

Minimization or Withdrawal of Immunosuppression in Liver Transplantation

Alberto Sánchez-Fueyo

Liver Unit, Hospital Clinic, University of Barcelona, IDIBAPS, Barcelona, Spain

Abstract

Human liver allografts have a lower susceptibility to rejection than other organs. In addition, in some liver transplant recipients immunosuppressive drugs can be completely withdrawn and these patients are considered as “operationally” tolerant. Accumulated clinical experience indicates that elective immunosuppressive drug weaning is feasible in almost 20% of selected liver transplant recipients. This is associated with an incidence of 12-76% of acute cellular rejection, but these episodes are commonly mild and often resolve by return to baseline immunosuppression, many times without the need to administer steroid boluses. The study of tolerance in liver transplantation has been hampered by the absence of prospective studies correlating the results of immune-monitoring assays and clinical outcome. Thus, we lack a clinically validated treatment-stopping rule capable of predicting the success of immunosuppression withdrawal and this procedure has to be performed on a trial and error basis. The search for an accurate means to identify allograft tolerance among immunosuppressed recipients should become a priority in liver-transplantation research. This information would provide a biologic basis for guiding immunosuppression-withdrawal protocols and for the implementation of tolerance-promoting strategies in liver transplantation. (Trends in Transplant 2007;1:15-23)

Corresponding author: Alberto Sánchez-Fueyo, AFUEYO@clinic.ub.es

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Correspondence to:

Alberto Sánchez-Fueyo
Liver Unit
Hospital Clinic, University of Barcelona, IDIBAPS
Villarroel, 170
08036 Barcelona, Spain
E-mail: AFUEYO@clinic.ub.es

Introduction

Liver allografts are unique in that indefinite survival in the absence of immunosuppressive therapy can be achieved in pigs, rats, and mice¹⁻³. In these animals, acute rejection occurs but resolves spontaneously, and recipients can accept organs from the same donor but not from third-party donors³. The unique immuno-privileged status of liver allografts is also evident in clinical transplantation, and has been exemplified in many clinical conditions such as:

- resistance to positive cross-match;
- irrelevance of human leukocyte antigen (HLA) matching;
- reduced incidence of hyperacute rejection;
- immunomodulating effect of the liver in case of combined hepatorenal grafting;
- occasional spontaneous recovery following a severe rejection episode;
- irrelevance of a single rejection episode in relation to later graft outcome;
- reduced incidence of chronic rejection and reversal of established chronic rejection in 30% of treated patients;
- achievement of very similar clinical results regardless of whether aggressive or simple immunosuppression schemes are employed;
- the ease with which the stage of steroid-free immunosuppression can be reached⁴⁻⁶.

Moreover, successful withdrawal of all immunosuppression is possible in selected patients, as has been shown “accidentally” in noncompliant patients, and purposely sought in patients presenting with posttransplant lymphoproliferative disease (PTLD), life-threatening infections, or in patients enrolled in carefully monitored prospective immunosuppression-weaning protocols. These patients off all immunosuppressive drugs have been shown to remain healthy in some cases for more than 30 years⁷ and are therefore considered as “operationally” tolerant. Altogether it is clear from the aforementioned clinical data that livers exhibit unique tolerogenic properties,

and are therefore the allografts most amenable to immunosuppression minimization or withdrawal and probably to tolerance induction.

The underlying mechanisms of this intrinsic tolerogenic ability are not fully understood and multiple factors have been proposed. The liver produces large amounts of soluble major histocompatibility complex (MHC) class I antigens⁸, and these soluble antigens have been shown to induce apoptosis of alloreactive CD8+ T-cells both *in vitro* and *in vivo*, leading to prolonged transplant survival^{9,10}. The exchange of migratory leukocytes between the graft and the recipient leading to long-term cellular microchimerism has also been proposed as a basis for the acceptance of liver allografts¹¹. However, although donor-cell microchimerism can occasionally be detected in humans long after organ transplantation¹², most studies have failed to establish a link between donor-cell persistence and tolerance maintenance¹³⁻¹⁵. A conceptually different hypothesis involving donor-derived “passenger” leukocytes postulates that tolerogenic dendritic cells (DC), generated from bone marrow-derived precursors contained within the graft, are central in the acceptance of liver allografts by suppressing cytopathic immune responses and promoting regulatory mechanisms¹⁶⁻²⁰. Similar effects could be mediated by other liver-derived antigen-presenting cells, including liver sinusoidal endothelial cells¹⁹ and liver macrophages (Kupffer cells). Furthermore, the size of the liver, its “un-barricaded” anatomical structure, and its anti-inflammatory cytokine microenvironment may add to its tolerogenic properties by promoting immune-cell trafficking and ensuring optimal opportunities for engagement with parenchymal and non-parenchymal liver cells. One of the downstream consequences of these tolerance-promoting mechanisms appears to be the extensive elimination by apoptosis of graft-infiltrating alloreactive T-cells²¹, a phenomenon widely documented in experimental models²²⁻²⁴. Other cellular elements required to ensure liver allograft acceptance in experimental models are naturally occurring CD4+ CD25+ regulatory T-cells. Despite this con-

siderable amount of data gathered from experimental models, none of the abovementioned mechanisms have been unambiguously validated in humans yet.

Definition of allograft tolerance

The use of the term “tolerance” in the field of transplantation is somewhat confusing, and multiple definitions have been employed over time in the clinical literature. From an immunologic point of view, tolerance defines a state of immune non-reactivity towards a specific set of antigens that is indefinitely maintained in the absence of ongoing immunosuppression. In experimental models, tolerance is induced through therapies aiming at the deletion of alloreactive cytopathic T-cells and/or generation of regulatory T-cells, and is formally proved by the demonstration that tolerant recipients accept same-donor second grafts without further immunosuppression, while rapidly rejecting third-party grafts. In addition, in tolerant experimental animals, T-cells often display anti-donor hyporesponsiveness, and in cases of peripheral tolerance in which regulatory T-cells are involved, tolerance can be transferred to naive hosts by T-cell adoptive transfer. These formal demonstrations of tolerance are obviously unsuitable for clinical application. Hence the term “operational” tolerance was chosen to designate the clinical situation in which a graft maintains a stable function without features of acute or chronic rejection and without the need for chronic immunosuppression. All intentional immunosuppression-weaning trials published so far have employed operational tolerance as their clinical endpoint. Although this is correct from a clinical perspective, it should not lead us to assume that operationally tolerant patients are immunologically equivalent to experimentally tolerant rodents. Indeed, we still have a very incomplete understanding of the mechanisms responsible for allograft tolerance in humans, and lack for instance a detailed picture of the nature of donor-specific immune responses in these patients. In fact, even the extent to which operation-

ally tolerant patients constitute an immunologically homogeneous population is unknown. A totally different concept is the notion of minimal immunosuppression, which is commonly applied to identify patients who, after having been treated mostly but not exclusively with potent T-cell depleting antibodies at the time of transplantation, are capable of maintaining stable graft function under the cover of very low doses of calcineurin inhibitors^{6,25,26}. Minimal immunosuppression is also known as “prope” or “near” tolerance, although it is not clear at all whether minimally immunosuppressed patients actually resemble operationally tolerant recipients, and whether they will ever be capable of completely discontinuing immunosuppression without developing rejection.

Clinical experience in immunosuppression withdrawal after liver transplantation

The first observations of drug-free tolerance in liver transplantation (LT) were reported in 1993 by Starzl, who described the cases of six noncompliant patients having normal liver function five to 13 years after transplantation¹². Since this landmark report, an increasing clinical experience has been gathered, although publications regarding immunosuppression withdrawal remain scarce. Critical evaluation of the actual reality of tolerance after LT requires that distinctions be made, first between temporary and definitive immunosuppression withdrawal, and second between non-elective weaning due to noncompliance, which involves non-selected patients, and elective or planned weaning, a slow process addressed to well-selected patients.

Clinical experience with temporary immunosuppression withdrawal

The published experience with immunosuppression discontinuation in the face of life-threatening infections or PTLD has been summarized in table 1. Collectively, the combined published ex-

perience amounts to 108 patients, mostly children²⁷⁻²⁹. One of the lessons that emerge from these reports is that temporary immunosuppression withdrawal is possible and in most cases is not immediately followed by acute rejection. Furthermore, although it is not uncommon for rejection to eventually occur (in around 35% of patients), it can be reversed by resumption or reinforcement of immunosuppression with a very small rate of graft loss. In fact, immunosuppression reinstatement is often not performed until rejection is formally proven. Most likely, inhibition of cellular immunity by severe sepsis or PTLD acts as a facilitator of successful immunosuppression withdrawal. In short, management of posttransplant life-threatening complications employing complete immunosuppression cessation may be warranted, although in most cases immunosuppression needs to be progressively reinitiated as the general condition of the recipient improves. In the group of patients suffering from Epstein-Barr virus (EBV)-related PTLD the proportion of successful immunosuppression weaning appears to be higher than in other circumstances. Whether less-aggressive immunosuppression reduction could achieve similar patient survival outcomes remains an open question.

Clinical experience with elective immunosuppression withdrawal

During the period 1993-2006, results from intentional immunosuppression withdrawal trials were reported by the Pittsburgh, King's College London, Kyoto, Murcia, New Orleans, Rome, and Miami groups^{14,30-35}. Data from these reports are summarized in table 2. Collectively, the accumulated experience amounts to 304 patients. Some of the particularities of each series are discussed below.

Pittsburgh experience

Immunosuppression was electively withdrawn in 95 (33% pediatric) selected recipients grafted between 1992 and 1996. At the time data

Table 1. Temporary immunosuppression withdrawal in liver transplantation

Year	Author	Reference number	No. of patients	Cause of IS withdrawal	Time between LT and IS withdrawal (days)	Follow-up after IS withdrawal	Maintenance IS at the time of IS withdrawal	Acute/chronic rejection	Graft loss due to rejection	Time between IS withdrawal and rejection
1994	Mañez R	27	18 (31)*	Life-threatening infection or PTLD	140 days (median)	17 days (median)	TAC + steroids (11) CsA + steroids (7)	22/11%	0	
1998	Massarollo PC	28	18 (adults)	Life-threatening infection	66 days (median)	32 days (mean)	CsA + steroids + AZA	25/0%	0	mean: 35 days range: 5-102 days
2001	Takatsuki M	41	34† (children)	PTLD in 30	NA	24 months (median)	TAC + steroids	35/3/0%	0	mean: 240 days range: 30-690 days
2004	Hurwitz M	29	38‡ (children)	PTLD in 19; EBV in 19	272 days (median)	51 months (mean)	TAC + steroids in > 90% patients	50/2.6%	2.6%	mean: 107 days range: 7-476 days

*This report provides information from 31 patients, but only 18 of them survived more than 20 days.

†15 patients (44%) remained off IS for a median of 24 months.

‡8 patients (21%) remained off IS for a mean of 4.2 years.

Table 2. Complete elective immunosuppression withdrawal in liver transplantation

Year	Author	Reference number	No. of patients	Selection criteria*	Maintenance IS	Time between LT and weaning (years)	Complete IS withdrawal	Follow-up after IS withdrawal (months)	Acute/Chronic rejection	Graft loss due to rejection
1997	Mazariegos JV	30	95 (30% children)	> 5 years post-LT > 2 years without rejection (biopsy proven)	13.7 % AZA 11.6% TAC 75% CsA	mean: 8.4 range: 1.7-25	19%	median: 35.5 range: 10-58	26 / 0 %	0
1998	Devlin R	14	18 (adults)	> 5 years post-LT Side effects of chronic IS	CsA and AZA	median: 7 range: 5-11	16.7%	120	28/5.6%	5.6%
2001	Takatsuki M	41	26 [†] (children)	> 2 years post-LT Normal liver function tests	TAC	> 2	23.8%	median: 21.9 range: 3-69	12/0%	0
2002	Oike F	40		> 1 year without rejection						
2003	Pons JA	31	9 (adults)	> 2 years post-LT	CsA	median: 5.2 range: 2-8.8	33%	range: 17-24	22/0%	0
2005	Eason JD	32	18 (adults)	> 6 months post-LT Normal liver function tests	TAC	> 0.5	5.6%	12	61/0%	0
2005	Tryphonopoulos P	35	104 (adults)	> 3 years post-LT > 12 months without rejection No autoimmune disease	85% TAC 14% CsA	mean: 4 range: 3.6-4.6	19%	mean: 25.8 range: 11-36	67%/1.9%	0.96%
2006	Tisone G	34	34 (adults)	> 1 year post-LT HCV-RNA positive Absence of rejection or cirrhosis on basal biopsy	CsA	mean: 5.25	23.4%	mean: 45.5 range: 15-44	76.4/0%	0

IS: immunosuppression; LT: liver transplantation; TAC: tacrolimus; CsA: cyclosporin A; AZA: azathioprine; HCV: hepatitis C virus.

[†]In all cases absence of recurrent original liver disease (other than HCV infection) was required for enrollment.

[‡]All patients were living donor liver transplant recipients.

were submitted for publication, results were as follows: 18 (19%) patients were off immunosuppression; 37 (39%) patients were on spaced immunosuppression; 28 (29%) patients underwent acute rejection; and 12 patients were withdrawn from the protocol. There was a highly significant advantage for azathioprine (AZA) or tacrolimus (TAC) baseline immunosuppression at the time of weaning as compared to cyclosporin A (CsA). It is important to note that although 46% of patients had liver-test elevations, acute rejection was present in only 25 of them (26%, 18 cases biopsy proven and seven diagnosed based on high clinical suspicion). The mean delay between the start of weaning and biopsy proven acute rejection was 13.2 months, most rejection episodes were mild, and in all cases they were easily reversed using steroid pulses (83%) or switch to TAC. No patient developed chronic rejection, although in three patients minor duct injuries were detected prompting immunosuppression resumption. All 18 patients off immunosuppression had a significantly improved health-related quality of life, and in patients developing rejection during the weaning procedure the average immunosuppression at the end of a three-year follow-up was less than at their entry in the study. A later report from the same group analyzing peripheral blood obtained from six pediatric liver recipients off immunosuppression showed that, compared to patients under maintenance immunosuppression, successfully weaned patients had increased numbers of potentially tolerogenic plasmacytoid dendritic cells (pDC), and decreased numbers of the theoretically more immunogenic myeloid dendritic cell (mDC) subset (increased pDC/mDC ratio)³⁶. This finding, however, remains to be validated in view of more recent reports showing that both increasing age and chronic immunosuppression diminish peripheral blood pDC numbers^{37,38}.

King's College experience

In 18 adults with an uneventful post-LT follow-up of more than five years, immunosuppression drugs were abruptly discontinued under

strict in-hospital monitoring¹⁴. After a follow-up of more than three years, five patients (27%) remained off immunosuppression, while in the remaining 13 elevated liver tests were detected and immunosuppression was eventually recommenced. Important again was the fact that out of these 13 patients with deranged liver tests only four had acute rejection on liver biopsy, with only one case of severe rejection among them. Acute rejection was easily reversed using steroid pulses (77%) or switch to TAC. In eight patients, liver biopsies revealed a hepatitis-like disorder with a mixed inflammatory portal-tract infiltrate, occasionally also affecting the lobules. Predictors of successful immunosuppression withdrawal were: lower incidence of early post-LT rejection, good HLA matching, non-autoimmune and nonviral primary liver diseases, but not presence of donor microchimerism. In a recent addendum to their first report, out of the five patients successfully withdrawn from immunosuppression, one patient originally transplanted for primary sclerosing cholangitis developed chronic rejection and required re-grafting, while in another one, immunosuppression was restarted due to low-grade rejection³⁹.

Kyoto experience

The Kyoto experience relates to a cohort of 26 pediatric, living, related LT recipients, having one HLA haplotype identity, in whom immunosuppression was electively weaned³³. Immunosuppression was successfully withdrawn in six patients (23%). At the end of follow-up 15.4% of patients had encountered acute rejection (successfully resolved in all cases with reintroduction of TAC or steroid boluses) and 36.5% were still being weaned. In a later update⁴⁰ the number of patients included in elective weaning was increased to 67 with almost identical results (23.8% success and 12% acute rejection). No cases of hepatitis-like disorders were reported. Neither the degree of HLA matching nor early episodes of rejection was found to predict success. In contrast, more recent reports from the same group have described that downregulation of intra-graft

TH1 cytokines⁴¹ and increased numbers of peripheral blood regulatory CD4+ CD25+ T-cells and delta-1 gamma-delta T-cells are associated with the immunosuppression-free tolerant state⁴². The Kyoto group has very recently communicated that in two out of 11 (18%) operationally tolerant liver recipients in whom protocol liver biopsies were performed an average of four years after weaning, substantial bile-duct atrophy was detected; reintroduction of TAC showed histologic recovery⁴³. This important observation should be compared with a previous report from Sebagh, indicating that among patients on maintenance immunosuppression more than 10 years after transplantation and exhibiting normal liver function tests, histologic signs of chronic rejection can be detected in 24% of cases⁴⁴. Altogether, these reports suggest that operationally tolerant liver recipients, similarly to patients requiring ongoing immunosuppression, might not be completely protected from the development of chronic rejection.

Murcia experience

Nine elective patients were included in a prospective immunosuppression-weaning protocol. Three patients could be completely taken off immunosuppression, while significant elevations of liver-function tests occurred in the remaining patients due to acute rejection (two patients) and a mixed inflammatory portal tract infiltrate (four patients)³¹. Liver endothelial cell chimerism was studied in five patients and not found to be associated to successful weaning.

New Orleans experience

The recent report by Eason is unusual in that sustained immunosuppression withdrawal was attained in only one (5.5%) out of 18 patients³². It must be highlighted that in this study immunosuppression weaning was started earlier than in any other study; indeed weaning was offered after just six months post-LT. In addition, the reasons for the discontinuation of the immu-

nosuppression-weaning procedure in some patients are not clearly reported. In this series there was one episode of steroid-resistant rejection that required thymoglobulin administration.

Miami experience

The Miami group investigated the effect of donor bone-marrow infusions administered early postoperatively on the complete withdrawal of immunosuppression at least three years after transplantation¹⁹. Successful weaning was achieved in 19% (20 patients out of 104) regardless of whether recipients had received donor bone marrow (45 patients) or not (59 patients). Two patients developed chronic rejection, and one of them required retransplantation for this reason. No significant differences were found in donor cell chimerism levels (performed in bone-marrow aspirates using PCR flow) between successfully weaned patients and those developing graft rejections. It should be noted that in most patients acute rejection was diagnosed on a clinical basis without the performance of liver biopsy.

Rome experience

This series is also particular in that it relates to 34 hepatitis C virus (HCV) positive patients with recurrent allograft disease³⁴. Immunosuppression withdrawal was successful in eight patients (23.5%). Among the remaining patients, 12 developed acute rejection during the weaning procedure, while in 14 patients acute rejection occurred during the eight months after immunosuppression withdrawal (in one of them rejection was detected 43 months after). No episodes of severe rejection were detected and no treatment other than CsA resumption was required. Remarkably, successful immunosuppression withdrawal was associated with stabilization or improvement of histologic fibrosis. Low CsA trough levels during the first week of LT and steroid-free immunosuppression were both found to be predictors of successful weaning.

Barcelona experience

At Hospital Clínic Barcelona we have recently reported the results of an immune-profiling study performed on a cohort of operationally tolerant liver recipients gathered from University Tor Vergata, UCL-Brussels, Hospital Vall d'Hebró, and our own Liver Transplant Unit. Our data confirm the results from the Kyoto group in terms of the increased numbers of peripheral blood CD4+ CD25+ T-cells and delta-1 gamma-delta T-cells. In addition, our report provides data on a gene expression pattern characteristic of the tolerant state. These results can be regarded as a first step in the search for a diagnostic test of operational tolerance in liver transplantation⁴⁵.

Concluding remarks

The available data indicate that elective immunosuppression withdrawal is possible in 19.4% of recipients. Favorable clinical markers for successful immunosuppression withdrawal appear to be at least two years of post-LT follow-up, low incidence of previous acute rejection episodes, non-autoimmune primary liver disease, and possibly minimized post-LT immunosuppression. There are, however, two main caveats that have to be kept in mind when interpreting these published studies. The first one has to do with the selection criteria employed for enrolment in immunosuppression withdrawal protocols. These criteria have differed depending on the series (Table 2), and in some but not all studies, patients have been selected for weaning precisely on the basis of likelihood of successful immunosuppression withdrawal. For this reason, and in the absence of an intent-to-treat weaning trial, it is difficult to accurately estimate the actual rate of operational tolerance in LT. The second limitation is the absence in many series of follow-up reports providing long-term clinical and histologic data. This information is critical in order to ascertain the robustness and duration of the tolerant state.

The incidence of documented acute cellular rejection during immunosuppression weaning rang-

es from 12 to 76%, but these episodes are in most cases mild, and often resolve by return to baseline immunosuppression with or without administration of steroid boluses. Reassuringly, only two cases of graft loss (due to chronic rejection) among patients involved in immunosuppression-weaning protocols have been reported. Considering the absence of biochemical predictors of rejection, liver biopsies are required at baseline and during the weaning procedure, and maybe even during the follow-up after successful immunosuppression withdrawal. The significance of the high incidence (up to 78% in some series) of hepatitis-like disorders detected in liver biopsies obtained during weaning is not clear. These findings, consisting of portal and lobular necroinflammation, were already reported more than ten years ago by Pappo in long-term LT recipients on maintenance immunosuppression⁴⁶, and probably constitute a form of late rejection, since many improve after immunosuppression resumption. In conclusion, immunosuppression weaning can be considered in stable and well-selected long-term patients and in patients presenting with immunosuppression-related life-threatening complications. However, since no reliable biomarkers are yet able to identify tolerance, this approach still needs to be performed on a trial and error basis. Thus, the generation of a robust, clinically applicable, diagnostic algorithm of allograft tolerance is urgently needed.

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