

Anti-CD20 Chimeric Monoclonal Antibody Treatment of Refractory Immune-Mediated Thrombocytopenia in a Patient with Chronic Graft-versus-Host Disease

Voravit Ratanatharathorn, MD; Erik Carson, MD; Christopher Reynolds, MD; Lois J. Ayash, MD; John Levine, MD; Gregory Yanik, MD; Samuel M. Silver, MD, PhD; James L.M. Ferrara, MD; and Joseph P. Uberti, MD, PhD

Background: Autoimmune thrombocytopenia in chronic graft-versus-host disease may represent an instance of B-cell dysregulation leading to clinical disease.

Objective: To attempt to treat refractory immune-mediated thrombocytopenia in a patient with chronic graft-versus-host disease by using anti-CD20 chimeric monoclonal antibody.

Design: Case report.

Setting: Academic medical center.

Patient: A patient with chronic graft-versus-host disease after allogeneic peripheral blood stem-cell transplantation who had severe refractory immune-mediated thrombocytopenia.

Intervention: Weekly infusion of rituximab, 375 mg/m², for 4 weeks.

Measurements: Platelet count, CD3⁺ cell count, and CD19⁺ cell count.

Results: Rituximab therapy resulted in marked depletion of B cells in the peripheral blood and decreased levels of platelet-associated antibody. The increase in platelet count persisted despite tapering and discontinuation of immunosuppressive therapy for chronic graft-versus-host disease.

Conclusion: The efficacy of rituximab for the treatment of immune-mediated thrombocytopenia suggests that this drug may have activity in other autoimmune diseases or chronic graft-versus-host disease.

Ann Intern Med. 2000;133:275-279.

For author affiliations, current addresses, and contributions, see end of text.

Chronic graft-versus-host disease occurs in approximately 50% of long-term survivors of transplantation with marrow from HLA-identical donors (1); the risk for this disease increases with use of peripheral blood stem cells (2). Chronic graft-versus-host disease shares many of the clinical manifestations of autoimmune collagen vascular diseases, including oral ulceration, lichen planus, xerostomia, keratoconjunctivitis sicca, polyserositis, esophagitis and esophageal stricture, vaginal ulceration and stricture, intrahepatic obstructive liver disease, obstructive pulmonary disease, scleroderma, morphea, fasciitis, and myositis (3). Cytopenias, particularly thrombocytopenia, are a common feature of both chronic graft-versus-host disease and collagen vascular disease, and thrombocytopenia in chronic graft-versus-host disease is associated with poorer survival (4, 5). Antiplatelet antibodies are frequently detected in patients with thrombocytopenia associated with chronic graft-versus-host disease (6). Most patients with chronic graft-versus-host disease have evidence of B-cell dysregulation, with a high prevalence of autoantibodies to several cell surface and intracellular antigens (7). The role of these autoantibodies in the pathogenesis of chronic graft-

versus-host disease is unclear. Autoimmune thrombocytopenia in chronic graft-versus-host disease may represent an instance of B-cell dysregulation leading to clinical disease.

Rituximab is a humanized murine monoclonal antibody commonly used to treat B-cell lymphomas (8). This antibody is highly effective for in vivo depletion of B cells. Circulating B cells become undetectable after a single 375-mg/m² infusion of rituximab; recovery of B cells begins at 6 to 9 months after treatment, and counts normalize by 9 to 12 months (9). Because of these biological properties of rituximab and the association of antiplatelet autoantibody with thrombocytopenia in patients with chronic graft-versus-host disease (6), we hypothesized that rituximab might have clinically significant activity in the treatment of refractory immune-mediated thrombocytopenia.

We describe a patient with chronic graft-versus-host disease who developed severe refractory thrombocytopenia that responded to anti-CD20 chimeric antibody therapy. The rationale for this treatment was to eliminate B cells producing autoantibodies and thereby reverse the thrombocytopenia.

Case Report

A 32-year-old woman, gravida 3 para 1, presented with blurred vision and was found to have retinal hemorrhages. Complete blood count showed a leukocyte count of 451×10^9 cells/L with 2% basophils. A diagnosis of chronic myelogenous leukemia was confirmed by the presence of the Philadelphia chromosome in the marrow sample. The patient began receiving hydroxyurea to reduce her leukocyte count and was referred for stem-cell transplantation. She received 16 doses of busulphan (1 mg/kg of body weight every 6 hours) followed by cyclophosphamide (60 mg/kg daily for 2 days) in preparation for transplantation. Filgrastim-mobilized peripheral blood stem cells were harvested from an HLA-matched brother and were infused into the patient 3 months after diagnosis. The patient's blood group was A+, and she was seropositive for cytomegalovirus; the donor's blood group was O+, and he was seronegative for cytomegalovirus.

Prophylaxis against graft-versus-host disease consisted of tacrolimus and methotrexate (10). The patient had prompt hematologic reconstitution, with an absolute neutrophil count of 0.5×10^9 cells/L on day 12 after transplantation and a platelet count greater than 100×10^9 cells/L on day 16 after transplantation. Therapy with ganciclovir, 5 mg/kg twice weekly, was started on day 16 as prophylaxis against cytomegalovirus infection.

On day 28 after transplantation, the patient developed a maculopapular rash of the upper torso and diarrhea. Skin biopsy confirmed acute graft-versus-host disease, and she began receiving methylprednisolone, 1 mg/kg daily. The ganciclovir dose was increased prophylactically to 5 mg/kg 5 times per week. Bone marrow aspirate on day 100 after transplantation showed a normal male karyotype, and full donor chimerism was confirmed by polymerase chain reaction of microsatellite markers. The patient responded well to steroid therapy for the skin and gastrointestinal symptoms of graft-versus-host disease, but symptoms of xerophthalmia and xerostomia developed and steroid therapy was continued through day 127. On day 142, she underwent punctal occlusion of both eyes for severe xerophthalmia. Other manifestations of chronic graft-versus-host disease were progressive xerostomia with lichenoid changes of the oral mucosa. During the patient's course of therapy with steroids and the increased dose of ganciclovir, her platelet count remained greater than 100×10^9 cells/L. On day 211, a complete blood count showed an absolute neutro-

phil count of 2.7×10^9 cells/L, hemoglobin value of 131 g/L, and platelet count of 178×10^9 cells/L.

On day 230, the patient's platelet count decreased to 88×10^9 cells/L. On day 238, it decreased further to 28×10^9 cells/L. No microangiopathic changes were seen on peripheral smear. Bone marrow aspiration and biopsy showed normal trilineage hematopoietic maturation with an adequate number of megakaryocytes. Flow cytometry showed the presence of platelet-associated IgG on washed, formalin-fixed platelets. Intravenous immunoglobulin, 500 mg/kg, was administered daily for 3 days; the platelet count increased to 159×10^9 cells/L, but for only 2 weeks. The patient then received 5 doses of intravenous immunoglobulins with methylprednisolone, 64 mg/d. On day 278, therapy with mycophenolate was started to provide additional immunosuppression. The combination of intravenous immunoglobulins, steroids, and mycophenolate resulted in an increase in platelet count to 114×10^9 cells/L by day 299.

On day 308, the patient's platelet count decreased to 51×10^9 cells/L. A dose of anti-D antibody (50 μ g/kg) was given, and the platelet count increased to 127×10^9 cells/L. However, the patient experienced significant hemolysis; her hemoglobin value decreased from 131 g/L to 78 g/L, an expected side effect related to the destruction of Rho (D)-positive red cells. On day 337, she underwent laparoscopic splenectomy; an accessory spleen was also removed. By day 341, the platelet count increased to 139×10^9 cells/L but decreased to 2×10^9 cells/L 1 week later. Intravenous vincristine, 2 mg, was administered in four weekly doses starting on day 356, but it did not produce a response. The patient subsequently received intravenous cyclophosphamide, 1.5 g/m², on day 384, again without response.

On day 404, the patient received the first of four weekly doses of anti-CD20 antibody (rituximab [Rituxan, Genentech/IDEC, South San Francisco, California]). Peripheral blood flow cytometry on day 377 showed a CD19⁺ cell count (B cells) of 0.116×10^9 cells/L; by day 474, after rituximab therapy, CD19⁺ cells were absent. A gradual but sustained increase in the platelet count was noted after 2 doses of rituximab. The dose of mycophenolate mofetil was tapered and therapy was discontinued 3 weeks after initiation of rituximab therapy. At the time of this report, the patient has no clinically significant signs or symptoms of chronic graft-versus-host disease despite continued tapering of the tacrolimus dose and discontinuation

Table. Chronological Clinical Course of the Patient*

Day after Transplantation	Platelet Count, $\times 10^9$ cells/L	Clinical Events and Interventions
0	240	Peripheral blood stem-cell transplantation from HLA-matched brother; prophylaxis with tacrolimus and methotrexate for GVHD given
16	141	Absolute neutrophil count, 1.3×10^9 cells/L; therapy with ganciclovir for cytomegalovirus prophylaxis begun
28	103	Acute GVHD involving skin and gastrointestinal tract; therapy with methylprednisolone, 1 mg/kg per day
62	123	Acute GVHD completely resolved; methylprednisolone dose tapered
97	97	Bone marrow aspiration and biopsy showed normal structure, male karyotype, and absence of Philadelphia chromosome
127	157	Xerophthalmia and xerostomia developed; bilateral punctal occlusion was done on day 147, with relief of symptoms
211	178	Bone marrow showed normal structure
230	88	Severe xerostomia; approximately 10 kg of weight lost; receiving tacrolimus, 3 mg/d
238	28	Bone marrow examination showed adequate megakaryocytes with occasional small hypolobulated forms; therapy with IVIG, 500 mg/kg daily for 3 days; positive for platelet-associated antibody
243	159	Responded to IVIG
257	2	Recurrence of thrombocytopenia; therapy with IVIG, 500 mg/kg daily for 5 days, and methylprednisolone, 64 mg/d; CD19 ⁺ count, 0.145×10^9 cells/L, and CD3 ⁺ count, 0.925×10^9 cells/L
278	29	Therapy with oral mycophenolate, 1.5 g twice daily; continued therapy with oral methylprednisolone, 32 mg/d, and tacrolimus, 3 mg/d
299	114	Methylprednisolone dose tapered by 4 mg/wk
308	51	Therapy with anti-D antibody, 50 μ g/kg, causing severe hemolysis (decrease in hemoglobin value from 131 g/L to 78 g/L 5 days later)
337	11	Splenectomy done; platelet count increased to 74×10^9 cells/L after splenectomy
348	6	Two doses of IVIG given without response
356	9	First of four weekly doses of intravenous vincristine, 2 mg
377	5	CD19 ⁺ count, 0.116×10^9 cells/L; CD3 ⁺ count, 4.964×10^9 cells/L
384	3	Indium-labeled platelet study showed no abnormal platelet pooling; therapy with intravenous cyclophosphamide, 1.5 g/m ² ; methylprednisolone dose tapered to discontinuation; continued therapy with tacrolimus, 2 mg/d, and mycophenolate, 500 mg twice daily
404	3	First dose of rituximab, 375 mg/m ² , started; continued therapy with tacrolimus, 2 mg/d, and mycophenolate, 500 mg twice daily
411	4	Second dose of rituximab
418	20	Third dose of rituximab
425	112	Fourth dose of rituximab; mycophenolate therapy discontinued; continued tacrolimus therapy, 2 mg/d
436	242	Tacrolimus dose decreased to 1 mg/d
474	422	CD19 ⁺ count, 0×10^9 cells/L; CD3 ⁺ count, 4.553×10^9 cells/L; positive for platelet-associated antibody
485	487	CD19 ⁺ count, 0×10^9 cells/L; CD3 ⁺ count, 2.729×10^9 cells/L; weakly positive for platelet-associated antibody
506	614	Transient increase in alkaline phosphatase level; tacrolimus dose increased to 4 mg/d and then gradually tapered
532	424	CD19 ⁺ count, 0×10^9 cells/L; CD3 ⁺ count, 4.007×10^9 cells/L; negative for platelet-associated antibody
590	381	CD19 ⁺ count, 0×10^9 cells/L; CD3 ⁺ count, 5.378×10^9 cells/L
638	458	CD19 ⁺ count, 0×10^9 cells/L; CD3 ⁺ count, 4.043×10^9 cells/L; negative for platelet-associated antibody
667	449	All immunosuppressive therapy discontinued
687	489	CD19 ⁺ count, 0×10^9 cells/L; CD3 ⁺ count, 5.225×10^9 cells/L
692	489	Doing well, with no active signs or symptoms of chronic GVHD

* GVHD = graft-versus-host disease; IVIG = intravenous immunoglobulins.

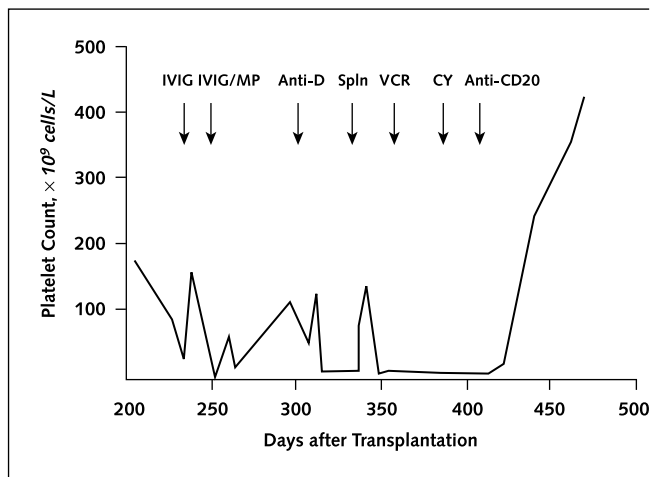
of this therapy on day 667. The platelet-associated antibody assay remained weakly positive at day 485 and became negative on day 532. The patient has now been followed for 11 months since initiation of rituximab therapy, and the platelet count has not decreased (Table and Figure).

Discussion

The reversal of our patient's immune-mediated thrombocytopenia was especially noteworthy because of the lack a sustained response to intensive immunosuppression, including treatment with steroids, mycophenolate, tacroli-

mus, vincristine, cyclophosphamide, intravenous immunoglobulins, anti-D antibody, and splenectomy. The improvement of thrombocytopenia was also associated with decreased levels of platelet-associated antibody and lack of progression of chronic graft-versus-host disease despite the discontinuation of immunosuppression. It is possible that cyclophosphamide therapy contributed in part to the platelet response. Reiner and colleagues (11) reported a series of 20 cases of refractory idiopathic thrombocytopenia purpura treated with pulse cyclophosphamide (1 to 1.5 g/m²), with complete remission in 65% of patients and partial remission in 20%. However, most patients required

Figure. Clinical course of a patient with chronic graft-versus-host disease in whom severe refractory immune-mediated thrombocytopenia responded to treatment with anti-CD20 monoclonal antibody.



Anti-CD20 = rituximab; Anti-D = anti-D antibody; CY = cyclophosphamide; IVIG = intravenous immunoglobulins; MP = methylprednisolone; Spln = splenectomy; VCR = vincristine.

multiple pulses (mean, 2; maximum, 4), and the mean time to response was 7 weeks. Considering the intensity of the immunosuppressive therapy that our patient received and the lack of platelet response at the time of neutrophil recovery, it is unlikely that cyclophosphamide contributed substantially.

Studies of B cells in patients with chronic graft-versus-host disease have not convincingly explained the clinical abnormalities, including reduced B-cell count, decreased Ig production, and increased spontaneous Ig production (12), seen in this condition. Clonal dysregulation of B cells in chronic graft-versus-host disease has been suggested by the presence of monoclonal gammopathy (13, 14) and elevated serum IgG and IgM levels (15). Antibody production is a prominent feature of many animal models of chronic graft-versus-host disease (16, 17). Mechanistic studies showed that secretion of Th2 cytokines, such as interleukin-4 and interleukin-5, by donor CD4⁺ cells are critical to B-cell activation and autoantibody production. Most of the autoantibodies are of the IgG subclass (16), and abnormal cross-linking of IgG receptors on the B-cell surface may be critical to production of such antibodies (18).

Another possible explanation of the pathogenesis of autoantibody production in chronic graft-versus-host disease is the activation or proliferation of the B-1 subset of B lymphocytes. Activation of this subset has been shown to

increase levels of anti-DNA antibodies and rheumatoid factor in a murine model, and it may be important in autoimmune disorders (19). The B-cell antigen CD19, a co-receptor that amplifies mitogen-activated protein kinase signaling by membrane-bound immunoglobulin during B-cell responses to T-cell-dependent antigens, seems to play a particularly important role in B-1 activation. Forced over-expression of CD19 in mice makes B lymphocytes hyper-responsive to antigen and promotes autoantibody development and breakdown of peripheral tolerance (19, 20). In our patient, the therapeutic effect of rituximab was associated with total depletion of B cells, and it is tempting to speculate that elimination of a particular autoreactive subset was especially beneficial. Further studies of B-cell subpopulations and the role of various signaling pathways in chronic graft-versus-host disease may allow more specific therapeutic interventions to be devised.

Because chronic graft-versus-host disease shares many clinical manifestations and immunopathogenesis with various autoimmune diseases, including idiopathic thrombocytopenia purpura, prospective clinical trials of rituximab in these autoimmune diseases are warranted.

From Blood and Marrow Stem Cell Transplant Program, University of Michigan Medical Center, Ann Arbor, Michigan.

Acknowledgments: The authors thank D. Rummler, T. Hunt, J. Wegner, K. Jacobi, H. Hinds, S. Anderson, M. Glasser, T. Norton, B. Wilson, and L. Lamirand for their dedicated patient care and A. Perrotta for his astute clinical skill in the management of patients with thrombocytopenia.

Requests for Single Reprints: Voravit Ratanatharathorn, MD, B1-207 Cancer Center, University of Michigan Medical Center, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109; e-mail, vratanat@umich.edu.

Requests To Purchase Bulk Reprints (minimum, 100 copies): Barbara Hudson, Reprints Coordinator; phone, 215-351-2657; e-mail, bhudson@mail.acponline.org.

Current Author Addresses: Drs. Ratanatharathorn, Carson, Reynolds, Ayash, Levine, Yanik, Silver, Ferrara, and Uberti: Blood and Marrow Stem Cell Transplant Program, Departments of Internal Medicine and Pediatrics, University of Michigan Medical Center, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109.

Author Contributions: Conception and design: V. Ratanatharathorn, S.M. Silver, J.P. Uberti.

Analysis and interpretation of the data: V. Ratanatharathorn, E. Carson, C. Reynolds, L.J. Ayash, J. Levine, G. Yanik, S.M. Silver, J.P. Uberti.

Drafting of the article: V. Ratanatharathorn, E. Carson, L.J. Ayash, J. Levine, G. Yanik, S.M. Silver, J.P. Uberti.

Critical revision of the article for important intellectual content: V. Ratanatharathorn, C. Reynolds, L.J. Ayash, J. Levine, G. Yanik, S.M. Silver, J.L.M. Ferrara, J.P. Uberti.

Final approval of the article: V. Ratanatharathorn, C. Reynolds, L.J. Ayash, S.M. Silver, J.P. Uberti.

Provision of study materials or patients: S.M. Silver, J.P. Uberti.

Administrative, technical, or logistic support: V. Ratanatharathorn, J.L.M. Ferrara, J.P. Uberti.

Collection and assembly of data: V. Ratanatharathorn, E. Carson, J.P. Uberti.

References

1. **Atkinson K, Horowitz MM, Gale RP, Lee MB, Rimm AA, Bortin MM.** Consensus among bone marrow transplanters for diagnosis, grading and treatment of chronic graft-versus-host disease. Committee of the International Bone Marrow Transplant Registry. *Bone Marrow Transplant.* 1989;4:247-54.
2. **Storek J, Gooley T, Siadak M, Bensinger WI, Maloney DG, Chauncey TR, et al.** Allogeneic peripheral blood stem cell transplantation may be associated with a high risk of chronic graft-versus-host disease. *Blood.* 1997;90:4705-9.
3. **Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S, et al.** Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol.* 1991;28:250-9.
4. **Sullivan KM, Witherspoon RP, Storb R, Deeg HJ, Dahlberg S, Sanders JE, et al.** Alternating-day cyclosporine and prednisone for treatment of high-risk chronic graft-v-host disease. *Blood.* 1988;72:555-61.
5. **Sullivan KM, Witherspoon RP, Storb R, Weiden P, Flournoy N, Dahlberg S, et al.** Prednisone and azathioprine compared with prednisone and placebo for treatment of chronic graft-v-host disease: prognostic influence of prolonged thrombocytopenia after allogeneic marrow transplantation. *Blood.* 1988;72:546-54.
6. **Anasetti C, Rybka W, Sullivan KM, Banaji M, Slichter SJ.** Graft-v-host disease is associated with autoimmune-like thrombocytopenia. *Blood.* 1989;73:1054-8.
7. **Kier P, Penner E, Bakos S, Kalhs P, Lechner K, Volc-Platzer B, et al.** Autoantibodies in chronic GVHD: high prevalence of antinucleolar antibodies. *Bone Marrow Transplant.* 1990;6:93-6.
8. **Leget GA, Czuczman MS.** Use of rituximab, the new FDA-approved antibody. *Curr Opin Oncol.* 1998;10:548-51.
9. **McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, et al.** Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol.* 1998;16:2825-33.
10. **Jacobson P, Uberti J, Davis W, Ratanatharathorn V.** Tacrolimus: a new agent for the prevention of graft-versus-host disease in hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 1998;22:217-25.
11. **Reiner A, Gernsheimer T, Slichter SJ.** Pulse cyclophosphamide therapy for refractory autoimmune thrombocytopenic purpura. *Blood.* 1995;85:351-8.
12. **Saxon A, McIntyre RE, Stevens RH, Gale RP.** Lymphocyte dysfunction in chronic graft-versus-host disease. *Blood.* 1981;58:746-51.
13. **Gerritsen EJ, van Tol MJ, Lankester AC, van der Weijden-Ragas CP, Jol-van der Zijde CM, Oudeman-Gruber NJ, et al.** Immunoglobulin levels and monoclonal gammopathies in children after bone marrow transplantation. *Blood.* 1993;82:3493-502.
14. **Mitus AJ, Stein R, Rappoport JM, Antin JH, Weinstein HJ, Alper CA, et al.** Monoclonal and oligoclonal gammopathy after bone marrow transplantation. *Blood.* 1989;74:2764-8.
15. **Storek J, Saxon A.** Reconstitution of B cell immunity following bone marrow transplantation. *Bone Marrow Transplant.* 1992;9:395-408.
16. **Rolink AG, Strasser A, Melchers F.** Autoimmune diseases induced by graft-vs-host disease. In: Burakoff SJ, Deeg HJ, Ferrara J, Atkinson K, eds. *Graft-vs.-Host Disease: Immunology, Pathophysiology, and Treatment.* New York: Marcel Dekker; 1990:161-75.
17. **Hakim FT, Mackall CL.** The immune system: effector and target of graft-vs.-host disease. In: Ferrara JLM, Deeg HJ, Burakoff SJ, eds. *Graft-vs.-Host Disease.* 2d ed. New York: Marcel Dekker; 1997:257-89.
18. **Goldman M, Druet P, Gleichmann E.** TH2 cells in systemic autoimmunity: insights from allogeneic diseases and chemically-induced autoimmunity. *Immunol Today.* 1991;12:223-7.
19. **Sato S, Ono N, Steeber DA, Pisetsky DS, Tedder TF.** CD19 regulates B lymphocyte signaling thresholds critical for the development of B-1 lineage cells and autoimmunity. *J Immunol.* 1996;157:4371-8.
20. **Inaoki M, Sato S, Weintraub BC, Goodnow CC, Tedder TF.** CD19-regulated signaling thresholds control peripheral tolerance and autoantibody production in B lymphocytes. *J Exp Med.* 1997;186:1923-31.

© 2000 American College of Physicians–American Society of Internal Medicine