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REVIEW

Minimization of immunosuppression after lung transplantation: current trends

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Summary

This paper reviews efforts that have been made to minimize immunosuppression in lung transplant patients. A brief history of tolerance and its potential application to lung transplantation is also discussed.

In 1963, the first single lung transplant was performed by Dr James D. Hardy at the University of Mississippi. Although the patient died within weeks, this was a tremendous success after decades of animal studies promising the feasibility of lung transplant in humans. Since this seminal surgery, refinements in surgical technique and the evolution of sophisticated immunosuppressive regimens have allowed for extended survival times. According to the 2007 Registry of the International Society of Heart and Lung Transplantation (ISHLT), patient survival rates for adult transplant recipients from 1994 through June 2005 at 1, 3 and 5 years were 78%, 62%, and 50% respectively [1]. As suggested by these statistics, lung transplant has been hindered by late graft failure because of chronic rejection. Bronchiolitis obliterans syndrome (BOS), a marker for chronic rejection, is the most significant long-term complication and is the most common cause of late death after lung transplant [2]. According to the 2007 Registry report of the ISHLT, the prevalence of BOS is 50% at 5.6 years. While immunosuppressive medications have reduced the incidence of acute rejection within the first year post-transplantation, they have not significantly affected long-term outcomes [3].

In the first year post-transplant, infection is the most common cause of death in the lung transplant patient [4] as the lung is directly exposed to a panoply of potential infections, and this infection risk is exacerbated by immunosuppressive medications. Also, within the first year of transplantation and somewhat later, post-transplant lymphoproliferative disease (PTLD) is a well-recognized complication of lung transplantation [5]. The standard approach to management of PTLD involves withdrawal or reduction of immunosuppressive medicines [6]. Finally, the side-effects of long-term immunosuppressive medications are sobering: diabetes, hypertension, weight gain, bone complications and elevated plasma cholesterol levels. As such, lung transplant physicians have begun to explore the boundaries of immunosuppression minimization, and initial animal studies involving induction of donor-specific immune tolerance offer hope of obviating the need for immunosuppressive medications altogether. The aim of this article was to highlight efforts that have been made to minimize immunosuppressive medicines in lung transplant patients and to review upcoming strategies, which may one day render immunosuppression therapy obsolete.

Borro *et al.* [7] selected 34 stable lung transplant patients on the basis of clinical, biochemical and histologic stability to be systematically tapered off steroids. The majority of the patients were maintained on cyclo-

sporine and azathioprine. In five patients, azathioprine was switched to mycophenolic acid because of nephrotoxicity. Eight patients had received tacrolimus as anti-calcineurin therapy. The authors did not mention whether these patients received induction therapy. The mean time for steroid withdrawal was 881 ± 237 days post-transplant. Although steroids had to be reinstituted in six patients, primarily for graft dysfunction, the remaining patients enjoyed lower blood pressures and reduction in plasma triglycerides. In two diabetic patients, insulin was withdrawn. While muscle strength was not a measured end point, the authors speculated that a potential benefit of steroid withdrawal would be an improvement of proximal muscle weakness and atrophy induced by chronic steroid use. The authors suggest that young patients and patients with major steroid side-effects are principal patients to be selected for steroid withdrawal protocols.

Shitrit *et al.* [8] attempted withdrawal of steroids on 35 patients who underwent heart–lung, double-lung or single-lung transplant who were taking a regimen of prednisone, azathioprine and cyclosporine, or a regimen of prednisone, mycophenolate mofetil and tacrolimus. The authors did not mention whether these patients received induction therapy. The patients were selected on the basis of 6 months of stability as indicated by pulmonary function tests and histologic criteria. The duration of taper was customized for each patient but all doses were sequentially reduced at 5-mg intervals. Eight patients were successfully tapered off steroids, most of them had emphysema, and one each had fibrosis and alpha-1 antitrypsin deficiency. Interestingly, the mean time from transplant to steroid withdrawal was significantly longer in patients who were successfully withdrawn from steroids compared with those patients who did not tolerate withdrawal. These investigators found significant differences in total cholesterol and weight in the two groups, but surprisingly, no differences in spirometry, glucose, or systemic blood pressure. That three out of the eight steroid withdrawal patients were on the tacrolimus regimen may explain the lack of significant difference for glucose levels or systemic blood pressure. No rejection was noted in patients who underwent successful steroid withdrawal.

The aforementioned studies have shown that withdrawal of steroids may be possible in select lung transplant patients, but specific assays that could allow for the selection of such patients are lacking [9]. Such assays are forthcoming, however. Bhorade *et al.* have used the Cylex Immune Cell Function Assay (ImmuKnow) to measure ATP levels as a marker for global immune response in lung transplant patients (S. M. Bhorade, personal communication). These levels may signal a state of over-immunosuppression and therefore an opportunity for reduction of immunosuppressive therapy. Immunofunction monitoring is an exciting shift from the current paradigm of measuring drug levels to monitor immunosuppression. Until such assays are available for widespread use, however, transplant physicians are left with indirect measures of immunosuppression, which likely miss the mark in determining which patients are appropriate for reduction of therapy. On the one hand, there may be circumstances brought on by side-effects and toxicities that force reduction of immunosuppression. The voluntary reduction of immunosuppression, on the other hand, should probably require a qualitative test of the patient's immune status prior to such reduction.

Chronic use of azathioprine, the antimetabolite derived from 6-mercaptopurine, is metabolized in part by the enzyme thiopurine S-methyltransferase (TPMT) [10]. Patients who are homozygous for low levels of TPMT activity or have no TPMT activity who received standard doses of azathioprine had greatly elevated concentrations of active metabolites, 6-thioguanine nucleotides, as well as a greatly increased risk of life-threatening, drug-induced myelosuppression [10]. This potential complication necessitates withdrawal or minimization of azathioprine in some patients. Kidney transplant patients have safely tolerated discontinuation of azathioprine and in fact, in kidney, heart, and liver transplant, it is common practice to switch to a double or single immunosuppression regimen after the initial postoperative period [11,12]. To investigate the feasibility of azathioprine withdrawal in lung transplant patients, Hoffmeyer *et al.* [13] abruptly withdrew Azathioprine from seven lung transplant recipients who were stable at least 4 years post-transplantation on a regimen of azathioprine, cyclosporine, and prednisone. Early induction therapy was not discussed. These seven patients were compared to a well matched control group on the basis of forced expiratory volume in 1 s (FEV_1). Despite similar FEV_1 at study entry, a significant difference emerged after 12 months. While only one of 17 patients in the control group ultimately evidenced progressive decline in FEV_1 suggesting bronchiolitis obliterans, four out of seven patients in the azathioprine withdrawal group showed a decline in FEV_1 of more than 10% at a mean of 262 days after azathioprine withdrawal. Unlike the patient in the control group who developed BOS in response to infection, decline of FEV_1 was not shown to be secondary to infection in these four patients, all of whom went on to develop progressive BOS despite reinstitution of azathioprine and augmentation of corticosteroid dose. Interestingly, the patients studied were low risk for the development of BOS; no patient in the study group had experienced more than two rejection episodes and more than one episode of cytomegalovirus infection at the time of entry into the study.

Withdrawal of azathioprine may have consequences beyond the sphere of rejection. Khan *et al.* [14] report a case of tumor lysis syndrome in a patient in whom azathioprine was abruptly discontinued after he developed post-transplantation lymphoproliferative disease 6½ years after receiving a single lung transplant for sarcoidosis and pulmonary fibrosis. The authors speculate the withdrawal of azathioprine to be the culprit, given the temporal course of the tumor lysis syndrome as well as the lack of specific treatment directed toward the patient's lymphoproliferative disease.

Compared with other solid organ transplants, such as of liver and kidney, minimization of immunosuppression in the lung transplant recipient is especially problematic. The incidence of acute and chronic rejection is increased after lung transplantation as compared with other solid organ transplants [2]. Lack of human leukocyte matching of donor and recipient, the large amount of donor antigen-presenting cells, which constantly process and present human leukocyte antigen alloantigens to recipient lymphocytes, and the lung's exposure to the external environment and therefore a variety of airborne infectious agents are likely explanations for differing rates of rejection in lung transplantation versus other solid organ transplants [15,16]. An additional caveat unique to lung transplant is that infection is difficult to discern from rejection [17]. Furthermore, it has been shown that the lung is more vigorously rejected than other allografts [18]. Unlike dialysis in case of kidney transplant graft dysfunction, there is no good alternative to allograft failure in lung transplantation; chronic mechanical ventilation and retransplantation are complicated and often unrealistic and rather prove to be only temporizing measures. Finally, the progressive improvement in graft survival makes it ethically difficult to minimize an immunosuppressant regimen without reliable systematic trials that support a successful alternative. Indeed, transplant physicians are often forced to reduce the dose of an immunosuppressive agent or exchange one immunosuppressive medicine for another because of toxicity or complications, but this does not translate to a systematic approach to minimization of immunosuppression. With regard to elimination of immunosuppression, there were no case reports found that report lung transplant patients who have tolerated complete withdrawal of immunosuppressive medicines.

The calcineurin inhibitors, cyclosporine A and tacrolimus bind to immunophilin proteins within lymphocytes. This binding ultimately inhibits calcineurin, thereby inhibiting inflammatory interleukins, tumor necrosis factor, granulocyte-macrophage colony stimulating factor, and the nuclear factor of activated T cells. [19,20]. The calcineurin inhibitors have become the cornerstone in long-term immunosuppression [1], but their success in immunosuppression has been marred by their problematic effects on the kidneys, both acute renal toxicity [21] as well as chronic and often irreversible nephrotoxicity [22]. Largely because of the nephrotoxicity, there has been extensive investigation into minimization of calcineurin inhibitors to resolve this predicament. The CEASAR study, for example, addressed cyclosporine minimization in combination with MMF in renal transplantation [23]. This multicenter randomized trial in 536 renal transplant recipients of low-dose cyclosporine followed by withdrawal versus continuous low-dose cyclosporine versus standard-dose cyclosporine in combination with daclizumab, MMF and steroids showed that the low cyclosporine regimen provided adequate immunosuppression but that the withdrawal regimen was associated with a an increased rate of acute rejection episodes compared to the low dose and standard dose regimens. While this study indicates that minimization of the calcineurin inhibitors is realistic, it should be emphasized that such studies pertain only to primarily kidney-, liver- and to a lesser extent, heart transplantation [24].

Everolimus and sirolimus and the rapamycin inhibitors, inhibit growth factor-stimulated proliferation of lymphocytes [25]. Snell *et al.* [26] performed a randomized, double-blind clinical trial of 213 BOS-free patients to receive either everolimus or azathioprine, in combination with cyclosporine and corticosteroids. The composite endpoint of efficacy failure, as defined by decline in FEV_1 >15%, graft loss, death or loss to follow up, was significantly lower in the everolimus group as compared with azathioprine group. At 36 months, the incidence of acute rejection remained significantly less in the everolimus group, while overall rates of efficacy failure was similar in both the groups.

Because of pharmacokinetic interactions, sirolimus and everolimus can worsen cyclosporine nephrotoxicity [27]. Animal studies have shown a positive synergistic interaction between everolimus and cyclosporine [28]. This synergy between the rapamycin inhibitors and cyclosporine theoretically allows for minimization of cyclosporine without compromising efficacy. Shitrit *et al.* [29] analyzed the effect of combined sirolimus with low-dose calcineurin inhibitors in eight, mostly emphysematous, lung transplant recipients with renal impairment and compared them to eight matched controls. All patients were entered into the study at a similar time frame after transplant. Immunosuppression regimens consisted of a combination of prednisone, azathioprine and cyclosporine, or prednisone, mycophenolate mofetil and tacrolimus. Tacrolimus and cyclosporine were tapered to trough levels of 4–8 and 80–120 ng/ml, respectively. All patients received statins and the doses of their other immunosuppressive medications were held at fixed levels. At

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18 months, change in FEV_1 was not statistically significant between the sirolimus group and matched controls. Two patients in the sirolimus group and one patient in the control group suffered acute rejection episodes, all three patients responded to pulse dose steroids. With regard to renal function, all but one patient in the sirolimus group enjoyed an improvement of creatinine clearance, while the renal function in control group continued to decline. This study is the first controlled study in lung transplantation to show that the addition of sirolimus allows for safe dose-reduction of calcineurin inhibitors to attain improved renal function. However, the addition of sirolimus represented the addition of a fourth immunosuppressive medication, and while this addition allowed for the reduction of dosing of the calcineurin inhibitor, this is not the same as immunosuppression minimization. Further studies are needed to test the safety of dose reduction on three-drug maintenance regimens.

Transplantation tolerance is the state of unresponsiveness to foreign antigens in the absence of ongoing immunosuppressive drugs, yet the immune system remains competent in response to infection [30]. The mechanism and basis of tolerance was first established by Billingham, Medawar and Brent in 1953 when transplantation tolerance was induced in neonatal mice by infusing immunologically immature recipients with allogeneic hematolymphopoietic cells from a histocompatible adult donor. The recipients, who now had donor chimerism, were subsequently able to accept skin grafts from mice of the donor strain, but not from other strains [31]. Years later, residual host cells found in the peripheral blood of allogeneic marrow transplant recipients [32] represented the coexistence of mutually nonreactive and collaboratively functional donor- and recipient immune-competent cells [33]. One of the first demonstrations of chimerism in human solid organ transplantation was the pathologic evidence of donor-specific hepatocytes and endothelium of major blood vessels in conjunction with recipient cell infiltration of the entire macrophage system in liver transplant patients [34].

Starzl and Zinkernagel have attributed the ability of donor leukocytes to induce both immune responses and tolerance mainly to their capacity to migrate to and persist in lymphoid organs. In essence, allograft acceptance involves widespread 'responses of co-existing donor and recipient immune cells, each to the other, causing reciprocal clonal expansion, followed by peripheral clonal deletion' [35,36]. This co-existence of donor and recipient immune cells is a dynamic relationship of host-versus-graft and graft-versus-host reactions that balance out to a state of equilibrium in the transplant recipient (Fig. 1) [37].

Zinkernagel *et al.* [36] outlined the interrelated changes that must take place in order for successful organ engraftment to occur: clonal deletion of the recipient's immune response, reciprocal deletion of the donor-leukocyte response, maintenance of clonal exhaustion, and a reduction in the immunogenicity of the donor-leukocyte-depleted organ. This clonal exhaustion-deletion hypothesis has been validated experimentally [37–39] and may, in fact, be the underlying mechanism that allows for drug-free tolerance in organ transplantation.

The large doses of immunosuppressive medicines given to recipients immediately post-transplant, the time of maximal leukocyte migration, may actually diminish the mechanism of tolerance by clonal exhaustion-deletion and thereby preclude the goal of immunosuppression withdrawal [33]. Two fundamental ways to avoid this consequence are first, pretreatment of the recipient with donor antigen, and second, extremely frugal administration of immunosuppressive therapy after transplantation [40].

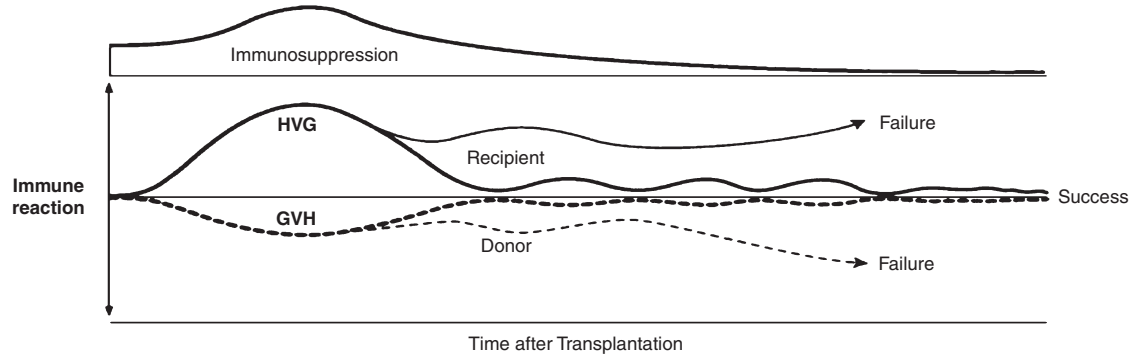

Figure 1 Contemporaneous host-versus-graft (HVG) and graft-versus-host (GVH) reactions after transplantation. Acute reciprocal clonal exhaustion after successful transplantation is subsequently maintained by chimerism-dependent low-grade stimulation of both leukocyte populations, which may wax and wane.

Pham *et al.* [41] have shown that mixed chimerism confers donor-specific tolerance to lung allografts in animal models. Until recently, however, the toxicity of sublethal preconditioning regimens necessary to create mixed chimerism has prevented incorporation of this approach to the clinical application of human organ transplants. Pham's group has now demonstrated that long-term donor-specific tolerance can be achieved in rodent lung allografts using mixed chimerism with a nontoxic conditioning regimen. This conditioning regimen consisting of a short course of tacrolimus, a single dose of anti-lymphocyte globulin, low dose irradiation and a single injection of T-cell depleted donor bone marrow was well tolerated in rats and achieved durable donor-specific tolerance to lung allografts without producing graft-versus-host disease. Importantly, this tolerance was achieved in major histocompatibility complex mismatched, high responder, adult combinations.

The aforementioned research in experimental rodent models has established principles in which new therapeutic approaches to clinical organ transplantation can be devised. Indeed, these experimental models will continue to provide a milieu for the exploration and refinement of approaches to induce tolerance in the organ recipient.

Principle to the success of lung transplantation is the treacherous balance sought between rejection and managing complications secondary to the use of nonspecific immunosuppressive agents to prevent rejection. Few studies are available to support the withdrawal of steroids in lung transplant patients. Those that have acknowledge that steroid withdrawal is likely appropriate only for a select few. Evidence exists that azathioprine withdrawal may not be safe in the lung transplant patient. Rapamycin inhibitors have been shown to allow for dose reduction of calcineurin inhibitors, but more studies need to be done to prove that this interaction will translate to a reduction in the number of immunosuppressive medications or total immunosuppression used. The assumption is that the state of tolerance is prerequisite to successful minimization of immunosuppression. Tolerance, however, is a process that takes shape early on in the post-transplant period and evidence suggests that current immunosuppressive regimens may weaken its development. Rather than pushing the limits of immunosuppression minimization, if immunotolerance is the goal, there is likely to be more clinical potential from relying on robust experimental models of tolerance induction and meticulously transitioning these principles to human clinical trials.

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