Late Progression to Diabetes and Evidence for Chronic β-Cell Autoimmunity in Identical Twins of Patients With Type I Diabetes

Charles F. Verge, Roberto Gianani, Liping Yu, Massimo Pietropaolo, Theresa Smith, Richard A. Jackson, J. Stuart Soeldner, and George S. Eisenbarth

Previous studies suggest that after 6 years of discordance, identical twin pairs rarely become concordant for type I diabetes. With up to 39 years of follow-up from the onset of diabetes in the index twin, we determined how many discordant twins have evidence of β-cell autoimmunity and how many develop overt diabetes. We longitudinally followed 23 pairs of identical twins (or triplets) that were selected from a total group of 30 pairs because they were discordant for type I diabetes when first ascertained. Seven developed diabetes after 3, 3, 7, 8, 9, 31, and 36 years of discordance. By survival analysis, the concordance after 10 years from the onset of diabetes in the index twin was estimated as 23% (95% confidence interval, 5–40%), increasing to 38% (95% confidence interval, 8–69%) after 31 years. Among 16 twins remaining nondiabetic at last follow-up (8–39 years of discordance), 12 were assessed with serial intravenous glucose tolerance tests and a total of 407 measurements by radioassay of antibodies against three defined autoantigens (glutamic acid decarboxylase, insulin, and the recently cloned molecule ICA512). Two-thirds (8 of 12) had evidence of β-cell autoimmunity (persistently positive autoantibody levels) and/or first-phase insulin release less than the 1st percentile of control subjects. In summary, identical twins may develop diabetes after a prolonged period of discordance and approximately two-thirds of long-term discordant twins have evidence of persistent β-cell autoimmunity and/or β-cell damage. The concordance for β-cell autoimmunity, therefore, is much higher than for overt diabetes. This suggests that additional environmental or non-Mendelian genetic factors or time are required for the development of type I diabetes. *Diabetes* 44: 1176–1179, 1995

From the Barbara Davis Center for Childhood Diabetes (C.F.V., R.G., L.Y., M.P., G.S.E.), University of Colorado Health Sciences Center, Denver, Colorado; the Joslin Diabetes Center (T.S., R.A.J.), Boston, Massachusetts; and the Division of Endocrinology and Metabolism (J.S.S.), University of California, Davis, Sacramento, California.

Address correspondence and reprint requests to Dr. George S. Eisenbarth, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, 4200 E. 9th Ave., Denver, CO 80262.

Received for publication 14 February 1995 and accepted in revised form 7 June 1995.

FPIR, first-phase insulin release; GAD, glutamic acid decarboxylase; IAA, insulin autoantibody; ICA, islet cell antibody; IVGTT, intravenous glucose tolerance test.

Type I diabetes is a complex autoimmune disease in which both genetic and environmental factors are implicated. Estimates of the concordance among identical twins are low, ranging from 13 to 36% (1–3). Data from the U.K. suggest that after 6 years of discordance identical twins rarely become concordant (1). We followed a group of identical twins initially discordant at the time of ascertainment to determine how many develop overt diabetes and how many have evidence of β-cell autoimmunity or damage, assessed with serial autoantibody measurements and intravenous glucose tolerance tests (IVGTTs). In addition to islet cell antibodies (ICAs) measured with an immunohistochemical assay, we used radioassays to measure antibodies against three defined autoantigens: glutamic acid decarboxylase (GAD), insulin, and ICA512 (4). We also used survival analysis to estimate the concordance for diabetes in this group of twins with long-term follow-up.

RESEARCH DESIGN AND METHODS

Subjects. Twenty-six identical twins and four identical triplets (from two families) of patients with type I diabetes have been followed at the Joslin Diabetes Center in a study starting in 1964 (5). Of these, seven were already concordant for diabetes when first ascertained (Fig. 1). For the remaining 23, the median length of discordance when first seen was 1.7 years (range 0.2–23.3). For simplicity, the triplets will be included in the term "twins." In all cases, type I diabetes was diagnosed in the index twin before the age of 30 (median 12.4 years, range 3.3–29.8). Monozygosity was confirmed by blood group analysis (ABO, Rh, Kell, Lewis, MNS, Lutheran, Duffy, Kidd, miscellaneous-Wr, Vel, and Yt−), the determination of four serum factors (haptoglobin, transferrin, Gm, and InV), and histocompatibility antigen testing. During longitudinal follow-up, serial IVGTTs were performed and serum samples were collected for autoantibody measurements with the aim of testing the discordant twins at yearly intervals. The diagnosis of diabetes was made according to adult National Diabetes Data Group criteria (6). Informed consent was obtained from all subjects, and the study protocol was approved by the local ethics committee.

IVGTTs. The first-phase insulin release (FPIR) was calculated as the sum of the insulin levels at 1 and 3 min after the end of the glucose infusion during an IVGTT. The 1st percentile for FPIR determined in 225 healthy, nonobese control subjects with no family history of diabetes (age range, 8–77 years) is 48 mU/l (7). We consider values below this cutoff to be abnormal. None of the twins studied was obese (>120% of ideal body weight). Before March 1990, the IVGTT was performed using 0.5 g glucose/kg body wt as a 20–25% solution of glucose in saline, administered by gravity drip over a 2- to 6-min period. After March 1990, the protocol was modified slightly to conform with the ICARUS consensus protocol (8), in which 0.5 g glucose/kg body wt as a 25% solution, up to a maximum of 35 g of glucose, is injected over a 3-min period (±15 s).

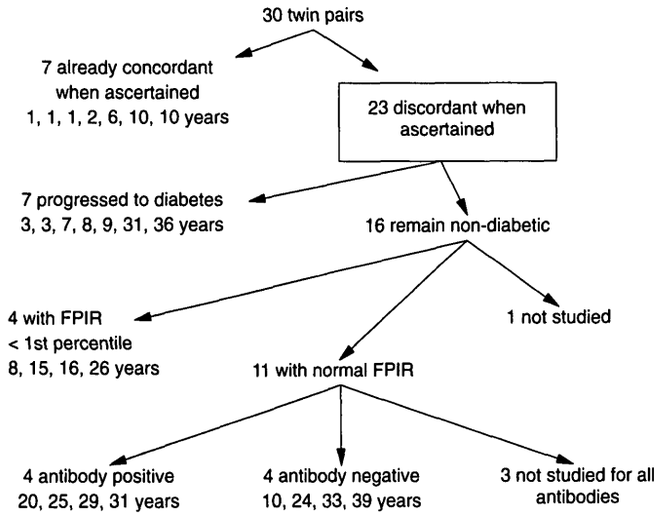

FIG. 1. Diagram showing the proportions of initially discordant monozygotic twins developing type I diabetes, developing low FPIR, and expressing autoantibodies. Numbers under each group show the length of discordance for each subject in years.

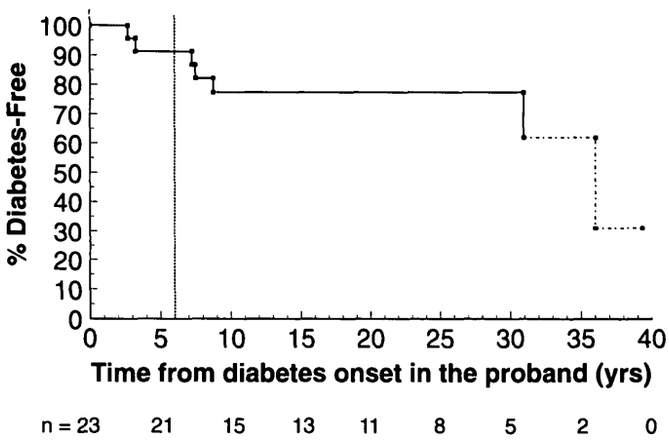

FIG. 2. The diabetes-free survival (measured from the time of onset of diabetes in the index twin) of 23 monozygotic twins who were discordant for type I diabetes when first ascertained. Most of the twins developing diabetes did so after more than 6 years (vertical line) had elapsed from the onset of diabetes in the index twin. A dashed line is used for the last part of the survival curve, for which fewer than five individuals are being followed.

Autoantibody assays. Serum samples were stored at −20°C before testing. ICAs and insulin autoantibodies (IAAs) were determined as described previously (9,10). The upper limit of normal for IAAs (39 nU/ml) was determined as the mean ± 3 SD in 74 healthy control subjects without any history of autoimmunity (11), and the interassay coefficient of variation is 10.3% at low positive levels (10). GAD autoantibodies were measured by radioassay, using in vitro synthesized recombinant human GAD_{65}, with the results expressed as an index calculated from the sample and control counts per minute (12). The interassay coefficient of variation in our laboratory is 6.5% (n = 10). ICA512 autoantibodies were measured using a similar assay format but with in vitro transcribed and translated ICA512 (13). The interassay coefficient of variation is 9.6% (n = 12). The upper limits of the normal ranges for GAD autoantibodies (index of 0.032) and ICA512 autoantibodies (index of 0.16) were established in our laboratory as the 99th percentile in 205 healthy control subjects tested for both autoantibodies. Some twins could not be tested for GAD and ICA512 autoantibodies because serum was no longer available (Fig. 1).

Statistical analysis. Concordance for type I diabetes was estimated using survival analysis (product-limit method, SAS software, SAS Institute, Cary, NC) to allow for the length of follow-up from the time of onset of diabetes in the index twin. To account for possible bias due to overascertainment of concordant pairs, we analyzed only twins who were discordant when they were first ascertained (n = 23) (Fig. 1).

RESULTS

Concordance. Figure 2 shows the development of diabetes among 23 twin pairs who were initially discordant when first ascertained. In this group, the pairwise concordance after 10 years had elapsed from the onset of diabetes in the index twin was 23% (95% confidence interval, 5–40%), increasing to 38% (95% confidence interval, 8–69%) by 31 years. Of the seven twins who developed diabetes, in five it developed after more than 7 years of discordance and in two it developed after more than 30 years of discordance. In the total group of 30 twins (including those who were already concordant when first ascertained) (Fig. 1), 14 twins developed diabetes and half of these did so after more than 6 years of discordance (median discordance at the onset of diabetes 6.6 years, range 1–36 years).

Autoimmunity in discordant twins. Sixteen twins remained nondiabetic at the last follow-up, after 8–39 years of discordance. Of these, 15 twins have been assessed with serial IVGTTs and 12 have been assessed with a total of 407 autoantibody measurements by radioassay (IAA, GAD, and ICA512). Four twins have FPIRs below the 1st percentile of control subjects, 11 twins have normal FPIRs, and one twin did not undergo an IVGTT (Fig. 1). Of the 11 twins with normal FPIRs, 4 are persistently positive for one or more autoantibodies. Therefore, we estimate that two-thirds of twins with long-term discordance (>7 years) have evidence of β-cell autoimmunity or damage (i.e., 8 of 12 for whom data on FPIR, IAA, GAD, and ICA512 autoantibodies are available (Fig. 1 and Table 1). Three twins (subjects ID 2196, 2578, and 916 in Table 1) had low-positive levels of IAA, GAD, or ICA512 autoantibodies on only a single occasion, and this was not deemed evidence of persistent β-cell autoimmunity (Table 1).

Figure 3 shows the time course of autoantibody levels and FPIRs for three representative initially discordant twins. The first, a triplet (ID 3047, Fig. 3*A*), remains nondiabetic after 29 and 10 years of discordance from the time of diabetes onset in his co-triplets. He has been persistently anti-GAD^+ from the first sample measured and, although initially IAA^-, developed this antibody during follow-up. The last sample obtained was ICA^+ (confirmed in two laboratories). The FPIR remains normal. The second (ID 916, Fig. 3*B*) remains nondiabetic after 39 years of discordance. Although autoantibody-negative (except for low level ICA512 at a single time point), he shows a slow decline in FPIR within the normal range. This was not deemed evidence of β-cell autoimmunity or damage by the above criteria. However, it is of interest because this pattern was not observed in any of 19 healthy control subjects (aged 7.3–54.7 years at the first test) who were studied in a similar manner with serial IVGTTs (3–12 IVGTTs over a period of 5–30 years, data not shown). The third twin (ID 1387, Fig. 3*C*) developed diabetes at the age of 41, after 31 years of discordance. Although a trend of decreasing FPIR was documented before the onset of diabetes, only the last serum sample, collected on the day of diagnosis, was autoantibody-positive. There was an interval of 6 years between this sample and the preceding one because of loss to follow-up.

TABLE 1
Antibody levels and FPIR for twins remaining discordant

Subject ID	Length of discordance (years)	Age at onset of diabetes in co-twin (years)	Anti-GAD (index)	IAA (nU/ml)	Anti-ICA512 (index)	ICA (JDF U)	FPIR (mU/l)
5063	7.5	12.4, 19.9*	0.002 (0/5)	−21 (1/7)	0.05 (0/4)	**40** (1/8)	**27**
5627	9.8	10.9	0.01 (0/14)	16 (0/16)	−0.09 (0/14)	**160** (1/16)	217
3120	15.1	18.6	**1.46** (5/5)	14 (0/4)	0.11 (0/5)	0 (3/5)	**34**
479	15.5	11.6	**0.35** (2/2)	19 (0/5)	0.06 (0/2)	0 (1/7)	**26†**
181	20.2	26.9	**0.09** (9/9)	24 (1/12)	−0.02 (0/9)	**10** (2/11)	126
2196	24.0	8.8	−0.01 (0/4)	**41** (1/4)	−0.06 (0/4)	0 (0/10)	280
5561	24.9	17.8	−0.002 (0/10)	19 (7/15)	−0.02 (1/10)	0 (7/22)	245
4141	25.7	18.0	−0.01 (0/11)	24 (0/7)	0.09 (1/11)	0 (0/11)	**36†**
3047	29.2	12.7, 21.5*	**0.78** (15/15)	**125** (7/31)	−0.04 (1/15)	**160** (1/32)	241
4516	31.1	29.8	**0.63** (6/8)	**52** (2/42)	0.04 (1/8)	0 (0/19)	130
2578	32.6	13.9	0.02 (1/10)	18 (0/12)	−0.03 (0/10)	0 (0/13)	99
916	39.4	16.7	0.02 (0/13)	9 (0/37)	−0.04 (1/13)	0 (0/21)	67

Autoantibody levels and FPIR (normal >48 mU/l) from the last time point on 12 identical twins remaining discordant at last follow-up and studied with serial measurements of autoantibodies: anti-GAD (normal ≤ 0.032), IAA (normal ≤ 39), anti-ICA512 (normal ≤ 0.16), and ICA. Abnormal values (above the 99th percentile of control subjects for autoantibodies and below the 1st percentile for FPIR) are shown in bold type. Figures in parentheses show the number of abnormal results for each autoantibody over the total number of determinations for each subject. Eight twins (with subject ID in bold type) were considered to have evidence of β-cell autoimmunity. JDF U, Juvenile Diabetes Foundation units. *Subject is a triplet. †Subject has impaired glucose tolerance by oral glucose tolerance test.

DISCUSSION

In most studies of type I diabetes in identical twins, the concordance is likely to be overestimated because of the tendency toward selective ascertainment of concordant twin pairs. Olmos et al. (1) partially compensated for this by excluding twin pairs that were not ascertained within 12 months of the onset of diabetes in the index twin. Using survival analysis, they estimated the lifetime concordance as 36%. Kumar et al. (3) obtained a similar figure of 32%, but this may also be biased by overascertainment of concordant pairs. Kaprio et al. (2) avoided problems with biased ascertainment by identifying all identical twins in Finland. They found a pairwise concordance of 13% and a probandwise concordance of 23% but did not use survival analysis to allow for varying lengths of time elapsed from the onset of diabetes in the index twin. Therefore, the Finnish study may have

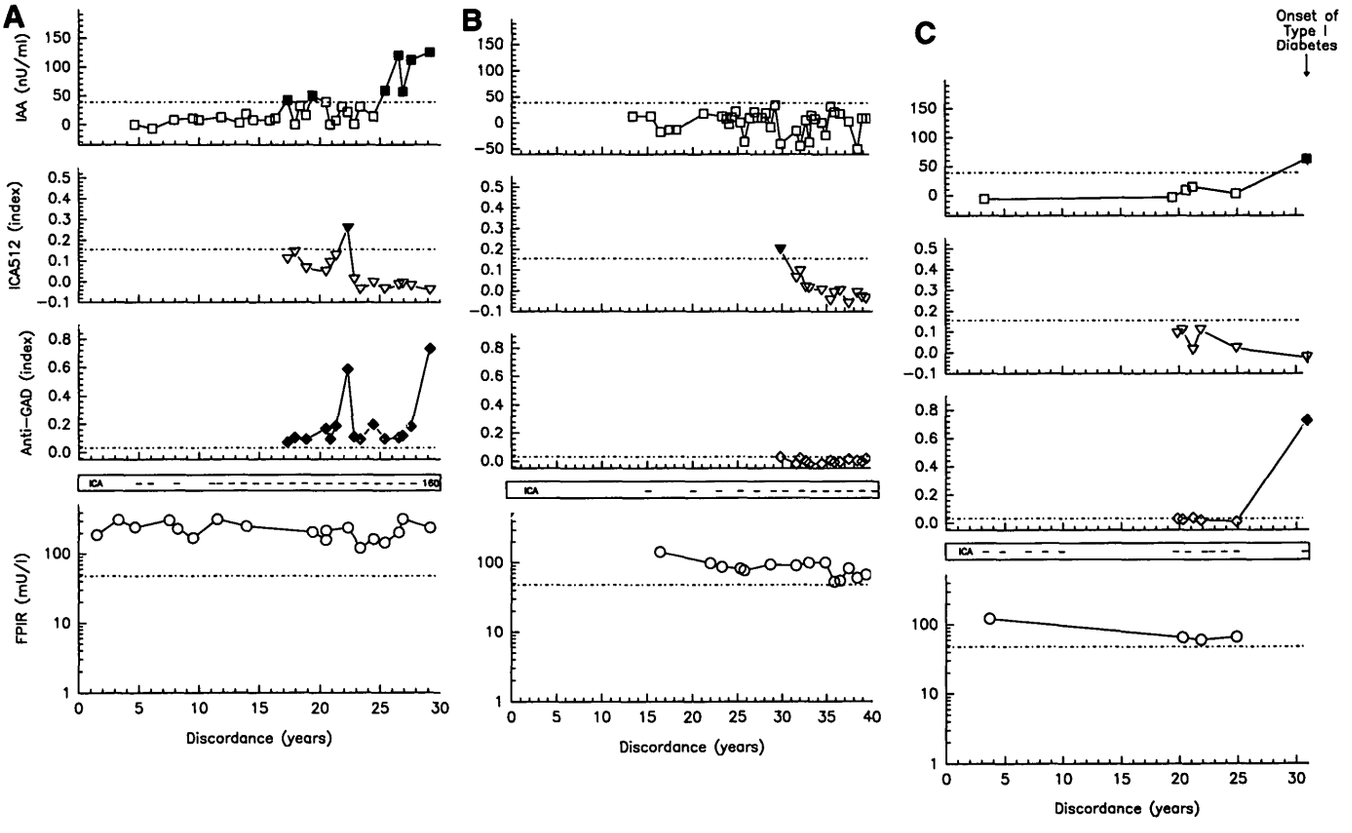

FIG. 3. The time course of autoantibody levels and FPIR for three initially discordant monozygotic twins (discussed in RESULTS). Dashed lines indicate the upper limit of normal for autoantibody levels or the first percentile in normal control subjects for FPIR. Abnormal values are shown with closed symbols. The FPIR is shown on a logarithmic scale.

underestimated the eventual concordance among identical twins with longer follow-up.

Our estimates of concordance are consistent with these studies after allowing for confidence intervals. They represent lower limits because we excluded twins who were already concordant when first ascertained. Olmos et al. (1) reported that the rate of development of diabetes fell with time after diagnosis of the index twin and that twins discordant for more than 6 years were unlikely to develop diabetes. In contrast, we found a significant rate of late progression to diabetes after 6 years of discordance. In the total group of twins who became concordant (including the 7 twins who were already concordant at the time of ascertainment), half did so after 6 years had elapsed from the time of diagnosis in the index twin. It is likely that the four discordant twins in our current series who have FPIRs below the first percentile of control subjects will also become diabetic with longer follow-up (14,15).

Our study indicates that the concordance of identical twins for β-cell autoimmunity is higher than that for overt diabetes. We found that approximately two-thirds of identical twins with long-term discordance (of 7 years or more) have evidence of β-cell autoimmunity or damage. This was indicated by the persistent presence of autoantibodies and/or the loss of FPIR.

In summary, these results indicate that identical twins may develop diabetes after a prolonged period of discordance (7–36 years). They also indicate that approximately two-thirds of long-term discordant identical twins have evidence of chronic β-cell autoimmunity. The concordance for β-cell autoimmunity, therefore, is much higher than that for overt diabetes. This suggests that autoimmunity is common in identical twins of patients with type I diabetes and that additional environmental or non-Mendelian genetic factors or time are required for the development of type I diabetes.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health Grants DK-43279 and DK-32083 and by grants from the Eleanor Dana Charitable Trust, the Blum-Kovler Foundation, the American Diabetes Association, and the Juvenile Diabetes Foundation. C.F.V. and M.P. are recipients of Post-Doctoral Fellowships (392268 and 393364) from the Juvenile Diabetes Foundation.

REFERENCES

1. Olmos P, A'Hearn R, Heaton DA, Risley D, Pyke DA, Leslie RDG: The significance of concordance rate of type I (insulin dependent) diabetes mellitus in identical twins. *Diabetologia* 31:747–750, 1988
2. Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengard J, Kesaniemi YA: Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia* 35:1060–1067, 1992
3. Kumar D, Gemayel NS, Deapen D, Kapadia D, Yamashita PH, Lee M, Dwyer JH, Roy-Burman P, Bray GA, Mack TM: North-American twins with IDDM: Genetic, etiological, and clinical significance of disease concordance according to age, zygosity, and the interval after diagnosis in first twin. *Diabetes* 42:1351–1363, 1993
4. Rabin DU, Pleasic SM, Shapiro JA, Yoo-Warren H, Oles J, Hicks JM, Goldstein DE, Rae PMM: Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. *J Immunol* 152: 3183–3188, 1994
5. Srikanta S, Ganda OP, Eisenbarth GS, Soeldner JS: Islet cell antibodies and beta-cell function in monozygotic triplets and twins initially discordant for type I diabetes mellitus. *N Engl J Med* 308:322–325, 1983
6. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–1057, 1979
7. Vardi P, Crisa L, Jackson RA, Herskowitz RD, Wolfsdorf JI, Einhorn D, Linarelli L, Dolinar R, Wentworth S, Brink SJ, Starkman H, Soeldner JS, Eisenbarth GS: Predictive value of intravenous glucose tolerance test insulin secretion less than or greater than the first percentile in islet cell antibody positive relatives of type I (insulin-dependent) diabetic patients. *Diabetologia* 34:93–102, 1991
8. Bingley PJ, Colman P, Eisenbarth GS, Jackson RA, McCulloch DK, Riley WJ, Gale EAM: Standardization of IVGTT to predict IDDM. *Diabetes Care* 15:1313–1316, 1992
9. Srikanta S, Rabizadeh A, Omar MAK, Eisenbarth GS: Assay for islet cell antibodies: protein A-monoclonal antibody method. *Diabetes* 34:300–305, 1985
10. Vardi P, Dib SA, Tuttleman M, Connelly JE, Grinbergs M, Radizabeh A, Riley WJ, Maclaren NK, Eisenbarth GS, Soeldner JS: Competitive insulin autoantibody assay: prospective evaluation of subjects at high risk for development of type I diabetes mellitus. *Diabetes* 36:1286–1291, 1987
11. Ziegler AG, Ziegler R, Vardi P, Jackson RA, Soeldner JS, Eisenbarth GS: Life table analysis of progression to diabetes of anti-insulin autoantibody-positive relatives of individuals with type I diabetes. *Diabetes* 38:1320–1325, 1989
12. Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Hagopian WA, Li L, Karlsen AE, Boel E, Michelsen B, Lernmark Å: A novel radiobinding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. *Diabetologia* 37:344–350, 1994
13. Gianani R, Yu L, Verge CF, Babu S, Pietropaolo M, Eisenbarth GS: Recombinant ICA512 radioassay (Abstract). *J Invest Med* 43 (Suppl.): 331A, 1995
14. Srikanta S, Ganda OP, Jackson RA, Gleason RE, Kaldany A, Garavoy MR, Milford EL, Carpenter CB, Soeldner JS, Eisenbarth GS: Type I diabetes mellitus in monozygotic twins: chronic progressive beta cell dysfunction. *Ann Intern Med* 99:320–326, 1983
15. Lo SSS, Hawa M, Beer SF, Pyke DA, Leslie RDG: Altered islet beta-cell function before the onset of type I (insulin-dependent) diabetes mellitus. *Diabetologia* 35:277–282, 1992