MOLECULAR RELATIONSHIP BETWEEN PRIVATE AND PUBLIC H-2 ANTIGENS AS DETERMINED BY ANTIGEN REDISTRIBUTION METHOD*

BY VĚRA HAUPTFELD AND JAN KLEIN

(From the Department of Microbiology, The University of Texas Southwestern Medical School, Dallas, Texas 75235)

Classical H-2 antigens can be divided into two, not very sharply defined groups, private and public. Private H-2 antigens (1) have a restricted strain distribution: among inbred strains they are found in only one haplotype of independent origin; public H-2 antigens (2), on the other hand, are shared by at least two, and quite often by a large number of unrelated haplotypes. Private H-2 antigens are believed to be controlled by alleles of two loci at the opposite ends of the H-2 complex, the H-2K locus in the K region, and the H-2D locus in the D region. In agreement with this belief, the antigens can be arranged in two series, where members of each series are mutually exclusive (3). No such arrangement is possible for the public H-2 antigens and the nature of the genetic control of these antigens has long been a matter of controversy.

According to the traditional interpretation of the H-2 complex (4), the public antigens are controlled by separate regions located between the K and D regions within the complex. According to the two-locus model (5), public H-2 antigens reside in the same molecules as the private ones and are controlled either by the H-2K or H-2D loci, or both. The two-locus model fits better the available data than the traditional multi-locus model (6). Also, the former model is supported by the results of transplantation analysis demonstrating that only the peripheral regions of the H-2 complex are involved in rapid skin-graft rejection (7). However, a formal proof that the serologically detectable public H-2 antigens are coded for by the K and D regions has never been provided. As a matter of fact, most recent developments (8, 9, cf. Discussion) suggest that an across the board assignment of public antigens to K and D regions could not be justified. Despite these developments, many investigators accept the two-locus model as an established fact and rely on it in their interpretation of the H-2 system. At this stage it is, therefore, critical to test whether the serologically detectable public H-2 antigens conform to the predictions of the two-locus model.

We have recently developed a method that can be used to examine molecular relationships among various cell membrane antigens. The method is based on the observation that under certain circumstances, antigens coated with alloanti-
bodies and then with xenoantibodies directed against the alloantibodies, redistribute in the cell membrane in such a way that the cell becomes resistant to subsequent exposure of the same alloantibodies and complement; however, the cell remains sensitive to antibodies (and complement) directed against antigens not physically associated with the coated antigens (10). Using this method, we were able to demonstrate that private H-2 antigens controlled by the K region redistribute independently of those controlled by the D region, and that H-2 antigens redistribute independently of la antigens and of membrane bound Ig (11). In this communication we extend these studies and examine the molecular relationship between private and public H-2 antigens.

Materials and Methods

**Mice.** All mice were bred in our colony at the University of Texas Health Science Center.

**Antisera.** H-2 alloantisera used and the antibodies they contain are listed in Table I. The K-series antisera were produced in our laboratory using an immunization regimen described previously (12). The D- and C-series antisera were produced at the Jackson Laboratories, Bar Harbor.

**Table I**

| Antiserum no. | Recipient Donor | H-2 haplotype combination | Antibodies present |
|---------------|----------------|--------------------------|-------------------|
| D-1b          | (C3H.SW × HTG)F₁ | C3H-H-2<sup>a</sup>       | (b/g)a 2          |
| K-302         | [B10.D₂ × A]F₁  | B10.A(2R)                | (d/a)h 2          |
| K-304         | (A.BY × B10.AKM)F₁ | B10.A                  | (b/m)a 4          |
| K-46          | (HTG × B10.D₂)F₁ | C57BL/10Sn              | (g/db) 5,33       |
| K-35          | C3H/Hae        | C3H.R3                   | (h)r 6,18         |
| D-8           | (B10 × A.SW)F₁ | B10.M                    | (b/s)f 8,9,37     |
| C-11A         | (B10.D₂ × C3H.NB)F₁ | LP.RIII               | (d/p)r 11,25     |
| D-13          | (B10 × LP.RIII)F₁ | B10.A(5R)               | (b/r)i 4,13,41,42,43,44 |
| K-26          | (B10 × A)F₁    | AQR                     | (b/a)y 17         |
| K-323         | [B10.RIII(71NS) × DBA/2]F₁ | B10.A               | (r/d)a 23         |
| K-325         | (DBA/2 × B10.GIF)F₁  | B10.RIII(71NS)         | (d/q)r 18,25     |
| D-28          | (A.CA × B10.BR)F₁ | A.SW                    | (f/k)s 12,19,36,42 |
| K-113         | (B10 × C3H)F₁  | B10.AKM                  | (b/k)m 30        |
| K-131         | (A × B10.AIF)₁ | B10.D₂                   | (a)d 31          |
| As-485        | [B10.A(2R) × C3H.SW]F₁ | C3H/Sn               | (h2/b)k 32,45,47 |
| K-30          | (B10.D₂ × A)F₁  | HTI                      | (d/a)i 33,39     |

K-series antisera were produced in this laboratory; D- and C-series were produced by Doctors M. Cherry and G. D. Snell at the Jackson Laboratories under a contract through the National Institutes of Health; As-485 was kindly supplied to us by Dr. G. D. Snell.

Maine by Doctors Marianna Cherry and George D. Snell under a contract from the National Institutes of Health. Specificity of all alloantisera was determined by testing them against a panel of inbred strains and congenic lines representing major H-2 haplotypes. The goat antimouse Ig(κ, γ) xenoantiserum (GAMIG) was a gift from Dr. Ellen S. Vitetta, of this department.

**Cell Suspensions.** Spleen was placed into a Petri dish containing Hanks' BSS and adhering fat

---

<sup>1</sup>Abbreviations used in this paper: ACT, ammonium chloride Tris; AMIRC, antibody-mediated induction of resistance to cytotoxicity; As, antiseraum; BSS, balanced salt solution; GAMIG, goat antimouse Ig serum; NMS, normal mouse serum; PBS, phosphate-buffered solution.
tissue was removed. The organ was then fragmentated with a spatula and cells released by teasing. The suspension was flashed back and forth in a Pasteur pipette, large fragments were allowed to sediment for 5 min in a test tube, and the supernate was transferred into another tube and centrifuged. Erythrocytes were destroyed by washing the cells twice in ammonium chloride Tris (ACT), followed by washing in HBSS. The cells were then counted and diluted to a required concentration. The whole procedure, except for the ACT treatment, was carried out in the cold.

Antibody-Mediated Induction of Resistance to Cytotoxicity (AMIRC) Technique. The method of AMIRC was described previously (11). Briefly, spleen cells were suspended in Hanks' BSS and divided into aliquots, each containing $5 \times 10^8$ cells. The suspensions were centrifuged, the supernates removed, the cells resuspended in H-2 alloantisera appropriately diluted in PBS and incubated for 2 h on ice. After the incubation, the cells were washed twice in the cold with BSS and incubated for 1 h on ice with GAMIG antiserum diluted in PBS. The cells were then washed twice in BSS, resuspended in RPMI medium containing 10% heat-activated normal mouse serum (pool) and cycloheximide (50 $\mu$g/ml) to give a final concentration of $2 \times 10^8$ cells/ml, and incubated for 1 h at 37°C. The suspension was then cooled to 0°C, washed twice with BSS, and the incubation with the GAMIG serum was repeated one more time. At the end of the second Ig treatment, the suspension was used without washing in the cytotoxicity test which was performed at room temperature (except for the incubation period with complement).

Cytotoxicity Test. Susceptibility or resistance to cytotoxicity after antisera treatment was determined by using the two-stage microcytotoxicity test of Amos and his co-workers (13), modified as described previously (14). Live and dead cells were distinguished by using an inverted phase-contrast microscope. Commercially available normal guinea pig serum (Grand Island Biological Co., Grand Island, N.Y.), diluted 1:2 or 1:4 was used as a source of complement.

Results

The most recent H-2 chart (6) lists 52 H-2 antigens. Of these, 18 are classified as private and the remaining 34 as public. Of the public antigens, those with a number lower than 33 have been known for some time and, consequently, are better characterized than most of the newer H-2 antigens. For this reason, we limited our study to the older antigens, namely to antigens $H-2.1, 5, 6, 8, 11, 13, 25, and 28$. In the following we shall deal with these antigens one by one.

$H-2.1$. This is one of the most versatile antigens in the whole H-2 system. Although $H-2.1$ antibodies cross-react with most unrelated H-2 haplotypes, the $H-2.1$ antigen of any particular haplotype appears to be slightly different from the $H-2.1$ antigen of another haplotype (15). Although sites reacting with $H-2.1$ antibodies have been postulated on both K and D molecules, the primordial $H-2.1$ site is believed to be controlled by the K region. The anti-$H-2.1$ serum which we used (D-1b) was produced in such a way as to contain only $H-2.1$ antibodies reacting with the K site. When we applied this antisera to B10.A($H-2^{a}$) cells, we observed that redistribution of antigen $H-2.1$ was always accompanied by redistribution of $H-2.23$, the private antigen controlled by the $K$ region of the $H-2^{a}$ haplotype (Table II). Similarly, redistribution of antigen $H-2.23$ was always accompanied by redistribution of antigen $H-2.1$. On the other hand, antigen $H-2.4$, controlled by the $D$ region of $H-2^{a}$, redistributed independently of both $H-2.1$ and $H-2.23$. These results suggest that in the cell membrane of $H-2^{a}$ mice, antigen $H-2.1$, as detected by antisera D-1b, is physically associated with the $H-2K$ molecule.

$H-2.5$. This antigen has been genetically mapped in the K region but according to the duplication model (16), a homologous genetic determinant is located also in the D end. Our data (Table III) confirm this prediction of the duplication model. The data show that in CBA/J ($H-2^{a}$) cells, redistribution of
antigen $H-2.5$ is accompanied by redistribution of not only $H-2.23$ (the $K$ region private antigen) but also of $H-2.32$ (the $D$ region private antigen). Surprisingly, treatment of CBA/J cells with anti-$H-2.23$, but not with anti-$H-2.32$, abolished the reactivity of these cells with $H-2.5$ antibodies. Thus, it appears that the $H-2.5$ antibodies are capable of redistributing both $K$ and $D$ molecules but mediate complement-dependent cytotoxicity reactions only when combined with $K$ molecules.

$H-2.6$. This is one of the least studied and least defined of the first 33 $H-2$ antigens. We were able to produce an antiserum (K-35) whose reactivity pattern approaches that of anti-$H-2.6$. In C57BL/6($H-2^d$) cells, the antigen detected by this antiserum redistributed together with $H-2.33$, the private antigen controlled by the $K$ region of the $H-2^d$ haplotype (Table IV). The $D^p$ region private antigen ($H-2.2$) redistributed independently of both $H-2.6$ and $H-2.33$. The physical association in the cell membrane of antigens $H-2.6$ and 33 contradicts the genetic mapping of antigen $H-2.6$ into the $D$ region. This discrepancy between genetic and topological mapping of $H-2.6$ means either that our antiserum detects a different antigen, or that there are $H-2.6$ sites on both $K$ and $D$ molecules and our antiserum reacts with only one of them.

$H-2.8$. Genetic mapping suggests that the determinant for antigen $H-2.8$ is at the $K$ end and the results of our redistribution experiments (Table V) confirm this conclusion. In B10.D2($H-2^d$) cells, redistribution of antigen $H-2.8$ was accompanied by the redistribution of the $K$ end antigen $H-2.31$, and vice versa, whereas, the $D$ end antigen $H-2.4$ redistributed independently of the two.

$K-2.11$. Redistribution of antigen $H-2.11$ in DBA/1J($H-2^k$) cells was always accompanied by the redistribution of $K$ region antigen $H-2.17$, and vice versa,
EXAMINATION OF THE TWO-LOCUS MODEL

### Table III
Redistribution of Antigens H-2.5(K-46), H-2.23(K-323), and H-2.32(As-485) After Pretreatment of CBA/J(H-2\(^k\)) Spleen Cells with H-2 Alloantisera and GAMIG

| Treated with As* | Tested with As | Dead cells at As dilution‡ |
|-----------------|----------------|---------------------------|
|                 |                | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 |
| —               | K-46           | 80  | 75  | 35  | 12   | 8    |
| K-46(n)         | K-46           | 20  | 20  | 18  | 18   |      |
| K-323(n)        | K-46           | 12  | 10  | 10  | 10   |      |
| As-485(1:16)    | K-46           | 80  | 70  | 40  | 12   | 12   |
| —               | K-323          | 95  | 95  | 82  | 60   | 35   |
| K-323(n)        | K-323          | 10  | 10  | 12  | 10   |      |
| K-46(n)         | K-323          | 25  | 28  | 25  | 25   |      |
| As-485(1:16)    | K-323          | 85  | 80  | 70  | 50   | 20   |
| —               | As-485         | 90  | 90  | 90  | 88   | 75   |
| As-485(1:16)    | As-485         | 12  | 15  | 12  | 12   |      |
| K-46(n)         | As-485         | 30  | 35  | 20  | 30   | 30   |
| K-323(n)        | As-485         | 85  | 80  | 80  | 70   | 68   |
| GAMIG           | NMS            | 12  | 12  | 15  | 12   | 15   |

* As = H-2 alloantiserum; dilution of antiserum in parentheses; n = undiluted.
‡ Measured in cytotoxicity test.

### Table IV
Redistribution of Antigens H-2.6(K-35), H-2.33(K-30), and H-2.22(K-302) After Pretreatment of C57BL/6(H-2\(^b\)) Spleen Cells with H-2 Alloantisera and GAMIG

| Treated with As* | Tested with As | Dead cells at As dilution‡ |
|-----------------|----------------|---------------------------|
|                 |                | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 |
| —               | K-35           | 70  | 70  | 42  | 10   | 8    |
| K-35(n)         | K-35           | 25  | 20  | 18  | 15   | 15   |
| K-30(n)         | K-35           | 40  | 35  | 15  | 12   |      |
| K-302(n)        | K-35           | 85  | 75  | 35  | 12   |      |
| —               | K-30           | 100 | 100 | 95  | 80   | 35   |
| K-30(n)         | K-30           | 15  | 18  | 15  | 10   |      |
| K-35(n)         | K-30           | 55  | 38  | 28  | 22   | 12   |
| K-302(n)        | K-30           | 95  | 95  | 88  | 60   | 20   |
| —               | K-302          | 95  | 90  | 50  | 22   | 10   |
| K-302(n)        | K-302          | 10  | 10  | 10  | 10   |      |
| K-35(n)         | K-302          | 75  | 72  | 65  | 60   | 28   |
| K-30(n)         | K-302          | 80  | 80  | 70  | 65   | 25   |
| GAMIG           | NMS            | 10  | 10  | 10  | 10   |      |

* As = H-2 alloantiserum; dilution of antiserum in parentheses; n = undiluted.
‡ Measured in cytotoxicity test.

whereas redistribution of D region antigen H-2.30 did not change the distribution of H-2.11 (Table VI). This result is in agreement with genetic mapping placing the determinant for antigen H-2.11 in the K region.

H-2.13. Tests with antigen H-2.13 inconclusively suggested association of
this antigen in DBA/1J(H-2\textsuperscript{a}) cells with the D region antigen H-2.30 and independence from K region antigen H-2.17 (Table VII). Cells treated with anti-H-2.13 (D-13) became resistant to subsequent action of anti-H-2.30 (K-113) while remaining susceptible to the action of anti-H-2.17(K-26). However,

**Table V**

Redistribution of Antigens H-2.8(D-8), H-2.31(K-131), and H-2.4(K-340) After Pretreatment of B10.D2(H-2\textsuperscript{a}) Spleen Cells with H-2 Alloantisera and GAMIG

| Treated with As* | Tested with As | Dead cells at As dilution‡ |
|------------------|----------------|---------------------------|
|                  |                | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 |
| —                | D-8            | 90  | 80  | 65  | 50   | 38   |
| D-8(n)           | D-8            | 30  | 22  | 18  | 20   | 20   |
| K-131(n)         | D-8            | 30  | 38  | 25  | 22   | 20   |
| K-304(n)         | D-8            | 85  | 80  | 65  | 60   | 50   |
| —                | K-131          | 95  | 88  | 85  | 70   | 60   |
| K-131(n)         | K-131          | 35  | 35  | 20  | 35   | 28   |
| D-8(n)           | K-131          | 58  | 60  | 60  | 45   | 40   |
| K-304(n)         | K-131          | 85  | 80  | 75  | 60   | 50   |
| —                | K-304          | 95  | 95  | 90  | 80   | 60   |
| K-304(n)         | K-304          | 38  | 35  | 25  | 25   | 28   |
| D-8(n)           | K-304          | 90  | 90  | 85  | 75   | 60   |
| K-131(n)         | K-304          | 90  | 90  | 85  | 80   | 80   |
| GAMIG            | NMS            | 15  | 20  | 20  | 15   | 20   |

* As = H-2 alloantiserum; dilution of antiserum in parentheses; n = undiluted.
‡ Measured in cytotoxicity test.

**Table VI**

Redistribution of Antigens H-2.11(C-11A), H-2.17(K-26), and H-2.30(K-113) After Pretreatment of DBA/1J(H-2\textsuperscript{a}) Spleen Cells with H-2 Alloantisera and GAMIG

| Treated with As* | Tested with As | Dead cells at As dilution‡ |
|------------------|----------------|---------------------------|
|                  |                | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 |
| —                | C-11A          | 95  | 90  | 80  | 50   | 35   |
| C-11A(n)         | C-11A          | 38  | 35  | 38  | 38   | 40   |
| K-26(n)          | C-11A          | 35  | 35  | 32  | 35   | 40   |
| K-113(n)         | C-11A          | 95  | 90  | 80  | 70   | 40   |
| —                | K-26           | 95  | 95  | 90  | 90   | 88   |
| K-26(n)          | K-26           | 22  | 25  | 22  | 25   | 22   |
| C-11A(n)         | K-26           | 30  | 35  | 35  | 35   | 30   |
| K-113(n)         | K-26           | 95  | 90  | 80  | 88   | 85   |
| —                | K-113          | 95  | 92  | 85  | 70   | 55   |
| K-113(n)         | K-113          | 50  | 50  | 40  | 38   | 40   |
| C-11A(n)         | K-113          | 90  | 88  | 85  | 78   | 70   |
| K-26(n)          | K-113          | 95  | 92  | 88  | 80   | 65   |
| GAMIG            | NMS            | 38  | 35  | 40  | 30   | 15   |

* As = H-2 alloantiserum; dilution of antiserum in parentheses; n = undiluted.
‡ Measured in cytotoxicity test.
anti-\(H-2.30\) treatment only slightly reduced the sensitivity of the DBA/1J cells to anti-\(H-2.13\) serum and complement. Whether the latter result was caused by the presence of an additional antibody in the D-13 serum could not be determined.

\(H-2.25\). CBA/J(\(H-2^d\)) lymphocytes treated with anti-\(H-2.25\) serum (K-325) became resistant not only to anti-\(H-2.25\) but also to anti-\(H-2.23\) (K-323), while remaining fully sensitive to anti-\(H-2.32\) (As-485, cf. Table VIII). Similarly, the same cells treated with anti-\(H-2.23\) became resistant to both anti-\(H-2.23\) and anti-\(H-2.25\), but not to anti-\(H-2.32\). This result, which suggests molecular association of antigen \(H-2.25\) with \(K\) region antigen \(H-2.23\), is in agreement with genetic mapping placing the \(H-2.25\) determinant in the \(K\) region.

\(H-2.28\). Antiserum D-28 contains several \(H-2\) antibodies. However, when tested against HTG(\(H-2^d\)) cells, it should react only with antigen \(H-2.28\). Treatment of HTG lymphocytes with D-28 makes the cells resistant not only to D-28, but also to K-131, an antiseraum specific for \(K\) region antigen \(H-2.31\) (Table IX). Similarly, treatment with K-131 makes HTG cells resistant to both K-131 and D-28, but not to K-302. Genetic mapping of \(H-2.28\) is somewhat ambiguous and it has been suggested (16) that the antigen might be controlled by duplicate sites in both the \(K\) and \(D\) regions. Our data indicate that in the membrane of HTG cells, antigen \(H-2.28\) is associated with \(K\) region antigen \(H-2.31\).

### Discussion

The data presented in this communication prove that in the cell membrane public \(H-2\) antigens 1, 5, 6, 8, 11, 13, 25, and 28 are physically associated with molecules carrying private \(H-2\) antigens coded for by the \(K\) or \(D\) regions. The data, however, do not provide an insight into the nature of this association. Theoretically, co-redistribution of two antigens in the membrane can occur either
when the two antigens are carried by the same molecule, or when the antigens are on two different molecules associated by noncovalent bonds in a supramolecular structure which redistributes as a unit. The distinction between the two possibilities can be made only through isolation and serological characterization.
EXAMINATION OF THE TWO-LOCUS MODEL

of purified H-2 molecules. Such characterization, so far, has been accomplished for only two public H-2 antigens. Cullen and her colleagues (17) demonstrated by differential immunoprecipitation of NP-40 solubilized membrane preparation that antigen H-2.3 was on the same fragment as antigen H-2.4, and that antigen H-2.5 was on the same fragment as antigen H-2.32. By inference, this data strongly suggest that the associations which we observed between public and private H-2 antigens are also caused by the two types of antigens being present on the same molecule.

Taken together with the biochemical data on antigen H-2.3, evidence is now available that all the public antigens previously assigned to separate regions of the H-2 complex are associated with antigens controlled by either the K or the D regions, or both. In other words, the data disprove the existence of separate regions A, C, and E, and thus substantiate the principal claim of the two-locus model.

Paradoxically, it is now clear that the two-locus model in its original form is an oversimplification. Since the proposal of the model, the following developments have taken place that complicated the interpretation of the H-2 complex. First, a class of antigens was discovered to be controlled by the I region (Ia antigen, cf. ref. 18, 19), which in some respects resembles the classical H-2 antigens, but the two classes differ in several characteristics (e.g., tissue distribution and molecular weight). It is likely that the H-2 and Ia antigens are related genetically and functionally. Second, in addition to the two strong histocompatibility loci positioned in the K and D regions, a third strong H locus has recently been discovered in the I region, close to H-2K (20). Furthermore, some unpublished data suggest that loci coding for weak transplantation antigens might be dispersed throughout the entire H-2 complex. Third, one of the public H-2 antigens (H-2.7) originally believed to be controlled by the K or D regions was demonstrated to be controlled by a separate region (G), located between S and D (8, 9).

Thus, the picture of the H-2 complex emerging from current studies is that of a large series of loci, most likely related in their function, and displaying considerable homology, but also some significant differences. In this array of loci, the two loci of the original two-locus model (H-2K and H-2D) still stand out as the most homologous. A direct support for the homology of the H-2K and H-2D loci is provided by our results on redistribution of antigen H-2.5. We observed that in H-2K cells, redistribution of antigen H-2.5 is accompanied by redistribution of both K (H-2.23) and D (H-2.32) region private antigens. This observation is first direct evidence for the validity of the duplication model of H-2.

The data presented in this communication also demonstrate that the technique of AMIRC is a powerful tool for evaluation of topological relationships among antigens in the cell membrane. In the absence of reliable biochemical data, it could provide the missing link between serology and genetics in the study of a complex antigenic system.

Summary

Molecular relationship of public H-2 antigens 1, 5, 6, 8, 11, 13, 25, and 28 to private antigens controlled by K and D regions was studied using the technique of antibody-induced resistance to complement-mediated cytotoxicity. The results indicate physical association in the cell membrane between H-2 antigens 1
and 23 of H-2\textsuperscript{a}, 8 and 31 of H-2\textsuperscript{b}, 11 and 17 of H-2\textsuperscript{d}, 13 and 30 of H-2\textsuperscript{b}, 25 and 23 of H-2\textsuperscript{a}, and 28 and 31 of H-2\textsuperscript{a}. These results are in agreement with genetic mapping placing the determinants of antigens H-2.8, 11, and 25 in the K region, the determinant of antigen H-2.13 in the D region, and the determinants of antigens H-2.1 and 28 in either the K or the D region. In contrast to genetic mapping placing the determinant for antigen H-2.6 in the D region, we found that in the H-2\textsuperscript{a} haplotype the antigen is associated with K region antigen H-2.33. We found antigen H-2.5 in the H-2\textsuperscript{a} associated with both H-2.33 and H-2.32, and interpreted this result as evidence for two homologous H-2.5 sites controlled by opposite ends of the H-2 complex. Although the data do not prove that public antigens are carried by the same molecules as private ones, they demonstrate a close physical association in the membrane between the two groups of antigens. The data provide strong experimental evidence that there are two groups of loci, K and D, coding for the first 33 classical H-2 antigens (with the exception of antigen H-2.7), and thus support the two-locus model. The data also support the duplication model of H-2 by demonstrating two homologous H-2.5 sites associated with K and D molecules.

We thank Dr. Ellen S. Vitetta for her generous gift of the goat antimouse Ig serum and Ms. JoAnne Tuttle for her help in preparing this manuscript.

Received for publication 1 April 1975.

References

1. Hoecker, G., S. Counce, and P. Smith. 1954. The antigens determined by the H-2 locus: a rhesus-like system in the mouse. Proc. Natl. Acad. Sci. U.S.A. 40:1040.
2. Klein, J. 1971. Private and public antigens of the mouse H-2 system. Nature (Lond.). 229:635.
3. Snell, G. D., M. Cherry, and P. Démant. 1971. Evidence that H-2 private specificities can be arranged in two mutually exclusive systems possibly homologous with two subsystems of HL-A. Transplant. Proc. 3:183.
4. Shreffler, D. C. 1970. Immunogenetics of the mouse H-2 system. In Blood and Tissue Antigens. D. Aminoff, editor. Academic Press, Inc., New York. 85.
5. Klein, J., and D. C. Shreffler. 1971. The H-2 model for major histocompatibility systems. Transplant. Rev. 6:3.
6. Klein, J. 1975. Biology of the Mouse Histocompatibility-2 Complex. Springer-Verlag, Inc., New York.
7. Klein, J., and D. C. Shreffler. 1972. Evidence supporting a two-gene model for the H-2 histocompatibility system of the mouse. J. Exp. Med. 135:924.
8. Klein, J. 1975. Evidence for a fifth (G) region in the H-2 complex of the mouse. Immunogenetics. 2:141.
9. David, C., J. H. Stimpfling, and D. C. Shreffler. 1975. Identification of specificity H-2.7 as an erythrocyte antigen. Control by an independent locus, H-2G, between the S and D regions. Immunogenetics. 2:131.
10. Bernoco, D., S. Cullen, G. Scudeller, G. Trinchieri, and R. Ceppellini. 1973. HL-A molecules at the cell surface. In Histocompatibility Testing (1972). J. Dausset and J. Colombani, editors. Munksgaard A/S, Copenhagen. 527.
11. Hauptfeld, V., M. Hauptfeld, and J. Klein. 1975. Induction of resistance to antibody-mediated cytotoxicity. H-2, Ia, and Ig antigens are independent entities in the membrane of mouse lymphocytes. J. Exp. Med. 141:1047.
12. Klein, J., D. Klein, and D. C. Shreffler. 1970. H-2 types of translocation stocks T(2; 9)138Ca, T(9; 13)190Ca, and an H-2 recombinant. Transplantation. 10:309.

13. Amos, D. B., H. Bashir, W. Boyle, M. MacQueen, and A. Tiilikainen. 1969. A simple microcytotoxicity test. Transplantation. 7:220.

14. Hauptfeld, V., M. Hauptfeld, and J. Klein. 1974. Tissue distribution of I region associated antigens in the mouse. J. Immunol. 113:181.

15. Snell, G. D., P. Demant, and M. Cherry. 1971. Hemagglutination and cytotoxic studies of H-2. I. H-2.1 and related specificities in the EK crossover regions. Transplantation. 11:210.

16. Snell, G. D., P. Demant, and M. Cherry. Hemagglutination and cytotoxic studies of H-2. V. The anti-27, 28, 29 family of antibodies. Folia Biol. (Praha). 20:145.

17. Cullen, S. E., B. D. Schwartz, and S. G. Nathenson. 1972. The distribution of alloantigenic specificities of native H-2 products. J. Immunol. 108:596.

18. Hauptfeld, V., D. Klein, and J. Klein. 1973. Serological detection of an Ir-region product. Science (Wash. D.C.). 181:167.

19. David, C. S., D. C. Shreffler, and J. A. Frelinger. 1973. New lymphocyte antigen system (Lna) controlled by the Ir region of the mouse H-2 complex. Proc. Natl. Acad. Sci. U.S.A. 70:2509.

20. Klein, J., M. Hauptfeld, and V. Hauptfeld. 1974. Evidence for a third, Ir-associated histocompatibility region in the H-2 complex of the mouse. Immunogenetics. 1:45.