Long-term follow-up of patients with moderate aplastic anemia and pure red cell aplasia treated with daclizumab

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ABSTRACT

Background
Pure red cell aplasia and moderate aplastic anemia are marrow failure states with an immune pathogenesis. Previously, we described short-term improvements in blood counts in two pilot studies treating moderate aplastic anemia (mAA) and pure red cell aplasia (PRCA) patients with daclizumab, a humanized monoclonal antibody to the interleukin-2 receptor; we now report our long-term experience with a larger cohort of patients.

Design and Methods
After a median follow-up period of 4.8 years, 19 of 45 (42%) evaluable mAA patients and 10 of 26 (38%) patients with PRCA responded by three months and 2 additional m-AA patients responded by six months following administration of the drug.

Results
Seven of 28 (25%) mAA patients achieved long-term packed red blood cell PRBC transfusion independence, and all PRCA responders achieved long-term transfusion PRBC transfusion independence.

Conclusions
Red cell transfusion-independence prior to treatment in mAA patients predicted response. The only significant adverse treatment-related events were transient rashes and arthralgias. Daclizumab is safe and effective, and produces lengthy remissions in patients with PRCA and mAA.

Key words: interleukin-2 receptors, immunosuppression, T-regulatory cells, marrow failure.

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The online version of this article has a Supplementary Appendix.

Introduction

Pure red cell aplasia and moderate aplastic anemia are acquired bone marrow failure syndromes characterized by an immune destruction of hematopoietic precursors. While immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine (CSA) is effective in treating severe aplastic anemia (AA), therapeutic decisions are more difficult in patients with moderate aplastic anemia (mAA), especially for those who require red cell or platelet transfusions. In pure red cell aplasia, many drugs including corticosteroids, azathioprine, cyclosphosphamide, as well as ATG and CSA, have produced variable responses.[1-3] However, these agents have acute toxicities and long-term complications, including infusion reactions, serum sickness, posterior reversible encephalopathy, and nephrotoxicity.[1,4-6] Less toxic but equally effective strategies potentially would avoid the need for hospitalization (as required for ATG administration), for monitoring of drug serum levels (as required for CSA), and potentially serious end organ damage.

To circumvent these complications, we treated a cohort of patients with daclizumab,[7,8] a genetically engineered humanized monoclonal IgG1 antibody that specifically recognizes an epitope of the 55-kDa α-subunit of the heterotrimeric interleukin 2 receptor (IL-2R). Our study population included patients with bone marrow failure syndromes including moderate aplastic anemia and pure red cell aplasia. Daclizumab was initially developed to block proliferation of virally transformed T lymphocytes in adult T-cell leukemia[9] because of its specificity for the IL-2R (CD25) T-lymphocyte activation marker. Subsequently, daclizumab has been widely used in solid organ transplantation to inhibit the activation of T lymphocytes resulting from MHC mismatched recognition.[10,11]

Recently, a phase I/II clinical trial demonstrated efficacy in preventing progression in patients with immune-mediated uveitis.[12] Phase II trials in multiple sclerosis have demonstrated positive clinical outcomes, including decreased relapse rates.[13,14] Although the effect of daclizumab is relatively short and reversible *in vitro*, the serum half-life is 20 days, and its administration results in prolonged saturation of CD25 on circulating lymphocytes.[15]

Previously, we reported the short-term effectiveness of daclizumab in a small group of patients with PRCA and mAA.[7,8] We now describe the safety and efficacy results of a long-term follow-up in a larger cohort of these patients.

Design and Methods

Patient populations

Eligible patients with mAA and PRCA were entered into the study after obtaining informed consent according to approved protocols by the Institutional Review Board of the National Heart, Lung and Blood Institute (Bethesda, MD) as previously described.[7,8] All patients were treated at the National Institutes of Health Clinical Center. Patients received 1 mg/kg daclizumab infused intravenously every other week for five doses. Response was defined at three months by the criteria defined previously.[8,16] The primary end-point was a hematologic response in at least one affected peripheral blood count parameter, as determined by three separate measurements in the first 12 weeks after completion of the infusion. CR was defined as achievement of normal blood counts, while PR was defined as any response less than a CR. Responding patients were retreated with a second course of daclizumab if they relapsed or showed evidence of decline in their counts. Normal blood counts were determined using hospital standards.

Response criteria

Subjects were considered a complete responder if their blood counts returned to normal on at least a minimum of 2 serial measurements at least one month apart. Transfusion independence is defined as no transfusions for more than eight weeks. Criteria for partial response have been detailed in previous publications[7,8] and are reviewed in the *Online Supplementary Table S1*.

Statistical methods

Patients' characteristics were presented separately for mAA and PRCA cohorts using means for continuous variables, such as disease duration, age and peripheral blood count parameters, and percents for discrete variables, such as sex, race and transfusion dependence status. The corresponding statistical inferences for these summary statistics were presented using 95% confidence intervals and *P* values based on two-sample t-tests comparing the two disease cohorts. The probability of response to treatment at three months was estimated separately for the two disease cohorts. The associations between baseline covariates and the probability of 3-month response were analyzed using univariate and multivariate logistic regression models. The multivariate logistic regression models were evaluated using the stepwise variable selection procedure in the statistical software package S-plus (TIBCO Software Inc., Palo Alto, CA, USA).

Coefficients of these logistic models describe the change of the log-scaled response probability associated with a unit change of the corresponding covariate, whereas a 0 coefficient would suggest that the covariate had no effect on the probability of response at three months. *P* values from the approximate t-tests were used to test the null hypotheses that the covariates were not associated with the probability of response at three months. The probabilities of overall survival were estimated separately for mAA and PRCA patients using the Kaplan-Meier method with the survival time defined to be the time of death of lost to follow-up in years since treatment. Among those patients who have survived the first three months since treatment, the effects of 3-month response on the probability of survival were analyzed using the Cox Proportional Hazard Model.

Results and Discussion

Patients' characteristics

Forty-seven patients with mAA and 29 patients with PRCA were enrolled between January 2000 and June 2009. Patients' characteristics are shown in the *Online Supplementary Table S2* for the two disease categories. Two patients with mAA were not evaluable for response at three months; one patient developed severe pancytopenia prior to the initial drug infusion and never received daclizumab (he was treated with ATG); one patient was lost to follow-up (left the country).

Two PRCA patients were not evaluable; one was lost to follow-up and one died of an unrelated vascular event before the 3-month evaluation period.

Responses of patients with mAA to daclizumab

Of the 45 evaluable mAA, 19 (42%) responded at three

months; 6 (14%) had a complete response (CR; normal counts) at three months, and an additional 2 who were non-responders at three months received another course of daclizumab and achieved a PR by six months (total response rate 21/45)[17]. Of the 28 mAA patients who were red cell transfusion dependent before treatment, 7 (25%) achieved transfusion independence.

Twelve of the 44 neutropenic mAA patients (27%) had a neutrophil response and 16 of 45 (36%) thrombocytopenic patients had a platelet response. Twelve patients with a PR received a second course of daclizumab three months following the previous last dose of daclizumab; four of the 12 had improvements in counts while five of the twelve had a CR (Figure 1A). Of the 26 non-responders (at three months), 12 later progressed to sAA and 8 received horse ATG. Two patients who had improvements in blood counts (but not sufficient to achieve a PR were retreated three months following the last dose of daclizumab, and both had partial responses. Five patients with mAA died: 3 deaths in non-responders were related to disease progression and 2 were unrelated (one patient died in a car accident, and one due to pre-existing polycystic kidney disease). When patients' characteristics were studied in univariate analysis, only lack of transfusion dependence prior to treatment in mAA correlated favorably with response (*Online Supplementary Table S3* and Figure 2). Median follow-up period was 5.4 years.

Responses of patients with PRCA

Ten of 27 evaluable patients (37%) with PRCA responded to a single course of daclizumab by the three month evaluation period and 6 (22%) experienced normalization of the hemoglobin (CR) (Figure 1C). One of the partial responders had a complete response by six months following treatment. Two patients who had improvement in counts (but not sufficient for a PR) were given a second course of therapy and neither responded. Of the 6 patients with CR after a single course of daclizumab, 4 relapsed within six months of treatment; all entered CR following a second course of therapy. Median follow-up of patients with CR is 5.1 years. Only one of these patients had a second relapse (five years post-daclizumab) following treatment for breast cancer, but responded within a month of an additional course of daclizumab. All are currently transfusion-independent. All complete and partial responders achieved transfusion-independence. Median follow-up period was 4.8 years. Cumulative response data are depicted in Figure 2.

Untoward effects of daclizumab

A severe generalized erythema, sometimes associated with arthralgia, developed in 15 patients in the period 20 to 70 days following the last dose of antibody (Figure 2A). Patients with cutaneous eruptions had severe pruritis and

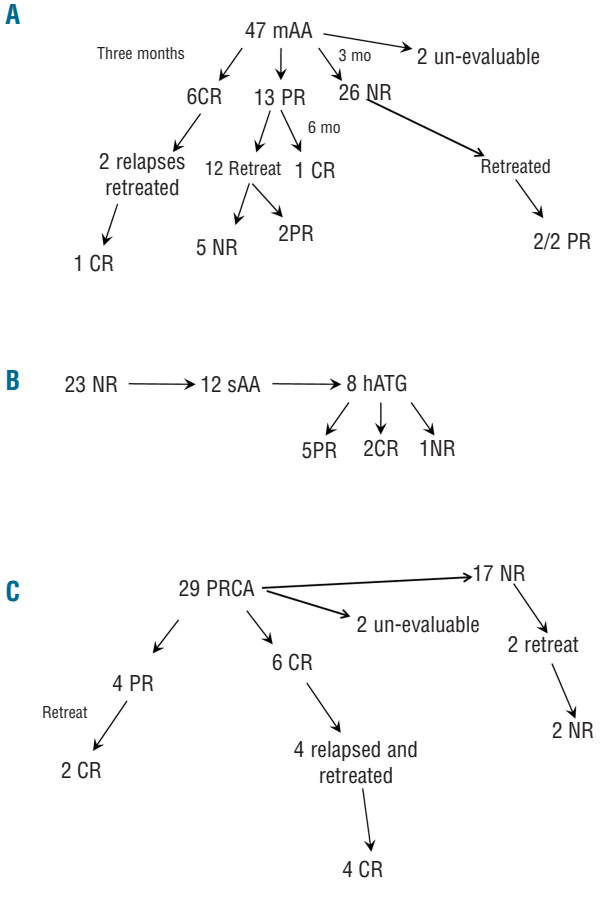

Figure 1. Response of patients with mAA and PRCA treated with daclizumab (A) Patients treated with one course of daclizumab infusions were assessed for response at three months following the last infusion and again at six months. Responses were determined as described in Design and Methods section. (A) Responses of mAA patients. (B) Fate of NR patients. (C) Responses of patients with PRCA.

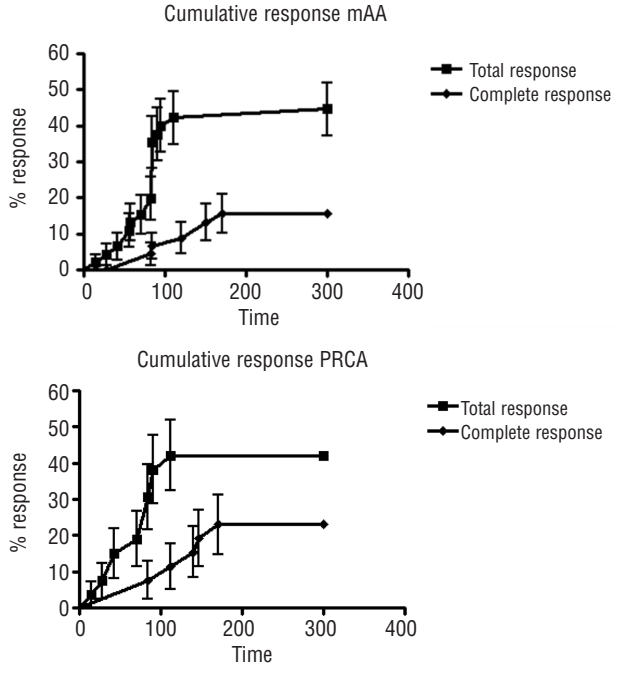

Figure 2. Cumulative response curves for patients with mAA and PRCA. Routine complete blood counts were obtained weekly on patients with mAA (top panel) and PRCA (bottom panel) for the first three months and every other week between three and six months. Cumulative response curves were generated from these blood counts.

intense erythematous inflammation with spongiotic papules and plaques, and were photosensitive (Figure 2B). Systemic corticosteroids for 2-4 weeks were required in symptomatic patients. One patient transiently tested positive for anti-nuclear antibody. Two patients with previous thymectomy for thymoma developed significant new autoimmune disease, which required continued IST for years following cessation of treatment. One patient developed myasthenia gravis and another patient developed rheumatoid arthritis and reactive airway disease. The third thymectomized patient, who had a history of arthrosclerosis, died of ischemic bowel disease (believed to be unrelated to daclizumab). Except for the patient who died, all thymectomized patients experienced the severe cutaneous eruption. Other than mild upper respiratory infections that did not require hospitalization, no patient developed a significant infection.

Survival of patients with PRCA and mAA following daclizumab treatment

The 5-year survivals for both groups were over 90% with no difference between responders and non-responders (Figure 4).

In this study, we expanded our original cohorts of patients with PRCA and mAA and included long-term follow-up for a median of 4.8 years (range 0.6-9.3 years). Daclizumab is effective in producing durable responses in patients with mAA and PRCA, without substantial acute or long-term toxicity. We chose to administer five infusions over the course of ten weeks with the option of a second course of therapy with relapse. However, it is unclear if the response rate would have been improved with more intensive therapy such as is utilized for uveitis.[12] The majority of patients with both mAA and PRCA showed improvement in blood counts within three months of initiating therapy. Most responding patients with PRCA required two cycles of daclizumab to achieve sustained improvements in hemoglobin, but all had durable responses following their second course of treatment. The mAA patients most likely to respond to daclizumab were those who were transfusion independent at time of enrollment. Daclizumab targets the IL-2 receptor on activated T cells.[9]

Binding of antibody to $CD25^+$ lymphocytes may result in the clearance of CD25-bearing effector cells via the reticuloendothelial system[18] or apoptosis due to IL2 deprivation.[19] Daclizumab transiently down-regulates the tyrosine phosphorylation events dependent upon the IL-2 ligand, including but not limited to the phosphorylation of Jak1, Jak3, and STAT5a/b 20. Daclizumab also may inhibit $CD25(^+)$ effector T-cell function *in vivo* by directly blocking CD40L expression.[21] One concern regarding daclizumab is that it would affect not only alloreactive $CD25^+$ cells, but also $CD4^+$ $CD25^+$ FoxP3 expressing T-regulatory cells.[22] Interleukin-2 (IL-2) stimulation is critical for the function of T-regulatory cells, and mice receiving IL-2R antibody develop autoimmune sequelae[23] which are believed to be related to transient loss of T-regulatory cells.

It is possible that the autoimmune symptoms including the rash are related to transient decreases in T-regulatory cells. Preliminary findings in our laboratory suggest that the rash coincides with loss of T-regulatory cells (*data not shown*).

Autoimmune complications have not been described in patients receiving daclizumab in conjunction with solid organ or allogeneic stem cell transplantation, or in patients

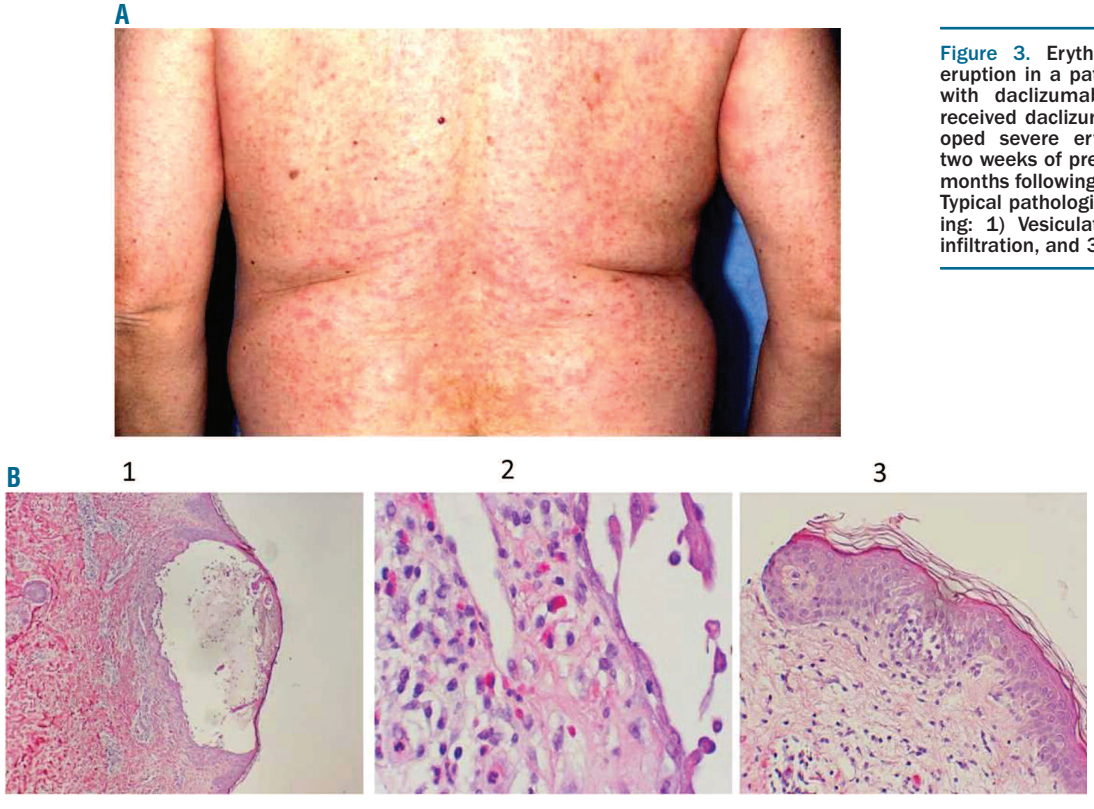

Figure 3. Erythematous cutaneous eruption in a patient after treatment with daclizumab. (A) Patient who received daclizumab for PRCA developed severe erthroderma requiring two weeks of prednisone therapy two months following the last infusion. (B) Typical pathological specimens showing: 1) Vesiculation, 2) Eosinophilic infiltration, and 3) Spongiosis.

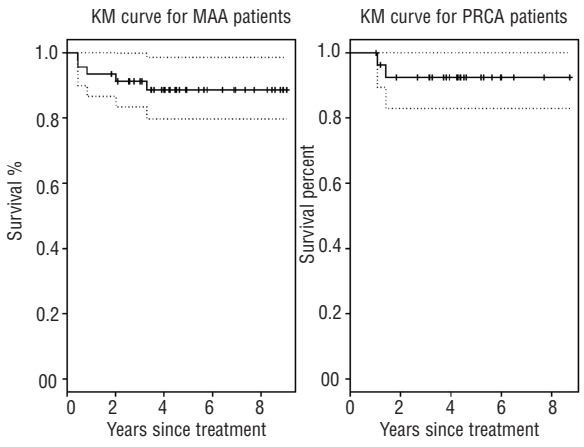

Figure 4. Survival curves for patients with PRCA and mAA treated with daclizumab. Five-year survival for daclizumab-treated mAA and PRCA patients was over 90%; however, there were too few deaths to demonstrate differences in survival between responders and non-responders.

with uveitis. Yet in our study, a severe generalized cutaneous eruption with or without significant arthralgias occurred in 20% of the patients over 50 years of age. Clinically, the cutaneous eruption was self-limited and easily treated with systemic corticosteroids. Differences in immunosuppressive regimens, as well as the schedules and length of daclizumab administration, may have been responsible for these differences. Under most circumstances, transplant patients also receive calcineurin blockers,[15,24-26] and most patients with uveitis received infusions of high doses of daclizumab (8 mg/kg at day 0 and 4 mg/kg at day 14) given every four weeks for a year.[12] It is of note that the patients with prior history of thymectomy were the only patients with long-standing autoimmune problems that were severe enough to require continued systemic immunosuppression. Foxp3$^+$ Treg cells generally arise from the thymus, but some may differentiate from peripheral CD4$^+$CD25$^-$ naive T cells in the periphery in response to TGF-β stimulation.[27]

These cells appear to be capable of suppressing T-cell proliferation and Th1 and Th2 cytokine production *in vitro*, but their activity *in vivo* has not been studied. The unique problems of thymectomized patients receiving daclizumab requires more careful study in order to be able to separate out these patients' natural predisposition for autoimmune problems from immunological phenomena occurring following treatment with daclizumab. Nonetheless, administration of daclizumab to this patient population should be carried out with caution.

Daclizumab was not associated with any infectious complications in either population of patients. This observation differs from the finding in bone marrow transplant recipients which reported a 95% incidence of opportunistic infections.[28] The use of steroids, as well as the fact that these patients had undergone transplantation (all but one was myeloablative), many from mismatched unrelated donors, probably acounts for the difference in the two studies. Also, was made up of the study group bone marrow transplantation patients with their innate immune deficiencies.

Despite the transient rash and autoimmune problems that occured in a minority of individuals, daclizumab proved an effective, non-toxic outpatient treatment both for patients with PRCA and for those with mAA. Further study with different treatment regimens may help clarify optimal dosing in these populations.

Authorship and Disclosures

EMS conceived and designed the study, collected and analyzed data, wrote the manuscript and approved the final approval of the version to be published; MJO collected and analyzed data, edited the manuscript and approved the final approval of the version to be published; BW and RN collected and analyzed data, edited the manuscript and approved the final approval of the version to be published; CW collected and analyzed data, approved the final approval of the version to be published; JM designed the study, edited the manuscript, approved the final approval of the version to be published; PS collected and analyzed data, edited the manuscript, approved the final approval of the version to be published; NSY conceived and designed the study, collected and analyzed data, contributed to the writing of the manuscrip, approved the final approval of the version to be published; SL analyzed the data and edited the manuscript.

The authors reported no potential conflicts of interest.

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