Specificity of Dot Hybridization Assay in the Presence of rRNA for Detection of Rotaviruses in Clinical Specimens

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Received 27 March 1987/Accepted 20 May 1987

Nucleic acid hybridization is used to identify viral genomic sequences in clinical and environmental samples. However, RNA virus genomes have been reported to hybridize to mammalian rRNA from uninfected cells under stringent conditions, and caution has therefore been advised in the use of nucleic acid probes for detection of RNA viruses. To evaluate the effect of rRNA on a diagnostic assay for an RNA virus, we tested the specificity of a rotavirus dot hybridization assay with clinical specimens which contained eucaryotic rRNA. The cDNA probe used in this assay contained sequences complementary to all 11 rotavirus genes. Preliminary experiments indicated that hybridization between rRNA and the cDNA probe occurred only with >50 ng of rRNA, and this interaction was easily distinguished from the hybridization of the rotavirus probe with homologous or heterologous strains of the same rotavirus group. When 95 clinical specimens were tested, the rotavirus dot hybridization assay had a specificity of 98.8%. The predictive value of a negative test was 94.2%, although nearly all of the specimens contained rRNA and also reacted with an rRNA probe. Although the specificity of all dot hybridization assays should be individually evaluated, we conclude that our dot hybridization assay for rotaviruses is highly specific even in the presence of quantities of rRNA that may be anticipated in extracts of fecal specimens.

Hybridization of clinical specimens with nucleic acid probes is proposed as a means of diagnosing infection with viral and bacterial pathogens (7). Nucleic acid hybridization permits viral diagnosis without purification of the pathogen or extensive biochemical or serologic confirmatory tests. Although the assays can be highly specific when used in a research laboratory, concern has been expressed about the specificity of these assays when they are used for diagnosis of infection. For example, McClure and Perrault found that eucaryotic rRNA hybridized under stringent conditions with viral RNA from enteroviruses, reovirus, Sindbis virus, and vesicular stomatitis virus (4, 5). In view of these findings, caution has been advised in the use of nucleic acid probes to detect RNA viruses in clinical specimens (5).

We have previously used a dot hybridization assay to identify rotaviruses (2). To assess the effect of rRNA on the specificity of this assay, we conducted experiments to measure the hybridization of rotavirus with rRNA and to evaluate the reactivity of our rotavirus probe with clinical specimens which contained rRNA.

Genomic double-stranded RNAs from tissue culture-adapted strains of group A rotaviruses (SA-11, NCDV, and WA) and reovirus type 1 were purified as previously described (2). rRNAs from bovine liver and *Escherichia coli* were purchased from a commercial supplier (Sigma Chemical Co., St. Louis, Mo.). Human fecal specimens were obtained from hospitalized infants at our institution during February 1986. After collection, specimens were kept at 4°C for up to 24 h. Specimens were then stored at -20°C as 10% (wt/vol) suspensions in phosphate-buffered saline which contained 1 mM CaCl₂ and 0.5 mM MgCl₂ (2). These fecal specimens were tested for reactivity in a sensitive immunoassay for detection of rotavirus (8). For dot hybridization, nucleic acid was extracted from samples of these specimens with phenol and chloroform. ³²P-labeled cDNA

probe was manufactured from RNA templates with reverse transcriptase and random primer as previously described (2). If rotavirus RNA served as a template in this reaction, the resulting cDNA contained sequences complementary to all 11 segments of the viral genome (2). Dot hybridization on nitrocellulose membranes was performed as previously described (2). The hybridization conditions were 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0), 42°C, 50% formamide, and 10% dextran sulfate for 18 h. After hybridization, the membranes were washed in 2× SSC at room temperature and 0.1 and 2× SSC at 50°C. Autoradiography was done with X-Omat AR X-ray film (Eastman Kodak Co., Rochester, N.Y.) and Cronex Lightning-Plus intensifying screens (Du Pont Co., Wilmington, Del.). The X-ray films were examined visually for evidence of exposure to radioactive probe. The appearance of any clearly outlined spot was considered a positive test.

DNA probes prepared from each of three serotypes of group A rotaviruses hybridized with 5 to 50 ng of RNA purified from the other serotypes (Fig. 1a to c). These rotavirus probes did not cross-react with rRNA or reovirus RNA under identical conditions of hybridization. A weak signal was obtained when the rotavirus probes were incubated with dots containing 500 ng of bovine rRNA, but this was easily distinguished from the signal obtained with dots of group A rotavirus genomic RNA.

Probes of ³²P-labeled cDNA were also prepared from rRNA and hybridized to viral RNA (Fig. 1e). Hybridization between the probe and the template RNA was easily observed. Again, only a very weak hybridization signal between the ribosomal nucleic acid probe and dots containing 500 ng of the rotavirus genomic RNA was noted.

Experiments were next performed to determine whether the specificity of the rotavirus dot hybridization assay was affected by the presence of rRNA in clinical specimens. Our intent was to evaluate the occurrence of false-positive results rather than to reevaluate the sensitivity of the assay.

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FIG. 1. Autoradiographs after hybridization of ^{32}P -labeled cDNA probes with purified viral RNA or rRNA immobilized on nitrocellulose papers. The hybridization conditions are described in the text. The RNA concentration is indicated at the top of each column. The identities of the immobilized RNAs are noted to the left

FIG. 2. Autoradiographs after hybridization of ^{32}P -labeled rotavirus cDNA with phenol extracts of human fecal specimens immobilized on nitrocellulose papers. Dot D4 contained 5 ng of bovine rRNA. Dot D5 contained 5 ng of SA11 rotavirus RNA. Dots D6 and D7 were blanks. The rest of the dots all contained human fecal extracts. Specimens reactive with the rotavirus immunoassay were contained in dots A4 to 7; B1, 2, 4, and 7; C1, 2, 4, and 6; and D1. The cDNA probes were derived from the following sources: a, bovine rRNA; b, WA rotavirus.

Therefore, most of the test specimens were obtained from infants without rotavirus infection as determined by means of a sensitive immunoassay (8). Fecal specimens were collected once a day from 21 hospitalized infants, 10 of whom had gastroenteritis, 10 of whom had no gastrointestinal symptoms, and 1 of whom shed rotavirus asymptotically. Of the patients with gastroenteritis, two shed rotavirus and the etiologic agent in the other eight was unknown. Of 95 specimens tested, 13 were reactive in the rotavirus immunoassay. To assess the presence of rRNA in the fecal specimens, dot hybridizations were first performed with the specimens and ^{32}P -labeled cDNA probe prepared from bovine rRNA. Comparison of the hybridization signals of the clinical specimens with a control dot of 5 ng of rRNA indicated that the fecal extracts all contained <5 ng of rRNA per μl . However, hybridization between the ribosomal cDNA probe and almost all of the fecal extracts was noted, regardless of whether the specimens contained rotavirus or were nonreactive in the rotavirus immunoassay (Fig. 2a is representative of these hybridizations). In contrast, the rotavirus cDNA probe hybridized with only 1 of 82 specimens nonreactive in the rotavirus immunoassay. Hybridization of this rotavirus probe was also observed with 8 of the 13 specimens which were reactive in the rotavirus immunoassay. A representative autoradiograph of the rotavirus dot hybridization is depicted in Fig. 2b. The specificity of the rotavirus probe was 98.8% when compared with the immunoassay, and the sensitivity was 61.5%. The predictive value of a positive reaction with the rotavirus probe was 88.9%, and the predictive value of a negative dot hybridiza-

of each panel. The cDNA probes were derived from the following sources: a, SA11 rotavirus (serotype 3, subgroup 1); b, WA rotavirus (serotype 1, subgroup 2); c, NCDV rotavirus (serotype 6, subgroup 1); d, reovirus type 1; e, bovine rRNA.

tion was 94.2% when compared with the immunoassay ($P < 0.01$ by chi-square analysis).

RNA genomes of poliovirus, vesicular stomatitis virus, Sindbis virus, and reoviruses have been reported to hybridize to each other as well as to eucaryotic 28S rRNA (4, 5). Complementary regions between the poliovirus genome and 28S RNA have also been identified. Consequently, caution has been advised in the use of nucleic acid hybridization for identification of RNA viruses in clinical specimens (4). Since we used a dot hybridization assay to detect rotaviruses, we decided to assess the effect of rRNA on the specificity of that assay. The rotavirus assay consisted of a cDNA probe hybridized with specimens dotted onto nitrocellulose, whereas the original reports of rRNA-RNA virus hybridization used RNA probes and electrophoretic transfer of specimens to nitrocellulose. Hybridization conditions were otherwise similar with respect to temperature, buffer, and formamide concentration. In our preliminary experiments, hybridization with the virus probe was indeed observed when a sufficient quantity of rRNA was dotted onto nitrocellulose. However, comparison of these quantitative results with previous reports was not possible since hybridization signals in the latter were measured only by densitometric scanning of autoradiographs.

Although rotavirus probes may hybridize with rRNA, rotavirus dot hybridization assays are said to be highly specific (1, 3). We also found that the rotavirus dot hybridization assay was highly specific (98.8%) when the cDNA probe was hybridized with fecal extracts from symptomatic or asymptomatic infants. These extracts contained rRNA, but false-positive reactions were not observed. It appears that false-positive reactions were not detected because the clinical specimens contained less than 5 ng of rRNA per μ l and were insufficient in quantity to generate a detectable signal.

Dot hybridization with either RNA or cDNA probes appears to be a sensitive means of detecting the presence of rotavirus genomic RNA (1, 3, 6). As little as 8 pg of purified, homologous viral RNA may be detected by use of nucleic acid probes (3), although detection of heterologous strains of rotavirus RNA is reported to require ≥ 0.5 to 31 ng of viral genomic RNA (1, 6). Thus, the dot hybridization assay appears to be slightly less sensitive than immunoassay or electron microscopy for detection of rotavirus in fecal specimens (1). We did not attempt to reassess the sensitivity of our dot hybridization assay in this report, although we did document that the detection of purified rotavirus RNA in our

assay was comparable to that of other published reports. Since we assayed specimens throughout the course of gastroenteritis and included low-titer specimens, it was not surprising that the sensitivity of our hybridization assay with the clinical samples was 61.5% when compared with our immunoassay.

We conclude that the technique of dot hybridization for detection of rotaviruses is highly specific. The presence of less than 5 ng of eucaryotic rRNA per μ l in phenol-extracted fecal specimens does not result in an appreciable increase in the number of false-positive results. However, in light of the reported interactions of rRNA and RNA viruses (4, 5), each probe should be carefully evaluated with respect to its specificity of reaction with clinical samples.

This work was supported in part by Public Health Service grant no. RO1AM33089, contract no. NO1AA15257N, from the National Institutes of Health and by the Thrasher Research Foundation, Salt Lake City, Utah.

LITERATURE CITED

1. Dimitrov, D. H., D. Y. Graham, and M. K. Estes. 1985. Detection of rotaviruses by nucleic acid hybridization with cloned DNA of simian rotavirus SA11 genes. *J. Infect. Dis.* **153**:293-300.
2. Eiden, J., S. Vonderfecht, K. Theil, A. Torres-Medina, and R. H. Yolken. 1986. Genetic and antigenic relatedness of human and animal strains of antigenically distinct rotaviruses. *J. Infect. Dis.* **154**:972-982.
3. Flores, J., R. H. Purcell, I. Perez, R. G. Wyatt, E. Boeggman, M. Sereno, L. White, R. M. Chanock, and A. Z. Kapikian. 1983. A dot hybridisation assay for detection of rotavirus. *Lancet* **i**: 555-558.
4. McClure, M. A., and J. Perrault. 1985. Poliovirus genome RNA hybridizes specifically to higher eucaryotic rRNAs. *Nucleic Acids Res.* **13**:6796-6816.
5. McClure, M. A., and J. Perrault. 1986. RNA virus genomes hybridize to cellular rRNAs and to each other. *J. Virol.* **57**: 917-921.
6. McCrae, M. A., and S. Pedley. 1984. A rapid screening assay for detecting individual RNA species in field isolates of rotaviruses. *J. Virol. Methods* **9**:173-181.
7. Palmer, L., and S. Falkow. 1985. Selection of DNA probes for use in the diagnosis of infectious disease, p. 211-218. *In* D. T. Kingsbury and S. Falkow (ed.), *Rapid detection and identification of infectious agents*. Academic Press, Inc., New York.
8. Yolken, R. H., R. Viscidi, F. Leister, C. Harris, and S.-K. Wee. 1986. Enzyme immunoassay for the detection of rotavirus antigen and antibody, p. 573-581. *In* N. R. Rose, H. Friedman, and J. L. Fahey (ed.), *Manual of clinical laboratory immunology*, 3rd ed. American Society for Microbiology, Washington, D.C.