Angiogenesis and Hemodynamics of Microvasculature of Transplanted Islets of Langerhans

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Transplantation of isolated islets of Langerhans is frequently followed by early loss of islet function. Because whether this is caused by insufficient vascularization or graft rejection is unknown, angiogenesis and microvascularization of islet grafts were studied in vivo by means of intravital microscopy. After transplantation of syngeneic islets in hamster dorsal skin-fold chambers, 97% (n = 66) of the islets exhibited the first signs of angiogenesis at days 2–4, characterized by sinusoidal sacculations and capillary sprouts. After 10 days, angiogenesis was completed, consisting of a microvascular network similar to those of islets in situ: arterial supply, afferent and efferent capillary loops, and venular drainage. Functional density of microvessels was 700.1 ± 127.0 cm^{-1}, and erythrocyte velocity was 0.58 ± 0.35 mm/s. Intracellular insulin was demonstrated immunohistochemically. Electron-microscopic studies revealed normal fine structure of the capillary wall. The model allows in vivo analysis of microvascular phenomena occurring in host-vs.-graft reaction after allogeneic and xenogeneic islet transplantation. Furthermore, it may be used to quantitatively assess immunosuppressive regimens.
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Since Moskalewsky (1) described an enzymatic method for isolation of islets of Langerhans in 1965, free transplantation of pancreatic islets has been discussed as a cure for diabetes. Major obstacles of clinical islet transplantation have included the isolation of a sufficient mass of islets and the management of graft rejection. Recently, a modified isolation technique was introduced, allowing isolation of >200,000 islets from one donor pancreas (Ricordi et al., this issue, p. 140). However, the problem of early loss of function after free islet transplantation has not been solved. In 1985, Sutherland and Kendall (2) reported on 166 cases of free pancreatic islet grafting in which none of the recipients was insulin independent.

Because whether these poor functional results are caused by insufficient vascularization or by graft rejection is unknown, studying the microvasculature of free islet grafts is helpful. Therefore, the aim of this study was to analyze angiogenesis and microvascularization of pancreatic islets after free transplantation.

MATERIALS AND METHODS

Islets of Langerhans were isolated from Syrian golden hamsters after vital staining with neutral red (Sigma, St. Louis, MO) by means of a modified collagenase digestion technique (3). A handpicking procedure guaranteed exocrine free islets for transplantation. Subsequently, the islets were transplanted into the dorsal skin-fold chamber of syngeneic hamsters, which allowed intravital microscopy in the awake animal over a prolonged period. The chamber and implantation procedure have been described by Endrich et al. (4). Briefly, under Nembutal anesthesia (50 mg/kg body wt; Abbott, North Chicago, IL), the hamsters (6–8 wk old; 60–80 g body wt) were fitted with two symmetrical Teflon-coated aluminum frames positioned on the dorsal skin fold so that they sandwiched the extended double layer of skin. One layer was completely removed in a circular area (15 mm diam), and the remaining layer, containing skin muscle and subcutaneous tissue, was covered with a removable cover glass incorporated into one of the aluminum frames.

Angiogenesis and microvascularization were observed for 10 days after transplantation. Quantitative analysis of microcirculatory hemodynamics (functional density of microvessels, capillary erythrocyte velocity and diameters) was performed on days 4, 6, and 10 by means of intravital microscopy, video techniques, and a computer-assisted microcirculation analysis system (CAMAS) (5). To better vis-

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TABLE 1
Functional density of microvessels and diameter of total microvascular network during angiogenesis of syngeneic free islet grafts

Days posttransplantation	Functional density of microvessels (cm^{-1})		Total microvascular network diam (μm)	
	Mean ± SE	*n*	Mean ± SE	*n*
4	430.6 ± 66.4	21	232.5 ± 37.8	21
6	495.7 ± 75.5	17	374.9 ± 56.6	17
10	700.1 ± 127.0	19	436.9 ± 59.6	19

TABLE 2
Erythrocyte velocities and mean diameters of capillaries during angiogenesis of syngeneic free islet grafts

Days posttransplantation	Capillary erythrocyte velocity (mm/s)		Capillary diam (μm)	
	Mean ± SE	*n*	Mean ± SE	*n*
4	0.32 ± 0.08	72	15.8 ± 4.2	76
6	0.43 ± 0.17	116	10.7 ± 3.3	124
10	0.58 ± 0.35	117	9.0 ± 2.8	120

ualize the microvasculature during intravital microscopy, contrast enhancement was provided by intravenous injection of 0.2 ml 5% fluorescein isothiocyanate–dextran of 150,000 M_r (Sigma). For histological examination, electron-microscopic studies were performed at day 10, and intracellular insulin was proved histochemically by aldehyde fuchsin and immunohistochemically by anti-insulin staining (6).

RESULTS

In 97% of syngeneic islet grafts (n = 66), the first signs of angiogenesis, characterized by sinusoidal sacculations and capillary sprouts originating from capillaries and postcapillary venules of the host tissue, were observed 2–4 days after transplantation. Subsequently, the capillary sprouts connected with terminal arterioles, which was followed by a change of direction and an increase in blood flow. At day 6, the islets exhibited a growing microvascular network; after 10 days, angiogenesis was completed, consisting of a glomerulum-like network of microvessels similar to those of islets in situ, i.e., arterial supply, afferent and efferent capillary loops, and venular drainage.

The quantitative analysis of microcirculatory hemodynamics revealed an increase in functional density of microvessels and diameter of the total microvascular network during the 10-day observation period (Table 1). Whereas the mean diameter of the microvessels decreased, an increase of capillary erythrocyte velocity up to ~0.60 mm/s at day 10 was observed (Table 2). Intracellular insulin could be demonstrated histochemically and immunohistochemically 10 days after transplantation; electron-microscopic studies revealed normal fine structure of the capillary endothelial wall.

DISCUSSION

Transplantation of insulin-secreting tissue as a free graft has the potential to become a safe and simple procedure to cure diabetes. However, clinical attempts have failed to achieve insulin independency. Whether these poor functional results are caused by insufficient vascularization or graft rejection is unknown. Adequate microvascular supply should play an important role for successful implantation and function of islet grafts.

Although several experimental studies have been performed on the microcirculation of pancreatic islets in situ by means of intravital microscopy (7–9), the microsphere technique (10–12), and vascular corrosion casting and scanning electron microscopy (8,13,14), very little is known about the microvasculature of free islet grafts. Recently, Sandler and Jansson (15) analyzed blood flow in autotransplanted pancreatic islets of rats with the microsphere technique. However, this technique does not allow in vivo analysis of the microhemodynamics or observation of the integration of the islets into the donor tissue, i.e., by angiogenesis and microvascularization.

For the first time, angiogenesis and microvascularization in pancreatic islet grafts were observed in this study in situ by means of fluorescence microscopy. Syngeneic grafts exhibit the first signs of angiogenesis after 2–4 days, and a glomerular microvascular network is completed within 10 days. These findings are in agreement with histological studies of Griffith et al. (16), who described complete vascularization of islet isografts in the rat by the 8th–11th day after transplantation. The morphology of the microvasculature of the islet grafts is very similar to the morphology of pancreatic islets in situ, evaluated by means of vascular corrosion cast and scanning electron microscopy (14).

In further studies we will investigate the microangiodynamics in allogeneic and xenogeneic islet grafts. The in vivo analysis of microvascular phenomena occurring in host-vs.-graft reaction may be achieved by staining the white blood cells with acridine orange (Sigma). With this technique the flow properties of white blood cells and their interaction with the endothelial surface will be quantitatively assessed with a computer-assisted image-analysis system. Because the endothelium of the microvasculature is the target of the immunologic rejection process, the model has the potential of a new approach for the analysis of the effects and mechanisms of action of immunosuppressive regimens.

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