REVIEW ARTICLE

# Contact System: A Vascular Biology Modulator With Anticoagulant, Profibrinolytic, Antiadhesive, and Proinflammatory Attributes

By Robert W. Colman and Alvin H. Schmaier

THE KALLIKREIN-KININ system was first recognized as a plasma and tissue proteolytic system responsible for the liberation of the vasoactive, proinflammatory mediator, bradykinin (BK).[1] BK, a nonapeptide released from kininogens by kallikreins, could reproduce many of the characteristics of an inflammatory state, such as changes in local blood pressure, edema, and pain, resulting in vasodilation and increased microvessel permeability. In 1975, three individuals were described with deficiency of high molecular weight kininogen (HK), a precursor of BK, all of whom had a prolonged activated partial thromboplastin time (APTT), a surface-activated coagulation protein screening test.[2-4] Despite the fact that none of these individuals had a hemorrhagic state, studies on the plasma kallikrein-kinin system focused on defining the procoagulant property of HK. In fact, it was already known that deficiency of the two zymogens, factor XII and prekallikrein, required for the enzymatic cleavage of HK, also did not lead to bleeding. These plasma proteins together were grouped as the contact system because they required contact with artificial, negatively charged surfaces for zymogen activation. Over the last 20 years, these proteins have been shown to have little influence on hemostasis. However, examination of their molecular, biochemical, biologic, and physiologic properties has shown that these proteins interact with a number of physiologic and pathophysiologic systems. Cloning and delineation of their structure-function relationships have shown new activities of these proteins such as protease inhibition, antithrombin function, and antiadhesive properties. Their specific interactions with biologic membranes of endothelial cells, platelets, neutrophils, and monocytes indicate that assembly and activation of this system takes place in a physiologic milieu, independent of negatively charged surfaces. In fact, it is correct to say that the so-called elusive physiologic, negatively charged surface for contact system activation is actually the assembly of these proteins on cell membranes. In vivo, a negatively charged surface is not needed for activation. One may argue that the term contact activation is a misnomer to describe this system. The proteins of the plasma contact system have anticoagulant, profibrinolytic, antiadhesive, and proinflammatory functions. This review presents a revitalized view of the contact system as a physiologic mediator of vascular biology and inflammatory reactions. We will first examine the current structure-function knowledge of each of the proteins of the system: HK, prekallikrein, and factor XII. We will next describe how this system assembles on cell membranes. The participation of these proteins in various biologic activities (eg, blood pressure regulation, inhibition of thrombin activation of cells, cellular fibrinolysis, and antiadhesion) then will be characterized in terms of their assembly and activation on cell membranes. Furthermore, we will describe both clinical examples and experimental models in which this system is activated. It is the goal of this review to clarify the contributions of this system to physiologic and pathophysiologic reactions of vascular biology. Last, this presentation will point to possible new therapeutic strategies to treat various diseases arising out of the knowledge of this system in physiologic and pathophysiologic states.

## STRUCTURE-FUNCTION CHARACTERISTICS OF THE CONTACT SYSTEM

### *High Molecular Weight Kininogen (Williams, Fitzgerald Factor)*

*Gene expression and regulation.* The two forms of plasma kininogens, HK and low molecular weight kininogen (LK), are the products of a single gene.[5,6] This gene maps to 3q26-qter, the location of the homologous $\alpha_2$HS-glycoprotein and histidine-rich glycoprotein.[7-9] The single kininogen gene of 11 exons consisting of 27 kb produces a unique mRNA for HK and LK by alternative splicing (Fig 1).[6] HK and LK share the coding region of the first nine exons, a part of exon 10 containing the BK sequence, and the first 12 amino acids after the carboxy-terminal sequence of BK. Exon 11 codes for a unique 4-kD light chain of LK. The complete exon 10 contains the full coding sequence for the unique 56-kD light chain of HK. A novel mechanism for alternative RNA processing has been characterized in the rat kininogen gene.[10] Splicing efficiency is controlled by the interaction of U1 small nuclear ribonucleoproteins and the U1 small nuclear RNA (snRNA)-complementary repetitive sequences of the kininogen pre-mRNA. The mRNA for LK and HK are 1.7 and 3.5 kb, respectively.

The molecular basis for one example of homozygous total kininogen deficiency, Williams trait, has been determined.[11] A C to T transition at nucleotide 587 occurred, changing a CGA (Arg) codon to TGA (Stop) mutation in exon 5 and resulting in prevention of synthesis of both HK and LK.[11] The phenotype of this defect is similar to that seen in Brown-Norway, Katholiek strain rats that have absent plasma kininogens, but the defect in the rats is due to a single point mutation, $Ala_{163}$ to Thr, which results in defective secretion from the liver.[12] Little is known about what regulates gene expression of kininogens. In the rat, ovariectomy results in a reduction of kininogen transcripts in the liver, whereas

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*Submitted December 27, 1996; accepted June 13, 1997.*

*Supported in part by National Institutes of Health Grants No. HL54894, DK43735, and HL47186 to R.W.C. and HL35553, HL52799, HL55907, and HL56415 to A.H.S.*

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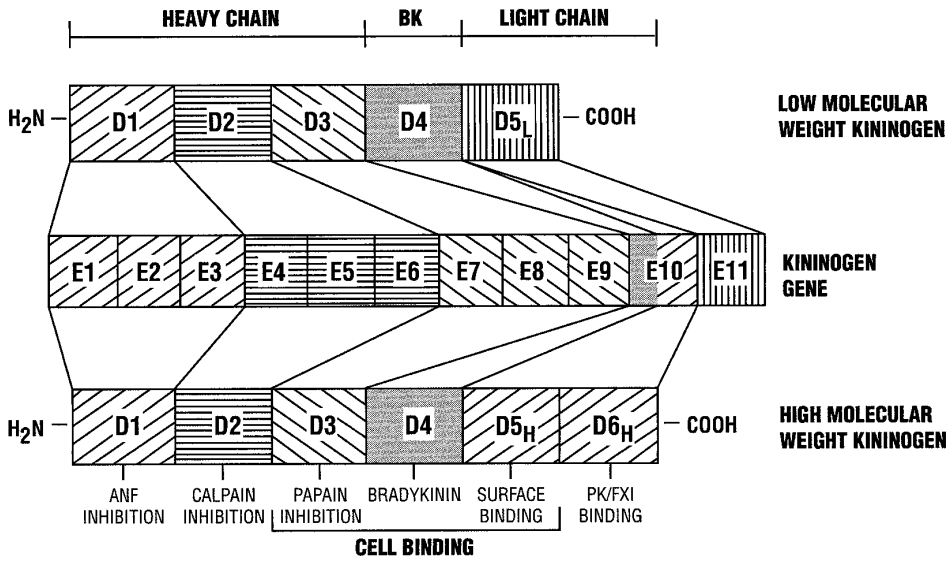


**Fig 1. The domain structure of the kininogens. The kininogens are produced by one gene with 11 exons (E1-E11). E1-E3 codes for domain 1 (D1) on both HK (high molecular weight kininogen) and LK (low molecular weight kininogen). Parts of domain 1 inhibit atrial naturetic factor. E4-E6 codes for domain 2 (D2), which has papain and unique calpain inhibitory sequences. E7-E9 codes for domain 3 (D3), which has papain inhibitory sequences. Domain 4 (D4) is coded by part of E10; it is the bradykinin sequence on kininogens and the first 12 amino acids of the light chains of HK and LK. The remainder of E10 codes for HK's light chain, which consists of domain 5 ($D5_H$) and domain 6 ($D6_H$). $D5_H$ is an artificial surface binding region; $D6_H$ has the prekallikrein and factor XI binding regions. Domains 3, 4, and 5 on HK also participate in cell binding. E11 codes for the remainder of the unique light chain of LK ($D5_L$).**

estrogens increase kininogen mRNA levels.[13] This result is consistent with the clinical observation that HK concentrations increase in pregnancy.[14] In contrast, progesterone treatment reduced kininogen gene expression, resulting in a slight reduction of plasma kininogen levels.[15] Murine fibroblasts synthesize and secrete kininogens in response to cyclic-AMP, forskolin, prostaglandin $E_2$, and tumor necrosis factor $\alpha$.[15] Similarly, tumor necrosis factor $\alpha$ has been recognized to increase kininogen expression in HEP G2 cells.[16] Little else is known to influence kininogen levels, only because this aspect of kininogens has not been studied extensively.

*Protein chemistry and structure of the kininogens*. The two mRNAs of the kininogens code for two separate proteins. LK is a 66-kD $\beta$-globulin with a plasma concentration of 160 $\mu$g/mL (2.4 $\mu$mol/L) and an isoelectric point of 4.7.[17,18] HK is a 120-kD $\alpha$-globulin with a plasma concentration of 80 $\mu$g/mL (0.67 $\mu$mol/L) and an isoelectric point of 4.3.[18,19] Human liver is a source for cDNA for both kininogens,[5,6] but human umbilical vein endothelial cells have been shown to contain HK mRNA and to synthesize the protein.[20] Kininogen antigen also has been found in platelets, granulocytes, renal tubular cells, and skin.[19-24] LK, until its cloning, was also known as an $\alpha_1$-cysteine protease inhibitor.[25] Both HK and LK are composed of globular units. LK gel filters at 66 kD and behaves as a true globular protein; HK, although 120 kD, gel filters at 220 kD, indicating a high axial ratio. Physical evidence for HK being a complex of globular units was obtained by electronmicroscopy studies.[26] On electron microscopy, HK appeared to be a linear array of three linked centralized globular regions, with the two ends thinly connected.[26] Cleavage of HK by plasma kallikrein leads to a striking change in conformation in HK. The central globular region is separated after bradykinin liberation and rearranged with the cysteine protease inhibitory region opposite the prekallikrein binding region.[26] The regions of kininogens are divided into domains (Fig 1). Separating these domains are serine protease sensitive regions.[27-29] As will be discussed below, contiguity of certain domains are important for some biologic functions of kininogens such as calpain inhibition and HK and LK binding to endothelial cells.[30-32] Alternatively, proteolytic cleavage of HK unmasks a new function, ie, its cell antiadhesive activity.[33] Of course, the major activity of kininogens, which is to deliver bradykinin, is programmed disruption of the protein, because bradykinin is not active as a biologic peptide unless liberated from its precursor.

*Domain structure of kininogens*. The kininogens are proteins composed of multiple domains, each with associated activities (Fig 1). Binding of kininogen to its cell receptors facilitates bradykinin liberation in a circumscribed environment in which the peptide can bind to bradykinin receptors and influence the local cellular milieu. Thus, one can view each function of the domains of the kininogens as participating in the whole protein's kinin delivery activity. The kininogens, in general, can be divided into three portions: the heavy chain that is common to both HK and LK, the bradykinin moiety, and the light chains that, as already stated, are unique to HK and LK, respectively (Fig 1). Domains 1 through 3 comprise kininogens' heavy chain. Domain 4 is the bradykinin region. Domain 5 for LK ($D5_L$) is its unique 4-kD light chain. Domains 5 and 6 of HK ($D5_H$ or $D6_H$) are unique to this protein and comprise its light chain.

Little is known about the function of domain 1 except to note that it has a low-affinity binding calcium binding site

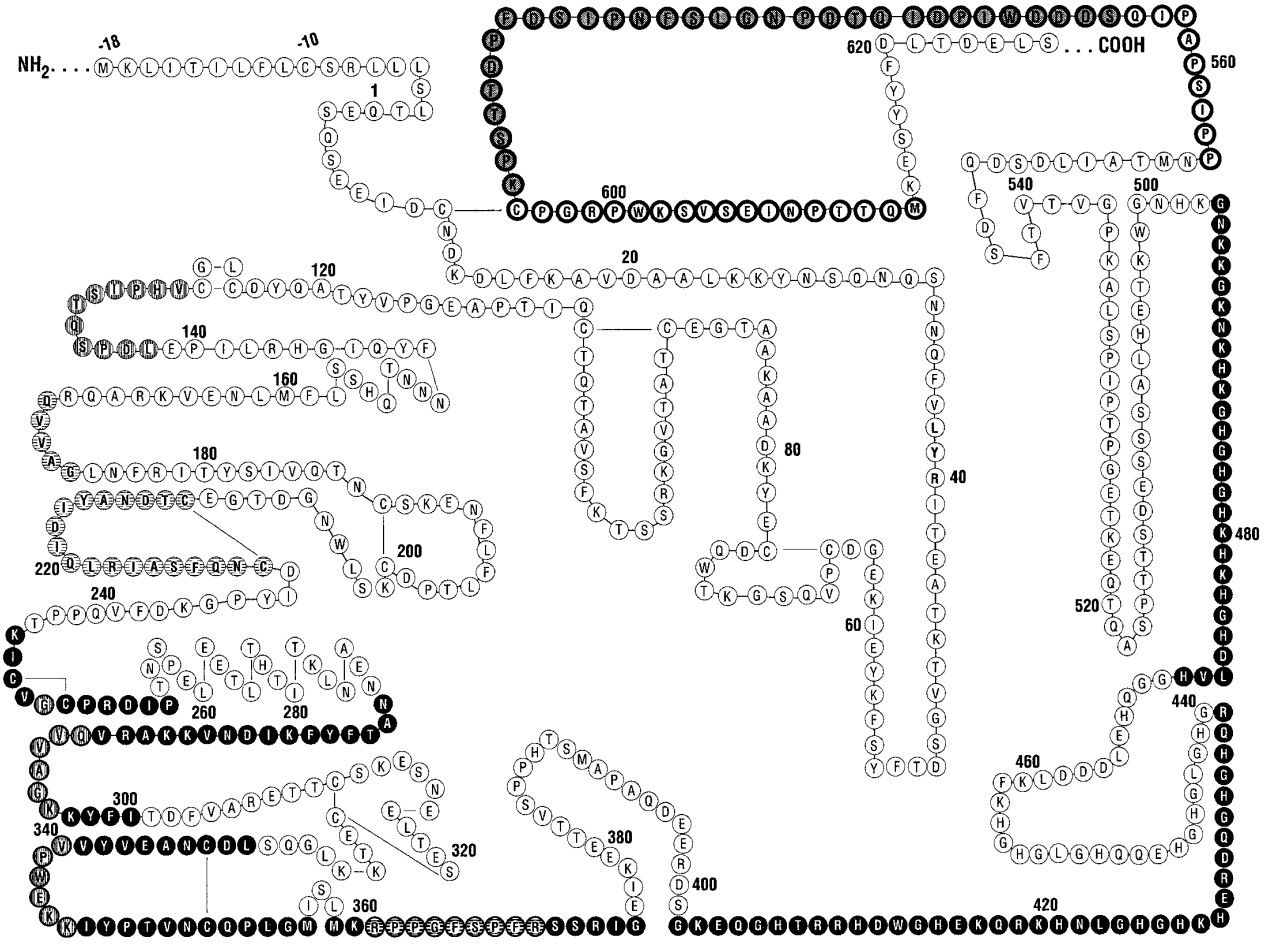

**Fig 2. The structure of high molecular weight kininogen. An amino acid sequence diagram of high molecular weight kininogen. A circle with thin vertical lines represents a papain inhibitory domain. A circle with thin horizontal lines represents a calpain inhibitory domain. A circle with a solid background represents a cell surface binding domain. A circle with thick vertical lines represents overlapping papain inhibitory activity and cell surface binding activity. A circle with thick horizontal lines represents bradykinin. A circle with a blank backround represents the factor XI binding domain. A circle with a shaded background represents overlapping prekallikrein and factor XI binding domain.**

whose role is unknown.[34] Although calcium ions are important for phorbol 12-myristate 13-acetate upregulation of LK and heavy chain binding to endothelial cells,[35] there is no good evidence that calcium ions participate in HK binding to cells,[31,36] contrary to other laboratories' work.[37,38] Recent evidence also indicates that a peptide from domain 1 inhibits atrial naturetic peptide.[39] Domains 2 and 3 contain the highly conserved amino acid sequence, QVVAG, found in cysteine protease inhibitors (Fig 2).[27] Both LK and HK are potent, tight-binding, reversible cysteine protease inhibitors with $K_i$s of 2 and 0.5 nmol/L, respectively, of platelet calpain.[18,40] Kininogens' calpain inhibitory region is exclusively found on domain 2[28,30,40,41], whereas papain and cathepsin L are effectively inhibited by regions on both domains 2 and 3.[27,30,42,43] Computer three-dimensional models of domain 2 were constructed using x-ray crystallographic coordinates of cystatin, which is 50% identical to domains 2 and 3.[30] Peptides from domain 2 of HK were selected and air-oxidized to form disulfide-bonded loops. A peptide containing $Q_{170}VVAG_{174}$ blocked HK inhibition of calpain and thus functioned as a binding site (Fig 2). Another peptide (C211-C229) C-terminal to this peptide was a direct inhibitor of calpain ($IC_{50}$ = 35 $\mu$mol/L). The two regions probably form a continuous binding site on the three-dimensional structure of kininogens (Fig 2). A third peptide (V128-L138), N-terminal to the QVVAG region, inhibited papain, but not calpain, indicating that the inhibitory sites on domain 2 for these two cysteine proteases are not identical (Fig 2). In contrast, the optimal inhibition of cathepsin B and H requires three loops of domain 3 (Fig 2).[43] Although an inhibitor of cysteine proteases, kininogens are also substrates of this class of enzyme when there is molar excess of enzyme to inhibitor.[18,44,45] Because kininogens are extracellular or within granules in platelets and granulocytes, it has been unclear how they interact with cellular cysteine proteases that, for the most part, are internal membrane or cytosolic in location.[19,21,46] However, when platelets are activated, calpain translocates to the external membrane in which it could be inhibited by plasma or externalized platelet $\alpha$-granule HK.[45-47]

Domain 3 of kininogens has other functions. The finding that LK and its isolated heavy chain bind to platelets and endothelial cells indicates that there is a cell binding region on kininogens' heavy chain.[35,48,49] This point was confirmed by direct studies using isolated and recombinant domain 3 that contained the heavy chain cell binding region on platelets[50] and neutrophils.[51] Using a computerized model of domain 3 also based on the structure of crystallized cystatin,[52] the sequential amino acid structure of domain 3 was drawn to show three surface-exposed regions: a disulfide loop connecting it to domain 2 and two hairpin loops (Fig 2). The cysteine protease inhibitory region of domain 3 consists of portions of these three surface-exposed loops. Using synthetic peptides of these surface-exposed regions, $K_{244}$ICVGCP-RDIP$_{254}$ (KIC11), $N_{276}$ATFYFKIDNVKKARVQVVAGK-KYFI$_{301}$ (NAT26), and $L_{331}$DCNAEVYVVPWEKKIYPT-VNC-QPLGM$_{357}$ (LDC27), studies were performed to determine if they inhibit HK binding to endothelial cells. KIC11, NAT26, and LDC27 inhibited biotin-HK binding to endothelial cells with $IC_{50}$ of 1,000, 258, and 60 $\mu$mol/L, respectively. The minimal sequence in LDC27 to inhibit binding was 13 amino acids, $C_{333}$NAEVYVVPWEKK$_{345}$ ($IC_{50}$ = 113 $\mu$mol/L).[53] Because papain blocked HK binding to endothelial cells, the cysteine protease inhibitory site overlaps with the cell binding site on domain 3.[53] Thus, the last 27 amino acids of domain 3, which are contiguous to domain 4, the bradykinin region, are an endothelial cell binding site. Thrombospondin (TSP), a platelet $\alpha$-granule protein secreted upon platelet stimulation, also binds to HK both to a site on the heavy chain requiring calcium ions and to the light chain independent of calcium ions.[54] TSP's interaction with kininogens' heavy chain may be on domain 3 overlapping the KIC11 sequence.[54]

The last function ascribed to domain 3 was kininogens' $\alpha$-thrombin inhibitory activity.[48,50,55] Isolated domain 3, prepared by tryptic digestion of LK in solution, inhibited $\alpha$-thrombin–induced platelet activation.[50] The thrombin inhibitory region was not the same as the platelet binding region because one monoclonal antibody (MoAb), which did not block cell binding, neutralized HK's ability to inhibit $\alpha$-thrombin's activation of platelets.[50] Furthermore, the $\alpha$-thrombin inhibitory region on kininogens was not one of the three cell binding regions, KIC11, NAT26, or LDC27.[53,56,57] Two other distinct sequences, one from domain 3 and another contiguous with domain 3 on domain 4, respectively, are capable of inhibiting thrombin-induced platelet activation by different mechanisms. Kunapuli et al[57] expressed domain 3 in *Escherichia coli*, G235-M357. The recombinant polypeptide inhibited thrombin-induced aggregation of platelets with an $IC_{50}$ of 4 $\mu$mol/L. It should be noted that this sequence, unlike the tryptic digest of LK, does not include any part of domain 4. The protein coded by exon 7, G235-Q292, showed an $IC_{50}$ of 13.4 $\mu$mol/L, and a recombinant peptide of 23 amino acids, K270-Q292, showed an $IC_{50}$ of 30 $\mu$mol/L. Finally, a synthetic heptapeptide located on domain 3, L271-A277 (LNAENNA), was the minimal sequence to inhibit $\alpha$-thrombin–induced platelet aggregation ($IC_{50}$ = 65 $\mu$mol/L). As will be described below, this sequence competes for thrombin binding to platelets by mimicking a GPIb sequence on platelets for binding thrombin.

Alternatively, Hasan et al[56] indicate that the kininogens' thrombin inhibitory activity previously ascribed to domain 3,[50] prepared by proteolytic cleavage, is really domain 4, or the kinin moiety remaining attached to the C-terminus of domain 3. When pure or plasma HK is cleaved by plasma kallikrein on an artificial surface, bradykinin is liberated from its parent protein in three ways.[58,59] The first cleavage yields a nicked kininogen composed of two disulfide-linked 64- and 56-kD chains. The second cleavage yields bradykinin (0.9 kD) and an intermediate kinin-free protein of approximately similar molecular weight to nicked HK. The third cleavage results in a stable, kinin-free protein composed of two disulfide-linked 64- and 46-kD chains. However, when kininogens are cleaved in solution without a surface, this sequence does not necessarily occur and bradykinin can remain attached to kininogens' heavy or light chain.[60] Because isolated domain 3 was prepared by proteolytic cleavage in solution, we examined both trypsin-cleaved LK and domain 3 prepared by tryptic digestion and found that the bradykinin moiety remained attached to LK's heavy chain and isolated domain 3.[32] Investigations were next performed to determine if bradykinin, analogs of bradykinin, and its breakdown products block $\alpha$-thrombin–induced platelets activation. In experiments to be described below, all of these domain 4 fragments were shown to be inhibitors of $\alpha$-thrombin–induced platelet aggregation by preventing $\alpha$-thrombin from cleaving its cloned receptor (PAR1).[56]

Certainly, domain 4, the bradykinin region, has many functions assigned to this nanopeptide in addition to its newest function, $\alpha$-thrombin inhibition.[56] In the liberation of bradykinin, HK is a better substrate of plasma kallikrein and LK is a better substrate of tissue kallikrein. However, both kininogens are substrates to both forms of kallikrein. Factor XIIa cleaves HK similarly to plasma kallikrein.[61] Factor XIa initially cleaves HK into 76- and 46-kD bands. Upon prolonged exposure to factor XIa, the 46-kD light chain of HK is proteolyzed into smaller, inactive fragments.[62] Elastase treatment of LK renders the protein a better substrate of plasma kallikrein to liberate bradykinin and Met-Lys-bradykinin,[63] although it destroys HK's procoagulant activity. Cathepsin D inactivates kininogens' cysteine protease inhibitory activity.[64] One last function of domain 4 is to serve as a cell binding region.[65] The carboxy terminal portion of bradykinin and the amino terminal portion of kininogen's common light chain participate as a low-affinity (kd = 1 mmol/L) binding site to endothelial cells. The importance of the domain 4 cell binding region is not its isolated affinity to the cell surface, but its ability to hold kininogens in the proper conformation for optimal cell binding.[26] For example, intact HK binds to endothelial cells maintained at 37°C with a kd of 7 nmol/L and $1 \times 10^7$ molecules/cell versus kinin-free kininogen, which binds to endothelial cells maintained at 4°C with a kd of 30 nmol/L and 1 to $2.6 \times 10^6$ molecules/cell.[31] These different data for intact or kinin-free HK's interaction with biologic surfaces are not surprising considering the major change in the shape of HK that occurs when it is cleaved on an artificial surface.[26]

LK's light chain is 4 kD and consists of one domain ($D5_L$). Its function is not known. HK's light chain is 56 kD and

consists of two domains, domains 5 ($D5_H$) and 6. $D5_H$ serves as an additional cell binding site on platelets, granulocytes, and endothelial cells.[35,49,51,66] Two areas of $D5_H$ were found to participate in cell binding.[67] One is on the amino terminal end of the domain and consisted of sequences $G_{402}$KEQGHTRRHDWGHEKQRK$_{420}$ (GKE19) and $H_{421}$NLGHGHKHERDQGHGHQRGH$_{441}$ (HNL21) (Fig 2). These peptides inhibit biotin-HK binding with $IC_{50}$ of 792 and 215 $\mu$mol/L, respectively.[67] The other region is on the carboxy terminal region of $D5_H$ and is subsumed in the region of two overlapping peptides $H_{479}$KHGHGHGKHKNKGKKNGKH$_{498}$ (HKH20) and $H_{471}$VLDHGHKHKHGHGHGKHKNKGKK$_{494}$ (HVL24) that inhibit HK binding with $IC_{50}$ of 0.23 and 0.8 $\mu$mol/L, respectively.[67] Preliminary evidence suggests that the region responsible for binding to neutrophils on D5 is localized to H420-H458, similar to HNL21.[68] Independent of its cell binding region, $D5_H$ has been recognized as HK's artificial surface binding region.[28,69,70] $D5_H$'s histidine- and glycine-rich regions have the ability to bind to anionic surfaces, zinc, and heparin.[69-72] Using an MoAb that blocks HK clotting and binding of cleaved HK to anionic surfaces,[28] a 7.3-kD peptide was isolated on an immunoaffinity column that was identified by N-terminal analysis as H441-K497.[72] This 57 amino acid peptide inhibited coagulant activity and had the ability to bind to anionic surfaces with an $IC_{50}$ of 30 $\mu$mol/L. $D5_H$ contains two histidine- and glycine-rich regions, one on its carboxy terminal side, which was also rich in lysine (H457-K502), similar to HKH20, and the other on its amino terminal side (K420-H458), similar to HNL21. Using a deletion mutagenesis strategy on $D5_H$, the anionic surface binding region was found to be associated with both histidine-glycine–rich regions of $D5_H$.[70] Either region was able to support coagulant activity provided it was associated with D6.[70] This question was examined further using synthetic peptides.[67] Peptides HKH20 and HVL24, which are found to be its high-affinity cell binding regions on the carboxy terminal side of $D5_H$, are also found to inhibit the procoagulant activity of HK.[67] No other peptides from $D5_H$, including HNL21, have this property. Furthermore, a polyclonal antibody reared to HKH20 is able to prolong the procoagulant activity of HK in plasma.[67] These data indicate that the endothelial cell and neutrophil cell binding regions and the artificial surface binding region on HK are contained within the same highly conserved region of $D5_H$. Furthermore, the endothelial cell and artificial surface binding regions are overlapping.[67] Peptide HKH20 and its parent HK have the additional ability to interact with M protein on *Streptococcus pyogenes*.[73] It is of interest that the highest affinity cell binding site for $D5_H$ turns out to be the artificial surface binding site. Efforts by many investigators over the last two decades to characterize HK binding to artificial surfaces indicated the location of HK's cell binding site. Last, when HK is bradykinin free, the residual kinin-free kininogen has the ability to prevent the adhesive interaction of vitronectin with tumor cells, endothelial cells, platelets, and monocytes.[33] This property is much weaker in intact, nonproteolyzed HK. This result was anticipated by the finding that, after the liberation of bradykinin from HK, the resulting kinin-free kininogen binds much more tightly to anionic surfaces than does the uncleaved HK.[74]

HK's domain 6 has a prekallikrein (S565-K595) and factor XI binding site (P556-M613) (Fig 2).[75-77] The affinity of prekallikrein for its binding site on the light chain of HK is about 17 nmol/L.[78,79] The prekallikrein and factor XI binding site consists of a 31-residue sequence that contains predominantly $\beta$-turn elements.[80] Although the 30 amino acid region (S565-K595) was shown to be sufficient for binding, more recent studies show that an N-terminally and C-terminally truncated 27-mer (W569-K595) has the essential structural elements for prekallikrein binding.[81,82] HK's procoagulant activity is dependent on two activities: (1) the ability to bind to anionic surfaces via $D5_H$ and (2) the ability to bind prekallikrein and factor XI to domain 6.[28] Inhibition of either interaction with MoAbs directed to these regions will inhibit HK's procoagulant activity.[28,83,84] HK's domain 6 serves as the acceptor protein for factor XI and prekallikrein binding to platelets, neutrophils, and endothelial cells.[37,85,86] As will be seen below, prekallikrein binding to bound HK initiates a sequence of events that leads to prekallikrein activation on biologic surfaces independent of factor XII activation.

### *Prekallikrein (Fletcher Factor)*

Prekallikrein (PK) is produced by a single gene that maps to chromosome 4.[87] PK's gene structure is similar to that of factor XI.[88] Its mRNA codes for a 371 amino acid heavy chain and a 248 amino acid light chain that are held together by a disulfide bond (Fig 3).[88] The amino acid sequence of PK has 58% homology to factor XI.[88] The protein has four tandem repeats in the amino-terminal portion of the molecule due to the linking of the first and sixth, second and fifth, and third and fourth half cysteines residues present in each repeat (Fig 3). This arrangement results in four groups of 90 or 91 amino acids that are arranged in so-called apple domains.[89,90] These same structures have been described in factor XI, suggesting a common ancestor genic duplication event for plasma prekallikrein and factor XI.[87,91]

In plasma, PK appears as a doublet of 85 and 88 kD, whether or not the protein has undergone reduction.[92,93] In plasma, PK is a fast $\gamma$-globulin (isolelectric point = 8.5 to 9.0) with a circulating concentration in blood estimated at 35 to 50 $\mu$g/mL (0.41 to 0.56 $\mu$mol/L).[94,95] Human liver has been shown to be a source for PK cDNA.[89] In liver disease, plasma PK is decreased.[94] Women on oral contraceptives have increased PK levels, but women in their second and third trimester of pregnancy do not.[14,94] When PK is activated to kallikrein ($\alpha$-kallikrein) by either factor XIIa or factor XIIf, the protein on reduced sodium dodecyl sulfate (SDS) gel electrophoresis has two subunits: a heavy chain of approximately 52 kD and two light chains variants of approximately 36 and 33 kD.[92,93] The active site of kallikrein is contained within its light chain because this region incorporates tritiated diisopropyl fluorophosphate in a covalent linkage with $serine_{559}$.[96] $Histidine_{415}$ and $asparatic\ acid_{464}$ comprise the other two amino acids involved in catalytic activity (Fig 3). Prolonged incubation of kallikrein with itself results in autodigestion of its heavy chain into 33- and 20-kD bands as seen on reduced SDS gel electrophoresis to yield a form termed $\beta$-kallikrein.[97] These cleavages occur through the tandem repeats in the heavy chain and result in a protein that cleaves HK more slowly and fails to activate neutrophils

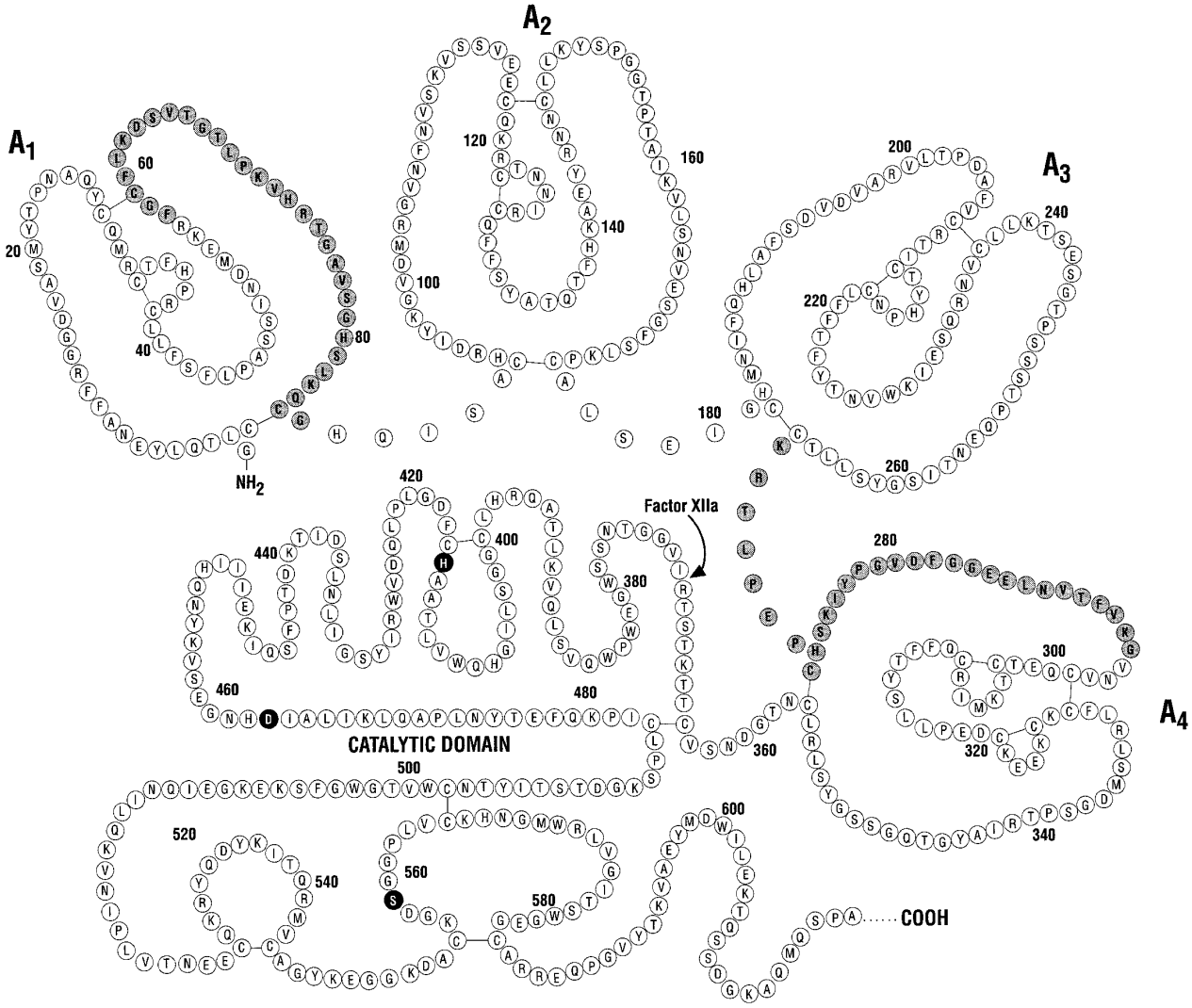


**Fig 3. The structure of prekallikrein. The letters $A_1$ through $A_4$ represent the apple domains of prekallikrein's heavy chain. The notation Factor XIIa and arrow at $arginine_{371}$ represent the factor XIIa activation site on prekallikrein. $Histidine_{415}$, $aspartic\ acid_{464}$, and $serine_{559}$ represent kallikrein's catalytic active site. A circle with a shaded background represents the regions involved in binding to high molecular weight kininogen. Adapted from Chung et al.[89]**

or induce their secretion of elastase.[97,98] Nonreduced SDS gel electrophoresis of artificial, negatively charged surface activation of plasma results in the appearance of kallikrein in complex with $\alpha_2$macroglobulin ($\alpha_2$M) and C1 inhibitor as well as the appearance of a 50-kD prekallikrein/kallikrein fragment containing a portion of the native protein's heavy chain.[14] At least 75% of PK circulates bound, noncovalently, to HK.[99] The binding regions on PK for HK are on apple domains 1 (F56-G86) and 4 (K266-G295) (Fig 3).[100-104] The prekallikrein binding regions for factor XII are localized in apple domains 3 and 4, but the specific sequence has not been delinated.[100]

The in vitro conversion of human plasma PK to kallikrein, its active form, is catalyzed by activated factor XII, on a surface augmented by HK, or by Hageman factor fragment ($\beta$FXIIa) in the fluid phase.[96] In the absence of factor XII, prekallikrein will not become activated on an artificial surface. It is because of this finding that this system is called the contact system. A single bond ($Arg_{371}$-$Ile_{372}$) is split, generating a heavy chain of 371 amino acids still linked to a light chain by a single disulfide bridge without a change in molecular weight. On the endothelial cell surface, this cleavage occurs in the absence of factor XII when PK is bound to HK.[86] The light chain of kallikrein reacts with protease inhibitors, principally $\alpha_2$M and C1 inhibitor (C1-INH). C1-INH forms a 1:1 stoichiometric complex with kallikrein,[105-109] resulting in loss of proteolytic and amidolytic activity. HK protects kallikrein from inhibition by C1-INH and $\alpha_2$M in a purified system,[109,110] suggesting a mechanism of substrate (HK) protection of the enzyme (kallikrein) from active site-directed protease inhibitors. $\alpha_2$M inhibits the kinin-forming activity but only partially inhibits the amidolytic

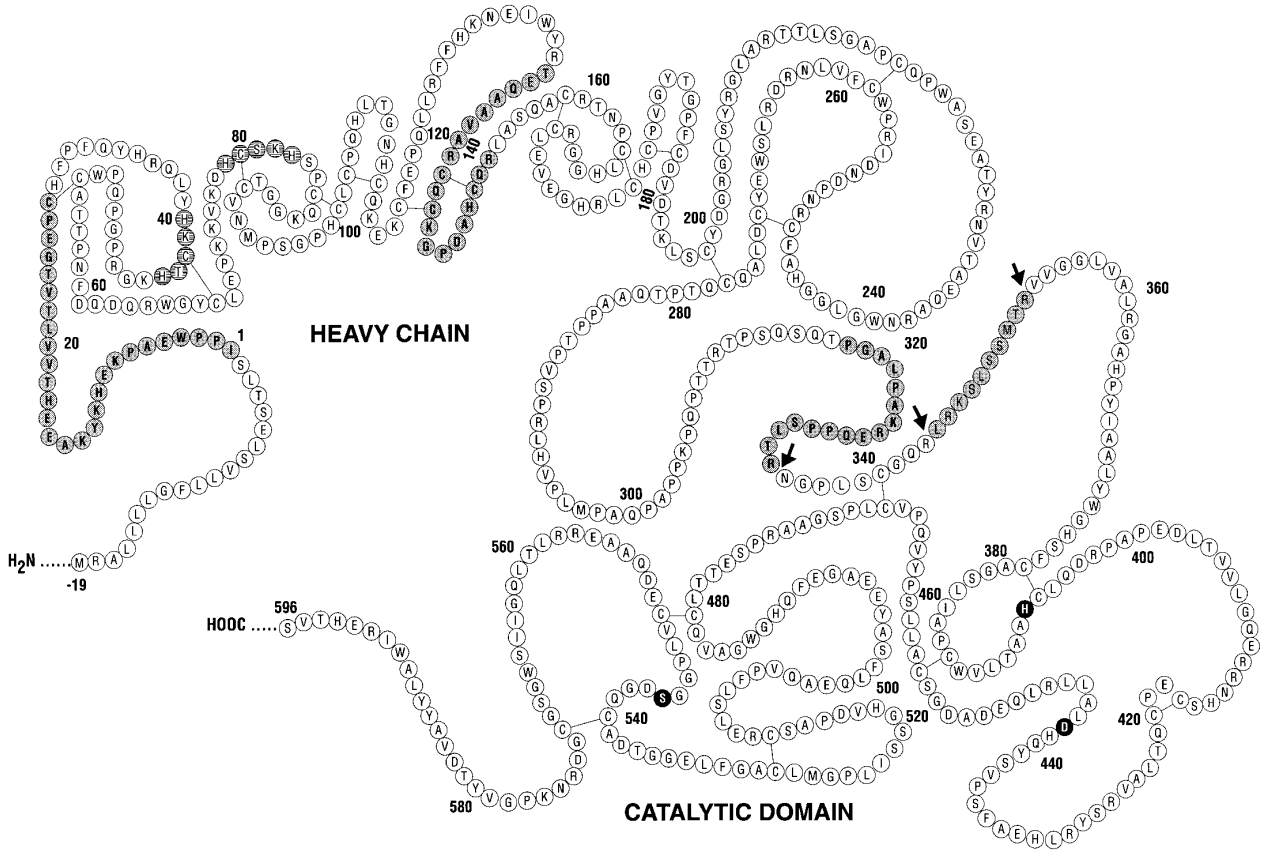


**Fig 4. The structure of factor XII. Proteolysis at arginines 334, 343, and 353 (see arrows) results in activated factor XII (β-factor XIIa). The catalytic triad of factor XIIa consists of histidine$_{393}$, aspartic acid$_{442}$, and serine$_{544}$. A circle with a shaded background represents the artificial surface binding domains on factor XII's heavy chain. A circle with horizontal lines represent two of factor XII's zinc binding domains. Adapted from Cool and MacGillivray.[119]**

activity of kallikrein[108] by forming a covalent complex. Although C1 inhibitor and $\alpha_2$M account for an equal amount of kallikrein inhibition in plasma, C1 inhibitor in plasma acts more rapidly than $\alpha_2$M.[111] Antithrombin III also inhibits kallikrein, but it does so slowly, even in the presence of heparin.[112] In the presence of HK, heparin, which binds to HK,[71,72] significantly accelerates the inhibition of kallikrein by antithrombin. Protein C inhibitor has also been recognized to be a potent inhibitor of kallikrein.[113,114] The major protein substrates of plasma kallikrein are factor XII, HK, and pro-urokinase.[115,116]

*Factor XII (Hageman Factor)*

Factor XII is produced by a single gene that maps to chromosome 5.[117,118] The gene for factor XII is 12 kb and is composed of 13 introns and 14 exons.[119] By both the complementary DNA (cDNA) and DNA sequence, factor XII has multiple domains with extensive sequence homology with regions of tissue-type plasminogen activator (tPA; the epidermal growth factor [EGF]-like region and the kringle region) and fibronectin (Fig 4).[119-121] The factor XII intron/exon gene is similar in organization to the serine protease family of tPA and urokinase-type plasminogen activator (uPA) genes, but is different from most other coagulation protein genes.[119] Its 2.4-kb mRNA codes for a 596 amino acid, single chain $\beta$-globulin with a molecular mass of 80 to 90 kD and an isoelectric point of 6.1 to 6.5 (Fig 4).[122] Its concentration in plasma is estimated to be 30 $\mu$g/mL (0.375 $\mu$mol/L; range, 15 to 47 $\mu$g/mL).[123,124] Human liver has been shown to be a source for factor XII DNA[120] and cultured rat hepatocytes synthesize factor XII.[125] In humans, estrogens administered to postmenopausal women and pregnant women elevate plasma levels of factor XII and its expression is enhanced in isolated livers of estrogen- and prolactin-treated rats.[126-128] Rat liver DNA has been shown to have a functional estrogen regulatory element contained in its 5′ untranslated region that is modulated by 17$\beta$-estradiol.[129] Factor XII, which contains an EGF domain, enhances HepG2 cell proliferation and thymidine and leucine incorporation, suggesting that it is a mitogen for these cells.[130] In fact, factor XII, through its EGF domain, functions as a mitogen and stimulates a signal transduction pathway by a mitogen-induced protein kinase.[131] This activity is independent of activated factor XII's proteolytic activity.

Factor XII can be divided into two regions, a heavy chain

and a light chain. The heavy chain contains two artificial surface binding regions, one at the distal amino terminal end (I1-C28) and another on its fibronectin type I region (T134-R153) (Fig 4).[132,133] Recent studies using recombinant deletion mutants of factor XII confirmed these findings and also indicated that a third region on factor XII's heavy chain, on the second EGF-like or kringle domain (P313-R334, L344-R353), also participated in artificial surface binding (Fig 4).[134] Upon contact with negatively charged surfaces, FXII is autoactivated (solid-phase activation).[135] Both the binding to the surface and the cleavage during autoactivation result in distinct, defined conformational changes.[136] Plasma proteinases, including plasma kallikrein and plasmin, activate factor XII (FXII) to FXIIa ($\alpha$FXIIa), cleaving the bond connecting $Arg_{353}$-$Val_{354}$ and generating a two-chain molecule composed of a heavy chain (353 residues) and a light chain (243 residues), held together by a disulfide bond.[120] The light chain of FXIIa is a typical serine proteinase containing the canonical $Asp_{442}$, $His_{393}$, and $Ser_{554}$ and is the site for inhibition by its major plasma inhibitor, C1-inhibitor.[137] Hageman factor fragments or FXII fragments (FXIIf, $\beta$FXIIa) (Mr = 30 kD) are produced by further proteolytic cleavage, resulting in a chain of 243 residues expressing catalytic activity attached to a fragment of the former heavy chain by a single disulfide bond. Defects in the light chain of factor XII result in disorders of the enzymatic activity of the protein. Coagulation factor XII Washington DC has a $Cys_{571}$-to-Ser substitution that results in complete loss of procoagulant activity.[138] Coagulation factor XII Bern is a protein that, when kallikrein-cleaved, is unable to activate factor XI or prekallikrein.[139] Contact activation arises from the activation of factor XII. Factor XII can be activated by contact with negatively charged surfaces or by the addition of a protease that produced enzymatic cleavage. These two mechanisms have been referred to as solid- and fluid-phase activation, respectively.[140]

The activation of factor XII that arises from binding with negatively charged surfaces[140-143] is termed autoactivation.[144-149] Some evidence suggests that $Zn^{2+}$ binding to factor XII induces a conformation change that makes the protein more susceptible for development of enzymic activity when associated with negatively charged surfaces.[150-152] There are four zinc binding sites, two of which have been identified (H40-H44 and H78-H82).[153] Although there are large number of candidate physiologic negatively charged surfaces that in vitro can be associated with factor XII autoactivation, the concept of autoactivation itself has never been a sufficiently convincing mechanism to explain activation of factor XII and associated contact system activation in vivo. Alternative mechanisms have been searched for factor XII activation in vivo. A rabbit endothelial cell activator of factor XII has been described, but there is no corresponding example in humans.[154] Our own studies indicate that incubation of zymogen factor XII with human umbilical vein endothelial cells does not result in human factor XII activation (unpublished data). However, assembly of PK bound to HK on human umbilical vein endothelial cells results in PK activation independent of factor XII by a cell-associated thiolprotease.[86] Furthermore, factor XII activation by this pathway can occur. In the absence of prekallikrein, factor XII does not activate on endothelial cells in a purified system or in plasma (unpublished data).

Enzymatic activation of factor XII gives rise to successively smaller proteins, each with the same active site serine site. Activation of zymogen factor XII by plasma kallikrein, trypsin, or plasmin results in an enzyme with a decreasing size, a decrease in its surface-binding properties, and a decrease in its coagulant activity. There are two major forms of activated Hageman factor: factor XIIa ($\alpha$XIIa), an 80-kD protein consisting of two disulfide-linked polypeptide chains, and factor XIIf (Hageman factor fragments, HFf, $\beta$XIIa), a 28- to 30-kD fragment derived from factor XIIa.[155-159] $\beta$-Factor XIIa results from cleavage at arginines 334, 343, and 353.[120] The 80-kD form of activated factor XII has the ability to bind to negatively charged surfaces[133,134] and activate factor XI. The 28- to 30-kD enzymatic form of factor XII has no surface-binding properties but retains its ability to activate prekallikrein and C1.[140,160,161]

The major plasma protease inhibitor of activated factor XIIa and XIIf is C1 inhibitor, accounting for greater than 90% of the inhibition of these proteases in plasma.[162-165] C1 inhibitor will bind both proteins and irreversibly inactivate them. When associated with a kaolin surface, factor XIIa is protected from C1 inhibitor inactivation.[166] Antithrombin III has some inhibitory activity on factor XIIa.[167,168] Plasminogen activator inhibitor-1 (PAI-1) also inhibits factor XIIa.[169] Endothelial cells may also produce a protein that impairs factor XII activation, but not its coagulant or amidolytic activity once formed.[170]

## EXPRESSION OF KININOGENS ON BIOLOGIC MEMBRANES

A major impediment to appreciate the contact system is the pervasive notion that the system has no biologic relevance because it is entirely activated on artificial surfaces. Although most studies to date only describe activation of this system on artificial surfaces and much work has been performed to describe physiologic, negatively charged surfaces (eg, acidic phospholipids, cholesterol sulfate, sulfatides, gout crystals, etc), none has been convincing as a single, unifying in vivo activator of this system. The physiologic, negatively charged surface for contact system activation is actually the assembly of these proteins on biologic surfaces, ie, cell membranes. In the protected milieu of cell membranes, we have now shown that the assembly of contact proteins on endothelial cell membranes leads to a multiprotein complex that results in prekallikrein activation independent of activated factor XII.[86] This mechanism will be discussed in a later section. Detailed investigations of the proteins of the contact system interacting with cells have led to this current hypothesis as to how this system is physiologically active. Although there are some individual cell differences, we will first discuss the common features of contact protein expression and interaction with cells in the intravascular compartment.

The pivotal protein for contact system assembly on cell membranes is HK. In addition to being contained within platelets, granulocytes, and endothelial cells, unoccupied binding sites for HK exists on each of these cells.[19-21,36-38,45,171,172] Why each of these cells contain kininogens and also have unoccupied binding sites for them is not known.

In platelets, less than 8% of total platelet HK is HK tightly bound to the platelet membrane.[19,45] Upon platelet activation, 40% of total platelet HK is secreted and another 40% of the total becomes expressed upon the activated platelet membrane.[45] The total platelet contribution to plasma HK is only 0.23%.[19,173] The local concentration of HK on or about the activated platelet membrane may exceed 10 times the plasma concentration of this protein because platelets excrete their granule contents by exocytosis.[19,45]

The majority of granulocyte-associated HK appears to be exogenous HK tightly bound and nonexchangeable with the granulocyte surface.[174] Granulocytes have the ability to assemble all of the proteins of the contact system.[174] Elastase liberated from granulocytes proteolyzes cell-bound HK.[175] Initial investigations suggested that human umbilical vein endothelial cells were able to internalize HK.[20,172] However, more recent detailed investigations indicate that there is no mechanism for HK internalization by endothelial cells.[31] The difference in the amount of HK associated with the endothelial cell membrane when cells are maintained at 4°C versus 37°C is that, at the higher temperature, there is increased expression of kininogen binding sites.[20,31,35,172,176]

There are characteristic features of kininogens binding to all cells. First, kininogen binding to cells has an absolute requirement for $Zn^{2+}$.[20,21,36,38,171,172] The requirement for $Zn^{2+}$ is probably not limited to mediate HK binding to the cells by its zinc binding region of domain 5.[69,72] LK binding to platelets and endothelial cells also has an absolute requirement for $Zn^{2+}$. These data indicate that $Zn^{2+}$ is necessary for the expression of the kininogen binding site, putative receptor.[35,48] Although some investigators have suggested that calcium is a cofactor for binding to endothelial cells and platelets, our investigations show that it does not influence HK binding to unstimulated platelets, endothelial cells, or granulocytes.[21,31,36] However, calcium was a requirement for maximal upregulation of LK or isolated heavy chain binding to endothelial cells after stimulation with phorbol esters.[35] When HK or LK binds to platelets, granulocytes, or endothelial cells, the affinity of binding are the similar (Table 1). Because the affinity of HK binding to cells in the intravascular compartment is between 7 and 52 nmol/L and the plasma concentration of HK is 670 nmol/L, we can postulate that all kininogen binding sites in the intravascular compartment are saturated in vivo. The number of binding sites for the kininogens on cells in the intravascular compartment varies with the cell type. Platelets have approximately 1,000 binding sites/cell; granulocytes have approximately 50,000 sites/cell; and endothelial cells have approximately 1,000,000 sites/cell when chilled to 4°C and approximately 10,000,000 sites/cell when maintained at 37°C (Table 1).[20,21,31,35,36,50]

The expression of kininogens on cell membranes is a complex process. As indicated above in previous sections, there appear to be multiple regions on kininogens that allow them to interact with its various cellular receptors. The first information that such was the case was the finding that HK binds to platelets, endothelial cells, and granulocytes by regions on their heavy and light chains.[35,49,51,66] HK actually has three domains that fit into the putative kininogen receptor(s) on endothelial cells.[65] The interaction sites between HK and its putative receptor may be multiple locations: 3 in domain 3, 1 in domain 4, and 2 in domain 5.[53,65,67] Clearly, the sequence of peptide LDC27 from domain 3 and HKH20 from domain 5 are the highest affinity binding regions on HK for endothelial cells.[53,67] It is important to appreciate that the binding of even a low-affinity sequence from domain 4, for example, will block whole HK from binding to endothelial cells.[65] This information suggests that HK and, presumably, LK have a very tight fit into its binding site(s), putative receptor(s). In fact, because the $K_i$ and $K_d$ calculated from binding studies for HK, LK, and all of their subunits are the same, the two chains of kininogens do not bind to cells in an optimal manner.[66,177] This kind of noncooperative interaction is characterized by a loss of entropy on binding and suggests that whole HK bends to fit into its binding site, putative receptor.[177] In support of this notion, when bradykinin is liberated from HK, kinin-free HK binds to endothelial cells with lower affinity and number of binding sites.[31,65] Likewise, when LK is cleaved between domains 1 and 2 such that there is a change in the conformation of the LK, there is decreased LK binding to endothelial cells compared with intact LK.[32] These changes in the biology of HK expression on cell membranes when bradykinin is removed from the protein are, in retrospect, predictable from the major conformational changes that take place between HK and kinin-free kininogen as shown in functional characteristics[74] in electronmicroscopy[26] and documented by circular dichroism.[178]

The kininogen binding site, putative receptor on endothelial cells appears to be a structure that can be regulated. First, treatment of endothelial cells with metabolic inhibitors to anaerobic and aerobic metabolism and the hexose monophosphate shunt abolish the ability of HK to bind to the cells.[31] Cycloheximide has no effect on HK binding to endothelial cells. Second, temperature or the bradykinin sequence in kininogens contributes to the level of kininogen binding to endothelial cells.[31,65] Third, bradykinin treatment of endothelial cells results in increased HK and LK binding and this pathway is mediated by protein kinase C and the endothelial cell B1 bradykinin receptor.[35] Fourth, heavy chain and LK

**Table 1. Kininogen Expression on Cells in the Intravascular Compartment**

| Cell Type | kd (nmol/L)* | No. of Sites |
|---|---|---|
| Platelets | | |
| $^{125}$I-HK | 15 ± 4† | 911 ± 239 |
| $^{125}$I-LK | 27 ± 2 | 647 ± 147 |
| $^{125}$I-D3 | 39 ± 8 | 1,227 ± 404 |
| Granulocytes | | |
| $^{125}$I-HK | 10 ± 1.3 | $4.8 \times 10^4$ |
| Endothelial cells | | |
| $^{125}$I-HK at 4°C | 52 ± 13 | $9.3 \times 10^5$ |
| $^{125}$I-LK at 4°C | 43 ± 8 | $9.7 \times 10^5$ |
| Biotin-HK at 4°C | 46 ± 8 | $2.6 \times 10^6$ |
| Biotin-HK at 37°C | 7 ± 3 | $1.0 \times 10^7$ |

* Values presented were determined by direct binding studies.

† Values presented represent the mean ± SD.

have a $Ca^{2+}$ requirement for phorbol 12-myristate 13-acetate 4-0-methyl ether upregulation of their endothelial cell binding site, whereas HK does not.[35] Fifth, angiotensin-converting enzyme inhibitors potentiate the effect of bradykinin on upregulating the HK binding site on endothelial cells.[35] Last, when HK binds to endothelial cells, it initiates a series of events that allow for an endothelial cell- or matrix-associated enzyme to activate prekallikrein bound to HK.[86] Thus, bradykinin upregulates kininogen binding on endothelial cells and kininogens can influence bradykinin formation.[35,86] These data indicate that this system is tightly controlled in an autocrine-like manner.

The combined data described above indicate that there should be a physiochemical receptor(s) for kininogens on blood and endothelial cells. Recent evidence proposes a number of candidate proteins to be the kininogen receptor(s). Antibody inhibition studies suggest that Mac-1 (CD11b/18) may be an HK binding site on granulocytes.[51] Fibrinogen has been shown to be a noncompetitive inhibitor of HK binding to granulocytes and ADP-stimulated platelets.[175] HK could bind directly to CD11b/18 on granulocytes or could interact with a receptor complexed to that integrin (see below). Herwald et al[180] have isolated on a HK affinity column from EA.hy926 cells, a human umbilical vein endothelial cell line,[179] a 33-kD protein that was identified as gC1qR. gC1qR is a known C1 receptor protein[181] that only binds HK and peptides from domain 5, but not LK or binding peptides from domain 3. Furthermore, its ability to bind HK does not require $Zn^{2+}$, although other workers claim that $Zn^{2+}$ is required for ligand blots.[182] Moreover, only a small portion of total endothelial cell gC1qR is found on the external membrane of endothelial cells.[183] These data indicate that gC1qR cannot explain all of the characteristics of the kininogen receptor. Factor XII blocks HK binding to qC1qR.[180] These data support the previous finding that factor XII partially blocks HK binding to endothelial cells.[184] The kininogen binding protein just described may form part of a multiprotein receptor complex to explain the features of HK and LK binding to cells. Recently, preliminary evidence has been presented that HKa also binds to the urokinase receptor on endothelial cells.[185] An antibody to domain 2/3 of the urokinase receptor completely inhibits HKa binding to endothelial cells, as does vitronectin, a ligand for this receptor domain. Soluble urokinase receptor markedly inhibits the binding of HKa and forms a zinc-dependent complex with it in a cell-free system. The finding that integrins are tightly associated with the urokinase receptor[186] and can enhance the binding of ligands to domain 2/3 of the urokinase receptor could be relevant to the interaction of kininogens with neutrophils, which display both integrins and the urokinase receptor. Recent evidence indicates that HKa binds directly to cells transfected with Mac-1 and to purified Mac-1.[186a] The interaction of the urokinase receptor with CD11b/18 could be a potential pathway by which kininogen binding could signal within cells. However, because platelets do not express the urokinase receptor, this candidate binding site also cannot be the major kininogen receptor on all cells. Recent evidence indicates that cytokeratin 1 is an additional kininogen (HK and LK) binding site on endothelial cells, platelets, and granulocytes.[187] Kininogen binding to cytokeratin 1 requires $Zn^{2+}$ and all cell binding domains of kininogens interact with it. gC1qR and suPAR block HK binding to cytokeratin, suggesting that these proteins participate in a multiprotein assembly on endothelial cells. These data along with the recent finding that cytokeratin 8 is a cellular plasminogen receptor suggest that cytokeratins may represent a new class of presentation receptors on cells.[188,189] Full characterization of the multiprotein kininogen receptor complex is the next challenge in this field.

## HK AND CELLULAR ACTIVATION

On endothelial cells and platelets, kininogen binding modulates activation of the contact system. Platelet and endothelial cell bound HK is protected from activation by exogenous plasma kallikrein.[66,190] Moreover, HK serves as the binding site or receptor for factor XI and prekallikrein on platelets and endothelial cells.[37,85,86,191] No evidence exists to date to indicate that platelet-associated factor XI is activated to factor XIa in any favorable fashion.[192] However, prekallikrein bound to HK on platelets or endothelial cells can result in its activation to kallikrein by a factor XIIa-dependent[85,193] or independent[86] mechanism. The factor XII-independent prekallikrein activation mechanism is due to a membrane- or matrix-associated thiolprotease whose activity is regulated by HK binding.[86] Both situations result in the generation of bradykinin.[86] Thus, cell membrane assembly of contact proteins through binding can result in a complex that can be activated through physiologic mechanisms to result in bradykinin liberation and the kinin-dependent activities.

In addition to the general characteristics of contact proteins interacting with cells of the intravascular compartment as described above, there are some unique protein-cell interactions as well. Kallikrein, but not PK, is chemotactic for neutrophils.[194] Exposure of neutrophils to concentrations of kallikrein capable of eliciting chemotaxis increased aerobic glycolysis and activity of the hexose-monophosphate shunt.[194] In the presence of calcium, neutrophils aggregate in response to kallikrein.[195] This interaction is associated with stimulation of the respiratory burst in neutrophils, as indicated by an increase in oxygen uptake.[195] Kallikrein also induces neutrophils to release human neutrophil elastase from their azurophilic granules[196] and primes neutrophils for superoxide production.[197]

In plasma, human neutrophils release elastase during blood coagulation,[198] but neutrophils resuspended in either PK- or FXII-deficient plasma release less than one-third of the amount of elastase released in normal human plasma.[196] A skin window technique that assesses the in vivo chemotaxis of leukocytes in response to tissue or microvascular injury shows a significant impairment in chemotaxis in FXII and PK-deficient patients.[199] This result suggests that both kallikrein and FXIIa are important in the release of elastase from neutrophils in plasma. In addition, kallikrein induces an in vitro release of elastase from neutrophils in a concentration-dependent fashion that requires the presence of both the active site of kallikrein (on its light chain) and an intact heavy chain.[200] The requirement for an uncleaved heavy chain can be explained by the requirement for both apple 1

and apple 4 sequences for binding of kallikrein to HK on neutrophils.[104,105] Kallikrein formation occurring in human sepsis and experimental arthritis and enterocolitis (see below) would also recruit neutrophils to participate in the body defenses. FXIIa has also been shown to cause neutrophil aggregation[201] and degranulation (release of elastase). FXIIf will not stimulate neutrophils, and, thus, a domain on the heavy chain is required. However, the catalytic activity of FXIIa is required because the active site inhibitors, D-Pro-Phe-Arg-$CH_2Cl$ and corn trypsin inhibitor, both abolish the reaction.

Factor XIIa can decrease the number of Fc$\gamma$R1 (Ig) receptors on monocytes without affecting its affinity. This interaction requires the heavy chain, but, in contrast to the effect of FXIIa on neutrophils, does not require the catalytic apparatus of the light chain.[202] The site on FXII responsible for the downregulation of Fc$\gamma$R1 may be within the N-terminal 18 amino acids,[203] and this decrease could impair the clearance of immune complexes. Toossi et al[204] have found that factor XII induced monocyte synthesis and secretion of interleukin-1 (IL-1) and IL-6. These investigators found that lipopolysaccharide-stimulated secretion of these interleukins is also potentiated by factor XII.

## BIOLOGIC INTERACTIONS OF CONTACT SYSTEM PROTEINS

The simple fact that a deficiency of HK, prekallikrein, and factor XII prolongs artificial surface-activated clotting without being associated with bleeding has obfusicated understanding the role of this system in vivo. The absence of hemostatic states associated with these proteins does not lessen their importance. The dicotomy between abnormal surface-activated screening laboratory tests for bleeding states and in vivo hemostasis should give us caution in interpreting laboratory tests as predictors of bleeding. Independent of its lack of effect on hemostasis, contact system activation modulates vascular biology. The multidomain kininogens have a number of biologic activities either within the intact protein or becoming manifest when the intact protein is proteolyzed by kallikreins or activated factor XII. This system is a potent local regulator of blood pressure through bradykinin delivery. It also has both selective antithrombin and profibrinolytic activity. Lastly, the cleaving of HK unmasks antiadhesive properties of the protein as well.

### *Bradykinin Delivery*

The first and most enduring function of the plasma kininogens is the delivery of bradykinin, a potent biologically active peptide.[1] In many ways, kininogens and bradykinin, an activation peptide from domain 4, contribute to vessel patency, increased blood flow, and anti-thrombotic/profibrinolytic activities (Table 2). Bradykinin itself is a potent stimulator of endothelial cell prostacyclin synthesis; an inhibitor of platelet function,[205,206] superoxide formation,[207] and tissue plasminogen activator release; and a stimulator of plasminogen activation,[208,209] nitric oxide formation,[210] and endothelial cell-dependent smooth muscle hyperpolarization factor formation.[211] Furthermore, bradykinin, through its ability to stimulate NO and cGMP formation in endothelial cells, provides a major stimulus to prevent subendothelial smooth muscle proliferation.[212,213] In the presence of an intact endothelium, kinins appear to prevent vascular smooth muscle growth and proliferation.[214,215] Alternatively, when vessels are injured, bradykinin stimulates protein kinase C and subsequently MAP kinases that can result in vascular smooth muscle growth and proliferation.[215-217] Thus, in an intact vessel, the sum of bradykinin activities is to keep blood flowing and vessels patent; in the absence of endothelium, bradykinin stimulates repair of vessels that could lead to smooth muscle proliferation and intimal hypertrophy.

**Table 2. Kininogens' Antithrombin, Antiadhesive, and Profibrinolytic Activities**

| Domain | Activity |
|---|---|
| Bradykinin | Stimulates prostacyclin formation |
| Bradykinin | Stimulates NO formation |
| Bradykinin | Stimulates superoxide formation |
| Bradykinin | Selectively stimulates tissue plasminogen activator secretion |
| RPPGF | Prevents $\alpha$-thrombin from cleaving its receptor (PAR1) |
| Domain 1 | Inhibits atrial naturetic factor |
| Domain 2 | Prevents calpain-related platelet aggregation |
| Domain 3 | Prevents $\alpha$-thrombin binding to platelets and endothelial cells |
| Domain 5 | Prevents cells from sticking to artificial surfaces |
| Domain 5 | Displaces fibrinogen from surfaces and cells |
| Domain 6 | Prekallikrein and factor XI receptor on endothelial cells and neutrophils |

Bradykinin effects its changes in the intravascular compartment by binding to at least two receptors, the B1 and B2 receptors.[218,219] Both of these receptors are G-coupled; thus, binding of bradykinin stimulates cellular signal transduction. Increased bradykinin results in increased cellular stimulation. Blocking of the B2 receptor with an antagonist Hoe 140 (D-Arg,[Hyp$^3$,Thi$^5$,D-Tic$^7$,Oic$^8$]-bradykinin) in developing rats results in higher blood pressures, heart rates, and body weights than controls.[220] The in vivo modulation of bradykinin levels by angiotensin converting enzyme (ACE) inhibitors is believed to be the basis for the cardioprotective attributes of these agents.[221,222] ACE inhibitors induced NO and prostacyclin formation in cultured bovine endothelial cells and protect isolated perfused hearts from ischemia.[223] The effects of ACE inhibitors to elevate NO and protect isolated ischemic hearts was abolished by the B2 receptor antagonist, Hoe 140.[224] ACE inhibitor treatment in spontaneously hypertensive rats prevented the development of hypertension and left ventricular hypertrophy and Hoe 140 blocks these effects.[225,226] These data in animals have been extended to humans, in whom ACE inhibitors have also been shown to be protective against myocardial infarction by increasing myocardial blood flow and decreasing ischemic changes. Although these cardioprotective effects of ACE inhibitors may be sufficiently explained alone by bradykinin's effect on vasculature, recent information that this peptide and its breakdown products are also selective inhibitors of $\alpha$-thrombin could contribute as well (see below).[56]

*Blood Pressure Regulation*

Although much is known about the physiologic effects of bradykinin and how its stimulates its response in cells, little is known on what regulates its liberation from kininogens by kallikreins. What regulates prekallikrein and tissue kallikrein activation on the vascular endothelium is not known. Regulation of these zymogen's activation is important because they directly modulate bradykinin liberation, which, in turn, has a direct effect on blood pressure in vivo. Dextran sulfate activation of rat plasma in vivo induces arterial hypotension, which can be blocked by a B2 receptor antagonist.[227] Transgenic mice overexpressing tissue kallikrein are hypotensive.[228] Intramuscular delivery of rat kallikrein-binding protein reverses hypertension in transgenic mice overexpressing human tissue kallikrein.[229] Rat kallikrein binding protein or kallistatin is the cognate SERPIN of tissue kallikrein.[230-232] Gene delivery of tissue kallikrein reduced mean blood pressure of spontaneous hypertensive rats and this inhibition was blocked by kallistatin.[233,234] These molecular genetic studies directly indicate that presumed tissue kallikrein induced bradykinin liberation directly modifies local and, if sufficiently diffuse, systemic blood pressure regulation.

*Thrombin Inhibition*

In addition to the salutory effects of kinins to maintain vessel patency, bradykinin's precursor proteins, the kininogens, have been shown to selectively inhibit $\alpha$-thrombin–induced platelet activation. There are at least three mechanisms by which kininogens influence $\alpha$-thrombin–induced platelet and endothelial cell activation[48,50,235] (Table 2). The first mechanism is an indirect one probably mediated by kininogen's ability to inhibit platelet calpain. When $\alpha$-thrombin activates platelets, cytosolic or internal membrane-associated platelet calpain translocates to the activated platelet surface.[46,47] Externalized platelet calpain is able to proteolyze platelet surface membrane glycoproteins such as glycoprotein Ib.[236] Platelet calpain also proteolyzes a putative platelet ADP receptor that exposes the platelet fibrinogen receptor and thus allows for platelet aggregation.[235,237] Thus, inhibition of externalized platelet calpain by leupeptin or HK, ie, inhibitors of calpains, results in inhibition of $\alpha$-thrombin–mediated platelet aggregation by preventing fibrinogen binding.[55,235] These data have been used to develop a group of compounds modeled after kininogens' domain 2 that prevent $\alpha$- and $\gamma$-thrombin–induced platelet aggregation without interfering with other platelet agonists and thrombin-induced intracellular platelet activation.[238] Selective thrombin-induced platelet aggregation inhibitors can be developed by making peptides modeled after kininogens' domain 2.

Additional studies suggested that there are more mechanisms by which kininogens inhibit $\alpha$-thrombin–induced platelet activation. HK and LK were found to inhibit $\alpha$-thrombin–induced platelet aggregation and secretion.[48,56] Because $\alpha$-thrombin–induced platelet secretion is independent of and occurs before platelet aggregation, kininogens must interfere with $\alpha$-thrombin–induced platelet activation by other mechanisms than just inhibition of calpain-related platelet aggregation.[239] HK, LK, and D3 were found to noncompetitively inhibit $\alpha$-thrombin, but not Phe-Pro-Arg-chloromethylketone–treated thrombin, from binding to the platelet high-affinity site and endothelial cells.[31,48,50,56,240] This finding was one explanation of how all platelet activation by $\alpha$-thrombin could be blocked by large molecular mass proteins such as HK and LK. Additional studies have recognized other mechanisms for inhibition.

Kunapuli et al[57] found that recombinant domain 3 (containing no residues of domain 4) inhibited thrombin-induced aggregation of platelets with only twofold less affinity than purified HK, indicating that at least one site for inhibition does reside in domain 3 (Fig 5). The minimal $\alpha$-thrombin inhibitory sequence was $Leu_{271}$-$Ala_{277}$.[57] $Leu_{271}$-$Ala_{277}$ did not inhibit platelet aggregation by ADP or collagen. It did not inhibit thrombin's amidolytic or clotting activity. $Leu_{271}$-$Ala_{277}$ also failed to inhibit platelet shape change and it did not inhibit SFLLRN from aggregating platelets. Bradford et al[241] have obtained evidence that $Leu_{271}$-$Ala_{277}$ and K270-Q292 inhibited thrombin-induced platelet activation at low thrombin concentrations by inhibiting the binding of thrombin to GPIb-IX-V complex. Furthermore, antibodies to and ligands of GPIb$\alpha$ inhibited HK binding to platelets and HK inhibited binding of antibodies to GPIb$\alpha$ to platelets. Moreover, domain 3 peptides directly inhibited high-affinity $^{125}$I–$\alpha$-thrombin binding to platelets. Finally, HK inhibited binding of thrombin to fibroblasts transfected with GPIb-IX-V. These findings suggested that domain 3 peptides may block $\alpha$-thrombin binding to its high-affinity site on GPIb$\alpha$. The sequence NAEN appears in HK, domain 3 peptides, and the ligand binding domain of GPIb$\alpha$. It is possible that HK's domain 3 may mimic this high-affinity binding site for thrombin. These findings do not necessarily imply that binding of thrombin to GPIb$\alpha$ itself results in platelet activation. Rather, GPIb$\alpha$ may serve to present thrombin to the G-protein–linked cloned thrombin receptor, thus lowering the concentration of thrombin necessary to cleave the latter receptor. Kininogens by blocking this interaction would then modulate thrombin-induced platelet activation.

A third mechanism of $\alpha$-thrombin inhibition has been described by Hasan et al.[56] Kininogens and peptides derived from it actually inhibit $\alpha$-thrombin–induced platelet activation by blocking the enzyme's ability to cleave the cloned thrombin receptor (PAR1).[56] In work already described above, purified domain 3 prepared from proteolytic cleavage actually had domain 4 attached to it.[32,56] Peptides from domain 4, BK, and related sequences were found to inhibit $\alpha$-thrombin–induced platelet activation (Fig 5). Although large molecular mass HK, LK, and D3 inhibited $\alpha$-thrombin binding to platelets, isolated domain 4, ie, bradykinin (RPPGFSPFR) and MKRPPGFSPFRSSRIG, did not inhibit binding.[48,50,56] These data indicated that another mechanism is operative for these peptides to block $\alpha$-thrombin–induced platelet activation. Like the parent proteins HK and LK, domain 4 peptides did not inhibit $\alpha$-thrombin's ability to cleave a tripeptide substrate or clot fibrinogen, suggesting that these peptides did not interact with $\alpha$-thrombin's active site or anion binding exosite.[31,48,50,56] Moreover, like HK and LK, these peptides were not substrates of $\alpha$-thrombin and they did not form complexes with $\alpha$-throm-

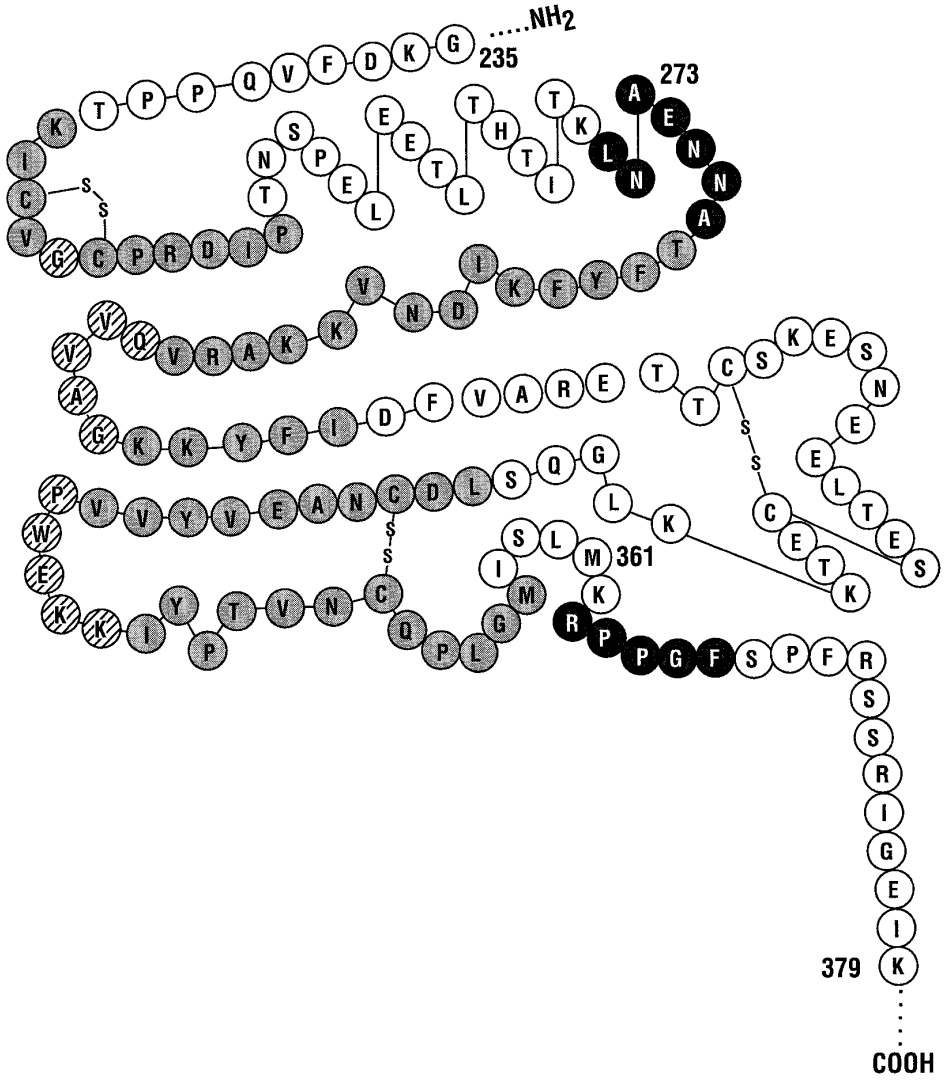


**Fig 5. Kininogens' thrombin inhibitory domains. A circle with a solid background represents thrombin inhibitory activity. A circle with a shaded background represents a cell membrane binding region. A circle with diagonal lines represents overlapping papain inhibitory activity and cell membrane binding activity.**

bin.[31,48,56] Domain 4 peptides did not block ADP-, collagen-, or U46619-induced platelet aggregation in vitro.[56] They did block $\alpha$-thrombin–induced calcium mobilization and $\gamma$-thrombin–induced platelet aggregation in plasma in vitro.[56] The minimal form of domain 4 that inhibited $\alpha$-thrombin–induced platelet activation was the peptide RPPGF (Fig 5).[56] RPPGF is the major angiotensin converting enzyme breakdown product of bradykinin in plasma with a metabolic degradation rate of 4.2 hours.[242,243] The mechanism by which RPPGF and related domain 4 peptides inhibit $\alpha$-thrombin–induced platelet activation is unique. RPPGF does not block the thrombin receptor peptide, SFLLRN, from inducing platelet activation.[56] Domain 4 peptides prevent $\alpha$-thrombin from cleaving the cloned thrombin receptor to initiate the activation process. This result means that domain 4 peptides actually prevented $\alpha$-thrombin from cleaving the cloned thrombin receptor after $\text{arginine}_{41}$, a critical step in $\alpha$-thrombin activation of cells through this receptor.[56] When a peptide was prepared that spanned the $\alpha$-thrombin cleavage site on the cloned thrombin receptor (NATLDPRSFLLR), RPPGF and HK actually prevented $\alpha$-thrombin from cleaving this peptide between the arginine and the serine.[56] RPPGF specifically interfered with thrombin's ability to cleave the cloned thrombin receptor to activate platelets without interfering with its procoagulant activity. These combined data indicate that domain 4 peptides and this same sequence in kininogens are selective, proteolytic inhibitors of $\alpha$-thrombin–induced platelet activation by being directed to $\alpha$-thrombin's substrate, the cloned thrombin receptor. Compounds based on the RPPGF sequence could represent a new class of thrombin inhibitors that achieve selectivity by being directed to the substrates of thrombin, rather than the enzyme itself.

### *Participation in Fibrinolysis*

In addition to these unique mechanisms of $\alpha$-thrombin inhibition, contact proteins participate in cellular fibrinolysis. From the time of recognition of HK deficiency, this protein has been ascribed to have a role in the fibrinolytic process, although the specific, physiologic mechanism has not been known.[2,4] It has been known for more than 35 years that contact activation can increase total plasma fibrinolysis.[244] Kallikrein, factor XIIa, and factor XIa cleave plasminogen directly, albeit much less efficiently than tPA or uPA.[245-248]

However, bradykinin has been characterized as a potent and selective in vivo inducer of tissue-type plasminogen activator release from endothelial cells in rabbits and humans.[208,209] Plasma kallikrein also has been characterized to be a kinetically favorable activator of single-chain urokinase in vitro.[116] More recent studies suggested single-chain urokinase activation by kallikrein can best occur on the platelet and endothelial cell surface.[85,193,249]

These studies prompted us to examine the relationship of prekallikrein assembly on endothelial cells and how it may participate in single-chain urokinase activation (Table 2).[86] When prekallikrein binds to HK on endothelial cells, the zymogen becomes activated to kallikrein, as indicated by elaboration of amidolytic activity, changes in the structure of prekallikrein to kallikrein on gel electrophoresis, and cleavage of HK.[86] Prekallikrein activation occurs independently of any activated forms of factor XII. The prekallikrein-activating enzyme(s) is not a serine protease, but a membrane-associated or matrix-associated thiolprotease.[86] Prekallikrein activation over endothelial cells is kinetically similar to prekallikrein activation by factor XII on an artificial surface. These data show for the first time that contact protein assembly on endothelial cells results in prekallikrein activation in the absence of factor XII and an artificial surface.[86] This assembly of contact proteins allows for a physiologic pathway for this system to be activated. The degree of prekallikrein activation is regulated by HK. Increasing HK concentrations upregulates the enzyme that activates cell-bound prekallikrein. Thus, HK regulates prekallikrein activation, which, in turn, liberates more bradykinin from cell-bound HK and removes HK from the surface to slow prekallikrein activation.[86] In support of this mechanism, we have recently shown that peptides derived from D6 of HK can downregulate plasmin formation by interfering with prekallikrein binding to HK on the endothelial cell surface.[250] Also, increased bradykinin increases kininogen binding, which decreases soluble kallikrein from cleaving HK to liberate more bradykinin.[35] Thus, there is a closely regulated pathway of prekallikrein activation and bradykinin liberation.

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.[208,209] Second, kallikrein induces kinetically favorable conversion of single-chain urokinase into two-chain urokinase in an environment in which there is constitutive molar excess secretion of endothelial cell plasminogen activator inhibitor-1.[86,250] Formation of two-chain urokinase results in a 4.3-fold increase in plasminogen activation. This system for plasminogen activation occurs in an environment in which there is no contribution by factor XIIa. This mechanism for single-chain urokinase activation is a pathway for cellular fibrinolysis that is either independent of or conjoined with single-chain urokinase activation associated with its binding to its receptor.[251] The possible binding of HK (and, thus, kallikrein) to domain 2/3 of the urokinase receptor[185] on the same molecule as prourokinase, which binds to domain 1 of the receptor, may allow for a very efficient cleavage of the latter by kallikrein. In addition, HK can compete with vitronectin, which also binds to domain 2/3 of the urokinase receptor, and displace vitronectin and its associated molecule, plasminogen activator inhibitor-1, thus enhancing fibrinolysis.

### *Antiadhesive Properties*

HK has been postulated to be an antiadhesive protein. This property has been observed under three different situations. First, cleaved, kinin-free kininogen (HKa) can compete for deposition with adhesive proteins on artificial negatively charged surfaces such as those that occur on biomaterials. Second, HKa can compete with adhesive proteins for binding to cells. Third, HK on surfaces or in solution can prevent cells from attaching to protein-covered surfaces.

Vroman and Adams[252] found that fibrinogen can be detected immunochemically on a negatively charged surface within seconds after normal human plasma contacts the surface, but, within minutes, is no longer detectable. We have shown that this phenomenon is due to the displacement of fibrinogen by HK after surface-dependent autoactivation of factor XII.[253,254] Factor XIIa, both directly and indirectly (through the formation of kallikrein), generates HKa from HK. HKa (but not HK or LK) displaces fibrinogen from the surface.[62] Therefore, the Vroman effect is due to the time- and surface-dependent generation of HKa, via contact activation of plasma, which results in the physical displacement of adherent fibrinogen from the surface.[254] Extensive proteolysis results in HKi,[62] which does not displace fibrinogen.[254]

We have also described a similar effect on blood cells, as HK and/or HKa can displace $^{125}$I-fibrinogen from both neutrophils and platelets.[175] Asakura et al[33] extended these results by showing that HKa, but not HK, HKi, or LK, inhibited the adhesion and spreading of human osteosarcoma cells to vitronectin-coated polystyrene plates. HKa inhibited the attachment of platelets and monocytes to extracellular matrix proteins, and the spread of bovine aortic endothelial cells on both fibrinogen and vitronectin.[33] The inhibition by HK of cell attachment to vitronectin may be explained by their competition for occupancy of domain 2/3 on the urokinase receptor.[185] Results from our laboratory also indicated that neutrophils in a flow system at a shear rate of 20 $s^{-1}$ adhere to a fibrinogen-coated surface linearly. In contrast, the rate of adherence to the same surface coated with HK is at least five times slower.[255] The possibility of passivating surfaces with kininogen or its peptides may provide a new approach to biocompatibility. Alternatively, ligands derived from HK could, by binding to neutrophils, prevent their adhesion to surfaces or other cells such as endothelial cells.

## DISEASE STATE INTERACTIONS

### *Hereditary Angioedema (HAE)*

HAE is a congenital condition associated with a deficiency or defect in C1 inhibitor (Table 3).[256,257] Acute attacks of HAE have been well-documented to be associated with contact system activation.[14,258-260] Characteristically, in acute attacks of HAE, there is reduced plasma prekallikrein activity with normal plasma prekallikrein antigen and reduced HK activity and antigen.[14,259,261] Contact activation arises due to

**Table 3. Diseases States and Conditions Associated With Contact System Activation**

| Disease* | PK | | HK | | C1 INH | | XI | XII | $\alpha$2M-Kal Complexes | C1 INH-Kal Complexes |
|---|---|---|---|---|---|---|---|---|---|---|
| | Act. | Ant. | Act. | Ant. | Act. | Ant. | (Act.) | (Act.) | (Ant.) | (Ant.) |
| HAE[14,259] | D | D | D | D | D | D | — | — | I | — |
| Sepsis[270,276] | D | — | — | — | D | — | — | D | I | — |
| Typhoid fever[272] | D | U | — | — | D | I | — | — | — | — |
| ARDS[269] | D | D | D | U | D | I | U | D | — | — |
| RMSF[274] | D | D | — | — | I | — | I | U | — | I |
| Low-dose endotoxin[277] | D | U | D | — | — | — | D | — | I | — |
| CPB[281-283] | — | — | — | — | — | — | — | — | — | I |

Abbreviations: D, decreased; U, unaffected; I, increased.

* The following diseases were investigated: HAE, hereditary angiodema; ARDS, adult respiratory distress syndrome; RMSF, Rocky Mountain spotted fever; CPB, cardiopulmonary bypass. The numbers after the disease category are references.

the absence of protease inhibition as a result of lowered C1 inhibitor levels. Bradykinin liberation is believed to be a major mediator of the edema seen in that condition.[262] The phenomena of cold activation of factor VII is result of cold inactivation of C1 inhibitor and factor XII activation in a tube with resultant factor VII activation.[263-265] Lowering temperatures to less than 37°C decreases the reactivity of C1 inhibitor for its enzymes.[266]

*Sepsis*

Contact system activation has been postulated to be one of the mediators of systemic inflammatory response syndrome (SIRS).[267] Contact activation of factor XII and prekallikrein in sepsis result in cleavages that activate them to enzymes that rapidly react with C1-inhibitor to form factor XIIa-C1-INH and kallikrein-C1-INH complexes (Table 3).[268] The result is depletion of functional prekallikrein and factor XII with persistence of normal levels of the corresponding antigens. Functional C1-inhibitor also declines, but its antigen remains constant or may even increase, suggesting that it behaves as a weak acute-phase reactant. As functional C1-INH decreases, $\alpha_2$M becomes a more important inhibitor of kallikrein and $\alpha_2$M-Kal complexes form.[14] The HK coagulant activity and antigen decrease in parallel.[269] Paradoxically, for unknown reasons, functional factor XI may increase.[269]

Investigations of patients with gram-negative sepsis showed that functional factor XII, prekallikrein, and C1-INH are decreased in patients with hypotensive septicemia.[270] Patients with disseminated intravascular coagulation (DIC) due to septicemia or viremia had decreased functional factor XII, prekallikrein, and C1-INH, but individuals with DIC secondary to neoplasia had no significant changes in the kallikrein-kinin system.[270] In patients with postoperative septicemia, decreased prekallikrein activity and elevated bradykinin were associated with positive blood cultures and hypotension.[271] In an experimental infection of humans with typhoid fever, all patients with typhoid fever showed a decrease in functional prekallikrein and C1-INH, but the corresponding antigens remained unaffected.[272] In the adult respiratory distress syndrome (ARDS), effected patients had reduced plasma levels of factor XII and prekallikrein.[269,273] HK and C1-INH activity were also decreased, but there were increased levels of C1-INH antigen. Decreased levels of prekallikrein also have been documented in patients with septicemia due to viruses, fungi, or Rickettsia. Patients with Rocky Mountain Spotted Fever have decreased prekallikrein levels but increased kallikrein-C1-inhibitor complexes.[274] Because, kallikrein-C1-inhibitor complexes are cleared rapidly in most cases of septic shock,[275] we developed a sandwich enzyme-linked immunosorbent assay for $\alpha_2$M-Kal complexes and found that, in septicemic hypotension, but not in septicemia alone, $\alpha_2$M-Kal complexes were elevated.[276]

None of the studies noted above indicated whether activation of the contact system is an early event or whether it is related to complications of sepsis such as hypotension and multiple organ failure. To address this question, normal human volunteers received a low dose of *E coli* endotoxin (0.4 ng/kg body weight). These individuals developed a flu-like illness associated with a hyperdynamic cardiovascular state lasting 24 hours.[277] Functional prekallikrein levels were significantly lower in the endotoxin group as compared with controls at 2 hours after infusion and remained low throughout the rest of the experimental protocol at 5 and 24 hours. The concentration of $\alpha_2$M-Kal complexes was significantly elevated fourfold in the endotoxin-treated group by 3 hours and fivefold by 5 hours, with a decrease to normal in the circulating levels of complexes by 24 hours. Thus, a low dose of endotoxin can induce a prolonged state of contact activation.

To prove that contact activation is related to either shock or DIC, animal studies were performed (Table 4). In an established experimental baboon model of bacteremia, two concentrations of *E coli* were used to produce lethal and nonlethal hypotension. The lethal group developed irreversible hypotension, which significantly correlated with both the decline in functional levels of HK and an increase in $\alpha_2$M-Kal complexes.[278] The nonlethal group experienced reversible hypotension, a less striking decline in HK, and only a slight elevation in $\alpha_2$M-Kal.[278] Irreversible hypotension correlated with activation of the contact system. Further investigations were performed to address the causality of contact activation in shock and hypotension. An MoAb to human factor XII that is able in vitro to inhibit factor XII coagulant activity in baboon plasma by 60% and slow kininogen cleavage in dextran sulfate-activated baboon plasma was infused

**Table 4. Experimental Diseases States for Which Contact System Activation Is Pathogenetic**

| Disease* | PK | | HK | | C1 INH | | XI | XII | α2M-Kal Complexes | C1 INH-Kal Complexes |
|---|---|---|---|---|---|---|---|---|---|---|
| | Act. | Ant. | Act. | Ant. | Act. | Ant. | (Act.) | (Act.) | (Ant.) | (Ant.) |
| Baboon sepsis[278,279] | D | — | D | — | — | — | I | — | I | — |
| Rat arthritis[284,286] | D | — | D | — | — | — | D | — | — | — |
| Rat enterocolitis[286,287] | D | — | D | — | — | — | D | — | — | — |

In these conditions, a role for contact activation in the pathogenesis of these disorders is shown by the finding that specific inhibitors of the contact system blocked the progression of the disease.

Abbreviations: D, decreased; I, increased.

* The numbers after the disease category are references.

into the lethal baboon group 30 minutes before the *E coli*.[279] Although the decline of factor V, fibrinogen, and platelets were similar in both groups and prekallikrein values were normal, there was a marked decline in HK in the untreated group, reaching 40% of the baseline levels by 300 minutes. In the group treated with the MoAb to factor XII, the HK remained stable and was significantly higher (110% of baseline) at 360 minutes. Furthermore, in the untreated group, there was a progressive increase of $\alpha_2$M-Kal complexes, which was highly significant and was completely blocked by the MoAb in the treated group. A significant decline of mean systemic arterial pressure was observed in both groups of animals between 60 and 120 minutes. A Kaplan-Meier plot showed that treated animals survived significantly longer than untreated animals. Inhibition of contact system activation with an MoAb to factor XII modulated the hypotension.[279]

### *Cardiopulmonary Bypass*

Clinical cardiopulmonary bypass (CPB) is performed on more than 350,000 Americans each year (Table 3). During CPB, there is extensive contact between blood anticoagulated with heparin and the synthetic surfaces of the extracorporeal circuit. Blood cell interactions and plasma protein alterations prolong the bleeding time, increase postoperative blood loss, and trigger a chemical and cellular whole body inflammatory response. Extracorporeal circulation has been associated with both qualitative and quantitative alterations of platelets, neutrophils and complement and contact systems. Heparin, which markedly accelerates inactivation of FXa and thrombin by antithrombin III, exhibits minimal enhancement of the inactivation of FXIIa and FXIa in CPB.[280] In simulated CPB, there is a significant increase in kallikrein-C1-INH complex formation.[281] The simultaneous formation of C1-C1-INH complexes suggested that factor XII activation occurred, which, in turn, activated both kallikrein and C1, thus triggering both the contact and classical complement pathways.[281] Further studies showed that aprotinin, an inhibitor of both plasmin and plasma kallikrein, reduced blood loss after cardiac operations and decreased the elevated postoperative bleeding time. In a simulated extracorporeal bypass model, in which no plasmin is found, aprotinin decreased both kallikrein-C1-inhibitor and C1-C1-inhibitor complexes, resulting in a marked inhibition of the release of neutrophil elastase.[282] Similar results were obtained with specific kallikrein inhibitors, Bz-Pro-Phe-boroArg-OH, Arg15-aprotinin, and Ala357-Arg358-$\alpha_1$-protease inhibitor.[283]

### *Experimental Arthritis in Genetically Susceptible Rats*

The role of the kallikrein-kinin system in inflammatory arthritis was investigated by a model of acute and chronic relapsing arthritis induced by intraperitoneal injection of proteoglycan-polysaccharide from group A streptococci (PG-APS) into rats (Table 4).[284] The mean joint diameter peaked at a maximum value of 8 at day 3, indicating an acute arthritis. After a decrease in the volume of the joint on days 9 through 12, the joint diameter spontaneously increased beginning at day 15 and then progressed with waxing and waning of individual joints, indicating reactivation leading to chronic synovitis and joint erosion. An increase in the acute-phase protein, T-kininogen, splenic enlargement, and the development of the anemia of chronic disease were consistently associated with the arthropathy. HK in rat plasma decreased on days 1, 5, and 15, but not at 30 minutes, day 23, or day 45. There is a striking inverse correlation between HK concentration and joint enlargment on day 5, with $r = .85$. Prekallikrein levels were significantly lower in PG-APS–injected animals compared with controls. Prekallikrein levels decreased as early as 30 minutes after injection, and the levels remained low throughout the experimental protocol. Further experiments showed that, when the rats were injected with PG-APS and received a specific, potent oral plasma kallikrein inhibitor, P8720 or Bz-Pro-Phe-boroArg-OH ($K_i = 0.15$ nmol/L, $K_{assoc} = 1.6 \times 10^6$ mol/Ls$^{-1}$), there was a significant decrease (61%) in joint swelling at 49 hours, with a disappearance of most of the dense infiltration of neutrophils and mononuclear cells.[285] Furthermore, there was no decrease in plasma HK. Lastly, the anemia, the increase of TK, and the splenic weight increase were largely inhibited. These data indicated that contact system activation mediates the arthritis and that its inhibition ameliorates all the manifestations of this disorder.

### *Acute and Chronic Enterocolitis in Genetically Susceptible Rats*

Further investigations examined the role of the contact system in inflammatory bowel disease using a model of acute and chronic enterocolitis induced by subserosal injection of PG-APS into the wall of the distal ileum and cecum (Table 4).[286] Acute intestinal inflammation in the Lewis rat and the Buffalo rat are characterized by edema, hemorrhage, thickening of the bowel wall, and mesentery and adhesions. However, genetically susceptible Lewis rats, but not resistant Buffalo rats, spontaneously develop chronic enterocolitis

with dense adhesions, thickening of intestinal wall, serosal nodules, enlarged mesenteric lymph nodes, histological changes consisting of mononuclear cell infiltration and crypt abscesses, and a markedly elevated intestinal myeloperoxidase that persists for at least 16 weeks. Furthermore, a marked disparity existed in the incidence of extraintestinal manifestations between Lewis and Buffalo rats. Arthritis and hepatic granulomas occurred in 73% of Lewis rats examined 14 days or more after PG-APS injection; however, only 4% of Buffalo rats developed hepatic granulomas, and arthritis was not evident in Buffalo rats. T-kininogen, the major acute-phase protein in the rat, increased in the Lewis rat but not in the Buffalo rat.

PK levels were significantly lower in PG-APS–treated Lewis rats compared with controls or Buffalo rats during both acute and chronic phases of inflammation. HK levels were significantly decreased in PG-APS–treated Lewis rats on days 5 and 42 from its respective control group. Buffalo rats injected with PG-APS had stable plasma HK concentrations at all time points. Treatment with the specific, oral plasma kallikrein inhibitor, Bz-Pro-Phe-boroArg-OH, in the acute phase of enterocolitis[287] in the Lewis rat decreased the increase of joint diameter, the gross gut score, and intestinal myeloperoxidase activity and prevented the decrease of factor XI and HK. The kallikrein inhibitor could block bradykinin release and, thus, pain, swelling, and vasodilation as well as neutrophil activation. Moreover, because factor XII can stimulate IL-1 expression, IL-1 induces IL-6 expression, and an MoAb to factor XIIa blocks IL-6 release, one can surmise that contact activation is involved in the acute and chronic phases of the enterocolitis.[204,288] Demonstration of a pathogenetic role of the kallikrein-kinin system in experimental enterocolitis, arthritis, and related systemic inflammation suggest a similar role in idiopathic human intestinal and joint inflammation and raise the possibility that selective kallikrein inhibitors may be useful in disorders such as Crohn's disease and rheumatoid arthritis.

### *Thrombosis Risk Factor*

Independent of the multiple mechanisms by which kininogens are selective antithrombins that modulate $\alpha$-thrombin's activation of platelets and endothelial cells in vitro and the proposed physiologic mechanism for cellular fibrinolysis due to assembly of HK and PK on endothelium, clinical observation suggests that deficiencies of these proteins may be additive risk factors for thrombosis. John Hageman (Hageman factor) and Mayme Williams (Williams trait) both died of pulmonary emboli. Although both of these patients had other reasons for pulmonary emboli, their contact protein deficiencies may have contributed an additional risk factor. Certainly, their deficiencies did not protect them from thrombosis. Numerous other clinical studies also 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,[289-293] an increased incidence of factor XII deficiency in patients with venous thrombosis, and acquired thrombotic disorders such as myocardial infarction[294] and re-thrombosis of coronary arteries after thrombolytic therapy.[295] Although these studies are interesting, contact protein deficiencies are relatively rare occurrences. It will require careful prospective investigations with age- and sex-matched controls to determine whether these factors contribute to the ever enlarging list of inherited risk factors for thrombosis.

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