Effects of Fresh-Frozen Plasma and Its Cryosupernatant Fraction on von Willebrand Factor Multimeric Forms in Chronic Relapsing Thrombotic Thrombocytopenic Purpura

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Remission plasma samples of some patients with chronic relapsing thrombotic thrombocytopenic purpura (TTP) contain unusually large von Willebrand factor (vWF) multimers similar to those produced by normal human endothelial cells in culture. The infusion of the cryosupernatant fraction of normal plasma is as effective as normal fresh-frozen plasma (FFP) in the treatment or prevention of TTP episodes in patients with the chronic relapsing form of TTP. Three patients with chronic relapsing TTP during remission have unusually large vWF multimers present in their plasma. Two of the patients were transfused once with FFP, one of the two received cryosupernatant on three occasions, and the third patient was studied before and immediately after plasma exchange. Unusually large vWF multimers decreased or disappeared from patient plasma samples within 1/2 to 1 1/2 hours following the transfusion of FFP (on two occasions) or cryosupernatant (on two of three occasions), and immediately after plasma exchange (on one occasion). The patient who received cryosupernatant was studied serially after the infusions.

During remission in patients with chronic relapsing thrombotic thrombocytopenic purpura (TTP), von Willebrand factor (vWF) multimers larger than those in normal plasma, and similar to those produced by normal human endothelial cells in culture, have been found. During relapses, the unusually large vWF multimers decrease or disappear from patient plasma transiently in association with intravascular platelet agglutination and thrombocytopenia. This may be because they attach preferentially to platelets in vivo in the presence of a putative inciting cofactor released in association with inflammation or tissue necrosis. The unusually large vWF multimers then return to patient plasma as recovery occurs.

The infusion of normal fresh-frozen plasma (FFP), often in association with plasmapheresis, is effective in controlling or preventing relapses in chronic relapsing TTP. In contrast, transfusions of normal platelets, albumin, γ globulin, or preparations of factor IX or fibronectin have been reported to be ineffective or harmful.

The transfusion of normal cryoprecipitate, which contains the largest vWF multimers present in normal plasma, is also ineffective. Transfusion of the cryosupernatant fraction of normal plasma, however, has been found to be at least as useful as normal plasma for prophylaxis against, or therapy for, TTP relapses in patients with chronic relapsing TTP. Cryosupernatant contains residual amounts of the smallest vWF multimers present in normal plasma.

In this report, we describe the effects of FFP and cryosupernatant infusion, as well as plasma exchange, on the plasma vWF multimer patterns in three patients with chronic relapsing TTP who were in complete or partial remission. The patients were studied during remission in order to allow a correlation to be made between therapy and any changes in plasma vWF multimer forms. It is not possible to make this correlation during relapses in chronic TTP, because during relapses the unusually large vWF multimer forms (and sometimes even the largest vWF multimers found normally in circulation) disappear from patient plasma in association with the in vivo platelet agglutination that is the pathophysiologic basis for the multifocal organ ischemia.
EFFECTS OF CRYOSUPERNATANT ON vWF IN TTP

PATIENTS

Patient 1
For five years, this 26-year-old woman has required transfusion of either FFP or cryosupernatant every three to four weeks to prevent TTP relapses. She is described as patient 5 in Byrnes and patient C in Moake et al. When the patient was in clinical remission (platelet counts, 190,000 to 335,000/μL) and when unusually large vWF multimers were present in her plasma (vWF antigen levels, 122 to 235 U/dL), she was transfused with 2 units of either FFP or the cryosupernatant fraction (~400 mL) of normal plasma. This was done as part of her regular prophylactic transfusion program at the University of Miami Medical Center. She understood the purpose of the study and gave informed consent for it to proceed.

Patient 2
This 34-year-old woman has had periodic episodes of TTP since infancy. She was once believed to have “thrombopoietin deficiency” and is patient B in Moake et al. She receives a single unit of FFP intermittently as prophylaxis against TTP relapses. On one of those occasions, she gave permission for blood samples to be taken before, and then 30 minutes after, completion of the infusion. At the time, she was in remission (platelets 308,000/μL) and unusually large vWF forms were found in her plasma (vWF antigen level, 383 U/dL).

Patient 3
This 21-year-old woman had chronic relapsing TTP. She is patient A in Moake et al. On one occasion, when she had partially recovered from a TTP relapse (platelets 110,000/μL), and when unusually large vWF multimers had reappeared in her plasma (vWF antigen, 168 U/dL), she had a 3-L exchange with normal FFP (postexchange vWF antigen, 143 U/dL). Venous blood samples were obtained from the patient before, and then immediately after, the procedure.

MATERIALS AND METHODS

The preparation of patient and normal pooled platelet-poor plasma (PPP) from citrated venous blood samples has been described. vWF antigen levels in patient and normal plasma were quantified by Laurell electroimmunoassay using 0.5% SeaKem HGT agarose and a continuous buffer system in a modification of the method of Ruggeri and Zimmerman. vWF forms were found in hen plasma (vWF antigen level, 383 U/dL).

RESULTS

Unusually large vWF multimers in the remission plasma of two chronic relapsing TTP patients were no longer detected, or were considerably reduced in relative amounts, in citrated PPP samples prepared from blood obtained 30 to 90 minutes after the transfusion of either normal cryosupernatant in patient 1 (Fig 1), or FFP in patients 1 and 2 (Fig 2). These changes in

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circulating vWF forms were observed following the transfusion of 2 units of FFP (on one occasion) or of cryosupernatant (on two of three occasions) in patient 1. Similar changes were also seen immediately after the transfusion of 1 unit of FFP in patient 2. Ten to 24 hours later the unusually large vWF patterns were the same as the pretransfusion patterns in patient 1, and remained unchanged thereafter as long as the patient was in remission.

Platelet counts did not decrease, plasma vWF antigen levels did not change detectably, and the clinical status of patients 1 and 2 was not altered following the transfusion of cryosupernatant or FFP. These results indicate that the disappearance of the unusually large vWF multimers was not the result of their attaching to platelets and causing agglutination in vivo.

Unusually large vWF multimers were not present in the plasma of patient 3 immediately following 3-L plasma exchange (Fig 3). This procedure was done during a period when her platelet count was progressively increasing and her clinical status had become normal. Disappearance of the unusually large vWF forms in patient 3 could have been the result both of their physical removal and their conversion in vivo to somewhat smaller multimers (as described below).

Unusually large vWF multimers did not disappear in vitro from remission plasma samples of patient 1 when they were incubated with normal cryosupernatant or normal plasma (Fig 4). Similar results were obtained when remission plasma samples of patient 3 were incubated with normal plasma. Unusually large vWF multimers also did not disappear in vitro from the medium removed from cultured human endothelial cells when this medium was incubated in the fluid phase with normal cryosupernatant (Fig 5). These in vitro results were not affected by the presence or absence of calcium (12.5 mmol/L), additional heparin (14.3 U/mL) or proteolytic inhibitors (2 U/mL hirudin + 100 U/mL aprotinin + 2 mmol/L diisopropylfluorophosphate (DFP) + 1 μmol/L p-amidinophenyl
EFFECTS OF CRYOSUPERNATANT ON vWF IN TTP

Fig 3. Venous blood samples were obtained from patient 3 before (Pre) and immediately after a 3-L plasma exchange (Post). n, normal pooled plasma; p, patient 3 plasma.

Fig 4. Patient remission plasma (TTP) was mixed with normal platelet-poor plasma (NP) or with normal cryosupernatant (CS) in the ratios indicated. The mixtures were either sampled immediately (0 time) or were incubated at 37°C for eight and 24 hours (8h and 24h) before sampling. EC, normal human endothelial cell culture medium.

Fig 5. Samples of normal human endothelial cell culture medium (EC) were removed and mixed with equal volumes of normal human cryosupernatant. The mixtures were either sampled immediately (0 time) or incubated at 37°C for one hour (1h). Some of the mixtures contained calcium (Ca) or calcium plus additional heparin (Ca + Hep), as indicated, and all in this experiment contained hirudin + aprotinin + DFP + p-APMSF. NP, normal pooled plasma; and CS, normal cryosupernatant.
methanesulfonyl fluoride (p-APMSF), or by extending the time of incubation to eight hours.

**DISCUSSION**

Within 30 to 90 minutes after the transfusion of FFP or cryosupernatant into two chronic relapsing TTP patients in remission, unusually large vWF forms were present in relatively reduced amounts, or had disappeared, from their plasma. Unusually large vWF multimers were also absent from the plasma of a third patient, who was in partial remission at the time, when venous blood samples were obtained immediately after exchange transfusions with FFP. The unusually large vWF multimers reappeared during the next ten to 24 hours in the plasma of the patient who was studied serially on different occasions. In contrast to the in vivo studies, the conversion of unusually large vWF forms did not occur in the fluid phase when normal cryosupernatant was mixed with (1) the medium removed previously from endothelial cells in culture or (2) with chronic relapsing TTP remission plasma.

One possible explanation for these observations is that the cryosupernatant fraction of normal plasma contains an activity that converts, or potentiates the conversion, of unusually large vWF multimers to smaller forms only on endothelial cell surfaces. This may occur as the unusually large multimers are secreted from endothelial cells into the circulation. A corollary of this explanation would be that the turnover of unusually large vWF multimers already in the circulation of the TTP patient at the time of plasma or cryosupernatant must be rapid.

A second possible explanation for our results, which cannot be excluded, is that the transfusion of FFP or cryosupernatant somehow accelerates the removal of unusually large vWF multimers from TTP patient plasma. Techniques that are capable of distinguishing between these two possibilities are not yet available.

Normal FFP and its cryosupernatant fraction may be useful in the treatment of relapses in chronic TTP because they contain an activity that promotes the breakdown or removal of unusually large vWF multimers before they can attach to agglutinating platelets.

The rapid dissipation of the effect of FFP or cryosupernatant on the metabolism of unusually large vWF forms indicates that the active substance that was transfused was short-lived in the patient studied. This short-lived effect may be an intrinsic property of the yet uncharacterized substance in FFP and its cryosupernatant fraction. Alternatively, an autoantibody in the blood of the chronic relapsing TTP patient may have inactivated within a few hours the effective substance in transfused normal FFP/cryosupernatant, or interfered with its attachment to endothelial cell surfaces. Although this is speculation, it is compatible with clinical observations that normal FFP often must be infused frequently (with or without plasmapheresis) for days in order to control relapses in some chronic relapsing TTP patients. It is also compatible with reports that agents with immunosuppressive properties (glucocorticoids, vincristine, azathioprine11) may have beneficial effects in the treatment of some patients with the chronic relapsing form of TTP.

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