Pathology of Liver Transplantation

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INTRODUCTION AND HISTORICAL PERSPECTIVE

Pioneers in the field of hepatic transplantation pathology include Kendrick Porter\textsuperscript{1-2} and Hank Fennel\textsuperscript{3-4} who, while working with Starzl\textsuperscript{5-11}, laid the foundation on which is based much of what is written in this chapter. Important early contributions were also made by Bernard Portmann\textsuperscript{12-14} and Derek Wight\textsuperscript{15-19} who, working with Sir Roy Calne\textsuperscript{20-24} helped to provide additional landmarks on the roadmap for those who would follow. More recent contributors to the field, who now have become too numerous to mention by name, have quickly come to realize that the pathology of liver transplantation covers almost every aspect of hepatic pathology. Just about every disease encountered in a native liver has, or will be, seen in an allograft. Moreover, an allogeneic liver is susceptible to new diseases, such as rejection, or provides interesting twists to the pathology of old ones, such as viral hepatitis. The broadness of the subject makes it difficult to cover adequately in one chapter, although Randall Lee\textsuperscript{25} and Derek Wight\textsuperscript{26} have done marvelous jobs!

The following chapter is based on personal experience in the practice of liver transplantation pathology and a review of the current literature. It is intended primarily for practicing histopathologists and clinically based physicians, who face difficult management problems and decisions daily. A discussion of the perceived role of the pathologist in a hepatic transplantation program is provided because it is somewhat different from that of a classic surgical or tumor pathologist. An overview of pathophysiological concepts is added to provide a conceptual framework that enhances our understanding of the histopathological findings. A brief review of possible clinical presentations is provided because the final histopathological interpretation or diagnosis is so often based on the clinicopathologic background. The diagnostic approaches to the different types of specimens, gross (if appropriate) and histopathologic findings, as well as their differential diagnoses are the main purposes of the chapter. We hope that the format is user-friendly and that any advice or perspectives given are helpful.

ROLE OF THE PATHOLOGIST IN A LIVER TRANSPLANTATION PROGRAM

The anatomical pathologist with a special interest in transplantation pathobiology can be an invaluable member of a large solid organ allograft program. Even at smaller centers or follow-up care facilities, pathologists will often find themselves active participants in, or at the center of, discussions regarding liver allograft candidacy or posttransplantation recipient care. The pathologist, therefore, is first and foremost a medical consultant; proficiency in this role requires a fundamental working knowledge of four basic areas.

A detailed understanding of liver pathophysiology is probably the most important prerequisite qualification. It will enable the pathologists to diagnose correctly original and recurrent disease and posttransplantation allograft syndromes and to anticipate problems that arise as a result of operative procedures or therapeutic interventions. Almost as important is a thorough understanding of clinical management problems and terminology. This allows the consulting pathologist to communicate the histopathological findings effectively and put them into perspective for patient management. Because recipients are immunosuppressed and thus susceptible to a wide variety of opportunistic infections and associated malignancies, recognition of hepatic and systemic manifestations of bacterial, fungal, and viral infections and the varied presentations of virus-associated malignancies is certainly helpful. Last, familiarity with immunopathology comes in handy when conducting and interpreting various immunobiological tests that measure effector mechanisms of allograft damage, such as immunofluorescent staining, crossmatch testing, and functional analysis of lymphocytes, including the mixed lymphocyte response and cell-mediated lympholysis.

In everyday practice, the histopathologist will be asked to render "opinions" given a detailed clinical scenario, a task that some practitioners may find particularly difficult. The following is a typical example. Three weeks after liver transplantation, a recipient becomes septic, necessi-
tating a dramatic lowering or even discontinuance of immunosuppression. Six days later, elevation of liver injury test results prompts a liver biopsy to rule out or confirm the strong clinical suspicion of rejection. Although a straightforward histopathological diagnosis of rejection may easily be rendered, the physician asks the consulting pathologist, "Do you think we can let this rejection go untreated for another couple of days until the sepsis has completely cleared?" Answering this question requires experience with similar situations in the past and willingness to participate in a decision-making process in which the pathologist will, at times, be proved wrong. This situation is foreign and, therefore, particularly uncomfortable for those of us who are used to pathologists' being the "final" checkpoint, responsible for the "correct" diagnosis. More than anything else, a willingness to participate in this type of decision-making process is what makes transplantation pathology different from classic surgical or tumor pathology. Perhaps the irreversibility of malignancy-associated therapy as opposed to the relative reversibility of transplant-related treatment has dictated this difference.

**APPROACH TO TISSUE SPECIMENS**

Tissue triage, diagnostic considerations, and clinicopathological correlation differ with the type of tissue specimen. The approach to each is discussed next.

**Pretransplant Biopsies and Outside Slides**

Most patients undergo hepatic replacement because of cirrhosis developing on a background of chronic inflammatory hepatic disease. Correct identification or confirmation of the origin of disease is most often accomplished by a review of consultation slides of core needle biopsies performed elsewhere. Familiarity with those liver disorders that commonly recur after transplantation and those that are frequently associated with hepatocellular carcinoma assists in recipient selection and determining the need for adjuvant therapy before or after transplantation. A brief clinical history must accompany such slides, including any previous operative or surgical pathology reports. Failure to adhere to these guidelines may result in patient or disease misidentification and the possibility of inappropriate operations. Academic uses of the native liver disease data are entirely dependent on a thorough pretransplantation work-up.

Patients with fulminant liver failure may be referred for transplantation without a firm diagnosis or thorough work-up. In such instances, pathologists may be requested to evaluate frozen sections or "rapidly processed" slides, or both, of the native liver obtained via a transjugular biopsy, in which the liver is intravascularly approached from the superior vena cava. The etiological and prognostic significance of microscopic architectural alterations may then be used to assess the need for transplantation. In some instances, knowing the pattern of liver injury associated with particular etiological agents will enable the pathologists to provide information regarding the potential reversibility and stage of the process. Reference to a standard liver pathology text for further information is suggested. It must be stressed that the pathologist is only part of a patient management team that includes a hepatologist, critical care and anesthesiology specialists, and a surgeon. The team members combine information gained from biopsy histopathological findings with those from liver injury tests and liver synthetic and cerebral function studies, but the recipient surgeon is ultimately responsible for the collation of this information and for the decision about and timing of transplantation.

**Native Hepatectomy Specimens**

In many centers the native hepatectomy specimen first becomes available for pathological evaluation during the early hours of the morning. Immediate tissue processing may on occasion be required to isolate RNA or tissue for measuring enzyme activity to identify the original disease (e.g., tyrosinemia) correctly. Although the majority of native hepatectomy specimens undergo triage the following morning, prompt tissue processing may also be required at odd hours to fulfill research requests for fresh RNA isolates or to establish cell cultures or lines from the native hepatectomy specimen. Such requests are best handled by in-house arrangements between the department of pathology and the investigators.

Access to the entire liver, including hilar and perihilar tissues, with the opportunity to choose grossly abnormal regenerative nodules in cirrhotic livers for microscopic evaluation, will result in significant additions or changes to the original diagnosis in about 10% of cases. Clinically undetected and unsuspected hepatocellular or bile duct carcinomas are not uncommon. Other common changes include the discovery of structural abnormalities in the hilar bile ducts or their accessory glands and the detection of metabolic diseases that were not apparent before transplantation.

Gross examination of native hepatectomy specimens should be done according to a predefined protocol. The hepatic artery, bile duct, and portal vein are identified and opened longitudinally starting at the resection line, and the resection margins are sampled. Any thrombi, vegetation, calculi, strictures, fibrosis, or tumors are noted. Next, the hepatic veins or vena cava, if present, is identified and the resection margins are sampled. The capsular surface of the organ is examined for nodularity and any obvious defects. The gallbladder is opened and routinely sampled. The liver is then serially sectioned in a horizontal plane at 1.0-cm intervals, yielding slices similar to those observed on a computed tomography scan. The precise location of any intrahepatic defect other than regenerative nodules (e.g., tumors, cysts, and abscesses) is recorded.

It is extremely important to slice the liver thinly (at approximately 1-cm intervals) and to sample any regenerative nodule that, by virtue of size or color, distinguishes itself from the surrounding cirrhotic parenchyma. Microscopic sections other than those suspicious nodules or obvious anatomical defects are taken according to a protocol. Routine samples should include a superficial and deep section of the right and left hepatic lobes; a section of the hepatic artery, portal vein and hepatic veins, and bile duct at the resection margins; and a deep hilar section. Bulk frozen, optimum cold-temperature compound embedded, and bulk formalin-fixed tissue are saved from each
case in an in-house tissue bank. Photographs or diagrams, or both, should accompany interesting or complicated cases.

Failed Allografts

The reasons for allograft failure differ depending on the time since transplantation. Statistics of graft failures based on autopsy analysis are often difficult to interpret because comorbid conditions are frequently present. The majority of allografts fail early after transplantation because of "preservation" injury, vascular thrombosis, and humoral rejection, or a combination of these. Acute rejection is a relatively uncommon cause of early graft failure unless immunosuppressive therapy was deliberately withdrawn. Late graft failures (>4 weeks) are due to chronic rejection, recurrent native disease (especially viral), vascular thrombosis, or delayed manifestations of early ischemic preservation injury.

The main goal of the pathological examination of allograft hepatectomy specimens is precise identification of the cause of failure, when possible. An attempt should be made to correlate the pathological findings and previous serial biopsies with the preceding clinical course. A pathological diagnosis of "widespread coagulative necrosis" provides little additional useful information without an accompanying statement or speculation of the cause. The sequence of the gross examination is the same as that used for native hepatectomy specimens. Particular attention, however, is paid to the inspection and dissection of hilar structures because technical problems with vascular and biliary anastomoses account for most allograft failures occurring during the first few postoperative months.

Microscopic sections are taken by the same protocol used in the native livers. They include sections of the "deep hilum," including second- and third-order branches of the hepatic artery, and peribiliary glands may be particularly informative in determining the cause of graft failure. Bulk frozen and formalin-fixed tissue is also saved in a tissue bank.

Posttransplantation Needle Allograft Biopsies

Posttransplantation needle allograft biopsies are the most common type of pathology specimens from liver allograft recipients and are usually obtained to determine the cause of graft dysfunction or to examine its status. Proper triage of the tissue specimen depends on the clinical differential diagnosis, which in turn depends on the time since transplantation (Table 69-1). Although most routine histopathological studies can be completed on formalin-fixed, paraffin-embedded tissues, we prefer that the fresh, sterile tissue specimen be submitted to the pathology department in sterile tissue culture medium, such as RPMI 1640. An information sheet requesting general patient data, results of pertinent diagnostic tests, and clinical differential diagnosis is completed by the clinician at this time. The handling of the specimen is based on this information (eg, culture for micro-organisms, and special fixation). Immunofluorescence staining requires fresh-frozen tissue. Rarely, alternative methods of tissue preservation may be required for particular diagnostic or research purposes.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Timing</th>
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<tr>
<td>&quot;Preservation&quot; injury</td>
<td>Immediate; worst during first week, but dysfunction may persist for 1 - 2 mo if initial insult is severe</td>
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<tr>
<td>Acute rejection</td>
<td>5 - 30 days; occasionally seen later in inadequately immunosuppressed patients</td>
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<tr>
<td>Hepatic artery thrombosis</td>
<td>0 - 4 wk with later increase seen between 18 - 36 mo (see text)</td>
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<tr>
<td>Biliary obstruction or stricturing</td>
<td>Variable</td>
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<tr>
<td>&quot;Opportunistic&quot; viral and fungal infection (eg, CMV, HSV, VZ, EBV, Candida, Aspergillus)</td>
<td>0 - 2 mo</td>
</tr>
<tr>
<td>Chronic rejection</td>
<td>Usually &gt; 60 days, but occasionally seen earlier</td>
</tr>
<tr>
<td>Recurrent or de novo viral hepatitis (eg, HBV, HCV, HDV)</td>
<td>&gt;4 - 6 wk</td>
</tr>
<tr>
<td>Recurrent nonviral inflammatory liver disease and malignancies</td>
<td>Usually &gt; 1 yr</td>
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CMV = cytomegalovirus; HSV = herpes simplex virus; VZ = varicella-zoster; EBV = Epstein-Barr virus; HBV = hepatitis B virus; HCV = hepatitis C virus; HDV = hepatitis D virus.

Only slides stained with hematoxylin and eosin (H&E) are routine in our practice; requests for all other special stains or procedures are based on the H&E findings.

Serial biopsy monitoring of recipients is particularly helpful in patient management, and any previous posttransplant specimens should be reviewed with the current one. This not only establishes a baseline for each allograft but also greatly assists in the interpretation of the effect of therapy or disease progression. We recommend that the initial histological review of any of the pathology specimens be performed methodically and blindly (ie, without a clinical history). This minimizes the introduction of bias, which may occur when clinical information or prejudices are known. However, the final interpretation must be based on complete clinicopathological correlation.

BIOPSY EVALUATION OF THE DONOR LIVER

Frozen Sections

The pathologist may be asked to evaluate a donor liver by frozen section before implantation to determine its suitability for transplantation. The requests are most often prompted by the macroscopic appearance of the organ, which raises uncertainties in the mind of the surgeon. In other instances, the clinical history surrounding the donor's death may prompt suspicion.

Gross inspection of the donor liver by the pathologist is helpful, and if possible, the pathologist should assist in choosing the biopsy site. A 1.0-cm² wedge or 2.0-cm long needle core from the anterior inferior edge of the liver is...
adequate in most cases when the anticipated changes are diffuse. The decision to perform direct biopsy sampling of localized defects is intuitive.

Donor diseases identified on frozen section of the donor liver at Pittsburgh have included metastatic carcinoma, nodular regenerative hyperplasia, focal nodular hyperplasia (Fig 69 - 1), hepatic granulomas, severe steatosis, probable alcohol injury, small subcapsular infarcts, and subcapsular scars. An operation may even be aborted because of frozen section findings, but this is uncommon. Transplantation is currently contraindicated when a malignant tumor or severe macrovesicular steatosis is detected. The severity of steatosis is roughly estimated on review of the H&E-stained slides; in our experience, fat stains are not necessary. The cutoff for disqualifying livers at the University of Pittsburgh is greater than \( \text{50\% macrovesicular steatosis} \), depending on the recipient circumstances (Fig 69 - 2). It is clear, however, that liver allografts with less severe macrovesicular steatosis are also susceptible to an increased incidence of dysfunction after transplantation. Microvesicular steatosis, on the other hand, is often found after a short period of warm ischemia or other insults, and in our experience usually does not adversely affect the clinical course after transplantation. In the absence of obvious contraindications or severe ischemic injury, the pathologist is unable to predict the adequacy of organ function after transplantation on the basis of frozen-section light microscopic evaluation before the operation.

"Backtable" Biopsies

In the absence of gross abnormalities, donor organs usually undergo biopsy on the backtable before implantation and are routinely processed for viewing the next day.

Figure 69 - 1 Frozen section of a donor liver. This needle biopsy from a 2.0-cm focal nodular hyperplasia lesion was found immediately subjacent to the capsular surface of the right lobe of a donor liver. The remainder of the liver was normal. Note the appearance of focal cirrhosis, and the large fibrous scar, containing proliferating bile ductules. The lesion was resected on the backtable, and the organ was used for transplantation without incident.

Figure 69 - 2 Frozen section of donor liver with severe steatosis. This needle biopsy shows about 60% macrovesicular steatosis, which, in our opinion, renders the organ suboptimal for transplantation.

These samples provide a baseline for each organ with which any future alterations can be compared. Rarely, an unrecognized donor disease may slip past all of the fail-safe points before implantation only to be detected after the organ has been placed into the recipient; \( \alpha_1 \)-antitrypsin deficiency and low-grade chronic hepatitis have been detected in this manner.

CAUSES OF GRAFT DYSFUNCTION AFTER TRANSPLANTATION

Preservation Injury and Primary Dysfunction

Preservation injury refers to a variety of insults resulting in allograft dysfunction that begin immediately after transplantation and are not readily explainable on the basis of a technical or vascular insult, such as arterial or venous thrombosis, alloimmunological reaction, and infection. The term, as used by the author, includes damage to an organ because of donor disease or insults arising during the agonal stages in the donor from hypotension, drugs, infections, or toxins; damage to the organ during the harvesting process, cold preservation, and implantation in the recipient; and damage incurred during or shortly after reperfusion. Experimental animal research has primarily focused on the injury that occurs during cold preservation, but investigators in this field have acknowledged that many donor and recipient factors contribute to poor early posttransplantation allograft function.

Pathophysiology. Clinical and experimental animal studies show that one of the most significant insults occurs during "cold preservation," when the donor organ is stored in a physiologically compatible preservation solution. Loss of sinusoidal endothelial attachment to the underlying extracellular matrix results in exposure of underlying ground substances and, thus, loss of the normal antithrombogenic milieu. Vascular reanastomosis and the reintroduction of blood then result in "reperfusion injury," when platelet aggregation and neu-
trophil sludging occur in the areas of denudation (Fig 69-3). Microvascular thrombosis and localized activation of neutrophils with release of oxidative enzymes act together to diminish blood flow and prevent adequate reoxygenation of the starved tissues. Activated coagulation proteins and immune complexes evoke neutrophilic inflammation that is usually preserved unless the damage is severe and the allograft is in danger of failure. In general, the trend after transplantation is toward improvement, unless another insult supervenes, but resolution of the syndrome and return of bilirubin values to normal levels may take several months, particularly if the initial damage was severe.

Reperfusion of a donor liver with pre-existing macrovesicular steatosis results in a characteristic intraoperative syndrome manifest as wound site bleeding and “oozing” from disrupted vessels, which makes it difficult to achieve hemostasis before abdominal closure. Microvascular thrombosis and localized activation of neutrophils with release of oxidative enzymes act together to diminish blood flow and prevent adequate reoxygenation of the starved tissues. Kupffer cells can also play an important role in exacerbating preservation injury through secretion of toxic cytokines and other metabolic substances that directly affect hepatocellular function. Kupffer cells can also play an important role in exacerbating preservation injury through secretion of toxic cytokines and other metabolic substances that directly affect hepatocellular function. Warm ischemia, if kept to less than 45 minutes, is usually minimized as a clinical problem, and the insult is primarily hepatocellular and mostly reversible. It manifests morphologically as macrovesicular steatosis, often prevalent in periporal hepatocytes. donor livers with pre-existing steatosis show an increased susceptibility to both warm and cold ischemic injury.

Unfortunately, routine light microscopic examination by frozen section or even after formalin fixation and paraffin embedding cannot be used before implantation to identify severely damaged grafts. Perfusion fixation and electron microscopy are required for proper evaluation of the sinusoidal microvasculature.

**Clinical Presentation.** The most reliable early signs of allograft dysfunction are poor bile production and persistent elevation of serum lactate after complete revascularization. A marked elevation of serum transaminases to levels greater than 1500 IU/ml is usual during the first few days after transplantation, and this is followed by a rapid normalization of the transaminases but a gradual rise of bilirubin level over the first week. Hepatic synthetic function is usually preserved unless the damage is severe and the allograft is in danger of failure. In general, the trend after transplantation is toward improvement, unless another insult supervenes, but resolution of the syndrome and return of bilirubin values to normal levels may take several months, particularly if the initial damage was severe. The decision to await the return of normal liver function after a prolonged hospital stay, often punctuated by other comorbid conditions, or to proceed with retransplantation is one of the most difficult in patient management.

Reperfusion of a donor liver with pre-existing macrovesicular steatosis results in a characteristic intraoperative syndrome manifest as wound site bleeding and “oozing” from disrupted vessels, which makes it difficult to achieve hemostasis before abdominal closure. Marked elevations of liver injury test results immediately after transplantation and early allograft dysfunction manifest as hyperbilirubinemia are also frequent findings. Recovery of recipients who were critically ill (United Network for Organ Sharing statuses III and IV) before transplantation seems to be more complicated and protracted when they receive a fatty liver allograft.

**Histopathological Findings.** Routine histopathological findings in biopsy specimens taken within hours of complete revascularization (ie, “reperfusion” biopsies) can, with reasonable accuracy, predict poor allograft function during the first few postoperative weeks. Indicators of severe preservation injury in reperfusion biopsies include zonal or confluent coagulative necrosis, particularly if it is periportal or bridging, and severe neutrophilic exudation. It must be emphasized, however, that the subcapsular parenchyma is most susceptible to damage in the perioperative period. A needle biopsy specimen taken from this area may show more severe damage than that in the deeper parenchyma. In addition, neutrophilia without necrosis can be seen because of manipulation of the liver alone. Therefore, as with any histopathological finding, final interpretation should consider the complete clinical profile.

Unless the liver is totally necrotic, the histopathological changes just described attributed to “ischemic” injury overlap with those of a regenerative response, which begins within 2 to 3 days. Usually, the regenerative response is in proportion to the severity of injury. Histopathological changes of less severe hepatocellular ischemic injury that is usually reversible include macrovesicular steatosis and hepatocellular cytoaggregation (ie, rounding...
up of hepatocyte cytoplasm with detachment from adjacent hepatocytes). If the initial damage is mild, regeneration is limited to hepatocellular mitosis, twinning of the plates, and nuclear enlargement. As the liver repairs from this mild insult, mild centriflobular hepatocellular swelling and hepatocanalicular cholestasis (Fig 69 - 4) are frequent findings.

If the necrosis is more severe but centriflobularly confined and the reticulin architecture is intact, regeneration by hepatocellular mitosis proceeds rapidly, and restoration of the normal architecture is orderly and complete. If, however, there is marked periportal or bridging necrosis with architectural collapse, cholangiolar proliferation occurs, which often results in periporal fibrosis; this may bridge between the triads and be accompanied by mild neutrophilic, lymphocytic, and plasmacytic portal inflammation (Fig 69 - 5). Centrilobular hepatocellular swelling and hepatocanalicular and cholangiolar cholestasis are typical lobular findings. With severe injury, the cholangiolar proliferation and fibrosis can take 1 to 2 months or more to resolve, but they eventually disappear if the liver recovers (unpublished observation). The appearance of blastic lymphocytes and eosinophils in the portal triads should arouse a suspicion of acute rejection.

Reperfusion of a donor liver with pre-existing macrovesicular steatosis results in lysis of some of the fat-containing hepatocytes. Release of the lipid into the sinusoids causes coalescence of the miscible fat globules, around which fibrin, neutrophils, and red cell congestion accumulate (Fig 69 - 6). The term lipopeliosis has been used to describe this lesion. Complete resolution of the large fat globules may take several weeks.

Differential Diagnosis. Once vascular patency has been confirmed, the clinical and pathological differential diagnoses include sepsis, humoral rejection, and superimposed acute rejection. Separating sepsis from preservation injury using histopathology alone is frequently impossible. Humoral rejection is also difficult to separate from preservation injury because the pathophysiological mechanisms of injury are similar to each other as well as to eclampsia and disseminated intravascular coagulation. Sinusoidal thrombosis with or without arterial spasm is the common pathway of injury with all of these insults, but cold storage initiates the damage in preservation injury, whereas antidonor antibodies precipitate the injury in humoral rejection. In addition, gradual architectural repair with return of good function usually occurs with the former, whereas both the damage and dysfunction are likely to worsen with the latter, particularly without augmentation of immunosuppression. The clinical history (e.g., presence of preformed antibodies and length of preservation time) and the results of immunofluorescent stains of the liver biopsy provide other information useful in separating these two entities (see under Humoral Rejection). Superimposed acute rejection is identified by using the same histopathological criteria for acute rejection in a liver allograft not also damaged by preservation injury. Mild to moderate mixed portal infiltrate containing blastic lymphocytes and eosinophils with infiltration and damage to bile ducts and portal and central veins are
the most useful distinguishing features of superimposed acute rejection.

Vascular Thrombosis

Unfortunately, arterial thrombosis is relatively common after transplantation and is a major cause of allograft dysfunction and early failure.\textsuperscript{11, 62} Portal vein and vena cava thrombosis are uncommon.\textsuperscript{11, 62} Arterial defects, such as intimal flaps or irregularities, iatrogenically induced acute angle branchings or kinks, intimal or medial tears and dramatic reductions in caliber across a suture line, introduced during surgical manipulation and reconstruction of the vasculature, directly cause or predispose to thrombosis.\textsuperscript{62} Any physiological insult that decreases hepatic arterial flow or raises intrahepatic vascular resistance is likely to further increase the risk of thrombosis at these sites. Arterial interposition grafts, often kept in cold storage in culture medium, are another unrecognized risk factor for arterial thrombosis, particularly if the wall is nonviable before implantation (unpublished observation). The increased risk of hepatic artery thrombosis in children\textsuperscript{62, 63} is likely attributable to smaller-caliber vessels and technically more difficult vascular anastomoses.

It is important to remember that the hepatic artery is the exclusive source of blood to the major hilar excretory ducts, intrahepatic bile ducts, hilar connective tissue, lymph nodes, and wall of the portal vein.\textsuperscript{64} Therefore, compromised arterial flow frequently leads to selective necrosis of these structures.\textsuperscript{30, 31} Also, the allograft liver appears to be more susceptible to injury from arterial thrombosis than are native livers because, at least early after transplantation, it is devoid of the natural cascade-type arterial collaterals that normally help to protect the liver from infarcts.

Clinical Presentation. Hepatic artery thrombosis usually occurs within the first few postoperative weeks,\textsuperscript{62, 65} although a second smaller peak in incidence is seen more than 1 year after transplantation (unpublished observation). Patients often present with symptoms related to the biliary tree, such as right upper abdominal pain or discomfort, intermittent fever, bacteremia or fungemia because of severe cholangitis and intrahepatic biliary abscesses, bile duct necrosis and bile peritonitis, biliary strictures, or obstruction.\textsuperscript{11, 62, 65} If large areas of the liver are infarcted, fulminant hepatic failure can be seen, whereas if sufficient collaterals have developed, which is not uncommon in the pediatric recipient, the patient may be asymptomatic. Ultrasonography is most often used as a screening test; the diagnosis is confirmed with selective angiography.\textsuperscript{11, 62, 65}

The second peak in the incidence of technically related arterial thrombosis alluded to previously occurs between 1 and 3 years after transplantation. In this subset of patients, an anastomosis that is less than perfect is thought to cause turbulent arterial flow immediately downstream to the suture line. Alternatively, an arterial interposition vascular graft can develop fibrointimal hyperplasia more quickly than the vasculature within the organ allograft. Eventually, these technically related problems culminate in accelerated atherosclerosis and arterial thrombosis, which present as biliary tract obstruction, ascending cholangitis, or both.\textsuperscript{30, 31, 62, 66} Careful attention to detail by the transplant surgeon during arterial reconstruction may decrease the frequency of this complication.
Portal vein thrombosis in a noncirrhotic allograft can be complete and result in catastrophic liver failure or can be partial and present with relapsing fever and miliary seeding of the liver with bacteria. The common presentations in these recipients are ascites, encephalopathy, and variceal bleeding. As expected, cirrhotic allografts are more susceptible to portal vein thrombosis than are noncirrhotic allografts.

**Histopathological Findings.** The gross pathological evaluation of the failed allograft with arterial compromise is of paramount importance.\(^{30, 31}\) First, identification of the arterial anatomy may require the assistance of the operative surgeon. The precise location of the thrombus and any relationship to suture lines, vascular injuries, or other obvious defects such as intramural dissections, intraluminal mural flaps, and mycotic aneurysms should be noted. Necrosis of hilar structures, especially the bile duct wall with bile leakage into the hilar connective tissue and superimposed bacterial and fungal infection (Fig 69–7), is common.\(^{14, 26, 28, 30, 31}\) Varying sizes of subcapsular infarcts may also be present.

Needle biopsy is often of little or no value in establishing the diagnosis of hepatic artery thrombosis.\(^{30, 31, 63}\) The histological changes are varied, and needle biopsies are subject to more sampling error than usual. A completely normal histological appearance, frank coagulative necrosis or marked centrilobular hepatocyte swelling, cholangiolar proliferation with or without bile plugs, and acute cholangiolitis, similar to that seen with ischemic preservation injury, may be observed.\(^{30, 31}\) In some cases, spotty acidophilic necrosis of hepatocytes, mimicking acute viral hepatitis, can also be seen. If the hilar bile ducts have become necrotic or filled with sludge, needle biopsies of the periphery can also show changes of acute or chronic obstructive cholangiopathy or even resemble chronic viral hepatitis. When necrotic tissue is encountered, Gram’s and Grocott’s stains are recommended because these foci often become seeded with micro-organisms.

Many of the areas prone to necrosis from arterial thrombosis are not accessible to direct visualization or needle biopsy sampling. An attempt to salvage an allograft by arterial thrombectomy may be made during surgical exploration because of the “viable” gross appearance to the

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*Figure 69–7*  A. Hepatic artery thrombosis frequently results in necrosis of the wall of the large bile ducts in the hilum of the liver. The lumen of the necrotic duct is filled with bile sludge and inflammation. B. The necrotic duct also contains colonies of gram-positive cocci. C. This necrotic arterial wall shows some inflammation, and a few fungi are evident on the hematoxylin and eosin stain (arrows). D. However, Grocott’s stain reveals numerous branching hyphae, indicative of *Aspergillus* overgrowth.
capsular surface. Not infrequently, a frozen section of a needle core from the periphery of the liver may be requested to “confirm” the impression of viability. Although the biopsy specimen may appear normal, the surgeon should be warned of the possibility of hilar necrosis despite the normal peripheral biopsy.

Isolated portal vein thrombosis in a noncirrhotic allograft after transplantation is uncommon in our experience. Several patients in whom this complication developed shortly after transplantation presented with liver failure, and the pathology showed widespread coagulative necrosis. In several patients presenting late after transplantation, the portal vein was only partially occluded, and the thrombus became seeded with gram-positive bacilli from the gastrointestinal tract. Needle biopsies taken before allograft hepatectomy in these cases revealed small miliary infarcts in the parenchyma associated with an intense neutrophilic exudate.

Differential Diagnosis. As mentioned, needle biopsy evaluation is usually of little or no value in establishing the diagnosis of hepatic arterial thrombosis. Therefore, in this circumstance, the biopsy may be more useful as a way to exclude other causes of dysfunction, such as viral hepatitis and acute rejection. The histopathological differential diagnosis for hepatic artery thrombosis is extensive because, in our experience, artery thrombosis can mimic almost every histopathological syndrome associated with graft dysfunction. Many times, the biopsy specimen is relatively unremarkable either because vascular collaterals have developed and the thrombosis is inconsequential or because there has been necrosis of hilar bile ducts and changes secondary to biliary sludging have not yet developed in the periphery. When peripheral ischemic injury is seen, it can take the form of coagulative necrosis or centrilobular hepatocellular swelling and cholangiolar proliferation resembling preservation injury. Review of previous biopsies is usually helpful in distinguishing between preservation injury and arterial thrombosis.

Late after transplantation, hepatic artery thrombosis or stenosis often presents with biliary tract obstruction or stricturing. In such cases, needle biopsies may show changes of acute or chronic obstructive cholangiopathy. Ludwig and colleagues coined the concise descriptive term ischemic cholangitis to use in this circumstance. When biliary tract disease is encountered, examination of the adequacy of hepatic arterial flow is standard practice. Last, changes of chronic obstructive cholangiopathy can mimic those of viral hepatitis. The presence of cholangitis rather than acute cholangiitis and the presence of lobular disarray, which is seen in viral hepatitis but not in cholangiopathy, are useful distinguishing features. Exclusion of viral pathogens by adjuvant testing procedures can be used to rule out viral infection.

Bile Duct Complications

Anastomotic breakdown, mural necrosis with subsequent bile leakage and abscess formations, ascending cholangitis, anastomotic or intrahepatic stricturing, obstruction, and biliary-vascular fistulas can affect the allograft biliary tree. The two most common factors underlying biliary tract complications are an iatrogenically introduced abnormal anatomy, which predisposes to inadequate drainage or inordinate reflux, and arterial ischemic injury. Both of these problems can eventually result in mechanical obstruction. Lack of innervation of the donor duct is also a consideration. There are numerous causes of biliary ischemia: hepatic artery thrombosis, prolonged cold ischemia, preformed antidonor antibodies (anti-major histocompatibility complex [MHC] or anti-ABO blood group), and unrecognized and inadvertent hepatic arterial branch ligation. Once all other causes of biliary strictures have been excluded with reasonable certainty, the possibility of recurrent primary sclerosing cholangitis (PSC) should be considered in patients who had PSC before transplantation.

Clinical Presentation. The diagnosis of biliary tract problems is most often made on the basis of selective elevation of liver injury tests such as gamma-glutamyl transpeptidase and alkaline phosphatase, the presence of clinical symptoms, and the results of biliary imaging studies. Restoration of continuity and drainage of the allograft biliary system is achieved by performing a duct-to-duct or a choledochojejunostomy anastomosis. The anastomotic site is temporarily stented using a suitable-sized T-tube and Silastic stent, respectively. In daily clinical practice, the biliary system is routinely evaluated by ultrasonography. T-tube cholangiograms are usually performed before clamping (1 week) and at the time of T-tube removal (3 months). In patients with clinical, biochemical, and ultrasonographic evidence of biliary obstruction, visualization of the entire biliary system is achieved using a T-tube cholangiogram or endoscopic retrograde cholangiopancreatography (duct to duct) or percutaneous transhepatic cholangiogram. During the first 2 to 3 weeks after transplantation, anastomotic leaks or breakdown with bile peritonitis, mechanical obstruction, and bile duct necrosis caused by arterial thrombosis with the development of the bile cast syndrome are the most frequently encountered biliary complications.

Thereafter, ready access to the biliary tree is more restricted. This forces the clinical physicians to rely on other clinical features or diagnostic tests such as ultrasonography and cholangiography to screen for biliary tract patency. The utility of using a needle biopsy is described next.

Histopathological Findings. In general, needle biopsy evaluation of a liver allograft recipient for biliary tract obstruction or stricturing is less useful than cholangiography because of a comparative insensitivity and, in some cases, a relative nonspecificity of histological findings. Although the histopathological findings seen in patients with duct problems are frequently classic and diagnostic, such as those described next, large duct obstruction or stricturing not infrequently will result in changes that mimic both acute and chronic rejection and chronic hepatitis (see discussion of differential diagnosis).

Biliary tract complications that have been histopathologically recognized include duct stricturing, obstruction, acute cholangitis, periductal hemorrhage, and biliary-vascular fistulas. The features of obstruction and acute
cholangitis are identical to those seen in the nonallograft liver. The most important of these include a predominantly neutrophilic portal inflammatory infiltrate, peri-ductal edema, intraepithelial and intraductal neutrophils, mild ductular and cholangiolar proliferation, centrilobular hepatocanalicular cholestasis, and small clusters of neutrophils throughout the lobules (Fig 69-8).

Finally, recognition of biliary-vascular fistulas requires alertness to the abnormal presence of red cells in bile duct lumens or, conversely, bile concretions surrounded by foreign body giant cells in blood vessels. Prompt surgical intervention is often needed when either finding is encountered. Last, the presence of red cells surrounding a small interlobular bile duct (Fig 69-9) is a common finding in a recipient who has just undergone transhepatic cholangiography. Resolution of this finding without clinical or pathological sequelae is the usual outcome.

Differential Diagnosis. Early after transplantation, the distinction between biliary obstruction and cholangitis from acute rejection may be difficult at times, particularly if the patient was treated with increased immunosuppression before biopsy. An important differential diagnostic feature is the neutrophilic predominance of the portal inflammation in duct-related disorders. Acute rejection, on the other hand, usually shows a mixed or even a predominantly mononuclear infiltrate. For the first several months after transplantation, the presence of a significant number of eosinophils in the portal infiltrate is most often related to acute rejection.

Six months or more after transplantation, portal eosinophilia, mild duct proliferation, and edema are more often the result of duct obstruction or stricture (see later discussion) than rejection, and distinguishing between obstructive cholangiopathy and acute or chronic rejection may be particularly difficult. Changes found in obstructive cholangiopathy that can mimic rejection include a mixed or even predominantly mononuclear portal infiltrate, duct damage (see Fig 69-8), and apparent subendothelial inflammation of the portal veins. Portal fibrosis with mild duct proliferation, mild portal neutrophilic or eosinophilic inflammation, and mild centrilobular cholestasis should suggest obstructive cholangiopathy. Other clues that biliary tract disease is masquerading as histologically classic acute rejection are (1) posttransplantation time of longer than 6 months and (2) presence of adequate immunosuppressive drug levels. In addition, mononuclear inflammation in and around the central vein, or "central venulitis," can be a feature of acute or chronic rejection and not of duct obstruction and, therefore, can be helpful in distinguishing between these insults.

Rejection

Rejection is an immune response primarily elicited by a genetic disparity between the donor and the recipient. Several different schemes are used to separate and classify rejection. Clinically, the time and rapidity of onset, apparent vigor of the reaction, and its potential for response to therapy are important distinguishing features. Immunopathologically, rejection can be categorized on the basis of the effector mechanisms responsible for tissue damage, such as antibody-mediated rejection, cellular rejection, or a combination of these two. Although all of the divisions are to some extent overly simplistic and artificial, the uni-

Figure 69-8 Obstructive cholangiopathy. A. Teitlae histopathological features of bile duct obstruction or stricture include portal edema (PT = portal tract), a mixed or predominantly neutrophilic portal infiltrate, and cholestasis of varying severity. B. Higher magnification shows that mononuclear inflammation may, on occasion, predominate in some or all of the triads and be associated with bile duct damage (arrow), making separation of obstructive cholangiopathy from late-onset acute and early chronic rejection difficult in some cases.

Figure 69-9 Periductal hemorrhage. Needle biopsies obtained shortly after cholangiographic studies can show red cells rimming the periphery of small bile ducts (arrows). The inset shows one of the affected ducts in greater detail.
formity of an accepted classification scheme brings with it an ability to communicate more effectively.

Recently, the World Gastroenterology Congresses sanctioned a group of investigators to construct a standardized nomenclature for liver allograft rejection that included definitions, common clinical and laboratory findings, and minimal histopathological criteria. The terminology and definitions proposed by that group are adhered to in this chapter.

Pathophysiology. As mentioned, rejection occurs primarily because of a genetic disparity between the donor and the recipient, which manifests as an immunological reaction elicited by foreign donor antigens in the recipient. Various inductive, effector, and regulatory immune mechanisms participate in the reaction, and, as is the case for other immune responses, the alloreaction demonstrates both specificity and memory. A previous encounter with the same antigens because of a pregnancy, blood transfusion, or organ transplant can result in a "pre-sensitized" state, so that re-exposure to that same antigen provokes a more rapid and vigorous response. Although an in-depth discussion of each mechanism is beyond the scope of this chapter, an overview of inductive and effector mechanisms will assist in the interpretation of the morphological manifestations of the various types of rejection.

Several findings have led to the development of new concepts that have greatly enriched our understanding of the complex immunological events that occur during the initial recognition of the allograft as foreign and then later, when some grafts are accepted without immunosuppression. Every allograft is composed of a number of different cell types, including parenchymal epithelial cells, endothelium, smooth muscle cells, fibroblasts, and cells of hemato lymphoid lineage. When an organ becomes an allograft, the hemato lymphoid cells are called "passenger leukocytes" because they are carried into the recipient along with the organ. The dendritic cell, a specialized cell of the monocyte-macrophage lineage, is the passenger leukocyte prototype and is the most immunogenic among these cells. However, the passenger leukocyte population in the liver also contains hematopoietic stem cells, T and B lymphocytes, Kupffer cells, natural killer cells, and conventional tissue macrophages.

Under normal circumstances, hematolymphoid cells continuously traffic into and out of organs via hematogenous and lymphatic pathways. When an organ becomes an allograft, complete vascular anastomosis re-establishes the hematogenous migratory routes that disseminate donor passenger leukocytes throughout the body of the recipient. Conversely, trafficking of recipient immune cells into the allograft also becomes possible. Arrival of the donor cells in recipient lymphoid tissues and influx of recipient cells into the allograft brings the two alloge neic hematolymphoid populations into physical contact. This results in the spontaneous formation of clusters between dendritic cells and alloge neic T cells. Twenty-four to 48 hours later, the lymphocytes within these clusters become activated, undergo blastogenesis, secrete a plethora of cytokines, and begin to proliferate.

The reaction just described represents one of the earliest phases of the rejection response. In the recipient lymphoid tissues, it is known as "central" sensitization. In the allograft, it is known as "peripheral" sensitization and is histopathologically recognized in needle biopsies as acute rejection. Most of the time, especially in liver allografts, acute rejection promptly responds to increased immunosuppression.

Until recently, it was thought that the donor passenger leukocytes that had emigrated into the recipient were rapidly destroyed or simply died out within days or weeks after transplantation. However, we, and now others, have conclusively shown that the donor cells can persist in the recipient for decades.

In experimental animal studies, the persistent donor cells are multilineage, and they preferentially home to the same physioanatomical destinations as their phenotypically identical recipient counterparts. We have, therefore, hypothesized that a small and mobile donor immune system has integrated into the overwhelmingly larger recipient network. Possible consequences of this transfer of donor immunocytes and immune system include graft-versus-host reactions, amelioration of hemato lymphoid-based metabolic diseases, conversion of ABO blood group typing from recipient to donor, and drug-free allograft acceptance.

Once the recipient has recognizes the allograft as foreign through contact with dendritic and other cells, maturation of helper and cytotoxic T lymphocytes, release of cytokines, and synthesis and secretion of antibodies directed at donor antigens, the effector phase of the rejection reaction commences. Immunosuppression is aimed at blunting these effector responses. The most important targets and widely studied donor antigen systems in rejection are those encoded by the MHC and the major ABO blood group antigens, which are expressed on the portal microvascular, portal and central vein, and hepatic artery endothelium and bile ducts (Table 69-2). Other vascular and tissue-specific antigen systems may also be involved.

Antigen expression by a cell, however, is a dynamic process, influenced by many factors (eg, drugs, lymphokines). For example, after transplantation, bile ducts can express class II MHC antigens when perturbed by any type of inflammation, and class I antigen expression is increased on hepatocytes as opposed to normal, nontransplanted livers. Although several changes have been detected in the expression of MHC antigens in the liver in association with certain graft syndromes, none to date appears to be entirely specific or clinically useful.

The liver also shows some unique properties in transplantation immunobiology. For example, experimental porcine and murine liver allografts are accepted without using any immunosuppression and induce a state of hyporesponsiveness to other tissue from the same donor. Thus, a state of "donor-specific tolerance" can be induced in animals by liver allografting. Moreover, both rat and human liver allografts have been shown to be relatively resistant to humoral rejection and are able to "protect" other nonhepatic allografts from the same donor, from both humoral and acute rejection. Although no spontaneous long-term liver allograft survival is seen in the dog, baboon, rhesus monkey, or human, immunosuppressive
drug requirements to prevent rejection are usually less than those required for kidney or heart allografts in the same recipients. Several groups have even documented "spontaneous reversal" of acute rejection in human liver allograft recipients without bolstered immunosuppressive therapy. Moreover, the liver seems to be resistant to chronic rejection as compared with kidney or heart allografts.

Several hypotheses have been presented to explain the immunological privilege of the liver allograft or so-called liver-induced tolerance. One of the most widely quoted has been the release of soluble class I MHC antigens by the donor hepatocytes, which renders the recipient immune system anergic, combined with clonal deletion of cytotoxic effector cells within the allograft. However, studies by Qian et al. showing liver allograft acceptance in the absence of class I mismatching and without evidence of clonal deletion, respectively, are not supportive of that viewpoint. We have proposed a more general hypothesis based on the discovery that donor hematolymphoid cells can persist in the recipient after transplantation. Rather than representing a specific phenomenon, the liver simply uncovers a general process whereby donor hematolymphoid cells from the allograft transfer the ability to the recipient to view the organ as "self." In essence, our hypothesis suggests that solid organ transplantation represents the transfer of two donor organ systems from the donor to the recipient. The first system is provided by the physiological functions of the transplanted organ, or liver, that assimilates with the gastrointestinal system of the recipient. The second is embodied in the mobile donor hematolymphoid cells that disseminate and integrate with the overwhelmingly larger recipient immune system. Once integrated, the mobile donor hematolymphoid cells have access to immunologically privileged sites such as the thymus and through stimulation, over time they alter the emergent properties of the recipient immune network. This hypothesis also explains the ability of a donor liver to change the recipient blood type after transplantation, the susceptibility to delayed-type hypersensitivity responses, and, possibly, the ability to withdraw immunosuppressive therapy and a lesser susceptibility to chronic rejection in liver allograft recipients. Further details are provided in Chapter 27.

### Humoral Rejection

Antidonor antibodies can be present in the recipient before transplantation (ie, preformed) or can develop afterward in response to the allograft (reviewed by Demetris et al.). They can cause failure of, enhance the survival of, or have no effect on the function of an allograft. The consequences of antidonor antibodies depend on the class, titer, timing of the antibody response, and the density and distribution of target antigens in the organ. Only those capable of causing damage are covered here.

Historically, it had been difficult to incriminate antibodies as mediators of allograft damage in liver transplantation. This was largely attributable to a well-documented resistance of the liver to humoral rejection in comparison with the case with kidney or heart allografts. In addition, many of the nonimmunological complications, such as preservation injury and sepsis, that affect early liver allograft function can also produce a clinicopathological syndrome similar to humoral rejection. Moreover, comparatively poor survival rates after liver transplantation made it difficult to separate out those who died or required retransplantation because of antibody-mediated injury. It is now clear, however, that the resistance of the liver to antibody-mediated damage is not insurmountable. In fact, antibodies directed against the major ABO blood group antigens and MHC antigens have been clearly shown to cause graft failure.

The first system to be described that predictably resulted in humoral hepatic allograft rejection is encountered when ABO blood group barriers are violated. Since recognizing this potentially catastrophic form of injury, violating the ABO blood group barriers in liver transplantation is generally avoided, and humoral rejection resulting from a major blood group incompatibility is rare. However, in candidates with fulminant hepatic failure, in

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**TABLE 69-2 Distribution of major histocompatibility complex and ABH antigens in normal human liver**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Hepatocytes</th>
<th>Bile Ducts</th>
<th>Sinusoidal Cells</th>
<th>Arteriovenous Endothelium</th>
<th>Capillary Endothelium</th>
<th>Dendritic Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>H</td>
<td>−</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>HLA-A, -B</td>
<td>+/−</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>−</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>HLA-DQ</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>HLA-DP</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

HLA = human leukocyte antigen; − = negative; + = expressed very weakly; ++ = expressed weakly; +++ = moderate expression; ++++ = strong expression.

Pathophysiology. Antibodies directed at antigens expressed on the vascular endothelium are the more worrisome and can be particularly destructive because vascular injury interferes with the blood supply. Included are antibodies reactive with the major ABO blood group antigens and class I MHC antigens, which are detectable in conventional blood typing and lymphocytotoxic crossmatch tests, respectfully. Less well-defined antigen systems, such as those shared by endothelial cells and monocyte-macrophages and minor blood group antigens, have been associated with nonliver allograft injury. In xenotransplantation, polysaccharide antigens on the surface of endothelial cells are a major barrier to successful engraftment.

The pathophysiology of the effector phases of humoral rejection have been well worked out. Antibody binding to the endothelium results in complement fixation, endothelial damage, the formation of platelet thrombi, initiation of the clotting cascade, subsequent microvascular thrombosis, and arterial vasospasm, all of which act in concert to ruin the microvasculature and impair blood flow, causing hemorrhagic necrosis. The well-known resistance of the liver to humoral rejection could be the result of several factors: secretion of soluble MHC class I antigens by the liver; Kupffer cell phagocytosis of immune complexes and activated platelet aggregates; the dual hepatic blood supply through the hepatic artery and portal veins; and the unique hepatic sinusoidal microvasculature, which is devoid of a conventional basement membrane (see Demetriz et al[60, 61]). The importance of Kupffer cells in offering protection from humoral rejection and in the shielding of extraneous organs from injury was shown by Gugenheim et al[48, 49] Houssin et al[50, 51] and Orosz et al[52].

More recently, observations in hepatic xenotransplantation have offered yet another explanation for resistance of the liver to humoral rejection[53, 54]. If the target cell and complement are from the same source (donor versus recipient species), complement-mediated lysis of the target is less effective. Therefore, by providing its own source of complement, a liver allograft may protect its own endothelial cells from complement-mediated lysis triggered by the preformed antibodies[55]. Whether this form of protection is operable in allografts has yet to be determined. However, despite all of the protective mechanisms that exist, Knecht et al[56] and Gubernatis et al[57] clearly showed in animals that they could be overridden by intense presensitization. As mentioned, the same was shown in clinical liver transplantation for ABO-incompatible organs and, more recently, for lymphocytotoxic antibodies.

Clinical Presentation. The syndrome that develops in unconditioned recipients of ABO-incompatible organs is the liver equivalent of "hyperacute" renal rejection, but it usually develops more slowly over a period of hours to days rather than minutes to hours[58, 59]. The first signs of serious injury or impending doom can develop in the operating room after vascular reanaastomosis and before abdominal closure. The liver can initially reperfuse uniformly and produce bile but then becomes hard and swollen before bile flow slows or stops altogether. Difficulty in achieving hemostasis and an inordinate need for platelets and blood component replacement therapy signal the initiation of an intrahepatic consumptive coagulopathy. Rarely are the intraoperative events serious enough to abort the procedure or undertake immediate retransplantation. However, a relentless rise in liver injury test results during the first several days after transplantation and other signs of impending hepatic failure signal the possibility that humoral rejection is occurring[58, 59, 138, 157].

Hepatic angiography used to rule out arterial thrombosis may show segmental narrowing, or a "sausage-link" appearance. Diffuse luminal narrowing with poor peripheral filling early after transplantation is also suggestive of immunologically mediated arterial vasospasm. Unfortunately, with ABO-incompatible organs, the marked rise in transaminase levels is followed, in 60% to 70% of untreated cases, by synthetic function failure, subsequent wound site bleeding, and other systemic signs of hepatic failure that necessitate retransplantation[58, 59, 138, 140, 157].

Lymphocytotoxic antibodies can cause a spectrum of clinicopathological syndromes, although, in general, they cause less serious injury than do the isoagglutinins[139, 140, 158]. The variability appears to be related to the antibody titer, specificity, and class[53, 132, 158] with the immunoglobulin (IgG) class being the most destructive[139, 142, 158]. Patients with low-titer IgG anti-MHC antibodies detected on the routine crossmatch usually have no adverse consequences, but recipients with high-titer (especially greater than 1:500) preformed IgG lymphocytotoxic antibodies before transplantation often encounter significant difficulties during and after the operation[53, 132, 158]. However, this risk should be kept in perspective. The rate of crossmatch positivity is in the range of 8 to 12% of all recipients and only 30% of those with crossmatch positivity have relatively high titers. Thus, the patient population at greatest risk is small[142, 145, 158, 159] and humoral rejection may be overlooked as a cause of dysfunction or failure if it is encountered only once or twice per year.

In those in whom problems develop, an increased intraoperative need for platelets has been described[56]. In rare cases, precipitous hemorrhagic necrosis similar to that seen with isoagglutinins can occur. More frequently, a persistent rise in serum bilirubin level during the first week after transplantation, accompanied by refractory throm-
bocytopenia, low complement activity, and a biopsy showing changes of "preservation" injury, is the characteristic scenario. This is usually followed by the onset of acute (cellular) rejection and the need for increased immunosuppression. Ischemic biliary necrosis later manifested as biliary sludge, obstructive cholangiopathy, and small bile duct loss are other serious late manifestations.

**Histopathological Findings.** The histopathological findings depend on the timing of the biopsy and the nature of the presensitized state. For ABO-incompatible organs, samples taken within 2 to 6 hours after the operation show a clustering of neutrophils and red cell sludging in the sinusoids and focal platelet-fibrin thrombi in portal and central veins. Hemorrhage into the space of Disse, hepatocellular cytoaggregation, and single-cell acidophilic necrosis may also be seen. Samples taken 1 to 2 days later begin to show clusters of hepatocytes undergoing coagulative necrosis, red cell congestion, and hemorrhage (Fig 69-10). The areas of necrosis may not show any particular lobular distribution. Veins often show circumferential fibrin deposition, whereas arteries are usually less severely affected; however, on occasion, they may show neutrophilic or necrotizing arteritis, or both. A mild neutrophilic portal exudate usually appears at 2 to 3 days, along with focal cholangiolar proliferation, the latter of which is interpreted as a sign of regeneration. The histological features up to this point are difficult to separate from those of preservation injury. Thereafter, progressive patchy hemorrhagic infarction of the organ is not unusual.

Because the hepatic injury is generally less florid in patients harboring preformed lymphocytotoxic antibodies, it is difficult to reconstruct the sequential histopathological findings from clinical needle biopsy samples. Therefore, a clinically relevant small animal model of humoral rejection caused by anti-class I MHC antibodies has been developed. The time sequence of the histopathological changes in that model is shown in Table 69-3.

In clinical samples, reperfusion biopsies from patients with a positive lymphocytotoxic crossmatch more often contain platelet aggregates in the portal or central veins than do those from crossmatch-negative controls (Fig 69-11). In the first week after transplantation, spotty acidophilic necrosis of hepatocytes, centrilobular hepatocellular swelling accompanied by cholangiolar proliferation, and hepatocanalicular cholestasis often appear. Neutrophilic or necrotizing arteritis is rare. Overall, the histopathological changes closely resemble those of preservation injury, except for subtle arterial changes, which may not be present in needle biopsy samples. These include endothelial hypertrophy, medial thickening, and medial myocyte vacuolization and are best observed in the perihilar region of allograft hepatectomy specimens. Other hilar changes in humoral rejection include congestion of the peribiliary vascular plexus, partially organized thrombi in arterial branches, and focal mural necrosis of large septal bile ducts.

If allograft failure does not occur, superimposed acute (cellular) rejection usually becomes evident within 5 to 7 days of transplantation, at which time the diagnosis of rejection becomes obvious. As mentioned, long-term sequelae of an early humoral insult can include biliary sludge and stricturing with obstructive cholangiopathy, obliterator arteriopathy, and loss of small bile ducts or chronic rejection.

The gross appearance of failed ABO-incompatible grafts at the time of retransplantation is similar to that of other organ allografts undergoing "hyperacute" rejection. They are often enlarged, cyanotic, and mottled with areas of necrosis. The capsule may or may not be ruptured. Hepatic artery and portal vein thrombosis is variably present. Microscopically, one observes large geographic areas of hemorrhagic necrosis. Focal fibrinoid necrosis of arteries may be seen but is present in only a minority of cases. More common vascular findings include arterial and venous endothelial cell hypertrophy, neutrophil sludging, and focal fibrin deposition around a partial circumference of the vessel, with a mass of fibrin extending...
TABLE 69–3 Routine histopathological findings in rodent liver allograft recipients presensitized with skin graft from the same donor

<table>
<thead>
<tr>
<th></th>
<th>1–3 Min</th>
<th>10–30 Min</th>
<th>1–3 Hr</th>
<th>6–24 Hr</th>
<th>36–48 Hr</th>
<th>72–96 Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet plugging</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endothelial hypertrophy or vacuolization</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Hepatocye necrosis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Sinusoidal neutrophilia</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neutrophilic portal venulitis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Portal artery thickening</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Arterial myocyte vacuolization</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cholangiolar proliferation (focal)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mononuclear inflammatory infiltrate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Biliary sludge</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>IgG</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sinusoids</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Portal and central veins</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

IgG = immunoglobulin G. The findings were semiquantitatively from – = not present to +++ = severe.


Figure 69–11 Humoral rejection of an ABO-compatible organ. A. A failed allograft removed 2–3 days after transplantation from a patient with a strongly positive (titer > 1:1024) immunoglobulin G lymphocytotoxic crossmatch shows perportal necrosis (arrows) and focal neutrophilia in the areas of damage. B. A higher magnification shows sludging of platelets in the portal vein (arrow) endothelial cell hypertrophy, and mild neutrophilic accumulation. C. A needle biopsy from a different patient with strong positive crossmatch obtained 1 week after transplantation shows central ballooning, cholestasis, and cholangiolar proliferation (lower left corner), changes that are difficult to separate from preservation injury.
into the lumen.\textsuperscript{58, 59} Arterial medial thickening and myocyte vacuolization are common and probably represent morphological manifestations of vasospasm.

Immunofluorescent and immunoperoxidase stains in ABO-incompatible organs will often reveal IgM, focal IgG, and complement components C3, C4, and C1q in an occasional artery and in the sinusoids. However, deposits of IgG, IgM, and C1q may be seen in a similar distribution in allografts with nonimmunologically mediated injury, and background fluorescence is usually a confounding problem. Elution studies can be performed to confirm the identity of the deposited antibodies.\textsuperscript{58, 59} The final diagnosis should be based on a complete clinicopathological analysis during which other nonimmunological causes of graft failure are excluded. The immunofluorescent findings in ABO-compatible organs with humoral rejection are discussed next.

**Differential Diagnosis.** As mentioned, humoral rejection in an ABO-incompatible organ can easily be confused or overlap with hemorrhagic liver necrosis precipitated by a severe hypotensive insult, sepsis, and vascular thrombosis.\textsuperscript{58, 59, 146, 163, 164} Simply knowing that the organ is ABO incompatible and recognizing the injury described previously are often sufficient to distinguish these entities, provided that the organ underwent a biopsy before becoming totally necrotic.

Under ABO-compatible circumstances, the presensitization state and clinical profile can provide useful information for the interpretation of the histopathological findings and vice versa. For example, a recipient who harbors high-titer (>1:32 - 500) preformed IgG lymphocytotoxic antidonor antibodies should be assumed to be at greater risk for humoral rejection compared with a recipient with low-titer (<1:32) antidonor antibodies, particularly when there is no other obvious technical cause of allograft dysfunction.\textsuperscript{142, 145, 158, 159} A diagnosis of humoral rejection is further supported when there is persistence of the preformed antibodies after transplantation, a drop in the platelet count and persistent thrombocytopenia below 50,000 mm\textsuperscript{3}, and hypocoomplementemia compared with normal pretransplantation values.\textsuperscript{158}

Subtle histopathological clues that humoral rejection is occurring include arterial endothelial cell hypertrophy, arterial medial thickening, and myocyte vacuolization with features suggestive of parenchymal ischemia, such as centrolobular hepatocellular swelling.\textsuperscript{146} Fibrinoid necrosis and inflammatory arteritis are more definite features of humoral rejection but are rarely seen in a needle biopsy. In addition, the cholangiolar proliferation, acute cholangiitis, centrolobular swelling, and hepatocanonical cholestasis in severe preservation injury generally improve over time, whereas these same changes generally worsen with time in serial biopsies from patients with humoral rejection. Moreover, humoral rejection is usually followed by acute (cellular) rejection.

In our experience, the immunofluorescence findings in ABO-compatible allografts are generally supportive, but alone they rarely are diagnostic unless the humoral rejection and immune deposits are florid. When detectable, deposits of IgG, C3, and C4 in the arteries and in the portal and hilar microvasculature, without heavy \(\alpha_2\)-macroglobulin or other macromolecule deposition, are more indicative of humoral rejection than deposits of IgM and C1q, which frequently become lodged in necrotic arterial walls, regardless of the cause of damage.

**Acute Rejection**

Acute rejection has been defined by the World Congresses of Gastroenterology international panel\textsuperscript{70} as "inflammation of the allograft, elicited by a genetic disparity between the donor and recipient, primarily affecting interlobular bile ducts and vascular endothelia, including portal and hepatic veins and occasionally the hepatic artery and its branches." "Cellular rejection" is an acceptable synonym, because the descriptive adjective highlights the most prominent biopsy feature. Other synonyms that appear in the literature include early rejection, nonductopenic rejection, rejection without duct loss, and reversible rejection. The panel does not encourage use of these latter terms.\textsuperscript{78}

**Pathophysiology.** As discussed in more detail later, the morphological manifestations of acute rejection can be directly related to the pathophysiological mechanisms responsible for development of the reaction. With vascular anastomoses restored, hematogenous migratory routes for passenger leukocytes are re-established, and some, but not all, of the donor hematolymphoid cells emigrate from the allograft into the tissues of the recipient.\textsuperscript{76, 87, 94, 128} There they elicit a strong proliferative immune response, also known as "central sensitization," that results in the formation of cytotoxic T lymphocytes and the development of an antidonor antibody response, both of which can damage the organ.\textsuperscript{76, 87, 94, 128} Conversely, recipient T cells entering the allograft spontaneously cluster with the remaining donor dendritic cells that are found exclusively in the portal triads and in the connective tissue immediately subjacent to terminal hepatic venules.\textsuperscript{78} These clusters result in T cell activation, blastogenesis, proliferation, and secretion of cytokines that upregulate MHC antigens and adhesion molecules on nearby vascular endothelium and act as chemotactic agents, which enhance the immigration of white cells into the tissues.\textsuperscript{76, 105, 106, 108} This reaction is histopathologically recognized as acute rejection and results in the development of cytotoxic T lymphocytes and secretion of other chemotaxins that recruit macrophages and neutrophils, which in turn release oxidative products, leading to nonspecific damage of structures targeted for injury. Targeted for injury are the portal microvasculature, portal and central vein endothelium, small bile ducts, and arterial endothelium. Hepatocytes seem relatively spared from direct assault. Untreated, the endothelial injury culminates in microvascular congestion, thrombosis, and ischemic necrosis of the organ. Fortunately, however, this reaction (acute rejection) is easily reversible with increased immunosuppression.

**Clinical Presentation.** Acute rejection usually first occurs between 5 and 30 days after transplantation, although earlier or later presentations can be seen in presensitized patients or in those who receive less than optimal baseline immunosuppression. Because
there is little controversy regarding acute rejection and most groups report similar if not identical findings.\textsuperscript{1,2,12,14-16,18,30,73,74,77,166-175} specific bibliographic references to particular features are not included at each juncture.

Clinical findings are often absent in early or mild acute rejection, although in late or severe cases fever, enlargement, cyanosis, and tenderness of the allograft frequently occur. Bile drainage from a T-tube often becomes thin and pale, and the flow is decreased. Occasionally, ascites develops because of liver swelling, increased intrahepatic pressure, and production of lymphatic fluid.\textsuperscript{78}

Liver dysfunction is usually manifest as concomitant, nonselective elevations of some or all of the standard liver injury test findings, including total bilirubin, alanine aminotransferase, aspartate aminotransferase, \( \gamma \)-glutamyl transpeptidase, and alkaline phosphatase levels.\textsuperscript{78} Peripheral blood leukocytosis and eosinophilia are also frequently present. Biochemical tests of the peripheral blood often show elevation of various interleukin levels or their receptors, neopterin, amyloid A protein, and antidonor class I MHC antibodies.\textsuperscript{25,26,60,70,77,172} Unfortunately, all of the clinical and laboratory findings lack sensitivity or specificity. The diagnosis is suspected on clinical grounds and is confirmed by examination of a core-needle biopsy specimen or a fine-needle aspirate of the allograft, the former of which has become the gold standard method of establishing the diagnosis.

**Histopathological Findings.** Acute rejection is characterized by (1) predominantly mononuclear but mixed portal inflammation containing blastic or activated lymphocytes, neutrophils, and eosinophils; (2) subendothelial inflammation of portal or terminal hepatic veins, or both; and (3) bile duct inflammation and damage.\textsuperscript{78} Minimal diagnostic criteria needed to establish the diagnosis of acute rejection include a least two of these histopathological findings (mixed portal infiltrate and clearly defined bile duct damage) and biochemical evidence of liver damage.\textsuperscript{78} The diagnosis is strengthened if greater than 50% of the ducts are damaged or if unequivocal endotheliitis of portal or terminal hepatic vein branches can be identified.\textsuperscript{78} As with all allografts, interference with blood flow at either the arterial or the microvascular level is one of the most serious manifestations of liver rejection. Findings that are associated with severe injury and used for histopathological grading include lobular inflammation, centrilobular necrosis, arteritis, and inflammatory bridging.

Immunophenotypic analysis of the inflammatory cells present during acute rejection reveals a predominance of T lymphocytes, dominated by CD8+ subset in the portal triads and more specifically, in the damaged bile ducts.\textsuperscript{105,176-178} However, B cells, macrophages, and other leukocytes are also present.\textsuperscript{105,106,108,176,179} A minority of cells demonstrating phenotypic characteristics of dendritic cells may also be present.\textsuperscript{105} Expansion of the infiltrative cells in vitro using interleukin-2 (IL-2) and subsequent functional analysis have demonstrated the presence of activated T lymphocytes displaying proliferative and cytotoxic activity directed at donor MHC antigens.\textsuperscript{178,180-184}

Infiltration and damage to small bile ducts are important features used to recognize acute rejection and therefore merit further description. The bile ducts show inflammatory cells inside the basement membrane, with evidence of damage to the epithelial cells such as paranuclear vacuolization, pyknosis, and cytoplasmic eosinophilia. Luminal disruption with breaks in the basement membrane can occur, but well-formed portal-based granulomas are not generally seen. In response to the injury (or microenvironment), the biliary epithelium also shows enlarged nuclei, an increased nucleus-to-cyttoplasm ratio, nucleoli, and mitosis.

Small portal tract arteries and arterioles are difficult to locate during acute rejection. Endothelial swelling and mural hypertrophy are most commonly observed when the arteries are found. Necrotizing or neutrophilic arteritis is rare. Lymphocytic inflammation can be seen but is a poorly reproducible finding\textsuperscript{185} and is present in less than 5% of cases. Although inflammatory arteritis may be seen with acute rejection, the vessels most commonly affected are located in the hilum and are not usually accessible by needle biopsy (Fig 69 - 12). Therefore, the low incidence of arteritis detected in needle biopsy samples is likely due to a sampling problem.

As rejection worsens, the portal infiltrate may spill over into the periphery of the lobule, associated with periportal hepatocyte necrosis (Fig 69 - 13). The remainder of the lobule usually shows mild Kupffer cell hypertrophy, a slight increase in inflammatory cells in the sinusoids, and infiltration of the connective tissue of the surrounding central vein by cells, similar to that seen in the triads. Zonal centrilobular hepatocyte swelling, necrosis, and hepatocanalicular cholestasis may also be present. Significant lobular disarray is unusual. Some of the lobular findings may be related to ischemic injury occurring before or as a consequence of rejection. Decreased hepatic blood flow and disruption of the sinusoidal microvasculature, particularly near the central vein,\textsuperscript{1,2,76,77} can also contribute to the "ischemic" lobular changes.

Treatment of acute rejection with additional immunosuppression before a biopsy specimen is obtained may

![Figure 69 - 12 Inflammatory arteritis associated with acute rejection.](image_url)
Figure 69-13 Acute rejection. A. Mild acute rejection shows mild mixed portal inflammation with evidence of duct damage and portal or central venulitis or endothelitis in some but not a majority of the triads. B. A higher magnification shows the bile duct damage (thick arrows) and subendothelial inflammation (thin arrow) of the portal vein. C. In moderate acute rejection the mixed infiltrate involves most or all of the triads, and the portal infiltrate may spill over into the edge of the triads, as shown here. However, there is no evidence of centrilobular necrosis, inflammation, and dropout. D. Severe acute rejection is characterized by a usually moderate to severe but variable portal infiltrate associated with moderate to severe lobular inflammation and necrosis (PT = portal tract; CV = central vein). E. A higher magnification of a central vein region shows hepatocyte dropout and inflammation. F. The failed allograft with severe acute rejection also showed subendothelial lymphocytic inflammation of large hilar arteries and early mild foam cell accumulation.
make the histopathological diagnosis more difficult because of subsequent loss of the subendothelial infiltration of veins and a relative decrease in the number of mononuclear inflammatory cells.

Currently, several well-known systems are used for grading acute rejection (Table 69-4). All of these systems include mild, moderate, and severe grades, and there are few substantial differences between them, except for the Minnesota scheme, which includes bile duct loss under severe acute rejection. Each of the grading systems listed in Table 69-4 has shown utility in terms of prognosis or prediction of liver function abnormalities at the home institution. However, the National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK) system has the additional advantage of proven reproducibility and prognostic value across three separate institutions and five separate pathologists. A challenge in the near future is to combine all of these schemes into one so that a common nomenclature and grading scale can be used.

Chronic Rejection

Chronic rejection is usually refractory to immunosuppressive therapy and is an important cause of late graft failure, although it may be decreasing in incidence. Chronic rejection has also been defined by the World Congresses of Gastroenterology as a “usually irreversible process defined by two main histopathological features: obliterative vasculopathy and loss of bile ducts. Although these two components usually coexist, they occasionally may occur independently. The process is elicited by a genetic disparity between the donor and the recipient, but other cofactors may be involved.” “Ductopenic rejection” is considered an acceptable synonym. But the panel discouraged the use of other names such as late rejection, irreversible rejection, rejection with duct loss, vanishing bile duct syndrome, acute vanishing bile duct syndrome, and chronic vascular rejection that appear in the literature as substitutes for chronic rejection. The consensus document just mentioned explains the conundrum associated with the term chronic:

Although the term “chronic” technically implies a time parameter, none is intended. Chronic rejection has been described as early as 2-3 weeks after transplantation and certainly may occur after this time. Although it is unusual before 60 days after transplantation. By convention, the term “chronic” has been used in other organ allografts to imply irreversible changes. The alternative terms mentioned—vanishing bile duct syndrome and ductopenic rejection—are descriptors of one of the major findings but loss of ducts is not a defining feature of an irreversible process. For the time being, by convention the term “chronic” provides a brief, understandable shorthand for a complex and incompletely understood process.

Pathophysiology. Risk factors for the development of chronic rejection reveal possible pathogenic mechanisms responsible for the development of this disorder. These include multiple or poorly controlled acute rejection episodes, chronic rejection in a previous failed allograft, a positive pretransplantation lymphocytotoxic crossmatch, anti-MHC antibodies that develop after transplantation, cytomegalovirus (CMV) infection, matching at the class II MHC locus and mismatching at the MHC class I locus, nonwhite recipient race, chronic viral hepatitis, and treatment with interferon-α. Most of these risk factors identify patients who either have been sensitized against the allograft or show strong immunological reactivity toward it. Hyperlipidemia, hypertension, diabetes, and preservation injury have, to our knowledge, not been associated with chronic rejection of the liver but are thought to contribute to the development of obliterative arteriopathy (OA) in other solid organ allografts.

The major defining feature of chronic rejection is OA, because bile duct loss alone can occur with several immunological or nonrejection-related disorders. Several groups have suggested that the pathogenic mechanisms involved in the development of OA include a “response to immunological injury,” the same hypothesis used to explain the development of atherosclerosis in the general population. Thus, attempts at classification are based on the nature of the initial insult. In an allograft, the cause of the injury appears to be primarily rooted in an allogeneic immune response. Likely arterial targets of this response include the endothelium, periarterial dendritic cells, and lymphatic capillary endothelium in the adventitia because disruption of the lymphatic vessels could cause mural edema and alter the intimal milieu. However, multiple other cofactors, including preservation injury, viral infections (especially CMV), hyperlipidemia, hypercholesterolemia, hypertension, and diabetes, could certainly contribute to the insult and exacerbate the arterial disease. In any event, the arterial injury triggers a cascade of intimal inflammation, growth, and repair explored in detail by Hayry and colleagues that eventually results in the characteristic OA.

The second major target for injury in chronic rejection, the bile ducts, are directly susceptible to immunological injury from invading inflammatory cells and indirectly susceptible to ischemic damage because of arterial occlusion and destruction of the peribiliary capillary plexus. Direct recognition of bile duct cells by activated recipient lymphocytes and injury via the development of cytotoxic T lymphocytes (CTLs) are usually noted ultrastructurally and histopathologically and are the defining features of both acute and chronic rejection. In addition, cytokines locally released by the invading lymphocytes can either directly injure the ducts or recruit neutrophils and macrophages via chemotaxins that indirectly cause damage through release of oxidative products. These same effector mechanisms, as well as antidonor antibodies, can also destroy the small portal arterioles and fine webbing of capillaries that are the final conduit of arterial blood to the ducts. In addition, this unique portal microvasculature can be plugged and ruined by platelets and neutrophils in presensitized patients, explaining the association between preformed antibodies and bile duct loss. Donaldson, O’Grady, and others suggested that bile ducts can also contribute to their own destruction by acting as antigen-presenting cells when their class II MHC antigens are matched with those of the recipient and when they are simultaneously mismatched for class I MHC or infected with CMV (see under Cytomegalovirus infection)
TABLE 69-4  Grading systems for acute liver allograft rejection

<table>
<thead>
<tr>
<th>Acute Rejection</th>
<th>NIDDK System(^\text{184,*})</th>
<th>Minnesota(^\text{173})</th>
<th>European(^\text{172,\dagger})</th>
<th>Williams et al(^{188})</th>
<th>Kemnitz et al(^{174})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Rejection infiltrate in some but not a majority of the triads confined within the portal spaces</td>
<td>Lymphocytic or mixed portal infiltrate with &lt;30% damaged bile ducts and endotheliitis of portal or central veins</td>
<td>Inflammatory changes are generally mild and patchily distributed within portal areas; bile duct damage and venous endothelial inflammation are both mild</td>
<td>Minimal infiltration of portal tracts and portal vein branches with or without involvement of interlobular bile ducts combined with minimal subendothelial infiltration of central veins and minimal infiltration of the hepatic parenchyma about central veins</td>
<td>Slight mononuclear, predominantly lymphocytic, or partially mixed cellular infiltration of the portal tracts; venous endotheliitis in portal, central, or both localizations; degenerative parenchymal changes up to necrosis with infiltrates of up to 10% of the hepatic parenchyma; bile duct damage</td>
</tr>
<tr>
<td>Moderate</td>
<td>Rejection infiltrate involving most or all of the triads with or without spillover into the lobule; no evidence of centrilobular necrosis, ballooning, or dropout</td>
<td>Lymphocytic or mixed portal infiltrate, &gt;50% damaged bile ducts, with or without endotheliitis</td>
<td>Inflammatory changes are more severe and widespread, with the majority of portal tracts involved; bile duct damage and venous endothelial inflammation are both conspicuous</td>
<td>More extensive infiltration of both portal tracts and parenchyma with focal, nonbridging necrosis of the hepatic parenchyma</td>
<td>More pronounced infiltration of the portal tracts, degenerative changes of the hepatic parenchyma and focal nonbridging necroses of the hepatic parenchyma, mixed but predominantly mononuclear infiltration affecting 10–30% of the whole hepatic parenchyma, portal and central venous endotheliitis, bile duct damage</td>
</tr>
<tr>
<td>Severe</td>
<td>Rejection infiltrate in some or all of the triads with or without spillover into the lobule with or without inflammatory linkage of triads; associated with moderate severe lobular inflammation and necrosis</td>
<td>Acute rejection plus arteritis, paucity of bile ducts, or central hepatocellular ballooning with confluent dropout of hepatocytes</td>
<td>All three classic features of acute rejection are present to a marked degree; they are sometimes accompanied by additional perisplenic, sinusoidal parenchymal, and vascular changes as outlined in footnote</td>
<td>Marked mononuclear infiltration of portal tracts and parenchyma with bridging hepatocellular necrosis</td>
<td>Marked mixed but predominantly mononuclear infiltration of the portal tracts and the hepatic parenchyma, with pronounced degenerative changes and necroses that affect &gt;30% of all hepatocytes and that are partly bridging; venous endotheliitis in portal and central localization; bile duct damage</td>
</tr>
</tbody>
</table>

*For all grades of rejection at least two of the three histopathological findings must be present: a predominantly mononuclear infiltrate, bile duct inflammation or damage, and subendothelial localization of mononuclear cells in the portal or central veins.

\(^{\text{1}}\)The severity of (1) portal inflammation, (2) bile duct damage, and (3) venous subendothelial inflammation are subjectively scored on a scale of 0 (none) to 3 (severe) and are collated to provide a final rejection grade. In some cases, other features that are inconsistently seen in acute rejection (eg, portal inflammatory spillover, sinusoidal endotheliitis, perivenular inflammation with or without necrosis, and arteritis) are used to upgrade the overall severity of acute rejection.

\(^{\text{184\,*}}\)NIDDK = National Institute of Diabetes and Digestive and Kidney Diseases.
Hepatitis). In vitro, we have shown that bile duct epithelium can present alloantigen to primed lymphocytes, but they are much less efficient at antigen presentation than are endothelial cells tested in the same assay. This implies, but does not prove, that arterial or microvascular destruction may be the critical lesion in chronic rejection.

**Clinical Presentation**

Chronic rejection usually does not occur before 2 months after transplantation and most frequently develops (1) after an unresolved episode of acute rejection, (2) after multiple episodes of acute rejection, or (3) indolently over a period of months to years, with few or no clinically apparent acute cellular rejection episodes. However, it has been observed as early as 2 weeks after transplantation. Often, unresolved or indolent rejection is apparent only because of a persistent elevation of liver injury test abnormalities in a patient with chronic rejection usually does not occur before 2 months after transplantation and most frequently develops (1) after an unresolved episode of acute rejection, (2) after multiple episodes of acute rejection, or (3) indolently over a period of months to years, with few or no clinically apparent acute cellular rejection episodes. However, it has been observed as early as 2 weeks after transplantation.

**Histopathological Findings**

The two histopathological findings that are indicative of chronic rejection are loss of small (<60 μm) bile ducts involving more than 50% of the triads and OA with foam cells, although arteries with pathognomonic changes are rarely present in needle biopsy specimens (Fig 69–14). Cases with either isolated bile duct loss or foam cell arteriopathy alone may occur, but usually both features occur together. Duct loss without arteriopathy may be seen in some patients as a result of non–rejection-related complications (eg, bile duct strictures, drug toxicity, and CMV infection), so appropriate caution and clinical correlation while establishing the diagnosis are suggested.

In a biopsy specimen, minimal diagnostic criteria for chronic rejection are the presence of foam cell OA or convincing evidence of bile duct loss (>50% of the triads) documented by the presence of hepatic artery branches without bile ducts or by the absence of both ducts and hepatic artery branches. The diagnosis is strengthened if the patient had documented episodes of acute rejection that had progressed to chronic disease and prolonged liver dysfunction that had not responded to appropriate antirejection therapy.

Duct loss is determined by calculating the ratio of the number of hepatic artery branches and the number of bile ducts within a portal tract (normal value is usually >0.7). The greater the number of triads counted, the more likely the count is to be valid, although Ludwig and colleagues suggested that at least 20 portal tracts should be included. Study of several sequential biopsy specimens, obtained over a period of 3 to 6 months, may be required to examine a sufficient number of triads. Recognition of portal triads may be difficult in cases in which the hepatic arterioles have also been destroyed.

The presence of foam cell arteriopathy can rarely be confirmed by a core needle biopsy (see Fig 69–14). Features in a peripheral needle biopsy that suggest but do not prove the presence of foam cell arteriopathy include loss of small arteries (<20 μm) in greater than 20% of the portal tracts, centrilobular hepatocellular swelling, perivenular sclerosis, and centrilobular hepatocyte dropout.

Portal inflammation can be mild in chronic rejection, but, despite a relative paucity of portal inflammation, intraepithelial lymphocytes located adjacent to pyknotic or apoptotic biliary epithelial cells are seen. Characteristic degenerative changes of the biliary epithelium include uneven spacing of individual epithelial cells, eosinophilic transformation of the cytoplasm similar to that observed in primary biliary cirrhosis, and ducts only partially lined by epithelial cells. As the lesion progresses, bile ducts become difficult to identify. Special stains (eg, trichrome, PAS-D, and cytokeratin) may be used to enhance their detection by highlighting the basement membrane or selectively staining the biliary epithelial cells. Eventually, the bile ducts are completely destroyed and are replaced by fibrous tissue; the smaller the duct, the more susceptible it is to damage and loss.

Other portal tract alterations in chronic rejection include "collagenization" of the connective tissue with loss of portal capillaries, small arteriole loss or mural thickening, subintimal foam cells, and fibrosis (see Fig 69–14). Significant ongoing, piecemeal necrosis or cirrhosis caused by chronic rejection is uncommon in our experience but has been observed by Wight.

Lobular changes in chronic rejection include centrilobular hepatocanicular cholestasis, intrasinusoidal foam cell clusters, mild spotty acidophilic necrosis of hepatocytes, centrilobular hepatocyte atrophy, and ballooning and perivenular sclerosis. The clusters of foamy macrophages, although common, may simply represent a nonspecific response to cholestasis; therefore, alone, they are not diagnostic of chronic rejection. The centrilobular degeneration and perivenular sclerosis may be related to either ischemia or damage from repeated bouts of "central venulitis" during acute rejection.

In an explanted failed allograft, the diagnosis of chronic rejection is easier to establish. The presence of foam cell OA should be seen in at least some of the muscular arteries...
in the hilum. Arteries affected by OA most commonly show luminal narrowing because of subintimal deposition of lipid-laden cells that derive from macrophages and intimal and medial myocytes (see Fig 69–14). However, lymphohistiocytic intimal inflammation, smooth muscle cell proliferation, disruption of the elastic lamina, and periadventitial and intramedial inflammation may also be present. Affected vessels may also contain immunoglobulin and complement deposits. Major hilar bile ducts may show sloughing of the epithelium, focal necrosis, papillary intraluminal hyperplasia, mural fibrosis, and acute and chronic inflammation. Foamy macrophages may also be seen around bile ducts and veins in the connective tissue.

The diagnosis of chronic rejection is difficult and uncertain in the early stages before overt bile duct loss involving greater than 50% of the triads is evident. Such cases usually show only mild portal inflammation, but the biliary epithelium shows eosinophilic transformation of the cytoplasm, intraepithelial lymphocytes, and uneven spacing of the nuclei, with only partial lining of the ductular circumference by epithelium. Cholestasis may not be present at this stage. The diagnosis in the earlier stages is strengthened by repeated biopsies and clinical correlation, which typically show a patient with documented acute rejection that has progressed to chronic injury and prolonged liver dysfunction that has not responded to appropriate antirejection therapy. Such cases would be categorized as “rejection, indefinite for duct loss” by the World Congresses of Gastroenterology consensus panel.

GRADING AND STAGING. A tentative scheme for the grading of liver allograft rejection, which is indefinite for bile duct loss (discussed later) and chronic rejection (Table 69–5) has been proposed by the NIDDK liver pathologists but has not yet been tested for reliability or prognostic significance. As in acute rejection, the arteries affected by rejection-related causes are usually not present in needle biopsies. Therefore, grading of severity is based on “surrogate” markers of OA.
TABLE 69–5  NIDDK definitions of grades for chronic rejection and for rejection, indefinite for chronicity (indefinite for bile duct loss) ²⁰⁸

<table>
<thead>
<tr>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
</table>
| Rejection, indefinite for Chronicity (Indefinite for Bile Duct Loss) | 1. No complicating lobular changes  
2. Lobular changes, including one of the findings: centrilobular cholestasis, perivenular sclerosis, hepatocellular ballooning or necrosis, dropout  
3. Bile duct loss with at least two of the following four findings: centrilobular cholestasis, perivenular sclerosis, hepatocellular ballooning or necrosis, dropout |

Chronic (Ductopenic) Rejection ²⁰⁹

1. Bile duct loss without centrilobular cholestasis, perivenular sclerosis, or hepatocyte ballooning or necrosis and dropout  
2. Bile duct loss with one of the following four findings: centrilobular cholestasis, perivenular sclerosis, hepatocellular ballooning or necrosis, dropout  
3. Bile duct loss with at least two of the following four findings: centrilobular cholestasis, perivenular sclerosis, hepatocellular ballooning or centrilobular necrosis, dropout

* Bile duct loss in > 50% of triads.  
NIDDK = National Institute of Diabetes and Digestive and Kidney Diseases.  

**Differential Diagnosis.** The differential diagnosis of chronic rejection is limited to a few conditions, but as described previously, establishing the diagnosis with certainty is difficult, particularly in the early stages. Moreover, focal bile duct loss in a single biopsy does not necessarily indicate a widespread process. Other conditions associated with bile duct loss and cholestasis in a liver allograft include biliary tract obstruction and drug- or virus-related bile duct injury and loss.²⁰⁸

An unequivocal diagnosis of chronic rejection can be rendered only if a medium-size artery is sampled that shows foam cell OA.¹⁸⁶ Unfortunately, this is an extremely rare occurrence. Therefore, one is forced to rely on bile duct loss associated with the cholestasis and other lobular changes described previously. The safest approach is to review prior biopsies and closely correlate the histopathological findings with the clinical course. Bile duct loss in a patient who has experienced multiple or refractory episodes of acute rejection, who experienced a previous failed graft from chronic rejection, or who harbored preformed lymphocytotoxic antibodies and showed a progressive decline in ducts and liver function is more likely to be due to chronic rejection.⁷⁸ In addition to the biopsy findings, the decision to proceed with retransplantation should be based on clinical parameters such as a progressive decline in synthetic function, superimposed hepatic artery thrombosis, and bile duct necrosis or biliary sludging. Primary reliance on decline of liver function and clinical morbidity is urged because bile duct loss and jaundice have spontaneously resolved without clinical intervention in some patients.¹⁸⁹,²⁰⁹,²¹⁵ In our FK506 rescue protocol,²¹⁶,²¹⁷ we found that patients whose bile duct loss exceeded 50% of the triads and whose bilirubin level was higher than 10 mg/dl responded to therapy for chronic rejection much less frequently than those with less severe damage.²⁰⁸

**Graft-Versus-Host Disease**

Acute GVHD is most commonly seen after allogeneic bone marrow transplantation, although reports in recipients of unirradiated blood products that contain allogeneic leukocytes are not uncommon. Prerequisites of this reaction include immunocompetent mature donor T cells and a defenseless, or at least weakened, recipient immune system.²¹⁸ The donor T cells, carried with the allograft in the interstitium and hilar lymph nodes,²¹⁹ respond to recipient MHC-bearing cells that include relatively immature epithelial cell populations in the basal layers of the skin, gastrointestinal tract, liver, and lung. A more in-depth discussion of GVHD is beyond the intended scope of this text.²¹⁸ Nevertheless, GVHD further suppresses recipient immunity and predisposes to both bacterial and viral infection. Moreover, donor B cells can produce antirecipient antibodies that cause lysis of recipient red blood cells and hematolymphoid cells.¹¹,²²⁰

**Clinical Presentation.** Patients usually present during the first 6 months after transplantation with a fever, rash, and, at times, diarrhea.⁸⁴,¹⁰⁰–¹⁰² In ABO-compatible but mismatched cases, GVHD disease can take the form of red cell hemolysis and pancytopenia.²²⁰ The diagnosis of the more conventional type of GVHD is confirmed by detailed analysis of a tissue biopsy specimen usually taken from the skin or gastrointestinal tract.²²¹,²²² “Humoral” GVHD can be diagnosed by determining the nature of the red cell antibody responsible for hemolysis.

**Histopathological Findings.** Of course, the liver allograft is not susceptible to GVHD in a liver allograft recipient. Histopathological findings of GVHD in nonhepatic tissues are identical to those described for GVHD after bone marrow transplantation. In the skin, a mild, mixed, lymphocytic, and eosinophilic infiltrate in the upper reticular and papillary dermis is typically associated with lymphocytic exocytosis, spongiosis, acidophilic necrosis of individual keratinocytes and epithelial cells surrounded by lymphocytes (“satellitosis”) (Fig 69 – 15). Unfortunately, indistinguishable histopathological findings may also be present because of a viral exanthem or an adverse drug reaction.²²¹ In the intestines, there is usually no increase in the number of inflammatory cells above those already present. Instead, one usually finds apoptosis in crypt epithelial cells without viral inclusions.²²² Disease severity progresses in a fashion similar to that described for bone marrow allograft recipients. More severe GVHD in the intestine manifests as crypt abscesses with focal areas of crypt destruction and ultimately areas of mucosal necrosis and sloughing; it manifests in the skin by formation of bullae and epidermal sloughing.

**Differential Diagnosis.** In the skin, the two most common syndromes mimicking acute GVHD are adverse drug reaction and a viral exanthem. In the gastrointestinal tract, GVHD is most similar to CMV enteritis without obvious inclusion bodies. Distinguishing GVHD from these entities using routine histopathological findings alone is extremely difficult if not impossible. In addition to a detailed clinical history (including a list of medications, pos-
BACTERIAL AND FUNGAL INFECTIONS

The first 2 months is the critical time for the most serious opportunistic fungal and viral infections.\textsuperscript{223} Thereafter, most infections are due to bacterial pathogens. Clinical histories that should also arouse the suspicion of an infection include anastomotic or wound dehiscence, retransplantation, fever, persistent abdominal pain, and vascular thrombosis. However, a high index of suspicion should always be maintained.

Any nonviable hilar tissue removed from the allograft or from the immediate vicinity is routinely subjected to special stains (Gram's and Grocott's) for the detection of micro-organisms. A caveat for the pathologist to remember is that inflammation may be mild or absent because of immunosuppression, and organisms are easily overlooked on H&E stains alone (see Fig 69-7).

The histopathological changes associated with bacterial or fungal infections of tissues are well known to most surgical pathologists and are not discussed. However, histopathologists involved with the care of transplant patients are encouraged to have a good working knowledge of the histopathological characteristics of superficial and deep fungal, bacterial, and viral infections, as referred to early in this chapter. The manifestations of these infections are not entirely different from those seen in the general population, with several exceptions alluded to in each of the sections on viral infections. In addition, common dermatophytes can cause deep infections of the skin or visceral organs (unpublished observation). In such cases, the final diagnosis was arrived at by being unable to classify the fungal pathogens on morphological criteria alone and on the basis of the growth of dermatophytes in cultures of the tissue specimens.

VIRAL INFECTIONS

Liver allograft recipients are highly susceptible to and frequently acquire viral hepatitis. Many have been chroni-
cally infected with the hepatic viruses, such as hepatitis B, C, and D viruses (HBV, HCV, HDV, respectively) before transplantation. After transplantation, they receive multiple blood products and require potent immunosuppression, which makes them susceptible to opportunistic hepatitis viruses, such as CMV, Epstein-Barr virus (EBV), herpes simplex virus (HSV), and varicella-zoster (VZ), that do not usually cause hepatitis in the general population.

In general, the histological appearance of viral hepatitis in the liver allograft is similar to that observed in non-grafted livers from immunosuppressed patients and to viral hepatitis in the general population. In addition, because of the potent immunosuppression and allogeneic liver, there are pathophysiological presentations of viral hepatitis that are unique to the liver allograft recipient.

**Clinical Presentation.** It is helpful to anticipate the time of onset of the different viral syndromes (see Table 69 - 1). It is also helpful to know that recipients who are seronegative for opportunistic viral pathogens like CMV, EBV, HSV-VZ, and adenovirus and receive seropositive organs show an increased incidence and severity of disease after transplantation. Furthermore, although blood product screening for HBV and HCV is routine, de novo infection with these pathogens in a liver allograft recipient is not rare. Treatment of opportunistic pathogens usually involves lowering of immunosuppression and addition of an effective antiviral drug, such as acyclovir. The "hepatic" viruses are a particular problem, because the current drug arsenal used to treat them is not very effective, and may precipitate rejection. Furthermore, lowering immunosuppression can, in some cases, exacerbate the disease.

**HEPATITIC VIRUSES**

**Hepatitis B and Delta Coinfection**

HBV infection with or without delta coinfection is largely restricted to patients whose original liver disease was caused by this virus.13, 224-234 Virtually all patients who show evidence of viral replication before transplantation (ie. hepatitis B antigen [HBAg] seropositive or HBV DNA-positive) will experience reinfection of their allograft and hepatitis after transplantation. Allograft reinfection and disease are less predictable in patients who had HBV-induced fulminant liver failure or in those with chronic liver disease who had become anti-HBc positive and serum HBV DNA and HBAg negative before transplantation.13, 224-234 Approximately 10 to 25% of the latter patients will not experience reinfection of the allograft, nor will they experience HBV disease. Despite donor and blood product screening, a small number of patients without previous HBV disease will acquire HBV infection during or after transplantation.

Because of the high incidence of recurrence, various treatment modalities have been used in an attempt to break the cycle of reinfection. These include hepatitis B immune globulin, active vaccination with hepatitis B surface antigen (HBsAg), INF-α, and human monoclonal anti-HBc. Although none of these therapies appears to be entirely effective in preventing reinfection, a later onset of disease recurrence and amelioration of liver injury have been reported with continuous high-dose anti-HBAg therapy.13, 224-234 The high infectivity of HBV and the documentation of extrahepatic reservoirs of HBV probably account for the difficulties encountered in eradicating the virus.

**Pathophysiology.** It is beyond the scope of this chapter to delve into a detailed discussion of the pathophysiology of HBV-induced liver disease; however, some pathophysiological presentations and observations unique to the allograft recipient could provide useful insights into the overall disease pathogenesis. Under normal circumstances, HBV is not thought to be cytopathic. Liver damage is thought to be at least partially attributable to the expression of hepatitis B core antigen (HBcAg) on the surface of hepatocytes, which then become targeted for destruction by MHC-restricted CTLs.235, 236 Alternatively, in the allograft, the processing of viral antigens by periporal recipient accessory cells and their MHC class II-restricted presentation to T lymphocytes in a fashion resembling a delayed-type hypersensitivity response may be involved.225, 237

It should be remembered that CTL lysis of virally infected parenchymal cells is MHC restricted, and identity between the donor and recipient is a matter of chance. There is no attempt at prospective MHC matching in clinical liver transplantation.225, 237 There are too few cases at this time to draw any definite conclusions about the relationship of MHC matching or mismatching and HBV disease pathogenesis. Massive viral replication in class II MHC-mismatched deteriorating liver allografts with little or no hepatic inflammation prompted several groups to suggest that, under special circumstances, the virus may be directly cytopathic.225, 227-229, 238, 239

**Clinical Presentation.** HBV hepatitis usually occurs 6 to 8 weeks after transplantation. The presentation varies from mild elevations of liver injury tests to nausea, vomiting, jaundice, and hepatic failure. The clinical syndrome, therefore, is not significantly different from viral hepatitis seen in other immunosuppressed or even nonimmunosuppressed patients in the general population.13, 224-234 Needle biopsy evaluation confirms the diagnosis.

**Histopathological Findings.** The histopathological presentation of hepatitis B infection in the hepatic allograft is similar to that seen in nonallograft livers, although local treatment policies can apparently influence the onset and severity of the histopathological findings.13, 224-232, 234, 238, 239 In the majority of patients who eventually experience chronic disease, there is a typical progression from an acute to a chronic hepatitis, and cirrhosis can develop with striking rapidity.13, 224-232, 234, 238, 239 However, occasional patients will show histopathological resolution of disease activity after a bout of acute hepatitis, and rare patients will actually "clear" the virus after transplantation. There are also several pathophysiological presentations of HBV unique to the liver allograft that are likely related to the potent immunosuppression and MHC nonidentity between the liver and recipient.
In the majority of patients, acute viral hepatitis type B presents in a predictable fashion, first manifest within several weeks after transplantation by the appearance of cytoplasmic hepatitis core antigen in an occasional hepatocyte. Characteristically, the infection, and thus core and surface antigens, spread throughout the liver to involve a large number of cells, and then lobular necroinflammatory activity, Kupffer cell hypertrophy, lobular disarray, and varying amounts of portal inflammation mark the onset of acute disease (Fig 69-17). Even though patients are immunosuppressed, a small number can experience bridging or even submassive necrosis at this stage, particularly if immunosuppression is withdrawn. A few other patients show resolution of the acute necroinflammatory activity but persistence of infection. A more common scenario is evolution into chronic disease. This is characterized by lymphoplasmacytic portal inflammation with relative sparing of the bile ducts and portal veins and varying degrees of piecemeal necrosis characterized by extension of lymphocytes and macrophages into the edge of the lobule combined with cholangiolar proliferation (Fig 69-18). Lobular findings typical of the chronic phase of infection include a large number of cells with a ground glass cytoplasm containing HBVAg and, on occasion, numerous hepatocytes with sanded nuclei filled with HBVAg. This is accompanied by varying degrees of lobular disarray, regeneration, Kupffer cell hypertrophy, and lobular necroinflammatory activity.

Several patterns of liver injury associated with HBV are not commonly encountered in the general population, and are probably (but not definitely) related to effects of immunosuppression and MHC nonidentity between the liver and recipient. The first is characterized by marked hepatocyte swelling, lobular disarray, and cholestasis, with only mild or no portal or lobular inflammation and varying degrees of cholangiolar proliferation. Such cases are usually marked by massive hepatocellular production of core and surface antigen, in association with the hepatocyte degenerative changes including cell swelling, steatosis, and necrosis (Fig 69 - 19). Follow-up biopsies may reveal progressive portal and periportal sinusoidal fibrosis and lobular collapse often without a significant inflammatory component. High levels of viral replication and antigen expression in these cases have led several groups to suggest that HBV may be directly cytopathic under the special circumstance of an allograft liver in an immunosuppressed patient. The Cambridge group suggested the term fibrosing cholestatic hepatitis to describe this lesion, whereas Benner et al preferred fibrosing cytolytic hepatitis to describe a similar, if not the same set of findings. Phillips et al also emphasized the heavy viral burden in liver allograft recipients, which caused swelling of the endoplasmic reticulum and hepatocellular degenerative changes. They drew particular attention to the presence of hepatocellular steatosis and also coined the terms steatoviral and fibroviral hepatitis.

Figure 69-17  A. Acute hepatitis type B is most often similar to the acute hepatitis seen in the general population. It is characterized by portal inflammation of varying severity, accompanied by lobular disarray, Kupffer cell hypertrophy, and spotty acidophilic necrosis of hepatocytes (arrows). B. A higher magnification shows the typical lobular findings associated with acute viral hepatitis. C. Occasionally, severe acute disease can result in bridging necrosis, as shown here, particularly if immunosuppression is withdrawn.
Chronic hepatitis type B often appears histopathologically similar to chronic hepatitis B in the general population, characterized by mononuclear portal inflammation with spillover of the infiltrate into the edge of the lobules and associated with perilobular hepatocyte necrosis and relative sparing of the bile ducts (arrows) (pt = portal tracts). A higher magnification illustrates the perilobular activity, intact bile duct (arrow), and expression of hepatitis B core antigen in the nuclei of hepatocytes (arrowhead). (A and B from Demetris AJ, Todo S, Van Thiel DH, et al. Evolution of hepatitis B virus liver disease after hepatic replacement: Practical and theoretical considerations. Am J Pathol 137:667–676, 1990.)

... accentuating the features of fat and fibrosis. The exact relationship between the new terms proposed by Phillip et al. and those proposed by Benner and Davies and their colleagues is uncertain, but in general they appear to describe a similar set of findings. More important, this relatively unique presentation in the allograft could provide useful insights into disease pathogenesis.

Finally, a massive overproduction of the surface antigen can be seen in the allograft liver, with the vast majority of hepatocytes containing a ground glass cytoplasm, similar to that in HBV transgenic mice. This results in marked cytological distortion of the hepatocytes, lobular disarray, and mild necroinflammatory lobular activity.

In patients with histopathological features compatible with one of these presentations, immunohistochemical staining for HBVAg and HBcAg using polyclonal reagents are utilized to detect viral antigens. Our experience with monoclonal anti-HBV reagents has yielded results of higher specificity but lower sensitivity. Patients with cytoplasmic HBcAg expression have experienced a more aggressive course in our experience (unpublished observation).
As in the general population, delta agent coinfection may complicate reinfec­tion of the allograft by HBV. Follow-up of such patients has yielded somewhat conflicting results, with reports of both more and less severe disease after transplantation.232, 234, 242-245 In addition, there are conflicting reports about the cytopathic effect of HDV after transplantation and its relationship to HBV replication. David et al246 noted that HDV associated with nonreplicative HBV infection resulted in hepatic lesions similar to those described as “fibrosing cholestatic,” “fibrosing cytolytic,” or “steatoviral” hepatitis but without HBAg expression in the liver. In contrast, when active HBV replication was present, the HBV plus HDV hepatitis in the allograft produced necroinflammatory activity similar to that seen in viral hepatitis types B and D in patients from the general population.246

**Differential Diagnosis.** Acute hepatitis B is most often confused with acute hepatitis caused by other viruses. Distinction is usually achieved with the aid of special studies to detect viral antigens or nucleic acids in the blood or tissues or antibody reactions to the virus. Acute rejection and acute hepatitis can also be confused with each other at times.224, 225 Histopathologically, the lobule is the focus of injury in acute hepatitis. Spotty hepatocyte necrosis, disarray, and lobular necroinflammatory activity with varying degrees of portal inflammation are the usual findings. In contrast, immune damage in acute rejection is primarily directed at the portal structures, including the portal vein and bile ducts and portal inflammation is invariably present.224, 225

Difficulties can be encountered when trying to separate late-onset acute and chronic rejection from chronic “persistent” or chronic hepatitis with low-grade activity in cases in which no ground glass hepatocytes or sanded nuclei are seen. Prominent piecemeal necrosis, damage of only an occasional bile duct, perportal bridging necrosis and lobular disarray, and necroinflammatory activity usually are not features of acute rejection and thus point toward hepatitis as the cause of malfunction. In contrast, damage of more than an occasional bile duct and bile duct loss are not generally seen in hepatitis and point toward rejection. There also are occasional cases in which the separation of acute rejection and chronic hepatitis will not be possible.71, 208, 215 In such cases, our policy is to err toward overtreating viral hepatitis as rejection.

Finally, infection of the allograft by HBV does not equate with HBV disease or, for that matter, exclude rejection.225 Detection of either the core or surface antigen by immunohistochemistry may be seen in an allograft that otherwise has all the features of acute or chronic rejection. Several patients have persistently harbored HBV in the allograft but have experienced graft failure because of bile duct loss and OA.225

**Hepatitis C Virus**

Recurrent and de novo HCV infection is becoming recognized as a more serious problem in liver transplantation than originally thought. Earlier studies evaluating rates of recurrent infection and disease severity were based on serological evidence of infection, which clearly underesti­mated the extent of the problem because of a poor immunological response to the virus after transplantation.247-251 The rate of recurrent infection, on the basis of reverse transcriptase polymerase chain reaction (RT-PCR) testing of serum and tissues, is extremely high, being 90% or more in some centers.252-257 Data on the incidence and severity of HCV-related disease in the posttransplant population are just beginning to accumulate.252-257 Currently, it is estimated that a majority of those reinfected experience acute hepatitis, whereas 2 to 60% go on to have chronic HCV-related disease activity.252-257 The difference in chronic disease severity at various centers is likely related to several factors, including immunosuppression policies, lengths of follow-up, methods of viral detection, use of antiviral therapy, and frequency of needle biopsy evaluation.

**Clinical Presentation.** The clinical presentation of HCV hepatitis is virtually identical to that seen in the general population. Many times, the early phases of the disease may be asymptomatic, detectable only by an elevation of liver injury test findings, which are frequently monitored in this patient population. Fatigue, nausea, jaundice, and other typical signs of acute hepatitis are less frequent, and fulminant liver failure is rare.252-257 Needle biopsy evaluation is used to confirm the diagnosis.254, 258

**Histopathological Findings.** The histopathology of viral hepatitis type C in the liver allograft recipient is not substantially different from that seen in the general population, although, as is the case for hepatitis B, there are pathophysiological presentations unique to the allograft recipient.11, 28, 31, 254, 258 Similar to HBV, the acute stage is characterized by findings typical of acute viral hepatitis such as lobular disarray, Kupffer cell hypertrophy, spotty acidophilic necrosis of hepatocytes, and variable portal and lobular inflammation.28, 30, 31, 254, 258 Findings of more severe acute disease include bridging and confluent necrosis, although submassive necrosis caused by HCV in liver allograft recipients is rare.

Unresolved cases of HCV can then evolve into the chronic phase characterized by nodular portal lymphoid aggregates (Fig 69-20), piecemeal necrosis, mild midzonal or periportal macrovesicular steatosis, sinusoidal lymphocytosis (beading), and damage to an occasional bile duct, similar to that described in the general population.254, 258-260 Atypical histopathological features of HCV described in liver allograft recipients include centrilobular hepatocellular swelling, ductular proliferation, and acute cholangiolsis with portal fibrosis (see Fig 69-20), similar to “fibrosing cholestatic hepatitis” described earlier, including the paucity of portal tract inflammation.254, 258

The diagnosis of type C viral hepatitis is suspected on the basis of histopathological findings and is confirmed by showing viral RNA in the liver tissue or serum by RT-PCR. At present, we have not had much success with immunohistochemical staining for viral antigens in routinely processed liver biopsies.

**Differential Diagnosis.** Acute HCV hepatitis must be separated from acute hepatitis caused by other viruses such as HBV, CMV, and EBV. CMV hepatitis usually
shows less lobular disarray, and instead of diffuse lobular inflammation or sinusoidal lymphocytosis, CMV hepatitis typically shows distinct clustering of the lobular infiltrate into microabscesses or microgranulomas. Nuclear and cytoplasmic inclusions are seen in CMV and not in HCV. EBV hepatitis in an allograft recipient is more difficult to separate from hepatitis C on the basis of histopathological findings alone. Both show sinusoidal lymphocytosis, although atypical lymphocytes are present in EBV and not in HCV. EBV hepatitis may also contain microgranulomas. Moreover, the clinical profile of patients with EBV hepatitis is different from that of HCV patients, and, fortunately, use of RT-PCR for HCV and in situ hybridization for EBV are useful for distinguishing between them in tissue sections.

The differential diagnosis for chronic HCV hepatitis includes acute and chronic rejection, recurrent non-HCV viral hepatitis, recurrent autoimmune chronic hepatitis, recurrent primary biliary cirrhosis, and recurrent primary sclerosing cholangitis as well as bile duct obstruction. Exclusion of chronic HCV liver disease is based on the absence of HCV by tissue RT-PCR. HBV is identified on the basis of viral antigens, which are invariably present and detected in the serum or with immunoperoxidase staining of tissue specimens. Recurrent primary biliary cirrhosis is recognized on the basis of portal-based granulomas, minimal lobular activity, and the evolution of "biliary" fibrosis, which is not seen with chronic HCV.

Cholestatic hepatitis is difficult to differentiate from bile duct obstruction and hepatic artery thrombosis. Portal edema and portal, rather than periportal, neutrophilia are common in duct obstruction and acute cholangitis, whereas cholangiolar proliferation and acute cholangitis without portal edema are more characteristic of cholestatic hepatitis. In addition, lobular disarray and marked hepatocellular swelling are more usual for viral hepatitis in contrast to duct obstruction.

Acute and chronic rejection may be difficult to separate from chronic viral hepatitis C, mostly because both are characterized by portal inflammation and bile duct damage. However, in acute or chronic rejection, bile duct damage and loss involve more than an occasional duct, whereas in HCV only an occasional bile duct is damaged. In addition, lobular disarray is unusual for rejection but is common in hepatitis. Furthermore, centrilobular inflammation, fibrosis, and hepatocellular dropout present in more than an occasional central vein, are more often seen in rejection than in viral hepatitis. Unfortunately, it is not always possible to separate rejection from hepatitis on the basis of the histopathological findings alone. In our experience, we prefer to err on the side of overdiagnosis of rejection.

Hepatitis Types A and E

We have not as yet identified hepatitis types A and E as causes of allograft dysfunction. However, Fagan et al. showed hepatic persistence or reinfection of a liver allograft of a recipient who required transplantation for ful-
minant hepatic failure because of the hepatitis A virus. Extrahepatic reservoirs of the virus were thought to account for reinfection and hepatitis of the allografted liver. On the basis of these and the prior observations for hepatitis types B, C, and D, it might be expected that hepatitis in a liver allograft caused by these viruses would appear similar to that seen in nonallografted livers.

Hepatitis Type F

Several patients identified at the University of Pittsburgh have experienced a classic chronic hepatic histopathological profile but without evidence of hepatitis B, C, or D infection, and other causes of allograft dysfunction were reasonably excluded. Therefore, it appears that there may be yet another hepatitis virus capable of reinfecting a liver allograft. At least one of these patients experienced liver failure in two successive allografts but was repeatedly RT-PCR negative for hepatitis C virus in liver tissue.

OPPORTUNISTIC VIRUSES

Cytomegalovirus Hepatitis

CMV is the most commonly encountered opportunistic viral infection of the liver allograft recipient. Symptomatic disease is usually encountered during or after bolstered immunosuppressive therapy (eg, treatment of rejection) between 3 and 8 weeks post transplant. The infection may be the result of reactivation in a carrier, transmission through blood products or the donor organ, or acquisition from other sources in the environment. Seronegative recipients who receive seropositive donor organs are at the greatest risk for symptomatic disease. Viral infection and latency in granulocytes or endothelial cells (D. Sedmak, personal communication, November 30, 1994) and monocytes may explain the early appearance of viral antigens in the sinusoidal cells.

Clinical Presentation. Signs and symptoms of active CMV infection include fever, leukopenia and modestly elevated liver injury test results, although any organ system can be involved depending on the extent of viral dissemination. More frequent complications include diarrhea, gastrointestinal ulcers, and hepatitis. Respiratory insufficiency and retinitis occur when the disease is severe. The morbidity and mortality of CMV infection are also associated with viral dissemination. Occasionally, the disease can mimic the posttransplant syndrome associated with the EBV infection, which includes mild liver function abnormalities, lymphadenopathy, fever, and atypical lymphocytosis on the peripheral blood smear.

Histopathological Findings. The histopathological manifestations of active CMV infection depend, in part, on the immune status of the host. In excessively immunosuppressed patients who have no prior serological evidence of CMV infection, any cell type of the liver may be infected, and viral inclusions are numerous. The cytomegalic cells contain a large eosinophilic intranuclear inclusion surrounded by a clear halo, and occasionally small basophilic or amphophilic cytoplasmic inclusions are also seen. Despite widespread CMV infestation of the liver allograft, fulminant liver failure from submassive or massive necrosis from CMV alone has never been seen.

With improved immunological monitoring, effective pharmacological therapy, and immunoprophylaxis, overwhelming CMV infections, like those described previously, are now uncommon. With the current pharmacological arsenal, CMV hepatitis is usually characterized by spotty lobular necrosis, Kupffer cell hypertrophy, mild lobular disarray, and microabscesses or microgranulomas scattered throughout the lobules (Fig 69-21). The necrotic hepatocytes or nearby cells may contain nuclear and cytoplasmic inclusions and are surrounded by neutrophils (microabscess) or cluster macrophages and lymphocytes (microgranulomas). Mild plasmacytic and lymphocytic portal inflammation, associated with bile duct cell infiltration and damage, may also be seen, mimicking, or actually associated with, acute or chronic rejection (see Fig 69-21). Duct loss has been associated with persistent infection of the allograft by CMV, On occasion, the characteristic parenchymal alterations described previously may be seen but without clear evidence of cytomegaly or a nuclear or cytoplasmic CMV inclusion. In such cases, immunoperoxidase staining for early viral antigen can be used to detect the infected cells.

In cases resembling EBV hepatitis, needle biopsy of the liver allograft will reveal a mild lymphoplasmacytoid portal and lobular infiltrate, with blastic and atypical lymphocytes present. Microgranulomas are usually seen in the lobules, although characteristic CMV inclusions are absent. Deeper cuts into the block, immunoperoxidase stains for EBV and CMV viral antigens, and in situ hybridization for EBV nucleic acids are usually required to distinguish between CMV and EBV in such cases.

“Activated” or rapidly dividing tissues such as young granulation tissue, proliferating cholangioles seen in ischemically damaged livers, edges of infarcts, abscesses, or other intraparenchymal defects are fertile soil for CMV growth. When such tissue is encountered, a more careful search for CMV is warranted.

Differential Diagnosis. Multinucleated cells may be seen in CMV hepatitis, simulating an HSV infection. However, CMV-infected cells may also contain small basophilic or amphophilic cytoplasmic inclusions, which are not seen in HSV. Also, the circumscribed zones of coagulative necrosis characteristic of HSV generally are not encountered with CMV.

CMV hepatitis is, for the most part, separable from acute hepatitis caused by hepatotrophic hepatitis B or C viruses. However, individual cases can pose considerable difficulties, especially when no CMV infections are detected. Moreover, the distinction is important because medical therapy for hepatotrophic versus opportunistic viral hepatitis is dramatically different; for CMV, ganciclovir is routinely used, whereas for the hepatotropic viruses, the usual treatment is INF-a. In general, CMV causes much less lobular disarray and hepatocyte swelling, and the lobular inflammation seen with hepatotrophic vi-
ruses is more diffuse, not being clustered in microabscesses or microgranulomas. In the end, reliance is placed on adjuvant techniques including viral cultures for CMV, RT-PCR assays for HCV, immunoperoxidase staining for viral antigens (CMV, HBV) or in situ hybridization for viral nucleic acids (EBV), all of which are quite helpful in identifying the cause of hepatitis.

A difficult challenge is determining whether the liver injury is due to residual CMV hepatitis or to the onset of acute or chronic rejection, because CMV hepatitis most commonly occurs in patients who are under treatment or have recently completed an augmented immunosuppressive regimen for rejection. Experience with renal transplantation has shown that CMV (and other infections) can precipitate an episode of rejection in the allograft. Further complicating the issue is the report by O'Grady et al.\(^\text{28}\) and others showing an association between CMV infection of liver allograft and chronic rejection.\(^\text{196, 198, 199, 285–287}\) Others have not seen this association.\(^\text{288}\) Difficulties arise in the pathological interpretation of biopsies in which obvious CMV inclusions are not detected, but staining for viral antigens is positive and other histopathological findings typically seen with acute rejection or loss of bile ducts are also seen. In our experience, the presence of CMV inclusions or antigens in the biopsy specimen is generally given priority, and the immunosuppressive therapy is lightened and ganciclovir is given. Follow-up biopsy after 1 to 2 weeks, if liver function abnormalities persist, is used to follow therapy. Unfortunately, bile duct loss may develop and persist in such patients.

**Herpes Simplex and Varicella-Zoster Viral Hepatitis**

Both subtypes of HSV (types 1 and 2) and VZ have been identified as causes of liver allograft hepatitis.\(^\text{28, 30, 31, 289}\) They have been seen as early as 3 days after transplantation but may occur anytime thereafter. The clinical presentation has included fever, vesicular rashes, fatigue, and body pain combined with serological evidence of hepatic injury. If HSV hepatitis goes unrecognized, it may rapidly lead to submassive or massive hepatic necrosis, hypotension, disseminated intravascular coagulation, and metabolic acidosis.\(^\text{289}\) Fulminant cases usually occur in patients without evidence of prior humoral immunity. Early recognition using needle biopsy sampling is particularly crucial because effective medical therapy is available.

**Histopathological Findings.** Two histopathological patterns of HSV hepatitis have been identified: localized and diffuse. However, distinction between the two may be more related to swiftness in establishing the diagnosis, level of immune competence, and evidence of prior immunity than to differences in the viral biology. The pattern of injury important to recognize is that of circumscribed areas of coagulative-type necrosis, showing no respect for the lobular architecture.\(^\text{28, 30, 31, 289}\) (Fig 69–22). Ghosts of hepatocytes, intermixed with neutrophils and nuclear debris, are seen in the center of the lesions. More viable hepatocytes at the periphery may be slightly enlarged and contain “smudgy” or ground glass nuclei or characteristic Cowdry type A eosinophilic inclusions (see Fig 69–22). We have not been able to distinguish reliably between HSV and VZ on the H&E slides alone. Multinucleate cells are occasionally present, but, not infrequently, no changes diagnostic of HSV or VZ will be detected on the H&E side. In such cases, immunoperoxidase stains for HSV antigens can be confirmatory. Some antibody preparations used to detect HSV subtypes show considerable cross-reactivity, making it difficult to separate HSV1 from HSV2 using immunohistochemistry. Separation of VZ from HSV using monoclonal antibodies is not usually a problem in our experience.

**Differential Diagnosis.** It can be difficult to distinguish the edge of an infarct from the periphery of a necrotic HSV lesion. The most obvious distinction is that inclusion...
Herpes simplex hepatitis (HSV). HSV and varicella-zoster hepatitis are characterized by large areas of coagulative-type necrosis. A. The center of the necrotic lesions contain neutrophils and nuclear debris, whereas more viable cells at the periphery (arrows) contain the inclusion bodies, when present. B. A higher magnification shows some multinucleated cells containing the characteristic Cowdry A type inclusions (arrow).

bodies are present in HSV and VZ hepatitis and absent at the periphery of an infarct. However, unequivocal HSV inclusions may not be present; only cells with a smudged nuclear chromatin may be found. In such cases, it is our policy to overdiagnose HSV hepatitis in patients with a compatible clinical profile because, without the highly effective treatment, it can rapidly cause liver failure and death.

Separation of HSV-VZ hepatitis from CMV hepatitis is occasionally a problem. HSV is associated with large areas of coagulative-type necrosis, whereas, alone, CMV rarely causes confluent hepatocyte necrosis. In addition, CMV hepatitis may show nuclear and cytoplasmic inclusions, whereas the inclusions of HSV are exclusively nuclear.

Epstein-Barr Virus

EBV latently infects a majority of the general population. It often becomes active after liver transplantation when 1 to 2% of recipients experience persistent or recurrent EBV disease. This can eventually result in the development of posttransplant lymphoproliferative disorder (PTLD), which is a proliferation of B cells that sometimes acts like an aggressive lymphoma. As with the other opportunistic viruses, the incidence of disease is higher and the severity of the complications is worse in patients who were seronegative before transplantation but who received an allograft from a seropositive donor.

EBV is known to infect and lay dormant in B lymphocytes and some epithelial cells. In vitro, in the absence of T cell regulatory influences, infection of B lymphocytes by EBV “immortalizes” the B cells, especially in the presence of immunosuppressive drugs. In vivo, maintenance of viral latency is the product of immune surveillance by T cells that keep viral replication and B cell proliferation in check. However, potent immunosuppressive therapy depresses immune surveillance and accounts for the various disease manifestations listed later. Recurrent or persistent disease can result in the emergence of oligoclonal or monoclonal B cell proliferations that sometimes act like aggressive lymphomas. It should be remembered that monoclonality alone does not necessarily mean that the lesion will behave autonomously and be free from immune surveillance or need treatment with chemotherapy.

A more detailed discussion of the pathophysiology of EBV in the allograft recipient is beyond the scope of this chapter. The interested reader is referred to several excellent reviews.

Clinical Presentation. The systemic viral syndrome associated with EBV often resembles that seen with classic infectious mononucleosis. Fever, lymphadenitis, pharyngitis, and jaundice are the typical findings, but atypical signs and symptoms in the form of jaw pain, arthralgia, joint space effusions, diarrhea, encephalitis, pneumonia, and mediastinal lymphadenopathy and ascites can also be seen. Laboratory investigation usually shows elevation of the levels of hepatocellular liver enzymes and circulating atypical lymphocytes in the peripheral blood. Pancytopenia is noted on occasion.

Unresolved or recurrent EBV syndromes often culminate in the development of a PTLD. Although PTLD can involve any site in the body, most commonly it affects the lymph nodes, hepatic allograft, and gastrointestinal tract. Signs and symptoms attributable to a mass lesion at the site of involvement are not uncommon. Withdrawal or a dramatic reduction in immunosuppression with the addition of antiviral agents such as acyclovir is the first line of therapy regardless of the histopathological appearance or clonality of the lesion. This maneuver is an attempt to restore immune regulation or surveillance, which, if unsuccessful, can be supplemented by conventional chemotherapy treatment in some circumstances.

Histopathological Findings. EBV hepatitis usually shows portal and perportal mononuclear infiltrates of varying severity (Fig 69-23) composed of small and blas-
tic lymphocytes, some of which are atypical, admixed with plasmacytoid lymphocytes and plasma cells. Eosinophils and neutrophils are much less common in the portal tracts than in acute rejection. Bile duct damage can be seen, but the severity and prevalence of duct damage are less than would be expected for rejection on the basis of the severity of the portal infiltrate. Subendothelial localization of lymphocytes can be seen in the portal or central veins.

The lobule typically shows features of a "reactive" or low-grade hepatitis taking the form of focal hepatocellular swelling, mild acidophilic necrosis of hepatocytes, and mild lobular disarray. Regenerative activity, including double-layered plates, pseudoacinar formation, and mitotic figures, is common. The sinusoids contain linearly aligned and focally aggregated mononuclear cells, which are cytologically similar to those seen in the portal triads. Occasional granulomatoid aggregates can also be seen.

In the liver, PTLD manifests as map-like enlargement of portal triads because of sheets of monomorphic atypical immunoblastic cells (see Fig 69–23), which obscure the normal architectural landmarks. Smaller aggregates composed of a similar cell population can be seen in the sinusoids, and on occasion, focal areas of necrosis are present. Cytologically, the infiltrate resembles an immunoblastic lymphoma, and there are usually many more cells with atypical cytological features, occasionally including Reed-Sternberg-like cells, than seen in EBV hepatitis. The diagnosis is confirmed by in situ hybridization for EBV RNA (EBER sequence). The reader is referred elsewhere for a detailed discussion of the extrahepatic manifestation of PTLD in lymph nodes and other tissues.

**Differential Diagnosis.** EBV hepatitis is most often confused with acute rejection and acute and chronic type C and CMV hepatitis. In addition to the clinicopathological profile, which is helpful in differentiating among these various syndromes, reliance is placed on adjuvant techniques to identify viral antigens and nucleic acids in the tissue. Histopathologically, both hepatitis C and EBV can show sinusoidal lymphocytosis or a linear alignment of mononuclear cells in the sinusoids. However, the sinusoids contain cytologically atypical cells in EBV, whereas in HCV small, round, inactive-appearing lymphocytes are present in the sinusoids and portal tracts.

Separation of EBV hepatitis or PTLD from acute rejection is also difficult at times. In acute rejection, the portal infiltrate typically is a mixed one, containing blastic and smaller lymphocytes, neutrophils, plasma cells, and often numerous eosinophils. In contrast, the portal infiltrate in EBV is less pleomorphic, consisting primarily of activated and immunoblastic mononuclear cells, many of which contain features of plasmacytic differentiation. Eosinophils and neutrophils are less common in EBV-related

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**Figure 69–23** Epstein-Barr virus (EBV) hepatitis and posttransplantation lymphoproliferative disorders. A. EBV hepatitis is characterized by a mononuclear portal infiltrate that sometimes is seen beneath the endothelium of the portal vein (arrow). There is also sinusoidal lymphocytosis (arrowheads) and mild lobular regenerative activity. B. Mistaken treatment of the patient whose biopsy is shown in A with increased immunosuppression resulted in the development of a posttransplantation lymphoproliferative disorder seen here as massive expansion of the triads by a population of atypical mononuclear cells (inset) showing features of plasmacytic differentiation. C. Discontinuation of the immunosuppression (and 1 day of cyclophosphamide treatment) in this patient resulted in resolution of the portal infiltrate and return of the normal architecture in 3 to 4 weeks.
disorders. Moreover, inflammatory infiltration and damage of bile ducts are common in acute rejection, whereas in EBV-related disorders, the bile duct damage is relatively mild, compared with the intense portal inflammation. 261–266

In the end, the diagnosis of EBV-related disorders is confirmed by in situ hybridization for the EBER RNA sequence of EBV, a technique that has greatly assisted in the management of allograft recipients. 261, 262, 293 Widespread availability of the EBER probe has led to a greater appreciation of the extent of EBV infection in this population, although the results have to be interpreted with caution. 293 It is known that occasional cells containing the EBER sequence are not uncommon in the general population and are found with increased frequency in an allograft recipient. The significance of occasional EBER-positive cells is open to debate. 293 However, in our opinion, clustering of such cells into aggregates or the presence of EBER-positive cells in tissues showing other histopathological features of EBV-associated disease (unpublished observation) points toward at least a transient defect in the ability to control viral replication and B cell proliferation. Caution with immunosuppression management in such patients is an appropriate course of action.

Adenoviral Hepatitis

Adenoviral infection and disease after liver transplantation are largely limited to the pediatric population. 294–296 Presumably most adults already have protective immunity and, thus, are less susceptible. Viral subtypes 1, 2, and 5 have been isolated from the lung, gastrointestinal tract, and liver in patients with fever, respiratory distress, diarrhea, and liver dysfunction. 294–296 The onset of disease usually occurs between 1 and 10 weeks after transplantation, and biopsy histopathological study is used to ascertain tissue disease. Hepatitis is most often caused by subtype 5, but subtypes 2, 11, and 16 have been associated with hepatitis in the general population and could be expected to infect and cause disease in liver allografts. 294–296

**Histopathological Findings.** Histopathologically, adenoviral hepatitis is distinctive, but some experience is required to establish the diagnosis with certainty. Most characteristic are the “pox-like” granulomas, consisting almost entirely of macrophages, which are spread randomly throughout the parenchyma, encompassing small groups of necrotic hepatocytes (Fig 69–24). The nuclei of hepatocytes located near the edge of the necrotic zones or granulomas often contain the distinctive adenoviral inclusions (see Fig 69–24). They are characterized by a crowding of chromatin toward the nuclear membrane, imparting a muffin-shape appearance to the nucleus. Immunohistochemical staining is confirmatory.

**Differential Diagnosis.** The histopathological differential diagnosis includes other causes of hepatic granulomas, such as deep fungal or mycobacterial infections, that can be excluded by microbiological cultures of the biopsy and negative special stains for granuloma-causing organisms. CMV and HSV-VZ should also be distinguished from adenovirus. The granulomas associated with adenovirus consist almost entirely of macrophages and are much larger than the “microgranulomas” of CMV, and multinucleate giant cells are rare. In contrast, CMV causes cytomegaly and produces both eosinophilic intranuclear inclusions surrounded by a clear halo and basophilic or amphophilic small cytoplasmic inclusions. Adenovirus does not cause cytomegaly, the nucleus appears smudgy, and there are no cytoplasmic inclusions. The hepatocyte necrosis associated with adenovirus is generally less than that seen with HSV or VZ hepatitis.

**RECURRENT DISEASE**

Recurrence of the native liver disease in the allograft is unfortunately common unless the patient originally required
transplantation for a liver-based metabolic disease. The
latter disorders are generally cured after transplantation
and include α1-antitrypsin deficiency, Wilson’s disease,
tyrosinemia, cholesterol low-density lipoprotein receptor
deficiency, glycogenesis types I and 4, factors VIII and
IX—deficient hemophilia, and familial amyloid polyneuropathy.11, 297 Liver transplantation has provided valuable
information about the pathogenesis of several of these
disorders.11 Reinvolvement of the liver by Gaucher’s disease
has been reported,298–300 but in several cases, an overall
improvement in patient health was better than expected
from liver transplantation alone.85, 300 Despite the associa-
tion of several different causative factors with giant cell
hepatitis, recurrence of the disease, which is more
common in children, has been reported in liver allografts.301

All of the virally induced cirrhotic, including hepatitis
types B, C, and D, covered in detail previously, have been
shown to recur after liver transplantation. In general, the
rate of recurrent viral infection is high and the severity of
posttransplant disease is often significant. Details as to the
incidence and severity of recurrent viral hepatitis are given
in the respective sections on those disorders.

In contrast, the incidences of recurrent autoimmune
disorders like primary biliary cirrhosis, “autoimmune
active chronic hepatitis, and sclerosing cholangitis
at present appear to be lower than for viral hep­
titis.123, 226, 269–271, 302–306 Moreover, recurrent autoim­
mune disease appears to be less severe than recurrent viral
disease or the same autoimmune syndrome before
transplantation.123, 226, 269–271, 302–306

Except for stage I (T1, NX, MX) hepatocellular carci­
nomas, other liver-based malignancies frequently recur
after transplantation,307–309 as does the Budd-Chiari
syndrome.11

Recurrent PBC and autoimmune hepatitis have been
the nonviral diseases studied in greatest de­
tail.71, 123, 226, 269–271, 302–306 The recurrence rate for these
two disorders varies from 0 to 90% after a follow-up period
of 1 to 19 years after liver transplantation (Table 69–6).
There are many possible reasons for these differences,
including pretransplant diagnosis, immunosuppressive
management policies, length of posttransplant follow-up,
operative techniques and other factors that would influ­
ence biliary tract physiology during or after transplan­
tation, methods of ascertaining the diagnosis, and the
influence of immunosuppression on the disease pro­
cess.71, 123, 226, 269–271, 302–306

The diagnosis of recurrent PBC after liver transplanta­
tion is primarily based on pathognomonic histopatho­
logical findings interpreted in the context of the clinical
profile. Re-elevation of antimitochondrial antibodies after
transplantation is almost universal, although the titer may
be lower.71, 123, 226, 270–272, 302–304 The diagnosis of autoim­
mune hepatitis is based on a combination of clinical, patho­
logical, and serological findings once HCV has been
excluded.270, 305, 306 In general, the histopathological mani­
festations of recurrent PBC are identical to those seen in
the native liver.71, 123, 226, 270–272, 302–304 Granulomatous
duct damage producing breaks in the ductal basement
membrane, or “florid duct lesions,” is a “diagnostic” find­
ing, although lymphocytic duct infiltration and damage
combined with a picture of an evolving biliary cirrhosis
have also been seen (Fig 69–25). The lobule usually
shows mild spotty necrosis, Kupffer cell hypertrophy, a
slight increase in sinusoidal lymphocytes, and Kupffer cell
granulomas. In the absence of pathognomonic findings,
the diagnosis of recurrent primary biliary cirrhosis be­
comes less certain.123

Confirmation of the diagnosis of autoimmune hepatitis
after transplantation is even more difficult than con­
firmation of recurrent PBC. A chronic hepatic histopathology,
combined with negative RT-PCR for hepatitis C and no
other evidence of viral hepatitis infection, in a patient who
had autoimmune hepatitis with the appropriate autoanti­
bodies before transplantation points toward recurrent dis­
ease as a possibility. However, the possibility that a non-A,
non-B, non-C, non-D viral hepatitis could cause a similar
or identical histopathological profile cannot be excluded
at this time.

Data on cases of probable recurrence of primary sclero­
sing cholangitis are just beginning to emerge.70, 71 It has
been tentatively identified from at least two institutions on
the basis of an exhaustive search for other causes of biliary
tract obstruction or strictureing.70, 71 However, this diag­
osis, perhaps more than other recurrent diseases, is
fraught with potential pitfalls. There are numerous insults
that could potentially lead to the development of biliary
tract obstruction or strictureing, including prolonged cold

<table>
<thead>
<tr>
<th>TABLE 69–6 Incidence of recurrent autoimmune liver diseases after liver transplantation</th>
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<tbody>
<tr>
<td><strong>Disease</strong></td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
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<tr>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Autoimmune CAH</td>
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</tbody>
</table>

NA = not applicable, original case reports; CAH = chronic active hepatitis.
Alcohol Injury

End-stage alcoholic liver disease is a leading indication for liver transplantation at many centers. Despite rigorous pretransplant screening programs that attempt to identify patients who are likely to relapse after transplantation, recurrent alcoholism can be a cause of allograft dysfunction. The exact incidence of recurrent alcohol use and abuse after transplantation in those who underwent transplantation for alcoholic liver disease is difficult to determine with certainty, but reported values range from 24 to 31%.310,311 Despite this, there is a relatively small impact on the patient and allograft survival.310-312 Discussion of the pathophysiologic mechanisms of liver injury associated with alcohol abuse is beyond the scope of this chapter.

Clinical Presentation. Problems with alcohol abuse recidivism are usually detected because of elevation of liver injury test findings that are routinely obtained in liver transplant populations or inappropriate social behavior.71 Isolated elevation of y-glutamyl transpeptidase in the absence of a concomitant rise in the alkaline phosphatase level can be seen, as in alcoholics from the general population.71 Compliance with immunosuppression can also be a problem in these patients.

Histopathological Findings. The histopathological findings of alcohol abuse in a liver allograft are virtually identical to those seen in the general population. The most common is mixed steatosis involving centrilobular hepatocytes, distributed in a distinctly zonal pattern (Fig 69–26). So-called foamy degeneration of the hepatocytes is not uncommon.71 This can be accompanied on occasion by the presence of megamitochondria, Mallory’s hyaline bodies, and the so-called alcoholic hepatitis lesion.29 Perivenular and subsinusoidal fibrosis, as well as increased iron deposition in the reticular endothelial cells and hepatocytes, without steatosis can also be seen.71

ischemia, arterial thrombosis or stricturing, positive lymphocytotoxic crossmatch, and numerous operative or technical difficulties with the biliary anastomosis. Therefore, when one observes histopathological findings suggestive of recurrent PSC, one should try to exclude, as best as possible, the many other insults that could lead to similar, if not identical, histopathological findings. Harrison et al78 noted that classic “fibro-obliterative duct lesions” were restricted to liver allograft recipients who had PSC before transplantation. Even after an exhaustive search to rule out other causes of biliary strictures is complete, there will still remain cases of unexplained biliary strictures in patients whose original disease was PSC. In such cases a diagnosis of recurrence seems justified.

Figure 69–25 Recurrent primary biliary cirrhosis. This needle biopsy was obtained in a 49-year-old woman 11/2 years after she underwent liver transplantation for primary biliary cirrhosis. Note the lymphogranulomatoid portal infiltrate and bile duct damage (inset), which was seen in only one or two of more than a dozen triads. Most all of the other triads were free from inflammation and bile duct damage.

Figure 69–26 Recurrent alcohol abuse. A, A return to alcohol abuse is most often characterized histopathologically by centrilobular steatosis, which is often severe. B, Higher magnification shows that the steatosis is mixed (microvesicular and macrovesicular), and occasionally, Mallory’s hyaline bodies (arrow) and acute “foamy” degeneration of hepatocytes are seen. Other causes of steatohepatitis should be excluded.
In our experience, evolution toward cirrhosis in liver allograft resulting from recurrent alcoholism appears in most cases to be a relatively slow process, although the data are too scarce to draw any conclusions at this point. In one patient who had particular difficulties with recurrent disease, bridging fibrosis developed within 5 years of the original transplantation procedure (unpublished observation).

**Differential Diagnosis.** The differential diagnosis of recurrent alcoholism includes all of the disorders known to cause steatohepatitis, including obesity, poorly controlled diabetes, intestinal bypass surgery, malabsorption, hyperlipidemia, and toxicities of several drugs. In the allograft recipient, we have also seen similar changes in patients with a portal vein “steal” syndrome, in which the nutrient-rich portal blood bypasses the liver and elicits centrilobular steatosis. Awareness of the original disease, detailed clinical history, and blood alcohol levels can further strengthen a diagnosis of recurrent alcohol abuse that was suspected on the biopsy findings. Appropriate caution is urged so that a mistaken accusation is not made.

**LONG-TERM CHANGES**

Several studies have examined the structural integrity of the allograft and causes of dysfunction in recipients who have survived from 1 to 19 years after liver transplantation (Table 69-7). Even though the recipient pool, immunosuppressive management policies, and study designs differed, the causes of allograft dysfunction were similar. Most remarkable was the relatively low incidence of acute and chronic rejection, which varied from 4 to 38%. Recurrence of the original disease, especially viral hepatitis, was a leading cause of dysfunction, and obstructive cholangiopathy also was surprisingly common. These observations support the contention that liver allografts are immunologically privileged. Pappo et al particularly noted that awareness of the original disease, recent change in immunosuppressive management policies, a review of previous biopsies, the clinical profile, and the result of any therapeutic or diagnostic tests or intervention should be incorporated with the biopsy findings to correctly identify the cause of late allograft dysfunction. They also emphasized that interpretation of liver allograft biopsies obtained from long-term survivors is often more difficult than interpretation of biopsies obtained early after transplantation.

**Clinical Presentation and Histopathological Findings.** Clinical presentation and histopathological findings of acute and chronic rejection, viral hepatitis, obstructive cholangiopathy, and recurrent autoimmune diseases were discussed in the respective sections and are not repeated here. However, several histopathological changes in long-surviving allografts could not be attributed to a specific pathological process, albeit they were minimal deviations from normal. These included mild lymphocytic portal inflammation without significant duct damage or venulitis; portal arterial and arteriolar thickening and hyalinization; and subtle intralobular regenerative change, characterized by thickening of the plates and pseudorosette formation. This resulted in a vague nodularity to many of the needle cores, although the findings were insufficient in most cases for an unequivocal diagnosis of nodular regenerative hyperplasia. The arterial changes were attributed to a combination of hypertension, diabetes, and injury. Because more centers may attempt drug withdrawal trials in the near future, protocol biopsies taken before stopping or lowering immunosuppression are encouraged.

**LIVER DISEASE ASSOCIATED WITH SYSTEMIC DISORDERS**

**Sepsis**

Sepsis and intra-abdominal infections are frequent occurrences during the first 2 months after liver transplantation, and alone, they can be the main insult responsible for allograft dysfunction. The usual clinical signs and symptoms of infection, such as fever and chills, are accompanied by liver dysfunction primarily manifest as hyperbilirubinemia. The underlying mechanism of liver dysfunction in this setting may be related to endotoxemia and cytokine release from Kupffer cells.

**Histopathological Findings.** Histopathological changes seen as a result of sepsis or endotoxemia are identical to those seen in the nonallograft liver. They include cholan-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Starzl et al7</th>
<th>Nakleh et al122</th>
<th>Hubscher et al123</th>
<th>Pappo et al11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up (yr)</td>
<td>1 - 7</td>
<td>3 - 4</td>
<td>1 - 8</td>
<td>5 - 19</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonspecific changes</td>
<td>NA</td>
<td>32%</td>
<td>63%</td>
<td>24%</td>
</tr>
<tr>
<td>Obstructive cholangiopathy</td>
<td>21%</td>
<td>7%</td>
<td>12%</td>
<td>6%</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>7%</td>
<td>34%</td>
<td>38%</td>
<td>35%</td>
</tr>
<tr>
<td>Acute and chronic rejection</td>
<td>38%</td>
<td>15%</td>
<td>4%</td>
<td>15%</td>
</tr>
<tr>
<td>Other</td>
<td>34%</td>
<td>13%</td>
<td>9%</td>
<td>20%</td>
</tr>
</tbody>
</table>

NA = not applicable.
giolar proliferation with bile plugging, acute cholangiolitis usually without cholangitis, hepatocanicular cholestasis, and occasionally megakaryocytes within the sinusoids.29-31 Kupffer cells are often hypertrophic, and small clusters of neutrophils, unassociated with viral inclusions or cytomegalic cells, can be observed in the sinusoids. Another finding can be the presence of extramedullary hematopoiesis. The histopathological differential diagnosis includes preservation injury, bile duct obstruction or strictureting, and humoral rejection.29-31

**Differential Diagnosis.** Blood or peritoneal fluid cultures positive for bacteria or fungi confirm the diagnosis of sepsis or intra-abdominal infection. The diagnosis of preservation injury is substantiated by review of the early post-transplantation clinical course and previous biopsies, if available. The presence of preformed antidonor antibodies, low platelet counts, hypocomplementemia, and IgG and C3 and C4 deposits in the liver biopsy favors a diagnosis of the humoral rejection. In some cases, it may be difficult if not impossible to differentiate between these possibilities on the basis of a single biopsy. The diagnosis becomes apparent only after reviewing serial biopsies during evolution of the clinical syndrome. In general, dysfunction attributable to sepsis improves with appropriate antimicrobial therapy, whereas preservation injury spontaneously improves without specific therapeutic intervention. In contrast, the histopathological changes and allograft dysfunction usually worsen over a period of 1 to 2 weeks, and acute (cellular) rejection appears if humoral rejection is the cause of dysfunction.

**DRUG AND TOXIC INJURY**

A discussion of the clinical presentation and histopathological changes associated with adverse drug reactions is beyond the scope of this chapter. However, in general, the morphological manifestations induced by a particular agent in an allograft are likely to be the same as those described for nonallograft livers. The exception may be drugs that induce an immunological response or cases in which altered self-antigens may precipitate or drive the reaction. Such reactions could be blunted or even less common because of the potent immunosuppression. The use of azathioprine as an immunosuppressant in liver allograft recipient has been associated with the development of central lobular necrosis and central vein and sinusoidal fibrosis in the short term, and nodular regenerative hyperplasia has been seen with long-term use. Because there are many insults that could potentially result in central necrosis and fibrosis, appropriate caution is urged when the lesion is attributed to azathioprine.

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**References**


