Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 7462–7466, June 1999
Medical Sciences

Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: A phase I/II clinical trial

ROBERT B. NUSSENBLATT*†, ERIC FORTIN*, RHETT SCHIFFMAN‡, LUIZ RIZZO*, JANINE SMITH*, PAUL VAN VELDHUISEN§, PUSHPA SRAN¶, ANITA YAFFE§, CAROLYN K. GOLDMAN‖, THOMAS A. WALDMANN‖, AND SCOTT M. WHITCUP‡

*Laboratory of Immunology, ‡Clinical Branch, §Ocular Genetics and Clinical Services Branch, National Eye Institute, ¶Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-1858; and ¶The EMMES Corporation, Potomac, MD 20854

Contributed by Thomas A. Waldmann, April 21, 1999

ABSTRACT **To evaluate the safety and potential therapeutic activity of humanized anti-IL-2 receptor mAb (Daclizumab) therapy in the treatment of patients with severe, sight-threatening, intermediate and posterior noninfectious uveitis, a nonrandomized, open-label, pilot study was performed. Patients with uveitis were treated with a minimum of 20 mg of prednisone, cyclosporine, antimetabolites, or any combination of these agents were eligible. Patients were weaned off their systemic immunosuppressive agents according to a standardized schedule, while ultimately receiving Daclizumab infusions every 4 weeks. Anti-IL-2 receptor antibody therapy, given intravenously with intervals of up to 4 weeks in lieu of standard immunosuppressive therapy, appeared to prevent the expression of severe sight-threatening intraocular inflammatory disease in 8 of 10 patients treated over a 12-month period, with noted improvements in visual acuity. One patient met a primary endpoint with a loss of vision of 10 letters or more from baseline in one eye and another patient discontinued therapy because of evidence of increased ocular inflammation. All patients were able to tolerate the study medications without the need for dose reduction. We report effective long-term use of anti-IL-2 therapy for an autoimmune indication. These initial findings would suggest that anti-IL-2 receptor therapy may be an effective therapeutic approach for uveitis and, by implication, other disorders with a predominant Th1 profile.**

Immunosuppressive therapy for serious, sight-threatening uveitis can be divided into two phases. The acute phase of the disease often can be treated successfully with aggressive use of pharmacologic agents such as corticosteroids. Once the acute inflammation is controlled, long-term immunotherapy frequently requires the treating physician to balance side effects with a continued therapeutic response. An increased understanding of the mechanisms that result in noninfectious uveitis has made it possible to consider the use of other means to abrogate the ocular immune response (1). The IL-2 receptor system is a well characterized lymphokine receptor system that plays a central role in the induction of immune responses, including uveitis. Observations in experimental autoimmune uveitis have delineated many of the features of the autoaggressive cells, which appear to play a central role in this disorder (2). In the experimental autoimmune uveitis model, these T cells have been shown to bear large numbers of IL-2 receptors on their surface (3) and these findings have been noted on the cells from uveitis patients as well (4).

A murine anti-IL-2 receptor mAb directed against the α or "Tac" portion of the receptor has been developed. A humanized form of this antibody combines the complimentary/determining regions and other selected residues of a murine antibody within the framework and constant regions of the human IgG1 antibody. Infusions of this anti-Tac antibody (Daclizumab) have been used in patients undergoing renal transplantation with an enhancement of graft survival when combined with other immunosuppressive agents (5). In experimental uveitis, Guex-Crosier and colleagues (6) demonstrated that infusion of the anti-IL-2 receptor antibody had a positive therapeutic effect on S antigen-induced experimental autoimmune uveitis in nonhuman primates. We report here results of the use of Daclizumab in the treatment of a chronic putative autoimmune disease, uveitis, and its use as a sole immunosuppressive agent in patients for a 12-month period.

METHODS

This nonrandomized, open-label study was initiated after internal review board approval and under an Investigational New Drug Application. The study was performed at the Clinical Center of the National Institutes of Health. Ten patients with chronic, noninfectious, bilateral, sight-threatening, intermediate or posterior uveitis who were 18 years or older and of either gender were enrolled in this study. Patients were required to have been treated for at least 3 months before enrollment with a minimum of 20 mg of orally administered prednisone, with any dose of cyclosporine or antimetabolite, or any combination of these. They were considered eligible if they were intolerant to the dosage of medication(s) needed to control their disease or if they were willing to be weaned off their current systemic immunosuppressive therapy. In addition, eligible patients were required to have a visual acuity of 20/63 or better in at least one eye and normal renal and liver function or evidence of only mild abnormalities as defined by the World Health Organization criteria. Patients were not eligible for entry into the study if they had received previous treatment with an IL-2 receptor-directed mAb or any other investigational agent that would interfere with the ability to evaluate the safety, efficacy, or pharmacokinetics of the humanized anti-Tac antibody. Additionally, patients could not have any significant active systemic infection or a history of cancer (other than nonmelanoma skin cancer) within the past 5 years. Pregnant or lactating patients and patients with Behçet's disease were not recruited.

After providing a written informed consent, patients were tapered off their systemic antiinflammatory medications over an 8-week interval according to a preestablished protocol (Fig. 1). This protocol was adhered to unless a study endpoint was reached. Patients could continue to use topical corticosteroids throughout the study. Infusions of Daclizumab began 2 weeks

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

†To whom reprint requests should be addressed at: Laboratory of Immunology, National Eye Institute, National Institutes of Health, Building 10, Room 10N202, 10 Center Drive, MSC 1858, Bethesda, MD 20892-1858. e-mail: DrBob@intra.nei.nih.gov.

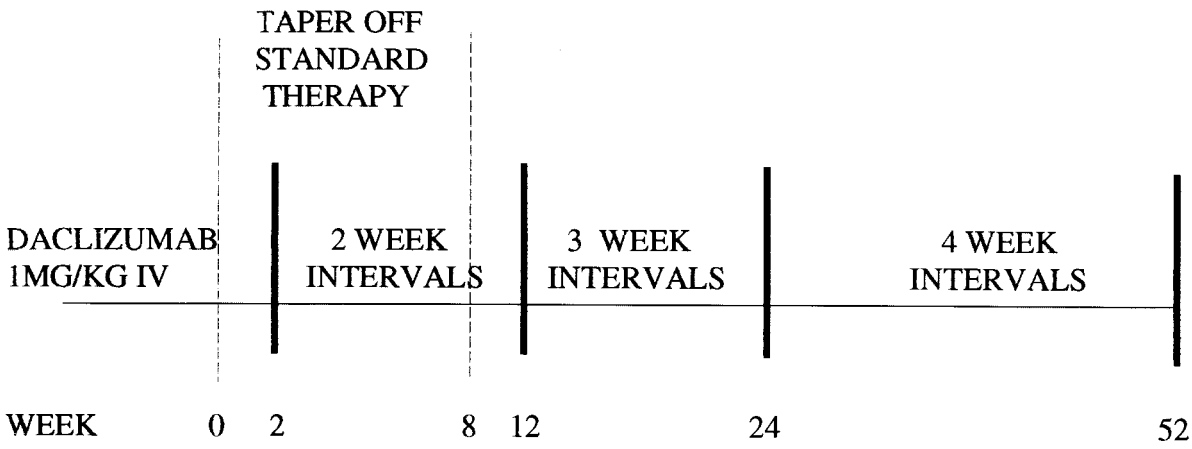

FIG. 1. Schematic outline of trial design. During the initial period, while patients were receiving infusions of Daclizumab every 2 weeks, patients were tapered off their standard immunosuppressive therapy that they were receiving for treatment of uveitis.

after the immunosuppressive taper initiation. Intervals between infusions were initially 2 weeks and were increased in a stepwise fashion until week 24, when patients were scheduled to receive infusions every 4 weeks for the duration of the time covered in this report. Patients were hospitalized for the infusions and treated with 1 mg/kg Daclizumab. The formulation given contains 5 mg/ml Daclizumab and 0.2 mg/ml polysorbate-80 in 67 mM phosphate buffer, pH adjusted to 6.9. The appropriate quantity of antibody solution at 5 mg/ml was diluted with 50 ml of normal saline in the minibag. Therapy was given intravenously in a slow drip over approximately 1 hour.

Patients were seen at regular intervals as designated by the protocol or sooner if the patient felt there was a change in his or her status. The patient's ocular inflammatory activity was graded at each visit by using standardized methods (7, 8). This evaluation included a best-corrected visual acuity, dilated ocular examination for evidence of ocular inflammation, and a physical examination. If, at a regular visit, a study endpoint had not been reached and no contraindication for an infusion was found, patients were infused with Daclizumab.

The primary endpoint for this study was a decrease in best-corrected visual acuity of 10 letters or more from baseline at a study visit. This visual acuity was obtained by using a standardized method for measuring visual acuity by using the ETDRS chart (9). Secondary endpoints included (*i*) an increase in vitreous haze of at least two grades from baseline by using standardized photographs of the vitreous (7); (*ii*) the need to restart systemic immunosuppressive therapy for recurrence of the patient's uveitis; and (*iii*) a patient's inability to be tapered off his or her systemic immunosuppression in an 8-week span. Data collection for this report was frozen when all patients had the potential to reach 12 months of follow-up.

Anti-IL-2 Receptor mAb Serum Level Determinations. Blood samples for measuring the serum concentration of anti-IL-2 receptor mAb levels were taken before each infusion. mAb levels were determined by using a solid-phase IL-2 receptor inhibition array (10).

Human Anti-HAT Antibody Levels. Human antibodies to the infused mAb were detected by using a two-arm capture quantitative ELISA assay. In brief, a measured amount of patient sera or standard was added to microtiter wells precoated with the infused mAb. After incubation for 16–20 hours at 2–8°C in a humid chamber and washing of the plate, biotinylated infused mAb was added to each well, followed by incubation for 2 hours at 35–39°C and washing. The plate was developed by using streptavidin–horseradish peroxidase conjugate and *p*-nitrophenyl phosphate substrate. After a 1-hour incubation at 35–39°C, the plates were read at 405 nm. Levels of specific antibody to the infused mAb in the patient sera were calculated by comparison to a standard curve prepared with affinity-purified specific antibodies from the sera of a hyperimmune goat immunized with the same infused mAb preparation.

Statistical Methods. For analysis of repeated outcomes over time for endpoints such as visual acuity and serum cholesterol, the generalized estimating equations method of Liang and Zeger (11, 12), unadjusted for covariates, was used. This method accounts for the correlation among measurements over time for each patient.

RESULTS

A total of 10 patients (5 female, 5 male; 5 Caucasian, 4 African-American, 1 Pacific Islander) were enrolled in the study. All patients had a history of severe bilateral uveitis, requiring systemic immunosuppression. The average age was 37.4 years (median 37.0 years, range 20–53 years). The diagnoses of the ocular disorders treated were as follows: sarcoidosis, 3 patients; idiopathic intermediate uveitis, 3 patients; Vogt–Koyanagi–Harada's disease (VKH), 2 patients; idiopathic panuveitis, 1 patient; and multifocal choroiditis, 1 patient.

All patients free of a study endpoint had at least 12 months of follow-up. Of the 10 patients who entered the study, 9 were treated with orally administered prednisone (Table 1), while 9 of the patients were receiving cyclosporine (either Sandimmune or Neoral). The one patient not administered prednisone at baseline was receiving Neoral and Methotrexate. Patients on prednisone were taking an average of 26 mg/day, and the average dose of cyclosporine was 3.6 $mg \cdot kg^{-1} \cdot day^{-1}$. All patients had immunosuppressive therapy stopped at 8 weeks, as prescribed by the protocol. During the first 12 months of follow-up, a total of 149 Daclizumab infusions was given among the 10 patients, with patients free of an endpoint receiving between 15 and 17 infusions. Each patient, on average, received 84.0 mg per infusion.

With 12 months of follow-up on all patients, 9 of the 10 patients receiving Daclizumab therapy did not reach a primary endpoint, with the 1 patient having a 10-letter drop from baseline in best-corrected visual acuity at week 12 after 5 infusions (Table 1, patient 2). One patient reached a secondary endpoint after 8 months of therapy because of bilateral flare-up of inflammation in the anterior and posterior segments of the eye and was reinitiated with standard systemic immunosuppressive therapy (Table 1, patient 9).

Although not initially designated as a primary outcome variable, mean visual acuity of patients over follow-up was also examined (Fig. 2). All 10 patients are included in this analysis,

Table 1. Patient baseline characteristics and visual outcome

Patient	Age	Diagnosis	Immunosuppressives			Inflammatory flareups, no.	Attempts to type immuno-suppressants, no.	Visual acuity			
								Baseline		12-Month	
			CSA, mg/kg	Predisone, mg	Months before study			Better eye	Worse eye	Better eye	Worse eye
1	35	Idiopathic intermediate	2.0	10	72	5	3	94 (20/13)	80 (20/25)	92 (20/16)	84 (20/20)
2*	23	Idiopathic intermediate	2.4	60	24	Data unavailable	Data unavailable	75 (20/32)	71 (20/40)	65 (20/50)	69 (20/40)
3	40	VKH	1.1	17.5	116	5	4	85 (20/20)	51 (20/100)	84 (20/20)	63 (20/50)
4	38	VKH	3.7	5	4	1	1	59 (20/63)	58 (20/63)	71 (20/40)	71 (20/40)
5	53	Idiopathic intermediate	3.3	20	113	7	6	61 (20/63)	41 (20/160)	59 (20/63)	44 (20/125)
6	43	Sarcoidosis	—	30	4	0	0	90 (20/16)	87 (20/20)	86 (20/20)	84 (20/20)
7	20	Sarcoidosis	2.8	17.5	115	8	6	75 (20/32)	41 (20/160)	74 (20/32)	55 (20/80)
8	51	Sarcoidosis	3.1	—	12†	2	0	59 (20/63)	54 (20/80)	70 (20/40)	79 (20/25)
9‡	36	Idiopathic panuveitis	8.1	50	31§	Data unavailable	Data unavailable	71 (20/40)	65 (20/50)	82 (20/25)	69 (20/40)
10	35	Multifocal choroiditis	3.9	20	18	2	2	60 (20/63)	58 (20/80)	67 (20/50)	65 (20/50)

Data for immunosuppressives given as daily oral dose unless otherwise indicated. Data for duration of pre-study systemic immunosuppression were obtained from chart review. Visual acuity values are given with approximate Snellen equivalent in parenthesis. For patients 2 and 9, the 12-month visual acuity refers to endpoint visual acuity. CSA, cyclosporine A; VKH, Vogt–Koyanagi–Harada's disease.
*Patient 2 met endpoint after 12 weeks.
†Patient 8 also received methotrexate (10 mg/week).
‡Patient 9 met endpoint after 36 weeks.
§Patient 9 also received azathioprine (125 mg).

with the 8 patients without an endpoint included throughout their 12-month visit. The visual acuity of the 2 patients who discontinued Daclizumab therapy is included through the visit the endpoint was observed. At baseline, the mean visual acuity in the better eye was 72.9 letters (approximate Snellen equivalent = 20/32) and 60.6 (approximate Snellen equivalent = 20/63) in the worse eye. As patients were tapered off their standard immunosuppressive therapy, a small but constant improvement in mean visual acuity over time was noted, particularly in the worse eye. This noted overall improvement in visual acuity over time is statistically significant in the worse eye ($P = 0.005$) but not in the better eye ($P = 0.94$). This improvement resulted in an average visual acuity at the 12-month visit of 75.4 letters (approximate Snellen equivalent = 20/32) for the better eye and 68.1 letters (approximate Snellen equivalent = 20/40) for the worse.

Inflammatory Activity. The anterior chamber of 3 of the 10 patients showed increased activity as compared with baseline ocular examinations at 10 weeks after initiation of the study, the first time at which patients were completely tapered off standard immunosuppressive medication. This inflammatory activity did not cause a drop in visual acuity and was treated with topical medication as permitted in the protocol. The anterior chamber of the 2 of the 10 patients at either the 12-month visit or the visit at which an endpoint occurred showed increased activity compared with baseline, whereas the other 8 patients showed either no change ($n = 3$) or an improvement over baseline ($n = 5$).

Vitreous haze, a secondary endpoint, was measured by using a series of standard photographs. All but one patient had a baseline grade of clear in both eyes, with one patient having a grade 1 vitreous haze in one eye. Aside from the one patient experiencing the primary endpoint, no patient experienced a worsening in vitreous haze from baseline when examining the patient's 12-month visit, or the visit in which an endpoint was observed if applicable.

Nonocular beneficial effects were also seen. All but one patient at entry into the study was taking prednisone, and nine patients required two or more agents for treatment of their intraocular inflammation. Many patients entered the study presenting with systemic hypertension, mildly abnormal creatinine clearances, and elevated cholesterol levels. Fig. 3 shows mean serum cholesterol over the first 24 weeks of follow-up, demonstrating a drop in the serum level associated with discontinuation of their systemic immunosuppressive therapy. This noted decrease from baseline over time is statistically significant ($P < 0.001$).

Daclizumab Levels. Circulating humanized anti-IL-2 receptor antibody levels were measured in patients before Daclizumab infusions to determine whether blood levels were in a therapeutic range. A total of 106 levels for the 2- ($n = 46$), 3- ($n = 16$), and 4- ($n = 44$) week infusion intervals was obtained. For each patient, interval-specific levels were averaged, resulting in a mean patient serum level 2 weeks after an infusion of 5,838 ng/ml (range: 4,528–8,168), well above a level thought to be therapeutic (13) (Table 2). The mean level 4 weeks after infusion was 1,716 ng/ml (range: 655–2,423), a level still believed capable of immunosuppressive effects.

Antibody Production Directed Against the Humanized Anti-Tac Antibody. An evaluation was performed before every infusion for the presence of antibodies directed against the humanized anti-IL-2 receptor antibody. A total of 104 determinations was made over the first year. Antibodies directed against this agent were not detected at any point during the study.

Adverse Effects. Patients were closely monitored for possible effects secondary to the administration of the anti-IL-2 receptor antibody. Over the first 12 months of the study, six patients had cutaneous lesions (either rashes or hives) that

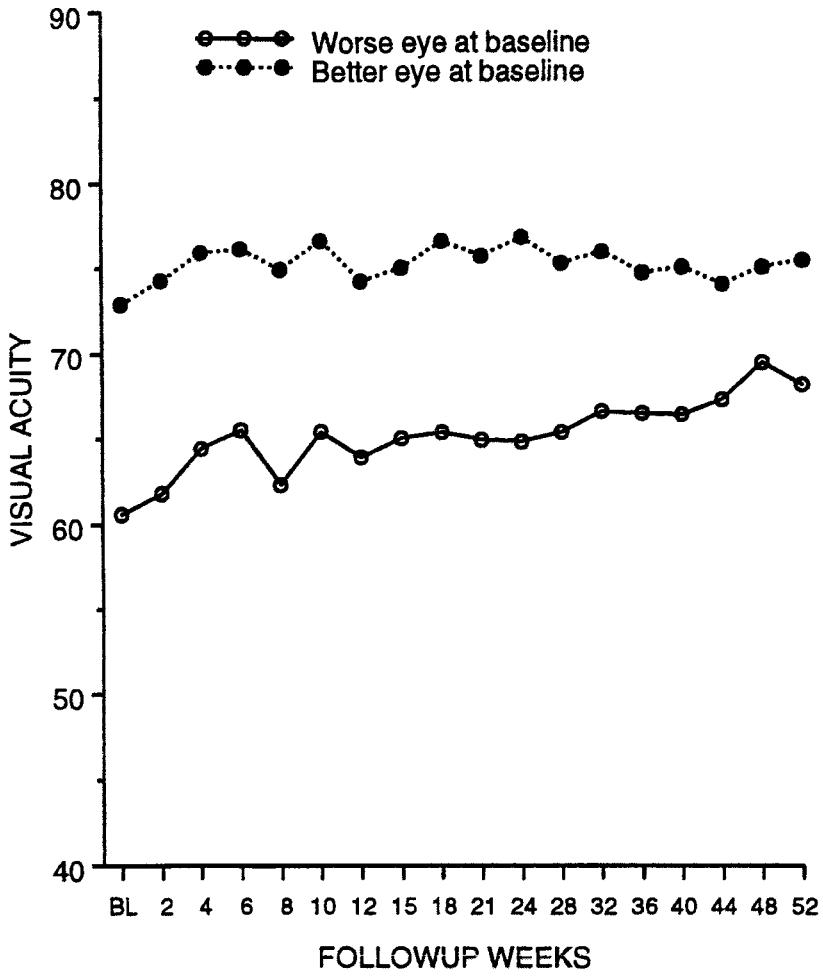

FIG. 2. The mean visual acuity of uveitis patients receiving Daclizumab therapy over a 12-month period. The two lines on the graph follow the visual acuities for the eye of each patient that had the worse visual acuity at baseline and the eye that had the better acuity. All patients in this study had two functional eyes. The BL point indicates the baseline visual acuity. The *y* axis indicates the number of letters read from the ETDRS chart. Therefore the greater the number of letters read, the better the visual acuity. This method of visual acuity analysis is standardized and is outlined in ref. 9. Patients reading 85 letters have an equivalent visual acuity of 20/20 in the Snellen acuity system.

appeared on their arms or trunks. Some of these lesions were treated with topical steroid ointments, although others did not require treatment. One patient with a diagnosis of sarcoidosis had slightly abnormal liver function tests on entry into the study. This patient had further increases in the abnormalities of his liver function tests. A liver biopsy was performed, which diagnosed a granulomatous hepatitis, which on consultation with the hepatology service at the National Institutes of Health Clinical Center was not thought to warrant therapy and was likely a preexisting condition. Two patients experienced edema of their lower extremities, with one also having mild edema of the arms and face. The development of the edema did not seem temporally related to their receiving therapy. One patient was treated with furosemide 10 mg orally twice a day with resolution of the edema but continues on this therapy, because there was a recurrence of edema when the diuretic was previously discontinued. The other patient did not require therapy and had spontaneous resolution of the problem. A complete work-up of cardiac, renal, hepatic, endocrine, and vascular causes of edema was negative in both patients, and both continue receiving anti-IL-2 receptor antibody therapy. Six patients experienced upper respiratory infections of presumed viral origin, some being described as a "cold" or bronchitis. One patient developed a localized herpes zoster patch on the upper arm, which responded to treatment with fanciclovir and did not recur. Four of these six patients (including the one patient with the zoster lesion) received antibiotic therapy with resolution of their conditions. They continued to receive anti-IL-2 receptor infusions. One patient experienced neuralgia of an upper extremity, which did not respond to oral steroids and disappeared after a period of 4 months. It is not clear whether all or some of these complications were directly related to the anti-IL-2 receptor antibody infusions.

All patients were tapered off their standard immunosuppressive medications as planned. However, two patients required low-dose prednisone therapy (5–7.5 mg maintenance) because of documented adrenal insufficiency, presumably because of long-term exogenous corticosteroid therapy.

DISCUSSION

We report here successful use of an anti-IL-2 receptor antibody in the long-term treatment of an autoimmune disease. In addition, this report documents the long-term efficacy of this therapeutic approach. Eight of 10 patients with chronic, severe sight-threatening intermediate and posterior uveitis have had their ocular disease controlled by using this agent over a 12-month period with a concomitant taper of their standard immunosuppressive agents.

The rationale for use of anti-IL-2 receptor therapy in this disorder has support from various sources. Experimental autoimmune uveitis is a model for human disease that has many characteristics seen in the human situation. Effective

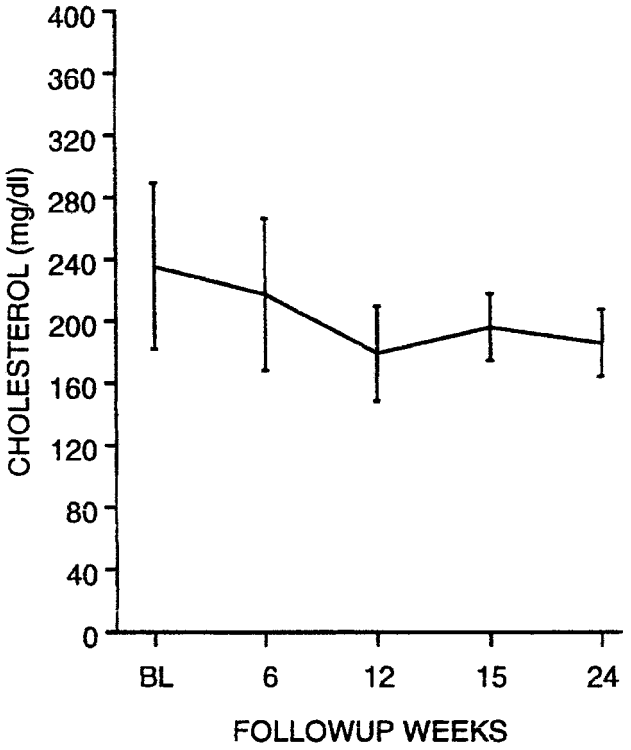

FIG. 3. The mean serum cholesterol levels (with standard deviations) of uveitis patients receiving Daclizumab therapy. The cholesterol levels are presented as mg/dl, with the normal serum levels reported between 100 and 200 mg/dl.

immunomodulating approaches in this model appear to be excellent candidates for therapy in patients with uveitis (1, 2). In this model, Caspi and colleagues (3) demonstrated that the autoaggressive T cells capable of inducing experimental uveitis on adoptive transfer were of the Th1 subtype bearing large numbers of IL-2 receptors on their cell surfaces. Furthermore, Feron *et al.* (4) used flow cytometry to show that both CD4+ and CD8+ peripheral lymphocytes from uveitis patients had a significant increase in the expression of the α-chain of the IL-2 receptor compared with controls. This α-chain is the chain to which the agent used in this study is directed. Nonhuman primates were treated with Daclizumab for experimentally induced uveitis, and based on the positive therapeutic response seen there (6), the first study in patients with uveitis was initiated. Daclizumab is directed against the α-chain of the high-affinity IL2 receptor and is specific in its action. Its effective control of intermediate and posterior uveitis would suggest that T cells would appear to play a dominant role in many types of such ocular inflammatory conditions. However, it is interesting to note that in some of the patients treated in this study, their anterior uveitis was not well controlled with this form of therapy. This phenomenon had been noted as well in some uveitic cases treated with cyclosporine. This would suggest that non-T cell mechanisms are important mediators of anterior segment inflammation.

Several parameters related to the infusion of Daclizumab were followed. Although Daclizumab is a humanized murine antibody, to maintain configurational integrity and the specific binding sites, $\approx$10% of the molecule is murine. This would then create a potential for an immune response by the host directed against the molecule. Human anti-HAT antibody levels were drawn before each infusion and stayed undetectable for the duration of the study. Furthermore, Daclizumab levels measured 4 weeks after the last infusion were, on average, at levels thought to be immunosuppressive. We believe that the therapeutic approach as outlined in this study—initial therapy with standard immunosuppressive agents followed by Daclizumab therapy—may be a useful template for the treatment of many putative autoimmune disorders. This is particularly the case where chronically administered immunosuppressive therapy becomes problematic because of cumulative adverse events.

Table 2. Summary of Daclizumab levels

	Infusion schedule		
	2-week (10–17 days)	3-week (18–23 days)	4-week (24–34 days)
Number of patients*	10	8	9
Mean	5,836.8	3,304.8	1,715.6
SD	1,178.4	1,130.7	547.6
Median	5,490.0	3,201.0	1,599.2
Min	4,528.3	1,840.0	655.0
Max	8,167.5	5,280.0	2,423.3

*Patients contribute one Daclizumab level per infusion interval, with the level being the patient's mean level within the interval.

Secondary effects of Daclizumab therapy did not require a readjustment of dose or necessitate stopping the medication. A granulomatous dermatitis was noted in two patients. This was not like the lichenoid dermatitis noted to occur with Ig therapy (14). These lesions cleared with topical steroid therapy. One could speculate that macrophage-mediated mechanisms, not controlled by Daclizumab therapy, resulted in this clinical condition. However, it is not clear why the condition has not, as of yet, returned.

We have demonstrated that Daclizumab can be administered long-term to uveitis patients with minimal secondary effects and without the need for other immunosuppressive agents. Although the dosing schedule is still being evaluated, infusions interspersed with 4-week intervals appear effective and relatively safe. These results suggest the need for a randomized controlled trial to evaluate whether such an approach would provide a major therapeutic advance to patients with uveitis.

1. Nussenblatt, R. B. (1991) *Invest. Ophthalmol. Vis. Sci.* **32,** 3131–3141.
2. Caspi, R. R. & Nussenblatt, R. B. (1994) in *Autoimmunity: Physiology and Disease*, eds. Coutinho, A. & Kazatchkine, M. (Wiley–Liss, New York), pp. 377–404.
3. Caspi, R. R., Roberge, F. G., McAlliser, C. G., el-Saied, M., Kuwabara, T., Gery, I., Hanna, E. & Nussenblatt, R. B. (1986) *J. Immunol.* **136,** 928–933.
4. Feron, E. J., Calder, V. L. & Lightman, S. L. (1992) *Curr. Eye Res.* **11 S**uppl., 167–172.
5. Vincenti, F., Kirkman, R., Light, S., Bumgardner, G., Pescovitz, M., Halloran, P., Neylan, J., Wilkinson, A., Ekberg, H., Gaston, R., *et al.* (1998) *N. Engl. J. Med.* **338,**161–165.
6. Guex-Crosier, Y., Raber, J., Chan, C. C., Kriete, M. S., Benichou, J., Pilson, R. S., Kerwin, J. A., Waldmann, T. A., Hakimi, J. & Roberge, F. G. (1997) *J. Immunol.* **158**, 452–458. .
7. Nussenblatt, R. B., Palestine, A. G., Chan, C. C. & Roberge, F. (1985) *Ophthalmology* **92,** 467–471.
8. Hogan, M. J., Kimura, S. J. & Thygensen, P. (1959) *Am. J. Ophthalmol.* **47,** 155–170.
9. Ferris, F. L., III, Kassoff, A., Bresnick, G. H. & Bailey, I. (1982) *Am. J. Ophthalmol.* **94,** 91–96
10. Hakimi, J., Seals, C., Anderson, L. E., Podlaski, F. J., Lin, P., Danho, W., Jenson, J. C., Perkins, A., Donadio, P. E., Familletti, P. C., *et al.* (1987) *J. Biol. Chem.* **262**, 17336–17341.
11. Liang, K. Y. & Zeger, S. L. (1986) *Biometrika* **73,**13–22.
12. Zeger, S. L. & Liang, K. Y. (1986) *Biometrics* **42,**121–130.
13. Depper, J. M., Leonard, W. J., Robb, R. J., Waldmann, T. A. & Green, W. C. (1983) *J. Immunol.* **131,** 690–696.
14. Yockey, S. M. & Ahmed, I. (1997) *Mayo Clin. Proc.* **72,** 1151–1152.