Major Histocompatibility Complex–specific Prolongation of Murine Skin and Cardiac Allograft Survival after In Vivo Depletion of Vβ+ T Cells

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Summary

The preferential usage of certain T cell receptor (TCR) Vβ genes has been well established in several major histocompatibility complex (MHC)–restricted immune responses. However, Vβ usage among allogeneic responses remains unclear. Because recent findings of ours and others indicate that Vβ8 predominates in certain L^d-restricted, peptide-specific responses, we examined the Vβ8 usage in allogeneic responses to L^d. To selectively recognize the L^d molecule, cells from BALB/c-H-2^{dm2} (dm2), the L^d-loss mutant mouse, were stimulated in vitro or in vivo with wild-type BALB/c cells. We report here that after the intraperitoneal administration of the anti-Vβ8 monoclonal antibody (mAb) F23.1, peripheral Vβ8 T cells were depleted from dm2 mice. This in vivo depletion abrogated the ability of dm2 splenocytes to mount a primary response to L^d molecules. This abrogation was specific, since the response of Vβ8-depleted dm2 cells to K^b/D^b antigens was the same as that of control nondepleted dm2 cells. Furthermore, in vivo depletion of Vβ8 cells was found to cause a dramatic prolongation of L^d-disparate skin grafts (mean survival time [MST] 22.1 ± 2.1 vs. 10.3 ± 1.1 d for saline-treated controls, or 10.9 ± 1.7 d for controls treated with mAb KJ23 to Vβ17). By contrast, Vβ8 depletion had no effect on recipients grafted with haplotype-mismatched skin or single D^k-locus-disparate skin. These findings demonstrate that Vβ8+ T cells predominate in allogeneic response to L^d but not other alloantigens. The effect of Vβ8 depletion was found to be even more dramatic on recipients grafted with L^d-disparate vascularized heart transplants (MST > 100 vs. 8.6 ± 0.5 d for controls). In total, these findings establish the efficacy of using mAb to the Vβ gene family to specifically and significantly enhance the survival of allografts. The implications of detecting Vβ8 usage in both alloreactive or MHC–restricted TCR responses to the same class I molecule are discussed.

The function of the immune system is to discriminate self from non-self. For example, the cellular arm of the immune system identifies virus-infected cells, malignant cells, or genetically disparate tissue transplants using CD8+ CTL. These CTL bear receptors (TCR) capable of specific identification of appropriate target cells that express foreign peptides bound to self–class I MHC molecules (1). Such immunogenic peptides are 8–9 amino acids long and are bound to the antigen binding groove formed by the α1 and α2 domains of the class I molecule (2). The TCR is a disulfide-linked αβ heterodimeric glycoprotein with a number of invariant associated molecules (3, 4). The heterogeneity of TCR molecules that is required to recognize the abundance of potential foreign antigens is achieved by somatic recombination of V-(D)-J segments to form a single functional transcriptional unit encoding each α or β TCR chain. Since there are several different alternative V-(D)-J segments, as well as additional junctional diversities, innumerable distinct TCR are generated, each with unique structures (5–7). Even though it is clear that structural differences in both TCR α and β chains determine their specific interaction with MHC–peptide complexes, the details have yet to be fully elucidated. Recent data support a model whereby distinct regions of each α and β chain interact with the MHC molecule or its bound peptide ligand (7).

Given the large number of different combinational TCR possibilities, as well as the various manners by which TCR and MHC–peptide complexes potentially interact, it might be expected that most immune responses would show diverse TCR usage. However, some interesting examples of limited TCR usage have recently been noted. For instance, the L^d-restricted immune response of a BALB/c mouse after CMV infection was shown to preferentially use Vβ8+ T cells (8). Similarly, predominant Vβ8 usage was also observed

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in studies of L^d-restricted CTL specific for the tumor-associated tum$^-$ peptide (Solheim, J. C., M. A. Alexander-Miller, J. M. Martinko, and J. M. Connolly, manuscript submitted for publication). In studies of K^d-restricted responses, CTL specific for a single *Plasmodium berghei* circumsporozoite-derived peptide displayed diverse TCR (9) in contrast to ones specific for an HLA-CW3 peptide that display limited TCR diversity (10). The structural basis for why particular immune responses have been found to show limited TCR usage remains unclear.

Our understanding of the diversity of TCR usage in allogeneic responses to class I, such as allograft rejection, is potentially important for both mechanistic and therapeutic reasons. However, allogeneic recognition of class I has an additional level of complexity compared with the syngeneic systems mentioned above. This uncertainty stems from the fact that it remains unclear whether alloreactive T cells recognize predominantly polymorphic MHC determinants or their bound ligands. If bound ligands play a key role in allorecognition, then tissue-specific antigens could significantly influence the diversity of alloreactive CTL involved in allograft rejection. Initial studies analyzing TCR gene usage during end-stage transplantation rejection episodes or in vitro allorecognition have been conflicting. Certain studies have demonstrated the preferential usage of specific TCR variable regions (11, 12), whereas others have reported high levels of diversified gene usage (13–15). It is difficult to understand the implication of these studies on TCR diversity because of the expression of multiple major and minor alloantigenic differences between host and donor tissue. To alleviate this problem, we have investigated the in vitro and in vivo response to a single class I alloantigen, the L^d molecule of the mouse. Studies of allorecognition using L^d have two notable advantages. First, L^d-specific responses can be generated using the L^d-loss mutant (16) mouse strain BALB/c-H-2^{dm2} (dm2)[1]. Second, we and others (8; Solheim et al., manuscript submitted for publication) have generated a background of relevant information concerning the TCR recognition of L^d–peptide complexes in syngeneic immune responses.

In this communication, we demonstrate that the in vivo depletion of the $V\beta8^+$ T cell subset results in a significant decrease in the dm2 anti-L^d allogeneic response. Furthermore, recipient dm2 mice depleted of $V\beta8^+$ cells show dramatic and specific prolongation of skin or heart allograft survival. These findings thus represent a clear example of dominant $V\beta$ usage in an in vivo primary response to a class I alloantigen. The implications of these findings on TCR recognition of allogeneic versus syngeneic class I–peptide complexes are discussed.

Materials and Methods

Animals. Female C57BL/6, BALB/c, B10.HTT, BALB.K, (B10.AKM × dm2)F_1, and BALB/c-H-2^{dm2} (dm2) mice (see Table 1) were bred and maintained in the animal facility of Donald C. Shreffler, Washington University School of Medicine. All mice were used at 6–12 wk of age and were cared for according to National Institutes of Health guidelines.

Tumor Target Cell Lines. The mastocytoma P815, lymphoma EL-4, thymoma R1.1, and L^d-transfected thymoma R1.1L^d (see Table 1) were maintained at 37°C, 95% air/5.0% CO_2 in DME (Gibco Laboratories, Grand Island, NY) containing 10% FCS (Hyclone Laboratories, Logan, UT), 2 mM L-glutamine, and 100 U/ml penicillin–streptomycin (Washington University Tissue Culture Center).

mAb Used for In Vivo Depletions. The anti-$V\beta8$ mAb F23.1 (17), and anti-$V\beta17$ mAb KJ23 (18) (generously provided by Dr. O. Kanagawa, Department of Pathology, Washington University) are mouse IgG2a isotype that were grown in our laboratory as culture supernatant and ascites fluid and were protein A affinity chromatography purified.

In Vivo Depletion of $V\beta8^+$ T Cells. Groups of three identically treated experimental dm2 mice received three independent 500 μg i.p. injections of protein A-purified F23.1 mAb in 0.5 ml normal saline (days 0, +2, and +4) before harvesting of splenocytes (day +7) for analysis via flow cytometry (FACS®; Becton Dickinson & Co., Mountain View, CA). Control dm2 mice received intraperitoneal injection of the vehicle, 0.5 ml 0.9% normal saline without F23.1 before splenocyte harvest and analysis.

Flow Cytometry. Briefly, 2–4 × 10^5 cells were plated in the wells of round-bottomed microtiter plates, washed once with HBSS (lacking phenol red) containing 0.2% FCS/0.1% sodium azide (FACS® medium), and were incubated with a saturating concentration of F23.1 mAb, biotinylated anti-CD8 (Ly2) (Pharmingen, San Diego, CA), or with FACS® medium alone for 30 min at 4°C. The cells were washed three times with FACS® medium, and then incubated with a saturating concentration of fluorescein-conjugated F $(ab')_2$ fragment of Fc-specific goat anti–mouse IgG, (Organon-Teknika-Cappel, Durham, NC) or Streptavidin-PE (Caltag Laboratories, South Francisco, CA) for 30 min at 4°C, washed with FACS® medium three times, and finally resuspended in 0.5 ml FACS® medium. Fluorescein and PE-labeled cells were analyzed using a FACScan® (Becton Dickinson & Co.) equipped with an argon laser tuned to 488 nm and operating at 150 mW of power. Fluorescent four-quadrant, two-color contour plots were generated with logarithmic amplification of fluorescence emitted by single viable cells. Each sample analyzed comprised a minimum of 2.5 × 10^4 cells and was statistically evaluated using the CONSORT 30 computer software (Becton Dickinson & Co.).

Generation of Specific dm2 Anti-H-2L^d CTL. Splenocytes used for the generation of primary dm2 anti-H-$2L^d$ CTL were harvested from control saline- or F23.1-treated dm2 mice 7 d after the initial intraperitoneal injection of the mAb F23.1. At the completion of BALB/c skin graft rejection, spleen cells used for the generation of secondary dm2 anti-H-$2L^d$ CTL were harvested from control saline- and F23.1-treated dm2 recipient mice. 7.5 × 10^6 responding spleen cells were cocultured in 24-well tissue culture plates (Costar Corp., Cambridge, MA) with 3.5 × 10^6 irradiated (2,000 rad) stimulating BALB/c spleen cells at 37°C, 95% air/5% CO_2 for 5 d in a final volume of 2 ml. At the completion of this incubation period, effector cells were harvested, washed, and resuspended in DME supplemented with 10% FCS.

^{51}Cr-Release Assay. 10^6 target cells were labeled with 200 μCi ^{51}Cr ($Na^{51}CrO_4$, 1 mCi/ml; Amersham Corp., Arlington Heights, IL) in 100 μl of DME supplemented with 10% FCS for 1 h at 37°C in 95% air/5% CO_2. Radiolabeled target cells were washed three times, counted, and resuspended. 2 × 10^3 cells in 100 μl DME with 10% FCS were added to V-bottomed microtiter plates

[1] *Abbreviation used in this paper:* dm2, L^d-loss mutant mouse strain BALB/c-H-2^{dm2}.

(ICN Biomedicals, Inc., Costa Mesa, CA). For antibody blocking, 10 μg of F23.1 was added 15 min before the addition of the respective targets and remained throughout the 4-h ^{51}Cr-release assay.

Effector cells were harvested, resuspended, diluted to the appropriate concentration, and dispensed into the V-bottomed plates. The plates were spun at 50 *g* for 2 min and incubated for 4 h at 37°C and 95% air/5% CO_2. At completion of the incubation period, the plates were spun at 500 *g* and 100 μl of the supernatant was harvested and counted on a gamma counter (model 1272 Clinigamma; LKB Instruments, Turku, Finland). The mean of triplicate samples was calculated and percent ^{51}Cr-release was determined according to the following equation: percent ^{51}Cr-release = 100× [(experimental ^{51}Cr-release − control ^{51}Cr-release)/(maximum ^{51}Cr-release − control ^{51}Cr-release)]. Where experimental ^{51}Cr-release represents counts from target cells mixed with effector cells, control ^{51}Cr-release represents counts from target cells incubated with medium alone (spontaneous release), and maximum ^{51}Cr-release represents counts from target cells exposed to 5% Triton X-100. For data presented in this paper, the SEM percent lysis was <5% of the value of the mean.

Skin Grafting. Female dm2 and (B10.AKM × dm2)F_1 mice were engrafted on the left and right dorsal thorax with full thickness abdominal B10.HTT, C57BL/6, BALB.K, or BALB/c skin grafts on day 0 according to an adaptation of the method of Billingham and Medawar (19). On days −2, 0, and +3, experimental dm2 mice were injected intraperitoneally with 500 μg affinity-purified F23.1 mAb, while control dm2 mice received an intraperitoneal injection of 0.5 ml normal saline without F23.1, or 500 μg of the isotype-matched mAb KJ23 (anti-Vβ17). Bandages were removed on day +7, and the grafts were scored daily until rejection was complete (defined as loss >90% of the grafted tissue).

Cardiac Transplantation. Intraabdominal heterotopic cardiac transplantation was performed according to a modified method of Corry et al. (20).

BALB/c donors received 300 U of heparin intravenously 10 min before surgery. The superior and inferior vena cavae were ligated and the heart perfused with 2 ml of 4°C lactated Ringer's solution via the inferior vena cava. The pulmonary artery was divided at its bifurcation and the ascending aorta transected proximal to the origin of the innominate artery. After mass ligation of the pulmonary veins, the heart was removed and stored in 4°C lactated Ringer's solution.

The donor's ascending aorta was anastomosed end to side to the recipient infrarenal abdominal aorta with a running 10–0 dermalon suture (courtesy of Davis & Geck, Co., Danbury, CT). The donor pulmonary artery was anastomosed end to side to the recipient inferior vena cava with a 10–0 dermalon suture in a running fashion. Graft ischemia was consistently <20 min, and all grafts began beating promptly after revascularization. Graft function was assessed by daily cardiac palpation, and graft rejection, defined as complete cessation of ventricular contraction, was confirmed histologically.

Statistical Analysis of Cardiac Allograft and Skin Graft Survival Data. Allograft survival data were evaluated for statistical significance by the Kaplan-Meier analysis, and considered significant at $p < 0.05$.

Results

Blocking of the In Vitro Response to L^d Using F23.1. Because of recent studies of ours (Solheim et al., manuscript submitted for publication) and others (8) implicating Vβ8 TCR in syngeneic CTL responses to L^d plus peptide, we sought to define the role of $V\beta8^+$ CTL in allogeneic responses. Primary CTL specific for L^d were generated using splenic lymphocytes from the L^d-loss mutant dm2 as responders and irradiated wild-type BALB/c splenocytes as stimulators. To determine whether $V\beta8^+$ CTL were involved in this response to L^d, the anti-Vβ8 mAb F23.1 was added to the 4-h ^{51}Cr-release assay to block target cell recognition. In the experiment shown in Fig. 1 *a*, the mAb F23.1 was found to block CTL recognition of L^d by ~80%. Furthermore, this blocking was specific since the dm2 response to K^b/D^b molecules was not blocked by F23.1 (Fig. 1 *b*). Even though specific blocking of L^d by F23.1 was observed in three of four experiments, the level of blocking was variable (range 30, 60, and 80%). It is noteworthy, however, that better blocking was observed if F23.1 mAb was added to both the sensitization and assay cultures (data not shown). This blocking suggests that a significant component of the primary response to L^d involves $V\beta8^+$ T cells. The basis for the variable blocking by F23.1 could reflect either variability in TCR usage among L^d-reactive allo-CTL, or that F23.1

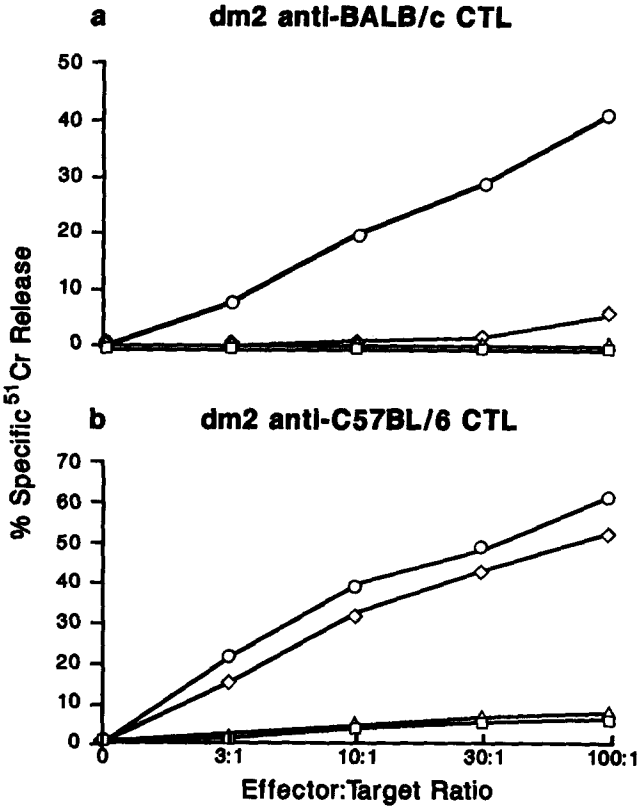

Figure 1. Inhibition of target cell recognition by in vitro primary allo-L^d CTL by antibody to Vβ8. (*a*) Primary L^d-specific CTL were assayed against R1.1L^d in the presence (◇) or absence (○) of mAb F23.1. Controls included unstimulated dm2 effector cells tested on R1.1L^d (△) and L^d-specific effector cells tested on R1.1 (□). (*b*) Primary K^b/D^b-specific CTL were analyzed against EL-4 in the presence (◇) and absence (○) of mAb F23.1. Controls consisted of unstimulated dm2 effector cells analyzed against EL-4 (△) and K^b/D^b-specific effector cells tested against R1.1 (□).

does not block efficiently. For these studies we utilized the L^d-transfected thymoma target R1.1 which is FcR negative, thus ruling out the possibility of FcR-mediated redirected lysis. Therefore, poor blocking could be attributed to either F23.1 having a lower affinity for Vβ8 than Vβ8 has for L^d, or that the F23.1 site on Vβ8 is sterically distinct from that involved in L^d recognition. To resolve this issue we used F23.1 in vivo depletion of Vβ8 cells (21) to determine the extent of the contribution of $V\beta8^+$ T cells to the generation of a primary response to L^d.

Effect of In Vivo Administration of the mAb F23.1. To in vivo deplete the $V\beta8^+$ T lymphocytes, dm2 mice were given three 500-μg i.p. injections of affinity-purified F23.1 mAb on days 0, 2, and 4. Approximately 72 h after the third injection, the spleen cells were harvested, pooled, and analyzed for the expression of the Vβ8 TCR and CD8. Whereas in control dm2 mice, 5–7% of the peripheral spleen cells were found to be $V\beta8^+$ $CD8^+$, the F23.1-treated dm2 mice showed virtually complete depletion of all $CD8^+$ $V\beta8^+$ T lymphocytes (Fig. 2). Similar results were seen in five independent cytometry experiments run as controls for subsequent CTL and allograft studies. It should be noted that after F23.1 treatment, staining with the secondary fluorescein-conjugated goat anti-mouse IgG alone was negative on $V\beta8^+$ T cells. This result indicates that the failure to detect $V\beta8^+$ T cells was not due to to antibody blocking by the F23.1 mAb as a result of the in vivo treatment. At present, it appears that the loss of $V\beta8^+$ T cells was a result of T cell depletion and not TCR modulation. FACS® profiles generated after the in vivo administration of F23.1 did not demonstrate downregulation of either the TCR alone or the TCR in combination with CD8 (Fig. 2), in apparent contrast to recent studies by Teh et al. (22) or Rocha and von Boehmer (23). The $V\beta8^+$ T lymphocytes did not begin to reappear in the spleen and axillary lymph nodes until ~6 wk after F23.1 administration, which was the longest interval evaluated (data not shown).

Effect of In Vivo Depletion of $V\beta8^+$ T Lymphocytes on the Primary Anti-H-2L^d Response. To define the effect of in vivo $V\beta8^+$ T lymphocyte depletion on the in vitro anti-H-2L^d and anti-H-2^b allogeneic immune responses, pooled spleen cells from F23.1-treated or control saline-treated dm2 mice were stimulated in vitro for 5 d with the L^d-disparate BALB/c or fully MHC-disparate C57BL/6 spleen cells. At the completion of this culture period, the CTL were analyzed for specific cytolytic activity. As shown in Fig. 3 *a*, depletion of the $F23.1^+$ lymphocytes resulted in a marked decrease in the anti-H-2L^d cytolytic response in comparison with the control dm2 CTL, which demonstrated strong cytolytic activity against the L^d-disparate targets. In contrast, depletion of the $V\beta8^+$ T lymphocytes did not alter the anti-H-2^b response (Fig. 3 *b*). These data demonstrate that the allogeneic dm2 anti-L^d primary response preferentially uses the Vβ8 gene family and that the in vivo depletion of these T cells abrogates this primary response. Additionally, this effect is L^d specific since the depletion of Vβ8 T cells did not affect the response to K^b/D^b antigens (Fig. 3 *b*).

Effect of In Vivo Depletion of $V\beta8^+$ T Lymphocytes on L^d-disparate Skin Allograft Survival. Because of these dramatic in vitro results, we wanted to determine the role of $V\beta8^+$

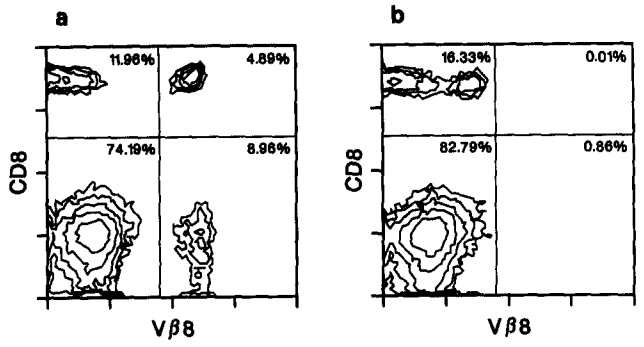

Figure 2. Depletion of $V\beta8^+$ T cells by in vivo treatment with F23.1. Two-color flow cytometry analysis of Vβ8 TCR and CD8 expression was performed on spleen cells from (*a*) control dm2 mice and (*b*) dm2 mice treated intraperitoneally with 1.5 mg F23.1 mAb. Cells were incubated with mAb F23.1 and developed with FITC-conjugated goat anti-mouse IgG. This was followed by incubation with biotinylated antibody to CD8, and developed with streptavidin PE.

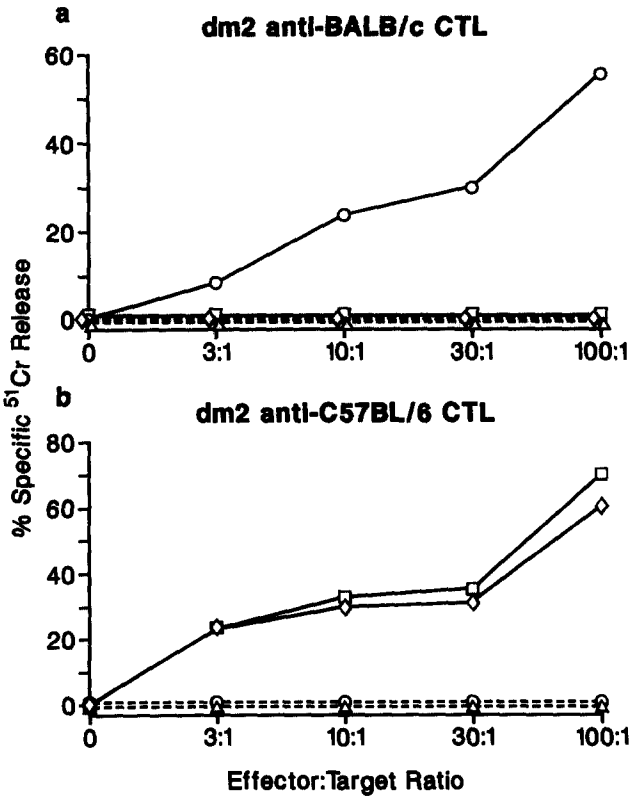

Figure 3. In vivo depletion of $V\beta8^+$ T cells specifically ablates the primary dm2 anti-H-2L^d response. (*a*) Primary L^d-specific CTL were assayed against P815 after in vivo treatment with saline (○) or 1.5 mg F23.1 i.p. (□). Controls consisted of saline-treated (◇) and F23.1-treated (△) L^d-specific CTL tested against the third party EL4 target. (*b*) Primary K^b/D^b-specific CTL were tested against EL-4 after in vivo treatment with saline (◇) or F23.1 (□). Controls include cytolytic activity against the third party target P815 by saline (○) and F23.1-treated (△) effector cells.

Table 1. *Mouse Strains and Tumor Target Cell Lines Used*

Mouse strain	Haplotype	Class I and II molecule expressed
BALB/c	H-2^d	$K^dA^dE^dD^dL^d$
BALB/c-H-2^{dm2}	H-2^{dm2}	$K^dA^dE^dD^{d-}$
BALB.K	H-2^k	$K^kA^kE^kD^k$
C57BL/6	H-2^b	$K^bA^bD^b$
B10.HTT	H-2^{t3}	$K^sA^sE^{s/k}D^dL^d$
B10.AKM	H-2^m	$K^kA^kE^kD^qL^q$
Cell Lines		
P815	H-2^d	$K^dD^dL^d$
EL-4	H-2^b	K^bD^b
R1.1	H-2^k	$K^kD^{k‡}$
R1.1L^d	H-2^k	$K^kD^kL^{d*‡}$

* L^d-transfected R1.1 target cell line.
‡ FcR-negative target cell line.

T lymphocytes in L^d-disparate, haplotype-mismatched and single D^k-locus-disparate skin graft rejection. Female control untreated dm2 mice and dm2 mice treated with 500 μg F23.1 (days −2, 0, and +3) received two skin grafts (Table 1). The right dorsal thorax was engrafted with a full thickness BALB/c (H-2^d) abdominal skin graft, and the left dorsal thorax was engrafted with a C57BL/6 (H-2^b) abdominal skin graft. Whereas F23.1-treated female dm2 mice rejected the H-2^b skin grafts in a time similar to control animals, the H-2^d skin graft survival was significantly prolonged by about 10 d in comparison with controls ($p < 0.01$) (Fig. 4, *A* and *B*). To properly control for nonspecific effects of the antibody treatment, dm2 female mice were treated with the isotype-matched mAb KJ23 that is specific for Vβ17. As shown in Fig. 4 *C*, mice treated with purified KJ23 antibody rejected L^d-disparate grafts in a fashion comparable with untreated control recipient mice. Thus, the above L^d-disparate graft prolongation observed with F23.1 was specific and attributable to the depletion of Vβ8$^+$ T lymphocytes. To further characterize the alloantigenic specificity of this graft prolongation, transplants across other MHC disparities were also tested. To determine whether grafts differing by L^d in addition to other class I and II alloantigens were affected by F23.1, treated, dm2 mice were grafted with B10.HTT (K^s, A^s, $E^{s/k}$, D^d, L^d) skin. These skin grafts were rejected by control dm2 mice in 11.2 ± 0.9 d, whereas the F23.1-treated dm2 mice rejected these B10.HTT skin grafts in 11.3 ± 0.8 d, which is not significantly different than controls (Fig. 5 *A*). Since there was no prolongation in skin allograft survival in this complete MHC-disparate combination, we next analyzed skin graft survival with F23.1 treatment in a strain combination that differed only at the MHC class I locus D^k. To confer specificity for only D^k alloantigens, we bred F_1 hybrids between dm2 and B10.AKM (K^k,A^k,E^k,D^q,L^q). These F_1 mice

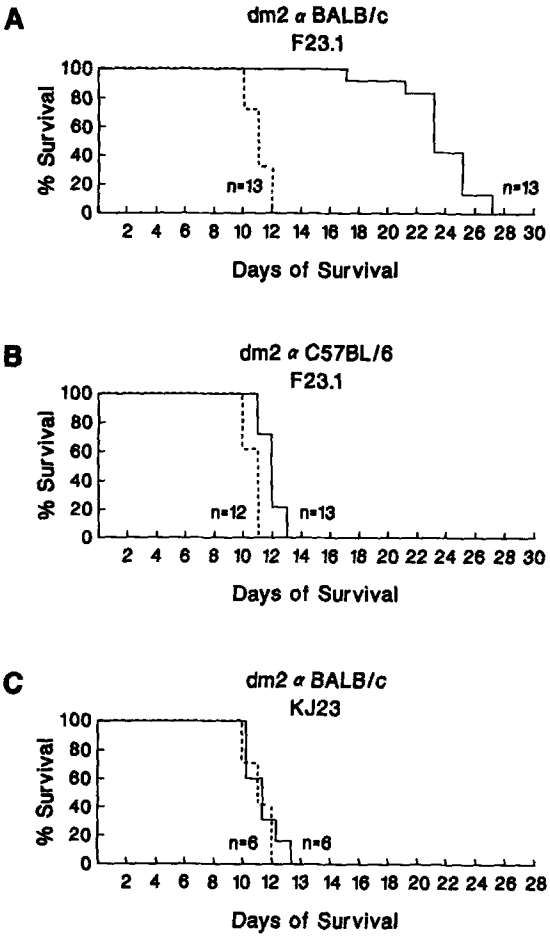

Figure 4. In vivo depletion of Vβ8$^+$ CTL specifically prolongs L^d-disparate skin allograft survival. (*A*) Control dm2 mice (- - -) rejected BALB/c skin allografts in 10.3 ± 1.1 d. BALB/c skin allograft survival was significantly ($p < 0.01$ vs. control) prolonged on dm2 mice treated with the mAb F23.1 (——) (MST 22.1 ± 2.1 d) (*B*). The in vivo administration of F23.1 to dm2 recipients does not prolong C57BL/6 skin allograft survival. Control dm2 (- - -) rejected C57BL/6 skin grafts in 9.7 ± 0.9 d, whereas F23.1-treated dm2 (——) rejected C57BL/6 skin grafts in 11.8 ± 1.9 d. (*C*) In vivo treatment of the dm2 recipient with the isotype-matched mAb KJ23 does not prolong BALB/c skin allograft survival. Control dm2 (- - -) and KJ23-treated (——) dm2 mice rejected BALB/c skin allografts in 11.1 ± 1.7 and 11.9 ± 1.5 d, respectively.

were grafted with skin from BALB.K (H-2^k). Thus the only genetic disparity between the F_1 recipients and the BALB.K donor is the D^k locus, because they are genetically matched at other class I, class II, and non-MHC loci. All BALB.K skin grafts were rejected by control and F23.1-treated (B10.AKM × dm2)F_1 mice in similar fashions, (control mice rejected the BALB.K skin grafts in 11.8 ± 1.0 d, whereas F23.1-treated mice rejected these grafts in 12.1 ± 1.2 d) (Fig. 5 *B*). These skin graft results indicate that the depletion of Vβ8$^+$ T lymphocytes does not affect allogeneic responses to all MHC antigens. Thus Vβ8$^+$ T lymphocytes play a central and specific role in the primary response to L^d alloantigens.

To assess the possibility that the Vβ8$^+$ T lymphocytes

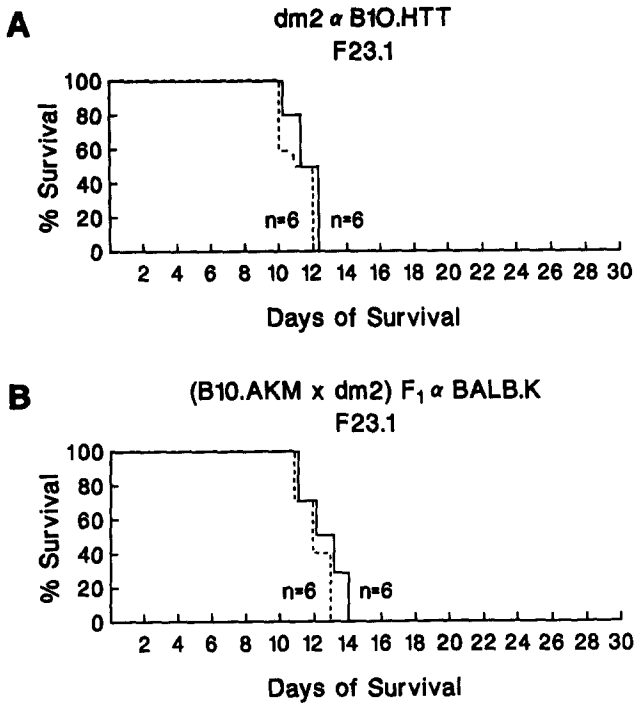

Figure 5. The in vivo depletion of Vβ8⁺ T lymphocytes does not prolong fully MHC-disparate B10.HTT skin allograft survival. (*A*) Control dm2 (- - -) mice rejected B10.HTT skin allografts in 11.2 ± 0.9 d. The survival time of these skin allografts is not prolonged by the in vivo administration of F23.1 to the dm2 recipient (——) (MST 11.3 ± 0.8 d). (*B*) In vivo F23.1 administration does not prolong the MHC class I-disparate rejection of BALB.K skin allografts by (B10.AKM × dm2)F_1 mice. The control (B10.AKM × dm2)F_1 (- - -) mice rejected BALB.K skin in 11.8 ± 1.0 d. F23.1 treated (B10.AKM × dm2)F_1 (——) rejected BALB.K skin allografts in a similar fashion (MST 12.1 ± 1.2 d).

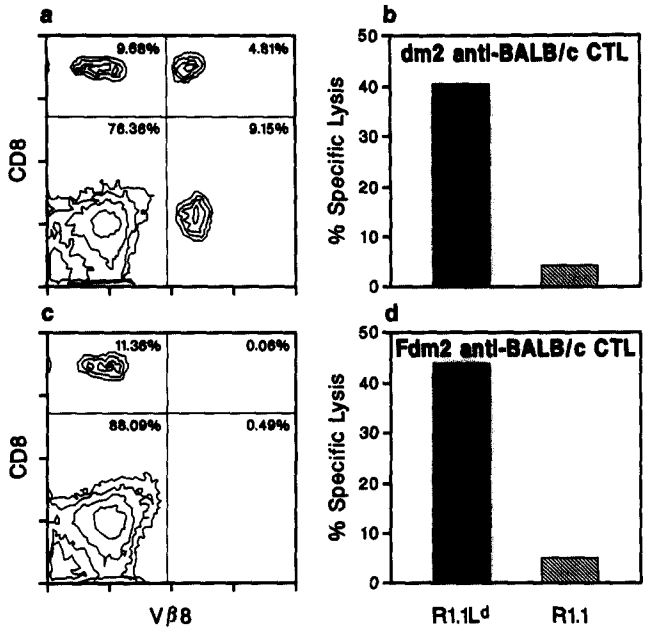

Figure 6. Secondary anti-L^d response generated by a Vβ8⁻ population. Two-color flow cytometry analysis of Vβ8 TCR and CD8 expression was performed at the completion of skin graft rejection on spleen cells from (*a*) control dm2 mice and (*c*) dm2 mice treated with F23.1. Cells were incubated with mAb F23.1 and developed with FITC-conjugated goat anti-mouse. This was followed by incubation with biotinylated antibody to CD8, developed with Streptavidin PE. Secondary L^d-specific CTL from (*b*) saline-treated control dm2 and (*d*) F23.1-treated dm2 mice (*Fdm²*) were assayed against R1.1L^d and R1.1 at an effector/target ratio of 100:1 at the completion of skin graft rejection.

had repopulated the peripheral lymphoid circulation and mediated the anti-H-2L^d skin allograft rejection response in the Vβ8-depleted recipients, flow cytometry evaluation of the peripheral lymphoid system was performed. At the completion of rejection of both the H-2^b and H-2^d skin grafts, spleen cells and draining axillary lymph node lymphocytes were harvested and evaluated for the presence of Vβ8⁺ T lymphocytes by flow cytometry. The control dm2 spleen cells contained 5–8% CD8⁺ Vβ8⁺ T cells (Fig. 6 *a*) whereas F23.1-treated dm2 mice, despite rejecting an H-2^d skin graft, did not possess CD8⁺ Vβ8⁺ T cells (Fig. 6 *c*). Identical results were obtained with axillary lymph node lymphocytes indicating that the Vβ8⁺ T cells were not concentrating in the region of the graft. These results demonstrate that the Vβ8⁺ T cell population plays a dominant role in the primary response to L^d and that, in the absence of a Vβ8 T cell response, a secondary T lymphocyte population develops after a delay and effects rejection of the L^d-disparate skin graft.

Effect of In Vivo Depletion of Vβ8⁺ T Lymphocytes on the Secondary Anti-H-2L^d Allogeneic Response. Since Vβ8-depleted dm2 mice did ultimately reject their L^d-disparate grafts, it was interesting to characterize the nature of this response. After the complete rejection of BALB/c skin grafts by untreated control and F23.1-treated dm2 mice, pooled spleen cells were stimulated with BALB/c irradiated spleen cells for 5 d. Secondary CTL were evaluated for anti-L^d cytolytic activity utilizing the L^d-transfected target cell line R1.1 and the nontransfected R1.1 cell line. Control and F23.1-treated secondary CTL demonstrated cytolytic activity against the L^d-transfected R1.1 cell line, but not the R1.1 cell line, as

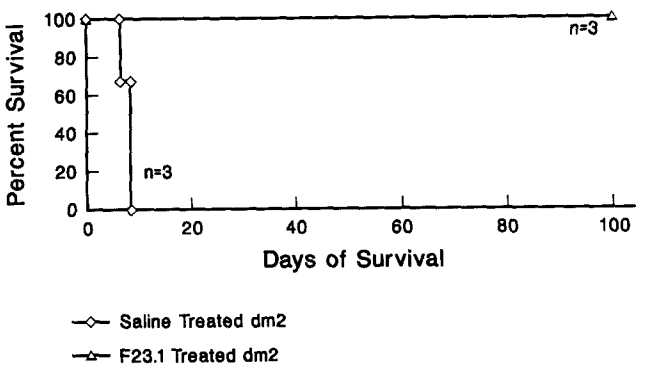

Figure 7. L^d-disparate cardiac allograft survival is prolonged by the in vivo depletion of Vβ8⁺ T lymphocytes. Control saline-treated dm2 mice (◇) rejected BALB/c cardiac allografts with a MST of 8.6 ± 0.5 d. In contrast, BALB/c allograft survival was significantly prolonged (MST > 100 d, $p < 0.01$) when Vβ8⁺ T lymphocytes were depleted in dm2 recipient mice using F23.1 (△).

shown in Fig. 6. These data demonstrating L^d-directed cytotoxicity in the $V\beta8^+$ T lymphocyte-depleted host confirm that the anti-L^d allogeneic response can occur after a prolonged exposure to antigen in the absence of $V\beta8^+$ T lymphocytes and suggest that lymphocytes expressing other T cell receptors not affected by the F23.1 mAb are playing a role. In support of this contention, anti-L^d CTL lines from F23.1-treated mice were propagated in vitro for an additional 4 wk and shown to lack the $V\beta8$ TCR by flow cytometric analysis (data not shown). It is noteworthy that the targets used in Fig. 6 are MHC class II-negative, thus the cytotoxicity observed was presumably $CD8^+$ CTL recognizing intact L^d and not $CD4^+$ CTL recognizing processed L^d.

Effect of In Vivo Depletion of $V\beta8^+$ T Lymphocytes on L^d-disparate Cardiac Allograft Survival. Because the in vivo depletion of $V\beta8^+$ T lymphocytes resulted in prolonged L^d-disparate skin graft survival, we next attempted to extend this observation to the less immunogenic vascularized cardiac allograft by utilizing a heterotopic cardiac transplantation model. Control untreated dm2 mice rejected L^d-disparate BALB/c cardiac allografts in 8.6 ± 0.5 d (Fig. 7). Histologic evaluation of BALB/c cardiac allografts removed at the complete cessation of ventricular contraction demonstrated a typical dense mononuclear cell infiltration and associated myofibril necrosis consistent with allograft rejection. In contrast, the mean survival time of BALB/c cardiac allografts in dm2 mice that were treated with 500 μg F23.1 on days −2, −1, +3, +25, +50, and +75 was prolonged to >100 d ($p < 0.01$ vs. control). Additional antibody was given to these animals on days 25, 50, and 75 to ensure that the $V\beta8^+$ T cells did not repopulate the circulation. Histologic evaluation of these BALB/c hearts removed 100 d after heterotopic transplantation demonstrated healthy cardiac myocytes without mononuclear cell infiltration or evidence of tissue damage. Therefore, in the less immunogenic vascularized cardiac allograft, removal of the $V\beta8^+$ T lymphocyte population eliminates the primary response and thereby allows dramatically prolonged survival of the allograft. The prolongation of BALB/c cardiac allograft survival by the depletion of $V\beta8^+$ T lymphocytes in the dm2 recipient thus extends the observation to a second organ and confirms that the $V\beta8$ TCR gene family plays a pivotal role in the primary immune response to H-$2L^d$ alloantigen.

Discussion

As a model system to study the TCR gene usage of a defined alloantigen, we have studied allorecognition of the murine L^d molecule. We report here that in vivo treatment with the $V\beta8$-specific mAb F23.1 eliminates all detectable peripheral $V\beta8^+$ T lymphocytes. This in vivo depletion was found to abrogate a subsequent primary in vitro response of dm2 cells to L^d. Additionally, the depletion of $V\beta8^+$ T lymphocytes significantly prolonged survival of L^d-disparate skin and even more dramatically prolonged vascularized heart allografts. This abrogation of the dm2 anti-L^d response was MHC specific in that in vivo depletion of $V\beta8^+$ CTL had no effect on the in vivo or in vitro response of dm2 homozygous or heterozygous mice to other class I alloantigens. Thus, the in vivo administration of the mAb F23.1 results in a substantial MHC-specific abrogation of the primary dm2 response to L^d alloantigens. These findings demonstrate that TCR with $V\beta8$ segments predominate in the in vivo and in vitro primary responses to the L^d alloantigen. This finding of a predominant TCR usage in allogeneic responses to MHC molecules is in agreement with certain studies (e.g., 11, 12), whereas others have reported finding high levels of TCR diversity (13–15). The reported diversity of alloresponsiveness could be explained by either structural differences among class I alloantigens (24) or their respective ligands (25). For example, the high level of polymorphism among class I molecules results in ~20% differences between the amino acid residues of different K, D, or L molecules (26). Furthermore, most of these differences are located in the $\alpha1/\alpha2$ domains at positions where they could affect TCR recognition of class I either directly or indirectly via ligand binding (27). Given that the L^d molecule is as different from D^d and K^d molecules as they are from each other (26), the lack of diversity in the TCR response to L^d alloantigens is probably not due to insufficient polymorphism. Another contributing factor in the complexity of the alloresponse to class I could be the role of the bound peptide ligand. Although still somewhat controversial, several studies indicate that certain alloreactive (28) and xenoreactive (29) TCR to class I can discriminate self-peptide ligands. Furthermore, a given class I molecule is clearly capable of binding numerous self-peptide ligands (30). Therefore, if the bound peptide ligand plays a role in allorecognition, diversity in the TCR gene family usage would be anticipated. Given that L^d molecules, like K and D alloantigens, have been shown to bind several different immunogenic or self-peptides (31–33), the lack of TCR diversity of L^d alloantigens is presumably not due to its inability to bind a variety of ligands. Thus, there are no obvious structural differences between L^d and other class I molecules and their respective ligands that might explain why the alloresponse of dm2 mice to L^d should show such a limited $V\beta8$ usage. However, based on the recent model of Davis and Bjorkman (7), speculations can be made that might explain the predominance of $V\beta8$ in TCR recognition of L^d.

Although the precise manner by which TCR and class I-peptide interact is unclear, a very intriguing model has been recently proposed. Even though TCR molecules have not yet been crystallized, based on structural similarities with Ig, their respective V domains are likely to display comparable folding. Furthermore, the three hypervariable regions of Ig (CDR1-3), known to form the principal contact points of Ig with antigen, are located at equivalent locations in TCR gene products. Given these similarities between TCR and Ig, Davis and Bjorkman (7) proposed a model of how MHC molecules and TCR might interact. In this model, the CDR1 and CDR2 regions of $V\alpha$ and $V\beta$ of the TCR would interact with the α helical portions of the $\alpha1/\alpha2$ class I molecule. Furthermore, the CDR3 region located in the V(D)J junction would interact with bound peptide (34). Indeed, there are several findings consistent with this model (cf 11, 12). In the context of this model, speculations can be made

as to why the L^d alloresponse might show a predominant Vβ8 usage. Perhaps the predominance of Vβ8 recognition of L^d is determined by the structure of its α helical regions independent of the bound peptide ligand. Fortuitously, the CDR1 and CDR2 regions of Vβ8 could have an affinity for surface residues in the α helical regions of L^d. This explanation is supported by the observations that Vβ8 predominates in both alloreactive (data presented here) and syngeneic peptide-specific responses restricted to L^d in BALB/c mice. In these studies of L^d-restricted syngeneic responses, Rodewald et al. (8) demonstrated a 3–5-fold higher L^d- restricted CTL precursor frequency in Vβ8$^+$ T lymphocytes compared with Vβ8$^-$ T lymphocytes after infection with CMV; and Solheim et al. (manuscript submitted for publication) found a dominant usage of Vβ8 by L^d-restricted CTL clones specific for the tum$^-$ peptide. Thus Vβ8 predominates in both allogeneic and at least certain peptide-specific responses restricted by L^d in mice of the BALB/c genetic background. It will be very interesting to determine whether this extends to mouse strains of other genetic backgrounds.

An alternative explanation for the predominance of Vβ8 usage is that it is determined by the peptide ligands bound to L^d. Although L^d presents many different peptides, perhaps a high proportion of alloreactive CTL to L^d recognize the same dominant self-peptide or peptides of a common structural motif. However, predominant Vβ8 usage has also been observed in syngeneic peptide-specific responses to L^d, and these latter CTL display exquisite peptide specificity. Furthermore, the primary region of the TCR thought to interact with the bound peptide is CDR3 which is located in the junctional region, not the V segment alone (34). Thus, it appears more likely that the predominant usage of Vβ8 found in responses restricted by L^d is independent of the bound peptide. Regardless of which of the above explanations is correct, the predominance of Vβ8 usage reported here provides an important insight into TCR recognition of an allogeneic class I molecule. It was noted by Davis and Bjorkman (7) that the relative sizes of the interactive surfaces of MHC and TCR would allow binding in different registers along the MHC α helices. Thus the V genes employed by different T cells restricted to the same MHC molecule would not be expected to be identical. By contrast, our data would suggest that at least Vβ8 and L^d interact primarily along a single register as defined by the predominance of Vβ8 in allogeneic and MHC–restricted responses.

In conclusion, the findings here may have clinical relevance for the design of transplantation immunotherapies. Previous studies of end stage rejection of transplanted tissues have demonstrated heterogeneous TCR usages. This heterogeneous response is predictable however, based on the fact that at end stage rejection there would be recruitment of large numbers of T lymphocyte specificities due to both nonspecific factors produced as a result of inflammatory processes and specific recruitment of T lymphocytes reactive with potentially tissue-specific cryptic epitopes exposed as a result of tissue destruction. Recent reports in the human have indicated that, similar to the data presented here, initial rejection episodes demonstrate a preferential TCR usage for the bulk of the response (12). Therefore, if specific TCR therapies could be designed to block the initial predominant rejection response, perhaps the second line of recruitment and rejection may be more easily managed with lower doses of conventional immunotherapies and thereby decrease host susceptibility to opportunistic infections and malignancies. In addition, EAE has been treated with TCR peptides derived from the predominantly selected variable regions (35, 36). Recent data reported by Lehmann et al. (37) supports our hypothesis that if the first line immune response is abrogated early, the secondary response may also be downregulated. Lehmann et al. (37) demonstrated in the experimental allergic encephalitis model specific peptide therapy during primary disease induction processes inactivates the primary TCR clonally restricted effector cells, abrogating the inflammatory reaction in the central nervous system, a prerequisite for second wave priming. However, after determinant spreading has occurred, as a result of central nervous system inflammatory processes, anticlonotypic therapy may no longer be effective in therapeutically managing the diversified autoreactive T cell pool in more advanced disease. Indeed, our preliminary studies indicate that a peptide derived from the CDR2 region of Vβ8.2 can prolong L^d-disparate allografts. Thus peptide therapy may also be effective for the prevention and treatment of allograft rejection when a predominant TCR gene family usage can be identified.

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