*Short communication***Autoantibodies to ICA12 (SOX-13) are not specific for Type I diabetes****H. Steinbrenner¹, T. Lohmann², B. Ostendorf¹, W. A. Scherbaum¹, J. Seissler¹**¹ German Diabetes Research Institute at the University of Duesseldorf, Germany² Department of Medicine III, University of Leipzig, Germany³ Department of Rheumatology, University of Duesseldorf, Germany**Abstract**

Aims/hypothesis. The majority of patients with Type I (insulin-dependent) diabetes mellitus has autoantibodies to insulin, glutamic acid decarboxylase and the tyrosine phosphatase-like protein IA-2. Some patients with Type I diabetes, in particular with adult onset diabetes, have, however, only cytoplasmic islet cell antibodies that could be directed to as yet unknown beta cell autoantigens. Recently, one potential antigen, ICA12, was identified as the high mobility group (HMG) box transcription factor SOX-13. Our aim was to evaluate the diagnostic sensitivity and specificity of autoantibodies to SOX-13 for autoimmune diabetes.

Methods. Full-length SOX-13 was cloned from human brain mRNA, expressed by vitro transcription/translation and used to detect autoantibodies by immunoprecipitation and radioligand assay in patients suffering from autoimmune diabetes or non-organ-specific autoimmune diseases.

Results. We found SOX-13 antibodies to be present in 11 of 125 (8.8 %) patients with newly diagnosed Type I diabetes and in 3 of 43 (7.0 %) patients with latent autoimmune diabetes in adults. There was no association with age, sex and a particular pattern of diabetes-specific autoantibodies. Similar frequencies of SOX-13 antibodies were found in 84 patients with rheumatic diseases (4.0–11.4 %) and 211 healthy control subjects (5.2 %).

Conclusions/interpretation. Our data indicate that SOX-13 represents a minor target of autoantibodies in patients with autoimmune diabetes. Because SOX-13 antibodies were found not to be disease-specific, we assume they are not helpful in improving the diagnosis and prediction of Type I diabetes. [Diabetologia (2000) 43: 1381–1384]

Keywords Autoantibodies, autoantigen, ICA12, SOX-13, Type I diabetes.

Type I (insulin-dependent) diabetes mellitus is caused by a chronic autoimmune process directed

against the pancreatic islets. Insulin, glutamic acid decarboxylase (GAD) and the protein tyrosine phosphatase-like molecule IA-2 have been shown to comprise the major target autoantigens in Type I diabetes [1]. Some patients, particularly subjects with adult-onset diabetes, have, however, cytoplasmic islet cell antibodies (ICA) in the absence of antibodies to recombinant antigens suggesting the presence of antibodies to antigens which are still not known [2, 3]. One potential candidate antigen is the islet cell antigen ICA12 which was identified by immunoscreening of an islet cDNA library [4]. Recently, the target protein of the ICA12 autoantibodies was described as the high mobility group (HMG)-box transcription

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Abbreviations: aa: Amino acids, GADA: antibodies to glutamic acid decarboxylase, ICA: cytoplasmic islet cell antibodies, IA-2A: antibodies to the tyrosine phosphatase-like protein IA-2, LADA: latent autoimmune diabetes in adults, SOX-13Ab: antibodies to SOX-13, IA-2: protein tyrosine phosphatase-like molecule IA-2, HMG: high mobility group, SOX: SRY-related HMG box.

factor SOX-13 (SRY-related HMG box). Up to now there is only one report on SOX-13 autoantibodies (SOX-13Ab) in patients with Type I diabetes suggesting that ICA12/SOX-13 is a new diabetes-specific autoantigen in Type I diabetes [5].

In this study, we cloned and expressed full-length human SOX-13 protein to analyse autoantibodies by immunoprecipitation and radioligand assay. To find whether or not SOX-13Ab are useful markers for Type I diabetes we investigated the sensitivity and specificity of SOX-13Ab in patients with Type I diabetes and latent autoimmune diabetes in adults (LADA) compared with patients with non-organ-specific autoimmune diseases.

Material and methods

Patients. Serum samples were obtained from 125 patients with newly diagnosed Type I diabetes (age 1–60, median 20 years, 67 females) and 43 patients with LADA (age 40–82, median 62 years, 24 females). Latent autoimmune diabetes in adults was classified according to clinical (absence of insulin deficiency within the first 6 months after diagnosis) and immunological criteria [positive ICA or antibodies to GAD (GADA) or both]. In addition, we studied 19 newly diagnosed diabetic patients (age 4–39, median 12 years, 9 women) who were positive for ICA only. Sera from 209 healthy subjects (age 20–40, median 31 years, 75 women), 24 patients with rheumatoid arthritis (age 25–78, median 56 years, 18 women), 25 patients with systemic lupus erythematosus (age 28–72, median 46 years, 18 women) and 35 patients with systemic sclerosis (age 18–72, median 53 years, 25 women) were used as controls.

Cloning of human SOX-13 and measurement of SOX-13 antibodies. Full-length human SOX-13 cDNA was amplified from brain mRNA (Clontech, Palo Alto, Calif., USA) by reverse-transcriptase polymerase chain reaction using primers designed from the published GenBank sequence (accession number: NM_005686). The PCR product was ligated into the pGEM-4Z vector (Promega, Madison, Wis., USA) and sequenced (ABI 310; Applied Biosystems, Foster City, Calif., USA). The SOX-13 was expressed by in-vitro transcription/translation (TNT Quick-Coupled-System, Promega) as described previously [2]. For immunoprecipitation, aliquots of [³⁵S]-methionine labelled SOX-13 (15000 cpm) were diluted in 100 µl TRIS-buffer (20 mmol/l TRIS, 150 mmol/l NaCl pH 7.4, 0.1% BSA, 5 mmol/l methionine, 5 mmol/l benzamidine, 2 mmol/l EDTA, 0.5% Triton X-100) and incubated with 20 µl serum overnight at 4°C. After addition of 100 µl protein-A-Sepharose (Pharmacia, Freiburg, Germany) for 2 h, adsorbed immunocomplexes were washed five times with TRIS-buffer, separated on 10% SDS-polyacrylamide gels and analysed by autoradiography.

To quantify SOX-13Ab concentrations, a radioligand assay was established similar to those assays used for the detection of GADA and IA-2A [2]. Aliquots of SOX-13 (20,000 cpm) were diluted in 50 µl TRIS-buffer and incubated with 5 µl serum overnight at 4°C. Then probes were incubated with 25 µl protein-A-Sepharose (2 h), washed with TRIS-buffer and measured in a beta-counter. Antibody concentrations were calculated as follows: (cpm test serum–cpm normal serum)/(cpm positive serum–cpm normal serum) × 100. Values above the 95th centile of healthy subjects (30.0 Units) were considered

Fig. 1. SOX-13 is not a diabetes-specific target of humoral autoimmunity. [³⁵S]-methionine labelled human SOX-13 was immunoprecipitated by sera from patients with Type I diabetes (lanes 1–3), patients with systemic sclerosis (lanes 4–6) and healthy control subjects (lanes 7–9). Immunocomplexes were separated on 10% SDS polyacrylamide gels. Gels were dried under vacuum and exposed to X-ray films at –80°C for 10 days. Molecular weight markers are indicated on the left margin

as antibody positive. The intra-assay and inter-assay coefficient of variation were 5.9% and 9.6%.

Detection of ICA and GADA and autoantibodies to IA-2 (IA-2A). We detected ICA, GADA and IA-2A as described previously [2]. The ICA test has 100% sensitivity and specificity in the ICA Proficiency Program. The GAD and IA-2 antibody assays had a diagnostic sensitivity of 81% and 74% and a specificity of 99%, respectively (Combinatorial Islet Autoantibody Workshop).

Results

Cloning and expression of human SOX-13/ICA12. The cDNA clone obtained by PCR amplification has 100% identity to the sequence of human SOX-13 submitted to the Genbank (NM_005686 and AF116571) encoding a protein of 622 amino acids (aa) with a predicted molecular weight of 69,200 M_r. Immunoprecipitation of the radiolabelled protein showed that 2 of 20 sera from patients with Type I diabetes strongly bound a polypeptide of about 70,000 M_r corresponding to human SOX-13 (Fig. 1). The same band was, however, also immunoprecipitated by 4 of 46 sera from healthy control subjects and patients with rheumatic disorders.

Prevalence of SOX-13 autoantibodies. Using the 95th centile of healthy control subjects as the experimental cut-off for the radioligand assay, 11 of 125 (8.8%) patients with Type I diabetes and 3 of 43 (7.0%) patients with LADA were found positive for SOX-13Ab compared with 11 of 211 (5.2%) healthy control subjects (Fig. 2). The overall frequency of SOX-13Ab was 7.1% in patients with non-organ-specific autoimmune diseases. The highest prevalence was found in scleroderma patients (11.4%) followed by patients with rheumatoid arthritis (4.2%) and systemic lupus erythematosus (4.0%). There was neither a correlation to age and sex nor any significant difference between the antibody concentrations in the different study groups.

Fig. 2 A, B. Concentrations of autoantibodies to SOX-13 in healthy control subjects, patients with Type I diabetes, latent autoimmune diabetes in adults (LADA) (**A**) and patients with rheumatic disorders (**B**). RA, rheumatoid arthritis; SSc, systemic sclerosis; SLE, systemic lupus erythematosus. The dotted line represents the cut-off of the assay

Correlation of SOX-13 autoantibodies with ICA, GADA and IA-2-A. Among SOX-13Ab-positive patients with Type I diabetes all subjects had positive ICA, ten (90.9%) were positive for GADA and nine (81.8%) had IA-2A. All three LADA sera with SOX-13Ab had ICA and one was also positive for GADA. In addition, SOX-13Ab were detected in only 1 of 19 (5.2%) diabetic patients who were positive for ICA only.

Discussion

The recent description of SOX-13 as the target of ICA12 antibodies made it a promising candidate as a new specific autoantigen to improve the diagnosis of Type I diabetes [5]. In our study we observed a low prevalence of SOX-13Ab in patients with newly diagnosed Type I diabetes (8.8%), LADA patients (7.0%) as well as in diabetic patients with single ICA (5.2%). The frequencies were only slightly in-

creased compared with healthy control subjects indicating that SOX-13 might not represent a major target antigen in autoimmune diabetes. Our data are in contrast to a study reporting on ICA12/SOX-13Ab in 18% of patients with Type I diabetes and 4.6% of healthy control subjects [5]. This discrepancy could be partly explained by differences in the composition of subjects studied (long-term vs newly diagnosed diabetes) and differences in the assays. We here used full-length SOX-13 (aa 1–622) instead of truncated proteins (aa 66–604 or a 115–604). It is important to note that the aa sequence described in the earlier study [5] missed the first 18 N-terminal residues deducted from the author's own cDNA clone (AF116571) and another Genbank report (NM_005686) [5]. Thus, the SOX-13 protein from that study corresponds to aa 84–622 and aa 133–622 of our full-length clone. Although it is unlikely that N-terminal truncation increases the diagnostic sensitivity of SOX-13Ab for Type I diabetes, we cannot completely exclude the presence of a cryptic epitope exposed after deletion of the N-terminus. Direct analysis of autoantigenic regions could be required to assess this. Our antibody assay format is identical to the tests used for sensitive and specific measurement of IA-2A and GADA [2, 3]. We did not observe a higher background reactivity compared with the GADA assay excluding the possibility that the low specificity is mediated by high unspecific binding to the solid phase.

The SOX-13 protein belongs to the family of SOX proteins containing a characteristic DNA binding domain of 80 aa, the HMG box. It is a member of group D SOX-proteins [6] and is ubiquitously expressed in human tissues with highest expressions in pancreas, placenta and kidney [5]. Because autoantibodies against proteins associated with DNA replication and transcription are well known to be present in patients with rheumatic diseases [7], we decided that sera from those patients could serve as optimal controls to determine the specificity of SOX-13Ab. We observed SOX-13Ab in 4.0–11.4% of those patients which clearly shows their non-diabetes specific nature.

Our data suggest that previous studies have overestimated the importance of ICA12/SOX-13Ab for Type I diabetes. We believe SOX-13 adds to the growing list of minor autoantigens being detected in Type I diabetes. Notably, various nuclear antigens such as topoisomerase II and jun-B have been described to be part of the autoimmune response in Type I diabetes [8, 9]. The broad spectrum of autoantibodies that can be shown in Type I diabetes could reflect the complex nature of the autoimmune response including beta-cell specific and non-specific autoantigens.

Autoantibodies against SOX-13 were found not to be specific for Type I diabetes in that they were also

present in patients with rheumatic diseases. Our findings imply that a SOX-13Ab cannot be regarded as an additional diagnostic marker for the classification or prediction of Type I diabetes.

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