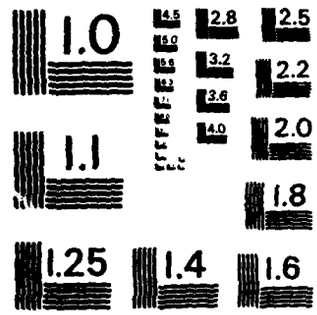
AD-A133 986 COLLECTION SEPARATION CRYOPRESERVATION AND 1/1
CHARACTERIZATION OF PERIPHERAL..(U) NAVAL BLOOD
RESEARCH LAB BOSTON MASS A J ROY ET AL. 01 OCT 83
UNCLASSIFIED N00014-79-C-0489 F/G 6/5 NL



(12)

OFFICE OF NAVAL RESEARCH

Contract N00014-79-C-0489

Task No. NR207-196

ANNUAL REPORT

June 1, 1982 to May 31,1983

COLLECTION, SEPARATION, CRYOPRESERVATION AND CHARACTERIZATION OF PERIPHERAL BLOOD AND BONE MARROW STEM CELLS AND THEIR USE IN TREATING LETHALLY IRRADIATED DOGS

Albert J. Roy, Ph.D.
Naval Blood Research Laboratory
Boston University School of Medicine
615 Albany Street
Boston, MA. 02118

AD-A133 196

1 October, 1983

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Annual Report No. 2	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): A Simplified Method for the Cryopreservation of Canine Peripheral Blood Mononuclear Cells Obtained as a By-product of Platelet Collection	5. TYPE OF REPORT & PERIOD COVERED: 6/1/82-5/31/83
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): A.J. Roy, A.J. Melaragno, L.T. Lavigne, Jr., A.D. Gray, J. Dittmer, and C.R. Valeri	8. CONTRACT OR GRANT NUMBER(s): ONR N00014-76-C-0489
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Boston University School of Medicine, Naval Blood Research Laboratory, Boston, MA 02118	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NR207-196, Code 444
11. CONTROLLING OFFICE NAME AND ADDRESS: Biological Sciences Division, Office of Naval Research (N00014), 800 N. Quincy Street, Arlington, VA 22217	12. REPORT DATE: 1 October 1983
	13. NUMBER OF PAGES: 27
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): Same as above	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE: NA

16. DISTRIBUTION STATEMENT (of this Report)
Distribution of this report is unlimited [redacted]

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)
Same as above

18. SUPPLEMENTARY NOTES
NA

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
blood mononuclear cells, cryopreservation, mononuclear cell harvest and preservation

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
Peripheral blood mononuclear cells (MNC) were obtained from buffy coats collected during 26 platelet apheresis procedures in the dog using the Haemonetics 30 Blood Processor. The number of mononuclear cells and platelets were determined for each procedure. After separation of the platelet rich plasma, dimethylsulfoxide (Me_2SO) in McCoy's medium was added to the residual buffy coats rapidly in 1 to 2 minutes or slowly over 15 to 20 minutes. The cell suspensions were frozen in polyolefin plastic bags at 2-3 C per minute by

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

10 to the 11th Power 1,340,000,000

placing the plastic bag in a -80 C freezer or at 1 C per minute by use of a graded freezing apparatus. The percentage of viable MNC's recovered was determined after thawing and washing by measurement of uptake of fluorescein deacetate and thidium bromide. The average harvest of platelets from a standardized four pass procedure was 1.26×10^{11} cells and of MNC's, ~~1.34×10^{9}~~ cells. Viable recovery of frozen, thawed and washed MNC's was similar when frozen at 1 C/minute with slow addition of the cryophylactic agent to those frozen at 2-3 C/minute with rapid addition.

A SIMPLIFIED METHOD FOR THE CRYOPRESERVATION OF CANINE PERIPHERAL BLOOD MONONUCLEAR CELLS OBTAINED AS A BY-PRODUCT OF PLATELET COLLECTION

A.J. ROY, A.J. MELARAGNO, L.T. LAVIGNE, JR.,A.D. GRAY,

J. DITTMER AND C.R. VALERI

NAVAL BLOOD RESEARCH LABORATORY
BOSTON UNIVERSITY SCHOOL OF MEDICINE
BOSTON, MA, 02118

Prepared for publication
in
Cryobiolgy

INTRODUCTION

Aplasia of the bone marrow has long been recognized as a potentially lethal sequela of disease of the blood forming organs and as a side-effect of total body irradiation. It was not until the possibility of widespread use of ionizing radiation, both in weapons systems and for therapy of malignant and other diseases as well as the first use of bone-marrow inhibiting antineoplastic chemicals in the middle to late forties that concerns about treatment of bone marrow aplasia received serious consideration. Only since the mid-fifties however, have a substantial number of reports describing bone-marrow transplantation appeared.[1-5] It is not coincidental that most of the early reports of successful transplants described the use of cryo-preserved marrow. Early trials of homologous transplants were largely unsuccessful because of the dearth of data concerning the immunologic requirements for establishing and maintaining stable allografts. The great majority of the reports of successful bone marrow engraftments in man or animal have been the result of autologous or isologous transplants.[6] Marrow for <u>autologous</u> infusion must be obtained before the insult to the bone marrow occurs, in the case of irradiation or while a patient is in remission in circumstances where the intention is to treat a patient in which a relapse of a hematologic abnormality is predictable.

In most instances, the cells must be stored for periods beyond which they can reasonably be expected to remain viable in the non-frozen state. A variety of cryoprotective media[7,8,9], freezing rates[10,11,12], freezing containers[7,12,13], etc. have been suggested as optimal for successful cryopreservation of bone marrow. Many of these methods have been successful, as measured by several *in vitro* and *in vivo* criteria.

There are a variety of problems associated with marrow transplants, however.[14, 15] For example, a surgical procedure is required which is not easily repeated; the number of cells obtained may be insufficient for engraftment; general anesthesia is required. There is great heterogeneity of bone marrow cells. Recent evidence suggests that stem cells may be obtained from the peripheral blood in quantities sufficient to repopulate a compromised bone marrow.[14,16-20] Compared to the use of autologous bone marrow transplantation the use of peripheral blood mononuclear cells to repopulate the bone marrow has several advantages:

(a) repeated cell collections are possible over a relatively short period of time.

(b) no anesthesia is required

(c) further purification for cryopreservation of cells destined for autologous therapy may not be necessary

(d) no surgical procedures are required

The same requirements obtain for transfusion of autologous mononuclear cells as for bone marrow with respect to the need for storage over a reasonably long period of time. The extensive results from studies of cryopreservation of bone marrow are now being applied to that of peripheral blood mononuclear cells. There are essentially no common protocols for cryopreservation of those cells. There is general agreement however,

that dimethylsuloxide (Me_2SO) in a concentration of about 10% is the best cryoprotective agent reported to date. The cells should be frozen at somewhere between 1 and 3 C/minute to at least -40 C. In most instances, a graded freezing unit is used to reduce the temperature at a constant rate. The freezing time may vary, therefore, between 15 and 45 minutes. There are some cooling regimens which are programmed in rather complicated patterns, e.g. 6 C/min (liquid phase), 90 sec phase transition time, 2 C/min down to -35 C, 20 C/min down to -100 C. This procedure required the design of an entirely new freezing device.[15]

Previous experience by this group in cryopreservation of platelets suggested that these cells could be successfully frozen without using a graded freezing apparatus.[21] When placed in a mechanical freezer at -80 C, there was a relatively constant freezing rate of 2 to 3 C/min. One of the objectives of the present study was to determine whether peripheral blood mononuclear cells could be successfully cryopreserved by this simple freezing technique.

In general, peripheral blood MNC's are separated from the contaminating red blood cells before freezing by sedimentation of the RBC's with dextran[12] or hydroxethyl starch[20] and often further purification by Ficoll-Hypaque gradient centrifugation.[22] Goldman, however, although using controlled rate freezing routinely froze buffy coats together with contaminating red cells[10] with apparently satisfactory results. A second objective of this study, therefore, focused on the recovery and viability of frozen/thawed peripheral blood MNC's without separation from the contaminating erythrocytes.

For practical purposes, a decision to repopulate the bone marrow of patients rendered aplastic either by irradiation or by marrow-inhibiting

drugs also dictates extensive blood component support. This includes several to many platelet transfusions to treat the concomitant thrombocytopenia. Previous experience in harvesting platelets with the Haemonetics 30 had shown that the residue obtained from separation of the platelet-rich plasma from contaminating red blood cells was relatively rich in mononuclear cells.[23] A third objective of this study was to determine whether it was possible to collect significant numbers of peripheral blood MNC's as a by-product of platelet collections.

MATERIALS AND METHODS

Isolation of Platelets and Buffy Coats by Discontinuous - Flow Centrifugation Using the Haemonetics - 30 Blood Processor

Healthy beagle dogs weighing 10 to 15 kg were studied. At least two weeks before their first apheresis procedure, an arterio-venous fistula was surgically created between the carotid artery and jugular vein to provide sufficient pressure in the vein to allow adequate blood flow to the blood processing apparatus. For blood component collection an outflow catheter (16 gauge) was inserted into the venous segment of the A.V. fistula. A return catheter (18 gauge) was inserted into a vein in the fore leg. A Haemonetics pediatric bowl (125 ml capacity) was used. Platelet collection was started when the platelet-containing ring was about 2 cm from the core of the bowl. Before beginning the platelet collection, blood was collected into the bowl at 60 ml/minute. During the platelet and buffy coat collection, the flow was reduced to 20 ml/minute and was continued for either 45 or 60 seconds after red blood cells appeared in the collection port. The excess plasma and red blood cells were returned to the dog. This was repeated three times, for a total of four "passes." Acid-citrate-dextrose (Formula A) in a ratio of 1:7 was used as the anticoagulant. A total of 120 to 200 ml was collected.

Separation of Cellular Components

A volume of ACD, Formula A, equivalent to 7.5% of the volume of the platelet and MNC-rich concentrate was added and the mixture was centrifuged at 160 g for 10 minutes in a Sorval R3CB centrifuge at room temperature. The platelet-rich plasma free of red blood cells and leukocytes was expressed into a separate 300 ml transfer pack and the platelets were

concentrated by centrifugation at 4500 g for 5 minutes. All but 30 ml of the platelet-poor plasma was expressed from the platelet concentrate into a second transfer pack (300 ml) .This platelet-poor plasma was frozen at -20 C for future use. The platelet concentrate was kept at room temperature for 90 minutes and then gently resuspended by manual agitation. A platelet count was obtained in duplicate using phase-contrast microscopy. The platelet yield was calculated by multiplying the mean of these two counts by the volume. A small sample of the MNC-rich residue was obtained for determination of the number of MNC's. White blood cell counts were determined in triplicate using the Coulter mod. ZF. Smears were prepared in duplicate for differential white blood cell counts. The number of MNC's was determined by multiplying the white blood cell count by the percentage of mononuclear cells on the smears and by the volume of the residue.

Cryopreservation of Buffy Coat MNC's

A cryopro tective solution in a volume equal to the volume of buffy coat to be frozen (30 to 40 ml) was prepared by addition of dimethylsulfoxide to McCoy's medium in a ratio of 80:20. The appropriate volume of cold McCoy's was pipetted into a sterile bottle which was in ice. The Me_2SO was injected into the McCoy's by syringe over about one minute with constant manual spinning of the bottle to dissipate the heat generated by an endothermic reaction. With use of an 18 gauge, 3½ inch spinal needle, the Me_2SO/McCoy's mixture was added to the cell suspension which had been transferred aseptically to a polyolefin (Del Med) freezing bag fitted with a sampling port (Fenwal # 4C2405).Addition of the cryoprotectant was accomplished in one of two ways:

(a) rapid addition in one to two minutes with constant agitation of the bag on ice

(b) slow addition by drip over 15 to 20 minutes with constant agitation at about 100 lateral oscillations per minute, on ice.

The bag was placed in an aluminum freezing container and frozen in one of two ways:

(a) by immediate placement into a -80 C mechanical freezer for 12 hours followed by placement in the gas phase of liquid nitrogen refrigerator (-150 C)

(b) freezing in a Cryo-Med graded freezing unit at 1 C/min from +5 to -40 C followed by placement in the gas phase of a liquid nitrogen refrigerator (-150 C)

Three of these combinations were used in this study:

(a) rapid addition of cryoprotectant followed by immediate placement in -80 C freezer (simple protocol)

(b) slow addition of cryoprotectant and immediate placement at -80 C

(c) slow addition of cryoprotectant and freezing at 1 C/min

Thawing and Washing Procedures

The concentrates were thawed by immersion of the bag in a 37 C water bath with constant manual agitation until the last ice particle had melted. This was generally accomplished in less than 30 seconds. An 0.5 ml sample was immediately withdrawn aseptically by syringe and needle for counts and 50 ml of McCoy's medium was added rapidly by needle through the sampling port. The dilute concentrate was transferred to a 300 ml polyvinylchloride bag and centrifuged at 4160 rpm for 5 minutes in a Sorval R3CB centrifuge. The supernatant was drawn off through a sterile Fenwal #4C2244 transfer set and replaced with 35 to 50 ml of homologous plasma. The cells were re-suspended by gentle manual agitation of the bag. A second 0.5 ml sample was withdrawn for testing purposes. In the late stages of the study,

a second wash with 50 ml McCoy's medium was interposed between the first wash and the final suspension in plasma. A sample was also obtained following this wash for counts.

The studies carried out after thawing and washing were:

(1) total WBC count in triplicate using the Coulter ZF

(2) differential WBC count by traditional methods on slides stained with Wright's-Giemsa

(3) viability index using fluorescein diacetate (FDA) (green fluorescence= viable) and ethidium bromide (EB) (red fluorescence = nonviable) For this, a Zeiss fluorescence microscope was used.

RESULTS

As seen in Table 1 the average harvest of platelets from a four pass apheresis procedure was 1.26×10^{11} cells. From the same collection, an average yield of 1.34×10^{9} peripheral blood mononuclear cells was obtained.

Recoveries of mononuclear cells after freezing and thawing were greatest when they had been frozen at 1 C/min with slow addition of the cryophylactic solution. (Table 2) However, the difference between those cells and the samples frozen by the simple protocol in the -80 C freezer, although measurable, were inconsequential. The lowest recoveries were obtained using slow addition of the Me_2SO solution and freezing in the -80 C freezer. However, the percentage viability of the recovered cells was greatest, although not substantially in the samples frozen in the -80 C freezer with rapid addition of the cryophylactic solution (simple protocol). In 19 of 21 individual experiments, the viability index was increased after washing the cells in McCoy's medium and suspension in plasma as compared with the cells measured immediately post-thaw. The remaining two samples showed essentially identical viabilities.

Calculation of the combined recovery and viability of frozen, thawed, and washed MNC's (Table 4) demonstrated no significant difference between those frozen at 1 C/min with slow addition of the cryophylactic agent and those frozen at -80 C with rapid addition. The lowest recovery of viable cells occured among those frozen at -80 C after slow addition of the DMSO/McCoy's solution.

Increasing the buffy coat collection from 45 to 60 seconds after red blood cells appeared in the collection port resulted in a slight increase in the collection of white blood cells (Table 5). However, this increase represented essentially all granulocytes and did not improve the collection of mononuclear cells.

DISCUSSION

Estimates of the number of cryopreserved autologous peripheral blood mononuclear cells required to repopulate the bone marrow of lethally irradiated animals or human beings range from 0.6 to 2.4 x 10^9 per kilogram of body weight[10,18,26-28]. In essentially every report of cryopreservation of these cells, a more or less complicated freezing regimen has been used. The harvesting of these MNC's has also, for the most part, been carried out exclusively for the collection of these cells apart from any other procedure for the collection of other blood cellular components for support of irradiated individuals during the period of bone marrow aplasia.

Our experience in supporting lethally irradiated dogs with platelets has suggested that as many as 12 to 15 units of platelets may be required to prevent bleeding for a period of time during which these animals are at risk from an aplastic bone marrow (unpublished observations). The studies reported here have demonstrated that it is possible to collect more than 1.3 x 10^9 peripheral blood MNC's as a by-product of each platelet collection from dogs weighing as little as 10 kg. By this means, while collecting sufficient numbers of platelets to support the irradiated dogs during their period of thrombocytopenia, it is possible to collect 16-19 x 10^9 mnc's from the peripheral blood simultaneously. This represents up to 1.9 x 10^9 MNC's per kg for a 10 kg dog or up to 1.3 x 10^9 cells/kg for a 15 kg animal. These numbers are well within the minimum limits for transfusion requirements as described by other investigators. At the rate of two apheresis procedures per week, six or seven weeks are required to collect sufficient numbers of MNC's and platelets

for support of a lethally irradiated dog using this regimen. Because of the time required for collecting these cells, cryopreservation of both the platelets and MNC's is required because liquid preservation of neither of these cells is possible for more than a few days.

The simplest freezing procedure consistent with the recovery of an optimal proportion of viable cellsis the goal of these investigations. The vast majority of the reports of cryopreservation of these cells have described, as a minimum, freezing at defined rates in a cryophylactic medium. In most instances, freezing at pre-determined rates requires the use of liquid nitrogen as well as expensive freezing instruments. A simple, effective freezing method, in addition to providing convenience to well equipped laboratories would make possible the cryopreservation of MNC's under less than ideal conditions, e.g., in developing nations, in institutions where the number of patients to be supported with MNC's is too few to justify expensive instrumentation, etc. The studies described here demonstrate that viable peripheral blood mononuclear cells, frozen as buffy coat in a mechanical freezer can be recovered in numbers not significantly less than those frozen at 1 C per minute in a graded-rate freezer. Viability was measured using uptake of fluorescein diacetate and exclusion of ethidium bromide, as described by Persidsky[24] and by Lionetti[25].

The recovery of viable peripheral blood mononuclear cells following our simplified feezing method compares favorably with reports of other investigators using more complicated freezing regimens[13,14,15,18,20].

TABLE 1

HARVEST OF PERIPHERAL BLOOD MONONUCLEAR CELLS AND PLATELETS FROM 26 APHERESIS PROCEDURES IN NORMAL DOGS

	WBC x 10^9	%MNC	Total MNC x 10^9	Platelets x 10^{11}
MEAN	2.03	67.1	1.34	1.26
S.D.	.43	14.5	.39	.45
RANGE	1.09 - 3.05	41.5-91.0	0.88-2.38	0.25-2.54

TABLE 2

RECOVERY* OF PERIPHERAL BLOOD MONONUCLEAR CELLS
FOLLOWING FREEZING, THAWING AND WASHING

METHOD OF FREEZING	NO. OF EXPTS	NO. OF CELLS FROZEN x 10^9	NO. OF CELLS RECOVERED POST-THAW x 10^9	% OF NO. FROZEN	NO. OF CELLS RECOVERED POST-WASH x 10^9	%OF NO. FROZEN
-80 (simple)	8	.74± .44	.63± .29	83 ± 18	.70 ± .42	84 ± 15
-80	6	.69± .14	.59± .14	85 ± 11	.51 ± .17	73 ± 19
1 C/min	7	.70± .10	.67± .14	89 ± 11	.72 ± .27	94 ± 9

*Relates only to countable cells and does not address viability.

TABLE 3

VIABILITY OF PERIPHERAL BLOOD MONONUCLEAR CELLS FOLLOWING FREEZING, THAWING AND WASHING

METHOD OF FREEZING	NO. OF EXPERIMENTS	% VIABILITY AFTER THAWING	% VIABILITY AFTER WASHING
-80 (simple)	8	62 ± 13	80 ± 9
-80	6	56 ± 16	72 ± 11
1 C/min	7	47 ± 19	74 ± 8

TABLE 4

RECOVERY OF VIABLE PERIPHERAL BLOOD MONONUCLEAR CELLS FROM DOG BUFFY COATS AFTER FREEZING, THAWING AND WASHING

METHOD OF FREEZING	-80(simple)	-80	1 C/min
NUMBER OF EXPERIMENTS	8	6	7
No.OF CELLS FROZEN x 10^9	.74 ± .44	.69 ± .14	.70 ± .10
No. VIABLE POST-THAW x 10^9	.41 ± .15	.32 ± .06	.34 ± .21
% VIABLE POST-THAW	56 ± 22	46 ± 11	48 ± 22
No. VIABLE POST-WASH x 10^9	.50 ± .23	.39 ± .11	.51 ± .20
% VIABLE POST-WASH	67 ± 20	57 ± 08	73 ± 16

TABLE 5

EFFECT OF LENGTH OF COLLECTION INTO THE BUFFY COAT DURING PLATELET APHERESIS ON THE NUMBER OF WHITE BLOOD CELLS AND MONONUCLEAR CELLS ISOLATED USING THE HAEMONETICS MODEL 30 BLOOD PROCESSOR

DURATION OF COLLECTION	NUMBER OF EXPERIMENTS	TOTAL WBC x 10^9	% MNC	TOTAL MNC x 10^9
60 seconds	13	2.03 $\pm$ .34	60 $\pm$ 16	1.24 $\pm$ .34
45 seconds	34	1.88 $\pm$ .52	73 $\pm$ 14	1.33 $\pm$ .30

REFERENCES

1. Thomas, ED, Lochte, HL, Lu, WC: Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy, N. Engl. J. Med., 257:491-496, 1957.

2. Porter, KA and Murray, JE: Successful homotransplantation of rabbit bone marrow after preservation in glycerol at -70 C. Cancer Research, 18:17, 1958.

3. Schwartz, IR, Repplinger, EF, Congdon, CC and Tocantins, LM: Transplantation of bone marrow after preservation at -70 C. J. Appl. Physiol., 2:22, 1957.

4. Ferrebee, JW and Thomas, ED: Transplantation of marrow in man. Arch. Int. Med., 106:523, 1960.

5. Kurnick, NB, Montano, A, Gerdes, JC and Feder, BH: Preliminary observations on the treatment of postirradiation hematopoietic depression in man by the infusion of stored autologous bone marrow. Ann. Int. Med., 49:973, 1958.

6. Graze, PR and Gale, RP: Autotransplantation for leukemia and solid tumors. Transplantation Proc., 10: 1978.

7. Thomas, ED, Ferrebee, JW: Prolonged storage of marrow and its use in the treatment of radiation injury. Transfusion, 2:115, 1962.

8. Buckner, CD, Storb, R, Dillingham, LA: Low temperature preservation of monkey marrow in dimethylsulfoxide. Cryobiology, 7:136-140, 1970.

9. Shlafer, M: Drugs that modify cellular response to low temperatures (cryoprotectants). Fed. Proc., 36:2950-2959, 1977.

10. Goldman, JM, Th'ng, KH, Park, DS, Spiers, AS, Lowenthal, RM and Runtin, T: Collection, cryopreservation, and subsequent viability of hematopoietic stem cells intended for treatment of chronic granulocytic leukemia in blast-cell transformation. Brit. J. Haematol., 40:185-195, 1978.

11. Porcellun, A, Manna A, Manna, M, Moretti, L, Agostinelli, F and Tucarelli, G: Cryopreservation and subsequent viability of human bone marrow in hematologic malignancies: comparison of two different methods of cell reconstitution. Cryobiology, 18:541-546, 1981.

12. Ma, DD, Johnson, LA, Chan, PM and Biggs, JC: Factors influencing myeloid stem cell (CFU-C) survival after cryopreservation of human marrow and chronic granulocytic leukemia cells. Cryobiology, 19: 1-9, 1982.

13. Ellis, WM, Autken, W and Dobrostanski, B: The effect of cryopreservation on committed stem cells (CFU-c's) in humans. Crybiology, 18:238-243, 1981.

14. Epstein, RB and Sarpel, SC: Procurement and preservation of circulating hematopoietic cell in dogs. Proc. Haemonetics Research Inst. Adv. Computer Sem., Boston, MA, 1978.

15. Scheiwe, MW, Pusztai-Markoc, ZS, Essers, U, Seelis, R, Raw, G, Korber, C, Sturner, KH, Jing, H and Liedtke, B: Cryopreservation of human lymphocytes and stem cells (CFU-c) in large units for cancer therapy - A report based on the data of more than 400 frozen units. Cryobiology, 18:344-356, 1981.

16. Richman, CM, Weiner, RS and Yankee, RA: Increase in circulating stem cells following chemotherapy in man. Blood, 47:1031, 1976.

17. Fleidner, JM, Korbling, M, Calvo, W: Cryopreservation of blood mononuclear leukocytes and stem cells suspended in a large fluid volume. Blut, 35:195, 1977.

18. Sarpel, SC, Zander, AR, Harvath, L and Epstein, RB: The collection, preservation and function of peripheral blood hematopoietic cells in dogs. Exp. Hemat., 7:113-120, 1979.

19. Diesseroth, A and Abrams, RA: The role of autologous stem cell reconstitution in intensive therapy for resistant neoplasms. Cancer Treat. Rep., 63:461-471, 1979.

20. McGann, LE, Turner, AR, Allalunis, MJ and Turc, JM: Cryopreservation of human peripheral blood stem cells: optimal cooling and warming conditions. Cryobiology, 18:469-472, 1981.

21. Melaragno, AJ, Doty, AJ, Dittmer, J, Vecchione, JJ and Valeri, CR: Therapeutic effectiveness of cryopreserved autologous platelets in the treatment of thrombocytopenic dogs. Tech. Report No. 82-06. Naval Blood Research Laboratory, Boston, MA, April, 1982.

22. Ash, RC, Detrick, RA and Zanjani, ED: Studies of human pluripotential hemopoietic stem cells in vitro. Blood, 58:309-316, 1981.

23. Roy, A: Status Report No. 1 to the Office of Naval Research, Jan. 18, 1982.

24. Persidsky, MD and Ballie, GS: Fluorometric test of cell membrane integrity. Cryobiology, 14:322-331, 1977.

25. Lionetti, FJ, Hunt, SM, Mattaliano, RJ and Valeri, CR: In vitro studies of cryopreserved baboon granulocytes. Transfusion, 19: 685-692, 1978.

26. Seelis, R, Essers, V, Scheive, MW,Pusgtoi-Maokos, ZS, and Sturner, KH: Reconstitution of hematopoiesis with cryopreserved peripheral hemopoietic stem cells after high dose cytostatic treatment. Cryobiology, 624, 198

27. Debelak-Fehir, KM, Catchatourian, R and Epstein, RB: Hemopoietic colony forming units in fresh and cryopreserved peripheral blood cells of canines and man. Exp. Hemat., 3:109-116, 1975.

28. Applebaum, FR: Hemopoietic reconstitution following autologous bone marrow and peripheral blood mononuclear cell infusion. Exp. Hemat., 7:Suppl 5, 7-11, 1979.

ACKNOWLEDGEMENTS

This research was supported by Contracts #N00014-79-C-0489 and #N00014-79-C-0163 from the Office of Naval Research.

OFFICE OF NAVAL RESEARCH
BIOLOGICAL SCIENCES DIVISION
BIOPHYSICS PROGRAM, Code 444
DISTRIBUTION LIST FOR TECHNICAL, ANNUAL AND FINAL REPORTS

Number of Copies	
(13)	Administrator, Defense Documentation Center Cameron Station Alexandria, Virginia 22314
(6)	Director, Naval Research Laboratory Attention: Technical Information Division Code 2627 Washington, D. C. 20375
(6)	Office of Naval Research Attention: Code 102IP (ONRL DOC) 800 N. Quincy Street Arlington, Virginia 22217
(3)	Office of Naval Research Biophysics Program Code 444 Arlington, Virginia 22217
(1)	Commanding Officer Naval Medical Research and Development Command National Naval Medical Center Bethesda, Maryland 20014
(1)	Chief, Bureau of Medicine and Surgery Department of the Navy Washington, D. C. 20375
(2)	Technical Reference Library Naval Medical Research Institute National Naval Medical Center Bethesda, Maryland 20014
(1)	Office of Naval Research Branch Office Building 114, Section D 666 Summer Street Boston, Massachusetts 02210
(1)	Office of Naval Research Branch Office 536 South Clark Street Chicago, Illinois 60605

(1) Office of Naval Research Branch Office
1030 East Green Street
Pasadena, California 91106

(1) Commanding Officer
Naval Medical Research Unit No. 2
Box 14
APO San Francisco 96263

(1) Commanding Officer
Naval Medical Research Unit No. 3
FPO New York 09527

(1) Officer in Charge
Submarine Medical Research Laboratory
Naval Submarine Base, New London
Groton, Connecticut 06342

(1) Scientific Library
Naval Aerospace Medical Research Institute
Naval Aerospace Medical Center
Pensacola, Florida 32512

(1) Commanding Officer
Naval Air Development Center
Attn: Aerospace Medical Research Department
Warminster, Pennsylvania 18974

(1) DIRECTOR
Naval Biosciences Laboratory
Building 844
Naval Supply Center
Oakland, California 94625

(1) Commander, Army Research Office
P. O. Box 12211
Research Triangle Park
North Carolina 27709

(1) DIRECTORATE OF LIFE SCIENCES
Air Force Office of Scientific Research
Bolling Air Force Base
Washington, D. C. 20332

(1) Commanding General
Army Medical Research and Development Command
Forrestal Building
Washington, D. C. 20314

(1) Department of the Army
U. S. Army Science and
Technology Center - Far East
APO San Francisco 96328

(1) Assistant Chief for Technology
Office of Naval Research, Code 200
800 N. Quincy Street
Arlington, Virginia 22217

END

DATE
FILMED

11-83

DTIC