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ORIGINAL ARTICLE

Clinical manifestation and islet β-cell function of a subtype of latent autoimmune diabetes in adults (LADA): positive for T cell responses in phenotypic type 2 diabetes

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Abstract

Aims To investigate the possibility of identifying a subtype of latent autoimmune diabetes in adults (LADA), T-LADA (T cell responses-positive and autoantibody-negative) from patients with phenotypic type 2 diabetes (T2D) by enzyme-linked immunospot (ELISPOT).

Methods Eighty-two patients with phenotypic T2D were studied. Autoantibodies against glutamic acid decarboxylase (GAD), insulinoma-associated protein-2 and zinc transporter 8 were measured by radioligand assay. Thirty-nine Ab^+ and 43 Ab^- patients with phenotypic T2D were enrolled for T cell assay of responses to GAD65 and C-peptide antigen by ELISPOT.

Results (1) Eleven of 43 Ab^- participants with phenotypic T2D were demonstrated interferon (IFN)-γ secreting T cells by ELISPOT, while 13 of 39 Ab^+ patients with phenotypic T2D were positive for T cells responses to islet antigens. (2) The onset ages of T $cell^+$ people with phenotypic T2D were younger than that of T $cell^-$ individuals (42.7 ± 9.3 vs. 48.2 ± 10.2 years, $P = 0.025$). Moreover, T $cell^+$ patients with T2D displayed a significantly lower fasting C-peptide (FCP) compared with T $cell^-$ participants [0.28 (0.02–0.84) vs. 0.42 (0.05–1.26) nmol/L, $P = 0.013$]. (3) Ab^-T^+ group had a significantly lower FCP compared with Ab^-T^- group [0.31 (0.13–0.84) vs. 0.51 (0.07–1.26) nmol/L, $P = 0.023$].

Conclusions By measuring T cell responses to islet antigens in patients with phenotypic T2D, we identified a specific subtype of LADA who may be associated with worse basal β-cell function than classic T2D (Ab^-T^-).

Keywords Latent autoimmune diabetes in adults (LADA) · Type 2 diabetes · T-LADA · Enzyme-linked immunospot (ELISPOT) · Islet β-cell function

Introduction

Latent autoimmune diabetes in adults (LADA) is considered as a T cell-mediated autoimmune disease with early clinical manifestations similar to type 2 diabetes (T2D), distinguished from T2D by the presence of circulating islet autoantibodies (Abs), including glutamic acid decarboxylase autoantibody (GADA), insulinoma-associated protein-2 autoantibody (IA-2A), zinc transporter 8 autoantibody (ZnT8A) and insulin autoantibody (IAA) [1–3], accounting for at least 5.9% of newly diagnosed T2D [4, 5]. However, the prevalence of islet autoimmunity in phenotypic T2D may be underestimated when detected by islet Abs alone.

In recent years, using cellular immunoblotting, Goel et al. [6] identified a group of patients in phenotypic T2D who have T cell reactive to multiple islet proteins but are negative for islet Abs. The researchers proposed these Ab^-T^+

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individuals with phenotypic T2D should belong to a unique subgroup of LADA, T-LADA [7]. These patients seem to have a more severe β-cell lesion [8] with a more rapid β-cell functional decline with follow-up [9]. Cellular immunoblot, however, is under debate owing to lack of control tissues or specific identification of target antigens. Hence, it is a high priority to confirm whether there are islet-antigen-specific T cell responses in T2D by other T cell assays. Alternatively, enzyme-linked immunospot (ELISPOT) assay is a promising technique, allowing detection of antigen-reactive T cells and their cytokine response qualitatively and quantitatively at single-cell resolution. Herein, we hypothesized that stratification of phenotypic T2D according to islet-specific T cell responses by ELISPOT combined with islet Abs could help us to identify a subtype of LADA (Ab^-T^+), who may be associated with worse β-cell function than classic T2D (Ab^-T^-).

Participants and methods

Participants

Patients were enrolled in the Department of Endocrinology at the Second Xiangya Hospital of Central South University. The inclusion criteria were as follows: (a) diagnosis of diabetes (World Health Organization [WHO] 1999 criteria) at age over 18 years old; (b) insulin independence (usage of insulin < 1 month) for at least 6 months postdiagnosis; (c) duration of diabetes within 5 years. The exclusion criteria included (a) gestational diabetes and other specific types of diabetes; (b) acute infection, ketosis or ketoacidosis within 2 weeks at the time of blood draw; (c) use of glucocorticoids or immunomodulator in half of a year; (d) history of any malignancy or other severe diseases; (e) women in pregnancy. C-peptide levels and serum glucose were measured before and 120 min after a standard 543.6-kcal mixed-meal tolerance test (44.4% of calories as carbohydrate, 47.7% as fat and 7.9% as protein). Clinical and laboratory data including age, gender, duration of diabetes, height, weight, waist circumferences, hip circumferences, total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-CH), low-density lipoprotein cholesterol (LDL-CH), triglyceride (TG), glycosylated hemoglobin A1c (HbA1c), fasting blood glucose (FBG), 2-h postprandial blood glucose (2hBG), fasting C-peptide (FCP) and 2-h postprandial C-peptide (2hCP) were collected with standardized methods by trained physicians and clinical biochemistry laboratories. GADA, IA-2A and ZnT8A were analyzed by radioligand assay in duplicate as previously described [10–12]. In the Islet Autoantibody Standardization Program (IASP) 2012, all our assays showed high sensitivity and specificity, i.e., 78.0% and 96.7% for GADA, 74.0% and 96.7% for IA-2A, and 70.0% and 98.9% for ZnT8A, respectively. There were 50 men and 32 women with phenotypic T2D enrolled in this study. The mean ± SD age was 48.1 ± 10.4 years and the mean ± SD duration of diabetes was 19 ± 15 months.

T cell assay: ELISPOT

The ELISPOT assay was performed as described previously [13]. Briefly, polyvinylidene difluoride (PVDF) ELISPOT plates (Millipore MSIPS4510, Ireland) were coated overnight with anti-interferon (IFN)-γ Ab (U-Cytech, Utrecht, the Netherlands). Plates were blocked with RPMI + 10% human AB serum. Along with 2.5U/mL recombinant human IL-2 (R&D, Minneapolis, USA), the following antigens were added in triplicate wells: GAD65 (25 μg/mL final concentration, Diamyd, Sweden) and its diluent (RPMI medium) as negative control as well as 1 μmol/mL C-peptide (GL Biochem, Shanghai, China). Pantaxim (2 μl/well, Sanofi Pasteur, France) was added in duplicate wells as positive control. PBMCs were seeded at 3×10^5 cells/well and cultured for 40–48 h. After PBMC removal, IFN-γ secretion was visualized with a biotinylated anti-IFN-γ antibody (U-Cytech), ExtrAvidin-Alkaline Phosphatase (Sigma, USA) and nitro blue tetrazolium-5-bromo-r-chloro-3-indolyl phosphate (NBT-BCIP) reagent (Roche, German). Spots were counted by an ELISPOT reader (CTL, USA). A stimulation index (SI) for each antigen was calculated as the ratio of mean number of spots in experimental wells divided by the mean number of spots in negative control wells. The lowest measured value (0.5) was substituted when the mean value of spots in negative control wells was zero as a ratio cannot be calculated with a denominator of zero [14]. According to the results in 79 control individuals and 37 patients with autoimmune type 1 diabetes (T1D), receiver-operator characteristic plot analysis was used and a response to GAD is considered positive when SI > 3.2 with a specificity of 92.4% and sensitivity of 37.8%. Based on the results in 79 control individuals and 39 people with autoimmune T1D, a response to CP is designated as positive if SI > 6.3 with a specificity of 91.1% and sensitivity of 35.9%.

Statistics

Statistical analysis was performed with SPSS 20.0 software. Data were presented as mean ± standard deviation (SD), median and range or as indicated. A *t*-test was used to examine the age, duration of diabetes, body mass index (BMI), waist-hip ratio (WHR), FBG, 2hBG, HbA1c, CHOL, HDL-CH and LDL-CH between groups. A Mann–Whitney *U* test was performed to determine statistical significance in C-peptide and TG between the patient groups. A Chi-square test was used to compare categorical variables. Two-sided

statistical tests were performed, and a P value of <0.05 was considered significant.

Results

Islet Abs

With islet Abs (GADA, IA-2A and ZnT8A) determined, 39 of 82 participants recruited in this study were defined as classic Ab^+ LADA. Among these 39 Ab^+ patients, 32 were GAD Ab^+ alone, three were positive for both GADA and IA-2A, and four were positive for three Abs. No statistical difference was observed in gender, age, duration of diabetes, BMI, WHR, FBG, 2hBG, HbA1c, CHOL, HDL-CH and LDL-CH between Ab^+ and Ab^- groups. However, Ab^- group had a significantly higher level of TG compared with Ab^+ group [1.07 (0.30–6.80) vs. 1.61 (0.57–7.13) mmol/L, $P=0.005$].

T cell responses to islet antigens

Eleven of 43 Ab^- patients with T2D phenotype were demonstrated IFN-γ secreting T cells by ELISPOT (four cases reactive to GAD alone, six responsive to CP alone and one reactive to both GAD and CP); Thirteen of 39 Ab^+ patients with phenotypic T2D positive for islet antigens (one reactive to GAD alone, ten responsive to CP alone and two reactive to both (see Fig. 1).

According to the results of T cell assay, 82 participants with T2D phenotype were divided into T^+ and T^- groups. No statistical difference was observed in gender, duration of diabetes, GADA positivity proportion, BMI, WHR, FBG, 2hBG, HbA1c as well as serum lipid profile between T^+ and T^- groups. However, the age as well as the onset age of T^+ group were significantly younger than that of T^- group (44.4 ± 9.1 vs. 49.7 ± 10.5 years, $P=0.033$; 42.7 ± 9.3 vs. 48.2 ± 10.2 years, $P=0.025$).

Islet β-cell function

Compared with Ab^- group, Ab^+ group had significantly lower levels of FCP [0.31 (0.02–0.71) vs. 0.48 (0.07–1.26) nmol/L, $P=0.001$] as well as 2hCP [0.98 (0.13–2.58) vs. 1.32 (0.23–3.09) nmol/L, $P=0.011$]. There was no statistical difference in ΔCP (2hCP-FCP) between Ab^+ and Ab^- groups. T^+ T2D group displayed a significantly lower fasting C-peptide (FCP) compared with T^- group [0.28 (0.02–0.84) vs. 0.42 (0.05–1.26) nmol/L, $P=0.013$] (Fig. 2). There was no statistical difference in 2hCP and ΔCP between T^+ and T^- groups.

To further investigate the Ab^- group, we compared the Ab^-T^+ and Ab^-T^- groups. No statistical difference was observed in gender, age, duration of diabetes, BMI, WHR, FBG, 2hBG, HbA1c as well as serum lipid profile between Ab^-T^+ and Ab^-T^- groups (Table 1). Ab^-T^+ group had a significantly lower FCP compared with Ab^-T^- group [0.31 (0.13–0.84) vs. 0.51 (0.07–1.26) nmol/L, $P=0.023$] (Fig. 3), while there was no statistical difference in 2hCP and ΔCP between these two groups.

To gain more information about Ab^-T^+ group, we also compared it with classic LADA, Ab^+ group. No statistical difference was observed in gender, age, duration of diabetes, BMI, WHR, FBG, 2hBG, HbA1c, CHOL, HDL-CH and LDL-CH between these two groups except that Ab^-T^+ group had a higher level of TG compared with Ab^+ group [1.07 (0.30–6.80) vs. 1.98 (0.57–4.99) mmol/L, $P=0.045$]. Besides, there was no statistical difference in FCP, 2hCP and ΔCP between Ab^-T^+ and Ab^+ groups.

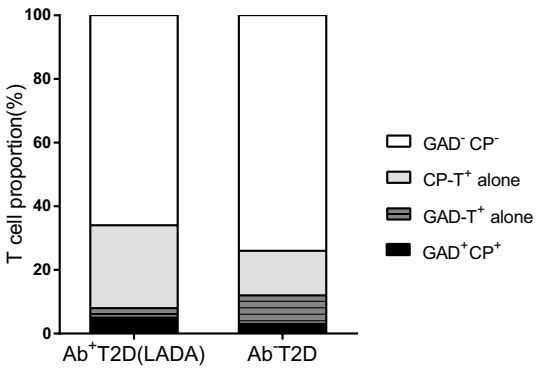

Fig. 1 Constituent ratio of T cell positivity in Ab^+ ($n=39$) and Ab^- ($n=43$) groups. *T2D* type 2 diabetes, *LADA* latent autoimmune diabetes in adults, *GAD* glutamic acid decarboxylase, *CP* C-peptide

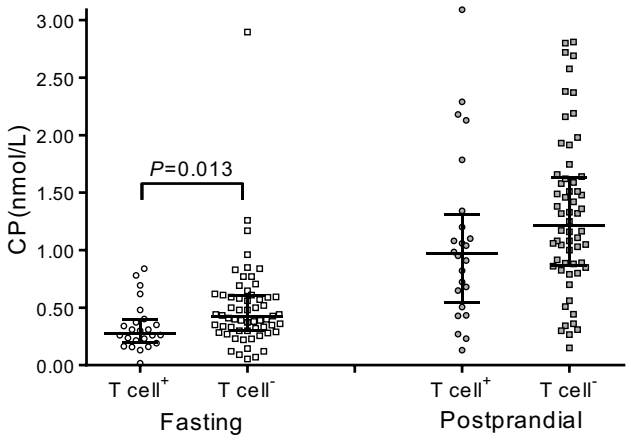

Fig. 2 Islet β-cell function in T^+ ($n=24$) and T^- ($n=58$) groups independent of autoantibody status. Data were presented as dot plots with indications of median, 25th and 75th percentiles. *CP* C-peptide

Table 1 Clinical characteristics of Ab^-T^+ and Ab^-T^-groups

	Ab^-T^+ ($n=11$)	Ab^-T^- ($n=32$)
Men/women	8/3	18/14
Age (years)	46.5 ± 9.4	48.6 ± 9.7
Age at diagnosis (years)	44.5 ± 10.0	47.4 ± 9.7
Duration of diabetes (years)	2.0 ± 1.5	1.2 ± 1.1
BMI (kg/m^2)	22.4 ± 3.2	23.4 ± 3.4
WHR	0.89 ± 0.04	0.90 ± 0.09
FBG (mmol/L)	8.2 ± 3.2	8.6 ± 2.8
2hBG (mmol/L)	11.8 ± 3.7	13.2 ± 5.2
HbA1c (%)	7.4 ± 2.0	7.3 ± 1.8
HbA1c (mmol/mol)	57 ± 21.9	56 ± 19.7
TG (mmol/L)	1.98 (0.57–4.99)	1.53 (0.66–7.13)
CHOL (mmol/L)	4.31 ± 1.11	4.93 ± 1.08
HDL-CH (mmol/L)	1.14 ± 0.53	1.20 ± 0.28
LDL-CH (mmol/L)	2.35 ± 0.86	3.02 ± 0.86

Data were expressed as mean ± standard deviation (SD), median (range) or frequency

BMI body mass index, *WHR* waist-hip ratio, *FBG* fasting blood glucose, *2hBG* 2-h postprandial blood glucose, *HbA1c* glycosylated hemoglobin A1c, TG triglyceride, *CHOL* total cholesterol, *HDL-CH* high-density lipoprotein cholesterol, *LDL-CH* low-density lipoprotein cholesterol

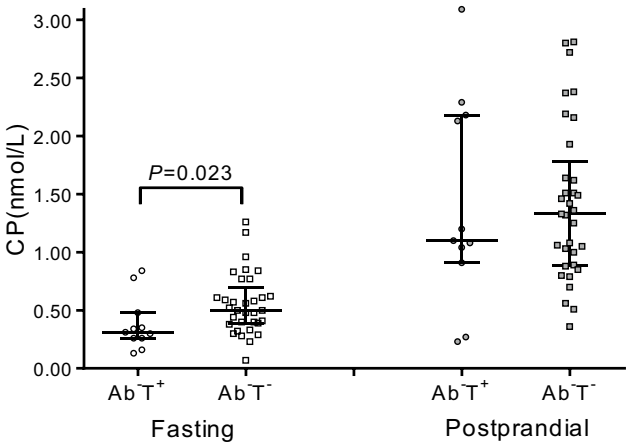

Fig. 3 Islet β-cell function in Ab^-T^+ ($n=11$) and Ab^-T^- ($n=32$) groups. Data were presented as dot plots with indications of median, 25th and 75th percentiles. *CP* C-peptide

Discussion

Previously, an epitope-specific ELISPOT assay in 15 patients with T2D and ten healthy donors showed that T2D subjects had more IFN-γ producing $CD4^+$ T cells than controls [15]. Brooks-Worrell et al. analyzed T cell responses in less than 40 people with phenotypic T2D by cellular immunoblot [6, 8], while our study enrolled 82 patients (including 39 Ab^+ and 43 Ab^- cases) with the largest sample size so far that gave evidence of T cell autoreactivity in phenotypic T2D. In our study, using ELISPOT, we confirmed that stratification of phenotypic T2D based on T cell responses and islet Abs help identification of a subtype of LADA (Ab^-T^+), who may be associated with lower basal β-cell function compared with classic T2D (Ab^-T^-).

Clinically, LADA is diagnosed routinely by islet Abs at present which are just fluid autoimmune biomarkers, not sufficient to induce β-cell lesions, even though the islet-antigen-specific T cells are considered as primary effectors of β-cell destruction. Additionally, the positive rate of Abs in diabetes is different as a result of distinct races, positive thresholds and so on [16–18], implying that patients with negative Abs could not exclude the possibility of LADA even if multiple Abs are analyzed. Since the decreasing rate of islet β-cell function in LADA is three times that of T2D as we have reported [19], early identification of LADA is of great importance for accurate therapeutic choice in clinical practice. Here, we observed that 11 of 43 (25.6%) Ab^- T2D patients had T cells responding to islet antigens, who could be called T-LADA as previously reported by Brooks-Worrell team using cellular immunoblot [20]. Unlike ELISPOT, cellular immunoblot utilizes human islet comprising the full spectrum of islet antigens, expected to be detected more readily because a larger pool of potentially responsive T cells is present in the blood. Therefore, it is understandable that Brooks-Worrell et al. [21] estimates the percentage of phenotypic T2D with islet autoimmunity may be higher than 30%. However, some limitations such as the difficult availability of human islets restrict the application of cellular immunoblot. Alternatively, by ELISPOT, we detected T cell responses to specific antigens GAD65 [22–24] in combination with C-peptide, key targets of autoreactive CD4 + T cells in T1D [25, 26]. In addition, allowing the HLA-unrestricted evaluation of T cell responses, full-length antigens GAD65 and C-peptide, instead of peptides, are of great value of clinical application.

In our study, T $cell^+$ T2D group displayed a significantly lower FCP than T $cell^-$ group independent of Ab status. Looking into the Ab^- T2D, T-LADA (Ab^-T^+) group had similar islet β-cell function compared with classic LADA, Ab^+ group, but a significantly lower FCP compared with Ab^-T^- group. In brief, islet autoimmunity in phenotypic T2D may be associated with poorer basal islet β-cell function. Accordingly, patients with T-LADA need more rigorous follow-up, as they are prone to the need for insulin therapy. In addition, this Ab^-T^+ group, namely T-LADA, may gain more benefits such as β-cell functional improvement from immune-modulating therapies as mentioned formerly [21, 27, 28]. Similarly, Brooks-Worrell et al. reported that people with T-LADA seem to have a more severe β-cell

lesion using cellular immunoblot [8]. Whereas, we did not observe statistical differences in 2hCP and ΔCP between T^+/T^- groups or Ab^-T^+/Ab^-T^-groups, which is not completely consistent with previous studies. Besides different inclusion criteria and T cell assays, the results of mixed-meal tolerance test (MMTT) we conducted and glucagon stimulation test (GST) in other studies should not be directly comparable, although studies indicated that the MMTT is more sensitive and more reproducible than the GST [29].

Limitations of our study include the lack of longitudinal follow-up. Our follow-up study under way will further determine the effect of T cell autoreactivity on the progressive β-cell dysfunction in T2D. In conclusion, by measuring islet-antigen-specific T cell responses in phenotypic T2D, we identified a specific group of patients with Ab^-T^+ LADA, T-LADA. Compared with classic T2D (Ab^-T^-), T-LADA may be associated with lower basal β-cell function. Further prospective studies are needed for confirmation in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (the Second Xiangya Hospital, Central South University, China) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Niechcial E, Rogowicz-Frontczak A, Pilacinski S et al (2018) Autoantibodies against zinc transporter 8 are related to age and metabolic state in patients with newly diagnosed autoimmune diabetes. Acta Diabetol 55(3):287–294. https://doi.org/10.1007/s00592-017-1091-x
2. Huang G, Wang X, Li Z, Li H, Li X, Zhou Z (2012) Insulin autoantibody could help to screen latent autoimmune diabetes in adults in phenotypic type 2 diabetes mellitus in Chinese. Acta Diabetol 49(5):327–331. https://doi.org/10.1007/s00592-010-0196-2
3. Jin P, Huang G, Lin J, Luo S, Zhou Z (2011) Epitope analysis of GAD65 autoantibodies in adult-onset type 1 diabetes and latent autoimmune diabetes in adults with thyroid autoimmunity. Acta Diabetol 48(2):149–155. https://doi.org/10.1007/s00592-010-0250-0
4. Zhou Z, Xiang Y, Ji L et al (2013) Frequency, immunogenetics, and clinical characteristics of latent autoimmune diabetes in China (LADA China study): a nationwide, multicenter, clinic-based cross-sectional study. Diabetes 62(2):543–550
5. Qi X, Sun J, Wang J et al (2011) Prevalence and correlates of latent autoimmune diabetes in adults in Tianjin, China: a population-based cross-sectional study. Diabetes Care 34(1):66–70. https://doi.org/10.2337/dc10-0488
6. Goel A, Chiu H, Felton J, Palmer JP, Brooks-Worrell B (2007) T-cell responses to islet antigens improves detection of autoimmune diabetes and identifies patients with more severe beta-cell lesions in phenotypic type 2 diabetes. Diabetes 56(8):2110–2115. https://doi.org/10.2337/db06-0552
7. Naik RG, Brooks-Worrell BM, Palmer JP (2009) Latent autoimmune diabetes in adults. J Clin Endocrinol Metab 94(12):4635–4644. https://doi.org/10.1210/jc.2009-1120
8. Brooks-Worrell BM, Reichow JL, Goel A, Ismail H, Palmer JP (2011) Identification of autoantibody-negative autoimmune type 2 diabetic patients. Diabetes Care 34(1):168–173. https://doi.org/10.2337/dc10-0579
9. Brooks-Worrell BM, Boyko EJ, Palmer JP (2014) Impact of islet autoimmunity on the progressive beta-cell functional decline in type 2 diabetes. Diabetes Care 37(12):3286–3293. https://doi.org/10.2337/dc14-0961
10. Petersen JS, Hejnaes KR, Moody A et al (1994) Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. Diabetes 43(3):459–467. https://doi.org/10.2337/diab.43.3.459
11. Yang L, Luo S, Huang G et al (2010) The diagnostic value of zinc transporter 8 autoantibody (ZnT8A) for type 1 diabetes in Chinese. Diabetes Metab Res Rev 26(7):579–584. https://doi.org/10.1002/dmrr.1128
12. Huang G, Xiang Y, Pan L, Li X, Luo S, Zhou Z (2013) Zinc transporter 8 autoantibody (ZnT8A) could help differentiate latent autoimmune diabetes in adults (LADA) from phenotypic type 2 diabetes mellitus. Diabetes Metab Res Rev 29(5):363–368
13. Zheng C, Zhou Z, Yang L et al (2011) Fulminant type 1 diabetes mellitus exhibits distinct clinical and autoimmunity features from classical type 1 diabetes mellitus in Chinese. Diabetes Metab Res Rev 27(1):70–78. https://doi.org/10.1002/dmrr.1148
14. Petrich dMLG, Fu J, Connor KJ et al (2010) IFN-gamma and IL-10 islet-antigen-specific T cell responses in autoantibody-negative first-degree relatives of patients with type 1 diabetes. Diabetologia 53(7):1451–1460
15. Sarikonda G, Pettus J, Phatak S et al (2014) CD8 T-cell reactivity to islet antigens is unique to type 1 while CD4 T-cell reactivity exists in both type 1 and type 2 diabetes. J Autoimmun 50:77–82
16. Xiang Y, Zhou Z, Deng C, Leslie RD (2013) Latent autoimmune diabetes in adults in Asians: similarities and differences between East and West. J Diabetes 5(2):118–126. https://doi.org/10.1111/1753-0407.12029
17. Xiang Y, Huang G, Shan Z et al (2015) Glutamic acid decarboxylase autoantibodies are dominant but insufficient to identify most Chinese with adult-onset non-insulin requiring autoimmune diabetes: LADA China study 5. Acta Diabetol 52(6):1121–1127. https://doi.org/10.1007/s00592-015-0799-8
18. Huang G, Yin M, Xiang Y et al (2016) Persistence of glutamic acid decarboxylase antibody (GADA) is associated with clinical characteristics of latent autoimmune diabetes in adults: a prospective study with 3-year follow-up. Diabetes Metab Res Rev 32(6):615–622. https://doi.org/10.1002/dmrr.2779
19. Yang L, Zhou ZG, Huang G, Ouyang LL, Li X, Yan X (2005) Six-year follow-up of pancreatic beta cell function in adults with latent autoimmune diabetes. World J Gastroenterol 11(19):2900–2905

20. Ismail H, Wotring M, Kimmie C, Au L, Palmer JP, Brooks-Worrell B (2007) "T Cell-positive antibody-negative" phenotypic type 2 patients, a unique subgroup of autoimmune diabetes. Diabetes 44:S24
21. Brooks-Worrell B, Narla R, Palmer JP (2013) Islet autoimmunity in phenotypic type 2 diabetes patients. Diabetes Obes Metab 15(Suppl 3):137–140
22. Yuan J, Cui Q, Tang W, Chao C, Zhou Z, Yang L (2017) Comparison of effects between IL-2 and IL-7 on glutamic acid decarboxylase 65 reactive T cell responses in patients with Type 1 diabetes. Zhong Nan Da Xue Xue Bao Yi Xue Ban 42(11):1257–1262. https://doi.org/10.11817/j.issn.1672-7347.2017.11.003
23. Brooks-Worrell B, Tree T, Mannering SI et al (2011) Comparison of cryopreservation methods on T-cell responses to islet and control antigens from type 1 diabetic patients and controls. Diabetes Metab Res Rev 27(8):737–745
24. Yang L, Chao C, Tang W et al (2013) Effect of cryopreservation method on islets specific T cell responses in type 1 diabetic patient. Zhong Nan Da Xue Xue Bao Yi Xue Ban 38(2):169–175. https://doi.org/10.3969/j.issn.1672-7347.2013.02.010
25. Endl J, Otto H, Jung G et al (1997) Identification of naturally processed T cell epitopes from glutamic acid decarboxylase presented in the context of HLA-DR alleles by T lymphocytes of recent onset IDDM patients. J Clin Invest 99(10):2405–2415
26. So M, Elso CM, Tresoldi E (2018) Proinsulin C-peptide is an autoantigen in people with type 1 diabetes. Proc Natl Acad Sci U S A 115(42):10732–10737. https://doi.org/10.1073/pnas.1809208115
27. Brooks-Worrell B, Narla R, Palmer JP et al (2012) Biomarkers and immune-modulating therapies for type 2 diabetes. Trends Immunol 33(11):546–553. https://doi.org/10.1016/j.it.2012.07.002
28. Brooks-Worrell BM, Palmer JP (2013) Attenuation of islet-specific T cell responses is associated with C-peptide improvement in autoimmune type 2 diabetes patients. Clin Exp Immunol 171(2):164–170. https://doi.org/10.1111/cei.12012
29. Greenbaum CJ, Mandrup-Poulsen T, McGee PF et al (2008) Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. Diabetes Care 31(10):1966–1971