predominant role of radioresistant thymic cells in positive selection of TCR V$_{\beta6}$ domains is consistent with most (but not all) classical functional studies of MHC restriction in radiation bone marrow chimeras (13). Furthermore, our data are compatible with recent reports indicating that both transgenic (1) and V$_{\beta17a}^+$ (5) TCR are positively selected by radioresistant thymic MHC components.

TABLE I

Characteristics of Mouse Strains Used in this Study

Strain	Mls-1	MHC class II		Percent V$_{\beta6}^+$CD4$^+$
		I-A	I-E	
B10.G	b	q	–	3.8 ± 0.2
B10.BR	b	k	k	8.8 ± 0.3
B10.D2	b	d	d	9.3 ± 0.2
(B10.G × B10.BR)F$_1$	b/b	q/k	–/k	9.5 ± 0.2
DBA/1	a	q	–	4.2 ± 0.5
DBA/2	a	d	d	0.4 ± 0.2

V$_{\beta6}^+$CD4$^+$ T cells (mean ± SD of at least three individual mice) were quantitated by two-color immunofluorescence. Most of these data have appeared previously (3).
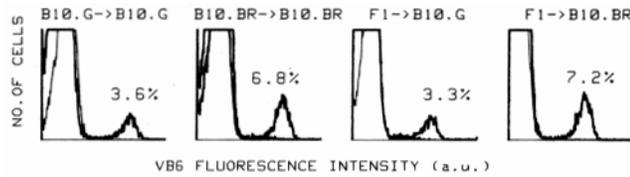
FIGURE 1. Positive selection of $V_{\beta 6}{}^+$ CD4$^+$ thymocytes in F$_1$ → parent chimeras. CD8-depleted thymocytes from the indicated radiation bone marrow chimeras were stained with anti-$V_{\beta 6}$ mAb 44-22-1 followed by fluorescent anti-rat Ig (*thick lines*) or with the conjugate alone (*thin lines*). Percent positive cells is indicated for individual mice tested in the same experiment. Complete data are summarized in Table II.

TABLE II

Positive Selection of $V_{\beta 6}{}^+$CD4$^+$ T Cells in F$_1$→Parent Irradiation Bone Marrow Chimeras

Tissue	Surface expression of	B10.G B10.G (5)	B10.BR B10.BR (5)	F$_1$ B10.G (5)	F$_1$ B10.BR (4)
Lymph node	$V_{\beta 6}$	3.7 ± 0.4	7.3 ± 0.8	4.9 ± 0.7	7.3 ± 0.6
	$V_{\beta 8}$	14.5 ± 0.7	13.2 ± 1.6	20.8 ± 2.1	12.4 ± 2.0
	H-2^q	96.6	0.3	95.0 ± 1.3	88.1 ± 2.3
	H-2^k	3.1	94.1	90.0 ± 4.0	87.2 ± 3.1
Thymus	$V_{\beta 6}$	3.8 ± 0.4	6.5 ± 0.5	3.8 ± 0.4	7.4 ± 0.2
	$V_{\beta 8}$	16.6 ± 1.0	13.5 ± 1.0	20.3 ± 1.0	12.5 ± 1.8
	H-2^q	ND	ND	99.1 ± 0.7	98.7 ± 0.6
	H-2^k	ND	ND	83.2 ± 0.6	89.3 ± 7.3

Upper strain is the donor and lower is the host. Numbers of mice are in parentheses. Data for V$_\beta$ expression are percent positive cells (mean ± SD normalized to total CD4$^+$ subset). Chimerism was assessed by MHC class I expression.

TABLE III

Clonal Deletion of CD4$^+$ T Cells Expressing $V_{\beta 6}$ or $V_{\beta 11}$ in Fully Allogeneic Irradiation Bone Marrow Chimeras

Tissue	Surface expression of	B10.G DBA/2 (4)	B10.G DBA/1 (4)	B10.D2 DBA/2 (4)	B10.D2 DBA/1 (4)
Lymph node	$V_{\beta 6}$	6.8 ± 0.9	3.4 ± 0.1	0.4 ± 0.5	1.0 ± 0.4
	$V_{\beta 8}$	13.3 ± 1.5	17.7 ± 1.1	13.9 ± 1.6	20.2 ± 0.9
	$V_{\beta 11}$	5.1 ± 1.4	6.5 ± 0.5	0.8 ± 0.1	0.9 ± 0.3
	H-2^q	97.2 ± 2.2	97.9 ± 0.4	1.9 ± 0.2	4.8 ± 3.4
	H-2^d	3.7 ± 3.2	1.9 ± 1.2	98.1 ± 0.8	96.8 ± 1.3
Thymus	$V_{\beta 6}$	7.3 ± 0.6	3.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.5
	$V_{\beta 8}$	10.8 ± 0.3	9.3 ± 0.3	11.0 ± 0.4	11.7 ± 1.3
	$V_{\beta 11}$	4.0 ± 0.4	4.6 ± 0.0	0.8 ± 0.1	1.0 ± 0.1

Upper strain is the donor and lower is the host. Numbers of mice are in parentheses. Data for V$_\beta$ expression are percent positive cells (mean ± SD normalized to total CD4$^+$ subset). Chimerism was assessed by MHC class I expression.

The nature of the cells involved in negative selection (clonal deletion) of $V_{\beta 6}{}^+$ TCR is more complicated to address because of a dual requirement for expression of Mls-1^a and I-E genes. However, we (15) and others (10) have recently shown that the Mls-1^a and I-E requirements can be genetically complemented in radiation bone marrow chimeras, such that $V_{\beta 6}{}^+$ cells are deleted in situations where deletion does

not occur in either donor or host genotype alone. Table III shows an example of such genetic complementation in a B10.D2 (Mls-1^b, I-E^d) → DBA/1 (Mls-1^a, I-E$^-$) chimera. The molecular mechanism underlying this phenomenon remains unclear, but the simplest interpretation would be that the host Mls-1^a gene product can be transferred to (and presented by) donor I-E$^+$ cells. A similar explanation could also account for the apparently MHC-unrestricted functional tolerance to minor histocompatibility antigens previously observed in some bone marrow chimeras (reviewed in reference 16).

When congenic I-E$^-$ (B10G) or I-E$^+$ (B10.D2) Mls-1^b bone marrow cells were used to reconstitute an I-E$^+$ Mls-1^a host (DBA/2), deletion of V$_{\beta 6}^+$ CD4$^+$ cells (either in thymus or periphery) was only observed in the latter instance (Table III). Since deletion was also seen in the B10.D2 → DBA/1 combination referred to above (where there is no I-E expression on radioresistant host cells), these data imply that I-E expression on radiosensitive donor cells is both necessary and sufficient to induce clonal deletion of V$_{\beta 6}^+$ cells in Mls-1^a mice, irrespective of the source of Mls-1^a. This phenomenon was not peculiar to the Mls-1^a model system, since deletion of V$_{\beta 11}^+$ CD4$^+$ cells (which depends upon I-E and an unknown minor antigen [11, 12]) followed exactly the same pattern as V$_{\beta 6}$ deletion (Table III).

It should be noted that consistent differences in V$_{\beta 8}$ expression were also observed in certain chimeras (Tables II and III); however, since no correlation has been established between expression of V$_{\beta 8.2}$ (the most frequently used V$_{\beta 8}$ family member recognized by KJ16 mAb) and reactivity to MHC or Mls antigens, it is not possible to conclude whether these biases reflect positive and/or negative selection events.

The formal requirement for I-E$^+$ radiosensitive (i.e., hematopoietic-derived) thymocytes to induce clonal deletion of V$_{\beta 6}^+$ T cells in Mls-1^a mice is again consistent with the majority of earlier chimera studies (13). Furthermore, the failure of radioresistant thymus cells to impose detectable clonal deletion in this system concurs with the ineffectiveness of transplanted thymic epithelial grafts to induce functional tolerance (13) or clonal deletion (17) with respect to the engrafted MHC antigens. In apparent contrast to these findings, however, is the recent report of Kisielow et al. (6), showing that male (HY)-specific H-2D^b-restricted transgenic TCR were deleted in irradiated H-2^b male mice reconstituted with transgenic H-2^b female bone marrow, leading the authors to conclude that deletion depended upon radioresistant thymic components expressing HY. If, however, the HY antigen (like Mls-1^a) can be transferred from one cell to another in vivo, as suggested by earlier studies (18), then these transgenic experiments would also be compatible with deletion being controlled by radiosensitive hematopoietic cells. Alternatively, it is possible that expression of the transgenic TCR at abnormally high levels may result in a nonphysiologic deletion of immature thymocytes upon encounter with epithelial thymic components. Reciprocal allogeneic chimeras (differing in H-2D^b expression) will be required to resolve this issue in the transgenic model.

Collectively, the data obtained in the V$_{\beta 6}$ system point to a model in which largely (if not exclusively) nonoverlapping populations of thymic cells deliver the signals necessary to initiate positive and negative selection events during development. Although not addressed directly here, it seems likely that the radioresistant component responsible for positive selection is a cortical epithelial cell (19), while the radiosensitive component required for clonal deletion is a medullary dendritic cell (20).

In fact, localization of $V_{\beta6}{}^+$ cells on thymic sections in congenic Mls-1^b or Mls-1^a mice is consistent with clonal deletion occurring at the corticomedullary junction (21), a region noted to be rich in cells of dendritic morphology (22). In the same study, no evidence was obtained for positive selection of $V_{\beta6}{}^+$ cells in the thymus corex; however, it is not clear whether the Mls-1^a antigen would contribute additional avidity for positive selection of $V_{\beta6}$ TCR not already provided by the I-E molecule itself (see reference 23 for discussion).

Finally, it should be noted that the compartmentalization of positive and negative selection events (involving distinct I-E-expressing cells) in the thymus does provide several potential explanations for the differing outcomes on developing T cells. In this context, differences in ligand (I-E and/or peptide) densities or production of different cofactors by the appropriate thymic component could induce distinct (positive or negative) signals upon TCR engagement. Alternatively (as originally proposed for B cells [24]), it is possible that positive and negative selection reflect programmed differences in the response of developing T cells to a conserved stimulus, and that the observed compartmentalization of selecting cells is due to anatomical (rather than mechanistic) constraints.

Summary

The role of thymic MHC class II–bearing cells in the selection of the TCR repertoire has been investigated in allogeneic radiation bone marrow chimeras. Positive selection of mature CD4$^+$ T lymphocytes expressing the $V_{\beta6}{}^+$ TCR domain was found to depend upon radioresistant (presumably epithelial) I-E$^+$ thymic cells. On the other hand, negative selection of CD4$^+$ $V_{\beta6}{}^+$ cells (which was additionally dependent upon expression of the Mls-1^a gene product) was controlled by a radiosensitive I-E$^+$ thymic component (most likely dendritic cells). These data argue in favor of a compartmentalization of positive and negative selection events during T cell development.

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