World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i36.5981

World J Gastroenterol 2013 September 28; 19(36): 5981-5987
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Tolerance and chimerism and allogeneic bone marrow/stem cell transplantation in liver transplantation

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Received: April 5, 2013 Revised: June 5, 2013
Accepted: July 18, 2013
Published online: September 28, 2013

Abstract

The liver has particular tolerogenic properties that allow its spontaneous acceptance in some animal species. Liver structure is considered to favor a tolerogenic environment. The peripheral tolerance mechanisms also play a role in spontaneous tolerance to liver graft. In a clinical setting, the main challenge nowadays facing liver transplantation is minimization of immunosuppression with the goal of donor-specific tolerance. Mechanisms involved in tolerance to transplanted organs are complex and partly unknown. A significant mechanism in tolerance induction is chimerism. Chimerism can be induced through transplantation of allogeneic donor bone marrow/stem cells under appropriate host conditioning. This review focuses on the tolerance mechanisms in liver transplantation and highlights the role of chimerism and allogeneic bone marrow/stem cell transplantation in tolerance development.

Key words: Immunotolerance; Chimerism; Bone marrow transplantation; Stem cell transplantation; Liver transplantation

Core tip: The liver is considered an immune privileged organ. The main challenge facing liver transplantation is to induce donor-specific tolerance. Numerous reports have documented the phenomenon of microchimerism in liver transplant recipients. Most have demonstrated that higher levels of chimerism in liver transplantation are associated with reduced incidence of acute rejection and better initial graft acceptance. Mechanisms involved in chimerism-induced tolerance have only been partly elucidated. Chimerism can be induced through transplantation of allogeneic donor bone marrow cells under appropriate host conditioning and represents a clinically feasible approach for the induction of durable liver transplantation tolerance.

Wu SL, Pan CE. Tolerance and chimerism and allogeneic bone marrow/stem cell transplantation in liver transplantation. *World J Gastroenterol* 2013; 19(36): 5981-5987 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i36/5981.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i36.5981

INTRODUCTION

The availability of non-specific immunosuppressive agents has allowed liver transplantation to become an established treatment of end-stage liver disease. With the introduction of new immunosuppressants the incidence of acute rejection has considerably decreased, and transplanted patient survival is now 83% and 70% after 1 and 5 years, respectively[1]. However, immunosuppressive drugs broadly suppress the immune system, and their use is associated with an increased risk of neoplasms, opportunistic infections, and end-organ toxicity[2,3]. Thus, the main challenge nowadays facing liver transplantation is minimization of immunosuppression with the goal of donor-specific tolerance. Tolerance is defined as a state of donor-specific hyporesponsiveness in the recipient in the absence of immunosuppression[4]. Mechanisms involved in tolerance to transplanted organs are complex and partly unknown. This review focuses on the tolerance mechanisms in liver transplantation and to highlight

the role of chimerism and allogeneic bone marrow/stem cell transplantation in tolerance development.

LIVER AS A PRIVILEGED IMMUNE ORGAN

It is generally recognized that after clinical liver transplantation, the incidence of chronic rejection is lower than after transplantation of other organ grafts. Among animal species such as pigs, and selected rat and mouse combinations, liver transplants may be performed with no immunosuppression[5-7]. In selected patients with liver transplantation, immunosuppressive therapy can be withdrawn without occurrence of graft rejection[8-10]. Furthermore, co-transplantation of a liver allograft can prevent rejection of other organ grafts from the same donor[11,12]. The transplanted liver may even behave as an immunosuppressor when transplanted to animals suffering rejection after pancreas transplantation[13]. Liver allografting also reverses ongoing rejection of the heart[14]. All of these facts had led people to consider the liver to be an immunologically privileged organ that may be tolerated with less immunosuppression after transplantation, and immunosuppressants can sometimes even be completely withdrawn[15].

TOLERANCE MECHANISMS IN LIVER TRANSPLANTATION

The mechanisms responsible for this relative tolerogenicity of the liver have only been partially elucidated, and liver structure is considered to have major implications for hepatic immune function. The liver is a place where gastrointestinal tract antigens and alloantigens in transplanted liver are presented to lymphocytes through a complex network of sinusoidal cells and antigen presenting cells (APCs)[16]. The context in which liver-resident antigens are presented to T cells favors a tolerogenic environment. Liver sinusoidal endothelial cells (LSECs) have a unique phenotype expressing myeloid cell markers (CD1, CD4 and CD11c), similar to immature dendritic cells (DCs). LSEC-activated $CD4^+$ lymphocytes cannot differentiate into T helper (Th)1 [interleukin (IL)-2-producing] cells, and express high immunosuppressive IL-10 levels[17]. Besides, LSEC-stimulated $CD8^+$ lymphocytes cannot respond to new antigenic stimuli[18].

A recent study demonstrated that the liver capacity to induce tolerance partly results from *in situ* T-cell activation. It is well known that hepatocytes, as non-professional APCs, may play key roles in regulating immune responses and facilitating tolerance induction[19]. Warren *et al*[20] showed intrahepatic lymphocytes and circulating naïve $CD8^+$ cells could interact with hepatocytes by means of cytoplasmic extensions capable of going through LSEC fenestrations. This local activation of T cells by hepatocytes provides the latter with a significant role as APCs and induces tolerance development in the liver[21].

A number of observations indicate that a high percentage of non-conventional lymphocytes-which only rarely are found in peripheral blood-in the liver graft may also play a relevant role in the induction of tolerance[22]. Natural killer (NK) cells represent up to 30%-40% of liver lymphocytes, but only 10%-15% of peripheral mononuclear cells. Liver NK cells contain perforin and granzymes, exert stronger cytotoxicity against K562 target cells when compared with blood NK cells, and secrete interferon (IFN)-γ, but no IL-10 or Th2 cytokines, upon stimulation with monokines. Liver NK cells seem to emit negative signals to the host T cells upon their migration into the liver following liver transplantation, thus contributing to liver graft tolerance[23].

The peripheral tolerance mechanisms also play a role in liver graft spontaneous tolerance. Bishop *et al*[24] demonstrated after liver transplantation that a large number of donor leukocytes rapidly migrate to secondary lymphoid organs and stimulate production of IL-2 and IFN-γ by host $CD4^+$ cells, which then become depleted and subsequently undergo apoptosis.

A distinct subset of T cells that play an important role in peripheral regulation is natural T regulatory cells (Tregs). They constitutively express CD25, and constitute 5%-10% of peripheral $CD4^+$ T cells in healthy mice and humans. The $CD4^+/CD25^{high}$ Tregs are best recognized by expression of the transcriptional regulator forkhead box (Fox)P3[25]. Dieckmann *et al*[26] showed that human peripheral blood $CD4^+CD25^+$ Tregs can suppress allogeneic responses through soluble mediators such as IL-10 and/or transforming growth factor-β. It was recently found that peripheral blood $CD4^+CD25^+Foxp3^+$ cells increased in patients with liver grafts and might contribute to spontaneous tolerance[27].

Compared with peripheral tolerance, central tolerance phenomena occur primarily in the thymus. So far, two experimental approaches exist in murine models that may generate highly stable central tolerance to allogeneic thymus transplantation or bone marrow transplantation (BMT). Induction of mixed chimerism through transplantation of allogeneic donor bone marrow/stem cells under appropriate host conditioning, is one of the most reliable strategies to induce transplantation tolerance and has been used in clinical practice[28]. Next, we focus on the mechanisms and applications of chimerism induced by allogeneic bone marrow/stem cell transplantation in tolerance introduction in liver transplantation.

CHIMERISM

Chimerism can be defined as a phenomenon in which cells from one individual are present in another individual. Two types of chimerism have been described: microchimerism and microchimerism. Microchimerism usually occurs when bone marrow/stem cells are transplanted in a conditioned recipient and the donor pluripotent hematopoietic stem cells (HSCs) engraft in the recipient and produce all its lineages, including the donor immune system. 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. Consequently, not all stem-cell-derived lineages are produced and low levels of donor cells are found in the recipient's blood[29].

CHIMERISM AND TOLERANCE IN LIVER TRANSPLANTATION

Tolerance induction with live donor leukocytes became the bedrock of modern transplantation after its first description in mice[30]. Numerous reports have documented that mononuclear cells of donor type migrate out of the graft after transplantation of solid organs and these cells can persist in the recipient over several years, thus leading to allogeneic microchimerism[31]. Jonsson *et al*[32] prospectively investigated the peak levels and kinetics of donor leukocyte chimerism in human recipients following liver transplantation. The peak levels of chimerism were observed within the first 48 h following transplantation and ranged from 0.15% to 20% of total peripheral blood mononuclear cells. In almost all patients, there was an early peak level of chimerism that declined over time such that donor leukocytes were only intermittently detectable after 3-4 wk. In another study, Verdonk *et al*[33] prospectively collected blood samples of 21 liver transplant recipients up to 3 mo after transplantation. They found donor chimerism in 71% of their liver transplant recipients and chimerism was most frequently found in the first month after transplantation. However, by polymerase chain reaction (PCR) for donor type DNA sequences, microchimersim has been described to be present in patients > 10 years after liver transplantation. Some of these patients were off immunosuppressive treatment for various periods of time and all had good graft functions[34]. Stable levels of donor chimerism, in the absence of other major clinical events, may be a marker of transplantation tolerance, and may help to tailor immunosuppressive treatment in liver transplantation[35]. As low as 1% donor chimerism is sufficient to induce robust tolerance to donor-specific organs, cells, and tissues[36].

So far, the mechanisms of induction of tolerance by microchimerism are still a matter of speculation. It has been suggested that donor leukocytes of bone marrow origin present in organ grafts represent a functional part of the donor immune system that is incorporated into the recipient's immune system, and a new hybrid immune system is thus established in the recipient and reciprocal bidirectional donor:host tolerance results[37]. Starzl *et al*[38] make a strong argument that allogenic microchimerism, in which the donor passenger leukocytes migrate widely into the recipient's lymphoid tissues, is essential for the maintenance of clonal exhaustion-deletion that is induced by the initial flood of passenger leukocytes during the first few weeks after transplantation, and the survival of passenger leukocytes is associated with long-term acceptance of the graft. It is not known what determines the release of donor leukocytes from the graft. These cells may be stimulated to migrate from the allograft by high local concentrations of tumor necrosis factor-α and IL-1 secreted early after transplantation. Alternatively, they may be mobilized or released by the organ procurement and reperfusion process. Cold preservation of liver allografts may result in injury to adhesion molecules of sinusoidal-lining cells, with the resultant sloughing of viable cells into the sinusoidal lumen[39]. Once donor stem cells have engrafted, they coexist and develop together with those of recipient origin giving rise to all hematopoietic cell types. Consequently, not only self-reactive but also donor-reactive thymocytes are intrathymic ally eliminated through negative selection, leading to a robust state of tolerance. Billingham *et al*[30] have shown that the induced tolerance is T cell mediated, and thymectomy of recipient mice before establishment of mixed chimerism results in failure to induce tolerance.

According to the concept of the two-way paradigm proposed by Starzl *et al*[40], clinical organ transplantation under immunosuppression involves a double-immune reaction that has host-versus-graft as well as graft-versus-host arms[41]. After transplantation, except for the migration of donor hematopoietic cells into host tissues, protecting the allograft, the host's own hematopoietic cells also repopulate the allograft, protecting it from autologous alloreactive T cells[42]. Chimeric cells can either be circulating or they can be integrated into the parenchyma, which was first described in transplanted organs in 1969[43]. Okabayashi *et al*[44] termed it reverse chimerism. In their model, reduced-size livers of the DA rat strain were transplanted into the allogenic green fluorescence protein (GFP) + Lewis recipients. In this strain combination, a combination of tacrolimus and plerixafor led to indefinite graft survival. Histological examination of the grafts showed a surprisingly high percentage of the liver parenchyma expressing GFP. This unexpected finding suggests that the host's HSCs were mobilized, repopulating the liver and converting to hepatocytes. In other studies, chimeric endothelium and duct epithelium were also found in transplanted livers[45,46]. Chimerism was also reported in other transplanted organs: recipient-derived endothelial cells were found in kidney grafts[47]; chimeric cardiomyocytes and smooth muscle cells were found in transplanted hearts[48]; and chimeric bronchial epithelium and type Ⅱ pneumocytes were found in transplanted lungs[49]. These results are of interest because of their potential clinical application in organ transplantation. It may be conceivable to perfuse a deceased donor allograft with recipient stem cells pretransplantation, thus reversing chimerism and inducing transplantation tolerance.

ALLOGENEIC BONE MARROW/ STEM CELL TRANSPLANTATION IN TOLERANCE INDUCTION IN LIVER TRANSPLANTATION

Many studies suggest that chimerism is essential for tolerance induction in transplantation, therefore, the logical

next step is to augment it. Chimerism can be induced through transplantation of allogeneic donor bone marrow/stem cells under appropriate host conditioning. To surmount physiological and immunological barriers for successful bone marrow cell (BMC) engraftment, various myeloablative or nonmyeloablative conditioning protocols have been developed involving the global elimination of recipient T cells[50]. Myeloablative irradiation leads to complete destruction of the hematopoietic repertoire of the host, which is then reconstituted by donor BMCs. Apart from the toxicity of this approach, full donor chimerism is associated with a higher incidence and severity of graft-versus-host disease and some degree of immunoincompetence for primary immune responses, and is therefore clinically undesirable[51]. Mixed chimerism can be achieved by nonmyeloablative doses of total body irradiation (TBI) combined with different medication protocols to overcome pre-existing alloreactive T cells in the periphery. Rahhal *et al*[52] prepared mixed chimeras by transplanting 10^8 T-cell-depleted allogeneic BMCs into Wistar Furth rats recipients conditioned with 300-600 cGy TBI. An 11-d course of tacrolimus and one dose of antilymphocyte serum were administered postoperatively. Mixed chimerism was initially achieved in almost all recipients and induced long-term acceptance of composite tissue allotransplants. Co-stimulation blockade, consisting of an anti-CD154 monoclonal antibody and the fusion protein cytotoxic T-lymphocyte antigen (CTLA)4-Ig inhibits CD40-CD40L and CD28-CD80/86 interactions between T cells and (allo)antigen-presenting cells[53]. Pree *et al*[28] outlined a nonmyeloablative murine BMT protocol including 3 Gy TBI on day 1, a conventional dose of fully mismatched BMCs on day 0, plus a single dose injection of anti-CD154 on day 0 and administration of CTLA4-Ig on day 2. With this protocol, high levels of mixed chimerism (20%-90%) in all tested lineages is induced and maintained for the length of follow-up, and donor skin is accepted permanently in the majority of chimeras, whereas third party skin is rejected promptly.

In humans, BMT-induced mixed chimerism has been shown to confer acceptance of donor-specific skin[54] and kidney allografts[55] without long-term immunosuppression. Similar results were also observed in the field of liver transplantation. In a pilot study in 1997, donor peripheral blood stem cell (DPBSC) infusions were performed in three recipients of living-related liver transplantation (LRLT). The results, at 20 wk post-transplant, suggested that the levels of donor cells detected in LRLT recipients treated with DPBSC infusions may be higher than that in recipients of cadaver donor liver allografts, indicating that administration of DPBSCs to recipients of liver transplants is a feasible procedure[56]. Since then, many human trials of HSC infusion before or after liver transplantation have been performed and tolerance induction observed in the patients[57-59]. Tryphonopoulos *et al*[60] perioperatively administered to liver transplant recipients unmodified cadaveric donor bone marrow infusions (DBMIs) (5×10^8/kg) in order to enhance chimerism. They found that patients who had DBMI tolerated withdrawal of tacrolimus or cyclosporine-based immunosuppression more often and had 5-6-fold more chimerism. Donckier *et al*[61] reported three patients prospectively enrolled in an original protocol designed to promote graft acceptance in living donor liver transplantation. The protocol relies on the use of donor stem cells administered after liver transplantation as tolerogenic/suppressive cells[62]. Post-transplant immunosuppression and conditioning included steroids, rapamycin and antithymocyte globulin. Donor $CD34^+$ stem cells were infused 7 d post-transplant. The clinical observations demonstrated that donor stem cell infusion combined with recipient conditioning may allow early immunosuppression withdrawal or minimization after liver transplantation.

All of these studies provide hope for liver transplant recipients to be off drugs for the rest of their lives. However, researchers have cautioned that only the healthiest patients will be able to withstand the conditioning regimens that allow donor stem cells to engraft, and for the comprehensive application of bone marrow/stem cell transplantation in solid organ transplantation, there is still a long way to go[63-66].

Establishment of chimerism in donor liver with recipient-type BMCs prior to liver transplantation is another strategy to induce tolerance to the liver graft. Sanada *et al*[14] established chimerism in rat donor liver by intraportal injection of recipient-type BMCs, followed by intramuscular administration of FK506 for 5 d. At 1-2 mo later, livers were harvested and transplanted. No immunosuppressants were used. They found that with livers from rats pretreated with recipient-type BMCs, survival of liver allografts was significantly extended. However, the significance of reverse microchimerism in liver transplantation is still controversial. In a recent study, Aini *et al*[67] compared the proportions of recipient-derived hepatocytes in long-term stable liver allografts and late dysfunctional allografts caused by chronic rejection. They found that hepatocyte chimerism was a constant event. However, the extent of engraftment of recipient-derived hepatocytes does not seem to correlate with the degree of hepatic injury in long-term liver allografts. More importantly, this protocol cannot itself be applicable to clinical allotransplantation because it needs donor preparation long before liver transplantation.

SUMMARY AND CONCLUSION

The liver has particular tolerogenic properties that allow its being spontaneously accepted in some animal species. Liver structure is considered to favor a tolerogenic environment. The peripheral tolerance mechanisms also play a role in liver graft spontaneous tolerance. A most significant mechanism in tolerance induction is chimerism. The mechanisms of induction of tolerance by microchimerism are still a matter of speculation. Chimerism can be induced through transplantation of allogeneic donor bone marrow/stem cell under appropriate host

conditioning. In humans, BMT-induced mixed chimerism has been shown to confer acceptance of donor liver allografts without long-term immunosuppression. However, recipients must be able to withstand the conditioning regimens that allow donor stem cells to engraft.

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ISSN 1007-9327