Treatment of whole blood with riboflavin plus ultraviolet light, an alternative to gamma irradiation in the prevention of transfusion-associated graft-versus-host disease?

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**BACKGROUND:** Exposure of blood products to gamma irradiation is currently the standard of care in the prevention of transfusion-associated graft-versus-host disease (TA-GVHD). Regulatory, technical, and clinical challenges associated with the use of gamma irradiators are driving efforts to develop alternatives. Pathogen reduction methods were initially developed to reduce the risk of microbial transmission by blood components. Through modifications of nucleic acids, these technologies interfere with the replication of both pathogens and white blood cells (WBCs). To date, systems for pathogen and WBC inactivation of products containing red blood cells are less well established than those for platelets and plasma.

**STUDY DESIGN AND METHODS:** In this study, the in vitro and in vivo function of WBCs present in whole blood after exposure to riboflavin plus ultraviolet light (Rb-UV) was examined and compared to responses of WBCs obtained from untreated or gamma-irradiated blood by measuring proliferation, cytokine production, activation, and antigen presentation and xenogeneic (X-)GVHD responses in an in vivo mouse model.

**RESULTS:** In vitro studies demonstrated that treatment of whole blood with Rb-UV was as effective as gamma irradiation in preventing WBC proliferation, but was more effective in preventing antigen presentation, cytokine production, and T-cell activation. Consistent with in vitro findings, treatment with Rb-UV was as effective as gamma irradiation in preventing X-GVHD, a mouse model for TA-GVHD.

**CONCLUSION:** The ability to effectively inactivate WBCs in fresh whole blood using Rb-UV, prior to separation into components, provides the transfusion medicine community with a potential alternative to gamma irradiation.

Functional white blood cells (WBCs) in blood components may be responsible for a number of adverse transfusion effects, including transfusion-associated graft-versus-host disease (TA-GVHD), alloimmunization, and alloimmune platelet (PLT) refractoriness. TA-GVHD occurs when functional WBCs are transfused into a patient who is unable to mount an immune response to the allogeneic donor cells due to human leukocyte antigen (HLA) compatibility or to immunosuppression. In contrast, in immunocompetent patients the generation of alloantibodies against HLA antigens on donor WBCs and PLTs is the major cause of refractoriness to PLT transfusions in patients receiving repeated blood transfusions. The induction of immunosuppressive-like reactions termed “transfusion-related immune modulation” or TRIM1 and the ability...
of donor cells to persist in the circulation of recipients despite leukoreduction (microchimerism) is just beginning to be explored in greater detail. Attempts to reduce these undesirable effects have included exposure of the blood products to gamma irradiation and the use of leukoreduction filters.

Studies have shown that exposure of PLT concentrates or plasma to riboflavin plus ultraviolet light (Rb-UV) causes irreversible modifications of nucleic acids that result in inactivation of a wide range of pathogens as well as inhibition of the immunologic responses mediated by WBCs present in the PLT concentrates. This system was further developed for the treatment of whole blood, providing a single pathogen reduction and WBC inactivation step, followed by the use of the product as whole blood or subsequent separation into components. The treatment of red blood cells (RBCs) or whole blood has been challenging due to the absorption of light by hemoglobin and therefore the UV light energy dose delivered to units of whole blood is normalized for RBC volume (J/mL RBC). As part of developing treatment of whole blood with Rb-UV, in vitro variables that may predict 24-hour survival of RBCs in vivo were established in a clinical evaluation. Whole blood units were exposed to 22, 33, and 44 J/mL RBC UV light in the presence of riboflavin, and the component quality and in vivo performance were assessed. This study showed that RBC quality variables, such as hemolysis and ATP concentration, may be predictive of their 24-hour recovery and T50 survival. These variables were used to modify the system including energy dose for treatment and storage duration. The current configuration exposes units of blood to 80 J/mL RBC UV light in the presence of riboflavin; this treatment energy was chosen to balance blood component quality and pathogen reduction.

The efficacy of this final configuration has been evaluated with tests of parasitic, viral, and bacterial reduction. Rb-UV treatment inactivates parasites associated with transfusion-transmitted diseases, reduces enveloped and nonenveloped viruses by approximately 3 to 4 log, and eliminates clinically relevant levels of contaminating bacteria. Based on the correlations of in vitro variables with clinical outcomes, RBC quality is expected to meet Food and Drug Administration (FDA) recovery requirements for licensure of new transfusion products. In addition, all toxicology studies performed to date have shown no adverse toxicologic effects with these treatments.

The objective of this study was to assess in vitro and in vivo WBC functionality after treatment of whole blood with Rb-UV in comparison to gamma irradiation. We also present WBC inactivation data from the clinical evaluation performed at lower UV energy doses to demonstrate the robustness of the system in inactivating WBCs.

MATERIALS AND METHODS

Whole blood collection and treatment

Initial samples treated at UV energy doses of 22, 33, or 44 J/mL RBC in the presence of riboflavin were obtained from the clinical evaluation performed at Hoxworth Blood Center in Cincinnati. The study was conducted with the approval of the University of Cincinnati institutional review board, in compliance with the Department of Defense’s requirements for human subjects research protocols and under the authorization of the FDA. Twelve healthy volunteers donated whole blood units that were assigned sequentially to undergo illumination at different energies. Treatment involved mixing 470 mL of whole blood with 35 mL riboflavin solution in the illumination bag, which was exposed to the desired dose of UV light in the Mirasol Illuminator (Mirasol System for Whole Blood, CaridianBCT, Lakewood, CO). The illumination bag (integratedly connected to a storage bag) is placed in the illuminator for exposure to UV-light (UV-A and UV-B, with a peak at 313 nm). The energy dose delivered is normalized for the volume of RBCs present in the bag. Values for hematocrit (Hct) of the illuminated mixtures were used to adjust the energy delivery to account for the volume of RBCs present. Whole blood samples were removed before and after treatment, and samples were shipped at room temperature to Rhode Island Hospital for peripheral blood mononuclear cell (PBMCs) separation and assessment.

For the in vitro and in vivo studies, performed with the final configuration using 80 mL/mL RBC, units of fresh human whole blood were purchased from Research Blood Components (Brighton, MA). The study was conducted with the approval of the Lifespan institutional review boards and in compliance with the Department of Defense’s requirements for animal research protocols. For in vitro studies, two aliquots of 50 mL blood were removed from the whole blood upon receipt. One aliquot was left untreated and the other aliquot received 25 Gy of gamma irradiation. The remaining blood (>400 mL) was treated within 6 hours of collection at 80 J/mL RBC, as described. Immediately after each treatment, PBMCs were isolated from the treated or untreated samples as previously described.

For the in vivo animal studies, at least 200 mL of fresh human whole blood was treated with Rb-UV and the remaining blood was split and left either untreated or subjected to gamma irradiation (25 Gy) within 6 hours of collection. The treatment of the 200-mL volume was performed at a condition that is equivalent to that used for full-size units. After treatment was completed, PBMCs were isolated from the entire volumes of blood.
In vitro characterization of PBMNC responses

Immuno phenotype
PBMNCs were stained with a panel of antibodies (iMK panel, BD Biosciences, San Jose, CA) to define various subpopulations and samples were analyzed using a flow cytometer (FACScan, BD Biosciences).

Proliferation
The ability of untreated or treated PBMNCs to proliferate in response to the mitogen phytohemagglutinin (PHA), to plate-bound anti-CD3 plus anti-CD28 (anti-T-cell receptor [TCR]) antibodies, or to allogeneic stimulator cells in mixed WBC culture (MLC assay) was conducted as previously described. In addition, the ability of untreated or treated cells to stimulate allogeneic responder PBMNCs was tested using the MLC assay. Proliferation was measured by addition of [3H]thymidine for 4 hours on Day 3 for the PHA and anti-TCR–stimulated cells or on Day 5 for the cells stimulated with allogeneic stimulator cells.

Activation
The ability of PBMNCs to be activated was measured by incubating cells in phorbol myristate acetate (PMA) or phosphate-buffered saline (PBS) for 4 hours at 37°C as previously described. Cells were then stained with anti-CD3, anti-CD8, and anti-CD69 to measure the up regulation of CD69 surface expression on T cells. The flow cytometric analysis was conducted by gating on CD3+ cells.

Cytokine production
To measure the ability of the untreated or treated PBMNCs to secrete cytokines, cells from the different treatment groups were stimulated with lipopolysaccharide (LPS, 100 ng/mL) for 24 hours or with bound anti-CD3 plus anti-CD28 for 72 hours. Supernatants were collected and stored at −20°C until assayed. Levels of inflammatory cytokines (interleukin [IL]-1β, IL-6, tumor necrosis factor [TNF]-α, IL-10, IL-8, and IL-12) produced in response to LPS or Th1/Th2 cytokines (interferon [IFN]-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10) produced in response to anti-CD3 and -CD28 were assayed using CBA assay kits (BD Biosciences) per manufacturer’s directions.

Limiting dilution assays
Limiting dilution assays were conducted to quantitate the effectiveness of the treatments on the ability of T cells to respond. To do this, varying numbers of untreated or treated PBMNCs were added to wells containing allogeneic CD3-depleted irradiated feeder cells and stimulatory factors including recombinant human IL-2 and the mitogen, PHA, in the medium and quantifying the presence of clones on Days 14 and 21. Allogeneic feeder cells were prepared by obtaining PBMNCs from one or two normal volunteers by Ficoll-Hypaque discontinuous centrifugation. The CD3+ cells were depleted from the PBMNCs using CD3 immunomagnetic particles per manufacturer’s instructions (Miltenyi Biotec, Auburn, CA). The CD3– cells then received 50 Gy of gamma irradiation. Feeder cells prepared from several different individuals were mixed in equal numbers. Purity of cells was always greater than 93%. Each well received $\times 10^5$ feeder cells and dilutions of the responder cells: untreated cells (40, 20, 10, 5, 2.5, 1.25, and 0.625 cells/well) or gamma-irradiated or Rb-UV–treated cells (1 × 10^5, 3.3 × 10^4, 1.1 × 10^4, 3700, 1200, 410, 140, and 46 cells/well). Twelve wells were used for each cell dilution and 12 control wells containing only feeder cells were set up. The medium used to set up the assay contained 16% fetal calf serum (FCS; Atlanta Biologicals, Atlanta, GA), 8 μg/mL PHA-M (Sigma, St Louis, MO), 50 units/mL recombinant human IL-2 (Zeptometrix, Buffalo, NY), and 10% T-cell growth factor (Zeptometrix). On Days 3, 7, and 14 each well was fed with 25 μL of feed medium containing 45% FCS, 45% T-cell growth factor, 500 U/mL recombinant human IL-2, 80 μg/mL PHA-M. To prevent evaporation, all plates were taped with CO2–permeable, water-impermeable tape (3M #483 polyethylene film tape, R.S. Hughes, Solon, OH) and incubated at 37°C, 10% CO_2_. A positive well was defined as one with at least one clone of cells when plates were scored on Day 14 and Day 21. The frequency (f) of responding T cells was calculated by minimum chi-square analysis based on Poisson distribution. For the Poisson distribution one can determine that 37% of the wells are on average negative when one specific WBC was plated per well at the beginning of the culture. The log T-cell reduction can be calculated as log(fcontrol/ftreated), where fcontrol is the T-cell frequency of control cells and ftreated is the T-cell frequency of the Mirasol-treated or gamma-irradiated cells.

Xenogeneic GVHD model
The in vivo function of PBMNCs was tested by measuring the xenogeneic (X-)GVHD response in nonobese diabetic-severe combined immunodeficient recipient mice lacking expression of the IL-2 receptor gamma chain gene (NOD-scid IL2rnull mice, The Jackson Laboratory, Bar Harbor, ME) after injection of treated or untreated human PBMNCs. Six- to 7-week-old female mice were exposed to 200 cGy of gamma irradiation 1 day before injection. Three groups of mice (10 mice/group) received equal numbers of PBMNCs (10 × 10^6-20 × 10^6) from any given donor (n = 6): untreated, 25 Gy gamma irradiated, or treated with Rb-UV. Three control mice, not injected with any PBMNCs, were also evaluated. Mice were observed and weighed a minimum of three times per week and were euthanized when the weight loss was above 15% and/or mice looked hunched with ruffled fur. All remaining mice were euthanized 2 weeks after 70% of the mice injected with untreated PBMNCs developed X-GVHD.
Blood, spleen, and marrow were collected from the mice at the time of euthanization. Blood was collected into heparinized syringes, transferred to a glass Wintrobe tube, and centrifuged for 15 minutes at 1000 \( \times \) g. The Hct was recorded and the plasma was collected and stored at \(-20^\circ C\) until assayed. The levels of human cytokines and immunoglobulins present in the plasma obtained from these mice were assayed using CBA reagents (BD Biosciences) per manufacturer’s instructions. Theuffy coat was added to 2 mL of RBC lysis solution (Invitrogen, Grand Island, NY), incubated for 15 minutes at room temperature, and centrifuged and the cell pellet was resuspended in HuFACS buffer (PBS containing 1% human serum albumin, 0.2 mg/mL human IgG [Polygam, Baxter Healthcare, Deerfield, IL], and 0.1% sodium azide). The spleen was dissociated, the single-cell suspension was centrifuged, and the pellet was resuspended in HuFACS buffer. The marrow cells were centrifuged and resuspended in a similar fashion. After being counted, cells from the blood, spleen, and marrow were stained with the IMK panel of antibodies as described. Samples from recipient mice that had received treated donor cells were assayed using CBA reagents (BD Biosciences) per manufacturer’s instructions. The Buffy coat was added to 2 mL of RBC lysis solution (Invitrogen, Grand Island, NY), incubated for 15 minutes at room temperature, and centrifuged and the cell pellet was resuspended in HuFACS buffer. The marrow cells were centrifuged and resuspended in a similar fashion. After being counted, cells from the blood, spleen, and marrow were stained with the IMK panel of antibodies as described. Samples from recipient mice that had received treated donor cells were assayed with an antibody to human CD45 (BD Biosciences) per manufacturer’s instructions. The Buffy coat was added to 2 mL of RBC lysis solution (Invitrogen, Grand Island, NY), incubated for 15 minutes at room temperature, and centrifuged and the cell pellet was resuspended in HuFACS buffer. The marrow cells were centrifuged and resuspended in a similar fashion. After being counted, cells from the blood, spleen, and marrow were stained with the IMK panel of antibodies as described. Samples from recipient mice that had received treated donor cells were assayed with an antibody to human CD45 (BD Biosciences). Since no human CD45-recipient mice that had received treated donor cells were assayed using CBA reagents (BD Biosciences) per manufacturer’s instructions. The Buffy coat was added to 2 mL of RBC lysis solution (Invitrogen, Grand Island, NY), incubated for 15 minutes at room temperature, and centrifuged and the cell pellet was resuspended in HuFACS buffer. The marrow cells were centrifuged and resuspended in a similar fashion. After being counted, cells from the blood, spleen, and marrow were stained with the IMK panel of antibodies as described. Samples from recipient mice that had received treated donor cells were assayed with an antibody to human CD45 (BD Biosciences). Since no human CD45+ cells were detected in these animals, no further staining with the IMK panel was performed. Samples were analyzed using a flow cytometer (FACScan, BD Biosciences).

Statistical analysis

Results are expressed as the mean \( \pm \) standard deviation (SD) unless otherwise indicated. Statistical comparison of treated and control samples was carried out using a paired t test, nonparametric Mann-Whitney test, or one-way analysis of variance using computer software (Prism 5, GraphPad Software, Inc., La Jolla, CA).

RESULTS

In vitro results

T-cell functionality was assessed in proliferation assays using stimulation by the mitogen PHA, anti-TCR, and in a mixed WBC culture containing allogeneic stimulator and responder cells. As shown in Fig. 1, Rb-UV treatment and gamma irradiation inhibited WBC proliferation in response to all of the tested stimuli (Figs. 1A-1C). The level of proliferation detected after Rb-UV treatment was at or below background levels. Gamma-irradiated lymphocytes did not show any signs of cell division in response to anti-TCR as assessed by light microscopy, but exhibited a residual amount of \([\text{H}]\)thymidine incorporation, suggesting that some DNA synthesis is still taking place in gamma-irradiated cells. Rb-UV treatment, but not gamma irradiation, inhibited stimulation of allogeneic responder cell (allostimulation, Fig. 1D), indicating that WBCs treated with Rb-UV lose their ability to act as antigen-presenting cells.

The limiting dilution assay provided a quantitative measure of the inhibitory capabilities of these two treatments. Whereas 1 in 5.55 untreated cells were able to proliferate in this assay, only 1 in 300,000 Rb-UV–treated or gamma-irradiated cells proliferated. Both, Rb-UV treatment and gamma irradiation were equally effective at preventing proliferation of lymphocytes, resulting in a 4.7-log reduction of viable T cells.

The effect of Rb-UV treatment and gamma irradiation on WBC phenotype and activation was tested. WBCs isolated from whole blood before and after treatment were stained with fluorescent antibodies against CD45, CD3, CD19, CD4, CD8, CD14, CD16, CD56, and HLA-DR. Samples were analyzed by flow cytometry, gating on the whole WBC population (ungated) or the lymphocyte population (B and T cells). Analysis of the un gated WBC population or gated lymphocyte population showed no changes in the surface marker expression profile after Rb-UV treatment or gamma irradiation (data not shown). The effect on lymphocyte activation was measured by quantifying the up regulation of CD69 expression after PMA stimulation. Rb-UV treatment inhibited induction of CD69 expression on T cells by 98% compared to untreated controls, whereas gamma irradiation only partially inhibited CD69 expression by 69%.

The production of cytokines by WBCs during storage of blood products can impact immune responses in recipients. The ability of treated cells to secrete cytokines, without a stimulus and in response to the stimulus LPS or to bound anti-TCR, was examined. Concentrations of most cytokines in unstimulated cells were below the detection limit of the assay, with the exception of IL-8. IL-8 is stored in large amounts in granules of WBCs and can be released without synthesis or further modifications. IL-8 levels in Rb-UV–treated cells were decreased compared to untreated and gamma-irradiated cells immediately after treatment (data not shown). After stimulation, levels of cytokines produced by gamma-irradiated cells were not significantly different than the amount of cytokines produced by untreated WBCs. In contrast, the production of cytokines by Rb-UV–treated cells was almost completely inhibited, with the exception of IL-8 and TNF-\( \alpha \) (Fig. 2). Background levels observed for IL-8 and TNF-\( \alpha \) represent the release of existing intracellular cytokines during storage. The results show that Rb-UV treatment impairs cytokine synthesis and results in greater than a 4-log reduction in cytokine production.

In vivo results

The in vitro studies demonstrated that while gamma irradiation was able to prevent proliferation of lymphocytes, it
was much less effective than treatment with Rb-UV in preventing lymphocyte activation, cytokine production, and the ability of cells to act as antigen-presenting cells. A model of TA-GVHD utilizing X-GVHD responses of human cells when injected into immunodeficient murine recipients (NOD-scid IL2rnull) was chosen to confirm the in vitro results. WBCs isolated from untreated, Rb-UV–treated, or gamma-irradiated (25 Gy) whole blood were injected intravenously into immunodeficient recipient mice. Mice were monitored regularly for the development of X-GVHD and were euthanized and analyzed once they became ill. Depending on the donor, mice that received $10^6$ to $20^6$ untreated cells uniformly developed X-GVHD symptoms within 9 to 12 days (Fig. 3). Remaining mice, not showing any symptoms of disease, were euthanized 2 weeks after the time when at least 70% of the mice receiving untreated cells had succumbed to GVHD (Days 24-31). Five of the 60 mice that were injected with untreated WBCs had not developed X-GVHD by the end of the study period. Three of these five mice exhibited good engraftment of human lymphocytes and appeared to be developing X-GVHD, but exhibited slower kinetics for unknown reasons. In contrast, none of the recipient mice injected with WBCs isolated from Rb-UV–treated or gamma-irradiated whole blood exhibited any engraftment of human WBCs or developed X-GVHD. Mice undergoing X-GVHD presented with decreased Hct in peripheral blood (Fig. 4). The four mice injected with untreated cells that did not show any decrease in Hct belonged to the group of mice with delayed engraftment of human WBCs.

Immunophenotypic analysis of the cells in blood, marrow, and spleen of mice that had received untreated donor cells revealed that these compartments were composed almost exclusively of human T and B lymphocytes. The majority of T lymphocytes expressed HLA-DR, indicating cellular activation. The ratio of CD4+ to CD8+ T lymphocytes was slightly above 1, indicating a bias toward

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**Fig. 1.** Effect of Rb-UV treatment using different energy levels on PHA and anti-TCR–induced proliferation (A and B) and allogeneic responder or stimulator cells in a MLC (C and D). Shown are mean values ± 1 SD. All values were corrected for the background of cells incubated in PBS, in the absence of any stimulus (untreated, 746 cpm; Rb-UV treated, 818 cpm; gamma irradiated, 352 cpm; allostimulation responder cells, 1542 cpm; allore cognition stimulator cells, 630 cpm), resulting in proliferation levels close to the detection limit of the assay for Rb-UV–treated samples. Gamma irradiation data are only shown for the experiments at 80 J/mLl asc. Significant differences compared to untreated controls (p < 0.01) or Rb-UV–treated samples (p < 0.01) are shown after applying a paired t test. (■) Untreated; (□) Rb-UV; (♂) gamma irradiated.
Interestingly, the percentage of CD3+CD19+ and CD4+CD8+ double-positive cells recovered from mice undergoing X-GVHD significantly increased compared to the amount present in the WBC preparation injected into the mice (Fig. 5). In organs of mice that had received Rb-UV–treated or gamma-irradiated donor cells, no human CD45+ lymphocytes were detected. The presence of human cytokines and human immunoglobulins in recipient mice is also a measure of human WBC engraftment. Increased production of cytokines, in particular, IFN-γ, IL-10, and sporadically IL-5 (Fig. 6), as well as increased levels of human IgG and IgM (Table 1), were detected in the plasma of recipients of untreated WBCs. In contrast, mice injected with Rb-UV–treated or gamma-irradiated WBCs did not contain any detectable human cytokines in their plasma. Some mice injected with Rb-UV–treated or gamma-irradiated WBCs displayed levels of IgG that were detectable, but at least 5 log lower than levels observed in mice that received untreated cells. This phenomenon appeared to be donor dependent and was observed for mice injected with Rb-UV–treated as well as gamma-irradiated WBCs.

**DISCUSSION**

The presence of viable WBCs in blood products can contribute to a variety of immunologic consequences of
transfusion, and currently gamma irradiation (25-30 Gy) is the accepted standard of care for transfusions in immunocompromised patients or patient populations that are particularly susceptible to TA-GVHD. The use of cesium source irradiators has become more difficult due to requirements implemented to reduce the risk of terrorist activity directed at obtaining and using cesium, and the US Nuclear Regulatory Commission has called for efforts to develop and use alternate forms of cesium that would reduce the safety risks. The challenge is that a technology replacing gamma irradiation must be able to treat all blood components, including RBCs.

This report presents studies comparing the effects of gamma irradiation to the effects of treating fresh whole blood with Rb-UV on WBC functionality. Gamma irradiation was found to be as effective as Rb-UV treatment in preventing lymphocyte proliferation and X-GVHD, but less effective in preventing cells from acting as antigen-presenting cells and producing cytokines. WBC inactivation performed at lower UV energy doses demonstrates the robustness of the system in inactivating WBCs. This evidence supports Rb-UV treatment as a potential alternative means of preventing TA-GVHD. The ability of the treatment to influence immune responses in the recipient, such as alloimmunization, is being investigated in the ongoing PREPAReS clinical trial. The ability to effectively treat whole blood would allow the transfusion medicine community to treat the blood with Rb-UV before separation into components and to cease gamma-irradiation of the derived components, thereby improving the cost-effectiveness ratio of the treatment. The fact that Rb-UV treatment prevents antigen-presenting cells from stimulating allogeneic responder cells suggests that this treatment may prevent alloimmunization and PLT refractoriness. This hypothesis was investigated in an animal model by Asano and colleagues and results from these studies suggest that the treatment may reduce or eliminate alloantibody generation and alloantibody-mediated cardiac graft rejection, observed as a result of transfusion of WBCs. Consistent with the observed difference in antigen presentation between groups is the finding that the use of gamma-irradiated blood units for transfusion does not alter the frequency of alloimmunization.

Our previous studies on X-GVHD responses utilized the Rag2γc−/− mouse that showed variable human WBC engraftment and GVHD development. A recently published report indicated that the use of the NOD-scid IL2γnull recipients results in more rapid and robust GVHD responses, regardless of the WBC donor. The use of NOD-scid IL2γnull recipient mice in our studies resulted in similar kinetics of GVHD onset as published when 20 × 10⁶ donor PBMCs were injected. Plasma, collected from mice showing signs of full-scale GVHD, contained high levels of IFN-γ and moderate levels of IL-10 and occasion-
ally IL-5, but no TNF-α, IL-2, or IL-4. Interestingly, while we were not able to detect TNF-α in the end stages of X-GVHD, King and coworkers18 showed that blocking TNF-α signaling in this model delayed onset and progression to GVHD, suggesting that TNF-α production plays an important role in the development of GVHD. The production of IFN-γ and IL-10 in mice undergoing GVHD has previously been associated with syngeneic GVHD development. Recent studies have shown that strong continued stimulation of CD8+ cells results in increased expression of cytolytic effector molecules and production of IL-10.19,20 Thus the presence of IL-10 in the plasma of mice with ongoing X-GVHD could reflect prolonged stimulation of xenoreactive T cells in the recipient mice and the finding of elevated HLA-DR expression is indicative of activated T cells.

We observed an increased percentage of CD19+CD3+ and CD4+CD8+ in the mice with X-GVHD. The presence of such cells has not been described for this mouse model before and we hypothesize that these antigens may have been acquired via trogocytosis, the exchange of surface antigen between interacting cells.21,22 The environment may also play a role and the interaction with xenogeneic cells could induce expression of these antigens on human cells.23 In hepatitis C virus–infected individuals, T-regulatory cells up regulate the expression of mRNA for B-cell antigens including CD19,24 indicating the potential for T cells to express CD19.

Subjecting blood components to gamma irradiation has been the almost universal means of preventing TA-GVHD for more than 40 years. Considerable expense and many technical requirements are associated with purchasing, installing, maintaining, and operating gamma irradiators. There are also clinical and regulatory concerns associated with the use of the gamma irradiators.14 The remaining risk of TA-GVHD is unknown and may vary depending on the use of leukoreduction or fresh blood components. While the likelihood of TA-GVHD is related to the number of transfused lymphocytes, the threshold dose is not known and the disease has been reported after the transfusion of leukoreduced nonirradiated components.25,26 We present here a technology capable of inactivating WBCs in whole blood, providing a treatment of all blood components, including RBCs. The benefits and the risks of Rb-UV treatment of whole blood need to be weighed in comparison to gamma irradiation to determine whether this treatment will provide a potential alternative to gamma irradiation for preventing TA-GVHD in the future. Recent approval by the FDA to test RBC function in human subjects after Rb-UV treatment in vivo will provide further data in support of the Mirasol System for Whole Blood.

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CONFLICT OF INTEREST

RPG, HLR, and SM are employees of CaridianBCT Technologies. The remaining authors declare no competing financial interests.

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