ANTIGENIC STRUCTURE OF LOCUS H-2 OF MICE BELONGING TO THE CC57BR AND CC57W STRAINS

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In our preceding study [1] we reported that mice of the CC57BR and CC57W strains have a genotype H-2^b. However the genetic test of the F_1 hybrids used in the investigation did not make it possible to elicit certain possible antigenic differences in the mice of these strains from the standard type H-2^b (C57BL/10Sn strain). As was indicated these differences can arise as a consequence of the origin of the CC57BR and CC57W mice from the hybrids BALB/c × C57BL or as a result of spontaneous mutation from the H-2 locus.

In the present study we used the serological method to elucidate the problem of the antigenic structure of locus H-2 of CC57BR and CC57W mice.

EXPERIMENTAL METHOD

As is known there are two methods for serological identification of antigens of the H-2 system of mice: 1) reciprocal immunization of the investigated strain and strains with a known set of H-2 antigens and determination of the antibodies in the obtained sera (absence of antibodies in the sera indicate an antigenic identity of the strains; the presence of antibodies in one or both sera proves that the studied strains have antigenic differences); 2) the production of antisera reacting with one or several determined antigens of the H-2 system, the exhaustion of these sera by tissues of the investigated and known strains, and determination of antibodies in the exhausted sera. Both methods were used in the present study.

In the experiment we used mice of the CC57BR strain of the 48th inbred generation, of the CC57W strain of the 48th inbred generation, and also mice of several inbred lines with a known H-2 genotype: A (H-2^b), BALB/cDe (H-2^b), C3H/Sn (H-2^k), C57BL/10 Sn (H-2^b). The antigenic structure of the mice of these strains was investigated in a number of works [2, 4, 5].

Immunization of the mice was carried out six times at week intervals by subcutaneous injection of live homologous cells of the spleen, an organ most rich in transplantation antigens [3], with 10 million cells per injection (4 times) and 20 million cells (2 times). The blood was sampled on the 19th day after the last injection, the serum was stored at −20°. The types of antisera used in the experiments are shown in Table 1.

The hemagglutination reaction in a dextran-human serum medium was set up by the Gorer and Mikulska method [4] with mouse erythrocytes of the donor strain, recipient strain (negative control), and A strain. The last were used in each experiment since it has been demonstrated that erythrocytes of A strain have a more complete set of isoantigens in comparison with erhtyrocytes of other strains and are a most sensitive object for hemagglutination [4]. In determining the hemagglutinins in the exhausted sera we set up a positive and negative control with erythrocytes of mice of strains having a corresponding H-2 genotype (see below).

For exhaustion, the antiserum, diluted 1:5 by a physiological salt solution, was mixed with a double volume of multiply washed precipitate of the liver homogenate of mice of the corresponding strains (0.2 ml of serum and 0.4 ml of precipitate), incubated 40 min at 37°, then 20 min at 4°, centrifuged 5 min at 2500 rpm, and the supernatant was used in further work.

TABLE 1. Immune Homologous Sera Used in Experiments

No. of serum	Strain of mice		Expected antigenic differences (see Table 4).
	Tissue donor	Recipient	
1	CC57BR	BALB/cDe	D^bEK^bV
2	Same	C57BL/10Sn	None
3	"	CC57W	"
4	CC57W	BALB/cDe	D^bEK^bV
5	Same	C57BL/10Sn	None
6	"	CC57BR	"
7	C57BL/10Sn	Same	"
8	Sarcoma Sa-1 of mice A	(C57Bl/10Sn x BALB/cDe) F_1	AKWY
9	Same	(C3H/Sn x C57BL/10Sn) F_1	DJM

TABLE 2. Results of the Reaction of Dextrin Hemagglutination of Immune Sera with Erythrocytes of Mice of Donor Strains and Strain A*

Immune sera		Erythrocytes of mice of strains			
		A	CC57BR	CC57W	C57BL/10Sn
BALB/cDe	Anti CC57BR	2,048†	8,192		
BALB/cDe	" CC57W	8,192		8,192	
CC57W	" CC57BR	None‡	None		
CC57BR	" CC57W	"		None	
C57BL/10Sn	" CC57BR	"	None		
C57BL/10Sn	" CC57W	"		None	
CC58BR	" C57BL/10Sn	"			None

*Reaction with erythrocytes of mice of the antiserum recipient strain was always negative.
†Denominator of antiserum titer.
‡Negative reaction with all antiserum dilutions starting with 1:4.

EXPERIMENTAL RESULTS

The hemagglutination reaction did not reveal any antibodies in the antisera of mice C57BL/10Sn immunized by spleen cells of mice CC57BR and CC57W (Table 2), which indicates the absence in these two strains of mice of any additional H-2 antigens in comparison with mice C57BL/10Sn ($H\text{-}2^b$). At the same time the sera of mice BALB/cDe $H\text{-}2^d$ immunized by spleen cells of mice CC57BR and CC57W contained antiboides that can be elicited in reactions with erythrocytes of mice of the donor strains and strain A in titers exceeding 1:8000.

The investigation of antisera of mice CC57BR immunized with spleen cells of mice CC57W and of mice CC57W immunized with cells of CC57BR showed the completer absence of antibodies in these antisera (see Table 2), which indicates the antigenic identity of mice of strains CC57BR and CC57W.

The antiserum of mice CC57BR immunized with spleen cells of mice C57BL/10Sn proved to be inactive in the reaction with erythrocytes of mice C57BR/10Sn and A. This experiment showed that mice of the CC57BR strain have all those antigens of the H-2 system which are present in the C57BL/10Sn mice. It is evident that CC57W mice also have all these antigens, since in a previous experiment their antigenic identity with CC57BR was elicited.

These data were also confirmed by experiments with exhaustion of sera No. 8 (anti AKWY) and No. 9 (anti DJM) by the liver of CC57BR and CC57W mice. As is apparent from Table 3, the liver of CC57BR and CC57W, just as of mice C57BL/10Sn (negative control), does not adsorb antibodies to antigens AKWY and DJM. At the same time the liver of BALB/cDe mice adsorbs only antibodies to DJM, and the liver of C3H/Sn mice adsorbs only the

TABLE 3. Results of the Dextran Hemagglutination of Immune Sera, Adsorbed by the Liver of Mice of Different Strains, with Erythrocytes of A Strain Mice

Immune sera	Antibodies to antigens	Non-adsorbed sera	Sera adsorbed by liver of mouse strains					
			A	BALB/cDe	C3H/Sn	C57BL/10Sn	CC57BR	CC57W
(C57BL/10Sn × BALB/cDe) F_1 Anti-A*	AKWY	10,240†	None‡	10,240	< 40	10,240	10,240	10,240
(C57BL/10Sn × C3H/Sn) F_1 Anti-A	DJM	10,240	"	40	10,240	10,240	10,240	10,240

*Immunization was performed with a sarcoma Sa-1 of the A strain

†Denominator of antiserum titer.

‡ Negative reaction with all antiserum dilutions beginning with 1:20.

TABLE 4. Antigenic Structure of Locus H-2 of the Mouse Strains Used in the Experiments*

Genotype	Strain	Antigens of locus H-2
H-2^a	A	A, C, D, E, F^1– H–^{2} J K M N–^{3} –^{4} R–^{5} – W Y Z A^1 B^1 C^1 –^{6}
H-2^b	C57Bl/10Sn†	– – D^bE, F – – – –K^b – N– – R– V – – Z A^1 B^1 C^1 –
H-2^d	BALB/cDe	– C D E^dF – H– –– M N– – R– – – – Z A^1 B^1 C^1 –
H-2^k	C2H/Sn	A C D^kE – – H– K – – – – –– – W Y Z – – – –

*The data are cited in conformity with results obtained by other authors [2, 5].

†Mice of the CC57BR and CC57W strains belong to this group; 1) Antigen c; 2) J; 3) P; 4) Q; 5) S; 6) D^1

antibodies to AKWY (positive control). Consequently, mice of the CC57BR and CC57W strains do not have antigens DJM and AKWY, which corresponds to the antigenic structure in the H-2^b genotype (Table 4).

Thus, the use of the genetic [1] and serological methods yielded similar results indicating that the mice of the CC57BR and CC57W strains have the genotype H-2^b and carry the corresponding antigens of the H-2 system.

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