Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6301–6305, August 1990
Immunology

Thymus: A direct target tissue in graft-versus-host reaction after allogeneic bone marrow transplantation that results in abrogation of induction of self-tolerance

(clonal elimination/autoreactive T cells/thymic stroma/secondary disease)

NORIKO FUKUSHI, HISASHI ARASE, BINGYAN WANG, KAZUMASA OGASAWARA, TOSHIHIKO GOTOHDA, ROBERT A. GOOD*, AND KAZUNORI ONOÉ†

Institute of Immunological Science, Hokkaido University, Sapporo 060, Japan; and *Department of Pediatrics, All Children's Hospital, University of South Florida, Saint Petersburg, FL 33701

Contributed by Robert A. Good, May 11, 1990

ABSTRACT **Graft-versus-host reaction (GVHR) following allogeneic bone marrow (BM) transplantation was investigated by analyzing expression of antigen receptors on T cells specific for recipient antigens. GVHR chimeras were prepared by transplanting mixtures of splenic T cells and T-cell-depleted BM cells from B10 (I-E$^-$, Mls-1^b) or B10.AQR (I-E$^+$, Mls-1^b) mice into lethally irradiated AKR/J (I-E$^+$, Mls-1^a) recipients. Increased proportions of $V_\beta6^+$ T cells reactive to recipient antigens (I-E and Mls-1^a) were observed in thymuses from such chimeras 1 or 5 wk after BM transplantation. $V_\beta6^+$ T cells observed 1 wk after BM transplantation were derived from mature T cells that had been inoculated into recipients. These cells responded to recipient antigens expressed in the thymus. After 5 wk, thymocytes brightly positive for $V_\beta6^+$ were shown not to descend from mature T cells but to differentiate from precursor cells present in the BM inocula. Since $V_\beta6^+$ T cells were eliminated in thymuses from non-GVHR chimeras 5 wk after BM transplantation using T-cell-depleted BM cells alone, it appears that GVHR occurring in the thymus at an early stage abrogates thymic stromal functions essential to induction of self-tolerance in the T-cell repertoire. These findings propose a mechanism (autoimmunity) to explain in part the pathogenesis of chronic GVHR.**

Graft-versus-host reaction (GVHR) has assumed importance with the expanding use of allogeneic bone marrow transplantation (ABMT) to treat diseases for which no alternative therapy exists (1–4). GVHR occurs when mature T cells in the bone marrow (BM) graft react immunologically against histocompatibility antigens of recipient type (5–9). GVHR may have diverse features, depending upon the genetics of donor and recipient, cell populations of donor, and recipient antigen systems (10, 11).

Donor CD4$^+$ and CD8$^+$ T cells respond vigorously to antigens of recipient (5, 6, 12), although these two subsets may play different roles in the pathogenesis of GVHR (6, 12, 13) and may also influence each other (12, 14). Despite the development of reagents for definition of T-cell subsets, analysis of GVHR in the combined histopathological and cellular-immunological perspective is hampered by difficulty in determining whether proliferating lymphocytes in different lymphoid regions represent immunologic reactions or whether this lymphoid proliferation merely reflects physiological lymphopoiesis *in situ*. Another difficulty is the complexity of processes that underlie histopathological changes observed in the lymphoid tissues (15, 16). It has been argued, for example, that during GVHR, stress-induced activation of the pituitary-adrenal axis may lead to hypersecretion of corticosteroid hormones and to thymic involution (17). To simplify further study of GVHR, we sought to determine whether thymus is a direct target of GVHR.

MATERIALS AND METHODS

Mice. AKR/J (AKR) (H-2^k, Mls-1^a) were obtained from The Jackson Laboratory; C57BL/10 (B10) (H-2^b, Mls-1^b) (AQR), and B10.BR(BR) (H-2^k, Mls-1^b) mice were purchased from Shizuoka, Hamamatsu, Japan. B10.AQR (AQR) (H-2^{y1}, Mls-1^b), [B10 × B10.A(4R) (H-2^{h4}, Mls-1^b)]F$_1$ (B10 × 4R)F$_1$, and (B10 × AQR)F$_1$ mice were maintained at Hokkaido University.

ABMT. Irradiation chimeras were prepared as described (18). BM cells were treated with anti-Thy1.2 (F7D5, Olac, Bichester, U.K.) plus selected rabbit C prior to i.v. injection of 2.5 × 10^7 cells into recipient mice after lethal [1000 R (0.258 mC/kg)] total body irradiation (36 R/min). GVHR chimeras were prepared by reconstituting irradiated AKR mice with a mixture of T-cell-depleted BM cells and nylon-wool-purified splenic T cells from various strains.

Surface Marker Analysis. Fluorescence-activated cell sorter (FACS) analysis was carried out according to Arase *et al.* (19). Thymuses and spleens were removed from chimeras 1 or 5 wk following ABMT. Thymocytes were treated with different monoclonal antibodies: 2C11 (anti-ε chain of the CD3 complex), F23.1 [anti-Vβ8.1, -8.2, -8.3 of T-cell receptor for antigen (TCR)], 44-22-1 [anti-Vβ6 of TCR (20)], or anti-Thy1.2 and then biotinylated anti-mouse or rat immunoglobulin (Vector Laboratories). Duochrome (Becton Dickinson) was added to cell suspensions. Binding sites of the monoclonal antibodies were blocked using purified mouse or rat immunoglobulin, and phycoerythrin (PE)-anti-CD4 and fluorescein isothiocyanate-anti-CD8 (Becton Dickinson) were added. F23.1 was purchased from American Type Culture Collection; 2C11 and 44-22-1 were provided by J. A. Bluestone (University of Chicago) and H. Hengartner (University Hospital, Zurich), respectively.

To study the origin of $V_\beta6$ thymocytes or splenocytes, cells were incubated with anti-K^k, -K^b, -D^d (Meiji Institute of Health, Kanagawa, Japan), Thy1-1 (T11D7e; Olac, Bichester, U.K.), or Thy1.2 and then FITC anti-mouse immunoglobulin. Nonspecific staining was blocked by adding mouse immunoglobulin, and then biotinylated $V_\beta6$ and PE-streptavidin (Biomeda, Foster City, CA) were added. $V_\beta6^+$ T cells were gated by red fluorescence. Stained cells were analyzed using FACScan and several kinds of gating. Dead

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ABMT, allogeneic bone marrow transplantation; BM, bone marrow; GVHR; graft-versus-host reaction; FACS, fluorescence-activated cell sorter; mAb, monoclonal antibody; PE, phycoerythrin; SP, single positive; TCR, T-cell receptor.

†To whom reprint requests should be addressed.

Table 1. Analysis of CD4, CD8 expression on thymocytes from [B10 → AKR] or [AQR → AKR] chimeras 1 wk after ABMT

Mice	Splenic T cells	Proportion of thymocyte subsets, %			
		$CD4^+CD8^-$	$CD4^+CD8^+$	$CD4^-CD8^-$	$CD4^-CD8^+$
[B10 → AKR]	—	19.0	16.3	48.7	16.0
[B10 → AKR]	1×10^5	41.2	6.5	29.8	22.5
[AQR → AKR]	—	6.3	39.1	47.7	6.9
[AQR → AKR]	1×10^5	13.3	24.0	49.9	12.8

Thymocytes were pooled from four chimeras of each donor/recipient combination. Approximately 1×10^6 or 1.2×10^6 cells were recovered from thymuses of control or GVHR [B10 → AKR] chimera, respectively.

cells and debris were excluded using forward and side light scatter and, in some cases, red fluorescence from propidium iodide staining (Sigma). The number of cells analyzed varied from 5000 to 50,000.

Immunohistology. Immunohistological examination was as described by van Ewijk *et al.* (21). Thymuses from BM chimeras were embedded in OCT compound (Miles) and frozen in liquid nitrogen. Sections were allowed to react with primary mAb (44-22-1) overnight at 4°C and then stained with avidin-biotin complex (ABC) kit (Vector) and counterstained with methyl green solution.

Statistical Analyses. Statistical analyses were carried out using Student's *t* test. *P* values > 0.05 were considered not significant.

RESULTS

Mature T Cells Appeared in the Thymus of GVHR Chimeras. Addition of >5% splenic T cells from B10 mice to T-cell-depleted B10 BM prior to i.v. injection caused severe GVHR and death in supralethally irradiated AKR recipients. AKR recipients given 2.5×10^4–1.5×10^5 splenic T cells in BM inocula showed chronic, mild GVHR symptoms (e.g., hunched posture, ruffled fur, and slight weight loss) 6 wk after ABMT. We thus chose to add 1×10^5 T cells per mouse T-cell-depleted B10 BM cells (2.5×10^7 per mouse) to prepare [B10 → AKR] chimeras (GVHR chimeras). AKR recipients given T-cell-depleted B10 BM alone (control recipients) showed no signs of GVHR (8, 9, 18, 19, 22–24).

T-cell subsets in thymuses from GVHR or control chimeras were analyzed 1 wk following ABMT. Table 1 compares representative data for expression of CD4 and/or CD8 on thymocytes pooled from 4 GVHR or control [B10 → AKR] chimeras. Higher proportions of $CD4^+$ or $CD8^+$ single positive (SP) cells were observed in thymuses from GVHR chimeras than from control chimeras. A similar but less marked difference was obtained with [B10.AQR → AKR] Ia-compatible chimeras in which irradiated AKR mice were transplanted with T-cell-depleted BM cells alone or mixtures of T-cell-depleted BM and B10.AQR splenic T cells.

Origin of $CD4^+$ or $CD8\ SP^+$ Cells in Thymuses of GVHR Chimeras. Cellular origins of the CD4 or CD8 SP populations in thymuses from [B10 → AKR] chimeras were analyzed using anti-Thy1 mAb and Duochrome (three-color analysis). Almost half the thymic SP cells from GVHR chimeras were of donor origin ($Thy1.2^+$) (Table 2, experiments 1 and 2). By contrast, none of the SP cells from control chimeras was of Thy1.2 phenotype 1 wk after ABMT. Most $Thy1.2^+$ cells from control thymuses were $CD4^-8^-$ at this point, whereas in GVHR chimeras the proportions of $CD4^+8^-$ and $CD4^-8^+$ SP cells among $Thy1.2^+$ cells were 53.5% and 28.5%, respectively (Fig. 1). Thus, virtually all $Thy1.2^+$ cells within $CD4^+$ or $CD8^+$ SP populations in thymuses from GVHR chimeras were splenic T cells that had been inoculated with BM cells.

Origin of $V_\beta6^+$ Cells in Thymuses of GVHR Chimeras. A high proportion of T cells expressing $V_\beta6$ of TCR was detected among $CD4^+$ and $CD8^+$ SP cell populations from GVHR [B10 → AKR] but not control chimeras (Table 2, experiments 1 and 2). Representative FACScan profiles (Fig. 2) suggest that the $V_\beta6^+$ cells were derived from splenic T cells of B10 mice. The proportion of $V_\beta6^+$ cells in the $CD4^+8^-$ SP population was more than half that of $Thy1.2^+$ cells (Table 2). Although the proportion of $V_\beta6^+$ cells was not as high among $CD4^-8^+$ cells as among $CD4^+8^-$ SP cells, the proportion of $V_\beta6^+$ cells in both $CD4^+8^-$ and $CD4^-8^+$ fractions was greater than in splenic T cells from normal mice (Table 2). By contrast, the proportion of $V_\beta8^+$ cells in GVHR [B10 → AKR] chimeras was only slightly higher than in control [B10 → AKR] chimeras. Similar results were obtained with [B10.AQR → AKR] chimeras (Table 2, experiment 3) or spleen cells from [B10 → AKR] chimeras (data not shown). Thus, a substantial number of T cells derived from donor mice can enter the thymus as mature cells, and $V_\beta6^+$ cells constitute the major population of these invading T cells.

Table 2. Analysis of T-cell subsets in $CD4^+8^-$ and $CD4^-8^+$ SP thymocytes from [B10 → AKR] or [AQR → AKR] chimeras 1 wk after ABMT

Exp.	Donor mice	*n*	Splenic T cells	$CD4^+8^-$				$CD4^-8^+$			
				% Thy1.2	% CD3	% $V_\beta8$	% $V_\beta6$	% Thy1.2	% CD3	% $V_\beta8$	% $V_\beta6$
1	B10	3	—	0.9	93.2	ND	0.6	0.3	63.2	ND	1.5
	B10	3	1×10^5	46.4	95.5	ND	26.2	47.1	86.1	ND	20.3
2	B10	4	—	0.4	96.9	20.9	0.5	1.8	65.5	16.4	0.1
	B10	4	1×10^5	39.9	99.0	23.1	22.4	47.7	94.1	19.7	13.6
3	AQR	3	—	3.0	79.5	ND	1.2	0.7	37.1	ND	0.0
	AQR	3	1×10^5	35.5	89.8	ND	28.3	48.6	64.0	ND	17.5
	Normal B10 spleen cells			98.6*	99.5*	21.3†	8.8*	99.4*	99.4*	22.6†	7.4†

$CD4^+8^-$ or $CD4^-8^+$ cells were computer-acquired from pooled thymocytes and analyzed for expression of surface antigens. *n* = Number of mice. ND, not determined.
*SE = 0.1.
†SE = 0.2.

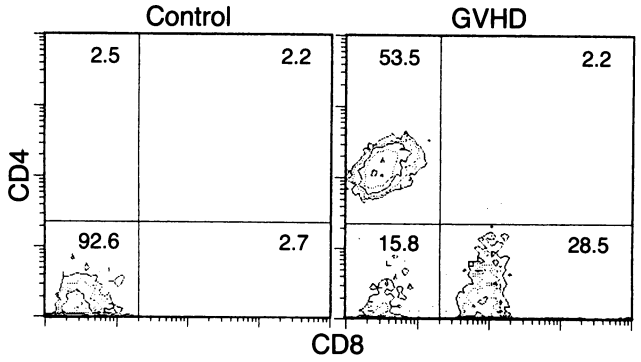

FIG. 1. Expression of CD4, CD8 antigens on donor-derived (Thy1.2$^+$) cells in thymuses of [B10 → AKR] chimeras 1 wk after ABMT. Thy1.2$^+$ cells were selected by computer software gating. Percentages of thymic subpopulations positive for CD4 and/or CD8 are shown for profiles containing 1×10^4 cells. GVHD, graft-versus-host disease.

Given that $V_\beta 6$ TCR expression is strongly associated with recognition of I-E and Mls-1^a antigen (18, 20, 25), the increased proportions of T cells in thymuses of GVHR chimeras may result from alloreactivity of donor T cells against recipient antigens (I-E and Mls-1^a) within the thymus.

$V_\beta 6^+$ T Cells in the Thymuses of GVHR Chimeras May Be Reacting Against Recipient (I-E and Mls-1^a) Within the Thymus. We examined thymuses from GVHR or control [B10 → AKR] chimeras to identify $V_\beta 6^+$ cells *in situ*. Significant numbers of $V_\beta 6^+$ T cells were present in the GVHR thymus tissue (Fig. 3), although their precise localization (cortex or medulla) could not be identified. These cells were not seen in thymuses from control chimeras. FACS analysis of these tissues showed that the cell size of the major population in $V_\beta 6^+$ T cells in thymuses and spleens was greater in GVHR chimeras than in normal donor (B10) mice (Fig. 4). Thus, most $V_\beta 6^+$ T cells are large blast-like cells that almost certainly have been stimulated by specific antigens of recipients.

Failure of Clonal Elimination of $V_\beta 6^+$ Mature Thymocytes in GVHR Chimeras. Expression of $V_\beta 6$ and $V_\beta 8$ on mature

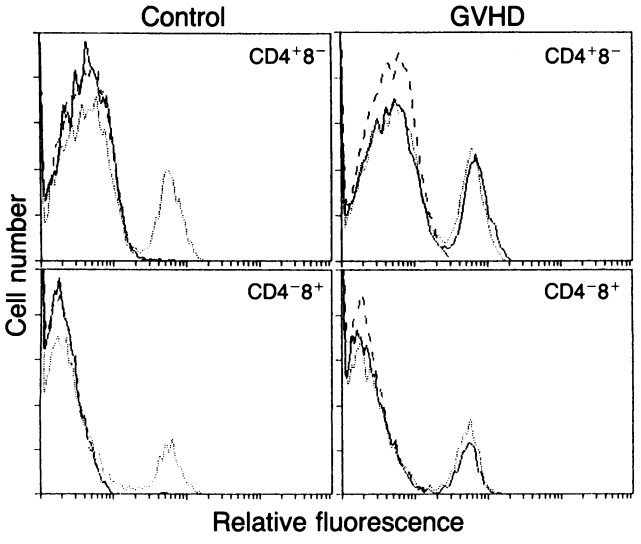

FIG. 2. Expression of $V_\beta 6$ and $V_\beta 8$ products on CD4$^+$8$^-$ or CD4$^-$8$^+$ SP cells in thymuses from [B10 → AKR] chimeras 1 wk after ABMT: $V_\beta 6$ (—), $V_\beta 8$ (····), control (---). Each profile represents data from 1×10^4 cells. Almost no thymic cells from control chimeras expressed $V_\beta 6$ products, whereas a high proportion of $V_\beta 6^+$ cells was detected among cells from GVHR chimeras. GVHD, graft-versus-host disease.

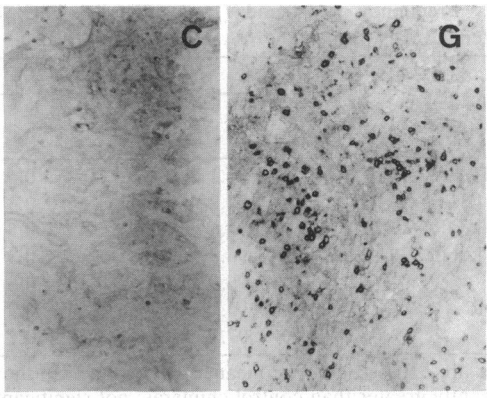

FIG. 3. Expression of $V_\beta 6$ products on thymus cells from [B10 → AKR] chimeras (C, control; G, GVHR) 1 wk after ABMT. Cryosections were stained with 44-22-1. (×160.)

thymocytes (CD4$^+$8$^-$ and CD4$^-$8$^+$ SP cells) from GVHR or control chimeras was compared 5 wk after ABMT. The numbers and proportions of CD4$^+$8$^-$ and CD4$^-$8$^+$ cells in thymuses from control or GVHR chimeras fell within ranges observed in normal donor mice at this stage. The proportion of $V_\beta 6^+$ cells among CD4$^+$8$^-$ or CD4$^-$8$^+$ SP thymocytes in GVHR chimeras was greater than in control [B10.AQR → AKR] chimeras (Table 3) but not different than among SP thymocytes of normal donors. The $V_\beta 6^+$ cells in thymuses from control [B10.AQR → AKR] chimeras were completely eliminated by means of clonal deletion and specific apoptosis (18, 25–28). However, $V_\beta 6^+$ cells in control [B10 → AKR] chimeras were only partly eliminated.

The substantial number of $V_\beta 6^+$ T cells seen in thymuses of GVHR chimeras 5 wk after ABMT could be attributed to a deficiency of the intrathymic system that otherwise induces self-tolerance in the T-cell repertoire. Alternatively, a substantial number of $V_\beta 6^+$ cells in the GVHR chimeras might result from proliferation of remaining mature T cells after stimulation in the thymus at an early stage of GVHR. Cell tracing studies were used to test these two possibilities.

$V_\beta 6^+$ SP Thymocytes in GVHR Chimeras 5 wk after ABMT Were Cells that Had Differentiated from Donor BM. Splenic T cells from (B10 × 4R)F_1 mice were mixed with T-cell-depleted BM cells from B10 mice and inoculated into AKR recipients (mixed chimeras). T cells from (B10 × 4R)F_1 mice were assumed not to respond against B10 BM cells or to exert greater influence on reconstitution of recipient thymuses by donor precursors than do B10 T cells. Five weeks following ABMT no differences in thymocyte subpopulations were observed between such mixed chimeras and GVHR chimeras (data not shown). Seven percent of CD4$^+$8$^-$ and 14.1% of CD4$^-$8$^+$ SP thymocytes were $V_\beta 6^+$ (Table 4), consistent with data presented in Table 3.

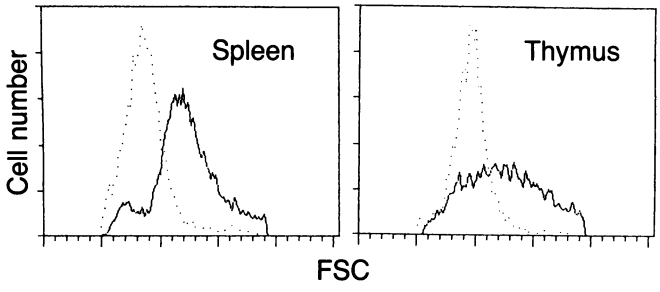

FIG. 4. Cell size (forward light scatter) analysis of $V_\beta 6^+$ cells in spleens and thymuses from GVHR chimeras (—) 1 wk after ABMT or from normal [B10 → AKR] chimeras (····). $V_\beta 6^+$ cells were selected using computer software gating. Dead cells were excluded using forward and side light scatter. FSC, forward light scatter.

Table 3. Frequency of $V_\beta 8$ and $V_\beta 6$ positive cells in $CD4^+8^-$ or $CD4^-8^+$ SP thymocytes from [B10 → AKR] or [AQR → AKR] chimeras 5 wk after ABMT

Mice	*n*	$CD4^+8^-$ % CD3	$CD4^+8^-$ % $V_\beta 8$/CD3	$CD4^+8^-$ % $V_\beta 6$/CD3	P	$CD4^-8^+$ % CD3	$CD4^-8^+$ % $V_\beta 8$/CD3	$CD4^-8^+$ % $V_\beta 6$/CD3	P
GVHR [B10 → AKR]	5	97.1 ± 0.4	20.2 ± 0.5	6.7 ± 0.2*	<0.05	77.8 ± 4.6	31.9 ± 1.8	12.1 ± 0.7*	<0.001
Control [B10 → AKR]	5	98.2 ± 0.2	18.6 ± 0.3	4.5 ± 0.6		79.1 ± 1.0	27.2 ± 0.4	2.5 ± 0.7	
GVHR [AQR → AKR]	5	95.6 ± 0.1	22.9 ± 0.5	8.6 ± 0.3*	<0.001	85.1 ± 1.3	32.0 ± 0.2	14.4 ± 0.9*	<0.001
Control [AQR → AKR]	5	96.8 ± 0.1	21.2 ± 0.3	0.9 ± 0.2		74.6 ± 1.5	25.3 ± 0.9	0.6 ± 0.2	
B10	3	96.9 ± 0.3	23.5 ± 0.6	7.2 ± 0.3		82.7 ± 3.5	28.9 ± 0.5	10.5 ± 0.3	
B10.AQR	3	95.1 ± 0.3	23.4 ± 0.9	8.9 ± 0.0		94.3 ± 2.5	28.9 ± 0.7	11.8 ± 0.8	
AKR†	3	97.1 ± 0.1	21.5 ± 0.2	0.3 ± 0.0		88.2 ± 1.4	23.7 ± 0.4	0.9 ± 0.0	

Values are expressed as the mean ± SE of the % in the $CD3^+$ population. *n* = Number of animals.
*Significantly greater than control chimeras; not significant versus normal donors.
†Normal control mice.

Expression of K^k, K^b, Thy1.1, or Thy1.2 antigens on $V_\beta 6^+$ bright positive cells from thymuses of GVHR chimeras was analyzed 1 or 5 wk after ABMT. Most $V_\beta 6^+$ cells were positive for K^k, K^b, and Thy1.2 antigens 1 wk after ABMT (Table 4) and thus were considered to be splenic T cells of (B10 × 4R)F_1 mice or their direct descendants. However, 5 wk following ABMT, >99% of cells were $Thy1.2^+$ and <1% were K^k positive, indicating that most $V_\beta 6^+$ cells in the recipient thymus were indeed derived from B10 BM. Almost identical results were obtained when $V_\beta 6^+$ cells in recipient spleens were analyzed 5 wk after ABMT, although a very small proportion of (B10 × 4R)F_1 cells persisted (Table 4). These results indicate that a substantial number of $V_\beta 6^+$ T cells observed in GVHR chimeras 5 wk following ABMT were not persisting F_1 T cells but were cells derived from BM cells that had differentiated in the recipient thymus. Essentially the same results were obtained when BM cells from B10.AQR and T cells from (B10 × AQR)F_1 mice were transplanted into AKR mice (Table 4).

DISCUSSION

The mechanism by which GVHR influences hematopoietic and lymphopoietic tissues remains a significant obstacle in the increasing use of ABMT (1–4). In this study of a T-cell subpopulation ($V_\beta 6^+$) that reacts against recipient AKR antigens (I-E and Mls-1^a) (18, 25), we demonstrate that the thymus functions as a direct target tissue in GVHR. When mature T cells from B10 mice or B10.AQR mice were transferred with T-cell-depleted BM cells to irradiated AKR mice, a large number of $V_\beta 6^+$ T cells were found among $CD4^+8^-$ or $CD4^-8^+$ SP cells in thymuses from recipient mice (GVHR chimeras) 1 wk following ABMT. The proportion of $V_\beta 6^+$ cells was considerably higher in GVHR chimeras than among peripheral T cells from normal donor mice. These T cells were shown to be large, blast-like cells and were established as the T cells that had been inoculated or their direct descendants. No $V_\beta 6^+$ T cells were present in thymuses of control chimeras at this stage. Thus, mature T cells that respond immunologically against recipient antigens (I-E and Mls-1^a) enter the recipient thymus and proliferate at this site. Similarly, a slight increase in the proportion of $V_\beta 8^+$ cells seen in thymuses from GVHR chimeras may be attributed to reactivity of $V_\beta 8.1^+$ T cells against Mls-1^a antigens (29).

At 5 wk following ABMT, the proportion of $V_\beta 6^+$ cells among SP thymocytes from GVHR chimeras was reduced but was similar to that in normal donors. Tracing of inoculated cells revealed that most of the cells brightly positive for $V_\beta 6^+$ were differentiated from precursor cells in the BM inocula. Thus, thymuses of AKR recipients did not appear to influence selection of $V_\beta 6^+$ cells. By contrast, $V_\beta 6^+$ cells were not seen in thymuses from control chimeras due to clonal deletion in the presence of donor-derived $I\text{-}E^+$ cells and Mls-1^a antigens of recipient (18, 25–28, 30). The partial but significant decrease in $V_\beta 6^+$ SP cells seen in thymuses from control [B10 → AKR] chimeras may relate to involvement of particular class II antigens (I-A) expressed on donor-derived macrophages and/or dendritic cells in the partial elimination of $V_\beta 6^+$ cells (18).

The present findings suggest that the GVHR observed soon after ABMT influences stromal components of recipient thymus that participate in elimination of self-reactive T cells (18, 30). Although the possibility that GVHR might influence indirectly thymic function (i.e., by means of corticosteroid hormones) (17) was not examined, abrogation of stromal function by donor T cells might best explain the appearance at later stages of a significant number of $V_\beta 6^+$ SP thymocytes derived from BM precursors. T cells activated by recipient antigens may directly damage recipient cells expressing Mls-

Table 4. Origin of $V_\beta 6^+$ cells in thymuses or spleens of GVHR chimeras

Mice	*n*	T cells added (1 × 10^5)	Time after ABMT, wk	Recovery site	Proportion of $V_\beta 6^+$ cells, % $CD4^+CD8^-$	Proportion of $V_\beta 6^+$ cells, % $CD4^-CD8^+$	Chimerism in $V_\beta 6^+$ bright positive cells, % K^k	K^b	Thy1.1	Thy1.2
[B10 → AKR]	3	(B10 × 4R)F_1	1	Thymus	ND	ND	95.5*	94.1	0.3	91.8
[B10 → AKR]	3	(B10 × 4R)F_1	5	Thymus	7.0 ± 0.3	14.1 ± 0.6	0.8 ± 0.3	80.8 ± 2.6	0.0 ± 0.0	99.3 ± 0.1
[B10 → AKR]	3	(B10 × 4R)F_1	5	Spleen	ND	ND	2.3 ± 1.0	97.2 ± 0.5	0.4 ± 0.3	95.2 ± 0.9
							K^b	D^d	Thy1.1	Thy1.2
[AQR → AKR]	3	(B10 × AQR)F_1	1	Thymus	ND	ND	94.2*	94.2	7.4	85.2
[AQR → AKR]	3	(B10 × AQR)F_1	7	Thymus	7.5 ± 0.5	11.8 ± 1.3	0.9 ± 0.1	98.6 ± 0.2	0.3 ± 0.0	99.3 ± 0.3
[AQR → AKR]	1	(B10 × AQR)F_1	7	Spleen	ND	ND	3.6	99.1	0.0	97.3

Lethally irradiated AKR mice (*n* = number of mice) were reconstituted with mixtures of 2.5 × 10^7 T-cell-depleted BM cells from B10 or AQR mice and 1 × 10^5 splenic T cells from (B10 × 4R)F_1 or (B10 × AQR)F_1 mice. ND, not determined.
*Data for $V_\beta 6^+$ thymocytes pooled from three chimeras.

1[a]. Alternatively, lymphokines secreted by activated T cells might influence donor-derived macrophages or dendritic cells that play a major role in clonal deletion of autoreactive thymocytes (18). Such responses might be followed by chronic immunological responses against recipient antigen by donor BM-derived T lymphocytes (31), perhaps representing a kind of autoimmunity, given that $V_\beta 6^+$ T cells obtained from GVHR [AQR → AKR] chimeras 5 wk after ABMT can mount proliferative responses to stimulation with anti-$V_\beta 6$ mAb *in vitro* (unpublished data).

A slight degree of GVHR may be of benefit in treatment of leukemias (graft-versus-leukemia phenomena) (2, 32, 33). Moreover, complete elimination of allogeneic T cells from donor BM inocula appears to increase the rate of failure in allogeneic engraftment (23, 34). Since chronic GVHR after ABMT is accompanied by complex autoimmunities and chronic immunodeficiencies, our findings may be relevant to the clinical use of ABMT. Given that alternate forms of tolerance induction by clonal anergy (35, 36) and suppressor mechanisms (37) against T cells reactive to recipient antigens have been demonstrated, the relationship among these mechanisms in induction and maintenance of self-tolerance after ABMT should be pursued.

We thank H. Hengartner for providing mAb 44-22-1 and Ms. M. Konishi for manuscript preparation. This research was supported by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan, the Nishimura Aging Fund, and National Institutes of Health Grants AG05628 and AI22360.

1. Good, R. A. (1982) *N. Engl. J. Med.* **306,** 421–423.
2. Gale, R. P. (1985) *Immunol. Rev.* **88,** 193–214.
3. Good, R. A., Kapoor, N. & Reisner, Y. (1983) *Cell. Immunol.* **82,** 36–54.
4. O'Reilly, R. J. (1983) *Blood* **62,** 941–964.
5. Korngold, R. & Sprent, J. (1978) *J. Exp. Med.* **148,** 1687–1698.
6. Sprent, J., Schaefer, M., Lo, D. & Korngold, R. (1986) *Immunol. Rev.* **91,** 195–218.
7. Storb, R. & Thomas, E. D. (1983) *Immunol. Rev.* **71,** 77–102.
8. Onoé, K., Fernandes, G., Shen, F. W. & Good, R. A. (1982) *Cell. Immunol.* **68,** 207–219.
9. Krown, S. E., Coico, R., Scheid, M. P., Fernandes, G. & Good, R. A. (1981) *Clin. Immunol. Immunopathol.* **19,** 268–283.
10. Piguet, P.-F. (1985) *J. Immunol.* **135,** 1637–1643.
11. Gleichmann, E., Pals, S. T., Rolink, A. G., Radaszkiewicz, T. & Gleichmann, H. (1984) *Immunol. Today* **5,** 324–332.
12. Sprent, J., Schaefer, M., Gao, E.-K. & Korngold, R. (1988) *J. Exp. Med.* **167,** 556–569.
13. Rolink, A. G., Pals, S. T. & Gleichmann, E. (1983) *J. Exp. Med.* **157,** 755–771.
14. Onoé, K., Iwabuchi, K., Katsume, C., Gotohda, T., Arase, H., Hatakeyama, S., Mishima, M., Good, R. A. & Ogasawara, K. (1989) *Acta Pathol. Jpn.* **39,** 101–110.
15. Van Rappard-Van der Veen, F. M., Kiesel, U., Poels, L., Schuler, W., Melief, C. J. M., Landegent, J. & Gleichmann, E. (1984) *J. Immunol.* **132,** 1814–1820.
16. Elkins, W. L., Kavathas, P. & Bach, F. H. (1973) *Transplant. Proc.* **5,** 1759–1762.
17. Heim, L. R., Martinez, C. & Good, R. A. (1967) *Nature (London)* **214,** 26–29.
18. Fukushi, N., Wang, B., Arase, H., Ogasawara, K., Good, R. A. & Onoé, K. *Eur. J. Immunol.*, in press.
19. Arase, H., Fukushi, N., Hatakeyama, S., Ogasawara, K., Iwabuchi, K., Iwabuchi, C., Negishi, I., Good, R. A. & Onoé, K. *Immunobiology*, in press.
20. Payne, J., Huber, B. T., Cannon, N. A., Schneider, R., Schilham, M. W., Acha-Orbea, H., MacDonald, H. R. & Hengartner, H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7695–7698.
21. Ewijk, W. V., Rouse, R. V. & Weissman, I. L. (1980) *J. Histochem. Cytochem.* **28,** 1089–1099.
22. Ogasawara, K., Fukushi, N., Arase, H., Iwabuchi, C., Hatakeyama, S., Iwabuchi, K., Good, R. A. & Onoé, K. (1989) *Immunobiology*, in press.
23. Onoé, K., Iwabuchi, K., Iwabuchi, C., Arase, H., Hatakeyama, S., Wambua, P. P., Fukushi, N., Negishi, I., Good, R. A. & Ogasawara, K. (1989) *Immunobiology* **179,** 172–189.
24. Onoé, K., Fernandes, G. & Good, R. A. (1980) *J. Exp. Med.* **151,** 115–132.
25. Hengartner, H., Bernhard, O., Schneider, R., Schneider, M., Wälle, G., MacDonald, H. R. & Zinkernagel, R. M. (1988) *Nature (London)* **336,** 388–390.
26. Yoshikai, Y., Ogimoto, M., Matsumoto, K., Sakumoto, M., Matsuzaki, G. & Nomoto, K. (1989) *Eur. J. Immunol.* **19,** 1009–1013.
27. Kisielow, P., Blüthmann, H., Staerz, U. D., Steinmetz, M. & von Boehmer, H. (1988) *Nature (London)* **333,** 742–746.
28. Kappler, J. W., Roehm, N. & Marrack, P. (1987) *Cell* **49,** 273–280.
29. Kappler, J. W., Staerz, U. D., White, J. & Marrack, P. (1988) *Nature (London)* **332,** 35–40.
30. Speiser, D. E., Schneider, R., Hengartner, H., MacDonald, H. R. & Zinkernagel, R. M. (1989) *J. Exp. Med.* **170,** 595–600.
31. Iwabuchi, K., Ogasawara, K., Ogasawara, M., Yasumizu, R., Noguchi, M., Geng, L., Fujita, M., Good, R. A. & Onoé, K. (1987) *J. Immunol.* **138,** 18–25.
32. Bortin, M. M. (1974) in *Clinical Immunology*, eds. Bach, F. H. & Good, R. A. (Academic, New York), pp. 287–306.
33. Truitt, R. L., Shih, C.-Y. & LaFever, A. V. (1983) *J. Immunol.* **131,** 2050–2058.
34. Sykes, M., Sheard, M. & Sachs, D. H. (1988) *J. Immunol.* **141,** 2282–2288.
35. Ramsdell, F., Lantz, T. & Fowlkes, B. J. (1989) *Science* **246,** 1038–1041.
36. Rammensee, H.-G., Kroschewski, R. & Frangoulis, B. (1989) *Nature (London)* **339,** 541–544.
37. Ogasawara, M., Iwabuchi, K., Good, R. A. & Onoé, K. (1988) *J. Immunol.* **141,** 3306–3312.