Quality control of blood irradiation: determination T cells radiosensitivity to cobalt-60 gamma rays

E.G. Góes, J.C. Borges, D.T. Covas, M.D. Orellana, P.V.B. Palma, F.R. Morais, and C.A. Pelá

BACKGROUND: To identify the most appropriate dose for the prevention of transfusion-associated graft-versus-host disease, the radiosensitivity of T cells has been determined in blood bags irradiated with X-rays produced by a linear accelerator and gamma rays derived from the cesium-137 source of a specific irradiator. In this study, the influence of doses ranging from 500 to 2500 cGy was investigated on T cells isolated from red blood cell (RBC) units preserved with ADSOL and irradiated with a cobalt teletherapy unit.

STUDY DESIGN AND METHODS: A thermal device consisting of acrylic and foam was constructed to store the blood bags during irradiation. Blood temperature was monitored with an automated data acquisition system. Dose distribution in the blood bags was analyzed based on isodose curves obtained with a polystyrene phantom constructed for this purpose. The influence of cobalt-60 gamma radiation on T cells was determined by limiting-dilution analysis, which measures clonable T cells. T-cell content of the mononuclear cell population plated was assessed by flow cytometry with a monoclonal antibody specific for CD3.

RESULTS: Blood temperature ranged from 2 to 4.5°C during irradiation. Dosimetry performed on the phantom showed a homogenous dose distribution when the phantom was irradiated with a parallel-opposite field. A radiation dose of 1500 cGy led to the inactivation of T cells by 4 log, but T-cell growth was observed in all experiments. At 2500 cGy, no T-cell growth was detected in any of the experiments and a greater than 5 log reduction in functional T cells was noted.

CONCLUSION: The results showed that a dose of 2500 cGy completely inactivates T cells in RBC units irradiated with cobalt-60 source.

Transfusion-associated graft-versus-host disease (TA-GVHD) is a rare, but fatal, potential complication that occurs when viable donor T lymphocytes proliferate and engraft in susceptible patients after transfusion of whole blood and cellular components.[1,2] TA-GVHD can occur in severely immunocompromised patients, including patients with congenital immunodeficiencies and marrow transplant recipients, as well as in patients with cancer treated with chemotherapy or radiotherapy.[3-9] TA-GVHD has also been reported in immunocompetent patients who had received blood from donors homozygous for shared human leukocyte antigen (HLA) haplotypes.[10] At least three factors appear to be directly related to the risk of TA-GVHD:[11] 1) the susceptibility of the patient's immune system to the engraftment, 2) the degree of HLA similarity between donor and recipient, and 3) the number of viable donor lymphocytes present in the transfused components. There is no effective treatment for TA-GVHD, and the irradiation of cellular blood components before transfusion has been the only proven method of preventing this reaction.[12]

ABBREVIATIONS: LDA = limiting-dilution analysis; TA-GVHD = transfusion-associated graft-versus-host disease; TLD(s) = thermoluminescent dosimeter.

From the Nuclear Engineering Program of Alberto Luiz Coimbra Institute, Federal University of Rio de Janeiro-RJ; Center for Cell-Based Therapy, School of Medicine of Ribeirão Preto; and Center of Instrumentation, Dosimetry and Radioprotection, School of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto-SP, Brazil, Ribeirão Preto, São Paulo University, Ribeirão Preto-SP, Brazil.

Address reprint requests to: Evamberto Garcia de Góes, PhD Hemocentro Regional de Ribeirão Preto, Laboratório Controle de Qualidade, Rua Tenente Catão Roxo, 2501, Ribeirão Preto-SP, CEP 14051-140, Brazil; e-mail: eggoes@terra.com.br.

This research was partially supported by FAPESP and CNPq.

Received for publication December 14, 2004; revision received April 11, 2005, and accepted June 4, 2005.

doi: 10.1111/j.1537-2995.2006.00669.x

TRANSFUSION 2006;46:34-40.

Blood irradiation can be performed with commercial irradiators designed specifically for this purpose, usually located in blood banks. Commercial irradiators incorporate one to four gamma ray sources, generally cesium-137 (^{137}Cs), of 22 to 89 TBq (600-2400 Ci) each, and typical initial dose rates range from 300 to 1000 cGy per minute. If a dedicated blood irradiator is not available, however, other alternatives such as X-rays produced by a linear accelerator or gamma rays derived from a cobalt teletherapy unit can be used for this purpose.[13-17] Gamma and X-rays, both representing ionizing radiation, damage DNA of T lymphocytes and arrest responses to allogeneic cells. Thus, these lymphocytes are unable to proliferate in the host and therefore cannot mediate TA-GVHD. The radiosensitivity of T cells and the best dose for blood irradiation able to eliminate the risk of TA-GVHD have been determined for blood irradiated with gamma rays derived from ^{137}Cs irradiators and X-rays generated by a linear accelerator.[18,19] This dose was determined by use of a limiting-dilution analysis (LDA) assay that provides quantitative data on very low frequencies of proliferating T cells.[20-23]

In this present study, an LDA assay was used to investigate the influence of 500 to 2500 cGy of gamma irradiation delivered by the cobalt-60 (^{60}Co) source of a cobalt teletherapy machine on T cells when delivered in situ to ADSOL-preserved red blood cell (RBC) units. To preserve the temperature of the blood during irradiation, a thermal device consisting of acrylic and foam was constructed to store blood bags. An automatic acquisition system, coupled to an amplifier and a thermal-sensitive probe, was used to check blood temperature. The dose distribution in blood bags was simulated with a homogenous phantom consisting of polystyrene plastic.

MATERIALS AND METHODS

Irradiation system used and calibration

In this study, all material was irradiated with a cobalt teletherapy unit (Gammatron S-80, Siemens, Munich, Germany). The apparatus contained a ^{60}Co source (effective energy of 1.25 MeV). The dose rate was determined with a calibrated clinical dosimeter consisting of an electrometer (DI4, PTW, Freiburg, Germany) and a ionization chamber (M23332, PTW). Dosimetry of the ^{60}Co beam was performed according to an international dosimetry protocol.[24]

Maintenance of the temperature of the RBC units during irradiation

The temperature of the RBC units was kept constant during irradiation by storing the blood bags in a thermal device (Fig. 1). The dimensions of the device ($20 \times 16 \times 6$ cm) were chosen to fit one or two blood bags during a single irradiation. The thermal device consisted of a double acrylic wall filled by a foam wall, each material measuring 5 mm in thickness. The lid of the device had the same composition and contained a central orifice for introducing a thermometer (Fig. 1).

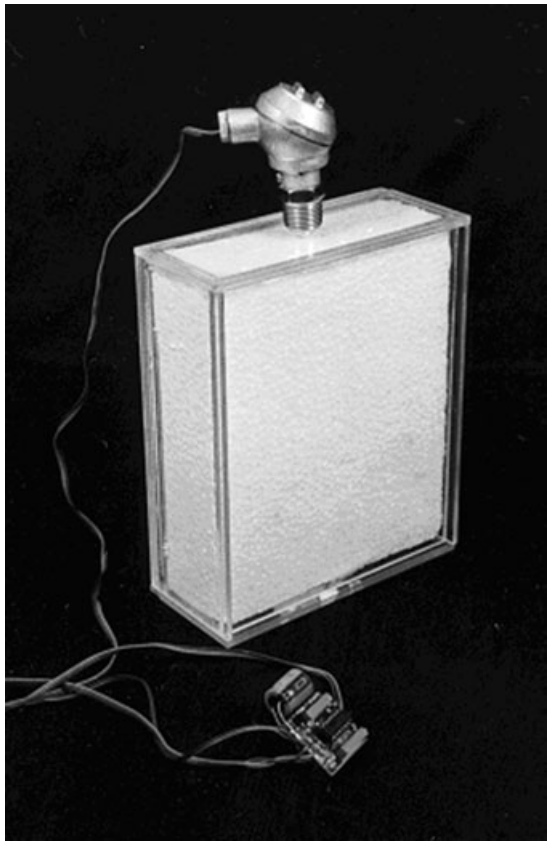

Fig. 1. The thermal device for blood bag storage and thermometric system.

The thermal performance of the device was evaluated with a PT-100 temperature sensor (cromel-alumel junction) consisting of an aluminum minihead and a stainless sheath (6-mm diameter and 200-mm long). An electronic circuit was constructed to amplify the exit signal and a computer program was developed to permit automatic data acquisition.

A blood bag was then stored in the thermal device filled with water at a temperature of 2°C, the sensor was coupled to the apparatus through the lid as shown in Fig. 1, and the water temperature was monitored automatically by the computer program. The experiment was performed with the thermal device exposed to an environmental temperature of approximately 23°C.

Study of the dose distribution in the blood bags

The dose distribution in a blood bag was simulated with a homogeneous clear polystyrene phantom with size

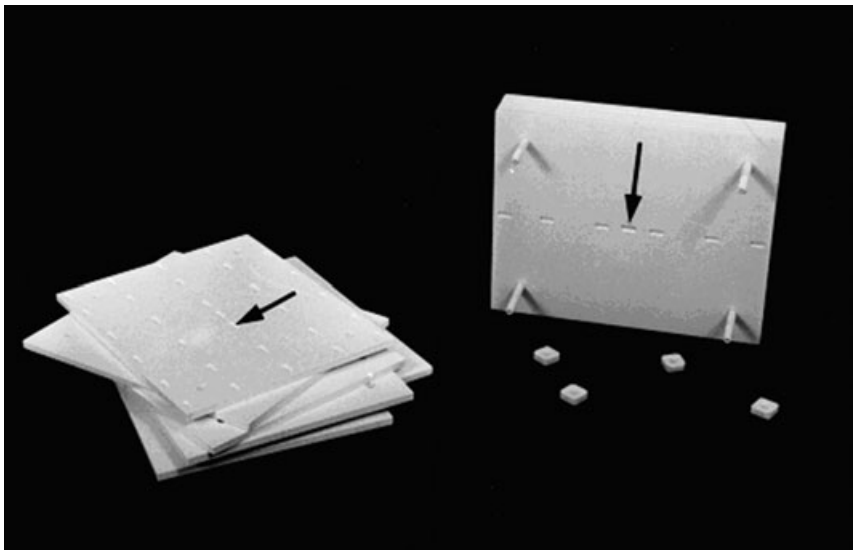

Fig. 2. Phantom plates assembled by four connecting screws. Arrows indicate cavities used to locate thermoluminescent dosimeters.

and shape matching those of the irradiation device (Fig. 2). Because the difference in linear attenuation coefficients of clear polystyrene plastic and whole blood for energy, normally used for blood irradiation, is lower than 5 percent, this type of material is recommended to represent blood for dosimetric purposes.[25] Plaques of clear polystyrene were cut according to the phantom dimensions and attached face to face with screws of the same material (Fig. 2). Thermoluminescent dosimeters (TLDs) were used to measure the absorbed dose. A total of 38 cavities ($9 \times 3 \times 1$ mm) containing three TLDs (TLD-100, Harshaw Chemical Co., Solon, OH) were used to measure the dose along the central plane of the phantom. The plaques were aligned and the phantom was then irradiated with a 30×30-cm^2 field at the surface of the phantom and a 80-cm source to phantom surface distance. Dose distributions in the phantom were studied with a single and parallel-opposed fields. The irradiation time was selected to obtain a dose of 200 cGy at the phantom volume center. A total of 120 TLDs were individually calibrated for each dose and separated in two groups, one for the phantom dosimetry and the other for the control. The TLD response was analyzed by use of a Harshaw reader, Model 2000-B/2000-C. The TLDs were annealed according to the manufacturer's recommendations (1 hr at 400°C followed by 2 hr at 100°C). For both single and parallel-opposed fields, the dose in each cavity was measured with three TLDs readings that were averaged and normalized to the maximum dose value. The results were represented in terms of isodose curves, with a cubic spline interpolation technique.[26]

Collection of RBC units and irradiation

RBC units were individually prepared from blood collected from three healthy adult volunteers. The RBC units were separated from whole blood by centrifugation and stored in PL-146 plastic bags (Baxter Healthcare Corp., Deerfield, IL) with ADSOL (Fenwal) as preservative. Each unit contained approximately 250 mL of RBCs from a single donor. Ten hours after blood collection, each blood bag was protected with a plastic wrap, stored individually in the thermal device filled with water at an initial temperature of 2°C, and irradiated with 2500 cGy with the cobalt teletherapy unit described above. Irradiation was performed with a 30×30-cm^2 parallel-opposite field at 80-cm source to device surface distance, based on the dose distribution results obtained previously for the phantom.

The dose was released in a staggered manner at 500, 1000, 1500, 2000, and 2500 cGy. To minimize biologic variability in the effect of radiation on T cells, an aliquot was removed from each blood bag at each 500-cGy incremental dose and compared to a control sample (not irradiated) obtained from the bag before irradiation. For each fraction, the released dose was confirmed with a TLD calibrated and annealed as described above.

LDA assay

Mononuclear cells (MNCs) were isolated from samples collected from the blood bags on a Ficoll-Hypaque density gradient (Sigma Chemical Co., St Louis, MO), washed three times, and resuspended in RPMI 1640 (Sigma) containing 5 percent fetal calf serum (FCS; Gibco, Grand Island, NY), penicillin-streptomycin (Gibco), and L-glutamine (Sigma Chemical Co.). Each sample was then plated according to a geometric series of dilutions of ratio 4 with six to eight series of dilution (12-24 wells per dilutions) on 96-well microculture plates (Corning, New York, NY). The dilutions were made based on the count of viable cells (determined by trypan blue exclusion) in a Neubauer chamber. For each dilution (number of cells per well), the starting concentration of cells was selected in such a way that some dilutions were informative; that is, all wells were not 100 percent positive or negative. Each culture volume (200 µL per well) was supplemented with 2.5 µg per mL phytohemagglutinin (Sigma Chemical Co.) and 100 units per mL human recombinant interleukin-2 (IL-2; Sigma Chemical Co.). Experimental cells were cocultured with the allostimulator with a pool of 10^5 irradiated cells (4000 cGy) obtained from an RBC unit from five donors. The cultures were incubated

at 37°C in a 5 percent CO_2 atmosphere for 4 weeks. At the end of each week, half the volume of culture medium in each well (100 µL) was removed and the same volume of RPMI (Sigma Chemical Co.) containing 10 percent FCS and 500 units per mL IL-2 was added. T-cell growth was monitored during the 4 weeks by an inverted phase microscope. Culture wells containing colonies of at least 10 cells were considered to be positive. For each LDA experiment involving irradiated samples, the following controls were performed: 1) one control experiment to establish the efficacy of radiation for that donor, 2) one experiment with cells obtained from the white blood cell pool to exclude growth of the allostimulator, and 3) one experiment with irradiated cells without the addition of any growth factor to determine the experimental background.

The frequency of T-cell proliferation was calculated at the end of the 4 weeks of incubation by chi-square minimization.[20] The data obtained for each group were treated with a computer program (L-Calc, Stem Cell Technologies, Vancouver, British Columbia, Canada), where $f_{\text{experimental}}$ is the frequency of T cells in irradiated samples and f_{control} is the frequency of T cells in nonirradiated samples. The reduction in T-cell proliferation as a function of the dose applied was expressed as[18]

$$\int \text{Log T cell reduction} = \text{Log}\left[\frac{f_{\text{experimental}}}{f_{\text{control}}}\right].$$

Flow cytometry

The cells in cultures containing 10^6 cells per well were analyzed on Day 0 by flow cytometry with a CD3-specific monoclonal antibody (MoAb) to detect the presence of mature, viable T cells. The cells were collected and incubated for 20 minutes in the presence of anti-CD3–phycoerythrin (PE) and anti-CD45–fluorescein isothiocyanate (FITC) MoAbs (Becton Dickinson, Mountain View, CA). Next, the cells were washed twice, resuspended in the presence of 50 µg per mL propidium iodide (PI; Becton Dickinson), and incubated for 10 minutes. The analysis of fluorescence as a function of cell size and external complexity was determined with a flow cytometer (FACSort, Becton Dickinson) equipped with a 488-nm argon laser. Viable T cells were identified by the anti-CD3 MoAb and gating of cells that exclude PI. The negative control consisted of nonspecific antibodies labeled with FITC, PE, and PERCP fluorochromes (Becton Dickinson). A total of 10,000 events were analyzed for samples collected from each well with computer software (Cell Quest, Becton Dickinson).

RESULTS

Temperature measurements during the irradiation of the blood bags

Evaluation of the performance of the thermal device showed that the temperature of the RBC units ranged from 2 to 4.5°C during the first 60 minutes, reaching 6°C after 150 minutes (Fig. 3). Irradiation of each blood bag lasted approximately 60 minutes. Thus, at the end of each irradiation the temperature of the RBC units had increased from 2 to 4.5°C. Room temperature remained at about 23°C during the irradiation procedures. The uncertainty associated with the temperature measurements was ±0.2°C.

Dose distribution in blood bags

Dose distributions in the volume of the phantom were determined by monitoring the dose in 38 cavities distributed among the two planes, with an uncertainty of less than 3 percent. Isodose curves were obtained for the central plane of the phantom aligned perpendicularly and parallel to the ^{60}Co beam. Results of dosimetry performed with the phantom irradiated with a single field showed significant heterogeneity in dose distribution (from 70% to 98%). Dose distributions ranged from 92 to 100 percent on the two planes when the phantom was irradiated with a parallel-opposed field. Figure 4 show this dose distribution in the central plane when aligned parallel to the radiation beam.

T-cells growth frequency versus dose

The mean percentage of T-cell content of the MNCs plated was 63 percent in the samples not irradiated and 57 percent in the samples irradiated. The data about T-cell viability on Day 0, determined by PI exclusion, are summarized in Fig. 5. Up to 1500 cGy the mean percentage of viable T cells varied from 97 to 99 percent and from 93 to 94 percent for the dose interval between 2000 and 2500 cGy.

The unirradiated control group yielded a mean T-cell growth frequency of 1 in 9 plated MNCs (range, 1:4-1:17). At 500 cGy, the mean growth decreased to 1 in 322 (range,

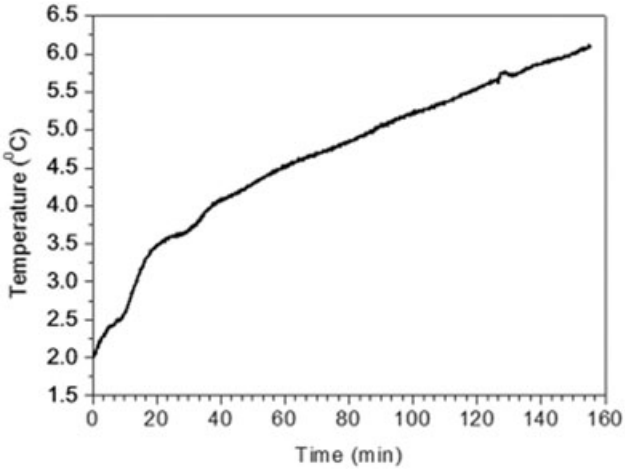

Fig. 3. Blood temperature during irradiation.

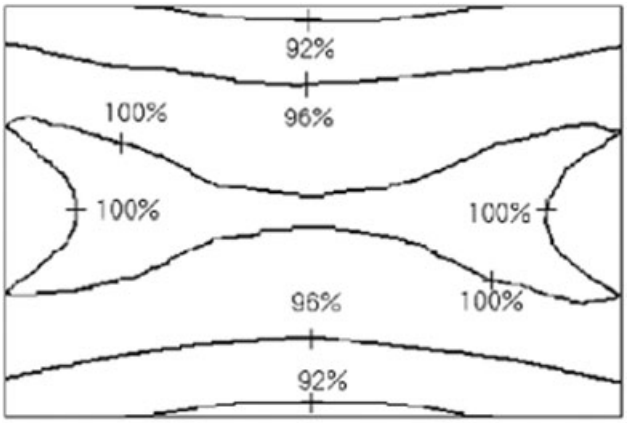

Fig. 4. Isodose curves obtained at the central plane when the phantom was irradiated with parallel-opposed fields.

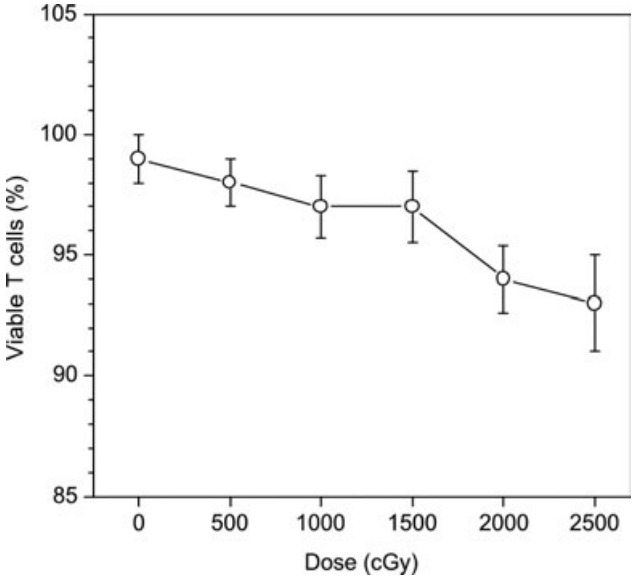

Fig. 5. Percentage of viable T cells (CD3+ cells) on Day 0 assessed by flow cytometry.

1:46-1:649). At 1000 cGy, the mean T-cell growth was 1 in 2372 (range, 1:1056-1:4336). When cells were irradiated with a dose of 1500 cGy, the mean growth decreased to 1 in 128,821 (range, 1:34,175–1:208,017), with T-cell growth being observed in all experiments. At 2000 cGy, no T-cell growth was observed in wells containing less than 2×10^6 cells per well in any of the three experiments, corresponding to a reduction in the growth potential of T cells of more than 5 log. No T-cell growth was observed in any of the three experiments at 2500 cGy. At this dose, the mean growth was less than 1 in 10^6 plated cells, corresponding to a reduction in the T-cell growth of more than 5.4 log. The results of T-cell inactivation as a function of the dose are summarized in Fig. 6.

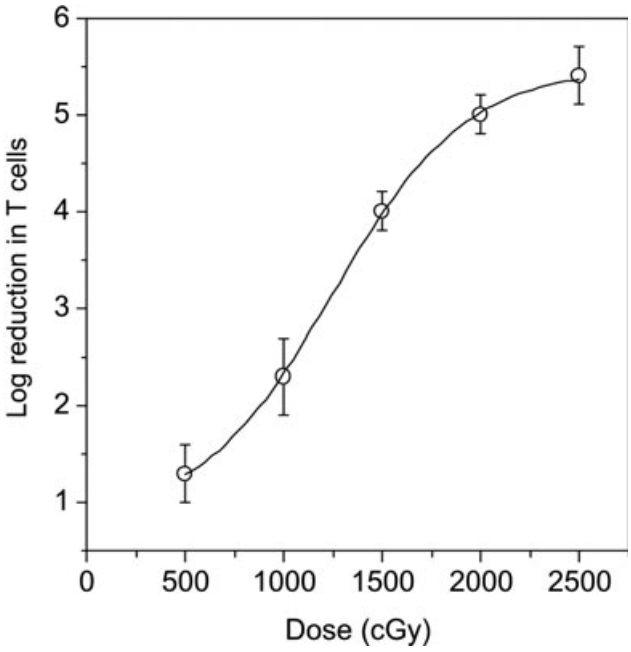

Fig. 6. Reduction in functional T cells, as a function of the radiation dose used, represented by a sigmoidal function.

DISCUSSION

The prevention of TA-GVHD by the irradiation of blood components before transfusion is based on the fact that irradiation produces T-cell damage at a magnitude sufficient to inhibit their proliferative abilities and therefore their ability to respond to allogeneic cells. The ideal dose necessary to prevent TA-GVHD while preserving the quality of other blood cells, excluding the lymphocytes, continues to be an issue. A dose as little as 500 cGy can attenuate the response of lymphocytes to allogeneic cells in a mixed lymphocyte culture, and 1500 cGy can reduce the response to mitogen-induced stimulation by 90-99 percent, representing a sensitivity lower than 2 log.[27-29] In the past, based on data about the elimination of allogeneic reactivity in the mixed lymphocyte culture, the use at least 1500 cGy was recommended.[29] Three cases of TA-GVHD have been reported in patients who received blood irradiated with doses between 1500 and 2000 cGy by use of a specific blood irradiator and a linear accelerator.[30-32] Dosimetric procedures necessary to verify the dose delivered in the blood bags, however, were not applied in these cases.

Recently, two studies with LDA assay indicated that 2500 cGy was the minimal dose necessary for TA-GVHD prevention.[18,19] One of those studies tested the effects of gamma rays emitted by the ^{137}Cs source of a blood irradiator, and X-rays emitted by an linear accelerator, on T cells when delivered in situ to ADSOL-preserved RBC units in PL-146 and PL-2209 plastic containers.[18] The

other study tested the effect of gamma rays emitted by the ^{137}Cs source of a blood irradiator on T cells when delivered in situ to plateletpheresis units in the PL-732 plastic container.[19]

In this study, we focused our attention on the determination of T-cell radiosensitivity to ^{60}Co gamma rays to better delineate the minimal dose needed to inactivate T cells when irradiating RBC with a cobalt teletherapy unit. We used the LDA assay, which is the most sensitive method previously employed to determine functional T cells after T-cell depletion for the prevention of GVHD in the setting of marrow transplantation.[20-23] In our studies, the phantom consisting of standard material provided information regarding the dose distribution in the irradiated blood volume and permitted us to establish in advance the geometry of irradiation that would minimize dose heterogeneity in the volume irradiated. The minimization of dose-distribution heterogeneity is important to avoid subdosage, or overdosage, of some of the blood cells.

The present LDA results demonstrated T-cell growth in all experiments for cells irradiated with a dose of 1500 cGy. As can be seen in Fig. 6, the slope of the curve at first increases slowly between 500 and 1000 cGy and then becomes fairly straight between 1000 and 1500 cGy (i.e., maximal reduction in functional T cells per dose range), and finally decreases at doses higher then 2000 cGy. At 2500 cGy, reduction in functional T cells reached a plateau, suggesting the detection limit of the LDA method. We found that a gamma irradiation dose of 2500 cGy may be required to completely inactivate T cells in RBC units when a ^{60}Co source is used. The LDA of T cells irradiated with a similar dose delivered either by the ^{137}Cs source of a specific irradiator or by X-rays produced by a linear accelerator has shown that a dose of 2500 cGy reduces functional T cells by more than 5 log.[18,19] These findings are in agreement with the present LDA results. Thus, the results obtained in this study on the radiosensitivity of T cells to gamma rays delivered by a ^{60}Co source support the use of 2500 cGy as the appropriate dose to prevent TA-GVHD.

When irradiation is performed with a specific blood irradiator, the dose necessary for TA-GVHD prevention is released within a few minutes. When a linear accelerator or cobalt teletherapy unit is used for this task, however, the entire irradiation process is completed within 30 to 60 minutes. In such cases, the blood components remain at room temperature (approx. 23°C) during the irradiation process. A thermal device that provides appropriate storage conditions during the irradiation of blood components with a teletherapy unit has been recently proposed.[17] Because of the dimensions of this device (20 cm in diameter and 20 cm in height), however, the dose distribution in the volume is more heterogeneous, and therefore we decided to build a more appropriate thermal device to store the blood bags during the process of irradiation used in this experiments. The method used to store the blood bags guaranteed that the temperature of the RBC units would be kept below 4.5°C during irradiation, ensuring that all damage caused to the cells studied was exclusively due to the action of radiation at the different doses applied.

ACKNOWLEDGMENTS

The authors thank Thomaz Ghilardi Netto, PhD, Elisa A. Reis, Maria Helena da Silva, Marcos Vasques, José Luiz Aziani, Dilma Freire, and Aurea T. Souza, for their technical assistance.

REFERENCES

1. Kerman NA, Collins NH, Juliano L, et al. Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-v-host disease. Blood 1986;68:770-3.
2. Przepiorka D, LeParc GF, Stovall MA, et al. Use of irradiated blood components: practice parameter. Am Clin Pathol 1996;106:6-11.
3. Thomaz ED, Herman EC, Greenough WB. Irradiation and marrow infusion in leukemia. Arch Intern Med 1961;107: 829-45.
4. Parkman R, Mosier D, Umansky I, et al. Graft-versus host disease after intrauterine and exchange transfusions for hemolytic disease of the newborn. N Engl J Med 1974; 290:359-63.
5. Von Fliedner V, Higby DJ, Kim U. Graft-versus-host reaction following blood transfusion. Am J Med 1982;72:951-9.
6. Brubaker DB. Human postransfusion graft-versus-host disease. Vox Sang 1983;45:401-20.
7. Anderson KC, Weinstein HJ. Transfusion-associated graft-versus-host disease. N Engl J Med 1990;332:315-21.
8. Greenbaum B. Transfusion-associated graft-versus-host disease: historical perspectives, incidence, and current use of irradiated blood products. J Clin Oncol 1991;9:1889-902.
9. Jamieson NV, Joysey V, Friend PJ, et al. Graft-versus-host disease in solid organ transplantation. Transplant Int 1991;4:67-71.
10. Thaler M, Shamiss A, Orgad S, et al. The role of blood from HLA-homozygous donors in fatal transfusion-associated graft-versus-host disease after open heart surgery. N Engl J Med 1989;321:25-8.
11. Ferrara JL, Deeg HJ. Graft-versus-host disease. N Engl J Med 1991;324:667-74.
12. Chapman J, Finney RD, Formam K. Guide on gamma irradiation of blood components for prevention of transfusion-associated graft-versus-host disease. Transfusion 1996;6:261-71.
13. Anderson KC, Goodnough LT, Sayers M, et al. Variation in blood component irradiation practice: implication for

prevention of transfusion-associated graft-versus-host disease. Blood 1991;77:2096-102.
14. Moroff G, Luban NLC. The irradiation of blood and blood components to prevent graft-versus-host disease: technical issues and guidelines. Transfus Med 1997;11:15-26.
15. Patton GA, Skowronski MG. Implementation of a blood irradiation program at a community cancer center. Transfusion 2001;41:1610-4.
16. Chen F, Covas DT, Baffa O. Dosimetry of blood irradiation using an alanine/ESR dosimeter. Appl Radiat Isot 2001; 55:13-6.
17. Góes EG, Covas DT, Haddad R, et al. Quality control system for blood irradiation using a teletherapy unit. Vox Sang 2004;86:105-10.
18. Pelszynski MM, Moroff G, Luban NL, et al. Effect of gamma irradiation of red blood cell units on T-cell inactivation as assessed by limiting dilution analysis: implication for preventing transfusion-associated graft-versus-host disease. Blood 1994;83:1683-9.
19. Luban NL, Drothler D, Moroff G, Quinones R. Irradiation of platelet components: inhibition of lymphocyte proliferation assessed by limiting-dilution analysis. Transfusion 2000; 40:348-52.
20. Taswell C. Limiting dilution assays for the determination of immunocompetent cell frequencies. I. Data analysis. J Immunol 1981;126:1614-9.
21. Kernan NA, Flomenberg N, Collins NH, et al. Quantitation of T lymphocytes in human bone marrow by a limiting dilution assay. Transplantation 1985;40:317-22.
22. Moretta A, Pantaleo G, Moretta L, et al. Direct demonstration of the clonogenic potential of every human peripheral blood T cell: clonal analysis of HLA-DR expression and cytolytic activity. J Exp Med 1985;157:743-54.
23. Quinones RR, Gutierrez RH, Dinndorf PA, et al. Extended-cycle elutriation to adjust T-cell content in HLA-disparate bonne marrow transplantation. Blood 1993;82:307-17.
24. Andreo P, Cunningham JR, Hohlfeld K, et al. Absorbed dose determination in photon and electron beams: an international code of practice. Technical Reports Series 277.Vienna: International Atomic Energy Agency; 1987.
25. White DR, Booz J, Griffith RV, et al. Tissue substitute in radiation dosimetry and measurement. Report 44. Bethesda: International Commission on Radiation Units and Measurements; 1989.
26. Press WH, Flannery BP, Teukolsky SA, et al. Numerical recipes in Pascal: the art of scientific computing. New York: Cambridge University Press; 1997.
27. Sprent J, Anderson RE, Miller JF. Radiosensitivity of T and B lymphocytes. II. Effect of irradiation on response of T cells to alloantigens. Eur J Imunol 1974;4:204-10.
28. Valerius NH, Johansen KS, Nielsen OS, et al. Effect of in vitro x-irradiation on lymphocyte and granulocyte function. Scand J Haematol 1981;27:9-18.
29. Rosen NR, Weidner JG, Boldt HD, et al. Prevention of transfusion-associated graft-versus-host disease: selection of an adequate dose of gamma irradiation. Transfusion 1993;33:125-7.
30. Dobrynski W, Thibodeau S, Truitt RL, et al. Third-party-mediated graft rejection and graft-versus-host disease after T-cell-depleted bone marrow transplantation as demonstrated by hypervariable DNA probes and HLA-DR polymorphism. Blood 1989;74:2285-94.
31. Lowenthal RM, Challis DR, Griffiths AE, et al. Transfusion-associated graft-versus-host disease: report of a case following administration of irradiated blood. Transfusion 1993;33:524-9.
32. Sproul AM, Chalmers EA, Mills KI, et al. Third party mediated graft rejection despite irradiation of blood products. Br J Haematol 1992;80:261-2.