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Gliadin, endomysial and thyroid antibodies in patients with latent autoimmune diabetes of adults (LADA)

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SUMMARY

Latent autoimmune diabetes of adults (LADA) manifested after the age of 35 is characterized by the presence of disease-specific autoantibodies (anti-glutamate decarboxylase GADAb, anti-IA2Ab). However, autoimmunity in Type 1 diabetes mellitus is not targeted only to pancreatic beta-cells. No data have so far been published concerning the antibodies associated with other autoimmune disease in LADA patients. The presence of anti-thyroglobulin (TGAb), anti-thyroid peroxidase (TPOAb), anti-gliadin IgA (AGAAb) and IgG (AGGAb) and endomysial antibodies (EMAb) in sera of 68 diabetics typed as LADA was compared with the antibody presence in sera of 85 patients with Type 2 diabetes. We found a significantly higher occurrence of gliadin antibodies in LADA patients: the rate of AGGAb was 19·1% in comparison with 3·5% in the T2DM group ($P = 0·0026$), the rate of AGAAb was 13·2% in comparison with 3·5% ($P = 0·035$). The prevalence of EMAb was very low in both groups (1·5% and 0). The two groups differed significantly in the TPOAb rate: 22·1% in LADA compared to 9·4% in T2DM ($P = 0·04$), whereas no significant difference was found in the presence of TGAb (8·8% and 3·5%, $P = 0·187$). In comparison with T2DM patients, LADA patients were found to express higher antibody activity against gluten-related antigens and against TPO.

Keywords anti-gliadin coeliac latent autoimmune diabetes of adults thyroidal

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is the result of an autoimmune destruction of beta cells of the pancreatic Langerhans islets with consequent insulin deficiency. Both humoral and cellular components participate in pathogenic autoimmune reactions. Humoral markers of the autoimmune reaction to beta cells of the pancreas include islet cell antibodies (ICAb), antibodies to insulin (IAb), antibodies to glutamic acid decarboxylase (GADAb) and antibodies to 'tyrosine phosphatase-like protein' (IA-2Ab) [1]. The presence of these markers before the development of overt disease can identify patients at risk. In adults, the onset of diabetes-specific autoantibodies is useful in diabetes classification and appropriate treatment; these antibodies can distinguish between diabetes mellitus Type 2 and slowly progressing T1DM (LADA: latent autoimmune diabetes of the adults) [2]. Immunologically mediated diabetes occurs commonly at any age, even in the 8th and 9th decades of life.

T1DM occurs often in association with other autoimmune diseases, both organ-specific and organ-non-specific (thyroid disease, Addison's disease, coeliac disease, rheumatic disease and others). The presence of various circulating autoantibodies has been reported frequently. The co-existence of coeliac disease (CD) and T1DM has been shown in several studies; the rates of co-existence are 1–7% [3] [4], much higher than rates in the normal population. The diagnosis of coeliac disease is based on typical antibody presence, clinical history and jejunal biopsy. Several reports show the presence of clinically milder, less symptomatic forms of CD in diabetic patients. Anti-gliadin antibodies (AGAb) have been used widely as a screening tool for CD, with sensitivity of approximately 75% and specificity of approximately 95% [5]. Apart from anti-gliadin antibodies and antibodies to endomysium, tissue transglutaminase and calreticulin are found in the sera of coeliac patients [6]. Endomysial antibodies (EMAb-IgA) have higher sensitivity and specificity than AGAb [7]. It was shown that the

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prevalence of AGAb may be high in patients with T1DM without symptoms of CD [8]. Most of the studies have focused on the association of childhood diabetes and coeliac disease, but the patterns of antibodies and the clinical course of the two diseases in adults seem to be different. To date, no studies related to the association of autoimmune diabetes with delayed onset and coeliac disease have been published.

Autoimmune thyroiditis (AT) is also a common autoimmune disease associated with T1DM, characterized by the presence of specific thyroid autoantibodies, thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TGAb). The prevalence of thyroid antibodies in T1DM adult patients has been reported to be between 20% and 30% [9–12]. TPOAb occur more often than TGAb. The prevalence of autoimmune thyroiditis seems to increase with age [13].

The association of autoimmune diseases in both human patients and animal models of autoimmunity is explained by the spreading of T cell response to new determinants during the course of autoimmunity development. This phenomenon of recruitment of additional T cell epitopes has been reported during the course of experimental autoimmune encephalomyelitis (reaction to myelin basic protein and subsequently to other myelin proteins). In NOD mice, the initial response to GAD determinants was spread to mycobacterial hsp 65 peptide, carboxypeptidase H and insulin. As proposed, the explanation can be in an up-regulated determinant presentation, the down-regulation of CD4 molecules or the presence of antibodies that bind to these epitopes on the same antigen [14]. To improve understanding of the combined presence of coeliac-related antibodies and thyroid-related antibodies in diabetics in older age, we focused on the presence of these antibodies in a population of LADA patients, compared with Type 2 diabetic patients (T2DM). The objective of our work was to study the prevalence of coeliac-associated antibodies (AGAb, EMAb) and antithyroidal antibodies (TPOAb, TGAb) in the population of patients with latent autoimmune diabetes of adults (LADA).

PATIENTS AND METHODS

Subjects

We tested 153 consecutive sera from diabetic patients over 35 years of age at diagnosis. These patients were selected from the epidemiological study of the Diabetes Center at the 3rd Medical Faculty, Charles University and several out-patient diabetes clinics in Prague and Melnik. The selection of patients was based on clinical parameters; patients with secondary diabetes were excluded and body mass index and fasting C peptide were not used as recruitment criteria. The study was approved by the Institutional Review Board of the 3rd Faculty of Medicine, Charles University and patients gave an informed consent.

Group A included 68 diabetic patients with the presence of glutamate decarboxylase antibodies (GADAb) and/or IA2 antibodies (IA2Ab), with disease onset after the age of 35 years (typed as LADA type diabetics). Group B included diabetic patients without the presence of diabetes-related autoantibodies (GADAb, IA-2Ab), with disease onset after the age of 35 years (considered as Type 2 diabetes). We used diagnostic criteria for T1DM according to a recent report [15]. The characteristics of both groups were the same in terms of male : female ratio, age and body mass index. The normal control group consisted of individuals selected randomly from common elderly population and from a senior's home with no signs of severe metabolic disease, matched by age and sex with the groups tested (Table 1).

METHODS

GAD antibodies were tested by commercial ELISA test (Roche Applied Science, Germany). A level of specific IgG below 32 ng/ml is considered negative. IA2 antibodies were tested by commercial ELISA set (Roche Applied Science, Germany); a level below 0·9 U/ml is considered negative. Anti-gliadin antibodies IgA and IgG were tested by a commercial ELISA set (Pharmacia Diagnostics) and an endomysial autoantibody test was performed by indirect immunofluorescence analysis according to Not *et al*. [16]. TPO and TG antibodies were tested by ELISA supplied by Milenia Biotec, Germany.

Statistical evaluation

Differences in antibody frequencies were tested by the χ^2 test with Yates's correction or Fisher's exact test. The trend of an antibody association was tested by χ^2 test of the trend. Numerical data were expressed as mean and standard deviation unless indicated otherwise and their significance was assessed by the Mann–Whitney test when appropriate, or by unpaired *t*-test. A probability value of less than 0·05 was considered statistically significant.

RESULTS

Among the 153 patients studied, we found only GADAb positivity in 36 subjects (23·5%), only IA2Ab positivity in 19 patients (12·4%) and double positivity of GADAb and IA2Ab in 13 subjects (8·5%). Eighty-five patients (55·5%) were without diabetes-related antibodies. In group A (with diabetes-related autoantibodies), the presence of anti-gliadin antibodies in IgA and IgG immunoglobulin classes was significantly higher (13·2% *versus* 3·5% for AGAAb ($P = 0·035$) or 19·1% *versus* 3·5% for AGGAb

Table 1. Clinical characteristics of adult diabetic patients and controls: Group A represents diabetic patients with the presence of either anti-GAD antibodies or anti-IA2 antibodies (LADA); group B represents diabetic patients without the presence of diabetes-related autoantibodies (GADAb, IA2Ab). The normal control group is selected randomly from common population

Mean/standard deviation	A	B	Normal controls	Statistical significance
Number of patients	68	85	62	NS
Men/women ratio	29/39 (42·6%)	37/48 (43·%)	26/36 (41·9%)	NS
Age (years)	64·4/10·0	65·4/8·4	63·8/8·2	NS
Disease duration (years)	10·6/7·6	11·9/6·5		NS

($P = 0{\cdot}0026$) than in group B (T2DM). The prevalence of endomysial antibodies was generally very low in group A (1·5%); it was found in only one patient who also had increased anti-gliadin antibodies. No EMAb were detected in group B (Table 2a).

The presence of TPOAb was significantly higher in group A than in group B (22·1% *versus* 9·4%, $P = 0{\cdot}04$). A difference between the value quantification was also detected (171·7 IU/ml *versus* 63·8 IU/ml, $P = 0{\cdot}041$). On the other hand, no difference was found between the groups in the presence of TGAb (Table 2b).

When we looked at the relationship between the presence of anti-gliadin antibodies and either IA2Ab or GADAb or combination of those antibodies to test the hypothesis 'more diabetes-related antibodies increase the risk of thyroid or gliadin antibodies', we found a significant trend of association. The relative risk for AGGAb increased from 1 (T2DM) to 9·44 in double-antibody positive T1DM patients and up to 7·08 in AGAAb (Table 3a). A similar trend was found for TPOAb; the relative risk for TPOAb increased to 7·08 in the group of T1DM patients with both GADAb and IA2Ab (Table 3b). No risk for TGAb production was found in these groups.

Table 2a. The rate of coeliac-associated antibodies in LADA group A and T2DM group B of patients in comparison with a normal population

	Group A $n = 68$	Statistical significance (A and B)	Group B $n = 85$	Statistical significance (controls and A)	Control group
AGA IgG	13/68 19·1%	$P = 0{\cdot}0026$	3/85 3·5%	$P = 0{\cdot}0038$	5/108 4·6%
AGA IgA	9/68 13·2%	$P = 0{\cdot}035$	3/85 3·5%	$P = 0{\cdot}0035$	2/108 1·85%
EMA IgA	1/68 1·5%	$P = 0{\cdot}50$	0/85 0%		NT

Table 2b. The rate of antithyroidal antibodies in LADA group A and T2DM group B of patients

	Group A	Group B	Statistical significance
TGAb			
Positive	6/68 (8·82%)	3/85 (3·53%)	$P = 0{\cdot}187$
Mean/s.d.	194·0/1295·6	82·6/391·0	$P = 0{\cdot}696$
TPOAb			
Positive	15/68 (22·1%)	8/85 (9·4%)	$P = 0{\cdot}04$
Mean/s.d.	171·7/930·8	63·8/258·0	$P = 0{\cdot}041$

Group A represents diabetic patients with the presence of either anti-GAD antibodies or anti-IA2 antibodies (LADA). Group B represents diabetic patients without the presence of diabetes-related autoantibodies (GADAb, IA2Ab). Controls represent a group of normal, age- and sex-matched populations. s.d.: standard deviation, NT: not tested.

Table 3a. Association between the presence of anti-gliadin antibodies and diabetes-related autoantibodies (the risk to develop anti-gliadin antibody dependent on the presence or absence of GADAb and/or IA2Ab)

Absolute/%	AGA IgG	Rel. risk (CI)	AGA IgA	Rel. risk (CI)
GADAb – IA2Ab$^-$	3/85 3·5%	1	3/85 3·5%	1
GADAb – IA2Ab$^+$	3/19 15·8%	4·47 (0·98–20·48)	2/19 10·5%	2·98 (0·53–16·63)
GADAb + IA2Ab$^-$	6/36 16·7%	4·72 (1·25–17·85)	4/36 11·1%	3·15 (0·74–13·36)
GADAb + IA2Ab$^+$	4/12 33·3%	9·44 (2·4–37·15)	3/12 25%	7·08 (1·61–31·17)
Trend (statistical significance)		$P = 0{\cdot}00054$		$P = 0{\cdot}0096$

Table 3b. Association between the presence of thyroidal and diabetes-related autoantibodies (the risk to develop thyroidal antibody dependent on the presence or absence of GADAb and/or IA2Ab)

Absolute/%	TPO	Rel. risk (CI)	TG	Rel. risk (CI)
GADAb – IA2Ab$^-$	3/85 9·4%	1	8/85 3·5%	1·0
GADAb – IA2Ab$^+$	3/19 15·8%	4·47 (0·98–20·5)	1/19 5·3%	0·56 (0·07–4·21)
GADAb + IA2Ab$^-$	9/36 25%	7·08 (2·08–24·65)	5/36 13·9%	1·48 (0·52–4·21)
GADAb + IA2Ab$^+$	3/12 25%	7·08 (1·61–31·17)	0/12 0%	0
Trend (statistical significance)		$P = 0{\cdot}00031$		$P = 0{\cdot}851$

DISCUSSION

Our study has shown a substantially increased prevalence of IgG and IgA anti-gliadin antibodies in the cohort of LADA patients (defined by the disease onset after 35 years of age and by the presence of diabetes-related autoantibodies) compared to patients with Type 2 diabetes (the average age of patients in our study was about 65 years). The higher prevalence of coeliac antibodies has been described previously by various authors in patients with Type 1 diabetes; however, the age of the patients in these studies varied. Lorini reported a 10% prevalence of AGAAb positivity in an Italian diabetic population [17], 3% has been reported in Australia [18], 9% of IgG or IgA AGAb in a Spanish childhood population [19], 6% of IgG AGAb in an Austrian population [20,21] and we have recently found 10·4% in diabetic children from Libya [22]. None of those patients were characterized as patients with LADA, and this fact can explain the different results of our present study (13·2% or 19·1% AGA positivity). EMAb positivity has been shown in the range of 3–8·2% in diabetic children [23], while our data show that only 1·5% of all LADA patients were positive for EMAb. The estimation of prevalence of CD-associated autoantibodies in a normal population in Sweden was 3% [24]. The prevalence of coeliac seropositivity (antibodies against gliadin or tissue transglutaminase and endomysium) in healthy blood donors in the Czech Republic was 0·4% [25]. In our study, we focused only on patients with distinct markers of autoimmune insulitis.

Our findings of the increased occurrence of IgA and IgG anti-gliadin antibodies in LADA patients combined with a very low incidence of EMAb positivity suggest that coeliac disease is not necessarily associated with LADA. Unfortunately we could not confirm this negativity by bioptic investigation, but EMA autoantibodies are usually present in coeliac patients. The presence of anti-gliadin antibodies suggests that the mucosal barrier function in these patients is impaired and dietary antigens can pass more freely to the circulation and induce an antibody response. Analysis of antibodies against other dietary components is under way. Environmental triggers of the intestinal immune system have been suggested in T1DM. Enteroviral infections and cow's milk protein were often considered and the mechanism of molecular mimicry has been suggested [26], although it was not proved completely in human studies. As most of these potential environmental triggers are present in the gut, the intestinal immune system is implied to provide a link between risk factors and T1DM [27]. In animal models of T1DM developing the disease spontaneously, i.e. in NOD mice and BB rats, diabetes could be inhibited by dietary manipulation. Oral tolerance induction by feeding autoantigens is currently used in clinical trials in efforts to prevent the disease [28]. Also our finding in NOD mice, demonstrating that a gluten-free diet prevents the onset of diabetes, points to the role of the gut and dietary components in the induction of diabetes [29]. Our finding of the trend of association of gliadin antibody production and the presence of diabetes-related autoantibodies can support the idea of tendency of higher food antigen exposure to the immune system and therefore higher appropriate antibody production in diabetic organism with high diabetes-related autoantibody production. Another piece of evidence suggesting the involvement of the intestinal immune system in T1DM is the expression of the same lymphocyte and endothelial adhesion molecules (which directs the migration of lymphocytes into a gut-associated lymphoid tissue) in the insulitis present in NOD mice and in human T1DM [30,31]. Moreover, jejunal mucosa of patients with TIDM was recently shown by immunohistological methods to express signs of activation [32]. We have found a higher prevalence of TPOAb in LADA patients in comparison with T2DM patients. This TPOAb prevalence is similar to the prevalence of TPOAb in adult patients with T1DM in a Belgian study (24·7%), where the average age was 40·8 years [33]. This higher prevalence of thyroid antibodies and the strong association of TPO autoantibodies with the number of diabetes-related autoantibodies is related to the idea of autoimmunity association on the basis of epitope spreading.

To summarize, we have observed that autoimmune diabetes with disease onset after the age of 35 is accompanied by the presence of IgG and IgA anti-gliadin antibodies and autoantibodies to thyroid peroxidase. These findings support the idea of autoimmunity association and suggest specific susceptibility to AGA antibody production in LADA patients, which could reflect changes in their mucosal barrier function.

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