

Radioimmunoassay for *Bacteroides fragilis* Infections

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Radioimmunoassay methods were evaluated for immunoglobulin G and immunoglobulin M antibodies against *Bacteroides fragilis* antigen. Of 12 serum samples from patients with *B. fragilis* infections, 9 had higher concentrations of immunoglobulin G antibodies than any from 11 control subjects. Of 9 serum samples from infected patients, 6 had higher concentrations of immunoglobulin M than any from control subjects. Six serum samples from patients with *Escherichia coli* bacteremia did not contain elevated concentrations of immunoglobulin G or immunoglobulin M antibodies against *B. fragilis* antigen.

Infection with *Bacteroides fragilis* has been recognized with increasing frequency as a result of improvements in anaerobic methodology. Cultural diagnosis of *B. fragilis* infections may require surgical exploration of intraabdominal abscesses, osteomyelitis, or intracranial abscesses. In addition, anaerobic organisms may not be cultured if good anaerobic methods are not available. Several serological procedures have been used for the study of *B. fragilis* infections, including hemagglutination, fluorescent antibody tests, gel precipitation, radioimmunoassay (RIA) with a tagged *B. fragilis* polysaccharide, and an enzyme-linked immunosorbent assay (1-16; W. L. Hoppes, S. C. Bruins, J. W. Smith, and A. White, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 407, 1978; R. D. Meyer, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, Atlanta, Ga., abstr. no. 169, 1977). In the current study, we have evaluated an RIA method for the detection of immunoglobulin G (IgG) and IgM antibodies against *B. fragilis* antigen.

MATERIALS AND METHODS

Methods for agar gel diffusion for *B. fragilis* antibodies and for preparing ultrasonic supernatants of *B. fragilis* have been published previously (14). A strain of *B. fragilis* obtained from a patient with intraabdominal abscesses was used as a source of antigen. Serum samples from 12 patients with *B. fragilis* infections, 11 uninfected control subjects, and 6 patients with bacteremia due to *Escherichia coli* secondary to pyelonephritis were tested by RIA method. All sera were obtained at least 1 week after the onset of symptoms, with a median of 18 days and a range of 7 to 46 days. For the RIA, 0.2 ml of a 1:10 dilution of the ultrasonic

supernatant of *B. fragilis* was used to coat plastic tubes (75 by 100 ml). After incubation overnight, unbound antigen was removed, and the tubes were washed three times. Bovine serum albumin (5%) was added to coat the remaining binding sites on the tube; after 1 h of incubation at 37°C and three washings, a 1:10 dilution of sera was added to the tube and incubated at 37°C for 1 h. After three washings, IgM or IgG antibodies were detected by goat antihuman IgM or goat antihuman IgG tagged with ¹²⁵I. After 1 h of incubation at 37°C and three washings, the tubes were counted in a gamma counter. The results were expressed as the ratio of the counts per minute of test sera to the counts per minute of a single control serum sample.

RESULTS

Serum samples from uninfected control subjects had a narrow range of IgG antibodies against *B. fragilis* antigen, ranging up to two times the values found for the single control serum sample (Fig. 1). Of 12 serum samples from patients infected with *B. fragilis*, 9 had counts higher than 2 times those of the controls; none of 5 serum samples from patients with *E. coli* bacteremia secondary to pyelonephritis had counts higher than those of the uninfected subjects. All of the sera from patients with *E. coli* bacteremia produced precipitins against *E. coli* extract detected by agar gel diffusion, but none of the uninfected subjects produced precipitins against this extract.

Nine serum samples from patients infected with *B. fragilis* were also tested for IgM antibodies; six of the nine serum samples had ratios of test sera counts per minute to control sera counts per minute of greater than three. None of the serum samples of the control subjects had ratios greater than three (Fig. 2). In addition, six serum samples obtained from patients with *E. coli* bacteremia did not have antibody titers against *B. fragilis* higher than those of serum

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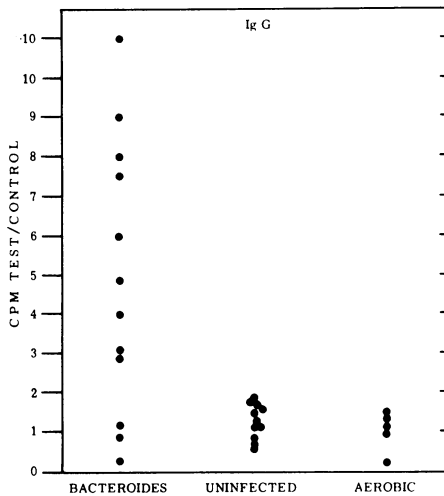


FIG. 1. IgG antibodies against *B. fragilis* in patients with *B. fragilis* infections and *E. coli* infections (aerobic) and in uninfected controls.

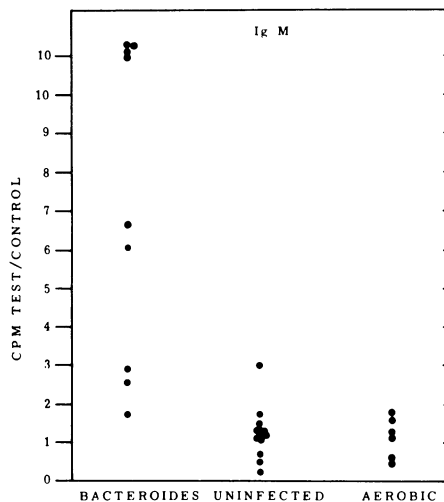


FIG. 2. IgM antibodies against *B. fragilis* in patients with *B. fragilis* infections and *E. coli* infections (aerobic) and in uninfected controls.

samples from uninfected subjects.

Precipitins against *B. fragilis* were detected by agar gel diffusion methods in 7 of 12 serum samples from patients with *B. fragilis* infections but not in 11 serum samples of uninfected subjects or in serum samples from patients with *E. coli* bacteremia.

DISCUSSION

Several authors have reported studies of antibodies against *B. fragilis* antigen with hemagglutination, precipitin tests, enzyme-linked immunosorbent assays, or an RIA with tagged *B.*

fragilis lipopolysaccharide. Hemagglutination methods are more effective in detecting IgM antibodies than in detecting IgG antibodies. Enzyme-linked immunosorbent assays also differentiate between infected and noninfected subjects. The RIA method allows the evaluation of both the IgG and IgM responses to the *B. fragilis* infection. The published enzyme-linked immunosorbent assay reports evaluated only IgG responses (9, 13). In addition, the RIA method is more rapid than precipitin test methods. The RIA results described here can be available in 3 h with precoated tubes, whereas precipitin tests by agar gel diffusion require 3 days.

Only limited data are available on the specificity of the RIA methods in this study. Six patients with *E. coli* bacteremia with antibodies against *E. coli* detected by agar gel diffusion did not have antibodies to *B. fragilis* antigen detected by the RIA. Unpublished studies from this laboratory have demonstrated that rabbit antisera against *Streptococcus sanguis*, *Streptococcus mitior*, *Streptococcus salivarius*, and *Streptococcus millieri*, as well as rabbit antisera against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter*, *Klebsiella pneumoniae*, and *E. coli*, did not have significantly higher titers of antibodies against *B. fragilis* than control sera, whereas rabbit antisera against *B. fragilis* did have higher titers of antibody. We have not tested rabbit antisera against other anaerobes. Rissing et al. (13) reported limited cross-reactivity between sera from patients infected with other *Bacteroides* species and the *B. fragilis* lipopolysaccharide. Further evaluation of the specificity of the RIA and the enzyme-linked immunosorbent assay require testing of sera from a large group of patients infected with other organisms, including other species of *Bacteroides*.

LITERATURE CITED

1. Brown, W. R., and E. Lee. 1974. Radioimmunological measurements of bacterial antibodies. II. Human serum antibodies reactive with *Bacteroides fragilis* and *Enterococcus* in gastrointestinal and immunological disorders. *Gastroenterology* **66**:1145-1153.
2. Danielsson, D., D. W. Lambe, Jr., and S. Persson. 1972. The immune response in a patient to an infection with *Bacteroides fragilis* ss. *fragilis* and *Clostridium difficile*. *Acta Pathol. Microbiol. Scand.* **80**:709-712.
3. Helphingstine, C. J., D. J. Hentges, B. J. Campbell, J. Butt, and J. T. Barrett. 1979. Antibodies detectable by counterimmunoelectrophoresis against *Bacteroides* antigens in serum of patients with inflammatory bowel disease. *J. Clin. Microbiol.* **9**:373-378.
4. Hofstad, T. 1974. Antibodies reacting with lipopolysaccharides from *Bacteroides melanogenicus*, *Bacteroides fragilis*, and *Fusobacterium nucleatum* in serum from normal human subjects. *J. Infect. Dis.* **129**:349-352.
5. Kasper, D. L. 1976. The polysaccharide capsule of *Bac-*

- teroides fragilis* subspecies *fragilis*: immunochemical and morphologic definition. *J. Infect. Dis.* **133**:79-87.
6. **Kasper, D. L., D. A. Eschenbach, M. E. Hayes, and K. K. Holmes.** 1978. Quantitative determination of the serum antibody response to the capsular polysaccharide of *Bacteroides fragilis* ss. *fragilis* in women with pelvic inflammatory disease. *J. Infect. Dis.* **138**:74-80.
 7. **Kasper, D. L., A. B. Onderdonk, and J. G. Bartlett.** 1977. Quantitative determination of the antibody response to the capsular polysaccharide of *Bacteroides fragilis* in an animal model of intraabdominal abscess formation. *J. Infect. Dis.* **136**:789-795.
 8. **Kasper, D. L., A. B. Onderdonk, B. F. Polk, and J. G. Bartlett.** 1979. Surface antigens as virulence factors in infection with *Bacteroides fragilis*. *Rev. Infect. Dis.* **1**: 278-288.
 9. **Lambe, J. W. Jr., D. Danielsson, D. H. Vroon, and R. K. Carver.** 1975. Immune response in eight patients infected with *Bacteroides fragilis*. *J. Infect. Dis.* **131**: 499-508.
 10. **Landay, M. E., and W. Baldwin.** 1979. Comparative serology of two clinical isolates of *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*. *J. Clin. Microbiol.* **9**:643-644.
 11. **Lindberg, A. A., A. Weintraub, and C. E. Nord.** 1979. The humoral antibody response to *Bacteroides fragilis* infections in humans. *Scand. J. Infect. Dis.* **19**(Suppl.): 46-51.
 12. **Quick, J. D., H. S. Goldberg, and A. C. Sonnenwirth.** 1972. Human antibody to *Bacteroidaceae*. *Am. J. Clin. Nutr.* **25**:1351-1356.
 13. **Rissing, J. P., T. B. Buxton, and H. T. Edmondson.** 1979. Detection of specific IgG antibody in sera from patients infected with *Bacteroides fragilis* by enzyme-linked immunosorbent assay. *J. Infect. Dis.* **140**:944-998.
 14. **Rissing, J. P., J. G. Crowder, J. W. Smith, and A. White.** 1974. Detection of *Bacteroides fragilis* infection by precipitin antibody. *J. Infect. Dis.* **130**:70-73.
 15. **Schwan, A., and A. C. Ryden.** 1978. Antibody response in thirteen patients with *Bacteroides fragilis* infections. *Scand. J. Infect. Dis.* **10**:213-218.
 16. **Sonnenwirth, A. C.** 1979. Antibody response to anaerobic bacteria. *Rev. Infect. Dis.* **1**:337-341.