Evidence of Islet Cell Autoimmunity in Elderly Patients With Type 2 Diabetes

Massimo Pietropaolo, Emma Barinas-Mitchell, Susan L. Pietropaolo, Lewis H. Kuller, and Massimo Trucco

In light of an occurring growth of elderly people affected by type 2 diabetes and recent observations indicating that type 2 diabetes may be a disease of the innate immune system, we evaluated whether signs of islet cell autoimmunity are associated with an abnormal glucose control, the presence of insulin requirement, or an activation of the acute-phase response in older individuals with type 2 diabetes. GAD65 and IA-2 autoantibodies along with the acute-phase response markers fibrinogen and C-reactive protein were tested in 196 serum samples from patients with type 2 diabetes and in 94 nondiabetic control subjects over the age of 65 years from the Pittsburgh cohort of the Cardiovascular Health Study. Of the diabetic patients, 12% (24 of 196) had autoantibodies against GAD65 and/or IA-2, a prevalence significantly higher than that found in nondiabetic individuals (1 of 94, 1.1%; P = 0.001). Type 2 diabetic patients who were positive for GAD65 and/or IA-2 autoantibodies (Ab+), as compared with those negative for these autoantibodies (Ab−), had an abnormal oral glucose tolerance test (OGTT) (P = 0.03) before and a higher frequency of oral hypoglycemic treatment (P = 0.003) at the time of autoantibody testing. No differences were seen in the percentage of insulin requirement in the two groups. Moreover, a statistically significant increase in fibrinogen (P = 0.005) and C-reactive protein levels (P = 0.025) was found in type 2 diabetic patients with high levels of GAD65 and/or IA-2 autoantibodies as compared with Ab− patients and control subjects. In conclusion, in type 2 diabetic subjects ≥65 years old, the presence of islet cell autoimmunity is associated with an impairment of the acute-phase insulin secretion, as revealed by the OGTT. A pronounced activation of the acute-phase response, found to be associated with islet cell autoimmunity, may in part explain this defect in insulin secretion. These findings not only have direct implications for adequate classification and treatment of diabetes in the elderly, but also for understanding the autoimmune/inflammatory mechanisms involved in the pathogenesis of hyperglycemia. Diabetes 49:32-38, 2000

Despite the fact that the number of elderly people with diabetes is estimated to increase dramatically in the next few decades (1-3), little is known concerning the prevalence and the significance of islet cell autoimmunity in elderly patients affected by type 2 diabetes. Circulating antibodies to islet cells (ICA) (4) and to the islet autoantigen GAD (5) can be detected in a subgroup of patients with type 2 diabetes (6-10). For the most part, in type 2 diabetic patients, positivity for ICA and GAD autoantibodies correlates with some of the phenotypic features consistent with those of type 1 diabetes, such as younger age at diagnosis, lower BMI, and a relentless loss of β-cell mass (11). Because of the peculiar characteristics of this subgroup of type 2 diabetes, the term latent autoimmune diabetes in adults (LADA) has been coined (12). Recently, this topic has been of peaking interest and has led to the inclusion of LADA into the proposal for the new World Health Organization (WHO) criteria for the diagnosis and classification of diabetes (11). In support of the role of islet cell autoimmunity in LADA, Shimada et al. (13) have recently reported their findings of a 65-year-old woman originally diagnosed as having type 2 diabetes with residual β-cell function who had a mononuclear cell infiltration in the pancreatic islets, predominantly CD4+ cells, as well as positivity for GAD and IA-2 autoantibodies. Islet cell autoimmunity appears to be present in 10-33% of subjects clinically diagnosed with type 2 diabetes (14). Therefore, up to 4 million Americans might have an unidentified autoimmune form of type 2 diabetes, a prevalence greater than that of recent-onset childhood diabetes. According to the classical definition, type 2 diabetes is a heterogeneous disease that results from a combination of abnormalities in both insulin secretion and insulin action (15). In addition, it has been proposed that type 2 diabetes is an acute-phase disease of the innate immune system in which increased levels of cytokines are released from many cells such as macrophages, adipose cells, or endothelial cells (16-21). Unknown factors such as age and overnutrition in genetically predisposed individuals may cause an increased secretion of the cytokines like interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6, which in turn induce the liver to release acute-phase proteins such as fibrinogen and C-reactive protein (CRP). In the pathogenesis of type 2 diabetes, long-term hypersecretion of IL-1β and TNF-α (20,21) may contribute to impaired β-cell insulin secretion (22) and insulin resistance (21), respectively, whereas IL-6 may be involved in the pathogenesis of cardiovascular complications (23).
The objective of this study was to establish whether islet cell autoimmunity is present in older individuals classified as having type 2 diabetes, to determine to what extent it is related to impaired glucose control, and to evaluate whether there is any relationship with the activation of the acute-phase response. Autoantibodies to the islet autoantigens GAD (65-kDa isofrom) and IA-2 (24,25) along with markers of activation of the acute-phase response (21) were evaluated in participants ≥65 years old from the Pittsburgh cohort of the Cardiovascular Health Study (CHS).

### RESEARCH DESIGN AND METHODS

The present study was designed to evaluate the prevalence of autoantibodies to GAD65 and/or IA-2 in the following groups of type 2 diabetic patients and randomly selected nondiabetic controls: participants ≥65 years old who participated in the Pittsburgh, Pennsylvania, clinic site of the CHS (26). After identifying type 2 diabetic subjects with and without evidence of islet cell autoimmunity, these groups were compared based on cross-sectional and retrospective assessments of glucose control and markers of inflammation.

#### Study population.

The CHS is a longitudinal study of risk factors and subclinical disease related to the incidence and natural history of cardiovascular disease (CVD) and stroke in noninstitutionalized adults 65 years of age and older (27). A detailed description of recruitment for CHS has been previously reported (26). Participants were recruited from four communities in the U.S.: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania (Allegheny County). A randomized systematic sampling from the Medicare eligibility lists of the Health Care Financing Administration (HCFA) in each site yielded a total of 5,201 eligible participants in the original cohort, including 2,955 women (153 African-American) and 2,246 men (91 African-American). The first CHS examination took place from June 1989 through May 1990 (year 2 of CHS). In 1992-1993 (year 5 of CHS), a new cohort of almost exclusively African-American individuals (98%) was enrolled in the CHS. A total of 687 (424 African-American women, 249 African-American men, and 14 participants of other races) participants were enrolled as part of this new cohort. An overview of the CHS dates pertinent to this ancillary study is presented in Table 1.

All CHS participants had a clinical examination and provided a medical history at baseline examination as defined for year 2 for the original cohort and year 5 for the new cohort (Table 1). The purpose of the extensive baseline examinations was to identify the presence and severity of CVD risk factors (e.g., hypertension and glucose intolerance), subclinical disease (e.g., carotid artery atherosclerosis), and clinically overt CVD in the elderly. Physical and laboratory evaluations were performed at baseline and repeated at year 5 for the original cohort. Anthropometric assessment, as well as resting blood pressure evaluation, is available for each year from baseline through year 9 of the study for both cohorts. After a 12-h fast, blood specimens were collected at baseline and year 5 for complete blood analysis of lipids, fasting glucose and insulin, albumin, creatinine, clotting factors, and inflammatory markers. Serum glucose and insulin levels after a 75-g oral glucose load were only measured at baseline. Diabetic patients treated with insulin or oral hypoglycemic agents were not administered the oral glucose tolerance test (OGTT). CRP was only measured at baseline visit for both cohorts. Blood specimens for the purpose of measuring cholesterol were collected at year 6 through year 8. A detailed description of laboratory techniques and quality assurance methods used in the CHS has been reported (28).

#### Subjects.

All subjects included in the present study were from the Pittsburgh, Pennsylvania, clinic site of the CHS. The entire Pittsburgh cohort of CHS consists of 1,499 participants, including 224 from the new cohort. For the present study, serum specimens from 196 type 2 diabetic patients and 94 randomly selected nondiabetic participants of the CHS were tested for autoantibodies against GAD65 and IA-2. The blood samples used for autoantibody testing in the present study were collected and stored at the Pittsburgh clinic during the years 1994-1995 (year 7 of CHS; Table 1). Available blood specimens from all surviving diabetic participants from the original cohort were used in the present study (n = 157). There were 39 diabetic patients from the new cohort included in this analysis, which represents 96.7% of diabetic participants surviving to year 7 from this cohort. The sample of control subjects was randomly selected from a prepared list of nondiabetic participants of the CHS (Pittsburgh cohort) with available blood specimens at year 7. Nondiabetic control subjects included in the present study did not differ from the total Pittsburgh population-based nondiabetic cohort of the CHS (n = 1,250) by age, sex, race, smoking status, and other baseline characteristics (i.e., fasting glucose and insulin levels, lipid levels, and BMI).

The diagnosis of diabetes was based on the 1985 WHO criteria for the classification of diabetes (29). Diabetes was defined as having one of the following: a positive medical history of diabetes, treatment with insulin or hypoglycemic agents, a fasting glucose >140 mg/dl, or a 2-h postchallenge glucose during an OGTT >200 mg/dl.

GAD65 and IA-2 autoantibody radioimmunoassays. Autoantibodies against GAD65 and IA-2 were detected in serum samples collected from CHS participants at year 7 of the study (Table 1). These autoantibodies were tested in triplicate using in vitro transcribed/retranslated recombinant human GAD (65-kDa isofrom), as described by Grubin et al. (30), and the recombinant human IA-2. The GAD65 construct used for this study was kindly donated by Dr. Åke Lernmark, and the IA-2 construct (ICASS2abcd) (31-33) was kindly provided by Dr. George Eisenbarth. The latter includes the amino acid residues 256–979 of the published IA-2 sequence (25). The results are expressed as an index (Index = sample counts per minute [cpm] - negative control cpm/positive control cpm - negative control cpm). The cutoff points for both assays were established as the 99th percentile of autoantibody levels calculated using 280 control subjects for both radioimmunoassays and corresponded to 0.069 and 0.032 for GAD65 and IA-2 autoantibodies, respectively. Both assays performed very well in the GAD and IA-2 Autoantibody Proficiency Workshops conducted by the University of Florida in Gainesville, Florida (100% sensitivity, specificity, and validity in the 1995, 1996, and 1997 GAD Autoantibody Proficiency Workshop; 78.5% specificity, 100% specificity, 87.5% validity, and 91.6% consistency in the 1996 GAD-2 Autoantibody Proficiency Workshop).

#### Methods of determination of fibrinogen, CRP, and albumin.

Plasma fibrinogen, CRP, and serum albumin were measured using blood specimens collected at baseline after a 12-h fast (28). Fibrinogen was measured in a BBL fibrinometer (Becton Dickinson, Cockeysville, MD) by the Clauss method (34) with Dade fibrinogen calibration reference (Baxter-Dade, Bedford, MA) and bovine thrombin (Parke-Davis, Littitz, PA). A Kodak Ektachem 700 analyzer with reagents (Eastman Kodak, Rochester, NY) was used for albumin assays (28.35). CRP was measured by colorimetric competitive immunoassays (CRP antibodies and antigens from Calbiochem, La Jolla, CA).

#### Statistical analysis.

Data were analyzed using Statistical Analysis System software on a Windows operating system (Release 6.12; SAS Institute, Cary, NC). Data are presented as mean ± SD for normally distributed variables, as percentages for categorical variables, or as median (interquartile range) for non-normal continuous variables. The interquartile range is presented as the 25th percentile and the 75th percentile of the distribution. Comparisons of means between two groups were performed using the unpaired t test for normally distributed variables and the Mann-Whitney test for non-normal variables. The χ² and Fisher’s exact test, when appropriate, were used to test between group differences for categorical variables.

### Table 1

<table>
<thead>
<tr>
<th>Timeline for the CHS and the present Pittsburgh site ancillary study</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHS</td>
</tr>
<tr>
<td>1989–1990 (year 2)</td>
</tr>
<tr>
<td>1992–1993 (year 5)*</td>
</tr>
<tr>
<td>1994–1995 (year 7)*</td>
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*No OGTT performed at this visit; the only biochemical variable measured was total serum cholesterol.

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TABLE 2
Baseline characteristics of CHS participants by diabetic status and GAD65 and/or IA-2 autoantibody status

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic participants</th>
<th>Diabetic participants</th>
<th>P value (Ab- vs. Ab+ diabetic subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>94</td>
<td>172</td>
<td>24</td>
</tr>
<tr>
<td>Sex (% men)</td>
<td>40.4</td>
<td>45.9</td>
<td>50.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.5 ± 4.6</td>
<td>72.4 ± 5.2</td>
<td>73.3 ± 6.1</td>
</tr>
<tr>
<td>Race (% Caucasian/African-American/other)</td>
<td>78.7/20.2/1.1</td>
<td>68.6/30.8/0.6</td>
<td>75.0/25.0/0.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 ± 4.5*</td>
<td>28.3 ± 4.9</td>
<td>29.9 ± 4.9</td>
</tr>
<tr>
<td>Current/ever/never smoker (%)</td>
<td>17.0/43.6/39.4</td>
<td>9.3/44.2/66.5</td>
<td>4.2/58.3/37.5</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>100.5 (95.0–108.0)*</td>
<td>140.0 (114.0–185.0)</td>
<td>168.5 (135.0–205.8)</td>
</tr>
<tr>
<td>2-h Glucose (mg/dl)†</td>
<td>124.0 (101.8–142.3)*</td>
<td>222.0 (202.0–270.0)</td>
<td>302.5 (217.3–378.3)</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)‡</td>
<td>—</td>
<td>16.0 (11.0–25.0)</td>
<td>16.0 (12.0–30.0)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136.9 ± 23.4</td>
<td>141.7 ± 19.4</td>
<td>134.6 ± 19.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72.9 ± 10.4</td>
<td>71.9 ± 10.1</td>
<td>70.1 ± 13.3</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>215.6 ± 36.6</td>
<td>208.3 ± 40.9</td>
<td>205.8 ± 38.9</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>135.0 ± 35.1</td>
<td>126.7 ± 37.8</td>
<td>130.9 ± 31.8</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>55.7 ± 14.6*</td>
<td>50.8 ± 14.3</td>
<td>45.5 ± 11.6</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>103.0 (85.8–143.5)*</td>
<td>139.0 (99.8–193.8)</td>
<td>99.0 (84.5–183.0)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>381.7 ± 58.4</td>
<td>323.9 ± 71.9</td>
<td>354.1 ± 81.1</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>4.1 ± 0.26</td>
<td>4.1 ± 0.30</td>
<td>3.9 ± 0.32</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.71 (0.72–3.05)*</td>
<td>2.53 (1.51–5.30)</td>
<td>2.98 (1.94–11.52)</td>
</tr>
<tr>
<td>Insulin treatment (%)‡</td>
<td>—</td>
<td>6.4</td>
<td>16.7</td>
</tr>
<tr>
<td>Use of oral hypoglycemic agents (%)‡</td>
<td>—</td>
<td>30.8</td>
<td>37.5</td>
</tr>
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</table>

Data are %, means ± SD, or medians (interquartile range) for non-normal variables. The cutoff points for both GAD65 and IA-2 autoantibodies were established as the 99th percentile of autoantibody levels calculated using 280 control subjects for both radioimmunoassays (40). Values shown in bold type are statistically significant. *Nondiabetic participants have statistically significant lower BMI, lower levels of fasting and 2-h glucose, fasting insulin, triglycerides, and CRP and higher levels of HDL cholesterol as compared with diabetic participants overall (P < 0.05); †new cohort participants and known diabetic patients using insulin or oral hypoglycemic agents were not administered the OGTT; therefore, 2-h glucose values were available for nondiabetic participants (n = 78) and Ab- (n = 95) and Ab+ (n = 12) diabetic participants; ‡based on 164 Ab- and 22 Ab+ type 2 diabetic patients.

ABLES. The McNemar test was used to test the change in percent use of oral hypoglycemic agents and insulin from baseline to year 7 within groups. For the McNemar test, eight autoantibody-negative (Ab-) and two autoantibody-positive (Ab+) diabetic subjects were excluded because of missing data for year 7, which resulted in 164 type 2 diabetic patients Ab- for GAD65 and/or IA-2 autoantibodies and 22 type 2 diabetic patients Ab+ for GAD65 and/or IA-2 autoantibodies for this analysis. Nonparametric analysis of variance (Kruskal-Wallis test) was applied to test the difference in levels of fibrinogen and CRP across autoantibody titer groups. All statistical tests were two-tailed, and P values of <0.05 were considered statistically significant.

RESULTS
Prevalence of GAD65 and IA-2 autoantibodies in elderly type 2 diabetic patients. The prevalence of antibodies to GAD65 and IA-2 was estimated in the Pittsburgh participants with type 2 diabetes from the CHS and compared with nondiabetic control subjects. All attended the year 7 clinic visit between 1994 and 1995 and provided a blood sample at that visit. Among 196 type 2 diabetic patients evaluated, GAD65 and IA-2 autoantibodies exceeded the limit of normal in 10.2% (20 of 196) and 2.6% (5 of 196), respectively. Autoantibodies to GAD65 and IA-2 exceeded the cutoff point for these two assays in 12.2% (24 of 196; P = 0.001) of the diabetic patients—a prevalence significantly higher than that of the nondiabetic control group, which was 1.1% (1 of 94).

Baseline characteristics of type 2 diabetic subjects and nondiabetic control subjects by autoantibody positivity status are presented in Table 2. The mean age of the study participants was 72 years. Of the participants, 80% of diabetic and 83% of nondiabetic individuals were from the original cohort of the CHS. A similar proportion of the diabetic and control subjects were men (46 and 40%, respectively) and Caucasian (69 and 79%, respectively). When compared with the nondiabetic control subjects, diabetic subjects had a significantly (P < 0.05) higher BMI (28.5 kg/m²) and levels of fasting glucose (142.0 mg/dl), fasting insulin (16.0 mU/ml), triglycerides (138.0 mg/dl), and CRP (2.56 mg/l), while they had lower levels of HDL cholesterol (50.2 mg/dl). Selected demographic and clinical characteristics and levels of antibodies to GAD65 and IA-2 of all individuals (n = 26) who tested positive for either autoantibody are presented in Table 3.

In addition, race-specific analysis revealed that autoantibodies to GAD65 and/or IA-2 were present in 13.2% (18 of 136) of Caucasian diabetic participants and 10.2% (6 of 59) of African-American diabetic participants. None of the African-American nondiabetic control subjects (0 of 19) exceeded the normal range for either autoantibody. Both Caucasian and African-American diabetic participants exhibited a higher prevalence of autoantibodies to GAD65 and/or IA-2 compared with nondiabetic control subjects (P = 0.001 for Caucasians, P = 0.014 for African Americans).

Characteristics of type 2 diabetic patients by GAD65 and/or IA-2 autoantibody positivity status. Comparisons of baseline data between type 2 diabetic patients with and without autoantibody positivity are presented in Table 2.
All the diabetic CHS participants were comparable with respect to sex, age, race, smoking status, fasting insulin, systolic and diastolic blood pressure, total and LDL cholesterol, and use of oral hypoglycemic agents measured at baseline. Fifty-eight percent of Ab+ (99 of 172) and 58.3% of Ab+ (14 of 24) diabetic participants self-reported past medical history of diabetes before enrollment in the CHS.

Ab+ type 2 diabetic patients had significantly higher 2-h blood glucose levels after an OGTT at baseline as compared with Ab− type 2 diabetic patients (P = 0.03; Table 2). Ab+ type 2 diabetic patients also had higher fasting glucose levels at baseline as compared with Ab− type 2 diabetic patients, although not statistically significant. At baseline (before serum sampling for autoantibody testing) 45.5% (10 of 22) of Ab+ diabetic participants and 36.6% (60 of 164) of Ab− diabetic participants were on insulin and/or oral hypoglycemic treatment (P = 0.42). However, at year 7, the proportions were increased to 68.2% (15 of 22) and 44.5% (73 of 164), respectively (P = 0.037). Among those not treated with insulin or oral hypoglycemic agents at baseline, the proportion that began treatment by year 7 was higher in Ab+ than Ab− participants (41.7% [5 of 12] as compared with 25% [26 of 104]). Moreover, among those treated at baseline, the proportion that discontinued treatment during follow-up was less in the Ab+ than Ab− participants (0% [0 of 10] and 21.7% [13 of 60], respectively). Neither of these differences is statistically significant, but the results suggest that autoantibody-positive patients with diabetes are less likely to be able to manage their diabetes consistently with diet alone than autoantibody-negative patients.

Type 2 diabetic patients with high levels of GAD65 and/or IA-2 autoantibodies appeared to have elevated levels of the acute-phase response proteins fibrinogen and CRP (Figs. 1 and 2; Table 3). Statistically significant higher levels of fibrinogen and CRP were found in type 2 diabetic patients with high titer of GAD65 and/or IA-2 autoantibody as compared with low titer (P = 0.004 and P = 0.03, respectively), autoantibody-negative diabetic patients (P = 0.006 and P = 0.03, respectively), and nondiabetic patients (P = 0.0006 and P = 0.001, respectively). Moreover, type 2 diabetic patients with evidence of islet cell autoimmunity had lower serum albumin levels as compared with autoantibody-negative patients (mean ± SD: Ab+, 3.91 ± 0.32; Ab−, 4.05 ± 0.26; P = 0.033; Table 2).

**DISCUSSION**

Diabetes is an important health issue for the elderly. A large body of evidence indicates that this population is the fastest growing portion in the U.S. (1,3), currently representing ~13% of the total population, and is estimated to nearly double in the next 20–30 years (1–3). Older diabetic patients have an increased prevalence of diabetes-related complications as compared with age-matched nondiabetic control subjects (2,36). Thus, the management of hyperglycemia in older diabetic subjects should be seriously considered (3,37,38).

To date, there have been no investigations, particularly in the U.S., concerning the prevalence and the clinical significance of islet cell autoimmunity in elderly patients with type 2 diabetes. We applied two of the most widely used markers for the diagnosis and prediction of type 1 diabetes, namely GAD65 and IA-2 autoantibodies (39–41), in a well-
characterized population of type 2 diabetic patients over the age of 65 years. Our observations indicate that the majority of type 2 diabetic patients with signs of islet cell autoimmunity had an impaired glucose tolerance and a higher frequency of oral hypoglycemic treatment as compared with patients without evidence of islet cell autoimmunity. These results are important for two reasons: 1) The fact that islet cell autoimmunity appears to be associated with poor diabetes control among elderly patients raises the possibility that these individuals may require aggressive intervention to reduce the risk of the complications related with hyperglycemia. 2) They provide new insight toward an adequate classification and treatment of a subgroup of LADA in the elderly.

Several studies have suggested that testing for markers of islet cell autoimmunity, such as GAD and ICA, may be useful in predicting the likelihood of insulin requirement, particularly in young and middle-aged patients diagnosed with type 2 diabetes (9). Unlike in the younger age-groups, in which the presence of GAD autoantibodies and/or ICA are considered major predictors of subsequent need for insulin therapy (9,42,43), our results indicate that in type 2 diabetic patients older than 65 years of age, the occurrence of either GAD65 or IA-2 autoantibodies was not associated with the need for insulin therapy. Likewise, in the U.K. Prospective Diabetes Study (9), it was reported that in type 2 diabetic patients ≥45 years of age, the presence of GAD or ICA alone was a weaker predictor of insulin requirement as compared with patients carrying both autoantibodies. However, the possibility that multiple rather than single antibodies to islet autoantigens (which were not found in this study) may correlate with a high risk of progression to insulin requirement cannot be excluded in elderly patients. Although we do not have data in

FIG. 1. Fibrinogen levels in relation to GAD65 and/or IA-2 autoantibody titer. High antibody titer was defined as threefold above the threshold for the GAD65 and IA-2 autoantibody assays. The median of fibrinogen values is represented by a horizontal bar. *Nonparametric analysis of variance (Kruskal-Wallis test) was used to test the difference in fibrinogen across the four groups (P < 0.05). A statistically significant increase in fibrinogen levels was found in high titer GAD65 and/or IA-2 autoantibody type 2 diabetic patients (range 328–518 mg/dl) compared with low titer (P = 0.004; range 235–490 mg/dl), autoantibody-negative diabetic patients (P = 0.006; range 185–695 mg/dl), and nondiabetic subjects (P = 0.0006; range 212–499 mg/dl).

FIG. 2. C-reactive protein levels in relation to GAD65 and/or IA-2 autoantibody titer. High antibody titer was defined as threefold above the threshold for the GAD65 and IA-2 autoantibody assays. The median of CRP values is represented by a horizontal bar. *Nonparametric analysis of variance (Kruskal-Wallis test) was used to test the difference in CRP across the four groups (P < 0.05). A statistically significant increase in CRP levels was found in high titer GAD65 and/or IA-2 autoantibody type 2 diabetic patients (range 2.93–86.36 mg/l) compared with low titer (P = 0.03; range 0.48–15.46 mg/l), autoantibody-negative diabetic patients (P = 0.03; range 0.18–62.79 mg/l), and nondiabetic subjects (P = 0.001; range 0.17–15.09 mg/l).
the present study on sequential evaluation determinations, there is solid evidence suggesting that GAD and IA-2 autoantibodies are stable for a number of years or even decades in both type 1 (44,45) and type 2 diabetes (43,46).

Type 2 diabetes has also been considered to have a cytokine-associated acute-phase reaction, which is part of the innate immune response (16,17,19,21,35,47–52). Previous investigations reported on the CHS population have indicated that there is a high prevalence of inflammatory proteins in type 2 diabetic subjects (53,54). The results recently reported by Schmidt et al. (55) on the Atherosclerosis Risk in Community Study have substantiated the concept that markers of inflammation may predict diabetes in adults.

We found that type 2 diabetic patients with evidence of high levels of GAD65 and IA-2 autoantibodies seem to have a more pronounced activation of the acute-phase response as compared with low titer and autoantibody-negative patients. In fact, both fibrinogen and CRP levels appeared to be increased in patients with high titer of GAD65 and/or IA-2 autoantibodies as compared with low titer and autoantibody-negative diabetic patients. It is possible that the enhanced activation of the acute-phase response that we found in high titer autoantibody-positive type 2 diabetic patients may partially account for either a defect in the induction of insulin resistance or a defect in insulin secretion. In support of these findings, there have been gathering observations (21) suggesting that in type 2 diabetes, cytokines like TNF-α might contribute to the development of insulin resistance by inhibiting the tyrosine kinase activity of the insulin receptor (21) and that IL-1β may promote an impairment of insulin secretion in pancreatic β-cells (22).

Additionally, we found that albumin levels were also significantly decreased in autoantibody-positive as compared with autoantibody-negative type 2 diabetic patients. Albumin, unlike fibrinogen, is a negative acute-phase protein, and its serum levels may be reduced as a result of an augmented vascular permeability during CVD and tissue damage (56). It is noteworthy that both low serum albumin and higher levels of fibrinogen and CRP may all be markers of inflammation and underlying CVD, and low serum albumin is predictive of coronary heart disease in elderly individuals with diabetes (47,50,53,57).

In view of our results, it is conceivable that inflammation may be part of the autoimmune reaction associated with the functional as well as structural deficit of pancreatic β-cells, which leads to hyperglycemia. However, because of the preliminary characteristics of the present data, further investigations are needed to explain the mechanisms underlying this enhanced activation of the acute-phase response in type 2 diabetic patients with signs of islet cell autoimmunity.

In summary, GAD65 and IA-2 autoantibodies may be useful markers in identifying a subgroup (~12%) of individuals with autoimmune diabetes in older patients with impaired glycemic control. This subgroup seems to have a marked activation of the acute-phase response. This estimate might be even higher when additional markers of islet cell autoimmunity and inflammation are applied to further define LADA in older populations. These observations may provide new insight to an adequate classification and treatment of type 2 diabetes and in turn lead to better knowledge and understanding of the autoimmune/inflammatory mechanisms involved in autoimmune diabetes.

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ISLET CELLCARIMMONITY IN TYPE 2 DIABETES


