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CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS IN IRAQ: ISOLATION, IDENTIFICATION AND ELECTRON MICROSCOPY

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Summary. — Crimean-Congo haemorrhagic fever (CCHF) virus was isolated for the first time in Iraq from the blood of three patients. It caused a cytopathic effect in lamb kidney and BHK-21 cell cultures. The virus particles were spherical, enveloped and had 90 nm in diameter; similar particles were found in ultrathin sections of the liver from two fatal cases. The isolated virus proved to be antigenically closely related to CCHF virus.

Key words: Crimean-Congo haemorrhagic fever; serology; electron microscopy

Introduction

In 1944, Crimean haemorrhagic fever was recognized in the Crimean peninsula in the U.S.S.R. This syndrome was described also in other areas of the Soviet Union (Chumakov *et al.*, 1968, 1970). The virus causing Crimean haemorrhagic fever was later shown to be antigenically and biologically closely related to Congo haemorrhagic fever virus which was first isolated in Congo and Zair (Simpson *et al.*, 1967; Casals, 1969). The disease was also reported from Bulgaria and Pakistan (Begum *et al.*, 1970). Antibodies against this virus were reported in man and among different animal species in Iran (Saidi *et al.*, 1975).

Sporadic cases of previously unreported viral haemorrhagic fever were recently reported in Baghdad, Iraq (WHO, 1979*a*, *b*). We are describing the results of isolation and identification of the causative virus.

Materials and Methods

Infectious materials. Whole blood was collected during the acute stage of the disease. Post mortem material (liver and brain) was collected from two fatal cases and used for virus isolation and electron microscopy.

Cell cultures. Lamb kidney, bovine thymus, HeLa and BHK-21 cell lines, and primary cultures of lamb kidney and testis cells and chick embryo cells (CEC) were used for virus isolation. Cells

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were grown in medium 199 supplemented with 10 % heat-inactivated foetal calf serum in 75 cm^2 plastic flasks.

Laboratory animals. Two-three-day-old suckling mice used for virus isolation were inoculated intracerebrally (i.c.) and/or intraperitoneally (i.p.) with 00.3 and 0.1 ml respectively, and kept under observation for 15 days. In some experiments, blind i.c. mouse passages were carried out.

Electron microscopy. Infected cell cultures with prominent cytopathic effect (CPE) at 10 days post inoculation (p.i.) were examined. Cell debris was removed by low speed centrifugation and the virus-containing supernatant was centrifuged at 30,000 rev/min for 2 hr in a Ti 60 rotor. The virus pellet was resuspended in 1/50 of the original volume in phosphate buffered saline, pH 7.2, and negatively stained with 2 % phosphotungstic acid. Ultrathin sections from post mortem liver and brain materials were stained with uranyl acetate and lead citrate and examined in a Phillips 300 electron microscope at an accelerating voltage of 80 kV.

The haemagglutinating activity of the isolate was tested against one-day-old chick red blood cell. at pH 7.2 and 37 °C.

Antiserum against Congo haemorrhagic fever virus strain 3010 was kindly supplied by Dr. D. Simpson, Center for Applied Microbiology and Research, Porton, England.

Complement fixation (CF) test. Antigens prepared from infected LK or primary lamb cell cultures by three cycles of freezing and thawing followed by clarification at 2000 rev/min for 20 min at 4 °C were used. The CF test was also used for the detection of antibodies against CCHF in patients and their relatives and contacts. The microtechnique was employed.

Results

Virus isolation in cell cultures was attempted from the plasma of three patients during the acute stage of the disease. Virus growth in the LK cell line and primary lamb kidney cell culture induced a CPE appearing on the 5th day p.i. Foci of cell rounding, cytoplasmic strainding, increased granularity and refractility of cells were noticed. The CPE progressed till the 11th day p.i. (Figs 1 and 2) when about 60 % of the cells were destroyed. The period required for the appearance of the CPE was shortened in the second passage of the virus, when the destruction of the monolayer was prominent 72 hr p.i. Fixed and haematoxylin-and-eosin-stained cell culture preparations showed intranuclear and cytoplasmic inclusions surrounded by a clear hallo (Fig. 3).

Neither virus growth nor a CPE was detected in the infected cultures of bovine embryo thymus cell line and primary cultures of CEC and lamb testis cells. Weak growth of the virus was observed in the BHK-21 cell line.

Virus isolation in animals was attempted with plasma and liver of patients. The virus induced fatal illness in 1—3 days old suckling mice. The inoculated animals appeared sluggish 48 hr p.i. Later they developed signs of nervous disorders, paralysis of the tail and hind limbs and enlargement of the liver; they died by the 4th—5th day p.i.

The isolated virus failed to agglutinate one-day-old chick red blood cells.

Negative staining of the virus pelleted from cell culture fluid revealed enveloped spherical virions measuring about 90 nm in diameter. Virus-like particles were detected in ultrathin sections of liver from three patients. Rounded particles measuring 40—50 nm in diameter were observed in the cytoplasm of the hepatocytes. Few particles with an electron-dense core were noticed.

The virus isolated in lamb kidney cells gave a positive reaction in the CF test with reference anti-Congo serum.

Sera from four recovered patients and 196 sera from their relatives, contacts as well as members of the virology laboratory team and other hospital staff were examined by the CF test. Examination of acute and convalescent sera of the four patients revealed a significant rise in antibody titres. Titres of 256 were recorded. Of the 196 other sera tested, 16 had significant titres (8—128) of CF antibodies against the isolated virus. One of the positive sera was from a shepherd with no previous contact with any of the recorded cases.

Discussion

The haemorrhagic fever syndrome was reported for the first in Iraq in 1979 (WHO, 1979*a*, *b*). The causative agent of the disease was now isolated in Baghdad, Iraq, in suckling mice in which it induced a fatal illness 4—5 days p.i. behaving in this respect similarly to viruses belonging to the CCHF group (Smirnova, 1979).

The virus was also successfully isolated in cell cultures. Lamb kidney cells were the most susceptible among the cultures tested. Although the virus grew in both LK and BHK-21 cells, the most prominent CPE was seen in LK cells. This might suggest that sheep in Iraq are the natural host of this virus as substantiated by the case history of the patients most of whom have had contact with sheep. Direct man-to-man transmission of the disease was noticed in three hospital attendants during the outbreaks in the autumn of 1979 and the spring of 1980.

Electron microscopy of the isolate revealed enveloped particles 90 nm in diameter, which is consistent with the known morphology of *Bunyaviridae*.

Results of the CF test revealed that the isolate is antigenically closely related to, if not identical with the Crimean-Congo haemorrhagic fever virus.

A rise in CF antibody titer of 4-fold or more was demonstrated in paired sera of recovered patients. This result together with the isolation and identification of the virus are indicative that the isolated virus was the aetiologic agent of the disease. A seroconversion in some of the contacts including laboratory and hospital staff is indicative of subclinical infection. It is suggested to designate this isolate as the "Yarmouk" strain of CCHF virus.

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Explanation of Micrographs (Plate XXXV):

Fig. 1. Lamb kidney cells showing a CPE 10 days p.i. with the isolated virus. 40×.
Fig. 2. BHK-21 cells showing cell rounding 5 days p.i. with the isolated virus. 40×.
Fig. 3. Lamb kidney cells showing intranuclear inclusions surrounded by a clear hallo with margination of the chromatin.
Haematoxylin and eosin, 160×.