

## NEW THERAPIES AIMED AT THE PRESERVATION OR RESTORATION OF BETA CELL FUNCTION IN TYPE 1 DIABETES

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### ABSTRACT

Type 1 diabetes is caused by an immune-mediated destruction of the insulin-secreting beta cells in the pancreas. The disease can become clinically apparent at any age. At diagnosis, there is invariably some residual beta cell function and more so in adults than in children. Recent studies - including one conducted mainly in Belgium - have provided proof of principle that short-term anti-T-cell antibody treatment is able to preserve residual beta cell function for at least 18 months. The resultant stabilizing effect on metabolic control is expected to delay or limit chronic complications in these patients. With a similar goal in mind, non-uremic C-peptide negative patients are offered beta cell transplantation. The outcome of these implants

looks promising but their final applicability hinges on finding ways to induce immune tolerance to the donor beta cells. A widespread application, however, will only occur if the shortage of viable human donor cells can be overcome. Both xenotransplantation and stem cell therapy provide possible strategies to solve this problem and represent areas of intense investigation. The ultimate goal is prevention of clinical disease. Studies by the Belgian Diabetes Registry and others in first degree family members of type 1 diabetic patients have refined the identification of individuals at very high risk of hyperglycaemia so that new immunological treatments can be tested in the prediabetic phase.

### INTRODUCTION

Type 1 diabetes is a disease of unknown origin, which can become clinically apparent at all ages (1). An important step forward in our understanding of the disease process was provided by Gepts' description of pancreatic islet infiltration by lymphocytes and macrophages (insulinitis) in children and young adults who died soon after clinical onset (2;3). Another hallmark is a selective loss of the insulin-producing beta cells in the pancreas (2-4). Nowadays it is assumed that this beta cell loss is the consequence of a T-cell-mediated autoimmune attack (5). The most classical form of type 1 diabetes is diagnosed weeks to months after the development of insulinopenic symptoms such as thirst, fatigue, polyuria and weight loss. At this clinical stage, life-long subcutaneous insulin treatment be-

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comes obligatory. These daily insulin injections present a heavy social burden to patients and their family. In fact, even with the so-called intensified insulin regimens, patients have to match insulin doses carefully with a (preferentially pre-planned) diet and exercise in order to avoid disabling hypoglycaemia and unacceptable blood glucose levels. If this is not achieved, the individual is at risk of developing chronic complications of diabetes (6;7). In Western countries, these microvascular and macrovascular complications are responsible for most of the diabetes-related morbidity and mortality. New experimental treatments aim to change the natural course of the disease in order to prevent or retard these devastating complications including blindness, kidney failure, diabetic foot and lower limb amputations, incapacitating or deadly cerebrovascular and myocardial infarction. The quality and power of these intervention trials depends largely on the inclusion of subjects at homogeneous risk of loss of beta cell function and the tests that are used to assess beta cell function.

### Tests of beta cell function in type 1 diabetes

Non-invasive methods for the measurement of beta cell mass in vivo are currently lacking in man. Presence of beta cells must therefore be assessed indirectly in insulin-treated subjects, through measurements of plasma C-peptide and proinsulin concentrations as indicators of beta cell function. Both hormones can be measured during fasting and after physiological or non-physiological stimulation. In most intervention and islet transplantation studies, levels of plasma C-peptide are examined, but variations in test conditions prevent the formation of reliable comparisons between studies (8).

Both the oral glucose tolerance test (OGTT) and the mixed meal tolerance test can to some extent be regarded as physiological tests of beta cell function in response to glucose but unfortunately their reproducibility is poor. The intravenous glucose tolerance test (IVGTT) represents a non-physiological test that was standardized in the nineties by the ICARUS group (9). The beta cell secretory response is usually represented as the sum of the plasma insulin at 1 and 3 min after the iv glucose load. It is generally felt that this test is more sensitive than the oral stimulation tests and can therefore detect subtle abnormalities at an earlier stage of the disease before clinical onset (10), but this test is difficult to standardize (9). Moreover, the first

phase insulin response is lost in most insulin-treated patients after onset of hyperglycaemia (11). Alternatively, a hyperglycaemic clamp can be used, which is regarded as the golden standard test for the measurement of residual beta cell function in type 2 diabetes (12). Negative aspects of this test are that it is time-consuming and necessitates experienced personnel and therefore cannot be done on a large scale. In addition to the insulin secretory capacity in the presence of glucose, the functional beta cell mass can also be studied in terms of response to cyclic AMP. This is usually done after a non-physiological glucagon or arginin iv injection. The glucagon test results depend largely on the prevailing glucose concentrations, making direct follow-up of beta cell function problematic (13).

### The natural history of type 1 diabetes

The natural history of type 1 diabetes after onset of hyperglycaemia is more heterogeneous than usually described in textbooks. Onset of hyperglycaemia is caused by the dropping of beta cell mass to a level that is unable to maintain pre- and postprandial glycaemia within the non-diabetic range. At this stage, beta cell mass is generally assumed to have decreased to 5-20% of levels in age-matched controls. However, most of these data were gathered at a time when diagnosis depended primarily on clinical suspicion, rather than "routine" blood glucose measurements which are used increasingly frequently, principally in family members of type 1 diabetic patients. For example, a subgroup of patients today is diagnosed solely because they have 2hr hyperglycaemia post oral glucose challenge (14). Furthermore, few quantitative measurements exist of the pancreatic beta cell mass at clinical onset in adult type 1 diabetic patients, although they represent the largest group. Residual beta cell function that is assessed with glucagon or arginin is higher than after acute intravenous glucose injection and therefore is in most cases maintained (15). Another important factor in test conditions is the duration of intensified insulin treatment. It is well established that residual beta cell function tends to recover to some extent after instauration of insulin treatment, especially in individuals with significant hyperglycaemia at diagnosis (16). Notwithstanding these caveats, most studies show a major decrease in residual beta cell secretion, especially in young children, which may suggest that type 1 diabetes follows a more aggressive course in this age category (17-19).

After clinical onset of the disease, continuation of beta cell destruction over months to years finally leads to undetectable plasma C-peptide levels (i.e. plasma C-peptide negativity) in most subjects. Whilst reviewing published data on beta cell mass in insulin-requiring type 1 diabetes, Pipeleers et al. noticed a marked age-dependent heterogeneity in the number of surviving beta cells in pancreata procured 1-30 years after diagnosis (4). In patients diagnosed before the age of 7 years, the residual beta cells invariably disappeared within the first years after clinical onset whereas in older patients, a more moderate and variable decrease in pancreatic beta cell mass was observed over time. In a recent study of patients aged 12-39 years, we documented on the loss of residual beta cell function during the 18 months after diagnosis of type 1 diabetes mellitus (20). Beta cell function was assessed through the C-peptide release during hyperglycaemic clamp, first in the absence and then in the presence of glucagon. Glucagon increased C-peptide release at all time points, indicating that beta cells maintained a secretory response to glucagon despite the diabetic state and previously imposed 2hr period of hyperglycaemia. Both indices of beta cell function progressively decreased following diagnosis, with an average reduction of 35% after 18 months. This reduction was accompanied by a 50% rise in mean daily insulin needs. Within the same age range, however, a variable loss of beta cell function was measured, demonstrating that other factors besides age contribute to the rate of decline of C-peptide levels after clinical onset. A multivariate analysis by Decochez et al. showed that random C-peptide levels 2 years after clinical onset were correlated with autoantibodies against unidentified islet cell cytoplasmic antigens (ICA) at diagnosis and to a lesser degree with age at diagnosis (19). More recently, Weets et al. described a more rapid decline of residual beta cell function in male type 1 diabetic patients (21). Another important consideration regarding C-peptide secretion is that an early implementation of intensive insulin therapy at diagnosis can slow down the loss of C-peptide, as shown in the Diabetes Control and Complications Trial (DCCT) (22).

While our understanding of beta cell function in insulin-requiring type 1 diabetes after clinical onset has improved during the last decade, there is little evidence from human data to support hypothetical cartoons showing the time sequence of decline in beta cell function prior to clinical onset. When high risk relatives are studied thoroughly as in the Diabetes Prevention Trial- type 1 (DPT-1 see later), in the majority

of cases a progressive deterioration of glucose tolerance leading to diabetes can be documented which is assumed to represent a simultaneous decline in beta cell function.(23).

### Primary prevention of type 1 diabetes

The most elegant strategy to tackle type 1 diabetes would be primary prevention of the disease, i.e. prevention of onset of beta cell destruction by avoiding the exposure of genetically predisposed subjects to putative environmental triggers. It is presumed that environmental agents initiate an autoimmune reaction, maybe via limited destruction of pancreatic beta cells. It should be emphasized that no environmental factors have been identified, except in a few individual cases. Viruses, especially enteroviruses such as Coxsackie B viruses are old suspects (24;25). Dietary factors may be alternative environmental insults (26). Controversial reports point to an early introduction of cow's milk as a putative trigger. To test this hypothesis, a primary prevention trial, the TRIGR study, is currently underway to determine whether the incidence of childhood type 1 diabetes can be reduced in first-degree relatives at genetic risk (27). After exclusive breastfeeding, infants in this study underwent a double-blind, randomised trial of either casein hydrolysate or conventional cow's milk-based formula until the age of 6 months. At the present time, a life-table analysis shows a significant protection by the intervention from positivity for ICA (27).

### Secondary prevention of type 1 diabetes

A second option is to arrest or delay beta cell destruction before the onset of hyperglycaemia (i.e. secondary prevention). It is assumed that this phase of the disease process can easily be diagnosed by the measurement of ICA, insulin, tyrosin phosphatase (IA-2), or glutamic acid decarboxylase (GAD65). It is well documented that these autoantibodies can be present in non-diabetic relatives of type 1 diabetic patients from months to decades before the onset of hyperglycaemia (1;28). In cases that are followed from birth, they appear to be already present at the age of 5 years. In these children, insulin antibodies tend to precede the formation of other autoantibodies (26).

As in other autoimmune endocrine diseases, however, not all subjects develop clinical disease (29). An-

other disturbance to the creation of more or less homogenous risk groups is that all mathematical possible combinations of autoantibodies can be measured at the same time and that changes may occur during follow-up. It is unknown today whether this heterogeneity in autoantibody positivity in the prediabetic phase mirrors a marked heterogeneity in residual beta cell function. Therefore at the present time, the additional use of a metabolic test of beta cell function is in the authors' view obligatory in intervention trials. Most studies have used the IVGTT (30,31).

During the previous years, two large secondary prevention trials, DPT-1 and the European Nicotinamide Diabetes Intervention Trial (ENDIT), received much attention. (30,31). ENDIT was a randomized double-blind trial that enrolled non-diabetic ICA-positive first-degree relatives of patients with type 1 diabetes. (30;32). Five hundred and forty nine participants were given high-dose oral nicotinamide or placebo. Nicotinamide was given to decrease DNA damage and subsequent apoptosis of beta cells. After 5 years of treatment, incidences of the onset of hyperglycaemia were identical in the nicotinamide-treated group and the placebo-group. Thirty-four percent of subjects had a low insulin response during IVGTT at base-line and the cumulative risk of progression to diabetes in the placebo group was lower than expected. The DPT-1 trial also failed to show any beneficial effect of an intervention which consisted of subcutaneous treatment with ultralente insulin twice daily in 170 subjects (31). The equally-sized control group did not receive any treatment. Participants were ICA-positive relatives with 2 abnormal IVGTT s and/or oral glucose intolerance. Subcutaneous insulin treatment was supposed to allow beta cells to rest and to decrease the autoimmune aggression. With the subcutaneous doses injected, however, complete beta cell rest was not achieved as evidenced by the non-suppressed plasma C-peptide levels. (33)

The outcomes of both trials were in stark contrast to those of the smaller human pilot studies (34-36) and those in the NOD mice (37), which is regarded as the best available animal model of human type 1 diabetes. The latter may partially be explained by the fact that it is very difficult to transfer the dose and schedule of the pharmacological treatment from mice to man. Fortunately, no clinically relevant serious adverse events were reported in either of the human trials (31;32) and participation in these trials appears to have benefited most subjects who developed clinical diabetes. Frequently subjects presented with asymptomatic hyperglycaemia at diagnosis, which sharply

contrasts with the classical presentation of diabetes in routine clinical care.

### **Tertiary prevention of type 1 diabetes in C-peptide positive patients at clinical onset**

During the last decade, encouraging outcomes in terms of beta cell function have been reported when experimental treatment is given at clinical onset of the disease. This prevention strategy aims to safeguard the patient from chronic complications of the disease. A substudy of the DCCT provided strong evidence that preservation of beta cell function not only results in improved glucose control, and consequently fewer chronic complications such as retinopathy and incipient nephropathy but also leads to a lower incidence of severe hypoglycaemic events (38).

Following the hypothesis that Type 1 diabetes is an autoimmune disorder that involves the killing of beta cells by cytotoxic T-lymphocytes, a wide range of immunosuppressive or immunomodulating agents have been tested at clinical onset in small, poorly controlled pilot trials. A demonstration of both the feasibility and effectiveness of this approach was first provided by the usage of cyclosporine. This calcineurin-inhibitor was the first immunosuppressive agent that was used in placebo-controlled, double-blind clinical trials (39;40). Beta cell function was preserved by a daily intake of cyclosporine during the first 12 months. However, the benefit was lost after withdrawal from the drug, implying a need for the indefinite administration of cyclosporine, with attendant risks of chronic immunosuppression and calcineurin nephrotoxicity.

An alternative approach consists of short-term intravenous administration of monoclonal antibodies directed at the CD3-complex that is present on all T-lymphocytes. The first on the market, OKT3, was a mouse IgG2a that was successfully used to treat and prevent renal allograft rejection (41). However, the associated major cytokine-release syndrome excluded its use in recent onset type 1 diabetic patients (42). Because of its high antigenic nature, OKT3 also stimulates the production of neutralizing antibodies that may decrease the efficiency of OKT3 after a few injections. Chatenoud et al. administered monoclonal antibodies against mouse CD3 during 6 consecutive days to NOD mice with recent overt hyperglycaemia and was thereby able to obtain a long-term remission in 80% of the animals. These studies provided the basis for the development of monoclonal anti-CD3 antibodies with a better safety profile (43). Human en-

gineered anti-CD3 antibodies that do not produce a full cytokine-release syndrome and that are less antigenic are now available. To achieve this, two mutations were introduced into the Fc portion of the humanised version of OKT3, giving rise to OKT3 $\gamma$ 1 Ala-Ala (44). Another antibody, ChAglyCD3, derived from the rat YTH 12.5 antibody, contains a single mutation that prevents glycosylation of its  $\gamma$ 1 Fc portion (45). With OKT3 $\gamma$ 1 Ala-Ala, a phase 1 study in children and adults was performed in the United States with promising results, although some concern was expressed since the study was not blinded (44). Because glycaemic control was not standardized and HbA1c concentrations were higher in the control group, residual beta cell function could have been underestimated in this group due to presumed glucotoxicity (44). The Belgian Diabetes Registry together with the Schwabing Hospital in Munich (A. Ziegler) therefore performed a multicentre placebo-controlled phase 2 trial including 80 patients randomly assigned to receive placebo or ChAglyCD3 for 6 consecutive days (20). Only autoantibody positive patients between the ages of 12 and 39 years who had been undergoing insulin treatment for less than 4 weeks, and who had basal C-peptide levels greater than 0.60 ng/ml, were included. These entry criteria ensured that patients with type 1 diabetes who had already experienced a major loss of beta cell function when diagnosed did not participate. Beta cell function was assessed with a hyperglycaemic clamp every 6 months. Results showed that up to 18 months post-treatment residual beta cell function was better maintained in antibody-treated patients than in those who were given placebo. Importantly, both treatment groups had comparable HbA1c concentrations of less than 7% during the whole treatment period. To maintain this level of glucose control, ChAglyCD3 treated patients needed less insulin than placebo-treated patients. Follow-up of this study, which has been extended to 48 months, will reveal whether the ChAglyCD3 treatment effect is maintained. Another interesting aspect of this study is the finding that ChAglyCD3 was particularly effective in those patients who initially had the highest residual beta cell function (20). The fact that ChAglyCD3 appears predominantly effective when substantial residual beta cell function is present may limit its routine clinical use, as injecting the CD3 monoclonal antibody with potential side-effects may be ineffective if the beta cell function is too low. Adverse events caused by anti-CD3 treatment will be better assessed in phase 3 trials that are underway. In the phase 2 study, 6 consecutive doses of 8 mg of ChAglyCD3

treatment resulted in a limited cytokine related flu syndrome in virtually all patients, especially after the first 2 infusions, but these side-effects never necessitated stopping or delay of treatment (20). Future studies should assess whether similarly effective anti-CD3 treatment can be obtained with lower doses in adults and in children. In the phase 2 study, which included only subjects with a history of Epstein Barr virus (EBV) infection, confirmed by their EBV IgG positive status, a reactivation of EBV was observed 10–20 days after the first injection. An increase in EBV copies in peripheral blood mononuclear cells was measured and 75% of patients had symptoms of infectious mononucleosis. Within 1–3 weeks all patients were asymptomatic and the number of EBV copies returned to normal baseline pre-treatment levels. Concomitantly, a cellular and humoral immune response specific to EBV was measured, comparable to that observed in normal subjects who develop acute infectious mononucleosis (20). At the present time, no lymphoma, PTLD-like disease or other serious adverse events related to treatment, have been reported. It is unclear whether the EBV-related disease resulting from anti-CD3 treatment is triggered by the cytokines released during the first days of treatment or by other mechanisms. The question of whether the induction of virus-antigen-specific CD8+ T cells is implicated in the maintenance of residual beta cell function must also be answered. The action mechanisms of human anti-CD3 antibodies remain unclear. From experiments in NOD mice, it can be speculated that induction of so-called regulatory CD4+CD25+ T cells play a decisive role and may help to restore self-tolerance to beta cell autoantigens without triggering generalized immune suppression (46).

### Tertiary prevention in C-peptide negative type 1 diabetic patients

Implantation of a metabolically adequate beta cell mass in these patients constitutes a potential strategy, whereby restoration of the insulin secretory responses to metabolic needs would correct glucose homeostasis and thus limit the incidence of hypoglycaemia and the severity of the chronic complications. Over the past 20 years, the surgical procedures for whole-pancreas transplantation have remarkably improved. This technique can now be performed with one-year success rates that are close to those for kidneys alone (47). Pancreas graft survival is almost invariably associated with normalized glucose levels and a state of insulin-

independence, which improve the quality of life for the recipients. However, these benefits do not occur without risks, especially in non-uremic patients (48). Firstly, the surgical procedure is still technically cumbersome and associated with morbidity. Moreover, the need for chronic immune suppression carries the same infectious and tumorigenic risks as other organ transplantations. The decision for pancreas transplantation is therefore usually delayed until late in the course of diabetes when renal failure increases the need for a kidney graft. At this later stage, simultaneous implantation of a pancreatic organ will not bring a major benefit in terms of prevention of secondary complications, as these are already advanced to an irreversible level. Pancreas transplantation at an early stage of the disease would certainly qualify as a cure if means to induce immune tolerance to transplanted organs became available. In their absence, more attention will probably remain necessary on methods for islet cell transplantation as a treatment modality for non-uremic patients.

Islet cell transplants have since long been proposed as a safer alternative to pancreas transplants. (49) Studies in rodents have demonstrated that islet grafts can be implanted in different sites using simple techniques (reviewed in (50)). In several models the islet transplants corrected diabetes rapidly and for extended periods. More importantly, pre-treatment of isolated rodent islet tissue or cells can reduce the immunogenicity of this donor material so that successful allografts can be performed without the need for permanent immune suppression (51-54). For many years, none of these promising features could be reproduced in type 1 diabetic patients. Reasons for this were probably multiple, ranging from technical difficulties in preparing viable and metabolically adequate grafts to biological obstacles of inflammatory and immune natures. Over the years, progress has been made in the isolation of human islet tissue and its use in auto- and allotransplantations (55). Human islet grafts were shown to correct diabetes in type 1 patients who had received a donor kidney prior to, or simultaneously with, the islet graft (56-58). However, the percentage of successful cases at one year after transplantation remained low (59). In a more recent study of non-uremic recipients, Shapiro et al. observed that insulin-independence was achieved in 80% of subjects after 1 year (60). This success was attributed mainly to the use of a new immunosuppressive regimen, although the improvement of selected organ procurement and processing conditions were probably more important contributors. This was documented recently in an international study that used this so-

called Edmonton protocol (61). In this study, there was a marked site-to-site heterogeneity in the proportion of patients that reached 1-year insulin-independence, varying from 0 to 100%. In a similar group of patients with another immune suppressive regimen, we were able to obtain 1-year insulin-independence in all patients who received at least twice 2 million beta cells per kg (Keymeulen et al. Proc Natl Acad Sci USA in press). The human islet cell transplantation procedure can also be considered to be safe. In our own program, islet beta cell grafts are infused by laparoscopic repermeabilization of the umbilical vein or percutaneous transhepatic puncture, so far without serious adverse effects (62;63). Through these encouraging results, the first step towards considering islet cell transplantation as a treatment for diabetes, namely, the ability to prepare islet cell grafts that can reproducibly correct diabetes in type 1 diabetic patients without major adverse events, has been realised. However, other hurdles still have to be taken before islet cell transplantation can be considered to be a cure. Firstly, most patients become again insulin-dependent by 2 to 5 years after transplantation. In a selected group of recipients, the insulin-independence rate with the Edmonton protocol was 20% after 5 years (64). The progressive loss of long-term graft function may be the consequence of a subtle interplay of many precipitating factors. Some of the immunosuppressants, such as the calcineurin-inhibitors, may be diabetogenic. Another possibility is the relative ineffectiveness of the commonly used immunosuppressive drugs, which can result in chronic rejection and may contribute to the loss of function of islet grafts with time. It is also unknown to what extent the immune suppressive regimens can suppress reactivation of the autoimmune destructive process against the beta cells (65,66). In the absence of immune suppression, this reactivation can be fast and vigorous, even in cases where it had been dormant for several years, as indicated by the rapid destruction of a pancreas transplant in non-immunosuppressed HLA identical twins (67). Another possibility is that the liver is not the most optimal site for maintaining long-term islet graft function, as documented in rodents (68). Lastly it cannot be excluded that the beta cell renewal capacity in human islet grafts is limited.

A second problem to be solved is that all human islet transplantation programs nowadays use non-specific immune suppressants. Each of these agents, but especially sirolimus, can be associated with side-effects, some with a serious impact on vital tissues (61). The immunosuppressive regimen that is used in our program consists of anti-thymocyte globulin (ATG)

as an induction treatment followed by mycophenolate mofetil and tacrolimus as a maintenance therapy (Keymeulen et al. Proc Natl Acad Sci USA in press). This regimen is better tolerated than the Edmonton immunosuppressive regimen but its long-term risks and efficacy remain to be determined. In organ transplantation, life-long maintenance immune suppression increases the risk for recurrent infections and certain cancers.

Another limitation of islet cell transplantation is the unfavourable donor-to-recipient ratio. In the successful series of islet transplantations carried out by the Edmonton group and in our series, metabolic correction required islet preparations from 2 to 5 pancreata or pancreatic segments. (61)

### Perspectives

Provided that anti-CD3 treatment is also safe in children, it may be reasonable to attempt this therapy in children and adults with a very high risk of developing clinical disease (29;69;70). The promising results of experimental interventions at clinical onset in insulin-requiring type 1 diabetes may also be of interest for a subgroup of diabetic patients that is frequently misdiagnosed as suffering from type 2 diabetes. These individuals have no insulinopenic symptoms at clinical diagnosis, are frequently older than 40 years of age and show evidence of autoantibodies (71;72). GADA is the most frequently occurring autoantibody, followed by ICA. The disease of these autoantibody-positive type 2 diabetics is often termed latent autoimmune diabetes in adults (LADA). This group of patients comprises approximately 10-15% of Caucasian "type 2" diabetic patients (73). In the United Kingdom Prospective Diabetes Study (UKPDS), the LADA patients entering this study were estimated to have approximately 50% of the residual beta cell secretory capacity. The natural course of these patients shows that C peptide will decrease with time in these patients in parallel with the curve for C peptide in classical type 1 diabetic patients. Most of the LADA patients will require insulin injections for appropriate glucose control within 6 years after clinical diagnosis (73; 74).

The future of islet beta cell transplantation will depend on the ability to establish long-term allograft function without the need for a continuous generalized immune suppressive treatment and on the safe use of new sources of islet beta cells.

The hypothetical "ideal" therapeutic strategy in

beta cell transplantation should result in a selective suppression of the patient's immune reactivity to both allo- and autoantigens of the beta cells. Operationally, this means the establishment of immunological tolerance in a mature immune system, in other words a state of sustained antigen-specific unresponsiveness in the absence of generalized immune suppression. Such strategy should avoid (1) the destruction of the islet implant by alloimmune reactions to donor-specific antigens, (2) a relapse of the autoimmune attack to beta cell-specific antigens, and (3) the need for continuous generalized immunosuppressive treatment. In long-term clinical allografts of liver, and rarely kidney, immune tolerance has been noticed. In some transplant recipients, the dosage of maintenance immune suppression could be drastically reduced, or even completely stopped, without a major loss in graft function (52). However, this could not be regularly implemented, since there is no immunological marker available that allows identification of patients in whom immune suppression can be decreased without graft loss. Better insights into the immune mechanisms mediating transplantation tolerance would, of course, help in the definition of such markers and in the design of clinical strategies. Over the last years, strategies towards tolerance induction have been developed in various experimental models, including nonhuman primates (75;76) but none of them are sufficiently safe for clinical use (77). Experimental induction of tolerance of allografts usually requires transient immune suppression of the recipient at the time of, and mostly also for some time after, transplantation. Antibodies to lymphocytes or to some of their membrane molecules have been instrumental to this effect. They are expected to suppress T cell activation that would lead to rapid graft destruction, while allowing formation of regulatory T cells that are needed for long-term graft survival in the absence of a sustained immunosuppressive therapy. It is clear that this transient immunosuppressive treatment should also succeed in suppressing a reactivation of the autoimmune destructive process. Interestingly, antilymphocyte antibodies have been found to exhibit such an effect in rodents (78) We have noticed that patients with a history of ATG treatment at the time of kidney transplantation did not develop diabetes-specific T cell autoreactivity after they received an islet cell allograft, as if the antibody treatment had removed this property (65). In these ATG pre-treated patients, the maintenance immune suppressive regimen also appeared sufficient to suppress autoimmune destruction of the graft (58). It is, of course, possible

that discontinuation of this immune therapy would result in a return of the autoimmune process.

Another hurdle to be taken in islet beta cell transplantation is the use of beta cells that are not of human cadaveric origin, as the number of potential recipients outnumbers by large the number of islet beta cell transplants that can be done from cadaveric human donors. Prenatal porcine islet cell grafts can be produced on a large scale and are able to normalize hyperglycaemia in immunocompetent mice (79;80). Studies with porcine xenografts in primates are underway and promising (81;82). Importantly, they were not associated with the transmission of porcine endogenous retroviruses (81). Porcine islet beta cell transplantation will necessitate, however, the introduction into clinical practice of new immunosuppressive agents that can be used safely.

It is hoped that one day therapeutic in vitro or in vivo islet beta cell regeneration or neofunction will become a clinical reality. This is a highly ambitious area of intense and active research that examines neofunction of beta cells from adult or embryonic stem cells, transdifferentiation from adult cells with another phenotype to cells with a beta cell phenotype and beta cell replication. (83)

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### SAMENVATTING

Type 1 diabetes kan klinisch op elke leeftijd tot uiting komen en wordt veroorzaakt door een immunogemedieerde vernietiging van de insuline-secreterende betacellen. Meestal kan er nog een residuele betacelfunctie worden gemeten wanneer voor het eerst hyperglycemie wordt gemeten. Recente studies waaronder één door het Belgisch Diabetes Register

hebben aangetoond dat een korte behandeling met een gehumaniseerd antilichaam gericht tegen de T-lymfocyten, deze residuele betacelfunctie gedurende minstens 18 maanden kan behouden. Bij placebo-behandelde patiënten daarentegen zal na de diagnose een progressieve daling van de residuele betacelfunctie worden gemeten. Bij behoud van residuele betacelfunctie hebben de patiënten een lager risico op ernstige hypoglycemie en de chronische complicaties van diabetes. In de nabije toekomst wenst men te onderzoeken of deze behandeling eveneens werkt in de preklinische fase bij verwanten van type 1 diabetespatiënten met een sterk verhoogd risico op het ontwikkelen van deze ziekte. Bij patiënten zonder residuele betacelfunctie kan betaceltransplantatie worden overwogen met als doel hypoglycemie en de chronische complicaties van diabetes te voorkomen. Momenteel kunnen betacel-enten echter enkel overleven onder chronische afweeronderdrukkende medicatie en kunnen er slechts een gering aantal transplantaties worden uitgevoerd door een tekort aan goede humane cadaverdonoren. Men hoopt dat in de toekomst de transplantatie van varkensbetacellen of stamcellen dit probleem kan oplossen.

### RÉSUMÉ

Le diabète type 1 est causé par une destruction auto-immune des cellules bêta du pancréas. La maladie peut devenir cliniquement apparente à n'importe quel âge. Au moment du diagnostic, il existe une activité résiduelle des cellules bêta, en général davantage chez les adultes que chez les enfants. Des études récentes, incluant une étude majeure effectuée en Belgique, ont apportés des preuves du principe qu'un traitement de courte durée par anticorps anti-cellules T est capable de préserver la fonction des cellules bêta pour une durée d'au moins 18 mois. La stabilisation sur le contrôle métabolique qui en résulte est censée retarder ou limiter les complications chroniques chez ces patients. Dans cette même perspective, on peut proposer à des patients non-urémiques qui sont C peptide négatifs une transplantation des cellules bêta. Le suivi de ces implants semble prometteur mais leur application finale dépend de la découverte de l'induction d'une tolérance immune aux cellules bêta du donneur. Une application large sera cependant seulement possible si l'on peut remédier au manque de donneurs de cellules humaines viables. Autant la xenotransplantation que la thérapie par cellules souches fournissent des stratégies possibles pour résoudre ce problème et

représentent des domaines d'intenses investigations. Le but ultime est la prévention de la manifestation clinique de la maladie. Des études faites par le registre Belge et d'autres chez des membres de famille au premier degré avec diabète de type 1 ont permis d'identifier des individus à très haut risque d'hyperglycémie de telle sorte que de nouveaux traitements immunologiques peuvent être testés dans la phase de pré-diabète.

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