

STUDY OF STRAIN-SPECIFIC ANTIGENS IN MICE OF STRAINS A AND C57BL

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Several authors have shown that homotransplantation of various tissues and organs can easily be accomplished in highly inbred animals. In noninbred animals, however, homotransplants are usually rejected. Certain facts shedding light on the mechanism of the rejection process have been established: the participation of the immune reaction of the organism in the rejection of homotransplants [2] has been shown. The survival of homotransplants in inbred animals has been attributed to the fact that there are no individual tissue antigenic differences between them. Hence, the differences between different strains must correspond to the individual antigenic differences in the tissues of noninbred animals.

Recently, many authors have found biochemical and antigenic differences in the tissues of different strains of inbred animals – in *Drosophila* [10,11], hens [6], and mice [3,4,5,7]. Using the agglutination reaction, Adler [3] established tissue antigenic differences in four highly inbred mice strains (BALB/c; C57B¹/₆; A/Jax; DBA²/₃). However, Adler's results give more evidence on the quantitative than the qualitative antigenic difference between these strains. At the same time, the data of Amos [4] on the isoimmunization of different strains of mice by blood cells, and the data of the same investigator and his colleagues [5] on skin grafts, indicates qualitative antigenic differences in different mice strains. Casperi [7] also showed the existence of qualitative biochemical, immunological, and cytological differences between inbred mice (C57BL and BALB). Recently, however, certain authors [1] have found no tissue antigenic differences between genetically heterogeneous inbred strains (A, CC57, C₃HA).

In view of the contradictory nature of the information communicated by different authors on this question, we decided to undertake a special study in order to discover the existence of strain-specific antigens in inbred mice.

Experimental Method

We used the anaphylaxis plus desensitization reaction on guinea pigs. Guinea pigs weighing 300-350 g each were sensitized subcutaneously by suspensions (20 mg each in first series of experiments, 40 mg each in second) of kidney, spleen, liver, skin tissues, or erythrocytes of highly inbred mice (C57BL and A). For the experiments we used only adult males, since, recently, information has been obtained which indicates immunological, biochemical, and cytological differences between males and females of inbred mice [7,9,12, and others]. It has also been shown that males of different strains (particularly C57BL and BALB) differ more than females of these strains.

On the 21st day after the sensitizing injection, all the animals of the first series of experiments were desensitized to species- and organ-specific antigens by subcutaneous and intraperitoneal injections of a saline

TABLE 1

Anaphylaxis Plus Desensitization Reaction in Guinea Pigs Sensitized by Suspensions of Tissues of Mice of One Strain and Desensitized by Extract of Same Tissues of Mice of Second Strain in Response to Injection of Shocking Dose of Tissue Extract from Mice of First Strain

No. of guinea pig	Sensitization (subcutaneous, 20 mg)	Desensitization (subcutaneous and intraperitoneal injections of 1000 mg of tissue extract)		Test for complete desensitization (intravenous injection of 1000 mg extract of corresponding tissues)			Shocking injection (intravenous injection of 1000 mg of tissue extract)	
	tissue antigens	tissue antigens	reaction	first reaction	second reaction	third reaction	tissue antigens	reaction
1	Kidney C57BL	Kidney A	+	++	—	.	Kidney C57BL	+
2	The same	The same	++	+	+	—	The same	±
3	The same	The same	+	—	.	.	The same	+
4	The same	The same	+	++	—	.	The same	±
15	Liver C57BL	Liver A	+	—	.	.	Liver C57BL	—
16	The same	The same	++	+++	++	—	The same	—
17	The same	The same	+	+	—	.	The same	—
18	The same	The same	+	++	+	.	The same	—
11	Spleen C57BL	Spleen A	+	+++	+	—	Spleen C57BL	—
12	The same	The same	++	+	—	.	The same	—
13	The same	The same	+	+++	+	—	The same	—
20	Erythrocytes C57BL	Erythrocytes A	+	+	—	.	Kidney C57BL	—
22	The same	The same	+	+	—	.	The same	—
32	The same	The same	++	—	.	.	The same	—
30	The same	The same	+	—	.	.	The same	—
21	The same	The same	++	—	.	.	Erythrocytes C57BL	—
24	The same	The same	++	—	.	.	The same	—
31	The same	The same	+	—	.	.	The same	—
23	The same	The same	++	—	.	.	The same	—
35	Kidney A	Kidney C57BL	+	++++	—	.	Kidney A	—
41	The same	The same	+	++	—	.	The same	—
42	The same	The same	++	++	—	.	The same	—
43	The same	The same	+	++	—	.	The same	—
19	Liver A	Liver C57BL	+	++	—	.	Liver A	—
34	The same	The same	++	—	.	.	The same	—
36	The same	The same	+	+++	+	—	The same	+
38	The same	The same	++	—	.	.	The same	±
26	Spleen A	Spleen C57BL	+	++++	—	—	Spleen A	—
25	The same	The same	+	++	+	—	The same	—
27	The same	The same	+	++	+	—	The same	—
29	The same	The same	++	++	++	—	The same	—

Note. ± indistinct symptoms of anaphylactic shock; + tremor, rubbing of nose and ears, ruffling of fur, dyspnea, slight drop in temperature; ++ the same symptoms, more pronounced, animal sneezes frequently; +++ the same symptoms, even more pronounced, convulsive leaps, coughing, lateral position, but animal survives; ++++ all symptoms very pronounced, animal dies; — no symptoms of anaphylactic shock; no injections made.

TABLE 2

Anaphylaxis Plus Desensitization Reaction in Guinea Pigs Sensitized by Suspensions of Tissues of Mice of One Strain and Desensitized by Liver Extract from Mice of Other Strain in Response to Injection of Shocking Dose of Liver Extract from Mice of First Strain

No. of guinea pig	Sensitization (subcutaneous, 40 mg)	Desensitization (subcutaneous and intraperitoneal injection of 1000 mg tissue extract)		Test for complete desensitization (intravenous injection 1000 mg extract of corr. tissues)		Shocking injection (intravenous injection 1000 mg tissue extract)	
	tissue antigens	tissue antigens	reaction	first reaction	second reaction	tissue antigens	reaction
45	Skin A	Liver C57BL	++	+++	—	Liver A	+
46	The same	The same	+	+	—	The same	+
47	The same	The same	++	±	—	The same	+
48	The same	The same	++	+++	—	The same	±
50	Erythrocytes A	The same	+	++	—	The same	±
51	The same	The same	++	±	—	The same	±
52	The same	The same	++	+++	—	The same	+
54	The same	The same	+	—	•	The same	—
55	The same	The same	++	±	—	The same	±
59	Kidney A	The same	+	—	•	The same	+
60	The same	The same	++	—	•	The same	+
61	The same	The same	+	—	•	The same	±
62	The same	The same	+	—	•	The same	+
57	Spleen A	The same	+	—	•	The same	±
58	The same	The same	++	—	•	The same	—
65	The same	The same	+	±	—	The same	+
66	The same	The same	+	—	•	The same	—
63	Skin C57BL	Liver A	+	++	—	Liver C57BL	+
64	The same	The same	++	++	—	The same	+
67	The same	The same	+	—	•	The same	+
68	The same	The same	+	++	—	The same	±
70	Erythrocytes C57BL	The same	++	—	•	The same	±
71	The same	The same	++	+++	—	The same	+
72	The same	The same	+	—	•	The same	+
73	The same	The same	++	±	—	The same	±
74	Kidney C57BL	The same	+	++	—	The same	++
75	The same	The same	++	—	•	The same	—
76	The same	The same	+	±	—	The same	±
77	The same	The same	++	+++	—	The same	++
78	Spleen C57BL	The same	++	—	•	The same	+
79	The same	The same	+	+	—	The same	+
80	The same	The same	+	±	—	The same	±

Note: Symbols as in Table 1.

extract of the same organs of mice of the other strain. In the second series of experiments, the guinea pigs were desensitized only to species-specific antigens by subcutaneous and intraperitoneal injections of saline extract of liver of mice of the other strain. On the following day we checked the completeness of desensitization (intravenously), and in the case of incomplete desensitization we conducted a second test. When desensitization was complete, we injected intravenously a shocking dose of antigen (saline extract of muscle tissues of the strain used for sensitization) after two hours. For the shocking injection in the first series of experiments we used antigens of the same organs which were used for immunization, and in the second series we used another organ (liver).

The extract was obtained *ex tempore* in the following way. The tissues washed free of blood were ground up with 9 volumes of physiological saline. The obtained suspension was centrifuged for 20 min at 3000 rpm, and the supernatant liquid was used as the extract.

Experimental Results

In the first series of experiments, all the guinea pigs sensitized by antigens of kidney, liver, spleen, or erythrocytes gave a strong anaphylactic reaction when desensitized by the same tissues of the other strain. It should be noted that we observed complete desensitization to species- and organ-specific antigens only after repeated injections of antigen (subcutaneous, intraperitoneal, and several intravenous injections). The majority of the animals showed no symptoms of anaphylactic shock in response to the injection of the shocking dose of antigen (Table 1). Only in three animals (Nos. 1, 3, 36) out of 31 did we observe a weak anaphylactic shock, which was rated as +; in the other animals (Nos. 2, 4, 38) the symptoms of anaphylactic shock were indistinct, and were rated \pm .

These results revealed no distinct antigenic differences between inbred mice of strains C57BL and A. However, taking into account the fact that desensitization to species- and organ-specific antigens sets in very slowly, after repeated injections accompanied by severe anaphylactic shock, we carried out a second series of experiments. In this series, the animals were desensitized only to species-specific antigens on the assumption that such an experiment would enable a clearer demonstration of antigenic strain differences. This assumption was based on the results of the first series of experiments, where some animals had responded to the injection of strain-specific antigens with a weak anaphylactic reaction. We surmized that, after prolonged desensitization to species- and organ-specific antigens, the sensitization to weak strain-specific antigens would be reduced to some extent, and the guinea pigs would not respond with a pronounced anaphylactic reaction to the injection of these antigens. Hence, in the second series of experiments, the animals were desensitized only to species-specific antigens, and organ-specific antigens were excluded. In addition, we doubled the sensitizing dose of antigen on the expectation that this would increase the sensitization to weak strain-specific antigens. The second series of experiments included the group of animals sensitized by skin antigens, since the studies of some authors have revealed antigenic differences when skin was grafted between inbred mice of one sex of different strains [5,8].

In the second series of experiments, the majority of the animals sensitized by antigens of kidney, spleen, erythrocytes, or skin, and after desensitization to species-specific antigens — by liver extract from mice the other strain, responded with an anaphylactic reaction to the injection of the shocking dose of antigen — liver extract from mice of the strain used for sensitization (Table 2).

In two animals (Nos. 77, 74) out of 32 the anaphylactic shock was rated as ++, in 15 as +, in 11 as \pm , and in 4 animals no symptoms of anaphylactic shock were observed. In animals sensitized by antigens of strain C57BL, the symptoms of anaphylactic shock in response to injection of the shocking dose were a little more pronounced than in animals sensitized by antigens of strain A. This might be due to the more complex composition of strain-specific antigens in mice of strain C57BL as compared with mice of strain A.

In addition, in our experiments we observed unequal sensitivity of guinea pigs sensitized to antigens of different organs. A rather more pronounced anaphylactic reaction was noted in guinea pigs sensitized by kidney antigens; a relatively weak reaction was observed in guinea pigs sensitized by antigens of skin, spleen, and erythrocytes. The results of these experiments showed that strain-specific antigens are apparently unevenly distributed quantitatively in different organs. This is in agreement with published information [3].

The results of the first series of experiments would appear to indicate the absence of antigenic differences between strains A and C57BL. This conclusion, in particular, was drawn by V. V. Gorodilova and L. V. Shersul'skaya [1]; the setup of their experiments on four groups of animals (15 guinea pigs) corresponded to the setup

of the first series of our experiments. However, the results of the second series of our experiments showed the presence of strain-specific antigens in mice of strains A and C57BL.

We attribute the difference in the results of these two series of experiments to the better sensitization of the animals to strain-specific antigens in the second series, owing to the larger sensitizing dose of antigen and the exclusion of the effect of organ-specific antigens on the results of the reaction.

Thus, as a result of these experiments, it has been established that the genetically different strains of mice C57BL and A differ qualitatively from one another in their antigenic properties as well. The tissues of inbred mice contain strain-specific antigens, which characterize the genetic homogeneity of each strain and demonstrate its qualitative difference from other strains.

SUMMARY

Antigenic strain-specific differences were shown in mice of two inbred strains (A and C57BL) by means of the anaphylaxis plus desensitization reaction on guinea pigs. Strain-specific antigens are present in various organs (kidneys, liver, spleen, skin) and in erythrocytes. They characterize the genetic antigenic uniformity of each inbred strain, and demonstrate its qualitative differences from the other ones. The antigenic strain-specificity of kidney and liver was more pronounced than that of the skin, spleen, and erythrocytes.

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