Immunosuppressants

I. OVERVIEW

The importance of the immune system in protecting the body against harmful foreign molecules is well recognized. However, this protection can result in serious problems. For example, rejection of the transplanted tissue. Transplantation of organs and tissues (for example, kidney, heart, or bone marrow) has become routine due to improved surgical techniques and better tissue typing. Also, drugs are now available that more selectively inhibit rejection of transplanted tissues while preventing the patient from becoming immunologically compromised. Earlier drugs were nonselective, and patients frequently succumbed to infection due to suppression of both the antibody-mediated (humoral) and cell-mediated arms of the immune system. Today, the principal approach to immunosuppressive therapy is to alter lymphocyte function using drugs or antibodies against immune proteins. Because of their severe toxicities when used as monotherapy, a combination of immunosuppressive agents, usually at lower doses, is generally employed. [Note: Immunosuppressive therapy is also used in the treatment of autoimmune diseases. For example, corticosteroids can control acute glomerulonephritis.]

Immunosuppressive drug regimens usually consist of anywhere from two to four agents with different mechanisms of action that disrupt various levels of T-cell activation. Immunosuppressive drugs can be categorized according to their mechanisms of action: 1) Some agents interfere with cytokine production or action; 2) others disrupt cell metabolism, preventing lymphocyte proliferation; and 3) monoclonal antibodies block T-cell surface molecules.

II. SELECTIVE INHIBITORS OF CYTOKINE PRODUCTION AND FUNCTION

Cytokines are soluble, antigen-nonspecific, signaling proteins that bind to cell surface receptors on a variety of cells. The term cytokine includes the molecules known as interleukins (ILs), interferons (IFNs), tumor necrosis factors (TNFs), transforming growth factors, and colony-stimulating factors. Of particular interest when discussing immunosuppressive drugs is IL-2, a growth factor that stimulates the proliferation of antigen-primed (helper) T cells, which subsequently produce more IL-2, IFN-γ, and TNF-α (Figure 40.2). These cytokines collectively activate natural killer cells, macrophages, and cytotoxic T lymphocytes. Clearly, drugs that interfere with the production or activity
of IL-2, such as cyclosporine, will significantly dampen the immune response and, thereby, decrease graft rejection.

A. Cyclosporine

Cyclosporine is a lipophilic cyclic polypeptide. The drug is extracted from the soil fungus Beauveria nivea. Cyclosporine is used to prevent rejection of kidney, liver, and cardiac allogeneic transplants. Cyclosporine is most effective in preventing acute rejection of transplanted organs when combined in a double-drug or triple-drug regimen with corticosteroids and an antimetabolite such as mycophenolate mofetil. Cyclosporine is an alternative to methotrexate for the treatment of severe, active rheumatoid arthritis. It can also be used for patients with recalcitrant psoriasis that does not respond to other therapies, and it is also used for keratoconjunctivitis. x

1. Mechanism of action: Cyclosporine preferentially suppresses cell-mediated immune reactions, whereas humoral immunity is affected to a far lesser extent. After diffusing into the T cell, cyclosporine binds to a cyclophilin (more generally called an immunophilin) to form a complex that binds to calcineurin. The latter is responsible for dephosphorylating NFATc (cytosolic Nuclear Factor of Activated T cells). Because the cyclosporine-calcineurin complex cannot perform this reaction, NFATc cannot enter the nucleus to promote the reactions that are required for the synthesis of a number of cytokines, including IL-2. The end result is a decrease in IL-2, which is the primary chemical stimulus for increasing the number of T lymphocytes.

2. Pharmacokinetics: Cyclosporine may be given either orally or by intravenous (IV) infusion. Oral absorption is variable. Interpatient variability may be due to metabolism by a cytochrome P450 (CYP3A4) in the gastrointestinal (GI) tract, where the drug is metabolized. Cyclosporine is also a substrate for P-glycoprotein (P-gp), a drug efflux pump, which limits cyclosporine absorption by transporting the drug back into the gut lumen. About 50 percent of the drug is associated with the blood fraction. Half of this is in the erythrocytes, and less than one tenth is bound to the lymphocytes. Excretion of the metabolites is through the biliary route, with only a small fraction of the parent drug appearing in the urine.

3. Adverse effects: Many of the adverse effects caused by cyclosporine are dose dependent. Therefore, it is important to monitor blood levels of the drug. Nephrotoxicity is the most common and important adverse effect of cyclosporine, and it is critical to
monitor kidney function. Reduction of the cyclosporine dosage can result in reversal of nephrotoxicity in most cases, although nephrotoxicity may be irreversible in 15 percent of patients. Hepatotoxicity can also occur, liver function should be periodically assessed. Infections in patients taking cyclosporine are common and may be life-threatening. Viral infections due to the herpes group and cytomegalovirus (CMV) are prevalent. Lymphoma may occur in all transplanted patients due to the net level of immunosuppression and has not been linked to any one particular agent. Anaphylactic reactions can occur on parenteral administration. Other toxicities include hypertension, hyperlipidemia, hyperkalemia (it is important not to use K+-sparing diuretics in these patients), tremor, hirsutism, glucose intolerance, and gum hyperplasia.

B. Tacrolimus

Tacrolimus (originally called FK506) is a macrolide that is isolated from the soil fungus Streptomyces -tsukubaensis. Tacrolimus is approved for the prevention of rejection of liver and kidney transplants and is given with a corticosteroid and/or an antimetabolite. This drug has found favor over cyclosporine, not only because of its potency and decreased episodes of rejection, but also because lower doses of corticosteroids can be used, thus reducing the likelihood of steroid-associated adverse effects. An ointment preparation has been approved for moderate to severe atopic dermatitis that does not respond to conventional therapies.

1. Mechanism of action: Tacrolimus exerts its immunosuppressive effect in the same manner as cyclosporine, except that it binds to a different immunophilin, FKBP-12 (FK-binding protein).

2. Pharmacokinetics: Tacrolimus may be administered orally or IV. Tacrolimus is subject to gut metabolism by CYP3A4/5 isoenzymes and is a substrate for P-gp. Together, both of these mechanisms limit the oral bioavailability of tacrolimus. Absorption is decreased if the drug is taken with high-fat or high-carbohydrate meals. Tacrolimus is from 10- to 100-fold more potent than cyclosporine. It is highly bound to serum proteins and is also concentrated in erythrocytes. Like cyclosporine, tacrolimus undergoes hepatic metabolism. At least one metabolite of tacrolimus has been shown to have immunosuppressive activity. Renal excretion is very low, and most of the drug and its metabolites are found in the feces.
3. Adverse effects: Nephrotoxicity and neurotoxicity (tremor, seizures, and hallucinations) tend to be more severe in patients who are treated with tacrolimus than in patients treated with cyclosporine. Development of posttransplant, insulin-dependent diabetes mellitus is a problem. Other toxicities are the same as those for cyclosporine, except that tacrolimus does not cause hirsutism or gingival hyperplasia. Compared with cyclosporine, tacrolimus has also been found to have a lower incidence of cardiovascular toxicities, such as hypertension and hyperlipidemia. Anaphylactoid reactions to the injection vehicle have been reported.

C. Sirolimus

Sirolimus is a macrolide obtained from fermentations of the soil mold Streptomyces hygroscopicus. The earlier name is rapamycin. Sirolimus is approved for use in renal transplantation, to be used together with cyclosporine and a corticosteroids, allowing lower doses of those medications to be used, thereby lowering their toxic potential. The combination of sirolimus and cyclosporine is apparently synergistic because sirolimus works later in the immune activation cascade. The antiproliferative action of sirolimus has found use in cardiology. Sirolimus-coated stents inserted into the cardiac vasculature inhibit restenosis of the blood vessels by reducing proliferation of the endothelial cells.

1. Mechanism of action: Sirolimus and tacrolimus bind to the same cytoplasmic FK-binding protein, but instead of forming a complex with calcineurin, sirolimus binds to mTOR. Binding of sirolimus to mTOR blocks the progression of activated T cells from the G1 to the S phase of the cell cycle and, consequently, the proliferation of these cells. Unlike cyclosporine and tacrolimus, sirolimus does not owe its effect to lowering IL-2 production but, rather, to inhibiting the cellular responses to IL-2.

2. Pharmacokinetics: The drug is available only as oral preparations. Although it is readily absorbed, high-fat meals can decrease the drug’s absorption. Sirolimus has a long half-life (57 to 62 hours) compared to those of cyclosporine and tacrolimus, and a loading dose is recommended at the time of initiation of therapy, but only requires once daily dosing. Sirolimus also increases the drug concentrations of cyclosporine, and careful blood level monitoring of both agents must be done to avoid harmful drug toxicities. The parent drug and its metabolites are predominantly eliminated in feces.

3. Adverse effects: A common side effect of sirolimus is hyperlipidemia. The combination of cyclosporine and sirolimus is more nephrotoxic than cyclosporine alone.
Although the administration of sirolimus and tacrolimus appears to be less nephrotoxic, sirolimus can still potentiate the nephrotoxicity of tacrolimus, and drug levels of both must be monitored closely. Other untoward problems are headache, nausea and diarrhea, leukopenia, and thrombocytopenia. Impaired wound healing has been noted with sirolimus in obese patients and those with diabetes.

D. Everolimus

Everolimus (another mTOR inhibitor) was recently approved by the U.S. Food and Drug Administration for use in renal transplantation in combination with low-dose cyclosporine and corticosteroids. It was originally approved in 2009 for second-line treatment in patients with advanced renal cell carcinoma.

1. Mechanism of action: Everolimus has the same mechanism of action as sirolimus. It inhibits activation of T cells by forming a complex with FKBP-12 and subsequently blocking mTOR.

2. Pharmacokinetics: Everolimus differs from sirolimus in its pharmacokinetic profile. Everolimus is rapidly absorbed, attaining maximal concentrations in 1 to 2 hours post dose, but absorption is decreased with high-fat meals. Everolimus is a substrate of CYP3A4 and P-gp and, thus, is subject to the same drug interactions as previously mentioned immunosuppressants. Everolimus avidly binds erythrocytes, and monitoring of whole blood trough concentrations is recommended. It has a much shorter half-life than does sirolimus at 30 ± 11 hours and requires twice-daily dosing. Everolimus increases drug concentrations of cyclosporine, thereby enhancing the nephrotoxic effects of cyclosporine, and is, therefore, recommended to be used with reduced doses of cyclosporine.

3. Adverse effects: Everolimus has similar side effects to sirolimus, including hyperlipidemia, impaired or delayed wound healing following transplantation, and enhanced nephrotoxicity in combination with higher doses of cyclosporine. An additional adverse effect noted with everolimus is angioedema, which may increase with concomitant use of angiotensin-converting enzyme inhibitors. There is also an increased risk of kidney arterial and venous thrombosis, resulting in graft loss, usually in the first 30 days posttransplantation.

III. IMMUNOSUPPRESSIVE ANTIMETABOLITES
Immunosuppressive antimetabolite agents are generally used in combination with corticosteroids and the calcineurin inhibitors, cyclosporine and tacrolimus.

A. Azathioprine

Azathioprine was the first agent to achieve widespread use in organ transplantation. It is a prodrug that is converted first to 6-mercaptopurine (6-MP) and then to the corresponding nucleotide, thioinosinic acid. The immunosuppressive effects of azathioprine are due to this nucleotide analog. The drug has little effect on suppressing a chronic immune response. Its major toxicity is bone marrow suppression. Concomitant use with angiotensin-converting enzyme inhibitors or cotrimoxazole in renal transplant patients can lead to an exaggerated leukopenic response. Allopurinol, an agent used to treat gout, significantly inhibits the metabolism of azathioprine. Therefore, the dose of azathioprine must be reduced by 60 to 75 percent. Nausea and vomiting are also encountered.

B. Mycophenolate mofetil

Mycophenolate mofetil has, for the most part, replaced azathioprine because of its safety and efficacy in prolonging graft survival. It has been successfully used in heart, kidney, and liver transplants. As an ester, it is rapidly hydrolyzed in the GI tract to mycophenolic acid. This is a potent, reversible, uncompetitive inhibitor of inosine monophosphate dehydrogenase, which blocks the de novo formation of guanosine phosphate. Thus, like 6-MP, it deprives the rapidly proliferating T and B cells of a key component of nucleic acids. Mycophenolic acid is quickly and almost completely absorbed after oral administration. Both mycophenolic acid and its glucuronidated metabolite are highly bound (greater than 90 percent) to plasma albumin. The glucuronide metabolite is excreted predominantly in urine. The most common adverse effects include diarrhea, nausea, vomiting, abdominal pain, leukopenia, and anemia. Higher doses of mycophenolate mofetil (3 g/day) were associated with a higher risk of CMV infection. [Note: mycophenolic acid is less mutagenic or carcinogenic than azathioprine.] Concomitant administration with antacids containing magnesium or aluminum, or with cholestyramine, can decrease absorption of the drug.

C. Enteric-coated mycophenolate sodium

In an effort to minimize the GI effects associated with mycophenolate mofetil, enteric-coated mycophenolate sodium was developed. The active drug (mycophenolic acid) is
contained within a delayed-release formulation designed to release in the neutral pH of the small intestine. The new formulation was found to be equivalent to mycophenolate mofetil in the prevention of acute rejection episodes in kidney transplant recipients. However, the rate of GI adverse events was similar to that with mycophenolate mofetil.

IV. ANTIBODIES

The use of antibodies plays a central role in prolonging allograft survival. The names of monoclonal antibodies conventionally contain “muro” if they are from a murine (mouse) source and “xi” or “zu” if they are chimerized or humanized, respectively. The suffix “mab” (monoclonal antibody) identifies the category of drug. The polyclonal antibodies, although relatively inexpensive to produce, are variable and less specific, which is in contrast to monoclonal antibodies, which are homogeneous and specific.

A. Antithymocyte globulins

They are primarily used, together with other immunosuppressive agents, at the time of transplantation to prevent early allograft rejection, or they may be used to treat severe rejection episodes or corticosteroid-resistant acute rejection. The antibody-bound cells are phagocytosed in the liver and spleen, resulting in lymphopenia and impaired T-cell responses. The antibodies are slowly infused intravenously, and their half-life extends from 3 to 9 days. Because the humoral antibody mechanism remains active, antibodies can be formed against these foreign proteins. [Note: This is less of a problem with the humanized antibodies.] Other adverse effects include chills and fever, leukopenia and thrombocytopenia, infections due to CMV or other viruses, and skin rashes.

B. Muromonab-CD3 (OKT3)

Muromonab-CD3 is a murine monoclonal antibody that is synthesized by hybridoma technology and directed against the glycoprotein CD3 antigen of human T cells. MuromonabCD3 is used for treatment of acute rejection of renal allografts as well as for corticosteroid-resistant acute allograft rejection in cardiac and hepatic transplant patients. It is also used to deplete T cells from donor bone marrow prior to transplantation.

Adverse effects: Anaphylactoid reactions may occur. Cytokine release syndrome may follow the first dose. The symptoms can range from a mild, flu-like illness to a life-threatening, shock-like reaction. High fever is common. Central nervous system effects, such as seizures, encephalopathy, cerebral edema, aseptic meningitis, and headache, may
occur. Infections can increase, including some due to CMV. Muromonab-CD3 is contraindicated in patients with a history of seizures, in those with uncompensated heart failure, in pregnant women, and in those who are breast-feeding. Because of these adverse effects and the improved tolerability of rabbit antithymocyte globulin and the IL-2 receptor antagonists, muromonab-CD3 is rarely used today.

C. IL-2-receptor antagonists

Basiliximab is said to be “chimerized” because it consists of 25 percent murine and 75 percent human protein. Daclizumab is 90 percent human protein, and is designated “humanized.” Both agents have been approved for prophylaxis of acute rejection in renal transplantation in combination with cyclosporine and corticosteroids. They are not used for the treatment of ongoing rejection. In late 2009, daclizumab was withdrawn from the U.S. market by the manufacturer due to a diminished demand for the product.

1. Mechanism of action: Both compounds are anti-CD25 antibodies and bind to the α chain of the IL-2 receptor on activated T cells. They thus interfere with the proliferation of these cells. Basiliximab is about 10-fold more potent than daclizumab as a blocker of IL-2 stimulated T-cell replication. Blockade of this receptor foils the ability of any antigenic stimulus to activate the T-cell response system.

2. Pharmacokinetics: Both antibodies are given IV. The serum half-life of daclizumab is about 20 days, and the blockade of the receptor is 120 days. Five doses of daclizumab are usually administered, the first at 24 hours before transplantation, and the next four doses at 14-day intervals. The serum half-life of basiliximab is about 7 days. Usually, two doses of this drug are administered, the first at 2 hours prior to transplantation, and the second at 4 days after the surgery.

3. Adverse effects: Both daclizumab and basiliximab are well tolerated. Their major toxicity is GI. No clinically relevant antibodies to the drugs have been detected, and malignancy does not appear to be a problem.

D. Alemtuzumab

Alemtuzumab, a humanized monoclonal antibody, exerts its effects by causing profound depletion of T cells from the peripheral circulation. This effect may last for up to 1 year. Alemtuzumab is currently approved for the treatment of refractory B-cell chronic lymphocytic leukemia. Although it is not currently approved for use in organ
transplantation, it is being used in combination with sirolimus and low-dose calcineurin inhibitors in corticosteroid-avoidance protocols at many transplant centers. Preliminary results are promising, with low rates of rejection with a prednisone-free regimen. Side effects include first-dose cytokine-release syndrome, requiring premedication with acetaminophen, diphenhydramine, and corticosteroids. Adverse effects include neutropenia, anemia, and, rarely, pancytopenia. Intermediate term results have shown an increase in B-cell mediated rejection and development of autoimmune disorders in a small number of patients and, thus, this agent should be used with caution.

A summary of the major immunosuppressive drugs is presented in Figure 40.8.

V. CORTICOSTEROIDS

The corticosteroids were the first pharmacologic agents to be used as immunosuppressives both in transplantation and in various autoimmune disorders. They are still one of the mainstays for attenuating rejection episodes. For transplantation, the most common agents are prednisone or methylprednisolone, whereas prednisone or prednisolone are used for autoimmune conditions. The steroids are used to suppress acute rejection of solid organ allografts and in chronic graft-versus-host disease. In addition, they are effective against a wide variety of autoimmune conditions, including refractory rheumatoid arthritis, systemic lupus erythematosus, temporal arthritis, and asthma. The exact mechanism responsible for the immunosuppressive action of the corticosteroids is unclear. The T lymphocytes are affected most. The steroids are able to rapidly reduce lymphocyte populations. They bind to the glucocorticoid receptor. The complex passes into the nucleus and regulates the translation of DNA. The use of these agents is associated with numerous adverse effects. For example, they are diabetogenic and can cause hypercholesterolemia, cataracts, osteoporosis, and hypertension with prolonged use.