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DIFFERENT ELECTROPHORETYPES OF HUMAN ROTAVIRUSES IN HUNGARY

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Summary. — Polyacrylamide gel electrophoresis of rotaviral double-stranded ribonucleic acid (RNA) extracted directly from faecal specimens collected in three different parts of Hungary was applied to characterize and distinguish 21 randomly selected viral isolates. This technique made it possible to define 7 different electrophoretypes. Of the isolates 11 exhibited an identical "long" electrophoretic migration pattern. "Short" RNA pattern was found in two cases, and one atypical rotavirus was also revealed. This is the first description of rotavirus RNA electrophoretypes in Hungary.

Key words: human rotavirus; electrophoretype; RNA; electrophoresis; Hungary

Introduction

Rotaviruses are known as a major cause of acute gastroenteritis in infants and young children. Since the rotaviral infections have been recognized all over the world, several diagnostic methods for detection of rotavirus infection have been developed. Recently, polyacrylamide gel electrophoresis provides a simple, suitable method of separating and distinguishing rotaviral isolates on the basis of 11 segments of their double-stranded ribonucleic acid (RNA) extracted directly from faecal specimens. In addition, the analysis of the migration pattern of genomic RNA segments seems to be one of the best procedures for molecular characterization of this important virus group (Estes *et al.*, 1984).

In the present paper, we report the use of RNA gel electrophoresis to identify human rotaviruses and describe their electropherotypes from infants and young children admitted to hospitals located three different parts of Hungary during 1982—1983.

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Materials and Methods

Faecal samples. During 1982 and 1983 faecal samples taken from children (all aged 4 years or younger) with symptoms of acute gastro enteritis in three different provinces of Hungary were collected and routinely analysed with EM and/or ELISA. Approximately 10% rotavirus-positive stool suspensions prepared in PBS (pH 7.3) were received from virus laboratories in Budapest (9 samples) and Pécs (10 samples), respectively, and 750 µl of trichlorotrifluorethan (Genetron 113, Fluka, Switzerland) extracted positive materials were sent from the laboratory in Szeged (7 samples). Supernatants of each suspension after centrifugation at 5000× g for 15 min and extracted samples were stored for 6—12 months at —20 °C. Of the 26 rotavirus-positive specimens, 21 yielded sufficient viral RNA for visualization on polyacrylamide gels.

Extraction of viral nucleic acid. The extraction of viral RNA directly from faecal specimens was performed according to techniques described by Rodger *et al.* (1981) and Nicolas *et al.* (1983) with minor modifications. Briefly, 1.5—2.0 ml of supernatants were extracted twice with Genetron 113. After centrifugation at 2000× g for 20 min the liquid phases were saved. All extracted samples were mixed with an equal volume of extraction medium (0.02 mol/l EDTA and 2% SDS in 0.01 mol/l Tris-HCl buffer, pH 7.5), incubated 15 min at 50 °C and then extracted twice with water-saturated phenol-chloroform isoamyl-alcohol (25 : 24 : 1). The aqueous phases were precipitated with 2 volumes of absolute ethanol at —70 °C for 2 hr. The RNA was then pelleted, dried, resuspended in 20—100 µl of Laemmli's sample buffer (Laemmli, 1970). Simian rotavirus SA11 viral RNA was purified from viruses cultured in MA-104 cells.

Polyacrylamide gel electrophoresis (PAGE). Samples after boiled for 2 min were loaded on to 10% polyacrylamide slab gels (with 4% stacking gels) using the Laemmli discontinuous buffer system. Electrophoresis was performed at room temperature for 18 hr at constant 20 mA. RNA visualization in gels was obtained by staining with ethidium bromide at a concentration of 2 µg/ml and photographed under UV light of 254 nm wavelength. For comparison, samples were coelectrophoresed in the same lane. SA11 viral RNA was included in all gels as an internal reference.

Results

In 26 samples received from three distinct parts of the country (about 200 km from each other), rotaviruses were detected by electron microscopy and/or ELISA prior to the determination of electrophoretypes. Of the Genetron extracted samples from laboratory in Szeged and of faecal suspensions from laboratory Budapest 3 and 2, respectively, did not yield sufficient viral RNA for visualization in polyacrylamide gels. Seven different electropherotypes were observed and designated as A, B, C etc. according to the resolution of the RNA segments (Fig. 1). Three readily identifiable features of RNA patterns based on the relative migration of segments 10 and 11 were resolved and referred to as "long" (from A to E), "short" (F) and "atypical" (G) comparing to the migrations of corresponding segments of SA11 (Espejo *et al.*, 1979 and Kalica *et al.*, 1981).

Of "long" patterns, 5 distinct electrophoretypes were identified. Comigration of segments 2 and 3 and of 7 and 8 was designated as A. With the patterns A 11 rotaviruses were found, 4 and 7 of samples from Budapest and Pécs, respectively. All specimens collected in Szeged contained a single electropherotype referred to as B. Segments 2 and 3 moved very close to each other but 7, 8 and 9 were clearly separated. Very similar pattern was observed in one sample from Pécs where the migration of segments 7, 8 and 9 was identical, but segments 2 and 3 did not comigrate (pattern C). The pattern designated D showed comigration of segments 2 and 3 and separatedly

migrating segments 7, 8 and 9. A faster migration of segment 9 was observed in this single case. There was one rotaviral RNA with comigration of segments 8 and 9 and with segments 2, 3 and 4 which were well resolved and were approximately in equal distance from each other (pattern E).

Using of the RNA-pattern of simian rotavirus SA11 as an internal reference, two "short" electrophoretypes (pattern F) were observed in samples received from Budapest. Differences between the RNA migration patterns designated "short" and that of isolates with the "long" pattern A were confirmed by coelectrophoresis. There was one sample of pattern G with atypical rotavirus profile. Migration of the segments was characteristic, namely, one of the segments 7, 8, 9 was heavier and migrated close to segment 6 showing an atypical 4 : 3 : 2 : 2 segment distribution as compared to the pattern 4 : 2 : 3 : 2 of conventional rotaviruses (e.g. group A rotaviruses with patterns A-E, F and SA11). Furthermore, segments 10 and 11 migrated faster than the corresponding segments of SA11. This virus showed rotavirus morphology by direct electron microscopy but its antigen(s) did not react in ELISA.

Discussion

Many different human rotavirus RNA electrophoretypes have been described all over the world (Arista *et al.*, 1983; Buitenwerf *et al.*, 1983; Dimitrov *et al.*, 1984; Forster *et al.*, 1983; Ushijima *et al.*, 1984). Various investigators have attempted to make arbitrary classification systems based on the major variations within RNA gene classes (Espejo *et al.*, 1980; Kalica *et al.*, 1981; Lourenco et al., 1981), however, Espejo drew attention of shifts in the electrophoretic pattern under different performance conditions (Espejo and Puerto, 1984). For convenience, we designated our electrophoretypes according to Espejo (1979) and Kalica *et al.* (1981). who divided human rotaviruses due to the mobility of their segment 10 as related to segment 11 into "long" and "short" types. With respect to the strict electrophoretic identity, coelectrophoresis was done in viral RNA preparations with patterns observed as different in the first separation in gel.

In our cases there were no relationships between patients and hospitals. Samples were collected from different individuals and from different places. Only faecal samples from Szeged were taken from children with association of an outbreak of rotavirus diarrhoea in a nursery room. We succeeded to identify seven different electrophoretypes among the 21 samples. Patterns A were found in 11 faecal specimens from Budapest and Pécs. The 4 rotaviral RNA with the pattern B originated from Szeged, all other patterns were observed in single cases. The two "short" patterns, which were found less frequently all over the world and considered typical of serological subgroup 1 of human rotaviruses, were detected in samples from two distinct cases in Budapest. Finally, a human rotavirus was isolated with viral RNA segments of different size classes. It was undetectable by ELISA but was morphologically identical to other rotaviruses. Similar isolates were reported from different countries and different designations (e.g. pararotavirus, group B

rotavirus, atypical rotavirus) were proposed to distinguish them (Dimitrov *et al.*, 1983; Espejo *et al.*, 1983; Hung *et al.*, 1984). According to Flewett, this type of atypical rotaviruses belongs to a distinct group recently classified as group C (Flewett *et al.*, 1984).

To our knowledge this study has been the first attempt to reveal different electrophoretypes of human rotaviruses in Hungary. Although randomly selected and limited to a few rotavirus-positive faecal specimens, our results are in good accordance with data in the literature about the considerable heterogeneity of human rotaviruses. Nevertheless, it is necessary to analyse a larger number of viral strains in Hungary to receive a more complete information on the circulation and epidemiology of these different rotaviruses.

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Explanation to Figure (Plate LIV):

Fig. 1. Representative electrophoretypes of 21 human rotaviruses isolated in three different parts of Hungary. Direction of the run and numbering of the segments is from the top to the bottom. Seven different patterns can be distinguished by RNA electrophoresis (slots A, B, through G). Rotaviruses with "short" pattern and with an "atypical" migration pattern can be seen in slots F and G, respectively.