Immunosuppression in heart transplantation: shooting ourselves in the foot!

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Kardiochirurgia i Torakochirurgia Polska 2006; 3 (3): 258–261

Background

Kidney transplantation began during a time when immunosuppression was not well understood, and achieved a modicum of success utilizing steroids and 6-mercaptopurine, later replaced by azathioprine. When heart transplantation began in 1967 [1], this two-drug combination was also employed [2, 3], with dismal results. Indeed by the early 1970s, many centers had abandoned heart transplantation, and were it not for the advent of cyclosporine A (CYA), heart transplantation would be a historical footnote in the literature about failed therapies for end-stage heart disease [4]. Instead, CYA was combined with steroids and azathioprine and triple therapy was born. The fact that 2 drugs did not produce acceptable protection from rejection has led to steadfast adherence to triple drug therapy for many years, and the logic of this approach has stifled efforts to examine less intense regimens. The purpose of this paper is to examine the changes that have occurred in heart transplant immunosuppression over the last 20 years, and consider the provocative question of whether immunosuppression prevents the development of partial tolerance.

Immunosuppressive regimens for heart transplantation

A triple combination of CYA, an anti-proliferative drug such as azathioprine, and corticosteroids has been the typical post-transplant regimen for more than 25 years. Over time, a variety of medications have been substituted for azathioprine and even CYA is less commonly used, with tacrolimus being substituted [5-7].

Newer elements of the immunosuppressive armamentarium include mycophenolate mofetil, which was shown to be superior to azathioprine in a randomized, controlled trial [8]. Other alternative cell cycle inhibitors include rapamycin, and everolimus, which are also substituted for azathioprine or mycophenolate in some patients. Tacrolimus has been increasingly used in heart transplants instead of cyclosporine [9], and has proven to be efficacious even in cases of refractory rejection [10-13].

Steroids are commonly maintained at some minimum dosage long-term, despite literature suggesting that withdrawal is safe and may carry significant benefits [14-17]. Long-term corticosteroid therapy is associated with numerous morbidities including hyperlipidemia, hypertension, obesity, cataract formation, osteoporosis, diabetes mellitus, and the development of Cushingoid facial features, just to name a few. Given these problems, it is remarkable that so little emphasis is placed on discontinuation of steroids. Certainly, in liver, and kidney transplantation, the use of steroids is minimized, and the use of single-agent immunosuppression (predominantly tacrolimus) is increasingly common [18-19]. Of course, liver and kidney transplant patients can be monitored for allograft dysfunction with simple, commonly followed blood tests, whereas the diagnosis of cardiac rejection requires invasive endomyocardial biopsy. Lastly, there is the sense (without evidence) that heart transplants are ”different”, and the literature of kidney and liver transplantation does not apply.

Steroid use is so entrenched that most clinical research trials mandate the use of a minimum dose, thus maintaining triple therapy. Recent multi-center trials report rates of ISHLT ≥3A rejection of 25 to 50% at 6 months, which is not insignificant. However, recent retrospective reports of tacrolimus monotherapy with rapid steroid discontinuation report similar rates, suggesting that steroids are not essential [20, 21]. Another concern is the development of adrenal insufficiency, which mandates permanent steroid therapy when it occurs. Nevertheless, there is very little written to guide the clinician how to wean steroids late after transplantation.

Clinicians may regard toxicities of immunosuppression as simply the ”price” of renewed life with a heart transplant. However, given that patients are living longer, with 50% survival exceeding 10 years, it is increasingly important to reduce long-term morbidities.

”Acceptance” of the allograft

It is well known that the incidence of allograft rejection is highest early after transplantation, but decreases steadily over the first year. Most clinicians routinely target

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lower calcineurin inhibitor trough levels over time due to this well known phenomenon. Indeed, some transplant groups do not conduct surveillance cardiac biopsies after one year due to the vanishingly small risk of rejection (in the absence of symptoms). This finding has been attributed to a variety of mechanisms including the process of "clonal deletion" where T-cells which recognize the allograft progressively die (perhaps since calcineurin-inhibitor based therapy partially prevents T-cell activation).

Another mechanism which is under intense scrutiny is the role of regulatory T-cells (tregs), which express CD-4 and CD-25 cell surface markers [22-27]. These cells are responsible for down-regulating immune responses, and therefore interest in exploiting this property is high. Evidence suggests that treg activation may be the mechanism which explains the protective effect of donor-specific blood transfusion (DSBT) at the time of transplantation in both human and animal models [28-30]. In addition, it is likely that the reduced frequency of allograft rejection with time is due to the activity of donor-specific tregs.

One of the issues surrounding this area of research is the difficulty in deriving a marker for the presence of tregs. Flow cytometry is simple and can identify CD-4+, CD-25+ T-cells but a minority of these cells are tregs. Other markers such as the transcription factor FOXP3 may identify tregs, but add additional complexity to diagnostic assays.

Effect of drugs on treg cells

Various studies have investigated the effects of commonly used immunosuppressants on treg production. Cyclosporine has been shown to have an inhibitory effect on treg production, particularly at high levels [31, 32]. Interestingly, the timing and dose of cyclosporine was shown to be critical in one paper [32]. Kawai and colleagues studied an established model of rat cardiac transplantation, with DSBT [32]. DSBT when given prior to transplant (more than one week) has been shown to lead to treg production and tolerance to the cardiac allograft (survival without immunosuppression). Interestingly, DSBT must be given days prior to transplantation or the tolerance effect is not observed.

Various combinations of DSBT and CYA were investigated. High dose CYA (50 mg/kg) along with pre-operative (>1 week) DSBT led to rejection, whereas low dose (10 mg/kg) CYA (without DSBT) was actually associated with a state of tolerance similar to that produced by pre-operative DSBT. However, the ineffective post-operative DSBT in combination with delayed low dose cyclosporine did result in tolerance. The important aspect of this rat model research is that the dose and timing of delivery of immunosuppression may be critically important in the stimulation of tregs.

Other investigators have examined the use of rapamycin and mycophenolate mofetil. Neither of these agents appears to be associated with reduction in treg production [31]. This may be due to the fact that these agents do not act via calcineurin inhibition.

Corticosteroids have been investigated as well in this regard, with experiments in a mouse tissue culture model demonstrating up-regulation of tregs (in this case with dexamethasone treatment) [33]. However, human studies demonstrate reduction in t-cell number [34] and specifically treg levels in association with corticosteroid use [35, 36].

The concept of operational or "prope" tolerance

Tolerance is defined as the state of engraftment which does not require any immunosuppression. To date, while this has been achieved in some animal models, it has not been realized in patients. Calne first proposed that an "almost" or "prope" tolerance could be achieved after observing that some renal transplant patients stop their immunosuppression and yet have long-term stable engraftment. Calne proposed in 1996 that achievement of this quasi-tolerant state could be facilitated by providing a "window of opportunity for immunologic engagement" (WOFIE) where the host is exposed to the foreign allograft without immunosuppression present, followed by commencement of anti-rejection therapy [37, 38]. Later, he reported on the application of this approach in renal transplant recipients, with promising results [38].

More recently, Calne and colleagues have studied the use of antibody preparations such as alemtuzumab which massively deplete the lymphocyte population when administered post-transplant to kidney recipients [38, 39]. The use of antibody induction allows the calcineurin therapy to be delayed for several days post-transplant. The principal reason clinicians favor delayed initiation of calcineurin inhibitors is their characteristic nephrotoxic properties. In addition, antibody use is associated with lower risks of graft rejection. The possibility that rejection may also be reduced by providing a "window of opportunity for immunologic engagement" is quite intriguing.

Dresske and colleagues have followed up the important work of Calne with a separate trial comparing delayed calcineurin inhibition in one group (WOFIE) with immediate calcineurin therapy in 40 renal transplant patients. They found that the WOFIE group had less rejection, and these patients were more likely to be withdrawn from steroids than the group with no delay in calcineurin blocker usage. Interestingly, the concentration of tregs was also higher in the WOFIE group, as shown by CD4+, CD25+ cell counts, as well as FOXP3 messenger RNA expression [40].

Recently, Pirenne and colleagues reported long-term follow-up on 4 intestinal transplant recipients treated with peri-operative DSBT, along with basiliximab induction, and minimal corticosteroid therapy (specifically avoiding steroid boluses), along with azathioprine and tacrolimus. Intestinal transplantation typically carries the highest risk of allograft rejection of all solid organs, and yet tacrolimus levels were maintained at levels of less than 5 ng/ml by 6 months post transplant, along with azathioprine 0.5 mg/kg and 4 mg oral methylprednisolone maintenance therapy. In more than 250 intestinal biopsies, no evidence of graft rejection

or graft-versus-host disease was seen. While this is a very small number of patients, this success in a high-risk organ transplant setting is quite encouraging.

Trials in human heart transplantation

For reasons described above, the impetus to reduce immunosuppression in heart transplant recipients has been limited. Baran et. al. first reported the use of tacrolimus without the use of azathioprine or mycophenolate mofetil, coupled with relatively rapid steroid weaning in 2001 [20]. The risk of rejection was comparable to other published studies, and the mortality very low in this first report. Lubitz et al. reported the long-term follow-up of this approach, with similar findings [21]. Superior survival was demonstrated for tacrolimus monotherapy patients as compared to other patients treated with more intense immunosuppression over the study period, along with an equivalent incidence of transplant-associated vasculopathy.

Opelz and colleagues recently reported a large cohort study of 1110 kidney transplant recipients and 450 heart transplant patients [41]. They showed significantly lower mortality for patients weaned from steroids, in both the kidney and heart groups. The mortality curves continued to separate after one year, suggesting that the hazards of corticosteroids extend beyond the initial few months post-transplant.

Lubitz recently reported a retrospective single center analysis of pharmacologic correlates of survival in 220 transplant recipients. Statin use, along with angiotensin receptor blocker, as well as steroid withdrawal were all shown to be independent predictors of enhanced survival.

Lastly, a prospective randomized study (Tacrolimus in Combination, Tacrolimus Alone Compared, the TICTAC study) is currently underway, comparing tacrolimus monotherapy with tacrolimus and mycophenolate mofetil. Patients in both groups are weaned from corticosteroid therapy in 2-3 months, which is the most rapid steroid weaning protocol that has been tested in heart transplant recipients to date. Preliminary results are expected soon.

Putting it all together

The continual expansion of knowledge in transplantation and immunology promises to deliver improved outcomes to patients worldwide. However, the inherent limitation of heart transplantation is that the number of procedures per year is small, and as long as allograft rejection carries the real possibility of death, research into reduced immunosuppression will continue to be restrained. Therefore, it is critical to examine the literature broadly, including basic science models, as well as other solid-organ models such as kidney and liver. In the end, the problems faced by the liver transplant recipient are likely to be similar to the heart or kidney patient, and we must learn what we can from our colleagues and patients, for the betterment of all.

The data on WOFIE and the papers on tregs suggest that minimizing immunosuppression might offer benefits beyond minimization of morbidities. Indeed, it is quite possible that logic is leading us astray. It is possible that the more we immunosuppress our patients, the more we prevent mechanisms of prope tolerance from being operative.

Whether we can successfully walk the tightrope and find a way to suppress the immune response to avoid rejection but allow partial tolerance remains to be seen. There is no doubt that innovative thinking will be required to move beyond our current paradigm of non-selective over-immunosuppression for the majority of patients. Instead of firing targeted bullets, I believe we are shooting ourselves in the foot while attempting to arrest rejection.

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