American Journal of Hematology 20:73–79 (1985)

Therapy of Chronic Relapsing Thrombotic Thrombocytopenic Purpura With Prednisone and Azathioprine

Joel L. Moake, Christine K. Rudy, Joseph H. Troll, Andrew I. Schafer, Mark J. Weinstein, Noreen M. Colannino, and Suchen L. Hong

William B. Castle Hematology Research Laboratory, Thorndike Memorial Laboratories, Boston City Hospital; Boston and West Roxbury V.A. Medical Centers; Boston University School of Medicine, Harvard Medical School, Massachusetts; and Baylor College of Medicine, Waco and Rice Universities, Houston, Texas

As a 51-year-old woman recovered from an initial acute episode of thrombotic thrombocytopenic purpura (TTP), her plasma was found to contain unusually large von Willebrand factor (vWF) multimers. Clinical, hematological, and vWF studies of her siblings and children were normal. The unusually large vWF forms were presumably derived from endothelial cells, persisted in her plasma after recovery, and were associated with recurrent episodes of TTP during the subsequent 6 months. After the last episode of relapse they disappeared from her plasma following 3½ weeks of therapy with prednisone and did not return during 17 months of treatment with prednisone and/or azathioprine. She is now receiving no drugs, has normal plasma vWF forms, and has not had any more episodes of TTP. We conclude that our patient had an acquired defect in the conversion of unusually large vWF multimers derived from endothelial cells to the somewhat smaller vWF forms usually present in circulation. The defect may have been immune-mediated, because it was eliminated during therapy with immunosuppressive drugs.

Key words: thrombotic thrombocytopenic purpura, von Willebrand factor, prednisone, azathioprine (Imuran), immunosuppression

INTRODUCTION

Factor VIII-related von Willebrand factor (vWF) multimers larger than those in normal plasma have been found during remission in patients with chronic relapsing thrombotic thrombocytopenic purpura (TTP) [1]. During relapses the unusually large vWF multimers (and sometimes also the largest plasma vWF forms) decrease or disappear in association with intravascular platelet agglutination and thrombocytopenia. During acute episodes of the hemolytic-uremic syndrome, the largest plasma vWF multimers also decrease in patient plasma [2].

In this report we describe the effects of prednisone and azathioprine on the clinical course and vWF multimeric forms of a patient with relapsing TTP.

Received for publication November 14, 1984; accepted January 31, 1985.

Address reprint requests to Joel L. Moake, M.D., Medical Hematology Section, Methodist Hospital—Mail Station 902, Main Bldg., 6565 Fannin, Houston, TX 77030.

METHODS

The preparation of patient and normal pooled platelet-poor plasma, the culture conditions used for human umbilical vein endothelial cells, the radioimmunoassays of plasma β-thromboglobulin (a platelet α-granule release product) and fibrinopeptide A (cleaved from fibrinogen by thrombin), and the electrophoretic techniques have been described [1,2]. Briefly, immunoelectrophoresis was into 0.5% agarose containing rabbit antihuman vWF antibodies. For two-dimensional studies normal pooled plasma was always run concurrently with each patient sample, which was diluted with barbital-acetate running buffer to the same 100% (100 units/dl) vWF antigen level present in normal plasma. Patients and normal plasma samples were electrophoresed in the first dimension from two different origin wells that were cut into the same 0.5% agarose gel slab. The first-dimensional gel lanes were then cut out and electrophoresed in the second dimension for the same time periods in the same electrophoresis chamber. Under the experimental conditions described, the relative positions of patient and normal pooled plasma vWF patterns were always reproducible. Dried Coomassie blue-stained individual gels of patient and normal plasma were superimposed after exact matching of the origin sample wells, taped together, and photographed. The vWF antigen in patient plasma was quantified by one-dimensional immunoelectrophoresis and by solid-phase immunoradiometric assay using unlabeled ^{125}I-labeled rabbit antihuman vWF. Plasma vWF multimers were separated by sodium dodecyl sulfate (SDS)-1% agarose gel electrophoresis, overlaid with rabbit ^{125}I-antihuman vWF IgG, and analyzed by autoradiography. For these studies, patient and normal pooled plasma samples were diluted to 15% (15 units/dl) vWF antigen levels. The vWF antigen in endothelial cell supernatants was 7.5% (7.5 units/dl).

PATIENT AND RESULTS

The patient is a 51-year-old woman who had a flulike illness followed by headache, confusion, fever, nausea, and numbness of the tongue and left hand. Admission laboratory data included the following: platelets, 6,000/μl; hematocrit, 22%, with intravascular hemolysis, reticulocytosis, schistocytosis, and a hypercellular bone marrow; normal renal function; and normal coagulation studies.

She received prednisone 50–60 mg/day, red blood cells, plasma exchange transfusions and fresh-frozen plasma. Twenty-one days after admission, she was clinically normal with hematocrit, 35%, and platelets, 254,000/μl. Two-dimensional immunoelectrophoretic and SDS-agarose autoradiographic analyses of vWF forms obtained at different times during her clinical course are in Figures 1 and 2. Slowly migrating vWF forms were not detected in the first sample obtained for testing on the third hospital day after she had received 3 units of fresh-frozen plasma and 3-liter plasma exchange transfusion (sample 1; vWF antigen, 217%). As she recovered from her initial acute TTP episode, slowly migrating, unusually large vWF multimers appeared in her plasma. Sample 2 was taken when she was well and on no therapy (vWF antigen, 319%).

The patient had a recurrence 4½ months later that was associated with decreased plasma levels of the slowly migrating, unusually large vWF multimers (sample 3; obtained before any therapeutic intervention; vWF antigen, 287%). During the first 3 days of this recurrent TTP episode, she received fresh-frozen plasma (without

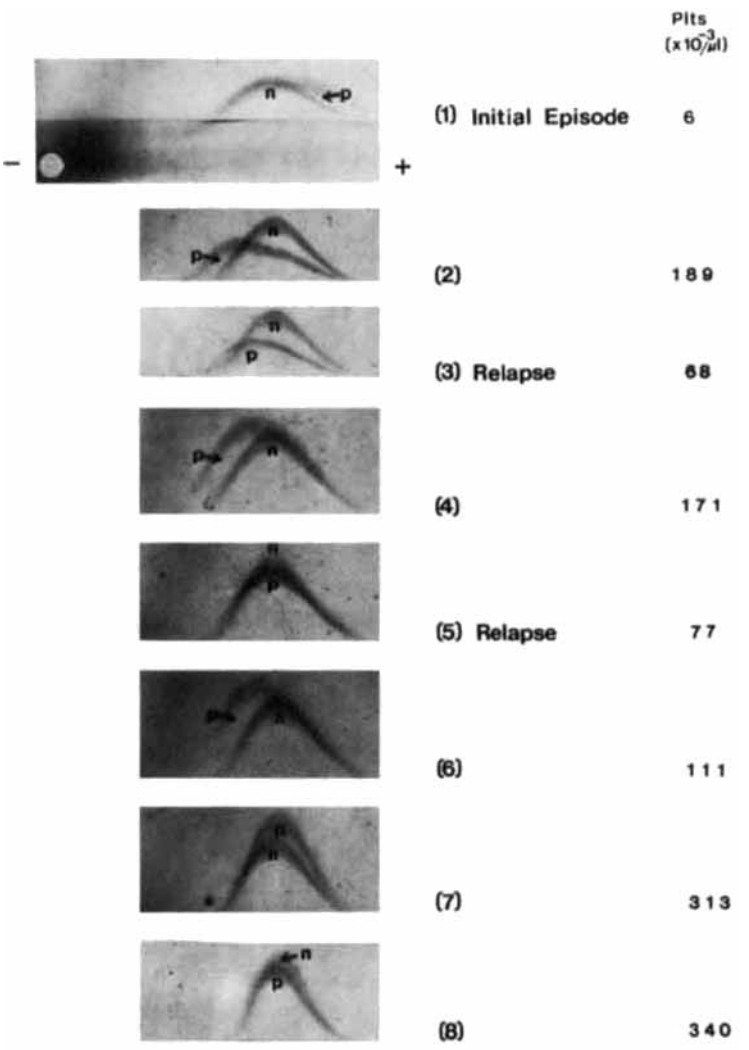

Fig. 1. Two-dimensional immunoelectrophoresis of patient plasma. n, normal pooled plasma. Patient plasma samples (p) 1–8 contained 217–460% vWF antigen (lowest value in sample 1). Sample 1 was obtained on the third day of her initial TTP episode; samples 3 and 5 during subsequent relapses; samples 2 and 4 between relapses when she was clinically well; samples 6, 7, and 8 after 1½, 3½, and 9½ weeks of glucocorticoids (prednisone plus azathioprine for sample 8).

plasmapheresis). Despite a transient hypersensitivity reaction, she recovered by the seventh day (sample 4; vWF antigen, 306%). Another TTP relapse then occurred within 24 hr and was associated with the disappearance of slowly migrating, unusually large vWF multimers from her plasma (sample 5; vWF antigen, 390%). She was given 40 mg/day of dexamethasone (without plasma infusion or exchange) and, as she improved 6 days later, unusually large vWF multimers reappeared in her plasma (sample 6; vWF antigen, 367%). Dexamethasone was discontinued, prednisone was substituted (120 mg/day, rapidly decreased to 40 mg/day), and she was discharged.

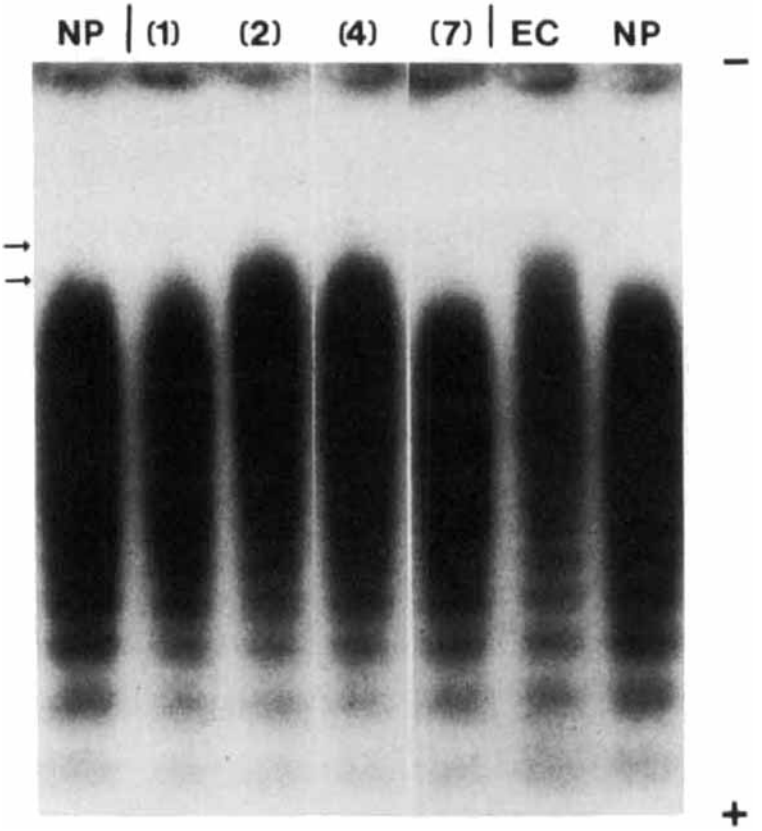

Fig. 2. SDS-1% agarose gel electrophoresis and autoradiography of vWF multimers in patient plasma. Sample numbers correspond to those in Figure 1. NP, normal pooled plasma; EC, normal human endothelial cell supernatant. The upper arrow indentifies the top of vWF patterns in lanes 2, 4, and EC. The lower arrow points out the top of vWF patterns in lanes NP, 1, and 7.

After 3½ weeks of glucocorticoid therapy, her vWF multimer pattern became normal (sample 7; vWF antigen, 460%). At this time, prednisone was continued in decreasing doses, and azathioprine (100–150 mg/day) was added. She received the combination of decreasing doses of prednisone and azathioprine for 6 months, and then azathioprine (100 mg/day) alone for 11 months. She has now been on no therapy for more than 10 months. vWF antigen levels have been 201–346% in plasma samples taken every 2–4 weeks during this period, and none has contained slowly migrating, unusually large vWF multimers. Plasma sample 8 is one example (vWF antigen, 232%). She has remained well and without recurrent thrombocytopenia, hemolytic anemia, or neurological disturbance.

β-Thromboglobulin and fibrinopeptide A levels were normal in both relapse and recovery plasma samples. Each of her five siblings and three children is in good health, with no history of TTP, and has normal hematocrit, platelet count, red cell morphology, plasma vWF antigen level, and plasma vWF forms (by two-dimensional immunoelectrophoresis and by SDS-1% agarose gel electrophoresis and autoradiography).

DISCUSSION

Previous observations that unusually large vWF multimers are in the circulation of patients with chronic relapsing TTP, and are most apparent between relapses [1], led us to predict correctly that TTP would recur in this patient following the initial episode.

Unusually large vWF multimers are synthesized and secreted by human endothelial cells [1,3]. vWF multimers, including unusually large forms, are also synthesized by megakaryocytes [4] and stored in platelet α-granules [5,6]. Platelet release of α-granule contents did not occur during or after any of the TTP episodes in our patient (β-thromboglobulin levels were normal). It is unlikely, therefore, that the unusually large vWF multimers in the remission plasma samples of our patient were derived from platelets. It is probable that she developed a persistent defect in the conversion of unusually large vWF multimers derived from endothelial cells into the somewhat smaller vWF forms normally present in circulation. This defect was almost certainly acquired because she had been healthy for 51 years, her siblings and children were all unaffected, and the defect was reversed during subsequent immunosuppressive therapy.

During her initial and recurrent TTP episodes, the unusually large vWF multimers were either undetectable or decreased relative to the unusually large vWF forms present in her plasma samples during periods of remission. We have suggested previously [1] that this may have been because they were attached to agglutinating platelets in vivo in response to intrusion into the blood of some other substance, as yet unidentified, during TTP attacks. Similar observations on vWF multimeric forms have been made recently in patients with relapsing TTP or a TTP-like syndrome by Rowe et al [7] and Miura et al [8]. The patient of Rowe et al [8] continues to have unusually large vWF multimers in her plasma months after splenectomy but has had no subsequent TTP relapses (at the time of this writing).

A recent abstract by Lian and Siddiqui [9] claimed that vWF was not involved in the platelet agglutination that is the pathophysiological basis for acute TTP episodes. This contention was based on experiments in which a platelet-clumping activity in the plasma obtained from four of five patients during TTP episodes was not reduced by preincubation of the plasma with either antibodies to vWF or a monoclonal antibody to glycoprotein 1b, which is a component of one of the sites of binding of vWF multimers on the platelet surface. These experiments are difficult to interpret because any unusually large vWF multimers, and in some patients even the largest plasma vWF forms (ie, the types of vWF multimers capable of binding to platelets), are likely to be decreased or absent in plasma obtained *during* TTP episodes. (The large vWF forms have probably already attached to patient platelets.) In the report by Lian and Siddiqui, vWF multimeric forms in the test plasma were not analyzed. Furthermore, these investigators obtained only modest reductions in vWF antigen (as little as 3%) after preincubation of patient plasma with anti-vWF antibodies. They did not determine if the preincubation of acute TTP plasma with antibodies to human immunoglobulins inhibited platelet clumping. No information was provided on the presence or absence of platelet isoantibodies in their patient plasma samples, or previous exposure of their patients to blood products.

In contrast to the abstract of Lian and Siddiqui, a recently published paper by Kelton et al [10] reported that the largest plasma vWF multimeric forms were depleted

in the plasma of patients during acute episodes of TTP. Kelton et al found that acute TTP serum that did not spontaneously agglutinate normal donor platelets could be made to do so by the addition of normal defibrinated cryoprecipitate containing the largest plasma vWF forms. Their study included 48 TTP patients who were a part of the Canadian Cooperative TTP Project. Their findings are also compatible with our previous suggestion [1] that a "vWF cofactor" may be present in the blood of patients during acute TTP episodes.

The patient reported in this paper, as well as two other patients studied previously by us [1,11], had systemic platelet agglutination *unaccompanied* by release of platelet α-granule contents. This is characteristic of vWF-mediated agglutination in fluids containing Ca^{2+}/Mg^{2+} (as circulating blood) [12].

vWF antigen levels remained elevated (201–460%) in plasma samples obtained from our patient while she has been in long-term continuous remission during therapy with prednisone alone, prednisone plus azathioprine, and azathioprine alone. It is unlikely, therefore, that the drugs interfered to any important extent with the synthesis or secretion of vWF multimers by endothelial cells.

Unusually large vWF multimers disappeared from her *remission* plasma samples during treatment with prednisone alone. It cannot be known for certain that this effect was related to prednisone therapy. During the preceding months, however, when the patient was receiving no therapy, all of her remission plasma samples contained unusually large vWF multimers. The unusually large vWF forms have not reappeared during long-term immunosuppression with prednisone plus azathioprine, azathioprine alone, or following the discontinuation of drug therapy. In the absence of unusually large vWF multimers in her plasma, she has had no subsequent episode of TTP.

Almost all patients who have survived episodes of TTP have received prednisone or related agents [13], and high doses of glucocorticoids were reported recently to be important in the successful treatment of TTP episodes [14]. We hypothesize that our patient may have developed an autoantibody that prevented the conversion of some of the unusually large vWF multimers emerging from endothelial cells into the somewhat smaller vWF forms normally in circulation. This activity is present in the cryosupernatant fraction of normal plasma, and apparently is not effective in the fluid phase [15]. It may be active only on endothelial cell surfaces. The putative autoantibody in this patient may have attached to endothelial cell surfaces [16] and interfered with the vWF conversion process for unusually large vWF forms.

Glucocorticoids enhance suppressor T-lymphocyte activity and inhibit autoantibody production [17]. Antibody production is also decreased by azathioprine [18]. Prednisone, and then prednisone plus azathioprine, may have suppressed the production of the autoantibody in our patient and, in association, the propensity for TTP relapses. A similar explanation may account for the previously described [19] therapeutic effectiveness in TTP of vincristine, another agent that has immunosuppressive properties.

ACKNOWLEDGMENTS

We are grateful for the clinical contributions of Drs. Mark Hornstein and Stephen Emerson, and the secretarial skills of Ms. Stevelyn M. Diczok and Ms. Andrea Bethel. We also appreciate the support and encouragement of Drs. John I.

Sandson, Carl Franzblau, William Hollander, Alfred I. Tauber, Alan S. Cohen, Leon J. Marks, and O.H.L. Bing.

This work was supported by research funds from the Veterans Administration, grants HL22355 and HL13262 from the National Institutes of Health, and the Dean's fund of the Boston University School of Medicine.

REFERENCES

1. Moake JL, Rudy CK, Troll JH, Weinstein MJ, Colannino NM, Azocar J, Seder RH, Hong SL, Deykin D: Unusually large plasma factor VIII: von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. N Engl J Med 307:1432, 1982.
2. Moake JL, Byrnes JJ, Troll JH, Rudy CK, Weinstein MJ, Colannino NM, Hong SL: Abnormal VIII:von Willebrand factor patterns in the plasma of patients with the hemolytic-uremic syndrome. Blood 64:592, 1984.
3. Hong SL, Weinstein MJ, Moake JL: von Willebrand factor multimer forms produced by human endothelial cells. Blood 62:5(Suppl 1):286a, 1983.
4. Nachman R, Levine R, Jaffe EA: Synthesis of factor VIII antigen by cultured guinea pig megakaryocytes. J Clin Invest 60:914, 1977.
5. Zucker MB, Broekman MJ, Kaplan K: Factor VIII-related antigen in human blood platelets. Localization and relase by thrombin and collagen. J Lab Clin Med 94:675, 1979.
6. Ruggeri ZM, Mannucci PM, Lombardi R, Federici AB, Zimmerman TS: Multimeric composition of factor VIII/von Willebrand factor following administration of DDAVP: Implications for pathophysiology and therapy of von Willebrand's disease subtypes. Blood 59:1272, 1982.
7. Rowe JM, Francis CW, Cryan E, Marder VJ: Splenectomy in thrombotic thrombocytopenic purpura. Clinical response and effect on von Willebrand protein multimers. Blood 64:5(Suppl 1):240a, 1984.
8. Miura M, Koizumi S, Nakamura K, Ohno T, Tachinami T, Yamagami M, Taniguchi N, Kinoshita S, Abildgaard CF: Efficacy of several plasma components in a young boy with chronic thrombocytopenia and hemolytic anemia who responds repeatedly to normal plasma infusions. Am J Hematol 17:307, 1984.
9. Lian EC-Y, Siddiqui FA: Investigation of the role of von Willebrand factor in thrombotic thrombocytopenic purpura. Blood 64:5(Suppl 1):238a.
10. Kelton JG, Moore J, Santos A, Sheridan D: Detection of a platelet-agglutinating factor in thrombotic thrombocytopenic purpura. Ann Intern Med 101:589, 1984.
11. Moake JL, Rudy CK, Troll JH, Weinstein MJ, Colannino NM, Hong SL, Koutcher JA, Melaragno AJ, Manner CE: von Willebrand factor abnormalities and endothelial cell perturbation in acute thrombotic thrombocytopenic purpura. Am J Med Sci, 1985 (in press).
12. Moake JL, Tang SS, Olson JD, Troll JH, Cimo PL, Davies PGA: Platelets, von Willebrand factor, and prostaglandin I_2. Am J Physiol 241:H54, 1981.
13. Ridolfi RL, Bell WR: Thrombotic thrombocytopenic purpura—Report of 25 cases and review of the literature. Medicine 60:413, 1981.
14. Pillai MV, Tucker HS-G, Sharp DE: Early plasma exchange and high dose corticosteriods—Decisive factors in successful treatment of thrombotic thrombocytopenic purpura. Blood 62:5(Suppl 1):189a, 1983.
15. Moake JL, Byrnes JJ, Troll JH, Rudy CK, Hong SL, Weinstein MJ, Colannino NM: Effects of fresh-frozen plasma and its cryosupernatant fraction on von Willebrand factor multimer forms in chronic relapsing thrombotic thrombocytopenic purpura. Blood, 65, 1985.
16. Burns ER, Zucker-Franklin D: Pathologic effects of plasma from patients with thrombotic thrombocytopenic purpura on platelets and cultured vascular endothelial cells. Blood 60:1030, 1982.
17. Nouri-Aria KT, Hegarty JE, Alexander GJM, Eddleston ALWF, Williams R: Effect of corticosteroids on suppressor-cell activity in autoimmune and viral chronic active hepatitis. N Engl J Med 307:1301, 1982.
18. Gabrielson AE, Good RA: Chemical suppression of adaptive immunity. Adv Immunol 6:92, 1967.
19. Gutterman LA, Stevenson TD: Treatment of thrombotic thrombocytopenic purpura with vincristine. J Am Med Assoc 247:1433, 1982.