ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 7, 2007

DOI: 10.1089/ars.2007.1610

Forum Review

Isletopathy in Type 2 Diabetes Mellitus: Implications of Islet RAS, Islet Fibrosis, Islet Amyloid, Remodeling, and Oxidative Stress

MELVIN R. HAYDEN[1,2,4] and JAMES R. SOWERS[1–4]

ABSTRACT

This review focuses primarily on islet structural and functional changes related to an activated islet renin–angiotensin system (RAS), islet oxidative–redox imbalance, the concurrence of islet fibrosis (pericapillary, intra- and peri-islet), and islet amyloid deposition (pericapillary, intra- and peri-islet). The islet–acinar–portal vascular pathway and the emerging important anatomical and functional region, the islet–exocrine interface, are also discussed. Because there is an associated histopathological islet disease in type 2 diabetes mellitus (T2DM), the term isletopathy is discussed in detail. The isletopathy in T2DM is equally important as the other complications of diabetes. Special stains and special lighting (bright field and crossed polarized light) are utilized, along with light and transmission electron microscopy, in order to better understand islet structural remodeling in T2DM. The importance of an isletopathy in T2DM is supported by numerous remodeling changes within the islet and the islet–exocrine interface. While some of the structural findings are only preliminary observations, additional investigation in this area should lead to the development of new pathophysiological concepts and new therapies regarding the prevention and treatment of T2DM. *Antioxid. Redox Signal.* 9, 891–910.

INTRODUCTION

TYPE 2 DIABETES mellitus (T2DM) has reached pandemic proportions, and current predictions are that this trend will continue, at least in the near future (1, 71). Even though there has been a vast amount of new information and treatments made available during the past decade, we as clinicians and researchers must continue our attempts to better understand the various mechanisms and to translate these newer findings and guidelines to our patients. Education of health care providers and new treatment paradigms are critical to empower us to deal with this pandemic.

In times of great societal change, including overnutrition and underexercise, and their association with the obesity and the T2DM epidemic, we need to consider new pathophysiologic concepts and understanding to address this pandemic "the diabesity epidemic."

It is imperative that we achieve a better understanding of this complex disease and its resultant complications involving the pathophysiology of end organ disease in T2DM. One new paradigm involves considering the islet as being an end organ disease that is affected very early in the development of T2DM, a process we term isletopathy, since the pancreatic islet has been shown to undergo significant remodeling concurrently or even earlier than other end organs in T2DM. Additionally, we refer to the multiple end organ remodeling in T2DM as diabetopathies. The following terms are used in each of the end organs affected in T2DM. For example, in the *M*yocardium: diabetic cardiomyopathy; the *I*ntima: diabetic intimopathy; the *N*erve: diabetic neuropathy; the *E*ndothelium: diabetic endotheliopathy with associated micro- and macro-vasculopathies; the *R*etina: diabetic retinopathy; and *R*enal tissue: diabetic nephropathy (MINER). In addition to this MINER acronym, it is now time to consider adding yet another -opathy to this

[1]University of Missouri School of Medicine Department of Internal Medicine, [2]Endocrinology Diabetes and Metabolism, [3]Pharmacology and Physiology, [4]Diabetes and Cardiovascular Disease Research Group, University of Missouri School of Medicine, Columbia, Missouri.

multiorgan list. We have recently coined the term isletopathy to describe the intra- and extracellular remodeling changes within the pancreatic islets of diabetic animal models and of course the most important model of all: the human patients with T2DM. Use of the term isletopathy might result in clinicians and researchers considering the protection and salvaging of the vulnerable islet similar to protecting the vulnerable plaque in atherosclerosis amidst the background of T2DM.

This review is intended to provide background information regarding the cellular and extracellular remodeling that takes place in the vulnerable islet by considering both the ongoing functional and structural changes. We demonstrate these findings through multiple images, which utilize light and transmission electron microscopy (TEM) as well as various types of transmitted light, including bright field and crossed polarized lighting methods. We employ special staining procedures including immunohistochemisty, periodic acid Shiff (PAS), Verhoeff Van Gieson (VVG), and picrosirius red and Congo red stains.

THE NATURAL HISTORY OF TYPE 2 DIABETES MELLITUS

T2DM is closely tried to obesity and there are at least three important diabetogenic factors in play during its development and progression. First, there is the polygenic component (involving multiple genetic defects or gene polymorphisms related to insulin sensitivity and/or insulin insensitivity); second, there is the environmental component (involving the societal change of overnutrition and underexercise); third, there are functional metabolic alterations and structural remodeling changes (involving islet fibrosis, islet amyloid and β-cell apoptosis) (28). These etiological factors interact with each other often in a synergistic fashion in the natural history of T2DM (Fig. 1). There are two central defects in T2DM: insulin resistance (IR) and β-cell dysfunction or loss in volume/mass, commonly referred to as β-cell failure (8, 13, 19). The final and absolute requirement for the development of overt T2DM in humans involves β-cell failure, a process that has been shown to be, in large part, due to β-cell apoptosis with reduction in β-cell mass and volume within the islets (8). Approximately 80% of the IR patients are capable of compensating, at least for a period of time, due to the β-cell capability to compensate with hyperinsulinemia to overcome the insulin resistance (33); however, over time the pancreas loses this compensatory ability.

As a result of cellular (β-cell apoptosis) and remodeling, there is both a loss of β-cells and a disruption or disorganization of the β-cell connectivity. This is important because the β-cell organization requires connectivity to act in a syncytial synergistic fashion to produce a prompt and appropriate first phase insulin response to nutrient stimuli. If this β-cell connectivity and syncytial quality is lost, first phase insulin response becomes impaired, resulting in the impaired glucose tolerance associated with prediabetes (Fig. 2) (55). The terms β-cell fatigue and failure have been used for some time; however, we now understand that these two processes are very complicated mechanisms, involving endoplasmic reticulum (ER) stress and the misfolding of amyloidogenic proteins, human islet amyloid polypeptide (hIAPP), or amylin and β-cell apoptosis (8, 24).

THE ROLE OF ISLET RAS AND REDOX IMBALANCE

Islet RAS

Recently, there have been multiple papers published regarding the role of an active renin–angiotensin system (RAS)

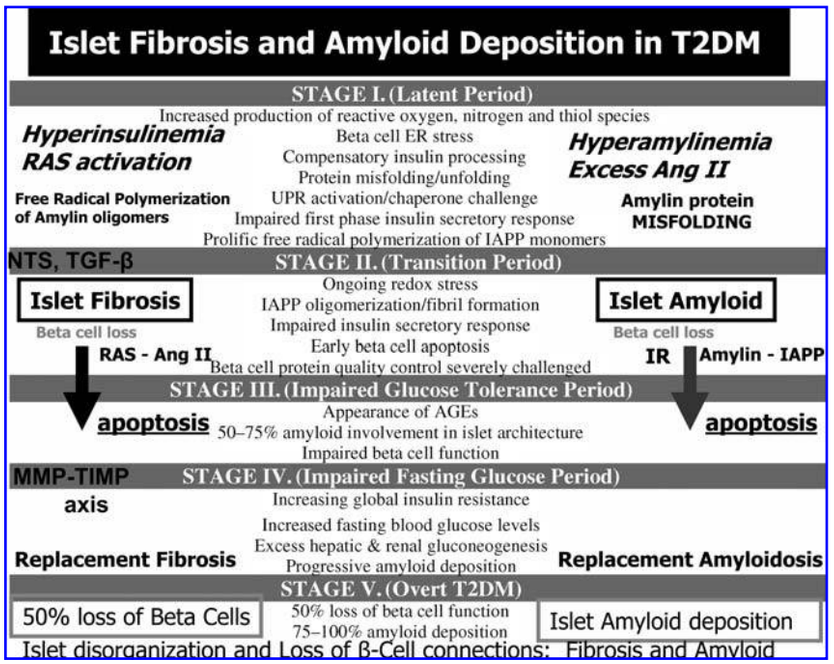

FIG. 1. The natural history of T2DM. Putative model of the five stages of type 2 diabetes mellitus (T2DM) with the combined role of islet fibrosis and amyloid remodeling. Ang II, angiotensin II; AGEs, advanced glycation endproducts; ER, endoplasmic reticulum; IAPP, islet amyloid polypeptide; IR, insulin resistance; MMP, matrix metalloproteinase; NTS, nitrotyrosine; RAS, renin angiotensin system; TGF-β, transforming growth factor beta; TIMP, tissue inhibitor of metalloproteinase; UPR, unfolded protein response.

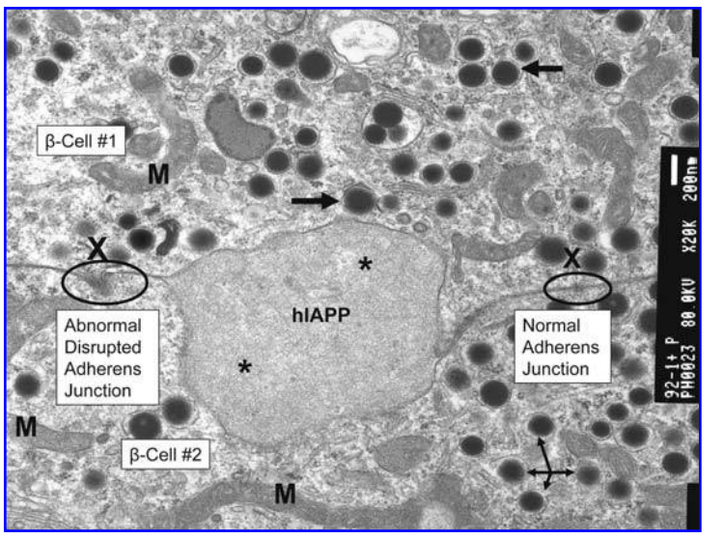

FIG. 2. Islet amyloid may disrupt β-cell connectivity and syncytial function. This TEM image demonstrates inter β-cell islet amyloid (*) and the possible association of abnormal adherens junctions (*X*) between two β-cells in the islet of the 4-month-old HIP rat model of T2DM. Note the mildly abnormal appearing adherens junction encircled (*X*) *on the left* as compared to the more normal one *to the right* of the inter β-cell amyloid deposition in the *center*. Mitochondria (M) in the β-cell appear more tubular and elongated when compared to other cell organs. Insulin secretory granules (*arrows*) appear normal in this image. HIP, human islet amyloid polypeptide; T2DM, type 2 diabetes mellitus; TEM, transmission electron microscope.

in the islet (Fig. 3) (9, 38, 40, 42, 43, 61, 64). The presence of the angiotensin type 1 (AT_1) receptor on the β-cells and islet capillary endothelium has provided evidence for angiotensin II (Ang II), and potential diabetic prevention roles for RAS blockade with angiotensin converting enzyme inhibitor(s) (ACEI) and the angiotensin type 1 antagonist(s) blocker(s) (ARBs) (9, 38, 40, 42, 43, 61, 64).

Ang II significantly impairs both pancreatic blood flow in isolated perfused rat pancreata, especially islet blood flow. Blood flow to islets is significantly impaired by systemic and locally generated Ang II, and this abnormality is improved with administration of RAS antagonist treatment (34). Additionally, Ang II has been shown to inhibit insulin release from isolated mouse islets in response to high glucose concentrations (38). This inhibitory action is partly a result of decreased proinsulin synthesis. Further, the ARB (losartan) treatment prevented this decrease in proinsulin synthesis (38), which is strongly suggestive that there are effects, in addition to those of reduced islet blood flow, that contribute to β-cell synthesis.

Ang II activates the nicotinamide adenine dinucleotide phosphate reduced [NAD(P)H] oxidase enzyme, which results in the excess production of reactive oxygen species (ROS) within the islet (16, 19, 21, 36, 59). The local increase in islet oxidative stress may result in the overconsumption of the local antioxidant defense mechanisms within the β-cell, with resultant net accumulation of unpaired electrons and oxidative stress in islets. The β-cells have limited ability to handle oxidative stress, and this makes the islet quite vulnerable to oxidative stress (23, 58, 66). For example, the two most commonly used potent oxidants (streptozotocin and alloxan) used to create diabetic models in rodents seem to

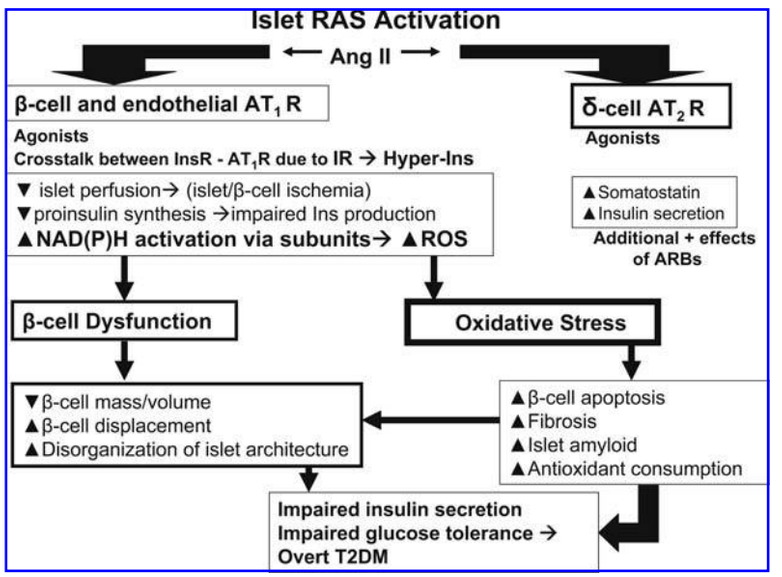

FIG. 3. Islet RAS activation. This image demonstrates the importance of islet RAS activation, Ang II, and the role of the AT-1 receptor agonism. Ang II, angiotensin II; ARB, angiotensin receptor blocker; AT-1R, angiotensin type 1 receptor; Ins, insulin; InsR, insulin receptor; IR, insulin resistance; NAD(P)H, nicotinamide adenine dinucleotide phosphate reduced, ROS, reactive oxygen species; T2DM, type 2 diabetes mellitus.

damage the islet β-cells more selectively than in other organs such as the kidney, heart, and liver. This observation in the oxidatively-damaged rodent diabetic models points strongly to the vulnerability of the islet and β-cell to oxidative stress.

IR results in elevated levels of insulin, proinsulin, and amylin (19). Each of these islet β-cell derived hormones is known to be capable of activating the RAS (12, 31). Later in the development of overt T2DM, hyperglycemia is capable of activating the islet RAS (19, 39) with resultant damage to vulnerable islets. Knowing that RAS expression is upregulated in the endocrine pancreas in T2DM and that RAS blockade with ACEI and ARBs results in improvement of both islet function and structure in animal models of T2DM and the recent human clinical trials delaying the development of T2DM in those patients with impaired glucose tolerance makes the islet RAS a prime target for drug development to prevent T2DM (64).

Islet oxidative–redox stress

The term redox stress is used along with oxidative stress in this review because hyperglycemia (glucotoxicity) is one of the most potent producers of ROS in T2DM. A great deal of ROS are produced via cytosolic reductive stress with an increased ratio of NADH/NAD^+, which has been termed pseudohypoxia (30, 68). There are multiple metabolic pathways and enzymes involved (Fig. 4), as well as multiple metabolic toxicities responsible for generating ROS in metabolic syndrome, prediabetes, and overt T2DM, which are grouped together in a acronym (A-FLIGHT-U) (Fig. 5).

The hyperglycemia involved in impaired glucose tolerance (prediabetes) and T2DM results in the formation of ROS within the islet, which are derived from the following sources: (a) oxidative phosphorylation in the mitochondria with an excessive electron leak of unpaired electrons and oxidative phosphorylation during anaerobic glycolysis; (b) the Schiff reaction during glycation reactions resulting in advanced glycation endproducts (AGE) and activation of its receptor (RAGE); (c) glucose autoxidation, and (d) hexosamine metabolism (Fig. 6) (4, 6, 56). Additionally, NAD(P)H oxidase is also capable of generating ROS not only within the islet vasculature but also the islet β-cell itself via a protein kinase C-dependent mechanism (51). The elevated tension of islet redox stress, coupled with a low endogenous antioxidant activity within the islet, allows ROS damage to proteins, nucleic acids, and lipids. In the islet and the β-cell, this results in damage and dysfunction to messenger proteins, nuclear proteins, and plasma membranes, leading to impaired insulin gene expression, signaling secretion, and ultimately apoptosis.

Reactive nitrogen species (RNS) and reactive thiols

RNS are increased in T2DM and could contribute to additional ROS and damage to the islet as well as the misfolding of native amylin within the endoplasmic reticulum. The ROS superoxide reacts with nitric oxide to result in the generation of peroxynitrite as well as other RNS, which are important in the development of T2DM. Peroxynitrite is a selective potent oxidant to tissues modifying tyrosine in proteins/polypeptides to create nitrotyrosine, which leaves behind an indelible fingerprint of nitrotyrosine staining *in vivo*, as noted in Fig. 11. Further nitrotyrosine and nitroarginine are capable of competing with the natural substrate L-arginine in the endothelial reaction, resulting in endothelial nitric oxide production via the endothelial nitric oxide synthase reaction. In turn the

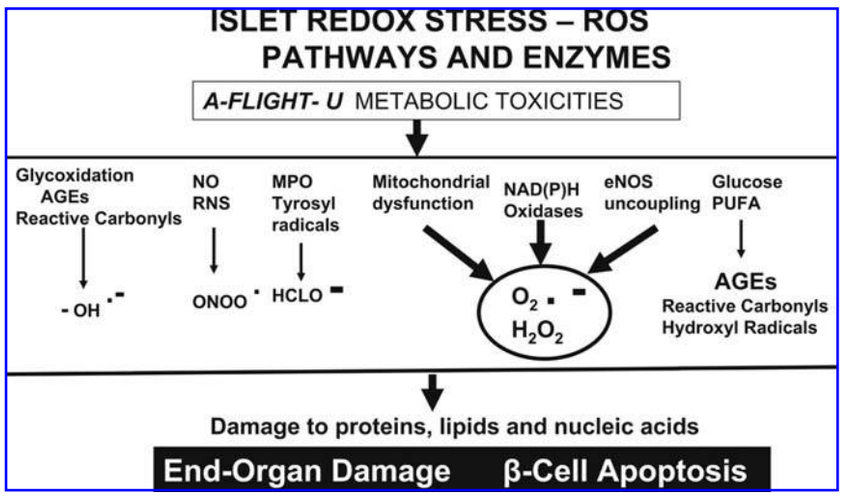

FIG. 4. Islet redox stress—ROS: pathways and enzymes. The possible metabolic pathways and enzymes involved in the production of islet reactive oxygen species are shown. AGE (advanced glycation end products) that are initially formed through the process of a glucose nucleophilic addition reaction with proteins forming a Schiff base, followed by the formation of an Amadori compound that undergoes further reactions, rearrangements, dehydrations, and cleavage, resulting in brown insoluble, cross linked complexes called AGEs. This process is thought to liberate H_2O_2 through two pathways: the first is the 1,2-enolization pathway, which leads to 3-deoxyglucosone forming H_2O_2 and glucosone; the second pathway is the 2,3-enolization pathway leading to 1-deoxyglucosone and putative 1,4-deoxyglucosone. Under oxidative conditions, the 2,3-enediol is thought to generate H_2O_2 and carboxymethyllysine; eNOS (endothelial nitric oxide synthase) uncoupling, MPO, myeloperoxidase, that needs to be explored in greater detail in the islet; NAD(P)H, nicotinamide adenine dinucleotide phosphate reduced, that results in increased ROS as a result of islet RAS and AT-1 activation; NO (nitric oxide) and its reaction with superoxide resulting in peroxynitrite; mitochondrial dysfunction with the known electron leak of superoxide and unpaired electrons, that is associated with a possible structural change in the elongated-tubular mitochondria noted in Fig. 2 to a more elliptical and rounded mitochondrial structure in animal models as noted by authors (unpublished data); PUFA,(polyunsaturated fatty acids), which are known to result in lipoxidation and the generation of even more damaging reactive oxygen species within the islet.

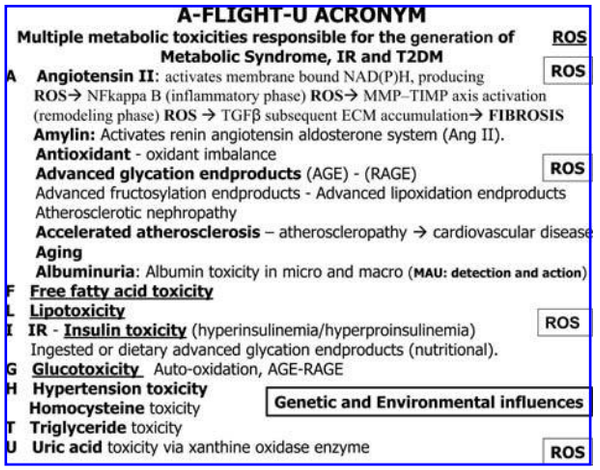

FIG. 5. A-FLIGHT-U acronym. The multiple metabolic toxicities in the metabolic syndrome and type 2 diabetes mellitus are illustrated. This figure demonstrates the multiple metabolic toxicities associated with the metabolic syndrome, prediabetes, and overt T2DM, that individually and synergistically contribute to the formation of the damaging ROS to the islet and β-cells. Ang II, angiotensin II; AGE, advanced glycation end products; ECM, extracellular matrix; IR, insulin resistance; MMP, matrix metalloproteinase; NAD(P)H, nicotinamide adenine dinucleotide phosphate reduced; NFkappa B, nuclear factor kappa B (23, 24); RAGE, receptor for advanced glycation endproducts, ROS, reactive oxygen species; T2DM, type 2 diabetes mellitus; TGF-β, transforming growth factor beta.

nitroarginine could compete with L-arginine of the eNOS reaction and result in decreased production of endothelial nitric oxide. Also, the overall increase in RNS could oxidize BH4 to BH3 and BH2, resulting in eNOS uncoupling discussed later in the section regarding the pericyte (23, 24).

Thiol species are generally viewed as nonenzymatic antioxidants; however, oxidized thiol species are considered to be reactive thiol species (RTS) and could also contribute to the overall oxidative stress within the islet and contribute to protein/polypeptide misfolding as occurs with amylin, resulting in islet amyloid oligomers and eventual fibril within the islet. In turn, when thiols react with other free radicals and H_2O_2, reactive thiols are also generated [where R = any protein or polypeptide] as in the following reactions generating not only reactive thiols (also termed thiyl radicals), but nonreactive oxidative species such as H_2O_2.

$$RSH + R' \rightarrow R'H + \underline{RS}^{\cdot}$$
$$RSH + O_2^{\cdot} + H+ \rightarrow \underline{RS}^{\cdot} + H_2O_2$$
$$2RSH + H_2O_2 \rightarrow \underline{2RS}^{\cdot} + H_2O$$
$$RSH + -OH^{\cdot} \rightarrow \underline{RS}^{\cdot} + H_2O$$

The thiol homocysteine (Hcy) is a classic example of a thiol capable of causing a tremendous amount of damage to proteins and various enzymes and cells, especially vascular mural cells such as the endothelium. The important role of oxidative–redox stress in metabolic syndrome, prediabetes, overt T2DM, and hyperhomocysteinemia is biologically plausible because Hcy promotes oxidant injury to vascular cells (particularly the endothelium and the eNOS enzyme reaction) through the auto-oxidation of Hcy, formation of Hcy mixed disulfides, interaction of Hcy thiolactones, and protein homocysteinylation (2). Oxidation of two Hcy molecules yields the oxidized disulfide (homocystine), two protons (H^+), and two electrons (e^-), while promoting the formation of reactive oxygen species (ROS).

Auto - oxidation of homocysteine

$2Hcy - SH ---- O_2 \text{-------} \rightarrow$ (Homocysteine) $\quad HcyS - SHcy + 2H^+ + 2e^- \rightarrow$ (Homocystine: oxidized disulfide) $\quad$ Generating Ros

Superoxide
Hydroxyl radical
Hydrogen peroxide

Also, formation of mixed disulfides contributes to the additional formation of ROS

$$Hcy\text{-}SH + R\text{-}SH ---- O_2 ---- \rightarrow Hcy\text{-}S\text{-}SR + H_2O \text{-}\rightarrow ROS$$
$$Hcy\text{-}SH + R1\text{-}S\text{-}S\text{-}R2 \rightarrow R1\text{-}S\text{-}S\text{-}Hcy + R2\text{-}SH \text{-}\rightarrow ROS$$

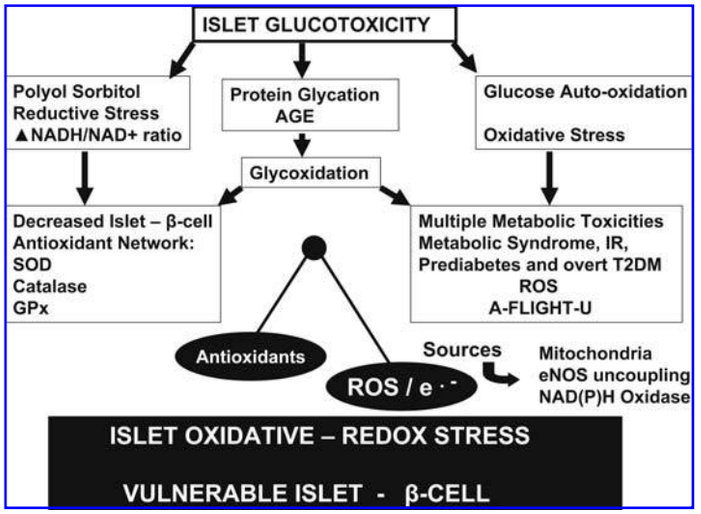

FIG. 6. Islet glucotoxicity. This figure depicts the various pathways in the production of reactive oxygen species due to glucotoxicity. In the *center* of the figure, a balancing scale demonstrates that the excess amounts of ROS and unpaired electrons tip this scale, which in health should remain balanced; however, in metabolic syndrome, insulin resistance, prediabetes, and overt T2DM, the oxidant–antioxidant scales become tipped, favoring a pro-oxidant milieu within the islet. AGE, advanced glycation endproducts; eNOS, endothelial nitric oxide synthase; GPx, glutathione; IR, insulin resistance; NADH, nicotinamide adenine dinucleotide reduced; NAD^+, nicotinamide adenine dinucleotide oxidized; NAD(P)H, nicotinamide adenine dinucleotide phosphate reduced; ROS, reactive oxygen species; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus.

where R equals any organic compound with a thiol group (-SH) accessible to react with Hcy, such as proteins, cysteine, glutathionine, gamma-glutamylcysteine, or cystinylglycine. In health, reactive thiol species are detoxified through the assistance of superoxide dismutase (SOD) and vitamin C with the aid of catalase within the cytosol. Unfortunately, SOD and catalase are known to be decreased in the T2DM patient and are also overwhelmed in the metabolic syndrome and prediabetes due to the A-FLIGHT-U generated ROS (Fig. 5). Redox stress, as manifested by increased ROS, RNS, and reactive thiol species, may significantly post-translationally modify amylin to the extent that protein misfolding is favored. Additionally, these redox stressors can overwhelm the ER folding complex of the β-cell, chaperone-induction signaling mechanism, lysosome–proteasome pathway, and attenuate the secretory capacity of this cell. These effects likely result in augmented beta cell apoptosis and the accumulation of islet amyloid as discussed below.

Islet and β-cell vulnerability due to diminished islet and β-cell antioxidant network

The islet and β-cell are susceptible to innate and intrinsic low levels of antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione (GPX) (15, 35, 62, 67). Due to the lower level of antioxidant capacity, the oxidant molecules preferentially destroy the β-cell prior to damaging renal, myocardium, or hepatic cells when creating diabetic animal models.

Intrinsic low levels of antioxidants within the islet and β-cell are compromised by continual overconsumption and diminished replenishment due increased ROS and unpaired electrons. In many instances, antioxidant enzymes are codependent on one another and this could result in a dysfunctional antioxidant network (23). Additionally, protein glycation and creation of dysfunctional antioxidant protein conformations, as well as the possibility of various gene polymorphisms in this antioxidant network also may contribute to this dysfunction. Regardless, this scenario (oxidant–antioxidant imbalance) places the islet and β-cell at extreme risk to excess ROS and results in damage to the vulnerable islets and β-cells.

THE ROLE OF ISLET FIBROSIS

It is commonly known and accepted in type 1 diabetes mellitus (T1DM) that islet fibrosis is an end stage finding associated with the extracellular matrix (ECM)/collagen and fibronectin deposition due to islet wounding and a resultant response-to-injury mechanism (20). This may be caused by autoimmune damage to β-cells and islets and other islet environmental factors. However, in T2DM the islet fibrosis story is not as readily known as it is in T1DM.

At autopsy when human pancreata are examined in patients with T2DM, the following fibrotic changes were noted: fibrotic tissues are irregularly distributed throughout the pancreas and associated with a subclinical pancreatitis in the exocrine portion, and this is frequently accompanied by islet amyloid deposition (69). The exocrine-acinar size is contracted with increased fibrosis, demonstrating variable intra- and peri-islet fibrosis (10, 17, 69).

Recently, a study of the Zucker obese rat model, which evolves through IR, prediabetes to overt T2DM, demonstrated a local islet RAS activation with the disruption of islet architecture, fibrosis, and β-cell apoptosis. This model also demonstrates increased islet nitrotyrosine, and transforming growth factor beta (TGF-β) staining. Further, RAS inhibition significantly attenuated islet damage and augmented β-cell mass, which was associated with a reduction in oxidative stress, apoptosis, and attenuation of TGF-β and profibrotic pathways (64). While blockade of the AT_1 receptor did not significantly influence fasting hyperglycemia or glycemic control by glycated hemoglobin measurements, it was associated with a significant improvement of first phase insulin secretion. This seminal study provides novel mechanisms that could partially explain the reduced incidence of new-onset T2DM that has been observed in clinical trials involving RAS blockade. Additionally, this study sheds new light on the role of Ang II in islet fibrosis and oxidative stress.

Pericapillary fibrosis

Recently, our laboratory observed a very early change of pericapillary fibrosis in the Zucker obese model of T2DM by TEM (21), and this has been demonstrated by one other group in the spontaneously diabetic db/db mouse model of T2DM (49). Further, these images strongly suggest impaired trafficking and definite impaired docking of the insulin secretory granule(s) (ISG) with islet capillaries (21), as compared to normal nondiabetic animal models (Figs. 7 and 8). It is interesting that in the various animal models we have studied including the Zucker obese model of T2DM and the Ren2 model of hypertension the ultrastructural finding of pericapillary fibrosis was an early change in the islet, kidney, heart, and liver (Fig. 9). This finding may be one of the earliest structural changes and also be a unifying ultrastructural morphological change in insulin resistance; metabolic syndrome, and T2DM (26).

Intra and peri-islet fibrosis

Intra and peri-islet fibrosis have been demonstrated in human studies of T2DM as well as the animal models previously discussed. In human studies of T2DM intra-islet fibrosis is routinely found to be present; however it has been reported as being variable and not as readily apparent as in the peri-islet areas (69). These observations could be due to the highly cellular content of islets prior to the development of β-cell loss due to apoptosis. In contrast to the variability of the intra-islet fibrosis, in the human islets we have studied there is a more intense, diffuse, and ubiquitous presence of peri-islet fibrosis as compared to intra-islet fibrosis (Fig. 10) (17).

It is interesting to note that the increase in peri-islet fibrosis is associated with increased staining for nitrotyrosine and TGF-β in the exact same peri-islet areas where there is increased peri-islet fibrosis (Figs. 10, 11, and 12). This spatial predilection for the deposition of peri-islet collagen, and its positive association with nitrotyrosine (an oxidative stress marker) and TGF-β (a profibrotic growth factor), indicate the islet is certainly contributing to this increase in peri-islet fibrosis. However, one cannot rule out the contribution of the ECM expansion of the exocrine pancreas to this spatially located peri-islet fibrosis, as the exocrine pancreas is known to harbor

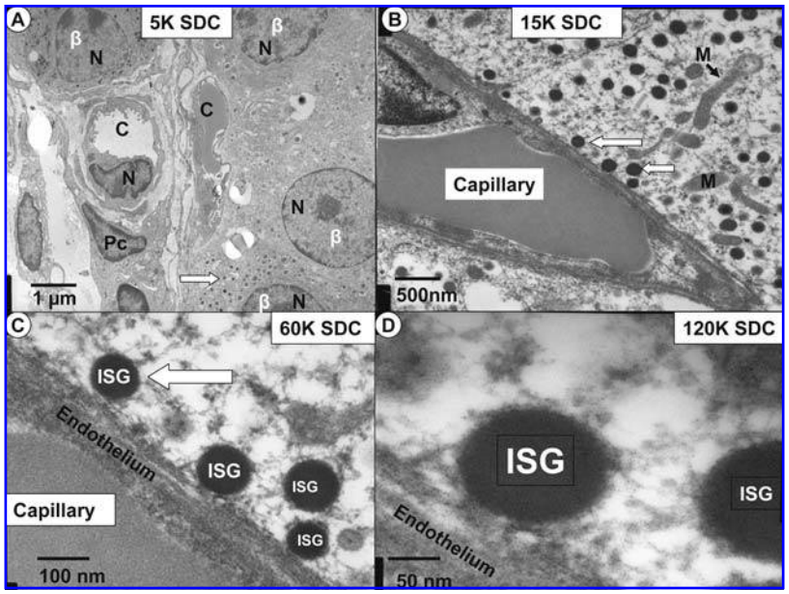

FIG. 7. Normal trafficking and docking of insulin secretory granules in control male animals. (**A**) Four β-cells (β) aligning immediately adjacent to capillaries with minimum separation for proper docking and absorption of the insulin secretory granules (ISG) (*white arrow*) in the 4-month-old male Sprague—Dawley control (SDC) model at 5K magnification. Just below the one endothelial cell of the capillary there is present a pericyte (Pc), which protects endothelial cells. N, nucleus; K, 1000; bar, 1 μm; (**B**) a central capillary endothelial cell in the same model with the β-cell abutting it at 15K magnification. Note how the ISG (*white arrows*) appear to be trafficking and docking with the endothelial cell of the capillary. M, mitochondria; bar, 500 nm; (**C**) a higher magnification (60K) of this trafficking and docking sequence of ISG (*white arrow*) in this same model. Bar, 100 nm; (**D**) an ISG at 120K magnification actually docking with the capillary endothelial cell in the same model. Bar, 50 nm.

a subclinical inflammatory response with increased interstitial fibrosis due to the subclinical pancreatitis associated with T2DM (Fig. 12) (10, 17, 69). Similar findings in others species such as the Zucker obese rat model of obesity, IR and T2DM and the early peri-islet fibrosis in the Ren2 rat model of hypertension and IR (transfected with the mouse renin gene and overexpressing Ang II at the local tissue level) suggest that similar mechanisms may be operative in these models. The Zucker obese rat model has been shown to harbor an increased staining for nitrotyrosine and TGF-β, as discussed previously (64). When this model was treated with ACEI (perindopril) and ARB (irbesartan), there was a significant reduction in nitrative stress as well as a significant reduction in the islet staining for TGF-β. These findings in the animal models suggest that similar mechanisms may be operative in the human studies of T2DM and the Zucker obese rat model. Therefore, the findings in the Zucker rat model may help to better understand the recent clinical trials with RAS blockade in human subjects,

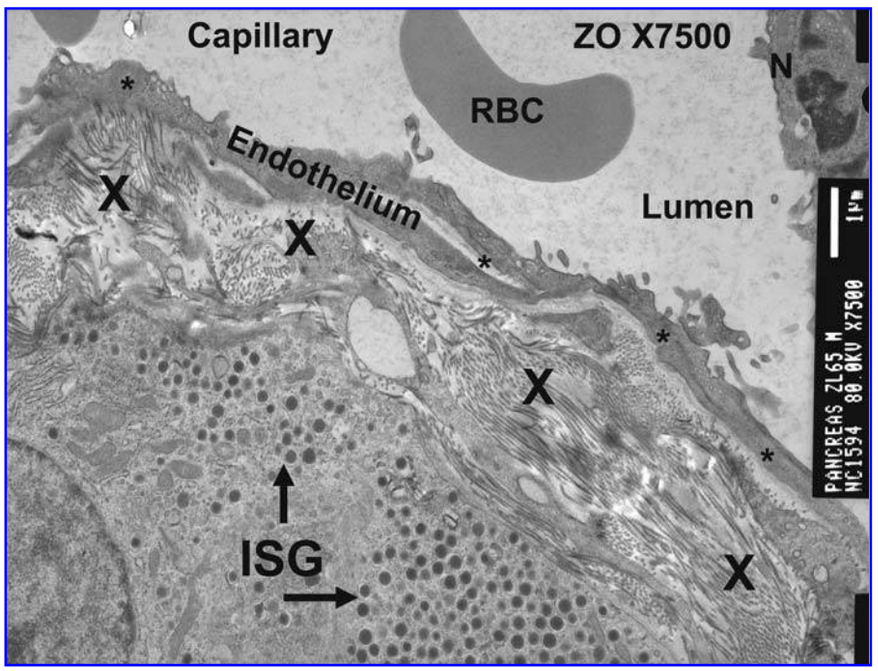

FIG. 8. Pericapillary fibrosis. This transmission electron micrograph demonstrates pericapillary fibrosis (*X*) separating the endothelium (*) from the adjacent β-cell in a 14-week-old male Zucker obese rat model of type 2 diabetes mellitus (magnification X7500; bar, 1 μm). Note how the insulin secretory granules (ISG) (*arrows*) abut the fibrotic tissue and not the endothelium. This pericapillary fibrotic tissue prevents the normal docking of the ISG to the endothelium as demonstrated in Fig. 7. The impaired docking of the ISG could contribute to the impaired first phase insulin secretion noted in the Zucker obese rat model of type 2 diabetes mellitus. N, endothelial cell nucleus; RBC, red blood cell.

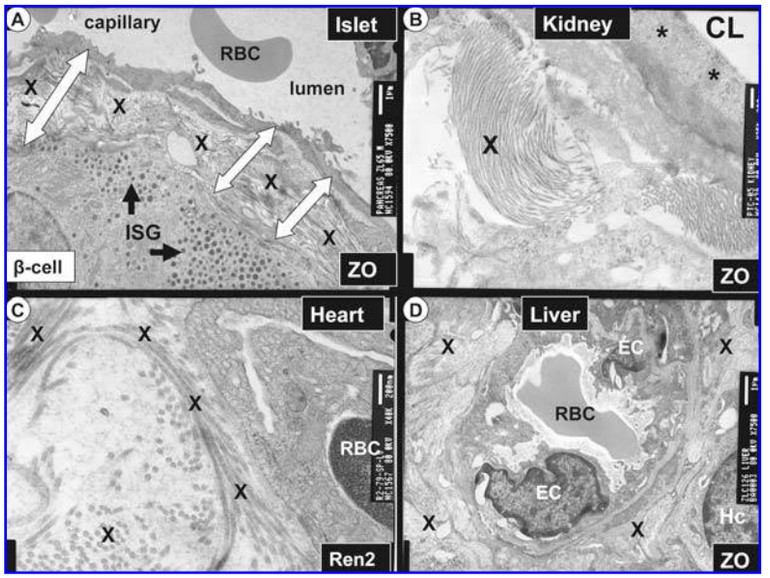

FIG. 9. Early pericapillary fibrosis in the islet, kidney, heart, and liver of animal models of type 2 diabetes mellitus and insulin resistance. (**A**) The same TEM image as in Fig. 8: the pericapillary fibrosis in the islet of the Zucker obese model of type 2 diabetes mellitus; (**B**) a similar pericapillary fibrosis (*X*) in the male 14-week-old Zucker obese model of type 2 diabetes mellitus in the tubulointerstitium of the kidney (magnification, 25K, bar, 200 nm). The capillary lumen (CL) is located in the *upper right hand corner*. Endothelium, (*); (**C**) a similar pericapillary fibrosis (*X*) in the male 11-week-old Ren2 rat model of hypertension and insulin resistance in the myocardium. The capillary lumen in this image is closed and contains a red blood cell (RBC). Magnification, 40K, bar, 200 nm; (**D**) a similar pericapillary–perisinusoidal fibrosis (*X*) in the male 14-week-old Zucker obese model of type 2 diabetes mellitus in the liver. These four different tissues represent early pericapillary fibrosis in four different organs, which may be the very earliest structural change associated with diabetes mellitus: pericapillary fibrosis. Magnification, X7500, bar, 1 μm.

which resulted in a delay in the onset of T2DM in high-risk patient groups with impaired glucose tolerance.

Peri-islet–perivascular fibrosis and the islet–acinar–portal pathway (IAP)

The peri-islet region is where the efferent venules drain the secretory products of the islet and contain the islet–acinar–portal (IAP) vascular pathway. The efferent vascular system is responsible for the delivery of islet contents to the liver and is important in providing the hormone insulin that is assessed with first phase insulin secretion.

The human studies of T2DM demonstrate a marked peri-islet fibrosis with picrosirius red staining and crossed polarized light (Fig. 10). This stain is specific for collagen types I and III and is birefringent with collagen type I staining yellow-golden and collagen type III staining a greenish color (Fig. 10). The IAP vascular pathway originates from the islet as the efferent venule and is responsible for the transport of islet hormones to the portal vein, the liver, and subsequently (via the inferior vena cava) to the heart and systemic circulation in humans. The adventitial area of the efferent vascular pathway (IAP) in the peri-islet area is subject to vascular fibrosis—stiffening could result in impaired delivery of islet contents. These fibrotic vascular

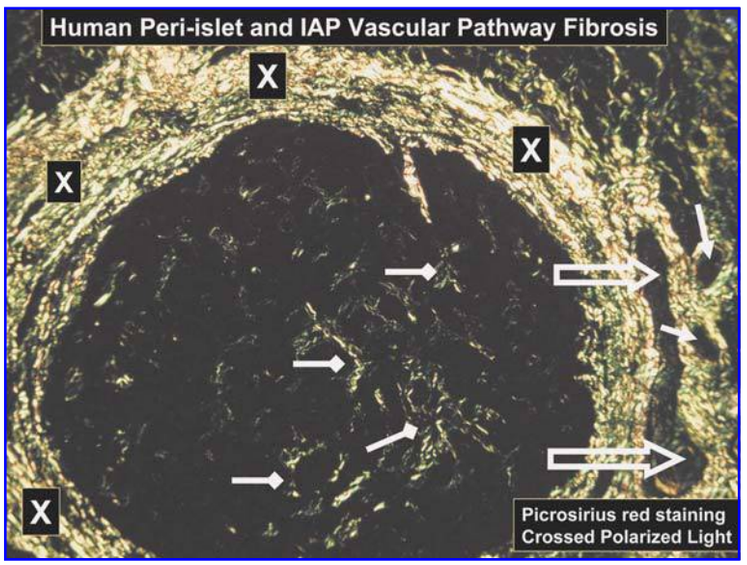

FIG. 10. Human peri-islet and IAP vascular pathway fibrosis. This image demonstrates peri-islet fibrosis (*X*) of a hypertrophic pancreatic islet in a 58-year-old Caucasian female patient who had type 2 diabetes mellitus for 6 years and was insulin dependent for the previous year. This micrograph was taken with from autopsied pancreatic tissue with light microscopy utilizing picrosirius red staining and crossed polarized light. Picrosirius red staining is specific for types I and III collagen and depicts apple-green birefringence. Intra-islet fibrosis (*diamond arrows*) is also present. *Open arrows* demonstrate the islet-acinar-portal (IAP) vascular pathway (venule) and *closed arrows* depict the other IAP vessels (arterioles). This human islet demonstrates both intra- and peri-islet fibrosis with predominate peri-islet fibrosis.

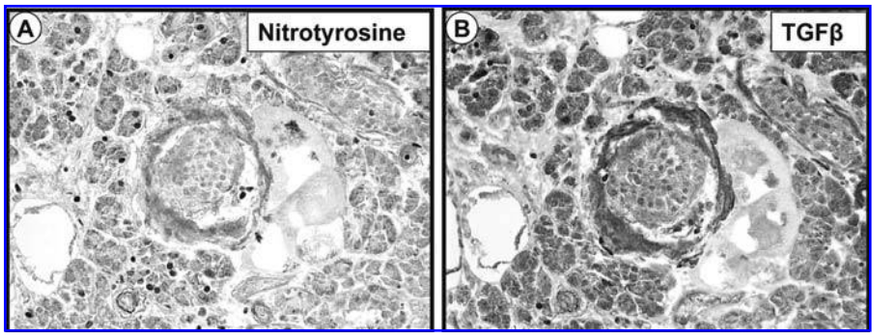

FIG. 11. Immunohistochemical staining of islets with nitrotyrosine and transforming growth factor beta. (**A**) depicts a different human islet than in Fig. 10, which demonstrates pronounced positive staining of nitrotyrosine at the peri-islet region of the islet. Nitrotyrosine is a specific fingerprint for the presence of oxidative stress and note that the peri-islet area of pronounced staining is in the same anatomical region where there is pronounced peri-islet fibrosis; (**B**) the same islet as in (**A**) stained for the presence of transforming growth factor beta. Here again there is intense staining of the peri-islet anatomical region of the islet. These two immunohistochemical stains demonstrate this very important anatomical region of the human islet.

changes of the IAP vessels, combined with the pericapillary fibrosis and impaired ISG docking, could independently and synergistically contribute to the clinical finding of impaired first phase insulin secretion (Figs. 8, 9A, 10, and 12A, B, C) (17).

Verhoeff Van Gieson (VVG) staining to further support islet fibrosis

VVG staining allows for the identification of fibrosis due to the ECM contents having differential staining properties against the background tissue, staining a magenta color under bright field examination (Figs. 12A, B, and C). In the human and Sprague–Dawley rodent transfected with human islet amyloid polypeptide (amylin) gene referred to as the HIP model of T2DM, we were able to identify ECM collagen deposition in the peri-islet region, and this stain will assist in making the HIP rodent model an even better model to study the remodeling effects of not only islet amyloid but also peri-islet fibrosis and the fibrosis of the islet acinar portal vascular pathway with light microscopy (Fig. 13D).

THE EMERGING ROLE OF ISLET AMYLOID

Islet amyloid, also termed amylin and islet amyloid polypeptide (IAPP), is found utilizing Congo red staining and cross polarized light (XPL) in up to 90% of all patients autopsied with T2DM (8, 10, 17, 23, 24, 28, 69). It is possible that the presence of islet amyloid may be as high as 100% if TEM were used because we have noted that there is pericapillary amyloid in islets even when Congo red staining does not demonstrate its presence in the novel human islet amyloid polypeptide (HIP) rat model of T2DM.

The novel HIP rat model was created in 2004 by transfecting the Sprague–Dawley rat with the human amylin gene

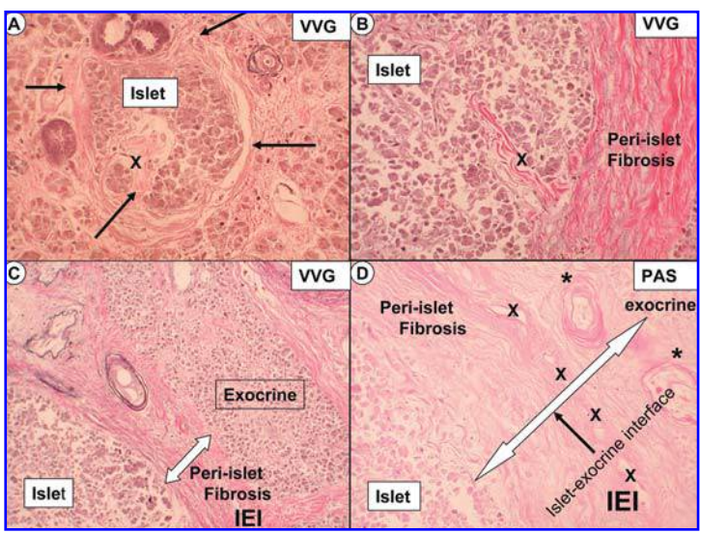

FIG. 12. Islet staining with VVG and PAS. (**A**) A pancreatic islet in the same patient described in Figs. 10 and 11. The *magenta staining* in the peri-islet region of the islet with Verhoeff Van Gieson (VVG) corresponds to the intense peri-islet fibrosis noted in Fig. 10. Note the infiltration of VVG^+ staining infiltrating the intra-islet region (*X* panels A and B) resulting in disorganization of the islet; (**B**) the peri-islet anatomical region of the same islet at higher magnification. Notice the absence of staining of the peri-islet region with VVG creating gaps, as this aids in the understanding of the presence of both collagen fibrosis and islet amyloid deposition in this region; (**C**) excessive fibrosis at the islet–exocrine interface (IEI) (*white double arrow*). The adjacent exocrine pancreas may contribute to the peri-islet fibrosis at the islet– exocrine interface; however, it cannot contribute to the peri-islet amylin-derived islet amyloid deposition as this is derived only from the β-cell within the islet; (**D**) deeper pink coloration of the periodic acid Schiff (PAS) positive staining, which is known to stain mucopolysaccharides, glycoproteins, and glycolipids. In addition to staining the outer peri-islet region incompletely, the PAS-positive staining seems to stain more intensely in the regions of the peri-islet vasculature of the insulino-acinar-portal (IAP) vascular pathway (*X*) and demonstrates an onion-skinning appearance of the more distant arterioles (*).

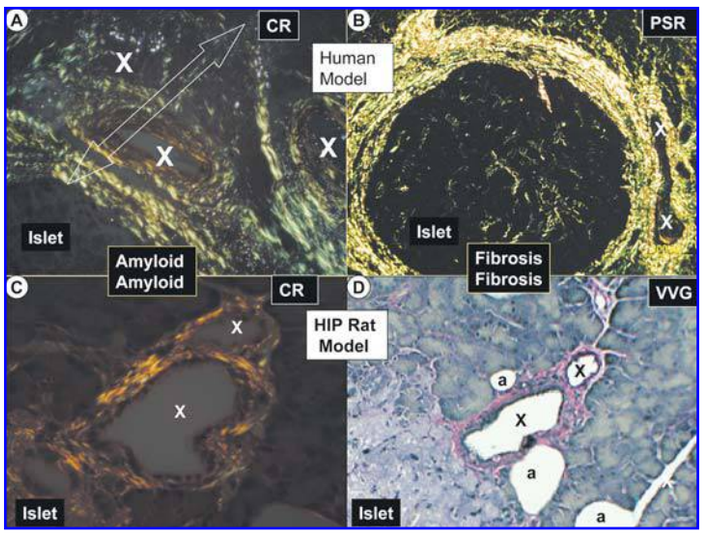

FIG. 13. Peri-islet insulo-acinar-portal vessel staining. (**A**) Apple-green birefringent staining with Congo red (CR) staining in the same human islet in Figs. 10–12 utilizing crossed polarized light. Congo red staining is specific for the birefringent staining of amyloid with crossed polarized light (XPL) of the peri-islet insulo-acinar-portal (IAP) vessels (*X*). This image demonstrates the presence of islet amyloid staining of the IAP vessels in the region of islet–exocrine-interface (IEI) (*open double arrow*); (**B**) birefringent staining with picrosirius red (PSR) staining of collagen in the same peri-islet IAP vessel (*X*) with crossed polarized light (XPL) in the previous Fig. 10; (**C**) apple-green birefringent staining with CR in the human islet amyloid polypeptide (HIP) male model of type 2 diabetes mellitus at 8 months of age. Note the positive staining of the peri-islet IAP vessels (*X*) with XPL. This image supports the finding in (**A**), in that both the human model and the HIP rat model of type 2 diabetes mellitus stain the IAP vessels positive for the presence of islet amyloid; (**D**) fibrosis with VVG staining of collagen in the same peri-islet IAP vessels (*magenta color*) (*X*) with bright field examination in the 8-month-old HIP model. Note the areas of fatty infiltration (*a*). This type of staining has also been noted in the Zucker obese model of T2DM at age 5 months (7). The images **A**–**D** demonstrate that the IAP vessels stain not only for the presence of types I and III collagen, but also islet amyloid, and could contribute to a stiffening and impairment of vascular function and be involved with a delay in first phase insulin secretion in these patients and animal models due to a remodeling structural defect. IAP, islet-acinar-portal; HIP, human islet amyloid polypeptide; T2DM, type 2 diabetes mellitus; VVG, Verhoeff van Gieson.

(7, 44, 45). Rodent models of T2DM do not develop diabetes spontaneously because rodent models do not possess an amyloidogenic amylin. Their amylin is rendered nonamyloidogenic due to proline substitutions at amino acid positions 25, 28, and 29 (Fig. 14). The HIP model is unique, in that it is the first animal model capable of developing islet amyloid deposition similar to humans. It has been previously demonstrated that the 2-, 5-, and 10-month-old HIP model progresses from no diabetes, impaired fasting glucose, and diabetes, respectively (7, 18, 44, 45).

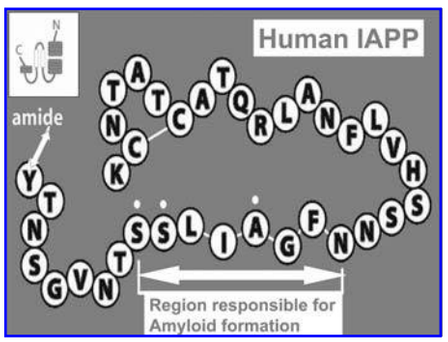

FIG. 14. The primary amino acid structure of human islet amyloid polypeptide hIAPP. Human islet amyloid polypeptide (hIAPP) is a 37 amino acid polypeptide with a known amyloidogenic region spelled commonly as NFGAILS positions 22–29. Proline substitutions at positions 25, 28, and 29 as found in rodent models of diabetes make this region nonamyloidogenic and therefore the rodent models, with the exception of the HIP model of T2DM, do not develop islet amyloid. The secondary structure consists of two alpha chains followed by two beta strands followed by a small alpha chain (*see inset upper left*). The other important area is the C2 and C7 disulfide bridge, which is quite sensitive to oxidative stress and allows the molecule to open, allowing it to be more vulnerable to misfolding within the endoplasmic reticulum.

The 4-month-old HIP model demonstrates an abundance of β-cells and ISGs with significant pericapillary and inter-β-cell islet amyloid deposition. The 8-month-old HIP model demonstrates extensive islet amyloid deposition and marked changes of β-cell apoptosis. The 14-month-old HIP model demonstrates islet and β-cell atrophy, with even greater amounts of extracellular islet amyloid as compared to the 4- and 8-month-old models. Functional beta cells were sparse in the 14-month-old HIP model and were associated with intra-islet adipose deposition. Only traces of intra-islet fibrosis was identified in the 14-month-old HIP model and it can only be hypothesized that the older models (18 and 20 months of age) may indeed demonstrate more significant intra- and peri-islet fibrosis as this animal model ages, similar to the human studies of T2DM (Fig. 15) (18).

Pericapillary amyloid

Similar to pericapillary fibrosis discussed previously, pericapillary islet amyloid seems to be the initial site of amyloid deposition in the HIP rat model of T2DM at 4 months of age, prior to the development of impaired glucose tolerance in the 5-month-old animal and overt T2DM at 10 months of age (Fig. 16) (18). Even at this early stage of 4 months, there appears to be impaired trafficking and definitely impaired docking of the ISGs, and it can be noted that ISGs seem to abut the islet amyloid instead of abutting the capillary endothelial cell as it does in the Sprague–Dawley and the nondiabetic

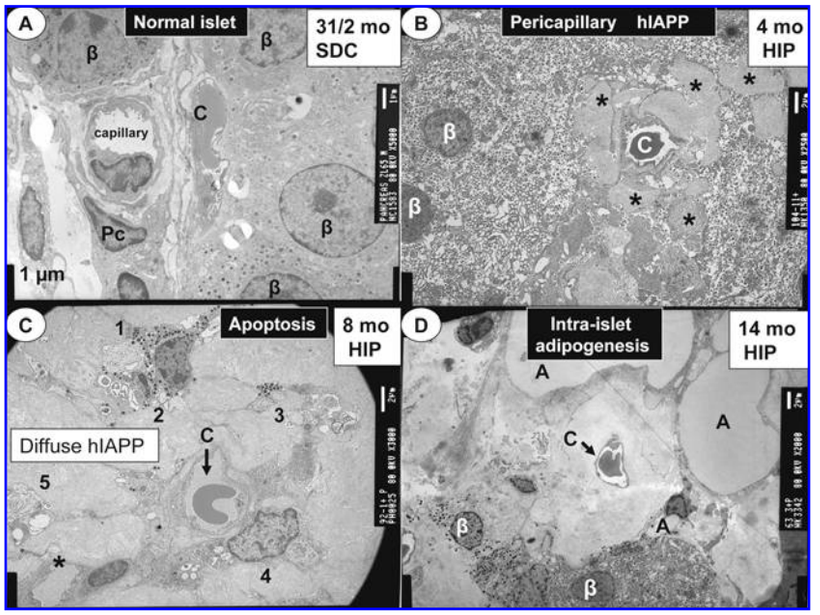

FIG. 15. Significant longitudinal events of the HIP rat model of T2DM. (**A**) Normal findings of the Sprague–Dawley nondiabetic control model and is the same image as in Fig. 7A and the same legend applies to this figure. Magnification, X5000, bar, 1 μm; (**B**) 4-month-old male HIP model of T2DM; the significant finding at this age is pericapillary islet amyloid (*) deposition and inter-β-cell amyloid deposition as in Fig. 2. We have come to term this pericapillary islet amyloid as the flowering or blooming stage of islet amyloid since it resembles the petals of a flower emanating from the capillary (**C**). The β-cells are numerous and comparable to the control models and contain normal numbers of insulin secretory granules as discussed in greater detail in Fig. 16. Magnification, X2500; bar, 2 μm; (**C**) 8-month-old male HIP model of T2DM; the significant finding at this age is β-cell apoptosis. This image demonstrates four to five β-cells in various stages of apoptosis highlighted numerically. Apoptosis is discussed in greater detail in Fig. 22. Magnification, X3000, bar, 2 μm; (**D**) 14-month-old male HIP model of T2DM; the significant finding at this age is much more diffuse amyloid deposition and the development of adipogenesis within the islet. When sparse functioning β-cells (β) could be found, they were routinely accompanied by nearby adipocytes (A) with varying amounts of lipid contents. Capillary (C). Magnification, X2000; bar, 2 μm.

control models in Fig. 4 (Fig. 16). These structural observations regarding the ISGs could certainly help explain the impaired first phase insulin secretion so commonly associated with impaired glucose tolerance in this model, as well as in humans (17). Additionally, this pericapillary islet amyloid at 16 weeks appears very similar structurally to pericapillary fibrosis at 14 weeks, since both pericapillary amyloid and pericapillary fibrosis appear to create a structural barrier, which interferes with the proper docking of the ISGs to the capillary endothelium.

Intra and peri-islet amyloid

The pericapillary region appears to be the very first area involved with intra-islet amyloid in the 4-month-old HIP model. However, the intra-islet amyloid was progressively

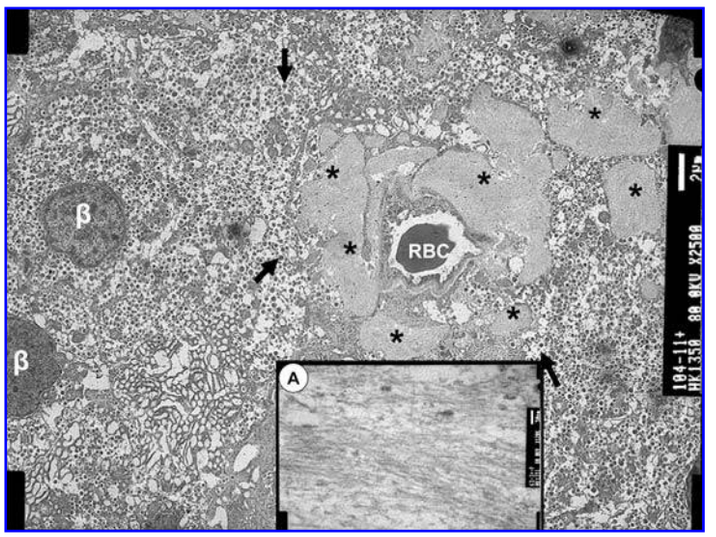

FIG. 16. Pericapillary islet amyloid: human islet amyloid polypeptide (hIAPP). This image depicts the 4-month-old male HIP model of type 2 diabetes mellitus and pericapillary islet amyloid (hIAPP) deposition denoted by asterisks (*). Note the abundance of insulin secretory granules (ISG), which are very small (electron dense) black dots measuring 100–200 nm in diameter. Note the absence of ISG docking with the endothelial cell of the centrally located capillary. Islet amyloid appears to structurally impair ISG docking to the endothelium and instead the ISG seem to abut the pericapillary islet amyloid. Magnification, X2500; bar, 2 μm. (**A, insert**) 120K image of islet amyloid to demonstrate the noncrossed banded parallel arrays and interlacing disordered fibrils with a diameter of 7–10 nm of islet amyloid, in contrast to collagen types I and III (Figs. 18 C, D and 19), which are banded with a diameter ~10-fold larger. X-ray diffraction reveals the adjacent amyloid fibrils to be organized as antiparallel crossed beta-pleated sheet conformations.

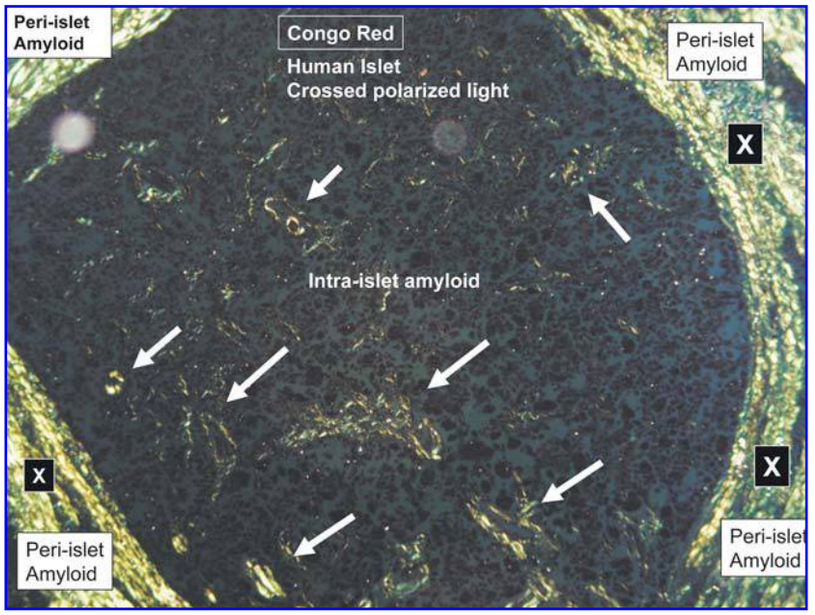

FIG. 17. Human peri-islet amyloid. This image demonstrates apple-green birefringent peri-islet amyloid deposition (*X*) in the same hypertrophic human model of type 2 diabetes mellitus as in Fig. 10. Intra-islet amyloid deposition is also demonstrated (*arrows*). This same islet was stained with Congo red (specific for amyloid) and viewed with crossed polarized light utilizing the light microscope. The staining of peri-islet amyloid occurs regionally in the same areas where peri-islet fibrosis, nitrotyrosine, and TGF-β stained so positive in Figs. 10 and 11. It appears that oxidative stress and the growth factor TGF-β result in the concurrent deposition of collagen (ECM)—fibrosis and islet amyloid.

more diffuse as this animal model aged from 4 to 14 months (Fig. 17). It should be noted that intra-islet amyloid is occupying different areas of the islet than the intra-islet fibrosis in human studies of T2DM in Fig. 7. One of the most important findings in human studies of T2DM is the finding of concurrent deposition of fibrosis and amyloid within the intra-islet, peri-islet, and the IAP vascular pathway.

Peri-islet–perivascular amyloid and the islet–acinar–portal (IAP) vascular pathway

Peri-islet amyloid deposition appears to be concurrent with peri-islet fibrosis and collagen deposition (Fig. 13) and peri-islet fibrotic tissue is laid down alongside the peri-islet amyloid in this region. This spatial and concurrent islet amyloid and fibrotic tissue contributes to the structural thickening of the islet–exocrine interface. This remodeling and thickening of the peri-islet region could interfere with crosstalk or communication (via paracrine or endocrine mechanisms) between the endocrine and exocrine portions of the pancreas.

The islet–acinar–portal (IAP) vascular pathway is important as it serves as a conduit to carry hormones and metabolic byproducts to the liver and eventually to the systemic circulation. Any impairment of this conduit, such as fibrosis or stiffening, could attenuate the delivery of islet hormones, including insulin, and could result in an impaired first phase insulin secretion so commonly associated with impaired glucose tolerance and T2DM. Additionally, in this same region (IAP vascular pathway) the staining with picrosirius red also demonstrates perivascular collagen deposition of both collagen types I and III (Fig. 13). When Zucker obese rats were treated with ACEI or ARB there was a reduction in islet fibrosis and an associated improvement in impaired glucose tolerance (64). While this group did not specifically examine the IAP pathway, their treatments with RAS blockade may have had a positive effect on reducing not only the fibrosis within the islet but also the fibrosis affecting the IAP vascular pathway as well.

THE EMERGING ROLE OF THE ISLET–EXOCRINE INTERFACE

The islet–exocrine interface (IEI) is an important anatomical/functional region in the pancreas, which contains a neurovascular supply and afferent and efferent vessels (IAP efferent venules) (Figs. 18 and 19). The importance of the IEI has become especially apparent during the past decade with the advent of islet transplantation and the need for purification of these islets for donation (29). The structural and functional aspects of the IEI deserve a through investigation due to its critical location. Indeed, this exciting area of study is a fertile soil for future research to better understand how these pancreatic subdivisions communicate and interact as a syncytium. No longer can we think of the pancreas as two separate entities within a single organ, but rather as an integrated organ with two interrelated systems (endocrine and exocrine) in constant communication and functioning synergistically (5, 41).

Significant peri-islet fibrosis and amyloid in this anatomical region occurring concurrently in T2DM could potentially interfere with their paracrine and endocrine communication between the endocrine and exocrine pancreas. Additionally, these remodeling changes could interfere with the afferent delivery of oxygen and nutrients to the islet and the efferent delivery of islet hormones (insulin) and metabolic byproducts of metabolism. Dysfunction of the neurovascular supply and/or vascular drainage of the islet due to remodeling changes could have detrimental results on islet and exocrine function, which could contribute to the delay of first phase insulin secretion and dysfunctional islet exocrine interaction.

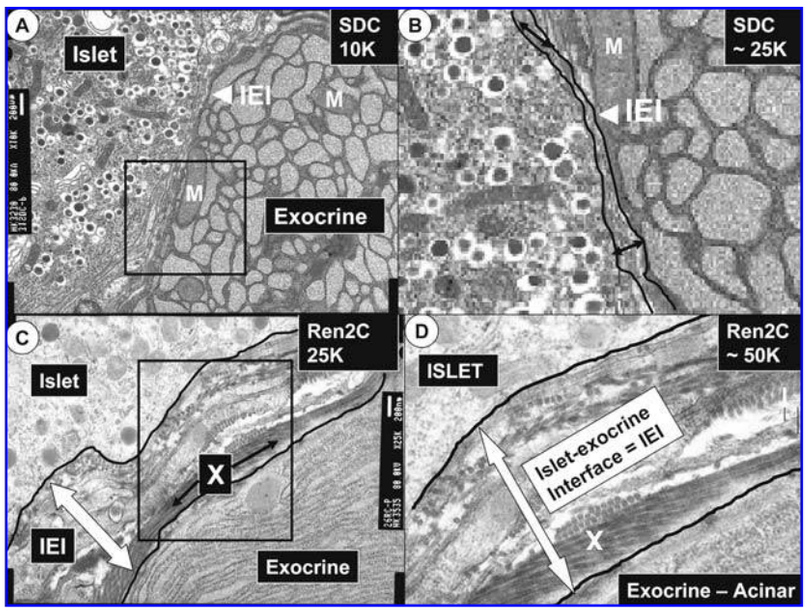

FIG. 18. Anatomical region of the islet–exocrine interface (IEI). (**A**) The anatomical region of the islet-exocrine interface (IEI) in the normal Sprague–Dawley nondiabetic control (SDC) model. Note the islet tissue is on the *left* marked and the exocrine tissue is on the *right* (marked). Further, note the fine line dividing these two completely different morphological regions of the pancreas marked IEI (*arrowhead*). One will note the electron dense secretory granules of the islet cell and in this peripheral location one cannot differentiate between beta, delta, or alpha cells but this cell leans to being an alpha cell morphologically. The acinar cell in the exocrine tissue has been depleted of its zymogen granules and the dense lines represent the endoplasmic reticulum. M, mitochondria; Magnification, 10K; bar, 2 μm; (**B**) an exploded view of (**A**) of the SDC animal model to highlight islet-exocrine interface outlined by hand-drawn dark lines and labeled islet exocrine interface (IEI). Magnification ~25K; bar, none, as this is an exploded view approximately twice the original view; (**C**) the IEI in the 11-week-old male Ren2 rat model of hypertension and insulin resistance. Note how much wider the IEI is in this model (*white closed double arrow*). The islet region on the upper left and the exocrine region of the pancreas are divided by the IEI and one will note the early deposition of banded collagen (*X*) in this interface (*arrows*). The *black box* represents the exploded view in (**D**). Magnification, 25K; bar, 200 nm. (**D**) represents an exploded view of (**C**) in order to further expand this area for better understanding of its relationships between the islet region and the exocrine regions of the pancreas. The background matrix in this area is known to consist of nonbanded type IV collagen and laminin but was not specifically stained for in these images. The IEI is labeled and boundaries depicted by dark hand drawn lines (*bidirectional closed arrow*). Magnification ~50 K as this is an exploded view and therefore bar length does not apply. This image is shown full size in Fig. 19 so that additional information can be shared with a visual background.

Vascular supply and microcirculation of the islet

The vascular supply to the islet is important in its remodeling characteristics and its communication with the local exocrine portion, as well as with the systemic circulation via its efferent venous portal and inferior vena cava connections. Islets vary in size from 40 to 900 μM and are not located randomly but in close parallel development to the pancreatic vascular supply. Each islet receives up to five afferent arterioles, which results in end capillaries reminiscent of a glomerulus, with a polar blood flow from the afferent pole to the efferent pole (46). Efferent arteriolar venules transport the capillary effluent via the IAP vascular pathway to the acinar and ductal portion of the exocrine pancreas. Flow is then to the venous portal system to the liver and eventually to the systemic circulation via the inferior vena cava (46–48). In addition to the local paracrine islet exocrine acinar cell communication, the IAP vascular pathway delivers signaling hormones and byproducts of islet metabolism to the acinar-ductal portion of the pancreas (5, 41, 46). This intricate vascular network aids in the communication between the islet and exocrine portion of the pancreas and is affected by remodeling changes in T2DM.

The islet inflammation paradox: possible role of islet–exocrine interface

The absence of an islet inflammatory response seems somewhat paradoxical in view of the dramatic fibrotic changes commonly observed in animal models and human patients with T2DM. Viewing pancreata in patients with T2DM with light microscopy does not usually reveal any intra-islet inflammation; however, subclinical pancreatitis and fibrosis is frequently observed (Fig. 12C). Further, the lack of islet microscopic inflammatory changes in large human autopsy studies of T2DM (10, 63) could lead to the conclusion that the inflammatory phase is not an important factor in the development of T2DM. However, it is commonly known that inflammation and fibrosis track together in a response to injury mechanism in most tissues. Since the innate immune system may be associated with an increased risk for developing T2DM and some have found evidence for innate immune markers such as white blood cell count, fibrinogen, sialic acid, orosomucoids, and highly sensitive C reactive protein to be elevated (14), then where is the inflammatory response in the islet?

One possible explanation is that the inflammatory phase of wound healing in the response to injury mechanism occurs prior to the granulation or fibrotic phase of wound healing, with observers merely missing the temporal inflammatory component of islet injury. The other explanation could be that the inflammatory reaction occurs in the exocrine portion of this mixed gland, and it is associated with the commonly found subclinical pancreatitis.

Recently, a seminal paper reported that there was an inflammatory response associated with the development of

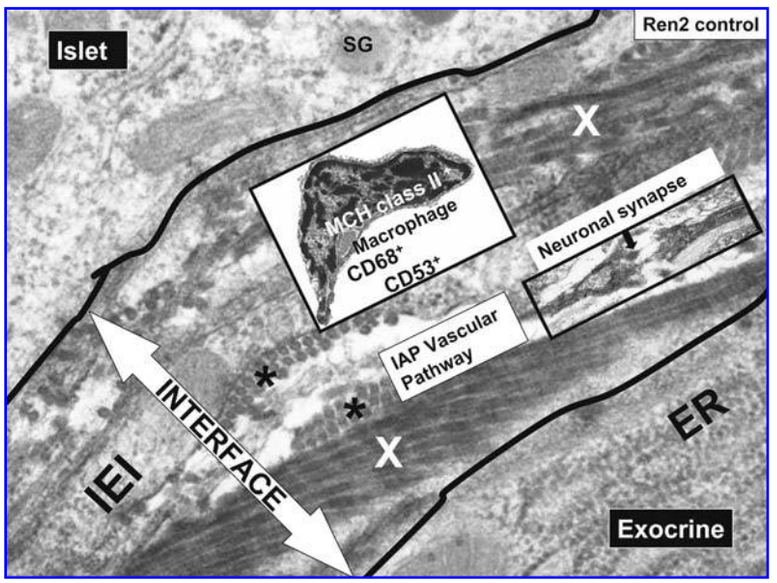

FIG. 19. Islet–exocrine interface in the Ren2 model with early fibrosis (Fig. 18 C). This image represents an exploded view of the 11-week-old male Ren2 Model of hypertension and insulin resistance to demonstrate the very early changes of fibrosis as compared to the Sprague–Dawley nondiabetic control model at 3.5 months of age. In this exploded view, one can easily visualize the islet-exocrine interface (IEI) (*bidirectional closed arrow*) and highlighted with *hand drawn dark lines*. The islet and exocrine-acinar region are labeled and the IEI is readily noted to divide these two anatomical regions of the pancreas. Banded longitudinal collagen (*X*) is readily noted, as well as cross sectional collagen bundles. This anatomical region is known to contain the islet-acinar-portal (IAP) vascular pathway vessels, and neuronal supply to the islets and adjacent exocrine-acinar portion of the pancreas. Recently, peri-islet inflammation in the Goto–Kokisake (GK) nonobese rat model of T2DM was shown to reside in this anatomical region (55). Two *boxed-in areas* represent the importation of a macrophage, demonstrating the positive linage of MHC class II, CD68, and CD53 from Ref. 27, and neuronal axons representing a synapse from other Ren2 animals we have noted and captured. These importations are meant for educational purposes only and were not in the original image. Magnification ~50 K, as the 25K image was double in sized to fit the full image portrayed, Bar has no pertinent meaning in an exploded view.

T2DM in the Goto–Kokisake (GK) nonobese rat model of T2DM. This GK model is obtained by selective inbreeding of mildly glucose-intolerant Wistar rats, and is known to develop intra- and peri-islet fibrosis (27). This model develops an extensive inflammatory response associated with fibrosis, and this inflammatory response was not in the intra-islet region but located specifically to the peri-islet regions of the islet (27). This pathophysiological process consisted of numerous macrophages, including major histocompatibility complex class II $^{+}$ (MHC class II), and CD68 and CD53 positive cells located in the peri–islet–islet endocrine interface.

The role of the innate immune system in the peri-islet-islet endocrine interface, coupled with the temporal and spatial location of fibrosis in this model, helps to explain why the inflammatory response was visually lacking in prior studies of islets in T2DM. Further, these findings in the GK model suggest that inflammation is indeed "part and parcel" of the islet fibrotic process and implicates the important anatomical region of the islet exocrine interface.

Islet–exocrine (islet–acinar–ductal) communication

The IEI contains the neurovascular pathway, which is the important vascular connection between the islet and the exocrine portion of the pancreas. This vascular communication coupled with the paracrine mechanisms has been termed the islet–acinar–ductal pathway (5). The islet–exocrine synergism could be disrupted due to remodeling (ECM fibrotic and amyloid deposition) within the IEI as demonstrated in both human and animal studies of T2DM. These important anatomical relationships within the pancreas could become dysfunctional, resulting in a cellular tissue uncoupling as previously described between the endothelium and cellular uncoupling (Fig. 9).

In summary, within the anatomical region of the IEI reside the neural and IAP vascular pathways, which allow for the cerebral neural communication and the islet vascular communication with the acinar and ductal elements of the exocrine portion of this mixed glandular organ. Therefore, the pancreas should be envisioned as an organ consisting of the triad of an endocrine–exocrine–ductal axis with active paracrine and endocrine (islet–acinar–ductal) communication and complex interactions, many of which remain to be discovered.

ISLETOPATHY

There are numerous histopathological abnormalities within the islets in patients with T2DM. Prior investigations of pancreata in patients with T2DM have demonstrated multiple histological abnormalities consisting of islet amyloid, decreased β-cell mass/volume, and increased β-cell apoptosis, arteriolosclerosis, and atherosclerosis. Adipogenesis occurs primarily in exocrine tissue but occasionally within islets, exocrine fibrosis with low-grade inflammation, and variable intra and peri-islet fibrosis (10, 69). Since these similar findings are present in other end organs affected by T2DM, it seems appropriate to use the term, isletopathy.

Concurrence of islet fibrosis and amyloid

The Congo red and picrosirius red stains, when viewed with crossed polarized light, demonstrate that islet and peri-islet remodeling occur concurrently (Fig. 13) in the HIP rat

model and human studies of T2DM. This concurrence of fibrosis and amyloid deposition is also found in the peri-islet vascular supply involving the afferent and efferent vessels or the IAP vascular supply within the IEI. This regional location of the IAP vascular pathway further points to the importance of this anatomical region of the pancreas.

Periodic acid Schiff (PAS) staining to further support an isletopathy in T2DM

Periodic acid Schiff (PAS) stains specifically for the presence of mucopolysacchrides, glycosaminoglycans, and glycolipids. PAS positive staining is observed to stain positive in the myocardium in those patients with diabetic cardiomyopathy and in the kidneys of patients with diabetic nephropathy. The presence of PAS positive staining within the peri-islet and islet-exocrine interface vessels, compatible with arteriolosclerosis of the human model of T2DM, supports comparable remodeling changes known to be present in at least two other diabetopathies (Fig. 12D).

The emerging role of the pericyte and the pancreatic stellate cell in isletopathy

The pericyte is a ubiquitous mesenchymal-derived mural cell found throughout the vasculature and serves both a supportive and protective role regarding the capillary endothelium (Fig. 20) (3, 50). There is a very close homology between the pericyte and the vascular smooth muscle cell and it is thought that vascular smooth muscle cells may give rise to pericytes and vice versa at times of vessel growth and remodeling. Additionally, these mesenchymal qualities make it difficult to differentiate precisely these two mural cell types *in vivo* and *in vitro* (50).

Pericyte foot processes are in close contact with the capillary endothelial cells and communicate directly by sharing their basement membranes through identifiable ultrastructures termed peg-sockets (Fig. 20C) (3). Pericytes seem to be injured prior to the endothelial cell upon exposure to oxidative stress and metabolic toxicities. This early injury results in cellular remodeling (hypertrophy and edema, increases in alpha smooth muscle actin, increased matrix metalloproteinase-9 (MMP-9), and even morphing of pericytes into other cells types, migration, and apoptosis). Injurious stimuli results in pericyte apoptosis prior to its vulnerable vascular companion: the endothelial cell.

The earliest vascular structural findings on TEM in diabetes are pericyte hypertrophy, loss of vascular foot processes, and eventually apoptosis. The pericyte will literally lay down its life for the endothelial cell and may be referred to as the guardian angel of the endothelial cell (20, 25). Pericytes are multipotential cells with stem cell-like properties and are capable of morphing into many other mesenchymal cells including fibroblasts, osteoblasts, chondrocytes, adipocytes, and possibly islet pancreatic stellate cells (11, 60). The vulnerability of the pericyte is reminiscent of the islet β-cell in that each of these cells is sensitive to an elevated tension of oxidative–redox stress (20).

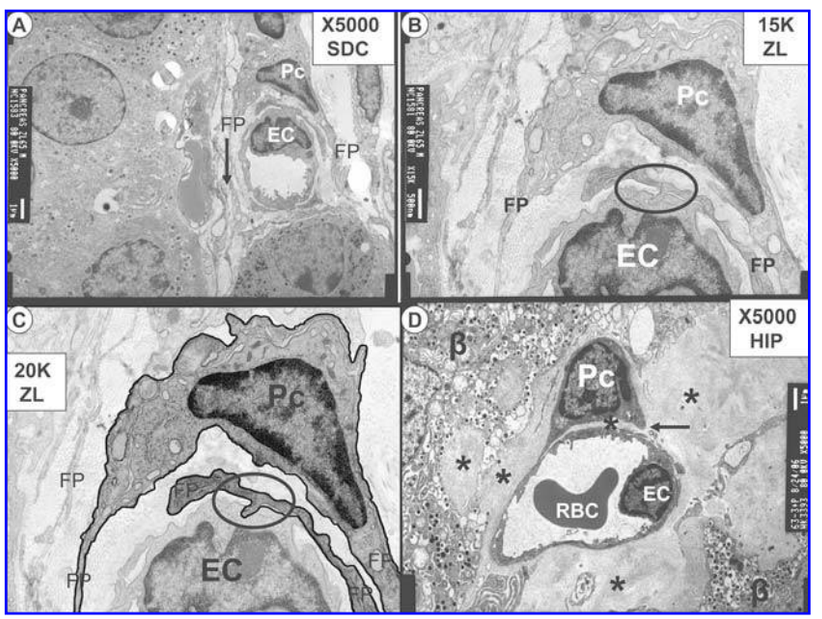

FIG. 20. Pericyte endothelial interaction diabetic compared to nondiabetic. (**A**) The nondiabetic Sprague–Dawley control (SDC) model; this image has been discussed previously in Figs. 15A and 17A. (**B**) 3.5-month-old male nondiabetic Zucker lean model control. This image depicts the close interaction of the pericyte (Pc) and the endothelial cell (EC) with the sharing of basement membranes and demonstrats the insertion of an extension of one of the foot process (FP) (*encircled*) of the Pc into an invagination of the EC and termed the "peg socket." This peg socket allows for the direct cellular communication between the two cells. The foot process encircles the endothelial cells in health. (**C**) is taken from a 20K image of the Zucker lean model in (**B**) and colored to highlight the pericyte (Pc) and to differentiate it from the endothelial cell (EC). This image highlights the peg socket where the Pc foot process extension enters into an invagination of the EC, allowing for direct cellular communication between these two important cells, close cellular communication, and direct crosstalk; (**D**) demonstrates the loss of the pericyte (Pc) foot process due to the islet oxidative stress and the diffuse amyloid (*) structural alterations of the islet and the Pc–EC interaction form a structural standpoint. Note how islet amyloid is occurring between the Pc and the EC (* *and arrow*) in the male 14-month-old HIP rat model of type 2 diabetes mellitus. Notice once again that the amyloid (hIAPP) disallows the insulin secretory granules (*electron dense black dots*) from docking with the endothelium similar to Figs. 15B and 16. No peg sockets were noted in any of the Pc–EC interactions in the 14-month-old HIP model during our investigations. This image almost gives the impression that the pericyte may undergo apoptosis in the very near future and that some of the apoptotic cells adjacent to the endothelial cell in Figs. 15C and 22A may well indeed be apoptotic Pc.

The pancreatic stellate cell is very important to the pancreatic fibrotic process, and it may have its origins from the multipotential pericyte within the islet and the islet exocrine interface of the pancreas (52). The pericyte is susceptible to oxidative damage as a result of its surrounding interstitial oxidative stress and also from its companion endothelial cell once its endothelial nitric oxide synthase (eNOS) enzyme uncouples and the vasculature becomes a net producer of ROS in the metabolic syndrome and T2DM due to the multiple metabolic toxicities (Fig. 5) and their excess generation of ROS (20, 22, 25). Once the pericyte is lost by either dysfunction or apoptosis, the capillary endothelium becomes very vulnerable to oxidative stress and is also highly vulnerable to the glucotoxicity associated with postprandial and sustained hyperglycemia of T2DM (25).

There exists in T2DM a diffuse endotheliopathy due to the associated multiple metabolic toxicities, including the reductive stress or pseudohypoxia (30, 68) associated with hyperglycemia or glucotoxicity, and this reductive vascular stress contributes to vascular damage resulting in the endothelium becoming a net producer of ROS instead of being a net producer of endothelial derived nitric oxide (NO) or eNOS uncoupling. The eNOS enzyme consists of three vulnerable arms as labeled below and is capable of uncoupling when exposed to excess oxidative-redox stress.

$$\begin{array}{c} O_2 \\ \text{NAD(P)H Oxidase} \\ \text{(2) eNOS} \\ \text{(1) L-arginine ---------- } \rightarrow \text{ NO + L-citrulline + NAD(P)}^{+} \\ \text{(3) } BH_4 \end{array}$$

The abundance of ROS and unpaired electrons, whether they be from the net effect of oxidation or reduction, interfere with the eNOS enzyme by primarily oxidizing the prerequisite cofactor tetrahydrobiopterin (BH_4) to BH_3 or BH_2 which will not run the eNOS reaction, resulting in the endothelium becoming a net producer of superoxide, termed eNOS uncoupling as follows.

$$\begin{array}{c} O_2 \\ \text{NAD(P)H ------- NAD(P)H Oxidase ------- } \rightarrow \text{ NAD(P)+ + } O_2^{\cdot -} \end{array}$$

The MMP–TIMP axis

Isletopathy necessitates a discussion of the matrix metalloproteinase(s) (MMP) tissue inhibitor of matrix metalloproteinase(s) (TIMP) axis due to the necessity of these important proteases: antiproteinases in ECM remodeling within the islet. In order to remodel any structure the body must first tear down the structure utilizing MMPs before it can rebuild the structure. Thus, there is both a temporal and spatial organization of the MMP–TIMP axis, and it is not unusual for both MMPs and TIMPs to demonstrate activity at the same time in an actively remodeling tissue such as the islet (Fig. 21). As a general rule in matrix remodeling: The more robust the signal for MMP activation (as in oxidative stress represented by the positive nitrotyrosine fingerprint) (Fig. 11A), the more extensive the accumulation of ECM resulting in fibrosis and scarring.

The MMP–TIMP axis plays an important but poorly understood role in islets of T2DM patients. In both animal and human models of T2DM there is evidence of an active MMP–TIMP axis within the islet (65, 70). MMP-2, and MMP-12 and -14 are upregulated in islets of the Zucker obese rat model of obesity and T2DM, which contribute to islet fibrosis. Further, when a broad-spectrum MMP inhibitor was given, it resulted in significant reduction in the density of islet fibrosis and prevention of overt T2DM (70). In human islets MMP-2, MMP-9, TIMP-1, and TIMP-2 have been identified and this axis may be both temporal and spatially activated (65). Regarding the human studies of T2DM imaged for the current review there was activation of MMP-2, MMP-9, and TIMP-1 (Fig. 21).

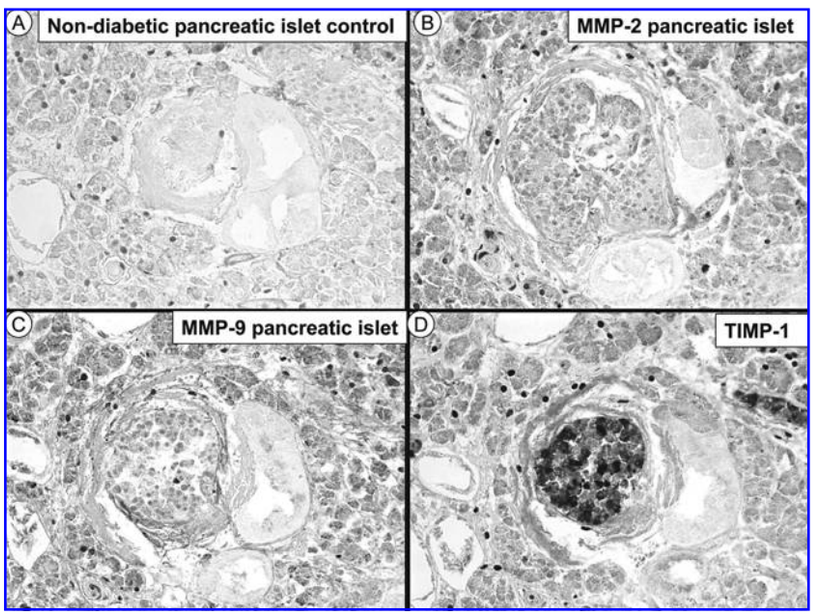

FIG. 21. An active islet MMP-TIMP-2 axis in the human model islet. (**A**) represents the background staining of a nondiabetic human pancreas. There are no significant signals in either the intra or peri-islet areas; (**B**) mild staining in the human model of type 2 diabetes in islets from the same pancreas as discussed in Figs. 10–13 and 17. The MMP-2 positive staining seems to be primarily in the peri-islet regions and also the adjacent exocrine tissue; (**C**) mild MMP-9 positive staining in the same human model in the peri-islet and adjacent exocrine tissue and pancreas as in (**A**); (**D**) very strong positive staining for TIMP-1 in the intra-islet areas and in some surrounding areas of the exocrine tissue. Little is known regarding the activity of the various MMP–TIMP axis in the islet, but what these images do demonstrate that there is, indeed an active MMP–TIMP-1 axis in this human patient with type 2 diabetes mellitus and gives reason to continue further study in this area.

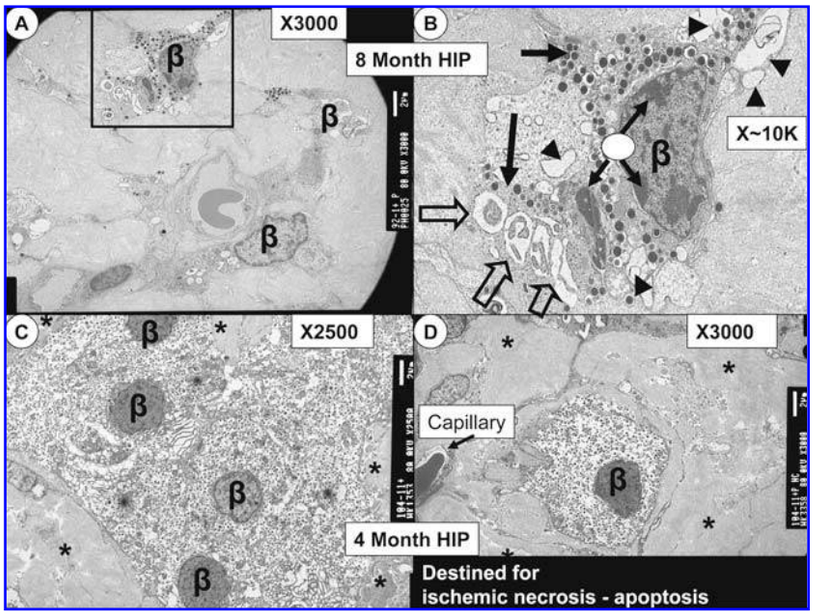

FIG. 22. β-Cell apoptosis in the HIP rat model of type 2 diabetes mellitus. (**A**) Multiple β-cells in various stages of apoptosis; diffuse nature of islet amyloid (hIAPP) during the time frame when this animal model is known to develop overt hyperglycemia and overt type 2 diabetes. This image was previously discussed in Fig. 15C. This image supports other findings that β-cell apoptosis is one of the primary causes for loss in β-cell mass and volume in the HIP model. Please note that the cells undergoing apoptosis adjacent to the endothelial cell could be apoptotic pericytes since some of them do not have insulin secretory granules present. The *boxed in area* is magnified in (**B**): an exploded image of two β-cells undergoing apoptosis. In this view one can still see many *electron dense black circles* representing insulin secretory granules present (*arrows*), classic apoptotic bodies (*open arrows*), chromatin clumping and membrane vesiculation with vacuoles (*arrowheads*); (**C**) at least four β-cells with encroachment of islet amyloid on three to four fronts; (**D**) a single β-cell in a sea of islet amyloid. This single β-cell is separated by the structural barrier of islet amyloid from its nearest capillary (*marked*) with an embedded red blood cell. This image causes fear for the survival of this lonely β-cell since it has completely lost its connectivity and is distanced from nourishment and oxygen.

β-*Cell apoptosis*

Apoptosis in β-cells results when there is an imbalance of pro-apoptotic death gene activation and anti-apoptotic survival mechanisms. Apoptosis is now believed to be the primary mechanism for the reduction of β-cell mass and volume in humans with T2DM (8).

Apoptosis of β-cells is known to be induced by excessive elevations in glucose (glucotoxicity), free fatty acids, human amylin (hIAPP), ROS, calcium leakage through plasma membranes, and the unfolded protein response (UPR) if chronically sustained as occurs in the increased demand on the β-cell during the sustained compensatory stage of insulin resistance and the requirement for sustained hyperinsulinemia (commonly referred to as β-cell fatigue (7, 24, 44, 45). Initially, this UPR is thought to be protective of the β-cell; however, over time and with sustained activation of the UPR this response may lead to apoptosis (24).

β-Cell apoptosis in T2DM may be thought of as a triple hit phenomenon (Fig. 22). The first-hit could result from and excess demand on the β-cells in order to meet the demands of increased insulin production inducing chronic and sustained ER stress and activation of the UPR. In turn, this could result initially in β-cell dysfunction and eventually cell death (2, 8, 24, 37, 53).

The second-hit could involve plasma membrane toxicity due to the vesicle formation as a result of soluble oligomers of islet amyloid (hIAPP–amylin) or protofibrils creating invaginations in β-cell plasma membranes. This would allow for calcium ion leakage within the cytosol and thus promote β-cell apoptosis (7, 24, 32, 44, 45). The third-hit could involve islet and β-cell toxicity from the multiple metabolic toxicities (A-FLIGHT-U) (Fig. 5) responsible for the production of excess ROS and especially the generation of ROS via glucotoxicity via auto-oxidation, glycation (AGE and its receptors RAGE) reactions, and reactive carbonyls (Figs. 4, 5, 6, and 22). Further, in the presence of glucotoxicity, lipotoxicity may add to the oxidative stress and ROS generation via an increase in oxidative stress, beta-oxidation of free fatty acids, and ceramide-associated lipotoxicity in redox stress associated β-cell apoptosis (54, 57).

Possible β-*cell necrosis*

β-Cell apoptosis is known to be induced by immature soluble oligomers of hIAPP–amylin and replicating β-cells are more susceptible (7, 44, 45, 55). Even though the insoluble mature hIAPP aggregates-fibrils of islet amyloid are not toxic to the β-cell, they may create a diffusion barrier, a secretory and absorptive defect, which could starve the remaining β-cells (Fig. 22C and D). The structural defect of islet amyloid could permit β-cells to suffer ischemic necrosis—apoptosis by impairing the transport of oxygen and allowing the accumulation of toxic metabolic byproducts of metabolism. The ultrastructural images in Fig. 22 support this notion in the novel HIP rat model of T2DM.

CONCLUSION AND PERSPECTIVE

Type 2 diabètes mellitus (T2DM) is a heterogeneous, multifactorial, polygenic disease characterized by a defect in insulin action (insulin resistance) and insulin secretion (including β-cell defects, islet structural changes of islet fibrosis and amyloid, and loss of β-cell mass-volume primarily through apoptosis). In order to briefly describe the relation between islet function and structure in T2DM, one might begin with a blank canvas and then proceed to paint in the various mechanisms.

Concerning the background of polygenic abnormalities (gene polymorphisms) and environmental stressors (overnutrition and underexercise), there is development of a peripheral resistance to endogenous insulin and a host of metabolic toxicities (A-FLIGHT-U) (Fig. 5), contributing to an excess production of ROS. In response to IR, there is a compensatory excess secretion from the β-cell via its insulin secretory granules of insulin, proinsulin, and amylin. Each of these excessive islet hormones is known to activate the renin–angiotensin system; recent publications have demonstrated that indeed there is a local islet RAS activation not only in animal models but also humans with T2DM. As glucose becomes elevated (even postprandial excursions), there is further activation of tissue islet RAS. The ROS generated from the multiple metabolic toxicities coupled with glucose and RAS activation results in even greater amounts of islet ROS.

The excess of ROS, coupled with an innate islet and β-cell deficient antioxidant network, result in islet wounding; in a response to injury mechanism, there is a recapitulation of embryonic genetic memory that is associated with activation of nuclear transcription and growth factors, resulting in islet wound repair. This wound repairing process results in a chronic wound healing response and fibrosis. In humans, nonhuman primates, feline species, and the rodent models transfected with human amylin (hIAPP) there is the concurrence of islet amyloid deposition with islet fibrosis due to their amyloidogenic amylin. Each of these remodeling changes seems to occur quite early in the pericapillary area, and over time quite diffusely in the periislet areas. There is a more gradual progression in the intra-islet deposition. In addition to this ECM remodeling, there are cellular remodeling changes that result in β-cell apoptosis due to the toxic effects of oligomers of hIAPP and ROS. Additionally, there may be a role for β-cell necrosis as progressive islet amyloid and fibrotic deposition physically surround and separate the β-cell from its nutrient capillary supply (Fig. 22D).

This brief summary of islet cellular and ECM remodeling due to islet amyloid and islet fibrosis helps to structurally and functionally better understand the islet wounding phenomenon and the body's response to injury, resulting in islet scarring and the hyalinization (islet amyloid deposition) of the islet as described over 100 years ago by Eugene Lindsey Opie during his examination of islets in adult diabetic patients (28).

The introduction of the islet-exocrine interface (IEI) as an important anatomical region in the islet may allow researchers to focus more on this exciting area of the islet, for further knowledge of its role in islet wounding and remodeling. The better understanding of this important anatomical region may help to bring gastroenterologists, pancreatic researchers, and endocrinologists closer together with an increase their collaborative studies.

A better understanding of islet RAS mechanisms may help in understanding how the various proteins such as neprilysin and ACE2 are involved in islet remodeling and function. In depth scientific studies of transgenic rodent models, which overexpress the renin gene, may prove to be very helpful in the future to assist in the better understanding of islet RAS and its clinical translation to human medicine.

If T2DM is viewed similar to other end organs affected by T2DM and thought of as a disease of the islet (isletopathy), then clinicians and researchers may be more inclined to focus their thoughts on the vulnerable islet and β-cell.

ACKNOWLEDGMENTS

The authors wish to acknowledge the Peter C Butler laboratory of the Larry Hillblom Islet Research Center, David Geffen School of Medicine, University of California, Los Angeles, California, who provided tissue specimens for morphologic study with TEM. Additionally, the authors would like to acknowledge the Electron Microscopic Core Center at the University of Missouri, Columbia, Missouri for their excellent help and tissue preparation of animal samples for viewing. The authors also wish to thank Poorna R. Karuparthi, M.D. for his tireless efforts in TEM image collection and analysis of these images and his critical appraisals and suggestions provided for this review. The authors greatly acknowledge James R. Turk, DVM, Ph.D., Professor, Department of Veterinary Pathobiology—College of Veterinary Medicine, University of Missouri, Columbia, Missouri for his excellent assistance regarding the immunohistochemistry images presented in this article.

This research was supported by the National Institutes of Health Grant R01 HL73101-01A1, the Veterans Affairs Merit System (0018), and Novartis Pharmaceuticals for James R Sowers, M.D.

ABBREVIATIONS

ACEI, angiotensin converting enzyme; AGE, advanced glycation endproducts; Ang II, angiotensin II; AT_1, angiotensin type 1; AT_1 R, angiotensin type 1 receptor; ARB, angiotensin receptor blocker; BH_4, tetrahydrobiopterin; ECM, extracellular matrix; eNO, endothelial nitric oxide; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; GPx, glutathione; hIAPP, human islet amyloid polypeptide; HIP, human islet amyloid polypeptide; IAP, islet-acinar-portal; IAPP, islet amyloid polypeptide; IEI, islet–exocrine interface; IR, insulin resistance; ISG, insulin secretory granule; MHC, major histocompatibility class; MMP, matrix metalloproteinase; NAC, *N*-acetyl-L-cysteine; NAD^+, nicotinamide adenine dinucleotide oxidized; NADH, nicotinamide adenine dinucleotide reduced; NAD(P)H, nicotinamide adenine dinucleotide phosphate reduced: PAS, periodic acid Schiff; RAGE, receptor for advanced glycation end products; RAS, renin angiotensin system; ROS, reactive oxygen species; SOD, superoxide dismutase; TEM, transmission electron microscopy; TGF-β, transforming growth factor beta; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TIMP, tissue inhibitor of matrix metalloproteinase; VVG, Verhoeff van Gieson; XPL, crossed polarized light.

REFERENCES

1. Amos AF, McCarty DJ, and Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med* 14: S1–S85, 1997.
2. Araki E, Oyadomari S, and Mori M. Impact of endoplasmic reticulum stress pathway on pancreatic beta-cells and diabetes mellitus. *Exp Biol Med* 228: 1213–1217, 2003.
3. Armulik A, Abramsson A, and Betsholtz C. Endothelial/pericyte interactions. *Circ Res* 97: 512–523, 2005.

4. Baynes JW and Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* 48: 1–9, 1999.
5. Bertelli E and Bendayan M. Association between endocrine pancreas and ductal system. More than an epiphenomenon of endocrine differentiation and development? *J Histochem Cytochem* 53: 1071–1086, 2005.
6. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 54: 1615–1625, 2005.
7. Butler AE, Jang J, Gurlo T, Carty MD, Soeller WC, and Butler PC. Diabetes due to a progressive defect in beta-cell mass in rats transgenic for human islet amyloid polypeptide (HIP Rat): a new model for type 2 diabetes. *Diabetes* 53: 1509–1516, 2004.
8. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, and Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52: 102–110, 2003.
9. Carlsson PO. The renin-angiotensin system in the endocrine pancreas. *JOP* 2: 26–32, 2001.
10. Clark A, de Koning EJ, Hattersley AT, Hansen BC, Yajnik CS, and Poulton J. Pancreatic pathology in non-insulin dependent diabetes (NIDDM). *Diabetes Res Clin Pract* Suppl: S39–47, 1995.
11. Collett GDM and Canfield AE. Angiogenesis and pericytes in the initiation of ectopic calcification. *Circ Res* 96: 930–938, 2005.
12. Cooper ME, McNally PG, Phillips PA, and Johnston CI. Amylin stimulates plasma renin concentrations in humans. *Hypertension* 26: 460–464, 1995.
13. DeFronzo RA. Lilly Lecture 1987: the triumvirate: cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes* 37: 667–687, 1988.
14. Duncan BB and Schmidt MI. Chronic activation of the innate immune system may underlie the metabolic syndrome. *Sao Paulo Med J* 199: 122–127, 2001.
15. Grankvist K, Marklund SL, and Taljedal IB. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. *Biochem J* 199: 393–398, 1981.
16. Griendling KK, Minieri CA, and Ollerenshaw JC. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 74: 1141–1148, 1994.
17. Hayden MR. Islet amyloid and fibrosis in cardiometabolic syndrome and type 2 diabetes mellitus. *J Cardiometabolic Syndr* 2: 70–75, 2007.
18. Hayden MR, Poorna R Karuparthi PR, Manrique CM, Lastra G, Habibi J, and Sowers JR. Longitudinal ultrastructure study of islet amyloid in the HIP rat model of type 2 diabetes mellitus. *Exp Biol Med* (in press), 2007.
19. Hayden MR and Sowers JR. Hypertension in type 2 diabetes mellitus. *Insulin* 1: 22–37, 2006.
20. Hayden MR, Sowers JR, and Tyagi SC. The central role of vascular extracellular matrix and basement membrane remodeling in metabolic syndrome and type 2 diabetes: the matrix preloaded. *Cardiovasc Diabetol* 4: 9, 2005.
21. Hayden MR, Stump C, and Sowers JR. Organ involvement in cardiometabolic syndrome. *J Cardiometabol Syndrome* 1: 16–24, 2006.
22. Hayden MR and Tyagi SC. Is type 2 diabetes mellitus a vascular disease (atheroscleropathy) with hyperglycemia a late manifestation? The role of NOS, NO, and redox stress. *Cardiovasc Diabetol* 2: 2, 2003.
23. Hayden MR and Tyagi SC. Islet redox stress: the manifold toxicities of insulin resistance, metabolic syndrome and amylin derived islet amyloid in type 2 diabetes mellitus. *JOP* 3: 86–108, 2002.
24. Hayden MR, Tyagi SC, Kerklo MM, and Nicolls MR. Type 2 diabetes mellitus as a conformational disease. *JOP* 6: 287–302, 2005.
25. Hayden MR, Tyagi SC, Kolb L, Sowers JR, and Khanna R. Vascular ossification-calcification in metabolic syndrome, type 2 diabetes mellitus, chronic kidney disease, and calciphylaxis-calcific uremic arteriolopathy: the emerging role of sodium thiosulfate. *Cardiovasc Diabetol* 4: 4, 2005.
26. Hayden MR, Whaley–Connell A, and Sowers JR. Role of angiotensin II in diabetic cardiovascular and renal disease. *Current Opinions Endocrinol Diabetes* 13: 135–140, 2006.
27. Homo–Delarche F, Calderari S, Irminger JC, Gangnerau MN, Coulaud J, Rickenbach K, Dolz M, Halban P, Portha B, and Serradas P. Islet inflammation and fibrosis in a spontaneous model of type 2 diabetes, the GK Rat. *Diabetes* 55: 1625–1633, 2006.
28. Hoppener JW, Ahren B, and Lips CJ. Islet amyloid and type 2 diabetes mellitus. *N Eng J Med* 343: 411–419, 2000.
29. Hughes SJ, Clark A, McShane P, Contractor HH, Gray DW, and Johnson PR. Characterization of collagen VI within the islet-exocrine interface of the human pancreas: implications for clinical islet isolation? *Transplantation* 81: 423–426, 2006.
30. Ido Y and Williamson JR. Hyperglycemic cytosolic reductive stress 'pseudohypoxia': implications for diabetic retinopathy. *Invest Ophthalmol Vis Sci* 38: 1467–1470, 1997.
31. Ikeda T, Iwata K, and Ochi H. Effect of insulin, proinsulin, and amylin on renin release from perfused rat kidney. *Metabolism* 50: 763–766, 2001.
32. Jaikaran ET and Clark A. Islet amyloid and type 2 diabetes. from molecular misfolding to islet pathophysiology. *Biochim Biophys Acta* 1537: 179–203, 2001.
33. Jones LC and Clark A. Beta cell neogenesis in type 2 diabetes mellitus. *Diabetes* 50: S186–S187, 2001.
34. Kampf C, Lau T, Olsson R, Leung PS, and Carlsson PO. Angiotensin II type 1 receptor inhibition markedly improves the blood perfusion, oxygen tension and first phase of glucose-stimulated insulin secretion in revascularised syngeneic mouse islet grafts. *Diabetologia* 48: 1159–1167, 2005.
35. Kralik PM, Xu B, and Epstein PN. Catalase transfection decreases hydrogen peroxide toxicity in a pancreatic beta cell line. *Endocr Res* 24: 79–87, 1998.
36. Lastra–Gonzalez G, Manrique CM, Govindarajan G, Whaley–Connell A, and Sowers JR. Insights into the emerging cardiometabolic prevention and management of diabetes mellitus. *Expert Opin Pharmacother* 6: 2209–2221, 2005.
37. Lastra G, Manrique CM, and Hayden MR. The role of beta cell dysfunction in the cardiometabolic syndrome. *J Cardiometabol Syndrome* 1: 41–46, 2006.
38. Lau T, Carlsson PO, and Leung PS. Evidence for a local angiotensin system and dose-dependent inhibition of glucose-stimulated insulin release by angiotensin II in isolated pancreatic islets. *Diabetologia* 47: 240–248, 2004.
39. Leung PS. The physiology of a local renin-angiotensin system in the pancreas. *J Physiol.* Jan 11; [Epub ahead of print], 2007.
40. Leung PS. Pancreatic renin-angiotensin system: a novel target for the potential treatment of pancreatic diseases? *JOP* 4: 89–91, 2003.
41. Leung PS and Carlsson PO. Pancreatic islet renin angiotensin system: its novel roles in islet function and in diabetes mellitus. *Pancreas* 30: 293–298, 2005.
42. Leung PS and Carlsson PO. Tissue renin-angiotensin system: its expression, localization, regulation and potential role in the pancreas. *J Mol Endocrinol* 26: 155–164, 2001.
43. Leung PS and Chappell MC. A local pancreatic renin-angiotensin system: endocrine and exocrine roles. *Int J Biochem Cell Biol* 35: 838–846, 2003.
44. Matveyenko AV and Butler PC. β-Cell deficit due to increased apoptosis in the human islet amyloid polypeptide transgenic (HIP) rat recapitulates the metabolic defects present in type 2 diabetes. *Diabetes* 55: 2106–2114, 2006.
45. Matveyenko AV and Butler PC. Islet amyloid polypeptide (IAPP) transgenic rodents as models for type 2 diabetes. *ILAR J* 47: 225–233, 2006.
46. Moldovan S and Brunicardi FC. Endocrine pancreas: summary of observations generated by surgical fellows. *World J Surg* 25: 468–473, 2001.
47. Murakami T, Fujita T, Taguchi T, Nonaka Y, and Orita K. The blood vascular bed of the human pancreas, with special reference to the insulo-acinar portal system. Scanning electron microscopy of corrosion casts. *Arch Histol Cytol* 55: 381–395, 1992.
48. Murakami T, Fujita T, Tanaka T, Tsubouchi M, Tsubouchi Y, Taguchi T, Ohtsuka A, and Kikuta A. Microcirculatory patterns in human pancreas: supplementary observations of vascular casts by scanning electron microscopy. *Arch Histol Cytol* 57: 9–16, 1994.
49. Nakamura M, Kitamura H, Konishi S, Nishimura M, Ono J, Ina K, Shimada T, and Takaki R. The endocrine pancreas of spontaneously

diabetic db/db mice: microangiopathy as revealed by transmission electron microscopy. *Diabetes Res Clin Pract* 30: 89–100, 1995.
50. Nehls V and Drenckhahn D. The versatility of microvascular pericytes: from mesenchyme to smooth muscle? *Histochemistry* 99: 1–12, 1993.
51. Oliveira HR, Verlengia R, Carvalho CR, Britto LR, Curi R, and Carpinelli AR. Pancreatic beta-cells express phagocyte-like NAD(P)H oxidase. *Diabetes* 52: 1457–1463, 2003.
52. Omary MB, Lugea A, Lowe AW, and Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. *J Clin Inves* 117: 50–59, 2007.
53. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, and Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306: 457–461, 2004.
54. Poitout V and Robertson RP. Minireview: Secondary beta-cell failure in type 2 diabetes—a convergence of glucotoxicity and lipotoxicity. *Endocrinology* 143: 339–342, 2002.
55. Ritzel RA, Meier JJ, Lin CY, Veldhuis JD, and Butler PC. Human islet amyloid polypeptide oligomers disrupt cell coupling, induce apoptosis, and impair insulin secretion in isolated human islets. *Diabetes* 56: 65–71, 2007.
56. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem* 279: 42351–42354, 2004.
57. Robertson RP, Harmon J, Tran PO, and Poitout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes* 53: S119–124, 2004.
58. Sakurai K, Katoh M, Someno K, and Fujimoto Y. Apoptosis and mitochondrial damage in INS-1 cells treated with alloxan. *Biol Pharm Bull* 24: 876–882, 2001.
59. Sowers JR. Hypertension, angiotensin II and oxidative stress. *N Engl J Med* 346: 1999–2001, 2002.
60. Sundberg C, Ivarsson M, Gerdin B, and Rubin K. Pericytes as collagen-producing cells in excessive dermal scarring. *Lab Invest* 74: 452–466, 1996.
61. Tahmasebi M, Puddefoot JR, Inwang ER, and Vinson GP. The tissue renin-angiotensin system in human pancreas. *J Endocrinol* 161: 317–322, 1999.
62. Tanaka Y, Tran PO, Harmon J, and Robertson RP. A role for glutathione peroxidase in protecting pancreatic beta cells against oxidative stress in a model of glucose toxicity. *Proc Natl Acad Sci USA* 99: 12363–12368, 2002.
63. Tikellis C, Cooper ME, and Thomas MC. Role of the renin-angiotensin system in the endocrine pancreas: implications for the development of diabetes. *Int J Biochem Cell Biol* 38: 737–751, 2006.
64. Tikellis C, Wookey PJ, Candido R, Andrikopoulos S, Thomas MC, and Cooper ME. Improved islet morphology after blockade of the renin-angiotensin system in the ZDF rat. *Diabetes* 53: 989–997, 2004.
65. Tomita T and Iwata K. Gelatinases and inhibitors of gelatinases in pancreatic islets and islet cell tumors. *Mod Pathol* 10: 47–54, 1997.
66. Turk J, Corbett JA, Ramanadham S, Bohrer A, and McDaniel ML. Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islet. *Biochem Biophys Res Commun* 197: 1458–1464, 1993.
67. Welsh N, Margulis B, Borg LA, Wiklund HJ, Saldeen J, Flodstrom M, Mello MA, Andersson A, Pipeleers DG, and Hellerstrom C, et al. Differences in the expression of heat-shock proteins and antioxidant enzymes between human and rodent pancreatic islets: implications for the pathogenesis of insulin-dependent diabetes mellitus. *Mol Med* 1: 806–820, 1995.
68. Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, Nyengaard JR, van den Enden M, Kilo C, and Tilton RG. Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes* 42: 801–813, 1993.
69. Zhao HL, Lai FM, Tong PC, Zhong DR, Yang D, Tomlinson B, and Chan JC. Prevalence and clinicopathological characteristics of islet amyloid in Chinese patients with type 2 diabetes. *Diabetes* 52: 2759–2766, 2003.
70. Zhou YP, Madjidi A, Wilson ME, Nothhelfer DA, Johnson JH, Palma JF, Schweitzer A, Burant C, Blume JE, and Johnson JD. Matrix metalloproteinases contribute to insulin insufficiency in Zucker diabetic fatty rats. *Diabetes* 54: 2612–2619, 2005.
71. Zimmet O. The burden of type 2 diabetes: are we doing enough? *Diabetes Metab* 29: 6S9–18, 2003.

Address reprint requests to:
Melvin R. Hayden, M.D.
Research Professor
Department of Internal Medicine
Endocrinology Diabetes and Metabolism
Diabetes and Cardiovascular Disease Research Group
Health Sciences Center, MA410, DC043.00
University of Missouri School of Medicine Columbia, Missouri
Columbia, Missouri 65212

E-mail: mrh29@usmo.com

Date of first submission to ARS Central, February 12, 2007; date of acceptance, February 26, 2007.

This article has been cited by:

1. Nils Welsh. 2012. Does the small tyrosine kinase inhibitor imatinib mesylate counteract diabetes by affecting pancreatic islet amyloidosis and fibrosis?. *Expert Opinion on Investigational Drugs* 1-8. [CrossRef]
2. Asma Kassab, Agnieszka Piwowar. 2012. Cell oxidant stress delivery and cell dysfunction onset in type 2 diabetes. *Biochimie* **94**:9, 1837-1848. [CrossRef]
3. Elias Darido, Timothy M. Farrell. 2012. Laparoscopic longitudinal gastrectomy and duodenojejunostomy for treatment of diabetic gastroparesis. *Surgery for Obesity and Related Diseases* . [CrossRef]
4. Muhammad Sajid Hamid Akash, Qi Shen, Kanwal Rehman, Shuqing Chen. 2012. Interleukin-1 receptor antagonist: A new therapy for type 2 diabetes mellitus. *Journal of Pharmaceutical Sciences* n/a-n/a. [CrossRef]
5. Esder Lee, Gyeong Ryul Ryu, Seung-Hyun Ko, Yu-Bae Ahn, Kun-Ho Yoon, Hunjoo Ha, Ki-Ho Song. 2011. Antioxidant treatment may protect pancreatic beta cells through the attenuation of islet fibrosis in an animal model of type 2 diabetes. *Biochemical and Biophysical Research Communications* . [CrossRef]
6. Matheni Sathananthan, Adrian VellaIs there a Role for Incretin-Based Therapy in Combination with Insulin? 91-95. [CrossRef]
7. William I. Sivitz , Mark A. Yorek . 2010. Mitochondrial Dysfunction in Diabetes: From Molecular Mechanisms to Functional Significance and Therapeutic Opportunities. *Antioxidants & Redox Signaling* **12**:4, 537-577. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
8. Danilo Milardi, Matteo Pappalardo, Martina Pannuzzo, Domenico M. Grasso, Carmelo La Rosa. 2008. The role of the Cys2-Cys7 disulfide bridge in the early steps of Islet amyloid polypeptide aggregation: A molecular dynamics study. *Chemical Physics Letters* **463**:4-6, 396-399. [CrossRef]
9. Melvin R. Hayden, Kamlesh Patel, Javad Habibi, Deepa Gupta, Seema S. Tekwani, Adam Whaley-Connell, James R. Sowers. 2008. Attenuation of Endocrine-Exocrine Pancreatic Communication in Type 2 Diabetes: Pancreatic Extracellular Matrix Ultrastructural Abnormalities. *Journal of the CardioMetabolic Syndrome* **3**:4, 234-243. [CrossRef]
10. M HAYDEN, J SOWERS. 2008. Treating hypertension while protecting the vulnerable islet in the cardiometabolic syndrome. *Journal of the American Society of Hypertension* **2**:4, 239-266. [CrossRef]
11. Melvin R. Hayden, James R. Sowers. 2008. Pancreatic Renin-Angiotensin-Aldosterone System in the Cardiometabolic Syndrome and Type 2 Diabetes Mellitus. *Journal of the CardioMetabolic Syndrome* **3**:3, 129-131. [CrossRef]
12. Benjamin Ward, Karen Walker, Christopher Exley. 2008. Copper(II) inhibits the formation of amylin amyloid in vitro. *Journal of Inorganic Biochemistry* **102**:2, 371-375. [CrossRef]
13. Melvin R. Hayden , James R. Sowers . 2007. Redox Imbalance in Diabetes. *Antioxidants & Redox Signaling* **9**:7, 865-867. [Citation] [Full Text PDF] [Full Text PDF with Links]