

ROTAVIRUS PERSISTENCE IN MALABSORPTION SYNDROME: VIRUS ASSESSMENT BY IMMUNOFLUORESCENCE IN SMALL INTESTINE BIOPSIES IN RELATION TO ANTIBODY TITRES

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Received January 16, 1986

Summary. — Results of quantitative immunofluorescence (IF) in small intestine biopsies and the serum antibody titres as detected by indirect Rotalex^R test in rotavirus infections were compared using regression and correlation analysis. Based on the significance of the statistically established correlation ($r = 0.76$; $n = 30$) between immunohistological identification of rotavirus antigen and determination of serum antibody levels, the indirect Rotalex^R test may be applied as a diagnostic tool to avoid biopsies in productive rotavirus persistence.

Key words: rotavirus; malabsorption syndrome; small intestine biopsy; immunofluorescence

Introduction

Rotavirus was the first time detected by Bishop *et al.* (1973) in biopsies of the duodenal mucosa from children affected with acute gastro-enteritis. Well-directed studies of antibody kinetics in comparison to excretion kinetics of human rotaviruses (HRV) are scarce in the literature. According to Weihmann (1980), the virus excretion, as a rule, seldom lasted for more than 8 days, with maximum demonstration of infectious agent between days 3 and 4 of the disease. In general, virus excretion lasts for 4 to 6 days, but in single cases more than for one month as stated by Maass *et al.* (1977). In immunodeficiency, Saulsbury *et al.* (1980) observed chronic HRV infection with virus detection for a period of more than 6 weeks. Virus-specific antibodies can be found in serum as early as on the day 5, the peak being reached 5 weeks after the onset of disease (Abe and Inouye, 1979). Houly *et al.* (1984) extracted rotavirus RNA from stool samples obtained from infants ($n = 203$; rotaviruses were detected by ELISA) with gastro-enteritis. With the aid of the polyacrylamide gel electrophoresis, bands characteristic for rotavirus double-stranded RNA were detected in 172 cases, i.e. in 85 per cent. Six different electrophoretotypes could be identified.

In our work we aimed at assessment of persistence kinetics of HRV in tissue specimens in relationship to demonstration of antibodies in corresponding sera. The question arose whether there is any statistically established relevance between the two methods of investigation.

Materials and Methods

Virus. Rotavirus strain SA11 (Friedrich-Loeffler-Institut, Akademie der Landwirtschaftswissenschaften der DDR, Insel Riems) was propagated in permanent monkey kidney cells (MK cells). To prepare the antigen, cells were infected at multiplicity (MOI) of 0.006 and 0.018, respectively. On day 3 (72 hr) post-inoculation (p.i.), when gross CPE was observed, the cultures were frozen and thawed, cells were harvested and centrifuged at $10,000 \times g$ for 10 min. To 10 ml of HRV solution, 1.6 g of polyethylene glycol was added at room temperature, left at 4°C for 45 min and centrifuged at $7,000 \times g$ for 20 min. The sediment was suspended in 0.3–0.4 ml of Rotalex^R buffer (pH 7.2). The infectious dose was between 6.4×10^2 to 1.3×10^3 TCID₅₀/ml (tested with Rotalex^R).

Cell culture. MK cells were grown in Eagle's medium (pH 7.2) containing 10 % of foetal calf serum (FCS) and antibiotics.

Immunofluorescence test. HRV was demonstrated in bioptic material from small intestine according to the technique described (Körting and Fiehring, 1983).

Antibody assay using the indirect Rotalex^R test. The patients sera were diluted geometrically in 6 wells of the microtiter plate (2^{-1} to 64^{+1}) starting from the mixture of 0.025 ml patients serum with 0.025 Rotalex^R buffer (pH 7.2). Each solution was mixed with the antigen (1×10^3 TCID₅₀/ml) in proportion of 1 : 2 and incubated for 1 hr at 37°C . Thereafter, 10 μl of each dilution mixture was removed for agglutination on a slide with 10 μl Rotalex^R latex reagent (rabbit antiserum to calf rotavirus). Agglutination was performed at room temperature for 2 to 5 min.

Absence of agglutination indicated in tested serum the presence of an amount of antibodies sufficient to bind the antigen supplied (positive result). The occurrence of agglutination indicated that no such antibodies were present in tested serum (negative result).

Results

In a group of patients ($n = 347$; 150 ♀ and 197 ♂) with malabsorption syndrome, 32 % ($n = 110$; 52 ♀ and 58 ♂) could be traced back to HRV infection according to examination. In 237 subjects (98 ♀ and 139 ♂) negative

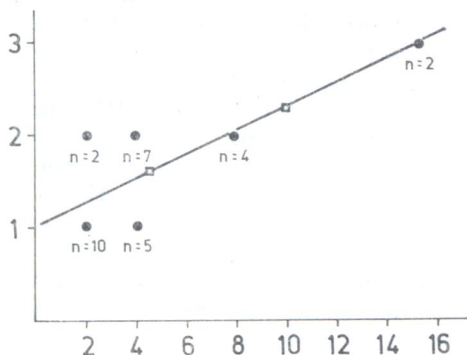


Fig. 1.

Comparison of quantitative determination of HRV antigen by IF in small intestine biopsies with antibody titres of corresponding sera by indirect Rotalex^R test.

Abscissa: antibody titre ($\times 10^{-1}$); ordinate: IF evaluation

Table 1. Immunohistological assay of rotavirus antigen small intestine biopsies using quantitative IF

Age (years)	Σn	%	Immunofluorescence					
			n	\pm	+	+/++	++	++/+++
< 3	74	67.3	3	42	1	25	0	3
3-6	17	15.5	2	10	0	5	0	0
6-14	14	12.7	1	6	0	5	1	1
> 14	5	4.5	0	2	0	3	0	0
$\Sigma 110$								

n = number of positive cases

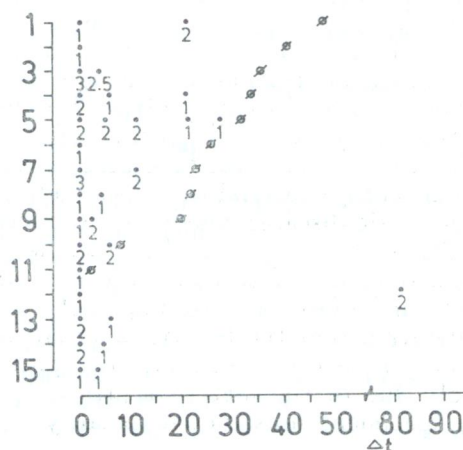
Total examined: 347; IF positive cases: 110; IF negative cases: 237

results were obtained. Tissue specimens (aspiration biopsies) were obtained from the upper digestive tract of the patients with preference from the jejunum, and tested by IF. In 15 patients repeated biopsies were performed at different time intervals to assess the persistence of rotaviruses in the tissue specimen (Fig. 2). Among 110 patients with signs of gastro-enteritis and established rotavirus infection (IF examination), the highest percentage of 67.3 % was found in the age group of "up to 3 years", and the lowest of 4.5 % in the group of ">14 years" (Table 1).

Fig. 1 shows the results obtained from selected patients ($n = 30$) with malabsorption syndrome in the age of 3 months to 16 years, comparing the extent of virus persistence as detected by IF (tissue specimen) and by indirect Rotalex^R test (serum). As controls 19 negative sera had been examined with the indirect Rotalex^R test. The comparison of IF in small intestine

Fig. 2.

Chronic course and persistence of rotavirus infections in patients with malabsorption syndrome as demonstrated by the quantitative IF at different time intervals (Δt) in weeks; full circles (●) signify measuring points; evaluation: 1 = 1-fold; 1.5 = 1.5-fold; 2 = 2-fold; 2.5 = 2.5-fold; 3 = 3-fold (compare Table 1). Abscissa: weeks of illness; ordinate: cases under observation



biopsies and of antibody titres revealed a correlation coefficient of $r = 0.76$ (Fig. 1), so that at $n = 30$ there was a significance of $r_{0.05;28} = 0.361$ or $r_{0.01;28} = 0.570$, respectively. It applies:

$$y = 0.13 \cdot x + 1,$$

wherein: y = IF evaluation and x = serum antibody titres. In contrast to the results described in the literature, HRV could be still found in the intestinal epithelium for up to 82 weeks (as related to the first biopsy). Nevertheless, in 11 cases the 2nd to 4th biopsy results were negative within 27 weeks on the average (Fig. 2). In 4 infants, the evidence of HRV in repeated biopsy specimens was positive at intervals of 81.7, 5.9, 5 and 3.4 weeks, respectively. In parallel to IF determinations, the antibody titres in corresponding sera were examined using the indirect Rotalex^R test. In addition, in one case out of 11 subjects the antigen was found in the excised material from the intestine and in stool as well, at an serum antibody titre of 1 : 4 as determined by comparison of IF, direct (stool) and indirect (serum) Rotalex^R tests.

Discussion

Abacterial gastro-enteritis in infants according to stool examinations (Schumacher *et al.*, 1985) in 47 % of cases is caused by rotavirus infection ($n = 104$; determined by ELISA, by electronmicroscopy and counterimmunoelectrophoresis). Based on antibody titre examinations in this study even 65 % of the cases were assigned to rotavirus infection ($n = 50$; using ELISA and counterimmunoelectrophoresis). According to Adameczyk *et al.* (1979) in 30 % of cases with signs of gastro-enteritis rotavirus infection could be demonstrated ($n = 101$; determined by KBR) in children aged up to 3 years (stool examinations). Fiehring *et al.* (1984a, b) and Körting and Fiehring (1983; 1984) found in infants and children aged up to 15 years with malabsorption syndrome rotavirus antigen in 43 % of cases ($n = 53$; determined by IF in the material excised from the jejunum).

Provided that faecal specimens were examined and the analytical methods were compared as performed as Ellens *et al.* (1978), counterimmunoelectrophoresis showed 31 %, IF and electron microscopy approximately 40 %, the complement fixation test 43 % and ELISA technique about 50 % diarrhoea cases to be caused by HRV ($n = 98$).

In this study 32 % of patients with malabsorption syndrome were found to be caused by rotavirus according to reliable identification of HRV in the jejunum biopsy material ($n = 347$; determined by IF). The statistic data in gastro-enteric diseases which had been claimed for pure rotavirus infections are influenced — as shown by examples from literature — by the number, age and selection of patients and by analytical methods and objects of the assay (source of excision, serum, stool, etc.). However, it should be taken into consideration that the bioptic material we used was a piece of tissue in the size of a pin head excised from the upper small intestine under radiographic control. Due to the fact that plaque spots show a dispersed pattern, the tissue specimens must not necessarily represent areas of intestinal mucosa

with virus-specific lesions. The unchanged clinical picture and the slow declining antibody titres underline this view.

In 11 cases a chronic course of a rotavirus infection was diagnosed in several biopsies. On average, at time interval of the first biopsy a negative result could be found only after 27 weeks by IF test. In one infant even at interval of 81.7 weeks after the first biopsy, HRV was positive by IF, which speaks for rotavirus persistence. Davidson *et al.* (1975), and later on also others who demonstrated rotavirus antigen in the cytoplasm of epithelial cells, expressed the opinion that virus detection was possible within first 4 days after occurrence of the clinical signs only. Such a view can no longer be sustained in respect of the above-mentioned results.

The comparison of IF examinations of the small intestine and serum titration with the aid of the indirect Rotalex^R test shows similar results despite of the great dissimilarity of the techniques applied. Based on the statistically established dependence of the IF results and antibody titres, the indirect Rotalex^R test may be used for the serological diagnosis of rotavirus infections. The possibility is given to avoid aspiration biopsy that is not problemless for infants and requires much time and effort.

Acknowledgement. We thank Mrs. Erika Schubert for her excellent technical assistance.

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