AD-A076 774 GEORGETOWN UNIV WASHINGTON DC LOMBARDI CANCER CENTER F/G 6/5
LD TYPING FOR BONE MARROW TRANSPLANTATION.(U)
SEP 79 P J ROMANO N00014-76-C-1173
UNCLASSIFIED NL

| OF |
AD
A076774

END
DATE
FILMED
12-79
DDC

13

OFFICE OF NAVAL RESEARCH

Contract (15) N000 14-76-C-1173
N000 14-77-C-0747

Task No. NR 207-067

(9) FINAL REPORT.
Sep 76 - Sep 78

(11) 5 September 1979

(12) 17

(6) LD TYPING FOR BONE MARROW TRANSPLANTATION.

By

(10) Paula J. Romano

Immunologic Oncology Division

Department of Pediatrics

Georgetown University School of Medicine

3900 Reservoir Road, N.W.

Washington, D.C. 20007

410 734 gw

LD TYPING FOR BONE MARROW TRANSPLANTATION

ONR Contract N000-14-76-C-1173

Background

In the course of performing relevant tasks Navy personnel are frequently faced with potentially hazardous duty. These hazards may take the form of physical trauma resulting in severe limb injury or exposure to toxic materials or irradiation, resulting in bone marrow depression and subsequent aplastic anemia. Military personnel are at greater risk of contact with bone marrow toxic agents than the civilian population, by virtue of working in hazardous areas. The necessity of using various toxic fuels and chemicals as well as potential exposure to irradiation, produce a hazard which requires the availability of bone marrow transplantation in the treatment of aplastic anemia from whatever etiologic source. Bone marrow transplantation in the case of aplastic anemia has become an accepted form of treatment and is currently funded in civilian institutions by health insurance plans. However, at this time, the only long-term survivors with this form of this therapy has occurred when HLA identical siblings have been used as the marrow donor. As only one third of the potential transplant recipients have matched sibling donors it is necessary to identify new methods of tissue matching and transplantation that will allow for the use of unrelated donors. Improved methods of tissue typing will be essential in the development of new methods for the broader use of bone marrow transplantation.

It is clear that the success of transplants, either organ or bone marrow, is greatest with the most compatible grafts, and is much less successful with poorly matched tissues. We have therefore initiated a program of tissue typing, in order to define the essential components of graft rejection and to seek ways to alter the body's natural rejection mechanisms.

In mid 1976 we received a contract to study the human histocompatibility system and to develop methods for large-scale, accurate tissue typing for support of ongoing kidney and bone marrow transplantation programs within the Navy.

At the present time tissue typing is essential for all current transplant programs. However, it is clear that typing of histocompatibility antigens must be extended and further refined to fulfill its potential as a major clinical tool. To outline the complexity of the HLA (Human Leukocyte Antigen) tissue typing system, it now appears that there are at least four genetic loci which control the cell surface glycoprotein antigens that are responsible for the rejection or acceptance of tissue grafts. These four loci have been called HLA-A, -B, -C and -D, and code for approximately 64 currently identified unique glycoprotein antigens. One of these, the HLA-D locus or LD (Lymphocyte defined) locus, is the least understood; however, it seems to play a dominant role in the acceptance or rejection of the tissue graft. The HLA-D products are the primary determinants of the in vitro proliferative response of leukocytes from one individual to leukocytes from a second (allogeneic) individual. This in vitro reaction to allogeneic leukocytes is called the mixed leukocyte reaction (MLR) and probably represents the body's major recognition system for foreign transplantation antigens. The use of HLA-D homozygous cells as stimulator cells in the MLR is the only currently available technique which defines the HLA-D region antigens. The research that we have initiated is directed toward the problem of examining this HLA-D system, defining the HLA-D locus antigens, and cataloging them in such a manner that individuals could be easily typed for these most important determinants. At the onset of the contract it was estimated that the seven accepted HLA-D antigen types represent about one fourth of the total, the remainder had not been identified. Currently, eleven types are recognized, in part, due to the work done at Georgetown.

Research Design

The initial plans for this contract were to follow a sequence of events.

1. Establishment of a contract facility with laboratory capacity to study the human histocompatibility system and do routine mixed lymphocyte culture testing.
2. Preparation of a panel of homozygous typing cells (HTCs) capable of identifying the common HLA-D specificities.
3. Cryopreserving and storing these reagents in such quantitites that they would allow typing of large numbers of individuals whenever necessary.

ACCOMPLISHMENTS

The LD Typing Contract was maintained for approximately two years, from September 1976 to September 1978. The primary goal of the contract was to establish a facility where HLA-D typing could be accomplished, and to identify and collect the essential reagents for large scale typing. During this period, an excellent lymphocyte typing facility was developed and 47 homozygous typing cells were identified and collected in large quantity. As the program developed, it was merged with a second ONR contract entitled, "Histocompatibility Typing" (N000-14-77-C-0747) which combined the advances of the intitial contract and extended the effort to include fundamental genetic studies as well as the development of large scale practical typing of random individuals and families.

More than 100 individuals were typed and cells from these donors cryopreserved in large number for later use. Unique methods of identifying HLA-D homozygous individuals were identified and new methods of cryopreserving and storing large numbers of viable lymphocytes were developed. Facilities and equipment were developed and organized in such a way so as to permit a small number of technicians to efficiently perform large experiments.

The Georgetown laboratory was a major participant in the 1977 International Histocompatibility Typing Workshop, as well as several American Histocompatibility Typing Workshops. In these cooperative workshops this laboratory made major contributions in identifying and testing new HLA-D typing reagents, and expanding the overall understanding of the HLA-D genetic region.

In conjunction with these accomplishments four scientific papers and four abstracts were accepted for publication and seven presentations were made.

When the contract was merged in September 1978, the LD typing program was well established and functioning at a high level. The work begun under this contract has proceeded, and the Laboratory is now internationally recognized as one of the 10 or 12 HLA-D typing centers in the world.

Data and Results

Outlined below are the cells collected and typed that were made available to our Navy investigators.

PUBLICATIONS

1. Hartzman, R.J., Ahmed, A., Strong, D.M., Pappas, F., Romano, P. and Sell, K.W. Generation of Highly Discriminate PLT Cells Using a Hybrid HTC-PLT System. Transplant. Proc. 9(4):1763, 1977.

2. Hartzman, R.J., Strong, D.M., Romano, P., Pappas, F. and Sell, K.W. Identification of HLA-D Specificities of a Random Population With Use of Homozygous Typing Cells (HTC). In Histocompatibility Testing 1977. W. Bodner (ed.), Copenhagen, Munksgaard pp. 536-539.

3. Hartzman, R.J. Summary of the First International Workshop on Human Primed LD Typing. Tissue Antigens. 13:203, 1979.

4. Hartzman, R.J., Pappas, F., Romano, P.J., Johnson, A.H., Ward, F.E. and Amos, D.B. Dissociation of HLA-D and HLA-DR Using Primed LD Typing. Transplantation Proceedings, 10(4):809, 1978.

5. Hartzman, R.J., Amos, D.B., Pappas, F., Johnson, A.H., Ward, F., Romano, P.J. and Sell K.W. Specificity of Primed LD Typing: The Major Reactions Transplantation Proceedings, 11(1):690, 1979.

ABSTRACTS

1. Romano, P.J., Jaffe, H. and Woody, J.N. HLA Restriction of Human Natural Killing Activity. Leukocyte Culture Conference, 1979.

2. Hartzman, R.J., Strong, D.M., Ahmed, A., Pappas, F. and Sell, K.W. Development of a Hybrid HTC-PLT System to Identify HLA-D Specificities. Abstract of the First International PLT Conference, January 10-12, 1977, Bethesda, Maryland.

3. Hartzman, R.J., Bailey, R.C., Ahmed, A., Strong, D.M., Pappas, F., Romano, P. and Sell, K.W. Genetics of the PLT. I. HLA-A, -B and-D Restrictions. Seventh Histocompatibility Workshop Abstract, Vol. 10, No. 3, Sept. 1977, p. 164.

4. Romano, P., Hartzman, R.J., Ahmed, A., Pappas, F., Strong, D.M. and Sell, K.W. In Vitro Characterization of the PLT Cell. Seventh Histocompatibility Workship Abstract, Tissue Antigens, Vol. 10, No. 3, Sept. 1977, p. 164.

5. Hartzman, R.J., Ahmed, A., Strong, D.M., Pappas, F., Romano, P. and Sell, K.W. Generation of Highly Discriminant PLT Cells Using a Hybrid HTC-PLT System. Submitted to American Association for Clinical Histocompatibility Testing, March, 1978.

6. Hartzman, R.J., Amos, D.B., Johnson, A., Ward, F., Pappas, F., Romano, P. and Sell, K.W. The Genetic Complexity of Primed LD Typing. Transplantation Congress, Sept. 1978.

PRESENTATIONS

1. Romano, P., HLA Restriction of Human Natural Killer Activity, Leukocyte Culture Conference, Ottawa, Canada, April, 1979.

2. Pappas, F., Development of Primed Lymphocyte Typing, American Association for Clinical Histocompatibility Testing, Boston, Mass., June, 1978.

3. Ayres, J., HLA-D Testing Using Homozygous Typing Cells, American Association for Clinical Histocompatibility Testing, June, 1978.

4. Pappas, F. Primed LD Typing for Clinical Testing, South East Organ Procurement Society meeting, Washington, D.C., May, 1978.

5. Ayres, J. HLA-D Typing for Transplantation, South East Organ Procurement Society meeting, Washington, D.C., May, 1978.

TABLE 1

HOMOZYGOUS TYPING CELLS IDENTIFIED

BY THE LD TYPING CONTRACT

CELL IDENTIFICATION		HLA-D SPECIFICITY
MLAU	(14)	1
GGRU	(201)	1
JREE	(53)	1
KCAR	(01)	2
TRAL	(02)	2
JBED	(07)	2
LAND	(75)	2
JFER	(85)	2
ELAB	(92)	2
MWEI	(299)	2
DCOR	(300)	2
RROU	(301)	2
MLEB	(03)	3
KSOL	(05)	3
EMYR	(10)	3
RKEL	(12)	3
EFIS	(28)	3
AFIS	(29)	3
LHOR	(250)	3
PLAN	(251)	3
DDOO	(302)	3
PLANG	(303)	3

CELL IDENTIFICATION (continued)		HLA-D SPECIFICITY (continued)
GYOD	(22)	4
LYOD	(21)	4
EYOD	(23)	4
AWHI	(91)	4
MJAR	(297)	4
MSCH	(298)	4
TFLE	(51)	5
KSTE	(94)	5
JSUM	(523)	5
CCAS	(569)	5
RSUM	(574)	5
BRAN	(157)	6
PBUR	(57)	7
TDOH	(118)	9
ESMO	(27)	11
PWIL	(40)	11
AKIN	(42)	11
KKIN	(42)	11
JKIN	(43)	11

CELL IDENTIFICATION (continued)		HLA-D SPECIFICITY (continued)	
PGED	(162)	LD 27a	(NEW)
JHAY	(295)	LD 40a	(NEW)
PHUM	(296)	LD 40a	(NEW)
VDIB	(64)	LD 40a	(NEW)
JMAT	(202)	LD 40b	(NEW)
ACOV	(26)	LD 45a	(NEW)

TABLE 2

Summary of Inventories of Frozen Cells Categorized by Serological HLA Types

Cells Homozygous for HLA-A and -B loci		No. Collected	Identification No.
HLA-A	HLA-B		
1	8	35	139, 110, 120, 106, 158, 17, 03, 99, 69, 147, 10, 144, 09, 146, 13, 70, 05, 16, 180, 129, 90, 213, 214, 29, 28, 06, 215, 226, 230, 234, 235, 240, 249, 250
2	12	23	161, 212, 183, 221, 190, 206, 14, 143, 111, 198, 53, 133, 94, 97, 91, 86, 105, 22, 238, 245, 257, 290, 204
3	7	16	76, 75, 07, 116, 134, 135, 130, 08, 119, 02, 04, 216, 117, 222, 239, 277
2	7	6	01, 140, 121, 92, 85, 231
29	12	7	09, 155, 104, 40, 27, 253, 74
2	40	6	151, 157, 154, 172, 149, 64
2	27	3	162, 153, 150
2	5	4	159, 14, 36, 24
11	35	2	148, 30
2	17	3	42, 43, 41
2	15	5	177, 15, 227, 229, 232
2	8	3	93, 24, 218
32	8	2	89
1	17	1	145
28	35	1	228
24	35	1	152
3	14	2	56, 118
9	12	1	201
1	35	2	195
3	35	2	223, 254
30	13	1	237
2	18	2	256
1	12	1	272
24	12	1	315
24	7	1	221

TABLE 3

HLA TYPED PANEL

Cells #	CODE	A locus	B locus	C locus	W4,W6	D locus
01	KCAR	2	7		W6	Dw2
02	TRAL	3	7		W6	Dw2
03	MLEB	1	8		W6	Dw3
04	GSNO	3	8			Dw3
05	KSOL	1	8		W6	Dw3
06	GHOV	1	8			Dw3
07	JBED	3	7		W6	Dw2
08	ROBR	3	7			Dw2
09	BRYD	1	8			Dw3
10	EMYR	1	8		W6	Dw3
11	RKEL	32	8			Dw3
12	BSHU	1	8			Dw3
13	MLAU	2	5		W4	Dw1
14	ECAR	2	w15.2	Cw3	W4,W6	Dw4,Dw2
15	DSUL	1	8			Dw3
16	DKOL	1	8			Dw3
17	JDES	1,28	8,18			Dw3,Dw7
18	DMCG	1,2	8,12			-
19	RBUD	2,11	44,w22	Cw3,5	W4,W6	Dw4,Dw9
20	LYOD	2	12			Dw4
21	GYOD	2	12		W4	Dw4
22	EYOD	2	12			Dw4
23	JWIL	2	8			Dw3
24	MLEV	2,w24	w35,w38	Cw4	W4,W6	Dw5,Dw9
25	ACOV	28	w45	Cw3	W6	LD45a
26	ESMD	29	12			Dw3
27	EFIS	1	12			Dw3
28	AFIS	1	8		W6	Dw3
29	ESMU	11	w35		W6	Dw1
30	SKRO	29	w15	C3	W4,W6	Dw1,Dw4
31	ISCH	2,11	35,52	Cw4	W4,W6	Dw1,Dw8
32	MGOL	26,32	7,44		W4,W6	-
33	GFRI	10,w32	w35,w18			-
34	ADAV	11,28	8,w22	Cw3	W6	Dw7,Dw9
35	PWIL	29	12		W4	Dw7
36	AKIN	2	w17			Dw11
37	KKIN	2	w17			Dw11
38	JKIN	2,9	w17			Dw11
39	TWIL	w26	44,38	Cw4	W4	Dw2,Dw6
40	MFOR	3,29	44,49		W4	Dw7,Dw3
41	JBUT	3,w24	7,w40			-
42	JHAR	2,Aw26	Bw38,27	Cw1	W4	Dw1
43	EFIN	Aw23,w25	Bw35,w37	Cw4	W4,W6	Dw6
44	WLET	2,Aw26	Bw16,w17			-

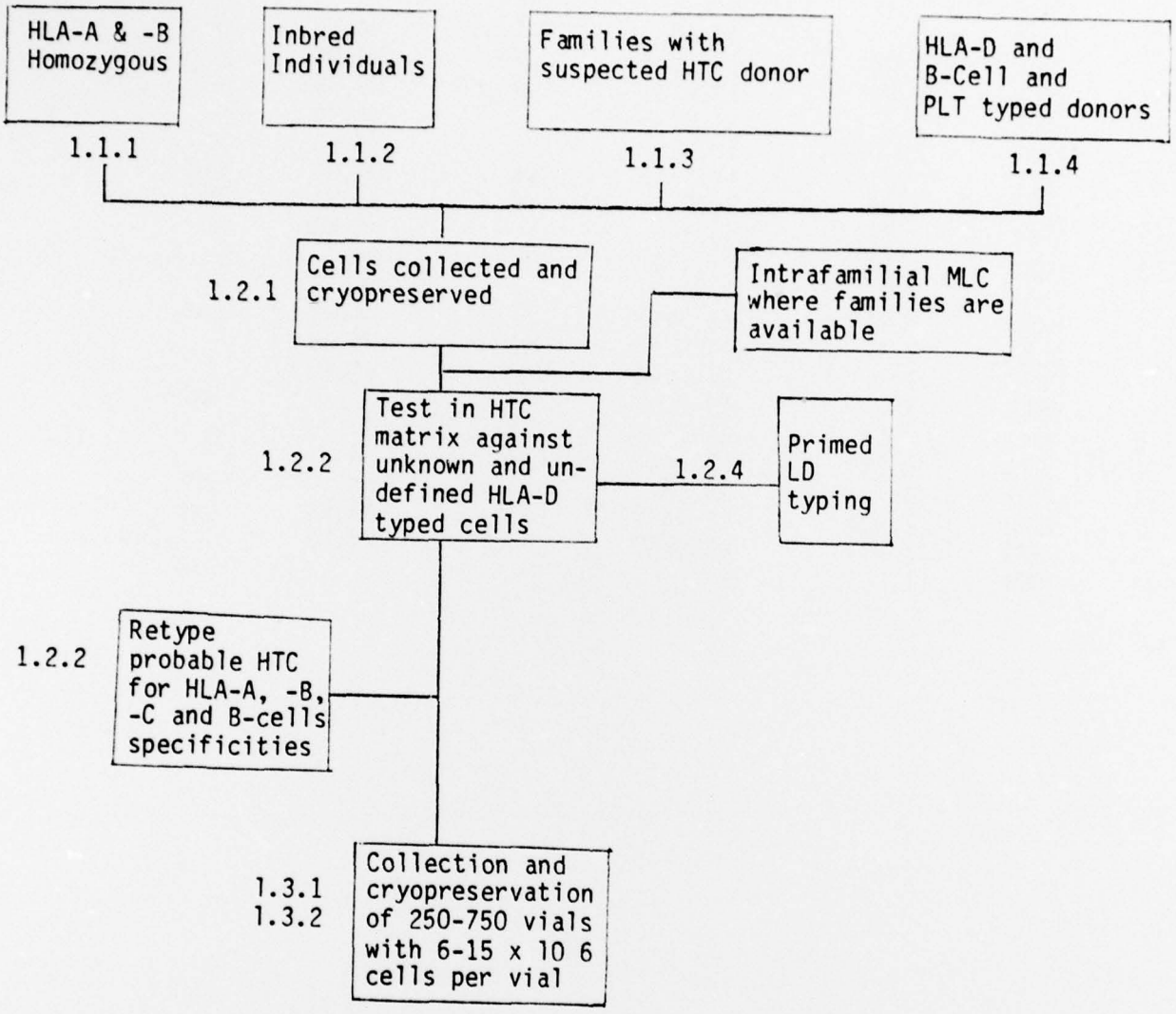
Cells #	CODE	A locus	B locus	C locus	W4,W6	D locus
45	TFLE	2,23	27,44	Cw2,Cw4	W4	Dw5
46	JSAU	1,w26	8,w38		W4,W6	Dw1,Dw3
47	JREE	2	12		W4	Dw1
48	HBUR	1,2	w16,14			Dw7
49	PBUR	1,2	14		W6	Dw7
50	JHAR	28,32	5,12	Cw3	W4	
51	RCAH	2,Aw32	7,44		W4,W6	Dw2
52	KSTE	1	w40	Cw3		Dw6
53	SLAI	1	w17,12			-
54	VDIB	2	w40	Cw3		D40a
55	LRYN	3,31	5,7	Cw4	W4,W6	Dw5
56	KGRO	w25,w26	7,w18			Dw2
57	SJOS	w30,w31	13,w49	Cw6	W4	Dw5
58	JPER	1,2	8,40	Cw3	W6	Dw4,w5
59	HDRE	2,24	44,35	Cw4	W4,W6	Dw5,Dw7
60	RBRO	1,11	8,13	Cw6	W4,W6	-
61	DVIL	24,33	17,35	Cw3,w4	W4,W6	Dw3,w7
62	LAND	3	7			Dw2
63	JKEE	1,w29	17,17	Cw4,w6		-
64	JHIL	29,30	22,44		W4,W6	Dw3,w7
65	JNAP	1,w30	7,8		W6	Dw3,w7
66	JLAM	2	12			Dw4
67	RKEL	32	8			Dw3
68	AWHI	2	44	Cw5	W4	Dw4
69	ELAB	2	7			Dw2
70	MWAS	2	8			Dw3
71	LSTE	2	12		W4	Dw5
72	JMOR	1,w25	8,w17		W4,W6	Dw3,w7
73	AWAL	2	w44		W4	Dw7
74	RHAR	2,26	17,41	Cw6	W4,W6	Dw5+ DW New
75	BCLA	3	7			Dw2
76	VQUE	3	7			Dw2
77	TDOH	3,w33	14		W6	Dw9
78	CCIS	2,w31	8,w15	Cw3	W6	Dw3,Dw9
79	TDAV	2,w25	44,18		W4,W6	Dw2,Dw4
80	DSTR	3,29	44,w35	Cw4	W4,W6	Dw7
81	HMCE	2	51,27	Cw2	W4	Dw4
82	DRIN	3,1	18,w51		W4,W6	Dw5
83	JBUD	1,32	14,8.1(long)		W6	Dw3,w7
84	RBRA	1,2	51,w15	Cw3,w4	W4,W6	Dw6,Dw10
85	MBUR	1,2	12,37	Cw5		Dw2,Dw6
86	GROB	25,29	12,18			-
87	ESHA	2	12	Cw5,w6	W4	Dw4,Dw2
88	MDUS	2,29	44,17	Cw5	W4	-
89	LGER	2,3	51		W4,W6	-
90	RGRI	2	7		W6	Dw2
91	JBAT	2,3	8,12			-
92	NLEE	1	8			Dw3
93	RSIM	2,23	w40,12	Cw3,w4	W4,W6	Dw5
94	IKLE	2	27			LD27a

95	LKAI	2	27			LD27a
96	BRAN	2	40			LD40a
97	TCOS	2,29	51,44		W4	-
98	PGED	2	27			LD27a
99	GRIE	2	w40			Dw1
100	AANS	2,26	w53,8		W4,W6	
101	WSEB	3,23	7,44	Cw4	W4,W6	Dw6
102	RWIL	1,26	7,40	Cw6	W4,W6	Dw4
103	JOST	2,3	7,40		W6	Dw2,Dw6
104	JMCK	3,11	7	Cw6	W6	-
105	VCHA	2	44,45	Cw5,w6	W4,W6	-
106	IWAL	2,26	15.2,44	Cw6	W4,W6	-
107	WCLU	2,28	13,w16	Cw39,w6	W4,W6	-
108	FMUL	3	44,w7		W4,W6	-
109	AGUS	18,w24	12,38		W4,W6	-
110	TBEL	1,11	17 long	Cw6	W4,W6	Dw7
111	RBAE	1,28	8,35	Cw4	W4,W6	Dw3
112	RWIT	3,29	40,44		W4,W6	-
113	MGRE	1,29	12,w35		W4	-
114	PMON	1,3	8,51		W4,W6	-
115	PROM	2,241	18,38		W4,W6	Dw5
116	SLEA	2,3	8,27	Cw2	W4,W6	-
117	CPIC	2	15.1,44	Cw3	W4,W6	-
118	GGRU	9	12			Dw1
119	JMAT	w31	w40	Cw3		-
120	JRYA	2,26	17,44	Cw6	W4	-
121	ADOR	3	49,53		W4	Dw6
122	WSHU	2,w24	15,44	Cw3	W4,W6	Dw4
123	DPAS	2,w32	w35,w40		W4,W6	Dw6,Dw8
124	LHOR	1	8			Dw3

TABLE 4

Schematic Diagram of Method of

Identification and Procurement of HTC



Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) LD Typing for Bone Marrow Transplantation		5. TYPE OF REPORT & PERIOD COVERED Final Report 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Paula J. Romano, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) N000-14-76-C-1173
9. PERFORMING ORGANIZATION NAME AND ADDRESS Immunologic Oncology Division Lombardi Cancer Center & Dept. of Pediatrics Georgetown University, Washington, D.C. 20007		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Task No. NR 207-067
11. CONTROLLING OFFICE NAME AND ADDRESS Office of Naval Research Biophysics (Code 444), 800 N. Quincy Street Arlington, VA 22217		12. REPORT DATE 5 September 1979 13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

See enclosed list.

DISTRIBUTION STATEMENT A

Approved for public release;
Distribution Unlimited

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

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Histocompatibility Typing, HLA-D, LD Typing, cryopreservation, homozygous typing cells, irradiation.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The primary goal of this contract was to develop a support facility for HLA-D typing. The major accomplishments were:

(1) Establishment of a facility for the performance of large scale sterile tissue culture;

(2) Development of new methods of collecting and cryoperserving large amounts of lymphoid cells to use as reagents for HLA-D typing;

(3) Development of techniques for irradiating cryopreserved lymphoid cells;

(over)

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

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

(4) Identification of 47 new homozygous typing cells (HTC);

(5) HLA-D typing of panel of 100 random individuals;

(6) Participation in the Seventh International Histocompatibility Typing Workshop (Oxford, England, 1977); and

(7) Organization of the First International Histocompatibility Typing Conference. Naval Medical Research Institutes, Bethesda, MD, 1977.