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IMMUNOSUPPRESSIVE THERAPY

New Concepts

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The first successful kidney transplant was performed on identical twins in 1954 without the need for immunosuppression.[100] Since then, the field of allogeneic solid organ transplantation has developed rapidly. The number of transplants has grown yearly, with improvement in outcomes and increasing indications for transplantation.[148]

The immune system of the body is capable of recognizing nonself (foreign) proteins. Transplantation of an organ among genetically nonidentical individuals can lead to its destruction by a process called *rejection*. Types of rejection vary. Hyperacute rejection occurs soon after reperfusion of the organ, and is characterized by rapid thrombotic occlusion of the graft microvasculature. This process is mediated by preexisting antibodies in the recipient that bind to the endothelium of the microvasculature of the donor organ. This binding activates the complement cascade, which in turn promotes intravascular thrombosis. Hyperacute rejection was first described in cases of ABO-incompatible organs. Subsequently, hyperacute rejection could also occur in cases in which IgM or IgG antibodies were directed against human leukocyte antigens (HLA) found on the donor organ. Acute rejection entails humoral or cellular mechanisms. In humoral rejection, endothelial cells of the donor microvasculature show changes of vasculitis without thrombi. With cellular-mediated acute rejection, an inflammatory change occurs in the

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allograft because of a mononuclear infiltrate, usually perivascular, that may result in distal ischemia if advanced. Chronic rejection is a slow process leading to fibrosis and obliterative arteriopathy and ischemia of the functional elements of the graft. This phenomenon is not yet clearly understood; however, recurrent bouts of acute cellular rejection can lead to rapid development of chronic rejection as soon as 6 to 12 weeks after transplantation. Rejections can be minimized by three different approaches: (1) using ABO-compatible and lymphocytotoxic crossmatch-negative allografts; (2) minimizing allogeneic differences between donor and recipient, such as HLA matched kidney transplantation; and (3) ensuring adequate induction immunosuppression.[1]

THE BASIS OF MODERN-DAY IMMUNOSUPPRESSION

Multiple steps are involved from the recognition of allograft to the development of effector mechanisms, such as cytotoxic T cells and alloantibody formation, which result in allograft rejection.[1, 141] Each step represents a potential site that can be targeted in an antirejection strategy.

- Anti-inflammatory: Corticosteroids
- Nonspecific inhibition of cell division: Cyclophosphamide, azathioprine
- Selective inhibition of de novo purine synthesis in lymphocytes: Mycophenolate mofetil, mizoribine
- Inhibition of pyrimidine synthesis: Brequinar
- Inhibition of interleukin (IL)-2 gene transcription: Cyclosporine and tacrolimus
- Inhibition of action of IL-2 on effector cells: sirolimus
- Monoclonal antibodies (MAb) with specific sites of action:
  1. Murine anti-cd3 MAb: Muromonab-cd3
  2. Anti-IL-2 receptor monoclonal antibodies:
     a. Chimeric IL-2R MAb: Basiliximab
     b. Humanized IL-2R MAb: daclizumab
  3. Anti-T cell receptor MAb:T10B9.1A-31
- Polyclonal antibodies against human thymocytes (antithymocyte globulin): antithymocyte globulin (ATGAM)
- Costimulatory pathway blockade (blocking B7 and cd28 co-stimulation): cytotoxic T lymphocyte antibody (CTLA4Ig), anti-cd40 MAb
- Adhesion molecule blockade: leukocyte function–associated antigen 1 (LFA)-1, intercellular adhesion molecule 1 (ICAM-1), VCAM, VLA-4

THE HISTORIC PERSPECTIVE

Activation of the immune system results in proliferation of various subsets of lymphocytes and other cellular mediators, which in turn target the transplanted organ. Therefore, chemotherapeutic agents such as mercaptopurine and cyclophosphamide, which interfere with synthe-

sis of DNA and RNA synthesis and thereby prevent the proliferation of dividing cells, were used along with corticosteroids in the 1960s to prevent rejection. Mercaptopurine was shown to inhibit skin graft rejection in rats and later delay it in canine renal transplantation.[21, 125, 160] Later, a derivative of mercaptopurine, azathioprine, with improved oral absorption was found to prolong human kidney homograft.[32]

Corticosteroids were shown to prolong skin graft survival in rabbits.[13, 97] Steroids have been useful in controlling ongoing acute episodes of rejection and also as prophylaxis in preventing rejection.[61] Although the serum half-life of the more commonly used corticosteroids (prednisone and methylprednisolone) is short (2–3 h),[145] their effect is prolonged for up to 24 hours. There are two proposed mechanisms of action: (1) selective lysis of immature cortical thymocytes along with certain T cells, and (2) blockade of cytokine gene transcription and cytokine secretion from mononuclear phagocytes.

Starzl et al[135] and Murray et al[99] in 1963 independently showed the benefit of a combination of corticosteroids and azathioprine to obtain meaningful survival following allogeneic kidney transplantation in humans; however, the doses of corticosteroids used in these regimens were associated with a high incidence of serious side effects, including hypertension, hyperglycemia, delayed wound healing, osteoporosis, increased risk for gastrointestinal ulceration, bleeding, and suppression of pituitary–adrenal axis.[15, 145] Thus, attempts to reduce corticosteroid doses with azathioprine required the development of another form of immunosuppression. In the late 1960s, antilymphocyte globulin antibodies (e.g., ATGAM) derived against human lymphocytes and thymocytes from horse, rabbit, or goat were also combined with azathioprine and corticosteroids to prevent acute rejection.[43]

THE ADVENT OF MODERN IMMUNOSUPPRESSIVE AGENTS

Great leaps in clinical outcomes for solid organ transplantation were achieved with the development of cyclosporine and tacrolimus. Both agents contributed to the success of solid organ transplantation and thrust these therapies into the realm of clinical applicability.

Cyclosporin A (CsA) is produced as a metabolite by the fungus species *Tolypocladium inflatum* with a molecular weight of 1202. Tacrolimus is derived from the soil fungus *Streptomyces tsukubaensis*, with a molecular weight of 822. Both drugs are virtually insoluble in water and hexane but soluble in methanol, ethanol, acetone, ethyl acetone, chloroform, and dimethyl ether. The chemical structures of CsA and tacrolimus are quite different. CsA is a neutral lipophilic cyclic polypeptide consisting of 11 amino acids, whereas tacrolimus is a macrolide lactone with a hemiacetal-masked diketoamide incorporated in a 23-member ring. It is surprising that, although both compounds are chemically unrelated, they have similar modes of action in preventing allograft

rejection. They both act through inhibition of the nuclear factor of activated T cells, which are responsible for activation of the transcription of IL-2 gene, thus preventing production of IL-2, essential for the proliferative response of T lymphocyte[44, 91, 123]; however, CsA and tacrolimus bind to different intracellular families of proteins, cyclophyllins for CsA and FK binding protein for tacrolimus. They form a drug-binding protein complex that specifically and competitively binds to and inhibits the phosphatase activity of calcineurin.[57, 80, 122, 153]

Following oral administration, both CsA and tacrolimus have unpredictably variable and incomplete absorption, and, therefore, monitoring of the concentration of these agents is recommended to adjust the dose for a given patient. Neoral is a microemulsion formulation of CsA. This formulation has a more predictable absorption, and intrapatient and interpatient variable in absorption is lower. Following oral administration, a higher peak at a shorter period of time and increased area under the concentration curve are observed compared with the older formulation of CsA.

Both drugs are metabolized by the liver cytochrome P450 system.[150, 158] In the event of hepatic dysfunction, metabolism of both drugs is slower, clearance of the drugs is slower, and this prolongs the half-life of these drugs. Drugs that stimulate cytochrome P450 activity (e.g., phenytoin [Dilantin]) promote the clearance of the drug and shorten the half-life. On the other hand, drugs that compete for P450 metabolism (e.g., ketoconazole and fluconazole) prolong the half-life of both drugs.[2, 63] Although the presence of bile is necessary for absorption of conventional formulation of CsA, tacrolimus can be absorbed in the absence of bile.[37, 63, 68, 150, 158] Neoral has better absorption characteristics than the conventional formulation of CsA in the absence of bile.[74, 104, 155]

Cyclosporine

In 1979, Calne et al[18, 19] introduced CsA in clinical trials. This was further refined by Starzl et al[133] when CsA was combined with corticosteroids. Subsequently, European and Canadian multicenter trials revealed significant improvement in 1-year patient and graft survival in renal transplantation.[23, 41] The sustained long-term benefits of CsA were maintained at 3 years in Canadian, and 5 years at European and other trials.[17, 22, 40, 69] The inherent nephrotoxicity of CsA was the impetus for lower doses of CsA to be combined with azathioprine and steroids[156]; however, recent reports have suggested that doses of CsA that are too low are associated with the development of chronic rejection and late acute rejection in renal transplant.[4, 38, 120] A maintenance dosage of CsA of more than 4 mg/kg/d has thus been recommended.[87, 88] The application of CsA to liver transplant by Starzl et al[133] was shown to almost double 1-year patient survival from 30% to 35% under azathioprine and steroids to 70% under CsA and steroids. Similar benefits were also observed in heart transplantation[55, 78, 83, 106] and lung transplantation.[16, 51]

Tacrolimus

Tacrolimus (FK506, Prograf) was introduced by Starzl et al in 1989. Tacrolimus was first used for liver allografts failing from either acute or chronic rejection under CsA.[35, 48, 134] Its ability to control ongoing steroid or orthoclone (OKT3) or muromonab-CD3–resistant acute rejection and prevent the progression of chronic rejection (or even reverse chronic rejection in some cases) was unprecedented and was the impetus for subsequent primary liver transplantation trials.[61, 142, 144] An improvement in the quality of life of the patient on tacrolimus has been reported by Felzer et al[42] following conversion from CsA to tacrolimus. The US Multicenter study group and other centers found similar clinical benefits[94, 149] for rescue of liver allografts failing from acute and chronic rejection under CsA.

Three separate prospective randomized trials have been conducted to study the efficacy of tacrolimus versus CsA in primary liver transplant recipients: (1) University of Pittsburgh (single center, 154 patients)[45–47]; (2) European Tacrolimus Multicenter (8 centers, 545 patients)[39]; and (3) US Multicenter Tacrolimus Liver Study Group (12 centers, 529 patients).[140] All three studies revealed a significantly lower incidence of rejection under tacrolimus and no significant difference in 1-year patient or graft survival. One-year patient and graft survivals in the European trial were 82.9% and 77.5% for tacrolimus versus 77.5% and 72.6% for CsA. The acute rejection-free rate was higher in the tacrolimus group: 56.6% versus 46.4% for CsA ($p = 0.004$). The refractory rejection rate was 0.8% with tacrolimus versus 5.6% with CsA ($p = 0.005$), and the chronic rejection rate was 1.5% with tacrolimus versus 5.3% with CsA ($p = 0.032$), despite higher concomitant use of corticosteroids or azathioprine in the CsA patients. In the US Multicenter Trial, the actuarial 1-year patient survival, by intent-to-treat analysis, was 88% for both groups of patients, whereas graft survival was 82% for tacrolimus-treated patients and 79% for CsA-treated patients. Overall, 22 CsA-treated patents with refractory rejection were switched to tacrolimus, 19 of whom survived with their original grafts. The rates of acute rejection, steroid-resistant rejection, and refractory rejection were 68%, 19%, and 3% with tacrolimus versus 76%, 36%, and 15% with CsA ($p < 0.002$, < 0.001, and < 0.001), respectively. Patients in the CsA arm received higher dosages of corticosteroids (at all 12 centers), and also received azathioprine (at 11 centers) with antilymphocyte preparation (at one center).[131] In total, 14.1% of patients receiving tacrolimus were withdrawn from the study, mainly for neurotoxicity and nephrotoxicity. In the Pittsburgh trial, the 1-year patient and graft survival were not different with intent-to-treat analysis, although a trend toward better survival in the tacrolimus group existed; however, in all three trials, a large percentage of patients were switched from CsA to tacrolimus, mainly because of persistent rejection. Reanalyses of the US Multicenter Trial for undesirable endpoints ([1] rejection; [2] rejection, retransplantation, or death; [3] rejection, retransplantation, death, or adverse event requiring withdrawal of the drug; or [4] rejection, retransplantation, death, or withdrawal of the drug for any reason)

showed significantly better results with tacrolimus compared with CsA.[131]

Longer-term follow-up on a large population from a single center has shown sustained benefit of tacrolimus for liver transplantation.[62, 65, 143] In addition, the rates of chronic rejection are also lower under tacrolimus.[14, 66] More recently, the longer-term follow-up of these studies has begun to demonstrate a divergence in both patient and graft survival. The long-term follow-up (> 2 y) revealed a modest increase in patient and graft survival. In a comparison of transplanted patient half-lives, tacrolimus-treated patients had a calculated half-life of 25.1 years compared with 15.2 years for CsA-treated patients. The freedom from rejection remained statistically greater in the tacrolimus-treated group than in the CsA-treated group over all periods studied. In addition, the freedom from steroid use, the lower incidence of hypertension, and less hypercholesterolemia and hypertriglyceridemia were prominent features of patients treated with tacrolimus.

Tacrolimus has also been demonstrated to have a special advantage in the pediatric population for both primary liver transplantation and for failing liver allografts under CsA for chronic and acute rejection.[62, 65, 118, 119, 143] The lack of hirsutism and gingival hyperplasia under tacrolimus therapy and the ability to reduce the dosage of corticosteroids after successful rescue therapy with tacrolimus, resulting in improvement of cushingoid facial appearance, has substantial cosmetic benefits in this population of patients.[71, 72] Successful pregnancies have been reported in the mothers following liver transplantation, both under CsA and tacrolimus. Although preterm delivery in more than 50% of the mothers has been observed under both drugs, the incidence of hypertension and preeclampsia was lower with tacrolimus compared with CsA.[67, 111, 112]

The introduction of tacrolimus in renal transplantation for steroid-resistant or OKT3-resistant acute rejection under CsA has shown response rates in as many as 70% of the patients.[71, 72] This drug was also reported to be effective in primary kidney transplantation with less severe rejection, less hypertension, and less hyperlipidemia compared with CsA.[33, 70, 102, 103, 121, 126, 132] The US Multicenter kidney transplant phase III trials revealed lower rates of rejection with increased incidence of insulin-dependent diabetes mellitus with tacrolimus compared with CsA.[95, 109] The impact of tacrolimus in preventing chronic rejection in kidney allografts has not been as clearly defined as in liver transplantation; however, Gjertson et al[49] analyzed the United States kidney transplant registry data from 1988 to 1994, consisting of 38,057 first cadaveric kidney transplants from 224 centers in the United States. They predict a significantly prolonged half-life of kidney allografts with tacrolimus (14 y) versus CsA (8–9 y); however, tacrolimus cannot reverse established chronic rejection of renal allografts under CsA.

Tacrolimus therapy has also been reported in thoracic organ transplantation. In primary heart transplantation under tacrolimus therapy, patient survival of 92% at 1 year in the adult population and 82% in the pediatric population at 1 to 3 years has been reported. Although these

survivals are comparable to those with CsA, freedom from rejection at 90 days was higher with tacrolimus (40% in adults and 60% in children), combined with a lower incidence of hypertension (54% with tacrolimus compared with 70% for CsA) in adults.[6, 8, 9, 107] An improved quality of life for the heart transplant patients under tacrolimus has been reported by Dew et al.[36] Similarly, steroid-resistant and antilymphocyte antibody–resistant cardiac rejection during CsA-based immunosuppression can be successfully rescued with tacrolimus in both children and adults.[6, 8, 107] The benefits of tacrolimus in reducing not only the rate of acute rejection and severity of rejection for lung transplantation,[52, 53, 79] but also significantly lower incidence of obliterative bronchiolitis (a histologic manifestation of chronic rejection), has been observed over time compared with CsA.

In pancreatic transplantation, lower incidence of acute rejection under tacrolimus without induction therapy with antilymphocyte preparation has been reported. Like other acutely rejecting organs with CsA, tacrolimus has the ability to halt the progression of rejection with CsA.[27, 28, 54, 56, 138]

The need for concomitant use of corticosteroids and antilymphocyte antibodies has been significantly lower in all primary liver,[39, 62, 93, 140] kidney,[121] heart,[7] and lung[53] transplant recipients receiving tacrolimus compared with CsA.

Almost all current immunosuppressive protocols are based on either CsA or tacrolimus; however, both drugs have neurotoxic, nephrotoxic, and diabetogenic potential and thus the search for new immunosuppressive agents continues.

THE EXPANDING ARMAMENTARIUM OF IMMUNOSUPPRESSIVE AGENTS

Mycophenolate Mofetil

Mycophenolate mofetil (MMF, RS 61443) is a semisynthetic derivative of mycophenolic acid. It is a new immunosuppressive agent that has recently been approved by the Food and Drug Administration for kidney transplantation with CsA and steroids. MMF inhibits the de novo purine nucleotide synthesis by noncompetitively and irreversibly inhibiting inosine monophosphate dehydrogenase and thereby DNA replication in T and B lymphocytes. It affects only the de novo purine synthesis in lymphocytes, which are unable to use alternate salvage pathways.[3]

Deierhoi et al[34] reported 100% patient survival at 2 years and 95% graft survival at 2 years following cadaveric kidney transplantation in 21 patients using MMF and CsA.[34] They also noted an ability to rescue kidney allografts with steroid or OKT3-resistant rejection with CsA in cadaveric kidney transplantation with a 54% graft survival. Sollinger et al[128, 129] also reported successful long-term rescue in 69% of patients with the addition of MMF for refractory rejection in kidney transplant in a multicenter study. Three large multicenter prospective, randomized dou-

ble-blinded clinical trials comparing CsA, steroids, and MMF with CsA, steroids, and placebo (or azathioprine) were conducted in the United States (n = 449), Europe-Canada-Australia (n = 503), and Europe (n = 491). Each revealed significantly lower episodes of rejection in kidney transplant recipients who were randomized to MMF. The principal toxicities observed in these trials were bone marrow suppression and gastrointestinal toxicity.[117, 130, 139]

Because MMF and tacrolimus have different mechanisms of action and toxicities, a prospective randomized trial in liver transplant recipients was conducted. It consisted of MMF, tacrolimus, and steroids (triple-drug regimen) versus tacrolimus and steroids. The interim results in liver transplantation have shown a slight benefit in the rate of rejection, a decrease in perioperative renal impairment, and some benefit in steroid reduction with triple-drug regimen; however, approximately one third of the liver transplant recipients have anemia or leukopenia or thrombocytopenia before transplantation and the rate of infection in the postoperative period was more than 50%. MMF also has gastrointestinal toxicity. This had led to discontinuation of MMF in almost two thirds of the recipients[64]; however, the withdrawal rate was lower when the drug was used to reduce the dose of tacrolimus in order to improve chronic nephrotoxicity.[60] Similar studies are now underway for kidney, pancreas, and heart transplantation.

Muromonab-cd3

Muromonab-cd3 (Orthoclone OKT3) is a murine monoclonal antibody targeted against the CD3 receptor, which is closely associated with the T-cell receptor. It has both in vitro and in vivo immunomodulatory activity. It has been used in clinical trials for steroid-resistant rejection and also for induction therapy.[12, 24, 29] With the first dose (and occasionally with the second), there may be a release of cytokines by the targeted CD3+ lymphocytes that can cause fever, chills, tachycardia, gastrointestinal disturbances, bronchospasm, and changes in systemic blood pressure. This is most likely mediated via tumor necrosis factor release and can be prevented by premedication with hydrocortisone and antihistamines 0.5 hour before OKT3. The recommended dose of OKT3 is 5 mL daily for 10 to 14 days in adults, and 2.5 mL in children. The main side effect is increased risk for viral infection and development of human antimurine antibodies that may limit the effectiveness of OKT3 in future treatments.[24]

Anti-Interleukin-2 Receptor Alpha Chain (CD25)

The IL-2 receptor has three subunits: (1) alpha, (2) beta, and (3) gamma. The alpha subunit is upregulated on activated T lymphocytes, converting the IL-2 receptor to a high affinity receptor for IL-2. It is a 55-kD peptide, also referred to as *CD25* or *T-cell activation antigen* (Tac). Murine monoclonal antibody against T-cell-activated antigen (anti-Tac)

was developed in 1983 and used in clinical trials in 1991. Forty renal transplant recipients received anti-Tac MAb with CsA and steroids and were compared with recipients (controls) who did not receive anti-Tac MAb treatment. The rate of rejection was significantly lower for those who received anti-Tac MAb treatment. The time to the first episode of rejection was also significantly delayed.[82]

A chimeric anti-IL-2R MAb (basiliximab, Simulect) was developed by Novartis Pharmaceutical Company (Basel, Switzerland). Phase 3 prospective European and Canadian multicenter double-blinded placebo-controlled clinical trials involving 21 centers were conducted. Both groups received microemulsion formulation CsA (Neoral) and steroids. Although the study arm (n = 190) received basiliximab, 20 mg intravenously, 2 hours before renal transplantation and on the fourth postoperative day, the control arm received placebo. At 12 months, no significant difference was found in patient or graft survival. Patients who received anti-IL-2R had significantly fewer and less severe rejection compared with controls.[101] Similar results are available from the US multicenter study group (21 centers), in which 173 patients received basiliximab, Neoral, and steroids compared with 173 controls who did not receive basiliximab. The rate of rejection was 28% lower for the study group (P = 0.15).[75] In both trials, no evidence showed infusion reaction, and no increase in malignancy or infectious complications was observed.

A humanized anti-IL-2R MAb (Daclizumab, Zenapax) was developed by Roche Pharmaceuticals (Hoffman-La Roche, Ltd, Basel, Switzerland). A phase 3 multicenter, double-blind, placebo-controlled clinical trial was conducted involving 17 centers in the United States, Canada, and Sweden. A total of 226 patients who received first cadaveric transplants were included in the study. All patients received CsA, azathioprine, and prednisone. In addition, 126 patients received humanized anti-IL-2 MAb, and 134 patients received placebo. The antibody was given intravenously 1 mg/kg (maximum, 100 mg) 24 hours before renal transplantation and at 2, 4, 6, and 8 weeks after transplantation. Significantly lower rates of rejection were observed in the group of patients who received Daclizumab compared with those who received placebo: 22% versus 35% (p = 0.03). A significantly lower number was also found in episodes of rejection per patient and steroid-resistant rejections in the group who received Daclizumab. The mean half-life of Daclizumab was 20 days. This offered a significant decrease in the percentage of circulating lymphocytes that stained with anti CD25 antibody starting from 10 hours after transplantation and lasting up to 4 months in the group of patients who received Daclizumab.[151]

Other Agents in Preclinical Testing or Clinical Trials

Murine anti-T-cell receptor MAb (T10B9.1A-31) entered phase 2 trials in 1992. A prospective, open-labeled, single-center, randomized trial compared the efficacy of T10B9.1A-31 (n = 18) with OKT3 (n =

20) in renal transplantation to control ongoing rejection. Reversal of acute rejection was obtained by T10B9.1A-31 and was comparable to OKT3. Side effects, such as fever, diarrhea, dyspnea, nausea, and vomiting, experienced by T10B9.1A-31 recipients were significantly lower than with OKT3.[152]

Murine anti-CD4 MAb was developed by Johnson Pharmaceutical Research Institute (Ortho), Raritan, NJ. Reports on a pilot study were published in 1997. Thirty patients entered the trial in three centers. Increasing doses were used starting with 0.5 mg/kg (n = 24) to 1.0 mg/kg (n = 3) and 2.0 mg/kg (n = 3) for 12 days. A high percentage of CD4 saturation with minimal side effects and a low rate of rejection at 3 months was observed with 2-year graft survival of 83% and patient survival of 95%.[26]

Blockade of T-cell/antigen-presenting cell costimulatory pathways mediated via CD28-B7 has been accomplished by both MAb (anti-CD40 MAb) and hybrid proteins (CTLA4Ig) to permit acceptance of highly sensitized skin graft following cardiac allograft in murine models[84] and in renal allografts in primates and has shown reversal of acute rejection with prolongation of graft survival,[81] and resolution of chronic rejection has been observed in mouse aortic allograft models.[137]

Adhesion molecules are important not only in the effector pathway of allograft rejection, but also in the sensitization phase of alloimmunization. LFA-1 is required in the adhesion of leukocytes to endothelial cells. Anti-LFA-1 (anti-CD11a and anti-CD18) monoclonal antibody was better tolerated and required less post-transplant dialysis compared with rabbit antithymocyte globulin in renal transplantation; however, the rate of rejection in both groups was similar.[58] Immunosuppression by inhibition of cellular adhesion mediated by LFA-1 and ICAM-1 has been reported to be associated with tolerance induction in murine cardiac allograft.[157]

Rapamycin (Sirolimus) is a natural fermentation product (macrolide antibiotic) produced by *Streptomyces hygroscopicus*. Its molecular weight is 914.2 and the chemical formula $C_5H_{79}NO_{13}$. Although rapamycin binds to FK-binding protein, it does not seem to modulate the calcineurin activity. It prevents the action of IL-2 on T cells and thymocyte proliferation and B-cell activation. The site of action of rapamycin is late in the G1 phase of the cell cycle. Phase 1 clinical trial included 30 post–kidney transplant patients under CsA-based immunosuppression who received rapamycin and 10 patients who received placebo. Rapamycin is reported to cause thrombocytopenia, leukopenia, and lipid abnormalities.[98] Phase 3 studies have been conducted to evaluate safety and efficacy of rapamycin and CsA combination in renal allograft recipients. A total of 450 patients were enrolled in one study; patient survival and graft survival were 98% and 97%, respectively. A synergistic effect with lower incidence of rejection has been reported in the group of patients who received rapamycin.[77]

SDZ RAD is a rapamycin analogue made by Novartis Pharmaceuticals that has been shown to be synergistic with CsA both in vitro and

in small animal models. SDZ RAD is currently in phases 2 and 3 kidney transplant trials.[124]

Mizoribine is an imidazole nucleoside that was isolated in 1974 from the soil fungus *Eupenicillium brefeldianum*.[146] It seems to inhibit inosine monophosphate dehydrogenase, an enzyme required for the synthesis of guanine nucleotides from ionosine monophosphate (IMP). In phase 2 prospective randomized trials, mizoribine was used with CsA and steroids and compared with azathioprine, CsA, and steroids in renal transplantation. Patients who received mizoribine had a lower incidence of rejection, a lower incidence of leukopenia, and more freedom from steroids compared with the CsA, azathioprine, and steroid group.[86] In another similar study from Japan involving living related kidney transplants, mizoribine showed similar patient survival, graft survival, and rate of rejection but a lower incidence of leukopenia.[96] Still another study compared mizoribine in combination with CsA and steroids (group 3); mizoribine, azathioprine, and steroids (group 2); and azathioprine and steroids (group 1). Bone marrow suppression and diabetes mellitus were significantly lower in groups 2 and 3.[92]

Brequinar sodium is a quinoline carboxylic acid analogue that inhibits its dihydrofolate dehydrogenase and subsequent inhibition of de novo pyrimidine biosynthesis that prevents cell proliferation. It has dose-dependent antineoplastic activity against mouse and human tumor models. In rat liver, heart, and kidney allograft models, this drug was found to be highly effective in preventing allograft rejection.[30] Kahan et al[76] demonstrated synergistic interaction in vitro and in vivo of brequinar sodium, CsA, or rapamycin.[76] The drug was used in phase 1 trials for patients with advanced tumors. The dose-limiting toxicities were thrombocytopenia, dermatitis, mucositis, nausea, vomiting, malaise, anorexia, diarrhea, phlebitis, and irreversible elevations in transaminases.[10]

SUMMARY

Although several new immunosuppressive medications have been developed in the past decade, many possible avenues are yet to be explored. Although the newer agents have not reflected any clear benefit in patient or graft survival over CsA or tacrolimus, they have been useful in reducing the incidence and severity of rejection, reducing the concomitant use of steroids, and decreasing the doses of CsA or tacrolimus to minimize their toxicity profile. The appearance of these new agents has given more options to clinicians, who can select the one with the least toxicity and most efficacy for individual patients. In the future, combinations of these agents, in conjunction with a strategy to induce tolerance of the donor organ without drug toxicity, will be the goal.

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