The Relationship of the Properties of Antihemophilic Factor (Factor VIII) That Support Ristocetin-Induced Platelet Agglutination (Factor VIII:R:RC) and Platelet Retention by Glass Beads as Demonstrated by a Monoclonal Antibody

By Kanji Ogata, Hidehiko Saito, and Oscar D. Ratnoff

A monoclonal antibody to human antihemophilic factor (AHF, factor VIII) was derived from BALB/c mouse spleen cells fused with P3x63Ag8 mouse plasmacytoma cells. This antibody, harvested from culture medium or ascites fluid, reacted with purified AHF and with plasmas from normal subjects or classic hemophiliacs, as measured by enzyme-linked immunosorbent assay (ELISA), but not with plasmas from patients with severe von Willebrand’s disease. The antibody possessed only IgG1 heavy chains and light chains. It blocked ristocetin-induced platelet agglutination and, to a lesser degree, platelet retention by glass bead columns, but it did not inhibit the procoagulant activity of AHF significantly. An amount of rabbit antiserum against AHF that provided equivalent inhibition of ristocetin-induced platelet agglutination inhibited glass bead retention much more effectively than the mouse monoclonal antibody. This difference was exaggerated in studies of the corresponding Fab fragments. These data suggest that the site or sites on the AHF complex molecule that are associated with ristocetin-induced platelet agglutination differ quantitatively or qualitatively from those associated with enhancement of platelet retention by glass beads. ELISA titers of immunoreactive AHF, using the monoclonal antibody, were closely correlated to those using rabbit antiserum against AHF in normal, hemophilic, and most von Willebrand’s disease plasmas.

Abbreviations used in this paper:
AHF (factor VIII) The molecular complex of antihemophilic factor that has procoagulant properties, contains antigens recognized both by homologous and heterologous antibodies against proteins related to AHF, and supports ristocetin-induced platelet agglutination and retention of platelets by glass bead columns.
Factor VIII:C The procoagulant property of AHF (factor VIII).
Factor VIII:R:RC The property of AHF-related proteins with cofactor activity in the ristocetin-induced agglutination of platelets. Current evidence equates factor VIII:R:RC (or factor VIII:RC) with factor VIII:VWF, that is, the agent, deficient in von Willebrand’s disease, that is required for vascular hemostasis.
Factor VIII:Ag AHF-related antigen, as detected by heterologous antiserum against AHF (factor VIII).
P-AHF Partially purified human antihemophilic factor.
ELISA Enzyme-linked immunosorbent assay.
PBS-Tween Phosphate-buffered saline (pH 7.2) containing 0.05\% (v/v) Tween 20.
BSA Bovine serum albumin
β-NF β-nerve growth factor
PL Platelet

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The collected void volume was concentrated to 4 ml. 

AHF Procoagulant Activity (Factor VIII:C) Assay

AHF procoagulant activity was measured as described earlier.7 One unit of factor VIII:C was arbitrarily defined as that amount present in 1 ml of pooled normal plasma.

Ristocetin Cofactor (Factor VIII:RC) Assay

Ristocetin cofactor (factor VIII:RC) in plasma was assayed by the method of Macfarlane et al.8 with the following modifications. Barbital-saline buffer was used to suspend formalin-fixed platelets. To increase the sensitivity of the aggrerometer (Platelet Aggregation Profiler, Bio Data Co., Horsham, Pa.), a ½ dilution of platelet suspension in buffer was used as a control (100% transmittance) rather than barbital-saline buffer alone. Ristocetin (Cutter Biological, Berkeley, Calif.) was used at a final concentration of 1 mg/ml in all studies. Inhibitory activity against factor VIII:RC was measured as described by Ruggeri et al.9 Pooled normal plasma, used as a control sample that had been diluted appropriately with barbital-saline buffer alone. Ristocetin (tetramethylpentadecane, Aldrich Chemical Company, Milwaukee, Wisc.) was injected into a 2-wk period, and 2 wk later, were injected intraperitoneally with a 2 x 10^6 cloned hybridoma cells per mouse.

Preparation of Partially Purified Human AHF

Partially purified human AHF was prepared by gel filtration of 150 U of commercial human AHF concentrate (FRACULATE, Armour Pharmaceutical Co., Kankakee, Ill.), dissolved in 10 ml imidazole-saline buffer, through a column (5 x 42 cm) of 4% agarose (Bio-Gel A15M, Bio-Rad Laboratories, Richmond, Calif.). The collected void volume was concentrated to 4 ml (175 μg protein/ml) by ultrafiltration, using an Amicon YM30 membrane (Amicon Corp., Lexington, Mass.). AHF to screen culture media was prepared in the same way, substituting Ultragel AcA 22 (LKB, Rockville, Md.) for Bio-Gel A15M.

Preparation of Monoclonal Antibodies

Murine monoclonal antibodies to partially purified human AHF were prepared essentially by Warren8's modification of the method of Kohler and Milstein.11 In brief, a 6-wk-old BALB/c mouse was injected intraperitoneally with 50 μg (0.29 ml) of partially purified AHF in an equal volume of complete Freund's adjuvant (GIBCO Laboratories, Grand Island, N.Y.) and reimmunized 3 wk later with 50 μg AHF emulsified in incomplete Freund's adjuvant (GIBCO). Four days later, single-cell suspensions derived from the mouse spleen were mixed with P3 x 63Ag8 mouse plasmacytoma cells and Lewis rat spleen cells immunologically primed with β-nerve growth factor (β-NGF). This monoclonal antibody binds to an antigenic determinant common to mouse β-NGF, snake venom (Naja naja)β-NGF, and human β-NGF.11

Rabbit Antiserum

Rabbit antiserum against human AHF and rabbit antiserum against human plasminogen were purchased from Calbiochem-Behring, LaJolla, Calif. and Behring Diagnostics, Somerville, NJ, respectively. Rabbit antiserum against mouse IgG k chains was obtained from Research Product International.

Preparation of IgG Fractions and Fab Fragments

In all experiments involving rabbit antiserum and monoclonal antibodies, IgG-rich fractions or Fab fragments were used instead of unfractionated antiserum or ascitic fluids. An IgG-rich fraction of rabbit antiserum against human AHF used to prepare alkaline-phosphatase-labeled antiserum was separated by octancionic acid extraction, 50% ammonium sulfate precipitation, and dialysis against barbital-saline buffer, as described earlier.14,15 For other purposes, IgG-rich fractions of murine ascitic fluids, rabbit antiserum against human AHF, and rabbit antiserum against human plasminogen were separated as follows. Two-milliliter aliquots were dialyzed against 0.02 M potassium phosphate buffer (pH 8) overnight at 4°C and chromatographed on 11 ml of DEAE Affi-Gel Blue (Bio-Rad) packed in a polypropylene syringe. The columns were eluted with the same buffer and proteins that were not adsorbed were pooled and concentrated by negative pressure dialysis against barbital-saline buffer and adsorbed with tricalcium phosphate (10 mg/ml) for 10 min at room temperature. After centrifugation at 2500 g for 10 min at 4°C, the supernatant was separated and heated at 56°C for 60 min. The amount of protein and IgG in the IgG fractions was assayed, respectively, by Lowry's
technique and by single radial immunodiffusion, using goat antiserum against mouse IgG (Miles-Yeda Ltd., Rehovoth, Israel) or goat antiserum against rabbit IgG (Antibodies, Inc., Davis, Calif.). Mouse IgG, purified by protein A-Sepharose column chromatography, was used as a standard. An IgG-rich fraction of rabbit antiserum against IgG κ chains, used to detect antibody-producing hybridoma clones, was prepared by affinity chromatography on mouse IgG-Sepharose. IgG was separated from normal mouse serum (Biologicals Inc., Rogers, Ark.) by affinity chromatography, using protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, N.J.). The normal mouse IgG was attached to cyanogen-bromide-activated Sepharose (Pharmacia) according to the instructions of the manufacturer. Rabbit antiserum against mouse IgG κ chains was adsorbed to and eluted from the IgG-Sepharose with 0.1 M glycine-HCl (pH 3.0), providing an IgG-rich fraction.

The Fab fragments of the IgG-rich fractions of murine monoclonal antibody and rabbit antiserum against AHF were prepared by published methods. Upon sodium deoxycholate polyacrylamide gel electrophoresis, both Fab fragments were found to be intact. The murine Fab contained 3.4 U antifactor VIIIR:RC/mg protein, and the rabbit Fab contained 4.0 U antifactor VIIIR:RC/mg protein.

**ELISA**

ELISA was used for selection of hybridoma clones that produced antibodies against AHF and for measurement of AHF-like antigens (factor VIIIR:Ag) in plasma. Alkaline phosphatase-labeled antibodies were prepared by the method of Engvall and Perlman from IgG-rich fractions of murine ascitic fluid, rabbit antiserum against human AHF, and rabbit antiserum against mouse IgG κ chains. The alkaline phosphatase was derived from calf intestine (type VII, Sigma Chemical Co., St. Louis, Mo.).

To detect the presence of antibodies against partially purified AHF in clones of hybridized cells, each well of a polystyrene microtiter plate (Flow Laboratories, Inc., Hamden, Conn.) was coated with 0.1 ml of partially purified AHF (80 μg protein/ml). Fifty microliters of the supernatant fluid of each clone was added to the wells, and antibodies that attached to the AHF were identified by addition of 70 μl of the alkaline phosphatase-labeled IgG-rich fraction of rabbit antiserum against mouse IgG κ chains (see above). The adherent alkaline phosphatase was measured by addition of 60 μl of p-nitrophenyl phosphate (Sigma), 1 mg/ml 10% diethanolamine buffer (pH 9.8).

AHF-like antigens (factor VIIIR:Ag) in plasma were measured by ELISA, using monoclonal mouse antibody or rabbit antibody, using the method of Voller et al. A polystyrene microtiter plate (Costar) was coated with 0.2 ml diluted IgG fraction in coating buffer (0.05 M sodium carbonate buffer, pH 9.6) for 2 hr at 37°C. The concentrations of IgG-rich fractions in the coating buffer were 100 μg/ml for rabbit antiserum and 25 μg/ml for murine monoclonal antibody. After washing 3 times with phosphate-buffered saline (pH 7.2) containing 0.15 M NaCl and 0.05% polyoxyethylene sorbitan monolaurate (Tween 20, Sigma) (PBS-Tween), 0.2 ml serially diluted normal pooled plasma or test plasma in PBS-Tween, with or without 6% BSA, was then added to each well and incubated at 37°C for 4 hr. The wells were washed 3 times with PBS-Tween. A quantity of 0.2 ml of the alkaline phosphatase-conjugated IgG fraction of either monoclonal antibody or rabbit antiserum, diluted in PBS-Tween, was put into each well and incubated at room temperature overnight. After washing with PBS-Tween 3 times, 0.2 ml of p-nitrophenyl phosphate was added to each well at room temperature. Fifteen minutes later, 0.05 ml 3 M NaOH was added to each well to stop the reaction, and absorbance at 405 nm was recorded spectrophotometrically. A linear relationship was observed between absorbance and the logarithm of the concentration of normal or test plasma. Each sample was assayed in triplicate at two different dilutions.

**Platelet Retention by Glass Bead Columns**

Platelet retention studies were performed by the method of Bowie et al. Lead glass beads (0.45–0.52 mm diameter, Arthur H. Thomas Co., Philadelphia, Pa.) were washed with distilled water and dried in a 37°C room for 2 days. Then, 2.6 g of glass beads were packed into a 29-cm long polyvinyl tube (0.133 x 0.022 inches, Insulon Medical, Haverhill, Mass.). A ml/mm adapter used in Thrombo-Screen Platelet Adhesion Columns (Cutter) and a plastic screen (No. 32800, Tetko Inc., Elmsford, N.Y.) were attached at each end of the column. All columns were stored in a dessicator for at least 24 hr at room temperature before use. Human venous blood (15.5 ml) was collected by a double syringe method through a 19-gauge butterfly needle (Abbott Hospitals, Inc., North Chicago, Ill.) into a 20-ml plastic syringe (JELCO Laboratories, Barritan, N.J.) containing 64 U of heparin sodium (beef lung, Upjohn Company, Kalamazoo, Mich.) and a predetermined amount of IgG fraction in 0.5 ml 0.15 M sodium chloride. The effect of monoclonal antibody was tested at 3 different final concentrations of anti-VIIIR:RC activity: 0.5 U, 0.2 U, and 0.03 U/ml of whole blood. The total amounts of IgG added to achieve these concentrations were 360 μg, 145 μg, and 20 μg, respectively. In control experiments, 360 μg, 154 μg, 81 μg, and 12 μg IgG derived from anti-flNGF were used. The effect of rabbit antibody was tested at a final anti-VIIIR:RC concentration of 0.03 U/ml. Similar experiments were performed substituting Fab fragments for the IgG-rich fractions of murine monoclonal antibody and rabbit antiserum.

After careful mixing with IgG, avoiding trapping of air, the blood was allowed to stand for 20 min at room temperature. Thereafter, 1 ml of blood was used for initial platelet counting and 3 ml of blood was passed through a glass bead column at a flow rate of 6.4 ml/min using a constant perfusion pump (Syringe Pump Model 341, Sage Instruments, Cambridge, Mass.). Each 1 ml of blood from the column was collected into a silicone-coated 10 x 75 mm polystyrene tube containing 0.1 ml of 4% dipotassium ethylenediaminetetraacetic acid (EDTA) in 0.15 M NaCl. The platelets in each sample of blood were enumerated in a Coulter Counter (Model 2BI, Coulter Electronics Inc., Hialeah, Fla.). Platelet (PL) retention was calculated as follows:

\[
\text{PL retention (\%)} = \frac{\text{Initial PL count} - \text{PL count in each tube}}{\text{Initial PL count}} \times 100
\]

**Buffers**

Barbital-saline buffer, pH 7.4, contained 2.76 g barbital, 2.06 g sodium barbital, and 7.3 g sodium chloride per liter. Phosphate-buffered saline (PBS), pH 7.2, contained 8.00 g sodium chloride, 0.20 g potassium chloride, 1.15 g disodium hydrogen phosphate, and 0.20 g potassium dihydrogen phosphate. Imidazole-saline buffer, pH 6.4, was 0.02 M imidazole in 0.15 M sodium chloride.

**RESULTS**

The Effect of Murine and Rabbit Antibodies on Ristocetin-Induced Platelet Agglutination (Factor VIIIR:RC)

Activity that inhibited ristocetin cofactor (factor VIIIR:RC) was present in the purified and concen-
The effect of antibodies on AHF procoagulant activity in normal plasma. Normal plasma (0.1 ml) was mixed with equal volumes of serially diluted monoclonal murine antibody or rabbit IgG antiserum in barbital-saline buffer and incubated at 37°C for 60 minutes. The residual factor VIII:C was then assayed and expressed as percent of the original activity. The concentration of the antibody is that before mixing with plasma.

Fig. 1. The effect of antibodies on AHF procoagulant activity in normal plasma. Normal plasma (0.1 ml) was mixed with equal volumes of serially diluted monoclonal murine antibody or rabbit IgG antiserum in barbital-saline buffer and incubated at 37°C for 60 min. The residual factor VIII:C was then assayed and expressed as percent of the original activity. The concentration of the antibody is that before mixing with plasma. (●—●) Monoclonal antibody, (○—○) rabbit antibody.
SOME PROPERTIES OF ANTIHEMOPHILIC FACTOR

Fig. 2. The effect of antibodies on factor VIII:Ag (ELISA) using rabbit antibody. Normal plasma, diluted 1/16 in PBS-Tween containing 0.1% BSA, was mixed with the same volume of serially diluted monoclonal antibody or rabbit IgG antiserum in PBS-Tween. After mixing, the titers of factor VIII:Ag in the mixtures were assayed by ELISA, using rabbit IgG antiserum to coat the wells and rabbit antiserum conjugated with alkaline phosphatase to detect antigen. The calibration curve for the quantitative measurement of factor VIII:Ag was made by serially diluting the normal plasma. The values of residual factor VIII:Ag are expressed as percent of original factor VIII:Ag. The concentration of the antibody is that before mixing with plasma. (•—•) Monoclonal antibody, (O—O) rabbit antibody.

The effect of murine and rabbit antibodies on platelet retention by glass bead columns. The addition of the IgG fraction of rabbit anti-AHF serum to whole blood at a final concentration of 0.03 U antifactor VIII:RC/ml decreased platelet retention by glass bead columns to 45% of the control value (p < 0.005 in the fourth and fifth milliliter filtered) (Fig. 4). When a similar experiment was performed using the IgG fraction of the murine monoclonal antibody, no significant effect on platelet retention was observed at a concentration of 0.03 U antifactor VIII:RC/ml (Fig. 5C). At higher antibody concentrations, however, the murine antibody blocked platelet retention significantly. A quantity of 0.2 U and 0.5 U of antifactor anti-AHF serum effectively blocked platelet retention in the assay system.

Fig. 3. The effect of antibodies on factor VIII:Ag (ELISA) using monoclonal antibody. The protocol was the same as in Fig. 2, but the study was performed using monoclonal murine antibody to coat the wells and, conjugated with alkaline phosphatase, to detect antigen. (•—•) Monoclonal antibody, (O—O) rabbit antibody.

Fig. 4. The effect of rabbit antiserum on platelet retention by a glass bead column. Heparinized whole blood of normal subjects mixed with rabbit IgG antibody against AHF or against plasminogen was filtered through a glass bead column (2.5 g beads) at a flow rate of 6.4 ml/min. Platelet retention was calculated in the second, fourth, and fifth aliquots of blood. "N" signifies the number of experiments. The vertical line represents the mean ± 1 SD. The final concentration of antifactor VIII:RC in the rabbit antiserum was 0.03 U (3.8 g IgG)/ml. The final concentration of IgG in the control rabbit antiserum against human plasminogen antiserum was 37 µg/ml. (•—•) Rabbit antiserum against AHF, (O—O) control antiserum against human plasminogen.
Fig. 5. The effect of monoclonal murine antibody on platelet retention by a glass bead column. The technique used was the same as in Fig. 4, using monoclonal murine antibody against AHF or against βNGF. The final concentrations of antibody against factor VIIIR:RC were (A) 0.5 U (24 μg IgG)/ml; (B) 0.2 U (10 μg)/ml; and (C) 0.03 U (1.3 μg)/ml. The final concentration of control monoclonal IgG (anti-βNGF) was (A) 10 μg/ml; (B) 5.4 μg/ml; and (C) 0.8 μg/ml. The mean values of the fourth and fifth ml filtered were compared statistically with control values. (●●●●) Monoclonal antibody against factor VIIIR:RC; (-----) control antibody against βNGF.

VIIIR:RC/ml whole blood decreased platelet retention to 76% (p < 0.01) and 36% (p < 0.005) of the control values, respectively (Fig. 5A and B). Thus, rabbit antiserum was much more potent in blocking platelet retention by glass bead columns than the monoclonal antibody when compared in terms of units of factor VIIIR:RC inhibition.

To determine whether the greater effect of rabbit antiserum was due to its precipitating nature, the Fab fragments of this antiserum and of monoclonal antibody were tested for their ability to suppress glass bead retention of platelets. At 0.5 U antifactor VIIIR:RC/ml whole blood, the murine monoclonal Fab did not block platelet retention at all, whereas the rabbit antiserum Fab, at a concentration of 0.2 U/ml, inhibited retention significantly under the same conditions. Similar results were obtained in duplicate experiments with fresh preparations of the two Fab fragments.

**Patient Studies Using Monoclonal Antibody**

The titers of factor VIIIR:Ag measured by monoclonal antibody ELISA using monoclonal antibody for coating and detection were compared with those of other factor VIII-related properties in the plasmas of the 15 normal subjects, 10 patients with classic hemophilia, and 16 patients with von Willebrand's disease. In normal subjects, all four titers were closely related to each other (Table 2). The levels of VIIIR:Ag measured by monoclonal antibody ELISA were very close to those measured by rabbit antibody ELISA (correlation coefficient r = 0.89, p < 0.001). In patients with von Willebrand's disease, the levels of factor VIII:C were usually higher than those of other AHF-related functions (Table 3). When analyzed as a group, the factor VIIIR:Ag levels as measured by ELISA using monoclonal antibody were closely related to the titer of factor VIIIR:RC and to factor VIIIR:Ag as measured by ELISA using rabbit antiserum. The correlation coefficient (r) between monoclonal antibody ELISA and rabbit antibody ELISA was 0.55 (p < 0.05). In 4 patients (nos. 1–4), however, the titer of factor VIIIR:Ag was much lower using monoclonal antibody than rabbit antiserum. In hemophiliacs, all titers except factor VIII:C were normal or elevated and correlated with each other (Table 3). The levels of factor VIIIR:Ag measured by monoclonal antibody ELISA were similar to those
Table 3. Comparisons of Factors VIII:C, VIIIR:RC, and VIIIR:Ag in Patients With von Willebrand’s Disease and Classic Hemophilia

<table>
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<th>Subject</th>
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<th>VIIIR:RC (U/dl)</th>
<th>Rabbit Ab (U/dl)</th>
<th>Monoclonal Ab (U/dl)</th>
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*Von Willebrand’s disease. LM and DM were related, as were DS and AT, SK and FK, and DI, MI, CI, and JI.

estimated by rabbit antiserum ELISA (correlation coefficient $r = 0.83$, $p < 0.001$).

DISCUSSION

The experiments reported here describe the properties of a monoclonal antibody against factor VIIIR:RC that was generated by stabilized and subcloned hybridoma cells produced by the fusion of a mouse myeloma cell and the spleen cell of a mouse that had been immunized with partially purified human antihemophilic factor (AHF). Assuming that a monoclonal antibody reacts with only one antigenic site, this monoclonal antibody appeared to be directed toward an antigen at or near the critical portion of the AHF molecule required for ristocetin-induced platelet agglutination.

A current view is that AHF is composed of two dissociable subcomponents, one of lower molecular weight with procoagulant activity, and another of higher molecular weight that contains the bulk of protein and supports ristocetin-induced platelet agglutination and platelet retention by glass bead columns. Much of our knowledge about the structure and function of AHF has been obtained with the use of specific heterologous antiserum and human circulating anticoagulants against AHF. Thus, a rabbit immunized with purified human AHF produces precipitating antibodies that block the procoagulant activity of AHF as well as ristocetin-induced platelet agglutination and platelet retention by glass bead columns. In contrast, human circulating anticoagulants inhibit the procoagulant properties of AHF, but not its platelet-associated functions. Furthermore, rare patients with severe von Willebrand’s disease develop a precipitating antibody directed against AHF that block ristocetin-induced agglutination. Whether this type of antibody blocks procoagulant activity has been debated.

The interrelationship between two platelet-related activities of AHF, the capacity to support platelet agglutination by ristocetin and the ability to support glass bead retention, has not been clarified by the use of polyclonal heterologous antibodies. In the present studies, we have investigated the effect of this monoclonal antibody upon several functions of AHF in comparison to rabbit antiserum against AHF. Their characteristics differed. Rabbit antisera contain precipitating antibodies and usually block the procoagulant activity of AHF (factor VIII:C). In contrast, the monoclonal antibody was nonprecipitating and did not inhibit AHF procoagulant activity significantly. Both the monoclonal antibody and rabbit antiserum inhibited ristocetin-induced platelet agglutination. Relative to this property, however, the rabbit antiserum was more effective than murine antibody in inhibiting platelet retention by glass bead columns. Conceivably, the greater inhibitory effect of rabbit antiserum in this assay was due to the precipitating nature of its constituent antibodies. In fact, the Fab fragments of rabbit antiserum inhibited platelet retention, while those of the murine antibody did not block this phenomenon at the concentrations tested. One explanation for this difference is that the rabbit antiserum contained antibodies directed against multiple sites on the AHF molecule that participate in the phenomenon of platelet retention. Thus, it is tempting to speculate that the site or sites on the AHF complex molecule that are associated with ristocetin-induced platelet agglutination are not identical to those associated with enhancement of platelet retention by glass bead columns. We recognize that there is a spectrum of rabbit antisera...
against AHF that contain different sets of antibodies and that we used one rabbit antiserum in the present study. We believe, however, that our conclusion is valid, since the monoclonal and rabbit antibodies were compared by their ability to interfere with glass bead column retention when used at equivalent potencies standardized by ristocetin-induced platelet agglutination.

We are familiar with several previously reported studies of monoclonal antibodies against AHF. Muller and colleagues27 successfully produced such antibodies against the procoagulant low molecular weight subcomponent of human AHF (factor VIII:C) and showed that these did not inhibit ristocetin-induced platelet agglutination. Katzman et al.28 prepared monoclonal antibodies to porcine von Willebrand’s factor. Bowie et al.29 demonstrated that the i.v. infusion of these antibodies prolonged the in vivo bleeding time without alteration of factor VIIIR:RC. Meyer et al.30 made 7 monoclonal antibodies against human AHF, none of which inhibited its biologic functions. More recently, she31 reported that antibodies directed against factor VIIIR:RC did not block the adhesion of platelets to exposed subendothelial surfaces. Taken together with the results reported here, Bowie and Meyer’s studies suggest that the property that supports ristocetin-induced platelet agglutination may differ in some respects from those involved in the adhesion of platelets to endothelial surfaces or in the retention of these cells by glass bead columns. Assuming that the antibodies are truly monoclonal, perhaps the expression of these various properties depends on the number or configuration of the subunits that make up the high molecular weight subcomponent of AHF. Alternatively, different portions of the complex molecule of AHF may be involved in the several platelet-related phenomena. These observations gain interest because of the occasional discordance between the bleeding time and the results of in vitro assays in von Willebrand’s disease.12

The levels of factor VIIIR:Ag measured by ELISA using monoclonal antibody were in good agreement with the titers of other factor VIII-related properties, factor VIII:C, factor VIIIR:Ag measured by ELISA employing rabbit antiserum, and factor VIIIR:RC. In patients with the usual form of von Willebrand’s disease, the levels of factor VIIIR:Ag measured by monoclonal antibody also correlated well with factor VIIIR:RC and factor VIIIR:Ag levels measured by rabbit antibody but not as closely with factor VIII:C. These von Willebrand’s disease patients had long bleeding times, suggesting that the monoclonal antibody reacted with the portion of AHF that is important for primary homostasis. In 4 patients with von Willebrand’s disease (nos. 1–4 in Table 3), the titer of factor VIIIR:Ag measured by monoclonal antibody was much lower than that by rabbit antibody. Although the reason for this discrepancy is not clear at present, it is possible that their factor VIIIR:Ag may be structurally different from those in usual von Willebrand’s disease patients. In hemophiliacs, the levels of factor VIIIR:Ag assayed by the monoclonal antibody and by rabbit antibody were similar. This suggests that it may be possible to use a monoclonal antibody against factor VIIIR:RC in the diagnosis of the carrier state of classic hemophilia.

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REFERENCES

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The relationship of the properties of antihemophilic factor (factor VIII) that support ristocetin-induced platelet agglutination (factor VIII R:RC) and platelet retention by glass beads as demonstrated by a monoclonal antibody

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