Role of Platelets in the Pathogenesis of Canine Endotoxin Shock

ARTHUR H. L. FROM,* JACK S. C. FONG, TAISAN CHIU, AND ROBERT A. GOOD

Departments of Medicine, Microbiology, Pathology, and Pediatrics, University of Minnesota School of Medicine, Minneapolis, Minnesota 55455, and Department of Veterinary Biology, University of Minnesota School of Veterinary Medicine, St. Paul, Minnesota 55108

Received for publication 8 January 1976

Endotoxin-platelet interactions are thought to be of major importance in the response of dogs and other species to bacterial endotoxin; the mechanisms postulated are: (i) the release of vasoactive substances, (ii) the formation of occlusive platelet aggregates, and (iii) induction of intravascular coagulation. The role of platelets in canine endotoxin shock was examined in animals with thrombocytopenia induced by estrogen pretreatment (<10,000 platelets/mm³) and in controls. After intravenously administered endotoxin, the hemodynamic responses, mortality, and gross necropsy findings were similar in both groups. These data indicate that endotoxin-platelet interactions are not determinative in the pathogenesis of canine endotoxin shock.

The importance of platelets in the hemodynamic response of several species of animals to endotoxin has been emphasized in recent years (4, 5, 8, 18). Three mechanisms by which endotoxin-platelet interactions may contribute to the syndrome of endotoxin shock have been proposed: (i) through the release of vasoactive substances such as histamine and serotonin (4, 18); (ii) through the formation of intravascular occlusive platelet aggregates (5); and (iii) through the induction of disseminated intravascular coagulation (15). The role of platelets in canine endotoxin shock is examined in this investigation through the use of dogs with marked thrombocytopenia induced by a single injection of estradiol cypionate (T. Chiu, Ph.D. thesis, Univ. of Minnesota, St. Paul, 1974). The data reported herein indicate that significant levels of circulating platelets are not crucial to the genesis of canine endotoxin shock.

This work was previously published in brief [Circulation 43(Suppl. IV):166, 1973].

MATERIALS AND METHODS

It has been established that treatment of dogs with pharmacological quantities of estrogenic substances will produce a severe marrow injury, the intermediate phase of which is manifested by marked thrombocytopenia and granulocytosis due to the virtual disappearance of megakaryocytes from the bone marrow and stimulation of granulopoiesis (1, 2) (Chiu, Ph.D. thesis). This effect has been noted with both natural and synthetic estrogens and is dependent upon dose but not upon mode of administration (1, 2) (Chiu, Ph.D. thesis). Though no species seems nearly as sensitive as the dog, hemato logical changes after estrogen administration have been noted in rodent species (rabbits, mice, and rats), with anemia being the most prominent finding though some degree of leukocytosis and thrombocytopenia has also been noted in rats and rabbits. Few changes have been noted in cats, monkeys, or chickens (Chiu, Ph.D. thesis).

Two groups of mongrel dogs weighing 10 to 12 kg were studied; a treatment group of four animals that had been given an intramuscular injection of estradiol cypionate in oil (1 mg/kg; Depo-estradiol cypionate; The Upjohn Co., Kalamazoo, Mich.) 9 to 12 days before the day of endotoxin challenge and a control group consisting of seven animals. The injected animals were followed with regular platelet counts until the level of circulating platelets was 10,000/mm³ or less. The animals did not demonstrate spontaneous bleeding, though special care was taken to minimize dissection and obtain good hemostasis at the time of the experiment.

The dogs were anesthetized with intravenously administered pentobarbital (30 mg/kg of body weight). Aortic pressures were continuously monitored by means of a strain gauge transducer and a catheter inserted in the aorta via the femoral artery. The pressures were recorded with an oscillographic recording system, and mean pressures were obtained electronically. A femoral vein catheter was also inserted for intravenous injections and blood sampling. All dogs received an intravenously injected bolus of Escherichia coli endotoxin (11) (0.9 mg/kg, an approximately 80% lethal dose) administered after stabilization. Using standard methods, blood samples were withdrawn at various time intervals to determine leukocyte and platelet levels (13). The volume of blood withdrawn was replaced with sterile saline. Statistical comparisons were carried out with Student's t test, and the data are expressed as mean ± standard error.
RESULTS

The responses of mean aortic blood pressure and leukocytes after endotoxin challenge were comparable with marked decreases in both the control and thrombocytopenic animals (Fig. 1A, B). It should be noted that the initial leucocyte count was higher in the estradiol-treated group, and this was consistent with the granulocyteosis usually induced by the drug at that point in the marrow response (Chiu, Ph.D. thesis). Thus, though the relative drop of leukocytes was similar, the absolute drop was greater in the estrogen-treated group. The circulating platelet levels dropped precipitously in the control group (the typical canine response to endotoxin) (Fig. 1C). The platelet levels of the thrombocytopenic estrogen-treated group (7,500 ± 1,400/mm³) dropped even further. The observed responses of the two groups after endotoxin challenge were also similar, with initial respiratory distress and later blood diarrhea.

The mortality in both groups was comparable, with all estrogen-treated animals dying within 8 h of endotoxin injection and all but one of the controls dying within 15 h (four animals died within 8 h of endotoxin administration).

Indeed, there were no major differences of statistical significance (Student's t test) in the variables measured between the control and estrogen-treated groups with the exception of the preexisting platelet and leucocyte numbers and late aortic pressures. The higher blood pressure seen in the controls during the latter portion of the observation period may have related in part to the early death during the experiment of two control animals and, in part, may have reflected a slight real difference in the course of the thrombocytopenic animals. Gross necropsy findings in both groups were comparable, demonstrating mucosal hemorrhage and necrosis in the small intestine.

DISCUSSION

The important observation made in this study is that even with severe chronic depletion of the circulating and noncirculating platelet pools after estrogen pretreatment (Chiu, Ph.D. thesis), neither the early hemodynamic response nor the mortality attendant to endotoxin administration in the dog was ameliorated. Of particular note, the rapid early fall in aortic blood pressure subsequent to bolus administration of endotoxin was not altered. This fall in...
blood pressure has been previously shown to be inhibited by a complement-depleting dose of cobra venom factor (6), thus suggesting a major role of complement activation by endotoxin with subsequent release of histamine (18) and other vasoactive substances (4) from platelets and other sources. The very fact that the hemodynamic response could occur unaltered in the extremely thrombocytopenic animals strongly suggests that the platelet is not the major effector limb of the complement pathway in canine endotoxin shock.

In criticizing this conclusion, it may be argued that the small number of platelets present may have served as an adequate source of vaso-depressor substances or occlusive aggregates, or, alternatively, that the remaining platelets were not able to respond normally to endotoxin. These possibilities seem unlikely as outlined below.

The number of platelets present in thrombocytopenic animals was well under 5% of the control group, and it would seem unlikely that platelet-mediated events would occur unimpeded under such conditions. Additional support for this concept and our basic conclusions comes from the observation in the rabbit that isolated thrombocytopenia of comparable magnitude is capable of blocking an endotoxin-induced Shwartzman reaction, a response known to be platelet dependent (12). Further, in the canine perfused lung preparation, the absence of platelets did not alter the pathological or physiological responses to endotoxin (10). Spink's observations in the endotoxin-immunized dog challenged with a lethal dose of endotoxin are also relevant to our conclusions in that he demonstrated a markedly increased humoral response (elevated histamine levels and lower complement levels) which was associated with a more severe early hemodynamic response but markedly reduced mortality in the immunized group; he further demonstrated that histamine pretreatment decreased mortality in the canine endotoxin shock model (17). Thus these studies would suggest that: (i) responses known to be dependent upon endotoxin-platelet interactions can be blocked by thrombocytopenia of the magnitude we induced; (ii) in dogs, major endotoxin-induced pathophysiological events seem to be independent of the presence of the platelets; and (iii) marked rises in the major amine released by the platelet do not correlate with mortality in endotoxin-challenged dogs with unaltered platelet function. The role of other vasoactive substances, kinins and catecholamines, has been demonstrated in the dog, but the release of these substances has not been linked to platelets, so they were not evaluated in this study.

With respect to the possibility that the remaining platelets in the thrombocytopenic animals were unable to respond to endotoxin normally, we can only note that: (i) their numbers dropped immediately postendotoxin and remained depressed for the duration of the experiment, and (ii) the animals in our study showed no petechial bleeding before use (or other major evidence of bleeding), an unlikely observation if the few remaining platelets were not grossly competent hemostatically. Such bleeding does occur in estrogen-treated thrombocytopenic dogs when higher dosages are used and platelet levels fall even lower than those present at the time of study (1).

The role of the platelet as an inducer of disseminated intravascular coagulation (a phenomenon that is of importance in the rabbit (15)) was not explored in these studies, because (i) we have shown that prior defibrination does not alter the course of canine endotoxin shock (7), (ii) prior heparinization has not been found to alter the course of endotoxin shock (16), and (iii) prior treatment of dogs with epsilon amino caproic acid, an agent known to impede fibrinolysis, actually enhances survival following an endotoxin challenge (17). The foregoing would also apply to the possibility that estrogen itself modified the coagulation sequence so as to induce disseminated intravascular coagulation chronically, and thus alter the mortality following endotoxin challenge. In addition, studies of the coagulation system in estrogen-treated dogs have been normal (other than the abnormalities induced by thrombocytopenia), and fibrinogen levels have also been normal, suggesting that no such process was occurring and that other major effects of estrogen in this model (exclusive of the usual target organ and hematological changes) have not been described (1) (Chiu, Ph.D. thesis).

The question can also be raised as to whether the low platelet levels noted reflected total animal platelet depletion since, if this were not the case, it might be possible for endotoxin to interact with a noncirculating pool directly or indirectly with physiological consequences. However, a sequential study of the hematological responses after estrogen treatment (Chiu, Ph.D. thesis) clearly demonstrated the virtual absence of bone marrow megakaryocytes as well as low peripheral platelet levels at the same point in time that our thrombocytopenic animals were studied, observations that would make a significant noncirculating platelet pool highly unlikely in view of the half-life of the platelet.

Unexplained are observations in dogs that
pretreatment with acetylsalicylic acid blocked much of the hemodynamic response and decreased mortality after a challenge dose of endotoxin comparable to our own (9). The current data suggest that the more general and only partially defined anti-inflammatory effects of acetylsalicylic acid underlie this modification of the response to endotoxin rather than the ability of that compound to inhibit endotoxin-platelet interactions.

Of interest also is the observation that the endotoxin-induced acute leukocyte response was preserved in the thrombocytopenic animals and indeed was greater because of the leukocytosis present in that group. This response had been felt to be of major importance in the pulmonary pathology induced by endotoxin in some species on the basis of pathological studies (3). In more recent work, however, the same workers have demonstrated in a perfused canine lung preparation that removal of leukocytes and platelets from the perfusate did not alter the hemodynamic or pathological responses to endotoxin (10). In another study in primates, it was also found that induced leukopenia did not block either the hemodynamic or pathological findings attendant to endotoxin shock (14). This would suggest that at least in these two species that endotoxin-leukocyte interactions may not be a critical component of endotoxin shock.

The current findings in dogs are in marked contrast to those in rabbits (5) in which prior treatment with either platelet-depleting agents or inhibitors of endotoxin-induced platelet aggregation prevented endotoxin-induced shock and histological changes. Thus, our data and those of others suggest that there are major species differences in the relative importance of the various components of the response to endotoxin assault. The current work does not demonstrate that the platelet has no deleterious role in the pathogenesis of the canine endotoxin shock syndrome, but rather indicates that endotoxin-platelet interactions and their consequences do not play the determinant role in the dog.

ACKNOWLEDGMENTS

We wish to express our gratitude to Wesley W. Spink for the gift of the endotoxin used in these studies, to Pat Gerardy for her excellent technical assistance, and to Frances Wallace for her skillful aid in the preparation of the manuscript.

This work was supported by grants from the Minnesota Medical Foundation, the Minnesota Heart Association, and the National Institute of Allergy and Infectious Diseases, National Institutes of Health (Public Health Service grant no. AI-11843-02).

LITERATURE CITED