Photochemical Treatment with S-59 Psoralen and Ultraviolet A Light to Control the Fate of Naive or Primed T Lymphocytes In Vivo After Allogeneic Bone Marrow Transplantation

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Donor leukocyte infusions after allogeneic bone marrow transplantation can provide a curative graft-vs-leukemia (GVL) effect, but there is a significant risk of graft-vs-host (GVH) disease. A simple and effective method for controlling the fate of naive or primed T-lymphocytes in vivo without eliminating their beneficial properties is needed. In this report, photochemical treatment (PCT) ex vivo with a synthetic psoralen (S-59) and UVA light was evaluated as a pharmacological approach to limiting the proliferation and GVL potential of naive and primed donor T cells in vivo. S-59 rapidly intercalates into and cross-links DNA on UVA illumination. The effects of PCT on T cells were found to be both S-59 and UVA dose dependent. With selected PCT regimens, treated T cells still expressed activation markers (CD25 and CD69) and secreted IL-2 on activation, but they showed limited proliferative capacity in vitro and in vivo. Clonal expansion of CTL in MLR was reduced after PCT, but short term lytic activity of primed CTL was not affected. In a murine model of MHC-mismatched bone marrow transplantation, the addition of PCT-treated T cells to T-depleted bone marrow facilitated donor engraftment and complete chimerism without causing acute or chronic graft-vs-host disease. Allospecific GVL reactivity was reduced but not eliminated after PCT treatment. In an MHC-matched model using host-presensitized donor T cells, PCT significantly reduced GVH-associated mortality without eliminating GVL reactivity. Thus, PCT ex vivo offers a simple, rapid, and inexpensive method by which to control the fate of naive and primed T cells in vivo. The Journal of Immunology, 1999, 163: 5145–5156.

Allogeneic bone marrow transplantation (BMT) is curative for a significant portion of patients with hematological malignancies (1–4). The antileukemic effect of BMT derives in part from the preparative regimens used to condition the host for transplant, typically high dose chemotherapy in combination with myeloablative radiotherapy, but there is clinical and experimental evidence to support an immunological mechanism as well (5, 6). This “graft-vs-leukemia” (GVL) effect is often associated with immunological reactivity of donor cells against histocompatibility Ags of the host, i.e., with graft-vs-host (GVH) activity (5, 7). GVH reactivity becomes a greater clinical problem as the degree of mismatch between donor and host within the MHC increases (2, 8–10), and there is an inverse relationship between the degree of MHC disparity of the donor/recipient combination and the probability of leukemia relapse posttransplant (5, 11).

Depletion of T cells from the donor BM significantly reduces the risk of graft-vs-host disease (GVHD) but increases the risk of leukemia relapse as well as the risk of graft rejection and graft failure (5, 12–14). Recent clinical and experimental studies have shown that delayed infusion of donor T cells (donor leukocyte infusion (DLI) therapy) can facilitate conversion of the transplant recipient to complete donor hematopoietic and lymphoid chimerism and provide a curative GVL effect (15, 16). Although there is some evidence for a decrease in the risk of GVHD after DLI therapy, it remains a significant and potentially lethal clinical complication in the absence of methods to selectively control the fate of T lymphocytes once they are infused. The potential benefit of DLI has led to clinical and experimental attempts to control GVHD by selective elimination of the causative T cells through the transduction of a “suicide” gene, i.e., the gene for herpes simplex virus thymidine kinase, which renders cells susceptible to the toxic effects of ganciclovir (17–19). However, the technical problems and labor-intensive protocols required to ensure acceptable levels of incorporation and expression of transduced genetic material into lymphocytes are daunting.

In the studies reported here, we took a pharmacological approach to the selective control of T cell activity in vivo by using photochemical treatment (PCT) with S-59 psoralen and long wavelength UVA light ex vivo. S-59 is a synthetic psoralen (m.w. 337.8) that reversibly intercalates into helical regions of DNA and RNA (20). Functional studies in vitro and in vivo have established that T cells are highly sensitive to inactivation with both natural and synthetic psoralens and UVA (reviewed in Ref. 21). On illumination with UVA light, psoralens react with pyrimidine bases to form covalent monoadducts and then to cross-link DNA (22), thereby preventing DNA replication, leading to inactivation (23–26) and apoptosis (27, 28). PCT has been used to treat cutaneous T cell lymphomas (29), suppress allograft rejection (30), block induction of autoimmune disease (31), and prevent or eliminate the risk of GVHD (32–34).
In this study, we sought to test the hypothesis that PCT ex vivo can limit the proliferation of donor T cells in vivo and decrease the risk of GVHD while retaining the ability of the T cells to facilitate engrafment of T-cell-depleted MHC-mismatched BM. In addition, we sought to determine whether PCT affected the beneficial GVL reaction associated with allogeneic BMT. Initially, it was necessary to establish the conditions under which ex vivo PCT modulated T cell activity and limited cell proliferation without immediate toxicity. We assessed the effects of PCT on proliferation, cytokine secretion, and expression of T cell activation markers in response to polyclonal and clonal stimulation in vitro. We also examined the effect of PCT on ability to generate alloantigen-specific CTL in MLR cultures as well as on the lytic activity of primed CTL effector cells. Using this information in the second phase, we evaluated the ability of PCT-treated T cells to facilitate engrafment of T-depleted BM from MHC-mismatched donors without causing GVHD and assessed the effect of ex vivo PCT on allospecific GVL reactivity in an MHC-mismatched murine model of BMT. Finally, we evaluated the effect of PCT on GVH and GVL reactivity of primed T cells in an MHC-matched BMT model. Collectively, the data indicate that photochemical treatment with S-59 psoralen and UVA ex vivo can restrict the clonogenic potential of naive and alloantigen-primed T cells in vivo without loss of the beneficial effect on engrafment and on antitumor reactivity, but that the therapeutic window for PCT is narrow.

Materials and Methods

**Mice**

C57BL/6 (B6; H-2b, Thy-1.2), B6.PL-Thy-1a (H-2b, Thy-1.1), B10.BR (H-2d, Thy-1.2), and AKR (H-2a, Thy-1.1) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed in the Animal Resource Facility of the Medical College of Wisconsin in filter-topped microisolator cages and given mouse chow and acidified, chlorinated water *ad libitum*. The facility is accredited by the American Association for Laboratory Animal Care, and animal protocols were approved by the Institutional Animal Care and Use Committee.

**Preparation of splenic T cells**

Splenes were processed into single-cell suspensions, and erythrocytes were removed by hypotonic lysis. The cells were washed with DMEM (Life Technologies, Grand Island, NY), and viability was checked by trypan blue dye exclusion. The MACS Cell Separator System (Miltenyi Biotec, Auburn, CA) was used to positively or negatively select for T cells. T cell-enriched suspensions were prepared by negative selection using anti-B220 microbeads (Miltenyi). One cycle of negative selection generally resulted in enrichment to ~90%-95% T cells isolated by positive selection using anti-Thy-1.2 microbeads (Miltenyi). Purity was assessed by flow cytometric (FC) analysis on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) after staining with FITC-anti-Thy-1.2 (CD90, Pharmingen), and PE-anti-CD4 (PharMingen), and FITC-anti-Ly-5 (CD45R/ B220, CalTag) mAbs.

**Photochemical treatment**

S-59, a synthetic psoralen provided by Ceres (Concord, CA), was diluted in sterile distilled water and added to the cell suspensions so that the desired final concentration was achieved. The structure and synthesis of S-59 have been described (35). S-59 is highly efficient at intercalating into DNA, minimizing the length of UV exposure required to achieve DNA cross-linking compared with other psoralens. Splenic T cells, suspended in PBS plus 5% FBS, were placed in 15 × 60-mm disposable plastic petri dishes, so that the volume-surface ratio resulted in a fluid layer of ~0.5 cm. The dishes were placed on a UVA (320–400 nm) illumination device with a nominal flux of 7 mW (Cole Parmer, Vernon Hill, IL). The delivered UVA ranged from 0.035 to 3.5 J/cm² [7 mW/s × s] × 1000]. After PCT, the treated cells were pelleted by centrifugation, washed once, resuspended in tissue culture medium in medium, and recounted using a hemocytometer. Viability was assessed by trypan blue dye exclusion and was usually >90% after PCT. Control cells in various experiments were either untreated, exposed to UVA alone, or treated with S-59 but not exposed to UVA.

**Activation of T cells in vitro**

T cells were polyclonally activated using immobilized anti-CD3 mAb (10 μg/ml; clone 145 2C11 from Pharmingen) in flat-bottom 24-macrowell plates containing 2–4 × 10⁶ T cells. The cells were suspended in DMEM supplemented as described elsewhere and containing 10% FBS (complete DMEM) (36). The cultures were incubated for 24–72 h at 37°C in humidified air plus 10% CO₂. Cell proliferation was measured by quantitative flow cytometry using the standard cell dilution analysis (SCDA) assay (37) and/or by pulse labeling with [³H]Thymidine (NEN Life Science Products, Boston, MA), during the final 18–24 h of culture. The expression of activation markers on live and dead T cells was examined as a function of time after PCT. Naive and activated cells were double stained with PE-CD3 mAb and FITC-labeled Abs to CD23 (IL-2-receptor α-chain) or CD69 (early activation Ag) (PharMingen). Propidium iodide (PI, 0.2 μg/ml) was added to discriminate between live (PI⁻ cells) and dead (PI⁺ cells) by three-color FC analysis. Live and dead leukocyte gates were drawn on the basis of forward light scatter (log amplification) and PI fluorescence (FL-3), excluding apoptotic bodies and debris, and then on the basis of CD3 expression (FL-2 fluorescence) to identify T cells.

**Standard cell dilution analysis (SCDA) assays**

An adaptation of the SCDA assay of Pechhold et al. (37) was used to quantitate the number of T lymphocytes in heterogeneous cell cultures. The modified procedure has been described by us elsewhere (38).

**Analysis of cytokine secretion**

Cytokine (IL-2, IFN-γ, IL-6, and IL-4) levels in culture supernatants were measured by ELISA at 24, 48, and 72 h after polyclonal activation of T cells on immobilized anti-CD3 mAbs. Capture and detection Abs were purchased from Pharmingen.

**Cell-mediated lympholysis assays**

B6 T cells (2 × 10⁶) were cocultured with irradiated (700 cGy) AKR B cell lymphoblasts (6 × 10⁶) in 24-well plates for 5 days and used as effectors in standard 3.5-h ⁵¹Cr release assays. The responder T cells were 1) untreated, 2) exposed to UVA alone, or 3) PCT-treated. Triplicate V-bottom microwells were seeded with 5000 ⁵¹Cr-labeled AKR target cells (Con A lymphoblasts) at E:T ratios between 50:1 and 1:6.1. Control wells contained targets alone (spontaneous release) or targets plus 7% detergent (maximum release). The percent specific lysis was calculated using the formula: 100 × [experimental ⁵¹Cr release − spontaneous ⁵¹Cr release] ÷ (maximum ⁵¹Cr release − spontaneous ⁵¹Cr release) To assess the effect of PCT on activated CTL, cells from MLR cultures established with untreated T cells were collected on day 5 and then exposed to UVA or PCT. The lytic units-40% (LU₄₀) for each CML culture were calculated from regression curves. One LU₄₀ represents the number of effector cells that lysed 40% of the target cells. Data were normalized for 10⁶ responder cells using the formula: 10⁶ cells × (E:T ratio yielding 40% lysis × 5000 target-wells), and the LU₄₀ per culture was calculated as follows: LU₄₀ per million cells × 2 × 10⁶ responder cells per culture × proportion of cells recovered on day 5.

**Limiting dilution analysis (LDA) assays**

LDA assays were used to estimate the frequency of proliferating T cells. Eight serial 2-fold dilutions of responder T cells (100 μl/well) were conducted in sterile round-bottom microwell plates with complete DMEM as diluent (24 replicate wells per dilution). Lymphocyte-conditioned medium (L-CM) (10% FBS, 40% Rat-T-Stim; Collaborative Biomedical Products, Bedford, MA) was added to each microwell as a source of exogenous growth factor(s) along with 20,000 irradiated (700 cGy) allogeneic B cells as stimulators. B cells were isolated by immunomagnetic separation using anti-B220 microbeads (Miltenyi) and were preactivated with Escherichia coli LPS (0111:B4, 2 μg/ml, Calbiochem, San Diego, CA) for 24 h to augment presentation of allogeneic stimulators. Controls contained stimulator cells in LCM (4 × 48 wells) but no responder cells. The culture plates were incubated at 37°C for 8 days. One-half of the medium in each well was replaced with fresh LCM after 4–5 days. Alloactivation (response in MLR) was assessed by the addition of 0.5 μCi [³H]Thymidine in 50 μl to each well for the last 20–24 h of incubation. [³H]Thymidine uptake was measured, and individual wells were scored as positive for proliferation when the mean exceeded the mean for 24 control wells by ≥7 SDs. Estimates of the frequency of proliferating cells were made by χ² minimization (39).

A “split well” LDA assay was used to assess proliferation and cytolytic activity in the same culture wells. After 8 days in culture, each microwell
was mixed, and 100 μl were transferred to a V-bottom microwells containing 5000 51Cr-labeled Con A-activated lymphoblasts in 100 μl complete DMEM. The remaining cells were labeled with 0.5 μCi [3H]thymidine and reincubated overnight to measure proliferation to alloantigen (MLR). Lytic activity was assessed by a 51Cr release CML assay as described above. Individual wells were scored as positive for lytic activity when the cpm exceeded the mean spontaneous 51Cr release release of 24 control wells by ≥3 SDs. The frequencies of alloresponsive (MLR) and cytolytic (CTL) cells were calculated using χ2 minimization (39).

Assays for graft-vs-host (GVH) and graft-vs-leukemia (GVL) reactivity

Two transplant models were used: naive B6 donors into MHC-mismatched AKR hosts (H2b into H2k); and presensitized B10.BR donors into MHC-matched AKR hosts (H2b into H2k with mismatches at multiple minor Ags). In both models, donor BM was flushed from the excised femurs of naive mice with cold DMEM and syringes fitted with 25-gauge needles. In the B6/AKR model, the BM cells were T cell depleted (TCD) ex vivo with allele-specific anti-Thy-1 mAb and complement. T cell depletion was confirmed by FC analysis. T-enriched spleen cells were prepared from naive B6 donors by negative selection with a MACS Cell Separator. BM in the MHC-matched model was not TCD because the frequency of alloreactive T cells in marrow from naive B10.BR donors was too low to cause GVHD. Primed splenocytes were obtained from B10.BR mice presensitized with three i.p. injections of 10 × 106 AKR spleen cells and used 1 week after the third injection. The spleen cells from B10.BR anti-AKR donors and T-enriched spleen cells from naive B6 mice were treated with PCT before being mixed with donor BM.

Host AKR mice were conditioned with a single dose of 1100 cGy total body irradiation (TBI) at a rate of ~89 cGy/min with a Shepherd Mark I cesium irradiator (J. L. Shepherd and Associates, San Fernando, CA). This dose was lethal to 100% of nontransplanted AKR mice (data not shown). Irradiated recipients received a single injection i.v. of 5 × 106 nucleated BM cells with or without added T cells within 24 h of TBI. The mice were observed for survival and clinical evidence of GVH disease. Body weights were recorded approximately twice a week. Change in body weight is an objective indicator of GVHD (40). Weight loss of 10–25% was considered severe GVHD. Mice were randomly selected for analysis of chimerism at various times or leukemia challenge. Mice sacrificed during an experiment were censored from the survival data at the time of death. The presence of leukemia was confirmed at necropsy by visual examination of target organs (spleen, lymph nodes, and thymus).

Leukemia

The leukemia used in these studies came from a male AKR mouse that developed acute T cell lymphoblastic leukemia/lymphoma spontaneously (41). A frozen stock of the leukemia, designated AKR-M2, was used in all experiments.

Assessment of donor engraftment and chimerism

In most experiments, FITC-anti-H2Kb was used with PE-Thy-1.1 to identify infused B6.PL-Thy-1 T cells and with PE-Thy-1.2, PE-B220, and PE-Mac-1 (all from PharMingen) to identify cells of the T, B, and monocytic lineages that were derived from precursors in the donor BM. H2Kb-negative populations expressing Thy-1.1, B220, or Mac-1 were considered to be residual host AKR cells. Persistence of host cells was confirmed with FITC-H2Kb and PE-Thy-1.1 mAbs. For analysis of thymic repopulation, FITC-CD8 and PE-CD4 mAbs were used to determine the relative proportions of single- and double-positive thymocytes, and double-staining with FITC-Thy-1.1 and PE-Thy-1.2 mAbs was used to distinguish BM-derived thymocytes (Thy-1.2+) from residual host AKR thymocytes (Thy-1.1+). Cells were pelleted into V-bottom microwells (0.5–1 × 105/well) and labeled with 10 μl of mAb at 4°C for 30 min. Stained cells were diluted and washed with PBS/azide (100 μl/well), pelleted by centrifugation, re-suspended in 400 μl of Isoton II (Fisher Scientific, Pittsburgh, PA), and analyzed on a FACScan flow cytometer using forward and side scatter to gate on the leukocytes. At least 10,000 events were captured when cell number permitted.

Statistical analysis

Data were analyzed by Student’s t test or Fisher’s exact test for significant differences between groups. Survival curves were analyzed by log rank comparison of life tables. p < 0.05 was considered significant; NS indicates p > 0.05.

FIGURE 1. Effect of PCT on T cell function in vitro. A. Dose-dependent effect of S-59 psoralen and UVA on T cell response to polyclonal activation. Negatively selected T cells were activated with immobilized anti-CD3 mAb for 72 h and labeled with [3H]thymidine to assess proliferation. UVA exposure was 5, 50, or 500 s at 7 mW/cm2 (0.035, 0.35, and 3.5 J/cm2, respectively). B. Kinetics of early cell death (PI uptake) in PCT-treated T cells after activation with anti-CD3 mAb. T cells received no treatment (●), 8 min (3.4 J/cm2) UVA alone (□), 0.1 nM/8 min PCT (+), 1 nM/8 min PCT (×), or 10 nM/8 min PCT (▲). C. Effect of PCT on clonogenicity of alloreactive (MLR) T cells as measured by LDA. B6 (H2b) T cells were untreated (●), treated with UVA alone (□), or PCT-treated with 1 nM/8 min (×), 10 nM/1 min (+), 10 nM/2 min (○), or 10 nM/8 min (▲). LDA assays were done as described in Materials and Methods with the use of AKR (H2k) B cells for allostimulation. Data are presented as the frequency (1/f) of cells proliferating in response to alloantigen and percent change from the “No Treatment” control.
Results

Effect of PCT on T cells is S-59 psoralen dose and UVA time dependent

Spleen cells were treated with various concentrations of S-59 psoralen and UVA light to determine the operational range of PCT. A typical example from one of several experiments with various combinations of S-59 and UVA is shown in Fig. 1A. Inhibition of T cells proliferation was dependent on both the dose of S-59 psoralen and the length of time that the treated cells were exposed to UVA light (or J/cm² UVA). Treatment with S-59 alone did not significantly affect T cell response to mitogenic stimulation (data not shown). For most experiments, PCT regimens consisting of 0.1, 1, and 10 nM S-59 with constant UVA exposure (8 min) were selected for study because they represented incomplete (~10%), nearly complete (~90%), and complete (100%) inactivation of T cell proliferation, respectively (Fig. 1A and additional data not shown). However, similar effects could be achieved by holding the dose of 10 nM S-59 constant and varying exposure to UVA light (Fig. 1A and additional data not shown). PCT was not immediately toxic to T cells (Fig. 1B). Cell death from apoptosis was evident within 20 h after T cell activation only with the most intense PCT regimen used in this study (i.e., 10 nM/8 min).

LDA assays were used to estimate the frequency of clonable T cells remaining after treatment with various PCT regimens. As shown in Fig. 1C, no clonable T cells were detected after treatment with 10 nM/8 min PCT and almost none after 10 nM/2 min. In contrast, when 1 nM/8 min and 10 nM/1 min PCT were used, 6% of the T cells remained clonogenic. Exposure to UVA alone did not significantly reduce the frequency of clonable T cells. S-59 alone was not tested because it has no effect in the absence of UVA-induced cross-linking.

PCT inhibits T cell proliferation without blocking cytokine synthesis and secretion

The next series of experiments examined the effects of PCT on cytokine synthesis and secretion by T cells after activation with immobilized anti-CD3 mAb. The number of viable cells in replicate cultures was determined via the SCDA assay after 24, 48, and 72 h of culture. DNA synthesis was measured by [3 H]thymidine uptake, and culture supernatants were tested for IL-2 by ELISA. Three concentrations of S-59 were used (0.1, 1, and 10 nM), and exposure to UVA was kept constant (8 min). Control cells were untreated or treated with UVA only. Representative results from one of three experiments are shown in Fig. 2.

PCT with the 10 nM/8 min regimen prevented DNA synthesis (Fig. 2A). Although a low level of IL-2 was detected in the supernatant, the cell number did not increase. IL-2 levels did not increase after the initial 24 h, suggesting that it was produced only during the earliest hours of culture. T cells treated with 1 nM/8 min PCT showed low levels of DNA synthesis but did not increase in number over 72 h, despite the fact that the level of secreted IL-2 at 48 h equaled that of control cells (cf. Fig. 2B with Fig. 2D). The concentration of IL-2 remained high at 72 h, indicating that it was not being consumed by activated PCT-treated T cells (Fig. 2B).
The least intense PCT regimen tested in this experiment (0.1 nM/8 min) had no effect on the proliferation, DNA synthesis, or IL-2 levels compared with UVA-treated cells (cf. Fig. 2C with Fig. 2D). Increased DNA synthesis at 72 h was paralleled by increased cell numbers in the culture. IL-2 was secreted at 24 h and increased after 48 h, but it rapidly disappeared by 72 h. This was attributed to its utilization by activated T cells. In other experiments (data not shown), we found that IFN-γ and IL10 were secreted at control levels by 1 nM/8 min PCT-treated T cells, but IL4 was not detected in either control or experimental cultures.

PCT does not prevent expression of T cell activation markers

Because of the decreased utilization of endogenously produced IL-2 by T cells treated with PCT, we examined IL-2 receptor (CD25) expression along with that of the very early activation Ag CD69 at 24, 48, and 72 h (Fig. 3). The kinetics of CD25 expression did not change after PCT with 1 nM/8 min PCT (Fig. 3A), nor did the level of expression as indicated by mean fluorescence intensity (data not shown). With 10 nM S-59, very few cells were viable at 48 h, but 74.5% of the viable cells were CD25⁺. The number and percentage of CD25⁺ T cells present in culture after treatment with 0.1 nM S-59 were not different from untreated or UVA control cultures. Similar results were obtained with CD69 (Fig. 3B). Naive T cells express very low levels of CD69, but it is up-regulated within hours after activation. Collectively, the results in Fig. 3 levels by 1 nM/8 min PCT-treated T cells, but IL4 was not detected in either control or experimental cultures.

### Table I. Effect of PCT on generation of alloreactive CTL in 5-day MLR cultures

<table>
<thead>
<tr>
<th>Cell Treatment (S-59/UVA)</th>
<th>Exp.</th>
<th>LU₄₀/Million Cells</th>
<th>Cell Recovery (%)</th>
<th>Total LU₄₀/Culture</th>
<th>Average LU₄₀/Culture</th>
<th>% Control</th>
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<tr>
<td>None</td>
<td>1</td>
<td>38</td>
<td>325</td>
<td>250</td>
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<tr>
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<td>83</td>
<td>273</td>
<td>453</td>
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<td>—</td>
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<tr>
<td>UVA (8 min)</td>
<td>1</td>
<td>113</td>
<td>267</td>
<td>603</td>
<td>399</td>
<td>113</td>
</tr>
<tr>
<td>UVA (8 min)</td>
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<td>74</td>
<td>132</td>
<td>194</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.1 nM/8 min</td>
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<td>49</td>
<td>236</td>
<td>232</td>
<td>247</td>
<td>70</td>
</tr>
<tr>
<td>0.1 nM/8 min</td>
<td>2</td>
<td>116</td>
<td>113</td>
<td>261</td>
<td>—</td>
<td>—</td>
</tr>
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<td>54</td>
<td>43</td>
<td>47</td>
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<td>0.05</td>
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<td>0</td>
<td>5</td>
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* B6 T cells were treated as indicated and cocultured with irradiated AKR B cell lymphoblasts in 24-well culture plates. After 5 days, the cells were collected and used as effector cells in ⁵¹Cr release assays at E/T ratios of 50:1–1.6:1. Mitogen-activated AKR lymphoblasts were used as target cells. One LU₄₀ = the number of effector cells required to lyse 40% of target cells. LU₄₀ per culture = the proportion of cells recovered from 5-day MLR cultures × 2 × 10⁶ cells/culture × LU₄₀/million cells. Data from experiments 1 and 2 were averaged in the last two columns.
differentiation of CTL (LU40 per million cells) and limited cell proliferation (low cell recovery). Few viable cells were recovered after PCT compared with 88% of the CTL treated with UVA only. By 44 h (days 6 and 7 of culture), 7–15% of the cells treated with 1 or 10 nM S-59 and UVA remained viable compared with 73% of those treated with 0.1 nM S-59/8 min UVA and 91% of the UVA control. Addition of exogenous IL-2 did not change the number of cells that survived relative to untreated control CTL.

PCT of donor T cells decreases GVH reactivity in MHC-mismatched chimeras

The in vitro studies described above established that PCT could be adjusted to allow for limited functional activity before T cell death occurred but that the effect was highly PCT dose dependent. We next sought to test the hypothesis that PCT-treated cells added to TCD BM would facilitate engraftment without causing significant GVHD. Experiments were done comparing PCT with 0.1, 1, or 10 nM S-59 and 8 min UVA, respectively, survived for 20 h after PCT compared with 88% of the CTL treated with UVA only. By 44 h (days 6 and 7 of culture), 7–15% of the cells treated with 1 or 10 nM S-59 and UVA remained viable compared with 73% of those treated with 0.1 nM S-59/8 min UVA and 91% of the UVA control. Addition of exogenous IL-2 did not change the number of cells that survived relative to untreated control CTL.

PCT inhibits the generation of CTL in MLR cultures in a dose-dependent manner but does not inhibit the lytic activity of CTL

Because PCT ex vivo limited the proliferation of T cells after activation without affecting cytokine secretion or expression of activation markers, we examined whether PCT affected the induction of CTL in vitro. CTL were generated in 5-day MLR cultures (H2b anti-H2k) and tested for lytic activity. UVA alone did not significantly affect CTL induction (Table I). T cells treated with low dose PCT (0.1 nM/8 min) generated CTL activity that was 70% of that generated in the untreated cultures. Use of 1 nM/8 min PCT limited CTL differentiation. Reduction in the total number of LU40 generated in the 1 nM/8 min culture (an average of 91% in two replicate experiments) was due to both a reduction in the differentiation of CTL (LU40 per million cells) and limited cell proliferation (low cell recovery). Few viable cells were recovered when 10 nM/1 min PCT was used, and CTL activity was virtually undetectable. PCT with 10 nM/8 min was not tested because too few cells survived to 72 h.

In the second phase of this experiment, alloantigen-primed CTL effector cells were collected from untreated MLR cultures on day 5 and then treated with PCT to determine whether there was any effect on lytic activity (Fig. 4). Lysis of the specific target cells was not affected by PCT of the effector cells. The LU40 per million cells was similar regardless of whether the CTL were untreated (83 LU40), exposed to 8 min UVA alone (87 LU40), or treated with PCT (63 to 83 LU40). The most intense PCT regimen affecting naive T cells (i.e., 10 nM/8 min) did not significantly alter lytic activity (74 LU40), suggesting that PCT may be a useful procedure for limiting survival of activated T cells without eliminating short term functional activity. In a replicate experiment, the treated CTL were maintained in culture for an additional 44 h. Using the SCDA assay, we found that 78, 48, and 26% of the cells treated with 0.1, 1, or 10 nM S-59 and 8 min UVA, respectively, survived for 20 h after PCT compared with 88% of the CTL treated with UVA only. By 44 h (days 6 and 7 of culture), 7–15% of the cells treated with 1 or 10 nM S-59 and UVA remained viable compared with 73% of those treated with 0.1 nM S-59/8 min UVA and 91% of the UVA control. Addition of exogenous IL-2 did not change the number of cells that survived relative to untreated control CTL.

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FIGURE 5. Ex vivo PCT of donor T cells can prevent acute GVHD in MHC-mismatched B6 into AKR chimeras. AKR hosts (H2k) were given TCD BM mixed with T cells treated with 1 nM S-59 and 8 min UVA because they represented GVH-positive control mice were given TCD BM alone (n = 10). GVH-negative control mice were given TCD BM mixed with either 0.3 × 106 (n = 10) or 3 × 106 (n = 25) untreated spleen cells from B6. PL-Thy-1a donors (H2b Thy-1.1). PCT chimeras were given TCD BM mixed with T cells treated with 1 nM S-59 and 8 min UVA (n = 47). A. Combined actuarial survival from two replicate experiments, except for the low GVH control group which was a single experiment. Symbols indicate either the day of death or the day mice were removed and censured from the data for FC analysis to assess chimeraism (see Table III). B. Change in body weight as a function of time posttransplant for one of the two replicate experiments shown in A.

---

indicate that up-regulation of activation markers still occurred after PCT.
termination at 90–100 days. In contrast, the addition of $3 \times 10^6$ untreated T cells to the TCD BM resulted in acute GVHD as evidenced by rapid body weight loss and death (high GVH control in Fig. 5, B and A, respectively). The survival rate and change in body weights of the low GVH control group that received $0.3 \times 10^6$ T cells that were untreated or PCT treated (1.0 nM/8 min). Two to four mice were sacrificed at the times indicated, and their spleens were pooled. Infused Thy-1.2+ cells were positively selected by immunomagnetic separation and used as responder cells in split well LDA assays (see Materials and Methods). Purity was 89–97%. Irradiated (700 cGy) AKR (H2k) host B cells were used as stimulator cells in the assays. Proliferation was measured by [3H]thymidine incorporation in MLR LDA assays. Thy-1+ cells were positively selected by immunomagnetic separation were 88–90% Thy-1.2+. Irradiated (100 cGy) AKR mice were injected i.v. with $5 \times 10^6$ TCD B6.PL-Thy-1a BM cells plus $3 \times 10^6$ T6 (Thy-1.2+) T cells that were untreated or PCT treated (1.0 nM/8 min). Two to four mice were sacrificed at the times indicated, and their spleens were stained and analyzed by FC as described in Materials and Methods. Values in parentheses are SDs. Data are pooled from two replicate experiments, except for the low GVH controls, which are from a single experiment.

**Table II.** Effect of PCT on the frequency of anti-host-specific proliferative (MLR) and cytolytic (CTL) T cells recovered from MHC-mismatched B6/AKR chimeras posttransplant as measured by LDA assays in vitro

<table>
<thead>
<tr>
<th>Days Post-BMT</th>
<th>No treatment</th>
<th>PCT treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MLR Cells/Million Thy-1.2+ Cells</td>
<td>CTL/Million Thy-1.2+ Cells</td>
</tr>
<tr>
<td>5</td>
<td>7,874 (5.342–10.413)</td>
<td>1,240 (963–1,520)</td>
</tr>
<tr>
<td>12</td>
<td>203 (681–918)</td>
<td>253 (207–300)</td>
</tr>
<tr>
<td>66</td>
<td>1,227 (957–1,497)</td>
<td>Dead</td>
</tr>
</tbody>
</table>

* Irradiated (1100 cGy) AKR mice were injected i.v. with $5 \times 10^6$ TCD B6.PL-Thy-1+ BM cells plus $3 \times 10^6$ T6 (Thy-1.2+) T cells that were untreated or mixed with $3 \times 10^6$ PCT-treated (1 nM/8 min) T cells. Chimeras were sacrificed at the times indicated, and their spleens were analyzed by FC as described in Materials and Methods. Values in parentheses are SDs. Data are pooled from two replicate experiments, except for the low GVH controls, which are from a single experiment.

**Table III.** Kinetics of donor hemopoietic and immune reconstitution in the spleens and thymuses of B6/AKR chimeras given TCD BM alone or with Thy-1.1+ T cells that were untreated or PCT treated

<table>
<thead>
<tr>
<th>Day Post-BMT</th>
<th>N</th>
<th>No. of Cells/Spleen ($\times 10^6$)</th>
<th>Chimerism</th>
<th>% Infused T Cells</th>
<th>Donor BM-Derived Cells (H2k)</th>
<th>% CD4+8+ Cells in Thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GVH-negative control (TCD BM only)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28–37</td>
<td>14</td>
<td>53.5 (± 22.6)</td>
<td>76.6 (± 28.4)</td>
<td>27.4 (± 30.6)</td>
<td>13.3 (± 10.7)</td>
<td>46.3 (± 27.5)</td>
</tr>
<tr>
<td>98–104</td>
<td>8</td>
<td>53.8 (± 40.8)</td>
<td>75.7 (± 40.5)</td>
<td>21.1 (± 34.8)</td>
<td>28.7 (± 20.5)</td>
<td>41.0 (± 26.9)</td>
</tr>
<tr>
<td>PCT chimeras (TCD BM + $3 \times 10^6$ PCT-treated T cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>1.9 (± 0.3)</td>
<td>77.6 (± 7.6)</td>
<td>20.8 (± 7.0)</td>
<td>65.7 (± 7.9)</td>
<td>1.6 (± 3.9)</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>27.7 (± 5.1)</td>
<td>99.6 (± 0.4)</td>
<td>0.3 (± 0.2)</td>
<td>14.7 (± 3.6)</td>
<td>1.9 (± 0.2)</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>23.1 (± 2.9)</td>
<td>97.6 (± 2.3)</td>
<td>0.8 (± 1.6)</td>
<td>12.5 (± 3.4)</td>
<td>3.8 (± 8.0)</td>
</tr>
<tr>
<td>27–34</td>
<td>6</td>
<td>28.8 (± 11.3)</td>
<td>99.8 (± 0.2)</td>
<td>0.1 (± 0.1)</td>
<td>5.3 (± 3.4)</td>
<td>23.9 (± 9.7)</td>
</tr>
<tr>
<td>97–98</td>
<td>19</td>
<td>56.1 (± 34.4)</td>
<td>98.9 (± 2.0)</td>
<td>0.3 (± 0.3)</td>
<td>1.2 (± 0.5)</td>
<td>30.9 (± 9.0)</td>
</tr>
<tr>
<td>Low GVH-positive control (TCD BM + $0.3 \times 10^6$ untreated T cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>4</td>
<td>46.4 (± 22.3)</td>
<td>97.6 (± 4.4)</td>
<td>0.3 (± 0.5)</td>
<td>1.0 (± 0.6)</td>
<td>25.9 (± 8.3)</td>
</tr>
<tr>
<td>High GVH-positive control (TCD BM + $3 \times 10^6$ untreated T cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>2.3 (± 0.7)</td>
<td>93.2 (± 3.2)</td>
<td>5.8 (± 4.1)</td>
<td>80.7 (± 3.0)</td>
<td>1.3 (± 0.4)</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>10.9 (± 8.7)</td>
<td>99.3 (± 0.2)</td>
<td>0.2 (± 0.2)</td>
<td>25.0 (± 6.2)</td>
<td>2.3 (± 6.7)</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>11.0 (± 8.0)</td>
<td>96.6 (± 3.2)</td>
<td>0.0 (± 1.4)</td>
<td>33.2 (± 14.9)</td>
<td>1.6 (± 0.6)</td>
</tr>
<tr>
<td>≥27</td>
<td>0</td>
<td>All dead from acute GVHD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Irradiated (1100 cGy) AKR mice (H2k, Thy-1.1+) were injected i.v. with $5 \times 10^6$ TCD BM cells from B6 donors (H2k, Thy-1.1+) alone or mixed with $0.3 \times 10^6$ B6.PL-Thy-1+ T6 (H2k, Thy-1.1+) cells that were untreated or $3 \times 10^6$ PCT-treated (1 nM/8 min) T cells. Chimeras were sacrificed at the times indicated, and their spleens were stained and analyzed by FC as described in Materials and Methods. Values in parentheses are SDs. Data are pooled from two replicate experiments, except for the low GVH controls, which are from a single experiment.
Most thymocytes were double-positive CD4+ cell precursors derived from the transplanted BM (data not shown). More intense PCT regimen (10 nM/8 min) were similar to the BM controls shown in Table III, suggesting loss of alloreactivity. Those given cells treated with the less intense regimen (0.1 nM/8 min PCT) were similar to the high GVH control group shown in Table III, suggesting insufficient T cell inactivation.

To assess long term effects, 19 PCT-chimeras were sacrificed at 97–98 days post-BMT for FC analysis (Table III). In contrast to mice given TCD BM, which were incomplete chimeras, all 19 PCT-mice were complete donor chimeras with normal ratios of BM-derived T cells, B cells, and Mac-1 cells. A minor population of PCT-treated B6.PL-Thy-1⁺ T cells persisted >90 days in the chimeric spleens (average, 1.2%). Seventeen of the 19 PCT-chimeras had phenotypically normal thymuses, containing an average of 170 × 10⁶ cells. Thus, most MHC-mismatched PCT-chimeras (17 of 19 = 89%) avoided acute GVHD, repopulated their thymic and peripheral lymphoid tissues normally, and survived long term. The two exceptions showed symptoms consistent with the late effects of GVH reactivity. Notably, reconstitution of lymphoid tissues in long term survivors from the experimental PCT chimeras was not significantly different from that of the low GVH control group given untreated T cells (Table III).

High GVH control chimeras were also analyzed at 6, 12, and 20 days posttransplant, but all mice were dead by day 27 (Table III). At 6 days, their spleens were populated primarily by infused B6.PL-Thy-1⁺ T cells. At 12 and 20 days, infused T cells and donor BM-derived macrophages (H2b Mac-1⁺) dominated the spleen, and there were relatively few donor-derived B cells. GVH reactivity suppresses B cell lymphopoiesis (42). The thymuses of high GVH chimeras did not repopulate normally (<12 × 10⁶ cells/thymus at 20 days (Table III)). In contrast, low GVH chimeras (given 0.3 × 10⁶ T cells), like PCT chimeras (given 3 × 10⁶ PCT-treated T cells), fully engrafted with donor cells and reconstituted their T, B, and macrophage compartments to near normal levels. This indicates that only small numbers of immunocompetent donor T cells are necessary to facilitate engraftment of TCD MHC-mismatched BM.

Effect of PCT on allospecific GVL reactivity is PCT regimen dependent

Resistance to leukemia challenge was used to monitor persistence of alloreactive T cells in vivo. In this model of MHC-mismatched BMT, GVL reactivity is directed toward host MHC class I determinants expressed on the acute T cell leukemia and mediated by CD8⁺ effector T cells; i.e., it is allospecific (43). To determine whether allospecific GVL reactivity was affected by ex vivo PCT,
the experiments presented in Table IV were done. In Experiment 1,
we examined the effect of using PCT of varying intensity on
leukemia resistance in irradiated AKR hosts. The chimeras were
challenged with 250 AKR-M2 leukemia cells on day 3 posttrans-
plant. In the absence of T cells (group 1), all mice died with pro-
gressive leukemia. Host mice given spleen cells treated with S-59
alone resisted the leukemia but developed moderately severe
GVHD (−19.8% body weight loss at 60 days; group 2). PCT with
10 nM/8 min or 10 nM/2 min eliminated GVH reactivity, but all
mice died with leukemia. Less intense PCT resulted in leukemiam-
free survival with differing intensity of GVHD as indicated by
body weight loss (−3.5% to −32.4% for groups 5 and 6,
respectively).

In Experiment 2 of Table IV, we attempted to estimate the mag-
nitude of GVL reactivity remaining after PCT treatment using the
most effective regimen from experiment 1 (10 nM/1 min). Control
mice given TCD B6 BM alone (group 1) had no GVH reactivity,
and all died with progressive leukemia 19 days after challenge.
Control mice given TCD BM plus S-59-treated cells that were not
exposed to UVA light (group 2) all developed lethal acute GVHD
(MST 32 days), regardless of the dose of leukemia given. They
showed no evidence of leukemia at necropsy. GVHD was less
severe, but not absent, in chimeras given PCT-treated spleen cells
(groups 3 and 4). PCT chimeras were able to resist a challenge with
500 but not 50,000 leukemia cells. Two of 6 mice challenged with
5,000 leukemia cells died within 60 days. Based on these
data, the leukemia LD₅₀ for PCT chimeras (groups 5–7) was esti-
ated to be ~11,000 cells compared with >50,000 cells for
GVH control chimeras (group 2).

Collectively, the experiments in Table IV document that GVL
reactivity of PCT-treated cells, like GVH reactivity, is quantita-
tively decreased after PCT depending on the regimen used. Per-
sistence of an allospecific GVL effect after ex vivo PCT was as-
associated with subclinical to mild GVHD and is most likely due to
the survival of clonogenic T cells after PCT. In a dose titration
experiment using naive B6 spleen cells and TCD BM chimeras
(data not shown), we found that 10⁷ untreated spleen cells (~3 ×
10⁷ T cells) were necessary to eliminate a challenge dose of 5000
leukemia cells given on day 3 post-BMT. Transplantation of 10⁷
naive B6 spleen cells resulted in leukemia progression, whereas
transplantation of 10⁵ spleen cells resulted in lethal GVHD. The
GVL effect of 10⁷ ex vivo PCT-treated cells (10 nM/1 min) ap-
proximated a 10-fold reduction (1 log₁₀ or −90%) in naive T cells.
This is similar to the reduction is allospecific T cells predicted
from in vitro LDA assays (Fig. 1C).

Effect of PCT on GVH and GVL reactivity of presensitized
donor T cells

Because PCT did not affect the lytic activity of CTL (Fig. 4), we
examined the effect of PCT on GVH and GVL reactivity of primed
t cells in vivo. MHC-matched B10.BR (H₂k) donors were pre-
sensitized to host AKR (H₂k) alloantigens in vivo. In this MHC-
matched model, low doses of naive B10.BR spleen cells do not
cause significant GVHD in irradiated AKR hosts, but comparable
doses of host-primed T cells cause lethal GVHD (43, 44). Naive
B10.BR BM did not cause GVHD (group 1, Table V); however,
the mice were unable to resist a challenge with low dose leukemia
on day 3 posttransplant (group 7). The addition of 5 × 10⁶ spleen
cells from primed B10.BR anti-AKR donors resulted in lethal,
acute GVHD, regardless of whether the mice were given leukemia
or not (groups 2 and 8; p < 0.01 vs group 1).

The effect of PCT on GVH/GVL reactivity of presensitized
donor cells ex vivo was PCT regimen dependent and correlated with
the predicted level of T cell inactivation. Treatment with the more
intense PCT regimens, i.e., 10 nM/4 min (group 3) or 10 nM/2 min
PCT (group 4), significantly reduced GVH-related mortality com-
pared with untreated controls (p < 0.01 vs group 2) but also elimi-
nated GVL reactivity (p = NS; groups 9 and 10 vs group 7). PCT
with 10 nM/1 min significantly diminished GVH-associated mor-
MST = median survival time; NA = none alive at day 75. Deaths in
in groups 7–12 were from leukemia except for group 8 in which all deaths were from GVHD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment of Spleen Cells (S-59/UVA)</th>
<th>Leukemia Dose</th>
<th>No. Dead/Total (%) at 75 Days</th>
<th>MST</th>
<th>Day of Death</th>
<th>% Body Weight Change at 28 days</th>
<th>71 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BM Only</td>
<td>None</td>
<td>0/5 (0)</td>
<td>&gt;75</td>
<td>None (5 &gt; 75 days)</td>
<td>+4.0</td>
<td>−2.9</td>
</tr>
<tr>
<td>2</td>
<td>Untreated</td>
<td>6/6 (100)</td>
<td>30</td>
<td>None (6 &gt; 75 days)</td>
<td>−25.5</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 nM/4 min</td>
<td>None</td>
<td>0/6 (0)</td>
<td>&gt;75</td>
<td>None (6 &gt; 75 days)</td>
<td>−9.8</td>
<td>−10.9</td>
</tr>
<tr>
<td>4</td>
<td>10 nM/2 min</td>
<td>None</td>
<td>0/6 (0)</td>
<td>&gt;75</td>
<td>None (6 &gt; 75 days)</td>
<td>−13.4</td>
<td>−15.9</td>
</tr>
<tr>
<td>5</td>
<td>10 nM/1 min</td>
<td>None</td>
<td>1/5 (20)</td>
<td>&gt;75</td>
<td>30 (4 &gt; 75 days)</td>
<td>−19.8</td>
<td>−13.2</td>
</tr>
<tr>
<td>6</td>
<td>1 nM/8 min</td>
<td>3/6 (50)</td>
<td>&gt;75</td>
<td>34, 34, 62 (3 &gt; 75 days)</td>
<td>−13.5</td>
<td>−12.6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>BM Only</td>
<td>6/6 (100)</td>
<td>24.5</td>
<td>21, 21, 23, 26, 26, 48</td>
<td>−1.4</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Untreated</td>
<td>6/6 (100)</td>
<td>30</td>
<td>28, 28, 30, 30, 34, 37</td>
<td>−24.6</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10 nM/4 min</td>
<td>5/5 (100)</td>
<td>21</td>
<td>20, 20, 21, 23, 23</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10 nM/2 min</td>
<td>6/6 (100)</td>
<td>20</td>
<td>20, 20, 20, 20, 20, 21</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>10 nM/1 min</td>
<td>1/6 (17)</td>
<td>&gt;75</td>
<td>29 (5 &gt; 75 days)</td>
<td>−16.7</td>
<td>−5.6</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1 nM/8 min</td>
<td>2/6 (33)</td>
<td>&gt;75</td>
<td>36, 37, 4 (4 &gt; 75 days)</td>
<td>−19.5</td>
<td>−14.7</td>
<td></td>
</tr>
</tbody>
</table>

* Irradiated (1100 cGy) AKR hosts were given 5 × 10⁶ BM from naive B10.BR (H₂k) donors alone or together with 5 × 10⁵ spleen cells from presensitized B10.BR anti-AKR donors. The spleen cells were left untreated or were treated with S-59 and UVA as indicated. Chimeras in each group were randomly selected for challenge i.v. with 500 AKR-M2 leukemia cells on day 3 post-BMT. Mice surviving >75 days were sacrificed and found to be leukemia free. MST = median survival time; NA = none alive at 75 days. Deaths in groups 7–12 were from leukemia except for group 8 in which all deaths were from GVHD.
Discussion

In this study, we demonstrated that PCT ex vivo can be used to limit the proliferation of donor T cells in vivo and decrease the risk of GVHD while retaining the ability of the T cells to facilitate engraftment of T cell-depleted MHC-mismatched BM and mediate an allospecific GVL reaction. Using qualitative (Fig. 1A) and quantitative (Fig. 1C) in vitro assays, we showed that inactivation of T cells with S-59 psoralen and UVA light was dependent on the dose of psoralen and joules per cm² of UVA exposure. Under appropriate PCT conditions, proliferation of T cells after polyclonal activation was inhibited without eliminating their ability to synthesize and secrete cytokines, including IL-2, IFN-γ, and IL-10 (Fig. 2 and data not shown). This inhibition was not due to immediate toxicity to the T cells (Fig. 1B). On activation, PCT-treated T cells up-regulated their IL-2 receptors (CD25) (Fig. 3A) and expressed CD69 (Fig. 3B) as well as CD28 and CD44 (data not shown). Furthermore, limited differentiation of allogeneic-specific CTL occurred in MLR cultures after PCT (Table I). Collectively, these data indicate that some transcriptional and translational activity persisted on activation of PCT T cells. Varying the PCT regimen could control the level of functional activity remaining after PCT.

The active component in this PCT system is the synthetic psoralen S-59 (20). Psoralens are planar organic compounds that can be found in nature, principally in plants (21). S-59, like natural psoralens, reversibly intercalates into helical regions of DNA and, on UVA illumination, reacts with pyrimidine bases to form covalent monoadducts and then cross-link DNA, preventing DNA replication (22). S-59 photochemistry is specific to nucleic acids, resulting in minimal damage to cell membranes and proteins (20). Photochemical treatment with 1 nM S-59 and 3.0 J/cm² UVA has been estimated to leave a photoadduct density of ~1 S-59 psoralen molecule per 10⁶ to 10⁸ base pairs in the genomic DNA (46). Because most eukaryotic genes, including introns and exons, are less than 10⁴ base pairs long, this adduct frequency may have only minor impact on the transcription and expression of genes. However, DNA replication after polyclonal or Ag-specific activation of PCT-modified T cells was disrupted and led to T cell death in the absence of DNA repair.

The severity and intensity of GVHD are proportional to the number of T cells infused. GVHD can be eliminated by removal of T cells (13) or significantly reduced by partial or selective depletion of T cells (47, 48). Complete removal of T cells increases the risk of marrow graft failure and leukemia relapse (13). These conditions were reproduced in our experimental models with T-depleted MHC-mismatched BM and leukemia challenge. We found that the addition of PCT T cells to TCD allogeneic BM resulted in complete donor engraftment without acute GVHD (Table III and Fig. 5), but the outcome was PCT dose dependent. MHC-mismatched PCT-chimeras showed normal hemopoietic and lymphoid repopulation in their spleens and thymuses with only a few exceptions (Table III). Allospecific GVL reactivity was quantitatively reduced but persisted in PCT chimeras depending on the intensity of the treatment (Table IV).

The mechanism by which PCT-treated T cells facilitated engraftment of donor BM and establishment of complete donor chimerism without causing lethal acute GVHD is not clear. There was a correlation between the clonogenic potential of infused PCT-treated T cells measured in vitro (Fig. 1C) and their ability to facilitate engraftment of donor BM (Table III and data not shown). Complete donor chimerism was achieved only when at least some infused PCT-treated T cells persisted in vivo. This suggests that survival of a small population of clonogenic T cells capable of responding to host alloantigen (but insufficient to cause acute or chronic GVHD) may account for the beneficial effects observed when PCT-treated T cells were added to the TCD BM. Such an explanation is consistent with the results obtained by adding a low number of untreated B6 T cells (0.3 × 10⁶) to the TCD BM (Table III). Virtually identical outcomes were observed between PCT chimeras and low GVH control chimeras with regard to survival (Fig. 5A), body weight change (Fig. 5B), and long term donor engraftment (Table III). We cannot exclude that coadministration of PCT-treated T cells with limited functional activity contributed to the induction of other mechanisms through veto-like effect (49), induction of negative-regulatory T cells (50), or alteration of cytokine profiles (51, 52). Anti-host-specific Thy-1.2° cells were recovered from chimeras infused with PCT-treated Thy-1.2° T cells (Table II), indicating that the treated cells that persisted in vivo were not anergic. Whether they contributed a regulatory (suppressor) function as a result of PCT treatment is not known. In the setting used here, our data are most consistent with a reduction in the frequency of alloreactive T cells as an explanation for the in vivo effects.

Among the key observations described herein was that the effector function of allogeneic CTL was not compromised by PCT ex vivo (Fig. 4) and that the GVL reactivity of host-primed donor T cells could be modulated without elimination of GVL reactivity (Table V). This suggests that PCT ex vivo might be used as a means to control the fate of primed or activated T cells in vivo. PCT with S-59 psoralen offers several advantages: it is nontoxic in the absence of UVA light; the phototoxic product has a half-life of milliseconds; and it is inexpensive, rapid acting, and simple to use. The technical simplicity makes it an attractive alternative to more elaborate procedures such as those that require gene insertion and selection for transduced lymphocytes (19). Ionizing radiation also has been used as a simple way to limit the functional activity of T cells in vivo (53). Waller et al. (54) reported preliminary data suggesting that irradiated T cells facilitate engraftment of MHC-mismatched BM. We do not have any direct data comparing PCT and irradiated cells.

There may be circumstances in which it would be advantageous to infuse naive T cells that are capable of secreting cytokines after alloactivation in vivo but are not capable of proliferating, generating CTL, or surviving long term in vivo. If CTL activity is not affected by PCT as suggested by the data in Fig. 4, a more practical application for PCT might be the inhibition of naive but potentially GVH-inducing T cells in heterogeneous T cell suspensions primed against a specific Ag, such as a viral or histocompatibility Ags. Yee et al. (55) have infused CMV-specific allogeneic CTL clones into marrow transplant patients to provide protection against CMV infection. To avoid the risk of infusing GVH-inducing T cells, it was necessary to isolate and expand CMV-specific clones in vitro. This is a time-intensive, labor-intensive, and costly procedure. Similar problems confront strategies using allogeneic T cells as adoptive cellular therapy for posttransplant lymphoproliferative disorders and EBV-associated lymphomas (56).

Our data suggest that PCT might allow for selective inactivation of the clonogenic potential of contaminating T cells without affecting the short term lytic activity of Ag-specific CTL within a heterogeneous cell population, including the possible use of CTL directed against histocompatibility Ags (57). How long PCT-treated CTL persist in vivo is likely to depend on the intensity of the PCT regimen. Mice infused with host-primed B10.BR cells treated with 10 nM/1 min were able to resist a leukemia challenge 3 days later, but mice given the same cells exposed to 10 nM/2 min were not (Table V). Using naive B10.BR cells labeled with the cell tracker dye PKH26, we were able to detect alloresponsive T cells
in vivo 72 h after infusion into AKR hosts when the cells were treated with 10 nM/1 min PCT, but not when treated with 10 nM/2 min PCT (R. Truitt, unpublished data). In contrast, both PCT populations persisted in near equivalent numbers for 72 h when injected into congenic B10.BR-Thy-1.1 mice instead of allogeneic AKR hosts. This suggests that alloactivation contributes to the elimination of PCT-treated cells in vivo, perhaps by initiating DNA synthesis in a setting where photoadducts block cell division, leading to apoptosis. Even if T cell survival in vivo is reduced by PCT, multiple infusions of PCT-treated naive T cells or Ag-specific CTL might be given if the risk of GVHD is sufficiently reduced.

In summary, PCT-treated T cells have limited functional activity in vitro and in vivo. PCT ex vivo limited the proliferation of donor T cells in vivo and decreased the risk of GVHD. The ability of PCT-treated naive T cells to facilitate engraftment of TCD MHC-mismatched marrow and to establish a state of complete donor chimerism without causing acute GVHD correlated with the persistence of a small population of clonogenic T cells, but a unique regulatory property of PCT-treated cells has not been ruled out. The therapeutic dose at which PCT prevented GVHD without eliminating the allospecific GVL effect was narrow. Despite this limitation, PCT ex vivo is a simple and rapid procedure that may be useful for selectively controlling the fate of naive T cells (or other cells), while preserving Ag-specific T cell activity in vivo.

References


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