

# Identical Pattern of Acute Rejection After Isolated Islet and Vascularized Whole-pancreas Transplantation in the Rat

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*The course of acute rejection of major histocompatibility complex (MHC)-incompatible isolated rat pancreatic islets transplanted under the kidney capsule was monitored functionally and histologically from day 1 to 10. The patterns observed were compared to those of vascularized whole-pancreas transplants with preserved and suppressed exocrine secretion. In addition the morphologic reactions after isogenic transplantation of islets or whole-pancreas transplants are described. The sequential patterns of acute rejection were found to be essentially identical in isolated islet and whole-pancreas allografts. This was also true for the process of I-A-like class II MHC antigen induction. Endocrine cell necrosis and reduced insulin content of beta cells were detected in isolated islets but not in whole-organ isografts. Thus ischemic damage may have occurred to the transplanted islets on days 1 and 2 because connections to the renal vasculature were not demonstrated before day 3. Islet cell loss was, however, functionally compensated by beta cell proliferation beginning on day 4. From the first day after transplantation, an altered spatial distribution of insulin- and glucagon-containing cells was present in islet isografts. This phenomenon was, however, not unique to islets, but also occurred in duct-ligated whole-organ isografts on days 6 to 10. (Am J Pathol 1990, 137:93-102)*

The transplantation of isolated pancreatic islets bears great clinical promise as a treatment for diabetes, but has met with limited success until now.<sup>1,2</sup> In addition to questions of appropriate immunosuppression and donor-recipient matching, the optimal site for allotransplantation of

isolated islets is still controversial.<sup>2</sup> Although experimental islet transplantation is well established in the rat, there are few detailed histologic studies on the fate of islet grafts in different locations.<sup>3-5</sup> Sequential morphologic comparisons between islet and vascularized whole-organ pancreas grafts have not been published until now.

We have found the subcapsular space of the kidney to be a particularly suitable site supporting long-term function of isografted islets in streptozotocin-diabetic rats. In this study we report that the course and histologic pattern of acute rejection are essentially identical in islet and whole-organ pancreas grafts in the major histocompatibility complex (MHC) mismatched LEW (RT1<sup>l</sup>) to LEW.1U (RT1<sup>u</sup>) strain combination. Ischemic damage of endocrine cells is initially observed in islet isografts but not in whole organs. This is apparently compensated by proliferation of beta cells after day 3.

## Materials and Methods

### Animals

All rats were operated on at an age of 2 to 3 months. Male LEW(RT1<sup>l</sup>) and LEW.1U(RT1<sup>u</sup>) rats were bred in the central animal house of Hanover Medical School.

### Study Design

The study comprised an analysis of the sequential histologic changes in isolated islet isografts and allografts. The findings were compared to those in duct-ligated and pancreaticoduodenal whole-pancreas grafts. On 10 consecutive days after transplantation, one of the animals was in-

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investigated in each experimental group. In addition, isografts were investigated on days 28 and 360 after transplantation. All isografts were performed using LEW (RT.1<sup>+</sup>) rats. In allotransplantation LEW rats served as donors to LEW.1U (RT.1<sup>-</sup>) recipients.

## *Transplantation Procedures*

### *Chemical Diabetes*

Prospective recipients of isolated islets of Langerhans or vascularized pancreas grafts were rendered diabetic by a single intravenous injection of 55 mg/kg streptozotocin, freshly dissolved in phosphate-buffered saline (PBS) adjusted to pH 4.5. Diabetes was confirmed by repetitive nonfasting blood glucose levels above 18 mmol/l (millimolar), polydipsia, polyuria, and weight loss.

### *Duct-ligated and Pancreaticoduodenal Transplantation of the Whole Pancreas*

Details of the microsurgical technique of vascularized pancreas transplantation in the rat have been reported elsewhere.<sup>6</sup> The arterial blood supply to the graft was provided by an aortic segment carrying the coeliac axis and the superior mesenteric artery anastomosed end-to-side to the recipient's infrarenal aorta. Venous outflow from the graft was accomplished by an end-to-side anastomosis of the donor's portal vein to that of the recipient. The exocrine secretion was either suppressed by ligation of the common bile duct or a pancreaticoduodenal composite graft was transplanted with subsequent duodenojejunostomy in the recipient.

### *Renal Subcapsular Transplantation of Isolated Islets of Langerhans*

In the donor, the common bile duct was ligated at the papilla of Vater and cannulated proximally at the liver hilus with a 26-gauge catheter. Collagenase (Worthington IV CLS, Worthington Biochemical, Type IV CLS, Freehold, NJ) was dissolved in 8 ml of Hank's solution (2 mg/ml) and was slowly injected into the bile duct, thus distending the whole pancreas. The pancreas was removed and stationarily incubated in a water bath at 38°C for 20 minutes. After three washes in Hank's solution the islets were separated by ficoll density gradient centrifugation at 800g for 10 minutes. Further purification was obtained by hand picking under a stereo microscope. Eighteen hundred islets, isolated from three donors, were then collected in a 100- $\mu$ l syringe. For renal subcapsular transplantation, a 26-gauge catheter was placed transrenally from the lower pole of the left kidney ventrally under the kidney capsule.

After injection of the islets, the outlet was electrocoagulated to prevent bleeding and reflux of the islets.

### *Monitoring of Graft Function*

Endocrine graft function was assessed by daily determination of nonfasting blood glucose levels and body weight. Rejection was defined by recurrence of hyperglycemia above 14 mmol/l on at least 2 consecutive days. On days 28, 90, 180, 270, and 360 after transplantation, an intravenous glucose tolerance test was performed. After a 6-hour fasting period starting at 8 A.M., 1 g/kg glucose was injected as an intravenous bolus. Blood samples were taken 10 and 5 minutes before the injection as well as 2, 5, 10, 20, 40, and 60 minutes afterward. The k values were calculated according to the method of Lundbaek.<sup>7</sup>

## *Histologic Evaluation*

### *Immunohistologic Demonstration of Insulin and Glucagon*

The organs were removed under ketamine anesthesia, cut into small pieces, and fixed in Bouin's solution for 24 hours. Paraffin-embedded specimens were cut at 5  $\mu$ m and routinely stained with hematoxylin and eosin. For demonstration of insulin, the dewaxed sections were incubated in 1% H<sub>2</sub>O<sub>2</sub> in PBS for 30 minutes to block endogenous peroxidatic activity. After appropriate washing, guinea pig anti-insulin serum (Miles-Yeda No. 65-101, obtained through ICN Biomedicals, Eschwege, FRG) or rabbit anti-synthetic glucagon serum (Miles No. 64-7961) was applied at a 1:800 dilution in PBS containing 0.1% NaN<sub>3</sub> and 1% BSA at 4°C overnight. As a secondary antibody, peroxidase-conjugated rabbit anti-guinea pig serum (DAKO P 141, Hamburg, FRG) or swine anti-rabbit serum (DAKO P 217) was used at 1:150 or 1:80, respectively, diluted in PBS with 5% inactivated normal rat serum for 1 hour at 4°C. Finally, the color reaction was produced by application of diaminobenzidine.

### *Immunohistologic Demonstration of Class II MHC Antigens*

Class II MHC antigens were detected by antibodies Ox6<sup>8</sup> and F17-23-2,<sup>9</sup> directed at a monomorphic and a polymorphic epitope, respectively, of I-A-like molecules in the rat. A separate specimen of each organ was immediately frozen in liquid nitrogen and 10- $\mu$ m sections were cut in a cryostat. The sections were lightly fixed in cold isopropanol for 10 minutes and incubated with Ox6 asc-

tes (Serotec No. MCA 46, obtained through Camon, Wiesbaden, FRG) at a 1:500 dilution in PBS containing 0.1% NaN<sub>3</sub> and 1% BSA or with F17-23-2 (Serotec No. MCA 95) undiluted supernatant for 1 hour at 4°C. After washing, the sections were overlaid with peroxidase conjugated rabbit anti-mouse immunoglobulin (DAKO P 161) diluted 1:30 in PBS with 5% inactivated normal rat serum for another hour at 4°C. For F17-23-2 a peroxidase-anti-peroxidase-complex (PAP) method was used, applying the PAP complex at 1:30 in a third stage. The color reaction was developed as described above. For F17-23-2, sections of normal LEW organs were always included as positive controls.

#### *Demonstration of Islet Vascularization*

A saturated solution of Berliner Blau (Chroma No 1B 193, Stuttgart, FRG) was prepared in distilled water, which was further diluted 1:2 in PBS. Two milliliters of the dye were injected into the renal artery of the anesthetized animal immediately before removal of the organ. Efflux of the dye was provided through the renal vein.

## **Results**

### *Sequential Histology of Islet Isografts with Special Reference to Alpha and Beta Cells*

On the first day after islet transplantation, the islets were found to be included in fibrin strands and edematous fluid under the kidney capsule. Moderate numbers of granulocytes and single macrophages were present in their surroundings. The islets appeared flattened, but otherwise their shape was normal. Immunostaining for insulin and glucagon, however, revealed that the spatial arrangement of alpha and beta cells was grossly altered. Normal LEW islets are composed of centrally situated insulin-containing cells surrounded by a more or less complete shell of glucagon-immunoreactive cells (Figure 1a). On the first day after transplantation, the insulin-containing cells appeared unaltered and of normal staining intensity. The glucagon cells, however, no longer occupied most of the islet periphery but were scattered as few single cells over the islet. In addition, they seemed to have aggregated into clusters of immunoreactive cells concentrated at one pole of the islet (Figure 1b).

On day 2, the amount of fibrin deposition and the number of granulocytes, macrophages, and fibroblastlike cells surrounding the islets increased. Among the islet cells, slight differences in insulin staining intensity were noted. Injection of Berliner Blau into the renal artery before removal of the kidney showed that capillaries between the

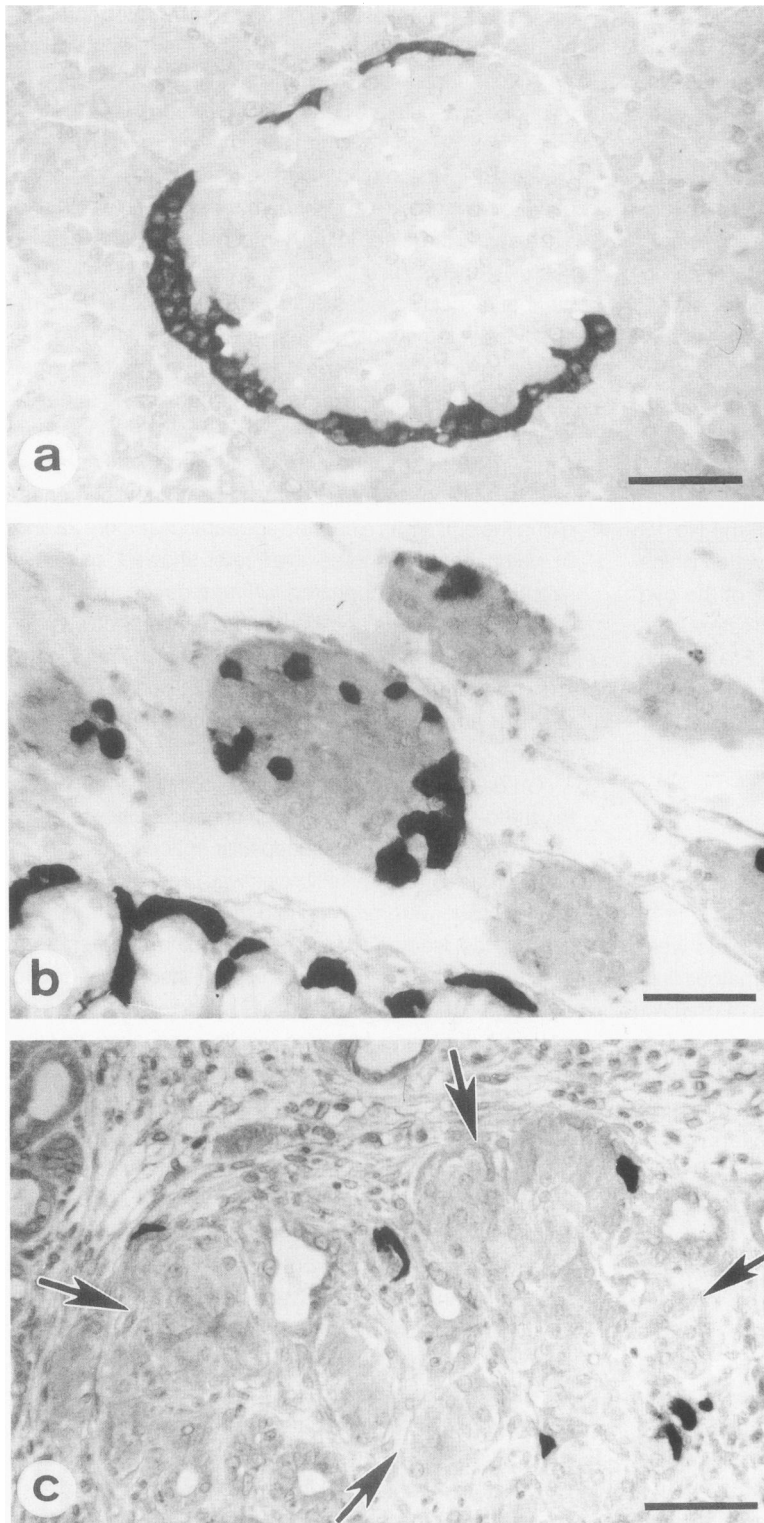
outer cortical tubules conspicuously protruded into the widened subcapsular space. However intraislet capillaries did not contain the dye, indicating that connections to the kidney vasculature had not yet formed.

On day 3, some of the islets showed major defects caused by invasion of connective tissue cells and macrophages from outside and by hemorrhage both within and surrounding the islets. In the center of some islets, cellular necrosis occurred, leaving only a rim of intact cells. The number of macrophages, fibroblasts, and occasional lymphocytes in the subcapsular space further increased, lifting the kidney capsule from the underlying parenchyma. On immunohistologic examination, the staining intensity for insulin showed considerable variation within the islets. Thus, in contrast to the normal situation, darkly stained cells lay immediately adjacent to almost unstained ones, giving the entire islet a spotted appearance (Figure 2a and b). Glucagon-immunoreactive cells showed uniformly strong staining but appeared further reduced in number. In some areas of the section plane an entire islet was devoid of glucagon cells. Injection of Berliner Blau clearly showed that the dye arrived in intraislet capillaries. Thus vascular anastomoses were established on day 3 (Figure 2b).

On days 4 to 10, and especially on day 6 and thereafter, the density of macrophages, connective tissue cells, and lymphocytes in the subcapsular space increased. In some areas a very dense fibrosis was present. Strands of fibrous tissue were growing into the islets and larger islet aggregates, leading to their distortion and fragmentation into cell clusters. Variable staining for insulin was found until day 10. In some islets all cells had a reduced staining intensity, indicating impaired insulin production or storage. However blood glucose levels were normal from day 2 and thereafter. Glucagon-containing cells appeared reduced and abnormal in spatial distribution at all dates.

Interestingly, on day 4 after transplantation, an enormous increase in mitotic activity of endocrine islet cells occurred. Single mitotic figures of endocrine cells already had been visible on day 3, but on day 4 three to four metaphase and anaphase cells were clearly visible in most islets (Figure 3). In addition, the islets tended to form larger aggregates on day 4. Immunostaining demonstrated that the mitotic cells contained insulin. They often appeared to have a reduced insulin content. The numbers of mitotic figures seemed to decrease slightly from day 5 to 10. However, even on day 10 they were still very visible.

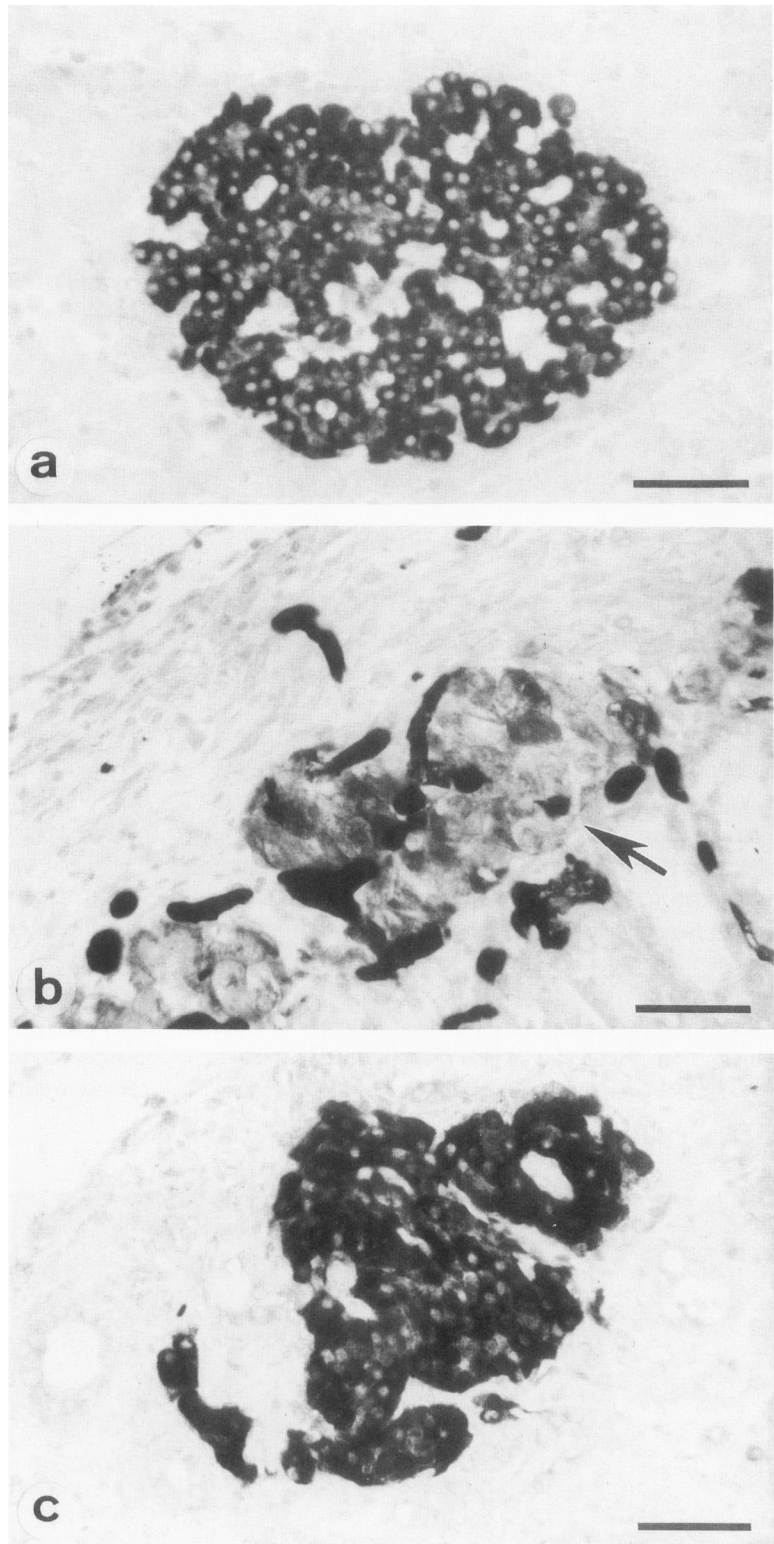
On day 28 the islets were still fragmented into smaller groups of cells or presented as large aggregates. However the infiltrate and fibrotic tissue under the kidney capsule had almost entirely disappeared. Also the differences in insulin immunoreactivity among single beta cells were no longer detectable.



**Figure 1.** *Glucagon immunoreactive cells in LEW rat islets. a: Normal pancreas—only a more or less complete peripheral rim of cells is stained in the islet. b: Islet isograft under the kidney capsule—on day 1 the stained cells are scattered across the islet and aggregate at one pole. In adjacent islets no glucagon-containing cells are sectioned. Dark structures in the lower left corner represent Berliner Blau in peritubular vessels. c: Duct-ligated pancreas isograft—arrows indicate an islet on day 10 that is deformed by outgrowing ducts and contains only few peripheral glucagon cells. Indirect immunoperoxidase staining of paraffin sections with bimalum counterstain. Bar = 50  $\mu$ m.*

One year after transplantation the situation had not changed. Small bands of fibrous tissue still subdivided the islets. However the intensity of infiltration and fibrosis was

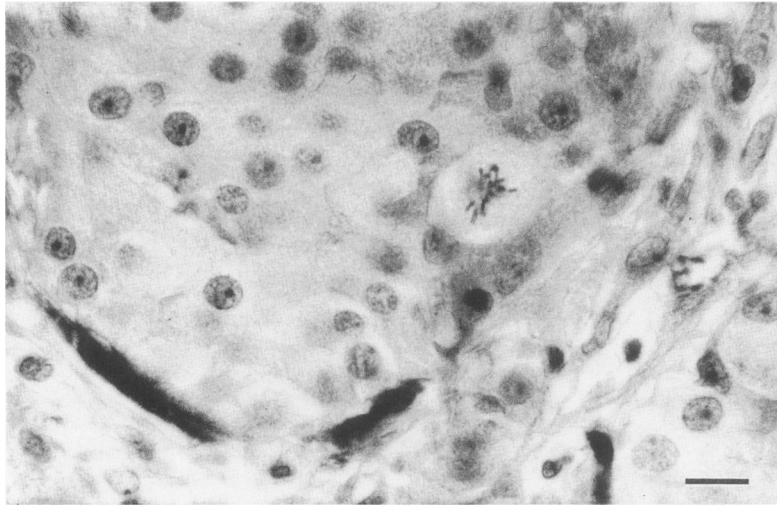
even more reduced in comparison to days 3 to 10. The islets showed a tendency to grow into the interstitial space between the outer cortical tubules (Figure 4). All



**Figure 2.** *Insulin immunoreactive cells in LEW rat islets. a: Normal pancreas—most of the centrally located endocrine cells contain insulin. b: Islet isograft—on day 3 staining intensity varies among single islet cells. Darkly stained structures represent islet vessels filled with Berliner Blau to demonstrate vascular anastomoses to the renal artery. Arrow indicates outer border of cortical kidney tubules. c: Duct-ligated pancreas isograft—on day 10 the islet is deformed by outgrowing ducts but consists of cells with normal staining intensity. Indirect immunoperoxidase staining of paraffin sections with hemalum counterstain. Bar = 50  $\mu$ m.*

islet cells had strong insulin immunoreactivity. The normal distribution of glucagon-containing cells was, however, not restored. By this time all recipients of islet isografts

were normoglycemic. The functional reserve of the graft, however, was markedly reduced, as revealed by the intravenous glucose tolerance tests.

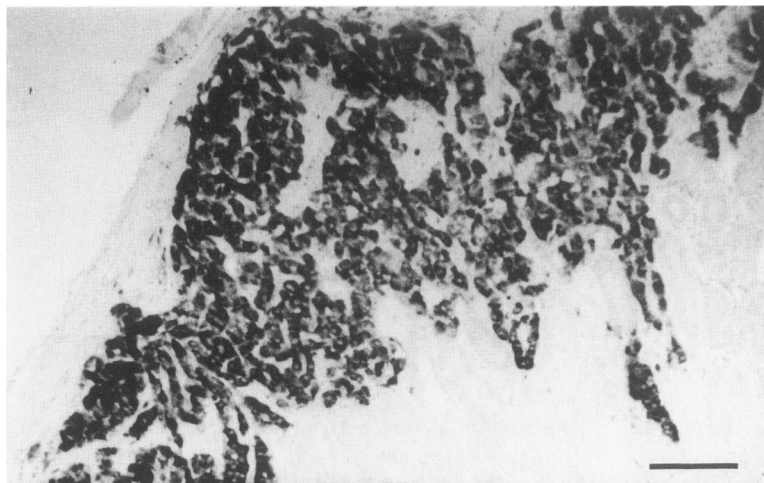


**Figure 3.** Endocrine islet cell in metaphase on day 4 after islet is transplantation. Hematoxylin and eosin staining of paraffin section. Bar = 10  $\mu$ m.

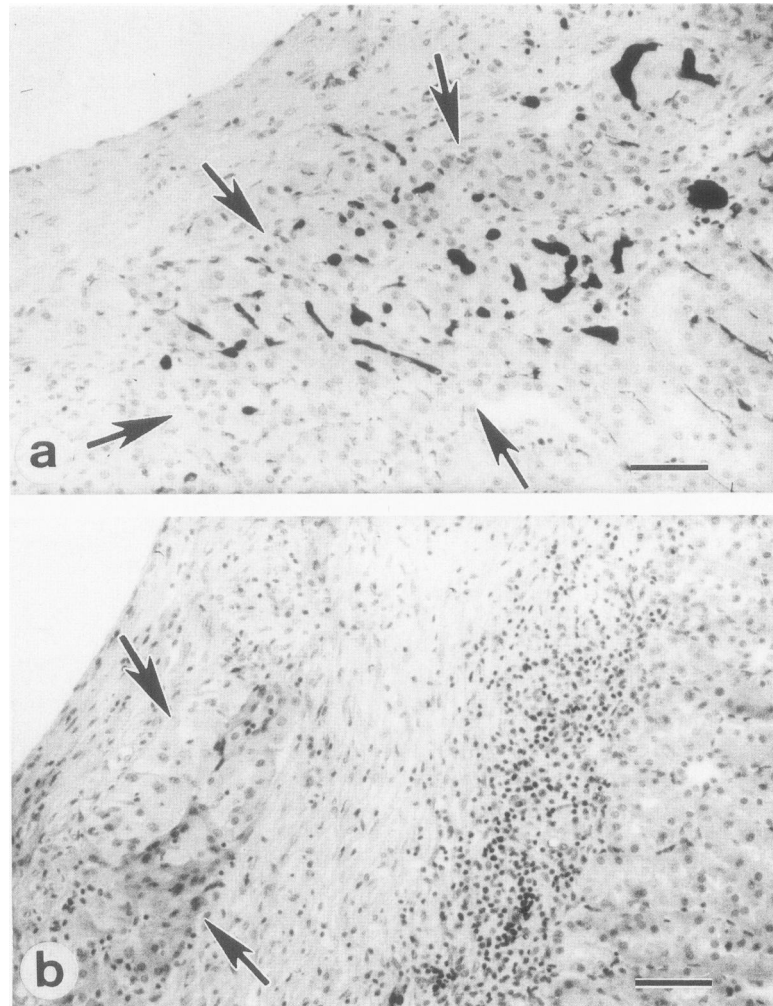
### *Sequential Histology of LEW to LEW.1U Islet Allografts with Special Reference to Alpha and Beta Cells*

From day 1 to day 4, no histologic differences were noted between LEW(RT1<sup>b</sup>) isografts and MHC disparate LEW(RT1<sup>b</sup>) to LEW.1U(RT1<sup>b</sup>) allografts. On day 5, however, the intensity of fibrosis and the number of small lymphocytes in the subcapsular infiltrate was larger in allografts than in isografts. On the following days the proportion of lymphocytes in the infiltrate and the density of fibrosis further increased in allografts. The lymphocytes were especially evident in the subcapsular space adjacent to the outer cortical kidney tubules (Figure 5b). Some lymphocytic infiltration was also present deep in the kidney parenchyma, surrounding arcuate vessels. Because

this phenomenon also occurred in isografts, it may be attributed to parenchymal lesions caused by the injection procedure. Excessive intraislet hemorrhage was present in allografts on days 6 to 8. Hemorrhage was also observed in isografts during the first days and thus was not specific for rejection. On day 8 a large increase in fibrosis and lymphocytic infiltration took place in allografts. At this date, unequivocal destruction of islets was visible. The islets were disrupted into small endocrine cell clusters by the infiltrate invading from the islet periphery (Figure 5a and b). On day 9 an increase in nonfasting blood glucose levels was noted from  $5.5 \pm 0.9$  mmol/l to  $17.6 \pm 5.6$  mmol/l, indicating functional rejection of the grafts. However the destructive process was not entirely complete on days 9 and 10 because clumps of insulin-reactive cells and also single glucagon cells were still found in the fibrotic tissue.



**Figure 4.** Insulin-containing cells in an islet isograft 1 year after transplantation. Islet aggregates grow into the space between the outer cortical kidney tubules. Indirect immunoperoxidase staining of paraffin section with hemalum counterstain. Bar = 100  $\mu$ m.



**Figure 5.** Histology of acute islet allograft rejection. **a:** Islet isograft on day 8. Darkly stained structures represent vessels filled with Berliner Blau. Arrows indicate islet borders. **b:** LEW to LEW.1U islet allograft on day 8. A dense lymphocytic infiltrate lies adjacent to outer cortical kidney tubules and a pronounced fibrosis is seen. Arrows indicate islet remnant. Hematoxylin and eosin staining of paraffin sections. Bar = 50  $\mu$ m.

### Sequential Analysis of Class II MHC Antigen Expression in Islet Isografts and Allografts

#### Isografts

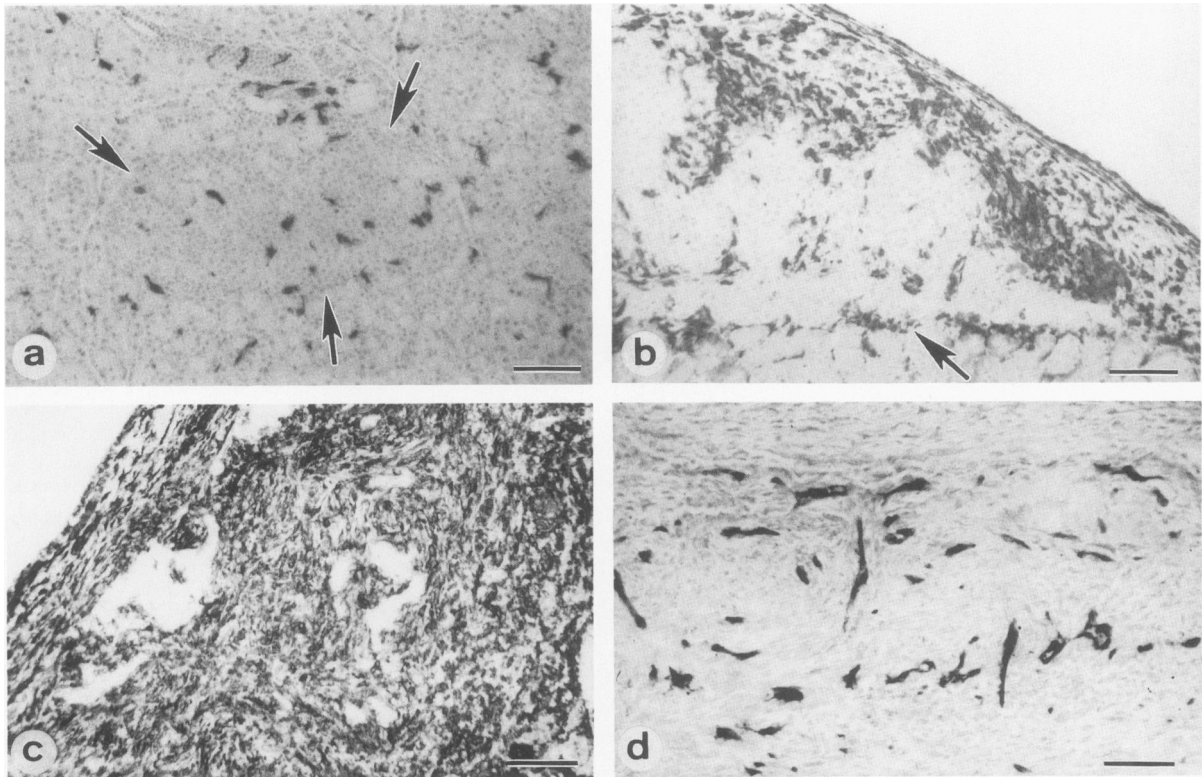
In normal LEW islets class II MHC antigens were only present in single dendritic-shaped cells (Figure 6a). After isotransplantation macrophagelike cells expressing I-A-like class II MHC antigens were present in the injection site surrounding the islets. Their numbers increased from day 1 to day 6 and appeared to diminish afterward. Within the transplanted islets only very few class II-positive cells were found. Oval and dendritic-shaped class II-positive cells amounted to almost 50% of the cells in the fibrotic tissue around the islets. In some areas large clumps of class II-positive cells were observed. Macrophagelike and dendritic cellular elements were the most prominent structures expressing class II antigens in islet isografts. Neither endocrine cells nor endothelia or ducts were stained at any date (Figure 6b).

#### Allografts

In LEW to LEW.1U islet allografts the antibody F17-23-2 was used in addition to Ox6 to selectively stain class II MHC antigens of the donor. F17-23-2 reacts with an epitope of I-A-like class II MHC molecules expressed in LEW but not in LEW.1U rats. Thus the demonstration of donor cells was possible without interference from the class II-positive recipient infiltrate.

Using the Ox6 antibody, which detects donor and recipient cells, always more class II-positive cells were found in the infiltrate surrounding the islets in allografts than in isografts on days 5 to 10. The majority of infiltrating cells in allografts had a dendritic shape and expressed class II MHC antigens. Typically the remnants of endocrine islet cells remained as class II-negative areas in the intensely positive surroundings until day 10 (Figure 6c).

On the first 5 days after allotransplantation, only few donor-type dendritic shaped cells were detected by F17-23-2 in the infiltrate outside the islets. Surprisingly



**Figure 6.** Expression of class II MHC antigens in LEW islets. **a:** Normal pancreas. Only single class II-positive dendritic-shaped cells are present within the islet (arrows). **b:** Islet isograft on day 8. Many dendritic shaped class II-positive cells are found in the infiltrate around the class II-negative islets. Arrow indicates border of outer cortical kidney tubules. **c:** LEW to LEW, 1U islet allograft on day 8. A dense infiltrate of class II-positive cells surrounds the islet remnants, which stay class II negative. **d:** LEW to LEW, 1U islet allograft on day 9. Isolated vascular elements and dendritic-shaped cells express class II MHC antigens of the donor type. Cryostat sections with bimalum counterstain. **a-c:** OX6 antibody, indirect immunoperoxidase staining. **d:** F17-23-2 antibody, PAP procedure. Bar = 100  $\mu$ m.

almost no donor class II-positive cells were found within the islets. On day 6 donor type dendritic cells increased in number in the infiltrate surrounding the islets. In addition, rare ductlike structures were stained by F17-23-2. On days 7 to 10, a third donor class II-positive cell type was detected at this site. These cells were smooth, elongated, and sometimes curved. On day 9 it was evident that they were surrounding lumens occasionally filled with leukocytes (Figure 6d). Thus the cells obviously represented endothelia of small vessels resembling arterioles and venules. However, these donor class II-positive endothelia did not form a vascular network but rather appeared as isolated structures within the fibrotic tissue. No class II-positive endothelia were seen in the transplanted islets. Also the endocrine cells were unreactive until day 10. In contrast to their surroundings there was no increase in donor class II-positive dendritic cells within the islets. Often no donor reactivity was observed at all in islet cross-sections during rejection.

### Sequential Immunohistology of Alpha and Beta Cells in Duct-ligated and Pancreaticoduodenal Whole-pancreas Isografts and Allografts

To analyze whether the altered arrangement of alpha and beta cells described above was unique to islet grafts as a consequence of the isolation or transplantation procedure, the composition of islets was also investigated sequentially in duct-ligated and pancreaticoduodenal whole-pancreas isografts. Duct ligation in pancreas grafts leads to an immediate atrophy of exocrine acinar cells with interstitial infiltration and an intense proliferation of small pancreatic ducts beginning on day 3, as described earlier.<sup>10</sup> All these changes did not, however, provoke an increase in mitotic activity of endocrine islet cells comparable to that observed after transplantation of isolated islets. In fact, only on day 4 after isotransplantation of a duct-ligated pancreas were single mitotic figures found in endocrine islet cells. On other days mitoses were not detected.



The islets were deformed by the outgrowing ducts, but uniform and strong insulin immunoreactivity was preserved from day 1 to day 10 (Figure 2c). The spatial relationship between insulin- and glucagon-containing cells remained normal until day 5. However, from day 6 and thereafter, changes occurred that corresponded to those after transplantation of isolated islets. The glucagon cells decreased and appeared to coalesce into peripheral aggregates in some islets (Figure 1c). Sometimes they seemed to form protrusions budding from the islet circumference. These alterations still persisted 100 days after transplantation.

When whole-pancreas isografts were transplanted as pancreaticoduodenal grafts with preserved exocrine secretion, the islets always remained perfectly normal.

In LEW to LEW.1U duct-ligated and pancreaticoduodenal whole-pancreas allografts, the first indications of rejection were observed on days 3 to 4 and the destruction of islets was complete on days 9 and 10.<sup>10,11</sup>

## Discussion

The histologic features of acute rejection in LEW(RT1<sup>b</sup>) to LEW.1U(RT1<sup>u</sup>) rat islet allografts transplanted under the renal capsule were evaluated sequentially and compared to LEW isografts. The findings were then analyzed further in comparison to vascularized whole-pancreas isografts and allografts, with suppressed and preserved exocrine secretion examined in previous studies.<sup>10,11</sup>

It is concluded that the histologic pattern of islet destruction does not differ, whether the islets are contained within an entire organ allograft or not. The sequence of events even appears to proceed somewhat more slowly in isolated transplanted islets. However in both allograft models the final destructive fibrotic process characterized by invasion and disruption of the islets from outside is initiated on day 8 after transplantation. The early events during rejection may be somewhat more difficult to observe in isolated islets than in whole organs because the islet transplantation procedure itself leads to infiltration and islet damage. Thus incipient rejection is histologically detectable in whole-organ grafts as early as day 3,<sup>10,11</sup> while this is not possible in isolated islets before day 5. Also in organ allografts, islets are no longer seen after day 8,<sup>10</sup> while after islet transplantation hormone-containing islet remnants remain until days 9 and 10. Thus in transplantation across an MHC barrier there is no morphologic indication of a more vigorous or different rejection process directed against islets in contrast to whole organs, as has been assumed by others.<sup>1,5</sup> This is also corroborated by an identical course of the sequential blood glucose values

in both models, with recurrence of hyperglycemia on day 9.

The only— and perhaps decisive—difference between whole organs and isolated transplanted islets was revealed by injection of Berliner Blau and by staining for insulin. It was shown that connections between the renal vasculature and the islets occurred on day 3 after isograft transplantation. At the same time islet cell necroses and a reduced staining for insulin were evident. This was not encountered in primarily vascularized organ isografts, indicating that ischemic damage had occurred to the islets during the first 48 hours after transplantation. A survey of the entire course of islet isografts, however, revealed that the loss and potential impairment of beta cells was compensated by beta cell proliferation beginning on day 4. This led to a long-term normalization of blood glucose values in recipients of islet isografts, despite an impaired glucose tolerance. The large proliferative potential of beta cells in transplanted islets has been described also by Griffith et al.<sup>3</sup> Proliferation may be precipitated by the functional challenge to an insufficient endocrine cell mass present in isolated islet grafts as compared to the primarily vascularized whole organ. It cannot be decided by light microscopic techniques whether the reduced insulin immunoreactivity in single islet cells on day 3 is due to ischemic damage or to functional hyperactivity with subsequent degranulation.

From the first day after islet transplantation the normal distribution of alpha cells was altered. The glucagon-containing cells appeared reduced and lost their mantlelike distribution. The reason for this phenomenon, which has also been described by others,<sup>12</sup> is unknown. It seemed to be correlated with disturbances in the tissue surrounding the islets and not with ischemia alone because it was also demonstrated late after duct ligation in whole-pancreas grafts. An active redistribution with accumulation of glucagon cells at certain areas of the islet periphery appeared to take place, but it remains enigmatic how this can be achieved within only 24 hours after islet transplantation.

Class II MHC antigens have been demonstrated to function as antigen-binding molecules, thus playing a decisive role in antigen recognition by immunocompetent T lymphocytes.<sup>13</sup> In addition, class II MHC antigens act as potent alloantigens *in vitro* and *in vivo*. Expression of class II MHC antigens can be induced in previously negative cells by mediator substances such as gamma interferon.<sup>14,15</sup> In the present study class II MHC-positive cells with macrophagelike and dendritic morphology formed a large proportion of the infiltrate. Most of these cells were recruited from the recipient. However donor-type class II-positive dendritic cells also were present in the islet surroundings. These cells apparently increased during the

rejection process, while the donor-type dendritic cells within the islets were diminished throughout. Later, donor-type reactivity also appeared in certain endothelia and ductlike structures. This is probably due to the cotransfer of dendritic cells, vascular and ductular fragments, together with the islets, despite hand picking. This contamination with nonendocrine tissue may be partially responsible for the fibrotic reaction observed in isografts, as demonstrated by others.<sup>16</sup> The extrainsular dendritic-shaped cells may be increased by induction of class II antigens in previously negative cells or by proliferation of positive ones. It is unlikely that the vascular structures represent outgrowths from the transplanted islets because all endocrine and endothelial cells within the islets remained entirely class II negative during rejection. This is in contrast to findings after combined application of gamma-interferon and tumor necrosis factor *in vitro* and to investigations in freshly diabetic BB rats *in vivo*.<sup>17,18</sup> Under these circumstances, class II MHC antigens were found in endocrine beta cells. However, also in acute whole pancreas allograft rejection in the rat, no expression of class II MHC antigens was observed in endocrine islet cells.<sup>11</sup> Obviously there is no difference between islet and whole-organ allograft rejection with respect to the induction of I-A-like class II MHC antigens.

In rats the histologic process of acute rejection thus appears to be identical in allografts of vascularized whole pancreas and of isolated islets transplanted under the kidney capsule. In isolated islets, hypoxic damage of endocrine cells occurs immediately after transplantation, which is, however, functionally compensated by beta cell proliferation.

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