

ANTI-CD3 MONOCLONAL ANTIBODY IN NEW-ONSET TYPE 1 DIABETES MELLITUS

KEVAN C. HEROLD, M.D., WILLIAM HAGOPIAN, M.D., PH.D., JULIE A. AUGER, B.A., ENA POUMIAN-RUIZ, B.S., LESLEY TAYLOR, B.A., DAVID DONALDSON, M.D., STEPHEN E. GITELMAN, M.D., DAVID M. HARLAN, M.D., DANLIN XU, PH.D., ROBERT A. ZIVIN, PH.D., AND JEFFREY A. BLUESTONE, PH.D.

ABSTRACT

Background Type 1 diabetes mellitus is a chronic autoimmune disease caused by the pathogenic action of T lymphocytes on insulin-producing beta cells. Previous clinical studies have shown that continuous immune suppression temporarily slows the loss of insulin production. Preclinical studies suggested that a monoclonal antibody against CD3 could reverse hyperglycemia at presentation and induce tolerance to recurrent disease.

Methods We studied the effects of a nonactivating humanized monoclonal antibody against CD3 — hOKT3 γ 1(Ala-Ala) — on the loss of insulin production in patients with type 1 diabetes mellitus. Within 6 weeks after diagnosis, 24 patients were randomly assigned to receive either a single 14-day course of treatment with the monoclonal antibody or no antibody and were studied during the first year of disease.

Results Treatment with the monoclonal antibody maintained or improved insulin production after one year in 9 of the 12 patients in the treatment group, whereas only 2 of the 12 controls had a sustained response ($P=0.01$). The treatment effect on insulin responses lasted for at least 12 months after diagnosis. Glycosylated hemoglobin levels and insulin doses were also reduced in the monoclonal-antibody group. No severe side effects occurred, and the most common side effects were fever, rash, and anemia. Clinical responses were associated with a change in the ratio of CD4+ T cells to CD8+ T cells 30 and 90 days after treatment.

Conclusions Treatment with hOKT3 γ 1(Ala-Ala) mitigates the deterioration in insulin production and improves metabolic control during the first year of type 1 diabetes mellitus in the majority of patients. The mechanism of action of the anti-CD3 monoclonal antibody may involve direct effects on pathogenic T cells, the induction of populations of regulatory cells, or both. (N Engl J Med 2002;346:1692-8)

Copyright © 2002 Massachusetts Medical Society.

TYPE 1 diabetes mellitus is a T-cell-mediated autoimmune disease that begins, in many cases, three to five years before the onset of clinical symptoms, continues after diagnosis, and can recur after islet transplantation.¹⁻³ The effector mechanisms responsible for the destruction of beta cells involve cytotoxic T cells as well as soluble T-cell products, such as interferon- γ , tumor ne-

crisis factor α , and others.⁴ Such observations have led to clinical trials with immunomodulatory drugs such as cyclosporine, azathioprine, prednisone, and antithymocyte globulin, which were shown to cause transient improvement in clinical measures and to enhance the rate of non-insulin-requiring remissions when initiated soon after diagnosis.⁵⁻⁸ Unfortunately, the toxic effects of such drugs, concern about the risk associated with immune suppression, and the need for continuous treatment in an otherwise healthy, young population limit the use of these agents.⁹

We,¹⁰ as well as Chatenoud et al.,¹¹⁻¹³ have reported that treatment of mice with a modified monoclonal antibody against CD3 that had been altered to prevent binding to the Fc receptor prevents or reverses diabetes in nonobese diabetic mice and other mouse models of type 1 diabetes mellitus. This antibody can be used without toxic effects such as the high fevers and hypotension that are typically associated with T-cell activation in vivo.¹⁰⁻¹³ Initial studies in which a humanized anti-CD3 molecule — that is, a monoclonal antibody called hOKT3 γ 1(Ala-Ala) that contains the binding region of OKT3 but a mutated Fc region that prevents it from binding to the Fc receptor — was used in patients with renal-allograft rejection demonstrated efficacy similar to that of OKT3 with markedly fewer side effects.^{14,15} On the basis of these observations, we initiated a randomized, controlled, phase 1-2 trial of this agent in patients with new-onset type 1 diabetes mellitus. In this report, we describe the results among patients who were followed for one year after treatment.

METHODS

Study Patients

Patients between 7½ and 30 years of age in whom type 1 diabetes mellitus had been diagnosed within the previous six weeks (or who had been discharged from the hospital within that period after receiving such a diagnosis) were eligible for participation. All

From the Naomi Berrie Diabetes Center and the Department of Medicine, Division of Endocrinology, College of Physicians and Surgeons, Columbia University, New York (K.C.H., E.P.-R., L.T.); Pacific Northwest Research Institute, Seattle (W.H.); the University of Chicago, Chicago (J.A.A.); the University of Utah, Salt Lake City (D.D.); the Departments of Pediatrics (S.E.G.) and Medicine (J.A.B.), University of California at San Francisco, San Francisco; the National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Md. (D.M.H.); and the R.W. Johnson Pharmaceutical Research Institute, Raritan, N.J. (D.X., R.A.Z.). Address reprint requests to Dr. Herold at Columbia University, 1150 St. Nicholas Ave., New York, NY 10032, or at kh318@columbia.edu.

patients had one or more of the following types of antibodies: anti-GAD (glutamic acid decarboxylase), anti-islet-cell antibody 512 (ICA512), and anti-insulin antibody. Patients were treated by their personal physicians, received at least three injections of short-acting or intermediate-acting insulin, and did not discontinue insulin therapy during the study period. The study was approved by the institutional review boards at Columbia Presbyterian Medical Center, the National Institute of Diabetes and Digestive and Kidney Diseases, the University of Utah, and the University of California at San Francisco. All patients or their parents provided written informed consent, and written assent was obtained from minor subjects.

Study Protocol

The data reported here were obtained between May 1999 and August 2001. Eligible patients were randomly assigned to the control group or the monoclonal-antibody group. Patients in the control group underwent metabolic and immunologic studies but did not receive monoclonal antibody and were not hospitalized. Blood samples were drawn for immunologic studies and measurement of glycosylated hemoglobin when the patient entered the study, and a four-hour mixed-meal tolerance test was performed after the morning dose of insulin and the previous evening's dose of long-acting insulin had been withheld.⁷

Nine patients in the monoclonal-antibody group were hospitalized, and the other three received monoclonal antibody on an outpatient basis. All 12 patients received a 14-day course of the anti-CD3 monoclonal antibody hOKT3 γ 1 (Ala-Ala) administered intravenously (1.42 μ g per kilogram of body weight on day 1; 5.67 μ g per kilogram on day 2; 11.3 μ g per kilogram on day 3; 22.6 μ g per kilogram on day 4; and 45.4 μ g per kilogram on days 5 through 14); the doses were based on those previously used for treatment of transplant rejection.¹⁵ The dosing resulted in median peak and trough serum monoclonal-antibody levels of 133 ng per milliliter (range, 68 to 275) and 51 ng per milliliter (range, 23 to 255), respectively. Flow cytometry was used for the enumeration of CD4+ T cells, CD8+ T cells, and non-T cells and for coating and modulation of the CD3 molecule.¹⁵ Coating of CD3+ cells was maximal (mean [\pm SD] percentage reduction in fluorescence, 69.2 \pm 2.9) by day 12 of monoclonal-antibody treatment. Modulation of the CD3 molecule reached a peak level of 54.0 \pm 3.1 percent by day 14.

Patients underwent physical examinations, blood counts, and blood chemistries and were questioned about side effects weekly for two weeks after discharge and every two to three months thereafter. Glycosylated hemoglobin was measured and a mixed-meal tolerance test was performed every six months.

Statistical Analysis

C-peptide levels were measured by radioimmunoassay at the Diabetes Research and Training Center at the University of Chicago.¹⁶ The C-peptide response to the mixed meal was expressed as the total area under the response curve or the incremental area under the curve formed by subtracting the fasting C-peptide level from the response at each time point.⁷ A change in the response was considered to have occurred if the response differed by more than 7.5 percent from the response at study entry (7.5 percent being half of the interassay coefficient of variation for the C-peptide assay). Changes in insulin secretion were evaluated by examining the slope of the line described by the three data points (at study entry, 6 months, and 12 months).

Anti-GAD antibody, anti-ICA512, and anti-insulin antibody were measured with radiobinding assays.¹⁷ For genotyping at the HLA-DQA and DQB loci, direct sequencing of exon 2 polymorphisms was used after polymerase-chain-reaction amplification.¹⁸

Cytokines were measured in serum by enzyme-linked immunosorbent assay (ELISA) (BioSource and ImmuneTech). Anti-idiotypic antibodies were identified by ELISA with the use of plate-

bound OKT3 or by flow cytometry to measure the blockade of binding of OKT3 fluorescein isothiocyanate to CD3.¹⁹ Glycosylated hemoglobin levels were measured by latex-agglutination inhibition tests (DCA 2000, Bayer) or by affinity chromatography (Isolab) in the three patients treated at the National Institutes of Health.

Data are expressed as means \pm SD. We used repeated-measures analysis of variance to compare the control group and the monoclonal-antibody group in terms of the response to the mixed-meal tolerance test, the glycosylated hemoglobin level, and the required dose of insulin. Comparisons between groups were made with the Mann-Whitney U test. Fisher's exact test was used to assess the effect of monoclonal-antibody treatment on the response to mixed-meal tolerance testing. Statistical analyses were performed with StatView software (SAS Institute).

RESULTS

Enrollment of Study Patients

The average age of patients in the control group was slightly higher than that in the monoclonal-antibody group, but there were no significant differences between the two groups at entry (Table 1). Autoantibodies against at least one type of biochemically defined autoantigen were present in all subjects.

Effects of Antibody Treatment on Circulating Lymphocytes

A transient reduction in the number of circulating lymphocytes occurred with monoclonal-antibody treatment. After the administration of the first full dose of monoclonal antibody on day 5, the absolute lymphocyte count reached a nadir of 26.5 \pm 9.0 percent of the base-line lymphocyte count. The changes in the absolute lymphocyte count were due to reductions in the numbers of CD4+ cells, CD8+ cells, and B cells (CD19+ cells) to 36.6 \pm 19.0 percent of their pretreatment levels. The reduction in the number of circulating lymphocytes was transient, however, and the number of circulating cells began to rise after the seventh day of treatment. By day 30 (two weeks after the last dose of the monoclonal antibody), the level of circulating lymphocytes reached 123.0 \pm 52.0 percent of the pretreatment level.

Release of Cytokines after Treatment

The levels of cytokines were measured in serum after the initial two doses of monoclonal antibody and after the first two full doses on days 5 and 6. Interleukin-6 was detectable in 8 of the patients treated with monoclonal antibody (range of levels, 14 to 225 pg per milliliter), and tumor necrosis factor α was detectable in all 12 patients (range of levels, 7 to 158 pg per milliliter). The circulating levels of these cytokines were maximal after the administration of the second dose of the monoclonal antibody but were considerably lower than levels previously reported in patients with the "cytokine-release syndrome" associated with the administration of OKT3; these levels were consistent with the mild clinical side effects.¹³ Interleukin-2 was not detectable in these patients,

TABLE 1. CHARACTERISTICS OF THE PATIENTS AT ENTRY.*

CHARACTERISTIC	MONOCLONAL- ANTIBODY GROUP (N=12)	CONTROL GROUP (N=12)	P VALUE
Sex (no.)	10	8	0.64
Male	2	4	
Female			
Age (yr)			0.15
Median	13	16	
Range	7–27	8–30	
Diabetic ketoacidosis at diagnosis (no.)	3	5	0.67
Glycosylated hemoglobin (%)	9.27±1.59	8.27±1.06	0.14
Fasting C-peptide level (nmol/liter)	0.20±0.13	0.21±0.07	0.77
Anti-GAD65 antibody			
Index	0.48±0.52	0.51±0.63	0.95
No. testing positive	8	9	
Anti-ICA512 antibody			
Index	0.34±0.44	0.35±0.44	0.84
No. testing positive	7	5	
Anti-insulin antibody			
Index	0.76±1.31	0.75±1.31	0.75
No. testing positive	7	8	
HLA-DQ haplotype†			0.40
No. with susceptible alleles	6	9	
No. with resistant alleles	6	3	

*Plus-minus values are means ±SD. GAD denotes glutamic acid decarboxylase, and anti-ICA512 anti-islet-cell antibody 512.

†Diabetes-resistant HLA-DQ (α,β) haplotypes included 0101/0601, 0101/0503, 0102/0602, 0103/0603, 0201/0201, 0201/0303, 0301/0301, and 0501/0301. A haplotype with a resistant allele was designated as a resistant haplotype, whether the other allele was susceptible, neutral, or resistant.

and interferon- γ was detectable in only one patient, whereas interleukin-5 was detected in the serum of nine of the antibody-treated patients (range of levels, 9 to 33 pg per milliliter) and interleukin-10 was detected in the serum of seven patients (range of levels, 5 to 316 pg per milliliter).

Side Effects of Antibody Treatment

Side effects of monoclonal-antibody infusions included mild and moderate fever in 9 of the 12 patients, generally on day 5; mild or moderate anemia in 9 of the 12 (which resolved after day 14); and nausea, vomiting, arthralgia, and headache in 1 patient each. A pruritic urticarial rash developed on the hands and occasionally the trunk and feet of 7 of the 12 patients. The rash appeared after the seventh day of treatment and resolved by day 30. A biopsy of this rash in two patients showed spongiosis consistent with eczematous dermatitis. There was no evidence of vasculitis. Antiidiotype antibodies developed in 6 of the 12 patients within the first month after treatment; but after six months, only 3 patients

still had antibodies, and at one year, only 1 had detectable levels. There has been no evidence of long-term toxic effects up to two years after antibody treatment.

Monoclonal-Antibody Treatment and Insulin Production

Antibody treatment significantly reduced the decline in the incremental and total C-peptide responses ($P=0.01$ for both comparisons) (Table 2 and Fig. 1). At the end of one year, the incremental C-peptide response in the monoclonal-antibody group was 109 ± 74 percent of the response to the mixed-meal tolerance test at entry and the total C-peptide response was 103 ± 53 percent of the base-line response, whereas the corresponding values in the control group were 42 ± 35 percent and 49 ± 33 percent of the base-line response. There was an average monthly decrease in the total C-peptide response of 5.52 ± 1.30 nmol per liter per four-hour test in the control group, as compared with an average monthly increase of 0.20 ± 1.86 nmol per liter per four-hour test in the monoclonal-antibody group ($P=0.006$). After one year, seven of the patients in the monoclonal-antibody group had no change or an increase (of more than 7.5 percent) from base line in the incremental response during the mixed-meal tolerance test; the other five had a decrease in the incremental response. By contrast, 11 of the 12 patients in the control group had a decrease in the incremental response ($P=0.03$). Nine of the 12 patients in the monoclonal-antibody group had no change or an increase in the total C-peptide response, whereas 10 of the 12 patients in the control group had a decrease in response ($P=0.01$).

Eleven of the 12 treated patients have been followed for more than 18 months. At 18 months, the mean incremental C-peptide response in these 11 patients was 90 ± 82 percent of the pretreatment level, and the total C-peptide response was 74 ± 39 percent of the base-line level. The incremental response was the same as the base-line response or greater in 6 of the 11 patients, and the total response was the same as the base-line response or greater in 5 of the 11 patients. By contrast, in 9 of the 12 controls studied, the incremental C-peptide response was 35 ± 38 percent of the base-line level ($P=0.07$ for the comparison with the monoclonal-antibody group), and the total C-peptide response was 42 ± 36 percent of the base-line level ($P=0.06$ for the comparison with the monoclonal-antibody group).

Metabolic Control of Diabetes

Antibody treatment resulted in a significant decrease in glycosylated hemoglobin levels ($P=0.008$). At study entry, the average glycosylated hemoglobin level was nonsignificantly higher in the monoclonal-antibody group, but the decline in glycosylated he-

TABLE 2. CHANGES IN THE INCREMENTAL AND TOTAL C-PEPTIDE RESPONSES DURING MIXED-MEAL TOLERANCE TESTING.*

VARIABLE	C-PEPTIDE RESPONSE DURING MIXED-MEAL TOLERANCE TESTING		
	STUDY ENTRY	6 MO	12 MO
	nmol/liter		
Monoclonal-antibody group			
Incremental response	63.1±33.0	69.0±51.2	67.7±62.3
Total response	111.5±50.2	121.1±79.7	114.2±90.6
Control group			
Incremental response	82.7±44.9	50.1±47.4	34.1±30.4
Total response	133.2±50.7	92.6±61.8	66.7±53.0

*Plus-minus values are means ±SD. The total response is the total area under the response curve, and the incremental response is the area under the curve formed by subtracting the fasting C-peptide level from the response at each time point. P=0.01 by repeated-measures analysis of variance for the comparison of the total response in the two groups.

moglobin levels between base line and six months was greater in that group (P=0.01) (Table 3). There were no severe hypoglycemic events in either group.

The improved glycemic control was not due to increased use of insulin in the monoclonal-antibody group. In fact, there was a significant decrease in the use of insulin in the monoclonal-antibody group as

compared with the control group (P=0.03) (Table 3). After one year, the average insulin dose in the monoclonal-antibody group was below the level that is considered to indicate clinical remission (0.5 U per kilogram per day).²⁰ Thus, monoclonal-antibody treatment resulted in improved metabolic control with reduced insulin usage during the first year after the diagnosis of type 1 diabetes mellitus.

Possible Predictors of Clinical Response

There were no differences between the patients with a response to monoclonal-antibody treatment and those with no response in terms of clinical presentation (including the presence or absence of patients with diabetic ketoacidosis), the titers of biochemically defined autoantibodies, the isotype subclasses of the autoantibodies, or the HLA-DQA1 and DQB1 genotypes. The mean fasting C-peptide level at study entry was 0.24±0.13 nmol per liter in subjects who had an increase or no change in the incremental C-peptide response to the mixed-meal tolerance test at six months, as compared with 0.12±0.09 nmol per liter in those who had a decline in the C-peptide response (P=0.13).

The pattern of T-cell repopulation after the nadir in the absolute lymphocyte count correlated with the response to monoclonal antibody. At 3 months (90 days), patients with a response to monoclonal-antibody treatment had a 68 percent increase in the

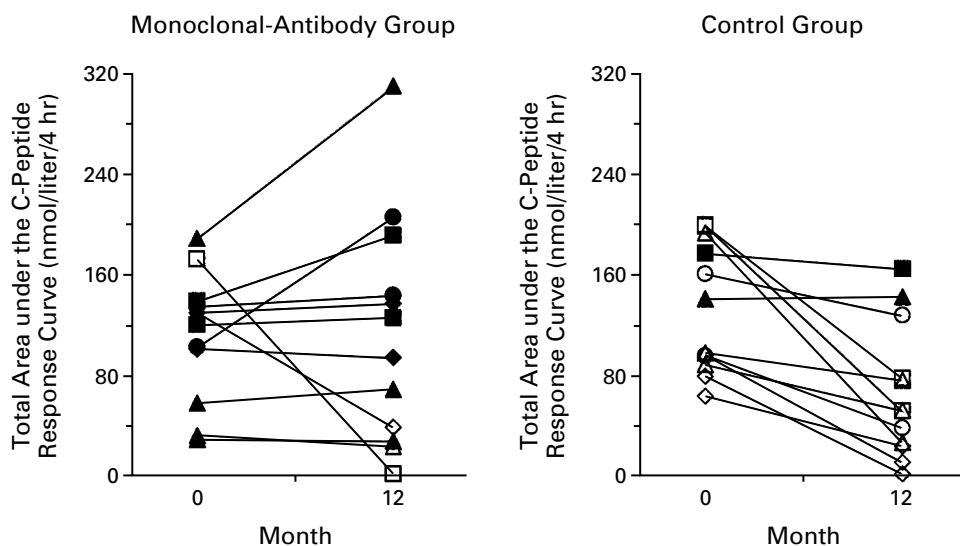


Figure 1. Changes from Study Entry to 12 Months in the Total C-Peptide Response to Mixed-Meal Tolerance Testing.

Data from each control and antibody-treated subject are shown. Solid symbols represent patients who had a sustained or increased C-peptide response, and open symbols represent patients who had a reduced response.

TABLE 3. EFFECTS OF TREATMENT WITH THE MONOCLONAL ANTIBODY hOKT3γ1(Ala-Ala) ON METABOLIC MEASURES.*

MEASURE	STUDY ENTRY	6 Mo	12 Mo
Glycosylated hemoglobin level (%)†			
Monoclonal-antibody group	9.27±1.59	6.23±0.86	6.98±1.70
Control group	8.20±1.05	7.65±1.41	7.53±1.27
Insulin dose (U/kg of body weight)			
Monoclonal-antibody group	0.57±0.17	0.36±0.26	0.49±0.28
Control group	0.44±0.25	0.52±0.21	0.59±0.17

*Plus-minus values are means ±SD. P=0.008 by repeated-measures analysis of variance for the comparison of the glycosylated hemoglobin level in the two groups, and P=0.03 by repeated-measures analysis of variance for the comparison of the insulin dose in the two groups.

†The normal range is 4.5 to 6.5 percent.

absolute number of repopulating CD8+ T cells, which was reflected in a reduction in the ratio of CD4+ T cells to CD8+ T cells (Fig. 2).

DISCUSSION

Treatment of new-onset type 1 diabetes mellitus with a single course of a monoclonal antibody against CD3 that does not bind to the Fc receptor appears to have arrested the loss of insulin responses during the first year after diagnosis in most, but not all, of the 12 patients we studied. One year after treatment, two thirds of the antibody-treated patients had a C-peptide response to the mixed-meal tolerance test that was the same as or greater than their response at study entry. In contrast, there was a consistent decline in the C-peptide response in 10 of the 12 untreated patients. The decline among control patients is somewhat surprising, since many of these patients entered a clinical “honeymoon” that has been thought to reflect improved insulin secretion after diagnosis. However, our metabolic studies, which used a four-hour provocative test rather than more abbreviated protocols, challenge this notion and suggest that a relentless decline is the natural history of the disease in the majority of patients. At the time of study entry, the control group was slightly older, had lower glycosylated hemoglobin levels, and had greater responses to the mixed-meal tolerance test than the monoclonal-antibody group. These differences between the two groups, although not statistically significant, would tend to bias the results against an effect of the antibody treatment, since patients younger than 18 years of age have generally been found to have more aggressive disease than patients 18 years of age or older.^{21,22} Thus, the true antibody effect may have been greater than is apparent from the comparison

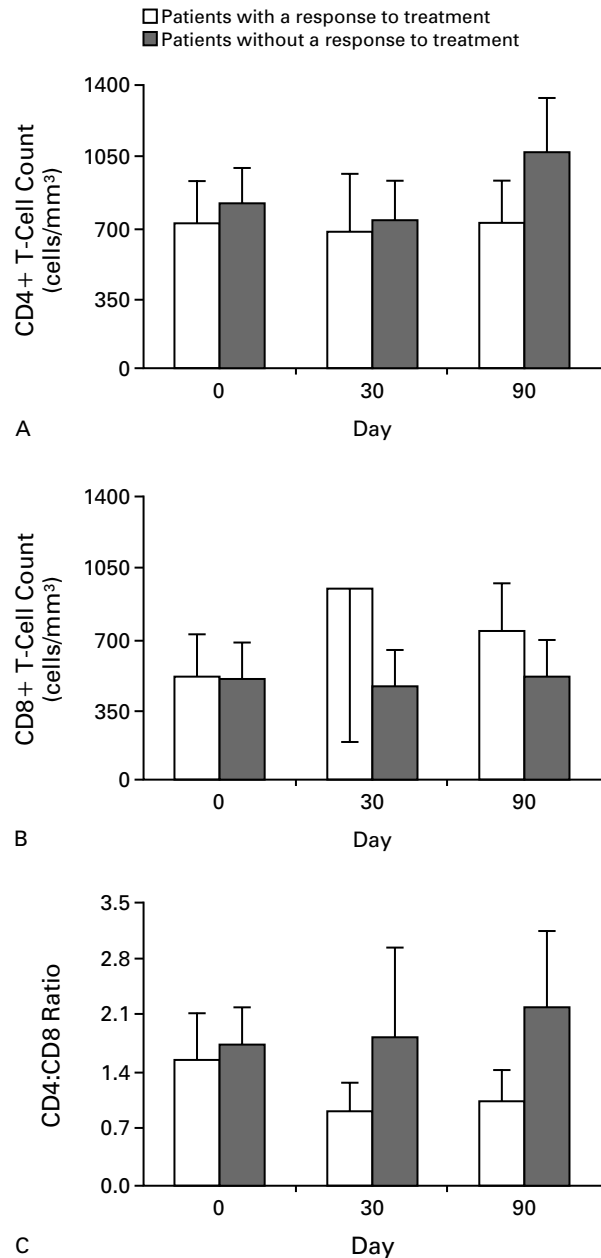


Figure 2. Mean CD4+ and CD8+ T-Cell Counts in the Monoclonal-Antibody Group According to the Presence or Absence of a Response to Treatment.

Panel A shows CD4+ T-cell counts, and Panel B CD8+ T-cell counts. The ratio of CD4+ T cells to CD8+ T cells (Panel C) was reduced in patients who had a clinical response to monoclonal-antibody treatment. The absolute number of each type of T cell was determined by multiplying the percentage of cells by the absolute lymphocyte count. The CD4:CD8 ratio was decreased in patients with a response to treatment who had an increase in the incremental C-peptide response at six months (P=0.03 by repeated-measures analysis of variance for the comparison with the patients with no response). The I bars represent standard deviations.

of these two groups. Furthermore, even after 18 months, the C-peptide response to the mixed-meal tolerance test was the same as or greater than that at diagnosis in 6 of the 11 antibody-treated patients who had been followed for that long.

Accumulated clinical experience, as well as results from the Diabetes Control and Complications Trial²³ and other studies,^{24,25} indicate that there is better metabolic control of type 1 diabetes mellitus in patients in whom some insulin secretion is retained. In the Diabetes Control and Complications Trial, a stimulated C-peptide level of more than 0.2 nmol per liter was associated with improved metabolic control, as reflected in the glycosylated hemoglobin level.²³ It is not surprising, therefore, that the improved insulin secretion was accompanied by an improvement in the glycosylated hemoglobin level and a reduction in the insulin needs of patients treated with monoclonal antibody.

Antibody treatment had a sustained effect on the disease in the absence of continued administration of the monoclonal antibody. The effects of this monoclonal antibody on T cells differ from those of previously tested immunosuppressive agents and may account for the more sustained response. Other immunosuppressive agents, including cyclosporine, azathioprine, and prednisone, work by blocking the effector phases of immune responses by interfering with the production of cytokines, the proliferation of T cells, or both. Preclinical studies by Bluestone and colleagues²⁶⁻²⁸ suggested that antibody against CD3 that does not bind to the Fc receptor has selective effects on specific populations of T cells. It kills or causes unresponsiveness in T cells that produce interleukin-2 or interferon- γ (type 1 helper T [Th1] cells), whereas T cells that produce interleukin-10 or interleukin-4 (type 2 helper T [Th2] cells) may be stimulated by the monoclonal antibody.²⁶⁻²⁸ This effect is seen only in activated T cells and not in naive T cells. The presence of interleukin-10 and interleukin-5 — but not interferon- γ or interleukin-2 — in serum after monoclonal-antibody treatment is consistent with these observations. Studies involving animal models support the importance of Th1 responses in the pathogenesis of type 1 diabetes mellitus, suggesting a mechanism for the effect of monoclonal-antibody treatment.^{4,29-32} Clearly, the drug binds all T cells that express the CD3 molecule. Therefore, the selectivity observed among subpopulations of T cells may relate to quantitative or qualitative differences in response to the signal delivered by the monoclonal antibody. This may be analogous to the differential response to altered-peptide ligands by various subpopulations of T cells.^{33,34} Thus, the effect of monoclonal-antibody therapy may be to shift the autoimmune response toward production of protec-

tive (Th2) cytokines. The rash that developed in most patients, with histologic features similar to those of eczematoid lesions, might be mediated by Th2 responses.³⁵

Subjects who had a response to the monoclonal-antibody treatment had an increase in the number of CD8+ T cells after treatment. Several reports have described subpopulations of CD8+ cells in rodents and humans that have immune-regulatory properties.³⁶⁻³⁸ Studies are under way to find cell-surface markers that can identify cells associated with a response to monoclonal-antibody treatment and that may indicate the presence of regulatory populations after such treatment.

We did not observe any changes in the titer or the isotypes of anti-GAD autoantibodies. It is possible that these autoantibody responses had already matured at the time of diagnosis and thus were not susceptible to change by circulating cytokines. Similarly, we failed to find an effect of monoclonal-antibody treatment on antirubella IgG titers (mean ratio of patient titers to standard titers at entry, 1.33 ± 0.62 ; mean ratio at six months, 1.38 ± 0.06), suggesting that established humoral responses were unaffected. Other immunologic markers, including HLA type and the titers and isotypes of autoantibodies, did not predict clinical response. The fasting C-peptide level was higher in the patients who had a response to treatment but was not an absolute predictor of a clinical response to the monoclonal antibody, as it was in the case of cyclosporine treatment of new-onset type 1 diabetes.⁵

Thus, treatment within the first six weeks after the onset of type 1 diabetes mellitus with a single course of anti-CD3 monoclonal antibody appeared to arrest the deterioration of insulin production in the majority of our 12 patients for at least the first year of disease. The mechanism of antibody action is under investigation, but we speculate that the monoclonal antibody may alter the immunologic response that causes type 1 diabetes mellitus, may induce a population of cells that can influence the disease process, or both.

Supported by grants from the National Institutes of Health (R01DK57846, U19A146132, M01 RR00645, M01 RR01271, and P60 DK20595) and the Juvenile Diabetes Research Foundation (Special Grant 4-1999-711 and Center Grant 4-1999-841). Dr. Bluestone has a financial interest in the monoclonal antibody hOKT3 γ 1(Ala-Ala) consisting of a patent application and a commercial agreement with Centocor and Johnson & Johnson Pharmaceuticals.

REFERENCES

1. Eisenbarth GS. Type I diabetes mellitus: a chronic autoimmune disease. *N Engl J Med* 1986;314:1360-8.
2. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 2001;358:221-9. [Erratum, *Lancet* 2001;358:766.]
3. Tydén G, Reinholdt FP, Sundkvist G, Bolinder J. Recurrence of autoim-

- mune diabetes mellitus in recipients of cadaveric pancreatic grafts. *N Engl J Med* 1996;335:860-3. [Erratum, *N Engl J Med* 1996;335:1778.]
4. Rabinovitch A, Suarez-Pinzon WL. Cytokines and their roles in pancreatic islet beta-cell destruction and insulin-dependent diabetes mellitus. *Biochem Pharmacol* 1998;55:1139-49.
 5. Bougneres PF, Carel JC, Castano L, et al. Factors associated with remission of type 1 diabetes in children treated with cyclosporine. *N Engl J Med* 1988;318:663-70.
 6. Silverstein J, Maclaren N, Riley W, Spillar R, Radjenovic D, Johnson S. Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. *N Engl J Med* 1988;319:599-604.
 7. Skyler JS, Rabinovitch A. Cyclosporine in recent onset type 1 diabetes mellitus: effects on islet beta cell function. *J Diabetes Complications* 1992;6:77-88.
 8. Stiller CR, Dupre J, Gent M, et al. Effects of cyclosporine immunosuppression in insulin-dependent diabetes mellitus of recent onset. *Science* 1984;223:1362-7.
 9. Parving HH, Tarnow L, Nielsen FS, et al. Cyclosporine nephrotoxicity in type 1 diabetic patients: a 7-year follow-up study. *Diabetes Care* 1999;22:478-83.
 10. Herold KC, Bluestone JA, Montag AG, et al. Prevention of autoimmune diabetes with nonactivating anti-CD3 monoclonal antibody. *Diabetes* 1992;41:385-91.
 11. Chatenoud L, Primo J, Bach JF. CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. *J Immunol* 1997;158:2947-54.
 12. Chatenoud L, Therivet E, Primo J, Bach JF. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc Natl Acad Sci U S A* 1994;91:123-7.
 13. Chatenoud L, Ferran C, Legendre C, et al. In vivo cell activation following OKT3 administration: systemic cytokine release and modulation by corticosteroids. *Transplantation* 1990;49:697-702.
 14. Woodle ES, Bluestone JA, Zivin RA, et al. Humanized, nonmitogenic OKT3 antibody, huOKT3 gamma(Ala-Ala): initial clinical experience. *Transplant Proc* 1998;30:1369-70.
 15. Woodle ES, Xu D, Zivin RA, et al. Phase I trial of a humanized, Fc receptor nonbinding OKT3 antibody, huOKT3gamma1(Ala-Ala) in the treatment of acute renal allograft rejection. *Transplantation* 1999;68:608-16.
 16. Faber OK, Binder C, Markussen J, et al. Characterization of seven C-peptide antisera. *Diabetes* 1978;27:Suppl 1:170-7.
 17. Woo W, LaGasse JM, Zhou Z, et al. A novel high-throughput method for accurate, rapid, and economical measurement of multiple type 1 diabetes autoantibodies. *J Immunol Methods* 2000;244:91-103.
 18. Erlich H, Bugawan T, Begovich AB, et al. HLA-DR, DQ and DP typing using PCR amplification and immobilized probes. *Eur J Immunogenet* 1991;18:33-55.
 19. Chatenoud L. Humoral immune response against OKT3. *Transplant Proc* 1993;25:Suppl 1:68-73.
 20. Yilmaz MT, Devrim AS, Biyal F, et al. Immunoprotection in spontaneous remission of type 1 diabetes: long-term follow-up results. *Diabetes Res Clin Pract* 1993;19:151-62.
 21. The DCCT Research Group. Effects of age, duration and treatment of insulin-dependent diabetes mellitus on residual beta-cell function: observations during eligibility testing for the Diabetes Control and Complications Trial (DCCT). *J Clin Endocrinol Metab* 1987;65:30-6.
 22. Karjalainen J, Salmela P, Ilonen J, Surcel HM, Knip M. A comparison of childhood and adult type 1 diabetes mellitus. *N Engl J Med* 1989;320:881-6.
 23. The Diabetes Control and Complications Trial Research Group. Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the Diabetes Control and Complications Trial: a randomized, controlled trial. *Ann Intern Med* 1998;128:517-23.
 24. Sjoberg S, Gjotterberg M, Berglund L, Moller E, Ostman J. Residual C-peptide excretion is associated with a better long-term glycemic control and slower progress of retinopathy in type 1 (insulin-dependent) diabetes mellitus. *J Diabetes Complications* 1991;5:18-22.
 25. Grajwer LA, Pildes RS, Horwitz DL, Rubenstein AH. Control of juvenile diabetes mellitus and its relationship to endogenous insulin secretion as measured by C-peptide immunoreactivity. *J Pediatr* 1977;90:42-8.
 26. Smith JA, Tso JY, Clark MR, Cole MS, Bluestone JA. Nonmitogenic anti-CD3 monoclonal antibodies deliver a partial T cell receptor signal and induce clonal anergy. *J Exp Med* 1997;185:1413-22.
 27. Smith JA, Tang Q, Bluestone JA. Partial TCR signals delivered by FcR-nonbinding anti-CD3 monoclonal antibodies differentially regulate individual Th subsets. *J Immunol* 1998;160:4841-9.
 28. Smith JA, Bluestone JA. T cell inactivation and cytokine deviation promoted by anti-CD3 mAbs. *Curr Opin Immunol* 1997;9:648-54.
 29. Katz JD, Benoist C, Mathis D. T helper cell subsets in insulin-dependent diabetes. *Science* 1995;268:1185-8.
 30. Herold KC, Vezys V, Sun Q, et al. Regulation of cytokine production during development of autoimmune diabetes induced with multiple low doses of streptozotocin. *J Immunol* 1996;156:3521-7.
 31. Zipris D, Greiner DL, Malkani S, Whalen B, Mordes JP, Rossini AA. Cytokine gene expression in islets and thyroids of BB rats: IFN-gamma and IL-12p40 mRNA increase with age in both diabetic and insulin-treated nondiabetic BB rats. *J Immunol* 1996;156:1315-21.
 32. Rapoport MJ, Jaramillo A, Zipris D, et al. Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in non-obese diabetic mice. *J Exp Med* 1993;178:87-99.
 33. Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. *Annu Rev Immunol* 1996;14:1-27.
 34. Sloan-Lancaster J, Evavold BD, Allen PM. Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. *Nature* 1993;363:156-9.
 35. Izuhara K, Shirakawa T. Signal transduction via the interleukin-4 receptor and its correlation with atopy. *Int J Mol Med* 1999;3:3-10.
 36. Ware R, Jiang H, Braunstein N, et al. Human CD8+ T lymphocyte clones specific for T cell receptor V beta families expressed on autologous CD4+ T cells. *Immunity* 1995;2:177-84.
 37. Liu Z, Tugulea S, Cortesini R, Suci-Foca N. Specific suppression of T helper alloreactivity by allo-MHD class I-restricted CD8+CD28- T cells. *Int Immunol* 1998;10:775-83.
 38. Jiang S, Tugulea S, Pennesi G, et al. Induction of MHC-class I restricted human suppressor T cells by peptide priming in vitro. *Hum Immunol* 1998;59:690-9.

Copyright © 2002 Massachusetts Medical Society.