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Immunosuppressive therapy after human lung transplantation

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Immunosuppressive therapy after human lung transplantation. C. Knoop, A. Haverich, S. Fischer. ©ERS Journals Ltd 2004.

ABSTRACT: In 2002, equal numbers of lung transplantation (LTx) were performed with or without induction therapy with antilymphocyte antibodies, monoclonal anti-CD3 antibody or anti-interleukin-2-receptor monoclonal antibodies. It remains to be established if induction therapy after LTx is beneficial or deleterious for long-term graft and patient survival.

The vast majority of lung transplant recipients receive a triple-drug maintenance regimen including a calcineurin inhibitor, a cell-cycle inhibitor and steroids. Equal proportions receive cyclosporin A (CsA) and tacrolimus (Tac). There is also a trend to prescribe mycophenolate mofetil (MMF) instead of azathioprine (Aza). Steroid withdrawal is uncommon even 5 yrs after transplantation.

The superiority of Tac over CsA as a maintenance agent has not been established to date, and the administration of MMF instead of Aza in combination with CsA and steroids did not improve graft or patient survival in a recent international, prospective, randomised, controlled trial.

Shift from cyclosporin A to tacrolimus has emerged as the first treatment step of refractory acute rejection followed by high-dose steroids or antilymphocyte agents, total lymphoid irradiation or photopheresis. The treatment of chronic rejection remains deceptive and includes varied strategies such as modification of the maintenance regimen, addition of inhaled immunosuppressants and/or total lymphoid irradiation and photopheresis.

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During the last two decades lung transplantation (LTx) has become a life-saving intervention for patients presenting with end-stage respiratory disease. A successful lung transplant may result in complete restoration of lung function and normal quality of life in these very ill patients. Unfortunately, medium- and long-term results are less spectacular. Indeed, lung transplant recipients may not consider themselves cured as 5-yr actuarial survival only approximates 50% [1]. The two main causes of death after LTx are bronchiolitis obliterans (BO), which is widely believed to be the expression of chronic rejection of the allograft bronchi/bronchioles, and infections [1]. Both complications are a sign of inadequate immunosuppression and, clearly, optimisation of the immunosuppressive strategy is of prime importance if medium- and long-term results are to be improved.

The discovery of cyclosporin A (CsA) has been a corner stone for the success of clinical LTx. LTx performed before the CsA era invariably failed, notably because the immune suppression provided by high-dose steroids in combination with azathioprine (Aza) resulted in prominent anastomotic complications and was insufficient to control acute rejection. As a result of this unfortunate experience and in line with immunosuppressive strategies then applied in renal and heart transplant recipients, the vast majority of patients, who underwent LTx from the early 1980s onwards, received a triple-drug maintenance regimen including CsA, Aza and steroids. Over time, the conservative approach of a triple-drug maintenance regimen has been maintained. In 2002, data from the Registry of the International Society for Heart and Lung Transplantation (ISHLT) indicated that >95% of patients receive a calcineurin inhibitor, 80% receive a cell-cycle inhibitor and >95% receive steroids even 5 yrs after transplantation [2].

This chapter reviews present knowledge on immunosuppressive strategies after LTx, but also highlights unanswered questions that will have to be resolved by multicentre, prospective, randomised, controlled trials.

Acute rejection after lung transplantation

The incidence of acute rejection

The incidence of acute rejection episodes is increased after LTx compared with other types of organ transplantation. Data from a recent international trial including 315 patients

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(receiving a triple-drug regimen including CsA, Aza or mycophenolate mofetil (MMF) and steroids) indicated that the incidence of acute rejection is 54% at 1 yr [3]. In comparison, the incidence of acute rejection at 1 yr is $\leqslant$40%, and can be as low as 17% in renal transplant patients [4].

There are multiple explications for the increased incidence of acute rejection after LTx. First, no prospective human leukocyte antigen (HLA) matching is or can be performed. Second, the lung graft, in contrast to all other transplanted organs, is in permanent contact with the external environment and is thus exposed to various inhaled agents, such as fumes, toxins and infectious agents, which may potentially cause local inflammation and trigger acute rejection. Finally, the lung graft contains a huge amount of donor antigen-presenting cells constantly processing and presenting HLA alloantigens to recipient lymphocytes that initiate a process of immune recognition.

The diagnosis of acute rejection

Acute rejection can be associated with fever, cough, dyspnoea and new adventitious lung sounds. These clinical signs are, however, nonspecific and do not distinguish between rejection and other causes of graft dysfunction. In addition, acute rejection may be clinically silent. Radiological abnormalities are only observed during the first few weeks. Thereafter, chest radiograph or high-resolution computed tomography are mainly unhelpful for the diagnosis of rejection.

Noninvasive diagnosis of acute rejection. Acute cellular rejection and infection typically produce an obstructive ventilatory defect [5–7]. The sensitivity of forced expiratory volume in one second (FEV1) measured at hospital for the detection of these complications ranges from 60–75% in recipients of heart and bilateral LTx [5–7], and from 48–72% in recipients of single LTx [8] when bronchoalveolar lavage (BAL) and transbronchial biopsies (TBB) are used as gold standard. On this basis, it has been recommended that lung transplant recipients perform daily measurements of FEV1 at home with a portable spirometer [9]. However, in a recent study, using daily monitoring of FEV1 and mid-expiratory flow at home with transmission of data to the hospital *via* the internet, the sensitivity of home spirometry for the detection of acute graft dysfunction was only 63% [10]. Thus, lung function is neither very sensitive nor specific for the diagnosis of acute rejection.

Two cross-sectional studies have investigated exhaled nitric oxide (eNO) as a tool for the detection of acute rejection. One study found that eNO increased during acute vascular rejection [11], while the other found an elevation during lymphocytic bronchiolitis but not during acute vascular rejection [12]. A recent study reported elevated levels of exhaled carbonyl sulphide in association with acute rejection, but data showed a large overlap with values obtained in stable patients [13].

Preliminary studies suggest that unlike BAL cellularity, BAL cytokine expression may show characteristic changes during acute rejection, but further studies are needed to confirm these results [14].

In conclusion, there is at present no sensitive and specific noninvasive tool available for the diagnosis of acute rejection, such that histology remains the gold standard.

Surveillance transbronchial biopsies: pros and cons. Owing to the impact of acute rejection on the development of chronic rejection and, thus, survival (see below), 66% of centres, which report data to the ISHLT registry, perform surveillance TBB at fixed intervals during the first postoperative year, in addition to TBB in the setting of new symptoms or signs [15]. The role of regularly scheduled TBB in completely asymptomatic lung transplant recipients, however, remains controversial.

Protagonists argue that clinical examination, chest radiography, spirometry and measures of gas exchange are nonsensitive and nonspecific for the diagnosis of acute rejection (see above). Previous studies have shown that surveillance TBB may show acute rejection histology in 22–73% of clinically and physiologically stable patients [16, 17]. Most importantly, the timely treatment of these silent episodes may eventually prove useful to uncouple the association between acute and chronic rejection [18].

Opponents retort that regularly scheduled TBB will only identify lower grades of acute rejection because grades 3 or 4 will not go without symptoms or signs. The impact of lower grade rejection on the development of chronic rejection, however, is not clear and the appropriate treatment of lower grade rejection remains controversial. Indeed, not all centres will give antirejection treatment for grades 1 or 2 acute rejection. Surveillance TBB will add risks and costs to the follow-up of lung transplant patients.

To date, there has been no prospective study to investigate the impact of surveillance TBB on the incidence of bronchiolitis obliterans syndrome (BOS) or survival. Some centres have even presented data supporting the fact that clinical outcome is satisfactory without surveillance TBB [19].

The questions of whether surveillance TBB should be performed and what is the impact of surveillance TBB on overall survival are clearly unsettled for the time being.

The relationship between acute and chronic rejection

Acute rejection, with a few exceptional cases, does not by itself cause death. However, it is a major determinant of long-term outcome as it (especially severe, recurrent and/or late acute rejection) has been identified as being the most significant risk factor for subsequent development of BOS/BO [20]. That said, the exact relationship between acute rejection and BOS remains unclear at present. Some patients with acute rejection develop progressive BOS without an intervening time interval. In other patients, a long time period intervenes between the occurrence and successful treatment of acute rejection and the development of BOS. Finally, other patients still develop BOS without any diagnosed episode of acute rejection [21–23]. In any case, successful prevention of acute rejection must be the main target of immunosuppression after LTx.

Prevention of acute rejection after lung transplantation

Induction therapy

Rationale for induction therapy. The efficacy of biological agents (table 1) inducing profound T-lymphocyte depletion, such as antithymocyte globulins (ATG) or monoclonal anti-CD3 antibody (OKT3), has led to their prophylactic use during the early postoperative period, a strategy called induction therapy. The rationale of induction therapy is to use the strongest immunosuppressive drugs at the time when the risk of rejection is highest, that is, in the first few weeks following transplantation [24]. Prospective, randomised studies in renal transplant recipients [25] and data provided by renal transplant registries [26] indicate that ATG or OKT3 induction therapy considerably reduces the incidence of acute rejection, and also increases long-term kidney graft survival by ~5% compared

Table 1. – Immunosuppressive drugs, their mechanisms of action and side-effects

Agent	Mechanism of action	Side-effects
ATG	Fixes numerous antigens on lymphoid cells; depletion of circulating lymphocytes	Cytokine release syndrome Leukopaenia, thrombopaenia
OKT3	Fixes CD3 present on T-lymphocytes; depletion of circulating lymphocytes	Cytokine release syndrome Pro-coagulation effect Possible sensitisation with loss of efficacy
Daclizumab	Binds the α-chain of IL-2 receptor; blocks the proliferation induced by IL-2	Unreported
Basiliximab	Binds the α-chain of IL-2 receptor; blocks the proliferation induced by IL-2	Unreported
Cyclosporin A	Binds cyclophylin; inhibits calcineurin; inhibits cytokine gene transcription	Nephrotoxicity Hypertension Hypercholesterolaemia Hypertrichosis Gingival hypertrophy
Tacrolimus	Binds FKBP-12; inhibits calcineurin; inhibits cytokine gene transcription	Nephrotoxicity Hypertension Neurotoxicity Diabetes mellitus Alopecia
Azathioprine	Inhibits purine biosynthesis and lymphocyte proliferation	Leukopaenia
Mycophenolate mofetil	Inhibits purine biosynthesis and lymphocyte proliferation	Diarrhoea Leukopaenia
Sirolimus/everolimus	Binds FKBP-12; inhibits the proliferative response to cytokines and growth factors	Hyperlipaemia Thrombocytopaenia Arthralgia

ATG: antithymocyte globulin; OKT3: monoclonal anti-CD3 antibody; IL: interleukin; FKBP: FK (tacrolimus)-binding protein.

with conventional therapy (CsA (Sandimmune®; Novartis Pharma AG, Basel, Switzerland), Aza and steroids). In patient groups that present a high risk of rejection, such as children, Black patients or patients with high levels of anti-HLA antibodies, the beneficial effect of these agents is even greater, allowing up to a 20% improvement in kidney graft survival after 3–5 yrs.

Data from the ISHLT registry indicate that ~45% of lung transplant patients receive some type of induction therapy (~20% polyclonal antilymphocyte preparations, ~20% anti-interleukin (IL)-2-receptor antibodies, ~4% OKT3) [2]. It has to be stressed that the type of product used, its dosage and the duration of administration vary widely. The rationale for induction therapy after LTx is triple. First, lung transplant recipients *per se* can be considered to be at a high risk of rejection (see above). Second, induction therapy leaves a comfortable time-frame to achieve target levels of calcineurin inhibitors without exposing the patient to the risk of early acute rejection. Third, induction therapy allows renal function to recover from operative stresses, such as hypovolaemia or negative effects of cardiopulmonary bypass without being exposed to the toxic effects of calcineurin inhibitors [27]. The use of induction therapy after LTx, however, remains controversial as its benefits have not been clearly established and there is a potential for increased post-transplant infections, especially cytomegalovirus (CMV) infection, and post-transplant malignancies.

Polyclonal antilymphocyte antibodies. ATG preparations are obtained by injecting rabbits or horses with human thymic cells or lymphocytes and the subsequent isolation and purification of antibodies against human cells. They contain antibodies targeting numerous membrane antigens, some of which are solely present on T-cells, such as CD3, CD4 or CD8, whereas others, like the adhesion molecules CD11b and CD18, are also found on other circulating cells [28]. Initially, the treatment induces a rapid and profound lymphopaenia through several Fc-receptor-dependent mechanisms, such as complement-dependent cytolysis, cell-mediated antibody-dependent cytolysis, as well as opsonisation and phagocytosis by macrophages. In addition, ATG preparations also modulate T-cell function through nondepletive mechanisms.

There has been only one single-centre, prospective, randomised study comparing ATG induction therapy *versus* conventional therapy alone (CsA, Aza, steroids) in 44 single- or bilateral-lung transplant patients. Induction therapy consisted of rabbit ATG (local preparation) 1.5 $mg \cdot kg^{-1} \cdot day^{-1}$ for 3 days. The incidence of biopsy-proven rejection (at least grade A2) was 23% at 1 yr in the ATG group *versus* 55% in the control group. In addition, there was a nonsignificant reduction in the incidence of BOS in the induction group at 3 yrs. The incidences of post-transplant infections and malignancies were not different between the two groups and actuarial survival was identical at 1 and 2 yrs follow-up [29]. These results are consistent with a previous retrospective study indicating that ATG induction may reduce the incidence of acute rejection without resulting in a concomitant improvement in survival [30].

Monitoring of peripheral CD3+ T-lymphocytes reduces the cost of ATG induction treatment, but also helps prevent overimmunosuppression and haematological complications in renal transplant recipients [31, 32]. KRASINSKAS *et al.* [33] applied this concept to 36 lung transplant patients, who received ATG either for induction therapy or for treatment of acute or chronic rejection, and whose absolute CD3+ T-cell counts were maintained between 50–100 $cells \cdot \mu L^{-1}$. The dose of ATG could be reduced from 10–15 $mg \cdot kg^{-1} \cdot day^{-1}$ to 1–5 $mg \cdot kg^{-1} \cdot day^{-1}$ without altering the efficacy of the treatment.

Monoclonal anti-CD3 antibody. OKT3 is a monoclonal mouse antibody directed against the CD3 complex, a series of proteins

associated with the T-lymphocyte antigen receptor (TCR). OKT3 binds to the CD3 complex and induces depletion of circulating T-lymphocytes, as well as modulation of the TCR-CD3 complex. The use of OKT3 for induction therapy after LTx has been very limited because the first dose of this agent frequently causes a cytokine release syndrome, which may induce cardiopulmonary instability [34, 35], and because of concerns regarding a possible increased risk of infections and malignancies.

WAIN *et al.* [23] treated 52 patients with a standard OKT3 dose of 5 $mg \cdot day^{-1}$ for 10 days combined with oral CsA and Aza; maintenance steroids were begun on postoperative day 8. Acute rejection was never seen during OKT3 administration, and 14 of 52 patients did not present any acute rejection episode during a median follow-up of 31 months. In the remaining patients, acute rejection episodes responded readily to steroids or ATG. Freedom from BOS was 69% at 36 months. These authors concluded that OKT3 is safe and effective for induction immunosuppressive therapy in lung transplant recipients, and that its use may limit the incidence of acute and chronic rejection. However, this conclusion is weakened by the absence of a control group [23].

Anti-interleukin-2-receptor monoclonal antibodies. IL-2 serves as a cell-cycle progression signal for T-lymphocytes stimulating their proliferation and differentiation. IL-2 binds to a high-affinity receptor located on T-cells, which is formed by three transmembrane proteins: CD25, CD122 and CD132. One of the earliest events after T-cell activation is upregulation of expression of CD25, after which the two other proteins rapidly associate with CD25, rendering T-cells exquisitely responsive to IL-2. Monoclonal antibodies (mAbs) directed against the IL-2 receptor will selectively block activated T-cells, whereas polyclonal ATG and OKT3 will nonselectively block and deplete both quiescent and activated T-cells. Rodent anti-IL-2-receptor mAbs have been associated with a lack of efficacy due to rapid development of neutralising antibodies and with a severe side-effect profile. The development of chimeric or humanised anti-IL-2-receptor mAbs (keeping from the murine parent antibody only the variable regions or the antigen-binding sequences, respectively) has circumvented these problems and has permitted their introduction into the clinic.

In renal transplant patients, the administration of anti-IL-2-receptor mAbs during the first weeks after transplantation has resulted in a decrease of acute rejection episodes when compared with conventional immunosuppression without any increase in toxicity and/or in bacterial or viral infections [36–38]. In heart transplant patients, the administration of five doses of daclizumab reduced the incidence of acute rejection when compared with a standard regimen including CsA, MMF and steroids, however, the beneficial effect was mainly observed during the first 3 months after transplantation [39].

In lung transplant patients, BROCK *et al.* [40] conducted a 4-yr prospective study enrolling 87 lung transplant recipients split into three groups: group 1 received OKT3, group 2 received ATG and group 3 received daclizumab. There was no difference in freedom from acute rejection or BOS at 2 yrs, and no difference in patient survival. Patients receiving OKT3 (n=30) had significantly more infections, mainly bacterial; this difference became significant only 2 months after transplantation [40]. GARRITY *et al.* [41] retrospectively compared the incidence of acute rejection in 27 patients who received daclizumab induction therapy with the incidence of acute rejection in a historical control group of 34 patients receiving tacrolimus (Tac), Aza and steroids. At 6 months after transplantation, 82% of patients were free of rejection compared with 52% in the control group. There was no increased incidence of infection [41].

Summary. Thus, there is some evidence that induction therapy after LTx may reduce and delay acute rejection episodes and also reduce the incidence of chronic rejection. Unfortunately, there are no large, prospective, randomised, placebo-controlled trials to establish the benefits of induction therapy compared with conventional immunosuppression convincingly and to compare different agents used for induction. From the available data (table 2), induction with ATG seems a good choice, induction with OKT3 is probably less safe, and more data on induction with anti-IL-2 receptor mAbs should be obtained. It also has to be stressed that, currently, there are no data to suggest that induction therapy after LTx should be abandoned.

Maintenance therapy

Maintenance immunosuppression aims at preventing acute rejection. Most lung transplant patients continue to receive triple-drug regimens in the long term [2]. These regimens are thought to be more effective than dual- or single-drug regimens, and the combination of three different drugs is expected to minimise side-effects of individual drugs. With the discovery of new drugs, combinations are now varied, but still always contain an association of a calcineurin inhibitor, a cell-cycle inhibitor and steroids.

Calcineurin inhibitors. Cyclosporin A. In 2002, the ISHLT registry indicated that 40–50% of all lung transplant recipients receive CsA as part of the maintenance immunosuppressive regimen at 1 and 5 yrs after transplantation [2].

The immunosuppressive properties of CsA were discovered in 1976 [45] and this revolutionised the practice of solid organ transplantation [46]. CsA is a cyclic peptide produced by the fungus *Tolypocladium inflatum*. CsA acquires its active form when penetrating into the cell and forming complexes with cytoplasmic proteins, called cyclophilins. The CsA-cyclophilin complexes bind to calcineurin in the cytoplasma. Calcineurin normally acts as a phosphatase that dephosphorylates nuclear regulatory proteins, such as the nuclear factor of activated T-cells, facilitating their passage through the nuclear membrane, where they act as transcription factors for the activation of the promoter regions of various cytokines. The CsA-cyclophilin complexes inactivate the enzymatic activity of calcineurin and, thus, ultimately inhibit the transcription of cytokines, namely IL-2, -3, -4 and -5, interferon-γ, tumour necrosis factor-α and granulocyte/macrophage colony-stimulating factor. The limited amount of calcineurin in immune cells compared with nonimmune cells, and the fact that calcineurin is critical to T-cell activation accounts for the exquisite sensitivity of T-lymphocytes to CsA [47]. In addition, CsA enhances, presumably by a distinct mechanism, the expression of transforming growth factor-β, which on one hand inhibits IL-2-stimulated T-cell proliferation and generation of cytotoxic T-lymphocytes, and on the other hand, exerts a profibrogenous effect.

CsA is a highly lipophilic molecule with wide interpatient and intrapatient variation in absorption and low oral bioavailability. A microemulsion formulation of CsA (Neoral®; Novartis Pharma AG), designed to increase the dispersion and solubility of CsA in the small bowel was introduced in 1996. This formulation reduces the effects of food and the presence of bile on absorption. It presents improved pharmacokinetic parameters compared with the original olive oil-based formulation (Sandimmune®) and assures: 1) a

Table 2. – Prospective, randomised, controlled trials of immunosuppressant drugs after lung transplantation

Comparator	In combination with			Patients n	Acute rejection	BOS	Survival	[Ref.]
ATG *versus* no induction	CsA	Aza	Steroids	44	Acute rejection (c+b) at 1 yr: 32% *versus* 64% Acute rejection (b) at 1 yr: 23% *versus* 55%	Incidence at 3 yrs: 20% *versus* 30%	68% *versus* 73% at 1 yr 64% *versus* 68% at 2 yrs	[29]
ATG *versus* OKT3	CsA	Aza	Steroids	87	Freedom from acute rejection (b) similar at 1 yr (~30%)	Freedom from BOS similar at 1, 2 and 3 yrs	Similar at 1 (~80%), 2 (68%) and 3 yrs (~65%)	[40]
CsA *versus* Tac	No induction	Aza	Steroids	133	Freedom from acute rejection (c+b): 11.5% *versus* 14%	Incidence of BOS: 22% *versus* 38% Delayed onset of BOS in the Tac group	71% *versus* 83% at 1 yr 66% *versus* 76% at 2 yrs	[42, 43]
CsA *versus* Tac	No induction	MMF	Steroids	Inclusion ongoing	NA	NA	NA	
Aza *versus* MMF	No induction	CsA	Steroids	81	Freedom from acute rejection (b) at 6 months: 58% *versus* 63%		82% *versus* 86% at 6 months	[44]
Aza *versus* MMF	Variable induction	CsA	Steroids	315	Acute rejection (c+b) at 1 yr: 54% *versus* 54%	Incidence of BOS at 3 yrs: 25% *versus* 27%	69% *versus* 75% at 3 yrs	[3]

BOS: bronchiolitis obliterans syndrome; ATG: antithymocyte globulin; OKT3: monoclonal anti-CD3 antibody; CsA: cyclosporin A; Tac: tacrolimus; Aza: azathioprine; MMF: mycophenolate mofetil; c: clinical; b: biopsy-proven; NA: not available.

shorter time to maximum blood concentration; 2) a higher maximum blood concentration; 3) a higher area under the concentration/time curve (AUC); and 4) a lower intrasubject variability for time to maximum concentration, maximum concentration, minimum blood concentration (Cmin or C0), AUC, and percentage peak-trough fluctuation [48].

For nearly two decades, CsA dosages have been adapted according to the morning trough level (Cmin or C0). Multiple studies have demonstrated, though, that C0 is poorly correlated with systemic exposure to CsA, as assessed by a full pharmacokinetic profile over 12 h (AUC0–12) or by a pharmacokinetic profile during the first 4 h after administration (AUC0–4) in recipients of kidney, liver and heart transplantation [49]. More importantly, low systemic exposure to CsA indicated by a low AUC0–4 has been recognised as a significant risk factor for acute rejection, whereas high systemic exposure has been associated with increased nephrotoxicity in renal transplant patients [50, 51]. The time-frame within which AUC0–4 target values are achieved also seems to be of prime importance. In a prospective trial, including 55 renal transplant patients, only 3% of patients who achieved a target AUC0–4 of 4,500–5,500 $ng \cdot h^{-1} \cdot mL^{-1}$ within 3 days of transplantation presented acute rejection during the first 3 months compared with 45% of patients who did not achieve this target [52]. Finally, high variability in systemic exposure has been associated with an increased risk of chronic rejection in renal transplant patients [53]. In the light of these observation, methods to assess systemic exposure to CsA in individual patients have been designed in order to optimise clinical outcomes.

The AUC0–12 gives a complete picture of CsA absorption, elimination and patient exposure. It is, however, impractical to obtain in the clinical setting because of inconvenience and cost. It also has to be stressed that the greatest variability in CsA absorption occurs in the first 4 h after administration. It has also been shown that the immunosuppressive effect of CsA is maximal and most consistent around the peak absorption time, which is within 2 h of the dose [54]. Thus, the determination of AUC0–4 will probably reflect most of the differences in exposure between, and within, individuals. However, the routine determination of AUC0–4 is also difficult to apply clinically. Fortunately, it has been established that sparse-sampling strategies may be very good predictors of AUC0–4 [55]. Measuring CsA levels 2 h after the morning dose (C2) is an especially excellent predictor of AUC0–4 in most adult renal, heart and liver transplant recipients [56]. Thus, it is expected that C2 monitoring will reduce the incidence of acute and chronic rejection in solid organ transplant patients on CsA. In addition, C2 monitoring may improve renal function and reduce the incidence of hypertension after transplantation by identifying patients who are receiving excessive doses of CsA. For all these reasons, the use of C2 monitoring is now strongly encouraged and target values for C2 according to the time interval from transplantation have been established for adult renal and liver transplant recipients [49].

Very little data on CsA monitoring after LTx are available. In a randomised trial, TRULL *et al.* [57] found that the AUC0–6 was better predicted by C2 than by C0. DUMONT *et al.* [58] studied 14 lung transplant recipients and showed that the AUC0–12 was best predicted by a sparse-sampling strategy including C0 and C3 [58]. These results, however, may not apply to all lung transplant recipients, in particular those with cystic fibrosis (CF) who may have a different pharmacokinetic profile of CsA, due to the presence of fat malabsorption associated with CF-related exocrine pancreatic insufficiency [59, 60]. Preliminary data from a few small, single-centre studies indicate that C2 is a valuable tool for predicting AUC0–12 or AUC0–4 in CF and non-CF patients

[61, 62]. These data have to be expanded to propose recommendations for optimal therapeutic drug monitoring of CsA in lung transplant recipients, and target values for AUC0–4 and C2 will have to be established.

Tacrolimus. Over the last few years there has been a clear trend to use Tac instead of CsA as part of the maintenance immunosuppressive regimen. In 1999, ~70% of lung transplant patients received CsA and 26% received Tac at 1 yr after transplantation; corresponding values at 4 yrs after surgery were ~60 and 36%, respectively [63]. In 2002, equal proportions of lung transplant recipients were receiving CsA or Tac, 1 and 5 yrs after transplantation [2].

Tac was discovered in 1984 and is a hydrophobic macrocyclic lactone derived from the actinomycete *Streptomyces tsukubaensis*. Tac suppresses the immune system by similar mechanisms to CsA. It binds in the cytoplasm with FK (Tac)-binding proteins (FKBPs), and the Tac-FKBP complexes associate with calcineurin. Tac is 50–100 times more potent than CsA *in vitro* due to differences in partition coefficients and increased binding affinity of Tac to FKBPs [47].

Tac, like CsA, has a narrow therapeutic window and significant systemic side-effects. Absorption after oral administration is poor, with maximum blood concentration occurring 4 h after intake. In contrast to CsA, the absorption of Tac is hindered by food but is completely independent of the presence of bile. The oral bioavailability and maximum concentration of Tac is highly variable between and within patients. The low and variable bioavailability of Tac is caused by its transformation in the gut wall by cytochrome P450 enzymes, and by a countertransport of the parent drug and metabolites by the multidrug resistance transporter p-glycoprotein.

Tac dosage is mainly adapted to C0 levels. The relationship between C0 and AUC varies widely in clinical trials with correlations ranging 0.11–0.92 [64]. Postdose-monitoring strategies will probably be developed in the near future. Some data on postdose-monitoring strategies of Tac after LTx are already available [65, 66]; these have to be expanded.

Cyclosporin A *versus* tacrolimus. Studies comparing CsA *versus* Tac in renal transplant patients have established different facts. First, CsA-Sandimmune® *versus* Tac in combination with OKT3 induction therapy, Aza and steroids resulted in a similar patient and graft survival at 5-yrs follow-up [67]. Second, CsA-Neoral® therapy *versus* Tac in combination with Aza and steroids resulted in a reduced incidence of biopsy-confirmed acute rejection in patients receiving Tac with no differences in patient or graft survival in the long run in a single centre [68], as well as at 6 months in a European multicentre study [69]. A meta-analysis of eight randomised trials comparing CsA with Tac confirmed that immunosuppression with Tac resulted in a significant decrease in episodes of acute rejection compared with CsA-based treatment. This analysis, however, also failed to show any positive effect of Tac on patient or graft survival at 1 yr after transplantation [70].

In heart transplant recipients, two prospective, randomised, open-label, multicentre, and two prospective, randomised, single-centre studies comparing CsA with Tac failed to show a significant reduction of acute rejection and/or a benefit in survival with Tac [71–74].

There are only three studies evaluating the efficacy of CsA *versus* Tac after LTx. KEENAN and co-workers [42, 43] randomised 133 recipients to receive either CsA or Tac combined with Aza and steroids. Less acute rejection episodes and significantly less BOS (38% *versus* 22%) were observed in the Tac group. There was also a nonsignificant trend to improved survival at 2 yrs [42] and a delayed development of BOS in the Tac group [43]. TREEDE *et al.* [75] evaluated CsA *versus* Tac in combination with MMF (2 g·day^{-1}) and steroids in a prospective, randomised, open, two-centre trial, including 50 lung transplant recipients. All patients also received induction therapy with rabbit antithymocyte globulin (RATG) for 3 days. Freedom from acute rejection tended to be higher in the Tac group at 6 and 12 months, but survival at 12 months was similar [75]. The same investigators had previously conducted a retrospective study including 78 consecutive patients receiving: 1) RATG induction, CsA, Aza and steroids (n=34); 2) Tac, Aza and steroids (n=30); or 3) Tac, MMF and steroids (n=12). The number of acute rejection episodes per patient was lower, and freedom from recurrent acute rejection was significantly higher in the Tac groups. However, after 3 yrs, there was no significant difference in the rate of development of chronic rejection in the first two groups. Survival was significantly better in the Tac, Aza and steroids′ group compared with the CsA, Aza and steroids′ group (93% *versus* 71% at 1 yr; 71% *versus* 51% at 3 yrs). The Tac, MMF and steroids′ group could not yet be evaluated for BOS and survival because of a limited follow-up. The significance of these interesting findings is, however, weakened by the small number of patients and the retrospective design of the study [76].

From these studies no firm conclusion can be drawn as to the respective merits of CsA and Tac for the prevention of acute and chronic rejection after LTx. An international, prospective, randomised trial comparing CsA *versus* Tac in combination with MMF (2–4 g·day^{-1} according to mycophenolic acid (MPA) trough levels) and steroids without induction therapy is currently under way. More than 200 patients have been included so far. The 1-yr interim analysis of this trial, due in autumn 2003, will hopefully provide some clues as to which calcineurin inhibitor is the most efficacious and/or less toxic in the setting of LTx. It should be remembered that CsA administration in this trial was guided by C0, a method that has been largely invalidated during the course of the trial (see above). Thus, if CsA turns out to be second choice compared with Tac, a follow-up trial comparing CsA monitored by sparse-sampling AUC strategies or single postdose levels *versus* Tac will probably be necessary.

Cell-cycle inhibitors. Azathioprine. Aza inhibits both deoxyribonucleic acid and ribonucleic acid synthesis, interferes with the precursors of purine synthesis and suppresses *de novo* purine synthesis. It has an effect on proliferation of T- and B-lymphocytes, but does not affect the production of cytokines. It also has an anti-inflammatory action, probably mainly due to its effect on proliferating cells.

Mycophenolate mofetil. The use of MMF instead of Aza has also increased since its commercialisation and testing in renal and heart transplantation. In 1999, 20% of lung transplant patients received MMF at 1 yr and 25% at 4 yrs after transplantation [63]. Corresponding values in 2002 are ~40% at 1 yr and ~35% at 5 yrs [2].

MMF is a pro-drug, a morpholinoethyl ester of MPA, which is the active compound. MPA was initially derived from *Penicillium* sp. cultures in 1896. MPA is a noncompetitive reversible inhibitor of inosine monophosphate dehydrogenase, which is the rate-limiting enzyme in the *de novo* pathway for purine synthesis. Resting lymphocytes rely on the salvage pathway for purine biosynthesis; activated lymphocytes rely on both the *de novo* and the salvage pathway. MPA suppresses T- and B-cell proliferation more potently than that

of other rapidly dividing cells, such as neutrophils and erythrocytes that can use the salvage purine synthesis pathway instead [47].

MMF is highly soluble at the lower pH of the upper gastrointestinal tract and is rapidly absorbed. The liver is the primary location for hydrolysis of MMF to MPA and an inactive metabolite. The inactive metabolite is excreted in the bile; some of it is reconverted to MPA by gut enzymes and undergoes enterohepatic recirculation. This recirculation causes secondary MPA peaks to appear in the plasma 6–12 h postdose.

MPA concentrations are lower in transplant recipients receiving MMF in combination with CsA than in patients receiving MMF with Tac. It is hypothesised that CsA attenuates the enterohepatic circulation of MPA and its glucuronide metabolite [77]. The standard dose of MMF in combination with CsA is thus 3 $g \cdot day^{-1}$, whereas in combination with Tac it is 2 $g \cdot day^{-1}$.

There are large interindividual and intraindividual variations in MPA pharmacokinetic parameters [64]. As with CsA, there is mounting evidence that systemic exposure to MPA, as measured by the AUC, is correlated to clinical events [78]. However, trough MPA levels seem poorly correlated to the 12-h AUC of MPA, so that therapeutic drug-monitoring strategies for MMF will also have to be refined in the near future [64]. Substantial intrasubject variability for AUC and maximum blood concentration has also been demonstrated in the pharmacokinetic substudy of the international trial comparing Aza with MMF after LTx (see below).

Azathioprine *versus* mycophenolate mofetil. The use of MMF has reduced the incidence of acute rejection in renal and heart transplant recipients. In renal transplant patients, a pooled efficacy analysis of three large, prospective, randomised, double-blind studies compared Aza with two different doses of MMF, 2 and 3 $g \cdot day^{-1}$. The incidence of acute rejection episodes at 1 yr was 41% with Aza, 20% with MMF 2 $g \cdot day^{-1}$ and 17% with MMF 3 $g \cdot day^{-1}$. Survival was 88, 90 and 89%, respectively [4]. In heart transplant recipients, a prospective, randomised, double-blind study, including 578 patients comparing Aza (1.5–3 $mg \cdot kg^{-1} \cdot day^{-1}$) to MMF (3 $g \cdot day^{-1}$) combined with CsA and steroids, showed a significant reduction in acute rejection episodes (74% Aza *versus* 66% MMF) and a significantly better survival (89% Aza *versus* 94% MMF) at 1 yr in the MMF group [79].

The role of MMF in the setting of LTx has been investigated in several studies. PALMER *et al.* [44] conducted a prospective, randomised, multicentre study in 81 consecutive lung transplant patients. Patients received CsA, steroids, and either Aza (2 $mg \cdot kg^{-1} \cdot day^{-1}$) or MMF (2 $g \cdot day^{-1}$). The incidence of biopsy-proven acute rejection ($\geqslant$ grade A2) was 58% in the Aza group *versus* 63% in the MMF group at 6 months. Rates of CMV infection were not significantly different at 6 months and survival was identical. The authors concluded that acute rejection rates and overall survival at 6 months were similar in patients treated with Aza or MMF [44]. In contrast, three small, nonrandomised studies suggested a decreased incidence of biopsy-proven acute rejection episodes with MMF and/or a reduced incidence of BOS, and/or a delayed onset of BOS. O'HAIR *et al.* [80] evaluated MMF as the primary immunosuppressant in 13 lung transplant recipients and observed a decrease in the acute rejection rate during the first 3 months compared with historical controls treated with Aza. ROSS *et al.* [81] conducted a two-centre, nonrandomised, concurrent cohort study including 22 patients receiving ATG induction, CsA, steroids and either Aza (1–2 $mg \cdot kg^{-1} \cdot day^{-1}$) or MMF (2 $g \cdot day^{-1}$). This study showed a decreased incidence of acute rejection in the MMF group and a nonsignificant, decreased prevalence of BOS at 12 months in the MMF group, with no significant differences in the incidence of infections [81]. ZUCKERMANN *et al.* [82] treated 38 consecutive patients with ATG induction therapy, CsA, steroids and MMF (2 $g \cdot day^{-1}$) and compared this group with a historical control group that had received Aza (1.5–2 $mg \cdot kg^{-1} \cdot day^{-1}$) instead of MMF. The data obtained at 6 months follow-up also indicated a significant reduction of acute rejection in the MMF group without any difference in the incidence of infections [82]. Thus, data from small studies using mainly historical controls suggest that MMF may be more efficient than Aza for the prevention of acute rejection in LTx, whereas the only prospective, randomised study of Aza *versus* MMF does not suggest any benefit.

Recently, an international trial was conducted comparing a combination of CsA, steroids and Aza (2 $mg \cdot kg^{-1} \cdot day^{-1}$) or MMF (3 $g \cdot day^{-1}$ for 3 months, then 2 $g \cdot day^{-1}$), in lung transplant recipients, in a prospective and randomised fashion. The administration of induction therapy was left to the discretion of the clinicians. The 3-yr analysis of this trial including 315 patients indicates that the incidence of acute rejection, delay to acute rejection, and the incidence of BOS and infections were similar in the two groups. Survival at 3 yrs was 69% in the Aza group and 75% in the MMF group, which was not statistically significant [3].

Thus, at the present time, there are no firm data that indicate the superiority of MMF over Aza in the setting of LTx, and there seems to be no other reason than its more specific mode of action to make MMF the first-choice, cell-cycle inhibitor.

Steroids. Most clinical lung transplant programmes rely on triple-agent immunosuppression including steroids. The use of steroids in the early postoperative phase has been controversial. Most physicians have adopted the use of a moderate dose of steroids; methylprednisolone 1–0.5 $mg \cdot kg^{-1} \cdot day^{-1}$ intravenously for several days before initiating an oral dose of prednisone 0.5 $mg \cdot kg^{-1} \cdot day^{-1}$. The withholding of steroids for the first few days seems to have neither beneficial nor adverse effects on bronchial healing. Steroid administration is gradually reduced to minimise the complications of long-term use [27, 83]. There are, at the present time, no formal reports either on strategies for weaning steroids or on altogether steroid-free immunosuppressive regimens after LTx.

Summary. At the present time, triple-drug maintenance therapy is still the norm after LTx. The benefits that novel therapeutic drug-monitoring strategies for CsA have procured, in terms of prevention of acute rejection and toxicity after renal and liver transplantation, will hopefully be reproduced in the setting of LTx. In any case, it is expected that the administration of CsA using these tools will be fine-tuned in lung transplant patients in the near future.

To date, it is still unknown if primary Tac maintenance therapy may result in better prevention of acute and chronic rejection than CsA, and whether Tac should preferably be associated to Aza or to MMF. It is clearly worthwhile to investigate if novel therapeutic drug-monitoring strategies, such as those developed for CsA, are also applicable to Tac.

MMF does not seem to procure benefits over Aza, at least when given at a dosage of 2 $g \cdot day^{-1}$ in combination with CsA and monitored according to C0.

Weaning of steroids surely cannot be advocated for each and every lung transplant patient in 2002.

Treatment of acute rejection

Uncomplicated acute rejection

Acute rejection is treated by intravenous steroid pulses, specifically by three to five doses of 1 g or 500 mg methylprednisolone per day, usually followed by an increase in the oral prednisone dose to 1.0 or 0.5 $mg \cdot kg^{-1} \cdot day^{-1}$ with a subsequent taper over 2–3 weeks [84].

Ongoing or recurrent acute rejection

Switch from cyclosporin A to tacrolimus. Tac has been successfully used in the treatment of intractable acute, and also chronic, rejection after kidney [85], liver [86] and heart transplantation [87].

Several small studies have assessed the effect of conversion from CsA to Tac on ongoing/recurrent acute rejection after LTx. GRIFFITH *et al.* [88] reported on 13 patients; recurrent acute rejection resolved in eight patients and improved in two patients. HORNING *et al.* [89] studied 14 patients. The switch from CsA to Tac resulted in a marked decrease in the number of episodes of rejection per patient and in the average histological grade of rejection. ONSAGER *et al.* [90] showed reversal of biopsy-proven acute rejection in eight patients and stabilisation of FEV1 in three additional patients after conversion, whereas four patients did not respond to Tac rescue therapy. VITULO *et al.* [91] investigated 20 lung transplant patients; conversion resulted in a reduced incidence and severity of further acute rejection episodes, in steroid sparing and in stabilisation or improvement of lung function.

An international retrospective study (not published) including data from 110 patients with ongoing/recurrent acute rejection indicated that conversion from CsA to Tac resulted in a remarkable reduction of the number of rejection episodes, whether acute rejection was diagnosed by histology or clinically, and in the number of steroid-pulse therapies. Indeed, 94% of patients experienced a decrease in the number of rejection episodes, and only 22% of patients presented documented acute rejection after the conversion from CsA to Tac.

No data are presently available regarding the incidence of BOS after the switch from CsA to Tac for recurrent acute rejection. The switch from CsA-based immunosuppression to Tac-based therapy has become the first step in the treatment of refractory acute rejection [92].

Polyclonal or monoclonal antilymphocyte antibodies. Before the advent of Tac, refractory acute rejection was usually treated with ATG or OKT3 with good clinical outcomes in some cases [93]. However, as a consequence of the multiple studies demonstrating the efficacy of Tac for the treatment of refractory acute rejection and the experience in cardiac transplant recipients, which indicated that Tac is a more efficacious and safer agent than OKT3 [94], these agents have now become second choice for the treatment of refractory acute rejection.

Other treatments. Other treatment modalities for refractory acute rejection include methotrexate (MTX) therapy [95], aerosolised CsA [96], inhaled steroids [97], total lymphoid irradiation (TLI) [98] or photopheresis [99]. However, data concerning these treatment modalities stem mainly from small single-centre studies; the effects of these therapies have not been investigated in large-scale investigations or multicentre trials. A treatment modality, which deserves a special mention, is the infusion of high-dose ($2\ g \cdot kg^{-1}$) intravenous immunoglobulins (IVIg). The efficacy of IVIg for reversing acute humoral rejection in renal and cardiac allograft recipients has been established [100]. IVIg have not been studied formally for the treatment of refractory acute rejection after LTx, but favourable outcomes have been seen occasionally and, compared with other treatment options, this therapy is largely nontoxic.

Summary. First-line treatment of an episode of acute rejection are high-dose intravenous steroid pulses. First-line treatment for ongoing or recurrent acute rejection is a switch from CsA to Tac. Second choice for refractory acute rejection is treatment with ATG or OKT3. In case of failure, high-dose IVIg are worth a trial.

Treatment of chronic rejection

Treatment of established chronic rejection is difficult and deceptive. Current strategies include: 1) changing medications within therapeutic classes; 2) adding inhaled immunosuppressants; 3) augmenting the net immune suppression; and/or 4) applying other immune-modulating therapies. Although each of these approaches has some support, the majority of reports are limited by the small numbers of patients treated, retrospective study design, short duration of follow-up after treatment and/or the absence of a control group.

Modification of maintenance immune suppression

Switch from cyclosporin A to tacrolimus. Several small studies have assessed the impact of conversion from Tac to CsA in patients with established BOS [101–104]. In each study, conversion from CsA to Tac significantly decreased the monthly rate of decline in FEV1 in each BOS group with stabilisation of FEV1 values. A recent retrospective study, pooling data from 13 lung transplant programmes, indicated that there was a statistically significant decrease in the rate of loss of FEV1 after conversion to Tac. Although encouraging, these results are limited by the fact that the rate of loss of lung function in many obstructive lung diseases is nonlinear, with the rate of decline of FEV1 decreasing as airflow obstruction becomes more severe. Therefore, the possibility exists that the same results may have been observed without changing medications. Interestingly, data obtained in patients who had a <19% decrease in FEV1 relative to baseline (pre-BOS group) showed a trend towards an increase in FEV1 values after the switch. This observation suggests that BOS may be partly reversible in some patients when the therapeutic intervention is made early in the course of the disease [105]. The updated classification of BOS, which now includes an early stage defined by a 10–19% decline in FEV1 and/or a >25% decline in the mid-expiratory flow rate, may help identify these patients.

Substituting mycophenolate mofetil for azathioprine. Some centres have substituted MMF for Aza in patients presenting BOS and have reported stabilisation of lung function in these patients [106, 107]. However, these reports concerned a total of 14 patients with no recent follow-up data. Thus, at the present time, there is no strong evidence for this approach.

Addition of inhaled immunsuppressants

Aerosolised cyclosporin A. It is possible that currently available maintenance immunosuppressive medications would prevent chronic rejection in more recipients if they could be given in higher doses. This has led to the concept of delivering these medications directly to the lower respiratory

tract by aerosol inhalation, thereby increasing drug delivery while decreasing systemic drug exposure. In this regard, IACONO *et al.* [108] have reported stabilisation of pulmonary function in seven of nine patients with chronic rejection after treatment with inhaled CsA. However, aerosolised CsA has been consistently unavailable for other investigators and there are no further data at the present time.

Inhaled steroids. In view of the prominent component of airway inflammation in BOS, it is possible that inhaled steroids would be of benefit. No data are available at the present time and large, placebo-controlled studies will be necessary to establish the impact of this approach.

Augmentation of the net immunosuppression

High-dose methylprednisolone and antilymphocyte antibodies. High-dose methylprednisolone is frequently given when the diagnosis of BOS is first evident. Unfortunately, this approach is entirely empirical and its benefits or absence of benefits have not been studied at all. ATG or OKT3 have also been frequently used to stabilise lung function in patients developing BOS before the introduction of novel immunosuppressants, such as Tac to the market. Three retrospective studies indicate that ATG may decrease or arrest the decline of lung function in patients presenting BOS for periods ranging from 3–12 months [109–111]. Significant improvement or long-term stabilisation of lung function have, however, not been reported and prospective, randomised trials evaluating ATG as a treatment for BOS are lacking. Thus, these agents should probably not be considered as a first choice for the treatment of BOS. Moreover, they may potentially induce infectious complications, which may be responsible for further deterioration of lung function.

Methotrexate and cyclophosphamide. MTX has been used to treat patients presenting BOS. The evidence is limited to one retrospective study concerning 10 patients presenting progressive BOS, who received conventional treatment plus MTX. Eight patients were evaluated: two patients improved, five patients stabilised with a follow-up of 6 months and one patient declined despite therapy [112].

Cyclophosphamide (Cyc) has also been used in seven patients to halt chronic rejection. In six of seven patients, FEV1 stabilised or increased [113].

No firm conclusion can be drawn from these small retrospective studies on MTX and Cyc. These drugs present a potentially high rate of toxicity in the already highly immunosuppressed lung transplant patient presenting BOS.

Other immunomodulatory treatments

Total lymphoid irradiation. TLI *via* mantle, para-aortic and inverted Y-field irradiation, delivered in fractions with a total dose of 8 Gy, has been used to induce donor-specific tolerance before transplantation and to treat refractory acute heart, heart-lung or lung rejection [98]. In heart transplant patients, it has even been shown that conversion to Tac or TLI are equipotent strategies for refractory acute rejection [114] and that MTX or TLI are equally effective [115].

DIAMOND *et al.* [116] have assessed the value of TLI in 11 patients presenting BOS. A subset of four patients experienced stabilisation of pulmonary function with a mean follow-up of 11 months. It has to be stressed that a positive response to TLI occurred mainly in patients who had a long time interval between transplant and onset of BOS, a preserved FEV1 at initiation of TLI and no pre-existing pulmonary infection. HABIB *et al.* [117] retrospectively compared conversion to Tac and TLI in the management of BOS stage 2 or 3 in 26 lung transplant recipients. In this series, efficacy of both procedures was equal, but conversion to Tac carried less risk of infection and less mortality. This group thus suggested that conversion to Tac should be used before TLI, at least in patients with correct renal function [117].

Photopheresis. Photopheresis includes a combination of leukopheresis and administration of the photosensitive drug 8-methoxypsoralen followed by extracorporeal photoirradiation with long-wavelength ultraviolet A. This therapy was developed initially to treat patients with erythrodermic cutaneous T-cell lymphoma; its underlying mechanism of action is believed to be modulation of T-cell-mediated immunity.

SLOVIS *et al.* [118] reported on three patients, presenting progressive BOS after single LTx despite steroid pulses and/or ATG treatment, who stabilised after initiation of photopheresis. SALERNO *et al.* [119] studied eight patients with progressive BOS; photopheresis allowed stabilisation of five. VILLANUEVA *et al.* [120] retrospectively reviewed their experience with 14 patients diagnosed with BOS who underwent this procedure. Five of eight patients presenting early BOS (stage 0-p or 1) were stabilised [120].

It has to be stressed that photopheresis does not seem to induce any noteworthy toxic side-effect.

Summary

Treatment of chronic rejection remains one of the most difficult tasks for the physician caring for lung transplant recipients.

Patients on a CsA-based immunosuppressive regimen diagnosed with BOS should probably be switched to Tac, at least for a period of 3–6 months. On the contrary, there are no data supporting a switch from Aza to MMF in this context. High-dose steroid pulses and ATG are still frequently used to stabilise patients unresponsive to the switch from CsA to Tac, which is effective in some. The available data do not support the addition of MTX or Cyc to the maintenance immunosuppressive regime. TLI and photopheresis, especially when initiated early, seem to stabilise some patients with early BOS. These procedures are, however, not so easy to organise in practice and are most often used as a third step when a switch to Tac and/or high-dose steroids/ATG therapy has failed.

Prospective, randomised, controlled trials to analyse the impact of these different therapies on BOS are cruelly lacking at the present time.

Prospects for the near future

Sirolimus and everolimus

Sirolimus (rapamycin) is a hydrophobic, macrocyclic lactone produced by the actinomycete *Streptomyces hygroscopicus*, with a structure remarkably similar to Tac. Everolimus (rapamycin derivative) is a macrolide synthesised to have an enhanced bioavailability compared with that reported for sirolimus. Unlike the calcineurin inhibitors, which inhibit transcriptional activation of early T-cell specific genes and, thus, inhibit the production of T-cell growth factors, sirolimus and everolimus exert their immunosuppressive effects by blocking growth factor-driven cell proliferation of both

haematopoietic and nonhaematopoietic cells, such as vascular and bronchial smooth muscle cells [44].

Preliminary data in animals specifically support the evaluation of these agents in lung transplant recipients, since everolimus prevents acute allograft rejection in rat models [121] and prevents epithelial destruction and luminal obliteration in a porcine heterotopic bronchial allograft model [122].

The safety and tolerability of everolimus in lung transplant recipients has been evaluated in a phase-I, multicentre, randomised, double-blind, two-period, two-sequence, cross-over study. Twenty stable, lung transplant patients (12 non-CF, eight CF) received single doses of everolimus (0.035 or 0.1 $mg \cdot kg^{-1} \cdot day^{-1}$) in combination with CsA, Aza and steroids. Everolimus was well tolerated at both dosages. Headache was the most common side-effect and there was a mild dose-dependent decrease in leukocyte and platelet counts. CF patients had significantly lower peak concentrations than non-CF patient, however, the overall exposure was similar in both patient groups. Concomitant administration of single doses of everolimus did not influence the steady-state pharmacokinetics of CsA, a notable finding given the fact that the two agents are intended to be administered simultaneously as part of the immunosuppressive regimen [123]. This is in contrast with sirolimus, which must be administered 4 h apart from CsA, due to significant pharmacokinetic interaction [124]. Phase-III studies are now ongoing to assess the long-term safety and efficacy of everolimus in lung and heart-lung transplant patients, including those with BOS.

FTY720

FTY720 is a synthetic analogue of a fungal metabolite, myriocin, which shows potent immunosuppressive activity *in vitro* and *in vivo*. FTY720′s mechanism of action is not fully characterised. It induces a significant reduction in the number of circulating lymphocytes, probably by altering lymphocyte trafficking and/or homing patterns through modulation of cell-surface adhesion receptors and ligands [125]. *In vivo*, FTY720 displays marked synergistic effects with CsA and/or rapamycin and, thus, may improve the therapeutic windows of drugs targeting cytokine synthesis or signal transduction.

In a murine airway model, combination treatment with FTY720 and cytotoxic T-lymphocyte antigen 4-immunoglobulin G preserves the respiratory epithelium and prevents obliterative airway disease [126]. As such, this compound is potentially interesting for human lung transplantation.

Conclusion and further prospects

LTx has come a long way since the discovery of CsA in the early 1980s. New immunosuppressant drugs have since been discovered and with each new discovery hopes have been fuelled that it may result in a marked benefit of survival for lung transplant patients. These new drugs have been added to or have replaced agents of the original immunosuppressive regimen combining CsA, Aza and steroids. However, their use has not been able to markedly improve survival; their use has only lessened toxic side-effects and allowed the adaptation of the immunosuppressive regimen to the patient′s comorbidities.

For the last 5 yrs, a huge international effort has been made to give rise to large-scale, multicentre, randomised, controlled trials. The first of these trials, the MMF trial, has now been completed with 315 patients included, and complete data will become available soon. A second trial comparing Csa *versus* Tac in combination with MMF and steroids is well under way. It is of the utmost importance that such trials continue to be organised and that researchers do not go back to small single-centre studies, which most often do not provide a robust answer to the initial question.

Finally, it is believed today that substantial improvement in long-term survival after lung transplantation will probably not come from any of these newly discovered immunosuppressants or their various combinations. Indeed, the authors believe that this goal will only be achieved when: 1) protocols for tolerance induction will become available for clinical solid organ transplantation; and/or 2) immunosuppression will to some extent be adapted according to the recipient′s risk factors for rejection; and/or 3) when the understanding of the pathogenesis of bronchiolitis obliterans syndrome will have progressed far enough to design specifically targeted immunological and/or pharmacological interventions to halt this process.

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