on the route of administration of cobalamin. Our patient is taking oral hydroxocobalamin in comparable dosage to other patients already described who are being treated parenterally, and we would stress again that 12.5 μg cyanocobalamin daily by mouth produced blood cobalamin levels of >1,000 μg/L and that 1 mg twice daily of hydroxocobalamin produced blood levels of 3,000 to 4,000 μg/L.

From our experience with this case and the previous literature, we believe that the total dose and the frequency of cobalamin administration are the most important factors in maintaining adequate cobalamin status in TC-II deficiency.

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VON WILLEBRAND FACTOR IN THROMBOTIC THROMBOCYTOPENIC PURPURA

To the Editor:

A report by Lian and Siddiqui in the Nov 1985 issue of Blood claimed to provide evidence that von Willebrand factor (vWF) is not involved in the pathophysiology of acute thrombotic thrombocytopenic purpura (TTP) episodes. The experiments described in the article did not justify the authors' conclusion. Lian and Siddiqui found a platelet clumping activity in the plasma obtained from five patients during TTP episodes. They reported that this clumping activity was not neutralized by preincubation of acute TTP patient plasma with either polyclonal antibody to vWF or by incubation of test platelets with monoclonal antibody to glycoprotein Ib. (Glycoprotein Ib is one of several externally exposed platelet membrane proteins to which vWF multimers will bind, or bind near, on the platelet surface. Others are the glycoprotein IIb-IIIa complex and a separate protein of mol wt 210,000.)

When large vWF multimers attach to platelets in vivo during acute TTP episodes, large vWF forms decrease or disappear from patient plasma. This has been found in samples obtained during acute TTP episodes in eight studies from four different laboratories. In the paper by Lian and Siddiqui, vWF multimeric forms in patient plasma were not reported. It is likely that large vWF multimeric forms had already decreased or disappeared from their patient samples before they were obtained. Interference with agglutination by antibodies against vWF would not, therefore, have been expected under their experimental conditions, i.e., the mixing of washed normal platelets, anti-vWF antibodies, and acute TTP plasma samples devoid of the large forms of vWF that bind to platelets.

Because of the probable relative absence of large vWF multimeric forms in the acute TTP plasma samples of Lian and Siddiqui's patients, I would also have expected that an antibody against one of the platelet glycoprotein receptors for large vWF multimers would have no effect on platelet clumping. By the same reasoning, antibody against the glycoprotein Ib-IIa complex would have been expected to have no effect under their particular test conditions. Nevertheless, it would have been important to determine if antibody directed against one of the other potential binding sites for large vWF multimers had any effect in their test system.

Lian and Siddiqui's experiments were based entirely on the in vitro clumping of platelets in the presence of several plasma samples obtained during acute TTP episodes. Platelet clumping in their test system was, apparently, relatively modest in the presence of acute TTP plasma alone. The actual extent of aggregation is not given in their article. However, 20% to 32% change in light transmission was reported in one of their previous publications (Table 1 of reference 12). Platelet aggregation of this limited extent is difficult to reproduce. This is especially true if (as in their system) the test platelets have been separated, washed, and resuspended in buffer before being suspended in TTP plasma.

The factor that causes the slight amount of platelet clumping in the plasma samples of Lian and Siddiqui is not clear. The authors present no direct evidence to indicate that it is not an isoantibody. It has long been known that plasma samples will clump normal donor platelets if the plasma contains isoantibodies directed against histo-compatibility antigens on the surface of test platelets. Platelet clumping tests performed on TTP plasma samples are probably useful only as relatively insensitive screening tests for the presence of platelet isoantibodies when the aggreometry studies are performed using plasma from patients who have previously been transfused with blood products, or who have previously been pregnant. Lian and Siddiqui do not indicate if their five TTP patients had ever been transfused or pregnant prior to the acquisition of their plasma samples for testing. They also do not mention if they have tested for the presence of HLA isoantibodies in the TTP patient plasma samples. Other investigators have observed that in vitro platelet clumping in some TTP plasma samples is probably caused by immunoglobulins. It is possible, therefore, that the platelet clumping test used by Lian and Siddiqui, which employs plasma from TTP patients and platelets from normal (non-HLA identical) donors, predominantly detects platelet isoantibodies in TTP patient plasma samples capable of clumping non-HLA-identical normal platelets in vitro. The authors do not indicate that they controlled for this possibility by incubating their TTP plasma samples with antihuman IgG, IgM, and IgA before performing platelet clumping studies.

Along with others, I am skeptical that the in vitro platelet clumping in the test of Lian and Siddiqui is related to the pathophysiology of TTP episodes in vivo. Platelet clumping observed in their test system, whether induced by acute TTP plasma or by fractions purified from it, is claimed by Lian et al to be inhibited by normal human IgG. Lian and colleagues have suggested, from the clinical course of one patient, that intravenous (IV) immunoglobulin may be useful in vivo in the treatment of acute episodes of TTP. However, Miuri et al as well as Dr J.J. Byrnes (Miami) and Dr J.G. Kelton (Hamilton, Ontario) in unpublished studies have found that concentrated IV IgG is of no therapeutic value in vivo during acute TTP episodes, and that IV IgG does not truncate or delay relapses in the chronic relapsing form of the disorder.

My colleagues and I, as well as Kelton et al and others have concluded that vWF is likely to be involved in the pathophysiology of TTP and closely related syndromes. The largest plasma vWF multimeric forms have been found to be depleted in the plasma of TTP patients tested during the in vivo platelet clumping and thrombocytopenia that characterize acute TTP episodes. Kelton et al have also observed that acute TTP serum, which does not directly agglutinate normal donor washed platelets, can be made to do so by the addition of normal defibrinated cryoprecipitate (containing the largest plasma vWF multimeric forms). They found that neither the smallest plasma vWF forms nor fibronectin supports platelet agglutination in the presence of acute TTP serum. In contrast to the report by Lian et al, platelet clumping in the test system of Kelton et al is not inhibited by normal IgG.

Plasma from which the large vWF multimeric forms have been
cytopenia and hemolytic anemia who responds repeatedly to normal of several plasma components in a young boy with chronic thrombo-
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We stated that von Willebrand factor (vWF) is unlikely to play a major role in the thrombotic thrombocytopenic purpura (TTP) plasma-induced platelet agglutination (or aggregation) and that glycoprotein (GP) Ib on the platelet membrane, to which vWF binds, is not involved in the development of TTP.1 We have not excluded the involvement of vWF in the pathophysiology of TTP. However, in addition to our data, there is some evidence presently available suggesting that vWF itself is less likely to assume the key role in the pathogenesis of TTP in some or the majority of patients afflicted with this disease. Most TTP patients respond to infusion or exchange with normal whole plasma.22 and, occasionally, even cryoprecipitate.I1 both contain vWF multimers. Contrary to the claim by Dr Moake that cryosupernatant is an effective treatment for acute episodes of TTP when other types of plasma manipulation
attachment to platelets of large vWF multimeric forms is of itself sufficient to induce platelet agglutination in vivo, or if fibrinogen, fibronectin, or other plasma proteins (or protein fragments) are also involved.

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therapy have failed, whole plasma works as well as cryosupernatant according to the literature cited.6-9 Furthermore, following infusion with plasma in patients with chronic TTP,4 the size of vWF was reduced for less than 24 hours; however, the remission lasted for 3 to 4 weeks. Apparently, infusion of "vWF conversion processing factor" present in the normal plasma cannot account for the prolonged remission. In clinical situations where platelet agglutination occurs as a result of hyperactive vWF or vWF receptor, such as in type IIb vWD and platelet type pseudo-vWD, typical TTP symptoms have not been reported. Murphy et al12 described that platelet agglutinating activity appeared when large vWF multimers were markedly reduced in acute episodes of TTP. This supports our findings that vWF is not essential in TTP plasma-induced agglutination.

The observation that large vWF forms decrease or disappear from patient plasma during acute TTP episodes4,9-11 is interesting and demands some explanation. It is an attractive hypothesis that large

REFERENCES


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von Willebrand factor in thrombotic thrombocytopenic purpura [letter]

JL Moake