

Prediction and Prevention of Type 1 Diabetes: Progress, Problems, and Prospects

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Type 1 diabetes mellitus (T1D) arises from selective immunologically mediated destruction of the insulin-producing β -cells in the pancreatic islets of Langerhans with consequent insulin deficiency.^{1,2} This occurs in genetically susceptible individuals and is a cellular-mediated process, presumably a specific reaction to one or more β -cell proteins (autoantigens), although probably initiated by some environmental factor(s). There is consequent progressive impairment of β -cell function and decline in β -cell mass. A secondary humoral immune response is characterized by the appearance of autoantibodies that serve as markers of the immune damage to β -cells.^{3–5} This insidious T1D disease process evolves over a period of years.⁶ The decline in β -cell function and mass is evidenced metabolically by loss of first-phase insulin response to an intravenous glucose challenge,^{7,8} and later by the appearance of impairment in glycemic regulation, manifested as dysglycemia—usually as impaired glucose tolerance, but occasionally as impaired fasting glucose.⁹ Ultimately, the clinical syndrome of T1D becomes evident when the majority of β -cells have been destroyed and frank hyperglycemia supervenes. Given this sequence of events, for which it is possible to envision intervention to interdict the process, it is not surprising that much research effort has been expended to identify individuals at risk of the disease.

PREDICTION OF DISEASE

Studies aimed at delay or prevention of clinical T1D are critically dependent on the ability to identify individuals at risk of the disease. Thus, prediction of T1D is crucial for prevention. Therefore, a significant amount of attention is given to identify potential risk markers and to quantify risk projection. The first large-scale studies of the prediction of T1D relied upon the detection of islet cell autoantibodies in relatives of individuals with T1D.^{3,4} This approach was also used in the general population.^{10,11} Although these studies

helped define our knowledge concerning progression to diabetes, the immunofluorescent assay is difficult to perform, varies widely among laboratories, and is dependent on the pancreas used for assessment. Modern approaches to antibody determination use radioassays for specific biochemical autoantigens, *e.g.*, insulin, GAD (glutamic acid decarboxylase), and IA-2 (an aborted tyrosine phosphatase).^{5,6} Prediction of development of T1D is based on risk assessment, which is accomplished using genetic, immunologic, and metabolic parameters. Prediction of T1D among relatives can be quite accurate, by combining screening of relatives by measurement of islet cell autoantibodies with subsequent assessment of insulin autoantibodies (IAAs), first-phase insulin response to intravenous glucose, and oral glucose tolerance, while excluding those relatives with the known protective genetic allele HLA-DQB1-0602.¹² Using this combination approach and screening approximately 100,000 relatives, it was possible to identify accurately two cohorts of relatives, one (339 individuals) with a projected 5-year risk of greater than 50% and actual 5-year risk of 60%,¹³ and another (372 individuals) with a projected 5-year risk of 25–50% and actual 5-year risk of 35%.¹⁴ It is obvious from the numbers cited that the effort is enormous and the yield is low. Moreover, only 10–15% of individuals with T1D have a known relative with the disease at the time they are diagnosed. Thus, screening of relatives will miss 85–90% of individuals developing the disease. Yet, to identify similar size cohorts from the general population would require screening 1–2 million people rather than 100,000.¹⁰ Thus, although it can be done, the effort is mammoth.

Some studies, particularly of infants, have used genetic screening of HLA to identify those with higher risk alleles (*e.g.*, DQA1*0301/DQB1*0302 or DQA1*0501/DQB1*0201) and have followed these individuals for development of autoantibodies and ultimately T1D.^{15,16} It is theoretically possible to screen all individuals at birth and thus identify those at risk. This approach would identify most (over 95%)

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Published online 28 March 2007. doi:10.1038/sj.cpt.6100179

of those destined to develop T1D, but would also identify a larger number of people who will not develop the disease.

Nonetheless, the studies performed to date have given us tremendous insight into the natural history of T1Ds.^{6,17,18} As a consequence, at present we can predict the development of T1D. Ideally, we would like to couple such prediction with prevention, but unfortunately we do not yet have a safe and effective preventive therapy.

PREVENTION STRATEGIES

Much investigation has been directed at interdicting the T1D disease process, both during the stage of evolution of the disease and at the time of disease onset.^{19–21} The goal of intervention before disease onset is to arrest the immune destruction and thus delay or prevent clinical disease.²¹ The goal of intervention at disease onset is to halt the destruction of remaining β -cells, perhaps allowing these residual β -cells to recover function, hopefully lessening the severity of clinical manifestations and disease progression.²² These studies attempt to preserve the residual β -cell function that remains. The goal is to modify severity of clinical manifestations, halt the destruction of β -cells, and perhaps allow residual β -cells to recover function. Success is measured by preservation of C-peptide.

The earliest studies of immune intervention were begun at or shortly after diagnosis of clinical T1D. Such studies have the advantage that research subjects have an unambiguous diagnosis, but have the disadvantage of having fewer β -cells to preserve. The use of immunosuppression at the time of onset of T1D was studied in randomized controlled clinical trials by several groups in the 1980s using cyclosporine^{23–25} and azathioprine (either alone or in combination with glucocorticoids).^{26–28} These studies clearly confirmed that T1D was immunologically mediated as they unequivocally demonstrated that such immune intervention prolonged β -cell function, and thus established that human T1D indeed is immune mediated. Unfortunately, the effects were not sustained when immune therapy was discontinued and the side effects, especially with cyclosporine, were not tolerable.^{29,30} There was also dissatisfaction with the magnitude of the improvement in β -cell function. This led to a dilemma. One possibility was that this type of potent immunosuppression might work better if initiated earlier—before the clinical onset of T1D. This would be earlier in the disease process when β -cell function and mass were better. Yet, to do so would run the risk of exposing to treatment some healthy individuals who might never develop T1D or might not develop it for many years. After much debate, it was felt that the best balance was to indeed initiate intervention earlier but to select interventions that were considered much safer. Thus, three large-scale studies were initiated—with the B vitamin nicotinamide, which since the 1940s had been shown to arrest development of T1D in experimental animals; and with insulin, both parenteral and oral. Parenteral insulin had been shown to prevent diabetes in two animal models of T1D, both BB rats^{31,32} and non-obese diabetic mice.³³ Small pilot

studies also suggested that it would work in human beings.^{34,35} The concept of oral tolerance was popular,³⁶ and oral insulin was effective in preventing diabetes in animal models of T1D.^{37–39}

Major collaborative efforts were initiated to study these interventions. The European-Canadian Nicotinamide Diabetes Intervention Trial (ENDIT) screened more than 30,000 eligible relatives through 354 clinical centers in 20 countries, and randomized 552 eligible participants.⁴⁰ The Diabetes Prevention Trial—Type 1 (DPT-1), undertaken to study both parenteral and oral insulin in two cohorts concomitantly identified, screened more than 97,000 eligible relatives at 360 locations in North America, and randomized 711 eligible participants—339 in the parenteral insulin study¹³ and 372 in the oral insulin study.¹⁴ In all three of these cohorts, T1D developed as was predicted from the baseline assessment (5-year rates of development of 30, 60, and 35%, respectively). Unfortunately, the rate of development on the treated and control groups within each of these studies was superimposable; there was no benefit of treatment in the cohorts as a whole. So, what went wrong?

The parenteral insulin study was based on very tiny pilot studies—only five treated subjects (one of whom would not have been eligible for the DPT-1 study because of protective HLA) in one study³⁴ and only seven treated subjects in the other study.³⁵ An important lesson is that clinical practice should not be altered solely on the basis of small pilot studies. During the course of conducting DPT-1, a number of individuals, either of their own accord or influenced by their physicians, declined randomization, as they believed that the pilot studies had already answered the question and that the DPT-1 trial was merely confirmatory. Well-designed, randomized, controlled clinical trials are crucial before implementation of clinical practice guidelines or public health practices.

The oral insulin trial had other issues. It was initiated without any pilot experience in human beings—could it have been that the theory was completely without basis? Not so. Although in the primary analysis of relatives selected and randomized in DPT-1, oral insulin did not delay or prevent development of diabetes, there was a subgroup in whom a potential beneficial effect was seen.¹⁴ Ironically, that subgroup was the very one that the investigators had set up the trial to study. Yet, owing to lagging enrollment, midway through recruitment the investigators initiated a protocol change that relaxed the entry criteria. The threshold for titer of IAAs eligible for inclusion was reduced from >80 nU/ml (5 SDs above the mean of healthy controls) to >39 nU/ml (3 SDs above the mean of healthy controls). However, during analysis it was noted that there was greater variability in the IAA assay for values 39–79 nU/ml than for values >80 nU/ml, particularly in confirmation of a positive result (98.7% overall confirmation for values >80 nU/ml compared to 70.6% for values 39–79 nU/ml). This prompted comparison of the rate of evolution of diabetes by entry IAA level. The cohort with confirmed IAA >80 nU/ml (the original entry

IAA criterion) progressed to diabetes at a faster rate than those subjects who did not have confirmed IAA > 80 nU/ml. In addition, those with confirmed IAA > 80 nU/ml had other risk characteristics that suggest more rapid evolution to diabetes, including younger age, greater likelihood of having other antibodies, and greater loss of β -cell function (lower levels of plasma C-peptide in response to several provocative challenges). It was then found that the effects of intervention in each of these two subgroups differed. The group with confirmed IAA > 80 nU/ml showed a beneficial effect of oral insulin – one that suggested a potential delay of development of diabetes of 4–5 years. Subsequent analysis showed that the treatment effect was even more dramatic in those with IAA > 300 nU/ml. Moreover, in an analysis confined to subjects randomized before the change in IAA criterion (all of whom had confirmed IAA > 80 nU/ml), the results were comparable to those seen in all subjects with confirmed IAA > 80 nU/ml. This is an obvious lesson for clinical trialists not to tamper with the trial design because enrollment is lagging. One might hypothesize that there might have been a clear beneficial result in the overall trial if the IAA entry criterion had not been changed. However, as none of these subgroup analyses were pre-specified, the results suggesting a potential beneficial effect in the subgroup with baseline confirmed IAA > 80 nU/ml can only be deemed hypothesis-generating and not a positive outcome. As a consequence, the successor study group to DPT-1, the Type 1 Diabetes TrialNet Clinical Trials Network, has initiated further studies to explore the potential role of oral insulin in delaying or preventing T1D in relatives with risk for diabetes similar to those in the DPT-1 subgroup. The problem is that it will take a worldwide screening effort to identify and quantify risk in 20,000 relatives a year for 5 years, with the study lasting another decade.

Other prevention studies are directed at newborns with genetic risk factors. On the basis of the concept that removal of cow milk proteins from the diet may protect β -cells, a multi-national randomized prospective study, the Trial to Reduce Incidence of Diabetes in Genetically at Risk (TRIGR), has been initiated to determine whether the frequency of T1D can be reduced by preventing exposure to cow milk proteins during early life.⁴¹ Before birth, mothers agree to randomization of their offspring—first-degree relatives with high-risk HLA alleles—either to a formula containing cow's milk or a formula with casein hydrolysate. Mothers in both groups are permitted *ad lib* breast feeding and introduce the formula only when they choose. The plan is to screen 6,800 babies and enroll approximately 2,400 with high-risk HLA alleles.

A pilot study, undertaken by the Type 1 Diabetes TrialNet Study Group, is examining whether addition to formula of the omega-3-fatty acid DHA (docosahexaenoic acid) can delay appearance of autoimmunity.

Meanwhile, there has been a resurgence of interest in studies in new onset T1D, for several reasons: (1) such individuals are readily identified; (2) there is the potential that prolonging the remission period will result in a milder

course of diabetes, in terms of both glycemic control and development of complications; (3) demonstration of some preservation of β -cell function with a given agent would give impetus to embarking on the more complicated prevention studies with that agent; (4) such studies help define risks and benefits.

Several studies have used short-term interventions designed to alter the immune response in a manner that might result in sustained beneficial effects without continuous exposure to the intervention. An immunomodulatory peptide from heat-shock protein-60 (p277 peptide) has shown suggestive evidence of better preservation of C-peptide in recent onset T1D.⁴² Likewise, a GAD vaccine showed promise in small pilot studies.⁴³ Two studies in new onset T1D with anti-CD3 monoclonal antibodies have shown preservation of β -cell function.^{44,45} The question with all of these is: will the effects be sustained beyond the initial period for which results have been reported? This is confounded by the relatively rapid decline in β -cell function in the control group in each of these studies, which results in statistically significant benefit, but uncertain clinical effectiveness.

Studies with newer immunosuppressive/immunomodulatory agents (e.g., mycophenolate mofetil, anti-IL2-receptor monoclonal antibodies, anti-CD20 monoclonal antibodies) also have been initiated. Additional studies are being conducted with various antigen-specific approaches.

Thus, the field is flush with clinical trials. There has been dramatic progress in the initiation of studies designed to interrupt the T1D disease process. Ultimately, this will lead to successful strategies to delay and prevent T1D.

CONFLICT OF INTEREST

The author declared no conflict of interest.

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