

# Histologic Analysis of the Porcine Pancreas to Improve Islet Yield and Integrity After Collagenase Digestion

K. Ulrichs, M. Bosse, H.-H. Wacker, A. Heiser, and W. Müller-Ruchholtz

**A** BASIC requirement for successful clinical xenotransplantation is to provide a well-preserved and viable xenograft. In the case of the isolated porcine pancreatic islet for curing type I diabetic patients, this requirement cannot yet be guaranteed to an extent that satisfies the clinician. This has to be admitted in spite of impressive first methodologic achievements, for example, a semiautomated method to facilitate collagenase digestion in mammalian species, including the pig,<sup>1</sup> and sophisticated training for isolation of large numbers of the porcine endocrine "miniorgan" for future clinical purposes.<sup>2</sup> The lack of success of attempts at isolation in the adult pig worldwide may be due not least to the particular fragility of the porcine islet for which morphologic parameters, for example, the collagen content of the connective tissue surrounding the islet ("islet capsule"), may be responsible. In extension of the only other report known so far,<sup>3</sup> it was the aim of this study to investigate the morphology of the porcine pancreas with particular regard to the islet of Langerhans. We wanted to obtain detailed information about islet shape, number, size, and volume density and the collagen content of the connective tissue. Therefore, hematoxylin-eosin, silver impregnation, and indirect immunoperoxidase histology with monoclonal antibodies (MAb) against the various collagen types were performed on pancreatic tissue from seven different domestic pig races and the wild boar.

## MATERIALS AND METHODS

### Animals and Pancreatic Tissue

Porcine pancreata were obtained from brain-dead animals, either from local slaughterhouses (HY, hybrid pig), from commercial breeders (GL, German Landrace; DU, Duroc; BL, Belgium Landrace; PI, Piétrain; HABL, Hampshire-x-Belgium Landrace (F1 offspring); MP, Minipig [Göttingen]), or from two game parks in the state of Schleswig-Holstein (WB, wild boar). Animals were either young (female, 7- to 12-month-old) or retired breeders (female, 3 years or older). With the exception of the hybrid pigs, which are mongrels of different pure breeds, the above races and the F1 offspring are pure bred. The porcine pancreata were cut to pieces. One half was stored in 5% formalin solution and embedded in paraffin 2 days later, the other half was embedded in freeze medium (Leica Instruments, Nussloch, Germany), shock frozen in liquid nitrogen, and then stored at  $-80^{\circ}\text{C}$  until further use.

### Histology

The histologic examinations were done on paraffin-embedded tissue sections stained with hematoxylin-eosin and silver impregnation. Immunoperoxidase histology was performed with frozen tissue sections, using three MAbs against collagen types I, III, and IV (Sanofi Diagnostics Pasteur, Freiburg, Germany). No anti-

collagen type II MAb was available. Rabbit anti-human collagen MAbs with proven crossreactivity were used because MAbs against porcine collagens were unavailable.

### Islet Isolation

Islets were isolated from the porcine pancreas by our modification<sup>4</sup> of the semiautomated method described by Ricordi et al.<sup>1</sup> For identification, they were stained with dithizone.<sup>5</sup>

### Microscopy and Documentation

Microscopy and photographic documentation were performed with an Olympus BH2 microscope. Islet size was evaluated using a grid ( $1.28\text{ mm} \times 1.28\text{ mm} = 1.638\text{ mm}^2 = \text{section area}$ ) inside the microscope. The total islet number per 20 grids was counted and converted into islets/cm<sup>2</sup>. Islet volume density (percent endocrine tissue of the total pancreas mass) was calculated according to the principle of Delesse<sup>6</sup>: Volume density (%) = areal density (%) =  $(\pi \Sigma r^2 / \text{section area}) \times 100$ .

## RESULTS

Analyzing the porcine pancreas (GL) histologically with hematoxylin-eosin for islet shape, number, and size, the following observations were made: (1) In shape, islets are round (42% of the total islet number in young individuals, 63% in adults), oval (50% and 43%), dumbbell-like (6% and 2.5%) or triangular (1.6% and 0.2%), and show all intermediate stages (results not documented in detail). These results indicate that the number of round islets increases with increasing age, whereas the number of oval, dumbbell-like, and triangular islets decreases. The four characteristic islet shapes were also discovered in high quality isolations using the semiautomated digestion procedure with collagenase, indicating their stability. (2) Table 1 documents the results of the analysis of the relative islet number (islets/cm<sup>2</sup> histologic section) in the various pig races. The main finding is that, of the eight pig races tested, including the nonpure bred hybrid pig, WB shows the highest number of islets ( $498 \pm 21.9$ ), followed by DU, GL, PI, MP and BL, HABL, and finally HY with the lowest number ( $190 \pm 24.7$ ). Not included in the table is the finding that young individuals have relatively more islets/cm<sup>2</sup> ( $388 \pm 78$ ) than adult individuals ( $242 \pm 29$ ).

From the Institute of Immunology, Medical School of the University, Kiel, Germany.

Address reprint requests to Dr Karin Ulrichs, Institute of Immunology, Medical School of the University, Brunswikerstr. 4, D-24105 Kiel, Germany.

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**Table 1. Histologic Analysis of the Pancreas of Seven Domestic Pig Races and the WB: Relative Islet Number, Islet Size Distribution, and Islet Volume Density**

Pig Races	WB (n = 3)	GL (n = 7)	DU (n = 3)	PI (n = 6)	BL (n = 5)	MP (n = 2)	HABL (n = 3)	HY (n = 4)
Islets/cm <sup>2</sup> tissue section (M ± SD)	498 ± 21.9	388 ± 69.9	401 ± 76.4	327 ± 178	252 ± 84.7	254 ± 110	226 ± 53.7	190 ± 24.7
Islet size distribution (%)								
<100 μm	85.84	60.93	68.91	66.61	68.25	60.36	65.04	59.84
100–150 μm	11.14	19.92	15.54	22.75	18.25	23.67	23.01	24.41
150–200 μm	3.02	10.25	9.38	6.61	7.14	8.88	6.19	7.87
200–250 μm	0	4.26	4.40	1.83	3.97	4.14	3.10	6.30
250–300 μm	0	2.13	1.47	0.73	1.59	1.78	1.77	0.79
>300 μm	0	2.51	0.29	1.47	0.79	1.18	0.88	0.79
Islet volume density (%)*	1.27	3.38	1.95	1.97	1.47	1.73	1.34	1.26

Note. Number of experiments shown in parentheses.  
\*Islet volume density was calculated according to the principle of Delesse.<sup>6</sup>

(3) Table 1 also documents the results of the analysis of the islet size within the different races: Although it has the highest relative number of islets, WB shows the highest percentage of small islets, namely 86% ≤ 100 μm, whereas GL shows the highest percentage of large islets, namely 2.5% ≥ 300 μm. (4) Based on these histologic findings, the islet volume density was calculated according to the formula of Delesse (see "Materials and Methods") and is documented in the table: The islet volume density is highest in GL (3.38%) and lowest in WB and HY (1.27% and 1.26%, respectively). (5) Indirect immunoperoxidase staining of the porcine pancreas (GL) to identify collagens around and within the islets was performed with rabbit anti-human collagen MAbs that crossreact with porcine tissue, for example, anti-collagen types I, III, and IV MAbs. The results, which are not documented in detail, indicate that the connective tissue surrounding porcine islets consists of type I, type III, and type IV collagens. No great quantitative differences in staining intensity could be detected. A similar staining pattern was obtained with silver impregnation, which detects argyrophilic collagen fibers. (6) Preliminary islet isolation results show that (a) adult individuals (HY) give better islet yields (islets/g digested organ) than young individuals (2930 ± 1450, n = 9, vs 837 ± 326, n = 7) and (b) that PI produces sufficient and, more importantly, reproducibly high islet numbers (2180 ± 317; n = 5).

**DISCUSSION**

To our knowledge this is the first report presenting detailed data on the morphology of the porcine pancreas with particular emphasis on the islet of Langerhans. This lack of data is surprising because the pig is considered a favorite donor species among those being discussed and already used for clinical xenotransplantation,<sup>7</sup> and because it has already been under study for large scale enzymatic islet isolations.<sup>1,2</sup> The semiautomated isolation technique probably represents the best available tool to date for this purpose. Nevertheless, since the introduction of this technique in our laboratory it has failed to fulfill our expectations of being able to isolate reliably high numbers of

structurally well-preserved and viable pig islets.<sup>4</sup> There may be various reasons for this limitation, among them the lack of analysis of (a) various pig races, because one race may be a better organ donor than another one, (b) the organ donor's age, although it has been suggested that this may be important,<sup>8</sup> and (c) the morphologic characteristics of the porcine pancreas. Although points (a) and (b) are still under investigation in our laboratory,<sup>4</sup> point (c) is the subject of this study.

The higher number of round islets with increasing donor age may be relevant for islet isolation, inasmuch as these islets resist the mechanical stress (shaking in the digestion chamber) better than, for example, the dumbbell-like islets that may break more easily. However, the low percentage of dumbbell-like islets indicates that they have little relevance for the final islet yield. The interesting observation that, although WB has the highest total number, it has significantly smaller islets than any other pig race tested so far, has no practical relevance unless the particular aim is to isolate and transplant small islets. This aspect may have to be reviewed in the context of graft physiology, when islets are transplanted into the portal system and embolize the venules.

The finding that young pigs show more islets/cm<sup>2</sup> than adult pigs may be misunderstood: The pancreas weight increases with increasing age by about a factor of 1.65. It is exactly this factor by which the islet number is reduced in adults compared with young individuals, that is, the individual islet may grow, but it does not divide. Only the islet volume density allows us to predict to a certain extent the final islet yield or islet mass, and thus it is a most valuable parameter for porcine islet isolation.

The observation that the porcine islet capsule consists of at least type I, III, and IV collagens may have relevance for the enzyme composition of collagenase preparations. These consist of varying compositions of collagenases and an unknown number of additional proteolytic enzymes that may vary not only from batch to batch but also from supplier to supplier.<sup>9</sup> Studies are under way to evaluate this important point for porcine islet isolation. The only other study dealing with the porcine islet capsule shows

that in comparison to rat, dog, cow, monkey, and human, the porcine islet capsule was the most poorly developed.<sup>3</sup> However, this finding obviously depends on the pig race as our data show.

In concordance with the morphologic parameters age and volume density, isolations from adult hybrid pigs and adult pure bred PI pigs gave the best islet yields so far. Further intensive analyses of morphologic parameters and their correlation with islet isolation results, as initiated in this study on a broad scale, will be helpful for the difficult task of developing the most effective porcine islet isolation technique.

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