Specific prolongation of allograft survival by a T-cell-receptor-derived peptide

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ABSTRACT Allograft rejection results from the specific recognition by host CD8+ T cells of allogeneic major histocompatibility complex (MHC) molecules on the tissue graft. The specificity of this cellular response is determined by the molecular interaction of the T-cell receptor (TCR) on host T cells with the MHC molecule and its bound ligand on the grafted tissue. To better understand the precise manner by which the TCR interacts with the MHC–peptide complex and how to therapeutically intervene, we have studied the allogeneic response to the mouse class I MHC molecule Ld. In this report, the therapeutic potential of a synthetic peptide derived from the TCR Vβ8 variable region that predominates in responses to Ld was tested. This Vβ8-derived peptide was found to dramatically and specifically block the in vitro and in vivo allogeneic response to Ld. Furthermore, this specific blocking is not dependent upon the presence of Vβ8+ effector cells nor does the Vβ8 peptide bind to the Ld ligand binding cleft. We propose that this peptide functions as an antagonist, competing with the native TCR for recognition of the Ld molecule.

During transplantation or allograft rejection, determinants encoded within the major histocompatibility complex (MHC) of the donor are recognized by T cells of the recipient. This recognition results in a potent immunological response in which the donor cells are rapidly and specifically killed and the allograft is destroyed (1, 2). It is generally accepted that T cells recognize foreign antigen in the form of peptides bound to self MHC molecules. However, the molecular basis of T-cell recognition of allogeneic cells remains controversial (e.g., the extent to which alloreactive T cells are peptide-specific). Furthermore, it has been proposed that chronic rejection may result from indirect T-cell recognition of an allogeneic MHC-derived peptide bound to a self MHC molecule (3). Thus MHC antigens clearly influence the fate of allograft rejection but the precise mechanism(s) remains unclear.

Initial studies analyzing T-cell receptor (TCR) usage during end-stage transplantation rejection episodes resulting from MHC disparities have been conflicting. Whereas certain studies have reported preferential TCR usage in alloreactive responses (4–6), other studies found diversified TCR usage (7–9). Recently, our laboratory has investigated (6) the allo- genetic response to the murine class I MHC molecule Ld and found that T cells carrying TCRs with Vβ8 variable (V) regions predominated in in vitro and in vivo T-cell responses to Ld. For example, monoclonal antibody (mAb) to Vβ8 was found to specifically and dramatically prolong Ld-disparate skin and heart allografts. This observation afforded us the unique opportunity to test the efficacy of using TCR-derived peptides to block the alloreactive response.

The use of peptide to manipulate the immune response was greatly facilitated by the elucidation of the three-dimensional structure of the MHC class I molecule (10). The crystal structure of class I molecules revealed α1 and α2 regions that fold to form a peptide binding cleft and helical regions that provide potential contact sites for the TCR (11). In addition, for certain murine models of autoimmune disease, it has been possible to identify TCR gene usage of the causative T cells (12). With this information, in vivo therapies using synthetic peptides have been successfully employed (13). However, to our knowledge, similar strategies have previously not been used in transplantation.

In this report we use a TCR-derived peptide to specifically block the in vitro and in vivo immune response to Ld alloantigens. Our experiments demonstrate that the TCR peptide does not induce a central regulatory mechanism nor does the TCR peptide bind the Ld ligand binding cleft. Based on these results and current models for the interaction of MHC with TCR, we propose that the TCR-derived peptide interacts with polymorphic sites on the helical regions of Ld and functionally competes with the TCR.

MATERIALS AND METHODS

Mice. Female C57BL/6, C57L, BALB/c, BALB.K, BAR-8, BALB/c-H-2dm2 (dm2), and (B10.AKM × dm2)F1 mice (Table 1) at 6–8 weeks of age were obtained from the animal facility of Donald C. Shreffler at Washington University (St. Louis).

Peptides. The amino acid sequences of the Vβ8 and Vβ14 TCR peptides correspond to residues 39–59 (Vβ8, DT-GHGLRLIHYSGAGSTEK; Vβ14, ATGGTLQQLFYSIGTVQVESV) of the murine Vβ segments (14). The amino acid sequences of the murine cytomegalovirus (MCMV) and lymphocytic choriomeningitis virus (LCMV) peptides correspond to residues 168–176 (YPFHFMPTNL) of the MCMV early protein pp89 (15) and residues 118–126 (RPQASGVYM) of the murine LCMV (16). The peptides were synthesized using Merrifield's solid-phase method (17) on an Applied Biosystems model 430A peptide synthesizer and t-butoxycarbonyl chemistry. Peptides were purified by reverse-phase HPLC as described (18).

mAbs. For detection of the Vβ8 TCR, mAb F23.1 was used (19). For detection of Ld and Kd molecules mAbs 28-14-8 and 11-4-1 were used, respectively (20, 21). All three mAbs are of the IgG2 isotype.

Flow Cytometry. Flow cytometry was performed as described (6). Briefly, cells were incubated with a saturating concentration of mAb, washed, and incubated with a satu-

Abbreviations: dm2, BALB/c-H-2dm2; CTL, cytotoxic T lymphocyte; EAE, experimental autoimmune encephalomyelitis; MHC, major histocompatibility complex; mAb, monoclonal antibody; TCR, T-cell receptor; MCMV, murine cytomegalovirus; LCMV, lymphocytic choriomeningitis virus; V, variable; CDR, complementarity-determining region.

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Table 1. Mouse strains and tumor target cell lines used

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Cell line

| P815          | H-2<sup>d</sup> | K<sup>d</sup> D<sup>d</sup> L<sup>d</sup> |
| EL-4          | H-2<sup>b</sup> | K<sup>b</sup> D<sup>b</sup> |
| R1.1          | H-2<sup>k</sup> | K<sup>k</sup> D<sup>k</sup> |
| R1.1-L<sup>d</sup> | H-2<sup>k</sup> | K<sup>k</sup> D<sup>k</sup> L<sup>d</sup> |

<sup>1</sup>L<sup>d</sup>-transfected R1.1 target cell line.

<sup>*</sup>Fc receptor-negative target cell line.

rating concentration of fluorescein-conjugated F(ab')<sub>2</sub> goat anti-mouse IgG, Fc-specific (Organon Teknika–Cappel). Labeled cells were analyzed with a FACScan (Becton Dickinson), and mean fluorescence was analyzed using the Consort 30 software.

Cytotoxic T-Lymphocyte (CTL) Assays. The in vitro generation and assay of CTLs has been described in detail (6). Briefly, responding dm2 or (B10.AKM x dm2)<sub>F1</sub> spleen cells were cocultured with irradiated (2000 rads; 1 rad = 0.01 Gy) L<sup>d</sup>-disparate BALB/c or D<sup>d</sup>-disparate BALB/K spleen cells for 5 days. Approximately 1 x 10<sup>6</sup> R1.1-L<sup>d</sup>, R1.1, and P815 target cells were labeled with 200 μCi of <sup>51</sup>Cr (Amersham; 1 Ci = 37 GBq) and 2 x 10<sup>5</sup> target cells were mixed with various concentrations of the effector cells for 4 h. For V<sub>v</sub>β<sub>8</sub> peptide blocking, 1 x 10<sup>4</sup> M V<sub>v</sub>β<sub>8</sub> peptide was included for the 5-day sensitization and/or during the 4-h <sup>51</sup>Cr-release assay. At the end of the 4-h assay, radioactivity in 100 μl of supernatant was measured in a LKB Clinigamma counter, and the percent <sup>51</sup>Cr release was determined by the following equation: percent <sup>51</sup>Cr release = 100 x [(experimental <sup>51</sup>Cr release – medium control <sup>51</sup>Cr release)/maximum <sup>51</sup>Cr release – medium control <sup>51</sup>Cr release].

Skin Grafting. Female dm2 and (B10.AKM x dm2)<sub>F1</sub> mice were grafted with abdominal C57BL/6, BALB/c, and BALB/K skin on day 0 (22). On days −2, 0, +3, and every 3 days thereafter until allograft rejection was complete, experimental mice were injected subcutaneously with 1 x 10<sup>4</sup> M V<sub>v</sub>β<sub>8</sub> or V<sub>v</sub>β<sub>14</sub> peptide, whereas control mice received injections of normal saline. Bandages were removed on day +7, and the grafts were scored daily until rejection was complete (defined as graft loss >90%). Allograft survival was analyzed for significance by the Kaplan–Meier analysis.

Surface Induction of L<sup>d</sup>. The L<sup>L</sup>-L<sup>d</sup> cell line was generated by introducing the L<sup>d</sup> gene into murine Ltk<sup>−</sup> DAP (H-2<sup>k</sup>) fibroblasts. Surface induction of L<sup>d</sup> was analyzed by incubating 5 x 10<sup>6</sup> cells per ml overnight in the presence of 2.5 x 10<sup>−4</sup> M V<sub>v</sub>β<sub>8</sub>, V<sub>v</sub>β<sub>14</sub>, or MCMV peptide. The cells were washed and analyzed for L<sup>d</sup> and H-2<sup>k</sup> expression by flow cytometry.

Competitive Inhibition Assay. Generation of MCMV-specific CTLs was performed as described (23). Competitive inhibition assays were performed as described (24) by pre-incubating <sup>51</sup>Cr-labeled P815 (H-2<sup>k</sup>) target cells for 1 h at 37°C with 100 μM competitor peptide in the presence or absence of 100 μM antigenic peptide, followed by analysis in a standard <sup>51</sup>Cr-release assay.

RESULTS

Synthetic V<sub>v</sub>β<sub>8</sub> TCR-derived peptide could prolong allograft survival, mice of the L<sup>d</sup>-loss strain dm2 (25) were injected subcutaneously with the V<sub>v</sub>β<sub>8</sub> peptide every 3 days until complete allograft rejection occurred. Peptide-treated dm2 mice were engrafted with two skin allografts, L<sup>d</sup>-disparate BALB/c skin and H-2<sup>b</sup>-disparate C57BL/6 skin. As shown in Fig. 1A, the V<sub>v</sub>β<sub>8</sub> peptide treatment prolonged the L<sup>d</sup>-disparate skin allografts =10 days beyond control mice injected with saline or the V<sub>v</sub>β<sub>14</sub> peptide. In contrast, V<sub>v</sub>β<sub>8</sub> peptide had no significant effect on H-2<sup>b</sup>-disparate allograft rejection (data not shown).

To more rigorously assess the MHC specificity of the V<sub>v</sub>β<sub>8</sub> peptide treatment, the peptide was tested in a donor-recipient strain combination differing by only D<sup>b</sup> or L<sup>d</sup>. As shown in Fig. 1B, the D<sup>b</sup>-disparate skin allografts were rejected by V<sub>v</sub>β<sub>8</sub>-peptide-treated and control mice in similar time frames, whereas L<sup>d</sup>-disparate skin allograft survival was significantly prolonged in V<sub>v</sub>β<sub>8</sub>-peptide-treated mice. Thus, the prolongation of L<sup>d</sup>-disparate allografts by the V<sub>v</sub>β<sub>8</sub> peptide is substantial in duration and MHC-specific. Lymphocytosis from peptide-treated mice showed no reduction in V<sub>v</sub>β<sub>8</sub> expression, indicating that graft prolongation did not result from depletion of V<sub>v</sub>β<sub>8</sub>-reactive T cells. In addition, serum from peptide-treated mice did not contain antibody capable of blocking CTL recognition of L<sup>d</sup>, indicating that peptide did not induce anti-idiotypic antibodies capable of down regulating the allogeneic response to L<sup>d</sup> (data not shown).

V<sub>v</sub>β<sub>8</sub> Peptide Abrogation of the in Vitro Allogeneic Immune Response to L<sup>d</sup>. To extend the above findings with allograft survival to in vitro assays, the V<sub>v</sub>β<sub>8</sub> peptide was tested for its ability to block cytotoxicity of dm2 spleen cells stimulated with BALB/c cells in vitro. When V<sub>v</sub>β<sub>8</sub> peptide was added throughout the entire culture period, as much as 80% inhibition of the response to L<sup>d</sup> was seen (Fig. 2). However, if V<sub>v</sub>β<sub>8</sub> peptide was added to the effector phase only

![Fig. 1](https://example.com/fig1.png)
CTLs could not be generated from responder cells cultured in the presence of the Vg8 peptide. However, Dk-specific alloreactive CTLs could still be effectively generated, thus demonstrating the Ld specificity of this treatment. The precursor frequencies of CTLs to Ld and Dk are not significantly different (data not shown), and thus the preferential blocking of Ld vs. Dk allogeneic responses is not due to quantitative differences in the number of cells that must be blocked. Therefore, the inhibition by the Vg8 peptide of the generation of an in vitro response to Ld alloantigens is MHC-specific.

Mechanistically, the specific blocking by the Vg8 peptide could occur at the level of the effector cell by acting selectively on Vg8+ T cells, or alternatively, the Vg8 peptide could be exerting its effect at the target cell level by binding to Ld. To discriminate between these two possibilities anti-Ld-reactive CTLs were generated using responder cells from the Vg8- mouse strain C57L (27). In data not shown, Vg8 responder cells mounted a significant anti-Ld response. Furthermore, comparable levels of Vg8 peptide-specific inhibition was observed with both Vg8+ (C57L) and control Vg8+ (C57BL/6) responder cells against Ld alloantigens. Thus, the Vg8 peptide blocking of allogreactivity to Ld is not dependent on the presence Vg8+ T cells and, therefore, must be functioning at the level of the MHC molecule.

Lack of Competitive Inhibition of MCMV by the Vg8 TCR Peptide. To determine the mechanism of the inhibition observed with the Vg8 peptide, we tested the ability of the peptide to bind Ld molecules on the target cell. Specifically, we addressed Vg8 occupancy of the peptide binding cleft by utilizing two binding assays. In the first assay, 5 × 10^5 Ld cells per ml were cultured overnight (16–18 h) in the presence of 2.5 × 10^-4 M Vg8 TCR peptide or synthetic MCMV peptide. As shown in Fig. 4, Ld surface expression was induced ~8-fold when Ld cells were cultured overnight.

FIG. 2. In vitro generation of Ld-specific alloreactive CTLs is specifically inhibited by in vitro treatment with Vg8 peptide. Primary Ld-specific CTLs were generated in the presence (Φ, Φ) or absence (Φ, Φ) of Vg8 peptide. Peptide (Φ, Φ) or saline (Φ, Φ) was also included in the 4-h 51Cr-release assay using R1.1-Ld target cells. R1.1-Ld is an H-2k thymoma transfected with the Ld gene.

(4-h 51Cr-release assay), no inhibition was observed. Furthermore, we have thus far been unable to block Vg8+ CTL clones or lines to Ld using the Vg8 peptide. These findings indicate that the Vg8 TCR peptide can inhibit the generation of an in vitro response to Ld alloantigen but cannot block established Ld-reactive CTLs. Perhaps this disparity in blockability by the Vg8 peptide reflects the fact that primary CTLs are more CD8-dependent than secondary CTLs (26). Thus primary CTLs maybe of generally lower affinity and thus more susceptible to blocking with the TCR-derived peptide.

To determine whether the in vitro blocking by the Vg8 peptide was specific to Ld, Dk-specific allogeneic responses were examined. Hybrid (B10.AKM × dm2)F1 responder cells were stimulated with Ld-disparate BALB/c or Dk-disparate BALB.K irradiated cells. As shown in Fig. 3, control F1 responder cells mounted a strong cytolytic response to both Ld and Dk alloantigens. In contrast, Ld-specific alloreactive

FIG. 3. Vg8 peptide specifically blocks the in vitro allogeneic response to Ld. Responder splenocytes from (B10.AKM × dm2)F1 mice were cultured with Ld-disparate BALB/c stimulators (A and C) or Dk-disparate BALB.K stimulators (B and D), in the presence (A and B) or absence (C and D) of peptide. Ld- and Dk-specific lysis was assayed on P815 (H-2d) or R1.1 (H-2d) target cells, respectively, in a 4-h 51Cr-release assay.

FIG. 4. Vg8 peptide treatment of Ld-Ld fibroblasts does not specifically induce the expression of Ld antigens. Ld-Ld fibroblasts were incubated overnight with the Ld ligand MCMV peptide (A and C) or the Vg8 peptide (B and D) at 2.5 × 10^-4 M. Ld expression was measured with mAb 28-14-8 (20, 21) and developed with fluorescein-conjugated goat anti-mouse IgG. Background (BKGD) represents fluorescence emitted from cells incubated with the developing reagent alone. Specificity of Ld induction was assessed by the measurement of Kk antigens on both MCMV- and Vg8-peptide-treated cells with the mAb 11-4-l (20, 21), which was developed with a fluorescein-conjugated goat anti-mouse IgG. Expression of Ld or Kk by cells not treated with peptide is indicated by a chained line, whereas expression of Ld or Kk by peptide-treated cells is indicated by a dotted line. The MCMV peptide gave a ~6-fold specific induction of Ld (A) and no increase in Kk expression (C). In contrast the TCR Vg8 peptide did not induce Ld (B) or Kk (D) expression.
with the Ld ligand MCMV, while there was no induction of H-2Kk molecules with the MCMV peptide. In contrast, cell surface expression of Ld was not induced after culture with the Vß8 peptide. As expected no induction of H-2Kk was observed. Thus the Vß8 peptide does not bind to the ligand binding groove of Ld, as measured by increased cell surface expression, as occurs with all other known ligands to Ld (28).

The second assay employed to determine whether the Vß8 peptide binds the Ld ligand binding site was a peptide competition assay using a CTL clone specific for the MCMV peptide bound to Ld (23). As shown in Fig. 5, the LCMV peptide, a known Ld ligand (16), showed significant inhibition at both 10 μM and 100 μM. In contrast, 100 μM Vß8 peptide or 100 μM Vβ14 peptide showed no inhibitor activity. Thus together with the inability to induce Ld cell surface expression, these data clearly demonstrate that the Vß8 peptide does not compete for the binding cleft of Ld. These findings indicate that the ability of the Vß8 peptide to inhibit alloreactivity to Ld does not reflect the displacement of endogenous ligands involved in allore cognition nor does the Vß8 peptide function as an agonistic Ld ligand capable of specifically down regulating T-cell function (29).

**DISCUSSION**

Allograft rejection remains a formidable problem in modern medicine. Transplantation recipients continue to receive lifelong high-dose nonspecific immunosuppression that renders them susceptible to a variety of infectious and neoplastic complications. Peptide therapies could represent a means by which the host response to allografted tissue could be specifically blocked and thus the number of posttransplantation complications could be decreased. Specific blockade of the alloreactive response requires intervention into the interaction between the TCR of host T cells and MHC molecules of the transplant. In the absence of the TCR crystal structure, the V regions of the TCR have been modeled based on structural similarities with immunoglobulin. Based on this modeling the V domains of the two molecules have been predicted to display similar folding. Furthermore, the three hypervariable regions of the immunoglobulin [complementarity-determining regions 1-3 (CDR1-3)] known to form the principal contact points of immunoglobulin with antigen are located at equivalent locations in the TCR. Given these similarities between TCR and immunoglobulin, Davis and Bjorkman (11) have proposed a model whereby the CDR1 and CDR2 regions of Vα and Vβ of the TCR interact with the helical portions of the α1/α2 domains of the MHC class I molecule (11) and the CDR3 region of the TCR interacts with the bound peptide. In the context of this model, strategies have been employed to specifically block TCR recognition of MHC by using synthetic peptides derived from TCR V regions implicated in autoimmune responses (12, 13).

The rodent autoimmune demyelinating disease, experimental autoimmune encephalomyelitis (EAE), was the first immune response in which the therapeutic potential of a synthetic TCR peptide was evaluated. It is recognized that in EAE self MHC molecules bind and present the self antigen myelin basic protein, followed by preferential recognition of these molecules by Vß8+ myelin basic protein-reactive T cells resulting in demyelination and paralysis (12, 13). The initial Vß8 TCR-derived peptide studies demonstrated that EAE could be prevented or reversed by treatment with a synthetic Vß8 peptide (12, 13). Mechanistic studies in EAE suggest that the Vß8 peptide binds to an MHC molecule (presumably class I) and induces a regulatory mechanism that selectively down regulates Vß8+ T cells (30).

Our present findings clearly demonstrate that specific alloreactivity to the murine MHC class I molecule Ld, which has been shown to predominantly use Vß8+ T cells (6), can be abrogated by in vitro treatment with the Vß8 peptide. Additionally, in vivo treatment significantly prolongs the survival of Ld-disparate skin allografts without altering the immune response to other MHC class I molecules, demonstrating that this immunological intervention is MHC-specific. Our mechanistic studies show that these findings are not due to regulatory cells or anti-idiotypic antibodies and are not dependent upon Vß8+ T cells. This would imply that Vß8 peptide is acting by a mechanism distinct from that proposed for EAE. Furthermore, the lack of ligand binding site competition indicates that the replacement of dominant alloreceptive peptides is not occurring. However, it remains possible that the Vß8 peptide binds an alternate region on Ld molecules such as the α1/α2 helical portion. It has been predicted that this Vß8 peptide is capable of attaining a secondary structure reflective of the native CDR2 region from which it was derived (31). In addition, based on computer modeling, it has been suggested that the CDR2 region of the TCR interacts with the α1 helical portion of the MHC molecule (11). Given the validity of these assumptions, it is attractive to speculate that the Vß8 peptide binds Ld, in a fashion similar to an intact TCR, and competes effectively for interaction with Ld. Such a mechanism is intriguing in light of the observation that peptides derived from the α2 region of HLA-A2 (32) or H-2Kk (33) molecules can also block in vitro allore cognition. However, the efficacy of these peptides in vivo is unknown. It is possible that both the MHC and TCR peptides could function as respective antagonists blocking the interaction of the TCR with the MHC. Indeed, in preliminary studies, we have found that the immune response to Ld can be abrogated by the in vivo administration of a peptide from the α2 region of Ld. These studies raise the possibility that peptide therapies can be customized to individual MHC molecules and used to specifically inhibit allore cognition.