

Tolerogenic Mechanisms in Liver Transplantation

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Abstract

The liver has unique tolerogenic properties which have been recognized since the beginning of liver transplantation. The liver allograft not only demonstrates a lower incidence of rejection compared with other solid organs, but it also has the ability to protect other organs from the same donor against rejection and graft loss. Up to 20% of liver transplant recipients have been successfully weaned from immunosuppressive therapy while maintaining stable allograft function. Furthermore, the liver has the ability to reverse ongoing rejection of other transplanted organs and counters the deleterious effects of preformed lymphocytotoxic antibodies. Various mechanisms have been proposed to explain the immunomodulatory properties of the liver. These include: (1) the transfer of donor-derived hematopoietic cells, called passenger leukocytes, with the liver graft and the creation of donor microchimerism; (2) the role of hepatocytes and non-parenchymal liver cells as tolerogenic antigen presenting cells; (3) the high-dose antigen effect leading to dilution of cytokines and clones of alloreactive T cells; and (4) the introduction of soluble and cell-bound major histocompatibility complex class I molecules by the liver graft. This article will examine the evidence underlying each of these hypotheses and assess their relative significance in the induction and maintenance of donor-specific hyporesponsiveness. An enhanced understanding of the immune processes responsible for transplantation tolerance may lead to the identification of biomarkers for the prediction of graft outcomes. More importantly, this knowledge may facilitate the development of therapeutic strategies to promote indefinite allograft acceptance, while eliminating or minimizing the need for immunosuppressive drugs.

Keywords: Transplantation tolerance; Liver transplantation; Chimerism; Antigen presentation; Soluble MHC molecules

Introduction

The tolerogenic capacity of the liver has been described since the earliest days of experimental Liver Transplantation (LT). In 1967, Roy Calne reported the prolonged survival of transplanted liver grafts between genetically disparate pigs [1]. Despite the absence of immunosuppression, the recipients remained immunologically unresponsive to the liver graft and other tissues from the same donor [2,3]. These findings were later confirmed in mice [4] and between certain strains of rats [5,6]. Furthermore, the liver graft can act as an immunosuppressive agent and reverse ongoing rejection of other transplanted organs [7-9].

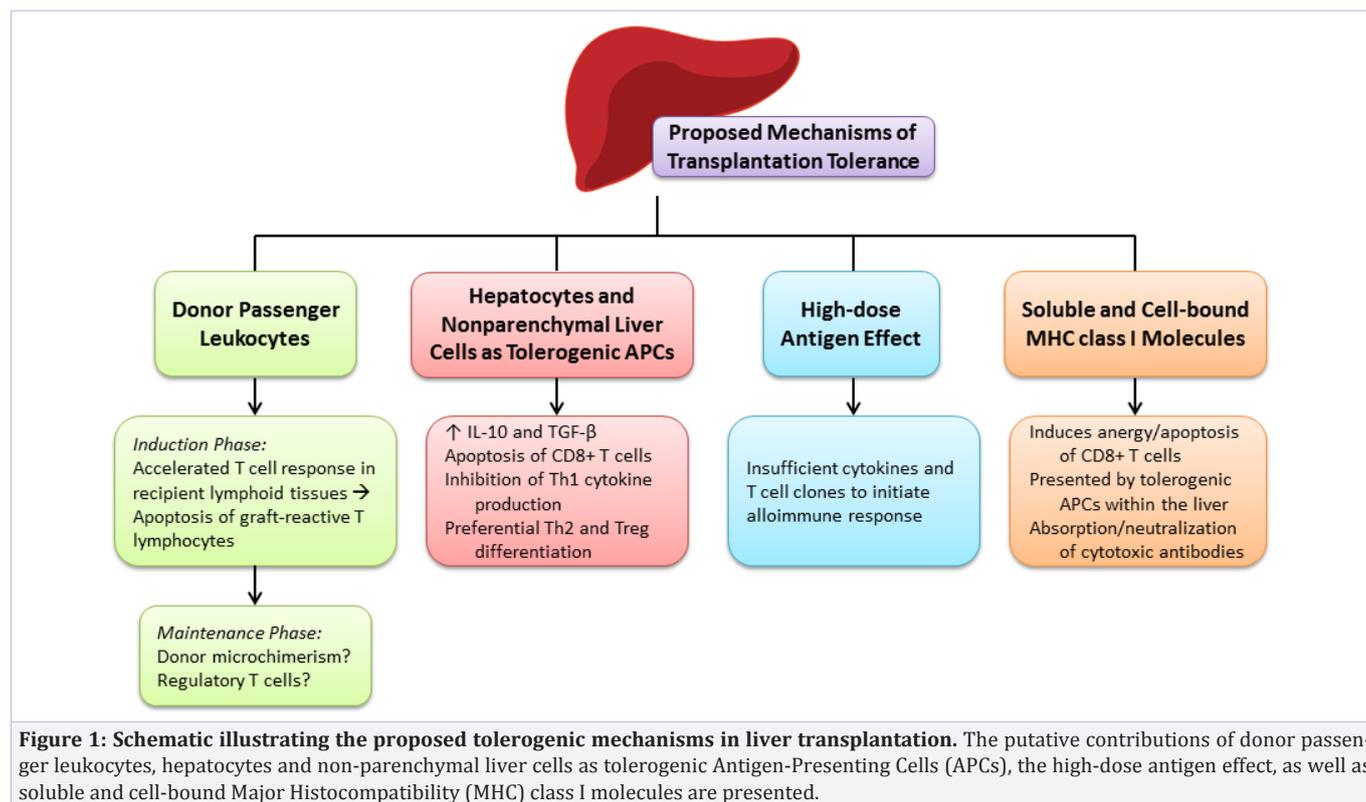
In clinical transplantation, the liver experiences a lower

incidence of rejection compared with other solid organs, and spontaneous acceptance of the liver graft with successful discontinuation of immunosuppression has been reported in nearly 20% of LT recipients [10,11]. Multiple studies have suggested the protective effect of the liver allograft in Simultaneous Liver-Kidney Transplantation (SLKT) compared with recipients of isolated renal transplants; SLKT recipients demonstrate a lower frequency of kidney rejection as well as improved long-term survival [12-14]. Among sensitized kidney transplant recipients with positive lymphocytotoxic crossmatches, inclusion of the liver graft reverted the crossmatch to negative within 1 hour of reperfusion, and prevented the occurrence of hyperacute rejection [15,16]. The protective effect of simultaneous LT extends to other solid organ grafts, as the observed incidence of intestinal allograft rejection is reduced in combined liver-intestine transplant recipients [17].

Various mechanisms have been proposed for the immunomodulatory properties of the transplanted liver (Figure 1). It has been shown that the liver harbors donor-derived hematopoietic cells, called passenger leukocytes, which are transferred to the recipient at the time of transplantation. Donor microchimerism, or the persistence of donor cells and nucleic acid in the blood and tissues of the recipient, has been postulated to promote long-term graft survival. Hepatocytes and nonparenchymal cells within the liver are thought to play an important role in the modulation of the T cell response which contributes to tolerance. An alternative hypothesis suggests that the large size of the liver graft creates a high antigen dose which overwhelms the recipient alloimmune response. Another attractive theory involves the absorption and/or neutralization of alloreactive antibodies by the membrane-bound and soluble forms of Major Histocompatibility Complex (MHC) class I molecules introduced by the liver allograft. In this review, we will examine the experimental and clinical evidence underlying each hypothesis, and integrate these concepts for an enhanced understanding of transplantation tolerance.

Passenger Leukocytes and Donor Microchimerism

The liver contains large numbers of donor-derived passenger leukocytes which migrate out of the graft immediately after transplantation and can persist in the recipient for some time



[18], creating a state of peripheral microchimerism. Donor microchimerism is frequently seen among LT recipients [19], leading to the hypothesis that chimerism plays an important role in tolerance induction. In the early 1990s, Starzl, et al. [20,21] demonstrated that microchimerism can persist in liver recipients greater than 10 years post-transplant, all of which maintained good graft function while some were able to discontinue immunosuppressive therapy.

Early evidence for the role of chimerism in allograft acceptance comes from experimental and clinical observations with donor-specific blood transfusions. Administration of donor whole blood into rat cardiac transplant recipients generated chimerism [22], and resulted in the complete suppression of rejection and induction of graft tolerance [23,24]. Similar results were obtained with a single injection of donor splenocytes at the time of transplantation, which causes downregulation of T-cell mediated alloreactivity within the allograft [25,26]. On the other hand, depletion of passenger leukocytes by donor irradiation abolishes tolerance and increases the risk of allograft rejection [27,28]. The use of chimeric rat liver grafts confirmed that, the presence of donor-derived passenger leukocytes is necessary for tolerance induction [29,30]. Attempts to promote chimerism in humans have been made using donor-derived bone marrow cell infusions. This strategy has reduced the incidence of acute and chronic rejection among solid organ transplant recipients [31], and afforded a modest improvement in the long-term survival of kidney allograft [32,33]. Clinical trials are now underway to further elucidate the effects of donor bone marrow infusion

in association with non-ablative conditioning in solid organ transplantation [34].

Passenger leukocytes in the induction of tolerance

Upon reperfusion of the liver allograft, passenger leukocytes migrate to recipient lymphoid tissues [35], where they trigger a rapid and vigorous alloimmune response. Host T cells are activated by means of direct antigen presentation pathway and proliferate in the draining lymph tissues [36,37], leading to marked increases in Interleukin-2 (IL-2) and Interferon- γ (IFN- γ) expression within the first day after transplantation [35,38]. This paradoxical early immune activation is more vigorous and greater in magnitude than the immune responses observed during rejection.

Host T cells activated in this manner are unable to initiate rejection and instead undergo apoptosis within the recipient lymphoid tissues. The remaining activated T lymphocytes travel back to the liver allograft, where they also undergo programmed cell death [37]. Evidence in support of this theory stems from the observation that large numbers of apoptotic leukocytes accumulate within the spleen and liver graft by day 2 post-transplant [39]. The end result of this process is the clonal deletion of donor-reactive T lymphocytes, and the induction of hyporesponsiveness towards donor-specific antigens [40].

The precise mechanisms leading to apoptosis of activated donor-reactive T cells remain elusive. Despite dramatic increases in the production of IL-2 and IFN- γ shortly after liver transplantation, some studies have suggested that the

concentrations of these cytokines are insufficient to support the vigorous host T cell response described above, leading to death by neglect of alloreactive T cells [40]. Other studies, however, indicate that the role of IL-2 in tolerance may be more complicated. For instance, there is evidence showing that IL-2 can sensitize T cells to Fas-mediated cell death [41,42]. While exogenous IL-2 has been shown to decrease T cell apoptosis and induce rejection in spontaneously accepted rodent liver allografts [43,44], the administration of an IL-2 receptor antagonist also prevented tolerance, possibly via the reduction of regulatory T (Treg) cells [45]. In clinical liver transplantation, multiple immunosuppressive agents are often given in combination during the immediate post-transplant period. Each of these agents can exert distinct effects on T cell activation and apoptosis. Cyclosporin and tacrolimus, commonly-used calcineurin inhibitors, reduce the transcription of IL-2 and related cytokines. On the other hand, the administration of corticosteroids during the induction phase decreases the production of IL-2 and IFN- γ , abrogates T cell apoptosis and impairs the development of donor-specific tolerance [35].

Despite the multitude of studies supporting the role of passenger leukocytes in tolerance induction, the particular donor cell types involved have not been clearly defined. Characterization of donor lymphocytes transferred with the liver graft revealed a predominance of partially activated T and Natural Killer (NK) cells, while smaller numbers of resting T and B cells are transmitted by the lymph nodes associated with the graft [46]. Experimental studies in rodents suggested that donor-derived T cells are needed to regulate tolerance induction [28,47], while other reports have implicated the involvement of Dendritic Cells (DC) [24,48]. Recently, Moroso, et al. [49] observed that the liver harbors an abundance of NK cells, which contain perforin and granzymes and exhibit potent cytolytic activity, suggesting that NK cells may play a role in the regulation of alloimmune responses. Further experiments from the same laboratory, however, showed that the depletion of hepatic NK cells failed to abrogate liver allograft acceptance [50].

Donor microchimerism and the maintenance of tolerance

Although Starzl, et al. [20] postulated that peripheral microchimerism is responsible for the long-term survival of liver allografts, the available literature does not show a clear association between chimerism and the recipient's immunological status [51]. For instance, a number of studies have reported the occurrence of rejection despite the presence of donor chimerism, and microchimerism does not necessarily correlate with long-term allograft survival [52-55]. Furthermore, while the depletion of donor passenger leukocytes on the day of transplantation (day 0) prevented tolerance induction, depletion on day 18 after transplant had no apparent effects on graft acceptance [56].

Some authors have proposed that the persistence of donor antigen, rather than microchimerism, is responsible for the maintenance of tolerance. The presence of donor antigen, not microchimerism, is a prerequisite for the induction of donor-

specific hyporesponsiveness [57]. Evidence from murine models of transplantation indicates that the continuous supply of antigen, provided by the allograft itself, is also essential during the maintenance phase [58,59]. In particular, removal of the allograft from tolerized animals leads to the eventual loss of tolerance [60]. Since CD4+ Treg cells require the continuous presence of donor antigens to survive in tolerance models, it has been suggested that Tregs may play a critical role in maintaining tolerance. Alloreactive T cells are likely to be suppressed by Tregs in the presence of donor antigen, whereas removal of the allograft decreases the number or activity of Treg cells, in turn favoring the activation or expansion of alloreactive T cells [61].

Data from recent immunosuppressive drug weaning or withdrawal trials have provided additional insight into the important processes critical for the maintenance of tolerance. To aid in the selection of patients eligible for immunosuppression withdrawal, gene expression analyses and immunophenotyping studies have been carried out to identify biomarkers of graft acceptance. Microarray profiling of peripheral blood samples has revealed the preferential expression of NK cell transcripts in tolerant LT recipients [62-64]. Pathways involved in iron homeostasis have also been implicated, as tolerant patients exhibit higher serum levels of hepcidin and ferritin, and demonstrate increased iron deposition within hepatocytes [65]. A selective expansion of $\gamma\delta$ T cells, a subset of innate-type lymphocytes that can exhibit a regulatory phenotype, have been reported among tolerant recipients [62,66,67]. Furthermore, peripheral blood concentrations of CD4+CD25+ Treg cells and Foxp3 expression increased upon the withdrawal of immunosuppression in tolerant patients, a phenomenon not observed in patients who suffered rejection [68-70].

Hepatocytes and Nonparenchymal Liver Cells

Experimental evidence from rodent models of liver transplantation suggests that donor passenger leukocytes alone are not sufficient to prolong graft survival indefinitely, and that the liver parenchyma itself participates in the induction of antigen-specific tolerance [29,71]. In addition to hepatocytes, the liver graft contains nonparenchymal cells such as Kupffer Cells (KC), Liver Sinusoidal Endothelial Cells (LSEC), resident DCs, and stellate cells. These cellular compartments are organized into a unique structure within the sinusoids which enables direct interaction between the hepatic cells and circulating lymphocytes (Figure 2). The fenestrated endothelium of liver sinusoids, along with the low velocity of blood flow, allows for antigen presentation by hepatocytes and other nonparenchymal liver cells to T lymphocytes that pass through the liver [72,73].

Hepatocytes

Hepatocytes constitutively express MHC I molecules at high levels, while MHC II expression can be induced following inflammation. Hepatocytes have been shown to act as efficient Antigen-Presenting Cells (APC) for naïve CD4+ and CD8+ T lymphocytes, but such interactions appear to be tolerogenic as they result in the loss of cytolytic function and premature T cell death [74-76].

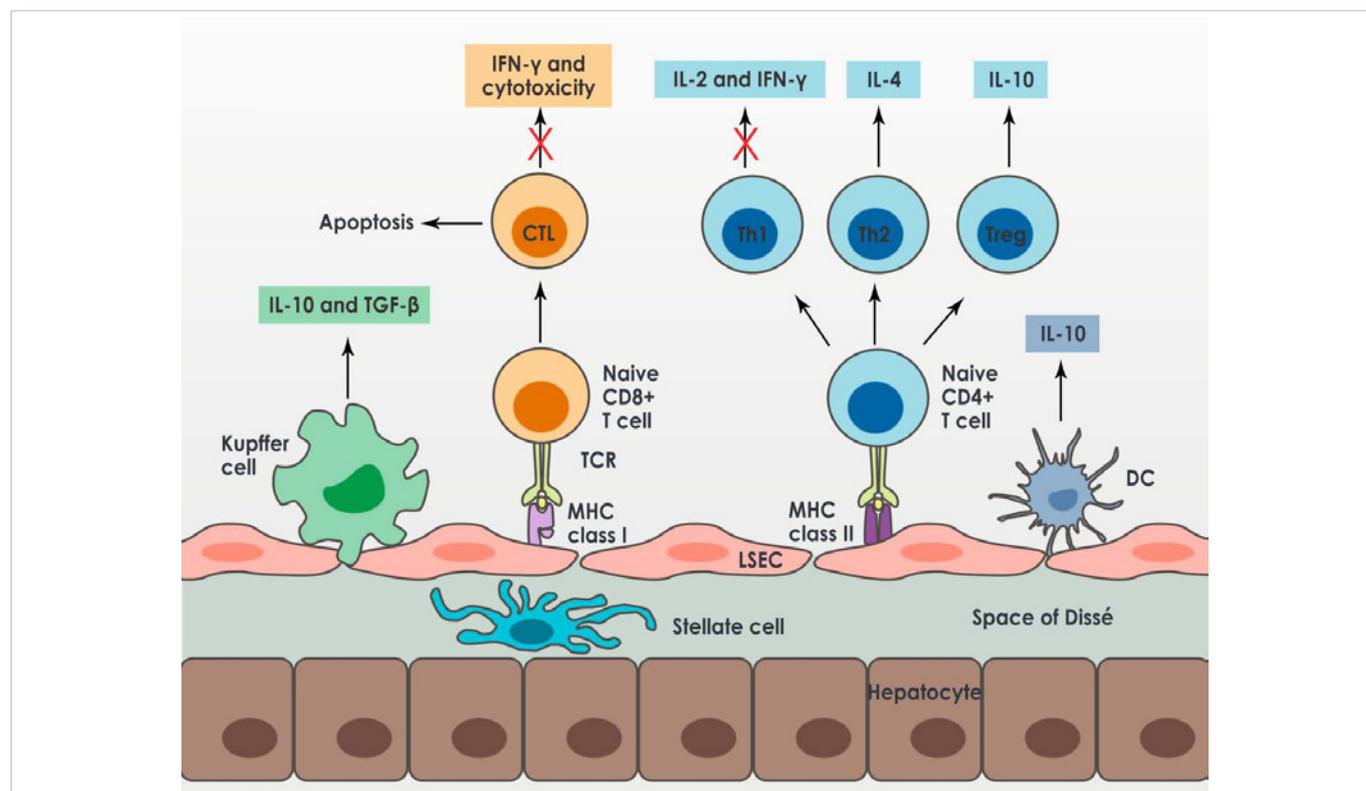


Figure 2: Cellular architecture and interactions within the liver. The fenestrated endothelium of the hepatic sinusoids allows the passage of large molecules into the subendothelial space of Dissé. Antigen presentation by Liver Sinusoidal Endothelial Cells (LSEC) to naïve CD8+ T cells leads to the development of Cytotoxic T Lymphocytes (CTL) deficient of cytotoxicity and a propensity for cell death by apoptosis. The interaction between LSEC and naïve CD4+ T cells favors differentiation towards Th2 and regulatory T (Treg) cell phenotypes, and suppresses cytokine production by Th1 cells. Kupffer cells, in conjunction with LSECs, produce IL-10 and TGF- β which contribute to the tolerogenic environment within the liver. Hepatic Dendritic Cells (DC) also secrete the immunomodulatory cytokine IL-10 and play an important role in tolerance induction.

Kupffer cells

KCs are hepatic macrophages residing within the sinusoidal lumen. *In vitro* evidence suggests that KCs express MHC class II and co-stimulatory molecules, and can function as APCs for allospecific T cells [77]. Shortly after liver allograft reperfusion, KCs secrete massive amounts of IL-10 [78-80], a dominant cytokine within the liver which exerts multiple immunomodulatory effects [81,82]. KCs have also been shown to release nitric oxide and prostaglandins which may suppress T cell activation [83,84]. Perhaps more importantly, KCs can initiate apoptosis of alloreactive T effector cells via the Fas/ FasL pathway. These findings were confirmed by the addition of anti-FasL blocking antibody to *in vitro* co-culture assays, which effectively abrogated T cell apoptosis. In a rat LT model, pretreatment of the recipient with gadolinium trichloride, a KC inhibitor, prompted allograft rejection by means of FasL suppression [85].

Liver sinusoidal endothelial cells

LSECs play a critical role in hepatic immune surveillance by clearing antigens in the blood, often in the form of immune complexes [82]. LSECs express MHC class II molecules and possess the ability to present antigens to CD4+ and CD8+ T

lymphocytes [86]. Naïve T cells activated by LSECs, however, do not differentiate into effector T cells and instead display a functional phenotype and cytokine profile compatible with tolerance. CD4+ T cell activation by LSEC does not lead to Th1 differentiation, but rather induces a regulatory phenotype characterized by IL-4 and IL-10 expression [87]. The adoptive transfer of transgenic CD4+ T cells into a murine model showed that LSECs selectively suppressed cytokine production by Th1 cells, while promoting the expansion of Th2 and regulatory T cells [88]. On the other hand, CD8+ T cells activated by LSECs show impaired proliferative ability, low expression of IL-2, and increased susceptibility to apoptosis [89,90]. More recent studies have revealed that the interaction of LSEC with naïve CD8+ T cells triggers LSEC maturation involving the expression of the negative co-stimulatory molecule programmed death-ligand 1 (PD-L1), which leads to the generation of tolerized CD8+ T cells devoid of cytotoxic activity [91].

Dendritic cells

DCs are professional APCs which have the capacity to effectively initiate immunity, but under certain conditions DCs can induce antigen-specific unresponsiveness [92]. KCs and LSECs within the liver constitutively secrete IL-10 and Transforming Growth Factor- β (TGF- β), creating a milieu which

subverts DC maturation and function [48,93,94]. Accordingly, freshly isolated resident hepatic DCs are phenotypically and functionally immature, expressing low levels of surface MHC and co-stimulatory molecules [95,96]. Antigen presentation mediated by immature DCs (iDC) is unable to initiate effective proliferation of alloreactive T cells, and instead induces donor-specific hyporesponsiveness [97,98]. At least four distinct DC subsets have been identified within the mouse liver, and variations in subtype composition have been proposed to account for the tolerogenic properties of hepatic DCs [99]. Plasmacytoid DCs, which have the capacity to induce tolerance in the steady state, are found more commonly in the liver than in the spleen. The immunostimulatory myeloid and lymphoid DCs, on the other hand, only account for approximately 20% of the liver DC population, whereas they make up the vast majority of spleen DCs [96].

Resident DCs in the liver inhibit CD8+ T cell effector function and facilitate Th1 cell apoptosis while promoting Th2 generation and Treg development in an IL-10-dependent manner [100-102]. Human monocytes differentiated into DCs following co-culture with rat LSECs secrete IL-10, and preferentially direct Th2 over Th1 responses [103]. Furthermore, the relative abundance of plasmacytoid DCs in the liver may promote the induction and expansion of Foxp3+ Treg cells [104,105]. Under steady state conditions, DCs also have been shown to mediate CD8+ T cell tolerance via the co-inhibitory molecules Programmed Death-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) [106].

The tolerizing effects of resident hepatic DCs have been demonstrated *in vivo* mostly in murine cardiac transplant models. iDCs administered to immunocompetent recipients 7 days prior to the transplantation were able to prolong cardiac allograft survival, which was accompanied by markedly blunted cytotoxic T lymphocyte reactivity. Expression of B7-1 and B7-2 molecules was up-regulated on donor-derived DCs within the recipient lymphoid tissues, implicating involvement of the CTLA-4 pathway [107]. The survival of heart allografts was further extended with the co-administration of donor-derived iDCs with anti-CD40 ligand antibody, illustrating the importance of co-stimulatory signals in the functional interaction between DCs and T cells [108]. On the contrary, enhancing the function of DCs by donor pretreatment with a hematopoietic growth factor (Flt3-ligand) elicited a potent allostimulatory response mediated by host T cells which led to allograft rejection [109].

Hepatic Stellate Cells (HSC)

HSCs constitute less than 8% of the total number of cells within the liver, but exhibit unique tolerogenic properties that deserve special mention. HSCs store fats and vitamin A, and have the ability to function as potent APCs for protein and lipid antigens [110]. In response to cellular stress, HSCs transform into myofibroblasts which are responsible for the development of liver fibrosis and cirrhosis [111]. Activated HSCs acquire immunoregulatory functions and promote T cell apoptosis via the PD-L1/PD-1 pathway [112]. Moreover, vitamin A (retinol)

and its active metabolite retinoic acid can modulate the immune response in a pleiotropic manner [113]. Of particular relevance in transplantation tolerance may be the induction of CD4+Foxp3+ Treg cells by HSCs in the presence of retinoic acid and TGF- β . When co-transplanted with hepatocytes, activated HSCs provided beneficial immunomodulatory effects and promoted transplanted cell engraftment in the liver [114]. HSCs have also been shown to protect pancreatic islet allografts from rejection in a murine islet transplantation model [115].

Taken together, the microenvironment of the liver allograft is a tolerogenic milieu rich in IL-10 and TGF- β . Under such conditions, resident hepatic DCs maintain a functionally immature phenotype. Antigen presentation by iDCs and other cellular subsets within the liver may lead to tolerance by the differentiation of naïve T cells into regulatory phenotypes, and by the apoptosis of recently activated CD4+ and CD8+ effector T cells. The end result of these mechanisms is the clonal deletion of graft-reactive T lymphocytes, and the induction of donor-specific hyporesponsiveness.

The High-Dose Antigen Effect

The liver is approximately 10 times larger than the heart or the kidney, and its large tissue mass has been postulated to dilute cytokines [116] and alloreactive T lymphocytes [117] leading to exhaustion of the host immune response. This hypothesis is predicated upon the assumption that a “critical mass” of graft-reactive T cells is needed to initiate immunity, and a high antigen dose dilutes the finite T cell clones such that the activation threshold is not reached [118].

The earliest evidence in support of this hypothesis stems from skin grafting experiments in animals. While larger skin grafts are more rapidly rejected compared with smaller grafts, drastically increasing the size of the skin graft has the paradoxical effects of reduced rejection rates and prolonged survival [119-121]. Subsequent studies in rats showed that the simultaneous transplantation of multiple organs, which increases antigen load, improves graft survival rates [122]. These findings were later affirmed in cyclosporin-treated swines, in which combined transplantation of the heart and kidney from the same donor prevented rejection of the cardiac graft [123].

In clinical transplantation, the antigen dose effect has been cited as a plausible explanation for the beneficial effects of combined liver-kidney transplantation on allograft outcomes. SLKT recipients have been shown to experience lower incidences of renal allograft rejection and enhanced survival in single-center [12,14] as well as larger registry studies [13,124]. The survival benefit associated with SLKT appears to be donor-specific, as kidney grafts from a third-party donor were not protected from rejection or rejection-related graft loss [125]. The antigen dose effect has also been demonstrated among pediatric renal transplant recipients, in whom the use of larger adult-sized kidneys conferred an immunologic advantage with prolongation of a rejection-free state and improved graft function [126]. Conversely, the use of reduced-size partial liver grafts in rats has been correlated with the occurrence of accelerated severe

rejection [127-129]. In human living donor LT, a positive T-cell crossmatch was of particular clinical relevance in the setting of small-for-size liver allografts, and independently predicted early postoperative mortality attributable to acute rejection episodes [130].

Further evidence in support of the antigen dose hypothesis originates from reports that the immunoprotective properties of simultaneous transplantation are not limited to the liver, and can be extended to other solid organs. For instance, the long-term survival of kidney grafts was equally high between recipients of liver-kidney and those receiving heart-kidney transplants [13]. The positive effects of dual-organ transplantation expand to heart, lung, and kidney allografts, with each organ being able to protect itself and one another from rejection [131-133]. In composite tissue transplantation, the entirety of allograft (including skin, subcutaneous tissues, muscle, bone, and blood vessels) elicits a lesser immune response compared with each of its individual components, and displays a lower rejection rate when compared with skin transplantation alone [134].

The high-dose antigen effect in isolation is likely not sufficient for tolerance induction. Some investigators have proposed that the number of donor-derived passenger leukocytes transferred is proportional to the size of the transplanted organ, and accordingly the benefits associated with larger grafts may be explained by the high number of donor leukocytes available. As mentioned previously, liver allograft acceptance in rats is contingent upon the presence of passenger leukocytes. When tissue mass is increased via the simultaneous transplantation of two hearts or two kidneys, the additional infusion of donor leukocytes was still necessary for successful tolerance induction [47]. Meanwhile, sensitized recipients of reduced-size liver allografts showed an increased risk of antibody-mediated rejection compared with recipients of full-size grafts, illustrating that the size effect is at least partially attributable to the liver's ability to neutralize lymphocytotoxic antibodies [135]. Taken together, these findings suggest that, alternative processes likely act in concert with the antigen dose effect to induce tolerance.

Soluble and Cell-Bound MHC Class I Molecules

The liver allograft releases large quantities of soluble MHC class I molecules, which persist in the recipient circulation at high concentrations as long as the graft is functional [136]. There is compelling experimental and clinical evidence in support of the role of soluble MHC on tolerance induction. The introduction of MHC class I alloantigen by intravenous administration of donor serum, or by genetically modified hepatocytes expressing MHC class I molecules, prevented rejection and prolonged allograft survival in rat liver and cardiac transplantation models [137-140]. Various paradigms have been proposed to explain the mechanisms underlying the immunoregulatory effects of MHC class I molecules. These include:

(1) Soluble MHC class I molecules can interact directly with the T-cell receptor on alloreactive CD8+ T lymphocytes. By selective stimulation of the T-cell receptor in the absence of a co-stimulatory signal, soluble MHC induces T cell apoptosis rather

than activation [141].

(2) Soluble MHC acts as a source for donor peptides which are processed by APCs and presented to allospecific T cells. As detailed above, many cellular subtypes within the liver graft can function as tolerogenic APCs, which alter the Th1/Th2 balance, shift T cell differentiation into regulatory phenotypes and facilitate the clonal deletion of graft-reactive T cells via apoptosis.

(3) Soluble MHC class I molecules may neutralize lymphocytotoxic alloantibodies by direct binding.

In the clinical arena, soluble MHC may account for the favorable outcomes following liver grafting in the presence of preformed alloantibodies. Among sensitized recipients undergoing SLKT, the liver allograft has been shown to reverse positive crossmatches and prevent the development of hyperacute rejection [15,16]. More recent studies reported that LT can be safely performed across preexisting antibodies directed against donor Human Leukocyte Antigens (HLA), and the spontaneous clearance of preformed antibodies is commonly observed after transplantation of the liver [142]. These observations lend support to the notion that the liver allograft mediates the absorption and/or neutralization of circulating antibodies. Dar, et al. [143] demonstrated that antibodies directed against donor MHC class I antigens are preferentially cleared compared with class II antigens among combined liver-kidney transplant recipients. These findings are in keeping with the release of soluble MHC class I, but not MHC class II, molecules by the liver graft. On the other hand, alloantibodies directed against class II HLA antigens are likely to persist after transplantation, and are associated with inferior patient and graft outcomes following SLKT [144].

Using an extracorporeal liver hemoperfusion system, Guggenheim, et al. [145,146] found that cell-bound MHC class I molecules may contribute to the neutralization of lymphocytotoxic antibodies. In sensitized rat cardiac allograft recipients, the application of liver hemoperfusion delayed hyperacute rejection and reduced the level of circulating antibodies. Histological examination of the liver revealed evidence of antibody deposition on KCs and LSECs, which express high levels of MHC antigens on their cell surfaces. Further experimentation showed that this process is donor-specific, as hemoperfusion with a third party liver failed to decrease the levels of circulating antibodies, and was associated with a markedly diminished prolongation of cardiac graft survival.

Human leukocyte antigen G

HLA-G is a non-classical MHC class I molecule with a multitude of immunomodulatory properties. Under physiologic conditions, HLA-G is expressed as a membrane-bound molecule on cell surfaces, and as a soluble form in bodily fluids. Several mechanisms have been proposed to explain the tolerogenic properties of HLA-G: inhibition of CD8+ T and NK cell cytotoxicity [147,148]; suppression of CD4+ T cell proliferation [149,150]; promotion of Th2 polarization [151]; inhibition of cell cycle progression in alloreactive T cells [152]; conversion of effector T

cells to a regulatory phenotype via cell-to-cell transfer of HLA-G [153]; and induction of tolerogenic DCs [154]. In autoimmune disorders such as multiple sclerosis [155] and rheumatoid arthritis [156], elevated levels of soluble HLA-G have been linked with disease remission. In contrast, the increased expression of HLA-G in malignancies may have deleterious consequences, as HLA-G may represent an escape mechanism by which tumor cells evade the host immune response. Markedly increased levels of soluble HLA-G have been detected in the serum of patients with various types of malignancies including breast and ovarian cancer, acute leukemia, malignant melanoma, and multiple myeloma [157].

In transplantation, HLA-G expression has been linked with the maintenance of allograft function and freedom from rejection. Renal transplant recipients with soluble HLA-G detected in the serum experienced lower incidences of acute rejection, chronic allograft nephropathy and subsequent graft failure [158,159]. Higher levels of HLA-G expression on peripheral blood CD4+ T lymphocytes were found among renal transplant recipients with stable function compared to recipients with rejection [160]. The detection of HLA-G in the serum and tissues of cardiac transplant recipients was also associated with a lower risk of acute and chronic rejection [161]. The administration of immunosuppressive drugs (including cyclosporin, tacrolimus, and corticosteroids) prompted a notable increase in soluble HLA-G levels which was associated with improved graft acceptance [162-164].

In liver transplantation, high levels of HLA-G expression in serum and tissue samples have been associated with reduced occurrences of acute rejection [165]. In a comparison of recipients with operational tolerance to those with stable liver function or acute rejection, tolerant patients were found to have significantly higher levels of serum HLA-G. The expression of HLA-G on circulating monocytoïd DCs of tolerant recipients was associated with enhanced Foxp3 expression, implicating the involvement of Treg cells in the induction of tolerance by HLA-G [166]. Recipients of combined liver-kidney transplants, but not kidney alone transplants, demonstrated high concentrations of serum HLA-G, which was associated with lower frequencies of hepatic and renal allograft rejection [167,168]. On account of the strong correlation between HLA-G expression and favorable outcomes after LT, HLA-G has been purported as a prognostic biomarker, a tool for immunosuppression monitoring, and as a potential molecular target for future therapeutic interventions [165,169].

Conclusion

In pursuit of transplantation tolerance, extensive efforts have been made to investigate the mechanisms responsible for the immunomodulatory properties of the liver graft. There is experimental and clinical evidence in support of each of the mechanisms described in this report, and it is likely that several of these processes act in concert to establish donor-specific tolerance. Common to these hypotheses is that the liver is a dynamic participant in the process of graft acceptance – whether

it is the transfer of donor-derived passenger leukocytes into recipient lymphoid tissues, or antigen presentation by hepatocytes and non-parenchymal liver cells, or the release of soluble MHC class I molecules leading to the suppression of alloimmune response. The dominant mechanism in each recipient may vary depending upon the conditions such as genetic compatibility with the donor, the immunologic status of the recipient, the degree of inflammation triggered by the peritransplant events, and the immunosuppressive agents administered.

During the induction phase of tolerance, donor-derived passenger leukocytes appear to play an important role via the initiation of an accelerated T cell response within recipient lymphoid tissues. The activated T lymphocytes then undergo apoptotic cell death within these lymphoid tissues and in the liver allograft. With a predominance of the immunomodulatory cytokine IL-10, APCs within the liver assume tolerogenic properties, and their interactions with circulating T lymphocytes lead to the preferential differentiation of naïve T cells into regulatory phenotypes and the induction of apoptosis. On the other hand, humoral immunity may be inhibited by the release of soluble MHC class I molecules which neutralize lymphocytotoxic antibodies. Additionally, antibodies may be absorbed by cells within the liver which contain an abundance of membrane-bound MHC molecules. Consistent with this phenomenon is the recent observation that antibodies directed against class II antigens are more likely to persist after LT compared with class I antibodies. As a consequence, the liver does not confer complete protection from preformed antibodies, particularly when class II antibodies are present.

In contrast to the process of tolerance induction, the mechanisms responsible for the maintenance of tolerance are less well elucidated. Initially, the persistence of donor microchimerism was thought to be an indicator of indefinite graft acceptance, but subsequent reports have failed to show a clear connection between chimerism and long-term graft survival. Biomarkers of graft acceptance in immunosuppressive drug minimization studies have implicated the involvement of NK and $\gamma\delta$ T cells. Tolerant transplant recipients also demonstrate a relative abundance of CD4+CD25+ Foxp3-expressing regulatory T cells. Further studies are needed to elucidate the contribution by each cellular compartment in maintaining tolerance.

Based on the role of donor-derived leukocytes in graft acceptance, clinical trials have been designed to study the effects of donor bone marrow infusions in solid organ transplantation. The available results have only demonstrated a modest benefit on long-term graft survival, indicating that alternative protocols are likely needed to achieve transplantation tolerance. A better understanding of the mechanisms leading to indefinite graft survival will facilitate the discovery of novel strategies for tolerance induction. In particular, HLA-G has been shown to possess multiple immunomodulatory properties and has been associated with favorable transplant outcomes. Although HLA-G has already been considered as a prognostic biomarker, its role in tolerance induction and potential as a therapeutic target warrants further investigation.

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