Photoinactivation of T-Cell Function with Psoralen and UVA Radiation Suppresses the Induction of Experimental Murine Graft-Versus-Host Disease Across Major Histocompatibility Barriers

Stephen E. Ullrich
Department of Immunology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, U.S.A.

Bone marrow transplantation is employed in the treatment of a number of hematologic and malignant diseases. A major complication is the induction of graft-versus-host disease. Whereas removal of T lymphocytes from the donor marrow effectively reduces the incidence of graft-versus-host disease, the incidence of graft failure often increases when T cells are depleted from the transplanted marrow. In the current study, photoinactivation of the donor cells with 8-methoxypsoralen coupled with exposure to long-wavelength ultraviolet radiation (PUVA therapy) was used to inactivate the response of the donor T cells against the host. PUVA therapy suppressed the ability of spleen cells to respond to alloantigen in the in vitro mixed lymphocyte reaction. The induction of acute graft-versus-host disease across complete major histocompatibility barriers in lethally X-irradiated mice was significantly suppressed after bone marrow transplantation with photoinactivated bone marrow cells. Long-term survivors demonstrated allogeneic reconstitution and partial restoration of T-cell function. Because PUVA therapy had no inhibitory effect on hematopoiesis, these data suggest that using phototherapy to inactivate the alloreactivity of T cells may provide an alternative to purging T cells from the donor marrow, thus suppressing both the incidence of graft-versus-host disease and the incidence of graft failure. *J Invest Dermatol 96:303–308, 1991*

Bone marrow transplantation has emerged as a promising treatment for a variety of hematologic, metabolic, and malignant disorders. One of the major complications of bone marrow transplantation is the development of graft-versus-host disease (GVHD) [1]. In genetically identical twins, where there are no immunologic barriers, GVHD does not develop. However, as the degree of genetic disparity between the donor and recipient increases, the incidence of severe lethal GVHD increases. Even in HLA-matched donor-recipient pairs, GVHD remains a major cause of morbidity and mortality following bone marrow transplantation [1]. The incidence of GVHD is decreased by depleting T cells from the donor bone marrow inoculum [2]. Patients receiving T-cell–depleted bone marrow, however, show an increased incidence of graft rejection, and in leukemic patients the recurrence of malignancy tends to be higher when T-cell–depleted bone marrow is transferred [3]. This has led some to suggest that T-cell depletion should be avoided and efforts should be directed at the management of GVHD [1]. Whereas this is feasible when a HLA-Dr–matched sibling donor is available, it becomes problematic when one considers that the severity and lethality of GVHD increases when non-sibling HLA mismatched donors are used. Donor registries have been established to help with this problem, but it is estimated that a pool size of at least 100,000 donors is required to match (HLA-A, B, C, Dr, and mixed lymphocyte culture compatible) 95% of the candidates [4], indicating the need for new approaches to suppress the induction of GVHD across allogeneic histocompatibility barriers.

The question that was addressed during the current study was whether inactivation of the T cells within the transplanted bone marrow, rather than T-cell depletion, can suppress GVHD without promoting graft failure. T-cell function is quite sensitive to inactivation with 8-methoxypsoralen (8-MOP) coupled with exposure to long wavelength (320–400 nm) ultraviolet A light (PUVA). In vitro experimentation has established that T lymphocytes can be functionally inactivated after treatment with 8-MOP and UVA radiation [5–7]. Patients undergoing PUVA therapy in the treatment of psoriasis have impaired T cell function [8]. Furthermore, extracorporeal photophoresis (in vitro exposure of leukocytes isolated from patients or animals previously treated with 8-MOP to UVA radiation) has been used to treat cutaneous T-cell lymphoma in patients [9] and to suppress allograft rejection [10] and the induction of autoimmune disease [11] in experimental animal systems. Also, Berger et al [6] have demonstrated that whereas the proliferation of normal T cells is inhibited by PUVA therapy, the proliferation of the promyelocytic leukemia cell line HL-60 was relatively resistant to photoinactivation with PUVA. Based on the sensitivity of T cells to PUVA treatment, an attempt was made to use phototherapy to

Manuscript received May 16, 1990; accepted for publication August 20, 1990.

A preliminary report of this work was presented at the 51st Annual Meeting of the Society for Investigative Dermatology, May 2–5, 1990, Washington, D.C.

This work was supported by grants from the Sunshine Kids Fund for Immunology Research and grant #RR5511-27 from the National Institutes of Health.

Reprint requests to: Dr. S. E. Ullrich, Department of Immunology, P. O. Box 178, The University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas, 77030.

Abbreviations:
GVHD: graft-versus-host disease
MLR: mixed lymphocyte reaction
8-MOP: 8 methoxypsoralen
PUVA: psoralen plus UVA radiation
UVA: ultraviolet A radiation (320–400 nm).

0022-202X/91/$03.50

inactivate donor-derived T lymphocytes and see what effect this procedure had on the induction of GVHD. The approach that was used was patterned after the extracorporeal photophoresis procedure described by Edelson and colleagues [9]. Donor mice were injected with psoralen and 2 h later their spleen and bone marrow cells were removed and exposed in vitro to UVA radiation. The data indicate that treatment of the donor cells with PUVA interferes with alloreactivity, but has no effect on hematopoiesis. Transplanting the PUVA-treated cells into lethally X-irradiated allogeneic recipients suppressed the induction of acute GVHD. Long-term survivors demonstrated allogeneic reconstitution with a partial restoration of T-cell function, suggesting that photoinactivation of donor bone marrow may provide a method of preventing GVHD across major histocompatibility barriers while avoiding the failure of engraftment frequently associated with T-cell depletion.

MATERIALS AND METHODS

Animals Specific-pathogen-free, young adult (7-10 weeks old) female C3H/HeN (MTV$^-$), BALB/c and C57B1/6 mice were obtained from the Animal Production Area, Frederick Cancer Research Facility, Frederick, MD. The animals were housed and cared for according to the "Guide for the care and use of laboratory animals" (DHHS publication No. [NIH] 78-23) in a AAALAC-accredited animal facility, and their use was approved by the Institutional Animal Care and Use Committee. The animals had free access to laboratory chow and chlorinated water and were housed in rooms where ambient lighting was regulated automatically on a 12-h light-dark cycle.

Extracorporeal Phototherapy A modification of the procedure described by Edelson et al [9] was used. 8-methoxypsoralen (Elder Pharmaceuticals, Bryan, OH) was diluted in 2% gelatin and 0.4 mg was injected into the peritoneal cavity of the donor mice, as described previously [12]. Two h later, the donor mice were killed and their spleens, tibiae, and femora removed. Single-cell suspensions were prepared, contaminating red blood cells were lysed with 0.83% ammonium chloride, the cells were resuspended in PBS, and plated in 100-mm tissue culture dishes (5 ml, at 1×10^7 cells/ml). The cells were exposed to UVA radiation supplied by a Dermalight 2001 equipped with an optical filter (H-1) to remove all wavelengths below 320 nm (Dermalight Systems, Studio City, CA). Approximately 99% of the energy emitted by this lamp was within the UVA range, as determined with an Optronics 742 Spectroradiometer (Optronics Laboratories Inc., Orlando, FL); the irradiance of the source averaged 94 J/m^2/s. After irradiation, the cells were harvested with a rubber policeman, washed in PBS, and counted. Control cells were obtained from normal mice and mice injected with the vehicle (0.2 ml of 70% ETOH diluted 1:5 in gelatin). In addition, some of the normal cells were exposed to UVA radiation. A portion of the cells isolated from the 8-MOP-injected mice were shielded from the UVA radiation (held in foil-covered test tubes) and used as an additional source of control cells.

Bone Marrow Transplantation and the Induction of GVHD A modification of the procedure described by Korngold and Sprent [13] was used. Lethally X-irradiated BALB/c mice (900 rads) were injected with a mixture of allogeneic (C3H) spleen and bone marrow cells. PUVA-treated or control spleen (1.25×10^6) and bone marrow cells (5×10^6) were mixed together and injected into the recipient mice via the tail vein. In addition, PUVA-treated C3H spleen and bone marrow cells were used to reconstitute lethally X-irradiated syngeneic C3H mice. The recipient animals were maintained on autoclaved food and bedding, and antibiotic-supplemented water. The animals were checked daily for morbidity and mortality and the median survival times were recorded. Controls that were not injected with spleen and bone marrow cells generally died 15-20 d post irradiation. There were 10 animals per group; statistically significant differences between the median survival times were determined by use of the Wilcoxon rank-sum test.

Mixed Lymphocyte Reaction Spleen cells from 8-MOP-injected or normal mice were isolated and exposed to UVA radiation. The cells were harvested and used as responders in a one-way MLR, as described previously [14]. Briefly, 2×10^5 responder cells were co-cultured with 2×10^5 gamma-irradiated (5000 rads) allogeneic stimulator cells in 96-well microtiter dishes in complete RPMI 1640 medium for 5 d. During the last 18 h of culture, 1 μCi of tritiated thymidine was added to each well. The proliferation of the responding cells was reflected by the incorporation of the radioisotope into newly synthesized DNA. This was determined by harvesting the cultures with a PHD cell harvester (Cambridge Technology Inc., Watertown, MA) followed by liquid scintillation counting. Cultures were run in triplicate, the mean counts per minute (CPM) and standard error of the mean were calculated, and statistically significant differences between the means were determined by use of the two-tailed Student t test.

Growth of Bone Marrow Stem Cells in Soft Agar A modification of the procedure described by Ananthaswamy [15] was used. C3H mice were injected with 8-methoxypsoralen and 2 h later their tibias and femurs were removed. The bone marrow was flushed out and single-cell suspensions were prepared. Contaminating red blood cells were lysed, and the cells were resuspended in PBS and exposed to various doses of UVA radiation. The cells were lifted off the plastic with a rubber policeman, washed, and counted. A 4-ml base layer of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 4.5 g/l D-glucose, glutamine, vitamins, sodium pyruvate (GIBCO, Grand Island, NY) and 0.6% Difco Noble agar was prepared and plated in 60-mm tissue culture dishes. This was overlayed with 0.3% top agar medium containing 10^5 bone marrow cells. Conditioned media (10% v/v) from the WEHI-3 cell line was used as a source of multipotential colony-stimulating factor. The dishes were incubated at 37°C in 8% CO_2 for 3-4 weeks. The resulting colonies were stained with p-iodonitritetrazolium [16] and counted. Triplicate cultures were run, the mean number of colonies per 10^5 input cells was calculated, and statistical differences were determined by use of the two-tailed Student t test.

Determination of Stem Cell Engraftment The procedure described by Kersey et al [17] was used to determine the haplotype of the peripheral blood lymphocytes of the long-term survivors following bone marrow transplantation. Blood was obtained by cardiac puncture and the mononuclear cells were isolated by buoyant density centrifugation using Ficoll-Hypaque. Mononuclear cells (4×10^4) were mixed with equal volumes of specific anti-H-2 antibody and complement (1:6 dilution of hybridoma supernatants), and incubated in triplicate in microcytotoxicity plates for 45 min at room temperature. The reaction was terminated by the addition of 2% EDTA and the viability of the cells was determined by trypan blue staining. Cytotoxicity was determined by comparing the viability seen after treatment with anti-H-2^k plus complement, to the viability seen after treatment with an irrelevant isotype matched antibody (anti-H-2^b) plus complement. Hybridomas producing monoclonal antibodies against H-2 K^kD^k (3-83P), H-2 K^dD^d (34-1-2S) and H-2 K^bD^b (28-8-6S) were obtained from the American Type Culture Collection, Rockville, MD. The cytotoxicity was calculated by the following formula: cytotoxicity = (% alive after treatment with anti-H2^k + complement) − (% alive after treatment with irrelevant antibody + complement)/(% alive after treatment with irrelevant antibody + complement) $\times 100$.

The level of circulating BALB/c cells was determined by substituting anti-H-2^d for the anti-H-2^k antibody. As a control for this experiment, mononuclear cells isolated from normal C3H or BALB/c mice were used.

RESULTS

Inhibition of Alloreactivity by PUVA Treatment The effect PUVA treatment had on the immune reaction to alloantigen was examined. Normal C3H mice were injected intraperitoneally with

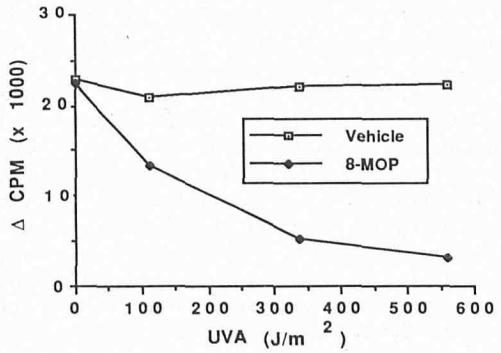

Figure 1. Suppression of the proliferative response to alloantigen by PUVA treatment. Spleen cells were isolated from C3H mice injected with 8-MOP or vehicle, exposed to various doses of UVA, and cultured with gamma-irradiated BALB/c stimulator cells. The proliferation of the C3H spleen cells was measured by incorporation of tritiated thymidine. The data is expressed as Δ cpm, the background response of the C3H cells cultured without stimulator cells has been subtracted. The Δ cpm response of normal cells was 24,863. Standard errors were less than 10%. This experiment was repeated twice with similar results; a representative experiment is shown.

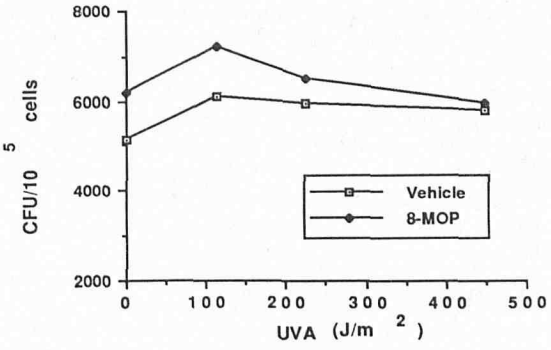

Figure 2. Effect of PUVA treatment on the hematopoietic potential of bone marrow stem cells. Bone marrow cells from 8-MOP or vehicle-injected mice were exposed to UVA and cultured in soft agar. Three–4 weeks after the initiation of the culture, 3–6 random fields were counted, and the number of colony-forming units (CFU) per 10^5 input cells was calculated. Normal C3H bone marrow yielded 5122 colonies/10^5 input cells. This experiment was repeated twice with similar results; a representative experiment is shown.

0.4 mg of 8-MOP. Two h later, their spleens were removed, single-cell suspensions were prepared, and the cells were exposed to various doses of UVA radiation. The ability of the PUVA-treated spleen cells to respond to alloantigen in a standard MLR was depressed (Fig 1). UVA treatment alone had no effect on the alloreactivity of spleen cells, as demonstrated by the comparable responses of normal spleen cells and cells from mice injected with vehicle and exposed to UVA. Also, injection of 8-MOP without subsequent UVA treatment did not impair the alloreactivity of the spleen cells. The decreased proliferative response to alloantigen by the PUVA-treated cells was not due to cell killing, because the viability of the PUVA-treated cells and normal cells after 1, 3, or 5 d of culture was similar (data not shown). Thus, these data demonstrate that extracorporeal PUVA treatment inactivates alloreactivity.

PUVA Treatment of Bone Marrow Cells does not Inhibit the Ability of Stem Cells to Proliferate in Response to Colony-Stimulating Factor The effect PUVA treatment had on hematopoiesis was measured. Normal mice were injected with 8-MOP, and 2 h later their bone marrow was removed and single-cell suspensions prepared. The cells were irradiated in vitro with UVA, and the effect this treatment had on colony formation was determined by measuring the growth of the cells in soft agar using multipotential colony-stimulating factor (interleukin-3) as the growth factor. As shown in Fig 2, PUVA treatment of bone marrow cells had no suppressive effect on their ability to form colonies in soft agar.

Suppression of GVHD by PUVA Treatment of the Donor Spleen and Bone Marrow Cells Because PUVA treatment inhibited alloreactivity in the MLR but did not affect stem cell hematopoiesis, we next measured the effect that PUVA treatment had on the induction of GVHD. Adult BALB/c mice were exposed to a lethal dose of X-irradiation (900 rads) and injected with allogeneic C3H spleen and bone marrow cells. A limitation of the murine model of bone marrow transplantation concerns the limited number of T cells found in mouse bone marrow. Human bone marrow usually contains between 10–20% T lymphocytes, whereas the number of T cells within murine bone marrow is approximately tenfold lower [18,19]. Therefore, to approximate the situation seen in human bone marrow transplantation, spleen cells were mixed with bone marrow cells, and the final concentration of T cells in the inoculum was 16% (as determined by indirect immunofluorescence staining with anti-Thy 1.2 antibody and fluorescein-conjugated goat anti-rat IgG). The C3H mice were injected with 0.4 mg of 8-MOP. Two h later, the mice were killed, spleen and bone marrow cells were obtained, exposed to 560 J/m² of UVA radiation, and injected into X-irradiated BALB/c recipients. The mice were monitored daily and survival times were recorded. Injecting X-irradiated recipient mice with normal allogeneic spleen and bone marrow cells resulted in the induction of acute GVHD with a median survival time (MST) of 13 d (Fig 3). When the recipient mice were injected with cells exposed to UVA, cells from donor mice injected with 8-MOP but not irradiated with UVA, or cells from vehicle-injected controls, the MST was not significantly different from the survival seen when the mice were injected with normal cells (MST of 11, 7, and 8, respectively, $P > 0.05$, *Wilcoxon rank sum test*). When the recipient mice were injected with PUVA-treated cells, a significant prolongation of survival was noted (MST, 44 days; $p < 0.005$) when compared to the MST of mice injected with normal cells. Injecting PUVA-treated cells into X-irradiated syngeneic C3H mice resulted in 100% survival, confirming the finding that PUVA treatment has no adverse affect on hematopoiesis. Thus, these data demonstrate that PUVA treatment of the donor cells inactivates T cell alloreactivity and suppresses the induction of GVHD.

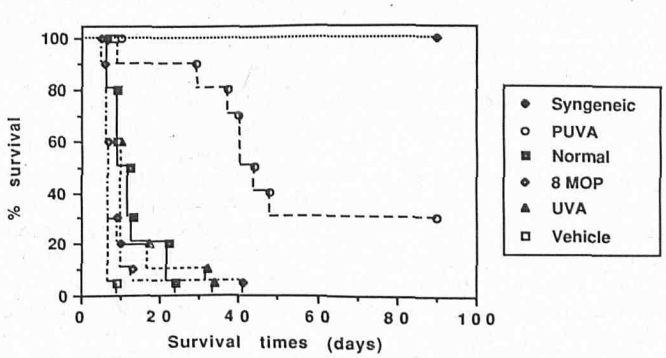

Figure 3. Effect of PUVA treatment on the induction of GVHD. Spleen and bone marrow cells from normal C3H mice, mice injected with vehicle, or mice injected with 8-MOP were injected into lethally X-irradiated BALB/c mice. *PUVA* cells are from mice injected with 8-MOP and exposed in vitro to UVA radiation, *UVA* are cells from normal mice exposed in vitro to UVA radiation, and *syngeneic* are PUVA-treated cells injected into X-irradiated syngeneic C3H mice. The mice were monitored daily; the experiment was terminated at 90 d. There were 10 mice per group. The individual survival times of the mice in the group injected with PUVA-treated spleen and bone marrow cells was 10, 29,37, 40, 43, 44, 48, >90, >90, >90. This experiment was repeated three times with similar results; a representative experiment is shown.

Table I. Haplotype of the Peripheral Blood Leukocytes Found in the Long-Term Survivors

Source of the PBL	% Cytotoxicity	
	αH-2^k	αH-2^d
Normal C3H	94	8
Normal BALB/c	3	90
GVH LTS$_1$[a]	88	16
GVH LTS$_2$	83	16

[a] Long-term survivor GVH experiment.

Effect of Extracorporeal PUVA Treatment on Engraftment The haplotype of the peripheral blood lymphocytes of the long-term survivors was determined. Between 83–88% of the peripheral blood lymphocytes isolated from the long-term survivors (>90 d) were of donor origin (H-2^k), as determined by an in vitro complement-dependent microcytotoxicity test (Table I), indicating that PUVA treatment of the bone marrow inoculum suppresses the induction of GVHD without suppressing stem-cell engraftment.

Partial Restoration of Alloreactivity in the Long-Term Survivors In order to determine whether there was any restoration of T cell function after PUVA therapy was used to suppress GVHD, spleen cells from two of the long-term survivors were used as responder cells in a one-way MLR (Table II). No proliferation was observed when spleen cells from the long-term survivors were cultured with either C3H or BALB/c alloantigen. The cells from the long-term survivors were able to mount a proliferative response (4 to 6 times increase in stimulation indices) to C57B1/6 cells, an irrelevant antigen. For the sake of comparison, the proliferative response of normal C3H and BALB/c spleen cells to alloantigen was also measured. Note that the normal cells showed a 7 to 16 times stimulation index when cultured with alloantigen. Thus, these data suggest that partial reconstitution of T cell function was achieved in animals reconstituted with photoinactivated bone marrow cells.

DISCUSSION

GVHD is a serious complication of bone marrow transplantation. Although removing T cells from the transferred marrow effectively suppresses the induction of GVHD, T-cell depletion increases the probability of graft failure and is associated with a greater risk of disease relapse, especially when bone marrow transplantation is used to treat leukemia. Thus, purging T cells from the donor marrow prior to bone marrow transplantation has been described as a "double-edged sword," GVHD is effectively suppressed, but graft rejection frequently occurs, accompanied by the relapse of disease. Because T cells have been shown to have a positive effect on hematopoiesis [20], the removal of these cells, and/or the colony-stimulating factors they produce, may explain the poor engraftment seen after T-cell depletion. On the other hand, T-cell depletion may remove a set of donor-derived regulatory T cells that suppress the residual host-versus-graft response mediated by radioresistant T cells or natural killer cells [21,22].

The transplantation surgeon, therefore, faces a dilemma: removal of the T cells from the transplanted marrow prevents GVHD, but enhances graft failure. The approach taken in these studies was to photoinactivate T-cell alloreactivity rather than deplete T cells from the graft and examine the effect this treatment had on the induction of GVHD. This approach was based on the observation of others that T cells, and T-cell function, is quite sensitive to photoinactivation with 8-MOP and UVA radiation [5–11]. These findings are supported by the data shown in Figs 1 and 2 of this report, which indicate that PUVA treatment effectively inhibits the MLR but has no effect on stem-cell colony formation. A trivial explanation for these findings would be that the drug, 8-MOP, does not reach the bone marrow. However, tissue distribution studies carried out by Muni and colleagues [23] suggest this is not the case. Within 4 h of oral administration of radiolabeled 8-MOP, peak levels of radioactivity were found in most of the organs of the body. Most of the drug was excreted by 24 h and little radioactivity remained at 96 h. In addition, previous data reported from this laboratory and others have shown that intraperitioneal injection of 8-MOP, followed by total body exposure to UVA, resulted in a systemic alteration of immune function [12,24]. These data, together with the finding that psoralen receptors are found on a wide variety of cells [25], suggest that limited distribution of the 8-MOP does not explain the effects reported here. Furthermore, Berger et al [6] have previously reported that myeloid cells are relatively resistant to the anti-proliferative effects of PUVA therapy. Therefore, we favor the view that T lymphocytes are more sensitive to inactivation by PUVA therapy and this is the reason for the inhibition of the MLR but not stem-cell colony-forming units, as found in Figs 1 and 2.

The data found in Fig 3 also support the concept that T cells are more susceptible to photoinactivation than bone marrow stem cells. Injecting normal cells, cells exposed to UVA, or cells isolated from 8-MOP–treated animals into X-irradiated allogeneic recipients resulted in an acute lethal graft-versus-host reaction. However, when the X-irradiated allogeneic recipients were injected with PUVA-treated spleen and bone marrow cells, the severity of GVHD was suppressed, as evidenced by a statistically significant increase in the MST and the fact that 30% of the animals were alive at the termination of the experiment. When the same cells were used to reconstitute lethally X-irradiated syngeneic recipient mice, 100% of the animals survived, suggesting that PUVA treatment did not adversely affect the colony-forming potential of the stem cells. On the other hand, the survival of the mice injected with the PUVA-treated spleen and bone marrow cells may be due to the large number of stem cells (5 million) used to reconstitute the recipient mice. It is possible that the PUVA treatment did cause some stem-cell toxicity, but a sufficient number of viable stem cells were present in the inoculum to allow reconstitution. Although the data presented in Fig 2 seem to rule out this possibility, this issue can only be resolved through the use of in vivo stem-cell engraftment assays [26].

Although an attempt was made to mimic the situation found in human bone marrow transplantation in regard to the proportion of T cells found in the bone marrow inoculum, it should be noted that the strains of mice used here are mismatched at both the major and minor histocompatibility loci. Thus, the donor and recipient pairs

Table II. Alloreactivity of Splenic Lymphocytes Isolated from Mice Reconstituted with PUVA-Treated Bone Marrow Cells

Cells Cultured	CPM ± SEM[a]	Stimulation Index[b]
BALB/c	2244 ± 117	
BALB/c + C3H[c]	15833 ± 2601	7.1
BALB/c + C57B1/6[c]	36291 ± 3547	16.2
C3H	3552 ± 412	
C3H + BALBc[c]	26644 ± 2893	7.5
C3H + C57B1/6[c]	38097 ± 1544	10.7
GVH LTS$_1$	3183 ± 751	
GVN LTS$_1$ + C3H[c]	4406 ± 644	1.4
GVH LTS$_1$ + BALB/c[c]	5053 ± 540	1.6
GVH LTS$_1$ + C57B1/6[c]	19732 ± 562	6.2
GVH$_2$LTS	3537 ± 494	
GVH LTS$_2$ + C3H[c]	3780 ± 254	1.1
GVH LTS$_2$ + BALB/c[c]	4076 ± 537	1.2
GVH LTS$_2$ + C57B1/6[c]	16100 ± 1604	4.6

[a] Mean CPM and the SEM was determined for cultures run in triplicate.

[b] Stimulation index was determined by the following formula: mean CPM of cultures containing stimulator + responder cells/mean CPM of cultures containing only responder cells.

[c] Stimulator cells were exposed to 5000 rads of gamma radiation.

used here represent the most stringent test for suppressing the induction of GVHD. It is not clear what would happen if there was partial identity between the donor and recipient, but it is not unreasonable to suggest that an even better suppression of GVHD may be achieved as the genetic disparity between the donor and recipient is decreased.

Examination of the haplotype of the peripheral blood leukocytes of the long-term survivors (Table I) indicates that suppression of GVHD can be achieved without an adverse affect on stem-cell engraftment. Eighty-three to 88% of the peripheral blood leukocytes of the long term survivors (>90 d) were of donor origin. Because the PUVA treatment used here is not lethal for the transplanted cells, it is possible that whereas alloreactivity of the T cells is suppressed, the ability of these cells to have a positive effect on hematopoiesis [20] is retained. Alternatively, PUVA treatment and, especially, extracorporeal photophoresis, have been shown to activate suppressor cells [10,12,24]; it is possible that suppressor cells within the graft inhibit a residual radioresistant host-versus-graft reaction, thus promoting engraftment.

Another advantage in using PUVA therapy to suppress acute GVHD across major histocompatibility barriers is pointed out by the data concerning the partial restoration of T-cell function found in the long-term survivors (Table II). Spleen cells from the long-term survivors were used as responder cells in a one-way MLR against BALB/c, C3H, and C57B1/6 stimulator cells. Little to no proliferation was seen when spleen cells from the long-term survivors were cultured with C3H stimulator cells, and because the data in Table I indicate that the lymphoid system of the long-term survivor was reconstituted with donor-derived cells, this result is to be expected. Similarly, spleen cells from the long-term survivors did not proliferate in response to the BALB/c stimulator cells. This "self" tolerance probably reflects the depletion of C3H anti-BALB/c clones during the development of the immune repertoire in the reconstituted X-irradiated BALB/c recipient mice. When spleen cells from the long-term recipient mice were cultured with an irrelevant antigen, C57B1/6 stimulator cells, a proliferative response was observed. Note, however, that the magnitude of the response generated by the long-term survivors against the C57B1/6 stimulator cells was approximately half of that seen when normal C3H cells were cultured with C57B1/6 stimulator cells (stimulation indices of 6.2 and 4.6 vs a stimulation index of 10.7 when normal responder cells were used). Due to the small sample size, both these results and those presented in Table I must be interpreted with caution. However, they certainly suggest positive engraftment and a partial restoration of T-cell function in the long-term survivors. Other immune parameters of the long-term survivors, such as the ability to generate a humoral immune response, generation of cytolytic T cells, or the development of a graft-versus-leukemia reaction, have not yet been examined.

Whereas others have shown that exposing human bone marrow cells to UVB and/or UVC radiation can suppress mitogen-induced proliferative responses without affecting stem-cell hematopoiesis [18], and UVB pretreatment of rat bone marrow cells suppresses the induction of GVHD [27], the results reported here are the first to demonstrate that PUVA treatment of allogeneic bone marrow cells can suppress the induction of GVHD. There may be some unique advantages in using extracorporeal PUVA treatment to condition donor bone marrow and suppress the induction of GVHD. First, psoralens are relatively inactive in the absence of long-wavelength UVA radiation. Second, the effective half-life of the UVA-activated psoralen is measured in milliseconds. Any drug remaining after irradiation returns to the inactive state, thus limiting adverse side effects. Third, the procedure used here is an adaptation of the extracorporeal photophoresis protocol that has been used in the clinic to treat cutaneous T-cell lymphoma [9]. The adverse side effects associated with this treatment include erythema and post-reinfusion febrile reactions. The side effects that are normally associated with standard chemotherapy (bone marrow suppression, hair loss, nausea, vomiting, gastrointestinal difficulties, and opportunistic infections) were absent when extracorporeal photophoresis was used to treat cutaneous T-cell lymphoma. Similar side effects, especially opportunistic infections, result from standard chemotheraputic protocols used to treat GVHD. An added advantage in using PUVA therapy to suppress the induction of GVHD comes from a potential reduction of these adverse side effects, especially opportunistic infections. Fourth, we have shown previously that donor-derived suppressor cells can inhibit the immunologic reaction of the graft against the host [28]. If extracorporeal PUVA treatment of human bone marrow induces suppressor cells in a manner similar to that reported with the murine model of photoinactivation [10], these cells may serve to further suppress GVHD.

The data presented here suggest that photoinactivation of donor T cell function with psoralen plus UVA radiation provides a novel method of suppressing GVHD. The suppression of GVHD is dramatic, in view of the fact that the donor and recipient mice used here differed across major histocompatibility barriers. Whether it will be possible to achieve better survival times if the donor and recipients are matched at the major histocompatibility loci, a situation comparable to what is seen during HLA-matched human bone marrow transplantation, remains to be seen.

I thank Dr. M. L. Kripke for her critical review of the manuscript, Dr. H. N. Ananthaswamy for his help with the soft agar cultures, and Ms. Amy Casagrande for technical assistance.

REFERENCES

1. Maraninchi DD, Blaise B, Rio V, Leblond F, Dreyfus E, Gluckman D, Guyotat JL, Pico M, Michallet N, Ifrah N, Bordigoni A: Impact of T-cell depletion on outcome of allogeneic bone-marrow transplantation for standard-risk leukemias. The Lancet II:175–178, 1987
2. Korngold R, Sprent J: Lethal graft-versus-host disease following bone marrow transplantation across minor histocompatibility barriers in mice. Prevention by removing mature T cells from marrow. J Exp Med 148:1687–1698, 1978
3. Gale RP, Reisner Y: Graft rejection and graft-versus-host disease: mirror images. The Lancet I:1469–1470, 1986
4. Beran M, Zander AR: Critical issues in autologous bone marrow transplantation in adult acute leukemia. Eur J Haematol 39:97–117, 1987
5. Kraemer KH, Waters HL, Cohen LF, Popescu NC, Amsbaugh SC, DiPaolo JA, Glaubiger D, Ellingson OL, Tarone RE: Effects of 8-methoxypsoralen and ultraviolet radiation on human lymphoid cells in vitro. J Invest Dermatol 78:80–87, 1981
6. Berger CL, Cantor C, Welsh J, Dervan P, Begley T, Grant S, Gasparro F, Edelson RL: Comparison of synthetic psoralen derivatives and 8-MOP in the inhibition of lymphocyte proliferation. Ann NY Acad Sci 446:80–90, 1985
7. Gasparro FP, Chan G, Edelson RL: Phototherapy and photopharmacology. Yale J Biol Med 58:519–534, 1985
8. Morison WL, Wimberly J, Parrish JA, Bloch KJ: Abnormal lymphocyte function following long-term PUVA therapy for psoriasis. Br J Dermatol 108:445–450, 1985
9. Edelson R, Berger C, Gasparro F, Jegasothy B, Heald P, Wintrob B, Vonderheid E, Knobler R, Wolff K, Plewig G, McKiernan G, Christiansen I, Oster M, Honigsmann H, Wilford H, Kokoschka E, Rehle T, Perez M, Stingl G, Laroche L: Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. N Engl J Med 316:297–303, 1987
10. Perez M, Edelson R, Laroche L, Berger C: Inhibition of antiskin allograft immunity by infusion with syngeneic photoinactivated effector lymphocytes. J Invest Dermatol 92:669–676, 1989
11. Berger CL, Perez M, Laroche L, Edelson R: Inhibition of autoimmune disease in a murine model of systemic lupus erythematosus induced by exposure to syngeneic photoinactivated lymphocytes. J Invest Dermatol 94:52–57, 1990
12. Ullrich SE, Kripke ML: Mechanisms in the suppression of tumor rejection produced in mice by repeated UV irradiation. J Immunol 133:2786–2790, 1984

13. Korngold R, Sprent J: Surface markers of T cells causing lethal graft-versus-host disease to class I vs. class II H-2 differences. J Immunol 135:3004–3010, 1985
14. Ullrich SE: Suppression of the immune response to allogeneic histocompatibility antigen by a single exposure to UV radiation. Transplantation 42:287–291, 1986
15. Ananthaswamy HN: Neoplastic transformation of neonatal mouse skin fibroblasts in culture after a single exposure to ultraviolet radiation in vivo. Photodermatology 1:106–113, 1984
16. Drebin JA, Link VC, Stern DF, Wienberg RA, Greene MI: Down-modulation of an oncogene protein and reversion of the transformed phenotype by monoclonal antibodies. Cell 41:697–706, 1985
17. Kersey JH, Kruger J, Song C, Kloster B: Prolonged bone marrow and skin allograft survival after pretransplant conditioning with cyclophosphamide and total lymphoid irradiation. Transplantation 29:388–391, 1980
18. Deeg JH, Balzer L, Sigaroudinia M, Cottler-Fox M: Ultraviolet B light inactivates bone marrow T lymphocytes but spares hematopoietic precursor cells. Blood 73:369–371, 1989
19. Vallera DA, Soderling C, Carlson GJ, Kersey JH: Bone marrow transplantation across major histocompatibility barriers in mice. II. T cell requirement for engraftment in total lymphoid irradiation-conditioned recipients. Transplantation 33:243–248, 1982
20. Navarro J, Touraine J-L: Promotion of fetal liver engraftment by T cells in a murine semiallogeneic model without graft-versus-host reaction. Transplantation 47:871–876, 1989
21. Cobbold S, Martin G, Waldmann H: Monoclonal antibodies for the prevention of graft-versus-host disease and marrow graft rejection. Transplantation 42:239–247, 1986
22. Murphy WJ, Kumar V, Bennett M: Acute rejection of murine bone marrow allografts by natural killer cells and T cells. J Exp Med 166:1499–1509, 1987
23. Muni IA, Schneider FH, Olsson TA, King M: Adsoprtion, distribution and excretion of 8-methoxypsoralen in HRA/Skh mice. Natl Cancer Inst Monogr 66:85–90, 1984
24. Kripke ML, Morison WL, Parrish JA: Systemic suppression of contact hypersensitivity in mice by psoralen plus UVA radiation (PUVA). J Invest Dermatol 81:87–92, 1983
25. Laskin JD, Lee E, Yurkow EJ, Laskin DL, Gallo MA: A possible mechanism of psoralen phototoxicity not involving direct interaction with DNA. Proc Natl Acad Sci USA 82:6158–6262, 1985
26. Murphy WJ, Kumar V, Cope J, Bennett M: An absence of T cells in murine bone marrow allografts leads to an increased susceptibility to rejection by natural killer cells and T cells. J Immunol 144:3305–3311, 1990
27. Chabot JA, Pepino P, Wasfie T, Stegall MD, Hardy MA: UVB pretreatment of rat bone marrow allografts. Prevention of GVHD and induction of allochimerism and donor-specific unresponsiveness. Transplantation 49:886–889, 1990
28. Ullrich SE, Magee MJ: Specific suppression of allograft rejection after treatment of recipient mice with UV radiation and allogeneic spleen cells. Transplantation 46:115–119, 1988

FOURTH WORLD CONGRESS ON CANCERS OF THE SKIN

The Fourth World Congress on Cancers of the Skin, sponsored jointly by The Skin Cancer Foundation and Memorial Sloan-Kettering Cancer Center, is scheduled to convene April 18–20, 1991, in New York City. The three-day international meeting will focus on recent advances in medical research and current concepts in the diagnosis and management of cutaneous malignancies.

The first day of the Congress will be devoted to viral oncogenesis and skin cancer, including sexually transmitted diseases and related malignancies. The second day's program will be concerned with malignant melanoma, and the final day will feature a series of round-table workshops on basal cell and squamous cell carcinomas.

Program chairmen for the World Congress are Alfred W. Kopf, M.D., and Perry Robins, M.D., members of the faculty of New York University School of Medicine. Co-chairmen are Bijan Safai, M.D., of Memorial Sloan-Kettering Cancer Center, and Philip Bailin, M.D., of the Cleveland Clinic Foundation. They will be joined by an international faculty of 46 medical and scientific authorities representing more than 10 medical specialties.

Information on registration may be obtained by writing to Memorial Sloan-Kettering Cancer Center, Continuing Medical Education Office, Box 31, 1275 York Avenue, New York, N.Y. 10021. Tel: (212)639-6754.