Precipitating Antibodies to Factor VIII/von Willebrand Factor in Von Willebrand’s Disease: Effects on Replacement Therapy

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Precipitating antibodies to factor VIII/von Willebrand factor can develop in patients with severe homozygous-like von Willebrand’s disease following multiple transfusions with blood derivatives. This study of 4 patients treated with cryoprecipitate for 13 different bleeding episodes demonstrates that the occurrence of such antibodies interferes with the management of the disease. The control of mucosal bleeding was poor, whereas more favorable responses were obtained in soft-tissue hemorrhages. These findings probably relate to failure of replacement therapy to shorten the prolonged bleeding time. Immediately after treatment, measurement of plasma factor VIII/von Willebrand factor-related antigen and ristocetin cofactor showed either no increase, or very low values, depending on the pre-infusion antibody titer. Levels of the factor VIII/von Willebrand factor-related procoagulant activity in the circulation were also lower than predicted and usually there was no evidence of the delayed and sustained rise typically observed in uncomplicated von Willebrand’s disease. An anamnestic rise in antibody titer appeared 6–15 days after treatment and showed no obvious relationship with the amount of cryoprecipitate infused. Replacement therapy invariably caused severe side effects during, or immediately after, concentrate infusion. The results of in vitro studies support the view that these reactions were due to the appearance of circulating immune complexes.

CIRCULATING INHIBITORS directed against factor VIII/von Willebrand factor (FVIII/vWF) have been described recently in nine patients with severe congenital von Willebrand’s disease (vWD).1–7 These inhibitors blocked the ristocetin cofactor activity expressed by human FVIII/vWF (VIIIIR:RCo), whereas factor VIII procoagulant activity (VIII:C) was partially inactivated in a nonspecific manner.7 The inhibitors showed the properties of heterologous IgG antibodies and a precipitin reaction between antibody and FVIII/vWF-related antigen (VIIIIR:Ag) was demonstrated in six of the reported cases.1–7 It is not well established whether these antibodies, as a consequence of their specific interaction with FVIII/vWF, complicate the management of patients with severe vWD. This report contains clinical information regarding the course of antibodies in 4 patients, and describes our experience in the treatment with cryoprecipitate of 13 bleeding episodes.

MATERIALS AND METHODS

Patients

Four patients with antibodies to FVIII/vWF were investigated: three (G.E., G.S., and G.T.) belonged to the same kindred, whereas the fourth patient (S.G.) was unrelated to them. They had a severe bleeding tendency characterized by frequent episodes of mucosal, joint, and soft-tissue bleeding that required multiple transfusions with whole blood, plasma, or cryoprecipitate. Prior to treatment, the patients had no measurable plasma levels of VIIIIR:Ag (<0.0001 U/ml) or VIIIIR:RCo (<0.03 U/ml), and very prolonged bleeding time (>30 mm); VIII:C was detectable but very reduced (0.02, 0.03, 0.03, and 0.02 U/ml in patient G.E., G.S., G.T., and S.G., respectively). Family findings and other details are reported elsewhere.7 Controls used in these studies were 3 patients with severe VWD presenting with clinical and laboratory findings similar to those of the 4 patients but without measurable antibody.

Clinical Studies

Patients with antibody were treated on 13 occasions with blood-bank or commercial lyophilized cryoprecipitate (Kabi AB, Stockholm, Sweden). These were employed for spontaneous bleeding episodes (2 hemarthroses, 2 muscular hematomas, 6 gastrointestinal hemorrhages, and 1 severe epistaxis) and before 2 dental extractions. During the period of the study, the control patients were similarly treated for 8 episodes of spontaneous bleeding (2 hematomas, 4 gastrointestinal hemorrhages, and 2 epistaxes similar in severity to those observed in patients with antibody).

The concentrates were reconstituted following the manufacturer instructions, assayed for content of FVIII/vWF related activities and administered intravenously through a 19-G scalp needle over 15–30 min. Blood specimens were obtained prior to, 30 mm, 6 hr, and 24 hr after the completion of each infusion. Additional samples were taken at varying intervals to study the effect of replacement therapy on circulating immune complexes and antibody titers. VIII:C, VIIIIR:Ag, and VIIIIR:RCo plasma levels measured 30 min after infusion were compared with the predicted values. It was assumed that there was a homogeneous distribution in plasma volume of the transfused FVIII/vWF-related activities. Plasma volume was calculated using a mean value of 41 ml/kg body weight corrected for the hematocrit (Documenta Geigy, Scientific Tables).

Methods

Preparation of plasma and serum; preparation of reference plasma; assays of VIII:C, VIIIIR:Ag, VIIIIR:RCo in cryoprecipitate and patient plasma and bleeding time have been described in detail in a previous publication.7

Assay of the antibody potency towards VIIIIR:RCo (anti-VIIIIR:RCo) was carried out as described by Ruggeri et al.7 In brief,

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pooled normal plasma used as a source of VIIIIR:RC0 was mixed with equal volumes of patient plasma or plasma from a patient with severe vWD without antibody. After incubation for 15 min at 37°C, residual VIIIIR:RC0 was assayed. A calibration curve was drawn for each antibody sample by plotting the values of residual VIIIIR:RC0 (expressed as percentage of the control mixtures) versus the logarithm of the corresponding plasma dilution. One unit of antibody (anti-VIIIIR:RC0) was arbitrarily defined as the antibody concentration leaving 25% residual VIIIIR:RC0 in these experimental conditions. The antibody potency towards VIIIIR:Ag (anti-VIIIIR:Ag) was defined by measuring the ability of test samples to prevent binding of VIIIIR:Ag to rabbit anti-FVIII/vWF antiserum. After incubation for 15 min at 37°C, the tubes were washed. 125I-labeled anti-FVIII/vWF was added and the IRMA procedure was followed as described. A linear relationship was observed between the logarithm of antibody plasma dilution and the amount of bound VIIIIR:Ag, expressed as a percentage of a control mixture containing plasma from a severe vWD patient without antibody. One unit of antibody (anti-VIIIIR:Ag) was arbitrarily defined as that concentration that blocks the binding of 50% of the initial VIIIIR:Ag. More details of the method were previously reported.

Circulating immune complexes were measured by a semiquantitative assay based on the precipitation of complexes from serum with 2% polyethylene glycol (PEG) followed by the measurement of the amount of IgG and Clq in the precipitate. In brief, diluted normal plasma was used as a source of VIIIIR:Ag. After incubation for 18 hr at 37°C, the tubes were washed. 125I-labeled anti-FVIII/vWF was added and the IRMA procedure was followed as described. A linear relationship was observed between the logarithm of antibody plasma dilution and the amount of bound VIIIIR:Ag, expressed as a percentage of a control mixture containing plasma from a severe vWD patient without antibody. One unit of antibody (anti-VIIIIR:Ag) was arbitrarily defined as that concentration that blocks the binding of 50% of the initial VIIIIR:Ag. More details of the method were previously reported.

RESULTS

Clinical Effects of Replacement Therapy

In the majority of 13 treated bleeding episodes the clinical response to replacement therapy was less prompt than that observed in patients with uncomplicated severe vWD treated for similar hemorrhages.

| Table 1. Response to Cryoprecipitate Infusion in Patients With Severe vWD and Precipitating Antibodies to FVIII/vWF |
|---|---|---|---|---|---|---|---|---|
| Patient Epistaxis Melena 22 16 10 32 21 0.04 <0.0001 Poor | 23 15 0.11 <0.0001 Poor | 0.50 | 0.96 | 0.76 |
| 3. Hemarthrosis left knee 10 31 28 0.10 <0.0001 Poor | 0.17 | 0.06 | 0.39 |
| 4. Extraction of 4 teeth 35 89 72 0.18 0.02 Poor | 0.77 | 1.97 | 1.60 |
| 5. Melena 120 44 15 32 25 0.11 <0.0001 Poor | 0.27 | 0.58 | 0.45 |
| 6. Melena 2 3 10 36 30 0.08 0.02 Poor | 0.18 | 0.61 | 0.54 |
| 7. Melena 2 1 40 77 53 0.51 0.32 Poor | 0.76 | 1.48 | 1.01 |
| 8. Deltoid hematoma 4 3 10 72 49 0.11 0.07 Good | 0.24 | 1.75 | 1.19 |
| 9. Epistaxis 3 2 25 ND ND 0.26 0.03 Poor | (0.52) | -- | -- |
| 10. Melena 17 8 20 ND ND 0.13 <0.0001 Poor | (0.37) | -- | -- |
| 11. Extraction of 2 teeth 62 36 50 89 72 0.11 0.09 Poor | 1.19 | 2.11 | 1.17 |
| 12. Hemarthrosis left knee 24 17 25 39 29 0.11 0.01 Poor | (0.60) | (0.96) | (0.70) |
| 13. Calf hematoma <0.8 <0.1 40 85 77 0.41 0.49 Poor | (0.97) | (2.07) | (1.87) |
| Control Hematoma (n = 2) <0.8 <0.1 10-40 32-96 31-61 0.18-0.92s 0.41-1.09 0.29-0.88 Poor | (0.22-0.97) | (0.53-1.47) | (0.47-1.08) |

*Figure in parentheses show the predicted values (see Clinical Studies in the text for more detail).
†Response was defined as "poor" when mucosal bleeding did not stop within 8-12 hr after treatment, hemoglobin levels continued to drop and more than 4 U of packed red cells were needed. Response to treatment of hematomas and hemarthroes was defined as "good" when swelling and pain subsided within 8-12 hr after treatment; in dental extractions a good response was characterized by no bleeding from the sockets 24 hr after surgery.
§Not done.
*Range of observed and predicted values (the letter are shown in parentheses).
There was a particularly poor response following seven episodes of mucosal bleeding (melena and epistaxis) and multiple transfusions of packed red cells were needed to control progressive anemia despite the administration of cryoprecipitate. In contrast, treatment of four episodes of soft-tissue bleeding (hematroses and muscle hematomas) showed a favorable clinical response with rapid decrease of swelling and pain. After dental extractions, formation of hemostatic clots was slightly slower than that observed in vWD patients without antibody and mild oozing from the sockets was observed for 12-16 hr postoperatively.

During cryoprecipitate infusion, patients with antibody experienced severe side effects characterized by lumbar and abdominal pain with hypotension (systolic blood pressure: 60–80 mm Hg). These symptoms were not accompanied by fever and skin manifestations, appeared shortly after the infusion was started, and persisted up to the end of infusion. The use of i.v. hydrocortisone (0.3–0.5 g) decreased the intensity of symptoms without completely abolishing them. Control patients without antibody did not experience similar symptoms after replacement therapy.

### Effects of Replacement Therapy on the FVIII/vWF-Related Activities

The 30-min postinfusion plasma levels of VIIIIR:Ag and VIIIIR:RCo were usually related both to the pre-infusion levels of anti-VIIIIR:Ag and anti-VIIIIR:RCo, and to the dose of cryoprecipitate administered. In patient G.E., for example, who presented with moderate to high levels of both anti-VIIIIR:Ag and anti-VIIIIR:RCo before treatment, there was little or no increase in plasma levels of VIIIIR:Ag and VIIIIR:RCo following administration of varied dosages of cryoprecipitate (Table 1 and Fig. 1). In patient G.S., measurable plasma levels of VIIIIR:Ag and VIIIIR:RCo were attained by administering large doses of VIIIIR:Ag and VIIIIR:RCo that probably neutralized the high inhibitor titers (bleeding episode 11). On the contrary, in patients G.T. and S.G., elevated plasma levels of VIIIIR:Ag and VIIIIR:RCo were obtained when preinfusion levels of both anti-VIIIIR:Ag and anti-VIIIIR:RCo were 2 U/ml or below (episodes 7 and 13, see Table 1 and Fig. 1). In all instances, the postinfusion levels of VIIIIR:Ag and...
VIIIIR:RCo were much lower than predicted from assaying these activities in cryoprecipitate. Similarly, they were lower than in vWD patients without antibody treated with comparable doses (Table 1).

Although the postinfusion increase in plasma VIII:C was more consistent than that of VIIIIR:Ag and VIIIIR:RCo, the immediate post-treatment levels were lower than predicted from the actual measurements of infused VIII:C (Table 1). VIII:C elevation was transient and a sustained rise was not observed in the majority of cases (Fig. 1). Following treatment, only patients G.T., S.G. and, to a lesser extent, G.S. (episodes 7, 13, and 11, respectively) showed a sustained increase of VIII:C at a time when measurable levels of VIIIIR:RCo were observed (Fig. 1).

In three severe vWD patients without antibody treated for eight bleeding episodes, post-infusion VIII:C, VIIIIR:Ag, and VIIIIR:RCo plasma levels were close to the predicted values (Table 1) and elevated VIII:C persisted for several hours (data not shown).

In all patients with antibody the bleeding time was always recorded as being longer than 30 min both before and half an hour after infusion, whereas it became normal (less than 7 min) in vWD patients without antibody.

Effects of Replacement Therapy on the Antibody Potency

Plasma levels of anti-FVIII/vWF antibodies were markedly influenced by replacement therapy. Since a similar pattern was observed in all patients, only two representative examples of short and long-term changes in antibody titers are illustrated in Fig. 2. Thirty minutes after infusion of cryoprecipitate there was a substantial, parallel decrease in the titers of both anti-VIIIIR:Ag and anti-VIIIIR:RCo (Fig. 2A). After a few hours the antibody titers rose slowly towards pretreatment levels, but an anamnestic rise was generally observed 3–6 days after infusion, with peak levels usually attained during the second week (Fig. 2A). In one instance (Patient G.E.; episode 5) an anamnestic rise was not observed when replacement therapy was given 13 days after a previous treatment and the antibody titer was approaching the peak of the previous anamnestic response (Fig. 2B). No clear relationship was found between the amount of FVIII/vWF related activities administered with cryoprecipitate and the degree of the anamnestic rise (Fig. 2B). In the absence of replacement therapy, both anti-VIIIIR:Ag and anti-VIIIIR:RCo levels declined slowly until the patient was again challenged with cryoprecipitate (Fig. 2B). In one patient (S.G.) a transfusion-free period of 23 mo was long enough for the antibody to fall to unmeasurable levels. A prompt anamnestic rise was observed, however, after the next treatment in this patient (episode 13, antibody levels not shown).

Effects of Replacement Therapy on Circulating Immune Complexes

The presence of IgG- and Clq-containing soluble immune complexes was serially tested in serum
and very low levels of anti-VIIIR:Ag antibody were detected prior to treatment. In contrast with patient G.S., relatively high levels of VIIIR:Ag were achieved after the infusion, coincident with the fact that little IgG-containing complexes and no Clq-containing complexes were present. By day seven, there was a 500-fold increase in anti-VIIIR:Ag titer coincident with the detection of both IgG and, to a lower extent, Clq-containing immune complexes. In 3 similar episodes in 3 severe vWD patients without antibody, PEG-precipitable IgG and Clq were not found in post-transfusion samples obtained at the same intervals (data not shown).

**DISCUSSION**

The appearance of antibodies directed against FVIII/VWF greatly complicates the management of bleeding episodes in severe vWD. In accordance with the transfusion studies performed in two patients by Stratton et al. and Egberg and Blomback, we found that, following infusion of cryoprecipitate, plasma levels of VIIIR:Ag and VIIIR:RCo showed either no increase or a lower than expected recovery, depending on the pre-infusion antibody titer. After treatment of 13 bleeding episodes in 4 patients with antibody, levels above 0.10 U/ml of either of the FVIII/VWF related activities were obtained in only 2 instances (episodes 7 and 13). These were both when specific antibody potencies were 2 arbitrary U/ml or less and relatively high dosages of cryoprecipitate were administered. VIIIR:RCo plasma levels were always lower than those of VIIIR:Ag, even when similar total amounts of VIIIR:RCo were infused. VIII:C levels, though less than expected, were higher than those of VIIIR:RCo and VIIIR:Ag despite a consistently lower total activity in the concentrates (Table 1 and Fig. 1). These clinical observations confirm previous in vitro findings on the different reactivities of the antibodies against the two moieties present in the FVIII/vWF complex.

From the clinical point of view, the management of soft-tissue or joint hemorrhages was satisfactory and appeared to be a result of the increase in VIII:C levels. The slightly excessive bleeding observed after dental extractions was compatible with the levels of VIII:C attained in plasma. The inability of replacement therapy to shorten the bleeding time was paralleled by a poor control of mucosal bleeding. These observations suggest that antibodies appearing in vWD block the FVIII/vWF activity involved in primary hemostasis. They further support previous suggestions that the bleeding time is the most critical parameter to monitor in the management of mucosal hemorrhages, whereas the outcome of soft-tissue and postoperative bleeding is mainly related to the achievement of critical VIII:C plasma levels.
The attainment of sufficient and persistent plasma levels of VIIIIR:RCo after cryoprecipitate infusion was essential for a sustained rise of VIII:C to occur. When the antibody levels were high enough to cause a rapid disappearance of this infused activity from the circulation, no sustained rise in VIII:C plasma levels was observed. In contrast, when the antibody level was lower and if enough concentrate was administered, measurable VIIIIR:RCo and sustained VIII:C levels were observed after infusion in patients G.T., S.G. and, to a lesser extent, G.S. These had previously failed to show such a response of the kind typically observed in vWD patients without antibody. In accordance with the in vitro studies of Weiss et al., our findings suggest that persistent levels of infused FVIII/vWF (or at least of the moiety carrying VIIIIR:RCo determinants) are necessary to stabilize VIII:C and prevent its inactivation in plasma. Alternatively, persistent circulating levels of infused FVIII/vWF might be necessary in VWD to stimulate the sustained production of VIII:C.

Our previous studies have shown that the anti-FVIII/vWF inhibitors appearing in VWD after replacement therapy are polyclonal IgG antibodies. The rise in antibody titer found in our patients 6–15 days after cryoprecipitate infusion is compatible with an anamnestic response and is additional evidence for the immunologic nature of such inhibitors. No clear relationship was observed between the magnitude of the antibody rise and the amount of the immunogen infused or the initial antibody titer. However, no further increase in antibody was seen when one patient (G.E.) was challenged with another dose of cryoprecipitate at the peak of the anamnestic response. Altogether, the immune response to FVIII/vWF observed in these VWD patients had the typical features of a secondary response to a foreign multideterminant antigen.

Severe side effects, such as lumbar and abdominal pain and hypotension, occurred in patients 10–15 min after the infusion was started and persisted up to 1–2 hr. Steroid therapy was effective in reducing the intensity of the symptoms. In vitro studies showed that the infusion of cryoprecipitate into a patient with high anti-FVIII/vWF titers resulted in the appearance of large amounts of 2% PEG precipitable IgG and Clq in serum, presumably immune complexes; in a fourfold decrease in antibody potency, and in a rapid clearance of VIIIIR:Ag from plasma. At the time of maximal Clq precipitation, complexes were in slight antibody excess. The persistence of IgG but not of Clq-containing complexes 36 hr after infusion may reflect a progressive shift to large antibody excess and the loss of the complement fixing ability of circulating complexes. In contrast, cryoprecipitate infusion into a patient with little circulating anti-FVIII/vWF antibody produced a higher VIIIIR:Ag level and significant amount of immune complexes were detected only when the secondary immune response occurred 6 days after infusion. The additional finding that complexes did not occur after cryoprecipitate infusion into three severe vWD patients without detectable antibody substantiates the relationship between in vivo immune complex formation and the simultaneous presence in the circulation of both VIIIIR:Ag and precipitating anti-VIIIIR:Ag antibody, and makes it unlikely that the complexes would involve some unrelated antigen present in the transfused concentrate.

Circulating immune complexes containing anti-VIIIIR:C antibodies have recently been demonstrated by several techniques in the serum of multitransfused patients with hemophilia A, and noncomplement fixing complexes have been found in some sera from patients with VWD without antibody. The lack of post-transfusional side effects in hemophiliacs could be due to the nonprecipitating character of the anti-VIIIIR:C antibodies. The unusually high incidence of side effects induced by replacement therapy in VWD patients with antibody most probably relates to the in vivo formation of complement-fixing immune complexes described in this study.

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