GAD IN METABOLIC & NEUROLOGIC DISEASE

DIABETES AND AUTOANTIGEN-SPECIFIC THERAPY

BREAKING NEWS:

GAD65 Phase II trials offer hope in autoimmune diabetes
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The potential for therapy in human autoimmune diseases has recently been demonstrated by the highly useful application of tumour necrosis factor (TNF) blockade in Rheumatoid Arthritis patients. The effects have been quite remarkable, patients’ quality of life improving immensely. However, as the number of patients treated has increased, unwanted side-effects, particularly in the form of susceptibility to infection, have become apparent with use of this ‘general’ immunosuppressive agent. This will always be a worry when tampering with general immune mechanisms that have been developed throughout centuries into their present-day forms. There is thus still clearly a need for disease-, organ- and autoantigen-specific therapies. The Diamyd™ vaccine is a good example of such a tailor-made approach for Type 1 diabetes.

Glutamic acid decarboxylase 65 (GAD65) is a candidate autoantigen implicated in autoimmune Type 1 diabetes. During the last 10 years, intensive efforts have led to development of high quality recombinant GAD65 proteins, which have been tested in Phase I and Phase II clinical trials. Diamyd Medical has recently finalized a successful Phase II study in Type 2 diabetes patients with GAD antibodies (Type 1.5 diabetes, LADA) that demonstrated clinical safety of Diamyd™, preservation of beta cell function and an increased time to insulin requirement. This improved clinical scenario was associated with induction of CD4+CD25+ regulatory T cells.

It is now clear to the immunological research community that there are different subsets of regulatory T cells and that a particular subset may be important in a particular organ and disease setting. That an autoantigen formulated with the adjuvant aluminium hydroxide can seemingly induce regulatory T cells is a novel finding that indicates the potential feasibility of this approach with other autoantigens in other human autoimmune diseases.

For those who champion an autoantigen-specific therapeutic approach, the Diamyd clinical trial should be regarded as a landmark in the field.
Diabetes and Autoantigen-specific Therapy

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DIABETES
Diabetes and its complications account for 1 out of 7 dollars spent on healthcare (more than US $100 billion p.a.) in North America. Presently, diabetes affects more than 17 million individuals in North America, about 90% of whom have Type 2 diabetes (T2D, insulin-independent) and 10% of whom have Type 1 diabetes (T1D, insulin-dependent). In 2003, the World Diabetes Foundation estimated there are 193 million individuals with diabetes (principally T2D) aged 20-79 in the world. The corresponding number of subjects with impaired glucose tolerance (IGT) is 314 million. The World Health Organization projects that by 2025 these figures will increase to 333 million and 472 million, respectively. The complications that arise from diabetes include heart disease, kidney disease, blindness, nervous system disorders, amputation and gum disease. Alarmingly, the total costs to the U.S. economy alone are projected to be $132 billion by 2010. Thus diabetes is one of the most costly diseases globally in both human and economic terms. Currently, a large and growing population is seeking prevention and cure of this debilitating medical condition. This has created a large critical unmet need for improved treatment strategies for the rapidly increasing number of patients with autoimmune diabetes that emerges in patients with T1D; recurrent T1D that develops in recipients of kidney/islet transplants; T2D that develop Latent Autoimmune Diabetes in Adults (LADA) and become insulin-dependent; and impaired glucose tolerance (IGT) that may give rise to T2D and LADA.

RECURRENT DISEASE
The major complications of T1D include failures in kidney and pancreatic islet function, and patients who experience this dysfunction are candidates for kidney and/or islet transplants. Recently, significant advances have been made in achieving successful kidney and islet transplantation in the clinic. However, T1D patients with advanced kidney and pancreatic disease who undergo kidney or islet transplantation need to be maintained on high dose anti-rejection drugs for the remainder of their lives. These drugs are required to suppress the immune system, not only to prevent rejection but also to block recurrent autoimmune islet destruction and T1D that often arises in post-transplant patients. Thus to avoid recurrent T1D, a therapy that modulates/protects against autoimmune responses targeted to islet beta cells and that minimizes/avoids the adverse effects (e.g. frequent infection) of currently used anti-rejection drugs will predictably have wide application in the prevention and treatment of T1D, recurrent T1D (transplant recipients), T2D (LADA patients) and IGT. Diamyd Medical AB’s (Diamyd) lead therapeutic product, Diamyd™, is a GAD65-based therapeutic (GAD65 formulated in alum) autoantigen that may benefit each of these market needs.

AUTOANTIGEN-SPECIFIC THERAPY
A major aim in studies of human T1D (and T2D with autoimmunity) is to identify the target autoantigen(s) that drives the pathogenic insulin-producing islet cell autoimmune T cell-mediated immune responses. To identify an autoantigen in T1D, clinical trials that test a given antigen-specific therapy are required in which tolerization to the autoantigen either protects against T1D prophylactically or prevents recurring autoimmunity in patients with established disease. Identification of an autoantigen that elicits this process is expected to considerably advance our understanding of the pathophysiology of this disease and the possibility of immunotherapeutic interventions.

“Diamyd’s ongoing GAD65-based clinical trials offer much hope that antigen-specific therapy may prove efficacious in the treatment and prevention of autoimmune pathology arising in patients with T1D and T2D.”

GAD65, which is expressed in all human islet cells, is like insulin the target of diagnostic serum autoantibodies in T1D. Support for the exciting possibility that GAD65 is a leading candidate islet autoantigen is provided by several encouraging observations from recent Phase II clinical trials conducted by Diamyd using their lead product Diamyd™ (GAD65 islet autoantigen) in attempts to tolerize and prevent further beta cell destruction in T2D patients with LADA, as outlined below.

MILESTONES ACHIEVED WITH DIAMYD™
During the last three years, Diamyd has achieved major milestones with the use of Diamyd™, including:

■ In 2003, Diamyd reported positive 6 month results from a Phase Ia clinical trial conducted with Diamyd™ administered to 47 patients with LADA. At 6 months, after only two treatments with Diamyd™, a significant improvement in beta cell function was observed. Moreover, an increased number of regulatory T cells (which may protect from disease) of the immune sys-
tem was found in the blood of these patients at this time.

Follow-up studies in 2004 recently showed that not only islet beta cell function significantly augmented, as reflected by increases in insulin and A1c levels, but importantly these levels were maintained for more than 2 years. Thus antigen-specific therapy achieved through treatment with Diamyd™ can enhance islet beta cell survival and function for at least 2 years. Conceivably, this may result in the administration of only a few injections of Diamyd™ during the lifetime of a patient with T2D, representing a considerable reduction in the number of insulin injections otherwise required by such T2D and LADA patients. These very promising results demonstrated that Diamyd™ is well tolerated, safe to administer and efficacious.

These findings were recently submitted for publication by Diamyd-associated investigators [Agardh et al., Clinical evidence for safety of GAD65 immunomodulation in adult-onset autoimmune diabetes, J. Diabetes Complications (2005), in press.]

In 2004, a Phase IIb clinical trial was approved by the Medical Products Agency (Uppsala, Sweden) and Institutional Review Board (Lund University; Malmo, Sweden) to be performed with Diamyd™ in 70 children newly diagnosed with T1D. The aim is to investigate whether the survival and function of residual insulin-producing islet beta cells at the time of onset of T1D can be maintained and/or enhanced in these children after two injections of Diamyd™. This trial is currently underway and may enlarge the market niche of Diamyd™ to include patients with recent onset T1D.

In 2005, based on the promising results from Diamyd’s Phase II clinical trial, Diamyd™ was entered into a double-blind, placebo-controlled Phase II/III clinical trial designed for the treatment of adult T2D patients and subjects with IGT. More than 2,000 T2D patients applied for entry into this trial. Screening of the 160 patients positive for serum GAD autoantibodies (LADA patients) to be enrolled is underway, and treatment of such positive patients with Diamyd™ has commenced. The aim of this trial is to demonstrate the ability of Diamyd™ to down-regulate the autoimmune attack that elicits islet beta cell destruction.

Thus, Diamyd’s ongoing GAD65-based clinical trials offer much hope that antigen-specific therapy may prove efficacious in the treatment and prevention of autoimmune pathology arising in patients with T1D and T2D.
Latent Autoimmune Diabetes in Adults (LADA)

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Latent Autoimmune Diabetes in Adults (LADA).

At least one-third of patients diagnosed with autoimmune diabetes are diagnosed after age 35 and 10%-15% of adult onset diabetes may be autoimmune. Whether LADA is a late-onset slowly progressive form of Type 1 diabetes or a separate clinical entity is disputed. What is not disputed is that LADA has genetic, autoimmune and clinical features that are different from those of early onset Type 1 diabetes.

Subjects with LADA have lower frequencies of the high risk HLA alleles (DR3/DQ*6201 and DR4/DQ*0302). LADA patients also have lower levels of islet cell and insulin antibodies but have varying titers of glutamic acid decarboxylase (GAD) antibodies. Clinically LADA usually presents in an insidious way which may be indistinguishable from the presentation of Type 2 diabetes.

LADA AND ANTI-GAD ANTIBODIES
The hallmark of LADA is the presence of anti-GAD antibodies. 1% of the non-diabetic population have anti-GAD antibodies simply because reference laboratories fix their upper limit of normal at that level. Therefore, 1 in 100 truly Type 2 diabetic subjects will have detectable anti-GAD antibodies. However, at least early in the course of Type 2 diabetes this will be accompanied by high normal or even elevated C-peptide levels (a measure of endogenous insulin production) whereas with the LADA patient the C-peptide levels will be low or low-normal.

In the United Kingdom Prospective Diabetes Study (UKPDS) of 3,762 white subjects with recently diagnosed Type 2 diabetes (based on clinical criteria) 10% had anti-GAD antibodies at the time of diagnosis. A Tasmanian study of 1232 patients with adult-onset diabetes reported that 36% of men and 34% of women were GAD antibody positive. In 102 Finns with an onset of diabetes after age 35, assumed to have Type 2 diabetes and therefore not treated with insulin, GAD antibodies were associated with subsequent early insulin deficiency and the need to utilize insulin. In those requiring insulin, 75% were GAD antibody positive compared with 12% in those who did not need insulin. A similar Swedish study showed 70 of 97 patients assumed to have Type 2 diabetes at onset after 6 years required insulin and GAD antibodies were present in 60% of these patients compared with 7% in those who did not require insulin.

Although studies of European populations have generally shown that 10%-20% of late onset diabetes is GAD positive, the presence of GAD antibodies is rare in Filipinos and subjects of African origin. Conversely, 16% of late-onset diabetes in Chinese subjects is GAD positive.

While LADAs in general have lower frequencies of the high risk HLA alleles present in early onset Type 1 diabetes, a study in Finland has shown a strong correlation between these alleles and GAD antibodies. Furthermore, T lymphocytes, the pathological hallmark of autoimmune diabetes, were observed in a pancreatic biopsy specimen of a GAD positive 65 year old with
late onset diabetes and retained endogenous insulin production.

THE CLINICAL PRESENTATION AND COURSE OF THE LADA PATIENT
In the UKPDS, the presence of GAD antibodies at onset was predictive of the need for insulin therapy at six years. In a New Zealand study of 1,148 subjects with what was presumed to be Type 2 diabetes within one year of diagnosis, 14.4% were GAD positive and of these 83% required insulin within a year of diagnosis. In Sweden, if GAD antibodies were positive in those with a typical Type 2 presentation 60% required insulin within 12 months.

Although most patients with LADA are thin, the disease can occur in patients of any body weight. Furthermore, because of the slower destruction of the beta cells, the patient with LADA can for some time be maintained in excellent glycemic control with lifestyle change (diet, exercise and weight loss) and/or oral anti-diabetic therapies. For example, a group of Finish women with LADA were maintained in reasonable glycemic control for as long as 10 years before insulin was needed.

Furthermore, as in the Type 2 diabetic patient, because of the slow destruction of the beta cells acute symptoms (polyuria, polydipsia and weight loss) are rare. Therefore, diabetes is present for several months or years before it is diagnosed. A common symptom in early diabetes is reactive hypoglycemia due to the loss of the first phase insulin response, hyperglycemia and delayed excessive insulin production. As with Type 2 diabetes, recurrent or recalcitrant yeast vaginitis may be a presenting feature. Realization of previous ill health and improved well being almost invariably occurs with glycemic control.

Therefore, the clinical presentation of LADA in most cases is indistinguishable from that of Type 2 diabetes. However, an acute onset especially in a non-obese subject should lead to the suspicion that the diagnosis is LADA rather than Type 2 diabetes.

DIAGNOSING LADA
The most sensitive test for the diagnosis of LADA is the presence of anti-GAD antibodies. If LADA is to be diagnosed this test should be performed in all subjects with adult onset diabetes since the clinical presentations of LADA and Type 2 diabetes are so similar. However, anti-GAD antibody testing is expensive and if another cheaper screening test could be utilized this would be more cost effective.

Our group has found that a random serum C-peptide level is an excellent screening test. C-peptide is the remainder of the proinsulin molecule when the alpha and beta chain of the insulin molecule separate and because of its longer half-life is a better measure of endogenous insulin production than a serum insulin level. On measuring a random C-peptide level we found that in GAD antibody positive subjects i.e. LADAs, C-peptide levels were low or in the normal range whereas in Type 2 patients i.e. GAD antibody negative, C-peptide levels were normal or high.

Therefore, a high C-peptide level which often occurs in Type 2 diabetes at onset rules out LADA and anti-GAD antibody levels need not be performed. However, if the C-peptide level is low or in the normal
range then an anti-GAD antibody level should be measured.

Another test that may be helpful is the HDL to triglyceride ratio. If this ratio exceeds 4 then the patient is likely to be insulin resistant and therefore less likely to have LADA. Behme et al have shown that insulin resistance is lower in LADA, similar to that found in long-term Type 1 diabetes and lower than that found in Type 2 diabetes.

WHEN LADA IS DIAGNOSED

If diagnosing LADA only resulted in the knowledge that insulin therapy would be needed at an earlier time than in the Type 2 patient irrespective of the therapy utilized, then diagnosing LADA would be no more than an academic exercise. However, if early utilization of insulin results in preservation of the insulin secreting pancreatic beta cells, resulting in better glycemic control and fewer diabetic complications, then diagnosing LADA is of the utmost importance.

The autoimmune attack on the pancreatic beta cells is only directed at the insulin producing cells. Therefore, by taking over the function of these cells by injecting exogenous insulin, endogenous insulin production declines or ceases protecting the beta cells from the autoimmune “attack”. Alternatively, if secretagogues, the most common therapy for Type 2 diabetes, are utilized the stimulated beta cells will be the subject of a more intense “autoimmune attack”, and will progress more quickly to the point of total destruction. Therefore, with secretagogues insulin not only will be needed at an earlier stage but glycemic control will worsen resulting in earlier and more severe diabetic complications.

The protective effect of insulin and the detrimental effect of secretagogues is based on the belief that only active beta cells are attacked and destroyed by the immune system. Animal studies have shown that stimulating insulin secretion results in the release of insulin-containing secretory granules which have antigenic properties. In the animal models of autoimmune diabetes (NOD mice and BB rats) administration of oral or subcutaneous insulin has been shown to prevent diabetes. In islet cell antibody positive diabetic subjects with the clinical features of Type 2 diabetes, islet cell destruction was slowed by daily administration of small doses of subcutaneous insulin. Early insulin administration may also be protective because insulin is an immunomodulator increasing the production of TH2 cytokines which protect the beta cell from autoimmune damage.

However, the evidence that insulin administration can suppress autoimmune destruction of beta cell in humans is far from proven or complete. In the Diabetes Prevention Trial, in antibody positive and endogenous insulin deficient relatives of Type 1 diabetic patients (a high risk group) insulin therapy did not prevent or even delay the clinical onset of Type 1 diabetes. However, with Type 1 diabetes there is a more intense and rapid autoimmune destruction of the pancreatic beta cells than that which occurs with LADA. Therefore, failure of insulin therapy to prevent Type 1 diabetes does not mean that insulin therapy will not avoid or delay the onset of complete beta cell destruction in the LADA patient.
Therefore, at this point in time and until proven otherwise or if potential antigen specific therapies such as for example a beta cell tolerising GAD-based vaccine becomes available I continue to screen for LADA and if found I will avoid secretagogues and utilize insulin in these “at risk” patients. I also in all of these subjects in combination with insulin utilize thiazolidinediones (TZDs) which are the oral agent of choice in the LADA patient. While it is well recognized that in Type 2 diabetes that TZDs preserve or even rejuvenate the pancreatic beta cell, the evidence of this protective effect is lacking in Type 1 diabetes. TZDs protective effect on the Type 2 diabetic beta cell is thought to be mediated through lowering of free fatty acid levels and their metabolites within the beta cell which results in decreased production of nitric oxide and suppression of the accelerated beta cell apoptosis. However, TZDs are also anti-inflammatory and therefore in the LADA patient could also potentially alter the course of beta cell damage.

Recently, Zhou et al, in a small placebo-controlled trial, reported beta cell preservation with rosiglitazone in subjects with LADA. Therefore, the LADA patient may benefit from the anti-inflammatory as well as the decreased beta cell free fatty acid content when TZDs are utilized.

In conclusion, LADA is associated with the presence of anti-GAD antibodies and lower endogenous insulin production than is seen in Type 2 diabetes though the clinical features are very similar. Screening for LADA should be through measuring a C-peptide level in later onset diabetes which if normal or low should precipitate measurement of a GAD antibody level. When LADA is diagnosed in the diabetic patient insulin therapy and TZD utilization should result in a better outcome and secretagogue therapy should be avoided.

REFERENCES

As discussed throughout this monograph, the body of evidence suggesting an association between self-reactive immune responses to the neuroinhibitory enzyme glutamic acid decarboxylase (GAD) and human disease continues to grow. While much attention has been given over the last 15 years to the role that GAD autoantibodies may have in disorders such as Type 1 diabetes and Stiff Persons syndrome, recent studies have also identified an autoimmune component in individuals with juvenile neuronal ceroid lipofuscinosis or Batten disease, thereby raising the question of whether anti-self immunity could represent a contributory element to the development of this disease. Combined with studies of degenerative diseases of the central nervous system such as progressive cerebellar ataxia and Rasmussen’s encephalitis, which have also been characterized by the presence of autoantibodies, this suggests that our long-held separations in thought to the origins of neurological and endocrine-based diseases may have been errant.

Type 1 diabetes in humans, suggest that the autoreactivity within Type 1 diabetes could possibly begin at the level of peri-islet Schwann cells and supports the potential of a neuronal element in Type 1 diabetes Autoimmunity. It will be interesting to see how, over the next few years, our understanding of the role of anti-GAD immunity in terms of autoimmune disorders including Type 1 diabetes matures.

Apart from issues of pathogenesis, additional interest has been directed at the development of therapies to prevent or reverse these disorders. Simply put, if we know that anti-GAD immunity is associated with diseases such as Type 1 diabetes, are there methods that could be taken advantage of to reverse or prevent the disease? Obviously, the notion of Type 1 diabetes prevention and reversal is currently a ‘hot topic’, with multiple groups attempting to use agents that non-specifically modulate the immune response (often involving immune suppression) in order to preserve the metabolic function of the insulin-producing beta cells, leading to Type 1 diabetes prevention or reversal. It is interesting that such non-specific agents have become fashionable, as in reality the notion for use of these agents dates back to the 1980s when cyclosporin was used in trials aimed at reversing Type 1 diabetes. Obviously, this movement relates in part to a number of issues, perhaps the most important being the failure of insulin administration (as was performed in the NIH DPT-1 Trial) to prevent Type 1 diabetes. However, with the failure of one form of trial (i.e. insulin) may come an opportunity for another, and in this case it may be that GAD administration, as seen with the positive preliminary data in the Latent Autoimmune Diabetes in Adults Trial, will prove more effective. Antigen-specific trials, such as those involving GAD, do offer several significant advantages over immunomodulating therapies involving immune suppression. Hence myself, and many others, look forward with interest to see what GAD therapy offers in terms of Type 1 diabetes prevention.
Despite the classification of diabetes into two major types, Type 1 (insulin-dependent) diabetes and Type 2 (non-insulin-dependent) diabetes, it is apparent that there are some forms of diabetes that do not fit comfortably into these categories. Indeed, there is one form of diabetes that appears to straddle the two major types, presenting with non-insulin requiring diabetes in adults, but with many of the genetic, immune and metabolic features of Type 1 diabetes and with a high risk of progression to insulin dependency. This form of latent autoimmune diabetes of adults (LADA) is found in about 10% of initially non-insulin requiring diabetes patients and is therefore probably far more prevalent than is classic Type 1 diabetes. A major European Union initiative (ACTIONLADA) plans to learn more about LADA and a preliminary study using glutamic acid decarboxylase (GAD) as a means of immunomodulation has successfully sought to limit the loss of insulin secretory capacity. In the meantime the management of LADA is similar to that of other patients with non-insulin requiring diabetes with some caveats, which are discussed.

BACKGROUND TO LADA

The development of the non-insulin requiring diabetes mellitus is projected to reach epidemic proportions over the next 10-20 years. WHO data indicate that in 1994 there were nearly 100 million affected individuals worldwide and that by the year 2010 this number will increase to over 215 million. In most Western societies, the overall prevalence has reached 4-6% and as high as 10-12% among the 60-70 year old individuals. Annual health costs caused by diabetes and its consequences are around 6-12% of overall health expenditures. While non-insulin requiring diabetes plays a major role in contributing to mortality and morbidity in most countries, a proportion of people (about 25%) defined with non-insulin requiring diabetes at diagnosis subsequently progress to insulin treatment. In some cases this progression results from the inadequacy of our therapy, but in many cases it is due to progression of the disease process with progressive and severe loss of insulin secretory capacity. Those people with non-insulin requiring diabetes and diabetes associated autoantibodies are defined as having LADA, and are at high risk of progression to insulin dependency (1-3). The prevalence of LADA has been estimated in a number of studies of both European and non-European populations. Wide variation has been described, partly depending on the markers chosen to define the condition, but also the characteristics of the subjects tested (for example, whether newly diagnosed or according to age at diagnosis). Within Europe, when defined as non-insulin requiring diabetes diagnosed in individuals aged 30 to 50 years with GAD autoantibodies, LADA is found in about 10% of cases. In populations outside Europe the frequency varies from zero in Papua New Guinea to 16% in the Congo and 16% in a Chinese population. Since Type 1 diabetes is not common, but Type 2 diabetes is common, and since an appreciable proportion of non-insulin requiring diabetic subjects have GAD autoantibodies, it follows that autoimmune non-insulin requiring diabetes, LADA, is probably substantially more prevalent than is classic Type 1 diabetes (1). The presence of GAD autoantibodies, which are also associated with Type 1 diabetes, partly defines LADA. But in individuals with LADA other diabetes-associated serum autoantibodies can occur, including islet cell autoantibodies and IA-2 autoantibodies. Those individuals with GAD and IA-2 autoantibodies progress more rapidly to insulin dependency than do those with GAD autoantibodies alone (2, 4).

CLINICAL FEATURES OF LADA

These genetic and immune features of LADA are consistent with these individuals having an immune-mediated disease process that resembles Type 1 diabetes. Subjects with LADA also show many of the clinical characteristics of Type 1 diabetes;
for example, they tend not to be obese and show a striking insulin secretory deficiency (3).

About 80% of individuals with recently diagnosed non-insulin requiring diabetes of adult age with GAD autoantibodies (i.e. LADA) progress to insulin requirement within 6 years. Metabolic decompensation to insulin therapy in LADA is accelerated compared with those with initially non-insulin requiring diabetes who do not have GAD autoantibodies. Even in those who progress to insulin therapy, the average interval between commencement of oral hypoglycaemic therapy and progression to requiring insulin is approximately 4 years in a LADA group but as long 8 years within the group that does not have diabetes-associated autoantibodies (4). At present, no treatment can stop this progression to insulin-requiring diabetes, but it is clearly of major public health importance since LADA is so prevalent (5).

MANAGEMENT OF LADA

There is no established management strategy for people diagnosed with LADA (5). The European Union has funded a major initiative (ACTIONLADA) to study the characteristics of LADA and to report on how to treat it. The potential value of identifying this group at high risk of progression to insulin dependence includes:

- Avoidance of using metformin treatment as glucose control deteriorates, given the theoretical associated risks of metformin in patients becoming insulin-dependent.
- Early introduction of insulin therapy.
- Application of intervention trials to arrest or reverse the destructive disease process.

For those people diagnosed with diabetes in which the primary defect is loss of insulin secretion, treatment should aim to restore islet insulin secretion. Therapy to prevent progression towards insulin dependency could include immunotherapy, insulin or oral hypoglycaemic drugs. The efficacy of sulphonylureas has not been formally tested but it is evident that they do not arrest progression to insulin dependency in subjects with LADA. Whether metformin is of benefit is unclear and the drug may be contraindicated in those with LADA as there is a theoretical risk of severe metabolic disturbance in individuals who progress to insulin dependency while receiving it.

Insulin replacement at an early stage remains an option but nothing is known about the value of such a strategy. Furthermore, the optimal insulin regime is also unclear. Given the broad loss of insulin secretory capacity it might be argued that the early introduction of a long-acting insulin preparation could be beneficial. Alternatively, the loss of rapid insulin release in these LADA patients suggests that replacement with a fast-acting insulin preparation could be beneficial, although benefits remain hypothetical.

Given the immune associations with Type 1 diabetes and LADA it remains possible that immunomodulation might prove valuable in protecting the loss of insulin secretory capacity. A Phase II study of LADA patients with GAD antibodies found that a tolerance induction plan using alum-formulated whole GAD had a significant and long-term effect on the C-peptide response to sustacal challenge, consistent with modulation of the aggressive process by the regime. This approach opens a new field in diabetes management that may be of substantial benefit in the future.

“This approach opens a new field in diabetes management that may be of substantial benefit in the future.”

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Among those with autoimmune diabetes, the rate of beta cell destruction is markedly age-dependent. In children it is usually rapid, whereas it can be considerably slower in adults (1). The combination of adult onset diabetes and a slow progression to insulin requirement in the presence of beta cell autoantibodies is described as latent autoimmune diabetes in adults (LADA) (2). One of the earliest descriptions was in 1986 by Groop et al (3). This highlighted a clinical course, which was characterized by initial adequate glycemic control by diet or oral hypoglycemic agents, but subsequent need for insulin. That study used identification of islet cell antibodies (ICA) as the only specific pancreatic marker of an autoimmune process.

Several reviews (4-6) and the specific studies referred to in them indicate the varying prevalence of LADA. This can be due to a number of differences, both in the real prevalence and the methodology employed in the studies. Inherent in the diagnosis of LADA, as described by the WHO (7), is that there are features of autoimmune-mediated destruction of the pancreatic beta cells. This can be demonstrated by any of a group of autoantibodies, particularly glutamic acid decarboxylase (GAD) antibodies and islet cell antibodies (ICAs) (7). In that there is no designated antibody that must be present, or concentration of such, there is considerable room for different prevalence to be detected.

Most importantly, the mode of subject selection is probably the major determinant of the prevalence of LADA estimated in a given study. It would appear that studies based on hospital or specialized clinics are more likely to observe a higher prevalence of LADA than are community- or population-based studies. This is probably due to the fact that those who are likely to need insulin are more frequently referred to secondary care than are those who do not seem to need insulin.

The highest documented prevalence of LADA as a proportion of those initially diagnosed as Type 2 diabetes was from an Italian study (8), which classified nearly 50% of their cohort as having LADA. The cohort for assessment was not randomly selected from adults newly diagnosed as having Type 2 diabetes, but were young (30-54 years) and lean (BMI<25 kg/m2), and therefore would be expected to be enriched for LADA. At just two months following diagnosis, 50% of those with at least one of GAD or ICA antibodies were receiving insulin, as compared to a quarter of those who were antibody negative. Even within the same country, a different population selection and relatively small sample numbers will lead to different prevalence estimates of LADA. Another Italian study (9) based on a random assessment of 600 adult diabetic patients attending an outpatient clinic found approximately 10% to be GAD antibody positive. GAD antibody positivity was strongly associated with insulin therapy and lower basal C-peptide levels. An even lower proportion of antibody presence was detected in a population-based Italian study in Cremona (10). This enabled antibody status to be determined according to glucose tolerance status. The definition of positive antibody status was based on an antibody concentration of more than two standard deviations above the mean among people with normal glucose tolerance. Combining the data for those newly and previously diagnosed with diabetes, 2.1% of adults with diabetes were classified as having LADA, which is markedly lower than the prevalence among the clinic-based populations, notwithstanding the lower cut-off mark for a positive antibody response. As diabetes was present in 9.4% of the survey population (11), the overall prevalence of LADA in the adult population was about 0.2%.

Another population-based Southern European study (12) from Pizarra, Spain, found only GAD antibodies to be more prevalent in diabetes. Whereas their prevalence in Type 2 diabetes was high (14.7% by ADA criteria (13)), antibodies suggestive of LADA were present in 3.7% of the diabetic population, suggestive of about 0.5% of the total adult population.

Among other population-based studies, the Dutch Hoorn study (14) was also based on a positive GAD response, although an older population (age 50-74) was surveyed in this case. When based on the 99th percentile of GAD concentrations within the whole population, 3.5% of those with previously diagnosed diabetes had LADA, but there were no cases among those newly diagnosed. These rates changed to 14.1%, and 4.5% if based on the 95th percentile, highlighting the importance of the diagnostic criterion of GAD antibody level in assessing LADA prevalence. In another Dutch study (15), GAD antibodies, using the 99th percentile were present in 2.8% of those with Type 2 diabetes. Islet cell antibodies (7.6%) were considerably more prevalent than were GAD antibodies (which is unusual), so that an estimate based solely on GAD antibodies could give a considerable underestimate of LADA.

The west Finland population study (16) determined GAD positivity in a slightly different way, based on 3 standard deviations greater than the mean of those with normal glucose tolerance. Despite the higher cut-off level of GAD, the overall prevalence of 9.3% in Type 2 diabetes was more than twice that among non-diabetic controls, and was strongly related to age of diagnosis, being 20% in those diagnosed between 36 and 45 years. The high prevalence of antibodies in the Finnish population (in those with and without diabetes) seems
consistent with that country’s extremely high incidence of Type 1 diabetes (16). Another Finnish study (17) (East Finland) included in the review by Schernthaner (5) found very comparable rates of LADA, although participant numbers were much lower and diagnostic methods similar. Population-based Australian data (18) incorporated both GAD and ICA status in an ethnically diverse but principally European population. Of those with clinically diagnosed Type 2 diabetes, GAD antibody prevalence was 3.6%. As in the East Finland study (17), fewer were positive for ICA than for GAD, although this assessment was made for only 20% of those with Type 2 diabetes. On the basis of GAD or ICA antibody presence, 10% of adults referred to a hospital diabetic clinic in Malmo (Sweden) (19) were deemed to have Type 1 diabetes or LADA.

A high LADA prevalence based on both GAD and IA-2 antibodies was observed in the follow-up of Pittsburgh members of a population-based cohort study of the elderly (aged 65 and over) (20). Using cut-offs based on the 99th percentile of autoantibody levels among controls, 10.2% of those labeled as having Type 2 diabetes were found to have antibodies to GAD, and 2.6% had antibodies to IA-2, with 12.2% having antibodies to either. There was no ethnic difference between Caucasian and African-American participants.

A survey in Korea of individuals found to have diabetes during a screening examination found GAD antibodies to be present in 2 out of 121 subjects (1.7%) (21). This low prevalence may be attributable to the ethnic background, in which Type 1 diabetes is much less common than in European populations, but may also relate to the fact that the subjects were diagnosed with diabetes in a single oral glucose tolerance test, and therefore differ from the clinically diagnosed patients included in most other studies.

One of the largest studies of the prevalence of autoantibodies in patients thought to have Type 2 diabetes is the UKPDS. This clinical trial of patients with recently diagnosed Type 2 diabetes in both primary and secondary care reported that out of 3672 subjects, 6% were ICA positive, 10% GADA positive, and 4% had both antibodies (22). The presence of autoantibodies was associated with a younger age at diagnosis, lower body mass index and reduced beta cell function.

A recent analysis of US data from a national population-based sample of over 1000 people with diabetes aged 40-90 reported that GAD antibodies were present (above the 99th percentile of a control population) in 6.3% of non-Hispanic whites, 3.7% of non-Hispanic blacks, and 1.2% of Mexican Americans (23). Unfortunately, the study did not attempt to exclude classical Type 1 diabetes, and so it is difficult to determine the prevalence of LADA from these data.

### Table 1

<table>
<thead>
<tr>
<th>Country</th>
<th>Age range (yrs)</th>
<th>Prevalence of LADA</th>
<th>Study numbers</th>
<th>Estimated national numbers with LADA age 20-79 (000s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia (18)</td>
<td>20-98</td>
<td>3.7%</td>
<td>1,252</td>
<td>30</td>
</tr>
<tr>
<td>Finland (16)</td>
<td>26+</td>
<td>9.3%</td>
<td>1,122</td>
<td>24</td>
</tr>
<tr>
<td>Italy (10)</td>
<td>40+</td>
<td>2.1%</td>
<td>193*</td>
<td>57</td>
</tr>
<tr>
<td>Netherlands (15)</td>
<td>40-96</td>
<td>2.8%</td>
<td>785</td>
<td>11</td>
</tr>
<tr>
<td>South Korea (21)</td>
<td>unspecified</td>
<td>1.7%</td>
<td>121</td>
<td>35</td>
</tr>
<tr>
<td>Spain (12)</td>
<td>18-65</td>
<td>3.7%</td>
<td>111*</td>
<td>105</td>
</tr>
<tr>
<td>Sweden (19)</td>
<td>40-69</td>
<td>6.2%</td>
<td>195</td>
<td>27</td>
</tr>
</tbody>
</table>

*Survey methodology does not indicate if persons with Type 1 diabetes were excluded from GAD estimates. Calculations are based on the assumption that they were.
CONCLUSION
In the same manner that overall diabetes prevalence by nation remains hard to precisely quantify, in that many rates are based on isolated studies in selected areas of the country using different methodologies, a quite widely differing prevalence of LADA is observed. Doubtless, much of this is real, as is the case with Type 1 incidence in children, but comparison of rates is also encumbered by the use of different methodologies. The factor most likely to affect the detected prevalence is the selection methodology, as younger and less obese persons are more likely to have autoimmune diabetes.

Determining the prevalence of LADA implies the need to exclude those with classical Type 1 diabetes, and to report the prevalence of antibody positivity in the remaining diabetic population. This often relies on physician diagnoses of type of diabetes, which are likely to vary. Some studies do not even attempt this, and it is notable that in the Italian study reporting a 50% antibody prevalence, 50% of those with positive antibodies at diagnosis were receiving insulin within 2 months of diagnosis as to which of these applies, and even whether a universal measurement could be employed, presents its own difficulties.

Nevertheless, some patterns emerge. Clinic-based studies of unselected patients seem to report an antibody prevalence of about 10%, with a lower prevalence being reported in most population-based studies. When populations are enriched for clinical features consistent with LADA, then, not surprisingly, a much higher prevalence is reported.

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Paul Zimmet is Director of the International Diabetes Institute and Professor of Diabetes, Monash University in Melbourne, Australia. Zimmet’s current research includes Type 1 diabetes etiology and the molecular mechanisms of Type 2 diabetes, insulin resistance and obesity and the effects of life-style change leading to diabetes, obesity, coronary heart disease and hypertension in developing countries in the Asia-Pacific region.
The Story of LADA in China

BY PROFESSOR ZHIGUANG ZHOU
PROFESSOR AND DIRECTOR OF THE DIABETES CENTER OF CENTRAL SOUTH UNIVERSITY IN CHANGSHA, CHINA

The first research paper describing LADA characteristics in China was by the late Professor Xiaoren Pan, whose patients samples for GAD-antibody (GAD-Ab) determination were performed in Paul Zimmet’s laboratory. In 1993 I was granted funds by the National Natural Science Foundation of China to conduct a GAD-Ab study. The foundation reviewer, Professor Rongli Qian, urged our own development of a method to measure GAD-Ab. We purified pig brain GAD and established an enzymatic assay for GAD-Ab screening during the following year. In collaboration with Thomas Dyrberg, our group set up the radioligand assay for GAD-Ab in 1995 and later reported that 14.8% of a group of Chinese adults diagnosed as Type 2 diabetes actually had LADA, which was confirmed by Professor Kunsan Xiang and other groups. We followed these patients for up to 8 years and found more than a 3-fold decrease in levels of fasting C-peptide every year in LADA as compared to in T2DM patients (15.8 % vs 5.2 %). Multivariate analysis showed that GAD-Ab titer was a major predictor for the progression of pancreatic beta-cell function.

Based on GAD-Ab titer alone these patients were classified into two clinically distinct subgroups - high titer or LADA Type 1 with more insulin-dependent phenotypes, and low titer or LADA Type 2 with more insulin-resistant phenotypes, as described by Lohmann. The cut-off value of GAD-Ab titer to identify these two subtypes was 175U/ml using receiver-operating characteristic (ROC) curves. The prevalence of LADA in the obese increased recently (3.5% in 1996 vs 28.8% in 2002, p=0.006).

Metabolic syndrome (MS) existed in 50% of LADA patients by WHO criteria and 40% by NECP ATP III criteria. The proportion of MS and its metabolic components were comparable between LADA and T2DM patients, except a lower frequency of hypertension in the former. LADA patients with MS had significantly higher HOMA-IR and hsCRP levels compared to those without MS. GAD-reactive T cells could be readily found by co-stimulation with a specific antigen (GAD) combined with a non-specific stimulator (IL-2) in LADA patients. The frequency of CD4+CD25+T cells decreased, but CD3+CD8+T cell number increased, and higher levels of Th1 cytokines (IL-2, IFN-_) as well as lower Th2 cytokine levels (IL-4) were recorded in PBMC supernatants from LADA patients.

In 1997 we started to administer the Chinese herb extract tripterygium (which is an oral immunosuppressive agent used for treatment of rheumatoid arthritis), to LADA patients. Their stimulated C-peptide
levels were maintained after 18 months of therapy, while mild hematological changes occurred. In 2001 we initiated a clinical trial to test whether rosiglitazone alone or combined with insulin for LADA Type 2 or Type 1 was effective in preserving beta-cells. The emerging results in the pilot study were promising, although it needed to be extended into a larger clinical trial.

With the aid of Bill Hagopian our GAD-Ab assay was evaluated by the Diabetes Autoantibody Standardization Program (DASP 2003), and was considered proficient. Recently, the ACTION-LADA project in Europe led by Professors David Leslie and Paolo Pozzilli sets a good example for organizing similar studies in China. We are happy to have the opportunity to participate in the international community, and hopefully look forward to seeing antigen-specific GAD vaccination successfully performed in LADA.

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“We found more than a 3-fold decrease in levels of fasting C-peptide every year in LADA as compared to in T2DM patients. We look forward to seeing antigen-specific GAD vaccination successfully performed in LADA.”
Beta Cell Preservation – its Impact on Diabetes Control and Complications

BY PROFESSOR PAUL ZIMMET & R. SICREE, J. SHAW
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Type 1 diabetes is a T cell-mediated autoimmune disease directed against the pancreatic islet cells resulting in beta cell loss (1). Type 2 diabetes is also characterized by eventual beta cell failure, albeit via different mechanisms. Even though insulin and or oral hypoglycemic medication regimens continue to improve, ideal glucose control and the prevention of later complications have been difficult to achieve.

Pancreatic beta cells produce both insulin and C-peptide in equimolar amounts (2). C-peptide levels are considered a reflection of beta cell activity (3) and as such, the American Diabetes Association has recommended that C-peptide measurement be used as the most suitable primary outcome for trials of therapies aimed at preserving or improving endogenous insulin secretion in Type 1 diabetes patients (1).

Beta cell function as measured by C-peptide levels declines with duration of diabetes (4). Madsbad et al found the prevalence of residual beta cell function in Type 1 diabetes declined from 100% in subjects with diabetes duration of up to two years to 15% after 15 years (5). Others have reported residual beta cell activity declining to 20% after only five years following diagnosis (6). In addition, age of onset has an effect, with the prevalence of beta cell activity being higher among late onset compared to those with early onset Type 1 diabetes (5,7). Latent autoimmune diabetes in adults (LADA) is similar to classical Type 1 diabetes in that it is characterized by autoimmune destruction of beta cells and consequent decline of beta cell function. However, for reasons that are poorly understood, the rate of decline of beta cell function is much slower than that evident in classical Type 1 diabetes, such that insulin dependence develops some time after diabetes diagnosis (8,9). This more gradual decline of beta cell function raises the important question of whether or not measures to preserve beta cell function in such patients might have long-term benefits.

Maintenance of beta cell function has been suggested to be valuable in allowing a more ‘physiological’ component of glycemic control to supplement the pharmacological methods usually used. Furthermore, it has been postulated that C-peptide itself has a physiological function. Thus, measures to maintain beta cell function might improve glycemic control and reduce the risk of diabetic complications. The evidence for these possibilities is reviewed below.

BETA CELL FUNCTION AND DIABETES COMPLICATIONS

A number of studies have examined the hypothesis that the risk of developing complications of diabetes is related to beta cell function. A study comprising 1533 subjects with Type 2 diabetes not receiving insulin examined the relationship between complications and quartiles of C-peptide (1st quartile: ≤0.43 pmol/ml, n=372; 2nd quartile: >0.43-<0.69 pmol/ml, n=375; 3rd quartile: >0.69-<0.89, n=393; 4th quartile: >0.89 pmol/ml, n=393), over 12 months of follow-up (10). Subjects in the lower quartiles of C-peptide secretion had a longer duration of diabetes and higher prevalence of retinopathy, higher mean albumin excretion rate and A1c, while those in the highest quartile had a greater mean BMI and blood pressure. Using a logistic regression model and adjusting for duration of diabetes, A1c, sex, BMI and hypertension, C-peptide was significantly associated with retinopathy (OR 0.82, 95% CI 0.72-0.94, p=0.0045) as well as the presence of the metabolic syndrome (OR 1.50, 95% CI 1.20-1.80, p=0.0007).

A Japanese study (11) with a mixed population of Type 1 and Type 2 diabetes (n=160; diabetes duration > 10 years) assessed beta cell function using urinary C-peptide measurements. Although the baseline prevalence of retinopathy was unrelated to beta cell function, the incidence of proliferative retinopathy over the next 10 years was highest in those with the lowest baseline C-peptide excretion. A Danish study of 533 patients with Type 1 diabetes found that the prevalence of retinopathy was higher in those with a reduced C-peptide response (to either glucagon or a meal), but this difference seemed to disap-
pear after accounting for diabetes duration (12). A small Swedish study of 44 patients with Type 1 diabetes found that reduced urinary C-peptide was associated with increased prevalence of retinopathy, microalbuminuria and proteinuria, but found no association with neuropathy (13).

In contrast, other studies examining Type 1 diabetes have failed to show that residual C-peptide levels influence rates of complications. The population-based Wisconsin study of diabetic retinopathy found no relationship between higher levels of C-peptide (>0.3 pmol/ml) at the time of diagnosis and prevalence of retinopathy at six years (14). Winocour et al examined people with longstanding Type 1 diabetes (mean 15.4 years) for the development of complications over the next two years (15). Although subjects with higher C-peptide concentrations (>0.100 pmol/ml) at entry required less insulin, there were no significant differences in the prevalences of neuropathy, nephropathy or coronary disease between this group and subjects with low or negligible C-peptide levels. A study from Brazil which examined adults with Type 1 diabetes (mean age 25 years, mean diabetes duration 9 years) over six months, has also demonstrated no association between baseline C-peptide levels and microalbuminuria and retinopathy (16).

The most recent and important study in this area was an analysis of the first six years of the Diabetes Control and Complications Trial (DCCT) by Steffes et al (17), which examined the incidence and progression of retinopathy and albuminuria according to baseline C-peptide status in more than 1400 patients with Type 1 diabetes. These results showed that the risk of complications increased progressively as C-peptide levels decreased, with the effects being more pronounced in the intensively-treated than in the conventional arm. Subjects were divided into groups according to their stimulated C-peptide at study entry. Those with more than minimal levels were further subdivided according to whether or not their response was sustained after one year. Within the intensive arm of the study, subjects with undetectable C-peptide at entry had four times the progression rate of retinopathy and albuminuria than did those in the sustained responder group (p ≤ 0.05) (Table 1).

TABLE 1

<table>
<thead>
<tr>
<th>Stimulated C-peptide group</th>
<th>Undetectable</th>
<th>Minimal</th>
<th>Baseline only</th>
<th>Sustained</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinopathy</td>
<td>6.5 ± 0.7 a</td>
<td>3.5 ± 0.3 b</td>
<td>1.8 ± 0.9 bc</td>
<td>1.4 ± 0.6 c</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>4.0 ± 0.5 a</td>
<td>2.3 ± 0.4 b</td>
<td>1.7 ± 0.9 bc</td>
<td>0.9 ± 0.5 c</td>
<td>2.9 ± 0.3</td>
</tr>
</tbody>
</table>

Data are rates ± SE per 100 participant-years. Rates were compared (horizontally) between stimulated C-peptide groups. For each comparison, rates with different letters were significantly different (P < 0.05), while rates sharing the same letter were indistinguishable.

Retinopathy defined as a single three-step change on the ETDRS scale.
Albuminuria defined as a single event >40 mg/24 h.
“Even though insulin and or oral hypoglycemic medication regimens continue to improve, ideal glucose control and the prevention of later complications have been difficult to achieve. Diabetic patients with preserved beta cell function appear to have better glycemic control, fewer episodes of hypoglycemia and a lower risk of developing complications.”

It remains unclear whether the benefits seen in those with greater beta cell function are due to the improved metabolic control associated with preserved beta cell function or a direct effect of C-peptide on the microvasculature (see below). In this context, it is interesting to note that in the DCCT data, the impact of C-peptide was independent of A1c, suggesting that much of the benefit is not mediated through improved glycemic control. Conversely, the risk reduction was more pronounced in the intensively treated arm, suggesting that the benefits associated with preserved beta cell function are more apparent in the face of tight glycemic control.

It has been suggested that C-peptide itself is not an inert by-product of insulin secretion (produced when pro-insulin is cleaved prior to release of insulin into the circulation from the beta cell), but may have an important physiological function. A series of small randomized controlled trials in Type 1 diabetes has indicated that an infusion of C-peptide can acutely improve autonomic function (19), and that 3 months of daily subcutaneous injections of C-peptide was associated with a reduction in urinary albumin excretion from 58 mcg/min to 34 mcg/min, with no such change in the placebo group (20). A 12 week study of subcutaneous injections of C-peptide also showed a significant improvement in nerve conduction velocity which was not apparent with placebo (21). The mechanism through which C-peptide may be acting is unclear, but we have recently demonstrated that C-peptide appears to improve endothelial function and has a vasodilatory effect in the skin (22). Similar effects in other tissues might ameliorate certain diabetic complications, and the retention of C-peptide secretion by beta cell preservation might contribute directly to a reduced risk of complications.

### BETA CELL FUNCTION AND METABOLIC CONTROL

In addition to the DCCT showing that intensive treatment lessened the decline in C-peptide and that subjects with residual C-peptide levels had fewer complications (17; 23-25), there is also evidence that those with preserved beta cell function had better metabolic control compared to those with undetectable C-peptide levels (25). The DCCT reported findings on 855 subjects with Type 1 diabetes divided into responders (n=303, C-peptide level 0.20-0.50 pmol/ml) and non-responders (n=552, C-peptide <0.2 pmol/ml). The two groups were equally divided between the intensive (responders n=138, non-responders n=274) and conventional therapy (responders n=165, non-responders n=278) arms. At study entry, responders had significantly lower median A1c levels compared to non-responders (intensive group: responders 7.1% versus non-responders 9.1%, p<0.01; conventional group: responders 8.0% versus non-responders 9.1%, p<0.05). These differences were maintained in the intensive treatment group for the next four years, but only for two years in the conventional treatment arm. However, by years eight and nine, responders in the conventional treatment arm had significantly higher median A1c levels compared to non-responders (responders 9.9% versus 9.2%, non-responders, p<0.01 at year nine).

By this stage the number of patients had declined to only 17 in the responder group and 37 in the non-responder group, so interpretation of A1c levels is difficult. It should be noted that the DCCT was not designed to examine preservation of beta cell function and therefore these observations have some limitations. First, subjects were recruited with diabetes of a mean duration of 2.7 years rather than within a year of diagnosis. Second, those with C-peptide levels >0.50 pmol/ml were excluded during screening. Third, C-peptide levels were not measured again if <0.20 pmol/ml, resulting in patients being classified as non-responders and so it is not possible to know if these subjects might have recovered C-peptide secretion if randomized into the intensive treatment arm.

In another large study conducted by Bott et al (26) it was also shown that C-peptide levels predict long-term glycemic control. This prospective study examined 697 subjects with Type 1 diabetes (newly diagnosed n=49, not new n=648) aged 15-40 years (mean age 26 ±7 years), who took part in a five day intensified insulin treatment and teaching program and were then assessed after one, two and three years. Using multiple logistic regression analyses the most significant (p<0.05) predictors of poor glycemic control were higher A1c levels before intervention, smoking, poor diabetes-related knowledge, less frequent home blood glucose monitoring, younger age at onset and negative C-peptide levels ≤0.1 pmol/ml at the baseline assessment. Of note is that C-peptide only reached significance in the regression model of the composite variable taking into account A1c levels and severe hypoglycemia.

Other, smaller, studies have failed to show a correlation between C-peptide levels and glycemic control. Salardi et al (27) conducted a retrospective review of severity of presentation of Type 1 diabetes in children in terms of residual beta cell function as measured by C-peptide levels and metabolic control. Sixty-six children aged 0.7 to 14.8 years at diagnosis were divided into groups depending on presentation and followed up for 10-32 years, with C-peptide being measured at diagno-
The ratio of insulin requirements between subjects with peptide excretion (nmol/kg/24 hours) or significant differences in median urinary C-management regimens. There were also no differences in endogenous insulin produ-
ction at two years or in glycemic control at five years post diagnosis between the two programs. There were no significant intensive family-based education and sup-
port program. There were no significant differences in C-peptide levels, another prospective study examined 151 newly diagnosed children with Type 1 diabetes (mean age 10.2 ±4.6 years) for three years looking at factors that predicted the course of beta cell function (29). Loss of C-peptide secretion was predicted by younger age (p=0.0001), male sex (p=0.003), ICA tit-
res (p=0.006), severity of clinical presentation (i.e. DKA (p=0.001)) and shorter symptom duration at diagnosis (p=0.002). However, there were no significant differ-
ces in A1c levels between subjects that did or did not secrete C-peptide during the 36 months follow-up period.

Forsander et al (30) randomly chose 38 children aged 3-15 years, with newly diagnosed Type 1 diabetes for conventional hospital management of diabetes or a more intensive family-based education and support program. There were no significant differences in endogenous insulin produ-
tion at two years or in glycemic control at five years post diagnosis between the two management regimens. There were also no significant differences in median urinary C-peptide excretion (nmol/kg/24 hours) or insulin requirements between subjects with good control (mean A1c <6.4%, insulin requirement 0.85U/kg/day) or poor control (mean A1c >8.3%, insulin require-
ment 1.0U/kg/day).

These studies highlight conflicting data regarding the impact of C-peptide levels on glycemic control. It is notable that the two largest studies (23-26) showed better gly-
cemic control in those with higher C-peptide levels, and that these studies were conducted in adults, whereas the studies that reported no benefit attributable to C-peptide secretion were smaller and were conducted in children. It is likely that LADA, which is a disease of adults, is more akin to adult-onset than to childhood-onset Type 1 diabetes.

In addition to the data alluded to above regarding glycemic control there is also evidence that patients with Type 1 diabetes who have preserved beta cell function have a lower risk of hypoglycemia. Among 684 patients with type 1 diabetes in a prospecti-
ve German study, C-peptide negativity was an important and independent risk factor for severe hypoglycemia (31). The DCCT also showed that those with a C-peptide response that was present at baseline and maintained at one year had a 30% reduc-
tion in their risk of hypoglycemia during the subsequent six years when compared to all other groups (17).

CONCLUSION

Intuitively, one would expect that preservation of a degree of natural insulin secretion would be beneficial. Unfortunately, the data are not all consistent, and this may reflect our inability to accurately measure beta cell function, as well as the variation in pathophysiological processes occurring at different stages of the disease and at diffe-
tent ages. Nevertheless, the balance of evidence seems to be in favor of the conclusion that better outcomes are evident in individuals with preserved beta cell function, as measured by a variety of parameters concern-
ing C-peptide secretion. Specifically, even after accounting for other factors such as diabetes duration, diabetic patients with preserved beta cell function appear to have better glycemic control, fewer episodes of hypoglycemia and a lower risk of developing complications.

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Diabetes mellitus has classically been divided into two clinical phenotypes. Whereas Type 1 diabetes is an autoimmune disease involving cellular mediators, Type 2 diabetes evolves from concurrent insulin resistance and a non-autoimmune-mediated insulin secretory deficiency. In addition, there is a subgroup of phenotypic Type 2 diabetic patients who demonstrate autoimmunity. This subgroup has been termed latent autoimmune diabetes in adults (LADA) or Type 1.5 diabetes (1).

Recently, we described the value of C-peptide measurements and the correlation with beta-cell function in Type 1 diabetic patients (2). A logical extension of this concept is the utility of C-peptide in the assessment of beta-cell function in LADA. In this manuscript we will review the strength of the evidence supporting the concept that the rate of decline of beta cell function in LADA patients is intermediate between Type 1 and Type 2 diabetes.

Landin-Olsson (3) measured random C-peptide levels in recent-onset Type 1 diabetic patients, Type 2 diabetic patients, and LADA over a three-year period. LADA patients had higher baseline C-peptide relative to Type 1 diabetic patients, but with time both exhibited parallel decreases in C-peptide. These two parallel slopes declined more rapidly relative to the rate of decline found in Type 2 diabetic patients.

Hosszufalusi et al. (4) assessed newly diagnosed LADA and Type 1 diabetic patients during a ten-year period. Though C-peptide values in both groups did not differ at diagnosis, within one year a more rapid fall in fasting C-peptide was noted in the Type 1 diabetic group relative to LADA, and this relationship remained statistically significant after ten years. Cerna et al. (5), in a study correlating HLA markers in autoimmune diabetes, also noted lower fasting C-peptide in Type 1 diabetic patients relative to LADA. Monge et al. (6), in a mass screening, observed a significantly lower fasting C-peptide level in LADA patients relative to Type 2 diabetic patients. GADA predicted a significantly lower fasting C-peptide, and this level diminished further when GADA was associated with ICA. Lohmann et al. (7) determined a negative correlation of GADA with fasting C-peptide and additive effects of ICA with respect to lower fasting C-peptide. Again, beta-cell function was lower in the LADA patients relative to Type 2 diabetic patients. Interestingly, in both of these studies the presence of ICA alone (unassociated with GADA) appeared to be associated with minimal effects on fasting C-peptide. Carlsson et al. (8) examined stimulated C-peptide values and in LADA patients found lower C-peptide values stimulated with glucose and arginine relative to Type 2 diabetic patients. Groop et al. (9) also found lower stimulated C-peptide values in LADA patients than Type 2 diabetic patients and noted that these LADA patients were prone to earlier insulin-dependence.

Tuomi et al. (10) reported a statistically significant negative correlation between fasting C-peptide levels and GADA levels (R = -0.25). The presence of GADA correlated with decreased insulin response to oral glucose relative to the absence of GADA. As greater GADA levels were asso-
associated with greater decreases in fasting C-peptide, GADA may prove to be useful to estimate the ‘severity’ of LADA. Among LADA patients, findings of a study by Li et al. (11) similarly correlated higher GADA titers with lower fasting C-peptide values.

Gottsäter et al. (12) examined fasting C-peptide values in LADA, Type 1 diabetic patients and Type 2 diabetic patients. LADA was defined solely by the presence of ICA. Initial C-peptide levels of LADA were intermediary between Type 1 diabetic patients, who demonstrated the lowest initial values, and Type 2 diabetics, who demonstrated the highest values. Interestingly, in the LADA patients, the fasting C-peptide fell to a greater extent over a three-year period relative to that of Type 1 diabetics. This statistically significant finding is somewhat contrary to other studies in suggesting that LADA is more aggressive than is Type 1 diabetes, but it should be noted that the numbers in the study were relatively small. Unequivocally, in this study and in a subsequent study (13), Gottsäter demonstrated the predictive worsening of beta-cell function using C-peptide measurement in LADA patients relative to Type 2 diabetic patients.

Based upon C-peptide levels, greater beta-cell dysfunction is usually recorded in both autoimmune diabetic entities, LADA and Type 1 diabetes, relative to Type 2 diabetes. The majority of evidence suggests a more slowly progressive course of beta-cell decline in LADA relative to Type 1 diabetic patients. Standardized measurements of stimulated C-peptide may provide fairly precise assessments of the rate of beta-cell decline, and may enable appropriate and timely interventions when means of immune modulation become available. GADA appears especially useful to identify the LADA population, and quantification of GADA may offer prognostic value as a way to estimate rate of decline in beta cell dysfunction.

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Clinical Trials in Type 1 Diabetes – Past and Present

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Currently there is no cure for any autoimmune disease. Existing potential therapies are either effective only in subgroups of patients or have associated adverse side-effects. The need for new therapies is thus obvious, not least for Type 1 diabetes mellitus (T1DM), which can be partly controlled through daily injections of insulin. In this short essay some of the efforts made over the last 10 years to stop the disease in its tracks are reviewed.

GLYCEMIC LEVELS

The discovery and use of insulin dates back to a report by Banting & Best in 1922, following which an otherwise fatal disease was transformed into one with a variety of long-term complications. Different insulin preparations have been developed since then, improving length of action, convenience of use and route of delivery. In parallel, accurate methods to study glycemic regulation facilitated the first large clinical trials aimed at intensive insulin treatment as a means of maintaining near-normal (nondiabetic) glycemic levels. The Diabetes Control and Complications Trial (DCCT; 1441 patients; 1993) and the Stockholm Diabetes Study (102 patients; Reichard et al., 1993) were the first controlled trials to address this therapeutic modality. The DCCT Research Group reported that intensive insulin therapy had a durable, uniform and major effect on three major diabetes-specific long-term complications (retinopathy, neuropathy, cardiovascular disease) for 6.5 years of follow-up and concluded that most T1DM patients should be treated as early as possible with intensive insulin therapy. Longer-term follow-up (additional 4 years; DCCT/EDIC, 2000) endorsed the protective effect, with more than 75% reduced complications compared to the control group.

Despite the range of alternative approaches to T1DM therapy as outlined in the following sections, further development of insulin preparations and dosing regimes is still being conducted. Multiple daily injection therapy with Humalog mixtures was recently compared to separately injected insulin lispro and NPH insulin in adults with T1DM in an open clinical trial (Roach et al., 2004). Similarly, the effectiveness of insulin (Levemir®, NovoNordisk) was recently reported to be superior to that of NPH insulin in an open clinical trial (STEADINESS study) of 408 T1DM patients (Home et al., 2004).

Additionally, a recent double-blind, placebo-controlled study of 56 children with newly diagnosed (within 1 week) T1DM treated with diazoxide, an ATP-sensitive K+ channel opener and inhibitor of insulin secretion, was reported (Örtqvist et al., 2004). In addition to daily insulin injections children received 7-7.5mg/kg daily for 3 months of diazoxide (Avondale, Rathdrum, Ireland). The study demonstrated that partial inhibition of insulin secretion suspended the remission period and transiently preserved residual insulin production, but the treatment was less well tolerated and efficacious than in a similar study performed in young adults in which diazoxide treatment induced long-term preservation of residual insulin secretion (Björk et al., 1996). Regulation of insulin levels thus still remains a key therapeutic area.

IMMUNOMODULATORS

BETA CELL-SPECIFIC AUTOANTIGENS

In light of unwanted side-effects with general immunotherapies, interest in specific beta cell therapy is increasing in the scientific community. At this time there are two autoantigens that have been evaluated in clinical Phase II studies, Glutamic Acid Decarboxylase (GAD65) and insulin.

GAD

While GAD is an important enzyme that converts the excitatory amino acid glutamate into the inhibitory neurotransmitter GABA – and imbalances in the conversion between these two molecules thereby may play an important role in neurologic and psychiatric disease – it is also a major autoantigen in autoimmune diabetes. GAD involvement in T1D has therefore been extensively studied by numerous scientific groups around the world. It has been shown in clinical Phase I and II studies (Agardh et al., 2005) that subcutaneous administration of GAD formulated with adjuvant (aluminium hydroxide) is safe and activates regulatory T cells that downregulate beta cell inflammation. Pivotal studies with GAD in alum (DiaMyd™) are now ongoing in adults (LADA) as well as in children (T1D) at 25 hospitals in Sweden.

INSULIN

In addition to GAD65, insulin is an autoantigen in T1DM as it is produced by pancreatic β-cells, which are the target of the destructive autoimmune process.

A randomized, controlled clinical trial (Diabetes Prevention Trial of Type 1 Diabetes (DPT-1 study, 2002)) was conducted to determine whether insulin could prevent or delay the onset of T1DM in islet antibody 339 positive relatives of patients with T1DM. Insulin was infused annually during a 4-day treatment in addition to twice-daily low dose insulin injection, and patients were monitored for 4 years. Using this dose regime, T1DM was neither prevented nor delayed in these high-risk relatives.

ORAL INSULIN

As double-blind, controlled clinical trial
was conducted in 82 recent onset T1DM patients (within 4 weeks of diagnosis) in which 5mg insulin/day was administered orally for a 12 month period in addition to intensive subcutaneous insulin therapy (IMDIAB VII study; Pozzilli et al., 2000). The results of this study indicate that at this dose, oral insulin therapy is ineffective in protecting from disease development and may even accelerate the disease course in the very young patient cohort. Additionally, recent results from the DPT-1 (DPT-1, 2005) involving 372 patients receiving oral insulin also failed to demonstrate that this treatment could delay or prevent Type 1 diabetes.

### OTHER IMMUNOMODULATORS

#### GENERAL IMMUNOSUPPRESSION

A number of general immunosuppressive agents have been tested in clinical trials of T1DM. Cyclosporine causes inhibition of calcium-dependent activation of T cells, blocking the signal to produce cytokines that are associated with pancreatic inflammation (Bougnères et al., 1988; CERCT study group, 1988; Skyler & Rabinovitch, 1992; Stiller et al., 1992). Azathioprine disrupts the synthesis of DNA and RNA, disrupting cell division of T cells (Silverstein et al., 1988; Cook et al., 1989). Prednisone is a catabolic glucocorticoid similar in action to corticosterone (Silverstein et al., 1988). Despite transient improvements in disease development these clinical trials were too small to be conclusive and were also associated with toxic side-effects. The need for more specific therapies is evident, and this has been the focus of clinical trials in T1DM during the last decade.

### HEAT-SHOCK PROTEIN PEPTIDE

**DIAPEP277**

Heat shock proteins (HSPs), also called stress proteins, are a group of proteins that are present in all cells in all life forms. They are induced when a cell undergoes various types of environmental stresses such as heat, cold and oxygen deprivation. Heat shock proteins are also present in cells under perfectly normal conditions, acting as ‘chaperones,’ making sure that the cell’s proteins are in the right shape and in the right place at the right time. HSP60 is an abundant protein that is constitutively expressed and is induced by environmental stress.

In a Phase I clinical trial with a modified peptide of HSP60 (DiaPep277) in 35 newly diagnosed T1DM patients, administration of 3x1mg peptide subcutaneously over a period of 6 months indicated that endogenous insulin was preserved in treated patients at 10 months (Raz et al., 2001). Additional clinical studies are ongoing.

### ANTI-CD3 MONOCLONAL ANTIBODY TREATMENT

The rationale of this approach is to delete T lymphocytes (T cells) in the host and thus halt pathogenesis. It is well established that islet antigen-specific T cells are among the cellular players that lead to islet beta cell destruction and resulting T1DM. The CD3 molecule is expressed on all T cells, and once bound by a specific antibody (anti-CD3), the T cells become unresponsive or killed, and can thus no longer contribute to pancreatic damage. In order that the administered antibody not have unwanted non-specific side-effects such as activation of other host cells, it has been appropriately modified (its human Fc portion can not ligate Fc receptors). Recent onset (within 6 weeks of diagnosis) T1DM patients received a course of anti-CD3 treatment (14 day, intravenous) in a recent randomized, controlled, Phase I/II clinical trial (Herold et al., 2002). This treatment was associated with a transient decrease in circulating T

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cells, and maintained or improved insulin production after one year in 9/12 treated patients, while only 2/12 controls has a sustained response. Glycosylated haemoglobin levels and insulin doses were also reduced in the antibody-treated group.

VITAMINS

NICOTINAMIDE
Nicotinamide is one of the two principal forms of the B-complex vitamin niacin. Through its major metabolite NAD++ (nicotinamide adenine dinucleotide), nicotinamide is involved in a wide range of biological processes, including the production of energy, the synthesis of fatty acids, cholesterol and steroids, signal transduction and the maintenance of the integrity of the genome. This latter function, or its antioxidative activity, is thought to explain the ability of nicotinamide to prevent diabetes in animal models.

A double blind, controlled clinical trial (IMDIAB III study; Pozzilli et al., 1995) in 56 newly-diagnosed IDDM patients was conducted treating with nicotinamide for 12 months at a dose of 25mg/kg body weight or placebo. Whether this treatment could protect against disease development was assessed during the year after diagnosis. In addition to nicotinamide or placebo, patients received daily insulin injections to optimize blood glucose levels. Whether nicotinamide could protect against T1D was assessed during the year after diagnosis. The results showed no significant difference between groups unless they were stratified for age, improved stimulated beta-cell function only being recorded in patients diagnosed after puberty.

Further controlled trials using nicotinamide at different doses (IMDIAB III study; Visalli et al., 1999), in combination with vitamin E in children less than 4 weeks since diagnosis (IMDIAB IX study; Crino et al., 2004), or at high dose orally in first-degree relatives (ENDIT study; Gale et al., 2004) have also reported no difference in development of diabetes through application of these treatment regimes.

ANTIOXIDANTS
The inflammatory process that results in pancreatic -cell destruction is associated with elevated levels of reactive oxygen and nitric radicals, high-energy molecules that damage cell membranes and protein structures. Vitamins are effective scavengers of such radicals, and thus have been employed in clinical trials (Vitamin E - IMDIAB IV study; Pozzilli et al., 1997) without major improvement in disease course.

As no single vitamin has been effective, treatment with a high oral dose combination of anti-oxidative agents (nicotinamide, Vitamin C, Vitamin E, beta carotene & Selenium) was tested in a double-blind, placebo-controlled clinical study in newly diagnosed T1DM children (Ludvigsson et al., 2001). There was no effect on either metabolic balance or preservation of -cell function through this treatment.

CONCLUDING REMARKS
The last decade has seen many attempts at modulation of the autoimmune process that results in T1DM. It is clear that the immunomodulatory treatment approach holds substantial promise in the future treatment ofT1D and LADA patients as well asT1D transplant recipients. The positive results from the ongoing Phase II trial with GAD65 indicate that GAD65 is likely to be a safe and specific therapy for autoimmune indications.

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LADA and GAD65 Phase IIb Study

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During my work as a clinician at the Department of Endocrinology in Malmö, I have taken a special interest in patients with LADA (Latent Autoimmune Diabetes in Adults). In my thesis, presented in April 2002, we could show that patients with LADA can be clinically, genetically and metabolically distinguished from classical Type 1 and Type 2 diabetes (1-6). LADA, defined as GADab (antibodies to glutamic acid decarboxylase) positivity, occurs in about 10% of patients diagnosed with Type 2 diabetes. Compared with Type 2 diabetes, LADA is characterized by impairment of beta-cell function and features of the metabolic syndrome. Compared with Type 1 diabetes, LADA show better residual beta cell function and more features of the metabolic syndrome (1,2). Some genotypic features distinguish LADA from Type 1 and Type 2 diabetes. The frequency of the Type 1 diabetes susceptibility genotype HLA-DQB1*0201/*0302 and genotypes containing the *0302 allele is increased in patients with LADA compared with Type 2 diabetes, but is lower than in Type 1 diabetes. Further, the frequency of the +/+ genotype of the Hph1-polymorphism in the insulin gene associated with Type 1 diabetes does not differ between LADA, Type 2 diabetes patients or controls, whereas it is higher in Type 1 diabetes (1,2). We proposed that LADA be defined as GADab positivity in a patient older than 35 years at onset of Type 2 diabetes and who initially do not (at least for six months) require insulin treatment (1,2). From a clinical standpoint, it is important to detect LADA in order to provide proper care to these patients. As a means of identifying patients with LADA I recommend routine screening for GADabs in persons diagnosed with Type 2 diabetes. An essential ambition is to preserve the beta-cell function. This may be obtained by early insulin treatment and/or different modes of immunomodulation. Interestingly, when we investigated differences in insulin secretion between non-diabetic GADab+ and GADab- individuals with autoimmunity thyroiditis with intravenous glucose and arginine test, which is considered to measure the maximal insulin secretory capacity, GADab positivity was associated with a decreased maximum capacity for insulin secretion (5). This could mean that GADab positivity is a marker for decreased insulin secretion even in non-diabetic subjects and if followed prospectively could indicate individuals who would benefit from preventive measures for diabetes.

Large clinical trials, such as the DCCT (Diabetes Control and Complications Trial) (7) and the UKPDS (8) have demonstrated the importance of metabolic control in the development of diabetic complications. To date, there are no indications that LADA patients differ in this respect. In the absence of sufficient endogenous insulin secretion, insulin treatment should be initiated, and it is assumed that intensive insulin therapy will reduce the risk for complications. Pharmacological therapy in Type 2 diabetes is aimed at both reducing insulin resistance and improving insulin release from the beta cells. There are no clear strategies for intervention in LADA. Theoretically, exogenous insulin aimed at allowing beta cells to rest could reduce the exposure of reactive T cells to beta-cell antigens (9,10). The action of insulin deteriorates with the degree of insulin deficiency in LADA patients who also show features of the metabolic syndrome, although less prominent than that seen in Type 2 diabetic patients. This implies that measures applied to improve insulin sensitivity could be beneficial in both LADA and Type 2 diabetes. Agents used to improve insulin release from the beta cells may actually aggravate beta-cell destruction, since stimulation of insulin secretion may induce the release of molecules that have antigenic properties (11). Residual beta-cell function in LADA patients offers the potential for “saving” the beta cells that may have long-term benefits. As reviewed by R. Sicree, J. Shaw and P. Zimmet in this GAD-Issue, maintenance of beta cell function might improve glycemic control and reduce the risk of diabetic complications. Therapies such as early insulin treatment, vaccination with specific antigens, and other types of immunomodulation is indeed interesting, not only in Type 1 diabetes but also from a “LADA perspective”.

As a physician, I have been involved in caring for the patients included in the study with the therapeutic use of GAD as an immunomodulant (Diamyd™) in a recently completed Phase II study. In the search of new therapies for autoimmune disease in general and diabetes in particular, the positive evidence for safety and efficacy, together with new insights into the mechanism of action of Diamyd™ (presented elsewhere in this GAD Issue) is both encouraging and exciting. Obviously, further carefully planned clinical trials must be performed to evaluate strategies for both the prevention and treatment of autoimmune diabetes.

“A vaccine to prevent beta cell destruction is an elegant way to tackle autoimmune diabetes”
The promising results from the Phase II trial with Diamyd™ are now being followed up in an enlarged trial. The study is a multicentre, randomised, placebo-controlled Phase II b trial. In total 160 LADA patients will be randomised in two parallel groups, receiving either 20 ìg Diamyd™ or placebo. After an initial eighteen months double-blind study period the patients will continue in a study extension requiring follow-up every six months for another three and a half years for progression to diabetes requiring insulin therapy. LADA patients are defined as non-insulin dependent patients with Type 2 diabetes (diagnosed within five years) with positive GADab titre. Women with no childbearing potential, and men aged between 30 and 70 years old are included.

Patients are recruited via advertisements in the local press. After initial information, patients who agree to participate are pre-screened for GADab titre. Women who give their consent to continue in the study are then vaccinated with the study drug. Diamyd™ or placebo is given subcutaneous as a prime injection and a booster thirty days later. As a safety precaution the patient is asked to stay in the hospital for three hours after the injection. Adverse events are recorded both by the patient and the investigator.

The study objective is to confirm the effect of indicated preservation of beta cell function (increase in fasting C-peptide, and decrease in A1c levels) best seen in LADA patients receiving 20 ìg in the previous Phase II trial with Diamyd™. In addition, the proposed study will provide further data to evaluate the safety of Diamyd™ treatment.

I believe that the development of a vaccine to prevent beta cell destruction and/or preserve beta cell function is an elegant way to tackle autoimmune diabetes. Indeed the indication for induction of a specific T cell population capable of down regulating autoimmunity is extremely interesting. Also of great importance, there seem to be no indications of aggravating beta cell destruction and Diamyd™ appear to be well tolerated. I anticipate future studies with Diamyd™ that offers a product that in addition to possible intervention in diabetes also offers valuable experiences in the developing field of pharmaceuticals in diseases with autoimmune pathogenesis.

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ÅsaLinda Lethagen, MD, Ph.D., is a specialist in Internal medicine and Endocrinology at the Department of Endocrinology, Malmö University Hospital in Sweden. Lethagen has been involved in diabetes research and clinical studies since 1995. She has shown that Latent Autoimmune Diabetes in Adults, LADA, can be clinically, genetically and metabolically distinguished from classical Type 1 and Type 2 diabetes. She is one of the investigators of Diamyd and its use in LADA patients.
When we in Linköping started treatment with plasmapheresis (1), the first immune intervention at diagnosis of diabetes ever tried in the world, we could not foresee the consequences. Plasmapheresis had positive effects on residual beta cell function in some patients although the effect was too small to motivate further clinical trials at that time. Other immune interventions with e.g. Cyclosporin A took over the mainstream interest.

However, our experiences with plasmapheresis had other good consequences: We generated litres of plasma, which not only later became the basis for JDRF and WHO-standards for autoantibodies (2), but also gave us the possibility to analyse more carefully the composition of plasma from diabetic children. A protein of molecular weight 64kD was identified in collaboration with Steinnun Baekkeskov and Åke Lernmark (3), and it is rewarding to experience how this protein, later identified as GAD, has become a key substance in the search for a cure of beta cell disease.

After having tried many different methods to intervene at the onset of Type 1 diabetes in children such as by using antioxidantia (4), photopheresis (5), Linomide (6) and Diazoxide (7), we have now returned to the substance we originally found in our Linköping patients, namely GAD. Based on the results in Diamyd Medical’s LADA patient Phase II clinical trial we have now chosen to give two vaccinations one month apart with 20µg GAD65 (Diamyd vaccine) in a double-blind, placebo-controlled randomised study. Based on power calculations we include 70 children aged 10-18 years who have been diagnosed with Type 1 diabetes during the previous 18 months and who still have residual beta cell function (fasting serum c-peptide 0.1 pmol/l or more) and positive GAD-antibodies (>67 Units). Patients younger than 10 years are excluded for several reasons: Previous immune intervention studies have shown no or little effect in younger children; ethical problems would arise when using a rather unknown drug in very young children; and practically it may be quite difficult to perform repeated sustacal loads in young children which might lead to drop-outs.

In our previous intervention studies we have only included newly-diagnosed patients, but as Diamyd™ vaccination showed a positive effect in LADA-patients, who are supposed to have a slowly progressive auto-

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Children aged 10-18 years who after up to 1.5 years after diagnosis of Type 1 diabetes still have reasonably good C-peptide should have a tendency of a slow progression, similar to LADA patients, and if they then in accordance with LADA patients also have GADA we mean that they ought to be suitable for the treatment.

Residual insulin secretion is evaluated with regular Sustacal loads at baseline and
then after 3, 9, 15, 21 and 30 months. The primary aim is to improve fasting serum C-peptide, but as secondary aim we also want to improve the beta cell response after stimulation, as well A1c and insulin dosage. If two vaccinations with 20µg Diamyd™ prevent or slow down the destruction of beta cells it is a breakthrough, as the vaccine seems to have no side-effects and no known risks. However, as most other immune interventions have had no or very limited effect in children and teenagers with a more aggressive disease process than in adults, our expectations should not be too high. Of course we can just make a qualified guess of what dose is needed in this type of patients, we do not know if two vaccinations will be sufficient or if a third should be added, and we do not know if other inclusion criteria should be used. However, we have just to do the best we can and hope for success. We may gradually learn that a certain form of GAD vaccination is an effective way to calm down the immune process and save residual beta cell function in Type 1 diabetes. Then the natural step will be to find out how to use this technique to prevent Type 1 diabetes.

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Safety and Efficacy of Diamyd™
Sustained at 24 months After Treatment

BY JOHN ROBERTSON, PH.D.
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Positive outcomes for safety and efficacy are apparent from the first clinical trial with Diamyd™. This review will summarise these critical outcomes and identify their implications for further commercial development of Diamyd™ as a therapeutic for treatment of autoimmune diabetes.

This Phase II trial was conducted at two centres in Sweden as a randomized, placebo-controlled, double-blinded, group comparison, dose-escalation study in patients previously diagnosed with Type 2 diabetes and shown to have antibodies to GAD. These patients are thereby identified as having ongoing autoimmunity and have a strong likelihood of beta cell destruction causing their subsequent progression to insulin requirement within six years (Turner R. et al 1997 Lancet 350:1288-1293).

A total of 47 patients was allocated to either one of four groups receiving 4 (n = 9), 20 (n = 8), 100 (n = 9), or 500 µg Diamyd™ (n = 8), or placebo (n = 13). The 4 µg dose was intended as a “no effect” dose level. Nine patients in each group were planned to receive Diamyd™ and three to receive placebo. Treatment was by subcutaneous administration as a “prime” dose followed by a “boost” dose (at the same dose level) one month later. Sequential immunization of each dose group was conducted once no safety issues were confirmed at lower doses. Interim safety evaluation to approve dose escalation was conducted by a separate safety committee 4 weeks after administration of the second Diamyd™ injection.

The wide range in dose levels was used to identify any dose-dependent effects, and particularly for effects that might only be seen at lower – rather than higher – doses. This was considered a distinct possibility in view of “low dose versus high dose tolerance” seen in certain animal models for treatment of other autoimmune diseases (Weiner H.L. Immunology Today 1997 18(7): 335-343).

During the initial 6 months of the (main) study each patient was followed at regular intervals as outpatients with a total 10 study visits, during which the assessment of immunological markers, diabetic status, fasting lipids, haematological and biochemical parameters, as well as physical examinations and reporting of concomitant medications and adverse events were performed. Clinical neurological assessment and EMG were performed pre-dose and after 6 months to detect any adverse effect on the neuromuscular system.

Study un-blinding and analysis after 6 months (i.e. at the end of the main study period) showed:

- None of the patients in any group had significant study-related adverse events. There were no sudden increases in A1c or plasma glucose or decreases in beta cell function observed in any of the dose groups.

- Fasting c-peptide levels at 24 weeks were increased compared to placebo (p=0.0015) in the 20 µg but not in the other dose groups. In addition, both fasting (p=0.0081) and stimulated (p=0.0236) c-peptide levels increased from baseline to 24 weeks in the 20 µg dose group.

- GADA log levels clearly increased (p=0.0002) in response to 500 µg Diamyd™.

- The CD4+/CD25+/CD4+CD25- cell ratio increased (p=0.0128) in the 20 µg group.

These results have been accepted for publication (Agardh C-D et al. J Diabetes & Its Complications, in press).

Since first announcement of these positive clinical outcomes from the main study in June 2003, patient monitoring in the 4.5 year “follow-up” phase of the study has been ongoing. The results from the 2 year follow-up have consolidated the main study outcomes as follows:

- Safety of treatment at all dose levels is demonstrated for up to 2 years after treatment.
The positive impact on diabetic status at 6 months (shown by the increases in C-peptide levels in the patient group receiving 20 µg Diamyd™) is not short-lived, but is sustained for 2 years.

C-PEPTIDE LEVELS IN THE 20 µg DIAMYD™ AND PLACEBO GROUPS OVER 24 MONTHS:

The decreasing trend in A1c observed at 6 months in patients receiving 20 µg Diamyd™ becomes statistically significant in this group alone after 12 months – thereby supporting the positive impact on C-peptide levels seen in patients in the same dose group.

A1C LEVELS IN THE 20 µg DIAMYD™ AND PLACEBO GROUPS OVER 24 MONTHS:

Insights into the mechanisms underlying these positive effects have been provided by immunoassays performed on blood samples analysed from patients in the study.

The fact that clinical safety is still maintained two years after Diamyd™ treatment is underscored. Demonstration of the safety of treatment and the absence of side effects in patients in this study are major factors that have facilitated our progression to further clinical trials.

The positive outcomes for C-peptide and A1c are even more striking, as our follow-up analyses show these improvements to persist for at least 2 years after treatment.

Our finding of statistically significant improvements in C-peptide levels has provided the first clinical evidence for Diamyd™ being therapeutically effective in patients. C-peptide has recently been recognized by the American Diabetes Association as the appropriate outcome measure for Type 1 diabetes clinical trials to preserve beta cell function (Palmer J. P. et al. 2004 Diabetes 53:250-264). It is exciting to note that the positive effect on C-peptide levels observed in the 20 µg dose group is associated with an increase in CD25+ T cells seen only in the same dose group. It is therefore tempting to speculate that the increase in C-peptide seen is mediated by an elevated number of T cells capable of down-regulating autoimmune attack on islet beta cells, and thereby giving rise to improved insulin (C-peptide) production.

The association of the above 2 positive effects in the 20 µg dose group together with a decrease compared to placebo in mean levels of glycated hemoglobin (A1c) after treatment – also only seen only in the 20 µg dose group – is compelling. We reason that the sustained increases in insulin levels seen in the 20 µg dose group (indicated by increases in C-peptide) result - after several months - in improved metabolic control reflected by a lowering in A1c. This finding is exceedingly important because A1c levels is widely used to evaluate the efficacy of diabetes treatments.

In summary, our successful demonstration of clinical safety and provision of evidence for therapeutic efficacy in the first clinical trial with Diamyd™ has provided the critical information required to enable further clinical development. Two more clinical trials are now under way in Sweden. The first of these is a double-blind trial in a greater number of patients in order to confirm efficacy in LADA patients, and the second is the first trial to investigate therapeutic efficacy in recent-onset Type 1 patients (the majority of whom are children or adolescents). We eagerly await the outcome of these further clinical trials with Diamyd™.

John A. Robertson Ph.D., has been VP and Director of Research and Development at Diamyd Medical since its inception in 1994. His responsibilities have included initiation and supervision of in-house product-related research (at the Karolinska Institute in Stockholm), as well as all out-sourced Manufacturing, Pre-clinical, and initial Phase I and II clinical development. Robertson is currently focusing on Manufacturing issues required to expedite further clinical development.
It is well documented that the pancreatic islet beta cells are the unfortunate targets of the autoimmune attack in type 1 diabetes. Only the beta cells are apparently destroyed sparing the neighboring alpha and delta cells. The destruction of beta cells are marked by the presence of autoantibodies to specific beta cell autoantigens such as insulin, IA-2 and GAD65. Although major advances have been made in the understanding of this disease there are still major gaps in knowledge that needs to be filled. These gaps are best filled in safe and carefully controlled clinical trials. All data so far in large clinical trials with insulin or nicotinamide (1, 2) in islet cell antibody-positive subjects at risk for type 1 diabetes showed no protective effect on diabetes development. These well conducted studies showed two things. One is that the type 1 diabetes disease process is not an easy fix. The autoimmune “enemy” is stronger and more vicious than to be affected by these two relatively innocuous treatment approaches. The second is that safe and well conducted studies can be carried out in subjects at risk including teenagers at the particular age when a majority of new patient are diagnosed.

The recent trial to demonstrate safety and beneficial effects of alum-formulated GAD65 is an important step to develop safe therapies that not only suppress or modulate the immune system but also focus on alleviating self reactivity to an autoantigen (3).

Finally, it should be noted that safe therapies that halt the Type 1 diabetes process will soon be needed since subjects at risk are identified in well-funded screening programs. In TEDDY (The Environmental Determinants in Diabetes of the Young) more than 200,000 children will be HLA typed at birth (http://www.teddystudy.org/). HLA high-risk children will be followed body-positive subjects at risk for type 1 diabetes showed no protective effect on diabetes development. These well conducted studies showed two things. One is that the type 1 diabetes disease process is not an easy fix. The autoimmune “enemy” is stronger and more vicious than to be affected by these two relatively innocuous treatment approaches. The second is that safe and well conducted studies can be carried out in subjects at risk including teenagers at the particular age when a majority of new patients are diagnosed.

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REFERENCES
Diamyd, Inc., Announces a New Nonisotopic Assay for GAD65 Autoantibodies (GADA), the Key Marker of Islet ß-Cell Autoimmunity and Predictor of Type 1 Diabetes.

A new, for research use, nonisotopic assay for detection of GAD65 autoantibodies will become available to the market this year.

The new assay, based on an easy-to-use ELISA format, displays equal or better sensitivity and specificity as present Diamyd RBA assay on the market, the Diamyd Anti-GAD65 RIA. The Diamyd Anti-GAD65 RIA was evaluated and proved excellent clinical efficacy by comparison with the reference or gold standard assay, the RBA with in vitro transcribed and translated 35S-GAD65, displaying identical sensitivity of 84% and specificity of 98%.

Classification of Type 1 diabetes, insulin dependent, has been improved by autoantibody testing. The combination of GADA and IA-2 testing is today used as a sensitive way to establish active ß-cell autoimmunity.

Measurement of GAD65 autoantibodies in type 2 diabetes patients is also becoming more important in the prediction of progression to insulin dependent diabetes, termed latent autoimmune diabetes in adults (LADA).

The new GADA assay utilizes the divalent properties of the antibody, which demands two specific binding events to give a true positive detection. This solves the historical problems related to non-specific binding when using ELISA based assay for autoantibody measurement, thus giving the new GADA assay detection of autoantibodies under similar physiochemical conditions to those in the RBA assays.

With the launch of the Diamyd assay, a nonisotopic, rapid, highly sensitive and specific ELISA based method will be available, easily automatable for large-scale screening of the major risk-assessment marker, GADA, for insulin-dependent diabetes mellitus (IDDM).

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Diamyd Medical is listed on the O-List of the Stockholm Stock Exchange (omx: DIAM B) and conducts selected pharmaceutical development based on the GAD (glutamine acid decarboxylase) technology platform. GAD is an important enzyme regulating the balance between excitatory and inhibitory neurotransmitters and is also a target antigen in autoimmune diabetes. Diamyd Medical’s most advanced project is the diabetes therapeutic Diamyd™, for which three clinical trials are currently in progress. Further information is available at www.diamyd.com.