論　文　内　容　要　旨

Sera from Septic Patients Contain the Inhibiting Activity of the Extracellular ATP-Dependent Inflammasome Pathway

(敗血症患者の血清には細胞外 ATP 依存性インフラマソーム経路の活性抑制物質が含まれる)

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Abstract

Sepsis, a systemic inflammatory response to infection, causes multi-organ failure, severe morbidity, and death; endothelial dysfunction, metabolic changes, and overwhelming or hyperinflammatory innate immune responses to pathogen-associated molecular patterns have been proposed to be the main underlying mechanisms of this condition. Sepsis-induced immunoparalysis, in which both innate and adaptive immunity are suppressed, is also an active focus of research.

Inflammatory responses mediated by innate immunity generally promote healing and microbial clearance, although the massive production of pro- and anti-inflammatory cytokines by macrophages might also lead to pathological inflammation. Primary inflammatory cytokines include tumor necrosis factor-α, interleukin-1β (IL-1β), and IL-6, which mediate acute inflammation but also cause cell injury and cell death. In turn, injured tissues and dying cells generate damage-associated molecular patterns (DAMPs), which further engage various inflammasomes to promote or suppress inflammation. Although the underlying mechanisms are unknown, damage-associated molecular pattern molecules (DAMPs) from septic tissues might be involved. DAMPs stimulate nucleotide oligomerization domain-like receptor (NLR) inflammasomes to activate caspase-1, which then cleaves pro-IL-1β to generate mature, bioactive IL-1β. One such NLRP3 inflammasome is particularly sensitive to a wide variety of DAMPs including adenosine triphosphate (ATP), crystalline substances, nucleic acids, and hyaluronan. In this study, we screened sera from septic patients for the ability to alter innate immune responses to DAMPs and inflammasomes, with the goal of defining mechanisms that drive immunoparalysis during sepsis.

Macrophages derived from THP-1 human acute monocytic leukemia cells are one of key points in innate immune response. THP-1-mediated macrophages were incubated with each DAMP, in the presence or absence of sera that were collected from critically ill patients. Sera from critical ill patients diagnosed with sepsis or trauma were collected at the time of admission to Emergency and Critical Care Center. Secreted cytokines in supernatant

were then quantified, and treated-cell lysates were assayed for relevant intracellular signaling mediators.

The results indicated that sera collected from septic patients at the time of admission tended to suppress the *in vitro* production of IL-1β by macrophages; however, sera from trauma patients or healthy volunteers did not. This result suggests that septic sera, collected at the time of patient admission, are likely predominantly anti-inflammatory, despite enhanced levels of both pro- and anti-inflammatory cytokines. Strikingly, sera from septic patients who ultimately did not survive significantly suppressed IL-1β production in response to extracellular adenosine triphosphate (ATP) only. This effect was most pronounced with sera collected on day 3, and persisted with sera collected on day 7. However, this effect was not observed when THP-1 macrophages were treated with sera from survivors of sepsis. Therefore, we hypothesize that sera from septic patients have inherent anti-inflammatory functions and might contain factor(s) that suppress the response to extracellular ATP by possibly mechanisms as ATP hydrolysis by ATPases in sera from septic patients, inhibition of $P2X_7$, or inhibition of NLRP3 inflammasome.

The further experiments were setting up for identifying mechanisms of the observed loss of ATP-induced IL-1β production. The results suggest that ATPase activity in sera from septic patients is negligible, and septic sera from non-survivors enhance $P2X_7$ activity. Then cell lysates were subjected to the detection of cytosolic mediators associated with the ATP-dependent inflammasome pathway. Results shown that septic sera collected at the time of admission (day 1) also diminished intracellular levels of inositol 1,4,5-triphosphate and cytosolic calcium ($P < 0.01$), both of which are essential for ATP signaling. Finally, activated caspase-1 was significantly diminished in cells exposed to sera collected on day 7 ($P < 0.05$). Taken together, these data suggest that activation of NLRP3 and caspase-1 in response to extracellular ATP is suppressed by factors that are present in sera from septic patients. This suppressive effect is clearly associated with prolonged immunoparalysis and poor clinical outcomes since it was found to be more pronounced in sera collected from patients who did not survive.

Our data indicated that sera from septic patients suppress IL-1β production in macrophages stimulated with extracellular ATP, likely by antagonizing upstream NLRP3 or pro-inflammatory pathways. This suppressive effect peaked on day 3 after admission and persisted until day 7 among patients who did not survive, but was lost by day 7 among patients who survived. These observations suggest a new mechanism through which sepsis mediates immunoparalysis, and partially support the prior hypothesis that persistent suppression of innate and adaptive immunity can cause late-stage death in septic patients. In conclusion, factors present in the sera of septic patients that persistently suppress the immune response to extracellular ATP might trigger adverse clinical outcomes.