Restoring a functional β-cell mass in diabetes

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Type 1 and type 2 diabetes have often been presented as disease forms that profoundly differ in the presence and pathogenic significance of a reduced β-cell mass. We review evidence indicating that the β-cell mass in type 1 diabetes is usually not decreased by at least 90% at clinical onset, and remains often detectable for years after diagnosis at age above 15 years. Clinical and experimental evidence also exists for a reduced β-cell mass in type 2 diabetes where it can be the cause for and/or the consequence of dysregulated β-cell functions. With β-cell mass defined as number of β-cells, these views face the limitation of insufficient data and methods for human organs. Because β-cells can occur under different phenotypes that vary with age and with environmental conditions, we propose to use the term functional β-cell mass as an assessment of a β-cell population by the number of β-cells and their phenotype or functional state. Assays exist to measure functional β-cell mass in isolated preparations. We selected a glucose-clamp test to evaluate functional β-cell mass in type 1 patients at clinical onset and in type 1 recipients following intraportal islet cell transplantation. Comparison of the data with those in non-diabetic controls helps targeting and monitoring of therapeutic interventions.

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Reduced β-Cell Mass in Type 1 and Type 2 Diabetes

Diabetes occurs under different clinical forms but all present a failure of the pancreatic β-cell population to provide sufficient insulin for glucose homeostasis. In type 1 diabetes, this failure is primarily attributed to a massive loss of β-cells prior to onset [1]. After diagnosis, the residual β-cell mass can be expected to further decline as a result of the ongoing autoimmune process [2]. There are, however, few quantitative data on the β-cell mass during the course of the disease, and those that are available may not always be representative for the organ as they have been collected from too little tissue which, in addition, came from unspecified or variable regions. Furthermore, data from age-, sex- and body-matched normal controls were often not reported making it difficult to express losses as a percentage. Finally, in non-diabetic patients of the same age a large between-subject variability has been noted because long known, thus further limiting quantitative comparisons [3].

In the past it has often been stated that 90% of the β-cells are destroyed when type 1 diabetes is diagnosed, and that the remaining part is usually lost during subsequent years. This common assumption probably originates from Gepts’ seminal paper in which he concluded that the number of β-cells in patients with acute juvenile diabetes is as a rule less than 10% of normal and that the residual β-cells completely disappear during the subsequent ‘chronic’ phase [1]. In reviewing the literature, we noticed that the loss during the disease process varied

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with age [4]. The massive depletion around clinical onset appeared only characteristic for children. The β-cell number appeared less reduced at onset above age 15, and this remained so during subsequent years. When different age groups were compared for their progression to a complete loss of β-cells, this occurred within 1 year for almost all children under age 7, whereas only in a small minority of older patients. When the disease was diagnosed between age 7 and 30 years, 40% of patients were β-cell positive when their pancreas was autopsied 1–30 years later [4]. These histopathological observations are consistent with measurements of β-cell function in type 1 diabetic patients of various ages at clinical diagnosis: residual C-peptide secretion rapidly disappears following diagnosis in children although it remains detectable for years in a significant proportion of onsets at adolescence and even more so in adults [5–7]. For patients diagnosed at age 30–40 years and followed for 15–34 years, 15% was found to maintain a residual C-peptide secretion. The Diabetes Control and Complications Trial (DCCT) initiative reported that 30% of type 1 patients diagnosed above age 13 years presented a meal-induced C-peptide secretion during 5 years after onset; beyond 5 years this percentage decreased to 11% for adult-onsets and to 0% for adolescent-onsets [8,9].

Cases with clinical onset under age 7 are thus characterized by a rapid depletion in β-cells. This could express the outcome of a more acute and massive inflammatory and immune process than at older age. It is also conceivable that β-cells of young children are more susceptible to damaging mediators, for example as a result of their state of differentiation or of their metabolic or hormonal environment. Another possibility is that β-cell loss in young subjects imposes a heavier metabolic load on the remaining β-cells in view of the higher insulin needs during growth [10]. During this period, the β-cell mass is also expected to markedly expand [11,12], a process that will be affected when β-cells with proliferating capacity are destroyed.

The course leading to rapid and complete deletion is, however, not the most prevalent one in the pathophysiology of type 1 diabetes. Use of autoantibody assays has indeed shown that diagnosis of the type 1 form is not restricted to children, and that in fact more cases are diagnosed after age 15 than before [13,14]. Consequently, patients whose β-cell mass is higher than 10% of normal at onset are more frequent, and those who maintain pancreatic β-cells for several years far from an exception. The question is of course whether this residual β-cell mass is functional, and clinically relevant, and whether it can be the target for therapeutic interventions aiming at its preservation and its growth to a size that can achieve again metabolic control. It will thus be necessary to define criteria of a functional β-cell mass, and seek ways to measure it (see next sections).

In type 2 diabetes, insulin’s failure to maintain glucose homeostasis is usually attributed to a combination of an insulin resistant state and a secretory dysfunction of the β-cells [15–18]. Debates have often focused on the sequence in which these pathogenic components appear rather than on a possible role of a reduced β-cell mass in the pathogenesis. The lower attention to the β-cell mass is explained by its in vivo inaccessibility to quantification, the difficulties in performing and interpreting in vitro measurements and the scarcity of data. A decrease in β-cell mass has been reported by several pathologists of the human pancreas [3,19–22] but according to one of them the reduction was only observed in the subgroup that was treated with insulin raising the question whether it was a feature of the more severe cases or a consequence of the insulin administration [21]. In more general terms it is difficult to determine whether a reduction in β-cell mass precedes the hyperglycaemic state or develops during the course of the disease. Because the diagnosis is usually made following an undetermined, and possibly long, period of glucose intolerance or hyperglycaemia, primary etiologic events are not easy to recognize and to dissect, and their influences not simple to distinguish from those of secondary factors such as the metabolic derangements in diabetes and their treatment. In more practical terms it seems in the first place important to know whether the β-cell mass is reduced in type 2 diabetic patients or in specific subgroups as this would then set a target for interventions that prevent a further decline and possibly restore it.

The first quantifications of β-cell mass in the diabetic pancreas have been reported more than 40 years ago [1,3,23]. The methods certainly had more limitations than those of today, and the classification of patients was mainly based on their age at diagnosis without use of markers to distinguish type 1 and type 2 forms, and often without much clinical information. It is nevertheless indicative that an average 30–50% reduction in β-cell mass was noticed in the organs of ‘mature-onset’ diabetes including those from obese patients [3,23]. It was, however, also reported that the measured β-cell mass varied considerably in non-diabetic controls, irrespective of age [3,19,24,25], which makes it of course more difficult to reach statistically significant differences in data from relatively small series of patients. In more recent analysis of the relative β-cell volume in pancreatic sections taken at autopsy, a 40 and 60%
decrease was measured in, respectively, lean and obese type 2 diabetic patients as compared to age- and Body Mass Index-matched non-diabetic controls [22]; this reduction was seen in both patients on insulin treatment and in those on oral hypoglycaemic agents; the duration of the disease was not mentioned, but the mean ages of 80 years in the lean group and 60–65 years in the obese group suggests that ageing of the β-cell mass should be considered as an additional component in the interpretation of the data. This study found an increased rate of apoptosis in β-cells from the 80-year-old lean type 2 diabetic patients but not in those from the younger obese patients; it is thus indeed conceivable that this higher death rate of β-cells contributes to the lower values of the relative β-cell area in this older age category but is not necessarily indicative for the processes that occur in type 2 diabetic patients at younger age. Further studies in younger age categories are thus needed before concluding that a higher rate of β-cell apoptosis is the major cause of a reduced β-cell mass in type 2 diabetes, rather than an increased ability to form new β-cells.

Among the possible causes for a reduced β-cell mass in type 2 diabetes, a prolonged or repeated exposure of β-cells to elevated nutrient levels is most often seen as the major component [26–30]. This view is mainly based on observations in rodents [31–35], isolated islets and cell lines [36–38] showing β-cell toxic effects of chronically high glucose and fatty acid levels. Studies were undertaken to investigate the underlying glucotoxic [31,39–41], lipotoxic [42–44] and/or glucolipotoxic [30,45–48] mechanisms in these laboratory models. It is not always clear whether the reported ‘toxicity’ consists of a direct cyto-destruction or of a loss of the typical β-cell functions as can occur following a phenotypic change. We do not favour use of the term ‘toxicity’ for conditions that cause phenotypic changes with dysregulated functions [49–52]. β-Cells undergoing a phenotypic alteration may face a shorter life span but they can also become more resistant to well-known cytotoxins, as was the case following culture with interleukin-1β [53]. Numerous studies have shown that β-cells loose their glucose-responsiveness and insulin provision function following prolonged exposure to high glucose levels but a direct cyto-destructive effect is controversial [26,49,50,54,55]. In our studies on isolated β-cells we instead found that low glucose levels caused β-cell death involving reactive oxygen species-production and induction of apoptosis [56,57]. Our data do, however, indicate that glucose recognition by β-cells serves as a survival factor and thus raise the possibility that loss of glucose recognition can reduce their life span (figure 1). Because individual β-cells differ in their glucose sensitivity, physiologic variations in glucose levels can be seen as a condition that keeps the functional heterogeneity that is needed to achieve the acute dose-dependent secretory responses to glucose as well as to maintain the size of the β-cell population. This heterogeneity can also be expected to allow a compensation in β-cell mass through changes in proliferation or apoptosis rates of β-cell subpopulations. Likewise, decompensation occurs when these adjustment mechanisms fail. This model is compatible with the view that type 2 diabetes can be considered as the ‘glucotoxic’ induced decompensation phase after the glucose-induced compensation phase has failed or become insufficient [58–60]. Along this line, the molecular mechanism underlying the reduction in β-cell mass in type 2 diabetes might also be operative in the residual β-cell mass of type 1 patients.

**Functional β-Cell Mass: Definition and Measurement**

With a reduced β-cell mass as cause of type 1 diabetes and as likely contributor to the progressive β-cell failure in type 2, it is evident that quantification of the β-cell mass would be useful for early diagnosis of the disease and for following its progression, as well as for monitoring interventions that aim at its preservation and correction. In its true sense, β-cell mass corresponds to the number of β-cells. This parameter can so far not be determined *in vivo* and has only been indirectly evaluated in pancreatic sections. Insulin or C-peptide release tests offer at present the only approach to an *in vivo* assessment of β-cell mass. Their amplitude is expected to vary with the number of β-cells but is not necessarily a quantitative index for it as it depends on the number of cells that have been stimulated by the test and by their capacity to respond. The latter properties reflect the functional state of the β-cells and thus their phenotype. This has brought us to consider functional β-cell mass as a term that expresses both the number of β-cells and their functional state (figure 2). This parameter can be determined in isolated cell preparations in which the number of β-cells can be counted as well as their functional state [49,50]. In order to approach it *in vivo*, a release test needs to be selected which reproducibly activates a high proportion of the β-cells in normal control individuals that will serve as reference for comparison. An acute stimulatory test seems less appropriate for this purpose as it assesses the responsiveness of the β-cell subpopulation that can be rapidly activated. *In vitro* studies on isolated β-cells showed that this is a subpopulation of variable size but that a 120-min incubation at 10 mM
glucose reproducibly recruited over 80% of β-cells into an activated state ([50], unpublished data). We therefore opted for a glucose intravenous clamp test to compare the functional β-cell mass in type 1 diabetic recipients of an islet cell graft and that of age- and sex-matched non-diabetic controls [61]; prior measurements in non-diabetic volunteers had shown a higher reproducibility of this test than the first phase insulin release after intravenous glucose administration (unpublished data). The test consists of a euglycaemic phase (60–90 mg/dl) followed by a 160-min hyperglycaemic (180 mg/dl) clamp during which 1 mg glucagon is injected at min 150. C-peptide release is measured as AUC/min during the euglycaemic phase and during the hyperglycaemic phase (from min 120 to 150 and from min 150 to 160). The data demonstrate that measuring the functional capacity of the β-cell mass during sustained activation allows a reproducible evaluation of the β-cell mass before and during therapeutic interventions. It will nevertheless be necessary to compare, in these studies, the utility of the presently used clamp test with that of the acute challenge tests which have been recently reviewed in the perspective of estimating β-cell mass [62].

**Functional β-Cell Mass in Type 1 Diabetic Recipients of Islet Cell Graft**

Restoring the β-cell mass is the main objective in programs aiming at a cure for type 1 diabetes. This therapy should also be beneficial for type 2 patients with reduced β-cell mass as it is expected to correct the dysfunctional state of the residual β-cells; in fact it seems more appropriate as goal than searching for drugs that increase insulin production capacity by an insufficient number of β-cells. In principle, transplantation of β-cells is thus not to be restricted to type 1 patients. However, under the limitations of current protocols, trials have so far only included type 1 patients who have progressed to diabetic complications, and most of which lack signs of a residual β-cell mass as judged by their plasma C-peptide negativity [61,63–65]. The trials consist of implanting an islet cell graft prepared from human donor pancreases and suppressing the immune system of the recipient. Several centres have achieved consistent success in correcting glucose control and achieving insulin independence. However, they are all hindered by the shortage in donor organs and all face the need to find immune-modulating regimens that do no longer require continuous administration of drugs with a risk for serious side-effects. Furthermore, most recipients show a progressive decrease in graft function during the first years post-transplantation making insulin treatment again necessary [65]. Many of them maintain a better metabolic control even after restarting daily insulin injections, but it is conceivable that this effect will be lost in time. These limitations and observations do not favour an extension of transplant trials to patients who still present signs of a residual β-cell mass in the pancreas. It should nevertheless be kept in mind that selecting C-peptide negative type 1 patients may represent the most difficult population to achieve long-term metabolic control and insulin independence. It is likely that these endpoints are more difficult to achieve as a larger β-cell mass needs to be installed and a more severe metabolic disturbance to be corrected than in patients who still benefit from a functional, be it insufficient, β-cell mass. In long-term type 1 patients, the presence of a residual β-cell mass might also be indicative for a less severe autoimmune reactivity at the time of clinical onset, and disappearance of its β-cell destructive effect with time.
Selection of C-peptide-negative patients for transplant trials allowed us to determine the number of β-cells that are needed to achieve a circulating sign of a functional β-cell mass, to induce a state of insulin-independence and to correct glucose control [61]. This study was conducted in non-uraemic type 1 patients with poor metabolic control despite intensive insulin treatment, as evidenced by a wide within person between-day variation in fasting glycaemia (coefficient of variation (CV) ≥ 25%), episodes of nocturnal hypoglycaemia, and HbA1c > 7%. They presented signs of chronic diabetes lesions such as hypoglycaemic unawareness, microalbuminuria despite optimal dose of angiotensin-converting-enzyme inhibitor and/or retinopathy. The immune-suppressive protocol consisted of one ATG course and maintenance doses of tacrolimus and mycophenolate mofetil; in case of a second transplant, no new antibody course was given nor were drug doses increased. We measured the

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**Fig. 2** Components and regulation of functional β-cell mass. The functional β-cell mass is determined by the number of β-cells and their phenotype(s) that define their functional state and responsiveness. Cell number and phenotype are subject to regulatory factors that reflect the metabolic state and thus adjust the functional β-cell mass so that it is capable of adequately responding to acute and chronic metabolic needs (A). Functional tests that evaluate this capacity are useful for early diagnosis as well as for monitoring therapeutic interventions in diabetes. Diabetes develops when the functional β-cell mass has become inadequate for metabolic control (B). This failure is the result of an insufficient number of β-cells or of a phenotypic alteration or of a combination of both. The diabetic metabolic state will change the regulation on β-cell number and phenotype. Indirect evidence indicates that these secondary influences cause a decrease in β-cell number and a shift in β-cell phenotype.
number of β-cells in cultured human islet cell grafts before implantation and examined – for a range between 0.5 and 5.0 million β-cells/kg recipient body weight – which numbers resulted at post-transplant (PT) month 2 in plasma C-peptide levels of minimally 0.5 ng/ml, a sign for a surviving graft. The functional significance of the implant was assessed by monitoring insulin doses, CV-glycaemia, number of hypoglycaemic episodes and HbA1c levels.

Plotting of plasma C-peptide levels at PT month 2 against the β-cell number in the graft indicated a dose-dependent correlation. The set criterion for graft function (C-peptide > 0.5 ng/ml) was only reached when grafts contained minimally 2 × 10⁶ β-cells/kg body weight; it was achieved for 16 out of 17 recipients of this β-cell mass. These 16 patients also presented a significantly lower CV of prebreakfast glycaemia, the values of which were also closely correlated with donor β-cell number. Considering the loss of β-cells that occurs during injection and engraftment, it can be concluded that a β-cell mass of 2 × 10⁶ β-cells/kg BW overestimates the number that is needed in situ to achieve signs of function. As could be expected, a larger number of β-cells was needed to achieve a state of insulin-independence: graft recipients who were insulin-independent at PT year 1 had received, on average, 4.6 × 10⁶ β-cells/kg BW, either in one or over two intraportal injections. Again taking into account the cell losses at different phases of this replacement therapy, these data suggest that a mass of 4.6 × 10⁶ β-cells/kg BW should certainly be sufficient in the pancreas to be considered as non-diabetic. This reflection does of course not mean that this is also the number of β-cells in normal controls. In fact we have no knowledge of studies that have counted the number of β-cells in the human pancreas.

In order to evaluate the size of the β-cell mass in normal graft recipients versus that in normal controls we performed the higher described glucose-clamp which we consider as an index for the functional β-cell mass. The test was carried out in recipients who were insulin-independent at PT year 1 and in non-diabetic controls that were matched for age and body weight. Graft recipients did not show a release above baseline during the first 10 min of glucose stimulation but did present a sustained response during the phase of prolonged hyperglycaemia (120–150 min). The amplitude of the response was, however, only 26% of that in normal controls; addition of glucagon increased this amplitude but did so to the same extent in the controls. When individual values were plotted, all graft recipients were found to exhibit a markedly lower β-cell secretory capacity than controls, both in absence and in presence of glucagon. Nine of the 10 recipients were clustered at 20% of the median control and 1 at 60%. These data indicate that ‘normalized’ graft recipients – at least those in our series – exhibit, 1 year after transplantation, a markedly lower functional β-cell mass than normal controls. In the presence of a marginal β-cell mass, individual β-cells are expected to be, or become, dysregulated; a sustained recruitment into biosynthetic and secretory activities could be one of the underlying mechanisms [49,50]. This deficiency in size may explain in itself the progressive decrease in graft function that has been observed following the first PT years [65]. This does not exclude detrimental influences by autoimmune and alloreactive processes or by immune-suppressive agents, but does indicate that these well-known immune-related aggressors are not necessarily the major cause and thus the prime target for interventions that aim a better long-term preservation of grafts that have achieved insulin-independence during the first year. The first goal is, in our opinion, to install a larger functional β-cell mass during the first year. Measurement of the functional β-cell mass in the isolated graft and during the first post-transplantation months should help identify how adaptations in graft composition, transplant site and immune-suppressive treatment can help decrease the difference with the functional β-cell mass in normal controls.

**Functional β-Cell Mass in Type 1 Diabetic Recipients at Clinical Onset**

The glucose-clamp test was also used to measure the functional β-cell mass at clinical onset of type 1 diabetes [66]. The purpose was to examine whether administration of humanized ChAglyCD3-antibody, a glycosylated human IgG1 antibody directed against CD3, was capable of preserving the functional β-cell mass during follow-up, using placebo-treated patients as control. The residual function at start was determined in 80 patients aged 12–39 years within 4 weeks after diagnosis; patients with C-peptide levels under 0.5 ng/ml were excluded (30%). When we compared the residual secretory capacity of selected patients with that in age- and body weight-matched non-diabetic controls, it averaged 25% of normal values [66]. This observation is consistent with the view that the residual β-cell mass at clinical onset of type 1 diabetes is higher than the 10% that is classically mentioned, at least in patients who are older than 12 years. This residual β-cell mass should not be neglected as it represents a target for interventions that aim its preservation and further natural growth. Although this may in itself not be sufficient for complete recovery of physiologic functions, it could help in metabolic control.
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