Role of Immunosuppression Minimization Protocols in Renal Transplantation, A Review

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ABSTRACT

The subject of transplant immunosuppression has generated significant interest in recent years. Excellent immunosuppression, advances in surgical technique, post-transplantation care, and infection control have resulted in excellent outcomes. There is widespread support for the notion that the fundamental objective in transplant immunology should be the achievement of specific graft tolerance. However, until this objective evolves into reality, investigators are in search of the "ideal immunosuppressant", which should target predominantly the immune system with minimal consequences for other tissues and minimal metabolic, cardiovascular and renal complications. While immunosuppressants have been associated with a tremendous trade-off in terms of morbidity, new agents have provided the investigators with the opportunity to formulate strategies that employ combination therapies with the goal of decreasing doses of individual agents and minimizing their toxicities. Multiple small studies have addressed the issue of minimizing immunosuppressants, but there is a need for well-designed clinical trials which should evaluate protocols that will reduce acute rejection, as well as chronic allograft nephropathy. They should address methods to identify subsets of patients who would maximally benefit from avoidance or withdrawal of steroids or calcineurin inhibitors. Other promising areas of research include tolerance studies among the sensitized recipients, and development of optimal immunosuppression based on genotype. In general, future trials must include a more diverse population of recipients, particularly the immunologically high risk groups.

Key words: Cyclosporine, Kidney Transplantation, Mycophenolate, Sirolimus, Tolerance, Tacrolimus

INTRODUCTION: TRANSPLANT IMMUNOSUPPRESSION, A DOUBLE-EDGED SWORD

The transplantation era, which was launched in the middle of the last century, has contributed significantly to development of the science of clinical immunology and immune pharmacology. Advances in the field have made manipulation of the immune system a tangible reality. The scientific community has intermittently shifted its focus of attention between approaches to minimize immunosuppression and strategies aimed at preventing and reducing the severity of acute rejection. This topic has received much deliberation and has generated significant interest in recent years. Availability of new immunosuppressive agents has provided the opportunity for investigators to formulate novel and distinctly tailored strategies that employ combination therapies with the goal of decreasing doses of individual agents and minimizing their toxicities. While the non-sensitized patient undergoing first transplant is expected to require only modest immunosuppression, the immunologically high-risk patient requires more intensive immunosuppression. This subset includes sensitized patients, repeat transplants, patients with delayed graft function, black recipients, and patients undergoing simultaneous kidney and pancreas transplants.

Approaches to minimize drug side effects and toxicities include the total elimination of steroids, elimination or reduction of calcineurin inhibitors (CIs), and the discontinuation of mycophenolate mofetil (MMF) after short-term use. The first section of this review will include an overview of the various currently available immunosuppressive agents. We will also aim to address whether newer agents have led to an improved outcome. In the second section of this review, strategies aimed at avoiding immunotoxicity will be discussed; more specifically, results of studies considering the avoidance and withdrawal of steroids and CIs will be elaborated upon. Although the predominant focus of this review is immunosuppression in renal transplantation, much of the information presented is also applicable to other solid organ transplants.

SECTION I. Overview of Transplant Immunosuppression

Following refinements in surgical techniques, the main focus of transplantation endeavor changed towards strategies to prevent graft rejection. Initial pharmacological manipulation consisted of using 6-mercaptopurine (6-MP), which proved to be beneficial in a small number of renal transplant patients. Subsequently, animal studies revealed that a related molecule, azathioprine, proved efficacious in transplantation. These studies led to the clinical application of azathioprine in 1962 (1). Subsequently, when combination of azathioprine and steroids were introduced into the arena of transplantation, a one-year graft survival rate of up to 50% was achieved (2). Although the majority of patients with rejection died following rejection, many deaths were attributed to infections secondary to the high steroid dosage. Active attention towards infection control and

refined overall care of the transplant patients led to an increase in 1-year graft survival of 60-70% in the early 1980's. The true breakthrough in transplantation immunosuppression, however, was realized when cyclosporine was approved for use (3). To prevent immune activation, one or both of two possible paths may be chosen. One type of modulation would be to influence intracellular pathways and the other is to target the cell surface. Chemical immunosuppression influences the first pathway, while antibodies constitute the latter group.

IA. Chemical immunosuppression

Azathioprine: This purine analog can inhibit nucleotide synthesis. A cascade of cell-to-cell interactions is required for the primary immune response, which eventually leads to lymphocyte proliferation. Azathioprine undergoes rapid hepatic metabolism with no need for dose adjustment in renal failure. Monitoring of blood levels is not required and dose adjustments are made by following white blood cell count. This agent is not effective in sensitized patients and has no role in the treatment of rejection. The main side adverse effect of azathioprine is bone marrow suppression, mainly in the form of leukopenia. Considering the interaction between allopurinol and azathioprine, which can lead to longer half-life of the latter, it would be prudent to avoid the combination to prevent severe leukopenia. Although azathioprine has been replaced by other more effective drugs in new transplants, it is still used in conjunction with steroids and cyclosporine in patients with successful transplants who had been maintained on it previously (4).

Corticosteroids*:* As discussed above, azathioprine was not deemed suitable to treat acute rejection episodes. Glucocorticoids were known to have significant anti-inflammatory properties, and their concurrent use along with azathioprine proved to enhance the overall outcome and prevention of rejection. Although steroids have been used for several decades, the exact mechanisms of immunosuppression are not totally clear. The original assumption was that steroids lead to immunosuppression mainly through mediating inflammation. Further investigations have yielded an improved comprehension of the mechanism of steroid immunosuppression. Pioneer studies by Woods et al. have demonstrated occurrence of lympholysis and subsequent lymphopenia following high dose steroid use (5). In addition, Fauci demonstrated sequestration of lymphocytes in the reticuloendothelial system following administration of high dose steroids (6). In the early 1990's it was demonstrated that a major component of steroid function is through blocking gene expression for lymphokines and cytokines essential for initiation of immune response (7,8). This could, in turn, lead to inhibition of the action of protein-1 and nuclear factor-B, thus inhibiting the induction of proinflammatory genes (9,10). The predominant opinion regarding the mechanism of action of steroids is that they are mainly effective on the macrophages and dendritic antigen-presenting cells through inhibition of both interleukin (IL)-1 and IL-6 by macrophages and monocytes (11-14). Steroids are capable of suppressing both the cellular and the humoral

immune systems, because IL-1 is a co-stimulus for T-helper cell activation, while IL-6 is a major B-cell inducer. In addition to the effects noted above, steroids are capable of inhibiting expression of IL-2, tumor necrosis factor (TNF), and interferon (IFN) gamma (15,16). As steroids enter the cell, they bind to receptors and migrate to the nucleus, binding regulatory regions of DNA. This subsequently leads to decrease activity of the IL-2 gene. While most other immunosuppressant agents reduce immune reactivity through specific mechanisms, steroids act at various sites along the immune response and have multifactorial immunosuppressant capabilities.

Mycophenolate mofetil (MMF): This agent was approved for clinical use in 1995. As previously noted, mycophenolate mofetil has replaced azathioprine as the "choice" inhibitor of nucleotide metabolism (17). Its main advantages over azathioprine are increased immunosuppressive effects and significantly lower bone marrow suppression. MMF is converted to mycophenolic acid (MPA), which inhibits inosine monophosphate dehydrogenase; this enzyme promotes synthesis of guanosine nucleotides and nucleosides. The inhibition of this enzyme leads to a selective block in T and B cell proliferation. Cells other than lymphocytes that have an alternate salvage pathway capable of bypassing blockade by MPA are spared. Another indication for MMF use is to replace azathioprine in patients requiring allopurinol for treatment of gout. Use of MMF has led to improve transplant outcome by reduction of acute rejection rates and preservation of renal function. Follow-up studies have shown that this has resulted in significant survival benefits (18). The most common side effects are related to gastrointestinal discomfort and diarrhea, and are usually responsive to dose reduction. Leukopenia, another potential side effect, is also responsive to dose reduction. Due to variable pharmacokinetics of the active form of the drug (MPA) blood level monitoring is usually neither helpful nor required.

Cyclosporine: This is a hydrophobic cyclic polypeptide containing 11 amino-acids. It was isolated from a Norwegian fungus in the 1970's and was noted to have immuno-suppressive properties. Animal studies showed the ability of cyclosporine to prevent acute tissue rejection and clinical trials followed. The discovery of cyclosporine as an effective and powerful immunosuppressant has had a significant impact on transplant medicine. This drug was approved for clinical use in 1983 and within two years, there was an impressively positive impact on graft survival. Initially, some centers used cyclosporine to replace azathioprine; however, the overall agreement was that utilization of cyclosporine in a triple therapy regimen (addition of cyclosporine to steroids and azathioprine) would allow lower of doses for each agent. The effect of cyclosporine, similar to azathioprine, is limited to prevention of rejection and it is not effective for the treatment of rejection. Cyclosporine use is associated with nephrotoxicity, hepatotoxicity, hypertension, hyperlipidemia, tremors, hirsutism, and gingival hypertrophy. Nephrotoxicity, which is dose-dependent and caused by the native molecule, has been a significant limiting factor for its use, and it is often clinically not discernable from

rejection. Although these side effects have limited its use, cyclosporine remains a valuable immunosuppressant.

Progress in understanding the roles of IL-2 and other cytokines in immunity led to productive studies on the mechanism of action of cyclosporine in the 1980s. When cyclosporine was added to antigen-stimulated cell cultures, T lymphocytes failed to secrete IL-2, IL-3, IL-4, IFN-gamma, and TNF-alpha, while this did not lead to any change in the production of transforming growth factor (TGF)-beta and IL-l by macrophages (19,20). Interestingly, addition of IL-2 led to proliferation of T cells. The molecular mechanism of action of cyclosporine is well defined in T cells; it inhibits critical signaling pathways that regulate T-cell activation and thus interferes with the activation and proliferation of cytotoxic T cells. The calcium-dependent pathway to NFkB is the intracellular pathway most influenced by cyclosporine. Cyclosporine is an inhibitor of calcineurin, which is an enzyme capable of T lymphocyte activation. When calcineurin is blocked, there is a significant decrease in the production of IL-2 and the other cytokines. The term "cyclophilins" was coined to identify cytosolic molecules that bind to cyclosporine. The cyclosporine-cyclophilin complex then binds to calcineurin, blocking its activity (21,22).

Cyclosporine has a narrow therapeutic index and its bioavailability is limited by the lipophilic nature of the molecule. This drawback led to the development of microemulsion cyclosporine and other CIs such as tacrolimus. The microemulsion formulation has a more predictable absorption and a more favorable pharmacokinetic. The absorption of this agent is affected by P-glycoprotein, as well as intestinal 3A4 activity. The former protein clears cyclosporine from cells into the intestinal lumen, where cyclosporine is metabolized via 3A4. In addition, cyclosporine undergoes hepatic metabolism mediated by 3A4. This renders cyclosporine the property of multiple potential drug-drug interactions. In addition, inhibitors and inducers of 3A4 and P-glycoprotein posses the potential to alter cyclosporine levels. Cyclosporine itself is an inhibitor of 3A4 and P-glycoprotein and may lead to altered levels of drugs dependent upon these for their metabolism. Ketoconazole, fluconazole, itraconazole, myconazole, clarithromycin, erythromycin, fluvoxamine, nefazodone, sertraline, verapamil, diltiazem, allopurinol, atorvastatin, simvastatin, losartan, and grapefruit juice lead to increased cyclosporine levels by inhibiting 3A4. Other drugs are also known to lead to an increase cyclosporine level, by decreasing its clearance. Acyclovir, valsartan, and alendronate are examples of such drugs; the exact nature of the pharmacokinetic interactions with these medications is not quite clear. Carbamazepine, phenobarbital, phenytoin, rifampin, sulfinpyrazone, and several herbal remedies are inducers of 3A4 or P-glycoprotein and are known to decrease levels of cyclosporine. There have been multiple case reports of transplant rejection secondary to decreased cyclosporine levels in patients using over the counter herbal medications. Other interactions have also been noted. Levels of lipid lowering agents in HMG-CoA reductase inhibitor (statins) class are increased by cyclosporine inhibition of 3A4. Several cases in the literature describe rhabdomyolysis presumed to be secondary to high levels of statin drugs when these drugs were given in combination with cyclosporine. The multitude of noted drug interactions mandates careful monitoring

of cyclosporine levels when drugs are added or deleted from a patient's regimen. In addition, it is imperative to carefully monitor drugs whose metabolism may be altered by cyclosporine to avoid toxicities.

Tacrolimus (TAC): Following the clinical success with cyclosporine, investigators probed other fungal sources for immunosuppressive drugs. The fermentation of streptomyces stukubaensis led to the isolation by Japanese investigators of tacrolimus (FK506) in 1985; it was first used in clinical transplantation in 1989 and was approved by the Food and Drug Administration for clinical use in the United States in 1994. This is another calcineurin inhibitor with some similarities and a few differences in property when compared with cyclosporine. Unlike the latter, it is a macrolide in structure, with a totally different binding protein (FKBP12) from cyclophilins. Interestingly, the FKBP12-tacrolimus complex binds to, and blocks, the effects of calcineurin (23), similarly to the effect of cyclosporine. The term "immunophilins" has been coined to refer to cyclophilins and the FKBP. The pharmacological behavior of tacrolimus is similar to cyclosporine, however, it has a more potent inhibitory effect on T lymphocyte activation and has a proven increased potency in preventing rejection (24); it has also been shown to reverse rejections in cyclosporine-treated patients. Tacrolimus has a more favorable side-effect profile compared with cyclosporine. Patients on tacrolimus have a lower propensity to develop hyperlipidemia and hypertension and do not develop gingival hyperplasia and hirsutism. Similar to cyclosporine, it is nephrotoxic and may induce diabetes; it can also intensify gastrointestinal side effects associated with MMF use.

Tacrolimus has been compared to cyclosporine in patients who were concomitantly treated with azathioprine and prednisone. It has been shown to be associated with a reduction in the rate of rejection, as well as a lower prevalence of graft dysfunction (25). In a study of long-term transplant recipients, Gill et al. (26) considered the effect of various immunosuppressive agents on the glomerular filtration rate. Tacrolimus use and no CI exposure proved to be the two "CI exposure" categories least associated with a decline in glomerular filtration rate compared to patients who received cyclosporine. In an attempt to evaluate the optimal immunosuppression, using the USRDS database, Gill, et al. (27) studied the outcomes of more than 40,000 kidney transplants. They determined that tacrolimus was the calcineurin inhibitor associated with the most favorable effects on rates of change in allograft function. Similarly, with regards to purine metabolism inhibitors, patients who received MMF had a less rapid decline in GFR compared with azathioprine. This is consistent with previous data, which reported a lower incidence of late acute rejection episodes in MMF compared to azathioprine treated patients (28). Recent 5-year graft survival rate analysis revealed a survival benefit of tacrolimus therapy compared with cyclosporine (29). To summarize, tacrolimus-based immunosuppressive therapy has been associated with reduction in acute rejection, improved graft function, and survival benefits.

Sirolimus (Rapamycin): The screening of fungi from Rapanui Island led to the

identification of another fungal product originally designated as “rapamycin” and found to possess immunosuppressive properties. Unlike the other two fungal derivatives (cyclosporine and tacrolimus), sirolimus has no effect on the calcineurin pathway. IL-2 and cytokine generation are preserved, but T cells are noted to be unresponsive to IL-2 (30). It possesses a macrolide structure, similar to tacrolimus, with a common binding site to FKBP12. The sirolimus-FKBP12 complex subsequently binds to a protein named “mammalian target of rapamycin” (mTOR), rather than to calcineurin. This large complex, sirolimus-FKBP12-mTOR, then binds and inhibits phosphorylation of p70S6 kinase, leading to blockade of cell cycle activity. This culminates in a rather indiscriminate effect, slowing B lymphocyte and fibroblast, as well as T-lymphocyte proliferation. One important property of sirolimus is its inhibitory effect on fibroblast proliferation. When coronary stents are coated with sirolimus, they have been shown to be less prone to neointimal proliferation (31). This has led investigators to study the potential role of sirolimus in the prevention of chronic allograft nephropathy (CAN). The relatively long half-life of sirolimus renders the benefit of once-daily oral administration in adults. The concomitant administration of sirolimus and cyclosporine has resulted in elevated serum creatinine levels (32). Investigators have addressed the use of sirolimus in the absence of CIs, as well as its combination with other agents in steroid-free regimens. Although sirolimus use has resulted in reduction of acute rejection, improved graft function is noted only when sirolimus is combined with cyclosporine (33,34). Side effects of sirolimus include anemia, thrombocytopenia, delayed wound healing, hyperlipidemia, and oral ulcers. In order to determine the optimal dose that produces effective therapy with minimal side effects, blood level monitoring is often useful.

IB. Biological immunosuppression

Polyclonal antibodies: Antibodies are another group of agents that have been developed to combat rejection. The polyclonal antibodies were mainly developed in horses or rabbits against human lymphocytes. Initial studies indicated that these antibodies were more efficacious than high dose steroids in reversing rejection. Rabbit antithymocyte globulin (ATG) is the most common polyclonal antibody used, having been proposed as an induction agent, as well as for treatment of rejection. Rabbits are immunized with human thymus tissue, and antibodies are collected from multiple rabbit donors. The use of induction therapy has the benefit of withholding cyclosporine in the immediate pre-operative period and thus avoiding nephrotoxicity. There is evidence of an advantage in using ATG intra-operatively rather than postoperatively (35,36).

Monoclonal antibodies: In contrast to the polyclonal antibodies, monoclonal antibodies are highly specific proteins. As an example, OKT3 is a monoclonal antibody that is specific for the T-cell receptor (TCR)/CD3 complex, preventing activation of T-lymphocytes (37). It was shown to be very effective in the reversal of acute rejections (including steroid-resistant cases), and was subsequently also used as induction therapy

(38). However, a major disadvantage is the often severe reaction resulting from massive cytokine release. These side effects consist of chills, fever, headache, pulmonary edema, aseptic meningitis and serum sickness. Comparisons of monoclonal OKT3 and polyclonal ATG revealed that the latter is better tolerated, while equally effective (39). Repeated courses of OKT3 are associated with a higher incidence of infection as well as development of post-transplant lymphoproliferative disorder.

Humanized monoclonal antibodies: Advances in genetic engineering led to the development of humanized versions of monoclonal antibodies in the 1990s. These chimeric molecules are expected to be much less immunogenic and with better intravascular survival time. Basiliximab and daclizumab are relatively new chimeric (human/murine) monoclonal antibodies that can provide effective induction with minimal side effects and have gained popularity as attractive options for the prevention of rejection. These agents have been utilized in combination with other immunosuppressants in the prevention of rejection. Patients on a combination consisting of these agents along with two or three other immunosuppressants have experienced significantly reduced acute rejection episodes compared with dual or triple immunotherapy. The side effect profile of humanized monoclonal antibodies is fairly benign, with no increase in the incidence of infection, malignancies, or post-transplant lymphoproliferative disorders. Hypersensitivity reactions have been rarely reported. Several other humanized monoclonal antibodies are being studied in clinical trials. One such antibody is Campath

SECTION II. Immunosuppression minimization protocols

IIA. Reduction and Elimination of Corticosteroids

Much of the success in organ transplantation is attributed to the use of corticosteroids in prevention of graft rejection, and these agents have been considered indispensable components of transplant immunosuppression. However, there has been a tremendous trade-off in terms of significant morbidity (40). The goal in this section of the review is not to elaborate upon the many very well known side effects of chronic steroid use, but rather to attempt a review of the evidence in favor of steroid-elimination. Various approaches have been implemented to eliminate steroids from the immunosuppressant regimen with mixed success. Some have completely avoided steroids, while others have eliminated steroids within the first two post-transplant weeks, and a third group has continued steroids only for up to three months.

In the early years of introduction of cyclosporine, there was much optimistic excitement regarding its possible use as a single agent and possibly avoiding steroids, completely. Since this required using very high (toxic) doses of cyclosporine, the results did not prove to be rewarding (41,42). In the following years, in order to use lower doses of cyclosporine in steroid-free regimens, azathioprine was added. Despite better outcomes, the concept of complete steroid avoidance was not very well-received (43,44). With

the advent of potent anti-T-cell antibodies, there has been renewed interest in developing strategies of complete steroid avoidance. Knechtle et al. (45) has illustrated that the use of alemtuzumab (Campath-1), an anti-CD52 monoclonal antibody, as induction therapy, along with maintenance sirolimus (as monotherapy) in 29 renal transplants resulted in fairly good short-term graft survival (3-29 months of follow-up), with no evidence of CAN. However, there was a relatively high incidence of humoral rejection (5 out of 29). Mahalati and colleagues (46) have also successfully attempted steroid withdrawal. In a pilot study, steroids were tapered at a median time of 415 days. Seventy-eight percent of the patients remained free of steroids at a mean follow-up of 3 years. The incidence of acute rejection was 4.5% and for chronic rejection it was 7.5%. In a large prospective trial, Squifflet et al. (47) studied outcomes in patients randomized to either MMF or steroid elimination at 3 months. All participants were initially on identical tacrolimus-based immunosuppression that included a steroid taper. The overall incidence of rejection at six months was low in both withdrawal groups (15.1% for the steroid elimination group and 14.8% for the MMF elimination group). This incidence was similar to that for patients who remained on a regimen of TAC + corticosteroids + MMF (17%). Thus, with regards to the incidence of acute rejection, steroid elimination was fairly successful. However, there was increased rate of recurrent rejection among patients in the steroid withdrawal group compared with the other groups. This implies that although successful weaning from steroids is quite feasible in the majority of patients, those who sustain an acute rejection episode are more likely to develop further sequels.

A less “radical” avoidance of steroids is to use steroids only in the immediate post-operative period for up to 2 weeks (early withdrawal). Stratta et al. first described this strategy in 1988 (48). They studied 52 living-donor renal transplant recipients for whom intentional early steroid withdrawal was employed. When compared to a control group on conventional steroid therapy, there was no difference in graft or patient survival, rejection, infection, or mean serum creatinine level. They concluded that early steroid withdrawal is a feasible option without exposing the graft or the patient to a survival disadvantage. More recently, Matas et al. (49) followed 51 low-risk kidney recipients on thymoglobulin induction, prednisone, MMF, and cyclosporine. Prednisone was rapidly tapered and discontinued on the sixth post-op day (rapid discontinuation of steroids: RDS group). There was no significant difference in 6- and 12-month patient or graft survival and rejection-free graft survival between recipients on the RDS protocol when compared with historical controls. They concluded that for low risk living donor kidney recipients, rapid discontinuation of steroids does not adversely influence graft or patient survival. In 1987, investigators at the University Hospitals of Cleveland and Case Western Reserve University developed an early steroid withdrawal protocol that included cadaveric donor, as well as live donor transplantations. Schulak published a summary of the results of these studies in March of 2004 (50). In this randomized trial, 67 patients were allocated to either continuous treatment with maintenance steroids or to discontinuation of prednisone within several weeks of transplantation. Induction

antilymphocyte globulin (ALG) and azathioprine therapy were used in all patients. They noted equivalent patient and graft survivals for the two treatment groups; however, early graft rejection rate was significantly higher in the early withdrawal group (80% vs. 50%). In addition, the number of severe rejection episodes was double in the early withdrawal group when compared to the standard treatment group. However, the overall numbers of rejection episodes were not significantly different between the two treatment groups (51 for the early withdrawal group and 35 for the conventional group). Due to the concern for late graft loss among patients with early rejection episodes, 60% of the patients in the early withdrawal group were returned to maintenance steroid therapy. This consisted of all patients with severe rejections requiring treatment with OKT3 and recipients who experienced repeated steroid-sensitive rejections. Considering the high incidence of early rejection, in 1989, these investigators changed the protocol such that steroids were eliminated six months after transplantation in patients with stable graft function. This phase of the study revealed that 79% of the late withdrawal patients experienced none or at most one rejection episode (steroid-sensitive), compared to the previously noted 40% rate for the early withdrawal. In univariate analysis, this strategy was found to be particularly more successful among white recipients and in those with donor-recipient racial match. Further analysis determined that renal function at initiation of steroid taper predicted success of withdrawal. Additional evaluations showed that a higher cyclosporine dose after steroid withdrawal (5.5 mg/kg/day vs. 4.5 mg/kg/day) was associated with better graft function, indicating that caution should be exercised with adequate dosing of the other immunosuppressants during steroid withdrawal.

IIB. Reduction and Elimination of Calcineurin Inhibitors

In the initial decades of experience with renal transplantation, the major emphasis was on short-term graft survival and the avoidance of acute rejection episodes. The introduction of cyclosporine in 1983 and MMF, tacrolimus, and sirolimus in the 1990's resulted in reduction of acute rejection and improvement in short-term graft survival. The emphasis of transplant protocols has now shifted towards improvement in long-term graft survival. Analysis of the United Network of Organ Sharing (UNOS) database published in 2000, revealed a significant improvement in long-term graft survival (51), attributable to a lower acute rejection rate. Patients who develop an acute rejection episode within the first post-transplant year have lower long-term graft survival rates, and those who develop acute rejection after the first post-transplant year have a ten-fold increase in graft failure (52). The frequency, severity, and type of rejection (vascular worse than cellular) are other important factors that influence long-term graft survival (53,54), and patients who respond poorly to treatment of acute rejection episodes have the worst long-term graft survival (55). Therefore, for any strategy that aims to enhance long-term graft survival, in addition to the goal of limiting drug nephrotoxicity, prevention of acute rejection is also imperative.

Since most transplant recipients establish a low baseline graft function, the choice of immunosuppressant regimen should be dictated at least partially by effect of the regimen

on renal function. Development of calcineurin inhibitors, currently, the major constituents of most regimens, has led to irrefutable improvement in graft survival. However, these agents have also led to significant renal toxicity. Their administration is often cited as a major factor in the development of graft fibrosis and CAN, the leading cause of long-term graft failure, with no currently available effective treatment (56). To minimize this potential toxicity, concurrent therapy with steroids and MMF has been used, allowing a lower overall dose of the CI. Introduction of newer agents such as sirolimus has led to interest in further decreasing and eliminating calcineurin inhibitors. It is anticipated that this will lead to a decrease in chronic nephropathy, hypertension, hyperlipidemia, and glucose intolerance, all thought to be potentiated with chronic CI use. The introduction of newer immunosuppressives, has led to development of protocols, which minimize or totally avoid calcineurin inhibitors. In devising these protocols, investigators are often faced with a dilemma: Calcineurin inhibitors are known to decrease the risk of acute rejection; acute rejection episodes are known to predispose to CAN, and at the same time, chronic CI use has also been associated with increased risk of development of CAN.

CI avoidance, antibody induction, MMF, and steroids: In a multicenter cohort study, Vincenti et al. (57) evaluated the safety and efficacy of daclizumab, MMF, and prednisone in 98 low risk kidney recipients on a CI sparing protocol. At one year, the patient survival was 97%, and the graft survival was 96%; the incidence of acute rejection was 53%. Due to a high incidence of acute rejection, CIs were initiated in 62% of the patients. At one year, patients who were rejection free and never received any CI had the lowest serum creatinine level. Protocol renal biopsy was performed at the end of the first year in 18 patients. Only 3 patients demonstrated evidence of CAN. These patients also had a significantly lower level of fibrogenic gene expression (transforming growth factor-β, fibronectin, and collagen), when compared with a cohort of historical controls treated with CIs. Using a similar protocol, except for a higher dose of prednisone, Tran et al. (58) assessed CI avoidance in 45 kidney recipients. With a median follow-up of 8 months, they found a patient survival rate of 100%, allograft survival of 95%, and incidence of acute rejection was 38%. Similar to the study by Vincenti, patients who were rejection free and CI free had the lowest serum creatinine, compared to patients with an episode of acute rejection, requiring initiation of cyclosporine. Grinyo et al. (59) conducted a cohort study on thirty patients, including those with suboptimal and non-heart-beating donors. They used a protocol similar to Tran et al., except for replacing rabbit ATG for daclizumab. Patient and graft survival rates were 94% and 83% at 1 year and 79% and 65% at 5 years, respectively. The cumulative incidence of acute rejection was 24% at 5 years. While 65% of the patients remained CI free at one year, at five years only 36% were CI free.

In summary, these studies reveal a high incidence of acute rejection; however, approximately half of the recipients were CI free without acute rejection and with excellent one-year graft function. In addition, it has been demonstrated that a CI-free

regimen is associated with low expression of fibrogenic genes, which are surrogate markers for CAN. The study by Grinyo and colleagues was an attempt to address the question of utility of CI avoidance in high risk populations, namely recipients of kidneys from marginal donors, with discouraging results. Although none of these three studies were randomized, however they are considered landmark studies that have paved the road for future randomized controlled trials. They have demonstrated that a protocol containing either daclizumab or rabbit ATG with MMF and steroids is associated with a high incidence of acute rejection (24% to 53%) and that this regimen should be avoided even in low risk patients. Therefore, it is necessary to add another non-nephrotoxic immunosuppressant.

CI avoidance, sirolimus, antibody induction, azathioprine/MMF, and steroids: Sirolimus is an immunosuppressive agent without nephrotoxic potential. This property has made it an attractive agent for inclusion in protocols that aim at eliminating CIs. There is, however, sufficient evidence that concomitant use of sirolimus with CIs can potentiate the latter's nephrotoxicity, while in animal models a synergistic effect in the prevention of CAN has been demonstrated when MMF is combined with sirolimus (60-66). The latter is thought to be due to the antiproliferative effect of the combination on smooth muscles and subsequent reduction in arterial intimal thickening. This concept has been evaluated in 3 randomized clinical studies. In a comparison between the sirolimus-azathioprine-prednisone combination and cyclosporine-azathioprine-prednisone combination, Groth et al. (67) studied 83 low risk cadaveric kidney transplant recipients in a multicenter study. Patient and graft survival, as well as incidence of rejection at 6 months, were similar in the two groups. The calculated GFR at one year, however, was significantly better in the sirolimus group compared with the cyclosporine group (70 mL/min vs. 59 mL/min). In a similar study design, replacing MMF for azathioprine, Kreis et al. (68) compared the sirolimus-MMF-prednisone combination (40 patients) with the cyclosporine-MMF-prednisone combination (38 patients) in a multicenter study. The two treatment groups were similar with regards to patient and graft survival, as well as acute rejection rates. In this study, the incidence of graft rejection was 28%, while in the study by Groth et al., where azathioprine was used, the rejection rate was 41%. Both Groth et al., and Kreis et al. reported a higher incidence of hyperlipidemia in the sirolimus group compared to the cyclosporine group. The sirolimus-MMF combination was also associated with a higher incidence of thrombocytopenia and diarrhea when compared with the cyclosporine-MMF combination. This indicates the need for close monitoring of mycophenolate level when administered in combination with sirolimus. In a recent pooled analysis of the above studies (69), a consistently lower mean serum creatinine level was noted in the sirolimus-treated patients compared to cyclosporine-treated patients; this difference occurred as early as the first two post-transplant months. The incidence of hypertension was also lower in the sirolimus group. Flechner et al. (70) conducted a single center randomized study to compare the sirolimus-MMF combination (31 patients) with the cyclosporine-MMF combination in a low risk

population consisting of 30 patients. The doses of sirolimus and cyclosporine were lower than those used in the studies by Groth et al. and Kreis et al. An additional innovation in this study was induction therapy with basiliximab. There were no differences in the patient and graft survival rates between the 2 groups. At 1 year, the sirolimus-MMF group had higher calculated GFR than patients in the cyclosporine-MMF group (81 mL/min vs. 61 mL/min). No statistical difference was noted between the two groups regarding cholesterol and triglyceride levels. In contrast to the two previous studies (67,68), the incidence of thrombocytopenia was similar between the 2 groups. This may be attributed to the lower sirolimus target level in this study. Pooling the data from these 3 randomized studies, the incidence of acute rejection is lowest (6%) in patients receiving the sirolimus-MMF combination with basiliximab induction, while patients receiving the sirolimus-azathioprine combination without induction demonstrated the highest (41%) rate of acute rejection. The sirolimus-MMF protocol was associated with higher incidences of hyperlipidemia and bone marrow toxicity than the cyclosporine-based protocol.

Previous reports have indicated that allograft survival is predicted by the serum creatinine at one year. Extrapolating from this information, it would be reasonable to conclude that the sirolimus-MMF combination will extend graft half-life from 9 to 14 years (71,72). However, before incorporating the sirolimus-MMF combination into routine clinical practice, it must be noted that all three of the studies that have demonstrated a benefit from sirolimus-based protocols have involved low risk recipients. Therefore, future studies should address the immunologically high risk population, including recipients with marginal donors. Also since the tacrolimus-MMF combination is the most common protocol in most transplant centers, another question that should be addressed is whether a sirolimus based protocol is superior to a tacrolimus-based protocol.

CI avoidance and induction of tolerance: Sirolimus, while inhibiting interleukin-2 receptor signaling, does not prevent T-cell signal transduction, and thus is not expected to interfere with the tolerance process. Three small case series have investigated the effect of the combination of sirolimus either with alemtuzumab or with rabbit ATG on achieving tolerance without CI or steroids (45,73,74). As also noted above, in a study by Knechtle et al. (45), sirolimus was combined with alemtuzumab induction in 29 kidney transplant recipients. Thirty minutes before each dose of alemtuzumab, 500 mg of methylprednisolone was given. Six of the first 24 patients had early acute humoral rejection. Subsequently, for the remaining 5 patients, rabbit ATG was administered on the first post-op day. Two of these patients also experienced acute rejection. Protocol biopsies were performed at six months for 20 patients and at one year for 13 patients. None showed evidence of CAN. The overall patient survival was 100%, allograft survival was 97%, and the incidence of acute rejection was 28%. At 1 year, the patients with prior rejection episodes had higher serum creatinine than patients without rejection (2.0 mg/dL vs. 1.6 mg/dL). The majority of patients (85%) required lipid-lowering

therapy. Swanson et al. (73) studied the combination of sirolimus and high-dose rabbit ATG in 12 kidney transplant recipients. They reported a one-year patient and graft survival of 100%, and an acute rejection incidence of 42%. All rejection episodes were steroid responsive and were associated with subtherapeutic sirolimus levels. Eighty-three percent of the patients required lipid-lowering therapy. Kirk et al. (74) studied the effect of combining sirolimus and alemtuzumab in 7 kidney recipients. All patients had an early acute rejection episode, responsive to steroids or muromonab-CD3. None of the patients experienced any late acute rejection episodes. This study demonstrates that maintenance immunosuppression is most likely required to prevent early acute rejection. In the lymphocyte depletion protocols outlined above, patient and graft survival was similar to conventional immunosuppression. Although there was a high incidence of acute rejection, the absence of evidence of CAN was encouraging. To further elucidate the implications of these protocols, randomized clinical trials are needed.

CI withdrawal using sirolimus as maintenance immunosuppressant:

Two multicenter, randomized controlled trials studied the outcomes of withdrawing CI after the early posttransplant period and before development of CI-associated nephrotoxicity or evidence of CAN. In the first study (75), 525 kidney transplant recipients received sirolimus, cyclosporine, and prednisone. At 3 months, 430 patients were randomized to either continue cyclosporine (n = 215) or withdraw from cyclosporine (n = 215). The patient and graft survival rates at one year were comparable between the 2 groups. The incidence of acute rejection after randomization in the cyclosporine-free group (9.8%) was higher than in the cyclosporine group (4.2%). Most of the acute rejection episodes in the cyclosporine-free group were attributed to subtherapeutic sirolimus trough levels. At 2 years, there were no further episodes of acute rejection in the cyclosporine-free group, and the improvement in kidney function was maintained at 2 years following cyclosporine withdrawal. There was a gradual loss of renal function in the patients on cyclosporine-sirolimus group, while in patients on the cyclosporine-free protocol had stable graft function. In a similar multicenter randomized study (76), 246 kidney transplant recipients were enrolled. Of these, 197 patients fulfilled the inclusion criteria. Ninety seven were randomly assigned to the control group (cyclosporine plus fixed dose sirolimus), and 100 were randomly assigned to the treatment group (reduced target level of cyclosporine and sirolimus with target trough levels 10-20 ng/mL). At 3 months, patients in the treatment group were considered for complete cyclosporine withdrawal. Of the patients in the treatment group, 82 were eligible for cyclosporine withdrawal; in 76 of these patients, cyclosporine was successfully withdrawn. The patient and graft survival, as well as the incidence of acute rejection were similar in the two groups. The 1-year serum creatinine was significantly lower in the cyclosporine-withdrawal group (1.54 mg/dL vs. 1.93 mg/dL), and the calculated GFR was also significantly higher in this group (65.3 mL/min vs. 56.4 mL/min). Both of the studies illustrate that patients had more favorable outcome following cyclosporine

withdrawal. Graft function improvements were evident starting at one month after cyclosporine withdrawal and continuation of cyclosporine was associated with progressive loss of renal function. In addition, as another likely contributor to improved long-term outcome, there was an improvement in blood pressure control following cyclosporine withdrawal. The incidence of acute rejection was low in both studies because of excluding patients at risk for acute rejection such as those with delayed graft function, previous episodes of acute rejection, or evidence of renal dysfunction at time of withdrawal. The use of sirolimus was associated with severe hyperlipidemia and bone marrow suppression. The major limitation of both studies is patient selection bias, implying that the findings in these studies cannot be extrapolated to most patients (75,76). Because of the pharmacokinetic interactions of cyclosporine and sirolimus, caution should be exercised when these two agents are used in combination. While sirolimus does not have nephrotoxic properties when used alone, there is an increased nephrotoxicity of cyclosporine when used in combination with sirolimus. Several mechanisms have been proposed to explain this phenomenon. A doubling of the bioavailability of sirolimus has been demonstrated when used concomitantly with cyclosporine, compared with when the 2 drugs are administered 4 hours apart (77). In addition, the intrarenal accumulation of cyclosporine is more significant when administered with sirolimus (78,79).

CI minimization using MMF as maintenance immunosuppressant:

In a single-center study, Weir et al. (80) compared 3 different regimens in patients with failing graft function secondary to CAN. MMF was either added or continued while the CI was either reduced or withdrawn. Graft survival was noted to be superior in patients withdrawn from CIs compared with patients who remained on low-dose tacrolimus or cyclosporine. A prospective, randomized, multicenter trial confirmed these results (81). In the 143 patients studied, there was a significant improvement in graft function in patients maintained only on MMF and prednisone compared with patients on cyclosporine. It seems reasonable to conclude that in patients with worsening renal function secondary to CAN, withdrawal of CI with the addition of MMF maintenance therapy appears to be a safe approach.

CONCLUSIONS: HORIZONS FOR FUTURE RESEARCH

While perhaps the ultimate goal in transplantation immunology is achievement of specific graft tolerance, investigators are in search of the “ideal immunosuppressant”; this should target predominantly the immune system with minimal consequences for other tissues and minimal metabolic, cardiovascular and renal complications. The goal of future research in immunosuppressive regimens is the development of protocols that will reduce acute rejection rates and CAN, improving patient and graft survival, while at the same time ensuring minimum toxicity and maximal tolerability. It seems clear

that none of the currently available agents is either individually, or even as part of a combination regimen, capable of fulfilling these criteria. Nevertheless, careful combination of chemical and biological agents, with judicious dosing and timing is expected to approach the optimal strategy. Protocols that utilize induction of tolerance remain an area in need of well-designed clinical trials. Caution is to be exercised when combining chemical immunosuppression with induction of tolerance, since animal models have revealed that chemical agents can lead to prevention of tolerance. Another area in need of research, particularly in tolerance studies, is the high risk, sensitized recipients. Finally, further elucidation of the effect of gene polymorphism on predisposition to immune injury will be valuable in determining optimal immunosuppression based on genotype.

With regards to minimizing immunosuppression, several issues are clear. In light of the widespread inhibitory effect on all immune responses and the multitude of significant side effects of steroids, their continuous use as maintenance therapy should not necessarily be considered an indispensable component of the immunosuppressant armamentarium. While in moderate-to-high risk patients, rapid steroid tapering may lead to rejection, among low risk patients, early withdrawal of steroids has not been demonstrated to adversely affect graft or patient survival. In order to initiate steroid withdrawal, the goals should be identification of low risk patients to optimally adjust their other immunosuppressants, and to successfully withdraw steroids. In order to minimize the effect of CI on development of CAN, protocols that avoid or attempt to withdraw calcineurin inhibitors are attractive, and patients on CI-free protocols have clearly demonstrated improved graft function. However, these protocols are also associated with a high incidence of acute rejection. To surmount this disadvantage, regimens consisting of basiliximab, sirolimus, MMF, and steroids have been employed. These regimens, however, have been associated with hyperlipidemia and bone marrow toxicity. Hyperlipidemia is of major concern because it is a significant risk factor for coronary artery disease. Therefore, future studies should aim to identify regimens that lead not only to preservation of graft function, but that are also cardioprotective. Future trials must also attempt to include a more diverse population of recipients, and should particularly include the immunologically high risk groups. Subsets of patients who would maximally benefit from avoidance or withdrawal of calcineurin inhibitors should be identified and targeted. Finally, these protocols are still in the development phase; caution and prudence should be exercised, while awaiting results from long-term survival analysis, and before incorporation preliminary results into daily clinical practice.

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