Advances in Diagnosis, Prevention, and Management of Hepatic Allograft Rejection
Russell H. Wiesner

Despite recent improvements in immunosuppressive therapy, hepatic allograft rejection remains a major cause of morbidity and late graft loss in patients undergoing liver transplantation. Although some biochemical tests suggest hepatic allograft damage, the gold standard for defining rejection remains based on morphologic findings. Acute cellular rejection usually occurs within the first 3 weeks posttransplantation and the incidence varies between 40% and 70%. Ductopenic rejection occurs in 5–10% of patients undergoing initial liver transplantation and usually occurs between 6 weeks and 6 months after the procedure. Induction and maintenance of immunosuppression with triple-drug therapy (cyclosporine, prednisone, and azathioprine) and other combinations that include antilymphocyte preparations have led to an overall decrease in the incidence of both cellular and ductopenic rejection. In addition, the availability of FK506 as a rescue therapy has saved grafts in some patients experiencing chronic (ductopenic) rejection. Overall, the correlation between the degree of biochemical liver dysfunction and the presence and severity of histologic rejection remains poor. Histologic severity of rejection, however, suggests which patients will require more immunosuppressive therapy and which patients may need antilymphocyte therapy to control the rejection episode. Some rejection episodes remain resistant to all known therapy and eventually lead to graft loss. New immunosuppressive agents and regimens are needed to improve graft and patient survival, decrease the incidence of cellular and ductopenic rejection, minimize drug-related side effects and complications, and reduce the high cost of immunosuppressive therapy.

Indexing Terms: liver transplants/immunosuppressive drugs

Hepatic allograft rejection after liver transplantation is common and remains a major cause of morbidity and late graft failure in the liver-transplant recipient (1–7). Rejection is broadly defined as the response of the recipient immune system to the transplanted allograft that leads to graft damage. The major targets of the immunologic response against the hepatic allograft include bile duct epithelial cells and the endothelium of hepatic arteries and veins; hepatocytes seem less vulnerable to rejection-related damage (8, 9).

In the liver-transplant recipient, rejection-associated damage has been classified primarily on the basis of patterns of histologic features, timing, response to therapy, and reversibility. Here, I examine our current understanding of cellular and ductopenic hepatic allograft rejection and specifically review definitions, incidence, timing, risk factors, and the relationship of rejection to biochemical abnormalities. In addition, I review immunosuppressive therapy used in liver transplantation for induction, maintenance, as well as specific anti-rejection treatment for episodes of hepatic allograft rejection.

Types of Rejection

Three types of hepatic allograft rejection have been recognized: hyperacute rejection related to preformed cytotoxic antibodies (humoral antibodies), cellular rejection, and ductopenic rejection. The last two types of rejection are primarily cell-mediated phenomena.

Hyperacute rejection. Hyperacute (humoral) rejection is a relatively uncommon form of allograft injury and subsequent graft dysfunction; it is primarily mediated by preformed cytotoxic antibodies and complement (10–13). This type of rejection usually occurs in the first 1 to 2 days after liver transplantation. The antibodies are either preformed or represent anti-donor antibodies (i.e., anti-major histocompatibility complex, anti-ABO blood group, or antiendothelial) that develop before or shortly after transplantation.

The clinical findings are often severe hepatic allograft dysfunction without obvious cause, occurring in the first several days after surgery. However, given the apparently wide spectrum of graft damage, it is frequently difficult to distinguish hyperacute rejection from preservation injury. The allograft may become swollen, cyanotic, or mottled and bile production may be slow or cease completely (12). The differential diagnosis includes preservation injury, hepatic artery thrombosis with necrosis, portal vein thrombosis, or venous outflow obstruction with graft congestion. Depending on the severity, the above findings may be accompanied by a consumptive coagulopathy and the need for excess blood product transfusions. In humorally mediated rejection, the platelet count frequently falls to <20,000/µL, and the amount of total serum complement is frequently undetectable. If searched for, circulating immune complexes can be found, supporting the diagnosis of humorally mediated injury of the hepatic allograft. Close study of hepatic allografts that have failed secondary to hyperacute rejection will reveal the presence of anti-donor antibodies.

The liver histology varies according to the severity of the rejection episode. Platelet and fibrin thrombi appear several hours after complete revascularization, primarily in perportal sinusoids. This is soon followed by neutrophilic exudation, red blood cell congestion, and coagulation necrosis. In severe cases, massive necrosis and hemorrhagic infarction may be noted (11) (Fig. 1). If
larger arteries are seen on liver biopsy, they reveal endothelial hypertrophy, focal endothelial denudation, and neutrophilic inflammation of the intima, along with nonocclusive fibrin thrombi and medial wall edema and thickening (13, 14). Immunofluorescence of the liver tissue would show linear deposits of IgG or IgM, with C1q, C3, C4, and fibrinogen being deposited in arteries, veins, and portal capillaries (15). In those patients experiencing a severe reaction, hepatic allograft failure usually occurs within hours and frequently leads to the need for regrafting.

Cellular rejection. Cellular rejection is characterized by the presence of portal or periportal hepatitis (or both), destructive or nondestructive nonsuppurative cholangitis, and endotheliitis and phlebitis of portal and hepatic vein branches (Fig. 2). In general, two or more of these findings should be present for a diagnosis of cellular rejection; however, some pathologists require the presence of all three (16). The presence of centrilobular necrosis is a nonobligatory feature that is part of the spectrum of cellular rejection; it can also be caused by severe preservation-induced injury or by hyperacute rejection, as noted above (17).

In the past, cellular rejection has been called acute rejection, which refers to the early timing of the event. Although cellular rejection usually occurs during the first 3 weeks after liver transplantation, investigators are now reporting late cellular rejection episodes, often associated with low cyclosporine concentrations after alterations or discontinuation of immunosuppressive therapy (3, 18, 19). Therefore, “acute” seems inappropriate for defining this type of rejection (Table 1). Furthermore, the term acute has numerous connotations that imply responsiveness to therapy and reversibility, connotations we now know are not always true. The phrase cellular rejection seems more appropriate, being based exclusively on hepatic histologic findings (still the gold standard for diagnosis of hepatic allograft rejection) (Table 1). Furthermore, a consensus exists about the histologic criteria for the diagnosis of cellular rejection, which have been found to be highly reproducible among experienced pathologists and appear to be generalizable (20). More recently, a scheme to grade histologic severity of cellular rejection has been developed that seems to be reproducible and prognostic (see below) (21).

Ductopenic rejection. Histologically, ductopenic rejection is characterized by the loss of interlobular and septal bile ducts; it often is associated with hepatic foam cell arteriopathy, but this is not a necessary component (1, 22) (Fig. 3). Ductopenic rejection can arise from two mechanisms: (a) direct immunologic damage to the bile duct epithelium, and (b) indirect damage to the biliary arterioles resulting in secondary ischemic damage to bile ducts (Fig. 4). In my opinion, frequently both components are present, ultimately leading to interlobular and septal bile duct destruction and loss. The diagnosis is quantified by counting portal tracts and determining what percentage of portal tracts with arteries have no interlobular and septal bile ducts. In this context, the use of cytokeratin staining is helpful for identifying bile duct epithelium within the dense portal cellular infiltrate. An inexact science, it still gives some objective measurement of the degree of bile duct loss. Investigators have suggested that at least 20 portal tracts should be available for study and that at least 50% should lack interlobular or septal bile ducts or both for a confident diagnosis (23). If foam cell arteriopathy is found, ductopenic rejection should be expected; however, the absence of arteriopathy does not exclude the diagnosis of ductopenic rejection because <10% of liver biopsy specimens from patients with confirmed ductopenic rejection have this histologic finding (24) (in part, because it is unusual to find a medium-sized artery in a routine liver biopsy specimen).

Ductopenic rejection has been referred to by several names, including chronic rejection, vanishing bile duct syndrome, vascular rejection, and irreversible rejection (22, 25, 26). The word chronic implies of long duration; however, ductopenic rejection can occur within the first 3 weeks after liver transplantation (acute vanishing bile duct syndrome); therefore, use of “chronic” in this
context seems inappropriate (23) (Table 1). Chronic rejection also connotes being unresponsive to therapy and irreversible despite maximal immunosuppressive therapy; however, these conditions do not always prevail (27, 28). Therefore, the name ductopenic rejection rather than chronic rejection is preferable, being based purely on morphologic findings and independent of duration, responsiveness to immunosuppressive therapy, and reversibility. Unfortunately, unlike cellular rejection, in which the assessment of histologic findings has been found to be highly reproducible within a group of experienced pathologists, the diagnosis of ductopenic rejection has been found to be less reproducible and reliable (20).

Incidence and Timing of Rejection

Humoral rejection. The incidence of humoral rejection is uncommon, occurring in <1% of patients undergoing liver transplantation, but when it does occur, it is usually within the first 3 days after the liver transplant procedure (12). The graft damage is often irreversible and frequently leads to the need for retransplantation; however, a spectrum of the graft damage that occurs is related to preformed cytotoxic antibodies, and not all damage related to these mechanisms is irreversible.

Cellular rejection. The incidence of cellular rejection is variable but reportedly occurs in 40–70% of liver transplant recipients (2–5). Some medical centers report greater incidence of cellular rejection in children than in adults—a finding that may be related to increased immune responsiveness in children (29–31). Cellular rejection usually occurs within the first 15 days after transplant (median 11 days), when cyclosporine-based immunosuppression is used, but can occur anytime after liver transplantation (3). When cellular rejection occurs late after liver transplantation, it is often associated with a decrease in or discontinuation of maintenance immunosuppressive therapy (19).

In patients treated with antilymphocyte preparations for induction immunosuppression, rejection usually occurs sometime later, most often during week 4 or 5 after

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**Table 1. Terminology and definitions associated with hepatic allograft rejection.**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Morphologic findings</th>
<th>Time after transplant</th>
<th>Additional immunosuppression</th>
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<tbody>
<tr>
<td><strong>Traditional</strong></td>
<td></td>
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<tr>
<td>Acute</td>
<td>Portal hepatitis, nonsuppurative cholangitis, and endothelitis</td>
<td>Implies short duration</td>
<td>Usually responsive</td>
</tr>
<tr>
<td>Cellular</td>
<td>Portal hepatitis, nonsuppurative cholangitis, and endothelitis</td>
<td>Independent of time</td>
<td>Independent of response to immunosuppression</td>
</tr>
<tr>
<td>Chronic</td>
<td>Duct loss with or without arteriopathy</td>
<td>Implies long duration</td>
<td>Usually unresponsive</td>
</tr>
<tr>
<td>Ductopenic</td>
<td>Duct loss with or without arteriopathy</td>
<td>Independent of time</td>
<td>Independent of response to immunosuppression</td>
</tr>
</tbody>
</table>

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**Fig. 4.** Mechanisms of bile duct destruction leading to ductopenic rejection.

The immunological attack shown on the right is a proven feature; the role of ischemia is controversial but may be important in some instances. Reprinted with permission from Wiesner et al. (1).
liver transplantation. However, the overall incidence of cellular rejection seems to be similar with or without use of induction antilymphocyte therapy (29, 32, 33).

**Ductopenic rejection.** The incidence of ductopenic rejection after liver transplantation occurs in 8–10% of adults who undergo this procedure (1); a similar incidence has been shown in children. However, some investigators believe the incidence in children is higher than in adults (34). Both figures represent the incidence of ductopenic rejection during the late 1980s in patients who were primarily treated with cyclosporine-based immunosuppression. The incidence of ductopenic rejection seems to be decreasing because of improved immunosuppressive therapy (35). The differences in the incidence of ductopenic rejection reported from various centers can be explained by the lack of agreement on diagnostic criteria in some instances, and in others by the lack of liver histology to support the diagnosis. Furthermore, the spectrum of ductopenic rejection ranges from mild loss of bile ducts and mild cholestasis, which are potentially reversible, to a severe category, in which most or all interlobular and septal bile ducts are lost and severe clinical and histologic cholestasis is found with impending irreversible failure of the graft (36) (Table 2). The latter condition is often referred to as vanishing bile duct syndrome (23, 25).

The timing of ductopenic rejection can also vary. Ductopenic rejection has been arbitrarily subclassified on the basis of clinical pathologic findings by its appearance within one of three posttransplant periods: early (<6 weeks); delayed (6 weeks to 6 months); or late-onset (>6 months after the procedure) (18, 37). Early ductopenic rejection usually occurs within 1 month after liver transplantation (23). The initial manifestation is that of cellular rejection with the onset of malaise, fever, and a sharp increase in results for liver function tests, particularly in serum concentrations of aminotransferases (Fig. 5). Typically, a liver biopsy specimen shows features of nonsuppurative destructive cholangitis along with moderate-to-severe portal inflammation. The rejection episode generally fails to respond to additional immunosuppressive therapy, and follow-up liver biopsy specimens show persistent rejection cholangitis with evidence of ongoing bile duct destruction. As the rejection process progresses without response to additional immunosuppressive therapy, bile ducts continue to disappear, portal tracts become fibrotic, and inflammatory infiltrates eventually subside (Fig. 6). Biochemically, the concentrations of aminotransferases decrease, but those of bilirubin, alkaline phosphatase, and γ-glutamyltransferase progressively increase. As cholestasis persists, the graft becomes irreversibly damaged, and retransplantation frequently becomes necessary as a lifesaving procedure.

Delayed ductopenic rejection (6 weeks to 6 months after transplantation) is the most common manifestation. In this setting, ductopenic rejection develops after one or more episodes of cellular rejection that ultimately fail to respond to maximum immunosuppressive therapy. Bile ducts are progressively lost, and cholestasis develops and slowly leads to failure of the graft. A final histologic pattern is similar to that described for early ductopenic rejection. Delayed ductopenic rejection must be distinguished from severe cholestasis, which can occur in the transplant setting with recurrence of hepatitis C (38).

In this laboratory, the least common presentation is late-onset ductopenia (>6 months after transplant). This condition occurs insidiously, often without a history of previously recognized episodes of cellular rejec-

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### Table 2. Relationship between ductopenia and severity of ductopenic rejection.

<table>
<thead>
<tr>
<th>Portal tracts with bile ducts, %*</th>
<th>Severity of ductopenic rejection</th>
</tr>
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<tbody>
<tr>
<td>&gt;80</td>
<td>Normal</td>
</tr>
<tr>
<td>50–8</td>
<td>Mild—reversible</td>
</tr>
<tr>
<td>&lt;50</td>
<td>Moderate—reversible (?)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>Severe—vanishing bile duct syndrome, usually irreversible</td>
</tr>
</tbody>
</table>

* Calculated as number of portal tracts with bile ducts/total number of portal tracts.

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Fig. 5. Characteristic laboratory findings in a patient with vanishing bile duct syndrome.

Note the fall of aminotransferase activity after anti-rejection therapy and the relentless increase of serum bilirubin and alkaline phosphatase. Liver biopsy days are indicated at the bottom of the graph; retransplantation was done on day 25. (Reprinted with permission from Wiesner et al. (1, p 726).)

Fig. 6. Schematic illustration of the histological evolution of hepatic allograft rejection, showing progression from a reversible manifestation to an irreversible condition (vanishing bile duct syndrome).
tion. Liver histologic findings frequently reveal ductopenia and degeneration of bile duct epithelial cells but without prominent portal inflammatory infiltrates (39). Progression is slow, over a period of months to years, but eventually leads to cholestatic failure of the graft. In our experience with >400 liver transplantations, we have encountered this condition in only 2 patients. Investigators have suggested that humoral mechanisms may also play a major role in the pathogenesis of this type of late-occurring ductopenic rejection (13, 40). Further evaluation of the mechanisms involved in the etiopathogenesis of bile duct loss based on timing should delineate whether they involve a common mechanism that leads to ductopenia.

Risk Factors

**Humoral rejection.** The presence of preformed donor-reactive antibodies (presensitization in the recipient before transplantation) appears to be the major risk factor for developing humoral type rejection. However, the presence of these antibodies does not necessarily indicate that a humoral-type rejection will occur. Other risk factors for humoral rejection have not been identified to date.

**Cellular rejection.** Clinical risk factors for cellular rejection have also been inadequately studied. Recent analysis of recipients of liver transplantation in the National Institutes of Health Liver Transplant Database Study has shed some light on this subject (2). In 377 consecutive patients from three liver-transplant centers (Mayo Clinic, University of California at San Francisco, and University of Nebraska), the risk factors identified for cellular rejection included a direct correlation with HLA DR mismatch and an inverse correlation with age (Fig. 7). Indeed, the incidence of cellular rejection in recipients of liver transplants who were older than 40 years was significantly less than in younger patients (37% vs 59%, respectively; P <0.004).

This study further identified that primary immunosuppression with cyclosporine and prednisone but without azathioprine was a risk factor. Such findings had also been previously reported for the Mayo Clinic experience, in which the incidence of rejection in triple-drug immunosuppression was 38%, compared with 64% in double-drug treatment (cyclosporine and prednisone; P <0.001) (36) (Fig. 8). This preliminary look at potential risk factors for cellular rejection introduces the possibility of an individualized approach to immunosuppressive therapy, particularly in patients who appear to be at high risk for the development of cellular rejection.

**Ductopenic rejection.** Although clinical risk factors for ductopenic rejection also remain poorly understood, several seem important. Primary sclerosing cholangitis as an underlying indication for liver transplantation, primary immunosuppression without azathioprine, and a positive lymphocytotoxic crossmatch all increase the risk for the development of ductopenic rejection (3, 41-43). Although the importance of a positive lymphocytotoxic crossmatch has been controversial in the past, recent data from other transplant centers seem to support these results (44, 45). Another important finding is that 90% of patients who underwent retransplantation because of ductopenic rejection had a recurrence of ductopenic rejection in the retransplanted hepatic allograft (46). However, a second study has reported a lower incidence (33%) of recurrent ductopenic rejection in retransplanted hepatic allografts (47). The role of HLA
DR mismatch—independently, or associated with cyto-
meagalovirus (CMV) infection—as a risk factor for duc-
topenic rejection has been reported by one center but
could not be confirmed at Mayo (48–50). Finally, at least
one investigator has identified the pediatric population
as having a greater risk of developing ductopenic rejec-
tion than does the adult population (34). These defined
risk factors are only preliminary, however, and need
further investigation, particularly now that many new
immunosuppressive agents are available to prevent and
treat rejection.

Histologic Grading of Cellular Rejection

Several grading systems for acute liver allograft re-
jection have evolved through the years. Although the
details may differ, most are derived from the concepts
originally developed for renal allografts, particularly
regarding the prognostic significance of parenchymal
necrosis and arteriolar inflammation. In the liver-trans-
plant recipient, centrilobular necrosis may be used as an
indicator of severe rejection in most of the published
grading schemes (17). As a result of the National Insti-
tutes of Health Liver Transplant Database Study, a new
grading system has been developed that represents a
merger and simplification of many of the previously
published systems. The new system also takes advan-
tage of proven reproducibility of certain histologic
findings. The grading of acute cellular rejection with this
histologic scheme is as follows:

1) Mild rejection—infiltrates in some but not all por-
tal triads, with inflammation confined within the portal
spaces.

2) Moderate rejection—infiltrates involving most or
all of the portal triads, with or without spillover of the
inflammatory infiltrate into the lobule, and with no
evidence of centrilobular necrosis, ballooning, or hepa-
tocellular dropout.

3) Severe rejection—infiltrates in some or all of the
portal triads, with or without spillover into the lobule,
and with or without inflammatory linkage of triads
associated with moderate-to-severe lobular inflamma-
tion and necrosis.

This grading scheme for histologic severity of rejec-
tion has been quite reproducible among a group of he-
patic pathologists having an interest in liver transplan-
tation and seems to have prognostic significance (2, 20).

Biochemical Dysfunction and Liver Histologic Findings

Various clinical and laboratory indicators have been
developed to diagnose cellular rejection in recipients of liver
transplants. Unfortunately, in most cases, these clinical
and laboratory findings lack sensitivity and specificity
(51–53). Furthermore, in general, the correlation be-
 tween histologic severity of rejection and the degree of
biochemical hepatic dysfunction has been poor (36, 51).
Our experience reveals that in liver-transplant recipi-
ents with early biochemical hepatic dysfunction (occur-
ing within the first 3 weeks and defined as increases in
serum alanine aminotransferase, bilirubin, or alkaline
phosphatase of ≥15%), histologic evidence for cellular
rejection could be found in only 58% of such patients. In
contrast, in patients who had normal, stable, or improving
hepatic biochemical findings in the early course after transplantation, histologic evidence for cellular
rejection (on the basis of protocol liver biopsies) was
unexpectedly present in 47%. Overall, 43% of such pa-
tients would have been misdiagnosed if only hepatic
biochemical studies had been used (36), an inaccuracy
that would have resulted in inappropriate immuno-
suppressive management. These data emphasize the pre-
sent importance of protocol liver biopsies as a guide for
initiating additional immunosuppressive therapy (53).

More recently, other biochemical tests have been per-
formed in an attempt to enhance our ability to diagnose
rejection without performing a liver biopsy. One study
found that dynamic tests of liver function, e.g., indocy-
amine green clearance, offered no benefit over conven-
tional liver biochemistry in diagnosing hepatic al-
lograft rejection (54). In addition, several studies have
shown that serum concentrations of soluble interleu-
kin-2 receptor, soluble CD8, and soluble intercellular
adhesion molecules might be indicators of hepatic al-
lograft rejection (55–57). However, in this laboratory we
have recently demonstrated considerable overlap with
patients who have other conditions posttransplantation,
such as CMV hepatitis. In fact, we showed that serum
concentrations of interleukin-2 receptor and soluble
CD8 are greatest in patients with CMV hepatitis (Fig. 9).
Other markers used to diagnose rejection include
increased concentrations of β2-microglobulin in both se-
rum and bile, of tissue necrosis factor-α in serum, of
secretory immune globulins and secretory component in
bile and serum, and of blood and tissue eosinophil
counts (58–62). One group has suggested that serum
alkaline phosphatase isofoms can be used for early
detection of complications of liver transplantation (63).
Still others have claimed that measuring serial serum
concentrations of delta unconjugated bilirubin can also
be of help in diagnosing early rejection and in assessing
response to therapy (63, 64). However, overall, these
markers, though sensitive, in many cases are quite non-
specific, which compels us to retain liver histology as the
gold standard for the diagnosis of cellular rejection. In
addition, many of these assays are quite inconvenient to
perform on a routine basis in most laboratories and have
long turnaround times; thus, their information is not
provided in a timely fashion for clinical decision making.

Induction and Maintenance Immunosuppression

Induction immunosuppression after liver transplan-
tation has mainly been based on regimens previously
used in renal transplantation. A combination of predni-
sone and azathioprine for liver transplant recipients
was used in the 1970s but was associated with a high
incidence of rejection, infection, and poor graft and pa-
tient survival (65). In the 1980s, the combination of
cyclosporine and prednisone was used for primary im-
munosuppression, which substantially increased sur-
vival and revitalized liver transplantation as a thera-
peutic modality for patients with end-stage liver disease.
Improvements in anesthetic technology, intensive-care management, and prophylaxis against infections further enhanced survival, which once again highlighted rejection as the major source of posttransplantation morbidity in the late 1980s. Indeed, 40–50% of late graft failure is a result of irreversible rejection (6, 7).

Furthermore, frequent side effects associated with cyclosporine led investigators to seek improved immunosuppressive regimens (67, 68). One such regimen was the triple-drug treatment: low-dose cyclosporine, prednisone, and azathioprine. A major advantage of this regimen was the ability to decrease the dose of cyclosporine to avoid renal toxicity and hypertension, both of which are commonly associated with cyclosporine therapy (69). This triple-drug regimen did seem to decrease the severity of nephrotoxicity and hypertension but did not totally eliminate these problems. In our experience, the main effect of triple-drug therapy was to decrease the incidence of both cellular rejection and ductopenic rejection (36) (Fig. 8). Because of the success of the triple-drug regimen, it continues to be widely used in liver transplantation programs throughout the US. Many investigators consider it the standard against which new immunosuppressive agents should be evaluated.

Other investigators have evaluated “sequential quadruple” immunosuppressive regimens, in which induction therapy—with OKT3—an antilymphocyte globulin, or anti-cytokine antibodies initiated after liver transplantation—is followed by triple-drug maintenance therapy with cyclosporine, prednisone, and azathioprine (70–73). In general, the antibody therapy is instituted for the first 5 to 10 days after liver transplantation, after which the patient is switched over to a triple- or double-drug maintenance regimen. The rationale for using sequential quadruple immunosuppressive therapy is that initiating cyclosporine therapy later and avoiding the early intravenous administration of cyclosporine might decrease or avoid the severity of nephrotoxicity, neurotoxicity, and hypertension. However, treatment trials evaluating sequential quadruple immunosuppression therapy show that the occurrence of rejection is not decreased but is delayed until week 5 or 6 after liver transplantation, whereas with the triple-drug immunosuppressive regimen the peak incidence of rejection is in week 2. With both regimens, the overall incidence of cellular rejection has, in general, been similar. Delaying the onset of initiation of cyclosporine by using quadruple immunosuppression seems to have little or no long-term effect on nephrotoxicity or glomerularfiltration rates (70–72). Furthermore, patients so treated often have to be readmitted to the hospital during week 5 or 6 after transplantation for treatment of rejection episodes. Frequently, such late episodes of rejection prolong the patient’s course and duration of stay at the liver transplant center. In some studies, the use of OKT3 for induction therapy has been associated with a higher incidence of bacterial, fungal, and CMV infections (74). Finally, the use of antilymphocyte preparations, for induction immunosuppression or treatment of episodes of rejection or both, may be associated with an increased incidence of lymphoproliferative disorders related to Epstein–Barr viral infections (75–77). Some investigators suggest that these disorders may be directly related to the total dose of OKT3 administered; others suggest that the use of the combination of antilymphocyte globulin and cyclosporine for induction immunosuppression may predispose patients to this complication (76). Nevertheless, excess immunosuppressant with any of these combinations may be associated with an increased risk of lymphoproliferative disorders (Table 3).

The data suggest, therefore, that induction immunosuppression with OKT3 or antilymphocyte globulin has few advantages over triple-drug immunosuppression because the incidence of cellular rejection and the degree of nephrotoxicity are similar. One study with a monoclonal antibody to interleukin-2 receptor demonstrated a decrease in the incidence of steroid-resistant rejection
associated with a significant increase in both patient and graft survival and no particular increased risk noted for bacterial, fungal, or CMV infections (73). On the contrary, patients undergoing induction immunosuppression with OKT3 have shown an increased risk of infection and possibly an increased risk of lymphoproliferative disorders (76; 78, 79).

In another approach to decrease or avoid cyclosporine-induced toxicity, we attempted to wean liver transplant recipients off of cyclosporine and to maintain immunosuppression with prednisone and azathioprine alone (80). We studied 12 patients 1 to 5 years after their liver transplantation. These 12 patients had normal results for liver-function tests, a normal liver biopsy, and no previous history of rejection, but their serum creatinine exceeded 20 mg/L or they were subject to hypertension uncontrolled with medical therapy, or both. In these patients, we attempted to taper the cyclosporine regimen slowly, decreasing the dose by 25 mg/day each week and closely monitoring results of the liver-function tests. Of the 12 patients, 6 (50%) had an episode of cellular rejection while we were tapering their dose of cyclosporine or shortly after its discontinuation. Four of these patients experienced corticosteroid-resistant rejection, which necessitated treatment with an antilymphocyte preparation. One patient responded to OKT3, but two eventually experienced graft failure because of uncontrollable rejection. A fourth patient responded to FK506 rescue therapy.

Although tapering of the dose of cyclosporine was associated with a high incidence of rejection, it seemed to have little or no effect on renal toxicity; the mean creatinine clearance increased from only 28 to 32 mL/min in the six patients in whom cyclosporine therapy was tapered and eventually discontinued and who were followed-up 4 months or more after discontinuation of cyclosporine. Only one of these six patients had enough improvement in hypertension to decrease the use of antihypertensive medications. Because of the high incidence and severity of episodes of rejection, we believe that continuation of our cyclosporine tapering study would be unethical; therefore, the study was stopped. We conclude that withdrawal of cyclosporine from a triple-drug immunosuppressive regimen is frequently associated with severe cellular rejection episodes; these episodes are often steroid-resistant and are associated in the short-term with only minimal improvement in renal function or control of hypertension.

Other investigators have attempted to withdraw steroids from long-term immunosuppression regimens in liver-allograft recipients. The University of Birmingham group reported corticosteroid withdrawal from the immunosuppressive regimen in 85% of liver-transplant recipients who survived for ≥3 months (81). The rejection rate after steroid withdrawal was only 4.5%; however, 3.9% developed chronic ductopenic rejection. The prevalence of side effects related to steroids also appeared to be lower, with 66% of patients not requiring antihypertensive medication and only 14% being infected. Padbury et al. concluded (81) that withdrawal of corticosteroids after 3 months can be done successfully in most of liver-allograft recipients and is associated with a low, acceptable rate of rejection and graft loss. Furthermore, steroid withdrawal appears to reduce the complications commonly associated with the triple-drug immunosuppressive regimen. Further studies on long-term graft outcome will be necessary before conclusions can be made.

Still more recently, the identification of microchimerism and its possible association with graft tolerance has been described. In these studies, several children were completely weaned off of all immunosuppressive agents and have not experienced a rejection episode (82). Obviously, further research will be needed to be able to identify in which patients immunosuppressive therapy can be tapered or discontinued.

The most recent combination therapy introduced for induction and maintenance immunosuppression for recipients of liver transplantation is FK506 and low-dose corticosteroid therapy. Recently, three major multicenter trials have been reported, two in the US and one in Europe (83). These studies indicated that FK506 and low-dose corticosteroid therapy is associated with a decrease in the incidence of cellular rejection and of steroid-resistant rejection. However, patient and graft survival in the FK506 group were similar to that in the group treated with the cyclosporine-based immunosuppression. In addition, FK506 therapy was associated with both acute and chronic renal toxicity; overall, FK506 seemed to have no long-term advantage over cyclosporine for decreasing the incidence and severity of nephrotoxicity (84). Similarly, the incidence and severity of neurotoxicity were similar between the two treatment groups (85). Preliminary studies suggest that FK506 is associated with a significantly lower incidence and severity of hypertension in comparison with cyclosporine therapy. One study suggests that this may be related to a decreased vasoconstriction effect of FK506 on the peripheral vasculature (86). Furthermore, treatment with FK506 and low-dose corticosteroid has been

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associated with a decrease in the incidence and severity of hypercholesterolemia and less weight gain (87). Whether this effect is secondary to the lower corticosteroid doses used in conjunction with FK506 or reflects inherent properties of FK506 remains to be determined.

Although FK506 and some of the more recent monoclonal antibodies aimed against lymphocytes and cytokines have made major inroads into increasing our immunosuppressive alternatives, these regimens are associated with significant toxicity and all are associated with a high infection rate. Therefore, the search continues for new, more-specific immunosuppressive agents that will be associated with a low incidence of infection and a low incidence of side effects.

Treatment of Rejection

**Cellular rejection.** The approach to treatment of episodes of cellular rejection after liver transplantation has also been derived from the past experience with renal transplantation. Bolus intravenous corticosteroid therapy is most commonly used to treat episodes of cellular rejection in liver-transplant recipients. The dose and duration of corticosteroid therapy, however, varies considerably among liver-transplant centers. Our experience suggests that early initiation of bolus intravenous corticosteroid therapy for cellular rejection by using two or three 1000-mg doses of methylprednisolone is associated with a significantly higher response rate than after a single 1000-mg intravenously administered dose of methylprednisolone followed by an oral recycle of prednisone (88) (Fig. 10). In comparing the treatment regimens, we saw no apparent increase in the incidence of infections, diabetes, or complications related to osteoporosis in those patients treated with three boluses instead of one bolus. Furthermore, those patients treated with a single 100-mg intravenously administered dose of methylprednisolone followed by an oral prednisone recycle experienced an increased incidence of OKT3-resistant rejection, which resulted in ductopenic rejection and graft failure. On the basis of these data, we recommend that, for maximal efficiency, episodes of cellular rejection be treated with three intravenous administered 1000-mg bolus doses of methylprednisolone on alternate days during a 5-day period. A follow-up liver biopsy is important for assessing therapeutic response (88).

A recent retrospective study has even questioned whether patients experiencing mild acute cellular rejection with mild hepatic dysfunction indeed require therapeutic intervention; several rejection episodes reportedly resolved spontaneously on baseline immunosuppression (89). For episodes of rejection that are unresponsive to bolus intravenous corticosteroid therapy, or for episodes in which severe histologic bile duct damage is demonstrated in the initial liver biopsy, some centers have used antilymphocyte preparations, including OKT3 and antilymphocyte globulins. In a recent study (90, 91), OKT3 was effective in a rescue protocol for steroid-resistant rejections: it was necessary to administer OKT3 to 25% of the patients, which successfully reversed 85% of these steroid-resistant rejection episodes. Failure to respond with OKT3 was a serious complication, and repeat courses of OKT3 resulted in a high incidence of infectious complications and lymphoproliferative disorders (90, 91). For those unfortunate patients whose rejection episode did not respond to bolus corticosteroids or OKT3, FK506 and other new immunosuppressive therapies have successfully rescued some grafts (92–97).

**Ductopenic rejection.** In patients who develop steroid-resistant rejection and OKT3-resistant rejection, a trial of FK506 rescue should be initiated. However, in the late stages of ductopenic rejection, when portal inflammatory infiltrates have receded, administration of additional high-dose immunosuppressive therapy is rarely helpful. Indeed, such treatment often leads to an excessively immunosuppressed state for the patient, which can be complicated by CMV, *Pneumocystis carinii*, and fungal infections. These infectious complications are associated with considerable morbidity and may be fatal. In patients who present with an insidious onset of ductopenic rejection and in whom liver biopsy specimens reveal little or no inflammatory response, additional antirejection therapy also appears to be of little benefit. Thus, administration of maximal immunosuppressive therapy to treat ductopenic rejection should be considered in the context of both the clinical and the histologic findings.

When ductopenic rejection becomes associated with a decrease in hepatic synthetic function, as manifested by an increasing prothrombin time and a decrease in serum albumin, retransplantation should be considered. Although, as noted above, recurrence of ductopenic rejection is common in patients undergoing retransplantation for the vanishing bile duct syndrome, new immunosuppressive options, particularly FK506 make it reasonable to proceed with retransplantation.

Major advances have been made in the diagnostic criteria as well as in the use of immunosuppressive therapy during the past 10 years, although utopia has not been achieved. With an ever-expanding number of new immunosuppressive agents becoming available,
Tailoring immunosuppressive therapy for the individual patient may become a reality. Decisions about which immunosuppressive regimens and agents to use will most likely be based on the risk factors in individual liver-transplant recipients. Goals for using new immunosuppressive agents and regimens should include improvement of graft and patient survival, but it will also be imperative to reduce the side effects and infectious complications. Finally, financial considerations appear to be increasing in decision-making choices for both induction and maintenance immunosuppressive therapy for liver-transplant recipients. Cost effectiveness will be a recurrent theme in diagnosing, preventing, and treating hepatic allograft rejection.

References

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