

Remission of Chronic Thrombotic Thrombocytopenic Purpura after Treatment with Cyclophosphamide and Rituximab

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Background: Thrombotic thrombocytopenic purpura (TTP) in adults is usually caused by autoantibody inhibitors of ADAMTS13. Treatment with plasma exchange is often effective but does not address the underlying autoimmune process.

Objective: To report the efficacy of intensive immunosuppressive therapy in refractory TTP.

Design: Case report.

Setting: University medical center.

Patient: 42-year-old woman with chronic relapsing TTP.

Intervention: Immunosuppression therapy with rituximab and cyclophosphamide.

Measurements: ADAMTS13 activity and inhibitors and hematologic variables for TTP.

Results: For 19 months, the patient had relapsing thrombotic microangiopathy despite plasma exchange; splenectomy; and therapy with vincristine, prednisone, and cyclosporine. ADAMTS13 activity was low, and tests detected an IgG inhibitor that recognized the metalloprotease domain of recombinant ADAMTS13. After treatment with rituximab and cyclophosphamide, the disease remitted, ADAMTS13 levels normalized, and the inhibitor was undetectable. The patient has required no treatment for 13 months.

Conclusion: Intensive immunosuppressive therapy can lead to sustained clinical remission in patients with refractory autoimmune TTP.

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Most adults with idiopathic thrombotic thrombocytopenic purpura (TTP) have autoantibodies that inhibit a von Willebrand factor–cleaving protease in plasma (1–3). This protease recently was cloned and identified as a new member of the ADAMTS metalloproteases, ADAMTS13 (4–8). Plasma exchange therapy has increased survival in patients with TTP from less than 10% to approximately 75% (9). However, plasma exchange does not directly address the underlying autoimmune process, and up to one third of patients have relapse after initially successful treatment (10). Anecdotal data suggest that immunosuppressive therapy can sometimes be beneficial, but this approach has not been tested systematically in patients known to have ADAMTS13 deficiency. We report the case of a woman with chronic relapsing TTP who had undetectable ADAMTS13 activity and elevated ADAMTS13 inhibitor titers. Treatment with rituximab and cyclophosphamide resulted in prompt disappearance of ADAMTS13 antibodies, normalization of ADAMTS13 activity, and remission of thrombotic microangiopathy.

CASE REPORT

A 42-year-old woman was hospitalized in May 1999 with gait disturbance, disorientation, and lethargy. She had been healthy, with three uncomplicated pregnancies and deliveries and no previous symptoms of autoimmune disease. Computed tomography showed many cerebral infarcts. The platelet count was 60×10^9 cells/L, the hemoglobin level was 96 g/L, and the fibrinogen level was normal. The serum lactate dehydrogenase level was 1157 U/L. The patient received intravenous immunoglobulin

but did not respond. She was transferred to our institution in June 1999 (Figure, day 1).

On admission, hematocrit was 0.231, platelet count was 24×10^9 cells/L, haptoglobin level was 0.09 g/L (normal range, 0.27 to 2.2 g/L), and serum creatinine concentration was 141 μ mol/L (1.6 mg/dL) (normal range, 50 to 125 μ mol/L [0.6 to 1.4 mg/dL]). Prothrombin time and partial thromboplastin time were normal. Results of direct and indirect antiglobulin tests were negative. The peripheral smear contained more than 10 schistocytes per high-power field. Thrombotic thrombocytopenic purpura was diagnosed, and plasma exchange (1.5 volumes daily) was begun (Figure, top). The platelet count normalized but decreased to 34×10^9 cells/L by day 22. Prednisone (1 mg/kg of body weight per day) and aspirin (325 mg/d) were added without sustained benefit. The platelet count improved transiently after laparoscopic splenectomy on day 36 but decreased to less than 100×10^9 cells/L by day 43. Two intravenous doses of vincristine, 2 mg each, resulted in gradual improvement. Frequency of plasma exchanges was decreased to every other day, and the patient was discharged on day 85 with a normal platelet count. Plasmapheresis was discontinued on day 97.

On day 105, the platelet count was 43×10^9 cells/L. Plasma exchange was resumed, and cyclosporine, 75 mg twice per day, was started. The platelet count increased, and plasma exchange was tapered over 3 weeks. During the next 4 months, blood counts were relatively stable. The daily prednisone dose was decreased to 40 mg. On day 248, the patient was admitted with cortical blindness and a platelet count of 58×10^9 cells/L. Plasma exchange was followed by an increase in platelet count but no improvement in vision. The patient was discharged after 12 days,

Context

Many adults with thrombotic thrombocytopenic purpura (TTP) have autoantibodies that promote clotting by inhibiting a von Willebrand factor–cleaving protease (ADAMTS13) in plasma.

Contribution

This 42-year-old woman had chronic relapsing TTP despite plasma exchange; splenectomy; and treatment with vincristine, prednisone, and cyclosporine. She had low to absent ADAMTS13 activity and an IgG inhibitor. After immunosuppressive therapy with rituximab and cyclophosphamide, the TTP remitted, ADAMTS13 levels normalized, and the inhibitor was undetectable.

Cautions

Intensive immunosuppressive therapy aimed at the autoimmune aspect of TTP should be tested in prospective trials before being used widely.

—The Editors

and plasma exchange was tapered over 2 weeks. During the next 11 months, the patient was hospitalized four times for relapsed TTP and each time was treated with plasma exchange. The prednisone dose was tapered to 5 mg/d.

On day 572, the patient was hospitalized with deafness and thrombocytopenia. The platelet count increased rapidly with plasma exchange. The serum creatinine concentration increased to 221 $\mu\text{mol/L}$ (2.5 mg/dL), and cyclosporine therapy was discontinued. The patient received two doses of intravenous rituximab (375 mg/m² weekly). The creatinine concentration decreased to 159 $\mu\text{mol/L}$ (1.8 mg/dL), the platelet count remained above 100×10^9 cells/L, and symptoms were stable for 5 months. On day 740, the platelet count was 69×10^9 cells/L. The patient received daily plasma exchange for 16 days, responded well, and was discharged. She was admitted twice during the next 4 months to receive plasma exchange for relapsing TTP.

Beginning on day 849, the patient received one dose of intravenous cyclophosphamide, 1 g/m², and four doses of intravenous rituximab, 375 mg/m², every 7 to 14 days. No side effects of rituximab were noted. During 10 months of follow-up, the patient has had a normal platelet count, stable hematocrit, normal lactate dehydrogenase level and creatinine concentration, and rare schistocytes. No other signs or symptoms of TTP have been noted. Her neurologic defects (blindness and deafness) persist unchanged.

METHODS

Informed consent was obtained from the patient and her family. ADAMTS13 was assayed as described elsewhere (11); however, the substrate was 10 μg of von Wil-

lebrand factor per mL in 5 mmol of Tris–HCl per L (pH, 8.0), 1.5 mol of urea per L, and 1 mmol of phenylmethanesulfonyl fluoride per L (Sigma, St. Louis, Missouri). Plasma samples were heat-treated at 56 °C for 30 minutes and were serially diluted in phosphate-buffered saline. Diluted plasma (5 μL) was added to normal human plasma (5 μL) and incubated at 37 °C for 30 minutes. Residual ADAMTS13 activity was measured as described earlier. One unit of inhibitor reduces the ADAMTS13 activity of an equal volume of normal plasma by 50%.

Recombinant ADAMTS13 (7) truncated after the metalloprotease (residues 1 to 289) was cloned in pcDNA3.1/V5-His TOPO (Invitrogen, Carlsbad, California), expressed in transiently transfected Chinese hamster ovary cells (CHO-S), and purified from conditioned medium on TALON metal affinity resin (BD Biosciences Clontech, Palo Alto, California). ADAMTS13 was immunoprecipitated with IgG adsorbed from plasma samples onto protein A agarose. Proteins were analyzed by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blot with monoclonal anti-V5 antibody (Invitrogen).

RESULTS

Early in her disease, the patient had complete deficiency of plasma ADAMTS13 activity and an inhibitor was detected (**Figure, bottom**). Plasma therapy over the next 19 months resulted in several short periods of remission, even though ADAMTS13 inhibitor titers decreased minimally and plasma ADAMTS13 activity remained low. The inhibitory activity was recovered in IgG purified from the patient's plasma and immunoprecipitated the metalloprotease domain of recombinant ADAMTS13 (data not shown).

For continuing autoimmune TTP, immunosuppressive therapy with rituximab was initiated. Rituximab is usually given at a dose of 375 mg/m², repeated weekly for four doses (12, 13). The patient received an abbreviated course of two doses, which was followed by prompt disappearance of ADAMTS13 inhibitor, normalization of ADAMTS13 activity, and a normal platelet count. The patient had the first of three additional clinical relapses 5 months after receiving rituximab. Before the second course of rituximab plus cyclophosphamide, ADAMTS13 activity was 33% of normal and no ADAMTS13 inhibitor was detected. These values were obtained after 9 days of daily plasmapheresis, which may have partially corrected the ADAMTS13 deficiency. Subsequently, symptoms of TTP resolved, platelet count and plasma ADAMTS13 activity increased, and the ADAMTS13 inhibitor remained undetectable. The most recent ADAMTS13 level was 17%, with no inhibitor detected.

DISCUSSION

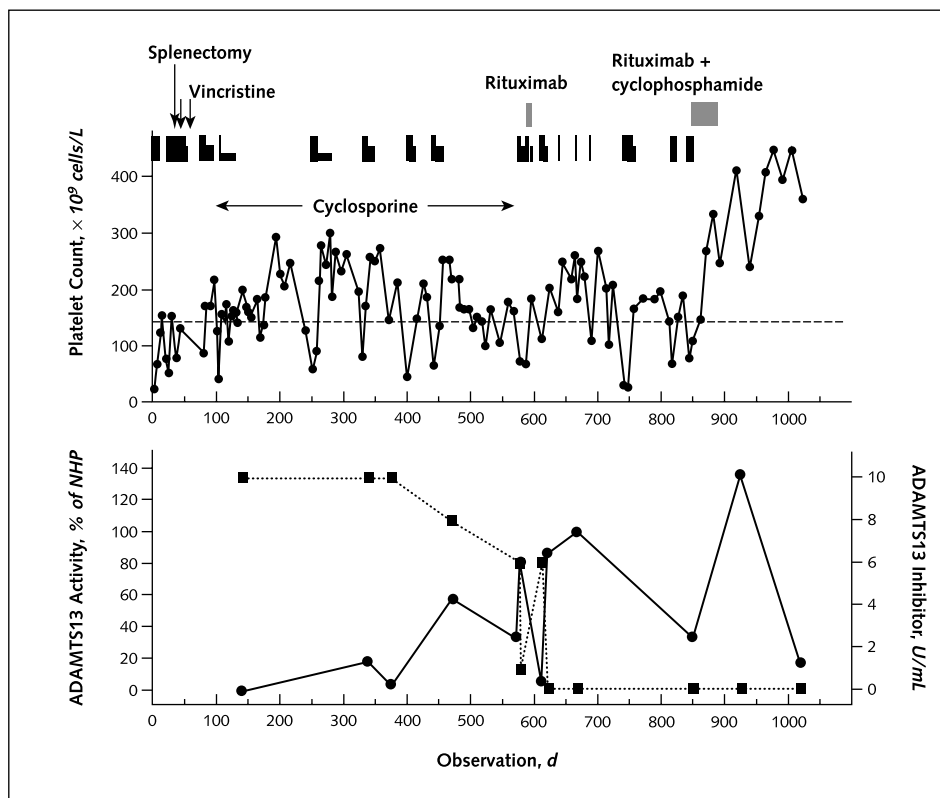
Although idiopathic TTP is usually an autoimmune disease (1–3), standard therapy does not address this underlying mechanism. Plasma exchange may increase patient survival because it removes deleterious antibodies and replenishes ADAMTS13 protein. Most patients have self-limited disease that remits after 1 to several weeks of plasma exchange. However, approximately one third of patients have a chronic relapsing course (10). Thus, TTP often behaves as an aggressive autoimmune disease, and some patients might benefit from additional therapy directed at B cells and antibody production.

Many immunosuppressive regimens have been tried in TTP, with encouraging but inconclusive results. Corticosteroids are often administered at the time of plasma exchange (14), but their efficacy has not been systematically evaluated (9, 10). Case reports and small series have described sustained responses to splenectomy (15), and responses to cyclosporine (16), vincristine, cyclophosphamide, and azathioprine (17, 18) have also been described. This anecdotal experience provides a rationale for testing additional immunosuppressive strategies in patients with relapsing or refractory TTP.

Rituximab is a chimeric anti-CD20 monoclonal antibody developed for treatment of non-Hodgkin lymphoma; CD20 is expressed on B cells. Rituximab has emerged as a promising treatment for autoimmune disorders, including autoimmune hemolytic anemia (12) and idiopathic thrombotic purpura (13). Common side effects observed during the treatment of lymphoma include transient hypotension and fever during the first infusion, probably related to tumor lysis. These side effects are uncommon in patients with autoimmune disorders, possibly because the mass of sensitive lymphocytes is lower (12, 13).

The results obtained with rituximab and cyclophosphamide in our patient are consistent with the autoimmune nature of idiopathic TTP and suggest that intensive immunosuppression may be appropriate for patients who do not respond to plasma exchange. While this manuscript was under review, qualitatively similar results were reported for two patients with refractory TTP treated with rituximab alone (19). A recent report also described remissions in two patients with refractory TTP who received rituximab alone, although ADAMTS activity and inhibitors were not studied (20). Because of the demographic characteristics of most patients with TTP, rituximab could

Figure. Platelet count (top) and ADAMTS13 activity (bottom) in a patient with thrombotic thrombocytopenic purpura during 2.5 years of observation.



Arrows indicate splenectomy and vincristine doses. Black bars indicate plasma exchange treatments; bars of full height, half height, and one-third height represent treatment daily, every other day, or twice per week, respectively. The dashed line in the top panel represents the lower limit of normal platelet count (140×10^9 cells/L). In the bottom panel, the solid line represents ADAMTS13 activity and the dotted line represents ADAMTS13 inhibitor. NHP = normal human plasma.

be preferable to other immunosuppressive approaches. Two thirds of adults with TTP are women with a mean age of approximately 40 years (9, 14). Consequently, most patients with refractory TTP are women of childbearing age, and presentation during pregnancy is common (14). In such patients, treatment with rituximab would avoid the potential infertility and teratogenicity caused by cytotoxic chemotherapy. Therefore, it may be useful to consider rituximab in prospective trials of immunosuppressive therapy for refractory TTP.

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