Histopathological and Immunohistochemical Analysis of the Endocrine and Exocrine Pancreas in Twelve Cattle with Insulin-Dependent Diabetes Mellitus (IDDM)

Hiroyuki TANIYAMA*, Kazuko HIRAYAMA, Yumiko KAGAWA, Takashi KUROSAWA, Masashi TAJIMA¹, Tomoo YOSHINO and Hidefumi FURUOKA²

Department of Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu 069–8501, ¹Laboratory of Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818, and ²Department of Veterinary Pathology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Japan

(Received 12 February 1999/Accepted 26 March 1999)

ABSTRACT. Histological and immunohistochemical studies were carried out on the pancreas of twelve cattle of insulin-dependent diabetes mellitus (IDDM). They showed clinical signs such as persistent hyperglycemia, glycosuria and decreased glucose tolerance, and some cases accompanied with or without ketonuria. Histopathologically, eight cattle were diagnosed as chronic IDDM, while others were acute IDDM. The most characteristic lesions of the pancreas in chronic IDDM showed a decrease in the size and number of pancreatic islets, interlobular and interacinar fibrosis, mild lymphocytic insulitis, and vacuolation of a few islets. Almost all cells in the atrophied islets had a small amount of ungranulated cytoplasm. Immunohistochemical examination revealed that the atrophied islet cells did not react to anti-insulin antibody, but occasionally reacted to anti-glucagon or somatostatin antibodies. A few solitary islets with mild lymphocytic infiltration, necrotic islets with occasional calcification, and atrophied islets with mild fibrosis were also observed. A few islets consisted of many islet cells with vacuolated cytoplasm including a small number of insulin-positive granules. Accumulation of glycogen granules was occasionally observed in these islets. Lymphocytic infiltration was frequently observed in the islets which consisted of many islet cells having karyorrhectic nuclei and vacuolated and severely degranulated cytoplasm. Immunohistochemically, islet cells with vacuolated cytoplasm had a small amount of insulin-positive granules, suggesting severe degranulation of β-cells. An increase in acinar-islet-cells and proliferation of ductal epithelial cells showing insulin-immunoreactivity were observed. Bovine IgG-immunoreactive islet cells were frequently seen in the vacuolated islets. In summary, pathological observations suggested that β-cells were being destroyed by an inflammatory process which selectively affected the pancreatic islets. Lymphocytic insulitis and anti-bovine immunoreactive islet cells were thought to be the most significant changes in determining anti-islet autoimmunity in this form of diabetes.—KEY WORDS: cattle, diabetes mellitus, IDDM, immunohistochemistry, insulitis.

It is believed that insulin-dependent diabetes mellitus (IDDM) results from the destruction of insulin producing β-cells induced by cellular and/or humoral autoimmune mechanisms. The inflammatory infiltration in the islet of Langerhans is one of the usual findings in the histopathology of spontaneous IDDM in humans and experimental animals [10, 12, 19, 27]. This phenomenon in the islets was called “insulitis” by von Meyenburg [35], because of the specific localization of the inflammation. The occurrence of insulitis has thus far been limited to patients with IDDM (formerly called juvenile diabetes) who died shortly after the onset of the disease [8, 10, 15, 27]. Although other remarkable changes such as a decrease in the number and size of islets, disappearance of β-cells, hyalinization, calcification, necrosis and fibrosis are seen in endocrine pancreatic tissues in long-standing diabetes, insulitis is considered to be a specific histopathological manifestation of IDDM. Furthermore, the pancreas is usually atrophied and exhibits increased firmness due to diffuse interlobular and interacinar fibrosis with atrophy of the exocrine pancreatic tissues in very long-standing diabetes in humans [13, 27].

In cattle, the occurrence of spontaneous diabetes mellitus is extremely rare in comparison with that in humans [3, 4, 23, 26, 39, 42, 43, 46]. Histological and immunohistochemical studies have not been thoroughly carried out on the pancreas in bovine diabetes mellitus; therefore, the details of the histopathologic characteristics of the disease in cattle are still unclear [23]. Recently, histopathological analysis of the pancreas done on a few cases of bovine diabetes mellitus revealed such morphological changes in the islets as a decrease in the number and size of islets, hydropic and/or vacuolar degeneration and degranulation of islet cells, lymphocytic insulitis, and fibrosis [4, 26, 39, 42, 43, 46].

In the present study, histopathological and immunohistochemical examinations were performed on the endocrine and exocrine pancreatic lesions of twelve cases comprised of eight IDDM cattle with ketonuria and four IDDM cattle without ketonuria.
MATERIALS AND METHODS

Animals and clinical findings: The twelve diabetic cattle included seven Japanese Black and five Holstein-Friesian breeds, eight females and four males from 6- to 48-months-old. All diabetic animals were persistently hyperglycemic (Table 1) [25]. During an intravenous glucose tolerance test (0.5 g glucose/kg) [26], plasma glucose levels returned to preinjection ones within 2 hr in the control cattle, but remained markedly elevated levels after 3 hr in the diabetic cattle. Urinalysis using a test stick (N-Multistix. Miles-Sankyo Co., Tokyo, Japan) showed acidity and glycosuria. Eight cattle were associated with ketonuria, and the others without ketonuria (Table 1).

Tissue preparation for histopathology: Tissue samples of pancreas were collected from each diabetic cattle necropsied immediately after death, and fixed for 24 hr in Bouin’s or 4% paraformaldehyde solution. For histopathologic examination, 4-µm paraffin sections were stained with hematoxylin and eosin (HE), aldehyde fuchsin and Masson-Goldner (AFMG) methods [16], Van Gieson, Masson trichrome, silver impregnation for reticulum, von Kossa for calcium, Berlin blue for iron, Congo red for amyloid, and periodic acid-Schiff reaction for glycogen granules.

Immunohistochemistry: Serial histologic sections fixed in 4% paraformaldehyde solution were used for immunohistochemical analysis by the avidin-biotin-peroxidase complex (ABC) procedure with Mayer’s hematoxylin counterstain (Vectastain Elite ABC Kit, Vector Labs, Burlingame, U.S.A.)[21]. Specific antisera used in this study were anti-porcine insulin (Immuno Nuclear Corp., U.S.A.), anti-porcine glucagon (Amersham International Plc. U.S.A.), anti-human somatostatin (donated by Dr. S. Ito, Japan), anti-bovine chromogranin (Immuno Nuclear Corp. U.S.A.), anti-bovine IgG (The Binding Site, Ltd., UK) antibodies, and anti-human collagen type I, III antibodies (Fuji Yakuhin, Japan).

Control animals: Pancreatic tissues collected from four nondiabetic Holstein-Friesian cattle (12 to 48 months old) served as the histopathologic and immunohistochemical controls.

RESULTS

Histopathological findings: Twelve diabetic cattle were divided into two groups; chronic IDDM and acute IDDM, on the base of the histopathological findings of the pancreas which were summarized in Table 2.

In eight chronic IDDM cattle, the most characteristic changes in the pancreas were a decrease in the size and number of pancreatic islets, interlobular and interacinar fibrosis, and mild lymphocytic infiltration in a few islets. Occasionally, the islets disappeared completely in some pancreatic lobes. Almost all cells in the atrophied islets did not contain AFMG-positive granules in their small amount of cytoplasm (Fig. 1). Mild lymphocytic infiltration at both the periphery and interior of the islets was observed in some lobes. The islets with lymphocytic infiltration contained necrotic islet cells and stromal concentrically laminated basophilic bodies (Fig. 2) which were suggested as calcification by the von Kossa method. Mild fibrosis with or without lymphocytic infiltration was observed in some atrophied islets. Residual islets consisted of many islet cells with vacuolated cytoplasm including a small number of aldehyde fuchsin-positive granules with AFMG staining, indicating that the cells were degranulated b-cells. Two such cases had large numbers of glycogen granules in their cytoplasm. An accumulation of glycogen granules was also observed in the cytoplasm of the pancreatic ductal epithelium in these cases. Using the silver impregnation method, interlobular and interacinar fibrosis was diffuse and often accompanied by atrophy of acinar cells with reduced numbers of secretory granules. Mild infiltration of lymphocytes was present around the small and large

<table>
<thead>
<tr>
<th>Cases No.</th>
<th>Breed</th>
<th>Age (months)</th>
<th>Sex</th>
<th>Blood glucose (mg/dl)</th>
<th>Urinary Glucose&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Ketonuria&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic IDDM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>JB&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>6</td>
<td>□</td>
<td>364</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>JB</td>
<td>10</td>
<td>□</td>
<td>207</td>
<td>+++&lt;</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>JB</td>
<td>48</td>
<td>□</td>
<td>184</td>
<td>+++&lt;</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>HF&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>27</td>
<td>□</td>
<td>252</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>HF</td>
<td>34</td>
<td>□</td>
<td>256</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>6</td>
<td>HF</td>
<td>36</td>
<td>□</td>
<td>782</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>7</td>
<td>HF</td>
<td>36</td>
<td>□</td>
<td>615</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>8</td>
<td>HF</td>
<td>42</td>
<td>□</td>
<td>280</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Acute IDDM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>JB</td>
<td>6</td>
<td>□</td>
<td>356</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>JB</td>
<td>8</td>
<td>□</td>
<td>350</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>JB</td>
<td>11</td>
<td>□</td>
<td>136</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>JB</td>
<td>48</td>
<td>□</td>
<td>221</td>
<td>+++</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup>) JB = Japanese-Black.  <sup>b</sup>) HF = Holstein-Friesian.  <sup>c</sup>) Urinary glucose: ++ = 0.5%; +++ 1%; +++< = more than 1%.
<sup>d</sup>) Ketonuria: +++ = more than 100 mg/dl; = 0 mg/dl.
pancreatic ducts, occasionally with mild or severe fibrosis. In four acute IDDM cattle, the major pancreatic islets consisted of islet cells having vacuolated cytoplasm with a small number of aldehyde fuchsin-positive granules under AFMG staining (Fig. 3). In addition, the islets contained many islet cells with increased eosinophilia and shrinkage of cytoplasm and karyorrhexis, indicating necrosis of \( \beta \)-cells (Fig. 4). Glycogen accumulation was not observed in the vacuolated cytoplasm of islet cells. In some cases, each pancreatic lobe occasionally had slightly atrophied islets composed of islet cells with poor cytoplasm to varying degrees. Mild or severe lymphocytic infiltration at both the periphery and interior of the islets was frequently observed in all cases (Fig. 5). Most inflammatory cells consisted of small lymphocytes, occasionally a few large mononuclear cells and plasma cells. In some lobes, mitoses were seen in the enlarged islets composed of hypertrophic cells. In the exocrine glands, focal lymphocytic infiltration was sometimes found in the interlobular connective tissues, mainly around the small-sized pancreatic ducts. Interlobular and interacinar fibrosis was not observed in any cases. Insular amyloidosis was not detected in sections stained with Congo red and PAS methods in both types of IDDM.

**Immunohistochemical findings:** The majority of cells in the atrophied islets in both types of IDDM were infrequently reactive to anti-insulin antibody. Only a few of the cells that possessed poorly granulated cytoplasm were faintly positive.}

---

**Table 2.** The results of histopathological examinations of the pancreas

| Cases No. | Endocrine pancreas | | | | | | Exocrine pancreas | | | |
|-----------|---------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|           | Atrophy | Vacuolation | Insulitis | Fibrosis | Glycogen | Calcification | Atrophy | Acinar islet-cells | Fibrosis |
| **Chronic IDDM** | | | | | | | | | |
| 1 | +++ | + | + | + | – | – | ++ | – | ++ |
| 2 | +++ | + | + | + | ++ | – | – | +++ | – | +++ |
| 3 | +++ | + | + | ++ | – | ++ | – | ++ | – | +++ |
| 4 | +++ | + | + | + | ++ | – | + | + | – | +++ |
| 5 | +++ | + | + | ++ | – | – | ++ | – | ++ |
| 6 | +++ | + | + | + | – | – | + | + | – | + |
| 7 | +++ | + | + | + | – | – | – | + | – | – |
| 8 | +++ | + | + | + | – | – | – | + | – | – |
| **Acute IDDM** | | | | | | | | | |
| 1 | – | +++ | +++ | – | – | – | – | + | – |
| 2 | – | +++ | +++ | – | – | – | – | + | – |
| 3 | + | +++ | +++ | – | – | – | – | + | – |
| 4 | + | +++ | +++ | – | – | – | – | + | – |

a) +++ = severe. b) ++ = moderate. c) + = mild. d) – = negative.

---

**Fig. 1.** Pancreatic islet in chronic IDDM. An atrophied islet is composed of small uniform cells with small amount of cytoplasm and round or oval nuclei (arrow). Almost all islet cells do not contained AFMG-positive granules in their cytoplasm, suggesting the disappearance of \( \beta \)-cells. Aldehyde fuchsin and Masson-Goldner method. × 350.

**Fig. 2.** Pancreatic islet in chronic IDDM. Necrosis of islet with lymphocytic infiltrates associates with concentrically laminated basophilic bodies (arrows). HE. × 350.
acute IDDM cattle (Fig. 7). Islets containing various numbers of bovine IgG-immunoreactive islet cells were more frequently observed in acute IDDM than in those of chronic IDDM. Furthermore, many islets with lymphocytic infiltration contained small numbers of plasma cells with cytoplasm reactive to anti-bovine IgG antibody (Fig. 8). Although not in all cases, some atrophied islets were composed of islet cells showing positive immunoreactivity to anti-bovine IgG antibody.

In the islets with fibrosis, fibrous connective tissues in both the periphery and interior of the islets reacted to both anti-collagen I and III antibodies (Fig. 9).

DISCUSSION

In bovine diabetes mellitus, the histologic changes have been briefly described and only referred to a reduction in the number and size of the pancreatic islets, and the disappearance and/or vacuolar degeneration of the β-cells occasionally accompanied by lymphocytic infiltration at the initial lesion [42, 43, 46]. Lymphocytic infiltration in the pancreatic islets was described only in a few cases in cattle [42, 43, 46]. However, hyalinization, calcification, necrosis, or fibrosis of the pancreatic islets were not described in the previous reports of bovine diabetes mellitus [4, 26, 39, 42, 43, 46].

In chronic human IDDM, histopathological changes of the pancreas are a decrease in the number and size of pancreatic islets, vacuolar degeneration of residual islet cells, and interlobular and interacinar fibrosis [12, 15]. In particular, a complete or almost complete absence of β-cells in the islets, and diffuse fibrosis in the exocrine pancreas are considered to be characteristic of the changes in very chronic IDDM [15]. Atrophy of the islets following

---

**Fig. 3.** Pancreatic islet in acute IDDM. An enlarged islet is composed of swollen islet cells having hydropic cytoplasm with a small number of aldehyde fuchsin-positive granules. Aldehyde fuchsin and Masson-Goldner method. × 860.

**Fig. 4.** Pancreatic islets in acute IDDM. Pancreatic islets contain many islet cells with increased eosinophilic and shrinkage of cytoplasm with karyopycnosis or karyorrhexis indicating necrosis of islet cells (arrows). HE. × 350.

**Fig. 5.** Pancreatic islet in acute IDDM. Insulitis with mild infiltration of small lymphocytes at periphery of the islet. HE. × 350.
hydropic degeneration, calcification, fibrosis and hyaline degeneration of the pancreatic islets may also be noted in individual cases [27]. In the present study, the disease of eight cattle with ketonuria was diagnosed as chronic IDDM, because the major histological changes in the pancreas were a decrease in the number and size of islets together with a complete or almost complete absence of β-cells and diffuse fibrosis in the exocrine pancreas [4, 26, 42, 46].
Furthermore, calcification, necrosis, and fibrosis of the pancreatic islets were found in individual cases, as is the case in chronic human IDDM [27]. A decrease in the number and size of islets is a constant characteristic of the prevailing type of islets in the pancreas of humans IDDM [12, 14, 15, 42].

Fibrosis of the islets, which is the most frequent lesion in human chronic IDDM, is related to interacinar fibrosis, and occasionally to diffuse pancreatic fibrosis [15, 27]. In our cases, immunohistochemical analysis using anti-human collagen type III antibody suggests that such increased connective tissue fibrils were rich in newly produced collagen type III fibers, indicating islet fibrosis of vascular origin [27]. Although the cause of islet fibrosis is still unclear, it occasionally suggests an inflammatory etiology when accompanied by lymphocytic infiltration [13, 27, 42].

Selective calcification of the islets of Langerhans is extremely rare [27, 35]. Calcium deposits are usually located in the fibrous stroma of islets. The calcification of the islet is of the dystrophic type, but the events leading to islet injury and necrosis are not clear [27]. Some residual islets contained many islet cells having an apparently empty cytoplasm on HE-stained sections due to glycogen deposits in two cases. In human IDDM, glycogen deposits in islet cells are regarded as secondary changes resulting from hyperglycemia [45].

In acute human IDDM, it is very difficult to analyze pathomorphological changes in the pancreas, because treatment for this type of disease is usually prolonged [27]. Thus, the histopathological properties of the pancreas in the early stages of IDDM are still unclear. However, pathological observations in acute IDDM suggest that in at least some cases β-cells are destroyed by an inflammatory process which selectively affects the pancreatic islets [8, 10, 12, 15, 24, 34]. In the majority of cases, the inflammatory infiltrate is composed of lymphocytes, occasionally with macrophages and mast cells [27]. In a few cases, the cellular infiltrate consists predominantly of polymorphonuclear leucocytes [31]. In our cases, lymphocytic infiltration was more common in the vacuolated islets than in the atrophied ones. The presence of bovine IgG immunoreactive islet cell antibodies (ICA), complement-fixing islet cell antibody (CF-ICA), or islet-cell surface antibody (ICSA) may have been produced by plasma cells [5, 18, 19, 30, 33, 34].

Recent descriptions of the histopathology of pancreas in human IDDM increasingly regard lymphocytic infiltration in the pancreatic islets (so-called insulitis) as the most characteristic lesion in acute IDDM. Furthermore, insulitis is typically found soon after the onset of the disease, and frequently found in recent-onset IDDM following certain viral infections such as coxsackie group B viruses, mumps virus, rubella virus, Epstein-Barr virus, cytomegalovirus, and herpes virus [1, 2, 22, 28, 36–38, 40, 41]. Therefore, this finding has had considerable influence on the concept of the etiology and pathogenesis of IDDM in humans [19, 27, 37, 41]. Insulitis is reported to occur in experimental infections with certain viruses such as coxsackie B4, encephalomyocarditis (EMC) virus, mengovirus-2T, rubella virus, and Kilham's rat virus [6, 17, 47]. Furthermore, inflammation of the pancreatic islets occurs after active immunization with insulin, as well as after injections of anti-insulin antibody [20, 33]. Based on intensive analysis of insulitis in animal models, autoimmune mechanisms are widely considered to play a role in the development of insulitis in human and bovine IDDM [7, 9, 19, 32–34, 43, 47].

Other changes recognized in the acute human IDDM are hydropic or vacuolar degeneration of major β-cells. The β-cells are swollen and show a complete degranulation and an apparently empty cytoplasm. It has been noted that hydropic or vacuolar changes in β-cells can either be the result of a harmless glycogen deposition in the cytoplasm [44] or represent a truly degenerative lesion (ballooning degeneration) [28]. In the pancreas of human diabetes, true hydropic or vacuolar degeneration is not easily distinguished from vacuolization due to the occasional appearance of glycogen deposits [13, 29].

Although islet regeneration or neoformation (nesidioblastosis) is not a specific characteristic of the diabetic pancreas, and it is more prominent in the pancreases of acute IDDM [12, 13, 15, 27]. It is represented by a proliferation and endocrine differentiation of ductular and acinar cells showing a large number of insulin positive granules in their cytoplasm immunohistochemically [12], as well as in our cases of acute IDDM. These phenomenon may be interpreted as an attempt to compensate for an insulin deficiency due to a progressive destruction of β-cells [11, 13].

**Fig. 9.** Pancreatic islet in chronic IDDM. Atrophied islet with fibrosis is rich in anti-collagen type III immunoreactive fibers. Avidin-biotin-peroxidase complex stain with Mayer’s hematoxylin counterstain. × 350.
In summary, diabetes in the present cases was IDDM characterized by a severe insulin deficiency resulting from a drastic numerical reduction in β-cells. Pathological observations suggest that in at least some cases β-cells were destroyed by an inflammatory process which selectively affects the pancreatic islets. Lymphocytic insulitis and anti-bovine IgG immunoreactive islet cells are thought to be the most significant changes in determining the etiology and pathogenesis of both bovine and human IDDM, and suggest a role of anti-islet autoimmunity in this form of diabetes.

REFERENCES