

Monoclonal Antibodies to Porcine Factor VIII Coagulant and Their Use in the Isolation of Active Coagulant Protein

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Partially purified preparations of porcine factor VIII:C were used to immunize mice and spleen cells from the immunized animals were fused to NS-1 mouse myeloma cells. The ability of hybrid culture fluids to bind factor VIII:C was detected with a radiolabelled, affinity-purified, human antihuman VIII:C inhibitor. Three cloned hybrid lines have been obtained that preferentially bind to VIII:C when compared to von Willebrand factor binding. Two of these monoclonal antibodies partially inhibit VIII:C coagulant activity. The third antibody does not inhibit VIII:C, but it can be used as an affinity reagent to absorb dissociated VIII:C out of solution. Active coagulant can be recovered by

elution in 50% ethylene glycol. The VIII:C obtained has a specific activity of 6 units/ μ g based on absorbance measurements. When analyzed on SDS gels, the unactivated VIII:C contains 3 bands of apparent molecular weight 166,000, 130,000 and 76,000. Thrombin treatment results in a 40 fold increase in activity and cleavage to products of 76,000, 67,000 and 50,000 and small amounts of lower molecular weight peptides. EDTA inactivation of the factor VIII:C results in the separation of the 166,000 and 130,000 chains from the 76,000 chain, suggesting a Ca^{++} dependent noncovalent interaction among the chains.

MONOCLONAL ANTIBODIES derived from hybridomas have proven useful in the isolation and purification of human factor V.^{1,2} An important aspect of monoclonal antibody technology is the freedom from needing a purified immunogen to obtain a specific reagent. Both the difficulty of purifying significant percentages of original activity³ and the low initial starting concentration³ of factor VIII:C make this clotting factor a good candidate for this approach. While the existing anti-VIII:C antibodies, both of human and rabbit origin, have provided excellent probes for the study of the VIII:C molecule and its activated and degraded forms,^{4,5} these reagents are likely to be polyclonal and of limited value for isolation of active coagulant and for separation and identification of cleavage fragments of the coagulant molecule.

Mice immunized with porcine factor VIII complex and boosted with a preparation enriched with the VIII:C moiety produced sera capable of inactivation of porcine factor VIII:C. Spleens of these animals provided cells which, when fused with NS-1 plasmacytoma cells, produced immunoglobulins which bound porcine factor VIII:C. Because the factor VIII complex involves more than one protein, one cannot take factor VIII:C binding as an indication of specificity for the coagulant portion of the VIII complex. Studies of Holmberg and Ljung,⁶ Tuddenham et al.,⁷ and Laverigne et al.⁸ have shown that immobilization of von

Willebrand factor results in concomitant binding of factor VIII:C. This phenomenon requires that VIII:C binding screens for monoclonal antibodies be considered only partial evidence for their specificity. We have used protein analysis by SDS polyacrylamide gel electrophoresis and radioimmunoassays to demonstrate that the proteins bound by the hybridoma antibodies are similar to those bound by naturally occurring human autoantibodies to factor VIII:C and that the binding can occur without the participation of von Willebrand factor. There are other circumstantial criteria for the identification of factor VIII:C antigen. The activation of factor VIII:C by thrombin has been well known in the absence of any agreement on the mechanism.⁹⁻¹¹ Recently several lines of evidence have developed which demonstrated that thrombin cleaves bovine factor VIII:C during activation³ and that the molecular dimensions of human factor VIII:C changed after thrombin treatment.^{4,5} Inactivation of factor VIII:C by EDTA has also been demonstrated and, because of this, EDTA has been suggested as a reagent useful to construct synthetic factor VIII:C deficient substrates.¹² The proteins bound by the mouse antibodies described below are thrombin sensitive during conditions which activate VIII:C and EDTA sensitive under conditions which inactivate VIII:C.

Two of the antibodies described in this report show partial inactivation of plasma VIII:C while another does not, suggesting that they are directed against different sites on the coagulant molecule. Neither separately nor together do these antibodies inactivate the VIII:C to the extent of the mouse serum obtained from the spleen cell donor. The above observation reflects on the future potential for obtaining a large number of different antibodies from an apparently polyclonal response of the mouse to heterologous factor VIII:C.

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MATERIALS AND METHODS

Proteins

The initial immunogen used to raise the anti-VIII:C antibodies in the mice was the partially purified intact factor VIII complex previously described.¹³ Later, the animals received an additional injection of the preparation of von Willebrand factor deficient VIII:C obtained from von Willebrand factor-agarose chromatography.¹⁴ It contained approximately 16 U/ml of VIII:C and 8×10^{-4} U/ml of von Willebrand factor antigen as measured by the immunoradiometric assay according to a modification of the procedure of Ruggeri.¹⁵

The human antihuman VIII:C used as a probe for the porcine VIII:C was an IgG fraction which binds to DEAE at pH 8.0 in 0.01 M sodium phosphate buffer and has protein A recognition sites. It is equivalent to the acidic fraction of anti-VIII:C described in a previous communication from this laboratory.¹⁴ The antibody was obtained from a 19 yr old hemophiliac and contained 800 Bethesda Inhibitory Units/ml versus human plasma and 145 Bethesda Inhibitory Units versus porcine. After protein A-agarose absorption of the acidic IgG the antibody was recovered from the protein A-Sepharose in 0.1 M glycine hydrochloride, 0.5 M sodium chloride pH 3.0. This IgG contained 1200 Bethesda Inhibitory Units/ml, 400 units/mg of IgG, versus human and 162 Bethesda Inhibitory Units/ml, and 62 units/mg IgG, versus porcine plasma. The construction of a von Willebrand factor-agarose resin for the affinity purification of VIII:C is also described in our study of the protein bound to human antihuman VIII:C.¹⁴

The procedure involved first coupling denatured bovine serum albumin to agarose by cyanogen bromide activation of the agarose, followed by water soluble carbodiimide activation of the bovine serum albumin agarose and coupling of porcine von Willebrand factor at a rate of 20 U/ml of resin.

The acidic human antihuman VIII:C Ig recovered from protein A-Sepharose (Sigma, St. Louis, Mo.) was iodinated by the procedure of Hunter and Greenwood.¹⁶ One hundred μ g of Ig was reacted with 1 mCi of carrier-free 125 I (Amersham-Searle, Arlington Heights, Ill.) and the iodinated protein was separated from reaction products by gel filtration in 0.04 M sodium phosphate buffer pH 7.4 containing 0.1% bovine serum albumin (RIA grade) (Sigma). The labeled Ig, containing approximately 200 μ Ci of 125 I, was affinity purified on a column of 30 U of porcine VIII:C which had been dissociated from the von Willebrand factor by 0.25 M CaCl_2 and separated from the von Willebrand factor by gel filtration on 4% agarose. This factor VIII:C preparation was bound to Sepharose 4B by the cyanogen bromide method of March et al.¹⁷ The labeled protein bound to this resin was recovered by elution with 0.1 M glycine HCl buffer, pH 2.8, containing 0.1% bovine serum albumin. The effluent was collected into tubes containing 0.1 volume of 1 M Tris hydroxymethylaminomethane. Fractions containing more than 10% of the radioactivity recovered were pooled and diluted to 37,500 counts/min/ml prior to use as a probe for bound porcine VIII:C. The undiluted 125 I was stored frozen at -20° for up to 3 mo.

Assays and Screens

The factor VIII:C activity was measured in a one stage APTT¹⁸ using human severe hemophilic plasma as a substrate (George King Biomedical, Inc., Overland Park, Ks.). Citrated platelet poor plasma from a selected normal pig was used as a standard. To measure thrombin activated factor VIII:C (VIII:C_a), the sample containing the procoagulant activity was incubated with bovine α -thrombin, 2700 U/mg specific activity, which was a gift from Dr. K. G. Mann, Hematology Research, Mayo Clinic. This thrombin was stored as an

ammonium sulfate suspension and was dissolved, assayed, and diluted in imidazole buffered saline¹⁸ immediately prior to use. The concentrations of thrombin and the times of incubation used were determined to give maximum VIII:C_a activity for each set of conditions encountered. Samples of intermediate purity in dilute buffer were exposed to 1 U/ml of thrombin for 1 min. Samples in buffer containing up to 50% ethylene glycol required 10 U/ml thrombin for 10 min to achieve full expression of VIII:C_a activity. To measure the anti-VIII:C activity of serum, Ig, culture fluid, and ascitic fluid, a modification of the "Bethesda" assay was carried out using normal porcine plasma diluted to 50% in imidazole buffered saline.¹⁹ Samples were heated to 56° for 30 min, adsorbed with 0.1 volume of aluminum hydroxide suspension for 10 min and clarified by centrifugation at $3,000 \times g$ for 10 min. One volume of a sample was added to the diluted plasma and incubated for 1 hr at 37° . The VIII:C activity remaining after 1 hr was corrected for losses from a sample containing buffer in place of inhibitor.

Screening assays to distinguish potential monoclonal antibodies to VIII:C were carried out in 12×75 polystyrene tubes coated with rabbit IgG antimouse Ig (Cappel Laboratories, Cochranville, Pa.) purified by DEAE chromatography as described previously.¹³ Two hundred μ l of culture supernatant or ascitic fluid dilutions were incubated in these tubes for 4–6 hr at 4° . After three rinses in 0.05 M Tris, 0.10 M NaCl, 0.02% NaN_3 , pH 7.3, 200 μ l of a preparation of von Willebrand factor deficient VIII:C from dextran SO_4 -agarose, (0.25 U/ml VIII:C, <0.0015 U/ml, ristocetin von Willebrand factor) was added to these tubes and incubated at 4° for 24 hr. Following an additional three rinses, 200 μ l of a dilution of the porcine reactive human anti-human VIII:C 125 I Ig was added and incubated for 24 hr at 4° . The tubes were then washed and surveyed for bound radioactivity. All dilutions of VIII:C and labeled anti-VIII:C probe were made in the above buffer containing 0.1% BSA. Duplicate screens were also carried out with addition of 3% porcine plasma as a source of von Willebrand factor. Bound antigen was detected by the subsequent addition of labelled rabbit antiporcine von Willebrand factor as described in an earlier study on the production of monoclonal antibodies to porcine Willebrand factor.¹³

Immunization, Fusion and Cloning

Full descriptions of the fusion and cloning procedures used for the production of antihuman factor V and antiporcine von Willebrand factor monoclonal antibodies have been provided elsewhere^{2,13} and are applicable to this study. The mice used for the production of anti-VIII:C antibody received intraperitoneal injections of partially purified factor VIII complex adsorbed to aluminum hydroxide. Three injections of 2 units of von Willebrand factor and 0.2 units of factor VIII:C were given 2 wk apart. After an intervening 4 wk, an injection of 3.3 units of factor VIII:C from von Willebrand factor agarose¹⁴ on aluminum hydroxide was given and 3 days later spleen cells were harvested for fusion. When putative antibodies to factor-VIII:C were found on preliminary screens of culture supernatants, these hybrid colonies were plated and cloned by limiting dilution and rescreened. Cloned hybridomas maintaining the characteristics sought were grown as ascitic tumors as previously described.^{2,13}

RESULTS

The fusion of one mouse spleen with NS-1 cells yielded growth in 64 of 200 wells seeded. Supernatants from these 64 wells were assayed by the binding assay described in "Methods" and 12 were scored as positive, binding at least 3 fold background levels ($\text{Bkg} = 299$ counts/min of 125 I anti-VIII:C). Negative controls

for this assay were provided by mouse monoclonal antihuman factor V antibody and culture medium.

The 12 hybrids producing materials of tentative interest were further assayed for their ability to bind von Willebrand factor. The von Willebrand factor contamination of the VIII:C antigen used in the assay could give rise to false positive results by binding to anti-von Willebrand factor mouse antibodies and, thereby, immobilize VIII:C through the carrier effect. The use of paired assays using ^{125}I anti-VIII:C and ^{125}I anti-von Willebrand factor, indeed, indicated that some positive results in the VIII:C binding screen were potentially caused by mouse anti-von Willebrand factor antibodies. These results, as counts per minute for each assay, are given in Table 1. It can be seen that several hybrids gave strong responses to anti-VIII:C with limited or no response to anti-von Willebrand factor. Hybrids W3-1 through W3-8 were chosen to be cloned by limiting dilution. Two of 41 cloned colonies from W3-3 showed a positive response for VIII:C binding. For W3-4, one of 31 and for W3-8, one of 11 were positive. No antibody positive clones were obtained from the other hybrids. Table 2 shows the results from binding assays on three clonal isolates from W3-3, W3-4, and W3-8. Also included is medium from a culture well in which no growth occurred and, for comparison, a previously described anti-von Willebrand monoclonal antibody.¹³ It can be seen that while W3-4 and W3-8 bind proteins which react with anti-VIII:C, they also bind protein which in turn, binds anti-von Willebrand antibody. The anti-von Willebrand monoclonal antibody gives a completely different pattern of reactivity binding much more antigen detected with anti-von Willebrand than

Table 1. Anti-VIII:C and Anti-von Willebrand Factor Screening Assays: Hybrid Culture Medium

Culture	Assay* - CPM	
	^{125}I anti-VIII:C	^{125}I anti-WF
medium	333	246
anti-V	265	104
W3-1	1,651	231
W3-2	1,432	207
W3-3	2,592	119
W3-4	3,538	2,171
W3-5	1,270	1,460
W3-6	2,529	1,563
W3-7	1,216	1,407
W3-8	2,361	1,569
W3-9	1,147	1,421
W3-10	1,014	1,280
W3-11	2,537	2,514
W3-12	1,229	1,679

*- The antigen used in these assays had an activity ratio of VIII:C to ristocetin-von Willebrand factor of at least 167, based upon the lower limit of sensitivity for ristocetin-induced platelet aggregation.

Table 2. Anti-VIII:C and Anti von Willebrand Factor Screening Assays: Clonal Isolate Culture Medium

Clone #	Assay - CPM	
	^{125}I anti-VIII:C	^{125}I anti-WF
W3-3	2,740	514
W3-4	3,339	2,597
W3-8	2,454	2,553
No growth	334	674
W1-23*	908	3,515

*W1-23 is an anti-von Willebrand factor hybridoma, and this sample is a 10^5 -fold dilution of ascitic fluid.

with anti-VIII:C probes. These subclones from W3-3, W3-4, and W3-8 were grown as ascitic tumors. The ascitic fluid harvested from these mice were positive in the VIII:C binding assay at a minimum dilution of 1:100. The ascitic fluid of W3-3 was further diluted and was used to generate a titration curve indicating 50% binding at a dilution of 10^6 to 10^7 .

Fluid phase inactivation assays with ascitic fluids (Fig. 1) showed little inactivation of plasmatc VIII:C by W3-3 even when the undiluted antibody was incubated with an equal volume (not shown) of normal porcine plasma for 1 hr at 37° . The W3-4 and W3-8 antibodies produced only partial inactivation even at concentrations which appear to saturate the inhibitory effect.

The proteins in the ascitic fluids derived from hybridoma bearing mice were coupled to CNBr activated Sepharose.^{2,17} Columns containing 2 ml of gel bearing

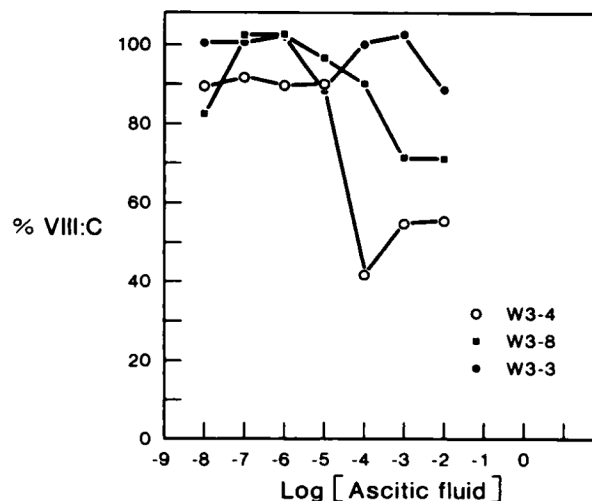


Fig. 1. Inhibition of plasma factor VIII:C (porcine) by ascitic fluid dilutions. The ascitic fluids were heated to 56° for 30 min and adsorbed with $\text{Al}(\text{OH})_3$ prior to dilution. The factor VIII:C remaining after 1 hr at 37° in the presence of ascitic fluid is plotted versus ascitic fluid concentration. Each point represents the average of two clotting time measurements, and each antibody is evaluated against an independent buffer control which corrects for nonimmunologic loss of factor VIII:C during incubation.

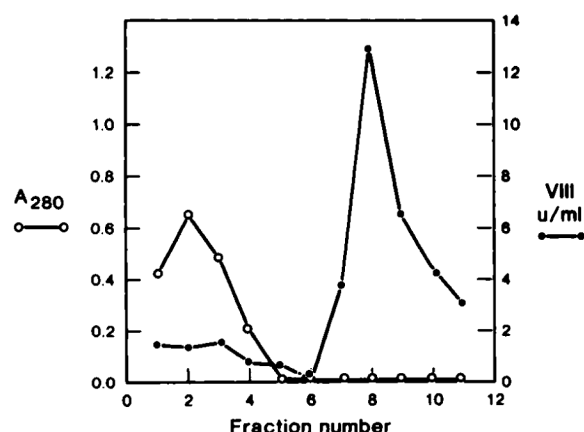


Fig. 2. The chromatography of porcine VIII:C on W3-3 agarose. The absorbance at 280 nm (○—○) and the level of unactivated factor VIII:C (●—●) are plotted versus fraction number. Fractions 1-6 contained 3.5 ml each and fractions 7-11 contained 1.6 ml each. A column containing 1 ml of resin (1 mg, W3-3) received 72 units of VIII:C in 8 ml of 0.25 M CaCl₂. Fraction 5 was a wash containing 2 M NaCl and fraction 6 was histidine buffer containing 0.1 M NaCl. Elution began with the application of 50% ethylene glycol in histidine buffer, after collection of tube 6. The flow rate was 15 ml/hr.

1 mg of protein per ml were exposed to partially purified VIII:C in 0.25 M CaCl₂. Over 95% of applied VIII:C activity, 18 ml containing 90 units, was bound by W3-3 in a single exposure at 15 ml per hr. Similar results were found with W3-4. The third antibody, W3-8, bound negligible VIII:C activity under these conditions. In other studies, W3-3 agarose could bind at least 60 units of factor VIII:C per ml of gel (Fig. 2). The active VIII:C bound to W3-3 in the presence of CaCl₂ could be recovered from the resin by elution with ethylene glycol 1:1 with 10 mM Histidine, 5 mM CaCl₂, 1 mM Benzamidine and 0.1 M NaCl pH 6.0 (Fig. 2). The activity recovered averaged 60% of that applied. Assuming a protein E₂₈₀^{1%} of 10, the peak of VIII:C activity from the W3-3 chromatography exhibited a specific activity of 6 U/μg. This represents a purification over plasma of about 3×10^5 . Gel electrophoretic analysis of the activity containing fractions showed protein bands similar to those seen in eluates from human anti-VIII:C-agarose columns.¹⁴ Further treatment of the W3-3 agarose with pH 2.8 glycine buffer¹⁴ removed a small additional amount of the same proteins. The column could be reused at least ten times.

The 50% ethylene glycol eluate contained protein staining bands with apparent molecular weights of 166,000, 130,000 and 76,000 (Fig. 3, gel A). Control agarose coupled with mouse monoclonal antihuman factor V released no protein bands upon elution with ethylene glycol or pH 2.8 glycine buffer. Treatment of the porcine VIII:C recovered by ethylene glycol elution

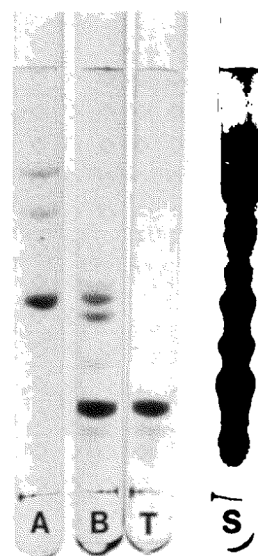


Fig. 3. SDS-polyacrylamide gel (5%) electrophoresis of material eluted from W3-3 agarose with 50% ethylene glycol. Gel A; 2 ml of ethylene glycol eluate containing 20 units of factor VIII:C activity. Gel B; similarly 2 ml of ethylene glycol eluate containing 20 units of factor VIII:C and 10 U/ml bovine II_a. Gel T; 20 units of bovine II_a. Gel S; ovalbumin 43,000, catalase 60,000, bovine serum albumin 69,000, β-galactosidase, 116,000 and myosin 210,000 daltons.

from W3-3 (diluted 1:40 in imidazole buffered saline) with one unit/ml of bovine thrombin for 1 min resulted in the enhancement of the clotting activity by 38 to 41 fold ($N = 3$). The factor VIII in ethylene glycol required additional thrombin and increased times of activation to reach full VIII:C_a activity. Electrophoretic analysis of the activated factor VIII:C by SDS polyacrylamide gel electrophoresis resulted in an altered banding pattern relative to unactivated factor VIII:C (Fig. 3, gel B). There was loss of the 130,000 and 166,000 dalton proteins. In addition to the persistent 76,000 dalton chain, a new major band was found at 67,000 and another band appeared at 50,000 daltons. The product precursor relationships, and the chain stoichiometries are not known with certainty. There is, however, good agreement with the pattern of protein chains seen with pH 2.8 elution of factor VIII:C_a from a column of human anti-VIII:C-agarose.¹⁴

Active VIII:C bound to W3-3 agarose could be inactivated by treatment of the resin for 10 min at room temperature with 10 mM histidine, 1 mM benzamidine, 5 mM EDTA, 100 mM NaCl pH 6.0. This EDTA buffer was collected and, after brief washing without EDTA, the column was eluted with 50% ethylene glycol as usual. The EDTA wash and ethylene glycol eluate were dialyzed against 0.2 M acetic acid, lyophilized, and analyzed by SDS polyacrylamide gel

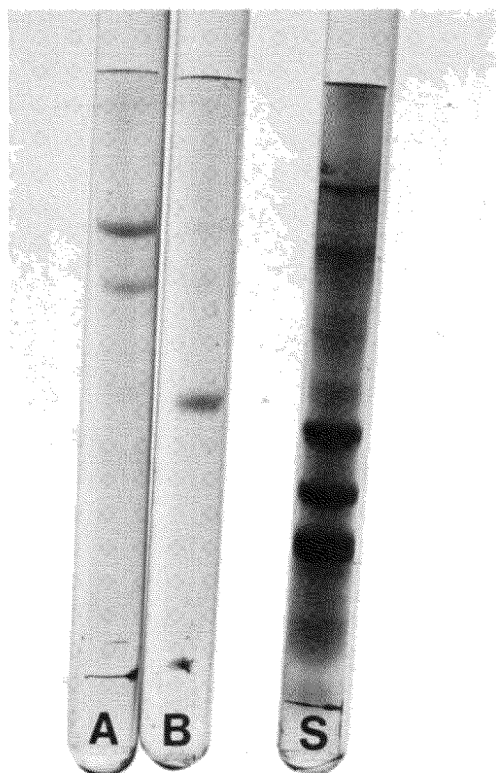


Fig. 4. SDS-polyacrylamide gel (5%) electrophoresis of material eluted from a 1 ml W3-3 agarose column. Thirty six units of factor VIII:C were bound by a 1 ml column of antibody agarose. Gel A; material eluted in four column volumes of 5 mM EDTA buffer. After the column was treated with EDTA, it was washed with four volumes of 2 M NaCl buffer and four volumes 0.1 M NaCl in histidine buffer. Gel B; the material eluted from the washed resin in eight column volumes of 50% ethylene glycol in histidine buffer. Gel S: ovalbumin 43,000, catalase 60,000, bovine serum albumin 69,000, β -galactosidase 116,000 and myosin 210,000 daltons.

electrophoresis. The 130,000 and 166,000 dalton chains were removed from the column by EDTA treatment (Fig. 4, gel A), while the 76,000 dalton chain was not. The 76,000 molecular weight peptide was eluted in 50% ethylene glycol (Fig. 4, gel B).

DISCUSSION

Three monoclonal antibodies to porcine factor VIII:C have been developed and detected through their binding of material reactive with human antifactor VIII:C antibody. The antigen used in the binding assay was a factor VIII:C preparation deficient in Willebrand factor.

One antibody (W3-3) does not inactivate VIII:C in porcine plasma. Although the monoclonal antibody is strongly positive in the VIII:C binding assay, it does not bind von Willebrand factor from porcine plasma.

This antibody reversibly binds VIII:C activity from von Willebrand factor deficient VIII:C. W3-4 partially inactivates VIII:C in porcine plasma, reacts strongly in the VIII:C screening assay, and binds some antigen recognized by anti-von Willebrand antibody. This antibody also binds VIII:C activity from solutions of dissociated VIII:C. A third antibody (W3-8) which gives screening results suggesting antiporcine VIII:C specificity does not remove VIII:C from solution although it does partially inactivate the coagulant in plasma. This antibody may be of low affinity, and we have not explored it fully. The mouse antibody, W3-3, binds the same protein species as are removed by human anti-VIII:C.¹⁴ The active VIII:C protein can be eluted with good yield by 50% ethylene glycol in buffer. This suggests the antigen antibody complex to be dependent to some significant degree on hydrophobic binding. The active eluted VIII:C is, on the average, 39 fold activatable by bovine thrombin. The activated protein is composed of peptide chains which are identical, within the resolution of SDS polyacrylamide gel analysis, with the VIII:C_a acid eluted from human antifactor VIII:C-agarose.¹⁴ Because the antiserum used in the previous experiments probably contained antibodies to all the chains, it could not be shown whether all the cleavage products remained noncovalently associated. Our evidence suggests that in porcine factor VIII:C the component chains (166,000, 130,000 and 76,000 daltons) are noncovalently associated through calcium bridges. The inactivation of VIII:C by EDTA is apparently analogous to that of factor V which has also been shown to dissociate when calcium is removed.²⁰

The isolation of the monoclonal antibodies and their preliminary use to probe the factor VIII:C structure has produced some interpretations not entirely consistent with previously reported VIII:C data. In gel filtration studies⁴ of human VIII:C antigen, an apparent molecular weight for the intact molecule has been estimated at 285,000. These data are in rough agreement with the SDS electrophoresis study of antigen-antibody complexes reported by Weinstein⁵ and the gel filtration data of purified bovine activity from Vehar and Davie.³ The three components which we see in SDS polyacrylamide gel electrophoresis comprise a molecular weight of 370,000 which is substantially larger than these others have reported. While this could be species difference, it is also possible that the 130,000 dalton chain in our preparation is a degradation product of the 166,000. Based upon dye binding in the gels, neither the 166,000 nor the 130,000 bands appear sufficient to achieve a 1:1 stoichiometry with the 76,000 molecular weight band. In addition, while we are certain that the 76,000 dalton chain contains a

unique antigenic site seen by W3-3, we do not have sufficient evidence to rule out antigenic similarity between the 130,000 and the 166,000. The porcine VIII:C prepared by our methods may then be a mixture of 166,000 + 76,000 and 130,000 + 76,000, the latter missing a 36,000 dalton region of the 166,000 dalton chain. What are perhaps profound species differences may become trivial if one accepts the 166,000 + 76,000 dalton structure of porcine VIII:C. A single, nearly symmetrical, cleavage in the 166,000 chain would yield a triplet similar to that seen in the bovine system.³ This possibility is supported by the structures of the thrombin activated protein. After thrombin cleavage similar chains are found in both species. The elucidation of subunit structure in the human protein has not been fully worked out. The gel filtration of dissociated human VIII:C and subsequent SDS polyacrylamide gel electrophoretic analysis of the active fractions has shown a major band at 79,000 and major VIII:C activation bands at 61,000, 51,000 and 18,000 daltons.²¹ The high molecular weight components are difficult to ascertain in the study reported by Switzer and McKee and the smaller thrombin cleavage products are not distinct in the gels which is a weakness of our study as well.

There have not been reports of investigations specifically designed to determine whether the immobilization of VIII:C from plasma causes the immobilization of some fraction of the von Willebrand factor. Knowledge of the interaction between these two molecules

would lead to this prediction, however. Immunologic studies on VIII:C inhibitors have suggested that heterologous antibody to factor VIII:C (coagulant inhibitor) contains specificities directed toward the von Willebrand factor.²² The binding of VIII:C by W3-3 does not appear to involve either of these phenomena as no von Willebrand factor is bound from plasma. The antibodies from W3-3 also do not bind VIII:C activity out of plasma, but rather potentially remove the activity from solutions of VIII:C from which the von Willebrand factor has been removed. These results suggest to us that the site of W3-3 interaction with the VIII:C (76,000 dalton chain) is at or near the site of binding of VIII:C to the von Willebrand factor.

The monoclonal antibodies described here were selected by methods not unique to the porcine protein and which should be applicable to the bovine and human system as well as additional specificities in the porcine protein. These reagents used in conjunction with our analytical approaches should aid in the isolation and understanding of the structure and function of factor VIII:C.

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