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Plasma Proteins and Inflammatory Disease*

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INFLAMMATION results from the interactions of plasma and cellular components that interact to generate enzyme activities, oxidative radicals, and injurious peptides. In plasma there are three major systems of proteins that are known to play a role in the inflammatory process: the complement, contact (Hageman factor), and clotting systems. The latter two share components, and cross activation is known to occur between them.

In this presentation, a brief description of the contact (Hageman factor) system of plasma will be given along with information regarding enzymes derived from cells that activate components of the contact system. A description of an inflammatory process in the lungs of humans will also be presented that exemplifies the interaction of these plasma systems with leukocytes to induce injury. Whereas much is known of the biochemistry and biological importance of the complement proteins of plasma, it has been only recently that meaningful data have been obtained on the contact system proteins. A presentation of the basic biochemistry of this system may therefore be of interest.

The components of the Hageman factor (HF) system are presented in figure 1, and an hypothesis of their assembly and activation on a negatively charged surface in figure 2. The physical characteristics and biochemistry of activation of the components in solution and on a negatively charged surface are the subject of a recent review from this laboratory (1). In brief, when plasma contacts a negatively charged surface, a group of proteins interacts on the surface to produce a sequence of conversions of proenzymes to enzymes. This occurs as a burst of activity lasting seconds. HF, prekallikrein (PK), high molecular weight kininogen (HMWK), and clotting factor XI are the principal molecules that undergo initial proteolytic cleavage as activation occurs. This cleavage appears to be essential for the rapid activation of each component. HF binds rapidly to the surface in whole plasma and the peptide chain is cleaved so as to produce chains of 28,000 and 52,000 MW that are held together by a small disulfide bridge. Cleavage then occurs on the N-terminal site of the disulfide bridge, allowing the smaller chain, which bears the enzymatic site of HF, to dissociate into the fluid phase (2). The larger fragment remains surface-bound.

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The enzyme in plasma responsible for the rapid cleavage of HF is kallikrein. Its precursor, prekallikrein (PK), is itself cleaved into heavy and light chains and activated by HF. Thus, a reciprocal activation by these two molecules was proposed as a control mechanism of activation of the contact system (3). The reciprocal enzymatic cleavage and activation of HF and PK is augmented because when bound to the surface HF is more than 100-fold more susceptible to enzymatic cleavage (4) than when in the fluid phase. In the absence of PK, HF is cleaved and activated slowly, which suggests that other enzymes may play a secondary role. However, in the absence of PK, the clotting and kinin generating activities are markedly retarded (5).

PK and factor XI exist in plasma as a complex with HMWK (6, 7). The HMWK, acting stoichiometrically with HF (8), brings PK and factor XI to the surface where they are able to interact with HF (9). The light chain of HMWK bears a domain extremely rich in histidine residues that apparently is responsible for the adherence of the molecule to negatively charged surfaces (10). A portion of the light chain is also responsible for the complexing of HMWK with PK and factor XI (11, 12). Thus, HMWK acts as a cofactor in a stoichiometric relation with HF to promote the reciprocal activation of PK and HF (8, 13) and the cleavage and activation of factor XI by HF.

Kallikrein rapidly dissociates from HMWK on the surface and cleaves and activates other surface-bound HF molecules (14). The dissociating kallikrein also rapidly cleaves HMWK both on the surface and in fluid phase.

The initial event that triggers the activation of HF and PK is not precisely understood. Although it has been thought for years that HF is activated upon binding to a surface, this has been questioned. In the absence of PK or HMWK, HF still becomes surface bound, but it does not activate and it does not undergo cleavage for several minutes (well after the burst of activity that is seen in normal plasma) (15). Single chain, zymogen HF binds [^{3}H]diisopropylphosphofluoridate (DFP) extremely slowly, and this is not influenced by contact with a surface (16, 17). Data supporting surface activation have been presented in which zymogen or activated HF were found to activate PK at the same rate (18). The PK was present in great excess (more than 60-fold higher concentration than that of normal plasma relative to the amount of HF used) and the possibility remained that rapid reciprocal cleavage and activation of the two molecules

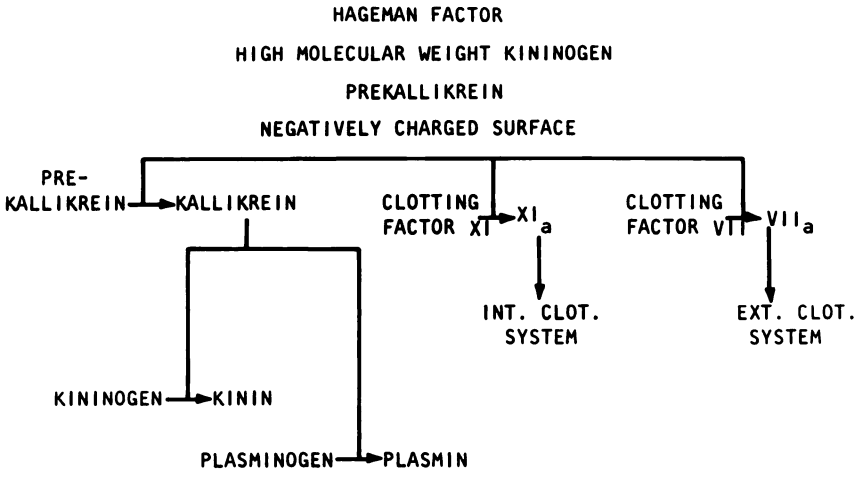

FIG. 1. Diagram of the sequences of activation of components of the Hageman factor system.

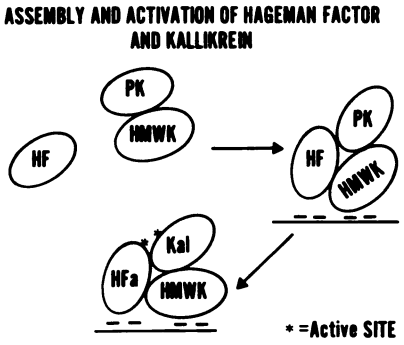

FIG. 2. Proposed mechanism of activation of Hageman factor (HF) and prekallikrein (PK), the cofactor, high molecular weight kininogen (HMWK). The proteins are seen in solution in the upper left. Upon contact with a negatively charged surface (right), the proteins are assembled, leading to activation of HF and PK; yielding HFa and Kal. HMWK is cleaved in the process. See text for details.

took place. This is especially true in view of the speed of reciprocal activation by kallikrein (14). The possibility exists that an undetected exogenous enzyme could activate either PK or HF, although no evidence for this has been obtained. And finally, the possibility that HF and PK are "active zymogens" has been raised (1). In support of this view are the findings that both HF and PK slowly take up [^{3}H]DFP in a manner similar to that of trypsinogen (1). A summary of the molecular assembly leading to the activation of HF is given in figure 2. At the upper left portion of the figure, HF and the complex of PK and HMWK are depicted in the plasma. It should be noted that factor XI and HMWK also exist in plasma as a complex. When presented with a negatively charged surface (right side of the figure), HF and the complex of PK and HMWK become bound to the surface. This occurs by virtue of positively charged residues on the heavy chain of HF and a histidine-rich portion (termed fragment 1-2) of the light chain of HMWK. The HF and PK are thus brought into apposition and activation of each takes place. This reaction is aided by apparent conformational changes occurring in the HF upon surface contact in that the surface-bound molecule is approximately 500-fold more sensitive to enzymatic cleavage than the unbound HF. The initial event in triggering activation could arise from an as yet undetected extraneous enzyme that could cleave and activate either HF or PK. However, HF and PK slowly take up [^{3}H]DFP into the active site of their light chains over a period of 24 hours, thereby rendering the molecules nonactivatable, and zymogen HF, when brought onto a negatively charged surface in apposition with other zymogen HF molecules, leads to cleavage and "autoactivation" of the population of surface-bound HF. This latter event fails to occur when the HF molecules are less densely dispersed on the surface. We therefore propose that HF and PK are "active zymogens" and, when brought into contact with each other, can induce a triggering event leading to proteolytic cleavage and activation. The kallikrein binds to HMWK with relatively low affinity and rapidly dissociates, leading to cleavage of HF molecules bound elsewhere on the surface. This movement of kallikrein in fluid phase probably accounts for a great majority of activation of the HF. The kallikrein at the same time cleaves HMWK in both fluid phase and on the surface.

Activation of Proteins of the Contact System by Cellular Enzymes

Several cells are now known to contain enzymes capable of cleaving and activating components of the HF system. Endothelial cells contain an enzyme(s) capable of cleaving HF into 52,000 and 28,000 MW fragments. When assessed for activity, the cleaved HF was found, in turn, to cleave and activate both PK and factor XI when bound to a negatively charged surface (19). The presence of the cofactor, HMWK, greatly enhanced the reaction with factor XI, presumably by bringing the factor XI to the surface where it encountered the cell enzyme-activated HF. The endothelial cell enzyme was associated with the 100,000 × g pellet of the cell, which contained cytoplasmic and microsomal membranes, and was in-

hibited by DFP (2 mM), but not soy bean trypsin inhibitor (50 μg/ml), hirudin (8 mg/ml), or purified antibodies to PK or plasmin. Thus this cell, which is located at the focal point of the initial inflammatory damage, contains an activator of the HF system.

Recent studies have revealed another pathway by which cellular constituents react with components of the HF system. In collaboration with Drs. Newball, Meier, Lichtenstein, and Kaplan, washed fragments of normal human lung were submitted to anaphylactic challenge and the supernatant fluid was examined for the presence of histamine and enzymes capable of cleaving and activating components of the HF system. Following such challenge, it was possible to isolate enzymes that cleaved HF, PK, factor XI, and HMWK, but not components of the complement system (C3, C5, C4, factor B) or plasminogen (20). The HMWK released kinin during the interaction with enzyme as revealed by contraction of the estrus rat uterus. The HF was cleaved in disulfide-linked fragments of 52,000 and approximately 28,000 MW that cleaved the HF substrate D-pro-phe-arg-p-nitroanalide. PK was cleaved by an enzyme, separable from the above enzymes on SP-Sephadex and DEAE-Sephacel, and was found in turn to cleave its peptide substrate Bz-pro-phe-arg-p-nitroanalide (which is not cleaved by activated HF). Other assays on PK activity have not been performed. Thus, during the anaphylactic response, enzymes are released that generate activity of the HF system and release kinin. The role played by these components in anaphylaxis or similar IgE-initiated reactions remains to be determined.

Another interaction was proposed by Kaplan and Austen (21) in which kallikrein induces chemotaxis of neutrophils, but not eosinophils, while PK or DFP-inactivated kallikrein did not possess activity. Our attempts to repeat these observations several times with peripheral blood neutrophils and kallikrein from both rabbit and human origin have not been successful. A wide range of dosage of kallikrein was employed, but kallikrein neither augmented nor inhibited chemotaxis induced by zymosan-treated serum (unpublished observations). Dr. R.C. Wiggins in our laboratory, together with Dr. P. Giclas, recently found that an intermediate component of plasma was essential for the chemotactic stimulation of rabbit neutrophils by rabbit kallikrein (22). Kallikrein, HF, and HMWK over a wide dose range, either alone or in combination, failed to stimulate chemotactic movement of neutrophils. However, in the presence of C5 kallikrein induced chemotaxis and release of β-glucuronidase. An anodally migrating species of C5 was generated by the interaction of kallikrein and C5. Thus, a novel activity of kallikrein on C5 was observed that generated a factor capable of stimulating both chemotaxis and exocytosis of neutrophils. Its relationship with C5a is not clear at present. In addition, a relationship of the C5-related chemotactic factor to the factor generated in the studies of Kaplan and Austen (21) is uncertain.

The Participation of the Contact System in Pulmonary Inflammatory Injury

We have extended these studies on the biological activities of the HF and complement system proteins to certain inflammatory conditions. I will report on one condition in which evidence suggests a participation of these proteins during development of the disease. Studies were initiated to examine fluids emanating from areas of inflammation of the lung for the presence of factors that mediate the injury. Accordingly, bronchoalveolar lavage (BAL) fluids were obtained from patients with acute and chronic pulmonary inflammatory disease and with non-inflammatory conditions, such as may be found with small solitary tumors. Among the inflammatory diseases studied have been adult respiratory distress syndrome (ARDS) and both active and inactive pulmonary fibrosis. Lavage fluid, obtained from such patients, was incubated with approximately 0.1 μg of [^{125}I]labelled HF, PK, plasminogen, HMWK and C3, C4, C5 and factor B of the complement system. After 20 minutes incubation, the [^{125}I]labelled proteins were assessed for proteolytic cleavage in reduced SDS-acrylamide electrophoresis (SDS-PAGE). Out of 24 cases of ARDS examined to date, 17 have shown the presence of free enzyme capable of cleaving each of the above-mentioned [^{125}I]labelled proteins (23). HF was cleaved into heavy and light chains along with PK and HMWK. A fragment of about 10,000 MW was cleaved from the α chain of C3. Plasminogen and the other complement components were cleaved into small fragments with the amounts of lavage fluid employed. The cleavage fragments of HF and HMWK differed in size from those produced by kallikrein. When the lavage-fluid-treated HF was tested for activity, by using cleavage of PK into heavy and light chains as an indication of HF activation, activity was observed. The activity was not as great as that produced by HF activated by kallikrein (and then separated from the kallikrein). Activity of the other components has not yet been assessed.

In seven of the patients, several lavage samples were obtained during the course of the illness. Of these at least one, and often three or four, of the samples did not contain active enzyme. Several that were negative on the day that ARDS was suspected, became positive on subsequent days, and several that were positive during the illness became negative as the patient recovered. In still others, fluids that contained detectable enzyme were intermixed with negative fluids on subsequent daily lavage samples.

The enzyme in the lavage fluids was neutrophil elastase. It solubilized elastin in elastin-agarose plates, cleaved the elastase-specific synthetic peptide methoxy-succinyl-ala-ala-pro-val-pNA, and reacted with antineutrophil elastase. The enzyme was inhibited with DFP (10^{-4}M) but not by chelating agents, which distinguished it from macrophage elastase. Those BAL fluids not containing free elastase were found to have as their major

inhibitor, active α-1-proteinase inhibitor (α-1-PI), present as determined by binding of [^{125}I]trypsin (which migrated in SDS-PAGE with a M_r (molecular weight) of the α-1-PI-trypsin complex of 80,000) and by immunological reaction with anti-α-1-PI.

Many of the BAL fluids that contained free enzyme also were found to have α-1-PI present. Investigation then revealed that the α-1-PI was inactive; it failed to bind [^{125}I]trypsin and did not inactivate porcine pancreatic elastase (unpublished observations).

The inactive α-1-PI was found to have been oxidized by an indirect experimental assay. When oxidized, a methionyl residue in the reactive site of α-1-PI is converted to methionyl sulfoxide. The molecule is then cleaved by porcine elastase to yield a 47,000 M_r fragment. Such was found to be the case with the α-1-PI obtained from the lavage fluids of the ARDS patient. As much as 300 μg of α-1-PI in the lavage fluids was found inactivated by this means.

These data showing the presence of oxidized, inactive α-1-PI suggested that a process of oxidation occurs in the pulmonary tissues of these patients. The source of oxidants may well be the neutrophils and alveolar macrophages that, when stimulated, generate superoxide anion. In fact, we have identified a strong stimulant of leukocytes, C5a, in the lavage fluid of several of the patients. The α-1-PI may act as an oxidant trap. It signals a second important potential pathogen, the oxidant activity in the lung beds.

The data suggest possible therapeutic approaches to the problem of adult respiratory distress syndrome: control of the elastase and blockade of the oxidant activity that may injure cells directly and inactivate the major control protein of elastase, α-1-PI.

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