



**HAL**  
open science

## Liver, rejection and tolerance

Daniel Eyraud, Victoria Lepere, Eric Savier, Pierre Coriat

► **To cite this version:**

Daniel Eyraud, Victoria Lepere, Eric Savier, Pierre Coriat. Liver, rejection and tolerance. Journal of Gastroenterology and Hepatology Research, 2016, 10.17554/j.issn.2224-3992.2015.05.594 . hal-01913977

**HAL Id: hal-01913977**

**<https://hal.science/hal-01913977v1>**

Submitted on 6 Nov 2018

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Liver, Rejection and Tolerance

Daniel Eyraud, Victoria Lepere, Eric Savier, Pierre Coriat

Daniel Eyraud, Victoria Lepere, Pierre Coriat, Department of Anesthesiology and Reanimation, Pitié-Salpêtrière University Hospital, Boulevard de l'Hôpital, Paris, France

Daniel Eyraud, Eric Savier, Department of Digestive, HPB Surgery, and Liver Transplantation, Pitié-Salpêtrière University Hospital, Boulevard de l'Hôpital, Paris, France

Correspondence to: Daniel Eyraud, MD, PhD, Department of Anesthesiology and Reanimation, Assistance Publique-Hôpitaux de Paris (AP-HP), Pitié-Salpêtrière University Hospital, Paris, France

Email: [daniel.eyraud@psl.ap-hop-paris.fr](mailto:daniel.eyraud@psl.ap-hop-paris.fr)

Telephone: +33-1-42175865 Fax: +33-1-42175613

Received: October 26, 2015 Revised: November 23, 2015

Accepted: November 28, 2015

Published online: January 21, 2016

### ABSTRACT

Innovative advances in the immunologic understanding of the relative resistance of the transplanted liver to rejection have continuously performed in the past decade as the results of experimental and clinical studies. Whereas mechanisms of the tolerogenic role of the liver were underlined, many evidences appeared that antibody mediated rejection (AMR) was probably not so negligible that we historically thought in liver transplantation (LT). This review will address to the spectrum of the mechanism of cellular and humoral liver rejection, the mechanisms of tolerance, as specific and not specific properties of the liver, and the immunomodulatory role of the liver in the combined solid organ transplantation.

© 2016 ACT. All rights reserved.

**Key words:** Liver transplantation; Rejection; Tolerance; Ab mediated rejection; Multiple transplantation

Eyraud D, Lepere V, Savier E, Coriat P. Liver, Rejection and Tolerance. *Journal of Gastroenterology and Hepatology Research* 2016; 5(1): 1880-1887 Available from: URL: <http://www.ghrnet.org/index.php/joghr/article/view/1556>

### INTRODUCTION

Clinical and experimental observations indicate that liver occupies a singular position relatively to other solid organs such as kidney, heart or lung. In some animal species as pig or some breeds of rat, liver transplantation can be performed without immunosuppression<sup>[1-3]</sup>. In humans, studies have been conducted where LT recipients were completely weaned off pharmacological immunosuppression<sup>[4]</sup> without rejection. Moreover, LT performed simultaneously with other solid organ of the same donor, prevent the rejection of this organ<sup>[5,6]</sup>. Liver can even reverse the rejection of another allograft, of another donor, transplanted a few days before the liver<sup>[7,8]</sup>. All these observations make us consider the liver to have inherent tolerogenic properties<sup>[9]</sup>. While this phenomenon was better understood, we discovered in the same time, that antibodies mediated rejection (AMR) was perhaps not as seldom in LT<sup>[10]</sup> as we thought, contrary to its fundamental role in other solid organ transplantations<sup>[11,12]</sup>. In this review, we will first expose the mechanisms of rejection and tolerance in LT, then we will describe in its complexity, the present knowledge of AMR in LT. At last, we will discuss the immunomodulatory role of the liver in multiple transplantations and expose the different hypothesis about its mechanism.

### I-PRELIMINARY CONSIDERATIONS ABOUT REJECTION IN LIVER TRANSPLANTATION

#### 1. Mechanisms and types of rejection in liver transplant

After solid organ transplantation, hyperacute vasculitis rejection can

occur in animal or human with preformed antibodies (Ab) against the donor major histocompatibility complex (MHC) class I-encoded antigens<sup>[13,14]</sup>. This Ab mediated attack of the recipients' cells leads to the necrosis of the organ in the hours after the reperfusion. In the case of the liver, this hyperacute rejection could occur in ABO incompatible transplantation because ABO antigens are present on the endothelial cells. In most other clinical situations, acute allograft rejection is initiated by recipient T cells that recognize donor antigens, mostly alloantigens encoded by the polymorphic MHC. Donor MHC molecules are internalized by donor and recipient antigen-presenting cells (APC). Molecular and cellular basis of graft rejection have been exhaustively reported elsewhere<sup>[15]</sup>. Rapidly, three different non exclusive pathways could be implicated in the allograft rejection<sup>[16]</sup>: first, the direct pathway: recipient T cells recognize intact allogenic MHC molecules (class I and II) on the surface of donor APCs, secondly the indirect pathway: recipients T-cell recognize donor antigen in recipient MHC molecule on the surface of recipient APC. These donors Ag are the product of the phagocytosis and trafficking of necrotic donor cells material in recipient APCs. Third, the semi direct pathway in which recipient APCs acquire intact donor MHC molecules following direct contact with donor APCs or through fusion with donor APC-derived exosomes. Acute cellular rejection is the best-characterized graft –specific presentation of immune rejection, especially in liver transplantation. It occurs most frequently in the period between 5 and 21 days after transplantation, its onset may be as early as three to four days or as late as several years following transplantation, in this case most frequently after interruption of immunosuppressive therapy. It is defined by an often –sudden deterioration in allograft function. Biopsy analysis of the transplanted tissue makes the diagnosis: in the liver, it shows infiltration by host T cells and other mononuclear leukocytes in portal veins, bile ducts and central vein endothelium<sup>[17]</sup>. Although patients with documented acute cellular rejection would undergo improvement in liver function tests and exhibit biopsy-proven reversal of acute rejection without administration of specific antirejection therapy<sup>[18]</sup>. Acute rejection should usually be treated, even in liver transplanted patients, to prevent the irreversible deterioration of the organ.

Beside the cellular mechanism of rejection, the production of antibodies is also associated with acute and chronic rejection in organ transplantation. The role of anti-donor MHC class I and II has been well identified in the past five years in the kidney<sup>[19,20]</sup>, cardiac<sup>[21]</sup> and pancreatic<sup>[22]</sup> transplantation. Antibodies can injure the graft by activating complement and monoclonal cells with Fc receptors that recognize the heavy chain of antibody. Fc receptor-expressing leukocytes can thereby be activated by antibodies-coated donor cells. Anti-donor antibody can also inhibit signaling cascades within endothelial cells<sup>[23]</sup> resulting in the common form of chronic rejection, the transplant vasculopathy. Over time, transplanted organ present inexorable deterioration of the function. Although this process is called chronic rejection, the donor-specific immune process is probably not the sole, and even not the primary cause in liver transplant<sup>[15]</sup>. In liver transplant, chronic rejection differs from acute rejection, in that it is more insidious in onset, does not respond to methylprednisolone boluses, and has a histopathologic picture that is characterized by loss of bile ducts and arteriole obstruction by foamy macrophages. Among the factors that are thought to increase the likelihood of chronic rejection are prior episodes of acute cellular rejection, chronic ischemia secondary to hepatic artery insufficiency or thrombosis; cytomegalovirus infection or recurrent infection with hepatitis C virus; and perhaps chronic antibody-mediated injury as

we will discuss this point. It is likely that chronic rejection occurs in grafts that are damaged or fail for many non-immunologic reasons and histologic analysis reveals fibrosis in the absence of immune cells infiltration.

## 2. Interaction between ischemia-reperfusion, inflammation and immune reaction

Newly engrafted organ is subject to intense inflammation. The injury to the graft, caused by donor disease (steatosis, stroke)<sup>[24]</sup> or hemodynamic instability, organ procurement or reanimation, cold and warm preservation, surgical trauma and reperfusion injury, leads to release of reactive oxygen species (ROS) and proinflammatory cytokines. Following liver ischaemia reperfusion, there is activation of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) by chemokine (CXCL) 10, interferon regulatory factor (IRF) and toll-like receptor (TLR) (innate immunity) in parallel. TNF- $\alpha$  activates downstream hepatocyte/sinusoidal endothelial cells (SEC) nuclear factor kb (NFkb) and CD4+ T cells separately which activate c-Jun N-terminal protein kinase-2 (JNK-2) and signal transducer activator of transcription-4 (STAT4), respectively leading to increased cell injury<sup>[25]</sup>. Innate natural killer, nucleophile polynuclears, macrophages, platelets and alloimmune (lymphocytes T and B, antigen presenting cells, dendritic cells) reactions are both stimulated. It has been demonstrated that ischemia-reperfusion protection of liver allografts was associated with a reduction in the expression of immune response genes and promotion of those involved in protection and repair<sup>[26]</sup>.

## II-TOLERANCE AND LIVER TRANSPLANTATION

### 1. Definitions

Immunologically, tolerance is defined as the absence of an immune response towards a specific antigen in the absence of immunosuppression. In contrast to clinical transplantation, animal models of organ transplantation allows a straightforward mean of detecting immunological tolerance<sup>[27]</sup>. An animal is formally proven to be tolerant when, in the absence of immunosuppression, a second graft from the same donor is accepted, while a graft from a third-party donor is rejected<sup>[28]</sup>. Since such unambiguous assays clearly cannot be used in clinical organ transplantation, the surrogate definition of operational tolerance has been established. Operational tolerance is defined as the absence of graft rejection without the use of immunosuppressive drugs<sup>[29]</sup>.

### 2. Mechanisms of tolerance

**2.1. Central tolerance:** In central thymic tolerance, thymocytes CD4<sup>+</sup>CD8<sup>+</sup> are initially positively selected to become CD4<sup>+</sup> or CD8<sup>+</sup> depending on their TCR interactions with thymic stroma cells (dendritic cells, macrophages, thymic epithelial cells) which strongly express MHC class I (CD8) and II (CD4) molecules. If MHC selection fails, the double positive thymocytes undergo apoptosis in 95 percent of case. Negative selection follows for single positive cells, in which most self-reactive CD4<sup>+</sup> or CD8<sup>+</sup> cells undergo TCR-induced death if they interact with MHC molecules on APCs carrying self-peptides. Alloreactive cells can also undergo negative selection in the thymus during the interaction of antigen-specific T cells with tolerogenic dendritic cells expressing varying levels of costimulatory molecules.

**2.2. Peripheral tolerance:** However, some CD4<sup>+</sup> or CD8<sup>+</sup> cells can escape negative selection and become auto/alloreactive T lymphocytes in the periphery (lymph nodes, blood, spleen). That can cause autoimmune disease or allograft rejection. Fortunately these high-reactive cells, escaping thymic deletion may be deleted

or regulated in the periphery, by activation-induced cell death, apoptosis or the suppressive action of T regulatory lymphocytes (Treg) and cytokines. Activated lymphocytes can also undergo apoptosis because absence of the antigen or the costimulatory factors. Moreover, cell surface receptors PD-1 and its inducible ligand PD-L1 down-regulate T cell activation, inducing tolerance. Another mechanism of peripheral concerns immature APCs such immature DC or plasmacytoid DC, that are poor presenters of alloantigens on MHC molecules, especially in tolerant environment (IL-10, IDO, PD-L1, Fas L, HO-1, HLA-G) resulting in deletion of Teff, induction of Treg and anergy/deletion of memory T<sup>30</sup>. However, a major drawback *in vivo* is the potential of tolerogenic DCs to mature during infections or inflammation, which would convert them into immunogenic cells<sup>31</sup>.

*In vitro* propagation of tolerogenic donor- or-recipient-derived DC has been used extensively as an experimental approach to target the pathways of allorecognition, with the aim of prolonging transplant survival, while reducing dependency on immunosuppressive drugs. The development of techniques to propagate large numbers of TOL (operationally tolerant patients) DC *in vitro* has provided the basis for ascertaining the ability of these cells to downregulate both host-versus-graft and graft-versus-host immune responses mediated by T cells (in the context of hematopoietic stem cell transplantation). DCs can be treated with pharmacologic agents before injection, which may attenuate their maturation *in vivo*. Genetically engineered DCs have also been tested. There have been numerous reports of indefinite murine allograft survival following infusion of either donor- or recipient-derived TOL DC<sup>32-34</sup>. Clinical trials in human including kidney transplantation are in progress.

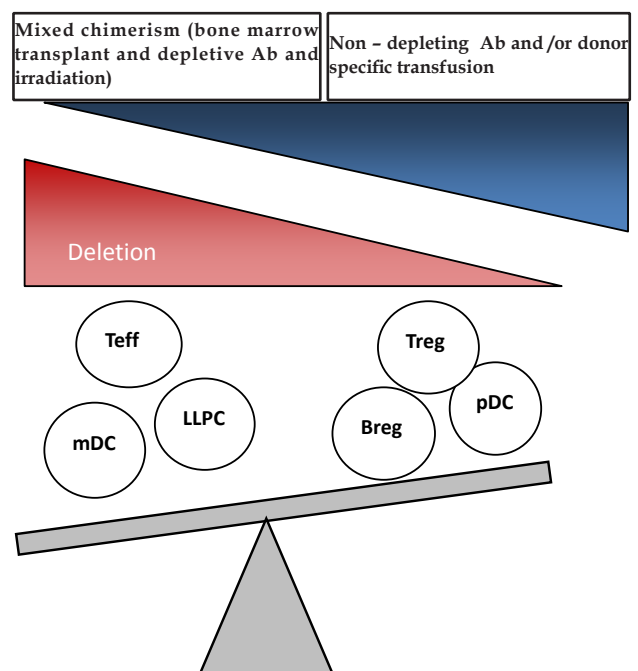
Interestingly, in a recent study<sup>35</sup>, B cell phenotypes were followed in blood of operationally tolerant kidney transplanted patients (TOL) and compared patients with stable graft function treated with immunosuppressive therapy. Apoptosis, proliferation, cytokine, immunoglobulin production and markers of differentiation were followed in blood of these operationally tolerant patients (TOL). Tolerant recipients show a higher frequency of CD20<sup>+</sup>CD24<sup>hi</sup> CD38<sup>hi</sup> transitional and CD20<sup>+</sup>CD24<sup>lo</sup> CD38<sup>lo</sup> naïve B cells compared to patients with stable graft function, correlating with a decreased frequency of CD20<sup>+</sup> CD38<sup>lo</sup>CD138<sup>lo</sup> differentiated plasma cells, suggestive of abnormal B cell differentiation. B cells from TOL proliferate normally but produce more IL-10. In addition, B cells from tolerant recipients exhibit a defective expression of factors of the end step of differentiation into plasma cells and show a higher propensity for cell death apoptosis compared to patients with stable graft function. This *in vitro* profile is consistent with down-regulation of B cell differentiation genes and anti-apoptotic B cell genes in these patients *in vivo*. These data suggest that a balance between B cells producing IL-10 and a deficiency in plasma cells may encourage an environment favorable to the tolerance maintenance.

Other authors studied the difference of the immune response in tolerant liver or kidney transplanted patients<sup>12</sup>. Whereas in liver transplant tolerance the major cell types involved appear to be NK cells and  $\gamma\delta$ TCR+T cells, in kidney transplantation the predominant cell type appears to be B cells. The term of B regulatory cells (Breg) for this type of B cells with tolerant properties, already defined<sup>36</sup> for auto-immune pathologies, was applied to organ transplantation<sup>37</sup>.

The induction and maintenance of immune tolerance is a complex mechanism involving not one cell subset alone but many types of cells (Figure 1), especially Tregs and iDC, with intricate interactions. Moreover, the immunological and inflammatory environment is crucial to determine the state of each cell type which will drive a tolerogenic or immunologic response. A major point in clinical care

for operationally tolerant transplant recipients is the ability to monitor for its stability. Several papers have already described transplant patients that lost their tolerant state towards the allograft and developed a rejection response<sup>38,39</sup>. Brouard *et al.*<sup>38</sup> showed that in two kidney transplanted patients the occurrence of rejection followed a loss of the tolerogenic phenotype. Some situation like infection may reverse tolerance but the mechanism responsible for the switch from tolerance to immunogenicity is not yet clarified in the field of liver immunology. For example, why any liver recipients with recurrent HCV (hepatitis C virus) infection and HCV specific T cells do not usually reject despite infiltration of the liver graft? In some of these cases, operational tolerance may have even occurred<sup>40</sup>.

The effects of the recurrences of HBV (hepatitis B virus), HCV or combined hepatitis virus & human immunodeficiency virus (HIV) on liver immunology are another interesting question. Concerning HBV recurrence, individualized modern prophylaxis regimen, based on an integrated approach, modern molecules (entecavir, tenofovir) and risk-assessment (genetic variations in the HBV) allow eradicating recurrences<sup>41</sup>. For HCV, the first difficulty is the overlapping features of clinical and histo-pathological characteristics between ACR and HCV recurrence. Regev *et al.*<sup>42</sup> evaluated more than 100 LT biopsies conducted by five liver expert pathologists and concluded that the histopathological differentiation of HCV recurrence and ACR post-LT had relatively low inter-observer and intra-observer agreement rates, showing concerning low reliability. More recently authors used interesting strategy, proteomic analysis in plasma samples, distributions of different types of immune cells in tissue samples, microarray technology allowing studying molecular profiles and regulatory mechanism, miRNA, permitting important advances in the identification of several potentially useful biomarkers to differentiate ACR and HCV recurrence<sup>43</sup>. Despite these interesting advances, large studies correlating tissue analysis, serum molecular biomarkers and clinical observations are lacking.



**Figure 1** Model of the allograft response and mechanism of tolerance, modified from (13). The outcome of graft rejection or tolerance depends on balance between effector T cells, mature dendritic cells (DC), long-lived plasma cells (LLPC) and antigen presenting cells (APC) and regulator T cells, regulator B cells and plasmacytoid DC (pDC).

The second difficulty is possible interaction between HCV recurrence and ACR. Many data confirm the pivotal role of T-cells, Tregs and DCs in the post-LT RHC setting<sup>[44]</sup>. So, the host immune response (mainly cellular mediated) appears to be crucial both in the control of HCV infection and in the genesis of rejection (paragraph I), and it is also strongly influenced by immunosuppressive treatment. No clear immunosuppressive strategy could be strongly recommended in HCV-positive recipients to prevent HCV recurrence. Nonetheless it seems that episodes of rejection and over-immunosuppression are more likely to enhance the risk of HCV recurrence through immunological mechanisms<sup>[45]</sup>. The past most common situation was an ACR episode (or a so-called post-LT increase in hepatic enzymes!), which was treated by increase in immunosuppressive therapy, with consecutive severe acute HCV recurrence.

Both complete prevention of rejection and optimization of immunosuppression represented the main goals towards reducing the rate of graft HCV reinfection. The present situation is much easier to manage with new hepatitis C treatments. These new interferon-free combinations can be used, either in pre transplant decompensated liver disease<sup>[46]</sup> or at every time after LT<sup>[47]</sup>. The eradication of the hepatitis C virus have favorable effect on the liver graft in double way: it permits to eliminate the differential diagnosis between ACR and HCV recurrence, and to increase IS therapy if ACR suspicion occurs, without the risk to trigger or worsen HCV recurrence concomitant with ACR.

### III-ANTIBODY-MEDIATED REACTION AND LIVER TRANSPLANTATION

Antibody-mediated rejection (AMR) is an increasingly problematic entity in solid organ transplantation that leads to graft dysfunction and loss. AMR often occurs late (>1 year) after kidney transplantation with an incidence of approximately 30%, and was recently attributed to cause half of all renal allograft failures (48). In liver transplantation, AMR is usually diagnosed on the basis of the presence of all 4 of the following strict criteria: (1) DSAs (DSA, donor specific antigen) in serum; (2) histopathological evidence of diffuse microvascular injury/microvasculitis consistent with antibody-mediated injury; (3) diffuse C4d staining in the portal microvasculature with or without staining in the sinusoids or central veins in at least 1 sample; and (4) the exclusion of other causes of a similar type of injury<sup>[49]</sup>.

One of the main reasons of renewed interest, especially in liver transplantation in which we considered this issue as minor or confined to ABO non compatible transplantation, is a recent technological advance that uses fast solid-phase assays and immobilized HLA to characterize HLA-specific antibodies<sup>[50]</sup>. The most widely adopted form of this is the single antigen bead assay (SABA), which although not perfect, has distinct advantages over the complement-dependent lymphocytotoxicity assay with which it is compared. SABAs identify antibody specificities to individual HLA alleles and can separate reactivity against different HLA loci, which is difficult to achieve using assays based on whole cells expressing multiple HLA in set combinations due to linkage disequilibrium. SABA also make sequential testing of multiple samples much easier to do

Although the frequency and the impact of AMR in liver allograft is much lesser than in other organ transplantations, < 10% of sensitized recipients, emerging literature suggests that DSA can be associated with more rapidly progressive fibrosis, especially in hepatitis C-positive recipients with recurrent hepatitis, diminished long-term graft and patient survival<sup>[51]</sup>. An understanding of how antibodies

cause graft injury and promote acute and chronic rejection is critical for the management of sensitized recipients and improvement of therapeutics. Studies have previously demonstrated that HLA I antibodies act as agonists, inducing molecular aggregation of HLA class I molecules to trigger intracellular signaling pathways that are critical in the regulation of cell survival, proliferation and migration in graft vascular cells<sup>[52]</sup>. Recent investigations have revealed additional functional responses of vascular cells to antibodies, including induction of inflammatory mediators by endothelial cells and recruitment of immune cells. Moreover, the relevance of complement fixing capacity of donor specific antibodies has received increasing attention<sup>[53]</sup> but some interesting studies counteract the relevance of sinusoidal deposition of complement 4d in liver allografts as proof of AMR<sup>[54]</sup>. We summarized in the table 1 the presumed role of different types of antibodies in liver AMR. However, the question of whether these antibodies are a cause or a consequence of the rejection process is unproven<sup>[55]</sup>. To confirm this point in liver transplantation, we should add to the definition<sup>[56]</sup> one last parameter: the therapeutic decrease in serum DSA improves liver function and decreases histologic injuries. Only, rare observation, in combined transplantation, demonstrated this last point<sup>[57]</sup>. In table 2, we summarize the possible role of DSA, according the different types of clinical rejection.

In state of the art, many meetings or consensus reports<sup>[48,50,51]</sup> insisted on different technical (especially, importance to detect HLA antibodies with solid phase immunoassay test) and no technical followed points: (1) The liver allograft may be partially resistant to antibody mediated damage; however, high-level DSA antibody may be associated with inferior outcomes and should be considered as a risk factor for graft dysfunction; (2) Pretransplantation screening for HLA antibodies is recommended in liver retransplant recipients and cross-match should be performed in sensitized recipients<sup>[51]</sup>; (3) Donor blood and tissue should be collected and stored in liver transplantation to a posteriori research DSA or complement deposition; (4) In sensitized recipients of combined liver-kidney transplantation, the liver may not confer full protection for preventing AMR in the kidney and should be included in risk assessment (confer next chapter).

### IV-THE LIVER AS IMMUNOREGULATORY ORGAN IN COMBINED ORGAN TRANSPLANTATION

Liver has been recognized as an immunoregulatory solid organ in the field of transplantation for a long time<sup>[58]</sup>. Some strains of pigs can tolerate allogenic MHC mismatched liver without

**Table 1** Different types of antibody related to liver graft injury and their relevance.

Auto versus allo	++	++
HLA vs non HLA	++	+?
De novo vs preformed	+	++
Class I vs Class II	+	++ (preformed)
IgG complement fixing vs not (IgG1,3 vs other Ig)	++	?
Flowcytometry crossmatches T vs B (MIFI)	+++ if > 5000	? if < 3000

**Table 2** Antibody-mediated rejection (AMR) and type of allograft rejection in liver or liver kidney transplantation. DSA: donor specific antigen.

	Circumstances
Hyperacute	ABO incompatible, very seldom
Acute	<1% recipients. In patients with preformed DSA
Chronic	Possible, in some patients after complete weaning immunosuppression (de novo DSA)
C4d-negative	Probably, under development

immunosuppressive therapy (IS). Moreover, in certain strains of rodents, instead of sensitizing the recipient, the enduring liver graft induce a state of donor-specific unresponsiveness in which subsequent grafts (skin or other organs) are accepted permanently without IS<sup>[59]</sup>. Tolerant recipients of liver allografts have a high blood concentration of Class I MHC molecules of donor type and injections of serum and lymph from such animals given daily successfully prolonged the survival of skin or heart grafts in naïve animals of appropriate strains<sup>[59,60]</sup>.

In human, many studies<sup>[9]</sup> reported that complete withdrawal of IS maintenance therapy is possible in a little percentage of well-selected recipients < 20%, with non-viral, nonimmune disease. The protective immunologic role of the liver was confirmed in combined heart-liver, intestine-liver, pancreas liver and kidney-liver transplantation<sup>[61]</sup>. However, in this large cohort provided by the United Network of Organ Sharing (UNOS), the reduction in allograft rejection was also observed in patients co-transplanted with two organs not including a liver: rejection-free survival of patients simultaneously kidney-heart or kidney-liver transplanted (from the same donor) was higher than the rejection-free survival of the patients transplanted with heart or liver alone; rejection-free survival of patients simultaneously transplanted with two lungs or two kidneys (from the same donor) was also better than patients transplanted with one single organ. Authors focused their explanations on the importance of high antigen load brought by simultaneous combined transplantation. In a more recent study<sup>[62]</sup>, also provided by the UNOS and interesting specifically in mortality and rejection, authors found that the mortality at 1, 3 or 5 years of patients undergoing combined heart-liver transplantation (respectively 84, 74 and 72%) was not different from patients undergoing isolated liver transplantation (respectively 85, 78 and 72%) and significantly better than patients undergoing isolated heart transplantation (respectively 83, 73 and 65%). Moreover, the incidence of acute cardiac rejection within 1 year for patients with cardiac graft survival greater than 1 year was 9% for those undergoing simultaneous transplantation compared with 24% for those undergoing heart transplantation alone ( $P=0.002$ ). The incidence of acute liver rejection within 1 year for patients with liver graft survival greater than 1 year was 5% for those undergoing simultaneous transplantation versus 12% for those undergoing liver transplantation alone ( $P=0.060$ ).

Concerning liver and kidney transplantation, Simpson *et al.*<sup>[63]</sup> showed that rejection-free graft survival of kidney after liver transplantation (KALT) patients was lesser than combined liver kidney transplantation (CLKT) patients. This difference was even more statistically significant when they considered patients with HLA mismatched grafts. Another study from the UNOS database<sup>[64]</sup> showed that there was no difference in recipient mortality between liver transplantation alone (LTA) and simultaneous liver-kidney transplantation (SLK). Recipient and graft survival in SLK was higher compared to both kidneys after liver transplantation (KALT) and liver transplantation after kidney (LAKT).

All these clinical studies have limitations: retrospective, comparison of medical selected patients and treatment and not randomized. However, all observed a better rejection free survival and a better “no liver” graft survival in patients simultaneously heart-liver or kidney-liver transplanted and seem to confirm experimental data. Many mechanisms have been reported in the past. Concerning acute cellular rejection, the main invoked mechanisms are: (1) Large cellular compartment with hematopoietic regulators ( $\gamma\delta$ T lymphocytes, NK and NKT cells, DC) and dilutional mass effect; (2) Mixed hematopoietic microchimerism; (3) Regulatory proteins/

cytokines secretion, for example HLA-G.

The first mechanism observed is that the liver is an especially large alloantigen transfer in recipient. The increased mass of tissue transplanted may exhaust the recipients' immune response. One mechanism for this exhaustion could be that there is a limited clone size of graft-reactive T cells which are unable to establish “critical mass”. This is analogous to a nuclear chain reaction, where fission does not occur until there is sufficient density of free neutrons<sup>[65]</sup>. Many experimental studies confirm this non-specific liver effect. Recently authors<sup>[66]</sup> studied in mice if recombinant adenoassociated virus (rAAV)- mediated expression of donor major histocompatibility complex in recipient livers could induce tolerance to donor-strain grafts. High-level expression of donor major histocompatibility complex in recipient livers promotes tolerance to skin allograft while low level accelerated the rejection. This effect of high dose was already described if donor MHC gene were transferred in hematopoietic cells before cardiac graft of the same donor<sup>[67]</sup> or into skeletal muscle before skin graft<sup>[68]</sup>.

The second main mechanism is microchimerism<sup>[69]</sup>. Microchimerism arises as a result of migration of passenger leukocytes from a transplanted allograft into an unconditioned recipient and donor pluripotent HSCs do not engraft, but alternatively hematopoietic-derived cells from the donor organ are produced and migrate systemically. Although this phenomenon probably does exist with all solid organ transplantations, but it is higher<sup>[70]</sup>, longer<sup>[71]</sup> and more frequent in liver transplantation<sup>[72]</sup>. Stable levels of donor chimerism may be a marker of transplantation tolerance, and may help to tailor immunosuppressive treatment in liver transplantation<sup>[73]</sup>. As low as 1% donor chimerism is sufficient to induce tolerance to donor-specific organs, cells, and tissues<sup>[74]</sup>. This level may occur more often when liver is present in combined solid organ transplantation. Already, Kawai *et al.*<sup>[75]</sup> achieved the establishment of chimerism in primates, without bone marrow transplantation, in a non myeloablative preconditioning protocol, opening promising possibility in human.

Concerning AMR, many reasons have been given to explain why liver can withstand and protect other organs from DSA<sup>[76]</sup>: (1) **Dilution**: Increase area of distribution: HLA Class I antigens are present on all liver cells, especially endothelial cells and liver capillary area is 100 times that of the heart or the kidney. HLA Class II is constitutively expressed only on dendritic cells and on other cells in the setting of inflammation. Moreover, platelets and complement factors are decreased in cirrhotic patients; (2) **Inactivation**: Role of Kupffer cells, able to removal immune complexes, activated complement and platelet aggregates, formed during AMR<sup>[77]</sup>; (3) **Characteristics of HLA alloantibodies**: class and subclass, Fc binding, complement fixation and antigen affinity, likely affect their injurious potential.

A last propriety of the liver to explain it withstand to both humoral and cellular rejection is its exceptional regenerative capacity. While other solid organs presenting acute rejection, are rapidly suffering from organ dysfunction, liver is able to function unless a very severe rejection occurs. Moreover, rapid treatment of the rejection will avoid anatomic and functional aftereffects.

## CONCLUSION

In conclusion, recent researches permitted a progression in the understanding of the better tolerance of the liver than other solid organs. The three major mechanisms, at this date, are microchimerism and tremendous antigen load, overpassing the

lymphocytes suppressing LT response, in cellular rejection and trapping of different products like, antibodies, immune complex and platelet aggregates in AMR. This better comprehension could offer perspectives not only in liver transplantation and combined liver-other solid organ transplantation but also in the other solid organs transplantation.

## CONFLICT OF INTERESTS

The authors declare that they do not have conflict of interests.

## REFERENCES

- 1 Calne RY, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, Binns RM, Davies DA. Induction of immunological tolerance by porcine liver allografts. *Nature* 1969; 223: 472-476
- 2 Qian S, Lu L, Fu F, Li Y, Li W, Starzl TE, Fung JJ, Thomson AW. Apoptosis within spontaneously accepted mouse liverallografts: evidence for deletion of cytotoxic T cells and implications for tolerance induction. *J Immunol* 1997; 158: 4654-4661
- 3 Farges O, Morris PJ, Dallman MJ. Spontaneous acceptance of liver allografts in the rat. Analysis of the immune response. *Transplantation* 1994; 57: 171-177
- 4 Lerut J, Sanchez-Fueyo A. An appraisal of tolerance in liver transplantation. *Am J Transplant* 2006; 6: 1774-1780
- 5 Rasmussen A, Davies HF, Jamieson NV, Evans DB, Calne RY. Combined transplantation of liver and kidney from the same donor protects the kidney from rejection and improves kidney graft survival. *Transplantation* 1995; 59: 919-921
- 6 Kamada N, Wight DG. Antigen-specific immunosuppression induced by liver transplantation in the rat. *Transplantation* 1984; 38: 217-221
- 7 Wang C, Sun J, Li L, Wang L, Dolan P, Sheil AG. Conversion of pancreas allograft rejection to acceptance by liver transplantation. *Transplantation* 1998; 65: 188-192
- 8 Sanada O, Fukuda Y, Sumimoto R, Hoshino S, Nishihara M, Kaneda K, Asahara T, Dohi K. Establishment of chimerism in donor liver with recipient-type bone marrow cells prior to liver transplantation produces marked suppression of allograft rejection in rats. *Transpl Int* 1998; 11 Suppl 1: S174-S178
- 9 Levitsky J. Operational Tolerance: Past lessons and future prospects liver transplantation. *Liver Transplantation* 2011; 17:222-232
- 10 Benseler V, McCaughan GW, Schlitt HJ, Bishop GA, Bowen DG, Bertolino P. The liver: a special case in transplantation tolerance. *Semin Liver Dis* 2007; 27: 194-213
- 11 Heidt S, Wood KJ, Phil D. Biomarkers of operational tolerance in solid organ transplantation. *Expert Opin Med Diagn.* 2012; 6(4):281-293.
- 12 O'Leary JG, Demetris AG, Friedman LS, Gebel HM, Halloran PF, Kirk AD, Knechtle SJ, McDiarmid SV, Shaked A, Terasaki PI, Tinckam KJ, Tomlanovich SJ, Wood KJ, Woodle ES, Zachary AA and Klintmalm G. The Role of Donor-Specific HLA Alloantibodies in Liver Transplantation. *Am J Transplant* 2014; 14:779-787
- 13 Knechtle S, Kolbeck PC, Tsuchimoto S, et al. Hepatic transplantation into sensitized recipients. *Transplantation* 1987;43:169-172
- 14 Williams GM, Hume DM, Hudson RP Jr, et al. "Hyperacute" renal homograft rejection in man. *N Engl J Med* 1968;278:642
- 15 Sanchez-Fueyo A, Strom BT. Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs. *Gastroenterology* 2011; 140:51-64
- 16 Afzali B, Lombardi G, Lechler RI. Pathways of major histocompatibility complex allrecognition. *Curr Opin Organ Transplant* 2008; 13:438-444
- 17 Anonymous. Banff schema for grading liver allograft rejection: an international consensus document. *HEPATOLOGY* 1997; 25:658-663
- 18 Dousset B, Hubscher SG, Padbury RT, Gunson BK, Buckels JA, Mayer AD, Elias E, McMaster P, Neuberger JM. Acute liver allograft rejection-is treatment allways necessary? *Transplantation* 1993; 55:529-34
- 19 Loupy A, Lefaucheur C, Vernerey D, Prugger C, Duong van Huynen JP, Mooney N, Suberbielle C, Frémeaux-Bacchi V, Méjean A, Desgrandchamps F, Anglicheau D, Nochy D, Charron D, Empana JP, Delahousse M, Legendre C, Glotz D, Hill GS, Zeevi A, Jouven X. Complement binding anti-HLA antibodies and kidney allograft survival. *N Engl J Med* 2013; 369: 1215.
- 20 Lefaucheur C, Loupy A, Vernerey D, Duong-Van-Huyen JP, Suberbielle C, Anglicheau D, Vérine J, Beuscart T, Nochy D, Bruneval P, Charron D, Delahousse M, Empana JP, Hill GS, Glotz D, Legendre C, Jouven X. Antibody-mediated vascular rejection of kidney allografts: a population based study. *Lancet* 2013; 381: 313.
- 21 Ho EK, Vlad ER, Vasilescu ER, de la Torre L, Colovai AI, Burke E, Deng M, Schwartz J, Marboe C, Mancini D, Opelz G, Suciufoca N. Pre- and posttransplantation allosensitization in heart allograft recipients: major impact of de novo alloantibody production on allograft survival. *Hum Immunol.* 2011 Jan; 72(1):5-10.
- 22 Cantarovich D, De Amicis S, Akl A, Devys A, Vistoli F, Karam G, Souillou JP. Post-transplant donor specific anti-HLA antibodies negatively impact pancreas transplantation outcome *Am J Transplant.* *Am J Transplant* 2011; 11(12):2737-46.
- 23 Li F, Atz ME, Reed EF. Human leucocyte antigen antibodies in chronic transplant vasculopathy-mecanisms and pathways. *Curr Opin Immunol* 2009; 21:557-62.
- 24 Chu MJ, Hickey AJR, Phillips AR and Bartlett AS. The Impact of Hepatic Steatosis on Hepatic Ischemia-Reperfusion Injury in Experimental Studies: A Systematic Review. *Biomed Res Int.* 2013;2013;192029. doi: 10.1155/2013/192029. Epub 2013 Aug 24
- 25 Datta G, Fuller BJ, Davidson BR. Molecular mechanisms of liver ischemia reperfusion injury: Insights from transgenic knockout models. *World J Gastroenterol* 2013 March 21; 19(11):1683-1698.
- 26 Jassem W, Fuggle S, Thompson R, Arno M, Taylor J, Byrne J, Heaton N, Rela M. Effect of Ischemic Preconditioning on the Genomic Response to Reperfusion Injury in Deceased Donor Liver Transplantation. *Liver Transplant* 2009; 15:1750-1765
- 27 Kingsley CI, Nadig SN, Wood KJ. Transplantation tolerance: lessons from experimental rodent models. *Transplant international : official journal of the European Society for Organ Transplantation.* Oct; 2007 20(10):828-41. [PubMed: 17711408]
- 28 Medawar PB, Woodruff MF. The induction of tolerance by skin allografts on newborn rats. *Immunology* 1958; 1:27-35
- 29 Auchincloss H Jr. In search of the elusive Holy Grail: the mechanisms and prospects for achieving clinical transplantation tolerance. *Am J Transplant* 2001; 1(1):6-12. [PubMed: 12095040]
- 30 Vassalli G. Dendritic Cell-Based Approaches for Therapeutic Immune Regulation in Solid-Organ Transplantation. *Journal of Transplantation* 2013; Volume 2013, Article ID 761429, <http://dx.doi.org/10.1155/2013/761429>
- 31 T. G. Berger, H. Schulze-Koops, M. Schäfer, E. Müller, and M.B. Lutz. Immature and maturation-resistant human dendritic cells generated from bone marrow require two stimulations to induce T cell anergy in vitro. *PLoS ONE*, 4( 8). Article ID e6645, 2009
- 32 Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. *Nat Rev Immunol.* 2007; 7:610-621. [PubMed: 17627284]
- 33 Ezzelarab M and Thomson AW. Tolerogenic dendritic cells and their role in transplantation. *Semin Immunol.* 2011 August ; 23(4): 252-263. doi:10.1016/j.smim.2011.06.007.
- 34 Xia MJ, Shan J, Li YP, Guo YJ, Sun GX, Wu WQ, Feng L. Adoptive transfusion of toerogenic cells prolongs the survival of liver allograft: a systematic review. *J Evid Based Med* 2014; 7(2): 135-

46. Doi: 10.1111/jebm.12094
- 35 Chesneau M, Pallier A, Braza F, Lacombe G, Le Gallou S, Baron D, Giral M, Danger R, Guerif P, Aubert-Wastiaux H, Ne'el A, Michel L, Laplaud D-A, Degauque N, Soullillou J-P, Tarte K and Brouard S. Unique B Cell Differentiation Profile in Tolerant Kidney Transplant Patients. *Am J Transplant* 2014; 14: 144–155
- 36 Lemoine S, Morva A, Youinou P, Jamin C. Regulatory B cells in autoimmune diseases: how do they work? *Ann NY Acad Sci* 2009; 1173: 260–267.
- 37 Nouël A, Ségalen I, Jamin C, Doucet L, Caillard S, Renaudineau Y, Pers JO, Le Meur Y, Hillion S. B cells display an abnormal distribution and an impaired suppressive function in patients with chronic antibody-mediated rejection. *Kidney Int.* 2014 Mar; 85(3):590-9. doi: 10.1038/ki.2013.457. Epub 2013 Nov 27.
- 38 Brouard S, Mansfield E, Braud C, Li L, Giral M, Hsieh SC, Baeten D, Zhang M, Ashton-Chess J, Braudeau C, Hsieh F, Dupont A, Pallier A, Moreau A, Louis S, Ruiz C, Salvatierra O, Soullillou JP, Sarwal M. Identification of a peripheral blood transcriptional biomarker panel associated with operational renal allograft tolerance. *Proc Natl Acad Sci U S A.* Sep 25; 2007 104(39):15448–53. [PubMed: 17873064]
- 39 Lozano JJ, Pallier A, Martinez-Llordella M, Danger R, López M, Giral M, Londoño MC, Rimola A, Soullillou JP, Brouard S, Sánchez-Fueyo A. Comparison of Transcriptional and Blood Cell-Phenotypic Markers Between Operationally Tolerant Liver and Kidney Recipients. *Am J Transplant* 2011; 11(9):1916-26. doi: 10.1111/j.1600-6143.2011.03638.x. Epub 2011 Aug 9.
- 40 Tisone G, Orlando G, Cardillo A, Palmieri G, Manzia TM, Baiocchi L, Lionetti R, Anselmo A, Toti L, Angelico M. Complete weaning off immunosuppression in HCV liver transplant recipients is feasible and favourably impacts on the progression of disease recurrence. *J Hepatol.* 2006;44(4):702-9. Epub 2006 Jan 4.
- 41 Ghaziani T, Sendi H, Shahraz S, Zamor P, Bonkovsky HL. Hepatitis B and liver transplantation: molecular and clinical features that influence recurrence and outcome. *World J Gastroenterol.* 2014; 20(39):14142-55. Doi: 10.3748/wjg.v20.i39.14142
- 42 Regev A, Molina E, Moura R, et al. Reliability of histopathologic assessment for the differentiation of recurrent hepatitis C from acute rejection after liver transplantation. *Liver transplant* 2004;10(10):1233-9
- 43 Gehrau RC, Mas RM, Suh JL, Maluf DG. Liver transplant complications in hepatitis C infected recipients: recurrence versus rejection. *Expert Rev Gastroenterol Hepatol;* 2014;8(5):453-6. Doi: 10.1586/17474124.2014.898562
- 44 Carpentier A, Conti F, Stenard F, Aoudjehane L, Miroux C, Podevin P, Morales O, Chouzenoux S, Scatton O, Groux H, Auriault C, Calmus Y, Pancre V, Delhem N. Increased expression of regulatory Tr1 cells in recurrent hepatitis C after liver transplantation. *Am J Transplant* 2009; 9: 2102-2112
- 45 Grassi A, Ballardini G. Post-liver transplant hepatitis C virus recurrence: an unresolved thorny problem. *World J Gastroenterol.* 2014;20(32):11095-115. doi: 10.3748/wjg.v20.i32.11095
- 46 Charlton M, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS Jr, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N; SOLAR-1 Investigators. Ledipasvir and Sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology.* 2015;149(3):649-59
- 47 Mariño Z, Londoño MC, Forns X. Hepatitis C treatment for patients post liver transplant. *Curr Opin Organ Transplant.* 2015 20(3):251-8
- 48 Haas M, Sis B, Racusen LC, Solez K, Glotz D, Colvin RB, Castro MCR, David DSR, David-Neto E, Bagnasco SM, Cendales LC, Cornell LD, Demetris AJ, Drachenberg CB, Farver CF, Farris III AB, Gibson IW, Kraus E, Liapis H, Loupy A, Nicleleit V, Randhawa P, Rodriguez ER, Rush ED, Smith RN, Tan CD, Wallace WD and Mengel M as the Banff meeting report writing committee. Banff 2013 Meeting Report: Inclusion of C4d-Negative Antibody-Mediated Rejection and Antibody-Associated Arterial Lesions. *Am J Transplant* 2014; 14: 272–28
- 49 O'Leary JG, Kaneku H, Demetris AJ, Marr JD, Shiller M, Susskind BM, Tillery GW, Terasaki PI, and Klintmalm GB. Antibody-Mediated Rejection as a Contributor to Previously Unexplained Early Liver Allograft Loss. *Liver Transplant* 20:218–227, 2014.
- 50 Tait BD, Süsal C, Gebel HM, Nickerson PW, Zachary AA, Claas FH, Reed EF, Bray RA, Campbell P, Chapman JR, Coates PT, Colvin RB, Cozzi E, Doxiadis II, Fuggle SV, Gill J, Glotz D, Lachmann N, Mohanakumar T, Suciú-Foca N, Sumitran-Holgersson S, Tanabe K, Taylor CJ, Tyán DB, Webster A, Zeevi A, Opelz G. Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation. *Transplantation* 2013; 95(1):19-47
- 51 O'Leary JG, A. Demetris D, Friedman LS, Gebel HM, Halloran PF, Kirk AD, Knechtle SJ, McDiarmid SV, Shaked A, Terasaki PI, Tinckam KJ, Tomlanovich SJ, Wood KJ, Woodle ES, Zachary AA and Klintman GB. Meeting Report. The Role of Donor-Specific HLA Alloantibodies in Liver Transplantation. *Am J Transplant* 2014; 14: 779–787
- 52 Valenzuela NM, Reed EF. Antibodies in transplantation: the effects of HLA and non-HLA antibody binding and mechanisms of injury. *Methods in molecular biology.* 2013; 1034:41–70.
- 53 Valenzuela NM, McNamara JT, and Reed EF. Antibody-Mediated Graft Injury: Complement-Dependent and Complement-Independent Mechanisms. *Curr Opin Organ Transplant.* 2014; 19(1): 33–40.
- 54 Dugum M, Askar M, Pai RK, Yerian L, Bennett A, McMahon J, Xie H, Eghtesad B, Hanouneh I, Liu X. Re-examination of sinusoidal deposition of complement 4d in liver allografts: experience from a single institution. *Int J Clin Exp Pathol* 2014;7(2):784-791 www.ijcep.com/ISSN:1936-2625/IJCEP1312033
- 55 Briggs D, Adams DH. Antibody-Associated Rejection in Liver Transplantation: Keep on Knocking, and the Door Will Be Opened to You. *Am J Transplant* 2011; 11: 1767–1768
- 56 Hadaya K, Ferrari-Lacraz S, Giostra E, Majno P, Moll S, Rubbia-Brandt L, Marangon N, Venetz JP, Bolle JF, Mentha G, Villard J. Humoral and cellular rejection after combined liver–kidney transplantation in low immunologic risk recipients. *Transplantation International* 2009 ; 22 242–246
- 57 Calne RY, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, Binns RM, Davies DA. Induction of immunological tolerance by porcine liver allografts. *Nature.* 1969 Aug 2;223(5205):472-6
- 58 Kamada N, Davies HFFS, Roser B. Reversal of transplantation immunity by liver grafting. *Nature* 1981; 292:840-42
- 59 Roser BJ. Immunosuppressive effect of experimental liver allografts. In: Calne RY, ed. 3. Liver transplantation. London: Grune & Stratton; 1987. p. 35–54.
- 60 Jiang M, Han D. Auxiliary liver heart transplantation in the rat: a new microsurgical model inducing tolerance. *Transplant Proc* 2004; 36: 1607-9
- 61 Rana A, Robles S, AB, Russo MJ, Halazun KJ, Woodland DC, Witkowski P, Ratner LE and Hardy MA. The Combined Organ Effect Protection Against Rejection? *Annals of Surgery* 2008;248 (5):871-79
- 62 Cannon RM, Hughes MG, Jones CM, Eng M and Marvin MR. A review of the United States experience with combined heart-liver transplantation. *Transplant International* 2012; 25:1223–1228
- 63 Simpson N, Cho YW, Cicciarelli JC, Selby RR, Fong TL. Comparison of Renal Allograft Outcomes in Combined Liver-Kidney Transplantation versus Subsequent Kidney Transplantation in Liver Transplant Recipients: Analysis of UNOS Database. *Trans-*



- plantation 2006; 82: 1298–1303
- 64 Martin EF, Huang J, Xiang Q, Klein JP, Bajaj J and Saeian K. Recipient and Graft Survival Are Not Diminished by Simultaneous Liver-Kidney Transplantation: An Analysis of the UNOS Database. *Liver Transpl.* 2012 ; 18(8): 914–929
  - 65 Cunningham EC, Sharland AF and Bishop GA. Liver Transplant Tolerance and Its Application to the Clinic: Can We Exploit the High Dose Effect? *Clin Dev Immunol.* 2013;2013:419692. doi: 10.1155/2013/419692. Epub 2013 Nov 6. Review.
  - 66 Cunningham EC, Tay SS, Wang C, Rtshiladze M, Wang ZZ, McGuffog C, Cubitt J, McCaughan GW, Alexander IE, Bertolino P, Sharland AF, Bowen DG, Bishop GA. Gene Therapy for Tolerance: High-Level Expression of Donor Major Histocompatibility Complex in the Liver Overcomes Naive and Memory Alloresponses to Skin Grafts? *Transplantation* 2013; 95(1):70-7. doi:10.1097/TP.0b013e318278d39a
  - 67 Madsen JC, Superina RA, Wood KJ, Morris PJ. Immunological unresponsiveness induced by recipient cells transfected with donor MHC genes. *Nature* 1988; 332: 161.
  - 68 Geissler EK, Wang J, Fechner JH Jr, Burlingham WJ, Knechtle SJ. Immunity to MHC class I antigen after direct DNA transfer into skeletal muscle. *J Immunol* 1994; 152: 413.
  - 69 Wu SL, Pan CE. Tolerance and chimerism and allogeneic bone marrow/stem cell transplantation in liver transplantation. *World J Gastroenterol* 2013; 19(36): 5981-598
  - 70 Jonsson JR, Hogan PG, Thomas R, Steadman C, Clouston AD, Balderson GA, Lynch SV, Strong RW, Powell EE. Peripheral blood chimerism following human liver transplantation. *HEPATOLOGY* 1997; 25: 1233-1236 [PMID: 9141443]
  - 71 Starzl TE, Zinkernagel RM. Transplantation tolerance from a historical perspective. *Nat Rev Immunol* 2001; 1: 233-239 [PMID: 11905833 DOI: 10.1038/35105088]
  - 72 Verdonk RC, Haagsma EB, Jongsma T, Porte RJ, Roozendaal C, van den Berg AP, Hepkema BG. A prospective analysis of the natural course of donor chimerism including the natural killer cell fraction after liver transplantation. *Transplantation* 2011; 92: e22-e24 [PMID: 21814126 DOI: 10.1097/TP.0b013e318225283e]
  - 73 Ayala R, Grande S, Albizua E, Crooke A, Meneu JC, Moreno A, Pérez B, Gilsanz F, Moreno E, Martínez-Lopez J. Longterm follow-up of donor chimerism and tolerance after human liver transplantation. *Liver Transplant* 2009; 15: 581-591 [PMID: 19479801 DOI: 10.1002/lt.21736]
  - 74 Ildstad ST, Sachs DH. Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. *Nature* 1984; 307: 168-170 [PMID: 6361574 DOI: 10.1038/307168a0]
  - 75 Kawai T, Sogawa H, Boskovic S, Abrahamian G, Smith RN, Wee SL, Andrews D, Nadazdin O, Koyama I, Sykes M, Winn HJ, Colvin RB, Sachs DH, Cosimi AB. CD154 blockade for induction of mixed chimerism and prolonged renal allograft survival in nonhuman primates. *Am J Transplant* 2004; 4: 1391-1398 [PMID: 15307826 DOI: 10.1111/j.1600-6143.2004.00523.x]
  - 76 Taner T, Stegall MD, Heimbach JK. Antibody-Mediated Rejection in Liver Transplantation: Current Controversies and Future Directions. *Liver Transplant* 2014; 20:514–527
  - 77 Dara W, Agarwala A, Watkins C, Gebelb HM, Brayb RA, Kokkoc KE, Pearson TC and Knechtlea SJ. Donor-Directed MHC Class I Antibody Is Preferentially Cleared from Sensitized Recipients of Combined Liver/Kidney Transplants. *Am J Transplant* 2011; 11: 841–847

**Peer reviewer:**