Clinical Immune Tolerance in Liver Transplantation: Present and Future

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Liver transplantation is the most effective treatment for end-stage liver diseases (ESLD) with satisfactory clinical results and so is considered as the treatment of choice for ESLD and early hepatocellular carcinoma with cirrhotic liver. Unfortunately, adverse effects of life-long immunosuppression prevent the development of alternative strategies to achieve better long-term outcome. Achieving clinical operational tolerance is one of the ultimate goals in the clinical transplantation field. Around 15% of liver transplantation recipients develop spontaneous operational tolerance after immunosuppression withdrawal, and the percentage may be even higher in pediatric living donor liver transplantation recipients. One of the possible explainable mechanisms is a T cell fatigue from large amount of antigen loaded. Despite continuing progress, clinical operational tolerance is still rare in liver transplantation. Reprogramming the recipient immune system by creating chimerism and utilizing regulatory cell therapies are among the newer promising means to achieve clinical liver transplantation tolerance in the future. In animal studies, administration of donor specific regulatory T cells allows a prolonged survival without immunosuppressive agents. In this review, proposed mechanisms for clinical tolerance will be offered and current experimental trial will be introduced.

Key Words: Liver Transplantation; Immune Tolerance; End Stage Liver Disease

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INTRODUCTION

Liver transplantation is the most effective treatment for end-stage liver diseases with satisfactory clinical results. Unfortunately, adverse effects of life-long immunosuppression prevent the development of alternative strategies to achieve better long-term outcome. Achieving clinical operational tolerance is the ultimate goal in the clinical transplantation field.

Around 15% of liver transplantation recipients develop spontaneous operational tolerance after immunosuppression withdrawal, and the percentage may be even higher in pediatric living donor liver transplantation recipients. Despite the progress, clinical operational tolerance is still rare in liver transplantation. Reprogramming the recipient immune system by creating chimerism and utilizing regulatory cell therapies are among the newer promising means to achieve clinical liver transplantation tolerance in the future. In this review, some proposed mechanism for clinical tolerance and current experimental trial will be introduced.

ANIMAL MODELS OF IMMUNE TOLERANCE IN LIVER TRANSPLANTATION

Spontaneous acceptance of a transplanted liver leads rapidly to liver donor-specific tolerance in many experimental models [1,2]. This tolerance is particularly robust and rapidly induces acceptance of skin grafts from the liver donor strain [1,3,4]. Clinical liver transplants also have a better outcome than transplants of other organs with a significant proportion of patients able to be removed from all immunosuppression [5,6]. There have been many proposed mechanisms for the ability of the transplanted liver to be accepted by the recipient. Initially, it was thought that the high levels of soluble major histocompatibility (MHC) molecules produc-

ed by the donor liver were responsible for liver tolerance. This has not subsequently been revealed and it appears that soluble MHC is at best a minor component of the liver tolerance effect [7,8]. Administration of donor leucocytes at the time of transplantation of a heart or kidney yielded considerable prolongation of survival in animal models. However, attempts to translate these findings to a clinical setting providing leucocytes, in the form of donor bone marrow, infused at the time of transplantation have shown only very modest improvement in outcomes [9-11]. A recently proposed mechanism for the ability of the liver to induce tolerance is based on the unique vascular architecture of the liver which allows intimate contact of circulating T cells with hepatocytes. This is facilitated by the fenestrated endothelium of the liver sinusoids, where small endothelial pores permit contact between recirculating T cells and hepatocytes. The intact lining and tight junctions of the endothelium in other organs prevents contact with parenchymal cells. The slow rate of blood flow in liver sinusoids further aids the establishment of contact between circulating CD8+ T cells and hepatocytes. Contact of T cells with hepatocytes leads to their engulfment by hepatocytes and degradation by a process termed "suicidal emperipolesis" or to their abortive activation and death [12-14]. Both of these processes lead to clonal deletion of liver-reactive T cells, a process that has been demonstrated to be responsible for liver transplant tolerance in animal models [15]. Despite these interesting findings, the process of suicidal emperipolesis has not yet been demonstrated in transplanted livers and its role in clinical liver transplantation has yet to be established. A further basis of liver transplant acceptance is the large size of the liver, approximately 10 times greater than that of a heart or a kidney. This mass of tissue can function as a cytokine sink and/or dilute the finite clones of alloreactive T cells and thus potentially exhaust the recipient's immune response. There is mounting experimental evidence that the volume of allogeneic tissue transplanted is an important contributor to tolerance, as increasing the mass of transplanted tissue prolongs survival. Of considerable interest, in clinical transplantation, there is convincing evidence from many studies that multiple organ transplants from the same donor to a single recipient have a better outcome than single organs alone [27]. As there has been no previous review of the dose effect in organ transplantation, the following sections will examine the experimental and clinical evidence for high dose tolerance and describe a gene therapy approach that can exploit it as a potential means to induce antigen-specific tolerance.

Studies in mouse skin transplant models with minor antigen mismatches gave rise to high dose effects. The first of these showed that if the donor and recipient were incompatible at loci other than H-2, larger grafts demonstrated prolonged survival. They also observed that while small secondary grafts underwent accelerated rejection, larger secondary grafts did not [16]. Since then, studies in the fully histo-incompatible rat transplant model donor to recipient have shown that increasing antigen load, by transplanting multiple organs, increases allograft survival rates. Transplantation of one heart or kidney in this model led to rejection in 9 and 8.5 days, respectively. Administration of donor leucocytes alone could not increase survival of a cardiac graft, but transplanting two hearts and two kidneys, with donor leucocytes, led to spontaneous acceptance and indefinite survival of the grafts [17]. Two or three hearts survived for 15.5 days, and two or three kidneys survived for 60 days and >100 days, respectively, while two hearts plus one or two kidneys prolonged their survival to >100 days [18,19]. This dose effect was also observed in an inbred miniature swine model where single MHC class I mismatched heart allografts were rejected within 55 days after transplanting into cyclosporine-treated recipients [20]. In contrast, hearts grafted into cyclosporine-treated recipients that also received a kidney from the same donor developed rapid and stable tolerance that resulted in long-term survival of the heart [21].

Cell-mediated cytotoxicity and alloantibody production were suppressed in combined recipients and there was no evidence of cardiac allograft vasculopathy. To address if this effect was specific to the kidney, the authors transplanted 2 hearts, MHC matched to each other, but class I mismatched to the host. These recipients also displayed significantly prolonged (>190 days) cardiac allograft survival [22]. Conversely, when the mass of an organ that would usually be accepted is reduced, graft survival declines. In experimental porcine liver transplants, rejection was more frequently observed in small accessory livers than large orthotopic livers [1].

One possible explanation for the prolonged survival of very large grafts is nonspecific immunosuppression due to the increased trauma associated with the surgery involved in transplanting massive or multiple grafts. Alternatively, survival prolongation could be due to the increased mass of tissue transplanted, which exhausts the recipient's 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". It had been suggested that this was due to the expression of tissue-specific anti-

gens on grafts but development of a mouse model allowing for examination of the response to different grafts without needing to consider tissue specific antigens showed that this was not the case [23]. In this model, T cells were depleted from the recipients, which were then reconstituted with a specific subset of T cells reactive against one MHC antigen, H-2Kb, expressed on the donor grafts.

T cell-depleted mice were immunocompromised and were unable to reject H-2Kb-expressing heart, skin, or islet grafts without the addition of the alloreactive T cells. Adoptive transfer of the H-2Kb-specific T cells resulted in rejection of all three types of graft although many more cells (-6,000-fold) were required to mediate rejection of the heart grafts than the skin and islet transplants, confirming that the latter two are more susceptible to rejection. Overall, these studies confirmed that a threshold number of cells seemed to be required for graft rejection and they suggested that larger grafts might be rendered resistant to rejection by exhausting the immune response.

A more recent study used the model described above [23], in which T cell-depleted recipients were reconstituted with H-2Kb-specific T cells, to examine the response in mice following transplantation of H-2Kb-expressing heart, kidney, and liver grafts [24]. Transfer of the same number of alloreactive T cells resulted in acceptance of the liver grafts but rejection of the kidney and heart allografts. They found that most of the alloreactive T cells had proliferated and differentiated into memory or effector cells after liver transplantation and were detected in the lymphoid tissues and the liver allograft. Some activation and proliferation were seen after kidney and heart transplantation, but naïve alloreactive cells remained in the lymphoid tissues, long term. The author concluded that following transplantation of a liver graft, the rapid and extensive T cell activation resulted in their clonal exhaustion or deletion.

Some recent work has examined whether this tolerance is due to exhaustion by examining the expression of various γ chain cytokines and their receptors in rat model of transplantation [25]. In this model, heart and kidney transplants are rejected in <10 days while liver transplants are accepted for >100 days. Cytokine levels (IL-2, IL-4, IL-7, IL- 9, IL-15, and IL-21) and their receptors (γc, IL-2R α, IL-2Rβ/IL-5Rβ, IL-4Rα, IL-7Rα, IL-9Rα, IL-15Rα, and IL-21Rα) were assessed by qPCR at days 3, 5, and 7 following grafting and, except for IL-21, the levels of the γ chain cytokines and receptors were lower in transplanted livers than in hearts or kidney. As γ chain-signaling is crucial for T cell survival, this indicated that the tolerance seen in this model may be due to a low level of signaling,

reflecting the "dilution" of a fixed number of alloreactive T cells in a large organ or tissue mass. The apparent importance of antigen dose in liver tolerance led us to use a liver-directed gene therapy approach to attempt to exploit this in a mouse skin transplant model [25]. Previously, induction of tolerance to a foreign protein has been shown to be facilitated by expression in the liver. In particular, it has been found that hepatocyte-restricted antigen expression, with no expression in professional antigen presenting cells [26], and higher levels of gene expression are important [27]. In one case, targeting the expression of a neural autoantigen to the liver was able to induce tolerance to subsequent neural autoimmunity in a mouse model of multiple sclerosis.

They employed a minimally immunogenic recombinant adeno-associated virus (rAAV) vector, designed to specifically express high levels of antigen in host livers [28] to assess tolerance induction in a mouse skin transplant model. They examined whether use of this system to express high levels of the mouse major histocompatibility locus (MHC) antigen H-2Kb in the recipient liver could induce long-term acceptance of skin grafts expressing this antigen. B10.BR (MHC k haplotype) mice were injected with rAAV-H-2Kb to induce specific expression of H-2Kb on their hepatocytes [25]. These recipients were grafted after 7 days with skin from mice that transgenically express H-2Kb on a k haplotype background. Therefore, they were able to express the single, mismatched MHC antigen, found on the donor skin graft, in the recipient's liver prior to transplantation.

EVIDENCE OF IMMUNE TOLERANCE IN CLINICAL TRANSPLANTATION

There have been a number of reports of tolerant patients, resulting from a variety of treatments, and up to 15% of liver transplant recipients have been shown to be able to completely discontinue immunosuppressive therapy [5,6]. In addition, ranges of clinical studies, analysing the survival of various transplanted organs, have confirmed the ability of simultaneous transplantation to protect organs from rejection. An analysis of United Network for Organ Sharing (UNOS) data from 1996 to 2003, of 1,136 combined liver-kidney transplant recipients and 352 patients receiving liver transplants, followed by kidney grafts from different donors, confirmed that the protective effect of the liver was donor specific [29]. There are also a number of reports of a combined transplant enabling successful kidney grafts, despite a positive cross-match between

donor and recipient, which usually results in hyperacute rejection of the kidney [30,31]. One study found that even a partial auxiliary liver transplant from the kidney donor can protect the kidney in a positive cross-match situation [32]. Some of these studies have also reported a lower rate of liver rejection in patients after combined liver-kidney transplantation, compared to those receiving livers alone [29]. There is also a growing body of evidence that this effect is not liver-specific but is instead related to the antigen load.

One of the most comprehensive analyses of the effect of combined organ transplantation to date looked at rejection rates in UNOS data for a total of 133,416 allograft recipients [33]. They found that heart, kidney, and liver grafts were protected and were able to protect each other, with lower rates of rejection and greater rejection-free survival of grafts apparent in the setting of combined transplantation. Specifically, the authors reported a lower rate of liver rejection in liver-kidney recipients, compared to patients receiving livers alone. There was also a reduced rate of kidney graft rejection in recipients of both heart-kidney and kidney-liver transplants, compared to kidneys transplanted alone, although they acknowledged a possible contribution of higher immunosuppression therapy to the reduction of the former. However, differences in immunosuppressive therapy were not responsible for the reduction in cardiac graft rejection seen in both heart-kidney and heart-liver transplants, when compared to heart transplants alone. The analyses also showed that recipients of double-lung and double-kidney transplants both had less rejection and improved rejection-free survival compared to single transplants, providing evidence that antigen load is an important factor.

Recently, in clinical composite tissue transplantation, where skin is transplanted as a component of a much larger tissue mass, skin survival is enhanced [34] compared to that of skin transplanted alone [35], supporting older anecdotal evidence that large experimental skin grafts in human burns patients survived considerably longer than small grafts [32,34]. Overall, it is becoming clearer from these smaller studies and large-scale analyses that reductions in rejection rates are not only associated with liver tolerogenicity, but may be related to the antigen load of organs transplanted as well.

CONCLUSION

The key to a potential major breakthrough in clinical transplantation tolerance relies largely on basic research to further clarify the mechanisms of tolerance. On the other hand, I would possibly focus more on learning the mechanisms of clinical operational tolerance and design new therapeutic strategies. All tolerance protocols learned from animal studies should be carefully tested and modified in large animal or human studies. Another major issue in clinical tolerance trials is inclusion criteria. The majority of clinical tolerance trials so far have excluded patients whose primary diseases are viral hepatitis or immune-mediated hepatic diseases, due to concerns of worsening primary illness or disease recurrence. Since these two major categories of liver diseases are the main indications for liver transplantation (LT), everywhere, excluding these patients from clinical tolerance trials will prevent a substantial number of recipients from achieving an immunosuppressive agent (IS) – free state. Given that preliminary clinical data showed that IS weaning in hepatitis C virus (HCV) infected patients does not worsen the outcome but it may slow down the progression of allograft fibrosis, and that viral hepatitis patients may benefit from minimization of immune suppression, future clinical trials with large population will be tried.

With more intimate collaborative efforts between basic researchers and clinicians, stable operational tolerance is expected to be realized in more generalized clinical LT recipients, perhaps even in the near future.

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