T-Cell Responses to Islet Antigens Improves Detection of Autoimmune Diabetes and Identifies Patients With More Severe β-Cell Lesions in Phenotypic Type 2 Diabetes

Amit Goel, Harvey Chiu, Jamie Felton, Jerry P. Palmer, and Barbara Brooks-Worrell

Latent autoimmune diabetes in adults or type 1.5 diabetes is considered to be a T-cell–mediated autoimmune disease. However, identification of patients is based commonly on autoantibody (Ab) detection. To determine whether measuring T-cell reactivity to islet proteins compared with measuring Abs improves detection of autoimmune diabetes and how β-cell function correlates with T-cell reactivity compared with Ab positivity, we assessed the T-cell proliferative responses and Ab responses (islet cell autoantibodies, insulin autoantibodies, insulinoma-associated protein-2 autoantibodies, and GAD Abs) to islet proteins of 36 phenotypic type 2 diabetic patients. To be considered Ab$^+$ or T-cell$^+$, patients were required to be positive for a minimum of two consecutive time points. β-Cell function was measured with fasting and glucagon-stimulated C-peptide. Independent of T-cell reactivity, Ab$^+$ and Ab$^-$ patients had comparable fasting and glucagon-stimulated C-peptide. Independent of Ab status, T-cell$^+$ patients demonstrated significantly lower glucagon-stimulated ($P <$ 0.003) C-peptide compared with T-cell$^-$ patients. These data suggest that measuring T-cell responses to multiple islet proteins in phenotypic type 2 diabetic patients improves identification of patients with autoimmune diabetes and delineates those who have a more severe β-cell lesion compared with Ab assessment alone. *Diabetes* 56:2110–2115, 2007

Diabetes in humans is classified clinically into two main types, type 1 and type 2 diabetes, with different underlying pathophysiologies. The disease process in type 1 diabetes is T-cell–mediated autoimmune destruction of the pancreatic β-cells (1). In contrast, the disease process in classic type 2 diabetes is not autoimmune. Decreased sensitivity to insulin action (2) is central to the disease process and a nonimmune-mediated β-cell lesion occurs, which diminishes insulin secretion (3). The diagnosis of type 1 versus type 2 diabetes is usually made using clinical criteria, but clinical distinction of type 1 and type 2 diabetes is recognized as being imperfect.

There is also a third group of patients who clinically are phenotypically similar to type 2 diabetic patients but who are also positive for one or more of the autoantibodies (islet cell autoantibodies [ICAs], GAD autoantibodies [Abs], insulin autoantibodies [IAAs], and insulinoma-associated protein-2 autoantibodies [IA-2Abs]) commonly seen in type 1 diabetes. This Ab$^+$ phenotypic type 2 diabetes has been termed (4,5) latent autoimmune disease in adults (LADA) or type 1.5 diabetes (6,7) or type 1½ diabetes (8). Type 1.5 diabetes is usually diagnosed after 35 years of age with the presence of one or more islet Abs, and there is no immediate need for insulin. Type 1.5 diabetes comprises ~10% of Caucasian adult phenotypic type 2 diabetic patients.

Like type 1 diabetes, type 1.5 diabetes is considered an autoimmune disease, mediated by autoreactive T-cells. T-cells reacting to various islet proteins in peripheral blood have been demonstrated in patients with both type 1 diabetes and LADA (9,10). Recently, we investigated T-cell responses to islet proteins and Ab responses using multiple assays and discovered that there appear to be antigenic differences in the proteins recognized by the T-cells and Abs of the type 1.5 diabetic patients versus patients with classic type 1 diabetes (11). Therefore, even though there are similarities between type 1 and type 1.5 diabetic patients, the pathogenesis appears to have some differences.

Although, type 1.5 diabetes is believed to be T-cell–mediated, studies identifying type 1.5 diabetic patients rely mainly on Abs (12–24). Therefore, in this study, we investigated whether β-cell dysfunction was more associated with Abs or T-cell reactivity to islet antigens in patients with type 1.5 diabetes. We categorized patients as Ab$^+$ or Ab$^-$ and T-cell$^+$ or T-cell$^-$. We then investigated whether antibody positivity or T-cell positivity would better identify patients with a more severe β-cell lesion (i.e., lower C-peptide).

RESEARCH DESIGN AND METHODS

Thirty-six phenotypic type 2 diabetic patients within 5 years of diagnosis were studied. The patients were obtained from a prospective study comparing the effect of rosiglitazone and glyburide on β-cell function. A type 2 diabetic phenotype required the onset of diabetes between ages 35 and 70 years, no history of ketonuria or ketoacidosis, and initially no requirement for insulin for glycemic control. Written informed consent was obtained from each

From the Veterans Affairs Puget Sound Health Care System, Department of Medicine, Division of Metabolism, Endocrinology, and Nutrition, University of Washington, Seattle, Washington.

Address correspondence and reprint requests to Barbara Brooks-Worrell, PhD, Veterans Affairs Puget Sound Health Care System, 1660 S. Columbian Way, Building 1, Room 609, Seattle, WA 98108. E-mail: bbrooks@u.washington.edu.

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Ab, autoantibody; IAA, insulin autoantibody; IA-2Ab, insulinoma-associated protein-2 autoantibody; ICA, islet cell autoantibody; IDS, Immunology of Diabetes Society; JDF, Juvenile Diabetes Foundation; LADA, latent autoimmune diabetes in adults; PBMC, peripheral blood mononuclear cell; *SI*, stimulation index.

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patient before study enrollment. Blood was drawn from patients after a 2-week washout period from all medication. All patients were required to have A1C between 6 and 10% and fasting C-peptide ≥0.80 ng/ml. The cut point of 0.8 ng/ml was chosen because we wanted patients in the drug study who, at least in the beginning of the study, could be treated with oral agents and not require insulin therapy. Exclusion criteria included a history of chronic pancreatitis or other secondary cause of diabetes, treatment with systemic corticosteroids, concurrent severe systemic illness, renal insufficiency, or hepatic dysfunction. Diabetes-associated Abs and T-cell proliferative responses to islet antigens were assessed to classify the patients. The Abs measured were GAD Abs, Abs to the tyrosine phosphatase IA-2 (IA-2Abs), ICAs, and IAAs. T-cell responses were measured using cellular immunoblotting. The patients are enrolled in a drug study and are seen every 3 months with blood samples taken. The definition for positivity or negativity is "for two consecutive time points." However, the C-peptide data presented in this article are from the baseline visit of the patients. At the baseline visit patients were taking no diabetes drugs (2-week washout period), and the C-peptide at this time point was used because the drugs to which the patients were randomly assigned could differentially affect their C-peptide results. The study is ongoing, and follow-up C-peptide data for these patients will be analyzed when the study is completed. β-Cell function was measured using fasting and glucagon-stimulated C-peptide. Demographic information such as age, weight, height, and duration of diabetes was also obtained.

IAA assay. This assay was performed as described previously (10,11). All sera with detectable ICAs were end point titered. The lower detection limit of our assay was 1 Juvenile Diabetes Foundation (JDF) unit, and the 95th percentile positivity threshold was established at 6 JDF units based on ~4,000 normal schoolchildren (25). Our laboratory had participated in a total of eight Immunology of Diabetes Society (IDS) Workshops and IDS-sponsored proficiency programs for ICAs with an average sensitivity of 80% and a specificity of 100% (in identification of patient versus control sera). In the IDS-sponsored Combined Antibody Workshop (26), our ICA assay had a specificity of 98% and a sensitivity of 76%. Our ICA assay had been validated in a serum exchange with the Diabetes Prevention Trial–Type 1 Diabetes (DPT-1) ICA core laboratory. In this exchange, the sensitivity of our assay was 85% with a specificity of 100%.

GAD Ab Assay. The determination of GAD Ab levels was performed at the Immunoassay Core of the Diabetes Endocrinology Research Center, University of Washington. GAD Abs were measured in a radiobinding immunoassay on coded serum samples as described previously (27). The levels of GAD Abs were expressed as a relative index (GAD index) using one positive serum (JDF World Standard for ICA) and three negative standard sera from healthy subjects. The GAD index was calculated, and a positive result was considered as ≥0.085, which is the 99th percentile based on 200 normal control subjects. Positive and negative controls are run in duplicate in each assay. The performance of the assays is monitored by a set of quality control standards and participation in external laboratory proficiency comparisons. The laboratory has participated in the Diabetes Antibody Standardization Program and scored 74% sensitivity and 97% specificity in 2005 (in identification of patient versus control sera). In the IDS-sponsored GAD Ab serum exchange and the Combined Antibody Workshop, our laboratory scored 100% for both sensitivity and specificity (28).

IA-2Ab Assay. Autoantibodies to IA-2 were measured under conditions identical to those described for GAD Abs (27) using the plasmid containing the cDNA coding for the cytoplasmic portion of IA-2. The IA-2Ab index for each sample was calculated using the same JDF standard serum and control sera that were used in the GAD Ab assay. An IA-2 index ≥0.017, the 99th percentile based on 200 normal control subjects, was the cutoff for positivity. The performance of the assays is monitored by a set of quality control standards and participation in external laboratory proficiency comparisons. The laboratory has participated in the Diabetes Antibody Standardization Program. In the 2001 evaluation, the laboratory scored 54% sensitivity and 96% specificity (28) (in identification of patient versus control sera). In the 2005 evaluation, the laboratory scored 62% sensitivity and 95% specificity.

C-peptide assay. Fasting and glucagon-stimulated C-peptide was used as a measure of endogenous β-cell function in all 36 patients at entry into the study. Stimulated C-peptide was measured 6 min after the intravenous injection of 1 mg glucagon. The C-peptide assay, which is a two-site immunoenzymometric assay, performed using a Tosoh 600 II autoanalyzer (Tosoh Bioscience, South San Francisco, CA), was completed in the Immunoassay Core of the Diabetes Endocrinology Research Center (DERC) at the University of Washington. Briefly, the C-peptide present in the sample is bound with a monoclonal antibody immobilized on a magnetic solid phase, and an enzyme-labeled monoclonal antibody is added. The magnetic beads are then washed to remove unbound enzyme–labeled monoclonal antibody and incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate. The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the C-peptide concentration in the patient sample. The interassay and intra-assay precision analyses show a coefficient of variation <10%. The assay has a sensitivity level of 0.04 ng/ml.

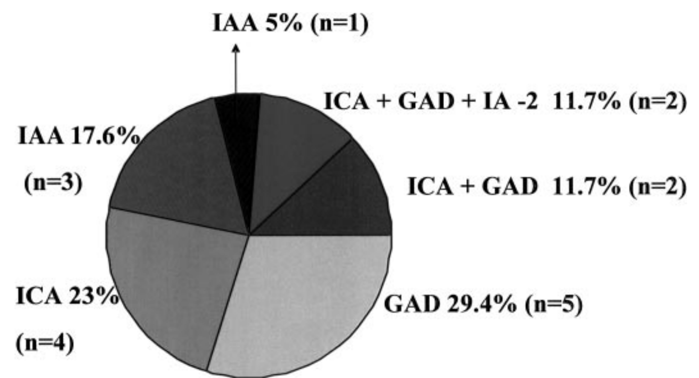

FIG. 1. Different autoantibodies in 17 Ab$^+$ patients.

Cellular immunoblotting. Cellular immunoblotting was performed as described previously for islets (9) and nonislet proteins (29,30). Human pancreata were obtained from the National Institutes of Health Islet Consortium. Islet cells were subjected to preparative 10% SDS-PAGE (31). After electrophoresis, the gels were electroblotted onto nitrocellulose (BioRad, Richmond, CA) at 30 mA overnight, nitrocellulose particles were prepared, and the nitrocellulose particles were used to stimulate peripheral blood mononuclear cells (PBMCs) in vitro.

A stimulation index (*SI*) for each molecular weight section was calculated as follows:

$$SI = \frac{\text{Mean counts per minute experimental wells}}{\text{Mean counts per minute control wells}}$$

Control wells contained nitrocellulose particles without antigen. Positive proliferation was considered to be an $SI > 2.0$, which corresponds to greater than the mean + 3 SD of control values (9). Antigen doses and specificity of PBMC responses of type 1 diabetic patients to the islet protein preparations and known islet autoantigens using cellular immunoblotting have been described previously (9). PBMC responses to tetanus toxoid were used as a control antigen along with the PBMC responses to mitogens, which were included to test for viability of the cultures. PBMC responses of type 1 diabetic patients, Ab$^+$ type 2 diabetic patients, Ab$^-$ type 2 diabetic patients, and control subjects to tetanus toxoid have been shown to be similar (9,11). Based on results in >60 control subjects and 60 patients with type 1 diabetes, cellular immunoblotting is considered positive when more than three blot sections have an $SI > 2.0$ (32). This assay has been validated by the Immune Tolerance Network; it was able to distinguish type 1 diabetic subjects from control subjects with a specificity of 83% and sensitivity of 91% (33).

Glucose assay. Analysis was performed enzymatically on a Hitachi 917 clinical chemistry autoanalyzer using the hexokinase method described by Peterson and Young (34).

Statistical analysis. Data were analyzed using an unpaired *t* test and the Mann-Whitney nonparametric test (StatView 4.0; Abacus Concepts).

RESULTS

General demographics. Among the 36 diabetic patients, 28 were male and 8 were female. Twenty-eight (77.8%) were Caucasians, 4 (11.1%) were African-Americans, and 4 (11.1%) were Asians or Native Americans. The mean ± SE duration of diabetes at the time of entry in the study was 30.2 ± 3.202 months (range 1–59 months).

Diabetes Abs. Seventeen of the 36 patients were Ab$^+$. Of these 17 patients, 9 (52.9%) were GAD Ab$^+$, 8 (47.1%) were ICA$^+$, 5 (29.4%) were IA-2Ab$^+$, and 1 (5.9%) was IAA$^+$ (Fig. 1). Five (29.4%) patients were GAD Ab$^+$ only, 4 (23.5%) were ICA$^+$ only, 3 (17.6%) were IA-2Ab$^+$ only, and 1 (5.9%) was IAA$^+$ only. Four patients (23.5%) were ICA$^+$ and GAD Ab$^+$, and 2 of these patients were also IA-2Ab$^+$.

To summarize, 13 patients (76.4%) were positive for one Ab, 2 (11.7%) were positive for two Abs, and 2 (11.7%) were positive for three Abs. None of the patients were positive for all four Abs.

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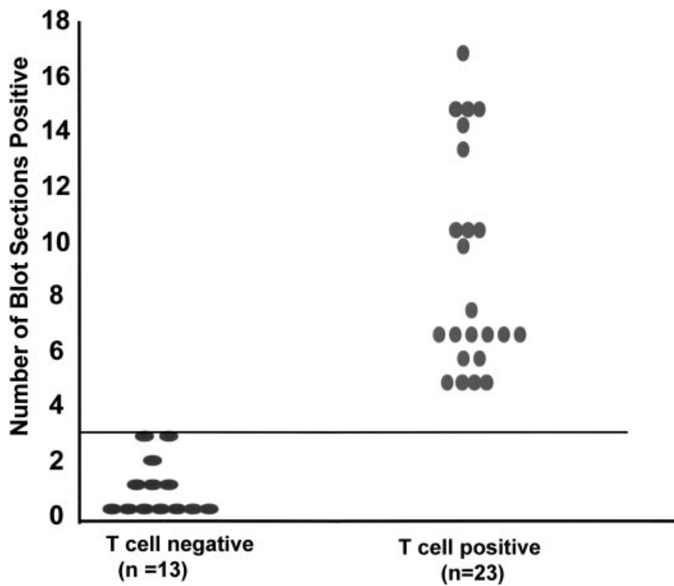

FIG. 2. Blot section positivity in 36 phenotypic type 2 diabetes patients using cellular immunoblotting.

T-cell proliferative responses. Of the 36 patients, 23 patients demonstrated positive PBMC responses to islet proteins (T-cell$^+$) and 13 showed negative PBMC responses (T-cell$^-$) (Fig. 2).

β-Cell function. Irrespective of T-cell proliferative responses, no statistical difference was observed in fasting and glucagon-stimulated C-peptide levels between the Ab$^-$ and Ab$^+$ groups (Fig. 3). Irrespective of autoantibody status, T-cell$^+$ patients had significantly lower glucagon-stimulated C-peptide compared with T-cell$^-$ patients, although no significant difference was observed in fasting C-peptide secretion between the two groups (Fig. 4). Data are summarized in Table 1.

We further divided the T-cell$^+$ patients into three categories (4–6 blots positive, 7–11 blots positive, and 14–18 blots positive); there were no significant differences in fasting or glucagon stimulated C-peptide levels in the patients within the three groups. If we divided the subjects into low (4–6 blots positive) or high (7–18 blots positive) categories, again there were no significant differences in the fasting or glucagon-stimulated C-peptide responses between the two groups.

Clinical characteristics. No statistical difference (*t* test or Mann-Whitney nonparametric test) was observed in fasting glucose, glycemic control (A1C as a percentage), BMI, age, and duration of diabetes between Ab$^-$ and Ab$^+$ groups and T-cell$^-$ and T-cell$^+$ groups (Table 2). We also investigated insulin resistance using the homeostasis model assessment (35,36) and did not find any significant differences between T-cell$^+$ and T-cell$^-$ subjects.

DISCUSSION

Type 1.5 diabetes, like type 1 diabetes, is considered an autoimmune disease characterized by the presence of Abs and autoreactive T-cells to islet proteins. Previously, using cellular immunoblotting, we demonstrated that T-cells in type 1 diabetic patients are responsive to a wide spectrum of islet proteins. This particular T-cell assay was developed in our laboratory for type 1 diabetic patients and, as mentioned above, has been validated by the Immune Tolerance Network where it was able to distinguish type 1 diabetic from control subjects with a specificity of 83% and a sensitivity of 91% (33). Using the same assay, we demonstrated that type 1.5 diabetic patients also show T-cell reactivity to islet proteins (11). In the study reported here, we investigated how β-cell function in type 1.5

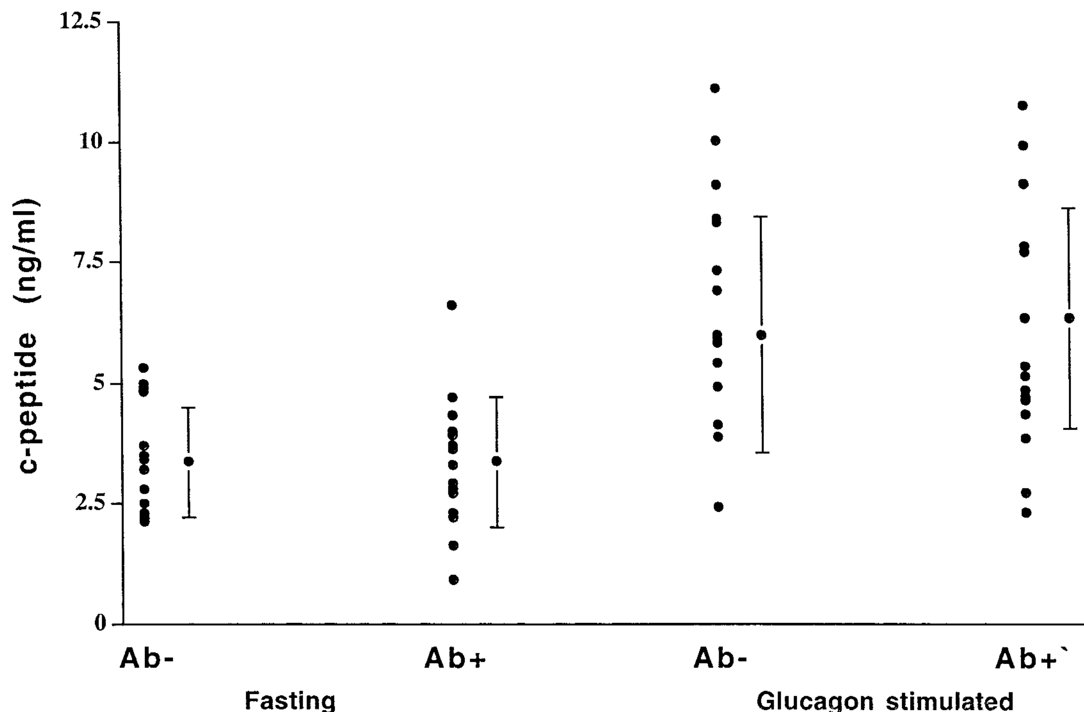

FIG. 3. Fasting and glucagon-stimulated plasma C-peptide in Ab$^-$ (n = 17) and Ab$^+$ (n = 19) patients independent of T-cell reactivity. Vertical bars with dots represent mean ± SD.

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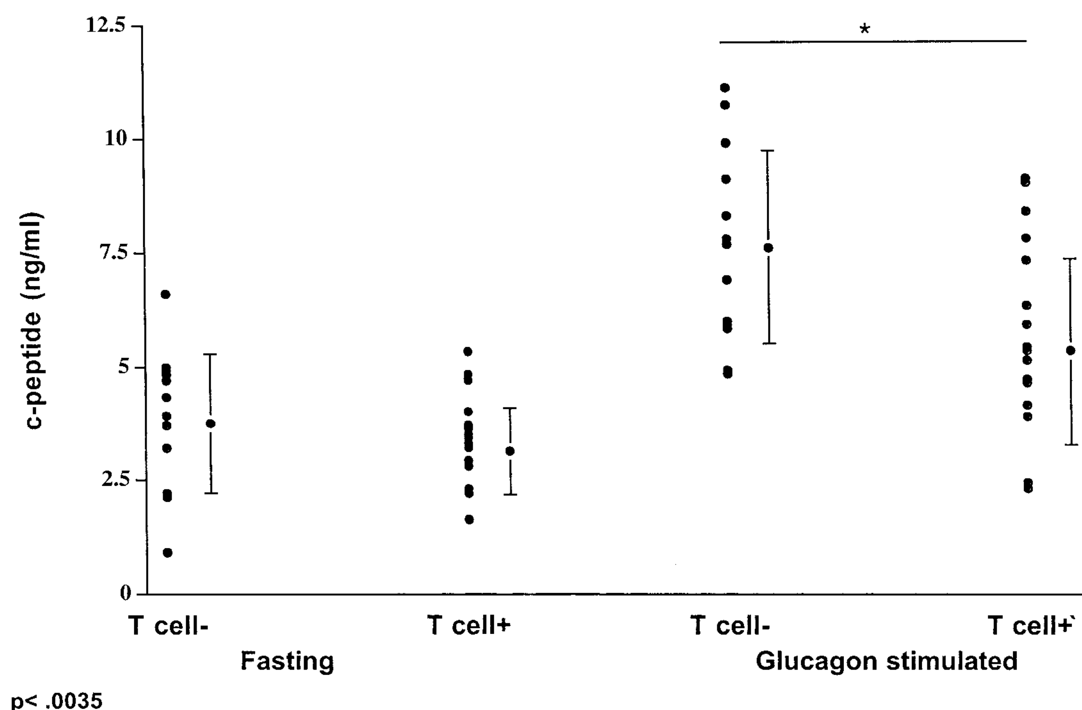

FIG. 4. Fasting and glucagon-stimulated plasma C-peptide in T-cell$^-$ (n = 13) and T-cell$^+$ (n = 23) patients independent of autoantibody status. Vertical bars with dots represent mean ± SD.

diabetes correlates with T-cell reactivity to islet proteins and Ab status.

In our study, T-cell reactivity and Abs were used to identify type 1.5 diabetic patients. First, we identified type 1.5 diabetes based on the presence of Abs. Of 36 phenotypic type 2 diabetic patients, 19 patients were negative and 17 were positive for Abs (ICAs, GAD Abs, IAAs, and IA-2Abs). No significant difference was observed in fasting and glucagon-stimulated C-peptide levels at baseline between the two groups. This is in agreement with some previous studies that also did not find a difference in C-peptide levels between type 1.5 and type 2 diabetes (20–24).

However, there are other studies that have reported differences in fasting C-peptide levels between type 1.5 and type 2 diabetes. These studies differ greatly from one another and from our study. Minimum age criteria for patients in the studies vary. For example, the studies by Gottsater et al. (12) and Torn et al. (13) included patients aged ≥15 years of age. The study by Borg et al. (14) had patients aged >20 years old, whereas Turner et al. (15) and Hosszufalusi et al. (16) included patients aged >25 years old. The study by Monge et al. (17) included patients aged >50 years. Because of the young age of the patients in some of the studies, it is possible that type 1 diabetic patients who are usually Ab$^+$ with lower β-cell function were included. In contrast, in our study, we included only phenotypic type 2 diabetic patients who were aged between 35 and 70 years to assess β-cell function in relation to Ab and T-cell reactivity status.

In our study we also included four autoantibodies to determine whether patients were Ab$^+$ or Ab$^-$. However, the Abs (ICAs, GAD Abs, IA-2Abs, and IAAs) were originally discovered in type 1 diabetic patients. We have reported differences in both Ab and T-cell reactivity to islet proteins between type 1 and type 1.5 diabetic patients (10). Therefore, there is still a possibility in our current study that patients identified as Ab$^-$ may indeed demonstrate Abs to unknown islet antigens. Although Ab reactivity to unidentified islet proteins is a definite possibility, the inclusion of ICAs helps address this issue. Sera positive for ICAs are recognized to frequently contain Abs to antigens other than GAD, IA-2, and insulin. Therefore, we believe that assaying patients for all four autoantibodies is the best attempt available at this time for identifying a patient's Ab status.

Furthermore, many of the patients in other studies (12,14,16,18,19) were taking insulin at the time of diagnosis or required insulin within a few years of diagnosis. Again, this could lead to skewing of results because the majority of insulin-treated subjects were in the Ab$^+$ group and probably had lower β-cell function that required

TABLE 1
β-Cell function in Ab$^+$/Ab$^-$ and T-cell$^+$/T-cell$^-$ groups

	Ab$^-$	Ab$^+$	*P* value (Ab$^+$/Ab$^-$)	T-cell$^-$	T-cell$^+$	*P* value (T-cell$^+$/T-cell$^-$)
n	19	17		13	23	
C-peptide (ng/ml)						
Fasting	3.4 ± 1.1	3.3 ± 1.3	0.9893	3.8 ± 0.1.5	3.1 ± 0.9	0.1379
Glucagon stimulated	6.3 ± 2.3	5.9 ± 2.5	0.6754	7.6 ± 2.1	5.3 ± 2.1	0.0035

Data are means ± SD.

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TABLE 2
Clinical characteristics of 36 phenotypic diabetic patients

Characteristic	Ab^-	Ab^+	T-cell$^-$	T-cell$^+$
n	19	17	13	23
Fasting glucose (mmol/l)	152 ± 53	181 ± 53	156 ± 57	171 ± 53
A1C (%)	7.0 ± 0.9	7.7 ± 1.3	7.2 ± 0.9	7.4 ± 1.3
BMI (kg/m^2)	34.1 ± 5.8	30.9 ± 5.2	34.0 ± 6.1	31.7 ± 5.4
Age (years)	55.5 ± 8.8	60.3 ± 6.8	55.6 ± 9.0	59.0 ± 7.5
Duration of diabetes (months)	34.9 ± 18.0	24.3 ± 18.4	27.6 ± 17.3	31.9 ± 19.7

Data are means ± SD.

treatment with insulin. In contrast, we excluded any patient who required insulin for glycemic control and patients with a history of ketoacidosis or ketonuria to avoid including any adult type 1 diabetic subjects.

Abs measured for classification of type 1.5 diabetes in previous studies were also not the same. Most studies measured GAD Abs and sometimes ICAs or IA-2Abs. The studies by Arikan et al. (19), Yang et al. (24), and Birkeland et al. (21) measured only GAD Abs. The U.K. Prospective Diabetes Study (UKPDS) (15) and the studies by Gottsater et al. (12) and Monge et al. (17) measured GAD Abs and ICAs. The studies by Borg et al. (14), Hosszufalusi et al. (16), and Tuomi et al. (18) measured three autoantibodies: GAD Abs, ICAs, and IA-2Abs. None of the studies measured all four autoantibodies. In all of the other studies, classification of type 1.5 diabetes was based on measurement of autoantibodies at only one point. In contrast, in our study, we checked patients for all four autoantibodies (GAD Abs, IA-2Abs, ICAs, and IAAs) to decrease misclassification of patients as having type 1.5 diabetes versus type 2 diabetes. We considered a patient Ab^+ if he or she had positive titers for the same antibody for two consecutive time points. We believe this to be a better way to ensure consistent Ab positivity in patients because we have seen that antibody titers can fluctuate, especially if they are borderline. We also restricted the duration of diabetes to 5 years in an attempt to maximize the sensitivity of antibody screening as a marker of type 1.5 diabetes. Restricting the analysis to patients with more recently diagnosed diabetes lessens the possibility of a misclassification of type 1.5 diabetes as type 2 diabetes because of reversion to an Ab^- status with increasing duration of diabetes. Such a reversion has been observed in type 1.5 diabetes over time (14).

Another confounding factor is the time from diagnosis of hyperglycemia. β-Cell function decreases with time and may decline relatively faster in Ab^+ patients compared with Ab^- patients. One large population-based study involving 1,122 type 2 diabetic patients performed by Tuomi et al. (18) showed that GAD Ab^+ patients had significantly lower fasting C-peptide levels than GAD Ab^- type 2 diabetic patients. The duration of diabetes was 81.6 and 68.4 months, respectively (18). This difference in duration of diabetes may account for at least some of the lower C-peptide levels in the GAD Ab^+ patients. In our study, all patients were required to have A1C between 6 and 10% and fasting C-peptide ≥0.80 ng/ml. The cut point of 0.8 ng/ml was chosen because we wanted patients in the drug study who, at least in the beginning of the study, could be treated with oral agents and not require insulin therapy. Moreover, the mean duration of diabetes was 30.0 and 30.2 months, respectively, in our patients. Therefore, the patients in this study are hypothesized to be earlier in the course of their disease progression based on higher A1C and C-peptide inclusion criteria. If this is the case, then our results would demonstrate that the T-cell responses can identify patients with a more severe β-cell lesion before detection by Abs. Because type 1.5 diabetes, like type 1 diabetes, is believed to be cell mediated, the fact that T-cells can identify patients with a more severe β-cell lesion earlier would be reasonable. Whether the C-peptide differences are detected by T-cells earlier than they are detected by Abs or irrespective of Abs remains to be decided. Future studies should help answer this question. However, the results in this study demonstrate that T-cells are able to identify patients with a more severe β-cell lesion irrespective of Abs.

In our study, we focused on the presence or absence of T-cell and Ab reactivity (positive for at least two consecutive time points) and the relationship to fasting and stimulated C-peptide. We found significantly lower ($P < 0.0035$) stimulated C-peptide responses correlated with T-cell positivity to islet proteins and not Ab positivity alone. We further investigated the relationship between the magnitude of T-cell and C-peptide responses by separating the patients with T-cell$^+$ responses into those with either low, medium, or high numbers of blot sections positive. We observed that neither fasting or glucagon-stimulated C-peptide correlated with the magnitude of T-cell responses but rather with the presence or absence of T-cell reactivity. Moreover, we further separated the patients into four groups on the basis of both their autoantibody status and T-cell status (Ab^-T-cell$^-$, Ab^+T-cell$^-$, Ab^-T-cell$^+$, and Ab^+T-cell$^+$): 8 patients were Ab^-T-cell$^-$, 5 patients were Ab^+T-cell$^-$, 11 patients were Ab^-T-cell$^+$, and 12 patients were Ab^+T-cell$^+$. The stimulated C-peptide responses demonstrated significant differences between the Ab^+T-cell$^+$ (mean 5.1 ng/ml) and Ab^+T-cell$^-$ (mean 8.2 ng/ml) patients with $P < 0.01$ and between Ab^+T-cell$^+$ (mean 5.1 ng/ml) and Ab^-T-cell$^-$ (mean 7.3 ng/ml) patients with $P < 0.03$. Stimulated C-peptide in Ab^+T-cell$^-$ and Ab^-T-cell$^-$ patients was not significantly different ($P = 0.47$). Thus, just the presence of autoimmunity as observed by Ab positivity alone did not identify the patients with more severe β-cell lesions.

We also questioned whether differences in insulin resistance (using homeostasis model assessment) might also contribute to our results. However, there was no significant difference between T-cell$^+$ or T-cell$^-$ subjects in their insulin sensitivity. Thus, a more severe β-cell lesion appears to correlate with the presence or absence of T-cell reactivity and cannot be explained in this study by differences in insulin sensitivity or magnitude of the T-cell response. We believe we have demonstrated that glucagon-stimulated C-peptide secretion is different in patients with and without autoimmunity as determined using T-cell assays, whereas this difference is not seen using only

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autoantibody assays. Therefore, we believe that cellular immunoblotting can identify patients with a more severe β-cell lesion irrespective of whether the patient is positive or negative for Abs to islet proteins. Consequently, we conclude that T-cell reactivity may be a better marker than Abs in detecting β-cell dysfunction in type 1.5 diabetes.

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