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Pancreatic beta-cell failure in the pathogenesis of type 1 diabetes

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In essence, it is the failure of the cell known as the pancreatic beta cell to make and secrete adequate insulin, that leads to the development of all forms of diabetes mellitus. Type 1 diabetes is caused by immune destruction of pancreatic beta cells. While multiple pancreatic beta-cell autoantibody positivity is strongly associated with the progression to diabetes, it is not clear whether autoantibodies can cause initial beta-cell destruction, or whether antibody production is only triggered after episodes of beta-cell death have already occurred leading to clinical onset of type 1 diabetes. Indeed even though many of the major T cell autoantigens are derived from the same proteins recognised by the immune cells known as B cells, it is currently thought that type 1 diabetes is mainly mediated by subsets of effector T cells. Documented epidemiological data suggest environmental factors act upon a background of genetic susceptibility to establish pancreatic islet inflammation known as 'insulitis' and subsequent beta-cell dysfunction and loss. Understanding the nature of the environmental factors contributing to insulitis, beta-cell loss and the development of type 1 diabetes may provide the key to developing interventions to prevent or delay onset in at-risk individuals. To date, no clinical trials including immune-based approaches aimed at preventing type 1 diabetes onset in high-risk individuals have proven effective. This chapter will focus upon the evidence for genetic susceptibility, and some of the putative environmental triggers in type 1 diabetes which may ultimately lead to methods to protect beta cells, and to stimulate residual beta-cell function or regeneration without ongoing immune destruction.

Type 1 diabetes mellitus is an autoimmune disease with very well-defined genetic susceptibility. Individuals with a strong genetic predisposition to type 1 diabetes typically develop one or more autoantibodies against islet antigens, and grumbling islet inflammation or insulitis ensues, with slow destruction of the islets and attrition of the islet cell mass over a number of years. In most cases, clinical diabetes develops before the complete destruction of islet beta cells has occurred, whereas absence of functioning beta cells is the rule in longstanding type 1 diabetes.

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Although this pathway represents the characteristic pattern of disease progression, many aspects of the pathogenesis of type 1 diabetes are incompletely understood. Not all individuals with a genetic predisposition develop diabetes. Disease incidence can be influenced by additional genes inherited concurrently with the known high-susceptibility alleles, and by environmental factors. Insulitis can be difficult to detect, and when present, is often patchy. Whilst most patients with type 1 diabetes have one or more pancreatic islet autoantibodies detectable at the time of diagnosis, and the risk of developing diabetes increases with the number of autoantibodies an individual is producing, a small number of patients never have detectable islet autoantibodies. The progression from susceptibility to autoantibody production and beta cell loss is usually steady and inexorable, yet some elements of this process may be reversible. Animal studies have demonstrated the potential for islet beta cells to regenerate after the onset of clinical diabetes – such regeneration may be possible in humans as well, and offers a potential target for therapeutic intervention. These and other areas of current interest and controversy will be discussed further in the following sections.

Genetic susceptibility to type 1 diabetes

Over 40 gene loci have now been identified as being linked to the risk of developing type 1 diabetes [1]. Consistent with the notion of type 1 diabetes as an autoimmune disease, essentially all these genes influence the immune system at some level, thus affecting its ability to mount a response against islet autoantigens. The strongest predisposition to diabetes development is conferred by genes found in the Human Leucocyte Antigen complex on the short arm of chromosome 6, particularly the HLA class II loci, DR and DQ [2]. The highest-risk genotype includes some combination of HLA DR3 or DR4 with HLA DQ2 or DQ8. This genotype is present in around 2.4% of newborns, but its representation is much greater amongst individuals with diabetes [3]. Infants with this gene combination have a greater than 50% chance of developing type 1 diabetes by age 12, and this risk is increased further if an HLA-identical sibling is already affected by diabetes [3]. Conversely, other class II alleles such as DQ 0602, are associated with dominant protection from diabetes, even amongst autoantibody-positive first-degree relatives of patients with type 1 diabetes [4]. The function of HLA DR and DQ molecules is to present antigens to CD4-positive helper T cells, thus initiating a cognate immune response. Structural and computational studies of susceptibility and protective alleles have revealed a number of shared features which modify the ability of these class II molecules to present peptides derived from islet autoantigens to responding T cells [5]. Charged residues at critical positions within the peptide binding groove allow diabetogenic peptide epitopes to bind strongly with slow dissociation kinetics, promoting the priming of a range of autoreactive $CD4^+$ T cells [5]. Consistent with these observations, possession of particular HLA DQ alleles is linked to antibody specificity, such that DQ8 is associated with the presence of insulin autoantibodies, and DQ2 is found in subjects with antibodies against glutamic acid decarboxylase (GAD65) [6]

HLA class I genes are also associated with diabetes susceptibility, and the presence of the common HLA A2 allele further increases the likelihood of developing diabetes in

individuals with high-risk class II alleles [7, 8] Possession of HLA B39 increases overall risk, and is linked with earlier onset of disease [9]. HLA class I molecules present antigens to $CD8^+$ T cells, the cell subset directly implicated in the final phase of islet destruction. A growing body of evidence supports a functional role for HLA-A2 in the display of antigenic peptides derived from insulin precursors and GAD on the beta-cell surface, thus triggering beta-cell killing by $CD8^+$ T cells which recognise these antigens [10–12].

Autoimmunity arises from a failure of development or maintenance of self-tolerance, where either the deletion of potentially autoreactive T cells in the thymus, or the generation of regulatory T cells which can suppress autoreactivity in the periphery, is inadequate. Two rare single gene (or monogenic) disorders which affect these processes result in severe generalised autoimmunity, with diabetes as one manifestation. These conditions are known as IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) in which regulatory T cells fail to develop appropriately due to a mutation in the FoxP3 transcription factor [13, 14], and APECED (autoimmune polyendocrinopathy – candidiasis – ectodermal dystrophy) where a defect in the protein AIRE prevents expression of otherwise tissue-specific genes (such as insulin) in the thymus, and blocks generation of self-tolerance to the proteins encoded by those genes [15]. Reminiscent of the mechanism underlying APECED, polymorphisms in the promoter of the insulin gene confer susceptibility to type 1 diabetes if they bind poorly to AIRE, and reduce insulin expression in the thymus [16, 17].

Recent advances in high-throughput sequencing technology have enabled genome-wide association studies (GWAS) to be performed in type 1 diabetes and other common disorders. These studies have confirmed that a number of other loci involved in the regulation of T cell function influence the likelihood of developing diabetes [18]. Such genes include PTPN 22, which encodes a tyrosine phosphatise which regulates T cell receptor signalling, and the genes encoding interleukin 2 receptor alpha chain and the co-inhibitory molecule CTLA4 [19]. Genes involved in innate immune responses to microorganisms have also been linked with diabetes susceptibility in recent studies, and intriguingly, these findings suggest one mechanism whereby genetic susceptibility can interact with environmental exposures to result in disease. IFIH1 is a gene which encodes the intracellular pathogen receptor MDA5 [19, 20]. MDA5 triggers immune responses to viral RNA derived from the enterovirus family, of which Coxsackie viruses are a member, and there is considerable circumstantial evidence linking Coxsackie infection with diabetes onset. Other intracellular nucleic acid receptors recently mapped in type 1 diabetes include the toll-like receptors TLR7 and TLR8 [21].

Environmental factors and type 1 diabetes

Whilst the growing epidemic of type 2 diabetes is common knowledge, it is less well appreciated that worldwide incidence of type 1 diabetes has been rising over the past few decades as well [22, 23]. Moreover, there has been a shift towards onset of clinical diabetes at an earlier age, such that the prevalence rate in individuals aged younger than 15 years is projected to increase by 70% between 2005 and 2020 [24]. These rapid changes in incidence and in age of onset, along with the lower than expected concordance between monozygotic

twins, are characteristic of the operation of environmental factors upon a background susceptibility [25]. Consistent with the idea that the highest-risk genotypes require the least environmental pressure to result in overt diabetes, the changes in incidence have been most pronounced amongst those with moderate, rather than extreme genetic susceptibility [26]. In Australia, the incidence of type 1 diabetes in children with the highest risk alleles has remained stable over time, as has age at diagnosis. Conversely, a greater proportion of children with moderate-risk alleles is developing diabetes, and is doing so at a younger age [27]. Other hallmarks of environmental influence, such as seasonal variations in onset of clinical diabetes, are also largely confined to those at moderate genetic risk [28]. Understanding the nature of the environmental factors contributing to the development of overt diabetes may provide the key to developing interventions to prevent or delay onset in at-risk individuals, and there is considerable research concentration in this area. In this section, we will discuss the evidence for some of the putative environmental triggers of diabetes.

Viral infections

Infections with several viruses have been plausibly associated with the development of type 1 diabetes. 22% of children infected with rubella *in utero* later developed type 1 diabetes [29], and cross-reactivity between T cells recognising both rubella and GAD-derived peptides was demonstrated [30]. However, it now appears that impairment of islet development following pancreatic infection with rubella, rather than islet autoimmunity, may be the mechanism of rubella-associated type 1 diabetes [31].

Data linking infection with enterovirus or rotavirus to diabetes onset are tantalising, and evidence to support a potential pathogenetic role for these infections exists. Nevertheless, these findings have not been consistent across all groups studied, perhaps reflecting the complexity of the interactions between individual susceptibility and environmental factors as well as differences in study methodology.

In the longitudinal Diabetes Prediction and Prevention (DIPP) study from Finland, enterovirus infections were reported in the majority of subjects developing islet autoantibodies in the six months preceding initial antibody detection, whereas significantly fewer children without autoantibodies had been infected [32]. Other large longitudinal studies of genetically at-risk cohorts did not confirm these findings [33, 34]. Seemingly conflicting results may arise because different substrains of the same virus may not be differentiated by routinely available serological testing, and yet may have different diabetogenic potential. In addition, identical viruses could have different effects upon progression if encountered at different stages in the natural history of the disease [35].

Fulminant type 1 diabetes is an entity described in Japan, where direct infection of the islets and exocrine pancreas with enterovirus leads to upregulation of chemokine and cytokine secretion by islets, triggering infiltration with aggressive T cells and macrophages, and islet destruction within a matter of days [36]. However, pancreatic infection with enterovirus family members is also reported in diabetes cases with a less dramatic onset. Coxsackie virus is tropic for human beta cells, and virus-positive beta cells have been demonstrated in

pancreas specimens from studies in children succumbing to severe metabolic complications of diabetes close to diagnosis, but not from control pancreata [37]. At symptom onset, multiple enterovirus nucleic acid sequences were identified in the peripheral blood cells of 50% of subjects, compared with 0% of age and sex-matched controls [38]. Moreover, T cells from a majority of recent-onset subjects proliferated in response to Coxsackie viral lysates [39]. One criticism of these data was that the controls were not matched with respect to diabetes risk, but recent reports from both the Diabetes Autoimmunity Study in the Young (DAISY) and DIPP studies have addressed these concerns. Amongst high-risk children with multiple islet autoantibodies, progression to clinical diabetes was significantly more frequent in those with evidence of a recent enterovirus infection than in those without [40, 41]. In addition to direct infection of the pancreas with enteroviruses such as Coxsackie, molecular mimicry between viral antigens and islet autoantigens has been postulated to trigger T cell reactivity against islets (discussed in [42]). The P2C non-structural protein of a diabetogenic strain of Coxsackie virus B4 shares extensive sequence similarity with GAD65, and virtually identical highly antigenic peptides can be derived from the two proteins [43].

Rotaviruses are double-stranded RNA viruses belonging to the Reovirus family. They are ubiquitous in the environment, and are the most frequent cause of gastroenteritis in young children [44]. The first indication that these agents may contribute to the development of type 1 diabetes came from studies demonstrating strong sequence homology between the rotavirus VP7 antigen and two epitopes recognised by islet-reactive T cells, one in GAD and one in IA-2 [45]. Longitudinal studies attempting to determine whether rotavirus exposure is associated with diabetes development have yielded varying results. The Australian BabyDiab study of at-risk children reported strong concordance between the appearance of islet autoantibodies and the detection of rotavirus-specific antibodies. Repeated rotavirus infection appeared to boost levels of anti-islet antibodies and coincided with epitope spreading and an increase in the number of autoantibody specificities present [46]. A later Finnish study failed to confirm these observations [47]. However, differences in the methodology used for determining rotavirus exposure may have contributed to this negative finding.

Lifestyle factors

Various other aspects of the modern lifestyle could be influencing the rise in incidence of type 1 diabetes (reviewed in [48]). Many of these (increased consumption of high-energy foods and foods containing trans-fatty acids, fructose or advanced glycation end-products, reduced energy expenditure due to declining levels of physical activity and maintenance of ambient temperatures within the thermoneutral zone for most of the time, reduced sleep duration) act through the mechanism of increasing insulin resistance, which will be discussed further below. In a seeming paradox, although exposure to some pathogenic viruses is linked to the development of type 1 diabetes, widespread adoption of clean living conditions with prevention or delay of exposure to environmental pathogens has also been implicated in the rise of autoimmune and allergic conditions, a theory termed the 'hygiene hypothesis' [49]. Changes in commensal gut bacteria, by increasing energy extraction

from the diet, may also predispose to obesity and insulin resistance [50]. Finally, exposure to sunlight and consequent generation of vitamin D has progressively declined in many developed and developing countries over the past few decades. Several epidemiological studies have shown an inverse correlation between vitamin D intake and the incidence of type 1 diabetes [51, 52], and this link has been strengthened by the demonstration of protection against type 1 diabetes development by vitamin D administration in primary prevention studies in at-risk populations [53, 54].

Insulin resistance and type 1 diabetes

Insulin resistance in children and adults in the developed and developing world has been increasing in parallel with the rise in overweight and obesity, and could be making a significant contribution to the rising incidence of type 1 diabetes, in addition to its acknowledged role in type 2 diabetes. Analyses of a number of independent cohorts from Europe, the US and Australia have demonstrated that age at onset of type 1 diabetes was inversely proportional to body mass index (BMI) [55]. Not surprisingly, BMI was higher in subjects with moderate genetic risk, compared to those with high risk, and direct measurements of insulin sensitivity during metabolic testing confirmed that insulin resistance was an independent risk factor for progression to clinical type 1 diabetes in individuals with islet autoimmunity [56]. There are several ways in which insulin resistance could accelerate the development of type 1 diabetes in those with an underlying genetic predisposition. Insulin resistance means that the beta cells are obliged to increase insulin production in order to maintain blood glucose within the physiological range. Metabolic upregulation results in increased production of insulin precursor proteins as well as the cellular enzymes GAD and IA-2. Increased production results in increased presentation of peptides derived from these molecules on the surface of the beta cell, thereby increasing the chances of the beta cell being destroyed by a cytotoxic T cell which recognises these peptides. Insulin resistance per se 'accelerates the rate of beta-cell apoptosis through glucotoxicity and lipotoxicity' [57]. Furthermore, patients would be expected to cross the threshold at which remaining beta-cell function is no longer able to maintain glucose homeostasis at an earlier stage of beta-cell loss, in the presence of insulin resistance.

Pathogenesis and disease progression

Beta cells and autoantibodies in type 1 diabetes

The principal autoantibodies in type 1 diabetes recognise four islet autoantigens: insulinoma-associated antigen-2 (I–A2), insulin (micro IAA or mIAA), the 65 kD isoform of the enzyme glutamic acid decarboxylase (GAD65) and zinc transporter 8 (ZnT8) [58]. Whilst autoantibody positivity is strongly associated with the progression to diabetes, especially when antibodies against multiple islet determinants are present [59], it is not clear whether autoantibodies can cause beta-cell destruction, or whether antibody production is only triggered after episodes of beta-cell death have already occurred [60]. Antibody-producing B cells may also play a role in the pathogenesis of type 1 diabetes as professional antigen-presenting cells for T cells. In this regard, B cells can use their surface antibody to capture islet autoantigens before processing and presenting them to $CD4^+$ T cells. B cells in

turn receive help from the T cells which culminates in increased antibody secretion. This role of B cells is consistent with the observation that many of the major T cell autoantigens are derived from the same proteins recognised by B cells [61–63].

Insulitis

Infiltration of the pancreatic islets with lymphocytes ('insulitis') in patients who succumbed to diabetic complications close to the time of presentation was first noted in the 1960s [64] and has since been considered a hallmark of type 1 diabetes. Cross-sectional studies of tissues obtained close to diagnosis can yield important information about the final pre-clinical stages of the disease. Advances in the management of metabolic complications of diabetes mean that patients now rarely die with ketoacidosis, and most data are derived from historical collections of tissue [65]. Painstaking re-analysis of one such large cohort affirmed earlier observations that the degree of insulitis varied not only between patients but between regions of the pancreas and between individual islets [63, 65–67]. The investigators subdivided islets according to the percentage of remaining insulin-positive cells. The numbers of infiltrating cells increased as insulin-positive cell numbers declined, reaching a peak when less than 10% of insulin-containing cells remained. The density of infiltrate was then dramatically reduced in islets lacking insulin positivity. Although this was a cross-sectional study, the different categories of islets were interpreted as representing stages in the progression to beta-cell destruction, which was proceeding asynchronously [68]. At all stages, $CD8^+$ T cells predominated in the infiltrate. B cells were preferentially found in more inflamed islets, whilst macrophages were a constant presence, albeit less frequent than $CD8^+$ T cells. In this study, at least some degree of insulitis was found in all pancreas specimens, and overall, insulitis was detected in 12% of islets examined [68]. The observation that the density of the infiltrate increases substantially once beta-cell loss has reached a critical threshold is consistent with the idea that the drive to maintain glucose homeostasis upregulates the metabolic activity of the remaining beta cells, causing antigenic peptides to be produced and displayed on the cell surface in increasing amounts. This increases beta-cell susceptibility to $CD8^+$ T cell-mediated killing and hastens the decline of the remaining beta-cell mass.

Much could be learned from the systematic histological study of the pancreas in at-risk and pre-diabetic individuals, as well as those with clinical diabetes of varying durations. However, obtaining tissue samples from these groups has previously been all but impossible. Recent initiatives have aimed to fill this void in our knowledge by screening organ donors for the presence of islet autoantibodies, and examining specimens of the pancreata of antibody-positive subjects. One such study of 62 antibody-positive donors found evidence of insulitis in only two individuals, a surprising result which led researchers to question the central role of islet inflammation in progression to diabetes [69]. Closer examination of the study cohort reveals that the two donors with insulitis were the only subjects with ≥3 autoantibodies, and also possessed high-risk HLA-DQ alleles. The increased age of this donor population compared with the usual age of onset of type 1 DM, single and low-titre antibody positive status of most of the subjects and relatively small number of islets

examined per pancreas may all have contributed to the paucity of insulitis noted, and may mean that these findings do not reflect the general situation in prediabetes.

Efforts to gather pancreatic tissue from deceased organ donors of all ages have continued, largely under the auspices of the network for pancreatic organ donors with diabetes (nPOD), a program established by the Juvenile Diabetes Research Foundation. One of the first studies to be reported as a result of this initiative revealed that 30% of pancreata from donors with longstanding type 1 diabetes still contained numerous insulin positive cells in at least some islets [70]. Two distinct histological patterns were detected – one with patchy distribution of insulin-positive cells in a fraction of islets, co-incident with the upregulation of pro-survival signals in these islets. The second pattern was characterised by the presence of residual insulin-positive cells in 100% of islets. Subjects with this pattern were typically islet autoantibody-negative, and lacked high-risk HLA class II alleles, yet their disease displayed metabolic hallmarks of type 1 diabetes, including episodes of ketoacidisis [70]. These studies suggest that the pathogenesis of type 1 diabetes may be more heterogeneous than previously appreciated, and, at least a subset of patients have the potential to benefit from treatment approaches which stimulate residual beta-cell function or regeneration. More than 60 projects are currently underway using tissues collected through nPOD, and hopes are high that further new insights into diabetes pathogenesis will soon emerge from these.

Non-invasive imaging and monitoring of T cell function

Imaging of pancreatic islets and cells *in vivo* has to date proven very difficult in humans. The capacity to non-invasively screen for and sequentially monitor islet inflammation in at-risk, pre-diabetic, or type 1 diabetes patients would allow much greater refinement of risk stratification as well as enabling direct and timely assessment of the effect of interventions. In animal models of type 1 diabetes, the onset of insulitis is accompanied by increased 'leakiness' of the small blood vessels supplying the islets [71]. Magnetic Resonance Imaging (MRI) can be used to capture signals from injected magnetic nanoparticles, which exit the circulation through the leaky vessels and are engulfed by infiltrating macrophages in the pancreas. A recent report demonstrates that this imaging method can be adapted for use in humans, and is able to differentiate between subjects with recent-onset diabetes and controls [71]. Further development and more widespread adoption of this and other non-invasive imaging techniques, such as positron-emission tomography [72], have the potential to greatly increase our understanding of type 1 diabetes pathogenesis.

Efforts are being made to develop and validate assays of cell-mediated anti-islet reactivity which might be used to monitor changes in T cell function in those at risk for type 1 diabetes development, and subjects in prevention and early intervention trials of immunomodulation. A recent workshop sponsored by the Immune Tolerance Network evaluated two such assays for $CD4^+$ T cells, a cellular immunoblot and a T cell proliferation assay [73]. Overall, these assays performed reasonably well in distinguishing patients with new-onset diabetes from normal controls with sensitivities of 94% and 58%, and specificities of 83% and 91%, respectively. Combination of the assays improved sensitivity, whilst maintaining specificity [73]. For $CD8^+$ T cells, an islet-specific ELISPOT assay has

been developed (ISL8SPOT, [74]). This assay measures interferon gamma-producing T cells in response to stimulation with a mixture of HLA-A2-restricted, beta cell-derived peptide epitopes. Beta cell-specific $CD8^+$ T cells can thus be quantitated directly from unfractionated peripheral blood mononuclear cells. ISL8SPOT responses were clearly detectable in newly diagnosed patients with type 1 diabetes, but waned rapidly over the following six to 12 months as the rate of beta-cell destruction declined. Autoantibody levels remained constant over this period, suggesting that the T cell functional assays were more likely to reflect the patients' current immunological status than autoantibody titres [75]. HLA-A2 tetramers loaded with beta cell antigenic peptides are another tool which can be used to detect and quantitate islet-reactive $CD8^+$ T cells [76]. These various assays are currently being evaluated for their suitability in monitoring responses to immunomodulatory treatments, and further information about these applications should soon become available.

On the opposite side of the T cell balance from the destructive effector cells are regulatory T cells, and attempts have also been made to assess regulatory T cell activity in type 1 diabetes. No consistent difference in the numbers of peripheral blood regulatory T cells have been identified between subjects with type 1 diabetes and normal individuals. Nonetheless, natural T regs isolated from type 1 diabetes patients are less potent suppressors than those isolated from control subjects, suggesting that function, rather than absolute number of these cells makes the paramount contribution to the disease state [77].

Interventions in at-risk individuals

The idea of being able to intervene in at risk or pre-diabetic individuals to prevent the onset of clinical diabetes is enormously appealing. Even with the most accurate predictive identification of high-risk groups, it should be remembered that not all members will develop diabetes. As a corollary, such trials must involve large numbers of subjects, and are very costly to conduct. Any interventions tested must be inherently very safe to maintain a balance between the risks and potential benefits to the participants. Interventions can be categorised as either non antigen-specific, or specific to various islet autoantigens. Dietary modifications are non antigen-specific interventions, and dietary substitutes and supplements studied in this way include hydrolysed cows' milk formula (Trial to Reduce IDDM in Genetically at Risk (TRIGR) study, [78]), vitamin D3 [79], Omega-3 fatty acids [80] and nicotinamide [81]. Another subgroup of prevention trials has aimed to induce specific tolerance to islet autoantigens by administering them via a route that is generally non-immunogenic. Accordingly, insulin has been given orally or intranasally to at-risk subjects. Whereas nicotinamide has been ineffective, early studies of vitamin D supplementation and of hydrolysed cow's milk formula have shown some benefit [82] and these interventions are now undergoing further evaluation. Results of insulin administration have been equivocal thus far [83].

The honeymoon phase and beta-cell regeneration

Up to 60% of patients newly diagnosed with type 1 diabetes achieve some improvement in functional beta-cell mass as measured by secretion of c-peptide and reduction in exogenous

insulin requirement, after the initiation of insulin replacement therapy [84]. This period is often referred to as the 'honeymoon phase'. The mechanisms governing this short-lived improvement in function are incompletely understood, but probably involve restoration of function to insulin-depleted beta cells, perhaps accompanied by some true expansion of beta-cell mass [85].

A subset of patients with type 1 diabetes maintains small populations of functioning beta cells for many years after the onset of clinical type 1 diabetes [85], and these patients may be particularly amenable to therapies which aim to restore or improven glucose homeostasis via regeneration of existing beta-cell mass. In mouse models, three different mechanisms have been shown by different groups to result in the generation of new beta cells [87]. Replication of existing beta cells was responsible for beta-cell regeneration after subtotal ablation of beta cells in a transgenic mouse model [88], while recent reports have demonstrated that both beta-cell neogenesis from ductal precursors [89] and transdifferentiation from glucagon-secreting islet alpha cells [90, 91] can also occur under some circumstances. In the human pancreas, staining for the nuclear proliferation marker Ki-67 suggests that mature, differentiated beta cells can replicate, though the basal rate of replication is low (reviewed in [92]). Data supporting the existence of the other two mechanisms in humans are scant.

Arresting autoimmune islet destruction may allow beta-cell regeneration to emerge, but in adult humans, it is not clear whether this alone could be sufficient to reconstitute beta-cell mass and restore glucose homeostasis. In order to harness the potential of beta-cell regeneration, it is essential to understand how beta-cell turnover is controlled, and whether this could be manipulated in such a way as to produce a net accumulation of functional beta-cell mass. Murine studies indicate that a critical driver of beta-cell replication is glycolytic flux in the beta cell itself, reflected in the activity of the enzyme glucokinase [93]. Under physiological conditions, this is proportional to blood glucose levels, but glucokinase activity can be modified by a novel class of small-molecule drugs, the glucokinase activators, and thus represents a potential target for intervention [94]. Glucokinase activators are currently under development for the treatment of type 2 diabetes. They act by increasing the glucose affinity and maximum velocity of glucokinase [94]. Administration of glucokinase activators could eventually be used to enhance the low basal rate of beta-cell replication *in vivo*, thus permitting gradual regeneration of sufficient beta-cell mass from the remaining viable beta cells after the onset of clinical type 1 diabetes.

Gastrointestinal hormones (incretins) also play a role in regulation of beta-cell mass [95]. Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are produced and secreted by intestinal cells in response to dietary fat and carbohydrate. They increase insulin secretion by the beta cell via binding to G protein-coupled receptors (discussed in [96]). GLP-1 reduces beta-cell apoptosis [97], and may also stimulate beta-cell proliferation via the mitogen-activated protein (MAP) kinase [98] and Wnt [99] pathways, thus shifting the balance of beta-cell turnover towards accumulation. Treatment with currently available GLP-1 agonists such as exendin-4, or with inhibitors of the enzyme dipeptidyl peptidase 4 (DPP-IV), which degrades GLP-1 and GIP, increase beta-cell mass

in diabetic rodents [100, 101]. These agents may have similar beneficial effects in humans if used in conjunction with treatments which target autoimmunity.

Conclusions

This is an exciting time for research in type 1 diabetes. Recent technological advances such as the ability to conduct genome-wide association studies or to undertake non-invasive imaging of the pancreas, coupled with initiatives such as nPOD, are greatly increasing our understanding of the aetiology and pathogenesis of type 1 diabetes. These insights will continue to suggest ways in which it might be possible to intervene in the course of the disease, both prior to and following the onset of clinical diabetes. Growing appreciation of the heterogeneity of type 1 diabetes will allow tailoring of treatment modalities to the particular subgroups of patients most likely to benefit from each type of intervention, while improved assays for monitoring anti-islet immune reactivity and response to treatment will inform clinical decisions and permit fine adjustment of immunomodulatory therapies.

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