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Differential Complement Activation and Susceptibility to Human Serum Bactericidal Action by *Vibrio* Species

M. L. TAMPLIN,[1]* S. SPECTER,[1] G. E. RODRICK,[2] AND H. FRIEDMAN[1]

Departments of Medical Microbiology and Immunology[1] and Comprehensive Medicine,[2] University of South Florida College of Medicine, Tampa, Florida 33612

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The ability of *Vibrio vulnificus* to resist human serum bactericidal action and to activate human complement was compared with similar cultures of *Vibrio cholerae* and *Vibrio parahaemolyticus*. Both *V. vulnificus* and *V. parahaemolyticus* had similar survival rates in sera and were much more resistant to killing than was *V. cholerae*. In contrast, *V. vulnificus* activated significantly less serum complement than did *V. cholerae* and *V. parahaemolyticus*. The relative ability of *V. vulnificus* to survive in serum and activate less complement than other *Vibrio* spp. tested may be related to its ability to cause chronic tissue infections and septicemias.

Vibrio vulnificus, a marine bacterium, can cause serious human disease resulting from contact with contaminated seawater or consumption of contaminated sealife (1, 2, 6, 8, 11, 17, 19, 26). However, serious infections usually occur in individuals with preexisting disease, especially liver disease (1, 2, 11). The predisposition of these individuals to *V. vulnificus* infections, plus the ability of the bacterium to invade tissues, suggests that this organism may have pathogenic properties differing from those of other *Vibrio* spp. normally causing limited gastrointestinal disease (1–4, 17, 24, 31).

Individuals with various types of liver disease are at greater risk of bacterial infections (5, 9, 10, 12, 15, 16, 23, 27–29). Possible reasons for this include depressed Küpffer cell activity (15, 16, 23), increased serum iron (31), and lower serum bactericidal action (9). Abnormalities in these host defenses might permit bacteria to persist in tissues. Moreover, if the bacterium resisted host defenses, the infection would likely progress.

In the present study, we report differences in complement activation (i.e., a reduction in the complement-mediated hemolytic titer of serum) and survival in human serum of *V. vulnificus* in comparison with *Vibrio cholerae* and *Vibrio parahaemolyticus*.

V. vulnificus was represented by three clinical isolates designated C1, C2, and C3. C1 was a blood isolate obtained from a Florida hospital, whereas C2 and C3 were isolates CDC 7184 and CDC 3457, respectively, kindly provided by Dannie Hollis of the Centers for Disease Control, Atlanta, Ga. *V. vulnificus* environmental isolates were designated 1, 14, 25, 85, and 86. *V. parahaemolyticus* environmental isolates were designated 2, 33, 37, 45, 46, 70, 87, and 89. *V. cholerae* isolates were represented by two clinical O-1 isolates serotyped Inaba (IN) and Ogawa (OG), and by two non O-1 environmental isolates designated 15 and 80.

For testing, bacteria were grown on brain heart infusion (BHI) agar (Difco Laboratories, Detroit, Mich.) for 15 min. at 37°C. Colonies were transferred from BHI plates into phosphate-buffered saline (PBS), pH 7.2, and washed by centrifugation at 1,500 × *g*. CFU and total counts were adjusted spectrophotometrically at 420 nm. Viable counts were determined on TCBS (thiosulfate-citrate-bile salts-sucrose agar) (Difco Laboratories). Total bacterial counts were measured with a Petroff-Hausser counting chamber (G. A. Hausser and Son, Philadelphia, Pa.). For some experiments, bacteria were grown at 37°C to mid-logarithmic growth phase in BHI broth (Difco Laboratories) or were resuscitated for 2 h at 37°C in peptone broth (Difco Laboratories), pH 8.4.

Human serum was obtained and pooled from at least 10 healthy individuals and stored at −70°C. Serum was used either untreated or treated by methods known to inactivate complement components as follows (22): (i) heat inactivation at 56°C for 30 min, (ii) incubation at 37°C for 60 min with 25 U of cobra venom factor (Cordis Laboratories, Inc., Miami, Fla.) per ml of serum, or (iii) incubation at 37°C for 60 min with 60 μl of goat anti-human C3 (Cordis Laboratories) per ml of serum. Heat inactivation, cobra venom factor, and anti-C3 treatments decreased the 50% serum complement-mediated hemolytic titer (CH_{50}) 99, 92, and 92%, respectively. For studies of the bactericidal action of

human serum, 1.0 ml of 75% serum in PBS was inoculated with 10^7 CFU in 0.05 ml of PBS and then incubated at 37°C. At selected time intervals, 0.1 ml of 10-fold serial dilutions were plated on TCBS agar.

Complement activation was measured as CH_{50} titers determined by a microtiter version of the method of Mayer (18). Fresh bacterial isolates from BHI agar, BHI broth, peptone broth, or after 1% Formalin treatment were suspended in PBS to a final concentration of 10^{10} bacteria per ml. One-tenth milliliter of bacterial suspension or PBS alone was added to 0.3 ml of undiluted serum and incubated for 2 h at 37°C (20). Complement activation was calculated for each isolate as 100 minus the percentage of the control CH_{50} remaining in the sample.

Bacterial microagglutinating antibody titers were determined by established techniques (7). Significant statistical differences in survival and complement activation of *Vibrio* spp. were determined by using Student's *t* test.

Serum bactericidal activity against both *V. vulnificus* and *V. parahaemolyticus* isolates was significantly lower than against *V. cholerae* isolates ($P < 0.05$) (Table 1). There was no significant difference between the former strains, whereas almost no *V. cholerae* survived the 1-h incubation with the serum. There was no difference in bactericidal action against *V. vulnificus* isolates from clinical versus environmental origins.

Figure 1 shows the effects of heat inactivation, cobra venom factor, and goat anti-human C3 on the bactericidal action of serum against clinical isolate C2 of *V. vulnificus*. All three treatments resulted in a significant reduction in bactericidal activity over that of the PBS controls ($P < 0.05$).

TABLE 1. Survival of vibrios in human serum

Bacteria tested[a]	Isolate designation	CFU/ml[b]	
		0 min ($\times 10^6$)	60 min ($\times 10^3$)
V. vulnificus	1	8.8 (0.29)	85.0 (1.50)
	25	8.0 (0.36)	5.2 (0.20)
	C1	7.9 (0.10)	44.0 (0.47)
	C3	6.1 (0.10)	0.07 (0.01)
V. parahaemolyticus	2	7.5 (0.09)	330.0 (2.10)
	33	8.1 (0.51)	34.0 (3.40)
	37	9.0 (0.09)	0.06 (0.01)
	70	6.8 (0.43)	180.0 (15.0)
V. cholerae	15	7.9 (0.04)	<0.01 (<0.01)
	80	5.8 (0.63)	<0.01 (<0.01)
	IN	9.2 (0.54)	ND[c]
	OG	7.2 (0.04)	ND

[a] 10^7 vibrios were incubated in 1.0 ml of 75% untreated human serum at 37°C.

[b] Average number of CFU per milliliter with standard deviation in parenthesis.

[c] ND, Not detected.

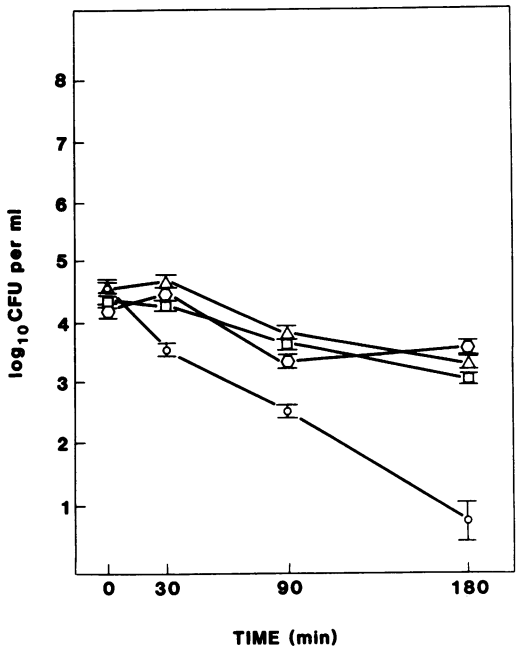

FIG. 1. Survival of a clinical isolate of *V. vulnificus* (C2) in untreated (○), heat-inactivated (◯), cobra venom factor (□), and anti-C3 (△)-treated sera.

Since serum complement appeared to significantly contribute to the killing of *V. vulnificus* (Fig. 1), species differences in complement activation were assessed. *V. vulnificus* isolates overall activated less serum complement than did *V. cholerae* ($P < 0.0005$) and *V. parahaemolyticus* isolates ($P < 0.0005$) (Table 2). Also, *V. parahaemolyticus* isolates activated more complement than did *V. cholerae* isolates ($P < 0.005$). It is of interest that *V. vulnificus* isolate 1 activated more complement under these test conditions than did the other *V. vulnificus* isolates. The reason(s) for this is unknown.

To determine whether greater complement activation by *V. parahaemolyticus* was due to more serum antibody, bacterial microagglutinating antibody titers of serum were measured for sera used in both survival and complement activation assays. No correlation existed between greater complement activation and higher bacterial microagglutinating antibody titers.

Although not shown, vibrio isolates killed with 1% Formalin showed the same species differences in complement activation as did the viable bacteria. Futhermore, resuscitating the bacteria or culturing them until mid-logarithmic growth phase did not affect species differences in complement activation.

TABLE 2. Complement activation by vibrio isolates

Bacteria tested	Vibrio isolate	Reduction in CH_{50} units[a] (SD)	Complement[b] activation (%)
Heat inactivation (positive control)		145.9 (1.3)	98.9
PBS (negative control)		0.0 (0.8)	0.0
V. vulnificus	1	93.3 (2.4)	63.3
	14	55.3 (1.7)	37.5
	25	58.6 (1.3)	39.8
	85	62.6 (2.3)	42.5
	86	71.0 (0.5)	48.2
	C1	53.3 (4.2)	36.2
	C2	48.5 (1.9)	32.3
	C3	44.2 (0.3)	30.0
V. parahaemolyticus	2	84.3 (2.8)	57.2
	33	147.4 (0.0)	100.0
	37	147.4 (0.0)	100.0
	45	147.4 (0.0)	100.0
	46	147.4 (0.0)	100.0
	70	147.4 (0.0)	100.0
	87	61.2 (3.4)	41.5
	89	147.4 (0.0)	100.0
V. cholerae	15	78.5 (4.1)	53.3
	80	81.8 (2.9)	55.5
	IN	85.2 (0.4)	57.8
	OG	77.2 (1.1)	52.4

[a] Average reduction in CH_{50} units from a control which received only PBS.

[b] Calculated as 100 − percentage of control CH_{50} remaining in sample.

These results suggest that there is less activation of the complement system with *V. vulnificus* than with *V. parahaemolyticus* or *V. cholerae*. In addition, *V. vulnificus* is more resistant to serum bactericidal action than is *V. cholerae*. These data are generally similar to those reported by Carruthers and Kabat for serum bactericidal activity against *V. vulnificus* and *V. parahaemolyticus* (4). However, these experiments were performed with different bacterial strains, serum pools, and conditions of assay, which might explain slight differences in results, since different strains of both species vary greatly in their ability to survive in serum (4). The studies of Wright et al. (31), which imply a need for complement for bactericidal activity of human serum, are further supported by our data (Fig. 1). Thus, these results are consistent with other studies indicating that both *V. vulnificus* and *V. parahaemolyticus* have relatively low sensitivity to serum bactericidal activity compared with *V. cholerae*.

Although bacterial-bound complement components were not quantitatively measured, bacteria-activating complement could be agglutinated with goat anti-human C3 serum, and in studies not reported here, C3b was found to bind to *V. vulnificus* and *V. parahaemolyticus* isolates, as measured by visible fluorescence after treating bacteria with human serum and fluorescein-conjugated anti-human C3b.

Previous investigators have shown that activation of the complement system leads to the binding of complement components to microbial surfaces (i.e., opsonization) (14, 21). Such opsonization then leads to efficient binding and uptake of the microbe by the reticuloendothelial system (14). Greater activation of complement then leads to more opsonization (30). Thus, lower complement activation by *V. vulnificus* could reduce opsonization. Preliminary results suggest that *V. vulnificus* is phagocytized much less efficiently than comparable numbers of *V. parahaemolyticus* are (13).

The results of these studies suggest that, in addition to the production of exotoxin (13) and collagenase (25) plus enhanced growth by elevated serum iron (31), a reduced ability to activate complement may contribute to the association of *V. vulnificus* with chronic tissue infections.

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