HISTOCOMPATIBILITY ANTIGENS CONTROLLED BY THE *I* REGION OF THE MURINE *H-2* COMPLEX*

I. Mapping of *H-2A* and *H-2C* Loci

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The *I* region of the mouse *H-2* complex was originally recognized because of the role it plays in the regulation of the immune response to thymus-dependent antigens (1). Later, the region was shown also to code for antigens capable of stimulating *(a)* T lymphocytes in the mixed lymphocyte reaction (cf. 2, 3) and in the graft-vs.-host reaction (cf. 4, 5), and *(b)* B lymphocytes toward the production of serologically detectable Ia antibodies (6, 7, and others). The *I*-region controlled antigens, however, were claimed to be incapable of stimulating the production of cytotoxic effector T cells (8) and, therefore, to differ fundamentally from antigens controlled by the *K* and *D* regions. The first finding contradicting the distinction of the *I* and *K(D)* regions, with respect to their capacity to generate T-effector cells, was the observation that *I*-region antigens can cause rapid rejection of skin grafts (9) and that lymphocytes obtained from mice after rejection of such grafts are capable of killing mitogen-stimulated blasts in the in vitro cell-mediated lymphocytotoxicity (CML)[1] assay (10). The latter observation recently has been confirmed and extended by several laboratories (11, 12).

In the meantime, data have been accumulated indicating genetic complexity of the *I* region and the division of this region into subregions (reviewed in reference 13). How many of these subregions actually exist is at present uncertain. Two subregions, *IA* and *IC*, have been defined by several criteria, and their existence can, therefore, be considered as firmly established. A third subregion, *IB*, has been defined by its capability to regulate the immune response to myeloma proteins (14), LDH_B (15), and staphylococcal nuclease (16); it was also claimed to control Ia antigens (17), but recently that claim has been withdrawn (18). The definition of the *IB* subregion is based on the typing of a single *H-2* recombinant, $H\text{-}2^{h4}$, and on the assumption that this recombinant was derived by a single, equal crossing-over event. Should the latter assumption prove to be incorrect, the *IB* subregion would cease to exist. In addition to *IA*, *IB*, and *IC*, the existence of other subregions has been claimed by several laboratories (19–22) with little data to support such claims. Although numerous *I* subregions may exist, the available data do not allow the definition of more than two or possibly three subregions.

The division of the *I* region into subregions, most likely containing distinct genetic loci, raises the question of the participation of different subregions in the production of effector T cells and the relationship of the *I*-region-associated

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[1] Abbreviations used in this paper: CML, cell-mediated lymphocytoxicity; Con A, concanavalin A; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; LPS, lipopolysaccharide; TNP, trinitrophenyl.

histocompatibility (H) antigens to the serologically-detectable Ia antigens. In this communication, we map a strong *H* locus into the *IA* subregion and a weak *H* locus into the *IC* subregion. In addition, we present some evidence suggesting that the H and the Ia determinants may be coded for by the same loci.

Materials and Methods

Mice. The mouse strains used in the present study are listed in Table I. They were all bred and produced in our colony originally at the University of Michigan, Ann Arbor, Mich. and now at the University of Texas Southwestern Medical School.

Skin Grafting. The majority of skin grafts were taken from tail and placed in the dorsolateral region of sex-matched recipients, following the technique described elsewhere (23). Bandages were removed between 7 and 9 days after grafting, and the grafts were inspected daily for the first 4 wk and once a week thereafter. In a few instances, the tail-to-tail grafting technique of Bailey and Usama (24) was used.

Cytotoxic Test. The presence of cytotoxic antibodies was determined using the microcytotoxic test described elsewhere (9). Either a mixture of 3:1 normal guinea pig serum and normal rabbit serum, or normal guinea pig serum alone, was used as a source of complement. The target cells were either splenocytes or epidermal cells.

Preparation of Epidermal Cells. The donor was killed by cervical dislocation, the hair on the abdomen and thorax was removed with clippers, and the mouse was dipped in 70% aqueous alcohol. Large pieces of skin were cut from the abdomen and thorax and minced into small fragments which were then incubated with gentle agitation by a magnetic stirrer for 30 min at room temperature in 50 ml of 0.25% trypsin (made in Ca^{++}- and Mg^{++}-free Hanks' balanced salt solution, HBSS, pH adjusted to 6.8–6.9). The supernate was decanted, and the remaining tissue was incubated three more times with fresh trypsin solution. The cells from the third and fourth decanted cell suspensions were concentrated by centrifugation at 220 *g* for 10 min, and then resuspended in HBSS containing 20% fetal calf serum (FCS). The suspension was filtered through a 20-ml syringe containing Leukopak fibers lightly dry-packed into a layer 4- to 5-mm thick. Live cells were counted, resuspended in HBSS-FCS, incubated for 90 min at 37°C, and then used in the cytotoxic test.

CML Assay. The CML assay was performed as described elsewhere (25). We used concanavalin A (ConA)- or lipopolysaccharide (LPS)-stimulated blasts as target cells. Since we found no difference in the degree of lysis of these two targets, we pooled the data from the corresponding experiments.

Results

Genetic Homogeneity of Strains Used. For the types of experiments described below, it is imperative to establish beforehand that any rejections observed are not caused by residual heterozygosity of the mouse lines used. For this reason, we randomly selected 10 sex-matched mice of each of the critical 7 strains (A.TL, A.TH, B10.AQR, 6R, 7R, 9R, and B10.HTT) and exchanged skin grafts between the individual animals in the manner of the reciprocal circle (26). None of the grafts was rejected during the observation period of 100 days, indicating that no histocompatibility loci were segregating in the seven lines.

Background Identity. Another important criterion that has to be satisfied before a genetic study of histocompatibility requirements can begin is the background identity of congenic lines used. To determine whether the congenic lines do indeed differ only in the *H-2* complex, as expected, the following F_1 hybrid tests were performed: A → (A.TL × B10.A)F_1, A → (A.TH × B10.A)F_1, B10.BR → (7R × C3H)F_1, B10.BR → (9R × C3H)F_1, B10.BR → (B10.HTT × C3H)F_1, B10.A → (6R × A)F_1, and B10.A → (B10.AQR × A)F_1. In each of these seven strain combinations, one parent of the F_1 hybrid provided the *H-2* haplo-

TABLE I
Mouse Strains Used

Strain	Abbreviation	*H-2* haplotype
B10.A		*a*
C57BL/10Sn	B10	*b*
B10.D2		*d*
B10.M		*f*
D2.GD		*g2*
B10.A(2R)	2R	*h2*
B10.A(4R)	4R	*h4*
C3H/HeJ	C3H	*k*
CBA/J	CBA	*k*
B10.BR		*k*
C3H.OL		*o1*
C3H.NB		*p*
B10.Q		*q*
B10.S		*s*
A.TL		*t1*
A.TH		*t2*
B10.S(7R)	7R	*t2*
B10.HTT		*t3*
B10.S(9R)	9R	*t4*
B10.AQR		*y1*
B10.T(6R)	6R	*y2*

type and the other the rest of the genome of the donor strain. Therefore, if, indeed, the seven tested congenic lines were to have the genome of either the A strain (lines A.TH and A.TL) or the B10 strain (the remaining 5 lines), grafts transplanted in these combinations should have survived permanently. 7–12 sex-matched mice in each of the seven strain combinations were grafted with tail skin. In six of the seven combinations no rejections occurred during the 100-day observation period; in one combination [B10.A → (B10.AQR × A)F_1], five grafts survived for over 100 days, whereas two grafts were rejected, one 73 and the other 85 days after transplantation. We conclude from these experiments that six of the seven tested congenic lines do not differ in any minor *H* loci from their inbred partners; the seventh line (B10.AQR) appears weakly histoincompatible with its inbred partner. The weak residual histoincompatibility of B10.AQR, however, did not interfere with the type of experiments described herein.

Mapping of the Strong I-Region Histocompatibility Locus. Once we established the genetic homogeneity and congenicity of the critical mouse lines, we attempted to map within the *I* region the strong *H* locus which we described previously (9). Summary of the mapping data is given in Table II. The first four strain combinations listed in this table confirm the presence of the strong *I*-region-associated *H* locus in four additional *H-2* haplotypes (*t1*, *t2*, *y1*, and *y2*). In pairs of congenic lines carrying these haplotypes and differing in central *H-2* regions, all exchanged skin grafts were rejected within 3 wk after grafting. The remaining two strain combinations in Table II map the strong *H* locus in the *IA* subregion. Since no strain combination differing only at the *IA* subregion is available, the mapping had to be indirect. It was based on comparing two strain

TABLE II

Survival of Skin Grafts Transplanted Across Central H-2 Region Barriers: Differences Involving the IA Subregion

Donor	Recipient	Sex	Response	*H-2* region difference								R/T*	Graft survival	
				K	*A*	*B*	*C*	*S*	*G*	*D*	*T*		MST ± SD‡	Range
B10.AQR	6R	♀	1°	–	*k*	*k*	*d*	*d*	*d*	–	–	10/10	12.8 ± 0.9	11–16
		♂	1°	–	*k*	*k*	*d*	*d*	*d*	–	–	5/5	15.2 ± 2.3	12–22
		♀	2°	–	*k*	*k*	*d*	*d*	*d*	–	–	7/7	8.3 ± 0.3	6–9
6R	B10.AQR	♀	1°	–	*q*	*q*	*q*	*q*	*q*	–	–	16/16	13.2 ± 1.0	11–17
		♂	1°	–	*q*	*q*	*q*	*q*	*q*	–	–	10/10	14.1 ± 1.0	12–19
		♀	2°	–	*q*	*q*	*q*	*q*	*q*	–	–	12/12	8.4 ± 0.4	6–9
A.TH	A.TL	♀	1°	–	*s*	*s*	*s*	*s*	*s*	–	*a*	12/12	13.1 ± 1.2	11–16
		♂	1°	–	*s*	*s*	*s*	*s*	*s*	–	*a*	10/10	13.8 ± 1.5	11–18
		♀	2°	–	*s*	*s*	*s*	*s*	*s*	–	*a*	8/8	8.0 ± 0.6	7–9
A.TL	A.TH	♀	1°	–	*k*	*k*	*k*	*k*	*k*	–	*c*	8/8	13.3 ± 1.5	11–17
		♂	1°	–	*k*	*k*	*k*	*k*	*k*	–	*c*	7/7	14.1 ± 0.9	12–16
		♀	2°	–	*k*	*k*	*k*	*k*	*k*	–	*c*	5/5	7.9 ± 0.5	6–8
B10.AQR	(B10.Q × B10.D2)F_1	♀	1°	–	*k*	*k*	–	–	–	–	*a*	7/7	12.1 ± 1.1	10–15
		♂	1°	–	*k*	*k*	–	–	–	–	*a*	5/5	13.5 ± 2.5	12–17
		♀	2°	–	*k*	*k*	–	–	–	–	*a*	7/7	7.5 ± 0.7	6–9
B10.A	(4R × B10.D2)F_1	♀	1°	–	–	*k*	–	–	–	–	*a*	3/5	37.3 ± 7.3	28–52
		♂	1°	–	–	*k*	–	–	–	–	*a*	4/4	36.8 ± 6.4	28–52

* Number of mice that rejected grafts/number of transplanted mice.

‡ Median survival time ± standard deviation.

combinations, one differing in *IA* + *IB* subregions and the other differing in the *IB* subregion only. In the former strain combination, all skin grafts were rapidly rejected (within 3 wk after transplantation); in the latter combination, two out of nine grafts survived for more than 80 days, while the remaining grafts were rejected but in a delayed and chronic fashion, indicating the presence of only a weak *H* barrier. Since in this strain combination the donor differs from the recipient also in the *T* region which is known to be associated with weak *H* loci (27), we consider it likely that the observed minor histoincompatibility is caused by the *T* region loci. We conclude, therefore, that the rapid rejection of skin allografts is associated with the *IA* subregion.

Mapping of the Weak I-Region Histocompatibility Locus. Table III lists eight donor-recipient combinations that are genetically compatible at *K*, *D*, and *A* regions but incompatible at *C*, *S*, and *G* regions; six of these combinations are also incompatible at the *T* region. In all eight combinations, skin-graft rejections were observed. Depending on the particular combination, the proportion of rejected grafts varied from between 30 and 100%. Although some of the first-set grafts were rejected as early as 2 wk after tranplantation, the rejection of the majority of grafts was delayed for a month or longer. Frequently, the rejection process was protracted over a period of several weeks. Some grafts that were never rejected, nevertheless, underwent a "cosmetic crisis" characterized by a temporary loss of hair crop and scaliness. The crisis lasted from 1 to 2 wk, but then the grafts recovered and remained healthy for the remainder of the observation period. After rejection of first-set grafts, recipients were regrafted with skin from the same donor strain. Majority of the second-set grafts were rejected in an accelerated manner, although, again, some grafts survived for the

TABLE III

Survival of Skin Grafts Transplanted Across Central H-2 Region Barriers: Differences Involving the IC Subregion

Donor	Recipient	Sex	Response	*H-2*-region difference								R/T*	Behavior of grafts	
				K	*A*	*B*	*C*	*S*	*G*	*D*	*T*		Grafts rejected at day	Grafts surviving at day
7R	9R	♂	1°	–	–	–	*s*	*s*	*s*	–	–	3/11	73, 84, 87	35, 48, 56, 100(5×)
		♂	2°	–	–	–	*s*	*s*	*s*	–	–	3/3	25, 30, 32	
9R	7R	♀	1°	–	–	–	*d*	*d*	*d*	–	–	3/10	20, 25, 48	60, 78, 100(5×)
		♂	1°	–	–	–	*d*	*d*	*d*	–	–	2/12	28, 63	54, 100(9×)
		♀	2°	–	–	–	*d*	*d*	*d*	–	–	5/7	15, 22, 23, 23, 28	50, 50
7R	B10.HTT	♀	1°	–	–	–	*s*	*s*	*s*	–	*a*	10/15	15, 19, 19, 22, 26, 32, 34, 36, 36, 80	50, 50, 65, 90, 120
		♀	2°	–	–	–	*s*	*s*	*s*	–	*a*	8/9	9, 10, 10, 12, 12, 12, 16, 20	90
B10.HTT	7R	♀	1°	–	–	–	*k*	*k*	*k*	–	*c*	5/5	14, 26, 36, 38, 39	
		♀	2°	–	–	–	*k*	*k*	*k*	–	*c*	22/22	9(2×), 10(9×), 11, 12(3×), 13, 14, 15(2×), 16, 17, 20	
9R	B10.HTT	♀	1°	–	–	–	*d*	*d*	*d*	–	*a*	8/8	17, 18, 25, 25, 31, 35, 38, 55	
		♂	1°	–	–	–	*d*	*d*	*d*	–	*a*	6/6	17, 21, 19, 19, 19, 32	
		♀	2°	–	–	–	*d*	*d*	*d*	–	*a*	6/6	8, 8, 12, 14, 14, 15	
		♂	2°	–	–	–	*d*	*d*	*d*	–	*a*	6/6	11, 11, 14, 15, 22, 22	
B10.HTT	9R	♀	1°	–	–	–	*k*	*k*	*k*	–	*c*	8/8	17, 18(3×), 19, 20, 25, 29	
		♀	2°	–	–	–	*k*	*k*	*k*	–	*c*	8/8	10, 11(2×), 12(4×), 13	
A.TL	(A.TH × 2R)F_1	♀	1°	–	–	–	*k*	*k*	*k*	–	*c*	1/3	13	127, 127
		♂	1°	–	–	–	*k*	*k*	*k*	–	*c*	3/6	35, 38, 40	127, 127, 127
A.TL	(A.TH × 4R)F_1	♀	1°	–	–	–	*k*	*k*	*k*	–	*c*	1/3	48	122, 122
		♂	2°	–	–	–	*k*	*k*	*k*	–	*c*	3/11	11, 17, 45	122(8×)
		♂	2°	–	–	–	*k*	*k*	*k*	–	*c*	3/3	15, 17, 19	
C3H.OL	(D2.GD × C3H)F_1	♀	2°	–	–	–	*d*	–	–	–	–	4/7	17, 23, 28, 30	55, 55

* Number of mice that rejected grafts/number of grafted mice.

entire observation period. This behavior of skin grafts is characteristic of weak histocompatibility barriers and thus indicates that the tested donor-recipient combinations differ at minor *H* loci. In six of the tested combinations, the rejections might have been caused by genetic differences at the *T* region which previously has been demonstrated to contain minor *H* loci (27). In the two reciprocal 7R ↔ 9R combinations, however, no difference at the *T* region was present, and yet the skin grafts were rejected. This result suggests that the portion of the *H-2* complex to the right of *IA* and to the left of *D* also contains a minor *H* locus. To map this locus further, we grafted C3H.OL skin on (D2.GD × C3H)F_1 hybrids. In this combination, the donor differs from the recipient only in the *IC* subregion. The rejection of skin grafts in this strain combination indicates that it is the *IC* subregion that contains the minor *H* locus.

The Involvement of the I Region in CML. To test whether antigens coded for

by the *IA* and *IC* subregions can also function as targets in the in vitro CML assay, we took some of the mice that rejected their skin grafts, restimulated their spleen cells in vitro with the donor cells, and then tested the effector cells against ^{51}Cr-labeled target cells (Con A or LPS blasts derived from donor lymphocytes). Most combinations were tested in three to four independent experiments with at least three replicas per each combination and with three effector:target cell ratios (50:1, 25:1, and 12:1). A typical experiment is shown in Fig. 1, and the data are summarized in Table IV.

In all combinations differing at the *IA* subregion, strong lympholysis was observed (see also reference 10); in all combinations differing in *IB, IC, S, G,* and T subregions, much weaker lympholysis was obtained, indicating that the strong killing was directed against antigens controlled by the *IA* subregion. Particularly informative in this respect are the combinations (B10.Q × B10.D2)F_1 anti-B10.AQR and (4R × B10.D2)F_1 anti-B10.A. The weak lympholysis observed in these two combinations was most likely directed against IC antigens (former combination) or T antigens (latter combination).

In all combinations differing at the *IC* subregion, a weak but significant killing was observed. In combinations 7R ↔ B10.HTT and 9R ↔ B10.HTT, this killing could have been caused by antigens coded for by *S* or *G* regions; but in the (D2.GD × C3H)F_1 anti-C3H.OL combination, the killing must have been directed against antigens of the *IC* subregion. We conclude, therefore, that *IC*-subregion antigens can function as targets in the CML assay.

To determine the nature of the killer cells responsible for the CML in these combinations, we exposed the effector cell suspension, before mixing it with target cells, to an AKR anti-C3H (anti-Thy-1.1) serum (dilution 1:5), incubated the cells for 30 min at room temperature, washed the suspension three times, incubated the cells with a 3:1 mixture of normal guinea pig and rabbit sera (as a source of complement), then incubated the cells for additional 30 min at room temperature, washed, and then resuspended them to the desired concentration. The treatment almost completely abolished the lympholytic capacity of the effector cell suspension (Fig. 2), indicating that the killer cells in this assay were T lymphocytes.

Production of Ia Antibodies in Skin-Grafted Mice. Some of the animals that received second- or third-set skin grafts in strain combinations listed in Table III were bled once a week for 8 wk and their sera individually tested in the cytotoxic test against spleen cells of the proper *H-2* haplotype. In two strain combinations, 7R anti-B10.HTT and (A.TH × 4R)F_1 anti-A.TL, antibodies were found in the recipients' sera (Table V). The antibodies appeared early after the second grafting (within 2 wk) and often persisted for at least 8 wk. The positive antisera were pooled, and the pools were then used for additional tests. Both antisera killed only about 60% of unfractionated spleen cells, almost 100 percent of B lymphocytes, but almost no T lymphocytes (Fig. 2), suggesting that the antibodies they contained were directed against Ia antigens. This suggestion was confirmed by testing the pooled antisera against a panel of *H-2* congenic lines and *H-2* recombinants (Table VI). The reactivity pattern of these antisera can be best explained by assuming that they are directed against antigens coded for by the *IC* subregion.

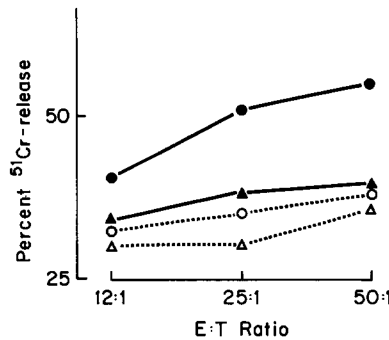

FIG. 1. The effect of effector-cell pretreatment with anti-Thy-1 serum and complement. (○- - -○) 9R (responder, untreated)–7R (stimulator)–9R (target); (△- - -△) 9R (responder, treated)–7R (stimulator)–9R (target); (●—●) 7R (responder, untreated)–9R (stimulator)–9R (target); (▲—▲) 7R (responder, treated)–9R (stimulator)–9R (target).

TABLE IV

Cell-Mediated Lysis in Strain Combinations Involving the IA and IC Subregions

Responder*	Stimulator	Target	*H-2* region difference‡								Mean percent release of ^{51}Cr ± SE§	Mean net release of ^{51}Cr
			K	*A*	*B*	*C*	*S*	*G*	*D*	*T*		
												%
B10.AQR	6R	B10.AQR	–	–	–	–	–	–	–	–	35.9 ± 3.1	–
6R	B10.AQR	B10.AQR	–	*k*	*k*	*d*	*d*	*d*	–	–	58.0 ± 3.9	22.1
6R	B10.AQR	6R	–	–	–	–	–	–	–	–	37.4 ± 3.2	–
B10.AQR	6R	6R	–	*q*	*q*	*q*	*q*	*q*	–	–	62.7 ± 4.1	25.3
B10.AQR	(B10.Q × B10.D2)F_1	B10.AQR	–	–	–	–	–	–	–	–	39.5 ± 3.9	–
(B10.Q × B10.D2)F_1	B10.AQR	B10.AQR	–	*k*	*k*	–	–	–	–	*a*	62.1 ± 4.2	22.6
B10.A	(4R × B10.D2)F_1	B10.A	–	–	–	–	–	–	–	–	31.3 ± 1.5	–
(4R × B10.D2)F_1	B10.A	B10.A	–	–	*k*	–	–	–	–	*a*	38.2 ± 1.9	6.9
7R	9R	7R	–	–	–	–	–	–	–	–	35.6 ± 3.2	–
9R	7R	7R	–	–	–	*s*	*s*	*s*	–	–	47.4 ± 3.3	11.8
9R	7R	9R	–	–	–	–	–	–	–	–	31.8 ± 2.5	–
7R	9R	9R	–	–	–	*d*	*d*	*d*	–	–	44.6 ± 1.6	12.8
B10.HTT	7R	B10.HTT	–	–	–	–	–	–	–	–	41.4 ± 1.3	–
7R	B10.HTT	B10.HTT	–	–	–	*k*	*k*	*k*	–	*c*	56.3 ± 1.9	15.2
7R	B10.HTT	7R	–	–	–	–	–	–	–	–	38.9 ± 3.6	–
B10.HTT	7R	7R	–	–	–	*s*	*s*	*s*	–	*d*	54.5 ± 3.9	14.6
9R	B10.HTT	9R	–	–	–	–	–	–	–	–	29.9 ± 1.5	–
B10.HTT	9R	9R	–	–	–	*d*	*d*	*d*	–	*a*	42.2 ± 3.1	12.3
B10.HTT	9R	B10.HTT	–	–	–	–	–	–	–	–	41.4 ± 2.4	–
9R	B10.HTT	B10.HTT	–	–	–	*k*	*k*	*k*	–	*c*	54.7 ± 2.9	13.3
C3H.OL	(D2.GD × C3H)F_1	C3H.OL	–	–	–	–	–	–	–	–	33.4 ± 3.1	–
(D2.GD × C3H)F_1	C3H.OL	C3H.OL	–	–	–	*d*	–	–	–	–	43.9 ± 2.8	10.5

* In all combinations the responding cells were obtained from a mouse that previously rejected a skin graft from the strain used as the source of stimulating cells.

‡ Theoretically, the effector cells can react against products of the indicated regions.

§ Effector:target cell ratio 50:1. The percent release of isotope was calculated according to the formula:

$$\text{Percent release of } ^{51}\text{Cr} = \frac{(\text{cpm supernate} - \text{background}) \times 2}{(\text{cpm supernate} + \text{pellet}) - 2 \times \text{background}} \times 100$$

Presence of IaC Antigens on Epidermal Cells. The antisera produced by skin grafting reacted in the cytotoxic test against CBA epidermal cells (Fig. 2; only data for the 7R anti-B10.HTT serum are shown). Absorption of the antisera by CBA spleen cells removed this reactivity, and absorption by equal number of CBA epidermal cells significantly reduced their reactivity against CBA spleen

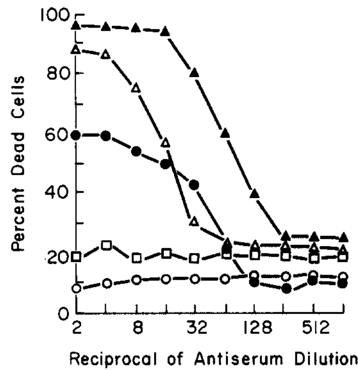

FIG. 2. Titration in the cytotoxic test of antiserum produced by grafting of 7R mice with B10.HTT skin. (●–●) C3H spleen cells; (△–△) CBA epidermal cells; (▲–▲) C3H B cells; (○–○) B10.S spleen cells; (□–□) C3H T cells.

TABLE V

Reactivity in the Cytotoxic Test of Sera from Mice Transplanted with Skin Grafts

Mouse no.	Reciprocal of titre* at days after transplantation						
	14	21	28	35	42	49	56
7R anti-B10.HTT							
1	(4)‡	32	32	8	4	2	0
2	0	(0)	0	0	0	0	0
3	(8)	8	4	0	0	0	0
4	(0)	128	128	32	32	32	
5	0	0	0	0	0	0	0
6	(32)	32	64	8	4	4	4
7	0	0	0	0	0	0	0
8	(128)	128	128	64	64	32	4
9	(16)	16	8	4	0	0	
(A.TH × 4R)F_1 anti-A.TL							
1	0	0	0	0	(0)§	0	0
2	0	(0)	8	8	2	0	0
3	0	(8)	8	8	8	4	4
4	0	0	0	0	0	(0)§	0
5	32	(64)	64	32	4	4	4

* Titre corresponds to the last serum dilution displaying above-background reactivity against CBA spleen cells (background varied between 10 and 20%).

‡ Parentheses indicate approximate time of second- or third-set graft rejection.

§ First-set grafts; all other grafts were of the second-set type.

cells (Fig. 3; again, only data for the 7R anti-B10.HTT serum are shown). These results indicate the presence on epidermal cells of Ia antigens controlled by the *IC* subregion.

Discussion

The data presented in this communication indicate that the *I* region of the *H-2* complex contains at least two *H* loci: a strong *H* locus in the *IA* subregion and a weak locus in the *IC* subregion. In an earlier communication (9), we designated

TABLE VI
Reactivity in the Cytotoxic Test of Pooled Sera from Mice Transplanted with Skin Grafts

Test cells		Reciprocal of titre* with antiserum	
Strain	*H-2* haplotype	K-477‡	K-517‡
B10.A	*a*	0	0
A.TFR5	*ap5*	0	0
B10	*b*	0	0
B10.D2	*d*	32	0
B10.M	*f*	32	0
B10.BR	*k*	64	16
C3H.NB	*p*	32	0
B10.Q	*q*	0	0
B10.S	*s*	0	0
A.TL	*t1*	32	32
A.TH	*t2*	0	0
B10.HTT	*t3*	62	16
B10.AQR	*y1*	0	0
6R	*y2*	0	0

* Titre corresponds to the last serum dilution displaying above-background reactivity against the indicated cells (background varied between 10 and 20%).
‡ K-477: 7R anti-B10.HTT; K-517: (A.TH × 4R)F_1 anti-A.TL.

the strong locus as *H-2I*, but in view of the mapping data presented here, a more proper designation is *H-2IA* or simply *H-2A*. The weak *H* locus can then be designated as *H-2IC* or *H-2C*. The localization of the *H-2A* locus in the *IA* subregion is based on the result of skin grafting in two strain combinations: the rapid rejection of B10.AQR grafts placed on (B10.Q × B10.D2)F_1 recipients (donor-recipient difference at *IA* + *IB* + *T* regions) and slow rejection of B10.A grafts on (4R × B10.D2)F_1 recipients (donor-recipient difference at *IB* + *T* regions). However, indirectly this mapping is supported by several additional combinations in which the donor differs from the recipient at all central *H-2* regions except *IA* and in which the grafts are rejected slowly (Table III). Thus the position of the *H-2A* locus in the *IA* subregion is relatively well established. The mapping of the *H-2C* locus in the *IC* subregion is based on the rejection of C3H.OL grafts placed on (D2.GD × C3H)F_1 hybrids (donor-recipient difference at the *IC* subregion only). However, this mapping, too, is supported by rejections in additional combinations in which the difference is in the *IC*, *S*, and G regions. In previous tests (28), we found no evidence for the *S* or G regions causing graft rejections and we assume, therefore, that in these *IC* + *S* + *G*-disparate combinations, the rejections are caused by *IC*-region antigens. Rejections of skin grafts in *IC* + *S* + *G*-disparate combinations was also observed by Stimpfling[2] and McKenzie and Henning (29).

The *H-2A* locus causes skin graft rejection within 3 wk (this communication) and heart-muscle rejection within 5 wk after transplantation.[3] Furthermore, the

[2] Stimpfling, J. H. 1971. Presentation at the Workshop on the *H-2* complex, Bar Harbor, Maine.
[3] Klein, J., C. Chiang, J. Lofgreen, D. Steinmuller. 1976. Participation of *H-2* regions in heart-transplant rejection. Submitted for publication.

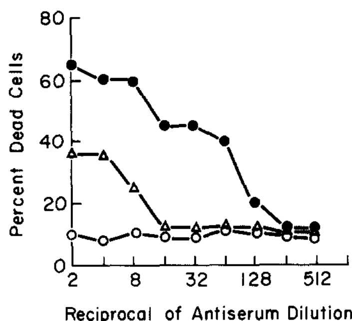

FIG. 3. Absorption of 7R anti-B10.HTT serum with CBA spleen and epidermal cells. (● – ●) unabsorbed antiserum; (△ – △) antiserum absorbed by epidermal cells; (○ – ○) antiserum absorbed by spleen cells. Antisera were absorbed by incubating them three times (spleen cells) and six times (epidermal cells) with equal volume of packed cells, each time 30 min at 37°C.

locus is apparently also responsible for rapid rejection of *I*-region-imcompatible lymphomas (30) and mammary adenocarcinomas (30 and J. Klein, unpublished data). Therefore, with regard to its strength, the locus is comparable to the *H-2K* or *H-2D* loci residing in the *K* and *D* regions, respectively. Testing of different *H-2* haplotypes indicated that at least four alleles exist at this locus, and there is every reason to believe that the locus is as polymorphic as the *H-2K* and *H-2D* loci. Antigens coded for by the *H-2A* locus serve as the targets in the in vitro CML assay when the assay is performed under optimal culture conditions (11, 12) or when spleens from in vivo presensitized animals are used as source of effector cells (10 and this communication). The mechanism of the *IA*-region killing in the CML assay is still under study, but the data available thus far indicate that the killing is mediated by T cells (12) and that it does not require *K*- or *D*-region compatibility (J. Klein and C. Chiang, unpublished data). This latter property distinguishes the H-2A antigens from all minor H antigens which have been shown by Bevan (31) to serve as targets in CML only when the target cell shares with the stimulating cell the same allele at either the *H-2K* or the *H-2D* locus. In this respect the *H-2A* locus is an equal partner to the *H-2K* and *H-2D* loci. In fact, the only major characteristic in which the *H-2A* locus differs from the *H-2K* and *H-2D* loci is its failure to mediate syngeneic lymphocytotoxicity against virus-infected or haptenated cells. Blanden et al. (32) reported that effector cells generated against syngeneic cells infected with the lymphocytic choriomeningitis or vaccinia virus will lyse virus-infected target cells in vitro, provided that the responding (and the stimulating) cell shares with the target cell either the *K* or the *D* region; sharing of *I* region is not sufficient to mediate the lympholysis. Similarly, Shearer et al. (33) and Forman (34) observed that sharing of *K* or *D* regions is necessary for lympholysis of trinitrophenyl (TNP)-modified target cells by effector cells generated against TNP-modified stimulating cells. Here again, sharing of the *I* region does not suffice for the lympholysis to occur. However, one should bear in mind that reproducible demonstration of allogeneic CML across the *I* region requires optimization of the culture conditions and it is, therefore, still possible that similar optimization will also be required for the demonstration of syngeneic

CML against neoantigens. At the time of this writing the problem of syngeneic *I*-region CML certainly must be considered as open to further investigation.

The *H-2C* locus is extremely weak and must, therefore, be classified as minor. Some first-set skin grafts transplanted across *H-2C* barriers remained healthy for at least 100 days, while others were rejected within this period of time. The rejection times range from between 14 and more than 80 days after grafting. The rejection process across *H-2C* barriers is protracted and often lasts for several weeks. Most second-set skin grafts are rejected in an accelerated manner. Whether the locus can cause rejection of grafts from tissues other than skin is not known.

By using in vivo sensitized mice as a source of effector cells, and by restimulating the effector cells in vitro, we were able to establish that the antigens coded for by the *H-2C* locus can also function as targets in the in vitro CML assay. Nabholz et al. (12) observed lympholysis by cells primed in vitro against antigens controlled by the right-hand portion of the central *H-2* segment. They suggested that the killing they observed was directed against antigens coded for by the *IC* subregion, but, in fact, the strain combinations that they tested also differed in the *S, G,* and *T* regions, and the authors did not exclude the possibility that the observed lympholysis might have been caused by these three regions. The observation of positive CML in the strain combination (D2.GD × C3H)F_1 anti-C3H.OL (Table IV) is thus the first unequivocal demonstration that IC antigens serve as targets in the CML assay. The critical question now is whether the IC lympholysis required compatibility at the *K* or the *D* region, in the same way as all minor *H* loci do (31). Experiments to answer this question are in progress. If it, indeed, turns out that *IC* killing were not to occur in *K-D* incompatible combinations, then the *H-2C* locus will have to be considered as another member of the growing family of minor *H* loci. Some 40 such loci have been identified in the mouse (13), and Bailey (35) has predicted that several hundreds of these loci may actually exist. Since minor *H* loci are distributed all over the mouse genome, it would not be surprising to find some of them within the *H-2* complex. On the other hand, if it were to turn out that the *IC* killing does not require *K-D* compatibility, as is the case of the *H-2A* locus, one will have to postulate that the *I*-region *H* loci differ from minor *H* loci in some special intrinsic properties.

The relationship of the *H-2A* and *H-2C* loci to the *Ia* loci is not clear. Three observations can be interpreted as indicating that the Ia products themselves may function as transplantation antigens. The first observation is that skin (9), heart,[3] and tumor (J. Klein, unpublished data) graft rejection across the *I*-region barrier is accompanied by the production of Ia antibodies. This production is particularly evident after grafting across *IA*-subregion differences. We found Ia antibodies in all *IA*-disparate combinations tested, usually after first-set gafting and around the time of the grafts' rejection. In *IC*-disparate graftings, Ia antibodies are found in only some combinations and usually can be detected only after exposure of the animal to second- or third-set grafts. However, we have not excluded the possibility that the Ia antibodies are formed against Ia antigens on passenger leukocytes present in the skin (36), and that they have nothing to do with the transplantation antigens against which the allograft reaction is directed. The second observation is that Ia antigens con-

trolled by *IA* and *IC* subregions are expressed on epidermal cells. The presence of IaA antigens on epidermal cells was first demonstrated by Hämmerling et al. (37), and we confirm this finding. The epidermal-cell expression of IaC antigens is for the first time demonstrated in this communication. The fact that two to three times as many epidermal cells, as compared with spleen cells, are needed to absorb Ia antibodies suggests that the concentration of Ia antigens is lower on epidermal cells than on lymphocytes. The third observation is that loci coding for transplantation antigens map in the same *I* subregions as the loci coding for Ia antigens. However, this last evidence is not very convincing since many strain combinations still have not been tested, and it is quite possible that thorough analysis designed specifically to look for minor *H* loci within the *H-2* complex will uncover additional such loci. On the other hand, it would not be surprising if the Ia antigens were to turn out to be also transplantation antigens. After all, they are expressed on the cell surface, and they are part of a histocompatibility complex.

Summary

Skin grafts were reciprocally exchanged in pairs of congenic lines identical in all genes except those located in the central portion of the *H-2* complex. Seven such lines were tested: 6R, B10.AQR, A.TL, A.TH, 7R, 9R, and B10.HTT. In all donor-recipient combinations at least some grafts were rejected. In combinations differing at the *IA* subregion (and other central *H-2* regions or subregions), all first-set grafts were rejected within 3 wk after transplantation, and all second-set grafts were rejected within 10 days. In combinations differing at the *IC* subregion (and other central regions, but not at the *IA* subregion) between 60 and 100% of first-set grafts were rejected, but some grafts survived for over 100 days. Most of the second-set grafts were rejected within 1 mo after grafting. This behavior of skin grafts indicated the presence of two histocompatibility loci in the *I* region, a strong one and a weak one. This conclusion was confirmed by genetic mapping which placed the strong locus in the *IA* subregion and the weak locus in the *IC* subregion. We designate the former locus *H-2A* and the latter *H-2C*. The same strain combinations used for the skin grafting were also used for determination of the capacity of *I*-region antigens to function as targets in the in vitro cell-mediated lymphocytotoxicity (CML) assay. Spleen cells from mice presensitized in vivo by skin grafting were restimulated in vitro and tested against ^{51}Cr-labeled concanavalin A or lipopolysaccharide blasts. The testing revealed the presence in the *I* region of two loci coding for CML-target antigens. The two loci comapped with the *H-2A* and *H-2C* loci and were most likely identical to them. As in the skin grafting test, in the CML test, the H-2A antigens evoked stronger response than the H-2C antigens. Rejection of skin grafts across the *H-2A* and *H-2C* loci was accompanied by the production of Ia antibodies. Direct cytotoxic and absorption tests with Ia antibodies directed against antigens coded for by the *IC* subregion revealed the presence of IaC antigens on epidermal cells. We suggest that the products of Ia loci might function as transplantation antigens.

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