

Insulinitis and Diabetes: A Perspective on Islet Inflammation

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Abstract

Immune cell infiltration into pancreatic islets (termed insulinitis) has been linked with destruction of pancreatic β -cells and thus with onset of diabetes mellitus. Recently published guidelines for reporting insulinitis may generate some deliberation on pancreatic islet inflammation and a re-examination of the role that immune cells play in the process of β -cell death and dysfunction. Herein, we offer the viewpoint that a mild insulinitis (e.g., 2-fold increase in leukocytic infiltration) would be sufficient to produce an adequate supply of inflammatory molecules capable of initiating and maintaining an inflammatory state within the pancreatic islets.

Recent guidelines recommend that a >2-fold increase in CD45+ immune cell infiltrates into islets be the minimum threshold for reporting insulinitis [1]. While standardization of the criteria for reporting insulinitis is an excellent idea, these new parameters may raise questions about whether a mild leukocyte infiltration (e.g., 2-fold increase) into the pancreas and targeted towards islets is responsible for reductions in β -cell mass and function. Since pro-inflammatory cytokines are a common link to both T1DM and T2DM [2-4], we propose herein that the secreted factors from the leukocytic infiltrates, in combination with the activity of the islet resident immune cells, ultimately determine the rate of decline of β -cell mass and function. Therefore, in our opinion, a 2-fold increase in insulinitis would be sufficient to promote losses in functional β -cell mass, if the immune cells present near or within islets were in a pro-inflammatory state. Thus, the quantity and array of pro-inflammatory mediators produced by the immune cell population within the islets will almost certainly produce alterations in insulin secretion, changes in β -cell mass, and the development of diabetes while the total immune cell numbers per se may or may not offer any specific indication of disease progression.

Based on the variable nature of insulinitis, several hypothetical models can be envisioned to correlate islet immune cell infiltration with pathophysiological outcomes. We envision four scenarios:

Large Quantitative Insulinitis with High Inflammatory Activity: Many immune cells detected within pancreatic islets, each producing moderate to high amounts of inflammatory mediators. This is most likely the situation observed in the female non-obese diabetic (NOD) mouse model.

Large Quantitative Insulinitis with Moderate Inflammatory Activity: Many immune cells detected within pancreatic islets but producing only low to moderate amounts of inflammatory mediators. Male (NOD) mice, which develop diabetes at a reduced frequency relative to their female counterparts, plausibly represent this situation.

Small Quantitative Insulinitis with High Inflammatory Activity: Fewer numbers of leukocytes detected within islets (e.g., >2-fold) but

capable of secreting moderate to high amounts of inflammatory mediators, perhaps producing a more sudden onset of diabetes. This scenario may explain the reported cases of fulminant diabetes, where destruction of β -cells and ensuing diabetes is rapid [5].

Small Quantitative Insulinitis with moderate inflammatory Activity: Fewer leukocytes with sustained, but moderate production of inflammatory mediators. This scenario may contribute to poor disposition index initially, but may also be adequate to produce overt diabetes over a period of many years.

We note that combinations of these possibilities may exist, such that any individual islet within the pancreas of one subject may reflect different levels of infiltration and thus the inflammatory state of a given islet may be highly variable. In addition, a variance in the sum of inflammatory mediators secreted by the discrete populations of leukocytes present within the islet is almost certainly a contributor to inflammation-associated pathologies targeting islet β -cells. This model fits with the asymmetry of β -cell destruction observed within islets of the same pancreas [6] as well as with the increasing heterogeneity observed in diabetes [7].

Neutrophils and macrophages, both capable of producing IL-1 β , are also both present in pancreas prior to and during diabetes [8-11]. Pancreatic β -cells express the IL-1RI at very high levels, some of the highest seen in any tissue, thus making them exquisitely sensitive to IL-1 β [12-14]. Consequently, even at submaximal levels of IL-1RI activation, β -cells activate inflammatory pathways, including NF- κ B, that initially re-program the cells at the transcriptional and metabolic levels followed by an eventual decline in their viability [15]. Due to the high expression of the IL-1RI, the pancreatic β -cell is sensitive to picomolar amounts of IL-1 β [13] and activation of this signaling mechanism is responsible for the expression of immunomodulatory chemokine genes [13,16,17]. A sustained release of chemokines from β -cells recruits immune cells into islets.

Experimental validation of the impact of chemokine synthesis and secretion directly from pancreatic β -cells is apparent in mice with transgenic production of CCL2 driven by the insulin promoter [18,19]. Interestingly, the insulinitis produced in these mouse models of CCL2 overexpression can be either non-destructive [19], associated with diabetes onset [18], or capable of reversing diabetes [20], depending on genetic background (and likely additional factors). If one chemokine, such as CCL2 in this case, can produce distinct outcomes associated with insulinitis, it seems likely that insulinitis per se is only an indirect readout of islet inflammation.

With the multitude of chemokines produced by β -cells after exposure to cytokines [16], the recruitment of discrete leukocyte populations and the crosstalk of these immune cells within the islets creates intriguing possibilities for dynamic regulation of β -cell function and quantity. Indeed, the heterogeneous clinical phenotypes

of T1DM and T2DM patients may be better explained by the potential variance in inflammatory mediators produced by immune cells infiltrating the islets (i.e., an inflammatory threshold) rather than by the total amount of insulinitis (i.e., how many immune cells are present within a group of islets). The latter is easier to quantify, offers important information, and therefore has been the standard approach.

The increasing degree of heterogeneity emerging across the major forms of diabetes is underscored by the diverse subgroups starting to be characterized [7]. As mentioned above, if an inflammatory threshold for islet inflammation could be established, new ideas and avenues for research leading to novel therapeutic options targeting the various forms of diabetes could emerge. As a starting point, and for the purposes of this perspective, there are some commonalities between both major forms of diabetes that will be considered:

- 1) There is measurable insulinitis in both T1DM and T2DM [4,21].
- 2) This insulinitis is variable between individuals and even between islets of the same individual ([1] and references therein).
- 3) Cytokines and chemokines are made and secreted from pancreatic β -cells [16,22-25].

We propose that the sum of the secreted products (cytokines, chemokines, inflammatory lipids such as prostaglandins, etc.) from the leukocyte population within islets, as well as directly from β -cells, are quantitatively more important for the losses in functional β -cell mass during progression to diabetes than the total given number of immune cells within a particular islet. As an example of the importance of secreted products, we note that diabetes is a disease that arises due to inflammation-associated dysfunction in multiple tissues, including adipocytes, liver, muscle, and pancreatic β -cells. TNF- α production increases during obesity and leads to both hepatic [26] and skeletal muscle insulin resistance [27]. TNF- α also has detrimental effects on pancreatic β -cells [28] yet anti-TNF- α therapy has had limited success when applied to diabetes [29].

Similarly, anti-IL-1 therapy in individuals with impaired glucose tolerance or overt diabetes reveals an improvement in β -cell secretory function, but does not appear to alleviate peripheral insulin resistance [30,31]. While improving β -cell function initially seems positive, it could lead to faster β -cell "burnout" (i.e., increased turnover due to metabolic overload). Moreover, additional cytokines (e.g., IFN- γ) often potentiate the TNF- α and IL-1 β -mediated losses in both insulin secretion and cytotoxicity [28, 32-34]. We therefore speculate that a failure of individual immunomodulatory approaches to treat diabetes is likely due to the vast array of immune cells capable of infiltrating islets [10] coupled with the multiplicity of inflammatory mediators secreted by such cells [35]. This combination of inflammatory events overwhelms any single intervention strategy and helps to explain why a 2-fold (or greater) increase in CD45+ infiltrates into islets can lead to pathophysiological outcomes.

As proof of concept, higher rates of glycolytic metabolism within an activated macrophage correlate with increased production of IL-1 β [36], meaning that the same number of macrophages, if exposed to an activating signal (e.g. lipopolysaccharide, saturated fats, etc.), will be secreting more cytokines. Since there are enough resident macrophages present within islets to produce inflammatory amounts of IL-1 β [32], it is plausible that very few additional infiltrating leukocytes are needed to initiate or maintain islet inflammation. Furthermore, a slow but quantitatively small influx of immune cells maintained by constant or pulsatile chemokine release from β -cells

may sustain a feed-forward auto-inflammatory response that drives chronic islet inflammation. At the same time, gradually rising blood glucose concentrations due to peripheral insulin resistance, coupled with production of cytokines (and chemokines) directly from the β -cell, would continue to be contributing factors to the islet inflammation. Thus, enhanced secretion of IL-1 β from highly glycolytic macrophages, coupled with glucose-induced production of IL-1 β within pancreatic β -cells [37], together with the large abundance of IL-1RI on the β -cell surface, exposes a situation sufficient to incite islet inflammation with a quantitatively small amount of insulinitis.

Finally, the timing of leukocytic infiltration into islets is almost certainly critical to any inflammation-associated decrease in functional β -cell mass. If β -cells are secreting soluble factors, such as chemokines, that promote leukocytic infiltration, then insulinitis prior to diabetes onset is probably driven by a slow, but steady, infiltration into islets as a reaction to the systemic availability of chemokines (released from β -cells). Prior to diabetes, there are numerous β -cells present, which are capable of the synthesis and secretion of such chemoattractant molecules. Release of many different chemokines into circulation could also prime multiple leukocyte populations for inflammatory actions, in addition to promoting their migration to islets. Conversely, once diabetes presents clinically, indicating that β -cell numbers are sufficiently diminished (e.g., 60-70% reduction) to allow blood glucose levels to rise, insulinitis should, in theory, start to decline. If true, this would explain why the pancreatic samples from rodents and humans analyzed thus far display little to no insulinitis once the insulin-positive β -cells are eliminated [38,39]. On the other hand, infiltrating immune cells that are largely inert or even anti-inflammatory would not be detrimental and there is the possibility that a population of leukocytes exist which may actually promote β -cell growth, proliferation, or regeneration [40]. How much of a back and forth between pro- and anti-inflammatory responses actually goes on within the islets is unclear at the present time.

In summary, the immunopathology contributions to insulinitis have thus far been carried out mostly on tissues of individuals already diagnosed with diabetes. While this data has been extremely valuable, the question of what happens prior to clinical presentation of diabetes has been difficult to address. Nevertheless, with the ongoing development of tools that allow for non-invasive approaches to quantify β cell death prior to diabetes onset [41], coupled with newly emerging imaging procedures [42], fresh approaches and insights will soon become a reality. The capability of addressing the relative contributions of total numbers of immune cells versus how active those immune cells are in terms of producing pro- (and anti-)inflammatory molecules will provide a greater understanding of immune-cell mediated destruction of β -cells. Understanding the more complicated molecular nuances associated with islet inflammation may also help to explain the variable nature of clinical presentation of T1DM and T2DM and hopefully provide new therapeutic approaches.

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