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# Editorial – Moving forward on the pathway of targeted immunotherapies for type 1 diabetes: the importance of disease heterogeneity

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Type 1 diabetes (T1D) is a chronic, organ-specific autoimmune disease resulting from the immune-mediated destruction of insulin-producing beta cells within the pancreatic islets, leading to lifelong dependence on exogenous insulin[1]. Although T1D has long been conceived as a disease arising from the complete loss of beta cells and subsequent absolute insulin deficiency[2], some patients with long-standing disease retain detectable levels of serum C-peptide[3-6] and exhibit the persistence of insulin-containing pancreatic islets even several decades after diagnosis[3,7-9]. These observations have therefore raised important questions, yet unanswered, about the existence of some subtypes of beta cells that are more resistant to autoimmune destruction, whether these cells are able to regenerate, and whether a decrease in the intensity of autoimmunity may occur over time[10].

Growing evidence suggests that T1D is characterized by a remarkable interindividual heterogeneity in terms of clinical and immunopathological features. Importantly, the age at diagnosis represents one of the most important variables underlying the heterogeneous rate of decline in insulin secretion among individuals with T1D. In particular, younger age at onset of T1D is often associated with lower residual beta-cell function[11,12], greater decline in stimulated C-peptide[13,14], and lower occurrence of the partial clinical remission phase ("honeymoon phase")[15,16]. Even though the exact mechanisms behind this heterogeneity are not fully understood, recent evidence suggests that variability in the extent of beta-cell destruction and residual beta-cell function may reflect, at least in part, the heterogeneity of blood and islet autoimmune response phenotypes observed among subjects with T1D. In this regard, Arif et al[17] distinguished two distinct patterns of immune infiltration within the pancreatic islets of 21 patients with T1D who died close to diagnosis: one pattern was characterized by large numbers of infiltrating immune cells, especially CD20+ B lymphocytes (also referred to as "hyperimmune CD20Hi" pattern), whereas the other pattern consisted of a relative paucity of immune cells with very low numbers of CD20+ B lymphocytes (also referred to as "pauci-immune CD20Lo" pattern). Interestingly, hyperimmune CD20Hi subjects had significantly lower mean age and fewer insulin-containing islets as a proportion of all islets identified, compared to pauci-immune CD20Lo subjects: mean age, 7.8 ± 1.7 *vs.* 13.0 ± 1.5 years, respectively ($p = 0.03$); mean proportion of insulin-containing islets 15.5 ± 4.8% *vs.* 38.3 ± 6.9%, respectively ($p = 0.02$)[17].

Thereafter, Leete et al[18] confirmed the existence of the aforementioned profiles of insulitis among different study cohorts, namely: a cohort of T1D patients who had died within 3 months of diagnosis (U.K.), a cohort from the Network for Pancreatic Organ Donors with Diabetes (US), and a cohort from the Diabetes Virus Detection (DiViD) study (Norway). Notably, all patients who received a diagnosis of T1D before the age of 7 years displayed the hyperimmune CD20Hi pattern, while all subjects who received a diagnosis beyond the age of 13 years exhibited the pauci-immune CD20Lo pattern. Furthermore, patients diagnosed with T1D at a younger age (<7 years of age) displayed a significantly lower proportion of residual insulin-containing islets compared to those who were diagnosed beyond the age of 13 years. Altogether, these findings suggest that patients diagnosed with T1D at a younger age display a more rapid and extensive beta-cell loss, as a likely consequence of the more aggressive insulitic profile (CD20Hi profile). On the other hand, the observation that patients receiving diagnosis of T1D

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in their teenage years (or beyond) exhibit a less severe insulitic profile and a higher proportion of insulin-containing islets might indicate that beta-cell dysfunction, rather than solely beta-cell loss, plays an important role in development of glucose intolerance and disease onset among those individuals[18].

In keeping with these findings, a recent survey of whole blood gene expression conducted by Dufort et al[14] revealed that newly diagnosed T1D patients exhibiting a faster decline in insulin secretion had immune phenotypes ("immunotypes") characterized by higher levels of B cell gene expression and lower levels of neutrophil gene expression. Also, B cell and neutrophil immune phenotypes showed a strong relationship with subject age, with high B cell gene expression predicting more rapid progression, although only in younger subjects (<20 years of age). This indicates that age, rate of decline in insulin secretion, and immunological characteristics may be all associated, particularly in young patients with T1D. Additionally, these findings well align with those observed by Leete et al[18], who found an increased islet B cell infiltration in younger T1D patients exhibiting more rapid beta-cell loss (as discussed above).

Overall, these data support the existence of different immune and islet pathology phenotypes in T1D, which appear to have a strong relationship with age at diagnosis and rate of decline in insulin secretion during the natural history of disease. These phenotypes may therefore account for the heterogeneity in clinical features and disease outcomes observed among individuals with T1D. Correspondingly, cell-type specific immune phenotypes in T1D could also contribute to the interindividual variability in response to immune intervention therapies. In this regard, a phase 2, randomized, double-blind study conducted on patients with newly diagnosed T1D using the anti-CD20 monoclonal antibody rituximab showed that younger individuals exhibited a better response than older individuals in terms of preservation of beta-cell function[19]. Using C-peptide and immunophenotyping data from this trial[19], Dufort et al[14] demonstrated that rituximab was most effective in delaying the decline in beta-cell function in younger T1D patients with high B cell gene expression prior to treatment.

In conclusion, preservation of beta-cell function represents a critical goal of clinical trials investigating the efficacy of disease-modifying agents for T1D[20]. Indeed, retention of endogenous insulin secretion after diagnosis of T1D has been associated with remarkable clinical benefits, including better glycemic control, lower risk of hypoglycemia, and fewer chronic complications[21,22]. In this context, clinical and immunopathological heterogeneity among individuals with T1D carries relevant implications for treatment and prevention strategies. First, early characterization of rapid disease "progressors" may allow for prioritization of treatment based on predicted severity of disease. Second, an appropriate stratification of T1D subjects according to their cell-type specific immune phenotypes (for which age at diagnosis appears to be a reliable surrogate) may be crucial for a proper interpretation of the efficacy outcomes of clinical trials investigating immune intervention therapies. Third, stratification of patients with T1D based on specific immunological features and/or age at diagnosis may facilitate the selection of targeted immunotherapeutic agents aimed to halt immune-mediated beta-cell damage. We conclude that future studies will be critical for a better comprehension of immunological markers able to accurately predict disease progression and treatment response in T1D, which may lead to the ultimate goal of targeted and personalized therapies for this challenging disease.

**Conflict of Interest**
The Authors declare that they have no conflict of interests.

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