Method for Accelerated Identification of Arboviruses After Inoculation of Mice

TAM. S. DAVID-WEST

Virus Research Laboratory,† Faculty of Medicine, University of Ibadan, Ibadan, Nigeria

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Sequential titration of infective virus and complement-fixing antigen in brain and liver of suckling mice infected with the following virus strains—Dugbe (a new arbovirus), Congo (related to Crimean hemorrhagic fever virus), yellow fever, dengue 1 and dengue 2—showed a progressive increase in titer after infection. High titers of both infective virus and complement-fixing antigen were demonstrated long before the mice showed clinical signs of infection. It is suggested that earlier isolation and identification of arboviruses from clinical and field specimens can be made if serological tests are done before mice are moribund.

In the course of studies of some aspects of pathogenesis of selected arboviruses, it was consistently observed that the titer of infective virus and complement-fixing (CF) antigen in the organs of infected suckling mice increased progressively to high levels long before the onset of morbidity (4, 5). Furthermore, histopathological examination also showed advanced encephalitis in these mice, even though there were no obvious clinical signs. It is the usual practice in attempts at isolating virus from field samples or clinical specimens by mice inoculation to wait until there are clinical manifestations of infection in the animals before beginning identification studies. This report, based on further studies on sequential production of infective virus and CF antigen with five arboviruses of clinical interest, shows that identification time can be lessened considerably if morbidity is not used as the only criterion for selecting inoculated mice for serological tests.

MATERIALS AND METHODS

Viruses. Viruses used were Dugbe (IB AR 1792), a new arbovirus (2); Congo (IB AR 7620), antigenically similar to the virus of Crimean hemorrhagic fever (1, 3); yellow fever (strain 17D); dengue 1 (Hawaiian strain); and dengue 2 (Trinidad strain). The passage history of the viruses in mice were: Dugbe, 21; Congo, 39; yellow fever, 7; dengue 1, 44; dengue 2, 21.

Infection of mice. Two-day-old Swiss white mice were infected intracerebrally (ic) with 100 median lethal doses (LD50) of either virus strain. The inoculum in each case was mouse brain suspension prepared in 0.75% bovine albumin (BA), pH 7.0. Four cages of mice containing six litters were used per virus strain. At daily intervals after infection a specimen mouse was taken from each cage. The mice were given light chloroform anesthetic. The brain and liver were dissected out and accurately weighed. Ten per cent (w/v) organ suspension was prepared in BA and used without centrifugation for LD50 determination as well as for the titration of CF antigen. The inoculated mice were observed for 14 days, after which the LD50 titers were calculated by the method of Karber. The end point of CF activity was taken as the reciprocal of the highest dilution of organ suspension giving at least two plus (50%) fixation with 1:8 dilution of specific mouse ascitic fluid.

Mouse ascitic fluid. The method of preparation was that described by Tikasingh et al. (6).

CF test. The microtiter technique as modified by Weinbren (7) was used.

Histopathology. Freshly dissected brain and liver specimens were fixed in 10% Formalin and Bouin’s fluid respectively. Sections were stained with hematoxylin and eosin.

RESULTS

The results of sequential production of infective virus and CF antigen in both brain and liver for the five viruses studied are shown in Fig. 1 (Dugbe), Fig. 2 (Congo), Fig. 3 (yellow fever), Fig. 4 (dengue 1), and Fig. 5 (dengue 2). The pattern of progressive increase of virus titer and CF antigen titer was similar for all five viruses. For dengue 1, a CF titer of ½8 was demonstrated in the brain suspension on the first day postinfection. This may have been due largely to residual activity in the inoc-
ulm. However, for all the viruses there was a definite rise in CF antigen in the brain by the third day. The titers were greater than \( \frac{1}{2} \) in all cases by the third day, except for yellow fever, where the titer was \( \frac{1}{2} \). In all cases except dengue 1, the liver proved to be a poor source of CF antigen.

In the light of the objective of this report, it is significant to stress that none of the infected mice showed any clinical signs of infection before the fifth day after inoculation. The dengue 1-infected mice were not sick even on the sixth day, and although the experiment was in effect terminated on day 6, some of the mice of this group were kept under observation until the first clinical signs of infection. These were manifest by slight and irregular tremor on day 10, followed later by flaccid paralysis starting from the hind limbs. There was progressive difficulty of movement, and by day 12 all the mice were moribund.

Upon histological examination of the organs of mice sacrificed on the third day of infection, pathological manifestation of infection in either brain or liver was evident with each of the viruses studied. These took the following general forms. With Dugbe, the target organ was the brain, with diffuse encephalitis; some neurons showed lytic necrosis. The meninges was not significantly affected. With Congo, the brain showed diffuse encephalitis with perivascular cuffing in some of the vessels. The liver was not remarkably affected. With yellow fever, the lesion was confined to the brain. There was encephalitis; some of the leptomeningeal vessels were congested. With dengue 1, the most striking lesions were in the liver. There were several discrete foci of hematopoietic cells in the sinusoids; some megakaryocytes were also seen. These foci were definitely less numerous in control sections. Degeneration of parenchyma cells appeared in much later sections.

**Fig. 1.** Sequential production of infective virus and complement-fixing antigen in brain and liver of suckling mice after intracerebral infection with Dugbe virus. Note the first signs of morbidity on day 5 postinfection. Solid line, brain titers; broken line, liver titers.

**Fig. 2.** Same experimental conditions as Fig. 1, but with Congo virus. Note the first signs of morbidity on day 6 postinfection. Solid line, brain titers; broken line, liver titers.
DISCUSSION

Public health virology principally involves attempts at isolating viruses from clinical specimens or field samples and subsequent identification of these viruses. There are several methods and techniques towards this end, and the choice of any one of them depends on the type of virus tentatively suspected through informed guesses. Among arboviruses the suckling mouse is the experimental host par excellence and is usually infected intracerebrally with processed specimens. It is the usual practice in this exercise to keep the infected mice under observation until the manifestation of clinical signs before proceeding with identification serological tests. Brain or liver samples of the sick animals are often employed as sources of virus in these tests.

With the exception of some group A arboviruses, the period of primary incubation may be about 1 week (e.g., yellow fever) and up to 2 weeks or more (dengue). The results reported here, using five relatively long-incubating viruses, show that the time of establishing identification can be lessened considerably if morbidity is not used as the sole limiting consideration of infection. The sequential titration of infective virus and CF antigen in the brain and liver showed that positive identification of the viruses tested could be made as early as the third day postinfection. This meant that in some cases identification could be made twice as fast, and in the case of the dengue 1 used, even four times as fast. The histological examination also showed that the characteristic pathological manifestations were evident by the third day after infection.

One obvious objection in the application of the results of this report to day-to-day public
health virology is that mouse-adapted strains of virus were used in the study. But this may not necessarily be a serious setback on the following grounds. Firstly, the average survival time of the infected mice in the experiments reported here was approximately equal to that obtained with the "wild" strains of the different viruses. The dengue 1 strain (Fig. 4) demonstrated this even better. The mice were not sick until day 12 after inoculation, but positive identification was possible as early as day 3. Secondly, similar patterns have been previously reported for a "wild" strain of yellow fever virus (5).

Instead of waiting until mice are moribund as in present routine methods, it is suggested that a pilot CF test be performed on the brain suspension of mice on the third day after inoculation with specimens under test. Since most arboviruses conform to the pattern of infection as reported here, it is conceivable that this method of serological identification might be applicable to most arbovirus infections.

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LITERATURE CITED