EVIDENCE SUPPORTING A TWO-GENE MODEL FOR THE H-2 HISTOCOMPATIBILITY SYSTEM OF THE MOUSE*

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The H-2 system of the IXth linkage group in the mouse determines cell membrane components which, in proper donor-recipient combinations, can induce both cellular and humoral responses. The H-2 antigens can therefore be studied by both transplantation and serological methods. The H-2 system is complex serologically (one H-2 chromosome determines more than one antigen) and genetically (crossing-over occurs within the system). The H-2 segment of the chromosome is divided into two regions, D and K, by the Ss locus (14). The Ss locus controls a serum protein(s) which can be recognized by xeno-(Ss) and allo-(Slp) antisera (12, 19). The locus is separable from the regions controlling the H-2 antigens by recombination (15). The Ss protein is not an integral part of the cell membrane and it does not behave like a transplantation antigen (20). At least one of its components (Slp) is subject to hormonal control (13). All this indicates that the Ss locus is genetically and functionally distinct from, and apparently unrelated to, the H-2 loci. The presence of an unrelated gene(s) in the middle of the H-2 segment requires that the two regions, H-2D and H-2K, be regarded as two distinct genetic entities. They could be either single genes or gene complexes. Until recently, genetic evidence seemed to support the latter possibility; serological analyses of intra-H-2 recombinants seemed to indicate further genetic divisions of the two regions, D into D, C, V, and E, and K into K and A (15, 16). The frequency of crossing-over between H-2D and H-2K was calculated to be of the order of 0.5% (15). Under normal circumstances, this is too high for intragenic crossing-over and thus the most likely explanation from this finding was that the D and K segments of the H-2 complex were each composed of several genes.

However, some new data have led to a reinterpretation of the genetic structure of the H-2 system and the proposal of a simplified model consisting of only two histocompatibility regions (loci) (10). The new interpretation is based on the assumption that there is serological cross-reactivity between the antigens determined by the H-2K locus and the antigens determined by the H-2D locus. The difference between the two interpretations can be illustrated by the following example.

Among the first intra-H-2 crossovers discovered were two called H-2h-o° and H-2h-o°. The difference between these two crossovers is that the H-2h-o° crossover occurred between the D and K segments, while the H-2h-o° crossover occurred within the D segment. The new interpretation suggests that these two crossovers represent the same genetic event, with the H-2h-o° crossover occurring within the D segment. This is consistent with the new model, which proposes that the D and K segments are each composed of several genes.

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1 Shreffler, D. C., and C. S. David. 1972. Studies on recombination within the mouse H-2 complex. I. Three recombinants which position the Ss locus within the complex. Submitted for publication.
Both were derived from F1 hybrids between the same two strains, A \((H-2^a)\) and C57BL \((H-2^b)\). The \(H-2^{b-ao}\) crossover received the \(H-2D\) region from \(H-2^b\) and the \(H-2K\) region from \(H-2^a\); the \(H-2^{i-ao}\) crossover received the \(H-2D\) region from \(H-2^a\) and the \(H-2K\) region from \(H-2^b\) (5). In both cases the crossing-over took place between the \(Ss-Slp\) locus and the \(H-2D\) region (16). (Both crossovers received the \(Ss-Slp\) locus together with the \(H-2K\) region.) When tested with a specific antiserum which defines antigen H-2.3, strain C57BL is negative; strain A and both crossovers are positive. Together these data can be interpreted as evidence for separation of an \(H-2C\) region, controlling antigen H-2.3, from the \(H-2D\) and \(Ss-Slp\) regions. It could be assumed that in \(H-2^{b-ao}\) the crossing-over took place between \(H-2D\) and \(H-2C\), and in \(H-2^{i-ao}\) between \(H-2C\) and \(Ss-Slp\), as diagrammed below:

\[
\begin{array}{c}
H-2^a \quad 11 \quad Slp^a \quad 3 \quad 4 \\
H-2^b \quad 33 \quad Slp^o \\
\end{array} \rightarrow \quad \begin{array}{c}
H-2^{b-ao} \quad 11 \quad Slp^a \quad 3 \quad 2 \\
\end{array}
\]

\[
\begin{array}{c}
H-2^a \quad 11 \quad Slp^o \\
H-2^b \quad 33 \quad Slp^o \\
\end{array} \rightarrow \quad \begin{array}{c}
H-2^{i-ao} \quad 33 \quad Slp^o \quad 3 \quad 4 \\
\end{array}
\]

In this way, three regions controlling histocompatibility antigens could be defined, \(K\), \(C\), and \(D\). However, the two crossovers could also be interpreted in a somewhat less orthodox way. Let us assume that anti-H-2.3 reacts with the \(H-2D\) product and cross-reacts with the \(H-2K\) product of the \(H-2^a\) chromosome. Then the two crossover events could also be interpreted as follows:

\[
\begin{array}{c}
H-2^a \quad (11 \quad "3") \quad Slp^a \quad (3 \quad 4) \\
H-2^b \quad 33 \quad Slp^o \\
\end{array} \rightarrow \quad \begin{array}{c}
H-2^{b-ao} \quad (11 \quad "3") \quad Slp^a \quad 2 \\
\end{array}
\]

\[
\begin{array}{c}
H-2^a \quad (11 \quad "3") \quad Slp^a \quad (3 \quad 4) \\
H-2^b \quad 33 \quad Slp^o \\
\end{array} \rightarrow \quad \begin{array}{c}
H-2^{i-ao} \quad 33 \quad Slp^o \quad (3 \quad 4) \\
\end{array}
\]

In this case, there would be no need to assign antigen H-2.3 to a region separate from regions \(H-2K\) and \(H-2D\).

Similar interpretations can also be applied to regions \(H-2E\) and \(H-2A\). Region \(H-2V\) is required only to code for antigen H-2.22. However, recent evidence seems to indicate that this antigen may be identical with antigen H-2.2, and therefore, there seems to be no need for a separate \(H-2V\) region. These, plus a number of additional, more complex considerations (18), led to the postulation of the new model. By eliminating regions \(A\), \(E\), \(V\), and \(C\), the only two regions remaining are \(K\) and \(D\), and the present data can be fitted to this concept in a much more satisfying way.

Thus, at the present time, one has a choice between two genetic interpretations of the H-2 system: A traditional one with no less than six separate regions controlling transplantation antigens, and an unorthodox one with only two histocompatibility regions. In this communication we present evidence supporting the latter interpretation.
Materials and Methods

H-2 Crossover Strains.—Over 35 intra-H-2 crossovers have been reported in the literature (15). Of these, 15 have been subjected to detailed serological analysis and have been shown to represent 12 different crossover types. In addition, at least two more H-2 chromosomes are thought (but not proven) to be derived from other known H-2 chromosomes by intra-H-2 recombination. This makes a total of 14 different crossover types, of which 12 were used in the present study. They were the following:

H-2a is a suspected crossover chromosome, possibly derived from chromosomes H-2d and H-2k. The crossover origin of H-2a was first suggested after it was shown that H-2d/H-2k hybrids accept H-2a grafts (24). The H-2a chromosome is now available on two inbred backgrounds as strains A and B10.A. In the present study, the strain B10.A was used.

H-2z is a proven crossover derived from chromosomes H-2d and H-2k (3). It differs from H-2a at the Ss-Slp locus (H-2a is SshSlp o; H-2z is SsPSlp o). The H-2z chromosome is available on the A strain background as congenic resistant (CR) line A.AL which was used in the present study.

H-2o is a crossover derived from hybrids between BALB/c (H-2d) and C57BL (H-2k) (5). Since its discovery it has been maintained as a separate line, HTG, mostly by sib-sib matings. A congenic line B10.H-2o is being produced by Dr. Frank Lilly, Department of Genetics, Albert Einstein College of Medicine, New York, but the line was not available at the time of this experiment. The line used by us is a descendant from the original Gorer line.

H-2ha chromosome is present in at least three independent crossover lines. All three lines were originally derived from hybrids between A (H-2d) and C57BL (H-2k) strains. The first H-2ha crossover was described by Gofer (5) and assigned a symbol H-2ha (26). The crossover chromosome is not available on any defined inbred background although the HTH line has been maintained mostly by sib-sib matings. The other two crossover chromosomes, H-2ha and H-2ha, are available on C57BL/10 background as CR lines B10.A(1R) and B10.A(2R), respectively (25, 26). Serologically there seems to be no difference between the products of the three H-2ha chromosomes. Since they all carry the Slp o allele at the Ss-Slp locus, we shall use here, an abbreviated symbol H-2ha for all three of them (a in the superscript standing for the Slp o allele). Of the three lines, only B10.A(2R) was employed in the experiment described below.

H-2ho was also derived from strains A and C57BL/10 (25, 26). It is now available as CR line B10.A(4R). The chromosome has been originally assigned a symbol H-2ho (26). In this paper, we abbreviate this to H-2ho since, at the present time, the only known difference between H-2ho and H-2ho is at the Ss-Slp locus. (Strain B10.A(4R) carries allele Slp o, see reference 20.)

H-2ho is the third of the three original Gofer crossovers (5). It was derived from strains A and C57BL. The recombinant chromosome was originally assigned symbol H-2o and later H-2ho (26). We abbreviate this symbol to H-2ho because the chromosome carries the Slp o allele at the Ss-Slp locus (20). The H-2ho chromosome is now present only in a line called HTI which is derived from the original Gofer crossover and has been maintained mostly by sib-sib matings. No CR line with the H-2ho chromosome is available at present.

H-2ha is present in at least two crossover lines, B10.A(3R) with chromosome H-2ha and B10.A(5R) with chromosome H-2ha (25, 26). Both lines are on C57BL/10 background. Since the H-2 products of these two lines are indistinguishable, and the H-2ho and H-2ha chromosomes are thought to be different, they were combined in the present study.

David, C. S., and D. C. Shreffler. 1972. Studies on recombination within the mouse H-2 complex. II. Serological analyses of four recombinants, H-2ho, H-2ha, H-2ho, and H-2ho. Submitted for publication.

Abbreviation used in this paper: CR, congenic resistant.
chromosomes differ from $H-2^{k}$ apparently only at the $Ss-Slp$ locus, we use the symbol $H-2^{a}$ (for $Ss^{a}$ or $Slp^{a}$) for both crossover lines. In the present experiment only line B10.A(5R) was used.

$H-2^{a}$ is another suspected crossover chromosome, possibly derived from chromosomes $H-2^{b}$ and $H-2^{k}$. Its crossover origin has been discussed by us previously (9). The chromosome is present on two inbred backgrounds: AKR, as a CR line AKR.M (22), and C57BL/10 as B10.AKM (25). We have used only the B10.AKM line.

$H-2^{ah}$ was derived from strains DBA/2 ($H-2^{b}$) and C3H ($H-2^{h}$) (17, 21). It is now available on C3H background in CR line C3H.OH.

$H-2^{ah}$ was derived from the same two strains as $H-2^{ah}$ (3). The two chromosomes apparently differ only at the $Ss-Slp$ locus ($H-2^{sh}$ is $Ss^{h}Slp^{a}$; $H-2^{ah}$ is $Ss^{a}Slp^{a}$). The $H-2^{ah}$ chromosome is now present on the C3H background in CR line C3H.OH.

$H-2^{k}$ was derived from chromosomes $H-2^{a}$ and $H-2^{h}$ (A.SW) (3). The $H-2^{k}$ chromosome is present on the A background in a CR line A.TL.

$H-2^{k}$ originated from a B10.A ($H-2^{a}$) x T138 ($H-2^{k}$) hybrid (9). It is present in non-inbred strain AQR. It is also being transferred to the B10 background but the B10.AQR line is not yet fully congenic with C57BL/10.

Origin of Mouse Strains Employed.—Breeding pairs of strains B10.A ($H-2^{a}$), C57BL/10Sn (-B10) ($H-2^{a}$), B10.D2 ($H-2^{a}$), B10.A(2R) ($H-2^{a}$), B10.A(5R) ($H-2^{a}$), and B10.BR ($H-2^{b}$) were obtained from the Jackson Laboratory, Bar Harbor, Maine. Breeding pairs of strains B10.A(4R) ($H-2^{b}$) and B10.AKM ($H-2^{a}$) were kindly supplied by Dr. J. H. Stimpfling, McLaughlin Research Institute, Columbus Hospital, Great Falls, Mont. All these strains have been maintained by sib-sib matings in the mouse colony of the senior author. Strains C3H.D ($H-2^{b}$), C3H.OH ($H-2^{ah}$), C3H.OH ($H-2^{ah}$), C3H.OH ($H-2^{ah}$), A.TL ($H-2^{a}$), HTG ($H-2^{a}$), HTI ($H-2^{ah}$), and C3H/He ($H-2^{k}$) are maintained by the junior author.

Skin Grafting.—The method of Billingham and Medawar (2), modified as described previously (7), was used. The grafts were taken from the ears of the donors. The recipients were inspected daily for the first 2 wk after the removal of the bandage (8 days postoperatively) and once a week thereafter. At 60 days postgrafting the experiment was terminated, since there was no reason to expect rejection due to $H-2$ incompatibilities after this period of time.

RESULTS

As mentioned above, there are presently two possible interpretations of the genetic organization of the $H-2$ system. We shall call them hypothesis I and hypothesis II. According to hypothesis I, the $H-2$ system is composed of a minimum of eight regions, six of which control transplantation antigens (regions $K$, $A$, $E$, $V$, $C$, and $D$), and there is no cross-reactivity between the different regions. The origin of the 14 well-defined $H-2$ crossovers, according to hypothesis I, is shown in Fig. 1. Hypothesis II, on the other hand, requires only four $H-2$ regions, of which only two control transplantation antigens (regions $H-2^{K}$ and $H-2^{D}$). However, hypothesis II requires that the products of the two histocompatibility regions cross-react extensively. The origin of the same fourteen $H-2$ crossovers, as interpreted by hypothesis II, is shown in Fig. 2. The two hypotheses lead to different predictions of the fate of skin grafts in certain donor-recipient combinations. An example of the different predictions is shown in Fig. 3. Crossovers $H-2^{ah}$ and $H-2^{ah}$ have both been derived from chromosomes $H-2^{a}$ and $H-2^{b}$. According to hypothesis I, crossover $H-2^{ah}$ received from the $H-2^{a}$ chromosome the $H-2D$ region but not regions $K$, $A$, $E$, $V$, and $C$. Crossover $H-2^{ah}$ received from the $H-2^{b}$ chromosome regions $K$ and $A$, but not regions...
FIG. 1. Origin of fourteen H-2 crossovers from six original H-2 chromosomes according to hypothesis I (see text). The two original H-2 chromosomes of each crossover are shown by the interconnecting lines. Light type divisions separate individual H-2 regions; heavy type divisions indicate the place where the hypothetical crossing-over event took place. The numbers inside each box represent major H-2 antigens assumed to be controlled by these regions.
Therefore, the $H-2^{a+i}/H-2^{e}$ heterozygote should not accept $H-2^b$ skin grafts. (It should react against the products of the regions $E$, $V$, and $C$ which it does not share with $H-2^b$.) According to hypothesis II, on the other hand, crossovers $H-2^{a+i}$ and $H-2^{e}$ received from $H-2^b$ the $H-2D$ and $H-2K$ regions, respectively, and since (under this hypothesis) there are no other regions controlling transplantation antigens in the $H-2$ system, the $H-2^{a+i}/H-2^{e}$ heterozygote should accept $H-2^b$ grafts, providing that there is no reaction against non-$H-2$ antigens. The 14 well-defined crossovers can be arranged into 82 different heterozygous combinations, some of which can be tested in a similar way (see Fig. 4, in which only combinations involving eight “basic” $H-2$ crossovers are shown; omitted are those which differ from the basic crossovers only at the $Ss-Slp$ locus). The combinations which cannot be tested in this way are the following: (a) combinations for which the required donor is not known. For example, heterozygote $H-2^{a+i}/H-2^{e}$ could have been tested against a donor whose $H-2$ chromosome had its $K$-end derived from $H-2^b$ and its $D$-end derived from $H-2^i$. However, such a chromosome is not presently known. (b) Combi-
nations in which the donor and the recipient are not available on the same inbred background. For example, the $H-2^b/H-2^s$ heterozygote cannot be tested against an $H-2^d$ graft because neither $H-2^b$ nor $H-2^s$ is on the same background as $H-2^d$. (c) Combinations in which the trans-configurations of the $H-2K$ and $H-2D$ regions in the $H-2$ heterozygote are identical with one or the other of the two cis-configurations of the parental $H-2$ chromosomes. For example, in heterozygote $H-2^b/H-2^s$, one trans-configuration, $H-2(KkD^o)$, is identical with $H-2^b$, and the other, $H-2(KkD^d)$, is identical with $H-2^s$.

When these three types of combinations are excluded, 41 combinations remain which can be tested. They are listed in Table I together with the predictions about the survival of the graft on the basis of hypotheses I and II. The predictions about the fate of the graft are based on the following assumptions:

(a) $Ss$, $Slp$, and $Ir-I$ differences do not induce cellular immunity. This assumption is relevant to both hypotheses I and II. The noninvolvement of $Ss-Slp$ in histocompatibility is well documented. For example, it is known that skin grafts exchanged between strains C3H.OH and C3H.OL, which differ at the $Ss-Slp$ locus only, survive permanently (D. C. Shreffler, unpublished data). There is no direct evidence as yet that the same assumption holds also for the $Ir-I$ differences, but indirectly the assumption is supported by the fact that there is no known relationship between $Ir-I$ and any of the H-2 antigens (6).

(b) Products of “silent” regions can induce cellular immunity. By a silent region we mean instances when a segment of an H-2 chromosome does not pro-
duce any serologically detectable antigen. For example, according to hypothesis I, the C-region of the H-2^a chromosome controls antigen H-2.3. The corresponding region in the H-2^b chromosome is silent, i.e., no antigen is known to be determined by this region. We assume, nevertheless, that the H-2^a recipient should react against a H-2C product of the H-2^b chromosome. This assumption is supported by the analogy with non-H-2 loci, most of which have to be considered as serologically silent. It is relevant to hypothesis I only.

(c) Products of serologically identical regions of different origin do not produce immunity. For example, the E-region in H-2^b and the E-region in H-2^k both control antigen H-2.5 and there is no other known serological difference between the two regions. Although the two E-regions in these two chromosomes are almost certainly unrelated and therefore probably nonidentical, we still assume, for the sake of simplicity, that the products of these regions will be the same. This assumption does not necessarily have to hold. However, if it is invalid, several additional combinations in Table I would be expected to reject grafts under the hypothesis I while the assumption has no bearing on hypothesis II.

(d) Under hypothesis I, the position of crossing-over in some cases is uncertain. In such cases, we take into consideration only one possibility. For example, according to hypothesis I, crossing-over which produced the H-2^{2a} chromosome

![Diagram of heterozygous combinations of H-2 crossovers and donors](image-url)
TABLE I

Expected and Observed Outcome of Skin Grafting in Strain Combinations Testing
Hypotheses I and II (See Text)

| Donor-recipient combination | H-2 type | Hypothesis I | Hypothesis II | Observed outcome |
|-----------------------------|----------|--------------|---------------|------------------|
|                            |          | Expected     | Expected       | Rejected         | Clusters         |
|                            |          | outcome      | grafts         |                  |                  |
| B10.D2 → (B10.A × HTG)     | d        | a/g          | A1; None       | A1               | 0/7§             | A (? )          |
| 2R → (B10.A × HTG)         | ha → a/g  | A     None   | A     0/13§     | A    (? )        |
| 4R → (B10.A × HTG)         | ho → a/g  | A (? ) Ss-Slp, E (? ) | A 0/13§ | A (? )         |
| B10.BR → (B10.A × C3H.OH)  | k → a/ob  | A     Ss-Slp | A     0/10     | A                |
| B10.D2 → (B10.A × C3H.OH)  | d → a/ob  | R     A, E   | A     0/15     | A                |
| B10.BR → (B10.A × C3H.OH)  | k → a/ol  | A (? ) C (? ) | A     0/5     | A                |
| B10.D2 → (HTG × 5R)        | d → g/ia  | A     None   | A     2/15§    | A (? )          |
| B10 → (HTG × 3R)           | b → g/ia  | A     Ss-Slp | A     0/7     | A (? )          |
| 2R → (HTG × B10.AKM)       | ha → g/m  | A (? ) C (? ) | A     2/14§    | A (? )          |
| 4R → (HTG × B10.AKM)       | ho → g/m  | A (? ) C (? E (? )) | A 1/8§ | A (? )        |
| B10.A → (2R × 5R)          | a → ha/ia | A     None   | A     0/8     | A                |
| B10.A → (4R × 3R)          | a → ho/ia | A     None   | A     0/6     | A                |
| 2R → (HTT × B10.AKM)       | a → ha/oa | A     None   | A     ND**     | —               |
| 4R → (HTT × B10.AKM)       | a → ho/oa | R     V, E, Ss-Slp | A ND  | —               |
| B10 → (2R × 5R)            | b → ha/ia | R     V, E, C, Ss-Slp | A 0/8 | A               |
| B10 → (3R × 5R)            | b → ho/ia | R     C     | A     0/6     | A                |
| 2R → (3R × 5R)             | b → ha/ia | R     C     | A     ND     | —               |
| 4R → (3R × 5R)             | b → ho/ia | R     C     | A     ND     | —               |
| B10.BR → (2R × C3H.OL)     | k → ha/ol | A     None   | A     0/8     | A                |
| 2R → (2R × C3H.OL)         | k → ho/ol | A     None   | A     0/4     | A                |
| 4R → (2R × A.TL)           | a → ha/ty | A     None   | A     0/10    | A                |
| B10.A → (2R × AQR)         | a → ha/ty | A     None   | A     0/7     | A                |
| 2R → (2R × AQR)            | a → ho/ty | A     None   | A     0/3     | A                |
| 4R → (2R × AQR)            | a → ho/ty | A     None   | A     0/12    | A                |
| B10.A → (3R × B10.AKM)     | a → ha/m  | A     None   | A     0/11    | A                |
| 2R → (3R × B10.AKM)        | a → ia/ol | R     A, E   | A     0/9     | A                |
| 4R → (3R × B10.AKM)        | a → ia/ol | R     A, E, V | A ND    | —               |
| B10.D2 → (3R × C3H.OH)     | d → ia/ol | R     A, E, V | A ND    | —               |
| C3H.D → (3R × C3H.OH)      | d → io/ol | R     A, E, V | A ND    | —               |
| 2R → (3R × C3H.OH)         | d → m/ol  | A     None   | A     0/7     | A                |
| B10.D2 → (3R × C3H.OH)     | d → m/ol  | A     None   | A     0/5     | A                |
| B10.A → (3R × A.TL)        | a → m/ol  | A     None   | A     0/8     | A                |
| 2R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/10    | A                |
| 4R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/7     | A                |
| B10.BR → (3R × C3H.OH)     | k → m/ol  | A     None   | A     0/7     | A                |
| 2R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/5     | A                |
| 4R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/3     | A                |
| B10.A → (3R × A.TL)        | a → m/ol  | A     None   | A     0/8     | A                |
| 2R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/10    | A                |
| 4R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/7     | A                |
| B10.BR → (3R × C3H.OH)     | k → m/ol  | A     None   | A     0/7     | A                |
| 2R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/5     | A                |
| 4R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/3     | A                |
| B10.A → (3R × A.TL)        | a → m/ol  | A     None   | A     0/8     | A                |
| 2R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/10    | A                |
| 4R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/7     | A                |
| B10.BR → (3R × C3H.OH)     | k → m/ol  | A     None   | A     0/7     | A                |
| 2R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/5     | A                |
| 4R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/3     | A                |
| B10.A → (3R × A.TL)        | a → m/ol  | A     None   | A     0/8     | A                |
| 2R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/10    | A                |
| 4R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/7     | A                |
| B10.BR → (3R × C3H.OH)     | k → m/ol  | A     None   | A     0/7     | A                |
| 2R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/5     | A                |
| 4R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/3     | A                |
| B10.A → (3R × A.TL)        | a → m/ol  | A     None   | A     0/8     | A                |
| 2R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/10    | A                |
| 4R → (3R × A.TL)           | a → m/ol  | A     None   | A     0/7     | A                |
| B10.BR → (3R × C3H.OH)     | k → m/ol  | A     None   | A     0/7     | A                |
| 2R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/5     | A                |
| 4R → (3R × C3H.OH)         | k → m/ol  | A     None   | A     0/3     | A                |
| B10.A → (3R × A.TL)        | a → m/ol  | A     None   | A     0/8     | A                |

2R = B10.A(2R); 4R = B10.A(4R); 5R = B10.A(5R); B10 = C57BL/10Sn.
* F1 hybrids. Arrow indicates direction of transplantation.
† A = accepted.
§ Some grafts developed signs of chronic rejection (see text).
¶ Rejected grafts/total number of grafts performed.
* R = Rejected.
** ND = not done.

could have occurred between regions D and C, C and V, or V and E. We take into account the last possibility only.

Of the 41 combinations which can be tested, 34 were actually tested and only those which involve strain HTI were omitted. In 10 of these 34 combinations,
hypothesis I predicts rejection of the donor skin. According to hypothesis II, grafts in all 34 combinations should survive permanently (see the left part of Table I). The number of skin grafts performed in each combination and the number of grafts rejected are shown in the right part of Table I. In all 10 combinations, in which, according to hypothesis I the grafts should have been rejected, all the grafts survived permanently. In some of the combinations in which according to both hypotheses the grafts should have been accepted, weak histoincompatibilities were detected. These were the following:

B10.D2 → (B10.A × HTG)F1: Five grafts were permanently accepted, two grafts showed signs of chronic rejection starting at day 45 and 50, respectively.

2R → (B10.A × HTG)F1: Ten grafts were healthy at day 60, one graft began to reject at day 27 but later recovered, and two grafts developed signs of chronic rejection between days 37 and 45, respectively.

4R → (B10.A × HTG)F1: Eleven grafts survived permanently without any signs of rejection and two grafts lost their hair crop before the termination of the experiment.

B10 → (HTG × 5R)F1: Six grafts remained healthy throughout the whole observation period and one graft began slow rejection at day 50.

B10.A → (B10.AKM × AQR)F1: Out of 15 grafts, 10 remained viable for the whole observation period and five began a prolonged rejection between days 40 and 50.

C3H.D → (C3H.OH × AQR)F1: One out of 15 grafts began prolonged rejection at day 30.

In none of the combinations mentioned thus far, was any graft completely rejected. The signs of rejection were limited to partial or complete loss of the hair crop, appearance of small scars, or swelling of the graft. In the following combinations, some grafts were completely destroyed:

B10.D2 → (HTG × 5R)F1: Two out of 15 grafts were rejected, one at day 45 and the other at day 57. Two grafts showed prolonged rejection starting with days 45 and 50, respectively. The rest of the grafts remained healthy.

2R → (HTG × B10.AKM)F1: Two grafts out of 14 rejected at day 57. The rest remained in good shape throughout the whole observation period.

4R → (HTG × B10.AKM)F1: One graft out of eight was rejected at day 57 and one showed prolonged rejection beginning at day 45.

All the combinations in which chronic or acute rejections were observed involve strains HTG and AQR, both not completely inbred. However, since the genetic backgrounds of these two strains should be irrelevant (in the particular combinations employed) to the outcome of the grafting, it is difficult to conceive how they can be responsible for the observed incompatibilities.

DISCUSSION

In this communication, we have shown that certain heterozygous combinations of H-2 crossovers, when challenged with donor grafts of the appropriate
TWO-GENE MODEL FOR THE H-2 HISTOCOMPATIBILITY SYSTEM

H-2 type, can be used for testing the two possible interpretations of the H-2 system which we have called here hypothesis I (system composed of at least six histocompatibility regions) and hypothesis II (system composed of only two histocompatibility regions). The outcome of the transplantation experiment is unequivocally in agreement with the predictions based on hypothesis II. In the critical ten donor-recipient combinations in which, according to hypothesis I, all the grafts should have been rejected, no rejection was observed. In addition to this evidence obtained by skin transplantation, evidence provided by serological, genetic, and biochemical analysis of the H-2 system can be also put forward in support of hypothesis II.

As early as 1955, Gorer and his coworkers (1, 5) observed that some H-2 antigens could be arranged into series in which antigens of the same series were mutually exclusive (i.e., presence of an antigen in a given H-2 type seemed to exclude presence of other antigens of the same series). Thus antigens D (4), D\(^b\) (2) and D\(^k\) (32), or antigens K (11) and K\(^b\) (33) never occurred together in any of the known H-2 types. Gorer's interpretation of this phenomenon was that antithetical antigens of the same series were controlled by alleles of the same gene. (This was reflected in the notation used by Gorer, in which the superscripts over the same capital letter designated allelic forms of a given antigen.) The idea of mutually exclusive series of H-2 antigens has again been stressed recently by Snell and coworkers (23). According to Snell, the private antigens of the H-2 system can be arranged into two such series, one determined by the K-end and the other by the D-end, relative to the Ss-Slp locus. The two series of antigens can be best explained by the assumption that they are controlled by two series of alleles, i.e., alleles at the H-2K locus and alleles at the H-2D locus. However, since the public H-2 antigens do not fit easily into this scheme, the serology alone does not prove that there are only two H-2 loci.

At the genetic level, the superiority of hypothesis II over hypothesis I is in its simplicity. Hypothesis II is virtually free of internal inconsistencies. This certainly cannot be claimed about hypothesis I. Several H-2 recombinants fit the multiregion map constructed on the basis of hypothesis I only with the provision that double or even triple crossing-over occurred inside the H-2 system (see Fig. 1). Such events are rather unlikely in a segment which is only 0.5 map units long and there is no evidence for increased frequency of double crossing-over in the chromosome carrying the IXth linkage group (8). On the basis of hypothesis II, all the H-2 crossovers can be explained by single recombinational event (see Fig. 2). Other inconsistencies associated with hypothesis I have been discussed in detail by one of us elsewhere (15).

The strongest evidence supporting hypothesis II, however, has been provided by biochemical analysis of the H-2 products. Solubilization and purification of the H-2 alloantigens produces two classes of glycopeptides, one which carries all the K-end specificities and another which carries all the D-end specificities (i.e., specificities which have been tested for) (11). This result is obtained regard-
less of the strain (H-2 type) involved and solubilization technique applied. It is exactly what one would expect if the H-2 alloantigens were controlled by only two loci (providing that the H-2 sites reside in proteins and not in carbohydrates). Moreover, the molecular size of each of the two classes of the H-2 glycoproteins (which is not more than 60,000 daltons, thus indicating that not more than 400 amino acid residues are present in each molecule) is approximately what one might expect from a product of an average size cistron (approximately 1000 nucleotide pairs). It can be argued, of course, that the H-2 products are "miniproteins" controlled by less than average size cistrons but then it still remains to be explained why such miniproteins resist separation from one another by the different isolation procedures which have been employed. Another possibility, is that the H-2K and H-2D regions are actually series of duplicated genes which control identical or very similar proteins. According to this interpretation, the purified H-2 preparations would consist of a population of molecules which would be products of several different cistrons. They would behave like a single homogeneous protein because of the similarity between the corresponding cistrons. However, such interpretation assumes a high degree of genetic conservatism in a system which is one of the most polymorphic systems known.

Thus the immunological (transplantation and serological), genetic, and biochemical findings can all be best explained by the assumption that the H-2 system consists of two histocompatibility loci separated by a chromosomal segment occupied by unrelated genes. We have suggested (10) that the two loci be called H-2D and H-2K, respectively, with superscripts indicating their chromosomal origin. Thus, for example, chromosome H-2a can also be written as H-2K^K^H-2D^K, chromosome H-2b as H-2K^H-2D^H, etc. (see Fig. 4). The symbolism can be simplified to H-2^K(K^KK^H-2D^K), etc. If necessary, the Ss-Slp and Ir-I loci can also be included in the compound symbols, such as H-2^K(K^KIr-I-Ss^Slp^Sp^D^K), etc.

In a recent article, Démant et al. (4) also tested the genetic relationship between different H-2 crossovers by transplantation methods. Although the design of their experiment and most of their donor-recipient combinations were different from ours, these authors reached the conclusion that "the antigenic specificities mapping into the central regions of the H-2 locus are secondary to those mapping into the D-region and into the K-region" (4). However, their data also fit the hypothesis that there are no "central" histocompatibility regions in the H-2 system.

**SUMMARY**

The genetic structure of the H-2 system has been traditionally interpreted as consisting of multiple regions controlling histocompatibility antigens. Recently however, many difficulties have been encountered in attempts to construct a single, consistent linear H-2 map on this basis. We have shown that the genetic,
serological, and biochemical findings on the H-2 system can be more readily explained by the assumption that there are only two histocompatibility regions (loci) in the H-2 system, \textit{H-2D} and \textit{H-2K}, which are separated by loci controlling serum proteins (Ss-Slp), immune response (\textit{Ir-1}), and perhaps others. Evidence supporting such an interpretation of the H-2 system was obtained by a transplantation analysis of the 14 well-defined \textit{H-2} crossovers. \textit{F1} hybrids between different \textit{H-2} crossovers were produced and challenged with skin grafts from third party strains. The donor-recipient relationships in these combinations were such that in at least 10 cases the skin grafts should have been rejected if the multiple-region H-2 map is correct but should survive permanently if the two-region model is correct. In all instances, the skin grafts survived permanently, providing further evidence for the two-region map of the H-2 complex.

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