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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

Robert L. Truitt,* Bryon D. Johnson,* Carrie Hanke,* Sohel Talib,† and John E. Hearst‡‡

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 GVH 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.

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logeneic 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 host histocompatibility Ags of the host, i.e., with graft-vs-host (GVH) reactivity (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 engraftment 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 engraftment 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 engraftment 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-2b, 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 Use Committee.

Preparation of splenic T cells

Spleens 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%. Thy-1.2+ T cells were isolated by positive selection using anti-Thy-1.2 microbeads (Miltenyi). Purity was assessed by flow cytometric (FC) analysis on a FACSscan flow cytometer (Becton Dickinson, Mountain View, CA) after staining with FITC-anti-Thy-1.2 (CD90, PharMingen, San Diego, CA), PE-anti-CD4 (PharMingen), and FITC-anti-Ly-5 (CD45R/CD220, CalTag) mAbs.

Photochemical treatment

S-59, a synthetic psoralen provided by Cerus (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, and the desired final concentration was achieved. The structure and synthesis of S-59 have been described (35). The concentration of S-59 used in these experiments was 0.5 μM. The photochemical treatment was performed in the dark at 37°C for 8 days. The cultures 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 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 at 37°C for 8 days. The enzyme was measured by the addition of 0.5 μCi (1)H)thymidine in 50 μl to each well for the last 20–24 h of incubation. (1)H)Thymidine uptake was measured, and individual wells were scored as positive for proliferation when the pmo 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 35Cr-labeled Con A-activated lymphoblasts in 100 μl complete DMEM. The remaining cells were labeled with 0.5 μCi [3H]thyminidine reincubated overnight to measure proliferation to alloantigen (MLR). Lytic activity was assessed by a 35Cr release CML assay as described above. Individual wells were scored as positive for lytic activity when the cpm exceeded the mean spontaneous 35Cr release release of 24 control wells by ≥3 SDs. The frequencies of alloresponsive (MLR) and cytolytic (CTL) cells were calculated using \( \chi^2 \) 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 × 10^6 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 × 10^6 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.

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 a T cells and with PE-Thy-1.2, PE-B220, and FITC-CD8 and PE-CD4 mAbs 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 × 10^5/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, resuspended 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/s (0.035, 0.35, and 3.5 J/cm^2, 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/cm^2) 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 (1/10^5) of cells proliferating in response to alloantigen and percent change from the “No Treatment” control.](http://www.jimmunol.org/asset/5147/5147F1.jpg)
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 [3H]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 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.

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![Figure 3](https://example.com/figure3.png)

**Figure 3.** Effect of PCT on up-regulation of CD25+ and CD69+ by polyclonally activated T cells. Spleen cells were stimulated with immobilized anti-CD3 mAb for 24 ( ), 48 ( ), and 72 ( ) h. A, Percent of viable T cells expressing CD25+. B, Percent of viable T cells expressing CD69+. Control cells were untreated (No Rx) or treated with 8 min of UVA alone (UVA). PCT cells were treated with 0.1, 1 or 10 nM S-59 and 8 min of UVA. ND, not done (too few cells); dotted line, unactivated cells.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** PCT did not affect the lytic activity of alloantigen-specific CTL effector cells. Untreated B6 CTL generated in 5-day MLR cultures (as described in Table I) were used as effector cells in 3HCr release cytotoxicity assays against MHC-mismatched AKR lymphoblasts. The effector cells were untreated ( ), exposed to 8 min of UVA alone ( ), or PCT-treated using 0.1 (×), 1.0 (+), or 10 ( ) nM S-59 plus 8 min UVA. Data from one of three experiments are shown.

![Table 1](https://example.com/table1.png)

**Table 1.** 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>LU100/Million Cells</th>
<th>Cell Recovery (%)</th>
<th>Total LU100/Culture</th>
<th>Average LU100/Culture</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>38</td>
<td>325</td>
<td>250</td>
<td>352</td>
<td>—</td>
</tr>
<tr>
<td>None</td>
<td>2</td>
<td>83</td>
<td>273</td>
<td>453</td>
<td>—</td>
<td>—</td>
</tr>
<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>
<td>2</td>
<td>74</td>
<td>132</td>
<td>194</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.1 nM/8 min</td>
<td>1</td>
<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>
<tr>
<td>1 nM/8 min</td>
<td>1</td>
<td>18</td>
<td>37</td>
<td>13</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>1 nM/8 min</td>
<td>2</td>
<td>54</td>
<td>43</td>
<td>47</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10 nM/1 min</td>
<td>1</td>
<td>0.05</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10 nM/1 min</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* 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 3HCr release assays at E:T ratios of 50:1–1.6:1. Mitogen-activated AKR lymphoblasts were used as target cells. One LU100 is the number of effector cells required to lyse 40% of target cells. LU100 per culture is the proportion of cells recovered from 5-day MLR cultures × 2 × 10⁶ cells/culture × LU100/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 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.

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 because they represented <10, ~90, and >99% inactivation, respectively (Fig. 1A and additional data not shown). The results indicated 1) that 0.1 nM/8 min PCT was ineffective at reducing GVHD, 2) that PCT with 10 nM/8 min completely eliminated GVHD as well as any beneficial effect on donor engraftment, and 3) that 1 nM/8 min PCT significantly reduced GVH-associated mortality and helped facilitate engraftment of TCD BM. Only data on the latter group are presented below; data for the other groups are not shown.

Thy-1 congenic B6 (Thy-1.2) and B6.PL-Thy-1⁺ (Thy-1.1) mice were used as donors of BM and T cells, respectively, so that the infused PCT-treated T cells could be distinguished from donor T cells arising de novo from precursors in the TCD BM. Two GVH-positive control groups were included: a “high GVH” control group given 3 × 10⁶ T cells, and a “low GVH” control group given 0.3 × 10⁶ T cells. The latter was used to simulate a 1 log₁₀ or 90% reduction in the number of clonogenic T cells infused with the TCD BM inoculum. AKR host mice given TCD B6 BM alone (GVH-negative controls) did not develop clinical GVHD and showed no significant weight loss during the first 2 mo postransplant (Fig. 5B). However, TCD BM-only chimeras lost significant body weight late after transplantation. This late weight loss, which was reproducible, may reflect onset of chronic GVHD or complications from poor immune reconstitution. The thymuses of long term survivors in the TCD BM-only group failed to fully repopulate (average of 28 × 10⁶ double-positive T cells per thymus vs 100 × 10⁶ for PCT chimeras; see data on long term survivors in Table III).

The addition of 3 × 10⁶ PCT-treated B6.PL-Thy-1⁺ T cells to the TCD BM inoculum did not cause significant acute GVHD (Fig. 5). Actuarial survival of the PCT chimeras was 95%, and after a transient decline in body weight, most of the mice thrived until...
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 $0.3 \times 10^6$ B6.PL-Thy-1 T-cells (H2b, Thy-1.1) were untreated or treated with 1 nM/8 min PCT. The T cells recovered from the chimeras by immunomagnetic selection were 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 LDR LDA assays. To study the effect of PCT on the frequency of anti-host-specific proliferative (MLR) and cytolytic (CTL) T cells in the spleens of AKR chimeras at 5, 12, and 26 days post-BMT (Table II). For this experiment only, irradiated AKR mice were transplanted with TCD BM from B6.PL-Thy-1 donors (Thy-1.1) and infused with T cells from B6 donors (Thy-1.2) so that the infused donor T cells could be separated from Thy-1.1 cells of the AKR host and BM donor. The infused donor B6 T cells were untreated or treated with 1 nM/8 min PCT. The T cells recovered from the chimeras by immunomagnetic cell separation were 88–90% Thy-1.2+ with negligible Thy-1.1+ contamination.

Transplantation of untreated T cells resulted in a high frequency of both proliferative and cytolytic anti-host-reactive T cells in the spleen on day 5 (Table II). PCT reduced the frequency of host-specific MLR-reactive donor T cells generated in vivo by 84% (from 7874 to 1240 per million) and host-specific CTL by 58%.

### 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+ Cells</td>
<td>CTL/Million Thy-1+ Cells</td>
</tr>
<tr>
<td>5</td>
<td>1,240 (963–1,520)</td>
<td>5,464 (4,027–6,873)</td>
</tr>
<tr>
<td>12</td>
<td>253 (207–300)</td>
<td>2,740 (1,971–3,479)</td>
</tr>
<tr>
<td>26</td>
<td>1,227 (957–1,497)</td>
<td>2,651 (1,417–2,786)</td>
</tr>
</tbody>
</table>

* Irradiated (1100 cGy) AKR mice were injected i.v. with $3 \times 10^6$ TCD B6.PL-Thy-1 BM cells from B6 donors (H2b, Thy-1.2) alone or mixed with $0.3 \times 10^6$ untreated T cells of AKR host and $3 \times 10^6$ 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.

### 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 (H2b)</th>
<th>% CD4+8+ Cells in Thymus</th>
</tr>
</thead>
<tbody>
<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 (± 30.6)</td>
<td>27.4</td>
<td>(± 10.7)</td>
<td>(± 8.1)</td>
</tr>
<tr>
<td>98–104</td>
<td>8</td>
<td>53.8 (± 40.5)</td>
<td>75.7 (± 34.8)</td>
<td>21.1</td>
<td>(± 20.5)</td>
<td>(± 4.1)</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>19.5 (± 0.3)</td>
<td>77.6 (± 7.6)</td>
<td>20.8</td>
<td>65.7 (± 7.9)</td>
<td>1.6 (± 0.3)</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>27.7 (± 5.1)</td>
<td>99.6 (± 0.3)</td>
<td>0.3</td>
<td>14.7 (± 3.6)</td>
<td>1.9 (± 4.1)</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>23.1 (± 2.3)</td>
<td>97.6 (± 1.6)</td>
<td>0.8</td>
<td>12.5 (± 3.4)</td>
<td>3.8 (± 0.7)</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</td>
<td>5.3 (± 14.9)</td>
<td>5.3 (± 9.7)</td>
</tr>
<tr>
<td>97–98</td>
<td>19</td>
<td>56.1 (± 3.4)</td>
<td>98.9 (± 2.0)</td>
<td>0.3</td>
<td>1.2 (± 0.5)</td>
<td>2.0 (± 0.9)</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 (± 23.3)</td>
<td>97.6 (± 4.4)</td>
<td>0.3</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>2</td>
<td>4.3 (± 0.7)</td>
<td>93.2 (± 3.2)</td>
<td>5.8</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</td>
<td>25.0 (± 6.2)</td>
<td>2.3 (± 0.7)</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>11.0 (± 8.0)</td>
<td>96.2 (± 2.0)</td>
<td>0.1</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>
Most thymocytes were double-positive CD4+CD8+ cell precursors derived from the transplanted BM (data not shown). By 20 days, the thymuses were fully reconstituted with T cells, engraftment of MHC-mismatched BM was inconsistent. Some BM control mice failed to engraft, some reverted to host chimerism, some became mixed chimeras, and others fully engrafted with donor cells. The percent donor H2b and host H2a cells in the spleen was variable with an average of 76.6% (±28.4%) donor and 27.4% (±30.6%) host at 28–37 days (Table III). Chimeras were randomly selected for FC analysis to assess the effect of adding PCT-treated T cells on the engraftment of TCD donor BM and on immune reconstitution (Table III). In the absence of T cells, engraftment of MHC-mismatched BM was inconsistent. Some BM control mice failed to engraft, some reverted to host chimerism, some became mixed chimeras, and others fully engrafted with donor cells. The percent donor H2b and host H2a cells in the spleen was variable with an average of 76.6% (±28.4%) donor and 27.4% (±30.6%) host at 28–37 days (Table III).

Hemopoietic engraftment and immune reconstitution were relatively normal when 1 nM/8 min PCT-treated T cells were added to the TCD BM (Table III). At 6 days, the spleens were dominated by infused PCT-treated T cells (H2b Thy-1.1+). Elimination of residual host cells was delayed by ~1 week in comparison with the high GVH controls. Donor hemopoietic cells appeared by day 12 with macrophages (H2b Mac-1+) predominating initially, then B cells (H2b B220+). Few BM-derived T cells (H2b Thy-1.2+) were detected before day 20 because the thymus had not yet repopulated. By 20 days, the thymuses were fully reconstituted with T cell precursors derived from the transplanted BM (data not shown). Most thymocytes were double-positive CD4+8+ cells (Table III). By 27 days, the PCT chimeras were stably and completely engrafted with MHC-mismatched donor-BM-derived T, B, and Mac-1 cells (~100% H2b) in proportions that approximate those found in a normal mouse. Data on chimeras given cells treated with 0.1 nM/8 min or 10 nM/8 min PCT are not shown. However, chimerism and immune recovery in mice given T cells treated the 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-1a 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-1a 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 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 PCT of varying intensity on leukemia resistance in irradiated AKR hosts. The chimeras were challenged with 250 AKR-M2 leukemia cells on day 3 posttransplant. In the absence of T cells (group 1), all mice died with progressive 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 leukemia-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 magnitude 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 estimated to be ~11,000 cells compared with >50,000 cells for GVH control chimeras (group 2).

Collectively, the experiments in Table IV document that GVH reactivity of PCT-treated cells, like GVH reactivity, is quantitatively decreased after PCT depending on the regimen used. Persistence of an allospecific GVL effect after ex vivo PCT was 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) approximated 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 presensitized 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 compared with untreated controls (p < 0.01 vs group 2) but also eliminated GVH reactivity (p = NS; groups 9 and 10 vs group 7). PCT with 10 nM/1 min significantly diminished GVH-associated mortality (from 100% to 20%; p < 0.02) without significantly compromising GVL reactivity (17% vs 100%; p < 0.01). GVH-associated mortality also was significantly reduced when 1 nM/8 min PCT was used (p < 0.02), but it was not statistically different from ex vivo treatment with 10 nM/1 min PCT (group 5). GVH-associated body weight loss with 10 nM/1 min and 1 nM/8 min PCT was mild (6% to 15% at 71 days posttransplant). Collectively, these results demonstrated that alloreactivity of presensitized MHC-matched T cells could be modulated using PCT. However, the therapeutic window at which PCT of donor T cells decreased GVH mortality without eliminating the beneficial antitumor effect was narrow.

### Table V. Effect of PCT on GVH and GVL reactivity of presensitized MHC-matched donor spleen cells

<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>% Body Weight Change at 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>None</td>
<td>6/6 (100)</td>
<td>30</td>
<td>30, 30, 30, 30, 34, 34</td>
<td>−25.5</td>
<td>NA</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>None</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>
</tr>
<tr>
<td>7</td>
<td>BM Only</td>
<td>500</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>
</tr>
<tr>
<td>8</td>
<td>Untreated</td>
<td>500</td>
<td>6/6 (100)</td>
<td>30</td>
<td>28, 28, 30, 30, 34, 37</td>
<td>−24.6</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>10 nM/4 min</td>
<td>500</td>
<td>5/5 (100)</td>
<td>21</td>
<td>20, 20, 21, 23, 23</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>10 nM/2 min</td>
<td>500</td>
<td>6/6 (100)</td>
<td>20</td>
<td>20, 20, 20, 20, 20, 21</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>10 nM/1 min</td>
<td>500</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>
</tr>
<tr>
<td>12</td>
<td>1 nM/8 min</td>
<td>500</td>
<td>2/6 (33)</td>
<td>&gt;75</td>
<td>36, 37 (4 &gt; 75 days)</td>
<td>−19.5</td>
<td>−14.7</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 × 10⁶ 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 allostrogenic-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 $10^6$ to $10^8$ base pairs in the genomic DNA (46). Because most eu karyotic genes, including introns and exons, are less than $10^4$ 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 reconstitution in their spleens and thymuses with only a few exceptions (Table III). Allospecific GVL activity 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 x $10^6$) 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 photoactive 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 photoactivates 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


