HLA Antigens, Cytoplasmic Islet Cell Antibodies, and Carbohydrate Tolerance in Families of Children with Insulin-dependent Diabetes Mellitus

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SUMMARY

Cytoplasmic pancreatic islet cell antibodies were found in 21% of 244 unaffected first degree relatives of type I diabetic patients. Twenty-five percent of HLA-identical, 35% of HLA-haploidentical, 16% of HLA-nonidentical siblings, and 14% of parents were ICA-positive. In the HLA-identical sibs, irrespective of ICA, and in the 18 ICA-positive parents but not the other groups, increased plasma glucose levels were observed after the administration of glucose. In most children, these were associated with reduced insulin levels, while in the adults elevated insulin responses were noted. In 48% of the ICA-positive children and 84% of the ICA-positive parents, other evidence of "autoimmunity" was obtained either by history or by testing for specific autoantibodies. Two of the originally unaffected HLA-identical and ICA-positive siblings developed diabetes during the course of the study. These findings, plus previously reported data in families with two diabetic sibs demonstrating that the empiric risk for developing IDDM is of the order of 30% for HLA-identical sibs but less than 5% for those that are HLA-haploidentical, suggest that HLA-identity may be a useful predictor of potential type I diabetes. The presence of ICA may, at times, portend the need for future antidiabetic therapy but prospective studies must be continued to fully elucidate this relationship.

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Recent investigations have indicated that type I, insulin-dependent diabetes mellitus (IDDM) is associated with specific HLA antigens as well as with the presence of circulating cytoplasmic pancreatic islet cell antibodies (ICA).[1–8] ICA have been reported in at least 85% of insulin-dependent diabetics at the time of onset of their disease with a gradual decrement in the prevalence thereafter.[9–12] The presence of ICA in non-insulin-dependent adult diabetics has been shown by at least one group to be associated with the eventual need for insulin therapy.[13] The presence of these antibodies many years after the onset of the disease has also been noted in association with autoimmune polyendocrine diseases, and with positivity for the HLA antigens B8 and DR3.[7,8] Despite these observations, the role of ICA in the development and pathogenesis of IDDM remains to be elucidated. The present studies were designed to investigate the prevalence of ICA, and the relationship between ICA, various HLA antigens and the development of glucose intolerance, and insulin secretory defects predating the clinical diagnosis of IDDM in a population at increased risk for its development: the siblings and parents of IDDM probands.[14]

MATERIALS AND METHODS

Sixty-four unrelated families with 2–6 (mean 3.0) children per family were included in the study. These families were recruited consecutively after consultation at the Pediatric Diabetes Unit of Mount Sinai Medical Center from 1978 through 1980. Fifty-six families had one child with IDDM, six families had two, and two families had three such children. Thus, 74 insulin-dependent diabetic children (35 girls, 39 boys, mean age 13.7 ± 1.5 yr), their 119 nondiabetic siblings (60 girls, 59 boys, ages 1–31 yr, mean age 12.8 ± 1.4 yr), and 128 parents (ages 24–55 yr, mean age 39.2 ± 2.1 yr) were studied. Twenty-seven families were Ashkenazi Jewish, 14 were Hispanic, 15 were Northern European, 5 were Southern European, and 3 were American black.

All propositi were documented as having had a typical onset of their diabetes before age 15 (mean age at onset 9.5 ± 0.4 yr) with ketosis and/or ketoacidosis and to have

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required insulin since the time of diagnosis. All were of normal weight (mean % ideal body wt 104 ± 5%). Twenty-four patients had diabetes for less than 1 yr, 29 for 1–5 yr, and 21 for longer than 5 yr. One mother, age 34, has had typical IDDM since age 16. A second mother, age 43, has been on oral antidiabetic medication for 5 yr but has recently required insulin therapy. One father, age 35, had a significantly elevated level of hemoglobin A_{1c} (7.0%), and subsequent study revealed definite fasting and postprandial hyperglycemia and hypoinsulinemia.

Blood samples were obtained from all members of a family on the same day. Lymphocytes were separated by standard techniques and were used for the determination of HLA types A, B, and C by the contrast fluorescence test and for DR with the two-color fluorescence test, modifications of the microcytotoxicity test, using 180 monospecific or oligospecific reagents.[15–17] Cytoplasmic islet cell antibodies (ICA) were measured by our recently described immunofluorescence method employing Bouin's fixed human pancreas from blood group O donors.[18,19] All positive sera were absorbed with insulin to remove insulin antibodies and retested. Control sera for ICA were obtained from 242 normal-weight children, adolescents, and young adults (mean age 14.4 ± 1.6 yr), with no family history of diabetes mellitus, no history of febrile illnesses within the preceding 2 mo, and no laboratory evidence of thyroid dysfunction. Sera were also examined for the presence of thyroid microsomal and thyroglobulin antibodies by hemagglutination and immunofluorescent techniques[20,21] and for adrenal, testicular, and gastric antibodies by immunofluorescence.[22]

Most nondiabetic members of each family were also tested for their response to glucose loads. Three-hour oral glucose tolerance tests (GTTs) were performed, for which the children received 1.75 g of glucose/kg ideal body wt as an iced, lemon-flavored 50% solution. Adults received 100 g of a similar glucose solution. Plasma glucose levels were measured in duplicate by the glucose-oxidase technique.[23] Serum levels of insulin were determined by radioimmunoassay[24] in the fasting state and at ½, 1, 2, and 3 h after glucose ingestion. Overall responses in serum insulin and plasma glucose were also quantified, respectively, as the sums of the five insulin and glucose levels measured during the GTTs. Control data for the sib GTTs were obtained in 48 (26 girls, 22 boys) normal-weight children and adolescents (mean % ideal body wt 103 ± 4%), ages 5–20 yr (mean 13.8 ± 1.7 yr) with no family history of diabetes mellitus. None of these control subjects had detectable islet cell antibodies.

The data were evaluated with standard statistical techniques. No significant differences were noticed in either HLA or ICA data obtained from families of the different racial origins in this sample (perhaps as a result of the small numbers involved). The results of all four ethnic groups were therefore combined.

RESULTS

ICA prevalence. The prevalence of ICA in the unaffected first degree relatives and their diabetic probands are summarized in Table 1. Twenty-five percent of 28 HLA-identical (19 of whom were male), 35% of 66 HLA-haploidentical (30 of whom were male), and 16% of 25 HLA-nonidentical sibs (9 of whom were male) were ICA-positive. The differences in the male/female ratio in the sibs sharing 2, 1, and 0 HLA haplotypes (19/9, 30/36, and 9/16) were statistically significant ($\chi^2_2 = 6.1$, $P < 0.05$). Three of the four ICA-positive, HLA-nonidentical sibs were children of parents who themselves had ICA. Parents of diabetic probands were ICA-positive less frequently (18/128) than were sibs ($\chi^2 = 7.81$, $P < 0.01$). In three of the families (5%) both parents were ICA-positive. A history of gestational diabetes mellitus was elicited in 7 of the 10 ICA-positive mothers. The three parents known to have diabetes were, however, ICA-negative. The presence of ICA in the parents did not influence the prevalence of ICA in the nondiabetic children (11/33 children of ICA-positive parents and 23/86 children of ICA-negative parents had ICA, $\chi^2 = 0.51$). There were no significant differences between the ages of the parents with and without antibodies (39.6 ± 3.2 vs. 38.4 ± 2.8 yr) or in their percent ideal body weights (118 ± 7 vs. 112 ± 8%). Furthermore, there were no significant differences (by Student's *t* test) between ICA-positive and ICA-negative sibs in current age (12.5 ± 1.1 vs. 13.2 ± 0.8 yr), in the number of years since the respective propositus developed diabetes (3.94 ± 0.62 vs. 3.81 ± 0.71 yr), or in age difference with the propositus (−0.31 ± 0.09 vs. +0.18 ± 0.08 yr). The mean age of the ICA-positive diabetics was 13.3 ± 0.5 yr and their mean duration of diabetes were 2.9 ± 0.4 yr, whereas the ages of ICA-negative diabetics were similar (14.4 ± 0.5 yr, $P > 0.1$) but their duration of diabetes was significantly longer (5.6 ± 0.5 yr, $P < 0.05$).

Segregation of HLA haplotypes. No significant deviation from Mendelian ratios was observed in the 119 sibs of pa-

TABLE 1
Relationship of ICA to HLA type in first degree relatives of diabetic probands

	Subjects studied			ICA+			ICA prevalence (%)
	Female	Male	Total	Female	Male	Total	
Diabetic probands*	35	39	74	24	24	48	65
Siblings (nondiabetic)	61	58	119	20	14	34	29
HLA-identical	9	19	28	4	3	7	25
HLA-haploidentical	36	30	66	13	10	23	35
HLA-nonidentical	16	9	25	3	1	4	16
Parents	64	64	128	10	8	18	14
Controls	118	124	242	1	0	1	<1

* Mean duration IDDM 3.1 yr.

TABLE 2
Prevalence of ICA according to HLA antigens

HLA	IDDM probands			Sibs			Parents		
	Total number with antigen	ICA+	%ICA+	Total number with antigen	ICA+	%ICA+	Total number with antigen	ICA+	%ICA+
B7	9	7	78	14	0	0*	15	1	7
B8	25	14	56	22	2	9*	30	5	17
B14	10	8	80	17	9	53*	18	4	22
B15	8	6	75	9	3	33*	9	0	0
B18	14	10	71	19	9	47	16	3	19
DR2	2	1	50	14	2	14	12	3	25
DR3	38	24	64	31	9	30	30	3	10
DR4	40	25	63	55	16	30	45	8	18
DR3,3	7	6	86	13	0	0	1	0	0
DR4,4	7	5	71	13	5	39	8	2	25
DR3,4	18	11	61	11	2	18	7	0	0

* Denotes significant difference in ICA prevalence for given HLA antigen by chi-square analysis.

tients: 28 shared two haplotypes with the respective patient, 66 shared one, and 25 shared none ($\chi^2 = 1.57$).

Relationship between HLA antigens and ICA prevalence. As shown in Table 2, islet cell antibodies were found in probands, sibs, and parents with various HLA antigens. DR3 and DR4 were the most common HLA-DR antigens in the probands with 38/48 (79%) ICA+ and 22/26 ICA− (88%) probands having at least one of these HLA antigens, giving a combined prevalence of 81%. Similarly, HLA-DR3 or DR4 was identified in 22/34 ICA+ (65%) and 62/85 ICA− sibs (73%), and 11/18 ICA+ (61%) and 57/110 ICA− parents (52%). On further breakdown of the ICA+ sibs, 5/7 HLA-identical (71%), 17/23 HLA-haploidentical (74%), and 1/4 HLA nonidentical (25%) were positive for HLA-DR3 or DR4. Thus, only minor deviations in the frequency of ICA according to HLA antigens were found in the subsets of patients, sibs, and parents. None of the differences was significant for any HLA-DR type. However, sibs with HLA-B7 ($\chi^2 = 6.4$, $P < 0.02$) and B8 ($\chi^2 = 5.0$, $P < 0.025$) had a significantly lower prevalence of ICA than the group of sibs as a whole, while those with B14 ($\chi^2 = 5.8$, $P < 0.02$) and B18 ($\chi^2 = 3.9$, $P < 0.05$) had a significantly higher prevalence. There were no significant differences in ICA frequency between DR3 and DR4-homozygous versus DR3- and DR4-heterozygous probands. DR3- and DR4-heterozygous sibs were equally frequently associated with ICA. Although no ICA-positives were found among the 13 DR3-homozygous sibs, this was not statistically significant.

Autoimmunity other than ICA. Information on the prevalence in the 74 families of autoimmune disorders other than ICA is summarized in Table 3. Among the ICA+ members, 84% of parents and 48% of sibs and IDDM probands had an autoimmune disorder. Among the ICA-negative members, only 5% of parents, 4% of sibs, and 16% of IDDM probands had such findings (the χ^2 is 85.3 for siblings and 34.3 for parents, both tests with $P < 0.001$). Of 37 first degree relatives (15%) with evidence of autoimmunity, 25 (69%) were female and 12 were male (31%) and 31 of them were ICA-positive (84%). Autoimmunity to the thyroid gland was most common in both ICA-positive and ICA-negative individuals affecting 17 sibs and 7 parents. In 15 ICA+ and 2 ICA− sibs there were detectable microsomal or thyroglobulin anti-

TABLE 3
Autoimmunity in first degree relative of diabetic probands

"Disease"	Parents				Sibs				IDDM Patients			
	ICA+		ICA−		ICA+		ICA−		ICA+		ICA−	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Multiple sclerosis	0	3	0	0	0	0	0	0	0	0	0	0
Rheumatoid arthritis	1	3	0	0	0	0	0	0	0	0	0	0
Regional ileitis	2	1	0	0	0	0	1	0	0	0	0	0
Thyroid dysfunction*	2	2	2	1	3	12	0	2	6	16	2	2
Testicular antibodies	0	0	0	0	1	0	0	0	0	0	0	0
Pemphigus	0	1	0	0	0	0	0	0	0	0	0	0
Prevalence of autoimmunity												
By sex	5/8	10/10	2/36	1/36	4/14	12/20	1/45	2/40	6/24	16/24	2/11	2/15
Total	15/18†		3/72		16/34		3/85		22/48		4/26	
Percent	84		5		48		4		48		16	

* Subjects with hypothyroidism and/or thyroglobulin or microsomal antibodies.
† Denominator is total number of individuals studied in each subgroup.

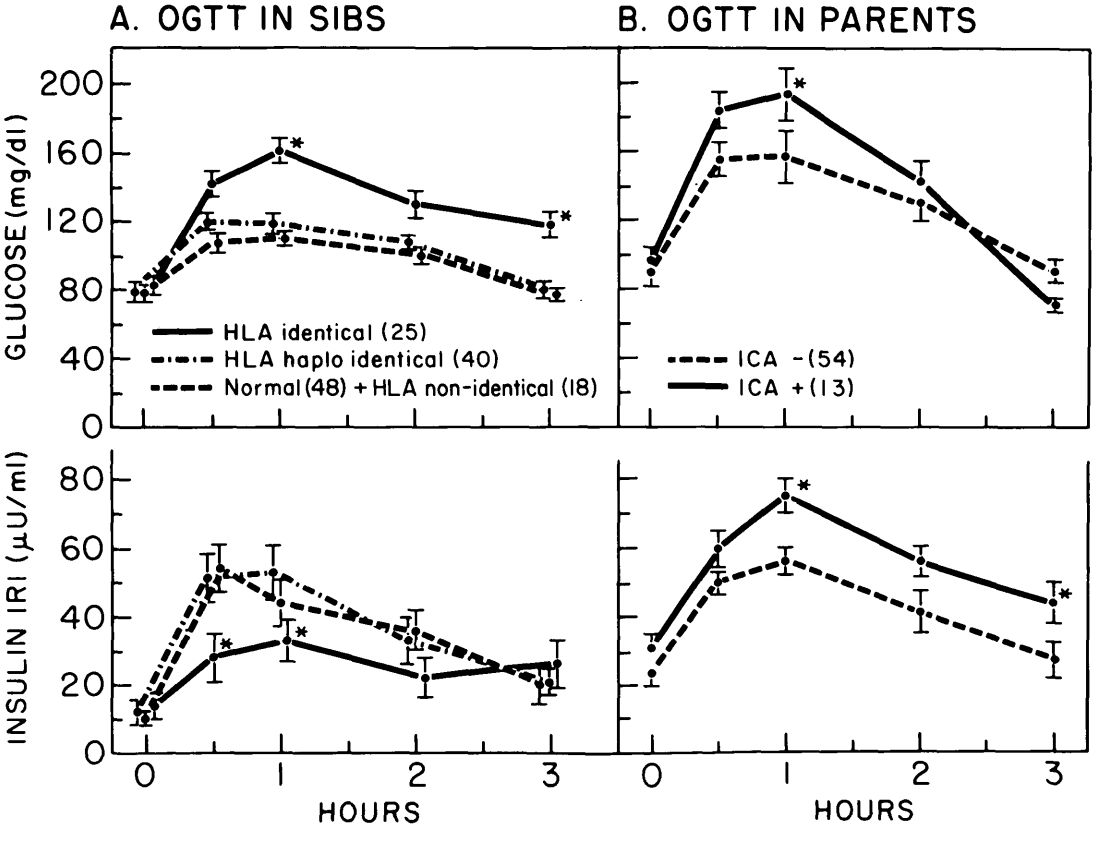
bodies. Two ICA− and three ICA+ parents also had such antibodies while two other parents (one ICA+ and one ICA−) presently had no detectable antibodies. These latter two were taking replacement doses of thyroid hormone and were documented as having had antithyroid antibodies as adolescents. In our family members, no adrenal or gastric antibodies could be detected. Furthermore, the prevalence of autoimmune disorders was similar among parents and HLA-identical, −haploidentical, and −nonidentical sibs with both HLA-DR3 and DR4.

THE RELATIONSHIP BETWEEN HLA TYPES, ICA, AND GLUCOSE HOMEOSTASIS

Sibs. As shown in Figure 1A glucose levels during the glucose tolerance tests of nondiabetic HLA-identical siblings, although not abnormal, were significantly increased ($P < 0.05$) at 1 and 3 h compared with values in HLA-haploidentical and nonidentical individuals as well as control children.[25] In addition, cumulative plasma glucose levels during the GTTs were also significantly increased ($P < 0.05$) in the HLA-identical sibs (613 ± 16 vs. 506 ± 12 in haploidentical, 480 ± 11 in nonidentical, and 485 ± 10 mg/dl in control children). There were no detectable differences between the 18 HLA nonidentical sibs and the 48 controls evaluated in terms of plasma glucose or serum insulin levels. They were therefore shown together in Figure 1 although statistics were applied to each group individually. The increases in serum levels of insulin after the administration of glucose were also significantly less ½ and 1 h after oral glucose ingestion ($P < 0.05$) than that observed in control and HLA nonidentical subjects in all but two of the HLA-identical sibs. The cumulative serum insulin response to the oral glucose challenge was, furthermore, significantly less in HLA-identical sibs than in control, HLA-haplo-, and HLA-nonidentical subjects (123 ± 11 vs. 162 ± 9, 169 ± 12, and 165 ± 10 μU/ml, respectively, $P < 0.05$).[25] However, two HLA-identical, ICA-negative sibs, ages 4 and 6, 136% and 132% of ideal body weights, respectively, had increased total serum insulin responses of 192 and 186 μU/ml. This was greater than two standard errors above the responses of normal controls. On the other hand, no differences in the glucose tolerance tests or serum insulin responses to glucose were observed, when ICA-positive and negative siblings were compared (data not shown).

Parents. As shown in Figure 1B ICA-positive parents had slightly increased but not abnormal plasma glucose concentrations at 0, ½, 1, and 2 h and decreased concentrations at 3 h. However, the only significant increase occurred 1 h postglucose ingestion ($P < 0.05$). In addition, cumulative plasma glucose levels were significantly higher (685 ± 14 vs. 619 ± 16 mg/dl, $P < 0.05$) in the ICA-positive parents. Insulin levels of ICA-positive parents were elevated at all test points, but the increases were significant ($P < 0.05$) only at 1 and 3 h after glucose ingestion. Cumu-

FIGURE 1. (A) Plasma glucose and serum insulin responses during oral glucose tolerance tests in sibs of insulin-dependent diabetics separated according to HLA relationships to proband. All values indicate the mean ± standard error of the mean. * Indicates differences between HLA-identical and nonidentical sibs significant at $P < 0.05$. N, number of subjects tested. (B) Plasma glucose and serum insulin responses during oral glucose tolerance tests in parents of insulin-dependent diabetics separated according to presence or absence of cytoplasmic islet cell antibodies (ICA). All values indicate the mean ± standard error of the mean. * Indicates differences between the two groups significant at $P < 0.05$. N, number of subjects tested.



lative serum insulin responses of these parents were also significantly increased (269 ± 11 vs. 196 ± 11 μU/ml in ICA-negative parents, $P < 0.05$). Postglucose increases in cumulative serum insulin responses remained significant (238 ± 10 vs. 174 ± 10 μU/ml, $P < 0.05$) even when corrected for the fasting hyperinsulinemia (31 ± 5 μU/ml; normal in our laboratory for adults <20 μU/ml) in the slightly overweight ICA-positive parents. The observed glucose intolerance was not associated with specific HLA-DR types.

The possibility that HLA-identical ICA-positive sibs of diabetic probands are at higher risk for developing diabetes suggested by retrospective studies[26,27] was borne out, in a prospective manner, by two of our cases. Both children were HLA-identical to the respective probands and had demonstrable ICA for at least 3 and 18 mo before developing glucosuria. The first, a 10-yr-old boy who possessed HLA DR3/DR4, had a fasting plasma glucose level of 115 mg/dl and a peak glucose level 3 h after glucose ingestion of 283 mg/dl, 1 day after glucosuria was first noted. His cumulative serum insulin levels during this GTT were 105 μU/ml, greater than 6 SE below those of our control subjects and more than 1 SE below the values of our other HLA-identical sibs. The second, a 3-year-old girl, who was homozygous for HLA DR4, had even more pronounced hypoinsulinemia, a total of 34 μU/ml during her 3-h GTT performed after glucosuria was first detected. Both these children are now receiving daily injections of insulin to control hyperglycemia.

DISCUSSION

These studies demonstrate the presence of cytoplasmic pancreatic islet cell antibodies in 65% of IDDM patients and in 32% of their normal sibs that share at least one HLA haplotype. In contrast, ICA were found in only 16% of HLA nonidentical sibs and in 14% of parents. No significant associations were found between ICA and specific HLA antigens, in contrast with previous reports linking persistent ICA with HLA-B8 and −DR3 in IDDM patients.[7,8,10] Other authors, however, have reported a similar lack of association in their series of IDDM ICA-positive patients.[11,28,29]

The prevalence of ICA in the nondiabetic, first degree relatives in our series is, however, considerably higher than previously reported by other investigators. Thus, Bottazzo et al.[29] reported ICA in 4% of family members with a striking predilection for those with "autoimmunity," Irvine et al.[8] reported ICA in 3%, and Barbosa et al.[30] reported ICA in 10%. On the other hand, the prevalences of ICA in our recently diagnosed and longer-term diabetic patients and in our nondiabetic control population are similar to those reported by other investigators.[1,7,8] The differences in prevalence among first degree relatives may be due to several possible factors. Most importantly, the ICA-positive family members in this series have an unusually high prevalence of other autoimmune diseases. Thus, almost half of the ICA-positive siblings and 84% of the ICA-positive parents have evidence of other organ-specific autoantibodies (primarily antithyroid), although its precise relationship with ICA remains to be elucidated. Other groups, however, have also found increased "autoimmunity" in families of insulin-dependent diabetics. Thus, Nissley et al. found that 17/99 parents and sibs had positive thyroid, adrenal, or gastric antibody titers,[31] Bottazzo et al. reported that 52/79 such families had at least one nondiabetic relative with organ-specific antibodies,[32] and Fialkow et al. found a 23% prevalence of thyroid autoimmunity in first degree relatives.[33] The high ICA prevalence rates found in our families are similar to data for the prevalence of surface cytotoxic islet cell antibodies (ICSA), which has previously been documented and published.[19] In that study, which included a small subset of the sample reported here, 3/5 HLA-identical, 5/14 HLA-haploidentical, 1/5 nonidentical sibs, 3/14 mothers, and 1/12 fathers had ICSA giving a total prevalence of 13/50 or 26% for ICSA. Second, the methods for detection of ICA differ. We used Bouin's-fixed tissue, while other laboratories use frozen pancreatic sections.[34] Our method may identify ICA at lower titers than do other methods. In addition, studies in progress in our laboratories reveal differences in ICA titers related to both time of storage of pancreatic sections and source of tissue. Finally, the recruitment of patients may be different. All our probands were recruited consecutively from a large Pediatric Diabetes Clinic, according to the criterion that the patients had developed IDDM before age 15, whereas such criteria may not have been followed by other groups. Since the time of appearance and duration of ICA in nondiabetic first degree relatives and probands are still unknown, the reported differences in prevalence might also be related to the younger ages of our subjects.

The presence of ICA does not appear to be, per se, a primary factor in determining abnormalities in glucose tolerance and/or insulin secretion in any of the three groups of sibs stratified according to the HLA relationship to the IDDM proband, since no significant differences were encountered. As previously mentioned, however, HLA-identity with the proband did associate with significant differences in glucose tolerance. Recently, however, Orchard and colleagues[35] as well as Barbosa et al.[30] reported increased but delayed peak-insulin values in nondiabetic HLA-identical sibs. Age and weight may be important factors since the two sibs in our series who had similarly increased insulin responses were both less than 6 yr of age and mildly obese, while older HLA-identical sibs had delayed but decreased peak-insulin values.

The presence of ICA in HLA-identical sibs might portend decompensation to IDDM in the near future since the two siblings who developed IDDM during the course of this study were HLA-identical to their respective probands and ICA-positive when initial blood tests were performed 3 and 18 mo before diagnosis. The prevalence of ICA in HLA-identical sibs may well be somewhat higher than the 25% (7/28) found, since 19 of the 28 HLA-identical siblings happened to be male (4/9 of HLA-identical female sibs and only 3/19 of such male sibs had ICA). This increased prevalence of ICA in female sibs is in line with the well-known female preponderance in autoimmune diseases. It is perhaps of interest that the frequency of ICA in the HLA-identical sibs in this series is of the same general magnitude as the highest values previously reported for concordance for IDDM in identical twins[36,37] and in HLA-identical siblings.[26]

Uncertainty as to the predictive value of positive ICA is highlighted by the haploidentical sibs, as only a few of these children are at risk for the development of IDDM; a recent estimate of about 5% has been made.[38] It is thus likely that certain ICA-positive individuals could develop diabetes later in life while others, perhaps most, might lose their ICA. For example, among the ICA-positive parents, the formerly

gestational diabetic mothers have already been shown to be at risk for the development of diabetes mellitus requiring insulin therapy.[39,40] In addition, the glucose tolerance tests of these ICA-positive parents demonstrate increased blood glucose and serum insulin levels, a characteristic noted in many adult chemical diabetics,[41] supporting other lines of evidence that positive ICA in adults can precede the development of diabetes.[13] Since Irvine et al. have reported relative hypoinsulinemia, in ICA-positive as compared with ICA-negative non-insulin-dependent diabetic adults,[42] follow-up studies in these parents will be of great importance in elucidating changing patterns of insulin secretion. The mild degree of obesity (118 ± 7% ideal body wt) of our ICA-positive parents may also contribute to their increased serum insulin levels.[41]

In HLA-nonidentical siblings, a smaller percentage (16%) also have ICA. In this instance too, a small subset may be expected to develop IDDM (recent estimate <1%).[38] This may result from the high frequency in the general population of the "IDDM" gene, coupled with its low penetrance, as already described.[27,43]

Thus, while HLA-identity to an IDDM sib may provide a good predictive marker for identifying those individuals who are at greater risk of developing this type of diabetes, the exact role of ICA in this connection remains unclear. It has recently been shown, however, that it is antibody to surface antigens on islet cells (ICSA) and not antibody to cytoplasmic antigens (i.e., ICA) that is cytotoxic for beta-cells in the presence of complement.[19] Thus, ICSA may turn out to be a more important marker for identifying individuals at risk than ICA. Our ongoing prospective studies in families in which cytotoxic ICSA as well as ICA are being detected will help in elucidating mechanisms in the development of IDDM in at-risk sibs and perhaps other relatives of probands.

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REFERENCES

[1] Nerup, J., Platz, P., Ryder, L. P., Thomsen, M., and Svejgaard, A.: HLA, islet cell antibodies and types of diabetes mellitus. Diabetes 27 (Suppl. 1):247–50, 1978.

[2] Cudworth, A. G., and Woodrow, J. C.: Genetic susceptibility in diabetes mellitus. Br. Med. J. 2:846–48, 1976.

[3] Cudworth, A. G., White, G. B. B., Woodrow, J. C., Gamble, D. R., Lendrum, R., and Bloom, A.: Etiology of juvenile onset diabetes: a prospective study. Lancet 1:385–88, 1977.

[4] Cudworth, A. G., and Woodrow, J. C.: HLA system and diabetes mellitus. Diabetes 24:345–49, 1975.

[5] Barbosa, J., Chern, M., Reinsmoen, N., Noreen, H., Ramsay, R., and Greenberg, L.: HLA-Dw antigens in unrelated juvenile, insulin-dependent diabetes. Tissue Antigens 14:426–36, 1979.

[6] Christy, M., Green, A., Christau, B., Kromann, H., Nerup, J., Platz, P., Thomsen, M., Ryder, L., and Svejgaard, A.: Studies of the HLA-system and insulin-dependent diabetes mellitus. Diabetes Care 2:209–14, 1979.

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

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

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

[10] Del Prete, G. F., Betterle, C., Padovan, D., Erle, G., Joffolo, A., and Bersani, G. Incidence and significance of islet cell autoantibodies in different types of diabetes mellitus. Diabetes 26:909–15, 1977.

[11] Richens, E. R., Quilley, J., and Hartog, M.: Immunological features of juvenile onset diabetic patients correlated to HLA type. Clin. Exp. Immunol. 36:198–204, 1979.

[12] Bottazzo, G. F., Florin-Christensen, A., and Doniach, D.: Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 2:1279–83, 1974.

[13] Irvine, W. J., Gray, R. S., McCallum, C. J., and Duncan, L. J. P.: Clinical and pathogenic significance of pancreatic islet cell antibodies in diabetics treated with oral hypoglycaemic agents. Lancet 1:1025–27, 1977.

[14] Burkeholder, J. N., Pickens, J. M., and Womack, W. S.: Oral glucose tolerance tests in siblings of children with diabetes mellitus. Diabetes 16: 156–60, 1967.

[15] Suciu-Foca, N., and Rubinstein, P.: Immunogenetics of HLA D:HTC and B-cell typing. *In* Histocompatability Testing: 1977. Bodner, W. F., Ed. Copenhagen, Munksgaard, 1978, pp. 545–47.

[16] Martel, J. L., Jaramillo, S., Allen, F. H., Jr., and Rubinstein, P.: Serology for automated cytotoxicity assays: contrast fluorescence test. Vox. Sang. 27:13–20, 1974.

[17] Van Rood, J. J., van Leeuwen, A., and Ploem, J. S.: Simultaneous detection of two cell populations by two-colour fluorescence and application to the recognition of B-cell determinants. Nature 262:795–97, 1976.

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

[19] Dobersen, M. J., Scharff, J. E., Ginsberg-Fellner, F., and Notkins, A. L.: Cytotoxic autoantibodies to beta cells in the serum of patients with insulin-dependent diabetes mellitus. N. Engl. J. Med. 303:1493–98, 1980.

[20] Bird, T., and Stephenson, J.: Evaluation of a tanned red cell technique for thyroid microsomal antibodies. J. Clin. Pathol. 26:623–29, 1973.

[21] Fulthorpe, A. J., Roitt, I. M., Doniach, D., and Coachman, K. G.: A stable sheep cell preparation for detecting thyroglobulin autoantibodies and its clinical applications. J. Clin. Pathol. 14:654–60, 1961.

[22] Blizzard, R. M., Chee, D., and Davis, W.: The incidence of adrenal and other antibodies in the sera of patients with idiopathic adrenal insufficiency (Addison's disease). Clin. Exp. Immunol. 2:19–30, 1967.

[23] Huggetts, A., and Nixon, D. A.: Use of glucose oxidase, peroxidase and o-dianisidine in determination of blood and urinary glucose. Lancet 2:368–70, 1957.

[24] Juan, C., and AvRuskin, T. W.: A combined immunoassay of human growth hormone and insulin: cumulative assessment of assay performance. J. Clin. Endocrinol. Metab. 33:150–57, 1971.

[25] Rosenbloom, A. L., Wheeler, L., Bianchi, R., Chin, F. T., Tiwary, C. M., and Grgic, A.: Age-adjusted analysis of insulin responses during normal and abnormal glucose tolerance tests in children and adolescents. Diabetes 24:820–28, 1975.

[26] Rubinstein, P., Suciu-Foca, N., and Nicholson, J. F.: Genetics of juvenile diabetes mellitus: a recessive gene closely linked to HLA-D and with 50 percent penetrance. N. Engl. J. Med. 297:1036–40, 1977.

[27] Rubinstein, P., Ginsberg-Fellner, F., and Falk, C.: Genetics of type I diabetes mellitus: a single recessive predisposition gene mapping between *HLA-B* and *GLO*. Am. J. Hum. Genet. 33:865–82, 1981.

[28] Lendrum, R., Walker, G., Cudworth, A. G., Woodrow, J. C., and Gamble, D. R.: HLA-linked genes and islet-cell antibodies in diabetes mellitus. Br. Med. J. 1:1565–67, 1976.

[29] Bottazzo, G. F., Gorsuch, A. N., Dean, B. M., Cudworth, A. G., and Doniach, D.: Complement-fixing islet-cell antibodies in Type-I diabetes: possible monitors of active beta-cell damage. Lancet 1:668–72, 1980.

[30] Barbosa, J., Chavers, B., Steffes, M., Szalapski, E., Cohen, R. A., Michael, A. F., Hoogwerf, B., and Mauer, M.: Muscle extracellular membrane immunofluorescence and HLA as possible markers of prediabetes. Lancet 2:330–33, 1980.

[31] Nissley, S. P., Drash, A. L., Blizzard, R. M., Sperling, M., and Childs, B.: Comparison of juvenile diabetics with positive and negative organ specific antibody titers: evidence for genetic heterogeneity. Diabetes 22:63–65, 1973.

[32] Bottazzo, G. F., Mann, J. I., Thorogood, M., Baum, J. D., and Doniach, D.: Autoimmunity in juvenile diabetics and their families. Br. Med. J. 2:165–68, 1978.

[33] Fialkow, P. J., Zavala, C., and Nielsen, R.: Thyroid autoimmunity: increased frequency in relatives of insulin-dependent diabetes patients. Ann. Intern. Med. 83:170–76, 1975.

[34] Lendrum, R., Walker, G., and Gamble, D. R.: Islet cell antibodies in juvenile diabetes mellitus of recent onset. Lancet 1:880–83, 1975.

[35] Orchard, T. J., Rabin, B. S., Wagener, D. K., Sales, M., Banks, M., and Drash, A. L.: Metabolic features associated with specific HLA types (B8, B15, B18) and sharing of haplotypes with a diabetic sibling. Diabetes 29 (Suppl. 2):83A, 1980. Abstract.

[36] Tattersall, R. B., and Pyke, D. A.: Diabetes in identical twins. Lancet 2:1120–22, 1972.

[37] Gottlieb, M. S., and Root, H. F.: Diabetes mellitus in twins. Diabetes *17*:693–99, 1972.

[38] Svejgaard, A., Platz, R., and Ryder, L. P.: Insulin-dependent diabetes mellitus (Joint Report). *In* Histocompatability Testing 1980. Terasaki, P. J., Ed. Los Angeles, Calif., UCLA Press, 1980, pp. 638–56.

[39] Ginsberg-Fellner, F., Dobersen, M., Mark, E. M., Nechemias, C., Hausknecht, R. U., Rubinstein, P., and Notkins, A. L.: Islet cell antibodies in gestational diabetes mellitus. Lancet *2*:362–63, 1980.

[40] Steel, J. M., Irvine, W. J., Feek, C. M., and Clarke, B. F.: The significance of pancreatic islet cell antibody and abnormal glucose tolerance during pregnancy. J. Clin. Lab. Immunol. *4*:83–85, 1980.

[41] Perley, M., and Kipnis, D. M.: Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects. Diabetes *15*:867–74, 1966.

[42] Gray, R. S., Irvine, W. J., Cameron, E. H. D., and Duncan, L. J. P.: Glucose and insulin responses to oral glucose in overt non-insulin-dependent diabetics with and without the islet cell antibody. Diabetes *29*:312–16, 1980.

[43] Rubinstein, P., Ginsberg-Fellner, F., and Falk, C. T.: Type I diabetes mellitus: a recessive disease? *In* Immunology of Diabetes. Irvine, W. James, Ed. Edinburgh, Teviot Scientific Publications, 1980, pp. 109–16.