Cholesterol in Pancreatic β-Cell Death and Dysfunction

Underlying Mechanisms and Pathological Implications

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Abstract: The mechanisms or causes of pancreatic β-cell death as well as impaired insulin secretion, which are the principal events of diabetic etiopathology, are largely unknown. Diabetic complications are known to be associated with abnormal plasma lipid profile, mainly elevated level of cholesterol and free fatty acids. However, in recent years, elevated plasma cholesterol has been implicated as a primary modulator of pancreatic β-cell functions as well as death. High-cholesterol diet in animal models or excess cholesterol in pancreatic β-cell causes transporter desensitization and results in morphometric changes in insulin granules. Moreover, cholesterol is also held responsible to cause oxidative stress, mitochondrial dysfunction, and activation of proapoptotic markers leading to β-cell death. The present review focuses on the pathways and molecularevents that occur in the β-cell under the influence of excess cholesterol that hampers the basal physiology of the cell leading to the progression of diabetes.

Key Words: hypercholesterolemia, insulin granule, ABC transporters, LXRs, amyloid protein, microRNA-33

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Pancreatic β-cells synthesize and secrete insulin, the proteinaceous chemical messenger, in response to rise in plasma glucose (hyperglycemia).[1,2] The loss of pancreatic β-cells occupies the central role in the pathogenesis of the most common noncommunicable disease—diabetes mellitus—which is also characterized by the body's inefficiency to produce insulin or to use insulin effectively, thus leading to hyperglycemia.[3,4] Diabetes is distinguished into 2 different types, based on its causes, as type 1 diabetes (T1D) and type 2 diabetes (T2D). Type 1 diabetes is characterized by insufficient insulin secretion due to death or reduced pancreatic β-cell mass, which is inadequate to meet the general insulin demand,[5] whereas in T2D, the pancreas produces adequate amount of insulin, but the target cells become resistant to the hormone.[6,7] However, declined β-cell mass as well as impaired and inadequate insulin secretion are also implicated as the major pathological features of T2D.[8,9] Of the several factors that cause death of β-cells, elevated levels of circulatory and/or cellular lipid-induced lipotoxicity are notable.[10,11] Lipotoxicity has also been reported as one of the major contributing factors to the gradual decrease in β-cell functional status, followed by impaired insulin secretion.[12,13] However, abnormal lipid profile, including elevated levels of triglyceride (TG) as well as small, dense, low-density lipoprotein (LDL) particles and low level of high-density lipoprotein (HDL) cholesterol, occurs as an early event in diabetes progression.[14–16] Besides elevated levels of free fatty acid, a well-known lipotoxic agent of diabetes,[12,17] abnormal lipid profile is also characterized by elevated level of total cholesterol in diabetic subjects.[16,18] Recent studies have implicated elevated levels of plasma cholesterol, a condition called hypercholesterolemia, as a primary modulator of β-cell functions as well as death.[19–21] The major pathological implications of elevated cholesterol on pancreas are composed of death and functional impairment of pancreatic β-cells.[22–25] The present review has collectively summarized the effects of excess cholesterol on the physiological status of pancreatic β-cells that might lead to the progression of diabetes and discussed the possible pathways and underlying molecular mechanisms.

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CHOLESTEROL AND ITS METABOLISM IN PANCREATIC β-CELL

Cholesterol, an amphipathic C-27 containing alcohol derivative of steroid, is a vital structural component of animal membranes that helps in maintaining membrane fluidity.[26,27] It is the starting ingredient of all other steroids in the body, including sex hormones, corticosteroids, bile acids, and vitamin D.[26,27] In plasma and tissues, it is present as free cholesterol or as cholesteryl ester, the storage form. All tissues containing nucleated cells are capable of synthesizing cholesterol from its precursor, acetyl coenzyme A (acetyl-CoA), in smooth endoplasmic reticulum by a series of enzymatic reactions, where hydroxymethylglutaryl-CoA reductase acts as the rate-limiting enzyme (Fig. 1).[28]

Apart from de novo biosynthesis, cholesterol is also obtained from diet and bile, which is absorbed from the small intestine. In the enterocytes of small intestine, cholesterol is esterified by acyl-CoA cholesterol acyltransferase and is packed into lipoproteins, chylomicrons, along with TG followed by its transport to the liver.[29,30] Because of its lipophilic nature, cholesterol is transported in circulation from its tissues of origin as free or esterified form crowded into lipoprotein particles. According to their density, lipoproteins are categorized into chylomicrons, very low density lipoprotein (VLDLs), intermediate density lipoproteins (IDLs), LDL, and HDL.[31] The liver secretes VLDLs into circulation, which get hydrolyzed by lipoprotein lipase,[32] leading to the formation of VLDL remnants and IDLs. The IDLs are either taken up by the liver or further hydrolyzed to LDLs, which are the main cholesterol carriers in the blood (Fig. 1).[33] Low-density lipoprotein is taken up by the hepatic or extrahepatic tissues, either independently or via the LDL receptor (LDL-R), which are expressed both in the liver as well as in peripheral tissues.[34,35] Excess cholesterol from extrahepatic tissues are packed into HDL particles and transported back to the liver by the process of reverse cholesterol transport, the only pathway of excess cholesterol elimination from extrahepatic tissues and organs (Fig. 1).[36,37] The excess cholesterol along with TG is transported to apolipoprotein A-I of

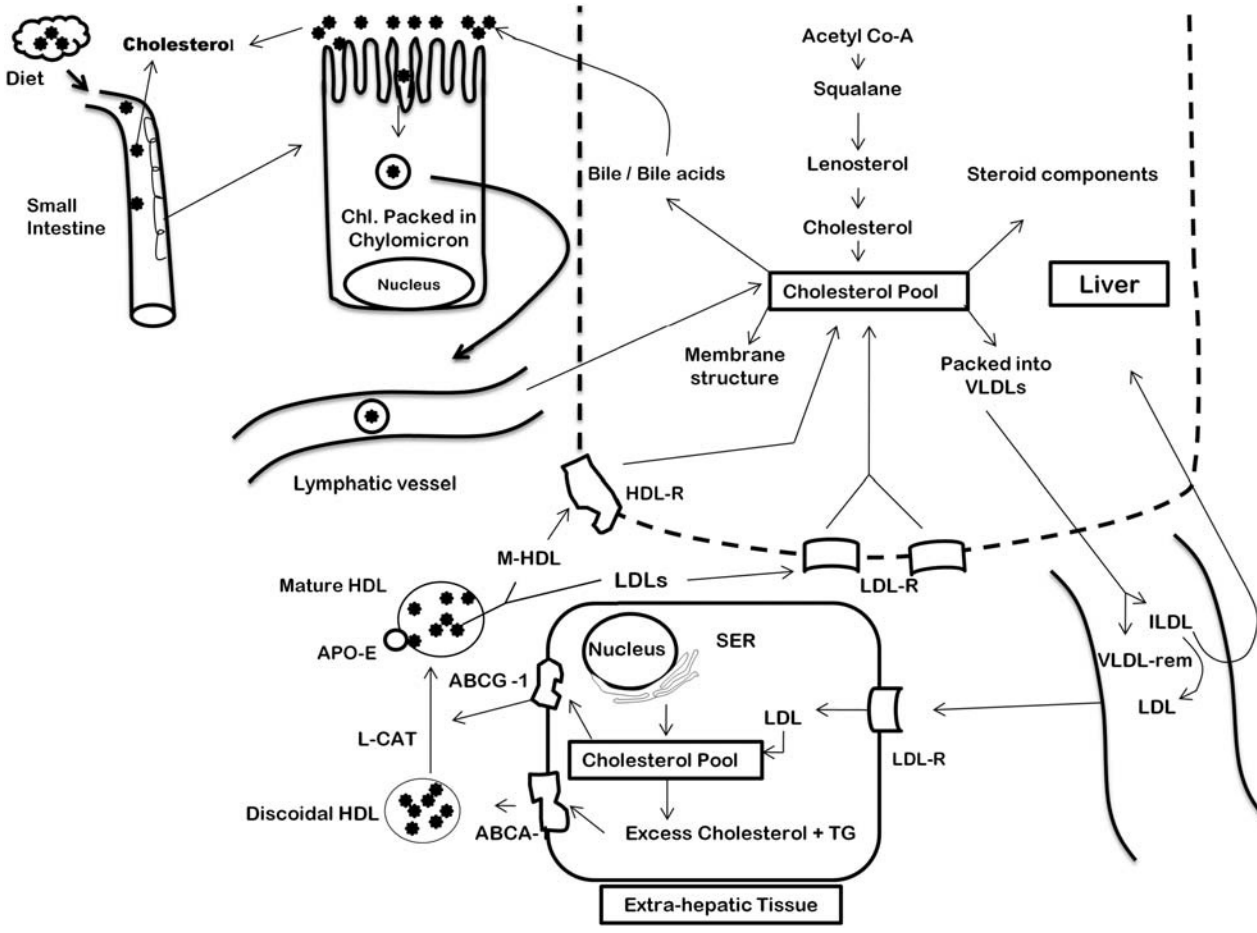

FIGURE 1. Schematic representation of cholesterol synthesis, absorption, and transport. Dietary cholesterol, after being absorbed by enterochromaphin cells of gut, streams to systemic organ (liver) via lymphatic vessels. Both transported and "de novo" synthesized cholesterol in the liver forms "cholesterol pool." Fractions of cholesterol from cholesterol pool are used during membrane structure formation and bile acid formation and in steroid component synthesis. The rest of the cholesterol undergoes packaging into VLDLs and enters circulation to yield ILDL and VLDL remnant. Among them, ILDL reenters into the hepatic cell, while another product of ILDL, that is, LDL, enters into extrahepatic tissue through LDL-R and forms cholesterol pool there. From the extrahepatic tissues, excess of cholesterol is transported out of the cells by ABCG1 or ABCA1 transporter, which further undergoes changes to form discoidal HDL. High-density lipoprotein undergoes successive morphological changes and finally enters hepatic tissue via HDL-R.

plasma via adenosine triphosphate (ATP)–binding cassette (ABC) transporter A1 and forms discoidal HDL, cholesterol of which becomes esterified and again receives excess cholesterol from extrahepatic tissues via ABCG1 and produces mature HDL (Fig. 1).[36]

Rodent and human pancreatic β-cells of rodents and human express high-affinity LDL-R, which facilitates cholesterol accumulation in β-cells.[19,38] The LDL-R, VLDL receptor, and LDL-R–related protein 1 are among the 3 LDL-Rs present on the β-cell membrane.[39] Two ABC transporters, ABCA1 and ABCG1, also present on β-cells membrane mediate the export of excess cellular cholesterol into HDL particles.[40,41] The scavenger receptor B1, a multiligand cell-surface receptor that plays a central role in HDL homeostasis,[42] helps in bidirectional movement of cholesterol from membrane to extracellular space and vice versa (Fig. 1).[39] The detailed process of cholesterol absorption, synthesis, metabolism, and transport has been diagrammatically represented in Figure 1.

IMPACT OF EXCESS CHOLESTEROL ON THE PHYSIOLOGY OF PANCREATIC β-CELL

Hypercholesterolemia is associated with several disease pathologies and is an independent risk factor of cardiovascular and cerebrovascular diseases.[43,44] Studies of last decades have implicated that hypercholesterolemia also hampers the normal physiological functioning of the pancreas, mainly the β-cells.[19,22,45] Here, we have discussed the pathological impacts of hypercholesterolemia-mediated accumulation of cholesterol or lipoprotein-mediated excess loading of cholesterol on the physiology of pancreatic β-cell.

Hypercholesterolemia on Pancreatic β-Cell Death

In hypercholesterolemic condition, excessive accumulation of cholesterol occurs in several cell types, including pancreatic β-cells.[46] High-fat diet in C57BL/6 J mice increases plasma cholesterol by 3 folds with a concomitant 1.5-fold elevation in pancreatic cholesterol content compared with normal animals.[25] Moreover, in cultured cells, excess cholesterol accumulation causes damage of pancreatic β-cells, due to oxidative stress, mitochondrial dysfunction, reduced cell viability, and increased rate of apoptosis.[19,22,23,45] When pancreatic β-cell–derived MIN6 cell lines are exposed to excess cholesterol, activation of proapoptotic enzyme, caspase 3, and elevated expression of p53 occur leading to cell death.[19,23] In MIN6 cell lines, excess cholesterol causes loading of extra cholesterol in plasma membrane, which leads to

the generation of reactive oxygen species as well as activation of a stress-induced protein kinase, p38, and mitogen-activated protein kinase signaling pathway,[22] thereby leading to apoptosis.[47] In a pancreatic β-cell line, NIT-1, hyperaccumulation of cholesterol caused 60% decline in cell viability with a concomitant 30% increase in apoptosis rate and significant decrease in the expression of antiapoptotic factor, B-cell lymphoma 2, as compared with untreated cells.[25] High fat diet–induced elevation of pancreatic cholesterol content in C57BL-6 mice causes a significant decrease in the mass of pancreas along with 2-fold decrease in the expression of pancreatic and duodenal homeobox-1, a critical molecule for pancreatic cell proliferation.[25,48]

At physiological concentration, LDL causes toxicity and death of islet β-cells isolated from rodent pancreas due to intracellular LDL oxidation.[19] One of the reasons of oxidative stress in pancreatic β-cells might be the diminished level of free radical scavenging enzymes, including catalase and glutathione peroxidase compared with other cell types.[49,50] Exposure of cultured β-cells to human VLDL, LDL, and oxidized LDL results in the inhibition of proliferation and impel apoptosis in dose-dependent manner.[39,51] Very low density lipoprotein and oxidized LDL prevail upon apoptosis of β-cells, which is mediated by caspase 3 and activation of c-Jun N-terminal kinase pathway, and these effects have been reported to be reversed upon HDL treatment.[39,51]

Hypercholesterolemia Causes Functional Impairment of β-Cell

The pancreatic β-cells secrete the antiglycemic and antilipolytic hormone, insulin in response to elevated plasma glucose level.[52] Circulating glucose enters β-cells by GLUT2 transporter and is quickly phosphorylated by glucokinase to glucose-6-phosphate, which undergoes further oxidation and forms pyruvate. After entering the mitochondria, the pyruvate produces ATP in tricarboxylic acid cycle. The elevated level of ATP blocks ATP-sensitive potassium (K^+-ATP) channels, and the resulting depolarized potential opens the voltage-dependent calcium (Ca^{2+}) channels in β-cell membrane (Fig. 2).[1,53,54] The influx of Ca^{2+} together

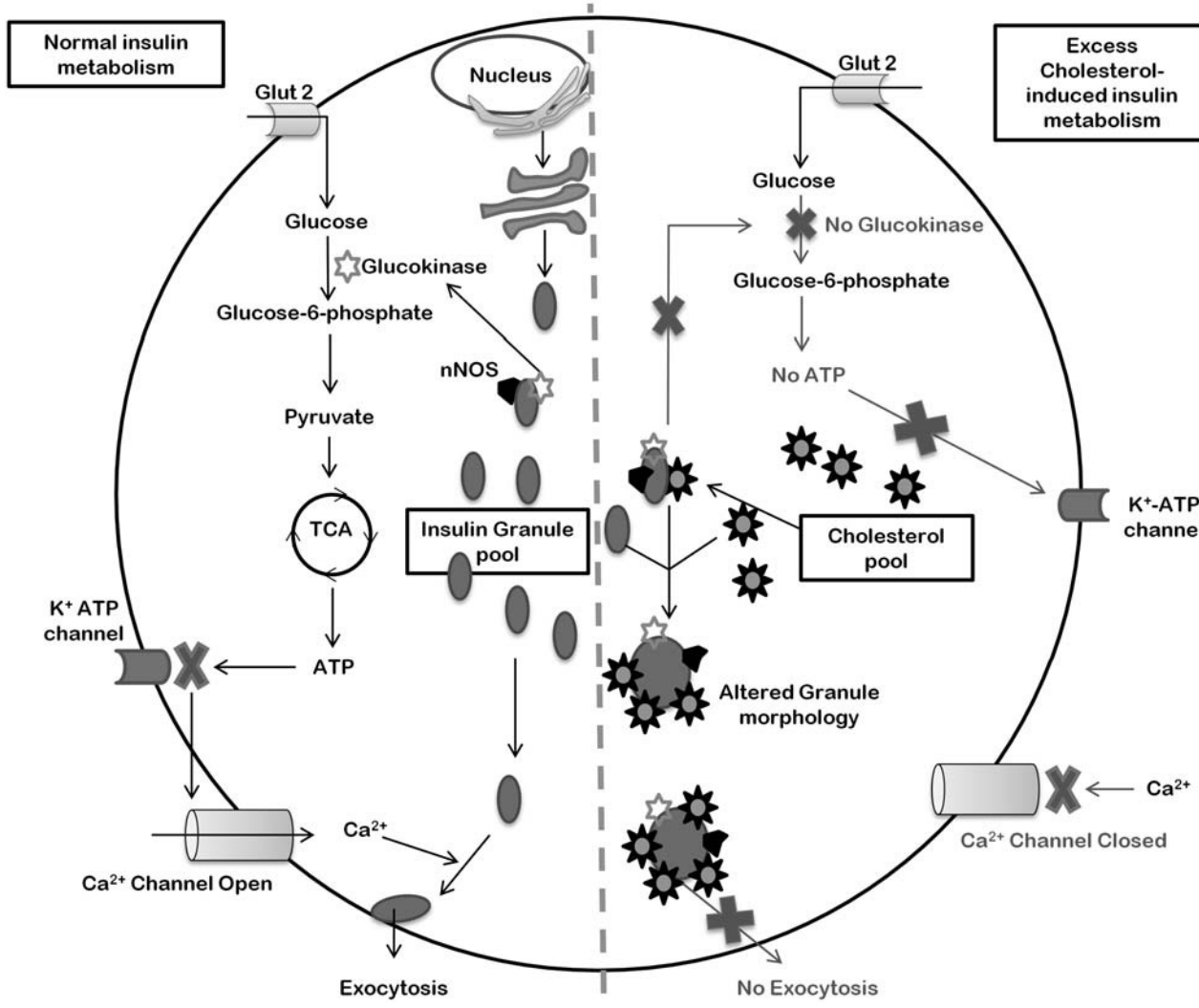

FIGURE 2. Schematic comparison between normal insulin secretion and cholesterol-induced hindrance in insulin secretion. Left, General mechanism of insulin secretion: glucokinase plays a crucial role in ATP production through glucose metabolism. Adenosine triphosphate blocks K^+ channels but opens Ca^{2+} channel, which is much needed for the exocytosis of insulin granules. Right, When cytosol is saturated with increasing quantity of cholesterol, it binds tightly with insulin granule with other 2 components, namely, nNOS and glucokinase. Hence, lack of ATP influences the K^+ channel opening and consequently impedes insulin secretion via blocking Ca^{2+} channel.

with the help of secretory apparatus consisting of the soluble N-ethylmaleimide–sensitive factor attachment protein receptor proteins syntaxin-1A, synaptosomal protein of 25 kDa, and synaptobrevin, facilitates the first phase of rapid exocytosis of insulin granules, followed by a slower and more sustained secretion of insulin, the second phase (Fig. 2).[55]

Cholesterol homeostasis is very essential for maintaining normal functioning and regulated insulin secretion from β-cells. The Ca^{2+} and K^{+}-ATP channels of β-cell link directly to SNARE proteins that associate β-cell ion channels closely with the insulin secretory vesicles.[56,57] The insulin secretory apparatus, as well as the Ca^{2+} and K^{+}-ATP channels, is located in cholesterol-rich microdomain of β-cells called lipid raft.[58] When β-cell cholesterol is lowered by 50%, insulin secretion has been reported to be reduced, probably because of inhibition of Ca^{2+} channels.[59] Moreover, excess accumulation of cholesterol in β-cells impairs granule morphology and hampers the process of exocytosis of insulin granules.[24,25] Sterol-responsive element-binding protein 2 (SREBP-2), a transcription factor, is an important regulator of β-cell cholesterol synthesis.[60] However, overexpression of SREBP-2 causes accumulation of cholesterol in β-cell and produces features of diabetes, including decreased β-cell mass and impaired insulin secretion.[61] In SREBP-2 overexpressed animals, the islet mass reduces because of abate expression of PDX-1 and BETA2, the transcription factors for β-cell development and survival.[61] Cholesterol accumulation in β-cell due to apoE deficiency in animal model impairs glucose-stimulated insulin secretion by inhibiting glucokinase.[62] In β-cell, glucokinase is bound to insulin-containing granules in the cytoplasm by dimeric neuronal nitric oxide synthase (nNOS).[63] Hao et al[62] demonstrated that increased β-cell cholesterol enhances nNOS dimerization and increases its interaction with glucokinase. This hampers glucose metabolism, thereby preventing exocytosis of insulin granules (Fig. 2). One of the major pathological contributions of elevated cholesterol to β-cell functional impairment comes from a substantial body of study demonstrating that insulin granules are the main sites of cholesterol accumulation in β-cells.[24] On the contrary, the recycling endosomes and trans-Golgi network are the major sites of intracellular cholesterol accumulation in other cells of nonsecretory nature.[64,65] Accumulation of excess cholesterol in insulin granules disturbs granule morphology and alters the distribution of some membrane proteins, thereby impeding exocytosis of granules (Fig. 2).[24] A recent significant study also demonstrated that elevated pancreatic β-cell cholesterol in C57BL/6 J mice leads to enlargement of insulin-positive granule area with irregular shape compared with the naïve animal.[25] Thus, elevated β-cell cholesterol induced by hypercholesterolemia impairs β-cell functional status mainly by modulating the insulin exocytosis process, as described in Figure 2.

Hypercholesterolemia Mediates Inflammation in β-Cell

Chronic cholesterol–rich high-fat diet increases the mass of adipose tissue, which in turn activates immune cells.[66] Diet-induced increased mass of white adipose tissue and related immune response have been reported in T2D subjects.[66,67] In T2D subjects, oxidized LDL-cholesterol–mediated inflammatory response is reported to be due to toll like receptor 4–mediated activation of certain cytokines like TNF-α, MIP-2, and monocyte chemoattractant protein-1.[68,69] Inflammatory activation of toll-like receptor 4 stimulates cholesterol accumulation and prevents efflux of cholesterol from the cell, due to inhibition of LXR-ABCA1 complex.[68] In addition, cholesterol-rich diet-induced inflammation, which has been reported to alter the level of adipokines, including adiponectin and resistin, plays a decisive role in insulin resistance and incidence of T2D.[70,71] Moreover, adiponectin enhances fatty acid oxidation and is also involved in the feedback sensitivity of insulin receptor.[72,73]

IMPACT OF CHOLESTEROL CARRIERS ON FUNCTIONAL STATUS OF PANCREATIC β-CELL

Role of ABC Transporter

ABCA1 and ABCG1 maintain pancreatic cholesterol homeostasis by exporting excess cholesterol from pancreatic islets to HDL particles.[40,74–77] However, lack of β-cell ABCA1 prevents the first phase of insulin granule secretion due to excess accumulation of cholesterol in membrane, which impairs exocytotic fusion of insulin granules, but without affecting β-cell mass and intracellular insulin content.[21,40] Again, increased expression of β-cell ABCA1 leads to excessive insulin secretion.[78] The major T2D features, including hyperglycemia as well as increased insulin level, have been reported to reduce the expression of β-cell ABCA1.[79,80] Moreover, a recent study demonstrated that lack of both ABCA1 and ABCG1 of pancreatic islets hampers insulin secretion and elevates the islets' cholesterol level in a much greater degree than either deletion of ABCG1 or ABCA1 in β-cells.[41] Gerin et al[81] reported that increased accumulation of cholesterol esters in pancreatic β-cell is attributed to the reduced expression of ABCA1 as well as ABCG1. Thus, cholesterol efflux through ABCA1 and ABCG1 is the critical regulator cholesterol content in islets as well as β-cell function. In both in vitro and in vivo systems, deletion of ABCG1 hampers the insulin secretion because loss of ABCG1 proteins located in insulin granules steered to altered insulin granule morphology and exhausts the granule cholesterol contents.[82]

Role of Liver X Receptor

The liver X receptor (LXR), a class of nuclear receptors that are suggested to have cholesterol sensing property, uses the sterol metabolites (oxysterols) as their ligand.[83] LXRα and LXRβ are the 2 subtypes of LXR; LXRα is being expressed mainly in metabolically active tissues, whereas LXRβ is expressed ubiquitously.[84] Excess accumulation of cellular cholesterol results in the rise of oxysterol level, which brings about LXR activation. Activated LXR stimulates transcription of several genes, including cholesterol transporter genes, that ultimately help in maintaining cellular cholesterol homeostasis.[83,84] In pancreatic islets, activated LXR improves glucose-stimulated insulin secretion, the most important parameter in determining functional status of pancreatic β-cells, and also stimulates lipogenic gene expression in β-cells.[85,86] However, increased expression and overstimulation of islets LXR reported to cause β-cell dysfunction, suggesting the involvement of chronic LXR dysregulation in β-cell failure during the progression of T2D in obese animals.[87] In cellular models (HIT-T15 and MIN6), LXR activation arrests the pancreatic β-cell at G1 phase and thereby inhibits the viability and proliferation, which is evidenced by the increased production of p27,[88] a vital regulator of cell cycle progression in pancreatic β-cell, overproduction of which leads to inhibition of β-cell proliferation.[89,90] However, a substantial body of work reported that LXR agonist in combination with 9-cis-retinoic acid (RXR), but not alone, inhibits the proliferation while induces apoptosis of pancreatic β-cell.[91] The underlying mechanism is reported to be due to activated LXR/RXR induced overproduction of mothers against decapentaplegic homolog 3 (SMAD3),[91] a protein known to inhibit progression of a cell from G1 to S phase and induce apoptosis.[92,93]

FUTURE PERSPECTIVES

Can Cholesterol Modulate Pancreatic β-Cell Function via Amyloid Protein?

The extracellular deposition and aggregation of toxic amyloid protein is one of the major causes of apoptosis and dysfunction of pancreatic β-cell in T2D patients.[94,95] Toxic amyloid is formed from a soluble polypeptide hormone of the pancreas, amylin, or islet amyloid precursor protein.[96,97] Islet amyloid precursor protein is processed in secretory insulin granules and secreted along with insulin and is implicated to play an important role in glucose homeostasis.[98,99] There exists a causal relationship between the aberrant cholesterol homeostasis and extracellular deposition of the toxic protein, amylin oligomers, in pancreatic β-cell.[100,101] From in vitro studies, Cho et al[101] demonstrated the inhibitory effect of cholesterol on aggregation and polymerization of amylin oligomers by forming a stable and soluble random coil structure of amylin. Studies with human and rodent pancreatic islets have shown that the reduction of membrane cholesterol resulted in increased extracellular deposition of amylin aggregates.[100] Again, replenishment of membrane cholesterol has been reported to prevent extracellular accumulation of amylin oligomers by lipid raft–mediated intracellular transport of amylin oligomers in the islets.[100] However, the effect of elevated membrane cholesterol on the formation and deposition of toxic amyloid in pancreatic β-cell is yet to be unveiled.

Cholesterol, microRNA-33, and Pancreatic β-Cell Dysfunction: Is There Any Pathological Link?

microRNA-33 (miR-33) is the constituent of the intronic part of the sterol regulatory element binding factor 2, a cholesterol synthesis regulator gene.[102,103] microRNA-33 has been reported to modulate cellular cholesterol homeostasis as well as hamper the insulin signaling.[102,104–106] In human and rodents, miR-33a modulates cholesterol homeostasis by silencing the genes encoding for ABCA1 and ABCG1 of the liver and macrophages.[102,104,107,108] Moreover, higher expression of ABCA1 in the liver and macrophages, and increased cholesterol efflux from the liver, has been reported in miR-33a knockout mice.[109] In human and mouse pancreatic islets, overexpression of miR-33a transcends to increased accumulation of islets' cholesterol due to reduced expression of ABCA1 accompanied with defective insulin secretion.[77] However, the role of hypercholesterolemia on expression of miR-33 in pancreatic islet cells and underlying pathological manifestations remain elusive.

Is Cholesterol Linked to Immune-Mediated Destruction of β-Cell?

More than 70% of the T1D are associated with self-antibodies–mediated damage of β-cells referred to as autoimmune diabetes.[110] The most prevalent markers of autoimmune diabetes are islet cell antibody, glutamic acid decarboxylase antibody (GADA), insulinoma-associated antigen-2 antibody, and zinc transporter 8 antibody.[110] Most forms of modified LDL cholesterols are reported to be immunogenic and induce the formation of autoantibodies in humans in the form of immune complexes (ICs).[111] In T1D patients, more than 90% of modified LDL circulates as soluble IC with high level of cholesterol.[112,113] The IC from T1D subjects predominantly contains proinflammatory classes of IgG having the potency to react with oxidized and modified LDL.[114,115] Abnormal lipid profile, including cholesterol-TG imbalance, has been reported in T1D patients,[116] where the cholesterol level elevates by 1.45-fold as compared with T2D and healthy individuals.[117] Conversely, higher level of good cholesterol (HDL) was reported in T1D and LADA (latent autoimmune diabetes of adults) patients compared with T2D patients.[118] Another study observed no association between cholesterol metabolism and autoimmune diabetes, as the values of TG and HDL cholesterol in GADA-positive as well as GADA-negative individuals did not change significantly.[119] Statins, the inhibitors of cholesterol biosynthesis, also have immunomodulatory properties.[120] In a mouse model of autoimmune diabetes, atorvastatin does not decrease or delay diabetes onset and fails to inhibit autoantigen-specific T-cells over time.[120,121] The effectiveness of atorvastatin in reducing declined β-cell function in T1D patients with higher levels of inflammation mediators such as C-reactive proteins has also been documented.[122] However, the impact of LDL immunogenicity particles on pancreatic β-cell functional status or on occurrence of autoimmune diabetes is still not clearly known. In addition, therapeutic approaches with statins have provided controversial results. Therefore, the effect of abnormal cholesterol metabolism on autoimmune diabetes, where pancreatic β-cells are destroyed, remains an unknown terrain in diabetic research.

CONCLUSION

Accumulating evidences suggest that increased cholesterol in β-cells can participate in diabetic complications, which then itself exaggerate the cell death or the signature pathologies of diabetes. Thus, the major pathological implication of hypercholesterolemia in diabetes has been attributed to its accumulation in β-cell due to transporter desensitization that ultimately results in impaired exocytosis of insulin granule by modulating granule morphology, which ultimately transcends to diabetic pathology. The overaccumulation of cholesterol in β-cell causes cell death by apoptotic mechanism. Thus, this review highlighted one of the causes of diabetes and supports the use statin or other therapies that can reduce cholesterol levels for the effective management and treatment of diabetes. In addition, we have appraised the role of cholesterol in modulating the pancreatic β-cell functions via pancreatic amyloid protein and miR-33.

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