Autoantibody associated disruption of kallikrein-kinin system in patients with recurrent pregnancy losses

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ABSTRACT

Factor XII, plasma prekallikrein and high molecular weight kininogen were first identified as coagulation proteins in the intrinsic pathway because patients deficient in these proteins had marked prolongation of in vitro surface-activated coagulation time. However, deficiencies of these proteins are not associated with clinical bleeding. Paradoxically, studies suggest that these proteins have anticoagulant and profibrinolytic activities. In fact, association between deficiencies of these proteins and thrombosis has been reported. Also, deficiencies of these proteins, autoantibodies to these proteins and anti-phospholipid antibodies are frequent haemostasis-related abnormalities found in unexplained recurrent aborters. Recently, evidence has accumulated for the presence of the kallikrein-kininogen-kinin system in the fetoplacental unit. Since contact proteins or kallikrein-kininogen-kinin system may play an important role in pregnancy especially in fetoplacental unit, autoantibodies to these proteins may be associated with pregnancy losses. These possibilities will be reviewed, the functions of the individual components will be summarized, and their role in blood coagulation and pregnancy discussed.

Key words: factor XII, antiphosphatidylethanolamine antibody, kininogen

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Contact activation

Factor XII is a single-chain zymogen with no detectable enzymatic activity. It has a molecular weight of 80 to 90 kDa, is synthesized in the liver, and circulates in plasma at a concentration of 30 μg/ml\(^1\).\(^2\). The protein has distinct domains homologous to fibronectin, plasminogen and plasminogen activators\(^3\).\(^4\).

Contact activation is initiated by the activation of factor XII. Factor XII can be autoactivated by contact with negatively charged surfaces or by exposure to proteases such as plasma kallikrein and plasmin, which produce enzymatic cleavage. These two mechanisms have been referred to as solid- and fluid-phase activation, respectively\(^5\). Although there are many candidate physiologic negatively charged surfaces that in vitro can induce factor XII autoactivation, the concept of autoactivation itself has never been a sufficiently convincing mechanism to explain contact system activation in vivo. The basement membrane of endothelial cell matrix may support contact activation, but this has not been demonstrated in vivo. Collagen, long thought to be an initiator, was proven to be ineffective and the activity reported was possibly due to contaminating matrix proteins\(^6\). Alternative mechanisms have been sought for factor XII activation in vivo. Recently, Motta et al.\(^6\) reported a novel mechanism for contact activation. Surprisingly, they reported that assembly of contact proteins, high molecular weight kininogen (HK) and prekallikrein, on cultured human endothelial cells resulted in prekallikrein activation independent of factor XII. Furthermore, factor XII activation by this pathway can occur\(^2\).

Activated factor XII (factor Xlla) converts prekallikrein to kallikrein and kallikrein digests HK to liberate the vasoactive, proinflammatory mediator, bradykinin. Factor Xlla also activates factor XI to continue the intrinsic coagulation cascade. The prekallikrein activation pathway on endothelial cells participates in two pathways for fibrinolysis. First, kallikrein cleaves HK to liberate bradykinin, which is the most potent and specific stimulator of endothelial cell tissue-type plasminogen activator liberation\(^7\). Second, kallikrein induces kinetically favorable conversion of single-chain urokinase into two-chain urokinase\(^8\).

Deficiencies of contact proteins are not associated with clinical bleeding despite marked prolonged activated partial thromboplastin time (aPTT), a surface-activated coagulation protein screening test. Paradoxically, studies suggest that contact proteins have anticoagulant, profibrinolytic functions in a physiologic milieu, on endothelial cells\(^8\)\(^9\)\(^10\)\(^11\). Numerous clinical studies suggest that contact protein deficiencies may be associated with impaired contact factor-dependent fibrinolysis. This result may contribute to an increased incidence of thrombosis in patients with congenital factor XII deficiency, an increased incidence of factor XII deficiency in patients with venous thrombosis, and acquired thrombotic disorders such as myocardial infarction and re-thrombosis of coronary arteries after thrombolytic therapy\(^12\)\(^13\)\(^14\). However, other studies suggest that factor XII plays no role in ischemic vascular disease\(^15\). Thus, it is unclear from existing literature whether a factor deficiency leads to thrombophilia.

Autoantibodies to contact proteins

Recently, numerous studies have suggested an association between contact protein deficiencies and recurrent pregnancy losses\(^17\)\(^18\)\(^19\), and between autoantibodies to contact proteins and recurrent pregnancy losses\(^20\)\(^21\). Sugi and McIntyre\(^21\) reported that certain antiphosphatidylethanolamine antibodies (aPE) are not specific for phosphatidylethanolamine (PE) per se, but are directed to PE-binding plasma proteins, kininogens. Sugi et al. tested recurrent pregnancy loss patients for aPE, especially those patients who lose during the embryonic period (<10 weeks' gestation).
They showed a strong association between recurrent pregnancy loss and aPE, the latter of which requires the presence of kininogen or other plasma proteins. In this study, 90.5% of the patients who were positive for plasma protein-dependent IgG aPE were kininogen-dependent. These data suggest that aPE may therefore represent a significant risk factor for early recurrent pregnancy loss.

Schved et al. reported the cases of three young women with a factor XII deficiency (two homozygous and one heterozygous) and a clinical history of spontaneous abortion. Braulke et al. reported on 8 patients with moderately reduced level of factor XII found among 43 patients with repeated abortions. Recently, Gris et al. reported the prevalence of haemostasis abnormalities in 500 unexplained primary recurrent aborters. They found 9.4% of the patients with an isolated factor XII deficiency. Gallimore and Winter reported a high incidence (20.9%) of apparently true factor XII deficiency in patients who were lupus anticoagulant (LA) positive. They have hypothesized that antibodies to factor XII might be present in some patients who are LA positive and that immune complexes may be formed leading to reduced levels of factor XII. They studied plasma samples from LA positive patients for the presence of antibodies to factor XII and reported that many patients were positive for antibodies to factor XII detected by ELISA and surface plasmon resonance. Jones et al. reported that when levels of factor XII were compared in patients with and without antibodies to factor XII, significantly lower levels of factor XII were seen in patients with antibodies to factor XII. This suggests that the immune complex formation and subsequent sequestration resulted in reduced levels of factor XII. They also reported that antibodies to factor XII showed a strong and statistically significant association with recurrent fetal loss (odds ratio 5.4, p=0.025). Autoantibodies to factor XII rather than factor XII deficiency may be a risk factor for thromboembolism and recurrent pregnancy losses.

Although some studies have identified factor XII deficiency as a risk factor for recurrent pregnancy loss, others failed to find such a relationship. Recently, Pauer et al. generated mice deficient for factor XII using a gene targeting approach. Homozygous factor XII knockout mice showed no factor XII plasma activity and had a markedly prolonged aPTT. Interestingly, they reported that matings of factor XII/− males and XII/− females resulted in normal litter sizes demonstrating that total factor XII deficiency in XII/− females does not affect pregnancy outcome. Iwaki et al. also reported that in female mice homozygous for a total factor XII deficiency, normal deliveries occurred with normal litter sizes. In contrast, Jones et al. reported that antibodies to factor XII showed a strong and statistical significant association with recurrent fetal loss (odds ratio 5.4, p=0.025). They reported that when levels of factor XII were compared in patients with and without antibodies to factor XII, significantly lower levels of factor XII were seen in patients with antibodies to factor XII. This suggests that the immune complex formation and subsequent sequestration resulted in reduced levels of factor XII. Autoantibodies to factor XII rather than factor XII deficiency may be a real risk factor for recurrent pregnancy losses.

HK inhibits thrombin-induced platelet aggregation by inhibiting the binding of thrombin to platelets. Domain 3 of HK is responsible. Recently, the binding site on platelets, which mediates this effect, was shown to be glycoprotein (GP) Ib-IX complex. It has been reported that HK and factor XII compete for the same binding site on endothelial cells. Bradford et al. reported that factor XIIa also inhibits thrombin interaction with platelets in a
mechanism also involving binding to the same receptor\(^{35}\). HK and factor XII both directly bind to glycopcalcin, the extra cellular subunit of GP Ib\(\alpha\), in a Zn\(^{2+}\)-dependent manner. They also reported that factor XII binding to platelets was inhibited by monoclonal antibody B7C9, whose noncontiguous epitopes have been mapped to amino acids 1-28 and an icosapeptide in the "finger region" of factor XII\(^{35}\). Interestingly, we reported that among plasmas from 17 recurrent pregnancy loss patients who were positive for autoantibodies to factor XII, 13 patients (76.5\%) recognized amino acids 1-30\(^{37}\). This suggests that autoantibodies to factor XII in patients with recurrent pregnancy losses may inhibit factor XII binding to platelets and may cause pregnancy loss.

The kininogens can inhibit platelet aggregation induced by thrombin. Domain 3 of the kininogen heavy chain was found to inhibit thrombin from binding to the platelet thrombin receptor. By using specific monoclonal antibodies, Jiang et al showed that it is the domain 3 region that is responsible for the inhibition of thrombin binding to platelets\(^{39}\). Kunapuli et al found that recombinant domain 3 inhibited thrombin-induced platelet aggregation\(^{38}\). Sugi and McIntyre\(^{21}\) reported that certain aPE are not specific for PE per se, but are directed to PE-binding plasma proteins, kininogens. Several studies report strong association of aPE with thrombosis and recurrent pregnancy losses\(^{22,23,39}\). Sugi and McIntyre hypothesized that when bound by aPE, the platelet-kininogen complex may no longer render the platelet refractory to thrombin activation, thus predisposing to aggregation and thrombosis. Their in vitro data\(^{40}\) support these observations as they demonstrated that kininogen-dependent IgG-aPE purified from several aPE-positive patient plasmas caused marked augmentation of thrombin-induced platelet aggregation, but did not affect ADP-induced platelet aggregation. Moreover, kininogen-independent IgG-aPE did not affect thrombin-induced platelet aggregation. For this to occur, it is possible that aPE may recognize the domain 3 region of kininogens subsequent to their binding platelet. Herwald et al\(^{41}\) reported that a monoclonal antibody to domain 3, HKH15, which interferes with the complex formation between kininogen and papa, also blocked the cell binding of kininogens and was directed to the extreme carboxyl-terminal portion of domain 3. The epitope of HKH15, which binds to domain 3 and blocks the binding of kininogens to platelets and endothelial cells, was mapped using synthetic peptides, which span the entire domain 3 sequence. They reported that one peptide, LDC27, specifically bound to HKH15. Fine mapping of the epitope of HKH15 has also revealed a minimal 13-residue segment in LDC27, named CNA13, to be the antibody-binding site. Katsunuma et al\(^{42}\) reported that among plasmas from 24 recurrent pregnancy loss patients who were positive for kininogen-dependent IgG-aPE, 17 (70.8\%) recognized the LDC27 peptide. They mapped the aPE-binding region to domain 3 using a plasma specimen from a recurrent pregnancy loss patient. Interestingly, the aPE of a patient recognized CNA13, which is identical to the epitope of HKH15. Leu331-Met357 (LDC27) and Cys333-Lys345 (CNA13) are located on the carboxyl-terminal portion of kininogen domain 3, which is known as the major kininogen heavy chain cell attachment site where it overlaps its cysteine protease inhibitory region. Because aPE interferes with the balance of hemostasis in vitro, aPE may therefore induce a similar condition in patients thereby causing thrombosis and recurrent pregnancy losses.

Many patients with recurrent pregnancy losses have both factor XII deficiency and aPE. In factor XII deficient patients with recurrent pregnancy losses, 32.4\% were positive for aPE (Sugi T, unpublished data). From our epitope mapping
studies, both autoantibodies to factor XII and kininogen-dependent aPTE may block factor XII-and kininogen-biding to GP Ib-IX-V complex and augment thrombin-induced platelet aggregation. Thus autoantibodies to factor XII and kininogens may cause thrombosis and recurrent pregnancy losses.

Recently, Harris et al reported the antigenic binding site(s) of antibodies to factor XII associated with the antiphospholipid syndrome (APS). They investigated plasma samples from 12 female patients with definite antiphospholipid syndrome for the presence of antibodies to factor XII. To investigate the antigenic binding site(s) of factor XII, 150 peptides of the known factor XII sequence were synthesized. Seven patients positive for factor XII antibodies were chosen and each patient's purified IgG or IgM was tested against each peptide. Plasma from only one of the seven patients showed binding to the synthetic peptides. In this patient, two regions were identified as possible antigenic binding site(s) for factor XII antibodies: one in the growth factor domain and the other in the catalytic domain. There was no convincing explanation on how these antibodies may inhibit the physiological function of factor XII and contribute to the clinical symptoms suffered by this patient group. In our recent study, we tested the antigenic binding site(s) of antibodies to factor XII in patients with recurrent pregnancy losses. We reported that among plasmas from 17 recurrent pregnancy loss patients who were positive for autoantibodies to factor XII, 13 patients (76.5%) recognized amino acids 1-30. A difference between their study and our study is no patients studied fulfilled criteria for definite antiphospholipid syndrome in our study.

**Kallikrein-kinin system**

The kallikrein-kininogen-kinin system is involved with inflammatory processes that occur in many tissues of the body. An acute phase induced inflammatory response is generally characterized by increased vascular permeability and tissue edema. Ovulation, endometrial proliferation, and decidualization/implantation evoke a similar inflammatory response, suggesting that activation of the kallikrein-kininogen-kinin system is involved with these biological events. Evidence for the presence of the kallikrein-kinin system in fetoplacental vessels has accumulated in several studies. Mutoh et al. indicated that a kinin generating activity of the kallikrein-kinin system is localized within the uteroplacental unit. Hermann et al. reported that kininogen and plasma prekallikrein/plasma kallikrein were present at the endothelial cells of placental villous capillaries. In larger placental blood vessels and umbilical cord, neither kininogens nor kallikreins were detected. The co-localization of kininogen and plasma prekallikrein/plasma kallikrein suggests that kinins could be generated locally in placental capillaries. Gene expression for plasma kallikrein, factor XII, and HK were detected in endometrium but not early conceptus tissues in the pig. Interestingly, although the liver is considered to be the major source of HK, plasma kallikrein, and factor XII, gene expression was detected in porcine endometrium. These results suggest that endometrium can exert a local effect on bradykinin release through endometrial synthesis of plasma kallikrein and factor XII. The functional spectrum of biologically active kinins, such as vasodilatation, vasoconstriction, smooth muscle contraction and relaxation, could influence placental blood flow regulation. With the indication that kinins induce increased microvascular permeability and vascular growth, the kallikrein-kininogen-kinin system may play a major role in uterine and placental angiogenesis essential for embryonic and fetal survival. Moreover, kinins could also have anti-thrombotic/profibrinolytic activities. Kinins, which are
referred within the placenta, may play a role in regulating placental blood flow and transplacental transport of substrates and metabolites. To influence placental circulation and nutrient supply to the fetus effectively, components of the kallikrein-kinin system should be situated within or close to the placental vasculature.

High molecular weight kininogen (HK) is divided into a heavy chain and a light chain. The heavy chain and light chain are linked by D4, which contains the sequence of bradykinin (BK). After releasing BK by proteolytic cleavage, the cleaved HK (HKa) contains a heavy chain and a light chain that remain connected by a single disulfide bond. Recent studies have revealed that HKa inhibits angiogenesis while BK and intact HK promotes angiogenesis. It has been known that HK binds to heparin, i.e., the mast cell-derived glycosaminoglycan (GAG). Recently it has been demonstrated that HK attaches to endothelial cell surfaces through Leu331-Met357 (LDC27) in domain 3 (D3) and His479-His498 in domain 5 (D5) by docking to the heparan sulfate (HS) and chondroitin sulfate (CS) chains of proteoglycans. Cell-bound HK was almost exclusively in the uncleaved form which is proangiogenic, because binding to GAG protects HK from proteolytic processing. Renne et al. reported that affinity-purified antibodies to LDC27 inhibited HK binding to HS. This strongly suggests that kininogen-dependent aPTE which also recognize LDC27 may inhibit HK binding to HS. Detachment of HK from cell surface GAG induces proteolytic processing and the products are HKa and BK. The t1/2 of BK is 30s while the t1/2 of HKa is 9h. Therefore, it may be possible that aPTE induce HKa generation which inhibit placental angiogenesis and may cause pregnancy loss.

Early gestation pregnancy loss often may be associated with the autoantibody-associated disruption of the plasma contact system or kallikrein-kinin system. Because the kallikrein-kinin system is localized within the uteroplacental unit, it may play a role in regulating placental blood flow and transplacental transport of substances and metabolites. Disruption of this system may be a risk factor for early gestational loss.

Acknowledgements

Support from the Japan Society for the Promotion of Science to T. Sugi (#18591815) is gratefully acknowledged.

References


23. Sugi T, Matsubayashi H, Inomo A, Dan L,


34. Reddigarri SR, Shibayama Y, Brunnee T, Kaplan AP. Human Hageman factor (factor XII) and high molecular weight kininogen compete for the same binding site on human umbilical vein endothelial cells. J Biol Chem 1993; 268: 11982-7.


as the only antiphospholipid antibodies found in patients with unexplained thromboses. Thromb Haemost 2001; 85: 800-5.


要旨

女性の生殖器系は、体内で2番目にキニノーゲンおよびその代謝産物の豊富な部位であると言われている。カリクリーヌン・キニン系は胎児、胎盤の血管に存在していることが最近明らかになってきている。胎盤の大きな血管や臓器ではなく、細毛の毛細血管内皮細胞にキニノーゲンやプレカリクリーヌン、カリクリーヌンが存在する事が報告されており、キニンが胎盤の毛細血管に限局して産生されていることが示唆されている。キニンは抗凝固、線溶促進作用だけでなく、血流を増加させるなどの生物学的活性をもったペプチドであり、胎盤内で放出され、胎盤の血流や代謝産物の経胎盤輸送などを調節する重要な役割を担っている可能性が指摘されている。また、プラジキニンの血管新生作用も報告されており、胎盤形成に重要な役割を果たしている可能性がある。つまり、カリクリーヌン・キニン系は、全身の血液凝固、線溶系のみならず、特に生殖の領域で非常に重要な位置を占めていると考えられる。

最近、第 XII 因子欠乏症などカリクリーヌン・キニン系の蛋白の欠乏と反復流産との関係が報告されている。また、キニノーゲン依存性抗Pエ抗体や、抗第 XII因子抗体など、カリクリーヌン・キニン系蛋白に対する自己抗体と反復流産との関係も報告されている。カリクリーヌン・キニン系は、妊娠維持に重要な役割を果たしていると考えられるので、その破壊は流産に結びつく可能性がある。

カリクリーヌン・キニン系の破壊による流産の特徴は、妊娠10週未満に起きる事である。現時点での治療は、低用量アスピリン療法やヘパリン療法などの抗凝固療法が挙げられる。しかしながら、最近筆者は、ヘパリンがELISA中で劇的に抗Pエ抗体の抗体価を低下させる事を報告しており、ヘパリンの抗体中和作用または吸着作用が重要である可能性がある。さらにヘパリンは、カリクリーヌン・キニン系、特にプラジキニンを介して血管新生、つまりは胎盤形成を促進するという報告もあり、直接胎盤に作用して流産を防止する可能性もある。ヘパリンは単に血液凝固系に作用するのみならず、妊娠維持に直接重要な役割を果たしている可能性がある。