IMMUNOCHEMICAL EVIDENCE FOR THREE Ia LOCI IN THE *I*-REGION OF THE *H-2* COMPLEX*

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Loci mapping in the *I*-region of the mouse *H-2* gene complex control a polymorphic group of membrane alloantigens, denoted as *I*-region associated (Ia) antigens (1). Initially, two distinct *Ia* loci were defined (map order *Ia-1, Ia-3*). Cross-overs on opposite sides of each marker locus define discrete segments of chromosome, designated as subregions (*I-A* and *I-C*, respectively).

Antisera detecting the product of either the *Ia-1* or *Ia-3* locus each precipitate a distinct Ia molecule (2, 3), which consists of two noncovalently associated glycopolypeptides of mol wt of approximately 35,000 daltons (α-subunit) and 28,000 daltons (β-subunit) (4). The demonstration that both α- and β-subunits from different haplotypes have distinct amino acid sequences suggests that both subunits are encoded by structural loci mapping in the *I*-region (5). It is not known whether anti-Ia sera are reactive with determinants on both or only one of the subunits.

One approach to estimating the number of *Ia* loci mapping in the *I*-region is dependent on determining the molecular association of different Ia antigenic specificities with distinct Ia molecules. Sequential immunoprecipitation analyses have thus far revealed that many of the defined Ia specificities are associated with one or the other of two separate Ia molecules (3, 6). These data, taken together with serological analyses of several intra-*I*-region recombinant strains, provide evidence for the existence of two *Ia* loci, i.e., *Ia-1* and *Ia-3* (2, 3, 6).

In this report, we present coprecipitation analyses performed with various antisera reactive with products of loci mapping in either the *I-A* or *I-C* subregions. The data will show that only a single molecule controlled by a locus mapping in the *I-A* subregion may be identified. A study of the *I-C* subregion will demonstrate that there exist two additional Ia molecules controlled by loci mapping between the *I-J* and *S* regions. These observations indicate the presence of at least three *I*-region loci which encode Ia molecules detectable by immunoprecipitation. Preliminary reports of these data have been previously presented (6–8).

* Supported by National Institutes of Health grant AI 07757 and Medical Research Council of Canada grant MA 5729.

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Materials and Methods

Mice. All strains of mice used in this study were maintained in our colonies at Stanford University and the University of Toronto. The haplotype origin of the *H-2* regions of these strains and additional strains used for antiserum production are presented in Table I.

Antisera. The antisera used in these studies and the respective *H-2* regions or subregions immunized against are as follows: A.TH anti-A.TL (anti-I^k, S^k, G^k); (B10.A(4R) × A.SW)F_1 anti-B10 (anti-K^b, $I\text{-}A^b$); (B10 × HTI)F_1 anti-B10.A(5R) (anti-$I\text{-}J^k$, $I\text{-}E^k$, $I\text{-}C^d$, S^d, G^d); B10.S(7R) anti-B10.HTT (anti-$I\text{-}E^k$, $I\text{-}C^k$, S^k, G^k); (B10.T(6R) × (B10.D2)F_1 anti-B10.AQR (anti-$I\text{-}A^k$, $I\text{-}B^k$, $I\text{-}J^k$, $I\text{-}E^k$). These antisera were raised by hyperimmunization of recipient mice with donor spleen and lymph node lymphocytes as previously described (9).

Preparation of ^{125}I-Labeled Spleen Cell Lysates. Spleen cell suspensions were made in Dulbecco's phosphate buffered saline containing 5% (vol/vol) fetal calf serum (D-PBS-FCS)[1] by the flushing out of spleens and the passage of cells through a nylon mesh as previously reported (10). After removal of the erythrocytes from suspensions by lysis with Tris-NH_4Cl (0.01 M Tris, pH 7.4, 0.85% NH_4Cl), the residual lymphocytes were routinely greater than 90% viable as judged by trypan blue dye exclusion. They were washed twice and resuspended with D-PBS at 5 × 10^7 cells/ml and their membrane associated proteins were labeled with ^{125}I (New England Nuclear, Boston, Mass., low pH iodine, carrier-free, 450 mCi/ml) by using the lactoperoxidase-catalyzed radioiodination technique (11). After radioiodination, the cells were washed three times with D-PBS, resuspended at 10^8 cells/ml in PBS (0.02 M phosphate buffer, pH 7.4, 0.15 M NaCl) containing 0.5% Non-idet P-40 (NP-40, Particle Data Systems, Chicago, Ill.) and 3500 Kallikrein Inactivator Units/ml of Trasylol (Sigma Chemical Co., St. Louis, Mo.), maintained for 30 min at 4°C, and the lysates then centrifuged for 1 h at 100,000 *g*.

Supernates from the above centrifugation were collected and either initially frozen at −70°C or immediately enriched for membrane glycoproteins by lentil lectin affinity chromatography (12). The latter technique was applied to the B10.A(5R) but not B10 lysates. Lentil lectin, prepared from brown kitchen lentiles (*Lens culinaris*), was a kind gift from Dr. M. Letarte, Department of Biological Research, University of Toronto, Toronto, Canada. It was coupled to cyanogen-bromide-activated Sepharose 4B (13) at a ratio of 6 mg lentil lectin/g Sepharose 4B. ^{125}I-labeled NP-40 lysates were fractionated at 4°C on a 1 x 5-cm lentil lectin column in the presence of PBS containing 0.3% NP-40. Approximately 5–10% of the total trichloroacetic acid precipitable radioactivity was recovered in the bound fraction, which was eluted with PBS-0.3% NP-40 containing 0.1 M α-methyl-D-glucopyranoside (Sigma Chemical Co.). Greater than 80% of immunoprecipitable Ia antigen activity was recovered in this fraction.

Immunoprecipitation and Gel Electrophoresis. Lentil lectin column bound fractions were precleared for radiolabeled Ig by treatment with goat anti-mouse IgG and normal mouse IgG as reported previously (10). Portions of this precleared sample, taken to represent a lysate derived from approximately 10^7 spleen lymphocytes, were treated with anti-Ia sera (10 μl) for 30 min at 4°C and goat anti-mouse IgG (100 μl) for a further 3–16 h at 4°C. Sequential precipitation analyses were performed by using two anti-Ia sera by treatment of the supernate of a precipitate formed with one anti-Ia serum and goat anti-mouse IgG with a second anti-Ia serum and goat anti-mouse IgG. Immunoprecipitates were washed three times with PBS-0.5% NP-40, dissolved, and electrophoresed under reducing conditions on 0.6 x 10-cm sodium dodecyl sulfate-12.5% polyacrylamide gels according to Laemmli (14), as previously described (10). The radioactivity in 2-mm gel slices was quantitated with a Searle gamma well counter (Searle Analytic Inc., Des Plaines, Ill.). The positions of migration of unlabeled marker mouse IgG heavy (H) and light (L) chains was determined on either the same or a parallel gel by using Coomassie Blue protein stain (14).

Results

The genetic organization of the *I*-region was examined by performing experiments designed to estimate the number of *Ia* genes present in the known *I*-

[1] *Abbreviations used in this paper:* D-PBS, Dulbecco's phosphate buffered saline; PBS, phosphate buffered saline (0.02 M phosphate buffer, pH 7.4, 0.15 M NaCl); FCS, fetal calf serum; H, heavy; L, light; NP-40, Non-idet P-40.

TABLE I
H-2 Haplotype Origin of Strains Used in This Study

Strain	Haplotype	Region* I								
		K	*A*	*B*	*J*	*E*	*C*	*S*	*G*	*D*
B10/Sn	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B10.D2/Sn	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
A.SW	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
B10.A (4R)/Sg	*h4*	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
HTI	*i*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*?*	*d*
B10.A (3R)/Sg	*i3*	*b*	*b*	*b*	*b*	*k*	*d*	*d*	*d*	*d*
B10.A (5R)/Sg	*i5*	*b*	*b*	*b*	*k*	*k*	*d*	*d*	*d*	*d*
A.TL/Sf	*t1*	*s*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*d*
A.TH/Sf, B10.S (7R)/Sg	*t2*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*d*
B10.HTT/Ph	*t3*	*s*	*s*	*s*	*s*	*k*	*k*	*k*	*k*	*d*
B10.AQR (N4)/Klj	*y1*	*q*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*d*
B10.T (6R)/Sg	*y2*	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*?*	*d*

* Haplotype origin of regions according to references 2 and 17.

subregions. Coprecipitation studies were conducted by treatment of B10, B10.A(5R), and B10.A(3R) radiolabeled spleen cell lysates with various anti-Ia sera. Samples were fractionated initially on a lentil lectin column to enrich for glycoprotein and to minimize nonspecific immunoprecipitation, and were then precleared for radiolabeled Ig. They were subsequently reacted with one anti-Ia serum, the precipitate was discarded, and the supernate further treated with a second anti-Ia serum. The latter precipitate was analyzed by gel electrophoresis. If treatment with the first antiserum removes all antigen precipitable by the second antiserum, then the Ia specificities detected by both sera are considered to be present on the same molecule. Alternatively, if the second antiserum precipitates a residual peak of radioactivity, then the specificities detected by the two antisera are considered to be present on separate Ia molecules.

In all of the experiments to be described below, when lysates were reacted initially with a normal mouse serum and the supernate of that precipitate was then treated with an anti-Ia serum, Ia peaks of activity in the 25,000–35,000 mol wt region were always observed (results not shown). Similarly, under the conditions employed, each antiserum quantitatively removed from the lysates all its Ia reactivity in a single precipitation. No Ia-like peaks were obtained when samples were first treated with a given anti-Ia serum, and the supernate of this precipitate was further reacted with the same anti-Ia serum (results not shown).

Evidence for a Single Ia Locus in the I-A Subregion. The molecular association of Ia specificities that map in the *I-A* subregion was analyzed by using B10 lysates and antisera reactive both with H-2 determinants controlled by K^b and Ia determinants controlled by $I\text{-}A^b$. The antisera employed were

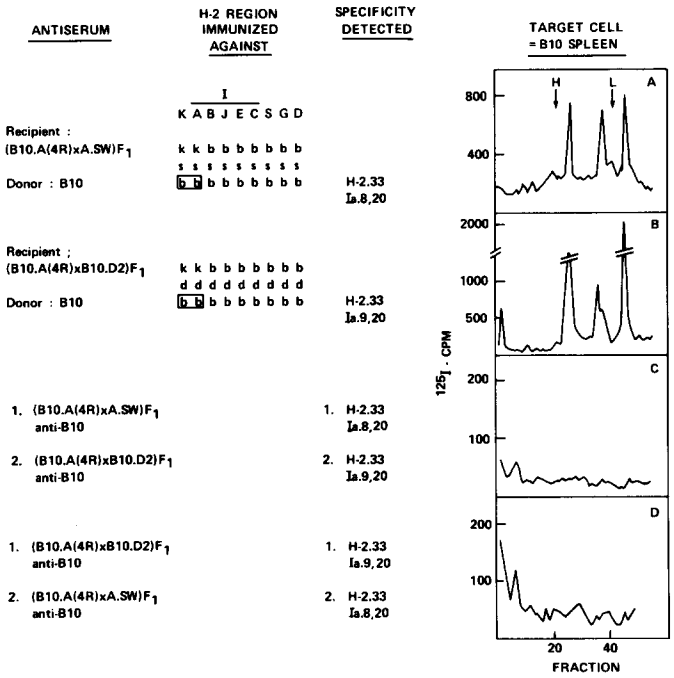


FIG. 1. Coprecipitation of specificities Ia.8 and Ia.9. B10 lysates were treated with either (B10.A(4R) × A.SW)F_1 anti-B10 (A) or (B10.A(4R) × B10.D2)F_1 anti-B10 (B). Sequential precipitations were performed in (C) and (D). The *H-2* regions detected by the antisera are enclosed. The *H-2* and Ia specificities detected on B10 target cells are listed. Marker immunoglobulin H and L chains are indicated by arrows.

(B10.A(4R) × A.SW)F_1 anti-B10 (anti-H-2.33, anti-Ia.8, Ia.20),[2] (B10.A(4R) × B10.D2)F_1 anti-B10(anti-H-2.33, anti-Ia.9, Ia.20),[2] and (B10.A(4R) × B10.HTT)F_1 anti-B10 (anti-H-2.33, anti-Ia.8, Ia.20).[2] As shown in Figs. 1A and 1B, three separate molecules were precipitated by the first two of the above antisera. These molecules correspond to proteins that have approximate mol wt of 45,000 daltons (H-2), 25,000–35,000 daltons (Ia), and 12,000 daltons (presumably β-2-microglobulin), respectively. A similar result was obtained with the (B10.A(4R) × B10.HTT)F_1 anti-B10 antiserum (T. Delovitch, unpublished observations). Each of these antisera yielded two peaks of activity in the 25,000–35,000 mol wt region, which are considered to represent the 35,000 mol wt (α-subunit) and 28,000 (β-subunit) mol wt subunits of Ia antigens (3–5). It should be noted, however, that the electrophoretic conditions used here have not permitted the optimum resolution of these Ia subunits. Reactivity of B10 extracts first with (B10.A(4R) × A.SW)F_1 anti-B10 and second with (B10.A(4R) × B10.D2)F_1 anti-B10 (Fig. 1C), or in the reverse order (Fig. 1D), resulted in the coprecipitation of the Ia antigens detected. A similar observation was made when (B10.A(4R) × B10.HTT)F_1 anti-B10 was substituted for (B10.A(4R) × A.SW)F_1 anti-B10 (results not shown). These data indicated that specificities Ia.8 and Ia.9, both

[2] Specificities detected by this antiserum are predicted from current Ia-specificity charts (15). Although the pattern of cytotoxic reactivity of this antiserum with a standard panel of cells is consistent with this assignment, formal absorption analyses have not been performed.

controlled by the *I-A* subregion, are present on either two separate but structurally homologous molecules or the same molecule. Similar findings have also been recently obtained by other investigators (16). Ia.20 has also been found to be in association with the same molecule as Ia.8 and Ia.9 (15). It may be concluded, therefore, that at least one *Ia* locus, *Ia-1*, maps in the *I-A* subregion.

Association of Ia.15 with an I-A Subregion Molecule. It was previously reported that the reactivity of an A.TH anti-A.TL serum with B10 target cells defined specificity Ia.3, the only specificity purportedly determined by the *I-B* subregion (1). It was thus of considerable interest to examine whether this specificity was present on a molecule distinct from that specified by the *I-A* subregion (7). However, it has been shown more recently that an A.TH anti-A.TL serum detects a second specificity, designated Ia.15 (6), and that strain B10 expresses this specificity (15). Absorption analysis of the activity of the A.TH anti-A.TL serum used in this study indicated that it reacts with Ia.15 and not Ia.3 on B10 cells. Strains B10(H-2^b), B10.D2(H-2^d), and B10.BR(H-2^k) clear antibody cytotoxic for B10 targets, while strains B10.M(H-2^f), B10.G(H-2^q), and B10.$_s$(H-2^s) fail to absorb this activity (D. Murphy, unpublished observations). An Ia peak of activity was obtained in this combination (Fig. 2 A). The association of Ia.15 with *I-A* subregion molecules was, therefore, examined.

B10 lysates were treated initially with A.TH anti-A.TL and then with (B10.A(4R) × A.SW)F_1 anti-B10. These antisera reciprocally removed the Ia reactivity for each other (Figs. 2 C and 2 D). The (B10.A(4R) × B10.D2)F_1 anti-B10 and (B10.A(4R) × B10.HTT)F_1 anti-B10 sera yielded identical results when each was tested in combination with the A.TH anti-A.TL serum (T. Delovitch, unpublished observations). These observations demonstrate that specificity Ia.15 is associated with a molecule controlled by the *Ia-1* locus, which maps in the *I-A* subregion.

Evidence for Two Ia Loci Mapping between the I-J and S Regions. The number of Ia molecules controlled by the *I-B---D* chromosomal segment was also investigated. B10.A(5R) lysates were treated with (B10 × HTI)F_1 anti-B10.A(5R) (anti-$I\text{-}J^k$, $I\text{-}E^k$, $I\text{-}C^d$, S^d, G^d?) and B10.S(7R) anti-B10.HTT(anti-$I\text{-}E^k$, $I\text{-}C^k$, S^k, G^k) antisera. Ia molecules were precipitated by both sera (see Figs. 3 A and 3 B). Two main peaks of radioactivity corresponding in molecular size to the α- and β-subunits of Ia molecules were observed. No peaks of radioactivity were precipitated from B10 extracts, thereby ruling out the presence of autoantibody and/or antibody crossreactive with *I-A* subregion determinants. When these experiments were conceived, it was assumed that both antisera potentially contained antibody against the products of loci mapping in the *I-C*, *S*, or *G* regions. Since no Ia-like molecule has been precipitated by an antiserum directed against determinants controlled by loci mapping in the *S* or *G* regions, it was initially deduced that the Ia polypeptides observed were controlled by the *Ia-3* locus, which marks the *I-C* subregion (6).

In view of the above deduction, the independent precipitation that occurred after treatment of a B10.A(5R) lysate first with B10.S(7R) anti-B10.HTT and second with (B10 × HTI)F_1 anti-B10.A(5R) was an unexpected result (Fig. 3 C). Coprecipitation occurred in the reverse direction (Fig. 3 D). This pattern of reactivity demonstrated the association of different determinants with two

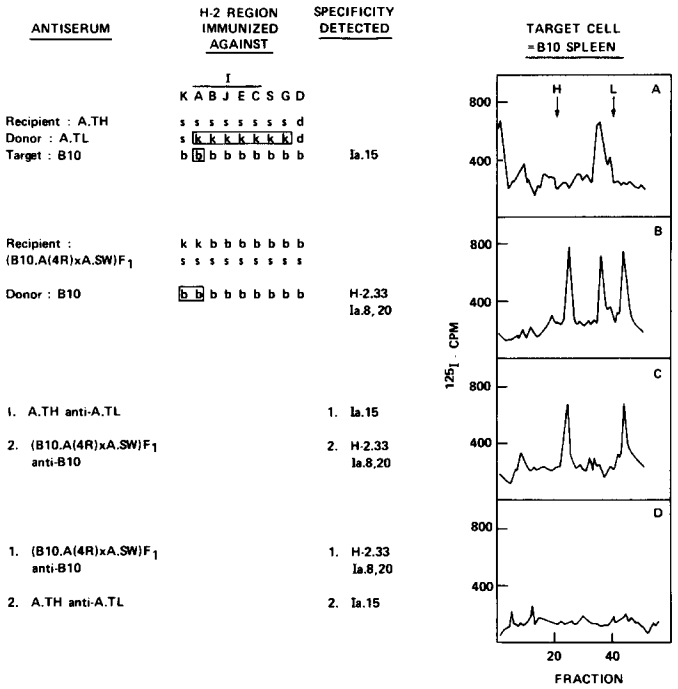
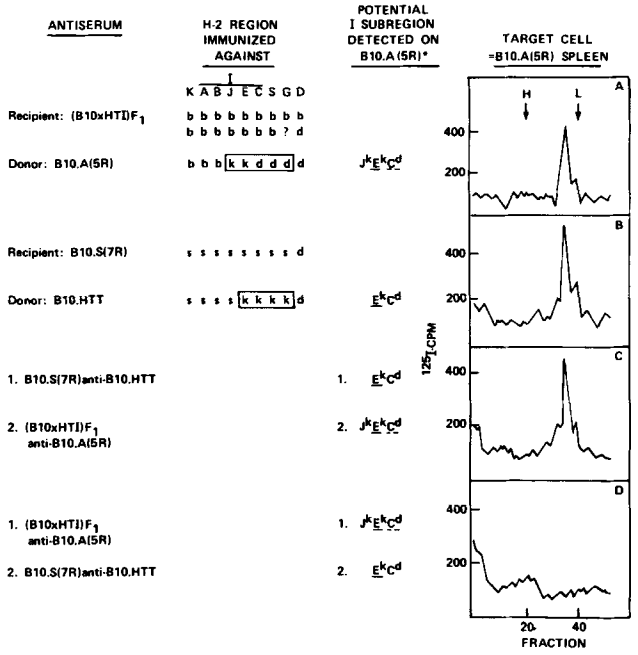


FIG. 2. Association of specificity Ia.15 with an $I\text{-}A^b$ subregion molecule. B10 lysates were treated with either A.TH anti-A.TL (A) or (B10.A(4R) × A.SW)F_1 anti-B10 (B). Sequential precipitations were performed in (C) and (D).

separate Ia molecules, i.e., one Ia molecule is detected by both antisera, while the second Ia molecule is detected only by the (B10 × HTI)F_1 anti-B10.A(5R) serum. Thus, these data suggested the localization of two *Ia* loci in the *I-C* subregion (6).

Recently, two additional *Ia* loci, which map between the *I-B* and *S* regions, have been identified. The *Ia-4* locus, which marks the *I-J* subregion, controls determinants that are selectively expressed by suppressor T lymphocytes (17). The immunochemical nature of the *Ia-4* product is unknown. The *Ia-5* locus, which marks the *I-E* subregion (intercalated between *I-J* and *I-C*), controls determinants expressed by splenic and lymph node lymphocytes (2, 18). The *Ia-5* product is a glycoprotein having a mol wt of 25,000–35,000 daltons. Since the (B10 × HTI)F_1 anti-B10.A(5R) serum used in the present analysis potentially contains antibody against both the *Ia-4* and *Ia-5* products, further analyses were performed to determine whether either of these products were being detected.

To test for the involvement of the *Ia-4* product, strain B10.A(3R) lysates were treated with the same two antisera used above (Fig. 4). Strain B10.A(3R) and strain B10.A(5R) differ only in the *I-J* subregion, i.e. strain B10.A(3R) carries the $Ia\text{-}4^b$ allele, while strain B10.A(5R) carries the $Ia\text{-}4^k$ allele (17). Since strains B10 and HTI both carry the $I\text{-}J^b$ subregion (17), the (B10 × HTI)F_1 anti-B10.A(5R) serum cannot detect $Ia\text{-}4^b$ determinants. Nevertheless, independent precipitation was again observed with B10.A(3R) lysates treated



*Underscore ___ indicates subregion detected based on cumulative data presented in this manuscript. Underscore _ _ indicates possible subregion detected. See text.

FIG. 3. Evidence for distinct Ia molecules determined by loci mapping between the *I-B* and *S* regions. B10.A(5R) lysates were reacted with either (B10 × HTI)F_1 anti-B10.A(5R) (A) or B10.S(7R) anti-B10.HTT (B). Sequential precipitations were performed in (C) and (D). The *I*-subregions potentially detected on B10.A(5R) target cells are listed.

first with the B10.S(7R) anti-B10.HTT serum and second with the (B10 × HTI)F_1 anti-B10.A(5R) serum (Fig. 4C). It may be concluded, therefore, that the *Ia-4* locus does not code for either of the two Ia molecules detected here.

The *Ia-5* product was initially detected by the reactivity of a (C3H.Q × B10.D2)F_1 anti-B10.AQR serum with B10.A(5R) target cells (2). Treatment of B10.A(5R) lysates with a similar antiserum, (B10.T(6R) × B10.D2)F_1 anti-B10.AQR, resulted in the precipitation of an Ia molecule (Fig. 5B). Pretreatment of B10.A(5R) lysates with this antiserum cleared all activity for the B10.S(7R) anti-B10.HTT serum and vice versa (Figs. 5C and 5D). In addition, as shown in Fig. 6, the (B10.T(6R) × B10.D2)F_1 anti-B10.AQR serum, when tested in combination with the (B10 × HTI)F_1 anti-B10.A(5R) serum, yielded similar results to those presented in Fig. 3. Therefore, both the (B10.T(6R) × B10.D2)F_1 anti-B10.AQR and B10.S(7R) anti-B10.HTT sera contain antibody reactive with the same Ia molecule determined by the *Ia-5* locus. It should also be noted that while the B10.S(7R) anti-B10.HTT serum could possibly contain antibody against *I-C* subregion determinants, it does not precipitate Ia molecules controlled by this subregion.

These data substantiate the claim for the presence of two *Ia* loci mapping between the *I-J* and *S* regions. Moreover, they indicate that one of the loci

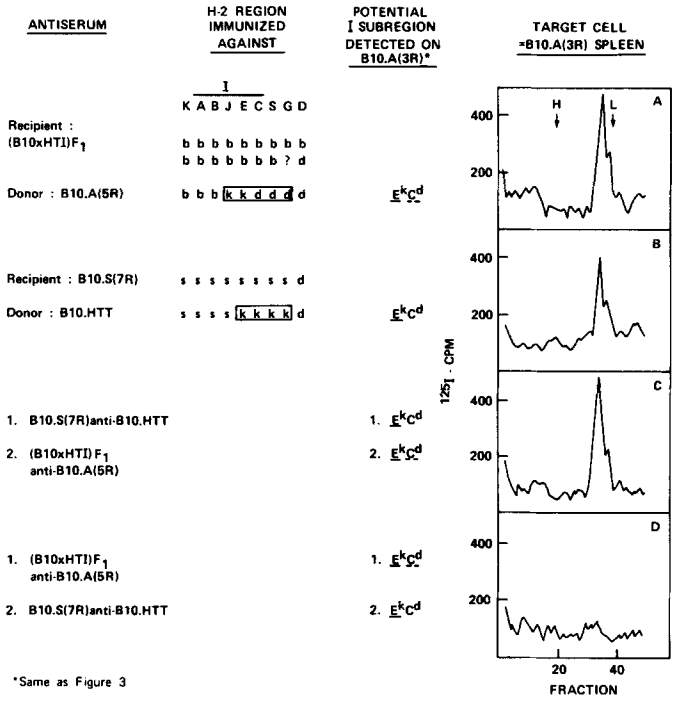


FIG. 4. Independent precipitation of Ia molecules controlled by two *Ia* loci in the *I-B--S* chromosomal segment. B10.A(3R) lysates were reacted with either (B10 × HTI)F_1 anti-B10.A(5R) or B10.S(7R) anti-B10.HTT (B). Sequential precipitations were performed in (C) and (D).

involved, *Ia-5*, maps in the *I-E* subregion. However, the data do not permit one to definitively map the second locus, most likely *Ia-3*, to a particular subregion. It could map in either the *I-E* or *I-C* subregion (see Discussion below).

Discussion

Coprecipitation analyses presented in this report show that different Ia determinants are associated with three separate molecules. Although no attempt has been made to optimally resolve the α- and β-subunits of these Ia molecules, or to determine whether either both or only one subunit is detected by the Ia antisera, these data suggest that at least three distinct *Ia* loci control determinants detectable by immunoprecipitation. According to the current *I*-region map, the data are consistent with the detection of products of the *Ia-1*, *Ia-5*, and *Ia-3* loci. These loci mark the *I-A*, *I-E*, and *I-C* subregions, respectively.

If the α- and β-subunits of the products of each of the above three *Ia* loci are encoded by *I*-region genes, then there exist a minimum of six structural *Ia* genes in this region. Coprecipitation of different Ia determinants controlled by a particular *I*-subregion may be predicted if they are present on either the same molecule or on separate but structurally homologous molecules. Serological crossreactivity between *I-A* and *I-C* subregion products, which suggests a

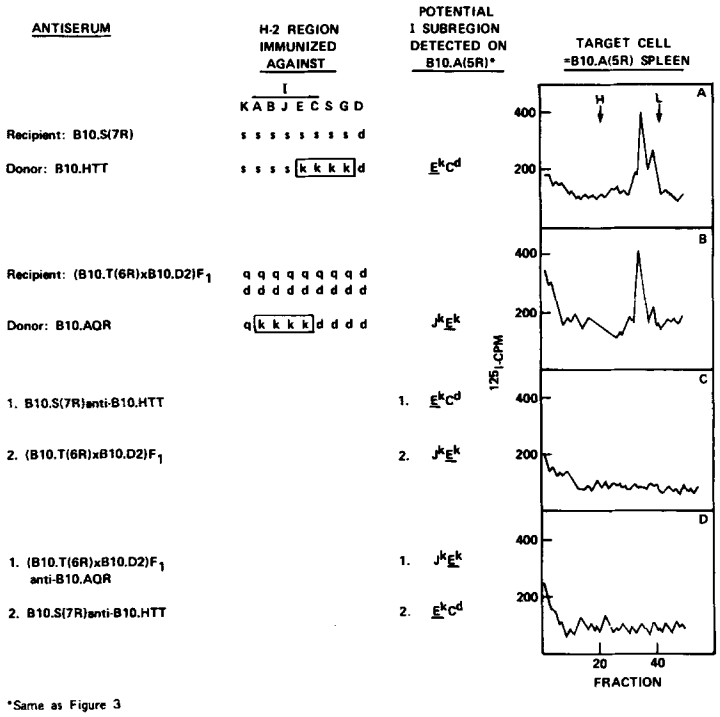
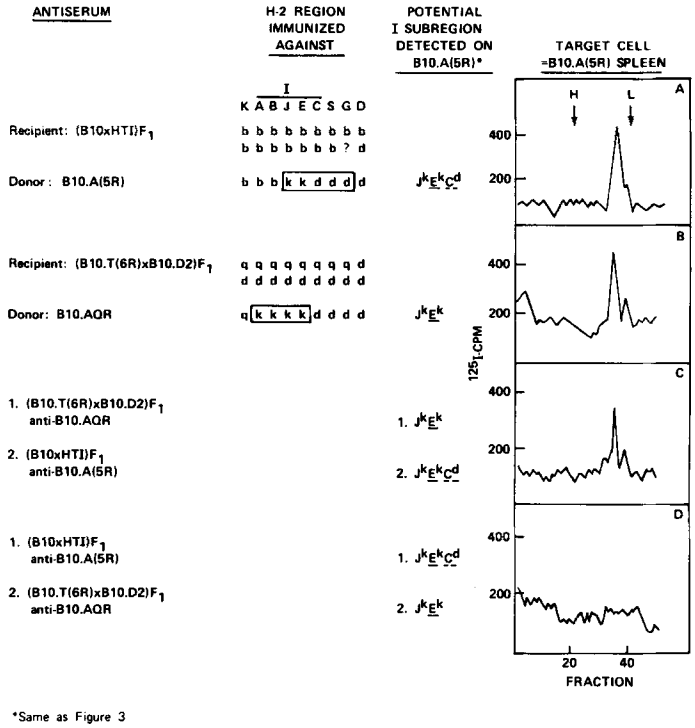


FIG. 5. Detection of the *Ia-5* locus product. B10.A(5R) lysates were reacted with either B10.S(7R) anti-B10.HTT (A) or (B10.T(6R) × B10.D2)F_1 anti-B10.AQR (B). Sequential precipitations were performed in (C) and (D).

possible structural homology between these products, has been previously reported (6, 19). It is possible that a given *I*-subregion codes for more than one Ia α- and β-chain, and that these subunits are structurally similar. Sequential precipitation studies may therefore underestimate the total number of detectable Ia molecules and may not indicate the overall extent of genetic complexity of the *I*-region.

We are presently unable to determine whether the two loci (*Ia-5* and *Ia-3*) mapping between the *I-J* and *S* regions are localized in the same or different *I*-subregions. Cross-over positions in strains B10.A(3R) and B10.HTT form the *K* end boundary of the *I-E* subregion, while the cross-over position in strain B10.A forms the *D* end boundary (2). By contrast, the cross-over position in strain B10.A forms the *K* end boundary of the *I-C* subregion, while strains BSVS and A.TFR5 form the *D* end boundary (1). The crossover in strain B10.A is therefore pivotal in postulating two subregions.

There are two problems in localizing the cross-over position in strain B10.A between the *Ia-5* and *Ia-3* loci. First, the key point in this assignment is the assumption that this strain carries the $Ia\text{-}3^d$ allele. Previously, David et al. (20) demonstrated that strain A.TL ($I\text{-}C^k$) failed to absorb B10.A(4R) anti-B10.A(2R) antibody reactive with B10.D2 or B10.A(2R) ($I\text{-}C^d$) target cells. The specificity which distinguished $Ia\text{-}3^d$ from $Ia\text{-}3^k$ was designated Ia.6. However,



*Same as Figure 3

FIG. 6. Independent precipitation of *Ia-5* and *Ia-3* locus products. B10.A(5R) lysates were reacted with either (B10 × HTI)F_1 anti-B10.A(5R) (A) or (B10.T(6R) × B10.D2)F_1 anti-B10.AQR(B). Sequential precipitations were performed in (C) and (D).

these and other investigators have been unable to produce additional sera which contain anti-Ia.6 activity. (All antibody present in the (B10 × HTI)F_1 anti-B10.A(5R) serum used in this study was absorbed with B10.BR lymphocytes, indicating the absence of Ia.6 antibody). It has therefore not been possible to confirm the assignment of the $Ia\text{-}3^d$ allele to strain B10.A.

Second, two dimensional gel electrophoretic analyses in which radiolabeled polypeptides are separated based on their net charge and molecular size (21) reveal unique spot patterns for products of loci mapping between the *I-J* and *S* regions of haplotypes $H\text{-}2^d$ and $H\text{-}2^k$ (T. Delovitch, unpublished observations, and Jones et al. [22]). The pattern observed with $H\text{-}2^a$ is identical to that observed with $H\text{-}2^k$; this implies that all spots observed are of $H\text{-}2^k$ origin. One of the antisera used in the two dimensional gel analysis was the subsequent bleed of the (B10 × HTI)F_1 anti-B10.A(5R) serum used in this study. The data are thus consistent with both loci examined in this study being of $H\text{-}2^k$ origin, which would localize both loci in the *I-E* subregion. Experiments are in progress to confirm this assignment.

Summary

Sequential immunoprecipitation analyses of Ia antigens were performed with ^{125}I-labeled B10, B10.A(3R), and B10.A(5R) spleen cell lysates. The results suggest the presence of one *I*-region associated (*Ia*) locus (*Ia-1*) in the *I-A*

subregion. In addition, they indicate that two *Ia* loci map between the *I-J* and *S* regions. One locus, *Ia-5*, maps in the *I-E* subregion. The second locus, most likely *Ia-3*, maps in either the *I-E* or *I-C* subregion. Taken together, the data demonstrate that at least three *Ia* loci in the *I*-region of the *H-2* complex code for molecules detectable by immunoprecipitation.

We thank Carol Ostrem and Judy Biggin for their superb technical assistance, and Patricia Camden for her cheerful secretarial assistance.

Received for publication 28 July 1977.

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