Monoclonal Antibodies to Human Coagulation Factor V and Factor Va

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BALB/c mice were immunized with human factor V. The immunogen was a mixture of procofactor (factor V) and thrombin-activated cofactor (factor Va). Spleen cells were obtained from an immunized animal and fused with NS-1 murine myeloma cells. Hybrid cell cultures were assayed for the production of antibodies to human factor V and factor Va by a solid-phase radioimmunoassay. Factor V and/or factor-Va-specific antibodies were detected in 38 of the 96 cultures assayed. The cells from 10 of these positive cultures were subcloned by limiting dilution and grown as ascites tumors in BALB/c mice. Ascitic fluids were obtained and characterized with respect to their binding interaction with human factor V and factor Va. Three hybridoma cell lines produce monoclonal antibodies that react equally well with factor V and factor Va. Another antibody reacts with both antigens, but the reactivity with factor V is better than with factor Va. An additional two antibodies react with factor Va better than factor V in the radioimmunoassay (RIA). The remaining four antibodies react exclusively with factor V. A previously described murine monoclonal antibody to human factor V (αHFV-1) has been used to study the peptides produced during the thrombin-catalyzed activation of human factor V. This antibody binds both factor V and factor Va, releases them at high ionic strength, and has an apparent dissociation constant for factor Va of $3 \times 10^{-6}$. When human factor V (mol wt 330,000) is activated by thrombin and passed over an αHFV-1-Sepharose affinity resin, factor Va binds and subsequently can be eluted. The eluate in 1.2 M NaCl contains two fragments of apparent mol wt 93,000 and 70,000. EDTA, which inactivates factor Va, promotes release of the mol wt 93,000 fragment from factor Va bound to the antibody. Subsequent elution with 1.2 M NaCl releases the mol wt 70,000 fragment. These observations indicate that human factor Va is a two subunit protein and that the epitope for αHFV-1 is on the mol wt 70,000 fragment.

Factor V is the high molecular weight (mol wt 330,000) single chain precursor to activated factor V (factor Va), a nonenzymatic cofactor required for the rapid conversion of prothrombin to thrombin. The isolation of bovine factor V has been described by our laboratory and that of Esmon. Further studies by Nesheim and Mann have demonstrated that the conversion of factor V to factor Va by thrombin results in at least a 400-fold enhancement of the rate of prothrombinase activity as compared to that rate observed with the single chain procofactor. This thrombin-catalyzed activation of bovine factor V proceeds via the formation of two high molecular weight intermediates (mol wt 205,000 and 150,000) to end-products of apparent molecular weights of 94,000, 92,000, 74,000, 71,000 and 31,000. It has been demonstrated that two of these fragments, the 94,000 component D and the 74,000 component E, are required for cofactor activity. In addition, the integrity of the cofactor activity of factor Va is calcium-dependent.

The isolation of human factor V, using an immobilized murine monoclonal antibody, was described by Katzmann et al. Kane and Majerus' and Suzuki et al. have reported the isolation of the procofactor using more conventional protein purification procedures. Suzuki et al. propose that the activation of human factor V by thrombin proceeds via discrete cleavages to end-products of mol wt 105,000, 71,000, 150,000, and 71,000/74,000. The mol wt 105,000 fragment D and the 71,000/74,000 fragment $F_1 F_2$ most likely constitute the active cofactor molecule. These observations are similar to those of Kane and Majerus.

We have previously described a library of murine monoclonal antibodies to bovine factor V(Va) that are being used to probe the cofactor molecule and its interactions with the other components of prothrombinase. In an effort to develop biospecific immunologic probes of human factor V structure and function, we have generated ten monoclonal antibodies reactive with human factor V and/or factor Va. This article is a report on the preparation and characterization of these monoclonal antibodies with respect to the binding interactions of the antibodies with factor V and/or factor Va. In addition, the interaction of the cofactor with a previously generated murine monoclonal antibody (αHFV-1) is described. This antibody is used to identify and isolate the products formed by the thrombin-catalyzed activation of human factor V.
MATERIALS AND METHODS
Isolation of Human Factor V

Human factor V was isolated from fresh-frozen plasma by a modification of the procedure described by Katzmann et al.1 and Nesheim et al.5 Plasma (approximately 1,000 ml) obtained from blood collected into citrate-phosphate-dextrose-adrenaline anticoagulant (0.16 M dextrose, 0.09 M trisodium citrate, 0.015 M citric acid, 0.016 M sodium biphosphate, 0.002 M adenine) was thawed slowly at room temperature, after which dextran-larginine Na-(3-ethyl-1,5-pentanediyl)amide (DAPA) and benzamidine - HCl were added to final concentrations of 30 pM and 10 mM, respectively. Solid polyethylene glycol 6000 (PEG-6000) was added slowly at 4°C with stirring to a final concentration of 11% (w/v). The solution was stirred for 30 min at 4°C and centrifuged at 10,000 g for 20 min. Solid PEG-6000 was added to the 21% PEG-6000 supernatant to a final concentration of 30 pM and 10 mM, respectively. Solid PEG-6000 was added to the 1% PEG-6000 supernatant to a final concentration of 21% (w/v). After stirring at 4°C for 30 min, the precipitate was collected by centrifugation. The supernatant was discarded and the 21% PEG-6000 pellet was dissolved in 0.02 M Tris - HCl, 0.15 M NaCl, pH 7.4 (approximately 200 ml) containing 10 pM DAPA and 10 mM benzamidine - HCl. This solution was then applied to a column (2 x 10 cm) of an immobilized monoclonal antibody to human factor V.1 Human factor V was eluted from this affinity resin as described previously. The pooled factor V was precipitated by the addition of solid ammonium sulfate (0.435 g/ml) at 4°C, and the precipitate was collected by centrifugation at 10,000 g, 4°C, 20 min. The pellet was redissolved in a minimal volume of 0.01 M Tris-0.01 M borate-0.001 M CaCl2-50% glycerol and stored at -20°C.

Radioiodination of Factor V

Human factor V was radioiodinated as described by Tracy et al.14,15 using Bolton-Hunter reagent.19 Following the separation of the 125I-factor V from the other products of the conjugation reaction by chromatography on QAE-cellulose,15 the labeled protein was dialyzed into 0.01 M Tris-0.01 M borate-0.001 M CaCl2-50% glycerol and stored at -20°C. The specific radioactivity of the radioiodinated factor V was typically 250-1,000 cpm/ng. Neither the specific activity nor the activation quotient of the antigen was altered significantly during the labeling procedures. 125I-factor Va was prepared by the proteolytic activation of 125I-factor V with catalytic amounts of bovine thrombin (1 NIH U/ml) at 37°C for 45 sec. 125I-factor Va was prepared immediately prior to use, and the reaction with thrombin terminated by the addition of disopropylfluorophosphate to a final concentration of 5 mM.

Assay of Antibodies to Factor V and Factor Va

A solid-phase radioimmunoassay was used for the detection of anti-factor-V and anti-factor-Va activities.10 For this purpose, the IgG fraction of rabbit anti-mouse immunoglobulin (RatMlg, Cappell Laboratories, Cochranville, PA) was adsorbed to polystyrene tubes by incubation in 0.05 M sodium carbonate, pH 9.5, at 4°C. Tubes were then washed with assay buffer (0.05 M Tris - HCl, 0.10 M NaCl, 0.02% azide, pH 7.3), and test samples were added to the RatMlg-coated tubes. Following a 6-hr incubation at 4°C, the tubes were again washed with assay buffer, and bound antibodies to human factor V or human factor Va were detected by the addition of 125I-factor V or 125I-factor Va in assay buffer containing 1 mg/ml RIA grade bovine serum albumin. Specific counts per minute bound were calculated by subtracting background nonspecific binding from the total cpm bound. (These values, determined by using fresh media, normal mouse serum, or buffer as test samples, were typically 10%-20% of the positive samples.) All data in this manuscript are reported as specific cpm bound.

Immunization, Cell Fusion, and Cloning

Antigen was prepared for immunization by the incubation of factor V (0.5 mg/ml in 0.02 M imidazole - HCl, 0.15 M NaCl, pH 7.4) with human α-thrombin (2 NIH U/ml) for 5 min at 37°C, during which time the activity (as measured by clotting assay) had attained a maximal value. Following the addition of CaCl2 and phospholipid vesicles (phosphatidylcholine-phosphatidylserine, 75:25) to final concentrations of 2 mM and 40 μM, respectively, human activated protein C (APC) was added to 2 μg/ml. The reaction of factor Va and APC was monitored by decrease in activity as measured in the clotting assay. After 30 min, the reaction with APC was terminated by the addition of citrate (5 mM final concentration). The activation of factor V by thrombin and the inactivation of factor Va by activated protein C proceeded as described by Canfield et al.17 and Walker and Esmon.18 BALB/c mice were initially immunized with the intraperitoneal injection of 100 μg of the antigen in complete Freund’s adjuvant. Two subsequent injections were made (100 μg each) in 0.02 M imidazole, 0.15 M NaCl, pH 7.4, at 3-wk intervals. Immunized spleen cells were fused with murine NS-1 myeloma cells in the presence of polyethylene glycol and distributed into the wells of four 24-well costar culture plates.1 Culture wells exhibiting hybrid cell growth were assayed for the production of antibodies to factor V and factor Va. The cells in the positive wells selected for further study were subcloned by limiting dilution as described previously and again screened for reactivity with 125I-factor V and 125I-factor Va in the RIA. Subeloned hybridoma cells were injected intraperitoneally into BALB/c mice (2.4 x 10^6 cells/mouse) for the production of antibody-rich ascites fluid as previously described.

Polyacrylamide Gel Electrophoresis

Protein samples were analyzed by polyacrylamide gel electrophoresis by the procedure described by Weber and Osborn,19 as modified by Mann et al.20 Proteins were detected with Coomassie brilliant blue R-250.

Isolation of Immunoglobulin Fraction of Murine Ascites Fluid

The immunoglobulin fraction of murine ascites fluids was isolated by gel filtration chromatography on Ultrogel AcA34 at 22°C in 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3, containing 0.02% sodium azide.10 The anti-factor V(Va)-containing fractions were precipitated by the addition of solid (NH4)2SO4 to 70% saturation. The precipitates were collected by centrifugation, dissolved in a minimal volume of 0.01 M Tris - borate, 5 mM CaCl2, 50% glycerol (w/v), and stored at -20°C.

Immobilization of aHFV-1 on Sepharose

Purified aHFV-1 immunoglobulin was immobilized on Sepharose 4B by the procedure of March et al.,27 using 0.2 M citrate, pH 6.5, for the coupling buffer. The resin was stored at 4°C in 0.05 M Tris - HCl, 0.10 M NaCl, pH 7.3, containing 0.02% sodium azide.

Isolation of Factor Va Subunits

Human factor V (85 μg) was dissolved in 1 ml 0.02 M Tris - HCl, 0.15 M NaCl, 5.0 mM CaCl2, pH 7.4, and applied slowly to a column of aHFV-1-Sepharose (1.0 x 1.0 cm in glass) at 22°C. One column volume of buffer containing bovine thrombin (2.0 NIH U/ml) was then applied to activate the bound factor V. Following
application of the thrombin, flow was stopped for 30 min. Flow was then started and fractions of 3 ml were collected. When the absorbance at 280 nm returned to baseline, one column volume of buffer containing 5 mM EDTA in place of CaCl₂ was added to dissociate factor Va subunits. Flow was halted for 20 mm and then restarted. When baseline absorbance was attained, elution was continued with buffer containing 1.2 M NaCl to disrupt the antigen–antibody interaction. The materials obtained were dialyzed separately against 0.2 M acetic acid, lyophilized, and analyzed by polyacrylamide gel electrophoresis in the presence of DodeSO₄.

Estimation of the Affinity of αHFV-1 for Factor Va

A solution was prepared consisting of bovine prothrombin (1.4 μM), human factor Va (3.3 nM), DAPA (3 μM), and phosphatidylcholine-phosphatidylinerine vesicles (13 μM) in 0.02 M Tris·HCl, 0.15 M NaCl, 2.0 mM CaCl₂, pH 7.4. Ten aliquots (1.5 ml each) were pipetted into separate fluorimeter cuvettes, and αHFV-1 was added at concentrations ranging from 0 to 50 nM. These solutions were incubated for 1 hr, after which factor Xa was added to final concentration of 18 nM. Under these circumstances, factor Va levels are linear with respect to the rate of prothrombin activation. The measured rates decreased with increasing antibody concentration and plateaued at a rate approximately two-thirds of the control rate, indicating that inhibition is saturable but only partial. The extent of inhibition between the observed extremes was assumed directly proportional to the fraction of factor Va bound, b, was expressed as in equation (1), where v₀ and vₙ are reaction rates in the absence of antibody and in its presence in the plateau, respectively, and ν is the reaction rate at intermediate concentration of added antibody.

\[ b = \frac{v_0 - v}{v_0} \]  

From the mass action expression,

\[ K_d = \frac{[Ab][Va]}{[Ab - Va]} = \frac{\int [Ab]}{b} = \frac{[Ab](1 - b)}{b} \]  

The concentration of free antibody (Ab) is given by equation (3), where \([Ab]₀\) is the total antibody concentration, \([Va]₀\) is the total factor Va concentration, and n is the stoichiometry of interaction (Ab/Va):

\[ [Ab] = [Ab]₀ - nb [Va]₀ \]

Substitution of equation (3) into equation (1) and (2) yields equation (4), below, which describes a linear (double reciprocal) relationship between the binding parameters K_d and n, the nominal concentrations \([Ab]₀\) and \([Va]₀\), and the inferred extent of binding, b.

\[ \frac{1}{f[Va]₀} = \frac{1}{K_d [Va]₀ b} - \frac{n}{K_d} \]  

Binding data inferred from inhibition were plotted according to equation (4), and the binding parameters K_d and n were extracted from the slope and vertical intercept of the plot.

RESULTS

Hybrid cell growth was present in all 96 culture wells generated from the single fusion experiment described. Each well was assayed for production of antibodies to human factor V and factor Va by the solid phase RIA. As indicated in Fig. 1, 38 of the 96 hybrid cell cultures were positive in the screening assays. Although the antibodies produced by most of these antibody-positive hybrid cell cultures were reactive with both factor V and factor Va in the RIA (Fig. 1), the antibodies detected in several culture wells exhibited preferential reactivity with either the single chain procofactor or the activated cofactor (e.g., Fig. 1, wells 5 and 6 versus wells 63 and 77). The cells in 13 of these antibody-positive cultures were subcloned by limiting dilution. The cell lines were selected for subcloning such that all patterns of reactivity with the antigens were represented. Four of these hybrids (wells 4, 5, 6, and 77, Fig. 1) did not yield cloned cells. In addition, the subcloned cells from one culture (Fig. 1, well 36) exhibited two distinct reactivity patterns with factor V and factor Va in the RIA, indicating that well 36 initially contained more than a single hybridoma that secreted anti-factor-V(Va) antibody. These two cell lines were expanded and grown separately. Cloned hybridoma cell lines were grown as ascites tumors, and aliquots of the ascites fluids were diluted and titrated.

![Fig. 1. Initial screen of hybrid cell cultures for antibodies to human factor V and factor Va. Culture supernatants from hybrid cell cultures (200 μl) were incubated with RoMig-coated polystyrene tubes for 6 hr at 4°C. The tubes were then washed 3 times with 0.5 ml of 0.05 M Tris·HCl, 0.10 M NaCl, 0.02% NaN₃, pH 7.3, and 125I-factor-V or thrombin-activated 125I-factor-V or thrombin-activated 125I-factor-V (125I-factor-Va) was added to each tube (20,000 cpm in 200 μl of 0.05 M Tris·HCl, 0.10 M NaCl, 0.02% NaN₃, pH 7.3, containing 1 mg/ml bovine serum albumin). Following a 6-hr incubation at 4°C, the tubes were washed 3 times with 0.5 ml 0.05 M Tris·HCl, 0.10 M NaCl, 0.02% NaN₃, pH 7.3, containing 1 mg/ml bovine serum albumin, and bound radioactivity was measured in a Beckman gamma counter.](#)
Fig. 2. Titration of anti-human factor V(Va) ascites fluids with factor V and factor Va. The ascites fluids were diluted in 0.05 M Tris - HCl, 0.10 M NaCl, 0.02% NaN₃, pH 7.3. added to RssMlg-coated tubes (200 µl) and incubated for 6 hr at 4°C. The tubes were washed 3 times with 0.5 ml 0.05 M Tris, 0.10 M NaCl, 0.02% NaN₃, pH 7.3, and bound specific antibodies to factor V or factor Va were detected as described in Fig. 1. The values obtained were corrected for nonspecific binding and plotted as specific cpm bound (vertical axis) versus the dilution of the ascites fluids (horizontal axis). (A) αHFV-3; (B) αHFV-5; (C) αHFV-4.
Table 1. Reactivity of aHFV(Va) Ascites Fluids With Factor V and Factor Va

<table>
<thead>
<tr>
<th>Well* No.</th>
<th>Clone</th>
<th>Anti-Factor V</th>
<th>Anti-Factor Va</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
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<td>aHFV-2</td>
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</tr>
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</tr>
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</tr>
<tr>
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<td>aHFV-9</td>
<td>170</td>
<td>3 x 10^7</td>
</tr>
<tr>
<td>21</td>
<td>aHFV-10</td>
<td>395</td>
<td>2 x 10^8</td>
</tr>
</tbody>
</table>

*Well no. corresponds to the well no. of the initial hybrid cell cultures as represented in Fig. 1.
†Maximum specific counts per minute bound in solid-phase RIA.
‡Amount of dilution required to reduce binding to half maximal in the RIA.
§aHFV-1 is the monoclonal antibody described by Katzmann et al.7
ND, not determined; no clear titer point was obtained.

with [125I]-factor V and [125I]-factor Va by RIA. Three representative titrations are shown in Fig. 2, where the specific bound radioactivity is plotted (vertical axis) versus the ascites fluid dilution (horizontal axis). Based on the total amount of radioactivity specifically bound in the RIA, the aHFV-3 ascites fluid is reactive with both factor V and factor Va (Fig. 2A). Similarly, aHFV-5 ascites fluid reacts with both antigens, but more radioactivity is bound in the assay with factor Va than with factor V (Fig. 2B). In contrast, aHFV-4 ascites fluid is reactive only with the single chain procofactor (Fig. 2C). The data from similar titrations of the remaining ascites fluids are summarized in Table 1. The ascites fluids are rich sources of these antibodies, requiring dilutions of 10^4 to greater than 10^6 to reduce the binding in the RIA to half maximum (Table 1).

Included in Table 1 is aHFV-1, which has been described previously.7 This antibody was used by Katzmann et al.7 for the isolation of human factor V, since the interaction of aHFV-1 with the antigen is disrupted by 1.2 M NaCl at neutral pH. As indicated in Fig. 3, this antibody (aHFV-1) partially inhibits factor Va activity in a system of purified components. The extent of inhibition was detected and quantified by antibody-induced changes in the rate of prothrombin activation. Rates of prothrombin activation, which are directly proportional to factor Va concentrations under these conditions, are expressed as moles of thrombin generated per minute per mole total factor Va by the

Fig. 3. Estimation of the affinity of aHFV-1 for human factor Va inferred from inhibition of factor Va activity. Variable amounts of purified aHFV-1 were added to factor Va (3.3 nM) and, after 1 hr, residual factor Va activity was determined as described in Materials and Methods. The inhibition was saturable but partial. An interpretation of the inhibition of activity as a binding interaction between factor Va and the antibody is indicated in the inset. The analysis suggests a dissociation constant of 3 x 10^-9 M.
units on the vertical axis. Antibody concentrations are indicated by the units of the horizontal axis, expressed relative to the factor Va concentration. An interpretation of these data in terms of a binding interaction between factor Va and the antibody is indicated in the inset. The analysis is described in Materials and Methods. The dissociation constant for the factor-Va-antibody interaction obtained by this analysis has a value of $3 \times 10^{-9} \text{M}$. These data, plus the observation that factor V or factor Va can be eluted from a column of immobilized $\alpha$HFV-1, indicate that the interaction of factor Va with $\alpha$HFV-1 is of high affinity and mediated most likely by an ionic mechanism.

When factor V is activated by thrombin (1 NIH U/ml, 37°C, 3 min) and passed through an $\alpha$HFV-1-Sepharose affinity resin, the cofactor activity is retained by the column. When the bound material was eluted with 1.2 M NaCl, DodSO₄ gel electrophoresis reveals two peptide fragments of apparent molecular weights 93,000 and 70,000. To determine on which of these components the $\alpha$HFV-1-reactive epitope is located, the studies represented in Fig. 4 were conducted. Human factor V was bound to the $\alpha$HFV-1-Sepharose affinity and thrombin was added (2 NIH U/ml of thrombin in 0.02 M Tris, 0.15 M NaCl, 5 mM CaCl₂, pH 7.4) to activate the bound factor V. After 30 min at 24°C, the column was washed with 0.02 M Tris · HCl, 0.15 M NaCl, 5 mM CaCl₂, pH 7.4, to elute activation fragments. Subsequently, the column was washed with the same buffer containing 5 mM EDTA in place of the CaCl₂ to elute the peptide fragment(s) bound by virtue of calcium-mediated interactions with the factor Va fragment containing the $\alpha$HFV-1-reactive epitope. The resin was then washed with 0.02 M Tris · HCl, 1.2 M NaCl, pH 7.4, to elute the epitope-containing fragment. In this experiment, the recovery of components initially bound was almost quantitative. DodSO₄ polyacrylamide gel electrophoresis analysis of the components eluted from the affinity column (Fig. 4) is shown in Fig. 5. The gel on the left was obtained with a sample of human factor V activated with thrombin. The next gel resulted from analysis of the material eluted with EDTA. The third gel was obtained with material eluted with 1.2 M NaCl. These latter two gels most likely represent the factor Va subunits, and the lower molecular weight subunit appears to possess the epitope recognized by $\alpha$HFV-1.

![Fig. 4. Activation of human factor V bound to $\alpha$HFV-1-Sepharose and isolation of factor Va subunits. Human factor V bound to $\alpha$HFV-1-Sepharose was treated with thrombin. Fractions were then eluted with 0.15 M NaCl (first peak), 0.15 M NaCl plus 5.0 mM EDTA (second peak), and finally, 1.2 M NaCl plus 5.0 mM EDTA (third peak). The total recovery of material was essentially quantitative.](image-url)
DISCUSSION

The immunizations described in this study were carried out with factor V proteolytically digested with both thrombin and activated protein C to enable us to generate antibodies selective for various states of factor V. We assayed the cell cultures described in this report for reactivity toward both factor V and factor Va.

With the exception of aHFV-1 reported previously,7 the monoclonal antibodies described in this report were generated from a single fusion experiment in which hyperimmune spleen cells were hybridized with NS-1 myeloma cells. The high percentage of antibody-positive hybrid cell cultures detected in the initial screen (38 of 96 cultures) suggests that the immunogen is highly antigenic; thus, the presence of more than one anti-factor-V(Va)-specific clone per culture well is probable. The existence of multiple anti-factor V secretory hybrid cell lines was inferred for wells 17, 21, and 36, as the reactivity patterns initially obtained with these wells changed upon the subcloning procedures that yielded aHFV-4, aHFV-9, and aHFV-10.

The data in Table 1 demonstrate that several of the monoclonal antibodies react differentially with factor V or factor Va. The ability of antibodies such as aHFV-2, aHFV-3, and aHFV-10 to interact with both factor V and factor Va suggests reactivities at epitopes present on factor V that are conserved during the proteolytic activation. The interactions of antibodies such as aHFV-4, aHFV-4ac, and aHFV-7, in contrast, suggest reactivities at epitopes that are either destroyed during the activation or are present on poorly radiolabeled activation fragments of factor V, which would not be detected by RIA. (Although the subunits of factor Va label with 125I by Bolton-Hunter reagent and stain with Coomassie blue similarly, activation peptides may exist which label poorly.4,9) Three of the ascites fluids (aHFV-2, aHFV-3, and aHFV-8) interact with both factor V and factor Va with equivalent titers (Table 1). aHFV-8 differs from the other two, however, in that it binds at saturation fewer counts with factor Va than with factor V. Thus, although the gross reactivity patterns of these three antibodies are similar, aHFV-8 clearly recognizes an epitope different from those recognized by aHFV-2 and aHFV-3.

The reactivity of aHFV-9 with factor V and factor Va is different from the other antibodies of Table 1 which interact with both antigens. Although this antibody reacts with both factor V and factor Va, the titers obtained with these antigens are different. This difference may represent a higher affinity for factor Va (and hence a higher titer) than for factor V. At low concentrations of reactants, aHFV-9 primarily detects factor Va, and thus, may be of potential use in the specific detection of the activated form of the cofactor. The antibody produced by aHFV-5 is reactive with both factor V and factor Va, and higher total counts are bound with factor Va. The equivalent titers with factor V and factor Va imply that the antibody interacts with both antigens with similar affinities, in contrast to the differential affinities inferred with aHFV-9.

aHFV-1 has proven useful in both isolating human factor V by affinity chromatography and separation of factor Va subunits under nondenaturing conditions. Its utility can be attributed both to its high affinity and its apparent ionic interaction with the antigen. The high affinity of the interaction permits quantitative binding in dilute solutions, and the ionic nature of the interaction permits recovery of the bound antigen at elevated ionic strength. With relatively simple techniques, the antibody has provided considerable insight into the products of the thrombin-catalyzed activation of human factor V and the subunit structure of factor Va. The results (Figs. 4 and 5) indicate that factor Va is composed of two subunits with apparent molecular weights of 93,000 and 70,000. The association of these subunits is disrupted by EDTA. Both the subunit structure and sensitivity to EDTA are similar to those observed with bovine factor Va.5,6,9,22 The data also indicate that the aHFV-1-reactive epitope is expressed on the mol wt 70,000 subunit. The sum of the apparent molecular weights of these subunits accounts for only half of the mass of factor V. The remaining mass may be represented by the poorly staining bands observed on gel 2. Fig. 5, the combined apparent mass of which is also equal to half the mass of factor V. The identification of these bands as poorly staining activation peptides is supported by the observation that they were released from bound factor V upon exposure to thrombin and represented approximately one-third of the absorbing (A280) material of the sample.

The results obtained with the antibodies described in this article indicate that they will be of further use as biospecific, immunochemical probes of the structure and function of human factor V and tools for the assessment of the role of factor V under normal and pathologic conditions.

REFERENCES

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WB Foster, MM Tucker, JA Katzmann, RS Miller, ME Nesheim and KG Mann