Prevention of Graft-Versus-Host Disease and Bone Marrow Rejection: 
Kinetics of Induction of Tolerance by UVB Modulation of Accessory 
Cells and T Cells in the Bone Marrow Inoculum

By Soji F. Oluwole, Kris Engelstad, and Tyshaun James

UVB irradiation (700 J/m²) of bone marrow cells (UVB-BMC) before transplantation into lethally gamma-irradiated (10.5 Gy) allogeneic rats prevents graft-versus-host disease (GVHD) and induces a stable complete lymphohematopoietic chimerism. To better understand the underlying mechanism of the development of stable chimerism and induction of tolerance to donor organs in this model, we examined if the addition of T cells or dendritic cells (DC), as antigen presenting cells (APC), would restore the immunogenicity of UVB-BMC in vitro mixed lymphocyte reaction (MLR) and induce in vivo bone marrow (BM) graft rejection. Whereas gamma-irradiated, unfractiooned BMC induce allogeneic T cells to proliferate, UVB irradiation of BMC abolishes the stimulatory capacity of such cells in a primary MLR. Addition of purified T cells, CD4⁺ T cells, CD8⁺ T cells or B cells, respectively, failed to restore the capacity of UVB-BMC to stimulate allogeneic T-cell proliferation. In contrast, the addition of only a small number of splenic accessory cells or purified DC, which by themselves were relatively ineffective in stimulating T-cell proliferation, restored the accessory function and the allostimulatory capacity of UVB-BMC. To define the molecular defect induced by UVB irradiation, cytokines were added as costimulatory factors to primary MLRs and the results showed that the addition of interleukin (IL)-2 or IL-6 but not IL-1 or interferon gamma (IFN-γ) restored the stimulatory capacity of UVB BMC. This finding suggests that UVB may alter the production, and/or utilization of IL-2 and IL-6 either at the membrane or cytoplasmic level. Parallel in vivo studies showed that addition of DC to UVB BM inoculum resulted in failure of BM engraftment, whereas addition of T cells led to development of fatal GVHD, thus suggesting that UVB modulation of accessory cells reduces graft immunogenicity and prevents BMT rejection, while modulation of T cells prevents GVHD. Our data provide evidence that UVB modulation of APC and mature T cells contained within BMC is potentially useful in preventing GVHD without endangering successful engraftment and may serve as a model for induction of adult chimeraism and tolerance without the development of GVHD.

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associated with complete allogeneic chimerism with 96% to 98% donor T cells.\textsuperscript{15,14,17}

Little consideration has been given to the presence of APC in the BM inoculum, which may play a role in recipient sensitization and BM rejection and/or nonengraftment. Although the underlying mechanisms of the failure of BM engraftment in TCD of BMT model remain unclear, many studies suggest that T cells in the BM inoculum may eliminate or modulate the residual radioresistant host lymphocytes that cause graft rejection.\textsuperscript{2,5} It appears that failure of BM engraftment in TCD of BMT is mediated by host T cells and natural killer (NK) cells, which are resistant to recipient conditioning regimens.\textsuperscript{2,5}

To better understand the underlying mechanisms of development of complete allogeneic chimerism in the UVB-BMT model, we have examined if addition of donor type T cells or DC as APC would restore the immunogenicity of UVB-BMT and induce GVHD or BM-engraftment failure. In addition, we examined the molecular defect induced by UVB irradiation of BMC. Many studies have shown that T-cell activation and proliferation require antigen recognition and costimulatory signals.\textsuperscript{18,19} These accessory signals have also been shown to be mediated by a class of molecules known as cytokines.\textsuperscript{18-20} Because we showed in our previous study\textsuperscript{6} that UVB-irradiated spleen cells (SC) do not stimulate allogeneic T cells in an MLR, despite continued expression of Ia antigens,\textsuperscript{10} we hypothesized that the alteration or defect in the immune response caused by UVB irradiation may be attributable to the inability of the metabolically disturbed APC to induce the secretion of cytokines, the costimulatory signals that are necessary for proper T-cell activation. To better understand the mechanisms of the failure of antigen presentation in this model of UVB immunomodulation, this study further examined the functional and molecular characterization of the accessory molecules required for the restoration of T-cell activation by UVB-irradiated BMC in MLR.

MATERIALS AND METHODS

Animals. Lewis (RT-\textsuperscript{1}), Wistar Furth (WF, RT-\textsuperscript{1}), and ACI (RT-\textsuperscript{1}) rats weighing 200 to 250 g were purchased from Harlan Sprague Dawley, Inc, Indianapolis, IN. Lethally irradiated Lewis rats were recipients of UVB-irradiated BMC and SC obtained from WF rats.

BMT. BMT was performed as we have previously described.\textsuperscript{14} Briefly, Lewis recipients were lethally gamma irradiated (10.5 Gy, \textsuperscript{173}Cs source at 1.1 Gy/min) and reconstituted within 16 to 20 hours with a UVB-irradiated admixture of BMC (10\textsuperscript{5} and SC (5 × 10\textsuperscript{5}). The BMC were obtained from the tibiae, femora, and humera and SC from the spleens of WF donors. Red blood cells in the BMC and SC were lysed with buffered tris-ammonium chloride (0.83%, pH 7.21), the cells were washed two times with RPMI 1640 (GIBCO Laboratories, Grand Island, NY) and then resuspended in phosphate-buffered saline (PBS) at a concentration of 10\textsuperscript{6} cells/mL. The cells were further diluted in PBS at a ratio of 1:50, placed into 250 mL petri dishes containing a magnetic stirring bar, and were UVB irradiated for 70 seconds at a rate of 10 J/m\textsuperscript{2}/sec (total dose of 700 J/m\textsuperscript{2}). The open petri dishes were placed at 10 cm distance from two Westinghouse FS-20 sunlamps emitting a flux of 1 mW/cm\textsuperscript{2} at 310 nmol/L as determined with a UV-X radiometer (Ultraviolet Products, San Gabriel, CA). The cells were washed two times in RPMI 1640 supplemented with 10% fetal calf serum (FCS). Viability of the nucleated BMC was consistently greater than 95% as determined by Trypan blue exclusion. Subsequently, 1 × 10\textsuperscript{6} nucleated BMC and 5 × 10\textsuperscript{5} SC in 1.0 mL of RPMI were injected intravenously (IV) into lethally gamma-irradiated Lewis recipients. The animals were housed in sterile microisolator cages where they received water containing tetracycline for 2 weeks.

Assay for chimerism using antibody-mediated cytotoxicity. Lewis anti-WF (donor-specific), WF anti-Lewis (recipient-specific) and Lewis anti-ACI (negative control) hyperimmune sera with donor-specific cytotoxic titers of 1:1024, 1:512, and 1:2048, respectively, were induced by three consecutive full-thickness skin grafts at 10-day intervals as previously described.\textsuperscript{21} These sera, in the presence of complement, were used to determine the relative proportions of donor- (WF) and host-derived (Lewis) lymphocytes in the thoracic duct lymphocytes (TDL) of Lewis chimeras and naive rats (controls) by a modified \textsuperscript{51}Cr release assay. Briefly, Con-A (Sigma Chemical Co, St Louis, MO) blasts were prepared by incubating 2.5 × 10\textsuperscript{5} TDL/well with 5 µg/mL Con-A in 24 round-bottomed microwells (Glassworks, Cornings, NY) for 48 hours at 37°C in 5% CO\textsubscript{2} and humidified air. The cells were harvested and labeled with \textsuperscript{51}Cr (100 µCi/106 cells). The \textsuperscript{51}Cr-labeled, Con-A stimulated, target cells (10\textsuperscript{6}/mL) were incubated in duplicate with 100 µL of hyperimmune serum serially diluted in PBS. Absorbed guinea pig complement, 100 U/mL (Accurate Chemicals, Westbury, NY), was added to one set of duplicate dilutions. The spontaneous \textsuperscript{51}Cr release was assessed by adding guinea pig complement to target cells without hyperimmune serum while the maximum release was obtained by the addition of NP40 (Sigma Chemical Co) to the target cells. Following 1 hour of incubation at 37°C in 5% CO\textsubscript{2} and humidified air, the plates were centrifuged at 500g for 5 minutes and 100 µL of supernatant was obtained from each well for liquid scintillation counting. The percent cytotoxicity was calculated using the formula:

\[
\frac{\text{Specific Release}}{\text{Maximum Release - Spontaneous Release}} \times 100 = \frac{\text{Experimental Release - Spontaneous Release}}{\text{Specific Release}}
\]

where the spontaneous release was consistently less than 10% of the maximum release.

Monoclonal antibodies (MoAbs). Murine MoAbs were purchased from Sera Lab (Accurate Chemicals). They consisted of OX8 (CD8) MoAb against rat cytotoxic/suppressor T-cell subsets, NK cells, and some monocytes/macrophages; W3/13 (CD43) MoAbs against all rat T cells, neutrophil, NK cells, and some plasma cells; W3/25 (CD4) MoAb against rat T-helper inducer subset and monocyt/macrophages; OX6 MoAb against rat MHC class II antigen; OX18 MoAb against rat MHC class I antigen; OX19 (CD4) MoAb against rat T cells; and OX33 MoAb against rat B cells.

Preparation of T-lymphocyte subsets. WF donor SC were obtained from naive donors as previously described.\textsuperscript{11} To isolate purified T-lymphocytes, 10\textsuperscript{9} splenic leukocytes were incubated with an equal mixture of W3/33 and OX6 MoAbs for 1 hour at 4°C with continuous rotation. The cells were washed three times with RPMI 1640 containing decomplemented 10% FCS (RPMI/FCS) and further incubated with goat anti-mouse IgG magnetic beads (Dynabead M-450, Dynal Inc, Great Neck, NY) for 1 hour at 4°C. Only negatively selected T cells were washed three times and incubated with saturating concentrations of W3/25 (for CD4 depletion) or OX8 (for CD8 depletion) MoAbs for 1 hour at 4°C with continuous rotation. The cells were washed with RPMI/FCS and subsequently incubated with goat anti-mouse IgG magnetic beads for 1 hour at 4°C. The negatively selected cells were washed three times and cell viability was consistently greater than 95% as determined by trypan blue exclusion.

Preparation of B lymphocytes. Splenic leukocytes (10\textsuperscript{9}) were incubated with equal mixtures of W3/13, W3/25, and OX8 MoAbs

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for 1 hour at 4°C with continuous rotation. The cells were washed three times with RPMI/FCS and incubated with goat anti-mouse IgG magnetic beads for 1 hour at 4°C. Only cells isolated by negative selection (nonadherent fraction) were recovered and their viability was greater than 95% as determined by Trypan blue exclusion.

**Dendritic cells.** An enriched population of DC was isolated from the thoracic duct lymph of lymphadenectomized and sublethally irradiated WF rats as we previously described. Briefly, following complete mesenteric lymphadenectomy (ie, mesenteric, cecal and portal lymph nodes), the animals were allowed to recover for 6 weeks at which time they received 7 Gy of subtotal total body irradiation from a 137Cs source at a rate of 1.1 Gy/min. Thoracic duct cannulation was performed 24 hours after irradiation and lymph was collected for 36 to 40 hours in RPMI 1640 containing 5 U/mL of heparin. The cells collected were then washed twice and enriched for DC by selection (nonadherent fraction) were recovered and their viability at which time they received 7 Gy of sublethal total body irradiation from a 137Cs source at a rate of 1.1 Gy/min. Thoracic duct cannulation was performed 24 hours after irradiation and lymph was collected for 36 to 40 hours in RPMI 1640 containing 5 U/mL of heparin. The cells collected were then washed twice and enriched for DC by high-density centrifugation. The enriched population of DC was 70% to 75% class II positive (OX6) as determined by fluorescence-activated cell sorter (FACS) and displayed DC morphology. A purified population of DC was obtained from enriched DC by incubating $2 \times 10^6$/mL enriched DC with saturating concentrations of W3/13, OX8, and W3/25 (for T-cell depletion), and OX33 (for B-cell depletion) for 1 hour at 4°C with continuous rotation. The cells were then washed three times and incubated with goat antimouse IgG magnetic beads. Purity of nonadherent DC was greater than 95% as determined by FACS.

**Analysis of cell markers.** Purity of fractionated T and B lymphocytes was checked by FACS analysis as we previously described. Briefly, aliquots of cell fractions were incubated separately with MoAbs OX6, OX8, OX18, OX19, OX33, and W3/25 for 1 hour at 4°C with continuous rotation. The cells were washed and resuspended in fluorescein isothiocyanate (FITC) conjugated to goat anti-mouse IgG (Cappel, Cooper Biomedical Inc, Malvern, PA) diluted in 25% heat-inactivated normal Lewis rat serum to avoid nonspecific cross-reactivity with surface Ig on B cells. The T cells were then greater than 98% positive for T-cell marker (Ox19), greater than 98% positive for class I marker (Ox18), and less than 2% positive for class II marker (Ox6). B cells showed greater than 98% positivity for Ox33 and greater than 97% positivity for MHC class II antigen marker (Ox6). The purity of CD4+ T cells and CD8+ T cells was greater than 97% for W3/25 and OX8, respectively.

**Interleukins and interferon-γ.** Recombinant human interleukin-1α and -1β (IL-1α and -1β), recombinant murine IL-2, and recombinant human IL-6 were purchased from Genzyme (Genzyme Corp, Boston, MA) and recombinant rat interferon-γ (IFN-γ) was purchased from Amgen (Amgen Biologicals, Thousand Oaks, CA).

**Mixed lymphocyte reaction (MLR).** MLR was performed as described elsewhere. Unmodified Lewis TDL (5 x 105) were used as responders and gamma irradiated (20 Gy) or UVB irradiated (700 J/m2) Lewis and WF SC (5 x 105), BMC (5 x 106) or DC (105) were used as stimulators. In some experiments, Lewis TDL (5 x 105) or purified Lewis CD4+ T cells (2 x 105), primed with 2.5 μg/mL phytohemmagglutinin (PHA; Difeo Laboratories, Detroit, MI), were used as responders to permit evaluation of the restoring capacity of IL-1 (200 U/mL), IL-2 (100 U/mL), IL-6 (100 U/mL) and IFN-γ (250 U/mL) on the proliferative response to UVB-irradiated stimulators.

**Responder TDL or CD4+ T cells and stimulator SC or DC were cultured in 96-well tissue culture plates (Falcon No. 3047, Oxnard, CA). Culture medium consisted of RPMI 1640 supplemented with 10% vol/vol decomplemented Lewis rat serum, L-glutamine, gentamicin, and mercaptoethanol. Plates were incubated at 37°C in 5% CO2 and 95% air for 4 days and then treated with an 18 hour 3H-Thymidine pulse before cell harvesting and scintillation counting.

**Assessment of GVHD.** The animals were weighed daily for 2 weeks and, thereafter, twice weekly. They were examined for clinical signs of GVHD. Clinical features of GVHD included erythema of the ears, snout, and paws; alopecia and diffuse dermatitis; hunched posture; diarrhea and weight loss. GVHD was scored as follows: grade I (mild, chronic) featured mild erythema of the ears, snout, and paws with no apparent weight loss; grade II (moderate) displayed mild erythema of the ears, snout, and paws, dermatitis, hair loss, diarrhea, and weight loss; and grade III (severe) included symptoms of severe erythema of the ears, snout, and paws, alopecia, profuse diarrhea, and marked weight loss before death.

**RESULTS**

**Survival and incidence of GVHD in recipients of UVB-irradiated BMC.** A dose of 700 J/m2 UVB was used in this study because previous reports from our laboratory indicate that this is the optimal dose of UVB to prevent GVHD without compromising BM engraftment in the rodent model. All lethally irradiated control animals (n = 10) that did not receive BMT died between 13 and 16 days. Nineteen of 20 (95%) lethally irradiated animals that received unmodified (non-UVB irradiated) admixture of BMC (5 x 106) and SC (5 x 106) developed acute GVHD and died (Table 1, group I). There was only one long-term survival (>100 days) in this group. In contrast, 27 of 30 (90%) lethally irradiated Lewis recipients that received UVB irradiated admixture of donor BMC and SC remained stable without any evidence of acute GVHD (Table 1, group II). The animals were fully hematologically reconstituted within 3 to 4 weeks following UVB-irradiated BMT as shown by the return of their white blood cell counts and hemoglobin to normal values similar to those observed in naive control rats.

**Assessment of chimerism after BMT.** Using a complement-dependent cytotoxicity assay, TDL obtained from lethally irradiated Lewis recipients of UVB-irradiated admixture of WF BMC and SC showed 96% ± 5% and 94% ± 7% donor type (WF) lymphocytes at 50 and 150 days after BMT, respectively. Recently, we have confirmed these results by using FACS analysis showing that lymph node lymphocytes obtained from Lewis chimeras that received UVB irradiated admixture of ACI, BMC, and SC expressed 98% MHC class I donor (ACI) antigen (with MoAb MN4 obtained from Sertoc) on days 90 and 180 after BMT, respectively. All lethally

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**Table 1. Survival and GVHD Incidence After UVB BMT (WF→Lewis)**

<table>
<thead>
<tr>
<th>Group</th>
<th>BMT</th>
<th>GVHD</th>
<th>MST ± SD</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admixture of naive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(BMC + SC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>13</td>
<td>32 ± 5.1</td>
<td>66</td>
</tr>
<tr>
<td>Moderate</td>
<td>6</td>
<td>48 ± 9.6</td>
<td>30</td>
</tr>
<tr>
<td>Chronic</td>
<td>1</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admixture of UVB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(BMC + SC)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No engraftment</td>
<td>27</td>
<td>360</td>
<td>90</td>
</tr>
<tr>
<td>Chronic</td>
<td>1</td>
<td>135</td>
<td>3.3</td>
</tr>
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</table>

Lethally gamma-irradiated (10.5 Gy) Lewis rats received an unmodified admixture of WF BMC (1 x 106) and SC (5 x 106) 16 hours after gamma irradiation in group I. Group II animals received UV-B (700 J/m2) admixture of WF BMC (1 x 106) and SC (5 x 106) 16 to 20 hours after lethal gamma irradiation (10.5 Gy).
irradiated recipients of UVB-irradiated BMC were complete donor-type chimeras.

Kinetic of allostimulatory capacity of BMC in MLR. Figure 1 shows that gamma-irradiated BMC have the ability to stimulate allogeneic T cells in MLR, although their allostimulatory capacity is not as potent as that of gamma-irradiated TDL. UVB irradiation totally abolished the allostimulatory capacity of BMC in a one-way MLR; thus suggesting that UVB irradiated stimulator cells do not lead to T-cell activation.

Effect of addition of mononuclear cells on MLR-response to UVB-BMC stimulators. To better understand the underlying mechanism of the state of anergy induced by UVB irradiated BMC in MLR, we began a systematic analysis of the various components of the BMC stimulators that might restore the stimulatory capacity of UVB-BMC. The addition of gamma-irradiated purified T cells, T-cell subsets, or B cells failed to restore the allostimulatory capacity of UVB-BMC stimulators (Fig 1). This finding suggests that these cells do not play an important role in recipient sensitization that may lead to BM rejection.

Restoration of allostimulatory capacity of UVB-BMC by antigen presenting cells (APC). We next examined if the addition of a small number of accessory cells, which by themselves fail to induce alloreactivity in MLR, could restore the APC function of UVB-BMC. Addition of a small number of unfraccionated splenic accessory cells, which alone were relatively ineffective in stimulating allogeneic T-cell proliferation, restored the allostimulatory capacity of UVB-BMC (Fig 2A). The addition of a small number of DC, which by themselves were relatively ineffective in stimulating T-cell proliferation, completely restored the allostimulatory capacity of UVB-BMC (Fig 2B), thus implicating APC within the BM inoculum in T-cell activation.

Reconstitution by ILs of allogeneic T cells proliferative response to UVB-BMC. Although the state of anergy induced by UVB-BMC can be restored by the addition of stimulator type APC, there remained the possibility that failure of production of essential accessory (costimulatory) molecules caused the anergic state. Therefore, we examined the capacity of purified responder CD4+ T cells to respond to UVB-irradiated BMC in the presence of ILs because T-cell activation and proliferation requires antigen recognition, T-cell receptor (TCR) occupancy, and ILs. It was determined that a complete deletion of endogenous accessory cells is essential in this reconstitution experiment because endogenous APC can induce syngeneic T-cell proliferation in the presence of interleukins. Absence of endogenous accessory cells in the purified CD4+ T cells was confirmed in a sensitive assay showing that PHA-primed (2.5 μg/mL) CD4+ T cells had similar response as nonprimed CD4+ T cells. Similarly, addition of IL-1, IL-2, IL-6, or IFN-γ to phytohemagglutinin (PHA)-primed CD4+ T cells failed to induce proliferation (Fig 3). However, the addition of IL-2 and IL-6, respectively, to coculture of PHA-primed allogeneic CD4+ T cells and UVB-BMC stimulators, restored the proliferative response to 120% and 106%, when compared with the response of primed CD4+ T cells to gamma-irradiated BMC. In contrast, addition of IL-1 and IFN-γ was relatively ineffective in restoring the allostimulatory capacity of UVB-BMC. Therefore, these results suggest that the state of anergy induced by UVB lies in the inability of accessory cells to mediate the secretion/production of IL-2 and IL-6 molecules, which are essential cosignals in T-cell activation.

Effect of reconstitution of lethally irradiated recipients with UVB-BMC and unmodified T cells. Lethally irradiated Lewis recipients reconstituted with a UVB irradiated admixture of WF BMC (10^6) and T cells (5 × 10^6) (Table 2, group II) developed stable, long lasting (>200 days), complete allogeneic chimerism (96 ± 5% donor T cells in TDL). In contrast, lethally irradiated recipients of UVB-irradiated BMC and unmodified T cells developed severe acute GVHD and 90% died at a mean survival time (MST) ± SD of 29 ± 3.0 days; only 1 of 10 animals survived indefinitely (>150 days) (Table 2, group III). This finding confirms that the presence of mature T cells in the BM inoculum is instrumental in GVHD induction.

Fig 1. Failure of addition of T cells, T-cell subsets, and B cells to restore the allostimulatory capacity of UVB-BMC in primary MLR. 2 × 10^6 responder Lewis CD4+ T cells were cocultured with UVB-irradiated (700 J/m²) WF BMC (5 × 10^6) stimulators with the addition of gamma irradiated (20 Gy) 5 × 10^6 T cells, CD4+ T cells, CD8+ T cells, and B cells, respectively. After 96 hours of incubation, 1.0 x 10^5 3H-thymidine was added to the culture and the cells were harvested 18 hours later for scintillation counting. The results are expressed as mean cpm ± SE of triplicate samples.
Effect of reconstitution of lethally irradiated recipients with UVB-BMC and unmodified DC. Lethally irradiated Lewis recipients of admixture of UVB irradiated WF BMC (10^9) and DC (10^9) developed long-lasting stable chimerism (Fig 4). In contrast, addition of unmodified donor DC to UVB-BMC led to failure of BM engraftment as shown by persistent anemia and low white blood cell counts observed in these animals before their death between 16 and 35 days (MST 22.4 ± 6.4 days) after transplantation. None of the animals showed clinical evidence of GVHD. This finding strongly suggests that presence of APC in the BM inoculum can result in failure of BM engraftment. These results complement the in vitro MLR studies showing that addition of donor-type APC restores the immunogenicity of UVB-BMC.

DISCUSSION

The risk of GVHD, which may be associated with a fatal outcome, has limited the therapeutic use of BMT. To date, TCD of BM inoculum remains the most effective way of preventing GVHD in clinical practice. The finding that TCD of BM inoculum prevents GVHD while increasing the incidence of engraftment failure suggests that low-grade GVHD may be beneficial in facilitating BM engraftment. Although the underlying mechanisms of BM engraftment failure are not clearly defined, available data suggests that mature T cells in the BM inoculum promote hematopoietic and immunologic reconstitution with sustained BM engraftment by virtue of their veto activity, by eliminating or modulating residual host T cells, and by the production of hematopoietic growth factors (IL-3, GM CSF). Because the presence of T cells in BM inoculum that promotes sustained BM engraftment induces GVHD, further studies are required to define the temporal relationship between the T-cell subsets that are involved in the induction and maintenance of tolerance to donor BMT and those that cause GVHD.
KINETICS OF ENGRAFTMENT OF UVB-IRRADIATED BM

Fig 3. Reconstitution by ILs of PHA-primed (2.5 μg/ml) Lewis CD4+ T-cells proliferative response to UVB-irradiated WF BMC. Primed responder Lewis CD4+ T cells (2 × 10⁶) were cocultured with UVB-irradiated (700 J/m²) WF stimulator BMC with the addition of IL-1 (20 U/mL), IL-2 (100 U/mL), IL-6 (100 U/mL), or IFN-γ (250 U/mL) at the initiation of culture. ³H-thymidine (1 μCi) was added to the culture at the end of 96 hours and harvested 18 hours later. The results are expressed as a mean cpm ± SE of triplicate counts.

Earlier studies, with the exception of a recent report by Dreger and Muller-Ruchholtz,¹⁷ have continued to focus on the role of donor T cells in the rejection of T-cell depleted BMT and have virtually ignored the presence of accessory cells in the BM inoculum despite growing evidence that presentation of foreign MHC molecules by donor APC to host T cells is an essential step in the host immune response during graft rejection.¹¹*¹² Thus, to define the underlying mechanism of action of ex vivo UVB irradiation of BMC that results in the development of stable allogeneic chimerism without the danger of acute GVHD, we examined the effects of reconstituting UVB-BMC with either T cells or DC. Our in vitro MLR studies suggest that resting T cells, B cells, and T-cell subsets do not play an important role in recipient sensitization. In contrast, the allostimulatory capacity of UVB-BMC was restored by the addition of only a small number of accessory cells, which by themselves were relatively ineffective in stimulating T-cell proliferation. This suggests that the accessory cells in BM inoculum play a critical role in graft immunogenicity, recipient sensitization, and graft rejection. In addition, the in vivo findings confirm that the addition of naive DC to UVB-BMC leads to rejection. These findings parallel the observation in the canine BMT model where animals that were pretreated with admixture of UVB donor-specific blood transfusions and unmodified donor DC rejected their BM grafts.³⁰ Thus, it appears that UVB modulation of APC in BM inoculum reduces or modulates graft immunogenicity and promotes sustained BM engraftment. The finding that animals that are reconstituted with UVB-irradiated admixture of BMC and SC develop complete allogeneic chimerism offers the explanation for the sustained BM engraftment in such animals. The donor-type DC generated from the graft see the BM-derived T cells as self and cannot mount an immune response to them.

Our data also confirm that UVB-irradiated admixture of BMC and SC, BMC and T cells, and BMC and DC, respectively, before transplantation into lethally irradiated allogeneic

Table 2. Incidence of GVHD After Reconstituting UVB-BMC With Naive Donor T Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>BMT</th>
<th>GVHD</th>
<th>MST ± SD</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(d)</td>
</tr>
<tr>
<td>I UVB-BMC</td>
<td>None</td>
<td>20</td>
<td>&gt;360</td>
</tr>
<tr>
<td>II UVB (BMC + T cells)</td>
<td>None</td>
<td>10</td>
<td>&gt;200</td>
</tr>
<tr>
<td>III UVB-BMC + naive T cells</td>
<td>Severe</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>52 ± 5</td>
</tr>
<tr>
<td></td>
<td>Chronic</td>
<td>1</td>
<td>&gt;150</td>
</tr>
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</table>

Lethally gamma-irradiated (10.5 Gy) Lewis rats received UVB (700 J/m²) WF BMC (1 × 10⁶) 16 to 20 hours after gamma irradiation in Group I. Group II animals received UVB (700 J/m²) admixture of WF BMC (1 × 10⁶) and T cells (5 × 10⁶) 16 to 20 hours after lethal gamma irradiation (10.5 Gy). Group III animals received UVB (700 J/m²) WF BMC reconstituted with 5 × 10⁶ unmodified (naive) syngeneic T cells.

Fig 4. Effect on BM engraftment by the addition of donor DC to UVB-irradiated BMC. Lethally gamma-irradiated (10.5 Gy) Lewis rats received either UVB-irradiated (700 J/m²) admixture of WF BMC (10⁶) and DC (10⁵) 16 to 20 hours after irradiation (○—○), or an admixture of UVB-irradiated (700 J/m²) BMC (10⁶) and naive donor-type DC (10⁵) 16 to 20 hours after irradiation (□—□).
rats results in stable complete allogeneic chimera without the risk of acute GVHD. This finding is in agreement with the earlier reports in the rat UVB-BMT model\textsuperscript{12,14} and the most recent reports\textsuperscript{17,21} in the murine BMT model of parental to F1 hybrid. To clarify the role of donor T cells in the UVB-BMT model, we added syngeneic T cells to UVB-BMC before transplantation into disparate allogeneic rats. The results show that the recipients developed severe GVHD with fatal outcome, thus further confirming that mature T cells in the BM inoculum cause acute GVHD. This finding strongly suggests that UVB modulation of T cells in BM inoculum, although similar in principle to the in vitro depletion of T cells from BMC, offers a more promising approach to the prevention of acute GVHD without endangering BM engraftment.

Effects of UVB irradiation on donor-host interactions by modulation of donor-graft immunogenicity and by the downregulation of host immune responses have recently been reviewed.\textsuperscript{22,33} The state of hyporesponsiveness or anergy induced by UVB irradiation is confirmed by the finding that UVB-irradiated BMC do not stimulate allogeneic T cells. In contrast, gamma-irradiated BMC are effective stimulators of T cells. The in vitro state of unresponsiveness induced by UVB is dependent, in part, on the failure of production of essential costimulatory molecules (IL-2 and IL-6). The reconstitution of allostimulatory capacity of UVB-BMC by IL-2 and IL-6 suggests that the primary defect caused by UVB lies in the inability of accessory cells to mediate the secretion/production of IL-2 and IL-6 molecules by T cells. Of interest is our result showing that IL-1 did not significantly alter the MLR response of primed CD4\(^+\) T cells to UVB-irradiated allogeneic BMC, whereas the addition of IL-2 or IL-6 restored the proliferation of primed CD4\(^+\) T cells. This finding suggests that IL-1 has a limited role as an accessory cosignal for primed allo-stimulated CD4\(^+\) T cells and agrees with other studies showing that exogenous IL-2 is more effective than IL-1 in restoring T-cell activation by UVB irradiated stimulator cells.\textsuperscript{34,35} This observation suggests that UVB-induced defect in certain, as yet unidentified, accessory signals required for T-cell activation aborts synthesis of IL-2 and other lymphokines. We speculate that these undefined accessory signals modulated by UVB may be adhesion molecules. This hypothesis receives strong support from our previous data showing that UVB irradiation abolishes the homing of T cells to high endothelial venule (HEV)-containing lymph nodes and prevents in vitro T-cell binding to frozen sections of cervical lymph nodes.\textsuperscript{40} Thus, these findings suggest that UVB modulates the expression or function of T-cell surface adhesion molecules (LFA-1 and VLA-4), which are necessary for HEV recognition and binding to their ligands (ICAM-1, ICAM-2, and VCAM-1) present on the surface of endothelial cells.\textsuperscript{36} Recent reports that UVB modulates ICAM-1 expression on murine Langerhans cells\textsuperscript{37} and on human monocytes\textsuperscript{38} complement our data on migration of UVB-irradiated cells.\textsuperscript{15,16}

It appears that the possible mechanisms of UVB modulation of BMT that lead to the induction of complete allogeneic chimera, with sustained engraftment and without any evidence of acute GVHD, involves modulation of mature T cells in the BM inoculum which, in turn, prevents allo-reactivity, migration of T cells to BM- and HEV-containing compartments in addition to changes in cellular interactions. On the other hand, UVB modulation of accessory cells in the BM inoculum prevents allosensitization, migration to BM compartments, and BM engraftment failure. Of interest is the observation that the result of UVB-BMT in the WF to Lewis rat strain combination is reproducible in the ACI to Lewis rat combination\textsuperscript{22,13} and in the murine parental to F1 hybrid\textsuperscript{17,29}, although the murine BMC appear to be more sensitive to UVB irradiation damage than rat BMC. It is apparent that further investigation into the functional and molecular alterations inherent in this model is required before applying this model to BMT in larger animals and man. We speculate that prevention of GVHD by UVB irradiation of BMC and the demonstration of hyporesponsiveness of T cells obtained from the chimeric rats to donor-type and recipient-type stimulator cells in MLR\textsuperscript{14} may limit the application of this new strategy to treatment of leukemic patients. UVB-irradiated BMC may prevent graft-versus-leukemic-effect and, therefore, predispose to relapse of leukemia. We hope that further studies will address this question. In conclusion, our data suggest that UVB modulation of BMT is an effective strategy in the successful induction of tolerance to BMC and donor organ allografts that might be applicable to the treatment of certain hematologic disorders and neoplastic diseases, and eventually to organ transplantation.

REFERENCES

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