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Comparative Immunogenicities of Chikungunya Vaccines Propagated in Monkey Kidney Monolayers and Chick Embryo Suspension Cultures

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A comparative study was made of Formalin-inactivated Chikungunya vaccines prepared from the virus propagated in African green monkey kidney monolayers and concentrated chick embryo suspension cultures. The vaccine prepared in the chick embryo suspension cultures was significantly more protective to mice against a live homologous virus challenge and stimulated the production of 4 to 5 times more circulating antibodies than the vaccine prepared with virus grown in African green monkey kidney monolayer cultures.

Chikungunya (CHIK) virus is a group A arbovirus which produces a denguelike febrile illness in man (12) and is widely disseminated throughout Africa and Asia (4, 8, 9, 11). The virus replicates in a variety of monolayer cultures (6-8, 10) and in at least one cell line in suspension cultures to high infective titers (1). CHIK vaccines that protect mice, guinea pigs, hamsters, and monkeys against live-virus challenge and produce neutralizing (N), hemagglutinating-inhibiting (HI) and complement-fixing (CF) antibodies have been produced in avian and mammalian cell cultures (2, 5). However, the vaccine produced from virus grown in chick embryo (CE) monolayer cultures was immunogenically weak due, at least in part, to low preinactivation infectivity and antigen titers (5). Thus, attempts were made to grow CHIK virus to high titers in concentrated CE suspension cultures for vaccine preparation, as has been done in this laboratory with Eastern equine encephalomyelitis virus (13). This report describes an immunogenical study of two Formalin-inactivated CHIK vaccines prepared from virus grown in African green monkey kidney (MK) monolayer cultures and in concentrated CE suspension cultures.

MATERIALS AND METHODS

African CHIK strain 168 was used in both vaccines. Lots of virus were propagated in MK monolayer cultures as previously described (2). Also, lots of virus were propagated in concentrated CE suspension cultures by the methods reported for Eastern equine virus (White et al., Arch. Gesamte Virusforch., **36**:13-17, 1972).

Vaccine preparation and potency assays. The vaccines from virus grown in MK monolayers and concentrated CE suspension cultures were prepared as previously described (2, 13). Potency assays of the vaccines were performed in 3- to 4-week-old Swiss Bagg strain male mice which were obtained from the Department of Laboratory Animals, Walter Reed Army Institute of Research, Washington, D.C. Vaccines were serially diluted, and groups of mice were inoculated intraperitoneally with 0.25 ml on days 0 and 7. All groups were challenged by the intracerebral route with a 100 to 1,000 mouse lethal dose$_{50}$ of the homologous strain of virus 7 days after the second vaccination. Effective dose$_{50}$ values were calculated by the method of probit analysis (3).

Antibody assays were performed on sera obtained from adult mice that had been given two 0.25-ml doses of vaccine intraperitoneally on days 0 and 7 and were bled 7 days after the last inoculation. The sera were tested for N, HI, and CF antibodies by methods previously described (13).

RESULTS

Pre- and postinactivation infectivity and antigenic activities of the vaccines are summarized in Table 1. As seen, the virus harvests from concentrated CE suspension cultures consisted of far greater numbers of infective and antigenic particles than the virus harvests from MK monolayer cultures. The differences in infectivity, hemagglutination, and CF activ-

TABLE 1. *Infectivity and antigenic activities of CHIK virus preparations before and after Formalin inactivation*

Source of virus	Infectivity[a]		HA[b]		CF[c]	
	Before	After	Before	After	Before	After
MK monolayer cultures	7.2	<0.1	64	<2	8	8
CE suspension cultures	11.0	<0.1	10,240	<2	128	128

[a] Mouse LD_{50} per ml.
[b] Reciprocal of highest antigen dilution agglutinating 0.4 ml of 0.25% goose red blood cells.
[c] Reciprocal of highest antigen dilution fixing 5 units of $complement_{50}$ when added to 0.3 ml of homologous antiserum.

TABLE 2. *Comparison of the two Formalin-inactivated CHIK vaccines as measured in mice*

Vaccines	ED_{50}[a]	HI[b]	CF[c]	N[d]
MK monolayer cultures	0.197	10	4	4
CE suspension cultures	0.044	40	32	32

[a] ED_{50} is the volume (in 0.5 ml) of vaccine protecting 50% of vaccinated mice against a lethal challenge.
[b] Reciprocal of the highest serum dilution inhibiting agglutination of 0.25% goose red blood cells by eight units of homologous antigen.
[c] Reciprocal of the highest serum dilution fixing 5 units of $complement_{50}$ when added to 0.3 ml of homologous antigen.
[d] Reciprocal of the highest serum dilution which protected 50% of the mice against 100 to 1,000 mouse LD_{50}.

ities were 10,000-, 160-, and 16-fold, respectively. It is apparent from the data presented in Table 2 that the vaccine propagated in CE suspension cultures is significantly more potent than the MK monolayer-propagated virus vaccine. Serological results were comparable to the potency results with the vaccine from CE suspension culture virus stimulating the production of 4 to 5 times more N, HI, and CF antibodies than the MK monolayer-grown virus vaccine.

DISCUSSION

From our study it may be concluded that a Formalin-inactivated vaccine prepared from virus propagated in concentrated CE suspension cultures was more effective in protecting mice against an intracerebral challenge than a vaccine prepared from virus propagated in MK cell monolayers. The differences in the immune response appear to be directly related to the quantitative difference in the physical mass of the virus populations, as shown by the preinactivation infective and antigenic activities. Thus, the use of concentrated CE suspension cultures provides readily available and inexpensive materials which support the growth of high-titered viral preparations that can be converted to potent vaccines and diagnostic antigens.

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