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CROSS-REACTIVITY BETWEEN *H-2K* AND *H-2D* PRODUCTS

I. Evidence for Extensive and Reciprocal Serological Cross-Reactivity*

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In the presently accepted view, the *H-2* gene complex is divisible by recombination into six regions, each marked by a genetic locus controlling a distinct function or product (Fig. 1) (1). Regions *K* and *D* determine the classical *H-2* alloantigens, defined serologically and by graft rejection (loci *H-2K* and *H-2D*) (2, 3). The *S* region controls qualitative and quantitative variations in a serum protein (*Ss-Slp* locus) (4). The capacity to respond to synthetic polypeptides and various other antigens is governed by regions *I-A* and *I-B* (loci *Ir-1A* and *Ir-1B*) (5–7). These latter two regions also determine a unique membrane alloantigenic system, called Ia (loci *Ia-1* and *Ia-2*) (8–11). A third region for the control of Ia antigens, *I-C*, has recently been postulated (*Ia-3* locus) (1, 12).

This current interpretation of the *H-2* system assumes that only two regions determine *H-2* antigens. Early maps of the *H-2* gene complex depicted multiple regions for the control of *H-2* specificities (3, 13, 14). These maps were constructed with the implicit assumption that no cross-reactivity occurred between the products of separate regions, i.e., that the genetic determinant for reactivity with a given antiserum always mapped in a single consistent position on the chromosome. In 1966, Shreffler et al. (15) noted certain inconsistencies in these linear maps, and suggested that genes controlling one particular *H-2* specificity might perhaps be localized at both the *H-2K* and *H-2D* ends of the complex. This theme was later elaborated (16), and a two-region model for the control of *H-2* determinants, based on the assumption of *H-2K-H-2D* cross-reactivity, was formally proposed to explain *H-2* recombinant data (17, 18). The assumption of cross-reactivity between *H-2K* and *H-2D* eliminated the necessity for additional "central" regions for control of *H-2* determinants. Demant et al. (19), Snell et al. (20), and Stimpfling (21) also proposed that genes coding for similar determinants might map at opposite ends of the complex, and Snell et al.

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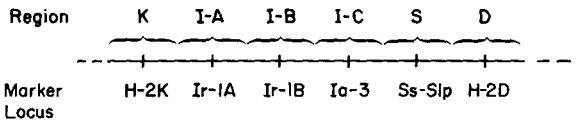

FIG. 1. Map of the *H-2* gene complex.

(22) pointed out in addition that the "private" *H-2* specificities could all be arranged into two segregant series.

Evidence favoring the two-region interpretation comes from three sources: (*a*) Skin grafts survive indefinitely when exchanged between combinations in which the multi-region model predicts incompatibility for regions other than *H-2K* or *H-2D* (21, 23–26). A weakness with this approach is that the multi-region maps were constructed on the basis of serological data, not skin graft data. (*b*) Antibody against an unrelated *H-2* source, but reactive with $H\text{-}2K^k$ determinants, can be absorbed by $H\text{-}2D^k$ antigen (20). This indirect demonstration of cross-reactivity has clearly shown *H-2.1*-like activity at opposite ends of the *H-2* complex. Other such serological data could likewise be interpreted as implicating *H-2K*-*H-2D* cross-reactivity (15, 16, 19, 20, 27, 28). (*c*) Biochemical data reveal only two *H-2* glycoprotein molecules, both of which express multiple specificities (29). Since antigenic activity resides in the protein moiety, and no evidence for subunit construction has been found, the *H-2* antigens appear to be single polypeptide products of two discrete structural genes.

The key assumption distinguishing the multi-region model from the two-region model for control of *H-2* determinants is that serological cross-reactivity occurs between the products of the *H-2K* and *H-2D* loci. In order to test this assumption, and provide further evidence supporting the two-region model, antisera were prepared against determinants controlled by one region (e.g. *H-2K*), and then tested in strain combinations which expressed determinants controlled by the other region (e.g. *H-2D*). Preliminary results with a few combinations have been reported (30). This paper presents extensive data which confirm the existence of *H-2K*-*H-2D* cross-reactivity in many combinations, and thus validate the assumption on which the two-region interpretation was predicated.

Materials and Methods

Mice. All mice utilized in this study are maintained at the University of Michigan. These lines, together with the abbreviations used in this paper, are listed in Table I.

Genetic Notation. This notation is based upon the recommendations of Klein et al. (32). *H-2K* and *H-2D* refer to genetic loci. Each locus is defined on the basis of control of a distinct function or product and separation from other loci in the *H-2* complex by recombination. For brevity in the text of this paper, the locus symbols are frequently shortened to *K* and *D*. Alleles at the *H-2K* and *H-2D* loci are normally designated by a superscript denoting the haplotype or origin (e.g. $H\text{-}2K^k$, $H\text{-}2D^d$). For convenience in this paper, the allele symbols will be abbreviated (e.g. K^k, D^d). The terms *H-2K* or *K* region and *H-2D* or *D* region refer to the segments of chromosome defined by intra-*H-2* recombinations which have separated the specific marker loci (i.e. *H-2K* or *H-2D*) from adjacent loci. The term *H-2K* or *K* end refers to the segment of chromosome situated to the left of the *Ss* gene, toward the *H-2K* marker gene, while *H-2D* or *D* end refers to the segment of chromosome situated to

TABLE I
List of Mouse Lines Used

Strain*	Abbreviation	*H-2* haplotype‡	Haplotype origin of region§					
			K	*I-A*	*I-B*	*I-C*	*S*	*D*
B10.A/SnSf	B10.A	a	k	k	k	d	d	d
B10/SnSf	B10	b	b	b	b	b	b	b
B10.A(2R)/Sg	2R	h2	k	k	k	d	d	b
B10.A(4R)/Sg	4R	h4	k	k	b	b	b	b
HTI/BoySf	HTI	i	b	b	b	b	b	d
B10.A(5R)/Sg	5R	i5	b	b	b	b	d	d
B10.D2/SnSf	B10.D2	d	d	d	d	d	d	d
B10.BR/SnSf	B10.BR	k	k	k	k	k	k	k
C3H/HeSf	C3H	k	k	k	k	k	k	k
C3H.OL/Sf	OL	o1	d	d	d	d	k	k
C3H.OH/Sf	OH	o2	d	d	d	d	d	k
B10.A/SnSf	B10.A	a	k	k	k	d	d	d
A.AL/Sf	A.AL	a1	k	k	k	k	k	d
D2.GD/Li	D2.GD	g2	d	d	b	b	b	b
B10.D2(R103)/Eg	R103	g3	d	d	d	d	d	b
AQR/K1j	AQR	y1	q	k	k	d	d	d
KBR/Sf	KBR	h1	k	k	k	k	k	b

* See Klein (31).

‡ See Klein et al. (32).

§ See Shreffler and David (1). Lower case letter indicates haplotype origin of each region, i.e., recombinant haplotype *h4* has the *K* region of *H-2*^{k}, *I-A* region of *H-2*^{k}, *I-B* region of *H-2*^{b}, etc.

the right of the *Ss* gene, toward the *H-2D* marker gene. The *H-2* haplotype is normally designated by a superscript lower case letter or combination of letter(s) and number (e.g. *H-2*^{k}, *H-2*^{h4}, etc.). For brevity and convenience in the text of this paper, haplotype symbols will also be designated simply by the italicized superscript symbol printed on line with the text (e.g. *k*, *h4*, etc.).

Immunizations. Animals, 15 to a group, were presensitized with a suspension of spleen and lymph node cells injected intraperitoneally (1 donor/15 recipients, approximately 10^7 cells/mouse). After 4 wk, the mice received three additional weekly injections, and were bled 1 wk after the fourth injection. Thereafter, animals were reimmunized every 3rd wk, and were bled 1 wk after each booster. A minimum of eight injections was given in each group. In order to maximize chances of detecting weak cross-reactions, bleedings from each individual mouse were assayed for cross-reactive cytotoxic activity. Those bleedings exhibiting strong or moderate activity were pooled and stored at −80°C until further tested. A list of antisera examined for *H-2K-H-2D* cross-reactivity can be found in Table II.

In F_1 control combinations designed to test for shared determinants, immunizations were performed with irradiated donor cells (15,000 R). This treatment was employed to prevent potential complicating antibodies resulting from graft-vs.-host reactions. The irradiated cells were found to be capable of eliciting a strong humoral response.

Dye Exclusion Microcytotoxic Test. A two-stage dye exclusion microcytotoxic test was performed as described by Amos et al. (33), with some modifications (34). Cell suspensions were prepared by teasing apart lymph nodes (axial, brachial, inguinal, mesenteric) in medium 199 fortified with 2% fetal calf serum and 1% antibiotic-antimycotic mixture (Grand Island Biological Co., Grand

TABLE II
Antisera Examined for Cross-Reactivity

Antiserum no.	Combination	Antiserum no.	Combination
AS-1	2R anti-B10.A	AS-51	OH anti-B10.D2
AS-10	5R anti-B10	AS-54	OL anti-C3H
AS-12	5R anti-B10.A	AS-63	B10.D2 anti-B10.A
AS-25	B10.A anti-5R	AS-66	4R anti-B10
AS-28	B10.A anti-2R	AS-73	C3H anti-A.AL
AS-37	B10 anti-5R	AS-77	B10.D2 anti-OH
AS-40	C3H anti-OL	AS-123	B10.D2 anti-5R
AS-43	B10.A anti-B10.D2	AS-131	(D2.GD × R103)F_1 anti-OH
AS-45	B10.A anti-B10.BR	AS-132	(D2.GD × R103)F_1 anti-B10.D2
AS-49	B10 anti-4R	AS-134	(D2.GD × R103)F_1 anti-B10

Island, N.Y.), separating on a Ficoll-isopaque gradient (35), and adjusting to a final cell concentration of 5×10^6 cells/ml. Antisera and nonimmune serum controls were titrated by doubling dilutions, and 2-μl aliquots transferred to wells in microtest tissue culture plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). All work was conducted at 4°C except where indicated. 2 μl of the cells were added to each well, and the plates incubated at 37°C for 15 min. A two-stage test was performed by washing with serum-free medium before the addition of complement. Rabbit complement, either unabsorbed or absorbed with agarose-EDTA (36), was used. After the addition of 2 μl of complement, the reaction mixture was incubated at 37°C for 30 min. This reaction was terminated by the addition of cold medium 199 (with fetal calf serum) in which the vital dye, Nigrosin, had been dissolved (0.1%). The number of live and dead cells was determined, and the percent cell lysis calculated. Nonimmune serum controls routinely yielded a baseline of 10% or less cell death. Antiserum titers were calculated as the dilution of serum giving 50% of the maximum specific lysis.

Hemagglutination. Red cell agglutination was assayed by the polyvinyl pyrrolidone (PVP) method (37).

Skin Grafting. Tail skin grafts (1 cm²) were placed on the right and/or left dorsal flanks of mice anesthesized with Nembutal according to the method of Billingham and Medawar (38). Grafts were secured with collodion, and protected with latex-gauze bandages. 1 wk after grafting, this dressing was removed, the excess collodion gently peeled off, and an ordinary Band-Aid applied for a few days to protect the graft from scratching. Graft survival was based on three criteria: (*a*) scale survival, (*b*) hair growth, and (*c*) pigmentation. Failure to exhibit all of these characteristics constituted the basis for classifying a graft as rejected. Chronic hair or scale loss implicated weak incompatibility.

Experimental Design. Representative recombinant and parental lines utilized in this analysis are graphically depicted in Fig. 2. (Only the *D*, *S*, and *K* regions of the complex are included). Two types of combinations can be set up to screen for serological cross-reactivity between the *K* and *D* ends. First, one can immunize parental lines (e.g. B10) with tissue from one of the recombinant strains (e.g. 4R), and then test this serum (e.g. B10 anti-4R) with tissue from the reciprocal recombinant line (e.g. HTI) (Fig. 2 A). In this combination, we are testing for the ability of antibody produced against determinants controlled by the *K* end of $H\text{-}2^k$ (anti-K^k) to react with antigen encoded by the *D* end of $H\text{-}2^d$ (D^d). Note that donor, recipient, and target strains all share the same S region; i.e., we are immunizing and testing across a "gap"[1] for S. Consequently, if one observes a reaction in this combination, one can assert that antibody directed against some determinant controlled by a gene to the left of *S* reacts with some determinant encoded by a gene to the right of *S*. A possible alternative to this interpretation is dealt with and excluded in the next section (Controls).

A second type of combination which can be examined involves immunizing a recombinant line (e.g. 2R) with tissue from one parent (e.g. B10.A), and then testing this antiserum (e.g. 2R

[1] The term "gap" is used here in the same sense as that introduced by Demant et al. (24).

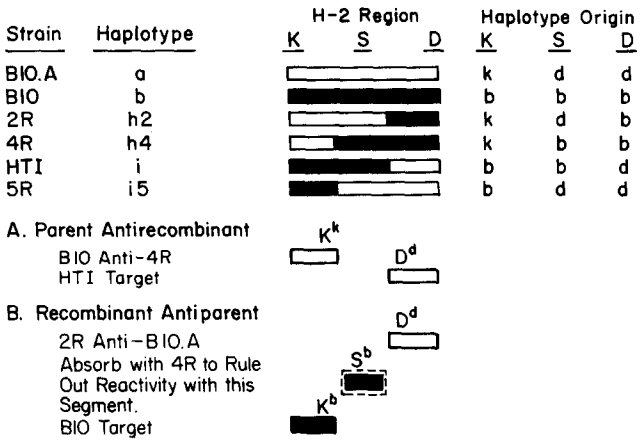

FIG. 2. Experimental design used to test for *H-2K-H-2D* cross-reactivity.

anti-B10.A) with target cells derived from the second parent (e.g. B10) (Fig. 2 B). Reactivity with this target strain could reflect (*a*) cross-reactivity between *D* and *K* end products, or (*b*) cross-reactivity between determinants controlled by separate genes both in the *S-D* interval. One can rule out reactivity with the *S* region in this combination, and artificially create a gap, by testing and absorbing the antiserum with strain 4R. A positive reaction of the absorbed antiserum could then be interpreted as evidence for a cross-reaction between anti-D^d antibody and K^b antigen.

A total of eight different test combinations can be constructed utilizing the four *a/b* recombinants and their parents. Employing the same approach, eight additional combinations were examined using the *d/k* recombinants, and three combinations with *d/b* recombinants.

Results

Controls. Most strain combinations in this analysis were selected such that immunization and testing took place across a gap for the *S* region. A positive reaction in this case would be interpreted as evidence for cross-reactivity between the *K* and *D* products. The only tenable alternative to this interpretation would be that donor and test strains share some histocompatibility gene missing from the recipient. This could occur if recombination had involved a double, triple, or unequal crossover event. For example, suppose haplotype *h4* carried by strain 4R was derived from a double or triple crossover (Fig. 3 A, B). One could then argue that reactivity of a B10 anti-5R (*b* anti-*i5*) serum with 4R target cells simply reflected the sharing of some common determinant, X^d, and not cross-reactivity between the *H-2D* and *H-2K* antigens. An unequal crossover, deleting some segment controlling membrane alloantigens from 4R, is also possible (Fig. 3 C). Reactivity of a 4R anti-B10 (*h4* anti-*b*) serum with B10.A (*a*) targets could in that case reflect a direct or cross-reaction between X^b and X^k products, and not *H-2D* and *H-2K* products.

Although any one of these rare chromosomal rearrangements is possible, data presented in Table III argue against such occurrences. Tissue from B10 donors is compatible with (4R × 5R)F_1 recipients (Group I). Skin grafts survive permanently, and no humoral immunity is detected in hyperimmunized animals. Thus,

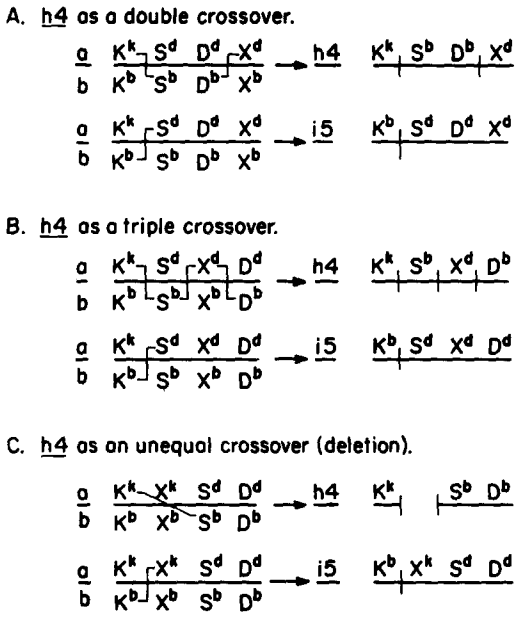

FIG. 3. Schematic representation of *h4* as a double, triple, or unequal crossover, and *i5* as a single crossover.

no detectable reaction is directed against the postulated X^b product, arguing against any double or triple crossover. Failure of (4R × 5R)F_1 hybrids to demonstrate reactivity against either parental B10 (Group I) or B10.A tissue (25) suggests that an unequal crossover in 4R, deleting a gene controlling some membrane determinant, is also unlikely. Lack of humoral or graft responses in the combinations included in Group I and Group II also argues against double, triple or unequal crossover origins for the 2R and 5R recombinants, and further implies that no *H*-gene mutation has occurred since the establishment of the 2R, 4R, 5R, B10, and B10.A strains.

Combinations included in Group III did produce cytotoxic, nonhemagglutinating antibody capable of lysing approximately 70% of lymph node targets. This behavior is unlike that of classical *H-2* antibody (see description in *K-D Cross-Reactivity* section). Reactivity in these two combinations has been attributed to a new *H-2*-linked antigen system called Ia.[2] The weak graft incompatibility observed with both combinations may implicate a minor transplantation role for some genes in the segment between *H-2K* and *H-2D*.

It was not possible to test the *d/k* recombinants for compatibility with parental hybrids in all combinations, since these haplotypes are on three different inbred strain backgrounds (Group IV and Group V). However, in those combinations tested, no serologically detectable responses occurred. Unexpectedly, multiple cases of weak graft incompatibility were observed. The most critical combination is (B10.BR × B10.D2)F_1 hybrids grafted with B10.A skin (Group IV). Two of 10

[2] David, C. S., S. E. Cullen, and D. B. Murphy. 1974. Serological and biochemical studies with the Ia system of the mouse *H-2* gene complex. Further evidence for an *I-C* region. *J. Immunol.* In press.

TABLE III
Control Combinations Designed to Test for Shared Determinants

Group	Host	Donor	Serological result*		Skin graft result	
			Cytotoxic	Hemagglutination	No. rejected	Days survived
I	(2R × 5R)F_1	B10	0	0	0/8	> 172
	(2R × 5R)F_1	B10.A	0	0	0/8	> 171
	(4R × 5R)F_1	B10	0	0	0/12	> 64
	(4R × HTI)F_1	B10	0	0	0/9	> 171
II	(B10.A × B10)F_1	2R	0	0	0/6	> 96
	(B10.A × B10)F_1	4R	0	0	0/6	> 96
	(B10.A × B10)F_1	5R	0	0	0/6	> 96
III	(4R × HTI)F_1	B10.A	80‡	0	1/7§	1 (145) 6 > 238
	(B10 × HTI)F_1	5R	320‡	0	4/17§	1 (160) 3 (239) 13 > 239
IV	(C3H × B10.D2)F_1	C3H.OH	0	0	ND	ND
	(C3H × B10.D2)F_1	C3H.OL	0	0	ND	ND
	(B10.BR × B10.D2)F_1	B10.A	0	0	2/10§	1 (48) 1 (90) 8 > 112
V	(B10.A × C3H.OH)F_1	C3H	0	0	0/7§	> 112
		B10.BR	ND	ND	3/10§	2 (71) 1 (113) 7 > 113
	(B10.A × C3H.OH)F_1	B10.D2	0	0	0/12	> 113
	(C3H.OL × A.AL)F_1	C3H	0	0	1/6§	1 (48) 5 > 112

* Reciprocal of antiserum titers.
‡ 70% maximum lysis with lymph node targets.
§ Some grafts show chronic hair and scale loss.

grafts were rejected at 48 and 90 days. One of the remaining eight grafts showed signs of chronic hair and scale loss, while the rest exhibited good viability. Since B10.A tissue is perfectly compatible with other B10 congenic recipients (Group I), and B10.D2 tissue is compatible with (B10.A × C3H.OH)F_1 hosts (Group V), it seems unlikely that this antigenic disparity resides in the B10 background of strain B10.A. The *a* haplotype is a postulated recombinant between *d* and *k* (13). Perhaps the haplotypes from which *a* was derived were slightly different from those in B10.D2 ($H\text{-}2^d$) and B10.BR ($H\text{-}2^k$), or perhaps mutation has since altered one of the haplotypes. We cannot distinguish between these possibilities.

The design used here to screen for possible shared determinants in certain combinations is similar to that employed by other investigators who tested for

skin graft compatibility across *H-2* barriers other than *H-2K* or *H-2D* (21, 23–26). According to the multi-region *H-2* model, one would predict that humoral immunity should be observed in the following combinations; (2R × 5R)F_1 anti-B10, (4R × 5R)F_1 anti-B10, (4R × HTI)F_1 anti-B10, and (B10.A × C3H.OH)F_1 anti-B10.D2. After prolonged immunization, no cytotoxic or hemagglutinating antibody was detected in any of these combinations (Table III). These serological data thus complement the previous skin graft data in failing to demonstrate immunity against those "central" *H-2* regions postulated under the older model.

In sum, these control immunizations suggest that no mutation or unusual recombinatory event (i.e. double, triple, or unequal cross-over) transpired in the establishment of the strains used in the experiments which follow. These observations imply further that the reactivity observed in the test combinations reported below is due to cross-reactivity between the products of two distinct genes, *H-2K* and *H-2D*, and not to direct reactions between the products of shared genes.

K-D Cross-Reactivity. Hemagglutination and cytotoxic data demonstrating extensive and reciprocal *K-D* cross-reactivity are summarized in Table IV (*a/b* recombinants), Table V (*d/k* recombinants), and Table VI (*d/b* recombinant). A plus (+) indicates a positive result in at least one of the two assays. In most cases,

TABLE IV

Antisera and Target Cells Tested for Cross-Reactivity, and Summary of Results (a/b Recombinants)

Antiserum	No.	Region immunized against	Region specifying antigen which might cross-react	Target cell	Result*	Gap
B10 anti-5R	AS-37	D^d	K^k	4R	+	I-B‡
B10 anti-4R	AS-49	K^k	D^d	HTI	+	I-B,I-C,S‡
B10.A anti-2R	AS-28	D^b	K^d	B10.D2	(+)‖	I-C,S‡
B10.A anti-2R	AS-28	D^b	K^b	5R	–	I-C,S‡
B10.A anti-5R	AS-25	K^b	D^b	2R	(+)‖	I-C,S‡
2R anti-B10.A	AS-1	D^d	K^b	B10	+	I-B,I-C,S§
4R anti-B10	AS-66	K^b	D^d	B10.A	+	I-B,I-C,S§
5R anti-B10	AS-10	D^b	K^k	B10.A	(+)‖	I-B,I-C,S§
5R anti-B10.A	AS-12	K^k	D^b	B10	–	I-B,I-C,S§

* A positive reaction in either the microcytotoxic or hemagglutination assays is scored as a plus (+). No reactivity in either assay system is scored as a minus (–).

‡ Donor, recipient, and target strains all share the same alleles in this segment of chromosome. In these combinations, one can immunize and test across a gap for this region. See text.

§ Absorption with a fourth strain rules out reactivity with determinants controlled by this segment of chromosome. In these combinations, one can by this method effectively immunize and test across a gap for this region. See text.

‖ Very weak but reproducible cytotoxic activity in this combination. See text.

TABLE V

Antisera and Target Cells Tested for Cross-Reactivity, and Summary of Results (d/k Recombinants)

Antiserum	No.	Region immunized against	Region specifying antigen which might cross-react	Target cell	Result*	Gap
C3H anti-A.AL	AS-73	D^d	K^d	C3H.OL	+	S‡
C3H anti-OL	AS-40	K^d	D^d	A.AL	+	S‡
B10.D2 anti-OH	AS-77	D^k	K^k	B10.A	+	I-C,S‡
B10.D2 anti-B10.A	AS-63	K^k	D^k	C3H.OH	+	I-C,S‡
B10.D2 anti-OH	AS-77	D^k	K^b	5R	+	I-C,S‡
B10.D2 anti-5R	AS-123	K^b	D^k	C3H.OH	+	I-C,S‡
OH anti-B10.D2	AS-51	D^d	K^k	C3H	+	S§
OL anti-C3H	AS-54	K^k	D^d	B10.D2	+	S§
B10.A anti-B10.BR	AS-45	D^k	K^d	B10.D2	−	I-C,S§
B10.A anti-B10.D2	AS-43	K^d	D^k	B10.BR	−	I-C,S§
B10.A anti-B10.D2	AS-43	K^d	D^b	2R	−	I-C,S‡

* A positive reaction in either the microcytotoxic or hemagglutination assays is scored as a plus (+). No reactivity in either assay system is scored as a minus (−).

‡ Donor, recipient, and target strains all share the same alleles in this segment of chromosome. In this combination, one can immunize and test across a gap for this region. See text.

§ Absorption with a fourth strain rules out reactivity with determinants controlled by this segment of chromosome. In this combination, one can by this method effectively immunize and test across a gap for this region. See text.

TABLE VI

Antisera and Target Cells Tested for Cross-Reactivity, and Summary of Results (d/b Recombinants)

Antiserum	No.	Region immunized against	Region specifying antigen which might cross-react	Target cell	Result*	Gap
(D2.GD × R103)F_1 anti-OH	AS-131	D^k	K^k	4R	+	I-B,I-C,S‡
		D^k	K^b	B10	+	I-B,I-C,S‡
(D2.GD × R103)F_1 anti-B10.D2	AS-132	D^d	K^k	4R	+	I-B,I-C,S‡
		D^d	K^b	B10	+	I-B,I-C,S‡
(D2.GD × R103)F_1 anti-B10	AS-134	K^b	D^k	OH	+	I-B,I-C,S‡
		K^b	D^d	B10.D2	+	I-B,I-C,S‡

* A positive reaction in either the microcytotoxic or hemagglutination assays is scored as a plus (+).

‡ Presence of certain regions in the F_1 recipient rules out reactivity with this segment of chromosome. In this combination, one can immunize and test across a gap for this region. See text.

both cytotoxic and hemagglutinating activity were detected. However, with some target cells, particularly those expressing K^b determinants, cytotoxic reactions but no red cell agglutination could be demonstrated. In addition to the eight basic combinations that can be tested with four recombinant and two parental types, secondary target strains which test for additional cross-reactions were also tested and these results are included in the tables.

Wherever a cross-reaction was observed, a gap exists at least for the *S* region, or, in the case of B10 anti-5R, a gap for *I-B*. In each case, one can infer that antibody directed against the product of some gene to the left of *S* or *I-B* reacts with an antigen controlled by a gene to the right of *S* or *I-B*, or vice versa. Several observations strongly suggest that the products of the *H-2K* and *H-2D* genes are involved in this reactivity, and not the closely linked *Ia* or *Tla* genes, which also control membrane alloantigens. First, no *Ia* determinants have been reported which map to the right of *S*. Second, in most combinations tested, greater than 90% kill of lymph node targets is observed. *Ia* antisera routinely give a maximum kill of 40-70% (8). Third, *Ia* determinants are not present on red cells (8, 10). Most cases of cross-reactivity are detected by hemagglutination as well as by cytotoxicity. Complications attributable to *Tla* are most unlikely, since *Tla* antigens are not expressed on lymph node lymphocytes, which were the target cells utilized in the cytotoxic assay (39). Identification of the cross-reacting specificities in these antisera as known *H-2* public specificities lends further credence to this interpretation. Absorption analyses have revealed cross-reacting *H-2.3*, *5*, *29*, and *36* antibodies in these sera.[3]

Raw data from both the hemagglutination and cytotoxic assays are included in Table VII (*a/b* recombinants), Table VIII (*d/k* recombinants), and Table IX (*d/b* recombinants). Antiserum titers are reported as the reciprocal of the endpoint dilution. Cytotoxic titers were determined by the dilution of antiserum giving 50% of the maximum specific lysis. Hemagglutination titers are based on the last antiserum dilution giving a positive reaction. In the discussion below, reciprocal antiserum combinations will be considered together to emphasize the generally reciprocal nature of the presence or absence of *K-D* cross-reactivity.

B10 anti-5R (AS-37), B10 Anti-4R (AS-49). Both antisera gave positive cytotoxic and hemagglutination cross-reactions with target cells from the appropriate strain (Table IV, Table VII). Preliminary results suggesting *D-K* cross-reactivity in the B10 anti-5R combination were reported previously (30). No cross-reactive antibody was found in early bleedings in the B10 anti-4R combination. Results with later bleedings demonstrated a positive reaction. These antisera thus demonstrate reciprocal K^k-D^d cross-reactivity.

B10.A Anti-2R (AS-28), B10.A Anti-5R (AS-25). When tested with 5R target cells, AS-28 exhibited no reactivity (Table IV, Table VII). However, this antiserum did give weak cytotoxicity with B10.D2 lymphocytes. The reciprocal antiserum, AS-25, gave a weak cytotoxic reaction with 2R target cells. These weak reactions probably reflect *H-2* cross-reactivity, unless Ia determinants are situated to the right of *S*. These data thus imply weak one-way cross-reactions of anti-D^b with the K^d product and of anti-K^b with the D^b product.

2R Anti-B10.A (AS-1), 4R Anti-B10 (AS-66). Evidence for K-D cross-reactivity is apparent with both antisera (Table IV, Table VII). As reported previously (30), AS-1 gave a positive cytotoxic reaction with B10. Since strain 4R did not react with this antiserum, and failed to absorb activity for B10 targets, we can infer that anti-D^d antibody reacts with K^b antigen. One of the most surprising

[3] Murphy, D. B., and D. C. Shreffler. 1974. Cross-reactivity between *H-2K* and *H-2D* products. II. Identification of the cross-reacting specificities. Manuscript submitted for publication.

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TABLE VII

Dye Exclusion and Hemagglutination Data with a/b Recombinant Combinations

Target	AS-37 B10 anti-5R	AS-49 B10 anti-4R	AS-28 B10.A anti-2R	AS-25 B10.A anti-5R	AS-1 2R anti-B10.A	AS-66 4R anti-B10	AS-66 4R anti-B10	AS-10 5R anti-B10	AS-12 5R anti-B10.A
Titers by dye exclusion*									
B10	0	0	—	—	230	1,000	250	2,560	0
B10.A	—§	—	0	0	20,480	350	50	60‖	8,500
2R	—	—	200	30‖	0	130‖	0	—	—
4R	520	3,700	—	—	0	0	—	—	—
5R	3,800	80	0	2,000	—	—	—	0	0
HTI	—	65	—	—	—	—	—	—	—
B10.D2	—	—	30‖	—	—	—	—	—	—
B10.BR	—	—	—	—	—	380‖	50‖	—	—
Titers by hemagglutination‡									
B10	0	0	—	—	0	0	—	160	0
B10.A	—	—	0	—	1,280	160	20	0	640
2R	—	—	80	0	0	0	—	—	—
4R	320	>2,560	—	—	0	0	—	—	—
5R	>2,560	80	0	0	—	—	—	0	0
HTI	—	320	—	—	—	—	—	—	—
B10.D2	—	—	0	—	—	—	—	—	—
B10.BR	—	—	—	—	—	0	—	—	—

* Reciprocal of antiserum dilution giving 50% of maximum specific lysis. Unless otherwise indicated, all antisera give a maximum kill of 90% or greater, with a nonimmune serum control of 10% or less.
‡ Reciprocal of last antiserum dilution giving agglutination.
§ Dash (—) indicates not tested.
‖ Maximum kill of approximately 50%, with nonimmune serum control of 10% or less.
¶ Absorbed in vivo in strain 2R. See text.

TABLE VIII

Dye Exclusion and Hemagglutination Data with d/k Recombinant Combinations

Target	AS-73 C3H anti-A.AL	AS-40 C3H anti-OL	AS-77 B10.D2 anti-OH	AS-63 B10.D2 anti-B10.A	AS-123 B10.D2 anti-5R	AS-51 OH anti-B10.D2	AS-54 OL anti-C3H	AS-45 B10.A anti-B10.BR	AS-43 B10.A anti-B10.D2
Titers by dye exclusion*									
C3H	0	0	—	—	—	220	1,900	—	—
B10.BR	—§	—	—	—	—	—	—	720	0
B10.D2	—	—	0	0	0	3,600	360	0	640
B10.A	—	—	500	15,000	—	—	—	0	0
A.AL	8,400	1,350	—	—	—	—	—	—	—
OH	—	—	150	180	120	0	0	—	—
OL	280	9,000	—	—	—	0	0	—	—
5R	—	—	110	—	155	—	—	—	—
2R	—	—	—	—	—	—	—	—	0
Titers by hemagglutination‡									
C3H	0	0	—	—	—	40	640	—	—
B10.BR	—	—	—	—	—	—	—	0	0
B10.D2	—	—	0	0	0	>2,560	160	0	80
B10.A	—	—	160	>2,560	—	—	—	0	0
A.AL	>2,560	640	—	—	—	—	—	—	—
OH	—	—	160	320	320	0	0	—	—
OL	160	Tr‖	—	—	—	0	0	—	—
5R	—	—	0	—	0	—	—	—	—
2R	—	—	—	—	—	—	—	—	0

* Reciprocal of antiserum dilution giving 50% of maximum specific lysis. Unless otherwise indicated, all antisera give a maximum kill of 90% or greater, with nonimmune serum control of 10% or less.
‡ Reciprocal of last antiserum dilution giving agglutination.
§ Dash (—) indicates not tested.
‖ Trace reaction.

TABLE IX
Dye Exclusion and Hemagglutination Data with d/b Recombinant Combinations

Target	AS-131 (D2.GD × R103)F$_1$ anti-OH	AS-132 n(D2.GD × R103)F$_1$ anti-B10.D2	AS-134 (D2.GD × R103)F$_1$ anti-B10
Titers by dye exclusion*			
(D2.GD × R103)F$_1$	0	0	0
OH	45	—§	40‖
B10	120	115	4,300
4R	130	23‖	—
B10.D2	—	780	19‖
Titers by hemagglutination‡			
(D2.GD × R103)F$_1$	0	0	0
OH	80	—	320
B10	0	0	0
4R	80	320	—
B10.D2	—	2,560	160

* Reciprocal of antiserum dilution giving 50% of maximum specific lysis. Unless otherwise indicated, all antisera give a maximum kill of 90% or greater, with a nonimmune serum control of 10% or less.
‡ Reciprocal of last antiserum dilution giving agglutination.
§ Dash (—) indicates not tested.
‖ Maximum kill of approximately 50%, with a nonimmune serum control of 10% or less.

results in this study was the cytotoxic reactivity of AS-66 with 2R lymphocytes (Table VII). Strains 2R and 4R are identical at the *H-2K* and *H-2D* loci (Table I). Therefore, this reaction should not be directed against the products of these genes. The low level of cytotoxic kill (50%), and lack of hemagglutination with 2R red cells, may be suggestive of Ia reactivity. Moderate cytotoxic killing and no hemagglutination was also obtained with cells from a second target strain, B10.BR (Table VII). Absorption in strain KBR cleared this reactivity, suggesting that the gene controlling the reactive determinant in B10.BR is positioned to the left of *H-2D*. Failure of 2R tissues to absorb for B10.BR lymphocytes placed this gene to the right of *I-B*. However, AS-66 also had both cytotoxic and hemagglutinating activity for target cells from B10.A, the test strain for *H-2* cross-reactivity. In vivo absorption in strain 2R did not clear this activity (Table VII). These data therefore also indicate a reciprocal K^b-D^d cross-reactivity.

5R Anti-B10 (AS-10), 5R Anti-B10.A (AS-12). A weak but reproducible cytotoxic cross-reaction was observed with AS-10 (Table IV, Table VII). In this particular strain combination, Ia antibody potentially reactive with B10.A could be present (anti-I-C^b). However, strain AQR failed to clear reactivity for B10.A, thus suggesting *H-2* reactivity. A negative reaction was observed in the reciprocal direction (AS-12). These data suggest a one-way cross-reaction of anti-D^b with K^k.

C3H Anti-A.AL (AS-73), C3H Anti-OL (AS-40). Both antisera reacted with appropriate targets cells (Tables V and VIII), implicating reciprocal K^d-D^d cross-reactivity.

B10.D2 Anti-OH (AS-77), B10.D2 Anti-B10.A (AS-63). Positive reactions

across a gap for the *S* region in both assay systems indicate reciprocal cross-reactivity between K^k and D^k (Table V, Table VIII).

B10.D2 Anti-5R (AS-123). This antiserum is included in Table V and Table VIII to demonstrate K^b-D^k reactivity, and represents a second reciprocal partner of AS-77.

OH Anti-B10.D2 (AS-51), OL Anti-C3H (AS-54). The reciprocal cross-reactivity with these combinations in both assay systems after absorption with OL (AS-51) and OH (AS-54) to create a gap situation (Table V, Table VIII) is comparable to that reported above with AS-37 and AS-49 (Table IV). We thus have a second, independent example of reciprocal K^k-D^d reactivity.

B10.A Anti-B10.BR (AS-45), B10.A Anti-B10.D2 (AS-43). When tested with B10.D2 and B10.BR respectively, no reaction was observed, suggesting the absence of cross-reactivity between D^k and K^d (Table V, Table VIII).

(D2.GD × R103)F_1 Anti-OH (AS-131), (D2.GD × R103)F_1 Anti-B10.D2 (AS-132), (D2.GD × R103)F_1 Anti-B10 (AS-134). Positive cytotoxic and/or hemagglutination reactions with all target strains tested provide additional examples of specific *K-D* cross-reactivity (Table VI, Table IX). For example, AS-132 gives a third, independent demonstration of cross-reactivity between anti-D^d antibody and K^k antigen (compare with AS-37) (Table IV) and AS-51 (Table V).

Unreactivity of K^b Cells in Hemagglutination. Poor reactivity of B10 red cells in the hemagglutination assay was recognized in early studies of *H-2* serology (40). In particular, anti-*H-2.5* antisera routinely fail to agglutinate K^b targets, regardless of strain background (41, 42). Data in this study further illustrate this phenomenon. Antisera against D^d or D^k all gave positive cytotoxic reactions but negative hemagglutination with K^b targets (AS-1, AS-77, AS-131, AS-132). Despite the fact that K^b tissue was used as the donor in the preparation of AS-25, AS-66, AS-123, and AS-134, no hemagglutinating activity was found with K^b red cells. These same antisera were cytotoxic for K^b target cells, however, and AS-66, AS-123, and AS-134 did have hemagglutinating activity for other red cell types. In contrast, when D^b tissue was used as the donor, good hemagglutinating activity was found with D^b targets (AS-10, AS-28).

Discussion

A summary of the *K-D* cross-reactivity detected this study is presented in Table X. Two points are striking. (*a*) The observed cross-reactivity is extensive. 13 of 18 possible comparisons exhibited cross-reactivity between *H-2K* and *H-2D*. In some cases, multiple combinations provide independent demonstrations of cross-reactivity. For example, AS-37, AS-51, and AS-132 all show reactivity between anti-D^d antibody and K^k antigen. On the basis of strong cytotoxic kill and/or positive red cell agglutination, 10 of these combinations revealed unequivocal reactivity between the products of the *H-2K* and *H-2D* genes. In these particular combinations, the cross-reactive entities have been identified as *H-2* public specificities *H-2.3, 5, 29,* and *36.*[3] Three combinations exhibited weak cytotoxic cross-reactivity. To date, no Ia-like activity has been mapped to

TABLE X
Summary of Cross-Reactivities Detected between the Products of H-2K and H-2D Alleles*

H-2K allele	*H-2D* allele		
	D^b	D^d	D^k
K^b	±	+	+
K^d	±	+	−
K^k	±	+	+

* (+) Denotes reciprocal cross-reaction, i.e. anti-D^d antibody reacts with K^b product, and anti-K^b with D^d product. (−) Denotes reciprocal absence of cross-reactivity. (±) Denotes weak one-way cytotoxic cross-reaction of an anti-*D* serum with a *K* product, but not vice versa. (+) Denotes weak one-way cytotoxic cross-reaction of an anti-*K* serum with a *D* product, but not vice versa.

the right of *S*. Consequently, the reactivity of these antisera is tentatively attributed to *H-2* antibody. (*b*) In most cases, the presence or absence of a cross-reaction is reciprocal in nature. Since the exceptions are weak, reactivity in the reciprocal direction could easily go undetected. Failure to find reactivity in five combinations may simply reflect the presence of certain determinants in the recipient. For example, AS-28, AS-43, and AS-45 were all produced in B10.A recipients. Both K^k and D^d cross-react extensively (Table X). Perhaps the presence of these highly cross-reactive determinants in the recipient blocked formation of potential cross-reactive antibody. As additional recombinants involving other haplotypes become available, cross-reactivity may also be found in these presently unreactive combinations.

Products of alternative alleles at the same locus (*H-2K* or *H-2D*) also, of course, share cross-reactive public specificities (2, 17). Dual comparisons between the different *H-2K* alleles included in this study (K^b, K^d, K^k) reveal that two out of three of these pairs share a common specificity. A similar result is found for comparisons between the different *H-2D* alleles studied (D^b, D^d, D^k) These estimates approximate that found in this report for cross-reactivity between the products of the *H-2K* and *H-2D* loci. If this cross-reactivity reflects structural homology, it would suggest that the similarity between the products of different alleles at the same locus is no greater than that between products of the two different loci. Biochemical studies seem to be consistent with this interpretation. Nathenson and Cullen (29) have reported that preliminary peptide maps reveal almost as much similarity between the products of the *H-2K* and *H-2D* genes as between the products of different alleles at *H-2D*. These data are precisely what one would expect if the *H-2K* and *H-2D* genes arose through duplication of a common ancestral gene and were both concomitantly modified by mutation. Complete amino acid sequencing of these gene products may be required to resolve this problem.

The cytotoxic reaction of 4R anti-B10 (AS-66, Table VII) with 2R target cells was unexpected. The 4R and 2R strains share the same *H-2K* and *H-2D* alleles. Reactivity therefore must be with determinants controlled by other genes which

map between these two loci. The absence of red cell agglutination and the maximum kill of 50% with 2R lymph node targets would be consistent with the behavior of an Ia reaction. Since no Ia determinants have been localized to the right of *S*, these data may reflect a cross-reaction of antibody directed against products of the *I-A* region with determinants controlled by *I-B* or *I-C*. Reactivity with the *I-C* region is implicated from results obtained with a second target strain, B10.BR. Alternatively, reactivity in this combination might reflect a cross-reaction between *H-2* antibody (anti-*H-2K*) and an Ia determinant (I-C antigen). This alternative appears less likely, since the molecules encoded by these two systems are biochemically distinct. Regardless of whether anti-Ia or anti-*H-2* antibody is involved, this reactivity suggests serological cross-reactivity with the product of a gene other than *H-2K* or *H-2D*.

Attempts to demonstrate *K-D* cross-reactivity by skin graft rejection have also been successful.[4] Prior sensitization of a recipient with tissue-bearing *H-2K* determinants results in the accelerated rejection of a graft expressing cross-reactive *H-2D* determinants, and vice versa. Using an experimental design comparable to that employed here, Nabholz et al. (43) have demonstrated a similar *K-D* cross-reaction in vitro in the cell-mediated lympholysis assay. Prior sensitization of lymphocytes with *H-2K* tissue can generate T-"killer" cells capable of destroying targets expressing cross-reactive *H-2D* determinants. This supports the idea that the accelerated graft rejection observed in vivo may be due to cross-sensitization at the cell-mediated level of immunity.

In conclusion, extensive and reciprocal serological cross-reactivity has been demonstrated between the products of the *H-2K* and *H-2D* loci. Controls designed to test for possible shared determinants in the combinations examined make alternative interpretations most unlikely. These data validate the key assumption underlying the current two-region interpretation for control of the classical *H-2* specifications. The observation of apparently comparable cross-reactivity between Ia determinants serves as a warning that the pitfalls encountered in early *H-2* serology may also appear in the analysis of this new antigenic system.

Summary

Serological cross-reactivity between the products of the *H-2K* and *H-2D* genes has been demonstrated by a design in which antibody was produced against determinants controlled by one locus (e.g. $H\text{-}2K^k$), and then tested against the product of the opposite locus (e.g. $H\text{-}2D^d$). A total of 13 out of 18 such test combinations exhibited *H-2K-H-2D* cross-reactivity. The presence or absence of cross-reactivity was reciprocal in most cases (i.e. antibody directed against the $H\text{-}2K^k$ gene product reacted with $H\text{-}2D^d$ determinants, and antibody directed against the $H\text{-}2D^d$ gene product reacted with $H\text{-}2K^k$ determinants). An Ia-like reaction was detected with one antiserum which implied possible cross-reactivity between the products of two discrete Ia genes.

[4] Murphy, D. B., and D. C. Shreffler. 1974. Cross-reactivity between *H-2K* and *H-2D* products. III. Effect of *H-2K-H-2D* cross-sensitization on skin graft survival. Manuscript submitted for publication.

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