Chapter 40 part 2

**III. IMMUNOSUPPRESSIVE ANTIMETABOLITES**

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

1. **Azathioprine**

Azathioprine [ay-za-THYE-oh-preen] was the first agent to achieve wide- spread 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. Because of their rapid proliferation in the immune response and their dependence on the de novo syn- thesis of purines required for cell division, lymphocytes are predominantly affected by the cytotoxic effects of azathioprine. [Note: The drug has little effect on suppressing a chronic immune response.] Its major nonimmune 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. (See p. 488 for a discussion of the mechanism of action, resistance, and pharmaco kinetics of 6-MP.)

1. **Mycophenolate mofetil**

Mycophenolate mofetil [mye-koe-FEN-oh-late MAW-fehtil] 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 (Figure 40.6). [Note: Lymphocytes lack the salvage pathway for purine synthesis and, therefore, are dependent on de novo purine pro- duction.] Mycophenolic acid is quickly and almost completely absorbed after oral administration. Both mycophenolic acid and its glucuronidat- ed metabolite are highly bound (greater than 90 percent) to plasma albumin, but no displacement-type interactions have been reported. 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 mycopheno- late mofetil (3 g/day) were associated with a higher risk of CMV infec- tion. [Note: mycophenolic acid is less mutagenic or carcinogenic than azathioprine.] Concomitant administration with antacids containing magnesium or aluminum, or with cholestyramine, can decrease absorp- tion of the drug.

1. **Enteric-coated mycophenolate sodium**

In an eff ort to minimize the GI eff ects associated with mycopheno- late 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. Enteric-coated mycophenolate sodium at 720 mg and mycophenolate mofetil at 1000 mg contain equivalent amounts of mycophenolic acid. In Phase III studies, 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. They are prepared either by immunization of rabbits or horses with human lymphoid cells (producing a mixture of polyclonal antibodies directed against a number of lymphocyte antigens), or by hybridoma technology (producing antigen-specifi c, monoclonal antibodies). [Note: Hybridomas are produced by fusing mouse antibody-producing cells with immortal, malignant plasma cells (Figure 40.7). Hybrid cells are selected and cloned, and the antibody specifi city of the clones is determined. Clones of inter- est can be cultured in large quantities to produce clinically useful amounts of the desired antibody. Recombinant DNA technology can also be used to replace part of the mouse gene sequence with human genetic material, thus “humanizing” the anti bodies produced, making them less antigenic.] 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 (see Figure 40.7). The suffi x “mab” (monoclonal anti- body) 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.

1. **Antithymocyte globulins**

Thymocytes are cells that develop in the thymus and serve as T-cell pre- cursors. The antibodies developed against them are prepared by immunization of large rabbits or horses with human lymphoid cells and, thus, are polyclonal. They are primarily used, together with other immuno- suppressive 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. Rabbit formulations of polyclonal antithymocyte globulin are more commonly used over the horse preparation due to greater potency. The antibodies bind to the surface of circulating T lymphocytes, which then undergo various reactions, such as complement-mediated destruction, antibody-dependent cytotoxicity, apoptosis, and opsonization. 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 human- ized antibodies.] Other adverse eff ects include chills and fever, leukopenia and thrombocytopenia, infections due to CMV or other viruses, and skin rashes.

1. **Muromonab-CD3 (OKT3)**

Muromonab-CD3 [myoo-roe-MOE-nab] is a murine monoclonal anti- body that is synthesized by hybridoma technology and directed against the glycoprotein CD3 antigen of human T cells. Muromo nab- CD3 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.

**1. Mechanism of action**: Binding to the CD3 protein results in a disruption of T-lymphocyte function, because access of antigen to the recognition site is blocked. Circulating T cells are depleted, thereby decreasing their participation in the immune response. Because muromonab-CD3 recognizes only one antigenic site, the immuno- suppression is less broad than that seen with the polyclonal anti- bodies. T cells usually return to normal within 48 hours of discontinuation of therapy.

**2. Pharmacokinetics**: The antibody is administered IV. Initial binding of muromonab-CD3 to the antigen transiently activates the T cell and results in cytokine release (cytokine storm ). It is, therefore, customary to premedicate the patient with methylprednisolone, diphenhydramine, and acetaminophen to alleviate the cytokine-release syndrome.

**3. 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 his- tory 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**

The antigenicity and short serum half-life of the murine monoclonal antibody have been averted by replacing most of the murine amino acid sequences with human ones by genetic engineering. Basiliximab [bah-si-LIK-si-mab] is said to be “chimerized” because it consists of 25 percent murine and 75 percent human protein. Daclizumab [dah-KLIZ- yoo-mab] 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 antibod- ies 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 abil- ity 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 [al-em-TOOZ-oo-mab], a humanized monoclonal anti- body directed against CD52, 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, rare- ly, 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 auto- immune conditions. [Note: In transplantation, they are used in combination with agents described previously in this chapter.] 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, system- ic 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 by lysis or redistribution. On entering cells, they bind to the glucocorticoid receptor. The complex passes into the nucleus and regulates the translation of DNA. Among the genes affect- ed are those involved in inflammatory responses. 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. Consequently, efforts are being directed toward reducing or eliminating the use of steroids in the maintenance of allografts.