Chapter 192
CLINICAL USE OF IMMUNOSUPPRESSANTS

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KEY POINTS

1. **Allograft rejection** is mediated primarily by the T cell in response to the presence of an antigen, which is processed by antigen-presenting cells (APC) and carried on the major histocompatibility complex (MHC) molecules to the T cell.
2. **The T-cell receptor (TCR),** in conjunction with accessory molecules such as CD3, CD4, and CD8, interacts with the antigen fragment on the MHC molecule and produces the growth factor interleukin-2 (IL-2) to activate the T cell and stimulate proliferation of the T cell.
3. **During allograft rejection,** cytokines attract various cells into rejecting allografts, stimulate the production of antibodies, and produce inflammation.
4. **Effective immunosuppressive protocols** combine multiple drugs targeted at different sites of the T-cell activation cascade.
5. **Corticosteroids block the early steps of T-cell activation;** they are used in tapering doses during the induction and maintenance phases of immunosuppressive protocols and in high, brief doses for the reversal of acute rejection episodes.
6. **The backbone of immunosuppressive protocols** are the calcineurin inhibitors cyclosporine and tacrolimus, which inhibit IL-2 production and subsequent T-cell activation and proliferation.
7. **Azathioprine and mycophenolate mofetil** inhibit purine synthesis, thereby disrupting the cell cycle and T-cell proliferation.
8. **Sirolimus** blocks the cellular response to IL-2 and inhibits the progression of the cell cycle, inhibiting T-cell proliferation.
9. **Antithymocyte globulin and monoclonal antibodies are potent cytotoxic compounds** that cause rapid, profound, and prolonged T-cell depletion; they are effectively used to reverse acute rejection episodes or as induction therapy before transplantation.
10. **Drug concentration monitoring is necessary** to maximize efficacy in preventing allograft rejection while minimizing the potential for significant adverse effects; monitoring aids in the management of drug interactions, particularly with cyclosporine, tacrolimus, and sirolimus therapy.

Advances in molecular biology and immunology have provided for greater understanding of the mechanisms involved in allograft rejection. Many of the key pathways of organ rejection are targeted by the growing armamentarium of immunosuppressive drugs available today. The vast array of immunosuppressive combinations has dramatically decreased the incidence of acute allograft rejection. However, very little ground has been gained with respect to the impact of chronic allograft rejection on long-term allograft survival. Furthermore, the relative nonselectivity of the current immunosuppressants with long-term use can lead to the development of malignancies and opportunistic infections. As we continue to explore different combinations of immunosuppressants and new immunosuppressive pathways, we will continue to grow in our comprehension of the immune system and come closer to true allograft acceptance.

BASIC PRINCIPLES OF IMMUNOSUPPRESSION

Optimal immunosuppression, as it relates to transplantation, is defined as the level of drug therapy that achieves graft acceptance with least suppression of systemic immunity. By optimizing immunosuppressive therapy, systemic toxicity (i.e., infection and malignancy) and other side effects can be minimized, albeit not entirely eliminated. Because monitoring of blood levels and titration of immunosuppression on this basis is possible with only a few agents, in practice, oversuppression or undersuppression almost invariably becomes apparent only in retrospect. Recently, monitoring of $CD3^+$ cell counts has provided an alternative means of measuring the degree of immunosuppression.

Current immunosuppression protocols typically use multiple drugs, each directed at a discrete site in the T-cell activation cascade.[1] Most immunosuppressive regimens combine drugs, often with differing modes of action and toxicities, allowing lower doses of each drug. Transplantation immunosuppression can be (1) *pharmacologic*, consisting of drugs such as corticosteroids, cytokine suppressive agents, and cell cycle inhibitors, or (2) *biologic*, consisting of monoclonal and polyclonal antilymphocyte antibodies and anticytokine receptor antibodies.[2]

The combination of cyclosporine or tacrolimus with a corticosteroid forms the backbone of most *maintenance immunosuppressive regimens* being used today. An antiproliferative agent may also be added. In general, the early postoperative period calls for the greatest degree of immunosuppression. As time goes on, many patients can maintain graft function with smaller doses of immunosuppressive agents.

If *acute cellular rejection* occurs, it is common to treat with a brief course of high-dose corticosteroid therapy, antilymphocyte antibodies, or both. Generally, high doses of a corticosteroid are used initially to reverse the acute attack on the allograft. Antilymphocyte antibody therapy with monoclonal or polyclonal antibodies is used for more severe rejection or if corticosteroid therapy fails.

Induction therapy, also called *prophylactic therapy*, refers to the use of antilymphocyte antibodies immediately after transplantation. This practice is based on the theory that early incapacitation of the immune system may reduce the likelihood of subsequent rejection. Claimed benefits are delayed onset of acute rejection, fewer episodes of rejection, and no significant increase in infectious complications.[3,4] The related concept of *sequential therapy* was introduced in response to the significant renal toxicity of cyclosporine observed in recipients of liver, heart, and kidney transplants. The practice is to use antibody therapy for the first 1 to 2 weeks after transplantation—the period in which renal injury is most likely to occur from a variety of insults. Cyclosporine therapy is not used during this period but is started later. The impact of this strategy on long-term renal function is much less clear.

This early intensification of immunosuppression is not universally accepted. Some experts voice concern because of the well-known association between antilymphocyte antibody therapy (and immunosuppression in general) and infection and malignancy.[5,6] Others describe no benefit, greater expense,[7] or the successful use of regimens that avoid induction altogether.[8] Intermediate strategies involve the use of induction only in high-risk patients or the use of just one dose of an antilymphocyte agent, followed by early evaluation of renal function.

Although some patients can tolerate complete withdrawal of immunosuppressive therapy without exhibiting rejection,[3] it is best done as a protocol-based strategy with patients under strict supervision. The current general approach is to minimize long-term immunosuppression. Various withdrawal protocols target individual components of the immunosuppressive regimen (e.g., corticosteroids, calcineurin inhibitors) in an attempt to decrease serious complications of immunosuppression; namely, infection, malignancy, and renal dysfunction.

OVERVIEW OF TRANSPLANTATION IMMUNOBIOLOGY

Antigen specificity is determined by an antigen-binding unit on the surface of the T cell called the T-cell receptor (TCR). The specificity and diversity of the TCR binding site result from variations in its amino acid composition among different T cells. The gene sequence coding for the TCR rearranges during development in the thymus, such that each T cell has a different TCR binding specificity. The result is a complex system that enables lymphocytes to discriminate between "self" and "nonself" or foreign antigen.

Once inside tissues or the circulation of the body, foreign antigen is presented to the lymphocytes by antigen-presenting cells (APCs), epitomized by dendritic cells. APCs phagocytose foreign proteins and cleave them enzymatically into small peptides that are 8 to 12 amino acids in length. These peptides are loaded onto a class of specialized carrier molecules, known as major histocompatibility complex (MHC) molecules. The MHC molecule carries the peptide fragment to the cell surface, where it is displayed to T cells in the host lymphoid organs. Thus, there are three essential requirements for the adaptive immune response known as rejection: (1) the presence of an antigen fragment or protein (a ligand) at the cell surface of the APC, (2) a receptor fit for the ligand, and (3) the activation of T cells.

The migration pattern of the antigen also is a critical factor. The only mobile antigen in organ transplantation consists of passenger leukocytes of bone marrow origin that are present in the graft and that migrate promptly and preferentially to host lymphoid organs.[9-11] These organs or organized heterotopic lymphoid collections provide the unique architectural structure and cellular milieu wherein factors that are necessary for progression from an immunogenic environment to a tolerogenic environment are present in abundance. These factors include cytokines, other molecules, cell–cell proximity, and homing mechanisms that ensure an efficient response to the antigen.[12] In the lymphoid organs, dendritic cells and other APCs that have captured and processed the antigen present the peptide fragment of the antigen to antigen-specific TCRs in the context of their upregulated host MHC peptide.

The efferent (effector) phase begins with the secretion of interleukin-2 (IL-2, or T-cell growth factor) and interferon-α (IFN-α) by activated lymphocytes. The antigen-specific immune activation and clonal expansion is aborted unless there is upregulation by the APCs of "accessory" cell-bound (costimulatory) molecules that sustain accelerated production of IL-2 and foster the secretion of numerous other cytokines (e.g., IL-1, IL-6, IL-9, IL-10, IFNs, tumor necrosis factor-α [TNF-α], TNF-β) and growth factors (granulocyte colony-stimulating factor [G-CSF] and granulocyte-macrophage colony-stimulating factor [GM-CSF]).[13] The sequential nature of the response amplification has been obscured by use of the term "costimulatory" to describe the accessory molecules, implying that the afferent and early effector phases are simultaneous.

The TCR is a cell surface molecule that associates with "accessory" molecules, including CD3, and either CD4 or CD8. The TCR-CD3 complex interacts with the peptide fragment carried by the MHC molecule of the APC. This complex is stabilized by the CD4 or CD8 molecule of the T cell. This interaction produces the signal that initiates activation of the T cell, leading to proliferation of a T-cell clone that recognizes the particular antigen fragments of the foreign protein. The basis for MHC-restricted antigen recognition requires antigen presentation by APCs bearing an MHC molecule specific to the host.

Antigen-directed proliferation of T-cell clones is absolutely essential for an effective immune response. The response is driven by a positive feedback loop. T cells that recognize antigen make the potent growth factor IL-2 and simultaneously become responsive to IL-2 by expressing the IL-2 receptor. This dual synthesis allows the cells to stimulate their own proliferation, as well as the proliferation of other T cells. Lymphocytes recirculate at a rate of 1% to 2% per hour, migrating through all tissues of the body. Specialized cell surface "homing" molecules on T lymphocytes mediate attachment to targeted alien tissues, with a special avidity for the endothelial cells of an allograft's vessels.

During an ongoing immune response, proliferating T cells recruit many other cell types and immune mechanisms into action. The cytokines can attract and activate

other leukocytes. For example, cytokines produced by CD4-positive helper T cells attract macrophages and CD8-bearing cytotoxic lymphocytes into rejecting allografts.[14] These cytokines also trigger macrophage activation and CD8+ T-lymphocyte cell maturation. The resulting multicellular tissue infiltration has traditionally been referred to as a *delayed-type hypersensitivity* response. Cytokines released by helper T cells also are responsible for the activation of B cells and thus, indirectly, for the majority of antibody production. Cytokines also upregulate both MHC molecules on tissues and adhesion molecules on endothelium. These events aid in the entry and accumulation of leukocytes. Finally, cytokines activate distant organ responses, such as the hepatic acute phase response, production of phagocytes in the bone marrow, and the hypothalamic-pituitary axis, producing the systemic signs of inflammation.

Once the antigen is consumed or removed, the process downregulates. If antigen removal is incomplete, continuously sensitized ("memory") T cells remain and contribute to a stronger secondary response on rechallenge with the same antigen. However, in some instances, if the antigen cannot be eliminated, the immune response can become exhausted and T cells deleted by mechanisms that are not fully understood but include Fas ligand–mediated apoptosis. Exhaustion-deletion in the first weeks or months after transplantation is never complete, but it can be maintained in a stable state by small numbers of persistent donor leukocytes.

Molecular insights regarding IL-2 gene transcription and the structure of the IL-2 receptor (IL-2R) have led to IL-2R–targeted therapy. As molecular knowledge has advanced, investigators have gained greater understanding of the workings of many immunosuppressants. New strategies guided by this knowledge have resulted in attempts to develop site-directed immunosuppression. Virtually every known step of the immune process can be targeted, and many new drugs are now in various stages of evolution.

SPECIFIC AGENTS

CORTICOSTEROIDS

Corticosteroids are extensively used in brief courses, at high doses, for the reversal of acute rejection episodes. These drugs are also used extensively in clinical immunosuppressive protocols, for both induction and maintenance phases.[15] Five glucocorticosteroids are commonly used in transplantation: hydrocortisone, prednisone, prednisolone, methylprednisolone, and dexamethasone.

Because hydrocortisone has the greatest mineralocorticoid activity per unit of glucocorticoid activity, its routine application in transplantation is relatively limited. The other four agents have more glucocorticoid activity in proportion to their mineralocorticoid activity.

Prednisone has an oral bioavailability of about 80%, and is metabolized in the liver to its active form, prednisolone. Oral prednisolone has a bioavailability of 100%. The serum half-life of both prednisone and methylprednisolone is 2 to hours.[16] The oral bioavailability of dexamethasone is 61%, a half-life of 2 hours.[17] However, the clinical activity of corticosteroids (i.e., suppression of cytokine production) per- for 24 hours or longer. In other words, the half-life for logic activity is much longer than the circulating half-life. ere is no universally accepted fixed dosing regimen orticosteroids. Rather, the dose is often dictated by local protocol. A preoperative dose of 250 to 1000 mg of methylprednisolone may be given, followed by 20 to 200 mg/day during the first week. Acute rejection may be treated with one to three large doses—250 to 1000 mg of methylprednisolone—or by a regimen starting at 200 mg/day of oral prednisone and tapering to baseline maintenance doses over 3 to 6 days. There is evidence that doses lower than those traditionally used can be equally effective. In combination regimens, steroid doses often can be reduced to 5 or 10 mg/day or less and perhaps given every other day.

Corticosteroids have broad effects on many cell types. These agents interfere with the production of IL-1 and IL-2, blocking the early steps of T-cell activation. Other pharmacologic effects related to immune function include the following:

1. Antagonism of inflammatory mechanisms by stabilization of leukocyte lysosomal membranes, reduction in capillary permeability, and inhibition of histamine release and the kinin and complement systems
2. Drastic reduction of lymphocyte traffic and circulating immunoglobulin levels and reduction in the number of neutrophils and eosinophils
3. Inhibition of leukocyte adhesion to endothelium

Prednisone and prednisolone have much less mineralocorticoid effect than the naturally occurring glucocorticoids do; however, sodium retention, edema, hypertension, potassium loss, and hypokalemic alkalosis can be seen with prolonged use of these drugs. Suppression of the pituitary-adrenal axis can be seen with all corticosteroids, but the magnitude of this effect varies among patients. Acute adrenal insufficiency can develop unexpectedly if patients are stressed, even as long as 12 months after steroids are withdrawn.

The adverse effects of corticosteroids are numerous and cause considerable morbidity. An increased incidence of serious infections is well documented. Impaired fibroblast growth and collagen synthesis contribute to poor wound healing. Hence, surgical wounds and anastomoses are at increased risk for dehiscence, and gastrointestinal ulcers tend to heal slowly, leading to increased risks of perforation and rebleeding. Spontaneous ulceration of the gastrointestinal tract occurs in approximately 2% of patients taking steroids. Because signs of inflammation are suppressed, the diagnosis of intraabdominal infection and peritonitis can be significantly delayed, sometimes with disastrous consequences.

Steroids impair glucose tolerance, often dramatically. For patients receiving large doses of steroids, it often is best to use "sliding-scale" insulin regimens to ensure adequate control of blood sugar levels. Some patients require long-term therapy with oral hypoglycemic agents or insulin to maintain adequate glucose control.

Central nervous system effects, such as euphoria and mood swings, are well known. These adverse effects are generally dose dependent and are seen most frequently early in the postoperative period or with therapy for acute rejection episodes, when higher doses of steroids are used. Central nervous system effects are usually self-limited and do not require treatment.

Long-term use of steroids can cause bone demineralization and lead to osteoporosis. Atherosclerosis may be accelerated. Prolonged administration of glucocorticoids is associated with an increased incidence of cataracts and elevated intraocular pressure (glaucoma). Soft-tissue and dermal changes (e.g., fat redistribution, skin atrophy, "moon face," striae) produce the characteristic cushingoid appearance.

To minimize the development of adverse sequelae, most immunosuppressive protocols attempt to reduce the dose of steroids over time to physiologic levels (equivalent to 5 mg/day or less of prednisone). However, corticosteroid doses must be reduced carefully to minimize side effects while maintaining adequate immunosuppression to prevent acute rejection of the allograft.

CYTOKINE INHIBITORS

Before the introduction of cyclosporine, immunosuppression protocols relied heavily on corticosteroids and cytotoxic drugs. These regimens had the disadvantage of producing broad suppression of the immune and inflammatory cascades. Cyclosporine introduced a new era of immunosuppression, because it provided potent, relatively specific, and noncytotoxic suppression of T-cell activation.

Cyclosporine

Cyclosporine is a lipophilic cyclic polypeptide with 11 amino acids and a molecular weight of 1202. On entering the T cell, cyclosporine binds to cyclophilin, a cytoplasmic immunophilin protein. The cyclosporine-cyclophilin complex inhibits the activity of calcineurin, which, in turn, inhibits transcription of several genes, including those transcribing IL-2, IL-3, IL-4, GM-CSF, IFN-γ, and TNF-α. One key action that results from blockade of calcineurin is inhibition of signaling via nuclear factor of activated T cells (NF-AT), which regulates activation of the IL-2 gene; this effect ultimately prevents the synthesis of IL-2.[18] Inhibition of the synthesis of IL-2, a potent T-cell growth factor, is the crucial activity of cyclosporine.

Cyclosporine is insoluble in water and therefore must be dissolved in an organic solvent. There currently exist two formulations: cyclosporine (Sandimmune, Novartis Pharmaceuticals, East Hanover, NJ) and cyclosporine for microemulsion (cyclosporine, modified; Neoral, Novartis Pharmaceuticals, and Gengraf, Abbott Laboratories, North Chicago, IL). The microemulsion formulation substantially increases cyclosporine absorption; the overall time to peak cyclosporine concentration is reduced, the peak concentration is higher, and the area under the curve (AUC) is increased. The lipophilicity of the conventional cyclosporine formulation is responsible for its variable bioavailability.

Oral bioavailability is about 30%, but there is much individual variability (range, 10% to 60%). Absorption in the small intestine decreases with bowel dysfunction or reduced bile flow.[19] The volume of distribution of cyclosporine is large and variable. Cyclosporine is metabolized in the liver via cytochrome P450 (CYP) 3A4 enzymes. It also is a substrate for the p-glycoprotein efflux pump. The mean terminal half-life with normal liver function is 19 hours. The microemulsion formulation of cyclosporine has superior pharmacokinetics, does not require bile excretion for its bioavailability, and is better dispersed and absorbed compared with conventional cyclosporine. The relative bioavailability of the microemulsion formulation is approximately 60%.[20] The total AUC is increased by 30% compared with the conventional formulation.[21]

At least 17 cyclosporine metabolites have been identified, and at least a few of them are immunosuppressive, although considerably so less than the parent compound. The half-life increases with hepatic failure and is changed significantly by coadministration of a large number of other drugs that can increase or decrease serum levels by induction or competitive inhibition of P450 (Table 192-1).[22] For all these reasons, it is essential that levels be monitored regularly and dosage adjusted accordingly.

TABLE 192–1. SOME OF THE DRUGS THAT ALTER CYCLOSPORINE AND TACROLIMUS CONCENTRATIONS

Increase	Decrease
Diltiazem	Rifampin
Nicardipine	Carbamazepine
Verapamil	Phenobarbital
Fluconazole	Phenytoin
Itraconazole	Ticlopidine
Ketoconazole	Nafcillin
Clarithromycin	
Erythromycin	
Methylprednisone (in large doses)	
Bromocriptine	
Danazol	
Protease inhibitors	

Monitoring of cyclosporine levels is not straightforward. Different results are obtained when cyclosporine concentrations in blood or plasma are determined by radioimmunoassay and by high-pressure liquid chromatography (HPLC). Neither method is clearly superior, and there are no universally accepted blood levels; target levels vary widely from center to center. Desired levels in serum or plasma, as measured by radioimmunoassay,[23] are 150 to 250 ng/mL at the time of transplantation, tapering to 50 to 100 ng/mL after 3 to 6 months. If the drug is measured in whole blood by HPLC, desired levels are 100 to 300 ng/mL initially, tapering to 80 to 200 ng/mL.

Recent literature suggests that AUC values and peak concentrations measured 2 hours after dosing (C_2) are more sensitive predictors of cyclosporine effects and may be better parameters to guide therapeutic monitoring of the microemulsion formulation of cyclosporine. Decreased bioavailability of cyclosporine has been correlated with acute rejection.[24] The first 4 hours after administration of a dose of cyclosporine represents the period of greatest variability in cyclosporine absorption.[25] Limited sampling techniques, consisting of two to five blood samples drawn within the first 4 hours after cyclosporine administration, are used to determine the AUC. AUC values greater than 4400 μg/L/hour correlate well with a low incidence of allograft rejection.[24,26] One study compared the correlation between the trough concentration, C_2, and the occurrence of rejection and concluded that trough concentrations lack predictive value; however, acute rejection did not occur in patients with C_2 values greater than 1200 μg/L.[27] Because of the convenience of a single blood sample compared with the multiple blood samples necessary for AUC measurements, C_2 monitoring is becoming a preferred way to adjust cyclosporine dosing. C_2 levels should range between 1.5 and 2.0 μg/mL for the first few months after transplantation and should be reduced to 0.8 μg/mL after 6 to 12 months of therapy.[26,28]

The typical daily intravenous dose of cyclosporine is 4 to 5 mg/kg. This amount can be given in two divided doses, each being delivered over 2 to 6 hours. Alternatively, some prefer to use a slow, continuous infusion over 24 hours. The changeover to oral dosing usually requires a dose three times higher, or about 12 to 15 mg/kg/day. Oral cyclosporine

should be administered every 12 hours. After 1 to 2 weeks, the dosage can be slowly tapered, once equilibration within body fat stores occurs. In many patients, the dose is tapered to as low as 3 mg/kg/day by 6 months after transplantation. Liver transplant recipients who have a T tube, which diverts some bile flow, require higher oral doses because of decreased absorption. Pediatric patients eliminate cyclosporine faster than adults do, and they require larger doses, typically about 5 to 6 mg/kg/day intravenously and 14 to 18 mg/kg/day orally. Some pediatric patients require doses up to 50% to 100% larger than adult doses.

Several adverse effects can occur early after initiation of cyclosporine therapy. Acute nephrotoxicity and hypertension are major problems. The mechanisms responsible for these adverse effects are controversial.[29,30] Nephrotoxicity may be the result of cyclosporine-induced afferent arteriolar vasoconstriction that results, in part, from an imbalance between the production of prostaglandin E_2, a vasodilator, and that of thromboxane A_2, a vasoconstrictor.[31,32] Other possible factors include endothelin-1–induced vasoconstriction and impaired nitric oxide production.[33] Cyclosporine-induced nephrotoxicity is transient and reversible with a decrease in dosage or discontinuation of the drug.[34] The incidence of nephrotoxicity varies from approximately 25% to 38%.[35]

Neurotoxicity associated with cyclosporine ranges from minor toxicity, manifesting as tremors, to severe complications, such as seizures or encephalopathy.[36] Tremors caused by cyclosporine are common (prevalence, 10% to 55%) and may improve over time without a change in therapy. The causal association between seizures and encephalopathy often is not clear.[36] Several reports have detailed a rare syndrome that is characterized by confusion and cortical blindness in both liver and bone marrow transplantation patients. Hypomagnesemia and hypocholesterolemia are believed to be risk factors for cyclosporine-induced neurotoxicity.[29]

Hypertension occurs frequently and usually begins within weeks after commencement of cyclosporine therapy. The incidence of hypertension varies widely in different patient populations, ranging from 10% to 80%.[35] It is hypothesized[37] that hypertension is caused by cyclosporine-induced vasoconstriction in the renal or systemic circulation, or both, perhaps as a result of antagonism of endothelium-derived relaxation factors or increased synthesis of endothelin-1, a vasoconstrictor. Hypertension responds to sodium restriction. Hypertension is best managed with diuretics or calcium channel blockers.[30]

Cyclosporine is diabetogenic, although analysis of this effect is confounded by the frequent concomitant use of steroids with cyclosporine. Other metabolic effects of cyclosporine include hypochloremic alkalosis and changes in serum concentrations of potassium, magnesium, prolactin, and testosterone. Hepatotoxicity, manifested by cholestatic jaundice, is common,[29] but intrahepatic cholestasis often resolves if the dose of cyclosporine is reduced. Connective tissue side effects of cyclosporine are common and can be distressing to the patient because of the cosmetic manifestations. These changes include hirsutism (seen within 2 to 4 weeks in 20% to 45% of patients receiving cyclosporine), gingival hyperplasia (in 4% to 16% of patients), and coarsening of facial features.[38] Long-term administration of cyclosporine is associated with irreversible nephrotoxicity. The incidence of this serious side effect is estimated to be 15% to 40%.[39] The pathologic lesion resembles nephrosclerosis.[40]

Tacrolimus

Tacrolimus (FK-506; Prograf, Fujisawa Healthcare, Deerfield, IL) is a macrolide antibiotic with immunosuppressive activity produced by the fungus *Streptomyces tsukubaensis*. It is approved by the U.S. Food and Drug Administration (FDA) for liver and kidney transplant recipients. It is also used extensively in small bowel, pancreas, heart, and lung transplantation. The molecular structure of tacrolimus is unrelated to that of cyclosporine, and the two drugs have different cytosolic binding sites.[41,42] Tacrolimus binds to the immunophilin called FK-binding protein-12 (FKBP12).[43] Like the cyclosporine-cyclophilin complex, the tacrolimus-FKBP12 complex binds to and inhibits the activity of calcineurin. As is the case with cyclosporine, inhibition of calcineurin by tacrolimus blocks the transcription of several genes, including the genes transcribing IL-2, IL-3, IL-4, GM-CSF, IFN-γ, and TNF-α. The effect of tacrolimus on TNF-β expression differs from that induced by cyclosporine. Tacrolimus-mediated inhibition of TNF-β expression may play a role in reducing chronic rejection,[43] although no clinical difference has been noted between the two drugs. Like cyclosporine, inhibition of calcineurin disrupts signaling via NF-AT, ultimately inhibiting the synthesis of the potent T-cell growth factor, IL-2; this is the key pharmacologic effect of tacrolimus. The immunosuppressive effects of tacrolimus also may involve other pathways that activate T cells.[44]

Tacrolimus is highly lipophilic and must be dissolved in an organic solvent. Oral bioavailability is highly variable and poor, reportedly ranging from 6% to 56%, with a mean of 25%.[45] The gastrointestinal absorption of tacrolimus, compared with that of cyclosporine, is less dependent on bile flow.[46] Tacrolimus is extensively bound to erythrocytes because of the high concentration of FKBP12 found in the red blood cells. Like cyclosporine, tacrolimus is metabolized in the liver via the cytochrome P450 enzyme system, primarily by CYP3A4, although other enzymes have been reported to be involved as well.[47] Tacrolimus metabolism, like that of cyclosporine, can be significantly altered by liver dysfunction or coadministration of other drugs that induce or competitively inhibit P450; these effects can decrease or increase circulating levels of tacrolimus (see Table 192-1). Tacrolimus is a substrate for the p-glycoprotein efflux pump. The mean terminal half-life of tacrolimus is 12 hours. At least 15 metabolites of tacrolimus have been identified.[43] Some of these metabolites have as much as 10% of the immunosuppressive activity of the parent compound.[47]

Therapeutic monitoring of circulating tacrolimus concentrations is essential for preventing toxicity while maintaining adequate immunosuppression. Plasma and whole-blood trough concentrations correlate with AUC as well as clinical outcomes and toxicities.[48] Because of the extensive binding of tacrolimus to erythrocytes, whole-blood tacrolimus concentrations are 10 to 30 times higher than the corresponding plasma concentrations.[47] The most commonly used tacrolimus assay is the microparticulate enzyme immunoassay, although HPLC and enzyme-linked immunosorbent assays are also readily available.[49] The therapeutic range for tacrolimus levels in whole blood is 5 to 20 ng/mL. Plasma tacrolimus levels should be maintained between 0.5 and 2 ng/mL.

The typical intravenous dose of tacrolimus is 0.05 to 0.1 mg/kg/day. The drug should be administered as a slow, continuous infusion over 24 hours. Oral doses are generally three to four times higher than intravenous doses and range

from 0.1 to 0.2 mg/kg/day, administered in two divided doses every 12 hours. Maintenance doses of tacrolimus range from 0.0125 to 0.5 mg/kg/day due to variability among patients with respect to absorption of the drug and requirements for immunosuppression.[47] No decrease in tacrolimus dose is needed when the T tube is clamped after liver transplantation. Tacrolimus clearance is faster in pediatric patients; therefore, larger doses may be required in children compared with adults.[47] Pediatric intravenous doses range from 0.03 to 0.05 mg/kg/day, and pediatric oral doses range from 0.15 to 0.3 mg/kg/day in divided doses.

Tacrolimus has a potential advantage over cyclosporine because of its ability to reverse ongoing acute rejection.[50-53] Experience with tacrolimus was first gained when the drug was used as rescue therapy in liver and kidney transplantation.[54-56] Today, tacrolimus is used as a primary immunosuppressive agent for all types of solid organ transplants.

The toxicity profile for tacrolimus is similar to that of cyclosporine, perhaps because they have a similar mechanism of action (i.e., calcineurin inhibition). As experience has been gained with tacrolimus, it is clear that many of the toxic side effects are dose related and are best managed by reducing the dose. Acute nephrotoxicity induced by tacrolimus is dose related. The incidence is not clearly defined in the literature, but it is similar to that of cyclosporine and most likely results from afferent arteriolar vasoconstriction. Nephrotoxicity resolves after the dose of tacrolimus is reduced or the drug is discontinued. As with cyclosporine, irreversible renal injury can occur after prolonged therapy with tacrolimus.[57]

Neurotoxicity is the most commonly reported adverse effect of tacrolimus. The reported incidence ranges from 3.6% to 32%.[58] This side effect can range from mild toxicity, such as tremors, headaches, paresthesias and insomnia, to severe complications including encephalopathy, coma, seizures, and psychosis. Usually, neurotoxicity associated with tacrolimus responds to a reduction of the dose; however, idiosyncratic reactions may require discontinuation of the drug.

The potential for tacrolimus to induce a diabetic state is similar to that for cyclosporine.[59,60] Increased fasting glucose levels and the development of overt diabetes mellitus are associated with elevated tacrolimus concentrations (greater than 15 ng/mL), acute rejection, and higher body mass index.[61] Tacrolimus-induced diabetes mellitus is reversible.[62]

Hyperkalemia and hypomagnesemia are commonly noted in patients receiving tacrolimus. Acute hyperkalemia can be managed with standard approaches, including administration of insulin and glucose and sodium bicarbonate or a cation exchange agent (sodium polystyrene sulfonate). Chronic hyperkalemia may require therapy with fludrocortisone acetate to increase renal potassium excretion. Hypomagnesemia often requires magnesium replacement to avoid complications.

The incidences of hypertension and hyperlipidemia associated with tacrolimus therapy appear to be lower than those reported with cyclosporine.[63-66] This more favorable adverse effect profile has been reported to translate into a decrease in the number of cardiovascular complications in patients treated with tacrolimus compared with cyclosporine.[66]

Tacrolimus is not associated with the connective tissue side effects seen with cyclosporine; therefore, cosmetic problems are not seen. Alopecia can be problematic for patients receiving tacrolimus, but this problem is reversible and usually does not require dosage adjustments.[67]

CELL CYCLE INHIBITORS

The precise mechanism of immunosuppression mediated by cytotoxic drugs is not known; however, the negative effect of these agents on the proliferation of lymphocytes is believed to inhibit the generation of antigen-specific T-cell clones. As one might expect, an increased risk of malignancies with the long-term use of these agents is a concern.

Azathioprine

Azathioprine (AZA; Imuran, Prometheus Laboratories, Greenville, NC), a thio analog of the purine adenine, inhibits purine metabolism. The parent drug is inactive but is rapidly converted to 6-mercaptopurine (6-MP) in red blood cells and subsequently to 6-thioinosine monophosphate, a purine analog, in vivo.[68] Both the de novo and the salvage pathways of purine synthesis are inhibited by azathioprine. 6-Thioguanine nucleotides interfere with DNA and RNA synthesis, rendering cells unable to function properly and allowing strand breaks in chromosomes. Azathioprine is most toxic to proliferating cells that are making new DNA.

Azathioprine can be used in maintenance immunosuppressive regimens; it has no usefulness for the treatment of acute rejection episodes.[69] The oral bioavailability of azathioprine is approximately 40%. Metabolism of 6-MP involves catabolism by xanthine oxidase in the liver and gut to inactive metabolites that are excreted by the kidneys. The 6-thioguanine nucleotides have a very long tissue half-life (approximately 13 days), permitting azathioprine to be administered by once-daily dosing. The inactive end metabolite is 6-thiouric acid, which is excreted by the kidneys. With congenital deficiency of the enzyme, thiopurine methyltransferase, (incidence, 1 in 300 patients), or with renal failure, accumulation of 6-thioguanine nucleotides causes increased toxicity.

The starting dose for azathioprine is 3 to 5 mg/kg once daily. The drug can be given intravenously at half the dose for brief periods. The typical maintenance oral dosage after transplantation is 2 to 3 mg/kg daily. Tapering of the dose to 1 to 2 mg/kg per day is often possible over time. In combination regimens, azathioprine can be reduced to as low as 0.25 to 0.5 mg/kg/day.

Dose-limiting myelosuppression usually occurs 1 to 2 weeks into therapy. Pancytopenia and thrombocytopenia with megaloblastic anemia is the pattern usually seen. White blood cell counts lower than 3000 cells/mm^3 warrant dose reduction or discontinuation of the drug. As with other antiproliferative drugs, nausea, vomiting, and hair loss may occur. Hepatic injury can occur in two patterns. One form is reversible hepatitis. The other form is rare but serious hepatic veno-occlusive disease, which can cause irreversible liver damage. Azathioprine therapy also has been associated with pancreatitis. Because of concerns about hepatotoxicity and pancreatitis, some transplantation experts questioned the value of azathioprine for immunosuppression.[70,71] Hypersensitivity to azathioprine has been reported to cause a variety of manifestations; diagnosis of these disorders is based largely on clinical findings.

Allopurinol inhibits xanthene oxidase, one of the enzymes involved in degradation of azathioprine metabolites, thereby increasing the toxicity of the parent compound. Accordingly, if therapy with allopurinol is indicated, this agent should be added cautiously to an immunosuppressive regimen containing azathioprine. If allopurinol must be used, the dose of azathioprine should be reduced by more than 50%.

Mycophenolate Mofetil

Mycophenolate mofetil (MMF; CellCept, Roche Laboratories, Nutley, NJ) is a prodrug of mycophenolic acid (MPA). MPA noncompetitively inhibits inosine monophosphate dehydrogenase (IMPDH), a key enzyme that regulates the purine nucleotide de novo synthesis pathway.[72] T and B lymphocytes are dependent on IMPDH and the de novo pathway for purine synthesis during proliferation. Other cell lines, including granulocytes, red blood cells, platelets and tissue cells, use both the de novo and the salvage pathways for purine synthesis.[73] For this reason, MPA is more selective for T and B lymphocytes, which results in a more favorable adverse effect profile. MPA also may induce apoptosis in activated T cells, and it may interfere with expression of adhesion molecules in leukocytes and lymphocyte recruitment.[74]

Mycophenolate mofetil is rapidly absorbed after oral administration and undergoes rapid first-pass metabolism in the liver to MPA, the active form of the drug. The bioavailability of MPA is 94%.[72] Maximum concentrations of MPA are reached approximately 1 hour after oral administration.[75] MPA binds to plasma albumin, and free MPA levels can be altered by fluctuations in albumin levels or other medications that compete for albumin binding. Metabolism of MPA occurs by glucuronidation in the liver and renal tubular cells, primarily to an inactive compound, mycophenolic acid glucuronide (MPAG), which is eliminated by the kidneys[72] and to a second acyl glucuronide (M-2), which has in vitro activity.[76]

The dose of mycophenolate needed to prevent rejection in kidney and liver transplant recipients is 2 g/day. Cardiac transplant recipients generally require higher levels of immunosuppression and should receive 3 g/day. The total daily dose should be administered over two dosing intervals. Patients who are unable to tolerate twice daily dosing may benefit from separation of the total daily dose into three or four dosing intervals.

The need for therapeutic monitoring of MPA levels remains controversial. Currently, two assays are available: HPLC and an enzyme-multiplied immunoassay technique (EMIT). HPLC can measure both MPA and metabolite concentrations and is sensitive enough to measure free MPA concentrations.[77] The active metabolite of MPA, M-2, cross-reacts with the EMIT assay, resulting in higher measured concentrations. A correlation between acute rejection and both total MPA AUC and trough MPA concentrations determined by HPLC has been demonstrated.[78] Acute rejection is predicted better by trough levels than by the AUC. However, the risk of adverse effects correlates better with the dose of MPA rather than circulating MPA concentrations.[79] The therapeutic range for total MPA AUC is 30 to 60 mg/h/L.[78] MPA trough levels should be maintained between 1 and 3.5 mg/L.[77] Another monitoring strategy is measurement of the early peak concentration (30 minutes after oral dose [C_{30}]).[80] Further studies are necessary to determine the most appropriate strategy for therapeutic monitoring of MPA.

The most common adverse effects are gastrointestinal. Mild effects include nausea, vomiting, diarrhea, constipation, and dyspepsia. Severe complications, including cholecystitis, large bowel perforation, and pancreatitis, are rare and have not been definitively related to treatment with MPA. Mild gastrointestinal effects usually are transient. Prolonged symptoms can be managed by either reducing the dose of MPA or increasing the number of dosing intervals from twice daily to three or four times daily.[81]

Hematologic adverse effects are rare and are manifested as bone marrow suppression. The most commonly reported features are leukopenia and anemia, but the side effect profile also can include thrombocytopenia and pancytopenia. The onset of myelosuppression typically occurs within the first 6 months after starting MPA therapy and may be dose related. Resolution occurs within 1 week after stopping the drug in most cases.[72]

Infections are frequently cited as adverse effects of MPA, but they are a complication of immunosuppression in general. The reported incidence of opportunistic infections was increased in patients receiving MPA in addition to cyclosporine and prednisone compared with those receiving cyclosporine and prednisone alone[81,83]; however, no difference was reported when the MPA-containing regimen was compared with cyclosporine, prednisone, and azathioprine.[84] Nephrotoxicity and hepatotoxicity have not been reported with MPA.

MPA is effective maintenance therapy for prevention of acute rejection of solid organ allografts in combination with other immunosuppressive agents, such as corticosteroids and cyclosporine[82-84] or tacrolimus.[85] MPA has been used to treat acute rejection of renal transplants[86] and, in refractory rejection, to reduce the use of antilymphocyte therapy.[87] In addition, MPA has been used as rescue therapy for acute and chronic rejection of cardiac transplants.[88] Recent studies have shown promise in combining MPA with sirolimus to eliminate the need for calcineurin inhibitors, thereby reducing the potential for nephrotoxicity.[89,90]

Sirolimus

Sirolimus (rapamycin; Rapa; Rapamune, Wyeth Laboratories, Philadelphia, PA) is a macrolide antibiotic that is structurally related to tacrolimus. Like tacrolimus, it also binds to FKBP12, but sirolimus does not inhibit calcineurin or block cytokine gene transcription in T cells; rather, it inhibits the mammalian targets of rapamycin (mTOR). When stimulated by IL-2 and other growth factors, mTOR activates kinases that translate cytokine messenger RNA, which ultimately progresses the cell cycle from G_1 to the S phase. By blocking mTOR, sirolimus inhibits the cellular response to IL-2 and inhibits progression of the cell cycle, thereby prohibiting T-cell proliferation.[91]

Sirolimus is insoluble in water and must be dissolved in an organic solvent. It has poor bioavailability (15%). Maximum concentrations are reached within 2 hours after oral administration.[92] Because of its high lipophilicity, sirolimus readily enters cells, producing a large volume of distribution. Sirolimus binds extensively to erythrocytes (95%) because of their high FKBP12 content; minimal binding occurs with other plasma proteins.[93] Like cyclosporine and tacrolimus, sirolimus is metabolized primarily in the liver by CYP3A4. Sirolimus is also a substrate for the p-glycoprotein efflux pump. *O*-demethylation and hydroxylation produce several metabolites. The metabolites of sirolimus have less than 10% of the immunosuppressive activity of the parent compound and are excreted via the bile into feces.[91]

Hepatic metabolism by the CYP3A4 enzymes creates the potential for significant changes in the half-life of sirolimus if other drugs affecting these enzymes are also administered. These changes can decrease or increase serum levels by induction or competitive inhibition of P450. Many of the same drugs that alter cyclosporine and tacrolimus levels can also alter sirolimus levels (see Table 192-1). Coadministration of

sirolimus with cyclosporine significantly increases the AUC and trough concentrations for sirolimus. Likewise, sirolimus also significantly increases the AUC and trough concentrations for cyclosporine. To minimize the interaction and potential toxicities of the two drugs, sirolimus administration should be separated from cyclosporine administration by 4 hours.[94]

Its long half-life of approximately 60 hours[95] makes sirolimus suitable for once-daily dosing. The two pivotal trials that led to the FDA-approval of sirolimus capitalized on the interaction that occurs with coadministration of cyclosporine and sirolimus. These studies demonstrated a reduction of acute rejection episodes in kidney transplant recipients when sirolimus was given using either of two fixed dosing regimens: a 6-mg loading dose followed by 2 mg daily or a 15-mg loading dose followed by 5 mg daily.[96,97] These results suggest that therapeutic drug monitoring is not necessary. However, clinical experience indicates that sirolimus therapy is optimized when doses are based on blood concentrations, particularly if sirolimus is used in the absence of cyclosporine synergy.[98]

Therapeutic monitoring of sirolimus should be based on whole-blood concentrations, because large amounts of the drug are sequestered in erythrocytes, resulting in undetectable concentrations in plasma.[99] HPLC with mass spectroscopy and ultraviolet detection are the most commonly used methods to measure sirolimus concentrations. A correlation between the trough level and the AUC for sirolimus has been established.[100,101] Furthermore, there is a strong correlation between the rate and severity of acute rejection and low trough levels, as well as between the occurrence of adverse effects and high trough levels. The therapeutic range is 5 to 15 ng/mL.[101] A microparticle enzyme immunoassay has been developed[102] and may be beneficial for analyzing multiple samples with more rapid turnaround.[103] Frequent monitoring of sirolimus levels is not warranted because of the long half-life of the drug. Sirolimus levels should be evaluated 5 to 7 days after initiation of therapy or a dose change, to allow sufficient time for drug levels to reach steady state.[100]

The adverse effect profile of sirolimus is different from that of other immunosuppressants. Unlike cyclosporine and tacrolimus, sirolimus rarely causes nephrotoxicity or neurotoxicity. Dose-dependent myelosuppression can be seen after initiation of sirolimus therapy. Thrombocytopenia commonly manifests within the first 2 weeks of therapy but improves with continued treatment. Leukopenia and anemia may also manifest shortly after initiation of therapy, but they are transient.[103] Thrombocytopenia and leukopenia are related to sirolimus trough concentrations greater than 15 ng/mL.[101]

Hyperlipidemia is commonly seen in patients receiving sirolimus; the findings are hypercholesterolemia and hypertriglyceridemia. This effect has been reported in virtually all clinical trials.[91] Peak levels of total cholesterol and triglycerides are dose related and usually are reached within 3 months after initiation of sirolimus, but the levels decrease after 1 year.[103] Both changes are reversible with dose reduction or discontinuation.[92] The cause of sirolimus-associated hyperlipidemia is thought to be overproduction of lipoproteins or inhibition of hepatic lipoprotein lipase, leading to decreased lipolysis.[103] Use of antihyperlipidemic agents, such as the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, is effective for treating hyperlipidemia in patients receiving sirolimus. Analysis of cholesterol values after 1 year of sirolimus therapy in the Framingham Model indicates that sirolimus should cause only a modest increase in the incidence of ischemic heart disease in kidney transplant recipients (2 to 3 new cases per 1000 persons per year).[103] Therefore, treatment with sirolimus should have only a minimal impact on the risk for cardiovascular disease. It has been proposed that the decreased incidence of hyperlipidemia associated with tacrolimus compared with cyclosporine may lessen the frequency and severity of hyperlipidemia in transplant recipients who receive tacrolimus- and sirolimus-based immunosuppressive therapy.[103]

Mouth ulcers have been reported with sirolimus; they appear to be more pronounced with the liquid formulation and may be dose related. Other adverse effects reported with sirolimus include elevated liver enzymes, lymphocele formation, hypertension, rash, acne, diarrhea, and arthralgia.

Sirolimus is effective as maintenance therapy for the prevention of acute rejection of solid organ allografts in combination with steroids and cyclosporine[96,97] or tacrolimus.[104] It also is effective in steroid-withdrawal regimens[105] or to spare cyclosporine in an attempt to minimize nephrotoxicity associated with this agent.[106,107] It is speculated that sirolimus may reduce the potential for chronic rejection by inhibiting growth factor–mediated cell proliferation and intimal hyperplasia associated with chronic rejection,[103] but longer follow-up is necessary to prove this theory.

BIOLOGIC AGENTS

Antithymocyte Globulin

Antilymphocyte antibodies such as antilymphocytic globulin (ALG) were first produced by immunization of animals against purified lymphocyte preparations, resulting in multispecific polyclonal antibodies. Antibodies that cross-reacted with other cellular molecules in blood were then removed by extensive adsorption to blood components. Because of variability among immunized animals, substantial amounts of ALG were pooled to produce a more homogeneous preparation.

Antibodies to surface molecules on lymphocytes interfere with lymphocyte function in the immune response by several possible mechanisms. Lymphocytes are removed from the circulation rapidly after treatment with antilymphocyte antibodies. In addition, lymphocytes are phenotypically and functionally altered. Thymocytes, unactivated lymphocytes, and T and B lymphoblasts are used to produce the equine polyclonal antibody, antithymocyte globulin (ATG; ATGAM, Pharmacia & Upjohn, Kalamazoo, MI). A newer rabbit preparation, RATG (Thymoglobulin, SangStat Medical Corporation, Fremont, CA), is less immunogenic and may have other advantages over the equine preparation. B lymphocytes are targeted to a lesser extent with RATG than with equine ATG,[108] helping to some extent to preserve infection-induced antibody production. Furthermore, CD4+ T lymphocytes are the predominant target of RATG,[109] and this agent has lesser effects on other leukocytes, compared with equine ATG. RATG-induced lymphocytopenia persists for a much longer time than with former antilymphocyte preparations. Surface molecules that serve as binding sites for RATG include the T-cell antigens, CD6, CD16, CD18, CD38, CD40, and CD58, among others. The result is inhibition of cellular function of other cell lines, including monocytes, thymocytes, natural killer cells, leukocytes, and dendritic cells.

Equine ATG is administered in a single daily dose (10 to 15 mg/kg). The dose of RATG, which is more potent, is 1 to 1.5 mg/kg given as a single daily dose. Therapy for acute

rejection usually is continued for 7 to 14 days. Induction therapy with polyclonal antibodies typically uses the same doses for 5 to 10 days of therapy. Polyclonal preparations cause a high incidence of febrile reactions with the first few doses. Antihistamines (usually diphenhydramine, 50 mg), antipyretics (i.e., acetaminophen, 650 mg), and corticosteroids are given as premedications.

Because of the lack of specificity of polyclonal antibodies, therapeutic drug monitoring generally is not useful. In addition, fixed weight-based dosing regimens reduce the need for drug concentration monitoring. Some advocate monitoring the number of CD3+ lymphocytes with flow cytometry as a gauge of immunosuppressive effect.

The effects of ATG on other cell types is the basis for adverse effects associated with these preparations. The most troublesome adverse effect is myelosuppression, manifested by leukopenia, anemia, and thrombocytopenia. These effects are dose related and can be managed by decreasing the dose or discontinuing the drug.

As described previously, the first few doses of ATG preparations are often accompanied by fever, which can be ameliorated with the use of appropriate premedications. Other adverse effects include anaphylactic reactions, hypotension, urticaria, and serum sickness, particularly with equine ATG. After approval of RATG, use of equine ATG declined considerably because of the better side effect profile of RATG and its increased efficacy in reducing acute rejection[110] and preventing rejection as part of induction therapy.[111]

The efficacy of ATGs in reversing solid organ allograft rejection has been well established. ATGs are frequently reserved for steroid-resistant allograft rejections. Prospective, controlled studies have demonstrated equal or superior efficacy for both equine and rabbit ATG in preventing rejection as induction therapy, compared with OKT3.[112,113] High doses of RATG are also being used in T cell–depleting regimens to induce tolerance and to allow for monotherapy after transplantation with subsequent weaning of immunosuppression.[114]

Anti-CD3 Monoclonal Antibody

Efforts to increase the potency and decrease the variability of ALGs led to development of single-specificity monoclonal antibodies. The first of these products was muromonab CD3 (OKT3; Orthoclone OKT3, OrthoBiotech Products, LP, Raritan, NJ). OKT3 is a purified murine-derived monoclonal antibody directed at the ε chain of the CD3 receptor,[115] which is found on all mature human T cells.[116] After administration, OKT3 binds to the CD3 receptor, opsonizing the cells and promoting their rapid removal from the circulation.[116,117]

Elimination of OKT3 occurs in two phases and is principally linked to T-cell binding. The first phase is elimination associated with rapid removal of the T cells bound to OKT3. The second, slower phase occurs days after initiation of therapy. The overall half-life for the agent is 18 hours.[117]

Dosing for OKT3 uses a fixed regimen of 5 mg/day for 10 to 14 days for treatment of acute rejection. Prophylactic induction regimens use the same dose for 7 to 10 days. After the first one or two doses, proinflammatory cytokines are released by opsonized lymphocytes, leading to clinical findings reminiscent of severe sepsis.[117] This *first-dose effect* frequently is associated with fever, chills, tachycardia, nausea, vomiting, diarrhea, bronchospasm, pulmonary edema, and elevation or depression of blood pressure. These effects can be ameliorated if the patient is pretreated with a 1-g intravenous bolus of methylprednisolone 15 to 60 minutes before OKT3 infusion.[118] Premedication often also includes antihistamines, diphenhydramine, and acetaminophen. Anaphylaxis occurs in fewer than 1% of patients; nonetheless, a skin test or test dose is recommended before OKT3 therapy is initiated.

The murine nature of the drug leads to anti-mouse immunoglobulin antibody formation. Individuals vary in the amount of endogenous antibody (directed against the mouse antibody) they form. This antibody production can be decreased by continuing other immunosuppressive treatments during monoclonal antibody administration. Human antimurine OKT3 antibodies usually peak after 1 to 2 weeks of therapy and can decrease the efficacy of future courses of therapy.[117] Repeat treatment with OKT3 is still successful in many cases, if larger doses of antibody are used for subsequent courses. Patients who produce very high antibody titers, probably about 5% to 20% of those receiving OKT3, fail to respond to subsequent doses of the drug even when the dose is increased. Some advocate monitoring of CD3+ T-cell counts with flow cytometry for patients receiving OKT3. If CD3+ cells reach 10%, it is recommended either that the dose of OKT3 be increased (to as much as 15 mg/day) or that treatment be discontinued. Others suggest monitoring anti-OKT3 antibody titers.

As described previously, OKT3 therapy produces a first-dose response that manifests within 45 to 60 minutes and must be managed with premedication. Because of the risk of severe pulmonary edema, fluid status should be evaluated if patients weigh more than 2% more than their usual body weight, and diuresis should be considered before proceeding with OKT3 therapy.

Septic meningitis also has been described as an early complication of OKT3 therapy, manifesting 2 to 7 days after initiation of OKT3. The common symptoms are fever, headache, and photophobia. The phenomenon appears to be self-limited and may be related to the release of cytokines early after OKT3 administration.

The potent suppression of T-lymphocyte populations is associated with an increased incidence of viral infections and lymphoproliferative disorders. It is not clear whether antibody therapy is worse in this regard than other approaches for achieving immunosuppression. Some evidence suggests that problems arise because antibodies are used for too long a time or too late in the course of resistant rejection, when the immunosuppression burden is already high.

The efficacy of OKT3 for treatment of acute rejection and induction strategies is well documented. However, OKT3 use has declined with the availability of better-tolerated antithymocyte preparations (i.e., RATG) that do not induce antibody production against the drug. OKT3 is often reserved as therapy for acute rejection that is resistant to steroids or other antilymphocyte preparations.

Anti–Interleukin-2 Receptor Monoclonal Antibodies

T-cell activation is characterized by the expression of IL-2 and high-affinity IL-2R by T cells. IL-2 exerts its effects on T lymphocytes by binding to the IL-2R. By binding to the α subunit of the IL-2R on activated T cells, anti–IL-2R antibodies inhibit IL-2–mediated T-cell activation and proliferation. Two anti–IL-2R monoclonal antibodies are currently available, daclizumab (Zenapax, Hoffman-LaRoche, Nutley, NJ) and basiliximab (Simulect, Novartis Pharmaceuticals). The important differences between the two drugs relate to the structure of the antibodies and the dosing strategies for each.

Daclizumab is a unique hybrid monoclonal antibody in which the variable region (binding site for the IL-2R) is murine but the remainder of the immunoglobulin molecule is human (immunoglobulin G_1). Only 10% of the hybrid molecule is of murine origin. As a result, antibody formation directed against the drug is decreased (e.g., in comparison with OKT3) and half-life is prolonged. Basiliximab is a chimeric anti–IL-2R antibody with a mechanism of action that is the same as daclizumab. In this monoclonal antibody, murine immunoglobulin amino acid sequences represent an even smaller fraction of the protein than is case for daclizumab.

Dosing strategies for anti–IL-2R monoclonal antibodies begin with administration of the first dose, before transplantation. A dose of 1 mg/kg of daclizumab is administered intravenously, and this dose is repeated every 14 days for a total of five doses. Newer dosing strategies use higher doses (2 mg/kg), or abbreviated schedules of two or three total doses, or both.[119] A 20 mg/kg dose of basiliximab is administered intravenously before transplantation, and this dose is repeated once more on day 4.

Anti–IL-2R monoclonal antibodies are effective in preventing acute rejection after transplantation. However, these agents are ineffective for reversing acute cellular rejection. Both drugs are well tolerated, with no differences in adverse effects reported in clinical trials between the drugs and placebo. Daclizumab and basiliximab have the reported beneficial effects of reducing delayed graft function and delaying calcineurin inhibitor use (to decrease nephrotoxicity).[120,121]

Anti-CD52 Monoclonal Antibody

CD52 is a surface marker found on mature T and B lymphocytes. It also is found to varying degrees on monocytes, macrophages, granulocytes, and natural killer cells. Alemtuzumab (Campath, ILEX Pharmaceuticals, LP, San Antonio, TX) is a humanized monoclonal antibody directed at the CD52 antigen that causes complete lympholysis, resulting in significant T-cell depletion. The early experience with alemtuzumab suggest that lower degrees of immunosuppression are needed after T-cell depletion following alemtuzumab infusion. Reports indicate that only single-drug therapy, usually with a calcineurin inhibitor (cyclosporine or tacrolimus) or sirolimus, is necessary after patients receive induction therapy with alemtuzumab.[122-124] Alemtuzumab also has been successfully used to treat acute rejection episodes.[125,126]

The dose of alemtuzumab administered in transplantation is 30 mg intravenously. Significant adverse effects are noted with administration of alemtuzumab, notably rigors, hypotension, fever, shortness of breath, bronchospasms, and chills. Premedication with diphenhydramine, acetaminophen, and corticosteroids is required before alemtuzumab administration to minimize the infusion-related effects. Other adverse effects noted after alemtuzumab therapy include neutropenia, anemia, thrombocytopenia, and pancytopenia.

IMMUNOSUPPRESSIVE AGENTS IN CLINICAL DEVELOPMENT

Everolimus

Everolimus (SDZ-RAD; Certican, Novartis Pharma AG, Basel, Switzerland) is an inhibitor of mTOR that is structurally similar to sirolimus and is currently approved for use in Europe. Everolimus produces the same inhibition of cell cycle progression and ultimate inhibition of T-cell proliferation. Dosing for everolimus is 3 mg daily. The half-life of everolimus is 16 to 19 hours, which is shorter than the half-life of sirolimus.[127] The adverse effects of everolimus are similar to those reported for sirolimus and include hypercholesterolemia, hypertriglyceridemia, and hematologic effects such as thrombocytopenia and anemia.

Mycophenolate Sodium

Mycophenolate sodium (Myfortic, Novartis Pharma AG) is an enteric-coated formulation of the sodium salt of mycophenolic acid that is currently approved for use in Europe. The enteric coating of mycophenolate sodium helps to minimize the gastrointestinal side effects that are associated with mycophenolate mofetil. Once in the small intestine, mycophenolic acid is released directly in the gastrointestinal tract for absorption. The immunosuppressive activity of mycophenolate sodium is identical to that of mycophenolic acid, the activated form of mycophenolate mofetil. The dose of mycophenolate sodium is 1.44 g/day, which is equivalent to 2 g/day of mycophenolate mofetil.

Leflunomide

Leflunomide (Avara, Aventis Pharmaceuticals, Kansas City, MO) is converted to an active metabolite, A77,1726. The latter compound inhibits de novo pyrimidine synthesis in T and B lymphocytes by inhibiting tyrosine kinase activity of the TCR or cytokine receptors. It is currently marketed as a treatment for rheumatoid arthritis. One study investigating leflunomide in liver and kidney transplant recipients administered a loading dose of 200 mg/day for 7 days, followed by a maintenance dose of 40 to 60 mg/day. Concentrations greater than 50 μg/mL allowed for lower doses of prednisone and calcineurin inhibitor, whereas concentrations of less than 80 μg/mL were associated with fewer adverse effects. Adverse effects included skin rash, anemia, and elevated liver enzymes.[128] Leflunomide was effective in reducing acute rejection and may show promise in reversing chronic rejection.[129]

FTY-720

FTY-720 is a novel immunosuppressant that does not affect T-cell activation but alters lymphocyte trafficking by altering the expression or function of adhesion molecules. The effect of treatment with this agent is the sequestration of T cells in secondary lymphoid organs (i.e., not in the allograft), producing peripheral lymphopenia.[130] FTY-720 is being investigated for the treatment and prevention of both acute and chronic rejection.

Mizoribine

Mizoribine is an imidazole nucleoside antibiotic that undergoes phosphorylation to inhibit both IMPDH and guanosine 5-monophosphate synthetase during purine synthesis.[131] The result is inhibition of RNA and DNA synthesis and consequent inhibition of both humoral and cellular immune responses. Limited clinical trials using mizoribine in place of azathioprine and with cyclosporine and corticosteroids have shown decreased graft loss to chronic rejection in renal transplant recipients.[132] Mizoribine has been used as a maintenance agent, in combination with cyclosporine and steroids, primarily in renal transplantation patients.[133] The drug appears to have advantages over azathioprine, in particular less myelotoxicity and hepatotoxicity.[134] Although it has not been compared in clinical trials, it is expected to have efficacy similar

to that of mycophenolate mofetil, because the two drugs have similar mechanisms of action.[135]

Mizoribine is administered once daily as an oral dose of 50 to 300 mg/day. Peak blood levels are achieved 2 to 3 hours after an oral dose. The major elimination pathway of mizoribine is renal (85% of the dose is excreted unchanged in the urine), and the half-life is 4 hours.

Brequinar

Brequinar is an antimetabolite with broad antineoplastic activity that has been tested in humans with cancer.[136,137] It inhibits the de novo pathway of pyrimidine synthesis, blocking RNA and DNA synthesis. Dose-limiting toxicities are thrombocytopenia and a severe desquamative dermatitis. The antiproliferative effects of the drug appear to be mediated by depletion of the pyrimidine precursors needed for DNA and RNA synthesis. Brequinar is a potent immunosuppressant in a rat model[138] and appears to act synergistically, at least in vitro, with cyclosporine and sirolimus.[139]

Anti-CD4 Antibody

Antibodies targeted against the CD4 receptor prevent the initiation of the immune response caused by presentation of MHC class II alloantigens. Selective disruption of the MHC class II–CD4 interaction can prolong allograft survival and induce tolerance in animal models.[140] These antibodies reduce synovial inflammation in rheumatoid arthritis and cause profound and long-term immunosuppression.[141] Use of anti-CD4 in conjunction with cytotoxic T lymphocyte-associated antigen 4 immunoglobulin (CTLA4-Ig) has been shown to prolong the survival of hamster liver xenografts in rats.[142] Use of murine OKT4 in cadaveric renal transplantation has not shown promise.[143] A human anti-mouse antibody (HAMA) response of more than 3 times the pretreatment level was observed in 84% of patients. An open-label pilot trial of murine OKT4A in humans produced mixed results.[144] The dose used in the study produced only partial CD4 saturation in all patients and was inadequate to reduce rejection. However, no HAMA response was observed. Other humanized anti-CD4 monoclonal antibodies are being evaluated.[2,144]

Anti-CD45 Antibody

The CD45 epitope plays a role in the regulation of T-cell activation. The CD45RB monoclonal antibody has been shown to effectively prevent allograft rejection in animal models and may have some applicability in humans in the future.

Anti-CD40–Ligand and Anti-CD40 Antibody

CD40 is a costimulatory molecule for MHC class II alloantigens that is present in large amounts on mature dendritic cells. Costimulation is necessary for T-cell sensitization and activation. Use of antibodies to prevent costimulation may induce tolerance by blocking activation of the T cell. In animal models, anti-CD40 or anti-CD40–ligand monoclonal antibodies have been shown to prevent and even reverse acute allograft rejection, leading to prolonged graft survival without the need for chronic maintenance immunosuppression[145,146] and to delayed onset of chronic rejection.[147] Other studies have demonstrated the tolerogenic potential of anti-CD40–ligand in animal models.[148]

Anti–Leukocyte Function–Associated Antigen-1

Leukocyte function–associated antigen-1 (LFA-1) plays an important role in adhesion of leukocytes to endothelial cells and to a variety of targets on T cells during the immune response. The immunosuppressive effect of anti–LFA-1 (anti-CD11a, anti-CD18) monoclonal antibody (odulimomab) was similar to that of RATG as induction therapy in renal allograft recipients.[149] Fewer patients required dialysis in the anti–LFA-1 monoclonal antibody group, possibly due to prevention of endothelial cell activation and the consequent protection of the allograft from ischemic damage. This same effect of protection from renal ischemia has been confirmed in animal models.[150] The combination of anti–intracellular adhesion molecule-1 (ICAM-1) antibody (enlimonab) and anti–LFA-1 monoclonal antibody has been shown to induce tolerance to murine cardiac allografts.[151,152]

Anti-CTLA4-Ig

CTLA4-Ig is a chimeric fusion protein that blocks the B7-CD28/CTLA4 pathway and thereby inhibits T-cell activation and IL-2 production. Development of chronic renal allograft rejection is prevented by CTLA4-Ig in animal models.[153] Current studies are investigating the use of this molecule with other costimulatory modulators, such as anti-CD40 ligand[147] and anti–LFA-1,[154] to induce tolerance in animal models.

Anti–Intracellular Adhesion Molecule-1

ICAM-1 is an immunoglobulin-like molecule that aids adhesion and migration of leukocytes in the vessels and also acts as a costimulatory molecule for T-cell activation. Use of anti–ICAM-1 monoclonal antibody (enlimomab) has not shown promise in reducing the incidence of acute rejection or delayed graft function in renal transplants.[155] However, anti–ICAM-1 in combination with anti–LFA-1 produced tolerance in animal models.[151,152]

FUTURE DIRECTIONS

The number of patients awaiting solid organ transplantation continues to grow each year. However, the number of organs available for donation shows very little change from year to year. Increased organ donation awareness among the public and increased use of organs from living donors have contributed to small annual increases in the number of organs available for transplantation, but the number still falls short of meeting the needs of the more than 80,000 candidates waiting for solid-organ transplants. Other strategies must be explored to try to meet the demand for organ transplantation.

Greater understanding of the way the immune system functions with respect to chronic allograft acceptance is vital to increasing the survival of transplanted allografts. Complete allograft acceptance without immunosuppressive therapy would eliminate the occurrence of long-term sequelae of immunosuppression, such as malignancies and opportunistic infections. Chimerism, a principle that is becoming better understood by transplantation immunologists, allows the donor and recipient leukocytes to stably coexist. Chimerism is essential for complete allograft acceptance with or without immunosuppression.

The principles of allograft tolerance build on the concept of chimerism in that the interaction between donor and recipient leukocytes allows for low-level activation of both populations, resulting in exhaustion of each species.[9,156,157] Tolerance can be induced with the use of T-cell–depleting regimens, followed by low doses of immunosuppressive medications.[114] Current research includes investigation of other means of modulating the immune response to induce

tolerance, including modulation of adhesion and costimulation pathways.

A better understanding of tolerance induction with immunosuppressive regimens will play a key role in permitting successful xenotransplantation. Use of xenoallografts can greatly increase the number of donor organs available for transplantation to help match the demand.

ANNOTATED REFERENCES

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The pharmacokinetics of mycophenolate mofetil is emphasized in this article, with an overview of the mechanism of action and pharmacodynamic properties of the drug. Clinical monitoring and the correlation of plasma concentrations with adverse and immunosuppressive effects are highlighted.

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