Chapter II

Overview of Renal Transplantation

Renal Transplantation has become the therapy of choice for most patients with end-stage renal disease. Azathioprine and corticosteroids were the first immunosuppressive agents used to prevent allograft rejection in the 1960s. (See table 2.1) However, after the introduction of cyclosporin A (CsA) in clinical transplantation in 1978, all forms of organ transplantation have improved (Lindholm, 1991). Since it was the first agent that produces relatively specific immunosuppression without causing bone marrow suppression, today CsA remains central part of most immunosuppressive protocols. With improvement in surgical procedures and immunosuppressive drugs, continued success in this area can be expected.

Table 2.1 Transplant Nomenclature

Term	Definition
Graft	Any organ, tissue, or object used for transplantation to replace a faulty part of the body
Autograft	A transplant of tissue or organ taken from the recipient (i.e., skin graft)
Syngraft	A transplant of tissue or organ taken from a genetically identical donor (i.e., monozygotic twins)
Allograft	A transplant of tissue or organ taken from a genetically different donor of the same species
Tolerance	Indefinite host unresponsiveness to an allograft without the need for long-term immunosuppression

Transplant Immunology

The present success of transplantation is based on an understanding of the immune system and the mechanisms involved in graft rejection. The host's immune system must be suppressed in order for allograft to survive. Medications are utilized to achieve a nonpermanent tolerance since permanent tolerance has not been achieved yet. A basic knowledge in immunology is requisite for understanding the clinical use of immunosuppressants.

Major histocompatibility complex (MHC)

Antigenic differences between members of a species are called alloantigens. When these play the major role in the rejection of allografts, they are called histocompatibility antigens. The major histocompatibility complex (MHC) refers to the genes of a chromosomal region of closely linked histocompatibility genes. MHC antigens play a pivotal role in immunity and in recognizing self versus nonself antigens. In human, the MHC is called the human leukocyte antigen (HLA) complex and is located on the short arm of chromosome 6. The response to these antigens is an influential factor of allograft survival.

There are three classes of antigens within the MHC: HLA classes I, II, and III based on their tissue distribution, structure, and function. Class III antigens are part of the compliment system and do not play a specific role in the graft rejection process. The HLA class I (HLA-A, HLA-B, and HLA-C) are presented in almost all tissue. These antigens promote the formation of antibody-producing B cells and are the primary target for cytotoxic T-lymphocyte reactions against allograft. The class II molecules consist of HLA-DR, HLA-DP, and HLA-DQ. They have a more restricted distribution and are located primarily on B-lymphocytes, activated T-lymphocytes antigen-presenting cells, and vascular endothelium. Class II antigens also trigger B cell generation and signal proliferation of helper T cells. The HLA-A, HLA-B, and HLA-DR antigens are considered the most important in clinical transplantation. The strongest positive effect for survival of a allograft is compatibility of the HLA-DR component, followed by HLA-B and HLA-A.

Histocompatibility testing of the donor and recipient is used to minimize donor-specific immune responses to a transplanted organ. Theoretically, the more antigens that match, the less likely rejection is to occur. ABO blood group compatibility is also considered an essential criterion for matching donor and recipient. The same rules as blood transfusion also apply to transplantation, for example, O to any non-O, A or B to AB (Haynes and Fauci, 1992).

Graft Rejection

When donor allograft is placed in a transplant recipient, the recipient's immune system recognizes the transplanted organ as a foreign body and initiates the immune response. Circulating macrophages serve as antigen-presenting cells that prepare allogeneic antigen for presentation to recipient T and B lymphocytes. This step provokes the lymphocytes to divide, proliferate, and finally generate both humoral and cell-mediated response. Interleukin-1,

which is produced by the activated macrophage, induces the release of lymphokines (e.g., interleukin-2 and interferon-γ) from helper-T lymphocytes.

The secretion of interleukin-2 is a vital step in the process of continuing response leading to the rejection. Interleukin-2 acts on activated T cells causing those cells to proliferate and demonstrate their differentiated function such as cytotoxicity. The cytotoxic T cells will bind directly to allogeneic cells and result in cell lysis. Interleukin-2 also causes the release of T-derived B cell growth factors. This leads to clonal expansion and differentiation of activated B cells, antibody production and secretion. A multitude of cytokines are released and cause a cascade of events such as increased macrophage infiltration, increased capillary permeability, fibrin-deposition, and platelet activation. This complex cascade results in destruction of the graft (Shaefer, 1992; Tsunoda and Aweeka, 1996a).

Immunosuppressive therapy

Immunosuppressive drugs are utilized to suppress the recipient's immune response in order to enable the recipient to accept a graft. Unfortunately, all of these agents currently available can not only inhibit the allograft rejection process, but also suppress all immune response. Therefore, it is necessary to balance the dose of immunosuppressive agents so that optimal immunosuppression is achieved and the risk of infection is minimized. This requires regular monitoring and adjustment in each patient. Combination of drugs with different side effects and different mechanisms of action is rational since the agents have potentially synergistic mechanisms. Moreover, the use of lower doses of individual agents is generally associated with fewer or less severe adverse effect.

A variety of regimens have been used. For example, monotherapy with cyclosporin (CsA); dual therapy with CsA and steroids or azathioprine; triple therapy with CsA, azathioprine, and steroids; and quadruple therapy with the addition of antilymphocyte globulin or muromonab - CD3 to the triple regimen. There is no universal immunosuppressive protocol. It varies with the organ transplanted, the transplant institutions, the time after transplantation, and the clinical studies at that time.

The first three months following surgery, relatively high doses of immunosuppressants are administered to prevent rejection during this high-risk period. After that, the doses are gradually reduced to minimize drug-related toxicity while they are adequate to prevent rejection since the risk decreases with time. Table 2.2 (Tsunoda and Aweeka, 1996a) summarizes the immunosuppressive drugs utilized most frequently in transplantation.

Table 2.2 Immunosuppressive drugs utilized most frequently in transplantation

Agent	*Primary Mechanism of Action*	*Usual Dose*	*Adverse Effect*	*Comments*
Corticosteroids	Blocks synthesis or response to IL-2, IL-1, prostaglandins and γ-interferon; reduces T-cell proliferative response to specific antigens	Initial prednisone: 0.5-2 mg/kg/day po Maintenance prednisone: 0.1-0.2 mg/kg/day po	Suppression of adrenal function, hypertension, fluid retention, hyperglycemia, psychosis, delayed wound healing, osteoporosis, cataracts	Administer with food or milk to minimize GI side effects
Azathioprine	Inhibits purine synthesis and metabolism, blocking DNA and RNA synthesis in response to antigenic stimulation	Initial: 1-4 mg/kg/day po or IV at the time of surgery Maintenance: 1-3 mg/kg/day	Bone marrow suppression megaloblastic anemia, nausea, vomiting, anorexia, diarrhea, drug fever, rash, alopecia, hepatotoxicity	Tablets may be administered with or after meals to minimize GI side effects
Cyclosporin	Inhibits T helper cell activity by decreasing IL-2 production and inhibiting T cell activation; also inhibits IL-1, IL-3, IL-5, and TNF-α	Initial: 0.5-5 mg/kg/day IV or 10-20 mg/kg/day po Maintenance: 2-5 mg/kg/day po; adjust dose based on measured trough levels and adverse reactions	Nephrotoxicity, hypertension, nausea, vomiting, diarrhea, hyperkalemia, hypomagnesemia, headache, tremors, paresthesias, hirsutism, gingival hyperplasia, seizures, hepatotoxicity, hyperuricemia	IV dose is ~ 1/3 the oral dose; IV administered as slow infusion (2-24 hr) ; Glass bottles only for administration of IV and oral solution; Oral IV solution has 12.5-32.9% alcohol, do not refrigerate
Tacrolimus	Inhibits T helper cell activity by decreasing IL-2 production; inhibits IL-3, IL-4, TNF-α, and IFN-γ	Initial: 0.05-0.1 mg/kg/day IV or 0.3 mg/kg/day po Maintenance: adjust based on measured trough levels and adverse reactions	Nephrotoxicity, insomnia, tremor, headache, tingling sensations, muscle aches, itching, fatigue, light sensitivity, nausea, vomiting, hypertension, hyperglycemia	IV dose is ~ 1/3 the oral dose; decrease dose in hepatic dysfunction
Mycophenolate mofetil	Inhibits de novo guanine synthesis; inhibits DNA proliferation of lymphocytes	Initial: 1 gm po bid	Gastrointestinal; diarrhea, nausea vomiting, loss of appetite, GI hemorrhage; anemia, leukopenia	Avoid co-administration of antacids, cholestyramine
Muromonab CD3 (OKT3)	Immediately decreases circulating T cells; interferes with antigen recognition by binding to CD3 cell surface	5 mg/day IV for 5-14 days	Fever, chills, tremor, headache, diarrhea, cramping, nausea, vomiting, hypotension, aseptic meningitis, pulmonary edema	Administer undiluted over 1 min; filter through a 0.22-micron filter
Antithymocyte globulin (ATG)	Complement-mediated lysis of lymphocytes, clearing of lymphocytes, clearing of lymphocytes, alteration of T cell function	10-30 mg/kg/day for 7-14 days	Fever, chills, malaise, arthralgia, nausea, vomiting, leukopenia, thrombocytopenia, rash	Dilute in 0.45 or 0.9% normal saline; must be filtered and administered through central line

Specific immunosuppressive agents

Glucocorticoids

Glucocorticoids were one of the first agents employed in transplantation and still being used in most immunosuppressive protocols. Glucocorticoids have several effects on the immune system. Their primary mechanism is by blocking interleukin - 1 (IL-1) and IL-6 production from macrophages, which results in decreased lymphocyte proliferative response to antigens. Other actions include decrease in the production of other cytokines such as IL-2, IL-4, and γ-interferon, reduction in the formation of eicosanoids and platelet-activating factor, and also decrease in complement components in the blood. All of these actions also contribute to the anti-inflammatory actions of glucocorticoids.

In addition to their immunosuppressive and anti-inflammatory activity, glucocorticoids also have metabolic effects, which are their unwanted side effects. Glucocorticoids cause both an increase in gluconeogenesis and a decrease in the uptake and utilization of glucose, resulting in a tendency to hyperglycemia which may develop into actual diabetes.

Glucocorticoids decrease protein synthesis and increase protein catabolism especially in muscle. In children, this metabolic effect may result in growth retardation. Prolonged systemic use may result in the redistribution of fat characteristic of Cushing's syndrome. Osteoporosis is one of the main side effects of these agents. This caused by glucocorticoids decrease calcium absorption in the gastrointestinal tract and increasing its excretion by the kidney.

The doses of steroids vary according to individual treatment protocols. Steroid doses are highest immediately after transplantation and then usually tapered to maintenance doses of less than 15 mg/d of prednisolone by 6 months after transplantation. To minimize steroid toxicity, alternate-day regimens are sometimes used for stable patients.

Azathioprine

Azathioprine is a prodrug of 6-mercaptopurine, which inhibits DNA synthesis. Azathioprine depresses both cell-mediated and antibody-mediated immune response because it inhibits early immune response by a cytotoxic action on dividing cells. Azathioprine also blocks bone marrow production of

lymphocytes, which is its serious toxicity. Patients receiving prolonged azathioprine therapy should be monitored blood count closely.

Since Azathioprine is converted to its active metabolites by liver enzymes, hepatic dysfunction and enzyme inducers/inhibitors affect its pharmacological response. Azathioprine is also metabolized by xanthine oxidase, thus when used combine with allopurinol, a xanthine oxidase inhibitor, the azathioprine dose should be reduced by 50-75% to avoid toxicity.

Mycophenolate Mofetil

Mycophenolate Mofetil is a prodrug of mycophenolic acid, a potent, noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase. While mycophenolate mofetil has many similarities to azathioprine as an antiproliferative agent, its mechanism is more specific for lymphocyte proliferation. Mycofenolate inhibits purine synthesis from the de novo pathway, which is specifically affect lymphocyte because lymphocyte proliferation depends on this pathway, whereas most other cells can use another pathway.

The adverse effects include neutropenia and thrombocytopenia may occur with mycophenolate mofetil. Gastrointestinal effects such as nausea, vomiting, and loss of appetite have been reported. The exact role of this drug in transplantation is not defined yet. Whether mycophenolate mofetil will replace azathioprine in triple regimens will depend on long-term clinical studies.

Cyclosporin

Cyclosporin has revolutionized the field of transplantation, significantly reducing the morbidity and incidence of rejection by its selective effects on lymphocytes. The action and pharmacokinetic of cyclosporin are described in chapter 3.

Tacrolimus

Tacrolimus, formerly called FK506, is a macrolide antibiotic extracted from fungus. Though structurally different from CsA, tacrolimus has a similar mechanism of action. Both tacrolimus and cyclosporin (CsA) inhibit interleukin-2 production in T lymphocytes, thereby inhibit proliferation of those cells.

Tacrolimus also exhibits highly variable pharmacokinetics like CsA. After oral administration, tacrolimus has poor and erratic absorption, its bioavailability ranges from 5 to 67% with a mean of 27%. In contrast to CsA, the oral absorption of tacrolimus does not appear to be reduced in patients with liver dysfunction or in patient with a T tube. Because of its high lipophilicity, tacrolimus is highly distributed into erythrocytes and tissues with a steady-state volume of distribution of 5-65 l/kg.

Tacrolimus is predominantly eliminated by hepatic and intestinal metabolism, thus alteration of hepatic function is expected to influence its elimination. Besides, drugs known to induce or inhibit cytochrome P-450 enzymes are likely to alter tacrolimus concentration in patients.

Tacrolimus has been shown similar toxicity profile to CsA. The common adverse effects appear to be nephrotoxicity, neurotoxicity, and diabetogenic effects. Some adverse effects reported with CsA appear to occur with reduced frequency with tacrolimus, including hirsutism, gingival hyperplasia, and hypertension. Other side effects such as hyperglycemia and tremor have been found to occur more frequently with tacrolimus than CsA.

The role of tacrolimus in clinical transplantation is not defined yet. The success of liver and intestinal transplantation suggests that tacrolimus may become the drug of choice for those recipients. However, the role in renal transplantation is less well defined because the high success rates routinely achieved from CsA therapy. Although, a major benefit of tacrolimus as compared with CsA is an enhance ability in patients to discontinue corticosteroids, long-term clinical trials are needed.

Anti-T-Cell Biological Products

Antilymphocyte/Antithymocyte Globulin (ALG/ATG) Therapy

Antilymphocyte or antithymocyte globulin is a sterile, nonpyrogenic solution of polyclonal immunoglobulins (IgG). ALG or ATG is derived from immunized animals with human lymphocytes or with foetal thymic tissue, respectively.

The major action is elimination of circulating T lymphocytes and decrease their formation. Like other animal products, a test dose of ALG or ATG should be administered before full dosing and monitored for an immunologic response.

The adverse events are mainly those to be expected with injection of foreign protein. The primary unwanted effects are fever, chills, myalgias, urticaria, nausea, vomiting, leukopenia, and thrombocytopenia. ALG or ATG should be infused into a large central vein to prevent thrombophlebitis. Furthermore, substantial batch-to-batch variability in preparations is a problem associated with the clinical use of these products.

Muromonab CD3 (OKT3)

After polyclonal antilymphocyte preparation were available, monoclonal antibody preparation for immunosuppressive therapy has been introduced by biotechnology advancement. Monoclonal antibody preparations have solved the batch-to-batch variability problem, and provided higher efficacy than polyclonal antibody preparations. While there are many monoclonal antibodies produced for diagnostic purposes, only OKT3 is commercially available for prevention and treatment of rejection.

OKT3 or muromonab CD3 is a murine monoclonal antibody that is directed against the CD3 antigen on the surface of T cells. It is a very potent immunosuppressant. Within minutes after intravenous administration of OKT3, circulating T lymphocytes become nearly undetectable. OKT3 is thought to bind to established cytotoxic T cells causing opsonization and removal by the reticuloendothelial system in the liver and spleen. It has shown that T-cell depletion is not the only mode of action. The other mechanism is modulation of the T-cell receptor. OKT3 cross-links with the CD3 antigen on T-cell surface, resulting in removal of all CD3 molecules including the T-cell receptor. The result is T lymphocytes loss the ability to function properly.

OKT3 therapy is used as induction therapy, as a first-line treatment of acute cellular rejection, and as a second-line therapy for the treatment of steroid-resistant rejection. The limitations for its use include expense, extensive T-cell suppression, which may cause an increase viral infections. Moreover, antibodies can develop against OKT3, preventing further use of the agent.

The initial administration of OKT3 is commonly associated with the risk of a first-dose reaction. This reaction may include fever, chills, headache, chest tightness, and can be accompanied by nausea, vomiting, and diarrhea. In addition, aseptic meningitis and pulmonary edema may occur with OKT3 (Rang, Dale and Ritter, 1995; Burckart, Venkataramanan, and Ptachcinski, 1996).