VOLUME 7

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J. Raymond Fletcher

ADVANCES IN SHOCK RESEARCH

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Preface

Volume 7 of ADVANCES IN SHOCK RESEARCH is a collection of papers derived from approximately half of the presentations made at the Fourth Annual Meeting of the Shock Society, held June 4–6, 1981, on Marco Island, Florida. In this volume, the focus is on the pathophysiology, as well as possible therapeutic interventions, in sepsis- and endotoxin-related studies. Volume 8 covers "Other Forms of Shock."

The first part of the book deals with pathophysiology and begins with a short review of the involvement of leukocytes and platelets in organ system function in sepsis. This paper was presented as part of a symposium, "Basic Mechanisms in Multiple Organ Failure With Sepsis." Several papers follow that touch on a variety of areas in septic shock. These include a proposed experimental model, survival characteristics, circulatory disturbances, myocardial dysfunction, enzyme changes, mitochondrial function, and glucose uptake during septic shock. Other studies are concerned with the role of opiate pathways, prostaglandins, and the reticuloendothelial system in endotoxin shock. The second part of the book deals with therapeutic intervention in endotoxin and septic shock, including studies on the beneficial use of vasodilating agents in combination with increases in circulating volume, alpha-adrenergic blockade, and alteration of thromboxane and prostaglandin concentrations in endotoxin shock.

These papers underscore the complexity of the sequence of events in sepsis. Today there are no effective clinical treatment modalities for septic shock, the greatest unresolved problem in the shock field. Although answers are not yet available, continual interchange between the basic scientist and the clinician will provide insight into areas of investigation that will benefit the patient with severe infection.

Sherwood M. Reichard
J. Raymond Fletcher

PATHOPHYSIOLOGY OF ENDOTOXIC AND SEPTIC SHOCK

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Advances in Shock Research 7:1-6 (1982)

Hematologic Disturbances During Sepsis: Platelets and Leukocytes

L. B. Hinshaw, L. T. Archer, and B. K. Beller-Todd

This brief review summarizes recent observations about leukocyte and platelet involvements during sepsis and septic shock. Endotoxins are known to exert significant effects on leukocytes and platelets as well as monocytes, macrophages, endothelial, and mast cells. The presence of endotoxin itself is reported to enhance the phagocytic and killing capacity of neutrophils. Transfusion of additional white blood cells has been shown to increase the probability of recovery from sepsis. Defects in neutrophil function, impaired opsonization, sequestration of neutrophils in pulmonary capillaries, and depressed metabolic states adversely affecting neutrophils may significantly contribute to the lethal outcome of septic shock. Platelet responses in septic shock are reported to include aggregation accompanied by release of several agents, including vasoactive amines, ADP, and platelet factor 3. In summary, leukocytes and platelets are known to perform significant roles in sepsis and septic shock although the precise mechanisms of their involvement remain to be clearly defined.

INTRODUCTION

Bacteria and endotoxin can exert multiple profound biologic effects when in contact with the blood of animals and man. In 1974, Thomas [1] commented that "these (endotoxin) macromolecules are read by our tissues as the very worst of bad news. When we sense lipopolysaccharide (endotoxin), we are likely to turn on every defense at our disposal." He further stated, "Our arsenals for fighting off bacteria are so powerful and involve so many different defense mechanisms, that we are in more danger from them than from the invaders." Morrison and Ulevitch [2] concluded in their extensive review of 1978 that endotoxins exert significant effects on polymorphonuclear leukocytes and platelets as well as monocytes, macrophages, endothelial cells, mast cells, coagulation, and complement.

POLYMORPHONUCLEAR LEUKOCYTES

The marked effect of endotoxins on circulating polymorphonuclear leukocyte concentration, resulting in immediate neutropenia followed by granulocytosis,

has been well documented in all species [2]. In 1961, Mechanic et al [3] documented that neutropenia took place within minutes following injection of endotoxin in humans and the recovery of normal neutrophil numbers occurred within six hours. Athens et al [4] hypothesized that the disappearance of neutrophils from the circulation of the patient was the result of their sequestration in capillaries, and their subsequent increase in concentration was accounted for by large numbers of immature neutrophils released from the bone marrow.

In 1972 Stossel [5] reiterated Metchnikoff's theory [6] by confirming the role of phagocytosis by polymorphonuclear leukocytes as the body's first line of defense and divided the process into seven stages: (1) The bone marrow produces and mobilizes phagocytes; (2) bacterial products or inflammatory mediators interact with serum factors, including complement, to attract phagocytes into injured or invaded areas by chemotaxis; (3) factors in normal serum, particularly gamma globulin and the third component of complement, opsonize microbes, rendering them tasty to the phagocytes; (4) the phagocyte ingests the opsonized microorganisms; (5) the cytoplasmic structures containing hydrolytic enzymes fuse with phagocytic vesicles and secretion of vesicular contents (a process called degranulation) occurs; (6) the phagocyte generates hydrogen peroxide, the most important antimicrobial agent within the phagocytic vesicles; and finally, (7) the phagocytes kill the microbes utilizing peroxidation.

Postel et al [7–9] and Meakins et al [10] have emphasized that circulating phagocytic cells, particularly the neutrophils, exert a potent antibacterial defense during septic shock. Neutrophils can also phagocytose endotoxin under certain conditions, as reported by several investigators including Cline [11] and Balis [12] and others. Morrison and Ulevitch [2] and Cohn and Morse [13] have reported that endotoxin significantly enhances the phagocytic and killing capacity of neutrophils.

There is evidence for protection against the adverse effects of sepsis by administering WBC transfusions. Graw and others [14] reported in 1977 a significant increase in the survival rate in neutropenic patients with gram-negative sepsis when they were transfused with granulocytes. Epstein et al [15,16] in 1974 confirmed their work by reporting the beneficial effects of transfused white blood cells as a treatment for experimental bacteremia in dogs.

Phagocytosis by leukocytes exerts a significant metabolic demand on the animal subjected to endotoxin shock or Escherichia coli-induced shock. Hinshaw et al [17] found that the additional demand occurred for three reasons: (1) There is a net increase in glucose utilization and lactic acid production by leukocytes after endotoxin is added to canine blood in vitro, indicating an increased glucose uptake per cell; (2) during granulocytosis, additional glucose is required because more leukocytes are present; and (3) the febrile response elicits a greater uptake of glucose based on the Q_{10} phenomenon. If additional metabolic demand contributes to hypoglycemia, the defense system may be inhibited in its ability to remove bacteria from the blood, as Postel's group has recently shown [9]. Concerning the importance of meeting metabolic requirements in human septic

shock, Meakins [10] pointed out that even though it is difficult to prove an association between malnutrition, the etiology of infection, and the failure of host defense mechanisms, much support for this probability is accumulating.

Neutrophil dysfunction has been associated with the presence of bacteria or endotoxin. Hellum and Solberg [18] stated that defects in neutrophil function caused by bacterial infection in patients may contribute to a fatal outcome of the disease. Weinstein and Young [19] concluded that impaired opsonization causes neutrophil dysfunction in patients during bacteremia. Hinshaw and others [17] documented an adverse effect of endotoxin on glucose uptake of leukocytes in vitro on the basis of depressed Q_{10} values.

The mortality rate of neutrophils is high in endotoxin shock or live E coli-induced shock, as we have shown in our laboratory [17,20]. Polymorphonuclear leukocytes have been found to be sequestered, degranulated, and fragmented in several vascular beds following three-hour infusions of live E coli in baboons according to the work of Coalson et al [21].

Neutrophils are also involved in functions other than phagocytosis. They are one of the sources of pyrogen responsible for the endotoxin-induced febrile response according to reports by Wood [22] in 1958 and Dinarello and Wolff [23] in 1978. The neutrophil may also participate in the pathogenesis of endotoxin-induced coagulative changes [2]. Studies by Miller et al [24] suggest that endotoxin-neutrophil interactions may lead to hemodynamic changes through the release of bradykinin. The work of Coalson and others [25] suggests that the sequestration of neutrophils in pulmonary capillaries produces pulmonary capillary injury leading to the "shock lung." Their work showed that the capillary endothelium of monkeys appeared damaged at areas of leukocyte attachment.

PLATELETS

Stetson, in 1951 [26], provided the first firm basis for the participation of platelets in the pathophysiologic effects of endotoxins in animals. In his experiments, histologic examination of tissues from rabbits administered endotoxins documented the deposition of leukocyte-platelet thrombi within the animals' small blood vessels. In 1957, Weil and Spink [27] demonstrated significant drops in the levels of circulating platelets within five minutes to one hour following the intravenous injection of endotoxin in dogs.

In 1961, Hinshaw et al [28] first assessed endotoxin effects on the level of circulating platelets in the primate, Cerococebus torquatus Atys (Sooty Mangebey). They demonstrated platelet decreases (up to 70%) in the primate within one to two hours following lethal injections of E coli endotoxin.

Along with the fall in platelet concentration after endotoxin administration in dogs, a rise in plasma serotonin levels was observed by Davis et al in 1960–1961 [29,30]. Horowitz et al [31] in 1962 reported transient increases in platelet factor 3 levels in rabbits given endotoxin which correlated with the rapid disappearance of platelets from the circulation.

In 1966 and 1967 Davis [32] and Spielvogel [33] found platelet aggregates and debris in pulmonary and peripheral vascular capillaries of rabbits after endotoxin administration.

The results of McKay and others [34] in 1967 suggested that platelets perform a role in the formation of microthrombi within one to two hours following the injection of lethal doses of endotoxin in rhesus monkeys. Balis et al [12], however, in 1974, were unable to detect either platelet aggregates or any ultrastructural alterations in platelets in the lungs of rhesus monkeys receiving lethal doses of E coli endotoxin.

The rapid drop in circulating platelets induced by endotoxin injection has been shown to be dependent on serum complement activation by Kitzmiller et al [35] using the cat model and by Garner et al [36] in 1974 studying dogs.

Coalson et al [21] in 1975 found that platelets were sequestered, degranulated, and fragmented in several vascular beds of the baboon following three-hour infusions of live E coli. In a report in 1978 Rowe et al [37] observed thrombocytopenia in piglets administered live E coli and they attributed the thrombocytopenia primarily to platelet injury and destruction. They also observed that only morphologically intact platelets took part in the aggregation and adhesion to the lining of blood vessels.

According to Morrison and Ulevitch in 1978 [2], the mechanisms of the platelet-endotoxin interaction depend on the presence or absence of immune adherence sites on the platelet membrane. They concluded that platelet responses are usually characterized by aggregation, or clumping, followed by platelet release of adenosine diphosphate (ADP), platelet factor 3, and vasoactive amines such as histamine and serotonin.

The pulmonary hypertensive response to infusion of E coli endotoxin in dogs has been shown by Bredenberg [38] in 1980 to be dependent in large measure on the presence of platelets: Dogs made thrombocytopenic with injections of goat antiplatelet serum demonstrated that the slowing of the pulmonary microcirculation after endotoxin was brief and mild. He concluded that sepsis appears to cause changes in pulmonary vascular membrane permeability and alterations in pulmonary vascular hemodynamics.

In conclusion, the foregoing illustrations of the involvements of leukocytes and platelets assure their significant roles in the fate of organ system function in sepsis and septic shock. The precise mechanisms of their involvement remain to be clearly defined.

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