Surface-mediated defense reactions. The plasma contact activation system.

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Research Article

Perspectives

Surface-mediated Defense Reactions

The Plasma Contact Activation System

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During the past two decades, considerable evidence has accrued that many of the body defenses against injury, specifically, blood coagulation, the inflammatory response, classical complement pathway activation, and fibrinolysis, are initiated by a common mechanism. Four proteins, namely Factor XII (XII,[1] Hageman factor), prekallikrein (PK, Fletcher factor), high molecular weight kininogen (HMWK, Williams, Fitzgerald, Flaujeac factor), and Factor XI, (XI, plasma thromboplastin antecedent) have been shown to be the major proteins required to initiate, amplify, and propagate surface-mediated defense reactions by activating $C\bar{1}$, Factor VII, prorenin (PR), and plasminogen, by stimulating neutrophils, and by releasing bradykinin (BK). Purification of each has allowed functional and immunochemical assays to be developed. The zymogens, XII (M_r of 80,000), PK (M_r of 88,000), and XI (M_r of 160,000) are converted by limited proteolysis into the active serine proteases XIIa, kallikrein (K), and XIa. HMWK is a nonenzymatic cofactor. The molecular events occurring during in vitro contact activation have begun to be described, and the role of these pathways in certain diseases has been tentatively probed. Despite advances, new questions have been raised in tandem with the answers provided. Striking laboratory abnormalities occur in surface-activated coagulation in individuals deficient in contact protein who otherwise appear asymptomatic. However, the proteolytic pathways initiated and amplified by these proteins are obviously important in host defense. In this review, new information will be summarized, problems that exist outlined, and approaches to their solution considered.

Initiation of the contact system (Fig. 1, left). The activation of the contact system is probably initiated by the binding of plasma XII to a negatively charged surface, where autoactivation (1) occurs; i. e., the inert zymogen XII, after a conformational change on the surface, is converted to the active serine protease, XIIa, by a small amount of XIIa. The origin of the trace XIIa remains controversial.

The substrates of XIIa, the zymogens PK and XI, can bind directly to activating surfaces, but, in the absence of HMWK, activation does not occur (2). Rather, PK (3) and XI (4) exist in bimolecular complexes with the contact system procofactor, HMWK. In normal plasma, HMWK binds to negatively charged surfaces, but little binding occurs in plasma deficient in XII (5). This observation suggests a means by which the adsorption of HMWK could be coordinated with the association of XII with the surface. The mechanism of initial binding of the PK-HMWK complex to the activating surface requires investigation. However, most of the PK and XI are bound while complexed with cleaved or "activated high molecular weight kininogen" (HMWKa) (5). An alternate possibility is that a few molecules of HMWK are cleaved to HMWKa by XIIa, as in a recently demonstrated reaction (6). The requirement of activation of HMWK for surface binding is analogous to the necessity of thrombin activation of V and VIII to Va and VIIIa for full cofactor activity.

On the surface XIIa cleaves PK to K, and XI to XIa; both are active serine proteases when each zymogen is complexed with HMWK. K during a reciprocal activation generates additional XIIa from XII, a reaction that proceeds several orders of magnitude faster than the autoactivation (1). K can also diffuse off the surface (4) and hydrolyze its substrate, HMWK, to form HMWKa composed of a heavy and light chain, which is linked by disulfide bonds. The light chain expresses the coagulant activity, since it contains both the surface-binding regions as well as the domain responsible for complex formation with PK and XI (2). The role of the heavy chain is still unknown, although preliminary evidence suggests it may augment binding of the light chain to negatively charged surfaces (7). Another product resulting from the cleavage of HMWK is the nonapeptide BK, one of the most potent mammalian vasodilators known. This activation peptide also induces pain and increases capillary permeability, thus mimicking many features of the inflammatory response. Thus, the net effect of the initial contact reactions is to release BK and position complexes of HMWKa-PK and HMWKa-XI on the surface in close proximity to XIIa (Fig. 1, left).

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1. *Abbreviations used in this paper:* α_2M,α_2-macroglobulin; XIIa, activated Factor XII; BK, bradykinin; C1 INH, C1 inhibitor; IX, Factor IX; XI, Factor XI; XII, Factor XII; XIIf, Factor XII fragment; HMWK, high molecular weight kininogen; HMWKi, inactivated HMWK; K, kallikrein; PK, prekallikrein; PR, prorenin; R, renin.

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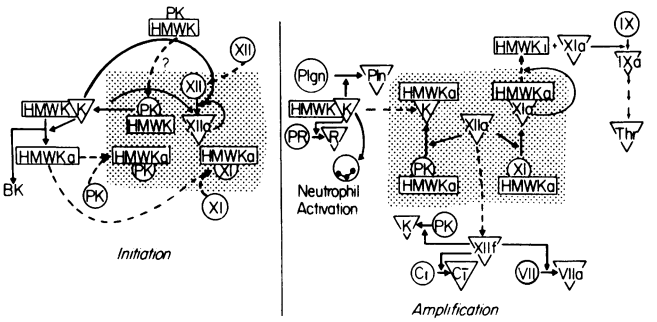

Figure 1. The contact activation system. Solid arrows designate conversion; dashed arrows, translocation; shaded area, activating surface; ○, zymogen; ▽, enzyme; and □, cofactor. Abbreviations as in text.

Most of the preceeding reactions have been studied using model negatively charged activating surfaces, such as glass or kaolin (8), as well as large soluble polymers such as dextran sulfate (9), and micelles such as sulfatides (10). However, the physiologic or pathologic in vivo activating surface is unknown, although the subendothelial vascular basement membrane is frequently suggested. The active component is probably not collagen. Although crude preparations of collagen have been reported to initiate the activation of XII (11), highly purified triple helical soluble collagen does not activate purified XII (12). Moreover, a variety of components of basement membrane including type I, III, and IV collagen alone or mixed with proteoglycans do not initiate XII activation in normal plasma (13). The specific in vivo activating surface remains elusive.

Amplification of the contact system (Fig. 1, right). XIIa acts on surface bound HMWKa-XI to form HMWKa-XIa, a reaction markedly enhanced by HMWKa. Unlike K, which does not bind tightly to HMWK and is released from the surface into the fluid phase after cleavage of PK, XIa remains tightly bound to HMWKa, and therefore remains associated with the surface. The association constant for XIa and HMWK in solution (14) is at least five times as tight as K for HMWK (15). The catalysis by XIa to convert IX to IXa (16) must take place either in the fluid phase or on the surface of a phospholipid micelle. Recently it has been found that XIa is also capable of cleaving HMWK (17). Although the initial cleavages result in the formation of a heavy chain and a light chain (containing the coagulant activity), as well as BK, XIa rapidly cleaves the light chain, which results in loss of biologic activity (HMWKi) (18). In addition to negative feedback control, it is attractive to postulate that formed HMWKi may lose its ability to bind XIa and K, thereby freeing XIa to activate IX and thus the coagulation cascade, and dissociating K to potentially participate in neutrophil activation, prorenin conversion, and fibrinolysis in plasma.

XIIa also converts PK to K on the surface at an accelerated rate compared with fluid-phase activation. K, in the fluid phase, has a variety of substrates besides HMWK. K directly converts plasminogen to plasmin (19), although the rate of reaction is too slow to account for the acceleration of clot lysis after exposure of plasma to activating surfaces. XIIa (20) and XIa (21) can also catalyze this reaction, but at well above plasma concentrations. The physiological or pathological significance of surface-activated fibrinolysis remains to be established. In vitro plasma K also converts PR to renin (R). However, this reaction requires prior exposure of plasma to acid to render the PR susceptible to K (22), which suggests that this pathway may not be physiologic. The susceptibility is not merely due to removal of inhibitors, since in plasma deficient in inhibitors of K ($C\bar{1}$ inhibitor ($C\bar{1}$ INH) and α_2-macroglobulin) no conversion of PR to R is observed when PK was activated to K or when K is added directly to plasma (23).

A function of K that may be more relevant for defensive reactions is its ability to activate neutrophils. Early studies (24) suggested that K was chemotactic for neutrophils and that an intact active site was required. More recently, K was demonstrated to aggregate neutrophils and stimulate oxygen consumption of these cells (25). Although in the rabbit the cleavage of C5 to C5a by K (26) has been shown to be the mechanism of activation, in the human system the aggregation of neutrophils by K is independent of C5a (25). The release of elastase from neutrophils in recalcified plasma (27) has recently been shown to be due to the ability of K to stimulate elastase secretion from neutrophil azurophilic granules (28). K also stimulates superoxide and H_2O_2 formation in human neutrophils (29). These observations expand the potential role for the contact system in pathologic states involving neutrophil activation.

XII in plasma is not only converted to XIIa, but also to XII fragments (XIIf), which retain the active site of XIIa while losing the surface-binding domain. Thus, XIIf diffuses back into plasma where it may encounter three potential substrates, PK, Factor VII, and $C\bar{1}$. Like XIIa, XIIf can cleave PK to K. Factor VII coagulant activity is enhanced 40-fold by XIIf which cleaves it into a two-chain molecule (30). However, unlike Xa (31), XIIf does not subsequently inactivate VIIa by further cleavage to a three-chain molecule. The shortening of the prothrombin time in glass compared with plastic (32) is due to Factor VII activation by activated XII. The "spontaneous" activation of Factor VII in the cold (33) is due to a combination of inactivation of the plasma protease inhibitor $C\bar{1}$ INH and surface activation of XII. Cold activation of Factor VII is more prominent in the plasma from women taking oral contraceptives, since their plasma has increased XII and decreased $C\bar{1}$ INH (34). The contribution of contact activation of VII by XIIf, compared with autoactivation or activation by Xa, is not known. Finally, XIIf has been demonstrated (35) to activate the first component of complement (C1).

Regulators of the contact system. Thus far the system has been approached to delineate activation pathways. The major regulators of this system, with its potentially explosive positive feedbacks, are the naturally occurring plasma protease inhibitors, $C\bar{1}$ INH, α_2-macroglobulin (α_2M), and α_1-protease inhibitor (α_1-antitrypsin). Although the importance of these proteins in the inactivation of activated XII, K, and XIa has been appreciated for two decades, it is only recently that quantitative in-

formation has been available as to the relative importance of various inhibitors towards the inhibition of contact system proteases. The suggestion (36) that $C\bar{1}$ INH was the major inhibitor of activated XII has been confirmed. $C\bar{1}$ INH contributes >90% of the inhibitory activity in plasma toward both XIIa (37) and XIIf (38). $C\bar{1}$ INH is also a major inhibitor of K, accounting for 42% of the ability of plasma to inactivate this enzyme (39). However, α_2M also contributes ~50% to the inhibition of K, as measured by residual activity on a tripeptide substrate. Although the α_2M-K complex contains 25% of the amidolytic activity of the uncomplexed enzyme (40), it has not yet been shown whether this complex has proteolytic activity. The rate of inhibition of K, XIIa, and XIIf in plasma is rapid, but XIa is inactivated an order of magnitude more slowly. The predominant inhibitor of XIa, α_1-protease inhibitor, accounts for two-thirds of the inhibitory activity of plasma toward XIa (14). Inhibition of the contact activation enzymes differs from the serine proteases in the later stages of the coagulation cascade, which are regulated by antithrombin III. Not only is antithrombin III a poor inhibitor of XIIa, XIa, and K, but heparin, which markedly accelerates inactivation of Xa and thrombin by antithrombin III, exhibits minimal enhancement of the inactivation of the contact enzymes (41, 42, 43). The molecular basis for this insensitivity to heparin is an unsolved problem. The inactivation of the contact system proteases is further modulated by the cofactor, HMWK, since in the fluid phase HMWK forms noncovalent complexes with K and XIa (K_d = 0.75 and 0.17 μM, respectively). It was hypothesized (44) and shown that the presence of the cofactor reduced the rate of inactivation of K and XIa in both purified systems (14, 15, 38) and in plasma (14, 39). This protection may occur because HMWK serves as a substrate for the proteases or because of complex formation (39, 40). This protective mechanism does not apply to XIIa or XIIf, which do not form such complexes.

Interaction of contact proteins and inhibitors with platelets. The prolonged blood clotting observed in vitro in the absence of clinical bleeding in deficiencies of XII, PK, and HMWK suggests that an alternative and more significant mode of activating the clotting system is present in vivo. As yet, no such pathway has been definitively demonstrated. The discovery that Factor VII, in the presence of tissue factor, can activate IX (45) suggests one possibility, but does not account for the occurrence of hemorrhage in XI deficiency. Participation of platelets in a putative bypass of the initial stages of contact activation was first suggested (46) because of the ability of activated platelets to correct coagulation defects in plasma deficient in XII and XI. Stimulated platelets have also been shown to promote proteolytic cleavage of XI in incubation mixtures containing XII, K, and HMWK (47). XI activity and XI antigen (48) in platelets with a different molecular weight than plasma has been demonstrated. The contact cofactor HMWK is not only present in platelets but can be secreted after stimulation (49), which raises the possibility that it functions similarly to Factor V in accelerating proteolysis on the platelet surface. Finally, the existence of the major inhibitors of the contact system $C\bar{1}$ INH (50), α_2M (51) and α_1-protease inhibitor, (52) in platelets suggests further regulatory possibilities.

Congenital deficiencies of contact system proteins and inhibitors. In the past 20 yr, congenital deficiencies of the contact system proteins and their inhibitors have been described. Thus, the physiological and pathological significance of this system might be inferred from the consequences of absent or defective proteins in individuals. It appears that XII, PK, and HMWK are not essential for physiologic blood coagulation, since a deficiency of each of these proteins does not lead to a hemorrhagic diathesis. The lack of participation of XII in normal hemostasis is underscored by the fact that patients with complete deficiency of this factor are not protected from thrombotic disorders. At least three individuals with Factor XII deficiency have experienced a myocardial infarction and seven have had venous thrombosis, while the index patient, Mr. Hageman, died of pulmonary embolism. Does the decrease of surface-activated fibrinolysis observed in vitro handicap the ability of these individuals to lyse clots in vivo?

Individuals with XII and PK deficiency have been demonstrated to have depressed neutrophil mobilization in a "skin window" (53). This finding may be the in vivo counterpart of the in vitro ability of K to activate neutrophils (25). Patients with XI deficiency frequently have a mild hemostatic disorder with exaggerated posttraumatic hemorrhage. One individual with total kininogen deficiency had an extremely sluggish response of R and angiotensin, to salt restriction, and to postural changes (54). Since this individual cannot form any BK, a role for this peptide may be indicated in the stimulation of the R-angiotensin system.

Deficiency of the major plasma inhibitor of the contact system $C\bar{1}$ INH gives rise to hereditary angioedema, the classic disease first described by Sir William Osler. Patients with hereditary angioedema are known to have in vivo activation of $C\bar{1}$, as assessed by diminished levels of C2 and C4, the physiologic substrates for $C\bar{1}$. However, the levels of C2 and C4 are decreased even during asymptomatic periods. Recently, evidence for contact-phase activation during abdominal and laryngeal attacks of hereditary angioedema has been reported (55). A decrease in functional PK (amidolytic and coagulant activity) without changes in PK-K antigen can best be explained by zymogen activation with the formation of K-inhibitor complexes that lose activity but retain antigenicity. In addition, during attacks of hereditary angioedema, a decrease in HMWK activity and antigen was noted, which might result from in vivo cleavage and increased clearance of the cleaved molecules (55). Such a reaction would form BK, which is a possible contributor to the attacks of localized edema.

Acquired disorders associated with activation of the contact system. A comprehensive review has appeared (56) of the early studies documenting changes in the contact system in such disorders as the carcinoid (57), postgastrectomy (58), and nephrotic syndromes (59), as well as in type IIa hyperlipoproteinemia (60) and in transfusion reactions (61). The role of surface-mediated defense reactions can be better illustrated by considering the

response of this system to the challenge of infection, hyperacute allograft rejection, and allergic reactions.

In septic shock, the peripheral vascular changes of BK, arteriolar dilatation, and venous constriction are manifest early in the course. In experimental animals, infusion of endotoxin leads to a decrease of plasma kininogen concentration and an increase of plasma BK (62), as well as a transient decrease in plasma PK and K inhibitors (63) and a similar pattern of changes is observed in the plasma of patients with hypotensive septicemia as well as decreased XII coagulant activity (63, 64). The sequence of events in human septic shock probably includes the following steps: activation of XII, resulting in a decrease in its plasma level; activation of PK, as evidenced by a drop in its concentration in plasma; formation of the active enzyme K and its complexes with $C\bar{1}$ INH; and release of BK into the circulation, with depletion of plasma HMWK. Early detection of these changes might allow intervention at a time when irreversible shock has not yet supervened. Many of these changes have been documented in the carefully controlled environment of vaccine trials. In typhoid fever (65), a fall in functional pre-K and K inhibitory activity is accompanied by normal PK and $C\bar{1}$ INH antigens, suggesting the presence of circulating K-$C\bar{1}$ INH complexes. These complexes were directly identified by crossed immunoelectrophoresis, and similar findings in infection have been demonstrated (66). Subtle evidence for contact activation has been gathered during a vaccine trial, in which prospective observations were made in Rocky Mountain Spotted fever treated 6 h after the onset of disease (67).

In patients with more flagrant disseminated intravascular coagulation thought to be due to endothelial injury by gram-negative or gram-positive sepsis, or viremia, decreased plasma XII coagulant activity, PK, and K inhibitory activity have been documented (63, 64, 68). This pattern would be expected if XII were activated. In contrast, patients with equally severe disseminated intravascular coagulation due to tissue injury or to the release of clot-promoting thromboplastin have not manifested these changes (68). The formation of K might be expected to enhance fibrinolysis. Study of the relative contributions of thrombin, plasmin, and K by measuring enzyme-inhibitor complexes would further understanding of the pathogenesis of disseminated intravascular coagulation.

Coagulation studies in hyperacute renal allograft rejection indicate that localized fibrin formation, platelet aggregation, and vasoconstriction are the primary changes leading to ischemia and graft death. In the presence of high doses of heparin, kidney grafts survive in individuals who have been sensitized earlier to the foreign kidney, and no fibrin thrombi can be detected in the allograft vessels. Marked fibrinolysis occurs along with activation of plasma K (69). Similar results have been obtained in studies of a primate model of hyperacute renal allograft rejection (70).

The role of kinins in allergic reactions has been suggested for many years because of their ability to stimulate most of the inflammatory changes, such as edema and vasodilation. The ability of antigen challenge to elicit IgE-mediated release of XII activators from human lung mast cells (71) and the occurrence of K-like activity in basophils (72) provide the experimental basis for the involvement of the K-kinin system in allergic disease. The fall in HMWK in human anaphylaxis (73) supports the involvement of the contact activation system. Recently, kinin generation was documented during a local allergic reaction in vivo (74). Intranasal antigen challenge in allergic individuals produced BK and lysyl BK in association with sneezing and decreased nasal airway conductance. Further studies will be necessary to define the mechanism of production of kinin and its role vs. other mediators in the allergic response.

These clinical examples of abnormalities of the contact activation system indicate the need for further application of biochemical knowledge and immunochemical techniques to develop more sensitive assays for activation of the contact system. Emphasis should be shifted from examining the participation of this system in hemostasis to the role of surface-mediated reactions as a defense system in inflammatory diseases.

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