Progression to Type I Diabetes in Autoimmune Endocrine Patients With Islet Cell Antibodies

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In an 11-yr screening program carried out on serum samples sent to an autoimmune serology laboratory, 158 patients with clinical or subclinical autoimmune endocrine manifestations and islet cell antibodies (ICAs) in the absence of overt diabetes were identified and followed for the development of insulin-dependent (type I) diabetes. Twenty-two (13.9%) developed type I diabetes in a follow-up of up to 12 yr (mean ± SE 4.8 ± 3.2 yr). The probability of being free of type I diabetes was 69.8% at 10 yr after the first detection of ICAs. Progression to disease was influenced by *1*) the amount of ICAs represented by high titers (63% of those with ICAs ≥20 Juvenile Diabetes Foundation units being free of type I diabetes at 10 yr), ICA persistency (59% being free of type I diabetes; $P <$ 0.02 vs. nonpersistent ICA), and complement-fixing (CF)-ICAs (63% being free of type I diabetes; $P <$ 0.05 vs. non–CF-ICA); *2*) the coexistence of insulin autoantibodies (IAAs) (25% being free of type I diabetes; $P < 0.005$ vs. IAA^-); and *3*) a positive family history (1st-degree relative) for type I diabetes (32% being free of type I diabetes; $P < 0.005$ vs. no family history). There was a trend for diabetes to develop earlier in males of a younger age. No relationships were found with the number, type, or clinical expression of the associated autoimmunities or with a family history of such disorders. These data confirm the predictive ability of ICAs and IAAs for type I diabetes and indicate that the events that lead to disease in individuals with ICAs are influenced by the level of the humoral response and by a familial association of type I diabetes. This study identified a large cohort of individuals with high ICA levels who have not progressed to type I diabetes. A study of these individuals will be valuable in identifying factors important for the ultimate progression to disease.
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Insulin-dependent (type I) diabetes mellitus is now accepted as an immunologically mediated destructive disorder that has a similar pathogenesis to other autoimmune organ-specific diseases (1,2). Islet cell antibodies (ICAs), the best recognized serological marker for pancreatic β-cell autoimmunity, are present in most type I diabetic patients at diagnosis, but whether they directly mediate β-cell destruction remains unclear (3,4).

ICAs have been detected months to several years before the clinical onset of insulin deficiency, demonstrating the existence of a long latency before diabetes becomes overtly manifest (5). ICAs have hence become predictive of type I diabetes, not only in populations at higher risk, e.g., unaffected first-degree relatives in diabetic families (6,7), discordant monozygotic twins (8) or triplets (9), and nondiabetic patients with organ-specific autoimmune manifestations (10), but also in sporadic cases identified within randomly selected school children (11). Insulin autoantibodies (IAAs) are the other immune serological marker of type I diabetes (12). Their detection may enhance prediction when present in association with ICAs (13–15), although more effort to standardize tests is required (16).

Type I diabetes is not uncommonly associated with other endocrine or organ-specific autoimmune disorders (17), and, indeed, ICAs were first identified in polyendocrine patients (18). Clinical and serological differences have been reported in polyendocrine diabetic patients when compared with those without accompanying autoimmunities, and possible heterogeneity within the type I diabetes syndrome has been suggested (19).

In this study, we describe 158 autoimmune polyendocrine patients who had ICAs without overt diabetes, and we report the value of ICAs and other immunological markers and clin-

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ical variables as possible predictors for progression to type I diabetes in these patients.

RESEARCH DESIGN AND METHODS

All serum samples (>90,000) sent to the autoimmune serology laboratory at Middlesex Hospital, London, between 1 January 1976 and 1 January 1987 were tested for ICAs independent of the original autoantibody request. Individuals in whom serum ICAs were detected, except those in whom ICA testing was requested because of diabetes or any disturbance of carbohydrate metabolism including impaired glucose tolerance, gestational diabetes, non-insulin-dependent diabetes, glycosuria, or transient hyperglycemia, were considered for recruitment in the study.

The collection of retrospective data and the prospective study began in mid-1984. Criteria for patient selection were *1*) ICAs detected on at least one occasion in the absence of diabetes and *2*) the documented presence of another organ-specific autoantibody and/or clinical expression of an organ-specific autoimmune disease. A total of 214 patients fulfilled the criteria. We were able to trace, contact, and enroll 158 of these individuals into the study (referred to as the polyendocrine study). They were all living in Southern England, mostly in London or surrounding areas. A detailed personal and family medical history and complete clinical information were obtained from questionnaires completed by both the patients and their doctors (general practitioners or consultants). One hundred and two (64.4%) patients were subsequently visited and interviewed by one of us (E.B., F.B., or R.W.). Clinical classification of diseases was based on the diagnosis given by the referring physician.

In each individual, the follow-up was calculated from the time when ICAs were first detected to the date of development of type I diabetes (start of insulin therapy) or of subsequent death, or, for the individuals still alive and nondiabetic, of the last contact or visit (up to October 1989). The mean ± SE follow-up was 4.8 ± 3.2 yr (range 0.5–12.8 yr). In 15 patients, ICAs were assayed in only one serum sample, whereas in all others, the test was performed on multiple occasions (mean ± SE 3.3 ± 2.7, range 2–17). In all patients, ICAs were detected in the first sample tested. Persistent ICAs were defined as ICAs detectable in all samples when two or more samples were assayed.

For the detection of ICAs and complement-fixing (CF)-ICAs, indirect immunofluorescence (IFL) on 4-μm cryostat sections of blood group O human pancreas was used with 25 μl of undiluted serum (20). ICAs and CF-ICAs were initially scored by intensity as negative, weakly positive, positive, or strongly positive. After the recommendations of the 2nd International Workshop on the Standardization of Cytoplasmic ICAs, quantification in Juvenile Diabetes Foundation (JDF) units was performed in early samples from 85 patients. Initial samples from the remaining 73 patients were unavailable for reassay. Sera were titrated to end point in parallel with 2–, 4–, 8–, 16–, 32–, and 80–JDF U standards. JDF units for the samples were calculated from the polynomial regression analysis for $\log_2$ end-point titer versus $\log_2$ JDF units of the standard, as recommended in the ICA Standardization Workshop report (21). The detection limit of the assay was 4 JDF U. Quantification of ICAs in JDF units was performed in the earliest sample available. This subgroup, in which quantification was performed, was representative of the whole group with comparable age, sex distribution, prevalence of associated autoimmune manifestations, duration of follow-up, and proportion of those who subsequently developed diabetes.

Insulin autoantibodies (IAAs) were measured by a radioimmunoassay with displacement with unlabeled insulin to correct for nonspecific binding (22). The corrected binding in 140 ICA^- control sera was 0.149 ± 0.298% with a normal distribution. The cutoff for positive sera was considered above the corrected value of 1.043% (mean + 3SD). IAAs were assayed in 123 individuals in the earliest sample available. In 69 of these, IAAs were also assayed in follow-up samples.

Gastric parietal cell and adrenal antibodies were tested in all samples by IFL on 4-μm cryostat sections of fresh frozen human blood group O organ tissues, and steroid-producing cell antibodies on cryostat sections of unfixed rat ovary and testis (23). Thyroglobulin (Tg) and thyroid microsomal (Mc) antibodies were measured by hemagglutination (HA) with commercial kits (Wellcome, UK). Titers of >1/10 for TgHA and >1/100 for McHA were considered positive. For all autoantibodies, sera were tested fresh or after storage at −20°C.

Data are reported as means ± SD or percent frequency. Differences between subgroups were tested with the χ^2-test with Yates' correction. The progression to type I diabetes was analyzed in all polyendocrine patients and in subgroups according to the different variables of risk with the life-table analysis of Cutler and Ederer (24). The results were expressed as the probability of remaining free of type I diabetes after the first ICA detection. Standard errors and 95% confidence intervals (CI) were calculated. Significance was calculated at 10 yr with the log-rank test. The Cox regression model was used to analyze the risk rank order of variables considered. Full information on the risk factors considered for developing type I diabetes was available as follows: CF-ICAs were determined in 156 patients; follow-up ICAs were tested in 137; quantification of ICAs (JDF U) were tested in 85; IAAs were determined in 123; and family history of type I diabetes was obtained in 102. Family histories for other organ-specific (thyroid, gastric, adrenal, gonadal, skin) autoimmune disorders were obtained in 100 patients.

RESULTS

Clinical and immunoserological characteristics of polyendocrine patients. Patients at the time when ICAs were first detected showed a large female preponderance (74.7%) and a wide age range (2.1–85.1 yr) with a predominance in mid-life (median 45.5 yr; Table 1). These sex and age distributions differed from those of the recruitment source (autoimmune serology laboratory samples), which showed a less pronounced female excess (64%) and a biphasic age distribution with peaks in the first and fourth decades of life.

Overall, 78.5% of patients had at least one clinical manifestation of the associated autoimmune disease, and the remainder had serological evidence alone. In addition to ICAs, 25.3% of individuals had one other autoimmune manifestation, 44.3% had two, 20.2% had three, and 10.1% had four or more. Thyroid and gastric were the most common

TABLE 1
Characteristics of polyendocrine patients and patients developing insulin-dependent (type I) diabetes

	Total (n = 158)		Patients developing type I diabetes (n = 22)		Predictive value for type I diabetes (%)
	n	%	n	%	
Sex (M/F)	40/118	25.3/74.7	7/15	31.8/68.2	17.5/2.7
Age (yr)					
0–9	2	1.3	0	0.0	0.0
10–19	11	7.0	3	13.6	27.3
20–29	18	11.4	2	9.1	11.1
30–39	31	19.6	7	31.8	22.6
40–49	32	20.2	3	13.6	9.4
50–59	25	15.8	3	13.6	12.0
60–69	23	14.6	3	13.6	13.0
70–79	11	7.0	1	4.5	9.1
80–89	5	3.1	0	0.0	0.0
First-degree relative with type I diabetes	15 of 102	14.7	6 of 19	31.6	40.0
First-degree relative with other autoimmune disorders	39 of 100	39.0	6 of 18	33.3	15.4
Clinical expression of associated autoimmunity	124	78.5	19	86.4	15.3
Associated autoimmunities					
Thyroid	119	75.3	19	86.4	16.0
Gastric	113	71.5	18	81.8	15.9
Thyrogastric	85	53.8	15	68.2	17.6
Adrenal	26	16.5	4	18.2	15.4
Gonadal	14	8.9	4	18.2	28.6
Skin (vitiligo)	19	12.0	2	9.1	10.5

Total mean ± SE age was 45.6 ± 18.5 yr; mean ± SE age was 41.8 ± 17.5 yr for patients developing type I diabetes. $P < 0.05$.

associated autoimmunities (75.3 and 71.5%, respectively), followed by adrenal (16.5%), skin (12%), and gonadal (8.9%) (Table 1). Of the 158 patients, 14.7% had a first-degree relative with type I diabetes and 39% had a first-degree relative affected by some other clinical autoimmune disease (Table 1).

The quantification of ICAs showed a substantial proportion (34.1%) of patients with high ICA levels (>80 JDF U; Table 2). In 27 (17.1%) patients, ICAs became undetectable in follow-up samples, and in 21 patients, a recent sample was unavailable (Fig. 1). ICA persistency was associated with higher ICA levels. All those in whom ICAs became undetectable in follow-up samples had ICAs <20 JDF U in their initial sample.

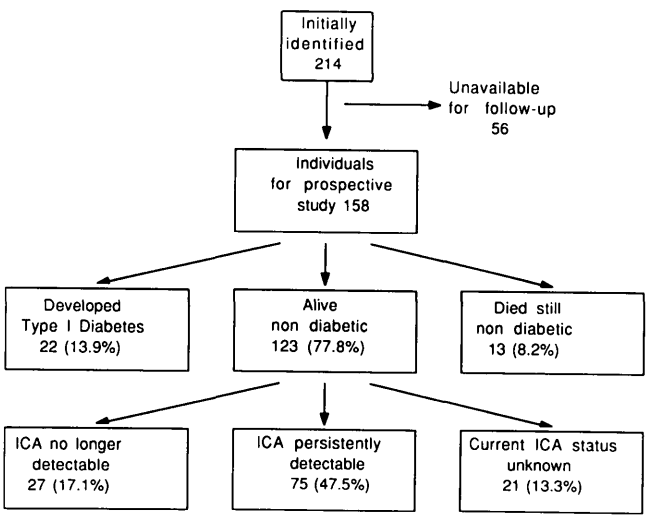

FIG. 1. Follow-up of 158 polyendocrine patients with islet cell antibodies (ICA) included in prospective study.

IAAs were detected in 9 (7.3%) of 123 patients (Table 2). In the 7 of these for whom multiple samples were available, IAAs were not persistent, being detected on no more than one occasion.

Progression to type I diabetes. Over the whole follow-up, 22 (13.9%) patients developed type I diabetes, 13 (8.2%) died still nondiabetic, and 123 (77.8%) were alive and nondiabetic (Fig. 1). Most of those who developed type I diabetes were female and >21 yr of age, being representative of the total cohort. There was no association with any particular organ-specific autoimmunity or the number of associated autoimmunities (Table 1).

The probability of being free of type I diabetes in the whole group of polyendocrine patients initially nondiabetic and with detectable ICAs was 96.5, 90, 87.7, 81.4, and 69.8% (95% CI 54.3–84.3) at 2, 4, 6, 8, and 10 yr, respectively, after the first detection of ICAs.

The analysis of the separate variables with the probability curves shown in Figs. 2 and 3 demonstrated that family history of type I diabetes, ICA titer, and IAA influenced the development of type I diabetes in this cohort. Polyendocrine patients with persistently positive ICAs had a probability of being free of type I diabetes at 10 yr of 59% ($P < 0.05$ vs. nonpersistent ICAs); the probability was 63% for those with CF-ICAs ($P < 0.05$ vs. non-CF-ICAs) and those with ICAs >20 JDF U (Table 3). In most of the patients with ICAs >20 JDF U, the ICAs were persistent and CF. Only 9 patients had

TABLE 2
Islet cell antibody (ICA) and insulin autoantibody (IAA) status of polyendocrine patients

	Total (n = 158)		Patients developing type I diabetes (n = 22)		Predictive value for type I diabetes (%)
	n	%	n	%	
Persistent ICA	110 of 137	80.3	22 of 22*	100.0	20.0
CF-ICA	132 of 156	84.6	22 of 22*	100.0	16.7
ICA	35 of 85	41.2	1 of 12	8.3	2.9
<20 JDF U					
20–79 JDF U	21 of 85	24.7	6 of 12	50.0	28.6
≥80 JDF U	29 of 85	34.1	5 of 12	41.7	17.2
IAA	9 of 123	7.3	4 of 16†	25.0	44.4

Type I, insulin-dependent diabetes mellitus; CF, complement-fixing; JDF, Juvenile Diabetes Foundation.
*$P < 0.05$.
†$P < 0.005$.

detectable IAAs, but the probability of being free of type I diabetes after 10 yr in these patients was 25% ($P < 0.005$ vs. IAA$^-$). The risk associated with a family history of type I diabetes was independent of ICA titer, and the presence of a first-degree relative with type I diabetes reduced the probability of being free of type I diabetes at 10 yr to 32% ($P <$ 0.01 vs. those with no affected 1st-degree relatives). Age, sex, clinical expression of the associated autoimmune disorders, and a first-degree family history of autoimmune diseases other than type I diabetes did not significantly influence progression to disease in the cohort (Table 3; Fig. 2).

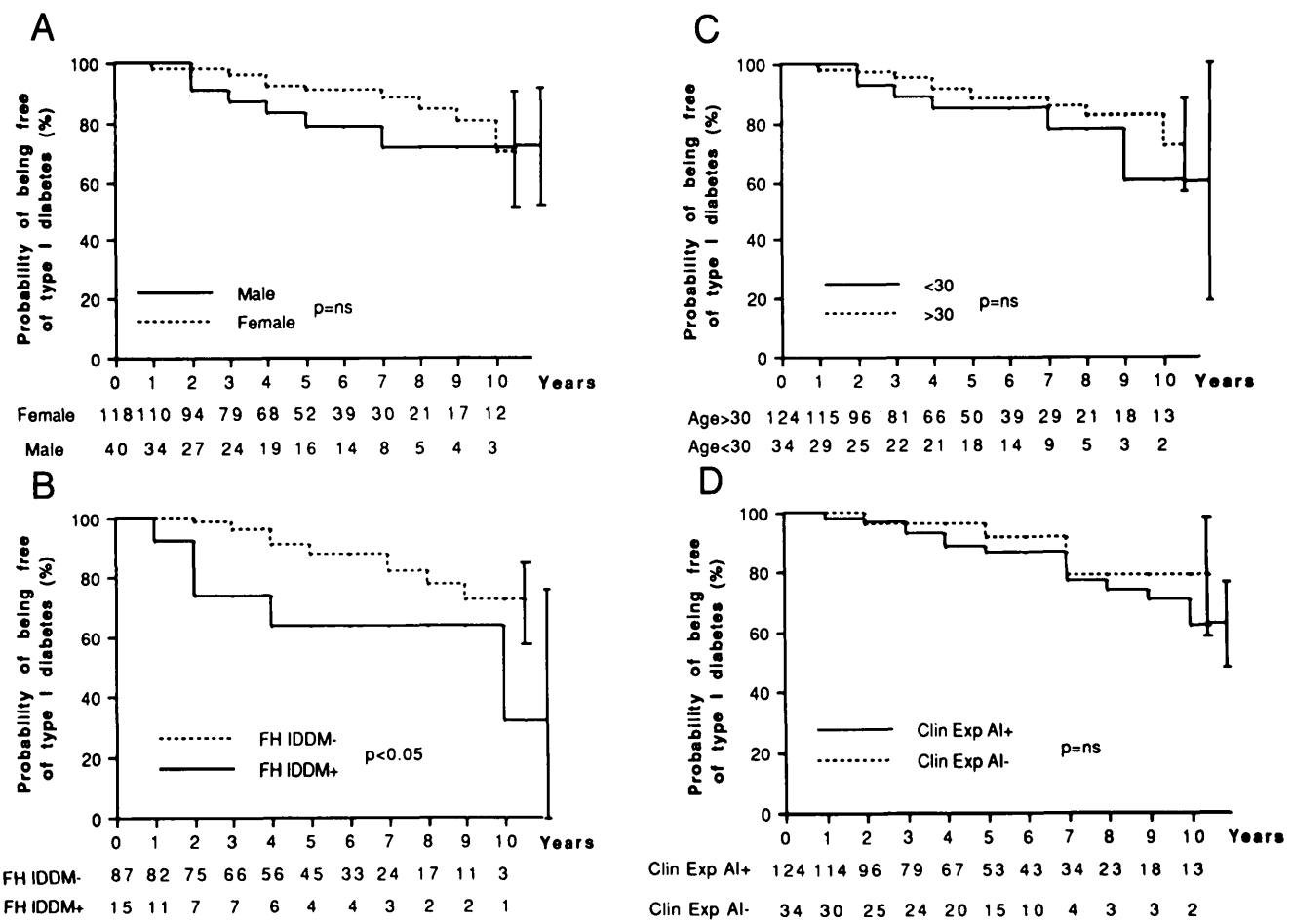

FIG. 2. Probability of being free of insulin-dependent (type I) diabetes in relation to sex (*A*), family history of type I diabetes (*B*), age (C), and clinical expression of other autoimmune diseases (*D*). Greater proportion of patients with first-degree family history (FH) of type I diabetes developed disease than those without ($P < 0.05$; log-rank test), but no significant differences were shown between male and female patients, young and old patients, or those with and without clinical expression of associated autoimmunities (AI). IDDM, insulin-dependent diabetes mellitus.

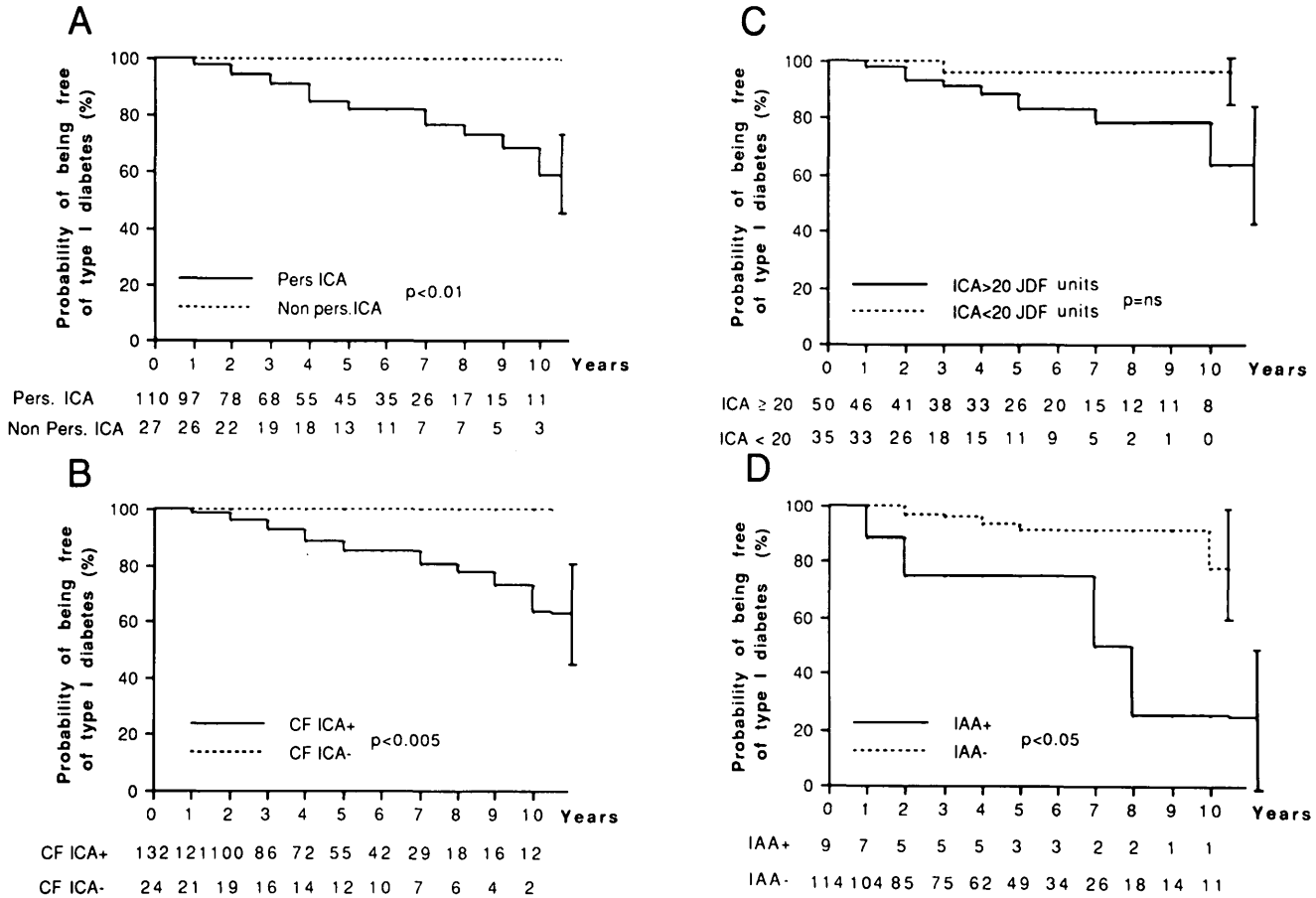

FIG. 3. Probability of being free of type I (insulin-dependent) diabetes in relation to islet cell antibody (ICA) and insulin autoantibody (IAA) status. Progression to type I diabetes was most frequent in patients with persistent (*A*), complement-fixing (CF) (*B*), or high-titer ICA (*C*) and in patients with additional detectable IAAs (*D*).

DISCUSSION

The ultimate aim of research into type I diabetes is to prevent the disease by devising safe forms of immunological intervention that can permanently halt β-cell damage. Before this can be achieved, we need not only reliable and reproducible tests for the identification of those at risk but also the enrollment of large cohorts of these individuals in properly designed prospective studies (25).

ICAs detected by IFL on cryostat sections of human pancreas remain the most reliable immunological predictive markers of type I diabetes. Standardization of the ICA-IFL assay with standard sera and standard curves has allowed quantification of ICA and expression of results in standard JDF units (21), thus providing a useful predictive test in unaffected first-degree relatives of type I diabetic patients (7,26). Screening of large populations is essential so that the final yield of individuals with ICAs, but without overt diabetes, will be of sufficient size to permit meaningful intervention trials. The regular screening of >90,000 samples has provided us with one of the largest cohorts of nondiabetic individuals with ICAs identified.

As in family studies (7,26), the risk for diabetes was increased in polyendocrine patients with high ICA levels or coexisting IAAs (13,14). The probability of being free of type I diabetes after 10 yr in polyendocrine patients with CF-ICAs was 63%, persistent ICAs 59%, and ICAs ≥20 JDF U 63%, compared with 100% for those in whom ICAs were non-CF or nonpersistent, and 96% for those with ICAs <20 JDF U. The prevalence of IAAs in the cohort tested was low (7.3%) and even less than in other groups of high-risk individuals (23–25). This could partly depend on differences in methodology and on the relatively small number of young patients in our study, because an inverse relationship of IAAs with age has been reported (27). Even so, IAAs were highly predictive in this small subgroup. The risk associated with IAAs may reflect increased ICA levels (14).

The clinical characteristics of our patients were varied but consistent with the classical features of organ-specific autoimmunity. As expected, most were female and middle-aged when ICAs were first detected. Although a direct comparison between this and other prospective studies cannot be done (mainly due to differences in ascertainment criteria, age, and sex distribution), the proportion of polyendocrine patients with ICAs who developed type I diabetes was substantially less than that observed in first-degree relatives in the Barts-Windsor Family Study (BWFS) that was conducted in parallel by our group (26). In the BWFS, all relatives with ICAs ≥80 JDF U progressed to type I diabetes within 8 yr, whereas in the polyendocrine group, 65% of those with ICAs ≥80 JDF U were unaffected at 10 yr. Furthermore, low ICA amounts (<20 JDF U) were predictive of type I diabetes in family members but not in polyendocrine patients. It is therefore not surprising that the strongest additional predictor of type I diabetes in the polyendocrine patients is a positive

TABLE 3
Progression to insulin-dependent (type I) diabetes in polyendocrine patients

Characteristic	*n*	Probability of being free of type I diabetes after 10 yr (%)	95% Confidence interval	*P*
n	158	69.8	54.3–84.3	
Sex (M/F)	40/118	71.7/69.9	52.3–91.1/52.6–87.3	
Age				
>30 yr	124	72.3	56.6–88.0	
<30 yr	34	60.4	29.5–91.4	
ICA				
Persistent	110	58.9	40.8–76.9	<0.05
Nonpersistent	27	100.0		
CF-ICA	132	63.2	46.6–79.8	<0.05
Non–CF-ICA	24	100.0		
ICA >20 JDF U	50	63.3	40.8–85.8	
ICA <20 JDF U	35	95.6	88.2–103	
IAA$^+$	9	24.9	0–65.8	<0.005
IAA$^-$	114	77.7	60.3–95.1	
Family history				
Type I diabetes	15	32.0	0–78.6	<0.01
No type I diabetes	87	72.6	57.5–87.7	
Clinical expression of autoimmunity	124	62.1	46.3–78.0	
Autoantibody only	34	78.9	58.8–99.1	

ICA, islet cell antibody; CF, complement fixing; JDF, Juvenile Diabetes Foundation; IAA, insulin autoantibody.

family history. The probability of being free of type I diabetes in those who had a first-degree relative with type I diabetes was 32%, which is similar to the 27% after 10 yr observed in first-degree relatives with ICAs >20 JDF U from the BWFS (26).

In contrast, only a few polyendocrine patients with ICAs but without a diabetic family history progressed to disease despite very high titers of ICAs and marked expression of other autoimmunities to several organs. This difference suggests that genetic factors beyond those governing the autoimmune process reflected by ICAs may be involved. This concept is also supported by studies in identical twins, where in discordant twins with ICAs there is almost 100% progression to type I diabetes within only 5 yr of the first twin becoming diabetic (8). This percentage is higher than in first-degree relatives with ICAs, where ~75% progress to disease within 10 yr and 37% within 5 yr. ICAs without the associated genetic susceptibility, e.g., family history, did not confer a very high risk for type I diabetes in our group of polyendocrine patients. Interestingly, in randomly screened children with no family history of diabetes, the predictive value of ICAs was confirmed, but the progression to type I diabetes in these so-called sporadic cases was only ~50% (11). Although the sample numbers in all the studies reported are relatively small, the data suggest that disease in individuals who have ICAs is most frequent in those with identical or similar genetic makeup and presumably more diabetogenic genes, declining in sporadic cases where the genetic similarities are less prominent and even less in older sporadic cases with associated autoimmunities. Such differences may reflect the higher prevalence of type I diabetes in co-twins and first-degree relatives of diabetic patients. Nevertheless, genetics, including genes other than in the major histocompatibility complex (MHC), is likely to be important for disease prediction in the sporadic cases of type I diabetes. Analysis of the MHC in the polyendocrine patients should help clarify this.

The slow progression toward overt diabetes in polyendocrine patients, despite the persistence of very high titers of ICAs, is intriguing. From one point, it gives further evidence that these autoantibodies alone are unlikely to cause β-cell destruction. On the other hand, it may also indicate that ICAs include several specificities, only some of which are relevant to β-cell destruction. Of interest are the recent findings in stiff-man syndrome, where most patients have autoantibodies to glutamic acid decarboxylase (GAD) in conjunction with autoantibodies to other endocrine glands, including ICAs (28). The frequency of associated type I diabetes in stiff-man syndrome and polyendocrine ICA$^+$ patients is 30%, which is similar to that observed in our polyendocrine ICA$^+$ patients. The GAD antigen is now recognized as one of the 64,000-M_r islet autoantigens found in type I diabetes (29), and antibodies to GAD have been detected in most of our polyendocrine patients (unpublished observations). Studying the diversity of autoantibody response to the recognized islet autoantigens in our cohort is likely to clarify which specificities are most relevant to disease. It is also possible that, in many polyendocrine patients, including stiff-man syndrome cases, the ICAs are a result of cross-reactive epitopes, with little or no autoimmunity directly in the islet. Such possibilities will be clarified once pancreas from nondiabetic polyendocrine individuals can be studied.

Factors other than autoantibodies are likely to be responsible for the β-cell destruction. Among these, relevant destructive mechanisms include cytotoxic T lymphocytes. This subpopulation of lymphocytes was found to be the most abundant infiltrating islets in a child who died at onset of the disease (30) and also in the recurrent insulitis after twin-to-

twin pancreas transplantation (31). These findings indicate that cytotoxic T lymphocytes must be a critical effector limb of the autoimmune response against pancreatic β-cells. If this is the case, their engagement into β-cell recognition is delayed compared to that of the helper T lymphocyte and B lymphocytes. Whether this is achieved through direct presentation of antigens by HLA class II–positive β-cells or by classical antigen-presenting cells remains unresolved. The event that might trigger cytotoxic T lymphocytes to express autoreactive T-lymphocyte receptors recognizing β-cell epitopes could be distinct from that of the other limbs of the autoimmune response (for review, see ref. 32). However, because final insulin requirement is correlated with ICA titer, such an event is likely to be related to the degree and specificity of autoantibody response, suggesting a role for specific suppressor mechanisms (33).

In summary, we have identified a heterogeneous population of endocrine autoimmune patients with ICAs. The risk for developing type I diabetes in these patients is significantly influenced by a positive family history, the ICA titer, and the detection of IAAs but not by the other concomitant autoimmunities. Sex and age of the polyendocrine patients may also influence the final outcome. The progression to type I diabetes among individuals with ICAs follows a hierarchy dependent on genetic susceptibility to the disease. Clearly, larger cohorts are required to adequately assess these and other variables. Pooling of data from several studies, perhaps through the establishment of an International Registry of asymptomatic individuals with ICAs, is likely to facilitate this and provide the basis for statistically ethical clinical prevention trials. Importantly, this study has identified a large cohort of patients with very high ICA levels but with no progression to overt diabetes, suggesting heterogeneity in the autoimmunity to islets. Therefore, the polyendocrine group of patients provides an invaluable cohort for identifying and separating those factors, including genetics, that ultimately govern the autoimmune processes involved in the initiation and progression to type I diabetes.

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