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Complement in Endotoxin Shock: Effect of Complement Depletion on the Early Hypotensive Phase

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The complement (C) inhibition caused by bacterial endotoxin is well known, but the relationship of this reaction to endotoxin shock is unclear. Anesthetized dogs were therefore given *Escherichia coli* endotoxin intravenously with or without prior C depletion by a purified cobra venom factor (CVF). Mean aortic blood pressures (MAP) and C levels were measured. Intravenous CVF usually caused an early transient drop of MAP and a profound, long-lasting drop in C. Bacterial lipopolysaccharide (LPS) alone always caused a sudden (within 2 min) drop in MAP which was followed by partial recovery and then more long-lasting depression. Moderate drops in C usually occurred. In animals pretreated with CVF so that C levels were markedly depressed (<25% of control), LPS did not elicit the immediate MAP drops; however, a later (after 5 to 20 min) MAP drop always occurred. CVF pretreatment did not modify LPS-induced mortality. CVF effects were not caused by LPS contamination. These data show that the early hemodynamic responses of the dog to LPS may be mediated through the complement system.

In the dog, the initial response to rapid injection of bacterial lipopolysaccharide (LPS) is a precipitous drop in systemic arterial blood pressure, an abrupt rise in portal vein and pulmonary artery pressures, and a transient elevation of blood histamine levels (24). It is possible that this response may be mediated humorally via the complement (C) system (5, 6, 12, 20, 21). In support of this hypothesis are the in vivo observation that intravenous LPS injection causes C levels to decline in the dog (20, 21) and the in vitro observations that LPS is capable of generating vasoactive substances in unheated dog serum containing platelets (23) and consuming mammalian complement with the generation of classical anaphylatoxin and chemotactic factor from the C3 component, C5 component, or both (4, 10, 18, 19). To define further the role of C in canine endotoxin shock, the responses to an LPS challenge were studied after acute decomplementation with the cobra venom C inhibitor (13, 14).

MATERIALS AND METHODS

Anesthetized (30 mg/kg of pentobarbital administered intravenously), 10- to 12-kg dogs were injected intravenously with a purified fraction of Egyptian cobra venom (CVF; 0.85 to 2.2 mg/kg; 17) and this was followed after 4 hr with an intravenous challenge dose of *Escherichia coli* endotoxin (0.75 mg/kg, approximately equivalent to LD_{80}; 11). Hemodynamic changes were continuously monitored during the experiments. Aortic pressure was measured with a strain gauge transducer via a catheter inserted in the carotid artery. The pressure and an electrocardiogram were recorded with an oscillographic system. Average pressure was obtained with an electrical meaning circuit. Drugs were injected through a jugular vein catheter. Frequent blood samples were drawn into heparinized syringes, and plasma total C levels were determined (15).

RESULTS AND DISCUSSION

Table 1 shows mean aortic pressure (MAP) changes after challenges with CVF alone, LPS alone, and LPS after CVF pretreatment; CVF caused a pronounced fall of C levels to less than 25% of control (average < 10%) within 4 hr in seven of nine animals. In CVF-treated animals not injected with LPS, the low levels of C persisted through the 6-hr observation period. An initial transitory fall in MAP to values less than 75% of control was noted within 2 min in five of these dogs. These animals became normotensive within 15 min after CVF administration and remained so until further manipulation.

LPS injection caused C titers to decline in all animals (average >60% of control), but not to the low levels achieved with CVF treatment. LPS in all control animals induced an initial marked drop in MAP within 2 min. However, in further

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TABLE 1. *Effect of cobra venom factor (CVF) and endotoxic lipopolysaccharide (LPS) on mean aortic blood pressure in normal and complement-depleted dogs*[a]

Treatment	No. of dogs	Serum complement[b]	Mean aortic pressure[c]				Survival greater than 72 hr
			2 min	5 min	15 min	90 min	
CVF	9	100	77.7 ± 24.9[d] (25–100)[f]	64.2 ± 23.5 (35–100)	93.2 ± 7.3 (78–100)	97.8 ± 6.4 (80–100)	—[e]
LPS	7	100	38.5 ± 15.1 (16–60)	34.3 ± 8.8 (16–47)	48.5 ± 15.0 (31–68)	61.7 ± 9.5 (48–76)	0
LPS post-CVF	6	<25	99.6 ± 0.8 (98–100)	86.5 ± 14.0 (57–100)	81.0 ± 14.4 (68–100)	59.1 ± 12.5 (41–75)	1

[a] Mean aortic pressure reductions in the animals receiving LPS after CVF-induced complement depletion, compared to the unmodified animals receiving LPS, were significantly altered at the 2-, 5-, and 15-min intervals ($P < 0.005$ using Student t test); the difference at 90 min was not significant ($P > 0.30$).

[b] Per cent of control at zero time.

[c] Per cent of control at given times post-treatment.

[d] Mean ± standard deviation.

[e] Most of these animals later were challenged with LPS; however previous investigations have shown that this amount of CVF itself is nonlethal (2).

[f] Range.

contrast with CVF, recovery of MAP, when seen, occurred more slowly, frequently was only partial, and usually was followed by a late recurrent hypotension. All seven animals expired within 48 hr.

When LPS was given after effective CVF-induced decomplementation (C < 25% of control), there was *no* significant early drop in MAP in any of the six animals tested, and it was not until later that a more gradual drop in pressure occurred and persisted (Table 1). At 2 min, the MAP averaged 38% of control in the unmodified dogs, whereas it was 99.6% of control in dogs in which C was depleted by CVF; at 5 min, these groups averaged 34.3 and 86.5% of control, respectively; and at 15 min their respective values were 48.5 and 81.0%. This CVF-induced alteration of the early MAP responses to LPS was highly significant ($P < 0.005$; Student t test). At later intervals, e.g. 90 min, the difference in MAP between CVF-treated and control groups was not significant ($P > 0.30$). In two additional animals, decomplementation after CVF administration was not profound at 4 hr (54 and 85% normal, respectively), and challenge with LPS induced the characteristic early pressure drop. In the adequately decomplemented animals, C remained at low levels through the 6-hr observation period after LPS injection. Only one animal in the decomplemented groups survived 48 hr, indicating that CVF pretreatment, even with adequate decomplementation, had little effect on the lethal outcome.

Figure 1 shows individual examples of the early hemodynamic effects of the various treatments. The marked contrast between the early responses of MAP to LPS in normal control dogs and those pretreated with CVF is evident.

It has been noted (Fig. 1, Table 1) that effective decomplementation with CVF did not always result in an early drop in MAP. Although the basis for this variation is not clear, other data suggest that the occurrence and amplitude of an MAP alteration depends on the initial rate of the CVF-induced C depletion.

The possibility that LPS contamination could have accounted for either the initial hypotensive effects of the CVF or the altered LPS challenge response after CVF was ruled out by three lines of evidence. First, effective lots of CVF were found to be nonpyrogenic in rabbits, indicating that there was little, if any, LPS contaminating them. Second, samples of CVF boiled for 2 hr lost all of their C-consuming activity in vitro, had no effects on either dog MAP or C levels in vivo, and did not block the early LPS hemodynamic effects. By contrast, the activities of LPS were unmodified by such boiling. This indicated that the effects induced by CVF could be attributed to a heat-labile substance rather than to contamination with LPS which was stable during boiling, and again that little, if any, LPS was present as a CVF contaminant. Third, treatment of two dogs with much smaller dosages (0.075 mg/kg) of LPS, which resulted in typical early drops in MAP, did not block the early MAP response to the standard LPS challenge (0.75 mg/kg) 4 hr later. This third experiment showed that even significant LPS contamination of CVF could not of itself abolish the early hemodynamic effects of subsequently administered LPS (Fig. 1).

It is interesting that the lower dose of LPS

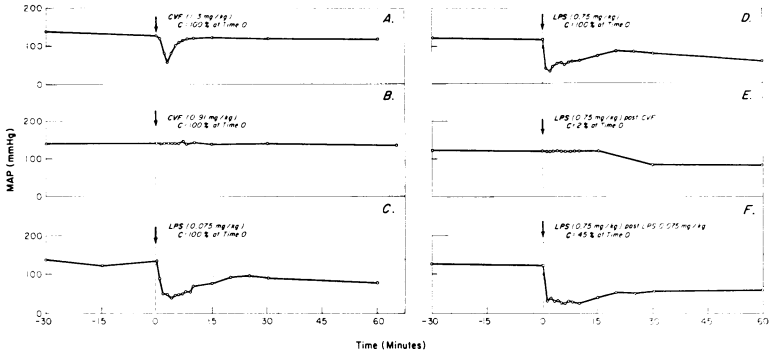

FIG. 1. *Examples of the immediate responses of MAP to the various CVF or LPS treatments, or both are shown. Panel A shows a dog responding, and panel B a dog failing to respond, to CVF. Panels C and D demonstrate that both "low" and "standard" doses of LPS induce almost identical responses. Panel E shows the marked modification of the early response to LPS after adequate venom-induced decomplementation and is to be contrasted with the usual LPS response shown in panel D above. Panel F shows that prior treatment with low doses of LPS does not modify the subsequent response to the standard dose.*

produced an early drop in MAP comparable to that caused by the larger dose, even though a dose of this magnitude did not induce as severe a late, prolonged MAP depression and presumably would be considerably less lethal. These data suggest that the early and late effects of LPS follow different dose-response curves. Other evidence supports the concept of dissociability of the early and late effects of LPS. As shown above, the early but not the later MAP drop is inhibited by CVF-induced C depletion. Further, treatment of LPS with NaOH and by membrane filtration (Millipore Corp., Bedford, Mass.) abolishes the initial hemodynamic effects but not the late effects or lethality in cats (7). This latter observation also would seem to relate the early effects to activation of C, since it has been shown in vitro that NaOH treatment of LPS abolishes anticomplementary activity (3).

The foregoing observations show that C activation is involved in the early hypotensive response of the dog to LPS. The role of C in the later phases of the endotoxin response is not yet clear, and our own data do not permit conclusions concerning this point.

Although a previous attempt to block LPS effects by decomplementation did not succeed (9), the complement levels did not fall below 50% of control, and, in particular, the residual terminal-complement component (i.e., C3 and later components) activity may have been adequate to support a typical endotoxin response.

Clearly, inoculation of endotoxic LPS leads to activation of multiple effector systems, including the complement, kinin, and coagulation systems (1, 5, 8, 12, 16, 22). The role each of these mediators play in the myriad of ensuing host responses is not yet clear. We do not even know whether their net effect is to the benefit or detriment of the host. However, it can be concluded that activation of the complement system is involved in, and likely accounts for, the early hemodynamic responses to LPS.

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