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State of Immunosuppressive Agents in Organ Transplantation

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TRANSPLANTATION has been built on the foundation of using drug therapy to nonspecifically suppress the recipient immune response. Failure to achieve adequate immunosuppression is associated with a high incidence of graft loss from rejection. The present goal of immunosuppressive regimens is to suppress the immune response to the allograft while preserving sufficient immunity to avoid opportunistic infections and malignancies. In addition, these must be achieved while minimizing the inherent adverse effects of a particular drug. The ultimate goal of immunomodulation is to achieve donor-specific tolerance without alterations in immunity to other antigens.

The purpose of this dissertation is to briefly summarize the mechanism and role of current and potential future immunosuppressive agents in solid organ transplantation. Since an exhaustive review of these agents is not possible, the reader is referred to a number of such reviews.[1-3]

CURRENT IMMUNOSUPPRESSIVE AGENTS

Corticosteroids possess an antirejection property and have been utilized in almost all baseline immunosuppressive regimens. Current baseline doses of steroids range around .1 to .2 mg/kg per day. Steroids are also utilized as the first line of treatment of acute rejection episodes, at higher doses approximating 10 to 15 mg/kg. The mechanisms of action are several-fold: (1) antiinflammatory, stabilizing lysosomal membranes, suppressing prostaglandin synthesis; (2) suppressing IL-1 synthesis by macrophages by inhibiting IL-1 gene transcription; (3) inhibiting IL-6 gene transcription; and (4) lympholysis. The side effects are numerous and are related to the total dose and duration of administration. These include mood swings, weight gain, hypertension, diabetes mellitus, ulcerogenesis, osteoporosis, acne, growth retardation in children, aseptic necrosis of the femoral head, glaucoma, and cataracts.

Antilymphocyte antibody preparations are heterologous antilymphocyte preparations. Either the serum fraction [antilymphocyte serum (ALS)] or the immunoglobulin fraction [antilymphocyte globulin (ALG)] can be utilized. The only FDA commercially available ALG is ATGAM (Upjohn), although MALG (University of Minnesota) has been utilized by a number of centers. ALS and ALG preparations are utilized for the treatment of rejection, generally those considered as steroid resistant, although induction therapy with these preparations have allowed for lowered doses of cyclosporine (CyA) in the early posttransplant period. The mechanism of action is probably by depletion of lymphocytes via antibody-mediated destruction, although other mechanisms may also be important. The side effects are related to the limitations of crude antisera preparations. These include fever, chills, GI distress, myalgias, arthralgias, anaphylactoid reactions, serum sickness, thrombocytopenia, anemia, and leukopenia.

Monoclonal anti-T-cell antibodies are the result of hybridoma technology, allowing for easy quantification while minimizing lot-to-lot variation. The antibody is of murine origin, with defined specificity to the CD3 receptor associated with the T-cell receptor. To date, only OKT3 (Ortho Pharmaceuticals) has been approved, but a number of other preparations are being tested in clinical trials (see next section). Like ALG or ALS preparations, the principle use of OKT3 has been for reversal of steroid-resistant acute cellular rejections. OKT3 has also been utilized in a number of induction protocols, again to minimize early use of CyA to prevent nephrotoxicity. The most important mechanism of OKT3 is its ability to modulate the antigen recognition unit of the T cell, thereby neutralizing lymphocyte function. While effective in the treatment of rejection, the development of human antibodies to mouse proteins limits the length of treatment. The side effects following OKT3 are similar to that of ALG or ALS, although thrombocytopenia, anemia, and leukopenia are not generally seen with OKT3 administration. An enhanced susceptibility to viral infections following OKT3 has been reported.

Azathioprine is an imidazole derivative of 6-mercaptopurine, which is the active metabolite following hepatic metabolism. 6-Mercaptopurine is a purine analog which inhibits a number of important purine nucleotide synthetic enzymes. Azathioprine (Imuran, Upjohn) was one of the first widely used immunosuppressive agents for clinical transplantation. When utilized as the principle immunosuppressive agent, relatively high doses of 3 to 5 mg/kg per day are required. When used as part of a combination regimen, maintenance doses of 1 to 2 mg/kg per day are used. The drug is readily absorbed after oral administration, and the intravenous (IV) dose is the same as that for oral dosing. The side effects of azathioprine are related to the DNA inhibitory properties. Myelosuppression is the limiting factor in its use and this effect is dose dependent.

CyA is a cyclic polypeptide derived from a soil fungus. Application of CyA to clinical transplantation has been considered to be the reason for a quantum improvement in

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patient and graft survival, when compared to azathioprine-based immunosuppression. CyA is a lipid-soluble compound, which requires bile salts for absorption. The oral bioavailability of CyA is approximately 30%, and dose changes must be made for IV dosing. Monitoring of trough levels is common, utilizing any of a number of different assay systems. CyA inhibits the transcription of IL-2 mRNA, thereby inhibiting T-cell proliferation. In addition, other cytokine gene expression is inhibited. Gamma-interferon and IL-3 secretion by T cells are also inhibited by CyA. This drug represents a newer generation of nonspecific immunosuppression, since the humoral arm of the immune response is relatively spared from the effects of CyA. There is no myelosuppressive side effects of the drug, although there are a number of other side effects associated with long-term CyA use. Nephrotoxicity, hypertension, hyperkalemia, hirsuitism, gingival hypertrophy, and tremors are relatively common side effects of CyA. Because of the relatively specific effect of CyA on T-cell activity, a higher incidence of posttransplant lymphoproliferative diseases is seen.

FUTURE IMMUNOSUPPRESSIVE AGENTS

Investigational immunosuppressive agents include: FK 506 (inhibits cytokine synthesis), rapamycin (inhibits cytokine synthesis), brequinar (inhibits enzymes of the pyrimidine nucleotide synthesis pathway), mycophenolic acid (inhibits enzymes of the purine salvage pathway), and a number of monoclonal antibodies with varying specificities. Agents with significant experience in ongoing clinical trials will be presented. Again, readers are referred to more comprehensive reviews on these newer immunosuppressive agents.[4–6]

Mycophenolic acid was used as an agent for the treatment of refractory psoriasis. This drug was relatively well tolerated, the principle side effects being: leukopenia, mucositis, and GI upset. There was reportedly a higher incidence of upper respiratory infections and, in long-term–treated patients, a higher incidence of skin cancers. RS61443, is an analogue of mycophenolic acid, with enhanced oral bioavailability. Like mycophenolic acid, RS61443 (Syntex Pharmaceuticals) inhibits inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, both key enzymes which regulate the purine nucleotide salvage pathway.[7] This agent has been studied in clinical trials, as primary therapy along with CyA and steroids, in kidney transplantation, as well as in "rescue" therapy in patients with refractory organ rejection.[6] Preliminary results suggest that this drug is relatively well tolerated as doses up to 3.5 to 4.0 g/d.

FK 506 is a macrolide antibiotic, derived from the fermentation product of *Streptomyces tsukubaensis*. FK 506 is a hydrophobic compound, so that pharmaceutical formulation requires a solubilizing agent. The oral bioavailability varies considerably among individuals, and averages 25%. Oral absorption is not dependent upon bile salt presence, which is an important factor in liver transplant patients. Metabolism is almost entirely by the liver by the cytochrome P-450 IA and IIIA families. FK 506 binds to specific intracellular receptors, termed FKBP (FK 506 binding proteins). Like cyclophylin (the receptor for CyA), these receptors possess peptidyl-prolyl *cis-trans* isomerases. FK 506 inhibits the calcium-dependent pathway of T-cell activation, and inhibits transcription of various cytokine mRNAs. FK 506 has been utilized in a number of clinical situations, both as "rescue" and as primary therapy.[9] It has been used in liver, kidney, heart, and lung transplantation. FK 506 appears to be a potent antirejection agent for reversing ongoing rejection, especially with acute rejection episodes, although it also appears effective in the treatment of chronic liver allograft rejection. The use of FK 506 appears to lower the steroid requirement, allowing monotherapy in approximately 30% to 50% of primary transplant recipients. FK 506–based immunosuppression is not associated with hirsuitism, or gingival hyperplasia, and appears to have a lower incidence of hypertension. The adverse effects of FK 506 include: nephrotoxicity, neurotoxicity (predominantly in liver transplant recipients), glucose intolerance, and hyperkalemia.

Monoclonal antibodies with varying specificities have been developed to target specific interactions in the alloimmune response. Anti-CD4 monoclonal antibodies have been thought to target the helper T cell involved in the initial allorecognition and expansion phase of the immune response. Anti–IL-2 receptor monoclonal antibodies have been developed to the p55 component of IL-2 receptor of activated T cells, which will block the IL-2–driven T-cell proliferation. BMA 031 and T10B9.1A-31 are monoclonal antibodies against a monomorphic determinant of the α/β chain of the T-cell receptor. Like OKT3, they appear effective in the treatment of acute cellular rejection in kidney allograft recipients, except that they appear to be better tolerated. A number of monoclonal antibodies against cellular adhesion molecules have also been prepared, such as ICAM-1 (CD54) and LFA-1 (CD11). Most of these monoclonal antibodies have shown promise in early clinical trials, although comparison to currently available antilymphocyte antibodies are underway.

LIMITATIONS OF IMMUNOSUPPRESSION

There are two limitations of the current and experimental immunosuppressive agents. The first is the potency of the agents, either alone or in combination with other agents, in preventing rejection. The other is the side effects associated with these agents. While each immunosuppressive agent is associated with specific side effects, a host of infectious and malignant complications arise from the use of nonspecific immunosuppressive agents.

Certain types of nonlymphoid, epithelial cancers have an increased incidence in patients on long-term immunosuppression. Azathioprine use is associated with a four-

fold increase in the incidence of skin cancers. Kaposi sarcoma is associated with long-term CyA immunosuppression. Reticulum cell sarcomas are increased approximately 350-fold, when compared to the general population. Posttransplant lymphoproliferative disease (PTLD) is an abnormality of lymphocyte proliferation in a setting of an immunosuppressed patient. The spectrum of PTLD can range from a benign lymphoid proliferation such as a mononucleosis syndrome to a frankly malignant lymphoid tumor. PTLD has been associated with all types of immunosuppressive therapy, but the incidence is higher with the use of T-cell–specific immunosuppressive agents, such as CyA and FK 506. The incidence of PTLD in the CyA era is generally estimated between 2% and 4%. Most (90%) of PTLD are B cell in origin, and most are associated with integration of Epstein-Barr virus (EBV) DNA into the genome of the B cell.

Cytomegalovirus is the most common opportunistic infection in the transplant patient, although the spectrum of opportunistic infections is quite large. Several factors determine the severity and development of CMV infections. The seronegativity and use of intensive immunosuppression are considered major contributing factors. The incidence of CMV infections in most series examining large numbers of transplant patients is between 20% and 50%. This figure is dependent on the definition of CMV infections, since shedding of CMV virus can be asymptomatic. Progression of disease to invasive CMV entails positive identification of the CMV virus or viral antigens in tissue. The sites of CMV infection, in decreasing order, include: GI tract (gastritis, enteritis, or colitis), liver, lungs, kidney, and eyes. The CNS can be affected by CMV invasion. The treatment of CMV is with the use of specific antiviral therapy and simultaneous reduction of immunosuppression.

The other limitation of immunosuppression is the potency of the agents, either alone or in combination, in the prevention of rejection. Until the advent of CyA, liver and heart allograft transplantation was only intermittently successful. Even with CyA, rejection rates are quite high (40% to 70%), and the use of other agents have been proposed to prevent or treat rejections seen with CyA-based immunosuppression. Transplantation of "forbidden organs," eg, small bowel, lungs, and xenografts, have not proven successful with CyA-based immunosuppression. FK 506 has been utilized for clinical small bowel transplantation, with early success.[10] FK 506 appears more potent in the application of xenografting in animal models,[11] but in combination therapy with other agents, eg, RS61443, it appears to be even more efficacious.

CONCLUSIONS

Current day immunosuppression has led to success in a number of clinical transplant situations. Nevertheless, limitations of these agents exist, including inherent drug toxicity and the consequences of long-term immunosuppression. Newer agents may overcome some, but not all, of these limitations. The addition of the newer agents will allow transplant physicians to tailor immunosuppressive regimens with less toxicity and enhanced efficacy.

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