

# The pancreas in human type 1 diabetes: providing new answers to age-old questions

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## Purpose of review

Although studies of pancreata from type 1 diabetes (T1D) patients largely fell dormant for a period of decades, research efforts have recently been 'rekindled' in this area to address, using modern techniques, many unanswered questions related to the pathogenesis of this disease.

## Recent findings

As historically noted, a pancreatic infiltrate commonly referred to as 'insulinitis' is present at the symptomatic onset of T1D. Recent studies have further characterized this infiltrate both in terms of its cellular composition as well as the mechanisms that likely underlie  $\beta$  cell death in T1D. In addition, the notion that the pancreas from T1D patients is completely devoid of insulin producing cells years after the onset of disease has been challenged, whereas the concepts of whether  $\beta$  cell regeneration or replication are present have also been subject to much debate. Novel concepts regarding the rate and degree of  $\beta$  cell loss throughout the natural history of the disease have also been put forward to aid in explaining the disorder's pathogenesis.

## Summary

Although answers to many long-standing questions in T1D have recently been addressed, perhaps the main finding has been one supporting a disease of remarkable heterogeneity. However, additional lessons remain to be learned from the pancreas in T1D. Hence, attempts aimed at organizing the scientific community to address these issues are ongoing, particularly those from collaborative efforts, including the Belgium Organ Donor Consortium and the Network for Pancreatic Organ Donors with Diabetes (nPOD).

## Keywords

autoimmunity, histology, islets, pancreas, type 1 diabetes

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## Introduction

Many research articles pertaining to type 1 diabetes (T1D) begin by noting the disorder results from an autoimmune destruction of pancreatic  $\beta$  cells; this, based on long-standing observations that a chronic inflammatory infiltrate (i.e., insulinitis) affecting primarily the insulin containing-islets is present in a majority of individuals at the symptomatic onset of their disease. Yet, much of our current view regarding the pathogenesis and natural history of T1D, as well as the means by which we address many ongoing etiological questions in this disease, derive from analysis of serum and peripheral blood lymphocytes from patients with the disorder. This is not indicated to suggest these latter sources are without value, as major knowledge gaps related to the genetics, immune response (e.g., T1D associated autoantibodies), and  $\beta$  cell function (e.g., C-peptide production) have derived from such a form of investigation. However, given that T1D is a disorder that targets pancreatic  $\beta$  cells, a growing

number of investigators have considered it highly valuable to overcome obvious obstacles and practical limitations for studies of the pancreas in patients with, or at increased-risk for the disease; this, for the purpose of contributing to our understanding of the disorder's pathogenesis. For this reason, the following review will consider recent lessons learned from examinations of the pancreatic pathology in T1D.

## Why is it so difficult to study the pancreas in human T1D?

Without question, the primary reason why studies of peripheral blood have predominated in T1D research relates to the fact that investigations of the pancreas in those with the disease are limited by the difficulty of obtaining suitable tissue. Historically, the most common source of pancreatic specimens from individuals with T1D has involved retrospective collections of pancreata obtained at autopsy from individuals who died at or near

the time of their disease diagnosis [1,2]. This approach is, however, hampered by several significant limitations; the pancreatic tissues might be subject to a degree of autolysis, and furthermore, these samples have most often been subject to formalin-fixation and paraffin embedding; a facet that restricts the range of investigative techniques available. These factors, combined with limited pancreatic sampling, poor knowledge of clinical histories, and little access to modern assessments of either  $\beta$  cell function (e.g., C-peptide production, insulin use) or metabolic control (e.g., HbA1C) significantly limit, from a historical perspective, the range of potential correlative information that could have been gleaned from studies on these otherwise highly valuable pancreata.

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### **How can this limitation be overcome?**

This situation has, however, been subject to a variety of changes that have and will, with time, dramatically improve our understanding of pancreatic pathology as it relates to T1D. Amongst these newer approaches, investigators have performed laparoscopic pancreatic biopsies on T1D patients with recent diagnosis of their disease [3]. Although controversial with respect to its risk-versus-reward potential, and subject to questions both in terms of its ethics and practicality, this protocol does, in fact, overcome many of the aforementioned limitations. The method of collection is, however, hampered by a significant disadvantage related to sampling bias wherein the biopsies are exceedingly small. Another approach was developed in the mid-1990's, involved analysis of pancreata removed at the time of organ donation from patients who died due to severe diabetic ketoacidosis at clinical onset of T1D [4]. This form of study inspired investigators to step up efforts to obtain pancreatic tissue from this source (i.e., organ donors). This process not only allows for removal of the pancreas for research studies immediately after death, but also has the potential to overcome additional limitations related to tissue preparation, quantity, and storage. Other methods recently developed to obtain more optimal pancreata include sampling of organs from T1D patients undergoing pancreatectomy for islet tumors or other pancreatic disease. Pancreatic tissue has also been sought from islet auto-antibody positive organ donors deemed at increased-risk for the development of T1D [5–7]. These methods, when combined with modern assessments of metabolic activity,  $\beta$  cell development, and immune function (as well as the potential future for in-vivo imaging) should allow for major improvements in our understanding of the human pancreas in T1D.

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### **What is 'insulinitis'?**

The proverbial 'hallmark' study of the pancreas in T1D, both in terms of patient cases and depth of description,

was that published in 1965 by Gepts [1]. This established two morphological factors that clearly distinguished what we now refer to as T1D from those of type 2 diabetes (T2D). In cases of T1D, a reduction of  $\beta$  cells was demonstrated, with many islets being completely devoid of them. We would note, parenthetically, that this study predated the technique of immunohistochemistry, so strictly speaking, the cells were not proven to be insulin secreting. Furthermore, a chronic inflammatory cell infiltrate affecting islets, termed 'insulinitis', was also noted in these individuals. Neither of these phenomena was observed in pancreata of those with T2D [8]. Furthermore, Gept's observation of pancreatic insulinitis in 15 of 22 cases of recent onset (i.e., less than 6 months of clinical duration) T1D led him to hypothesize that the  $\beta$  cells were undergoing destruction by either viral infection or through a self-directed immune (i.e., autoimmune) process. Interestingly, a wide body of evidence acquired in the ensuing 40 years has supported, to some degree, both of these early suggestions regarding disease pathogenesis.

Alongside noting a potential autoimmune basis for T1D, similar early studies have provided other valuable insights. Among those, in individuals having T1D for more than five years, a majority of the remaining islets were noted as insulin deficient; containing a normal complement of the other hormone secreting cells (i.e., glucagon secreting  $\alpha$  cells, somatostatin secreting  $\delta$  cells, and pancreatic polypeptide secreting PP cells) [9]. Thus, the disorder clearly represents a disease involving the selective loss of  $\beta$  cells. It is important to emphasize that these historical studies also suggested that the natural history of  $\beta$  cell loss represented a variable response, subject to marked heterogeneity in both the degree as well as the patterns of destruction.

Three forms of islet were noted in pancreases of recent onset T1D patients [1,9]. First, approximately 70% of the islets in recent onset T1D pancreata displayed complete insulin absence (i.e., similar to those observed in T1D patients with prolonged disease). Second, approximately 20% of insulin containing islets, as opposed to only 1% of insulin deficient islets, were inflamed (i.e., insulinitis) [9]. Third, in many pancreases, noninflamed insulin-containing islets were present that appeared essentially normal. Thus, within a given pancreas, near the time of clinical presentation, there are islets where the  $\beta$  cells have been destroyed, islets where  $\beta$  cells are being destroyed, and islets where the  $\beta$  cells have yet to be destroyed (i.e., insulin-deficient islets, inflamed islets, and normal islets, respectively).

In the years since immunohistochemistry has been available, a limited number of reports suggested that T cells were the predominant inflammatory cell within the insulinitis lesion, followed by lesser numbers of macrophages

and B-lymphocytes [10]. For the purpose of this current review, an important question to address is, 'has any recent evidence changed this model?'. The answer is both 'yes' and 'no'. One recent analysis of postmortem pancreata from 29 patients agreed with these studies by suggesting that CD8+ T cells were indeed the most abundant population, with far fewer CD4+ T cells, and B-lymphocytes present in only small numbers in early insulinitis [11]. Interestingly, FoxP3<sup>+</sup> regulatory T cells, a cellular phenotype subject to more recent designation and interest, were detected in the islets of only a single patient within this study, whereas natural killer cells were detected rarely, even in heavily inflamed islets. Taken together, these results support a notion for a defined sequence of immune cell recruitment in T1D. Recent efforts also support a notion for disease diversity (i.e., heterogeneity) in T1D. Specifically, studies in Japan involving individuals with either 'autoimmune' T1D, or so-called 'fulminant' T1D, observed that both  $\beta$  and  $\alpha$  cells were decreased in fulminant T1D, but only  $\beta$  cells were significantly decreased in autoimmune T1D [12<sup>\*</sup>]. Lymphocytic infiltration to the exocrine pancreatic tissue was only observed in fulminant T1D, whereas a number of immunologically abnormal findings (e.g., increased expression of MHC class I molecules, Fas antigen in islet cells, Fas-ligand expression in infiltrating lymphocytes) were only detected in those thought to have autoimmune T1D. From these findings, it was hypothesized that in autoimmune T1D,  $\beta$  cells are destroyed through a long-standing autoimmune process, whereas in fulminant T1D, the cells seem to be destroyed very rapidly through actions that remain to be defined. This latter finding does, however, highlight that a clear need also remains for understanding the mechanisms of  $\beta$  cell death in T1D. To that void, one recent characterization of interest noted a modest increase in the degree of  $\beta$  cell apoptosis in patients with recent onset T1D [13]. This, from assessments of autopsy-based specimens collected from nine individuals having a T1D diagnosis of less than three years in duration.

### The pancreas before and after clinical onset of type 1 diabetes

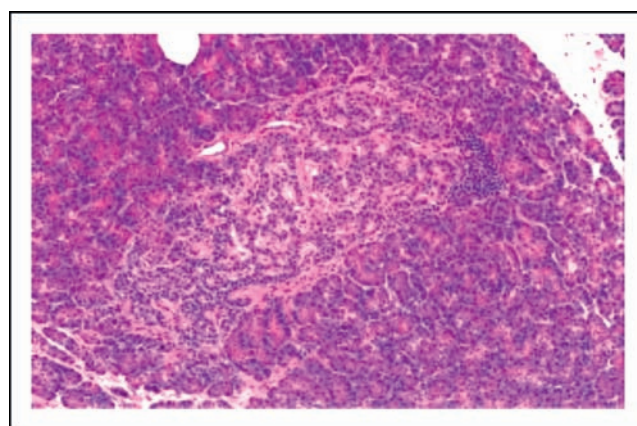
An important point to note is that the pancreas before clinical presentation, at clinical onset of disease, and for a few years after clinical presentation may be qualitatively similar, with all three types of islet present, although in vastly variable proportions [14]. For example, one classical study noted that insulinitis affecting insulin-containing islets could be observed in T1D cases up to 6 years following clinical presentation and in such a pancreas; approximately 95% of islets were insulin deficient [2]. By contrast, in a prediabetic-T1D pancreas, less than four percent of islets were noted as insulin deficient, yet insulinitis was present [15]. Pathologic examinations would

suggest that clinical presentation occurs when approximately two-thirds of the islets are insulin deficient [2]. To that thought, we would note that this percent is routinely subject to a degree of variance that could only be ascribed to 'artistic freedom', with figures of up to 95% destruction often being cited, as will be discussed later in this review). The importance of this lies in the fact that if the pathogenesis of T1D within the pancreas involves progression from a normal insulin-containing islet to an inflamed insulin-containing islet, and thence to an insulin-deficient islet, then the mechanisms involved may be studied in pancreases obtained at clinical presentation, as all three islet types are usually present in such organs [15]. For many years, investigators have not considered it practical or ethical to obtain pancreatic biopsies from living individuals at increased-risk for developing T1D.

To address this problem, recent investigations have attempted to use the aforementioned strategy of collecting pancreatic tissue from organ donors with serological evidence of islet autoimmunity, a subset of whom would presumably have developed T1D had they survived. Examples include a consortium of investigators organized in Belgium [16], as well as the Network for Pancreatic Organ Donors with Diabetes (nPOD) ([www.jdrfnpod.org](http://www.jdrfnpod.org)); both having developed large programs to screen asymptomatic organ donors for islet autoantibodies. nPOD also analyses individuals with T1D of various disease durations, efforts that have allowed for in depth and heretofore unaddressed questions regarding disease pathogenesis and insulinitis (Fig. 1). Here again, from these and other efforts, a key question is one of 'what has changed?'

The available evidence now supports the notion that there are several degrees of islet autoimmunity in the

**Figure 1** The insulinitis lesion in human T1D



Hematoxylin and eosin staining of a pancreatic section, from the nPOD effort, of a 12-year-old with T1D of less than 1-year duration. The histology reveals a diffuse and polarized insulitis, with typical islet architecture. Photo courtesy of Dr Martha Campbell-Thompson.

prediabetic state, which can (albeit rarely) be associated with insulinitis, and phenotyped by the number of autoantibodies for which an individual is positive. Specifically, the Belgium Organ Donor consortium retrospectively analyzed pancreatic tissue from 1507 organ donors, identifying 62 individuals as positive for islet autoantibodies (i.e., ICA, GAD, IA-2 or insulin antibodies) [16]. Insulinitis, defined in this study as more than 15 inflammatory cells per islet section, was detected in only two cases, both of which were positive for three autoantibodies. It is important to note that only three of the 62 individuals were positive for three autoantibodies, whereas the remaining 59 were positive for only one autoantibody. One caveat of this study is that only a relatively small quantity of pancreas was sampled; hence the potential exists that the prevalence of insulinitis was underestimated due to under-sampling of focal areas subject to islet inflammation. A second study involving analysis of pancreas obtained from an organ donor identified as positive for IA-2 autoantibodies also failed to detect insulinitis; this, despite numerous sections of pancreas being subject to examination [6]. Consistent with this, a postmortem study of one Finnish child who repeatedly tested positive for cytoplasmic islet cell antibodies (ICA) in serial measurements noted that the pancreas did not appear overtly different from normal settings, with no reduction in  $\beta$  cell numbers nor any sign of insulinitis [17<sup>•</sup>]. In yet another effort, investigators testing 135 organ donors for T1D associated autoantibodies (i.e., GAD, IA-2, and insulin autoantibodies) noted four were positive. Pancreata from these individuals were not subjected to histologic analysis, but were used in clinical transplantation [18<sup>••</sup>]. Interestingly, single autoantibody positivity did not affect the therapeutic outcome of pancreas transplantation.

With these four efforts, it is feasible to speculate that individuals possessing only one autoantibody will not show significant insulinitis as the degree of immunity does not reach a threshold reflective of widespread islet infiltration. It is also possible that secondary events (e.g., viral islet infection, endocrine stress, etc.) are necessary to trigger full-blown islet mononuclear cell infiltration, even in the presence of established islet autoimmunity.

In terms of the period at or following disease diagnosis, and how metabolic changes based on  $\beta$  cell mass *in vivo* occur, this has been the subject of a recent in-depth review [19]. In short, a growing body of evidence lends support for the notion that the 'honeymoon' period of T1D likely represents a phase of recovery wherein  $\beta$  cells that had been degranulated (but present at the time of diagnosis), return with some degree of function. This finding, if true, supports the intriguing possibility that if autoimmunity and insulinitis are arrested at or near onset, as is being attempted by multiple means currently [20],

the potential for  $\beta$  cell recovery may be realized. As noted previously, on the basis of a series of histopathology studies of patients with recent onset of the disease, it is commonly stated that the onset of clinical symptoms corresponds to an 80–95% reduction in  $\beta$  cell mass. Motivated by the importance of this question, a meta-analysis was recently used to determine the validity of this common wisdom [21<sup>•</sup>]. The results for 105 patients stratified by duration of T1D and age at onset suggested that the percentage reduction in  $\beta$  cell mass was highly correlated with the age of onset with the greatest reduction in  $\beta$  cell mass in the youngest patients. Interestingly, the severity in  $\beta$  cell reduction at onset decreased with age where, on average, a 40% reduction in  $\beta$  cell mass was sufficient to precipitate clinical symptoms by 20 years of age. Hence, factors relating to body weight, insulin demands, and  $\beta$  cell mass may be critical to when symptoms of the disease occur.

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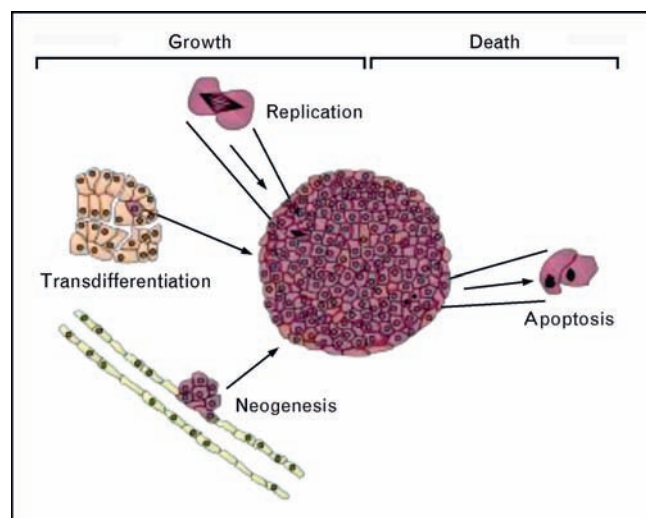
### Regeneration in the pancreas of human type 1 diabetes: fact or fiction?

The often quoted notion portending ' $\beta$  cell extinction' did not develop without some basis of fact. This list would include an absolute loss in C-peptide for most T1D patients, lack of spontaneous remissions from disease, and histological examinations suggesting that a healthy islet cell mass is not readily evident in long-standing disease. However, this concept has recently been challenged by the concept of pancreatic  $\beta$  cell regeneration; a process for which, if a functional basis is indeed present, the underlying mechanism remains unclear (Fig. 2) (reviewed in [22]).

This challenge, to a large extent, was derived from a study that performed immunocytochemical detection of insulin-producing cells in pancreata obtained from individuals with a T1D duration of 4 to 67 years [23]. The pancreases were also examined for many features including inflammation, periductal fibrosis, as well as  $\beta$  cell apoptosis and replication. The study reported, in contrast to established dogma, that 88% of T1D patients had insulin-producing cells and this feature was unrelated to disease duration or age at death [23]. Having said this, it should also be pointed out that the beta cell mass in these 42 cases was only 2.3% that of normal control pancreases.  $\beta$  cell apoptosis was studied in the four T1D patients who had sufficient  $\beta$  cell mass for calculation, and 6% of  $\beta$  cells were noted to express the apoptosis marker, cleaved caspase 3. This is a remarkably high figure, given the transient nature of apoptotic cells, and would imply an extremely high turnover of  $\beta$  cells to provide a stable long-term population.

The same group also studied the pancreas of an 89 year-old patient with recent-onset T1D who had undergone a

**Figure 2 Contributors to the maintenance of pancreatic islet beta cell mass: a balance between growth and death**



Net pancreatic beta cell growth can be influenced by several parameters (left-hand side), including replication of existing islet beta cells, neogenesis and transdifferentiation, and is balanced by the incidence of islet beta cell death, usually by apoptosis (right-hand side). Reproduced with permission [22].

distal pancreatectomy for removal of pancreatic intra-epithelial neoplasia [24]. The tumor-free tissue showed reduced fractional  $\beta$  cell area, islet infiltration with CD3+ positive T cells and macrophages, increased  $\beta$  cell apoptosis, and a marked increase in  $\beta$  cells undergoing replication (i.e., Ki67+  $\beta$  cells) [24]. This publication was followed up by that group [13] examining autopsy pancreata from nine T1D patients (age 11.5–38 years) who died of ketoacidosis and whose disease was less than 3 years in duration. They reported a ‘surprisingly’ modest increase in  $\beta$  cell apoptosis, as assessed by the TUNEL technique, and no evidence of  $\beta$  cell regeneration. It is possible that the crucial difference between the two situations is represented by the fact that in the first setting, the single patient had developed T1D very late in life, possibly as a result of a protracted struggle between the forces of  $\beta$  cell destruction (i.e. lymphocytes and macrophages) and the forces of regeneration (i.e.,  $\beta$  cell replication).

Another potential ‘blow’, for lack of a better word, to the notion of regeneration involved a recent study addressing whether hematopoietic stem cells could serve as a potential source of  $\beta$  cell regeneration [25]. In 31 pancreata obtained at autopsy from hematopoietic stem cell transplant recipients who had received their transplant from a donor of the opposite sex, no pancreatic  $\beta$  cells were identified that were derived from donor hematopoietic stem cells, leading to the conclusion that hematopoietic stem cells derived from adult donors contribute mini-

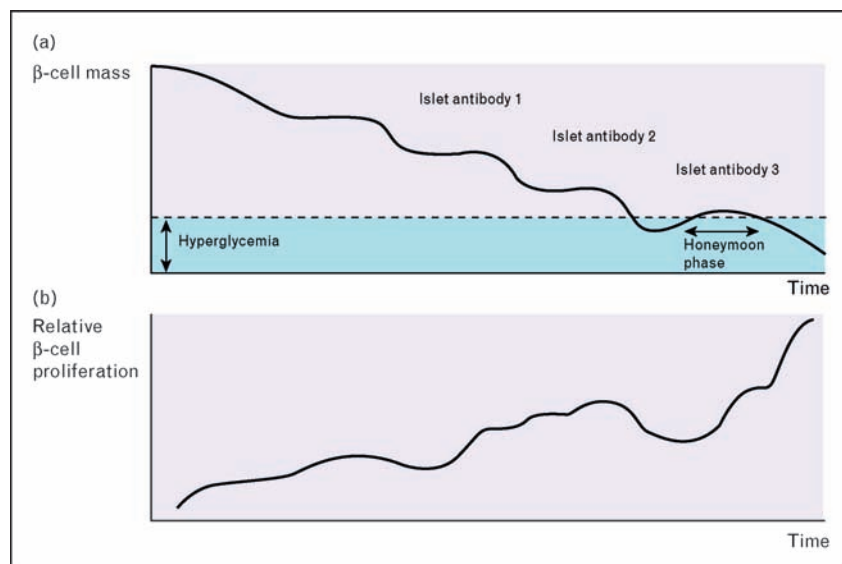
mally to pancreatic  $\beta$  cells in nondiabetic adult humans. Indeed, a growing body of evidence implies that regulation of  $\beta$  cell replication during infancy plays a major role in  $\beta$  cell mass in adults. This, based on studies of abdominal computer tomographies in 135 children aged 4 weeks to 20 years, and morphometric analyses were performed in human pancreatic tissue obtained at autopsy from 46 children aged 2 weeks to 21 years [26]. These studies suggested that  $\beta$  cell mass expands by several-fold from birth to adulthood, that the relative rate of  $\beta$  cell growth is highest in infancy and gradually declines thereafter to adulthood with no secondary accelerated growth phase during adolescence, and that a high rate of  $\beta$  cell replication is coincident with the major postnatal expansion of  $\beta$  cell mass. Despite this growing body of evidence against the potential for  $\beta$  cell replication, at least one study in humans does lend some support for this potential [27\*]. In a study of resected operative pancreatic specimens from patients diagnosed with primary adenocarcinoma (with or without chronic severe pancreatitis) or gastrinoma, patients with pancreatic adenocarcinoma and localized chronic severe pancreatitis displayed significant increases in the number of single  $\beta$  cells, as well as increased  $\beta$  cell replication rates. Patients with gastrinoma demonstrated significant increases in the number of single beta cells, but the  $\beta$  cell replication rate and islet differentiation factor levels were similar to those in controls. These findings indicate that chronic severe pancreatic inflammation can be associated with significant effects on  $\beta$  cell number or replication rates.

However, these reports exemplify the difficulties involved in the study of the pancreas in human T1D and form the basis wherein rodent models of pancreatic  $\beta$  cell regeneration are often subject to investigation [28\*]. However, great caution must be considered when extending findings of  $\beta$  cell regeneration from animal models to human T1D. Until then, examinations of many more human cases are required to substantiate the concept of  $\beta$  cell regeneration since, as for now, it remains an unproven concept for human T1D. That said, it is a notion of great importance, given its potential means as a therapeutic intervention for the disease [29\*,13].

## Conclusion

We believe a strong case has been presented that much more in the way of research involving human pancreas in T1D should be performed, with emphasis on elucidating the etiology of the process underlying the activation of the immune system, the natural history of  $\beta$  cell loss, and questions related to  $\beta$  cell regeneration. Address of these areas should not only provide much in the way of truly understanding the natural history of T1D but in addition, address the question of why the disorder develops. Another interesting concept that should be addressed

Figure 3 Type 1 diabetes as a relapsing–remitting disease?



(a) Graph showing the stepwise, nonlinear decline of  $\beta$  cell mass over time, as well as the development of autoantibodies that are associated with hyperglycemia; that is, the onset of T1D. (b)  $\beta$  cell proliferation increases in a cyclical fashion over time. This figure indirectly depicts the biological trends of the development of T1D, which may be attributed to the cyclical nature of the immunological events that lead to the attack or protection of  $\beta$  cells. In addition, as  $\beta$  cell mass declines, the pressure on each  $\beta$  cell to produce insulin increases, which may be sufficient to alter the recognition of  $\beta$  cells by the immune system and to alter their ability to regenerate and increase insulin production. Reproduced and modified with permission [30\*\*].

in future studies is whether the natural history of T1D is relapsing–remitting in nature (Fig. 3) [30\*\*]. That the progressive loss of  $\beta$  mass during the development of T1D could be nonlinear, would, if true, find some form of pathological equivalence in the immunological processes that underlie persistent viral infections and autoimmune disorders such as multiple sclerosis, which can be relapsing–remitting in nature. The field can also garner hope that new methods for imaging the pancreas on the horizon; ones that will certainly aid in the evaluation of  $\beta$  cell mass as well as inflammation. Examples include the use of  $^{11}\text{C}$ -dihydrotrabenazine PET that identifies type 2 vesicular monoamine transporter (VMAT2) expressed in  $\beta$  cells [31\*]; albeit at least one recent work (in part) questions its specificity for islet  $\beta$  cells, suggesting it may also reside in pancreatic polypeptide cells [32]. An improved understanding of events within the pancreas should also provide information relevant to the design of novel therapeutics capable of preventing or reversing T1D and potentially, the development of improved markers capable of monitoring the processes destroying  $\beta$  cells.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 330).

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