

Review

A Review on Molecular Basis of the Role of Psychological Stress in the Development and Progression of Type 1 and Type 2 Diabetes Mellitus

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Prolonged stress has long been shown to have major effects on the development of both type of diabetes mellitus, type 1 and type 2. This paper reviews the interrelationship between stress and diabetes. Chronic physical or emotional stress can activate the Hypothalamus-pituitary-adrenal (HPA) axis to induce production of the stress hormone glucocorticoids. Glucocorticoids cause abrupt thymus involution and result in a failure or breakdown in immunological tolerance. As a result of breakdown in immunological tolerance autoreactive T-cells are escaped from thymus microenvironment which ensures self/non-self education and selection of mature T-cells before being exported to the periphery. Though regulatory T-cells (Treg) are present in the circulation, they are unable to suppress the autoreactive T-cells from initiating the destruction of β -cells and the subsequent development of autoimmune type 1 diabetes. The destruction of β -cells can be mediated by various mechanisms including Fas-FasL, perforin/granzymes, reactive oxygen species, and cytokines (e.g., IL- 1β , IFN- γ). Oxidative stress leads to development of type 2 diabetes either by the activation of multiple stress-sensitive serine/threonine (Ser/Thr) kinase signaling cascades such as c-Jun NH₂terminal kinase (JNK) pathway resulting in insulin resistance and/or by causing defect in insulin gene expression due to the loss of at least two critical proteins {pancreatic and duodenal homeobox factor-1 (PDX-1) and musculoaponeurotic fibrosarcoma oncogene homolog A (MafA)} that activate the insulin promoter.

Key words: Psychological Stress, Reactive Oxygen Species, β -cells, pancreatic and duodenal homeobox factor-1, musculoaponeurotic fibrosarcoma oncogene homolog A, Diabetes.

INTRODUCTION

The immune system operates as an interconnected network, responding automatically to anything that invades or disrupts the body. It is critical that an individual be able to elicit strong immune responses to foreign antigens, yet not to react to a "self" antigen. This lack of an immune response to self when responses to environmental antigens are retained is due to immunological tolerance. Immunological tolerance is the process by which the immune system does not respond to an antigen and is important for preventing autoimmunity. T-cells development begin with the migration of bone marrow-derived, early thymic progenitor cells to the thymus (Allman et al., 2003),

where they will complete their self/non-self education and selection before being exported to the periphery. This process, known as thymopoiesis, ensures the establishment of central T-cell tolerance in the host (Haynes et al., 1989; Kaye, 2000) Peripheral tolerance occurs when mature lymphocytes, escaped from negative selection during ontogeny, encounter self antigens in secondary lymphoid organs and undergo anergy, deletion or suppression.

Stress disrupts the homeostatic balance of the immune system and causes acute thymic involution. Environmental stressors such as prolonged physical or emotional stress can activate the HPA axis to induce production of the stress hormone cortisol, causing abrupt thymus involution and a result in a drop in thymopoiesis (Haynes et al., 2000). The epithelial network of the thymus disrupts severely, with preferential loss of cortical

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epithelium which results in a breakdown of immunological tolerance. Autoimmunity is the result of a failure or breakdown of one or more of the mechanisms responsible for maintaining self-tolerance (Romagnani, 2006). Type 1 diabetes is caused by an autoimmune process in which the insulin-producing β -cells in the Islets of Langerhans are destroyed by the body's own immune system (Palmer et al., 1991). In this case, autoreactivity ensues as a result of the escape of self-reactive T cells that recognize β -cells antigens.

Stress hormone glucocorticoid may cause a shift from a T helper cell 1 (Th1) immune driven response to a T helper cell 2 (Th2), thus glucocorticoids favor a Th2 (Weigers et al., 2005). Pancreatic expression of Th2 cytokines accelerates autoimmune destruction of the pancreas (Lee et al., 1994; Mueller et al., 1997), suggesting that stress is responsible for the upregulation of autoimmune type 1 diabetes.

Stress hormone glucocorticoid also stimulates gluconeogenesis, particularly in liver. The pathogenic effect of high glucose in the development of type 2 diabetes is mediated to a significant extent via increased production of reactive oxygen species (ROS) e.g., $\cdot\text{O}_2^-$, HO^\cdot , H_2O_2 and the subsequent development of oxidative stress. Oxidative stress refers to the harmful condition that occurs when there is an excess of free radicals, a decrease in antioxidant levels, or both. Oxidative stress may take two routes for the development of type 2 diabetes: cells may become resistance to insulin and/or causes defective insulin gene expression. For the former case, oxidative stress leads to the activation of multiple stress-sensitive serine/threonine (Ser/Thr) kinase signaling cascades such as c-Jun NH₂-terminal kinase (JNK) (Joseph et al., 2003) which phosphorylates multiple targets, e.g., the insulin receptor (IR) and insulin receptor substrate (IRS) proteins, that decreases the extent of insulin-stimulated tyrosine phosphorylation (pY) (Paz et al., 1997; Birnbaum et al., 2001). The serine/threonine phosphorylated forms of IRS molecules are less able to associate with the IR and downstream target molecules, especially phosphatidylinositol 3-kinase (PI3K) (Paz et al., 1997; Paz et al., 1996) resulting in reduced insulin action or insulin resistance due to the lack of translocation of glucose transporter (GLUT4) to the cell surface.

On the other hand, oxidative stress causes defect in insulin gene expression due to the loss of at least two critical proteins that activate the insulin promoter (PDX-1 and MafA) (Robertson et al., 2004). Chronic exposure of β -cells to excess glucose and ROS levels causes loss of PDX-1 gene expression and MafA protein which inevitably results in diminished insulin synthesis, decreased insulin content, and defects in insulin secretion ensue.

Stress

Psychological stress refers to the emotional and

physiological reactions experienced when an individual confronts a situation in which the demands go beyond their coping resources (Venes et al., 2001). In medical terms stress is described as, "a physical or psychological stimulus that can produce mental tension or physiological reactions that may lead to illness". A stress condition seems relative in nature. Extreme stress conditions, psychologists say, are detrimental to human health but in moderation stress is normal and, in many cases, proves useful. Stress, nonetheless, is synonymous with negative conditions. Today, with the rapid diversification of human activity, we come face to face with numerous causes of stress and the symptoms of stress and depression.

Diabetes

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels that result from defects in insulin secretion, or action, or both. Type 1 or insulin-dependent diabetes mellitus (IDDM) is caused by autoimmune destruction of the insulin-producing β -cells of the pancreatic islets of Langerhans (Bach, 1994; Tisch et al., 1996). Symptoms seen in the type 1 diabetic patient include: polyuria, thirst, weight loss, neurotoxicity and ketoacidosis. Type 2 diabetes or non-insulin-dependent diabetes (NIDDM) which accounts for approximately 90–95% of those with diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Symptoms may include: fatigue, frequent urination, blurred vision and slow healing of wounds.

Mechanism of tolerance

Autoimmune diseases are characterized by the immune system attacking self-tissue. type 1 diabetes mellitus is a T-cell-mediated, organ-specific autoimmune disorder, in which the body's own immune system attacks β -cells of islets of Langerhans and damages them sufficiently resulting in reduced insulin production. In this case autoreactivity ensues as a result of the escape of self-reactive T-cells that recognize beta cell antigens. There is a break in immunological tolerance to allow this to happen. Defects in either central or peripheral tolerance may result in T-cell mediated autoimmune Type 1 diabetes. Therefore, the role of tolerance, or lack of tolerance, is important to the understanding of any autoimmune diseases.

T-cell development is tightly controlled by thymus. The process of self/nonself education, known as thymopoiesis, ensures the establishment of central T-cell tolerance in the host (Haynes et al., 1989; Kaye, 2000; Haynes et al., 1998) In thymus, medullary epithelial cells (MEC) present enormous number of self-peptides from

diverse tissues to thymocytes by the expression promiscuous gene (Kyewski et al., 2004). Thymocyte interaction with thymic epithelial cells during fetal development establishes a robust and organized environment in which distinct cortical and medullary thymic compartments are formed to provide the architectural framework necessary for thymopoiesis and subsequent export of naive T-cells to the peripheral circulation (Manley, 2000).

The recruitment of blood-borne migrant precursors to the thymus, as well as the subsequent migratory processes of thymocytes to the different thymic areas and also the migration of mature selected T-lymphocytes to the peripheral lymphoid organs, is an ordered process, which is regulated by chemotactic cytokines (chemokines) (Annunziato et al., 2001). In thymus, maturing thymocytes begin as CD4⁻/CD8⁻ double negative populations, before up-regulating CD4⁺ and CD8⁺ to become double positive (DP) thymocytes (Boyd et al., 1991). Over 90% of developing thymocytes will be of the DP phenotype. DP thymocytes undergo a rigorous selection process and eventually become CD4⁺ single positive (SP) or CD8⁺ SP thymocytes, which exhibit MHC Class II or MHC Class I restriction, respectively (Boyd et al., 1991). For example, auto-reactive CD4⁺ and CD8⁺ T-cells are clonally deleted during negative selection in the thymus, establishing central tolerance. The unique expression of the autoimmune regulator (AIRE) gene in medullary thymic epithelial cells results in expression of a broad array of tissue-specific antigens. Thymocytes bearing T-cell receptors that bind to these tissue-specific antigens are clonally deleted. At the end of the process, self-reactive cells failing negative selection are removed via apoptotic pathways, and mature, nonself-reactive SP thymocytes are exported to the periphery as antigenic, naive Th-cells (CD4) or cytotoxic T-cells (CD8) which ensure self-tolerance.

Stress-induced defects in central tolerance and Type 1 Diabetes

Stress disrupts the homeostatic balance of the immune system and causes thymic involution through physiologic conditions, such as malnutrition, emotional distress, or pregnancy, or pathological conditions, such as infection, disease, clinical cancer treatments, or preparative regimens, for bone marrow transplant. Environmental stressors such as prolonged physical or emotional stress can activate the HPA axis to induce production of the stress hormone cortisol, causing abrupt thymus involution and result in a drop in thymopoiesis (Haynes et al., 2000). Cortisol also plays a critical role in sepsis which causes massive lymphocyte apoptosis that impairs host T and B cell responses (Roivainen, 2006; Szopa et al., 1990) and causes severe thymic atrophy (Jahromi et al., 2007). Alterations in thymic stromal architecture affect T-cells maturation and the development of self-tolerance

(Zuklys et al., 2000), resulting in aberrant clonal deletion of thymocytes which allows the escape of self-reactive T-cells. Though Treg are present in the circulation, they are unable to suppress the autoreactive lymphocytes from initiating the destruction of β -cells (Zuklys et al., 2000). In the mouse model it is shown that the natural Treg (nTreg) exit from the thymus starts later than that of conventional T-cells (Tcon) explaining why early thymectomy may abate nTreg function and result in autoimmunity (Rouse, 2007).

Several small animal models exist using various stressors capable of inducing thymic involution. For example, stress from starvation (Mandrup-Poulsen, 1987) and physical restraint (Appels et al., 1989) increases glucocorticoid levels, which mediate thymocyte apoptosis. Besides this, stress hormone glucocorticoid shifts Th1/Th2 balance favoring to Th2. Pancreatic expression of Th2 cytokines were shown to be involved in IDDM pathogenesis through facilitation of pancreatic mononuclear-cell infiltration (Healey et al., 1995; Tian et al., 1997) and acceleration of islet β -cell destruction (Lee et al., 1996). Moreover, glucocorticoid hormones can modulate β -cell autoantigen Glutamic acid decarboxylase (GAD) expression by the transcriptional activation of the GAD promoter and may influence the development of autoimmune diabetes (Kim et al., 2002).

Mechanism of T-cell mediated destruction of β -cell

Type 1 diabetes is a T-cell-mediated autoimmune disease characterized by the destruction of insulin-producing pancreatic β -cells located in the pancreatic islets of Langerhans (Bach, 1994; Tisch et al., 1996; Steinman, 1995). Such destruction requires both CD4⁺ and CD8⁺ T cell subsets (O'Reilly et al., 1991; Shimizu et al., 1993; Nagata et al., 1994). β -cell autoantigens (released from the β -cells during spontaneous turnover or insult by viral infection) processed by macrophages and presented to helper T-cells in association with MHC class II molecules is considered to be the first step in the initiation of the disease process. Macrophages release IL-12, which activates Th1-type CD4⁺ T-cells. While this process is taking place, β -cell-specific precytotoxic T-cells may be recruited to the islets. These precytotoxic T-cells may be induced by IL-2 and other cytokines released by CD4⁺ helper T-cells to differentiate into effector T-cells. IFN- γ released by helper T-cells may cause macrophages to become cytotoxic. These cytotoxic macrophages release substantial amounts of β -cell-toxic cytokines (including IL-1 β , TNF- α , and INF- γ) and free radicals. In addition, the helper T-cells secretes interleukins that activate other helper T-cells, B lymphocytes, and cytotoxic T-cells. The autoantigen-specific CD8⁺ cytotoxic T-cells, as final effectors, may recognize the autoantigens expressed on many unaffected β -cells, in association with MHC class I molecules. Two major molecular pathways of CD8⁺ T

cell-mediated cytotoxicity have been defined: 1) the exocytosis of granules containing perforin and granzyme molecules, and 2) the ligation of Fas ligand (FasL) on T-cells with the apoptosis-inducing Fas molecule on target cells (Kägi et al., 1994; Walsh et al., 1994; Kojima et al., 1994). In this way, macrophages, T-cells, and cytokines synergistically destroy β -cells, resulting in the development of autoimmune type 1 diabetes.

Stress-induced insulin resistance and β -cell dysfunction

Stress causes metabolic disturbances including altered hepatic glucose metabolism, increased peripheral insulin resistance and hyperglycemia. Stress is a potential contributor to chronic hyperglycemia in diabetes. In stressful condition, glucocorticoid hormones (mainly cortisol) are produced in the adrenal cortex under the control of the HPA axis. Glucocorticoid induction of gluconeogenesis results in the synthesis of glucose from non-hexose substrates such as amino acids, lactic acids and glycerol from triglyceride breakdown. Enhancing the expression of enzymes, especially the phosphoenolpyruvate carboxykinase (PEPCK), involved in gluconeogenesis is probably the best-known metabolic function of glucocorticoids. The induction of the PEPCK gene which is accomplished through a glucocorticoid response unit (GRU) comprised of two non-consensus glucocorticoid receptor binding sites (GR1, GR2) and at least three accessory factor elements (gAF1-3) that bind HNF4/COUP-TF, HNF3, and COUP-TF, respectively (Imai et al., 1990; Scott et al., 1996; Wang et al., 1996). This PEPCK catalyzes the rate-limiting step of gluconeogenesis. The conversion of oxaloacetate to phosphoenolpyruvate (PEP) by PEPCK has long been thought to play a key role in this process (Granner et al., 1990; Hanson et al., 1972), as the enzyme provides the only known path whereby tricarboxylic acid (TCA) cycle intermediates can be converted to glucose.

The development of type 2 diabetes is usually associated with a combination of pancreatic β -cell dysfunction and insulin resistance (Saltiel et al., 2001; Shulman, 2000). Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion; once hyperglycemia becomes apparent, β -cell function gradually deteriorates and insulin resistance aggravates (Weir et al., 2001; Robertson et al., 1994). This process is called "glucose toxicity". There are different biochemical pathways and mechanisms of action for glucose toxicity include: glucose autoxidation, protein kinase C activation, methylglyoxal formation and glycation, hexosamine metabolism, sorbitol formation, and oxidative phosphorylation etc. All these pathways have in common the formation of ROS that, in excess and over time, cause chronic oxidative stress, which in turn causes insulin resistance as well as defective insulin gene expression.

Oxidative stress and insulin resistance

Insulin action is initiated by an interaction of insulin with its cell surface receptor (Shulman, 1999). The insulin receptor is a heterotetrameric protein that consists of two extracellular α subunits and two transmembrane β subunits connected by disulfide bridges (Ullrich et al., 1985; Ebina et al., 1985; Seino et al., 1989). Insulin binding to the extracellular α subunit induces conformational changes of the insulin receptor that activate the tyrosine kinase domain of the intracellular portion of the β subunit (Wilden et al., 1992; De Meyts et al., 1996; Rhodes et al., 2002). Activation of the tyrosine kinase of the insulin receptor leads to a rapid tyrosine phosphorylation of the IRS-1 that, in turn, activates PI3K. Consequently, PI3K pathway stimulates translocation of GLUT4 to the cell surface, an event that is crucial for glucose uptake by skeletal muscle and fat.

Chronic oxidative stress leads to the activation of multiple stress-sensitive serine/threonine (Ser/Thr) kinase signaling cascades such as JNK (Kaneto et al., 1997). Activation of the JNK pathway suppresses insulin biosynthesis and interferes with insulin action (Kaneto et al., 1997). Indeed, suppression of the JNK pathway in diabetic mice improves insulin resistance and ameliorates glucose tolerance. Once activated, these kinases are able to phosphorylate multiple targets, such as the IR and IRS proteins (including IRS-1 and IRS-2). Increased phosphorylation of IR or IRS proteins on discrete serine or threonine sites (pS/T) decreases the extent of insulin-stimulated tyrosine phosphorylation (pY) (Paz et al., 1997; Birnbaum, 2001). The serine/threonine phosphorylated forms of IRS molecules are less able to associate with the IR and downstream target molecules, especially PI3K (Paz et al., 1997; Paz et al., 1996), resulting in reduced insulin action or insulin resistance.

Oxidative stress and beta cell dysfunction

β -cells are sensitive to ROS because they are low in free-radical quenching (antioxidant) enzymes such as superoxide dismutase, glutathione peroxidase, and catalase (Tiedge et al., 1997), as well as ROS-scavenging proteins such as thioredoxin (Hotta et al., 2000). Chronic exposure of the beta cell to oxidative stress causes defective insulin gene expression accompanied by marked decreases in insulin content and abnormal insulin secretion. The defect in insulin gene expression is due to the loss of at least two critical proteins that activate the insulin promoter. One is pancreatic and duodenal homeobox factor-1 (PDX-1) (Olson et al., 1993; Olson et al., 1995) and the other is (musculoaponeurotic fibrosarcoma oncogene homolog A) MafA (Matsuoka et al., 2003). PDX-1 is a member of the homeodomain-containing transcription factor family. PDX-1 is expressed in the pancreas and duodenum and plays a crucial role in pancreas development (Holland et

al., 2002), β -cell differentiation/ regeneration (Miyazaki et al., 2004; Koizumi et al., 2004), and in maintaining normal β -cell function by regulating many important β -cell genes, including insulin, GLUT2, and glucokinase (Chakrabarti et al., 2002; Kulkarni et al., 2004). Apart from PDX-1 mRNA, oxidative stress also interferes with the action of MafA, a critical protein that activates the insulin promoter. These two transcription factors are essential to normal levels of insulin promoter activity. Chronic exposure of β -cells to excess glucose and ROS levels causes loss of PDX-1 gene expression and MafA protein which inevitably results in diminished insulin synthesis, decreased insulin content, and defects in insulin secretion that ultimately lead to the development of type 2 diabetes.

DISCUSSION

The brain and immune system continuously signal each other, often along the same pathways, which may explain how state of mind influences health. The immune system operates as an interconnected network whose activity is affected by stress. It has been shown that stress-induced changes in the immune system and in the metabolic activities are responsible for the progression and development of autoimmune mediated type 1 diabetes and the non-insulin dependent type 2 diabetes, respectively.

Autoimmunity is the result of a failure or breakdown of one or more of the mechanisms responsible for maintaining self-tolerance. Stress disrupts the homeostatic balance of the immune system and causes acute thymic involution through physiologic conditions, such as malnutrition, emotional distress, or pregnancy, or pathological conditions, such as viral infection. Stress-induced thymic atrophy is a complication from many environmental stressors in which transient reduction in thymus function persists until the physiological stressor is removed. Environmental stressors such as prolonged physical or emotional stress can activate the HPA axis to induce production of the stress hormone cortisol, causing abrupt thymus involution and result in a drop in thymopoiesis. Stress-induced thymic atrophy results in aberrant clonal deletion of thymocytes which allows the escape of self-reactive T-cells. These autoreactive T-cells then initiate the T-cell mediated autoimmune destruction of β -cell, resulting in the development of type 1 diabetes. The development of type 2 diabetes is usually associated with a combination of pancreatic β -cell dysfunction and insulin resistance. Stress causes metabolic disturbances including altered hepatic glucose metabolism, increased peripheral insulin resistance and hyperglycemia. Stress hormone glucocorticoid causes gluconeogenesis. This is usually done by enhancing the expression of phosphoenolpyruvate carboxykinase (PEPCK)-the rate limiting enzyme in gluconeogenesis. Chronic hyperglycemia results in oxidative stress via the

generation of reactive oxygen species (ROS). Oxidative stress activates serine/threonine (Ser/Thr) kinase signaling cascades such as c-Jun NH₂ terminal kinase (JNK) (Joseph et al., 2003). JNK phosphorylates IRS-1. Increased phosphorylation of IRS-1 decreases the action of phosphatidylinositol 3-kinase (PI3K) to translocate glucose transporter (GLUT), resulting in reduced insulin action or insulin resistance.

β -cells are sensitive to ROS because they are low in free-radical quenching (antioxidant) enzymes such as superoxide dismutase, glutathione peroxidase (Tiedge et al., 1997) which causes defective gene expression. The defect in insulin gene expression is due to the loss of at least two critical proteins that activate the insulin promoter- PDX-1, MafA. Chronic exposure of beta cells to excess glucose and ROS levels causes loss of PDX-1 (Olson et al., 1995) gene expression and MafA (Matsuoka et al., 2003) proteins which inevitably results in diminished insulin synthesis, decreased insulin content, and defects in insulin secretion occur.

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