Short Communications

Alterations of Exocrine Pancreatic Enzymes in Virus-Induced Diabetic Cattle as Revealed by Immunohistochemistry

M. Bendayan*, S. Ito** and I. Manocchio¹

Institute of Histology and Embryology, University of Geneva Medical School, Geneva, Switzerland and ¹Istituto di Patologia Generale e Anatomia Patologica Veterinaria, Università degli Studi, Perugia, Italy

Summary. The pancreatic tissue of normal and virus-induced diabetic cattle was investigated by the indirect immunofluorescence technique. Seven secretory proteins (chymotrypsinogen A, trypsinogen, carboxypeptidase A, RNase, DNase, α -amylase and lipase) were localized in normal bovine pancreatic acinar cells but in diabetic animals amylase, lipase and carboxypeptidase were either not detectable or markedly diminished.

Decrease in amylase content has been reported previously in other diabetic animals. The diminution of the three pancreatic enzymes may be related to the destruction of pancreatic endocrine tissue that occurs in these diabetic animals.

Key words: Bovine exocrine pancreas, pancreatic enzymes, diabetes, immunofluorescence, cattle.

In previous studies [7, 8], we were able to reveal different digestive enzymes in the pancreatic acinar cells of the normal rat. By using immunofluorescence, alterations of the endocrine pancreas in diabetes have been documented [1, 15, 19] but, in contrast, little is known about the changes occurring in the exocrine pancreas. In diabetic patients the exocrine secretion is impaired [9, 20], and in alloxan-diabetic rats, Ben Abdeljlil et al. [4] reported that alloxan reduces the amylase content in the pancreas but increases the level of chymotrypsinogen. In the present study, normal and virus-induced diabetic bovine exocrine pancreases were investigated using specific anti-enzyme antisera and an immunofluorescence technique described previously [7].

Materials and Methods

Preparation of the Antisera

Seven pancreatic enzymes of bovine (chymotrypsinogen A, trypsinogen, carboxypeptidase A, RNase and DNase) or porcine (α -amylase and lipase) origins were obtained from Worthington Biochemicals, Freehold, New Jersey, USA and used as antigens. The antisera were

raised in guinea pigs by subcutaneous injections of the emulsified antigens as described previously [7]. Each antiserum obtained was tested against the seven enzymes by the double-immunodiffusion technique on agar gel plates [7, 16]. The antisera were also tested against normal bovine pancreatic extract.

Preparation of the Tissues

Pancreatic tissues from seven healthy cattle were obtained at the Abattoir Municipal, Geneva, just after slaughter. Samples from the splenic and duodenal parts of the pancreas were fixed by immersion in Bouin's solution. Small samples from the splenic part of the pancreas of nine diabetic cattle were obtained from the Istituto di Patologia Generale e Anatomia Patologica Veterinaria, Università degli Studi de Perugia, Italy. Diabetes had been induced in these animals by infection with the foot- and mouth virus [2, 3], and was characterized by emaciation, anorexia, polyuria, glycosuria, ketonuria and hyperglycaemia (12.98 ± 0.38 versus 4.05 ± 0.22 mmol/l). Clinical findings and laboratory analysis which brought about the diagnosis of diabetes in these animals have been reported previously [2, 3]. The animals were slaughtered at different time points after the beginning of the infection and their pancreases were fixed by immersion in Bouin's solution.

Immunofluorescence Technique

Normal and diabetic bovine pancreatic tissues fixed in Bouin's fluid for 24 h were embedded in paraffin. Sections (5 μ) were deparaffinized, rehydrated and processed for the immunofluorescence technique as described previously [7]. The seven anti-exocrine enzyme antisera were applied on successive serial sections of the pancreatic tissue of each animal. The antisera were used at the following dilutions in 10 mmol phosphate buffered saline: anti-amylase 1:50, anti-chymotrypsinogen 1:100, anti-trypsinogen 1:100, anti-lipase 1:20, anti-carboxypeptidase A 1:20, anti-RNase 1:20, anti-DNase 1:20.

* Present address: Department of Anatomy, Université de Montréal, Montréal, Canada

** Present address: First Department of Internal Medicine Niigata University, Niigata, Japan

Fig. 1 a–b. Normal bovine pancreatic tissue incubated with **a** anti-chymotrypsinogen and **b** anti-amylase antisera. The fluorescent staining intensity is similar in most of the acinar cells in **a**, but in **b** the acini located around the islets of Langerhans (*IL*) are more brightly stained than the others. (X150). Parts **c** and **d** show serial sections of diabetic bovine pancreas incubated with **c** anti-trypsinogen and **d** anti-amylase antisera respectively. The acinar cells in **c** are brightly fluorescent while the same acini in **d** show only a weak staining. Surrounding the acinar cells is a non-reactive tissue which corresponds to loose connective tissue and tubular neoformations ($\times 250$)

The specificity of the immuno-staining was checked: (1) by adsorbing the antibodies with an excess of the corresponding antigen before applying to the sections; (2) by incubating the antisera with a saline extract of normal bovine pancreas for 24 h at 4 °C before applying to the sections; (3) by using non-immunized guinea-pig serum in the first step; and (4) by omitting the first incubation.

Results

The specificities of the anti-exocrine enzyme antibodies as shown previously with immunofluorescence [7] and immunoelectron microscopy [8, 17] were confirmed by

Table 1. Fluorescence intensity in exocrine acinar cells of the diabetic bovine pancreas

Diabetic pancreas		Enzymes						
Animal number	Days from the infection	Amylase	Lipase	Carboxy-peptidase	Chymo-trypsinogen	Trypsinogen	RNase	DNase
1210	45	—	weak	weak	+	+	+	+
1211	65	weak or —	weak or —	weak	+	+	+	+
1212	70	—	weak	+	+	+	+	+
1213	62	—	weak	+	+	+	+	+
1245	21	—	weak	weak	+	+	—	—
1246	16	weak or —	—	+	+	+	+	+
1247	12	weak or —	weak or —	weak or —	+	+	+	+
1249	15	weak or —	weak	—	+	+	+	+
1250	15	—	weak	weak	+	+	weak	+

Fig. 2. Diabetic bovine pancreatic tissue showing lymphocyte infiltrations at the site of the endocrine tissue (arrows). The exocrine acinar tissue appears well preserved. (hematoxylin-eosin) ($\times 150$)

the double-immunodiffusion tests. A precipitin line was present at the site of reaction between the antibody and the specific antigen; a weak precipitin line was also present between the antibody and the pancreatic extract. No cross-reaction with the other antigens was observed.

By immunofluorescence, the acinar cells of the normal bovine pancreas showed positive reactions. The staining was restricted to the cell cytoplasm (Fig. 1 a and b) and complete inhibition of the staining was obtained in the different control conditions. The endocrine cells of the islets of Langerhans were consistently negative (Fig. 1 b). In the tissue of some animals, the majority of acinar cells showed a positive reaction of similar intensity but in most cases the staining pattern was not homogeneous; the acini located close to the endocrine tissue were brightly fluorescent while others further from the islets showed a weak or even a negative reaction (Fig. 1 b). This inhomogeneity was found for all seven enzymes tested, and in both the splenic and duodenal parts of the pancreas.

In the pancreatic tissue of the diabetic cattle, chymotrypsinogen and trypsinogen were constantly present by immunofluorescence in the acinar cells while amylase, lipase and carboxypeptidase A gave a weak or negative

reaction. DNase and RNase were present in all but one diabetic pancreas. Figures 1 c and 1 d illustrate two serial sections of pancreatic tissue from a diabetic animal, exposed to anti-trypsinogen, and anti-amylase antisera respectively. The bright fluorescence obtained with the anti-trypsinogen (Fig. 1 c) was patchy due to unreactive regions corresponding to the oedematous and infiltrated interstitial tissue; the same acinar cells were only weakly stained by the anti-amylase antiserum (Fig. 1 d). A semi-quantitative evaluation summarizing these results is reported in Table 1. The pancreatic tissue of these different diabetic animals was characterized histologically by the disappearance of most of the islet structures and the presence of an inflammatory reaction with lymphocyte infiltration at the site of the endocrine tissue (Fig. 2). No evident correlation was found between these alterations, the disappearance of enzymes and the duration of the infection.

Discussion

When the enzyme content of normal and diabetic bovine pancreas was examined by the indirect immunofluores-

cent technique, major differences were found with respect to three enzymes, amylase, lipase and carboxypeptidase. The results from normal pancreas confirm previous work [12, 14, 17, 21], showing the presence of several secretory proteins in acinar cells and extend these data to include lipase and amylase. Since our amylase and lipase antigens were of porcine origin, the present results indicate cross-reactivity between the porcine and bovine enzymes. In the normal bovine pancreas, as in rat pancreas [7], islets of Langerhans were surrounded by a halo of brightly fluorescent cells while those more distant from the islet were less intensely fluorescent. This may indicate the existence of a functional partition of the exocrine pancreas into peri- and tele-insular regions [7, 10, 13]. Furthermore, intercellular junctional specializations were found between endocrine and exocrine pancreatic cells, implying that both types of cells are structurally and functionally associated [5].

In contrast, the acinar cells of the diabetic bovine pancreas had markedly reduced contents of amylase, lipase and carboxypeptidase, as reflected by a weak immunofluorescent staining for these enzymes. These results are consistent with our previous finding that, in the pancreas of streptozotocin-diabetic rats, the peri-insular exocrine tissue is devoid of amylase while chymotrypsinogen is normally distributed [6]. Biochemical studies have shown that the pancreatic amylase content decreases in alloxan diabetic rats [4, 18] and it is well known that insulin plays a key role in the synthesis of amylase [4, 18]. Recently, this effect has been shown to be due to a decreased cellular content of amylase messenger RNA in streptozotocin-diabetic rats – the normal mRNA content being restored by insulin administration to these animals [11]. From our results alone, however, it is not possible to decide whether the observed alterations in pancreatic enzyme content are due to a destruction of the endocrine tissue or whether there is a direct viral effect on the exocrine cells.

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Dr. M. Bendayan
Department of Anatomy
Faculty of Medicine
Université de Montréal
C.P. 6128, Succ. A
Montréal, Québec
Canada, H3C 3J7