COMPARATIVE IMMUNOGENICITY AND ENHANCEABILITY OF INDIVIDUAL *H-2K* AND *H-2D* SPECIFICITIES OF THE MURINE HISTOCOMPATIBILITY-2 COMPLEX*

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Histocompatibility-2 or *H-2*[1] is the major histocompatibility system of the mouse (*Mus musculus*), and is probably homologous with the *HL-A* system of man (1–3). It is generally regarded as consisting of two loci, *H-2K* and *H-2D* (2, 3), though there is some evidence for other loci in the same chromosome region, producing antigens nonreactive or very weakly reactive with *H-2* antibodies, but with effects on histocompatibility and/or the mixed lymphocyte reaction (4). Since *H-2K* and *H-2D* show about 0.5% recombination (3) there doubtless are other loci between them. Two such loci have been identified. Shreffler and co-workers (5) found, in this intermediate region, a locus or loci (*Ss-Slp*) determining the quantity and structure of a serum protein (5–7). This has proved to be a useful marker in crossover studies. Also a major immune response gene or group of genes (*Ir-1*) has been located between *H-2K* and *Ss-Slp* (8).

Only the separate forms of *H-2K* and *H-2D* and not of the total *H-2* region are properly referred to as alleles. Nevertheless, it is convenient to have a term for the separate forms of the chromosome segment as a whole, since, because of the low crossover frequency, it is usually transmitted intact. The term "haplotype" is used in this sense in *HL-A* studies, and we shall adopt this usuage here. The *H-2K* and *H-2D* loci each occur in several and perhaps many allelic forms, and each allele determines a number of serologically defined antigenic specificities. These specificities can be divided into private and public groups, the former occurring (with some possible exceptions) in a single allelic form of the *H-2K* or *H-2D* antigens, the latter occurring

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; *H-2*, histocompatibility-2; Ir, immune response; MST, median survival time.

in more than one form (2, 3). Seven of the known private specificities (*H-2.15*, *17*, *19*, *20*, *23*, *31*, *33*) can be assigned definitely to *H-2K*, five (*H-2.2*, *4*, *12*, *30*, *32*) to *H-2D*. The remaining four (*9*, *16*, *18*, *21*), because of the absence of the appropriate crossover, cannot yet be assigned to either locus, quite possibly when the necessary crossovers become available each will split into *K* and *D* components. The private *H-2* specificities thus comprise two mutually exclusive series, one determined by *H-2K*, the other by *H-2D*. They are generally strong, especially when tested by lymphocytotoxicity, though there may be some exceptions (2).

Through the use of *H-2* recombinants of congenic strain pairs differing one from another only at *H-2* (9), and of hybrid recipients, it is possible to get donor-recipient pairs which differ by only a single private specificity, and to produce monospecific antisera to these specificities. Of necessity, antisera against the public specificities contain also an antibody against a private specificity, and sometimes to both *H-2K* and *H-2D* private specificities. In some cases, it is impossible, with the haplotypes currently available, to selectively eliminate all the public specificities from strain combinations differing by only a single private specificity.

One possible source of information concerning the *H-2K* and *H-2D* loci is studies of the alloantigenicity of their respective products. Several investigations have suggested that the two antigens differ in immunogenicity, the products of the *H-2D* locus appearing less immunogenic than those of the *H-2K* locus. Thus, antibodies against *H-2K* private specificities generally show a higher cytotoxic titer than antibodies against *H-2D* private specificities (2), and lymphoma (10) and skin (11, 12) grafts differing from the recipient by private or combined private and public *H-2K* specificities are more rapidly rejected than those differing by *H-2D* specificities. The difference in the immune response to *H-2K* and *H-2D* antigens was also seen in an heterologous antiserum made to mouse lymphocytes (13). Likewise, graft-vs.-host disease (11, 14, 15) and mixed lymphocyte reactions (16–18) are stronger in strain combinations with *H-2K* differences than in those with *H-2D* differences, and tolerance induction is easier (11). Most of these tests have used only a few of the possible *H-2K* and *H-2D* combinations, and often public specificities have not been excluded. A partial reversal of the situation with respect to the relative strengths of *H-2K* and *H-2D* may be seen in the case of marrow grafts, where a postulated *Hh-1* (hybrid histocompatibility) locus at the *D* end of the *H-2* complex is responsible for the rejection of parental bone marrow grafts made to irradiated F_1 recipients (19). Recent evidence, however, indicates that this affect may not be confined to the *D* end (20).

In most of these experiments, multiple *H-2* antigenic differences were present between donor and recipient. However, when a single *H-2K* antigenic difference was examined, specificity *H-2.31*, a number of factors affecting the intensity of the ensuing immune response became apparent. For instance, when a single *H-2* antigenic difference was examined, enhancement could be produced for skin grafts (21) and a gene dose effect demonstrated (22), neither being obtained when multiple *H-2* differences were present. We have therefore examined individual private *H-2K* and *H-2D* antigenic specificities to determine their role in immune responses. Using this model, it is apparent that the *K* and *D* regions of the *H-2* complex give rise to antigens of consistently differing immunogenicity

for cell-mediated and humoral responses, and that they differ in their response to passive immunological enhancement.

Materials and Methods

Mice.—All mice were from inbred strains maintained at the Jackson Laboratory, Bar Harbor, Maine, except that mice of strain B10.S were kindly provided by Dr. J. Stimpfling, McLaughlin Laboratories, Great Falls, Mo. The standard inbred strains and the congenic resistant partner strains employed were: A/WySn, A.CA, A.SW, AKR/J, AKR.M, C57BL/10ScSn, (hereinafter abbreviated B10), B10.A, B10.A(2R), B10.A(5R), B10.AKM, B10.BR, B10.D2/n, B10.M, B10.RIII(71NS), B10.S, B10.WB, C3H/HeDiSn, C3H.SW, C3H-*H-2*^{o}, DBA/1J, D1.C, LP.RIII, and SJL/J. The haplotypes of these strains are shown in the tables. 13 haplotypes were represented, of which 6 (*H-2*^{a}, *H-2*^{h}, *H-2*^{i}, *H-2*^{ja}, *H-2*^{m}, and *H-2*^{o}) are known or presumed recombinants. Strain A/J was sometimes substituted for A/WySn and C57BL/6J for C57BL/10ScSn. Minor non-*H-2* differences between the latter two strains have been revealed by tail skin grafts (D. W. Bailey, personal communication), but we have noted little differences using body skin grafts (21). Strain C3H-*H-2*^{o} was originally backcrossed to C3H/HeJSt, but the last two backcrosses were to C3H/HeDiSn. We shall hereafter refer to the strains without the substrain symbols and for strains C57BL/10ScSn and C57BL/6J use the abbreviations B10 and B6, respectively.

Selection of Recipient-Donor Combinations.—Information concerning *H-2* serotypes and *H-2* recombinants, used in selecting recipient-donor combinations, came from reviews by Shreffler (23) and Klein and Shreffler (3). We also made use of an unpublished *H-2* chart prepared by P. Demant, and of the classification of public and private specificities proposed by Snell et al. (2). In so far as possible, recipient-donor combinations were chosen so that donor and recipient differed only by an *H-2K* private specificity or an *H-2D* private specificity. This entailed the use of donor and recipient *H-2* haplotypes in congenic backgrounds to eliminate non-*H-2* differences, and of a recombinant for either the donor or one parent of the recipient, or both, to restrict the differences to one end of *H-2*. It also usually entailed the use of hybrid recipients so as to restrict the response to the one chosen specificity. In a few cases public (presumably cross-reacting?) specificities could not be excluded; these are indicated in the tables. The essentially monospecific private specificity differences thus achieved were: for *H-2D*, *2*, *4*, *30*, *32*; for *H-2K*, *15*, *17*, *23*, *31*, *33*. Combinations differing for private specificities *9*, *18*, *19* were also used. The first two of these have not been localized; probably they represent the summation of *D* and *K* end private specificities. *H-2.19* has been localized in *H-2K*^{s}, its companion *H-2D*^{s} specificity is *H-2.12*, but because the *H-2K*^{qp} recombinant haplotype which provided the information concerning *19* is not yet available in a congenic strain, the combinations which we used differed by both *12* and *19*.

Skin Grafts.—Skin grafts were performed by the method of Billingham and Medawar (24). The grafts were considered to be rejected when no viable epithelium remained on macroscopic examination of the graft. Second set grafts were placed on the left side of the recipient, 14 days after rejection of a first set skin graft on the right side. Male mice, 8–12-wk old, were used in all skin graft experiments, and there were 10–15 mice in each experimental group. The statistical analyses were done according the Litchfield (25).

Production of Alloantisera.—Recipients for the production of alloantibodies were of mixed sexes and, with the exceptions noted below, at least 8-wk old; usually they were F_1 hybrids. Immunizing injections were given intraperitoneally. The first two injections were thymocytes, one young donor per 25 recipients, given 40 days apart. Subsequent injections of cells pooled from thymus, spleen, and submaxillary gland, one donor per 10 recipients, given at weekly or two weekly intervals. The first bleeding was usually after the fourth injection. The rationale for this immunization schedule has been given elsewhere (26). Once a titer was established,

two bleedings on day 7 and 10 after the last injection were alternated with single injections. Bleedings were made from the tail. Antisera were stored in a freezer at −60°C.

Antisera C-2, C-9, C-17, C-19, C-30, C-32, and C-33 were made available at the Jackson Laboratory under a National Institutes of Health contract, and were made available through the Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Mice used in the production of these antisera were 5–8-wk old at the time of the first injection rather than 8+ wk. On the other hand, the recipients in most cases received more than four injections before the first bleeding; the first bleedings of C-2 and C-32 were made after 13 injections. These antisera were made approximately 5 yr before their use in these tests. AS-377 was approximately 2-yr old when used. All other antisera were less than 1-yr old.

Antisera were also obtained by bleedings on days 10, 15, 20, 25, and 30 following first set skin grafts. Four mice were bled on each of these days and the sera tested individually by the ^{51}Cr cytotoxic method.

Hemagglutinating and Cytotoxic Tests.—Hemagglutination tests were performed by the PVP method of Stimpfling (27) with the addition of 0.1% bovine serum albumin (BSA). Details of the method as we use it are given in the Catalog of Mouse Alloantisera (28).

Cytotoxic tests were performed using the ^{51}Cr method to measure cell lysis (29). Spleen cells were used as the target and were prepared free of red cells by flushing with Tris-NH_4Cl and incubating at 37°C for 5 min. 50 μl of ^{51}Cr-labeled cells (at a concentration of 10^6/ml) were mixed with 50 μl of antibody diluted in L15 medium (Microbiological Associates Inc., Bethesda, Md.) and 50 μl of rabbit complement added. Antiserum titers were calculated using semilog probit paper, the titer being defined as the antibody dilutions which caused the release of 50% of the maximum ^{51}Cr available for release. The same pool of complement was used for all tests, which were performed in duplicate.

Enhancement Studies.—To produce passive enhancement of skin grafts, 0.1 ml of the alloantibody pool, reactive with the skin graft donor antigens, was given by the intraperitoneal route on days 0, 2, and 4 after the skin grafts. This regimen of antiserum administration had previously proved to be effective for the enhancement of skin grafts with an *H-2K.31* difference (21). Antibody production by mice bearing enhanced grafts was also studied by the ^{51}Cr method described above.

RESULTS

First Set Skin Graft Rejections (*Tables I and II*).—When *D* region and *K* region private specificities were compared, a difference in the time required for rejection was apparent. *D* region specificities produced slower rejections than did *K* region specificities. In recipient-donor combinations in male mice differing only by *D* region specificities (Table I), median survival times (MST's) ranged from 13.8 ± 1.1 days for *H-2.4* to 18.6 ± 1.6 days for *H-2.32*. For *K* region differences, the range was 9.3 ± 0.8 days for *H-2.33* to 14.5 ± 1.0 for one of several *H-2.31* combinations. Of the *D* region specificities, *H-2.4* gave rise to the strongest response, while *H-2.32* in one donor-recipient combination (AKR into AKR.M) gave the weakest. If the antigens are ranked in order of immunogenic potency, then at the *D* region, the specificities are *H-2.4* > *H-2.30* > *H-2.2* = *H-2.32*. *H-2D* specificity *32* and *H-2K* specificity *31* were each tested in a number of recipient-donor combinations. Each showed considerable variation. For *32*, the MST's ranged from 14.5 ± 1.3 to 18.6 ± 1.6; for *31* they ranged from 9.8 ± 1.0 to 14.5 ± 1.0. In the discussion we shall consider some of the factors that may be responsible for this variability.

TABLE I

Cytotoxic and Hemagglutinating Antibody Formation and First and Second Set Skin Graft Rejection in Recipient-Donor Combinations Differing by Private H-2D Specificities

Private *H-2* specificity	Public *H-2* specificities	Strain and haplotype of		Antiserum no.‡	Cytotoxic antibody titer	Hemagglutinating antibody titer	Survival of skin grafts	
		Donor	Recipient*				First set	Second set
							MST ± *SD*	
H-2.2	—	B10.A(2R) *H-2*^{h}	B10.D2 × A/J *H-2*^{d} × *H-2*^{a}	C-2	1/32	1/640	17.8 ± 1.2	9.3 ± 0.7
H-2.4	*H-2.41, 42, 43, ?47*§	B10.A *H-2*^{a}	B10 × AKR.M *H-2*^{b} × *H-2*^{m}	AS-377	1/128–1/256	1/640	13.8 ± 1.2	8.0 ± 0.6
H-2.30	—	B10.AKM *H-2*^{m}	B6 × A/J *H-2*^{a} × *H-2*^{b}	ASM-80	1/128	1/512	15.5 ± 1.0	8.5 ± 0.7
	—	B10.AKM *H-2*^{m}	B10.A × LD *H-2*^{a} × *H-2*^{b}	C-30	1/32	1/1,280	NT	NT
	H-2.13	AKR.M *H-2*^{m}	AKR × B10 *H-2*^{k} × *H-2*^{b}	AS-312	1/64	1/320	NT	NT
H-2.32	—	B10.BR *H-2*^{k}	B10 × A/J *H-2*^{b} × *H-2*^{a}	C-32	1/90	1/1,280	16.0 ± 1.2	7.8 ± 1.2
	—	C3H *H-2*^{k}	C3H.SW × A/J *H-2*^{a} × *H-2*^{b}	AS-513	1/64	0	16.8 ± 1.1	8.8 ± 0.6
	?H-2.47	B10.BR *H-2*^{k}	B10.A(2R) × C3H.SW *H-2*^{b} × *H-2*^{h}	AS-541	1/90	0	14.5 ± 1.3	8.2 ± 0.4
H-2.32	—	C3H *H-2*^{k}	B10.A(2R) × C3H.SW *H-2*^{a} × *H-2*^{b}	AS-485	1/128	0-tr	15.0 ± 1.1	8.5 ± 1.6
H-2.32	*?H-2.47*	AKR *H-2*^{k}	AKR.M *H-2*^{m}	AS-542	1/64	0	18.6 ± 1.6	9.5 ± 1.8
	?H-2.47	B10.BR *H-2*^{k}	B10.AKM *H-2*^{m}	AS-543	0	0	15.5 ± 1.1	8.3 ± 0.8

* Groups of 15–20 male mice were used as recipients in each experiment.

‡ The abbreviations used: C, mouse antisera from Catalogue of Mouse Alloantisera; AS, sera produced at The Jackson Laboratory; ASM, sera produced in the Department of Surgery, Massachusetts General Hospital by I. McK; NT, not tested; tr, trace.

§ The presence of *H-2.47* in these sera is uncertain, as the recipient or a parent of the recipient has not been typed for this specificity.

In the combination for AS-541, B10.BR → C3H.SW × B10.A(2R), there is also an antigenic difference of TL. 1,2,3, as B10.BR is TL(+), and the recipients are TL(−). (37)

Second Set Skin Graft Rejections (Tables I and II).—The difference between *D* and *K* region antigenic specificities was also observed, but to a lesser extent, when second-set skin grafts were performed 10 days after the first grafts were rejected. When there was a *D* region difference, second set grafts showed an MST of 7.8–9.5 days. When there was a *K* region difference, the values were 6.8–8.2 days. While the *H-2D* rejections were slower than were those for *H-2K*, the shortening in graft survival time observed after priming with a first set skin graft was greater. Second set grafts performed in the presence of a *D* region antigenic difference were rejected up to 9 days earlier than a first set graft; when there was a *K* region difference grafts were rejected 2–4 days earlier.

Skin Graft Rejection in Hyperimmunized Mice.—The F_1 hybrid female mice

TABLE II

*Cytotoxic and Hemagglutinating Antibody Formation and First and Second Set Skin Graft Rejection in Recipient-Donor Combinations Differing by Private H-2K Specificities**

Private *H-2* specificity	Public *H-2* specificities	Strain and haplotype of		Antiserum no.	Cytotoxic antibody titer	Hemagglutinating antibody titer	Survival of skin graft	
		Donor	Recipient				First set	Second set
							MST ± *SD*	
H-2.9‡	*H-2.37*, *39*	B10.M *H-2*^{f}	B10.D2 × SJL/J *H-2*^{d} × *H-2*^{s}	AS-544	1/512	1/320	10.8 ± 1.1	7.2 ± 0.5
	H-2.37, *39*	B10.M *H-2*^{f}	B10.D2 × A.SW *H-2*^{d} × *H-2*^{s}	C-9	1/540	1/640	10.2 ± 1.1	NT
H-2.15	*H-2.38*, ?47	B10.WB *H-2*^{ja}	B10.A(2R) × A.CA *H-2*^{h} × *H-2*^{f}	AS-537	1/1,080	1/1,280	9.8 ± 1.1	7.0 ± 1.1
H-2.17	—	DBA/1J *H-2*^{q}	D1.C × AKR.M *H-2*^{d} × *H-2*^{m}	AS-545	1/1,024	0	10.5 ± 0.1	7.2 ± 1.2
	—	DBA/1J *H-2*^{q}	D1.C × B10.AKM *H-2*^{d} × *H-2*^{m}	C-17	1/512	1/640w§	12.5 ± 1.0	7.4 ± 1.3
H-2.18‡	—	B10.RIII(71NS) *H-2*^{r}	B10 × A/J *H-2*^{b} × *H-2*^{a}	ASM-81	1/2,048	1/256	9.6 ± 1.1	6.8 ± 1.2
	—	B10.RIII(71NS) *H-2*^{r}	B10.A(2R) × C3H.NB *H-2*^{h} × *H-2*^{p}	AS-381	1/256	1/1,250	NT	NT
	H-2.25	B10.RIII(71NS) *H-2*^{r}	B10.D2 × DBA/1J *H-2*^{d} × *H-2*^{q}	AS-538	1/300	1/1,280	11.2 ± 1.2	7.0 ± 0.9
H-2.19	—	A.SW *H-2*^{s}	A.CA × B10.A *H-2*^{f} × *H-2*^{a}	C-19	1/128	1/640	9.8 ± 1.2	7.0 ± 1.0
	—	B10.S *H-2*^{s}	A.CA × B10.A *H-2*^{f} × *H-2*^{a}	C-19	1/128	1/640	10.3 ± 1.2	7.4 ± 1.5
H-2.23	—	B10.A *H-2*^{a}	B10.RIII(71NS) × PL/J *H-2*^{r} × *H-2*^{u}	AS-512	1/70	1/640	11.5 ± 1.1	7.5 ± 1.2
	—	B10.A(2R) *H-2*^{h}	B10 × LP.RIII *H-2*^{b} × *H-2*^{r}	ASM-179	1/256	NT	10.2 ± 0.8	NT

H-2.31	*H-2.34*	B10.D2 $H\text{-}2^d$	B10 × A/J $H\text{-}2^a \times H\text{-}2^b$	AS-539	1/1,024	0	10.3 ± 1.3	7.2 ± 1.4
	H-2.34	B10.D2 $H\text{-}2^d$	B6 × A/J $H\text{-}2^a \times H\text{-}2^b$	—	—	—	10.5 ± 1.4	NT
	H-2.34	B10.D2 $H\text{-}2^d$	B10.A $H\text{-}2^a$	—	—	—	11.8 ± 1.0	NT
	H-2.34	B10.D2 $H\text{-}2^d$	B10 × B10.A $H\text{-}2^b \times H\text{-}2^a$	—	—	—	14.5 ± 1.0	NT
	—	D1.C $H\text{-}2^d$	DBA/1J × B10.A $H\text{-}2^a \times H\text{-}2^q$	AS-562	1/32	0	12.2 ± 1.1	8.2 ± 0.8
	—	B10.D2 $H\text{-}2^d$	B10.A × DBA/1J $H\text{-}2^a \times H\text{-}2^q$	AS-563	1/32	0	11.4 ± 1.0	7.0 ± 0.8
	—	C3H.$H\text{-}2^o$ $H\text{-}2^o$	C3H/DiSn × B10 $H\text{-}2^k \times H\text{-}2^b$	AS-561	1/512	0	9.8 ± 1.0	7.1 ± 0.4
H-2.33	*H-2.39*	B10.A(5R) $H\text{-}2^i$	B10.D2 × A/J $H\text{-}2^d \times H\text{-}2^a$	C-33	1/512	0	9.3 ± 0.8	6.8 ± 0.7

* See footnotes for Table I.

‡ Only provisionally localized in the *K* region (see text).

§ W, weak.

that were used to produce antibody, and which had therefore received 13–15 injections of donor lymphoid cells, were used as recipients for donor skin grafts. In all cases, irrespective of the antigens involved, the grafts were rejected between 6.5 and 8.0 days. No graft prolongation or active enhancement was seen, and no difference in the rate of rejection for *K* or *D* region antigenic differences.

Cytotoxic and Hemagglutinating Antibody Formation after Immunizations with Lymphoid Cells (Tables I and II).—*D* region antigens gave lower titers of cytotoxic antibodies than *K* region antigens. As with the skin graft survival, *H-2.4* appeared to be the most immunogenic *D* region specificity and *H-2.2* the least. With the possible exceptions of AS-377 (anti-*H-2.4*), cytotoxic titers to *D* region antigens were not greater than $\frac{1}{128}$. AS-543, a (B10.AKM anti-B10.BR) antiserum did not form a detectable cytotoxic anti-*H-2.32* antibody, even after 15 injections of lymphoid cells. This is possibly an example of an Ir (immune response) effect conferred by the C57BL/10 genetic background (McKenzie and Snell, unpublished data). Five other anti-*32* antisera gave cytotoxic titers ranging from $\frac{1}{64}$ to $\frac{1}{128}$.

At the *K* region, cytotoxic antibody titers ranged from $\frac{1}{32}$ to $\frac{1}{2,048}$. Only 4 of 16 titers were $\frac{1}{128}$ or less, hence overlapping the *D* region range. As we shall point out in the Discussion, some of the variability was probably due to identifiable factors other than the properties of the antigen itself.

The hemagglutinating titers were difficult to interpret, as several *H-2* specificities, notably *31*, *32*, and *33*, do not usually lead to hemagglutinating activity. However, several late bleeds after prolonged immunization contained antibody to *H-2.32* (C-32 and AS-485). In addition, when *H-2.17* was made in one combination (AS-545), no hemagglutinating antibody was detected. The difference between *H-2D* and *H-2K* region specificities in the titers of cytotoxic antibody and in skin graft survival was not reflected in the titers for hemagglutinating antibodies. Antigenic specificities from both regions led to hemagglutinating antibody titers of 0 to $\frac{1}{1,280}$.

Cytotoxic Antibody Formation after a First Set Skin Graft (Table III).—The humoral response engendered by a skin graft was much more pronounced for *H-2K* than for *H-2D* differences. Cytotoxic antibodies were found consistently on day 10, the approximate time of rejection of skin grafts where *K* region specificities were present. The titers subsequently rose to a maximum of $\frac{1}{64}$ to $\frac{1}{256}$ on days 15–20, and then began to fall. By contrast, when there was an antigenic difference of a *D* region specificity, no cytotoxic antibody was detectable in six of nine combinations. In the other three, titers of $\frac{1}{4}$ to $\frac{1}{8}$ were present on days 10 or 15. No tests were run for hemagglutinating antibodies in these sera. Clearly, skin grafts performed when there is an *H-2D* specificity difference give rise to relatively poor humoral as well as poor cellular immune responses.

Enhancement of Skin Grafts by the Passive Administration of Alloantibody to the Private H-2K and H-2D Specificities (Tables IV and V).—Although *H-2K*

TABLE III

Cytotoxic Antibody Titers Formed after a Skin Graft in Alloantibody Treated and Untreated Mice

Region	*H-2* specificity	Donor	Recipient	Antibody treatment	Cytotoxic antibody titer*				
					10	15	20	25	30
							day		
H-2D	*H-2.2*	B10.A(2R)	B10.D2 × A/J	None	0	0	0	0	0
				C-2	0	0	0	0	0
	H-2.4	B10.A	B10 × AKR.M	None	0	1/8	0	0	0
				AS-377	1/8	0	w§	0	0
	H-2.30	B10.RIII (71NS)	B6 × A/J	None	0	0	0	0	0
				ASM-80	0	0	0	0	0
	H-2.32	B10.BR	B10 × A/J	None	0	0	0	0	0
				C-32	0	0	0	0	0
	H-2.32	C3H/DiSn	C3H.SW × A/J	None	0	0	0	0	0
				AS-513	1/4	0	0	0	0
	H-2.32	B10.BR	B10.A(2R) × C3H.SW	None	0	0	0	0	0
				AS-541	0	0	0	0	0
	H-2.32	C3H/DiSn	B10.A(2R) × C3H.SW	None	0	0	0	0	0
				AS-485	1/6	0	0	0	0
	H-2.32	AKR/J	AKR.M	None	0	0	0	0	0
				AS-452	0	0	0	0	0
	H-2.32	B10.BR	B10.AKM	None	0	0	0	0	0
				AS-543	0	0	0	0	0
H-2K	*H-2.9*	B10.M	B10.D2 × A.SW	None	1/16	1/128	1/128	NT	1/64
				C-9	0	1/16	1/64	1/128	1/64
	H-2.15	B10.WB	B10.A(2R) × A.CA	None	1/8	1/64	1/128	1/128	NT
				AS-537	1/6	0	1/32	1/64	1/128
	H-2.17	DBA/1J	D1.C × B10.AKM	None	1/64	1/64	1/64	1/64	NT
				C-17	0	0	1/16	1/64	1/64
	H-2.18	B10.RIII (71NS)	B10 × A/J	None	1/32	1/64	1/128	1/64	NT
				ASM-81	1/8	0	1/16	1/128	NT
	H-2.18	B10.RIII (71NS)	B10.D2 × DBA/1J	None	w	1/64	NT	NT	
				AS-512	0	0	1/8	NT	NT
	H-2.31	C3H.*H-2*^{o}	C3H/DiSn × B10	None	1/4	1/64	1/128	1/128	NT
				AS-561	0	1/16	1/64	1/256	1/128
	H-2.31	B10.D2	B10 × A/J	None	0	1/64	1/128	1/256	1/256
				AS-539	1/8	0	1/64	1/128	1/128
	H-2.33	B10.A(5R)	B10.D2 × A/J	None	w	1/64	1/256	1/128	NT
				C-33	1/8	0	0	1/64	1/128

* Cytotoxic antibody was measured by the ^{51}Cr cytotoxic test. The titers are the approximate means obtained from testing, separately, the serum obtained from four mice.

‡ Alloantibody reactive with donor antigens was given 0.1 ml on days 0, 2, and 4 after skin grafting to a group of 10–15 mice, the other group received no serum. Bleedings were performed on days 10, 15, 20, 25, and 30, after the graft

§ W, weak.

specificities appear to be more immunogenic for both cell-mediated and humoral immunities than are *H-2D* specificities, enhancement of skin grafts was far greater in the presence of *H-2K* than of *H-2D* region differences. Thus, graft prolongation of 2.4 to 6.7 days occurred with *K* region specificities, but of only 0.2 to 1.5 days with *D* region specificities. This is illustrated in Fig. 1, which shows the survival time of skin grafts in mice, both with and without alloantibody administration, for the two *H-2*^{b} private specificities *H-2D.2* and *H-2K.33*. The skin grafts bearing *H-2.33* (*H-2K* region) from the B10.A(5R) donor, had an earlier rejection than the grafts bearing *H-2.2* (*H-2D* region)

TABLE IV

Passive Enhancement of Skin Grafts in Mice Differing by Single Private Specificities at the H-2D Locus

H-2 Specificity	Donor	Recipient	Antibody treatment*	Skin graft survival	Prolongation
				MST ± *SD, days*	*days*
H-2.2	B10.A(2R)	B10D2 × A/J	None	17.8 ± 1.2	0.4
			C-2	18.2 ± 1.3	
H-2.4	B10.A	B10 × AKR.M	None	13.8 ± 1.1	0.7
			AS-377	14.5 ± 1.1	
H-2.30	B10.AKM	B10 × A/J	None	15.5 ± 1.1	0.7
			ASM-80	16.2 ± 1.2	
H-2.32	B10.BR	B10 × A/J	None	16.0 ± 1.2	0.8
			C-32	16.8 ± 1.4	
H-2.32	C3H/DiSn	C3H.SW × A/J	None	16.8 ± 1.1	1.5
			AS-513	18.3 ± 1.1	
H-2.32	B10.BR	B10.A(2R) × C3H.SW	None	14.5 ± 1.3	0.2
			AS-541	14.7 ± 1.2	
H-2.32	C3H/DiSn	C3H.SW × B10.A(2R)	None	15.0 ± 1.1	1.0
			AS-485	16.0 ± 1.1	
H-2.32	AKR/J	AKR.M	None	18.6 ± 1.6	0.3
			AS-542	18.9 ± 1.9	
H-2.32	B10.BR	B10.AKM	None	15.9 ± 1.1	0.7
			AS-543	16.8 ± 1.1	

* Alloantibody given 0.1 ml i.p. on days 0, 2, and 3, after skin graft.

from the B10.A(2R) donor, but this early rejection could be delayed by passively administered antiserum to *H-2.33*. The graft bearing the weaker *H-2.2* (*H-2D* region) showed a late rejection, but this was scarcely affected by passively administered anti-*H-2.2*. The degree of enhancement was similar for all *K* region specificities and was similar to that previously reported, for the *K* region specificity *H-2.31* (21).

Although the earlier studies did not suggest that prolonged dosage affected the degree of enhancement attained (21), it still seemed possible that the lack of enhancement for *H-2D* region antigens was due to the long interval between the last dose of antibody (4 days after the skin was grafted) and graft rejection, with a consequent disappearance of antibody. For example, for the *D* region specificity *H-2.2*, 14 days elapsed between the last dose of antibody and first set skin graft rejection, whereas for *K* region specificity *H-2.31* the elapsed time was only 6 days. To exclude a possible insufficiency of antibody, 0.2 ml of C-32, anti-*H-2.32*, was given every second day until graft rejection occurred in the combination B10.BR into (B10 × A)F_1. Enhancement was not increased, the grafts being rejected at the same time as with the standard three-dose regimen. Similarly, a dose of 0.5 ml on days 0, 2, and 4 had no effect on the inability to produce enhancement in this combination.

TABLE V

Passive Enhancement of Skin Grafts in Mice Differing by Single Private Specificities at the H-2K Locus

H-2K Specificity	Donor	Recipient	Antibody treatment*	Skin graft survival	Prolongation
				MST ± *SD, days*	*days*
H-2.9	B10.M	B10.D2 × A.SW	0	10.2 ± 1.2	5.1
			C-9	15.3 ± 1.4	
H-2.9	B10.M	B10.D2 × SJL/J	0	10.8 ± 1.1	3.4
			AS-544	14.2 ± 1.2	
H-2.15	B10.WB	A.CA × B10.A(2R)	0	9.8 ± 1.1	4.8
			AS-537	14.6 ± 1.2	
H-2.17	DBA/1J	D1.C × AKR.M	0	10.5 ± 0.1	4.0
			AS-545	14.5 ± 0.1	
H-2.17	DBA/1J	D1.C × B10.AKM	0	12.5 ± 1.0	4.0
			C-17	16.5 ± 1.2	
H-2.18	B10.RIII(71NS)	B10 × A/J	0	9.6 ± 1.1	4.2
	B10.RIII(71NS)	B10 × A/J	ASM-81	13.8 ± 1.1	
	B10.RIII(71NS)	B10 × A/J	AS-381	12.5 ± 0.8	3.1
	B10.RIII(71NS)	B10D2 × DBA/1J	AS-538	12.0 ± 0.5	2.4
H-2.19	A.SW	A.CA × B10.A	0	9.8 ± 1.2	4.4
			C-19	14.2 ± 1.2	
H-2.19	B10.S	A.CA × B10.A	0	10.3 ± 1.2	4.7
			C-19	15.0 ± 1.5	
H-2.23	B10.A	B10.RIII(71NS) × PL/J	0	11.5 ± 1.1	3.7
			AS-512	15.2 ± 1.1	
H-2.31	B10.D2	B10 × A/J	0	10.3 ± 1.3	6.7
			AS-539	17.0 ± 1.1	
H-2.31	B10.D2	B6 × A/J	0	10.8 ± 1.4	5.2
			AS-539	16.0 ± 1.5	
H-2.31	B10.D2	B10.A	0	11.8 ± 1.1	4.2
			AS-539	16.0 ± 1.2	
H-2.31	B10.D2	B10 × B10.A	0	14.5 ± 1.1	2.3
			AS-539	16.8 ± 1.5	
H-2.31	D1.C	B10.A × DBA/1J	0	12.2 ± 1.1	4.2
			AS-562	16.4 ± 1.2	
H-2.31	B10.D2	B10.A × DBA/1J	0	11.4 ± 1.0	4.2
			AS-563	16.4 ± 1.2	
H-2.31	C3H.*H-2*^{o}	C3H/DiSn × B10	0	9.8 ± 1.0	4.8
			AS-561	14.6 ± 1.0	
H-2.33	B10.A(5R)	B10D2 × A	0	9.3 ± 0.8	6.0
			C-33	15.3 ± 1.4	

* Alloantibody given 0.1 ml i.p. on days 0, 2, and 4.

Although the specificity of the enhancement of skin grafts had been demonstrated in an earlier study, a check was included here. A group of 10 (B6 × A)F_1 mice received a B10.BR skin graft (an antigenic difference of *H-2D.32*) and 0.1 ml of anti-*H-2K.31*, intraperitoneally, on days 0, 2, and 4. The grafts were rejected in normal fashion with no prolongation.

Antibody-Mediated Suppression of Alloantibody Formation (Table III).—For *K* region antigens, the administration of passive antibody on days 0, 2, and 4 after skin grafting delayed the appearance of cytotoxic antibody for 5–15 days. However, in most cases, antibody appeared approximately at the same time that the graft was rejected. In mice receiving antibody passively, antibody pre-

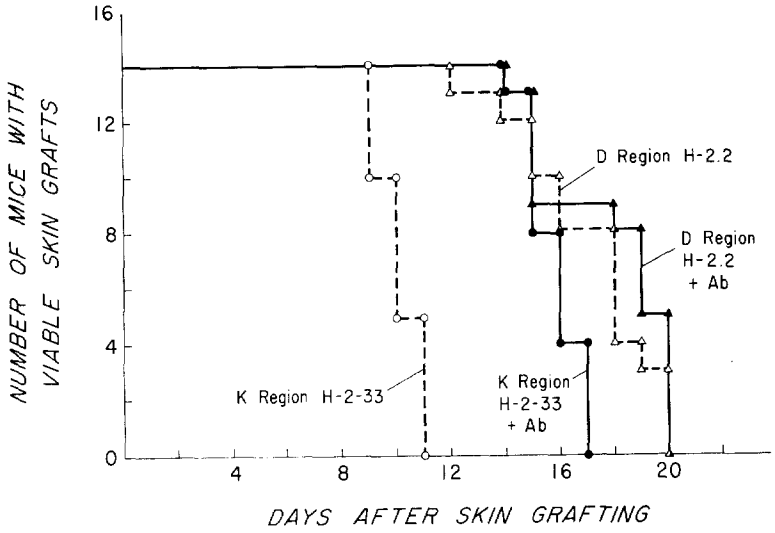

FIG. 1. The MST for skin grafts is shown for the two private antigens of the $H\text{-}2^b$ haplotype: *H-2.33* (*H-2K*) and *H-2.2* (*H-2D*). In both cases, male (B10.D2 × A)F_1 recipients were used and received a B10.A(2R) graft (for *H-2.2*) or a B10.A(5R) graft (for *H-2.33*). The basic differences in the immunogenicity of the skin graft is illustrated when the *H-2.2* graft is rejected later than the *H-2.33* graft. By contrast, enhancement could be produced for *H-2.33* but not with *H-2.2*.

sumed to be residual antibody, was still detectable on day 10, but absent on day 15. The studies of *D* region specificities could reveal no suppressive effect, there being little antibody formed even in untreated mice.

DISCUSSION

The *H-2* system of the mouse is generally regarded as consisting of two alloantigen-determining loci, *H-2K* and *H-2D*. By appropriate choices of recipient-donor combinations, it has been possible to study various manifestations of the immune response where recipient and donor differed by single, private *H-2K* or single, private *H-2D* specificities. The responses measured included the rejection times of first and second-set skin grafts, the lymphocytotoxic and hemagglutinating antibody responses engendered by multiple injections of donor tissue, the lymphocytotoxic response engendered by first-set skin grafts, and the degree to which immunological enhancement could be induced by the passive transfer of alloantibody. By all tests except the production of hemagglutinating antibody, where no *K-D* difference was observed, the *K* antigen appeared to be stronger than the *D* antigen. Thus the MST of grafts ranged from 9.3 to 14.5 days for *K* end differences, and from 13.8 to 18.6 for *D* end differences. On the other hand, enhancement was much greater for the stronger *K* end differences than for the weaker *D* end differences. For the former, it ranged from 2.4 to 6.7 days, for the latter from 0.2 to 1.5 days.

Differences in the immunogenic strength of the *H-2K* and *H-2D* alloantigens similar to those reported here, have been observed also with heart (30) and kidney (31) transplants.

There was considerable consistency in both the *H-2K* and *H-2D* responses. Nevertheless, there is reason to believe that there were in both sets of tests a number of identifiable variables which, if they could have been eliminated, would have made the results at each locus more uniform and the differences between the loci even more striking. Some of these variables are as follows.

The *Tla* locus is closely linked with *H-2*, and some *H-2* congenic stocks consequently differ at *Tla* as well as *H-2*. While *Tla* is primarily demonstrable through the cytotoxicity of thymic lymphocytes, it has recently been shown that it causes, or is associated with, a weak histocompatibility (32, 33). In our *D* end *H-2.32* combination, B10.BR into B10.A(2R) × C3H.SW (Table I), the donor was *Tla-1, 2, 3* and the recipient *Tla*-negative. The MST for this combination, 14.5 ± 1.3 days, was the second lowest. In our two *H-2.9* combinations (Table II), the donor was probably *Tla-1, 2, 3*, the recipients *Tla-2*/*Tla*-negative. The effect of *Tla* on histocompatibility in this particular combination has not been determined. The MST's were within the normal range.

There are a number of *H-2* private specificities which include two or three public specificities. These can be interpreted as the manifestation of cross-reactions. Thus anti-*H-2.25* may be an antibody reacting with both *H-2.18* and *H-2.23*. If there are indeed such cross-reactions, one might expect that strain combinations in which the antigens of recipient and donor are cross-reacting, and hence antigenically related, will give relatively weak immune responses. The two *H-2.23* combinations (Table II) are a possible example, since *H-2.18* was present in the recipient. The first of the two combinations, B10.A into B10.RIII(71NS) × PL/J, moreover, probably blocks a second cross-reaction. Strain PL/J (*H-2*^{u}) has been shown to react with anti-23 antisera (34, no symbol has been assigned). Not surprisingly, this combination showed both a slow rejection for a *K* end difference (MST 11.5 ± 1.1) and a particularly weak cytotoxic titer (1/70). The second combination, sharing only *H-2.25*, gave a somewhat weak antibody (titer 1/256) but a normal MST (10.2 ± 0.8). It is also noteworthy that *H-2.4*, which appeared to be the strongest of all *D* end specificities, was studied in a combination which failed to exclude three or four potential cross-reactions, though this may merely mean that one of the determinants of antigenic "strength" is the capacity to trigger a diversity of antigen-reactive cell clones. In one *H-2.18* combination, B10.RIII (71NS) into B10 × A/J, in which cross-reacting antibody *H-2.25* was blocked, the immune responses appeared particularly strong. But *H-2.18* is exceptional in that it is presumably the sum of both *K* and *D* private specificities.

It has been reported that DBA/1 and DBA/1 hybrids mount a particularly weak resistance to skin and tumor allografts (35, 46). In five cases involving specificities *H-2.17*, *H-2.18*, and *H-2.31* (Table II), DBA/1 or a DBA/1 congenic line was one parent of the recipient. In four of these five cases the other parent was B10 or a B10 congenic. These four cases showed relatively slow graft rejection for *K* end combinations (MST's of 12.5, 11.2, 12.2, and 11.4) and relatively poor antibody production (cytotoxic titers of 1/512, 1/300, 1/32, 1/32). In the fifth combination, DBA/1 into D1.C × AKR.M, graft rejection and cytotoxic antibody production were slightly stronger than average.

In four of the donor-recipient combinations in Tables I and II, the recipients were either nonhybrids or a hybrid between congenic partners. With the one exception B10.BR into B10.AKM, where graft rejection was rapid, these combinations showed slow rejections and low antibody titers. This could mean that absence of hybrid vigor weakens the immune response. Alternatively, or in addition, the unhybridized B10 genetic background may be unfavorable to some *H-2* immune responses.

As noted under Methods, the seven "C" antisera were produced with a somewhat different immunization schedule than the "AS" or "ASM" antisera, and were much older when used. The cytotoxic titers were generally somewhat low.

H-2 is the prototype of a "strong" histocompatibility "locus" (37). Now that it is known to consist of linked loci, which can be studied separately, the question of its strength is open to reexamination. *H-2K* still appears as a uniquely strong locus, in terms of skin graft rejections, almost as strong as the undivided *H-2* complex. It is very clear from this study that *H-2D* is somewhat weaker. But compared with non-*H-2* loci, for which the shortest MST's are of the order of 20–25 days (38), it still appears as relatively strong.

H-2K and *H-2D* are unique among histocompatibility loci in the ease with which they are demonstrated by serological as well as transplantation techniques. They are closely linked and perhaps the product of reduplication of a single, ancestral locus (34, 39). Antisera made against the antigen of one sometimes cross-react with the antigen of the other (3, 40, Snell and Cherry, unpublished data). The *H-2K* and *H-2D* antigens show considerable chemical similarity (41). With all these indications of similarity, why is one locus associated with stronger immune responses than the other?

It is possible that the difference is in part an artifact, the product of unfavorable immune response genes in some or all of the combinations in which *H-2D* has been studied. Lilly et al. (42) showed that B10 and some of its congenic partner strains are poor responders to *D* end specificity *H-2.2*, and Stimpfling and Durham (43) demonstrated a role of the *H-2* region itself on the response to *H-2.2*. This may apply also to other *D* end specificities (44, McKenzie, Cherry, and Snell, unpublished data). As a case in point, AS-543, B10.AKM anti-B10.BR, did not contain the anticipated anti-32. *H-2D* has usually been studied in combinations in which B10 and its congenics are present. But when the best combinations which we have encountered for the production of anti-*H-2D* are compared with the best combinations for the production of *H-2K*, the difference still remains substantial.

Despite the demonstrable chemical similarities of *H-2K* and *H-2D*, it is possible that undetected chemical differences account for the differences in antigenic strength. But this is no direct evidence in support of such a possibility.

The *H-2* complex plays a major role in the mixed lymphocyte reactions (45, 46). Lymphocytes differing at *H-2* regularly show stimulation in mixed cultures, whereas, with a few major exceptions, non-*H-2* combinations do not stimulate or stimulate weakly. Most of the mixed lymphocyte reactivity of the *H-2* complex resides in the *K* end, the *D* end playing only a minor role (4, 16–18,

47, 48). Originally, it was assumed that the *H-2* loci themselves were the responsible agents, but recent evidence, based on the use of certain critical recombinants, indicates that the effect maybe due primarily to other components of the complex (4, 47). A major part of the activity seems to be due to *Ir-1*, or some locus not now separable from it, but there is a weaker center of activity near to but independent of *H-2D*.

The critical recombinant haplotypes with respect to *K* end mixed lymphocyte reactivity are $H\text{-}2^{y\text{-}K^{lj}}$ and $H\text{-}2^{y\text{-}S_g}$. These are identical with respect to *H-2K* and *H-2D* but, as a result of recombinations at the extreme left and right ends, respectively, of the complex, differ at *Ir-1* and *SsSlp*. $H\text{-}2^{y\text{-}K^{lj}}$ arose from a rare crossover between *H-2K* and *Ir-1*, thus providing a source of information as to the effect of these loci separately. These particular recombinant haplotypes were not used in our tests.

Another example of unexplained reactivity associated with the *K* end of *H-2* is provided by the *H-2* mutant, $H\text{-}2^{ba}$ (49). Skin grafts exchanged between the original and the mutant haplotypes are strongly rejected, but numerous tests failed to reveal any serological difference. F_1 tests with appropriate recombinant haplotypes localized the effect at the *K* end. Whether the change is at *H-2K* itself, or at some closely linked, heretofore undetected histocompatibility locus is unknown.

There are thus, at the *K* end of the *H-2* region, known or postulated loci, other than *H-2K* itself, which can engender immune or at least blastogenic responses. This makes it tempting to assume that part of the unusual strength of *H-2K* as compared to *H-2D* is due to these companion loci. From what is known of these loci, it can plausibly be supposed that they contribute part of the potential for rapid graft rejection associated with the *K* end. However, since it is a primary characteristic of these loci that they are not demonstrable serologically, they do not serve to explain the strong serological responses associated with the *K* end.

Perhaps the most surprising property of the *K* end revealed in these studies is the relative success of the enhancement of graft survival by passively transferred antibody. Graft prolongation was generally less than 1 day in the *D* end tests, generally more than 4 days in the *K* end tests.

The poor response of *H-2D* seems not to be due to some intrinsic resistance of the *H-2D* alloantigenic specificities to immunosuppression. Demant et al. (50) have shown that rabbit antimouse lymphocyte serum more than doubles the survival of skin grafts with an *H-2D* difference. Grafts with an *H-2K* difference responded less effectively. Also, host irradiation can substantially prolong the survival of grafts across the *H-2D* barrier (51).

If the *K* end loci other than *H-2K* do not determine antibody-inducing antigens, they cannot provide an explanation of the efficiency of *K* end enhancement. Another possibility is that *K* end antibodies enhanced better simply because they were stronger. However, there was little correlation between antibody titer and enhancement. Thus *K* end antibody AS-563, anti-*H-2.31*, with a cytotoxic titer of $\frac{1}{32}$, produced a prolongation of 4.2 days, whereas *D* end

antibody AS-377, anti-*H-2.4*, with a cytotoxic titer of $\frac{1}{128}$, produced a prolongation of only 0.7 days. Also we have previously demonstrated enhancement with an anti-*H-2.31* (*K* end) with a dose as low as 0.1 μl (52). It is of course possible that cytotoxic titers are a poor measure of the concentration of enhancing antibody, but there is no direct evidence for this. At the moment there seems to be no good explanation of the relative enhanceability of grafts with an *H-2K* difference.

SUMMARY

The immunogenicity of single, private *H-2* specificities has been tested. In most cases, the specificity was known to be confined either to *H-2K* or to *H-2D*. This was accomplished by the appropriate choice, as donor and recipient, or parent of the F_1 hybrid recipient, of congenic strains differing at *H-2* only, and of recombinant haplotypes in donor and/or recipient.

By nearly all tests, the *H-2K* antigen appeared to be a stronger immunogen than the *H-2D* antigen. Skin grafts with an *H-2K* difference showed median survival times (MST) of 9.3–14.5 days; for *H-2D* the values were 13.8–18.6. The difference was also present, though narrowed, for second-set grafts. *H-2K* grafts regularly engendered a demonstrable cytotoxic antibody response; with *H-2D* differences the response was absent or very weak. *K* end cytotoxic titers after multiple immunizations with lymphoid tissues ranged from $\frac{1}{32}$ to $\frac{1}{2,048}$, *D* end titers from 0 to $\frac{1}{128}$. Hemagglutination titers showed no clear difference.

The results of passive enhancement of skin grafts with *H-2* alloantibody produced in donor recipient combinations, identical to those used for the skin grafts showed a different pattern. *H-2K*, despite its greater immunogenic strength was more easily enhanced than *H-2D*. Prolongation of MST's for *H-2K* was 2.4–6.7 days, for *H-2D* grafts, 0.2–1.5 days.

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