Bone marrow cells from ten normal donors were exposed to ultraviolet (UVC) or UVB light for total exposures of 0.1 to 100 mJ/cm², and assayed for granulocyte-macrophage colony-forming units (CFU-GM), erythroid burst-forming units (BFU-E), and phytohemagglutinin (PHA)-stimulated proliferative responses. After exposure to UVC CFU-GM, BFU-E and PHA responses showed a UV dose-dependent sharp decrease to levels <1% of controls with 0.5, 2.0, and 10 mJ/cm², respectively. With UVB, PHA responses were most sensitive, declining to <1% at 5 mJ/cm². BFU-E decreased to <1% of control with 15 mJ/cm² UVB. CFU-GM, at UVB doses of 0.1 to 2.0 mJ/cm², increased to 125% to 150% of control and decreased to <1% only at exposures >20 mJ/cm². Thus, these studies show that UVB, but not UVC light, can be used to inactivate bone marrow T lymphocytes selectively while sparing hematopoietic precursor cells. The data suggest that UVB irradiation can be used for T-lymphocyte purging for allogeneic marrow transplantation.

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**BIOLOGIC EFFECTS** of electromagnetic energy are dependent on numerous parameters, including wavelength and composition of target tissues. Ultraviolet (UV) light (200 to 400 nm) has little depth penetration. Nevertheless, studies aimed at evaluating the influence of UV light on skin and skin-associated immune cells also showed systemic effects. For example, lymphocytes obtained from human individuals within hours of UV exposure show reduced mitogenic capacity. In vitro treatment of immunocompetent cells with UV light in the B (280 to 320 nm) or C range (200 to 280 nm) abrogates the ability of lymphocytes to stimulate in mixed leukocyte culture, eliminates their accessory function in mitogen-stimulated cultures, and prevents development of cytotoxic effector cells. Lymphocytes stimulated by UV-treated accessory cells apparently express the interleukin-2 (IL-2) receptor but do not proliferate without the addition of exogenous IL-2. In vivo studies in a canine model demonstrated that UV irradiation of blood prior to transfusion completely prevents sensitization of the recipient. The present study was designed to investigate whether bone marrow T lymphocytes, similar to peripheral lymphocytes, could be inactivated by UV light. If this were possible without interfering with hematopoiesis, it might be an approach to T-cell purging of marrow.

**MATERIALS AND METHODS**

**Bone marrow.** Bone marrow was obtained from normal individuals serving as bone marrow donors for marrow transplant recipients. During marrow harvesting a separate aspirate into a syringe containing heparinized tissue culture medium was obtained from the posterior iliac crest. Some samples were obtained from normal volunteers. Donors had given informed consent on forms approved by the Institutional Review Board of Georgetown University Hospital.

**Cell separation.** Bone marrow cells were diluted 1:1 with culture medium RPMI 1640 containing 10% fetal calf serum (FCS). The suspension was layered on Ficoll LSM (Organon Technique, Durham, NC) and centrifuged for 15 minutes at 1,500 g. The interface cells were collected and washed three times in complete culture medium by centrifugation at 180 g for five minutes and resuspended in medium. The lymphocyte content of bone marrow as determined with an anti-CD3 reactive antibody ranged from 13% to 23%.

**UV irradiation.** UV irradiation was performed as described previously. For UVC irradiation, a UVC lamp (Spectrafine XX-15F, Spectronics Corporation, Westbury, NY) with a maximum emission (85%) at 254 nm was used. For UVB irradiation (280 to 320 nm), a UVB lamp (Spectrafine XX-15B, Spectronics) was used with a spectrum of 280 to 320 nm and maximum emission at 302 nm. Cells were suspended in PBS and placed in open Petri dishes at a maximum depth of 1.5 mm and exposed to UV doses of 0.1 to 100 mJ/cm². Different doses were achieved by exposing cells for different time periods. Exposure for UVC was determined by a Spectraline DRC-100 UV meter equipped with a DIX 254 sensor set for 254 nm (resolution 10 μW/cm²), and exposure for UVB was determined with a Spectraline DM 300× UV meter reading at 280 to 320 nm. Irradiated cells were washed and resuspended in medium before use. Viability was determined by trypan blue dye exclusion.

**Lectin stimulation.** Phytohemagglutinin (PHA)-stimulated cultures were carried out as described. Cells (1 x 10⁵) were plated in triplicate in round-bottom wells (Costar, Cambridge, MA), and PHA was added for final concentrations of 2%, 0.2%, or 0.02%. Cultures were incubated at 37°C in a humidified 5% CO₂/air atmosphere. After 72 hours, 1 μCi of [³²P]thymidine with a specific activity of 25 Ci/mmol (Difco Laboratories, Detroit) was added to each well. The cells were harvested 18 hours later. ³H Activity was counted in a liquid scintillation counter. Results were expressed in cpn. Unirradiated marrow cells served as control.

**Assays for CFU-GM and BFUE.** Bone marrow cells were isolated as described above, washed, and suspended in Iscove's modified Dulbecco's medium (IMDM) at 2.25 x 10⁷/mL. Cells were suspended at 5 x 10⁶/mL in 2-mercaptoethanol (30 μmol/L), erythropoietin, (1 U/mL, Toyoba), IMDM, methyl cellulose (1.25% wt/vol), fetal bovine serum (FBS, 30%, vol/vol), conditioned medium from PHA-stimulated peripheral blood mononuclear cells as a source of growth factor (10%, vol/vol), gentamycin sulfate (50 μg/mL), and amphotericin B (2.5 μg/mL). The samples were distributed into 35-mm Petri dishes (in triplicates, 1 mL per dish), and the soft suspension cultures were incubated for 14 days at 37°C in a 5% CO₂ atmosphere. Cultures were then scored for BFU-E and CFU-GM.

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CFU-GM. Colony numbers are expressed per 10³ cells. Nonirradiated marrow cells served as controls.

Presentation of results. In evaluating the effect of UV light on the response of marrow cells to PHA, responses of nonirradiated marrow mononuclear cells were considered as 100%. The responses of UV-treated cells were corrected for background proliferation in cells cultured in medium alone and the delta value was expressed as percent of control. Similarly, for BFU-E and CFU-GM, the numbers of colonies per 10³ cells obtained with nonirradiated cells served as the 100% reference values. The numbers of colonies obtained with cells exposed to various doses of UV light were expressed as percentage of control.

RESULTS

Results are summarized in Fig 1A and B. After exposure to UVC light, marrow cells showed a rapid decrease in functional ability of marrow cells. PHA responses (shown are results with 0.2% PHA), CFU-GM and BFU-E decreased to 50% of control with UVC doses of 0.2 to 0.4 mJ/cm². These functions were reduced to <1% with ~0.5 mJ/cm² for BFU-E, 2 mJ/cm² for CFU-GM and 10 mJ/cm² for PHA stimulation.

With UVB light, longer exposure (i.e., higher energy) was necessary for cell inactivation. Cell viability following UV exposure was ≥ 98%. The most UV-sensitive function was the response to PHA (shown are results with 0.2%), which declined to 50% after an exposure of 1 to 2 mJ/cm², and was reduced to <1% at 5 mJ/cm². These cells were also unable to generate cytotoxic effector cells against allogeneic targets (data not shown). BFU-E formation was reduced to 50% with an exposure of 3 mJ/cm² and to <1% with 15 mJ/cm². Most strikingly, CFU-GMs increased by 25% to 30% relative to control with UVB doses of 0.1 to 2 mJ/cm². More than 20 mJ/cm² was necessary to reduce the number of CFU-GM to <1%.

Thus, the amount of energy required overall for cell inactivation with UVB was about tenfold higher than with UVC. With UVC exposure, BFU-Es and CFU-GMs were as sensitive as or more sensitive than PHA responses. However, UVB exposure rapidly eliminated proliferative responses to PHA, whereas hematopoietic precursors (BFU-E, CFU-GM) maintained their colony-forming ability.

DISCUSSION

We showed in this study that UV light in the intermediate (UVB) but not short-wave (UVC) range can be used to selectively inactivate bone marrow T lymphocytes while preserving the function of hematopoietic precursors as determined by in vitro colony-forming assays. These data are consistent with work by other investigators showing that UVC treatment is mostly cytocidal, whereas UVB modulates cell function. The results indicate furthermore that different cell populations have differential sensitivity to UVB light. This could be due to a relative resistance to UV exposure, possibly mediated by differences in cell membrane or cytoplasmic components, or it could result from different repair processes in the cells; hematopoietic precursor cells but not T lymphocytes may be equipped to bypass UV-induced damage or repair it quickly.

We previously showed that among peripheral blood leukocytes T cells are more sensitive than B cells, and among T lymphocytes, CD8⁺ cells are more sensitive than CD4⁺ cells. UV sensitivity in peripheral blood leukocytes is manifested by an increase in intracellular calcium in unstimulated cells and an unresponsiveness of calcium mobilization to mitogenic stimuli. Similar studies in bone marrow cells may shed additional light on the mechanisms involved.

Fig 1. Bone marrow mononuclear cells were UV irradiated and assayed for CFU-GM, BFU-E, and PHA-induced proliferation as described in the Materials and Methods section. Results are expressed relative to control ( sham irradiated 100%). (A) UVC, ultraviolet light (200 to 280 nm) maximum energy at 254 nm. Shown are the means of ten separate experiments; SD ranged from 3% to 34% for PHA responses and from 2% to 22% for CFU-GM, and BFU-E. (B) UV, ultraviolet light (280 to 320 nm) maximum energy at 302 nm. Shown are the means of ten experiments; SD ranged from 15% to 35% for PHA responses and from 6% to 27% for CFU-GM and BFU-E.
Our results are of interest in the context of murine data showing that UV treatment of lymphocytes added to hematopoietic precursor cells prevented graft-v-host (GVH) reaction. The present study indicates that human marrow can be directly exposed to UV light, thus inactivating T cells contained in the marrow inoculum used for transplantation. Ongoing investigations in an in vivo model show that murine spleen cells can be treated with UV so that lymphocyte reactivity is abrogated while ability for hematopoietic reconstitution is spared (J. Deeg et al, unpublished observations).

In recent years, there has been a keen interest in use of photodynamic therapy for inactivation of cells in peripheral blood or in bone marrow, using chemicals such as merocyanine 540, methoxypсорalen, or phthalocyanine to sensitize cells to inactivation by visible or UV light. The current approach does not require any manipulation other than UV irradiation. These data are encouraging insofar as they suggest a novel approach to the prevention of GVS disease (GVHD).

REFERENCES