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Immunosuppression for Lung Transplantation

Evidence to Date

Gregory I. Snell and *Glen P. Westall*

Lung Transplant Service, Alfred Hospital and Monash University, Melbourne, Victoria, Australia

Abstract

With the introduction of ciclosporin (cyclosporine) into routine clinical practice 20 years ago, lung transplantation has become an established treatment for patients with advanced lung disease. Most lung transplant recipients routinely continue to receive a triple-drug maintenance immunosuppressive regimen consisting of a calcineurin inhibitor, an antimetabolite and corticosteroids. The use of antibody-based induction therapy remains common, although there has been a shift away from T cell-depleting agents, such as antithymocyte globulin, towards anti-interleukin-2 receptor monoclonal antibodies. Recent years have seen the introduction of sirolimus and everolimus, immunosuppressive drugs that act by blocking growth factor-driven cell proliferation. While the newer immunosuppressive drugs have been rigorously evaluated in large randomised trials in kidney, liver and cardiac transplantation, such studies are lacking in lung transplantation. Despite a shift towards more potent immunosuppressive regimens that incorporate tacrolimus and mycophenolate mofetil, the development of chronic allograft rejection, as manifested by the bronchiolitis obliterans syndrome continues to negatively impact on the long-term survival of lung transplant recipients. This article reviews the evidence for the immunosuppressive regimens used during induction and maintenance of patients undergoing lung transplantation, and discusses current strategies in the management of chronic rejection.

Lung transplantation is now an established therapy for the treatment of endstage pulmonary parenchymal and vascular diseases, with >25 000 procedures performed from >210 centres worldwide since 1983.[1] Chronic allograft rejection (in the form of the bronchiolitis obliterans syndrome [BOS]) and infection remain the most significant threats to long-term survival and quality of life.[1] Additionally, with a wide range of indications for lung transplantation, including cystic fibrosis, chronic obstructive pulmonary disease, interstitial lung diseases and pulmonary vascular diseases, recipients are of varying ages and have a varying intrinsic tolerance to immunosuppressive agents and their adverse effects.[1] These factors interweave and interact to a great extent, mandating that immunosuppressive strategies in lung transplantation will always represent a critical individualised balance between the risks of rejection and infection.

Current practice has been derived from other solid organ transplant trials, evolving clinical practice and the limited clinical trial material actually generated from lung transplantations. Many of the randomised clinical trials performed involve small

numbers of patients, such that much of our current clinical practice is based on retrospective case series. Therefore, it is not surprising that the International Society for Heart and Lung Transplantation (ISHLT) Registry describes a wide variety of maintenance immunosuppressive therapies currently in use (almost invariably in combination with a corticosteroid; 2002–5),[1] as follows:

- Tacrolimus + mycophenolate mofetil: 33% of patients at year 1 after lung transplantation, 26% at year 5
- Tacrolimus + azathioprine: 20% at year 1, 18% at year 5
- Ciclosporin + mycophenolate mofetil: 13% at year 1, 14% at year 5
- Ciclosporin + azathioprine: 12% at year 1, 16% at year 5
- Tacrolimus: 9% at year 1, 8% at year 5
- Sirolimus + calcineurin inhibitor: 6% at year 1, 7% at year 5
- Other agents: 7% at year 1, 11% at year 5.

With acute rejection rates in the first year of 50%, and chronic rejection (BOS) rates of 45% by 5 years, clinicians undertake significant switching between immunosuppressive regimens in an attempt to maximise efficacy and avoid toxicity.[1-3]

1. Clinical Trials of Lung Transplantation Immunosuppression

1.1 Induction Therapy

On the basis of other solid organ transplant results,[4] induction therapy in lung transplantation has potential benefits that could include lower rates of acute rejection, protection from nephrotoxicity due to the delayed introduction of a calcineurin inhibitor, and a decrease in the occurrence of BOS. The polyclonal agents, anti-lymphocyte/anti-thymocyte globulins (ALG/ATG), induce a rapid and profound generalised lymphopenia through Fc receptor-dependent mechanisms, including complement-related cytolysis and cell-mediated antibody-related cytolysis.[5] Monoclonal antibodies to CD25 inhibit activation of the interleukin (IL)-2 receptor and thereby selectively target activated T cells. A small number of centres use muromonab-CD3 (OKT3) or campath (monoclonal anti-CD52 antibody) for induction immunosuppression. The use of initial induction therapy with ALG/ATG has decreased to ≈12% of all lung transplantations, while the use of IL-2 receptor antagonists (IL-2RA) has risen to 33%.[1,5] However, the reality is that in lung transplantation, with few randomised trials and the limited available trials and case series showing conflicting or inconclusive results, no firm conclusions are possible. Induction therapy remains an area of active research.

Registry data from the ISHLT suggest that induction therapy with a polyclonal ATG significantly reduced the incidence of acute rejection in the first year after transplantation compared with either no induction or an IL-2RA.[1] In a 44-patient, randomised, single-centre study of rabbit ATG versus conventional triple immunosuppression, Palmer and coworkers[6] noted less acute rejection at 1 year but no difference in 2-year infection, malignancy or survival. Other studies have examined the efficacy of the IL-2RAs versus conventional immunosuppression. There were only subtle effects on acute rejection when daclizumab was used in two studies with historical controls.[7,8] Similarly, basiliximab has been very recently studied in a 121-patient randomised double-blind trial versus placebo, with no clinically significant differences in outcomes at 1 year.[9] Interestingly, limited head-to-head data comparing ATG and basiliximab suggest ATG may have superiority in terms of reducing acute rejection and BOS.[10,11] Hopefully these conflicting results will be clarified as part of the outcomes of large multicentre blinded ATG studies currently in progress.[12]

1.2 Maintenance Immunosuppression Therapy

Most lung transplant recipients receive a triple-drug maintenance regimen. The calcineurin inhibitors, ciclosporin (cyclosporine) or tacrolimus remain the cornerstone of long-term immunosuppression.[1] As an antimetabolite, mycophenolate mofetil (MMF) has been increasingly favoured over the use of azathioprine. Although corticosteroids are univer-

sally used, there have been limited reports of successful corticosteroid withdrawal years after transplant.[5,13]

1.2.1 Ciclosporin

The discovery of the immunosuppressive properties of ciclosporin in 1976 heralded the arrival of the modern era of solid organ transplantation.[14] Ciclosporin is a fungal polypeptide that forms complexes with intracytoplasmic proteins to inhibit calcineurin. Calcineurin is involved in T-cell activation and IL-2 production.

Immediately post-transplant, ciclosporin may be given in an intravenous form, which has a bioavailability 3-fold that of oral ciclosporin. Thus, the dose must be increased when the patient starts oral therapy. Of note, patients with cystic fibrosis (CF) show variable ciclosporin absorption compared with non-CF patients and therefore require different dosage regimens and an oral dose five times the intravenous dose. The calcineurin inhibitors interact with a number of drugs, including the antifungal azoles, necessitating a significant dose reduction of the calcineurin inhibitors when they are co-administered.

A number of generic ciclosporin formulations are available. Although these different formulations have similar bioavailability, brand changes should be undertaken cautiously.

Ciclosporin administration is monitored with blood concentrations. The targeted concentration varies according to time from transplantation, episodes of rejection and adverse effects experienced. Trough concentrations (C_0) correlate poorly with actual systemic exposure, and measuring concentrations within 2 hours of the dose (C_2) better reflect the full pharmacokinetic profile (area under the concentration-time curve from 0 to 12 hours; AUC_{12}).[15] In a study of 50 lung transplantation recipients (20 CF and 30 non-CF patients), achieving and maintaining targeted C_2 (>800 μg/L within 48 hours; 1200 μg/L from week 1 to month 1; >1000 μg/L in month 2; >800 μg/L in month 3; >700 μg/L in month 3–6; and >600 μg/L thereafter) was associated with reduced rates of acute rejection, BOS and renal dysfunction compared with historical controls.[16] Measuring C_2 is particularly useful in CF patients and in patients where toxicity is suspected, and the C_0 appears acceptable.

1.2.2 Tacrolimus

Tacrolimus (also known as FK506), a macrolide antibiotic, is 10–100 times more immunosuppressive *in vitro* than ciclosporin.[17] Although structurally unrelated, tacrolimus, like ciclosporin, inhibits the synthesis of IL-2, albeit through the binding of a different immunophilin, the FK506 binding protein-12.[18]

Over recent years there has been a clear trend towards the increased use of tacrolimus over ciclosporin.[1] Tacrolimus is typically administered orally, although similar bioavailability can be achieved sublingually.[19] Intravenous tacrolimus (target concentrations 12–15 ng/mL) can be used in the early post-lung transplantation period,[20] although there are concerns regarding neurotoxicity and nephrotoxicity when administered by this route.

In combination with either azathioprine or MMF, the efficacy of tacrolimus has been compared with ciclosporin in a limited number of studies.[21-23] In a prospective, open-label, single-centre trial, Keenan and colleagues[21] randomised 133 lung transplant recipients to receive either tacrolimus or ciclosporin together with azathioprine and prednisolone. While 1- and 2-year survival rates were similar, fewer patients in the tacrolimus group developed BOS (22% vs 38%; $p = 0.025$). Significantly, in this study, a large number of patients receiving ciclosporin required conversion to the better tolerated tacrolimus. In a smaller two-centre prospectively randomised trial, patients received corticosteroids, MMF and antibody induction, in combination with either tacrolimus (n = 26) or ciclosporin (n = 24). While the number of treated rejection episodes per 100 patients-days was significantly less in the tacrolimus group (0.225 vs 0.426; $p < 0.05$), there was no difference in survival at 6 months, 1 year or 3 years.[22,24] The same group reported similar results when the study cohort was expanded to 74 lung transplant recipients.[25] A large, randomised multicentre trial of 250 patients comparing tacrolimus with ciclosporin in combination with MMF and corticosteroids is currently nearing completion. A 1-

year interim analysis of 110 patients demonstrated a non-significant trend towards reduced episodes of acute rejection and BOS in the tacrolimus-treated patients.[23] These encouraging early results will need to be validated by the long-term data gathered from all enrolled patients.

In a multicentre retrospective study, the effect of switching calcineurin inhibitors was assessed in lung transplant recipients with either recurrent, ongoing rejection (n = 110) or BOS (n = 134).[26] The conversion from ciclosporin to tacrolimus was associated with reversal of recurrent, ongoing rejection, and allowed short-term stabilisation of lung function in patients with BOS. While this study is limited by the absence of a control arm, the results have translated into clinical practice; most transplant centres change baseline maintenance immunosuppression in those patients with recurrent acute rejection or who develop BOS.

1.2.3 Antimetabolites

The preferred use of MMF over azathioprine following lung transplantation has increased in recent years.[1] Azathioprine, through its inhibition of DNA, RNA and *de novo* purine synthesis, limits the proliferation of T and B lymphocytes. MMF also inhibits lymphocyte proliferation, but offers increased selectivity and decreased toxicity compared with azathioprine. It is a prodrug of the active compound mycophenolic acid (MPA), an inhibitor of the enzyme inosine monophosphate dehydrogenase, which is involved in *de novo* synthesis of guanosine monophosphate.

Reflecting the differential effects of calcineurin inhibitors on the enterohepatic circulation of MPA, higher doses of MMF need to be given when combined with ciclosporin (3 g/day) compared with tacrolimus (2 g/day).[27] There is significant variability in the pharmacokinetics of MMF and MPA, but the therapeutic monitoring of MPA concentrations is not as well established as for ciclosporin.[28] Enteric-coated mycophenolate sodium has proven efficacious and safely used in renal and heart transplantation, but similar data in lung transplant recipients are lacking.

Recently, following a number of small retrospective studies suggesting improved clinical outcomes in lung transplant recipients receiving MMF rather than azathioprine,[29-31] two prospective studies comparing the two drugs have been published. Palmer and colleagues[32] randomised patients to receive either azathioprine 2 mg/kg/day (n = 38) or MMF 2 g/day (n = 43), in combination with ciclosporin and corticosteroids. The primary endpoint of biopsy-proven acute allograft rejection was not significantly different between the two groups (63% MMF group vs 56% azathioprine group; p = 0.82) There was also no difference between the two groups in 6-month survival, rates of cytomegalovirus infection or adverse events.

In a larger multicentre international study, 320 patients were randomised to receive either azathioprine (2 mg/kg/day) or mycophenolate mofetil (3 g/day for 3 months, then 2 g/day), with ciclosporin and corticosteroids.[33] All patients received at least one dose of ATG (1–2 mg/kg/dose). Further use of induction therapy was left to the discretion of the individual centres. At both the 1- and 3-year analysis, there was no significant difference between the two groups in the incidence of acute rejection, time to first rejection, development of BOS or survival.

The results from these two trials are contrary to similar trials in kidney, liver and heart transplantation demonstrating improved efficacy of immunosuppressive regimens that include MMF. Of note, therapeutic monitoring of MMF and MPA concentrations was not performed in either of these two trials, and it is possible that patients were underdosed and not receiving therapeutic MMF concentrations. In the larger international trial, there was also a high withdrawal rate, particularly in patients receiving azathioprine, resulting in reduced follow-up time in this group. This may partly explain why there was no observed difference in clinical outcomes between the two groups.

Therefore, despite widespread use, there are currently no clinical data suggesting that MMF should be favoured over azathioprine, when used in combi-

nation with calcineurin inhibitors and corticosteroids, in patients undergoing lung transplantation.

1.2.4 Everolimus

Everolimus, a rapamycin derivative, is a macrocyclic lactone that inhibits growth factor-stimulated proliferation of lymphocytes and mesenchymal cells.[34] It inhibited the proliferation of human lung fibroblasts *in vitro*, [35] and prevented epithelial destruction and luminal obliteration in a swine lung transplantation model of obliterative bronchiolitis (the pathological correlate of BOS).[36] A synergistic interaction between everolimus and ciclosporin has been documented in experimental studies,[37] and the combination has shown excellent immunosuppressive activity in clinical trials in cardiac[38] and renal[39] transplant recipients.

In a randomised, double-blind clinical trial, 213 BOS-free patients received either everolimus or azathioprine, in combination with ciclosporin and corticosteroids.[40,41] The prospectively defined primary endpoint was the incidence of efficacy failure (a composite of a decline in forced expiratory volume in 1 second [FEV_1] >15%, graft loss, death or loss to follow-up) at 12 months. The incidence of efficacy failure at 12 months was significantly lower in the everolimus group compared with the azathioprine group (21.8% vs 33.9%; $p < 0.05$). The everolimus group also had significantly reduced incidences of a change in FEV_1 >15%, a change in FEV_1 >15% with BOS, and acute rejection. During long-term follow-up at 36 months, rates of efficacy failure became similar between the groups, although the incidence of acute rejection remained significantly less in the everolimus group. Treatment discontinuations (particularly those due to adverse events) were more frequent with everolimus than with azathioprine.

The reasonably complete 12-month data are promising, this drug being the first to significantly slow the loss in lung function that typically characterises lung transplantation BOS. Weakened by study withdrawal, the data are less compelling at 36 months and potentially only a subset of patients – those who start on everolimus early and can be kept on prolonged maintenance treatment – may actually benefit from replacing azathioprine with everolimus.[41] A subsequent, large, 36-month, multicentre, open-label study of everolimus is currently underway to evaluate the use of therapeutic concentration targeted everolimus versus MMF (in combination with ciclosporin and corticosteroids).[42]

1.2.5 Sirolimus

Sirolimus is another macrocycline lactone that has been used in lung transplantation. Predominantly, it has had a role in 'rescuing' patients where other immunosuppressants are contraindicated or ineffective.[43,44] This has proved particularly the case in renal impairment, where sirolimus substitution allows the calcineurin inhibitor to be stopped. Its role in the prevention of BOS progression remains unclear, with small case series showing mixed results.[43,45]

Importantly, however, sirolimus has been associated with interstitial pneumonitis in lung transplantation.[46] This reversible condition mimics acute rejection and is typically associated with higher blood concentrations.[46] Wound dehiscence has also been described with sirolimus.[47] A large 36-month multicentre open-label trial of sirolimus versus azathioprine (in addition to tacrolimus and corticosteroids) is currently underway,[48] to start at 3 months post-lung transplantation.

1.2.6 Inhaled Corticosteroids

Inhaled corticosteroids are now considered as significant therapies in asthma and chronic obstructive pulmonary disease.[49] There are potentially major similarities between the pathophysiology of these diseases and lung transplantation BOS.

There is a case report,[50] a small series,[51] and a moderate-sized, randomised, double-blind trial[52] investigating the role of inhaled corticosteroids in lung transplantation. The smaller studies showed improved lung function. Although demonstrating good tolerability, the randomised trial showed no advantage of inhaled corticosteroids in terms of acute or chronic rejection prophylaxis.[52]

1.2.7 Inhaled Ciclosporin

On the basis of evidence suggesting that inhaled ciclosporin may prevent acute rejection,[53] a recent randomised, double-blind, multicentre trial of inhaled ciclosporin versus no ciclosporin has suggested that it increased time spent free of chronic rejection and decreased overall survival.[54] The study had an accompanying editorial[55] noting several methodological issues, including (i) recruitment (58 lung transplant recipients were recruited versus a planned enrolment of 120, with some imbalance between the study arms); (ii) study completion rate (26 of the 58 completed 2 years); and (iii) failure to reach the actual primary efficacy endpoint (prevention of acute rejection). Although access to this formulation is proving difficult,[56] these results clearly warrant further study.

1.3 Treatment of Acute Rejection

Despite the absence of an evidence base, most centres treat acute rejection with intravenous corticosteroid pulses over 3 days (intravenous methylprednisolone 10 mg/kg/day). Repeat bronchoscopy is usually performed to confirm resolution of acute rejection. Ongoing or recurrent acute rejection is managed by changing baseline immunosuppression – a practice that is supported by a number of studies.[57-59] Other strategies for refractory acute rejection, which have not been formally evaluated by large multicentre studies, include the use of ATG,[60] methotrexate,[61] inhaled ciclosporin,[62] inhaled corticosteroids[63] and total lymphoid irradiation.[64]

The contributing role of humoral rejection to graft dysfunction following lung transplantation remains contentious. The treatment of humoral rejection aims both to remove preformed allo-specific antibodies and to inhibit further production of antibodies, and includes plasmapheresis, intravenous immunoglobulin, MMF, cyclophosphamide and rituximab.[65]

1.4 Management of Bronchiolitis Obliterans Syndrome

Baseline immunosuppression is often augmented or modified when BOS is first diagnosed, and there is some evidence suggesting that such an approach is efficacious.[43] A short course of high-dose intravenous methylprednisolone is typically given in this setting, although there is minimal evidence supporting this approach.[66] There have been three small retrospective studies suggesting that ATG may temporarily delay the progression of BOS;[66-68] however, such a strategy has not been rigorously appraised in a prospective, randomised and blinded study.

Other treatments that have been considered in an attempt to reverse or arrest the loss of lung function associated with the development of BOS include methotrexate,[69] cyclophosphamide,[70] photopheresis[71] and total lymphoid irradiation.[72] Reflecting that these treatment suggestions arise from small retrospective studies, none of these modalities can be recommended as routine management of BOS.

Recent interest has focused on the immunomodulatory properties of the macrolide azithromycin as a treatment for patients with established BOS. Two small case series have documented modest improvements in lung function in patients with established BOS.[73-75] These reports are limited by the small numbers of patients studied and the short period of follow-up, but nevertheless represent exciting developments in a hitherto treatment-unresponsive condition. HMG-CoA reductase inhibitors (statins), also appear to have immunomodulatory properties that show promise in slowing progression to BOS.[76]

2. Summary, Conclusions and Future Directions

The modern era of transplantation arose out of the discovery of the calcineurin inhibitor ciclosporin. Since then, most lung transplant recipients have received a 'triple immunosuppressive' regimen consisting of a calcineurin inhibitor (ciclosporin or tacrolimus), an antimetabolite (azathioprine or MMF) and corticosteroids. While newer immunosuppressants (sirolimus and everolimus) have been discovered and are prescribed, their use has not been demonstrated to improve survival. Importantly, as can be seen from this review, observations from other solid organ transplantation settings

that certain immunosuppressive drug combinations are particularly efficacious cannot automatically be extended to lung transplant recipients.

The commonly used immunosuppressants continue to have significant drawbacks. They nonspecifically inhibit the activation of lymphocytes, thereby increasing the risk of opportunistic infections, and through their adverse effects add to the morbidity and mortality of lung transplantation. Future immunosuppressants would ideally selectively target allo-specific T cells, disrupt the pathways that lead to BOS and induce tolerance to the lung allograft. As new drugs and generic alternatives enter the clinical field, it is imperative upon the lung transplant community to evaluate their role rigorously via large, randomised, multicentre trials. Clearly, much work remains to be done.

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Correspondence: Associate Professor *Gregory I. Snell*, Department of Allergy, Immunology and Respiratory Medicine, Lung Transplant Service, Alfred Hospital, Commercial Road, Melbourne, VIC 3004, Australia.
E-mail: g.snell@alfred.org.au