

Prediction and Prevention of IDDM—1991

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Although we can now identify some nondiabetic individuals who will subsequently develop clinical insulin-dependent diabetes mellitus (IDDM), our ability to predict subsequent clinical IDDM is far from perfect. In this article, we discuss the status of knowledge regarding the natural history of preclinical IDDM and discuss, especially in relation to predicting IDDM, the genetic, immunologic, and metabolic components of the IDDM disease process. *Diabetes* 40:943–47, 1991

In insulin-dependent diabetes mellitus (IDDM), the pancreatic β -cells are destroyed by an immune-mediated process, which usually occurs over an extended period. A large percentage of the functioning β -cells must be lost before hyperglycemia ensues, and in most cases, this β -cell destruction occurs over several years. During this time, patients are completely asymptomatic and euglycemic.

A major point of controversy is whether the immune-mediated β -cell destruction, once initiated, is relentless and progressive and always culminates in clinical IDDM or whether the disease process may wax and wane and sometimes go into temporary or permanent remission. Clinical IDDM would not develop in individuals with permanent remissions in the β -cell-destructive process. Eisenbarth et al. (1) have proposed that, in most individuals, the β -cell-destructive process in IDDM does not wax and wane but is linear, and they have developed a model for predicting the time of onset of clinical IDDM in islet cell antibody-positive (ICA⁺) first-degree relatives of IDDM patients. In contrast, our family study and data from several other groups strongly

suggest that prolonged and possibly permanent remissions in the IDDM disease process are as common as progressive β -cell loss leading to clinical IDDM.

The Barts-Windsor family study probably provided the first evidence that the IDDM disease process is not always progressive. During the initial 5 yr of follow-up, over half of the nondiabetic first-degree relatives of IDDM children who had initially been positive for complement-fixing ICAs became negative without developing IDDM (2). Although this observation was initially contested (3,4), we and others using standardized ICA assays have observed fluctuations in ICA (5). The Lyon study noted loss of ICAs in approximately one third of relatives after 6 mo of follow-up (6). Chase et al. (7) also reported loss of ICAs during 2 yr of follow-up. ICAs were found in nine children from the control population for the Swedish Childhood Diabetes Study; during 20–34 mo of follow-up, IDDM developed in two, but seven of nine became ICA⁻ and remained healthy (8). In the 10-yr follow-up report on nondiabetic patients with organ-specific autoimmune disease, such as Graves' disease, who were initially ICA⁺, ICA was persistently positive in only 61% (9). Most of the prospective studies have concentrated on individuals who were ICA⁺ on initial screening. In contrast, Spinass et al. (10) measured ICAs at 0, 6, 12, 24, 36, 48, and 60 mo of follow-up in 179 nondiabetic first-degree relatives of IDDM patients enrolled without knowledge of ICA status. Thirteen percent of the relatives were ICA⁺ at some time point over the 5 yr, but only 2.7% were positive throughout (10). These fluctuations in ICAs may reflect underlying fluctuations in the activity of the IDDM disease process similar to other autoimmune diseases such as rheumatoid arthritis and Hashimoto's thyroiditis in which antibody titer correlates with disease activity. This hypothesis is supported by the data of Kobayashi et al. (11). They identified a group of patients who were clinically non-insulin-dependent but who were ICA⁺. Some of them became ICA⁻ during prospective follow-up. In these subjects, C-peptide and 2-h blood glucose levels during oral glucose tolerance tests improved significantly, whereas, this was not observed in the patients who remained ICA⁺ (11).

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Comparable data showing improved β -cell function with loss of ICA positivity during prospective evaluation in the pre-clinical period of IDDM are not available. However, the natural history of β -cell destruction appears to be faster in children (12), and a slow and remitting course is more likely in older individuals.

Studies in identical twins probably provide the strongest evidence that remissions in the IDDM disease process are relatively common. Only 30–40% of identical twins are concordant for IDDM, but discordant identical twins are not normal immunologically or in terms of β -cell function, and the risk for concordance decreases dramatically with the duration of discordance (13). This temporal pattern is the opposite of what would be expected if the β -cell-destructive process were ongoing or progressive and strongly suggests the concept of a period of “attack” and then remission. These twin studies also provide evidence that immune changes associated with IDDM may remit without causing disease. Increased activated T lymphocytes, ICAs, or both were found in 85% of nondiabetic identical twins of recently diagnosed IDDM patients (<5 yr), whereas, these immune markers were uncommon in nondiabetic identical twins of IDDM patients discordant for ≥ 11 yr (14). β -Cell function has also been evaluated in nondiabetic identical twins of patients with IDDM selected for having been ICA⁺ in the past and for long-term discordance. Because these twins were unlikely to develop IDDM, the observed β -cell dysfunction suggests prior β -cell damage, which is nonprogressive (15). These observations strongly support the conclusions we reached from our family study. By using detailed evaluation of β -cell function, which takes into account each person’s insulin sensitivity, we have shown that a degree of subclinical β -cell dysfunction is in fact very common among first-degree relatives of IDDM probands but that, in many cases, it is nonprogressive (16). In the animal models of IDDM, progressive β -cell destruction appears to be controlled by the relative balance between immunologic effector and suppressor mechanisms. In some strains of animals, the suppressor mechanisms dominate to prevent diabetes (17). In first-degree relatives of IDDM patients who have been discordant for a long period, who are positive for only one antibody, especially if in low titer, and who have relatively normal residual β -cell function, it is probable that similar suppressor mechanisms are operative, and progression to clinical IDDM is unlikely.

In summary, although it is possible that the decline in β -cell function may be relentless and linear in some individuals, we believe that this is probably uncommon. More often, clinical IDDM is the consequence of a series of attacks on the β -cell or of periods of activity and quiescence in the disease process. In fact, in many genetically susceptible individuals (50–70% for monozygotic twins), the attack becomes nonprogressive and does not progress to clinical IDDM.

GENETIC MARKERS

More than 90% of IDDM patients are either DR3 and/or DR4, and even stronger associations have been found for certain alleles at the DQ locus. There are two postulated mechanisms to account for the strong association between certain HLA types and IDDM. T-lymphocyte receptors (TCRs) recognize antigens in conjunction with HLA class II molecules,

and hence, many investigators believe that the ability of macrophages to present the critical antigens of the IDDM disease process to helper T lymphocytes depends on which HLA class II molecules the macrophages are genetically programmed to express (18). This interaction of antigen with HLA class II molecule is critically dependent on the three-dimensional structure of the HLA molecules, and this in turn is dependent on the amino acid composition of its α - and β -chains. A strong association of IDDM with the absence of one particular amino acid at position 57 on the DQ B chain has been found (19), but more recent data suggest that, although this amino acid is important, so are other amino acids, especially in the A chain (20). In addition, there may be alterations in the regulatory regions of the A- and B-chain genes, which modify expression of the protein molecules. The recent proposal that HLA-DR is primarily utilized for antigen presentation to helper T lymphocytes, whereas HLA-DQ is utilized primarily for presentation to suppressor/inducer cells adds further complexity to this area (21). Nepom (22) recently proposed a fascinating explanation for the varying susceptibilities associated with HLA antigens. He suggests that certain HLA alleles may confer protection by binding the diabetogenic antigens intracellularly with high affinity. This would prevent their surface expression and subsequent presentation to the immune system. It may be that, in addition to inheriting a particular susceptibility gene, it is the lack of one or more crucial protective genes that allows the autoimmune destructive process to become persistent.

The alternative hypothesis to explain the close association of HLA and IDDM is that other genes are closely associated with HLA, and it is these genes that actually predispose to IDDM. The genes for heat shock proteins and tumor necrosis factor are located within the HLA region (23). Tumor necrosis factor has multiple immunologic effects and has direct effects on β -cells, and some investigators believe heat shock proteins are important β -cell antigens in the IDDM process (24). Of course, it is possible that both mechanisms may be important, but currently, we favor the role of class II molecules in antigen presentation.

Although certain HLA types (DR3 and/or DR4) are essentially required for IDDM, they are not sufficient; consequently, the clinical utility of HLA determinations in predicting subsequent clinical IDDM is minimal. All of the HLA haplotypes and alleles associated with IDDM are common in the general population, and because IDDM is relatively rare, knowing a person’s HLA type has little predictive value for subsequent clinical IDDM. But, HLA typing might be useful for identifying a population for screening with immune markers (discussed further under THERAPEUTIC DILEMMA). In contrast, because nearly all IDDM patients are DR3 and/or DR4, the absence of these alleles could be reliably used to predict that an individual otherwise thought to be at high risk will not develop clinical IDDM.

IMMUNE MARKERS

The first autoantibody found to be predictive of IDDM was ICA, and except for minor modifications, the assay is still performed as originally described (25). Two major limitations of this assay must be remembered when evaluating results. Even with standardized procedures for preparing the pancreas, there is still considerable variability between pancre-

ases in how well they stain in the ICA assay. This results in variability between laboratories because they invariably use different pancreas specimens. Individual laboratories also frequently experience variability when they are forced to change from one pancreas to another. The other major difficulty with the current ICA methodology is that the reading of the immunofluorescence is subjective and therefore prone to variability between readers. A series of international workshops sponsored by the International Diabetes Workshop (IDW) and the Juvenile Diabetes Foundation (JDF) have improved the standardization of the ICA assay (5). End point titers are compared to a single international reference standard, and values are reported in JDF units. Nonetheless, because of the intrinsic limitations of the assay, precise quantitation is less than ideal.

Several prospective family studies agree that, in nondiabetic first-degree relatives of IDDM patients, the presence of ICAs is associated with an increased risk of subsequent clinical IDDM. Three aspects of ICAs profoundly affect the magnitude of this increased risk, i.e., the predictive power of ICAs. For example, high-titer ICA (>80 JDF U) confers a much greater risk than low-titer ICA (<20 JDF U) (26). In addition, ICA appears to be more predictive in children than in adults. Furthermore, being persistently positive for ICA is much more predictive of subsequent clinical IDDM than being intermittently positive (27). Because of this temporal variability in ICA, and because of the technical limitations of the assay described above, all positive values should be repeated and assessed over an extended period (≥ 6 mo).

The other major immunologic marker available to identify individuals at high risk of subsequent clinical IDDM is insulin autoantibodies (IAAs). Nondiabetic first-degree relatives of IDDM patients with IAAs in addition to ICAs have a higher risk of IDDM than individuals who are ICA⁺ alone (28). Similar to ICAs, high-titer IAAs confer a higher risk of IDDM than low-titer IAAs (29). But in marked contrast to ICAs, IAAs alone appear to confer little risk of subsequent clinical IDDM (29). For several years, IAA has been measured by fluid-phase radioimmunoassays (RIAs) and by solid-phase enzyme-linked immunosorbent assays (ELISAs) with disparate results. The last IAA international workshop evaluated whether IAAs measured by ELISA versus RIA were more closely associated with IDDM. The results clearly showed that the IAAs found in newly diagnosed IDDM patients and prediabetic subjects could only be reliably detected by fluid-phase RIA (30).

Antibodies to a 64,000-M_r islet protein have also been found to be predictive of subsequent clinical IDDM; in fact, some data support this antibody as having greater predictive power than either ICAs or IAAs (31). The assay for measuring this antibody is complicated and technically very demanding; consequently, only a few investigators are able to perform these measurements. Glutamic acid decarboxylase has recently been identified as the 64,000-M_r islet protein (32). This should allow simplified assays for this antibody to be developed, and then this measurement will probably become clinically available.

METABOLIC MARKERS

Precise and reliable prediction of IDDM in some individuals may be very difficult because of variability in the natural

history of preclinical IDDM and may require sequential β -cell function testing to determine whether progressive β -cell destruction is occurring (33). Oral glucose tolerance tests are of little utility for this purpose, and detailed β -cell function tests with intravenous stimulation are required. We recently showed in an animal model of subclinical β -cell dysfunction that in vivo measurements of insulin secretion in response to intravenous glucose and arginine accurately reflect the reduction in pancreatic β -cell mass (34). One surprising finding from this study, however, was that the acute insulin response to glucose may be undetectable when 40–50% of the β -cells are still present, which argues against the dogma that 90% of β -cells need to be destroyed before clinical IDDM develops (34). Currently, these intravenous β -cell function tests are very poorly standardized between laboratories, and interpretation is hampered by the wide range of normal values reflecting, in part, the physiological differences in insulin release due to variability in insulin sensitivity between individuals. Our laboratory and several others are now simultaneously measuring insulin sensitivity and β -cell function in order to separate physiological from pathological changes. For example, if an individual is found to have a low insulin response to intravenous glucose, this could be a normal physiological response if he/she is a lean athletic individual who is highly insulin sensitive. Alternatively, the low insulin response could be a pathological consequence of β -cell damage. The only way to distinguish these would be to measure insulin sensitivity at the same time as insulin secretion. We have shown in normoglycemic adolescent baboons given streptozocin that periods of superimposed insulin resistance cause frank hyperglycemia (35). This again suggests that the degree of β -cell destruction necessary to produce clinical IDDM will vary depending on the subject's insulin sensitivity (Fig. 1, shaded area).

In summary, because the pancreatic β -cell is the target organ of the IDDM disease process, β -cell function tests are likely to become more standardized and more widely utilized as interest in screening for individuals at risk of subsequent IDDM increases.

THE THERAPEUTIC DILEMMA

The implications of all of these observations for the prevention of clinical IDDM are summarized in Fig. 1. It would clearly

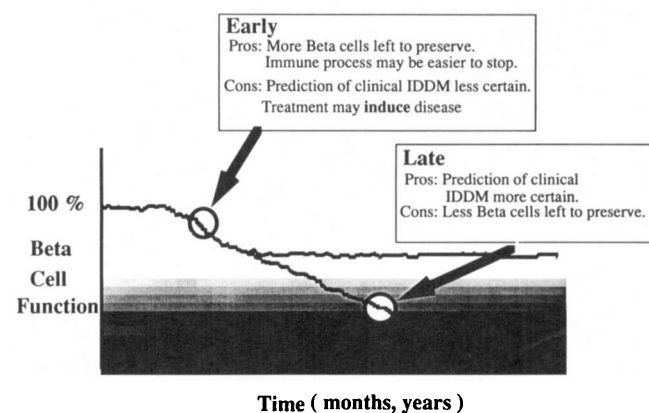


FIG. 1. Therapeutic dilemma for preclinical treatment to prevent insulin-dependent diabetes mellitus (IDDM).

be advantageous to intervene at an early stage in the disease process. There would be more β -cells to preserve, and it is likely that the autoimmune destructive process would be easier to stop. However, in this early period, with currently available techniques, immune markers may be absent, of low titer, or only intermittently positive. β -Cell function may be only slightly abnormal and nonprogressive. Intervention at this stage would certainly mean treating many people who would not have developed clinical IDDM if they had been left alone. This would only seem reasonable if such intervention were relatively innocuous. There is also concern that treatment at this stage might actually induce progression to clinical IDDM in someone who would otherwise have remained with nonprogressive subclinical β -cell dysfunction, as has been demonstrated in BB rats and NOD mice (36,37).

An alternative approach, proposed by a recently issued American Diabetes Association policy statement regarding preclinical IDDM, is to restrict intervention to a high-risk group (mainly 1st-degree relatives of IDDM probands) in whom immune markers are persistently present, in high titer and in combination, and in whom β -cell function is extremely low (38). It has been estimated that the likelihood of such individuals developing clinical IDDM within the next 3 yr probably approaches 90%. We believe that it is certainly justified to include such individuals in research protocols, especially immune-intervention studies, where detailed follow-up and evaluation will continue to improve our understanding of the pathogenesis of IDDM. However, the obvious disadvantage of such late intervention is that there is less β -cell mass to preserve and that the autoimmune process may be harder to stop at this stage. The disappointing long-term results from the cyclosporine trials in newly diagnosed IDDM patients underscore the problems of intervening too late (39,40).

One additional problem facing those of us wishing to prevent IDDM by preclinical intervention is how to move from selected high-risk groups (twins and 1st-degree relatives) to the general population, where 90% of new cases of IDDM arise. It has been calculated that, by use of a combination of tests (high-titer ICA, quantified IAA with fluid-phase assays, and 1st-phase insulin release after i.v. glucose <1st percentile), we can achieve a specificity of 99.75% and a sensitivity of 60% (41). When such tests are applied to first-degree relatives of IDDM probands, where the prevalence of clinical IDDM is ~3%, then the positive predictive value (true positives/false positives + true positives) is 88%. Indeed, calculations of this type constitute a large part of the justification for the American Diabetes Association's recent policy statement. However, if those same criteria were applied to the general population where the disease prevalence is ~0.3%, then the positive predictive value would fall to 42% (41). For every 100,000 people screened (Fig. 2), we would identify 180 for whom intervention is justified. However, we would fail to treat a further 120 who would be missed as false negatives. Probably worse, we would wrongly treat 249 individuals identified as false positives. Obviously, before prevention of IDDM becomes a reality on a large scale for the general population, our ability to predict clinical IDDM needs to improve substantially, and our intervention needs to be safe as well as effective. Regarding improved prediction, genetic screening of the general population may be-

For a population of 100,000 where 0.3% will get disease, if a test has 60% sensitivity and 99.75% specificity...

100,000	Number with CLINICAL DISEASE 300	Number without CLINICAL DISEASE 99,700
IDENTIFYING TEST +	TRUE POSITIVES 180	FALSE POSITIVES 249
-	FALSE NEGATIVES 120	TRUE NEGATIVES 99,451

POSITIVE PREDICTIVE VALUE = 42%

FIG. 2. Epidemiologic problem in predicting insulin-dependent diabetes mellitus in general population (derived from data in ref. 41).

come more common to exclude the $\geq 90\%$ who do not carry high-risk alleles. Regarding those who do have significant genetic risk, the continuing improvements in the specificity and sensitivity of immune markers should allow the identification of those on whom prospective β -cell function testing should be offered.

Until now, the main therapeutic strategies for prevention have used broad spectrum immunosuppression with agents such as azathioprine, cyclosporine, or nicotinamide. Although these approaches may be necessary in the later stages of preclinical IDDM, if intervention were applied much earlier, it might be possible to use more specific or targeted treatment. If glutamic acid decarboxylase is indeed the initiating antigen (32), techniques to block its presentation to the immune system might prevent IDDM. Therapies stimulating suppressor mechanisms that block or inhibit the IDDM disease process may become reality. There is evidence that suppressor T lymphocytes recognize TCRs on helper T lymphocytes rather than the antigen itself (42). The ability to prevent an animal model of multiple sclerosis by immunizing animals against the TCR, which recognizes the crucial antigen, offers promise for other autoimmune diseases such as IDDM (43). With continued efforts in many of the directions outlined in this article, we believe that the prediction and prevention of clinical IDDM will become a practical reality in the fairly near future.

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