

Immunosuppression in lung transplantation

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Abstract: Lung transplantation can be a life-saving procedure for those with end-stage lung diseases. Unfortunately, long term graft and patient survival are limited by both acute and chronic allograft rejection, with a median survival of just over 6 years. Immunosuppressive regimens are employed to reduce the rate of rejection, and while protocols vary from center to center, conventional maintenance therapy consists of triple drug therapy with a calcineurin inhibitor (cyclosporine or tacrolimus), antiproliferative agents [azathioprine (AZA), mycophenolate, sirolimus (srl), everolimus (evl)], and corticosteroids (CS). Roughly 50% of lung transplant centers also utilize induction therapy, with polyclonal antibody preparations [equine or rabbit anti-thymocyte globulin (ATG)], interleukin 2 receptor antagonists (IL2RAs) (daclizumab or basiliximab), or alemtuzumab. This review summarizes these agents and the data surrounding their use in lung transplantation, as well as additional common and novel therapies in lung transplantation. Despite the progression of the management of lung transplant recipients, they continue to be at high risk of treatment-related complications, and poor graft and patient survival. Randomized clinical trials are needed to allow for the development of better agents, regimens and techniques to address above mentioned issues and reduce morbidity and mortality among lung transplant recipients.

Keywords: Lung transplantation; immunosuppression; review

Submitted Feb 04, 2014. Accepted for publication Apr 16, 2014.

doi: 10.3978/j.issn.2072-1439.2014.04.23

View this article at: <http://dx.doi.org/10.3978/j.issn.2072-1439.2014.04.23>

Lung transplantation can be a life-saving procedure for those with end-stage lung diseases. Unfortunately, long term graft and patient survival are limited by both acute and chronic allograft rejection, with a median survival of just over 6 years (1). Immunosuppressive regimens are employed to reduce the rate of rejection, and while protocols vary from center to center, conventional maintenance therapy consists of triple drug therapy with a calcineurin inhibitor (cyclosporine or tacrolimus), antiproliferative agent [azathioprine (AZA), mycophenolate, sirolimus (srl), everolimus (evl)], and corticosteroids (CS). Roughly 50% of lung transplant centers also utilize induction therapy, with polyclonal antibody preparations [equine or rabbit anti-thymocyte globulin (ATG)], interleukin 2 receptor antagonists (IL2RAs) (daclizumab or basiliximab), or alemtuzumab (2). While these agents are used to prevent acute and chronic rejection, they are not without adverse effects, including drug-specific toxicities, as well as

opportunistic infections and malignancy. This review will summarize these agents and the data surrounding their use in lung transplantation, as well as additional common and novel therapies in lung transplantation.

Induction immunosuppression

Induction therapy is intensive immunosuppressant therapy given perioperatively to reduce the risk of acute rejection and also serves to delay initiation of maintenance immunosuppression, most notably the nephrotoxic calcineurin inhibitors. These agents primarily target T lymphocytes, which are considered the effector cells in cell-mediated rejection.

According to the most recent registry report of the International Society for Heart and Lung Transplantation (ISHLT), of the centers that utilize induction, majority use an IL2RA (2). Both daclizumab and basiliximab are non-

Table 1 Induction immunosuppression

Citation	Immunosuppressant	N	Methods	Outcomes
Palmer <i>et al.</i> 1999 (7)	ATG vs. no induction	44	Prospective RCT	≥ A2 AR: 23% vs. 55%, P=0.03 BOS: 20% vs. 38% Survival, 1-yr: 68% vs. 73% Survival, 2-yr: 64% vs. 68% No difference in infection or malignancy
Garritty <i>et al.</i> 2001 (8)	Daclizumab vs. no induction	61	Retrospective	≥ A2 AR: 18% vs. 48%, P<0.04 No difference in infection or PTLT
Borro <i>et al.</i> 2005 (9)	Basiliximab vs. no induction	15	Retrospective	AR: 13% vs. 38.5%, P=0.19 BOS: 20% vs. 38.5%, P=0.4 Survival, 2-yr: 80% vs. 54%, P=0.14 No difference in infection or malignancy
Hachem <i>et al.</i> 2005 (10)	Basiliximab vs. ATG	157	Retrospective	Cumulative A AR Score higher at 3-, 6-, 12-month with basiliximab, P=0.003, 0.004, 0.033 respectively BOS stage 1 at 2-yr: 36% vs. 26%
Burton <i>et al.</i> 2006 (11)	Daclizumab vs. ATG	335	Retrospective	Freedom from ≥ A2 AR, 3-month: 9% vs. 32% Freedom from ≥ A2 AR, 2-yr: 0% vs. 26% P<0.0001
Mullen <i>et al.</i> 2007 (12)	Daclizumab vs. ATG	50	RCT	No difference in AR or BOS at 1 year Survival: 96% vs. 88%
Ailawadi <i>et al.</i> 2008 (13)	Daclizumab vs. ATG	163	Retrospective	AR: 9% vs. 28%, P=0.002 BOS: 6.4% vs. 23%, P=0.02 Survival: 94% vs. 83%, P=0.05
Hartwig <i>et al.</i> 2008 (14)	ATG vs. no induction	44	Prospective RCT	AR: 62% vs. 68%, P=0.52 Early AR: 5% vs. 41%, P=0.01 Graft survival: 36% vs. 23%, P=0.048
Clinckart <i>et al.</i> 2009 (15)	Basiliximab vs. ATG	37	Retrospective	AR: 52.4% vs. 43.8%

RCT, randomized controlled trial; AR, acute rejection; BOS, bronchiolitis obliterans syndrome; PTLT, posttransplant lymphoproliferative disorder; ATG, anti-thymocyte globulin; yr, year.

depleting monoclonal antibodies that bind to the alpha subunit of the interleukin 2 (IL-2) receptor (CD25) present on activated T lymphocytes, thereby preventing T cell activation and proliferation (3,4). Daclizumab is a humanized (90% human, 10% murine) (3) monoclonal antibody that was removed from the US market in 2009 (FDA), thus making basiliximab the only IL2RA available for use. Basiliximab is a chimeric (75% human, 25% murine) monoclonal antibody and is generally well tolerated, with adverse effects similar to that of placebo (4). ATG is the second most commonly used induction agent, used by roughly 20% of centers that utilize induction (2). ATG is a polyclonal antibody preparation isolated from either rabbit (rATG, Thymoglobulin®) or horse (equine ATG, ATGAM®) sera which contain

antibodies toward human thymocytes and cause significant T cell depletion (5,6). Adverse effects associated with these agents include fever, chills, rash, arthralgia, diarrhea, leukopenia, and thrombocytopenia. Pre-medication with acetaminophen, anti-histamines, and CS are usually required and help minimize these reactions. Serum sickness and anaphylaxis have also been reported, in addition to increased rates of infection and malignancy.

Data for the use of induction in lung transplantation are presented in *Table 1*. Overall it appears that induction with either ATG or an IL2RA reduces or delays the incidence of acute rejection, bronchiolitis obliterans syndrome (BOS), and may improve graft and patient survival compared to no induction (7-9,14). Studies comparing IL2RAs and ATG

show inconclusive results; one study indicated IL2RAs are associated with lower rates of acute rejection and BOS, as well as improved survival (13); three studies showed lower acute rejection and BOS and improved survival with ATG (10,11,15), while still another showed no difference (12). In 2008, Hachem and colleagues published a registry report that retrospectively analyzed 3,970 adult lung transplant recipients. Four year graft survival in those who received induction with an IL2RA, ATG, or no induction were 64%, 60%, and 57% ($P=0.0067$), respectively (16). Reasons for such variability in outcomes relate to the size and retrospective nature of these studies, potential differences in patient population and management, duration of followup, and variability in maintenance immunosuppression regimens. More recently, alemtuzumab, a humanized monoclonal antibody targeting CD52, has been used as an induction agent. The CD52 antigen is found on T and B lymphocytes, as well as natural killer cells, monocytes and macrophages (17). Upon binding, alemtuzumab induces cellular lysis and causes significant and prolonged depletion, with B cell recovery occurring within 3-6 months and T cell recovery >12 months (18,19). This profound and prolonged lymphocyte depletion associated with alemtuzumab may allow for the possibility of reduced maintenance immunosuppression. Loenhout and colleagues published their findings using alemtuzumab induction in 20 lung transplant recipients with reduced maintenance immunosuppression in 2010. Compared to 20 historical controls who received standard maintenance immunosuppression, there were no statistical differences between 6- or 12-month survival (95% *vs.* 90%, 76% *vs.* 95%), episodes of acute rejection (2/16 *vs.* 5/20), or bacterial, viral or fungal infections (20). Subsequently, Shyu and colleagues published 5 year outcomes using alemtuzumab induction with reduced-intensity maintenance immunosuppression. Their retrospective analysis grouped patients according to induction type: alemtuzumab ($n=127$), ATG ($n=43$), daclizumab ($n=73$), or none ($n=93$). Graft survival differed by group: 59%, 44%, 41%, 47%, respectively; as did freedom from acute rejection: 30%, 20%, 19%, 18%, respectively; freedom from lymphocytic bronchiolitis: 82%, 54%, 55%, 70% respectively; and freedom from BOS: 54%, 27%, 43%, 46% respectively (21). While alemtuzumab induction with reduced maintenance immunosuppression thus far demonstrates similar if not improved overall outcomes compared to other induction regimens, the optimal induction and maintenance regimen still needs to be elucidated by large, randomized controlled trials. Though 50% of centers currently utilize induction,

enhanced immunosuppression must be weighed against adverse effects, including infection and malignancy. Large, randomized controlled trials measuring the difference in acute rejection, BOS, graft and patient survival, infection and malignancy comparing no induction, IL2RAs, ATG, and alemtuzumab are needed to better understand the effect of the agents and to identify the optimal regimen for lung transplant recipients.

Maintenance immunosuppression

Maintenance immunosuppression is lifelong immunosuppressive therapy that is given to prevent both acute and chronic rejection. The goal is to not only to prevent and minimize immune-mediated injury to the allograft but also to minimize adverse effects associated with the medications used. Conventional maintenance immunosuppressive regimens consist of triple drug therapy with a calcineurin inhibitor, antiproliferative agent, and CS. Historically cyclosporine and AZA were used along with prednisone, but over time additional agents have emerged on the market, including tacrolimus, mycophenolate, and the mammalian target of rapamycin (mTOR) inhibitors, sirolimus and everolimus. Despite the addition of these agents to the armamentarium of immunosuppression for lung transplant recipients, acute rejection and BOS remain obstacles to long-term survival. Additionally, minimization and management of adverse effects continue to be challenging. Selection of regimens is largely protocolized and based on studies from other types of organ transplantation as well as currently available literature in lung transplant, and center-specific outcomes and provider experience.

Calcineurin inhibitors

Cyclosporine was the first calcineurin inhibitor available for use, first approved by the FDA in 1983. It is a lipophilic compound that binds to intracellular cyclophilin in T lymphocytes, forming a complex that prevents transcription of interleukin 2, thereby decreasing activation and proliferation of T lymphocytes (22). Oral absorption of cyclosporine (Sandimmune[®]) is poor and variable (10-89%). A modified cyclosporine formulation was subsequently developed and approved by the FDA in 1997 (Neoral[®]) with enhanced bioavailability, with approximately 50-150% increases in area under the curve (AUC) and C_{max} (23,24). Sandimmune and Neoral are not interchangeable but both are available in capsules, oral solution, and intravenous

formulations. Therapeutic drug monitoring of cyclosporine consists of measuring trough (C0) values, AUC calculations, or 2-hour post-dose (C2) levels. In renal transplantation, AUC measurements have demonstrated superiority over troughs (25), however this requires multiple samples to estimate AUC, which is time consuming, cumbersome and impractical. A limited sampling strategy (LSS) may be employed as an alternative, measuring 2 post-dose levels (26), but this method still requires multiple samples and a calculation to estimate AUC. Therefore most centers utilize either C0 or C2 levels. Studies in lung transplant recipients indicate that C2 is a better correlate with AUC than C0 (27) and may reduce short-term nephrotoxicity associated with cyclosporine compared with C0, without compromising lung function (28). Target ranges vary according to center-specific protocols and practices, and take into account patient characteristics, such as time post-transplant and rejection and infection history. Generally, target trough levels range from 100-450 ng/mL, or C2 levels 800-1,400 ng/mL. Major adverse effects of cyclosporine include nephrotoxicity (acute and chronic), hypertension, hypercholesterolemia, electrolyte abnormalities (hyperkalemia, hypomagnesemia), neurotoxicity (posterior reversible encephalopathic syndrome, seizures, headache, tremor), diabetes, hirsutism, and gingival hyperplasia. A second calcineurin inhibitor, tacrolimus (previously known as FK506) (Prograf[®]) became available for use in 1997. It is 10-100 times more potent than cyclosporine. Tacrolimus binds to intracellular FKBP12, forming a complex that prevents transcription of cytokines, including interleukin 2, and ultimately prevents T lymphocyte activation and proliferation (29). Like cyclosporine, tacrolimus has poor and variable absorption, 17-23% (29). Tacrolimus is available in oral capsules and as an intravenous formulation. There is no commercially available oral suspension however formulas for pharmaceutical compounding are available. Sublingual administration of tacrolimus capsules at half of the oral dose is an option for those who are unable to tolerate oral therapy and wish to avoid intravenous tacrolimus due to significant toxicity (30). A once-daily extended-release formulation of tacrolimus, marketed under the trade name Astragraf XL[®] was approved by the FDA in 2013. No studies have yet been performed in lung transplant recipients; however they may be available in the future. Despite multiple studies indicating post-dose levels to more accurately predict AUC, most centers utilize trough concentrations for therapeutic drug monitoring (31,32). Target ranges vary according to center-specific protocols

and practices, and take into account patient characteristics, such as time post-transplant and rejection and infection history. Generally, target trough concentrations range from 5-15 ng/mL. Tacrolimus displays similar adverse effects to cyclosporine, with perhaps less hypertension and hypercholesterolemia, but more neurotoxicity and diabetes (33-39). Thrombotic thrombocytopenia purpura and hemolytic uremic syndrome have been reported with both cyclosporine and tacrolimus (40). Both cyclosporine and tacrolimus undergo metabolism via the hepatic cytochrome (CYP) P450 3A4 and 3A5 enzymes and p-glycoprotein efflux pumps present on intestinal mucosa, leading to significant drug interactions with CYP inducers (e.g., rifampin, phenobarbital, carbamazepine, phenytoin) and inhibitors (e.g., azoles, macrolides, calcium channel blockers). Additional drug interactions exist for cyclosporine, as it is not only a substrate of CYP 3A4 but also a moderate inhibitor (statins).

Selected data comparing cyclosporine and tacrolimus are shown in Table 2. Majority of the trials are small, prospective, randomized studies showing no statistical differences in acute rejection or survival between those treated with cyclosporine or tacrolimus, whether receiving no induction or ATG, AZA or mycophenolate. The most recent study published in 2012 by Treede *et al.* is the largest study to date and showed no difference between cyclosporine and tacrolimus in acute rejection or survival at 3-year, however there was a higher incidence of BOS stage 1 or greater with cyclosporine and it was also shown to be a risk factor for the development of BOS by univariate analysis (46). According to the most recent ISHLT Registry report, tacrolimus was the most frequently used calcineurin inhibitor, 83% at one year post-transplant, 77% at 5 years post-transplant (2).

Anti-proliferative agents

AZA was the first anti-proliferative agent available for use. AZA is converted to 6-mercaptopurine (6-MP) in vivo which then is converted into several compounds that get incorporated into the DNA of replicating cells and halt proliferation (47). AZA is associated with significant leukopenia, thrombocytopenia, anemia, hepatotoxicity (transaminitis and cholestasis), and rarely pancreatitis. Caution must be used when using AZA with xanthine oxidase (XO) inhibitors (e.g., allopurinol). XO is thought to be responsible for converting 6-MP to metabolites. The combination results in significant bone marrow suppression

Table 2 Maintenance immunosuppression

Citation	Immunosuppressant	N	Methods	Outcomes
Griffith <i>et al.</i> 1994 (41)	FK506 vs. CsA	74	Prospective, randomized	AR: 1.2 vs. 2 episodes per 100 patient days, $P < 0.05$ Survival, 1-yr: no difference Bacterial infection: 0.6 vs. 1.5 episodes per 100 patient days, $P = \text{NS}$
Treede <i>et al.</i> 2001 (42)	Tac vs. CsA	50	Prospective, randomized	Freedom from AR, 1 yr: 50% vs. 33.3%, $P = \text{NS}$ Treated episodes of AR/100 patient days: 0.225 vs. 0.426, $P < 0.05$ Survival, 1 yr: 73.1% vs. 79.2%, $P = \text{NS}$ No difference in infection
Zuckerman <i>et al.</i> 2003 (43)	Tac vs. CsA	74	Prospective, randomized	Freedom from AR, 1-yr: 46% vs. 35%, $P = 0.774$ Treated episodes of AR/100 patient days: 0.22 vs. 0.32, $P = 0.097$ Survival, 1-yr: 71% vs. 82%, $P = 0.748$ Infections: 0.55 vs. 0.7, $P = 0.059$
Hachem <i>et al.</i> 2007 (44)	Tac vs. CsA	90	Prospective RCT	Composite (Cumulative $\geq$ A3 AR, $\geq$ B4 LB, BOS 0-p): 50% vs. 84.8%, $P = 0.002$ AR or LB: 41% vs. 63%, $P = 0.036$ Freedom from BOS 0-p: Tac > CsA, $P = 0.1$
Neurohr <i>et al.</i> 2009 (45)	Tac + MMF	155	Retrospective	Freedom from AR, 1-yr: 74.6% Freedom from AR, 5-yr: 59.5% Freedom from BOS, 1-yr: 95.6% Freedom from BOS, 5-yr: 69.5% Survival, 1-yr: 86.4% Survival, 5-yr: 60.3%
Treede <i>et al.</i> 2012 (46)	Tac vs. CsA	249	Prospective, randomized	AR, 3-yr: 67.4% vs. 74.9%, $P = 0.118$ BOS $\geq$ stage 1-, 3-yr: 11.6% vs. 21.3%, $P = 0.037$ Survival, 1-yr: 84.6% vs. 88.6% (NS) Survival, 3-yr: 78.7% vs. 82.8% (NS) No difference in infection
FK506, tacrolimus; CsA, cyclosporine; AR, acute rejection; NS, not statistically significant; Tac, tacrolimus; RCT, randomized controlled trial; LB, lymphocytic bronchiolitis; BOS, bronchiolitis obliterans syndrome; MMF, mycophenolate mofetil; yr: year.				

and a 75% dose reduction of AZA in combination with XO inhibitors is generally recommended. The typical starting dose is 2 mg/kg IV or orally daily.

Mycophenolate is the most frequently used antiproliferative agent used according to the most recent ISHLT Registry report (2). Mycophenolate mofetil and mycophenolate sodium are converted to the active metabolite, mycophenolic acid (MPA), which inhibits inosine monophosphate dehydrogenase (IMPDH), the enzyme responsible for T and B lymphocyte production. Inhibiting this enzyme results in decreased T and B lymphocyte proliferation. Because lymphocytes lack the ability to utilize salvage

pathways for nucleotide synthesis and thus rely on the IMPDH pathway, mycophenolate is selective for T and B lymphocyte proliferation inhibition (47). Mycophenolate undergoes rapid absorption and conversion to MPA. MPA is metabolized hepatically into mycophenolic acid glucuronide (MPAG). MPAG is excreted via bile into the intestines, where it is converted back to the active metabolite, MPA, resulting in a second peak concentration in the plasma. Doses range from 1-1.5 g IV or oral twice daily. Therapeutic drug monitoring is available for mycophenolate, with AUC being the optimal parameter for measuring treatment response. Trough values have

shown poor predictive response (48-50). LSS calculations for estimation of AUC in lung transplant patients are also available; however, therapeutic drug monitoring has not been firmly established (51). Principle adverse effects of mycophenolate are leukopenia, thrombocytopenia, and gastrointestinal disturbances (diarrhea, abdominal pain, nausea, vomiting). Initial use of mycophenolate involved rescue therapy following development of BOS, with stabilization of pulmonary function testing after switching from AZA (52). In a prospective, randomized trial of 81 lung transplant recipients comparing azathioprine to mycophenolate in combination with cyclosporine and CS, there were no differences in biopsy-proven or clinical rejection, survival, infection, or adverse drug events at 6-month (53). A subsequent prospective, randomized multicenter study comprising 315 lung transplant recipients also showed no difference between AZA and mycophenolate when used in combination with cyclosporine and CS in the outcomes of acute rejection, BOS, and survival at 3-year, however a greater percentage of patients discontinued AZA than mycophenolate (59.6% *vs.* 46.5%) (54).

Srl and evl are two newer antiproliferatives in the mTOR inhibitor class. Both bind to intracellular immunophilin FK506 binding protein like tacrolimus, however unlike tacrolimus the complexes they form do not inhibit calcineurin but instead bind to mTOR, which is a signaling pathway needed to promote progression of the cell cycle from G1 to S phase. The end effect of mTOR inhibitors is a decrease in T lymphocyte activation and proliferation (47). Srl is available as oral tablets and an oral solution. Doses range from 0.5-6 mg daily, with target trough values ranging 5-15 ng/mL. Evl is available as oral tablets. Doses range from 0.25-3 mg twice daily, with target trough values ranging 5-15 ng/mL. Notable adverse effects include decreased wound healing, leukopenia, thrombocytopenia, hypertriglyceridemia, proteinuria, and pneumonitis. Both are metabolized by CYP 3A4 and therefore have similar drug interactions as tacrolimus. The role of mTOR inhibitors in lung transplant is still being identified. They may be used in conjunction with or substituted for either calcineurin inhibitors or other antiproliferative agents. The most common reasons for use include kidney dysfunction due to calcineurin inhibitors, onset of BOS, and malignancy (55-57). For those who exhibit kidney dysfunction, adding an mTOR inhibitor and reducing the calcineurin inhibitor dose has been shown to improve kidney function (55,58,59). Additionally, due to their antiproliferative and anti-fibroblast effects (60), mTOR inhibitors have been used in lung transplant recipients with

BOS to help slow progression. Indeed small, retrospective studies have shown stabilization or improvement in pulmonary function testing in lung transplant recipients with BOS (55,56,61,62). Two studies used srl immediately post-transplant and reported significant wound dehiscence and airway complications, leading to death in some patients (63,64), so mTOR inhibitors should not be used until the anastomosis and airways have healed. In 2006, Snell and colleagues performed a prospective randomized controlled trial comparing AZA and 3th month conversion to evl in 213 lung transplant recipients also maintained on cyclosporine and CS. The composite endpoint of efficacy failure (>15% FEV₁ decline from baseline, graft loss, death or loss to follow up) occurred in 33.9% *vs.* 21.8% of patients at 12-month (*P*=0.046), however there was no difference in this composite endpoint at 24-month. The authors concluded that evl did demonstrate a slowing in loss of pulmonary function over time (65). Most recently, Sacher and colleagues published data on 24 lung transplant recipients who were converted to srl prophylactically *vs.* AZA/MMF, one year post-transplant. Of the 19 patients who remained on long-term srl, a trend toward a reduction in the incidence of BOS and improved survival was reported (66). Larger, randomized controlled trials are needed to more fully elucidate the effect of mTOR inhibitors in the prevention of BOS.

Corticosteroids (CS)

CS have been used in solid organ transplant since the very beginning and have not only remained a corner stone of both induction and maintenance immunosuppression but they are also used to treat acute cellular rejection (ACR) as well. The most commonly used CS in solid organ transplant are methylprednisolone and prednisone. CS are known to have antiinflammatory properties and exert their effects in a variety of ways, including inhibiting the NFκB pathway, preventing T cell proliferation, decreasing macrophage activation, inhibiting cytokine production and altering lymphocyte migration (67). According to the most recent ISHLT registry report, CS continue to be used by almost all transplant centers, at one and five years post-transplant. Initial doses range from 500-1,000 mg given intraoperatively, and are gradually tapered over weeks to months to 5-10 mg per day for maintenance. Short and long term use of CS is associated with significant side effects, including hypertension, weight gain, hyperlipidemia, hyperglycemia and diabetes mellitus, osteoporosis and increased risk of fractures, increased risk of cataracts, poor

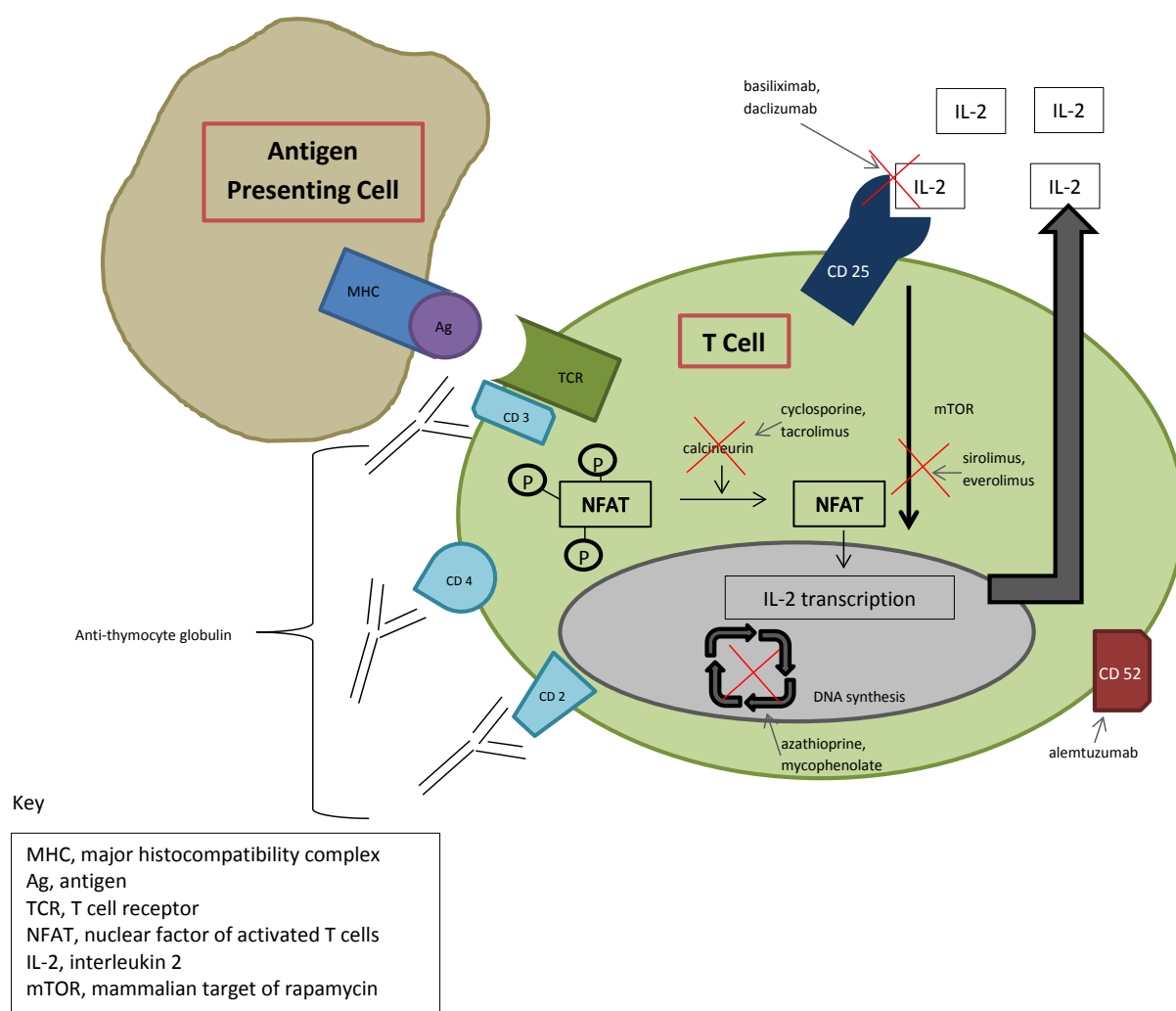


Figure 1 Mechanisms of action of immunosuppressive agents.

wound healing, psychiatric disturbances and infectious complications. Data on steroid-free regimens in lung transplantation is lacking and at best shows limited success (68,69). Complete steroid-withdrawal should be avoided at the present time, owing to a significant risk of allograft dysfunction; however, doses should be lowered as quickly and as safely as possible, and maintain the lowest possible doses with the goal of stable and optimal lung function while avoiding and minimizing drug-related adverse effects (Figure 1).

Antihumoral therapy

Generally immunosuppression is employed to suppress cell mediated immunity by targeting T cell function

and proliferation as rejection is usually a cell mediated phenomenon. However the role of humoral or antibody-mediated rejection (AMR) in solid organ transplant recipients has become more evident over the years. Antibody mediated rejection has been identified and characterized in other organs but remains poorly defined in lung transplant recipients. No agreed upon pathologic criteria exists to date in lung transplantation (70,71). Mechanisms by which anti bodies, which usually are donor specific antibodies (DSA), produce injury are not yet well described. Injury may be complement mediated or complement independent (72). No universally agreed upon management strategy exists for these antibodies. Use of intra venous immunoglobulin (IVIG), one of most commonly used treatments with a relatively low side effect

profile, with or without plasmapheresis, peritransplant and after development of DSA post-transplant resulted in improvement in certain parameters such as acute rejection and BOS at a single institution (73). In a study reported by Hachem and colleagues, use of IVIG combined with rituximab, a monoclonal anti CD20 antibody, *vs.* IVIG to clear newly acquired DSA showed improved survival and freedom from BOS in patients who cleared DSA after treatment. However there was no improvement in clearance of DSA with addition of rituximab to IVIG (74). Plasmapheresis is mainly used for antibody removal from circulation in suspected cases of humoral rejection which do not respond to steroids, leading to clinical improvement (75). Bortezomib, an inhibitor of 26S proteasome that leads to plasma cell apoptosis, has been used successfully in case reports to treat possible acute humoral rejection in lung transplant recipients (76,77). Hyperacute rejection due to pre formed antibodies against donor HLA antigens has become uncommon due to ongoing cross match screening. Treatment with IVIG, plasmapheresis, rituximab, antithymocyte globulin and eculizumab has been described in various case reports with variable degree of success (78-80).

Novel approaches

Aerosolized calcineurin inhibitors

A number of reports have been published regarding the use of aerosolized cyclosporine. In 1996, Iacono and colleagues published a report of histologic improvement of obliterative bronchiolitis (OB) and stabilization of pulmonary function testing in 7 lung transplant recipients who received aerosolized cyclosporine as rescue therapy (81). Shortly thereafter, the use of aerosolized cyclosporine to treat refractory acute rejection in 9 lung transplant recipients was associated with histologic improvement in 8 of 9 subjects, improvement in pulmonary function testing, a reduction in cycles of pulse dose CS and ATG, reduction in oral prednisone dose, and reduction in episodes of pneumonia was also observed, compared to 22 historical controls (82). Both reports showed no additional renal or hepatic toxicity with the use of aerosolized cyclosporine. A larger case-control study was subsequently undertaken and demonstrated a survival advantage in lung transplant recipients with biopsy-documented OB compared to conventional immunosuppression (83). While the most well-studied randomized placebo-controlled trial of aerosolized cyclosporine did not show a reduction in the primary endpoint of rate of ACR, it also demonstrated

a survival advantage compared with conventional immunosuppression, and showed an improvement in chronic rejection-free survival (84). Despite these results, an FDA-approved formulation of aerosolized cyclosporine is still currently unavailable. Animal studies aiming to characterize aerosolized tacrolimus pharmacokinetics and safety have been published (85-87). The first case report of using tacrolimus via inhalation in a human lung transplant recipient with BOS was recently published demonstrating improved functional capacity and oxygenation after one week of therapy (88). More data are needed to determine the optimal use of aerosolized calcineurin inhibitors but this therapeutic approach seems promising.

Azithromycin

Azithromycin is a macrolide antibiotic with anti-inflammatory and immunomodulatory effects (89). These effects, in conjunction with the beneficial effects of maintenance azithromycin seen in cystic fibrosis patients led to pilot studies of azithromycin in lung transplant recipients with BOS (90-93). In 5 of 6 patients, thrice-weekly azithromycin for 13 weeks demonstrated an average 17% improvement in FEV₁ (92) and an average 18% improvement in FEV₁ after 12 weeks of therapy in 8 others (93). A retrospective analysis of 20 lung transplant recipients also demonstrated an improvement in FEV₁ after 12 weeks of azithromycin therapy (average 110 mL from baseline) (94). However, not all patients respond to azithromycin therapy (95-97). Evidence suggests airway neutrophilia and elevated interleukin-8 bronchoalveolar (BAL) concentration may be predictors of response (95,97,98). Furthermore, studies have indicated that early initiation of azithromycin, e.g., BOS 0-p, may have more of an impact on preventing disease progression and may improve survival (97,99,100). In a randomized, placebo-controlled trial of 83 lung transplant recipients, there was a significant reduction in the incidence of BOS at 2-year in those who received azithromycin prophylactically compared to those who did not (12.5% *vs.* 44.2%, $P=0.0017$) (101). There was also a significant difference in BOS-free survival (HR 0.27, $P=0.020$), although overall survival was similar between groups. Collectively these data suggest early initiation of azithromycin in lung transplant recipients may prevent the incidence of BOS and prolong BOS-free survival, and may improve or stabilize pulmonary function after the onset of BOS, particularly in those with neutrophil- and IL-8-predominant BAL.

Extracorporeal photopheresis (ECP)

ECP was developed initially for treatment of cutaneous T cell lymphoma but has been utilized in variety of disease states including solid organ transplantation. The process involves leukopheresis followed by incubation of the isolated cells with 8-methoxypsoralen (8-MOP) and subsequent activation of 8-MOP with ultraviolet A radiation. These cells are then reinfused into the patient. 8-MOP activation causes DNA cross linkage and apoptosis. Reinfusion of these apoptotic cells generate T regulatory cells (T regs) and increased production of IL-10 and transforming growth factor beta. Exact mechanisms by which these immunomodulatory effects are produced are not well understood. At present, clinical studies assessing efficacy of ECP in lung transplant recipients are limited to retrospective single center studies done in patients showing declining lung function. No trials to assess the prophylactic effect of ECP on development of BOS by starting ECP immediately post-transplant have been done to date. In a study by Morrell and colleagues, 60 lung transplant patients received ECP in addition to conventional immunosuppression for treatment of progressive BOS. Fifteen patients (25%) showed an improvement in FEV₁ and rest showed a reduction in rate of decline in FEV₁ which persisted at 12 months after initiation of ECP (102). Another study done by Jaksch and colleagues, 51 lung transplant recipients who developed BOS and did not respond to augmentation of immunosuppression and azithromycin, received ECP. Thirty-one patients (61%) showed improvement or stabilization of lung function while 20 patients (39%) had continued decline in lung function and did not respond to ECP. Survival rate after start of BOS at 1, 3 and 5 years was significantly better in treatment responsive group (103). These studies did not identify any significant characteristics among lung transplant recipients that could predict the response to ECP. Recently a retrospective single center study done by Greer and colleagues assessed clinical efficacy of ECP treatment in lung transplant recipients with azithromycin-refractory chronic lung allograft dysfunction (CLAD) and attempted to associate clinical response to several CLAD phenotypes. Sixty-five lung transplant recipients were diagnosed and graded for graft dysfunction in accordance with ISHLT BOS criteria and were started on ECP treatment while showing deterioration or no improvement despite taking azithromycin which was started after reversible causes of graft dysfunction were excluded. Thirty-five patients

(54%) showed improvement or stabilization of FEV₁ while 30 patients showed >10% decline in FEV₁. Three CLAD phenotypes, restrictive allograft syndrome, defined by TLC ≤90% of baseline, non neutrophilic CLAD, patients demonstrating BAL neutrophilia <15% and rapid decliners, patients suffering a >100 mL/month decline in FEV₁ before ECP initiation showed that they were less likely to benefit from ECP treatment. Significant survival benefit was noted in the ECP responsive group when compared to the ECP refractory group (104). Randomized clinical trials are needed to better evaluate the benefit and possibility of early use of ECP after onset of CLAD in lung transplant recipients.

Statins

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, have been shown to have properties which may have a potential beneficial impact on lung allograft function post-transplant. They have been shown to reduce the gamma interferon induced expression of major histocompatibility molecules on cells, increase the number of CD4⁺CD25⁺ T regs, inhibit growth factor expression in lung fibroblasts and inhibit the development of obliterative airway disease in animal models (105-108).

These abovementioned immunomodulatory and anti-fibroproliferative properties have potential benefit for lung transplant recipients. However, clinical evidence in lung transplant recipients is limited to retrospective single center studies only. Johnson and colleagues showed improved 6-year survival in statin group compared to controls, 91% vs. 54%, as well as reduced rates of acute rejection and BOS (109). Li and colleagues showed improved survival and maintenance of lung function associated with post-transplant use of simvastatin in a single center cohort analysis of 502 lung transplant recipients (110). Prospective randomized trials are needed to confirm these findings, compare different statins and determine the optimal dose.

Pirfenidone

Pirfenidone is an anti-fibrotic agent used to treat pulmonary fibrosis. It inhibits growth-factor dependent proliferation of fibroblasts, T cell proliferation and activation, and may inhibit dendritic cell activation and function (111-115), and may be a potential therapeutic strategy for the treatment of CLAD. Thus far two case reports of pirfenidone use in human lung transplant have been published (116,117). The first reported a mild increase in FEV₁ following progressive

Table 3 Summary of stages and types of therapy

Induction immunosuppressants (Goal: prevent acute cellular and antibody-mediated rejection; delay initiation of nephrotoxic immunosuppressants)
Interleukin 2 receptor antagonists (non-depleting monoclonal antibody)
Daclizumab (Zenapax [®])
Basiliximab (Simulect [®])
Anti-thymocyte globulin (cell depleting polyclonal antibody preparation)
Equine (ATGAM [®])
Rabbit (Thymoglobulin [®])
Anti-CD 52 monoclonal antibody (cell-depleting)
Alemtuzumab (Campath [®])
Maintenance immunosuppressants (Goal: prevent acute cellular antibody-mediated rejection; prevent chronic lung allograft dysfunction)
Calcineurin inhibitors
Cyclosporine (Sandimmune [®] , Neoral [®])
Tacrolimus (Prograf [®])
Anti-proliferative agents
Azathioprine (Imuran [®])
Mycophenolatemofetil (CellCept [®])
mTOR inhibitors
Sirolimus (Rapamune [®])
Everolimus (Zortress [®])
Corticosteroids
Methylprednisolone (Solu-Medrol [®] , Medrol [®])
Prednisone (Deltasone [®])
Acute cellular rejection, treatment
Methylprednisolone (Solu-Medrol [®] , Medrol [®])
Anti-thymocyte globulin (Thymoglobulin [®])
Alemtuzumab (Campath [®])
Antibody-mediated rejection, treatment
Plasmapheresis
IVIg
Rituximab (Rituxan [®])
Bortezomib (Velcade [®])
Chronic lung allograft dysfunction, treatment
Azithromycin (Zithromax [®])
Extracorporeal photopheresis
Statins
Pirfenidone
IVIg, intra venous immunoglobulin.

decline with no evidence of infection or rejection and failure to respond to azithromycin, montelukast and fundoplication (116). The second reported a slower rate of decline in forced vital capacity, FEV₁, and a mild increase in total lung capacity in a lung transplant recipient with restrictive allograft syndrome (117). Given these findings, further study of pirfenidone in human lung transplantation is warranted.

Treatment

ACR, AMR and CLAD are discussed in-depth elsewhere. Specific treatment protocols vary from center to center, but options are limited to high-dose or “pulse” CS (e.g., methylprednisolone 10-15 mg/kg IV daily × 3-5 days), particularly for initial treatment or minimal-mild grade ACR; ATG (1.5 mg/kg IV daily × 3-5 days) or alemtuzumab (30 mg IV once) for moderate-severe grade ACR or steroid-resistant/steroid-refractory ACR. Therapies available for treatment of AMR include plasmapheresis (5-6 cycles), IVIG (1-2 g/kg over 3-6 days), rituximab (375 mg/m² IV weekly × 4 doses or 1,000 mg IV every 2 weeks × 2 doses), and/or bortezomib (1-1.3 mg/m² every 72 hours × 4 doses). Treatment options for CLAD are even more limited, and there is currently no agent available to date that reverses that process and restores lung function, other than re-transplant when available. Therapies targeting the processes of CLAD either prevent the onset of CLAD, or prevent and delay its progression. These include azithromycin, ECP, the statins, and pirfenidone. Augmentation of immunosuppression with ATG, alemtuzumab, addition or substitution of an mTOR inhibitor to the maintenance regimen, substitution of mycophenolate for AZA or of tacrolimus for cyclosporine, are additional strategies that have been employed with varying success (*Table 3*).

Summary

Our understanding of the underlying mechanisms and clinical presentation of acute allograft rejection and CLAD continue to evolve. Immunosuppressive regimens have significantly contributed to the improvement of the survival of lung transplant recipients. Despite the progress in the management of lung transplant recipients, they continue to be at high risk of treatment-related complications, poor allograft and patient survival. Randomized clinical trials are needed to allow the development of better agents, regimens and techniques to address above mentioned issues

and reduce morbidity and mortality among lung transplant recipients.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

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Cite this article as: Scheffert JL, Raza K. Immunosuppression in lung transplantation. *J Thorac Dis* 2014;6(8):1039-1053. doi: 10.3978/j.issn.2072-1439.2014.04.23