Monoclonal Antibody to Human High-Molecular-Weight Kininogen Recognizes Its Prekallikrein Binding Site and Inhibits Its Coagulant Activity

By Sesha R. Reddigari and Allen P. Kaplan

We developed a mouse monoclonal antibody (MoAb 115-21) to human high-molecular-weight kininogen (HK) that recognizes its prekallikrein binding site (residues 565 through 595 of HK). The corresponding synthesized 31-amino acid peptide (peptide IV) was recently shown to retain native HK’s prekallikrein binding property. The same peptide bound factor XI also, although less avidly. Our MoAb recognizes purified HK, peptide IV, and the light chain moiety of HK (where the peptide IV resides), as shown by enzyme-linked immunosorbent assay (ELISA) and Western blotting experiments. The apparent dissociation constant for the HK and MoAb 115-21 interaction was 2.2 nmol/L. It does not recognize low-molecular-weight kininogen (LK) with which HK shares its heavy chain moiety or any antigens in human plasma congenitally deficient in kininogens. The binding of MoAb 115-21 to purified light chain of HK was competitively inhibited by peptide IV. In addition, the antibody inhibits HK-dependent clotting activity of normal human plasma and dextran sulfate-mediated activation of prekallikrein in plasma and retards cleavage of HK in normal plasma after contact activation with dextran sulfate. Also, purified Fab fragments of MoAb 115-21 inhibited the HK-dependent coagulant activity and dextran sulfate-mediated prekallikrein activation in normal plasma. Since the kd for HK-MoAb 115-21 interaction is ten times lower than that of HK-prekallikrein, our data suggest that binding of MoAb 115-21 to HK’s peptide IV site increases the free prekallikrein concentration in plasma and thus results in the decreased efficiency of factor Xlla-mediated activation of prekallikrein. Decreased levels of kallikrein thus formed may be responsible for the inhibition of HK-dependent clotting activity and the decrease in rate and extent of HK cleavage in normal plasma on contact activation with dextran sulfate. MoAb 115-21 may thus prove very useful, especially with its high affinity for HK, in further delineation of the role of HK and prekallikrein in contact activation and kinin-related human pathology.

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From the Division of Allergy, Rheumatology, and Clinical Immunology, Department of Medicine, Health Sciences Center, SUNY, Stony Brook. Synthesis was performed on a Biosearch SAM TWO peptide synthesizer with standard programs and t-butoxycarbonyl amino acids on 4-methylbenzhydrylamine resin (Biosearch, San Rafael, CA). The peptide was further purified by gel filtration on Biogel P200 (BioRad, Richmond, CA) columns in PBS. Protein and peptide concentrations were determined according to the method of Lowry et al. 13

Plasma. Normal plasma was prepared by pooling blood from ten normal volunteers by venipuncture. Blood was drawn into syringes containing 40% citrate (final citrate concentration 0.4%). Immediately after the cells were centrifuged, plasma was aliquoted, quick frozen, and stored at −70°C until used. Kininogen-deficient plasma (Williams trait), and factor XII-deficient plasma were obtained from George King Biomedical, Overland Park, KS. All incubations involving plasma were performed in 1.5-mL plastic microfuge tubes.

Coagulation assays. HK and factor XII-deficient coagulant activities were determined as described previously. 14 Coagulant activity of the test samples was quantified against a standard curve obtained from serially diluted normal plasma.

MoAbs. MoAbs to HK were prepared as described previously 15 according to the method of Lipsch et al. 16 Antibodies were purified from mouse ascites fluid by ammonium sulfate precipitation and diethylaminoethanol (DEAE)-cellulose (DE-52; Whatman, Maidstone, England) chromatography. HK-dependent clotting activity was measured as inhibition of factor Xlla-mediated activation of HK in plasma and dextran sulfate-mediated activation of HK in normal plasma after contact activation with dextran sulfate. The publication costs of this article were defrayed in part by charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

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0006-4971/89/7402-0005$3.00/0

Peak fractions containing anti-HK activity were pooled, concentrated, and stored at −70°C. MoAbs 115-21 and 371-28 are directed against the light chain of HK, and MoAb 11-2 is directed against the heavy chain of HK. MoAb 115-21 for kD determinations was purified from hybridomas cultured in serum-free media in large volumes (1L), concentrated, and further purified by DEAE chromatography as described above. Antibody isotyping was performed with a commercial kit obtained from Southern Biotechnologies, Birmingham, AL.

**Fab fragments from MoAb 115-21.** Ten milligrams MoAbs 115-21 purified from serum-free media were digested with 100 µg papain (Sigma, St Louis) as described by Veloso et al. After the digestion, the digest was extensively dialyzed against 0.01 mol/L Tris-HCl + 0.02% sodium azide, 1 mmol/L EDTA and applied to a 1 × 5-cm DE-52 (Whatman) column. The flowthrough and wash were collected and concentrated to 400 µg/mL and stored at −20°C. The purity of Fab fragments was checked by enzyme-linked immunosorbent assay (ELISA) (Table 1). Microtiter plates were coated with 100 µL 0.2 µg/mL purified whole IgG (115-21) or purified Fab fragments overnight at 4°C. After blocking with bovine serum albumin (BSA), the wells were incubated with alkaline phosphatase conjugated to either goat anti-mouse IgG(Fc) or rabbit anti-mouse IgG(Fab) antibodies (Jackson Immunoresearch, West Grove, PA) and developed as described below under ELISA.

**ELISA.** ELISA was performed by standard procedures with 96-well microtiter plates (Immulon-2 plates; Dynatech, Alexandria, VA). Wells were coated with the proteins in 0.05 mol/L NaHCO₃ buffer, pH 9.6, was added to the wells, and absorbance was read at 405 nm on a Dynatech Automatic ELISA reader.

**Iodination of proteins.** MoAb 371-28 to the LC of HK (200 µg in 200 µL PBS) was labeled with 0.1 mCi ¹²⁵I (Na¹²⁵I was from Amer sham, Arlington, Heights, IL) with (two) iodobeads (Pierce 405 nmol/L) for four hours. Nonspecific binding was measured by adding 2% radioimmunoassay (RIA) grade bovine serum albumin to the sample and the acrylamide polymerization mixtures included in the sample and the acrylamide polymerization mixtures included in the sample and the acrylamide polymerization mixtures.

**Activation of plasma.** Dextran sulfate activation of plasma was performed on ice according to the method of Van der Graaf et al. ¹⁷ Plasma kallikrein activity. Plasma was incubated with or without MoAbs at room temperature for one hour, chilled on ice, and then activated with 20 µg/mL dextran sulfate at 0°C, and kallikrein activity (initial rate) at various incubation times was determined with benzoyl-Pro-Arg-para-nitroanilide-acetate (Chromozym-PK) as substrate according to the method of Silverberg and Kaplan. ¹⁸ Initial rates were calculated on an automated system in which a Gilford Spectrophotometer equipped with a cell temperature controller was interfaced with an Apple IIe computer.

**Cleavage of HK in plasma after contact activation.** Normal plasma was incubated with or without MoAbs at room temperature (Ag/MoAb ratio = 1:10, wt/wt, assuming a plasma HK concentration of 80 µg/mL). At the end of the incubation period, samples were chilled at 0°C for at least 15 minutes. Dextran sulfate was then added to a final concentration of 20 µg/mL, and the incubation was continued on ice; 10-µL aliquots were withdrawn at indicated time intervals, denatured immediately by addition of 20 µL 5 x SDS-gel sample buffer plus 70-µL distilled water, and heated for five minutes in boiling water. Samples equivalent to 1 µL plasma were subjected to SDS-PAGE. After electrophoresis, the gels were electrophotoblotted and the blots were probed with ¹²⁵I-labeled MoAb 371-28; the resulting blots were subjected to autoradiography.

**PAGE.** Discontinuous PAGE (7.5%) of plasma under nondissociating conditions was performed according to the method of Davis ¹⁹ on a BioRad Protein II slab gel apparatus. Tween-20 (0.05%) was included in the sample and the acrylamide polymerization mixtures for better separation of plasma proteins under nondissociating conditions. PAGE (7.5%) under dissociating conditions (nonreducing) in the presence of SDS was performed according to the method of Laemmli. ²⁰

**Immunoblotting.** Proteins were electrophoretically transferred to nitrocellulose sheets (Schleicher and Schuell, Keene, NH, pore size 0.1 µm) according to the method of Towbin et al. ²¹ in a BioRad Transblot apparatus for 16 hours at 30 V followed by one hour at 60 V. The nitrocellulose sheet was air-dried for ten minutes, and the unoccupied sites were blocked with 5% nonfat dry milk in PBS containing 0.01% thimerosal and 0.001% antifoam A (BLOTTO) ²² for one hour by gentle rocking. Next, the blots were treated with either unlabeled MoAb 115-21 (5 µg/mL) or ¹²⁵I-labeled MoAb 371-28 at 3 µg/mL in BLOTTO for two hours. Blots treated with 115-21 were then washed for 30 minutes with BLOTTO three times (ten minutes each wash), treated with goat anti-mouse IgG-F(ab′)₂ (Jackson Immunoresearch) at a dilution of 1:1,000 in BLOTTO, and washed for one hour with BLOTTO four times (15 minutes each wash). Bands were developed with a substrate mixture containing 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (kits available from Kirkegaard & Perry, Gaithersburg, MD.) Blots treated with ¹²⁵I-labeled MoAb 371-28 were washed with BLOTTO for one hour (four changes), dried, and then subjected to autoradiography using preflashed Kodak XOMAT-AR film (Kodak, Rochester, NY) and Dupont Cronex intensifying screens.

**Dissociation constant determination.** For the kd for HK, MoAb 115-21 interaction was determined by direct binding measurements as described by Schmaier et al. ²² Microtiter plates (Costar, Cambridge, MA, Strip Plate-8) were coated with 100 µL HK (20 mmol/L) in PBS at 4°C overnight. After being washed with PBS-Tween, wells were blocked with 2% radioimmunooassay (RIA) grade BSA in PBS (200 µL) for four hours at 37°C. The wells were then washed with PBS-Tween and incubated with the purified and ¹²⁵I-labeled MoAb 115-21 in PBS-Tween (100 µL; 0.4 to 25.6 nmol/L) for four hours. Nonspecific binding was measured by incubations in the presence of 50-fold molar excess of unlabeled antibody. Data were analyzed according to the method of Scatchard. ²⁴

**RESULTS**

**Anti-HK MoAb 115-21.** MoAb 115-21 was developed as described in the Materials and Methods section by previously
INHIBITORY MONOCLONAL ANTIBODY TO KININOGEN

published procedures. Balb/c mice were immunized with purified HK. MoAb 115-21 is one of the 22 anti–light-chain antibodies previously described. The antibody was isotyped with a commercial ELISA kit as described in the Materials and Methods section: it was an IgG1-λ.

Specificity of the MoAb. Proof of specificity was obtained by ELISA experiments. Microtiter plates were coated with peptide IV, purified LC or alkylated LC of HK, and incubated with the anti–light-chain MoAbs 371-28, 115-21, or anti–heavy-chain MoAb 11-2 (both 371-28 and 11-2 were described previously and were used in the present study as controls). As shown in Table 2, 115-21 recognized the peptide IV and the purified LC of HK, whereas the other anti–LC MoAbs, 371-28, recognized only purified LC. The anti–HC MoAb, 11-2, recognized only the LC since the HC of HK is identical to the HC of LK.

Further proof of specificity was obtained by Western blotting experiments. Normal plasma and kininogen-deficient plasma were subjected to discontinuous PAGE under nondissociating conditions and immunoblotted with MoAb 115-21 as described in the Materials and Methods section. As shown in Fig 1, 115-21 recognized HK in normal plasma and did not recognize any antigen(s) in kininogen-deficient plasma.

Dissociation constant determination. The affinity of MoAb 115-21 to HK was determined as described in the Materials and Methods section by direct binding method. This method of determining the affinities of MoAbs and their antigens was established previously and yielded dependable dissociation constants. Our data indicate that the HK–MoAb 115-21 interaction was very tight, with a Kd of 2.2 nmol/L (Fig 2).

Structure confirmation of peptide IV. Sequence of the peptide used in our experiments was published previously, and was synthesized. The primary structure of this peptide was verified by resequencing. As shown in Table 3, sequence of the first 28 of the 31 residues in the peptide was identical; further comparison during resequencing was rendered difficult by the presence of proline in position 30. However, amino acid composition analysis of the same sample was identical to that of peptide IV (data not shown).

To confirm the functional identity of our peptide with peptide IV, we incubated normal plasma with increasing amounts of our peptide and determined its effect on HK-dependent coagulant activity of normal plasma as described in the Materials and Methods section. Data shown in Fig 3 indicate that the peptide clearly inhibited HK’s coagulant activity. Together, these data indicate that MoAb 115-21 recognizes peptide IV of Tait and Fujikawa.

Inhibition of binding of MoAb 115-21 to LC of HK by peptide IV. We next examined the effect of incubating the MoAbs with increasing concentrations of peptide IV on their binding to the purified LC of HK. Microtiter plates were coated with purified alkylated LC and, after blocking with R1A-grade BSA, the wells were incubated with a preincubated mixture of indicated amounts of peptide IV and MoAbs 115-21 and 371-28 at 3 μg/mL in PBS-0.05% Tween-20. Linear regression plots shown in Fig 4 clearly indicated that the peptide IV inhibited binding of MoAb 115-21 but not of MoAb 371-28 to the LC of HK.

Inhibition of HK-dependent coagulant activity of normal plasma by MoAb 115-21. Next we examined the effect of antibodies on HK-dependent coagulant activity of normal plasma. Normal plasma was incubated with MoAbs 115-21, 371-28, or 11-2 at room temperature; its HK-LC-dependent coagulant activity was then determined as compared with a control in which normal plasma was incubated with PBS. Although the inhibition by the anti-LC antibody 371-28 was

Table 2. Specificity of Anti-HK MoAbs by ELISA

<table>
<thead>
<tr>
<th>Antigen</th>
<th>371-28</th>
<th>11-2</th>
<th>115-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide IV</td>
<td>0.046</td>
<td>0.022</td>
<td>0.92</td>
</tr>
<tr>
<td>LK</td>
<td>0.033</td>
<td>0.613</td>
<td>0.356</td>
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<tr>
<td>LC of HK</td>
<td>1.21</td>
<td>0.062</td>
<td>0.532</td>
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</table>

Microtiter plates were coated with peptide IV (at 2 μg/mL), LK (0.2 μg/mL), or purified light chain of HK (0.2 μg/mL) at room temperature for 72 hours. After blocking, wells were incubated (n = 4) with MoAbs and developed with goat anti-mouse IgG-alkaline phosphatase conjugate as described in the Materials and Methods section. Numbers are mean absorbance (SD) at 405 nm.
only 25%, inhibition by 115-21 was much higher, to 73% as shown in Table 4. The anti-HC MoAb did not inhibit the kininogen-dependent coagulant activity.

In a separate experiment, normal human plasma (NHP) was incubated with 0, 1.98, 3.96, 5.94, and 7.92 μmol/L purified Fab fragments of MoAb 115-21 overnight at room temperature, and its HK-dependent coagulant activity was determined. As shown in Fig 5, addition of Fab fragments drastically inhibited HK coagulant activity. To show the specificity of this inhibition by Fab fragments, we determined factor XII coagulant activity in the same samples. As shown in Fig 5, the samples retained 100% of factor XII activity, whereas only 12% of HK-dependent activity was retained.

**Effect of MoAbs 115-21 and 371-28 on activation of prekallikrein and cleavage of HK in activated plasma.** Next, we incubated normal plasma with these antibodies and then activated it with 20 μg/mL dextran sulfate. Aliquots were withdrawn at various time intervals, denatured in SDS-PAGE sample buffer, and immunoblotted after SDS-PAGE under nonreducing conditions. 125I-Labeled MoAb 371-28 was used as the probe. HK (120 Kd; Fig 6A, lane 1) was cleaved in a sequential manner in the absence of MoAb 115-21 (Fig 6A); the first cleavage product of HK at 103 kd appeared between four and eight minutes (lane 4). All HK was converted to 103 kd by eight minutes (lane 5). As activation continued, traces of the second cleavage product at 96 Kd appeared at ten minutes (lane 6); this latter band continued to increase in intensity while the 103 kd band decreased in intensity with further incubation (Fig 6A, lanes 6 through 9). Activation in the presence of MoAb 115-21 appeared to retard cleavage of native HK (Fig 6B). The 103-kd band did not appear until 15 minutes (lane 7), and only traces of the 96-Kd band were observed at 20 minutes.

The above data closely parallel activation of prekallikrein in plasma by dextran sulfate. Normal plasma was incubated with or without MoAbs 115-21 or 371-28 and activated with dextran sulfate as described above. Aliquots were withdrawn at various time intervals and assayed for kallikrein activity with chromozym-PK as substrate. The same substrate was used under similar conditions by Kluft. The data shown in Fig 7 indicate that without antibodies, plasma kallikrein activity reached a peak at six to seven minutes after an initial lag period of two minutes and then started to decrease gradually. This evolution of kallikrein activity coincided with initial cleavage of HK in immunoblotting experiments (Fig 6A, lane 4). With MoAb 115-21 added, the initial lag period for activation of prekallikrein was much longer than in the control. Kallikrein first appeared at ~12 minutes, peaked at

![Graph](https://via.placeholder.com/150)

**Fig 3.** Inhibition of HK-dependent coagulant activity by peptide IV; 25 μL normal plasma diluted 1:10 was incubated with 25 μL 0, 20, 40, 60, 80, and 100 μg/mL peptide IV in TBS at 25°C for 30 minutes, and 50 μL of the mixture was assayed for HK clotting activity. Plot shows activity from triplicate experiments (SD) v microgram of peptide per assay.

![Graph](https://via.placeholder.com/150)

**Fig 4.** Effect of peptide IV on binding of MoAbs to the light chain of HK: 96-well microtiter plates were coated with 100 μL purified light chain (0.2 μg/mL) for 24 hours at 25°C, blocked, and treated for two hours at 37°C with 100 μL preincubated mixture of MoAbs (3 μg/mL) and indicated amounts of peptide IV in PBS-Tween. After washing with PBS-Tween, bound MoAb was determined with goat anti-mouse IgG-alkaline phosphatase. Mean of triplicate determinations is plotted by linear regression with SD. MoAb 371-28 (○); MoAb 115-21 (●).

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**Table 3. Amino-Acid Sequence of the Peptide**

<table>
<thead>
<tr>
<th>Sequence</th>
<th>(Synthesized for us)</th>
</tr>
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<td>S-D-D-W-I-P-D-I-Q-T-D-P-N-G-L-S-F-N-P-I-S-D-F-P-D-T-T-S-P-K</td>
<td>(Tait and Fujikawa)</td>
</tr>
</tbody>
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**Fig 5.** Inhibition of HK-dependent coagulant activity by peptide IV; 25 μL normal plasma diluted 1:10 was incubated with 25 μL 0, 20, 40, 60, 80, and 100 μg/mL peptide IV in TBS at 25°C for 30 minutes, and 50 μL of the mixture was assayed for HK clotting activity. Plot shows activity from triplicate experiments (SD) v microgram of peptide per assay.
activity did not reach the same maximum as in the control. Addition, with MoAb 15-21, the initial rate of kallikrein activity in normal plasma with MoAb 15-21 added (Fig 6B, lane 7). In this coincided with initial cleavage of native HK in normal plasma. With the other anti-LC MoAb 37-28, the initial lag time for activity in normal pooled plasma was incubated with 35 µg purified IgG of MoAb 15-21 or purified Fab fragments of I15-21 in a total of 100%. Data are the mean of triplicate measurements.

Normal plasma (25 µL) was incubated with 35 µg purified MoAbs in 50 µL PBS for two hours at room temperature and assayed for HK-dependent coagulant activity as described in the Materials and Methods section. Numbers in parentheses indicate SD from triplicate determinations.

In a separate experiment, we tested the effect of purified Fab fragments of MoAb 115-21 on evolution of kallikrein activity in normal plasma on contact activation: 50 µL normal pooled plasma was incubated with 35 µg purified MoAb 115-21 or purified Fab fragments of 115-21 in a total volume of 100 µL overnight and then activated with dextran sulfate. Aliquots were assayed at seven minutes for kallikrein activity. Data shown in Fig 8 indicate that all IgG of MoAb 115-21 was completely inhibitory (>94%) and the Fab fragments were inhibitory to 62% as compared with the control. Kallikrein activity in similarly activated HK-deficient plasma was minimal both with and without Fab fragments, indicating that HK is essential for generation of dextran sulfate-mediated kallikrein activity in normal plasma.

DISCUSSION
We developed a MoAb to HK (MoAb 115-21) directed against the peptide region spanning residues 565 through 595 of HK (total residues in HK = 626), corresponding to residues 194 through 224 of its light-chain moiety. This peptide region was recently shown to be responsible for binding of HK to prekallikrein and factor XI. Chemically synthesized peptide IV bound to prekallikrein with the same affinity as that of native HK.

The primary structure of the peptide synthesized for our experiments was compared with that of the original peptide IV. Sequence analyses indicated that sequence of the first 28 residues (of 31) was identical to that of peptide IV (Table 3). Sequence of residues 29 through 31 could not be compared in our system due to the presence of proline at position 30. However, the amino-acid composition of our peptide was identical to that of Tait and Fujikawa's peptide IV. The identity of the two sequences was further established by inhibition of HK-dependent coagulant activity of normal plasma by the peptide used in our studies (Fig 3).

As expected, MoAb 115-21 recognized purified alkylated LC of HK and peptide IV, but not purified LK, which shares the HC moiety with HK (Table 2). The other anti-LC MoAb (371-28) did not recognize peptide IV, indicating that it is directed against a different epitope on the light chain. Competition binding experiments confirmed the nonidentity of LC epitopes recognized by these two antibodies (Fig 4). Further proof of specificity of 115-21 was obtained by immunoblotting of normal plasma and kininogen-deficient plasma after PAGE under nondissociating conditions. As shown in Fig 1, the antibody recognized HK in normal plasma and no antigens were recognized in kininogen-deficient plasma. These observations indicate that 115-21 is directed against the prekallikrein binding site of HK. Since PAGE of either purified HK or normal plasma under dissociating conditions (with SDS) resulted in poor recognition of HK by 115-21 on Western blots (data not shown), we suspect that 115-21 recognizes the prekallikrein binding site of HK in its nondenatured conformation. MoAb 371-28, on the other hand, recognizes HK very well after SDS-PAGE. This interaction of MoAb 115-21 with the native prekallikrein binding site of HK may be responsible for its inhibition of HK-dependent coagulant activity of normal plasma (Table 4). MoAb 371-28 inhibited HK coagulant activity by only 27%, whereas MoAb 11-2 (anti-HC) was not inhibitory at all (Table 4). Yet the interaction of MoAb 371-28 with HK was not inhibited by peptide IV (Fig 4). Thus, the effects of MoAb 371-28 may be steric. On the other hand, such simple steric effects could be precluded as in our system due to the presence of proline at position 30. However, the amino-acid composition of our peptide was identical to that of Tait and Fujikawa's peptide IV. The identity of the two sequences was further established by inhibition of HK-dependent coagulant activity of normal plasma by the peptide used in our studies (Fig 3).
In addition to its effect on the coagulant activity of HK, MoAb 115-21 appears to influence the cleavage of HK in normal plasma after contact activation. The role of dextran sulfate in the activation process was first investigated by Kluft and van der Graaf et al., who showed that factor XII, HK, and prekallikrein are essential for the activation. Optimal conversion of normal plasma with dextran sulfate results in a rapid and complete conversion of prekallikrein to kallikrein, and in immunoblotting experiments with MoAb 371-28, we previously showed that kallikrein-digested purified HK yields a similar pattern on cleavage by purified kallikrein.

In addition to its effect on the coagulant activity of HK, MoAb 115-21 seems to affect the cleavage of HK in normal plasma after contact activation. Normal plasma was incubated without (A) and with (B) MoAb 115-21 as described in the Materials and Methods section and activated with dextran sulfate. Samples were immunoblotted and autoradiographed.

Fig 6. Effect of MoAb 115-21 on kinetics of HK cleavage in normal plasma after contact activation. Normal plasma was incubated with MoAb 115-21 as described in the Materials and Methods section and activated with dextran sulfate. Samples were immunoblotted and autoradiographed.

Fig 7. Effect of MoAbs 115-21 and 371-28 on activation of prekallikrein in normal plasma on contact activation. Normal plasma (400 µL) was incubated with 12 Ci of MoAbs 115-21 (control) or 12 Ci of MoAbs 371-28 for one hour at 25°C and then placed on ice for 15 minutes. Aliquots (5 µL) were withdrawn at indicated times and assayed for kallikrein activity with Benz-Pro-Phe-Arg-para-nitroanilide as substrate. Initial rates, represented by absorbance change at 405 nm/min, are plotted against contact activation time.

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INHIBITORY MONOCLONAL ANTIBODY TO KININOGEN

Kininogens are identical with α,-cysteine protease inhibitors.

Kotnik M, Turk V, Kellerman J, Lottspeich F: Human plasma and L-thiol protease inhibitors: Inhibition of papain and cathepsins B, H, Kominami Y, Methods section. triplicate aliquots were assayed for kallikrein with dextran sulfate as described in the Materials and minutes;LL overnight at 25°C. Samples were then (B) or 35 pg Fab of MoAb 1 1 5-21 (C and E) in a with the light chain of kinin-free high molecular weight kininogen. J gil normal plasma (A. B. and C) or HK-deficient plasma (D and E) were incubated with 0 g (A and D) or 35 sg ofplasma. [62x74]I-r

yields two cleavage products under nonreducing conditions, at 103 Kd and 96 Kd, respectively. 3 In addition to confirming these observations, our current data suggest that MoAb 115-21 interferes with this cleavage pattern (Fig 6A and B) perhaps by inhibiting binding of prekallikrein to HK in plasma, which would slow down its subsequent activation to kallikrein by factor X1a. This was confirmed by direct assays for liberation of kallikrein in normal plasma on contact activation with MoAb 115-21 added. Kallikrein activity reached a peak at six to eight minutes without the MoAb whereas in its presence no kallikrein activity occurred at that time interval (Fig 7). These observations agree with those of van der Graaf et al., 14 who reported complete inhibition of prekallikrein activation in plasma by goat anti-HK polyclonal antibodies for seven minutes after contact activation. Longer times of contact activation in the presence of those antibodies were not reported in that communication. 15 With MoAb 115-21, kallikrein activity begins to appear at 12 minutes and peaks at 16 minutes but does not reach the same maximum as in the control (Fig 7). Since purified Fab fragments of this inhibitory antibody also significantly inhibited kallikrein activity of normal plasma on contact activation (Fig 8), simple steric effects may be ruled out as the cause of such inhibition.

Most plasma prekallikrein circulates as a complex with HK under normal conditions (kd for the HK-prekallikrein interaction = 20 nmol/L), 11 and complex formation is necessary for the juxtaposition of prekallikrein and factor XII on the activating surface to achieve optimal reciprocal activation of both factor XII and prekallikrein during contact activation. 26-31 Addition of MoAb 115-21 to normal plasma may be shifting the HK + prekallikrein = HK-prekallikrein equilibrium to favor free prekallikrein (kd of HK: MoAb 115-21 = 2.2 nmol/L). This would reduce the efficiency of the contact activation mechanism, resulting in a decrease in the rate of prekallikrein activation, which would result in turn in a decreased rate of HK cleavage; ie, addition of MoAb 115-21 to normal plasma could be simulating HK deficiency, since dextran sulfate activation of HK-deficient plasma yields only 8% of normal kallikrein activity. 17 Our control experiments confirmed this observation (Fig 8).

An anti-HK MoAb that (a) is directed against its prekallikrein binding site; (b) has a very low dissociation constant of 2.2 nmol/L; and (c) inhibits HK-dependent coagulant activity and surface-mediated activation of prekallikrein in whole plasma may prove very useful in further studies of the role of HK in contact activation and kinin-related pathology.

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Monoclonal antibody to human high-molecular-weight kininogen recognizes its prekallikrein binding site and inhibits its coagulant activity

SR Reddigari and AP Kaplan