P-19

The new sepsis marker, sCD14-ST (PRESEPSIN), induction mechanism in the rabbit sepsis models

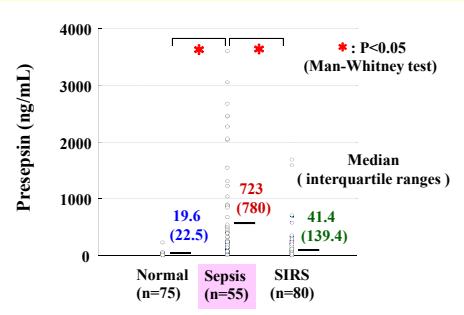
Katsuki Naitoh, Kamon Shirakawa, Jiro Hirose, Masaki Nakamura, Takashi Takeuchi, Yoshitaka Hosaka, Shoji Furusako

Discovery Research, Mochida Pharmaceutical Co., Ltd., Gotemba, Japan

Background

- ✓ CD14 is a high-affinity endotoxin receptor expressed on the immune cells and an attractive target for research on infectious diseases.
- ✓ sCD14-ST (Presepsin) is a truncated form of CD14. We generated an anti-presepsin antibody and developed the new immunoassay to measure a presepsin level as a diagnostic marker of sepsis and septic shock.
- ✓ The levels of presepsin were significantly higher in the septic patients than in the SIRS patients and healthy controls (Fig.1).
- ✓ The production mechanism of presepsin has been investigated with *in vitro* and *in vivo* system and our hypothesis is discussed in the poster presentation.

Fig.1 Presepsin levels were elevated in septic patients.



The concentrations of presepsin were measured in plasma samples from normal controls, septic patients, and SIRS patients by the 1st generation kit [1].

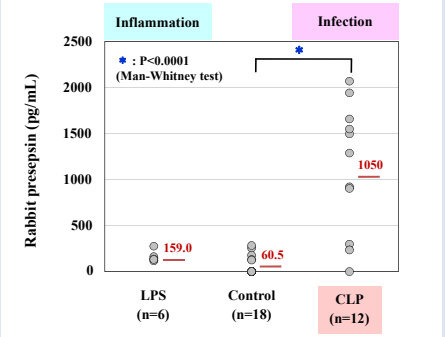
1) J Infect Chemother 2005;11:1234-38.

(1) Remarkable rise of presepsin level in CLP model, but not in LPS-induced model

In vivo

- LPS-induced sepsis model
- Cecal ligation and puncture (CLP) sepsis model

Fig.2 Presepsin levels in two experimental sepsis models.

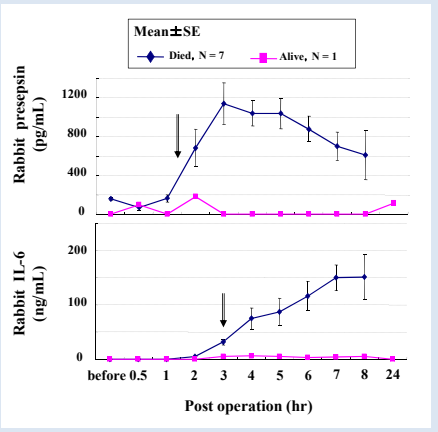


LPS induced systemic inflammation however CLP induced polymicrobial infection[2,3].

- ✓ The presepsin level was significantly increased in the CLP model, but not in the LPS one.
- ✓ Thus, it was speculated that the infectious stimulus led to the elevation of presepsin levels.

2) Kidney International 2003;64:1620-31
3) Shock 2005;24:52-7

Fig.3 The time course of presepsin and IL-6 levels in the CLP model.



- ✓ The presepsin levels were elevated in dead individuals within 24 hr, but not in alive individuals.
- ✓ The presepsin levels were elevated sharply prior to the inflammation marker, IL-6.

(2) Secretion of presepsin in rabbit peritoneal leukocytes.

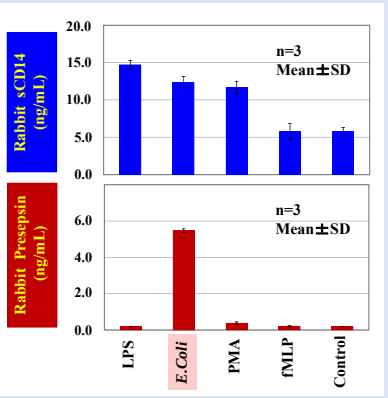
In vitro (cell assay)

- Induction of presepsin in rabbit peritoneal leukocyte
- Effect of phagocytosis inhibitors on presepsin secretion
- Effect of protease inhibitors on presepsin secretion

Preparation of rabbit peritoneal leukocytes

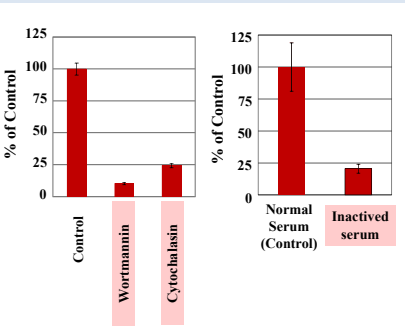
- ➢ Peritoneal leukocytes were prepared from NZW rabbit by injection of 0.1% glycogen.
- ➢ The peritoneal exudated cells were collected and suspended in the assay buffer containing 2% normal rabbit serum.
- ➢ The peritoneal leukocytes were treated with several stimulators (Fig.4) and inhibitors (Fig.5, 6)
- ➢ The rabbit presepsin levels in culture supernatants were measured by ELISA.

Fig.4 Secretion of sCD14 and presepsin in peritoneal leukocytes.



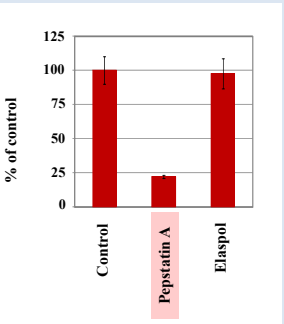
- ✓ The sCD14 level was elevated in LPS, *E.Coli* and PMA treated groups.
- ✓ The presepsin level was increased specifically by the treatment of *E. Coli*, demonstrating that infection was required for the secretion of presepsin.

Fig.5 Effects of phagocytosis inhibitors and inactivated serum on *E.coli*-induced presepsin secretion.



- ✓ The *E. coli* –induced presepsin secretion was suppressed by the addition of wortmannin or cytochalasin, and also in the inactived serum.
- ✓ The results indicated that phagocytosis was involved in the secretion of presepsin .

Fig.6 Effects of protease inhibitors on *E. coli* –induced presepsin secretion.



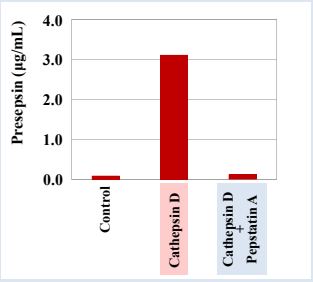
- ✓ An asparatic protease inhibitor, pepstatin A suppressed the *E. coli* – induced presepsin secretion while an elastase inhibitor, elaspol did not.
- ✓ It was speculated that the aspartic protease cleaved CD14 to generate presepsin.

(3) Production of presensin by the cleavage of CD14 with Cathepsin D.

In vitro (Protein)

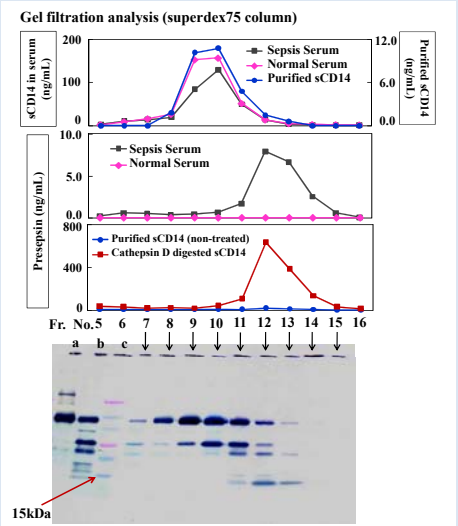
- Digestion of CD14 with a lysozomal enzyme
- Analysis of digested product with gel filtration analysis and western blotting

Fig.7 Generation of presepsin from sCD14 by cathepsin D and suppression by its inhibitor.



- ✓ Human sCD14 was purified from human serum. Treatment of sCD14 by cathepsin D produced presepsin over 30-fold (Fig.7).
- ✓ The presepsin produced by cathepsin D was identical to the fractions detected in the sepsis serum (Fig. 8).
- ✓ An estimated molecular weight of the presepsin produced by cathepsin D was 13kDa including N-terminal of CD14.

Fig.8 Analysis of CD14 derived products digested with cathepsin D.

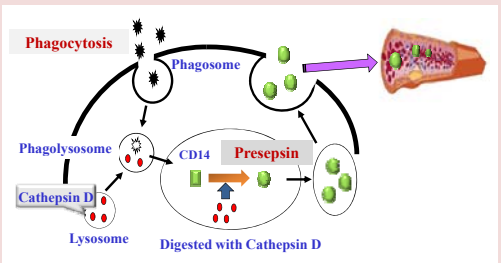


a : Purified sCD14 (non-treated)
b : Cathepsin D digested products
c : Molecular Weight Marker

Detection Antibody

- ✓ Anti-CD14 N-terminal antibody

A putative mechanism of presepsin secretion



Conclusions

- ✓ Presepsin markedly increased in the septic patients.
- ✓ Microbial infection caused the elevation of the presepsin level in rabbits model, but not LPS challenge.
- ✓ *E.Coli* cells induced the secretion of presepsin from peritoneal leukocytes, but not LPS.
- ✓ Phagocytosis inhibitors or aspartic protease inhibitors suppressed the secretion of presepsin in peritoneal leukocytes.
- ✓ Human presepsin is produced by cleavage of CD14 with cathepsin D.

4) Infect Immun. 1997; 65: 4747–4753

- ✓ Secretion of presepsin might be triggered by microbial infection.
- ✓ Phagocytosis process and cleavage with lysozomal enzymes are involved in its secretion.
- ✓ Presepsin might be a useful biomarker for infectious diseases including sepsis and septic shock.