Factor VII-activating protease: Mechanism and regulation of nucleosome release from dead cells
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Complexes of factor VII-activating protease with plasminogen activator inhibitor-1 in human sepsis

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Dear Sirs,

Systemic inflammation in sepsis is characterized by extensive cell death (1) resulting in the release of cellular structures, e.g. cell-free DNA and DNA-binding proteins such as histones (damage associated molecular patterns, DAMPs). DAMPs have been demonstrated to be highly pro-inflammatory and therefore crucially involved in the pathogenesis of sepsis (2). Circulating extracellular DNA, nucleosomes and histones correlate with disease severity and fatality in sepsis (2-5). At present the precise mechanism involved in DAMP release and how it is regulated is unclear. We have previously shown that factor VII-activating protease (FSAP) releases nucleosomes from late apoptotic cells and, in concert with DNase I, also from necrotic cells (6). FSAP is a serine protease which circulates in plasma as an inactive single-chain molecule and is proteolytically converted into its active two-chain form (7). FSAP in plasma binds to apoptotic and necrotic cells and is subsequently activated (8). We have demonstrated the presence of complexes of FSAP and its inhibitors α2-antiplasmin (AP) and C1-inhibitor (C1inh) in plasma of patients suffering from severe sepsis, septic shock and meningococcal sepsis (8). In purified systems plasminogen activator inhibitor-1 (PAI-1) has been shown to inhibit FSAP (9). Previously, FSAP-PAI complexes have been detected by immunoblotting in bronchoalveolar lavage fluids from patients with acute respiratory distress syndrome, suggesting that FSAP inhibition by PAI-1 also occurs in vivo (9). In sepsis PAI-1 plasma levels have been shown to increase with disease severity and are a strong predictor for fatality (10;11). A polymorphism in the PAI-1 gene, associated with higher PAI-1 levels, has been demonstrated to correlate with poor outcome in meningococcal sepsis (12). The adverse effects of increased PAI-1 levels in sepsis are based on its procoagulant and pro-inflammatory properties due to complex formation with tissue-type plasminogen activator (t-PA) and activated protein C (APC), respectively (13).

In our current study, we established an ELISA to measure FSAP-PAI-1 complexes in plasma of patients with severe sepsis and septic shock as well as in children suffering from meningococcal sepsis. We used a monoclonal anti-FSAP antibody (6) as a catching antibody and a polyclonal antibody to PAI-1 for detection (14). As positive control for this ELISA, FSAP in plasma was activated by co-incubation with late apoptotic cells. Since PAI-1 levels in healthy individuals are very low, recombinant PAI-1 (a kind gift of Paul Declerck, Leuven) (15) was added to control plasma before incubation with late apoptotic Jurkat cells (Figure 1A). In the next step we measured FSAP-PAI-1 complexes in 40 surgical and medical patients at the time point of diagnosis with severe sepsis (n=32) or septic shock (n=8) following the ACCP guidelines (16) and in 20 healthy controls. The clinical characteristics of these 40 sepsis patients were described in detail elsewhere (4). FSAP complexes with PAI-1 could be measured in 25% of the sepsis patients. Although FSAP-PAI-1 complex levels were significantly (p=0.01) higher in the
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sepsis patients when compared to the controls, no difference could be found in patients with severe sepsis and septic shock and between survivors and non-survivors (p>0.05; Figure 1B). By using Spearman’s rank correlation (corrected for multiple testing), significant correlations of FSAP-PAI-1 complexes with FSAP-AP (r=0.63, p<0.0001), FSAP-C1inh complexes (r=0.73, p<0.0001) and nucleosome levels (r=0.44, p<0.005) could be found. As PAI-1 and C3a are both predictive parameters for outcome, these significantly correlated with FSAP-PAI complexes (r>0.49, p<0.001). No correlation of FSAP-PAI complexes with organ dysfunction scores and cytokines could be found.

Next, we measured FSAP-PAI-1 complexes in 38 children with meningococcal sepsis (17). From these 38 patients, 9 died as a result of the disease (non-survivors). On admission, plasma samples were available from 35 patients. Increased FSAP-PAI-1 complexes were found in 91.4% of the patients on admission (Figure 1C). Highest FSAP-PAI-1 levels have been measured during acute disease and correlated with fatality (Figure 1D). Significant correlations with FSAP-AP (r=0.52, p<0.001), FSAP-C1inh (r=0.41 p=0.014) and nucleosomes (r=0.80 p<0.0001) could be found. FSAP-PAI complexes significantly correlated with organ dysfunction scores (r>0.53, p<0.001), pro-inflammatory cytokines (r>0.65 p<0.0001), coagulation and fibrinolysis markers (r>0.56, p<0.001) as well as with PAI-1 levels in those patients (r=0.871, p<0.0001). FSAP antigen levels are stable in plasma of all patients and comparable to healthy controls (~12 μg/ml). However, levels of PAI-1 are 5-10 times higher in meningococcal sepsis patients (~5 μg/ml (17)) compared to sepsis patients (~0.65 μg/ml (4)).

In this study we demonstrate the presence of FSAP-PAI-1 complexes in the plasma of patients suffering from severe sepsis, septic shock or meningococcal sepsis by means of ELISA. With increasing severity, FSAP-PAI-1 complexes increase and significantly correlate with organ dysfunction scores and clinical parameters for outcome, a dynamic comparable with PAI-1 levels in the plasma of these patients. Interestingly, the course of the FSAP-PAI-1 complex levels in time differs from the FSAP-C1inh and FSAP-AP complexes in the meningococcal non-survivors (8). The FSAP-C1inh and FSAP-AP complex levels are increasing in time, while the FSAP-PAI-1 levels are decreasing. The dynamics of complex formation of FSAP with different serine protease inhibitors (serpins) reflects the presence of serpins in different disease states. FSAP was demonstrated to cleave high-molecular kininogen (HMWK) resulting in the release bradykinin (BK), a nonapeptide essential in the induction of hypotension in sepsis (18;19). Since PAI-1 levels increase during sepsis, inhibition of FSAP by PAI-1 might contribute to the prevention of BK release and hence improve hypotension in sepsis patients. On the other hand, complex formation of FSAP with PAI-1 neutralizes PAI-1, since serpins form an irreversible covalent complex (20). PAI-1 levels closely correlate with disseminated
Figure 1: FSAP-PAI-1 complexes

FSAP-PAI-1 complexes were measured by ELISA using anti-FSAP4 (2 µg/ml) as catching antibody and a biotinylated polyclonal rabbit antibody against PAI-1 (dilution 1:1000 v/v) for detection. (A) Complexes were measured in recalcified plasma (r-plasma) of a healthy donor to which recombinant PAI-1 (1 µg/ml) was added before incubation with late apoptotic Jurkat apoptotic cells. Apoptosis was induced by incubation for 48h with etoposide (200 µM). This r-plasma was used as standard and arbitrarily set to 50 AU/ml since plasma was diluted 1:2 (v/v) with apoptotic cells. Results are given as mean±SEM, (n=3). (B) FSAP-PAI-1 complexes were measured in citrated plasma from 40 patients with severe sepsis or septic shock. Closed circles represent patients with severe sepsis and open circles represent patients with septic shock. Plasma of 20 healthy donors was taken as a control. FSAP-PAI-1 complex levels were expressed as AU/ml. (C) FSAP-PAI-1 complexes were measured in citrated plasma from 38 meningococcal sepsis patients. Blood was sampled at 0 h, 12 h, 24 h and 3 months. Plasma of 20 healthy donors was taken as a control. FSAP-PAI-1 complex levels were expressed as AU/ml. (D) FSAP-PAI-1 complexes were measured in citrated plasma from survivors and non-survivors of meningococcal sepsis. Blood was sampled at 0 h, 12 h and 24 h. FSAP-PAI-1 complex levels were expressed as AU/ml. Results are given as mean±SEM. Median values have been compared by using Mann Whitney Rank Sum test. *** indicates a p<0.001, ** indicates a p<0.01, * indicates p<0.05.
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intravascular coagulation and the severity of disease and are predictive for outcome (11;21-23). This inhibitory mechanism might be especially relevant in severe inflammation since both FSAP activation and PAI-1 levels increase with disease severity. Based on these results we speculate on a dual role for the interaction of FSAP and PAI-1. On the one hand, complex formation with PAI-1 will limit FSAP-mediated BK formation in sepsis. On the other hand FSAP-PAI-1 complex formation may attenuate the procoagulant and pro-inflammatory effects of PAI-1.

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Chapter 3

References

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