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Histological features of acute pancreatic allograft rejection after pancreaticoduodenal transplantation in the rat

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Abstract. For characterization of histopathological changes during pancreas graft rejection, pancreaticoduodenal transplants were performed in three groups: (1) Brown Norway into diabetic Lewis rats without immunosuppression, (2) Brown Norway into diabetic Lewis rats with cyclosporin A, and (3) Lewis into Lewis rats. Diffuse inflammatory infiltration of the acini by mononuclear cells indicated the onset of rejection (stage I). Shortly after acinar infiltration, damage to small and large interlobular excretion ducts occurred. This took the form of florid circumferential inflammation and vacuolar degeneration of epithelium similar to the bile duct damage seen in primary biliary cirrhosis, graft-versus-host disease, and liver allograft rejection (stage II). Thereafter, endothelialitis and destruction of islets were evident, consistent with a more advanced and irreversible stage of rejection (stage III). Acinar inflammation and moderate duct lesions were not prevented by immunosuppression but were delayed. Nonetheless, severe vascular changes and loss of islets were avoided. We conclude that duct lesions are a reliable criterion for pancreas allograft rejection. They are more sensitive than vascular changes and more specific than cellular infiltration of acinar tissue, which may also occur in infection.

Key words: Pancreas transplantation, rat, rejection – Rejection histology, rat, pancreas transplantation – Pancreatic ducts, in experimental rejection

Despite the fact that 1-year survival rates after pancreas transplantation in some centers nowadays exceed 80% [9], the lack of a reliable method of diagnosing rejection remains one of the major problems associated with this type of transplantation. Since the pancreas is, in most instances, transplanted together with a kidney from the same donor, the renal allograft is used for monitoring of the pancreas transplant as well [21]. In the future, however, the trend will be toward single pancreas transplants, performed at a stage of diabetes at which other organs, such as the kidney, have not been damaged. For this purpose, useful markers indicative of rejection are needed. It is known that monitoring of the exocrine pancreas graft function [2, 12] and, in particular, juice cytology represents a most helpful diagnostic tool [6, 16]. A major drawback of this latter method is its requirement of pure pancreatic secretion. On the other hand, it has been shown that percutaneous pancreas graft biopsies can be carried out safely [1], and investigation of cystoscopically directed needle biopsy specimens from pancreaticoduodenal allografts has revealed distinctive changes during the rejection process [3].

In order to gain more detailed information regarding the histopathology of pancreas allograft rejection, an animal model can provide information that cannot be obtained from clinical cases. To evaluate morphological criteria typical of various stages and degrees of pancreas graft rejection, a setting with drainage of pancreas secretion, rather than duct occlusion, is required, causing severe morphological changes [17]. Our model for juice collection [7] drains this juice into a catheter-reservoir system containing antibiotics and, hence, prevents ascending infection. As a result, changes in the excretory duct system caused by rejection can be better distinguished from infectious complications. Previous studies in the rat have shown that only minor differences in pancreas allograft rejection exist between a major histocompatibility complex (MHC)-compatible, but non-MHC incompatible group, and a group that is both MHC and non-MHC incompatible [22]. Thus, a comparison was made between one allogeneic group of rejecting animals, one group of immunosuppressed, allogeneic animals, and a control group of isogeneic grafted animals in order to provide information on: (1) histological criteria of rejection, (2) the influence of immunosuppression on graft survival, (3) changes caused by rejection and/or infection, and (4) the reliability of different histological parameters in rejection diagnosis.

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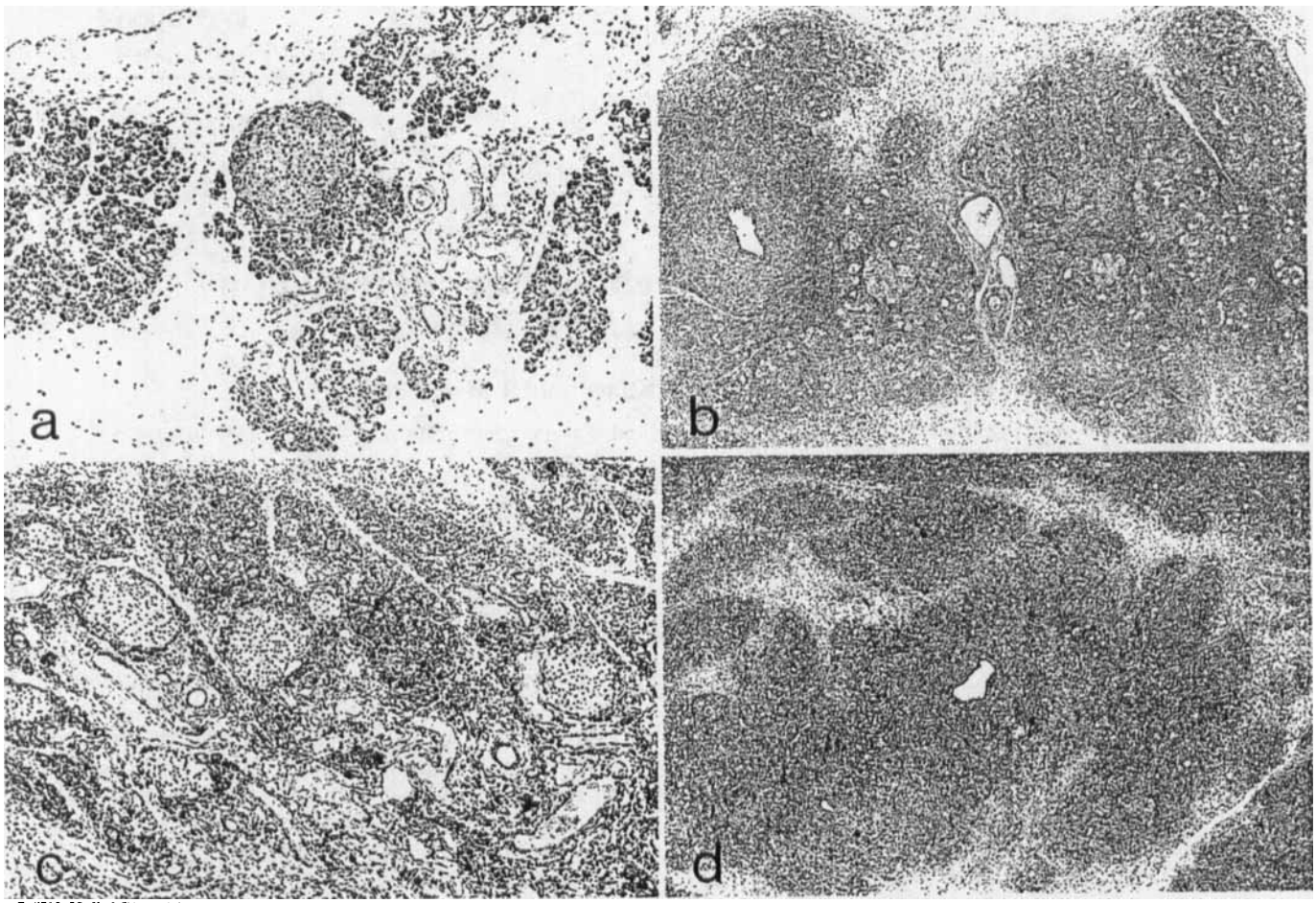

Fig. 1. **a** Interstitial edema with slight mononuclear infiltration. Acini, ducts, and islets are well preserved (BN-LEW/CyA, day 6, H&E, × 100). **b** Severe inflamed graft due to acute cellular rejection with intact islets but extensive duct lesions and almost complete loss of acini (BN-LEW, day 6, H&E, × 80). **c** Loss of acini by a destructive interstitial inflammation after infection. Ducts and islets remain intact (LEW-LEW, day 22, H&E, × 30). **d** Onset of fibrous replacement of the graft. Only remnants of larger ducts are discernible (BN-LEW, day 8, H&E, × 30)

Subjects and methods

Pancreaticoduodenal transplants were performed from Brown Norway (BN) to Lewis (LEW) rats (Zentralinstitut für Versuchstierzucht, Hannover, FRG; Tierversuchsgenehmigung GZ 68205/377-12/88) as previously described [7]. Recipients were rendered diabetic by means of streptozotocin (60 mg/kg body weight). No immunosuppression was given to 16 animals (BN-LEW), while 10 animals (BN-LEW/CyA) received 12 mg/kg body weight cyclosporin A orally. The control group consisted of 10 animals, each of which received an isograft (LEW-LEW). Animals in the BN-LEW group were killed between day 4 and day 12 (days 4 and 12: $n = 1$; days 5–11: $n = 2$), animals in the BN-LEW/CyA and LEW-LEW groups between days 6 and 35 (days 6, 10, 21, and 35: $n = 1$; day 14: $n = 6$) by an overdose of anesthesia.

The grafts and samples of other organs (pancreas, liver, spleen, gut, lymph nodes, skin, kidney, lung, and heart) were examined macroscopically and then immediately fixed in 10% neutral formalin. Small probes were snap-frozen in liquid nitrogen and stored at −70°C. Immunohistochemistry was performed by applying the peroxidase antiperoxidase technique on frozen sections (6 µm) using the monoclonal antibodies MAB 1441 (Chemicon International) recognizing MHC class I (rat RT1) and MCA 50 (Serotec) for MHC class II (rat RT2) at a dilution of 1:100 each without pretreatment.

Paraffin embedding was carried out as usual by automatic tissue processing, and sections were obtained at a range of 4–5 µm. Staining procedures included H&E, chromotrope anilin blue (CAB), and periodic acid-Schiff reaction (PAS).

Some of the animals were included in a study focusing primarily on pancreatic juice cytology and its correlation with histological changes. A significant increase in juice lymphocytes was found in the untreated allogeneic group, whereas in both the isogeneic control group and the CyA-treated allogeneic group, no such increase in mononuclear cells was detectable [8].

Results

Macroscopic examination

Between days 5 and 8, the BN-LEW rats showed edematous swelling of the pancreatic graft with a three to four-fold increase in size and a fibrinous exudate at the serosal surface. Subsequently, induration occurred, and on days 10–12 some firm, necrotic areas were discerned. The exudate formed a shell around the graft and protected the abdominal cavity from a diffuse spreading of the inflammation. The immunosuppressed (BN-LEW/CyA) animals revealed a significantly less severe swelling of the graft, which reached its maximum after 2 weeks. The size subsequently decreased and by day 35 the pancreas had

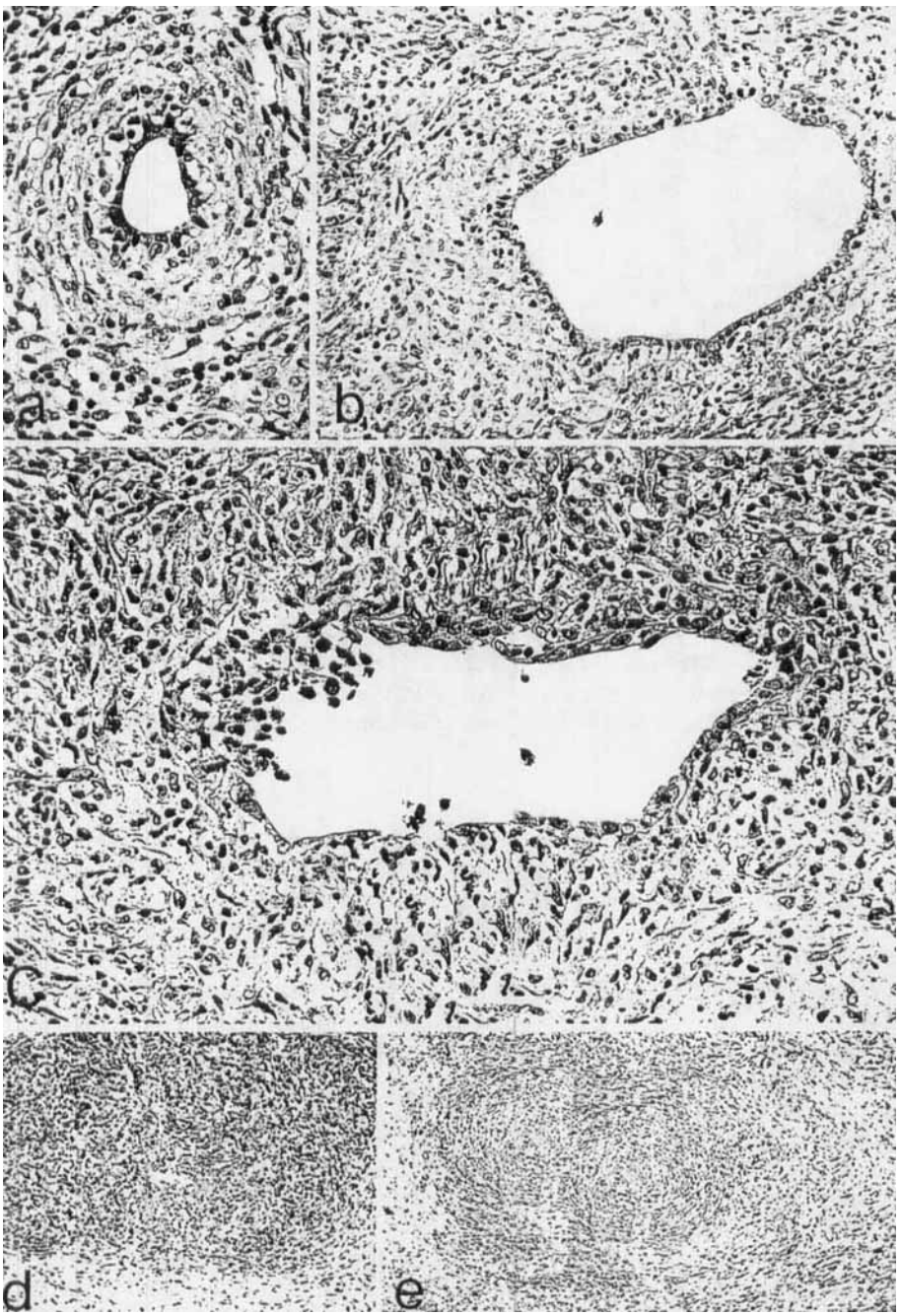

Fig. 2a–e. Spectrum of duct lesion:
a small duct with invasion by mononuclear cells, vacuolation, and flattening of the epithelium (BN-LEW/CyA, day 10, H&E, ×400);
b identical and more pronounced changes in a large duct (BN-LEW/CyA, day 16, H&E, ×250);
c duct ulcer with disruption and loss of basement membrane (BN-LEW, day 6, CAB, ×400);
d remnants and **e** fibrous obliteration of ducts without immunosuppression (BN-LEW, days 8 and 10, H&E, ×140)

returned to its normal volume but appeared slightly fibrotic. LEW-LEW combinations showed features similar to those of immunosuppressed animals, with moderate swelling for up to 10 days. Thereafter, some grafts became almost normal with an inconspicuous, smooth cut surface. Others remained enlarged with areas of tryptic pancreatitis, sometimes extending to the surrounding structures.

Immunohistological examination

In rejecting pancreatic grafts after day 4, staining for MHC class I antigens revealed positivity in ducts, periductal connective tissue, and within the acini, reacting predominantly within inflamed areas. Endothelial cells proved positive within exocrine and endocrine tissue, whereas staining of islet cells remained poor. Isogeneic controls scarcely differed from allogeneic grafted animals when there were foci of pancreatitis caused by suture granulomas. MHC class II antigen was shown predominant within interstitial dendritic cells and, to a lesser degree, in ducts and acini of rejecting grafts. Positive staining of endothelia occurred in some small and large vessels and spread to the capillaries of exocrine and endocrine tissue. Isogeneic grafts showed a similar pattern of MHC class II antigen expression on mesenchymal cells, while epithelial cells remained negative or only weakly positive in the vicinity of inflammation.

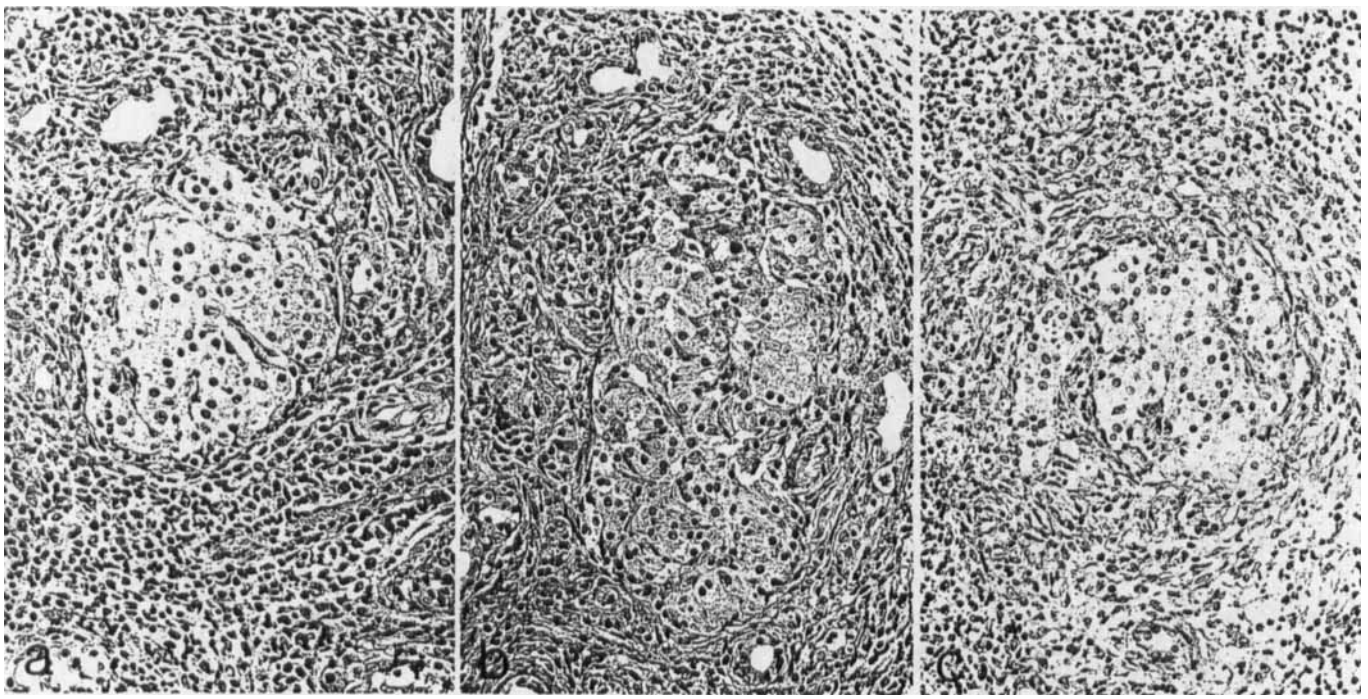

Fig. 3a–c. Rejection of islets: **a** intact islet despite dense interstitial inflammation and lesions of the small neighboring ducts (BN-LEW, day 6, CAB, ×250); **b** ingrowth of capillaries and fibroblasts (BN-LEW, day 8, CAB, ×250); **c** disintegration of islets with endocrine cell loss and onset of fibrous replacement (BN-LEW, day 9, H&E, ×250)

Histological examination

The acinar epithelium was found to be most sensitive to immunological damage by immigrating mononuclear cells. In the BN-LEW group, acinar structures were preserved on days 4 and 5, but a diffuse cellular infiltrate with invasion of the epithelium had evolved and the content of zymogen granules of the epithelium was diminished. Simultaneously, damage to the interlobular ducts occurred and progressed to complete duct loss within the following 5 days. Duct lesion was characterized by a florid, circumferential, predominantly mononuclear or mixed cellular infiltrate and vacuolar degeneration of epithelium. Thereafter, a flattening of the duct epithelium and ulcerous destruction, including the basement membrane, was seen. This process resembled bile duct injury of primary biliary cirrhosis (PBC) [11], graft-versus-host disease (GVHD) [13], or hepatic allograft rejection [4]. Without immunosuppression, duct damage led to complete fibrous replacement of the duct, revealing only a scar with an onion skinlike arrangement of fibrous tissue. Vascular changes included endothelialitis and vascular necrosis and progressed rapidly from day 7 onwards. At that time, an increasing proliferation of fibroblasts replaced the entire exocrine pancreas and destroyed the islets by splitting them into singular strands of endocrine cells. After day 12 only remnants of larger arteries were identified within proliferating connective tissue and areas of fibrinoid necrosis. Immunosuppresison was able to delay graft loss. The acinar epithelium was destroyed in all BN-LEW/CyA animals within 2 weeks in a more protracted way than in the BN-LEW group. Changes in the interlobular ducts displaying PBC-like features were evident in nine-tenths of the immunosuppressed rats and remained demonstrable until day 35. The intensity of duct lesions varied according to the degree of rejection; correspondingly, arterial endothelialitis was evident to a minor degree in four-tenths of the animals. Isogeneic grafted animals (LEW-LEW) lacked duct lesions and vascular changes attributable to rejection. Foci of pancreatitis, however, caused a perifocal mononuclear or mixed cellular infiltrate, destroying large areas of acinar tissue in a pattern similar to allograft rejection. In those cases, gram-positive rods *(Corynebacterium)* could be demonstrated within the inflammatory infiltrate.

Within the untreated allogeneic group, no variation of different histological features was found. In the isogeneic group, as well as in the immunosuppressed allogeneic group, however, the cellular infiltrate varied to some degree, as did the PBC-like lesions in the latter (Table 1).

To summarize, three stages of allograft rejection were distinguished. The first is a stage of acinar lesion, stage I. This process evolves constantly, with or without immunosuppression. It is the earliest and most sensitive criterion of immunological damage afflicting the acinar epithelium and small intralobular ducts. Acinar rejection can only be attenuated by immunosuppression; it cannot be prevented (Fig. 1).

Stage II is characterized by duct lesion. The small, medium, and large interlobular ducts are damaged by the rejection process in an identical manner. This lesion occurs with maximum activity 1–2 days after peak acinar inflammation. The character of duct lesion is similar to bile duct damage of the liver in various immunologically mediated pathological conditions and, hence, implicates the term PBC-like duct lesion. Without immunosuppression, duct

Table 1. Histological changes in various pancreatic structures in the different groups

	BN-LEW	BN-LEW/CyA	LEW-LEW
Acini	Diffuse interstitial infiltration (severe)	Diffuse interstitial infiltration (varying)	Focal interstitial infiltration (varying)
Ducts	PBC-like lesions, diffuse, severe	PBC-like lesions, diffuse, varying	Intact, single, PBC-like lesion
Arteries	Endothelitis and vascular necrosis	Endothelitis (4/10)	No abnormalities
Islets	Destroyed (10 days)	Diminished (> 20 days)	Intact

damage leads to fibrous replacement of the duct, comparable with vanishing bile duct (VBD) disease in irreversible rejection of liver allografts [10]. Immunosuppression protects against duct loss, but cannot postpone duct lesions (Fig. 2).

In stage III there is vascular and islet lesion. Endothelialitis of veins precedes arterial changes. The latter develops initially in a more focally accentuated distribution and less rapidly than exocrine parenchymal damage. Islets are affected by infiltration of lymphocytes, followed by ingrowth of fibroblasts along the intervening capillaries. Untreated rejection leads to vascular necrosis and fibrous replacement of islets after 10 days. Immunosuppression prevents complete loss of islets but does not prevent the decrease of size and number of islets. Endothelialitis may occur to a minor degree, indicating underimmunosuppression (Fig. 3).

Discussion

Graft monitoring is particularly important in the early post-transplant period and decisive for graft outcome since lost endocrine tissue can never be replaced, despite reversal of rejection [14, 20]. Assuming the kidney and pancreas possess a similar immunogenicity, functional parameters and histology of the renal allograft are also used as rejection markers for the pancreas in cases of combined transplants. In contrast to endocrine pancreatic function, including serum insulin and C-peptide, monitoring of the exocrine secretion and, in particular, pancreatic juice cytology has proved to be of great diagnostic value [2, 6, 12, 16]. On the other hand, it has been shown that percutaneous graft biopsies of pancreas grafts can be carried out safely [1]. Although histology is regarded as the gold standard of rejection diagnosis in most solid organs, cytology has a special advantage over biopsies: juice collection from the common duct provides insight into the entire exocrine tissue, while biopsies can only display a small area of grafted tissue. Therefore, it is of paramount importance to define a structure that is most probably encountered in small tissue specimens and that will reveal characteristic changes in case of rejection.

The experience gained from this rat model shows that three stages of pancreatic allograft rejection can be distinguished that do not necessarily reflect a certain course of events. Destructive interstitial inflammation of acini (stage I) seems to be the most sensitive and the earliest sign of rejection; however, it lacks specificity. Although acinar epithelium does not primarily express the MHC class I antigen, the early switch to MHC class I positivity within the second day after grafting [18] may dispose acini for destruction by immigrating cytotoxic cells. Correspondingly, inflammation unrelated to rejection, such as perifocal inflammation in the vicinity of infectious pancreatitis, suture granulomas, etc., can mimic rejection if only a limited amount of tissue is available for investigation, as in biopsy specimens. Immunohistochemistry does not contribute to the solution of this problem since increasing MHC antigen expression on epithelium in inflamed acini was found both in rejecting animals and in infections in nonrejecting isogeneic controls. Thus, acinar changes are of little diagnostic value unless combined with additional histological findings. Arterial endothelialitis and disintegration of islets (stage III) are indicative of rejection. In contrast, venous endothelialitis is of less diagnostic value when the thin vascular wall is affected by an interstitial inflammation process. Sampling error of the biopsy and focal distribution of arterial changes can cause these changes to be absent. Their presence, however, indicates an advanced stage of rejection.

A PBC-like duct lesion in the form of a nonsuppurative destructive inflammation (stage II) appears to be the most consistent finding in rejecting pancreatic allografts. Evolving in small and large ducts alike, it is found in a high percentage of biopsies. The constant expression of MHC class I and the induction of MHC class II antigens several days after grafting makes duct epithelium susceptible to cell-mediated damage, although duct lesions are somewhat attenuated by the periductal connective tissue compared with the more intensive acinar changes. Almost as sensitive but much more specific than acinar inflammation, PBC-like duct lesions indicate a status of rejection prior to islet damage, the latter developing surprisingly late, in spite of positive expression of MHC class I antigen on islet cells [5, 18, 19].

For diagnostic reasons, the typical histological aspect of PBC-like duct lesions closely resembling bile duct lesions in liver allograft rejection, GVHD, and PBC may easily be recognized. Hence, its early onset and regular distribution can be used as a guideline in the diagnosis of

Table 2. Proposed schedule for grading of acute pancreatic allograft rejection

Diagnosis	Histological features
Consistent with rejection (grade 0)	Destructive interstitial inflammation of acini
Acute rejection, grade 1	Minor, nonsuppurative (PBC-like) duct lesions
Acute rejection, grade 2	Intensive duct lesions
Acute rejection, grade 3	Arterial endothelialitis with or without islet damage

rejection. This suggests that one can apply the classification of acute cellular rejection of liver allografts [15] to pancreatic allograft rejection using a similar histological scoring system. Acinar inflammation without additional findings is consistent with rejection. PBC-like duct lesions of varying degress correlate with slight or moderate rejection. Arterial endothelialitis with or without islet damage indicates severe rejection (Table 2).

References

1. Allen RDM, Wilson TG, Grierson JM, Greenberg ML, Earl MJ, Stewart JH, Little JM, Chapman JR (1990) Percutaneous pancreas transplant fine needle aspiration and needle core biopsies are useful and safe. Transplant Proc 22: 663–664
2. Brattström C, Tyden G, Malmborg AS, Lundgren G, Öst L, Groth CG (1987) Studies of the exocrine secretion of segmental pancreatic grafts with special reference to the diagnosis of rejection and to the penetration of drugs into the pancreatic juice. Transplant Proc 19: 2332–2335
3. Carpenter HA, Engen DE, Munn SR, Barr D, Marsh CL, Ludwig J, Perkins JD (1990) Histologic diagnosis of rejection by using cystoscopically directed needle biopsy specimens from dysfunctional pancreatoduodenal allografts with exocrine drainage into the bladder. Am J Surg Pathol 14: 837–846
4. Fenell RH Jr, Shikes RH, Vierling JM (1983) Relationship of pretransplant hepatobiliary disease to that of bile duct damage occurring in the liver allograft. Hepatology 3: 84–89
5. Gaudecker B von, Ulrichs K, Müller-Ruchholtz W (1989) Immunoelectron microscopic localization of MHC structures in isolated pancreatic rat islets. Diabetes 38 [Suppl 1]: 150–153
6. Klima G, Margreiter R (1989) Pancreatic juice cytology in the monitoring of pancreas allografts. Transplantation 48: 980–985
7. Königsrainer A, Habringer C, Krausler R, Margreiter R (1990) A technique of pancreas transplantation in the rat securing pancreatic juice for monitoring. Transplant Int 3: 181–182
8. Königsrainer A, Dietze O, Habringer C, Krausler R, Klima G, Margreiter R (1991) Morphology of acute rejection and corresponding cytological findings in exocrine secretion after pancreas transplantation in the rat. Transplantation (in press)
9. Melzer JS, D'Allessandro AM, Kalayoglu M, Pirsch JD, Belzer FO, Sollinger HW (1990) The use of OKT3 in combined pancreas-kidney allotransplantation. Transplant Proc 22: 634–635
10. Portmann B, Neuberger JM, Williams R (1983) Intrahepatic bile duct lesions. In: Calne RY (ed) Liver transplantation. Grune and Stratton, London, pp 279–287
11. Portmann B, Popper H, Neuberger J, Williams R (1985) Sequential and diagnostic features in primary biliary cirrhosis, based on serial histologic study in 209 patients. Gastroenterology 88: 1777–1790
12. Prieto M, Sutherland DER, Fernandez-Cruz L, Heil J, Najarian JS (1987) Experimental and clinical experience with urine amylase monitoring for early diagnosis of rejection in pancreas transplantation. Transplantation 43: 73–79
13. Shulman HM, McDonald GB (1984) Liver disease after marrow transplantation. In: Sale GE, Shulman HM (eds) The pathology of bone marrow transplantation. Masson, New York, pp 104–135
14. Sibley RK, Sutherland DER (1987) Pancreas transplantation. Am J Pathol 128: 151–170
15. Snover DC, Freese DK, Sharp HL, Bloomer JR, Najarian JS, Ascher NL (1987) Liver allograft rejection. An analysis of the use of biopsy in determining outcome of rejection. Am J Surg Pathol 11: 1–10
16. Steiner E, Klima G, Niederwieser G, Königsrainer A, Herold M, Margreiter R (1987) Monitoring of the pancreatic allograft by analysis of exocrine secretion. Transplant Proc 19: 2336–2338
17. Steiniger B, Klempnauer J (1986) Distinct histologic patterns of acute, prolonged and chronic rejection in vascularized rat pancreas allografts. Am J Pathol 124: 253–262
18. Steiniger B, Klempnauer J, Wonigeit K (1985) Altered distribution of class I and class II MHC antigens during acute pancreas allograft rejection in the rat. Transplantation 40: 234–239
19. Steiniger B, Hiller WFA, Klempnauer J (1990) Identical pattern of acute rejection after isolated islet and vascularized whole-pancreas transplantation in the rat. Am J Pathol 137: 93–102
20. Sutherland DER, Casanova D, Sibley RK (1987) Role of pancreas graft biopsies in the diagnosis and treatment of rejection after pancreas transplantation. Transplant Proc 19: 2329–2331
21. Sutherland DER, Dunn DL, Goetz FC, Kennedy W, Ramsay RC, Steffes MW, Mauer SM, Gruessner R, Moudry-Munns KC, Morel P, Viste A (1989) A 10-year experience with 290 pancreas transplantations at a single institution. Ann Surg 210: 274–288
22. Timmermann W, Schang T, Thiede A (1985) Modelle und Perspektiven der Pankreastransplantation bei der Ratte. II. Der Abstoßungsverlauf von pankreaticoduodenalen Transplantaten verschiedener Histokompatibilität und unter dem Einfluß temporärer Therapie mit Ciclosporin (Sandimmun). Langenbecks Arch Chir 363: 235–243