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DPD epitope-specific glutamic acid decarboxylase (GAD)65 autoantibodies in children with Type 1 diabetes

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Abstract

Aim—To study whether DPD epitope-specific glutamate decarboxylase autoantibodies are found more frequently in children with milder forms of Type 1 diabetes.

Methods—We prospectively evaluated 75 children with new-onset autoimmune Type 1 diabetes, in whom we collected demographic, anthropometric and clinical data and measured islet autoantibodies. Glutamate decarboxylase 65 autoantibody-positive samples were analysed for epitope specificities using recombinant Fab against the DPD-defined epitope of glutamate decarboxylase 65.

Results—After adjustment for age, positive DPD epitope recognition was significantly associated with higher C-peptide levels at onset ($P=0.02$, $r^2=0.21$, $n=35$), and high DPD recognition in the highest quartile tended to be associated with HbA_{1c} 53 mmol/mol (7%) at the last follow-up [mean (SD) follow-up 1.3 (0.4) years; $P=0.07$; for the model, $P=0.044$, $n=30$]. Age- and sex-adjusted BMI percentile was significantly correlated with recognition of the DPD-defined epitope ($P<0.03$, $r^2=0.14$, $n=34$), but this correlation was driven by the older age group (age 10 years; $P=0.016$, $r^2=0.27$, $n=21$) and was not significant in younger children ($P=0.93$, $n=13$). There were no independent associations with sex, race/ethnicity, diabetic ketoacidosis, HbA_{1c} , HLA DR3-DQ2/DR4-DQ8 or autoantibody number.

Conclusions—Our findings suggest that recognition of the DPD-defined glutamate decarboxylase 65 autoantibody epitope at Type 1 diabetes onset is directly associated with β -cell function, BMI and age, which supports the hypothesis that immunological factors contribute to the

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Competing interests

None declared.

clinical heterogeneity of Type 1 diabetes. Larger studies relating epitope-specific glutamate decarboxylase 65 autoantibody to clinical phenotype in children with Type 1 diabetes are warranted.

Introduction

Type 1 diabetes is predominantly a T-cell-mediated autoimmune disease characterized by selective destruction of pancreatic β cells. Type 1 diabetes is phenotypically heterogeneous with regard to age at onset, disease presentation, rate of β -cell function loss, responsiveness to immunotherapies and islet pathology [1–5], suggesting that its pathogenesis is complex and variable. Supporting this hypothesis, forms of autoimmune diabetes as different as fulminant diabetes, which has a rapid onset, and latent autoimmune diabetes in adults (LADA), a characteristic of which is slow progression of β -cell destruction, have been described [6,7]. These different forms of autoimmune diabetes are characterized by distinct clinical, genetic, immunological and metabolic characteristics.

We and others have compared islet-cell autoantibodies directed against the smaller isoform of glutamate decarboxylase (GAD)65 among patients with different clinical phenotypes of autoimmune diabetes, and have found distinct differences with respect to GAD65 autoantibody (GAD65Ab) epitope recognition [8–10]. These results indicate that the autoimmune response associated with different clinical phenotypes of autoimmune diabetes is reflected in GAD65Ab epitope specificity. Specifically, GAD65Ab present in patients with LADA recognized an epitope defined by monoclonal GAD65Ab DPD more frequently than GAD65Ab in patients with early-onset, more rapidly progressing autoimmune Type 1 diabetes [11]. The monoclonal GAD65Ab DPD was isolated from a patient with Type 1 diabetes [12] and binds to a conformational epitope located in the N-terminal region of GAD65 (amino acid residues 96–173) [13]. These DPD epitope-specific GAD65Abs are also present in high frequency in a distinct group of adult patients with ketosis-prone diabetes who express islet autoantibodies (A+) and maintain β -cell functional reserve (β +) [14].

Based on this association between GAD65Ab epitope specificity and β -cell function, we hypothesized that DPD epitope-specific GAD65Abs are more frequently present in children with milder forms of Type 1 diabetes. Moreover, we hypothesized that development of Type 1 diabetes in children with DPD-defined GAD65Abs occurs more frequently in association with other diabetogenic factors, such as obesity. To test these hypotheses, we correlated recognition of the DPD-defined GAD65Ab epitope and individual clinical variables in a longitudinally followed cohort of children with Type 1 diabetes.

Patients and methods

Patients

We prospectively evaluated 103 children who presented with new-onset autoimmune Type 1 diabetes at Texas Children's Hospital between October 2010 and October 2011, and followed them until October 2012. The study was approved by the institutional review board at Baylor College of Medicine/Texas Children's Hospital. Written informed consent and

assent, as applicable, was obtained from all patients. In this analysis we included all patients with available GAD65Ab data ($n=75$), with median (range) age at diagnosis of 10.5 (1.3–17.9) years.

Data collection

Study data were collected at diagnosis, 7 weeks [mean (SD) 7.2 (3.1) weeks] after onset and at the last available follow-up visit [mean (SD) 1.3 (0.4) years]. Insulin autoantibodies were measured within 10 days of diagnosis (before the development of antibodies to exogenous insulin). Other islet autoantibodies—to GAD65, islet cell autoantigen 512 (ICA512/IA2) and zinc transporter 8 (ZnT8)—were measured at the 7-week visit. Demographic information included date of birth, date of diagnosis, sex and race/ethnicity. Anthropometric information included weight and height measured at the first clinical visit [mean (SD) 9.9 weeks] to avoid the effect of dehydration on weight at the time of diagnosis. Biochemical data included glucose, HbA_{1c}, pH, bicarbonate and β -hydroxybutyrate measured at diagnosis. BMI was calculated in patients aged > 2 years based on height and weight and was categorized using sex-specific and age-specific percentiles according to the Centers for Disease Control and Prevention criteria [15]. Obesity was defined as BMI ≥ 95th sex-specific and age-specific BMI centile, and overweight as BMI centile ≥ 85th to <95th. For ages <2 years, BMI was considered a missing value because of the lack of standardized age-adjusted and gender-adjusted BMI data for ages 0–2 years. Ketoacidosis was defined as venous blood pH < 7.3 and bicarbonate < 15 mEq/l.

Laboratory methods

Autoantibodies—The GAD65, ICA512/IA2, and ZnT8 autoantibodies were measured using a radioligand binding assay as previously described [16,17] and as standardized in the International Combined Autoantibody Workshop [18]. The threshold for GAD65Ab positivity was set at 45 U/ml, established as the 98th percentile for healthy controls. Samples were considered ICA512/IA2 autoantibody (ICA512/IA2Ab)-positive if binding exceeded that of the 98th centile for healthy controls (30 RU/ml). For ZnT8 autoantibodies (ZnT8Ab), a threshold was set at 15 RU/ml for autoantibodies for ZnT8Arg and 26 RU/ml for ZnT8Trp, based on the 98th centile observed in 50 healthy human control serum samples. Samples were considered ZnT8Ab-positive if binding to either ZnT8Arg or ZnT8Trp was detected. Our laboratory participated in the Diabetes Autoantibody Standardization Program [19] workshop, and the GAD65Ab assay showed 86% sensitivity and 93% specificity, while the ICA512/IA2Ab assay showed 66% sensitivity and 98% specificity. ZnT8 autoantibodies were not included in the workshop. Insulin autoantibodies were measured by the Quest Diagnostics Nichols Institute (San Juan Capistrano, CA, USA) by radioimmunoassay, with clinical sensitivity and specificity of 50 and 99%, respectively (positivity > 0.4 U/mL).

Epitope-specific GAD65Ab assay—GAD65Ab-positive samples were analysed for epitope specificities using recombinant Fab against the DPD-defined epitope of GAD65. Only samples with a GAD65Ab titre above 70 U/ml could be analysed because, for samples with lower GAD65Ab titres, the decreased signal to noise ratio interferes with the accuracy of the assay, which rendered eight samples unsuitable for DPD epitope specificity measurement. Epitope mapping of GAD65Ab was performed using competitive

radiobinding assays as previously described [10]. The threshold for specific competition was determined to be >12.5% by using negative control rFab D1.3 (specific to hen egg lysozyme) to determine maximum non-specific competition.

C-peptide measurement—Random serum C-peptide levels were obtained at diagnosis and measured using a highly specific radioimmunoassay (Human C-Peptide RIA kit, Millipore Research Inc., St Louis, MO, USA).

Statistical analyses

Statistical analyses were conducted using STATA 12 (StataCorp 2007, Stata Statistical Software Release 10; StataCorp LP, College Station, TX, USA). To compare proportions among groups, we used the chi-squared test. To compare means we used *t*-tests, Mann–Whitney *U*-tests or one-way ANOVA, as appropriate. We used Spearman's correlation to evaluate the relationship between continuous variables, as appropriate. To control for confounders, multiple linear and logistic regression models were used, as appropriate. Serum C-peptide levels were log-transformed because of the non-normality of its distribution. Sample size estimation was not performed *a priori* for the present pilot study, but instead all available cases were used. Throughout the manuscript, data are expressed as mean (SD) values unless otherwise stated. *P* values <0.05 were considered statistically significant.

Results

The demographic, clinical and immunological characteristics of the patients included in the analysis are shown in Table 1. As per the eligibility criteria, all study participants had at least one islet autoantibody (either GAD65Ab, IA-2Ab, ZnT8Ab or insulin autoantibodies). DPD epitope specificity was tested in the patients with GAD65Ab titre above 70U/ml, and found to be above the threshold in 31.4% (11/35) of these patients.

In univariate analyses, among patients who were GAD65Ab-positive, those with DPD epitope recognition above the threshold ($n=11$), compared with those below the threshold ($n=24$), were significantly younger at diagnosis of Type 1 diabetes [mean (SD) 9.9 (3.3) vs 12.6 (3.25) years; $P=0.03$], more likely to be female (72.7 vs 33.3%; $P=0.03$) and had a higher BMI percentile [mean (SD) 85.7th (11.0) vs 72.1th (20.0) percentile; $P=0.0451$]. C-peptide levels tended to be higher in patients with positive DPD recognition [mean (SD) 0.64 (0.33) ng/ml] compared with patients with negative DPD recognition [mean (SD) 0.45 (0.24) ng/ml; $P=0.0514$]. These and other comparisons between patients with positive and negative DPD epitope recognition are shown in Table 2. There was no significant correlation between GAD65Ab titres and DPD epitope recognition ($P=0.73$). Mean (SD) GAD65Ab titres were 0.59 (0.51) in patients with and 0.68 (0.73) in patients without DPD epitope recognition ($P=0.73$).

Multivariate analyses showed that patients with positive DPD epitope recognition had higher C-peptide levels at diagnosis compared with patients without DPD epitope recognition, after adjustment for age ($P=0.011$; for the model, $P=0.022$, $r^2=0.21$, $n=35$). BMI was not an independent factor in this association. Furthermore, among patients with a follow-up HbA_{1c}

measurement at least 6 months after onset, those with DPD epitope recognition in the highest quartile tended to achieve the target of HbA_{1c} $\leq$ 53 mmol/mol (7%) more frequently than their counterparts with lower recognition, after adjustment for age ($P=0.07$; for the model, $n=30$, $P=0.044$).

The BMI percentile was significantly correlated with recognition of the DPD-defined epitope ($P<0.03$, $r^2=0.14$, $n=34$), but this correlation was driven by the older patients (>10 years; $P=0.016$, $r^2=0.27$, $n=21$) and was not significant in younger patients ($P=0.93$, $n=13$). Among patients aged ≤ 10 years, all five patients with DPD epitope recognition above the threshold were obese or overweight (100%) compared with five out of 16 (31.3%) patients without DPD recognition ($P=0.007$). There were no independent associations with sex, race/ethnicity, diabetic ketoacidosis, HbA_{1c}, HLA DR3 DR3-DQ2/DR4-DQ8 or autoantibody number.

Discussion

In the present study, we observed that recognition of the DPD-defined epitope was associated with higher serum C-peptide levels at diagnosis, indicating a greater level of residual β -cell function. In addition, having DPD epitope recognition in the highest quartile tended to be associated with a greater likelihood of achieving HbA_{1c} $\leq$ 53 mmol/mol (7%), suggesting a clinically milder disease. We also noted that the correlation between recognition of DPD-defined epitope and BMI in patients with new-onset diabetes was largely driven by older patients (aged >10 years). Taken together, these findings suggest that the phenotypic heterogeneity of paediatric Type 1 diabetes (1–6) reflects, at least in part, different forms of islet autoimmunity, manifested by variable GAD65Ab epitope specificity. GAD65Ab recognition of the DPD-defined epitope has been previously associated with milder progressive autoimmune diabetes in patients with LADA [9,11] and in those with the A+B+ subtype of ketosis-prone diabetes [10]. The present results indicate that, in children, this epitope-specificity of GAD65Ab is associated with characteristics reflective of milder disease, such as a greater level of residual β -cell function at diagnosis, and better glycaemic control on follow-up.

Obesity may contribute to diabetogenesis by increasing insulin needs. It could also contribute to β -cell death through endoplasmic reticulum stress [20]. By these or other mechanisms, obesity may serve as a secondary risk factor that increases disease penetrance in individuals with only mild islet autoimmunity, who thus progress to overt diabetes in childhood. The relevance of studies to understand the impact of obesity on the pathogenesis of Type 1 diabetes is underscored by the fact that 20% of children are obese or overweight at diagnosis of Type 1 diabetes despite the weight loss that often accompanies diabetes onset [18,19]. We have previously shown that obese and overweight children have greater β -cell function, as exhibited by a higher C-peptide level at diagnosis [21]. In this study, we observed that patients with stronger DPD epitope recognition tended to have higher C-peptide levels at diagnosis and lower HbA_{1c} ($\leq$ 53 mmol/mol) at the last follow-up.

The present study is the first to report baseline and longitudinal clinical characteristics associated with GAD65Ab recognition of the DPD-defined epitope in paediatric Type 1

diabetes. The relatively small sample size is a limitation of the study that may have contributed to the lack of statistical significance in the correlation between GAD65Ab epitope recognition and C-peptide level as continuous variables. Although the generalizability of the present results may be limited in populations with different racial or ethnic distribution, we did not find an association with race/ethnicity and, in previous studies, we did not observe any ethnicity effect [22]. Serum C-peptide levels were obtained as a random measure at diagnosis of Type 1 diabetes. While mixed-meal stimulated C-peptide is the preferred method to assess β -cell function, C-peptide stimulated by hyperglycaemia at diagnosis has been successfully used as an indicator of clinically relevant residual β -cell function. We have previously used random C-peptide at diagnosis and islet autoantibody positivity to define four groups of paediatric diabetes with distinct characteristics at diagnosis and 2 years later [23]. In a separate study, higher random C-peptide level at diagnosis was associated with better HbA_{1c} and lower ketoacidosis risk [21]. Similarly, Ludvigsson *et al.* [24] used random C-peptide at diagnosis in the classification of paediatric diabetes. Random C-peptide has also been used in other studies to assess β -cell function [25–27].

Finally, longer follow-up of the cohort would allow the study of changes in the relationship between DPD recognition and HbA_{1c}.

Although there are studies that show that GAD65Ab affect GAD65 antigen presentation in epitope-specific ways [28–30], islet autoantibodies are currently thought not to play a major pathogenic role in Type 1 diabetes but rather to be a reflection of the underlying autoimmune response. Accordingly, GAD65Ab recognition of the DPD epitope seems to be a marker of milder autoimmune response.

Taken together, the results of the present study suggest that recognition of the DPD-defined GAD65Ab epitope at onset of Type 1 diabetes is associated with β -cell function, BMI and age, which supports the hypothesis that the clinical heterogeneity of paediatric Type 1 diabetes has a basis in variable immunological response, reflected in the epitope specificity of GAD65Ab. Larger studies relating epitope-specific GAD65Ab to clinical phenotype in children with Type 1 diabetes are warranted.

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What's new?

- This is the first study to report the recognition of the DPD-defined glutamic acid decarboxylase 65 autoantibody (GAD65Ab) epitope in children with Type 1 diabetes.
- Recognition of DPD-defined GAD65Ab epitope is directly correlated with β -cell function, BMI and age in children with newly diagnosed Type 1 diabetes.
- Differential GAD65Ab epitope recognition may reflect immunological heterogeneity in Type 1 diabetes and perhaps explain the clinical heterogeneity of the disease.

FIGURE 1.

Glutamic acid decarboxylase 65 autoantibody (GAD65Ab) DPD recognition correlates with age- and sex-adjusted BMI percentile in children aged 10 years. ($P<0.02$, $r^2=0.27$, $n=21$).

Table 1Participant characteristics (*n*=75)

Mean (SD) age at diagnosis, years	10.5 (4.1)
Male gender, % (<i>n</i> / <i>N</i>)	58.7 (44/75)
Race/ethnicity, % (<i>n</i> / <i>N</i>)	
Non-Hispanic white	50 (39/75)
Hispanic/Latino	34.7 (26/75)
African-American	9.3 (7/75)
Other	4.0 (3/75)
Ketoacidosis at diagnosis, % (<i>n</i> / <i>N</i>)	41.9 (31/74)
HLA DR3-DQ2/DR4/DQ8, % (<i>n</i> / <i>N</i>)	22.9 (16/70)
Mean (SD) BMI percentile	75.5 (22.0)
HbA _{1c} , mmol/mol	
At diagnosis	106 (24.0)
At 7 weeks	61 (18.6)
At last follow-up *	73 (23.0)
C-peptide, ng/ml	0.55 (0.55)
Islet autoantibody positivity, % (<i>n</i> / <i>N</i>)	
GAD65Ab	57.3 (43/75)
GAD65Ab DPD recognition	31.4 (11/35)
ICA512/IA-2Ab	72.0 (54/74)
Insulin autoantibody	41.9 (34/74)
ZnT8Ab	62.7 (47/75)
Single autoantibody	18.9 (14/74)

GAD65Ab, glutamic acid decarboxylase 65 autoantibody; ICA512/IA-2Ab, islet cell autoantigen 512 autoantibody; ZnT8Ab, ZnT8 autoantibody.

* Only participants with follow-up >6 months (median 1.4 years, *n*=74).

Table 2

Comparison of characteristics in glutamic acid decarboxylase 65 autoantibody-positive patients with and without positive DPD epitope recognition ($n=35$)

	DPD epitope-positive	DPD epitope-negative	<i>P</i>
Mean (SD) age at diagnosis, years	9.9 (3.3)	12.6 (3.3)	0.03
Male gender, % (<i>n/N</i>)	27.3 (3/11)	66.7 (16/24)	0.03
Race/Ethnicity, % (<i>n/N</i>)			0.58
Non-Hispanic white	63.6 (7/11)	41.7 (10/24)	
Hispanic/Latino	27.3 (3/11)	37.5 (9/24)	
African-American	9.1 (1/11)	12.5 (3/24)	
Other	0	8.3 (2/24)	
Ketoacidosis at diagnosis, % (<i>n/N</i>)	36.4 (4/11)	43.5 (10/23)	0.69
HLA DR3-DQ2/DR4/DQ8, % (<i>n/N</i>)	27.3 (3/11)	31.8 (7/22)	0.78
BMI percentile	85.7 (11.0)	72.1 (20.0)	0.0451
HbA _{1c} , mmol/mol			
At diagnosis	11.7 (2.4)	12.5 (2.2)	0.364
At 7 weeks	7.8 (1.2)	7.9 (2.2)	0.97
At last follow-up *	8.6 (2.1)	9.0 (2.1)	0.65
Mean (SD) C-peptide, ng/ml	64 (0.33)	0.45 (0.24)	0.051
Islet autoantibody positivity, % (<i>n/N</i>)			
GAD65 Autoantibody	100% (11/11)	100% (24/24)	N/A
GAD65Ab DPD Recognition	100% (11/11)	0% (0/24)	N/A
ICA512/IA-2Ab	81.8 (9/11)	66.7 (16/24)	0.36
Insulin autoantibody	27.3 (9/23)	39.1 (3/11)	0.50
ZnT8Ab	45.5 (5/11)	70.8 (17/24)	0.15
Single autoantibody	9.1 (1/11)	13.0 (3/23)	0.74

GAD65Ab, glutamic acid decarboxylase 65 autoantibody; ICA512/IA-2Ab, islet cell autoantigen 512 autoantibody; ZnT8Ab, ZnT8 autoantibody.

* Only participants with follow-up > 6 months (median 1.4 years, $n=74$).