REVIEW

Predicting Type I Diabetes

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Currently, there are three markers that are being studied with the potential to give a high positive predictive value for the development of type I diabetes (insulin-dependent diabetes caused by autoimmune β-cell destruction) and that can be utilized to predict the disease in susceptible relatives: *1*) high-titer cytoplasmic islet cell antibodies, *2*) insulin autoantibodies detected with fluid-phase radiobinding assays, and *3*) first-phase insulin release after intravenous glucose <1st percentile. With the combination of these assays, it seems to be possible to identify first-degree relatives with a high probability of developing type I diabetes within a limited time span (i.e., <10 yr). The ability to predict type I diabetes with selected assays will allow trials for prevention of diabetes and trials to assess whether prediction will decrease morbidity and mortality at onset of diabetes. *Diabetes Care* 13:762–75, 1990

In recent years, understanding of the pathophysiology of diabetes has increased. Consequently, we now prefer to reserve the term *type I diabetes* to refer to insulin deficiency caused by autoimmune β-cell destruction rather than applying it to all forms of insulin dependence. Type I diabetes occurs mainly in genetically susceptible individuals carrying high-risk HLA alleles such as DR3 and DR4. Incidence of type I diabetes varies between countries, and in Europe, there is a north-south gradient in diabetes risk. In addition, the frequency of type I diabetes seems to be increasing (1,2). As we discuss, at onset of diabetes, one or more types of anti-islet autoantibodies are usually present. Variations in disease course appear to reflect the rate of the underlying autoimmune process, which in turn is often inversely related to the age at which diabetes develops. Immunologic assays are likely to play a growing role in the diagnosis of early type I (as opposed to type II [non-insulin-dependent]) diabetes in new-onset diabetic patients and to help predict the development of type I diabetes and the rate or intensity of the autoimmune process. We review available strategies for such prediction of type I diabetes. Although only a minority of type I diabetic patients have a first-degree relative with diabetes, most of the studies we review have evaluated first-degree relatives of patients with type I diabetes; similar studies in the general population are in early stages.

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RATIONALE FOR PREDICTION

The most important reason for identifying an asymptomatic individual at risk for developing diabetes is the availability of intervention during the prodromal phase, which can prevent subsequent morbidity or mortality. Researchers have begun to study therapies to prevent the autoimmune β-cell destruction that leads to type I diabetes (3–5). However, type I diabetes mellitus cannot yet be safely prevented. Until such therapy is available, screening individuals developing type I diabetes is undertaken *1*) to potentially prevent morbidity and mortality at the acute onset of severe hyperglycemia, *2*) to

avoid situations that would create an unacceptable risk should type I diabetes develop in the future (e.g., potential pancreatic segment– or kidney-transplant donors), 3) to aid in career and family planning, and 4) in research settings. The major disadvantage of identifying individuals at high risk for future diabetes is the stress of realizing that one's risk of developing diabetes is much higher than the overall risk for unscreened first-degree relatives (3–5%) or the general population (0.3%). One study has assessed the psychological impact of screening and has documented a small increase in anxiety level but a good level of coping (S. Johnson, unpublished observations).

With accurate screening assays, we believe that the advantages of the knowledge gained outweigh the disadvantages for most individuals, although some families may choose to be tested and others may not. However, widespread clinical screening is unlikely to become standard clinical practice until current assays are improved and standardized and reliable preventive therapy is available.

USE OF PREDICTIVE SCREENING TESTS

The usefulness of a surveillance of the general population and of unaffected relatives of patients with type I diabetes for prediabetes depends on the accuracy of the available screening tests for disease susceptibility. Both the prevalence with which a disease develops in a given population and the specificity and sensitivity of the assays affect the clinical applicability of screening programs (Fig. 1). Let's examine the result of screening 100,000 individuals without a family history of diabetes by use of a predictive assay with 98% specificity and 90% sensitivity for diabetes. Among the general population, 3 of 1000 or 300 individuals will develop diabetes. This assay would detect 270 of these 300 individuals and miss 30 prediabetic individuals. Unfortunately, from the 99,700 who will not develop diabetes, the same assay would identify 1994 as antibody positive and potentially diabetic, giving a positive predictive value of only 12% [270/(1994 + 270)]. Apply-

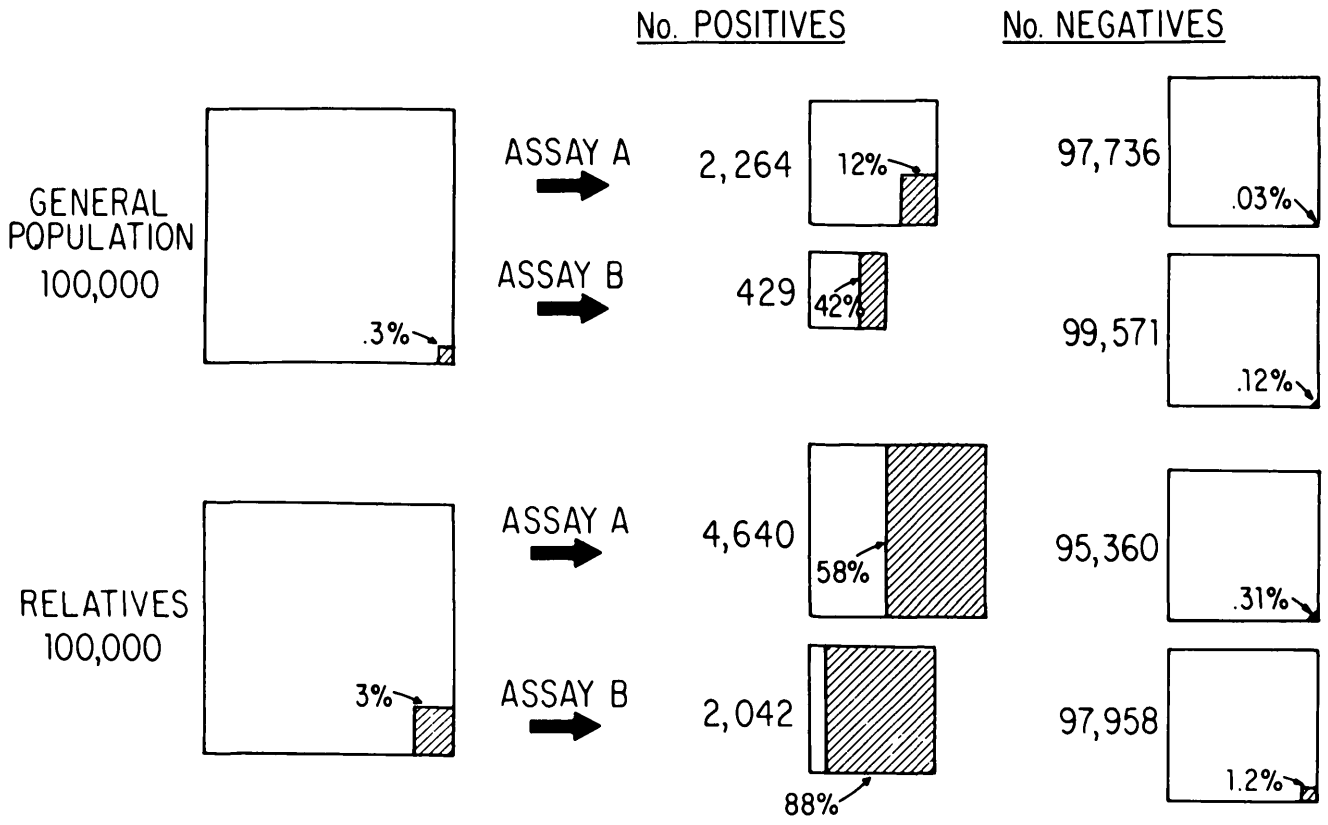

FIG. 1. Bayes' theorem and its influence on predictive assays for type I diabetes (insulin-dependent diabetes caused by autoimmune β-cell destruction). *Top left panel,* number of individuals from general population who will develop diabetes (300 of 100,000; *hatched area*); *bottom left panel,* number of 1st-degree relatives of patients with type I diabetes who will develop diabetes (~3000 of 100,000; *hatched area*). If 2 assays, assay A and assay B (which differ in their specificity [98% for assay A, 99.75% for assay B] and sensitivity [90% for assay A, 60% for assay B]), for anti-islet antibodies are applied to these populations, number of antibody-positive individuals who actually develop diabetes will vary dramatically. *Middle panels,* all antibody-positive individuals; *hatched areas,* number of antibody-positive individuals who will actually develop diabetes. *Right panels,* assay-negative individuals and their development of diabetes.

ing the same assay to a population of 100,000 first-degree relatives of type I diabetic patients, of whom 3 of 100 or 3000 will develop diabetes, 2700 will be appropriately identified as prediabetic, but 1940 who will not develop diabetes would also be identified, and the resulting positive predictive value is 58% [2700/(1940 + 2700)]. With such a high incidence of individuals falsely identified as prediabetic, the stress of identification is not balanced by prevention of morbidity.

In many instances, sensitivity can be sacrificed and the positive predictive value of a test can be increased by defining positivity at a higher cutoff point. As indicated by the Barts-Windsor study, this is the case with immunologic assays associated with diabetes (6). For example, an assay with 99.75% specificity but 60% sensitivity (e.g., current protein A and complement-fixing [CF] cytoplasmic islet cell antibody [ICA] assays; Table 1) can be used to screen 100,000 relatives. We would identify 1800 of the 3000 who will develop diabetes, whereas 242 relatives would be incorrectly identified as at risk. The positive predictive value would be 88% [1800/(1800 + 242)] and would approach clinical utility. In fact, it is likely that a combination of assays will have to be used to accurately identify individuals at risk for type I diabetes.

GENETIC MARKERS

Ninety-five precent of Whites developing type I diabetes express either the serologically defined HLA-DR3 or HLA-DR4 histocompatibility antigens (7). HLA-DR3/4 heterozygosity is associated with the highest risk of developing type I diabetes. However, at least 40% of the nondiabetic population also express these alleles. Furthermore, it has become clear that HLA-DR4 represents a serological specificity present on structurally distinct class II antigen molecules. Studies reported by Nepom et al. (8) have identified a variant of the DQ β-chain (DQw3.2), which serves as a genomic marker more tightly associated with type I diabetes than DR4. More than 95% of $DR4^+$ diabetic patients express the DQw3.2 allele. Identical findings have also been reported by several other groups with restriction endonucleases and oligonucleotide probes (9–11). Thomsen et al. (12) found 100% of $DR4^+$ diabetic subjects and 68% of the $DR4^+$ control population to be $DQw3.2^+$. They also studied haploidentical $DR4^+$ siblings of DQw3.2 patients with type I diabetes. As expected, siblings also carry DQw3.2 alleles regardless of whether they have developed clinical diabetes (12). Thus, HLA haplotypes and specific allele variants may indicate increased risk of type I diabetes; although their presence may be a prerequisite, it is not sufficient to cause disease.

In a further attempt to identify the diabetes-associated genes, Todd et al. (13) sequenced the four major polymorphic class II gene products (DRβ1, DRβ3, DQβ, and DQα) expressed in three type I diabetic patients and nine healthy nondiabetic control subjects (13). All sequences found in diabetic patients were also found in nondiabetic control subjects. Thus, the autoimmune process of type I diabetes is not due to mutant HLA class II alleles but is probably HLA restricted by certain class II alleles that occur in a significant proportion of nondiabetic individuals. Nevertheless, by comparing DNA from control subjects and diabetic patients, it was found that replacement of aspartic acid at position 57 of the DQ β-chain by alanine, valine, or serine is strongly cor-

TABLE 1
Islet cell antibodies (ICAs) in first-degree relatives, new-onset diabetic patients, and nondiabetic relatives

Program	Refs.	Assay	ICA+		Mean follow-up (yr)	Diabetic (DM/patient-yr)		Current positive predictive value (%)	ICA+ new-onset diabetic		ICA+ nondiabetic control	
			n	%		*n*	%		*n*	%	*n*	%
Boston												
Sacramento	27–31	Protein A	75	1.7	2.6	22	0.11	29	54	54	1 of 442	0.2
Barts-Windsor	22–25	CF-ICA	24	3.3	4.1	13	0.13	54	118	60	1 of 322	0.3
Padua*	26	CF-ICA	10		7.0	7	0.10	70	5	22	NR	
Dusseldorf	39	CF-ICA							159	57	NR	
Mean ± SD				2.5 ± 0.8	4.6 ± 1.3		0.110 ± 0.009	51 ± 12		48 ± 9		0.25 ± 0.05
Gainesville	35–37	F-ICA	125	2.4	3.2	17	0.04	14	26	68	2 of 292	0.7
Barts-Windsor	22–25	F-ICA	54	7.5	5.3	1	0.006†	3†	152	77	5 of 322	1.6
Padua	26	F-ICA	21		7.0	1	0.007†	9†	22	96	NR	
Düsseldorf	39	F-ICA							576	85	NR	
Malmo	74	F-ICA		•					316	81	9 of 321	2.8
Mean ± SD				4.9 ± 2.6	5.1 ± 1.5		0.02 ± 0.01†	9 ± 3†		81 ± 6		1.7 ± 0.6

DM, progressed to diabetes mellitus; protein A, peroxidase- or fluorescein-conjugated protein A ICA; CF-ICA, complement-fixing ICA; F-ICA, fluorescein-conjugated anti-antibody ICA; NR, not reported.

*In Padua, screening was performed in organ-specific autoimmune patients not in 1st-degree relatives of patients with type I (insulin-dependent diabetes caused by autoimmune β-cell destruction) diabetes.

†Predictive value calculated for $CF\text{-}ICA^-$, ICA^+ individuals.

related with susceptibility to type I diabetes in Whites, whereas aspartic acid is usually present at position 57 in DQβ alleles, which are neutral or negatively associated with the disease (DR7 is an exception). Thus, it appears that the amino acid at position 57 may play a critical function in the DQ molecule of type I diabetes. This hypothesis can be directly tested in NOD mice, which lack aspartic acid in position 57 of their I-A β-chain.

Even though considerable advances have been made in our understanding of genetic predisposition to type I diabetes, current genetic information cannot be used in population studies to predict development of diabetes. Almost 40% of the general population express DR3, DR4, or DQw3.2. At the DNA sequence level too, diabetogenic class II genes are frequently found in the general population. Thus, these genes appear necessary but not sufficient for the development of type I diabetes. It is likely that genetic probes will shortly allow the identification of individuals within the general population whose risk of developing diabetes approaches that of first-degree relatives (3–5%). This statement is based on the work of Sheehy et al. (14) in their Wisconsin population studies, where definition of specific DQβ alleles and DRβ alleles in combination appears to identify individuals with an increased risk of developing type I diabetes (14). Such a population of heterozygote haplotypes may constitute as many as 10% of future diabetic patients, and further screening of this group may be feasible, as indicated below.

Among siblings of diabetic patients who themselves develop diabetes, 55–60% share both HLA haplotypes with the proband, 37% share one haplotype, and <5% share none. The risk for developing diabetes in the sibling of a diabetic patient has been estimated to be 17% if the individual is HLA identical to the proband, 5% if sharing one HLA haplotype, and 1% if sharing none. The question remains whether other genes can be identified that are not HLA linked and that could be used to detect increased risk for diabetes. Because the concordance rate among monozygotic twins is ~30–50% (15–19), the positive predictive value for type I diabetes would be ~50% (compared to 17% for HLA typing) if all diabetes-associated genes could be identified. Therefore, if a second linkage group outside of the MHC is discovered for type I diabetes, genetic prediction will become clinically more important for the general population. Until such time, autoantibody assays and metabolic parameters will provide most of the relevant clinical prediction data as outlined below.

AUTOANTIBODIES

Currently, there are three markers, which, when measured in highly specific assays (<1 of 300 nondiabetic control subjects positive), give a high positive predictive value for development of type I diabetes: cytoplasmic ICAs >40 Juvenile Diabetes Foundation (JDF) units, insulin autoantibodies (IAAs) detected with fluid-phase radiobinding assays, and first-phase insulin release <1st percentile during an intravenous glucose tolerance test (IVGTT). It is likely that antibodies to a 64,000-*M*, islet protein (64K), which have been detected in ~75% of prediabetic individuals (56), will eventually be used for screening, but current assay techniques are too cumbersome for large-scale analysis. A number of other assays and immunologic abnormalities are not discussed in this review, because their false-positive rate among relatives of diabetic patients is so high that it severely limits their use for prediction of diabetes (e.g., Bouin's fixed pancreas for cytoplasmic ICA screening, anti-islet surface assays, cytotoxic assays, enzyme-linked immunosorbent assays [ELISAs] with RIN islet tumors, and T-lymphocyte–subset abnormalities).

CYTOPLASMIC ICAs

Cytoplasmic ICAs are IgG immunoglobulins capable of binding to all islet cells in frozen sections. The designation *cytoplasmic* may be a misnomer, because ultrastructural studies are not available and the antibodies detected may bind to the plasma membrane of islet cells, even though they appear to give cytoplasmic staining on frozen sections. During the past decade, cytoplasmic ICAs have been evaluated as possible predictive markers of β-cell dysfunction or impending type I diabetes. Significant variations are seen between different laboratories regarding the prevalence and predictive value of ICA positivity. In nondiabetic individuals who have a first-degree relative with type I diabetes, the reported prevalence of ICA varies between 0.9 and 9.0%. Some of these variations result from differences in assay technique, differences in the human pancreas used as substrate, and differences in the subjective interpretation of fluorescence staining.

Several workshops have been held in an effort to standardize ICA assays (20,21). The latest ICA workshop, held in New York City in October 1987, set out to compare the reproducibility, specificity, and sensitivity (i.e., threshold of detection) of the assays performed in different laboratories. Most of the assays had acceptable levels of precision in identifying standard ICA$^+$ serums, but many laboratories identified one or more of the control blood donor serums as ICA$^+$. Some laboratories even found most of these control samples to have low titers of ICA positivity.

The detection limit for the assays varied widely. With an arbitrary JDF unit based on a single standard serum, some assays can detect as little as 5 JDF units; others have a detection limit of 40–80 JDF units. Assays such as the CF and protein A assays, which have a higher detection limit (20–40 JDF units), appear to have a higher positive predictive value; they are highly specific to patients with increased risk of developing type I diabetes (see below). Such assays also have more false negatives. On the other hand, highly sensitive assays

can help define the cutoff titer (such as 40 JDF units) at which the positive predictive value of a positive result has a high positive predictive value.

Table 1 summarizes the results of five different laboratories utilizing ICA assays to screen first-degree relatives of type I diabetic and new-onset diabetic patients and nondiabetic control subjects (22–27,31–39). With CF or protein A assays, the prevalence of ICAs in nondiabetic control subjects was 0.25% compared to 1.7% with standard ICA assays (a 7-fold difference); in nondiabetic first-degree relatives, prevalence of ICA was 2.5 ± 0.8% with CF or protein A assays vs. 4.9 ± 2.6% in relatives screened with standard ICA assays. Individuals identified with the CF and protein A assays developed diabetes five to six times more often (mean incidence of becoming diabetic [DM] 0.11 ± 0.009 DM/patient-yr of follow-up) than individuals who were ICA$^+$ but CF-ICA$^-$ (0.02 ± 0.01 DM/patient-yr). Therefore, these assays have a fivefold higher positive predictive value than standard ICA assays (mean ± SE 51 ± 12 vs. 9 ± 3%). With CF and protein A assays, ICA positivity was less common at onset of diabetes (48 ± 9%) than with standard assays (81 ± 6%). This is consistent with their higher detection limit.

The Barts-Windsor study used two different ICA assays with different sensitivities: a standard ICA assay with fluorescein-conjugated anti-antibody with a low detection limit and a CF-ICA assay with a high detection limit (22–25). Only the CF assay had a high positive predictive value of 54% (actual percentage, not life-table projection, which is higher) for the development of overt diabetes (Table 1; Fig. 2). Relatives who were ICA$^+$ with the anti-immunoglobulin assay but were negative by CF-ICA had a low risk of developing diabetes (3%). Fluctuating autoimmunity before diabetes was observed primarily with the lower specificity assay. By life-table analysis of subjects with CF-ICA, 76% were projected to be diabetic after 8 yr of known positivity compared to 3.4% of those with standard fluorescein-conjugated ICA alone and 0.3% (2 of 665) of ICA$^-$ relatives (Fig. 2). Thus, the positive predictive value with their current follow-up and by life-table analysis of ICA$^+$ (the more sensitive assay) but CF-ICA$^-$ relatives is negligible, with 1 of 30 developing diabetes. The question arises, even though the positive predictive value of low-titer ICA is poor, would a negative result with the more sensitive assay give clinical information in terms of diabetes not developing (25)? We believe the data do not support such a conclusion because the difference in the negative predictive value for CF-ICA$^-$ relatives versus regular ICA$^-$ relatives is very small. For example, negative predictive value for CF-ICA$^-$ is 99.58% (0.43% DM [3 of 695]), and negative predictive value for ICA$^-$ is 99.72% (0.30% DM [2 of 665]). The difference between 0.43% and 0.30% of antibody-negative relatives developing diabetes at a clinical level does not seem to justify identifying 29 false-positive individuals with the current follow-up, although further research in this area and longer follow-up of patients with low-titer ICA is of research interest.

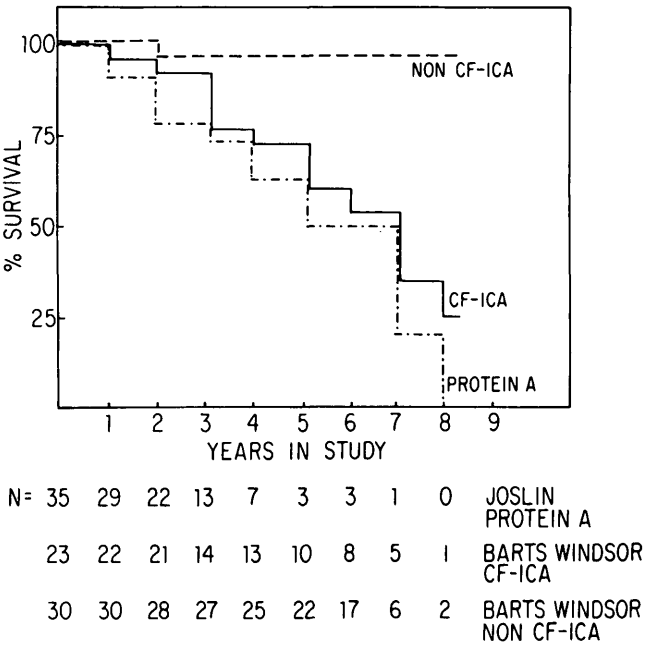

FIG. 2. Life-table analysis of cumulative risk of type I diabetes (insulin-dependent diabetes caused by autoimmune β-cell destruction) in islet cell antibody–positive (ICA$^+$) 1st-degree relatives of Barts-Windsor and Joslin cohort (only 35 relatives had been identified for ICA positivity and included by time figure was drawn). Complement-fixing (CF)-ICA$^+$ group and protein A$^+$ group differ from CF$^-$ standard ICA$^+$ group. From Jackson et al. (27).

Similar results to the Barts-Windsor study were obtained by Betterle et al. (26), who followed 21 nondiabetic subjects with other organ-specific autoimmune diseases for a mean period of 7 yr. Ten probands were persistently CF-ICA$^+$. Of these, 70% developed diabetes within 2 to 60 mo. A comparison group consisted of 11 CF-ICA$^-$ individuals who had low ICA titers (persistent, transient, or fluctuating) by a standard assay. Only 1 of these subjects developed diabetes, and this subject was later found to have high levels of IAAs (Table 1).

In our laboratory, we use a protein A assay with human or rat pancreas as substrate with interchangeable results (27–30; Fig. 3). With our technique, ICA positivity (defined positivity limit >40 JDF units) is strongly associated with progression to diabetes (31–34). To date, 22 of 75 ICA$^+$ first-degree relatives (29%) have progressed to overt type I diabetes during an average follow-up period of 2.6 yr (Table 1), and we have life-table results essentially identical to the CF-ICA$^+$ Barts-Windsor cohort (27; Fig. 2). Approximately 11% of ICA$^+$ first-degree relatives develop diabetes per year (0.11 DM/patient-yr of follow-up), and we have observed an interval of as long as 8 yr between detection of ICA and onset of diabetes (Fig. 2). For some individuals, the time to onset of diabetes is likely to be more

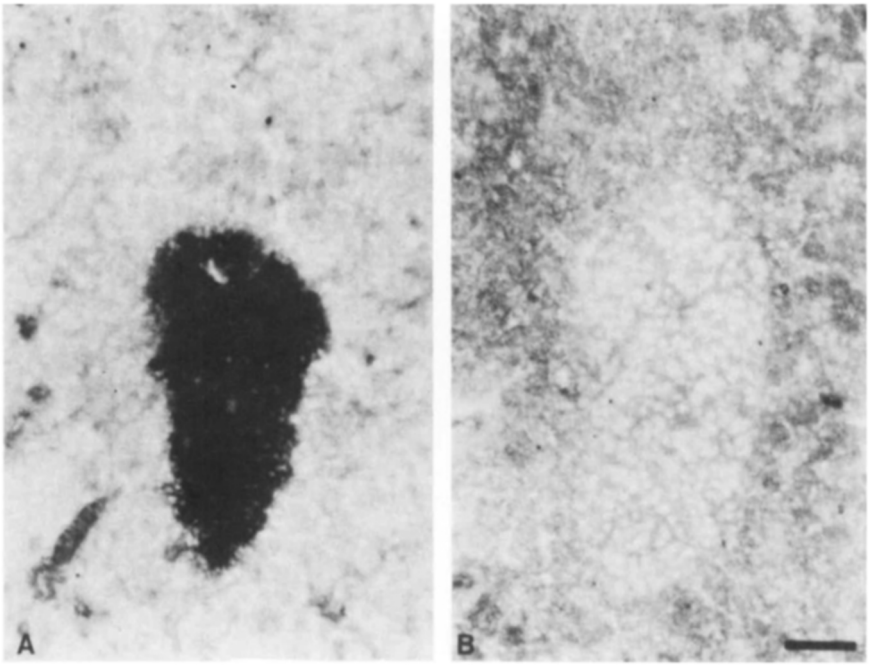

FIG. 3. Anti-islet autoantibodies identified before development of type I diabetes (insulin-dependent diabetes caused by autoimmune β-cell destruction) reacting with frozen sections of nondiabetic rat pancreas and detected with protein A coupled with immunoperoxidase. *A*: positive serums. *B*: negative serums. *Bar* = 50 μm. From Colman et al. (29).

than two decades, and some individuals may not develop diabetes within their lifetime, depending on the rate of the autoimmune process. Follow-up of larger cohorts for longer periods of time is essential to define the limits of the positive predictive value more accurately.

Riley and co-workers (35,36) in Gainesville have screened >5000 first-degree relatives with fluorescein-conjugated antibody assay with an intermediate detection limit; 2.4% were ICA^+, and 0.4% have developed diabetes, 78% of whom were ICA^+ (Table 1). Furthermore, the nondiabetic ICA^+ relatives have lower first-phase insulin secretion in response to intravenous glucose than the ICA^- relatives. The same group then started to study the feasibility of using ICAs as a marker for latent type I diabetes in the general population (37). They detected 0.82% of school children and school personnel to be ICA^+, 89% of whom carried the HLA-DR3 or HLA-DR4 allele, and concluded that the occurrence of ICAs in the general population is restricted to "genetically susceptible" subjects and might be associated with the development of diabetes in later life. To date, two of these ICA^+ children have become diabetic with <3 yr of follow-up.

It seems clear then that ICA levels >40–80 JDF units are highly predictive of future development of type I diabetes in nondiabetic individuals who have a first-degree relative with type I diabetes. In contrast, relatives with low-titer ICA rarely progress to diabetes. Spencer et al. (38) observed at least one patient who progressed to diabetes with an ICA level of 5 JDF units. The same group also reported that ICA levels of 5–10 JDF units may disappear. In the Schwabing City Hospital in the Federal Republic of Germany, three relatives with ICA levels of 5–10 JDF units had a significant decrease of first-phase insulin response compared with the control population, but longer follow-up and further data are required to confirm these observations. A major limitation with low-titer ICA assays remains the high incidence of false-positive results in terms of eventual development of diabetes. It may be that low-titer ICAs detect antibody binding to antigens that are unrelated to the destructive process of the anti-islet autoimmunity and therefore provide little clinically relevant information. Although such low-titer antibodies may provide clues relative to the pathogenesis of type I diabetes, available data suggest that detection of low-titer ICA or low-titer fluctuating ICA levels has a positive predictive value as low as 3% with a maximum of 8 yr of follow-up (Fig. 2).

IAAs

IAAs have been detected in many patients with new-onset type I diabetes before insulin therapy and also patients with other immune disorders such as the insulin autoimmune syndrome (40–45). Two general types of assays have been used to measure these antibodies: *1*) fluid-phase radiobinding assays with ^{125}I-labeled insulin, in which antibody-bound insulin is precipitated with polyethylene glycol, and *2*) ELISAs, in which insulin is adsorbed to plastic wells and insulin-binding autoantibodies are detected with anti-antibody reagents. The most recent workshop comparing insulin autoantibody assays (Immunology of Diabetes Workshop, Auckland, New Zealand, 1988) indicates that most radiobinding assays are linearly correlated but differ in sensitivity.

Table 2 summarizes the results obtained from screen-

TABLE 2
Insulin autoantibodies (IAAs) in islet cell antibody–negative (ICA⁻) first-degree relatives, new-onset diabetic patients, and nondiabetic subjects

Program	Refs.	Assay	ICA⁻ relatives		New-onset diabetic (% IAA⁺)	Nondiabetic (*n* IAA⁺/*n* studied)
			n IAA⁺	% IAA⁺		
Boston						
Sacramento	43,47,48	Radiobinding	29 of 1086	2.7	53	0 of 155 (0%)
Gainesville	49	Radiobinding	9 of 220	4	37	4 of 292 (1.4%)
Mean ± SD				3.4 ± 0.7	45 ± 8	
Padua	50	ELISA (IgG or IgM)	32 of 114	28	48	0 of 65 (0%)
Southampton	51	ELISA	14 of 39 (twins)	36	38	0 of 100 (0%)
London	42,54	ELISA	9 of 57	16	NR	5 of 73 (6.8%)
Düsseldorf	52	ELISA	33 of 292	11	NR	NR
Giessen	53	ELISA	68 of 611	11	NR	NR
Mean ± SD				20 ± 5* 16 ± 4†	44 ± 6	

ELISA, enzyme-linked immunosorbent assay; NR, not reported.
*Includes twins studied in Southampton.
†Excludes twins studied in Southampton.

ing first-degree relatives, new-onset diabetic patients, and a nondiabetic control population with radiobinding assays or ELISA techniques (42,43,47–54). Two laboratories using radiobinding assays found 3.4% of nondiabetic first-degree relatives to be IAA⁺ versus 45% of new-onset diabetic patients before insulin therapy. By use of ELISA techniques, five laboratories found a mean of 20% (16% excluding twins) positivity in nondiabetic first-degree relatives versus 44% positivity in new-onset diabetic patients. Because only ~3% of all first-degree relatives eventually develop diabetes, most of the "ELISA-positive" relatives are likely to remain free of the disease. Three laboratories measured IAAs in a nondiabetic control population separate from the one that defined the upper limit of normal of their assay. Insulin antibodies were detected in 0 and 1.4% of individuals by two different radiobinding assays and in 6.8% by one ELISA. However, in general, the findings with radiobinding assays for IAAs differ from those with ELISA techniques. Wilkin et al. (51) found insulin antibodies (ELISA) in 47% of discordant identical twins of type I diabetic patients whose follow-up time is long past the high-risk period for developing diabetes (Table 2). On repeat determination, antibodies frequently disappeared and reappeared. With a competitive ELISA assay, Kuglin et al. (52) detected insulin antibodies in 11.3% (33 of 292) of first-degree relatives (Table 2). Becker et al. (53) identified 8% of parents and 15% of siblings of patients with insulin-dependent diabetes mellitus (IDDM) as insulin-antibody positive (Table 2). These values far exceed the observed risk of developing diabetes in siblings and parents of diabetic probands. These studies suggest that not all ELISA assays for insulin antibodies can be applied to the prediction of diabetes. Recent workshop comparisons of identical serums assayed for IAAs by radiobinding assay and ELISA indicate that the two assay formats may actually measure qualitatively different antibodies (45; Immunology of Diabetes Workshop, Auckland, New Zealand, 1988).

In our laboratory, we determine antibodies to insulin with a competitive fluid-phase radiobinding assay with results reported in nanounits per milliliter (1 nU/ml insulin = 7.18 fM) of insulin precipitated as competitive IAAs (CIAAs). Control tubes are incubated with an excess of unlabeled insulin to compete with labeled insulin for antibody-binding sites (43). Of 100 patients with new-onset diabetes, we found 53 (53%) to be IAA⁺ (>39 nU/ml; 47). The concentration of CIAA is strikingly dependent on the age at which type I diabetes develops (46–48; Fig. 4). Not only are the concentrations of CIAAs extremely elevated in younger patients (note that in Fig. 4 the *y*-axis is a logarithmic scale), but the 18 (100%) children studied to date and diagnosed with diabetes at <5 yr of age had elevated CIAAs. In comparison, 30 of 48 (62%) children between 5 and 15 yr old and only 5 of 34 (14.7%) adolescents and adults had CIAAs exceeding the upper limit of normal. In contrast, we have not seen any correlation of cytoplasmic ICAs with age (ICAs were found in 44% of children ≤5 yr old and 54% of the overall patients). Therefore, in children, CIAAs may have a higher sensitivity for type I diabetes than high-titer ICA (>40 JDF units). Combining the determination of CIAAs with that of ICAs, most (56 of 66 [85%]) of the children who developed diabetes at <15 yr of age were found to have immunologic abnormalities. Why IAAs are present and why they are age related is not clear; we believe that the concentrations of CIAAs may reflect the virulence of autoimmunity to β-cells and can be used to predict the rate of β-cell destruction (see below). Note, however, that studies in new-onset type I diabetic patients may be a special case only specific to the last stages of the prediabetic state. It is conceivable that a previous CIAA⁺ subject could become CIAA⁻ because the site of antigen production

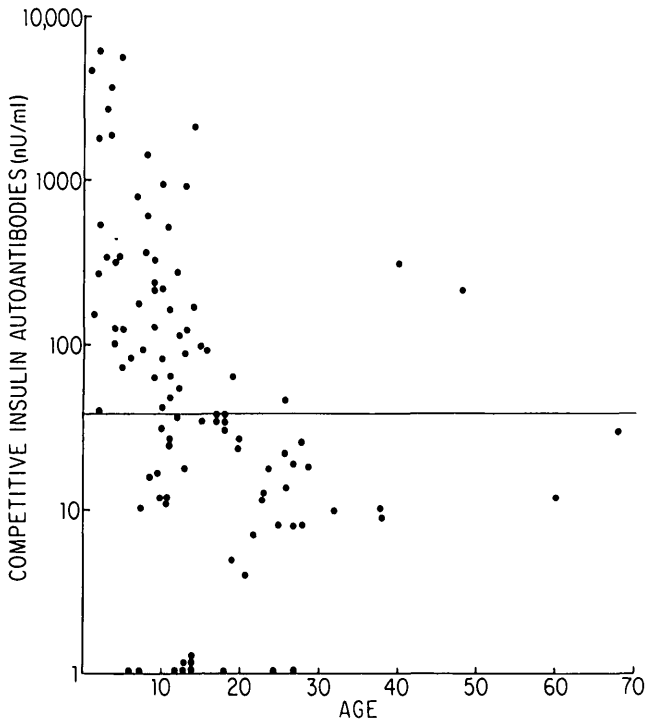

FIG. 4. Insulin autoantibodies versus age at diagnosis of 100 new-onset diabetic individuals before insulin therapy (each data point represents 1 individual). *Horizontal line*, upper limit of normal in previously studied control subjects. $r = -0.3$, $P < 0.002$.

becomes depleted and specific antibody levels become too low to be detected. Prospective studies throughout the prediabetic state will be required to clarify this circumstance.

Turning from new-onset diabetic patients to a nondiabetic population, we found CIAAs to be present in 53% (20 of 38) of ICA^+ and 2.7% (29 of 1086) of ICA^- nondiabetic individuals who have a first-degree relative with type I diabetes (47). Concentrations of CIAAs were once again highest in children <3 yr old; if present, they were usually >1000 nU/ml. The question of whether these antibodies appear in susceptible first-degree relatives at birth and disappear in later life is unanswered. Therefore, note that we observed one child who at 9 mo of age was $CIAA^-$, later converted to $CIAA^+$, and has remained $CIAA^+$ into later childhood. We also observed an adult twin whose IAAs and ICAs were consistently negative for 7 yr until the nearly simultaneous appearance of ICAs and antibodies to the 64K antigen (55,56). At this time, he also began to produce IAAs, and these remained elevated for 5 yr until he developed diabetes (40; Fig. 5).

As observed in our laboratory and by others, ICA^+ relatives of type I diabetic patients are more likely to become diabetic if they have IAAs than if IAAs are not present (49,57).

With our competitive radiobinding assay for insulin antibodies, we have infrequently found conversion (either from negative to positive or from positive to negative) of CIAA values over time before diabetes (e.g., once a patient has abnormally high CIAAs, these levels usually remain abnormally high; Fig. 5). In contrast, other investigators have noted random or systematic fluctuation of IAA levels. With different assays, McEvoy reported that relatives who progressed to diabetes only began producing IAAs shortly before onset of diabetes (58), whereas Leslie et al. (59) observed a decrease in IAA levels before onset in several identical twins who progressed to diabetes.

We believe it is likely that differences between the conclusions of different laboratories reflect major differences between ELISA and radiobinding assays and between radiobinding assays that do or do not assess specific anti-insulin binding by competition with unlabeled insulin. As discussed subsequently, we have reported a linear regression model, incorporating as independent variables the level of CIAAs and first-phase insulin secretion, for prediction of the time of onset of type I diabetes among ICA^+ first-degree relatives (57). The lack of systematic change of CIAA concentrations in prediabetic individuals in the glucose-tolerant phase over time with our assay is central to this model, which can utilize either initial or average IAA concentrations.

IVGTT INSULIN SECRETION

We reported that abnormalities of first-phase insulin secretion in response to intravenous glucose can precede

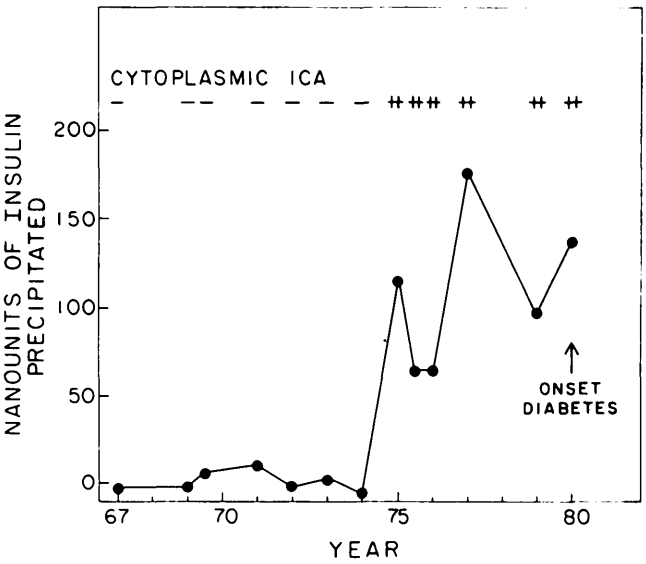

FIG. 5. Serial measurements of insulin autoantibodies and islet cell antibodies (ICAs) plotted in relation to year of study before onset of overt type I diabetes (insulin-dependent diabetes caused by autoimmune β-cell destruction). From Soeldner et al. (40). Reprinted by permission of the *New England Journal of Medicine*.

type I diabetes in both ICA$^+$ and ICA$^-$ relatives; this finding helps us assess their risk of progression to overt diabetes (32–34,60). Two other centers have now evaluated this test in a significant number of ICA$^+$ relatives. Chase et al. (61) reported that when insulin secretion (sum of 1-min + 3-min insulin after intravenous glucose) falls below 25 μU/ml, overt diabetes is imminent in ICA$^+$ children but not in adults (61). Riley et al. (36) found that the first-phase insulin release (1 min + 3 min) after intravenous glucose was lower in siblings and parents who had ICAs than in those who did not. However, they observed considerable individual variation of insulin responses over time. Wide variability of insulin responses after intravenous glucose was also shown in nondiabetic individuals (63,64). Some of this variability may be explained by the large differences found in the insulin responses of children and adults of different ages and stages of development. Smith et al. (65) reported that first-phase insulin secretion increases during puberty, declines after puberty, and appears constant thereafter (65). Decreasing insulin response after puberty was also suggested by Lindgren et al. (66). This fall after puberty is an important observation and should not be mistaken in an individual for early β-cell failure unless autoimmune abnormalities are present. Another group observed a significant reduction of insulin secretion during an oral glucose tolerance test (OGTT) in prediabetic children when plasma glucose and HbA_{1c} were clearly normal (67).

The Boston-Sacramento family study has performed IVGTTs on 43 nondiabetic ICA$^+$ (>40 JDF units) first-degree relatives (mean age 24 yr) with a mean follow-up period of >3 yr. The sum of the 1- + 3-min insulin values after a rapid infusion of glucose (0.5 mg/kg body wt i.v. as a 25% solution) is used as an index of first-phase insulin response. The results are expressed as a percentile of the response in 225 healthy nonobese individuals with no family history of diabetes.

With our insulin radioimmunoassay, the 1st percentile for the sum of 1-min + 3-min insulin is 48 μU/ml. Nineteen of the 43 ICA$^+$ relatives who underwent an IVGTT have progressed to diabetes (Table 3). Thirteen of the 43 ICA$^+$ relatives had an IVGTT result <1st percentile at the initial test; 5 more relatives who had IVGTT values >1st percentile at the initial test reached the 1st percentile on follow-up. To date, 15 of these 18 (83%) relatives whose first-phase insulin release was <1st percentile have progressed to overt diabetes (11 of 13 [88%] with 1-min + 3-min insulin <1st percentile at initial test and 4 of 5 [80%] of relatives who reached the 1st percentile on follow-up) compared to only 4 of 25 (16%) individuals with a normal insulin response on IVGTT (Table 3). Of 4 individuals who progressed to diabetes without a documented IVGTT <1st percentile, 1 was last tested 3 yr before overt diabetes; the other 3 were children whose most recent IVGTT was done between 7 and 12 mo before diabetes. All 3 had a progressive rise in fasting blood glucose during the months preceding IDDM (see below). Thus, for an ICA$^+$ relative with IVGTT <1st percentile, the relative risk of progressing to overt diabetes versus ICA$^+$ relatives with IVGTT insulin response >1st percentile is 15 ($P < 0.001$). In ICA$^+$ children, progression to diabetes may be more rapid than in adults. Therefore, with youngsters, it may be necessary to repeat the IVGTT as frequently as at 3-mo intervals to document the loss of first-phase insulin release, which heralds imminent overt diabetes.

Once the first-phase IVGTT response reached the 1st percentile, there was little further variability, and the mean time to development of overt diabetes was 744 days (range 71–1533 days), although young children progressed more rapidly than adults. In our family study, 3 of 32 (9.4%) ICA$^+$ relatives not studied with an IVGTT and 8 of 4247 (0.19%) ICA$^-$ relatives have progressed to diabetes. To date, the number of new diabetic patients per patient-year of follow-up was 0.4 DM/patient-yr for ICA$^+$ relatives whose initial 1-min + 3-min IVGTT response was <1st percentile, 0.04 DM/patient-yr for ICA$^+$ relatives with an initial 1-min + 3-min IVGTT >1st percentile, and 0.0005 DM/patient-yr for ICA$^-$ first-degree relatives.

TABLE 3
Islet cell antibody positivity (ICA$^+$) and intravenous glucose tolerance test (IVGTT) results in Boston-Sacramento study of first-degree relatives (Δ = 4342) of type I diabetic patients

	n	Mean follow-up (yr)	DM	DM/patient yr
ICA$^+$ (1.7)				
No IVGTT	32	2.6	3 of 32 (9)	0.0400
Initial IVGTT				
<1st percentile	13	2.2	11 of 13 (86)	0.4000
>1st percentile	30	3.4	8 of 30 (23)*	0.0700
ICA$^-$ (98.3)	4267	2.7	8 of 4267 (0.19)†	0.0006

Percentages in parentheses. Type I, insulin-dependent diabetes caused by autoimmune β-cell destruction; DM, progression to diabetes. In subjects where no IVGTT was performed, subjects developed diabetes before IVGTT was scheduled, were not contacted, or refused test.
*Five relatives were >1st percentile at initial screening but reached 1st percentile during follow-up, and 4 of 5 (80%; 0.2 DM/patient-yr) progressed to diabetes.
†Two ICA$^-$ relatives who progressed to diabetes were positive for competitive insulin autoantibodies on their serum-screening sample.

Five ICA$^+$ first-degree relatives were more intensively studied with multiple IVGTT evaluations before overt type I diabetes (68). A total of 55 IVGTTs and 84 fasting blood glucose determinations have been made in these five patients over a period of up to 4 yr before diabetes. Although remaining within the normal range, fasting blood glucose exhibited a striking linear rise over time (P = 0.0001), as did the blood glucose level at 60 min on IVGTT (P = 0.002); this increase began ~1.5 yr before the onset of overt hyperglycemia in these patients, whereas no major change was seen in either value during the earlier period between 1.5 and 4 yr before overt diabetes. The 12 individuals with fasting blood glucose >6 mM developed overt diabetes within 1.5 yr (positive predictive value 100%). This suggests that the development of type I diabetes in ICA$^+$ relatives is a chronic metabolic process with progressive rise of glucose in most but not all ICA$^+$ relatives in the last 1.5 yr.

TRANSIENT HYPERGLYCEMIA IN CHILDHOOD

In a child, stress hyperglycemia or asymptomatic glycosuria may represent the earliest clinical manifestation of impaired β-cell function. Subclinical abnormalities of glucose tolerance were previously defined by various criteria as chemical diabetes. In a review of 212 such children, Rosenbloom et al. (69) found that only 10% progressed to IDDM during 1–17 yr of follow-up. Subsequently, the National Diabetes Data Group adopted a classification that demands more stringent criteria for diagnosing diabetes (70). The designation of impaired glucose tolerance was reserved for children with a fasting venous plasma glucose concentration <7.84 mM but whose plasma glucose level remained >7.84 mM 2 h after a standard oral glucose load. One study described an incidence of development of overt IDDM of 17% within 14 mo among children with impaired glucose tolerance (71). This risk is further increased to 26% when impaired glucose tolerance occurs in siblings of patients with IDDM (72). However, many of the children in these studies were evaluated because of symptoms suggestive of hypoglycemia rather than because of transient hyperglycemia.

We evaluated 30 children, all ascertained because of transient hyperglycemia, to determine the predictive value of immunologic markers of insulitis and two standard tests of β-cell function: OGTT and IVGTT (73). This patient population consisted of children 2–17 yr old referred to the Joslin Diabetes Center because of transient hyperglycemia (documented laboratory blood glucose >8.4 mM and follow-up fasting blood glucose <6.7 mM). IDDM developed in 8 of 30 (27%) of these children over a follow-up period of up to 4 yr; when diabetes developed, it did so within 1 yr. Cytoplasmic ICAs were measured in all children. Antibodies were detected in 2 of 30. Both of these children had a positive family history for diabetes, and both subsequently developed IDDM. We were able to test for IAAs (CIAAs) in 25 of the children. Two (8%) had levels above normal (49 and 235 nU/ml), and both developed IDDM, including 1 ICA$^-$ child. Of the CIAA$^-$ individuals, 3 of 23 (13%) developed IDDM. In all, of the 6 children who developed IDDM and in whom both ICAs and CIAAs were measured, 3 (50%) were found to have elevated antibody levels on either or both assays.

First-phase insulin release during IVGTT, measured as the sum of 1-min + 3-min serum insulin levels, was determined in 21 children. Five had a first-phase insulin release <1st percentile. One of these had the test repeated 1 yr later, reached a first-phase insulin release at the 3rd percentile, and remained well during 15 mo of follow-up. The other 4 (80%) developed diabetes mellitus within 9 mo. In contrast, none of the 16 children whose first-phase insulin secretion was >1st percentile developed diabetes in up to 48 mo of follow-up (mean ± SD 19 ± 9 mo). Nine of the 16 children with intact first-phase insulin release underwent repeated testing after 1–3 yr; all maintained their first-phase insulin release >1st percentile and have continued to be ICA$^-$ and CIAA$^-$. All children with immunologic abnormalities also had reduced first-phase insulin release when measured, and all developed IDDM within 10 mo. Impaired glucose tolerance, on the other hand, was not necessarily associated with a reduced glucose disappearance (K rate) nor did either test necessarily predict the development of IDDM. Table 4 summarizes the quantitative predictive value of the tests we evaluated. Although the immunologic markers had the highest positive predictive value, first-phase insulin release had the greatest overall accuracy (95% for a single IVGTT) and the highest negative predictive value (100%).

DUAL-PARAMETER MODEL FOR PREDICTION OF DIABETES ONSET

As discussed earlier, the marked relationship of the concentrations of IAAs to age at onset of diabetes suggested that the concentrations of these antibodies might reflect the rate of β-cell destruction in ICA$^+$ relatives. In addition, the loss of first-phase insulin secretion appeared to reflect the degree of β-cell destruction. If the above parameters correlated with the rate and degree of a destructive process, they could aid in predicting the time of onset of overt diabetes. We determined first-phase insulin secretion and IAA concentrations in 19 ICA$^+$ relatives who progressed to diabetes. Linear regression of these two variables (1 set of initial values for each relative who progressed to diabetes) versus the time to onset of overt diabetes was analyzed, and a linear correlation model for the prediction of diabetes was calculated (57).

The application of such a model to the development of type I diabetes implies that the rate of development of diabetes is functionally constant for each individual over long periods of time, with different relatives having

TABLE 4
Summary of predictive parameters in children with transient hyperglycemia

	Positive predictive value	Negative predictive value	Overall accuracy (%)
No tests done	8 of 30 (27)	22 of 30 (73)	
ICA$^+$	2 of 2 (100)	22 of 28 (78)	80
CIAA$^+$ (>39 nU/ml)	2 of 2 (100)	20 of 23 (87)	88
OGTT impaired*	6 of 11 (55)	13 of 14 (93)	77
IVGTT†			
$K < 1.2$	3 of 4 (75)	12 of 13 (92)	88
1- + 3-min insulin (<48 μU/ml)	4 of 5 (80)	16 of 16 (100)	95

Percentages in parentheses. ICA, islet cell antibody; CIAA, competitive insulin autoantibodies; OGTT, oral glucose tolerance test; IVGTT, intravenous glucose tolerance test. The positive predictive value is the number who became diabetic with an abnormal test result. The negative predictive value is the number who remained nondiabetic with a normal test result. Overall accuracy is the number who became diabetic with an abnormal test result and the number who remained nondiabetic with a normal test result for all tests.
*Glucose levels by National Diabetes Data Group criteria.
†Single initial IVGTT results.

different rates, and that the route to diabetes is similar in all patients despite their wide age range (from 4 to 68 yr).

CONCLUSIONS

Currently available immunoassays are beginning to be standardized. In laboratories where cytoplasmic ICA assays detect >40 JDF units, a positive result is highly predictive of autoimmune islet cell destruction. IAA assays are not yet standardized, but current ELISAs for insulin antibodies generally appear to give low positive predictive values, whereas detection of IAAs by radiobinding assays is associated with more rapid progression to diabetes in ICA$^+$ relatives.

Individuals identified by a combination of tests (>40 JDF units ICAs and/or radiobinding assay–determined IAAs) as being at high risk for the presence of autoimmune islet cell destruction can be further studied with a rapid IVGTT, because the loss of first-phase insulin release appears to reflect the degree of islet cell destruction. This information, together with an estimate of the apparent rate of ongoing destruction derived from the level of IAAs, appears to allow for prediction of an individual's approximate time for development of diabetes.

This combination of two highly specific immunologic tests with a measurement of first-phase insulin release during an IVGTT can be used to identify individuals at high risk of developing diabetes within 3 yr. In such a population, we can then evaluate the effectiveness of treatment protocols aimed at halting further β-cell destruction. Several immunotherapeutic modalities have been used in the past, but none have proved effective. When used alone, plasmapheresis, monoclonal antibodies, antithymocyte γ-globulins, prednisone, azathioprine, and niacinamide have all had partial and/or temporary effects at best. Although promising to be more effective, cyclosporin A has been associated with nephrotoxicity. When initiated after diagnosis of overt diabetes, treatment with low-dose cyclosporin A may not provide lasting benefit. With an improved predictive ability, it becomes possible to evaluate fairly rapidly (within 3 yr) whether a new treatment plan is effective in preventing overt diabetes.

In summary, assays are now available that have a high positive predictive value for autoimmune β-cell destruction. Standardization will be required before large-scale screening can be instituted, but currently, antibody testing in selected individuals has clinical utility (e.g., potential kidney- or pancreas-transplant donors, differentiating early type I from type II [non-insulin-dependent] diabetes) and can alert relatives to a higher risk for diabetes, potentially obviating severe metabolic decompensation as the initial presentation with diabetes. As immunotherapeutic protocols are developed, safe prevention of diabetes becomes a current research goal but is not yet a clinical reality.

Many of the assays we have discussed are not yet commercially available. A number of research programs such as ours screen first-degree relatives of type I diabetic individuals within the United States for autoantibodies and insulin levels without charge and also will perform and/or assist in performing rapid IVGTTs, also without charge. In addition, a number of research laboratories and commercial laboratories offer clinical ICA testing for a fee. Unfortunately, for most such assays, information is not available relative to the positive or negative predictive value of the assay utilized. The Immunology of Diabetes Workshop Committee with Dr. Noel Maclaren (University of Florida, Gainesville) is beginning to supply characterized ICA-containing serums on a routine basis for quality control of ICA assays and IAA assays. This will hopefully foster the availability of better-characterized assays. The Diabetes Clinical Research Unit (J.S.S.) at the University of California Davis Medical Center, Sacramento, is willing to provide reference serums for insulin radioimmunoassay calibration.

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