Two Murine γ-Globulin Allotypic Specificities Identified by Ascitic Fluid Isoprecipitins and Determined by Allelic Genes. (28411)

SHELDON DRAY, ROSE LIEBERMAN, and HAROLD A. HOFFMAN
(Introduced by M. Landy)
Laboratories of Immunology and of Clinical Investigation, NIAID, and Laboratory Aids Branch, DRS, NIH, Bethesda, Md.

Serum proteins of individuals of the same species may be antigenic for other members of that species and yield precipitating antibodies(1-3). These isoantibodies identify allotypic (antigenic) specificities on protein molecules and can distinguish differences among serum proteins, not separable by other immunochemical criteria. In the rabbit, several of the allotypic specificities were found to be associated with 7S-γ-globulins(4-7).

Kelus and Moor-Jankowski(8) extended the study of γ-globulin allotypic specificities to mice by preparing an isoprecipitin in C57BL mice which identified an allotypic specificity in BALB/c mice. Wunderlich and Herzenberg(9), as well as Dubiski and Cinader(10), prepared isoprecipitins in BALB/c mice, which identified an allotypic specificity in the C57BL/6J mice.

This paper presents an immunochemical and genetic study of 2 murine γ-globulin allotypic specificities(11). The technique to induce ascites(12) is applied to the preparation of isoprecipitins so that much larger quantities of antibody would be obtainable from individual mice. Individual mice of 42 inbred strains and substrains, F_1 progeny, F_2 progeny, and progeny from the F_1 backcross to parent strains were tested for the presence or absence of these 2 allotypic specificities.

Materials and methods. Most strains of mice were obtained from the Laboratory Aids Branch, National Institutes of Health (N) and Roscoe B. Jackson Memorial Laboratory (J). Some strains were obtained from colonies of Dr.'s Andervont (An), Deringer (De), Heston (He), and Law (Lw) at Nat. Inst. Health. Backcross and F_2 progeny were produced in our own laboratory.

To induce ascites, 4 intraperitoneal injections, each containing 0.5 ml of an emulsion of equal parts of physiologic saline and incomplete Freund's adjuvant were given at 3-4-day intervals to each mouse(12). Eight to 10 weeks after the first injection, donor mice were selected as a source of allotypes (antigens) and recipient mice were selected for preparation of isoantibodies.

Donor mice with ascites were immunized intraperitoneally with a heat-killed suspension of *Salmonella typhi* to produce anti-*S. typhi* ascitic fluid. The anti-*S. typhi* ascitic fluid (0.1 ml) obtained from either individual mice of inbred strains or from the pooled samples of random bred strains was mixed with 20×10^9 packed typhoid bacilli to prepare agglutinates containing donor allotypes.

Recipient mice with ascites were injected intraperitoneally 3 times with the washed agglutinates at 3-4-day intervals followed by monthly boosters of similar amounts.

Ascitic fluids from recipient mice were tested for isoantibodies to allotypes in the sera and ascitic fluids of donor mice by the agar gel methods of single diffusion in tubes(13) and double diffusion in plates(14). The allotypes were characterized by immunoelectrophoresis(15). Absorption methods in agar gel plates were also used as a means of identification of allotypes(16).

Mice were bled from the infraorbital sinus. Progeny were at least 6 weeks of age before their sera were tested so as to minimize the possibility of the presence of maternal γ-globulins.

Results. Figure 1 is a photograph of immunoelectrophoretic plates showing the precipitin reactions of 2 isoimmune ascitic fluids with 2 different murine γ-globulin allotypes. The 2 allotypic specificities are designated Asa1 and Asa2. Figure 1 shows the reactions of anti-Asa1 (N240-C57BL/6N) and anti-Asa2 (N63-C57L/N) ascitic fluids with the electrophoresed normal murine sera containing the appropriate allotype. The position of the precipitin bands compared with those of the reference rabbit anti-mouse serum system indicates that the 2 allotypes are γ-globulins.

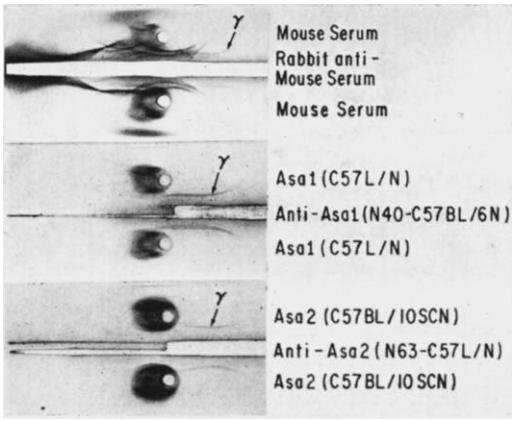

FIG. 1. Immunoelectrophoretic plate showing precipitin reactions of anti-Asa1 and anti-Asa2 with the γ-globulins of murine sera. A rabbit anti-mouse serum system is shown for reference.

Figure 2 is a photograph of a typical micro-Ouchterlony plate illustrating the criteria used in identifying each allotypic specificity. An immune ascitic fluid (+) and a reference ascitic fluid (*) are placed in alternate wells of the center row as shown in each diagram; the precipitin band between these alternate wells serve as reference bands. The top and bottom 6 wells of each diagram are filled with sera of normal mice to be tested. A mouse is considered to have the allotype if a precipitin band forms which coalesces with the reference band.

Specifically, in Fig. 2, two anti-Asa2 ascitic fluids, N65 (from a single C57L/N mouse) and N46 (from a single DBA/2N mouse) are compared in their reactions with the pooled sera from each of 36 inbred strains and substrains shown in Table I. The reference serum

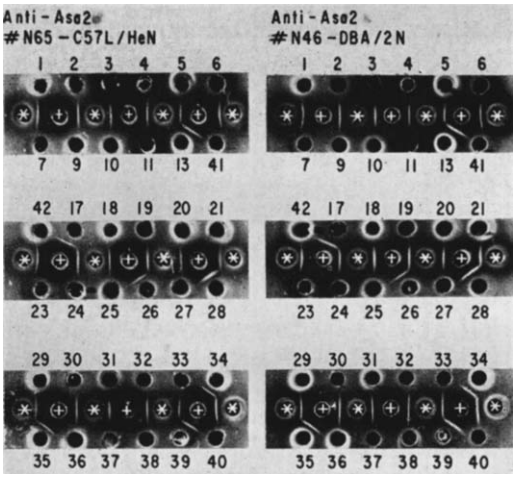

FIG. 2. Ouchterlony plate illustrating method used for identification of allotypic specificities in sera of inbred strains.

TABLE I. Presence of γ-Globulin Allotypic Specificities Asa1 or Asa2 in Sera of Individual Mice from 42 Inbred Strains and Substrains.

	Inbred strain	No. tested	Allotypic specificity Asa1	Asa2
1	A/HeN	11	+	—
2	AKR/N	10	+	—
3	AL/N	11	+	—
4	BALB/cAnN	9	+	—
5	BDP/J	10	+	—
6	BL/LyDe	13	+	—
7	BRSUNT/N	18	+	—
8	CBAf/Lw	6	+	—
9	CBA/J	15	+	—
10	CE/J	10	+	—
11	C3H/HeN	12	+	—
12	C3Hf/HeN	10	+	—
13	C57BL/He	7	—	+
14	C57BL/6J	20	—	+
15	C57BL/6N	10	—	+
16	C57BL/10ScN	4	—	+
17	C57BL/10H2dN	3	—	+
18	C57BR/cdJ	15	+	—
19	C57L/N	16	+	—
20	C58/J	15	+	—
21	DBA/1J	15	+	—
22	DBA/2J	20	+	—
23	DBA/2N	11	+	—
24	DD/He	12	+	—
25	DE/J	9	+	—
26	HR/De	12	—	+
27	I/An	11	+	—
28	LP/J	13	—	+
29	MA/J	10	+	—
30	NH/Lw	13	+	—
31	PL/J	9	+	—
32	RF/J	10	+	—
33	RIII/An	13	+	—
34	SJL/J	10	—	+
35	SM/J	9	—	+
36	ST/J	10	+	—
37	STOLI/Lw	7	+	—
38	STR/N	10	+	—
39	STR/1N	5	—	+
40	SWR/LyDe	10	+	—
41	YBR/He	10	+	—
42	129/J	15	+	—

{Substrains within a strain.

(*) was a C57BL/10ScN pool. In Fig. 2, the sera from strains 13, 17, 26, 28, 34, 35, and 39 (Table I) have allotypic specificity Asa2 while sera from other strains lack Asa2. Two other immune ascitic fluids N63 (C57L/N) and N52 (DBA/2N) as well as

TABLE II. Progeny Tests for Segregation and Assortment at the As_a Locus.

Cross	Total	As_a^1/As_a^1	As_a^1/As_a^2	As_a^2/As_a^2	Chi square	P value
C57BL/6 × DBA/2	20	0	20	0		
DBA/2 × B6D2F1*	58	23	35	0	2.48	.10-.20
male	29	8	21	0	3.53	.05-.10
female	29	15	14	0		
brown	33	13	20	0	.003	.95-.98
black	25	10	15	0		
dilute	23	8	15	0	.36	.50-.80
dense	35	15	20	0		
C57BL/6 × B6D2F1	56	0	28	28	.00	.99
male	33	0	16	17	.07	.50-.80
female	23	0	12	11		
B6D2F1 × B6D2F1	71	18	42	11	3.76	.10-.20

* B6D2F1 = F_1 Hybrid from C57BL/6 and DBA/2.

a pooled antiserum (BALB/c)* gave identical results (not shown).

Identification of the allotypic specificity Asa1 was established in a similar manner by immune ascitic fluid prepared in several C57BL/6N recipient mice.

Table I shows the results of testing the sera of individual mice from 42 inbred strains and substrains for the presence of allotypic specificities Asa1 and Asa2 with anti-Asa1 (N75-C57BL/6N) and with anti-Asa2 (N63-C57L/N) ascitic fluids. All sera from individuals of the same substrain exhibited one and the same allotypic specificity. This was also true for substrains within a strain except for STR/N and STR/1N; STR/N exhibited Asa1 and STR/1N exhibited Asa2. Pools of individual sera of each substrain when tested with the same immune ascitic fluids gave the same results as the sera from individual mice. The same results were obtained with other anti-Asa1 and anti-Asa2 ascitic fluids. The finding that each of 42 inbred colonies exhibited only one or the other allotypic specificity suggested that the Asa1 and Asa2 phenotypes are determined by allelic genes.

Table II shows the results of progeny tests for the segregation ratios of the allotypic specificities. All F_1 mice exhibited both specificities. The progeny of the backcross to each parental strain showed 1:1 segregation ratios (P = .10 - .20 and P = .99) indicating a one to one relationship of gene to allotypic specificity. The F_2 progeny showed a 1:2:1 segregation ratio (P = .10 - .20); none of the F_2 progeny lack both specificities. The F_1 and F_2 progeny data are consistent with the hypothesis that the allotypic specificities Asa1 and Asa2 are under the genetic control of a pair of codominant alleles. The presence of both Asa1 and Asa2 in the F_1 mice, all of which were males, suggests that the As_a locus is not sex-linked. That the As_a locus is not sex-linked is further indicated by the backcross progeny tests (P = .05 - .10 and P = .50 - .80). The backcross progeny tests also suggest independent assortment of the As_a locus with the black-brown locus on linkage group VIII (P = .95 - .98) and the dilute-dense locus on linkage group II (P = .50 - .80).

Discussion. Other investigators identified and tested for the presence of only one allotypic specificity in either four(8), eight(9), or 17(10) strains and substrains. Wherever similar strains of mice were tested for allotypic specificities by other laboratories and by our laboratory, the results of others corresponded with those for either Asa1 or Asa2 as follows: The γB^A (MuA1) of Kelus and Moor-Jankowski (3 strains compared) corresponded with Asa1; the allotypic specificity of Wunderlich and Herzenberg (8 strains compared) and the MuA2 of Dubiski and Cinader (15 strains compared) corresponded with Asa2. However, the correspondence of allotypic specificities identified in different laboratories requires confirmation by comparison of results in additional strains, by agar gel immunochemical analysis of the antibody reagents, and by further genetic studies.

* The pooled isoimmune serum prepared in BALB/c mice was obtained from Dr. Michael Potter, Nat. Inst. Health.

We have adopted a notation for allotypic specificities in mice similar to that employed for the rabbit(17); *As* is substituted for *A* as an abbreviation of *allotypic specificity* to avoid confusion with the *A* of the Agouti gene. The loci are designated by small letters; the allotypic specificities are designated by arabic numbers(17). The allelic genes at the *a* locus described in this paper are designated As_a^1 and As_a^2. We plan to use the prefix *Mu* to differentiate allotypic specificities in the mouse from those of other species. This is consistent with the notation MuA1 and MuA2 used by Dubiski and Cinader(10) for the allotypic specificities identified by them and by Kelus and Moor-Jankowski(8). Since it is of prime importance to adopt a uniform notation(17), we strongly urge that new allotypic specificities be given tentative notations until they can be compared with reagents from other laboratories and/or until the genetic control is known.

Although mice within an inbred strain were immunized in the identical way, not all of the mice showed precipitins by Oudin or Ouchterlony tests. In some mice, more than one precipitin band was observed. The ability to select individual mice with ascitic fluid exhibiting a single precipitin line was an advantage for accurate identification of the allotypes.

In contrast to the experience of other investigators who reported that massive immunization over long periods of time was required to produce isoantibodies in mice(8-10), we frequently found isoantibodies to allotypes as early as 3 weeks following the first injection of the mice with immunizing material. It remains to be determined whether this rapid response involves injection into the peritoneal site containing, as it does, ascitic fluid and a population of numerous mononuclear cells (18).

Since an inbred strain is established from a single pair of animals, differences or similarities between strains, depending on their common ancestry, would primarily be due to gene segregation in the original non-inbred stocks. This is further supported by consideration of the origin of the inbred strains and the distribution of allotypes among them. For example (Table I), the C57BL sublines all exhibit Asa2 whereas the A, BALB/c, CBA, C3H and DBA strains exhibit Asa1. The latter group of inbred strains have common origins and are not related to the C57BL substrains(19). The C57BL, C57BR and C58 strains were started from the same non-inbred stock whereas the C57L strain was established after the C57BR was inbred. Differences in allotypes among these strains are probably due to segregation of the As_a^1 and As_a^2 alleles in the non-inbred foundation stock. The HR/De, LP/J, SJL/J, and SM/J strains have no known association among themselves or with the C57BL substrains(20,21).

The STR/N and the STR/1N were the only substrains within a strain exhibiting different allotypic specificities. The STR/1N substrain presents the best example of a possible mutation at the As_a locus since the STR/1N subline was started after the STR/N strain completed 28 generations of brother by sister mating. The STR/1N was established because of a mutation at the piebald locus.

In the rabbit, 3 alleles at each of 2 loci have been found which determine γ-globulin allotypic specificities(17). Additional alleles and loci will probably be found in mice.

In many ways, the mouse is the most suitable species for the study of allotypy. Many genetic systems have been well characterized in this species, several inbred strains are readily obtainable, and mice bearing tumors are available for the study of protein synthesis. The usefulness of allotypic specificities as gene markers for the study of protein structure and as cell markers for cell transfer studies has already been demonstrated(22,23). With information obtained from the study of allotypy, new approaches are available for genetic studies in transplantation, antibody formation, maternal-fetal interaction and protein structure.

Conclusions. 1. Two murine γ-globulin allotypic specificities, Asa1 and Asa2 were identified by ascitic fluid isoprecipitins using agar gel immunochemical methods. 2. Only one allotypic specificity, either Asa1 or Asa2, was found in each of 42 inbred strains and substrains of mice. 3. The Asa1 and Asa2 allotypic specificities are inherited by codominant

autosomal alleles which are not closely linked with the brown-black locus (linkage group VIII) and the dilute-dense locus (linkage group II). 4. Substrain STR/N has the As_a^1 allele whereas STR/1N has the As_a^2 allele indicating the second known gene difference between these two substrains.

We gratefully acknowledge the technical assistance of Mr. William Humphrey, Jr.

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Received May 6, 1963. P.S.E.B.M., 1963, v113.

Anatomical Changes in Middle Ear and Cochlea of the Rat Following Stimulation with High Intensity Sound. (28412)

ROBERT I. HENKIN* (Introduced by F. C. Bartter)

Department of Anatomy, University of California School of Medicine, Los Angeles

High intensity sound is a potent stressor. As such it affects experimental animals by producing an increased secretion of adrenal cortical hormone(1-3). This effect might be mediated through (1) physical damage to the animal, *e.g.*, through trauma to the acoustical apparatus, (2) production of epileptiform seizures, and/or (3) activation of a complex neuroendocrine mechanism. This last interaction has been demonstrated in a previous study which showed that high intensity sound, like other stressors (immobilization, scalding) produces marked changes in the secretion rate of corticosterone in the rat(3,4). A marked increase of secretion is observed after 2 or 48 hours of sound exposure, while a marked decrease in corticosterone secretion is observed after 12 hours of exposure(3). This effect was noted without the occurrence of any type of seizure (3). As the increase of corticosterone secretion could be prevented by destruction of the middle ear bilaterally, it is clear that intense sound is effective only through the auditory apparatus(3). The present study was done to evaluate anatomical effects of high intensity sound on the middle ear and

* Present address: Nat. Inst. Health, Bethesda, Md.