ANTIGENIC DIFFERENCES BETWEEN MALE AND FEMALE MICE OF THE INBRED C57BL LINE

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In 1955, Eichwald and Silmser [4] reported that skin grafts from males failed to take in females of the inbred lines A and C57BL of mice, yet grafts of skin from females took satisfactorily in males. These findings have been confirmed by many workers, and it has since been stated that this phenomenon is to some extent characteristic of most inbred lines of mice, and that the various lines differ from each other only in its degree [2, 3, 6, 12, 14, 17]. Several writers have postulated that the rejection of the male skin graft by the female of the same inbred line is due to their genetic and, consequently, antigenic differences, associated with the presence of the Y-chromosome in the female [5, 9, 17].

Homo- and isotransplantation experiments have shown that the localization of sex-linked antigens is not confined to the skin; they are also present in other mouse organs, especially the liver and spleen [15], thymus [10], and other tissues [7]. Sex-linked antigens have been shown to be very weak, weaker than antigens connected with loci H1, H2, and H3. They do not cause noticeable hemagglutinin formation, nor do they produce cytotoxic antibodies detectable by means of the complement fixation reaction [15]. Antigenic differences between females and males have also been described in experiments with Drosophila [8, 11].

Since much of the information concerning the existence of sex-linked antigens in mice has been obtained by means of methods involving homo- and isotransplantation of skin, we decided to make a special investigation to seek antigenic differences between male and female inbred mice detectable by a direct experiment.

EXPERIMENTAL METHOD

We used the anaphylaxis-with-desensitization reaction in guinea pigs. Animals weighing 300-350 g were sensitized by subcutaneous injection of a kidney tissue suspension (40 mg per animal) from adult male (series 1) or female (series 2) mice of the highly inbred line C57BL.

On the 21st day after the sensitizing injection, all the animals in series 1 were desensitized to species- and line-specific antigens (see table) by subcutaneous and intraperitoneal injection of a saline extract of female liver, and in series 2—of male liver. Next day, the completeness of desensitization was verified (intravenously), and if it was incomplete, a second trial was made. If desensitization was complete, 2 h later the reacting dose of antigen was given intravenously (a saline extract of the liver of a mouse of the same sex as that used for sensitization). The extract was prepared when required, as follows. Liver tissue was washed free from blood and homogenized with 9 volumes of physiological saline. The resulting suspension was centrifuged for 20 min at 3000 rpm and the supernatant fluid was used as extract.

EXPERIMENTAL RESULTS

In the first series of experiments 15 guinea pigs, sensitized by male kidney antigens, were desensitized to species- and line-specific antigens by injection of an extract of female liver tissue. In response to the injection of the reacting dose of antigen (extract of male liver) most guinea pigs showed signs of anaphylactic shock. In 4 of the 15 animals (see table, guinea pigs Nos. 3, 8, 11, 13) the signs of anaphylactic shock were assessed at ++, in 10 animals as +, and in only one animal (No. 9) as ±. The results of this series of experiments clearly demonstrated the presence of sex-linked antigens in the tissues of the males.

Anaphylaxis with Desensitization Reaction of Guinea Pigs

Animal No.	Desensitization (40 mg subcutaneously)	Desensitization (1000 mg of extract subcutaneously and intraperitoneally)		Test of completeness of desensitization (1000 mg of extract intravenously)		Reacting dose (1000 mg of extract intravenously)	
	tissue antigens	tissue antigens	reaction	first reaction	second reaction	tissue antigens	reaction
1	Kidney ♂ C57BL	Liver ♀ C57BL	++	++	–	Liver ♂ C57BL	+
2	The same	The same	++	+	–	The same	+
3	" "	" "	+	–	0	" "	++
4	" "	" "	++	+	–	" "	+
5	" "	" "	+	–	0	" "	+
6	" "	" "	++	+	–	" "	+
7	" "	" "	+	+	–	" "	+
8	" "	" "	++	–	0	" "	++
9	" "	" "	++	+	–	" "	±
10	" "	" "	+	–	0	"	+
11	" "	" "	++	–	0	" "	++
12	" "	" "	+	–	0	" "	+
13	" "	" "	++	+	–	" "	++
14	" "	" "	+	–	0	" "	+
15	" "	" "	++	–	0	" "	+
16	Kidney ♀ C57BL	Liver ♂ C57BL	++	+	–	Liver ♀ C57BL	±
17	The same	The same	+	+	–	The same	±
18	" "	" "	++	–	0	" "	–
19	" "	" "	++	–	0	" "	±
20	" "	" "	+	–	0	" "	+
21	" "	" "	++	+	–	" "	±
22	" "	" "	++	–	0	" "	+
23	" "	" "	++	+	–	" "	–
24	" "	" "	+	–	0	" "	+
25	" "	" "	++	+	–	" "	+
26	" "	" "	+	–	0	" "	±
27	" "	" "	++	–	0	" "	–
28	" "	" "	++	–	0	" "	+
29	" "	" "	+	+	–	" "	+
30	" "	" "	++	–	0	" "	±

Legend: ± vague signs of anaphylactic shock; + tremor, scratching of nose and ears, hair disheveled, dyspnea, slight fall of temperature, ++ the same signs but more marked, animal often sneezes; –no signs of anaphylactic shock; 0 no injection given.

In the second series of experiments 15 guinea pigs, sensitized with female kidney antigens, were desensitized to species- and line-specific antigens by injection of an extract of male liver tissue. In this series of experiments, in response to injection of the reacting dose of antigen (extract of female liver) most guinea pigs showed no clear signs of anaphylactic shock. In 6 of the 15 animals the signs of anaphylactic shock were assessed as +, in 6 as ±, and in the other three as –.

Consequently, the results of the second series of experiments showed that the tissues of female mice contain small amounts of specific antigens not present in male tissues. These very slight antigenic differences between the female and male tissues could be due to the hormonal peculiarities of the female. Meanwhile, the results of the

first series of experiments undoubtedly showed the presence in male tissues of specific antigens absent from female tissues, thus indicating a genetic difference between the male and female.

Our findings are in agreement with the results of numerous skin homo- and isotransplantation experiments. As we have mentioned above, in most cases the transplantation of skin from males to females is unsuccessful, while transplantation of female skin to males is more successful. XXY and XO females have been found among mice. It has been shown that grafts of male skin survive in XXY females and are rejected by XX females of the same inbred line [13]. These results confirm the hypothesis that the presence of sex-linked antigens in male tissues is associated with the presence of the Y-chromosome in males.

Hence our experiments have demonstrated the presence of sex-linked antigens in the tissues of male mice of the inbred C57BL line. At the same time, they also suggest that very weak specific antigens may also be present in females. Sex-linked antigens are weak antigens, and are similar to line-specific antigens in their immunogenic activity [1].

SUMMARY

A method of anaphylaxis with desensitization was used to show on guinea pigs the presence of sex-linked antigens in the kidney and liver tissues of male mice (C57BL inbred strain). In the majority of the guinea pigs there occurred anaphylactic shock symptoms in response to the injection of the antigen of male mice. Tissues of female mice caused no distinct anaphylactic shock symptoms in guinea pigs in the same experimental conditions. Since a number of guinea pigs exhibited a weak anaphylactic reaction evidently female tissues also contained antigens specific to the female organism, but in low quantities.

LITERATURE CITED

1. B. V. Konyukhov and D. M. Glukharev, Byull. Éksper. Biol. 1, 97 (1961).
2. S. E. Bernstein, A. A. Silvers, and W. K. Silvers, J. nat. Cancer Inst. 1958, v. 20, p. 577.
3. R. E. Billingham and W. K. Silvers, Science, 1958, v. 128, p. 780.
4. E. J. Eichwald and C. R. Silmser, Transplant. Bull. 1955, v. 2, p. 148.
5. E. J. Eichwald, C. R. Silmser, and N. Wheeler, Ann. N. Y. Acad. Sci. 1957, v. 64, p. 737.
6. E. J Eichwald, C. R. Silmser, and I. Weissman, J. nat. Cancer Inst. 1958, v. 20, p. 563.
7. M. Feldman, Transplant. Bull. 1958, v. 5, p. 15.
8. A. S. Fox, Transplant. Bull. 1956, v. 3, p. 131.
9. T. S. Hauschka, Transplant. Bull. 1955, v. 2, p. 154.
10. B. B. Hirsch, Transplant. Bull. 1957, v. 4, p. 58.
11. S. G. Levit, S. G. Ginsburg, V. S. Kalinin, and R. G. Feinberg, Nature, 1936, v. 138, p. 78.
12. T. Mariani, C. Martinez, J. M. Smith, et al., Proc. Soc. exp. Biol. (New York, 1958), v. 99, p. 287.
13. S. Ohno, W. D. Kaplan, and R. Kinosita, Exp. Cell. Res. 1959, v. 18, p. 382.
14. R. T. Prehn and J. Main, J. nat. Cancer Inst. 1956, v. 17, p. 35.
15. L. Sachs and E. Heller, J. nat. Cancer Inst. 1958, v. 20, p. 555.
16. B. F. Short and W. R. Sobey, Transplant. Bull. 1957, v. 5, p. 110.
17. G. D. Snell, Transplant. Bull. 1956, v. 3, p. 29.
