Rejection is the major cause of graft failure, and if the injury to the tubules and glomeruli is severe, the kidney may not recover. It is therefore important to diagnose acute rejection as soon as possible to institute prompt antirejection therapy. Generally, the success with which rejection can be reversed by immunosuppressive agents determines the chance of long-term success of the transplant [1,2].
Mechanisms of Renal Allograft Rejection

A. Overview of rejection events. T cells recognize foreign antigens only when the antigen or an immunogenic peptide is associated with a self-HLA molecule on the surface of an accessory cell called the antigen-presenting cell (APC). Helper T cells (CD4) are activated to proliferate, differentiate, and secrete a variety of cytokines. These cytokines increase expression of HLA class II antigens on engrafted tissues, stimulate B lymphocytes to produce antibodies against the allograft, and help cytotoxic T cells, macrophages, and natural killer cells develop cytotoxicity against the graft.

B. Possible mechanisms for allorecognition by host T cells. In the direct pathway, T cells recognize intact allo-MHC on the surface of donor cells. The T-cell response that results in early acute cellular rejection is caused mainly by direct allorecognition. In the indirect pathway, T cells recognize processed alloantigens in the context of self-APCs. Indirect presentation may be important in maintaining and amplifying the rejection response, especially in chronic rejection.

IFN-γ—interferon gamma; IL-1—interleukin-1; IL-2R—interleukin-2 receptor; NK—natural killer. (Panel A adapted from [3]; with permission; panel C adapted from [4]; with permission.)
Classification of Rejection

A. VARIETIES OF REJECTION

<table>
<thead>
<tr>
<th>Types of rejection</th>
<th>Time taken</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperacute</td>
<td>Minutes to hours</td>
<td>Preformed antidonor antibodies and complement</td>
</tr>
<tr>
<td>Accelerated</td>
<td>Days</td>
<td>Reactivation of sensitized T cells</td>
</tr>
<tr>
<td>Acute</td>
<td>Days to weeks</td>
<td>Primary activation of T cells</td>
</tr>
<tr>
<td>Chronic</td>
<td>Months to years</td>
<td>Both immunologic and nonimmunologic factors</td>
</tr>
</tbody>
</table>

B. IMMUNE MECHANISMS OF RENAL ALLOGRAFT REJECTION

<table>
<thead>
<tr>
<th>Type</th>
<th>Humoral</th>
<th>Cellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperacute</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Accelerated</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Acute</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Cellular</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Vascular</td>
<td>++</td>
<td>+?</td>
</tr>
<tr>
<td>Chronic</td>
<td>++</td>
<td>+?</td>
</tr>
</tbody>
</table>

FIGURE 9-2
Varieties of rejection (panel A) and immune mechanisms (panel B). On the basis of the pathologic process and the kinetics of the rejection response, rejection of renal allografts can be commonly divided into hyperacute, accelerated, acute, and chronic types.

FIGURE 9-3 (See Color Plate)
Histologic features of hyperacute rejection. Hyperacute rejection is very rare and is caused by antibody-mediated damage to the graft. The clinical manifestation of hyperacute rejection is a failure of the kidney to perfuse properly on release of the vascular clamps just after vascular anastomosis is completed. The kidney initially becomes firm and then rapidly turns blue, spotted, and flabby. The presence of neutrophils in the glomeruli and peritubular capillaries in the kidney biopsy confirms the diagnosis. A, Hematoxylin and eosin stain of biopsy showing interstitial hemorrhage and extensive coagulative necrosis of tubules and glomeruli, with scattered interstitial inflammatory cells and neutrophils. B, Immunofluorescence stain of kidney with hyperacute rejection showing positive staining of fibrins.
Transplantation as Treatment of End-Stage Renal Disease

**Figure 9-4**
Histologic features of acute accelerated rejection. **A** and **B**, Photomicrographs showing histologic features of acute accelerated vascular rejection. Glomerular and vascular endothelial infiltrates and swelling are visible. An accelerated rejection, which may start on the second or third day, tends to occur in the previously sensitized patient in whom preformed anti-HLA antibodies are present. This type of rejection occurs in patients who have had a previous graft and presents with a decrease in renal function; the clinical picture is similar to that for hyperacute rejection.

**Figure 9-5**
Histologic features of acute cellular rejection. **A**, Mild tubulitis. **B**, Moderate to severe tubulitis. Acute rejection episodes may occur as early as 5 to 7 days, but are generally seen between 1 and 4 weeks after transplantation. The classic acute rejection episode of the earlier era (i.e., azathioprine-prednisolone) was accompanied by swelling and tenderness of the kidney and the onset of oliguria with an associated rise in serum creatinine; these symptoms were usually accompanied by a significant fever. However, in patients who have been treated with cyclosporine, the clinical features of an acute rejection are really quite minimal in that there is perhaps some swelling of the kidney, usually no tenderness, and there may be a minimal to moderate degree of fever. Because such an acute rejection may occur at a time when there is a distinct possibility of acute cyclosporine toxicity, the differentiation between the two entities may be extremely difficult.

The differential diagnosis of acute rejection, acute tubular necrosis, and cyclosporine nephrotoxicity may be difficult, especially in the early posttransplant period when more than one cause of dysfunction can occur together [2]. Knowledge of the natural history of several clinical entities is extremely helpful in limiting the differential diagnosis. Reversible medical and mechanical causes should be excluded first. Percutaneous biopsy of the renal allograft using real-time ultrasound guide is a safe procedure. It provides histologic confirmation of the diagnosis of rejection, aids in the differential diagnosis of graft dysfunction, and allows for assessment of the likelihood of a response to antirejection treatment.
C. CHRONIC ALLOGRAFT REJECTION

Typical clinical presentation
Gradual increase in creatinine (months)
Non-nephrotic range proteinuria
No recent nephrotoxic events
Key pathologic features
Interstitial fibrosis
Arterial fibrosis and intimal thickening

D. Hypothetical schema for chronic rejection

- Acute rejection
  - Antibody deposition
  - Oxidized LDL
  - Infection

- T cells
  - Macrophages
  - Platelet aggregates

- Cytokines/growth factors

- Cell proliferation
- Fibrosis

- Vascular injury
  - Arteriosclerosis

- Tubulointerstitial injury
  - Glomerular sclerosis

- Reduced nephron mass

- Graft loss

FIGURE 9-6
Features of chronic rejection. A, Arterial fibrosis and intimal thickening. B, Interstitial fibrosis and tubular atrophy. C, Typical presentation and pathologic features. Chronic rejection occurs during a span of months to years. It appears to be unresponsive to current treatment and has emerged as the major problem facing transplantation [5]. Because chronic rejection is thought to be the end result of uncontrolled repetitive acute rejection episodes or a slowly progressive inflammatory process, its onset may be as early as the first few weeks after transplantation or any time thereafter.

D, The likely sequence of events in chronic rejection and potential mediating factors for key steps. Progressive azotemia, proteinuria, and hypertension are the clinical hallmarks of chronic rejection. Immunologic and nonimmunologic mechanisms are thought to play a role in the pathogenesis of this entity. Immunologic mechanisms include antibody-mediated tissue destruction that occurs possibly secondary to antibody-dependent cellular cytotoxicity leading to obliterative arteritis, growth factors derived from macrophages and platelets leading to fibrotic degeneration, and glomerular hypertension with hyperfiltration injury due to reduced nephron mass leading to progressive glomerular sclerosis. Nonimmunologic causes can also contribute to the decline in renal function. Atheromatous renovascular disease of the transplant kidney may also be responsible for a significant number of cases of progressive graft failure.

(Continued on next page)
**BANFF CLASSIFICATION OF RENAL ALLOGRAFT REJECTION**

- **Normal**
  - Patchy mononuclear cell infiltrates without tubulitis is not uncommon
  - Borderline changes
    - No intimal arteritis; mild tubulitis and endocapillary glomerulitis
  - Acute rejection
    - Grade I: tubulitis ++
    - Grade II: tubulitis with glomerulitis
    - Grade III: intimal arteritis, interstitial hemorrhage, fibrinoid, thrombosis

**FIGURE 9-6 (Continued)**

E. Diagnostic and therapeutic approach to chronic rejection.
ATG — antithymocyte globulin; ATN — acute tubular necrosis; BP — blood pressure; CsA — cyclosporine; LDL — low-density lipoprotein.

**FIGURE 9-7**
The Banff classification of renal allograft rejection. This schema is an internationally agreed on standardized classification of renal allograft pathology that regards intimal arteritis and tubulitis as the main lesions indicative of acute rejection [6].
Transplant Rejection and its Treatment

New techniques

FIGURE 9-8
Fine-needle aspiration cytology technique for the transplanted kidney. A 23- or 25-gauge spinal needle is used under aseptic conditions. A 20-mL syringe containing 5 mL of RPMI 1-1640 tissue culture medium is connected to the needle. Ultrasound guidance may be used on the rare occasions when the graft is not easily palpable [8].

Monitoring of other products of inflammation such as neopterin and lymphokines continues to be explored. It has been shown that acute rejection is associated with elevated plasma interleukin (IL)-1 in azathioprine-treated patients and IL-2 in cyclosporine-treated patients. IL-6 is also increased in the serum and urine immediately after transplantation and during acute rejection episodes. The major problem, however, is that infection, particularly viral, can also elevate cytokine levels. Recently, polymerase chain reaction (PCR) has also been used to detect mRNA for IL-2 in fine-needle aspirate of human transplant kidney [7,8]. Using the PCR approach, IL-2 could be detected 2 days before rejection was apparent by histologic or clinical criteria. Reverse transcriptase-PCR has also been used to identify intrarenal expression of cytotoxic molecules (granzyme B and perforin) and immunoregulatory cytokines (IL-2, -4, -10, interferon gamma, and transforming growth factor-β1) in human renal allograft biopsy specimens [9]. Molecular analyses revealed that intragraft display of mRNA encoding granzyme B, IL-10, or IL-2 correlates with acute rejection, and intrarenal expression of transforming growth factor (TGF)-β1 mRNA is associated with chronic rejection. These data suggest that therapeutic strategies directed at the molecular correlates of rejection might refine existing antirejection regimens.

Treatment

IMMUNOSUPPRESSION PROTOCOLS

Induction protocols
Maintenance protocols
Early posttransplantation
Late posttransplantation
Antirejection therapy

FIGURE 9-9
Immunosuppressive therapy protocols. Standard immunosuppressive therapy in renal transplant recipient consists of 1) baseline therapy to prevent rejection, and 2) short courses of antirejection therapy using high-dose methylprednisolone, monoclonal antibodies or polyclonal antisera such as antilymphocyte globulin (ALG) and antithymocyte globulin (ATG).

Antilymphocyte globulin is prepared by immunizing rabbits or horses with human lymphoid cells derived from the thymus or cultured B-cell lines. Disadvantages of using polyclonal ALS include lot-to-lot variability, cumbersome production and purification, nonselective targeting of all lymphocytes, and the need to administer the medication via central venous access. Despite these limitations, ALG has been used both for prophylaxis against and for the primary treatment of acute rejection. A typical recommended dose for acute rejection is 10 to 15 mg/kg daily for 7 to 10 days. The reversal rate has been between 75% and 100% in different series. In contrast to murine monoclonal antibodies (eg, OKT3), ALS does not generally induce a host antibody response to the rabbit or horse serum. As a result, there is a greater opportunity for successful readministration.


**FIGURE 9-10**

Induction (panel A) and maintenance (panel B) immunosuppression protocols. These immunosuppressive protocols differ from center to center. There are numerous variations, but the essential features are 1) the prednisone dosage is high initially and then reduced to a maintenance dose of 10 to 15 mg/d over 6 to 9 months, and 2) the cyclosporine dosage is 8 to 12 mg/kg/d given as a single or twice daily dose, and dosage is adjusted according to trough plasma and serum blood levels. To maintain immunosuppression provided by cyclosporine and to reduce the incidence of cyclosporine side effects, azathioprine or mycophenolate has also been used with lower dosages of cyclosporine. The results of this triple therapy are excellent, with first-year graft survival greater than 85% reported in most instances and with a substantial number of patients having no rejection at all. Although this type of regimen was the most common, there have been a number of exceptions [2,10]. Recently, mycophenolate mofetil has been approved by the US Food and Drug Administration for prophylaxis of renal transplant rejection [11]. This agent was developed as a replacement to azathioprine for maintenance immunosuppression. FK 506 is a new immunosuppressive agent that has been approved by the FDA. FK 506 is similar to cyclosporine in its mode of action, efficacy, and toxicity profile. The drug has been used in kidney transplantation. FK 506 may be beneficial in renal transplantation as rescue therapy in patients taking cyclosporine who have recurrent or resistant rejection episodes [12–14].

**FIGURE 9-11**

Mechanism of action of immunosuppressive drugs. A, The sites of action of the commonly used immunosuppressive drugs. Immunosuppressive drugs interfere with allograft rejection at various sites in the rejection pathways. Glucocorticoids block the release of interleukin (IL)-1 by macrophages, cyclosporine (CsA) and FK 506 interfere with IL-2 production from activated helper T cells, and azathioprine (AZA) and mycophenolate mofetil (MPA) prevent proliferation of cytotoxic and helper T cells.

(Continued on next page)
A. ANTIREFLECTION THERAPY REGIMENS

- Intravenous methylprednisolone, 0.5 or 1 g x 3 d
- OKT3
- Antithymocyte gamma globulin
- Rabbit antithymocyte globulin
- Humanized anti-CD25 (IL-2 receptor) intravenously every 2 wk
- Anti–ICAM-1 and anti–LFA-1 antibodies

B, Mechanism of action of CsA, FK506, and rapamycin (RPM). CsA and FK506 block the transduction of the signal from the T-cell receptor (TCR) after it has recognized antigen, which leads to the production of lymphokines such as IL-2, whereas RPM blocks the lymphokine receptor signal, e.g., IL-2 plus IL-2 receptor (IL-2R), which leads to cell proliferation.

The addition of a prophylactic course of antithymocyte globulin (ATG) or OKT3 with delay of the administration of CsA or FK506 during the initial postoperative periods has been advocated by some groups. OKT3 prophylaxis was associated with a lower rate of early acute rejection and fewer rejection episodes per patient. Prophylactic use of these agents appears to be most effective in high-risk cadaver transplant recipients, including those who are sensitized or who have two HLA-DR mismatches or a prolonged cold ischemia time [2,10]. IFN-γ—interferon gamma; TNF-α—tumor necrosis factor-α.

FIGURE 9-11 (Continued)

B. Mechanism of action of CsA, FK506, and rapamycin (RPM). CsA and FK506 block the transduction of the signal from the T-cell receptor (TCR) after it has recognized antigen, which leads to the production of lymphokines such as IL-2, whereas RPM blocks the lymphokine receptor signal, e.g., IL-2 plus IL-2 receptor (IL-2R), which leads to cell proliferation.

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FIGURE 9-12

Treatment of acute rejection. A, Typical antirejection therapy regimens. B. Treatment algorithm. A biopsy should be performed whenever possible. The first-line treatment for acute rejection in most centers is pulse methylprednisolone, 500 to 1000 mg, given intravenously daily for 3 to 5 days. The expected reversal rate for the first episode of acute cellular rejection is 60% to 70% with this regimen [15–17]. Steroid-resistant rejection is defined as a lack of improvement in urine output or the plasma creatinine concentration within 3 to 4 days. In this setting, OKT3 or polyclonal anti–T-cell antibodies should be considered [18]. The use of these potent therapies should be confined to acute rejections with acute components that are potentially reversible, e.g., mononuclear interstitial cell infiltrate with tubulitis or endovasculitis with acute inflammatory endothelial infiltrate [19,21]. ATG—antithymocyte globulin; ICAM-1—intercellular adhesion molecule-1; LFA-1—leukocyte function-associated antigen-1.
9.10 Transplantation as Treatment of End-Stage Renal Disease

A. MAJOR SIDE EFFECTS OF IMMUNOSUPPRESSIVE AGENTS

<table>
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<tr>
<th></th>
<th>Cyclosporine</th>
<th>FK506</th>
<th>Azathioprine</th>
<th>Mycophenolate mofetil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrotoxicity</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>+</td>
<td>++</td>
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<td></td>
</tr>
<tr>
<td>Hirsutism</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gingival hypertrophy</td>
<td>+++</td>
<td>0</td>
<td>+</td>
<td>O</td>
</tr>
<tr>
<td>Hypertension</td>
<td>+++</td>
<td>+</td>
<td>?</td>
<td>Neoplastic</td>
</tr>
</tbody>
</table>

FIGURE 9-13
Side effects of immunosuppressive agents. A. The major side effects of several immunosuppressive agents. The major complication of pulse steroids is increased susceptibility to infection. Other potential problems include acute hyperglycemia, hypertension, peptic ulcer disease, and psychiatric disturbances including euphoria and depression. B. Vasoconstriction of the afferent arteriole (AA) caused by cyclosporine. (From English et al. [22]; with permission.)

FIGURE 9-14
The making of a polyclonal antilymphocyte preparation. Antilymphocyte globulin (ALG) or antithymocyte globulin (ATG) are polyclonal antisera derived from immunization of lymphocytes, lymphoblasts, or thymocytes into rabbits, goats, or horses. These agents have been used prophylactically as induction therapy during the early posttransplantation period and for treatment of acute rejection. Most centers reduce concomitant immunosuppression (e.g., stop cyclosporine and lower azathioprine dose) to decrease infectious complications. Antithymocyte gamma globulin (ATGAM) is the only FDA-approved polyclonal preparation. Two rabbit immunoglobulin preparations, raised by immunization with thymocytes or with a human lymphoblastoid line, are scheduled for phase III multicenter testing versus ATGAM or OKT3, respectively. Potential side effects include fever, chills, erythema, thrombocytopenia, local phlebitis, serum sickness, and anaphylaxis. The potential for development of host anti-ALG antibodies has not been a significant problem because of the use of less immunogenic preparations and probably because ALG suppresses the immune response to the foreign protein itself [2,10].
OKT3 is a mouse monoclonal antibody directed against the CD3 molecule of the T lymphocyte. OKT3 has been used either from the time of transplantation to prevent rejection or to treat an acute rejection episode. It has been shown in a randomized clinical trial to reverse 95% of primary rejection episodes compared with 75% with high-dose steroids in patients who received azathioprine-prednisone immunosuppression. In patients receiving triple therapy (cyclosporine-azathioprine-prednisone), 82% of primary rejection episodes were successfully reversed by OKT3 versus 63% with high-dose steroids. Like antilymphocyte globulin (ALG), reduction of concomitant immunosuppression (discontinuation of cyclosporine and reduction of azathioprine or mycophenolate mofetil dose) decreases the incidence of infectious complications. Side effects include fever, rigors, diarrhea, myalgia, arthralgia, aseptic meningitis, dyspnea, and wheezing, but these rarely persist beyond the second day of therapy.

Release of tumor necrosis factor (TNF), interleukin-2, and interferon gamma in serum are found after OKT3 injection. The acute pulmonary compromise due to a capillary leak syndrome rarely has been seen because patients are brought to within 3% of dry weight before initiation of OKT3 treatment. Infectious complications, particularly infection with cytomegalovirus, are increased after multiple courses of OKT3.

A. RECOMMENDED PROTOCOL FOR OKT3 TREATMENT

- Evaluation and treatment before administration
  - Physical examination
  - Laboratory tests including complete blood count
  - Monitor intake and output; record weight changes
  - Chest radiograph
  - Hemodialysis or ultrafiltration for volume overload
- Premedication on day 0 and 1
  - Methylprednisolone, 250-500 mg IV given 1 h prior to dose
  - Methylprednisolone or hydrocortisone sodium succinate, 250-500 mg IV given 30 min after the dose
  - Diphenhydramine, 50 mg IV 30 min prior to dose daily
  - Acetaminophen, 650 mg PO 30 min prior to dose
  - Discontinue cyclosporine, maintain azathioprine at 25 mg/d
- Administer OKT3, 5 mg/d IV, days 0–13
- Monitor clinical course
  - Check CD3 level on day 3
  - Increase OKT3 dosage to 10 mg/d if either:
    - Anti-OKT3 antibody is high
    - OKT3 level is low
    - CD3 level is not low

(Continued on next page)
**FIGURE 9-16 (Continued)**

B. Monitoring of peripheral blood T cells in a patient receiving OKT3 treatment. The absence of CD3+ cells from the circulation is the best parameter for monitoring the effectiveness of OKT3. Failure of the CD-positive percentage to fall or a fall followed by a rapid rise indicates the appearance of blocking antibodies. Approximately 50% to 60% of patients who receive OKT3 will produce human antimouse antibodies (HAMA), generally in low titers (<1:100). Low antibody titers do not affect the response to retreatment (reversal rate almost 100%) if the rejection episode occurs within 90 days after transplantation. Conversely, titers above 1:100 or recurrent rejection beyond 90 days is associated with a reversal rate of less than 25%. The reversal rate is essentially zero when both high HAMA titers and late rejection are present.

**FIGURE 9-17**

New immunosuppressive agents. New agents such as mycophenolate mofetil, FK506, and rapamycin are currently under evaluation for refractory acute rejection. In addition, both mycophenolate and rapamycin prevent chronic allograft rejection in experimental animals. Whether this important observation is reproducible in humans remains to be determined by long-term study.

A. Humanized monoclonal antibodies. The development of genetically engineered humanized monoclonal antibodies will largely eliminate the anti-antibody response, thereby increasing the utility of anti-T-cell antibodies in the treatment of recurrent rejection. Experimental antibody therapies are now being designed to directly target the CD4 molecule, the interleukin-2 receptor, the CD3 molecule by a humanized form of monoclonal anti-CD3, and adhesion molecules such as intercellular adhesion molecule-1 or leukocyte function-associated antigen-1 [23]. Humanized monoclonal antibodies are essentially human immunoglobulin G (IgG), nonimmunologic with a long half-life, and potentially can be administered intravenously about every 2 weeks. Humanized anti-CD25 (IL-2 receptor, chain) monoclonal antibodies has been shown to be effective in lowering the incidence of acute renal allograft rejection. Its role in the treatment of rejection, however, has not been explored. With increasing specificity for lymphocytes, these new agents are likely to have fewer toxicities and better efficacy.

B. Therapeutic application of CTLA4Ig to transplant rejection. APC—antigen-presenting cell; MHC—major histocompatibility complex; TCR—T-cell receptor.
References