J Arch Virol (1992) 127: 399–402

Archives of Virology

Hepatitis E virus in cultivated cells

Brief Report

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Accepted February 19, 1992

Summary. Hepatitis E virus (RNA and antigen) was detected in serial passages of FRhK-4 cells after they had been co-cultivated with primary kidney cells derived from cynomolgus monkeys experimentally infected with this virus.

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Hepatitis E virus (HEV) is the etiological agent of enterically transmitted human hepatitis E, acute jaundice disease widely spread in many tropical countries [2, 4]. Under laboratory conditions the infection has been reproduced in several species of non-human primates after inoculation with HEV-containing clinical specimens [1, 5, 8, 11]. Numerous attempts to grow this virus in cultivated cells using conventional cell culture inoculation techniques have so far proved unsuccessful. In this paper we report the establishment of cell lines which harbour the HEV.

The experiments were performed as follows: (1) Cynomolgus monkeys (*Macaca fascicularis*) were inoculated with the HEV (2598 Osh strain originated from Soviet Central Asia) as described earlier [1, 3]. (2) As soon as the levels of serum alanine aminotransferase became pathologically elevated the animals were sacrified; the kidneys were removed aseptically and used for preparation of primary cell monolayers following the usual procedure [6]. The cells were cultivated for 14 days at 37 °C in 500 ml flat glass flasks in 0.5% lactalbumin hydrolysate medium supplemented with 15% of fetal calf serum (Flow). (3) The primary cells were sub-passaged 1–2 times in the Eagle's MEM with 10% of fetal calf serum at 7–8 day intervals. (4) These cells were allowed to proliferate until sub-confluent monolayers had been formed, then the medium was decanted and a suspension of fresh fetal rhesus monkey kidney (FRhK-4) cells [13]

(2×10^6 cells per flask, ratio approximately 1 : 1) was added. Mixed cultures were further cultivated in Eagle's MEM with 10% of fetal calf serum at 37 °C. (5) Additional 7–9 passages were made reaching the total passage number of 11–13, the cells being transferred every 7–8 days.

In order to detect the HEV RNA total RNA from the harvested cells (approximately 5×10^6 cells per sample) were extracted with guanidine-phenol essentially as in [10], spotted onto the Hybond N membranes (Amersham), and hybridized with the ET 1.1 probe [9]. This probe was obtained from the ET 1.1 HEV cDNA (a kind gift of Dr. G. Reyes, GeneLabs Inc., Redwood City, U.S.A) by radiolabelling with Nick Translation Kit N.5000 (Amersham). Hybridization was conducted overnight (50% formamide, 42 °C) according to the instructions of membrane manufacturer.

For the detection in HEV antigen the cells grown on glass coverslips for 3–4 days were fixed with ice-cold acetone and stained with FITC-conjugated anti-HEV antibodies in humid chamber. Alternatively, an indirect fluorescent test was employed using commercial anti-human IgG FITC conjugate (Sigma). Hepatitis E convalescent serum ("Fausta") from the Mexican hepatitis E outbreak [12], kindly provided by Dr. D. Bradley (CDC, Atlanta, U.S.A.), was used throughout the study as a source of anti-HEV antibodies. FRhK-4 cells either non-infected or infected with hepatitis A virus (HAV) were taken as negative controls.

Altogether four cell lines carrying HEV RNA and antigen were obtained (Table 1). Neither primary cell cultures from non-infected monkeys nor non-infected FRhK-4 cells cultivated in similar conditions demonstrated the presence of substances capable of reacting with ET 1.1 HEV cDNA probe or anti-HEV

Table 1. Detection of HEV antigen and RNA in serial passages of infected cell cultures

Cell substrate	Total passage number	1944/F line		2954/F line		5/F line		16/F line	
		antigen[a]	RNA[b]	antigen	RNA	antigen	RNA	antigen	RNA
Trypsinized kidney cells	0	nd	+	nd	+	nd	nd	nd	nd
Primary culture	1	nd	nd	nd	nd	nd	nd	−	++
Sub-culture	2–3	+	++	+	++	+	+++	+	nd
Sub-culture + FRhK-4	3–4	+	++	nd	nd	+	nd	nd	+
	5–7	nd	nd	+	−	nd	+	+	+
	8–10	+	++	+	+++	+	+	−	++
	11–13	+	+	+	+++	nd	+	+	nd

[a] Presence (+) or absence (−) of HEV antigen in cells as detected by immunofluorescence

[b] Number of crosses corresponds to the intensity of hybridization signal

nd Not done

antibodies. The cDNA probes used for detection of HEV in cell cultures did not react with as much as 100 pg of HAV RNA under identical conditions. The hybridization signals from a number of infected cell samples were significantly higher as compared with the negative controls (Fig. 1). Granular immunofluorescence in the HEV-containing cultivated cells (Fig. 2) was similar to that observed in hepatocytes of HEV-infected cynomolgus monkeys [3, 7], particularly in respect to the size of granules and their intracellular localization.

The detection of HEV in primary cell cultures as well as in pre-cultivated trypsinized kidney cells provides convincing evidence that the HEV found in cultivated cells did originate from the infected monkey's organ.

In conclusion, the persistence of HEV RNA and antigen in serially passaged FRhK-4 cells after they had been co-cultivated with primary kidney cells derived from HEV-infected monkeys can be considered as an evidence for virus replication, taking into account that the original HEV from monkey's kidneys should become undetectable after 3–4 passages due to the serial dilutions in the cell transfer procedure. It is believed that the co-cultivation of HEV-carrying primary kidney cells with fresh FRhK-4 cells is a critical step in adaptation of HEV to grow in a continuous cell line, though the intrinsic mechanism of this process remains obscure. Experiments are currently in progress to determine whether the HEV grown in tissue culture remains capable of producing hepatitis in monkeys.

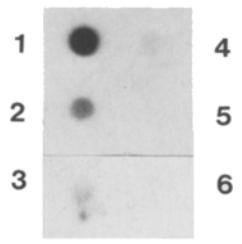

Fig. 1

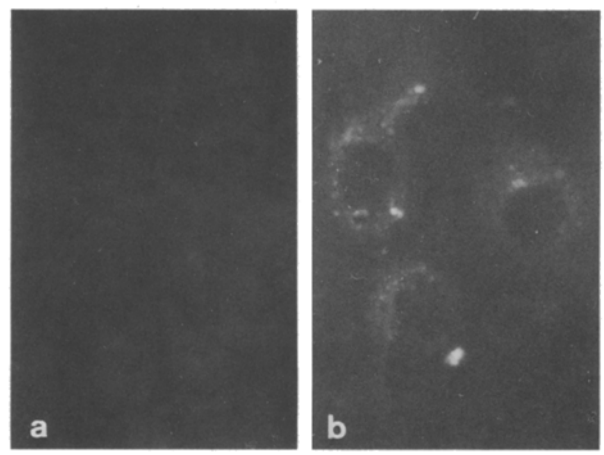

Fig. 2

Fig. 1. Dot blot hybridization with ET 1.1 HEV cDNA probe. Positive controls: ET 1.1 DNA, 100 pg (*1*), 10 pg (*2*), and 1 pg (*4*); negative controls: HAV RNA, 100 pg (*5*), and RNA from non-infected FRhK-4 cells (*6*); RNA from 16/F cells (Table 1), passage 10, 7 days after transfer (*3*)

Fig. 2. Granular immunofluorescence of HEV antigen in cytoplasm of cultivated cells: direct staining with FITC-labelled "Fausta" serum. **a** Non-infected FRhK-4 cell culture as a negative control. × 20. **b** 16/F culture (Table 1), passage 6, 3 days after transfer. × 90

Acknowledgements

This study received financial support from the WHO Programme for Vaccine Development.

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Received October 16, 1991