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ADAPTATION OF HUMAN ROTAVIRUS TO CELL CULTURE AND CHARACTERIZATION OF THE ISOLATED STRAIN

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Summary. – Human rotavirus strain 649 was isolated from a child with clinical picture of acute intestinal infection in the West European part of the U.S.S.R. This strain was adapted to MA-104 (rhesus monkey kidney) cells. By electrophoresis and haemagglutination it differed from the human rotavirus strain Wa and from the simian strain Sa-11. The productive reproduction of human rotavirus strain 649 may allow its use for the preparation of antigens for diagnostic, immunoprophylaxis, and treatment.

Key words: human rotavirus; cell culture; biological properties

Rotavirus infections are widespread in children, that is why the development of diagnostic methods for prophylactic and treatment are so essential. As rotaviruses are markedly heterogeneic, obviously the most effective approach will be to use strains pathogenic for man and endemic for certain areas. However, the difficulties connected with human rotavirus cultivation *in vitro* hamper such developments. Therefore, we aimed at adaptation to cell culture of some human rotavirus (HRV) strains circulating in the western areas of the European part of the U.S.S.R.

Detection of HRV in faeces and cell cultures was performed by enzyme immunoassay (EIA), counter immunoelectroosmophoresis (CIEOP) and electron microscopy (EM) with domestic diagnostic substances (Gudkov *et al.*, 1985). Cell cultures were inoculated with freon-113 or chlorophorm-treated rotavirus-containing faecal suspensions clarified by low speed centrifugation.

Virus adaptation was carried out in the continuous cell line MA-104 (rhesus monkey embryonic kidney) provided by the Belorussian Institute of Epidemiology and Microbiology. Four-days cell culture monolayer was grown in penicillin flasks containing medium 199 supplemented with 10% foetal calf serum. Before inoculation the virus-containing faeces suspension was pretreated with trypsin (5–15 μm/ml for 30 min) and centrifuged in an angle rotor (at 1000 g for 1 hr). The supernatant was removed and serum-free medium (equal parts of medium 199 and Eagle's medium) containing trypsin (2.5 μm/ml) was added. The infected cells were incubated at 37 ^{0}C in rolling flasks ant the CPE was determined or the results there checked by EM and EIA.

Each virus-containing faecal sample underwent no less than 5 successive passages under development of maximal CPE within 4–5 days. The virus was cultured in 0.5 l-roller flasks under the same conditions. To enhance virus release (at damage of 90–100% cells) the content of the flasks was frozen and thawed 3 times, cell debris was removed by low speed centrifugation. If necessary,

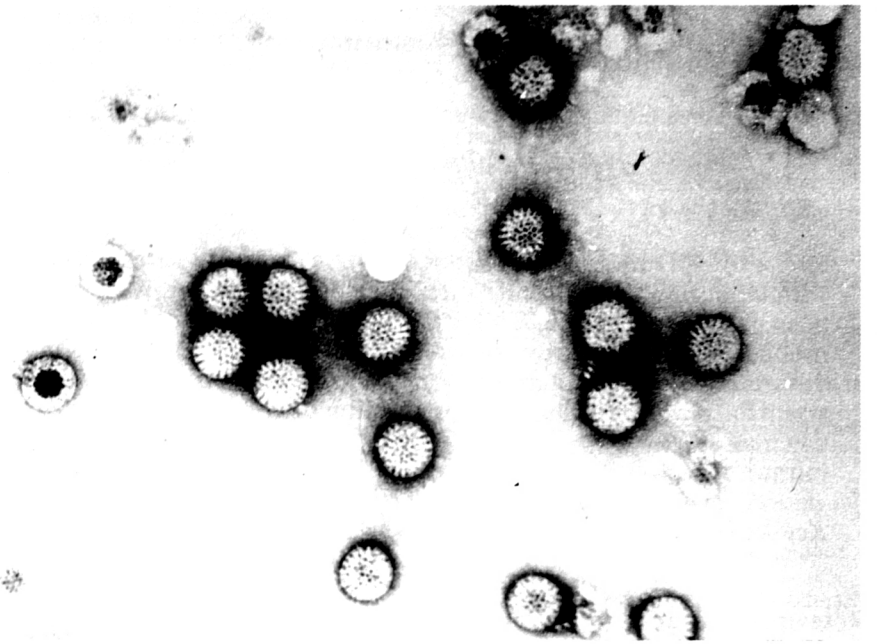

Fig. 1
Rotavirus virions. Human rotavirus 649 strain (negative contrast), x 90 000.

the virus was concentrated by ultracentrifugation (UCP-65, RPU-35 rotor at 30,000 rev/min for 2 hr) or by precipitation with 10% polyethylenglycol (mol. mass 6 000–8 000 for 18 hr at 4 ^{0}C) with subsequent centrifugation (8 000 g for 1 hr).

Immune serum to simian rotavirus SA-11 and to the isolate of HRV were produced by 3–4-fold immunization of guinea pigs and rabbits using the Freund's adjuvant.

RNA from purified and concentrated virus suspension was prepared in 2 main stages: disintegration of viral particles with 1% solution of dodecylsulphate and deproteinization of ribonucleoprotein by successive RNA extraction with water-saturated phenol, chlorophorm, and ether. The RNA obtained was stored -20 ^{0}C until electrophoresis.

Rotavirus RNA fragments were separated by electrophoresis in 7.5% polyacrylamide gel in the discontinuous system of Laemmli (1970), using 10 μA current for 16 hr. The gel was stained with silver nitrate solution according to the standard procedure (Dolan *et al.*, 1985).

Selection of rotavirus-containing faeces was performed by examination of children hospitalized with clinical signs of acute intestinal infection at Minsk infectious hospital in January-February 1986. By means of CIEOP and EM rotaviruses were discovered in 14 out of 46 samples investigated.

Twelve rotavirus strains were cell-adapted during 6 successive passages. All the strains grew in MA-104 cells in the first passage, 4 of them – in the second

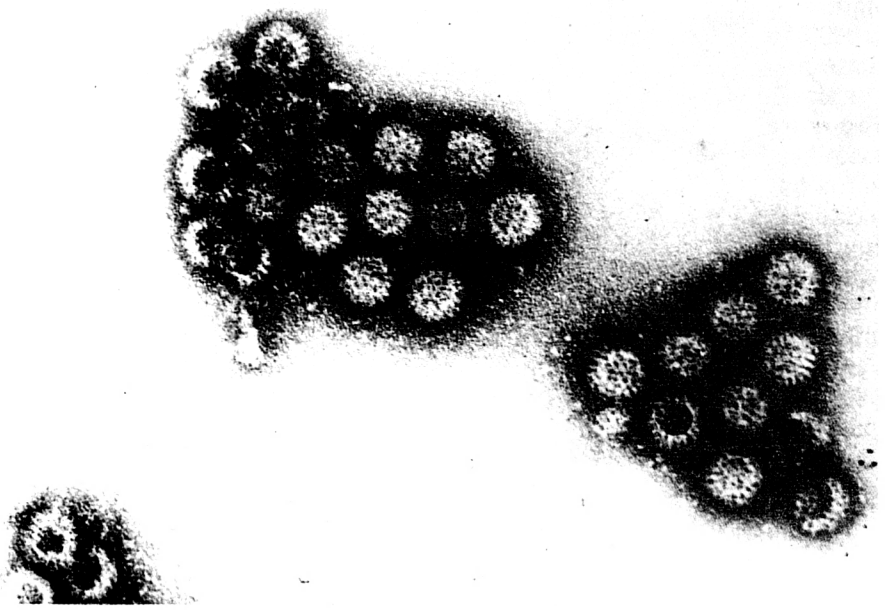

Fig. 2
Immune complexes with human rotavirus 649 strain (negative contrast), x 108 000.

passage, with CPE involving no more than 25–50% of the monolayer only days 4–5 post infection (p. i.). During further passages only one rotavirus strain (649) multiplied in cell culture so well that after 4 passages it constantly affected 75–100% cells already on day 2 p. i. By this marker the given HRV was not different from the standard SA-11 strain of simian rotavirus. In the course of further 9 (from 5 to 13) successive passages the pattern of reproduction of the adapted HRV strain remained unchanged as shown by the character of CPE development and the mean virus titre was 1.2×10^9 CPE_{50}/ml.

To obtain more information of the accepted HRV strain it was investigated by means of EM, EIA, CIEOP, haemagglutination in comparison with human WA and SA-11 simian rotavirus strains using antisera to the isolate and to SA-11. The electrophoretic mobility of human and simian rotavirus genome fragments were compared as well. Negative contrast staining of strain 649 revealed virions of 65–70 nm in diameter with typical rotavirus structure. As shown by calculations, the concentration of virions in the culture medium reached 10 log/ml. The core of the majority of viral particles was inpenetrable for the contrasting substance, however, in some of them it filled the virion centre indicating that they were so called empty uninfectious particles (Fig. 1).

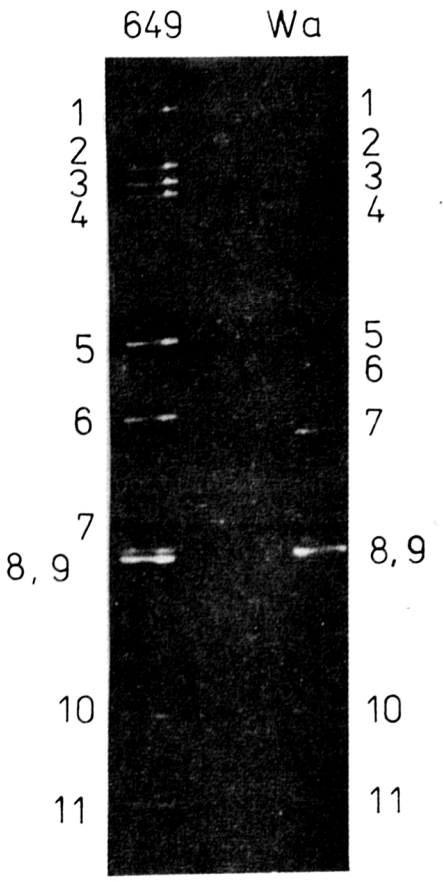

Fig. 3
Electropherogram of 649 HRV (a) and Wa (b) RNA fragments (separation in polyacrylamide gel)

Immunoelectron microscopy showed specific complexes formed with both SA-11 and 649 strains in dilutions ranging from 1:50 to 1:1000 (Fig. 2), whereas nonimmune control serum failed to form complexes with any of the viral strains.

The antigenic activity of strain 649 was also tested in EIA and CIEOP. In the latter test marked specific precipitation lanes were formed between the 649 HRV strain and SA-11 on the one hand, and respective antisera on the other; their specificity was documented by the absence of such precipitates between nonimmune serum and virus and between immune serum and the cells used for virus replication. The titre of immune serum ranged between 1:64 - 1:128 and did not much depend on the viral strain. In EIA based on 649 strain antigen, 2-fold dilutions (from 1:50 to 1: 25 000) of 649 and Wa strains were used starting at the same initial concentration (1.5 mg protein ml) and the same methods of protein virus cultivation, purification, and concentration. The titra-

tion results showed that the minimal antigenic activity of Wa strain was at 1:64 000 dilution, the ratio of extinction value of an experimental sample to that of control (P/N) was 2.37. The strain 649 was still detected in 1:25 000 dilution (P/N = 2.65). This 4-fold excess of the antigen titre of the isolated strain as compared to the prototype can be accounted for being homologous to the former. At the same time, final determination of the extent of antigenic differences between the isolated and prototype strain will require cross-reaction assays with strictly specific, e. g. monoclonal antisera to the tested strains.

One of the biological properties of certain rotavirus strains is their ability to cause agglutination of erythrocytes of 1(0) blood group of man and of guinea pigs. Comparative studies with 649 and simian rotavirus SA-11 strains showed that in contrast to SA-11 the isolate did not agglutinate the 0.5% suspension of human and guinea pig erythrocytes. The degree of electrophoretic mobility of RNA rotavirus fragments is important for intraspecies differentiation. The electrophoretype of 649 strain RNA was examined by electrophoresis in polyacrylamide gel in the presence of Wa strain RNA. The electrophoregram (Fig. 3) demonstrates that the former belongs to the so-called long phoretype. The differences in the mobility of fragments 4, 6, and 7 of 649 and Wa strains were most pronouced, fragments 3, 8, 9 also exhibited some differences. Particularly important appear the differences between fragments 4, 6, 9 as it is known from the literature that they code proteins determining the antigenic properties of the virus (Bukrinskaya, 1986).

The results of our studies demonstrate that the isolated strain is undoubtedly distinct from another Wa prototype strain of HRV which was at our disposal, and from SA-11 strain of simian rotavirus.

Thus, 649 strain of HRV isolated from a patient in the Western European part of the U.S.S.R. has been adapted to continuous cell culture MA-104. Some characteristics of this strain were studied: it was shown to be distinct from the Wa and SA-11 strains. Highly efficient permissive system for reproduction of the isolated strain can be helpful for the development and manufacture of antigenic preparations, useful for diagnostic, treatment, and prevention.

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