Antibodies to GAD65 and a Tyrosine Phosphatase–Like Molecule IA-2ic in Filipino Type 1 Diabetic Patients

FRANCESCO MEDICI, MD
MOHAMMED I. HAWA, BSC
ANGELA GIORGINI, BSC
ARACELI PANELO, MD
CHRISTINE M. SOLFELIX, MD
RICHARD D.G. LESLIE, MD
PAOLO POZZILLI, MD

OBJECTIVE — Type 1 diabetes is more prevalent in Europeans than it is in Asians. The disease is associated with autoantibodies to GAD65 and a protein tyrosine phosphatase–like molecule (IA-2). The frequency of GAD antibodies in Asian patients with type 1 diabetes may be lower than that in Europeans. No data are available on IA-2 antibodies in Asians. We tested antibodies to GAD65 and IA-2ic (the intracellular fragment containing the antibody epitope) in Filipino diabetic patients because this population has mixed European and aboriginal racial origins.

RESEARCH DESIGN AND METHODS — A cross-sectional study of antibodies to GAD65 and IA-2ic was performed on a consecutive series of 91 type 1 diabetic patients, 74 type 2 diabetic patients, and 100 control subjects attending a diabetes clinic in Manila, the Republic of the Philippines. All subjects were <40 years of age, with a mean age ± SD of 24.8 ± 9.8, 34.3 ± 5.8, and 25.8 ± 8.0 years, respectively. Diagnosis of type 1 diabetes was determined clinically and confirmed by baseline C-peptide.

RESULTS — Of 91 type 1 diabetic patients, antibodies to GAD65 were detected in 25 (27.4%), but antibodies to IA-2ic were found in only 8 (8.8%) ($P = 0.002$); neither autoantibody was detected in either the type 2 diabetic or control subjects. Of the 25 recently diagnosed type 1 diabetic patients (disease duration <2.0 years), autoantibodies to GAD65 were detected in 14 (56%), but those to IA-2ic in only 4 (16%) ($P = 0.007$); GAD65 antibodies were detected in only 4 (6%) of 66 patients with a longer disease duration ($P = 0.0004$). Comparison with recently diagnosed European type 1 diabetic patients of age and disease duration similar to that of the Filipinos indicated that IA-2ic antibodies, unlike GAD antibodies, were significantly less prevalent in Filipino type 1 diabetic patients ($P = 0.0007$).

CONCLUSIONS — This is the first study investigating the prevalence and pattern of humoral immune response in type 1 diabetic patients from the Philippines. Antibodies to IA-2ic, unlike GAD antibodies, were infrequent. Patterns of immune responses to type 1 diabetes–associated antigens may differ worldwide, with important implications for prediction of the disease and the potential for antigen-specific therapy.

Diabetes Care 22:1458–1461, 1999

Type 1 diabetes is less frequent in Asia than in Europe (1,2). The incidence of type 1 diabetes in Japan and China is ~1 case per 100,000 people per year and in South India, ~10.5 per 100,000 people per year (3). The prevalence of diabetes in the Philippines in 1975 was 4.1%, with most of the patients probably having type 2 diabetes. As in other Asian countries, the frequency of type 1 diabetes in the Philippines is thought to be low, although the exact level is unknown. The history of the Philippines differs from that of most Asian countries. The Philippines are islands, isolated from the mainland, and are, therefore, less prone to genetic influences due to migration, as occurs in other Asian countries. The Philippines were colonized by the Spanish in the 16th century, and their influence prevailed for several centuries, e.g., ~90% of the population is Catholic. Thus, in contrast to other Asian countries, the people of the Philippines have mixed Spanish and aboriginal racial origins.

Type 1 diabetes is associated with antibodies to pancreatic islet cell antigens, including GAD65, the tyrosine phosphatase–like molecule IA-2, and insulin (4,5). In patients of European origin with recently diagnosed type 1 diabetes, up to 95% have autoantibodies to either GAD65 or IA-2 (6–9).

Asian type 1 diabetic patients also have antibodies to GAD65. Reported frequencies for GAD65 antibodies in Asian type 1 diabetic patients range from 30% in Korea (10) and 40% in China (11) to 50% in Thailand (12) and 57% in South India (13). As with European patients, the frequency of GAD65 antibodies in Asian patients depends on the time from diagnosis; as many as 70% of Japanese type 1 diabetic patients of recent onset had GAD65 antibodies (14–17). Little is known about the frequency of IA-2 antibodies in Asians with type 1 diabetes. Among Europeans, antibodies to IA-2 recognize the intracellular fragment (IA-2ic) (4). In a small Japanese study of 30 type 1 diabetic patients of short disease duration, antibodies to ICA512 (itself a part of the intracellular fragment of IA-2) were detected in 73% of the patients (15); there are no studies of IA-2 or IA-2ic antibodies in Asians, nor are there any studies of either GAD65 or IA-2ic antibodies in Filipino patients. Therefore, in this study, we determined the frequency of antibodies to GAD65 and IA-2ic in Filipino diabetic patients.

From the Department of Diabetes and Metabolism (F.M., M.I.H., A.G., R.D.G.L., P.P.), St. Bartholomew's Hospital, London, U.K.; the Institute for Studies on Diabetes (A.P., C.M.S.), Manila, the Republic of the Philippines; and the University of Rome Tor Vergata and Campus Biomedico (P.P.), Rome, Italy.

Address correspondence and reprint requests to P. Pozzilli, MD, Dept. of Diabetes and Metabolism, St. Bartholomew's Hospital, London, U.K. E-mail: p.p.pozzilli@mds.qmw.ac.uk.

Received for publication 18 December 1998 and accepted in revised form 12 May 1999.

F.M. and M.I.H. contributed equally to this work.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Downloaded from http://diabetesjournals.org/care/article-pdf/22/9/1458/450240/10480509.pdf by guest on 12 August 2022

RESEARCH DESIGN AND METHODS

Subjects

A series of 165 consecutive outpatients attending a diabetes clinic in Manila were studied. All patients (both type 1 and type 2 diabetic) were included if they were born in and living in the Philippines and were aged <40 years. Their diagnosis and classification of diabetes was based on the National Diabetes Database Criteria (18). We studied three groups of subjects: *1*) type 1 diabetic patients, *2*) type 2 diabetic patients, and 3) normal healthy control subjects. Informed consent was given by all subjects, and the study was approved by the local ethics committee.

Type 1 diabetic patients. Our study included 91 type 1 diabetic patients (mean age ± SD 24.8 ± 9.8 years, 29 males). All type 1 diabetic patients were on insulin treatment and had no past history of oral hypoglycemic therapy. Diagnosis of type 1 diabetes was confirmed by measuring nonfasting serum C-peptide: levels <1 ng/ml were considered to be consistent with insulin dependence. To assess the impact of diabetes duration on the frequency of the antibodies, these patients were further divided in two subgroups according to their diabetes duration: *1*) 25 patients with diabetes duration of <2 years (age 23.1 ± 10.7 years, diabetes duration 0.96 ± 0.6 years, 10 males) and *2*) 66 patients with diabetes duration of >2 years (age 25.2 ± 7 years, diabetes duration 3.6 ± 1.4 years, 19 males). Overall, the mean age of onset of type 1 diabetes was 17.6 ± 10.0 years.

Type 2 diabetic patients. The study included 74 type 2 diabetic patients (age 34.3 ± 5.8 years, 40 males). Classification of type 2 diabetes was based on the following criteria: *1*) adequate control of diabetes on diet alone or oral hypoglycemic agents, or on insulin with a baseline serum C-peptide level >1 ng/ml and *2*) no history of diabetic ketoacidosis.

Normal control subjects. A control group of 100 normal healthy subjects (age 25.7 ± 7.9 years, 40 males) were studied. The subjects were obtained from the local community and had no family history of diabetes and no current illness. All the subjects had normal fasting blood glucose values (i.e., <7.0 mmol/l).

Methods

Blood samples were collected from all subjects included in the study. Serum samples were stored at −20°C. C-peptide was measured using a commercially available radioimmunoassay kit (Incstar, Stillwater, MN). The radioimmunoprecipitation assays for GAD65 and IA-2ic were carried out on batched samples by observers blinded to the clinical status of the subjects.

The radioimmunoprecipitation assays for GAD65 and IA-2ic both use in vitro transcription and translation systems (Promega, Madison, WI). Human full-length IA-2ic cDNA in pGEM-4Z vector (Invitrogen, San Diego, CA) and human islet GAD65 cDNA in the vector pB-1882 (gift of Dr. Thomas Dyrberg, Novo Nordisk, Copenhagen, Denmark) were used to transcribe and translate the proteins, according to the manufacturer's instructions. For both antibody assays between 0.8 and 1.0 µg, DNA was transcribed and translated with SP6 (IA-2ic) and T7 (GAD65) RNA polymerase in a coupled transcription and translation rabbit reticulocyte lysate system (Promega) in the presence of [^{35}S]methionine (0.8 mCi/ml) (Amersham International, Amersham, U.K.). Incorporated radioactivity was determined by precipitation with 10% trichloroacetic acid and scintillation counting. For the immunoprecipitation in each assay, 50 µl aliquots of [^{35}S]methionine (50,000–75,000 counts/min) labeled antigen were incubated overnight with 2 µl serum (final dilution 1:25) in Tris-buffered saline solution.

The immunocomplexes were isolated by adding 1 mg protein A-Sepharose and were counted on a multiwell Wallac counter. All samples were tested in duplicate including positive and negative control standard sera. Each assay for GAD65 antibodies included serially diluted sera from a Stiff Man Syndrome patient and a prediabetic individual with GAD65 antibodies to further evaluate the cut-off level for positivity for GAD65. Values >3 SD above the control population (>1:8,000 dilution of both Stiff Man Syndrome and prediabetic sera for GAD65) were taken as positive. In the latest IA-2ic (M.I.H., R.D.G.L., unpublished observations) and GAD65 Antibody Proficiency Workshops, our assays had a sensitivity, specificity, validity, and consistency of 100% (19).

Statistical analysis

Data regarding the frequency of the individual antibodies and their combination are expressed as *n* (%) unless otherwise specified. Characteristics of subjects are expressed as means ± SD. Differences between groups were assessed using two-tailed Fisher's exact test with Yates' correction. CIs of the odds ratios were calculated using the approximation of Woolf. *P* values <0.05 were considered to be statistically significant.

RESULTS — The results of this study are summarized in Table 1. Of 91 clinically identified type 1 diabetic patients, none had C-peptide levels >1 ng/ml, indicating that the diagnosis was probably correct despite the usually mild presentation of type 1 diabetes in Filipino patients, in whom ketoacidosis is unusual. Some 28 (30.7%) type 1 diabetic patients had at least one antibody, but none of the type 2 diabetic or control subjects did. In type 1 diabetic patients, antibodies to GAD65 and IA-2ic were detected in 25 (27.4%) and 8 (8.8%), respectively.

Of 25 type 1 diabetic patients with diabetes duration <2 years, antibodies to GAD65 were detected in 14 (56%), whereas antibodies to IA-2ic were found in only 4 (16%) (odds ratio [CI] 6.6 [1.8–25.3], P = 0.0007). The frequency of GAD65 antibodies was significantly higher in 25 recently diagnosed type 1 diabetes cases compared with that in 66 patients with longer disease duration (56.0 vs. 16.6%, 6.3 [2.3–17.6], P = 0.0004). There was no significant difference in the frequency of

Table 1—*Frequency of antibodies to GAD65 and IA-2ic in diabetic patients and normal control subjects*

	n	GAD65	IA-2ic
Type 1 diabetes			
Total	91	25 (27.4)	8 (8.8)
Duration <2 years	25	14 (56.0)	4 (16.0)
Duration >2 years	66	11 (6.6)	4 (6.0)
Type 2 diabetes	74	0 (0)	0 (0)
Normal control subjects	100	0 (0)	0 (0)

Data are *n* (%) of diabetic patients and normal control subjects positive for each antibody.

Downloaded from http://diabetesjournals.org/care/article-pdf/22/9/1458/450240/10480509.pdf by guest on 12 August 2022

Table 2—*Antibodies to GAD65 and IA-2ic in recently diagnosed type 1 diabetic patients from the Philippines and Europe*

	n	GAD65	IA-2ic
European patients	60	46 (75)	34 (57)
Filipino patients	25	14 (56)	4 (16)

Data are *n* (%). Type 1 diabetic patients from Europe of comparable age and disease duration (mean age 15.5 ± 7.5 years, mean diabetes duration 1.0 ± 0.2 years, 37 males) show a significantly higher frequency of antibodies to IA-2ic ($P = 0.0007$) but not GAD65 (21).

IA-2ic antibodies in recently diagnosed cases and patients with longer disease duration (Table 1). IA-2ic antibodies were much less frequent in type 1 diabetic Filipinos than in Europeans of comparable age and disease duration (Table 2).

CONCLUSIONS — Autoantibodies to GAD65 and IA-2ic, which are associated with type 1 diabetes in patients of European origin, were also detected in Filipino type 1 diabetic patients. GAD65 antibodies were more prevalent in recently diagnosed type 1 diabetic patients than were IA-2ic antibodies. None of the Filipino patients with type 2 diabetes had either GAD65 or IA-2ic antibodies.

To reduce errors of ascertainment noted in previous studies of Asian patients, we examined a consecutive series of patients attending a diabetes clinic. In addition, we estimated C-peptide levels to avoid misclassification, which is an important problem in Asians because type 2 diabetes may present at a relatively young age. Indeed, of the patients that we studied with diabetes under the age of 40 years, 45% had type 2 diabetes. While none of our 74 Filipino type 2 diabetic patients had GAD65 or IA-2ic antibodies, a study of 508 British type 2 diabetic patients aged 35–44 years found islet cell antibodies (ICA) or GAD65 antibodies in 16% (20).

We detected similar frequencies of GAD65 antibodies in recently diagnosed Filipino and European type 1 diabetic patients of comparable disease duration and age using the same GAD antibody assay. Autoantibodies to GAD65 are observed in 50–80% of newly diagnosed European type 1 diabetic patients (17,21). Variations in the antibody frequency may be, in part, due to differences in the method of detection, age (reported to show inverse association with age), sex (reported to have higher percentage in males), and HLA type (more frequent in HLA DR3) of the subjects (5,8,22). GAD65 autoantibodies in relatives of European type 1 diabetic patients can predict the disease with a high degree of certainty (7,9,21). The observation that GAD65 antibodies are prevalent in recently diagnosed type 1 diabetic Filipinos suggests that these autoantibodies may be as valuable in predicting type 1 diabetes in Filipinos as they are in European subjects.

The frequency of IA-2ic antibodies in the Filipinos, in contrast to that found in the European patients, was much lower than the frequency of GAD65 antibodies. In our present study, only 16% of recently diagnosed type 1 diabetes Filipino patients had IA-2ic antibodies, compared with 57% of European patients of comparable disease duration, age range, and sex (21). The low frequency of IA-2ic antibodies in Filipinos with type 1 diabetes raises the possibility that IA-2ic antibodies are less predictive of type 1 diabetes in this population, and possibly in other Asian populations, than they are in Europeans (7,21).

Why was there such a striking difference in frequency of IA-2ic antibodies between the two populations of Filipinos and Europeans with type 1 diabetes? The differences in autoantibody frequency cannot be due to the assay because we used the same IA-2ic antibody assay for both populations, nor can it be related to age or disease duration because both were comparable in the two populations. The difference could be genetically determined, i.e., the European population may have a higher prevalence of the HLA DQA1*0301-DQB1*0302 genotype than the Filipinos, and IA-2ic antibody positivity is associated with this genotype (8). Furthermore, the environmental factors inducing type 1 diabetes–associated autoimmunity may differ between European and Asian populations, although the frequency of GAD65 antibodies in the Filipinos was similar to that in European patients.

In conclusion, our study shows a relatively low frequency of autoantibodies to IA-2ic, but not to GAD65, in type 1 diabetic patients from the Philippines. The frequency of IA-2ic is significantly lower than that in European populations of comparable age and duration. The low prevalence of IA-2 antibodies in Filipino type 1 diabetic patients suggests that strategies for the prediction of type 1 diabetes based on the combination of GAD65 and IA-2ic antibodies may be inappropriate in some populations. Other disease-associated antigens probably remain to be discovered, and Asian patients with type 1 diabetes could be a fruitful group to study. Moreover, future attempts to prevent type 1 diabetes using antigen-specific therapy will have to take into account population differences in the immune response to type 1 diabetes–associated antigens (23).

Acknowledgments — This study was supported by grants from the University of Rome, Tor Vergata (P.P.), the British Diabetic Association (R.D.G.L.), Action Research (R.D.G.L.), and the Joint Research Board of St. Bartholomew's Hospital (R.D.G.L.).

References

1. Karvonen M, Tuomilehto I, Libman I, LaPorte R, Group of the World Health Organization DIAMOND Project Group: A review of the recent epidemiological data on the worldwide incidence of type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 36:883–892, 1993
2. Green A, Gale EAM, Patterson CC, for the EURODIAB ACE Study Group: Incidence of childhood-onset insulin-dependent diabetes mellitus: the EURODIAB ACE Study. *Lancet* 339:905–909, 1992
3. Ramachandran A, Snehalatha C, Krishnaswamy CV, Madras IDDM Registry Group: Incidence of IDDM in children in urban population in southern India. *Diabetes Res Clin Pract* 34:79–82, 1996
4. Zhang BW, Lan MS, Notkins AL: Autoantibodies to IA-2 in IDDM: location of major antigenic determinants. *Diabetes* 46:40–43, 1997
5. Gorus KF, Goubert P, Semakula C, Vandevalle CL, Schepper JD, Scheen A, Christie MR, Pipeleers DG, the Belgian Diabetes Registry: IA-2 autoantibodies complement GAD65 autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings. *Diabetologia* 40:95–99, 1997
6. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, Chase HP, Eisenbarth GS: Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD and ICA512bdc/IA-2 autoantibodies. *Diabetes* 45:926–933, 1996

Downloaded from http://diabetesjournals.org/care/article-pdf/22/9/1458/450240/10480509.pdf by guest on 12 August 2022

7. Seissler I, Morgenthaler NG, Achenbach P, Lampeter EF, Glawe D, Payton M, Christie M, Scherbaum WA, the DENIS Study Group: Combined screening for autoantibodies to IA-2 and antibodies to glutamic acid decarboxylase in first degree relatives of patients with IDDM. *Diabetologia* 39:1351–1356, 1996
8. Genovese S, Bonfanti R, Bazzigaluppi E, Lampasona V, Benazzi E, Bosi E, Chiumello G, Bonifacio: Association of IA-2 autoantibodies with HLA DR4 phenotypes in IDDM. *Diabetologia* 39:1223–1226, 1996
9. Kulmala P, Savola K, Petersen JS, Vahasalo P, Karjalainen J, Lopponen T, Dyrberg T, Akerblom HK, Knip M: Prediction of insulin-dependent-diabetes mellitus in siblings of children with diabetes: a population based study. *J Clin Invest* 101:327–336, 1998
10. Tuomi T, Zimmet P, Rowley MJ, Min HK, Vichayanrat A, Lee HK, Rhee BD, Vannasaeng S, Humphrey AR, Mackay IR: Differing frequency of autoantibodies to glutamic acid decarboxylase among Koreans, Thais, and Australians with diabetes mellitus. *Clin Immunol Immunopathol* 74:202–206, 1995
11. Thai AC, Ng WY, Loke KY, Lee WRW, Lui KF, Cheah JS: Anti GAD antibodies in Chinese patients with youth and adult-onset IDDM and NIDDM. *Diabetologia* 40: 1425–1430, 1997
12. Rattarasarn C, Aguilar-Diosdado M, Soonthornpun S, Patarakijvanich N, Jaruratanasirikul S: GAD antibodies in IDDM in Thailand. *Diabetes Care* 19:674–675, 1996
13. Sanjeevi CB, Shtauvere A, Ramachandran A, Snehalatha C, Falorni A: Prevalence of GAD65 autoantibodies in South Indian patients with insulin-dependent diabetes mellitus, and in their parents. *Diab Nutr Metab* 10:60–64, 1997
14. Ozawa Y, Kasuga A, Marayuma T, Kitamura Y, Amemiya S, Ishihara T, Suzuki R, Saruta T: Antibodies to the 37,000-Mr tryptic fragment of islet antigen were detected in Japanese insulin dependent diabetes mellitus patients. *Endocr J* 43:615–620, 1996
15. Akamine HS, Komiya I, Shimabokuro T, Asawa T, Tanaka H, Yagi N, Taira T, Nagata K, Arakaki K, Wakugami T, Takasu N, Powell MJ, Furmaniak J, Smith BR: High prevalence of GAD65 (and IA-2) antibodies in Japanese IDDM patients by a new immunoprecipitation assay based on recombinant human GAD65. *Diabet Med* 14:778–784, 1997
16. Zimmet PZ: The pathogenesis and prevention of diabetes in adults: genes, autoimmunity and demography. *Diabetes Care* 18:1050–1056, 1995
17. Zimmet PZ, Rowley MJ, Mackay IR, Mackay IR, Knowles WJ, Chen QY, Chapman LH, Serjeantson SW: The ethnic distribution of antibodies to glutamic acid decarboxylase: presence and levels in insulin-dependent diabetes mellitus in Europoid and Asian subjects. *J Diabet Complications* 7:1–7, 1993
18. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–1057, 1979
19. Schmidli RS, Colman PG, Bonifacio E, and participating laboratories: Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies. *Diabetes* 44:636–640, 1995
20. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R, UKPDS 25: Autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. *Lancet* 350:1288–1293, 1997
21. Hawa M, Rowe R, Lan MS, Notkins AL, Pozzilli P, Christie MR, Leslie RD: Value of antibodies to islet protein tyrosine phosphatase–like molecule in predicting type l diabetes. *Diabetes* 48:1270–1275, 1997
22. Sanjeevi CB, Falorni A, Kockum I, Hagopian WA, Lernmark A: HLA and glutamic acid decarboxylase in human insulin-dependent diabetes mellitus. *Diabet Med* 13:209–217, 1996
23. Pozzilli P: Prevention of type 1 diabetes. *Diabetes Metab Rev* 14:69–84, 1998

Downloaded from http://diabetesjournals.org/care/article-pdf/22/9/1458/450240/10480509.pdf by guest on 12 August 2022