795

A Review of Adult and Pediatric Post-Transplant Liver Pathology

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The operative techniques of liver transplantation were pioneered by Starzl in the 1950s and early 1960s through extensive animal experimentation.[1-3] Starzl began human orthotopic liver transplants in 1963 and has since accumulated vast experience in this procedure.[4-7] Calne and Williams initiated the procedure in 1968 at Cambridge and King's College in London, and through the years have published on their experience.[5-10] Although early clinical results were disappointing, advances in surgical technique and immunosuppressive regimens have made the procedure an acceptable therapeutic modality for patients with many forms of end stage liver disease. Presently, liver transplantation is performed in over 20 centers in the United States and Europe.

Pathologic studies on early animal and human liver allografts were done by Porter[11,12] in collaboration with Starzl. Initial pathologic studies are frequently the most difficult, since they are fraught with many confounding factors. Porter's meticulously detailed observations throughout the years have formed the basis for our understanding of the pathology of liver transplantation.

The current review is based on experience acquired in examining over 900 surgical specimens from more than 260 adult and 200 pediatric patients who underwent orthotopic liver transplantation at the University of Pittsburgh from 1981 to 1986. The spectrum of primary liver disease for which transplantation was performed, is shown in Table 1.

The pathologist plays a pivotal role in the precise identification of the primary disease and assists in determining the causes of allograft dysfunction. Needle biopsies of the allografted liver have become a routine aspect of

TABLE 1. ORIGINAL DISEASE PRIOR TO TRANSPLANTATION

	No. (%)
ADULT: Non-neoplastic	
Primary biliary cirrhosis	75 (29)
Cirrhosis[a] (cause uncertain)	64 (25)
Sclerosing cholangitis	38 (15)
Hepatitis B	
Chronic active	10 (4)
Acute fulminant	2 (<1)
Wilson's disease	10 (4)
Budd–Chiari syndrome	7 (3)
Alpha-1-antitrypsin deficiency	6 (2)
Secondary biliary cirrhosis	5 (2)
Hemochromatosis	3 (1)
Caroli's disease	4 (2)
Toxin	2 (<1)
Other[b]	8 (3)
ADULT: Neoplastic	
Hepatocellular	
Carcinoma	10 (4)
Fibrolamellar	6 (2)
Adenomas	2 (<1)
Cholangiolar carcinoma	4 (2)
Epithelioid hemangioendothelioma	3 (1)
Angiosarcoma	1 (<1)
PEDIATRIC	
Extrahepatic biliary atresia	84 (41)
Intrahepatic biliary atresia[c]	18 (9)
Alpha-1-antitrypsin deficiency	28 (14)
Cirrhosis "posthepatitic"	17 (8)
Familial cholestasis[d]	16 (8)
Cirrhosis (cryptogenic)	8 (4)
Wilson's disease	7 (3)
Tyrosinemia[e]	5 (2)
Acute hepatic necrosis (drug toxicity)	4 (2)
Congenital hepatic fibrosis	4 (2)
Glycogen storage disease (type IV)	4 (2)
Choledochal cyst with cirrhosis	2 (1)
Hyperlipidemia	2 (1)
Others[f]	5 (2)

[a]Includes cases of non-A, non-B hepatitis, "autoimmune" hepatitis, heterozygous alpha-1-antitrypsin deficiency.
[b]Includes trauma, alcohol abuse, hyperlipidemia, cryptococcal cholangitis, and cystic fibrosis.
[c]Syndromatic and sporadic, two patients had combined intra- and extrahepatic atresia.
[d]Includes Byler's disease.
[e]All patients had coexistent hepatocellular carcinomas.
[f]Includes type I glycogen and neurovisceral storage diseases, inflammatory pseudotumor, cirrhosis secondary to histiocytosis X, and methotrexate-induced cirrhosis.

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patient care. The following is intended as a guide to histopathologic and pathophysiologic interpretation based on observations made in the allograft biopsies, failed allografts, and autopsy specimens from these patients.

HANDLING OF PATHOLOGIC SPECIMENS

The varied functions of the liver combined with the diverse etiologies of primary disease processes cut across disciplinary boundaries in medicine and biology. This is manifested by frequent requests we receive for liver tissue from primary resections and post-transplant specimens from these patients. The advent of a transplantation unit can therefore place a strain not only on the diagnostic facilities, but also on facilities for the handling of tissues. Arrangements for freezing, cataloguing, storage, retrieval, and shipping to other investigators should be anticipated and plans made to share the expense. The rapid handling of liver tissue for research purposes when a hepatectomy occurs at 2 AM, presents its own logistic problems.

Primary Resections

Special handling of the original hepatectomy specimens is often required. Livers that require particular attention, such as those with metabolic disorders, must be identified in advance to alert the pathologist and other laboratories to specific needs. Special procedures for handling tissue from primary resections as well as from post-transplant specimens are listed in Table 2.

Recourse to previous biopsy material, surgical pathology, and operative reports is needed in order to establish the primary diagnosis. This is espe-

TABLE 2. SPECIAL PROCEDURES FOR HANDLING PRIMARY RESECTIONS, POST-TRANSPLANT BIOPSIES,[a] FAILED GRAFTS, AND AUTOPSY SPECIMENS

1. Procurement of fresh sterile tissue for microbiologic cultures, parenchymal and lymphoid cell cultures, biochemical or gene cloning studies.
2. Immediate bulk freezing of tissue for biochemical or gene cloning studies, e.g., metabolic, genetic, or viral diseases.
3. Freezing of small samples in frozen section[b] compound or special fixation for immunohistopathologic or electron microscopic studies.
4. Special gross dissection[c] with/without x-ray contrast studies
   a. Porta hepatis in extrahepatic biliary atresia and intrahepatic bile duct paucity syndromes and cystic disorders of bile ducts.
   b. Hepatic vein examination in venous outflow obstruction and hepatic tumors.
   c. Gross determination of anastomotic patency and or breakdown in failed allografts and autopsy specimens.

[a]When tissue samples are limited, routine fixation and H&E histologic examination should take precedence over investigational studies.
[b]OCT.
[c]Photographs or diagrams should document interesting cases.

cially true where operative intervention has changed the underlying anatomy, e.g., where Kasai-type procedures have been performed on livers with *intra*hepatic atresia. As a courtesy, referring institutions should be made aware of diagnoses established after resection in particular where there is disagreement with the original diagnosis or where information comes to light that was not apparent before the transplantation.

Needle Biopsies

Ideally, needle biopsy specimens should be delivered fresh to the surgical pathology department moist and as soon as possible after the procedure (see Table 2). Accompanying the biopsy there must be appropriate clinical information on the surgical requisition form to answer the questions that most frequently arise. This is most easily accomplished by providing an information sheet to be filled out by the clinical physician when the biopsy is submitted. The information, at a minimum, other than the general demographic data that are required, includes the following: the patient's original disease; the date of transplantation(s); the results of the studies of anastomotic patency, i.e., cholangiogram, ultrasound, arteriography; general condition of the graft at the time of transplantation, if less than 3 weeks post-transplant; and current immunosuppressive therapy, especially if additional immunosuppressives have recently been given. Some of this information may already be on file from the primary resection and prior biopsies, the most recent of which should be reviewed with the current specimen. Directed clinical questions are important to the triage and handling of the tissue, e.g., ?cholangitis, ?viral disease, ?recurrent hepatitis, since they will direct particular attention to relevant diagnostic procedures. Ideally, biopsy results should be communicated directly to the attending physician as this facilitates a useful exchange of information.

Our specimens are sectioned at 4 to 6 microns and routinely stained with hematoxylin and eosin (H&E), trichrome, periodic acid-Schiff after diastase digestion (PAS-D), and reticulin when indicated. The trichrome and PAS-D stains are of particular value in demonstrating the integrity of the bile ducts and ductules. Other stains that we have found particularly useful are those for iron and elastin, and immunoperoxidase techniques for identification of the hepatitis B core and surface antigens, herpes simplex virus, cytomegalovirus (CMV), and alpha-1-antitrypsin accumulation.

Failed Allografts and Autopsy Specimens

Failed allografts removed at the time of retransplantation and autopsy specimens should be handled using guidelines set up according to a defined protocol. Considerations for handling of the tissue specimens are similar to those outlined for primary resections and biopsies. However, the diagnostic approach must be modified to answer specific questions pertaining to the reasons for graft failure, e.g., primary or secondary failure. Very importantly, therefore, close cooperation between the surgeons and pathologist is needed in assigning diagnostic categories for liver allograft failure since pertinent

information such as vascular patency may not be obvious in the specimen, or even submitted for examination.

The gross examination of the failed allograft at retransplant or autopsy should include a thorough examination of the hilar region. Particular attention should be given to all the biliary and vascular anastomotic sites, initially via a complete gross dissection of the arterial supply, portal vein, and biliary system. This is most easily accomplished in autopsy specimens by approaching the anastomotic sites from the recipient's side beginning in the aorta, portal vein, gastrointestinal tract, or inferior vena cava. Obviously this approach is not possible in surgical specimens, where with a basic knowledge of the gross anatomy of the hilum of the liver, the vessels and bile ducts can be identified and dissected.

Following the gross examination of the hilum, a horizontal cross-section of the superior aspect of the liver across each of the hepatic lobes reveals the openings of the hepatic veins. Further sectioning of the liver in a parallel plane is used to identify gross intrahepatic defects such as infarcts or abscesses.

Histologic sections from areas other than grossly evident defects should be taken according to a defined protocol in order to compare sections from similar locations from case to case. We routinely submit several sections from the hilum taken in a plane perpendicular to the long axis of the major hilar structures at 4 to 6 mm intervals. It is not uncommon to discover significant pathologic alterations in these hilar sections especially in grafts that failed during the first several months post-transplant (Fig. 1).

Frozen Sections

Frozen section examination of post-transplant needle biopsy specimens may at times be useful but should be limited because of the prime importance of good quality histology. Nevertheless, in selected instances judicious use of the frozen section can be used to identify changes associated with ischemia, necrosis, rejection, and, in some cases, cytomegaloviral (CMV) hepatitis. In fact, the eosinophilia of damaged and necrotic cells is accentuated on frozen sections and can lead to overestimation of the amount of irreversible damage. Bile stasis is also more conspicuous on frozen than on embedded sections. Our institution uses rapid processing, i.e., 5- to 6-hour turnaround time, of specimens when expedient results are needed. There are considerations of 7-day coverage to be dealt with by each institution.

POST-TRANSPLANT HISTOPATHOLOGY

Donor Disease

Prior donor disease has been seen but is not common. A donor with unsuspected alpha-1-antitrypsin deficiency and mild fibrosis was detected for the first time at biopsy several days following transplantation. One patient re-

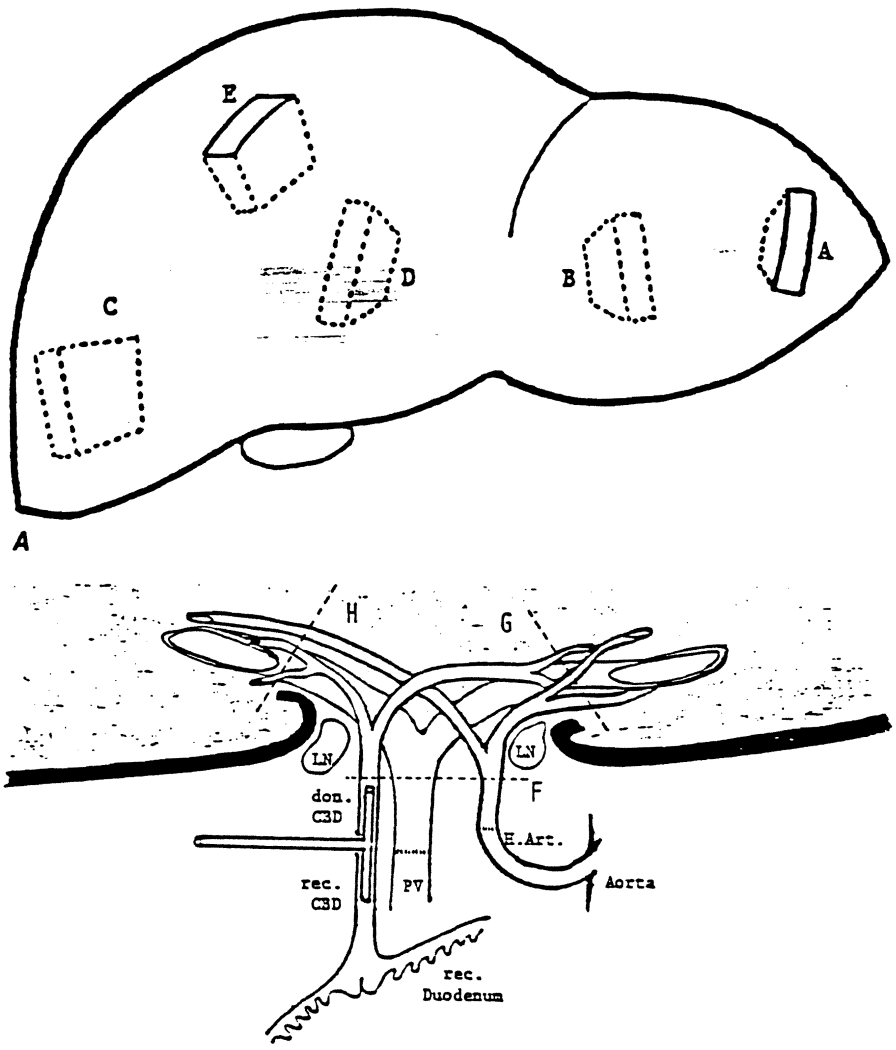

Figure 1. Schematic of gross and microscopic sectioning protocol. ***A.*** A–E shows the location of protocol microscopic sections. Sections A and E include the capsule. ***B.*** Representation of primary duct-to-duct anastomosis (T-tube in place), hilar anatomy, and further protocol microscopic sections (F–H).

ceived an allograft from a donor with Fabry's disease. There was little abnormality seen by light microscopy aside from a small number of PAS-D-positive foamy endothelial and pericytes of small portal tract arterioles, which by electron microscopy revealed characteristic lamellar intracellular storage lipid. In one instance there was prominent central vein and subsinusoidal fibrosis suggestive of alcohol abuse by the donor.

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Biopsy of donor livers before transplantation has shown that some of the early postoperative changes are due to damage done to the liver before transplantation. Such damage may have been due to cardiac failure, hypotension, drugs, or toxins. Subcapsular hematomas, focal subcapsular necrosis, areas of infarction, and freeze burns from prolonged transport have been noted and confirmed microscopically. It is important to be aware of the focal nature of some of these defects especially those in a subcapsular location, since they may lead to sampling errors in early post-transplant biopsies.

Harvesting Changes (Nonimmunologically Mediated Damage)

An organ donated for transplantation can suffer injury from a variety of noxious influences before or during insertion in the recipient (Table 3). Although all of the factors mentioned may be involved in graft injury, damage secondary to hypoxia before harvesting and during preservation is likely to be the most significant. The histologic changes associated with harvesting injury are best approached by considering separately those associated with injury (degenerative) from those of recovery (regeneration) even though overlap because of the regenerative capacity of the liver is obvious.

The changes of graft injury related to harvesting are primarily based in the lobule. The mildest and most common changes are microsteatosis, hepatocanalicular cholestasis, and hepatocellular ballooning accentuated centrilobularly, with spotty acidophilic degeneration of hepatocytes throughout the lobule. Kupffer cell hypertrophy, mild centrilobular or portal tract interstitial hemorrhage may also be seen early. Centrilobular hepatocellular coagulative necrosis without collapse of the reticulin architecture may be seen as the degree of injury increases. Centrilobular dropout of hepatocytes with reticulin collapse appears quite severe because of the associated hemorrhage into the area. Severe ischemia may lead to a peculiar periportal hepatocellular necrosis, and be associated with subcapsular necrosis and focal infarcts.[13] The degree of injury is gauged by the area of the lobule that is affected as well as the severity of hepatocellular damage. Polymorphonuclear inflammation of the central vein may be a feature of harvesting damage. Examples of differing degrees of harvesting injury are shown in Figure 2.

The mucosal health of the donor gallbladder was assessed in the pediatric population in an attempt to prognosticate graft survival. Although the epithelium showed changes of marked damage and repair with inflammation in some

TABLE 3. HARVESTING INJURY: NONIMMUNOLOGIC DAMAGE OF THE ALLOGRAFT PRIOR TO REVASCULARIZATION IN THE HOST

Insults while in the donor. Ischemia, shock, cardiac failure, drugs, toxins, alcohol, traumatic graft injury, and donor disease
Insults during harvesting and transport. Prolonged cold ischemia and freeze burns
Insults during implantation. Bench time (vascular trimming), hypotension, warm perfusion, and anesthetic agents

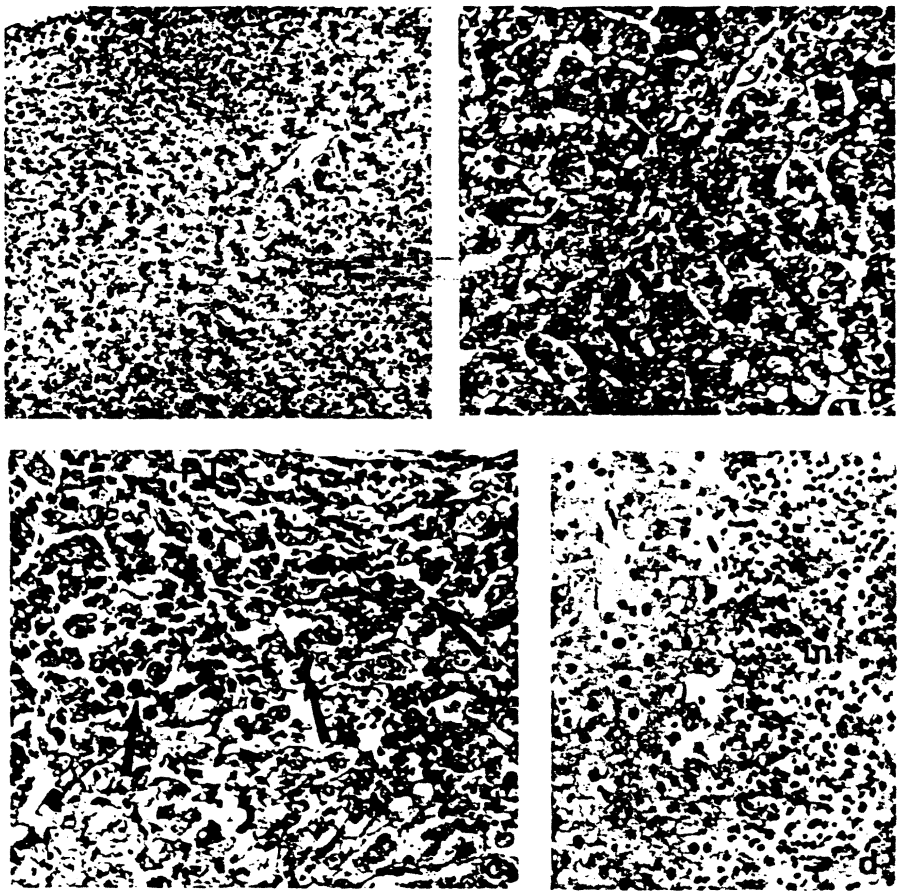
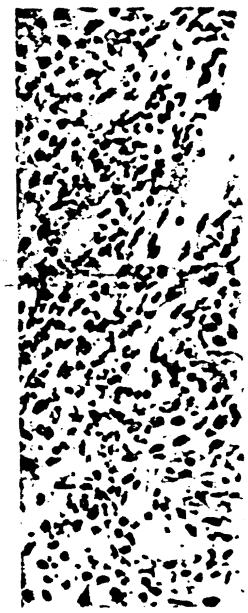


Figure 2. Harvesting injury. Needle biopsies (first 2 weeks post-transplant) demonstrating: **a.** Centrilobular ballooning of hepatocytes associated with hepatocanalicular cholestasis. **b.** Steatosis with random acidophilic degeneration of hepatocytes (*arrows*). **c.** Periportal hepatocellular necrosis (PT = portal tract, necrotic area is outlined by arrows). **d.** Edge of infarct (inf).

patients, this did not correspond to ischemic damage present within the graft.[91]

Regenerative activity becomes evident within the first 2 to 3 days post-transplantation. The microscopic findings in general reflect the degree of injury. Mild lobular disarray with pseudorosetting of hepatocytes, nuclear enlargement with prominent nucleoli, and hepatocellular mitosis are indicative of mild regenerative changes. Phagocytosis of cellular debris with Kupffer cell hypertrophy is also observed.

Prominent ductular proliferation at the edge of the limiting plate surrounded by a mild predominantly polymorphonuclear inflammatory infiltrate (cholangiolitis, Fig. 3) may be the result of severe injury with extensive necro-



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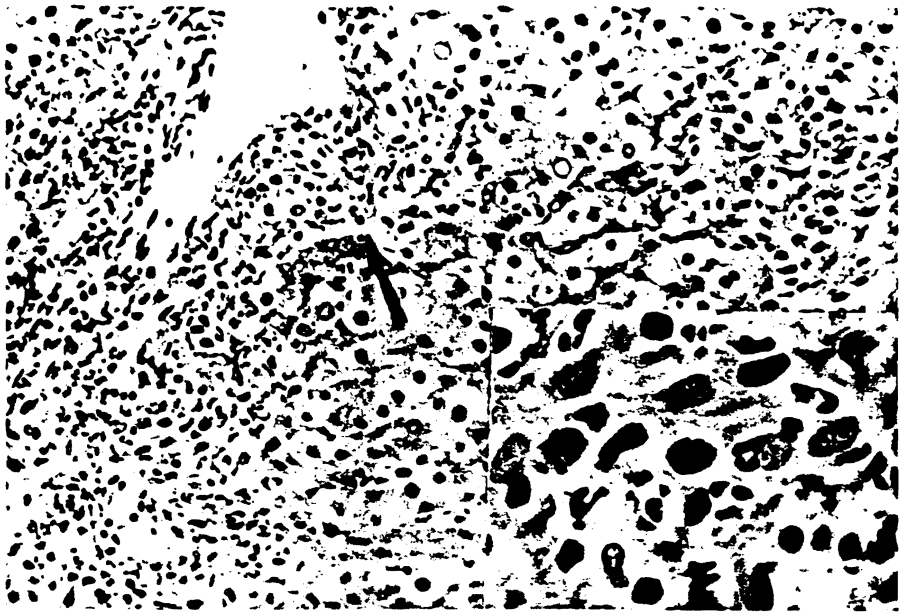

Figure 3. Cholangiolitis associated with repair of harvesting injury. Note the cholangiolar proliferation at the edge of the limiting plate surrounded by neutrophils (*inset*). This histologic picture can be easily confused with the early changes of duct obstruction and/or ascending cholangitis (see text).

sis. This may be accompanied by areas of infarction, abscess formation, bile leaks, and graft failure. Cholangiolar cholestasis may also develop in severe ischemic injury simulating large duct obstruction[14,15] or cholangitis complicated by sepsis, which was frequent in early animal models[16] and in humans.[4,8] A useful histologic criterion for distinguishing ischemic injury from duct obstruction and cholangitis is the presence of significant hepatocellular injury, i.e., centrilobular coagulative necrosis, seen in harvesting injury, whereas cholangitis, rather than cholangiolitis, is seen in duct obstruction. Useful indicators of the degree of harvesting injury as well as of the presence of duct obstruction, other than the histopathology, include the surgeon's opinion of the viability of the graft at the time of implantation, the rising or falling trend as well as the level of cytosolic hepatocellular enzymes immediately and shortly after transplantation,[13] the presence of T-tube bile drainage, the results of sonographic and cholangiographic studies of the graft, and, obviously, the timing of the biopsy. However, these two processes—ischemia and cholangitis with sepsis—may be intimately related.[7,17,18]

Findings in follow-up biopsies done on patients whose initial specimen demonstrated harvesting injury depend on the balance between the injury and regenerative capacity of the graft. Biopsy of livers manifesting changes of mild injury show quick resolution with minimal residua. Those with a more severe insult may show a gradual normalization of the biopsy findings usually

over a period of 2 to 3 weeks if the initial damage permits orderly restructuring of the hepatic architecture. This implies only mild intralobular collapse of the reticulin architecture and modest cholangiolar proliferation. The hepatocellular ballooning may, however, persist for several weeks and the hepatocanalicular cholestasis for 1 to 2 months. If the centrilobular reticulin pattern is intact, but only collapsed due to hepatocyte dropout, then regeneration is rapid and complete. If, however, the initial damage is accompanied by more severe interlobular architectural collapse and cholangiolar proliferation, graft failure or residual damage such as pericentral and portal fibrosis may develop as a consequence.

Although the histologic damage may appear quite severe, it is important to stress that histology can overestimate the damage and underestimate the potential for repair, especially when clinical and biochemical indicators of liver function are improving. One must also realize the subcapsular sampling problem can further confound the picture.[13]

The pathophysiologic mechanisms which lead to the morphologic manifestations of graft injury related to harvesting are yet to be precisely defined. The pathologic changes are similar to those described in other studies of liver allografts (as well as in nongrafted livers), injured by a variety of mechanisms.[15,19–23] Several clinicopathologic studies of liver damage due to the toxic shock syndrome and heat stroke[21,22] which manifest similar pathologic alterations have, in addition to ischemic injury, suggested a role for bacterial endotoxin. Brettschneider et al[17] documented colonization of the hepatic parenchyma, portal vein and peripheral blood, and bile with organisms from the intestinal tract in ischemically damaged animal liver allografts. These findings offer a reasonable explanation for the presence of both cholangiolitis and cholangitis associated with ischemic injury. Starzl points out the need to protect the ischemically injured graft from bacterial seeding by the use of appropriate antibiotic therapy and from rejection using immunosuppressive therapy.[7,17,18]

Although many factors may be involved in harvesting damage, it is apparent that the injury related to ischemia, bacterial seeding, and the operative procedure play key roles. This is further supported by the observation of similar findings in allografts which have undergone arterial thrombosis or severe hypotension after initial satisfactory function. Although the pathologic changes of ischemic damage may not be absolutely diagnostic, their separation from those of coexistent rejection can be recognized. The most important distinguishing feature is the predominantly mononuclear nature of the infiltrate seen with early or acute cellular rejection (see section on Rejection). Therefore, the biopsy in the early postoperative phase can be of great value in distinguishing between rejection and harvesting changes and may be useful in guiding further diagnostic studies or theraputic intervention (Fig. 4).

Whether coexistent toxic insults from pharmaceuticals play an additional role is uncertain since in the early postoperative period these patients have been exposed to anesthetic agents[24] and are receiving intravenous ste-

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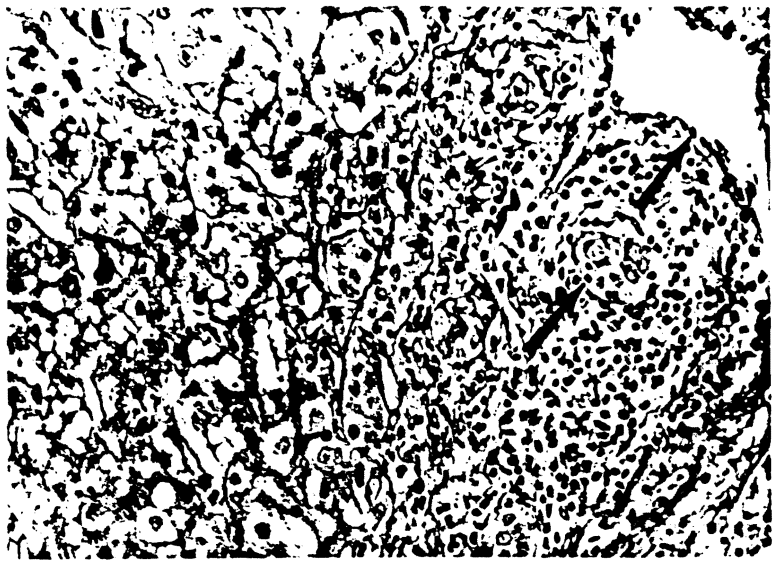


Figure 4. Harvesting injury with early cellular rejection. There is residual lobular steatosis and ballooning with mild ductular proliferation as features of harvesting injury. Note however, the mononuclear predominance in the portal infiltrate along with early subendothelial and bile ductular infiltration (*arrows*).

roids,[24–26] cyclosporine,[27] and antibiotics.[24] However, similar changes are not consistently observed in biopsies from patients treated with supplemental intravenous steroids for a rejection event temporally remote from the operative procedure and not complicated by ischemia. Therefore, although one cannot entirely disregard toxic influences, it seems clear that factors related to prolonged ischemia and the operative procedure are major etiologic factors in harvesting injury.

Rejection

Rejection of an allografted organ has been broadly defined as an "immunologic" reaction against the graft with the potential to lead to graft damage and eventual failure. Defining the histologic characteristics of such a reaction in the liver has been a particularly difficult task since many disease processes affecting the liver can be broadly defined as "immunologic" and a myriad of technical complications may be seen.[5,7–9] Therefore, up to the present, many of the pathologic lesions attributed to rejection have been reinforced by clinical observations in the recipient so that the diagnosis of rejection has been based on the exclusion of alternative reasons for graft dysfunction combined with a consistent hepatic histopathology.

The primary antigenic targets of rejection reactions in any organ are the major histocompatibility complex (MHC) antigens, which are cell surface glycoproteins expressed on the cytoplasmic membranes of certain cellular sub-

sets within an organ.[28] Other tissue-specific antigen systems such as those encountered on endothelial cells[29,30] and bile ductules may also play a role. The MHC antigens are broadly divided into classes I and II, which are thought to be important in cytotoxic T-cell reactions and proliferative mixed lymphocyte reactions, respectively.[25] Both classes may serve as targets for cytotoxic cells.[31,32] It is known that class I antigens are expressed to at least some degree on all nucleated cells,[28] although the density of antigen expression may vary considerably, as is seen in the liver.[33] Normally (nonallografted, nondiseased livers), class I antigens are strongly expressed in endothelial, reticuloendothelial, and bile ductular epithelial cells and only very weakly on hepatocytes. Class II antigens are expressed strongly on sinusoidal cells, endothelial cells of small capillary-sized vessels, and dendritic cells which may be closely associated with bile ductules in the portal tracts. Following transplantation, class I antigens are induced on hepatocytes and enhanced on bile ductules, and class II antigens become detectable on the larger vessel endothelial cells and in biliary epithelium.[34,35] This induced MHC expression may result in enchanced immunogenic potential since antigen density on targeted cells is thought to play a role in recognition.[31,32]

Effector mechanisms mediating allograft rejection encompass a variety of immunologic pathways mediated by mononuclear cells, immunoglobulins, complement, coagulation proteins, and platelets.[32,36] Although antibody and complement deposition (humoral rejection) have been seen in animal and human liver allografts during rejection, their role in mediating graft destruction is thought to be less important than in the kidney.[37] Porter emphasized their appearance after, rather than before, damage mediated by cellular rejection.[12,38] Undoubtedly as more experience becomes available, the role of humoral factors in hepatic rejection will be more precisely defined. The following discussion of the morphologic findings will be limited to cellular rejection that occurs under the influence of cyclosporine and steroid maintenance immunosuppressive therapy.

Hyperacute Rejection. The diagnosis of "hyperacute rejection" of an hepatic allograft has not been made in this series of patients despite the presence of transplantation across major blood group incompatibilities or positive lymphocytotoxic cross-matches. Some ischemically damaged livers may show marked deposition of immunoglobulins.[13] Several patients have been identified who have undergone an accelerated deterioration of graft function (<1 week) which appears to have been immunologically mediated. However, staining for immunoglobulin and complement deposition was either negative or difficult to interpret. Clearly more investigational work is needed in this area.

Acute Rejection. The earliest changes observed during a rejection reaction within the liver have been extensively documented by Porter and others.[11,12,39-44] Mononuclear cells accumulate in the interstitium of the portal tracts often immediately beneath swollen endothelial cells of capillary-sized

vessels, small veins, and near connective tissue dendritic cells. The endothelial cells may be lifted away from the underlying connective tissue. This usually occurs between 7 and 10 days and 2 to 3 months post-transplant in the immunosuppressed host but has been observed as early as 3 to 4 days. The early lesion may be very focal, making the early diagnosis of rejection susceptible to sampling error. The infiltrate consists of an admixture of large (blastoid) and small lymphocytes, some of which have plasmacytoid cytologic features, monocytoid cells, and lesser numbers of neutrophils and eosinophils which accumulate in the portal tracts and lead to portal expansion. Immunologic staining for phenotypic characteristics has shown that the majority of infiltrative cells express T-cell antigens as would be expected, although B-cells, macrophages, neutrophils, and eosinophils are also present.[34] The number of eosinophils varies but can be quite striking, and may simulate an allergic drug reaction.[24] The portal inflammatory cells are seen within the basement membrane and occasionally within the lumen of bile ductules. Ductular cell hyperplasia with enlargement of the cells and an increase in the nuclear to cytoplasmic ratio is the most frequent finding in specimens with rejection obtained within 2 months of transplantation; however, frank biliary epithelial cell damage is also coexistent. The degenerative changes seen in the biliary epithelium are subnuclear vacuolization, nuclear pyknosis or karyorrhexis, eosinophilic degeneration or frank luminal disruption. A proliferative response with neocholangiolar formation at the edge of the limiting plate is not a prominent feature, even in the face of extensive ductular damage. Although in early episodes of rejection the limiting plate is generally intact, "spillover" of the mononuclear cells into the periphery of the lobule with periportal hepatocyte necrosis can also be seen. Scattered mononuclear cells similar in appearance to those seen in the portal tracts can also be seen within the sinusoids or traversing the space of Disse. Mononuclear cells may also be seen beneath and around the endothelium of central veins and may be associated with centrilobular hepatocyte necrosis. Concomitant ischemia before or associated with rejection[7,38] may also contribute to the hepatocellular injury in the periportal and centrilobular areas or, alternatively, the periportal and pericentral hepatocytes are uniquely susceptible to a variety of insults.

Medium-to-large vessel damage is occasionally present but is found predominantly in the hilar vessels which are not present in biopsy specimens. Inflammatory vasculitis of veins or arteries, with or without associated fibrinoid necrosis and thrombosis, can occur but is not uniformly present. Arteriolar inflammation and necrosis are uncommonly detected in biopsy specimens. Inflammatory cell infiltration around and into peripheral nerves trunks in the hilum may be related to the expression of MHC antigens by these structures.

The histologic grading of acute cellular rejection is based on a subjective impression of the degree of exuberance of the portal inflammation combined with the presence or absence of centrilobular hepatocellular necrosis present in the biopsy (Figs. 5 and 6). It is difficult, however, to predict the degree of elevation of serum liver enzyme levels based on the histologic grading. It also

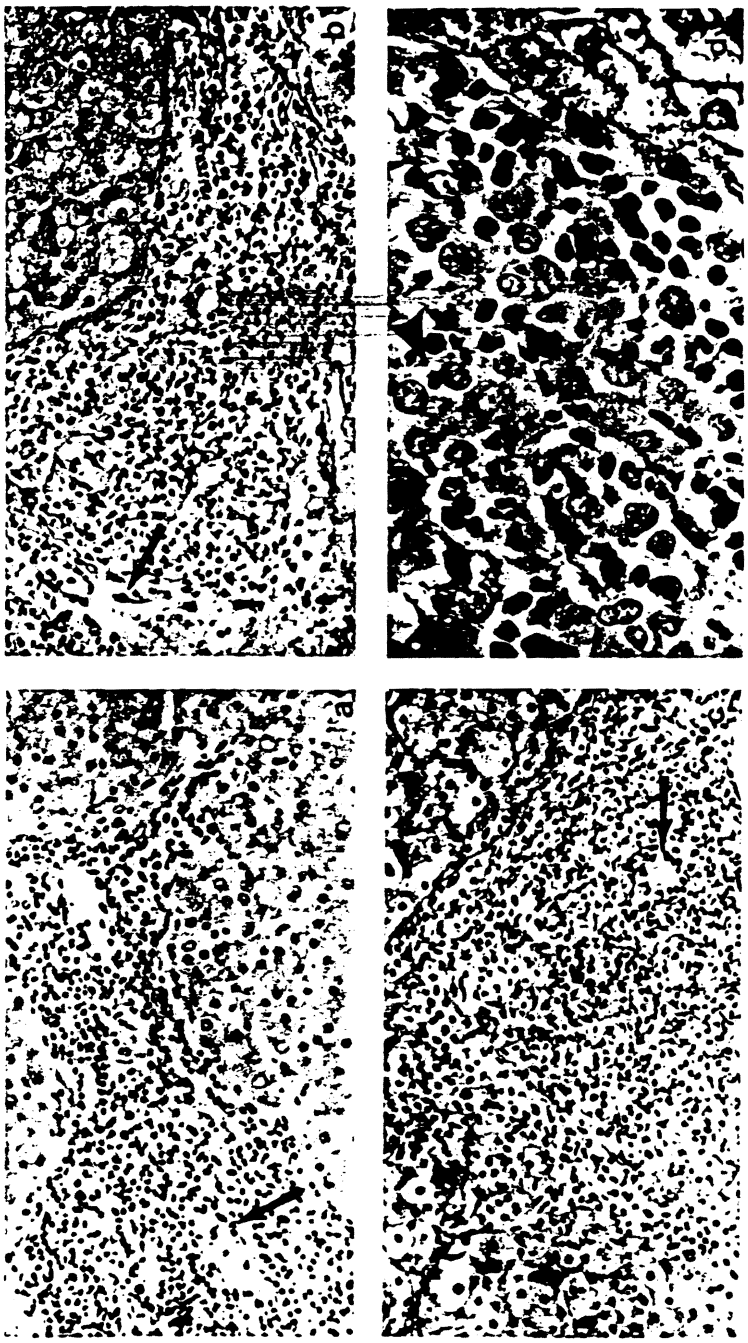
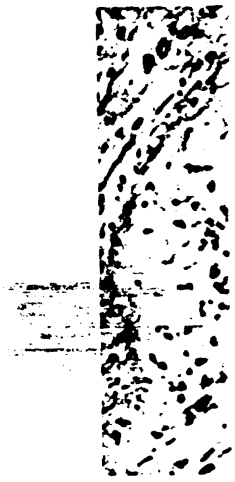


Figure 5. Histologic features and grading of acute cellular rejection: mild (**a**), moderate (**b**), and severe (**c**) acute cellular rejection, in the last of which there was portal-to-portal linkage and centrilobular necrosis. Note the subendothelial and intraductal mononuclear inflammatory cells (*arrows*) associated with endothelial and biliary epithelial cell hypertrophy and reactive atypia (*arrowhead*) (**d**).



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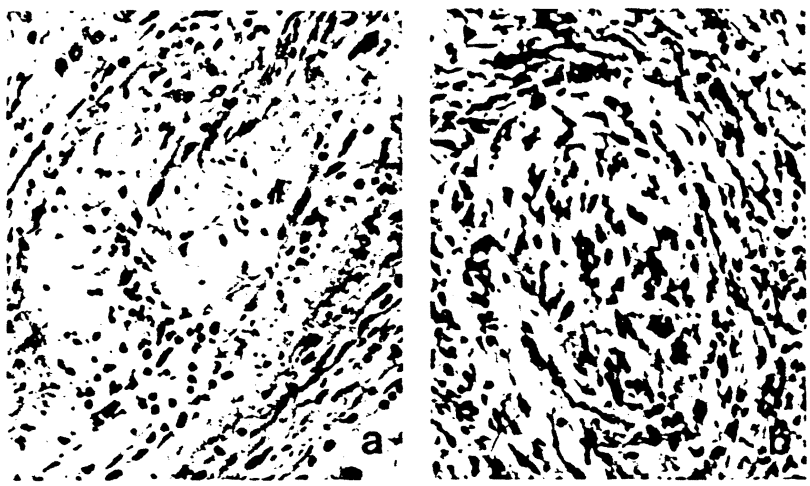

Figure 6. Additional features of acute cellular rejection. **a.** Acute arteritis, a rare finding in biopsy specimens, and although more frequent in failed grafts this feature is not common. **b.** Infiltration of hilar nerve trunk.

Figure 5. Histologic features and grading of acute cellular rejection: mild (**a**), moderate (b), and severe (c) acute cellular rejection, in the last of which there was portal-to-portal linkage and centrilobular necrosis. Note the subendothelial and intraductal mononuclear inflammatory cells (*arrows*) associated with endothelial and biliary epithelial cell hypertrophy and reactive atypia (*arrowhead*) (d).

appears that in most instances, regardless of histologic grading, acute cellular rejection is reversible with bolstered immunosuppressive therapy. This is particularly true with the advent of monoclonal antibody therapy used to treat acute cellular rejection.[45] A retrospective review of hepatectomies for refractory rejection did not show that "severe" cellular rejection was a necessary antecedent (see section on Chronic Rejection).

Rebiopsy after a period of 1 to 2 weeks following successful treatment of rejection shows a diminution of the inflammation, particularly the mononuclear cell fraction with residual neutrophils, plasma cells, and eosinophils; mild portal edema; mild ductular proliferation with or without ductal cholestasis; and at times a mild increase in portal connective tissue. The endothelial, biliary epithelial, and portal tract connective tissue cell nuclei may remain prominent. The findings in biopsy specimens obtained during or after treatment of suspected rejection may easily be confused with the early manifestations of biliary tract obstruction or acute cholangitis because of the paucity of lymphoid cells while other changes persist. However, the presence of hypertrophic endothelial and epithelial cell nuclei and a portal tract infiltrate weighted toward plasma cells and eosinophils with a lesser number of neutrophils are suggestive of prior treatment of rejection. Therefore awareness that recent antirejection therapy has been administered before obtaining the biopsy is critical to interpretation and cannot be overemphasized. A diagnosis of "partially treated rejection" is rendered on those biopsies in which a previous diagnosis of rejection is followed, on a subsequent biopsy, by a picture of diminished cellularity but still having the endothelial and epithelial changes (Fig. 7). We are not able to make prognostic inferences about further poten-

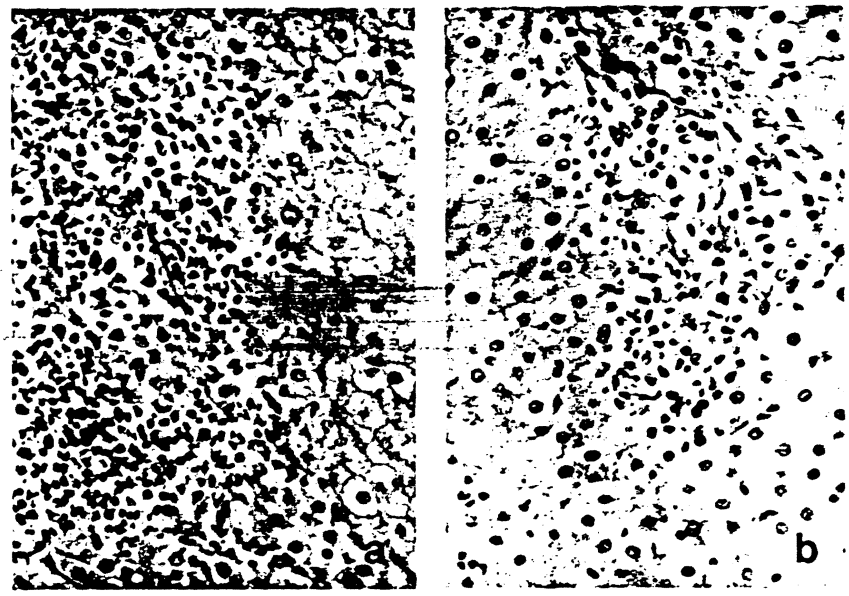

Figure 7. Treatment of acute cellular rejection. **a.** Mild-to-moderate acute cellular rejection (10 days post-transplant). **b.** Rebiopsy after 15 days of treatment with OKT3 monoclonal antibody (Ortho Pharmaceuticals, Raritan, NJ). Note the diminished portal cellularity, with residual endothelial and epithelial changes.

tial responses to antirejection therapy based on the response seen in the biopsy.

We have seen three cases where there has been objective improvement in the histologic features of an acute rejection episode without any alteration of the immunosuppressive therapy. Also, incidental biopsies taken during abdominal surgery have shown prominent rejection changes with a relative paucity of clinical or biochemical manifestations of liver injury. Long-term follow-up of these confounding instances is lacking and their meaning is not clear. Identical observations have been made in experimental animals and in several human liver allograft series.[3,42,46,47]

Chronic Rejection. Although the separation of allograft rejection into acute and chronic forms may be somewhat artificial and established by convention, there are some differences in the histologic appearance of rejection episodes. These different forms of rejection cannot be separated solely on a chronologic basis. However, in general, those episodes occurring within the first 2 postoperative months generally appear as described above (see section on Acute Cellular Rejection) and those occurring at later time periods[39–41,43] as described below for "chronic rejection." Many of the antigenic targets in the graft are the same since the bile ducts, endothelial cells, and hepatocytes remain of donor origin and continue to express foreign MHC antigens[48] (un-

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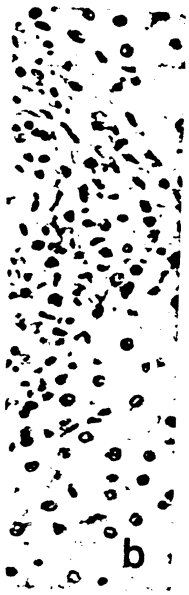

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The morphologic features in "chronic rejection" can best be described by comparing and contrasting the appearance of this type of rejection with those of acute rejection. The characteristic lesion consists of the following triad of morphologic findings: (1) a mild-to-moderate mononuclear portal tract infiltrate, (2) damage and loss of small bile ductules, and (3) arteriolar thickening or hyalinization or large vessel changes as described below. Subendothelial inflammation of portal venules is less striking. Adherence to the presence of the complete triad of findings is helpful since lack of even one may diminish the diagnostic sensitivity of the biopsy. Note, however, that the diagnosis of chronic rejection can be made in the absence of a significant lymphoid infiltrate, particularly when the other changes, i.e., duct loss and vascular lesions, are present. Features of chronic rejection are shown in Figures 8 and 9. The composition of the infiltrate differs somewhat from that found in earlier episodes of rejection in that there are fewer large or "blastoid" lymphocytes, neutrophils, and eosinophils with some subjective increase in plasma cells. In fact, if a significant number of "blastoid" lymphocytes are present in a biopsy obtained later than 2 months post-transplant, one must consider the possibility of discontinuation of immunosuppressive therapy, Epstein–Barr virus (EBV) or CMV infection, or a lymphoproliferative disorder (Fig. 10).[49] Over a period of time which varies from patient to patient, the portal tracts may become devoid

Figure 8. Chronic rejection. Failed graft (4 months post-transplant) demonstrating portal tract (PT) lacking bile ductules, secondary centrilobular hepatocanalicular cholestasis, mild hepatocellular ballooning, and lack of "true cirrhosis."

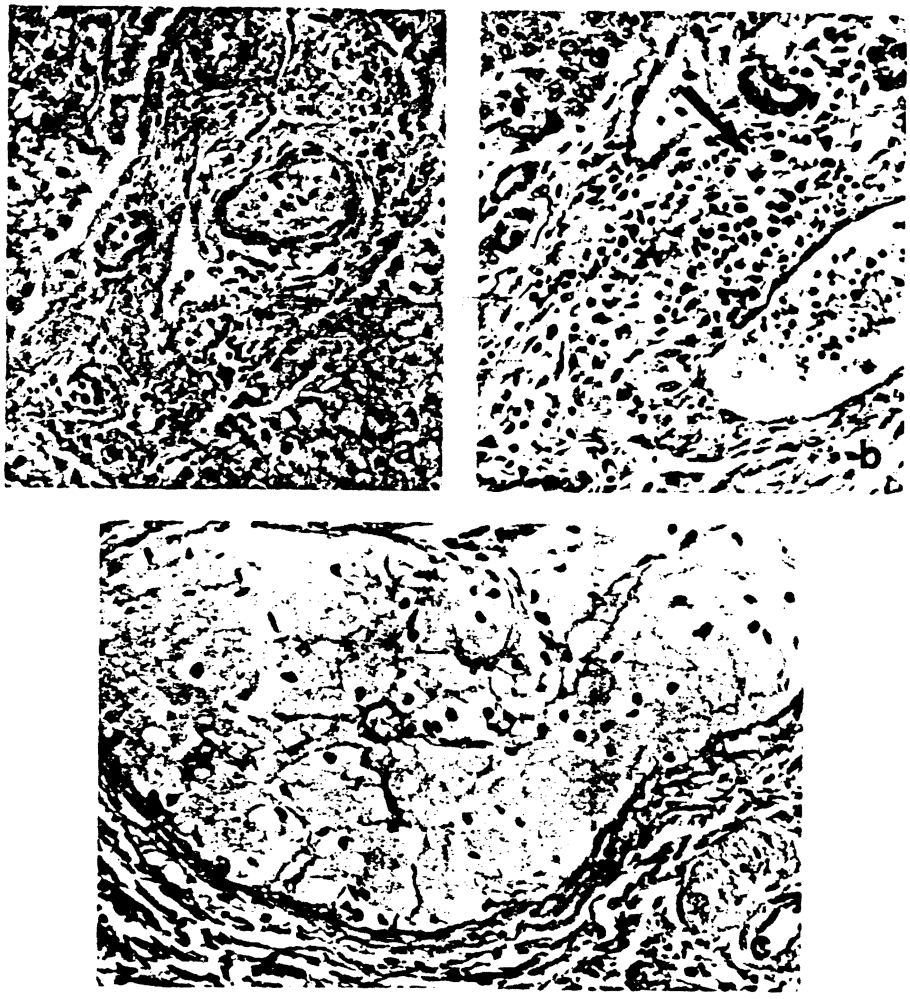

Figure 9. Chronic rejection. **a.** Needle biopsy (10 months post-transplant). **b, c.** Failed allograft (13 years post-transplant). Note the mild portal inflammation in **a** and **b**, the residual biliary epithelial cells in **b** and marked luminal narrowing of a portal vein by subintimal foam cells in **c**. Identical chronic vascular lesions can be seen in arteries, most often in hilar sections of failed grafts.

of small ductules and slightly expanded by fibrosis. A true portal-to-portal cirrhosis with inflammatory cell activity at the edge of the limiting plate is rarely seen in end stage rejection. The limiting plate may have a "moth-eaten" appearance due to the lining up of inflammatory cells near the edge of the lobule. However, extension of the inflammatory cells deep into the lobule with extensive piecemeal necrosis is not a prominent feature. Lobular alterations include mild regenerative changes, slight anisocytosis and anisonucleosis.

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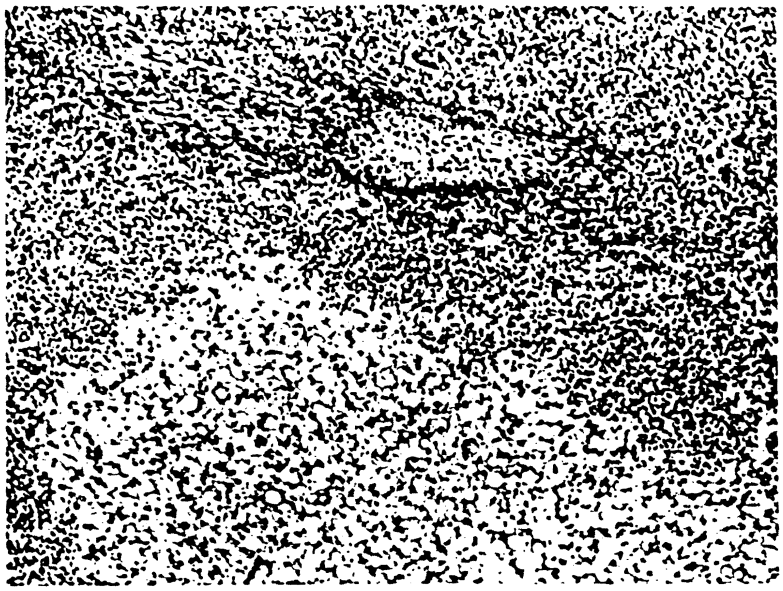

Figure 10. Post-transplant lymphoproliferative disorder involving the liver. Failed graft (13 years post-transplant), note the massive portal expansion by a relatively monomorphic population of large lymphoplasmacytoid cells. The infiltrate overruns the portal landmarks.

Kupffer cell prominence, focal mild acidophilic hepatocyte dengeneration, a progressive secondary centrilobular hepatocanalicular cholestasis, atrophy of the centrilobular hepatocytes resulting in an apparent widening of the sinusoids, and randomly distributed small clusters of sinusoidal foam cells. A fine perisinusoidal and central vein fibrosis is also frequently present.

A useful guide for the determination of loss of bile ductules associated with late cholestasis can be derived from Nakanuma and Ohta's[50] histometric study of normal livers compared to those with primary biliary cirrhosis. Normally, ductules and arterioles run in parallel in the portal tract. Arteries with a lumen less than 95 microns are accompanied by ductules of a similar size within a radius of three times the arterial diameter 70 to 80 percent of the time. Utilizing these figures for a normal adult control population one can get some idea as to the presence or absence of ductular loss.

The findings described above may be quite subtle and require close histologic examination but nonetheless predict a progressive graft deterioration refractory to bolstered immunosuppression and eventual graft failure. Surprisingly, in allografts removed after longstanding chronic rejection, the inflammatory infiltrate may be inconspicuous. However, one also observes a paucity of ductules. Perhaps one of the prime antigenic targets has been removed.

Vascular changes found in the hepatectomy specimen of grafts after long-

standing and indolent rejection include arteriolar thickening, fibrointimal hyperplasia, and deposition of subintimal foam cells in hilar arterial and venous channels. The arterial changes may be severe resulting in marked luminal narrowing with vascular compromise to structures supplied by the arterial tree. Deterioration of the septal bile duct walls with sloughing of the epithelium has been seen in association with these chronic vascular lesions. The vascular lesions may therefore contribute to loss of biliary epithelium and, possibly, biliary stricture formation.

In contrast to the situation outlined for acute cellular rejection, the presence of histologic findings consistent with chronic rejection usually correlates well with the presence of an obstructive serum liver enzyme pattern. Also, chronic rejection is less responsive to bolstered immunosuppressive therapy, suggesting that some of the immunologic damage is irreversible.

Considerable overlap exists in the morphologic patterns of rejection outlined above, particularly partially treated and chronic rejection, and therefore when reviewing biopsy specimens one may not be able to precisely categorize each one. However, an estimate of the degree of potentially irreversible damage (i.e., loss of ductules, arterial sclerosis, fibrosis) and potentially reversible (i.e., actively destructive cellular infiltrate) is useful information for guiding immunosuppressive therapy. Adding to the complexity of the situation is the fact that cases categorized as "chronic rejection" may be seen in the first several months post-transplant and those diagnosed as "acute rejection" much later.

Donor-Derived Hilar Lymph Nodes

Hilar tissues in failed allografts often contain enlarged lymph nodes, particularly in grafts that failed during the first month. The nodes can be identified as being of donor origin by virtue of their location and by immunologic staining using type-specific anti-MHC antibodies. Anatomically, the afferent vasculature and lymphatics should remain intact after the procedure and therefore be a site of host sensitization to donor immune cells.[51]

Nodes from grafts in residence for a few days show hypertrophy of the sinusoidal lining cells and endothelial cells of the paracortical high endothelial venules (HEV). There is dilatation of the peripheral and nodal sinuses, which contain edema fluid, large lymphoid cells, pigmented macrophages, some with phagocytized debris, and neutrophils. Thereafter, during the first week, large or blastic lymphocytes are observed, particularly beneath and surrounding paracortical HEVs and within the sinusoids. Lymphoid proliferation ensues leading to marked expansion of the paracortex by a variety of immunoblasts, some with plasmacytoid features, smaller lymphocytes, and monocytoid cells. Mitotic activity and immunoblastic transformation may be quite prominent in this area. The endothelial and sinusoidal lining cells may become undermined by the lymphoid cells and lifted from the underlying connective tissue, similar to the findings described in liver rejection. Germinal center formation is either inconspicuous or absent. These findings are similar

to those described by Porter,[11,12] except for the uniform presence of markedly enlarged and active germinal centers which we have not observed. It is probable that the type of immunosuppressive agent is responsible for this difference since our patients receive cyclosporine and Porter described patients receiving azathioprine. Nodal tissue in residence for longer periods of time tends to be much smaller and sometimes shows a relative paucity of lymphocytes and an increase in connective tissue and in the number of sinusoidal reticuloendothelial cells.

Other pathologic changes involving the nodes have included infarction, marked sinusoidal dilatation, and CMV, bacterial, or fungal infections.

Immunoperoxidase staining of the nodes using type-specific anti-MHC antibodies for which there was a donor–recipient mismatch have shown a gradual transition of the lymphocyte population within the nodes from that of donor to that of recipient.[51] Nodal tissue removed from two patients 1 and 2 years post-transplant has shown the basic reticular architecture and vasculature of the node to be of donor origin whereas a majority of the resident lymphocytes were of recipient origin. Interestingly, the basic T- and B-cell segregation of the lymphocytes was similar to that seen in normal nodes.

Functional Studies

Concepts concerning the histopathologic findings associated with hepatic rejection are supported by in vitro functional analysis of T-lymphocytes obtained from needle biopsy and failed allograft specimens. Lymphocytes extracted from specimens showing the histologic features of ischemic injury either failed to expand in culture or demonstrate donor-specific proliferative or cytotoxic activity. However, those diagnosed as rejection uniformly expressed donor-specific reactivity directed at class I and class II MHC antigens which are preferentially expressed on structures targeted by the rejection reaction.[52]

Viral Hepatitis

The morphologic manifestations of viral hepatitidies in liver allograft biopsies are not unlike those described in immunosuppressed patients without liver allografts. The following descriptions of viral hepatitis occurring in allografts are presented in their order of frequency.

Cytomegalovirus. CMV is the most frequently diagnosed viral hepatitis in this population of liver allograft patients and liver biopsy is the definitive means of diagnosis.[53] Clinically, patients with CMV hepatitis present with prolonged pyrexia and cannot be distinguished from patients with rejection. This infection occurs most frequently 4 to 5 weeks after initiation of the immunosuppressive regimen (range 15 to 113 days). It is quite unusual to see CMV earlier than 3 weeks post-transplant, unless the patient has been previously immunosuppressed. Early reinfection, before 7 days, has been seen after retransplantation where the resected allograft was infected.

The diagnosis of CMV hepatitis rests upon the identification of nuclear or

cytoplasmic inclusion bodies, but awareness of the characteristic response to CMV will prompt a search for the virus when inclusions are sparse.

The characteristic histopathologic pattern which should initiate a search for CMV inclusions is the presence of small "microabscesses or microgranulomas" scattered at random throughout the lobule. These are composed usually of small collections of neutrophils (10 to 20 cells) or a mixed collection of inflammatory cells surrounding a necrotic hepatocyte, in which, or in nearby levels, inclusions are found. A somewhat similar pattern of neutrophil aggregates can be seen in bacterial sepsis or subsequent to intra-abdominal surgery, but obviously without the CMV inclusions. In a given section, the number of cells with intracytoplasmic virus exceeds the number of cells with nuclear inclusions, so that it is important to recognize cytoplasmic virus when it is present. Immunostaining for viral antigens, in general, shows more than can be recognized on H&E sections. CMV debris may be recognized in neutrophilic clusters even when inclusions or other evidence of a cytopathic effect are lacking.

Damaged tissue with active rapidly dividing cells such as the reactive or granulation tissue near infarcts, abscesses, and suture lines is also fertile soil for CMV growth. Therefore, when such tissue is encountered a more careful search is warranted.

Subsequent biopsies from patients who initially manifest CMV hepatitis and in whom the infestation by CMV increased, showed that there was little inflammatory cellular reaction to the infected cells. Conversely, in situations where the CMV infestation was less severe in a repeat biopsy, the neutrophilic response to the virus was obvious. The implication is that when the virus is being contained, the neutrophil clusters remove the damaged cells.

CMV and rejection can be diagnosed separately and independently on the same biopsy. The presence of CMV is recognized by the characteristic cytomegalic changes with inclusion bodies and in some instances may be located in portal tract structures. Nonetheless, CMV does not cause portal tract changes mimicking rejection. Rejection on the other hand is recognized by the characteristic portal findings. Although a precise definition of the role each plays in graft damage may not be possible, we generally communicate the "severity" of CMV infection based on the number of inclusion bodies found and the severity of the rejection based on the degree of destructive portal inflammation (Fig. 11).

Treatment of patients whose biopsies manifest changes of both CMV and rejection has been individualized depending on which lesion appeared the most severe. Immunosuppression was curtailed when CMV infestation was high and increased when the infestation was low.[53]

Hepatitis B. All liver allograft patients to date with chronic active hepatitis B (HBsAg positive serologically) before transplantation surviving more than 3 months (eight total) have had recurrence of the B viral infection in the graft. These recurrences occurred over a period of time similar to that seen in

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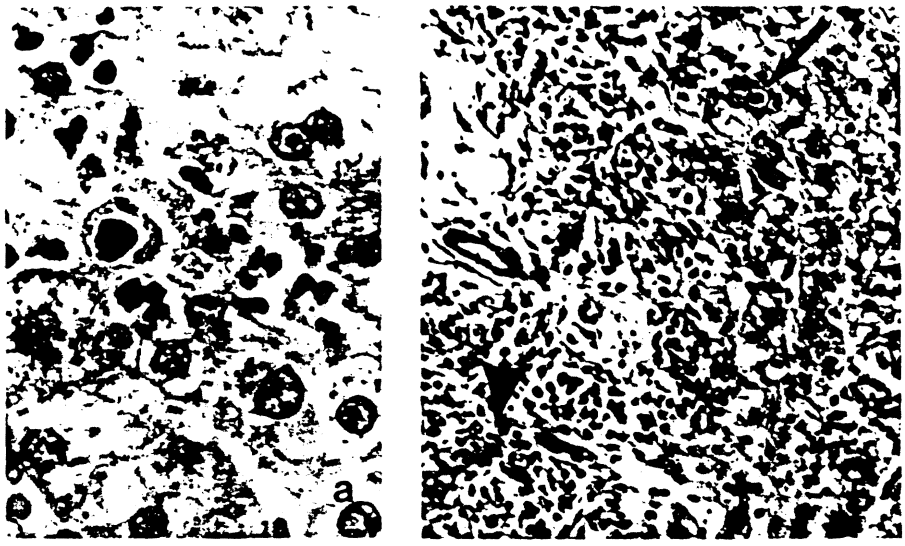

Figure 11. CMV hepatitis with and without rejection. **a.** Needle biopsy (4 weeks post-transplant) showing the characteristic "microabscess" response to CMV-infected hepatocytes. **b.** Needle biopsy (5 weeks post-transplant) showing the portal tract changes of acute cellular rejection with infiltration and damage of the biliary epithelium (*arrowhead*) along with a CMV-infected hepatocyte at the edge of the lobule (*arrow*).

primary acquisition of the disease (longer than 8 weeks). The one exception was a patient transplanted during acute fulminant hepatitis B with massive hepatic necrosis who developed serologic evidence of viral immunity (positive anti-HBs and anti-HBc) postoperatively. A detailed description of these cases with the histopathologic findings has been published elsewhere.[54] The patients most commonly presented with nausea, malaise, jaundice, and an increase of hepatocellular enzymes (ALT and AST) coincident with the reappearance of HBeAg in the serum and HBcAg in tissue. The diagnoses were confirmed by needle biopsies.

The histopathologic manifestations of HBV infection of the allograft liver may lead to a variety of alterations. Three basic patterns of allograft pathology have been seen in association with recurrent HB: (1) prominent lobular disarray, ballooning, inflammation, and necrosis; (2) mild lobular disarray with minimal inflammation and conspicuous acidophilic necrosis of hepatocytes; and (3) moderate lobular disarray with portal inflammation, active piecemeal necrosis, and little to no bile ductular or portal venous damage. Common to all the patterns was the evidence of a preferential lobular insult with minimal to no damage to structures targeted by rejection (bile ducts and venous endothelium) (Fig. 12). Therefore, the appearance is not dissimilar to HB seen in immunosuppressed patients who have not had liver allografts. Immunoperoxidase staining (particularly HBcAg) and serologic studies are absolutely essen-

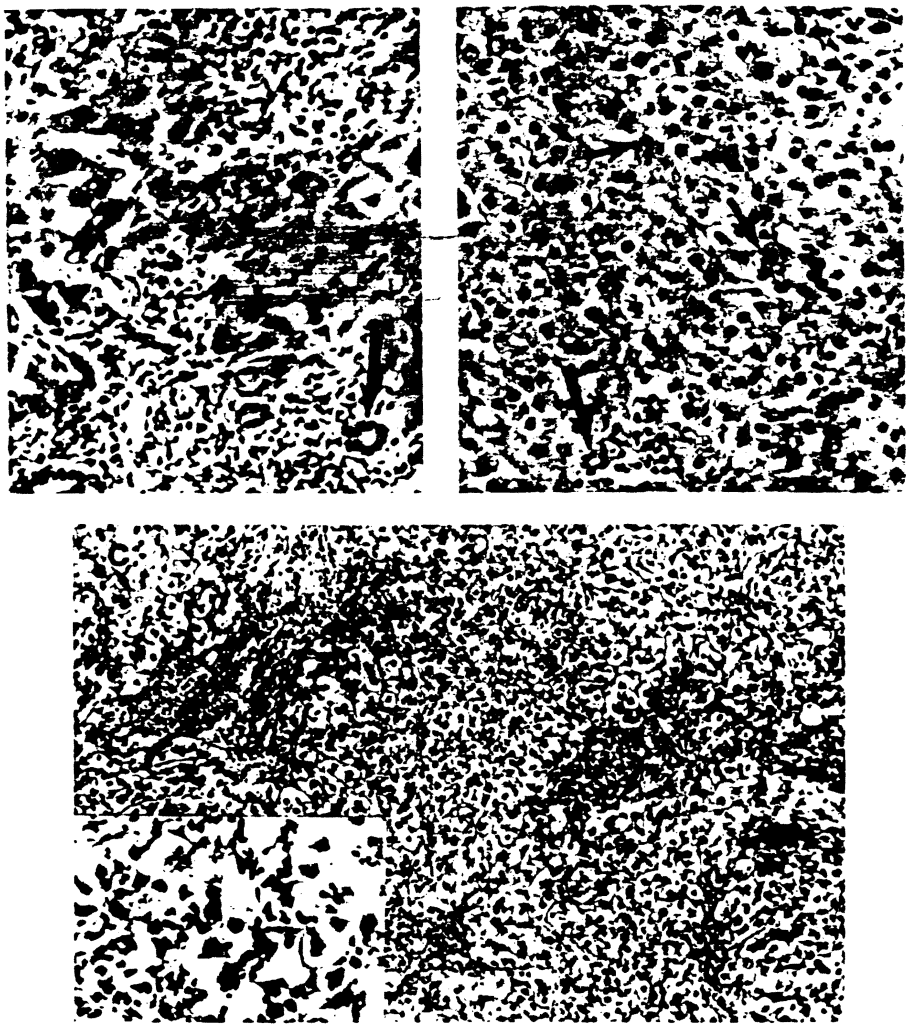

Figure 12. Recurrent hepatitis B in the liver allograft patient. **a.** Needle biopsy (7 months post-transplant) showing typical acute viral hepatitis with prominent lobular inflammation and spotty hepatocyte necrosis. **b.** Needle biopsy (6 months post-transplant) showing individual acidophilic necrosis of hepatocytes (*small arrows*) with little portal or lobular inflammation. **c.** Needle biopsy (10 months post-transplant) showing marked lobular disarray, ballooning of hepatocytes, and inflammatory cell infiltration at the periphery of the lobule surrounding ballooned hepatocytes (*inset*). Common to all the specimens is a preferential lobular or hepatocellular insult with little damage to bile ductules (*large arrows*).

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Treatment of recurrent hepatitis B with increased immunosuppression has resulted in death secondary to sepsis in two cases. In these two cases, the autopsy specimens showed less inflammation than the previous biopsies. Decreased suppression has resulted in acute self-limited graft dysfunction without viral clearing or fulminant hepatitis requiring retransplantation (one case each). Where there has been no change in therapy, the result has been a self-limited acute disease without viral clearing (one case) or maintenance of a low-grade chronic disease in three instances.

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Herpes Simplex Hepatitis. Herpes simplex hepatitis is most frequently seen more than 2 weeks post-transplant and is related temporally to augmented antirejection therapy. The pathologic lesions in biopsy specimens are quite characteristic and similar to those seen in nonimmunosuppressed patients. There are well-demarcated areas of coagulative necrosis of variable size without respect for the lobular architecture. Neutrophils, nuclear debris, hepatocytes with characteristic ground glass intranuclear inclusions (Cowdry type A), and occasional multinucleated giant cells can be seen within and at the periphery of these necrotic zones. Although these findings may be confused with adenoviral hepatitis, immunoperoxidase staining for herpes simplex viral antigens is confirmatory (Fig. 13).

Adenoviral Hepatitis. Adenovirus has been identified as the cause of viral hepatitis in three pediatric patients. The hepatic lesions seen were similar to those described for herpes simplex except for a lesser degree of hepatocellular necrosis and a more granulomatous response. Adenovirus was confirmed by culture and viral particles demonstrated by electron microscopy. CMV inclusions were present simultaneously in one of these cases. One was noted on biopsy and in an allograft resection, but did not recur in the subsequent liver transplant. The others were noted on biopsy, and both of these patients survived (Fig. 14).

Non-A, Non-B Viral Hepatitis. The diagnosis of non-A, non-B hepatitis is quite difficult if not impossible to substantiate at this time in a liver allograft biopsy, because, as is the case in nonallografts, the diagnosis is one of exclusion.[56,57] Nevertheless, we have seen biopsy specimens with a predominantly lobular alteration with no serologic or morphologic evidence of currently identified

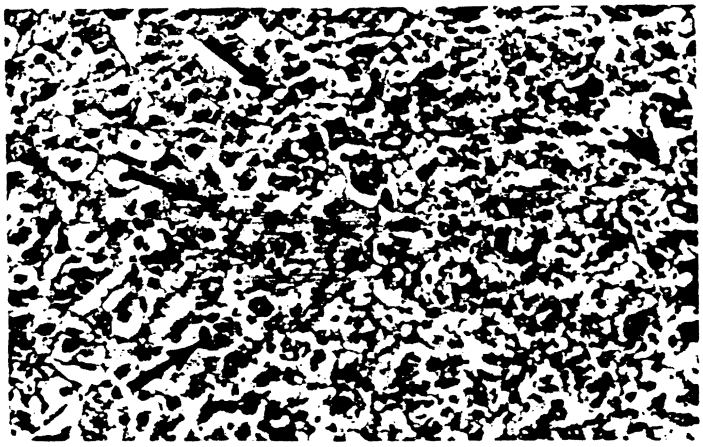

Figure 13. Herpes simplex viral hepatitis. Needle biopsy (5 weeks post-transplant) showing well-demarcated area of coagulative hepatocellular necrosis, which contains neutrophils and cellular debris.

viral liver pathogens or evidence of adverse drug reactions. Several of these patients had chronic active hepatitis with cirrhosis of undetermined etiology as their original disease. The biopsy specimens have shown active piecemeal necrosis without damage of vessels or bile ducts and mild lobular disarray. Prominent spotty acidophilic degeneration of hepatocytes with only a mild inflammatory infiltrate similar to that described with HB has also been seen.

Although undoubtedly the allografted patient is susceptible to this type of viral hepatitis, further identification of the pathogen(s) must be made to substantiate such a diagnosis in this group of patients. Nevertheless, combining experience gained from the study of viral HB with published studies on non-A, non-B hepatitis in nonimmunosuppressed hosts,[36,37] it is likely that the allografted liver would be susceptible to reinfection and potentially therefore to disease. A similar incubation period to the original disease and a preferential hepatocellular or lobular insult by light microscopy is to be expected.

Vascular Thrombosis

The diagnosis of hepatic artery or portal vein thrombosis at this institution has largely been based on a combination of clinical, operative, sonographic, and radiographic findings. The clinical manifestations range from minimal effects to fulminant hepatic necrosis, delayed biliary leak, and relapsing bacteremia.[38] Biopsy pathology may or may not yield a conclusive diagnosis because of the variety of parenchymal changes seen in association with vascular thrombosis. Therefore, although biopsy pathology may be helpful in some instances, the major contribution of pathology has come from an examination of the removed failed allograft.

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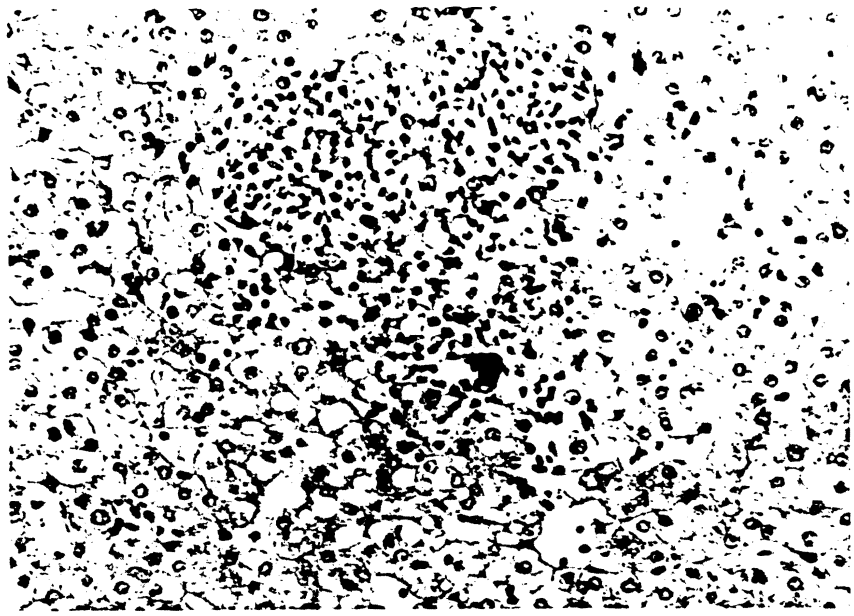

Figure 14. Adenoviral hepatitis (type 5). Needle biopsy (4 weeks post-transplant) with "granulomatous, punched-out" lobular lesion with less hepatocellular necrosis than is seen with herpes hepatitis.

The hepatic artery supplies approximately 30 percent of the oxygenated blood to the hepatic parenchyma but is the major source of blood supply to the hilar and portal tract structures including the arterial walls, major bile ducts, and branches of the portal vein.[59-62] Therefore with compromise of arterial flow one may expect damage to these structures. Also, since the allograft is devoid of nerves and most naturally occurring arterial collaterals, it may be more sensitive to the effects of arterial compromise.

The most common post-transplant vascular accident is thrombosis of the arterial anastomosis or a major branch of the hepatic artery, occurring in approximately 6 to 7 percent of the adult allograft recipients. This occurs most often within the first several months post-transplant and quite commonly within the first 2 weeks. Isolated major portal vein thrombi are much less common (two cases) but can be seen in association with arterial thrombosis. The exact site of initiation of the thrombus is at times difficult to determine precisely but it is frequently found near a site of arterial wall damage such as a mural suture with surrounding granulation tissue, devitalized arterial wall, or traumatic mural dissection. Most, but not all, thrombi seen have been fairly recently deposited as evidenced by a lack of extensive organization, which in general reflects the gravity of the clinical situation. Others have been extensively organized suggesting partial compensation of the deficient blood supply to maintain hepatic function. One young adult

male is currently 4 to 5 months posthepatic artery thrombosis and has retained the graft with adequate function.

Acute cellular rejection may be associated with vascular thrombosis. The flow of both arterial and venous blood through the liver slows during rejection episodes and there is an increase in intrahepatic pressures.[35] Therefore, when acute cellular rejection is occurring within the hepatic parenchyma the possibility of thrombosis at a site of endothelial damage such as a suture line is enhanced because of slowed vascular flow.

Arterial thrombosis may lead to coagulative necrosis of hilar structures as would be expected since the hepatic artery supplies hilar connective tissue, the bile ducts, vein walls, and lymph nodes. There may be infarction of the major bile duct[59,62] leading to bile leaks with digestion of the hilar soft tissues, explaining the delayed biliary leaks observed clinically. The vein walls may also show necrosis and are frequently filled with loosely organized fibrin and leukocyte stasis, which may extend into the small venous radicles. The venous thrombi seen in association with arterial ones are generally less well organized, giving an indication as to the sequence of events.

Pathologic findings within the hepatic lobes may vary considerably from a normal appearance to diffuse steatosis, centrilobular hepatocellular ballooning, atrophy or coagulative necrosis, focal or diffuse infarction. All of these may coexist with cellular rejection of varying degrees. Foci of dystrophic calcification in mummified hepatocytes signify previous ischemic damage. The remaining viable periportal parenchyma or tissue near infarcts may show ductular proliferation accompanied by polymorphonuclear cholangiolitis, with or without bile stasis similar to that seen in association with operative ischemic injury. As mentioned previously, the latter observations may be confused with large duct obstruction with or without cholangitis. However, in ascending cholangitis without ischemic injury coagulative hepatocellular necrosis is not generally observed. Necrotic areas on the other hand, often become seeded with bacterial and fungal organisms forming abscesses, which is not surprising in light of the studies by Brettschneider et al[17] and would explain the septicemia seen in these patients. Therefore, stains for microorganisms in such a situation may be the first evidence of a potentially systemic bacterial or fungal infection, and in that respect may be quite helpful. It is important to point out, however, that because of an uneven distribution of damage throughout the liver, the biopsy process is subject to more sampling error than usual. In fact, a small number of biopsies taken when there has been documented occlusion of the hepatic artery have shown little to no pathologic change. Illustrations of arterial thrombosis and its consequences are shown in Figures 15 and 16.

Biliary Tract Complications

The biliary tract has frequently been the site of complications, i.e., leaks, intrahepatic or anastomotic strictures, early or late obstruction, cholangitis or biliary–vascular fistulas, after liver transplantation particularly in early series

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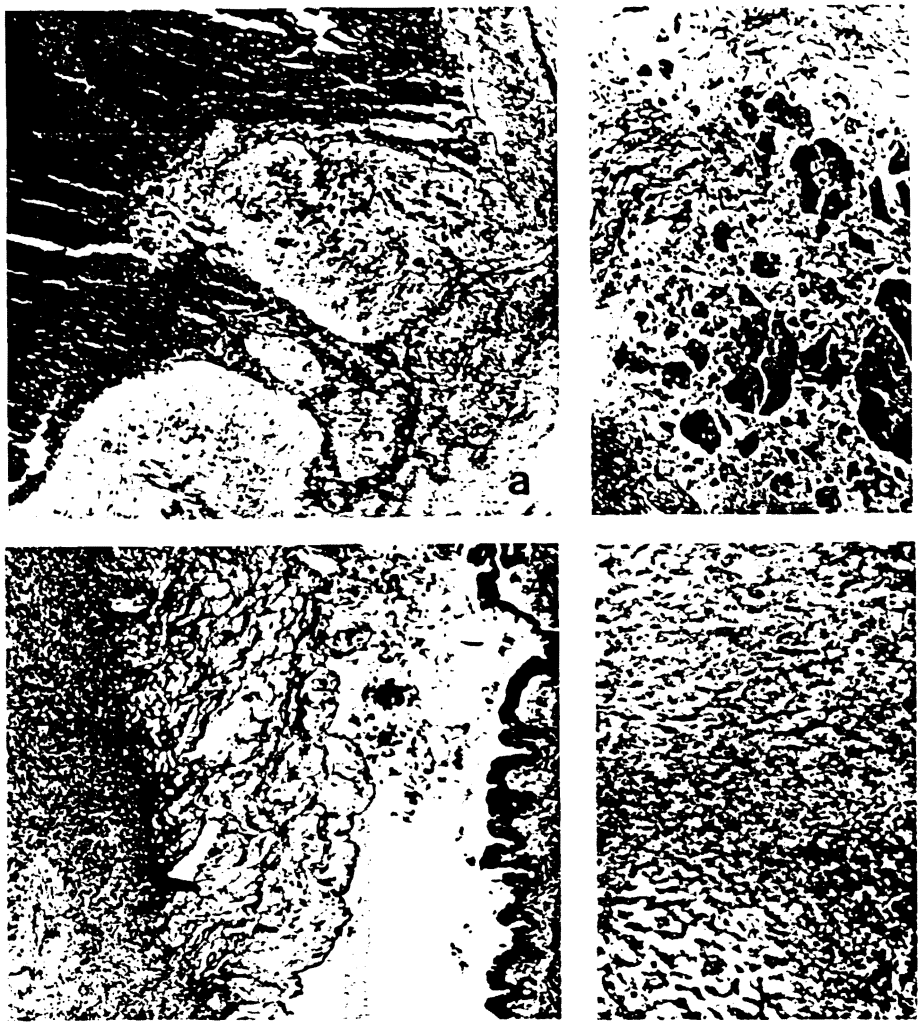

Figure 15. Arterial thrombosis. **a.** Failed allograft specimen with an acute thrombus at the site of vascular anastomosis. **b, c.** Sections of the major bile ducts from the same graft demonstrating necrosis of the duct wall with bile leakage. **d.** Subcapsular section of the same graft showing large parenchymal infarct (*left side of photo*).

of patients.[7,8,63–65] The biliary complications were in large part attributed to failure of surgical techniques.[63–66] Accordingly, as techniques improved,[66] biliary complications have become less frequent. The preferred type of biliary anastomosis seen in this population of patients has been a primary duct to duct anastomosis with a T-tube stent or choledochojejunostomy to a Roux-en-Y loop. The donor gallbladder is removed in both procedures.

The early postoperative biliary tract complications are usually due to

Figure 16. Hepatic artery thrombosis. Needle biopsy (1 month post-transplant) with ballooning of the centrilobular hepatocytes and mild ductular proliferation, cholangiolitis, and bile stasis. Note the similarity to "harvesting injury." The patient retained the graft and rebiopsy 5 months later showed only mild portal fibrosis and centrilobular cholestasis.

technical problems with the surgery or ischemic injury and have been diagnosed clinically or by radiologic procedures.[63–65] Biopsy pathology in these instances has not been the major means of diagnosis; however, when access to the biliary tree is not readily available it may provide useful information in guiding further investigative or therapeutic procedures.

Changes related to large duct obstruction (total or partial due to strictures), acute cholangitis, and biliary–vascular fistula formation are the most frequent complication recognized histopathologically. The morphologic changes associated with these complications are identical to those in nonallograft livers.[14,15] Pathognomonic changes of large duct obstruction in biopsy specimens which may take up to a week of total obstruction to develop[14,67] are not frequently seen since prompt clinical recognition and surgical correction of this problem is essential.[7] In fact, intraoperative biopsies obtained during an operative revision of an obstructed biliary anastomosis have in some instances failed to show evidence of duct obstruction. Nevertheless, several histopathologic findings can be used to identify ductal obstruction or cholangitis and to distinguish it from rejection. The most useful criteria for recognizing large duct obstruction and cholangitis are identical to those outlined by Gall and others[14,15,67] and include bile lakes; suppurative cholangitis: dilated, proliferated, and tortuous ducts with periductal edema and periductal lamellar fibrosis. The predominantly polymorphonuclear nature of the portal infiltrate with the presence of neutrophils within the wall and lumen of bile ducts with periductal edema is particularly helpful in differentiating early duct obstruction or cholangitis from rejection in which the infiltrate has a higher content of mononuclear cells. Augmented immunosuppressive therapy given before obtaining a biopsy alters the morphology and therefore adds to the confusion (see discussion of partially treated rejection). Suppurative cholangitis when severe may lead to duct damage, destruction, and focal abscess formation. Bile and blood cultures may be helpful in identifying pathogenic organisms in these instances. However, bile cultures from T-tube drainage may be positive for enteric bacteria without clinical or pathologic evidence of sepsis or cholan-

gitis. Unfortunately, in some instances, the pathologic findings may not be diagnostic but only be suggestive of duct obstruction or cholangitis. Further assessment of the clinical situation or diagnostic tests such as cholangiograms or ultrasonography must be then performed.

Biliary–vascular fistula formation occurred in three instances and demonstrated quite inconspicuous histopathologic findings if one were not attuned to the abnormal presence of red blood cells within bile ductules.

Later biliary tract complications (>6 months) are usually related to established graft pathology such as arterial compromise and intra- or extrahepatic biliary strictures associated with cholangitis. Although strictures at the operative anastomosis are not entirely unexpected, those occurring in the hepatic parenchyma, some of which are associated with biliary sludge, are more difficult to explain.

Late onset (>6 months) intrahepatic bile strictures associated with biliary sludge were much more common before adopting as the preferred type primary duct-to-duct biliary anastomosis.[64–66,68] Since the switch from cholecystoduodenostomy to choledochocholedochostomy as the procedure of choice, the incidence of intrahepatic bile sludge has decreased dramatically[7,66] but has been seen in one pediatric patient. The biopsy features were those of biliary obstruction (Fig. 17).

Three other patients have developed delayed onset intrahepatic strictures. Evidence of chronic rejection with severe vascular lesions and superimposed thrombosis with multiple intrahepatic abscesses was present in one

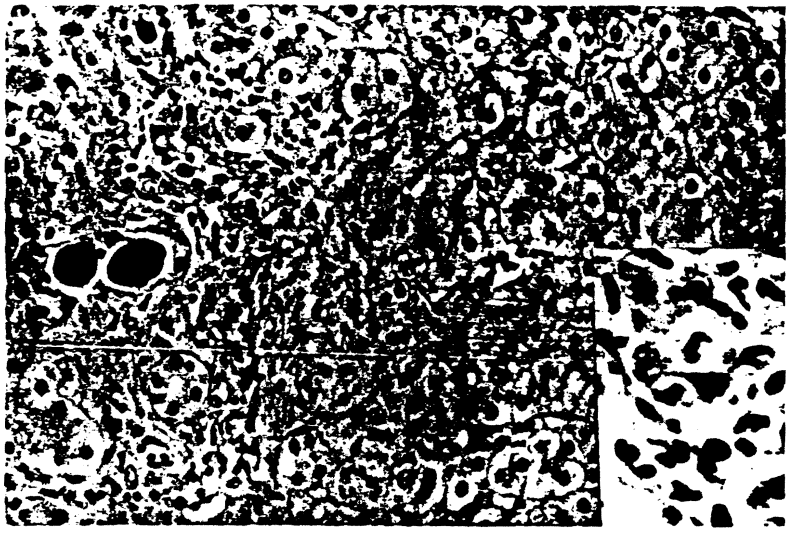

Figure 17. Duct obstruction/cholangitis. Needle biopsy (7 weeks post-transplant) with large duct obstruction. Changes of duct obstruction in the allograft liver are identical to those seen in nongrafts (see text). Note the bile plugs, mildly proliferated ducts and acute cholangitis (*inset*).

patient at autopsy (see section on Chronic Rejection). Another patient was found to have a thrombosed hepatic artery, which was thought to contribute to or cause the strictures. A third patient, whose original disease was sclerosing cholangitis, developed intrahepatic biliary strictures documented by cholangiography. However, the extrahepatic biliary tree was relatively spared in contrast to the patient's original disease. In a series of four percutaneous biopsies obtained over a period of 1 year, there was progressive portal fibrosis: ductular widening and proliferation; mild cholestasis; and, in the last biopsy, a single lymphogranulomatoid inflammatory aggregate near but not involving a septal duct (Fig. 18). There was minimal portal inflammation otherwise. The graft is still functional at this time which precludes a more detailed examination of the organ to determine the possible cause of the strictures. Although recurrent disease is possible, many confounding factors such as ischemic injury make such a diagnosis impossible to confirm.

Recurrent Original Disease

The possibility of recurrent primary disease is of prime consideration in the field of transplantation. Documented recurrences of the original disease in adult patients studied at this institution include HB,[54,69] hepatic malignancies,[70,71] and the Budd–Chiari syndrome.[72] Reported recurrences at other institutions in addition to HB and hepatic malignancies include primary biliary cirrhosis (PBC)[73] and "autoimmune" hepatitis.[74] Recurrence in the pediatric population has not been a problem to date, except for an instance of neurovisceral storage with ophthalmoplegia,[75] in which there was recurrent deposition in the new liver and progressive neurologic disease. In fact a number of metabolic disorders have been "cured," by transplantation: alpha-1-antitrypsin deficiency, Wilson's disease, tyrosinemia, cholesterol LDL receptor deficiency, hemophilia.[76–78]

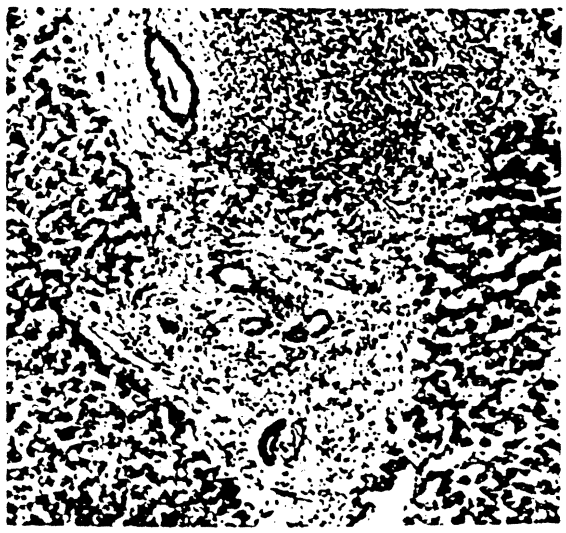

Figure 18. Late onset intrahepatic strictures. Needle biopsy (1 year post-transplant); the patient's original disease was sclerosing cholangitis. Note the paraductal lymphoid aggregate and portal fibrosis (see text).

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**TABLE 4. HISTOPATH
CHRONIC REJECTION**

Granulomas
Lymphoid nodules
Loss of ducts
Cholestasis
Copper deposition
Cirrhosis
Marginal ductular prolife
Chronic vascular lesion
Lobular foam cells
Mallory's hyaline

*Minimal deposition seen ir

ceptor deficiency, glycogenosis types 1 and 4, and Factor VIII-deficient hemophilia.[76–78]

Although HB, malignancies, and Budd–Chiari syndrome are not difficult to differentiate from rejection or complications arising as a result of transplantation, the same cannot be said of PBC, non-A, non-B hepatitis, or sclerosing cholangitis. The difficulty arises because of the apparent pathophysiologic similarities of PBC and chronic rejection,[34,79–82] the lack of a specific marker for non-A, non-B hepatitis and the problem with postoperative biliary strictures for sclerosing cholangitis.

Neuberger et al[73] reported the recurrence of PBC in three patients post-transplantation based on the characteristics of the clinical course, reappearance of elevated serum titers of antimitochondrial antibodies, and the presence of granulomas and increased copper deposition in needle biopsies obtained from those patients. It is of note that the recurrences were documented 3 to 4 years after the procedure. A detailed analysis of 19 patients with PBC as the primary diagnosis in whom tissue specimens were available (from 2 months to 4 years post-transplant), has failed to show a recurrence based on the aforementioned criteria. It is of note, however, that only three of the patients have tissue samples after 2 years. All of the patients had recurrence of elevated titers of antimitochondrial antibodies; however, none of the patients developed pathologic of clinical manifestations of recurrent PBC. Histopathologic features of end stage PBC were compared to those seen in chronic rejection in failed allografts from patients with and without PBC as the original disease. The similarities and differences are shown in Table 4.

If the pathogenesis of PBC is indeed an autoimmune disease with cell-mediated cytotoxicity directed at self-MHC or other biliary antigens,[34,73,51,82] changing the target organ should have some effect on the recurrence of the pathogenic sequence unless the originally recognized biliary or MHC antigens are present in the graft. Also, since the allograft rejection reaction may involve destructive immunologic mechanisms similar to PBC,[34,82] separation of the two processes may be quite difficult. Further confounding the analysis is

TABLE 4. HISTOPATHOLOGIC COMPARISON OF END STAGE PBC AND CHRONIC REJECTION

	End Stage PBC	Chronic Rejection
Granulomas	+	–
Lymphoid nodules	+++	+
Loss of ducts	+++	+++
Cholestasis	++/peripheral	++/central
Copper deposition	++	–/+[a]
Cirrhosis	+++	+/–
Marginal ductular proliferation	++	–
Chronic vascular lesions	–	++
Lobular foam cells	+	+/++
Mallory's hyaline	+	–

[a]Minimal deposition seen in occasional centrilobular hepatocyte.

the fact that other antigen systems (foreign MHC and endothelial) are introduced with the graft making superimposed rejection changes likely. The possibility of an "overlap" syndrome of rejection and recurrent PBC or accelerated duct destruction in PBC patients whose immune system may be sensitized to biliary antigens cannot be disregarded.

We previously suggested the potential difficulty in separating non-A, non-B hepatitis from chronic allograft rejection, especially in light of the reports of the presence of bile duct lesions in the former.[36,37] Although separation of the two processes may not always be possible, the presence of prominent lobular alterations in hepatitis similar to those caused by the B virus may be helpful. Also, the bile duct lesions described in non-A, non-B hepatitis appear to more frequently involve medium-sized ducts associated with a lymphoid nodule, and are reported to be less prevalent and less widespread, i.e., involving fewer bile ducts, than in rejection. Duct loss similar to that seen in chronic rejection has not been described for chronic viral hepatitis.

Drug Toxicities

The essential criteria used for identification of adverse drug reactions (ADR) were summarized by Irey[83] and he presented an algorithm for the application of these criteria. Irey pointed out that "the disease indicator of an ADR should be chosen such that it is not affected by either the basic disease of the patient or by any concurrent comorbid state." One quickly realizes that strict application of this method is particularly difficult in the liver transplant patient when biochemical and morphologic indicators of liver injury are used as the disease marker of an adverse drug reaction (ADR).

Presently, the diagnosis of an ADR is only rarely based on the histopathologic findings present in a liver allograft biopsy.

The histopathologic manifestations of specific hepatotoxic drug reactions are reviewed in detail elsewhere[24] and only those agents which are likely to play a major role in such reactions in liver allograft patients are discussed.

Cyclosporine (Cys) is known to be hepatotoxic in renal,[84] bone marrow,[85] heart,[86] and liver transplant patients[27] as well as in nontransplanted individuals who receive this drug. Most studies report modest hyperbilirubinemia with mild elevations of the transaminases which uniformly resolved after a lowering of the dosage. Unfortunately, all of these studies are based on biochemical evidence of liver dysfunction coincident with elevated blood levels of cyclosporine with no mention of hepatic morphology. Therefore, information about the structural alterations which may be secondary to the drug is limited to individual case accounts and animal studies.[87] Histopathologic findings in humans which may have been secondary to Cys toxicity include cholestasis and random acidophilic degeneration of hepatocytes.[27] Centrilobular steatosis in addition to the above findings have been reported in animal studies.[87]

Clinically, Cys is notorious for its adverse affect on renal function, which along with blood levels and clinical symptomatology serve as guides for therapeutic monitoring.

Glucocorticoids are known to induce hepatic steatosis in man[24] even in relatively small doses (15 to 29 mg prednisone/day). The effects of much larger doses such as those received by transplant patients immediately after surgery and for treatment of cellular rejection are unknown. However, man is reported to be a "relatively glucocorticoid-resistant" species.[25,26] Animal studies on the effect of glucocorticoids on hepatic function and morphology confirm that high doses administered to rabbits, rats, dogs, and mice can produce ballooning, vacuolization, glycogen accumulation, and focal necrosis (in rabbits).[25,26] Whether a similar effect can be seen in man is uncertain and further study is necessary.

Azathioprine and other cytotoxic drugs have been associated with "venosclerosis" within the liver.[55] One must therefore be aware of the potential hepatotoxic side effects of patients receiving these drugs as part of the immunosuppressive regimen.

Other therapeutic agents have been reported to produce histopathologic changes which may appear similar to immunologic or infective complications seen in allograft biopsies. Reference to texts dealing with this subject is suggested.[24]

Hyperalimentation

The morbidity associated with the surgery may necessitate prolonged peripheral alimentation which may produce structural alterations within the liver, which are not significantly different from those described in nongraft livers.[59] The changes include hepatocanalicular cholestasis, cholangiolar proliferation with ductal cholestasis, steatosis, pigment deposition, and sinusoidal fibrosis. It is important to recognize the changes so that the alterations are not imputed to other processes.

THE ROLE OF LIVER BIOPSY IN PATIENT CARE

The liver biopsy has come to play a vital role in patient management during the postoperative clinical course. Not uncommonly, a specific diagnosis for a cause of graft dysfunction can be rendered, which may or may not have been clinically apparent. Alternatively, but less frequently, a clinical diagnosis of graft dysfunction may be apparent without pathologic confirmation. Most frequently, the two are in agreement.

Although the biopsy findings are reliable and can be used to guide therapy and other diagnostic procedures, one must be aware of the limitations of the procedure. Many if not all of the limitations of allograft biopsy are identical to those outlined many years ago for liver needle biopsies in general.[90] The liver may react nonspecifically to a variety of noxious stimuli. This shortcoming is particularly evident when attempting to diagnose drug toxicities in these patients in whom so many other factors are involved which may lead to similar if not identical histologic alterations. Another is the focal nature of

some of the graft syndromes described previously which may lead to sampling problems. This is particularly evident in early rejection which may be quite focal—focal subcapsular infarcts, intrahepatic abscesses, or the changes associated with vascular thrombosis. Also, graft dysfunction which may be detectable by other means may not be demonstrated histopathologically, such as bile duct or vascular obstruction, especially if it is partial or of short duration before biopsy. Perhaps the most significant limitation, which is a consequence of the factors mentioned above, is the rate of false-negative findings on biopsy specimens. Although we have not statistically evaluated the frequency of occurrence of false-negative findings, they are not rare. In these instances, review with the clinical physician is invaluable.

CONCLUSIONS

This review was intended as a general guide to the interpretation of the morphologic representations of the pathophysiologic events that occur in the liver after transplantation. We are far from understanding all the various lesions that are seen in biopsy specimens, particularly those occurring years after the procedure since experience with these types of specimens is limited. Also, knowledge of the impact of the primary disease on the postoperative course is limited. However, we hope this review will prove useful to those pathologists faced with interpretation of post-transplant liver pathology.

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Tumor Cell
Prognosis

S.D. Greenberg,

The incidence of lun
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