Published in final edited form as:

Crit Care Med. 2014 January ; 42(1): 219–220. doi:10.1097/01.ccm.0000435680.21801.0f.

Recombinant Human Annexin A5: A Novel Drug Candidate for Treatment of Sepsis?

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Keywords

annexin A5; cytokine; inflammation; lipopolysaccharide; nuclear factor κ B; sepsis

Sepsis is characterized by activation of the innate immune response with production of massive inflammatory cytokines, which contributes to multiple organ failure and death. Pattern recognition receptors (PRRs) sense pathogen-associated molecular patterns and endogenous stress signals termed danger-associated molecular patterns to initiate a signaling cascade leading to immune responses. Toll-like receptors (TLRs) are the best characterized PRRs and importantly contribute to the innate immune response to bacterial and viral infections. Bacterial lipopolysaccharide (LPS), the major structural component of the outer wall of all Gram-negative bacteria, is recognized by TLR4/myeloid differentiation factor 2 (MD-2)/CD14 complex to initiate myeloid differentiation factor (MyD88)-dependent and MyD88-independent signaling pathways to activate inflammatory gene expression (1, 2). Recruitment of the adaptor protein MyD88 initiates the early activation of nuclear factor (NF)- κ B and mitogen-activated protein kinase (MAPK) which in turn induce release of proinflammatory cytokines, such as tumor necrosis factor (TNF)- α and interleukin (IL)-6. In parallel, the MyD88-independent pathway leads to delayed NF- κ B activation but rapid activation of interferon regulatory factor 3. MyD88 promotes association with IL-1 receptor-associated kinase (IRAK)-4 and IRAK-1. TNF-associated factor (TRAF)-6 is recruited to IRAK-1. The complex IRAK-4/IRAK-1/TRAF-6 dissociates from the receptor and then interacts with transforming growth factor- β -activated kinase (TAK1) complex. TAK1 activates I κ B kinase, leading to phosphorylation and degradation of I κ B, and consequent release of NF- κ B (2, 3). Once translocated into the nucleus, NF- κ B induces the expression of inflammatory chemokines and cytokines. MAPK-mediated activation of transcription factors activator protein-1 and cAMP response element-binding protein also coordinates the induction of many genes encoding adhesion molecules and inflammatory mediators (1–3).

Management of severe sepsis and septic shock includes source control, early antimicrobial therapy, supportive, and adjunctive therapies as there is no specific treatment strategy for the inflammation of sepsis (4). The pathophysiology of sepsis entails a complex host immune response to infection, involving initial cytokine-mediated hyperinflammation followed by immunosuppression (5). Activation of TLR4 signaling not only induces inflammatory cytokine production which plays a pathogenic role in the early stage of sepsis but also is required to combat bacterial and viral infection. Consequently, appropriate modulation of

excessive inflammatory response through inhibition of TLR4 signaling pathways may have considerable potential as therapeutics for inflammatory disorders (6). In the past two decades, independent efforts have been made to improve the outcome of patients with sepsis by means of blocking inflammation and immune activation. Unfortunately, all clinical trials of agents that are designed to interfere with proinflammatory mediators conducted to date have failed to show efficacy (7). It is worthy of note that among all anti-inflammatory agents, MD-2/TLR4 inhibitor Eritoran recently also failed to show reduced mortality in patients with severe sepsis and septic shock in the ACCESS trial (phase III) (8). However, a more recent study provided compelling evidence that Eritoran improved influenza-induced lethality, lung injury, clinical symptoms, and cytokine levels in mice (9).

Annexins A5 (or annexin V) is a member of a highly conserved and ubiquitous phospholipid-binding protein superfamily, the annexins. It binds reversibly to phosphatidylserine-expressing membranes with high affinity in the presence of Ca^{2+} . Annexin A5 has been widely accepted as an early marker of apoptosis because it recognizes phosphatidylserine exposed on the apoptotic cell surface resulting from loss of membrane asymmetry. Although annexin A5-deficient mice are viable and reveal no significant alterations in the biochemical variables characteristic for metabolic or functional defects (10), evidence has accumulated that annexin A5 acts as a critical regulator of antithrombotic function, Ca^{2+} homeostasis, endocytosis, and phagocytosis (11, 12). Annexin A5 has recently been shown to prevent wound expansion and promoted membrane resealing via its binding to torn membrane edges (12). Furthermore, annexin A5 was able to bind to bacteria and LPS and attenuated LPS-induced TNF- α production (13). These findings suggest that annexin A5 may play an important role in the pathogenesis and progression of sepsis.

In this issue of *Critical Care Medicine*, Arnold et al (14) investigated the potential therapeutic effect of recombinant human annexin A5 on sepsis in a murine endotoxin model. Their results showed that recombinant human annexin A5 significantly attenuated myocardial inflammation and improved cardiac function and animal survival via its suppression of TLR4 signaling. Annexin A5 precludes the engagement of LPS with the TLR4/MD-2 receptor complex in myocardial cells, leading to inhibition of both MAPK and PI3K/Akt signaling pathways, which finally block NF- κ B activation and subsequent release of proinflammatory cytokines TNF- α and IL-1 β . Therefore, it is likely that recombinant human annexin A5 may offer potential life-saving treatment for sepsis.

Several interesting and important questions remain unanswered. In this study, Arnold et al (14) used a mouse model of LPS-induced endotoxemia to address the effects of recombinant human annexin A5 on Gram-negative sepsis. It is unclear whether recombinant human annexin A5 also has therapeutic effects on cardiac function and animal survival in a clinically relevant model of cecal ligation and puncture-induced polymicrobial sepsis (15). Furthermore, endotoxemia caused by LPS can induce multiple organ injury and dysfunction, which relates closely to animal death. It will be important to determine the potential protective effects of recombinant human annexin A5 on other vital organs (lung, liver, and kidney) during sepsis. In addition, the molecular mechanisms by which annexin A5 regulates TLR4 signaling pathway remain further investigation. Although the current finding indicates the association between annexin A5 and TLR4, it is not known how this

association blocks TLR4 signaling. Whether annexin A5 directly regulates downstream signaling molecules is not clear. Despite these limitations, Arnold et al (14) have taken an important step in bringing an exciting arena of investigation to the treatment of patients with sepsis. This work and further investigation sure to follow will undoubtedly help us gain new insights into the role and mechanism of recombinant human A5 in the treatment of sepsis and the potential for a novel therapeutic drug. Systematical and delicate animal experiments will be required to define whether these promising observations can be translated into human trials.

Acknowledgments

Dr. Hu received support for article research from the National Institutes of Health.

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