The Prevalence of Islet Cell Antibodies in Japanese Insulin-dependent and Non-insulin-dependent Diabetic Patients Studied by Indirect Immunofluorescence and by a New Method

TETSURO KOBAYASHI, TADAO SUGIMOTO, TOKUJI ITOH, KINORI KOSAKA, TOSHIAKI TANAKA, SEIZO SUWA, KAORU SATO, AND KIMIYOSHI TSUJI

SUMMARY

Islet cell antibodies (ICA) were measured in Japanese patients with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) by a standard, indirect immunofluorescence method (IF method) and by a newly established, three-layer immunofluorescence method applying a biotin-avidin system (BAS method). In addition, the relationship between ICA and HLA was studied in IDDM patients.

ICA titers detected by the BAS method correlated well with those determined by the standard IF method (r_s = 0.987, $P < 0.01$). The BAS method had about an eightfold higher sensitivity for ICA than the IF method.

The overall prevalence of ICA detected by the BAS method (ICA-BAS) versus that by the IF method (ICA-IF) was 41% (82/198) versus 28% (56/198) in IDDM patients and 3% (19/593) versus 2% (14/593) in patients with NIDDM. In IDDM patients, ICA-BAS was all positive <1 mo after the onset of diabetes, while the prevalence of ICA-IF was 83% (20/24) during the same period. The prevalence of ICA-IF decreased rapidly with the duration of disease, reaching a value of 6% (3/55) in the patients with a disease duration of 10 yr or more. The incidence of ICA-BAS also decreased with the duration of disease, although to a lesser degree than ICA-IF. No association was found between HLA types and persistence of ICA-BAS or -IF.

These results suggest that pancreatic autoimmune processes occur in almost all Japanese IDDM patients. Although IDDM is less common in Japan than among Caucasians, the prevalence of ICA seems to be the same. The higher sensitivity of the BAS method may be of significant diagnostic value, especially in patients with a long duration of disease. DIABETES 1986; 35:335–40.

From the Department of Endocrinology and Metabolism (T.K., T.S., T.I., K.K.) and the Department of Pediatrics (T.T.), Toranomon Hospital, Okinaka Memorial Institute for Medical Research, 2-2-2 Toranomon, Minato-ku, Tokyo 105; the Department of Pediatrics (S.S.), Division of Endocrinology, Kanagawa Children's Medical Center, Yokohama 232; and the Department of Transplantation (K.S., K.T.), School of Medicine, Tokai University, Isehara, Kanagawa 259-11, Japan.

Address reprint requests to Dr. T. Kobayashi, Department of Endocrinology and Metabolism, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105, Japan.

Received for publication 22 July 1985 and in revised form 24 September 1985.

It is well documented that cytoplasmic islet cell antibodies (ICA) are present in a large proportion of patients with insulin-dependent diabetes mellitus (IDDM) among Caucasians in Europe[1–4] and North America.[5–8] In these studies, the prevalence of ICA at the onset of IDDM was as high as 65–85% and decreased gradually as the disease progressed.[2,3] The prolonged persistence of ICA appeared to correlate with HLA-B8 and/or -DR3,[2,9–12] although these findings could not be confirmed by Barbosa et al.[7] and Riley et al.[8] These HLA antigens are associated with several autoimmune endocrinopathies, suggesting that many cases of IDDM involve autoimmune processes. On the other hand, the Japanese apparently have a weaker autoimmune predisposition. In fact, the prevalence and the annual incidence of IDDM in Japanese are less than 20/10^5 and 0.4/yr/10^5, respectively.[13,14] These values are merely $\frac{1}{10}$ to $\frac{1}{20}$ of those of Caucasians. In addition, IDDM associated with autoimmune polyendocrinopathies seems to be rather rare and a matter of case report in the Japanese population.[15,16] On the other hand, Graves' disease (HLA-DR5 related[17]) and rheumatoid arthritis (HLA-DR4 related[18]) are rather common[19,20] in Japan, with prevalence values of 80/10^5 and 300/10^5, respectively. Few reports have been published in Japan[21,22] and in Europe[23] on the prevalence of ICA in Japanese IDDM, and its value was lower than that in Caucasians.[2–5,7] Recently, a multicenter family study[24] including our institution demonstrated that IDDM associated with autoimmune polyendocrinopathies was related to the HLA haplotype Bw61-DRw9, corresponding to B8-DR3 in Caucasians. However, possible relationships between ICA and HLA in the Japanese IDDM population remain unclear.

In the present study, we investigated the prevalence of ICA in 198 insulin-dependent and 593 non-insulin-dependent diabetic patients by the indirect immunofluorescence method as well as by a highly sensitive and specific method using

a biotin-avidin system, which produces the most intense staining with least background in comparison with the unlabeled antibodies (peroxidase-antiperoxidase, PAP) or the protein-A techniques.[25,26] Furthermore, the relationship between ICA positivity and HLA were studied on the largest number of IDDM patients to characterize the nature of ICA detected by the new method as well as by the standard method.

MATERIALS AND METHODS

SUBJECTS

We studied 198 cases of IDDM [88 males and 110 females; mean age at onset 21.3 (range: 1–66) yr; mean duration of disease 6.7 (range: 0–28) yr], 593 cases of non-insulin-dependent diabetes mellitus (NIDDM) [379 males and 214 females; mean age at onset 44.0 (range: 18–72) yr; mean duration of disease 8.0 (range: 0–21) yr], and 177 nondiabetic controls [82 males and 95 females; mean age 29.6 (range: 2–80) yr]. Because IDDM is rare in Japan, we established a collaborative study to recruit sufficient numbers of IDDM cases. All subjects were residents of the Tokyo and Yokohama areas. The diabetic patients were classified as IDDM or NIDDM based on the criteria of the National Diabetes Data Group.[27] All 198 IDDM patients were ketosis prone and showed no C-peptide immunoreactivity (CPR) to an oral glucose tolerance test. Among the IDDM cases, six (3%) had autoimmune diseases: three Graves' disease, two Hashimoto thyroiditis, and one primary biliary cirrhosis. Ten (2%) NIDDM cases were complicated by autoimmune diseases: eight Hashimoto thyroiditis and two Graves' disease.

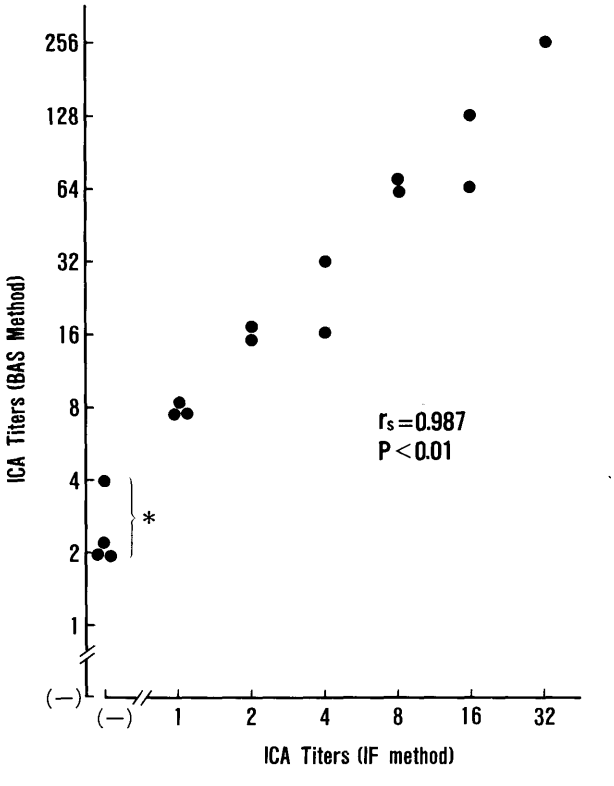

FIGURE 1. Correlation between the ICA titer determined by a standard immunofluorescence method (IF method) and that determined by a method applying a biotin-avidin system (BAS method). A significant correlation was demonstrated between the ICA titer obtained by the IF method and that by the BAS method. *Indicates the results of the samples, which were obtained from four IDDM patients <1 mo after disease onset.

TABLE 1
Overall prevalence of ICA detected by the BAS and the IF methods

Subjects	BAS method		IF method	
	No.	%	No.	%
IDDM	82/198	41.4	56/198	28.3
NIDDM	19/593	3.2	14/593	2.4
Treated with				
Diet	8/281	2.8	5/281	1.8
OHA*	4/127	3.1	2/127	1.6
Insulin	7/185	3.7	7/185	3.7
Nondiabetic controls	1/177	0.6	0/177	0

*OHA, oral hypoglycemic agents.

ICA DETECTION METHODS

Sera. Sera were obtained at the time of diagnosis from patients with IDDM or NIDDM. The sera were frozen until determinations were performed. Normal sera were obtained from nondiabetic controls and were likewise frozen until use.

Pancreas section. Fresh human group O pancreata were surgically removed from nondiabetic gastric carcinoma patients as the tissue substrate. The tissue was embedded in Tissue-Tek II O.C.T. Compound (Lab Tec, Naperville, Illinois), frozen in liquid nitrogen immediately after removal, sectioned at 5 μm on a cryostat, and mounted on slides. The ischemic time of the pancreata before freezing was kept as short as possible (<20 min). The sections were stored at −80°C until staining. Three pancreata were used throughout this study.

Immunofluorescence staining of ICA by the biotin-avidin system (BAS method). All sera were diluted 1:2 and 1:4 in 10 mM phosphate-buffered saline (PBS), pH 7.2, applied to the pancreatic sections, and incubated at room temperature for 30 min in a moist chamber. The slides were washed three times over 5-min intervals in PBS. Biotinated goat anti-human IgG (H + L) serum (Vector Labs, Burlingame, California) diluted 1:150 in PBS was then applied to the tissue section, which was then incubated at room temperature for 30 min. The section was then washed three times for 5-min intervals in PBS. Fluorescein-isothiocyanate (FITC)-conjugated avidin-D (Vector Labs) diluted 1:150 in PBS was applied to the section, which was then incubated at room temperature for 30 min. The section was then washed three times for 5 min in PBS.

Indirect immunofluorescence (IF) staining of ICA by the standard method (IF method). Each section was overlayed with undiluted sera for 30 min at room temperature in a moist chamber. After washing three times with PBS for 5 min, the sections were stained with FITC-conjugated goat anti-human immunoglobulin sera (Meloy Labs, Springfield, Virginia) diluted 1:20 for 30 min at room temperature.

After staining by the BAS or IF method, the section was finally mounted in 90% glycerol buffered with 0.5 M carbonate buffer (pH 9.5). The section was read using a Nikon Optiphot with epiillumination.

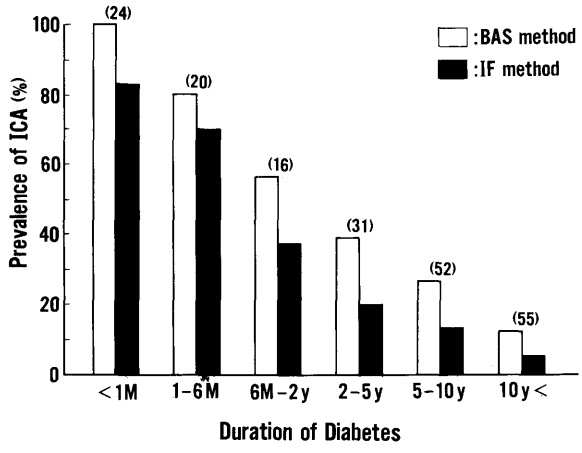

FIGURE 2. The prevalence of ICA detected by an indirect immunofluorescence method (IF method) or a method applying a biotin-avidin system (BAS method) in patients with IDDM, based on the duration of disease. The decline in ICA detected by the BAS method was less marked than that in ICA detected by the IF method with the duration of disease. The numbers in parentheses indicate the number of patients tested for ICA.

With both the BAS and IF methods, the following procedures were carried out to ensure uniform and nonbiased evaluation for ICA. The results were evaluated as negative, trace, 1+, or 2+ by two or three investigators in a double-blind manner, and the ICA titers were determined by double dilution. Positive (titer 1:1 and 1:4 by IF method) and negative control samples were included in each assay.

Sensitivity and specificity of ICA detected by the BAS or IF methods. The sensitivity of the ICA assay was expressed as the number of times a positive standard sample (titer 1:1 by IF method) was read as positive divided by the number of times the sample was tested. The specificity of the ICA assay was expressed as the number of times a negative control sample was read as negative divided by the number of times the sample was tested.

HLA TYPING

Analysis of persistence of ICA versus HLA type was done in 153 IDDM patients. HLA typing for the A, B, C, and DR antigens was performed by a standard microcytotoxicity test.[28] The HLA antigens typed were A1, A2, A3, A11, A24, A26, A31, Aw33, B7, B8, B13, B17, Bw35, Bw40, Bw44, Bw46, Bw48, Bw51, Bw52, Bw54, Bw55, Bw56, Bw59, Bw60, Bw61, Cw1, Cw2, Cw3, Cw4, Cw5, Cw6, DR1, DR2, DR3, DR4, DR5, DR7, DRw8, and DRw9. HLA antigens of 171 normal subjects, who were residents of the Tokyo and Yokohama areas, were employed as normal controls. All patients and all controls were of Japanese ethnic stock.

STATISTICAL ANALYSIS

Spearman's correlation test was applied to compare ICA titers determined by the BAS method and the IF method. Fisher's exact test was used to compare the prevalence of ICA and HLA.

RESULTS

Correlation between ICA determined by the standard IF method (ICA-IF) and that determined by the BAS method (ICA-BAS). A significant linear correlation was demonstrated between the ICA titers obtained by the standard IF method and by the BAS method ($r_s = 0.987$, $P < 0.01$, $N = 16$, Figure 1). All four ICA-negative (by the standard IF method) sera, which were obtained from IDDM patients <1 mo after onset, were found to be positive with titers of 2–4 by the BAS method (Figure 1).

Sensitivity and specificity of ICA detected by the BAS or IF method. The sensitivity was 96% (99/103) and 97% (109/112) by the BAS and IF methods, respectively; the specificity was 95% (98/103) and 94% (105/112), respectively.

Prevalence of ICA-IF and ICA-BAS. The overall prevalence of ICA in IDDM patients detected by the BAS and the IF methods are listed in Table 1. All 70 sera positive for ICA-IF were positive for ICA-BAS.

ICA-IF were detected in 83% (20/24) of IDDM patients whose onset of disease was <1 mo (Figure 2). The prevalence of ICA-IF decreased thereafter, dropping to only 5.5% (3/55) in patients with a duration of 10 yr or more. In 20 ICA-IF-positive diabetic patients tested <1 mo after the onset of disease, the titer of ICA-IF ranged from 1:1 to 1:1024 (mode: 1:8).

ICA-BAS were positive in all 24 IDDM patients whose duration of disease was <1 mo (Figure 2). The decline in ICA-BAS was less marked than that in ICA-IF with the duration of

TABLE 2
Frequencies of islet cell antibodies determined by the BAS method versus HLA types and duration of diabetes (DOD)

		DOD ≤5 yr		DOD >5 yr		All	
		No.	%	No.	%	No.	%
Bw	54/X*	14/15	93	4/28	14	18/43	42
	54/61	1/5	20	2/2	100	3/7	43
	61/X	7/10	70	3/15	20	10/25	40
	X/X	22/35	63	8/43	19	30/78	38
	All	44/65	68	17/88	19	61/153	40
DR(w)	4/X†	24/35	69	10/47	21	34/82	41
	4/9	6/8	75	5/19	26	11/27	41
	9/X	13/17	76	1/6	17	14/23	61
	X/X	1/5	20	1/16	6	2/21	10
	All	44/65	68	17/88	19	61/153	40

*X is any antigen other than 54 or 61.
†X is any antigen other than 4 or 9.

disease. The prevalence of ICA-BAS in IDDM patients with disease duration of >2 yr was two to three times higher than that of ICA-IF (Figure 2).

As listed in Table 1, ICA were also detected in NIDDM patients. The frequency of ICA in NIDDM patients was higher by the BAS method than by the standard method, similar to the results in IDDM.

HLA and ICA. No association was found between HLA and the persistence of ICA when the frequencies of ICA-BAS or -IF were compared in IDDM patients with a disease duration of ≤5 yr and in those with a disease duration of >5 yr (Table 2). ICA-BAS positivity in patients with HLA-Bw61 or -DRw9, which seems to be related to autoimmune-complicated IDDM in Japan,[24] did not differ from the value in patients with HLA-Bw54 or -DR4, which is also related to IDDM in Japan[24] (Table 2). HLA frequencies in IDDM patients and in controls are listed in the APPENDIX.

DISCUSSION

Many workers[5,29–31] have considered the possibility that the heterogeneity of IDDM might be expressed as differences in the prevalence of ICA. Indeed, the prevalence of ICA in IDDM patients is lower in American Blacks[5] and Mexican-Americans[30] than in Caucasians.[3–5,7] The prevalence of ICA in Japanese IDDM patients reported by Irvine[23] (14% within 1 yr after diagnosis) and Sakurami et al.[22] (5% in overall prevalence) was very low, although recently Sakurami and co-workers,[32] in a preliminary report, found a higher overall prevalence of 24%. In the present study, we demonstrated that, immediately after the onset of disease, the prevalence of ICA-IF detected by a standard immunofluorescence method in Japanese IDDM patients was as high as that in Caucasians.[2–8] This is the first and the largest Japanese study of ICA in IDDM that confirms Caucasian studies.[1–8] The most common titer of ICA immediately after the onset of IDDM and the prevalence of ICA in NIDDM and controls were comparable to those in Caucasians,[2,3] suggesting that the sensitivity of our standard IF method was similar to that used in Caucasian studies.[1–8] The high reproducibility of our ICA detecting method indicates that our study is reliable. In another series of experiments, we measured ICA in four positive and two negative samples, which were also assayed in the laboratory of Dr. G. F. Bottazzo (Middlesex Hospital, London United Kingdom), and found concordant results (unpublished data in collaboration with Dr. Y. Sakamoto, Jikei Medical School, Japan). The paucity of ICA in Irvine's study[23] in the Japanese population could be explained by the small number of patients who were measured for ICA immediately after the onset of IDDM, because ICA-IF decreased rapidly with prolonged duration of disease (Figure 2).

Although ICA have become an important research tool for studying the pathogenesis of IDDM, there has been considerable concern[33] about the sensitivity and standardization of the method most widely used, namely, the indirect immunofluorescence test. We describe here a new method applying a biotin-avidin immunofluorescence system and show that it compares favorably with the indirect immunofluorescence method. Several new methods,[34–39] including immunoenzyme histochemical methods other than the standard IF method, have been reported for the detection of ICA. Generally, the three-layer technique [i.e., the method applying peroxidase-antiperoxidase (PAP), the protein-A, or the biotin-avidin complex] is more sensitive than the indirect method.[40] With the three-layer method, the biotin-avidin immunoenzyme technique is considered to produce more intense staining and lesser background than the PAP or protein-A techniques.[25,26] These conditions are prerequisite for evaluating the positivity of ICA.[37] Moreover, we adopted the immunofluorescence method rather than the immunoperoxidase method[39] for the staining of pancreatic sections, because the immunofluorescence method is easy to read and free from interference by endogenous peroxidase. In fact, our ICA detection method applying a biotin-avidin immunofluorescence system (BAS method) was about eight times more sensitive than the standard IF method (Figure 1); high specificity could also be attained. The prevalence of ICA-BAS was 100% immediately after the onset of IDDM, and this value subsequently decreased less prominently than ICA-IF, indicating higher diagnostic value of the former especially in long duration of disease. Furthermore, this BAS method may be useful for predicting the deterioration of B-cell function in genetically predisposed relatives of IDDM patients in whom ICA sometimes fluctuate or disappear,[41] possibly because of insufficient sensitivity of the standard IF method.[33] In fact, we recently observed several mild diabetic subjects who progressed from ICA positive to ICA negative by the standard IF method, but remained ICA positive by the BAS method and progressed slowly to an insulin-dependent state (unpublished data).

Our results showed no association between any type of HLA and the persistence of ICA in IDDM patients. Some European reports[2,9–12] indicate a closer relationship between the persistence of ICA and HLA-B8 and/or -DR3; however, Barbosa et al.[7] did not show any relationship between ICA and HLA. One possible reason for this effect may be due to small clusterings of IDDM with overt autoimmune polyendocrinopathies in our study, although a paucity of autoimmune-related disorders is one of the characteristics of Japanese IDDM.[15,16] In European studies[2,10] the percentage of autoimmune-related IDDM with autoimmune diseases was around 9% and 24%, while in our study it was only 3%. Further studies in other Japanese populations with a higher risk of autoimmune polyendocrinopathies may clarify the autoimmune aspects of IDDM in Japan. The possibility cannot be excluded that there were not enough patients with IDDM for >5 yr to test the association between ICA and HLA types.

There is a paucity of information of HLA in the Japanese population. Our study clearly demonstrated the negative correlation between HLA-DR2 and IDDM patients (APPENDIX), while a previous study[42] failed to show this effect in the Japanese population.

ACKNOWLEDGMENTS

The authors wish to acknowledge Kazuhiko Kobayashi for his assistance with the statistical analysis and Mitsue Amatatsu for her excellent secretarial help.

This work was supported in part by research grants from the Ministry of Education, Science and Culture of Japan (no. 60770932) and from the Insulin Research Foundation, Tokyo, Japan.

APPENDIX

HLA-B and -DR antigen frequencies in patients with IDDM and in normal controls

Antigens	IDDM (N = (153)		Controls (N = 171)		Corrected P-value	Relative risk
	N	%	N	%		
B7	18	11.8	17	9.9		
B8	0	0	1	0.6		
B13	2	1.3	4	2.3		
B17	0	0	1	0.6		
Bw35	21	13.7	27	15.8		
Bw40	3	2.0	0	0		
Bw44	24	15.7	28	16.4		
Bw46	7	4.6	12	7.0		
Bw48	8	5.2	9	5.3		
Bw51	15	9.8	26	15.2		
Bw52	9	5.9	43	25.1	0.00078	0.19
Bw54	50	32.7	19	11.1	0.00305	3.88
Bw55	2	1.3	10	5.8		
Bw56	2	1.3	2	1.2		
Bw59	6	3.9	6	3.9		
Bw60	22	14.4	19	11.1		
Bw61	33	21.6	31	18.0		
DR1	11	7.2	16	9.4		
DR2	13	8.5	67	39.2	0.011×10^{-4}	0.14
DR3	0	0	2	1.2		
DR4	109	71.3	72	42.1	0.0263	3.41
DR5	7	4.6	18	10.5		
DR7	1	0.7	3	1.8		
DRw8	25	16.3	22	12.9		
DRw9	49	32.0	45	26.3		
DR4/DRw8	13	8.4	7	4.1	NS	2.18
DR4/DRw9	26	17.0	12	7.0	0.01*	2.71

NS, not significant; *uncorrected P-value.
Relative risk was calculated as follows: (a) × (d)/(b) × (c), where (a) = no. of patients positive for antigen, (b) = no. of patients negative for antigen, (c) = no. of controls positive for antigen, and (d) = no. of controls negative for antigen.

REFERENCES

[1] Bottazzo, G., Christensen, A., and Doniach, D.: Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974; 2:1279–82.

[2] Irvine, W., McCallum, C., Gray, R., Campbell, C., Ducan, L., Farquhar, J., Vaughan, H., and Morris, P.: Pancreatic islet cell antibodies in diabetes mellitus correlated with the duration and type of diabetes, coexistent autoimmune disease, and HLA type. Diabetes 1977; 26:138–47.

[3] Lendrum, R., Walker, G., Cudworth, A., Theophanides, C., Pyke, D., Bloom, A., and Gamble, D.: Islet cell antibodies in diabetes mellitus. Lancet 1976; 2:1273–76.

[4] Del Prete, G., Betterle, C., Padovan, D., Erle, G., Toffolo, G., and Bersahi, G.: Incidence and significance of islet cell autoantibodies in different types of diabetes mellitus. Diabetes 1977; 26:909–15.

[5] Neufeld, M., MacLaren, N., Riley, J., Lezotte, D., McLaughlin, J., Silverstein, J., and Rosenbloom, A.: Islet cell and other organ-specific antibodies in U.S. Caucasians and Blacks with insulin-dependent diabetes mellitus. Diabetes 1980; 29:589–92.

[6] West, R., Colle, E., Belmonte, M., Tingle, A., Guttman, R., Hynie, I., Thomas, D., Wilkins, J., Poirier, R., and Crepeau, P.: Prospective study of insulin-dependent diabetes mellitus. Diabetes 1981; 30:584–89.

[7] Barbosa, J., Chavers, B., Dunsworth, T., and Michael, A.: Islet cell antibodies and histocompatibility antigens (HLA) in insulin-dependent diabetics and their first-degree relatives. Diabetes 1982; 31:585–88.

[8] Riley, W., Winter, W., Spillar, R., Rosenbloom, E., and Glancey, L.: Cytoplasmic islet cell autoantibodies (ICA) and insulin-dependent diabetes (IDD). Abstract. Diabetes 1983; 32 (Suppl.):46A.

[9] Morris, P., Vaughan, H., Irvine, W., McCallum, G., Gray, R., Campbell, C., Duncan, L., and Farquhar, J.: HLA and pancreatic islet cell antibodies in diabetes. Lancet 1976; 2:652–53.

[10] Irvine, W., Mario, U., Feek, C., Gray, R., Ting, A., Morris, P., and Ducan, L.: Autoimmunity and HLA antigens in insulin-dependent (type I) diabetes. J. Clin. Lab. Immunol. 1978; 1:107–10.

[11] Bottazzo, G., Cudworth, A., Moul, D., Doniach, D., and Festenstein, H.: Evidence for a primary autoimmune type of diabetes mellitus. Br. Med. J. 1978; 2:1253–55.

[12] Ludwig, H., Schernthaner, G., and Mayr, W.: Ia-type alloantigens and humoral autoimmune responsiveness in insulin-dependent diabetes. Metabolism 1979; 28:797–800.

[13] World Health Organization: WHO Expert Commitee on Diabetes Mellitus. Geneva, WHO Technical Series, 1980: 646:18.

[14] Kitagawa, T., Owada, M., Mano, T., and Fujita, H.: The epidemiology of childhood diabetes mellitus in Japan. *In* Clinico-genetic Genesis of Diabetes Mellitus. Mimura, G., Baba, S., Goto, Y., and Köbberling, J., Eds. Amsterdam, Excerpta Medica, 1982:51–57.

[15] Kitamuro, F., and Abe, T.: Coexistence of diabetes mellitus and primary hypothyroidism. J. Jpn. Diabetes Soc. 1978; 21:583–88.

[16] Miwa, U., Sakato, S., Yoshimitsu, K., Sato, T., Mori, K., Haseka, Y., Yoshino, K., Yamamoto, H., Onoe, T., Ohka, T., and Kinoshita, T.: Two cases of hyperthyroidism complicated with diabetic ketoacidotic coma. J. Jpn. Diabetes Soc. 1980; 23:879–88.

[17] Uno, H., Sasazuki, T., Tamai, H., and Matsumoto, H.: Two major genes, linked to HLA and GM, control susceptibility to Graves' disease. Nature 1981; 292:768–70.

[18] Nakai, Y., Wakisaka, A., Aizawa, M., Itakura, K., Nakai, H., and Ohashi, A.: HLA and rheumatoid arthritis in the Japanese. Arthritis Rheum. 1981; 24:722–25.

[19] Izumi, M., Kudo, I., and Nagataki, S.: Graves' disease. Nihon Rinsho 1983; 41 (Suppl.):797–803 (in Japanese).

[20] Azuma, T.: Rheumatoid arthritis. Nihon Rinsho 1983; 41 (Suppl.): 1147–58 (in Japanese).

[21] Kobayashi, T., Sawano, S., Itoh, T., Sugimoto, T., Tanaka, T., and Suwa, S.: The prevalence of islet cell antibodies and complement-fixing islet cell antibodies in Japanese diabetics. Endocrinol. Jpn. 1981; 28:429–33.

[22] Sakurami, T., Nagaoka, K., Nabeya, N., Ueno, Y., Imura, H., and Kuno, S.: HLA specificities and islet cell antibodies in Japanese insulin-dependent diabetics. *In* Genetic Environmental Interaction in Diabetes Mellitus. Hanna, M., and Baba, S., Eds. Amsterdam, Excerpta Medica, 1982:155–60.

[23] Irvine, W. J.: Immunological aspects of diabetes mellitus. *In* Immunology of Diabetes. Irvine, W. J., Ed. Edinburgh, Teviot Scientific Publications, 1980:24.

[24] Kida, K., Mimura, G., Kobayashi, T., Nakamura, K., Sonoda, S., Inoue, H., and Tsuji, K.: HLA haplotype study of Japanese type I diabetes mellitus

families. *In* Proceedings of Diabetes Mellitus in Asia. Mimura, G., and Baba, S., Eds. In press. Amsterdam, Excerpta Medica.

[25] Hsu, S. M., Raine, L., and Fanger, H.: The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 1981; 29:577–81.

[26] Hsu, S. M., and Raine, L.: Protein-A, avidin, and biotin in immunohistochemistry. J. Histochem. Cytochem. 1981; 29:1349–53.

[27] National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979; 28:1039–57.

[28] Terasaki, P. I.: HLA-DR joint report. *In* Histocompatibility Testing, 1980. Terasaki, P. I., Ed. Los Angeles, UCLA Tissue Typing Laboratory, 1980: 506.

[29] Oli, J., Bottazzo, G., and Doniach, D.: Islet cell antibodies in Nigerian diabetics. Lancet 1980; 1:1090.

[30] Zeidler, A., Frasier, S., Penny, R., Stein, R., and Nicoloff, J.: Pancreatic islet cell and thyroid antibodies, and islet cell function in diabetic patients of Mexican-American origin. J. Clin. Endocrinol. Metab. 1982; 54:949–54.

[31] Irvine, W. J.: The immunology and genetics of autoimmune endocrine disease. *In* Genetic Control of Autoimmune Disease. Rose, N. R., Bigazzi, P. E., and Warner, N. L., Eds. Amsterdam, Elsevier, 1978:77.

[32] Notsu, K., Oka, Y., Kuno, S., and Sakurami, T.: Organ-specific autoantibodies and classification of diabetes mellitus. Abstract. J. Jpn. Diabetes Soc. 1985; 28:183 (in Japanese).

[33] Riley, W., and MacLaren, N.: Islet cell antibodies are seldom transient. Lancet 1984; 1:1351–52.

[34] Dobersen, M. J., Bell, A. M., Jenson, A. B., Notkins, A. L., and Fellner, F. G.: Detection of antibodies to islet cells and insulin with paraffin-embedded pancreas as antigen. Lancet 1979; 2:1078.

[35] Krell, J., and Rabin, B. S.: Comparison of an immunohistochemical and immunofluorescence procedure to detect antibody to pancreatic islets. Diabetes 1984; 33:709–11.

[36] Pilcher, C., and Elliott, R. B.: Improved sensitivity of islet cell cytoplasmic antibody assay in diabetics. Lancet 1984; 1:1352.

[37] Srikanta, S., Rabizadeh, A., Omar, K. A. M., and Eisenbarth, G. S.: Assay for islet cell antibodies: protein-A-monoclonal antibody method. Diabetes 1985; 34:300–305.

[38] Yagihashi, S., Suzuki, H., Dobersen, M. J., Onodera, T., Notkins, A. L., and Fellner, F. G.: Autoantibodies to islet cell: comparison of methods. Lancet 1982; 2:1218.

[39] Taub, F., and Scharff, J.: Assay of islet cell antibodies (ICA), a more sensitive method. Abstract. Diabetes 1984; 33 (Suppl. 1): 63A.

[40] Bergroth, V.: Comparison of various immunohistochemical methods. Histochemistry 1983; 77:177–84.

[41] Spencer, K. M., Tarn, A., Dean, B. M., Lister, J., and Bottazzo, G., F.: Fluctuating islet cell autoimmunity in unaffected relatives of patients with insulin-dependent diabetes. Lancet 1984; 1:764–66.

[42] Sakurami, T., Ueno, Y., Nagaoka, K., Kuno, S., Iwaki, Y., Park, M. S., and Terasaki, P. I.: HLA-DR specifications in Japanese with juvenile-onset insulin-dependent diabetes mellitus. Diabetes 1982; 31:105–106.