Modulation of Leukocytes of the Innate Arm of the Immune System as a Potential Approach to Prevent the Onset and Progression of Type 1 Diabetes

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Diabetes 2021;70:313–322 | https://doi.org/10.2337/dbi20-0026

Type 1 diabetes (T1D) is characterized by insulin deficiency resulting from the selective destruction of pancreatic β-cells by self-reactive T cells. Recent evidence demonstrates that innate immune responses substantially contribute to the pathogenesis of T1D, as they represent a first line of response to danger/damage signals. Here we discuss evidence on how, in a relapsing-remitting pattern, pancreas remodeling, diet, microbiota, gut permeability, and viral/bacterial infections induce the accumulation of leukocytes of the innate arm of the immune system throughout the pancreas. The subsequent acquisition and presentation of endocrine and exocrine antigens to the adaptive arm of the immune system results in a chronic progression of pancreatic damage. This process provides for the generation of self-reactive T-cell responses; however, the relative weight that genetic and environmental factors have on the etiopathogenesis of T1D is endotype imprinted and patient specific. With this Perspectives in Diabetes, our goal is to encourage the scientific community to rethink mechanisms underlying T1D pathogenesis and to consider therapeutic approaches that focus on these processes in intervention trials within new-onset disease as well as in efforts seeking the disorder's prevention in individuals at high risk.

Type 1 diabetes (T1D) results from a selective loss of insulin-producing pancreatic β-cells (1). T1D has long been considered solely the result of autoimmunity, but increasing evidence supports a role for potentially the entire pancreas as influencing the disorder's pathogenesis (2–4). While the precise interactions between genetic susceptibilities and environmental factors contributing to T1D remain to be fully elucidated, both appear to influence the natural history of the disease, albeit with variances in both depending on the geographic population subject to analysis (5). An improved understanding of these complexities in pathogenesis is vital for the development of an effective means to cure the disease and, ultimately, see its prevention.

The long-held notion of an autoimmune-driven pathogenesis of T1D has inspired decades of investigations phenotyping both adaptive and innate immune responses. Most predominant have been efforts evaluating cells of the T- and B-cell linage, yet a variety of non-T-cell immune aspects have also been associated with T1D including myeloid and plasmacytoid dendritic cells (DC), natural killer cells, and neutrophils. Unfortunately, achieving "consensus" view on disease pathogenesis has historically been difficult regarding which cell population(s) are key, how they interact, and whether assessments in peripheral blood are reflective of activity within the pancreas. This is likely, at least in part, due to variations in study cohorts (e.g., geographic region, age at onset, disease duration, methodological differences in assessment, phenotypic markers utilized) as well as the emerging notion of disease "endotypes" (5). One can think of T1D endotypes as a dynamically changing intersection of causal and manifestational biological, genetic, and epigenetic variables that define and inform a patient-specific entity of a clinical syndrome. We propose that the progression of inflammation, first by

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Received 10 August 2020 and accepted 15 November 2020

leukocytes of the innate arm of the immune system, is a function of endotypes that affect not only the molecular and anatomic development of the postnatal pancreas but also the characteristics of the innate leukocytes and their range of potential responses to aberrations in postnatal pancreatic development. This situation could be influenced by a number of aggravating factors including but not limited to pathology-driving changes in gut microbiota, gut permeability, and the sequelae of infections by, and responses to, infectious pathogens. These, however, are not necessarily the only possible aggravating factors to an already "inborn" endotype-imprinted aberration of postnatal pancreas remodeling that stimulates an inflammatory response by leukocytes of the innate arm of the immune system, which we, herein, propose as a major driver in the etiopathogenesis of T1D. In this article, we limit our regarding of aggravating factors to the microbiome, gut permeability, and infectious pathogens, but are fully cognizant that these are not the only possible aggravating factors to T1D endotypes.

While the contributions of adaptive immunity to T1D development are well appreciated, increasing attention has recently been given to innate immunity as well as the potential role of the nonendocrine component of the pancreas, which, surprising to the modern researcher, was suggested in the 1940s, only to be recently resurrected by emerging data (4). Independent of autoimmunity, innate immune cells are among the earliest responders to infection, neoplastic transformation, or cellular stress. In settings of T1D, alterations in innate immune cell numbers (reduced or increased), function, receptor expression, and responsiveness have all been noted. A closer examination of these features, as well as their potential contributions toward the pathogenesis of T1D, forms the subject of this Perspectives in Diabetes.

DC and Macrophages Are the Sentinels, Guardians, and Nurturers of a Physiologic Pancreas

Like all organs, the pancreas is endowed with tissue-resident DC and macrophages (6). Tissue-resident antigen-presenting cells (APC) generally act as sentinels for "danger" and "damage," enabling a trophic environment and repair processes aimed at maintaining homeostasis (7). With respect to T1D, much of our knowledge has been derived from studies of T1D animal models. For example, in 2- to 3-week-old nonobese diabetic (NOD) mice, the predominant rodent model for T1D, postnatal pancreas DC and macrophages exhibit a state of activation (6). Table 1 lists the phenotypes of these cells and their currently understood immunobiology. Unfortunately, it remains unknown whether there are similar cell populations and events in postnatal humans with high genetic risk for T1D.

DC enable seeding of insulin-reactive $CD4^+$ T cells in 3-week-old NOD mouse pancreas. Indeed, Unanue and colleagues (8) elegantly demonstrated that T cells from Batf3-deficient mice are fully competent but unable to initiate diabetogenesis in the absence of $Batf3^+$ $CD103^+$ DC. Importantly, T cells from NOD.Batf3-deficent mice transferred diabetes into NOD.Rag1-deficient mice (8). Although it is not clear how $CD103^+$ DC prime β-cell-reactive T cells (i.e., inside the islets, peri-islet, intrapancreatic, or pancreatic lymph node T-cell priming), accumulating evidence indicates that these DC present islet antigens in a class II MHC-dependent manner to T cells following the uptake of secretory granules (termed "crinosomes") containing insulin and possibly other autoantigens from islet β-cells to which the $CD103^+$ DC are physically coupled (9).

Given the large body of evidence supporting DC as orchestrating the β-cell reactivity in the onset of T1D autoimmunity, it was somewhat surprising that islet-resident macrophages (10) were of equipotent relevance in the process. Distinct islet-resident macrophages (Table 1) with unique gene signatures throughout T1D development (11) include rare cells with filopodia extending into the microvascular circulation, allowing for uptake of insulin-containing crinosomes. In the absence of a danger/damage signal, the role of these DC and macrophages was thought to be homeostatic, noninflammatory, and possibly trophic to the maturing endocrine pancreatic regions and their substrata. Under these conditions, such cells are in a resting state, but a danger/damage signal could condition a phenotypic shift toward immunologic alarm.

Beyond the obvious potential role of pathogens causing such a shift, we believe it is important to revisit a concept, first proposed by Trudeau and Finegold some 20 years ago (12), that an aberration in the natural process of early postnatal physical remodeling of the pancreas might act as the trigger of T1D autoimmunity. Of course, pathogen incursion and aberrant postnatal remodeling need not be mutually exclusive (see WHY DO INNATE CELLS ACCUMULATE INSIDE THE PANCREAS?). It will be important for future studies to test the hypothesis of whether tissue maturation and remodeling occurs under $Batf3^+$ $CD103^+$ DC- and macrophage-selective depletion. Indeed, it is also possible that homeostatic pancreatic remodeling is not restricted to the islets and peri-islet architecture but, rather, also extends to the nonendocrine component.

Neutrophils Respond to Intrapancreatic Damage and Danger

The role of neutrophils in autoimmune diseases, including T1D, has gained renewed interest in recent years as reports have highlighted their large phenotypic and functional heterogeneity. Circulating neutrophil numbers are reduced in presymptomatic stages of T1D and are associated with worsening β-cell function (13). In the absence of evidence for impaired neutrophil output from the bone marrow or increased peripheral destruction mediated by anti-neutrophil antibodies, these findings corroborate the hypothesis of neutrophil sequestration at the pancreatic level. Neutrophils have been detected in the pancreas of donors with T1D but not donors without diabetes (14). Interestingly, in autoantibody-positive donors, neutrophils have been

Table 1—Leukocytes observed inside the pancreas prior to T-cell accumulation

Leukocytes	Specific for T1D but not T2D	Activated	Localized in the pancreatic islets	Localized in the exocrine pancreas	Elevated in subjects at risk for T1D
Macrophages		X	X		
DC	X	X	X		
Neutrophils	X	X		X	X
Natural killer cells	X	X	X	X	

found primarily in the exocrine pancreas and not within islets (15), supporting the notion that the exocrine pancreas tissue is affected and potentially contributes to T1D pathogenesis. Indeed, pancreas weight and volume appear to be reduced even in presymptomatic stages of the disease (2,3) alongside of impaired pancreatic exocrine function (16). A comprehensive mapping of immune cell localization using imaging mass cytometry in pancreas from patients with T1D showed that neutrophils are more abundant in the exocrine compartment than in islets and accumulate at a later disease stage (17). It may be possible that neutrophil infiltration in the pancreas has an intermittent course, with peaks starting long before the clinical onset and persisting until the later stages of the disease. Neutrophils engage in complex interactions with other tissue-resident and infiltrating immune cells. In NOD mice, β-cell death–induced recruitment and activation of neutrophils and plasmacytoid DC occur as early as 3–4 weeks of age (18). Of note, neutrophils have been shown to exhibit APC activity, being able to present antigen in class II MHC and to provide costimulation to naïve and memory, as well as autoreactive, T cells (19). Accordingly, early neutrophil depletion reduced the diabetogenic T-cell response and inhibited T1D development in NOD mice (18). Furthermore, with use of this animal model, cross talk between macrophages and β-cells was found to be responsible for neutrophil infiltration in the pancreas during the initiation phase of autoimmune diabetes (20). These data reveal that cross talk between innate immune cells takes place in the pancreas long before T1D onset and is required for initiation of the disease. Taken together, the evidence strongly indicates that neutrophils preferentially localize in the human exocrine pancreas in the presymptomatic phase of T1D, thus preceding autoreactive T-cell accumulation.

Why Do Innate Cells Accumulate Inside the Pancreas?

It stands to reason that intrapancreatic DC, macrophages, migratory neutrophils, and possibly B-1a cells will not, on their own, activate into a proinflammatory state absent a danger/damage trigger, the most obvious being a pathogenic microbial/microbiome trigger, or conceivably aberrant tissue remodeling (see below). Individuals with pre-T1D exhibit altered gut permeability (21), and autoantibody-positive subjects who progressed to overt T1D display higher gut permeability than nonprogressors (22). A leaky gut barrier induces systemic inflammation by allowing translocation of microbes and their products into the circulation, and this has been associated with the formation of neutrophil extracellular trap (NET) (23), suggesting a tight link between gut inflammation and neutrophil activation. Translocation of bacteria and their molecular components may influence organ-specific autoimmunity by fostering the differentiation of proinflammatory APC and the generation of antigen-specific effectors. While the exact processes by which immunity is activated against an initiating event outside the draining lymphoid organs may not yet be fully clear, our model—in addition to fitting well inside the "danger/damage" hypothesis—can also fit quite well inside the cellular frustration algorithm conceived by de Abreu and Mostardinha (24) as well as the associative recognition models presented by Bretscher and Cohn (rev. in 25). However, while these two other models can explain the events that result in the peripheral activation of autoreactive leukocytes of the adaptive arm of the immune system, they do not provide any mechanistic basis to answer the question of why, absent any evident initiating event, innate leukocytes are activated en route to, and accumulate inside, anatomically distinct regions of specific tissues and organs that eventually become physical targets of autoreactive effector leukocytes of the adaptive arm. At this time, a reconciliation of the "danger" model with those of de Abreu and Bretscher and Cohn is beyond the scope of this Perspectives in Diabetes, even though the "danger" model adequately and elegantly offers a rational basis for our model.

Twenty years ago, Finegood and colleagues (12) reported that NOD mice and BioBreeding (BB) rats, which both spontaneously develop progressive β-cell-targeting autoimmune diabetes, exhibit a significant wave of developmental β-cell apoptosis in the pancreata, peaking at 2 weeks of age, that is nonpathologic per se. In the absence of autoreactive lymphocytes, such remodeling—even if it exposed damage-associated molecular patterns (DAMPs)—would not be expected to provoke anything other than a tissue-specific, self-regulating, and transient inflammation. On a background of autoreactive pancreas antigen-selective lymphocytes, such a process would instead activate pancreas-resident APC (macrophages and/or DC) (26) resulting in the activation of autoreactive T and B lymphocytes. Hence, we propose a model (Fig. 1) that is not exclusive of any of the possible microbial

pathogen-triggered mechanisms and that can accommodate the known time at onset of the initiation of β-cell-directed autoimmunity in NOD mice and BB rats (15 days of age) (1).

T1D As a Heterogeneous, Complex, and Possibly "Patient-Specific" Pancreatic Condition

Inflammation in human T1D pancreata not only is limited to an insulitis but also affects nonendocrine tissue (2–4). A growing body of evidence indicates that this is also observed in NOD mice (18,20), though it remains unknown whether nonendocrine pancreatic inflammation persists past 5–6 weeks of age (18,20) and whether it is part of a continuous/sustained or relapsing-remitting inflammation, in line with the growing observations in human pancreata. This would compel a significant revision of consensus on how T1D evolves and the role of nonendocrine tissue as a potential participant and target in the overall process, further strengthening the argument that T1D may not be a condition with a limited number of etiopathogenic variables that distinctly affect islets of Langerhans and β-cells (5).

The high demands of energy generation required by endocrine and exocrine cells to sense circulating nutrients and produce and secrete regulatory hormones and enzymes can result in immune cell activating signals, from excess free radicals and extracellular ATP, to the unfolded protein response. One can think of this as an endotype variable that can constitute a danger/damage trigger for innate leukocytes, the trigger of pancreatic secretory cell dysfunction and/or loss. Indeed, accumulating data indicate that prior to T1D onset, exocrine damage is present and that immune cell infiltration can be more abundant in the exocrine compartment, rather than peri-/intraislet, in some individuals (2,13,15). This infiltration induces not an acute pancreatitis but, rather, a sustained chronic pancreatopathy ending up in a subclinical exocrine dysfunction. The following is still unknown: *1*) whether this damage occurs prior to or after β-cell death and *2*) whether exocrine pancreas dysfunction occurs in all patients progressing to diabetes or contributes to the mechanism underlying T1D pathogenesis in a subgroup of patients with a specific disease "endotype" (see below: ARE THERE DIFFERENT KINDS OF T1D?) (5).

Studies in pancreata from human T1D organ donors have confirmed that islet β-cell mass is not substantially infiltrated by lymphocytes at the time of clinical onset. Unexpectedly, significant residual β-cell mass exists, even in the absence of insulitis, in individuals with long-standing established disease (>5 years) (2,17). Pharmacologic insulin replacement, diet, and lifestyle most certainly are expected to affect inflammation, directly and indirectly.

When considered together, these observations also compel a reassessment of what is, and what could, underlie and control the "honeymoon period" (27) and whether there are certain endotypes that confer a favorable honeymoon period outcome (i.e., extension). By the time this apparently favorable event is observed, there is no question that islet cell–selective lymphocytes are the main drivers of β-cell impairment and damage (27). However, it is unknown whether the damage is restricted to islets or extends to nonendocrine cells and whether it is a singular event that leads to an inexorable outcome or a relapsing-remitting process. Furthermore, it is unknown to what extent intrapancreatic leukocytes of the innate arm license and maintain the detrimental lymphocytes, and it is also unknown whether and how leukocytes like DC, macrophages, and neutrophils of an inherent, or acquired, tolerogenic ability can maintain some balance in favor of restraining the autoreactive effectors either directly or indirectly (e.g., via regulatory lymphocyte populations) during this period. If any of these mechanisms imprint the length of the honeymoon period, it stands to reason that modifying the activity of cells of the innate arm (as well as processes that underlie pathologic nonimmune anatomic and physiologic changes of the pancreas) offers a unique opportunity and a novel approach to delay the time to pharmacologic insulin requirement. The resulting improved preservation of a larger mass of β-cells, permitting a longer time for natural production of insulin, could also likely delay the mechanisms underlying the onset of diabetes complications (28). One could envisage approaches that restrain the accumulation of proinflammatory neutrophils into the pancreas or methods to shift the phenotypes and activities of intrapancreatic innate leukocytes in favor of "regulatory" capabilities (e.g., M2 macrophages, N2 neutrophils, naturally tolerogenic DC). We predict that the endotype, genetically influenced as well as environmentally conditioned, will also determine the degree to which such cells can acquire and retain these beneficial features as well as any remodeling response and process of the damaged endocrine and nonendocrine pancreas. The honeymoon period and the mechanisms that govern its persistence, in these contexts, have never been explored and represent an exciting area for discovery of mechanisms that can better inform how we view the progression of T1D as well as new therapeutic targets that can restrain and limit the process at the honeymoon period.

Are There Different Kinds of T1D?

In the mid-1970s, when T1D was initially classified as an autoimmune disease, and for most of the years since, the vast majority of clinical research efforts approached questions of pathogenesis with the view that at least in childhood, adolescence, and early adulthood, the disorder largely represented a singular disease. In the time since, we have learned that many other forms of diabetes can occur in this age range that were once diagnosed as T1D. As noted above, there is an emerging view that apart from these nonautoimmune forms of diabetes, T1D itself involves different endotypes (5). While the details are still subject to debate and no endotypes have been officially adopted by an authoritative organizational body, aspects such as age at onset (<7 years, 7–15 years, >15 years),

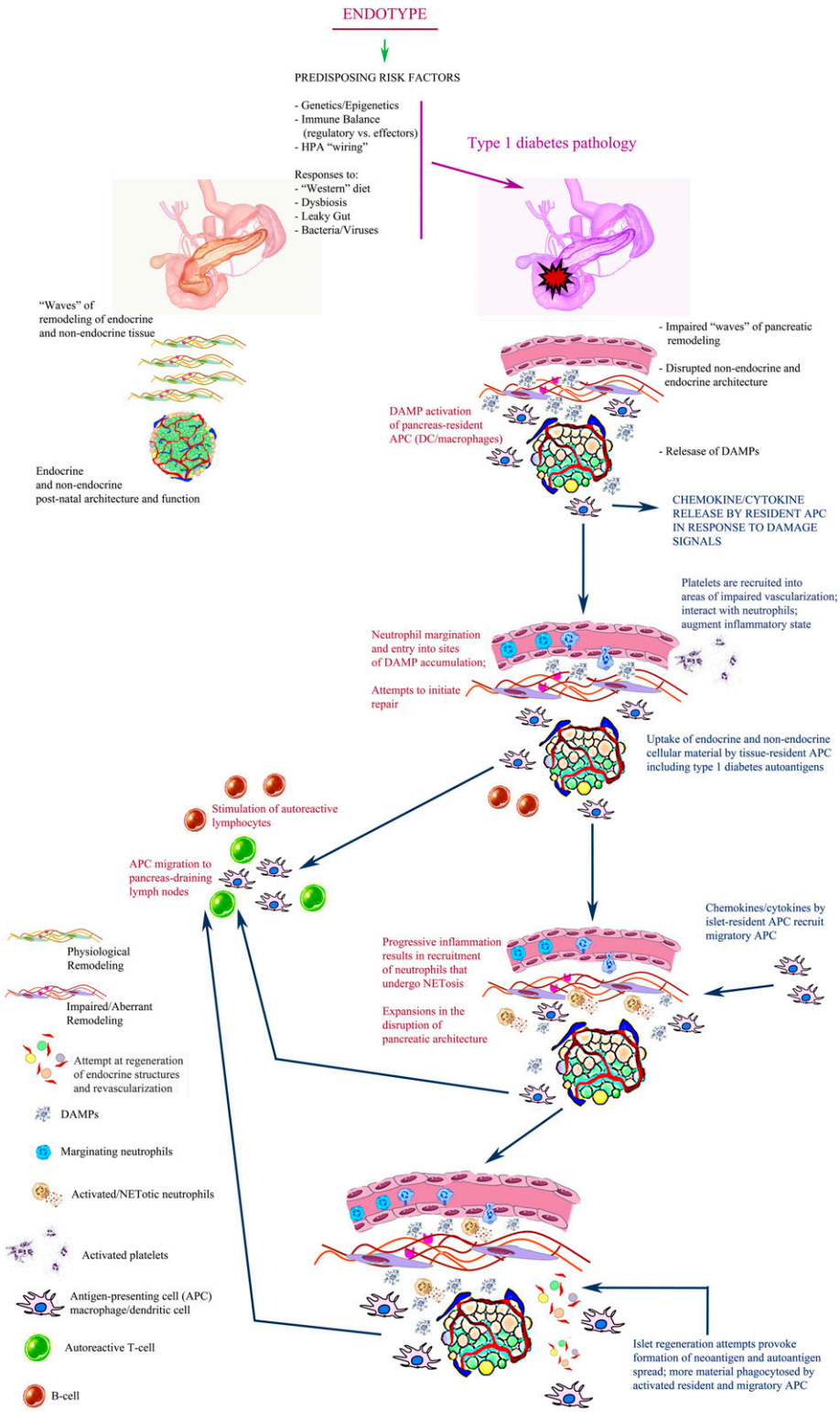

Figure 1—Proposed model for T1D pathogenesis. It is possible that an obesogenic diet combined with genetic susceptibility may promote dysbiosis of the microbiome and gut leakage, resulting in pathologic accumulation and translocation of bacteria and/or viruses (including potential reactivation of endogenous retrovirus elements) that indirectly incites a wave of β-cell apoptosis. In the process, the vasculature could be damaged, and an attempt at remodeling of the vasculature could be occurring, resulting in changes in blood vessel diameter and thereby affecting shear flow, which would facilitate the recruitment of platelets at sites of high shear as well as those exhibiting shear stress–dependent and/or –independent endothelial damage. As these events unfold, DAMPs would be sensed by neutrophils, which then accumulate inside the pancreatic tissue following arrest at P-selectin^{+} cell regions. The formation of platelet-neutrophil aggregates activates neutrophils to release NETs and promotes neutrophil extravasation. NETosis would be expected at this time, activating tissue-resident APCs, which would then migrate in the pancreatic lymph nodes and prime β-cell-reactive T cells. HPA, hypothalamic-pituitary axis.

autoantibody type (insulin [IAA] vs. GAD [GADA]), autoantibody number (single vs. multiple), degree of genetic susceptibility (genetic risk score), pancreas pathology (as described above), and others are being considered as potential features for endotypic classification (5). This notion of endotypes is important in determining the role for innate immunity in that its contribution to T1D may vary from individual to individual depending on the endotype in question.

Therapeutic Strategies to Short-circuit the Actions of the Innate Immune System to Prevent or Delay the Onset and Progression of Pancreatic Pathology

If proinflammatory action of pancreas-resident and pancreas-accumulating innate leukocytes (i.e., neutrophils, macrophages, DC) triggers and promotes the eventual activation of autoreactive lymphocytes in humans during the first months to years of life, extending beyond the postnatal period as observed in the NOD mouse (29,30), a reasonable window of opportunity exists to intervene, potentially when impaired glucose tolerance is first observed, reflecting β-cell dysfunction in genetically at-risk first-degree relatives of patients with T1D (31). The endocrinologist's objective, here, could be aimed at maintaining a suppressed state of intrapancreatic inflammation for as long as possible to mitigate innate leukocyte-driven damage to the pancreatic endocrine and nonendocrine components, to thereby delay the activation of autoreactive T and B cells.

We believe that a reasonable number of viable approaches to safely target these cells may exist, as summarized below and in Table 2. These individual approaches, which target proinflammatory pathways common to neutrophils, macrophages, and DC, could be combined for more broad suppression. In the circulation of T1D subjects, there is an increase in NETotic neutrophils that also exhibit different IFN signatures compared with healthy subjects (13). Huang et al. (32) have also shown a reduced neutrophil migration in subjects with T1D. Outside of the circulation, the frequency of proinflammatory innate leukocytes (e.g., neutrophils) is increased inside the pancreas of humans with T1D and animal strains that exhibit type 1 diabetes in comparisons with normal control subjects (33). Even though the agents that we propose below can be characterized as systemic immunosuppressives (depending on route of administration), their action is most effective inside inflamed tissues where leukocytes exhibit high activity in the indicated molecular pathway, with little to no bioactivity in tissues where there are few leukocytes and/or leukocytes with low activity in the indicated pathway.

Targeting Inflammasomes

A number of inflammasome inhibitors are currently in late preclinical development (rev. in 34). These include (as of June 2020) NLRP3-targeting small-molecule drugs like OLT1177 (35), inzomelid (clinical trial reg. no. NCT04015076, ClinicalTrials.gov), and IZD334 (NCT04086602). The dietary agent withaferin A is also a potential "nutraceutical" of interest, as it disintegrates the inflammasome complex and modulates multiple cytokines and chemokines associated with inflammation and cancer (36).

Chemokine Antagonism

One attractive means to mitigate innate leukocyte-induced intrapancreatic inflammation is to impair the accumulation of innate leukocytes via chemokine blockade or chemokine

Table 2—Intrapancreatic DC, macrophages, and B cells in NOD mice

	Phenotype	Demonstrated or proposed role/function	Concomitant events inside pancreas
DC	XCR1$^+$ CD103$^+$ Batf3$^+$	Increase in numbers beginning at 3 weeks of age	Insulin-reactive CD4$^+$ T cells evident inside the pancreas and around the islets
		CD103$^+$ DC cross present class I MHC epitopes to CD8$^+$ T cells, and Batf3 is necessary for this function	Insulin-reactive T cells are in tight physical contact with the CD103$^+$ DC, which exhibit an interferon-inducible gene expression signature
	Batf3-deficient NOD mice		XCR1$^+$ CD103$^+$ DC were absent in the islets, transgenic mice remained diabetes free and without evidence of islet-reactive T cells; poor priming of diabetogenic CD4 and CD8 T-cell responses
Macrophages	Derive from hematopoietic progenitors, slow replicating, not replaced by circulating monocytic precursors	Filopodia extend into the microvasculature	Deletion of islet-resident macrophages eliminated T-cell entry into islets and reduced diabetes incidence in NOD mice
	Exhibit a proinflammatory gene signature	Sense blood-borne molecules	
	Resemble lung "barrier macrophages" with high lysosomal content and activity	Physically adjacent to β-cells and take up insulin-containing crinosomes	

Table 3—Potential targets to inhibit the activation and proinflammatory effects of innate leukocytes known to be involved in the onset and progression of T1D

Pathway	Candidate agents
Inflammasomes	OLT1177, inzomelid, IZD334, withaferin-A
Chemokines	CXCL12 antibodies, SB225002, SB656933, reparixin, ladarixin, navarixin, danirixin, AZD5069, AZD8309
Formation of NETs (NETosis)	GSK484
Enzymes secreted by innate leukocytes	
NE	AAT, silvelestat, AZD9668, BAY-678, BAY 85-8501
MPO	AZD5904, AZD3421, PF-06282999

receptor antagonism. In T1D, a key role for Cxcr2 was shown in mice, where neutrophils were identified as the first immune cells to enter islets in NOD mice as early as 2 weeks of age (18,20). A follow-up study demonstrated that intraislet macrophages and islet β-cells produced Cxcl1 and Cxcl2, recruiting Cxcr2-expressing neutrophils from the blood to the islets. Blockade of neutrophil recruitment with a selective Cxcr2 antagonist (SB225002) as early as 3 weeks of age attenuated diabetogenic T-cell responses and development of autoimmune diabetes (20). Citro et al. (37) demonstrated that CXCR1 and CXCR2 inhibitors (reparixin and ladarixin) attenuated insulitis; reduced the frequency of neutrophil, macrophage, and T-cell infiltration inside the pancreas; and prevented low-dose streptozotocin-induced T1D in NOD mice. In the transplantation setting, blockade of the CXCR1/2 pathway with use of reparixin improved islet engraftment in murine (38) and human islet transplant recipients (39). While these inhibitors are potentially attractive as monotherapies to possibly delay the accumulation of a significant number of islet-autoreactive lymphocytes in individuals at high risk, they can induce mild neutropenia; hence, their therapeutic index requires careful assessment prior to clinical use. Nevertheless, these agents could be coupled to nano-/microparticle (NMP) drug delivery systems that, by facilitating slow/sustained drug release and targeting selective anatomic sites, are expected to minimize off-target effects and the induction of systemic neutropenia (see DRUG DELIVERY, below).

NETosis Suppression

NETosis is a specific form of cell death in granulocytes, especially neutrophils, that differs from apoptosis and necrosis (40). NETs can activate plasmacytoid DC (41) and macrophages (42). Self-reactive B cells can be activated by DNA inside NETs in the form of LL37-DNA complex, triggering B-cell activation in a TLR9-dependent manner (43). Thus, it is conceivable that B-cell-derived immune complexes may activate neutrophils by binding the Fc γ receptor IIIb, inducing further NET formation (44) and, thus, a feedback loop promoting autoimmunity and disease progression. The molecular pathways that lead to the formation of NETs, as well as the constituent enzymes on NETs, are therefore attractive therapeutic targets. These include PAD4, a nuclear enzyme that mediates NET formation by histone hypercitrullination and thereby contributes to chromatin decondensation (45). A number of PAD4 inhibitors have been under development (46), but currently, only GSK484 has demonstrated consistent activity in vivo in animal models of inflammatory disease (47).

Inhibition of Enzymes Secreted by Proinflammatory Innate Immune Cells

These include neutrophil elastase (NE) and myeloperoxidase (MPO), which are often components of NETs (48). NE is quickly released from neutrophil granules and is highly concentrated at inflammation sites. NE activates matrix metalloproteinases, inactivates their inhibitors, and has the ability to degrade components of the coagulation and fibrinolytic pathways. NE also activates and processes proinflammatory cytokines (49).

Among the most obvious NE inhibitors are the naturally occurring serpin inhibitors (50). Imbalances between NE and its endogenous inhibitors have been implicated in the pathogenesis of a wide range of progressive or chronic inflammatory disorders (50). In preclinical models of autoimmunity and transplantation, α-1-antitrypsin (AAT) therapy prevented or reversed autoimmune disease and graft loss, and these effects were accompanied by tolerogenic changes in cytokine and transcriptional profiles and T-cell subsets (51). AAT (e.g., prolastin, aralast, zemaira) could be paired with elafin (tiprelestat) as possible biotherapies.

A number of nonbiologic NE inhibitors have been developed and tested in human subjects. NE inhibition, however, must be balanced by the possibility that it can also induce endothelial cell damage, which could, in turn, promote the release of autoantigens, exacerbating the progression of autoimmunity (50).

MPO is an enzyme expressed primarily by phagocytic leukocytes, especially neutrophils, and plays an essential role in the inflammatory response by catalyzing the formation of reactive oxygen species involved in microbial killing (52). A number of MPO inhibitors have completed early-stage clinical trials; however, all have failed to meet their primary clinical end points (53). The failure of these agents is thought to be due not to their inability to inhibit the enzyme (54) but, rather, to the overlapping proinflammatory pathways that innate leukocytes use

Table 4—Areas for future investigation

	Questions
Species/system, organ	
Rodent, circulation	• Is there a specific transcriptome/epigenome signature in circulating leukocytes of the innate arm of the immune system that marks the onset of pancreatic inflammation (e.g., in NOD mice, BB rats)? • Are there extracellular vesicles in the circulation of NOD mice and BB rats with protein and/or nucleic acid content indicative of the onset of pancreatic inflammation? • Is methylated insulin gene DNA present in the circulation prior to the onset of lymphocyte inflammation inside the pancreas?
Rodent, pancreas	• Is there a specific transcriptome/epigenome signature in the cells of peri-islet vasculature that is associated with the eventual, or concomitant, accumulation of intrapancreatic proinflammatory leukocytes of the innate arm of the immune system (e.g., in NOD mice, BB rats)? • Do neutrophils coincide with intrapancreatic DC that exhibit an activated state?
Human, circulation, physiology	• In individuals without diabetes who are first-degree relatives of patients with type 1 diabetes, beginning at 3–4 years of age, is there a specific transcriptome/epigenome signature in circulating leukocytes of the innate arm of the immune system that informs *1*) HLA-associated or HLA-independent risk of dysglycemia, *2*) age at onset of dysglycemia, *3*) risk of T1D, or *4*) age at onset of dysglycemia and/or clinical disease? • Barriers: modify study protocols in international blood collection endeavors to *1*) enrich blood into more defined leukocyte populations and *2*) purify extracellular vesicles and automate protein and nucleic acid identification/sequences/modifications (DNA-histone methylation, acetylation; protein phosphorylation, glycosylation)
Human, pancreas	• In pancreatic tissue in the nPOD and DiViD tissue banks, *1*) identify upregulated genes encoding druggable products (enzymes, proteins) in intrapancreatic leukocytes of the innate arm that ordinarily confer a proinflammatory state to these cells • Barriers: need single-cell resolution that requires accuracy in the microanatomic location of sought-after intrapancreatic leukocyte(s); require evolution of optical-mechanical instrumentation to overcome this barrier
Clinical trials	• Test drugs (those with favorable safety profiles) that target the products identified above alone or in combination in first-degree relatives (dysglycemic) of patients with type 1 diabetes, e.g., inflammasome inhibitors, chemokine antagonists/decoys, NET formation inhibitors, NE/MPO inhibitors • Barriers: repurposable agents exist, but their safety needs to be demonstrated in dysglycemic individuals

when activated. Therefore, it is not surprising that MPO inhibitor monotherapy was unable to achieve a beneficial pharmaceutical outcome. Like the aforementioned drugs, MPO inhibitors could be useful in an adjunctive approach.

Drug Delivery

Targeted delivery to the inflamed pancreas alone or together with selective targeting to inflamed peri-islet anatomy can be one way to minimize the systemic immunosuppression that may occur if the aforementioned agents are administered by mouth or by intravenous route. NMP can be mobilized to specifically target defined macrophage and neutrophil populations based on unique cell-surface proteins (55). The surface of NMP can be equipped with a ligand or antibodies for cell receptors, which serve as a targeting vector (e.g., CD15 [56], CXCR2, and/or CXCR4 for targeting neutrophils), whereas the core can be loaded with one or more tailored drugs, allowing for their selective delivery with tunable release kinetics (57). Similarly, one can take advantage of the expression of cell adhesion molecules on the lymphoid tissue that subserves inflamed anatomical areas (e.g., peripheral node addressin; PNAd; selectins) and decorate the delivery vehicle with antibodies that recognize these molecules (58,59). The result is the retardation of these particles selectively at the site of inflammation and release of the payload at that site. The distribution of NMP to different organs is known to strongly depend on the size of the particles. For instance, particles sizing from 10 to 250 nm mostly distribute to liver and spleen, and NMP could be designed based on these physical characteristics to be selectively retained in these organs.

Conclusions, Recommendations, and Future Areas for Exploration and Investigation

Emerging data raise many questions that challenge the dogmatic, historical view of T1D as a lymphocyte-driven disease, mostly by β-cell-reactive T cells, where clinical onset of impaired glucose tolerance becomes clinically evident consequent to the physical eradication of a substantial mass of β-cells. The evolving view, which we support, is that the disease is a function of endotype and that the etiopathogenesis is much more complex, involving the exocrine pancreas very early on in the process. Like others, we propose that innate immune cell subsets serve as the first line of response to danger signals and that the disease endotype largely determines which of the variables is more responsible in the process that results in DAMP production (e.g., genetic/epigenetic conditioning of pancreas remodeling, pancreas response to changing microbiota,

viral/bacterial infection, diet). The marginalization and activation of neutrophils and platelets, activation of tissue-resident APC, and recruitment of migratory APC and presentation of endocrine and nonendocrine antigens to T cells are now appreciated to be key players for the generation of self-reactive T cells and immune-mediated β-cell impairment that may or may not always result in physical eradication. This then compels an urgent and better understanding of the role of tissue-resident and recirculating innate immune cells in subjects with prediabetes, which would allow for a clearer picture to emerge about the heterogeneous, endotype-imprinted pathogenesis of T1D. Furthermore, heterogeneity in the natural history of dysglycemia, in terms of time to onset and time in state, also suggests that the process is closer to a relapsing-remitting pattern of chronic inflammation involving the whole pancreas, which promotes tissue damage and the generation of autoreactive responses, instead of the historical view that the process follows a linear, checkpoint-regulated lymphocyte-driven β-cell functional decline. As the endotype concept gains more acceptance in the T1D field, one question that arises concerns how this would affect the issue of which of the treatment options we listed earlier would be better suited for an early-stage preventive approach. Although, currently, there are no data to guide a definitive response, we expect in the near future to be able to identify the endotype of the patient in the form of a combination of peripheral innate leukocyte immune signature (transcriptome, epigenome) together with circulating structures that can give insight about the "damage" ongoing inside the pancreas (e.g., extracellular vesicles coloaded with pancreas-specific proteins and proteins/nucleic acids indicating cellular stress and impairment/damage [e.g., heat shock proteins, nucleosomes, unmethylated insulin gene DNA]). Then, together with physiologic data such as data on dysglycemia and/or exocrine pancreas function, this would inform the stage of pancreas impairment and also the most likely cellular and molecular mechanism underlying the inflammation at that moment. This would then help decide which single or combination drug approach would be most effective at arresting the intrapancreatic inflammatory process. As further research is informed by this emerging model of disease etiopathogenesis, there are more targets available, beyond restoration of tolerance, to delay the progression and stabilize the honeymoon phase, and this is where cells and molecular pathways of the innate arm of the immune system become attractive therapeutic targets. Together with a better understanding of the variables that imprint the disease endotype, these agents can be deployed in a patient-specific manner, as the relapsing-remitting nature of the progression is expected to differ among different individuals.

Acknowledgments. A considerable and rich body of literature exists and is emerging that complements our perspective. Due to article length restrictions, we were unfortunately unable to reference all of the outstanding and novel primary research that we summarize inside this article; where possible, we instead provide comprehensive reviews.

Funding. Studies related to the concepts reported herein were supported by the National Institutes of Health (P01AI042288 to M.A.A., R21 TR001728-01 to N.G.), JDRF (SRA-2016-318-S-B to N.G., 3-APF-2019-744-A-N to A.P.), and the Network for Pancreatic Organ donors with Diabetes (nPOD) (RRID-SCR_014541), a collaborative T1D research project sponsored by JDRF (nPOD: 5-SRA-2018-557-Q-R), The Leona M. and Harry B. Helmsley Charitable Trust (grant 2018PG-T1D053), and the McNair Medical Institute at The Robert and Janice McNair Foundation (to M.P.).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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