Production and Characterization of a Murine Monoclonal Antibody Against a Heavy Chain of Hageman Factor (Factor XII)

By Hidehiko Saito, Toshiki Ishihara, Hisamitsu Suzuki, and Takeshi Watanabe

A murine hybridoma cell line that produces a monoclonal antibody to human Hageman factor (HF, factor XII) is described. The antibody (P 5-2-1) consists of mouse IgG\sub{2a}, heavy chains and \lambda light chains, selectively neutralizes HF procoagulant activity, and prevents the proteolytic cleavage of HF during contact activation in plasma. When HF is exposed to P 5-2-1 before the absorption of HF to kaolin, HF procoagulant activity is markedly inhibited. In contrast, P 5-2-1 does not interfere with HF activity after the adsorption of HF to kaolin. P 5-2-1 does not inactivate the prekallikrein-activating activity of 28,000-mol wt HF fragments (HF\_s). P 5-2-1 binds exclusively to the 40,000-mol wt portion of a heavy chain of HF and inhibits the adsorption of HF to negatively charged surfaces. P 5-2-1 immobilized on Sepharose can be used to deplete HF from normal human plasma. This immunoaffinity-depleted plasma is indistinguishable from congenital HF-deficient plasma and can be used as the substrate for HF procoagulant activity assay.

© 1985 by Grune & Stratton, Inc.

MATERIALS AND METHODS

Materials

The following reagents were obtained from sources as indicated: goat antibodies against mouse Fab were made in our laboratory and purified by an affinity column coupled with Fab fragment of mouse immunoglobulin G1; rabbit antibodies against subtypes of mouse heavy and light chains (Miles Lab, Elkhart, Ind); kaolin (Fisher Scientific Co, Fairlawn, NJ); calcium phosphate, chloramine T, and potassium thiocyanate (Wako Pure Chemicals Co, Japan); trypsin (230 U/mg protein; Millipore Corp, Freehold, NJ); lima bean trypsin inhibitor (LBTI, Worthington Biochem Corp, Freehold, NJ); N\textsuperscript{a}-succinimidyl 3-(2-pyridylthio)propionate (SPDP, Amersham International, England); CNBr-activated Sepharose 4 B (Pharmacia Fine Chemicals Inc, Piscataway, NJ); x-ray film (Fuji medical imaging film, type NC, Fuji Photo Film Co, LTD, Japan); polyethylene glycol (PEG, mol wt 4,000, Sigma Chemical Co, St Louis); RPMI 1640 culture medium (Flow Labs, Inc, MacLean, Va); fetal calf serum (FCS, Microbiological Assoc, Bethesda, Md). Other chemicals were reagent grade or better.

Monoclonal antibodies produced by hybridoma technology\textsuperscript{7} are a sensitive means for probing a specific structural epitope of a protein. In the past several years, a number of murine monoclonal antibodies against various clotting factors have been prepared.\textsuperscript{8-12} Monoclonal antibodies to HF, however, have not yet been reported. In the present paper, we describe the preparation and characterization of a murine monoclonal antibody against a heavy chain of human HF. In addition, we demonstrate the application of this antibody to the production of human plasma deficient in HF.

Isolation of Corn Hageman Factor Inhibitor (CHFI)

CHFI was prepared as reported except that the last step (isoelectric focusing) was omitted. The preparation showed a single component with an apparent mol wt of 14,000 on SDS-PAGE and inhibited the amidolytic activity of HF\_s.\textsuperscript{16}

Production of Monoclonal Antibodies

Murine monoclonal antibodies to human HF were prepared by a modification\textsuperscript{17} of the method of Köhl er and Milstein.\textsuperscript{7} In brief, female BALB/c mice were immunized by intraperitoneal injection...
of 10 μg of purified HF mixed with complete Freund's adjuvant on day 1, and 10 μg HF in incomplete Freund's adjuvant on day 16. On day 37, 20 μg HF was given intravenously. Three days later, immune mouse spleen cells (2.5 x 10^7) were fused with P3X63Ag8Ul mouse myeloma cells (2.3 x 10^7) in the presence of 1 mL 50% PEG-4,000. Cells were seeded in wells of 24-well culture plates (Nunc, Roskilde, Denmark) and grown on selective medium (hygromycin-aminopterin-thymidine [HAT] medium). After 12 days, supernatants were tested for reactivity against purified HF by a solid-phase radioimmunoassay using 125I-labeled anti-mouse Fab. Briefly, each well of 96-well plastic flexible plates was coated with purified HF radioimmunoassay using '25I-labeled anti-mouse Fab. Briefly, each well of 96-well plastic flexible plates was coated with purified HF (10 μg/mL) and blocked by 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Then, supernatants of hybridomas were added to the wells. After incubation at 4 °C overnight, wells were washed three times and 125I-labeled anti-mouse Fab (20,000 to 40,000 cpm per well) was added. After incubation at room temperature for four hours, the wells were again washed and the radioactivity of each plastic cup was counted by a Packard (Downers Grove, IL) autogamma-scintillation spectrometer.

The cells in the positive wells were subcloned by limited dilution and again screened for the production of antibodies to HF. Subcloned hybridoma cells secreting antibody to HF were injected intraperitoneally into BALB/c mice for the production of ascites fluid. Eleven cloned ascites fluid-containing enriched anti-HF were obtained, and one of them (clone P 5-2-1) was used in the present study.

Isolation of Immunoglobulin Fraction of Murine Ascites Fluid

Ascites fluid was adsorbed with calcium phosphate (10 mg/mL) and kaolin (100 mg/mL), and the supernatant was heated at 56 °C for 30 minutes. The immunoglobulin fraction was then separated by 45% ammonium sulfate precipitation, reprecipitated again, and then dialyzed extensively against barbital-saline buffer (BS, 0.025 mol/L sodium barbital in 0.125 mol/L NaCl, pH 7.4). A murine monoclonal antibody to human platelet that had been produced in our laboratory was used in the present experiments as a control for P 5-2-1.

Clotting Assays

The procoagulant activities of HF, factor XI, prekallikrein, high mol wt kininogen, factor IX, and factor VIII were measured by a modified partial thromboplastin time, using congenital deficient plasmas as substrates. The activities of prothrombin, factor V, factor VII, and factor X were assayed by a modified one-stage prothrombin time method. HF antigen was determined by a radioimmunoassay.

Radioiodination of HF

HF was radioiodinated with 125I by a chloramine T method. 125I-HF had 2.0 x 10^6 cpm/μg HF and retained its clotting activity during the radiolabeling procedure.

Immobilization of P 5-2-1 on Sepharose

The immunoglobulin fraction of P 5-2-1 (6.3 mg/g dry gel) or control antibody was covalently coupled to CNBr-activated Sepharose 4B according to the manufacturer's instruction.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

SDS-PAGE was performed according to the method of Laemmli. SDS-PAGE was performed according to the method of Laemmli. The acrylamide concentration was 3% in the spacer gel and 10% in the separating gel. After electrophoresis, gels were fixed in 10% acetic acid and 50% methanol overnight and dried for autoradiography or stained with Coomassie Brilliant Blue R (Sigma). Molecular weights were determined by running proteins of known mol wt (a low mol wt calibration kit, Pharmacia Fine Chemicals) on gels with the samples.

The Cleavage of 125I-HF During the Contact Activation of Plasma in the Presence or Absence of P 5-2-1

This was studied by the method of Revak et al. 125I-HF (0.5 ng in 7 μL) was mixed with 10 μL of normal plasma, 11 μL of either P 5-2-1 (10 μg) or control antibody (10 μg) and 172 μL of BS buffer in a polystyrene tube. The final plasma dilution was 1:20, and the radiolabeled protein that was added did not increase the plasma content of HF by more than 1%. Forty microliters of this mixture was added to 10 x 75-mm glass tubes and shaken at 22 °C for five minutes. The reactions were terminated by the addition of 20 μL 4% SDS with or without 2-mercaptoethanol, and the samples were then subjected to SDS-PAGE after boiling for three minutes. The zero-time sample was obtained by adding the sample directly to the SDS solution. After SDS-PAGE, the slab gels were fixed, dried, and subjected to autoradiography using a Fuji medical imaging film (type NC) with an intensifying screen at −20 °C.

The Effect of P 5-2-1 on the Binding of HF to Kaolin

125I-HF (10 μL) was incubated with 10 μL of normal pooled plasma, 40 μL of either P 5-2-1 (0.02 to 1.10 mg/mL) or control antibody (1.10 mg/mL) and 40 μL of BS buffer in a polystyrene tube for 15 minutes at 22 °C. Then 40-μL aliquots were mixed with equal amounts of kaolin suspension (10 mg/mL) for two minutes at 22 °C in 1-mL polystyrene Fisher centrifuge tubes. The tube was then centrifuged for 30 seconds at 5,000 g, and the pellet was counted for the radioactivity. Binding was expressed as the percentage of the total counts that were found in the pellet. Under these conditions, no significant binding of HF to the polystyrene tubes was observed.

The Effect of P 5-2-1 on HF Procoagulant Activity Before and After Kaolin Adsorption

Fifty microliters HF (1.10 μg/mL) was incubated with equal amounts of either P 5-2-1 (0.88 mg/mL) or control antibody (0.90 mg/mL) for five minutes at 37 °C in a 10 x 75-mm polystyrene tube. Then, 100 μL kaolin (10 mg/mL) was added to the mixture. After two minutes, 50 μL of either control antibody, P 5-2-1 or BS was added, respectively, and the mixture was assayed for HF activity. The results were expressed as the percentage of activity relative to that found in the control (control antibody and BS).

The Effect of P 5-2-1 on the Prekallikrein-Activating Activity of HF Fragments (HF₃)

Fifteen microliter HF₃ (31 to 124 ng in 0.025 mol/L BS buffer, pH 7.4) was incubated with equal amounts of either P 5-2-1 (4 mg/mL BS buffer) or control antibody (4 mg/mL) for ten minutes at 22 °C. Twenty-microliter aliquots were then mixed with 50 μL HF-deficient plasma for two minutes at 0 °C. After the action of HF₃, the sample was blocked by the addition of 5 μL CHF1 (40 μg/mL BS buffer), a 20-μL portion was incubated with 1 mL of 0.5 mmol/L H-D-Pro-Phe-Arg-pNA in BS buffer (5-2302, Kabi, Stockholm) for five minutes at 37 °C. The reaction was terminated by the addition of acetic acid, and absorbance was read at 405 nm. Preliminary experiment showed that the activation of prekallikrein in HF-deficient plasma by HF₃ proceeded linearly under these conditions.
**Binding of P 5-2-1 to a Heavy Chain of HF Molecule**

Fifty microliters purified HF (21 μg in BS buffer) was cleaved with 4 μL trypsin (0.4 μg) in a total volume of 67 μL at 37 °C for three minutes. After the action of trypsin had been blocked by addition of 40 μL LBTI (0.8 μg in BS buffer), the mixture was divided into two equal portions. One aliquot was incubated with 20 μL Sepharose P 5-2-1 equilibrated with BS buffer and the other with 20 μL Sepharose-control antibody at 0 °C for 30 minutes in a 1-mL polystyrene Fisher centrifuge tube with occasional stirring. The tubes were then centrifuged at 4,000 g for one minute, and the supernatant was separated. The pellets were washed with BS three times and eluted with 4% SDS and 2% 2-mercaptoethanol at 100 °C for three minutes. Both the supernatants and eluates were analyzed by SDS-PAGE. The slab gels were stained with Coomassie Brilliant Blue R.

**RESULTS**

**Characterization of a Monoclonal Anti-HF (P 5-2-1)**

P 5-2-1 antibody was found to be IgG2b, λ on immunodiffusion with rabbit antibodies specific for subtypes of mouse heavy and light chains. P 5-2-1 was a homogeneous IgG preparation as analyzed by SDS-PAGE and was derived from a single clone by limiting dilutions. The highest dilution of the IgG fraction (8.8 mg/mL) of P 5-2-1 that gave an unequivocal positive reaction with HF in a solid-phase radioimmunoassay (RIA) was 1.5 x 10⁻⁶.

When the binding of P 5-2-1 to HF was examined by a solid-phase RIA using ¹²⁵I-labeled anti-mouse Fab as described in Materials and Methods, the prior incubation of P 5-2-1 with purified HF or normal plasma inhibited the binding of P 5-2-1 to HF. In contrast, the prior incubation of P 5-2-1 with congenital HF-deficient plasma did not block the binding.

**Inhibition of HF Procoagulant Activity by P 5-2-1**

When normal pooled plasma was incubated with equal amounts of P 5-2-1 (1 mg/mL) for one hour at 37 °C, the HF clot-promoting activity was reduced to 24% of the control mixture (Table 1). In contrast, the activities of factor XI, prekallikrein, high mol wt-kininogen, factor IX, and factor VIII were not significantly changed. Similarly, the activities of other clotting factors (prothrombin, factor V, factor VII, and factor X) were not affected at all (data not shown). The inhibition of HF activity by P 5-2-1 was dose dependent (5% and 46% of the control at 4.0 mg/mL and 0.1 mg/mL, respectively) and occurred instantaneously without prior incubation.

To investigate the mode of the inhibition of HF activity, the cleavage of ¹²⁵I-HF during the contact activation in plasma was examined in the presence or absence of P 5-2-1. ¹²⁵I-HF in plasma before glass contact showed a single band (80,000 mol wt) with or without 2-mercaptoethanol (Fig 1, lanes 5, 6). Following a five-minute contact to glass in the presence of the control antibody, ¹²⁵I-HF was cleaved from its native 80,000-mol wt form to yield a heavy chain (H', 52,000 mol wt) and a light chain (L', 28,000 mol wt) as shown in Fig 1, lanes 7 and 8. There was some cleavage of ¹²⁵I-HF without reduction in lane 7, suggesting the formation of HF-fragments (β-XII) during glass contact. This pattern of the fragmentation is in good agreement with that previously reported for normal HF. In the presence of P 5-2-1, however, ¹²⁵I-HF remained as a native 80,000-mol wt form after five minute glass contact, and no cleavage was observed (lanes 3, 4). Thus, it appears that P 5-2-1 prevents the proteolytic cleavage of HF on the contact activation.

**The Effect of P 5-2-1 on the Binding of HF to Kaolin**

The binding of HF to negatively charged surfaces such as glass and kaolin is a prerequisite for its cleavage and activation. Binding of ¹²⁵I-HF from normal plasma to kaolin was studied in the presence or absence of various concentrations of P 5-2-1 (Table 2). Only 12% of HF was bound to kaolin in the presence of P 5-2-1 (1.1 mg/mL), whereas 51% to 52% was bound in the presence of the control antibody (1.1 mg/mL) or buffer (BS). The inhibitory effect of P 5-2-1 was dose dependent as shown in Table 2 and the difference between each concentration of P 5-2-1 was statistically significant (P < .01). Thus, P 5-2-1 appears to inhibit significantly the binding of HF to kaolin.

**The Effect of P 5-2-1 on HF Procoagulant Activity Before and After Kaolin Adsorption**

We examined whether or not P 5-2-1 influences the activity of surface-bound HF (Table 3). The addition of P 5-2-1 to purified HF before kaolin adsorption resulted in the marked reduction of HF activity (12%). On the other hand, when P 5-2-1 was added after kaolin adsorption, there was no significant alteration of HF activity (97%). These experiments suggest that P 5-2-1 does not interfere with HF activity after the adsorption of HF to negatively charged surfaces.

---

**Table 1. The Effect of P 5-2-1 on the Activities of Some Clotting Factors**

<table>
<thead>
<tr>
<th>Clotting Factors</th>
<th>Activity [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P 5-2-1 + Normal Plasma</td>
</tr>
<tr>
<td>XII (HF)</td>
<td>24</td>
</tr>
<tr>
<td>XI</td>
<td>98</td>
</tr>
<tr>
<td>Prekallikrein</td>
<td>100</td>
</tr>
<tr>
<td>High mol wt kininogen</td>
<td>95</td>
</tr>
<tr>
<td>IX</td>
<td>94</td>
</tr>
<tr>
<td>VIII</td>
<td>92</td>
</tr>
</tbody>
</table>

In a 10 x 75-mm polystyrene tube 50 μL normal pooled plasma was incubated with equal amounts of either P 5-2-1 (1 mg/mL) or control antibody (1 mg/mL) at 37 °C for one hour. The mixture was then diluted ten-fold with BS and was assayed for various clotting factors. The results were expressed as the percentage relative to that of the control mixture.
The Effect of P 5-2-1 on the Prekallikrein-Activating Activity of HF Fragments (HFf)

HFf is one form of activated HF (β-XIIa) that retains the active site of XIIa, while losing the surface-binding sites. HFf is a weak coagulant enzyme, but a potent activator of prekallikrein.22 We tested whether or not P 5-2-1 influences the activity of HFf. In this experiment, HFf activated prekallikrein in HF-deficient plasma to kallikrein, whose activity was then estimated by the use of a synthetic tripeptide substrate [16g. lanes 1 to 4) or control antibody (10 μg. lanes 5 to 8) in a total volume of 200 μL. Forty microliters of this mixture was added to glass tubes and shaken at room temperature for five minutes. The reactions were terminated by the addition of SDS with or without 2-mercaptoethanol, and the samples were analyzed by SDS-PAGE. The slab gels were fixed, dried, and subjected to autoradiography. The odd lanes were nonreduced, and the even lanes were reduced samples. The anode is to the bottom. Lanes 1 and 2, P 5-2-1 and no exposure to glass; lanes 3 and 4, P 5-2-1 and five-minute exposure to glass; lanes 5 and 6, control antibody and no exposure to glass; lanes 7 and 8, control antibody and five-minute exposure to glass. H' and L' indicate the heavy and light chain of HF.

Binding of P 5-2-1 to a Heavy Chain of HF Molecule

In an attempt to localize the epitope of HF molecule to which P 5-2-1 binds, the following experiment was performed. Purified HF was cleaved by trypsin into three fragments,3,15 and after trypsin was blocked by LBTI, the trypptic digest was incubated with Sepharose-P 5-2-1 or Sepharose-control antibody. The mixture was then centrifuged, and both the supernatant and eluate from the precipitates were examined by SDS-PAGE (Fig 2). The supernatant from the mixture of native HF and Sepharose-control antibody showed a single band at 80,000 mol wt (Fig 2, lane 3). The supernatant of the mixture of the trypsin-digested HF and Sepharose-control antibody demonstrated an amino-terminal fragment (40,000 mol wt) of a heavy chain and a light chain (28,000 mol wt) (Fig 2, lane 4).

Depletion of HF From Normal Plasma by P 5-2-1 Coupled to Sepharose 4B Column

HF could be totally removed from 40 mL of normal pooled plasma by passage over a 3-mL column of Sepharose 4B covalently coupled with P 5-2-1. The drop-through plasma contained less than 1% of HF activity, but essentially normal amounts of all other

Table 2. Effect of P 5-2-1 on the Binding of HF to Kaolin

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Concentration (μg/mL)</th>
<th>Bound HF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control antibody</td>
<td>1.10</td>
<td>52.1 ± 1.8</td>
</tr>
<tr>
<td>P 5-2-1</td>
<td>1.10</td>
<td>12.0 ± 2.4</td>
</tr>
<tr>
<td>P 5-2-1</td>
<td>0.28</td>
<td>15.7 ± 2.0</td>
</tr>
<tr>
<td>P 5-2-1</td>
<td>0.07</td>
<td>19.8 ± 1.0</td>
</tr>
<tr>
<td>P 5-2-1</td>
<td>0.02</td>
<td>27.3 ± 0.9</td>
</tr>
</tbody>
</table>

*Mean ± SD of four separate experiments.

The other fragment (12,000 mol wt) was not observed on the gels, probably due to poor staining of the fragment by the dye.15 The supernatant from the mixture of the native HF and Sepharose-P 5-2-1 showed no stained band (Fig 2, lane 1), and the supernatant from the mixture of the trypsin-digested HF and Sepharose-P 5-2-1 demonstrated only a light chain (HFf, Fig 2, lane 2). The missing native HF in Fig 2, lane 1, and a 40,000-mol wt fragment in lane 2 were found in the eluate of Sepharose-P 5-2-1 (Fig 2, lanes 5, 6, respectively). The other two stained bands in lanes 5 and 6 were derived from the monoclonal antibody (P 5-2-1) itself (a heavy and light chain of IgG). These data are consistent with the interpretation that P 5-2-1 binds to an amino-terminal 40,000-mol wt fragment of a heavy chain of HF molecule.

Table 3. The Effect of P 5-2-1 on HF Activity Before and After Kaolin Adsorption

<table>
<thead>
<tr>
<th>Mixtures</th>
<th>HF Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HF + P 5-2-1 → kaolin → control antibody</td>
<td>12</td>
</tr>
<tr>
<td>2. HF + control antibody → kaolin → P 5-2-1</td>
<td>97</td>
</tr>
<tr>
<td>3. HF + control antibody → kaolin → BS</td>
<td>100</td>
</tr>
</tbody>
</table>

Fifty microliters HF (1.10 μg/mL) was incubated with equal amounts of either (1) P 5-2-1 (0.88 mg/mL) or (2) and (3) control antibody (0.90 mg/mL) for five minutes at 37 °C. Then, 100 μL kaolin (10 mg/mL) was added to the mixture. After two minutes, 50 μL of either (1) control antibody, (2) P 5-2-1, or (3) BS was added, and the mixture was assayed for HF procoagulant activity. The results were expressed as the percentage of activity relative to that found in the control (3).

Fig 1. Autoradiogram of SDS-PAGE of the cleavage of 125I-HF during the contact activation in plasma. 125I-HF (0.5 ng in 7 μL) was mixed with 10 μL of normal plasma and 11 μL of either P 5-2-1 (10 μg, lanes 1 to 4) or control antibody (10 μg, lanes 5 to 8) in a total volume of 200 μL. Forty microliters of this mixture was added to glass tubes and shaken at room temperature for five minutes. The reactions were terminated by the addition of SDS with or without 2-mercaptoethanol, and the samples were analyzed by SDS-PAGE. The slab gels were fixed, dried, and subjected to autoradiography. The odd lanes were nonreduced, and the even lanes were reduced samples. The anode is to the bottom. Lanes 1 and 2, P 5-2-1 and no exposure to glass; lanes 3 and 4, P 5-2-1 and five-minute exposure to glass; lanes 5 and 6, control antibody and no exposure to glass; lanes 7 and 8, control antibody and five-minute exposure to glass. H' and L' indicate the heavy and light chain of HF.
Table 4. Effect of P 5-2-1 on the Prekallikrein-Activating Activity of HF Fragments (HF₄)

<table>
<thead>
<tr>
<th>Test Sample</th>
<th>Absorbance at 405 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF, 124 ng + P 5-2-1</td>
<td>0.459</td>
</tr>
<tr>
<td>HF, 124 ng + control antibody</td>
<td>0.457</td>
</tr>
<tr>
<td>HF, 62 ng + control antibody</td>
<td>0.273</td>
</tr>
<tr>
<td>HF, 31 ng + control antibody</td>
<td>0.172</td>
</tr>
</tbody>
</table>

Fifteen microliters HF₄ (31 to 124 ng) was incubated with equal amounts of either P 5-2-1 (4 mg/mL) or control antibody (4 mg/mL) for ten minutes at room temperature. Twenty-microliter aliquots were then mixed with 50 μL HF-deficient plasma for two minutes at 0 °C. After the action of HF, was blocked by CHF₁, the plasma kallikrein activity generated was estimated on H-o-Pro-Phe-Arg-pNA as described in Materials and Methods.

clotting factor activities. HF that had been bound to the column was not eluted by 2 mol/L NaCl. It was partially recovered by washing the column with 3 mol/L potassium isothiocyanate. Under these conditions, however, HF seems to be denatured, since it had approximately one third of the procoagulant activity, as compared with the HF antigen measured by a radioimmunoassay.

HF-deficient plasma obtained by an immunoaffinity column was used as an artificial substrate for the measurement of HF activity, and the results were compared with those obtained with congenital HF-deficient plasma (a natural substrate). Plasma from 25 normal individuals and plasma from five individuals with heterozygous HF deficiency were assayed with both substrates (Fig 3). There was good correlation between the assays (r = .926).

DISCUSSION

The present article describes the production and properties of a murine monoclonal antibody (P 5-2-1) against a heavy chain of human HF. This antibody consists of mouse IgG₂b heavy chains and λ light chains and selectively inhibits HF procoagulant activity but not the prekallikrein-activating activity of HF₄.

When normal blood comes into contact with negatively charged surfaces, HF, factor XI, prekallikrein, and high mol wt-kininogen are all adsorbed to the surface and HF becomes enzymatically active. Although the initial event that triggers the contact activation is not yet completely clear, HF undergoes, on the surface, specific limited proteolytic cleavage yielding 52,000-mol wt (a heavy chain) and 28,000-mol wt fragments (a light chain). With prolonged contact activation, a heavy chain appears to be cleaved further into 40,000-mol wt fragments, and a light chain is digested to yield HF₁ (β-XII₄). It has been shown that trypsin also cleaves HF into three domains (40,000, 28,000, 12,000 mol wt) and that the 28,000-mol wt portion of the molecule seems to contain an
active site, while the 40,000-mol wt portion is related to surface binding.

The mechanisms of inhibition of HF activity by P 5-2-1 were studied. At least two possibilities may be considered as responsible for the action of P 5-2-1 on HF: (1) P 5-2-1 interferes with the binding of HF to surfaces or (2) it blocks the activation and/or activity of the surface-bound HF. When purified HF was mixed with P 5-2-1 before the adsorption of HF to kaolin, HF activity was markedly inhibited. In contrast, P 5-2-1 did not interfere with HF procoagulant activity when HF was exposed to the antibody after the adsorption of HF to kaolin. The failure to inhibit the activity of surface-bound HF by P 5-2-1 could be sterical. This possibility, however, was excluded, because P 5-2-1 did not block the activity of HF without surface-binding sites. Furthermore, it was found that P 5-2-1 binds to the 40,000-mol wt portion of a heavy chain of HF and interferes with the adsorption of HF to kaolin. These data are consistent with the first possibility and further extend the observation that 40,000-mol wt fragments are related to the binding of HF to negatively charged surfaces.

The precise localization of the binding sites in 40,000-mol wt fragments remains to be studied. In this regard, it is noteworthy that the cell-attachment site of fibronectin was successfully identified by the use of monoclonal antibodies and proteolytic fragments of the molecule. P 5-2-1 would be a useful tool for elucidating the epitope of HF, which is essential for surface binding.

P 5-2-1 was covalently coupled to agarose and was used to selectively deplete HF from normal plasma. The performance of such affinity-depleted plasma in a coagulant assay for HF is as good as that seen with plasma from congenitally deficient patients. No evidence of microheterogeneity of normal HF has been found so far, since all HF was removed from normal pooled plasma (a pool of 24 normal male plasmas). The affinity of P 5-2-1 for HF appears high, since HF was not eluted under mild conditions. Therefore, it was not possible to use the immunoaffinity column for the isolation of HF.

The use of monoclonal antibodies as biospecific and immunochemical probes will be helpful in the study of the structure and function of HF and other coagulation factors.

REFERENCES

Production and characterization of a murine monoclonal antibody against a heavy chain of Hageman factor (factor XII)

H Saito, T Ishihara, H Suzuki and T Watanabe