Heterogeneity of Patients With Latent Autoimmune Diabetes in Adults: Linkage to Autoimmunity Is Apparent Only in Those With Perceived Need for Insulin Treatment

Results from the Nord-Trøndelag Health (HUNT) study

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OBJECTIVE — Subjects with the diagnosis of latent autoimmune diabetes in adults (LADA) are more prone to need insulin treatment than those with type 2 diabetes. However, not all patients with LADA develop the need for insulin treatment, indicating the heterogeneity of LADA. We investigated this heterogeneity by comparing phenotypes of LADA with and without perceived need for insulin treatment (data obtained at times when diagnosis of LADA was not investigated) and also compared LADA and type 2 diabetes phenotypes.

RESEARCH DESIGN AND METHODS — We used data from the all population–based Nord-Trøndelag Health study ($n = 64,931$), performed in 1995–1997. Data were assembled for individuals with LADA ($n = 106$) and type 2 diabetes ($n = 943$).

RESULTS — In the comparison of individuals with LADA both with and without the need for insulin, insulin-treated subjects had higher titers of GAD antibodies ($P < 0.001$) and lower fasting C-peptide levels ($P < 0.001$). GAD antibodies and C-peptide correlated negatively ($r = -0.40$; $P = 0.009$). In the comparison of individuals with LADA and type 2 diabetes, all without the need for insulin, markers of metabolic syndrome were equally prevalent and pronounced. Age, C-peptide, and glucose levels were also similar. In the comparison of insulin-treated individuals with LADA and type 2 diabetes, more patients with LADA received insulin (40 vs. 22%, $P < 0.001$) and C-peptide levels were lower ($P < 0.001$). Patients with LADA were leaner but were still overweight (mean BMI 28.7 vs. 30.9 kg/m^2 in type 2 diabetes, $P < 0.001$). In the comparison of type 2 diabetic patients with and without insulin, insulin-treated subjects were more obese and had higher A1C and lower C-peptide levels ($P < 0.001$).

CONCLUSIONS — Our conclusions are that 1) the need for insulin treatment in LADA is linked to the degree of autoimmunity and β-cell failure, 2) subjects with LADA and type 2 diabetes without the need for insulin treatment are phenotypically similar, and 3) insulin treatment in type 2 diabetic patients is associated with both insulin resistance and β-cell insufficiency.

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A utoimmunity is the major cause of type 1 diabetes. Autoimmunity is also assumed to be the major cause of latent autoimmune diabetes in adults (LADA) because this category of diabetes shares biochemical markers of β-cell–directed autoimmunity with "classic" type 1 diabetes. In clinical practice, autoimmunity in classic type 1 diabetes as well as in LADA is usually documented by antibodies against GAD.

LADA is considered a "mild" form of type 1 diabetes. Mild indicates the fact that patients with LADA do not by clinical judgment need insulin from the time of diagnosis. However, within the first few years after the diagnosis of diabetes a need for insulin treatment develops in many patients with LADA. This distinguishes patients with LADA from those with non-autoimmune type 2 diabetes in whom a need for insulin treatment develops later than in those with LADA (1). An early need for insulin treatment in patients with LADA points to ongoing autoimmune-mediated destruction of β-cells in these patients.

Risk factors for type 2 diabetes, such as age, obesity, and lack of physical activity, are associated with the development of LADA (2). In fact, odds ratios in terms of risk for diabetes for these factors of insulin resistance were the same for LADA and type 2 diabetes. This raises the question of the relative importance of autoimmunity vis-à-vis insulin resistance for the etiology of LADA and whether etiology differs among patients with LADA. Support for etiological heterogeneity comes from the large UK Prospective Diabetes Study (UKPDS), in which only a minority of patients with the diagnosis of LADA, when defined solely as GAD antibody positivity, developed the need for insulin treatment during 6 years of follow-up (1). The heterogeneity of LADA is also evident when one compares phenotypes of patients with LADA in different studies (3–6).

Heterogeneity of patients with LADA could be further elucidated by comparing phenotypes of insulin-treated patients with LADA with those not treated with insulin. However, several conditions have to be met for such comparisons to be valid. First, groups of diabetic subjects to be compared must be drawn from the same geographically defined population. A population-based study is necessary to avoid the possible biases incurred by studying referral patients or patients selected for inclusion in clinical intervention studies. Second, the perceived need for insulin treatment has to be unbiased by LADA classification, because it has been shown that insulin treatment is initiated earlier when prior knowledge of autoimmunity, measured as GAD antibodies, is avail-

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able (7). Third, valid comparison groups of insulin-treated and non–insulin-treated type 2 diabetic patients from the same population must be at hand.

We had the opportunity to study patients with LADA and type 2 diabetes fulfilling the above-mentioned conditions, who were identified and characterized in the Nord-Trøndelag (HUNT) study. The HUNT study was performed between 1995 and 1997. The study was open to all adult subjects (~90,000) living in a geographically defined area and had a high attendance rate (70%). To our knowledge, the patients with LADA and type 2 diabetes defined in the HUNT study did not participate in any study at the time of diagnosis and initiation of treatment, thereby minimizing any deviation from standard clinical practice and obviating increased attentiveness for signs of insufficient metabolic control and insulin requirement. Further, autoantibodies, such as GAD antibodies, were not measured by doctors (general practitioners) responsible for treatment in the region as part of the diabetes workup before and at the time of assembling data in 1995–1997. Thus, patients with autoimmune diabetes not requiring insulin at time of diagnosis were regarded and treated as type 2 diabetic patients. This assumption provided a unique opportunity to use the need for insulin as clinically perceived as a parameter for subclassifying patients with LADA and to compare phenotypes of insulin-treated and non–insulin-treated patients with LADA with respective groups of type 2 diabetic patients. Specifically, we wished to assess the influence of autoimmunity and β-cell insufficiency vis-à-vis insulin resistance factors for the perceived need for insulin treatment in patients with LADA.

RESEARCH DESIGN AND METHODS — The second Nord-Trøndelag Health (HUNT 2) study was conducted between 1995 and 1997. All inhabitants aged 20 years or older ($n =$ 92,703) in Nord-Trøndelag County, Norway, were invited to participate in the study, and 65,282 (70.4%) accepted the invitation and attended a clinical examination. The participants were asked to fill out self-administered questionnaires with health- and lifestyle-related items, such as general health, smoking, marital status, and education. The clinical examination included measurements of blood pressure (Dinamap 845XT; Critikon), heart rate, height, weight, and waist circumference.

Measurements were performed by a specially trained nurse or technician.

A nonfasting blood sample was taken from all participants for analyses in sera. Remaining sera were stored at −70°C.

Blood sampling and questionnaire in diabetic patients

The 1,952 participants who reported diabetes in the initial questionnaire were asked to fill out an additional questionnaire with specific questions on diabetes such as duration of known disease and antidiabetes and other medication. For insulin treatment, the questionnaire asked for starting date and the current dosage of insulin. The questionnaire contained three questions regarding cardiovascular disease: "Have you ever suffered from angina pectoris?", "Have you ever suffered from myocardial infarction?", and "Have you ever suffered from stroke?", with the alternatives of answering being "yes" or "no." A history of cardiovascular disease was defined as positive when at least one of the three questions was answered with "yes."

In addition, subjects were asked to submit a blood sample after an overnight fast. Blood was drawn for analyses of glucose, C-peptide, and GAD antibodies. Of the 1,511 diabetic subjects who participated in both the questionnaire and the supplemental blood sampling part of the study, 128 were classified as having LADA 1,133 as having type 2 diabetes, and 123 as having type 1 diabetes. In addition, 64 subjects were classified as having gestational diabetes mellitus or mature-onset diabetes of the young or not having diabetes. An equal number of subjects could not be classified because they did not fit into any of the predefined categories of diabetes. Further, of the classifiable subjects, 22 (17%) of those classified as having LADA and 190 (16.8%) of those classified as having type 2 diabetes were excluded from our study because of the lack of the necessary information on insulin treatment.

Assays

A1C was analyzed with the Architect ci8200/c8000 system and fasting glucose with a HemoCue monitor at Levanger Hospital, Levanger, Norway. Serum levels of C-peptide in the overnight-fasted state and GAD antibodies were analyzed at Aker Hospital, Oslo, Norway. C-peptide was measured with a radioimmunoassay method (Diagnostic System Laboratories, Webster, TX). GAD antibodies were mea-

sured by immunoprecipitation, using [^{3}H]leucine translation-labeled GAD65 as an indicator. The level of GAD antibodies was expressed as an index value relative to a standard serum. An index ≥0.08 was considered as positive. The method including the antibody used was adapted from Peterson et al. (8). Proficiency was tested at Aker Hospital in a diabetes autoantibody standardization program. The specimen for analysis was then blinded, with 100 samples from control subjects and 50 samples from type 1 diabetic subjects being analyzed. At the cutoff level of >0.08, the sensitivity was 0.64 and the specificity was 1.00. Cholesterol and HDL cholesterol were measured in the nonfasting state by routine methods.

Classification of diabetes

Diabetes was classified as follows:

LADA: GAD antibody titer ≥0.08 *and* lifestyle and oral therapy *or* insulin treatment started later than 12 months after diagnosis *or* insulin therapy started before 12 months after diagnosis, but with fasting C-peptide >150 pmol/l. (Six subjects were classified as having LADA according to the latter criterion; their C-peptide levels ranged between 158 and 2,366 pmol/l.)

Type 2 diabetes: Lifestyle and oral therapy *or* insulin treatment started later than 12 months after diagnosis *and* GAD antibody titer <0.08.

Type 1 diabetes (excluded from this study): Insulin treatment started before 12 months after diagnosis, either with a GAD antibody titer ≥0.08 *or* GAD antibody titer <0.08 *and* C-peptide <150 pmol/l.

Statistical analyses

We reported chronological age, age at diagnosis of diabetes, diabetes duration, GAD antibodies, insulin dosage, and years before the start of insulin as an unadjusted mean ± SEM. We compared mean values with 95% CIs, adjusted for age, sex, and diabetes duration for data on blood pressure, BMI, waist circumference, total cholesterol–to–HDL cholesterol ratio, fasting glucose, A1C, and fasting C-peptide, using a general linear model. Levels of C-peptide were not normally distributed and therefore were log transformed before calculation of the adjusted mean value. Results were considered significant when $P < 0.05$. Correlation tests were performed using Spearman's rank correlation. All analyses were performed using the statistical

Table 1—Characteristics of subjects treated with insulin or not

	LADA with insulin	LADA without insulin	Type 2 diabetes with insulin	Type 2 diabetes without insulin	P		
					Difference between LADA with and without insulin	Difference between LADA and type 2 diabetes with insulin	Difference between type 2 diabetes with and without insulin
n (men/women)	42 (22/20)	64 (36/28)	203 (102/101)	740 (379/361)	<0.001	NA	<0.001
Age at onset (years)	67.7 ± 1.7	66.6 ± 1.4	67.5 ± 0.8	68.0 ± 0.4	0.586	0.900	0.573
Diabetes duration (years)	14.0 ± 1.1	8.1 ± 0.9	13.1 ± 0.5	7.6 ± 0.3	<0.001	0.478	<0.001
GAD antibody titer (units)	0.54 ± 0.03	0.29 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	<0.001	NA	NA
Insulin (units)	46 ± 10	—	62 ± 5	—	NA	0.190	NA
Time of disease before start of insulin treatment (years)	5.6 ± 1.1	—	7.3 ± 0.5	—	NA	0.157	NA

Data are means ± SEM. No significant differences between LADA and type 2 diabetes not treated with insulin. NA, not applicable.

software SPSS 13.0 for Windows (SPSS, Chicago, IL).

Consent

Participation was voluntary, and each participant provided written consent. The studies were approved by the regional ethics committee and by the Norwegian Data Inspectorate.

RESULTS

Comparison of insulin-treated and non–insulin-treated subjects with LADA

Age and sex distribution were comparable between the insulin- and the non–insulin-treated subjects with LADA. Diabetes duration was longer in those receiving insulin (Table 1). Fasting blood glucose and A1C were significantly higher and fasting C-peptide was significantly lower in the insulin-treated subjects. The GAD antibody titer was significantly higher in the insulin-treated subjects. There was a tendency for a correlation between the GAD antibody titer and diabetes duration before the start of insulin treatment ($P = 0.07$). There was a negative correlation between the GAD antibody titer and C-peptide in the insulin-treated LADA group (correlation coefficient, $r = -0.40$; $P = 0.009$). Such a correlation was not readily apparent within the LADA group without insulin treatment ($r = -0.22$; $P = 0.09$). The degree of overweight measured as BMI and waist circumference did not differ between groups nor did blood pressure.

Comparison of non–insulin-treated subjects with LADA with non–insulin-treated subjects with type 2 diabetes

Age, sex, and disease duration did not differ between these two groups (Table 1). There was no difference in blood pressure, lipid status, or adiposity (Table 2). The proportion of sulfonylurea-treated patients was identical. The use of other oral antidiabetic medications also was evenly distributed in the two groups (supplemental Table 1, available in an online appendix at http://dx.doi.org/10.2337/dc08-1468).

Further, there were no differences in fasting C-peptide or fasting glucose between groups. Also, the frequency of history of cardiovascular disease did not differ significantly (present in 25% of patients with LADA vs. 29% of patients with type 2 diabetes). Results were similar when we excluded subjects receiving sulfonylurea treatment (Table 2).

Comparison between insulin-treated patients with LADA and insulin-treated patients with type 2 diabetes

Age and sex were comparable in insulin-treated patients with LADA and insulin-treated patients with type 2 diabetes. There was no significant difference in diabetes duration. A larger percentage of patients with LADA were treated with insulin (40% of those with LADA vs. 22% of those with type 2 diabetes; $P < 0.001$) (Table 1). The mean duration between diabetes diagnosis and start of insulin treatment was not significantly shorter in the patients with LADA ($P = 0.157$), and the mean daily dosage of insulin was not significantly different either ($P = 0.195$ for lower dosage in patients with LADA).

Insulin-treated patients with type 2 diabetes had higher BMI, waist circumference, and cholesterol-to–HDL cholesterol ratios than the insulin-treated LADA group. Fasting C-peptide was higher than that in the insulin-treated LADA group. The frequency of microalbuminuria was not significantly different (37% of patients with type 2 diabetes vs. 27% of patients with LADA; $P = 0.072$), whereas a history of cardiovascular disease was more frequent in the insulin-treated type 2 diabetes group (37 vs. 29%; $P = 0.031$) despite a comparable degree of smoking.

Comparison between insulin-treated and non–insulin-treated patients with type 2 diabetes

Type 2 diabetic patients who were treated with insulin were more obese and had higher A1C and lower C-peptide levels than subjects who were not treated with insulin (Table 2). The insulin-treated subjects had a more frequent history of cardiovascular disease (36 vs. 28%; $P = 0.031$).

CONCLUSIONS — Our study shows strong evidence for an overriding role of autoimmunity, assessed from GAD antibodies, behind a perceived need for insulin treatment in patients with LADA. In contrast, we find no phenotypic influence of autoimmunity in those patients with LADA who were not treated with insulin.

A major role for autoimmunity behind a need for insulin is indicated by insulin-treated patients with LADA having higher GAD antibody titers than patients not treated with insulin. Further, we find a strong negative correlation be-

Table 2—Features of metabolic syndrome and parameters of glycemic control and β-cell function

	LADA with insulin	LADA without insulin	Type 2 diabetes with insulin	Type 2 diabetes without insulin	*P*		
					Difference between LADA with and without insulin	Difference between LADA and type 2 diabetes with insulin	Difference between type 2 diabetes with and without insulin
Systolic blood pressure (mmHg)	150 (144–157)	158 (152–163)	157 (154–160)	157 (155–158)	0.102	0.086	0.897
Diastolic blood pressure (mmHg)	81 (77–85)	84 (81–88)	85 (83–87)	86 (85–87)	0.199	0.082	0.256
BMI (kg/m²)	28.7 (27.4–30.1)	28.4 (27.3–29.5)	30.9 (30.2–31.5)	29.5 (28.8–29.5)	0.696	<0.001	<0.001
Waist circumference (cm)	94.5 (91.1–97.9)	93.7 (90.9–96.4)	99.8 (98.2–101.4)	95.8 (95.0–96.6)	0.721	0.005	0.001
Cholesterol-to-HDL cholesterol ratio	4.7 (4.1–5.3)	5.5 (5.0–6.0)	5.8 (5.5–6.0)	5.6 (5.4–5.7)	0.054	0.001	0.235
Fasting blood glucose	11.3 (10.1–12.5)	9.8 (8.9–10.7)	11.5 (11.0–12.0)	8.9 (8.7–9.2)	0.049	0.746	<0.001
A1C	9.1 (8.7–9.7)	8.0 (7.6–8.4)	8.8 (8.6–9.0)	7.7 (7.6–7.8)	0.001	0.175	<0.001
C-peptide (pmol/l)	130 (105–160)	682 (577–806)	377 (343–416)	787 (749–827)	<0.001	<0.001	<0.001
C-peptide in subjects without sulfonylurea (pmol/l)	122 (97–152)	700 (542–904)	368 (332–409)	796 (736–862)	<0.001	<0.001	<0.001

Data are means (95% CI), adjusted for age, sex, and diabetes duration. No significant differences were found between LADA and type 2 diabetes not treated with insulin.

tween the GAD antibody titer on the one hand and levels of C-peptide on the other in the insulin-treated LADA group. A major role for autoimmunity is also supported by the lack of evidence for insulin resistance factors being operative. Thus, neither adiposity nor other parameters associated with metabolic syndrome, such as hypertension or dyslipidemia, were more pronounced in insulin-treated versus non–insulin-treated patients with LADA. This result was in contrast with findings in the corresponding groups of type 2 diabetic patients in whom obesity was more marked in those with a perceived need for insulin treatment. However, patients with LADA receiving insulin were overweight. Therefore, it remains possible that insulin resistance (obesity being a marker thereof) acts as a permissive factor for the need for insulin treatment in these patients.

Our results are in line with those of Genovese et al. (4), who found that patients with a high GAD antibody titer were more frequently receiving insulin therapy. Also, Buzzetti et al. (9) found that patients with high titers had more prominent traits of insulin deficiency and a profile of more severe autoimmunity. Interestingly, the latter study reported a bimodal distribution of GAD antibody titers that identified distinct subgroups of patients. We did not find a bimodal distribution (results not shown). Differences in study populations could possibly explain this discrepancy: Buzzetti et al. recruited their patients from diabetes centers, whereas our patients were recruited from an all population–inclusive survey.

We found no significant difference in phenotypes between non–insulin-treated patients with LADA and non–insulin-treated patients with type 2 diabetes. We did not verify GAD antibody positivity by repeat measurements; however, the high specificity of our GAD antibody assay strongly indicates that the presence of GAD antibodies is indeed a marker of autoimmunity. The question arises whether GAD antibody positivity in non–insulin-treated patients with LADA was of any clinical significance at the time of collection or later. The observation that up to 4% of a nondiabetic population harbors GAD antibodies (10–12) suggests that autoimmunity as measured by GAD antibodies toward insulin-producing β-cells can exist without clinically measurable effects. If so, the genetics of diabetes in our non–insulin-treated patients with LADA should be similar or identical to that of

type 2 diabetic patients. This notion agrees with our previous finding that a family history of diabetes was as strong a risk factor for LADA as for type 2 diabetes but was a lesser risk factor for type 1 diabetes (13). Furthermore, genes associated with type 2 diabetes have recently been found to be associated also with LADA (14). A genetic background similar to that for type 2 diabetes could possibly be the most influential factor in patients with LADA who were not treated with insulin. If so, this could explain a seemingly paradoxical finding of ours (13) and others' (15), namely that GAD antibody titers are negatively associated with a family history of diabetes in patients with LADA.

A comparison of insulin-treated patients with LADA and insulin-treated type 2 diabetic patients revealed lower fasting C-peptide levels in insulin-treated patients with LADA. Also BMI and waist circumference were lower in the insulin-treated LADA group than in the corresponding type 2 diabetic group. These findings suggest that the decision to start insulin treatment in type 2 diabetic patients was, in contrast to the situation in patients with LADA, based to some extent on insulin resistance–induced poor metabolic control. This notion is supported by more marked adiposity in insulin-treated than in non–insulin-treated type 2 diabetic patients. One should, however, also recognize a strong element of insulinopenia in the insulin-treated type 2 diabetic patients. Thus, C-peptide levels were significantly lower in these patients than in those with type 2 diabetes without insulin treatment, even after taking into account the increase in insulin secretion due to sulfonylurea treatment in the latter group. These findings are in line with the successive β-cell deterioration known to occur with increasing duration of type 2 diabetes (16,17).

Previous studies, including the large UKPDS (1), demonstrated a markedly shorter time interval from diagnosis to insulin treatment in patients with LADA than in type 2 diabetic patients. In our study population we found only a tendency for a shortening of duration before insulin. Two explanations may be offered for the partial discrepancy with earlier studies. First, the fact that our patients were not part of any study may have delayed the decision to start insulin treatment. Second, and most important, our classification of LADA required a longer time without insulin treatment, i.e., a whole year after diagnosis, than that in other studies, such as the UKPDS. The rationale for our classification was to obtain a clear separation between LADA and classic type 1 diabetes. Were we to include patients with the perceived need for insulin already between 6 and 12 months after diagnosis of diabetes (16 individuals in all), then the difference in time to insulin treatment was significant between LADA and type 2 diabetes (results not shown).

Our study has strengths and limitations. A strength and prerequisite for the validity of the present comparisons is that we have studied diabetic subjects who were recognized and categorized as part of a large geographically defined and all population–inclusive survey. To the best of our knowledge, our study is unique in this respect. Also, the participation rate was comparatively high and probably representative of all diabetes in Nord-Trøndelag. In this context, it is of interest that measurements of GAD antibodies in 330 of the unclassified diabetic subjects detected 17 subjects with diabetes onset >35 years of age who were GAD antibody positive (E. Petterson, K. M., V. G., unpublished observations). Had we included this small number of patients with probable LADA, the conclusions of our study would not have changed. On the other hand, the cross-sectional design of our study is an obvious limitation and should be kept in mind when the present results are interpreted.

In summary, our study suggests that autoimmunity plays a major role in the perceived need for insulin in patients with LADA despite the fact that a certain degree of overweight and insulin resistance is present. Further, we did not find phenotypic differences between those patients with LADA and type 2 diabetes who were not receiving insulin. These findings highlight heterogeneity in patients with LADA as currently defined.

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