Vascular Endothelial Growth Factor–Mediated Islet Hypervascularization and Inflammation Contribute to Progressive Reduction of β-Cell Mass

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Type 2 diabetes (T2D) results from insulin resistance and inadequate insulin secretion. Insulin resistance initially causes compensatory islet hyperplasia that progresses to islet disorganization and altered vascularization, inflammation, and, finally, decreased functional β-cell mass and hyperglycemia. The precise mechanism(s) underlying β-cell failure remain to be elucidated. In this study, we show that in insulin-resistant high-fat diet-fed mice, the enhanced islet vascularization and inflammation was parallel to an increased expression of vascular endothelial growth factor A (VEGF). To elucidate the role of VEGF in these processes, we have genetically engineered β-cells to overexpress VEGF (in transgenic mice or after adeno-associated viral vector-mediated gene transfer). We found that sustained increases in β-cell VEGF levels led to disorganized, hypervascularized, and fibrotic islets, progressive macrophage infiltration, and proinflamma-
tory cytokine production, including tumor necrosis factor-α and interleukin-1β. This resulted in impaired insulin secretion, decreased β-cell mass, and hyperglycemia with age. These results indicate that sustained VEGF upregulation may participate in the initiation of a process leading to β-cell failure and further suggest that compensatory islet hyperplasia and hypervascularization may contribute to progressive inflammation and β-cell mass loss during T2D. With islet inflammation and increased proinflammatory cytokine production (6–9). However, the mechanisms underpinning increased β-cell death remain to be elucidated.

In the early stages of T2D, expansion of β-cell mass is a key adaptive response to compensate for insulin resistance (2). During this period, islet vasculature also needs to expand to perfuse the new β-cells (10). The formation of vessels in adult organisms (angiogenesis) occurs through a multistep process requiring vascular endothelial growth factor A (VEGF) and other soluble factors (11,12). VEGF recruits circulating monocytes and macrophages, which are required for active angiogenesis (13). In adults, VEGF is highly expressed and secreted by insulin-producing β-cells and is responsible for the rich islet vasculature (14–16). VEGF deficiency in β-cells does not modify β-cell mass, but leads to insufficient islet vascularization, which results in defective insulin secretion and glucose intolerance (14,17). However, this deficiency does not impair β-cell mass growth during a high-fat diet (HFD); contrarily, it results in a slightly increased β-cell mass (18). Furthermore, islet vascular abnormalities have been described in several animal models of T2D. Prior to developing hyperglycemia, islets from Zucker diabetic fatty rats show vessel remodeling with expansion of endothelial cells and higher VEGF secretion (19). Similarly, pancreatic islets from spontaneously diabetic Torii rats are fibrotic with vascular alterations preceding hyperglycemia (20). Goto-Kakizaki (RK) rats also show endothelial hypertrophy with increased expression of vessel extracellular matrix components (21), and Otsuka Long-Evans Tokushima fatty rats display fibrosis and vascular abnormalities in islets (22). Finally, db/db mice develop irregular vessels with increased mean capillary size, edema, and fibrosis (23). In mouse islets, endothelial cells synthesize extracellular matrix (ECM) components surrounding β-cells (24–26). ECM accumulation surrounding islet vessels in T2D models can progress to fibrosis that disrupts islet structure (21). Taken together, these studies suggest that alterations in islet vasculature may precede and/or be involved in β-cell dysfunction and death. Nevertheless, the role of chronic islet hypervascularization in β-cell function and loss in T2D is still not fully understood. In this study, by genetically engineering β-cells to overexpress VEGF, we demonstrate that sustained increases in VEGF levels lead to islet hypervascularization, fibrosis, and inflammation, resulting in β-cell death and hyperglycemia.

RESEARCH DESIGN AND METHODS

Animals. C57Bl6/SJL transgenic mice expressing murine VEGF165 (provided by P.A. D’Amore, Boston, MA) under the control of the rat insulin promoter-I...
(RIP1)-I were obtained by embryo pronuclear microinjection. Both VEGFlox- and VEGFloxConf Griffen transgenic mice were born at the expected frequencies and fertile, and they did not develop tumors when old (>14 months). Two- month-old C57Bl6/SJL mice were used for adeno-associated viral (AAV) vector treatment. Mice were anesthetized with Ketamine (100 mg/kg) and Xylazine (10 mg/kg). A cecal vein labeled with an HFD for 4 months. HFD-fed mice developed in- creased vessel area/islet area ratio, and an increase in macrophage infiltration, with a compensation islet hyperplasia. Islets from these mice showed hypervascularization with thicker basal membranes after staining for the endothelial cell marker CD31 and the basement membrane marker collagen IV (Fig. 1A and B). To investigate whether intrasit islet vascularization was functional, a FITC-conjugated dextran solution was intravenously injected in these mice. An increased vessel area/islet area ratio (~25%) was observed in HFD-fed mice, indicating higher islet vascularization (Fig. 1C and D). This suggests that in hyperplastic islets, angiogenesis might have increased to perfuse new β-cells. This was parallel to the increased VEGF content (about twofold) in HFD islets (Fig. 1E and F). Because VEGF can induce collagen IV synthesis by endothelial cells (28), VEGF upregulation was probably responsible for the increased amount of endothelial ECM of these islets (Fig. 1A). Because fibrosis and VEGF upregulation are also usually found during inflammatory processes (11), parietal sections were stained for the macrophage cell marker Mac-2, and an increase in macrophage infiltration in the islets of HFD-induced insulin resistant mice was observed. 

RESULTS

Increased islet vascularization and inflammation parallels enhanced VEGF production in type 2 prediabetic mice. Islet vascularization and VEGF expression were determined in 6-month-old C57Bl6 mice fed with an HFD for 4 months. HFD-fed mice developed insulin resistance, as they were normoglycemic (chow, 136 ± 13.5 vs. HFD, 143 ± 17.5 mg/dL) and highly hyperinsulinemic (chow, 2.3 ± 0.4 vs. HFD, 8.1 ± 1.7 ng/mL) and showed altered insulin tolerance (Supplementary Fig. 1). In contrast to mice fed a chow diet, islets from HFD-fed mice were larger (chow, 290 ± 65 vs. HFD, 435 ± 110 μm2, P < 0.05), indicating compensatory islet hyperplasia. Islets from these mice showed hypervascularization with thicker basal membranes after staining for the endothelial cell marker CD31 and the basement membrane marker collagen IV (Fig. 1A and B). To investigate whether intrasit islet vascularization was functional, a FITC-conjugated dextran solution was intravenously injected in these mice. An increased vessel area/islet area ratio (~25%) was observed in HFD-fed mice, indicating higher islet vascularization (Fig. 1C and D). This suggests that in hyperplastic islets, angiogenesis might have increased to perfuse new β-cells. This was parallel to the increased VEGF content (about twofold) in HFD islets (Fig. 1E and F). Because VEGF can induce collagen IV synthesis by endothelial cells (28), VEGF upregulation was probably responsible for the increased amount of endothelial ECM of these islets (Fig. 1A). Because fibrosis and VEGF upregulation are also usually found during inflammatory processes (11), parietal sections were stained for the macrophage cell marker Mac-2, and an increase in macrophage infiltration in the islets of HFD-induced insulin resistant mice was observed.
observed (Fig. 1G and H). These results suggest that insulin resistance induces compensatory islet hyperplasia together with increased islet VEGF expression, angiogenesis, and macrophage recruitment.

**VEGF overexpression in islets leads to hypervascularization, altered morphology, and inflammation.** To elucidate the role of VEGF in islet angiogenesis and β-cell function, transgenic mice overexpressing VEGF in β-cells under the control of the RIP-1 were generated. Two transgenic lines, VEGFlow and VEGFhigh, with a 2.7- and 17-fold increase in VEGF expression (Fig. 2A and B), respectively, were obtained. VEGFlow mice showed an increase in VEGF comparable to that observed in insulin-resistant mice (Fig. 1F) and rats (19). VEGF serum concentrations remained unchanged (wild-type, 221 ± 27.8; VEGFlow, 202 ± 29.3; and VEGFhigh, 180 ± 25.1 ng/dL), suggesting an autocrine/paracrine effect of VEGF in the pancreas. Compared with wild-type, 2-month-old VEGFlow and VEGFhigh islets displayed hypervascularization and basal membrane thickening, as shown by both CD31 and collagen IV immunostaining (Fig. 2C). Intravascular injection of FITC-conjugated dextran confirmed the increased vessel area/islet area ratio of functional capillaries (Fig. 2C and D).

Islets from 2-month-old VEGFlow and VEGFhigh mice showed altered distribution of α and β-cells (Fig. 3A). Although the basal membrane of islet endothelial cells provides signals that promote β-cell proliferation (15) and transgenic islets were hypervascularized, the β-cell mass was similar in wild-type, VEGFlow, and VEGFhigh mice (Fig. 3B). This was consistent with normal rates of β-cell replication and apoptosis (Fig. 3C–F). This altered islet morphology was parallel to decreased levels of E-cadherin (Fig. 3G and H), a key protein in maintaining islet endocrine cell contacts (29,30).

**Long-term β-cell overexpression of VEGF leads to glucose intolerance and hyperglycemia.** Two-month-old wild-type, VEGFlow, and VEGFhigh mice presented similar glycemia, insulinemia (Fig. 4A and B), and glucose tolerance (Fig. 4C). In addition, comparable in vivo insulin secretion profiles were noted in all groups (Fig. 4D). This was consistent with normal GLUT-2 expression in VEGFlow islets (Fig. 4E and F). However, VEGFhigh islets showed a decrease in GLUT-2 expression without a major change in insulin secretion (Fig. 4E and F). Therefore, although transgenic islets were disorganized and hypervascularized, glucose homeostasis in young transgenic mice was nevertheless unaltered, indicating normal islet function, in agreement with the normal β-cell mass.
VEGFlow mice remained normoglycemic up to 12 months of age, when they developed mild hyperglycemia and hypoinsulinemia (Fig. 4A and B), altered glucose tolerance, and decreased glucose-stimulated insulin release (Fig. 5A and B), but normal insulin sensitivity (data not shown). In contrast, VEGF<sup>high</sup> mice, which expressed higher VEGF levels, developed glucose intolerance and decreased insulin secretion after a glucose load earlier, at 5 to 6 months of age (Fig. 5C and D). Moreover, 8-month-old VEGF<sup>high</sup> mice displayed overt hyperglycemia, hypoinsulinemia (Fig. 4A and B), and glucose intolerance (Fig. 5E).

Morphometric analysis of pancreatic sections from VEGFlow mice showed a trend toward decreased β-cell mass only at the age of 12 months (Fig. 5F and G), although this

FIG. 2. Increased VEGF expression in VEGFlow and VEGF<sup>high</sup> transgenic β-cells leads to islet hypervascularization. A: Representative Western blot showing higher expression of VEGF in both VEGF<sup>high</sup> and VEGFlow. B: A 2.7- and 17-fold increase in VEGF protein levels were found in islets from 2-month-old VEGFlow and VEGF<sup>high</sup> mice measured by ELISA, wild-type (WT) mice (white bar), transgenic VEGFlow mice (gray bar), and transgenic VEGF<sup>high</sup> mice (black bar). *P < 0.05 transgenic vs. WT (n = 8–15/group). C: Top panel: detection of endothelial cells by CD31 immunostaining. Middle panel: collagen IV (red) and insulin (green) immunohistochemical analysis of VEGFlow and VEGF<sup>high</sup> islets. Bottom panel: FITC-dextran (green) together with insulin (red) immunostaining was used to label functional islet vessels. D: Morphometric analysis revealed an increase in the vessel area in both VEGFlow and VEGF<sup>high</sup> mice (n = 3/group): WT mice (white bar), transgenic VEGFlow mice (gray bar), and transgenic VEGF<sup>high</sup> mice (black bar). Scale bars, 100 μm. *P < 0.05 transgenic vs. WT. (A high-quality digital representation of this figure is available in the online issue.)
FIG. 3. Two-month-old VEGF$_{\text{low}}$ and VEGF$_{\text{high}}$ mice showed disorganization of islet architecture, but normal β-cell mass. A: Top panel: immunohistochemical analysis of insulin (green) and glucagon (red). In transgenic mice, islets appeared disorganized with α-cells in the core. Bottom panel: insulin immunostaining used to visualize islet architecture.

B: β-Cell mass was measured in 2-month-old mice (n = 4-7/group): wild-type (WT) mice (white bar), transgenic VEGF$_{\text{low}}$ mice (gray bar), and transgenic VEGF$_{\text{high}}$ mice (black bar).

C: Ki67 (green) and insulin (red) immunostaining were used to label proliferating β-cells (arrow).

D: Percentage of Ki67-positive (replicative) β-cells.

E: TUNEL (green) and insulin (red) immunostaining showed apoptotic nuclei (arrow).

F: Quantification of TUNEL-positive (apoptotic) β-cells.

G and H: Western blot analysis of E-cadherin using islet homogenates from 2-month-old mice. G: A representative immunoblot is shown. H: Densitometric analysis of three different immunoblots: WT mice (white bar), transgenic VEGF$_{\text{low}}$ mice (gray bar), and transgenic VEGF$_{\text{high}}$ mice (black bar). Scale bars, 100 μm. *P < 0.05 transgenic vs. WT. (A high-quality digital representation of this figure is available in the online issue.)
did not reach statistical significance \( (P = 0.059) \). In contrast, 8-month-old VEGF\textsubscript{high} mice displayed a marked loss of \( \beta \)-cell mass (\( \sim 80\% \)) (Fig. 5\textit{F} and \textit{G}). In VEGF\textsubscript{high} pancreata, insulin- and glucagon-positive cells were scattered and surrounded by abundant collagen IV-positive staining (Fig. 5\textit{F}). VEGF overexpression leads to islet inflammation and increased cytokine production. In addition to promoting angiogenesis, VEGF is involved in macrophage recruitment (13). Mac-2–positive cells were detected in islets from 2- to 12-month-old VEGF mice (Fig. 6\textit{A} and \textit{B}). Macrophage infiltration progressively increased as animals aged and in parallel with VEGF expression, being higher in VEGF\textsubscript{high} than in VEGF\textsubscript{low} islets (Fig. 6\textit{A} and \textit{B}). These results suggest that chronic overexpression of VEGF progressively increased macrophage recruitment. Consistent with this, a statistically significant increase in the expression of CCL-2 (monocyte chemoattractant protein-1), IL-1\( \beta \), and TNF-\( \alpha \) was observed in islets of 12-month-old VEGF\textsubscript{low} mice and 5-month-old VEGF\textsubscript{high} mice, although a tendency toward increased expression was already noted in 2-month-old mice (Fig. 6\textit{C}–\textit{E}). Thus, the development of hyperglycemia and decrease in \( \beta \)-cell mass were concomitant with progressive macrophage infiltration, cytokine production, and fibrosis.

In vivo AAV-mediated VEGF overexpression in \( \beta \)-cells of adult mice results in islet hypertovascularization and inflammation. To avoid potentially undesirable effects of VEGF overexpression during embryonic development and to verify a direct effect of VEGF in endocrine pancreas inflammation, AAV vectors were used to overexpress VEGF in islets of adult mice. Intraductal delivery of AAV vectors of serotype 9 (AAV9) leads to highly efficient and long-term transduction of \( \beta \)-cells and exocrine pancreas (31). To restrict transgene expression to \( \beta \)-cells, the RIP-I promoter was used. Thus, AAV9 vectors carrying a RIP-I/VEGF transgene were intraductally injected into 2-month-old mice, and glucose homeostasis and islet morphology were studied within 2 months after AAV injection. Compared with AAV9-null–treated mice, AAV9-VEGF–treated animals showed about a twofold increase (AAV9-VEGF: \( 2.2 \pm 0.8 \)-fold increase vs. AAV9-null) in islet content of VEGF, similar to the increases detected in islets from HFD-fed mice, VEGF\textsubscript{low} transgenic mice, or Zucker rats (19).

This value was, however, variable among mice paralleling
differential efficiency of transduction. Despite the heterogeneity, all AAV9-VEGF-treated mice showed between 30 and 80% hypervascularized and disorganized islets, with increased collagen IV accumulation (Fig. 7A). These alterations were already observed 10 days after vector administration (Fig. 7A) and were also present at 40 days (Supplementary Fig. 2). Islet inflammation, determined by Mac-2 immunostaining, was increased in AAV9-VEGF-treated mice at 10 (Fig. 7A and B) and 40 days (Supplementary Fig. 2) after AAV injection. Moreover, increased VEGF expression in β-cells induced a progressive increase in glycemia (Fig. 7C), along with glucose intolerance (Fig. 7D). Thus, these results confirm that VEGF overexpression in islets of adult mice can induce hypervascularization, islet disorganization, and inflammation that result in im-
paired glucose homeostasis.

DISCUSSION

In the preliminary stages of T2D, expansion of β-cell mass is a key adaptive response to compensate for insulin resistance (2). During this period, islet vasculature also needs to expand to perfuse the newly formed β-cells. The development of vessels in adult organisms (angiogenesis) occurs through a multistep process requiring VEGF and other soluble factors (32). In this study, we found that islets from obese, insulin-resistant, HFD-fed mice displayed increased vascularization that paralleled increased VEGF levels. Similar observations were described in insulin-resistant prediabetic Zucker rats (32), in which islets of these rats showed increased vascularization and enhanced VEGF secretion compared with non-insulin-resistant rats. This may indicate that VEGF upregulation represents a compensatory response to support angiogenesis during β-cell
expansion. To discern the role of increased VEGF and islet vascularization during T2D development, VEGF low and VEGF high transgenic mice were generated. Similar to HFD-fed mice, VEGF low and VEGF high mice displayed islet hypervascularization with irregular capillaries and thicker basal membranes. These vascular alterations resembled those observed in islets from HFD-fed mice and were consistent with pathologic angiogenesis. Similarly, it has been

FIG. 6. VEGF-overexpressing islets showed progressive inflammation. A: Macrophage infiltration was determined by Mac-2 immunostaining in islets from 2-month-old wild-type (WT), VEGF low, and VEGF high mice (top panel) and 12-month-old WT and VEGF low and 8-month-old VEGF high mice (bottom panel). B: Quantification of Mac-2-positive area in pancreas sections from 2-, 8-, and 12-month-old WT, in 2-, 8-, and 12-month-old VEGF low, and in 2- and 8-month-old VEGF high mice (n = 4 animals/group): WT mice (white bar), transgenic VEGF low mice (gray bar), and transgenic VEGF high mice (black bar). C–E: Islets from VEGF low and VEGF high mice displayed increased expression of key inflammatory cytokines. CCL-2 (C), IL-1β (D), and TNF-α (E) were measured by qPCR from isolated islets from 2- and 12-month-old VEGF low and 2- and 5-month-old VEGF high mice (n = 4 pools of islets from three mice per pool): WT mice (white bar), transgenic VEGF low mice (gray bar), and transgenic VEGF high mice (black bar). Scale bars, 100 μm. *P < 0.05 vs. age-matched WT mice. (A high-quality digital representation of this figure is available in the online issue.)
shown in tumors that prolonged deregulated expression of VEGF leads to aberrant immature vessels that often invade the surrounding tissue (33,34). In contrast, normal vasculature spreads by physiologic angiogenesis, requiring proper regulation of the dose and timing of VEGF production (11,32).

Islets from transgenic VEGFlow and VEGFhigh mice showed thickening of the vessel basal membrane compared with AAV-null. FITC-dextran (green) together with insulin (red) immunostaining was used to label functional blood vessels (top middle panel). Insulin (green) and glucagon (red) expression showed islet disorganization (bottom middle panel). Macrophage infiltration in AAV-VEGF–treated animals was determined by Mac-2 immunostaining 10 days after AAV injection (bottom panel). Scale bars, 100 μm. B: AAV-VEGF–injected animals showed increased Mac-2–positive area/islet area when compared with AAV-null–treated mice as early as 10 days after injection: AAV-null–treated mice (white bar) and AAV-VEGF–treated mice (black bar) (n = 4 mice/group). C: Fed blood glucose levels were determined before AAV injection (day 0) and at several time points thereafter: AAV-null–treated mice (white circle) and AAV-VEGF–treated mice (black square) (n = 10 animals/group). *P < 0.05 VEGF vs. null. (A high-quality digital representation of this figure is available in the online issue.)

FIG. 7. AAV-mediated VEGF overexpression in β-cells increased islet vascularization and inflammation. Two-month-old wild-type (WT) mice were injected with VEGF-expressing (AAV9-VEGF) or nonexpressing (null) AAV9 vectors (10^12 vector genomes/mouse). A: Ten days after AAV injection vasculature structure was revealed by immunostaining for collagen IV (red) and insulin (green) (top panel). VEGF-treated islets showed increased basement membrane compared with AAV-null. FITC-dextran (green) together with insulin (red) immunostaining was used to label functional blood vessels (top middle panel). Insulin (green) and glucagon (red) expression showed islet disorganization (bottom middle panel). Macrophage infiltration in AAV-VEGF–treated animals was determined by Mac-2 immunostaining 10 days after AAV injection (bottom panel). Scale bars, 100 μm. B: AAV-VEGF–injected animals showed increased Mac-2–positive area/islet area when compared with AAV-null–treated mice as early as 10 days after injection: AAV-null–treated mice (white bar) and AAV-VEGF–treated mice (black bar) (n = 4 mice/group). C: Fed blood glucose levels were determined before AAV injection (day 0) and at several time points thereafter: AAV-null–treated mice (white circle) and AAV-VEGF–treated mice (black square) (n = 10 animals/group). *P < 0.05 VEGF vs. null. (A high-quality digital representation of this figure is available in the online issue.)

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also been described in islets of patients with T2D and in various spontaneous rodent models of T2D, such as db/db mice and Zucker and GK rats, in which it is involved in islet destruction (21,36–38). Thus, during T2D, chronic VEGF upregulation in islets may be responsible for ECM accumulation in surrounding islet vessels, leading to fibrosis.

Two-month-old VEGFlow and VEGFhigh mice also displayed disorganized islets with α-cells scattered in the islet core. The Ca2+-dependent cell adhesion molecule E-cadherin, which is highly expressed in pancreatic islets, mediates β-cell-to-β-cell contacts, controlling islet architecture and function (29,30). The expression of E-cadherin was reduced in VEGFlow and VEGFhigh islets, consistent with decreased numbers of direct β-cell-to-β-cell contacts and islet disorganization. It is widely accepted that proper insulin secretion requires the coordinated function of the β-cells that form an islet (29). Moreover, islet disorganization is considered a hallmark of islet dysfunction (39). However, despite altered islet architecture, 2-month-old VEGFlow and VEGFhigh mice displayed normal in vivo insulin release and glucose tolerance. Therefore, these transgenic mice indicate that abnormal islet morphology is not directly responsible for impaired β-cell function. Because the alteration of insulin secretion developed in parallel with the severity of inflammation, our results suggest that islet inflammation, rather than islet disorganization alone, was probably the proximate cause of islet failure.

Although the precise mechanism or more likely multiple mechanisms underlying decreased β-cell mass and function in T2D remain to be elucidated, islets from patients with T2D display an inflammatory process characterized by the presence of immune cell infiltration, inflammatory cytokines, apoptotic cells, and, eventually, fibrosis. VEGF transgenic mice partially recapitulate those processes that occur specifically in the islets without the development of insulin resistance. VEGF is a powerful chemoattractant for macrophages and other leukocytes at the sites of angiogenesis, where these cells play an important role in vessel formation and tissue remodeling (13,40). Islets from HFD-fed mice showed infiltrating macrophages, comparable to those described in islets from several type 2 diabetic animal models and human patients (7,38,41,42). Increased numbers of macrophages are detectable very early in islets, before the onset of diabetes (41). Normoglycemic insulin-resistant HFD-fed mice showed islet macrophage infiltration concomitant with VEGF upregulation. Similarly, islets from both VEGFlow and VEGFhigh mice were progressively infiltrated by macrophages. Moreover, Mac-2+ insulin grade and VEGF upregulation level in VEGFlow mice were similar to those observed in prediabetic HFD-fed mice. Finally, VEGF transgenic islets showed macrophage infiltration even before developing impaired glucose homeostasis, suggesting that infiltration is not a consequence of β-cell death. Therefore, this study suggests that macrophage infiltration may already be induced during islet hyperplasia and play a role in initiating and/or accelerating β-cell failure. This hypothesis is supported by the AAV-mediated overexpression of VEGF in islets of adult mice, because, as early as 10 days after vector administration, mice already showed islet disorganization and inflammation, which subsequently progressed to hyperglycemia and glucose intolerance.

VEGF-expressing islets showed macrophage infiltration and increased expression of IL-1β, CCL-2, and TNF-α. A significant increase in cytokine expression was detected at a slightly delayed time compared with macrophage infiltration, but both inflammatory processes increased with age and VEGF levels. Macrophages are able to secrete a broad range of cytokines. In RIP-CCL2 transgenic mice, myeloid cell infiltration of islets is able to induce β-cell death and diabetes (43). Moreover, it has been shown that high glucose concentrations can induce β-cell expression of several cytokines, such as IL-1β and CCL-2 (44–46). Thus, during development of T2D, VEGF and inflammatory cytokines may be secreted and attract macrophages, and, in turn, macrophages can produce higher levels of proinflammatory cytokines. These processes probably stimulate each other in a positive-feedback manner, and, together with other processes such as endoplasmic reticulum (ER) stress, may contribute to β-cell failure and death. During T2D, higher insulin requirements and high circulating levels of free fatty acids and glucose can lead to the activation of the unfolding protein response and ER stress within the β-cells (47). Moreover, several studies have proposed that proinflammatory cytokines are also able to induce β-cell apoptosis by means of ER stress (47–49). Because VEGF overexpression in islets from our transgenic mice resulted in enhanced cytokine production, this may also contribute to β-cell loss through ER stress.

It is of interest that although previous reports demonstrated that decreased VEGF expression in β-cells resulted in insufficient vascularization leading to impaired insulin secretion and glucose intolerance (14), our results suggest that excessive islet VEGF production also triggers architectural abnormalities and impaired β-cell function. Thus, VEGF levels need to be finely and tightly regulated to avoid development of undesirable hypervascularization, fibrosis, and inflammation.

Finally, although no direct links between angiogenic factor genetic variants and diabetes have been reported, allelic variants of VEGF (50) and the VEGF-inducer transcription factor hypoxia inducible factor-1α have been described in T2D patients (51). Moreover, polymorphisms in the VEGF-A gene have been reported in patients with micro and macrovascular diabetic secondary complications (52,53). These data further suggest that dysregulation of VEGF could be involved in the pathogenesis of T2D.

In summary, the current study demonstrates a crucial role for islet vascularization and inflammation in the development of β-cell failure, suggesting that macrophage recruitment and sustained islet cytokine production may result from increased angiogenesis and/or VEGF upregulation and that this inflammatory process may contribute to connect insulin resistance with β-cell death and T2D.
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REFERENCES
1. Mathis D, Vence L, Benoist C. beta-Cell death during progression to diabetes. Nature 2001;414:792–798
2. Rhodes CJ. Type 2 diabetes—a matter of beta-cell life and death? Science 2005;307:380–384
3. Kahn SE, Haffner S, Mykkanen L, et al. Homeostasis model assessment: a novel approach to characterizing insulin resistance. Diabetes Care 1998;21:646–650
4. Capelli C, Deidda A, Deidda S, et al. Low dietary fiber intake and the risk of type 2 diabetes: a prospective study of 275,106 Italian men and women. Am J Epidemiol 2005;161:385–394
5. Williams MA, Best MI, Zimmet PZ, et al. The epidemic of type 2 diabetes: burden and cost. Diabetes Res Clin Pract 2005;68:1–8
6. Wolf M, Gudala S, Kim Y, et al. The role of beta-cell inactivation in the onset of type 2 diabetes. Diabetologia 2003;46:1001–1007
7. Li X, Zhang L, Meshinchi S, et al. Ilet microvascularization in islet hyperplasia and failure in a model of type 2 diabetes. Diabetes 2006;55:2974–2978
8. Masuyama T, Kameda K, Hara A, et al. Chronological characterization of diabetes development in male Spontaneously Diabetic Torii rats. Biochem Biophys Res Commun 2004;324:570–577
9. Homo-Delechere F, Calderari S, Irming J-C, et al. Islet inflammation and fibrosis in a spontaneous model of type 2 diabetes, the GK rat. Diabetes 2006;55:1625–1633
10. Ko SH, Kwon HS, Kim SR, et al. Ramipril treatment suppresses islet fibrosis in the GK rat. Diabetes Res Clin Pract 1995;30:89–90
11. Otomokoski T, Banerjee M, Korsgren O, Thornell LE, Virtanen I. Unique basement membrane structure of human pancreatic islets implications for beta-cell growth and differentiation. Diabetes Obes Metab 2008;10(Suppl. 4):119–127
12. Shchors K, Brochu E, Cnop M, et al. Diabetic retinopathy. Curr Opin Hematol 2006;13:220–226
13. Tal MG. Type 2 diabetes: Microvascular ischemia of pancreatic islets? Med Hypotheses 2008;70:65–70
14. Lin CH, Horvath R, Sato M, et al. Impaired pancreatic beta-cell function in rats with high fat diet-induced obesity. Diabetologia 2006;49:813–820
15. Jhun B, Yoon J, Kim KC, et al. Type 2 diabetes: pancreatic mesenchymal cell ischemia and increased cell death. PLoS One 2009;4:e4606
16. Liu F, Qian Y, Li H, et al. Genetic variants on chromosome 6p22.2 are associated with type 2 diabetes risk: a case-control study in Han Chinese. J Hum Genet 2012;57:320–325
17. Yamada N, Horikawa Y, Oda N, et al. Genetic variation in the hypoinsulinemic factor-lipase gene is associated with type 2 diabetes in Japanese. J Clin Endocrinol Metab 2005;90:5841–5847
18. Tavakkoly-Bazaz J, Amoli MM, Pravica V, et al. VEGF gene polymorphism association with diabetic nephropathy. Mol Biol Rep 2010;37:3625–3630
19. Chun MY, Hwang HS, Cho HY, et al. Association of vascular endothelial growth factor polymorphisms with nonproliferative and proliferative diabetic retinopathy. J Clin Endocrinol Metab 2010;95:3547–3551

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28. Infanger M, Grosse J, Westphal K, et al. Vascular endothelial growth factor induces extracellular matrix proteins and osteopontin in the umbilical artery. Ann Vasc Surg 2008;22:273–284
29. Rogers GJ, Hodgkin MN, Squires PE. E-cadherin and cell adhesion: a role in architecture and function in the pancreatic islet. Cell Physiol Biochem 2007;20:987–994
30. Jain R, Lammert E. Cell–cell interactions in the endocrine pancreas. Diabetes Obes Metab 2009;11(Suppl. 4):150–167
31. Jimenez V, Ayuso E, Mallo C, et al. In vivo genetic engineering of murine pancreatic beta cells mediated by single-stranded adeno-associated viral vectors of serotypes 6, 8 and 9. Diabetologia 2011;54:1075–1086
32. Camelliti P. Angiogenesis in life, disease and medicine. Nature 2005;438:932–936
33. Camelliti P. VEGF as a key mediator of angiogenesis in cancer. Oncology 2005;69(Suppl. 3):4–10
34. van Beijnum JR, Petersen K, Griffioen AW. Tumor endothelium is characterized by a matrix remodeling signature. Front Biosci (Schol Ed) 2009;4:1216–225
35. Lacruz G, Giroix MH, Kassam N, et al. Islet endothelial activation and oxidative stress gene expression induced by IL-1Ra treatment in the type 2 diabetic GK rat. PLoS ONE 2009;4:e6963
36. Portba H, Lacruz G, Kergout M, et al. The GK rat beta-cell: a prototype for the diseased human beta-cell in type 2 diabetes? Mol Cell Endocrinol 2009;297:73–85
37. Tal MG. Type 2 diabetes: Microvascular ischemia of pancreatic islets? Med Hypotheses 2008;70:357–358
38. Bonsi PA, Boni-Schuetzler M, Faulenbach M, Donath MY. Macrophages, cytokines and beta-cell death in Type 2 diabetes. Biochem Soc Trans 2008;36:340–342
39. Shih DQ, Heimesaat M, Kuwajima S, Stein R, Wright CV, Stoffel M. Found defects in pancreatic beta-cell function in mice with combined heterozygous mutations in Pdx-1, Hnf-1alpha, and Hnf-3beta. Proc Natl Acad Sci USA 2002;99:3818–3823
40. Sourcek L, Lawlor ER, Soto D, Seegers K, Swigart LB, Evan GL. Mast cells are required for angiogenesis and macroscopic expansion of MYC-induced pancreatic islet tumors. Nat Med 2007;13:1211–1218
41. Elsjes PA, Perren T, et al. Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 2007;56:2356–2370
42. Grunnet LG, Akins R, Tonnesen MF, et al. Proinflammatory cytokines activate the intrinsic apoptotic pathway in beta-cells. Diabetes 2009;58:1807–1815
43. Martin AP, Rankin S, Pitchford S, Charo IF, Furtado GC, Lira SA. Increased expression of CCL2 in insulin producing cells of transgenic mice promotes mobilization of myeloid cells from the bone marrow, marked insulitis, and diabetes. Diabetes 2008;57:3025–3033
44. Maedler K, Sergeev P, Rfs P, et al. Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. J Clin Invest 2002;110:851–860
45. Maedler K, Sterling J, Stirua J, et al. Glucose- and interleukin-1beta-induced beta-cell apoptosis requires Ca2+ influx and extracellular signal-regulated kinase (ERK) 1/2 activation and is prevented by a sulfonylurea receptor 1/inwardly rectifying K+ channel 6.2 (SUR/Kir6.2) selective potassium channel opener in human islets. Diabetes 2004;53:1706–1713
46. Jonas JC, Bensellam M, Duprez J, Eliou S, Gout G, Pascale SM. Glucose regulation of islet stress responses and beta-cell failure in type 2 diabetes. Diabetes Obes Metab 2009;11(Suppl. 4):65–81
47. Kharroubi I, Ladrère I, Cardozo AK, Dogan Z, Cnop M, Eizirik DL. Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress. Endocrinology 2004;145:5087–5096
48. Tersey SA, Nishiki Y, Templin AT, et al. Islet mass in a mouse model. Diabetes 2012;61:818–827