Disorders of Platelets

Anti-interleukin-2 receptor antibody (daclizumab) treatment of corticosteroid-refractory autoimmune thrombocytopenic purpura

We administered daclizumab, a humanized monoclonal anti-interleukin-2 receptor (IL-2R) antibody, to 11 patients with corticosteroid-refractory autoimmune thrombocytopenic purpura (AITP) every 2 weeks for five treatments. Of nine evaluable patients, one individual experienced a partial response. Lymphocyte phenotyping by flow cytometry indicated post-treatment binding of IL-2Rα by daclizumab in all patients. Mid-study serum soluble IL-2R levels in all patients increased 4-15 –fold over baseline values (p=0.004). Despite these measurable immunologic effects, blockade of the IL-2/IL-2R axis did not effectively abrogate the autoimmune response in this group of patients with corticosteroid-refractory AITP.

haematologica 2006; 91:277-278
(*http://www.haematologica.org/journal/2006/02/277.html*)

Autoimmune thrombocytopenic purpura (AITP) is an acquired disorder in which anti-platelet antibodies result in platelet destruction.[1] Although autoantibody production is B lymphocyte mediated, a concomitant T-cell activation is also present in patients with AITP.[2] In chronic AITP, serum levels of the T-cell regulatory cytokine interleukin-2 (IL-2) are increased[3] and soluble IL-2 receptor levels (sIL-2R; a product of cytoplasmic IL-2R expression and cleavage) correlate inversely with the platelet count.[4] Daclizumab is a selectively modified humanized anti-IL-2R monoclonal antibody that is specific for the 55-kd α-chain (CD25) of the high-affinity IL-2R, which is expressed predominantly on activated T cells.[5] Treatment with daclizumab induces a prolonged saturation of CD25 receptors, thereby impairing interaction with IL-2 and reducing the viability of CD25-expressing cells.[6] Although pharmacologic blockade of CD25 has principally been applied in organ transplantation for prevention of rejection, presumably via inhibition of antigen-naïve T cells,[7] the use of daclizumab has also resulted in clinical improvement in chronic disorders of autoimmunity such as epidermolysis bullosa[8] and autoimmune uveitis,[9] indicating potential alternative immunomodulatory effects.

To test whether CD25 blockade might lead to clinical remission in AITP, we administered daclizumab to 11 patients (Table 1) with corticosteroid-refractory AITP (defined as failure to maintain a platelet count of at least 30×10^9/L using prednisone at a dose of at least 10mg per day for at least 6 weeks). All patients were required to have an average pre-enrollment platelet count of $<30\times10^9$/L. Concomitant use of stable doses of standard immunomodulatory medications was permitted, but initiation of new immunomodulatory agents within two months prior to study entry was not. The protocol was approved by the Institutional Review Board of the National Heart, Lung and Blood Institute, and all subjects provided written informed consent. Daclizumab (Protein Design Labs, Fremont, California, USA), was administered at a dose of 1 mg/kg intravenously over 15 minutes every 2 weeks for five treatments. Nine of 11 patients received all five doses of daclizumab and were evaluable at 12 and 20 weeks post-enrollment (4 and 8 weeks after the last dose of daclizumab, respectively; Table 1).

In one patient (patient n. 3), the platelet count increased from a baseline value of 15×10^9/L to 91×10^9/L two weeks after the first infusion of drug, after which point it slowly declined and stabilized in the $30\text{-}40\times10^9$/L range off all other therapies (Table 1). None of the other patients had a response. Infusions of the agent were well-tolerated and no grade 3-4 toxicities were observed. Grade 1-2 toxicities included (number of events) mild hypertension (6), upper respiratory/flu-like symptoms (4), transient hypotension (1), decreased appetite (1), conjunctival hemorrhage (1), fever (1), lightheadedness (1), vertigo (1), cerebral hemorrhage (1), neuropathy (1), and dyspnea (1). Binding of daclizumab to T cells was assessed at baseline and at weeks 12 and 20 by flow cytometry. Whereas the absolute number of lymphocytes expressing CD4 and CD8 remained relatively constant throughout the study period, the mean number of $CD4^+$ T lymphocytes bound by the antibody 2A3 (BD Pharmingen, San Diego, CA, USA),

Table 1. Patients' characteristics and platelet response.

Patient	Age (years)	Sex	Time since diagnosis (years)	Splenectomy	Prior treatments	Treatments during administration of study drug	Baseline platelet count ($\times10^9$/L)	Week 12 platelet count ($\times10^9$/L)
1	69	F	8	Yes	Pred, anti-D, vcr, cytox, ritux, aza	Pred 10/15 mg/day	9	6
2	57	F	18	No	Pred, dan, anti-d, IVIg, ritux	IVIg	5	14
3*	52	F	1	No	Pred, anti-D	Pred 5 mg/day	15	34
4	62	F	12	Yes	Pred, IVIg, anti-D, HiDex, dan, ritux, aza, vcr	Pred 5 mg/day, aza 50 mg/day	14	17
5°	57	F	20	Yes	Pred, IVIG, HiDex, colch, aza, ritux	–	22	ND
6	49	F	14	Yes	Pred, HIDex, anti-D, csa, dan, IFN, aza, oral cy, plas, ritux	Pred 15 mg/day, IVIg	21	28
7	53	F	9	Yes	Pred, IVIg, HiDex, ritux	IVIg	21	22
8	28	M	5	Yes	Pred, IVIg, anti-D, dan, oral cy, autotx, ritux	–	5	6
9	30	F	22	Yes	Pred, dan, oral cy, aza, vcr, colch	–	2	9
10†	18	F	1	No	Pred	–	26	ND
11	52	M	5	Yes	Pred, oral cy, ritux	Pred 10 mg/day	16	14

*Pred: prednisone; Vcr: vincristine; cytox: intravenous cyclophosphamide; ritux: rituximab; 6-MP: 6-mercaptopurine; HiDex: high-dose dexamethasone; colch: colchicine; dan: danazol; CSA: cyclosporine; IFN: interferon-α; IVIg: intravenous immune globulin; aza: azathioprine; oral cy: oral cyclophosphamide; plas: plasmapheresis; autotx: autologous transplantation; ND: not done. *The platelet count of patient n. 3 increased to 91×10^9/L following the first infusion of daclizumab and gradually declined to the $30\text{-}40\times10^9$/L range by week 32 of the study. °Patient 5 withdrew for non-medical reasons after receiving one infusion of daclizumab. †Patient 10 received corticosteroids following the week 8 infusion of daclizumab and became ineligible for follow-up at week 12.*

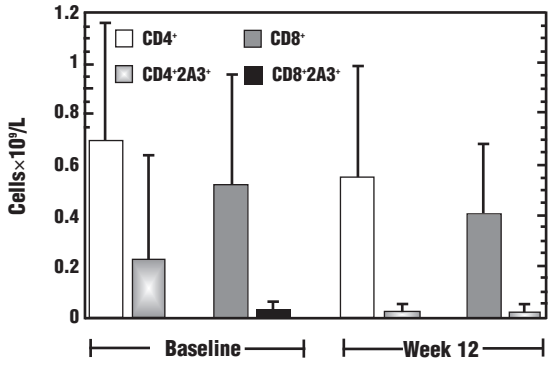

Figure 1. Absolute counts of CD4⁺ and CD8⁺ lymphocytes at baseline and in week 12 of the study. The number of CD4⁺ and CD8⁺ lymphocytes bound by the anti-CD25 antibody 2A3 at each time point is also indicated. The reduction in binding of 2A3 at week 12 in both CD4⁺ and CD8⁺ lymphocytes suggests that IL-2Rα is bound to daclizumab and is unable to bind 2A3.

which engages the same epitope as daclizumab, had decreased dramatically by week 12 in all patients (p=0.004; 225×10⁶/L at baseline and 2.0×10⁶/L at week 12), suggesting near-complete and persistent occupancy of CD25 by daclizumab (Figure 1). A similar decrease occurred in the mean number of CD8-positive T lymphocytes bound by 2A3 (p=0.004;19.5×10⁶/L at baseline and 0.6×10⁶/L at week 12). By week 20, 2A3 binding to lymphocytes positive for CD4 and CD8 had begun to return to pre-treatment values in most patients (*data not shown*), suggesting gradual disengagement of daclizumab from CD25 or clearance of daclizumab-bound CD25⁺ lymphocytes. Serum sIL-2R levels (Quantikine Immunoassay ELISA, R&D Systems, Minneapolis, MN, USA) at week 12 increased 4-15 –fold over baseline values (p=0.004; median 866 pg/mL at baseline and 7010 pg/mL at week 12) and had begun to return to their baseline values by week 20. Neither serum thrombopoietin levels nor antiplatelet antibody titers correlated with platelet counts, CD25 expression on T lymphocytes, or sIL-2R levels (*data not shown*).

In summary, only one individual in our series experienced a partial response to daclizumab, despite laboratory evidence suggesting efficacious IL-2Rα blockade in all patients. The responding patient had the lowest CD25 expression on CD4⁺ and CD8⁺ lymphocytes prior to treatment, though the significance of this finding is unknown. These data show that administration of an IL-2Rα-blocking antibody in this dose schedule does not significantly modulate the antiplatelet autoimmune response in most patients with AITP.

Patrick F. Fogarty, Ruth Seggewiss,° Donna Jo McCloskey,* Carol A. Boss,° Cynthia E. Dunbar,° Margaret E. Rick**

**Hematology Service, Department of Laboratory Medicine, Clinical Center and °Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland, USA*

Acknowledgments: the authors thank Protein Design Labs, Inc. for supplying daclizumab and Robert Wesley, Ph.D. for his assistance with the statistical analysis.

Key words: immune thrombocytopenic purpura, daclizumab, platelets, autoimmune disease.

Correspondence: Patrick F. Fogarty, M.D., Hematology Division, University of California, San Francisco, 505 Parnassus Ave., Box 1270, San Francisco, CA 94143-1270. Phone: international +415.4769608. Fax: international + 415.4760624. E-mail: pfogarty@medicine.ucsf.edu

References

1. Berchtold P, Wenger M. Autoantibodies against platelet glycoproteins in autoimmune thrombocytopenic purpura: their clinical significance and response to treatment. Blood 1993;81:1246-50.
2. Semple JW, Freedman J. Increased antiplatelet T helper lymphocyte reactivity in patients with autoimmune thrombocytopenia. Blood 1991;78:2619-25.
3. Semple JW, Milev Y, Cosgrave D, Mody M, Hornstein A, Blanchette V, et al. Differences in serum cytokine levels in acute and chronic autoimmune thrombocytopenia purpura: relationship to platelet phenotype and antiplatlelet T-cell reactivity. Blood 1996;87:45-54.
4. Erduran E, Aslan Y, Aliyazicioglu Y, Mocan H, Gedik Y. Plasma soluble interleukin-2 receptor levels in patients with idiopathic thrombocytopenic purpura. Am J Hematol 1998;57:119-23.
5. Taniguchi T, Minami Y. The IL-2/IL-2 receptor system: a current overview. Cell 1993;73:5-8.
6. Vincenti F, Nashan B, Light S. Daclizumab: outcome of phase III trials and mechanism of action. Double therapy and the Triple Therapy Study Groups. Transplant Proc 1998;30:2155-8.
7. Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. N Engl J Med 1998;338:161-5.
8. Nussenblatt RB, Fortin E, Schiffman R, Rizzo L, Smith J, Van Veldhuisen P, et al. Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: a phase I/II clinical trial. Proc Natl Acad Sci 1999;96:7462-6.
9. Egan CA, Brown M, White JD, Yancey KB Treatment of epidermolysis bullosa acquisita with the humanized anti-Tac mAb daclizumab. Clin Immunol 2001;101:146-51.