ULTRASTRUCTURAL CHANGES OF PANCREATIC ISLETS MICROCIRCULATION IN NONOBESE DIABETIC (NOD) MICE

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Absract. The objective of this study was to investigate the ultrastructural changes of vascular pancreatic islets using a transmission electron microscopic technique. The major ultrastructural changes of microvessel in NOD mice are indicated by the swelling and vacuolization of the endothelial cell. Swollen cells are the first noticeable lesion of the cell response in reversible degeneration that is caused by the failure of homeostatic control. Loss in endothelial cell homeostasis is primarily a marker of endothelial dysfunction that plays a key role in the pathogenesis of diabetic vascular disease by losing the control of vascular tone. Diabetes also associates with an increased generation of oxygen-derived free radicals that may impair vasodilatation through the inactivation of vasodilators. In conclusion, consistent with a hypothesis that loss of the modulatory role of the endothelium may be a critical and initiating factor in the development of diabetic vascular disease, the ultrastructural changes in this study may indicate the first sign of endothelial dysfunction. This dysfunction correlates to the relationship between diabetes and reversible lesions of vessels in NOD mice, making for a better understanding of the pathophysiology of diabetic vascular disease to set the stage for further investigation to restore endothelial dysfunction in diabetes.

INTRODUCTION

Insulin-dependent diabetes mellitus (IDDM), or human type I diabetes, is in most cases caused by autoimmune destruction of the insulin-producing beta cells within the pancreatic islets of Langerhans. The mononuclear cells infiltrate the islets-which results in the development of insulitis, a prerequisite step for the development of diabetes-are primarily composed of T cells. T cells play an important role in initiating and propagating an autoimmune process to destroy beta cells. IDDM is a chronic disorder and its complications are major causes of morbidity and mortality in patients. Some of its complications include atherosclerotic events, such as myocardial infarction, cerebrovascular accidents, gangrene of the lower extremity, and renal insufficiency (Robbins *et al*, 1991). This athero-sclerotic risk is related to vascular injury (Semenkovich and Heinecke, 1997). There is a hypothesized loss of the modulatory role of the endothelium that may be a critical and initiating factor in the development of diabetic vascular disease (De Vriese *et al*, 2000).

The obstacles to the study of diabetic vascular lesions in humans have been overcome by the use of nonobese diabetic (NOD) mice. Inbred colonies of NOD mice have become widely established since the publication of the initial report describing this strain's susceptibility to the spontaneous development of autoimmune type 1 insulin-dependent diabetes mellitus (IDDM) (Makino *et al*, 1980a). The NOD/Shi originated from subsequent selective breeding of glycosuric mice from a control line. The incidence of diabetes in NOD/Shi mice in the specific-pathogen-free (SPF) source colony was a 70-80% incidence in females versus a 20% incidence in males (Kikutani and Makino, 1992). This gender dimorphism is controlled, in part, by gonadal sex steroids (Makino *et al*, 1980b).

Diabetes development in NOD mice is characterized by insulitis, which is a leukocytic infiltrate of the pancreatic islets. A pervasive leukocytic infiltrate emanating from the pancreatic vasculature and secretory ducts is first observed at a time when the islets are free of lesions. Large numbers of leukocytes aggregate at the periphery of islets and develop insulitis between 5 and 7 weeks of age in females and several weeks later in males (Tarui *et al*, 1986). Marked decreases in pancreatic insulin content are demonstrable in NOD female mice at around 12 weeks of age and several weeks later in males. This finding correlates with the histological profile of selective destruction of beta, but not non-beta, islet cells (Harano *et al*, 1986).

NOD mice that are homozygous for the severe

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combined immune deficiency spontaneous mutation (NOD/SCID) are characterized by an absence of functional T cells and B cells, including defective antigen-presenting cells and NK cells. NOD/SCID mice are both insulitis- and diabetes-free throughout life and serve as diabetes-free controls for comparisons to NOD mice (Shultz *et al*, 1995; Custer *et al*, 1985).

The experiment was performed to test the hypothesis that a loss of the modulatory role of the endothelium may be a critical and initiating factor in the development of diabetic vascular disease. The objective of this experiment was to investigate the morphological changes of endothelial cell concerning the diabetic vascular lesions in pancreatic islets of the animal model of human type I diabetes, or nonobese diabetic (NOD) mice, using a transmission electron microscopic technique.

MATERIALS AND METHODS

Mice

Four NOD/Shi jic (NOD) mice and four NOD/LtSz-*Prkdc*^scid (NOD/SCID) mice, females, age 10-12 weeks were used in this study. Mice were bred and maintained at the Laboratory Animal Center, National Institute of Health, Nonthaburi, Thailand, under specific-pathogen-free conditions. Mice were fed with irradiated pellets and sterile water *ad libitum*, and maintained at 22±1 °C on a 12-hour light-dark cycle. The mice were euthanized and the pancreas was removed. The experiment was approved by the Institutional Animal Care and Use Committee of the National Institute of Health, Thailand.

Blood glucose determination

NOD mice tail blood was collected and checked for glucose using an automatic biochemistry analyzer (Vitros 250 chemistry system). Mice with non-fasting blood glucose levels that were higher than 250 mg/dl were selected for use in this study.

Serum immunoglobulin determination

NOD/SCID mice tail blood was collected and checked for immunoglobulin (Ig) G and M concentrations by the sandwich enzyme-linked immunosorbent assay (ELISA) method. Mice with total IgG and IgM concentrations of less than 0.2 µg/ml were selected for use in this study.

Preparation for transmission electron microscopy

For transmission electron microscopy observation, small pieces of mice pancreas were immediately fixed in 2.5% (V/V) glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 2 hours. Samples were washed three times in 0.1 M phosphate buffer and post-fixed with 1% (W/V) aqueous osmium tetroxide in 0.1 M phosphate buffer for 1 hour at room temperature. Samples were washed three times with phosphate buffer and dehydrated through graded ethanols (30-100% ethanol, 15 minutes per step). Tissues were infiltrated with propylene oxide and embedded in plastic embedding media (Epon-812). Ultrathin sections (150 nm) were cut with an Ultracut E (Reichert-Jung) ultramicrotome. Copper grids (200-mesh squares) were used to collect the ultrathin sections that were stained with lead citrate and uranyl acetate, following Reynold's method, and examined by a Hitachi H-7000 transmission electron microscope.
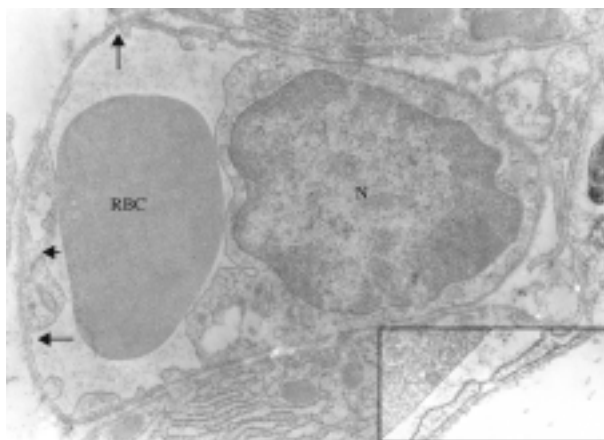

Fig 1- Electron micrograph shows a normal thin-walled blood capillary of pancreatic islets in NOD/SCID mice. Within the lumen (L) lies an erythrocyte (RBC). The nucleus (N), the cytoplasm of endothelial cell in flatten shape, typical with pore between cells – fenestration (arrows) bridged by thin diaphragm (inset), the endothelial cell junction (arrowhead) and micropinocytic vesicles (asterisk) are noticeable. 10,000 ×.
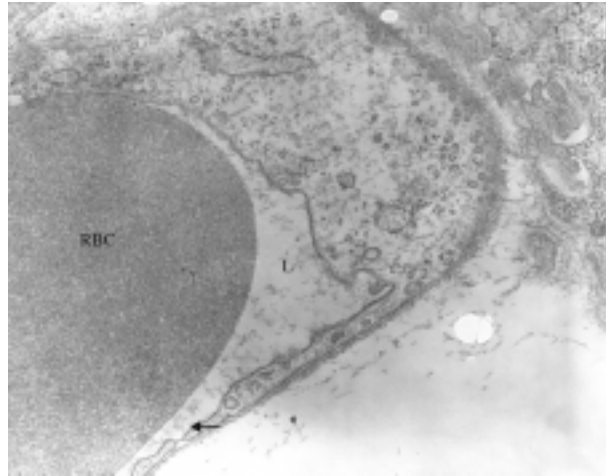
Fig 2- Electron micrograph showing a swollen endothelial cell in NOD mice . Notice the normal endothelial cell with fenestration in some areas (arrow). An erythrocyte (RBC) is in the lumen (L) of capillary. 17,000 ×.
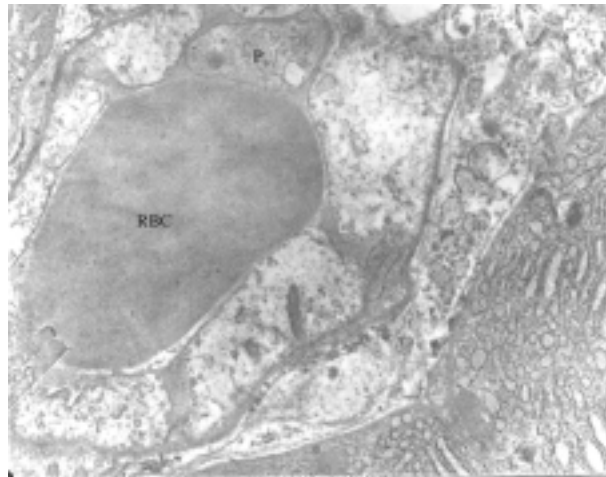

Fig 3- Electron micrograph showing a markedly swollen endothelial cell in NOD mice with an erythrocyte (RBC) and platelet (P) in lumen. 10,000 ×.

RESULTS

The transmission electron microscope observation of pancreatic islets vessels of mice demonstrated that the capillaries in NOD/SCID mice showed no sign of abnormality (Fig 1). The major ultrastructural changes in NOD mice were indicated by swelling and vacuolization of endothelial cells (Figs 2-5). Some insulin-producing beta cells were destroyed, but some cells were normally structured with insulin-secretory granules. There was no change of the pancreatic acinar cell. Pancreatic ducts within the islets and in the surrounding acinar tissue were observed in a few islets.

DISCUSSION

Susceptibility to IDDM in NOD mice is polygenic, and the environment including housing conditions, health status, and diet exerts a strong effect on penetrance of immune cells. Female NOD mice show a heterogeneity of pancreatic islet morphology, with some islets demonstrating heavy infiltration of immune cells, and others were untouched. Seventy percent of NOD mice at 13 weeks had diabetes controlled by NOD/SCID mice (Sreenan *et al*, 1999). Therefore, NOD mice with a high level of blood glucose were selected to study pancreatic islet vessels, and NOD/
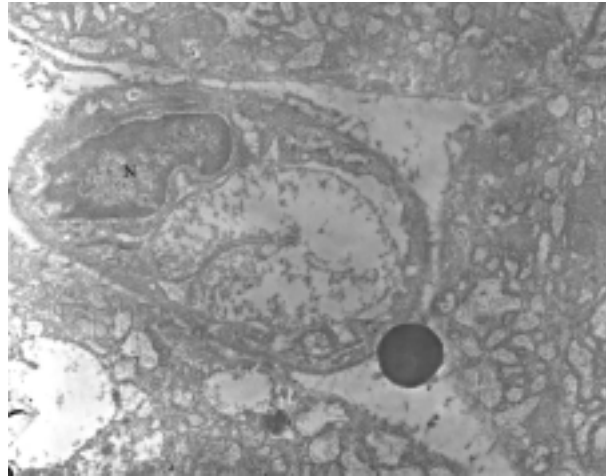
Fig 4- Electron micrograph shows a swollen, protruded endothelial cell in NOD mice, almost obstructing the lumen with its cytoplasm. 9,000 ×.
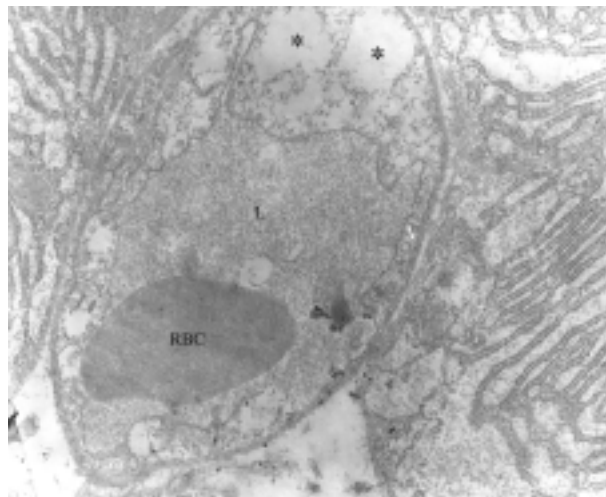

Fig 5- Electron micrograph shows a swollen endothelial cell with vacuolization (asterisks) in NOD mice. 12,000 ×.

SCID mice with low immunoglobulin level were selected as controls.

The major ultrastructural changes of vascular pancreatic islets in NOD mice were indicated by swelling and vacuolization of the endothelial cell. A swollen cell is the first noticeable lesion of cell response in reversible degeneration, caused by the failure of homeostatic control (Robbins *et al*, 1991). Loss of endothelial cell homeostasis is primarily a marker of endothelial dysfunction that plays a key role in the pathogenesis of diabetic vascular disease by losing the control of vascular tone. The endothelium controls the tone of the underlying vascular smooth muscle through the production of vasodilator mediators. The endothelium-derived relaxing factors comprise nitric oxide, prostacyclin, and a still elusive endothelium-derived hyperpolarizing factor (De Vriese *et al*, 2000). Nitric oxide generated by endothelial cells is a major regulator of vascular tone in muscular arteries. It also exerts other potent biological effects such as inhibition of platelet aggregation and of monocyte adhesion to the endothelium. Endothelial cells from different vascular beds exhibit metabolic and structural differences and may be affected differentially by hyperglycemia (Sobrevia and Mann, 1997).

Evidence implicates oxidative stress as an important pathogenic element in diabetic endothelial dysfunction. Oxidative stress is defined as an increase in the steady-

state levels of reactive oxygen species and may occur as a result of increased free radical generation and/or decreased antioxidant defense mechanisms. Several studies have reported decreased plasma or tissue concentrations of superoxide dismutase, catalase, glutathione and ascorbic acid in both clinical and experimental diabetes. In addition, diabetes has been associated with an increased generation of oxygen-derived free radicals (Giugliano *et al*, 1996). Oxygen-derived free radicals may impair endothelium-dependent vasodilatation through inactivation of nitric oxide or by serving as an endothelium-derived constricting factor (Rubanyi and Vanhoutte, 1986).

The disordered endothelium-dependent vaso-dilatation is primarily a marker of endothelial dysfunction and concerns adverse effects on endothelial cell homeostasis in diabetes. In addition, the susceptibility of tissues to the damaging effects of hyperglycemia may vary, altered vascular reactivity may influence target organ functioning. The mechanisms of endothelium-dependent vasodilatation may be quite different according to the size of the vessel and its anatomical location (De Vriese *et al*, 2000). The ultrastructural changes in this study, vacuolization and swollen endothelial cell, indicated that there was a relationship between diabetes and lesions of vascular pancreatic islets in NOD mice.

In conclusion, with regards to the hypothesis that the loss of modulatory role of the endothelium may be a critical and initiating factor in the development of diabetic vascular disease. The ultrastructural changes in this study may suggest that the first sign of endothelial dysfunction correlates to the relationship between diabetes and reversible lesion of vessels in NOD mice. This provides a better understanding of the pathophysiology of diabetic vascular disease; to set the stage for further investigation to restore endothelial dysfunction in diabetes.

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