ORIGINAL ARTICLE

Insulin Needs after CD3-Antibody Therapy in New-Onset Type 1 Diabetes

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ABSTRACT

BACKGROUND

Type 1 diabetes mellitus is a T-cell–mediated autoimmune disease that leads to a major loss of insulin-secreting beta cells. The further decline of beta-cell function after clinical onset might be prevented by treatment with CD3 monoclonal antibodies, as suggested by the results of a phase 1 study. To provide proof of this therapeutic principle at the metabolic level, we initiated a phase 2 placebo-controlled trial with a humanized antibody, an aglycosylated human IgG1 antibody directed against CD3 (ChAglyCD3).

METHODS

In a multicenter study, 80 patients with new-onset type 1 diabetes were randomly assigned to receive placebo or ChAglyCD3 for six consecutive days. Patients were followed for 18 months, during which their daily insulin needs and residual beta-cell function were assessed according to glucose-clamp–induced C-peptide release before and after the administration of glucagon.

RESULTS

At 6, 12, and 18 months, residual beta-cell function was better maintained with ChAglyCD3 than with placebo. The insulin dose increased in the placebo group but not in the ChAglyCD3 group. This effect of ChAglyCD3 was most pronounced among patients with initial residual beta-cell function at or above the 50th percentile of the 80 patients. In this subgroup, the mean insulin dose at 18 months was 0.22 IU per kilogram of body weight per day with ChAglyCD3, as compared with 0.61 IU per kilogram with placebo ($P<0.001$). In this subgroup, 12 of 16 patients who received ChAglyCD3 (75 percent) received minimal doses of insulin (≤0.25 IU per kilogram per day) as compared with none of the 21 patients who received placebo. Administration of ChAglyCD3 was associated with a moderate "flu-like" syndrome and transient symptoms of Epstein–Barr viral mononucleosis.

CONCLUSIONS

Short-term treatment with CD3 antibody preserves residual beta-cell function for at least 18 months in patients with recent-onset type 1 diabetes.

The T-cell–mediated autoimmune origin of type 1 diabetes mellitus has prompted efforts to prevent the progression of disease by targeting T lymphocytes.[1] This approach was first tested with the use of cyclosporine.[2-4] However, the benefit was lost after the withdrawal of cyclosporine, implying a need for the indefinite administration of this agent, with the attendant risks of chronic immunosuppression. Studies in nonobese diabetic mice indicated that short-term treatment with monoclonal antibodies against CD3 induced long-term remission of established diabetes[5,6] through the induction of immune tolerance that involved transforming growth factor β–dependent regulatory T cells.[7] The translation to use in the clinical setting necessitated the development of CD3 antibodies that were devoid of the toxicity that accompanies mitogenic antibodies such as muromonab-CD3 (OKT3), which produce a cytokine-related "flu-like" syndrome.[8-10] Two humanized, nonmitogenic, Fc-mutated CD3 antibodies have been available — hOKT3γ1(Ala-Ala)[11,12] and ChAglyCD3.[13-15] The first showed promise in an open-label phase 1 trial in which 12 patients who were treated with the antibody had better beta-cell function after one year and a lower insulin dosage at six months than did 12 patients who did not receive the antibody.[16] To assess the long-term metabolic efficacy of this strategy, we conducted a multicenter, randomized, phase 2 placebo-controlled trial involving the ChAglyCD3 antibody.

METHODS

PATIENT SELECTION

Patients with type 1 diabetes mellitus of recent onset were selected according to the following criteria: they were 12 to 39 years of age; had been treated with insulin for less than four weeks; had a positive result on testing for islet-cell autoantibodies, glutamic acid decarboxylase autoantibodies, or both; had a random plasma C-peptide level of more than 0.20 nmol per liter and a plasma glucose level of 180 to 250 mg per deciliter (10.0 to 13.9 mmol per liter); had had polyuria for less than six months; and had lost less than 10 percent of their body weight in the previous six months. Patients negative for Epstein–Barr virus (EBV) IgG were excluded. Details can be found in Supplementary Appendix 1 (available with the full text of this article at www.nejm.org). Written informed consent was obtained from each patient. The trial was approved by the Belgian Diabetes Registry and the ethics committee at each center.

STUDY DESIGN

Treatment with ChAglyCD3 was administered to 40 patients, and 40 patients received placebo. Patients received intensive insulin therapy (i.e., at least three injections per day) on the basis of blood glucose levels, as measured by the patient at home, to maintain levels of between 80 and 140 mg per deciliter and glycosylated hemoglobin values of less than 7.0 percent. Randomization was balanced according to center (five sites), age (less than 15 years or 15 years and older), and the presence of islet-cell antibodies.[17] A centralized minimization procedure was used. A third-party member made treatment assignments (see Supplementary Appendix 1).

The core facility of the Belgian Diabetes Registry maintained the database and performed all measurements of C peptide (results were not communicated to physicians) and of glycosylated hemoglobin (results were communicated if they were 7.0 percent or higher). Referring physicians regularly performed local glycosylated hemoglobin assays for the adjustment of insulin doses toward optimal metabolic control.

ADMINISTRATION OF ANTIBODY

ChAglyCD3 is an aglycosylated recombinant antibody (human γ1) with identical specificity to a previously described humanized aglycosylated CD3.[13-15] ChAglyCD3 was manufactured under Good Manufacturing Practice conditions[18] and formulated in phosphate-buffered saline; the formulation buffer was used as placebo.

Patients were hospitalized for the administration of ChAglyCD3 or placebo (given in an intravenous infusion over two to four hours) for six consecutive days. On the basis of a previous study protocol,[15] the first nine patients received a first dose of 24 mg, followed by infusions of 8 mg per day; four of these patients had severe headache, vomiting, or both after the first therapeutic dose. Consequently, the remaining 71 patients received six consecutive infusions of 8 mg of ChAglyCD3 or placebo per day.

EFFICACY TESTS

Residual beta-cell function was analyzed according to the measurement of C-peptide release as induced by a two-phase glucose-clamp procedure. During the first phase, before glucose infusion

(−180 to 0 minutes), euglycemia (blood glucose level, 60 to 90 mg per deciliter [3.3 to 5.0 mmol per liter]) was maintained by intravenous insulin infusion; during the second phase (0 to 146 minutes), blood glucose levels were increased and maintained at 180 to 250 mg per deciliter (10.0 to 13.9 mmol per liter); at 140 minutes, 1 mg of glucagon was injected intravenously.[19,20] Plasma samples were obtained for C-peptide measurements at 60, 90, 120, 140 and 146 minutes. The C-peptide level was measured with the use of a time-resolved fluorescence immunoassay (PerkinElmer). The area under the curve (AUC) was calculated for the induced release of C peptide before glucagon injection (at 60, 90, 120, and 140 minutes) and afterward (at 140 and 146 minutes). To compare the induced release of C peptide in both periods, we expressed the AUC value per minute for each. Every three months, the daily insulin dose, body weight, and blood glucose level as monitored by the patient were recorded, and the glycosylated hemoglobin level was measured with the use of high-performance liquid chromatography.[21]

SAFETY TESTS

During hospitalization and at each outpatient visit, patients underwent history taking, physical examination, and blood analysis as a means of monitoring for adverse events. Questionnaires were used to record acute symptoms. DNA extracted from peripheral white cells was used to screen for herpesviruses (herpes simplex virux [HSV] type 1 [HSV-1] and HSV-2, cytomegalovirus, EBV, varicella–zoster virus, and human herpesvirus 6) with the use of qualitative multiplex polymerase chain reaction (PCR) (Herpes consensus generic kit 67-090 and Herpes Hybridowell 67-050, Argene Biosoft). In 32 patients (22 in the ChAglyCD3 group and 10 in the placebo group), serial screening for EBV was also performed with the use of quantitative PCR[22]; these tests were conducted retrospectively.

IMMUNOLOGIC MONITORING

HLA-DQA and HLA-DQB genotyping and assays for islet-cell antibody and antibody against glutamic acid decarboxylase were performed at the Belgian Diabetes Registry.[23,24] Flow cytometry was used to quantify peripheral lymphocyte subsets.[8,25] Serum samples were analyzed for cytokine levels,[9] antibodies to ChAglyCD3,[26] and ChAglyCD3 concentrations.[27] EBV-specific antibodies were measured by enzyme-linked immunosorbent assay. EBV-specific CD8+ T cells were detected by immunofluorescence assay with the use of HLA class I–specific tetramers.[28,29]

STATISTICAL ANALYSIS

Data analysis was conducted according to the intention-to-treat principle and included all 80 randomized patients. The analysis compared residual beta-cell function, daily insulin dose (in IU per kilogram of body weight), glycosylated hemoglobin level, body weight, and body-mass index immediately before treatment (baseline) and at 6, 12, and 18 months. The primary end point was the change in residual beta-cell function between baseline and six months. Two-sided statistical tests were performed, with P values below 0.05 indicating significance. Comparisons of the two treatment groups were performed with the use of the t-test or Mann–Whitney test for quantitative variables and Fisher's exact test for binary variables. Comparison of the quantitative efficacy variables in the two groups was performed with the use of an analysis-of-variance model with center, treatment, and treatment-by-center interaction as factors, with the baseline value as the covariate. The Cochran–Mantel–Haenszel test was used in case there were nonnormal residuals in the model, with control for center effects. Multiple regression analysis was performed, as detailed in Supplementary Appendix 2. No adjustments were made for multiplicity.

All data shown are means ±SD. The clinical coordinator, Dr. Keymeulen, assisted by Dr. Vandemeulebroucke, the senior investigators, Drs. Pipeleers, Bach, Mathieu, and Ziegler, and the principal investigator, Dr. Chatenoud, collectively designed the protocol, vouch for the data, and wrote the manuscript. The statistician, Dr. Kaufman, was responsible for the statistical analysis. None of the coauthors involved in providing ChAglyCD3 and placebo — Dr. Hale, Ms. Rebello, Dr. Bird, Dr. Berrie, Mr. Frewin, and Dr. Waldmann, Oxford, United Kingdom — participated in data accrual or statistical analysis. Drs. Waldmann and Hale participated in the study design. Additional persons and institutions that contributed to the trial are listed in the Appendix.

RESULTS

CHARACTERISTICS OF THE PATIENTS

Of 210 patients with newly diagnosed type 1 diabetes who were screened between June 2000 and

March 2003, 39 declined to participate and 91 did not meet the inclusion criteria. Of the remaining 80 patients, 40 were randomly assigned to the ChAglyCD3 group and 40 to the placebo group. The two groups did not differ statistically in terms of clinical or laboratory characteristics (Table 1) or in the expression of susceptible or protective HLA haplotypes (data not shown).

Table 1. Characteristics of the Patients at Diagnosis, Screening, and Start of Treatment.*

Characteristic	Placebo Group (N=40)	ChAglyCD3 Group (N=40)
Demographic		
Sex (male/female)	26/14	25/15
Age (yr)	26±7	27±7
At diagnosis		
Patients with diabetic ketoacidosis (%)	19	29
Patients with polyuria and weight loss (%)	51	74
Weeks since onset of symptoms		
Median	3	4
Interquartile range	2–8	3–10
At screening		
Days since diagnosis		
Median	8	9
Interquartile range	5–16	3–18
Patients with anti–islet-cell autoantibodies (%)	75	80
Patients with glutamic acid decarboxylase antibodies (%)	90	85
At start of treatment		
Days since diagnosis		
Median	25	21
Interquartile range	19–27	18–24
Days of insulin treatment		
Median	20	19
Interquartile range	13–25	17–24
Insulin dose (IU/kg/day)	0.38±0.20	0.46±0.27
Body weight (kg)	67±12	70±15
Body-mass index†		
Median	21.1	22.1
Interquartile range	19.4–23.9	20.2–24.1

* Plus–minus values are means ±SD. Values are expressed as medians and interquartile ranges for variables for which there was a significant deviation from the normal distribution. There were no statistically significant differences between the two groups.

† The body-mass index is the weight in kilograms divided by the square of the height in meters.

EFFECTS OF TREATMENT

Before treatment, patients in the two groups had similar levels of glucose-clamp–induced C-peptide release before and after the administration of glucagon (Fig. 1A). Among patients in the placebo group, the values for C-peptide release both before and after the administration of glucagon progressively decreased over the 18 months of follow-up, reaching levels that were 33 and 37 percent lower, respectively, than at baseline (Fig. 1A). In the patients treated with ChAglyCD3, both variables increased at 6 months and returned to pretreatment levels by month 18 (Fig. 1A). The differences between treatment groups in the changes from baseline were statistically significant at all time points. Both in the absence and presence of glucagon, the patients in the ChAglyCD3 group had a net gain in C-peptide release over those in the placebo group at all time points (Table 2).

The progressive decline in residual beta-cell function in the placebo group was accompanied by a progressive rise in insulin dose, which was 50 percent higher at 18 months than at baseline (Fig. 1A). In the ChAglyCD3 group, the mean insulin dose at 18 months was 12 percent lower than at baseline (Fig. 1A). The mean differences between the groups in the change in the insulin doses from baseline were significant at all time points (Table 2). In both patient groups, there was a significant negative correlation between insulin dose and C-peptide release, both before treatment and at month 18. The Pearson's correlation coefficient was somewhat stronger at 18 months ($r=-0.60$ for placebo, $P<0.001$; $r=-0.64$ for ChAglyCD3, $P<0.001$) than before treatment ($r=-0.43$ for placebo, $P=0.006$; $r=-0.55$ for ChAglyCD3, $P<0.001$).

During the study period, the changes in mean body weight and body-mass index did not differ between the two groups (data not shown), nor did the glycosylated hemoglobin levels (Fig. 1A and Table 2), indicating that the differences in residual beta-cell function and insulin dose were not caused by inadequate insulin treatment in either group.[30] At 18 months, the glycosylated hemoglobin levels in the ChAglyCD3 group (6.9±1.3 percent) and the placebo group (6.9±1.0 percent) were almost identical; body weight was 71.8±14.5 kg and 72.3±12.9 kg, respectively.

INFLUENCE OF BETA-CELL FUNCTION AT BASELINE

In the placebo group, residual beta-cell function at baseline was the only significant factor predictive of outcome, whereas in the ChAglyCD3 group, none of the variables tested (in a stepwise, multiple linear regression model) were predictive of the response. To examine further the effect of residual beta-cell function at baseline, the patients in each group were

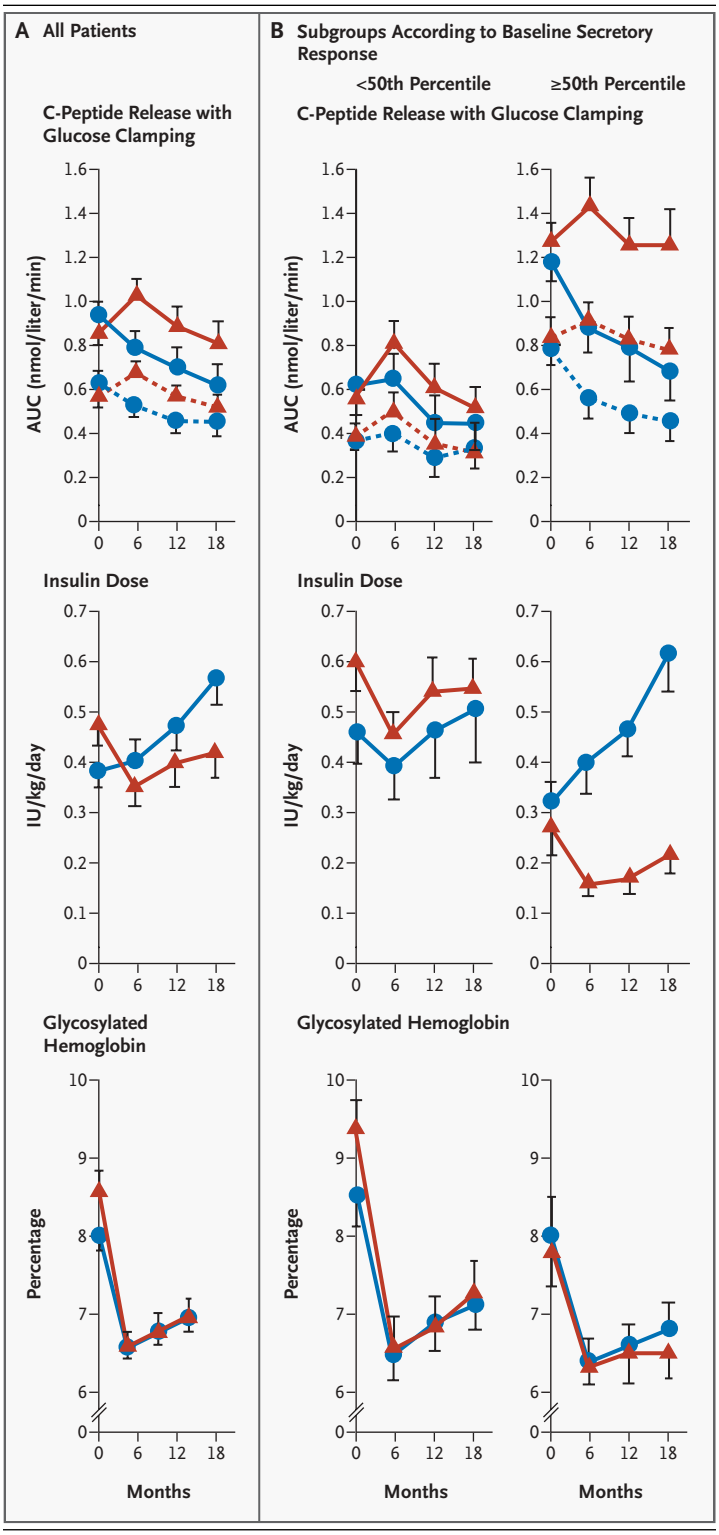

Figure 1. Efficacy Tests in the Two Study Groups.

Blue circles represent the placebo group, and red triangles the ChAglyCD3 group. Panel A shows the glucose-clamp–induced C-peptide release before glucagon injection (dashed lines) and afterward, the daily insulin needs, and the glycosylated hemoglobin levels at baseline, 6, 12, and 18 months for all patients in both groups. Panel B shows the values for these same factors according to whether the glucose-clamp–induced C-peptide release at baseline was below (left-hand side of panel) or at or above (right-hand side) the median (50th percentile) of all patients at baseline. There were 16 patients in the placebo group and 24 in the ChAglyCD3 group in whom the initial secretory response was below the 50th percentile and 24 in the placebo group and 16 in the ChAglyCD3 group in whom the response was at or above the 50th percentile. All values are means ±SE. AUC denotes area under the curve.

divided into two subgroups according to whether the initial glucose-clamp–induced C-peptide release was lower or higher than the median value of the entire population at baseline — that is, the 50th percentile, which was denoted as P50 (AUC, 0.52 nmol per liter per minute). P50 was approximately 75 percent lower than the value measured in 20 healthy, age-matched control subjects (data not shown). The demographic, clinical, and laboratory characteristics were similar in the respective subgroups (as shown in Supplementary Appendix 3).

Among the 16 patients in the placebo group in whom the initial C-peptide release was below P50, the residual beta-cell function (AUC) at 18 months remained low (values were 15 percent lower than at baseline); patients in whom the values were at or above P50 had a 41 percent decrease during this period (Fig. 1B). The mean insulin dose among patients in the placebo group who had values below P50 did not increase over the 18-month follow-up period, whereas the dose progressively increased among the 24 patients with values at or above P50, reaching values that were 1.9 times as high as those at baseline (Fig. 1B). This increase did not occur among the 16 patients treated with ChAglyCD3 in whom the initial C-peptide release was at or above P50; the residual beta-cell function was also preserved among these patients over this period (Fig. 1B).

At 18 months, the mean insulin dose among the 24 patients in the ChAglyCD3 group with baseline

Table 2. Comparison of Changes in Efficacy Tests in the Two Groups over Time.*

Efficacy Test	Difference between Groups at Baseline (CD3 minus Placebo)	Difference between Groups in Changes from Baseline					
		At Month 6	P Value	At Month 12	P Value	At Month 18	P Value
C-peptide release with glucose clamping (nmol/liter/min)							
Without glucagon	−0.12 (−0.26 to +0.03)	+0.22 (+0.06 to +0.38)	0.009	+0.22 (+0.05 to +0.39)	0.01	+0.20 (+0 to +0.38)	0.02
With glucagon	−0.20 (−0.43 to +0.02)	+0.39 (+0.12 to +0.65)	0.006	+0.40 (+0.12 to +0.69)	0.01	+0.43 (+0.13 to +0.73)	0.006
Insulin dose (IU/kg/day)	+0.07 (−0.05 to +0.19)	−0.07 (−0.19 to +0.05)	0.003	−0.12 (−0.26 to +0.03)	0.005	−0.18 (−0.35 to −0.02)	0.03
Glycosylated hemoglobin (%)	+0.77 (−0.02 to +1.56)	−0.16 (−0.72 to +0.40)	0.48	−0.17 (−0.80 to +0.47)	0.31	−0.07 (−0.77 to +0.63)	0.21

* For each group, the change in efficacy tests was calculated by subtracting the value at baseline from the value at months 6, 12, and 18. The change in each value in the placebo group was subtracted from the change in that in the ChAglyCD3 group, with the difference given here as the mean (with 95 percent confidence interval). For glucose-clamp–induced C-peptide release, positive differences indicate better results for ChAglyCD3 than for placebo. For insulin dose and glycosylated hemoglobin, negative differences indicate better results for ChAglyCD3 than for placebo.

values below P50 did not differ significantly from the dose in the comparable placebo-treated subgroup (0.54±0.30 vs. 0.51±0.33 IU per kilogram per day, respectively; P=0.73). In contrast, the mean insulin dose among the patients in the ChAglyCD3 group who had values at or above P50 at 18 months (0.22±0.11 IU per kilogram per day) was significantly lower than the dose among comparable patients in the placebo group (0.61±0.29 IU per kilogram per day, P<0.001). Glycosylated hemoglobin levels indicated that these differences were not caused by inappropriate insulin treatment (among patients with values at or above P50, 6.4±0.8 percent in the ChAglyCD3 group and 6.7±1.0 percent in the placebo group; P=0.25) (Fig. 1B). At this time point, the glycosylated hemoglobin levels in the ChAglyCD3 group were significantly lower among those with baseline C-peptide values at or above P50 than among those with values below P50 (7.2±1.4 percent, P=0.04); no difference in these levels was seen between the two subgroups within the placebo group. Analysis showed a significant treatment effect (P=0.01) as well as a significant interaction between treatment and AUC of glucose-clamp–induced C-peptide release before treatment (P=0.003).

The effect of ChAglyCD3 on insulin needs was also observed when individual patients were followed from baseline until the 18-month follow-up visit. Among the patients in the ChAglyCD3 group with values at or above P50, the percentage of patients with an insulin dosage of 0.25 IU per kilogram per day or less increased from 50 percent at baseline to 75 percent at 18 months; the percentage of patients with this dosage fell from 38 percent to 0 percent among patients in the placebo group with values at or above P50 (Fig. 2). Moreover, no patients with baseline C-peptide values at or above P50 required an insulin dosage of more than 0.75 IU per kilogram per day in the ChAglyCD3 group, whereas 30 percent of those in the placebo group required these higher dosages (Fig. 2).

IMMUNOLOGIC TESTS

Lymphocyte Counts

Treatment with ChAglyCD3 induced a transient decrease in peripheral CD2+ lymphocyte counts, which was most pronounced at day 2 and week 2, with a partial recovery occurring between these times (Fig. 3). Absolute B-lymphocyte counts (CD19+) were also transiently decreased. The recovery phase after week 2 was associated with a CD8+ T-cell lymphocytosis. This led to an inversion of the ratio of CD4+ to CD8+ lymphocytes (Fig. 3).

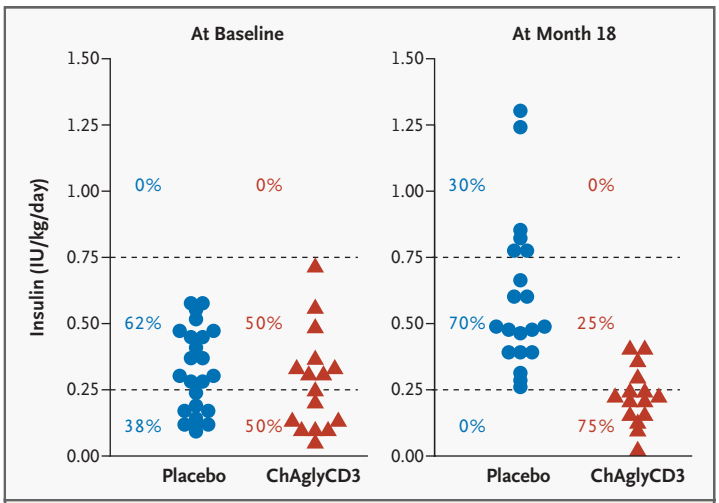

Figure 2. Comparison of Individual Insulin Doses at Baseline and 18 Months in Patients with an Initial Secretory Response at or above the 50th Percentile.
Circles represent the placebo group, and triangles the ChAglyCD3 group.

This inversion was observed in 29 of 37 patients at month 3, in 15 of 30 patients at month 6, in 10 of 33 patients at month 12, and in 9 of 31 patients at month 18 ($P<0.001$ for all, as compared with baseline).

Antigenic Modulation

During treatment with ChAglyCD3, T lymphocytes underwent antigenic modulation — that is, transient disappearance of the CD3 complex.[8,31] Thus, during treatment, most circulating T cells were CD2+CD3−CD4+ or CD2+CD3−CD8+. By day 14, T cells again expressed normal levels of CD3. Cell coating, assessed on staining with an isotype-specific antihuman IgG1 antibody, was only sporadically observed (in <1 percent of T cells) in a few patients.

Circulating ChAglyCD3 Levels

In patients who received a cumulative dose of 48 mg of ChAglyCD3, trough ChAglyCD3 concentrations increased to approximately 2 μg per milliliter (Fig. 3). By week 2, this level had decreased to less than 0.1 μg per milliliter.

Circulating Cytokine Levels

In patients who received a cumulative dose of 48 mg of ChAglyCD3, serum levels of tumor necrosis factor-α (TNF-α), interleukin-6, and interferon-γ rose after the first infusion (median peak concentrations: TNF-α, 527 pg per milliliter at one hour; interleukin-6, 1213 pg per milliliter at four hours; and interferon-γ, 4.4 IU per milliliter at four hours) and returned to normal before the third infusion. Serum interleukin-10 concentrations also increased after the first infusion (median peak concentration, 124.1 IU per milliliter at four hours) and returned to baseline levels before the third infusion. In 17 percent of patients, low interleukin-2 levels were noticed after the first or second infusion (range, 0.2 to 2.2 IU per milliliter).

Antiglobulin Responses to ChAglyCD3

Antibody responses to ChAglyCD3, including the presence of anti-idiotypic antibodies, were detected in 21 patients (53 percent), with a median time to appearance of four weeks (interquartile range, four to six weeks).

Correlation between Immunologic Tests and Treatment

No significant correlation was found between the effect of ChAglyCD3 and any of the immunologic factors tested, including the CD4:CD8 ratio after treatment, the titer of islet-cell antibodies and antibodies to glutamic acid decarboxylase before or after treatment, and HLA class II haplotypes.

ADVERSE EVENTS

All patients who received ChAglyCD3 had transient adverse events, as shown in Table 3. These included fever (see Supplementary Appendix 4), headache, gastrointestinal symptoms, arthralgia, and myalgia. The administration of ChAglyCD3 was neither delayed nor stopped in any patient because of these symptoms.

Twenty-nine of the 40 patients treated with ChAglyCD3 and 2 of the 40 treated with placebo had a rash on the palms, the trunk, or both, starting 1 week after the last infusion and lasting 5 to 18 days ($P<0.001$).

Of the 40 patients treated with ChAglyCD3, 30 had a syndrome similar to acute mononucleosis, with sore throat, fever, cervical adenopathy, or all of these, starting between day 16 and day 21 after the first infusion and resolving within 7 to 12 days ($P<0.001$ for the comparison with placebo). In 21 of 22 patients in the ChAglyCD3 group for whom samples were available, a transient increase appeared in EBV DNA copies on testing with quantitative PCR ($P<0.001$). It is important to note that all values returned to pretreatment levels by week

6 to week 12. Samples were available for testing for EBV-specific antibody in 37 patients treated with ChAglyCD3. An increase in viral-capsid-antigen–specific IgG was detected in 35 of these patients ($P<0.001$), an increase in viral-capsid-antigen–specific IgM in 13 ($P=0.04$), and an increase in early-antigen–specific IgG in 16 ($P=0.02$); all antibody levels peaked by day 28 after the first infusion. In all 16 patients treated with ChAglyCD3 for whom samples were available, an increased proportion of EBV-specific CD8+ T cells was detected with the use of tetramers that presented HLA-A2–restricted peptides, HLA-B8–restricted peptides, or both, from the lytic-cycle proteins BMLF1, BMRF1, and BZLF1.[28,29] Peak levels were observed by day 14 after the first infusion, at the time of the CD8+ T-cell lymphocytosis.

No patient had a positive result on qualitative PCR for any of the other herpes viruses for which we tested. During longer follow-up periods (24 to 57 months; median, 35 months), none of the patients treated with ChAglyCD3 had lymphoma akin to post-transplantation lymphoproliferative disease or clinical or biologic symptoms related to a syndrome of this type. Patients in both groups will continue to be monitored for possible late-onset side effects of the antibody treatment. Few episodes of severe hypoglycemia were detected, with no difference between patients receiving placebo and those receiving ChAglyCD3 (two episodes over 18 months).

DISCUSSION

This study documents that further loss of residual beta-cell function occurs during the 18 months after a diagnosis of type 1 diabetes mellitus in patients 12 to 39 years of age. Beta-cell function was assessed through the release of C peptide during prolonged glucose stimulation, first in the absence of an exogenous glucagon stimulus and then in the presence of this stimulus. Glucagon significantly increased the release of C peptide (by 46 to 65 percent) at all time points, indicating that beta cells maintained a secretory response to glucagon despite the diabetic state and a prior two-hour exposure to frank hyperglycemia. Both indexes of beta-cell function progressively decreased after diagnosis, with an average reduction of 35 percent after 18 months. This reduction was accompanied by a 50 percent rise in mean daily insulin needs.

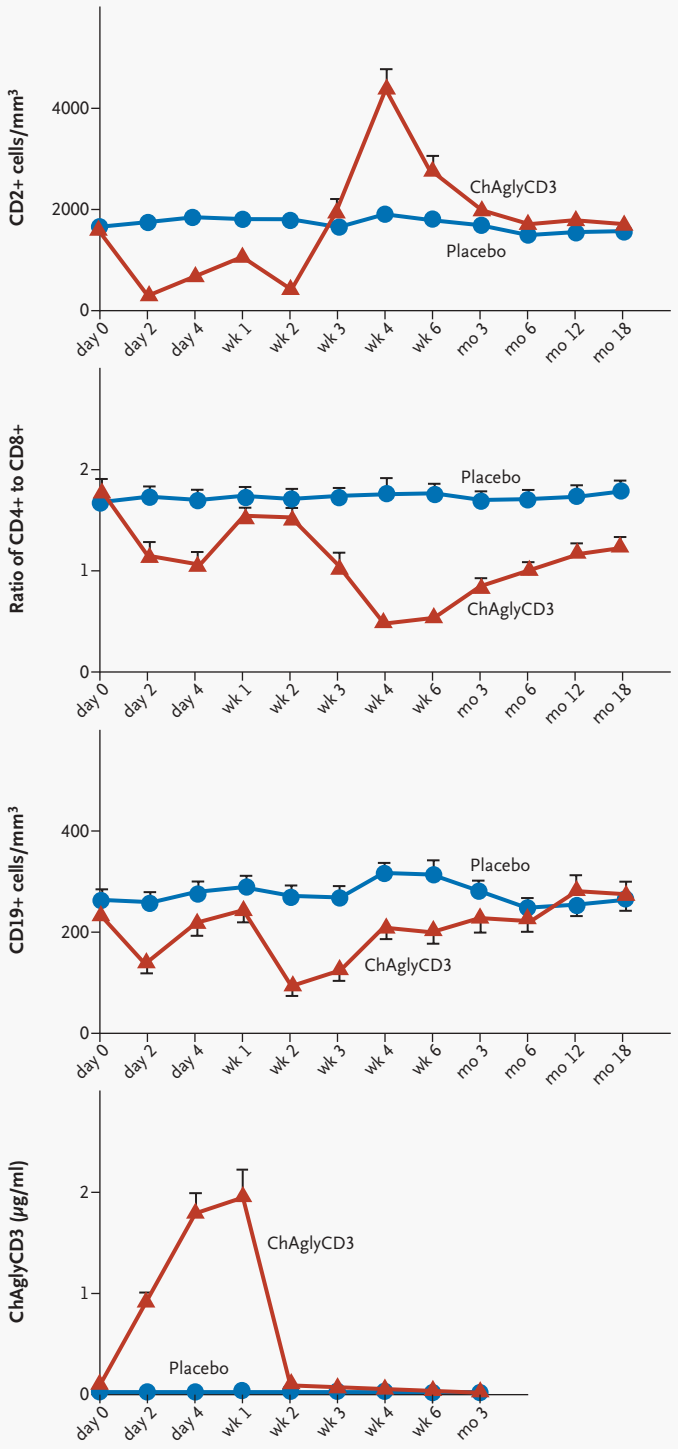

Figure 3. Immunologic Tests among Patients in the Two Groups.
All values are means ±SE. Circles represent the placebo group, and triangles the ChAglyCD3 group.

Table 3. Adverse Events among Patients in the Two Groups.*

Sign or Symptom	ChAglyCD3 (N=40)	Placebo (N=40)	P Value
	no. of patients		
During treatment			
Fever (>38.0°C)	38	1	<0.001
Headache	40	14	<0.001
Gastrointestinal symptoms	39	7	<0.001
Arthralgia	40	2	<0.001
Myalgia	35	8	<0.001
Onset at day 11–15			
Rash	29	2	<0.001
Onset at day 16–21			
Acute mononucleosis-like syndrome			
Sore throat	30	3	<0.001
Fever	13	1	0.001
Cervical adenopathy	10	3	0.07

* The cumulative dose of ChAglyCD3 was 48 mg in 35 patients, 64 mg in 4 patients, and 24 mg in 1 patient (treatment was discontinued after three 8-mg doses in this patient because of catheter sepsis).

The results of this trial indicate that short-term treatment with ChAglyCD3 ameliorates this reduction in residual beta-cell function. No rise in the daily insulin dose was observed, indicating that the short-term antibody treatment exerts a durable effect for at least 18 months. It is interesting to note that the protection induced by ChAglyCD3 was much less pronounced in patients with baseline residual beta-cell function that was lower than that for the 50th percentile of the study population; the insulin dose in these patients increased only marginally over 18 months if they were in the placebo group, and ChAglyCD3 treatment did not lead to a lower mean insulin dose. Patients with higher residual function at baseline (P50 or more of the study population) required less insulin initially, but in those receiving placebo, insulin requirements almost doubled during the first 18 months. This increase in the insulin requirement was associated with a 47 percent reduction in residual beta-cell function, indicating that patients with higher residual beta-cell function at diagnosis lose an important part of this function during subsequent months, probably as the result of a further decline in beta-cell mass.

Treatment with ChAglyCD3 prevented this loss in residual beta-cell function, and consequently, daily insulin needs remained stable over the 18-month follow-up period. At month 18, the majority of patients in the ChAglyCD3 group who had higher beta-cell function at baseline needed a daily insulin dose of 0.25 IU per kilogram per day or less, whereas this low dose was not sufficient to maintain glucose control in any of the patients in the placebo group. This subgroup also had the lowest glycosylated hemoglobin levels — 6.4 percent at month 18, a surrogate for good metabolic control. Thus, treatment with ChAglyCD3 confers a metabolic benefit that is most apparent in patients with higher residual beta-cell function. This effect might be explained by the administration of the antibody at an earlier stage of the disease. Our data extend the findings of an open-label trial with another humanized anti-CD3 antibody, hOKT3γ1(Ala-Ala), which showed positive results at 12 months.[16] Entry criteria in the present trial were more stringent, with a clear definition of metabolic state, which perhaps selected for a subgroup of patients who would respond to antibody treatment.

The therapeutic potential of ChAglyCD3 will depend on its safety. Symptoms observed after the first infusions were compatible with transient cytokine release. The transient rashes were similar to those previously described.[16] Clinical symptoms of infectious mononucleosis were observed in 75 percent of patients and coincided with transiently positive results on EBV-specific quantitative PCR. It is important to note that the values for EBV-specific PCR returned to baseline levels in all patients within 5 to 10 weeks after treatment; this was associated with the development of an antibody response and a CD8+ T-cell response specific for EBV antigens of the lytic cycle. This pattern differs from that observed in cases of EBV reactivation in patients with chronic immunosuppression, potentially leading to post-transplantation lymphoproliferative disease. Although a return to baseline values occurred in all patients who received ChAglyCD3, long-term follow-up will be important.

In conclusion, this placebo-controlled study demonstrates a sustained metabolic benefit from a short course of ChAglyCD3 in patients with recent-onset type 1 diabetes. The treatment appears to preserve residual beta-cell function, thereby preventing an increase in exogenous-insulin needs for at least 18 months. This effect appears to be most pronounced in persons with a higher initial residual beta-cell function. Treatment with ChAglyCD3 was

accompanied by significant, but seemingly transient, side effects, including fever after the start of infusions and rash and acute mononucleosis-like syndrome after the end of treatment.

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APPENDIX

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