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)F, → (B6 × CBA)F, (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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Allogeneic 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. 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. 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. 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.

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of irradiated (B6 × CBA)F1 mice with a donor bone marrow inoculum supplemented with either 5 × 10⁷ unseparated or 1 × 10⁸ 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-mPGPtide 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 (H2k⁺)] and (B6 × CBA)F1, [(B6CB)F1 (H2k⁻)], 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 glutamine, 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,²⁴ 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 600E system controller, Waters 490E programmable multi-wavelength detector; Millipore Corp, Bedford, MA) before use. The sequences of the synthesized peptides were as follows: rD-mPGPtide (CPGPEEKNELEC, all D-amino acids) and scrambled rD-mPGPtide (Scr-PGPtide; CEPKNELPERGEC, 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 Gy exposure from a GammaCell 133Cs source (116 cGy/min).

MoAb. Ascites fluid for anti-Thy-1.2 (J1, rat IgM)²⁶ and anti-CD8 (3.168, rat IgM)²⁸ 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 containing 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 then 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 solution containing 0.7% NaCl for removal of RBC, and pancreatin 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.²⁴ 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 × 10⁶ 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 × 10⁵ responder cells were incubated with 8 × 10⁵ 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-(B6D2)F1 only CPM - 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.²⁵ Briefly, 1 × 10⁵ 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 μCi [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 10⁷ (B6D2)F1, CD4⁺ T cells by insertion of an Intramedic PE 50 tube into a fistula perforated in the cysterna chyli, as previously described.²⁶ 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 × 10⁵ 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 day 60 posttransplantation. Median survival times (MST) were calculated as previously described.²⁷ 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 and tissues 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.

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RESULTS

**rD-mPGPtide inhibits alloseactivity 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-mPGPtide. 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 2 x 10^8 (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 post-BMT. Treatment with these regimens of rD-mPGPtide increased the MST of these mice to 32, 30, and 28 days, respectively. Compared with the untreated group, 27% of the untreated mice 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) by 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 × 10^8 spleen and LN cells from rD-mPGPtide–treated (B6CB)F1, mice more than 80 days posttransplantation of 10^8 (B6D2)F1 CD4^+ T cells were transferred to de novo-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.
INHIBITION OF GVHD BY A CD4-CDR3 PEPTIDE ANALOG

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 10^6) cells alone, or with unseparated donor T cells (5 x 10^6). 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 10^6) 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.
Fig 4. Ear skin (A through C) and small intestine (D and E) of irradiated (950 cGy) (B6CB)F1, mice 27 days after the transplantation of either (B6D2)F1 ATBM (2 × 10^6) alone (A and D) or with 1 × 10^6 (B6D2)F1, CD4+ T cells, and left untreated (B and E) or rD-mPGPptide–treated (0.5 mg) on days 0, 3, and 6 (C and F). Two mice per group were examined. The epidermal layer and intestinal epithelium of mice receiving only ATBM (A and D) were devoid of cellular injury, whereas numerous dyskeratotic and necrotic cells (arrows) were observed in the untreated positive GVHD controls (B and E). Also note the cellular infiltrate in lamina propria of positive control intestine (E). Peptide-treated animals failed to exhibit significant skin (C) and gut (F) pathology, and resembled ATBM controls. (Final magnification: A-C, ×800; D-F, ×500).

of both the skin and gut, suggesting GVHD-related cell death. In contrast, the tissue samples from the rD-mPGPptide–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-mPGPptide significantly reduced the clinical manifestations of GVHD, as evidenced by histological sampling.

**Effect of rD-mPGPptide on activation antigen expression in vivo—day 4 TDL.** To begin investigating the mechanism by which rD-mPGPptide 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 10^7 (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-mPGPptide 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 × 10^5 cells/mL versus 7 × 10^5 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 very high (90% for blast cells and 60% for non-blast cells) in the mice undergoing GVHD, suggesting that the allogeneic donor CD4+ T cells were reacting to the host alloantigens. Treatment of the mice with rD-mPGPptide resulted in a 30% reduction in the percentage of non-blast cells expressing IL-2R and a 10% reduction in the percentage of blast cells expressing IL-2R. More notable, were the changes in the mean antigen expression of IL-2R on both the blast cells and the non-blast cells. Nearly a three-fold reduction was seen for both populations (Fig 6B). Tr-R expression was also affected in a similar manner; however, Fas appeared to be expressed at a level of 50% in both cell populations and remains unchanged 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-

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Fig 6. Activation phenotype of TDL cells collected from mice undergoing GVHD is altered by treatment with rD-mPGPptide. (B6C3F1 mice (five mice per group) were lethally irradiated (950 cGy) and transplanted with allogeneic (B6D2)F1, CD4+ T cells (1 × 10^6). Recipients were either left untreated or rD-mPGPptide (0.5 mg) was administered IV on days 0 and 3. Mice were cannulated on day 4 and TDL cells collected over an 8- to 10-hour period. (A) Expression of CD25, CD71, and CD95 as a percentage of the small lymphocytes and the blasting lymphocytes. (B) Flow cytometric profile of the CD4+ TDL cells for expression of IL-2 receptor (CD25). The negative control samples for the control and peptide-treated lymphocytes were 0% positive for CD25 with a mean fluorescence of 0.153 and 0.158, respectively. The negative control samples for the control and peptide-treated blast cells were 9% and 7% positive for CD25, respectively, with a mean fluorescence of 0.226 and 0.232, respectively.
ulated 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-mPGP tide--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 alloantigen 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 proliferation of the culture supernatants. This proliferation was neutralized by anti-IL-2 MoAb suggesting little or no IL-4 production by the TDL in response to the alloantigen stimulation. The TDL from the rD-mPGP tide--treated mice demonstrated slightly reduced cytokine production (P > .01), which was neutralized by anti-IL-2 MoAb as well, as compared with the untreated TDL. These results suggested that the proliferation defect of the TDL from peptide-treated mice may not be entirely accounted for by a reduced capacity to produce IL-2.

**DISCUSSION**

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-mPGP tide. Experiments performed both in vitro and in vivo have demonstrated a potential for rD-mPGP tide 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
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-mPGPptide at the time of transplant was effective in delaying the onset of disease mediated by CD4+ T cells. The protective response of the rD-mPGPptide 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-mPGPptide 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-mPGPptide 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. 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-mPGPptide-treated recipients. The anti-host specific donor T cells, themselves, are mostly likely rendered dysfunctional in their ability to respond to alloantigen. This is supported by several observations, including the reduction of in vitro alloseactivity and activation antigen expression by the positively selected TDL collected from mice treated with rD-mPGPptide. In vitro alloseactivities 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 of GVHD mice (Fig 6A), whereas the TDL from the rD-mPGPptide–treated mice exhibited a marked reduction in the expression of IL-2R and Tr-R, yet no reduction in the expression of Fas. These findings may hold a clue to the mechanism by which this CD4-CDR3 peptide analog mediates inhibition of GVHD. It has been shown previously that the expression of Fas is required for the induction of T-cell apoptosis on incomplete activation, while the lack of Fas expression leads to T-cell anergy. The continued high expression of Fas on the TDL from the peptide-treated mice may allow for the induction of apoptosis of the allo-specific T cells on incomplete activation in the presence of peptide. Further investigation into the peptide mechanism is necessary to clarify this issue.

Several recent reports propose that alterations in the immune response can be induced which generate a protective effect against various immunologically based disorders. These approaches include the polarization in cytokine production from Th1-like to Th2-like phenotypes, potentiation of other specific cytokines (e.g., TGFβ), inhibition of T-cell trafficking to target organs, or the generation of non-lethal regulatory cells in vivo. The switching of cytokine phenotype from Th1 to Th2 has been reported to be induced by several agents including cytokines and anti-CD4 MoAb. The induction of a Th1 immune response in GVHD has been correlated with lethality and a switch in the response to a Th2 phenotype has been shown to significantly prolong survival. In this regard, we investigated whether or not rD-mPGPptide was inducing such a cytokine switch leading to protection of the transplanted mice. TDL taken from these peptide-treated mice demonstrated no appreciable difference in the IL-2 and IL-4 production profiles when stimulated ex vivo by alloantigen (Fig 7B). Therefore, our data do not support a rD-mPGPptide-induced cytokine switch phenomenon in alloreactive T cells during the development of GVHD to account for the inhibitory effects of the peptide.

In order for the T cells to cause GVHD, they must be able to traffic to the appropriate target organs including the skin, gut, and liver. Inhibiting the trafficking of these alloreactive T cells could also lead to a reduction in the symptoms of GVHD. The expression of adhesion molecules such as CD44, ICAM-1, VCAM-1, and LFA-1 on the surface of T cells play an important role in controlling the trafficking of these cells to their target tissues. Histopathological analysis of mice undergoing GVHD suggested that rD-mPGPptide was inhibiting the infiltration and subsequent destruction of epithelial tissue in the skin and gastrointestinal tract (Fig 4). In addition to the possible inhibition of proliferation and expansion of alloreactive T cells, this apparent inhibition of trafficking could also be a result of decreased expression of adhesion molecules on the surface of the activated T cells or insufficient upregulation of the ligand molecules on vascular tissues. These possibilities are currently under investigation.

The generation of regulatory cells controlling immune responses in various mouse models of autoimmunity has been previously reported. 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-mPGPptide–treated (B6CB)F1 mice more than 80 days posttransplantation of 106 (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-mPGPptide in these mice undergoing GVHD.

As a therapeutic modality, the rD-mPGPptide 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\(^1\) 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 D\(\mu\)mPGPride. 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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