Comparative Serology of Two Clinical Isolates of *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*

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Antiserum prepared in rabbits against *Bacteroides fragilis* showed numerous bands when reacted with *B. fragilis* antigen in Ouchterlony plates. This antiserum also reacted with *Bacteroides thetaiotaomicron* and showed one band of apparent identity with *B. fragilis*. The band of identity common for both organisms was lost if the antigen was heated at 80°C for 30 min. Antisera prepared against *B. thetaiotaomicron* did not react with *B. fragilis*.

An increasing number of infections with *Bacteroides* species are being detected. Identification of these organisms by cultural methods may require up to 4 days, but serological diagnosis would be more rapid. Because a serological method of identification of *Bacteroides* species does not now exist, we initiated a study of the comparative serology of *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*.

The immunogen was prepared from cells of a clinical isolate of *B. fragilis* (homologous strain) and from cells of *B. thetaiotaomicron* ATCC 8492 (6). Clinical isolates were identified by Analytab Products, Inc., Plainview, N.Y. Antisera were prepared in two groups of New Zealand white rabbits by giving them six intravenous injections of the respective antigens. Sera were obtained by cardiac puncture 6 days after the last injection of antigen (6).

Antigens were derived from two clinical isolates of *B. fragilis*, the homologous strain mentioned above and a heterologous one, and from *B. thetaiotaomicron* ATCC 8492. The cells were disrupted with glass beads (4) and centrifuged in a refrigerated centrifuge at 13,000 × *g* for 10 min. The supernatant was collected and used as the crude antigen. To determine heat lability, crude antigens were heated at 60 or 80°C for 30 min. Protein concentrations of the crude antigens were determined with a refractometer and adjusted to 8 mg of protein per ml with physiological saline.

Purified *B. fragilis* and *B. thetaiotaomicron* antigens were prepared by gel filtration of 1 ml of crude antigen through an EM gel SI 1000Å column. Fractions 2 through 6 from the column were one peak and were pooled, whereas the remaining fractions exhibited only background optical density. Pooled fractions 2 through 6

were lyophilized and stored at -4°C until reconstituted with distilled water. After reconstitution the sample was dialyzed against physiological saline at 4°C so that the antigen was contained in 1 ml of physiological saline.

Reactions between antigens and antisera were assayed by Ouchterlony plates obtained from Meloy Laboratories, Inc., Springfield, Va.

Tests with *B. fragilis* antisera. Five bands were seen in the *B. fragilis* crude antigen preparation. Four of these bands were stable to heating at 80°C for 30 min, and one band, which showed a line of apparent identity with *B. thetaiotaomicron*, was not stable to heating. However, this line was stable at 60°C for 30 min (Fig. 1).

Heterologous *B. fragilis* showed one band of complete identity and several bands of partial identity with the homologous strain (Fig. 2). Both antigens shared one line with *B. thetaiotaomicron*, which was later seen to be the heat-labile band.

When the homologous *B. fragilis* antigen was purified by gel filtration, three reactive bands were seen. One of these bands, which was heat labile, showed a line of apparent identity with *B. fragilis* and *B. thetaiotaomicron*.

Tests with *B. thetaiotaomicron* antisera. *B. thetaiotaomicron* crude antigen showed three heat-stable bands, and *B. fragilis* crude antigens and purified fractions 2 through 6 failed to react with antisera (data not shown).

In conclusion, the presence of heat-labile soluble antigen was demonstrated in two isolates of *B. fragilis*. Previous reports (3) indicate that the agglutinating antigens of this organism are heat stable. Conceivably the heat-labile band was a sequestered surface polymer. We suggest this polymeric nature because of the exclusion limit

FIG. 1. Center well, *B. fragilis* pooled antiserum. Outside wells: well 1, *B. thetaiotaomicron* crude extract heated at 60°C; well 2, *B. thetaiotaomicron* crude extract heated at 80°C; well 3, *B. fragilis* crude extract heated at 80°C; well 4, *B. fragilis* crude extract heated at 60°C; well 5, *B. fragilis* crude extract; and well 6, *B. thetaiotaomicron* crude extract.

FIG. 2. Center well, *B. fragilis* pooled antiserum. Outside wells: well 1, *B. fragilis* crude extract (homologous strain); well 2, *B. fragilis* crude extract (heterologous strain); well 3, *B. thetaiotaomicron* crude extract.

for the column (molecular weight, 1,000,000) and because the antigen came off the column just after the void volume.

There is confusion concerning the cross-reactivity between *B. fragilis* and *B. thetaiotaomicron*. Some authors claim cross-reaction with sonic extracts (5), whereas others see no cross-reaction (1, 7). This study demonstrated that

the cross-reaction with *B. thetaiotaomicron* was present and that when these antigens were prepared with glass beads, the antigens were heat labile.

As *B. fragilis* is one of the most common anaerobic clinical pathogens, a concentrated, purified preparation of the heat-labile band might be useful for detection of infection caused by this organism. The crude *B. thetaiotaomicron* antigen could be reacted with *B. fragilis* antiserum, and in this way the heat-labile band may be purified and subsequently coupled to a particle for an agglutination test.

Kasper (2) shows that numerous strains of *B. fragilis* contain a high-molecular-weight capsular polysaccharide. This is demonstrated by using material obtained after trypsinization. Because the antigen we have described was heat labile, lost reactivity after several months at 4°C, and appeared to be trypsin sensitive, we proposed that this antigen was probably a protein and was possibly eliminated by Kasper during trypsin treatment (2). However, all other bands in this study were heat stable, stable to storage at 4°C for several months, resistant to trypsin treatment, and were possibly polysaccharide in nature. This correlates well with the work of Kasper (2). The nature of the heat-labile antigen is currently under investigation in our laboratories.

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