1474

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Islet Cell Allotransplantation in Diabetic Patients

Histologic Findings in Four Adults Simultaneously Receiving Kidney or Liver Transplants

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Refined methods of islet cell purification have led to unprecedented success of islet cell allotransplantation via portal vein infusion in diabetic patients, resulting in marked reduction of exogenous insulin requirements and recently even insulin independence. The authors report the histologic findings of islet cell allografts in the liver of four patients who had undergone combined kidney-islet or liver-islet transplantation. Islet cell clusters were detected in subcapsular location at the edge of portal triads. The early post-transplant period was characterized by patchy mixed portal infiltrates. Only minimal inflammation but decreased islet cell granulation was observed in one patient 6 months after transplantation. As histologic detection of transplanted islet cells becomes available, additional parameters for evaluation of graft survival might be defined by morphologic assessment. (Am J Pathol 1992, 140:1255–1260)

Despite successful treatment with exogenous insulin diabetic patients suffer from a myriad of long-term complications other than deranged blood sugar concentrations. This has sustained efforts to transplant pancreatic islets either in the form of complete or segmental pancreatic allografts or as purified islet cell isolates.[1,2] Since full organ pancreatic transplantation is associated with considerable morbidity and mortality, the low risk procedure of islet cell transplantation is an attractive alternative and has been conducted experimentally for years. Until recently, the main obstacles to long-term success have been insufficient yield of functional islets and allograft rejection.[3–7] Within the last 2–3 years, refined purification techniques and better control of rejection have led to a number of successful transplants in type I insulin dependent diabetics with some patients achieving insulin independence.[7–10] At our own institution, treatment with the powerful immunosuppressive agent FK506 has contributed to successful islet cell transplantation in patients receiving multiorgan transplants after upper abdominal exenteration.[11–13] During the past year an additional number of patients with type I insulin-dependent diabetes mellitus received islet cell transplants simultaneously with either liver or kidney allografts. Graft survival in these patients is further complicated by potential recurrence of the primary disease similar to that reported in patients receiving pancreas allografts.[14] To date, histologic examination of successful islet cell transplants has been largely confined to animal studies.[15–17] Little is known about morphologic findings in human islet cell transplantation via portal vein infusion, which could potentially add valuable information in addition to functional assays. In four of our patients, liver biopsy specimens or autopsy tissues containing islet cells were available and the histologic findings are presented in this study.

Patients and Methods

Tissues

Liver tissue was examined from one needle biopsy taken at 14 days post-transplant, one wedge biopsy taken 2 days post-transplant and two entire organs obtained at autopsy 5 days and 6 months post-transplant, respectively.

Immunohistochemical Techniques

Immunohistochemical stains were performed on formalin-fixed, paraffin-embedded tissue using the avidin-

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biotin complex (ABC) method as described by Hsu.[18] A panel of antibodies was used with antibodies directed against insulin, glucagon, chromogranin (all Biogenex, San Ramon, CA), UCHL-1 (DAKO, Santa Barbara, CA), and LN3/HLA-DR (Biotest Diagnostics, Denville, NJ) as summarized in Table 1.

Patients

All patients underwent islet cell transplantation via portal vein infusion as previously described.[12] Each patient received purified isolates prepared from one or two donors as previously described.[19,20] In addition, two of the patients received a cadaveric renal transplant and the other two received liver transplants. Immunosuppression was achieved with FK506 and variable amounts of prednisone in all patients. Details of the clinical course and islet cell function of all four patients are published as part of a larger series of 22 patients receiving islet cell allotransplantation.[21]

Case 1

A 48-year-old man was admitted with end-stage liver disease due to alcohol abuse and a history of upper GI bleeding. In addition he had been an insulin-dependent diabetic requiring four daily injections ranging from 40 to 80 units of insulin. He underwent orthotopic liver transplant and simultaneous islet-cell transplant with islets prepared from one donor pancreas. Fourteen days after the operation, a liver needle biopsy was done to evaluate the liver allograft; 6 months after the combined transplant he died of hepatitis B and sepsis. No postmortem tissue was available for additional studies.

Case 2

A 34-year old man was admitted with end-stage renal disease secondary to diabetes mellitus. He underwent cadaveric renal transplant and simultaneous islet-cell transplant with islets prepared from pancreas of two donors. Postoperatively, he was taken to the operating room again for evacuation of a perinephric hematoma. A liver wedge biopsy was done at the same time. Since the amount of islet cells he had received initially was considered suboptimal, an additional isolate from a third donor was infused 5 days later. To date, he still requires 16 units of insulin per day; however, this represents an 80% reduction compared with pretransplant requirements.

Table 1. *Specificity, Working Dilution, and Source of the Antibodies Used in the Study*

Antibody	Dilution	Source
anti-insulin	1:1067	Biogenex
anti-glucagon	prediluted	Biogenex
anti-chromogranin	1:320	Biogenex
UCHL-1 (anti-CD45R)	1:10	DAKO
LN3 (anti-HLA-DR)	1:5	Biotest Diagnostics

Case 3

A 46-year-old man was admitted with end-stage renal disease secondary to diabetes mellitus. He underwent cadaveric renal transplant and simultaneous islet transplant with islets prepared from one donor pancreas. Three days after transplant he suffered an episode of aspiration with prolonged cardiac arrest. He was transferred to the intensive care unit, and immunosuppression was stopped. He did not recover and died 5 days after transplant. An autopsy was performed, which showed diffuse alveolar damage and early focal bronchopneumonia but no cardiac findings, specifically, no myocardial infarction could be found. Other findings are described under Results.

Case 4

A 31-year-old woman was admitted with end-stage liver disease secondary to cystic fibrosis. In addition she had a history of diabetes mellitus, diagnosed at age 15. She underwent orthotopic liver transplant and simultaneous islet-cell transplant with islets prepared from pancreas of two donors. Blood obtained during the operation before infusion of islets revealed high levels of C-peptide, indicating endogenous insulin production and therefore an insulin resistant form of diabetes mellitus. There was no significant reduction of insulin requirements, and she had multiple complications in the months after transplantation, including renal failure, marginal respiratory function, and multiple infections. She died with clinical signs of sepsis 9 months after transplant at an outside hospital, where permission was granted for an autopsy. Blocks from formalin-fixed, paraffin-embedded liver tissue obtained at autopsy were sent to our institution for analysis.

Results

In all patients, islet cells were found predominantly in a subcapsular location. In the first two patients that underwent liver biopsies, only insulin and glucagon stains were performed. In patient 1, an islet cluster was situated at the edge of a portal triad (Figure 1). The inflammatory infiltrate consisted predominantly of neutrophils and did not seem to be particularly concentrated around this cluster but was scattered diffusely throughout the triads that were visualized during the biopsy.

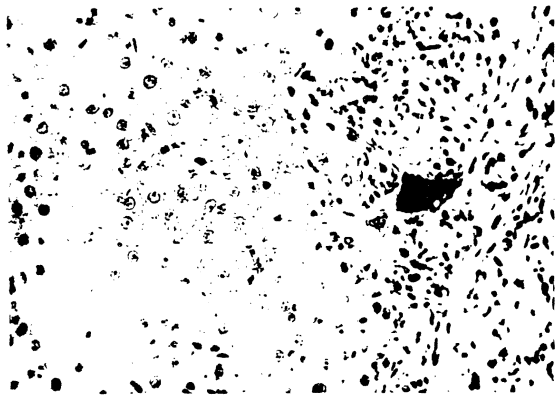

Figure 1. *Immunoperoxidase stain for insulin in liver-needle biopsy specimen from patient 1, obtained 14 days after combined liver-islet transplantation. The darkly staining cell cluster at the edge of a portal triad represents islet cells engrafted within a small a venule. The predominantly neutrophilic infiltrate reflects the clinical state of sepsis at the time and was seen in other triads that did not contain islets (×350).*

The second patient showed an intraportal thrombus composed of a cluster of cells and some fibrin strands, consistent with engrafting islet cells (Figure 2). The cluster was seen on the initial H&E stained section and was lost on deeper cuts stained with anti-insulin. An attempt at performing immunoperoxidase staining on the original slide was unsuccessful, most likely due to destruction of the epitope by the preceding destaining procedure. A second triad in this biopsy showed loosely cohesive cells surrounded by a dense infiltrate of lymphocytes and macrophages similar to the infiltrates observed in the third patient (Figure 3) except for the additional presence of scattered eosinophils. No staining for insulin was observed on deeper sections.

In the liver obtained from the third patient at autopsy, islet cells were identified morphologically in almost every section from the anterior inferior edge. All blocks on which immunoperoxidase staining was performed demonstrated islet cells containing insulin granules, mostly located within the portal triads (Figure 3a). Approximately 20% of cells within the same cluster stained positive for glucagon (not shown). An occasional single insulin-containing cell was detected within the lobule along the sinusoidal lining. Approximately half of the islet clusters were associated with a mild predominantly lymphocytic infiltrate. The other half was associated with a dense mixed infiltrate of lymphocytes, macrophages, and few eosinophils (Figure 3b,c). Most of the lymphocytes stained positive with the T-cell marker UCHL-1. Although a fair number of lymphocytes were also positive for HLA-DR, this rather signifies activation of T cells than B-cell origin. Some of the strongly HLA-DR positive histiocytic cells showed dendritic morphology and possibly represent antigen-presenting cells. Several clusters of loosely cohesive cells did not stain with any of the antibodies and could represent acinar cells from the allograft cell suspensions. A PAS stain after diastase digestion was negative, but this could simply reflect degranulation of zymogen granules. Alternatively, these cells could be multinucleated, foreign body giant cells in response to cellular debris from acinar cells or minute particles such as plastic flakes from culture flasks generated during the islet purification procedure. The allograft kidney showed moderate patchy lymphocytic infiltrates in interstitial and perivenular location, consistent with early acute cellular rejection.

Figure 2. *High-power view of liver-wedge biopsy specimen from patient 2, obtained 2 days after combined kidney-islet transplant. The cluster of cells within the portal vein, accompanied by some fibrin strands, most likely represents an islet cell "thrombus." A mild reactive mononuclear is present. The arteriole in the triad shows changes of diabetic arteriopathy (H&E, ×350).*

The largest cohesive clusters were found in the liver of the fourth patient. Again, clusters were situated preferentially along the anterior inferior edge of the liver and microscopically at the edge of the portal triad. Staining for insulin was only weakly positive and indicates degranulation which correlates with the clinical situation of hyperalimentation and sepsis at the time of death. Staining of deeper sections with antiglucagon showed scattered positive cells and confirmed the endocrine nature of the cell clusters. As in the triad shown in Figure 4, inflammation was minimal and consisted of occasional small lymphocytes. The allograft liver did not show any signs of acute or chronic rejection.

Discussion

Islet-cell transplantation traditionally has been monitored by production of C-peptide and blood glucose levels. As with other allografts, however, histologic findings may not always correlate with functional parameters and could possibly add valuable information to guide clinical management. One of the first crucial steps is correct tissue sampling and the small series presented in this study indicates that the anterior inferior edge of the liver is a

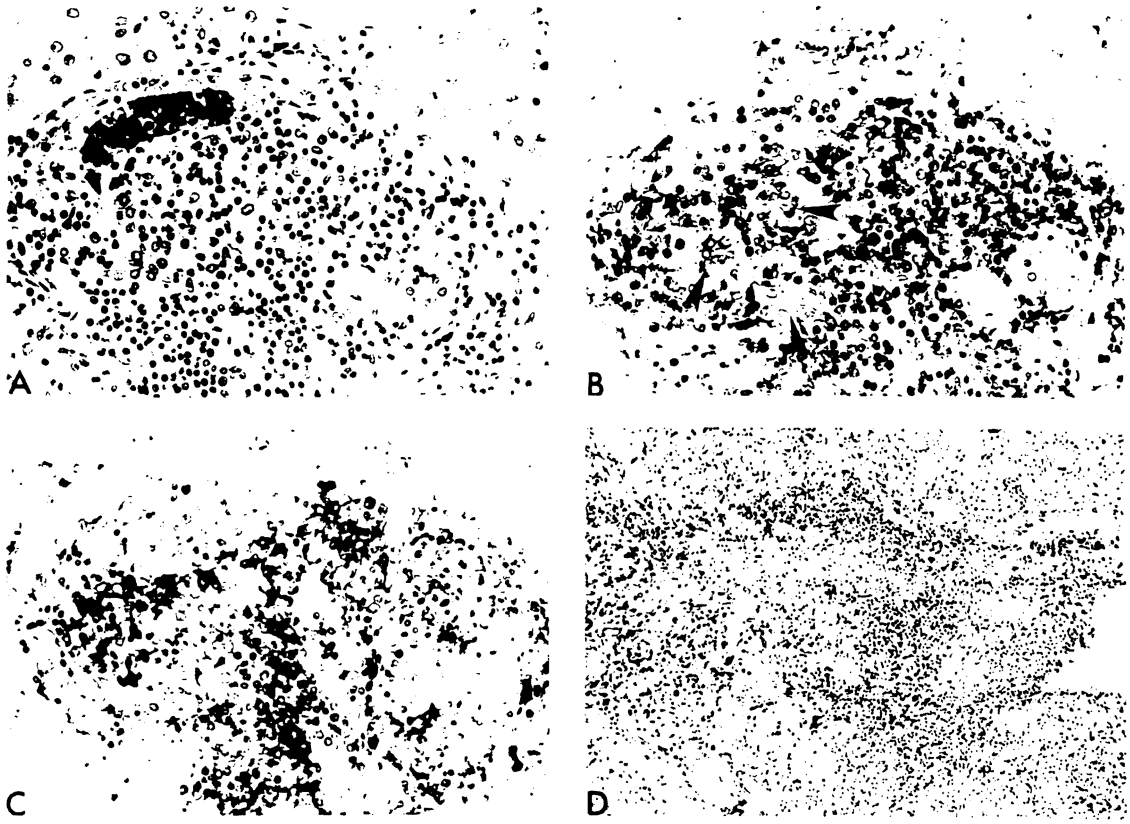

Figure 3. A–C: *Section of liver from patient 3, obtained at autopsy 5 days after combined kidney-islet transplant.* A: *Islet cell cluster with typical granular cytoplasmic staining for insulin (anti-insulin immunoperoxidase, ×350)* B: *Identical area stained for activated T-cell marker. Most of the small round lymphocytes are positive. The cell clusters indicated by arrowheads could represent foreign body giant cells clearing up cellular debris from acinar cells or other noncellular contaminants generated during the isolation procedure (UCHL-1 immunoperoxidase, ×350)* C: *Identical area stained for class II antigen. Several strongly positive histiocytic cells with dendritic cytoplasmic projections could represent antigen presenting cells. Many small lymphocytes also stain positive, indicating T-cell activation rather than B-cell lineage (anti-HLA-DR LN3 immunoperoxidase, ×350)* D: *Section of allograft kidney from same patient, showing a patchy, mild to moderate lymphocytic infiltrate, consistent with early acute cellular rejection (H&E, ×140).*

preferential location for islet cell engrafting. The same observations are being reported in two other patients who had undergone islet cell transplantation after upper abdominal exenteration and liver replacement[12,13] and leave little doubt as to where sampling should be performed. These findings are similar to intraportal islet cell transplants in rats and dogs, which also resulted in predominantly subcapsular location of endocrine cells.[15,17] In our material islet cell clusters were mostly confined to locations within and at the edge of portal triads, whereas only rare single subsinusoidal insulin positive cells were observed within the lobule. In canine autografts, most of the reported microscopic findings are similar with the exception of slightly more numerous endocrine cells within the lobule.[17] This could reflect subtle differences in the dispersion of islets during the purification procedure and could result in altered function and survival of a small subset of grafted cells. The fact that islet cells could even be found on a liver needle biopsy from one of our patients suggests that large numbers of islets do survive the transplantation procedure. Even in the immediate post-transplant period inflammation around the islet cell thrombi can be minimal as in our second patient and insulin stains are helpful for detection of small islet clus-

Figure 4. *Section of liver from patient 4, obtained at autopsy 9 months after combined liver-islet transplant. Several clusters of islet cells, outlined by arrowheads, stain only weakly with insulin. No significant inflammatory infiltrate is visible (anti-insulin immunoperoxidase, ×350).*

ters. In our hands the stain for insulin was consistently more intense than for chromogranin and is preferred as a survey stain. Glucagon-secreting cells could be demonstrated in larger islet clusters in the third and fourth patient. Small clusters were usually lost on deeper cuts. The intense portal inflammation which was seen 2 and 5 days post-transplant in some triads of the second and third patient is likely to be a reaction to nonislet cell contaminants such as acinar cells, soft tissue and hematolymphoid elements. Our own studies on the composition of islet-cell isolates have revealed that even the most highly purified preparations contain approximately 10–20% other cell elements and can be as high as 60%.[22] Islet cells have been shown to engraft in a great variety of sites such as forearm muscle, omentum, peritoneum, spleen, kidney,[1,3,4,6] but the liver has emerged as an especially favorable location once the infusate was reduced to such a low volume as to prevent portal hypertension. A significant change of portal vein pressure was not observed in any of the four patients, although islet-cell thrombi could be demonstrated histologically. The portal inflammation as seen in the second (not illustrated) and third patient, 2 and 5 days, respectively, after islet-cell infusion, could represent clearing up of degenerating acinar and other transplanted non-islet cells. Nevertheless, an additional component of early cellular rejection cannot be ruled out in the third patient, especially since immunosuppression had been withdrawn 2 days before the patient's death and changes consistent with early cellular rejection were found in his allograft kidney (Figure 3d). Despite the pronounced inflammation in many triads, islet cells appear well granulated and clinically no exogenous insulin was required to maintain normal blood glucose levels on the day of the patient's death. Mononuclear infiltrates within islet cell clusters such as described in rejection of whole organ pancreas transplants (isletitis) were not seen.[14] More material needs to be studied to determine morphologic correlates of rejection more accurately. In the fourth patient, the weak staining of islet cell clusters for insulin (Figure 4) is interpreted as excessive degranulation which correlates with the clinical situation of insulin resistant diabetes and parenteral hyperalimentation, both of which chronically stimulate sustained insulin release. Degenerative changes such as described in failed canine islet grafts were not observed.[17] Although the patient did not benefit from the islet transplant, the findings document the long-term survival of morphologically intact allograft islets in sufficient numbers to be detected on histologic examination. In summary, the early posttransplant period in diabetic patients receiving a combined kidney-islet or liver-islet allograft is characterized by focal portal inflammation consisting of lymphocytes, macrophages, and occasional eosinophils, which probably represents clean-up of decaying nonendocrine allograft components and cannot be reliably distinguished from early rejection at this point. Findings 6 months after transplantation included large islet clusters at the edge of portal triads with no or minimal inflammation and only weak granulation. As more material becomes available for histologic examination, morphologic findings might provide valuable information for prognosis and management of islet transplant recipients.

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