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The Role of Complement in Limiting Bacteremia Caused by Streptococcal L-Phase Variants (40905)[1]

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Abstract. Bacterial L-phase variants may participate in the pathogenesis of some infectious diseases. Previous studies performed *in vitro* have demonstrated that L-phase variants of *Streptococcus faecalis* are able to activate both the classical and alternative pathways of complement and that this activation results in death of the organism. The present study was performed in order to determine whether the complement system serves a protective role, *in vivo*, against L-phase variants of *S. faecalis*. Mice were depleted of the third component of complement (C3) by treatment with purified cobra venom factor. The C3 depleted mice had a significantly greater magnitude of bacteremia at 1 and 3 hr after the intraperitoneal injection of L-phase variants of *S. faecalis* than did control animals. Both groups of animals yielded sterile blood cultures at 8 hr. These studies suggest that the complement system participates in the host's defense against L-phase variants of bacteria.

There is some evidence that bacterial L-phase variants may participate in the pathogenesis of infectious diseases. A number of studies have implicated these cell wall deficient organisms in the persistence or recurrence of some bacterial infections (1–3). Despite a great deal of knowledge concerning the structure and metabolism of L-phase variants, relatively little is known about the host's defense against these oragnisms.

The complement system is known to function as an important defense against bacterial infections (4). However, its role in the host's defense against infections caused by L-phase variants is less clearly defined. Previous studies have demonstrated that L-phase variants of *Streptococcus faecalis* are able to activate both the classical and the alternative pathways of complement and that this activation results in death of the organism (5, 6). These *in vitro* studies have suggested that the complement system has the potential to participate in the host's defense against bacterial L-phase variants. Accordingly, the present study was performed in order to determine whether the complement system serves a protective role, *in vivo*, against L-phase variants of *S. faecalis*.

Materials and methods. Bacterial L-phase variants of *S. faecalis*, strain GK-L (ATCC 23242), were cultured and quantitated as previously described (5). Media was prepared using 37 g of brain-heart-infusion broth (Difco), 5 g of yeast extract (Difco), 9.3 g of NaCl, 97.3 g of sucrose, and 0.5 ml of 50% $MgSO_4$ per liter and supplemented with 10% γ-globulin free horse serum (Gibco) and 500 units/ml of penicillin G (Pfizer). Solid media were prepared by the addition of 13.3 g of agar per liter (Difco).

Cobra venom factor (CoVF) was purified from the venom of the Siamese cobra, *Naja naja* (Ross Allen Reptile Institute, Silver Springs, Fla.), by the sequential use of DEAE-cellulose chromatography and preparative polyacrylamide gel electrophoresis as previously described (7). Three micrograms of this purified CoVF preparation reduced the functional hemolytic titer of C3 in a 1/10 dilution of guinea pig serum by over 95% after incubation at 37°C for 30 min.

Swiss–Webster mice, weighing approximately 17 g each, were depleted of the

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third component of complement (C3) by the intraperitoneal injection of 10 μg of purified CoVF in 0.1 ml of saline at the beginning of each experiment and again after 48 hr as previously described (8). Control mice were injected with 0.1 ml of saline alone. Serum C3 was determined by use of a functional hemolytic assay (9). Serum C3 was undetectable, in the mice used in these experiments, within 2 hr after injection of CoVF and remained undetectable for a minimum of 4 days thereafter. Mice were challenged with L-phase organisms from 18-hr broth cultures, or dilutions thereof, 3 hr after treatment with CoVF.

Results. The intraperitoneal injection of *S. faecalis* L-phase variants in numbers ranging from 1.5×10^4 to 1.5×10^9 viable organisms resulted in neither death nor discernable morbidity in any of the CoVF treated or control mice for up to 4 weeks following challenge with the L-phase variants.

In another series of experiments, mice were given 3×10^9 viable *S. faecalis* L-phase organisms intraperitoneally and at the desired interval, a 10-μl sample of blood was obtained aseptically from the mouse's tail and placed on solid media for quantitation of bacteremia. As can be seen in Fig. 1, the magnitude of bacteremia in the CoVF treated mice was significantly greater at 1 hr ($P < 0.01$) and at 3 hr ($P < 0.02$) than in the control group of mice. There was no significant difference in the magnitude of bacteremia at 5 hr, and both groups of animals yielded sterile blood cultures at 8 hr.

There was no evidence of L-phase reversion to bacterial phase organisms or bacterial contaminants in any of the mice when simultaneous blood samples were cultured on 5% sheep blood agar plates. Furthermore, blood cultures taken from separate groups of mice injected intraperitoneally with sterile broth only, CoVF only, or CoVF plus sterile broth were all sterile.

Discussion. The results of the present study demonstrate that the complement system has the potential to participate in the host's defense against L-phase variants of *S. faecalis in vivo*. Animals depleted of C3 had a significantly greater magnitude of bacteremia than control animals after the intraperitoneal inoculation of these cell wall deficient organisms.

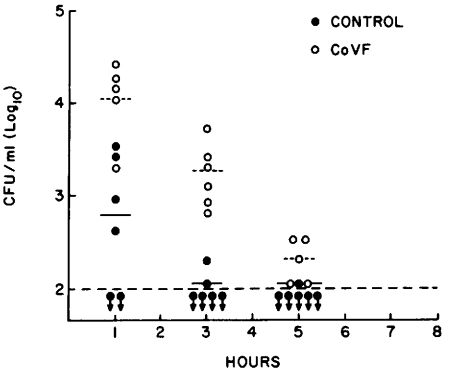

FIG. 1. The log of colony forming units (CFU)/ml of blood recovered from CoVF treated mice (○) and control mice (●) at various time intervals following the intraperitoneal inoculation of 3×10^9 *S. faecalis*. The horizontal bars signify the geometric mean CFU/ml at each time interval for the CoVF treated mice (dashes) and control mice (solid lines).

The reason for the increased magnitude of bacteremia in the CoVF treated mice may be accounted for in several ways. Animals injected with CoVF were depleted of C3. As a result, those complement mediated activities directly dependent on the activation of C3 would be markedly diminished. In addition, since C3 forms part of the enzyme responsible for the activation of C5-9, those activities dependent on the activation of C5-9 would also be markedly diminished. Normally, activation of C3-9 leads to the generation of chemotactic, opsonic and bactericidal activities, each of which may aid the host in its defense against infection (4). Animals injected with CoVF would therefore be unable to generate these complement mediated activities (8). Thus, a diminished peritoneal inflammatory response in the complement depleted animals may have permitted the intraperitoneal survival and multiplication of more organisms resulting in a greater number of L-phase variants entering the blood stream. In addition, decreased serum opsonic activity in the animals may have resulted in inefficient reticuloendothelial clearance of the organisms and thus a greater magnitude of bacteremia. Finally, depletion of C3 would preclude effective

complement mediated lysis of the organisms (5, 6) and thus may have permitted the intravascular survival of a greater number of L-phase variants.

The L-phase variants were ultimately eliminated from the circulation of both the complement depleted and normal animals. It should be emphasized, therefore, that factors other than the complement system also play an important role in the host's defense against these organisms. Previous studies involving mice have attested to the relative lack of virulence of a variety of L-phase variants (10–13). However, conflicting data exist regarding the ability of L-phase variants to persist *in vivo*. Schmitt-Slomska *et al.* were able to recover L-phase variants of group A streptococci for up to 25 days following intraperitoneal injection in normal mice (13). On the other hand, these same organisms were eliminated very rapidly following intravenous injection (13). Likewise, Fernandes and Panos demonstrated prolonged survival of *Streptococcus pyogenes* L-phase variants in methylprednisolone treated mice, but rapid clearance of the same organism in normal mice (11). Finally, Clasener *et al.* reported rapid clearance of osmotically stable and unstable L-phase variants of streptococci when they were injected either intravenously or intraperitoneally into mice (10). Thus, results vary depending on the particular L-phase variant employed and the route of inoculation.

Although previous studies have provided valuable information regarding the biology of L-phase variants, there has been little information on the specific aspects of the immune system involved in the host's defense against these organisms. The results of the present *in vivo* study, along with the results of previous *in vitro* studies (5, 6) suggest that the complement system has the potential to participate in the host's defense against L-phase variants of bacteria.

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