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SHORT COMMUNICATION

IMMUNOLOGICAL PROPERTIES OF CHIKUNGUNYA VIRUS AND ITS COMPONENTS

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When the protein components of purified Chikungunya virus (ChV) were analyzed, two major structural proteins were found on electrophoresis in polyacrylamide gel containing sodium dodecylsulfate. One protein was associated with hemagglutinin (HAnin) and the other with the core of the ChV particle. When HAnin was obtained from purified ChV by Tween 80-ether treatment followed by CsCl density gradient centrifugation, it was shown to contain only the HAnin protein. While the intracellular nucleoprotein core, formerly named "X", contained only the core protein of ChV (Igarashi et al., 1970). The antibody responses to these structural components of ChV and purified virus were studied.

Purified virus, HAnin and the "X"-component were prepared as reported before (Igarashi et al., 1970). The purified virus was obtained by sucrose gradient sedimentation, it was diluted with PBS (Dulbecco and Vogt, 1954) and concentrated by ultracentrifugation. The precipitate was resuspended in PBS at a protein concentration of 400 μg protein/ml, determined by the method of Lowry et al. (1951). The protein contents of HAnin and the "X" component determined were found to be 150 μg/ml before dialysis against PBS (for HAnin) and KTM[3] (for "X") in a cold room overnight. Then the concentrated purified ChV and the two dialyzed components, were each mixed with an equal volume of 0.5 M glycine containing 0.02 % of gelatin. To samples of ChV and "X", formalin was added at a final concentration of 0.05 %, and the mixture was kept for 4 weeks in a refrigerator at 4 C before immunization. Before immunization, the resiudal infectivity of all antigens was checked by inoculation into BHK21 cell cultures. No infectivity was detected.

One ml of each antigen was emulsified with an equal volume of Freund's complete adjuvant and injected intramuscularly into 2 kg female rabbit.

A preimmune serum specimen was taken from each rabbit, and samples of blood were

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3 KTM: 0.01M KCl, 0.01M Tris-HCl, 0.0001 M $MgCl_2$, pH 7.6.

4 Virus diluent: 5% bovine serum in YLE (0.5% lactalbumin hydrolysate, 0.1% yeast extract in Earle's balanced salt solution).

removed one month later. These paired serum specimens from each rabbit were tested for antibodies against " X ", HAnin and ChV by the hemagglutination-inhibition (HI), complement fixation (CF) and neutralization (N) tests. The HI and CF tests were carried out according to the methods of Clarke and Casals (1958) and Hammon and Work (1964), modified for use in a microtiter system (Sever, 1962). The N test by the 50% plaque reduction method was performed as follows: ten fold dilutions of the test sera in virus diluent[4] were mixed with an equal volume of 200 PFU/0.2 ml of fresh ChV at 37 C for 90 min. The mixture was then inoculated onto monolayer cultures of VERO cells to detect plaques (Igarashi and Tuchinda, 1967). The plaque reduction rate was plotted on a probit chart (Finley, 1952) to estimate the serum dilution that reduced the plaque count to 50% of the control.

TABLE 1. *Serological tests on serum specimens taken from test rabbits before immunization.*

Rabbit No.	HI	N	CF (SMB[a])
2	<20	<10	<4
3	<20	<10	<4
5	<20	<10	<4
6	<20	<10	<4
7	<20	<10	<4
8	<20	<10	<4
9	<20	<10	<4

a The antigen was prepared from infected suckling mouse brains by sucrose-aceton extraction method of Clarke and Casals (1958).

The results of serological tests are shown in Tables 1 and 2. Preimmune sera had no detectable antibody in any rabbit (Table 1). The sera from rabbits immunized with the " X"-component showed no homologous antibody detectable by the HI, N and CF tests using HAnin as antigen, but they gave a positive CF reaction when antigen from infected suckling mouse brain or " X " was used.

The sera from rabbits immunized with HAnin showed a significant positive reaction by the HI, N and CF tests with HAnin or antigen from infected suckling mouse brain but their response against " X " was not so high in the CF test. Similar results were obtained when rabbits were immunized with formalin-treated purified ChV.

These results show that immunization with HAnin is sufficient to evoke an antibody response detectable by HI and N tests in the arbovirus system as in the influenza system (Webster and Laver, 1967).

Viral antigen prepared from infected suckling mouse brains reacted with the three types of immune sera, showing that the former con-

TABLE 2. *Serological tests on serum specimens taken from test rabbits one month after immunization.*

Immunogen	Rabbit No.	HI	N	CF[a]		
				SMB	HAnin	" X "
" X", formalin-treated	2	<20	<10	64	8	32
	3	<20	20	64	8	64
HAnin	5	640	$10^{4.35}$	128	128	8
	6	1280	$10^{4.01}$	256	256	16
Purified virus, formalin-treated	7	640	$10^{3.46}$	256	128	8
	8	640	$10^{4.36}$	256	128	8
	9	640	$10^{4.24}$	256	64	32

a Three kinds of antigens were used in the CF tests.

tained both HAnin and "X" antigens. This seems significant in connection with the cross reactivity of the arboviruses, because antigens from infected suckling mouse brains are routinely used in standard serological tests. Formalin-treated vaccines of group A arbovirus have been reported to be potentially dangerous because in practice it is rather difficult to inactivate the virus particles completely in this way (Sutton and Brooke, 1954: Smith et al., 1956). Thus, if a vaccine is developed using HAnin as antigen, especially a highly virulent arbovirus, it may be more reliable and effective than the formalin-treated virus vaccine because the HAnin is free from the viral nucleoprotein core.

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