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Analysis of the Recovery of Cryopreserved and Thawed CD34+ and CD3+ Cells Collected for Hematopoietic Transplantation

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Abstract

Background—Cryopreservation is often used to store cellular therapies, but little is known about how well CD3+ or CD34+ cells tolerate this process.

Study Design—Viable CD34+ cell recoveries were analyzed from related and unrelated donor G-CSF-mobilized peripheral blood stem cell (PBSC) products and viable CD3+ cell recoveries from G-CSF-mobilized and non-mobilized apheresis products from related and unrelated donors. All products were cryopreserved with 5% dimethyl sulfoxide and 6% pentastarch using a controlled-rate freezer and were stored in liquid nitrogen. Related donor products were cryopreserved immediately after collection and unrelated donor products greater than 12 hours post-collection.

Results—The post-thaw recovery of CD34+ cells from related donor PBSCs was high (n=86; 97.5±23.1%) and there was no difference in post-thaw CD34+ cell recovery from unrelated donor PBSCs (n=14; 98.8±37.2%; p=0.863). In related donor lymphocyte products the post-thaw CD3+ cell recovery (n=48, 90.7±21.4%) was greater than that of unrelated donor products (n=14, 66.6±35.8%, p=0.00251). All unrelated donor lymphocyte products were from G-CSF mobilized products, while most related donor lymphocyte products were from non-mobilized products. A comparison of the CD3+ cell recovery from related donor G-CSF-mobilized products (n=19, 85.0±29.2%) with that of unrelated donor products found no significant difference (p=0.137).

CONCLUSIONS—The post-thaw recovery of CD34+ cells was high in both related and unrelated donor products, but the recovery of CD3+ cells in unrelated donor G-CSF-mobilized products was lower. G-CSF-mobilized unrelated donor products may contain less CD3+ cells than non-G-CSF exposed products upon thaw and, when indicated, cell doses should be monitored.

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The authors have no conflicts of interest

Keywords

T cells; cryopreservation; donor lymphocyte infusions; hematopoietic stem cell transplantation

Introduction

Cellular therapies have a short life-span when stored at room temperature and as a result they are often cryopreserved and stored frozen. Hematopoietic stem cells (HSCs) collected for autologous transplantation are almost always collected and cryopreserved while the patient undergoes pre-transplant chemotherapy. Most allogeneic transplants HSCs are administered within a few hours of collection and processing, however, our institution often cryopreserves HSC products collected from HLA-matched sibling donors and later thaws and infuses them for transplantation. Transplanting cryopreserved rather than fresh HSCs ensures that an adequate quantity of HSCs have been collected prior to beginning pre-transplant conditioning therapy. Several studies have found that when HSC products are cryopreserved shortly after collection the viability, function and engraftment potential of the thawed HSCs are well maintained.¹⁻¹²

Peripheral blood mononuclear cells (PBMC) products that are rich in CD3+ T cells are often collected by apheresis from HLA-matched sibling donors for use as donor lymphocyte infusions (DLIs). These DLI products are used to treat leukemia relapse following transplantation or improve T cell engraftment. When used as DLIs, PBMC products may be infused immediately after collection, but often, they are cryopreserved within a few hours of collection. Much less is known about how well CD3+ cells tolerate freezing and thawing compared to HSCs, but one study of a wide variety of cryopreserved related donor leukocyte products found that the CD3+ cell post-thaw recovery ranged from 76% to 86%¹³ and other found the CD3+ cell post-thaw recovery to be approximately 81%.¹⁴

Most DLI products are collected from donors who have not been given any HSC mobilizing agents, but sometimes aliquots from G-CSF-mobilized PBSC products collected for transplantation are removed and cryopreserved for use as DLIs. In addition, at our center when CD34+ cells are selected from G-CSF-mobilized PBSC concentrates, donor leukocytes are added back to the selected CD34+ cells to achieve a target CD3+ cell dose in order to ensure engraftment.¹⁵ The leukocytes used for the add-back are often from the G-CSF-mobilized PBSC product. Both the selected CD34+ cells and a leukocyte-rich aliquot is cryopreserved. At the time of transplantation, they are thawed and the CD34+ cells and a portion of the leukocytes are given to the recipient.

Occasionally, G-CSF-mobilized PBSC components collected from unrelated donors are cryopreserved for a short period of time in order to accommodate differences in the timing of the transplant conditioning and donor availability. In addition, aliquots from G-CSF-mobilized unrelated donor PBSC products are sometimes cryopreserved for later use as DLIs. While PBSC and DLI products collected for transplants involving HLA-matched sibling donors are processed, and if indicated, cryopreserved within a few hours of collection, this is not the case with products collected from unrelated donors. Unrelated donor grafts are almost always collected at one center and are transported to a cell therapy

laboratory in another city for processing. Because of the duration of time required to transport the cells, processing of unrelated donor products typically begins 12 to 48 hours after the cells are collected.

Several studies have found that HSC products can be stored 48 hours or more without significant loss of viability of CD34+ cells if the products are stored at 4°C and cell concentrations are not too high.^{16–19} However, little is known about the effects of prolonged storage and transportation of G-CSF-mobilized PBSC products on the post-thaw recovery of CD34+ or CD3+ cells. We hypothesized that a storage lesion in CD34+ and CD3+ cells that has minimal effects after 24 to 48 hours of liquid storage at 4°C might become apparent following cryopreservation and thawing. This study compared the post-thaw recovery of CD34+ and CD3+ cells in related and unrelated donor apheresis components and assessed the effects of G-CSF on the recovery of CD3+ cells.

Methods

Study design

The results of all post thaw quality assurance testing of unmanipulated G-CSF-mobilized PBSC and lymphocyte clinical products thawed between January 1, 2010 and June 20, 2012 were reviewed. The products were cryopreserved from 2005–2012 and thawed in 2010–2012. CD34+ cell recoveries were assessed in unmanipulated G-CSF-mobilized PBSC products used for transplants involving HLA-compatible related and unrelated donors. Post-thaw CD3+ cell recoveries were assessed in apheresis products used for DLIs or as lymphocyte add back products. The related donor products were collected from our apheresis clinic (Dowling Clinic, Department of Transfusion Medicine, Clinical Center, NIH, Bethesda, Maryland) and the unrelated PBSC products were collected by other institutions and shipped at 2–10°C for approximately 12 to 48 hours. These studies were approved by a NIH institutional review board.

Cryopreservation

All products were cryopreserved using a controlled rate freezer (Kryosave, Integra, Planer plc, Sunbury-on-Thames, UK) and 5% DMSO and 6% pentastarch with 4% human serum albumin as a cryoprotectant. The products were stored in aliquots with a final volume of 4.5, 25 or 50 mL; aliquots whose final volume was 4.5 mL were stored in vials (Nunc Cryotube Vials, Roskilde, Denmark) and aliquots of 25 and 50 mL were stored in polyfluoroethylene polyfluoropropylene bags (KryoSure, American Fluoroseal Corporation, Gaithersburg MD). The cell concentration of products frozen in vials ranged from 1×10^6 per mL to 1×10^8 per mL while that of products frozen in bags ranged from 2×10^7 to 3×10^8 cells per mL. The cryopreserved bags and vials were stored in both the vapor and liquid phases of liquid nitrogen.

Thawing PBSC and leukocyte products

The products were thawed in a water bath at 37°C. Immediately after thawing, the products that had been cryopreserved in vials were diluted to a final volume of 30 mL with Plasma-Lyte A (Baxter Healthcare) containing 10 units per mL of preservative free heparin. When

the 25 mL bags were thawed, 6 mL of Plasma-Lyte A was added and 12 mL of Plasma-Lyte A was added to 50 mL aliquots cryopreserved in bags.

Post-thaw quality control testing

An aliquot of the product was diluted 5-fold in Plasma-Lyte A immediately after thawing. Cell counts were performed using an automated cell counter (Abbott CellDyn 3500) and the cells were analyzed by flow cytometry (BD Biosciences, San Jose, CA) with anti-CD3 and CD45 (BD Biosciences). Viability was assessed by flow cytometry and 7-AAD staining. Each product was tested once.

Statistical analysis

For each sample the TNC recovery was equal to $(\text{post-cryopreservation TNC} \times \text{post-thaw product volume}) / (\text{pre-thaw TNC} \times \text{pre-thaw volume}) \times 100$ and the CD3+ cell recovery was $(\text{post-cryopreservation TNC} \times \text{post-cryopreservation CD3\%} \times \text{product volume}) / (\text{pre-thaw TNC} \times \text{pre-thaw CD3\%} \times \text{pre-thaw volume}) \times 100$. The CD34+ cell recovery was calculated in a similar manner. All data were expressed as mean $\pm$ one standard deviation. Groups were compared using t-tests or correlation coefficients (Excel, Microsoft, Redmond, Washington).

Results

Post-thaw recovery of CD34+ cells from G-CSF-mobilized PBSC products collected from related and unrelated donors

When 86 related donor and 14 unrelated donor G-CSF-mobilized PBSC products were compared, prior to cryopreservation the CD34+ cell concentration was similar ($0.71 \pm 0.38\%$ versus $0.88 \pm 0.36\%$, respectively $p=0.13$), but the nucleated cell viability was slightly greater in the related donor products ($99.0 \pm 0.5\%$ versus $97.4 \pm 1.4\%$, $p < 6.15 \times 10^{-6}$). Post-thaw, the recovery of viable CD34+ cells was similar among the related and unrelated donor products (Table 1). The TNC recovery was greater in unrelated donor products but the TNC viability was greater in related donor products (Table 1).

Post-thaw recovery of CD3+ cells from related and unrelated donor apheresis products

A comparison of leukocyte products from related donors used as DLI or lymphocyte add-back products ($n=48$) and unrelated donor products used as DLIs ($n=14$) revealed that pre-cryopreservation there was no difference in portion of CD3+ cells in related and unrelated donor products ($39.1 \pm 18.1\%$ versus $31.4 \pm 15.1\%$, $p=0.154$). However, the viability of nucleated cells was slightly greater in related donor products ($98.9 \pm 1.6\%$ versus $96.9 \pm 2.6\%$, $p=0.0008$). A comparison of the post-thaw recovery of CD3+ cells from all 62 lymphocyte products with the post-thaw recovery of CD34+ cells from all 100 PBSC products revealed that the CD3+ cells recovery was less than the CD34+ cell recovery ($85.3 \pm 27.0\%$ versus $97.7 \pm 25.3\%$, $p=0.00354$). When related and unrelated donor lymphocyte products were compared, the CD3+ cell recovery was less in unrelated donor product as was the post-thaw viability of TNCs and proportion of CD3+ cells, but there was no difference in TNC recovery (Table 2).

Post-thaw analysis of lymphocyte products collected from related donors given and related donors not given G-CSF

Among the 48 related donor leukocyte products, 19 were from donors given G-CSF and 29 from donors who were not receiving G-CSF. To determine if G-CSF administration might have affected the recovery of cryopreserved CD3+ cells we compared products from related donors given G-CSF and from those who did not receive G-CSF. Prior to cryopreservation the CD3+ cell content of the products was less in donors given G-CSF ($20.6 \pm 5.8\%$ versus $51.2 \pm 12.0\%$, $p=0.00154$), but there was no difference in the viability of TNC ($98.9 \pm 1.6\%$ versus $96.9 \pm 2.6\%$, $p=0.249$). The TNC post-thaw viability and CD3+ cell content of the products from donors given G-CSF was less than that of products from donors not given G-CSF, but the post-thaw TNC recovery was similar between the two types of products (Table 3). The CD3+ cell recovery tended to be less in related donors given G-CSF, but the difference was not significant (Table 3). Similarly, a comparison of the 19 leukocyte products from related donors given G-CSF and 14 products from unrelated donors given G-CSF found no difference in CD3+ cell content, TNC recovery or CD3+ cell recovery between the related donor and unrelated donor products; however, the post-thaw TNC viability was less in unrelated donor products ($p=0.038$). A comparison of products from unrelated donors given G-CSF and related donors who were not given G-CSF found that the unrelated donor products had reduced CD3+ cell recovery ($p < 1 \times 10^{-3}$) as well as the TNC viability ($p < 1 \times 10^{-5}$) and proportion of CD3+ cells ($p < 1 \times 10^{-6}$).

Discussion

We compared post-thaw viability and recovery of CD34+ and CD3+ cells from cellular therapies cryopreserved in 5% DMSO and 6% pentastarch and stored in liquid nitrogen. These studies confirmed that CD34+ cells in G-CSF-mobilized PBSC concentrates cryopreserved shortly after collection (within 48 hours) tolerate cryopreservation and storage well. We found the transport and many hours of storage associated with the shipment of PBSC concentrates collected from unrelated donors had no effect on the post-thaw recovery viable CD34+ cells in G-CSF-mobilized PBSC products.

In contrast, CD3+ cells did not tolerate cryopreservation and storage as well as CD34+ cells. The post-thaw recovery of CD3+ cells was less than that of CD34+ cells and when related and unrelated donor products were compared, the CD3+ cell recovery was less in unrelated donor products compared to related donor products. However, there were two major differences between the related and unrelated donor lymphocyte products studied: (1) all of the unrelated lymphocyte product donors had been given G-CSF while only about 40% of the related lymphocyte product donors were given G-CSF and (2) the unrelated donor products had been stored longer prior to cryopreservation. G-CSF is known to modulate lymphocyte function^{20,21} and it is possible that G-CSF-induced changes in CD3+ cells could affect their tolerance of cryopreservation and thawing. It is also possible that prolonged liquid storage of the products prior to cryopreservation could damage CD3+ cells, thereby, reducing their ability to survive the cryopreservation and thawing process. Alternatively, other cells in the lymphocyte products, such as granulocytes, could have been damaged or stimulated by prolonged liquid storage causing them to release proteolytic enzymes,

cytokines and chemokines that affected T cells. Our study did not evaluate which if any of these factors were responsible for the reduced CD3+ cell post-thaw viability.

DLI therapy using leukocytes that have been collected from donors who have not been given G-CSF requires a separate apheresis collection which is dependent on donor availability and willingness to donate. This can delay the treatment, particularly when cells are needed from unrelated donors. For related donor transplants at some centers at the time of transplant lymphocyte products that can be used as DLIs are collected from donors who have not been given G-CSF and the cells are cryopreserved within a few hours of collection. In this situation we found that the CD3+ cell recovery was high and similar to that of CD34+ cells ($94.5 \pm 13.1\%$ versus $97.5 \pm 23.1\%$). In contrast, for unrelated donor transplants using G-CSF mobilized PBSCs when excess cells are collected some centers routinely cryopreserve these cells for DLI therapy.²² However, we found that when aliquots from unrelated donor G-CSF-mobilized PBSC concentrates are used as DLI products, the CD3+ recovery was much less than that of products collected from related donors who were not given G-CSF.

The quantity of CD3+ cells in DLI products is important to their safety and effectiveness. Infusing too many CD3+ cells may cause acute GVHD while infusing too few may result in an ineffective graft versus leukemia effect.^{22,23} Our data suggests that when patients are treated with cryopreserved DLIs collected after G-CSF-mobilization from unrelated donors the recipient may not be getting as many CD3+ cells as expected. Based on these data, we recommend that the CD3+ post thaw recovery be measured on all such unrelated donor G-CSF-mobilized products used for DLIs and if clinically indicated additional cells should be given.

In conclusion, G-CSF-mobilized PBSC concentrates that have been collected from unrelated donors, stored and transported can be cryopreserved without a significant loss of CD34+ cells. However, in marked contrast, under the same conditions, there is a significant reduction in viable CD3+ cells. Further studies will be necessary to address the mechanism(s) responsible for the reduction in recovery of CD3+ cells. Most importantly, these data indicate that when unrelated donor G-CSF mobilized PBSC concentrates are used as DLI products, reduction in post-thaw CD3+ cell content may have clinical implications.

Acknowledgments

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Reference List

1. Galmes A, Besalduch J, Bargay J, Matamoros N, Duran MA, Morey M, Alvarez F, Mascaro M. Cryopreservation of hematopoietic progenitor cells with 5-percent dimethyl sulfoxide at -80 degrees C without rate-controlled freezing. *Transfusion*. 1996 Sep; 36(9):794–797. [PubMed: 8823452]
2. Galmes A, Besalduch J, Bargay J, Novo A, Morey M, Guerra JM, Duran MA. Long-term storage at -80 degrees C of hematopoietic progenitor cells with 5-percent dimethyl sulfoxide as the sole cryoprotectant. *Transfusion*. 1999 Jan; 39(1):70–73. [PubMed: 9920169]
3. Abrahamsen JF, Rusten L, Bakken AM, Bruserud O. Better preservation of early hematopoietic progenitor cells when human peripheral blood progenitor cells are cryopreserved with 5 percent dimethylsulfoxide instead of 10 percent dimethylsulfoxide. *Transfusion*. 2004 May; 44(5):785–789. [PubMed: 15104663]

4. Liseth K, Abrahamsen JF, Bjorsvik S, Grottebo K, Bruserud O. The viability of cryopreserved PBPC depends on the DMSO concentration and the concentration of nucleated cells in the graft. *Cytotherapy*. 2005; 7(4):328–333. [PubMed: 16162454]
5. Liseth K, Ersvaer E, Abrahamsen JF, Nesthus I, Rynningen A, Bruserud O. Long-term cryopreservation of autologous stem cell grafts: a clinical and experimental study of hematopoietic and immunocompetent cells. *Transfusion*. 2009 Apr 20.
6. Akkoc CA, Liseth K, Nesthus I, Lokeland T, Tefre K, Bruserud O, Abrahamsen JF. Autologous peripheral blood progenitor cells cryopreserved with 5 and 10 percent dimethyl sulfoxide alone give comparable hematopoietic reconstitution after transplantation. *Transfusion*. 2008 May; 48(5):877–883. [PubMed: 18298599]
7. McCullough J, Haley R, Clay M, Hubel A, Lindgren B, Moroff G. Long-term storage of peripheral blood stem cells frozen and stored with a conventional liquid nitrogen technique compared with cells frozen and stored in a mechanical freezer. *Transfusion*. 2010 Apr; 50(4):808–819. [PubMed: 19912586]
8. Valeri CR, Pivacek LE. Effects of the temperature, the duration of frozen storage, and the freezing container on in vitro measurements in human peripheral blood mononuclear cells. *Transfusion*. 1996 Apr; 36(4):303–308. [PubMed: 8623128]
9. Broxmeyer HE, Cooper S. High-efficiency recovery of immature haematopoietic progenitor cells with extensive proliferative capacity from human cord blood cryopreserved for 10 years. *Clin.Exp.Immunol*. 1997 Jan; 107(Suppl 1):45–53. [PubMed: 9020936]
10. Antoniewicz-Papis J, Lachert E, Wozniak J, Janik K, Letowska M. Methods of freezing cord blood hematopoietic stem cells. *Transfusion*. 2013 Apr 29.
11. Abbruzzese L, Agostini F, Durante C, Toffola RT, Rupolo M, Rossi FM, Lleshi A, Zanolin S, Michieli M, Mazzucato M. Long term cryopreservation in 5% DMSO maintains unchanged CD34(+) cells viability and allows satisfactory hematological engraftment after peripheral blood stem cell transplantation. *Vox Sang*. 2013 Jul; 105(1):77–80. [PubMed: 23384290]
12. Castelhana MV, Reis-Alves SC, Vigorito AC, Rocha FF, Pereira-Cunha FG, De Souza CA, Lorand-Metze I. Quantifying loss of CD34+ cells collected by apheresis after processing for freezing and post-thaw. *Transfus.Apher.Sci*. 2013 Apr; 48(2):241–246. [PubMed: 23394728]
13. Stroncek DF, Xing L, Chau Q, Zia N, McKelvy A, Pracht L, Sabatino M, Jin P. Stability of cryopreserved white blood cells (WBCs) prepared for donor WBC infusions. *Transfusion*. 2011 Dec; 51(12):2647–2655. [PubMed: 21658051]
14. Steininger PA, Smith R, Geier C, Zimmermann R, Eckstein R, Strasser EF. Leukapheresis in non-cytokine-stimulated donors with a new apheresis system: first-time collection results and evaluation of subsequent cryopreservation. *Transfusion*. 2013 Apr; 53(4):747–756. [PubMed: 22803702]
15. McIver Z, Melenhorst JJ, Wu C, Grim A, Ito S, Cho I, Hensel N, Battiwalla M, Barrett AJ. Donor lymphocyte count and thymic activity predict lymphocyte recovery and outcomes after matched-sibling hematopoietic stem cell transplant. *Haematologica*. 2013 Mar; 98(3):346–352. [PubMed: 23065508]
16. Jansen J, Nolan PL, Reeves MI, Akard LP, Thompson JM, Dugan MJ, Hanks SG. Transportation of peripheral blood progenitor cell products: effects of time, temperature and cell concentration. *Cytotherapy*. 2009; 11(1):79–85. [PubMed: 19153854]
17. Lazarus HM, Kan F, Tarima S, Champlin RE, Confer DL, Frey N, Gee AP, Wagner JE, Horowitz MM, Eapen M. Rapid transport and infusion of hematopoietic cells is associated with improved outcome after myeloablative therapy and unrelated donor transplant. *Biol.Blood Marrow Transplant*. 2009 May; 15(5):589–596. [PubMed: 19361751]
18. Antonenas V, Garvin F, Webb M, Sartor M, Bradstock KF, Gottlieb D. Fresh PBSC harvests, but not BM, show temperature-related loss of CD34 viability during storage and transport. *Cytotherapy*. 2006; 8(2):158–165. [PubMed: 16698689]
19. Kao GS, Kim HT, Daley H, Ritz J, Burger SR, Kelley L, Vierra-Green C, Flesch S, Spellman S, Miller J, et al. Validation of short-term handling and storage conditions for marrow and peripheral blood stem cell products. *Transfusion*. 2010 Jul 1.

20. Sloan EM, Kim S, Maciejewski JP, Van Rhee F, Chaudhuri A, Barrett J, Young NS. Pharmacologic doses of granulocyte colony-stimulating factor affect cytokine production by lymphocytes in vitro and in vivo. *Blood*. 2000 Apr 1; 95(7):2269–2274. [PubMed: 10733495]
21. Tayebi H, Kuttler F, Saas P, Lienard A, Petracca B, Lapierre V, Ferrand C, Fest T, Cahn J, Blaise D, et al. Effect of granulocyte colony-stimulating factor mobilization on phenotypical and functional properties of immune cells. *Exp.Hematol*. 2001 Apr; 29(4):458–470. [PubMed: 11301186]
22. Roddie C, Peggs KS. Donor lymphocyte infusion following allogeneic hematopoietic stem cell transplantation. *Expert.Opin.Biol.Ther*. 2011 Apr; 11(4):473–487. [PubMed: 21269237]
23. Deol A, Lum LG. Role of donor lymphocyte infusions in relapsed hematological malignancies after stem cell transplantation revisited. *Cancer Treat.Rev*. 2010 Nov; 36(7):528–538. [PubMed: 20381970]

Table 1

Results of the post-thaw analysis of related and unrelated donor G-CSF-mobilized PBSC products

	Related Donors (n=86)	Unrelated Donors (n=14)	p
TNC Viability	78.7±8.3%	64.2±19.0%	4.58×10 ⁻⁶
CD34+ cell content	0.81±0.44%	0.88±0.40%	0.544
TNC recovery	84.6±11.9%	95.9±20.8%	0.00271
CD34+ cell recovery	97.5±23.1%	98.8±37.2%	0.86

Table 2

Results of the post-thaw analysis of related and unrelated donor apheresis products used for donor lymphocyte infusions.

	Related Donors (n=48)	Unrelated Donors (n=14)	p
TNC Viability	85.6±9.7%	70.2±16.5%	0.000045
CD3+ Cell Content	36.2±17.8%	20.7±15.5%	0.0046
TNC Recovery	99.8±11.9%	101.7±18.7%	0.655
CD3+ Cell Recovery	90.7±21.4%	66.6±35.8%	0.00251

Table 3

Comparison of post-thaw analysis of lymphocyte products collected from related donors given and not given G-CSF

	Related Donors Not Given G-CSF (n=29)	Related Donors Given G-CSF (n=19)	p
TNC Viability	89.4±8.5%	79.7±8.4%	0.00033
CD3+ Cell Content	47.8±11.6%	18.6±8.4%	2.5×10^{-12}
TNC Recovery	100.7±12.5%	98.4±11.0%	0.522
CD3+ Cell Recovery	94.5±13.4%	85.0±29.2%	0.137