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Insulitis and Diabetes: A Perspective on Islet Inflammation

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Abstract

Immune cell infiltration into pancreatic islets (termed insulitis) has been linked with destruction of pancreatic β-cells and thus with onset of diabetes mellitus. Recently published guidelines for reporting insulitis 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 insulitis (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 insulitis [1]. While standardization of the criteria for reporting insulitis 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 insulitis 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 insulitis, several hypothetical models can be envisioned to correlate islet immune cell infiltration with pathophysiological outcomes. We envision four scenarios:

Large Quantitative Insulitis 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 Insulitis 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 Insulitis 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 Insulitis 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-1β, 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-1β [12-14]. Consequently, even at submaximal levels of IL-1RI activation, β-cells activate inflammatory pathways, including NF-κB, 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-1β [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 insulitis 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 insulitis, it seems likely that insulitis 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 insulitis (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 insulitis in both T1DM and T2DM [4,21].

2)	This insulitis 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-1β-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-1β [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-1β [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-1β from highly glycolytic macrophages, coupled with glucose-induced production of IL-1β 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 insulitis.

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 insulitis 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, insulitis 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 insulitis 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 insulitis 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.

References

1.	Campbell-Thompson ML, Atkinson MA, Butler AE, Chapman NM, Frisk G, et al. (2013) The diagnosis of insulitis in human type 1 diabetes. Diabetologia 56: 2541-2543.

2.	Donath MY, Böni-Schnetzler M, Ellingsgaard H, Ehses JA (2009) Islet inflammation impairs the pancreatic beta-cell in type 2 diabetes. Physiology (Bethesda) 24: 325-331.

3.	Padgett LE, Broniowska KA, Hansen PA, Corbett JA, Tse HM (2013) The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis. Ann NY Acad Sci 1281:16-35.

4. Böni-Schnetzler M, Ehses JA, Faulenbach M, Donath MY (2008) Insulitis in type 2 diabetes. Diabetes Obes Metab 4: 201-204.

5. Tanaka S, Kobayashi T, Momotsu T (2000) A novel subtype of type 1 diabetes mellitus. N Engl J Med 342: 1835-1837.

6. Gepts W (1965) Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 14: 619-633.

7. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, et al. (2014) The many faces of diabetes: a disease with increasing heterogeneity. Lancet 383: 1084-1094.

8. Battaglia M (2014) Neutrophils and type 1 autoimmune diabetes. Curr Opin Hematol 21: 8-15.

9. Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA (2007) Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 56: 2356-2370.

10. Lehuen A, Diana J, Zaccone P, Cooke A (2010) Immune cell crosstalk in type 1 diabetes. Nat Rev Immunol 10: 501- 513.

11. Valle A, Giamporcaro GM, Scavini M, Stabilini A, Grogan P, et al. (2013) Reduction of circulating neutrophils precedes and accompanies type 1 diabetes. Diabetes 62: 2072-2077.

12. Böni-Schnetzler M, Boller S, Debray S, Bouzakri K, Meier DT, et al. (2009) Free fatty acids induce a proinflammatory response in islets via the abundantly expressed interleukin-1 receptor I. Endocrinology 150: 5218-5229.

13. Burke SJ, Lu D, Sparer TE, Masi T, Goff MR, et al. (2014) NF-kappaB and STAT1 control CXCL1 and CXCL2 gene transcription. Am J Physiol Endocrinol Metab 306: E131-149.

14. Scarim AL, Arnush M, Hill JR, Marshall CA, Baldwin A, et al. (1997) Evidence for the presence of type I IL-1 receptors on beta-cells of islets of Langerhans. Biochim Biophys Acta 1361: 313-320.

15. Collier JJ, Burke SJ, Eisenhauer ME, Lu D, Sapp RC, et al. (2011) Pancreatic beta-Cell Death in Response to Pro-Inflammatory Cytokines Is Distinct from Genuine Apoptosis. PLoS One 6 e22485.

16. Sarkar SA, Lee CE, Victorino F, Nguyen TT, Walters JA, et al. (2012) Expression and regulation of chemokines in murine and human type 1 diabetes. Diabetes 61: 436-446.

17. Burke SJ, Goff MR, Lu D, Proud D, Karlstad MD, et al. (2013) Synergistic Expression of the CXCL10 Gene in Response to IL-1beta and IFN-gamma Involves NF-kappaB, Phosphorylation of STAT1 at Tyr701, and Acetylation of Histones H3 and H4. J Immunol 191: 323-336.

18. Martin AP, Rankin S, Pitchford S, Charo IF, Furtado GC, et al. (2008) Increased expression of CCL2 in insulin-producing cells of transgenic mice promotes mobilization of myeloid cells from the bone marrow, marked insulitis, and diabetes. Diabetes 57: 3025-3033.

19. Grewal IS, Rutledge BJ, Fiorillo JA, Gu L, Gladue RP, et al. (1997) Transgenic monocyte chemoattractant protein-1 (MCP-1) in pancreatic islets produces monocyte-rich insulitis without diabetes: abrogation by a second transgene expressing systemic MCP-1. J Immunol 159: 401-408.

20. Kriegel MA, Rathinam C, Flavell RA (2012) Pancreatic islet expression of chemokine CCL2 suppresses autoimmune diabetes via tolerogenic CD11c+ CD11b+ dendritic cells. Proc Natl Acad Sci U S A, 109: 3457-3462.

21. Atkinson MA, Eisenbarth GS, Michels AW (2014) Type 1 diabetes. Lancet 383: 69-82.

22. Donath MY, Böni-Schnetzler M, Ellingsgaard H, Halban PA, Ehses JA (2010) Cytokine production by islets in health and diabetes: cellular origin, regulation and function. Trends Endocrinol Metab 21: 261-267.

23. Frigerio S, Junt T, Lu B, Gerard C, Zumsteg U, et al. (2002) Beta cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. Nat Med 8: 1414-1420.

24. Corbett JA, McDaniel ML (1995) Intraislet release of interleukin 1 inhibits beta cell function by inducing beta cell expression of inducible nitric oxide synthase. J Exp Med 181: 559-568.

25. Arnush M, Heitmeier MR, Scarim AL, Marino MH, Manning PT (1998) IL-1 produced and released endogenously within human islets inhibits beta cell function. J Clin Invest 102: 516-526.

26. Cheung AT, Wang J, Ree D, Kolls JK, Bryer-Ash M (2000) Tumor necrosis factor-alpha induces hepatic insulin resistance in obese Zucker (fa/fa) rats via interaction of leukocyte antigen-related tyrosine phosphatase with focal adhesion kinase. Diabetes 49: 810-819.

27. Gregor MF, Hotamisligil GS (2011) Inflammatory mechanisms in obesity. Annu Rev Immunol 29: 415-445.

28. Pukel C, Baquerizo H, Rabinovitch A (1988) Destruction of rat islet cell monolayers by cytokines. Synergistic interactions of interferon-gamma, tumor necrosis factor, lymphotoxin, and interleukin 1. Diabetes 37: 133-136.

29. [Mastrandrea L, Yu J, Behrens T, Buchlis J, Albini C Quattrin, et al. (2009) Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. Diabetes Care 32: 1244-1249.

30. Larsen CM, Faulenbach M, Vaag A, Vølund A, Ehses JA, et al. (2007) Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 356: 1517-1526.

31. van Asseldonk EJ, Stienstra R, Koenen TB, Joosten LA, Netea MG, et al. (2011) Treatment with Anakinra improves disposition index but not insulin sensitivity in nondiabetic subjects with the metabolic syndrome: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab, 96: 2119-2126.

32. Heitmeier MR, Scarim AL, Corbett JA, (1997) Interferon-gamma increases the sensitivity of islets of Langerhans for inducible nitric-oxide synthase expression induced by interleukin 1. J Biol Chem 272: 13697-13704.

33. Collier JJ, Fueger PT, Hohmeier HE, Newgard CB (2006) Pro- and antiapoptotic proteins regulate apoptosis but do not protect against cytokine-mediated cytotoxicity in rat islets and beta-cell lines. Diabetes 55: 1398-1406.

34. Mandrup-Poulsen T, Bendtzen K, Dinarello CA, Nerup J (1987) Human tumor necrosis factor potentiates human interleukin 1-mediated rat pancreatic beta-cell cytotoxicity. J Immunol 139: 4077-4082.

35. Nathan C (2002) Points of control in inflammation. Nature 420: 846-852.

36. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF (2013) Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature 496: 238-242.

37. Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, et al. (2002) Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. J Clin Invest 110: 851-860.

38. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG (2009) Analysis of islet inflammation in human type 1 diabetes. Clin Exp Immunol 155: 173-181.

39. Novikova L, Smirnova IV, Rawal S, Dotson AL, Benedict SH, et al. (2013) Variations in rodent models of type 1 diabetes: islet morphology. Journal of diabetes research 965832.

40. Dirice E, Kahraman S, Jiang W, El Ouaamari A, De Jesus DF (2014) Soluble factors secreted by T cells promote beta-cell proliferation. Diabetes 63: 188-202.

41. EM Akirav, JLebastchi, Galvan EM, Henegariu O, Akirav M, et al. (2011) Detection of beta cell death in diabetes using differentially methylated circulating DNA. Proc Natl Acad Sci U S A 108: 19018-19023.

42. Lebastchi J, Herold KC (2012) Immunologic and metabolic biomarkers of beta-cell destruction in the diagnosis of type 1 diabetes. Cold Spring Harb Perspect Med 2: a007708.