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Review Article

Insulitis in the pathogenesis of type 1 diabetes

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Type 1 diabetes (T1D) is a chronic autoimmune disease in which autoreactive T-cells and inflammation cause severe loss of pancreatic beta cells. Insulitis, the pathologic hallmark of T1D, is an inflammatory lesion consisting of immune cell infiltrates around and within the islets. New research initiatives and methodologies are advancing our understanding of pancreas pathology. Studies have revealed the predominant cellular types that infiltrate the islets, novel molecular aspects associated with insulitis, and the coexistence of additional pathological abnormalities. While insulitis is a critical element of T1D pathology and pathogenesis, it is typically present only in a modest proportion of islets at any given time, even at diagnosis, with overall limited relation to disease duration. Thus, the relative importance of insulitis as a determining factor of diabetes symptoms at disease onset appears to have been overestimated; growing evidence also shows that beta cell loss at diagnosis is more modest than previously thought. Thus, the sole targeting of the immune system may not afford full therapeutic efficacy if dysfunction affects beta cells that are not under immune attack and this is a key contributor to symptoms. Combination therapies that promote both immunoregulation and address beta cell dysfunction should be more effective in treating this chronic disease process. It remains a major goal to clarify the relation of insulitis with the dynamics of beta cell loss and coexisting mechanisms of dysfunction, according to clinical stage; such improved understanding is key to design therapeutic strategies that target multiple pathogenic mechanisms.

Alberto Pugliese

Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL, USA

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Corresponding author: Alberto Pugliese, MD,
Diabetes Research Institute,
Department of Medicine,
Division of Diabetes, Endocrinology and Metabolism,
Department of Microbiology and Immunology
University of Miami Miller School of Medicine
1450 NW 10th Avenue,
Miami, FL 33136
USA.
Tel: (305) 243-5348;
fax: (305) 243-4404;
e-mail: apuglies@med.miami.edu

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Type 1 diabetes (T1D) is a multifactorial disease in which both genetic predisposition and environmental factors promote the triggering of autoimmune responses against pancreatic beta cells, which ultimately result in beta cell destruction and severe impairment of insulin secretion (1). Disease symptoms often manifest in children and adolescents, but patients are also diagnosed in adult age (2). Natural history studies have shown that the autoimmune process is triggered months and most often years prior to diagnosis (3). In studies of newborns from families with an affected individual or otherwise at genetic risk, the triggering of the autoimmune responses often occurs in early life, as assessed by the seroconversion for autoantibodies against islet cell autoantigens. Especially, the presence of multiple autoantibodies is a strong predictor of future T1D. Differences in the natural history are being appreciated in relation to age, and seroconversion may also occur later in life (4, 5). Thus, the clinical diagnosis represents a moment in the disease natural history at which the consequences of the autoimmune responses are severe enough that the disease becomes clinically manifest.

T1D is considered a T-cell-mediated autoimmune disease, and there are many lines of experimental, clinical, and pathological evidence supporting this view (1). The pathologic hallmark of T1D has long been considered the inflammatory lesion of the pancreatic islets, which is termed insulitis and is characterized by the presence of immune and inflammatory cells within and around the pancreatic islets (6). Insulitis is the manifestation of the autoimmune attack against beta

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cells. Although Gepts described insulitis more than 60 yr ago (7), studies of insulitis and other features of pancreas pathology in T1D have been limited by the scarce access to pancreata from T1D patients. The most extensive information about T1D pancreas pathology derives from the study of autopsy specimens obtained from patients near the time of diagnosis in the UK (8) and of biopsy specimens of living patients [the (Diabetes Virus Detection) DiViD Study from Norway, and earlier studies from Japan] (9, 10); during the last decade, the Juvenile Diabetes Research Foundation (JDRF) has supported the launch of an organized effort in the USA, (nPOD), to recover pancreas and other tissues from organ donors with T1D (11). This effort has allowed examining the pancreas with T1D throughout a much wider range of disease duration (12).

A consensus definition of the insulitis lesion has been recently reported and should help focusing research efforts in the future (13); key elements of this definition are the presence of a predominantly lymphocytic infiltration of the islets of Langerhans, consisting of at least 15 CD45+ cells/islet and present in a minimum of three islets. Insulitis can be found in the islet periphery (peri-insulitis, often showing focal aggregation at one pole of the islet and in contact with the islet periphery) or within the islet parenchyma (intra-insulitis); several studies indicate that the predominant form of insulitis in the human pancreas is peri-insulitis, and it is much less severe than in experimental mouse models (12, 14, 15). Insulitis is most often detected in islets containing insulin-positive beta cells, but critically the same pancreas will also show pseudo-atrophic islets devoid of insulin-positive cells.

Characteristics of the insulitis

Investigators have examined the relative proportions of various infiltrating cell types in the insulitis lesion and suggest that heterogeneous profiles may underlie disease severity and progression. While both T and B lymphocytes are reported in insulitis lesions, cytotoxic CD8 T-cells appear to be the predominant population and could target beta cells expressing elevated levels of Human Leucocyte Antigen (HLA) class I molecules (8, 16–18); of note, hyper-expression of class I molecules (and class II molecules) may be associated with viral infections postulated to play a key role in T1D pathogenesis (19). Hyper-expression of HLA molecules represents another key feature in the pathology of the T1D that highlights a chronic inflammatory state; this is often associated with insulitis (20). It is presently unknown whether islet-infiltrating CD8 T-cells can target viral epitopes presented by infected beta cells on their HLA class I molecules. However, studies of nPOD pancreata have demonstrated autoantigen-specific T-cells in the insulitis lesion (21); these observations directly connect those autoreactive CD8 T-cells to the insulitis and to disease pathogenesis. These studies also compared the diversity of the islet-infiltrating T-cell populations in relation to T1D duration, and reported increased diversity in the antigen specificity of the infiltrating CD8 T-cells in patients with longer disease duration. Thus, the autoimmune response appears to evolve with time, even after diagnosis; these results also provide evidence for the chronicity of the process. Two distinct patterns of insulitis were described in autopsy samples from UK patients with recent onset T1D according to the prevalence of CD20 B-cells (CD20Hi and CD20Lo) (22); these findings were replicated in the nPOD and DiViD cohorts (23); the CD20Hi profile appears associated with an early age of diagnosis (7 yr) while those diagnosed after age 13 have a CD20Lo insulitis. Thus, the presence of higher proportions of B-cells in the insulitis lesion may be a marker of early triggering of autoimmunity or of a more rapid rate of beta cell loss.

The study of nPOD pancreata has identified some key molecular changes typical of insulitis: these include the accumulation of hyaluronan, a key constituent of the extra-cellular matrix, and hyaluronan binding proteins, around islet cells and infiltrating lymphocytes in islets affected by insulitis (24). These molecules may play a role in insulitis by promoting lymphocyte adhesion and migration, and antagonizing hyaluronan deposition prevents diabetes in experimental mice (25–27). Moreover, cathepsins were found in the insulitis lesion near the areas of disruption of the peri-islet basement membrane, and may favor the penetration of lymphocytes inside the islets (28).

Insulitis in human type 1 diabetes

Overall, the proportion of islets showing insulitis in the human T1D pancreas is quite low (~10–30%); this may vary to some extent by age and disease duration. Insulitis appears more prevalent in younger patients and in those whose pancreas was examined nearer to diagnosis. For example, reports indicate that about 30% of insulin-positive islets are infiltrated in young patients who were recently diagnosed (6). In a study of biopsies performed in six patients with recent onset T1D (DiViD Study), aged 24–25 yr, the proportions of islets affected by insulitis varied significantly, ranging between 5 and 58%; of note, only one of the six patients had greater than 50% of the islets affected by insulitis, and that occurred in one of the two sections analyzed (9, 14); on average, only 11% of the islets examined had insulitis. Among 80 nPOD organ donors with variable duration of T1D, 17 had insulitis with disease duration extending from diagnosis to 12 yr, and age of onset ranging

between 4 and 28 yr. The frequency of insulitis had limited inverse correlation with diabetes duration and no correlation with age at onset, or age at demise. Insulitis predominantly affected insulin-positive islets (33% vs. only 2% of insulin-negative islets). Importantly, insulitis was observed in many patients, as well as residual beta cells, even many years after diagnosis (12).

Collectively, the available findings emphasize the chronicity of islet autoimmunity, which is present for several years after diagnosis in both children and young adults. All studies concur that insulitis does not affect all islets at the same time, suggesting that this is a process that evolves over time. Moreover, both a meta-analysis of earlier data (29, 30) and recent findings argue that the long held belief that 90% of the beta cell mass is lost at diagnosis is no longer a tenable concept. Younger children are expected to have a greater loss, but those diagnosed when teenagers or older were reported to have at least 40–60% of their islets staining positive for insulin (23, 29), including results from the UK, nPOD and DiViD cohorts. Six DiViD biopsies were performed in adult patients with recent onset T1D: insulin staining was found in 18–66% and on average in 36% of the islets examined (14). In the analysis of the 80 T1D nPOD donors, of whom only a few had disease duration less than 1 yr (12), residual beta cells were observed in all T1D donors with insulitis, who had, on average, a roughly 10-fold higher beta cell mass compared with those without insulitis; there was no correlation of beta cell mass with insulitis, disease duration and age of onset.

Thus, there appears to be some disconnect between the severe diabetes symptoms and impairment of insulin secretion at diagnosis and the emerging evidence suggesting limited insulitis and only partial physical beta cell loss. This limited pathology has also been noted in recipients of pancreas transplants who had developed T1D recurrence in their grafts (31). There is increasing evidence that inflammation and beta cell dysfunction may be important pathogenic mechanisms at the time of initial disease manifestation and contribute to cause the symptoms of severe hyperglycemia (32–36). Moreover, islet function may be recoverable (32), as shown by the DiViD study: islets isolated from the pancreas obtained via biopsy in newly diagnosed T1D recovered function in culture. This recognition has important implications for the design of therapeutic strategies to reverse diabetes at diagnosis. In fact, the low prevalence of insulitis at diagnosis suggests that even at that time, a therapy that attempts to deplete autoreactive T-cells may only be treating those islets with insulitis, and therefore therapeutic efficacy may be limited. Combinatorial therapies should therefore address pathogenic cofactors, attempt to mitigate inflammation and correct beta cell dysfunction (37).

The above findings also suggest that beta cell destruction is quite heterogeneous and is not likely to be completed until several years after diagnosis. Studies have reported persistence of insulin-positive beta cells even decades after diagnosis (38–40) and that glucose transporters continue to be expressed (21, 41). There is evidence in some patients with long disease duration that beta cells express the survivin molecule, possibly a factor in the persistence of the beta cell phenotype (42). Low levels of beta cell apoptosis have been noted in the pancreas of patients with long disease duration, implying the existence of some beta cell turnover (38–40). The above observations are usually in the context of chronic signs of islet inflammation, including insulitis and increased expression of HLA class I molecules (20).

Concordant with these observations, the assessment of functional beta cell mass through metabolic testing shows that stimulated C-peptide responses are often partially reduced in living patients (43–47), again with younger children showing more severe impairment. A prospective, postdiagnosis evaluation of C-peptide responses is being conducted in newly diagnosed patients by the T1D TrialNet; results so far have shown significant persistence of C-peptide, with about 80% of patients at 1 yr and 60% at 2 yr responding to a mixed meal with a peak C-peptide that would be suitable for randomization in a clinical trial at diagnosis; there is a slower decline in the second year after diagnosis, and not all patients experienced further decline of C-peptide secretion during the follow-up period (46). Several studies have reported that patients with decades of disease duration may secrete low amount of C-peptide and also respond with increased levels to stimulation (40, 48–50). Thus, both pathology data about insulitis and beta cell mass and clinical data suggest that T1D is due to a chronic disease process, which affects islets asynchronously continues its course over a long period of time after diagnosis.

The disease process in preclinical type 1 diabetes

There is much less information regarding the pathology of T1D during the prediabetic period. In fact, the relationships of insulitis to autoantibody conversion and beta cell destruction during the prodromic period are essentially unknown. It would be critically important to establish if insulitis is closely associated with the triggering or autoantibodies and/or circulating autoreactive T-cells; this would be of direct relevance to the design of prevention trials. Given that many individuals express autoantibodies for years prior to the eventual development of

T1D, we need to understand whether this chronicity is due to a slow destructive process affecting a minority of islets over time or if at some point critical factors act as precipitants and may trigger insulitis at a later stage. Prospective studies in relatives reveal that the triggering of islet autoimmune responses (autoantibodies) is eventually followed by the progressive impairment of insulin secretion and glucose metabolism; however, autoantibody-positive relatives may have normal metabolic measures for years, with abnormalities becoming apparent closer to diagnosis (51–53). Perhaps, the actual destruction of beta cells is a late event that is triggered nearer to diagnosis, and perhaps autoantibody positivity *per se* does not necessarily imply the presence of insulitis and beta cell loss (54). Such key questions may only be addressed if robust measures of islet inflammation, insulitis, beta cell mass, and death can be developed an applied in longitudinal studies. Addressing these questions will require a combination on laboratory and imaging approaches (55–57).

Efforts are presently ongoing to identify pancreata from general population organ donors who test positive for T1D-associated autoantibodies, to determine the relationship of autoantibody positivity to pancreas pathology and especially the presence or absence of insulitis. Such efforts are quite challenging, as the proportion of autoantibody-positive donors in the general population is very small. After pioneering studies demonstrated the feasibility of screening organ donors for autoantibodies (58, 59), the JDRF nPOD has launched a prospective, large-scale screening effort across the USA (11) So far, pancreata from 18 donors who tested positive for autoantibodies have been recovered, of whom 13 expressed a single autoantibody, typically against the glutamic acid decarboxylase autoantigen (12). Donors with a single autoantibody do not typically carry HLA genes associated with increased T1D risk and do not appear to have insulitis (12, 60). Thus, it is unclear if such donors should be considered as true prediabetic subjects, but there are increasing reports that their pancreas may show other abnormalities (11). nPOD identified five donors with multiple autoantibodies, and two had insulitis in association with high-risk HLA types (12). Parallel efforts in Europe have conducted retrospective autoantibody screening of donors whose pancreas had been used for islet cell isolation, which typically would allow pathology evaluation of a small portion of the pancreas. One such study from Belgium identified 62 autoantibody-positive donors, and insulitis was only found in 2 of the 3 donors with multiple autoantibodies, who also carried high-risk HLA types (61). Another study from Scandinavia identified 32 donors with autoantibodies, 9 of whom had multiple autoantibodies; yet none had high-risk HLA types, and no insulitis was found (62). We know that the presence of multiple autoantibodies is associated with much higher risk of progression to overt disease than positivity for a single autoantibody, and thus these findings may be related to the presence or absence of insulitis. The specificity of the autoimmune response could also be important, but the number of autoantibody-positive donors reported in the literature is too small to fully inform as whether particular autoantibody responses are closely associated with insulitis. There is a need to study more of these rare cases, as the relation between insulitis, beta cell loss, genetic predisposition, and autoantibodies is critical to understand during the prediabetic period. Addressing these questions needs to account for the genetic, environmental, and immunological heterogeneity of T1D.

Conclusions

In closing, the launch of programs aiming at the recovery and study of T1D and autoantibody-positive pancreata is leading to new discoveries about the pathology of human T1D; these findings also help identifying new therapeutic targets. Insulitis remains a key element of T1D pathology, but it needs to be recognized that it is not the only pathogenic element; this has relevance for the design of clinical trials for more effective targeting of pathogenic mechanisms. Combination therapies that promote both immunoregulation and address beta cell dysfunction should be more effective in treating this chronic disease process. Future studies will reveal more of the phenotypic features of infiltrating cells in the insulitis lesion, also in relation to other abnormalities, as well as information about gene expression and epigenetic regulation of the beta cells, which is likely to be a major contributor to their dysfunction and ultimate fate. If the field stays the course, these efforts and improved clinical assessments in natural history studies will advance our understanding of the disease pathogenesis.

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