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H-2 IMMUNOGENICITY OF LIVER CELL MEMBRANES IN THE MOUSE*

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Introduction. A recent report from this laboratory described the isolation of a lipoprotein (membrane) fraction from mouse liver which contained the bulk of the *H-2* serological activity of that organ (1). Brent, Medawer, and Ruskiewicz (2) and Davies and Hutchison (3) similarly reported the use of serological assays for *H-2* antigen purifications. Brent et al. reported that the material they obtained from spleen was capable of eliciting both a second-set homograft reaction and circulating isohemagglutinins (2). The following is a report on the immunogenicity of isolated cell membrane fractions from liver with respect to circulating hemagglutinin induction.

Materials and methods. Mouse strains. A/J ($H\text{-}2^a$), BAF_1, (C57BL/6J × A/J F_1, $H\text{-}2^b/H\text{-}2^a$), C57BL/6J ($H\text{-}2^b$), C3H. SW (C3H/Sn-$H\text{-}2^b$), DBA/2J ($H\text{-}2^d$), C3H/Sn ($H\text{-}2^k$).

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Preparation of membrane fraction. Liver membrane fraction was prepared from 5-month-old female BAF_1 mice according to the method already described (1) with the exception that the first supernatant was recentrifuged at 1100 × *g* for 5 min and the sediments recombined for further steps.

TABLE 1

*Immune response**

Animal No.	Hemagglutination Reactions at Indicated Log₂ Dilution of Antiserum										Reciprocal of Titer
	2	3	4	5	6	7	8	9	10	11	
3826	1†	2	3	2	2	2	2	2	3	2	>2048
3828	1	±	—	—	—	—	—	—	2§	—	4
3829	2	2	2	2	2	2	2	1	±	1	512
3830	—	—	—	—	±	—	—	±	—	—	<4
3831	2	2	2	1	2	2	1	2	1	±	1024
3832	1	2	2	2	1	1	—	1	±	±	128
3833	—	2	2	3	2	2	2	2	2	2	>2048
3834	—	—	—	—	—	—	—	—	—	—	<4
3835	—	1	2	3	2	1	2	1	1	±	1024

* Three month old, male and female, C3H/Sn mice were injected twice, 3 weeks apart, with 1–2 mg of BAF_1, antigen in one ml PBS. Sera tested against $H\text{-}2^a$ erythrocytes. The following hemagglutination controls were negative: Normal C3H/Sn serum and A/J erythrocytes, C3H anti BAF_1, antiserum and C3H/Sn erythrocytes.

† Numerical value in hemagglutination dilution indicates strength of agglutination as previously described (1).

§ False positives are occasionally seen.

Immunization. 3-month-old male and female C3H/Sn mice were given 2 ip injections, 3 weeks apart, of antigen suspended in one ml of phosphate buffer saline (PBS) (4). Two weeks after the second injection, individual fresh serum samples were collected and tested within 24 hours for hemagglutination titer.

Serological techniques. Hemagglutinins were determined by the method of Stimpfling (4) except that the incubation time was 60 min and the pH 6.5. Absorption and inhibition of hemagglutinin activity were as previously described (1) (5). Erythrocytes were prepared fresh daily as described (4) and resuspended to 2% in PBS.

Protein determination. By method of Lowry *et al* (6).

Experimental. Immune response. Two groups of C3H/Sn mice were challenged twice, 3 weeks apart, with a fresh preparation of the membrane fraction from BAF_1 liver. Each animal in Group I received 1.1 mg protein for the first injection and 1.6 mg protein for the second injection while each animal in Group II received 0.1 mg protein for the first injection and 0.08 mg protein for the second injection. (One to two mg protein is the approximate yield from 500 mg fresh weight of liver.) On day 14 after the second injection, sera of six of the nine animals in group I agglutinated $H\text{-}2^a$ erythrocytes (titers ranging from 1/128 to 1/2048, see Table 1). Sera from the remaining 3 animals in group I, as well as those (5) in group II (who received only 5–10% as much membrane fraction)

TABLE 2

Serological activity of membrane preparations

Preparation	Antiserum	Erythrocyte	Titer	Amount (in μg protein) sufficient:	
				To reduce by absorption the titer of 0.2 ml sera to 1/40*	To inhibit agglutination completely†
I	(4920C) $H\text{-}2^k$ anti-$H\text{-}2^a$	$H\text{-}2^a$	1/5120	560	‡
	(27H) $H\text{-}2^b$ anti-$H\text{-}2^d$	$H\text{-}2^a$	1/5120	‡	3.5
	(28) $H\text{-}2^d$ anti-$H\text{-}2^b$	$H\text{-}2^b$	1/5120	‡	12.5§
II	(46) $H\text{-}2^k$ anti-$H\text{-}2^a$	$H\text{-}2^a$	1/40960	2150	3.3
	(28) $H\text{-}2^d$ anti-$H\text{-}2^b$	$H\text{-}2^b$	1/5120	‡	25§

* Initial serum dilution 1/20 for absorption.

† Initial serum dilution 1/1000 for inhibition.

‡ Not tested.

§ Preparation used had been frozen and stored at −20°C.

failed to show any response. The results of group II accord with our experience that equivalent small doses of liver homogenate (25–50 mg) are also non-immunizing after 2 injections. None of the sera tested agglutinated $H\text{-}2^b$ erythrocytes although they might have been expected to do so as the membranes were from $H\text{-}2^a/H\text{-}2^b$ hybrids. Absorption studies with pooled serum from group I animals indicate that the only antibodies produced in detectable titer were those directed against antigenic components determined by the $H\text{-}2^a$ allele. Absorbing 0.20 ml of serum, diluted 1/20, with 100 mg (fresh weight) of $H\text{-}2^b$ (C57BL/6J and C3H.SW) liver failed to diminish the titer, whereas the same amount of $H\text{-}2^a$ (A/J) or $H\text{-}2^d$ (DBA/2J) liver removed all hemagglutinating activity. This is as expected since the known $H\text{-}2^a$ (A/J) components immunogenic in $H\text{-}2^k$ (C3H/Sn) mice are common to $H\text{-}2^d$ (DBA/2J) mice as well.

Serological activity of membrane preparation. The data in Table 2 show that the preparations of membranes used for immunization contained both $H\text{-}2^a$ and $H\text{-}2^b$ serological activity. Both preparations inhibited hemagglutination of $H\text{-}2^a$ and $H\text{-}2^b$ erythrocytes and absorbed hemagglutinins for $H\text{-}2^a$ erythrocytes (absorption of anti-$H\text{-}2^b$ antiserum was not done). The antiserum used to test $H\text{-}2^b$ activity had isoantibodies against $H\text{-}2^b$ components not removed by absorption *in vivo* in C3H/Sn mice. One cannot conclude that significantly different levels of serological activity of $H\text{-}2^a$ or $H\text{-}2^b$ are present in the two preparations, due to the different sera used for testing.

Summary and conclusions. A liver cell membrane (lipoprotein) fraction, showing in vitro antigenic activity, when injected twice at a dose of 1–2 mg protein (obtainable from 500 mg fresh liver) induces the formation of a high titer of agglutinating antibodies against A/J erythrocytes.

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