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Short communications

Antibodies to tissue transglutaminase C in Type I diabetes

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Abstract

Aims/hypothesis. Silent coeliac disease is a gluten driven autoimmune disease which is relatively frequent in patients with Type I (insulin-dependent) diabetes mellitus. To determine the extent of gluten associated autoimmunity in Type I diabetes, autoantibodies to tissue transglutaminase C, a major autoantigen in coeliac disease, were measured in patients with new-onset Type I diabetes.
Methods. We measured IgG and IgA tissue transglutaminase C autoantibodies using human recombinant antigen and radio-binding assays in a cohort of 287 patients with new-onset Type I diabetes, 119 with Type II (non-insulin-dependent) diabetes mellitus and in 213 control subjects.
Results. We found IgA and IgG tissue transglutaminase C antibodies in 24 (8 %) patients with Type I diabetes; 97 (33 %) patients had IgG antibodies only and 1 IgA antibodies only. Antibody concentrations were highest in those with both IgA and IgG antibodies. Only 2 (2 %) patients with Type II diabetes and 2 (1 %) control subjects had either IgG or IgA tissue transglutaminase C antibodies. Patients with HLA *DRB1*04* alleles had the highest prevalence of IgG tissue transglutaminase C antibodies.
Conclusion/Interpretation. These data show that almost 10 % of patients have autoimmunity typical of coeliac disease and that another 30 % have low level tissue transglutaminase C antibody binding. This high prevalence suggests either involvement of the gut in the pathogenesis of Type I diabetes or that transglutaminase is a secondary autoantigen resulting from beta-cell destruction. [Diabetologia (1999) 42: 1195–1198]

Keywords Type I diabetes, autoantibodies, coeliac disease, transglutaminase.

Autoimmunity is the hallmark of Type I (insulin-dependent) diabetes mellitus which is most clearly expressed through the presence of circulating antibodies to islets proteins. Type I diabetes is also often associated with the presence of other autoimmune diseases or autoantibodies. In particular, it is reported that coeliac disease, in which antibodies to dietary gluten and autoantibodies to endomisium (EMA) are present, occurs in around 5 % of patients with Type I diabetes [1]. This relatively high concomitant disease prevalence can be at least in part explained by the shared genetic susceptibility provided by the HLA *DQB1*02* allele [2], but could indicate an underlying gut-associated pathogenesis in some patients with Type I diabetes. This is supported by studies in animal models of Type I diabetes which have shown decreased disease incidences with the modification of diet and epidemiological studies in humans which suggest that variations in early infant diet are associated with differing disease incidences [3, 4].

To determine the extent of gluten associated autoimmunity in Type I diabetes we have cloned the re-

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Abbreviations: EMA, Endomisial autoantibodies; TGC, tissue transglutaminase C; TGCA, tissue transglutaminase C autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; IA2A, autoantibodies to the protein phosphatase-like IA-2.

cently identified autoantigen target of EMA, tissue transglutaminase C (TGC) [5] and measured IgG and IgA autoantibodies (TGCA) using novel radiobinding assays in a cohort of patients with new-onset Type I diabetes, Type II (non-insulin-dependent) diabetes mellitus and in control subjects.

Subjects and methods

Subjects. Serum samples at onset of Type I diabetes were obtained from 287 patients. All were diagnosed according to the World Health Organization criteria. The median age of patients was 10 years (range 0.8–33 years), 169 were males. Two patients had coeliac disease before the onset of Type I diabetes. Coeliac disease was diagnosed by intestinal biopsy which showed mucosal flattening according to the European Society for Paediatric Gastroenterology and Nutrition criteria. Serum samples were obtained from 119 patients with Type II diabetes with median age 65 years (range 42–87 years), 65 were males. Control serum samples were obtained from 213 children with no family history of diabetes (median age 4.4 years; range 0.1–11.2; 108 males). All patients and control subjects were from the Lombardy region of northern Italy.

Autoantibody measurements. We measured IgG and IgA antibodies to in vitro transcribed/translated ^{35}S-methionine labelled human tissue transglutaminase C (TGCA) by radiobinding assay as described previously [6]. Results for each assay were expressed as arbitrary units derived from standard curves of serial dilutions of a serum with both IgG and IgA TGCA tested in each assay. The threshold for positivity was the upper first centile of normal controls, respectively 0.9 units for the IgG TGCA assay and 0.3 units for the IgA TGCA assay. Endomisial antibodies [6] and antibodies to glutamic acid decarboxylase (GADA) and antibodies to protein phosphatase like IA-2 (IA2A) were measured in serum as described previously [7].

HLA typing. Human leucocyte antigen typing was carried out either using the standard microcytotoxicity test on lymphocytes isolated from blood samples by immuno-magnetic beads (Dynal, Oslo, Norway) or by sequence specific polymerase chain reaction on DNA extracted from peripheral blood mononuclear cells [8]. Subjects were grouped as DR3/X, DR4/Y, DR3/4 or DRX/Y, where X is an allele other than *DRB1*04* and Y is an allele other than *DRB1*03*.

Statistical analysis. Prevalence of TGCA in Type I diabetes, Type II diabetes and control subjects were compared using the chi-squared test with Yates' correction. Association between antibody prevalence and HLA-DR phenotype was tested with the chi-squared test with Yates' correction.

Results

TGC antibodies in Type I diabetes. Autoantibodies to tissue transglutaminase C above the 99th centiles of control subjects were detected in 122 of 287 patients with Type I diabetes at onset of disease (43%; CI 37–48%) (Fig. 1). Of the patients 25 (9%; CI 6–13%) had raised levels of IgA TGCA and 121 (42%; CI 36–48%) had IgG TGCA. In 24 patients

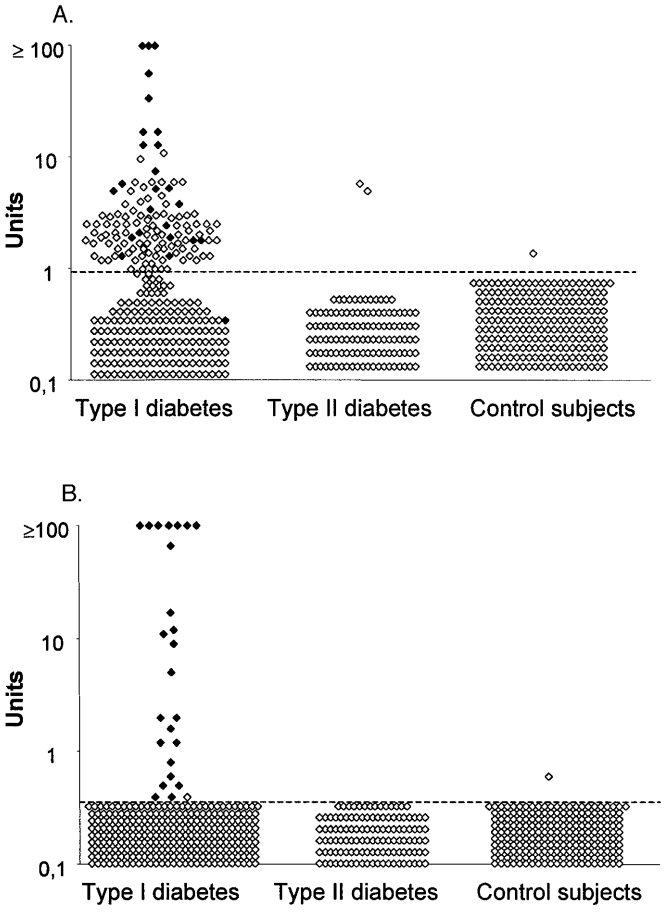

Fig. 1 A, B. Scatter plots of units obtained in the IgG (**A**) or IgA (**B**) TGCA radio-binding assay in sera from 287 patients with Type I diabetes, 119 patients with Type II diabetes and 213 control subjects. The threshold for positivity is indicated by the broken line. Samples with both IgG and IgA TGCA are indicated with filled symbols

(8%; CI 5–12%) both IgA and IgG TGCA were found. We found IgG TGCA in the absence of IgA TGCA in 97 patients (34%; CI 28–40%). Levels of IgG TGCA antibodies were higher in those with both IgG and IgA TGCA antibodies (median 5.2, range 1.3–100) than in those with raised IgG TGCA only (median 2.0, range 1–11 $p < 0.0001$). In the Type II diabetes cohort two patients had low levels of IgG TGCA only. We found IgA TGCA in the absence of IgG TGCA in 1 of 213 control subjects and 1 control subject had IgG TGCA only.

TGCA, HLA and other autoantibodies. Human leucocyte antigen typing was obtained for 128 patients with Type I diabetes. Of these, 34 had the DR3/4 genotype, 41 DR3/X, 38 DR4/Y and 15 DRX/Y. Raised IgG TGCA were found in 22 (65% CI 46–80%) with the DR 3/4 genotype, 19 of those with DR4/Y (50% CI 33–67%), 16 with DR3/X (39% CI 24–55%) and in 3 with DRX/Y (20% CI 4–48%), suggesting an association between HLA *DRB1*04*

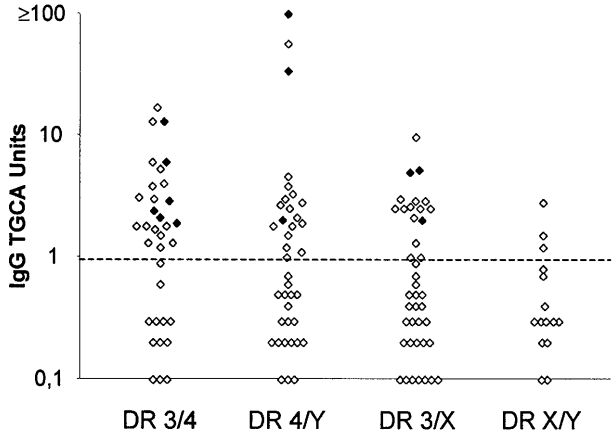

Fig. 2. Scatter plots of units obtained in the IgG anti-tissue transglutaminase antibody (TGCA) radio-binding assay in sera from patients with Type I diabetes with respect to HLA DR phenotype. The threshold for positivity is indicated by the broken line. Samples with both IgG and IgA TGCA are indicated with filled symbols

and the presence of IgG TGCA ($p < 0.02$, *DRB1*04* vs non-*DRB1*04*) (Fig. 2). We found IgA TGCA in five patients with DR3/4 (14% CI 5–31%), three with HLA-DR4/Y (8% CI 2–21%), three with HLA-DR3/X (7% CI 2–20%) and none of the DRX/Y patients. No association was found between the presence of TGCA and that of either GADA or IA2A (data not shown). Previously IgA EMA had been measured in 178 of the patients at onset of Type I diabetes and found present in 11 of 17 with both IgG and IgA TGCA, in 1 of 54 with IgG TGCA only, in 0 of 1 with IgA TGCA only and in 2 of 106 without TGCA. There were no differences in HbA_{1c} values or C-peptide concentrations between patients with and without TGCA antibodies (median HbA_{1c}: 11.5% vs 11.4%; median C-peptide: 0.13 vs 0.12 nmol/l). Follow-up samples one year after diabetes onset were available and tested in 13 patients with IgG TGCA only at diabetes onset and in none of these were IgG or IgA TGCA detected in these follow-up samples.

Discussion

An increased prevalence of coeliac disease in patients with Type I diabetes is well established. We found a prevalence of IgA antibodies to transglutaminase, a major autoantigen target of the traditional marker of coeliac disease IgA EMA, of around 8% at onset of Type I diabetes. All but one of these patients had also IgG TGCA and in seven of nine who also had intestinal biopsy, the histological changes typical of coeliac disease were confirmed (data not shown). Most, but not all, had the coeliac disease and Type I diabetes susceptible HLA *DRB1*03* allele. These findings are similar to those of previous studies which report IgA EMA and coeliac disease in around 5% of patients with Type I diabetes [1].

A striking finding of this study was that IgG TGCA were found in 42% of patients. The levels of IgG TGCA were high in patients who had also IgA TGCA and in the remaining 32% were generally low. These antibodies were infrequent in patients with Type II diabetes and in control subjects. The IgG antibodies to coeliac disease associated antigens are thought to be less specific for coeliac disease than IgA antibodies and, although prospective studies to validate this have not been reported, their detection in the absence of IgA EMA or IgA deficiency is thought not to confer a high risk for coeliac disease. Such a response in Type I diabetes was more associated with HLA *DRB1*04* than with *DRB1*03*. It might be expected, therefore that autoimmunity in these cases is possibly not mediated directly through gluten as suggested for coeliac disease but by other mechanisms. Indeed, anti-gliadin antibodies were rarely found in subjects with IgG TGCA only (data not shown).

Autoimmunity to transglutaminase outside the typical coeliac disease associated form could be postulated to arise through intestinal immunisation of some other form, direct immunisation during beta-cell inflammation, or cross-reactivity between transglutaminase and islet autoantibodies. Evidence for intestinal immunisation includes reports of an increased intestinal permeability in patients with Type I diabetes [9], and the numerous reports of increased immunity to dietary proteins in patients [4] and although coeliac-like immunisation is perhaps unlikely, we cannot exclude that low level IgG TGCA does not have a gut origin.

The possibility of a direct immunisation during beta-cell destruction is perhaps more appealing. It could be envisaged that during active inflammation and beta-cell destruction transglutaminase C, which is expressed in islets, is presented in an immunogenic form, and in view of the very high prevalence of IgG TGCA in patients with HLA *DRB1*04*, this presentation might be facilitated by these alleles. Alternatively, tissue transglutaminase could bind autoantigens such as GAD, IA-2 or insulin and subsequently be recognised through a mechanism of determinant spreading. Cross-linking by tissue transglutaminase of intracellular proteins released during apoptosis has been shown and an impairment of this function has been associated with the development of IgG TGCA in an animal model of systemic *lupus erythematosus* [10]. Low level IgG TGCA could, therefore, be a more general feature of destructive autoimmunity and examination in other autoimmune diseases is warranted. Of note is the loss of the antibody reactivity in the few patients tested one year after diabetes onset, supporting a transient autoimmunity associated with active beta-cell destruction.

Finally, it is possible that antibody binding to transglutaminase occurs non-specifically or indirectly as a result of the presence of antibodies to islet autoantigens such as GAD and IA-2 either through the recognition of cross-reactive epitopes, or cross-linking with antigen-antibody complexes found in the serum of patients with Type I diabetes. No correlation of IgG TGCA with antibodies to GAD or IA-2 was, however, found to support this hypothesis nor was binding to transglutaminase affected by addition of unlabelled GAD or IA-2 (data not shown).

In conclusion, we show that around 8 % of patients with Type I diabetes have autoimmunity typical of coeliac disease at diabetes onset and that a further 32 % have low level IgG autoantibodies to coeliac disease associated autoantigen, transglutaminase. These findings suggest that either the gut has a role in the autoimmune pathogenesis of Type I diabetes or transglutaminase is a secondary antigen during beta-cell destruction.

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