A GENE LOCUS CONCERNED WITH AN ANTIGENIC SERUM SUBSTANCE IN *MUS MUSCULUS*

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SEVERAL loci concerned with the production of serum proteins of the mouse have been identified. These include loci controlling immune globulins (KELUS and MOOR-JANKOWSKI 1961; WUNDERLICH and HERZENBERG 1963), transferrin (COHEN 1960; SHREFFLER 1960), a hemolytic complement component (CINADER, DUBISKI and WARDLAW 1964; ERICKSON, TACHIBANA, HERZENBERG and ROSENBERG 1964), two as yet unidentified esterases (POPP and POPP 1962; PETRAS 1963), a prealbumin (SHREFFLER 1964) and a protein associated with the *H-2* locus (SHREFFLER 1964a).

This paper presents evidence identifying a locus independent of those previously described which is concerned with another but as yet incompletely characterized serum substance.

MATERIALS AND METHODS

Mouse strains: The following strains were maintained in the Department of Genetics, Stanford University. C57BL/10Hz, RF/J, B10.D2(old)Hz, A/J, C3H/Hz, (C3H.SW/Hz × C57BL/10Hz) (CSW B10)F_1 and F_2 and the backcross to C3H.SW. In addition, CST a noninbred stock was also maintained and used for crossing with A/J to produce (CST × A/J)F_1 and a backcross to A/J.

Normal sera: Mice from the colony were bled from the tail. Serum samples were stored at —20°C. Samples of sera from strains not available in the colony were obtained from the sources indicated by the substrain symbol.

Antisera: Three parts of B10–D2(old)Hz serum were emulsified in complete Freund's adjuvant containing 8 mg/ml of *Mycobacterium tuberculum*. Each of ten adult female RF/J mice were injected intraperitoneally with 0.15 ml of the emulsion. Four weeks and eight weeks later they were reinjected with a similar emulsion utilizing incomplete Freund's adjuvant. Animals were bled at five, seven and nine weeks. The A/J anti C57BL/6J, B10–D2(old) anti B10–D2 (new) and BALB/C anti C57BL/6 sera have been described previously (ERICKSON, TACHIBANA, HERZENBERG and ROSENBERG 1964; WUNDERLICH and HERZENBERG 1963).

Agar diffusion: A modification of the OUCHTERLONY (OUCHTERLONY 1962) technique was employed using 6 ml of 1% Ionagar 2 in pH 7.15 phosphate buffered saline with 0.2% sodium azide on a 3 × 2 inch glass slide; the SCHEIDEGGER (SCHEIDEGGER 1955) microimmunoelectrophoresis method with Veronal buffer pH 8.2 0.05 M was used. Slides were developed for 24 hr at room temperature, dried, washed and stained with amido black.

Density gradient ultracentrifugation: A 37% to 10% sucrose gradient was made with a device similar to that described by BRITTEN and ROBERTS (1960). 0.5 ml of a mixture of equal parts of 10% sucrose in saline and serum was layered over a 4.8 ml gradient. Using a SW-39

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rotor the samples were spun for 15 to 18 hr at 35,000 rpm in a Spinco model L ultracentrifuge. Four-drop fractions were collected through a hole punched through the bottom with a 19 gauge needle.

RESULTS

Eight of the ten immunized RF/J animals developed a single precipitin line when their sera were tested by agar double diffusion with B10-D2(old) serum; two were negative. When tested on a single pattern it was seen that all of the lines fused completely, indicating that each individual serum had precipitating antibody directed against the same single class of molecules. All positive sera for each of the three bleeds were therefore pooled.

The pooled antiserum was tested against several individuals from each of several strains. The results of these tests are shown in Table 1. All individual sera from an inbred strain were either positive or negative. However, it was seen that with some positive sera the precipitin line formed was very close to the antigen well where it was not consistently distinguished from nonspecific staining. For this reason all negative sera were always retested against antisera that had been diluted in normal RF/J serum to ½ and, if necessary, to ¼ and ⅛. It was also found that B10-D2(old) serum, when concentrated by negative pressure dialysis to ½ its original volume, gave a much stronger reaction. Similar testing of a negative serum, DBA/2, did not reveal a positive reaction.

Sera from the progeny of appropriate crosses were examined to test whether the absence or presence of the antigenic serum substance was under genetic control. The results of these tests are shown in Table 2.

The F_1 progeny from a cross of animals belonging to a negative and positive

TABLE 1

Precipitin reactions of RF/J anti B10–D2(old) with individual sera

Mouse strain	Number +	Number —
DBA/2J	0	20
B10–D2(old)/Hz male	12	0
B10–D2(old)/Hz female	8	0
C57BL/10Hz male	12	0
C3H.SW	0	20

TABLE 2

Precipitin reactions of RF/J anti B10–D2(old) with the F_1 and F_2 progeny derived from a positive (C57/BL/10Hz) and a negative (CSW/Hz) strain

Cross	Number +	Number —	Chi-square*	P
(CSW B10)F_1	20	0	. .	. . . . .
(CSW B10)F_2	68	33	3.4	0.05–0.1
Backcross to positive	33	46	2.2	0.1–0.2

* For hypothesis of a single locus with dominance or codominance of the positive allele.

strain were all positive. The frequency of positive and negative individuals in the F_2 progeny and in the offspring of the backcross to the negative strain was compatible with the hypothesis of control of antigenic type at a single locus, with the positive allele dominant or codominant.

It was possible to test for independent segregation of the antigenic serum substance from loci concerned with a hemolytic complement component (*Hc*), the H chain of gamma$_{2a}$ (*Ig-1*), agouti (*A*), and coat color (*C*), using the sera from the progeny of the crosses in Table 2, as well as sera from the progeny of the cross of animals heterozygous for *Hc*, *C*, and the gene for antigenic serum substance. The results in Table 3 show the absence of close linkage between the locus of antigenic serum substance and the other loci examined. In the case of *Ig-1* this would probably imply independence from *Ig-2*, the locus specifying the H polypeptide chain of mouse β2A globulin, since *Ig-1* and *Ig-2* are closely linked (HERZENBERG 1964).

The antiserum pool was tested against sera from several strains. The results of these tests are shown in Table 4. Also shown, where available, are the *H-2*, *Ig-1* and *Hc* phenotypes.

Of several other mouse antisera to mouse sera available for testing, two have shown precipitin reactions with the antigenic serum substance. One of these antisera is an A/J anti C57BL/6 previously reported to develop three precipitin lines on double diffusion (ERICKSON, TACHIBANA, HERZENBERG and ROSENBERG 1964). The other is an NZB/B1 anti C57BL/10 B1 made available by DR. LESLIE NORINS. Both these sera develop a precipitin line with B10–D2 serum, which

TABLE 3

Test of linkage between the Sas *locus and* Ig-1, A, Hc, C

Cross	Genotypes†	Test	Chi-square*	P
CWBT × CSW	$\frac{Sas\text{-}1^a}{Sas\text{-}1^0}\frac{Ig\text{-}1^b}{Ig\text{-}1^a} \times \frac{Sas\text{-}1^0}{Sas\text{-}1^0}\frac{Ig\text{-}1^a}{Ig\text{-}1^a}$		1.0	0.3–0.5
		Ig-1^b		
		+ —		
		Sas + 4 5		
		Sas — 10 6		
(CWBT)F_2	$\frac{Sas\text{-}1^a}{Sas\text{-}1^0}\frac{A}{a} \times \frac{Sas\text{-}1^a}{Sas\text{-}1^0}\frac{A}{a}$		5.1	0.1–0.2
		A *a*		
		Sas + 45 23		
		Sas — 25 8		
(CST‡ × A/J)F_1 × A/J	$\frac{Sas\text{-}1^a}{Sas\text{-}1^0}\frac{Hc^1}{Hc^0} \times \frac{Sas\text{-}1^0}{Sas\text{-}1^0}\frac{Hc^0}{Hc^0}$		0.7	0.3–0.5
		Hc		
		+ —		
		Sas + 11 12		
		Sas — 17 12		
(CST × A/J)F_1 × A/J	$\frac{Sas\text{-}1^a}{Sas\text{-}1^0}\frac{C}{c} \times \frac{Sas\text{-}1^0}{Sas\text{-}1^0}\frac{c}{c}$		1.8	0.1–0.2
		C *c*		
		Sas + 14 9		
		Sas — 9 13		

* Hypothesis of independent segregation (without correction for small numbers).
† See DISCUSSION for designation of genotypes.
‡ CST is a stock (noninbred) from Jackson Laboratory. The parents of the F_1 used here were homozygous for *C* and *Hc*^{1}.

TABLE 4

Tests of the antiserum pool against sera from various strains

Strain	Serum antigenic substance	*Hc* type*	*Ig-1*† type	*H-2* type‡
A/J	—	0	e	a
AKR/J	+	0§	d	k
AL/N	+	.	d	a
BALB/CGa	+	1	a	d
BDP/J	+	1	h	p(?)
BSL/Di	+	.	h	.
B10–D2(new)Hz	+	1	b	d
B10–D2(old)Hz	+	0	b	d
CBA/J	—	1‖	a	k
CE/J	—	0	f	k
C3H/Hz	—	1	a	k
C3H.SW/SnHz	—	1	a	b
C57BR/cdJ	+	1	a	b
C57BL/Ka	+	1	b	b
C57BL/6J	+	1	b	b
C57BL/10Hz	+	1	b	b
C57L/J	+	1	a	b
C58/J	+	1	a	k(?)
DA/Hu	—	.	g	.
DBA/1J	—	1	c	q
DBA/2J	—	0	c	d
DD/He	—	0	.	.
DE/J	—	0	f	.
Fz/Di	—	1	g	.
LP/J	+	1	b	b
NZB/Bl	—	0	e	.
PL/J	—	1	a	.
RF/J	—	0	c	k
RIII/J	+	1	g	r
SJL/J	—	1	b	s
SM/J	—	1	b	.
ST/J	+	0	a	k
STR/N	+	1	a	.
SWR/J	+	0	c	b
58N/Sn	+	.	b	.
101/R1	+	1	b	k
129/RrGa	+	1	a	b

* Erickson, Tachibana, Herzenberg and Rosenberg 1964.
† Herzenberg, Warner and Herzenberg 1965.
‡ Snell, Hoecker, Amos and Stimpfling 1964; Staats 1964.
§ AKR/J previously reported as *Hc-1*.
‖ CBA/J previously reported as *Hc-0*.

forms a line of identity with the line formed by the RF/J anti B10–D2 sera. Furthermore, no such line appears when either of these antisera is tested against sera known to be negative for the antigenic serum substance. Similar sera prepared in one positive strain against another (101/R1 anti SWR/J), and in a positive strain against one that was negative (C57BL/10 Hz anti C3H/Hz), have

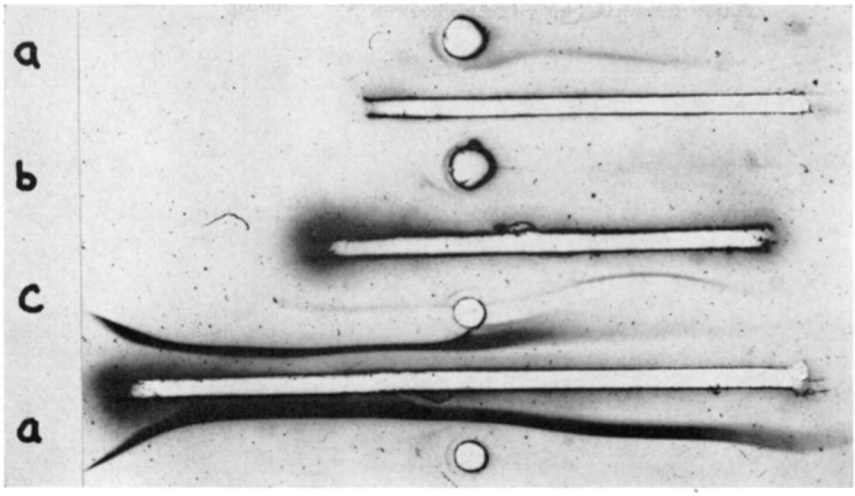

FIGURE 1.—Immunoelectrophoresis of mouse sera showing *Sas*, *Hc-1*, and *Ig-1*^{b}. (a) Concentrated B10.D2(old)Hz. (b) Concentrated DBA/2J. (c) C57BL/10SnHz. The top trough contained anti-*Sas* (RF/J anti B10.D2[old]), the middle anti *Hc-1* and the bottom anti *Ig-1*^{b}. The anode was to the right. The cathodal extension of the *Hc-1* line is due to a reaction with the *Hc-1* within the bottom antiserum.

not shown precipitin lines when tested by double diffusion. Thus, no product of another allele at this locus has been identified.

When C57BL/10 Hz serum was tested simultaneously with RF anti B10–D2, an anti-hemolytic complement component (Hc-1)(B10–D2[old] anti B10–D2 [new], and an anti gamma globulin (BALB/C anti C57B1/6), the single precipitin lines which formed spurred completely over each other indicating no cross reactions.

The electrophoretic mobility of the antigenic serum substance is shown in Figure 1. It can be seen that the RF anti B10–D2 serum produces a single precipitin line that runs in the fast β region, slightly anodal to *Hc*-1.

Several density gradient centrifugations were run. A typical result is shown

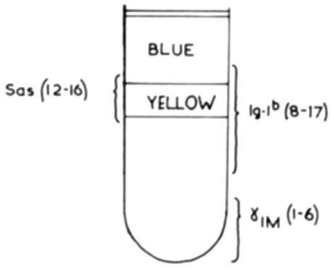

FIGURE 2.—Sucrose gradient of C57BL/10SnHz serum. Twenty-three fractions of four drops each were collected. Albumin was marked with brom phenol blue. Hemoglobin appeared as a yellow region. Fractions were tested by double diffusion against each antiserum. γ_{1M} was identified with a rabbit anti-mouse serum supplied by DR. RICHARD WISTAR.

diagrammatically in Figure 2. It can be seen that the antigenic serum substance sediments in a region below the albumin peak and above the gamma globulin area.

The precipitin line formed by RF anti B10–D2 with concentrated B10–D2 serum failed to stain with Sudan Black (URIEL and GRABAR 1956). It also failed to develop a positive reaction when incubated with α napthyl acetate (URIEL 1961). These results argue against the antigenic serum substance being a lipoprotein or an esterase.

DISCUSSION

By isoimmunization procedures a mouse antiserum that precipitates a mouse serum substance has been produced. A single locus concerned with the production of this substance has been demonstrated. We propose that the locus be designated *Sas-1* (Serum antigenic substance) and that the allele associated with a positive reaction be designated as *Sas-1*^{a}. Since no allelic product has been found, the designation *Sas-1*^{0} is proposed for the allele associated with a negative reaction.

No evidence relating *Sas-1* to the *Ig-1* or *Hc* systems has been obtained. In fact the genetic evidence, absence of cross reactivity on double diffusion, and differences in electrophoretic mobility all argue for independent systems.

The antigenic serum substance remains to be characterised. A more intensive search for allelic products also remains to be made.

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SUMMARY

An antigenic serum substance in mouse has been identified by an isoantiserum. It has been shown to be controlled by a single gene locus for which the symbol *Sas-1* is proposed. The serum substance migrates in the β region on agar electrophoresis and sediments slightly more rapidly than albumin in sucrose density gradient centrifugation. Evidence is presented that it is antigenically and genetically distinct from several other loci concerned with serum substances in mouse. Close linkage of *Sas-1* to the *A*, *C*, *Ig-1* and *Hc* loci can be ruled out.

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