JOURNAL OF CLINICAL MICROBIOLOGY, June 1982, p. 991–993
0095-1137/82/060991-03$00.00/0

Vol. 15, No. 6

Enzyme-Linked Immunosorbent Assay for Measurement of Antibody to Type III Group B Streptococci

RICHARD A. POLIN,[1]* STEVEN D. DOUGLAS,[1] DENNIS L. KASPER,[2] AND CAROL J. BAKER[3]

Department of Pediatrics, Joseph Stokes Jr. Research Institute, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104[1]; Channing Laboratory, Department of Medicine, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts 02115[2]; and Myers-Black Section of Infectious Diseases and Department of Pediatrics, Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030[3]

Received 30 November 1981/Accepted 25 January 1982

Neonates at risk for fulminant type III, group B streptococcal (III GBS) infection are those who lack antibody to the capsular polysaccharide. A newly developed enzyme-linked immunosorbent assay (ELISA) was compared with a standard radioactive antigen-binding assay (RABA) for quantitation of III GBS antibody in human sera. Although there was a significant correlation between the ELISA and RABA ($r = 0.81$; $P < 0.001$) in general, the ELISA detected antibody both to core and native antigens of III GBS, whereas the RABA detected antibody to native polysaccharide exclusively. The results of the two assays were discordant when sera which had only high native or core antibody (not both) were assessed. Although the ELISA was reproducible and required <1 μl of serum, interpretation of data obtained by the assay should be viewed with caution since only antibody to native III GBS has been correlated with human immunity.

Group B *Streptococcus* (GBS) remains one of the leading causes of neonatal mortality and morbidity (1, 5). Although each of the five serotypes of GBS has been associated with invasive disease in neonates, type III strains are the single most frequent isolates. Host immunity to type III GBS is complex and believed to require the interaction of the classical and alternate complement pathways (4), polymorphonuclear leukocytes (9), and antibody against the native polysaccharide determinant of type III GBS (2, 7). Baker and Kasper have demonstrated that mothers with moderate to high levels of immunoglobulin G antibody to type III GBS frequently deliver infants who become colonized with this organism but who remain asymptomatic (3). In contrast, infants with invasive disease uniformly lack serum antibody against the native type III polysaccharide antigen (2, 7), a condition resulting from either low maternal levels (term infants) or failure of placental transport of maternal antibody (preterm infants) (2). A discrepancy clearly exists between the protective capacity of antibodies directed to the native antigen and antibody directed to the core antigen. The latter antigen lacks a terminal sialic acid residue found in the native antigen (7). Only antibody to the native antigen has been correlated with immunity (7).

Numerous assays have been described which quantitate antibody or opsonins or both to type III GBS (3, 6, 8, 10–12). Using whole type III GBS as an antigen, we have developed an enzyme immunoassay to quantitate antibody to type III GBS. This assay is easily performed, requires less than 1 μl of serum, and eliminates the need for radioactivity or complex purification of polysaccharides. In this report, the methodology used in the enzyme-linked immunosorbent assay (ELISA) is presented and the results are correlated with the standardized radioactive antigen-binding assay (RABA).

MATERIALS AND METHODS

Antigen preparation. Type III GBS (strain D136C) was kindly provided by the late Rebecca Lancefield. The bacterial strain was grown overnight in 200 ml of Todd-Hewitt broth. To prepare type III antigen, the cells were resuspended in 6 ml of formalinized saline, and the pH of the suspension was lowered to 2 with sterile HCl. Pepsin was added to a concentration of 0.05%, and the mixture was incubated for 2 h at 37°C. The cell suspension was neutralized with 1 N NaOH, and 0.2% formalinized saline was added to a final volume of 10 ml. Bacteria were refrigerated at 4°C.

Sera. Specimens were obtained from three groups of individuals. Group 1 consisted of serum specimens from 20 adult volunteers. Each of the 20 specimens from group 1 was analyzed with both the ELISA and the RABA. Group 2 comprised 10 mother-infant (cord blood) pairs which were tested only with the ELISA. Group 3 consisted of serum specimens from six individuals known to have high native antibody (8.32 to 175 μg/ml) and low core antibody (33.3 to 163 ng/ml) concentrations and six specimens from individuals with high core antibody (510 to 1,154 ng/ml) and low

native antibody concentrations (0.648 to 1.18 μg/ml). These specimens were analyzed with both the RABA and the ELISA. All samples were assayed in quadruplicate on three separate occasions.

ELISA. (i) Fixation of bacteria to polyvinylchloride plates. Fifty microliters of a poly-L-lysine solution (0.001 g/100 ml of phosphate-buffered saline [PBS], pH 7.2) was placed into each well of a 96-well, flat-bottomed polyvinylchloride plate and incubated for 2 h at room temperature. The poly-L-lysine was removed by flicking the plate, and 2×10^6 bacteria suspended in 50 μl of a 0.5% glutaraldehyde solution (Sigma Chemical Co.) in cold PBS was placed into each well. After a 15-min incubation at room temperature, all wells were washed twice by immersion in PBS and flicking off the remaining fluid. Each well was then filled with a solution of 100 mM glycine in 1% bovine serum albumin (pH 7.6) and incubated for 30 min at room temperature. The plates were washed in PBS, dried under a heat lamp, and stored at room temperature until use within 6 months.

(ii) Assay. Each well which had been precoated with type III GBS was filled with a PBS solution (pH 7.2) containing 1% bovine serum albumin. The plate was incubated for 2 h at room temperature and washed twice with PBS. Excess PBS was removed by flicking the plate. Serum specimens were diluted (1/10 to 1/1,000) in PBS containing gelatin (0.01 g/dl) and Tween 20 (0.05 ml/dl), and 100 μl of each serum dilution was added to the plate in quadruplicate. The plate was covered and incubated overnight at 4°C and washed six times the following morning with PBS-Tween-gelatin solution. The excess wash solution was removed, and 100 μl of peroxidase-labeled anti-human immunoglobulin G (diluted 1/2,000 in PBS-Tween-gelatin; Cappel Laboratories) was added to each well. The plate was incubated for 1 h at room temperature and washed 10 times with the PBS-Tween-gelatin solution. The remaining wash solution was removed by flicking, and 100 μl of substrate (10 ml of citrate buffer [pH 4.5], 10 mg of orthophenylenediamene [Sigma], and 2 μl of 30% hydrogen peroxide) was added. The plates were incubated for 30 min in the dark, and the reaction was stopped by adding 50 μl of 0.1 M sodium fluoride to each well. To obtain quantitative readings, all plates were read on an eight-channel photometer (Titertek-Multiscan).

RABA. The RABA was performed as previously described, using ^{3}H-labeled native (EDTA-extracted) type III polysaccharide as the antigen (3). Antibody to the core polysaccharide of type III GBS, which is identical to the pneumococcal type XIV polysaccharide, was kindly determined by Gerald Schiffman for select sera.

Statistical analysis. Coefficient of correlation was determined by linear regression analysis. The reproducibility of the method was determined by comparing the slopes of replicate enzyme assays by the Student independent *t* test.

RESULTS

ELISA. Serum dilutions ranging from 1/10 to 1/1,000 were tested. A dilution of 1/400 was optimal and yielded optical densities ranging from 0.238 to 0.472. Serum dilutions less than 1/200 were more difficult to interpret because of nonspecific binding of serum to the bottom of the test well.

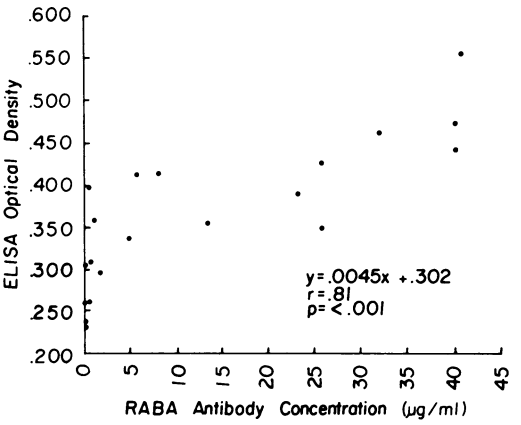

FIG. 1. Correlation of ELISA with RABA. Comparison of results of ELISA and RABA determinations of III GBS antibody in sera from 20 normal adults is shown.

Comparison of ELISA and RABA. There was a significant correlation between the ELISA and RABA determinations for sera from 20 adult volunteers ($r = 0.81$; $P < 0.001$ [Fig. 1]). To evaluate the reproducibility of the ELISA, each of the 20 sera specimens from the adult volunteers was tested on five separate occasions. There were no significant differences in the slopes of curves produced by each replicate run of the assay with these 20 specimens, signifying that the method was highly reproducible. However, adults with high levels of antibody to

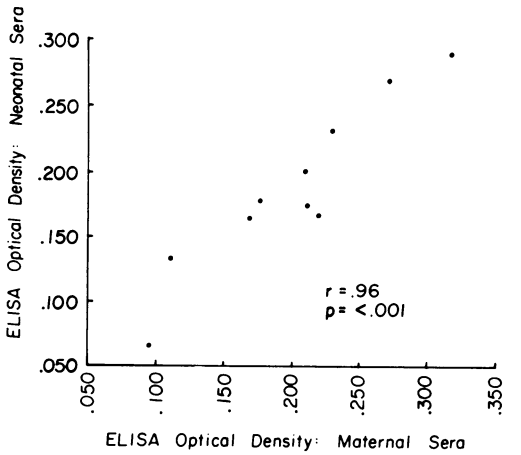

FIG. 2. Comparison of GBS antibody concentrations in maternal and infant sera. ELISA results of III GBS antibody in sera from 10 matched mother-neonate pairs are shown.

native III GBS often have concomitant elevation of antibody to the core antigen of III GBS in their sera (7). Therefore, sera were selected for high levels of antibody to native or core antigens, but not both, to determine the antigenic specificity of the whole-organism ELISA assay. Six sera from individuals with low levels of native antibody and high levels of core antibody (defined by radioimmunoassay with purified polysaccharide antigens) were tested. Five of these six sera had measurable levels of antibody to III GBS as detected by the ELISA (optical density range, 0.145 to 0.428). Conversely, when six sera with high levels of antibody to native antigen and low levels of antibody to core antigen were assessed, each demonstrated III GBS antibody by ELISA (optical density range, 0.298 to 0.546).

Comparison of antibody concentrations in maternal and infant sera. There was a direct relationship between the quantity of antibody to type III GBS in 10 matched maternal and cord blood specimens ($r = 0.96$; $P < 0.001$ [Fig. 2]) when tested in the ELISA. These sera were not tested in the RABA.

DISCUSSION

We have developed an enzyme immunoassay for detection of antibody to III GBS. The data presented in this paper indicate a significant correlation between antibody to the native type III antigen of GBS in sera as detected by the RABA and the whole-bacterial-cell ELISA assay. The specificity of this assay was compared with that of the RABA, employing defined sera and purified polysaccharide antigens. Although the ELISA was able to detect antibody to III GBS in all sera with high levels of antibody to native antigen, it also detected antibody to the core antigen. This difference in specificity is important because the core antigen of the III GBS is identical to pneumococcal type XIV polysaccharide. Human infection with type III GBS has been correlated with low levels of antibody to native, but not core, III GBS polysaccharide (7). Therefore, when sera tested in this ELISA assay are found to have high levels of antibody, this may reflect antibody to pneumococcal type XIV polysaccharide, an antibody which does not correlate with protection against III GBS disease in neonates (7).

ACKNOWLEDGMENTS

We thank Barbara Erwins for her editorial assistance and Judith Bailey for her technical assistance.

This work was supported in part by Public Health Service grant RR-05506-1 from the National Institutes of Health.

LITERATURE CITED

1. **Baker, C. J., F. F. Barrett, R. C. Gordon, and M. D. Yow.** 1973. Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. J. Pediatr. **82**:724–729.
2. **Baker, C. J., M. S. Edwards, and D. L. Kasper.** 1981. Role of antibody to native type III polysaccharide of group B *Streptococcus* in infant infection. Pediatrics **68**:544–549.
3. **Baker, C. J., and D. L. Kasper.** 1976. Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection. N. Engl. J. Med. **294**:753–756.
4. **Edwards, M. S., A. Nicholson-Weller, C. J. Baker, and D. L. Kasper.** 1980. The role of specific antibody in alternative complement pathway mediated opsonophagocytosis of type III group B streptococcus. J. Exp. Med. **151**:1275–1287.
5. **Franciosi, R. A., J. D. Knostman, and R. A. Zimmerman.** 1973. Group B streptococcal neonatal and infant infections. J. Pediatr. **82**:707–718.
6. **Hemming, V. G., R. T. Hall, P. G. Rhodes, A. O. Shigeoka, and H. R. Hill.** 1976. Assessment of group B streptococcal opsonins in human and rabbit serum by neutrophil chemiluminescence. J. Clin. Invest. **58**:1379–1387.
7. **Kasper, D. L., C. J. Baker, R. S. Baltimore, J. H. Crabbe, G. Schiffman, and H. J. Jennings.** 1979. Immunodeterminant specificity of human immunity to type III group B streptococcus. J. Exp. Med. **149**:327–339.
8. **Rote, N. S., N. L. Taylor, A. O. Shigeoka, J. R. Scott, and H. R. Hill.** 1980. Enzyme-linked immunosorbent assay for group B streptococcal antibodies. Infect. Immun. **27**:118–123.
9. **Shigeoka, A. O., J. I. Santos, and H. R. Hill.** 1979. Protection against group B streptococcal infection by administration of leukocytes or antibody containing serum. Pediatr. Res. **13**:468.
10. **Tieffenberg, J., L. Vogel, R. R. Kretschmer, D. Padnos, and S. P. Gotoff.** 1978. A chick embryo model for type III group B beta-hemolytic streptococcal septicemia. Infect. Immun. **19**:481–485.
11. **Vogel, L. C., R. R. Kretschmer, K. M. Boyer, D. M. Padnos, C. A. Gadzala, S. P. Gotoff.** 1979. Human immunity to group B streptococci measured by indirect immunofluorescence: correlation with chicken embryo protection. J. Infect. Dis. **140**:628–689.
12. **Wilkinson, H. W., and W. L. Jones.** 1976. Radioimmunoassay for measuring antibodies specific for group B streptococcal types Ia, Ib, Ic, II, and III. J. Clin. Microbiol. **3**:480–485.