Role of Ia-Like Products of the Main Histocompatibility Complex in Conditioning Skin Allograft Survival in Man

J. DAUSSET, L. CONTU, L. LEGRAND, A. MARCELLI-BARGE, T. MEO, and F. T. RAPAPORT, *Research Unit on Human Transplantation and Immunogenetics, Institut National de la Santé et de la Recherche Medicale, Unit 93, Hôpital Saint-Louis, Paris 75010, France, and Department of Surgery, State University of New York at Stony Brook, Stony Brook, New York 11794*

ABSTRACT This report correlates the survival time of 93 intrafamilial skin allografts performed under conditions of main histocompatibility complex (HLA) haploidentity with donor-recipient compatibility for products of the HLA-A, -B, -C, and -DR, as well as C3 proactivator, Glyoxalase I, and P loci located on the human 6th chromosome. Incompatibilities for HLA-A and -B (and to a lesser extent for HLA-C) and(or) for HLA-DR products exerted a strong influence upon the fate of skin allografts. When HLA-A and -B were considered alone, the most compatible group of grafts had a mean survival time of 15.8 d, as compared with 11.3 d for the most incompatible transplants. HLA-DR compatibility alone was associated with a mean survival time of 15.3 d, whereas HLA-DR-incompatible grafts had a mean survival time of 11.5 d. Incompatibilities for C3 proactivator, Glyoxalase I, and P did not have a significant effect upon graft survival.

There was no evidence of an association between donor-recipient incompatibility at HLA-A, -B, or -C or at HLA-DR; such incompatibilities occurred independently of each other, in spite of the state of linkage disequilibrium known to exist between HLA-B and -DR. Incompatibilities for HLA-A, -B, and for HLA-DR exerted a potent additive effect upon graft survival. Skin grafts bearing one, two, or three incompatibilities had a mean survival time of 16.2, 13.7, and 10.7 d, respectively ($P < 0.0005$).

The results point to the important role played by the Ia-like products of the HLA complex (HLA-DR) in conditioning skin allograft survival in man. This consideration may be of direct relevance to the potential clinical usefulness of in vitro serological techniques for the detection of donor-recipient compatibility for HLA-DR.

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INTRODUCTION

The influence of the main histocompatibility complex (HLA)[1] allogenic differences on the fate of human skin grafts was established independently by Dausset et al. (1) and van Rood et al. (2) in 1965, in studies performed with preimmunized recipients. Subsequent intrafamilial skin grafting studies under conditions of ABO-compatibility were performed (3, 4). Skin allografts exchanged by HLA-identical siblings were accorded particularly prolonged survival times (5, 6). Further detailed studies of the individual roles of HLA-A and -B incompatibilities in skin allograft rejection were performed in a series of families of known HLA genotypes (7, 8). For this purpose, 238 skin grafts were performed under conditions of donor-recipient HLA haploidentity. Such allografts were exchanged between parents and siblings, and occasionally between pairs of siblings in the volunteer families available to the study since 1969 (7, 8).

Subsequent progress in the immunogenetics of the HLA complex has resulted in the accumulation of a considerable array of new data regarding the occurrence of additional markers of the HLA complex in the same family units. During the past 8 yr, such families have been typed for human Ia-like determinants (the Ly-Li system, now termed HLA-DR) (9–11) as well as for HLA-C, C3 proactivator (Bf) (12), and C2 (13). Data have also been obtained on other markers present on

[1] *Abbreviations used in this paper*: Bf, C3 proactivator; GLO, glyoxalase I; HLA, main histocompatibility complex; MLC, mixed lymphocyte culture; MST, mean survival time; *t*, Student's *t* test.

TABLE I

Data on 77 Haploidentical Skin Grafts

Sex	Donors				Recipients								Survival time
	Nonshared haplotype				Nonshared haplotype				Shared haplotype				
	DR	B	C	A	DR	B	C	A	DR	B	C	A	
													d
M	1*	35	4	26	X‡	8	X	1	4	49	7	3	6.0
F	7	14	X§	26	5	27	2	11	4	7	2	24	7.0
F	5	15	3	2	2	37	7	1	7	18	X	25	7.0
M	4	5	7	26	X	18	X	24	5	27	1	32	8.0
M	X	35	4	24	X	37	X	1	6	14	X	X	8.0
M	X	27	1	1	4	27	2	11	5	44	1	2	8.0
M	7	44	X	29	5	7	X	24	3	8	X	1	8.0
F	X	39	X	2	2	44	5	11	X	18	X	2	8.0
F	5	40	2	X	7	44	5	2	X	52	X	28	8.0
M	5	40	2	X	X	52	X	28	7	44	5	2	8.5
M	5	35	X	3	5	49	X	24	3	X	4	1	9.0
F	5	51	1	2	X	8	X	30	X	18	X	3	9.0
M	7	44	X	29	5	27	1	32	X	18	X	24	9.0
F	6	35	4	3	5	40	X	2	2	52	X	24	9.0
F	6	15	3	28	3	X	X	11	X	14	X	3	9.0
M	5	12	5	28	3	X	X	11	X	14	X	3	9.0
M‖	5	12	5	28	6	15	3	28	X	14	X	3	9.0
M	2	12	3	X	X	17	X	9	3	X	X	11	9.0
M	6	35	4	2	5	27	2	11	4	7	2	24	9.0
F	6	35	4	2	4	7	2	24	5	27	2	11	9.0
M	5	35	X	3	3	X	X	1	5	49	4	23	10.0
M	1	7	X	29	X	45	5	X	5	15.1	2	2	10.0
F	5	12	5	X	X	14	X	3	3	X	X	11	10.0
M	2	37	4	26	7	44	5	2	X	52	X	28	10.0
M	2	7	X	2	4	14	X	32	7	44	X	23	10.0
F	2	7	X	2	7	44	X	23	4	14	X	32	10.0
F	6	18	X	2	4	14	X	32	7	44	X	23	10.0
F	2	7	X	3	3	8	X	26	X	18	7	32	10.0
F	5	7	X	24	7	44	X	29	3	8	X	1	10.0
M¶	4	14	X	32	6	18	X	2	7	44	X	23	10.0
M	X	35	7	32	7	44	X	23	3	8	4	1	10.0
M	4	27	X	26	X	45	X	29	7	27	2	3	10.0
M	5	X	X	2	2	37	7	1	7	18	X	25	10.0
F	3	8	X	1	2	7	X	2	6	35	4	3	10.0
M	3	44	5	2	2	7	X	24	4	18	X	3	10.5
M	3	8	X	1	6	35	4	3	6	7	X	2	10.5
M¶	X	18	X	24	4	51	7	26	5	27	1	32	11.0
M	1	27	1	2	5	14	X	28	7	13	7	30	11.0
M	4	27	2	24	2	44	X	24	X	7	X	26	11.0
F	3	15	3	11	X	13	7	30	5	7	X	24	11.0

TABLE I (*Continued*)

Sex	Donors: Nonshared haplotype DR	B	C	A	Recipients: Nonshared haplotype DR	B	C	A	Shared haplotype DR	B	C	A	Survival time
													d
M	4	14	X	11	5	40	X	2	2	52	X	24	11.0
F	1	27	1	2	7	13	7	30	5	14	X	28	11.0
M	1	5	X	2	X	22	1	2	5	15.1	3	2	11.5
M	1	5	X	2	5	15.1	3	2	X	22	1	2	11.5
M	X	18	5	30	3	8	X	26	5	18	7	32	12.0
F	3	18	5	30	5	15.2	3	32	7	17	X	2	12.0
F	5	44	4	2	3	8	4	1	7	44	4	23	12.0
F	3	38	X	26	2	51	7	2	6	35	4	28	12.0
F	1	35	4	2	4	40	3	28	2	7	X	1	12.5
M	5	41	X	26	5	35	4	32	3	41	X	32	13.0
F	2	51	X	2	X	7	X	26	2	44	X	24	13.0
F	6	51	2	2	X	13	7	30	5	7	X	24	13.0
M‖	5	13	7	30	7	14	X	28	1	27	1	2	14.0
F	1	15.1	3	2	5	15.1	3	2	X	22	1	2	14.0
M	X	40	3	2	4	50	X	2	X	X	5	23	14.0
M	2	8	X	1	2	35	4	X	5	18	X	24	15.0
M	5	8	X	1	6	40	X	32	X	8	X	1	15.0
F	5	8	X	1	X	8	X	1	6	40	X	32	15.0
M	5	41	X	26	3	41	X	32	5	35	4	32	15.0
M	3	8	X	2	5	14	X	31	5	40	3	25	15.5
M	X	40	3	30	X	44	2	2	6	35	4	3	16.0
F	X	8	X	2	6	35	4	28	2	51	7	2	16.5
M‖	7	14	X	28	7	13	7	30	1	27	1	2	16.5
M	7	44	X	29	7	37	4	1	6	35	X	3	17.0
F	5	51	3	24	7	39	X	24	X	51	X	31	17.0
M	4	40	3	28	6	35	4	2	2	7	X	1	17.0
M	3	44	X	23	4	22.1	3	11	1	35	4	1	17.5
F	6	15	3	28	2	44	5	28	X	14	X	3	17.5
F	2	7	X	11	7	17	X	2	5	15	3	32	17.5
F	5	8	X	1	6	8	X	1	6	7	X	3	18.0
M	X	50	X	2	X	5	3	24	1	35	4	2	18.0
F	5	38	X	26	5	49	X	23	5	14	5	3	19.0
F	X	22.1	1	11	5	49	X	30	2	7	X	24	20.0
F	7	45	4	23	X	51	X	31	7	39	X	24	20.0
M	2	8	7	1	X	51	7	29	6	45	X	1	23.0
M	6	18	3	1	6	45	3	1	X	51	7	29	23.0
F	X	15	X	26	6	35	X	1	X	15	7	23	29.0

* The incompatibilities are indicated by antigens underlined.
‡ X means antigen unknown (blank) or not assignated and is not considered.
§ X underlined is an incompatibility.

‖ Grafts between haploidentical siblings (always male).
¶ Grafts between fathers to offspring (always males).

the same chromosome, such as glyoxalase I (GLO) (14), and probably the P erythrocyte system (15).

The purpose of this report is to correlate the results of skin grafting in the family units under study with these new data. The results indicate that HLA-DR (i.e., Ia-like factors) incompatibilities exert a major influence upon the tempo and intensity of skin allograft rejection in man. This effect does not appear to be a consequence of the linkage disequilibrium known to occur between HLA-B and -DR, and is independent of other factors studied. The data suggest, however, that HLA-A, -B, and -DR incompatibilities exert an additive effect in decreasing skin allograft survival and underline the importance of the HLA-D locus (16).

METHODS

This study is based upon the results of 238 intrafamilial skin allografts performed in the course of an earlier histocompatibility study of 103 families of known HLA-A and -B genotypes (8). 39 of these families, including the results of 93 skin grafts, were available for a follow-up study designed to provide data on the products of the HLA-C, HLA-DR, GLO, C2, Bf, and P loci for these individuals. All skin grafts were performed by a standard technique (19), under conditions of donor-recipient HLA haploidentity. There were 86 grafts from offspring to their fathers, 3 grafts from fathers to offspring, and 4 grafts between haploidentical siblings. In 10 instances the same recipient was given two or more skin haploidentical grafts from HLA-identical donors. The survival time of HLA-identical grafts was identical in seven of these and different, but very close, in three instances. One graft was chosen by chance in these cases and included in this series. As a result, 77 skin allografts performed under various conditions of donor-recipient compatibility were available for analysis (Table I).

Typing for HLA-A, -B, and -C products was performed by the standard National Institutes of Health lymphocytotoxicity technique (8). Serological identification of HLA-DR produced was based on lymphocytotoxicity tests with the 7th Histocompatability Testing Workshop sera (20) and local sera using B-cell-enriched suspensions of lymphocytes that were isolated by the Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) -Isopaque (Winthrop Laboratories, New York) technique after removal of T cells by E-rosetting (9, 10). The C2 and GLO alleles were detected by a standard electrophoresis technique (13, 14) by one of the authors of the report (T. Meo), and Bf products were defined by the standard method (12). In evaluation of the donor/recipient compatibility state, the blank was considered as an unknown antigen different from all defined antigens at the same locus. When it is borne by the common haplotype, it was considered (ignoring the possible crossing-over) that donor and recipient have the same unknown antigen. The combinations in which the donor was blank for the two haplotypes, and where donor and recipient were both blanks for the antigen of the non-shared haplotype, were not taken into account. The combinations in which the donor was homozygous, or heterozygous-identical to the recipient, were noted as compatibles. All other donor/recipient combinations were noted as incompatibles.

For some part of analysis, the survival times observed in the 77 skin allografts under consideration were divided into five groups. These included survivals of (*a*) 6–<9 d; (*b*) 9–<12 d; (*c*) 12–<15 d; (*d*) 15–<18 d; and (*e*) ≥18 d. Standard chi-square tests were used with the Yates' correction. The difference of the mean survival time (MST) was analyzed on the basis of the Student's *t* test (*t*).

RESULTS

Influence of the products of individual loci at or near the HLA complex. Table II correlates the MST (and standard deviations) of skin grafts and the presence or absence of donor-recipient incompatibilities for alleles of loci HLA-A, -B, -C, -DR, Bf, GLO, or P. Each locus has been analyzed on an individual basis. The most profound influence upon graft survival time appears to have been exerted by products of HLA-A and -DR loci ($P < 0.005$). HLA-DR-compatible grafts had a mean survival time of 15.3 d (±4.1 d), in comparison to 11.5 d (±3.5 d) for grafts performed in the presence of serologically detectable HLA-DR incompatibilities. The effect of HLA-B or -C in-

TABLE II

Relationship of Skin Graft Survival to Donor-Recipient Compatibility for the Products of Seven Polymorphic Loci; Each Locus is Considered Separately

	Number of incompatibilities					
	No incompatibility		One incompatibility		Statistical significance of differences in survival time	
Loci	Number of grafts	MST	Number of grafts	MST		
		d		*d*	*t*	*P*
HLA-A	16	15.1±4.0	61	11.6±4.2	3.10	<0.005
HLA-B	12	14.8±5.2	65	11.9±4.1	2.23	<0.05
HLA-C	5	16.0±6.7	44	11.7±3.5	2.56	<0.02
HLA-DR	11	15.3±4.1	60	11.5±3.5	3.06	<0.005
Bf	62	12.1±3.7	15	11.9±5.3	0.24	NS
GLO	42	12.6±4.8	23	12.6±3.4	0.00	NS
P	21	10.5±3.1	14	12.2±2.6	1.64	NS

TABLE III
Effects of HLA-A, -B, and -C Compatibility upon Skin Graft Survival

Number of incompatible antigens	Loci considered: HLA-A and -B — Number of cases	HLA-A and -B — MST	HLA-A, -B, and -C — Number of cases	HLA-A, -B, and -C — MST
		d		*d*
0	5	15.8±1.6	1	14.0
1	18	14.5±4.4	5	16.6±6.6
2	54	11.3±4.2	9	13.4±3.2
3			34	11.2±3.6

compatibilities appeared to be somewhat less pronounced, and Bf, GLO, or P incompatibilities did not have a significant influence upon skin survival.

Table III outlines the effects of compatibility at HLA-A, and -B with or without HLA-C upon skin graft survival. In this instance, graft survival times are grouped in accordance with the presence of zero, one, two, or three donor-recipient incompatibilities at these loci. The results are divided into two categories in this table. The first includes only HLA-A and -B incompatibilities, and the second includes incompatibilities at HLA-A, -B, and -C. In both instances, skin graft survivals decreased in inverse relationship to the total number of incompatibilities present. Statistically significant differences ($t = 2.36$; $P < 0.053$) in graft survival times were observed when the MST of skin grafts, performed in the absence of any HLA-A or -B incompatibility (15.8±1.6 d), was compared with the survival of grafts done in the presence of two incompatibilities (11.3 ±4.2 d), as well as when the survival time of grafts performed across one incompatibility (14.5±4.4 d) was compared with grafts performed across two incompatibilities ($t = 2.73$; $P < 0.01$). When all three loci (HLA-A, -B, and -C) were considered together, a statistically significant difference ($t = 2.67$; $P < 0.02$) was observed between the survival of skin grafts performed across one incompatibility (16.6±6.6 d) vs. three incompatibilities (11.2±3.6 d).

Additive effect of HLA-A, -B, -C, and -DR determinants. As shown in Table IV, the MST of 11 grafts performed across HLA-A, -B, or HLA-A and -B incompatibilities was 15.5 d; it was 15.80 d for five HLA-DR-incompatible grafts. In contrast, grafts transplanted in the presence of HLA-A, -B, and -DR incompatibility (all such grafts were also HLA-C incompatibles) have a mean survival time of 10.71 d. This additive effect is demonstrated further in Table V, where skin grafts are ranked in descending order of HLA-A, -B, and -DR compatibility. The shortest MST occurred with three incompatibilities (10.71 d), i.e., HLA-A, -B, and -DR;

TABLE IV
Role of HLA-A and(or) -B vs. HLA-DR Incompatibilities

Incompatible antigens under consideration	Number of cases	MST	
			d
A, B, DR	44		10.71*
A	2	14.00	15.50‡
B	1	23.00	
A, B	8	14.93	
DR	5		15.80§

* For HLA-A, -B, and -DR.
‡ For HLA-A and -B alone.
§ For HLA-DR alone.

the longest survivals (16.2 d) occurred with one incompatibility (HLA-A, -B, or -DR); and grafts bearing two incompatibilities (HLA-A and -B; HLA-A and -DR; or HLA-B and -DR) had an intermediate survival time (13.76 d).

Statistical analysis of the significance of the differences in survival times observed in the face of HLA-A, -B, and -C incompatibilities with and without HLA-DR incompatibilities is provided in Table VI. The results indicate that the addition of an HLA-DR incompatibility has a regularly predictable unfavorable influence upon graft survival time.

Independent effects of HLA-DR and HLA-A, -B, and -C incompatibilities. Table VII lists the incidence of donor-recipient compatibility and incompatibility for HLA-A, -B, and -C according to the presence or absence of incompatibility at HLA-DR locus. There was no relationship between compatibility at HLA-A,

TABLE V
Additive Effect Incompatibilities for HLA-A, -B, and -DR

Incompatible antigens under consideration	Number of cases	MST
		d
A, B, DR	44	10.71*
A, B A, DR B, DR	19	13.76‡
A B DR	8	16.25§

* For three inconsistancies.
‡ For two inconsistancies.
§ For one inconsistancy.

TABLE VI
Additive Effects of HLA-DR and -A, -B and(or) -C Incompatibilities

Number of incompatible antigens present	HLA loci under consideration									
	HLA-DR + -A		HLA-DR + -B		HLA-DR + -C		HLA-DR + -A and -B		HLA-DR + -A, -B, and -C	
	Number of cases	MST	Number of cases	MST	Number of cases	MST	Number of cases	MST	Number of cases	MST
		d		*d*		*d*		*d*		*d*
0	1	23.0	2	14.0±0.8	1	23.0	0		0	
1	22	14.8±3.5	18	14.6±3.9	6	15.8±5.4	8	16.2±3.3	2	18.5±6.3
2	48	10.6±3.2	51	11.2±3.5	38	11.5±3.2	19	13.7±4.1	4	15.0±6.4
3							44	10.7±3.0	9	13.9±3.7
4									30	10.7±3.2

t for HLA-DR plus -A; comparison of MST of grafts bearing one vs. two incompatibilities = $P < 0.001$. *t* for HLA-DR plus -B; comparison of MST of grafts bearing one vs. two incompatibilities = $P < 0.005$. *t* for HLA-DR plus -C; comparison of MST of grafts bearing one vs. two incompatibilities = $P < 0.02$. *t* for HLA-DR plus -A, and -B; comparison of MST of grafts bearing one vs. three incompatibilities = $P < 0.001$. *t* for HLA-DR plus -A, and -B; comparison of MST of grafts bearing two vs. three incompatibilities = $P < 0.005$. *t* for HLA-DR plus -A, -B, and -C; comparison of MST of grafts bearing two vs. four incompatibilities = $P < 0.05$. *t* for HLA-DR plus -A, -B, and -C; comparison of MST of grafts bearing three vs. four incompatibilities = $P < 0.002$.

-B, or -C and at HLA-DR; the incidence of HLA-DR incompatibilities varied from 80 to 92.85%, regardless of the state of donor-recipient compatibility for HLA-A, -B, or -C. The same conclusions can be drawn if HLA-A and -B loci are considered together, vis-à-vis HLA-DR. A chi-square test for the independence of HLA-DR from HLA-A and -B also demonstrates the lack of any significant relationship between these factors (Table VII) concerning the compatibility state.

The survival time of 52 skin grafts performed across two HLA-A and -B incompatibilities is correlated in Fig. 1 with the state of donor-recipient compatibility for HLA-DR. The results show an inverse relationship between the number of HLA-DR incompatibilities and the duration of graft survival. A similar effect was observed in the groups of skin allografts performed across HLA-A or HLA-B incompatibility (data not shown). In the reverse situation, illustrated in Fig. 2, the survival of 60 grafts performed across one HLA-DR incompatibility was charted in relationship to the proportion of HLA-A or -B incompatibilities in this group. The incidence of HLA-A or -B incompatibility decreased in direct proportion to increases in graft survival time (the number of HLA-A and -B compatible grafts available for this study was not sufficient to permit an analysis of the behavior of HLA-DR products under such conditions).

TABLE VII
Relationship between Donor-Recipient HLA-DR Incompatibility and HLA-A, -B, and -C or HLA-A plus -B Compatibility or Incompatibility State

Locus under consideration	Incompatibility	Number of cases	Significance	
			%	*P*
HLA-A + -B*	0	5/5	100.0	0.2
	1	11/14	78.5	
	2	44/52	84.6	
HLA-A	0	12/13	92.3	0.26
	1	48/58	82.8	
HLA-B	0	10/12	83.3	0.03
	1	51/59	86.4	
HLA-C	0	4/5	80	0.01
	1	37/40	95	

* Chi-square for independence between DR and HLA-A plus -B = 0.2 with 2 D.F.

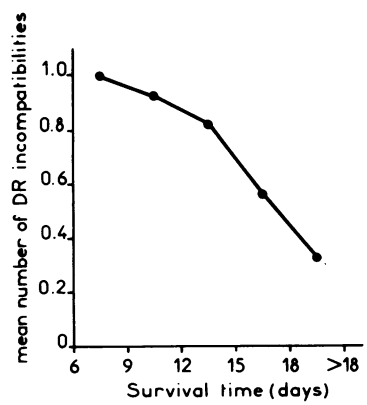

FIGURE 1 52 skin grafts in haploidentical situations; all with two HLA (HLA-A and -B) incompatibilities. DR = HLA-DR.

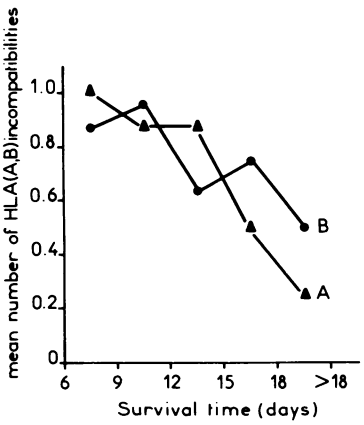

FIGURE 2 60 skin grafts in haploidentical situations; all with one HLA-DR incompatibility. A = HLA-A; B = HLA-B.

DISCUSSION

The availability of a large body of data on the behavior of skin allografts performed under conditions of donor-recipient HLA haploidentity, based upon the earlier reports of Dausset et al. (7, 8), has facilitated an assessment of the role of currently detectable markers of the HLA complex, and of HLA-DR in particular, in transplantation. The data presented in this report confirm and extend earlier evidence of a direct correlation between HLA-A and -B compatibility and the duration of survival of skin allografts. The results also point to an equally important role for products of the HLA-DR locus(*i*). Compatibility for HLA-DR has been shown to favor prolonged skin graft survival, and this effect appears to be as pronounced as the influence of HLA-A and -B compatability. The effect(s) of HLA-A and -B and of HLA-DR upon graft survival appear to be exerted independently of each other. The association of HLA-A, -B, and -DR incompatibility is additive, however, in causing significantly decreased skin graft survivals.

The mechanism(s) responsible for the influence of HLA-DR incompatibility upon graft survival are not clear at present. The restricted localization of the HLA-DR products and their biochemical differences from the HLA-A, -B, and -C products point to the particular importance of this question. It is generally agreed, for example, that the HLA-DR specificities probably exist in close association with the mixed lymphocyte culture (MLC) -stimulating products of the HLA-D locus(*i*) on the cell membrane (19); it is not yet known, however, whether the two entities are identical or different, and whether they are carried by one or two distinct molecules (20). Should the HLA-D and -DR products be identical, the results of this study would be in keeping with earlier findings regarding the influence of HLA-D compatibility upon kidney allograft survival in man (21–24) and in Rhesus monkeys (25). In Rhesus monkey, kidney grafts Ia-like-identical-MLC-positive have the same survival time as Ia-like-nonidentical-MLC-positive grafts (25). It may be relevant to note in this regard that the appearance of anti-HLA-DR antibodies has been associated with an unfavorable renal allograft prognosis (26, 27); this finding, in turn, raises the possibility that the HLA-DR antigen(s) may be present on the surface of some of the cellular components of the kidney. The presence of preformed HLA-DR antibodies (in response to transfusions and[or] pregnancy) has not, however, been shown to have an obvious effect upon the fate of transplanted kidneys (28, 29). Further detailed studies will be required to clarify this point with particular regard to the possible relationship of HLA-DR antibodies to the beneficial effects of blood tranfusions before transplantation. The results of this study may also be of relevance to earlier reports on the influence of HLA-D compatibilities upon the survival of skin (16, 30, 31) and bone marrow (32) allografts in man.

Taken together, these data point to the role of products of the HLA-D region (HLA-D and[or] -DR) in conditioning allograft survival. It is not known, however, whether this effect is the consequence of an amplification of thymus-derived (T)-effector lymphocyte function by T-helper cells, or lysis of target cells by immune T cells and(or) specific anti-HLA-DR antibody. In the murine species, where the H-2 complex bears so many similarities to HLA, the I subregion (Ia and Ic), which has the major MLC-stimulating power, is also the source of the determinants which govern the serologically detectable Ia products (33). It is interesting, in this regard, that skin graft incompatibilities at the murine Ia subregion result in acute rejection and formation of anti-Ia antibodies as well as effector T-lymphocytes detectable by the cell-mediated lympholysis technique (34). On the other hand, Ic incompatibilities produce chronic rejection of first-set grafts and effector T-cells active in cell-mediated lympholysis (35). One possible target may be epidermal cells, which have been shown to possess Ia determinants (36). A number of other properties of anti-Ia antibodies are of interest. These include (*a*) the ability to inhibit MLC responses, especially when the antibodies are directed specifically against the stimulating cells (37); and (*b*) the enhancement of organ graft survival when Ia antibodies are administered to the host before transplantation (38, 39). If Ia antibodies are injected after transplantation into T-cell-depleted mice, they no longer have the capacity to induce graft rejection (40). All of these data point to the existence in the murine species of specific targets coded by the Ia and Ic subregions of the H-2 complex. Such determinants are readily detectable after sensitization in vitro (41) or

in vivo (34, 35). The existence of these types of targets on cell surfaces does not, however, rule out mechanisms of action based upon a helper effect determined by the same subregion. It also does not eliminate the additional possibility that the specific target(s) is not Ia, but another product closely linked to Ia. Indeed, information obtained in the course of studies of mutant mice suggests the possible existence of at least three distinct products of the Ia and Ic subregion, including the MLC-stimulating factor(s), target(s) for cell-mediated lympholysis and the serologically detectable Ia products (40).

It may be relevant to note in this regard that cogent albeit not completely conclusive evidence supporting a difference between the HLA-D and -DR products has been reported (30), although the HLA-D and -DR series are very closely linked. The primary MLC reaction is conditioned mainly by HLA-D differences between stimulatory and responding cells. HLA-DR incompatibilities, however, have a slight but additive effect (42). In contrast, the secondary MLC reaction, as expressed in the primed lymphocyte test, is principally, if not exclusively, under the control of HLA-DR incompatibilities (30). It is extremely difficult, in general, to dissociate the HLA-D helper effect from the HLA-DR target effect, principally because the extremely close linkage between HLA-D and -DR products makes it difficult to locate pairs of donors and recipients in whom these effects are separable.

The lack of correlation between HLA-DR and HLA-A, -B, and -C incompatibilities observed in this study may be of special interest. In view of the well-documented linkage disequilibrium between HLA-B and HLA-DR, the opposite effect might have been anticipated; in keeping, for example, with the linkage disequilibrium between HLA-A and -B, which has been shown to exert a favorable influence in renal transplantation (43). Indeed, studies of the same family units presented in this report have demonstrated that this linkage disequilibrium can be extended from HLA-A to HLA-DR, and occasionally to GLO (11, 44). However, in our study no selection between donors and recipients has been done. Thus the alleles in disequilibrium should be found by chance only in both the donor and the recipient. This situation has been encountered only three times in our 77 grafts both between HLA-A and -B and between HLA-B and -DR. Of course in an hypothetical population with a high frequency of one or several haplotypes in disequilibrium, the incompatibilities at each series would no longer be independent. These conclusions must be regarded, however, in the light of the high proportion of as yet unknown HLA-DR (25.2%) gene products. Beyond these theoretical considerations, and in spite of the limited number of skin grafts studied, the results of this study highlight the importance of HLA-DR in transplantation and point to the potential usefulness of serological tests for the detection of products of this region, in the selection of optimally compatible donor-recipient combinations for clinical transplantation.

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