# High Level of Concordance Between Assays for Glutamic Acid Decarboxylase Antibodies

## The First International Glutamic Acid Decarboxylase Antibody Workshop

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**Glutamic acid decarboxylase antibodies (GADAbs) are being increasingly used in clinical and research programs for the prediction and classification of insulin-dependent diabetes mellitus (IDDM). A number of different assay formats for the measurement of GADAbs have been reported, but the degree of concordance between assays is unknown. In this study, GADAbs were measured on 16 coded sera in 34 assays to examine concordance between GADAb assays and establish the feasibility of an international GADAb standard of measurement unit. The 16 lyophilized coded samples consisted of sera from healthy control subjects ($n$ = 2), IDDM patients ($n$ = 3), a patient with polyendocrine autoimmunity ($n$ = 1), and duplicate dilutions of plasmapheresis serum from a patient with stiff-man syndrome (SMS). A high level of concordance was found in the ranking of GADAb levels ($P$ = 0.99, Friedman's test) in the samples. Thirteen (38%) assays could reproducibly distinguish dilutions of SMS serum and detect GADAbs in all IDDM and polyendocrine autoimmunity sera tested. Although assessed on only four samples, disease specificity was 100% in 29 assays. The majority of assays that immunoprecipitated radiolabeled GAD gave high results for sensitivity and specificity. Enzyme-linked immunosorbent assays and assays using immunofluorescence were generally less sensitive. Several assays, in particular those measuring GAD enzymatic activity immunoprecipitated in fluid phase from rat brain homogenate, showed a prozone-like phenomenon in the SMS dilution curve. Interpolation of results from a standard curve into workshop units resulted in relatively low scatter in samples with lower levels of GADAbs. Hence, the use of an international reference serum to enable comparison of results between laboratories appears feasible. A second serum exchange is currently in progress to explore differences in sensitivity and specificity using larger numbers of disease and control sera.** ***Diabetes*** **43:1005–1009, 1994**

Insulin-dependent diabetes (IDDM) is an autoimmune disease in which serum antibodies against islet antigens have been recognized. These include insulin antibodies (IAAs) (1), cytoplasmic islet cell antibodies (ICAs) (2), and antibodies to a 64-kDa antigen (3). The demonstration of glutamic acid decarboxylase (GAD) as a major autoantigen in the rare neurological disease stiff-man syndrome (SMS), which is known to be clinically associated with IDDM (4), led to the identification of this antigen as the 64-kDa antigen in IDDM (5). GAD exists as two proteins encoded by separate genes of predicted molecular weights of 65 kDa and 67 kDa, which are 65% identical at the amino acid level (6). The 65-kDa GAD ($GAD_{65}$), the predominant form found in human islets (7), has been shown to be the major target of autoantibodies (8,9).

In addition to their presence in recently diagnosed IDDM (10), glutamic acid decarboxylase antibodies (GADAbs) have been detected in 60% of subjects with SMS, of which a third also had IDDM (11). In addition, these antibodies have been described in patients with a variety of autoimmune polyendocrine syndromes, not necessarily associated with overt IDDM (12–14). In these latter groups, the titer of GADAbs was significantly higher than that of control subjects (13,14). Proposed applications of these assays include identification of subjects at high risk of developing IDDM (15), classification of diabetes type as IDDM or non-insulin-dependent diabetes (16,17), monitoring of subjects undergoing immunotherapy, and classification of SMS.

A number of assays for GADAbs have been developed including immunoprecipitation (IP) followed by measurement of enzymatic activity (5,15,18–22), IP of radiolabeled native (20,23) or recombinant (9,16,24,25) GAD, IP followed by Western blotting (5), enzyme-linked immunosorbent assay (ELISA) (19,26), and immunofluorescence (27). With these assays, the reported GADAb frequency in recently diagnosed IDDM ranges from 25 (18) to 79% (8,20). This suggests major differences in assay sensitivity (13) and in definition of normal ranges.

Experience with multicenter serum exchanges for ICA (28) and IAA (29) assays has shown a low level of concordance

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IDDM, insulin-dependent diabetes mellitus; IAA, insulin antibody; ICA, islet cell antibody; GAD, glutamic acid decarboxylase; SMS, stiff-man syndrome; GADAb, glutamic acid decarboxylase antibody; IP, immunoprecipitation; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; PAGE, polyacrylamide gel electrophoresis; IFL, indirect immunofluorescence.

between laboratories in early exchanges but considerable improvement after standardization of units of measurement and assay methods (30,31). In the case of these assays, use of international standards has allowed comparison of results between laboratories and pooling of results into multicenter studies.

No attempt has yet been made to compare GADAb methods on a large scale or to standardize units of measure. Accordingly, the first GADAb workshop was held with the aims of examining assay methods, comparing assay results, and exploring the feasibility of an international unit of GADAb measurement.

## RESEARCH DESIGN AND METHODS

**Samples and questionnaire.** Samples were sent to 54 laboratories, which were chosen because of previous publications reporting measurement of GADAbs, involvement in previous ICA or IAA workshops, or interest in participating in the current workshop. Results were submitted from 29 laboratories, using a total of 34 assays. Sixteen coded sera were sent to participating laboratories. Samples sent to participants were sera from healthy subjects with no family or personal history of diabetes ($n$ = 2), newly diagnosed IDDM sera ($n$ = 3), nondiabetic polyendocrine autoimmunity serum with known strongly positive GADAbs ($n$ = 1), duplicate pooled normal serum from 6 healthy volunteers ($n$ = 1), and duplicate dilutions of plasmapheresis serum from a patient with SMS; undiluted and diluted 1:500, 1:100, and 1:50 in pooled normal serum. Serum (200 μl) was lyophilized in glass containers, and no problems were reported with reconstitution of samples. Each laboratory was asked to provide details of the assay methods used, to rate samples as positive or negative, and to express results using their usual method of calculation. Results from qualitative assays were assigned a result of 0 if negative and 1 if positive.

**Statistics.** Healthy control and pooled normal sera were arbitrarily assigned a status of negative, and IDDM serum, the polyendocrine autoimmunity serum, and SMS serum dilutions were regarded as positive, for the purpose of determining sensitivity (number of correct positives/number of positives) and specificity (number of correct negatives/number of negatives). Results were ranked from the lowest to the highest for each assay, and the median rank of each sample was calculated for all assays. Concordance between assays was measured using Friedman's test, based on ranks, using the null hypothesis that the assays did not differ in the order that they ranked samples. Standard curves were constructed for each assay using the SMS serum dilutions and pooled normal serum, with 1,000 workshop units assigned to the undiluted SMS serum, 20 workshop units to the 1:50 sample dilution, 10 workshop units to the 1:100 sample dilution, 2 workshop units to the 1:500 sample dilution, and 0 workshop units to the pooled normal serum. The three sera from patients with recently diagnosed IDDM and the serum from the patient with polyendocrine autoimmunity were interpolated using the individual laboratory's curve, and results were expressed as workshop units.

## RESULTS

**Assay methods: GAD substrates.** Of the 34 assays, 13 used preparations of recombinant human $GAD_{65}$, 1 used recombinant rat $GAD_{65}$, and 2 used recombinant rat $GAD_{67}$. Homogenates of brain (seven rat, four pig, one fetal pig, and one mouse) were used as the antigen source in another 13 assays and of rat pancreas in 1 assay. Cryostat sections of human pancreas were used in one assay, and for three assays the antigen source was not specified.

**Assay methods: GADAb detection.** Three main assay formats were used: IP ($n$ = 26), ELISA ($n$ = 6), and immunofluorescence ($n$ = 2). In 24 of the 26 IP assays, test serum was incubated with the GAD preparation, followed by precipitation of immune complexes (liquid phase IP). In the two remaining IP assays (assays 20 and 21; Table 1), serum immunoglobulins (Igs) were bound onto the precipitation reagent before incubation with the GAD preparation (solid phase IP). Protein A-Sepharose was used as the precipitation reagent in 25 of the 26 IP assays, and anti-human IgG antibody was used in one (assay 14; Table 1). Radiolabeled GAD was used in 16 of the IP assays; in 12, recombinant GAD was labeled with [$^{35}$S]methionine and in 4, purified native GAD was labeled with $^{125}$I. Quantification in these 16 assays was carried out using either counting of radioactivity in the immunoprecipitates or fluorography after polyacrylamide gel electrophoresis (PAGE). In the remaining 10 IP assays, precipitated GAD was quantified by Western blotting (2 assays) or by enzymatic assay (8 assays). In these latter assays, precipitates from brain homogenates were incubated with $^{14}$C-labeled glutamate, and released $CO_2$ was trapped and quantified by scintillation counting.

In the six ELISAs, GAD was bound directly to the plate in five and captured onto the plate with $GAD_6$ monoclonal antibody (32) in one (assay 26; Table 1). After incubation with test serum, bound antibodies were detected with anti-human enzyme conjugate (horseradish peroxidase in assays 27, 28, and 32, alkaline phosphatase in assays 29 and 30, and unspecified in assay 31; Table 1).

In one of the two immunofluorescence assays (assay 33; Table 1), ICAs were measured by immunofluorescence before and after adsorption with rat brain homogenate and scored positive if ICAs became negative (27). No method was stated for the other immunofluorescence assay.

**Sensitivity, specificity, and sample ranking.** The sensitivity and specificity of each assay are shown in Table 1. Sensitivity ranged from 0% in 1 assay to 100% in 13 assays; 25 assays had a sensitivity >50%. Of the 10 assays that used IP of radiolabeled recombinant human $GAD_{65}$, 8 had a sensitivity of 100%; all had a specificity of 100%. The two assays that used recombinant rat $GAD_{67}$ had sensitivities of 75 and 42% and appeared to detect high levels of antibodies only. Of the 12 assays that immunoprecipitated GAD from brain homogenates, 5 had sensitivities of 100% and 2 had sensitivities <50%. All ELISAs and immunofluorescence assays had sensitivities ≤75%.

Specificity was 100% in 29 assays. The methods in the five assays with specificity <100% were IP of recombinant $GAD_{65}$ produced in *Escherichia coli* (sensitivity: 50%), IP of GAD enzymatic activity from pig brain (75%), IP of pig brain GAD revealed by Western blotting (25%), ELISA using crude rat pancreas (50%), and immunofluorescence (50%).

The median rankings for all assays ranked the serial dilutions of the SMS serum from the most dilute to the most concentrated. The IDDM serum samples 14 and 15 and the polyendocrine autoimmunity serum 16 were ranked between the 1:50 and undiluted SMS dilutions, and sample 13 was ranked between the 1:500 and 1:100 dilutions (Table 2). A high level of concordance was found between ranking of samples in all assays ($P$ = 0.99, Friedman's test, adjusted for ties).

**Dilution curves.** Thirteen assays ranked the SMS sample dilutions in the expected order (example shown in Fig. 1*A*). These included 8 of the 10 assays that immunoprecipitated radiolabeled recombinant $GAD_{65}$. Five assays produced a prozone-like dilution curve (assays 10, 17, 18, 19, and 20; Table 1, Fig. 1*B*), including four assays that measured GAD enzymatic activity immunoprecipitated from rat brain homogenate. The effect was confined to assays that used the fluid-phase IP method, in which serum and GAD substrate were incubated followed by precipitation with protein A-

TABLE 1
Assays methods, sensitivity, and specificity

| No. | GAD source | Method | Label | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| 1 | $rHGAD_{65}$ | IP | [$^{35}$S]methionine | 100 | 100 |
| 2 | $rHGAD_{65}$ | IP | [$^{35}$S]methionine | 100 | 100 |
| 3 | $rHGAD_{65}$ | IP | [$^{35}$S]methionine | 100 | 100 |
| 4 | $rHGAD_{65}$ | IP | [$^{35}$S]methionine | 41.7 | 100 |
| 5 | $rHGAD_{65}$ | IP | [$^{35}$S]methionine | 83.3 | 50 |
| 6 | $rHGAD_{65}$ | IP | $^{125}$I | 100 | 100 |
| 7 | $rHGAD_{65}$ | IP + PAGE | [$^{35}$S]methionine | 100 | 100 |
| 8 | $rHGAD_{65}$ | IP + PAGE | [$^{35}$S]methionine | 83.3 | 100 |
| 9 | $rHGAD_{65}$ | IP + PAGE | [$^{35}$S]methionine | 100 | 100 |
| 10 | $rHGAD_{65}$ | IP + PAGE | [$^{35}$S]methionine | 100 | 100 |
| 11 | $rRatGAD_{65}$ | IP | [$^{35}$S]methionine | 100 | 100 |
| 12 | $rRatGAD_{67}$ | IP | [$^{35}$S]methionine | 41.7 | 100 |
| 13 | $rRatGAD_{67}$ | IP | [$^{35}$S]methionine | 75 | 100 |
| 14 | Pig brain | IP | $^{125}$I | 100 | 100 |
| 15 | Pig brain | IP | $^{125}$I | 50 | 100 |
| 16 | Pig brain | IP | $^{125}$I | 100 | 100 |
| 17 | Rat brain | IP + Enzymatic assay | $^{14}CO_2$ | 91.7 | 100 |
| 18 | Rat brain | IP + Enzymatic assay | $^{14}CO_2$ | 100 | 100 |
| 19 | Rat brain | IP + Enzymatic assay | $^{14}CO_2$ | 83.3 | 100 |
| 20 | Rat brain | IP + Enzymatic assay | $^{14}CO_2$ | 100 | 100 |
| 21 | Rat brain | IP + Enzymatic assay | $^{14}CO_2$ | 33.3 | 100 |
| 22 | Rat brain | IP + Enzymatic assay | $^{14}CO_2$ | 58.3 | 100 |
| 23 | Rat brain | IP + Enzymatic assay | $^{14}CO_2$ | 33.3 | 75 |
| 24 | Fetal pig brain | IP + Enzymatic assay | $^{14}CO_2$ | 100 | 100 |
| 25 | $rHGAD_{65}$ | IP + Western blot | | 83.3 | 100 |
| 26 | Pig brain | IP + Western blot | | 66.7 | 25 |
| 27 | $rHGAD_{65}$ | ELISA | | 33.3 | 100 |
| 28 | $rHGAD_{65}$ | ELISA | | 33.3 | 100 |
| 29 | Mouse brain | ELISA | | 75 | 100 |
| 30 | Rat pancreas | ELISA | | 58.3 | 50 |
| 31 | ? | ELISA | | 8.3 | 100 |
| 32 | ? | ELISA | | 0 | 100 |
| 33 | Human pancreas | IFL | | 41.7 | 100 |
| 34 | ? | IFL | | 58.3 | 50 |

H, human; r, recombinant.

Sepharose. Ten assays either did not show a dilution curve (Fig. 1*C*) or had low sensitivity (Fig. 1*D*) and could not distinguish all SMS serum dilutions. These included both immunofluorescence assays and three ELISAs.

**Interpolation of results from standard curve.** Standard curves, constructed for each assay using the SMS serum diluted in pooled normal serum, were used to interpolate results for the three IDDM sera and the polyendocrine autoimmunity serum into workshop units (Fig. 2). The degree of concordance appeared higher in samples 13 and 14 than in samples 15 and 16. For sample 13, more than half of the results ranged between 5 and 7 workshop units, and for sample 14, two-thirds of the results ranged between 20 and 40 workshop units. Because samples 13 and 14 were ranked lower than samples 15 and 16 (Table 2), concordance appeared to be higher at lower GADAb levels.

TABLE 2
Median ranking of samples by all assays

| Sample | Description | Median rank | % positive |
|---|---|---|---|
| 1 | Healthy control | 2.5 | 2.9 |
| 4 | Healthy control | 3.0 | 5.9 |
| 6 | Pooled normal | 3.5 | 8.8 |
| 11 | Pooled normal | 4.0 | 11.8 |
| 12 | SMS, 1:500* | 5.3 | 44.1 |
| 2 | SMS, 1:500 | 6.0 | 50.0 |
| 13 | IDDM | 7.0 | 58.8 |
| 5 | SMS, 1:100 | 8.5 | 67.6 |
| 7 | SMS, 1:100 | 9.0 | 70.6 |
| 3 | SMS, 1:50 | 11.0 | 73.5 |
| 10 | SMS, 1:50 | 11.0 | 76.5 |
| 14 | IDDM | 12.0 | 76.5 |
| 16 | Polyendocrine autoimmunity | 13.0 | 88.2 |
| 15 | IDDM | 14.0 | 88.2 |
| 8 | SMS, 1:1 | 15.0 | 82.4 |
| 9 | SMS, 1:1 | 15.0 | 82.4 |

*Plasmapheresis serum from a patient with SMS and IDDM.

## DISCUSSION

This first international GADAb workshop has evaluated a wide variety of assay formats which, surprisingly, showed considerable concordance in their ranking of GADAb levels in different samples. This is in contrast with the early ICA and IAA workshops, in which a marked variation in results was observed even when apparently identical assay methods were used (28,29).

A number of assays reproducibly distinguished dilutions of SMS serum and detected GADAbs in all IDDM sera tested. The specificity was high in most assays, although this was assessed on only four samples. The limited number of samples tested does not allow conclusions with respect to the best assay format. However, 10 of the 16 assays using IP of radiolabeled GAD had a sensitivity and specificity of 100%, and in none of the ELISA or immunofluorescence assays was sensitivity >75%. In some assays, reducing the threshold of positivity increased the sensitivity without a corresponding loss of specificity. Clearly, however, thresholds need to be

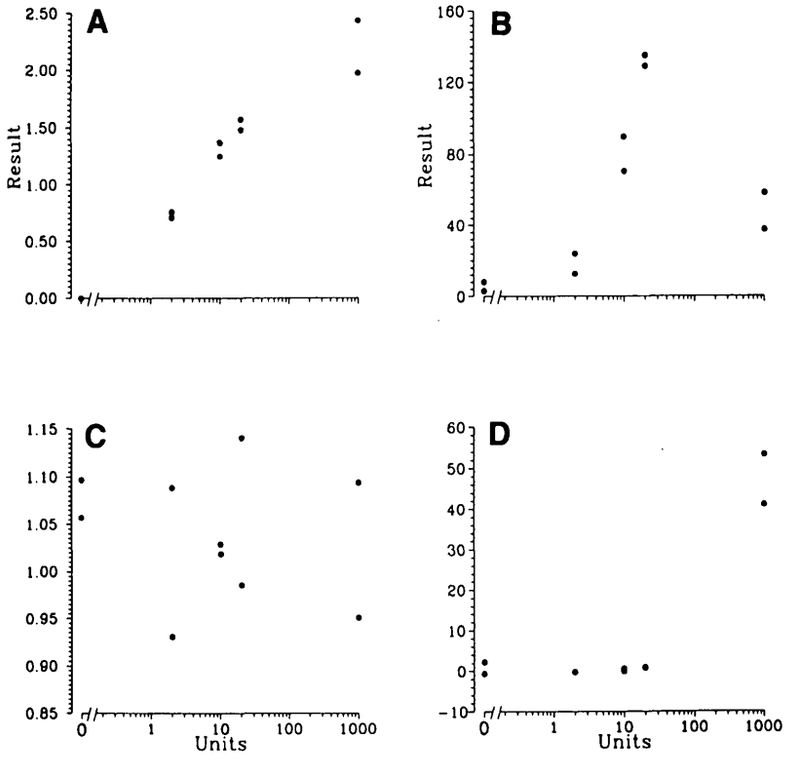


**FIG. 1. Representative dilution curves of SMS serum. Workshop units (log scale) plotted on the abscissa are plotted against the laboratory's result on the ordinate. *A*: example of an expected dilution curve. *B*: prozone-like effect in dilution curve. *C*: dilution curve showing inability to distinguish SMS serum dilutions. *D*: dilution curve from an assay with low sensitivity.**

determined using many more control sera than included in this serum exchange.

Several assays, in particular those measuring the GAD enzymatic activity immunoprecipitated in the fluid phase from rat brain homogenate, showed a prozone-like effect in the SMS serum dilution curve. The prozone effect is an incomplete antigen-antibody reaction despite adequate levels of antibody in the test medium (33). Recently, serum from subjects with SMS has been shown to inhibit GAD enzymatic activity (12), suggesting that this prozone-like effect may involve inhibition or depletion of GAD activity by the SMS serum. The prozone-like effect did not result in any false negative results in this workshop, but in assays in which the effect is known to occur, quantification of GADAbs will require the use of diluted and undiluted serum.

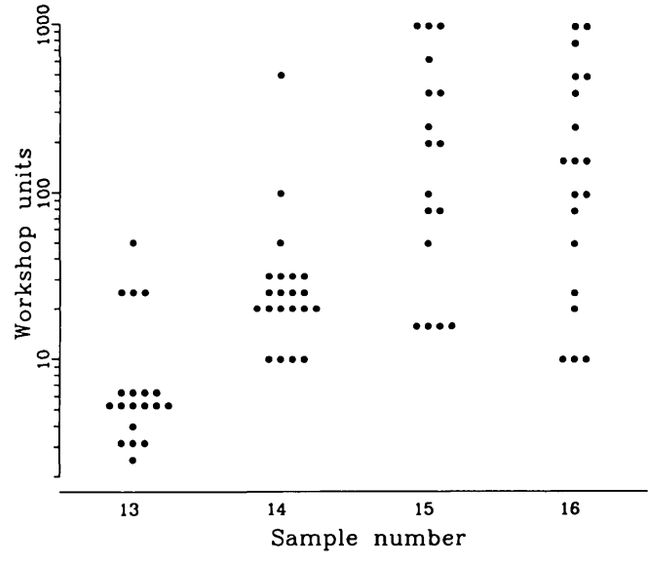


**FIG. 2. Effect of conversion of results from the standard curves on samples 13, 14, 15, and 16. Standard curves were constructed for each assay, and results were expressed as workshop units (log scale). The 10 assays that could not distinguish the SMS serum dilutions, 1 assay with a marked prozone dilution curve, and the 2 assays using recombinant rat $GAD_{67}$ were excluded from this analysis. In five assays with a prozone-type dilution curve, a standard curve eliminating the 1,000 unit standard was used.**

The use of serial dilutions of a standard positive serum should allow units of measure to be standardized and comparisons to be made between laboratories. The SMS serum used in this workshop is available in large quantities and is of high titer, permitting accurate interpolation of most results. However, both the titer of GADAbs and the pattern of epitope recognition in SMS may differ from that in IDDM. Thus, the titer of GADAbs in SMS sera is 10–200 times that in IDDM sera, and SMS sera frequently recognize denatured GAD protein, whereas IDDM sera rarely do (5). Additionally, GADAb-positive SMS sera immunoprecipitate both $GAD_{67}$ and $GAD_{65}$ (34), whereas IDDM sera uncommonly precipitate $GAD_{67}$ (8,9). In this study, interpolation of results from a standard curve into workshop units resulted in relatively low scatter in samples with lower levels of GADAbs. The large scatter seen for samples with very high GADAb levels may be contributed to by the prozone-like effect seen in some assays. Despite these possible limitations, the use of an international reference serum to enable comparison of results between laboratories appears feasible. Ultimately, the use of purified GAD antigen may allow an absolute GADAb standard to be defined as an alternative to a unit based on dilutions of a positive serum sample. This approach is currently limited by the availability of suitable soluble purified GAD antigen.

Because only a limited number of sera were included, many of the conclusions are preliminary. However, this workshop has shown a high level of concordance between a number of different assay formats, and results indicate that the use of an international reference serum is feasible. A second serum exchange is currently in progress to explore

differences in sensitivity and specificity using larger numbers of disease-related and control sera.

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## APPENDIX

Participating laboratories: A. Arnaiz-Villena (Madrid, Spain), S. Baekkeskov (San Francisco, CA), C. Betterle (Padua, Italy), E. Bosi (Milan, Italy), G.F. Bottazzo (London, U.K.), M. Bouanani (Montpellier, France), P.G. Colman (Melbourne, Australia), M. Delamaire (Rennes, France), T. Dyrberg (Bagsvaerd, Denmark), G. Eisenbarth (Denver, CO), W. Hagopian (Seattle, WA), L.C. Harrison (Melbourne, Australia), R.L. Humbel (Luxembourg, Belgium), D. Kaufman (Los Angeles, CA), T. Kobayashi (Tokyo, Japan), Å. Lernmark (Stockholm, Sweden), R.D.G. Leslie (London, U.K.), M. Marshall (Bagsvaerd, Denmark), S. Nagataki (Nagasaki, Japan), C. Muller (Freiburg, Germany), A. Powers (Nashville, TN), G. Pagano (Torino, Italy), J.S. Petersen (Gentofte, Denmark), M.J. Rowley (Melbourne, Australia), S.V. Rao (Newport Beach, CA), R. Ricciotti (Ancona, Italy), W. Scherbaum (Ulm, Germany), C. Thivolet (Lyon, France), A.G. Ziegler (Munich, Germany).

## REFERENCES

1. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL: Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science* 222:1337–1339, 1983
2. Bottazzo GF, Florin-Christensen A, Doniach D: Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* 2:1279–1283, 1974
3. Baekkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark Å: Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature* 298:167–169, 1982
4. Solimena M, Folli F, Denis-Donini S, Comi GC, Pozza G, DeCamilli P, Vicari AM: Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus. *N Engl J Med* 318:1012–1020, 1988
5. Baekkeskov S, Aanstoot H-J, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, DeCamilli P: Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 347:151–156, 1990
6. Erlander MG, Tillakaratne NJK, Feldblum S, Patel N, Tobin AJ: Two genes encode distinct glutamate decarboxylases. *Neuron* 7:91–100, 1991
7. Karlsen AE, Hagopian WA, Petersen JS, Boel E, Dyrberg T, Grubin CE, Michelsen BK, Madsen OD, Lernmark Å: Recombinant glutamic acid decarboxylase (representing the single isoform expressed in human islets) detects IDDM-associated 64,000-$M_r$ autoantibodies. *Diabetes* 41: 1355–1359, 1992
8. Hagopian WA, Michelsen B, Karlsen AE, Larsen F, Moody A, Grubin CE, Rowe R, Petersen J, McEvoy R, Lernmark Å: Autoantibodies in IDDM primarily recognize the 65,000-$M_r$ rather than the 67,000-$M_r$ isoform of glutamic acid decarboxylase. *Diabetes* 42:631–636, 1993
9. Velloso LA, Kämpe O, Hallberg A, Christmanson L, Betsholtz C, Karlsson FA: Demonstration of GAD-65 as the main immunogenic isoform of glutamate decarboxylase in type 1 diabetes and determination of autoantibodies using a radioligand produced by eukaryotic expression. *J Clin Invest* 91:2084–2090, 1993
10. Bottazzo GF: On the honey disease: a dialogue with Socrates. *Diabetes* 42:778–800, 1993
11. Solimena M, Folli F, Aparisi R, Pozza G, DeCamilli P: Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. *N Engl J Med* 322:1555–1560, 1990
12. Björk E, Velloso LA, Kämpe O, Karlsson FA: GAD autoantibodies in IDDM, stiff-man syndrome, and autoimmune polyendocrine syndrome type I recognize different epitopes. *Diabetes* 43:161–165, 1994
13. Schmidli RS, DeAizpurua HJ, Harrison LC, Colman PG: Antibodies to glutamic acid decarboxylase in at-risk and clinical insulin-dependent diabetic subjects: relationship to age, sex, and islet cell antibody status, and temporal profile. *J Autoimmun* 7:55–66, 1994
14. Wagner R, Genovese S, Bosi E, Becker F, Bingley PJ, Bonifacio E, Miles KA, Christie MR, Bottazzo GF, Gale EAM: Slow metabolic deterioration towards diabetes in ICA-positive patients with autoimmune polyendocrine disease. *Diabetologia* 37:365–371, 1994
15. Thivolet CH, Tappaz M, Durand A, Petersen J, Stefanutti A, Chatelain P, Vialettes B, Scherbaum W, Orgiazzi J: Glutamic acid decarboxylase (GAD) autoantibodies are additional predictive markers of type 1 (insulin-dependent) diabetes in high-risk individuals. *Diabetologia* 35:570–576, 1992
16. Hagopian WA, Karlsen AE, Gottsäter A, Landin-Olsson M, Grubin CE, Sundkvist G, Petersen JS, Boel E, Dyrberg T, Lernmark Å: Quantitative assay using recombinant human islet glutamic acid decarboxylase (GAD65) shows that 64K autoantibody positivity at onset predicts diabetes type. *J Clin Invest* 91:368–374, 1993
17. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR: Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. *Diabetes* 42:359–362, 1993
18. Martino GV, Tappaz ML, Braghi S, Dozio N, Canal N, Pozza G, Bottazzo GF, Grimaldi LME, Bosi E: Autoantibodies to glutamic acid decarboxylase (GAD) detected by an immuno-trapping enzyme activity assay: relation to insulin-dependent diabetes mellitus and islet cell antibodies. *J Autoimmun* 4:915–923, 1991
19. DeAizpurua HJ, Wilson YM, Harrison LC: Glutamic acid decarboxylase autoantibodies in pre-clinical insulin-dependent diabetes. *Proc Natl Acad Sci USA* 89:9841–9845, 1992
20. Rowley MJ, Mackay IR, Chen Q-Y, Knowles WJ, Zimmet PZ: Antibodies to glutamic acid decarboxylase discriminate major types of diabetes mellitus. *Diabetes* 41:548–551, 1992
21. Harrison LC, Honeyman MC, DeAizpurua HJ, Schmidli RS, Colman PG, Tait BD, Cram DS: Inverse relation between humoral and cellular immunity to glutamic acid decarboxylase in subjects at risk of insulin-dependent diabetes. *Lancet* 341:1365–1369, 1993
22. Christie MR, Brown TJ, Cassidy D: Binding of antibodies in sera of type 1 (insulin-dependent) diabetic patients to glutamate decarboxylase from rat tissues: evidence for antigenic and non-antigenic forms of the enzyme. *Diabetologia* 35:380–384, 1992
23. Kawasaki E, Takino H, Yano M, Uotani S, Matsumoto K, Takao Y, Yamaguchi Y, Akazawa S, Nagataki S: Autoantibodies to glutamic acid decarboxylase in patients with IDDM and autoimmune thyroid disease. *Diabetes* 43:80–86, 1994
24. Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AL: Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest* 89:283–292, 1992
25. Mauch L, Seissler J, Haubruck H, Cook NJ, Abney CC, Berthold H, Wirbelauer C, Liedvogel B, Scherbaum WA, Northemann W: Baculovirus-mediated expression of human 65 kDa and 67 kDa glutamic acid decarboxylases in SF9 insect cells and their relevance in diagnosis of insulin-dependent diabetes mellitus. *J Biochem* 113:699–704, 1993
26. DeAizpurua HJ, Harrison LC, Cram DS: An ELISA for antibodies to recombinant glutamic acid decarboxylase in IDDM. *Diabetes* 41:1182–1187, 1992
27. Genovese S, Bonifacio E, McNally JM, Dean BM, Wagner R, Bosi E, Gale EAM, Bottazzo GF: Distinct cytoplasmic islet cell antibodies with different risks for type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 35:385–388, 1992
28. Bottazzo GF, Gleichmann H: Immunology and diabetes workshops: report of the first international workshop on the standardisation of cytoplasmic islet cell antibodies. *Diabetologia* 29:125–126, 1986
29. Wilkin TJ, Schoenfeld SL, Diaz J-L, Kruse V, Bonifacio E, Palmer JP: Systematic variation and differences in insulin-autoantibody measurements. *Diabetes* 38:172–181, 1989
30. Bonifacio E, Lernmark Å, Dawkins RL: Serum exchange and use of dilutions have improved precision of measurement of islet cell antibodies. *J Immunol Methods* 106:83–88, 1988
31. Gleichmann H, Bottazzo GF: Progress toward standardization of cytoplasmic islet cell-antibody assay. *Diabetes* 36:578–584, 1987
32. Chang Y-C, Gottlieb DI: Characterization of the proteins purified with monoclonal antibodies to glutamic acid decarboxylase. *J Neurosci* 8:2123–2130, 1988
33. Nichols WS, Nakamura RM: Agglutination and agglutination inhibition assays. In *Manual of Clinical Laboratory Immunology*. 3rd ed. Rose NR, Friedman H, Fahey JL, Eds. Washington, DC, American Society of Microbiology, 1986
34. Butler MH, Solimena M, Dirkx R, Hayday A, DeCamilli P: Identification of a dominant epitope of glutamic acid decarboxylase (GAD-65) recognized by autoantibodies in stiff-man syndrome. *J Exp Med* 178:2097–2106, 1993