Factor VII-activating protease: Mechanism and regulation of nucleosome release from dead cells
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Citation for published version (APA):

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Cell death is indispensable for tissue homeostasis and a fundamental principle of inflammation and lymphocyte generation. Phagocytes engulf dead cells, which are recognized as dead by virtue of a characteristic “eat me” signal exposed on their surface. Rapid removal and destruction of the cellular corpses is critical and insufficient removal may lead to the release of potentially harmful cytotoxic and immunogenic cellular content into the environment. These immunogenic “host-self” molecules (damage-associated molecular patterns (DAMPS)) might induce a pro-inflammatory response, autoantibody formation, and development of autoimmune diseases, such as systemic lupus erythematosus (SLE). Nucleosomes might also function as endogenous danger signals. Recently, factor VII-activating protease (FSAP) was identified as the plasma serine protease responsible for nucleosome release from late apoptotic cells (1,2). In this thesis the mechanism of nucleosome release and its regulation was investigated. We studied FSAP activation and discuss the possible role of FSAP in health and disease.

### FSAP activation in health and disease and its regulation by plasma inhibitors

In *chapter 2* we have used Western blot to show that FSAP in plasma is activated by late apoptotic as well as necrotic cells, whereas living cells do not activate FSAP. In addition, FSAP strongly binds to late apoptotic as well as to necrotic cells and no binding to living cells was seen. In addition to our nucleosome releasing assay, we were interested in measuring FSAP activation in plasma. Direct measurement of FSAP enzyme activity in plasma is complicated due to the lack of specific substrates. Moreover, the predicted half-life of active enzyme is extremely short, due to the presence of high plasma levels of inhibitors of FSAP. By use of affinity purification and mass spectrometry analysis we have shown that activated FSAP in plasma rapidly forms a complex with α2-antiplasmin (AP) upon exposure to apoptotic cells.

In order to set up a fast and sensitive method to detect FSAP activation we have developed an ELISA to measure FSAP-AP complexes. Another plasma inhibitor of FSAP that has been described is C1-inhibitor (C1inh) (3). Although we were not able to detect FSAP-C1inh complexes after affinity purification followed by SDS-PAGE, we were able to detect these complexes by ELISA. This may indicate that ELISA is a more sensitive method than affinity purification and SDS-PAGE or, alternatively, that the FSAP-C1inh complex dissociates upon SDS-PAGE. Thus, we have demonstrated that FSAP activation in plasma can be monitored by assays detecting complexes of FSAP with its target serpins AP and C1inh. We then used these FSAP-inhibitor complex ELISAs to demonstrate FSAP activation in post-surgery patients, patients suffering from severe sepsis, septic shock and meningococcal sepsis. FSAP activation clearly correlated with nucleosome levels and severity in these patients.

In *chapter 3* we determined complex formation of FSAP with a third inhibitor, plasminogen activator inhibitor-1 (PAI-1). We were able to show the presence of FSAP-PAI-1 complexes in
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the plasma of patients suffering from severe sepsis, septic shock or meningococcal sepsis by means of a newly developed ELISA. FSAP-PAI-1 complexes could not be detected in plasma of healthy controls after activation of FSAP or in plasma of post-surgery patients. This is in line with the low PAI-1 concentrations in plasma of these patients or controls. In the meningococcal non-survivors the course of the FSAP-PAI-1 complex levels in time diverged from the FSAP-C1inh and FSAP-AP complexes. The FSAP-C1inh and FSAP-AP complex levels increased up to 24 hours after inclusion, whilst the FSAP-PAI-1 levels decreased simultaneously. The decrease of FSAP-PAI-1 complexes may be contributed to a reduction of PAI-1 in the plasma of these patients (4). Whether the increase in FSAP-C1inh and FSAP-AP complexes can be merely attributed to the concomitant decrease in FSAP-PAI-1 or is also a result of an increase in FSAP activation over time is presently not known. FSAP-inhibitor complex levels in meningococcal sepsis were significantly higher in survivors than in non-survivors, although the non-survivor group was small. FSAP-inhibitor complex levels in sepsis increased with the severity of inflammation as shown by significant correlations with inflammatory markers but did not discriminate for fatality. We observed a discrepancy between sepsis and meningococcal sepsis which may be explained by the fact that the sepsis patients formed a heterogeneous group, whereas the patients suffering from meningococcal sepsis were derived from a more homogenous population with a clear and more synchronized onset of sepsis.

In Chapter 4 we have demonstrated that besides the serine protease inhibitors (serpins) AP, C1inh and PAI-1, also the kunitz-type protease inhibitor Tissue Factor Pathway Inhibitor (TFPI) is an inhibitor of FSAP. TFPI was found to be a more efficient inhibitor than AP and C1inh in both plasma and a purified system. The efficient inhibition by TFPI might be attributable to the fact that it is a kunitz-type protease inhibitor that has a different inhibitory mechanism than the serpins AP and C1inh. TFPI is a canonical protein inhibitor and forms a tight, non-covalent interaction similar to the classical enzyme-substrate Michaelis complex that results in direct blockage of the active site and does not induce conformational changes (5). In contrast, serpins form an irreversible covalent acyl-enzyme complex resulting in large conformational changes in the inhibitor and disruption of the protease active site (6). TFPI binds to surfaces and is therefore considered to be an efficient surface-bound inhibitor of proteases. However, we found that also in a cell-free system TFPI was an efficient inhibitor of FSAP activity. Using monoclonal antibodies to the various domains of TFPI in combination with TFPI mutants we have demonstrated that both the K2 domain and the Cter of TFPI are involved in the inhibition of FSAP activity. Since the main function that is contributed to the Cter domain of TFPI is its ability to bind to cell surfaces and heparin-like structures (7;8) and we found that it was involved in the inhibition of FSAP even in the absence of a cell surface, we have also tested whether TFPI binds to FSAP. Indeed, TFPI directly binds to FSAP and this interaction was partially abrogated by addition of an anti-Cter antibody.
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FSAP has been reported to down-regulate TFPI and thereby propagates FXa activity and potentiates thrombin generation (9, thesis Dienava-Verdoold 2012). Kanse et al. has demonstrated that proteolytic degradation of TFPI was strongly dependent on FSAP binding to the TFPI Cter (9). This is in line with our results showing that FSAP inhibition by TFPI is dependent on binding of FSAP to the Cter of TFPI. Proteolytic degradation of TFPI might potentially provide a mechanism of how FSAP is inhibited by TFPI in our system. As such, TFPI would serve as a substrate for FSAP and hence compete with the chromogenic substrate or target proteases of FSAP. However, when the inhibition curves of the amidolytic activity after 30 and 60 minutes incubation are studied in detail no discernable difference was observed, indicating that there was no substantial loss of TFPI activity, which one would expect if proteolytic degradation of TFPI would occur.

TFPI is mainly produced by and bound to vascular endothelial cells (10;11). Therefore, the plasma concentration of TFPI does not reflect the local concentration of full-length TFPI on cellular surfaces. Administration of recombinant full-length TFPI has been demonstrated to attenuate the inflammatory response in animal and human sepsis (12-14). The plasma levels obtained in the human studies after therapeutic administration are in the same range as the concentrations we have used in vitro (12;15;16). Circulating histones and nucleosomes were shown to induce a potential fatal inflammatory response in sepsis (17;18). One might therefore hypothesize that FSAP has a pro-inflammatory role in sepsis. Inhibition of FSAP-induced nucleosome release might, in part, explain the anti-inflammatory effects of high-dose administration of recombinant TFPI observed in animal and human sepsis (12-14).

Rapid inactivation of activated FSAP via complex formation with its inhibitors might be of critical importance in order to prevent dissemination of activated FSAP in the circulation. FSAP was demonstrated to cleave high-molecular kininogen (HMWK) resulting in the release of bradykinin (BK), a nonapeptide essential in the induction of hypotension in sepsis (19;20). FSAP inactivation in plasma during sepsis and other diseases might be essential in order to control HMWK cleavage and BK release in plasma.

Some general questions concerning the regulation of FSAP by plasma inhibitors remain to be elucidated. It is not known how much FSAP remains associated with dead cells and how much is released during inflammation, free or in complex with its inhibitors. In order to estimate the fraction of single-chain FSAP and activated two-chain FSAP, we used an approach where FSAP was activated in vitro by incubating plasma with late apoptotic cells. We subsequently performed size-exclusion chromatography and found that approximately 30% of FSAP did not form complexes with inhibitors. These results suggest that 30% of the FSAP remains in its inactive single-chain form, whereas 70% is activated upon incubation with dead cells and forms complexes with its inhibitors (data not shown).
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Another open question is what specific determinants affect inhibitor specificity. The dynamics of complex formation of FSAP with different serine protease inhibitors may reflect the presence of distinct inhibitor subsets in different disease states. On the other hand, some inhibitors of FSAP are present in low concentrations and complex formation with activated FSAP might neutralize the inhibitor. For instance PAI-1, a serpin which forms an irreversible covalent complex with FSAP (6), is present at low levels during sepsis. PAI-1 levels correlate closely with the severity of disseminated intravascular coagulation and disease, but are also predictive of final outcome (21-24). This inhibitory mechanism might be especially relevant in severe inflammation since both FSAP activation and PAI-1 levels increase with disease severity. FSAP-PAI-1 complex formation may attenuate the procoagulant and pro-inflammatory effects of PAI-1.

In search of the activator of FSAP

FSAP was reported to have a dual role in hemostasis by activating coagulation FVII and activating scu-PA in vitro (25;26). However, these findings were mostly based on results from purified systems. We have shown in chapter 2 that when in plasma or serum, scFSAP is a rather stable molecule. Similarly, we could not detect FSAP-inhibitor complexes in serum of healthy donors indicating that there is no FSAP activation upon coagulation activation via FXII. Moreover we observed that activation of coagulation by tissue factor did not lead to FSAP activation (data not shown). When FSAP is purified, it becomes very susceptible to autoactivation (2). For a molecule described to be involved in coagulation and fibrinolysis it seems striking that coagulation of blood does not lead to FSAP activation. In addition, Moreover, FVII has been reported to be remarkably resistant to activation by recombinant FSAP (27).

We have however demonstrated that FSAP gets activated upon contact with late apoptotic and also necrotic cells. For our initial experiments a Jurkat cell line was used (chapter 2). To investigate the activation of FSAP in serum by different types of primary cells, necrotic peripheral blood mononuclear cells (PBMCs) and polymorphonuclear cells (PMNs) were also analyzed for their ability to induce FSAP activation and FSAP-inhibitor complex formation (Chapter 5). Although dead PBMCs were able to activate FSAP, dead PMNs failed to activate FSAP in serum. Consequently FSAP was not able to remove nucleosomes from the neutrophils. Neutrophil are the most abundant but also very short-lived human white blood cells with a high turnover rate. We speculate that when dead neutrophils would be able to activate FSAP, this may possibly lead to continuous FSAP activation. Furthermore, NETosis is a function of neutrophils leading to the release of neutrophil extracellular traps (NETs) in response to various stimuli. These NETs are web-like structures of DNA, histones and proteases of the
neutrophil and have been found to trap and kill microbes extracellularly (28). When these NETs would be immediately broken down by FSAP due to nucleosome release, this would possibly abrogate the antimicrobial role of the NET.

Neutrophils differ from PBMCs in their nuclear morphology in the way that they have multilobed nuclei. Moreover, during neutrophil maturation the number of mitochondria and ribosomes decreases and neutrophils contain a small Golgi apparatus and the rough endoplasmic reticulum is absent. Like PMNs, human erythrocytes also lack cytoplasmic organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus and ribosomes. In addition, mammalian erythrocytes enucleate during maturation. We found that, similar to PMNs, also dead erythrocytes fail to activate FSAP whereas dead avian erythrocytes, containing a nucleus and cytoplasmic organelles, did activate FSAP (chapter 5). These results suggest that structures in the nucleus or one of the missing organelles in neutrophils and erythrocytes may lie at the origin of FSAP activation by dead cells.

Negatively charged polyanions, such as heparin, polyphosphates, DNA and RNA have been demonstrated to enhance autoproteolysis of purified scFSAP (25;29-33). Also polycations, such as polyamine and histones have been demonstrated to activate scFSAP (20;34). We found that FSAP bound to fixed HEp2 cells displayed a nucleolar and cytoplasmic binding pattern upon detection by confocal microscopy. This nucleolar pattern showed similarities with an antinuclear antibody (ANA) staining pattern observed in patients with scleroderma or CREST syndrome (35). The nucleolar staining pattern has been associated with many different antibodies including anti-Scl-70 (anti-topoisomerase 1), anti-PM-Scl (anti-exosome), anti-fibrillarin, anti-Th/To ribonucleoprotein and anti-RNAPI/III (anti-RNA polymerase I/III) (36-40). Most of the antigens recognized by these antibodies are RNA-binding proteins or consist of RNA. The nucleolus is the site of rRNA transcription and processing, and of ribosome assembly. The nucleoli are composed of ribosomal proteins and RNA. Nakazawa et al. have identified cell-derived RNA species as potent cofactors for the autoactivation of FSAP (32). They have shown that several forms of RNA were able to autoactivate the isolated proenzyme of FSAP. In contrast, we have not detected activation of FSAP in plasma by total RNA, transfer RNA or synthetic RNA. This discrepancy in results may be explained by the fact that we use a plasma system. Purified FSAP may be more susceptible for activation by RNA compared to scFSAP present in plasma, or factors plasma may bind and neutralize the RNA. Another difference could be the purity of the several forms of RNA. Furthermore, we routinely used RNase-treated cells to induce FSAP activation. We have found that RNase treatment improves the specificity of the propidium iodide staining used in our nucleosome releasing factor assay. After RNase treatment of the cells, we could still observe nucleosome release by FSAP, suggesting that FSAP activation by RNA in our system is rather unlikely. The
nucleolar binding of FSAP and the fact that FSAP activation is unaffected by RNase treatment suggest that a ribosomal protein may potentially be involved in FSAP activation.

A possible role for FSAP in the clearance of dead cells and autoimmunity

Impaired clearance of dead cells can lead to the induction of inflammation, autoantibody formation and development of autoimmune diseases e.g. systemic lupus erythematosus (SLE) (41-43). Plasma proteins, such as components of the classical pathway of complement (e.g. C1q, C2 and C4) play an important role as these facilitate the phagocytosis of dead cells. Complement deficiencies within the classical pathway have been shown to predispose to the development of SLE (44-47). One of the main characteristics of SLE is the formation of antinuclear antibodies (ANAs) directed against ubiquitous intracellular antigens, e.g. double stranded (ds)-DNA and histones. These ANAs form immune complexes, which contribute to the pathogenesis of SLE upon deposition. Several of the immunogenic endogenous molecules are named damage-associated molecular pattern molecules (DAMPs). Several DAMPs such as DNA, RNA, histones and the DNA-binding protein HMGB1 are known to stimulate Toll-like receptors (TLRs) and are critically involved in the pathogenesis of SLE (48-55). As such, circulating nucleosomes will also function as endogenous danger signals. High levels of circulating nucleosomes and anti-nucleosome antibodies have been found in patients with SLE (56-58). Furthermore, nucleosome-specific autoantibodies have been detected in murine lupus models, before the onset of autoantibodies with other specificities (58). We have shown that FSAP removes nucleosomes from late apoptotic cells (2). In contrast to apoptosis, upon necrosis the integrity of the cell membrane is distorted without cleavage of DNA by intracellular nucleases. We have observed that DNA release from necrotic cells seems to be a multistep process. Whereas the addition of serum was essential to induce removal of nucleosomes from necrotic cells, purified FSAP alone was not able to induce nucleosome release. In chapter 6 we have shown that FSAP cooperates with DNase I in the degradation and removal of DNA from necrotic cells. Decreased activity of serum DNase I has been shown in patients with SLE (59;60) and lupus-prone NZB/NZW mice had significantly lower serum and urine concentrations of DNase I than normal mice (61). Napirei et al. have shown that mouse serum DNase I functions together with the serine protease plasmin in the chromatin breakdown from necrotic cells (62). Plasmin was identified as the plasma protease responsible for chromatin breakdown, since inhibitors, including aprotinin, α2-antiplasmin and PAI-1, were able to inhibit chromatin breakdown. FSAP is a serine protease with structural and functional similarities to plasmin and inactivated by some of the same inhibitors. Plasminogen, the proenzyme of plasmin, is present in the circulation. However, for activation of plasminogen into plasmin to occur, plasminogen activators need to cleave a unique bond in the serine protease domain. Similar to plasminogen, FSAP circulates in its inactive single-chain form and
has to be activated resulting in two chains connected by a disulfide bond. We have shown that FSAP gets activated upon contact with dead cells. In contrast, plasminogen activation requires the presence of specific plasminogen activators, which need to be released (e.g. t-PA from the endothelium). We have found that plasminogen is not activated upon contact with dead cells in our system. Since no additional factor apart from the necrotic cells is required for FSAP activation, the role of FSAP in the release of nucleosomes from necrotic cells appears of physiological significance. This is further supported by our finding that FSAP-deficient serum or serum in which FSAP is inhibited by an inhibitory antibody is not able to release nucleosomes from necrotic cells. Complement factor C1q has also been demonstrated to release nucleosomes from necrotic cells in cooperation with serum DNase (63). However, we were unable to detect differences when using C1q deficient plasma and normal plasma in our nucleosome release assay.

In our assay, where late apoptotic cells were incubated with serum, we have used a 30 minutes incubation period. However, when necrotic cells were incubated with serum for 30 minutes, no nucleosome release was observed. Since Napirei et al. demonstrated that mouse serum was able to generate nucleosomes in necrotic cells after a much longer incubation period (62) we have adjusted our assay system to a 48 hours incubation period. Using this longer incubation period we have found that serum alone can indeed release nucleosomes from necrotic cells. The increased incubation time after necrosis when compared to apoptosis may be required for efficient DNA cleavage by serum DNase I after necrosis, whereas intracellular endonucleases already confer DNA cleavage during the process of apoptosis.

We have not unraveled the precise mechanism by which DNase I contribute to FSAP-induced nucleosome release. DNase I is able to cleave the DNA of necrotic cells in a random manner as is illustrated by a HMW DNA smear that can be observed after DNA isolation and agarose gel electrophoresis. However, this cleaved DNA is not released from the cells in the form of nucleosomes. One interpretation could be that the DNA fragments are too large to be released from the cells or that the DNA remains associated with the necrotic cell via a DNA-binding protein. Interestingly, however, when we analyzed the isolated DNA from necrotic cells treated with a combination of FSAP and DNase I by agarose gel electrophoresis, LMW bands were visible, which we recognize as a typical hallmark of nucleosomes. This indicates that in presence of FSAP, internucleosomal cleavage occurs by DNase I. Several explanations for the observed cleavage pattern can be hypothesized. One could be that the FSAP cleaves a DNA-binding protein, thereby changing the conformation of the chromatin resulting in increased accessibility for DNase I. This hypothesis is supported by the fact that histone H1 is degraded upon incubation of necrotic cells with FSAP. Cleavage of histone H1 induces a more open structure of the DNA at the linker region of the nucleosomes and thereby might allow internucleosomal DNA degradation. Another possible explanation could be that inhibitors of
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DNase I are cleaved by FSAP, resulting in an increased activity of DNase I. Further investigation is needed to elucidate the precise mechanism by which FSAP and DNase I function together in the degradation and release of DNA from necrotic cells.

In absence of serum, nucleosomes remain bound to dead cells. Circulating phagocytes may not be very efficient in taking up these large fragments of cellular material. The removal of nucleosomes from late apoptotic cells by FSAP and of necrotic cells by FSAP in cooperation with serum DNase I may aid in the phagocytosis of the cell remnants and prevent exposition of the immunogenic intracellular content. In chapter 7 we have demonstrated that serum of a subset of SLE patients, with high disease activity and high anti-DNA levels, displayed a decreased release of nucleosomes from late apoptotic cells. We found that this difference in nucleosome release appeared to be unrelated to changes in FSAP levels and activation. The decrease in nucleosome release correlated with disease activity and anti-DNA levels in the sera. We observed that antibodies in the sera mediate the reduction in nucleosome release.

These antibodies are probably directed against (parts of) nucleosomes since a number of SLE sera interfered with the detection of nucleosomes by ELISA. Mouse monoclonal antinuclear antibodies (ANAs) directed against DNA, histone H3 and the nucleosome were all found to inhibit the nucleosome releasing activity of serum. In addition, Fab fragments of monoclonal ANAs did not inhibit nucleosome release, which suggests that the antibodies do not block a specific target of FSAP. Inhibition of nucleosome release was restored by artificially crosslinking Fab fragments through a monoclonal anti-light chain antibody. Together, these results support the concept that crosslinking of nucleosomes forms the main mechanism through which ANAs in SLE patients inhibit the release of nucleosomes by FSAP.

The nucleosomes that are released into the circulation are rapidly cleared by hepatocytes (64). We speculate that when FSAP is not able to remove the nucleosomes from dead cells, the nucleosomes, which stay attached to the cells, persist longer in the circulation, can become immunogenic and pathogenic in the development of lupus nephritis. This may lead to a vicious cycle leading to more anti-nucleosome autoantibodies and exacerbation of the disease.

Recent data indicate that DAMP-mediated TLR activation may drive the immune response and autoantibody production in several SLE models (48-53;55). Urbonaviciute et al. have demonstrated that HMGB1, a DNA-binding protein and pro-inflammatory mediator, remained bound to nucleosomes released from late apoptotic cells and induced pro-inflammatory response and anti-dsDNA/anti-histone IgG responses in a TLR2–dependent manner in vitro (54). HMGB1–nucleosome complexes were also detected in plasma from SLE patients (65). HMGB1-free nucleosomes from viable cells did not induce cytokine production or dendritic cell activation. Nucleosomes that are removed from late apoptotic cells by FSAP might lack the HMGB1 and thereby become less immunogenic. It would be interesting to investigate
whether the nucleosomes released from late apoptotic cells by recombinant/purified FSAP still contain HMGB1. FSAP might directly cleave HMGB1 or a different protein, e.g. histon H1, by which the nucleosomes lose the HMGB1. By losing its DNA-binding proteins the nucleosomes may become less pro-inflammatory.

Recently, it has been shown that serum of a group of SLE patients lacks the ability to degrade NETs. Two mechanisms are described as causative in this impaired NET degradation: (i) the presence of DNase I inhibitors or (ii) anti-NET antibodies that prevent DNase I access to NETs (66). The second mechanism might be comparable to the mechanism that is causing the decreased nucleosome release from late apoptotic cells by serum of SLE patients. To our knowledge DNase is the only factor described to play a role in this NET degradation. However, FSAP might also have a function in the degradation of NETs. We have found that in the chromatin breakdown and release of nucleosomes from necrotic cells FSAP cooperates with serum DNase I. A similar mechanism might serve in the degradation of NETs. Leffler et al. have demonstrated that NETs activate complement in vitro and SLE patients that have reduced ability to degrade NETs displayed lower levels of complement proteins C3 and C4 (67). On the other hand SLE is associated with complement activation and deposition of complement proteins in inflamed tissues. We show significant lower levels of C3 and C4 in the serum of SLE patients with reduced nucleosome release. It is tempting to speculate, that in analogy to the undegraded NETs that activate complement, late apoptotic cells that have not disposed of their nucleosomes can activate complement giving rise to the observed decrease in C3 and C4 levels respectively. Therefore it would be interesting to compare complement activation by late apoptotic cells of which the nucleosomes are released by FSAP with cells that still contain their nucleosomes.

The role of FSAP in dead cell clearance is still not well understood. FSAP seems to be involved in inflammation since a) it is activated by cellular debris resulting from the pro-inflammatory response, b) FSAP removes nucleosomes from apoptotic cells and c) it modulates vascular permeability both, directly and indirectly. However, whether FSAP activation upon inflammation is beneficial or detrimental remains an open question. One might speculate that the release of nucleosomes by FSAP upon inflammation might be an epiphenomenon reflecting the severity of the inflammatory response. On the other hand FSAP-induced nucleosome release might be harmful propagating the pro-inflammatory response. If FSAP contributes significantly to the hypotension observed in sepsis its activation might be a fatal event in the pathogenesis. In contrast, complex formation with PAI-1 in sepsis may attenuate the procoagulant and pro-inflammatory effects of PAI-1. Moreover, nucleosome release might be essential in SLE to prevent persistence of the nucleosomes in the circulation leading to a vicious cycle of more anti-nucleosome autoantibodies and exacerbation of the disease.
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The putative role of FSAP in inflammation is summarized in Figure 1. It would be interesting to investigate whether the nucleosomes released from dead cells by FSAP are pro-inflammatory or anti-inflammatory and compare these nucleosomes with nucleosomes from dead cells that are not treated with FSAP. Furthermore intervention studies in animals are warranted to elucidate the role of FSAP in the inflammatory response.

Figure 1: Role of FSAP in inflammation
Model on the role of FSAP in inflammation. FSAP is activated by cellular debris resulting from the pro-inflammatory response and can remove nucleosomes from dead cells. It can modulate vascular permeability via proteolytic cleavage of high molecular weight kininogen (HMWK) resulting in the release of the highly vasoactive bradykinin. Bradykinin is an important mediator of vasodilatation in severe sepsis and septic shock contributing to the development of the potentially fatal septic hypotension. FSAP-induced nucleosome release might be harmful propagating the pro-inflammatory response. Nucleosomes might also function as endogenous danger signals inducing a pro-inflammatory response. On the other hand nucleosome release by FSAP might be essential to facilitate phagocytosis and prevent persistence of the nucleosomes in the circulation leading to autoimmunity.

Abbreviations: BK: bradykinin; HMWK: high molecular weight kininogen; MODS: multiorgan dysfunction system; scFSAP: single-chain FSAP; tcFSAP: two-chain FSAP; PAI-1: plasminogen activator inhibitor-1; TFPI: tissue factor pathway inhibitor
In summary, our data show that FSAP is able to remove nucleosomes from necrotic cells as well as late apoptotic cells. The nucleosome removal from necrotic cells occurs in cooperation with DNase I. We set up novel assays to measure FSAP complexes with its inhibitors in plasma as indirect assay method to screen for FSAP activation. Using these assays we were able to determine that FSAP is activated in patients with several inflammatory conditions and that levels of FSAP-inhibitor complexes correlate with nucleosome levels and severity of disease. FSAP activation may thus serve as a sensor for cell death in circulation. We have further shown that serum from a subset of SLE patients displays an impaired nucleosome releasing activity. We envisage that impaired nucleosome release may in turn result in persistence of the nucleosomes in the circulation leading to a vicious cycle of more anti-nucleosome autoantibodies and exacerbation of the disease.
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