Vibrio vulnificus (Lactose-Positive Vibrio) and Vibrio parahaemolyticus Differ in Their Susceptibilities to Human Serum

MARY M. CARRUTHERS* AND WILLIAM J. KABAT
Veterans Administration Lakeside Medical Center and Department of Medicine, Northwestern University Medical School, Chicago, Illinois 60611

Eleven Vibrio vulnificus (lactose-positive vibrio) strains were less susceptible to the bactericidal activity of normal human serum or serum treated with magnesium-ethyleneglycol-bis(β-aminoethyl ether)-N,N'-tetraacetic acid than were six Vibrio parahaemolyticus strains.

Vibrio parahaemolyticus and Vibrio vulnificus (this nomenclature for V. vulnificus has been agreed upon, as reported recently by Baumann[1]) (lactose-positive vibrio, Beneckea vulnifica) are biochemically and morphologically similar bacteria which cause quite different diseases when they infect humans. The two bacteria were not differentiated in some early reports, but it now appears that V. parahaemolyticus usually causes gastrointestinal infection, and rarely wound infection, but it almost never causes bacteremia. On the other hand, about half of the reported cases of V. vulnificus disease are wound infections, and the remainder are bacteremias, of which about three-quarters occur in individuals with preexisting hepatic disease (2). The association of V. vulnificus, but not V. parahaemolyticus, with systemic infection makes it likely that the two bacteria differ in their susceptibilities to host defense mechanisms.

Complement-mediated bactericidal activity is considered a primary defense against gram-negative bacteria, and conversely, bacteria isolated from bloodstream infections are frequently serum resistant (9). The reported deficiency of cirrhotic sera in bactericidal activity against serum-sensitive Escherichia coli (3) prompted us to explore whether differences between V. parahaemolyticus and V. vulnificus in their susceptibilities to serum might explain their different pathogenicities. We examined the susceptibilities of 6 V. parahaemolyticus and 11 V. vulnificus strains to killing by normal human serum and magnesium-ethyleneglycol-bis(β-aminoethyl ether)-N,N'-tetraacetic acid (Mg-EGTA)-treated human serum to evaluate the contribution of the classical and alternative pathways of complement activation to bactericidal activity.

V. parahaemolyticus strains examined were M5242-1, 33C10, MY001, WP1, and MY74-001, all Kanagawa-positive human fecal isolates, and M5253J1, a Kanagawa-negative seafood isolate. Strains M5242-1 and M5253J1 were supplied by the Centers for Disease Control, Atlanta, Ga., strain 33C10 was supplied by the Food and Drug Administration, Washington, D.C., and strains MY001, WP1, and MY74-001 were supplied by Yoshifumi Takeda, Osaka, Japan. V. vulnificus strains were blood isolates A3308, A6694, C7184, C8806, E6184, E6185, soft-tissue isolates A1402, B3547, C2756, D9889, and oyster isolate D4473. All strains were originally supplied by Dannie Hollis of the Centers for Disease Control. Some strains were transmitted to us by James D. Oliver of the University of North Carolina, Charlotte, N.C. Bacteria were stored on cystine tryptic agar slants. Bacteria were streaked for purity, inoculated into veal infusion broth with NaCl added to 2%, and incubated at 37°C for 4 to 5 h in a shake culture.

Blood was obtained by venipuncture of three or more healthy individuals without history of vibrio infection or contact. Pooled sera were separated and used immediately or stored in aliquots at −70°C. Fresh or freshly thawed serum was used unaltered and heated at 56°C for 30 min and/or made approximately 10 mM Mg-EGTA by the addition of a stock solution of 100 mM Mg-EGTA prepared by the method of Fine (4). Serum-sensitive strain A1402 was used as a control for each experiment.

The bacterial inoculum (approximately 1 × 10⁷ colony-forming units) in 0.35 ml of phosphate-buffered saline and 0.65 ml of treated or untreated serum were mixed in a plastic tube; 0.1 ml of the mixture was withdrawn for an initial viable count, and the remainder was incubated at 37°C for 30 or 60 min. Tenfold dilutions were made in phosphate-buffered saline, inoculated on duplicate plates of brain heart infusion agar containing 2% NaCl, incubated overnight at 37°C, and read. More than 1 log₁₀ reduction in colony-forming units was consid-
ered to represent significant bactericidal activity because the decrease in tubes containing heat-inactivated normal serum or heat-inactivated, Mg-EGTA-treated serum was less. For strain A1402, log₁₀ reduction in colony-forming units in heat-inactivated normal serum was 0.55 ± 0.16 at 30 min and 0.42 ± 0.12 at 60 min. In heat-inactivated, Mg-EDTA-treated serum, log₁₀ reduction was 0.45 ± 0.17 at 60 min. Bacterial growth by any of the vibrio strains in heat-inactivated serum occurred during the experimental period only once in more than 100 observations.

The *V. parahaemolyticus* strains were uniformly serum sensitive (Fig. 1), whereas *V. vulnificus* strains varied in their sensitivities. The five *V. vulnificus* strains isolated originally from blood were somewhat less sensitive than the five soft-tissue isolates or the single environmental isolate, but sensitive and resistant strains were found in each group (Fig. 2).

Bactericidal tests were run in parallel with normal serum and Mg-EGTA-treated serum on the six *V. parahaemolyticus* and six serum-sensitive *V. vulnificus* strains. Bactericidal activity was determined at 30 and 60 min with the Mg-EGTA-treated serum because of the delayed activation of the alternative pathway observed with other bacterial genera (5). Bactericidal activity in Mg-EGTA-treated serum was more rapid and more complete against *V. parahaemolyticus* strains than against *V. vulnificus* strains. Bactericidal activity is shown in Fig. 3 for one strain of each bacterium, but each strain was typical of its group.

These results suggest that some strains of *V. vulnificus* are not susceptible to the bactericidal action of normal serum by either pathway of complement activation, whereas the remaining strains activate the classical pathway well and the alternative pathway less effectively. These findings are of interest in light of the observations of Fierer and Finley on sera from cirrhotic individuals (3). Cirrhotic sera had an intact alternative pathway of complement-mediated bactericidal activity against *E. coli* but often had suboptimal classical pathway activity. Thus, if cirrhotic individuals are dependent primarily on alternative pathway activation for bactericidal activity against gram-negative bacteria, they might be more vulnerable to systemic invasion by bacteria, such as *V. vulnificus* which are less efficient activators of the alternative pathway.

These results support the concept, previously advanced from animal studies by Poole and Oliver (8), that *V. vulnificus* has more potential for systemic invasion than does *V. parahaemolyticus*. We examined the three *V. vulnificus* strains used by those investigators and found...
FIG. 3. Bactericidal activity of normal human serum (NHS) and Mg-EDTA-treated serum on (---) *V. parahaemolyticus* M5242-1 and on (---, ---, ---) *V. vulnificus* E6184.

the increasing serum susceptibilities of the strains to parallel the increase in 50% lethal doses observed in their mouse studies. Murine serum has been reported to lack bactericidal activity (6), although murine alternative pathway activity has been demonstrated recently in a hemolytic system (7).

**LITERATURE CITED**


