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A Mouse Model for Studying Intrahepatic Islet Transplantation

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Abstract

Intrahepatic human islet transplantation has raised hopes for a cure for diabetes mellitus, especially in patients with type 1 diabetes; however, the need for a substantial amount of islets and, in many instances, repeated transplantations demonstrates underlying problems with this procedure, such as failure of angiogenesis and immunologic rejection. Studies using rodent models may be helpful in improving the success of islet transplantation. However, most of the studies using rodents for islet transplantation have been under the kidney capsule rather than the liver. Using islets from transgenic mice expressing green fluorescent protein under the control of mouse insulin I promoter, the authors have developed a method with which to visualize histologic and pathologic changes in intraportally transplanted islets and surrounding hepatic tissue using reflected light confocal imaging. Initial events 24 hr after islet transplantation in the liver include β -cell loss and hepatic ischemic injuries.

Keywords

Islet transplantation; Green fluorescent protein; Insulin; Liver

The success of the clinical application of human islet transplantation into the liver has opened up a way to cure diabetes mellitus (1,2). However, the requirement for a large number of transplanted islets to achieve normoglycemia and the loss of engraftment necessitating multiple transplantations suggest underlying problems associated with the survival of implanted islets. Rodent models have been widely used to study the causal mechanisms of failure of islet transplantation (3,4). Although in a clinical setting islet transplantation under the human kidney capsule is considered to be technically difficult and physiologically inferior to intraportal transplantation, the kidney capsule has been the site of choice in most of the studies using rodent models. The islets transplanted under the kidney capsule are protected from dynamic environmental changes such as continuous postgastrointestinal tract blood flow as found in the liver; thus, studies using this model are not comparable to those after intraportal injection. This has prompted us to develop a simple method with which to visualize intraportally transplanted islets together with surrounding hepatic tissue to facilitate studies of islet transplantation into the liver in the mouse.

We have previously reported the generation and characterization of transgenic mice expressing green fluorescent protein (GFP) in pancreatic β -cells under the control of mouse

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insulin I promoter (Tg[MIP-GFP]6729Hara) (5). The MIP-GFP mice develop normally, and specific expression of GFP in β -cells does not impair β -cell function or control of blood glucose levels. We isolated pancreatic islets from the MIP-GFP mice using a modification of the procedure originally described by Lacy and Kostianovsky (6). Briefly, the pancreas was inflated with a solution containing 0.3 mg/mL collagenase (type XI; Sigma-Aldrich, St. Louis, MO) in Hanks' balanced salt solution, injected through the pancreatic duct. The inflated pancreas was removed, incubated at 37°C for 10 min, and shaken vigorously to disrupt the tissue. After differential centrifugation through a Ficoll gradient to separate islets from acinar tissue, the islets were washed and then handpicked.

Approximately 200 islets isolated from the MIP-GFP mice were transplanted into the liver through the portal vein (7). The recipient animals were killed 24 hr after transplantation and stereomicroscopic images of the whole liver were taken using an Olympus SZX12 microscope (Olympus, Melville, NY). The liver was then removed, fixed in 4% paraformaldehyde at 4°C overnight, permeabilized in 1% Triton X-100 (Sigma-Aldrich) in phosphate-buffered saline overnight at room temperature, and treated with the tissue-clearing reagent FocusClear (Pacgen Biopharmaceuticals, Inc., Burnaby, BC, Canada) for 2 days at room temperature. Each lobe was placed in a glass-bottom dish and confocal laser scanning images were captured using an Olympus SZX-RFL3 microscope. Image analysis and postprocessing were performed using ImageJ (<http://rsb.info.nih.gov/ij/>). Three-dimensional reconstructions were created with MetaMorph (Universal Imaging Corp., Downingtown, PA) or Voxx software (8). The procedures involving mice were approved by the University of Chicago Institutional Animal Care and Use Committee.

Intraportally transplanted islets from MIP-GFP mice were readily visualized within the intact liver under the stereomicroscope (Fig. 1). GFP-expressing islets were implanted in all the lobes of the liver (Fig. 1A and B). Note that bile in the gallbladder gives strong autofluorescence (Fig. 1A and B). Necrotic regions in the peripheral lobes can be seen in the bright-field images (Fig. 1B–D). The transplanted islets reside in the blood vessels (Fig. 1C). Some islets were observed in the proximity of necrotic regions, suggesting that the infarction was caused by the transplanted islet emboli (Fig. 1D) (see also Supplement 1).

To further examine the location of transplanted islets in the liver and to detect morphologic changes in the islets and surrounding hepatic tissue, we applied confocal laser scanning microscopy. The images of GFP-expressing islet were captured using blue excitation (488 nm wavelength) and a narrow emission filter bandwidth (510–530 nm). The surrounding hepatic tissue was simultaneously visualized by reflected laser light imaging using a red laser (633 nm) with no emission filter and images were false-colored with red. The signals detected by this technique report the density of structures and it does not require immunohistochemical staining. Dense structures function as reflectors, back-scattering laser light and forming bright image pixels. Less dense structures such as the vasculature and nuclei appear as elongated dark structures and black circles in the hepatic tissue, respectively (Fig. 2A). In reflected laser light images, the islet cells strongly reflect the laser light because of their dense cytoplasmic contents. Intense β -cell backscatter could obscure fine details of GFP signals from the islet when these two sets of images are merged. The brightness of reflected images was compensated for by using threshold functions within ImageJ by subtracting the GFP images from the reflected laser light images. A montage of processed images in Figure 2(B) and Supplement 1 shows islet thrombus. A top-down movie created using the stack of z-series images can be seen in Video 1. The reflected light images produced high detail for up to 120 μ m into intact liver tissue treated with FocusClear.

We then performed three-dimensional reconstruction of the site of islet implant (Fig. 2C). The signals in the reflected laser light images were inverted (black for white) using ImageJ because less dense structures are more abundant in the liver. Contrast inversion aided three-dimensional volume rendition, because three-dimensional modeling is biased toward rare, bright objects in a darker background volume. As a consequence, structures such as nuclei and the vasculature are now visualized as bright, red objects. The z-axis of the three-dimensional reconstructed image shows the descending blood vessel in which the islet is lodged (Fig. 2D). The branches of blood vessels can be seen throughout the site of the islet transplant, and the surrounding hepatocytes appear to be healthy. An animation was created by capturing real-time motion of the reconstructed image and is shown in Video 2.

Pathologic changes of a transplanted islet and surrounding tissue can also be captured using this technique (Fig. 3). The transmitted light image at low magnification shows the blood vessel in which an islet was lodged (Fig. 3A). The edge of the lobe shows a wedge-shaped rough surface texture resulting from infarction caused by the islet emboli (Fig. 3A), which is also shown in bright-field images in Figure 1. (Note that other islets in the central lobe may also be responsible for the large area of necrosis.) A confocal image reveals the boundary where the blood supply was disrupted by the transplanted islet (Fig. 3B). The surrounding hepatocytes show abnormal cell shapes and texture compared with the lower left side of the tissue, which appears healthy. The loss of blood supply (or immunologic response, or both) appeared to have affected the transplanted islet itself (Fig. 3B), because the β -cells appear fragmented, suggesting they are undergoing apoptosis. The optical sectioning of this site of transplantation demonstrates the complete blockage of the blood vessel by the islet (Fig. 3C, Supplement 2, and Video 3). This infarction is visualized in three-dimensional reconstruction of the top-down view by inverting the signals (Fig. 3D). An animation of this site of transplantation is shown in Video 4. The three-dimensional reconstructions of this stack of images show the loss of blood supply downstream from the islet (Fig. 3E). The infarction can be seen in the different site of the islet transplantation that shows the loss of blood supply downstream from the islet (Fig. 3E) (see also Video 5). A tiny islet containing a few GFP-expressing β -cells lodged in the vasculature was captured (Fig. 3F).

In summary, we have demonstrated that islets from MIP-GFP transgenic mice can be used to study the pathophysiology of intrahepatic islet transplantation in mice. The method we have developed using reflected light imaging enables us to visualize histologic and pathologic changes of the intraportally transplanted islets in the liver and the surrounding hepatic tissue without using conventional immunohistochemistry, which is time-consuming and provides limited information in a two-dimensional context. Here, we show that it is possible to obtain three-dimensional information about the islet graft using reflected light confocal microscopy and readily available software packages such as ImageJ and MetaMorph. The technique can also be applied to other organs such as lung and heart (M.H. and V.P.B, unpublished data). We believe that the method we have developed to examine the pathophysiologic changes at the site of islet transplantation by three-dimensional representations may be useful for islet transplantation research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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FIGURE 1.

Macroscopic visualization of MIP-GFP islets in the liver 24 hr posttransplantation. (*left*) Bright-field images; (*middle*) GFP fluorescence; (*right*) merged images. (A) Intact liver showing transplanted islets in all lobes. Note that bile in the gallbladder (gb) shows strong autofluorescence. Scale bar = 3 mm. (B) Ventral view of the liver in situ showing ischemic regions in the peripheral lobes (*left*). The autofluorescence from the gallbladder is evident. Scale bar = 3 mm. (C) The right medial lobe of the liver in A. Merged image (*right*) shows islets lodged in the blood vessels. Scale bar = 1 mm. (D) Proximity of some islets and necrotic regions suggest embolism caused by the islets. Scale bar = 0.5 mm.

FIGURE 2.

Visualization of the site of islet transplantation. (A) Orthogonal views through merged laser scanning confocal images of GFP fluorescence (green) and reflected light (red). (*crosshairs*) Same locations in face (XY) and lateral views through the tissue volume (XZ and YZ image reconstructions). Note the detailed morphology of the surrounding hepatic tissue at the site of islet implantation including the blood vessel (*v*) and nuclei (*n*) that are visualized as dark structures. Scale bar = 50 μm . (B) Optical sectioning showing top-down view (0-, 20-, and 34- μm slices are shown) of the site of islet implantation in A. Note the formation of a thrombus (*t*) (see also Supplement 1 and Video 1). (C) Three-dimensional reconstruction using a stack of images shown in B. Note that the signals in reflected light images (red in B)

were inverted and the branches of blood vessels can be seen throughout the site of the islet transplant. (D) The z-axis of the three-dimensional reconstructed image showing the descending blood vessel (v) in which the islet is lodged. Nuclei appear as red circles. Scale bar = 50 μm (see also Video 2).

FIGURE 3.

Pathologic changes of a transplanted islet and surrounding tissue. (A) Transmitted light image merged with fluorescent image showing the transplanted MIP-GFP islet in green (black arrow). The islet appears to be trapped in the blood vessel and to have disrupted the downstream blood supply. The necrotic region in the hepatic tissue shows a wedge-shaped rough surface (yellow arrows) compared with the smooth texture of the center of the lobe. Scale bar = 200 μm . (B) A merged confocal laser scanning images of a fluorescent image (green) and a reflected light image (red) showing the site of transplantation of the islet in A. The islet-caused embolism shows the clear boundary where the blood supply has been blocked (upper right; v, vasculature). The hepatocytes in the ischemic region show abnormal cell shapes and texture. The β -cells in the islets show fragmentation caused by the loss of blood supply, suggesting that the islet was undergoing apoptosis or degenerative necrosis (inset). Scale bar = 50 μm . (C) A confocal image from top-down view of the site of islet implantation in A and B (see also Supplement 2 and Video 3) showing the complete

blockage of the blood vessel by the islet. Scale bar = 25 μm . (D) The islet-caused embolism is visualized in a three-dimensional reconstruction of the top-down view of the site of islet implantation. Note that the brightness in reflected light images (red in C, *asterisk*) was inverted to show the blood vessels and nuclei in bright red. Necrotic cells reflect more laser light. (E) Animated image created by capturing real-time motion of image reconstructions using image stacks shown in C. The branched blood vessels were clearly seen in the right side of the site of islet implant but disappeared in the necrotic region (see also Videos 4 and 5). (F) Three-dimensional reconstruction of a tiny islet lodged in the vasculature of the liver. This islet contained only three GFP-expressing β -cells. Scale bar = 50 μm .