

Wei Li, M.D., Ph.D.

- Mechanism of organ transplant tolerance and rejection: The role of regulatory T cells (Treg), dendritic cells (DC), and costimulatory molecules on tolerance induction



New immunosuppressive drugs improve the short-term survival of organ transplant recipients. However, long-term survival remains comparatively poor. This is likely due to the fact that immunosuppressive strategies are not tolerogenic. Transplant tolerance is likely to arise not from improved immunosuppressive regimens, but from improved understanding of the normal mechanisms that generate and maintain self-tolerance, and the ability to manipulate these mechanisms for the prevention and treatment of transplant rejection. The overall goal of my research is to define mechanisms of peripheral tolerance induction in order to develop new strategies to guide clinical therapy in transplant recipients. I am currently focusing on studying the cellular and molecular basis of immune mechanisms of organ transplant tolerance and rejection using our unique mouse orthotopic liver transplant (OLTx), heterotopic heart transplant (HTx), skin transplant (STx), or islet transplant (ITx) models. Our research uses the characteristics of TCR transgenic or gene knockout mice and costimulatory molecule blocking reagents to define and characterize the dominant factors involved in organ transplant tolerance induction. These factors include T cell subsets (including T regulatory cells [Treg]), the signals or pathways between antigen presenting cells (APC) (such as dendritic cells [DC]) and alloreactive T cells, both locally (in grafts) and systemically (in the spleen and lymph nodes), and the cytokines which modulate T cell activations and differentiations.

The goals of our research are:

- to further ascertain the mechanisms of organ transplant tolerance;
- to examine the ability of tolerogenic dendritic cells to induce Treg, *in vivo* and *in vitro*, and to study the cytokines or costimulatory molecules that modulate this activity;

- to assess and maximize the therapeutic potential of DC and Treg in promoting tolerance induction in organ transplantation.

Mechanisms of murine spontaneous liver transplant tolerance and the role of regulatory T cells

It has been previously demonstrated that murine liver grafts are accepted spontaneously across all MHC barriers and induce donor-specific tolerance without immunosuppressive therapy (hepatic tolerance). The tolerance induced by a liver allograft can further induce the tolerance of subsequent organs such as a heart or kidney from the same donor origin. The tolerance is transferable to the naïve syngeneic mice by spleen or liver graft infiltrating cells obtained from long-term liver allograft recipients. Despite *in vivo* hyporesponsiveness to the liver allografts and to subsequent grafts from the same donor, *in vitro* mixed lymphocyte response (MLR) and cytotoxic lympholysis (CTL) assays showed unimpaired antidonor reactivity (split tolerance).

By contrast, livers from donors treated with Flt3 ligand (FL), which dramatically increases hepatic functional mature DC, are rejected acutely. This switch from tolerance to rejection is associated with marked reduction in apoptotic activity of graft infiltrating T cells, enhancement in costimulation between donor APCs, major DC and recipient T cells, and increased production of IL-12, IFN- γ , and IL-10. The mechanism of liver tolerance continues to be extensively investigated and is considered by many to be due to the tolerogenicity induced by liver DC. Apoptosis of mature T cells in the liver, but with persistence of their precursors in the periphery, was suggested to be the explanation for split tolerance.

However, apoptosis alone cannot explain liver-induced tolerance to subsequent other organ grafts from the same donor strain. The liver tolerance seems to be an active process and one which is mediated by regulatory T cells. We hypothesize

that inducing activated T cell apoptosis and Treg production are both critical to liver tolerance. Liver immature DC may be a key factor to induce Treg cell production and mediate activated T cell apoptosis. Co-stimulation between donor DC and recipient T-cells contribute to the T cell immune deviation, alloreactive T cell apoptosis, and function of regulatory T cells. To test our hypothesis, we treated liver donors or recipients with depleting anti-CD25 mAb. For the first time, we confirmed that depletion of recipient, but not donor, CD4⁺CD25⁺ regulatory T cells prevented spontaneous liver transplant tolerance. It was associated with enhanced anti-donor immune responses (MLR, CTL, NK activities, and Th1 cytokines IL-2 & IFN- γ production) and decreased alloreactive T cells, particular in CD8 T cells apoptosis.

This suggests that recipient CD4⁺CD25⁺ regulatory T cells play a very important role in spontaneous

other hand, B7/CD28, B7H/ICOS, CD40L/CD40, 4-1BBL/4-1BB, and OX40L/OX40 interactions provide a positive signal to the T cells, promote T cell proliferation and IL-2 production, and induce immunity. Each of these costimulatory pathways may function independently or cooperatively with each other.

To examine the mechanistic relationships among these signals and precisely assess which signal is critical for transplant tolerance induction and rejection, our approach was a comprehensive investigation of their molecular constituents and functions on the alloimmune response. Using a model of orthotopic liver transplantation and heterotopic heart transplantation in mice with a costimulatory pathway deficiency, we analyzed the expression profiles of those genes and the outcome of the allografts. These studies on the role of these new accessory molecules and their effect on tolerance induction, activated T

The tolerance induced by a liver allograft can further induce the tolerance of subsequent organs such as a heart or kidney from the same donor origin.

liver transplant tolerance induction, and this Treg may mainly affect on indirect pathway of antigen recognition. Further studies on other potential mechanisms of CD4⁺CD25⁺ Treg on liver tolerance induction are undertaken in our laboratory.

The role of costimulatory molecules on tolerance induction

T cell activation requires two distinct signals: Signal 1 is antigen specific, mediated via the T cell receptors, and delivered in the context of donor MHC class II; Signal 2, the costimulatory signal, is not antigen specific. Costimulatory molecules, in particular the B7/CD28 super family, have recently been extensively studied. A number of new members have been discovered and characterized, including B7/CD28, B7/CTLA4, CD40/CD40L, and most recently PD-L/PD-1, B7H/ICOS, OX40L/OX40, 4-1BBL/4-1BB, CD30L/CD30, and Tim3L/Tim3. It has already been known that B7/CTLA4, PD-L/PD-1, and Tim3L/Tim3 interactions provide a negative signal to the T cell, inhibit T cell activation and IL-2 production, and induce tolerance. On the

cell apoptosis, and possible promotion of Treg may provide crucial implications for designing a target for a trial of DC, antibody, or gene based therapy in patients receiving organ transplants.

We have recently tested costimulation blockade on liver DC and T cell interaction by using CTLA4 Ig and anti-CTLA4 mAb. The results showed that blocking both B7-CD28/ B7-CTLA4 signals using CTLA4 Ig promoted liver allograft survival from FL pretreated donors. It was associated with increased alloreactive T cell apoptosis in the liver graft and recipient spleen, and increased IL-10, decreased IFN- γ levels in the recipient serum. In contrast, blocking CTLA4 signal using anti-CTLA4 mAb, which was defined as a negative signal to the T cells, broke the liver spontaneous tolerance and induced liver allograft acute rejection. This was associated with decreased alloreactive T cell apoptosis in the liver grafts and recipient spleens, and increased IL-2, IFN- γ , decreased IL-4 production, and decreased the CD4⁺CD25⁺ regulatory T cells in the recipient spleens.

The role of dendritic cells (DC) in organ transplantation

DC, professional antigen presenting cells of the immune system, have been considered having the potential to either stimulate or inhibit immune responses. Exploiting the immune-regulatory and tolerogenic capacities of DC hold great promise for the treatment of cancer, autoimmune disease, and prevention of transplant rejection. We have reported that liver immature DC play a critical role in the liver transplant spontaneous tolerance. We also reported that the immunoregulatory cytokine, IL-10 induces Treg both *in vivo* and *in vitro* and promotes heart allograft survival in mice.

A recent report revealed that DC is capable of inducing CD4⁺CD25⁺ Treg which express CTLA4 and produce immunosuppressive cytokines IL-10 and TGFβ, downregulating alloimmune responses.

Costimulation between donor DC and recipient T-cells may not only contribute to T cell immune deviation and alloreactive T cell apoptosis, but also may lead to production of regulatory T cells. Thus, treating the allograft recipient with immature donor DC in the presence of IL-10 or TGFβ may drive regulatory T cells generation *in vivo* and promote organ transplant tolerance. We will challenge DC-treated recipients with allogeneic heart transplants or islet transplants (in NOD mice or STZ treated diabetes mice) to assess the therapeutic potential of DC-induced alloantigen specific tolerance.

We believe that these studies will provide better understanding of the mechanism of transplant tolerance and rejection, and facilitate novel therapeutic strategies to combat organ rejection and even autoimmune disorders such as diabetes.

RELATED PUBLICATIONS

1. Lu L, Li W, Fu F, Chambers FG, Qian S, Fung JJ, and Thomson AW. Blockade of the CD40-CD40L pathway potentiates the capacity of donor-derived dendritic cell progenitors to induce long-term cardiac allografts survival. *Transplantation* 64(12): 1808-1815, 1997.
2. Li W, Fu F, Lu L, Narula SK, Fung JJ, Thomson AW, Qian S. Systemic administration of anti-IL-10 antibodies prolongs mouse allograft survival in normal and presensitized recipients. *Transplantation* 66(12): 1587-1596, 1998.
3. Li W, Fu F, Lu L, Narula SK, Fung JJ, Thomson AW, Qian S. Recipient pretreatment with cellular IL-10 prolongs cardiac allograft survival in mice. *Transplantation* 68:1402, 1999.
4. Steptoe R, Li W, Fu F, O'Connell PJ, and Thomson AW. Trafficking of APC from liver allografts of Flt3L-treated donors. *Transplant Immunology* 7:51-57, 1999.
5. Lu L, Li W, Zhong C, Qian S, Fung JJ, Thomson AW, and Starzl TE. Increased apoptosis of immunoreactive host cells and augmented leukocyte chimerism, not sustained inhibition of B7 molecule expression is associated with prolonged cardiac allograft survival in mice preconditioned with immature donor dendritic cells plus anti-CD40L mAb. *Transplantation*. 68:747-757, 1999.
6. Li W, Lu L, Wang Z, Wang L, Fung JJ, Thomson AW, and Qian S. IL-12 antagonism enhances apoptotic death of T cells within hepatic allografts from Flt3-treated donors and promotes the restoration of transplant tolerance. *J. Immunology*, 166:5619-5628, 2001.
7. Li W, Lu L, Wang Z, Wang Z, Fung JJ, Thomson AW, and Qian S. Costimulation blockade promotes the apoptotic death of graft infiltrating T cells and prolongs survival of hepatic allografts from Flt3L-treated donors. *Transplantation*, 72:1423-1432, 2001.
8. O'Connell PJ, Li W, Wang Z, Logar AJ and Thomson AW. Immature and mature CD8a+ dendritic cells prolong the survival of vascularized heart allografts. *J. Immunology*, 168:143, 2002.
9. Li W, Chou S, Wang C, Kuhr C, Perkins J. The role of the liver in peripheral tolerance: induction through oral antigen feeding. *American Journal of Transplantation*, 4:1574-1582, 2004.
10. Li W, Zheng XX, Kuhr CS, Perkins JD. CTLA4 engagement is required for induction of mouse liver transplant spontaneous tolerance. *American Journal of Transplantation*, 5:978-986, 2005.

DEPARTMENT CO-INVESTIGATORS

Christian S. Kuhr, M.D. / Adam E. Levy, M.D. / James D. Perkins, M.D. / Jorge D. Reyes, M.D.

OTHER CO-INVESTIGATORS

Yvette Latchman, Ph.D.; UW Department of Medicine / David L. Perkins, M.D.; University of California San Diego / Xin Xiao Zheng, M.D.; Harvard University
