The Role of the Spleen in Regulating the Plasma Levels of Factor VIII—von Willebrand's Factor After DDAVP

By Vicente Vicente Garcia, Raffaella Coppola, and Pier Mannuccio Mannucci

Organ transplantation and perfusion studies indicate that the spleen plays an important role in the regulation of plasma levels of factor VIII-von Willebrand's factor (FVIII-vWF). To better understand the mechanisms that regulate the FVIII-vWF increases after infusion of 1-deamino-8-D-arginine vasopressin (DDAVP), we have measured factor VIII coagulant activity (FVIII:C) and antigen (FVIII:CAg) and von Willebrand's factor antigen (vWF:Ag) and ristocetin cofactor (vWF:RCof) in 9 asplenic subjects with normal baseline concentrations, in 7 asplenic subjects with high concentrations, and in 14 normal controls with intact spleens. In "normal" asplenics, all the FVIII-vWF-related measurements increased significantly over baseline values, indicating that responsiveness to DDAVP is not abolished by splenectomy. The maximal vWF:Ag and vWF:RCof responses were no different from those of normal controls, suggesting that DDAVP releases vWF from storage sites other than the spleen. The FVIII:C response was significantly lower than in normal controls, but FVIII:CAg did not differ, making FVIII:CAg higher than FVIII:C in "normal" asplenics. These findings suggest that the spleen, rather than being a storage site for FVIII, is the organ in which a partially inactive form of FVIII acquires full coagulant activity. In "high" asplenics, all the FVIII-vWF-related measurements increased less than in "normal" splens, indicating that long-term elevations of plasma concentrations of FVIII-vWF are accompanied by decreased release from those storage pool(s) mobilized by DDAVP.

THERE ARE FUNCTIONAL, genetic, immunologic, and biochemical data to support the view that plasma factor VIII-von Willebrand's factor (FVIII-vWF) exists in plasma as a macromolecular complex of two related but nonidentical glycoproteins (see the review of Hoyer). Both factor VIII (FVIII), measured either as coagulant activity (FVIII:C) or as coagulant antigen (FVIII:CAg), and von Willebrand's factor (vWF), measured either immunologically (vWF:Ag) or as a ristocetin cofactor or (vWF:RCof) are known to increase in a variety of clinical and experimental situations. Postoperatively, in malignancy and in many other subacute and chronic conditions (reviewed by Brozovic), the relatively slow and long-lasting increases of both are compatible with increased synthesis, probably an acute phase reaction to tissue injury and repair or to neoplastic growth. vWF usually increases more than FVIII, and a number of possible reasons for such a discrepancy are discussed elsewhere. By contrast, the rapid increase in FVIII-vWF following strenuous muscular exercise or infusion of such drugs as adrenaline or 1-deamino-8-D-arginine vasopressin (DDAVP) were short-lived and not accompanied by changes in other proteins. Increased synthesis, therefore, is unlikely to account for that change, and release of autologous FVIII-vWF from storage sites appears to be more likely. In contrast to long-term changes, FVIII increased more than vWF after acute stimuli. In other studies, however, there have been parallel increases of these moieties.

To better understand the mechanisms that regulate the plasma levels of FVIII-vWF and the changes after acute stimuli, we elected to study the effect of DDAVP infusion in asplenic subjects compared with normal volunteers with intact spleens. Among possible acute stimuli, DDAVP was chosen because it has been widely given to human volunteers and patients with hemophilia A and von Willebrand's disease without causing the unpleasant side effects associated with infusion of adrenaline. Moreover, stimulation of FVIII-vWF by DDAVP is easier to standardize than by strenuous muscular exercise. Asplenic subjects were chosen because many organ transplantation and perfusion studies have indicated that the spleen plays an important role in the regulation of plasma FVIII:C levels, either as a storage reservoir or as a site of synthesis in conjunction with the liver.

MATERIALS AND METHODS

Sixteen asplenic subjects (median age 30 yr; range 18-51) were investigated. All subjects were aware of the experimental nature of the studies and gave informed consent according to the Declaration of Helsinki. Normal controls were 7 male and 7 female healthy volunteers, matched for sex and age with asplenic subjects. The underlying conditions that led to splenectomy were immune thrombocytopenic purpura (ITP) in 6 cases (5 women and 1 man), hereditary spherocytosis (HS) in 6 cases (2 women and 4 men), Hodgkin's disease (HD) in 3 cases (1 woman and 2 men), and non-Hodgkin's lymphoma (NHL) in 1 woman. The median time interval between splenectomy and this study was 15 mo (range 8-28). All the patients were in clinical and hematologic remission at the time of the study; in particular, platelet counts were higher than 120 x 10^9/liter in all instances.

DDAVP Administration

After a rest period of at least 30 min, 0.4 μg/kg of DDAVP (supplied by Valeas, Milano) were added to 50 ml of physiologic

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saline and infused over a period of 20 min into normal or asplenic subjects. Blood pressure was monitored during the infusions and no significant changes were detected. A 30%–40% increase in pulse rate was observed in 6 asplenic and 7 healthy subjects. No sign of water retention was observed in spite of the potent diuretic action of DDAVP. Mild facial flushing was usually seen during and immediately after the infusion.

Blood Collection and Plasma Preparation

Blood samples were collected by venipuncture through a 19-gauge needle immediately before infusion (time 0) and then 30, 120, and 360 min after the infusion was started. Nine parts of blood were added to plastic tubes containing one part of 0.13 M trisodium citrate and inhibitors of proteolysis (0.04 M epsilon-aminocaproic acid and 200 U/ml aprotinin). Platelet-poor plasma was obtained after centrifugation at 3000 g for 20 min and used fresh or kept frozen at −80°C until tested.

Assay Methods

FVIII:C was assayed in fresh samples by a one-stage method based on the partial thromboplastin time. Deficient plasma obtained by plasmapheresis from a patient with severe hemophilia was used as a substrate. Pretreatment FVIII:C concentrations were expressed in U/dl and referred to a pooled normal plasma (10 male and 10 female donors) calibrated against the Second International Standard for Factor VIII (kindly supplied by Dr. T. W. Barrow-cliffe). Changes after DDAVP were expressed in percentage of pretreatment values set at 100%.

FVIII:CAg was assayed by the two-site immunoradiometric assay of Holmberg et al., using two homologous nonhemophilic antibodies kindly provided by Dr. Lars Holmberg. Since no international standard for FVIII:CAg was available at the time of this study, FVIII:CAg concentrations were expressed in U/dl with reference to the same pooled plasma used for FVIII:C assays, assigning it a content of 1 U/dl of FVIII:CAg. Changes after DDAVP were also expressed in percentage of pretreatment values set at 100%.

vWF:Ag was assayed by quantitative immunoelectrophoresis using a commercial monospecific antiserum (Istituto Behring, Sco-pitto, Aquila).

vWF:RCof was assayed with formalin-fixed platelets, as previously described. vWF:Ag and vWF:RCof were expressed in U/dl or in percentage of pretreatment values as described for FVIII:CAg.

Statistical Analysis

The chi-square test was used for exact testing of the normality of data distributions. Since they were not normally distributed, the values were transformed logarithmically before testing the significance of the differences within and between groups by means of analysis of variance and the Student’s t test for paired or unpaired data, as appropriate. The results are given as geometric means and geometric standard errors (SE) or as the 95% ranges.

RESULTS

Baseline Concentrations of the FVIII-vWF-Related Measurements

Table 1 shows the baseline concentrations for FVIII:C, FVIII:CAg, vWF:Ag and vWF:RCof in 14 normal controls and 16 asplenic subjects. In the asplenics, all measurements were significantly higher than in controls (p < 0.01). Inspection of the individual values enabled us to identify a subgroup of 9 subjects (4 with ITP, 3 with HS, 1 with HD and 1 with NHL) in whom FVIII:C, FVIII:CAg, vWF:Ag, and vWF:RCof were within the 95% range for normal controls. They were all above the upper limit in the remaining 7 asplenic subjects (2 with ITP, 3 with HS, and 2 with HD). Table 1 shows the geometric mean concentrations and 95% ranges for these subgroups. The differences between the mean values for the asplenic subgroup with normal values for FVIII-vWF-related measurements and those of the subgroup with elevated concentrations were significant (p < 0.01).

Since the magnitude of FVIII-vWF responses to DDAVP might be affected by differences in baseline concentrations, the “normal” and “high” asplenics were analyzed separately throughout this study. To compensate for remaining differences between subjects within the normal controls and both asplenic subgroups, changes after DDAVP were expressed as percentage of baseline values, set at 100%.

Changes in FVIII-vWF-Related Measurements After DDAVP

Figure 1 shows that in normal controls and in both the subgroups of asplenic subjects, FVIII-vWF-related measurements increased significantly over the baseline 30 min after starting the infusion (p < 0.01) and

<table>
<thead>
<tr>
<th>Table 1. Baseline Concentrations of FVIII-vWF-Related Measurements in Normal Controls and Asplenic Subjects</th>
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<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Normal controls</td>
</tr>
<tr>
<td>(n = 14)</td>
</tr>
<tr>
<td>All asplenic subjects</td>
</tr>
<tr>
<td>(n = 16)</td>
</tr>
<tr>
<td>With normal FVIII-vWF-related measurements (n = 9)</td>
</tr>
<tr>
<td>(71-178)</td>
</tr>
<tr>
<td>With high FVIII-vWF-related measurements (n = 7)</td>
</tr>
<tr>
<td>(91-436)</td>
</tr>
</tbody>
</table>

*Geometric means and 95% ranges (between parentheses). Values are expressed in U/dl.

**p < 0.01.

†p < 0.001.

NS, not significant.
Fig. 1. Changes in plasma levels of FVIII-vWF-related measurements after DDAVP in normal controls (closed circles connected by the solid line), “normal” asplenics (closed squares connected by the dash-and-point line), and “high” asplenics (open squares connected by the dashed line). Geometric mean values are shown for each time interval after starting DDAVP infusion (time 0). Bars indicate the geometric standard errors about the mean.

described thereafter. Table 2 shows the maximal responses 30 min after DDAVP (see also Fig. 1). In “normal” asplenics, the FVIII:C response after DDAVP was significantly lower ($p < 0.001$), whereas the FVIII:CAg, vWF:Ag, and vWF:RCoF responses did not differ from those in normal controls. In “high” asplenics, all the responses were significantly less than in normal controls ($p < 0.01$). Comparison of the two subgroups of asplenic subjects showed that “high” asplenics also responded less than “normal” asplenics in all FVIII-vWF-related measurements (Table 2).

DISCUSSION

Since the spleen is thought to be the most important storage site for FVIII:C, we evaluated whether splenectomy affected the FVIII-vWF responses to DDAVP, and compared the magnitude of these responses in asplenic subjects and normal controls with spleens.

In “normal asplenics,” all the FVIII-vWF-related measurements showed significant increases over baseline values, indicating that responsiveness to DDAVP is not abolished by splenectomy. The maximal responses in vWF:Ag and vWF:RCoF were the same as in normal controls, suggesting that DDAVP releases vWF from storage sites other than the spleen (possibly the endothelial cells of the vessel wall). While the FVIII:C response was significantly lower than in normal controls, the response of another FVIII-related measurement, FVIII:CAg, was not different. Accordingly, FVIII:C and FVIII:CA did not change proportionally after DDAVP, as they did in normal controls, with FVIII:CAg increasing more than FVIII:C. This suggests that in asplenic subjects, some of the FVIII molecules released by DDAVP lack coagulant activity (FVIII:C) but retain antigenic determinants (FVIII:CAg). Perhaps the spleen, rather than being a storage site for FVIII, is the organ in which a partially inactive form of FVIII released by DDAVP acquires full coagulant activity. This interpretation is consistent with the results of earlier perfusion experiments of Benson and Dodds, who showed that liver perfusates contained 4 times as much FVIII:CAg (measured by an antibody neutralization assay) as FVIII:C before being passed through the spleen, thus acquiring additional coagulant activity. An alternative explanation for our findings would be a relative instability of FVIII released in the asplenic subject by DDAVP, resulting in more rapid inactivation of FVIII:C than of FVIII:CAg.

The study of “high” asplenics with elevated baseline concentrations of FVIII-vWF-related measurements has allowed us to evaluate whether the responses to DDAVP are different in subjects who have increased FVIII-vWF from those in subjects with normal baseline concentrations. The smaller increases of all the

Table 2. Maximal Increases of FVIII-vWF-Related Measurements After DDAVP in Normal Controls and Asplenic Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>FVIII:C</th>
<th>FVIII:CAg</th>
<th>vWF:Ag</th>
<th>vWF:RCoF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>431*</td>
<td>480</td>
<td>314</td>
<td>271</td>
</tr>
<tr>
<td>(n = 14)</td>
<td>(289-644)</td>
<td>(255-904)</td>
<td>(211-528)</td>
<td>(182-298)</td>
</tr>
<tr>
<td>Normal asplenic†</td>
<td>288</td>
<td>448</td>
<td>294</td>
<td>288</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>(158-524)</td>
<td>(277-702)</td>
<td>(217-398)</td>
<td>(182-456)</td>
</tr>
<tr>
<td>High asplenic‡</td>
<td>214</td>
<td>266</td>
<td>165</td>
<td>203</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>(106-320)</td>
<td>(124-486)</td>
<td>(102-267)</td>
<td>(128-363)</td>
</tr>
</tbody>
</table>

*Values are expressed in percentage of baseline concentrations taken as 100% (geometric means and 95% range).
†Asplenic subjects with normal baseline concentrations of FVIII-vWF-related measurements.
‡Asplenic subjects with elevated baseline concentrations of FVIII-vWF-related measurements.

<table>
<thead>
<tr>
<th>$p &lt; 0.05$</th>
<th>$p &lt; 0.01$</th>
<th>$p &lt; 0.001$</th>
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<tbody>
<tr>
<td>NS, not significant.</td>
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FVIII-vWF-related measurements we saw in "high" asplenic subjects, as compared with both "normal" asplenic subjects and controls with spleens, clearly indicate that elevated plasma concentrations of FVIII-vWF, probably resulting from increased synthesis, are accompanied by decreased release from those storage pool(s) mobilized by DDAVP. It is likely, therefore, that some feedback mechanism regulates the equilibrium between the storage and plasma compartments of these moieties.

From these observations it is possible to construct a working hypothesis about the mechanisms that govern the plasma increase of FVIII-vWF after DDAVP. Both the components of the complex are rapidly released from still undefined storage sites, but the magnitude of the response is affected by the presence of elevated baseline blood levels of FVIII-vWF. Whether this decreased responsiveness is due to depletion of the stores in conditions in which synthesis is increased or to a negative feedback effect on release remains to be established. We do not know whether the storage sites for FVIII and vWF are the same, but it is clear that the spleen is not the main storage organ for either. The spleen, however, plays a major role in the full expression or the stabilization of the coagulant activity of FVIII, because an excess of partially inactive FVIII circulates in asplenic subjects after DDAVP. It remains to be understood why this should only occur in asplenic subjects under conditions of rapid and massive FVIII release from the stores, since the baseline values of FVIII:Cag were not higher than those of FVIII:C (see Table 1). These and other aspects of the synthesis and regulation of FVIII-vWF should be studied further by repeating and extending earlier perfusion and transplantation experiments in the light of more recent knowledge, using the newer assay methods for these moieties.

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