

## Immunosuppression for lung transplantation

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### 1. ABSTRACT

As a result of advances in surgical techniques, immunosuppressive therapy, and postoperative management, lung transplantation has become an established therapeutic option for individuals with a variety of end-stage lung diseases. The current 1-year actuarial survival rate following lung transplantation is approaching 80%. However, the 5- year actuarial survival rate has remained virtually unchanged at approximately 50% over the last 15 years due to the processes of acute and chronic lung allograft rejection (1). Clinicians still rely on a vast array of immunosuppressive agents to suppress the process of graft rejection, but find themselves limited by an inescapable therapeutic paradox. Insufficient

immunosuppression results in graft loss due to rejection, while excess immunosuppression results in increased morbidity and mortality from opportunistic infections and malignancies. Indeed, graft rejection, infection, and malignancy are the three principal causes of mortality for the lung transplant recipient. One should also keep in mind that graft loss in a lung transplant recipient is usually a fatal event, since there is no practical means of long-term mechanical support, and since the prospects of re-transplantation are low, given the shortage of acceptable donor grafts. This chapter reviews the current state of immunosuppressive therapy for lung transplantation and suggests alternative paradigms for the management of future lung transplant recipients.

### 2. INTRODUCTION

At the turn of the last century, the rejection of solid-organ allografts was generally believed to result from 'malnutrition' of the graft, perhaps related to an inadequacy of its blood supply. It was not until the pioneering work of physicians such as Karl Landsteiner, Alexis Carrel, and Charles Guthrie that an immunologic basis for graft rejection was hypothesized. In his 1912 Nobel Address, Carrel aptly summarized the challenge to be met so that organ transplantation could become a clinical reality:

"...the power of the organism to eliminate foreign tissue was due to organs such as the spleen or bone marrow...all our efforts must now be directed toward the biological methods which will prevent the response of the organism against foreign tissue..."

Despite this prescient observation, it was almost a half of a century later before azathioprine (AZA), a derivative of 6-mercaptopurine (6-MP), became the first immunosuppressive agent to be in widespread clinical use. The introduction of AZA, along with corticosteroids, finally pushed the field of transplantation beyond renal transplantation between monozygotic twins.

In the early 1980s, the advent of cyclosporine A (CsA), the first maintenance immunosuppressive drug with T-cell specificity, allowed renal transplantation to be performed with clinical reliability, thus enabling thoracic organ transplantation to move out of the experimental arena. Current immunosuppressive management in lung transplantation still relies on AZA, CsA, and related compounds, although a number of novel agents are now entering clinical practice.

In this chapter, the current state of immunosuppressive therapy for lung transplantation will be examined in light of our increased understanding of the mechanisms that underlie graft rejection. Ultimately, however, the field of transplantation will need to undergo a paradigm shift away from traditional pharmacologic immunosuppression, if we are to circumvent the inevitable conflict that exists between the risks of excess immunosuppression and the consequences inadequate therapy.

### 3. MODES OF GRAFT REJECTION

Graft rejection has historically been classified into hyperacute rejection (HAR), acute rejection (AR), and chronic rejection (CR).

#### 3.1. Hyperacute rejection

HAR is an uncommon form of rejection that occurs when a recipient has preformed antibodies to antigens present on the donor tissue (alloantigen). From a clinical standpoint, HAR is only encountered when a solid organ graft is inadvertently transplanted across an ABO blood group barrier or when the recipient has been sensitized to alloantigen from previous exposures to blood products, past pregnancies, or a failed transplant. Because

HAR is driven by preformed antibodies, HAR occurs within minutes of reperfusion. The principal pathologic manifestation of HAR is hemorrhage and thrombosis within the graft, as antibodies fix complement resulting in an acute loss of vascular integrity. The clinical approach to this problem is based on prevention through appropriate donor-recipient matching, although under unusual circumstances, techniques such as plasma exchange, rituximab (an anti-B-cell agent) and splenectomy may be employed.

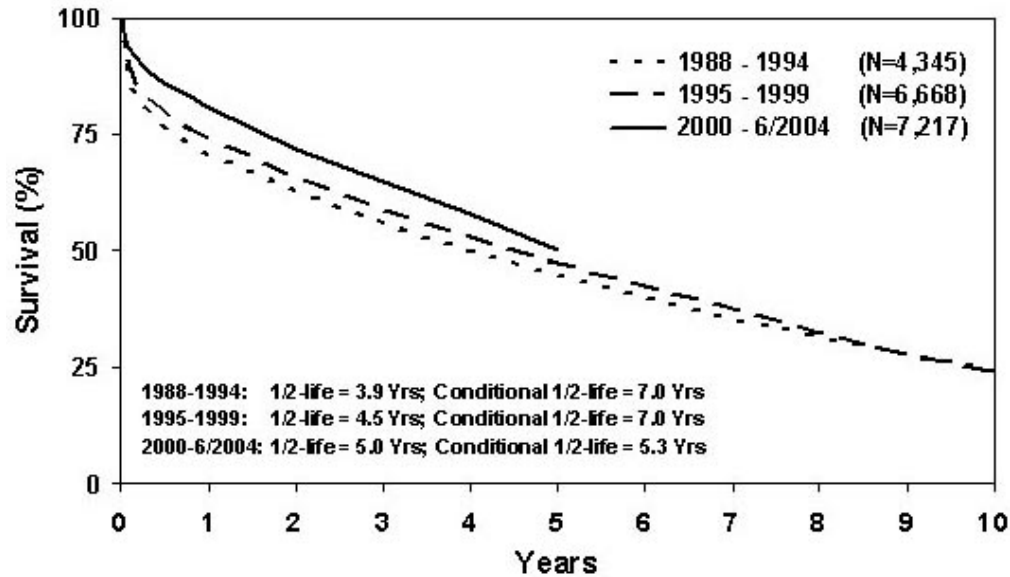
#### 3.2. Acute rejection

AR is a ubiquitous form of graft rejection that occurs primarily due to the development of a robust T-cell response of the recipient to the graft. In the absence of immunosuppression, most grafts would succumb to AR within a matter of days to weeks. The principal histologic manifestation of AR is the presence of perivascular and intraparenchymal mononuclear cellular infiltrates. Over the past quarter of a century, considerable progress has been made in the prevention and treatment of AR, primarily through the use of immunosuppressive drugs. As discussed below, AR is to a great extent a time-limited phenomenon, which occurs primarily in the first post-transplant year. Advances in the treatment of AR, as well as improvements in surgical technique and post-operative management, are mainly responsible for the reduction in early mortality that has been observed over time (Figure 1).

#### 3.3. Chronic rejection

Chronic rejection (CR) is the biggest obstacle to successful lung transplantation. The process of CR, regardless of the organ involved, is histologically characterized by a fibrotic replacement of the organ parenchyma as the final common pathway following a repeated sequence of immune and non-immune injury and inflammation. Gradually over time, these repeated insults to the graft lead to an exhaustion of beneficial repair mechanisms, ultimately resulting in fibrosis. This fibrotic scarring seems to have a predilection for narrowing and obliterating the endothelial- and epithelial-lined tubular structures in a graft.

The principal manifestation of CR in the lung is a pathological entity known as obliterative bronchiolitis (OB). Over half of all lung transplant recipients develop OB within 5 years of transplantation, and OB is currently the leading cause of graft loss and mortality after the first post-transplant year (1, 2). Pathologically, OB is a concentric fibrosis of the membranous and respiratory bronchioles that results in an obstructive defect to airflow (3). In chronically rejecting lung allografts, this fibrosis can also extend into the peribronchiolar interstitium and may involve the pulmonary vasculature in a process similar to cardiac allograft vasculopathy. Owing to the limited quantity of tissue that can be retrieved by bronchoscopy, the clinical diagnosis of OB and chronic lung rejection is often made by identifying the bronchiolitis obliterans syndrome (BOS), characterized by progressive limitation in spirometric airflow (4) and by correlative radiographic findings (5).



**Figure 1.** Adult lung transplantation Kaplan-Meier survival by era (redrawn from [1]).

Unfortunately, CR is clearly a multifactorial disease, and the immunologic mechanisms underlying its development are still poorly understood. If one examines the slopes of the survival curves in Figure 1 after the first post-transplant year, it becomes apparent that very few strides have been made in addressing the problem of CR, which is the principal contributor to the 6% annual fall-off in survival of lung transplant recipients (1).

#### 4. MECHANISMS OF GRAFT REJECTION

The CD4<sup>+</sup> T cell plays a pivotal role in orchestrating the immune response to an allograft. These helper T lymphocytes direct the cytotoxic activity of CD8<sup>+</sup> T cells and support the B lymphocyte in the production of alloantibody that can fix complement and mediate antibody-dependent cellular cytotoxicity. CD4<sup>+</sup> T-cell activation also initiates a plethora of immunologic events that lead to the production of a variety of cytokines and chemokines, which can either be injurious or beneficial to the graft. Over the last decade, significant progress has been made in understanding the pathways by which alloreactive T cells recognize foreign antigen and become activated.

##### 4.1. Alloantigen recognition

Two recognition pathways (which are not mutually exclusive) have been described: direct allorecognition and indirect allorecognition (6). Direct allorecognition occurs when the recipient's CD4<sup>+</sup> T cell recognizes intact donor major histocompatibility complex (MHC) molecules on antigen presenting cells (APCs) of donor origin. This mode of allorecognition has long been the focus of transplantation immunology, and appears to be particularly relevant to the process of AR. In the immediate post-transplant period, there are many viable donor APCs in the graft, available for direct recognition by recipient T cells. These donor APCs (expressing allogeneic

MHC class II antigens) and the high precursor frequency of recipient T cells capable of recognizing allo-MHC molecules without the requirement of priming make the direct alloresponse the dominant mode of immune recognition underlying AR (7). In fact, in a recent murine study where donor-type APCs were replaced by recipient-type APCs by bone marrow transplantation prior to allografting, AR was markedly attenuated, while CR persisted (8).

Over time, a downregulation of the direct alloresponse occurs as the population of donor APCs residing in and emigrating from the graft diminishes, leading to a diminution in the incidence and severity of AR. At this point, the other pathway of T-cell activation, indirect allorecognition, becomes dominant (9). Indirect allorecognition occurs when recipient APCs process alloantigens in the conventional manner, and the resultant peptide fragments are presented to recipient CD4<sup>+</sup> T cells in the context of self-MHC molecules. Because the T-cell precursor frequency for indirectly recognized allopeptides is several orders of magnitude less than that for directly recognized MHC antigens, the indirect immune response represents a more indolent immune reaction, consistent with the natural history of CR. However, because virtually all of the parenchymal cells of an allograft can serve as substrate for antigen processing by recipient APCs, indirect allorecognition is an ever-present mechanism in the long-term alloresponse of a host to a graft.

It is only in the last several years that scientists and clinicians have begun to appreciate the critical role that indirect allorecognition plays in the pathogenesis of CR. The first experimental evidence that indirect allorecognition was sufficient to mediate graft rejection came from studies of skin grafting between MHC class I- and class II-knockout mice in which the skin grafts were rejected in an experimental construct where direct

allorecognition was not operative (10). Analysis of lymphocytes extracted from recipient nodal tissue in these experimental constructs confirmed the presence of a small population of self-restricted T lymphocytes that were able to respond to donor-derived peptides (11). More recent experimental studies have shown that pre-transplant immunization with donor-derived MHC allopeptides can accelerate heart and lung allograft rejection and vasculopathy in both murine (12 - 14) and porcine systems (15, 16). In the clinical arena, many investigators have demonstrated a positive correlation between T-cell reactivity to synthetic allopeptides derived from the donor MHC and chronic graft rejection and dysfunction. This general finding has been seen in recipients of renal (17-19), heart (20), and most recently lung allografts (21).

### 4.2. Generation of an immune response

In 1970, Bretscher and Cohn proposed a multi-signal model of lymphocyte activation (22). This model was originally described in terms of B lymphocyte activation, but later evolved to describe the activation of T cells as well. In the context of organ transplantation, lymphocyte activation first requires the recognition of foreign antigen via one of the modes of allorecognition discussed above. The binding of the T-cell receptor (TCR) with foreign antigen presented in the context of MHC molecules is commonly referred to as 'Signal 1'. Next, a variety of complementary molecules expressed on the APC and the T cell must associate. This process ('Signal 2') is more commonly known by the term 'costimulation' (23). Finally, a third stimulus ('Signal 3') for full immune activation is provided by the induction of stimulatory cytokines (principally interleukin-2 [IL-2]), which act in both autocrine and paracrine fashions to promote the clonal expansion and differentiation of T cells specific for the alloantigen that was encountered.

Following immune activation, AR and CR rejection proceed through a variety of redundant effector mechanisms. Activated dendritic cells secrete IL-2 and other pro-inflammatory cytokines, which in turn promote the differentiation of CD8+ T cells into cytotoxic lymphocytes. IL-2 also promotes the differentiation of CD4+ T cells into a pro-inflammatory (Th1) phenotype that is capable of recruiting and inducing a variety of other immune effector cells and supporting the production of alloantibody. These events ultimately lead to the chemoattraction and activation of macrophages, natural killer cells, and the production of alloantibody.

Currently, the majority of immunosuppressive regimens in clinical use exert their effect either 1) by blocking the pathways involved in the clonal expansion of alloreactive T cells, or 2) by cytoreducing the T-cell population, so as to reduce the intensity of the immune response.

## 5. CURRENT IMMUNOSUPPRESSIVE STRATEGIES

Immunosuppression for lung transplantation can be considered under three clinical contexts: 1) maintenance

immunosuppression, 2) induction therapy, and 3) anti-rejection treatment.

### 5.1. Maintenance immunosuppression

While there has been some recent interest in identifying that small subset of patients who become tolerant to their graft, the vast majority of thoracic organ transplant recipients are maintained on life-long pharmacologic immunosuppression. For almost two decades, triple-drug therapy with a calcineurin inhibitor (CNI), an antimetabolite, and a corticosteroid has been the standard of care in maintenance immunosuppression. Like most other multi-drug regimens, the clinician's intent is to take advantage of drug synergies, while limiting the toxicity of any single agent. Each of the components of standard triple-drug therapy will be considered in turn below.

#### 5.1.1. Calcineurin inhibitors

Currently, two CNIs are in clinical use, CsA and tacrolimus (TAC). CsA is a cyclic polypeptide derived from the fungus *Tolypocladium inflatum*, and exerts its effects by interfering with IL-2 gene transcription, thereby limiting the clonal expansion of activated T cells. CsA was approved for clinical use by the FDA in 1983, and gained widespread acceptance following strikingly positive results in several clinical trials in renal transplantation. In particular, CsA dramatically decreased the incidence of AR, without the myelosuppressive properties of other existing agents.

TAC (also known as FK506) is a macrolide antibiotic that differs structurally from CsA, but has a mechanism of action that is quite similar to that of CsA. TAC entered clinical use in 1995, and has been shown to be more effective than CsA in reducing AR in renal allografts (24-27). In the arena of lung transplantation, TAC became the preferred CNI in the year 2000, based on data from the Scientific Registry of Transplant Recipients (SRTR) (28). Currently, TAC accounts for 70% of all CNI-based maintenance immunosuppression, both at discharge and at 3 years post-transplant (28). This preference for TAC is supported by the extrapolation of data from other solid-organ transplants, and is at least not refuted by interim results from a prospective two-center European trial comparing the efficacy of CsA and TAC, when used in conjunction with mycophenolate mofetil (MMF) (29).

Despite a growing clinical preference for the use of tacrolimus in maintenance therapy for lung transplant recipients, there has been renewed interest in utilizing cyclosporine as an inhalational agent. The obvious theoretical advantage of this approach is that it should be possible to achieve high intragraft levels of immunosuppression without significant systemic side effects. In a recent single-center, double-blind, placebo-controlled trial, inhaled cyclosporine did not reduce the rate of acute rejection, but it did improve survival and extend periods of chronic rejection-free survival (30). Similar studies involving the safety and efficacy of inhaled tacrolimus are currently underway.

### 5.1.2 Antimetabolites

Antimetabolites have also been a mainstay of maintenance immunosuppression since the early days of transplantation, when 6-MP was brought into clinical use. Currently, two nucleotide analogs are in common clinical use, AZA and mycophenolate mofetil (MMF).

Murray and colleagues brought AZA into the clinical arena, which has a greater margin of safety than 6-MP, into clinical use in 1963 (31). AZA undergoes *in vivo* reduction to 6-MP, which is able to inhibit both RNA and DNA synthesis, leading to a decrease in the proliferation of reactive immune cells. Multiple studies have shown that AZA has potent anti-inflammatory and immunosuppressive effects, and is especially useful in blunting AR when given in conjunction with a CNI.

MMF, the morpholinoethyl ester of mycophenolic acid, is a pro-drug that is rapidly converted by plasma esterases into mycophenolic acid after oral administration. It overtook AZA in clinical usage in 2000, based on SRTR data (28). MMF inhibits the proliferation of T- and B cells, and the production of antibody (32). The positive effect of MMF on early renal allograft rejection has been demonstrated in three randomized, double-blind clinical trials (33-35). All three studies revealed that MMF lowered the incidence of acute rejection at six months by approximately 50 percent. However, three years after transplantation, only a limited beneficial effect of MMF on graft survival was observed in these trials. Using data from the U.S. Renal Transplant Scientific Registry, Ojo and colleagues showed that MMF significantly reduced the incidence of chronic allograft failure in renal transplantation (36). The retrospective analysis on renal transplant recipients, who were either treated with MMF (n=8,435) or with azathioprine (n=48,436), demonstrated that the incidence of chronic allograft failure was reduced by 27% (risk ratio 0.73,  $P<0.001$ ), and this effect appeared to be unrelated to the effect on acute rejection (36). This latter result is in agreement with experimental data from rodent studies indicating that MMF prevents chronic rejection (37). MMF, in combination with sirolimus (SRL), has recently been shown to attenuate the progression of bronchiolitis obliterans syndrome in lung transplant recipients with established OB following conversion from CNI-based immunosuppression (38).

### 5.1.3. Mammalian target of rapamycin inhibitors

Mammalian target of rapamycin (mTOR) inhibitors that are in clinical use include sirolimus (SRL) and everolimus (ERL). This new class of drugs acts by interfering with T-cell proliferation by blocking a kinase causing cell cycle arrest (39, 40). As such, these new agents have found utility in replacing the traditional antimetabolites (AZA and MMF) in triple-drug regimens. It has been shown in heart transplant recipients that treatment with cyclosporine and everolimus resulted in significantly less CR at 12 months, compared to treatment with cyclosporine and azathioprine (41). This combination of cyclosporine and everolimus has been recently shown in a randomized double blind clinical trial to significantly slow the loss of lung function in lung transplant recipients

at 1 year (42). In another randomized trial, rapamycin was shown to halt progression of established and severe CR in heart transplant patients, resulting in fewer adverse cardiac events over a two-year span (43). More recently, in a multicenter, prospective, randomized, double-blind study, everolimus was compared to mycophenolate mofetil in the context of triple-drug maintenance therapy after lung transplantation. At 12 months, the everolimus-treated group had a lower incidence of pulmonary function decrements, BOS, and acute rejection. However, at 24 months, only the incidence of acute rejection remained significantly less in the everolimus group (44). Longer follow-up and confirmation of these results will be forthcoming.

### 5.1.4. Corticosteroids

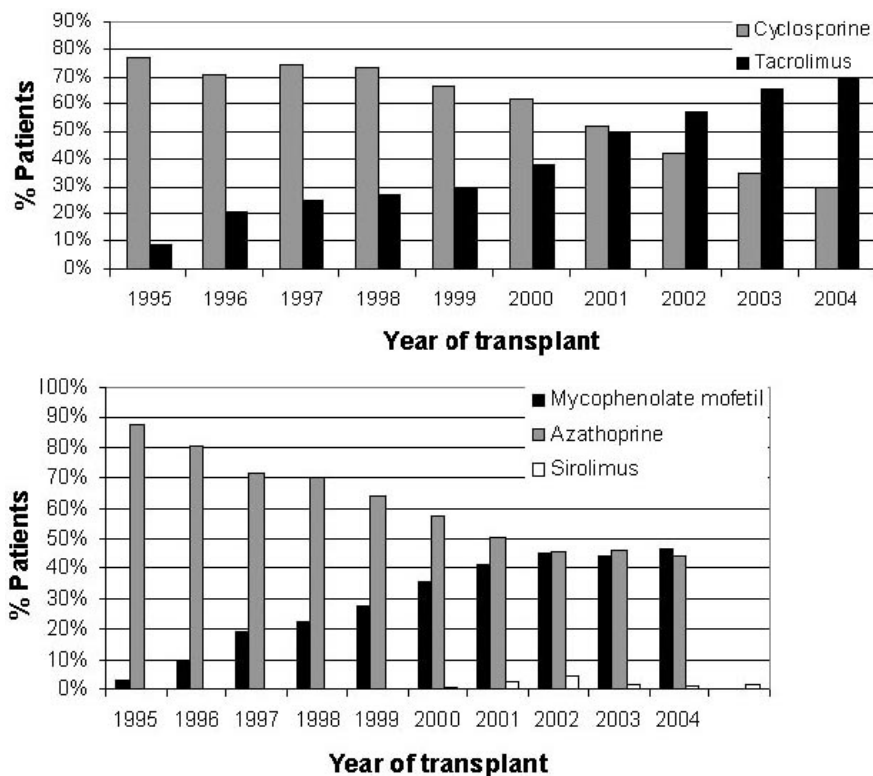
Corticosteroids have been in clinical use for almost 80 years. In the area of solid organ transplantation, steroids exert a variety of anti-inflammatory and immunosuppressive effects. Cytosolic corticosteroid receptors are expressed ubiquitously, and their translocation to the nucleus results in the interruption of multiple steps in the presentation of antigen, the production of cytokines, and the initiation of a proliferative response. Pharmacologic doses of steroids also have a redistribution effect on circulating lymphocytes and monocytes, causing them to be sequestered into lymph nodes and secondary lymphoid organs. The resulting cytopenic state greatly decreases the extent to which circulating lymphocytes encounter alloantigen. Steroids also reduce the production of prostaglandins and other inflammatory mediators, which otherwise would enhance the recruitment of immune effector cells to the graft and up-regulate the expression of alloantigen and costimulatory molecules.

Nearly all lung transplant recipients (98-100%, based on SRTR data [28]) are maintained on some level of steroid therapy. However, in renal allografting, there has been considerable interest in steroid reduction and steroid withdrawal, typically beginning in the middle of the first post-transplant year. Often the early withdrawal of steroids in renal recipients has been in association with the use of polyclonal or monoclonal anti-lymphocyte antibody induction therapy. Aggressive steroid withdrawal has not been broadly attempted in the field of thoracic transplantation, where the consequences of graft loss are dire. Nonetheless, steroid withdrawal has been successfully achieved in a number of cardiac allograft recipients, although it is difficult to determine *a priori* who will benefit from such an attempt (45).

Steroid avoidance, at least up to 2 years, was achieved in a group of lung transplant recipients that had induction therapy with ATG or Campath-1H (humanized anti-CD52 monoclonal antibody) followed by minimal post-transplant immunosuppression with tacrolimus monotherapy or near monotherapy (46).

Maintenance immunosuppression for lung transplantation at discharge has evolved over the last 10 years with TAC/MMF combination with corticosteroids being the most common regimen having taken over from

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**Figure 2.** Trends in maintenance immunosuppression prior to discharge for lung transplantation: a) calcineurin inhibitors and b) antimetabolites.

CsA/AZA combination (28) (Figure 2). This pattern is maintained up to 3 years post-transplant (Figure 3).

### 5.2. Induction therapy

Induction agents consist of several classes of antibodies that exhibit protective effects (principally from AR) when administered in the peri-transplant period. Polyclonal and monoclonal anti-T-cell antibodies have existed throughout the modern era of solid-organ transplantation, and exert their effect principally by cytoreducing alloreactive T-cells at a time when there is a high passenger (donor) leukocyte (APCs) load. The long-term utility of these cytolytic agents is limited by the tendency of patients to develop neutralizing antibodies to these animal-derived proteins. More recently, several anti-IL2 receptor antibodies, which inhibit IL-2 mediated T-cell proliferation, have been approved for clinical use. Over the past decade, there has been a tremendous increase in use of induction therapies. Currently, approximately half of all lung transplant centers routinely use some form of induction therapy, with anti-IL-2 receptor antibodies and polyclonal antibodies being the agents of choice (28) (Figure 4).

#### 5.2.1. Polyclonal antibodies

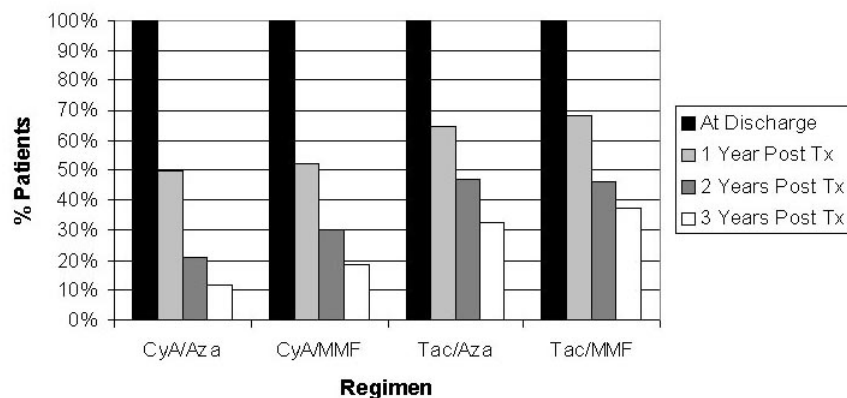
Polyclonal antibodies are derived from animals that have been immunized with human lymphocytes. Currently, two preparations (an equine anti-thymocyte globulin (ATG) and a rabbit-derived ATG) are available for clinical use. Polyclonal antibodies have the dual action of

depleting circulating T cells and modulating the expression of cell surface receptors in a manner that renders the T cell inert. These agents are generally well tolerated on administration, with the exception of some febrile reactions. However, these antibodies have a low specificity to the immunizing antigen (the T lymphocyte), and therefore can have immunosuppressive and leukopenic effects in excess of what are clinically desired. Patients receiving polyclonal antibodies are also at a slightly higher risk of infections and malignancies due to decreased immune surveillance.

#### 5.2.2. Monoclonal antibodies

Monoclonal antibodies are derived from the fusion of a murine myeloma cell with an antibody-producing B cell to create an immortalized hybridoma cell line that produces an antibody with a single specificity. At present, muromonab CD-3 (OKT3) is the only classic monoclonal antibody in clinical use, and its initial popularity is waning rapidly. The principal drawback of OKT3 is its propensity to cause a 'cytokine release syndrome' upon initial administration. This syndrome is characterized by the presence of a systemic inflammatory state with an attendant loss of capillary integrity. The cytokine release syndrome is particularly troublesome in pulmonary transplantation, where the allografted lungs have already been injured to some degree by ischemia and reperfusion. Like all animal-derived antibodies, OKT3 is recognized as a foreign protein by the host immune system, which can respond with the formation of neutralizing

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**Figure 3.** Percentage of lung transplant patients still on original discharge regimens at 1, 2 and 3 years post-transplant, for the four most common regimen in 2001.

antibodies that can lead to serum sickness and make prolonged or repeated administration ineffective.

### 5.2.3. Interleukin-2 blocking antibodies

Two new anti-interleukin(IL)-2 receptor antibodies are also available for clinical use, daclizumab and basiliximab. Like several other immunosuppressive drugs, these antibodies inhibit T-cell proliferation by blocking the autocrine and paracrine actions of the IL-2 signal. These monoclonal antibodies also are similar in that much of their murine protein structures have been replaced with human amino acid sequences through genetic engineering. This greatly reduces the antigenicity of these antibodies, extending their pharmacologic half-lives, and preserving their efficacy on repeat administration. Both of these agents have been shown to reduce AR in large studies of renal allografting (47). On the contrary, a recent retrospective analysis of 335 lung transplant patients from a single center, comparing anti-thymocyte globulin with daclizumab as induction therapy agent demonstrated ATG induction to be superior to daclizumab induction in the reduction in the incidence and severity of acute cellular rejection (48).

## 5.3. Anti-rejection treatment

### 5.3.1. Acute rejection

The treatment of suspected or confirmed episodes of AR is generally one of intensification of immunosuppression. In addition to optimizing CNI levels and antimetabolite therapy, most clinicians will treat AR initially with a 'steroid pulse' typically consisting of three days of high-dose intravenous corticosteroids (*i.e.*, methylprednisolone), followed by a slow steroid taper back to previous levels with close clinical monitoring. Refractory AR is typically treated with the use of induction agents, with IL-2 receptor antibodies and rabbit-derived ATG being the most common choices. Other less common approaches to treating refractory AR include the use of total lymphoid irradiation and photopheresis.

### 5.3.2. Chronic rejection

The treatment of CR in lung transplantation remains the greatest challenge to the field. As mentioned

earlier, much clinical success has been made in the prevention and treatment of AR, but little progress has been made in the treatment of CR. While an immune disparity is the *sine qua non* of CR, we now know that a large number of antigen-independent, *i.e.* non-immunologic factors can exacerbate CR (Table 1). In fact, the multifactorial nature of CR is the principal reason that CR has been so refractory to treatment. The scant progress that has been made in the treatment of CR, unfortunately, has focused on the prevention of vasculopathy, which is not the dominant clinical manifestation of CR in the lung. It remains to be seen whether treatments that reduce vasculopathy and parenchymal scarring in other solid-organ allografts will have a similar effect on OB.

When faced with CR in the lung transplant recipient, most clinicians attempt to both intensify and modify the patient's immunosuppression regimen (59). For patients maintained on older drugs (*e.g.*, AZA and CsA), this may entail switching to TAC and/or MMF. Several small studies have shown stabilization of CR with such changes (38, 60). However, these studies suffer from a lack of prospective control; and the transient stabilization of lung function typically observed might be the result of intensified medical care in general, rather than being attributable to a single pharmacologic manipulation.

A variety of other experimental agents in the treatment of CR are currently under investigation in a number of animal models (Table 2). While these agents may hold some clinical promise, it is more likely that CR will remain a persistent problem until the immunologic basis of CR can be circumvented.

## 6. CHANGING THE PARADIGM OF IMMUNOSUPPRESSIVE THERAPY

While it is likely that there will continue to be small incremental advances in pharmacologic immunosuppressive therapy, immunosuppression in the classic sense will always render the patient at risk for AR, CR, opportunistic infection, and malignancy. In the long run, success in organ transplantation is likely to be

**Table 1.** Contributors to chronic lung allograft rejection

Factor	References
Acute Rejection	1, 49, 50, 51
Ischemia-reperfusion injury	1, 52
Brain death of organ donor	53, 54
Toxicity of CNIs	55
Viral infections	56, 57
Gastroesophageal reflux	58

**Table 2.** Novel pharmacologic treatment of chronic rejection

Agent	Mechanism of Action	Studies	References
Leflunomide and analogs	Inhibition of pyrimidine synthesis	Pre-clinical: rat renal allografts Clinical: chronic nephropathy	61-64
Pirfenidone	Inhibition of TGF- $\beta$ 1 mediated fibrosis and fibroblast proliferation	Pre-clinical: murine heterotopic tracheal allograft	65
Carbon monoxide, Cobalt-protoporphyrin, Heme-oxygenase gene therapy	Anti-oxidant, anti-platelet, anti-apoptotic, and vasodilatory effects	Pre-clinical: rat renal allograft, mouse/rat aortic allograft models	66-68
M-T7 anti-MIG/CXCL9	Modulation of chemokine function	Pre-clinical: rat renal allograft, mouse cardiac allograft	69, 70
STI571 imatinib	Inhibition of PDGF receptor tyrosine kinase	Pre-clinical: rat renal allograft	71
FTY720	Alteration of lymphocyte trafficking	Pre-clinical: rat cardiac allograft	72

dependent on our ability to achieve a state of transplantation tolerance. Transplantation tolerance can be defined as the stable long-term engraftment of an organ in the absence of immunosuppression in a recipient who remains otherwise immunocompetent. Depending on whether tolerance is induced inside or outside the thymus, these mechanisms are further divided in *central* and *peripheral*, respectively (73).

## 6.1. Central tolerance

As compared to the immune response to conventional antigens, where the fraction of reactive T cells is approximately  $1/10^5$  to  $1/10^6$  of the total T-lymphocyte pool, approximately 7% of all T cells can respond to the direct presentation of alloantigen (74). This difference in T-cell precursor frequency suggests that mechanisms sufficient to regulate normal immune function and prevent pathologic autoimmune responses may be insufficient to control the quantitatively larger alloimmune response. Consequently, it is now widely believed that achieving robust central deletional tolerance (where allogeneic dendritic cells residing in the host thymus help to negatively select alloreactive T cells) will be a critical component in any strategy to induce transplantation tolerance.

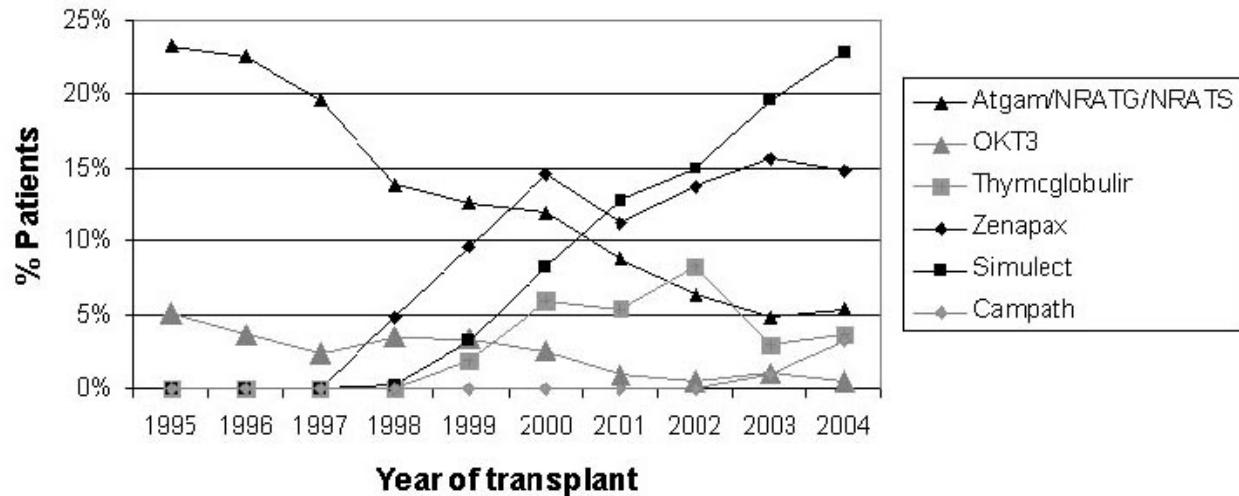
The full application of this mechanism to transplantation depends on the ability to achieve stable mixed-chimerism in a host, which has been accomplished in a variety of rodent (75) and swine (76, 77) models. However, recent studies in non-human primates have shown that transient thymic chimerism may afford an immunologic window during which tolerance can be achieved (78, 79). The principal impediments to the widespread clinical use of this technique include 1) the toxicity of the conditioning regimens necessary to prepare the recipient for engraftment, and 2) the risk of inducing graft-versus-host disease and hematologic malignancies (80). Nonetheless, this strategy has already enjoyed limited clinical success in selected patients (81, 82).

## 6.2. Peripheral tolerance

Peripheral tolerance includes mechanisms such as anergy, regulation (suppression), and peripheral deletion, either through passive cell death or apoptotic activation-induced cell death (83). As discussed previously, full T-cell activation requires both a primary signal from the engagement of the TCR and a second signal that arises from the interaction of a variety of costimulatory molecules. If TCR engagement is present without a concomitant costimulatory signal, the T cell can become specifically non-reactive (anergic) (84) or undergo programmed cell death consequent to cytokine withdrawal (85). Recent data has shown that selective blockade of these costimulatory signals, using either CTLA4Ig (a fusion protein of CTLA-4 and human Ig that competitively binds CD80 and CD86) or a blocking monoclonal antibody to CD154, can induce a state of peripheral tolerance through mechanisms that involve anergy, deletion, and regulation in rodent models of cardiac, hepatic, islet, renal, bone marrow and skin transplantation (86 - 95).

Recent interest has also been shown in the role of dendritic cells (DCs) in the induction of peripheral tolerance. DCs are responsible for priming and maintaining both direct and indirect alloresponses. New research suggests that the immunostimulatory properties of DCs are linked to their state of maturation, and that immature or tolerogenic DCs can induce peripheral tolerance by the induction of regulatory T cells (96, 97) and this provide the basis for targeting or using these cells to promote tolerance to bone marrow transplants or organ allograft (98-100). Moreover, many anti-inflammatory or immunosuppressive drugs commonly used in transplantation, inhibit the maturation of DCs and potentiate their tolerogenicity (101). Plasmacytoid DCs represent a recently characterized DC subset, the precursor of which can enhance allogeneic hematopoietic stem cell engraftment and promote donor-specific tolerance to skin grafts in mice (102). This ability to tolerize the indirect pathway may be particularly important for the prevention of chronic rejection.





**Figure 4.** Trends in induction immunosuppression for lung transplantation, 1995 – 2004.

Finally, there has been recent interest in another peripheral mechanism of tolerance known as immunologic ignorance. Typically, the process of allorecognition occurs in secondary lymphoid organs (such as regional lymph nodes, the spleen, and mucosal-associated lymphoid tissue) after an antigen is processed by an APC and transported to these sites. When an antigen-specific T cell encounters the relevant alloantigen outside this environment, the alloantigen may be ignored (103). This phenomenon suggests that agents that interfere with lymphocyte tracking, such as FTY720 (72), may also hold clinical promise.

## 7. BARRIERS TO TRANSPLANTATION TOLERANCE

### 7.1. Homeostatic proliferation

The depletional strategies increasingly used for induction immunosuppression are known to dramatically alter the circulating lymphocyte population for prolonged periods of time. There is evidence that, upon reconstitution of the lymphocyte compartment, an inverse CD4:CD8 ratio develops and persists for many years (104). The phenotype of these cells appears to be that of memory rather than naïve cells. A key feature of memory T cells is that their threshold of activation is lower and they utilize different costimulatory pathways for functional activation than naïve T cells. Hence these alloreactive memory T cells are resistant to standard immunosuppression and represent the major barrier to transplantation tolerance in humans.

### 7.2. Inhibition of regulatory T cells (Treg)

In most rodent models of transplant tolerance, except mixed chimerism, there is strong evidence that Treg cells play an important role. Their identification in tolerated skin allograft or vascularized organ allografts suggests that these Treg cells function locally to suppress anti-allograft responses (105). Normally, Treg cells respond to antigenic stimulation by inhibiting the proliferation of naïve antigen specific cells by direct cell-to-cell contact or elaboration of

soluble factors. Generation of donor-specific regulatory cells requires that lymphocyte activation occur in an appropriate, conducive milieu. Most immunosuppressive agents inhibit T-cell activation and therefore may simultaneously inhibit the generation of regulatory T cells. Experimental work has shown that calcineurin inhibitors diminish or abrogate regulatory activity, whereas mTOR inhibitors do not (106,107).

### 7.3. Antibody-mediated rejection

The traditional focus of immunosuppression has been to control activation and proliferation of T cells. Ever since crossmatching protocols largely eliminated hyperacute rejection, antibody-mediated processes driven by B cells were considered to be infrequent and unimportant. Recently, however, it has become known that antibody-mediated rejection (AMR) is a frequent component of acute cellular rejection and, less frequently, can occur independently.

The target of AMR for all transplanted organs is thought to be endothelial cells, but the histological picture varies with the transplanted organ (108). Recipients of kidney and heart allograft have the highest incidence of documented AMR. The most reliable histological finding of AMR is demonstration of C4d deposition in capillary endothelium (109).

### 7.4. Natural killer cells

Natural killer (NK) cells are a primary component of the innate immune response and, therefore, do not require prior antigen exposure or sensitization to antigen in order to activate. It is thought that self-MHC antigens prevent NK-cell activation, while the absence of 'self' MHC promotes activation. Teleologically, this mechanism was intended to protect against infectious agents. Consequently, the transplant setting might represent a constant and powerful stimulus for NK-cell activation. NK-cell activation and elaboration of cytokines have been implicated in the pathogenesis of chronic allograft damage

in a variety of solid organs (110). Inhibition of the mammalian target of rapamycin (mTOR) may mitigate the development of these conditions (111).

### 8. CONCLUSION

While we are likely to see continued incremental advances in pharmacologic immunosuppression, it is important to realize that the problem of graft rejection will not be fully solved by approaches that lack antigenic specificity. Our ability to control and direct the immune response is dependent upon the development of a mechanistic understanding of the pathways of rejection and tolerance. Ultimately, the achievement of transplantation tolerance will begin a new era in organ transplantation.

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