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Role of the Hepatic Parenchyma in Liver Transplant Tolerance: A Paradigm Revisited

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Abstract
Unlike other solid organs, liver transplants are spontaneously accepted in a wide range of animal models. In the clinic, transplanted livers also display privileged immunological properties allowing weaning of immunosuppression therapy in up to 20% of selected patients. To explain this phenomenon, many studies have focused on the role of donor-derived 'passenger' leukocytes that are thought to induce antigen-specific tolerance by migrating from the graft into recipient secondary lymphoid tissues. Although convincing evidence exists that these cells are able to elicit antiallograft T cell hyporesponsiveness, several studies argue against an exclusive role for this cell population and even question whether it is critical in conferring donor MHC-specific tolerance. Instead, these studies suggest that the hepatic parenchyma plays a more critical role in this phenomenon. In this review we will reinterpret the results of old and more recent literature in light of recent advances in the field of liver immunology to explain the contribution of both passenger leukocytes and liver tissue in the liver tolerance effect.

Introduction

Liver diseases represent an increasing health burden and an enormous human and financial cost to the community, and liver transplantation is currently the only long-term therapeutic option for patients with end-stage liver disease. While immunosuppressive medication prevents allograft rejection by suppressing the patient's immune responses toward the donor liver, these drugs place the recipient at risk of cardiovascular disease, malignancy and infection. Clinicians strive to minimize immunosuppressive medication without increasing the risk of rejection. Ultimately, the goal is to achieve transplantation tolerance, a state whereby the recipient, in the absence of immunosuppression, does not mount an immune response against the donor organ, while maintaining the capacity to respond to pathogens and tumors.

Liver transplants are unique compared to other solid organ grafts: they are spontaneously accepted in a wide range of animal models, including across completely MHC-mismatched mouse and rat inbred strain combinations [1]. Liver transplants can also reverse ongoing rejection of previous organ allografts from the same donor strain, including heart [2], pancreas [3] and skin [4, 5].

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Human liver transplants require less immunosuppression than other organs [6] and, unlike renal transplants, early rejection episodes do not affect long-term graft survival [7]. In about 20% of selected liver recipients, immunosuppression can even be weaned off completely without rejection of the allograft [8–10], although specific biomarkers to identify such patients are still lacking [11].

Various models have been proposed to explain liver transplant tolerance. Most studies in rats have highlighted both the role of donor passenger leukocytes and the liver tissue in this process, but their relative contribution and mechanisms are still unknown. Passenger leukocytes migrate to secondary lymphoid tissues where they are thought to induce an 'abnormal' activation of graft-reactive T cells, leading to tolerance [12, 13]. The mechanisms by which the liver tissue induces tolerance are even more enigmatic. In this review, we will reinterpret old and recent findings to explain how the liver tissue contributes to the spontaneous acceptance of liver allografts.

Liver Transplantation Is Associated with the Induction of Tolerance

In 1969, Sir Roy Calne [14, 15] reported for the first time that liver transplants were accepted in outbred pigs in the absence of immunosuppressive drugs, opening a new area of research in transplantation immunology. Liver acceptance between outbred individuals has been confirmed in several other species [5, 16–19]. Using inbred mouse and rat strains, it was confirmed that liver transplants were accepted between several complete MHC mismatch barrier combinations [5, 16–18]. In contrast, kidneys were rejected at day 12 [20], while hearts underwent rejection after 8 days [21]. Likewise, in clinical practice, unlike renal transplants, HLA-matching of liver transplant recipients and donors is not necessary [22–25].

A decade after the pioneering observation by Calne, Kamada and colleagues were able to demonstrate, in rats, that recipients of liver grafts also accepted subsequent skin or heart transplants from the same donor strain while rejecting third-party grafts [5, 16–18]. Liver transplants were also able to reverse severe ongoing rejection of previous organ transplants from the same strain, including heart [2], pancreas [3] and skin [4, 5, 17]. Liver transplants could reverse ongoing rejection even 6 days after transplantation of the heart, but only in 50% of cases, while both organs were rejected when the liver was transplanted after 6 days [26], indicating that there was an 'all-or-nothing' threshold between rejection and acceptance. These findings are consistent with the clinical observation that the combined transplantation of a liver with another organ from the same donor results in fewer rejection episodes of the organ, including kidney [27], heart [28], lung and multiorgan recipients [29]. These experiments reinforced the view that liver grafts were not ignored by the immune system, but rather induced antigen-specific tolerance.

Histology of Tolerant and Rejecting Liver Grafts

Several studies have followed to dissect mechanisms of tolerance associated with spontaneous liver transplant acceptance. In mice, the surgery is extremely difficult. For this reason, most studies have been performed in rats in which both tolerant and rejecting strain combinations have been reported. PVG rat strain livers transplanted to completely MHC-mismatched DA-strain recipients (PVG→DA) did not reject the liver for more than 100 days, even though they received no immunosuppression [18, 30]. In contrast, PVG→Lewis liver allografts were rapidly rejected [18]. Spontaneous acceptance of the PVG liver into the DA recipient was associated with hyporesponsiveness of the recipient to another graft from the same donor while rejecting a third-party organ at a normal pace, suggesting that it had induced donor-specific tolerance. Many groups have used these strain combinations to determine whether there are differences between tolerant and rejecting combinations.

The presence of cellular infiltrates and focal necrosis of hepatocytes in tolerant liver grafts during the first 2 weeks confirmed that tolerant liver transplants were not ignored by the recipient's immune system, but instead tolerance was associated with vigorous activation of the adaptive immunity [26]. Interestingly, experiments in the rat allograft model indicated that allograft damage occurred early in the post-transplant period, irrespective of whether the allograft was rejected or accepted [26], raising the question of how immune activation and allograft damage could alter the final outcome. By comparing the composition of infiltrates in the liver graft of accepting and rejecting strain combinations, Sun et al. [31] showed that there were minor differences in the proportion of CD11b+ (macrophages) and CD3+ cells (T cells). However, the liver of tolerant recipients contained a higher proportion of IgD+ cells (B cells). The significance of this difference was not clear. Other studies have confirmed that there is little difference in liver T cell infiltrates between rejecting and accepting strain combinations [32].

Cellular infiltration in liver grafts has often been associated with apoptosis of both hepatocytes and infiltrating leukocytes in animal models, but also in clinical liver transplantation [33–35]. Interestingly, apoptosis of lymphocytes also occurred in parallel in lymphoid tissues [36, 37], suggesting that recipient T cells might became apoptotic as a result of their activation. In acute rejection, the level of apoptosis within the graft correlated with the severity of the rejection [33, 35], and reducing apoptosis by knocking down IL-2 prevented rejection in the LEW to BN rat strain combination [38]. On the other hand, apoptosis was also observed in liver transplants that were accepted, and in the study by Qian et al. [39], reducing apoptosis in the liver by IL-2 treatment converted liver transplant tolerance to rejection [39, 40]. To date, no definitive study has been performed to determine if it is apoptosis of the liver tissue, apoptosis of liver infiltrates or apoptosis of leukocytes in secondary lymphoid organs that best correlates with liver transplant tolerance.

Role of Donor-Derived Leukocytes in Liver Transplantation Tolerance

Passenger Leukocytes and Microchimerism

The liver contains both resident leukocytes (e.g. Kupffer cells) and leukocytes that are able to recirculate to lymphoid tissues following liver transplantation [also known as passenger leukocytes (PLs)]. By migrating to lymphoid tissues, PLs are thought to induce abortive activation of recipient leukocytes leading to tolerance [41]. It was proposed that the liver would be more prone to induce tolerance than other solid organs due to its larger size [42] and/or because it harbors a higher number of migratory donor PLs [41]. Consistent with this proposal, several organs from the same donor transplanted at the same time survived longer than single organ transplants in both small animals [42] as well as in humans [43–45]. The mechanisms and exact contribution of this particular process to the spontaneous acceptance of liver grafts and liver tolerance 'effect' remain unclear. Depletion of donor PLs induced rejection [46–48], while the reconstitution of donor PLs either through i.v. infusion [42, 47] or parking the liver in a donor strain animal [46] was able to restore tolerance. These experiments led some investigators to suggest that this is the major pathway by which liver PLs induced tolerance.

This view was consolidated when, in 1992, Starzl et al. [49] demonstrated that liver transplant patients completely weaned off all immunosuppression drugs displayed long-term donor cell chimerism. The model of 'microchimerism', which describes the existence of a very low (<1%) frequency of hematopoietic donor cells within the recipients blood and tissue, was born. According to this model, the outcome of liver transplants is determined by a limited graft-versus-host and host-versus-graft response [50]. These 'two-way mixed lymphocyte reaction' would reach a balance after several months during which immunosuppressive treatment could be decreased and eventually completely stopped [51]. However, microchimerism alone could not explain spontaneous acceptance of liver grafts, as the existence of microchimerism failed to identify patients suitable for successful weaning of immunosuppressive therapy [52, 53]. Instead, it has been postulated that microchimerism could represent a consequence of tolerance rather than its cause [54].

Evidence supporting a role for donor PLs has also been provided in a rat liver transplant model, where the donor liver was retransplanted into a recipient after being 'parked' in another recipient of the same strain for several weeks. The livers which were composed of donor-derived parenchymal and liver-restricted leukocytes and recipient-derived PLs, were rejected following retransplantation [55]. Similar results were obtained by depletion of donor leukocytes within the graft using irradiation [48], while reconstitution of an irradiated liver by 'parking' it in a recipient rat of the donor strain for 36 h before transplanting into an allogeneic host could restore tolerance [46, 56]. In this context, it is important to note that irradiation might do more than depleting leukocytes. For example, irradiation induces some inflammation and upregulation of adhesion molecules [57]. In addition, NKG2D ligands MICA/B and ULBP1–3 proteins have been described as being upregulated following inflammation [58]. These changes might alter the immune response independently of PLs.

Use of Donor Splenocytes to Mimic the Role of PLs

Microchimerism and its mechanisms are still debated [1], but this model has inspired several investigators to examine the role of PLs in inducing antigen-specific T cell tolerance in a transplant setting. Infusion of donor leukocytes at the time of transplantation (to mimic PLs migrating out of the graft) has been used in several mouse and rat transplant models to prolong graft survival [59, 60]. These studies recapitulate the ability of donor PBMC to induce tolerance (the so-called 'blood transfusion effect' [61]) and have demonstrated some (sometimes limited) success in extending graft survival. This was highly

dependent on the nature of the transplanted organ, the species and the number of PLs adoptively transferred. In rats, donor-derived splenocytes administered just before liver transplantation induced donor-specific tolerance [42, 47] and led to acceptance of liver allografts that were normally rejected [62]. Administration of donor splenocytes was also able to induce tolerance to rat kidney allografts [62]. However, administration of donor splenocytes was unable to convert skin allograft rejection into acceptance [42], nor heart allograft rejection into tolerance [59]. In most cases, donor leukocytes alone were not able to induce long-term acceptance of allogeneic transplants. This could only be achieved when the recipients also received other treatments either pharmacologically (e.g. calcineurin-inhibitors) [59, 63] or by blocking costimulatory molecules, signal 2 in T cell activation, using antibodies (e.g. CD154 mAb) [64].

The nature of the donor splenocytes contributing to inducing tolerance or prolongation of graft survival has been reviewed elsewhere and is not the focus of this review [1]. In summary, although T cells [42, 55], B cells [65, 66] and dendritic cells (DCs) [67] have been shown to play some role in inducing tolerance, the nature of the cellular subset, and whether the spleen and lymph nodes play a similar role in activating and/or deleting alloreactive T cells remains unsolved. Although they are able to induce tolerance, it is also not clear whether the injection of donor splenocytes into transplant recipients is representative of the migration of liver PLs after transplantation.

Intrahepatic Leukocytes

The liver harbors different subsets of lymphocytes that differ from those of the blood and spleen, including hematopoietic stem cells, unique subsets of DCs [67, 68], and a high proportion of natural killer (NK) and natural killer T (NKT) cells [69]. It is thus possible that it is the qualities of liver leukocytes, rather than the quantity, that induces tolerance.

Role of Liver DCs

The total number of DCs in a normal liver is up to fivefold [70] higher than in other solid organs, but the volume density is the lowest of all organs [70]. Most studies agree that freshly isolated hepatic DCs are immature and are less immunogenic than splenic DCs [71, 72]. They express low levels of MHC class II and costimulatory molecules (CD80 and CD86), a finding consistent with their poor allostimulatory ability in MLR assays [73, 74]. They also secrete IL-10 [75] and display a higher threshold for activation than splenic DCs due to decreased expression of Toll-like receptor 4 [76]. It has been proposed that hepatic DCs induce apoptosis, possess immune regulatory/suppressive functions [77] and might participate in the generation of regulatory T cell (T_{reg}) populations [78, 79]. However, their immature phenotype and location in the portal tracts argue against their role in inducing spontaneous acceptance of liver transplants. Consistent with limited DC contribution in the liver tolerance effect, administration of the Flt3 ligand, which increases the number of DCs, caused rejection of liver allografts [80]. In humans, BDCA-1+ DCs were the most prevalent DC subset in the liver in contrast to CD16+ DCs in the blood. In direct comparison, between human liver and blood, DCs produced large amounts of IL-10 and induced FoxP3-positive T_{reg} and IL-4 producing Th2 cells, while being less effective in inducing proliferation of allogenic T cells [81].

Although the role of hepatic DCs in inducing spontaneous tolerance to liver allografts remains speculative, manipulation of DCs has the potential to be used as a therapy. Several investigators have shown that human and mouse DC manipulated ex vivo with cytokines such as IL-10 [82], TGF-β [83] and TNF-α [56], or pharmacological reagents like dexamethasone or mitomycin C [84] were able to promote antigen-specific hyporesponsiveness.

In addition to DCs, the liver contains sufficient hematopoietic stem cells to reconstitute lethally irradiated recipients [85, 86]. These cells have been suggested to play some role in tolerance by migrating into the recipient thymus and inducing negative selection of donor-derived T cells [87, 88]. Although this might occur and be important for the long-term maintenance of tolerance and the establishment of microchimerism, it is unlikely that it contributes significantly to establish tolerance, as thymectomy does not interfere with liver transplant tolerance [41, 89].

Role of NK/NKT Cells

NK and CD8+ CD69+ lymphocytes expressing high levels of MHC class II and CD25 are detected in portal tracts and perisinusoidal areas of liver transplants [90], indicating that they might play some role in inducing tolerance by interacting with resident recipient leukocytes [91].

Some in vitro experiments indicate that both NK and NKT cells display some indirect tolerogenic properties that require the presence of hepatocytes. NK cell interaction with hepatocytes in vitro led to NKG2A-dependent secretion of TGF-β, generating tolerogenic DCs which in

turn induced CD4+ CD25+ T_{reg} [92]. Likewise, hepatocytes induced NKT cells to secrete IFN-β and tolerogenic CD8+ T cells secreting IL-10 [93].

In vivo studies suggest, however, that NK/NKT cells are more involved in rejection than in protecting the graft. Administration of an antibody that depleted almost all NK and NKT cells prior to transplantation of a fully MHC-mismatched DA liver transplant into a Lewis recipient rat prolonged allograft survival from 10 to 19 days, while depletion of host CD8+ T cells did not prevent liver allograft rejection [94]. It is interesting to note that in this model, all intrahepatic NK cells were of host origin as soon as day 3 after transplantation, indicating that at least in this model, rejection was mediated by host NK and/or NKT cells rather than donor NK/NKT cells. However, a recent study showed, by depleting donor NK cells before transplant, that donor liver NK cells were not vitally important for induction of liver transplant tolerance in the PVG to DA rat strain combination [95]. Other studies support a role of NK cells in inducing rejection [96], and in the clinic matching HLA-C (a major inhibitory ligand for NK cells) has also been associated with a better long-term survival of liver transplants [97], thus supporting the hypothesis that NK/NKT cells can induce rejection.

Role of the Hepatic Parenchyma in Liver Transplant Tolerance

The concept that donor PLs play the most critical role in spontaneous liver transplant acceptance has been relatively popular due to the attractive model of microchimerism and experiments using adoptively transferred donor leukocytes. However, this mechanism alone is not sufficient to explain why liver grafts are accepted spontaneously. As mentioned before, microchimerism is not always associated with graft survival. Furthermore, donor splenocyte administration only prolongs the survival of kidney allografts, but has no effect on the survival of other organs (such as skin and heart) [42, 55].

Evidence for a Role of the Liver Tissue in Spontaneous Acceptance of Liver Grafts

Experiments performed by Calne and colleagues [56] have also emphasized the secondary role of PLs. In these experiments, the donor graft was a chimeric liver in which the migratory PLs were the only cells expressing the alloantigen. This liver was obtained by performing two successive transplants: a PVG strain liver was transplanted into a DA recipient and 20 days later, when the PVG liver was repopulated with DA leukocytes, the liver was retransplanted into a secondary PVG recipient. Although now containing passenger DA leucocytes, the chimeric transplanted liver grafts completely failed to prolong the survival of subsequent DA skin grafts, suggesting that PLs alone were unable to induce tolerance. To examine the role of the nonmigratory cells (liver tissue including hepatocytes, liver sinusoidal endothelial cells, Kupffer cells and stellate cells) in this process, the same investigators generated the reverse transplant chimera, in which a DA liver reconstituted with passenger PVG leukocytes into a PVG rat. In contrast to the other chimeras in which activation was induced by PLs, the survival of DA skin grafts on these chimeric transplant recipients was prolonged in the absence of DA PLs, suggesting that parenchymal cells were able to induce tolerance.

Chiba et al. [55] also confirmed the important role of the liver tissue by reporting a very intriguing finding. They repeated the experiments performed by Sun et al. [42, 46] showing that irradiation of a DA liver before transplantation into a PVG host, 24 h later, abrogated the spontaneous acceptance normally observed in this combination. They confirmed that if they reconstituted the donor just after irradiation with DA splenocytes, they could restore spontaneous acceptance of the liver graft. The surprising result was that if they reconstituted the donor with third-party (BN) splenocytes expressing a different allo-MHC molecule, acceptance was also restored. Recipient rats accepting the DA liver graft and injected with DA or BN splenocytes rejected a BN heart, but did not reject a DA heart, indicating that splenocytes restored tolerance regardless of their MHC haplotype and that the specificity of the tolerance depended on the MHC of the liver tissue rather than on that of splenocytes [55]. By injecting purified T and B cells instead of splenocytes, the authors of this study showed that restoration of the tolerance effect was conferred by T cells and suggested that T cells secreting immunomodulatory cytokines (potentially T_{reg}) were involved.

These results point out the critical role played by parenchymal cells and the liver tissue in spontaneous acceptance of liver allografts. Similar results were obtained by Kreisel et al. [98] by creating bone marrow chimeric rats: third-party bone marrow was as effective as the bone marrow of donor origin in inducing acceptance of the liver transplant. Again, in these experiments, the liver tissue was important in conferring the MHC specificity [98], arguing against a critical role for PLs in inducing

donor-specific tolerance. Similar results were obtained using chimeric mouse models [99].

In summary, there is substantial data in the literature to suggest that the non-bone marrow-derived component of the liver graft (including hepatocytes, liver sinusoidal endothelial cells and potentially stellate cells) play a critical role in inducing donor-specific tolerance. Two non-exclusive models could explain the role of MHC-restriction in this tolerance: soluble MHC molecules and direct T cell activation by liver cells.

The Role of Liver Tissue in Secreting Soluble MHC Molecules

The liver secretes soluble MHC class I molecules, and following transplantation, high amounts of these molecules are found in the serum [17, 100]. This has led some investigators to speculate that soluble MHC molecules might play a role in inducing spontaneous acceptance of liver transplants and be responsible for the effect of the liver tissue (in particular hepatocytes) in this phenomenon. Transplantation of livers from MHC class I-deficient donors did not prevent acceptance of the liver [101], but these studies are hard to interpret as allogeneic T cells would also ignore MHC class I-deficient liver.

Studies in which recipients were administered soluble MHC molecules are more convincing. Initial studies were disappointing and demonstrated that large quantities of soluble RT1a class I molecule from the DA strain, given intravenously to PVG recipients of DA cardiac allografts by a variety of protocols, did not have any effect on graft survival [102]. Similar results were obtained by other investigators [103, 104]. Soluble MHC molecules were only effective in prolonging graft survival in combination with cyclosporine treatment [103]. However, these experiments were performed using a single soluble MHC molecule in a complete mismatch combination.

Using a single mismatch MHC molecule combination, Sumimoto and Kamada [105] reported that the daily injection of a DA rat serum (MHC haplotype RT1a) into a PVG (RT1c) rat receiving a heterotopic PVG (RT1a) donor heart allograft prolonged the survival of the graft. Removal of the soluble MHC class I molecules by affinity chromatography abolished the immunosuppressive effect, indicating that MHC molecules were responsible for this result. Moreover, continuous infusion of purified soluble class I antigen from DA rat liver, even from day 4 after heart grafting, induced a significant prolongation of graft survival in a donor-specific manner. A mixture of monoclonal anti-class I (RT1a) antibody with DA serum by continuous infusion amplified the immunosuppressive effect [105]. Wang et al. [106] also reported that RT1Aa heavy chain proteins injected into the thymus or into the portal vein 14 days before transplantation induced indefinite survival of ACI liver allografts in Lewis (RT1l) recipients, but only when they were coadministered with anti-T cell receptor mAb [106]. Further experiments have confirmed the immunosuppressive effect of soluble MHC molecules. Geissler et al. [107] transfected primary cultured Lewis hepatocytes so that they expressed either soluble or membrane-bound MHC class I molecules. By transplanting an ACI (RT1Aa) liver into a Lewis (RT1l) recipient, they demonstrated that hepatocytes secreting RT1Aa molecules injected into the portal vein extended liver allograft survival and decreased CTL activity. Interestingly, in contrast, recipients injected with hepatocytes expressing membrane-bound RT1Aa demonstrated accelerated graft rejection and primed CTLs [107].

The mechanism by which soluble MHC molecules delay graft rejection is still a matter of debate, but it is thought that they play an important role at the very early stages after transplantation by preventing hyperacute graft rejection induced by alloreactive antibodies [108]. Soluble MHC would neutralize these antibodies, hence reducing organ damage and prolonging graft survival. A role for soluble MHC molecules in promoting apoptosis of alloreactive cytotoxic T cells [109] has also been reported. Alternatively, it has been hypothesized that immature host DCs take up peptides derived from donor soluble MHC molecules and present them to recipient T cells, leading to tolerance [110, 111]. Consistent with this hypothesis, soluble donor MHC bound to a monoclonal antibody (so that it would be picked up by macrophages and DCs) was more effective in prolonging allograft survival than infusion of soluble donor MHC alone [105]. However, there are also reports demonstrating that this indirect pathway of antigen presentation failed to induce tolerance to subsequent skin transplants [112].

Collectively, these experiments suggest that administration of soluble MHC class I alone has a minimal effect on graft survival by delaying rejection by a few days and, thus, cannot completely account for the role of the parenchyma to induce tolerance. In addition, human studies have shown no correlation between the large quantity of donor HLA in the sera, graft function [113] or a state of tolerance [114]. On the contrary, increased levels of soluble MHC class I has been reported in the setting of rejection or infection [115].

The Role of the Liver Tissue in Inducing Abortive Primary T Cell Activation

Homing and entry of naïve and activated T cells into lymphoid tissues and organs including the liver are determined by complex molecular interactions involving adhesion molecules that recognize their ligands on endothelial cells [116, 117]. Naïve T lymphocytes do not normally have access to the parenchyma of most organs, as they do not express adhesion molecules and chemokine receptors required for adhesion to endothelial cells or subsequent transendothelial migration [116, 117]. However, naïve T cells express L-selectin (CD62L) which binds to peripheral LN-specific vascular addressins and has been shown to play a critical role in initial binding (tethering) and subsequent rolling of the lymphocytes through the high endothelial venules of the LNs under normal conditions of flow [118]. Chemokines and intercellular adhesion molecule-1/leukocyte function-associated antigen-1 interactions also play an important role in these adhesion steps [118]. In the LNs, naïve T cells interact with professional APCs, in particular DCs, expressing relevant peptide/MHC complexes. This contact induces T cell activation resulting in expression and upregulation of adhesion molecules, which allows activated T cells to undergo transendothelial migration and to infiltrate the tissues.

T cells can enter the liver via the sinusoidal endothelium [119]. Sinusoids are formed by monolayers of hepatocytes defining narrow channels approximately 10 μm in diameter that are lined by a layer of specialized endothelial cells and liver resident Kupffer cells [120]. Unlike other endothelial cells, liver sinusoidal endothelial cells are perforated by multiple 120-nm diameter holes (fenestrations), and do not form tight junctions with adjacent endothelial cells [121]. Entry via liver sinusoids is unique as it does not require selectins [122, 123]. Lack of selectin requirement is thought to be due to a slower or intermittent blood flow that favors intimate contact between leucocytes and liver cells [121]. These unique conditions of slow blood flow, in combination with the narrow diameter of the hepatic sinusoids and their unusual structure favors contact between lymphocytes and liver cells resulting in their retention. Early studies have established that while in the absence of their cognate antigen, naïve T cells recirculate through the liver without being retained [124–127], and activated CD8+ [124, 126–128] and CD4+ T cells [125] are very efficiently retained in a nonantigen-specific manner, even in the absence of intrahepatically expressed cognate antigen.

We have suggested that the ability of the liver to induce tolerance is associated with its unique capability to retain and activate naïve alloreactive or antigen-specific CD8+ T cells [123, 126, 127, 129]. In transplantation, such activation is likely to be mediated by donor liver sinusoidal endothelial cells and Kupffer cells that directly line the sinusoidal lumen [71] and are able to directly contact recipient T cells. Hepatocytes that are located underneath the physical barrier formed by liver sinusoidal endothelial cells could be less accessible, although we have demonstrated that hepatocyte/T cell contacts nevertheless occur via liver sinusoidal endothelial cell fenestrations [120]. Retention and activation of antigen-specific naïve CD8+ T cells has been shown in the livers of transgenic mice specifically expressing the cognate antigen in hepatocytes, in the absence of antigen expression in lymphoid tissues. Transgenic T cells were specifically retained in the livers of recipient mice within minutes and became activated within 2 h [126], suggesting that the liver is an exception to the general rule of T cell activation and recirculation, which predicts that naïve T cells recirculate via the lymph and blood, but do not enter peripheral tissues prior to activation in secondary lymphoid organs. Antigen-presentation by hepatocytes to naïve CD8+ T cells was insufficient to promote activation and proliferation of the T cells, which subsequently died by death-by-neglect in a Bim-dependent pathway in vitro and in vivo [130, 131]. We predict that if this retention occurred in transplantation, it would result in the selective depletion of alloreactive T cells, leading to deletional tolerance.

Due to the difficulty of tracking very rare alloreactive T cells amongst the T cell repertoire, there has been no study reported to date investigating the retention of alloreactive T cells within hours after transplantation. However, some of the data in the literature is consistent with a model in which alloreactive T cells die by neglect. Treatment of rat liver transplant recipients with IL-2, a treatment that overcomes death-by-neglect induced by hepatocyte activation in vitro [130, 131] and which is able to rescue anergic T cells, was able to induce rejection of liver grafts [48].

Conclusion

Although there is convincing evidence that donor PLs play an important role in the liver tolerance effect, there is also evidence suggesting that the liver tissue itself plays a pivotal role in the antigen-specificity of this phenomenon. It appears that both components, PLs and the paren-

chyma, are needed to achieve a state of hyporesponsiveness, as neither the liver parenchyma alone nor donor leukocytes could induce antigen-specific tolerance in robust rodent models. In addition, pre-existing factors like preformed antibodies, prolonged ischemia times or cross-reacting memory T cells might complicate the dissection of liver transplant tolerance in humans and explain why conclusions from small animals are often hard to translate into the clinic.

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