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Cytoplasmic Islet Cell Antibodies Recognize Distinct Islet Antigens in IDDM But Not in Stiff Man Syndrome

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Cytoplasmic islet cell antibodies are well-established predictive markers of IDDM. Although target molecules of ICA have been suggested to be gangliosides, human monoclonal ICA of the immunoglobulin G class (MICA 1–6) produced from a patient with newly diagnosed IDDM recognized glutamate decarboxylase as a target antigen. Here we analyzed the possible heterogeneity of target antigens of ICA by subtracting the GAD-specific ICA staining from total ICA staining of sera. This was achieved *1*) by preabsorption of ICA$^+$ sera with recombinant GAD_{65} and/or GAD_{67} expressed in a baculovirus system and *2*) by ICA analysis of sera on mouse pancreas, as GAD antibodies do not stain mouse islets in the immunofluorescence test. We show that 24 of 25 sera from newly diagnosed patients with IDDM recognize islet antigens besides GAD. In contrast, GAD was the only islet antigen recognized by ICA from 7 sera from patients with stiff man syndrome. Two of these sera, however, recognized antigens besides GAD in Purkinje cells. In patients with IDDM, non-GAD ICA were diverse. One group, found in 64% of the sera, stained human and mouse islets, whereas the other group of non-GAD ICA was human specific. Therefore, mouse islets distinguish two groups of non-GAD ICA and lack additional target epitopes of ICA besides GAD. Longitudinal analysis of 6 sera from nondiabetic ICA$^+$ individuals revealed that mouse-reactive ICA may appear closer to clinical onset of IDDM in some individuals. Mouse-reactive ICAs, however, remained absent in 36% of the patients at diagnosis of IDDM.
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IDDM, insulin-dependent diabetes mellitus; ICA, islet cell antibody; GAD, glutamate decarboxylase; SMS, stiff man syndrome; JDF U, Juvenile Diabetes Foundation units; GABA, γ-aminobutyric acid; IVGTT, intravenous glucose tolerance test.

The humoral immune response to islet cells in IDDM has been extensively studied, and two major groups of autoantibodies were established as highly predictive markers for the development of the disease. Cytoplasmic ICAs were defined by an indirect immunofluorescence test on pancreas sections (1) and 64,000-M_r antibodies were evaluated by an immunoprecipitation assay on in vitro–labeled islet cells (2). The 64,000-M_r islet antigen was identified as the enzyme GAD (3), whereas islet antigens recognized by ICA were suggested to be monosialogangliosides (4,5). The first evidence that ICA and 64,000-M_r antibodies were partly identical was demonstrated by the analysis of human monoclonal ICA of the IgG class (MICA 1–6) derived from a patient with newly diagnosed IDDM (6). These human monoclonal autoantibodies revealed typical features of both ICA and 64,000-M_r antibodies and demonstrated that GAD is a target antigen of ICA (7). Whereas some ICA$^+$ IDDM sera, however, stained all cells of the islet, the GAD-specific MICA recognized predominantly β-cells, consistent with the β-cell–specific expression of GAD in islets (8,9). This indicated a heterogeneous composition of ICA in serum from patients with IDDM. A heterogenous composition of ICA was also suggested by a study describing species-restricted ICA with different predictive value for development of IDDM (10) and by a study describing β-cell–selective and –nonselective staining patterns of ICA$^+$ sera (11).

Apart from IDDM, ICA and GAD-reactive autoantibodies were described in sera from patients with the rare neurological disorder SMS, where GABA-ergic neurons rather than β-cells are affected by the disease. Patients with SMS are frequently ICA$^+$ and some of them develop IDDM during the progress of the autoimmune disease (12).

The aim of this study was to evaluate and further

characterize the heterogeneity of ICA in sera from newly diagnosed patients with IDDM and from patients with SMS. We analyzed ICA reactivity by *1*) preabsorption of sera with human recombinant GAD_{65} and GAD_{67} before ICA analysis and *2*) by analyzing reactivity with mouse pancreatic islets, which express low levels of GAD (7,13). The GAD-specific MICA had demonstrated that mouse islets express GAD at a level that is below the detection limit of the immunofluorescence test (24). We therefore assessed whether ICA analysis on mouse pancreas could be used as a short and simple method to distinguish GAD-reactive ICA from non–GAD-reactive ICA in sera from patients with IDDM. We found a heterogeneous reactivity of ICA^+ sera to mouse islets, which did not correlate with the GAD-blocking data. Only a subgroup of non–GAD-reactive ICA were detected in mouse islets, which, we concluded, must lack additional typical target epitopes of ICA besides GAD. GAD-reactive ICA as well as two different groups of non–GAD-reactive ICA may, therefore, coexist in sera from patients with IDDM.

RESEARCH DESIGN AND METHODS

Sera were obtained with informed consent from 25 patients with newly diagnosed IDDM (mean age of 20.7 ± 8.0 yr; range 11–33 yr) and from 7 ICA^+ patients with SMS (mean age 53.3 ± 12.6 yr; range 30–69 yr). One SMS patient (No. 4 in Table 1) had developed IDDM 4 yr before the serum sample was taken. Serial serum samples were analyzed from 6 ICA^+ individuals (mean age 15.7 ± 2.4 yr; range 10–17 yr) over a period of 32 mo. The individuals were characterized previously elsewhere (14), and 3 of them developed IDDM during this time of follow-up.

Antibody analyses. ICA were detected in patients' sera by the classical ICA test of indirect immunofluorescence on unfixed sections of human pancreas (blood group 0) as described previously (15). Quantification in JDF U was performed according to recommendations of the Second International ICA Workshop. Values >80 JDF U were calculated by extrapolation of the standard curve. ICA analysis on Balb/c mouse pancreas was perfomed with undiluted patients' sera as well as with 20 normal sera. Test specificity was 95%, and test sensitivity with the IDDM sera was 64%.

Antibodies to GAD were determined in an immunoprecipitation assay (16). Recombinant baculovirus encoding for full-length human GAD_{65} and GAD_{67} was derived as described elsewhere (17). GAD was expressed in *Spodoptera frugiperda* (Sf9) cells by infection with recombinant virus. At 48 h after infection, Sf9 cells were labeled for 2 h with [^{35}S]methionine in methionine-free Grace's medium. Cells were lysed with 20 mM Tris/HCl, pH 7.4, 1 mM phenyl-methyl-sulfonyl-fluoride, 1 mM amino-ethyl-isothiouronium-bromide, 0.02 mM pyridoxal-phosphate, 1% Triton X100 and 2 μg/ml of the proteinase inhibitors leupeptin, aprotinin, bestatin, and pepstatin and incubated for 30 min on ice. After a spin at 33,000 *g* for 30 min at 4°C, supernatant was precleared with Protein A Sepharose and normal human serum followed by specific precipitation of GAD by the patients' serum as described previously (16). The expressed GAD proteins were derived in a native conformation and a fully restored autoantigenicity (17). In the first GAD antibody workshop, our assay revealed a sensitivity of 83.3% and a specificity of 100%.

Blocking analyses. Blocking analyses were performed with baculovirus-expressed GAD_{65} and GAD_{67} unmixed or in a mixture of 3:1. Virus-infected cells were lysed 48 h after infection with 20 mM KH_2PO_4, 2 mM ethylen-diamin-tetra-acetate, 2 mM amino-ethyl-isothiouronium-bromide, 0.2 mM pyridoxalphosphate, and 1 mM phenyl-methyl-sulfonyl-fluoride. Cell debris was removed at 33,000 *g* for 30 min at 4°C, and supernatant was checked for protein content (18). Specific GAD enzyme activity of the supernatant was determined by a modification of the method described by Albers and Brady (19). Aliquots from homogenates were added to tubes containing 200 μl of 50 mM KH_2PO_4, 2 mM amino-ethyl-isothiouronium-bromide, 0.2 mM pyridoxal-phosphate, 1 mM L-Glu, and 0.1 μCi L-[1-^{14}C]Glu (59 mCi/mM) (Amersham, Amersham, UK). A filter paper soaked with 50 μl of 1 M hyamine hydroxide was placed in the tubes. The tubes were closed immediately and incubated at 37°C for 60 min. The reaction was terminated by injecting 1 ml of 5 N H_2SO_4. After a 1-h equilibration period, the $^{14}CO_2$ absorbed in the filter paper was counted in a liquid scintillation counter. One unit of GAD was defined as 1 μmol $^{14}CO_2$ formed per minute per milligram of protein.

For preincubation of sera before ICA analysis, 10 μl of serum were incubated with optimized amounts of GAD_{65}, GAD_{67}, or a mixture of both (3:1) for 1 h at room temperature under gentle rotation. Corresponding volumes of supernatant isolated from Sf9 cells infected with wild-type baculovirus expressing no GAD were used as a control. Twenty microliters of the blocking mixture were then applied in the indirect immunofluorescence test to evaluate residual ICA staining. SMS sera with titers >2560 JDF U were diluted 1:30 before blocking or for staining of mouse pancreas.

RESULTS

Blocking of ICA^+ sera with GAD_{65} and GAD_{67}. For subtraction of GAD reactivity from total ICA staining, we established a blocking assay requiring minimal dilution of sera by the blocking solution. The human monoclonal anti-GAD_{65} antibody MICA 4 and a high-titered ICA^+ serum (SMS serum No. 3) positive for GAD_{65} and GAD_{67} in the immunoprecipitation test were used to optimize blocking conditions. MICA 4 and the SMS serum were preabsorbed with increasing volumes of a mixture of GAD_{65} and GAD_{67} (3:1) before their ICA reactivity was quantitated by titration on human pancreas (Fig. 1). The titer of the SMS serum decreased rapidly with increasing amounts of GAD and was ablated by preincubation with 20 μU of the GAD mixture. The amount of GAD sufficient to block the ICA staining of MICA 4 was 5 μU; 40 μU of GAD was selected as the optimal blocking condition for further experiments. This required a dilution of the sera by the blocking mixture of only 1:4.

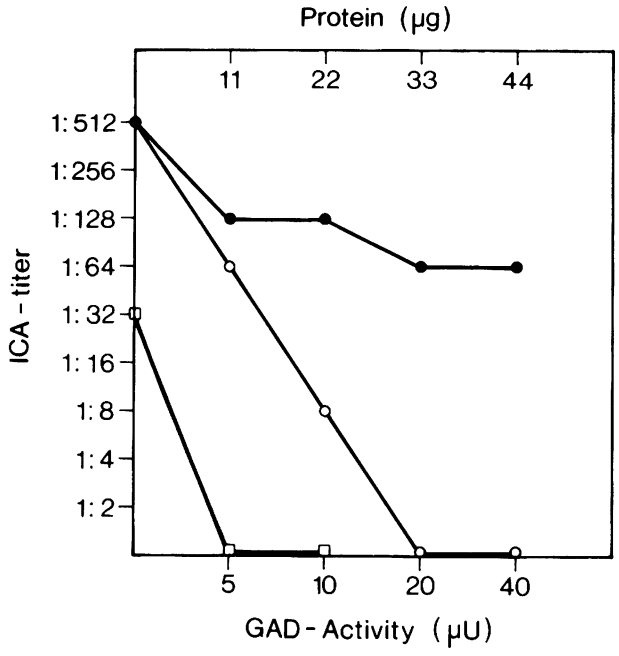

FIG. 1. The GAD-specific human monoclonal antibody MICA 4 and an ICA$^+$ serum (SMS serum No. 3 in Table 1) were preabsorbed with increasing volumes of a mixture of GAD_{65} and GAD_{67} (3:1) as described in METHODS. ICA reactivity was quantitated on human pancreas. ICA reactivity of MICA 4 (□) was blocked by preincubation with 5 μU of GAD. The titer of the SMS serum decreased rapidly after preincubation with increasing amounts of GAD extracts (○). The control incubation of the SMS serum with GAD-free baculovirus extracts (●) reflects the dilution effect attributable to increasing volumes of blocking solution applied in the preincubation step (control not shown for MICA 4).

Prevalence of GAD-reactive antibodies. In 25 ICA$^+$ sera from patients with newly diagnosed IDDM, prevalence of GAD antibodies was determined in an immunoprecipitation assay using human recombinant GAD_{65} and GAD_{67} expressed in a baculovirus system. Of the sera, 15 were positive for GAD_{65} and 3 were additionally positive for GAD_{67}. ICA staining of only 1 of 25 sera could be blocked completely by preincubation with mixtures of GAD_{65} and GAD_{67}. Therefore, in all but 1 serum, non–GAD-reactive ICA were detected. In contrast, the 7 sera from patients with SMS were all positive for GAD_{65} and for GAD_{67} in the immunoprecipitation assay, and ICA staining of all of them was completely blocked by preincubation with a mixture of GAD_{65} and GAD_{67} (Table 1). However, when we modified the assay according to a procedure applied by Atkinson et al. (20) and diluted the GAD-positive IDDM sera before their end-point titer before blocking with GAD, we observed that 3 additional sera were completely blocked by GAD (IDDM sera no. 11, 20, and 22). This indicates that GAD-reactive and non–GAD-reactive ICA are present in different titers in ICA$^+$ sera and reveals that GAD-reactive ICA represent the high titered ICA in 3 of the tested sera. In sum, GAD is the only islet antigen recognized by ICA from sera of patients with SMS, whereas additional islet antigens besides GAD are recognized by 96% of ICA$^+$ sera from newly diagnosed patients with IDDM. The serum of the only patient who had developed both SMS and IDDM behaved like all other SMS sera and was completely blocked by GAD.

Isoform-specific blocking on islets and brain. All SMS sera and the single IDDM serum, which was completely blocked by a combination of GAD_{65} and GAD_{67} on islets, were analyzed for isoform-specific blocking on islets and cerebellum. Preincubation with GAD_{65} resulted in a complete ablation of ICA staining in islets for the IDDM serum, consistent with its GAD_{65} specificity. Although all SMS sera were strongly positive for GAD_{67} in the immunoprecipitation test, GAD_{65} was sufficient to block their staining on islets, whereas preblocking with GAD_{67} did not ablate their ICA staining. However, when the blocking assay was performed on sections of rat cerebellum, where both GAD forms are expressed in neurons positive for GABA (21), none of the SMS sera was completely blocked either by preincubation with GAD_{65} or by preincubation with GAD_{67} alone. Only a combination of both GAD forms ablated the staining of the GABA-ergic neurons in 5 of 7 SMS sera. This demonstrated that GAD_{65}-specific as well as GAD_{67}-specific antibodies were present in at least 5 SMS sera and that 2 SMS sera contained additional brain-specific antibodies that recognized antigen(s) different from GAD in GABA-ergic neurons. These antigens were not detected in human islet cells. The inconsistency of results obtained with preabsorbed SMS sera on brain and islets can only be explained by differences in the expression of both GAD forms in these tissues. As GAD_{67}-specific antibodies escaped detection in human islets, no GAD_{67} seems to be expressed in human β-cells. These data are consistent with a report from Karlsen et al. (22) that detected only GAD_{65} in human islets, which confirms our own results (J. Kim, W. R., S. Baekkeskov, unpublished observations) that only GAD_{65} is expressed in human islets.

Heterogenous reactivity of ICA with mouse islets. Because of its low expression of both GAD_{65} and GAD_{67}, mouse pancreas is suitable for subtracting GAD reactivity from ICA$^+$ sera directly in the immunofluorescence test. Therefore we analyzed ICA reactivity of the IDDM and the SMS sera on mouse pancreas in comparison with ICA$^-$ normal human sera. The majority of sera revealed an unspecific background staining of exocrine cells of mouse pancreas that was independent of the conjugated secondary antibody used, the disease status, or the positivity of islets. This background staining seemed to be a limitation of mouse pancreas as the same unspecific staining was observed when the sera were analyzed on NOD mouse pancreas. Islets were evaluated as positive in our test when their staining was brighter than the dark cells of the exocrine pancreas. According to this criterion, 1 normal control serum was weakly ICA$^+$ when 20 ICA$^-$ normal human sera were analyzed on mouse pancreas. Among the ICA$^+$ SMS and IDDM sera analyzed, all SMS sera were ICA$^-$ on mouse islets (compare Fig. 2*B* and *D*), and IDDM sera behaved heterogenously. Only 16 of 25 ICA$^+$ IDDM sera (64%) remained ICA$^+$ when tested on mouse tissue (Table 1 and Fig. 2*A* and *B*). For the SMS sera, ICA analysis on mouse pancreas confirmed the data obtained by blocking; only GAD-reactive ICA contributed to the ICA staining of these sera on

TABLE 1
Antibody characteristics of ICA$^+$ sera and subtraction of GAD-specific staining

		Antibodies			ICA staining	
	Age (yr)	ICA (JDF U)	GAD_{65}*	GAD_{67}*	Blocked by GAD_{65} plus GAD_{67}	On mouse islets
IDDM subject						
1	16	20	+	−	−	+
2	38	20	+	−	+	−
3	33	40	+	+	−	+
4	11	40	−	−	−	+
5	22	80	−	−	−	−
6	18	80	−	−	−	+
7	19	80	+	−	−	+
8	17	80	+	−	−	+
9	36	80	+	−	−	+
10	19	160	−	−	−	−
11	16	160	−	−	−	−
12	18	320	−	−	−	+
13	20	320	−	−	−	−
14	18	320	−	−	−	+
15	14	320	−	−	−	−
16	11	320	+	+	−	−
17	33	320	+	−	−	+
18	18	320	+	−	−	+
19	12	320	+	−	−	+
20	31	320	+	−	−	−
21	11	640	−	−	−	+
22	14	640	+	−	−	−
23	25	640	+	−	−	+
24	26	1280	+	−	−	+
25	22	1280	+	+	−	+
SMS subject						
1	56	40	+	+	+	−
2	53	320	+	+	+	−
3	69	2560	+	+	+	−
4	11	2560	+	+	+	−
5	30	3200	+	+	+	−
6	59	5120	+	+	+	−
7	45	10240	+	+	+	−

+, positive; −, negative.
*Determined by immunoprecipitation of GAD_{65} or GAD_{67} from baculovirus extracts.

human pancreas. Among the diabetic sera, recognition of epitopes besides GAD became directly evident in 64% of the sera by their reactivity with mouse islets. In the remaining sera, being negative on mouse islets, only 1 serum was blocked by GAD in the blocking test. Non–GAD-reactive ICA must, therefore, exist as well in the majority of IDDM sera, which do not show ICA reactivity on mouse islets. These data clearly demonstrate the existence of non-GAD ICA that do not react with mouse pancreas. In conclusion, mouse islets must lack additional target epitopes of ICA outside of GAD. Non-GAD ICA in individual sera of patients with IDDM may, therefore, be heterogeneously composed, and multiple islet antigens seem to provoke a humoral immune response during the course of β-cell destruction in IDDM.

Longitudinal analysis of ICA$^+$ sera. We followed the heterogeneity of ICA in 6 ICA$^+$ individuals, 3 of which became diabetic during the 32-mo follow-up. To assess the natural history of ICA reactivity in these sera, GAD reactivity was analyzed by immunoprecipitation, and ICA staining was followed on mouse and human pancreas with the indirect immunofluorescence test. The results are summarized in Table 2. All individuals were GAD-antibody positive and remained ICA$^+$ on human pancreas throughout the follow-up. A heterogeneous reactivity of the sera was observed in the ICA test on mouse pancreas. Reactivity with mouse islets remained constantly negative or positive during the follow-up in 4 individuals, 3 of which were tested around the onset of IDDM. One individual, however, was initially ICA$^-$ on mouse pancreas and became strongly positive after 1 yr. During this period, GAD antibodies disappeared from the serum. This individual revealed a pathological IVGTT (first-phase insulin response below the first percentile) throughout the observation period but did not progress to overt diabetes yet. In a second individual, a weak reactivity on mouse pancreas turned into a strong positivity over a 6-mo period during which GAD antibodies remained stable. So far, no pathological first-phase insulin response in the IVGTT was observed in this individual.

DISCUSSION

For a long time, ICA and 64,000-M_r antibodies were defined only by the method by which they were detected. According to the disconcordant prevalence of the two

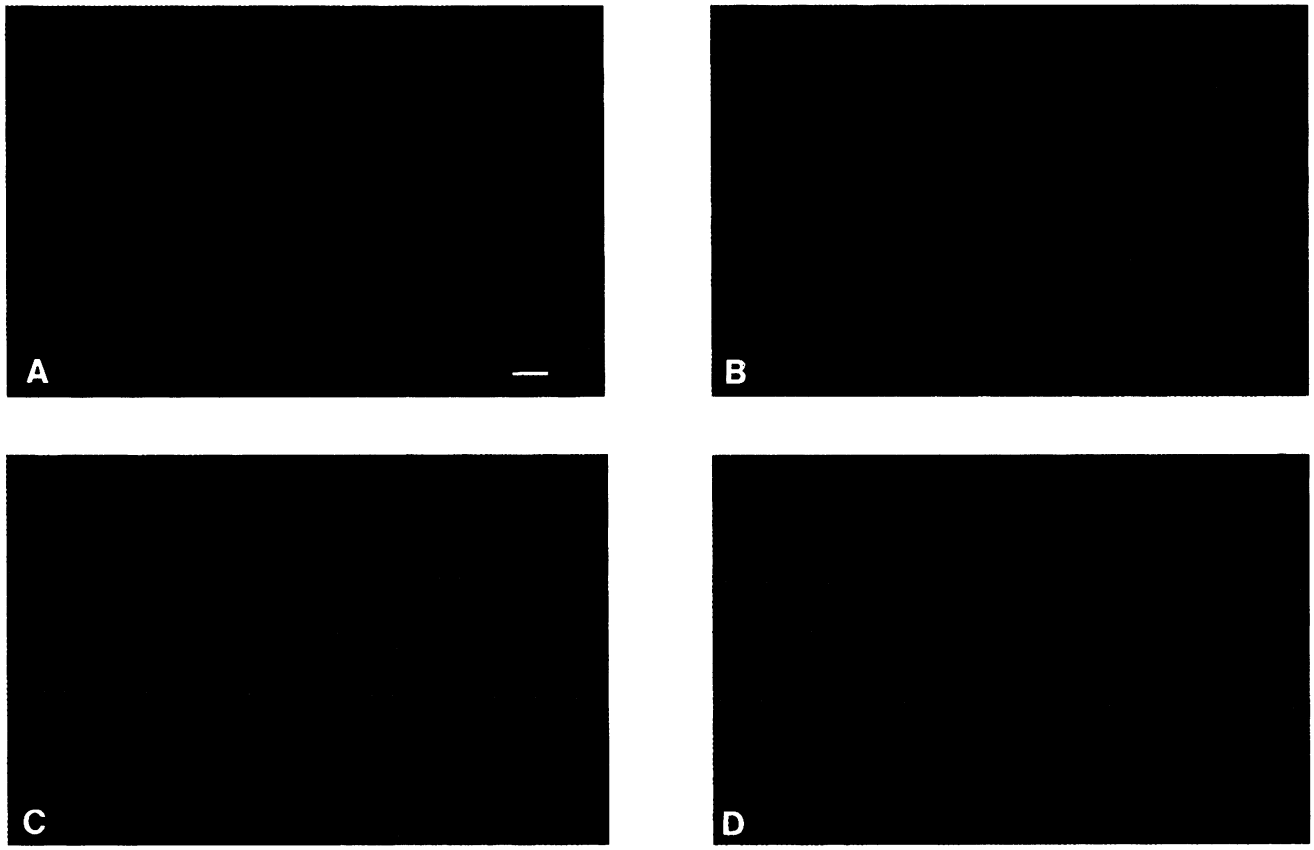

FIG. 2. Indirect immunofluorescence staining performed on unfixed cryostat sections of human pancreas (*A* and *B*) and mouse pancreas (*C* and *D*) obtained with serum from an ICA$^+$ patient with IDDM and from a patient with SMS. The serum from the patient with IDDM was positive on human and mouse islet cells (*A* and *C*). The SMS serum, which was positive for GAD_{65} and GAD_{67} in the immunoprecipitation assay, was positive on human islets (*B*) but failed to stain mouse islet cells (*D*). Scale bar 100 μm (*A*, *B*, and *D*); scale bar 64 μm (*C*).

antibody groups in sera, they were considered distinct entities with 64,000-M_r antibodies recognizing an islet protein and cytoplasmic ICA recognizing a monosialoglycolipid (2,4). Several lines of evidence have now accumulated to a more complex view of these markers of autoimmune insulitis.

Partial identity of ICA and 64,000-M_r antibodies became evident from analysis of human monoclonal ICA of the IgG class (MICA 1–6) derived from a patient with IDDM (6). MICA 1–6 shared common features of ICA and 64,000-M_r antibodies and identified GAD as a target antigen of ICA (7,8). In addition, heterogeneity of ICA became obvious by the predominantly β-cell–specific staining pattern of the MICA in contrast to a staining of all islet cells in some ICA$^+$ sera (8). Heterogeneity of ICA was also suggested by a study distinguishing ICA$^+$ sera by staining pattern. A diffuse staining of all islet cells was described for some ICA$^+$ sera, whereas other ICA$^+$ sera gave a granular staining pattern of only β-cells (11). Another report described species-restricted, β-cell–specific ICA that did not react with mouse pancreas and revealed a lower degree of risk for progression to overt diabetes than other ICA called nonrestricted ICA. These nonrestricted ICA, in contrast, stained all islet cells and reacted with mouse pancreas (10). As our GAD-specific MICA stained predominantly β-cells in islets (8) and lacked a reactivity with mouse islets (24), we speculated that GAD-reactive ICA may contribute for the β-cell–restricted subgroups of ICA observed in these two studies.

In this study, therefore, we investigated the possible hetereogeneity of ICA and the GAD reactivity of ICA in 25 ICA$^+$ sera from newly diagnosed patients with IDDM. By combining three different tests we characterized three distinct subgroups of ICA present in sera from patients with IDDM. In addition to the GAD antibodies that were detected in 60% of these sera by an immunoprecipitation assay, we identified non-GAD ICA in 96% of the individuals using a newly established GAD-blocking assay. By ICA analysis on mouse and human pancreas we further distinguished two different groups of non–GAD-reactive ICA in !DDM sera. One group, present in 64% of the sera, was reactive with mouse and human pancreas, whereas another group, present in at least 32% of the sera, did not recognize antigens in mouse islets. One possible explanation for these data is that one additional target antigen of ICA besides GAD exists in both mouse and human islet cells. Non-GAD ICA reactive with mouse and human pancreas would then recognize mouse-human cross-reactive epitopes in this antigen, whereas the other non-GAD ICA bind only human-specific epitopes in this antigen. Alternatively, ICA from IDDM sera recognize

TABLE 2
Antibody characteristics of 3 prediabetic and 3 ICA$^+$ nondiabetic individuals followed over a 32-mo period

Subject	Sample (mo of follow-up)	Anti-GAD_{65}	ICA	
			Human (JDF U)	Mouse
A	0	+	160	+
	8	+	40	+
	24*	+	160	+
B	0	+	40	−
	12	+	10	−
	32	−	40	+
C	0	+	80	−
	18*	+	10	−
	32	+	40	−
D	0	+	160	+
	12	+	160	+
	32	+	160	+
E	0	+	80	(+)
	6	+	80	++
	27	+	160	++
F	0	+	10	+
	9	+	5	+
	30*	+	10	+

−, negative; (+), weakly positive; +, positive; ++, strongly positive.
*Time of onset of IDDM in prediabetic individuals.

several additional antigens besides GAD, some of which are differentially expressed in mouse and human islet cells. A major difference in expression levels between mouse and human islets was already demonstrated for GAD (13). In sum, our results demonstrate that non-GAD ICA in IDDM are diverse and coexist in a single serum. The low expression of GAD_{65} and GAD_{67} in mouse pancreas does not provide a simple opportunity to distinguish GAD reactivity from all other ICA reactivities observed in IDDM sera.

By using a blocking assay requiring minimal dilution of sera, we observed a 96% prevalence of non–GAD-reactive ICA in IDDM sera. As the baculovirus-expressed GAD used for blocking demonstrated a fully restored antigenicity (17), the lack of correlation between blocking data and ICA staining of mouse islets is valid and not a result of uncorrect folding of the GAD used for blocking. In a previous study, Atkinson et al. (20) observed a prevalence of non–GAD-reactive ICA in 72% of new-onset IDDM patients with the use of a blocking test, in which the sera were diluted before their end point before blocking with GAD. Applying such conditions for our IDDM sera, the detection of non–GAD-reactive ICA decreased to 84%. We conclude that different ICA exist at different titers in the sera. As non–GAD-reactive ICA were diluted out in 3 sera by dilution of the sera before blocking, GAD-reactive ICA contributed to the high titered ICA in these sera.

ICA and GAD reactivity have been described in sera from patients with SMS, a condition that is sometimes associated with IDDM (12). Antibody reactivity in ICA$^+$ sera from patients with SMS was completely different from IDDM sera. All SMS sera reacted with both GAD_{65} and GAD_{67}, and all ICA observed were GAD reactive. In IDDM, in contrast, both GAD forms were recognized by only 8% of the sera, and non–GAD-reactive ICA existed in 96% of the sera. A major difference in antibody status was therefore observed in the two diseases. Coincident with the lack of GAD_{67} expression in human islets (22), GAD_{67}-reactive antibodies were rare in IDDM sera. In contrast, all SMS sera were autoreactive toward both forms of GAD, whereas GAD_{67} and GAD_{65} are both expressed in GABA-positive neurons (23). In addition, 2 SMS sera contained additional neuron-specific antibodies besides GAD antibodies. Although the number of patients with SMS tested is limited, these results indicate a heterogeneity of autoreactive antibodies occurring preferentially toward antigenic targets expressed at the site affected by the autoimmune disease: toward islets in IDDM and toward GABA-ergic neurons in SMS. Whether cross-reactivity of autoantibodies plays a role when patients with SMS develop IDDM is an interesting feature to be addressed. We analyzed ICA from serum of a patient with SMS who also suffered from IDDM and detected only GAD-reactive ICA. Because this serum was derived 4 yr after diagnosis of IDDM and no serum before or around onset of IDDM was available from this patient, it remains to be determined whether insulitis and precipitation of IDDM temporarily affect the heterogeneity of ICA in serum from patients with SMS.

ICA cross-reactivity between mouse and human have been reported to stain all islet cells and to indicate a high risk for the development of IDDM in first-degree relatives of patients with IDDM (10). ICA lacking reactivity with mouse islets were, in contrast, shown to be β-cell–specific and to indicate a lower risk for progression to overt diabetes. In our study on ICA$^+$ newly diagnosed patients with IDDM, frequency of mouse-reactive ICA was only 64%. ICA detected on mouse islets therefore represented less sensitive markers for progression to overt disease than ICA detected on human islets. Because the previous study compared ICA reactivity on mouse and rat pancreas and we compared ICA reactivity on human and mouse tissue, the observed differences may relate to the substrate chosen.

By natural history analysis of 6 ICA$^+$ individuals, we demonstrated that mouse-reactive ICAs may appear as additional markers closer to onset of IDDM but remain absent in some of the individuals that progress to overt diabetes. Atkinson et al. (20) observed in a longitudinal study on 3 individuals that GAD-reactive ICA turned into non–GAD-reactive ICA nearer to clinical onset of IDDM. The researchers therefore suggested that GAD antibodies are markers of early β-cell destruction, and non-GAD ICA may provide a marker for a more advanced lesion of islet cell destruction. Interestingly in one of our ICA$^+$ individuals with a pathological IVGTT, a conversion from GAD-reactive ICA to mouse-reactive non-GAD ICA was observed as well. This person has not, however, progressed to overt diabetes yet. Because mouse-reactive ICA in patients at onset of IDDM were more frequently present in GAD-positive sera than in those sera negative for GAD, this seems to be no general phenomenon. Identification of further target antigens of ICA will allow us to determine the significance that the diverse non-GAD–

reactive ICAs have as markers for autoimmune insulitis and as potential contributors to the development of IDDM.

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