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Targeting caspase-1 in sepsis: a novel approach to an old problem

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More than 500,000 people develop sepsis each year in the USA alone, and approximately 175,000 of them die [1, 2]. Moreover, the incidence of sepsis has been increasing at an annualized rate of 8.7% [1]. Sepsis is one of the most common risk factors for acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS), accounting for 89% of all ALI/ARDS cases [3]. Approximately 30–50% of patients with ALI/ARDS who die have sepsis [4]. Understandably, the identification of small molecules that can be used as therapeutic agents in the treatment of sepsis has been a major research priority for the past several years.

Sepsis is associated with a systemic inflammatory response thought to be orchestrated by pro-inflammatory cytokines. One of the most important pro-inflammatory cytokines produced in sepsis is interleukin-1 β (IL-1 β), an early cytokine produced by monocytes and macrophages in response to inflammatory stimuli such as LPS [5, 6]. IL-1 β can mediate its own production and also induce the expression of many pro-inflammatory cytokines such as IL-6, IL-8 and TNF- α [7, 8]. IL-1 β is produced as a 31-kDa precursor that must be cleaved into a 17-kDa form in order to become bioactive. The conversion of

pre-IL-1 β into bioactive IL-1 β can be accomplished by the enzyme caspase-1 [9, 10]. Caspase-1 is also involved in the activation of other pro-inflammatory cytokines, most notably IL-18. Caspase-1, therefore, is an obvious target for blockade in studies aimed at finding novel therapeutic strategies for sepsis.

In this issue, Boost et al. [11] report the use of the caspase-1 inhibitor Ac-YVAD-CHO (YVAD) in ventilated rats rendered septic by the intravenous administration of *E. coli* LPS. YVAD is a tetrapeptide aldehyde that reversibly inhibits caspase-1 with a $K_i = 0.76$ nM [12]. YVAD can also inhibit other caspases in vitro, but because the dissociation constants are at least one order of magnitude higher (e.g., 163 nM for caspase-5 and 353 nM for caspase-8), YVAD is considered a relatively specific inhibitor of caspase-1 [12]. A novel aspect of Boost's study is that YVAD was administered by nebulization, and this is important because nebulization is potentially less expensive than the intravenous administration of drugs. Despite the fact that YVAD was delivered exclusively to the lungs, Boost et al. observed a decrease in the concentrations of IL-1 β and IL-18 in plasma, indicating that local delivery to the lungs was effective in inducing systemic inhibition of the cytokine response. Furthermore, YVAD treatment reduced the expression of the pro-inflammatory enzyme COX-2 in the lungs, suggesting a possible protective effect against local lung inflammation. These results are encouraging, and raise the possibility that local delivery of caspase-1 inhibitors to the lungs could be protective of both the pulmonary and systemic manifestations of sepsis. However, it is well known that a large randomized controlled trial testing the role of IL-1 blockade in humans with sepsis failed to show a protective effect [13]. Why, then, insist on IL-1 blocking strategies as a therapeutic approach to sepsis?

The surprising answer is that the protective effect of caspase-1 blockade appears to be independent

of its role as the main activator of IL-1 β . Although macrophages from IL-1 β ^{-/-} mice have impaired production of cytokines in response to LPS in vitro, the IL-1 β knockout mice are not protected from endotoxic shock in vivo. After the intraperitoneal instillation of 5 mg/kg of *E. coli* LPS, IL-1 β ^{-/-} mice show similar mortality as the wild-type mice, and also similar concentrations of IL-1 α , IL-6 or TNF- α [14]. In contrast, mice with a targeted deletion of caspase-1 are resistant to endotoxemic shock [15]. Furthermore, caspase-1^{-/-} mice are protected from death in a model of live bacterial sepsis induced by the intraperitoneal injection of live *E. coli*, 5×10^8 cfu/kg [2]. The same dose of bacteria is lethal for IL-1 β ^{-/-} mice and for also for IL-1 β /IL-18 double knockout mice, confirming that the protection conferred by the lack of caspase-1 is independent of its role as IL-1 β -converting enzyme [2]. But, if the protective effect of caspase-1 deletion is not due to decreased production of IL-1 β , what is the mechanism?

Caspase-1 is one of the 15 known members of the cysteine-aspartic proteases family of proteins, all of which are involved in processing proteins critical to major changes in cell state [16]. Caspases recognize tetrapeptide sequences in proximity to an aspartic residue [17]. Protein substrates of caspases include nuclear proteins, transcription factors, cell cycle regulators, kinases and cytoskeletal proteins [16]. Although these proteins are involved in a number of different cell functions, caspases are best known for their role in the production of inflammatory cytokines and in apoptosis. Accordingly, caspases have been divided into functional groups. The “inflammatory” caspases, composed of caspase-1, -4 and -5, contain an N-terminal caspase recruitment domain (CARD) and are primarily involved in the activation of inflammatory cytokines. The “initiator” caspases (caspase-8, -9, -10, and -2) and the “executioner” caspases (caspase-3, -6 and -7) are primarily involved with the induction of apoptosis. However, activation of the prototypical inflammatory caspase, caspase-1, can induce apoptosis of macrophages and fibroblasts [18–20]. Alternatively, activation of the prototypical executioner caspase, caspase-3, has been associated with activation of activator protein-1 (AP-1) and chemokine production in the liver in vivo [21]. Thus, it is clear that the distinction between “inflammatory” and “apoptotic” caspases is not absolute, and the actual role in vivo of specific caspases in apoptosis or inflammation may differ from the established observations in vitro.

Accordingly, Sarkar et al. have proposed that the protective effect of caspase-1 deletion in sepsis is due to inhibition of lymphocyte apoptosis [2]. This is based on their observation that sepsis induces marked lymphocyte apoptosis in wild-type mice and also in IL-1 β ^{-/-} mice, but not in caspase-1^{-/-} mice. These observations are compatible with the “lymphocyte paralysis” apoptosis proposed by Hotchkiss et al., according to which “mortality in sepsis results from an impaired immune response due to excessive death of immune effector cells”, in particular lymphocytes (reviewed in [22]). This hypothesis is supported by studies showing that sepsis is associated with lymphocyte apoptosis, that adoptive transfer of apoptotic splenic lymphocytes worsens survival in experimental models of sepsis, and that blockade of lymphocyte apoptosis with broad caspase inhibitors is protective in a model of cecal ligation and puncture [23–25]. Although Boost and coworkers [11] did not measure lymphocyte apoptosis in their study, their findings are compatible with the possibility that caspase-1 inhibition exerted its protective effects by inhibiting lymphocyte apoptosis.

An alternative hypothesis to explain the protective effect of caspase-1 blockade in sepsis is disruption of the “inflammasome”. The inflammasome is a multiple adaptor complex that acts as an information-processing node coupling signals generated from inflammatory stimuli (such as LPS) with responses such as cytokine production and apoptosis [26, 27]. The inflammasome appears to have the ability to target a response depending on the individual adaptors that are being activated [26]. Disruption of inflammasome components is associated with resistance to LPS-induced sepsis [26], whereas gene mutations associated with dysregulation of the inflammasome result in the Muckle-Wells syndrome of recurrent fever [28]. Caspase-1 is a key component of the inflammasome, raising the possibility that caspase-1 deletion protects from sepsis by disrupting the processing of information by the inflammasome.

In summary, the study by Boost et al. published in this issue of *Intensive Care Medicine* adds to the growing body of evidence suggesting that caspase-1 blockade is protective in experimental sepsis. Furthermore, the Boost study [11] suggests that local administration of a caspase-1 inhibitor by inhaled aerosol is sufficient to exert a systemic protective effect at the cytokine level. Further studies are necessary to identify the mechanisms linking caspase-1 blockade with protection in sepsis, and to determine the relevance for human disease.

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