Enzyme-Linked Immunosorbent Assays for Measurement of Reovirus Immunoglobulin G, A, and M Levels in Serum

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Enzyme-linked immunosorbent assays were developed for the quantitation of anti-reo virus immunoglobulin G (IgG), IgA, and IgM in serum. The assays were specific, sensitive, and reproducible and measured antibodies to the reovirus group antigen. They should permit diagnosis of recent reovirus infection and longitudinal study of the development of reovirus group antibodies.

Until recently, reovirus infections in humans had not been associated with any one particular disease (12, 13). Consequently, serological studies of the antibody response to reovirus infection in particular patient groups have been limited. The relatively recent implication of reovirus serotype 3 in the etiology of extrahepatic biliary atresia in infants and neonatal hepatitis (1, 5, 11) requires the development of assays to quantitate the class-specific serum antibody response to reovirus infection.

To develop class-specific enzyme immunosassays, reovirus strains representing each of the three reovirus serotypes were grown in thymidine kinase-deficient mouse L fibroblast cells (LTK−) (7). Strains used were RCH1144 (reovirus serotype 1 [reo 1]) and RCH1147 (reo 2), identified by using neutralization tests with standard reovirus antisera produced against prototype reovirus strains; and the Dearing strain of reovirus serotype 3 (reo 3). LTK− cells were routinely cultivated and inoculated with reovirus. Virus was extracted when maximum cytopathic effect was observed, usually after 4 days. Reovirus was concentrated by centrifugation at 125,000 × g for 80 min at 4°C. Pellets were suspended in 0.01 M Tris buffer, divided into aliquots, and stored at −70°C. Negative control antigen was prepared in the same manner, using uninfected LTK− cells. The optimal dilution of reovirus and control antigen to be used in each assay was determined by checkerboard titration.

Assays for anti-reo 1, 2, and 3 immunoglobulin G (IgG) used an indirect enzyme-linked immunosorbent assay (ELISA) technique, whereas those for anti-reo 3 IgA and IgM used a capture ELISA technique. Quantitation of anti-reovirus IgG and IgA used units derived from a standard curve (3). Quantitation of anti-reo 3 IgM was by endpoint titration. The indirect assay method was similar to that previously described (3), and reactions were detected by using a rabbit anti-human IgG conjugate (DAKO Immunoglobulins, Glostrup, Denmark). The IgA and IgM capture tests used disposable certified immunoplates (NUNC, Denmark) coated with goat antibodies to human IgA (α-chain specific) or human IgM (μ-chain specific) purified by affinity chromatography (TAGO, Inc., Immunodiagnostic Reagents), and reactions were detected by using a guinea pig antiserum to reovirus (CSL, Melbourne, Australia) conjugated to horseradish peroxidase (14). All assays used tetramethylbenzidine substrate (Sigma Chemical Co., St. Louis, Mo.) (2), and the optical density of each well was measured at 450 nm with a Titertek Multiskan reader.

The anti-reovirus IgG and IgA assays included one standard serum used at five different dilutions during each assay to construct a standard curve of optical density values against units of antibody in each dilution. These assays also included a positive serum at a single dilution assessed as lying on the midpoint of the standard curve and a negative serum at a single dilution. The standard serum used in the IgG assay had an endpoint titer for IgG to reo 3 of greater than 1:12,800 and was arbitrarily considered to contain 1,000 U of anti-reo 3 IgG per ml of undiluted serum. The IgA assay incorporated a standard serum with an endpoint titer for IgA to reo 3 of greater than 1:51,200 and which when undiluted was arbitrarily assigned a content of 8,000 U of anti-reo 3 IgA per ml. The negative control serum for the IgG assay was a pooled serum from five children aged between 5 and 26 months selected after the initial screening of 87 serum samples. This serum had an endpoint titer for IgG to reo 3 of less than 1:100. The negative control serum used in the anti-reo 3 IgA ELISA was obtained from a hospitalized patient with agammaglobulinemia and had an endpoint titer for IgA to reo 3 of less than 1:400. Unit values of anti-reovirus IgG and IgA in these control sera and in test sera were calculated by reference to the standard curve in each assay. Reactions with sera were regarded as specific for reovirus antigen only if the optical density value exceeded 2.5 times that of the corresponding well containing control antigen.

Inter- and intrarun coefficients of variation inherent in each standard-curve ELISA were calculated as previously described (3). If either of the two control serum samples gave a unit result which fell outside twice the intrarun coefficient of variation calculated for that sample (95% confidence limits), then all the results of that assay were discarded. For each standard-curve ELISA, the positive-negative cutoff value for units of antibody to reovirus was defined as the largest antibody unit value of the negative control serum which was within the 95% confidence limits. This cutoff unit value was calculated to be 129 U/ml for anti-reo 1 IgG, 103 U/ml for anti-reo 2 IgG, 130 U/ml for anti-reo 3 IgG, and 20 U/ml for anti-reo 3 IgA. The intrarun coefficient of variation of reovirus antibody unit values was calculated to be 20% of the anti-reovirus IgG ELISA and 22% for the anti-reo 3 IgA ELISA.

A seroconversion was defined as an increase in ELISA antibody units per milliliter of serum of more than twice the intrarun coefficient of variation in consecutive serum samples from the same child (3), i.e., an increase in anti-reo 1, 2, or 3 IgG units of greater than 40% and an increase in anti-reo 3 IgA units of greater than 22%.
3 IgA units of greater than 44%. The assay was validated by using consecutive serum samples from a child aged 13 months admitted to the Royal Children's Hospital, Parkville, Melbourne, with symptoms of chronic chest infection of unknown etiology and from whom reo 2 had been isolated coincidentally from a urine specimen. Acute-phase serum was obtained on admission, and convalescent-phase serum was obtained 7 weeks later. Seroconversions in IgG units to reo 1, 2, and 3 and in IgA units to reo 3 were detected by assays of these paired serum samples (Table 1).

The anti-reo 3 IgM assay incorporated two positive control serum samples and one negative control serum sample. The positive control sera had endpoint titers for IgM to reo 3 of 1:10,240 and 1:1,280, while the negative control serum had an endpoint titer for IgM to reo 3 of 1:160. In each IgM assay, the endpoint of titration for each control and test serum was defined as the last serum sample dilution at which the specific absorbance to reo 3 antigen was >2.5 times the nonspecific absorbance to control antigen. Results of each assay were rejected if any of the three control serum samples showed a greater-than-twofold variation from the expected endpoint titer. To determine a positive-negative endpoint antibody titer for the IgM assay, the endpoint antibody titers of sera obtained from 44 infants with no evidence of recent virus infection were calculated. A serum sample was thus considered to be positive for anti-reo 3 IgM if its ELISA endpoint titer was greater than 1:160.

A seroconversion detected by endpoint titration in the anti-reo 3 IgM ELISA was defined as a fourfold-or-greater increase in endpoint titer between consecutive serum samples from the same child. The test was validated by using serum samples collected from a healthy child under surveillance in a prospective study who was shown to excrete reovirus particles in feces at age 22 months. Consecutive serum samples obtained during and after reovirus excretion showed fourfold increases in anti-reo 3 IgM titer from 1:320 to 1:1,280 (Fig. 1).

It is likely that the IgG, IgA, and IgM assays predominately measured antibodies to the reovirus group antigen thought to reside in the core of the virus particle and shared by the three mammalian reovirus serotypes (4, 6, 8, 9). Paired serum samples from the child with a known reo 2 infection (Table 1) showed simultaneous seroconversions in IgG to all three serotypes. In addition, comparison of the amounts of IgG, IgA, and IgM to reo 1, 2, and 3 measured in 260 serum samples collected for another study (S. C. Richardson et al., manuscript in preparation) showed that levels of antibody to each of the three reovirus serotypes were similar in each serum. Some degree of serotype-specific antibody response may be measured in these assays, however, since the units of anti-reo2 IgG in the convalescent-phase serum sample from the child infected with reo 2 was approximately double that of anti-reo 1 IgG or anti-reo 3 IgG (Table 1). Similar assays for rotavirus antibodies have been shown to detect antibody to the common group antigen (10). Thus, while the ELISA technique allows the detection of specific classes of antiviral immunoglobulin, the assay is limited in its capacity to measure a serotype-specific antibody response.

The development of ELISA systems to detect class-specific antibodies permits further investigation of the involvement of reovirus infections in the etiology of diseases such as extrahepatic biliary atresia in infants and neonatal hepatitis (5, 11). Availability of these assays should also facilitate diagnosis of symptomatic and asymptomatic reovirus infections in the general community.

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LITERATURE CITED


