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Human monoclonal islet specific autoantibodies share features of islet cell and 64 kDa antibodies

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Summary. The first human monoclonal islet cell antibodies of the IgG class (MICA 1–6) obtained from an individual with Type 1 (insulin-dependent) diabetes mellitus were cytoplasmic islet cell antibodies selected by the indirect immunofluorescence test on pancreas sections. Surprisingly, they all recognized the 64 kDa autoantigen glutamate decarboxylase. In this study we investigated which typical features of cytoplasmic islet cell antibodies are represented by these monoclonals. We show by double immunofluorescence testing that MICA 1–6 stain pancreatic beta cells which is in agreement with the beta-cell specific expression of glutamate decarboxylase. In contrast an islet-reactive IgM monoclonal antibody obtained from a pre-diabetic individual stained all islet cells but lacked the tissue specificity of MICA 1–6 and must therefore be considered as a polyreactive IgM-antibody. We further demonstrate that MICA 1–6 revealed typical features of epitope sensitivity to biochemical treatment of the target tissue which has been demonstrated for islet cell antibodies, and which has been used to argue for a lipid rather than a protein nature of target antigens. Our results provide direct evidence that the epitopes recognized by the MICA are destroyed by methanol/chloroform treatment but reveal a high stability to Pronase digestion compared to proinsulin epitopes. Conformational protein epitopes in glutamate decarboxylase therefore show a sensitivity to biochemical treatment of sections such as ganglioside epitopes. MICA 1–6 share typical features of islet cell and 64 kDa antibodies and reveal that glutamate decarboxylase-reactive islet cell antibodies represent a subgroup of islet cell antibodies present in islet cell antibody-positive sera.

Key words. Islet cell antibodies, 64 kDa antibodies, human monoclonal antibodies, glutamate decarboxylase, gangliosides, Type 1 (insulin-dependent) diabetes mellitus, islet cell antigens.

The humoral autoimmune response to islet specific antigens in Type 1 (insulin-dependent) diabetes mellitus comprises a variety of islet-specific autoantibodies circulating in the serum of affected individuals. Most target molecules of the autoimmune response to islets were unknown for a long time. Therefore the variant islet cell autoantibodies were classified according to the method by which they were detected. Cytoplasmic islet cell antibodies (ICA) have been defined by a cytoplasmic staining pattern of all islet cells in histochemical tests [1, 2] and 64 kDa antibodies were defined by immunoprecipitation experiments on in vitro labelled islet cells [3]. Whereas 64 kDa antibodies were shown to recognize the beta-cell specific enzyme protein glutamate decarboxylase (GAD) [4] ICA have been suggested to recognize gangliosides and not proteins [5, 6]. Evidence supporting this claim included the similarity in biochemical properties of antigens recognized by anti-ganglioside monoclonal antibodies with that of ICA-positive sera [5], and the observation that ICA binding to islet cells is inhibited by glycolipid extracts from human pancreas [6]. Our group recently isolated the first human monoclonal ICA of the IgG-class (MICA 1–6) derived from a patient with newly-diagnosed Type 1 diabetes [7]. MICA 1–6, which represent five different circulating primary B-lymphocyte clones from blood [7, 8], were selected by the histochemical ICA test and surprisingly recognized GAD as a target antigen. In addition, blocking studies with ICA-positive sera provided clear evidence that GAD reactivity of ICA was not limited to the single individual from whom the MICA were derived [7]. Our data were controversial to the suggested ganglioside nature of target antigens of ICA [5, 6].

In this study we, therefore, addressed the question which of the typical features of ICA or 64 kDa antibodies are represented by MICA 1–6. We analysed the reactivity of the MICA within the islet by double immunofluorescence staining with anti-hormone-specific antibodies and compared their tissue specificity with that of FAE7, a new

Table 1. Reactivity of MICA 1–6 and FAE7 with various human tissues and islets of different mammalian species

Human tissue[a]	MICA 1–6	FAE7	Islet cells[b]	MICA 1–6	FAE7
Pancreatic Islets	+	+	Human	+	+
Thyroid	–	+	Rat	+	–
Adrenal cortex	–	–	Pig	+	–
Adrenal medulla	–	–	Cow	+	–
Pituitary	–	+	Monkey	+	–
Stomach	–	–			
Intestine	–	–			

[a] Reactivity assessed by peroxidase conjugated pre-formed immune complexes.
[b] Reactivity assessed by indirect immunofluorescence staining.
MICA, Human monoclonal IgG-islet cell antibody.
FAE 7, Human monoclonal islet cell antibody of the IgM-class

human monoclonal ICA of the IgM-class which we derived from a pre-diabetic individual. We further analysed whether the MICA epitopes in GAD show the typical sensitivity of ICA epitopes reported previously for a panel of ICA-positive sera and for anti-ganglioside antibodies [5]. Completely different biochemical properties between GAD and antigens recognized by ICA from sera would suggest that GAD-reactive ICA are a minor subgroup of ICA, not relevant for the histochemical staining pattern obtained with ICA-positive sera from patients with Type 1 diabetes.

Subjects and methods

Generation of the human monoclonal IgM-ICA FAE7. Blood was obtained after informed consent from an ICA-positive pre-diabetic individual who acquired Type 1 diabetes 1 year after blood donation. B lymphocytes were immortalized by Epstein-Barr virus infection as described [9] and supernatants of the cultured cells were screened for ICA after 3–4 weeks by the classic indirect immunofluorescence test using fluorescein isothiocyanate (FITC)-labelled goat anti-human IgG and IgM antibodies (Dianova, Hamburg, Germany) [10]. Single cell cloning and stabilisation of the monoclonal line was performed as described for the MICA [7].

Immunohistochemical characterization. Species specificity and cross-reactivity of the monoclonal antibodies with different human tissues was assessed on cryostat sections using either preformed complexes of the individual MICA with peroxidase conjugated anti-human Ig antibodies [11] or indirect immunofluorescence testing [10]. Double immunofluorescence staining was performed by incubating a mouse monoclonal anti-proinsulin specific antibody (Novo, Bagsvaerd, Denmark) diluted 1:5 in supernatant of the different MICA on cryostat sections of human pancreas (1 h, 20 °C). After washing in phosphate buffered saline (PBS) (30 min) the presence of antibodies was assessed by FITC-conjugated goat anti-human IgG (preabsorbed with mouse serum proteins, Dianova) and rhodamine-conjugated goat anti-mouse IgG (preabsorbed with human serum proteins, Dianova) diluted 1:30 in PBS (60 min, 20 °C). For glucagon- or somatostatin-specific staining a rabbit anti-glucagon antibody or rabbit anti-somatostatin antibody (Dako, Glostrup, Denmark) and donkey anti-rabbit conjugate (Dianova) were used.

Pre-treatment of pancreas sections. Cryostat sections of human pancreas from a donor with blood group 0 were processed as described [5]. Sections were fixed in acetone (5 min, 20 °C) or incubated in methanol/chloroform (1:2) (5 min, 20 °C), or, after acetone fixation, washed for 10 min in PBS and incubated with Pronase 0.1 mg/ml in PBS (Boehringer, Mannheim, Mannheim, Germany) for 10 min at 20 °C or with neuraminidase 0.5 U/ml in 50 mmol/l sodium citrate pH 5.5 (Boehringer) for 60 min at 37 °C. A mouse monoclonal anti-human proinsulin antibody (1:10 in PBS; Novo) was used to optimize the duration of the Pronase incubation step. The monoclonal antibody A2B5, recognizing a ganglioside antigen in islets [12] was used as a control in the neuraminidase incubation step. After pre-treatment sections were washed for 15 min in PBS and staining of the antibodies was assessed by the indirect immunofluorescence test.

Immunoblotting. GAD was purified from the cytosolic fraction of pig brain homogenates (10% weight/volume in 20 mmol/l KH_2PO_4 pH 7.4, 1 mmol/l phenyl-methyl-sulphonyl-fluoride, 1 mmol/l amino-ethyl-isothiouronium-bromide, 0.02 mmol/l pyridoxal phosphate). The 33.000 × *g* supernatant was purified by gelfiltration on a Sepharose Q column and fractions with GAD enzyme activity were pooled and spotted on nitrocellulose membranes (30 µl/slot of dilutions 1:10, 1:100 and 1:500) (Hybond; Amersham, Amersham, Bucks., UK). Part of the blots were incubated in methanol/chloroform (1:2) for 5 min (20 °C) or in Pronase (0.1 mg/ml 10 min, 20 °C). After washing the nitrocellulose membranes were immunostained with supernatant of MICA 4 (2 µg/ml), a pool of all MICA (2 µg/ml), an irrelevant human monoclonal antibody (2 µg/ml), or the GAD 65-specific rabbit antiserum 1267 (donated by J. Peterson, Copenhagen, Denmark). Reactivity was detected in an enhanced chemiluminescence assay (Amersham) on Kodak XO Mat film. Membranes were then washed in PBS and stained with Coomassie brilliant blue.

Results

Immunohistochemical characterization. FAE7, a human monoclonal ICA of the IgM class was derived from a pre-diabetic individual using the above-mentioned methods. Reactivity of FAE7 and MICA 1–6 was analysed on different human organs and on pancreas sections of different mammalian species. In contrast to the MICA, FAE7 was not restricted to islet cells and did not cross-react with pancreas sections of the other mammalian species tested. Therefore, only MICA 1–6 exhibited a reactivity typical for ICA (Table 1).

Cell staining within islets. As judged by double immunofluorescence staining of the monoclonal antibodies and anti-hormone specific antibodies the MICA showed a beta-cell restricted staining pattern in many islets which overlapped almost completely with that of the anti-proinsulin antibody. However, some single cells lying at the periphery of some islets, which were negative with the proinsulin antibody were weakly stained by the MICA, especially when a pooled supernatant of MICA 1–6 was applied (Fig. 1 a–c). Cells positive for anti-glucagon or anti-somatostatin antibodies were negative for the MICA when judged within the same section (Fig. 1 d, e and results not shown). FAE7, in contrast, reacted with all cells of the islet and in addition with single cells of the exocrine pancreas as well as with pancreatic ducts (Fig. 1 f).

Biochemical stability of the antigenic epitopes of MICA 1–6. We compared the biochemical stability of the MICA epitopes with that of epitopes recognized by ICA from serum. MICA 3 and MICA 4 were selected because they were shown to recognize distinct epitopes in GAD which

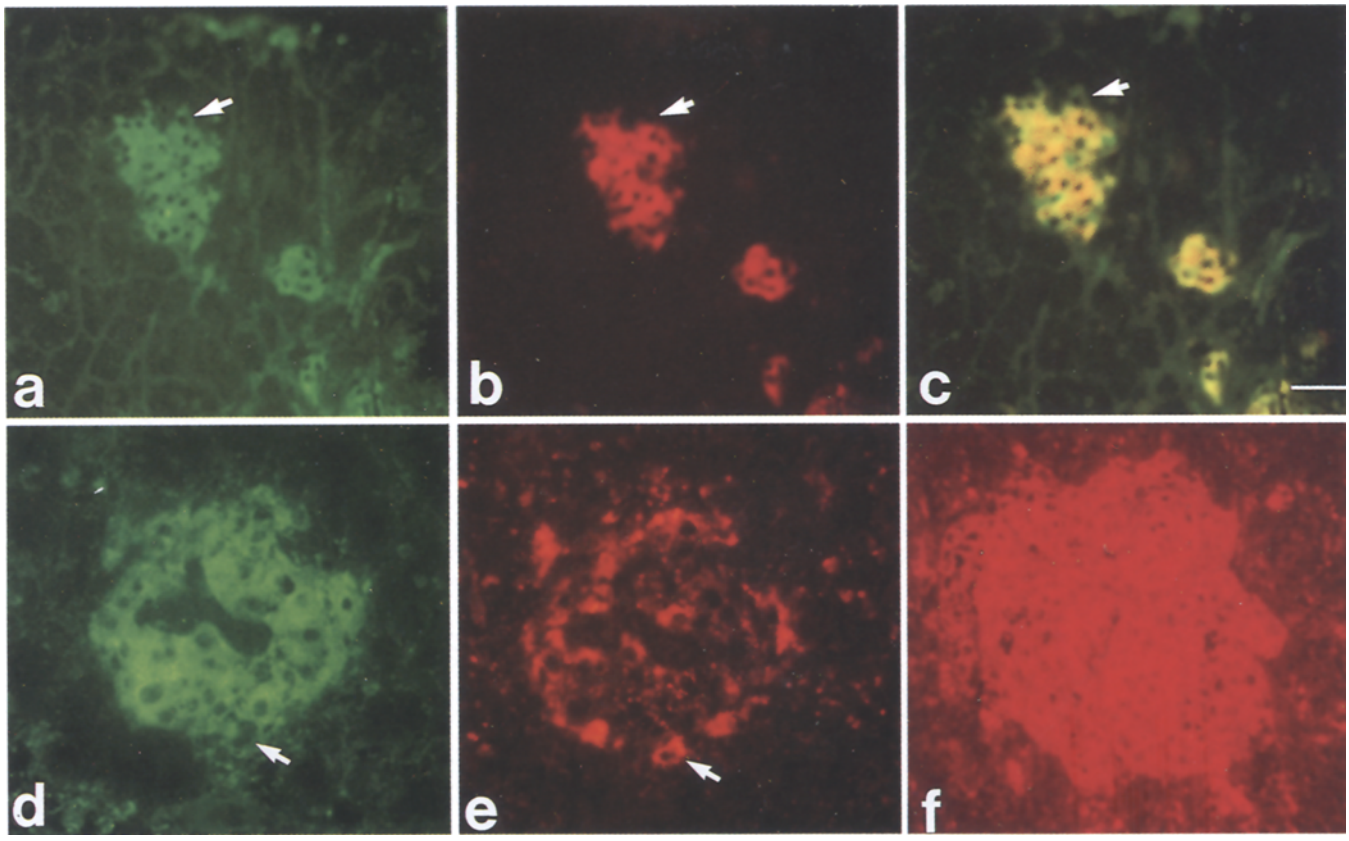

Fig. 1 (a–f). Double-fluorochrome immunofluorescence staining of a pool of MICA 1–6 and a mouse monoclonal proinsulin-specific antibody within the same section of human pancreas (**a–c**). MICA 1–6 were detected with FITC-conjugated anti-human IgG (**a**) and the anti-proinsulin antibody with rhodamine-conjugated anti-mouse IgG (**b**). Double exposure photography of picture **a** and **b** is shown in **c**. The yellow colour in **c** is given by the superimposed green and red colour in beta cells. Note that, in addition, there are some green-coloured cells visible at the periphery of the islets (indicated by the arrow) which are weakly stained by the MICA 1–6, but are negative for proinsulin. An islet double stained with MICA 1–6 (green in **d**) and an anti-glucagon-cell specific antibody (red in **e**) indicates glucagon-positive cells negative for the MICA (compare arrows in **d** and **e**). FAE7 gives a whole islet cell staining pattern in islets (**f**). Scale bar corresponds to 100 μm in **a, b, c,** and **f** and to 62 μm in **d** and **e**

are both common for sera from patients with Type 1 diabetes [8]. The anti-proinsulin specific monoclonal antibody and the monoclonal antibody A2B5 were included as examples of anti-protein and anti-ganglioside specific monoclonal antibodies, respectively. Binding of the ICA-positive serum and of MICA 3 and MICA 4 was not affected by treatment of the sections with acetone or Pronase. However, reactivity with islets was completely abolished when sections were pre-treated with methanol/chloroform. Neuraminidase treatment of the tissue optimized to ablate binding of the anti-ganglioside antibody A2B5 decreased the staining of ICA-positive serum and of MICA 3 and 4. This reduction of binding, however, was non-specific since the same result was achieved when the sections were pre-incubated with the control buffer alone (50 mmol/l sodium citrate, 37 °C, pH 5.5). In contrast, A2B5 staining was not affected by the buffer control incubation (Table 2). The biochemical stability of the epitopes recognized by MICA 3 and 4 was in line with the biochemical stability of the epitopes recognized by ICA from serum. Except for neuraminidase treatment, MICA 3 and 4 behaved similarly to the anti-ganglioside antibody A2B5. Despite recognizing protein epitopes, MICA 3 and 4 behaved differently from the anti-proinsulin antibody.

GAD was less sensitive to Pronase than proinsulin. To test whether GAD is insensitive to Pronase digestion, various concentrations and incubation times of Pronase were applied on tissue sections. Titration studies revealed a rapid degradation of proinsulin by 0.1 mg/ml Pronase in 10–15 min, whereas GAD epitopes recognized by the MICA remained stable during that time. A 1-h incubation with this Pronase concentration was necessary to degrade epitopes recognized by the MICA (Fig. 2). Alternatively a 10-fold increase in Pronase concentration degraded the MICA epitopes within 15 min and proinsulin epitopes within 2 min. GAD localized in the tissue, therefore, was much less sensitive to unspecific proteolysis by Pronase than proinsulin, but was not completely stable.

Methanol/chloroform treatment destroyed the MICA epitopes. We investigated whether the conformational GAD epitopes recognized by MICA 3 and MICA 4 were destroyed by incubation with methanol/chloroform or whether elution of GAD from the tissue contributed to the

Table 2. Sensitivity of islet cell epitopes to treatment with fixatives and enzymes: evaluation of islet cell staining on pre-treated sections

Treatment of sections	Immunostaining with[a]				
	MICA 3	MICA 4	ICA-positive serum	Anti-pro-insulin	A2B5
Untreated	+	+	+ +	+ +	+ +
Acetone	+	+	+ +	+ +	+ +
Methanol/ Chloroform	–	–	–	+ +	–
Pronase	+	+	+ +	–	+ +
Neuraminidase	–	(+)	+	+ +	–
Control buffer[b]	–	(+)	+	+ +	+ +

[a] Staining was performed with the indirect immunofluorescence test on cryostat sections of human pancreas.
[b] Buffer control incubation of the neuraminidase experiment: 50 mmol/l sodium citrate buffer pH 5.5 for 1 h at 37 °C.
MICA, Human monoclonal IgG-islet cell antibody. ICA, cytoplasmic islet cell antibody.
Semiquantitative evaluation of staining: + + very strong; + strong, (+) weak, – no staining

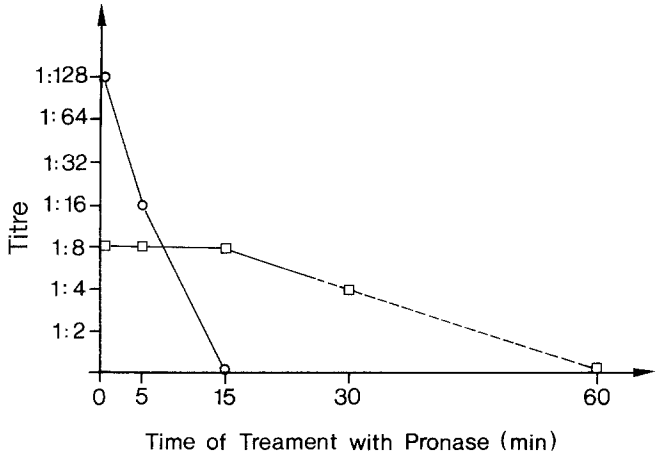

Fig. 2. Sensitivity of proinsulin and glutamate decarboxylase epitopes to Pronase digestion of the tissue section was assessed. Cryostat sections of human pancreas were incubated with Pronase (0.1 mg/ml) and after washing an indirect immunofluorescence test was performed on pre-treated sections. The titre of a monoclonal anti-human proinsulin antibody (○) and of a pool of the human monoclonal islet cell IgG antibodies MICA 3 and 4 (□) was assessed in relation with the time of Pronase digestion of the tissue. Antibody titres were not affected by the buffer control incubations without Pronase (not shown)

lack of reactivity after methanol/chloroform treatment. GAD purified from pig brain was immobilized on nitrocellulose and an immunoblotting assay was performed. Part of the blots were pre-treated with methanol/chloroform or Pronase. GAD pre-treated with methanol/chloroform was no longer recognized by MICA 3 and 4, whereas untreated or Pronase-treated GAD was still reactive (Fig. 3). An irrelevant human monoclonal antibody of the IgG class did not react with any of the slots (not shown). To exclude that GAD was not eluted from the nitrocellulose membrane during methanol/chloroform treatment, parallel blots were stained with a polyclonal serum (1267) raised against

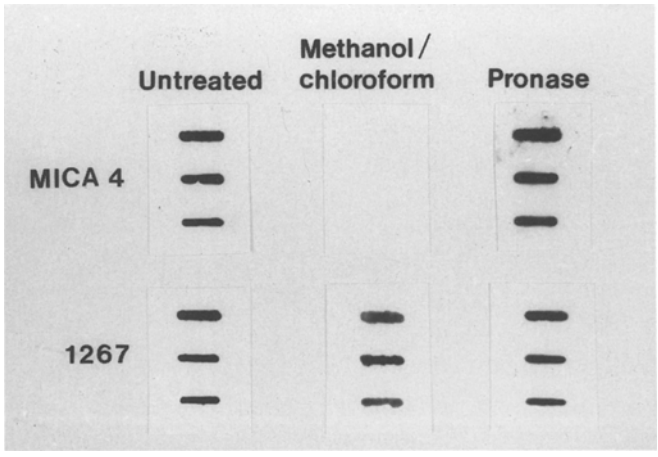

Fig. 3. Immunoblotting of GAD purified from pig brain. GAD (1 mg/ml) was spotted on nitrocellulose at dilutions 1:10, 1:100 and 1:500 (top to bottom) and blots left untreated or incubated with methanol/chloroform (5 min, 20 °C) or Pronase (0.1 mg/ml, 10 min, 20 °C). After washing blots were immunostained by MICA 4 or a polyclonal serum raised against a linear epitope in GAD (1267). An enhanced chemiluminescence assay was used to detect the peroxidase staining on an X-ray film

a linear epitope in GAD. An identical staining intensity with methanol/chloroform-treated and untreated GAD blots was observed confirming that no elution of GAD occurred. Therefore, the conformational epitopes recognized by MICA 3 and 4 must have been destroyed by methanol/chloroform treatment whereas the linear 1267 epitope in GAD remained unaffected.

Discussion

Human monoclonal antibodies are an invaluable tool with which to study the nature of the humoral immune response associated with pancreatic beta-cell destruction and development of Type 1 diabetes. We recently obtained the first human monoclonal ICA of the IgG-class (MICA 1–6) derived from a patient with Type 1 diabetes. MICA 1–6, selected by the classic ICA test of indirect immunofluorescence all recognized the Type 1 diabetes specific 64 kDa autoantigen GAD [7]. The epitopes recognized by the MICA were multiple and common for ICA from serum [7, 8]. We here asked the question whether the islet-reactive IgG monoclonals MICA 1–6 demonstrate all typical features of ICA. We further investigated whether FAE7, a new human monoclonal IgM-ICA isolated from a pre-diabetic individual fits in this panel of disease-related ICA.

FAE7 was derived from blood obtained 1 year before onset of Type 1 diabetes, but the antibody did not represent the typical features known for ICA-positive sera. Due to its cross-reactivity with human pituitary cells, thyroid, exocrine pancreatic epithelia, and pancreas ducts, FAE7 must be considered as a polyreactive IgM antibody like the previous islet-reactive human monoclonal IgM-antibodies derived from patients with Type 1 diabetes [13]. Such polyreactive IgM-antibodies also occur in nor-

mal individuals unrelated to autoimmune diseases [14]. Therefore, FAE7 can give no hints on ICA-reactive targets during earlier stages of beta-cell destruction in Type 1 diabetes.

Although MICA 1–6 were isolated using the conventional test for detection of ICA, they demonstrate all the known features of GAD antibodies. Beside the precipitation of a 64 kDa protein from islets and the immunotrapping of GAD-enzyme activity from brain extracts shown previously [7] MICA 1–6 revealed here a predominantly beta-cell specific staining pattern in islets which is consistent with the beta-cell specific expression of GAD [15, 16]. In some islets, a few single cells lying in the periphery were negative for proinsulin, but were weakly stained by the MICA. As glucagon or somatostatin staining did not overlap with the MICA staining, these single cells may be identical with the GAD-containing neural cells identified recently by Sorenson et al. [17]. These neurons were reported to be localized in the islet mantle and to extend nerve cell processes into the islet. We did not, however, test the PP-cell population and cannot exclude that PP cells contribute to this staining pattern.

Only some features typical for ICA were observed for the MICA. Whereas the specificity for islet cells and the cross-reactivity with islets of different mammalian species was typical for ICA, MICA 1–6 did not show the whole islet cell staining pattern reported for many ICA-positive sera [2]. Beta-cell specific GAD-reactive ICA, therefore, exist in at least some ICA-positive sera. Since many sera from newly-diagnosed patients with Type 1 diabetes are positive for both ICA and 64 kDa antibodies [3, 18], GAD-reactive ICA may be very common in ICA-positive sera.

Nayak and co-workers [5] have suggested that the islet antigen responsible for the ICA staining is a monosialoganglioside. When we analysed the biochemical properties of the MICA 3 and MICA 4 epitopes in GAD they revealed the same sensitivity to chemical pre-treatment of the tissue as epitopes recognized by ICA from serum. In addition, the stability of the MICA epitopes, except for the neuraminidase sensitivity, was identical to the anti-ganglioside antibody A2B5 and to the panel of ICA positive sera analysed by Nayak and colleagues [5]. The anti-protein control antibody directed to proinsulin behaved differently. We were able to show that GAD was very stable to Pronase digestion of tissue sections. Using GAD immobilized on nitrocellulose membranes we provided clear evidence that the conformational MICA epitopes in GAD were destroyed by treatment with methanol/chloroform. No elution of GAD from nitrocellulose membranes due to methanol/chloroform treatment was evident. However, it cannot be excluded that under normal conditions in the tissue sections elution of GAD may occur as an additional effect of methanol/chloroform treatment beside epitope destruction.

Together our data demonstrate that conformational protein epitopes can share sensitivity to chemical pretreatment of the target tissue with ganglioside epitopes. Therefore, chemical pre-treatment of the tissue used in the past was not suitable to characterize the chemical nature of target antigens recognized by ICA. The IgG monoclonal antibodies MICA 1–6 share features of both ICA and 64 kDa antibodies and must be considered as beta-cell specific GAD-reactive ICA. These data together with data from others [19, 20] strongly suggest a heterogeneous composition of ICA in ICA-positive sera from patients with Type 1 diabetes. We demonstrated with the MICA that the protein GAD is one of the autoantigens recognized by ICA and we suggest that proteins as well as gangliosides are potential candidates for additional target antigens of ICA beside GAD.

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References

1. Bottazzo GF, Florin-Christensen A, Doniach D (1974) Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet II: 1279–1283
2. Bottazzo GF, Doniach D (1978) Islet-cell antibodies (ICA) in diabetes mellitus. Evidence of an autoantigen common to all cells in the islet of Langerhans. La Ricerca Clin Lab 8: 29–38
3. Baekkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark Å (1982) Autoantibodies in newly diagnosed diabetic children immunoprecipitate pancreatic islet cell proteins. Nature 298: 167–169
4. Baekkeskov S, Aanstoot HJ, Christgau S et al. (1990) Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347: 151–156
5. Nayak RC, Omar AK, Rabizadeh A, Srikanta S, Eisenbarth GS (1985) Cytoplasmic islet cell antibodies: evidence that the target antigen is a sialoglycoconjugate. Diabetes 34: 617–619
6. Colman PG, Nayak RC, Campbell IL, Eisenbarth GS (1988) Binding of cytoplasmic islet cell antibodies is blocked by human pancreatic glycolipid extracts. Diabetes 37: 645–652
7. Richter W, Endl J, Eiermann TH et al. (1992) Human monoclonal islet cell antibodies from a patient with insulin-dependent diabetes mellitus reveal glutamate decarboxylase as the cytoplasmic islet cell antigen. Proc Natl Acad Sci USA 89: 8467–8471
8. Richter W, Shi Y, Baekkeskov S (1993) Autoreactive epitopes defined by diabetes associated human monoclonal antibodies. Proc Natl Acad Sci USA: 90: 2832–2836
9. Richter W, Eiermann TH, Graf G, Glueck M, Scherbaum WA, Pfeiffer EF (1989) Isolation of IgG islet cell antibody-producing B-lymphocytes from the peripheral blood of type I diabetic patients and an ICA-positive nondiabetic individual. Horm Metabol Res 21: 686–688
10. Scherbaum WA, Mirakian R, Pujol-Borrell R, Dean BM, Bottazzo GF (1986) Immunocytochemistry in the study and diagnosis of organ-specific autoimmune diseases. In: Polak M, Van Noorden S (eds) Immunocytochemistry modern methods and applications. Wright, Bristol, pp 456–476
11. Tuson RJ, Pascoe EW, Jacob DA (1990) A novel immunohistochemical technique for demonstration of specific binding of human monoclonal antibodies to human cryostat tissue sections. J Histochem Cytochem 38: 923–926
12. Eisenbarth GS, Shimizu K, Bowring MA, Wells S (1982) Expression of receptors for tetanus toxin and monoclonal antibody A2B5 by pancreatic islet cells. Proc Natl Acad Sci USA 79: 5066–5070
13. Satoh J, Prabhakar BS, Haspel MV, Ginsberg-Fellner F, Notkins AL (1983) Human monoclonal autoantibodies that react with multiple endocrine organs. New Engl J Med 309: 217–220
14. Casali P, Notkins AL (1990) Probing the human B-cell repertoire with EBV: polyreactive antibodies and CD5 + B lymphocytes. Ann Rev Immunol 7: 513–535

15. Christie MR, Pipeleers DG, Lernmark Å, Baekkeskov S (1990) Cellular and subcellular localization of an M_r 64.000 protein autoantigen in insulin-dependent diabetes. J Biol Chem 265: 376–381
16. Garry DJ, Sorenson RL, Elde RP, Maley BE, Madsen A (1986) Immunohistochemical colocalization of GABA and insulin in β-cells of rat islets. Diabetes 35: 1090–1095
17. Sorenson RL, Garry DG, Brelje TC (1991) Structural and functional considerations of GABA in islets of Langerhans. Diabetes 40: 1365–1374
18. Atkinson MA, MacLaren NK, Scharp DW, Lacy PE, Riley WJ (1990) 64.000 M_r autoantibodies as predictors of insulin-dependent diabetes. Lancet 335: 1357–1360
19. Gianani R, Pugliese A, Bonner-Weir S et al. (1992) Prognostically significant heterogeneity of cytoplasmic islet cell antibodies in relatives of patients with type 1 diabetes. Diabetes 41: 347–353
20. Genovese S, Bonifacio E, McNally JM (1992) Distinct cytoplasmic islet cell antibodies with different risks for type 1 (insulin-dependent) diabetes mellitus. Diabetologia 35: 385–388

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