Manipulation of the Immunoinflammatory Cascade in Sepsis: Facts and Perspectives

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Introduction

Septic shock continues to be a frequently encountered problem carrying a high mortality [1]. The invading organism interacts with the host to induce a complex array of responses, the initiating event being the release of microbial toxins. These have been divided into two broad classes: exotoxins (products of *Staphylococcus aureus* and *Clostridium perfringens*), and endotoxins (LPS) which originate from the cell wall of gram-negative bacteria. Circulating LPS of bacterial origin continue to have biological effects, even in absence of actively proliferating bacteria.

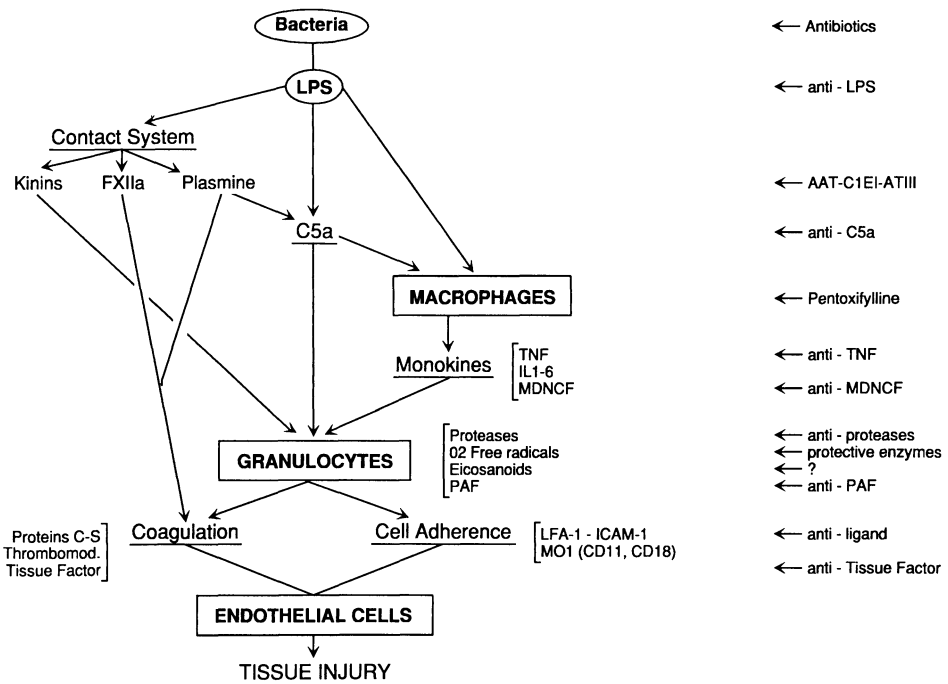

Fig. 1. The immunoinflammatory cascade in sepsis. Abbreviations: *AAT* = α_1-antitrypsin; C_1E_1 = C1 esterase inhibitor; *ATIII* = anti-thrombin III; *Thrombo.* = thrombomodulin

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Indeed, release of LPS and other bacterial cellular debris, immune complexes, virus... activate various humoral and cellular host defense systems, which include complement, kinin and clotting cascades with activation of various leukocytes (mononuclear phagocytes and granulocytes). These cells, in turn, release several potent mediators, such as tumor necrosis factor (TNF), and will express new cell surface adhesion molecules. The coordinate activation of both humoral and cellular limbs of the immunoinflammatory cascade (Fig. 1) results in an effective defense against infection. However, in many cases, this response becomes exaggerated, causing tissue damage, thereby threatening the host [2, 3]. Despite the development of antibiotics capable of killing a wide variety of gram-negative bacteria, mortality from gram-negative infections remains high (60 to 80%). In fact, with a few notable exceptions such as colistin and polymyxin [4], antibiotics have little effect on the specific effects of LPS, and may even promote the release of LPS from bacteria [5]. Alternative approaches are clearly needed.

In this brief review, we will concentrate on the steps in the cascade for which there exist therapeutic interventions. Antibodies recognizing LPS and the various mediators involved in the cascade, pharmacological agents that inhibit mediator production, recombinant protease inhibitors, as well as protective enzymes and oxygen-derived free radical scavengers have the potential of reducing the severity of the inflammatory response in vivo and improve the outcome of bacterial infections.

Role of the Acute Phase Response in Neutralizing LPS

An important yet confusing aspect of the immunoinflammatory cascade is the so-called non-specific host defense mechanism [3]. It had been known for decades that animals or humans given a single or repetitive doses of LPS become tolerant to the effects of a subsequent challenge. Early studies [6] reported that the reticuloendothelial system was activated and that clearance and/or metabolism of LPS was enhanced. However, blockade of the reticuloendothelial system with thorotrast did not lead to loss of tolerance once acquired [7]. Injection of large amounts of sera from a tolerant animal devoid of LPS antibodies to a non-tolerant animal renders it tolerant [8]. Furthermore, sera from patients with fever, bacteremia, or gram-negative shock had a 10-fold greater capacity to induce tolerance to LPS than sera from normal controls [3]. The injection of activated macrophage supernatants and human rIL-1 also induces increased neutralizing activity, suggesting that detoxification of LPS may be controlled by inducible macrophage factors [3]. The mechanism of the detoxification of LPS is incompletely understood. LPS is first disaggregated by a serum component and secondary binds to serum lipoprotein. It seems that the detoxification is related to the presence of a heat-stable esterase associated with LDL, as well as an inducible esterase present in HDL [3].

The clearance and subsequent distribution of LPS from the bloodstream into the body tissues occur in two phases: an initial rapid clearance (minutes) into the reticuloendothelial cells and a slower phase (hours), during which LPS bound to HDL circulates and is taken up by HDL receptors. There is a targeting of LPS to

cholesterol-rich tissues such as the adrenal gland [9], a process that may be related to the adrenal damage occasionally observed in fulminant septicemia. Antibody to the O-polysaccharide chain enhances the clearance of both LPS and preformed LPS-liprotein complexes by the liver and spleen and inhibits the binding of LPS to HDL. Cellular processes may also detoxify LPS. Recently, an enzyme has been isolated from human granulocytes that releases the nonhydroxylated fatty acids from the lipid A moiety of LPS [10].

The mechanisms involved are complex and incompletely understood, and it has not yet been possible to take advantage of them clinically.

Antibodies Against LPS

Antisera have been primarily used in the past for the treatment of typhoid, rabies, tetanus, diphteria, and pneumococcal pneumonia. Because there exists a large number of gram-negative organisms serotypes, it is impratical to prepare type-specific antisera to target all possible organisms. Monoclonal antibodies (MoAbs) against LPS are being developed with the aim of preventing or reversing the effects of LPS and facilitating the removal of gram-negative bacteria from bloodstream by the reticuloendothelial system.

The Concept of Anti-Core Glycolipid Antibodies

The structures of several LPS are now completely known. Each LPS-containing bacterial species is distinguished by its so-called "O" or somatic antigens which are repeating oligosaccharide subunits. Connected to this region is the LPS "core", composed of a group of sugars highly conserved across bacterial species and the lipid A biologically the portion most toxic of the LPS molecule.

Several investigators have shown that survival of patients with bacteremia due to various gram-negative bacilli was related to the titers of strain-specific and anti-core glycolipid antibodies present at the onset of bacteremia [11], suggesting that passive immunotherapy by strain-specific as well as anti-core glycolipid antibodies might be of benefit.

The *E. coli* (J5) and *Salmonella minnesota* (Re) mutants possess enzyme defects that render them incapable of incorporating the immunodominant oligosaccharide side chains to the core region of the LPS molecule [11]. LPS of these strains have been used to develop polyclonal and MoAbs which have the additional characteristic of being reactive to the antigenic core-lipid A moiety common to most gram-negative bacteria [11]. These MoAbs have now been generated in substantial quantities and clinical trials are underway in the United States [12] and Europe.

However, it should be noted that not all investigators have been able to demonstrate an in vivo protective effect of core-reactive antibodies in animal model of gram-negative sepsis. Ziegler [13], like Baumgartner and Glauser in the following chapter, give an excellent critical review of these studies.

Core Versus Type-Specific Antibodies

The protective effects of type-specific antibodies recognizing the "O" antigens determinants have been convincingly demonstrated, and when directly compared in the same model by the same investigator, the antibodies have been shown to be much more potent than the core-reactive antibodies [14]. These antibodies belong to both IgM and IgG classes and function mainly as opsonins and, at times, bacteriocidins. These observations have been extended to MoAbs [15].

In 1983, Ziegler et al. [16] reported a crucial clinical study of the efficacy of J5 antisera in the treatment of gram-negative sepsis. Because they could not relate protection to titers of the antiserum administrated, regardless of the immune status of the donor, no attempt was made to demonstrate that antibodies to LPS were the active principle in these sera. As noted above, "tolerant" sera contain a number of ill-defined mediators and substances that are apparently capable of neutralizing LPS. Several investigators have shown that LPS immunization can cause a polyclonal type-specific response. Two other human studies using J5 anti-sera prophylactically have not been conclusive. Recently, Baumgartner and Glauser [11] were unable to demonstrate a protective effect of a purified anti-J5 IgG. A clinical trial of purified high titer anti-Re antibodies is underway in Europe. Because low dose type-specific antibodies have been shown to be much more potent than the core-reactive antibodies, and serologic analyses have suggested that only a restricted number of serotypes cause the most serious bacteremias, Larrick [2] has suggested treatment with a cocktail of type-specific MoAB.

LPS-Induced Mediator Release

LPS interacts with both humoral and cellular elements. These include complement, kinin and clotting cascades. LPS and mediators released from the humoral systems act together to activate phagocytes and granulocytes.

Serum Mediators: The Complement, Kinin and Clotting Cascade

Contact activation has only been recently recognized [17]. At least four proteins, coagulation factor XII (Hageman factor), prekallikrein (PKK), high molecular weight kininogen (HMwK) and coagulation factor XI are known to participate in a contact activation. This activation of proenzymes occurs when plasma contacts negatively charged surfaces, leading to a burst of proteolytic activation: kallikrein is generated, bradykinin is released, complement is activated through the first component, the fibrinolytic system is activated through activation of plasminogen, and the intrinsic coagulation cascade is initiated through the action of FXIIa on FXI. Enzymes activated during contact activation are closely regulated by protease inhibitors (C_1-esterase inhibitor, α_2-macroglobulin, α_1-antitrypsin, antithrombin III ...). In septic shock, patients have low concentrations

of both contact activation proteins and protease inhibitors, especially in those who died [18].

The plasma kallikrein-kinin system is closely interrelated to other protease systems [19]. Polymorphonuclear leukocytes contain proteases with proteolytic activity directed against coagulation and complement factors. Elastase release from granulocytes was shown in patients with septicemia. α_1-antitrypsin-elastase and α_1-proteinase inhibitor-elastase complexes are increased in these plasma. Plasma kallikrein probably contributes to the neutrophil activation in vivo, since it has been shown in vitro to induce release of elastase from human neutrophils, even in the absence of C5 [20]. In addition, endotoxin leads to the generation of tissue factor, the cofactor of the extrinsic pathway, from endothelial cells and macrophages [21]. These observations underscore the role of plasma proteolysis in the pathophysiology of sepsis.

Several classes of inhibitors have potential as treatment of endotoxemic states [22]. MoAbs have been developed against each protein of the contact system and are currently being tested in animal models of sepsis. Genetically engineered protease inhibitors are another therapeutic possibility. A naturally occurring variant, designated α_1-antitrypsin Pittsburgh, changes the protein from an elastase inhibitor to an extremely potent inactivator of coagulation proteases. In an animal model of septicemia, pretreatment by recombinant α_1-antitrypsin Pittsburgh prolonged survival [21]. Site-directed mutation, resulting in the change of the amino-acid in P2 position to alanine, yields an inhibitor that has a 20-fold greater affinity for kallikrein than thrombin, capable of modulating hypotension experimentally induced by infusion of activated factor XII. Other recombinant or synthetic proteolytic inhibitors are in various stages of development.

Elevated levels of C5a have been associated with sepsis and the development of ARDS [23]. Stevens et al. [24] showed that neutralizing anti-C5a rabbit antibodies protected against a lethal challenge of *E. coli* bacteria. Interference with the coagulation process may block the inflammatory cascade. Both activated protein C [25] and anti-thrombin III [26] have shown excellent results in baboons. Another promising approach is the inhibition of tissue factor with MoAbs [27].

Monokines

Macrophages are known to produce a plethora of mediators following activation. Among the most important released by these cells during endotoxemia are TNF, interleukin-1 (IL-1), interleukin-6 (IL-6), and macrophage-derived neutrophil chemotaxis factor (MDNCF) [28].

TNF appears to play a pivotal role in the orchestration of the inflammatory cascade [29–31]. Tracey et al. [32] demonstrated that a neutralizing murine anti-TNF fragments MoAbs administered to baboons one hour before lethal *E. coli*-challenge protected against shock, but did not prevent multiple organ failure. However, complete protection was conferred by administration of MoAbs two hours before bacterial infusion. A clinical trial of a murine anti-rTNF MoAB is in progress in Europe.

The production of prostaglandins of the E type induced by LPS inhibit the generation of the TNF and IL-1 genes [33]. PGE_2 activates adenyl cyclase, leading to elevated levels of cytoplasmic cAMP. cAMP mediates inhibition of many cell functions. Pharmacologic agents, such as pentoxifylline, that inhibit phosphodiesterase, the enzyme that hydrolyzes cAMP, are now known to protect animals from the deleterious effects of endotoxemia. In addition, pentoxifylline decreases neutrophil adherence and superoxide production [34].

A particularly interesting phenomenon is the marked synergism observed between some cytokines. TNF induces IL-1 transcription and acts with this cytokine in LPS-induced organ injury [35–37]. Interferon (INF) gamma released from T cells stimulates mononuclear phagocytes that release TNF and IL-1 [38, 39]. MDNCF is released from LPS, TNF or IL-1-stimulated monocytes [40]. Inhibition of MDNCF activity with MoAbs has been shown to decrease the elimination of granulocyte influx into foci of inflammation and markedly reduces the magnitude of organ injury.

LPS-Mediated Microvascular Damage

Several pathways and mediators activate endothelial and inflammatory cells (Fig. 1). During this process, these cells express increased number of leukocyte adherence molecules of the CD11–CD18 complex consisting of the LFA-1 and MO-1 molecules. These molecules mediate the ability of leukocytes to adhere to each other and to endothelial cells. The ligand of LFA-1 is the intercellular adhesion molecule, ICAM-1 induced by IL-1 and INF gamma [41, 42]. MoAbs that bind to these molecules inhibit the function of inflammatory cells, and decrease tissue damage.

Tissue damage through activated granulocyte is mainly due to toxic oxygen products, neutral proteases, arachidonic acid metabolites, kinins and platelet activating factor (PAF) [28]. Phospholipids of the endothelial membranes are especially vulnerable to the oxydizing action of free oxygen radicals [43, 44]. This endothelial damage can be blocked either by the substitution of protective enzymes, such as superoxide dismutase and catalase, which are normally present in large concentrations in the cells, or by treatment with the so-called free radical scavengers (N-acetylcystein) [45]. These treatments only attenuate organ injury after endotoxin injection. Probably free radicals are partly involved in the development of organ failure, and cyclooxygenase metabolites and proteinases may also mediate endotoxin-induced injury. Pharmacological interventions in the arachidonic acid cascade have led to contradictory results [46]. It appears, however, that thromboxane contributes to pulmonary hypertension, dilating prostaglandins are beneficial early in septic shock [47], and cyclooxygenase inhibitors are not suitable for therapy [48].

Several other mediators have been implicated in the immunoinflammatory cascade, including endorphins [49, 50] and PAF [51, 52] for which specific receptor antagonists are available. Animal studies have indicated that organic inhibitors of PAF can reverse the vascular effects of LPS. Lastly, despite beneficial

effects in attenuating inflammatory reaction in several animal studies [53, 54], corticosteroids have failed to improve the survival of patients with septic shock [55].

Conclusion

Several steps in the immunoinflammatory cascade may be neutralized by MoAbs, or protease inhibitors, or modulated by pharmacologic agents. Because cascades are activated at different periods during sepsis, with consequent immunoregulation and feedbacks, exact timing of administration of agents will probably prove to be important. These new approaches appear promising although limited data is available at the present time as to how effective these will be. MoAbs offer several advantages, but are not without their problems; they are immunogenic and relatively expensive.

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