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SHORT COMMUNICATION

PASSIVE IMMUNIZATION OF MICE WITH RABBIT ANTISERA AGAINST CHIKUNGUNYA VIRUS AND ITS COMPONENTS

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In the previous work (Igarashi, Nithiuthai and Rojanasuphot, 1970), rabbits were immunized with antigens of purified, formalin-inactivated Chikungunya virus (ChV), with hemagglutinin (HA) extracted with Tween 80 and ether and the intracellular nucleoprotein core of ChV (X-component). Serological examination of the immune sera revealed that anti-HA and anti-ChV showed almost the same titers of hemagglutination-inhibition (HI) and neutralization in plaque reduction (N) tests, whereas anti-X gave scarcely any reactions in these tests, though it contained complement-fixing (CF) antibody. In this work, to see their protective effects on fatal encephalitis caused by intracerebral challenge with ChV, these three kinds of antisera were passively transferred to suckling mice, which are susceptible host to ChV.

Chikungunya virus (ChV), African strain, at the 175th passage level in suckling mouse brain was used as the challenge virus. A 10% (w/w) homogenate of infected brains was prepared in borate saline¹. Virus was diluted serially 10 fold with virus diluent². Volumes

of 0.01 ml of each dilution were inoculated intracerebrally into 1 to 2 day old suckling mice. The mice used were an inbred strain of C3H, which was kindly supplied by Dr. S. Tanabe, Department of Bacteriology, Medical School of Osaka University. Infected mice were observed for 2 weeks and the LD₅₀ titer of the virus was calculated by Reed and Muench's method (1938). The rabbit antisera used and their titers in serological reactoins were as reported previously (Igarashi, et al., 1970). Antisera were serially diluted 10 fold with virus diluent, and 0.05 ml volumes of each were administered intraperitoneally to suckling mice.

Table 1 shows that suckling mice were protected from fatal encephalitis caused by intracerebral challenge with ChV, by previous passive immunization of anti-HA or anti-Virus. Immunization with anti-X serum was without effect. This protective effect seemed to be only prophylactic, that is, the protective effect of the antiserum decreased with increase in the period between intracerebral inoculation of ChV and injection of antiserum, when antiserum was administrated later than ChV (Table 2). These results indicate that HA derived from purified ChV serves as a protective antigen in fatal encephalitis in mice caused by ChV infection, while the nucleoprotein core

1 Borate saline: 0.12 M NaCl, 0.05 M borate buffer, pH 9.

2 Virus diluent: 0.2% gelatin in YLE (0.1% yeast extract, 0.5% lactalbumin hydrolysate in Earle's balanced salt solution).

of ChV does not. Similar results were obtained with different lots of antisera and, or with adult mice.

Bose and Sagik (1970) reported that neutralizing antibodies were directed against antigens in the viral envelope in the Sindbis virus system. Eckels, Harrison and Hetrick (1970) showed that Chikungunya virus vaccines prepared by Tween 80 and ether inactivation of virus were as immunogenic as formalin-in-

activated vaccines judging by the results of serological and direct challenge tests in actively immunized mice. In consideration of the structural components of ChV (Igarashi, et al., 1970), these data support the idea that HA vaccine might be simpler than formalin-inactivated whole virus vaccine in the arbovirus system, as suggested before, because HA obtained by Tween 80-ether extraction followed by CsCl density gradient centrifugation con-

TABLE 1. *Protective effects of rabbit antisera against ChV and its components as revealed by passive immunization of suckling mice followed by intracerebral challenge with the virus*

Kind (lot)	Serum				Dead/Inoculated
	HI	N	CF ^a	Dilution	
Anti-X (No. 2)	<20	<10	64	10 ⁻⁰	7/8
Anti-HA (No. 6)	1280	10 ^{4.35}	256	10 ⁻⁰	0/8
				10 ⁻¹	0/8
				10 ⁻²	0/8
				10 ⁻³	8/8
Anti-virus (No. 8)	640	10 ^{4.36}	128	10 ⁻⁰	0/8
Control					14/14

One to 2 day old suckling mice were passively immunized by intraperitoneal administration of 0.05 ml of each serum. After 8 hr these immunized mice and control mice were challenged intracerebrally with 20 LD₅₀ of ChV. ^a Antigen was extracted from infected suckling mouse brains by the sucrose and acetone method (Clarke and Casals, 1958).

TABLE 2. *Influence of the time of serum administration of its protective effect against intracerebral challenge with ChV in suckling mice*

Time from challenge to serum administration hr	Dead/Inoculated
0	0/8
8	4/8
16	6/8
24	8/8

One to 2 day old suckling mice were intracerebrally inoculated with 120 LD₅₀ of ChV. After the intervals indicated, they were intraperitoneally administered with 0.05 ml of undiluted anti-HA serum (No. 6 in Table 1).

tains only one structural protein and is free from nucleoprotein core and possibly free also from membranes containing phospholipid.

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