ANNALS of Hepatology

CASE REPORT

July-September, Vol. 10 No.3, 2011: 361-364

Operational tolerance after liver transplantation, more common than we think: A case report(◆)

Carlos Benítez,* Juan Pablo Arancibia,* Marco Arrese,* Alejandro Soza,*
Pilar Domínguez,** Nicolás Jarufe,** Jorge Martínez,** Rosa María Pérez-Ayuso*

* Department of Gastroenterology, Medical School, Pontificia Universidad Católica de Chile. Santiago, Chile.
** Department of Surgery, Medical School, Pontificia Universidad Católica de Chile. Santiago, Chile.

ABSTRACT

Operational tolerance after liver transplantation has been described in around 20% of the recipients. These patients are able to maintain a normal graft function in the absence of immunosuppressive drugs, thus being free of adverse effects that are common and frequently severe. Here we present a well-documented case of operational tolerance after liver transplantation and discuss current concepts on this topic with emphasis on recent findings that will potentially allow for identifying graft-tolerant patients.

Key words. Operational tolerance. Liver transplantation. Biomarkers. Gene expression.

INTRODUCTION

Attention in improving long-term survival after liver transplantation (LT)[1] has shifted to later complications that limit survival expectancy and are frequently related to the chronic use of immunosuppressive agents (ISA). The most common complications are arterial hypertension, renal failure, diabetes mellitus, increased cardiovascular risk, various types of neoplasias, dyslipidemia, severe infections and neurotoxicity.[2] Since these conditions may impact on patient survival, it would be ideal to achieve an ISA-free state after LT. Over the last twenty years, it has become evident that a significant proportion of LT recipients (20%) develop operational tolerance (OT), defined as normal graft function in complete absence of ISA.[3,5] Here we report a case of OT after LT and discuss relevant concepts on this emerging topic.

Correspondence and reprint request: Carlos Benítez, MD
Departamento de Gastroenterología, Facultad de Medicina
Pontificia Universidad Católica de Chile. Marcoleta # 367. 833-0024, Santiago, Chile. Phone/Fax: 56-2-6397780.
E-mail: cbenitez@med.puc.cl

(◆) This paper was partially funded by the FONDECYT project No. 11100113.

Manuscript received: January 10, 2011.
Manuscript accepted: March 28, 2011.

CASE REPORT

A 28-year-old man developed acute liver failure secondary to a hepatitis A virus infection in November 1996. He underwent an urgent LT and received a cadaveric liver graft from a donor with the same blood group (0 Rh positive). Cold ischemia time was 8 h and 40 min. The patient had an uneventful postsurgical evolution and treatment with ISA was started, including steroids, mycophenolate mofetil and cyclosporine. Ten days after LT, a moderate cholestasis was detected. Liver function tests revealed:

- Total bilirubin (TB) 14.1 mg/dL (0-1 mg/dL).
- Direct bilirubin (DB) 7-04 mg/dL (0-0.2 mg/dL).
- Gamma-glutamyl transpeptidase (GGT) 272 mg/dL (4-50 mg/dL).
- Alkaline phosphatase (AP) 167 mg/dL (45-126 mg/dL).
- Aspartate aminotransferase (AST) 490 mg/dL (10-40 mg/dL).
- Alanine aminotransferase (ALT) 1,480 mg/dL (10-55 mg/dL)
- INR 1.2.

An endoscopic cholangiography revealed a normal biliary tree, and a liver biopsy showed mild cellular rejection that was treated with methyprednisolone 250 mg iv q.d. for three days with good biochemical

response. At day 24 the patient was discharged using prednisone 20 mg q.d. and cyclosporine 200 mg twice a day. Afterwards, the recipient's evolution was uneventful. By the end of the first year after LT, he was receiving cyclosporine monotherapy with mean trough levels of 180 ng/mL. For the next four years he remained asymptomatic and his liver tests were normal. We lost track of the patient in 2000, but regained contact with him in April 2010. The patient was completely asymptomatic and declared that after 2000 he gradually reduced his use of cyclosporine, from 100 mg twice a day to a complete withdrawal in 2008. During this period he remained asymptomatic.

The results of liver function tests in 2010 were as follows:

- TB 0.4 mg/dL (0-1 mg/dL).
- DB 0.1 mg/dL (0-0.2 mg/dL).
- AP 146 mg/dL (45-126 mg/dL).
- GGT 28 mg/dL (4-50 mg/dL)
- AST 32 mg/dL (10-40 mg/dL).
- ALT 32 mg/dL (10-55 mg/dL)
- INR 1.1.

Suspecting that the patient had developed OT, we decided to perform a liver biopsy to be sure that there was no evidence of alloimmnunity in the graft. Apart from a 10% steatosis, the liver biopsy was completely normal, showing no evidence of rejection, fibrosis or biliary duct loss. On that basis, we concluded that the patient indeed had developed OT. Therefore we recommended not adding ISA to his current therapy, with a clinical and biochemical follow-up every 4 to 6 months.

DISCUSSION

Immunologic tolerance (or operational tolerance in clinical practice) has been an unfulfilled goal of immunologists and transplant physicians. Since the liver has been considered an immunologically privileged organ, the possibility of acceptance of allogenic livers without ISA is theoretically more likely. In fact, since the early 1990s, several prospective cohorts of elective ISA withdrawal from Europe and USA have been published[6-15] and, from this data, the prevalence of OT has been estimated in around 20% of liver recipients and, probably, it is more frequent on children and late after liver transplantation.[16,17] Besides, ISA withdrawal seems to be a safe procedure, in fact, graft loss is a very infrequent side effect.[6-15] To perform a liver biopsy on those recipients that seems to be operationally tolerant contributes to properly establish this condition. Liver biopsy can show a subclinical rejection which can be present even with stable liver function.[18] Besides, it has been shown that in some liver recipients considered as tolerant, protocolized liver biopsies on the follow up are able to identify a group of recipients that develop fibrosis that can be reversed after the reintroduction of the IS drugs.[19] Although the mechanism of this phenomenon is unknown, it seems to be antigen related. Thus, the recipients that develop fibrosis after IS withdrawal may not be not truly tolerant recipients.

An important lesson from the previous studies is that OT does not seem to be related to an immune-suppressed state, but rather to immune regulation.[20] Tanaka, *et al.* have shown that potentially reactive T cells remain in the immune repertoire but seem to be specifically suppressed by a mechanism that is not yet well understood.[21] It must be considered, however, that these findings do not exclude the role of other factors that could also participate in tolerance development, such as the presence in the graft of natural killer cells (NK), NKT cells and regulatory T (Treg) cells, the ability of Kupffer cells to produce the anti-inflammatory cytokine, interleukin-10 after its exposure to lipopolysaccharide, the release of soluble HLA molecules by the liver, the presence of fenestrated sinusoidal endothelium that allows direct contact of hepatocytes with the blood and chimerism.[22] Non-immune-mediated liver disease, fewer donor-recipient HLA mismatches and the absence of previous acute rejection seem to be related to a successful ISA withdrawal.[23] However, it is notable that the previous history of acute cellular rejection was not an important obstacle to the development of OT in our patient.

Since tolerant recipients present reduced ISA related morbidity, require fewer medications and have a better quality of life,[10] attempting an ISA withdrawal is attractive, but not an easy decision considering an 80% chance of failure. For that reason, there are intensive research efforts devoted to identifying biomarkers able to predict successful ISA withdrawal. Li, *et al.*, for instance, compared pediatric operational tolerant liver recipients (OTLR) with age-matched liver recipients and non-transplanted volunteers. The study found that the frequency of CD4+ CD25 high Treg cells, B cells and Vδ1/Vδ2 (Vdelta1/Vdelta2) γδ (gamma delta) T cells ratio was higher among operationally tolerant liver recipients, but the NK cells frequency was lower in those patients.[24]

In a recent study, Martínez-Llordella, *et al.*, also found a higher frequency of CD4+ CD25+ Treg cells, as well as Vδ1+ T cells, among OTLR compared to healthy individuals and transplant recipients on ISA.[25] Yet more recently, another study from Li showed that Foxp3 mRNA expression was higher in the graft of OTLR than in the graft of liver recipients on ISA, although this tendency was not significant. However, the number of Foxp3+ cells was significantly higher among OTLR compared to recipients on ISA. The authors conclude that a prospective study is necessary to validate these findings.[26]

Among the different strategies used to dissect the immune system of OTLR, one of the most frequently used is gene expression profiling of peripheral blood samples. Two studies of renal transplant recipients have demonstrated that gene expression evaluation by microarrays can accurately discriminate among tolerant recipients, chronic rejecters and healthy volunteers.[27,28] This strategy has also been used to characterize OTLR.

The first study using microarray technology on this set of patients was published in 2007 by Martínez-Llordella, *et al.* In this study, peripheral blood mononuclear cell (PBMC) samples were taken from OTLR and from recipients who previously failed an ISA withdrawal attempt because of rejection (Non-Tol). A whole genome expression array was performed. The comparison of the two groups of patients showed 628 differentially expressed genes. A selected group of 22 genes was validated using qPCR. Among the most informative of the identified genes were γδ T cells specific transcripts and several NK related genes.[25]

Subsequently, Kawasaki, *et al.* published a second report comparing the expression profiles of PBMC samples from OTLR and healthy volunteers (HV), using a cDNA array for 12,814 probes. The authors found 717 differentially expressed genes, many of them involved in immune responses.[29]

In a second report from Martínez-Llordella, whole genome Affymetrix microarrays were conducted on PBMC samples from 17 OTLR and 21 non-TOL patients, showing that 1,932 genes were differentially expressed. The authors selected a group of 74 genes to be validated using qPCR, demonstrating that 33 of these genes were differentially expressed between OTLR and Non-TOL patients. Three predictors (using 2, 6 and 7 genes) were generated that accurately discriminated between OTLR and Non-TOL patients from an independent cohort of patients.[30] At present, the same team is completing a prospective study involving a similar transcriptional evaluation before gradually discontinuing ISA. The aim of the study is to prospectively validate the predictors generated in the previous study, to evaluate their ability to predict successful withdrawal of ISA, hence generating new knowledge about clinical variables associated with the development of tolerance.

Until now, no intervention has proven useful in inducing OT. Interestingly, simultaneous bone marrow and liver transplantation appears as a potentially useful alternative, at least in isolated reports.[31,32] The same holds true for the infusion of donor stem cells. In fact, Tryphonopoulos, *et al.* published a study were an attempt at ISA withdrawal was made with two groups of liver recipients, with bone marrow cell infusion, performed perioperatively on the intervention group. The rate of successful ISA withdrawal was the same in both groups, so the authors defined their results as negative.[13] Although several attempts have been made to induce OT with the administration of agents such as lymphocyte depleting antibodies, no pharmacologic strategy has as yet been proven effective.[5-20] There has been only one randomized trial that evaluated the rate of successful IS withdrawal during the first year post-LT, employing standard IS (tacrolimus monotherapy plus steroids) *vs.* ATG-fressenius, plus low-dose tacrolimus and no steroids.[33] In this study, no patients in the intervention group were able to complete the IS withdrawal without developing rejection. Although this trial only evaluated a specific strategy, it made evident that OT is not easy to induce, at least during the first year after LT, and new strategies must also be prospectively evaluated. Indeed, the accurate identification of operationally tolerant liver recipients is particularly important if we consider that currently there is no strategy (either pharmacologic or cellular-based) that can be considered consistently successful in inducing OT. However, promising data has been found through diagnostic yield of transcriptional biomarkers among prospective ISA withdrawal cohorts.[16] Considering this, it is possible that within a few years we will be able to properly recognize operationally tolerant patients in order to wean them off ISA with minimum risk and long-term benefits.

REFERENCES

1. European Liver Transplant Registry. Available from: http://www.eltr.org/ [September 2010].
2. Mells G, Neuberger J. Long-term care of the liver allograft recipient. *Semin Liver Dis* 2009; 29: 102-20.
3. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature* 1953; 172: 603-6.

4. Lerut J, Sanchez-Fueyo A. *Am J Transplant* 2006; 6: 1774-80.
5. Sánchez-Fueyo A, Strom T. Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs. *Gastroenterology* 2011; 140: 51-64.
6. Devlin J, Doherty D, Thomson L, Wong T, Donaldson P, Portmann B, et al. Defining the outcome of immunosuppression withdrawal after liver transplantation. *Hepatology* 1998; 27: 926-33.
7. Mazariegos GV, Reyes J, Marino IR, Demetris AJ, Flynn B, Irish W, et al. Weaning of immunosuppression in liver transplant recipients. *Transplantation* 1997; 63: 243-9.
8. Pons JA, Revilla-Nuin B, Baroja-Mazo A, Ramírez P, Martínez-Alarcón L, Sanchez-Bueno F, et al. FoxP3 in peripheral blood is associated with operational tolerance in liver transplant patients during immunosuppression withdrawal. *Transplantation* 2008; 86: 1370-8.
9. Pons JA, Yélamos J, Ramírez P, Oliver-Bonet M, Sánchez A, Rodríguez-Gago M, et al. Endothelial cell chimerism does not influence allograft tolerance in liver transplant patients after withdrawal of immunosuppression. *Transplantation* 2003; 75: 1045-7.
10. Orlando G, Manzia T, Baiocchi, Sanchez-Fueyo A, Angelico M, Tisone G. The Tor Vergata weaning off immunosuppression protocol **in stable** HCV liver transplant patients: the updated follow up at 78 months. *Transpl Immunol* 2008; 20: 43-7.
11. Eason JD, Cohen AJ, Nair S, Alcantera T, Loss GE. Tolerance: is it worth the risk? *Transplantation* 2005; 79: 1157-9.
12. Takatsuki M, Uemoto S, Inomata Y, Egawa H, Kiuchi T, Fujita S, et al. Weaning of immunosuppression in living donor liver transplant recipients. *Transplantation* 2001; 72: 449-54.
13. Tryphonopoulos P, Tzakis AG, Weppler D, García-Morales R, Kato T, Madariaga JR, et al. The role of donor bone marrow infusions in withdrawal of immunosuppression in adult liver allotransplantation. *Am J transplant* 2005; 5: 608-13.
14. Girlanda R, Rela M, Williams R, O'Grady JG, Heaton ND. Long-term outcome of immunosuppression withdrawal after liver transplantation. *Transplant Proc* 2005; 37: 1708-9.
15. Assy N, Adams PC, Myers P, Simon V, Minuk GY, Wall W, et al. Randomized controlled trial of total immunosuppression withdrawal in liver transplant recipients: role of ursodeoxycholic acid. *Transplantation* 2007; 83: 1571-6.
16. Benitez C, Lozano JJ, Martinez-Llordella M, Puig-Pey I, Lopez M, Rodriguez A, et al. Use of transcriptional biomarkers to identify liver transplant recipients who can successfully discontinue immunesuppressive therapy (abstract 213). Paper presented at: American Transplant Congress. May 30-Jun 03, 2009; Boston, MA. *Am J Transplant* 2009; 9: 252.
17. Feng S, Ekong U, Lobritto S, Demetris A, Rosenthal P, Alonso E, et al. ITN029ST: Immunosuppresion withdrawal in pediatric recipients of parental living donor liver transplants: preliminary results of a pilot study (abstract 189). Paper presented at: American Transplant Congress; Boston, MA; May 30-Jun 03, 2009. *Am J Transplant* 2009; 9: 244.
18. Mells G, Mann C, Hubscher S, Neuberger J. Late protocol liver biopsies in the liver allograft: a neglected investigations? *Liver transplantation* 2009; 15: 931-8.
19. Yoshitomi M, Koshiba T, Haga H, Li Y, Zhao X, Cheng D, et al. Requirement of protocol biopsy before and after complete cessation of immunosuppression after liver transplantation. *Transplantation* 2009; 87: 606-14.
20. Orlando G, Soker S, Wood K. Operational tolerance after liver transplantation. *J Hepatol* 2009; 50: 1247-57.
21. Koshiba T, Li Y, Takemura M, Wu Y, Sakaguchi S, Minato N, et al. Clinical, immunological, and pathological aspects of operational tolerance after pediatric living-donor liver transplantation. *Transpl Immunol* 2007; 17: 94-7.
22. Turka L, Lechler R. Towards the identification of biomarkers of transplantation tolerance. *Nat Rev Immunol* 2009; 9: 521-6.
23. Girlanda R, Rela M, Williams R, O'Grady JG, Heaton ND. Long-term outcome of immunesuppression withdrawal after liver transplantation. *Transplant Proc* 2005; 37: 1708-9.
24. Li Y, Koshiba T, Yoshizawa A, Yonekawa Y, Masuda K, Ito A, et al. *Am J Transplant* 2004; 4: 2118-25.
25. Martínez-Llordella M, Puig-Pey I, Orlando G, Ramoni M, Tisone G, Rimola A, et al. *Am J Transplant* 2007; 7: 309-19.
26. Li Y, Zhao X, Cheng D, Haga H, Tsuruyama T, Wood K, et al. *Transplantation* 2008; 86: 1837-43.
27. Brouard S, Mansfield E, Braud C, Li L, Giral M, Hsieh SC, et al. Identification of peripheral blood transcriptional biomarker panel associated with operational renal allograft tolerance. *Proc Natl Acad Sci USA* 2007; 104: 15448-53.
28. Braud C, Baeten D, Giral M, Pallier A, Ashton-Chess J, Braudeau C, et al. Immunosuppressive drug-free operational immune tolerance in human kidney transplant recipients: Part I. Blood gene expression statistical analysis. *J Cell Biochem* 2008; 103: 1681-92.
29. Kawasaki M, Iwasaki M, Koshiba T, Fujino M, Hara Y, Kitazawa Y, et al. Gene expression profile analysis of peripheral blood mononuclear cells from tolerant living-donor liver transplant recipients. *Int Surg* 2007; 92: 276-86.
30. Martínez-Llordella M, Lozano J, Puig-Pey I, Orlando G, Tisone G, Lerut J, et al. Using a transcriptional profilling to develop a diagnostic test of operational tolerance in liver transplant recipients. *J Clin Invest* 2008; 188: 2845-57.
31. Alexander SI, Smith N, Hu M, Verran D, Shun A, Dorney S, et al. Chimerism and tolerance in a recipient of a deceased-donor liver transplant. *N Engl J Med* 2008; 358: 369-74.
32. Scandling JD, Busque S, Dejbakhsh-Jones S, Benike C, Millan MT, Shizuru JA, et al. Tolerance and chimerism after renal and hematopoietic-cell transplantation. *N Engl J Med* 2008; 358: 362-8.
33. Benítez CE, Puig-Pey I, López M, Martínez-Llordella M, Lozano JJ, Bohne F, et al. ATG-Fresenius treatment and low-dose tacrolimus: results of a randomized controlled trial in liver transplantation. *Am J Transplant* 2010; 10: 2296-304.