Open access • Journal Article • DOI:10.1056/NEJMOA065213

Interleukin-1-receptor antagonist in type 2 diabetes mellitus. — Source link

Claus M. Larsen, Mirjam V. Faulenbach, Allan Vaag, Allan Vaag ...+6 more authors

Institutions: Steno Diabetes Center, University of Zurich, Lund University, Novo Nordisk ...+1 more institutions

Published on: 12 Apr 2007 - The New England Journal of Medicine (Massachusetts Medical Society)

Topics: Insulin resistance, Glycated hemoglobin, Glucose tolerance test, Insulin and Interleukin 1 receptor antagonist

Interleukin-1-receptor antagonist in type 2 diabetes mellitus

Larsen, C M; Faulenbach, M; Vaag, A; Vølund, A; Ehses, J A; Seifert, B; Mandrup-Poulsen, T; Donath, M Y

Interleukin-1-receptor antagonist in type 2 diabetes mellitus

Abstract

BACKGROUND: The expression of interleukin-1-receptor antagonist is reduced in pancreatic islets of patients with type 2 diabetes mellitus, and high glucose concentrations induce the production of interleukin-1beta in human pancreatic beta cells, leading to impaired insulin secretion, decreased cell proliferation, and apoptosis. METHODS: In this double-blind, parallel-group trial involving 70 patients with type 2 diabetes, we randomly assigned 34 patients to receive 100 mg of anakinra (a recombinant human interleukin-1-receptor antagonist) subcutaneously once daily for 13 weeks and 36 patients to receive placebo. At baseline and at 13 weeks, all patients underwent an oral glucose-tolerance test, followed by an intravenous bolus of 0.3 g of glucose per kilogram of body weight, 0.5 mg of glucagon, and 5 g of arginine. In addition, 35 patients underwent a hyperinsulinemic-euglycemic clamp study. The primary end point was a change in the level of glycated hemoglobin, and secondary end points were changes in beta-cell function, insulin sensitivity, and inflammatory markers. RESULTS: At 13 weeks, in the anakinra group, the glycated hemoglobin level was 0.46 percentage point lower than in the placebo group (P=0.03); C-peptide secretion was enhanced (P=0.05), and there were reductions in the ratio of proinsulin to insulin (P=0.005) and in levels of interleukin-6 (P<0.001) and C-reactive protein (P=0.002). Insulin resistance, insulin-regulated gene expression in skeletal muscle, serum adipokine levels, and the body-mass index were similar in the two study groups. Symptomatic hypoglycemia was not observed, and there were no apparent drug-related serious adverse events. CONCLUSIONS: The blockade of interleukin-1 with anakinra improved glycemia and beta-cell secretory function and reduced markers of systemic inflammation. (ClinicalTrials.gov number, NCT00303394 [ClinicalTrials.gov].).

ORIGINAL ARTICLE

Interleukin-1–Receptor Antagonist in Type 2 Diabetes Mellitus

Claus M. Larsen, M.D., Mirjam Faulenbach, M.D., Allan Vaag, M.D., Ph.D., Aage Vølund, M.Sc., Jan A. Ehses, Ph.D., Burkhardt Seifert, Ph.D., Thomas Mandrup-Poulsen, M.D., Ph.D., and Marc Y. Donath, M.D.

ABSTRACT

BACKGROUND

The expression of interleukin-1–receptor antagonist is reduced in pancreatic islets of patients with type 2 diabetes mellitus, and high glucose concentrations induce the production of interleukin-1β in human pancreatic beta cells, leading to impaired insulin secretion, decreased cell proliferation, and apoptosis.

METHODS

In this double-blind, parallel-group trial involving 70 patients with type 2 diabetes, we randomly assigned 34 patients to receive 100 mg of anakinra (a recombinant human interleukin-1–receptor antagonist) subcutaneously once daily for 13 weeks and 36 patients to receive placebo. At baseline and at 13 weeks, all patients underwent an oral glucose-tolerance test, followed by an intravenous bolus of 0.3 g of glucose per kilogram of body weight, 0.5 mg of glucagon, and 5 g of arginine. In addition, 35 patients underwent a hyperinsulinemic–euglycemic clamp study. The primary end point was a change in the level of glycated hemoglobin, and secondary end points were changes in beta-cell function, insulin sensitivity, and inflammatory markers.

RESULTS

At 13 weeks, in the anakinra group, the glycated hemoglobin level was 0.46 percentage point lower than in the placebo group ($P=0.03$); C-peptide secretion was enhanced ($P=0.05$), and there were reductions in the ratio of proinsulin to insulin ($P=0.005$) and in levels of interleukin-6 ($P<0.001$) and C-reactive protein ($P=0.002$). Insulin resistance, insulin-regulated gene expression in skeletal muscle, serum adipokine levels, and the body-mass index were similar in the two study groups. Symptomatic hypoglycemia was not observed, and there were no apparent drug-related serious adverse events.

CONCLUSIONS

The blockade of interleukin-1 with anakinra improved glycemia and beta-cell secretory function and reduced markers of systemic inflammation. (ClinicalTrials.gov number, NCT00303394.)

From the Steno Diabetes Center, Gentofte (C.M.L., A. Vaag, T.M.-P.), and Biostatistics, Novo Nordisk, Bagsvaerd (A. Vølund) — both in Denmark; the Clinic for Endocrinology and Diabetes, University Hospital Zurich and Center for Integrative Human Physiology (M.F., J.A.E., M.Y.D.), and the Department of Biostatistics, University of Zurich (B.S.) — both in Zurich; and the University of Lund, Lund (A. Vaag), and the Karolinska Institute, Stockholm (T.M.-P.) — both in Sweden. Address reprint requests to Dr. Donath at the Division of Endocrinology and Diabetes, University Hospital, CH-8091 Zurich, Switzerland, or at marc.donath@usz.ch.

Drs. Larsen, Faulenbach, Mandrup-Poulsen, and Donath contributed equally to this article.

N Engl J Med 2007;356:1517-26.

TYPE 2 DIABETES MELLITUS OCCURS WHEN beta-cell function fails to compensate for insulin resistance.[1,2] Beta-cell function progressively deteriorates with an increasing duration of diabetes,[3] partly because of beta-cell demise through apoptosis.[4-6]

Interleukin-1β, a proinflammatory cytokine[7] implicated as an effector molecule of inflammatory beta-cell destruction leading to type 1 diabetes,[8] inhibits the function and promotes the apoptosis of beta cells.[9] Beta cells producing interleukin-1β have been observed in pancreatic sections obtained from patients with type 2 diabetes.[10] Depending on culture conditions, high glucose levels increase beta-cell production and the release of interleukin-1β, followed by functional impairment and apoptosis.[10-13] These findings suggest that intra-islet production of inflammatory mediators has a role in the pathogenesis of type 2 diabetes and that interleukin-1β is a potential therapeutic target for preserving beta-cell mass and function in patients with this condition.

Interleukin-1–receptor antagonist, a naturally occurring competitive inhibitor of interleukin-1 binding to the type I receptor,[7,14] protects human beta cells from glucose-induced functional impairment and apoptosis.[10] Interleukin-1–receptor antagonist is reported to have no agonistic activity.[7,15] The expression of interleukin-1–receptor antagonist is decreased in beta cells obtained from patients with type 2 diabetes.[16] Given these observations, we hypothesized that intervening in the islet balance between interleukin-1–receptor antagonist and interleukin-1β might improve beta-cell function and glycemic control in patients with type 2 diabetes.

METHODS

STUDY DESIGN

This placebo-controlled, double-blind, parallel-group study involved 70 patients with type 2 diabetes at two centers (31 patients in Denmark and 39 patients in Switzerland) who were recruited from January 2004 to March 2005. Patients received either once-daily recombinant human interleukin-1–receptor antagonist (100 mg of anakinra [Kineret] donated by Amgen) or placebo by subcutaneous self-administration in the morning for 13 weeks. Patients continued their baseline antidiabetic therapy, dietary habits, and other lifestyle habits.

The County Pharmacy of Zurich was responsible for the blinding and randomization procedure; the latter was performed with the use of permuted blocks within the recruiting center. The authors designed the study, gathered and analyzed the data, wrote the manuscript, and vouch for the accuracy and completeness of the data and the analysis. There was no confidentiality agreement between the authors or their institutions and Amgen, which provided the study drugs.

PATIENTS

We conducted the study in accordance with the ethical guidelines of the Declaration of Helsinki II; the study design was approved by regional and institutional review boards. Written informed consent was obtained from all patients before randomization.

The inclusion criteria were an age of 20 years or more, type 2 diabetes diagnosed according to American Diabetes Association criteria[17] with a duration of more than 3 months, a body-mass index (BMI, the weight in kilograms divided by the square of the height in meters) of more than 27, and a glycated hemoglobin level of more than 7.5% (upper limit of the normal range, 6.4%). Eligible patients had had no changes in either types or doses of medications during the 3-month period preceding the study.

The exclusion criteria were the presence of autoantibodies to glutamic acid decarboxylase 65 or islet-cell autoantibody-2; a glycated hemoglobin level of more than 12%; a fasting C-peptide level of less than 400 pmol per liter; current treatment with antiinflammatory drugs, including corticosteroids and nonsteroidal antiinflammatory drugs (100 mg or less of aspirin per day was allowed); signs of current infection, including a C-reactive protein level of more than 30 mg per liter, fever, current treatment with antibiotics, chronic granulomatous infections (e.g., a previous diagnosis of tuberculosis or a current diagnosis based on chest radiography or a Mantoux test); a history of recurrent infection or a predisposition to infection; neutropenia (a leukocyte count of less than 2000 per cubic millimeter) or anemia (a hemoglobin level of less than 11 g per deciliter for men and less than 10 g per deciliter for women); pregnancy or breast-feeding (contraception for at least 3 months before inclusion was required for fertile women); liver or renal disease (a level of aspartate aminotransferase or alanine aminotransferase of more than three times the upper

limit of the normal range and a serum creatinine level of more than 130 μmol per liter); ongoing or previous cancer; the use of any other investigational drug within 30 days before enrollment or within five half-lives of the medication used in the other study, whichever was the longer period; and immunosuppressive treatment or immunodeficiency.

STUDY PROCEDURES

At baseline and 13 weeks, a 2-hour oral glucose-tolerance test was performed with 75 g of glucose, followed by measurements of plasma glucose, proinsulin, insulin, and C-peptide 10 minutes before and during the test and measurements of plasma glucose, insulin, and C-peptide 30, 60, 90, and 120 minutes after the test. Immediately after the end of the oral glucose-tolerance test, an intravenous injection of 0.3 g of glucose per kilogram of body weight, 0.5 mg of glucagon, and 5 g of arginine was administered. Blood was then sampled at 0, 3, 6, 9, and 12 minutes. The patients were asked to forgo their antidiabetic medication and to fast for 8 to 9 hours before the test.

At baseline and 13 weeks, euglycemic–hyperinsulinemic clamp studies[18] and muscle biopsies were performed. All patients were asked to participate in the clamp and muscle-biopsy studies, although participation in these studies was not a requirement for remaining in the overall study. The patients were required to forgo antidiabetic treatment for 8 to 9 hours and to avoid strenuous physical exercise for 24 hours before the clamp study. The clamp procedure was performed 2 to 7 days after the oral glucose-tolerance test. Indirect calorimetry was performed during the insulin-stimulated 30-minute steady-state period. The insulin-sensitivity index during the clamp procedure was calculated by dividing the M value (the glucose infusion rate during the steady-state period divided by the total body weight) by the steady-state plasma insulin level.

Biopsy specimens were obtained from the vastus lateralis muscle immediately after the administration of the intravenous bolus at the end of the oral glucose-tolerance test. Messenger RNA (mRNA) from glucose transporter 4 (GLUT4) and peroxisome-proliferator–activated receptor γ coactivator 1α (PGC-1α)[19,20] was quantified as described in the Supplementary Appendix (available with the full text of this article at www.nejm.org).

Levels of C peptide, insulin, and proinsulin were determined centrally at the Steno Diabetes Center. Insulin and proinsulin were assessed by enzyme-linked immunosorbent assays,[21,22] and C-peptide levels were determined by a time-resolved fluoroimmunoassay.[23] Measurements of glycated hemoglobin levels and routine clinical laboratory tests were performed in the central laboratory units of the two participating centers. For glycated hemoglobin, 22 coded samples from patients were tested at both laboratories. Regression analysis showed an r^2 value of 0.97 for agreement between the test results ($P<0.001$). Serum adipokines were assayed with the use of Luminex technology (Millipore) according to the manufacturer's instructions.

A physical examination and blood tests were performed at baseline and at 4 and 13 weeks. Fasting plasma glucose levels were measured weekly at home by the patients. At baseline and 13 weeks, funduscopy was performed, and urinary albumin excretion, creatinine clearance, and (in women) human chorionic gonadotropin were measured.

STUDY END POINTS

The predefined primary end point was the change in the glycated hemoglobin level between baseline and 13 weeks. Predefined secondary end points included a change by week 13 in the area under the concentration–time curve (AUC) for stimulated C-peptide during an oral glucose-tolerance test and after intravenous stimulation, a change in the ratio of fasting proinsulin to insulin, a change in the insulin-sensitivity index derived from the oral glucose-tolerance test,[24] a change in serum adipokine levels, a change in insulin sensitivity as assessed by a euglycemic–hyperinsulinemic clamp study (in the 35 patients who provided consent for this procedure), a change in insulin-regulated genes in biopsy specimens obtained from skeletal muscle, and changes in levels of fasting plasma glucose, interleukin-6, and C-reactive protein as markers of systemic inflammation.

STATISTICAL ANALYSIS

No interim analyses were carried out in this study. Data from all patients were analyzed. Values are expressed as means (±SD) unless otherwise specified. Differences were tested with the use of an unpaired t-test and with a Wilcoxon rank-sum test in the case of non-normal distribution. For categorical end points, Pearson's chi-square test was used. Correlations were performed by regression

Figure 1. Enrollment and Outcomes.

A total of 312 patients with type 2 diabetes mellitus were recruited either in the outpatient clinics of the two participating centers or through newspaper advertisements. Of those, 188 patients did not respond, were not interested, or were not eligible on the basis of their medical history. Of the 124 patients who underwent physical and biochemical screening, 54 were found to be ineligible according to entry criteria, in most cases because they had either a glycated hemoglobin level of less than 7.5% or a fasting C-peptide level of less than 400 pmol per liter. Seventy patients were randomly assigned to receive either anakinra or placebo. In the placebo group, one patient was excluded before starting study medication and two patients withdrew after 4 and 5 weeks of treatment. A total of 67 patients completed the 13-week treatment and evaluation of study end points.

analysis. To analyze the effect of baseline characteristics on the primary end point, bivariate regression analyses including the treatment effect were performed. A P value of less than 0.05 was considered to indicate statistical significance. All reported P values are two-sided and have not been adjusted for multiple testing.

RESULTS

BASELINE CHARACTERISTICS AND ADHERENCE

Seventy of the 124 subjects who underwent initial screening were randomly assigned to receive either anakinra or placebo (Fig. 1). In the placebo group, a late positive Mantoux reaction developed in one patient, suggesting a reactivation of tuberculosis, and the patient was excluded before starting study medication. Table 1 shows the baseline characteristics of the two study groups after randomization.

All 34 patients receiving anakinra completed the study, as did 33 of 35 patients receiving placebo. In the placebo group, a phlegmonous foot infection developed in one patient after 4 weeks, and one patient identified his study-group assignment by performing a biochemical analysis of the study drug after 5 weeks. Two patients in the anakinra group and one in the placebo group reduced the dose of their antidiabetic medication during the intervention because of improvement in glycemia but were not excluded from the study, according to the intention-to-treat principle. The remaining patients did not change their antidiabetic therapy during the 13-week study period, as reflected by recorded types and doses of antidiabetic medication at baseline and at 4 and 13 weeks (data not shown). Thirty-five subjects (16 in the anakinra group and 19 in the placebo group) gave informed consent to participate in the clamp and muscle-biopsy studies. The patients who provided consent did not differ in their baseline characteristics from those who declined (data not shown). The peak serum levels of interleukin-1–receptor antagonist, measured 1 to 2 hours after the last injection of anakinra or placebo at 13 weeks, were 1256±958 μg per liter in the anakinra group and 0.6±0.4 μg per liter in the placebo group ($P<0.001$).

PRIMARY END POINT

The average absolute difference in glycated hemoglobin levels between baseline and 13 weeks was a reduction of 0.33 percentage point (from 8.69±0.17 to 8.37±0.21) in the anakinra group and an increase of 0.13 percentage point (from 8.23±0.28 to 8.37±0.46) in the placebo group, yielding a between-group difference of 0.46 percentage point (95% confidence interval [CI], 0.01 to 0.90; $P=0.03$) (Fig. 2A). The number of patients who had any reduction in glycated hemoglobin levels

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Anakinra (N=34)	Placebo (N=35)	P Value
Age (yr)	60.6±8.7	60.3±8.6	0.89
Sex (no.)			
Male	23	27	
Female	11	8	0.39
Body-mass index	31.5±5.2	31.8±4.4	0.82
Weight (kg)	94.5±16.7	97.0±9.0	0.43
Glycated hemoglobin (%)	8.7±1.0	8.2±0.9	0.05
Fasting plasma glucose (mmol/liter)	10.8±2.7	10.0±3.1	0.24
Fasting C-peptide (pmol/liter)	958±312	907±508	0.62
Diabetes duration (yr)	10.6±6.2	11.0±7.3	0.82
Smoking status (%)			
Current	15	17	
Former	41	33	0.79
Cholesterol (mmol/liter)			
Total	4.65±1.12	4.86±1.42	0.50
HDL	1.35±1.27	1.09±0.33	0.25
Creatinine (μmol/liter)	91.5±21.5	94.3±21.9	0.60
Blood pressure (mm Hg)			
Systolic	145.8±15.9	146.4±18.7	0.89
Diastolic	83.4±7.9	82.7±8.2	0.75
Use of antihypertensive medication (%)	71	74	0.73
History of cardiovascular disease (%)	47	23	0.04
Albumin excretion rate >30 mg/liter/24 hr (%)	33	32	0.93
Retinopathy (%)	21	31	0.31
Antidiabetic treatment (%)			0.53
Diet	9	6	
Oral drug	53	43	
Insulin (alone or with oral drug)	38	51	

* Plus–minus values are means ±SD. The body-mass index is the weight in kilograms divided by the square of the height in meters. To convert cholesterol values to milligrams per deciliter, divide by 0.02586. To convert creatinine values to milligrams per deciliter, divide by 88.4. HDL denotes high-density lipoprotein.

at 13 weeks was 21 of 34 patients in the anakinra group, as compared with 10 of 33 patients in the placebo group (P<0.001). Glycated hemoglobin levels were significantly lower in the anakinra group after 4 weeks than in the placebo group (an absolute reduction of 0.36%; 95% CI, 0.11 to 0.60; P=0.004) (Fig. 2A). When the patients were stratified into three equal groups according to body-surface area as a surrogate for drug distribution volume, the difference in glycated hemoglobin levels in the anakinra group was a reduction of 0.84% in the lowest third (<2.00 m^2), a reduction of 0.30% in the intermediate group (2.00 to 2.24 m^2), and an increase of 0.11% in the highest third (>2.24 m^2) (P=0.02 for all comparisons) (Fig. 2C). Accordingly, body-surface area correlated with the changes in glycated hemoglobin levels in the anakinra group (r^2=0.22, P=0.007) but not in the placebo group (r^2=0.001, P=0.87).

SECONDARY END POINTS

Glycemia

Fasting plasma glucose levels that were measured by the patients at home once weekly were consistently lower in the anakinra group than in the placebo group (Fig. 2B). At 13 weeks, plasma glu-

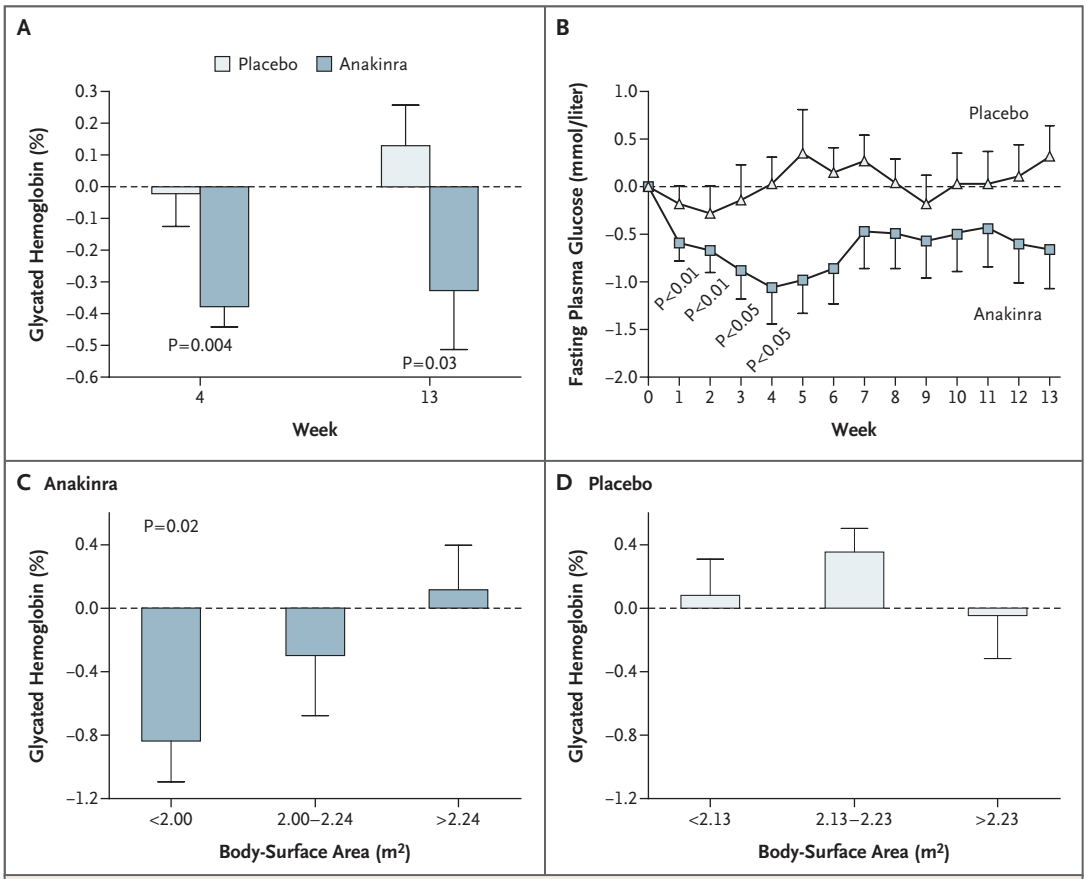

Figure 2. Changes in Glycated Hemoglobin and Fasting Plasma Glucose Levels during the 13-Week Study Period.

Panel A shows the average absolute difference in glycated hemoglobin levels between baseline and 4 and 13 weeks in each study group. Panel B shows the average absolute differences in fasting plasma glucose levels between the baseline value and weekly measurements performed by the patients at home during the 13-week study period. Panels C and D show the average absolute differences in glycated hemoglobin levels at 13 weeks in each study group stratified into thirds according to body-surface area. In the placebo group, the analysis included 35 patients at week 4, 34 at week 5, and 33 at week 13. In the anakinra group, the analysis included 34 patients at each weekly measure. The I bars indicate standard errors.

cose levels at the beginning and end of a 2-hour oral glucose-tolerance test were reduced by 0.6 mM (95% CI, 0.05 to 1.60; P=0.29) and 1.3 mM (95% CI, 0.00 to 2.6; P=0.05), respectively, in the anakinra group, as compared with the placebo group.

Beta-Cell Secretory Function

At 13 weeks, beta-cell function increased in the anakinra group and decreased in the placebo group (Fig. 3). In particular, the ratio of proinsulin to insulin was markedly lower in the anakinra group (P=0.005). The changes in beta-cell function assessed on the basis of the AUC from the oral glucose-tolerance test and intravenous tests were significantly correlated (P<0.001) (data not shown). The change in beta-cell function was correlated with changes in glycated hemoglobin levels in patients in the anakinra group but not in the placebo group. The correlation between the change in glycated hemoglobin levels and the AUC for stimulated C-peptide during the oral glucose-tolerance test was $r^2=0.13$ (P=0.04) in the anakinra group and $r^2=0.04$ (P=0.76) in the placebo group; the correlation for intravenous stimulation was $r^2=0.32$ (P<0.001) in the anakinra group and $r^2=0.03$ (P=0.29) in the placebo group; and the correlation for the two tests combined was $r^2=0.18$ (P=0.02) in the anakinra group and $r^2=0.01$ (P=0.59) in

the placebo group. Baseline glycated hemoglobin values did not correlate with the beta-cell secretion tests in patients in the anakinra group. The correlation between the starting glycated hemoglobin level and the AUC for stimulated C-peptide during the oral glucose-tolerance test was $r^2=0.05$ ($P=0.22$), the correlation for intravenous stimulation was $r^2=0.09$ ($P=0.10$), and the correlation for both tests combined was $r^2=0.06$ ($P=0.17$).

Insulin Sensitivity

With the dose of anakinra used in this study, no differences in insulin sensitivity were found at 13 weeks. The M value divided by the plasma insulin level during the steady-state period in the euglycemic–hyperinsulinemic clamp study was reduced by 0.13±1.76 μg of glucose per kilogram of body weight per picomole of insulin per minute in the anakinra group and by 0.56±2.52 μg of glucose per kilogram of body weight per picomole of insulin per minute in the placebo group ($P=0.58$). The insulin-sensitivity index, as calculated by the homeostasis model assessment (HOMA) on the basis of the oral glucose-tolerance test in all patients, increased by 0.003±0.179 square liter per milliunit of insulin per millimole of glucose in the anakinra group and by 0.029±0.228 square liter per milliunit of insulin per millimole of glucose in the placebo group ($P=0.60$). The insulin-sensitivity index on the basis of data from the oral glucose-tolerance test strongly correlated with the clamp data ($r^2=0.53$, $P<0.001$). There was no correlation between insulin sensitivity as determined by the clamp method and the primary end point at 13 weeks ($r^2=0.005$, $P=0.60$) and no change in oxidation rates of glucose, with an increase of 0.02±0.7 mg of oxidized glucose per kilogram per minute in the anakinra group and a decrease of 0.15±0.37 mg per kilogram per minute in the placebo group ($P=0.44$) or of fat, with a decrease of 0.05±0.26 mg per kilogram per minute in the anakinra group and an increase of 0.03±0.2 mg per kilogram per minute in the placebo group ($P=0.36$).

Markers of Systemic Inflammation

Levels of C-reactive protein were significantly lower after 4 and 13 weeks in the anakinra group than in the placebo group ($P=0.02$ after 4 weeks and $P=0.002$ after 13 weeks) (Fig. 4A). Similar declines in interleukin-6 levels were observed ($P<0.001$ after both 4 and 13 weeks) (Fig. 4B). Neither baseline values nor changes in levels of C-reactive protein or interleukin-6 were significantly correlated with changes in glycated hemoglobin in the anakinra group: $r^2<0.001$ ($P=0.89$) for baseline C-reactive protein levels, $r^2<0.001$ ($P=0.95$) for changes in C-reactive protein, $r^2=0.005$ ($P=0.70$) for baseline interleukin-6, and $r^2=0.001$ ($P=0.86$) for changes in interleukin-6. As previously observed,[25] neutrophil and platelet counts were slightly and reversibly decreased during anakinra therapy. Neutrophil counts decreased by 1200±1400

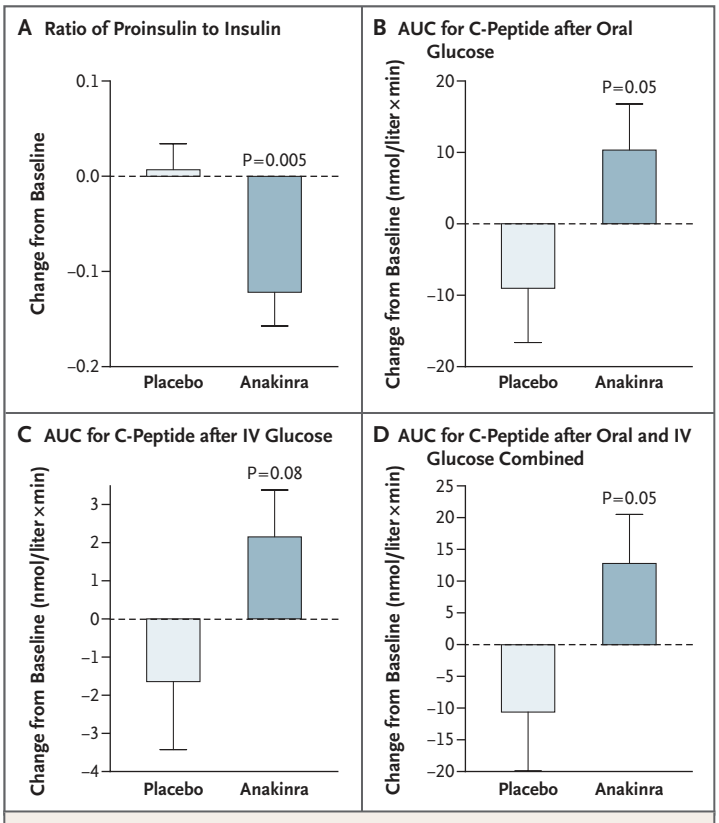

Figure 3. Beta-Cell Secretory Function.

Beta-cell secretory function was assessed by analysis of the ratio of proinsulin to insulin and by a 2-hour oral glucose-tolerance test, followed by intravenous stimulation with 0.3 g of glucose per kilogram of body weight, 0.5 mg of glucagon, and 5 g of arginine. Average absolute differences between baseline and 13 weeks in each study group are shown, including the change in the ratio of proinsulin to insulin (Panel A), the change in the area under the concentration–time curve (AUC) for C-peptide in response to a 2-hour oral glucose-tolerance test (Panel B), the change in the AUC for C-peptide in response to an intravenous (IV) stimulation test (Panel C), and the change in the AUC for the combination of oral and intravenous stimulation (Panel D). The analysis included 33 patients in the placebo group and 34 in the anakinra group. The I bars indicate standard errors.

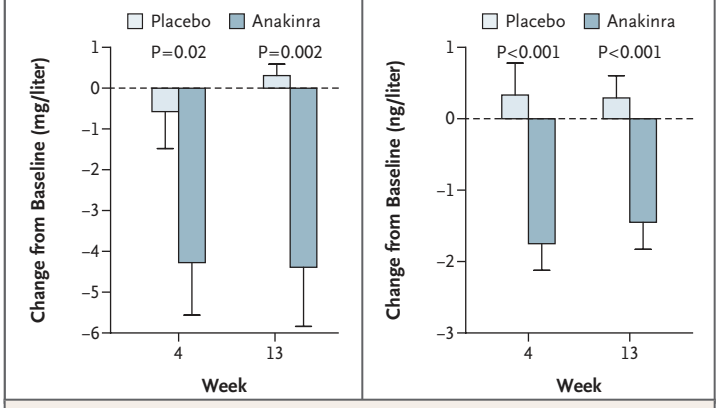

Figure 4. Markers of Systemic Inflammation.

The average absolute differences between baseline and 4 and 13 weeks in each study group are shown for levels of circulating C-reactive protein (Panel A) and interleukin-6 (Panel B). In the placebo group, the analysis included 35 patients at week 4 and 33 at week 13. In the anakinra group, the analysis included 34 patients at weeks 4 and 13. The I bars indicate standard errors.

per cubic millimeter in the anakinra group and by 100±1300 per cubic millimeter in the placebo group; platelet counts decreased by 16,000±25,400 per cubic millimeter in the anakinra group and increased by 3000±23,000 per cubic millimeter in the placebo group (P<0.001).

Insulin-Regulated Gene Expression

Treatment with anakinra for 13 weeks did not significantly change the mRNA expression levels of the insulin-regulated genes *GLUT4* (P=0.81) and *PGC-1α* (P=0.26) in biopsy specimens of skeletal muscle, with decreases of 0.23±1.43 for the relative expression levels of GLUT4 and 0.12±0.87 for PGC-1α normalized to the housekeeping gene cyclophilin A in the anakinra group, as compared with decreases of 0.34±0.72 in the expression levels of GLUT4 and 0.52±0.79 for PGC-1α in the placebo group.

Serum Adipokines

Levels of serum adipokines were not significantly changed at 13 weeks in either the anakinra group or the placebo group. The levels changed as follows for the comparison between the study groups: leptin, a decrease of 0.40±6.86 ng per milliliter in the anakinra group and an increase of 2.81±10.98 ng per milliliter in the placebo group (P=0.16); adiponectin, an increase of 3.69±11.36 μg per milliliter in the anakinra group and a decrease of 1.00±8.59 μg per milliliter in the placebo group (P=0.07); resistin, a decrease of 2.80±8.56 ng per milliliter in the anakinra group and an increase of 0.27±7.65 ng per milliliter in the placebo group (P=0.14); tumor necrosis factor α, a decrease of 0.13±1.44 pg per milliliter in the anakinra group and an increase of 0.11±1.56 pg per milliliter in the placebo group (P=0.52); monocyte chemotactic factor 1, an increase of 9.2±50.8 pg per milliliter in the anakinra group and a decrease of 3.3±93.0 pg per milliliter in the placebo group (P=0.50); and interleukin-8, a decrease of 0.81±4.55 pg per milliliter in the anakinra group and a decrease of 0.08±1.17 pg per milliliter in the placebo group (P=0.40).

OTHER OUTCOMES

The baseline body weight and BMI were significantly correlated with changes in glycated hemoglobin levels (covariate effect, P=0.003 and P=0.004, respectively; treatment effect, P=0.08 and P=0.05, respectively; and combined effect, P=0.001 and P=0.002, respectively). This correlation was not observed for the other baseline variables.

ADVERSE EVENTS

There were no significant changes in BMI in either group from baseline to 13 weeks, with levels changing from 31.5±5.2 to 31.8±5.6 in the anakinra group (P=0.82) and 31.8±4.4 to 32.0±4.4 in the placebo group (P=0.85) (P=0.38 for the comparison between study groups). No patient withdrew from the study because of drug-related adverse events. In particular, symptomatic hypoglycemia was not observed, even in patients with a marked improvement in glycated hemoglobin levels. All reported adverse events are listed in Table 2. In the anakinra group, the improvement in glycated hemoglobin levels did not differ between patients who had injection-site reactions and those who did not. There was a decrease of 0.24±0.18% in the glycated hemoglobin level in patients with injection-site reactions and a decrease of 0.64±0.32% in those without injection-site reactions (P=0.54). No changes were observed in blood pressure or heart rate or in levels of serum sodium, potassium, aspartate aminotransferase, alanine aminotransferase, lipids (including free fatty acids, total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides), cystatin C, or creatinine or in creatinine clearance, 24-hour urinary albumin excretion, or retinal fundus. Total hemoglo-

bin was unchanged, a finding that made changes in erythrocyte turnover unlikely as a confounder.

DISCUSSION

Our study shows that antagonism of interleukin-1 signaling with anakinra improved glycemic control in patients with type 2 diabetes, most likely through enhanced beta-cell secretory function. Indeed, improved glycemia in patients who received anakinra correlated with improved measures of beta-cell secretory capacity. There were no alterations in insulin sensitivity on the basis of insulin clamp studies, insulin sensitivity indexes modeled on the oral glucose-tolerance test, insulin-regulated gene expression in skeletal muscle, or serum adipokine levels. Finally, the BMIs of patients remained stable, thus excluding an anorexigenic effect of anakinra. However, we cannot exclude the possibility that higher doses of anakinra might improve insulin sensitivity.

Neither baseline levels of C-reactive protein or interleukin-6 nor changes in the levels correlated with changes in glycated hemoglobin levels, suggesting that reduced systemic inflammation did not play an important part in improved insulin secretion. Genetic ablation of systemic interleukin-1 action causes obesity.[26,27] It has therefore been proposed that interleukin-1 regulates body composition and fat distribution, mainly through the regulation of feeding behavior, satiety regulation, and energy metabolism, including thermogenesis. The integral physiological measure of these actions is body weight. In our study, the inhibition of the action of interleukin-1 by anakinra did not increase body weight.

Limitations of our study include its short duration and the lack of dose finding. Considering the short half-life of anakinra (6 to 8 hours), it is possible that higher doses or longer-lasting antagonism of interleukin-1 might improve the outcome.[28] The association between body weight or body-surface area at baseline and the glycemic outcome suggests that the anakinra dose might be increased according to the drug-distribution volume.

Apart from self-limited local reactions at the injection site, we observed no clear difference in the frequency of adverse events between the anakinra group and the placebo group. No patient stopped treatment because of adverse reactions. Symptomatic hypoglycemia was not reported by any patient. A potential concern with the use of interleukin-1 blockade is the inhibition of innate immunity and the occurrence of infection. However, in more than 100,000 patients with rheumatoid arthritis who underwent long-term treatment with anakinra, there was no significant increase in the incidence of infectious disease, despite concomitant immunosuppression.[28]

In summary, our study suggests that antagonism of interleukin-1 has possible therapeutic potential in the treatment of type 2 diabetes. Further studies are needed to test higher doses of anakinra, to evaluate its long-term use, and to test interleukin-1 antagonists that have a prolonged half-life, with the aim of preventing beta-cell destruction and promoting beta-cell regeneration in type 2 diabetes.

Table 2. Adverse Events.

Event	Anakinra (N=34)	Placebo (N=35)
	no. of patients	
Transient injection-site reactions*	17	0
Minor injection-site hematoma	1	0
Mild nausea	1	0
Transient peripheral facial-nerve palsy (day 40)	1	0
Urinary tract infection (days 26–28)	1	0
Upper respiratory tract infection (days 41–45)	1	0
Transient mild elevation of aspartate aminotransferase (day 87)	1	0
Dry cough	0	1
Peroneal-nerve palsy	0	1
Calf pain	0	1
Phlegmonous foot infection	0	1

* The reactions were first seen between days 6 and 14 and disappeared between days 19 and 38.

Supported by the Bonizzi-Theler and Hartmann-Müller Foundations, the Center of Clinical Studies at the University of Zurich, the Swiss National Science Foundation, the European Union, and Novo Nordisk. Amgen provided the study drugs at no cost.

Dr. Donath is listed as the inventor on a patent (WO6709) filed in 2003 for the use of an interleukin-1–receptor antagonist for the treatment of or prophylaxis against type 2 diabetes. The patent is owned by the University of Zurich, and Dr. Donath has no financial interest in the patent. Dr. Vølund reports being an employee of Novo Nordisk. Drs. Larsen, Vaag, Vølund, and Mandrup-Poulsen report having an equity interest in Novo Nordisk. Drs. Vaag and Mandrup-Poulsen report receiving grant support from Novo Nordisk. No other potential conflict of interest relevant to this article was reported.

We thank Erol Cerasi and Steven Kahn for their advice and critical reading of the manuscript; study nurses Erika Wettstein and Regula Messmer; laboratory technicians Hanne Foght, Helle Niebling, Charlotte Leth, Marianne Modest, and Margaretha Siegfried-Kellenberger; and study administrators Karen Kruse and Michèle Rothfuchs.

REFERENCES

1. Cerasi E, Luft R. Insulin response to glucose infusion in diabetic and non-diabetic monozygotic twin pairs: genetic control of insulin response? Acta Endocrinol (Copenh) 1967;55:330-45.
2. DeFronzo RA. Lilly Lecture 1987. The triumvirate: beta-cell, muscle, liver — a collusion responsible for NIDDM. Diabetes 1988;37:667-87.
3. U.K. Prospective Diabetes Study Group. U.K. Prospective Diabetes Study 16 — overview of 6 years' therapy of type II diabetes: a progressive disease. Diabetes 1995;44:1249-58. [Erratum, Diabetes 1996; 45:1655.]
4. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003;52:102-10.
5. Hellerstrom C. The life story of the pancreatic B cell. Diabetologia 1984;26: 393-400.
6. Donath MY, Halban PA. Decreased beta-cell mass in diabetes: significance, mechanisms and therapeutic implications. Diabetologia 2004;47:581-9.
7. Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996;87:2095-147.
8. Mandrup-Poulsen T. The role of interleukin-1 in the pathogenesis of IDDM. Diabetologia 1996;39:1005-29.
9. Bendtzen K, Mandrup-Poulsen T, Nerup J, Nielsen JH, Dinarello CA, Svenson M. Cytotoxicity of human pI 7 interleukin-1 for pancreatic islets of Langerhans. Science 1986;232:1545-7.
10. Maedler K, Sergeev P, Ris F, et al. Glucose-induced beta-cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. J Clin Invest 2002;110:851-60.
11. Maedler K, Spinas GA, Lehmann R, et al. Glucose induces beta-cell apoptosis via upregulation of the Fas-receptor in human islets. Diabetes 2001;50:1683-90.
12. Maedler K, Storling J, Sturis J, et al. Glucose- and interleukin-1beta-induced beta-cell apoptosis requires Ca2+ influx and extracellular signal-regulated kinase (ERK) 1/2 activation and is prevented by a sulfonylurea receptor 1/inwardly rectifying K+ channel 6.2 (SUR/Kir6.2) selective potassium channel opener in human islets. Diabetes 2004;53:1706-13.
13. Welsh N, Cnop M, Kharroubi I, et al. Is there a role for locally produced interleukin-1 in the deleterious effects of high glucose or the type 2 diabetes milieu to human pancreatic islets? Diabetes 2005;54: 3238-44.
14. Dinarello CA. The role of the interleukin-1–receptor antagonist in blocking inflammation mediated by interleukin-1. N Engl J Med 2000;343:732-4.
15. Zumsteg U, Reimers JI, Pociot F, et al. Differential interleukin-1 receptor antagonism on pancreatic beta and alpha cells: studies in rodent and human islets and in normal rats. Diabetologia 1993;36:759-66.
16. Maedler K, Sergeev P, Ehses JA, et al. Leptin modulates beta cell expression of IL-1 receptor antagonist and release of IL-1beta in human islets. Proc Natl Acad Sci U S A 2004;101:8138-43.
17. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2006;29:Suppl 1:S43-S48.
18. Vaag A, Alford F, Henriksen FL, Christopher M, Beck-Nielsen H. Multiple defects of both hepatic and peripheral intracellular glucose processing contribute to the hyperglycaemia of NIDDM. Diabetologia 1995;38:326-36.
19. Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003;34:267-73.
20. Ling C, Poulsen P, Carlsson E, et al. Multiple environmental and genetic factors influence skeletal muscle PGC-1alpha and PGC-1beta gene expression in twins. J Clin Invest 2004;114:1518-26.
21. Andersen L, Dinesen B, Jorgensen PN, Poulsen F, Roder ME. Enzyme immunoassay for intact human insulin in serum or plasma. Clin Chem 1993;39:578-82.
22. Hartling SG, Dinesen B, Kappelgard AM, Faber OK, Binder C. ELISA for human proinsulin. Clin Chim Acta 1986;156: 289-97.
23. Heding LG. Radioimmunological determination of human C-peptide in serum. Diabetologia 1975;11:541-8.
24. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487-95.
25. Goldbach-Mansky R, Dailey NJ, Canna SW, et al. Neonatal-onset multisystem inflammatory disease responsive to interleukin-1β inhibition. N Engl J Med 2006; 355:581-92.
26. Garcia MC, Wernstedt I, Berndtsson A, et al. Mature-onset obesity in interleukin-1 receptor I knockout mice. Diabetes 2006;55:1205-13.
27. Chida D, Osaka T, Hashimoto O, Iwakura Y. Combined interleukin-6 and interleukin-1 deficiency causes obesity in young mice. Diabetes 2006;55:971-7.
28. Dinarello CA. The many worlds of reducing interleukin-1. Arthritis Rheum 2005;52:1960-7.