Inhibitory Effect of a CD4-CDR3 Peptide Analog on Graft-Versus-Host Disease Across a Major Histocompatibility Complex-Haploidentical Barrier

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A structure-based designed peptide has been engineered to exhibit the same molecular surface as a portion of the CDR3-like region in domain 1 of the murine CD4 molecule. Earlier in vitro experiments indicated that this analog, known as rD-mPGPtide, inhibited T-cell proliferation in mixed lymphocyte reactions and blocked activation of both normal CD4+ T cells and T-cell lines after T-cell receptor triggering. In addition, rD-mPGPtide proved to be a potent inhibitor in vivo of CD4+ T-cell-mediated experimental allergic encephalomyelitis disease in the SJL mouse model. In this current report, we have evaluated the potential of rD-mPGPtide for suppressing the development of graft-versus-host disease (GVHD) in an irradiated major histocompatibility complex (MHC)-haploidentical murine bone marrow transplantation (BMT) model [(B6 × DBA/2)F1 → (B6 × CBA)F1, (950 cGy)]. Our results indicated that early administration of rD-mPGPtide was effective in the inhibition of alloreactive responses of the donor T cells against the host and thus prevented or reversed the onset of GVHD. The median survival time of animals treated with rD-mPGPtide was enhanced as much as four-fold with as little as a single dose of peptide at the time of transplant. Decreased alloreactivity was indicated by phenotypic and functional analysis of positively selected thoracic duct lymphocytes 4 days after transplant and by histopathological examination of skin and gastrointestinal tissue samples 4 weeks later. Therefore, the administration of a CD4-CDR3 peptide is an efficacious approach against the development of GVHD during allogeneic BMT.

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A LLOGENEIC BONE MARROW transplantation (BMT) is currently being used as a treatment for a number of disease states including several types of leukemia, aplastic anemia, and severe combined immunodeficiency, among others.1 The major complications that impede the overall success of this treatment include the development of graft-versus-host disease (GVHD), marrow graft rejection, chronic immuno-incompetence, and leukemic relapse (in the case of BMT for the treatment of leukemias). Acute and chronic GVHD is caused by residual mature donor T cells in the bone marrow graft and leads to significant morbidity and mortality.2 Removal of the mature T cells from the graft before engraftment reduces or prevents GVHD; however, this T-cell depletion also leads to reduced engraftment along with increased leukemia relapse rates.3,4 These observations suggest the importance of a T-cell component in a successful BMT, although it is not clear whether GVHD reactive T cells can be separated completely from either the antileukemia effect or from enhanced hematopoietic engraftment. This question can only be approached by highly selective means of inhibiting those host-allospecific GVHD-reactive T cells, while allowing for the potential development of antileukemia-specific responses and for protection from opportunistic infections.

The CD4 molecule on the surface of helper T cells, in association with the T-cell receptor (TCR)-CD3 complex that recognizes specific antigen in the context of MHC class II, plays a critical role in the transmembrane and intracellular signaling pathways required for T-cell activation.6-11 It has been well-established in murine models that CD4+ T cells are capable of mediating GVHD, primarily across MHC class II barriers,12,13 but also in some cases with minor histocompatibility antigenic differences.14,15 Inhibiting CD4+ T-cell responses by treatment of recipient mice with monoclonal antibodies (MoAb) directed against the CD4 molecule has effectively decreased the incidence of GVHD following BMT.16-19 However, MoAb therapy has several limitations for potential clinical use, including but not limited to total subset depletion and immunogenicity of the MoAb itself.18-21

In previous reports, we have described the design and production of a peptide that specifically mimics the CDR3-like region in the D1 immunoglobulin domain of the murine CD4 molecule.22-24 This peptide analog consists of thirteen amino acids (CELENRKEEPGPC) taken from the p86-94 sequence of the CD4 molecule with the addition of a proline-glycine-cysteine sequence to the carboxyl terminus to allow cyclization and tertiary structural constraint. In order to make the peptide more resistant to protease degradation, it was synthesized with D-amino residues, necessitating the reversal of the amino acid order so that side chain presentation would be similar to the native molecule.23 As a result of these adaptations, this CD4 peptide analog is referred to as reverse D amino acid mouse proline-glycine-proline peptide (rD-mPGPtide). The rD-mPGPtide is neither T-cell subset depletive nor immunogenic and thus has advantages over the use of anti-CD4 MoAb.

Our earlier reports documented that rD-mPGPtide is a potent inhibitor of certain types of CD4+ T-cell-mediated immune responses both in vitro and in vivo. In this report, we demonstrate the potential of rD-mPGPtide for inhibiting the in vivo alloreactive responses associated with the onset of GVHD in a major histocompatibility complex (MHC) haploidentical murine BMT model (B6 × DBA/2)F1 → (B6 × CBA)F1 (950 cGy). In this model, the donor and recipient mice possess both class I and class II differences. Injection

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of irradiated (B6 × CBA)F1 mice with a donor bone marrow inoculum supplemented with either 5 × 10^6 unseparated or 1 × 10^6 CD4^+ enriched donor T cells induces an acute form of GVHD, which leads to fatality within 2 to 3 weeks. We show here that administration of rD-mPGPptide to recipient mice at varying times within the first week of transplantation significantly increased the median survival time of mice undergoing GVHD.

MATERIALS AND METHODS

**Mice.** Mice, (B6 × DBA/2)F1, [(B6D2)F1 (H2^k^6^)] and (B6 × CBA)F1, [(B6CB)F1 (H2^k^6^)], were purchased from the Jackson Laboratory (Bar Harbor, ME). Male mice were used as donors between the ages of 7 to 12 weeks and as recipients between the ages of 9 to 16 weeks. Mice were kept in a sterile environment in microisolators at all times and were provided with acidified water and autoclaved food.

**Media.** Buffered saline solution (BSS) supplemented with 0.1% bovine serum albumin (BSA) (Hyclone, Logan, UT) was used for all in vitro manipulations of the donor bone marrow and lymphocytes. For injection, cells were resuspended in BSS alone. RPMI 1640 (Mediatek, Herndon, VA) supplemented with 10% fetal calf serum (FCS) (Sigma, St Louis, MO) and 10 U/mL glutamate, 10 U/mL penicillin and streptomycin, and 0.05 mmol/L β-mercaptoethanol (Mediatek) was used for all in vitro mixed lymphocyte responses.

**Peptides.** The peptides were designed as previously described, synthesized on an Applied Biosystems 430A peptide synthesizer (Foster City, CA) using standard Fmoc chemistry, refolded to enrich for intramolecular disulfide bonding, and purified by HPLC (Waters, Milford, MA) using standard Fmoc chemistry. The sequences of the synthesized peptides were as follows: rD-mPGPptide (CPGPEEKRELEC, all D-amino acids) and scrambled rD-mPGPptide (Scr-PGPptide; CEPKNEPERGEC, all D-amino acids). For treatment of GVHD, peptides were reconstituted in PBS and injected at the appropriate dose and time into mice intravenously (IV) in a volume of 0.25 mL.

**Irradiation.** All recipient mice received a 950 cGy exposure from a Gammascan 117cs source (116 cGy/min).

**MoAb.** Ascites fluid for anti-Thy-1.2 (J1), rat IgM) and anti-CD8 (3.168, rat IgM) MoAb were used for cell preparations. In addition, goat antimouse IgG (whole molecule) antibodies were purchased from Cappel-Organon Teknika (Westchester, PA). Guinea pig serum prepared in our laboratory was used as a source of C for all MoAb treatments. For phenotypic analysis of cells by flow cytometry, anti-murine CD4 (FITC-conjugated or biotinylated, as appropriate), CD25, CD71, CD95 (all biotinylated), and rat IgG (FITC- or PE-conjugated, as appropriate) standard control antibodies were purchased from Pharmingen (San Diego, CA).

**Preparation of cells.** Bone marrow cells were obtained from the femora and tibiae of donor mice by flushing with BSS alone. For injection, cells were resuspended in BSS with 0.1% bovine serum albumin (BSA). To prepare anti-Thy-1.2-treated (T-cell-depleted) bone marrow (ATBM), cells were incubated with J1 MoAb (at 1:100 dilution) and C (1:25) for 45 minutes at 37°C and were washed four times. T-cell enriched donor cell populations were prepared by treating pooled spleen and lymph node (LN) cells with: Gey’s balanced salt lysing solution containing 0.7% NH4Cl for removal of RBC, and panning on a plastic petri dish pre-coated with a 5 μg/mL solution of goat antimouse IgG for 1 hour at 37°C to remove B cells. These treatments resulted in populations of 90% to 95% CD3^+ cells, as quantitated by flow cytometric analysis. Further purification of T cells into CD4^+ cells was performed as described previously. These procedures resulted in highly purified populations of CD4^+ cells (>90%) with no detectable presence of the inappropriate subset.

**Flow cytometric analysis.** In a 96-well plate, 2 × 10^5 cells/sample were incubated and washed with BSS containing 1% fetal bovine serum (FBS) and 0.05% NaN3 (FACS buffer). Antibodies, conjugated to either FITC or biotin, were added to the appropriate wells in a volume of 25 μL for 30 minutes at 4°C then washed three times in FACS buffer and fixed overnight at 4°C in PBS containing 1% paraformaldehyde. In the case of the biotin conjugated antibodies, PE-streptavidin (Caltag, San Francisco, CA) was added (1:100 dilution) before fixation with paraformaldehyde and incubated for an additional 30 minutes at 4°C, then washed three times in FACS buffer and fixed with paraformaldehyde. Samples were analyzed on a Coulter Epics Profile II (Coulter Corp, Hialeah, FL).

**In vitro mixed lymphocyte reaction (MLR).** Single cell suspensions of responder cells for the murine MLR were obtained from either spleen and lymph nodes or TDL, as indicated. Stimulator cells were obtained from the spleens of indicated mice, irradiated with 15 Gy and washed three times with medium. In a 96-well plate 4 × 10^3 responder cells were incubated with 8 × 10^3 stimulator cells or medium alone for the indicated period of time at 37°C, 5% CO2. Cultures were incubated with 1 μCi [3H]TdR/well for the final 24 hours, harvested, and counted. The percent response was calculated in the following manner: experimental CPM [3H]TdR minus medium CPM/(anti-(B6CB)F1, only CPM [3H]TdR minus medium CPM). Responses indices were calculated as a ratio of the experimental CPM [3H]TdR to the anti-(B6D2)F1, only CPM [3H] TdR. When indicated, culture supernatants were removed and tested for cytokine production by CTLL bioassay, as previously described. Briefly, 1 × 10^5 CTLL cells in 25 μL medium were added to 100 μL of culture supernatant in a 96-well plate. Anti-murine interleukin-2 (IL-2) MoAb was added at 2 μg/mL in 25 μL to appropriate wells. Cells were cultured for 24 hours and incubated with 1% [3H]TdR/well for the final 6 hours, harvested, and counted. Experimental results were compared with a standard curve of mL-2 and IL-4. Statistical comparisons between experimental groups for proliferation responses were performed by the Student’s t-test analysis, using SYSTAT 5.2 software.

**Collection of thoracic duct lymphocytes (TDL).** Anesthetized mice were cannulated 4 days after injection of 10^7 (B6D2)F1, CD4^+ T cells by insertion of an Intramedic PE 50 tube into a fistula perforated in the cysterma clyae, as previously described. The mice were then placed on an apparatus which allows the mice exercise and access to food while they are being infused IV with physiologic saline. The lymph was collected for 8 to 10 hours in 15-mL tubes containing 2 mL RPMI 1640 medium supplemented with 10% FBS, 1 U/mL heparin, and kept at 4°C until assay.

**Mortality assay for GVHD.** Recipient mice were irradiated with 950 cGy and approximately 6 hours later were injected IV (in a maximum volume of 0.5 mL of BSS) with either 2 × 10^6 donor ATBM cells alone, as a negative control, or a mixture of ATBM plus donor T cells, as indicated. Mice were checked daily for morbidity and mortality until the experiments were terminated at 60 posttransplantation. Median survival times (MST) were calculated as previously described. Statistical comparisons between experimental groups for mortality curves were performed by the nonparametric Wilcoxon signed rank analysis, using SYSTAT 5.2 software.

**Histopathological analysis.** Two mice per experimental group were killed on day 27 post-BMT and organs were removed and fixed with 4% paraformaldehyde. Ear skin and gut were then processed for embedding in paraffin. Paraffin sections (6 μ) were cut and stained with hematoxylin and eosin (H&E). Sections were examined microscopically as indicated for the presence of inflammatory infiltrates and dyskeratotic or necrotic cells.
RESULTS

**rD-mPGPtide inhibits alloreactivity in vitro.** Lymph node cells taken from (B6D2)F1 mice were stimulated in vitro with irradiated (15 Gy) spleen cells from (B6CB)F1 mice and the proliferative response was measured by [3H]-Tdr incorporation of triplicate wells. The rD-mPGPtide was added at 200, 100, and 50 μmol/L to the appropriate wells and rhIL-2 was added at 100 U/mL to the appropriate wells.

**Fig 1.** Inhibition of in vitro allogeneic MLR by rD-nPGPtide. The data shown are representative of three separate experiments and expressed as mean percent response [3H]-Tdr incorporation of triplicate wells. The rD-mPGPtide was added at 200, 100, and 50 μmol/L to the appropriate wells and rhIL-2 was added at 100 U/mL to the appropriate wells.

**rD-mPGPtide inhibits acute GVHD directed across a MHC barrier.** An acute form of GVHD was induced in lethally irradiated (950 cGy) (B6CB)F1 mice by IV administration of 5 X 10^6 (B6D2)F1 CD4+ T cells along with 2 X 10^6 (B6D2)F1 ATBM. In this GVHD model, the untreated mice exhibited 80% fatality by day 20 posttransplant with a MST of 13 days (Fig 2). Transplanted mice were also administered three different regimens of rD-mPGPtide treatment, including: (1) daily injections (0.5 mg/injection) from days 0 to 6 post-BMT; (2) alternate days during this same time period (days 0, 2, 4, 6); and (3) every third day (days 0, 3, 6). Treatment with these regimens of rD-mPGPtide increased the MST of these mice to 32, 30, and 28 days, respectively. The observed increases were statistically significant as compared with the untreated group (P < .03 for all of the rD-mPGPtide–treated groups), although there was little difference between the three different regimens among themselves (P > .05). With time, all of the peptide-treated mice that received donor T cells eventually succumbed to GVHD. Both CD8+ and CD4+ T cells are likely to play a role in the development of GVHD in this strain combination due to the fact that the donor and recipient mice differ at both class I and class II MHC loci. Since the donor T cell subsets were unseparated, there was a potential development of a CD8+ T-cell–mediated GVHD, against which the rD-mPGPtide would be expected to have little effect.

**rD-mPGPtide inhibits acute GVHD mediated by MHC allogeneic CD4+ T cells.** To specifically evaluate the effect of rD-mPGPtide on CD4+ T cells during the GVHD response, an acute form of GVHD was again induced in irradiated (950 cGy) (B6CB)F1 mice by administration of 1 X 10^6 (B6D2)F1 CD4+ T cells along with 2 X 10^6 (B6D2)F1 ATBM. As shown in Fig 3, the MST for those mice left untreated was 25 days. Mice treated with rD-mPGPtide on days 0, 3, and 6 exhibited a significant increase in the MST to >60 days post-BMT (P < .02). In this case, 82% of the mice treated with rD-mPGPtide survived for the duration of the experiment, as compared with 27% of the untreated GVHD mice and 91% of the control mice transplanted with only ATBM (P < .02). A single injection of rD-mPGPtide also resulted in a significant increase in the MST (P < .05) with 80% of the mice surviving past 60 days. A cyclized control scrambled peptide (Scr-PGPtide) was also tested (0.5 mg administered on days 0, 3, and 6) to ensure specificity of the rD-mPGPtide and did not significantly affect survival as compared with the untreated mice (P > .99), with only 33% surviving past 60 days (Fig 3). The body weights of surviving animals at the conclusion of the experiment (day 60) exhibited little differences between groups: 30.0 ± 1.0 g for the ATBM group, 27.9 ± 2.4 g for the GVHD group, 26.6 ± 5 g for the rD-mPGPtide day 0, 3, 6-treated group, 28.1 ± 1.3 g for the rD-mPGPtide day 0-treated group, and 26.6 ± 3.3 g for the Scr-PGPtide–treated group. These data suggested that a chronic form of GVHD was absent in these surviving mice. To examine the possibility that regulatory cells were being generated during peptide treatment, 1.25 X 10^6 spleen and LN cells from rD-mPGPtide–treated (B6CB)F1, mice more than 80 days posttransplantation of 10^6 (B6D2)F1 CD4+ T cells were transferred to donor irradiated (B6CB)F1 mice undergoing GVHD by the same conditions. No enhancement of survival of the mice receiving adoptively transferred lymphoid cells (MST = 16 days; percent survival = 20%) was observed, as compared with the GVHD mice receiving just donor CD4+ T cells (MST = 13 days; percent survival = 0%).

**Histopathological analysis.** To evaluate the peptide’s effect on the clinical manifestation of GVHD in the target tissues, histology sections were prepared 27 days post-BMT from rD-mPGPtide–treated or untreated mice undergoing GVHD. Tissue samples were taken from ear skin and the gastrointestinal tract and examined for morphological changes such as swelling, cellular damage, and the presence of inflammatory infiltrates. In the ear skin and small intestinal tissue of the untreated GVHD mice (Fig 4B and E) there was significant inflammatory infiltration in comparison with the ATBM controls (Fig 4A and D). Numerous dyskeratotic and necrotic cells were also observed in the epidermal layers.
Fig 2. Survival of mice undergoing GVHD across a MHC-haploidentical barrier is enhanced by varying treatments with rD-mPGP tide. (B6CB)F1 mice were lethally irradiated (950 cGy) and transplanted with allogeneic (B6D2)F1 ATBM (2 x 10^5) cells alone, or with unseparated donor T cells (5 x 10^5). Recipients were either left untreated (positive GVHD control), injected IV with rD-mPGP tide (0.5 mg) daily, every other day, or every third day between days 0 and 6. The data are representative of three separate experiments and the numbers of mice used per group are indicated in the figure.

Fig 3. Survival of mice undergoing CD4+ T-cell–mediated GVHD across a MHC-haploidentical barrier is enhanced by treatments with rD-mPGP tide. (B6CB)F1 mice were lethally irradiated (950 cGy) and transplanted with allogeneic (B6D2)F1 ATBM (2 x 10^5) cells alone, or with CD4+ T cells (1 x 10^6). The rD-mPGP tide (0.5 mg) was administered IV on either day 0 alone or on days 0, 3, and 6. Scr-PGP tide (0.5 mg) was injected on days 0, 3, and 6 and had no significant effect on survival. Data are pooled from two separate experiments and the total numbers of mice used per group are indicated in the figure.
of both the skin and gut, suggesting GVHD-related cell death. In contrast, the tissue samples from the rD-mPGPtide–treated mice (Fig 4C and F) exhibited limited inflammatory infiltrates and significantly fewer dyskeratotic cells. The differences between the groups were quantitated by counting the number of dyskeratotic cells per linear millimeter of epidermis (Fig 5). The samples from peptide-treated mice displayed a two- to three-fold decrease in the frequency of dyskeratotic cells as compared with those from the GVHD control mice. These combined data suggested that prophylactic treatment of transplanted mice with rD-mPGPtide significantly reduced the clinical manifestations of GVHD, as evidenced by histological sampling.

Effect of rD-mPGPtide on activation antigen expression in vivo—day 4 TD L. To begin investigating the mechanism by which rD-mPGPtide prevents the onset of GVHD, we examined the cell surface of CD4+ T cells from transplanted mice for the expression of activation antigens including IL-2-receptor (IL-2R; CD25), Fas (CD95), and Transferrin-receptor (Tr-R; CD71). Irradiated (950 cGy) (B6CB)F1 mice were transplanted with 10⁷ (B6D2)F1 CD4+ T cells, and on day 4 posttransplant their thoracic ducts were cannulated,
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TDL were collected over an 8 to 10 hour period, and flow cytometric analysis was performed on the retrieved cells. Transplanted mice (3 per group) were either left untreated or were injected IV with 0.5 mg rD-mPGPtide on days 0 and 3. The TDL collected from each group were exclusively donor-type CD4⁺ T cells; however, the flow yield of TDL collected from peptide-treated mice was significantly less than those of the untreated mice, 4 × 10⁵ cells/mL versus 7 × 10⁵ cells/mL, respectively. Both experimental groups of TDL contained a high percentage of blast-like cells (26% to 28%), and were analyzed as a separate population from the remaining small lymphocytes. As shown in Fig 6A, the percentage of TDL cells expressing the activation antigen CD25 was significantly reduced in the peptide-treated group (Fig 6A).

Alloreactivity of GVHD TDL cells. As a measure of alloreactivity, MLR cultures were established using the TDL collected above as responder cells. These cultures were stim-
In this report, we have described a novel therapeutic approach for the treatment of murine GVHD across a MHC barrier using a peptide analog of the CDR3 region of the murine CD4 molecule, rD-mPGPtide. Experiments performed both in vitro and in vivo have demonstrated a potential for rD-mPGPtide to affect immune responses to alloantigens. The inhibition of the alloreactive immune response was indicated in vivo primarily by the prolongation of survival of mice undergoing GVHD and reduced tissue destruction in treatment with either irradiated (15 Gy) (B6CB)F1 splenocytes or media alone for 48 hours and pulsed with [3H]TdR as a measure of proliferation for the final 24 hours. As shown in Fig 7A, [3H]TdR incorporation by TDL from untreated control mice was enhanced in response to (B6CB)F1 stimulator cells, as compared with the syngeneic (B6D2)F1 stimulator cells. TDL collected from the rD-mPGPtide-treated mice exhibited a 50% reduction in proliferation when incubated with the allostimulator cells as compared with the untreated TDL proliferation (P < .01). Furthermore, proliferation responses to third party alloantigens (SJL spleen cells) remains intact following peptide treatment (P > .05). These results suggested that proliferative responses to alloantigens are impaired in the peptide-treated mice. To further analyze this proliferative response, culture supernatants from another representative experiment were removed after 24 hours and analyzed for cytokine content by a CTLL bioassay. As shown in Fig 7B, supernatants from the untreated control TDL cultured with media alone were not capable of supporting CTLL proliferation. Incubation of TDL with (B6CB)F1 stimulator cells significantly enhanced the CTLL prolifera-

**DISCUSSION**

Fig 7. Alloreactivity of TDL cells collected from mice undergoing GVHD is reduced by treatment with rD-mPGPtide. The TDL cells were collected in the same manner as described in Fig 6 and the data are representative of three similar experiments. (A) MLR proliferation responses to irradiated host (B6CB)F1 splenocytes were performed as described in the Materials and Methods section. Data is expressed as the response index of proliferation ± SEM. (B) CTLL proliferation supported by culture supernatants from TDL alloresponses. Data is expressed as CPM [3H]TdR incorporation ± SEM.
these mice. This enhanced survival was evident for GVHD induced by both unseparated T cells and purified CD4+ T cells (Figs 2 and 3). Even a single injection of rD-mPGPtide at the time of transplant was effective in delaying the onset of disease mediated by CD4+ T cells. The protective response of the rD-mPGPtide appears to be specific as the control scrambled peptide failed to exhibit significant enhancement of survival as compared with untreated mice in this model (Fig 3).

Upon comparing the two experimental conditions tested, unseparated T cells and purified CD4+ T cells, it is apparent that the rD-mPGPtide was more effective in preventing the GVHD induced by the purified CD4+ T cells. We hypothesize that in the case of the unseparated T cells, the rD-mPGPtide has limited effect on the CD8+ T-cell–mediated component of GVHD directed to MHC class I antigens, a large portion of which may actually be CD4-independent.13 Yet, even under these arduous conditions, the CD4-CDR3 peptide could prolong the survival time of recipient mice by at least 2 weeks.

Based on the animal survival data, it is apparent that the alloreactive (B6D2)F1, CD4+ T cells from the donor are less potent at mediating GVHD in the rD-mPGPtide–treated recipient. The anti-host specific donor T cells, themselves, are most likely rendered dysfunctional in their ability to respond to alloantigen. This is supported by several observations, including the reduction of in vitro alloreactivity and activation antigen expression by the positively selected TDL collected from mice treated with rD-mPGPtide. In vitro alloantigens and cytokine production were evident in the TDL of the mice undergoing GVHD (Fig 7, A and B); however, the TDL from the peptide-treated mice exhibited a 50% reduction in the proliferative response to alloantigen, but only a 15% reduction in IL-2 production. This reduced proliferative capacity could be due to either an inability of the T cells to recognize alloantigen or the presence of fewer alloreactive T cells due to deletion and/or inhibition of expansion. However, these results suggest that the observed inhibition of proliferation is relatively independent of IL-2 production. Furthermore, expression of all three activation antigens tested (IL-2R, Tr-R, and Fas) were increased on the TDL from the rD-mPGPtide–treated mice. The generation of regulatory cells controlling immune responses in various mouse models of autoimmunity has been previously reported.35-38 Adoptive transfer of these cells from protected animals to unprotected autoimmune-prone animals can lead to protection from disease and is one way of demonstrating the existence of these regulatory populations of cells. We examined this possibility by transferring spleen and lymph node cells from rD-mPGPtide–treated (B6CB)F1 mice more than 80 days posttransplantation of 10^6 (B6D2)F1, CD4+ T cells to de novo-irradiated (B6CB)F1 mice undergoing GVHD by the same conditions. We observed no enhancement of survival of the mice receiving adoptively transferred lymphoid cells as compared with the GVHD mice receiving just donor CD4+ T cells. Thus, it appears that the generation of a regulatory population of cells is not responsible for the observed protective effect of rD-mPGPtide in these mice undergoing GVHD.

As a therapeutic modality, the rD-mPGPtide peptide mimic of the CD4-CDR3 molecular site appears to be an effective agent for the prevention of GVHD. We believe that the peptide primarily affects the alloreactive T cells that are being activated early after transplantation, rendering them...
incapable of inducing GVHD. Results from recent EAE studies with the peptide provide strong evidence that inhibition is highly specific for CD4 T-cell responses to antigens present at the time of exposure to the α-mannosidase. One, 2, or 14 days after treatment, there is no diminution in any of the lymphoid cellular compartments, including the CD4 T-cell subset. In addition, lymph node T cells are fully functional in their capacity to respond to both recall antigens and to third-party alloantigens. The half-life retention of peptide in serum in mice is approximately 25 minutes and responsiveness to any type of antigen stimulation is significantly inhibited for up to 6 hours after administration, but has virtually no effect by 12 hours. Noting this short window of effect of the CD4-CDR3 peptide, it is a most intriguing possibility that if administered only within the first week of transplant, the peptide could leave the remaining non-alloreactive CD4 T-cell population intact for subsequent development of responses to opportunistic infections or potential leukemic relapse. Further studies are planned to clarify these issues.

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