Infection

**Presepsin (ng/mL)**


- 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.

### 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 immunosassay 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.

<table>
<thead>
<tr>
<th>Presepsin (ng/mL)</th>
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<tbody>
<tr>
<td>Normal (n=75)</td>
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<tr>
<td>Sepsis (n=55)</td>
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<td>SIRS (n=30)</td>
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The concentrations of presepsin were measured in plasma samples from normal controls, septic patients, and SIRS patients by the 1st generation kit 1), 2) Kidney International 2003;64:1620-31 3) Shock 2005;24:52-7

### (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.

![Presepsin levels in two experimental sepsis models](image)

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

### (2) Secretion of presepsin in rabbit peritoneal leukocytes.

**In vitro**

- 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.

**In vitro**

- Digestion of CD14 by 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.

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

<table>
<thead>
<tr>
<th>Gel filtration analysis (superdex75 column)</th>
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<tbody>
<tr>
<td>Sepsis Serum</td>
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<tr>
<td>Normal Serum</td>
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<tr>
<td>Purified sCD14 (non-treated)</td>
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<tr>
<td>Cathepsin D digested sCD14</td>
</tr>
</tbody>
</table>

**A putative mechanism of presepsin secretion**

- Phagocytosis
- Phagosome
- CD14
- Presepsin
- Lysozome
- Digested with Cathepsin D

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

- 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.

### Conclusions

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