Heterogeneity and endotypes in type 1 diabetes mellitus

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Abstract

Despite major advances over the past decade, prevention and treatment of type 1 diabetes mellitus (T1DM) remain suboptimal, with large and unexplained variations in individual responses to interventions. The current classification schema for diabetes mellitus does not capture the complexity of this disease or guide clinical management effectively. One of the approaches to achieve the goal of applying precision medicine in diabetes mellitus is to identify endotypes (that is, well-defined subtypes) of the disease each of which has a distinct aetiopathogenesis that might be amenable to specific interventions. Here, we describe epidemiological, clinical, genetic, immunological, histological and metabolic differences within T1DM that, together, suggest heterogeneity in its aetiology and pathogenesis. We then present the emerging endotypes and their impact on T1DM prediction, prevention and treatment.

Type 1 diabetes mellitus (T1DM) is currently diagnosed in individuals with insulin deficiency attributed to islet autoimmunity[1]. Major actors in the autoimmune attack against β-cells are antigen-specific autoreactive T cells, present in serum and islets of affected individuals[2]; regulatory T cells that fail to control effector cell populations[3]; and β-cell abnormalities that support autoimmunity. These β-cell abnormalities include HLA overexpression[4] and increased protein misfolding leading to neoantigen generation[5,6]. B cells, neutrophils, macrophages, dendritic cells and NK cells have also been implicated in T1DM pathogenesis[7]. The involvement of circulating B cells is unsurprising given that most people with T1DM produce autoantibodies, which are secreted by plasma cells expanded from B cell precursors. However, the involvement of B cells within pancreatic islets themselves is less common in T1DM and is still poorly understood. It has been hypothesized, however, that these B cells might serve as antigen presenting cells operating at the site of tissue inflammation[8].

Islet autoantibodies, although not pathogenic, are used as biomarkers for T1DM prediction and diagnosis because they are detectable in serum before, at and, often, for years after clinical onset. The 10-year risk of developing clinical (also known as stage 3) T1DM increased from 15% with single positive autoantibody to 70% with multiple positive autoantibodies (stage 2 T1DM) in an analysis of children enrolled from birth in the Colorado Diabetes Autoimmune Study in the Young (DAISY) study, the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study and the German BABYDIAB and BABYDIET studies[9]. While elevations of glucose during an oral glucose tolerance test are not sufficient for a definition of diabetes mellitus (stage 2

T1DM), once these abnormalities appear, the lifetime risk of clinical T1DM is virtually 100%[10].

Both genetics and environmental factors contribute to the initiation of islet autoimmunity and its progression to clinical T1DM. Half of the genetic risk of developing T1DM is linked to the HLA region, particularly class II (HLA-DR, HLA-DQ, HLA-DP) and class I (HLA-A, HLA-B, HLA-C) genes. The strongest association occurs with the HLA-DR4 allele, with an odds ratio (OR) of 6.81 and with HLA-DR3 allele (OR = 3.54)[11]. The strongest protective alleles are HLA-DR2 (OR=0.21), HLA-DR5 (OR=0.30), and HLA-DR7 (OR=0.24)[11]. Of note, opposite to their European counterparts, the African-specific DR3 and DR7 haplotypes confer, respectively, protection and susceptibility, while, in Asians, DRB1*09:01 imparts high risk[12]. There are >75 non-HLA loci associated with T1DM, with most regulating immune functions[13] although the majority are also present in β-cells, which reinforces the prominent role of the β-cell in the pathogenesis of T1DM. Out of these non-HLA loci, the insulin variable number of tandem repeats (*INS*-VNTR) polymorphism found in the *INS* promoter displays the strongest association with T1DM and regulates thymic immune tolerance to insulin. Other robustly linked genes are *CTLA4*, *PTPN22* and *IL2RA*[14]. However, for many diabetes mellitus-associated single nucleotide polymorphisms (SNPs), the relevant gene is unknown[15].

Among the various environmental exposures implicated in T1DM, coxsackievirus infection appears strongly influential[16] and variations in the microbiome are also increasingly reported[17]. Similar to genetic factors, the environmental factors that promote islet autoimmunity can vary from those mediating disease progression. Moreover, environment and genetics can

interact[18], complicating studies on etiology and prediction. Nevertheless, algorithms that incorporate demographic, genetic, immunologic and metabolic factors accurately predict T1DM[19,20] and, strategies to screen the general population for T1DM risk in a cost-effective manner are being proposed, although most studies still focus on genetically predisposed individuals [21,22].

Although insulin secretory capacity, measured as serum levels of C-peptide, shows early signs of insulin secretory capacity loss (measured as low C-peptide levels) can often be seen when islet autoantibodies first appear in serum[23]., this decline begins to accelerate around two years before diagnosis[24], After diagnosis of clinical T1DM, treatment with insulin improves glycaemia, and this is often followed by a partial recovery of endogenous insulin secretory capacity (partial remission, or 'honeymoon'). However, this partial remission is only transient as the underlying β-cell demise continues. In a long term study, the exponential decline in C-peptide levels slowed down and stabilized about seven years after onset[25]. Although most individuals with long-standing T1DM do not produce clinically significant amounts of insulin, some insulin secretion can persist for several decades[26]. The decline in C-peptide after the onset of T1DM is at least partially prevented by treatment with the anti-CD3 humanized monoclonal antibody teplizumab[27], the anti-CD20 monoclonal antibody rituximab[28], the anti-TNF monoclonal antibody golimumab[29], low dose anti-thymocyte globulin (ATG)[30,31], and the calcium channel blocker verapamil[32] among other agents. Teplizumab has proven to be safe and effective at delaying the progression to clinical T1DM in autoantibody-positive individuals[33,34]. However, responses to teplizumab, rituximab, golimumab, ATG, verapamil and other drugs aiming to modify the natural course of T1DM,

before or after the diagnosis of T1DM are variable for reasons still poorly understood[35].

T1DM is a highly heterogeneous disease as demonstrated by wide differences in epidemiology, etiopathogenesis, clinical course and response to intervention. This heterogeneity poses challenges to disease prediction, prevention, diagnosis and treatment. More broadly, the current classification schema for diabetes mellitus does not capture its complexity nor guide clinical management effectively[36,37]. The goal of applying precision medicine in diabetes mellitus (defined as “the right treatment, for each patient, at the right time”[38]) requires its heterogeneity to be shaped into ‘endotypes’, that is, subtypes of disease with distinct etiopathogenesis that might be amenable to specific intervention[39,40]. In this Review, we will consider the heterogeneity of T1DM, describe the evidence that supports the emerging concept of T1DM endotypes, discuss the implications of this concept and ongoing efforts in the field.

[H1] Heterogeneity of T1DM

[H2] Epidemiology and clinical characteristics

The global incidence of T1DM is estimated as 15 cases per 100,000 people and the prevalence as 5.9 cases per 10,000 people (95% CI=0.07-0.12) but there are large geographical differences between countries[41] that could reflect varying environmental triggers and genetic predisposition[42]. Clinically, perhaps the most extreme variation in T1DM manifestation is seen with fulminant T1DM in India and east Asia, which often presents with negative islet autoantibodies,

and association with HLA-DRB1*04:05-DQB1*04:01 and global pancreatic inflammation[43,44]. On the other hand, the most common form of diabetes mellitus in Japan is slowly progressive insulin dependent diabetes mellitus (SPIDDM), which is an autoimmune form of diabetes mellitus that progresses at a slower pace than typical T1DM[45]. Slower loss of β-cell function is also seen in latent autoimmune diabetes mellitus in adults (LADA)[46], although, unlike SPIDDM, this entity is by definition diagnosed in individuals >30 years of age. Milder differences between ethnic groups include those seen in the US, where Hispanic or non-Hispanic Black children develop islet autoantibodies at an older age, have lower risk and slower rates of progression[47] and, at the onset of T1DM, are older, have higher BMI and higher blood levels of glucose than non-Hispanic White children[48,49]. A study published this year has shown that certain African populations display a form of T1DM in which the autoantibodies found classically in European populations are not present[50].

T1DM shows profound differences by age [51,52]. Age at diagnosis partly reflects the rate of progression through pre-clinical stages of T1DM, which occurs more quickly with earlier initiation of islet autoimmunity. Furthermore, clinical presentation is more severe, with greater frequency of diabetic ketoacidosis, in children than in adults[53]. Genetic predisposition can also differ between childhood and adult onset T1DM[54,55]. T1DM-associated HLA genotypes are less frequent and the burden of T1DM-related genetic regions (as measured by T1DM genetic risk scores, a weighted combination of T1DM-associated SNPs in HLA and non-HLA regions) becomes lower as age at diagnosis increases[56,57]. Single autoantibody positivity is more frequent in adult onset than pediatric T1DM[58]). Although it is now recognized that more cases

are diagnosed during adulthood than childhood, adult onset T1DM is still understudied and, in the clinical setting, it is often misclassified as T2DM [57,59].

[H2] Islet autoimmunity

Islet autoimmunity is evident by the presence of both infiltrating immune cells in and around pancreatic islets (Figure 1), and circulating islet autoantibodies (for example, to GAD65, IA-2 (also known as ICA512), ZnT8 and insulin) although the presence of circulating islet autoantibodies not universal in T1DM[60,61]. Plausible explanations for the small percentage of individuals who present without autoantibodies include islet dysfunction resulting from cellular autoimmunity without circulating humoral markers or resulting from non-autoimmune causes, such as mutations in unidentified genes[62,63]. Ketosis-prone diabetes mellitus, which is more common in individuals of African or Asian ancestry than in people of European ancestry, displays intermittent insulin dependence, and can lack autoantibodies and typical HLA associations[64].

Both the time-course and the specificity of seroconversion to autoantibody positivity is heterogeneous among children and seroconversion often occurs within the first 6 years of age, even in people who develop T1DM much later in life[21,65,66]. This fact might imply an element of stochasticity in autoantibody generation, although more specific aetiologies associated with age-related endotypes have been proposed[51,67]. The fact that antibody generation often occurs early in life implies that the triggering events associated with progression to islet autoimmunity can be active at the youngest ages,

possibly in the first two years even if these do not lead immediately to disease onset. Thus, the age at which these events are initiated is not, itself, a direct determinant of disease progression, implying that other parameters must dictate the rate at which β-cell dysfunction and death ensue (such as which endotype is manifest). Whether those who develop T1DM later in life also had a much earlier development of islet autoantibodies (consistent with early initiation of the autoimmune process) is an area that requires further investigation in longitudinal studies.

Several commonly detected autoantigens (including insulin, IA-2 and ZnT8) are synthesized in coordination with each other and localised within insulin secretory granules[68], hinting that some facet of β-cell physiology might underpin initial autoimmunity. Anti-insulin antibodies tend to develop within the first 2 years of life and their epitope specificity and binding affinities are markedly heterogeneous[69]. A second major β-cell autoantigen, the 65KDa isoform of glutamate decarboxylase (GAD65) bucks the secretory granule protein trend since, in β cells, this resides in small membrane-enclosed vesicles[68,70]. GAD65 antibodies directed against the central and C-terminal regions of the GAD65 protein (known as 'truncated-GAD' antibodies) are most predictive of whether a patient will progress to clinical T1DM compared with other GAD65 antibodies[71].

In the TEDDY study, clustering 370 children according to their profiles of autoreactivity to insulin, GAD and IA-2 revealed stratification of likelihood of progression to clinical T1DM based on their age at seroconversion to each of these autoantibodies[72]. More granular analysis revealed that earlier age at seroconversion was the single most important discriminatory feature in

determining probable progression to disease, irrespective of the absolute profiles of autoantibody specificity. A classification and regression tree (CART) approach to residual C-peptide stratification of young people <20 years old was taken in the German “DiMelli” study[73], where autoantibody status was considered in parallel with age at T1DM onset, glucose control indices and BMI. Ten different subgroups emerged, among which, seven were autoantibody positive and contained 1,088 (91.2%) of the 1,192 individuals studied. Age at seroconversion was a critical factor in providing a basis for stratification. It was concluded that mechanisms driven by elevations in levels of IFNγ, IL-10 and TNF are influential in driving autoimmunity in children younger than 8 years of age. A further subset was characterised by a more benign inflammatory milieu, compared with the subset of younger children in which elevated levels of triglycerides and insulin resistance were primary features. Overall, young age and early seroconversion were associated with more intense inflammatory responses. This finding aligns, in part, with previous evidence that early age at T1DM onset is associated with a strongly proinflammatory signature mediated by IFNγ[74]. By contrast, older age at onset, extending into the teenage years, is associated with a less intense inflammatory milieu, characterised by a primarily IL-10 signature. A 2022 study monitoring longitudinal T-cell responses in a small cohort of children at high genetic risk of T1DM again revealed two profiles[75]. The first featured an increased IFNγ response when T cells were exposed to proinsulin or insulin-derived peptides and was detectable up to 6 months prior to T1DM onset. The second was characterised by an enhanced regulatory IL-10 response which, at least during the period of study, occurred in children who did not progress to T1DM.

[H2] Metabolic factors

Some people with T1DM also display features that are typically associated with T2DM, such as insulin resistance, obesity or specific genetic associations[58,76,77]. This observation is not surprising because T2DM is common in the general population. High BMI has been shown to accelerate the onset of clinical T1DM [76,78,79], and this effect was more pronounced in Hispanic (400% increase in risk) than in non-Hispanic white children (34% increase) enrolled in the Type 1 Diabetes TrialNet Study[47] (Figure 2) In the TrialNet study, overweight or obesity, older age and having a single positive autoantibody are associated with lower Index60, indicating relatively well preserved C-peptide levels in relation to levels of glucose[80,81]. Furthermore, using area-under-the-curve C-peptide and glucose measurements derived from oral glucose tolerance tests to classify autoantibody-positive TrialNet participants into 25 metabolic zones revealed that, for the same level of glucose, participants with higher levels of C-peptide had higher insulin resistance, older age and fewer immunological and genetic markers of T1DM than participants with lower levels of C-peptide[82]. The heterogeneity observed among these 25 metabolic zones also support the concept that, even within autoantibody-positive individuals without obvious T2DM risk factors, there is wide variation in the preclinical progression of β-cell dysfunction and endoplasmic reticulum (ER) stress (reviewed in [83]).

Furthermore, obesity might influence the progression of islet autoimmunity through inflammation, ER stress and β-cell apoptosis[84]. In T2DM, elevation of T_H17 T cells-to-T_{reg} cells ratio[85], proinflammatory cytokines (such as, CCL2 and TNF)[86], islet-reactive T-cells[87] and islet autoantibodies[87] have

been described. In a 2022 study, cellular islet autoimmunity, humoral islet autoimmunity or both were observed in, respectively, 41.3%, 13.5% and 5.3% of participants in the Glycemia Reduction Approaches in Diabetes: A Comparative Effectiveness Study (GRADE)[88]. Accordingly, among adolescents and children older than >9 years of age, without the high risk HLA DR4-DQ8 or DR3-DQ2 haplotypes, islet autoimmunity was more likely to progress inthose with overweight or obesity than in those without it. [89].

[H2] Genetics

Higher T1DM genetic risk score (indicating a higher genetic burden for the disease) correlates with higher T1DM risk and more rapid progression through the pre-clinical stages[19]. The ability of genetics to predict risk of progression to T1DM weakens progressively from the initiation of islet autoimmunity with a single autoantibody to the transition to multiple positivity and then to T1DM diagnosis[90], and the specific genetic regions involved in each step vary[91].

T1DM-associated HLA genotypes were less frequent in individuals with multiple positive autoantibodies who did not progress to T1DM within 10 years after seroconversion as compared with children who presented with clinical T1DM under the age of 5 years[92]. Moreover, geographical differences can be explained, at least in part, by differences in HLA alleles and haplotypes among populations[42] (Figure 3). Although most genetic studies of T1DM have been conducted in individuals of European ancestry, emerging cohorts from other ethnicities are revealing variation in the genetic risk conferred by HLA alleles between ethnic groups[12]. DR3 and DR4 alleles at the HLA class II locus are differentially associated with both age at onset and first autoantibody

generation. Children in the TEDDY study who carried HLA-DR3-DQ2 haplotypes were older at diagnosis and were more likely to feature primary autoreactivity against GAD65, whereas those carrying HLA-DR4-DQ8 were diagnosed earlier in childhood and were more likely to display autoreactivity against insulin as an initial response[93].

Further supporting the concept that pathogenic mechanisms typically associated with T1DM or T2DM can combine and interact in the same individual, it has been shown that *TCF7L2* genetic variants that confer risk for T2DM also modify the natural course[94] and presentation of T1DM[77,95]. Individuals with new onset T1DM and a single positive autoantibody (that is, only mild autoimmunity) are more likely to have insulin resistance and, among adolescents and adults, carry the T2DM-associated allele in the *TCF7L2* SNP than those with multiple autoantibody positivity[77]. Consistent with these findings, among donors from the Network for Pancreatic Organ Donors with Diabetes (nPOD) with T1DM, those with the *TCF7L2* risk allele, compared with donors without the allele, had higher frequency of residual insulin-containing islets after adjustment for age at onset, diabetes mellitus duration, BMI Z-score, sex and African American race[96]. In addition, participants in the T1DM Exchange with established T1DM carrying the T2DM-associated *TCF7L2* SNP, compared with those who did not carry it, were less likely to have T1D-associated HLA genotypes[97].

[H1] T1DM endotypes

Disease endotypes are defined as having intrinsically different pathologic processes from each other that necessitate specific treatment approaches and have prognostic implications[39]. Endotypes differ from phenotypes, which represent observable characteristics or traits of a disease that do not always entail a unique mechanism. For example, different degrees of severity or rates of progression of a disease are phenotypic features that do not necessarily imply an idiosyncratic pathogenesis. Similarly, age or sex of the patient might modulate the expression of a phenotype without the result qualifying as an endotype.

On the other hand, a distinct phenotype is often the first indicator of a different pathogenic mechanism and, when a distinctive molecular or cellular mechanism can be attributed, and/or effectiveness of a specific treatment proven, the phenotype is better identified as an endotype or a separate disease. For example, maturity onset of diabetes in the young (MODY) was recognized as a separate entity after a distinct phenotype (that is, young people with mild diabetes mellitus and a family history of diabetes mellitus consistent with Mendelian inheritance) was observed[98], then found to respond to non-insulin treatments[99,100] and decades later attributed to specific genetic mutations[101]. Further research led to the discovery of multiple unique genetic mutations causing specific molecular defects that respond to precise therapeutic approaches, such as a particular drug or absence of medical treatment (reviewed in [102]).

The strategy of aiming to develop disease taxonomy based on discrete biological signatures (endotypes) (reviewed in [103]) has proven useful to advance targeted therapies in the field of asthma and is also being tested to

dissect rheumatologic diseases[104]. In summary, phenotypic heterogeneity can support pathogenic heterogeneity but proving the existence of separate endotypes requires the identification of distinct pathogenic mechanisms that are amenable to specific treatment.

[H2] T1DM endotypes 1 and 2

Arguably the most substantial impediment to an improved understanding of the heterogeneity of T1DM is the inability to monitor the disease process in humans in real time at the site of tissue damage. Currently, studies rely mainly on pancreatic tissue from people with T1DM recovered *post mortem* either at the time of organ donation or at autopsy, although six pancreas biopsies from living patients newly diagnosed with T1DM have also been highly informative[105]. Mercifully, few individuals now die close to the diagnosis of T1DM; consequently, the availability of pancreas recovered from people (especially young children) with recent onset disease is severely limited[106]. The most extensive collection recovered from children under 10 years of age at T1DM onset was compiled within the UK by Foulis and colleagues [107] and is now curated as part of the larger Exeter Archival Diabetes Biobank[108]. The Network for Pancreatic Organ Donors with Diabetes (nPOD), supported by Juvenile Diabetes Research Foundation, also holds an extensive contemporary collection[109,110], with most donors of pancreatic tissue with recent-onset T1DM being >10 years of age. Study of these samples, together with specimens available from a Belgian collection [111,112] has revealed marked heterogeneity of T1DM on multiple levels.

The first level of heterogeneity lies in the realisation that islets within a given pancreas are subject to immune-mediated attack at variable rates over time[113-115]. Examination of the tissue reveals distinct foci of β cell destruction with islets in some regions apparently untouched while others, often in close proximity, are devoid of insulin immunopositivity. Again, age is important since children <10 years at disease onset display the least heterogeneity, with the majority of their β-cells destroyed and most residual insulin-containing islets under active inflammatory assault[116]. By contrast, those who develop T1DM in their teenage years display a much less aggressive disease profile with many insulin-containing islets retained (frequently >50%), most of which are devoid of inflammatory infiltrates. It has proven difficult to assign these differences to any underlying genetic architecture, but hints have emerged that certain predisposing SNPs in genes including *IKZF3* and *IL-10* are associated with children diagnosed under age 7 years[117].

Aligned with a variability in the proportions of inflamed islets are substantial variations in the magnitude and composition of the infiltrating immune cells. In the very youngest children (<7 years) the islet-associated inflammatory infiltrates comprise large numbers of both $CD8^+$ T cells and $CD20^+$ B cells, whereas, in older children ($\geq$13 years) the absolute number of infiltrating $CD8^+$ T cells is typically much lower and very few $CD20^+$ B cells are detected cells[8,116,118]. Importantly, these two immunological profiles segregate with age at diagnosis, and they do not represent a continuum. Inevitably, however, although the age dependence of the immune profiles is strict for those <7 years or >12 years of age, there is overlap within the intermediate (8–12 year) group. As a result, both profiles can be found among children at these intermediate

ages. Nevertheless, the two profiles remain fully segregated in that all pancreata examined from children in the 8–12 year-old range display either one pancreatic immunological profile or the other. This finding supports fully the proposition that the two immune cell profiles reflect differences in disease etiology. Accordingly, two immunological endotypes have been proposed: “T1D endotype 1” (T1DE1) and “T1D endotype 2” (T1DE2)[119] (Table 1). Despite this evidence, the concept of endotypes in T1DM remains controversial and, in this Review, we will strive to present both perspectives.

Additional histological analysis of pancreatic tissue from people with T1DM has revealed marked variation in the ability of residual β-cells to process insulin correctly between the two proposed endotypes. In children defined as having T1DE1, the majority of islets display evidence of aberrant proinsulin processing, leading to a marked increase in the circulating ratio of proinsulin to C-peptide. By contrast, in T1DE2, most islets retain apparently normal proinsulin processing and the circulating ratio of proinsulin to C-peptide is correspondingly lower[119] Measurement of this ratio offers a potential means to differentiate between T1DE1 and T1DE2 among children who develop T1DM between the ages of 8–12 years, where either pancreatic endotype can be found (Figure 4). Of course, increases in circulating levels of proinsulin are not confined solely to T1DM, since increased levels of proinsulin can also occur in T2DM (and in older people with T1DM) but both our own analysis[119] and that of others[120,121], indicate that the elevation in proinsulin to C-peptide ratio is particularly enhanced in young children with T1DM (classified as T1DE1). This observation supports histological evidence implying that the process of proinsulin processing is affected to a much greater extent in these patients.

When analysing proinsulin processing in children ultimately defined as having T1DE2, a particularly important finding was that these children had two different populations of pancreatic islets [119]. The first of these populations had apparently normal segregation of insulin and proinsulin within β-cells while the second population displayed aberrant insulin processing equivalent to that seen in T1DE1. More strikingly still, when pancreas samples from patients with longer duration T1DM were studied, this latter population of islets was missing, suggesting that these islets might have been targeted selectively during the autoimmune attack. The residual insulin-containing islets in these patients (which represented a much higher proportion of the total number of islets than in T1DE1, as judged by co-immunostaining with an anti-glucagon antibody) did not display aberrant proinsulin processing. Thus, differences in immune cell profiles, proinsulin processing, the proportion of residual insulin-containing islets and the extent of β-cell loss all differ between T1DE1 and T1DE2 (Table 1).

It must be acknowledged that many of these initial studies were undertaken by sampling only a proportion (sometimes a small proportion) of the islets present in each pancreas section. This limitation was an inevitable consequence of the time-consuming nature of manual assessment.. To ensure that the conclusions are fully representative of the wider islet population and with the advent of whole section scanning and software-based analysis, the data have now been expanded and identical outcomes obtained from many thousands of islets rather than a few tens to hundreds, as had been studied previously (Wyatt, Morgan & Richardson, in preparation).

Work from 2022 has revealed that islets of the youngest children, under 4 years of age, progressing to T1DM can selectively express a variant of Neuropilin 1, which renders their β-cells refractory to VEGF signalling and thereby impairs islet maturation[122]. By contrast, the β-cells of children who are older at onset can express a VEGF-responsive isoform of Neuropilin 1 and this difference has been proposed as a potential molecular basis for the development of T1DM endotypes in children. This hypothesis remains to be verified and it would be of immediate interest to establish whether the islets of children with T1DE1 express the variant form of Neuropilin 1 preferentially.

In summarizing the histological evidence that has spawned the endotype concept in T1DM, it must be accepted that the existence of endotypes has been concluded based on a relatively small number of cases. However, there is good reason for this fact because few pancreas samples taken from people with recent-onset T1DM exist worldwide. Moreover, many of these samples come from people with older-onset disease and few researchers have had the opportunity to study recent-onset disease in the pancreata of young children. This lack of available pancreatic samples can be a cause of scepticism of the endotype concept as few histopathologists have seen, at first hand, the differences in immune profile between children defined as T1DE1 versus those dubbed T1DE2. Inevitably, this can lead to questioning of the import of any differences reported and to the notion that they are likely to reflect changes in the intensity of the immune attack rather than materially different underlying mechanisms. It is also the case that some may consider that the variation between cases represents a continuum of heterogeneity rather than pointing to distinct endotypes. We do not subscribe to these views and would emphasise the

absolute separation of the proposed endotypes, T1DE1 and T1DE2, among children <8 years of age versus those >13 years old. Even if this current differentiation turns out to be an over-simplification, this fact does not exclude our firm conclusion that the pancreatic immune profiles define different disease aetiologies. Rather, we emphasize the adage that “the exception proves (tests) the rule” but, so far, no exceptions have been found.

[H2] Non-classical forms of T1DM with slow decline of β-cell function

Although T1DE2, the endotype observed in adolescent-onset and adult-onset T1DM, has a slower progression of loss of β-cell function (both before and after diagnosis) than that in very young children with T1DE1 (Figure 5, panels a and b), the severity and rate of autoimmune islet destruction vary among individuals with T1DE2. Many of these individuals develop T1DM with classical features, that is, rapid loss of beta-cell function, probably corresponding to the cluster identified as “severe autoimmune diabetes” (SAID) by Ahlqvist et al[123]. At the other extreme of the spectrum are those with SPIDDM, which has been well described in the Japanese population[124], and LADA[46], which is, by definition, diagnosed in individuals > 30 years old. We propose that SPIDDM and LADA are similar to T1DE2 as they also have especially mild islet autoimmunity compared with T1DE1. This concept] is supported by the observation that individuals with LADA or SPIDDM[124] often develop positivity to only one islet autoantigen, most commonly GADA. Compared with the aggressive attack on β-cells that occurs in classical T1DM, themild islet autoimmunity characteristic of LADA and SPIDDM takes a relatively long time to destroy β-cells to the point of causing clinical diabetes mellitus. Patients with LADA or SPIDDM initially have sufficient β-cell function

to maintain insulin independence even after diagnosis, but this phase is only transient as the disease progresses and more β-cells are lost (Figure 6). **In** contrast with SPIDDM and LADA, in classical T1DM, progression to diabetes mellitus is faster and the thresholds for clinical diabetes mellitus and insulin dependence are crossed almost simultaneously. SPIDDM and LADA have strong commonalities with classical T1DM, including similar HLA and non-HLA genetic regions, positive islet autoantibodies, increased personal and family history of other autoimmune conditions, and decline in β-cell function. However, the characteristically slow decline in β-cell function in SPIDDM and LADA leads to older age of diabetes mellitus onset or even absence of progression to clinical diabetes mellitus except in the presence of additional diabetogenic factors (Figure 6, panel C). These additional diabetogenic causes are most often a T2DM-related factor, which are, collectively, very prevalent in the general population. Indeed, although the genetic architecture of LADA is closest to that of T1DM, it has some T2DM burden[55] such as *TCF7L2* SNPs or the T2DM associated *HNF1A* locus. The phenotype that results from the combination of T1DM and T2DM pathogenic mechanisms has been given different names in the literature, including double diabetes mellitus (DDM) or type 1.5 diabetes mellitus [125] (see Box 1).

Persistent residual β-cell function, although not sustained, underlies the proposed response of LADA to non-insulin therapies such as certain thiazolidinediones[126], dipeptidylpeptidase IV inhibitors[127], disease modifying therapies such as alum-formulated recombinant GAD65 (GAD-alum)[128], and other agents (reviewed in [129]) but their respective efficacies require more detailed verification. Although it has been proposed that LADA represents a

further T1DM endotype[130], in our view, it is a particularly slowly progressive form of the T1DE2 endotype. Adding to the ongoing controversy, other investigators argue that LADA represents a mix of two subsets of individuals with either T1DM or T2DM[131].

[H2] T2DM pathogenic mechanisms in individuals with T1DE2: A double diabetes mellitus endotype?

Insulin resistance, obesity or specific T2DM genetic associations[58,76,77] can accompany not only LADA or SPIDDM but also classical T1DM [58,76,77,80,89,95,132]. Since T2DM rarely develops in prepubertal children[133], it most frequently coexists with T1DE2, which develops after age 7, as opposed to T1DE1, seen in children younger than 13 years of age (Figures 4 and 5, panels C and D). As reviewed in the section “Heterogeneity of T1DM”, insulin resistance, obesity and genes that are typically associated with T2DM can modify the progression of islet autoimmunity, accelerate the diagnosis of T1DM and modify its clinical presentation and course[134]. Furthermore, insulin resistance is an underlying pathogenic feature that can be targeted for treatment. Indeed, metformin, as an adjunct to insulin therapy, improved insulin resistance in adolescents with T1DM and obesity[135,136]. Given that endotypes are defined as disease subtypes with unique pathogenic mechanisms that could warrant specific preventive and therapeutic strategies, DDM might qualify as a T1DM endotype. An advantage of defining an endotype is that it facilitates its recognition and treatment. Moreover, in the case of DDM, this view emphasizes the interaction between underlying mechanisms (for example, influence of obesity on the initiation and progression of autoimmunity[89]). On the other hand, as opposed to the clear distinction between T1DE1 and T1DE2 at the individual level, T2DM factors

overlap and are continuous variables without sharp cutoffs. Therefore, rigid classification will continue leaving heterogeneity within and overlaps between categories[36] because the classifying factors (such as insulin resistance) are continuous variables and establishing criteria for cutoffs could be challenging. Therefore, an alternative approach is to evade further categorization of diabetes mellitus and simply evaluate, in each individual with T1DM, whether the presence and degree of T2DM mechanisms warrant specific treatment or preventive measures.

[H1] Implications

The findings we summarise here have potentially important implications for interventional immunotherapy, as illustrated when considering the efficacy of the anti-CD20 monoclonal antibody reagent, Rituximab[28,137]. Clinical trial data hint that a delay in disease onset is achieved most readily in young children, as might be predicted if $CD20^+$ B cells have a more critical role in driving β-cell destruction at younger ages, as seen in T1DE1. Similar considerations can also be extended to the anti-CD3 humanized monoclonal antibody Teplizumab[34,138] which might be predicted to be particularly effective in patients with the most intense islet inflammation, which is also a characteristic of T1DE1. Based on histologically defined endotypes, such individuals who respond to Teplizumab would be those with the greatest elevation in circulating proinsulin to C-peptide ratios. Consistent with a stratified response, participants at risk for T1DM carrying HLA-DR4-DQ8 (more common in people with T1DE1) those who did not and, conversely, those without HLA-DR3-DQ2 (more common in people with T1DE2) responded better than those carrying this haplotype[33] In contrast, GAD-alum therapy might be more effective in participants with HLA DR3-

DQ2[139]. Unfortunately, the initial results that higher insulin autoantibody titers might predict response to oral insulin[140] were not confirmed by a follow-up trial[141], while post-hoc analyses of the follow-up trial[140] suggested that elevated Diabetes Prevention Trial Risk Score (DPTRS) (a metabolic score) was associated with response to oral insulin therapy[142].

Clearly, an important goal is to link pancreatic endotypes with readily measured parameters in the circulation and, as noted, proinsulin C-peptide ratio is one such measure. Autoantibody profiles might be another and some important trends are emerging. Among these is the observation that the early development (<2 years old) of autoantibodies against insulin and IA-2 that then persist is highly correlated with the subsequent onset of diabetes mellitus within 5 years (that is, by 7 years of age)[72]. This would suggest that these children correspond to those defined by the pancreatic endotype T1DE1. Children in whom IA-2 antibodies did not persist were fewer in number and it seems premature to assign these to a specific subgroup. Nevertheless, it is of interest to note that these children developed T1DM slightly later compared with children with other autoantibody patterns, indicating that they could be included among children in whom the islet immunological profile is categorised as T1DE1 but who develop the symptoms of T1DM in the intermediate age range between 8-12 years of age. Further studies will be required to substantiate this notion. A group of children in whom stable anti-GAD antibodies persisted in the absence of antibodies to IA-2, developed T1DM at still later ages during childhood and might, then be equated more closely with T1DE2.

Superficially, these findings seem to provide a cohesive set of relationships, where autoantibody types are differentially associated with

clusters of HLA haplotypes, histopathological findings, T1D risk and ages at presentation but it is also clear that many children lie outside the assigned autoantibody groups. We conclude, therefore, that the definition of pancreatic endotypes according to autoantibody status is not (yet) a reliable index. This caveat might seem disappointing but, in our view, it should not deter the field from undertaking additional work designed to verify (or refute) the endotype concept. The “holy grail” of the endotype proposal does not lie in the achievement of an ever more complex system of disease classification but, rather, it seeks to facilitate the design of targeted (immuno)therapies, in particular immunotherapies, that are tailored mechanistically to the precise disease etiology in each person.

It has been suggested that the finding that T1DM disease proceeds more quickly in children below 7 years of age than in those who are older does not necessarily imply that different therapeutic approaches are warranted for these two groups. Researchers have expressed concerns that further subdivision of an already modestly remunerative disease area (T1DM), could lead to reduced therapeutic investment, particularly from pharmaceutical companies. However, by predicting responders to a specific agent, endotypes would improve the risk to benefit ratio of disease modifying therapies and decrease the number-needed-to-treat[143], a concept proven useful to assist in decision making.

There is also a question about the possibility that age itself is an important variable and that islet autoimmunity develops in children at a time when their immune systems (and their pancreatic islets) are undergoing radical change. In the view of some, these caveats are sufficiently important that the notion that endotypes of T1DM exist is an obfuscation driven by underlying

tissue remodelling. We do not favour this view but would argue that it is incumbent on the field to press on with the important task of defining disease etiologies in T1DM. Only when these are understood more fully, will the therapeutic relevance of the endotype concept be established.

[H1] Conclusion

From within the wide heterogeneity of T1DM, endotypes are emerging that can be used to improve prediction of T1DM development and response to preventive and therapeutic intervention. In this review, we have presented evidence of variability within T1D in aspects ranging from epidemiology to clinical presentations such as pediatric, adult-onset, slowly progressing forms (SPIDDM or LADA) or fulminant onset. Heterogeneous histopathologic, immunological and genetic features cluster into two major subgroups that, to date, are best defined on the basis of age when islet autoantibodies become measurable or clinical diabetes develops. In addition, T2DM-related pathological processes that can develop in adolescents and adults influence T1DM progression and presentation. We described the rationale for the identification of endotypes, that is, pathogenically unique disease subtypes that require specific treatment. We presented our views, based on our interpretation of the data available to date, on the conceivable existence of two immunological endotypes, T1DE1 and T1DE2. T1DE1, which appears in children younger than 13 years of age, is characterized by near-total loss of beta-cells, profuse inflammatory infiltrate of CD8+ T cells and CD20+ B cells and aberrant

proinsulin processing leading to elevated proinsulin to C-peptide ratio, all of which are markedly milder in T1DE2, observed after 7 years of age. Children between the ages of 7 and 13 seem to develop one or the other endotype. Under this framework, SPIDDM and LADA can be considered special cases of the T1DE2 endotype with particularly protracted course. Moreover, while it can be argued that the disease that results from the combination of T1DM and T2DM meets the definition of a DDM endotype, this categorization is hindered by the lack of rigid cutoffs to define the presence or absence of T2DM factors. The implications of T1DE1 and T1DE2 on prevention and treatment are beginning to be observed in the differential response to disease modifying therapies, such as rituximab, teplizumab or GAD-Alum. It must be noted that there are detractors of the concept of endotypes and that, overall, there is agreement that more data are needed to address outstanding questions.

The Precision Medicine in Diabetes Initiative supported by the American Diabetes Association and the European Association for the Study of Diabetes has engaged a large number of international experts in diabetes mellitus to conduct an extensive review of the literature on precision medicine in prediction, prevention, diagnosis and prevention in T1DM and other diabetes mellitus types. With most of the knowledge on T1DM heterogeneity stemming from post-hoc and secondary analyses, validation of findings in adequately powered, prospective studies and in clinical trials is among the most pressing issues. In addition, before the concept of endotypes can be used in clinical practice, biomarkers that identify the driving pathogenic process and monitor response to treatment must be developed and validated[144]. It is expected that

these and other efforts will bring clarity to the concept of T1DM endotypes and facilitate translation and implementation in clinical and research practices.

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Key Points

- Type 1 diabetes mellitus (T1DM) is heterogeneous; defining endotypes, or disease subtypes with unique etiopathogenesis that is amenable to intervention, will help apply precision medicine to the prediction, prevention, diagnosis and treatment of T1DM.
- T1DM endotype 1 (T1DE1) includes T1DM diagnosed in early childhood (typically <7 years of age) and characterized by extensive, early, β-cell destruction, aggressive insulitis with abundant $CD8^+$ T and $CD20^+$ B

cells, aberrant proinsulin processing and elevated circulating proinsulin-to-C-peptide ratio.

- T1DM endotype 2 (T1DE2) includes T1DM diagnosed in adolescence and characterized by retention of many residual insulin-containing islets and without insulitis, fewer infiltrating $CD8^+$ T cells, few $CD20^+$ B cells, normal proinsulin processing and lower proinsulin to C-peptide ratio compared with T1DE2.
- Evidence is emerging that T1DE1 might respond better than T1DE2 to interventional immunotherapy with agents targeted to specific immune cell subsets such as the anti-CD20 reagent, rituximab, while GAD-alum therapy might be effective for treating T1DE2.
- The T1DE2 endotype could underlie a spectrum of phenotypes with different degrees of severity of the autoimmune attack and thus, different rates of progression to insulin dependence.
- Whether T1DM endotypes exist is still a matter of debate, but data are accumulating that support this framework, which will benefit from further research to improve characterization of endotypes and test interventions directed to their underlying etiopathogenesis

Table 1. Type 1 diabetes mellitus endotypes 1 (T1DE1) and 2 (T1DE2). The third column illustrates the characteristics of double diabetes mellitus (DDM) as a combination of T1DE2 and T2DM.

Endotype	T1DE1	T1DE2	DDM
Primary genetic association	HLA DR4-DQ8	HLA DR3-DQ2	HLA DR3-DQ2
			Can have T2DM loci (e.g., *TCF7L2*)
Islet autoantibodies	IAA first (<2 years old). Then, IA2A, with or without GADA autoantibodies	GADA first. Then, with or without other autoantibodies	GADA first. Then, with or without other autoantibodies
	High percentage IAA+ and IAA titers at onset		
Other immunological findings	High percentage of islet-infiltrating $CD8^+$ T cells, high percentage of $CD20^+$ B cells	Low percentage of $CD8^+$ T cells compared with T1DE1, very low percentage of $CD20^+$ B cells compared with T1DE1	Low percentage of $CD8^+$ T cells compared with T1DE1, very low percentage of $CD20^+$ B cells compared with T1DE1
			With or without obesity-induced inflammation

β-cell abnormalities	High proinsulin to C-peptide ratio	Higher percentage of insulin containing islets compared with T1DE1	• Higher percentage of insulin containing islets compared with T1DE1
	Low percentage of insulin containing cells		With or without obesity-induced ER stress and β-cell apoptosis
	Abnormal β-cell maturation		
Systemic insulin resistance	No	No	Secondary to obesity, puberty, pregnancy and ageing
Response to immunomodulators	Yes	Less responsive than T1DE1	Less responsive than T1DE1
Other disease associations	Coeliac disease	Thyroid autoimmunity	Thyroid autoimmunity
			T2DM-associated

Figure Legends

Figure 1: Immunological heterogeneity in T1DM

Islets from two individuals with recent-onset T1DM were immunostained and imaged to display their endocrine cells (insulin: yellow; glucagon: blue) and associated lymphocytic infiltrates ($CD45^+$ cells; red). Distinct patterns are seen. **a|** Immunofluorescent image of a pancreatic sample taken from a patient with

T1DMdiagnosed in adulthood. This image reveals the retention of large numbers of β-cells with minimal lymphocytic infiltration. Most are arrayed peripherally and very few lymphocytes have migrated into the core of the islet. b| Immunofluorescent image of a pancreatic sample from a patient with T1DM diagnosed at <2 years of age. By contrast with the sample in panel a, the two islets in this sample are heavily infiltrated by lymphocytes. These lymphocytes have breached the islet capsule and many are in close proximity to β-cells, consistent with an aggressive autoimmune attack. These strikingly different patterns are typical of those seen among patients developing diabetes mellitus at different ages. [*Images courtesy of Dr Pia Leete*].

Figure 2. Influence of T2DM-related factors on T1DM development, and the effect of ethnicity

This graph shows data from the Type 1 Diabetes TrialNet study of 4,873 autoantibody-positive relatives of individuals with T1DM. Among children younger than 12 years of age, having overweight or obesity increased the risk of T1DM by 36% in non-Hispanic white (NHW) children (HR=1.36, p=0.024) while the risk was almost quadrupled in Hispanic children (HR=3.8, p=0.0026) after adjustment for confounders. Solid line, lean NHW children; dashed line, NHW children with overweight or obesity; dotted line, lean Hispanic children; dash–dotted line, Hispanic children with overweight or obesity (from Tosur et al, Diabetologia 2018)[47].

Figure 3. Geographical variation of T1DM genetic risk

The prevalence of five HLA-DR-DQ haplotypes that are associated with susceptibility or resistance to T1DM varies across geographical regions (from Redondo et al. Lancet Diabetes & Endocrinology, 2022[42])

Figure 4 Illustration of the conceptual model for the distribution of T1DM endotypes by age at onset

T1DM can develop as either T1DM endotype 1 (T1DE1, represented in orange) or endotype 2 (T1DE2, represented in yellow). T1DE1 is the predominant endotype in children diagnosed with T1DM before age 7 and its prevalence decreases sharply in individuals diagnosed at older ages. T1DE2 is the predominant type after age 7 and increases with age, becoming the predominant T1DM endotype after age 13. In the intermediate age group (7–13 years of age), some children have T1DE2 and other children have T1DE2. T2DM, which is highly prevalent in adults but also appears in younger individuals, can co-exist with T1DE2 and modify its features, resulting in double diabetes mellitus (DDM); this interaction is represented here in green.

Figure 5. Illustration of the conceptual model for the variability in trajectory of insulin secretory capacity using the T1DM endotype framework

a| In T1DE1, β-cell function declines rapidly and clinical diabetes mellitus develops in early childhood. **b|** In T1DE2, β-cell function declines more slowly and clinical diabetes mellitus develops later in life compared with T1DE1. **c|**

Latent autoimmune diabetes of adults (LADA) and Slowly progressive type 1 diabetes mellitus (SPIDDM) are special cases of T1DE2 with slower loss of β-cell function than classical T1DM. Thus, progression to clinical diabetes mellitus, if it happens, occurs late in life, such as adolescence or adult life. The presence of additional diabetogenic factors (such as insulin resistance, non-autoimmune insulin secretion defects, among others) accelerates the development of an imbalance between insulin needs and production, which causes diabetes mellitus. Therefore, the combination of T1DM and T2DM risk factors (that is, double diabetes mellitus) is common in individuals with slowly progressive islet autoimmunity who progress to diabetes mellitus. **d|** T2DM risk factors can co-exist with islet autoimmunity, accelerating the progression to clinical diabetes mellitus through insulin resistance, inflammation, ER stress and other factors. The effect of these T2DM risk factors on the preclinical progression to diabetes mellitus is more appreciable in individuals with T1DE2 than T1DE1, where the tempo of β-cell loss is much faster than the action of T2DM factors.

[bH1] Box 1: Non-classical forms of diabetes mellitus with T1DM and T2DM characteristics

[bH2] Latent autoimmune diabetes of adults (LADA): T1DM resulting from a slowly progressive attack on β-cells that requires longer time to reach the threshold for diabetes mellitus than classical T1DM (hence LADA cannot be diagnosed before >30 years) and/or requires other diabetogenic factors (such as insulin resistance and/or insufficient beta-cell function) Patients cross the threshold for diabetes mellitus but are still insulin independent (as in T2DM) for at least 6 months (by definition) until further loss of β-cells causes insulin dependence (as in classical T1DM).

[bH2] Slowly progressive insulin dependent diabetes mellitus (SPIDDM): Caused by slowly progressive autoimmune destruction of β-cells and therefore, progression to clinical diabetes mellitus takes longer than in typical T1DM, as in LADA. Clinical diabetes mellitus develops at an older age than in typical T1DM and often does so with the aid of additional T2DM-related mechanisms. Unlike LADA, the definition of SPIDDM is not confined to individuals >30 years who had at least 6 months of insulin independence.

[bH2] Double diabetes mellitus (DDM): Diabetes mellitus that results from a combination of T1DM-associated and T2DM-associated mechanisms (for example, islet autoimmunity and insulin resistance), which can develop at any age. The aggressive destruction of β-cells observed in children with T1DE1 or some individuals with T1DE2 makes the effect of T2DM pathways, which work more slowly, negligible. The milder and slower the autoimmune attack on β-

cells, the more apparent the influence of T2DM processes are. Therefore, DDM is often seen in patients with slowly progressive autoimmune diabetes mellitus such as LADA and SPIDDM[145]. Whether DDM constitutes an endotype is unclear.

[bH2] Type 1.5 diabetes mellitus: Typically refers to diabetes mellitus that initially presents as non-insulin dependent (as in T2DM) but rapidly progresses to insulin dependence and islet autoantibody positivity, resulting in a diagnosis of T1DM; and/or has elements of both T1DM (islet autoimmunity) and T2DM (such as obesity and insulin resistance). Often used as a synonym of DDM.