Organ-specific Differences in Achieving Tolerance

Maria Lucia L. Madariaga, M.D.[1], **Daniel Kreisel, M.D., Ph.D.**[2], and **Joren C. Madsen, M.D. D.Phil.**[1,3,4]

[1]Center for Transplantation Science, Department of Surgery, Massachusetts General Hospital, Boston, MA

[2]Division of Cardiothoracic Surgery, Department of Surgery, Washington University School of Medicine, St Louis, MO

[3]Division of Cardiac Surgery, Department of Surgery, Massachusetts General Hospital, Boston, MA

Abstract

Purpose of review—When it comes to tolerance induction, kidney allografts behave differently from heart allografts which behave differently from lung allografts. Here, we examine how and why different organ allografts respond differently to the same tolerance induction protocol.

Recent findings—Allograft tolerance has been achieved in experimental and clinical kidney transplantation. However, inducing tolerance in experimental recipients of heart and lung allografts has proven to be more challenging. New protocols being developed in nonhuman primates based on mixed chimerism and co-transplantation of tolerogenic organs may provide mechanistic insights to help overcome these challenges.

Summary—Tolerance induction protocols that are successful in patients transplanted with "tolerance-prone" organs such as kidneys and livers will most likely not succeed in recipients of "tolerance-resistant" organs such as hearts and lungs. Separate clinical trials using more robust tolerance protocols will be required to achieve tolerance in heart and lung recipients.

Keywords

Transplantation; tolerance; heart; lung; kidney; organ-specificity

INTRODUCTION

Induction of immune tolerance is the ultimate goal in the field of organ transplantation. Achieving a state of tolerance would lead to indefinite graft survival without the need for chronic immunosuppression and, would theoretically prevent chronic rejection. Tolerance of *kidney* allografts has been achieved in non-human primates (NHPs)(1-3) and in humans (4-7) by using a combination of nonmyeloablative conditioning and donor bone marrow

[4]Corresponding author: Joren C. Madsen, M.D., DPhil, WHT-05-510C, Massachusetts General Hospital, 55 Fruit St., Boston, MA 02114. 617-726-6506. jcmadsen@mgh.harvard.edu.

transplantation that results in mixed chimerism. However, mixed chimerism protocols that achieve long-term tolerance of kidney allografts in NHPs fail to induce tolerance in heart recipients (8).

The reasons for this organ-specific difference are not clear. However, it is clear that all transplanted organs are *not* created equal. Not only does the strength of the immune response to a particular organ vary with the organ transplanted but the nature of response itself, rejection versus tolerance, varies from organ to organ. In most experimental transplant models, kidney and liver allografts evoke a weaker rejection response than heart and lung allografts. Moreover, kidney and liver allografts can actively participate in the induction and maintenance of tolerance and thus, can be considered "tolerance-prone" organs. The same cannot be said for heart and lung allografts which are, for the most part, "tolerance-resistant." Finally, kidney and liver allografts also possess the unique ability to confer unresponsiveness upon co-transplanted, tolerance-resistant organs like hearts.

Mechanisms underlying these organ-specific differences are unclear but understanding them could contribute to the development of strategies that extend tolerance to recipients of tolerance-resistant organs. Here, we review organ-specific differences in tolerance induction, focusing on the dissimilarities between tolerogenic kidney allografts and the more stringent heart and lung allografts.

Organ-specific differences in spontaneous tolerance

Different organ allografts have different propensities to be spontaneously accepted without any treatment. Murine skin, hearts, intestines, lungs and hepatocytes are largely rejected when transplanted across MHC barriers (9-13). In contrast, kidneys and livers are commonly accepted across the same MHC barriers (10,14,15). Zhang et al. (10) compared liver, kidney and heart transplantation in three different MHC disparate mouse strain combinations without treatment. The majority of liver allografts (57-72%) in each strain combination were spontaneously accepted long term, whereas hearts transplanted across identical histocompatibility barriers all rejected in less than 10 days. The pattern of kidney allograft rejection was mixed with 20% to 50% organs surviving long term (10). Our results (13,16) and others (17-19) in mice support the fact that kidneys have a much greater propensity for spontaneous acceptance compared to hearts transplanted across the same MHC barrier.

Spontaneously accepted kidney allografts show prominent peri-arterial lymphoid sheaths containing nodules of CD3$^+$Foxp3$^+$ T cells, CD4$^+$ T cells, DCs, B cells and indoleamine-pyrrole 2,3-dioxygenase positive cells (16). These regulatory T cell-rich organized lymphoid structures, which we termed "TOLs", were distinct from tertiary lymphoid organs (TLOs) found in chronic inflammation in that they lacked high endothelial venules (20). Similar structures have been identified in tolerant pig and nonhuman primate kidney allografts (Farkash, et al., unpublished data). We have also shown that Foxp3$^+$ regulatory T cells (Tregs) are necessary to maintain unresponsiveness in spontaneously accepted MHC mismatched mouse kidney allografts (16). Treg depletion in long term surviving kidney allograft recipients triggered disintegration of TOLs and acute cellular rejection (16).

Liver allografts are also spontaneously accepted across certain mouse and rat strain combinations (10,21-24). Like kidney allografts, however, depletion of Tregs (using an anti-CD25 antibody) induces acute liver rejection (22).

Among higher-order animals, swine is the only species in which spontaneous tolerance has been reported after liver (25) or kidney (26) transplantation. Currently, there are no specific agents to deplete Tregs in swine, however, other studies suggest that Tregs play an important role in this effect (27).

Although a somewhat different phenomenon, spontaneous graft acceptance after cessation of immunosuppression (operational tolerance) has been observed in human kidney and liver recipients. Examination of the natural history of 27 kidney transplant patients rendered operationally tolerant after withdrawing immunosuppression found that 70% maintained stable graft function for an average of 9 years after transplantation (28). In adult liver transplantation, 8% to 33% of patients who withdraw from immunosuppression exhibit operational tolerance (29-32), although in the pediatric population the incidence is much higher (60%)(33). In contrast, there exist only anecdotal cases of operational tolerance in a lung recipient and heart recipients (34).

Organ-specific effects in calcineurin inhibitor-based tolerance protocols

Perhaps the most compelling evidence for the organ-specificity in tolerance induction comes from studies of heart, kidney and lung transplantation in MHC inbred miniature swine. When porcine recipients were transplanted with MHC class I disparate *hearts* and treated with 12 days of cyclosporine (CyA), they all rejected within 55 days and exhibited cardiac allograft vasculopathy (CAV) (35). In contrast, when swine were transplanted with class I disparate *kidneys* allografts and treated with the same course of CyA, they all became tolerant to donor antigen and maintained excellent renal function long-term, in some instances for over 2 years (36). The survival of *lungs* transplanted in an analogous setting was in between that of hearts and kidneys with graft survival ranging from 67 to >605 days and two thirds developing obliterative bronchiolitis (OB) (37).

A similar hierarchy was observed when organs were transplanted across a full MHC barrier and FK506 was substituted for CyA in the swine model. Long term tolerance was easily induced in renal allografts (38), whereas lung survival ranged from 100 to >500 days (39) and hearts only survived 18-35 days (40)(Fig 1).

Thus, clear differences exist between organs that dramatically affect the ability of a particular treatment protocol to induce tolerance of that organ. Kidneys have a greater propensity for tolerance induction than lungs which have a greater propensity than hearts, at least in swine. These observations emphasize that tolerance induction protocols are not necessarily transferable between organs and therefore, that preclinical and clinical testing of tolerance protocols for human transplantation must proceed in an organ-specific manner (41).

Even more striking than the apparent hierarchy in organ tolerogenicity, is the observation that tolerance-prone organs are able to confer unresponsiveness upon tolerance-resistant

organs procured from the same donor. Using MHC inbred miniature swine, we have shown that instead of rejecting their hearts, recipients co-transplanted with hearts and kidneys from the same class I disparate (42) or fully MHC mismatched (40) donor developed stable tolerance. The result was the long-term, rejection-free survival of *both* kidney and heart allografts (Fig 1) with no alloantibody formation or evidence of CAV. Neither heart nor kidney allograft survival was affected by the placement of donor-specific and third party skin grafts despite the return of anti-donor CML reactivity, which suggests the effects of ongoing T cell regulation (43,44). We have termed this phenomenon kidney-induced cardiac allograft tolerance (KICAT).

We found that the donor kidney must remain in the recipient for over 8 days to achieve KICAT (45) and that a radiosensitive cell population within the donor kidney appeared necessary for the development of KICAT (46). We also found that the host thymus played a critical role during the induction phase of KICAT (47) as did MHC matching of the heart and kidney allografts (48). We have examined the phenotypes of graft infiltrating lymphocytes (GILs) retrieved from isolated kidneys transplanted across a class I barrier which accepted versus rejected (49). The number of cells expressing the phenotypic markers of Tregs was substantially higher in acceptor than in rejector transplants at all time points. We went on to show that CD25^{+} T cells in peripheral blood leukocytes from heart/kidney recipients but not isolated heart recipients could suppress the anti-donor response of naïve-matched T cells in co-culture CML assays (50). Based on these results and others (20-22), we hypothesize that cells or cell products intrinsic to kidney, but not heart, allografts promote the activation/expansion of host Tregs which induce tolerance of heart grafts (reviewed in (27)).

Of note, a similar effect has been described in human recipients of liver transplants (25,51-54). Recipients of combined heart-liver transplants require less immunosuppression than recipients of isolated hearts or kidneys (55) and despite the lower immunosuppression, rejection rates for heart and liver are low (13% and 10%, respectively) (56,57). In combined liver/lung transplantation, acute rejection of the lung graft was observed at comparatively low rates (58,59) although definitive conclusions about a potential liver-protective effect cannot be drawn from these small series. Several clinical studies have examined outcomes between combined heart/kidney transplants versus heart alone transplants. Although there does not appear to be any difference in patient survival, some groups report decreased rejection episodes and incidence of CAV in heart/kidney recipients (60-64). Chronic immunosuppression in these patients probably masks any tolerogenic effects of the kidney or liver allograft.

Organ-specific effects in mixed chimerism-based tolerance protocols

We have extended the study of KICAT to cynomolgus monkeys using a mixed chimerism tolerance induction strategy instead of a short course of CyA or FK506. Tolerance of isolated MHC disparate kidneys allografts in cynomolgus monkeys has been previously reported from our laboratory using mixed chimerism-based protocols that included T cell depletion, nonmyeloablative total body irradiation, thymic irradiation, donor bone marrow infusion and a short course of calcineurin inhibitor (1-3). However, the same mixed

chimerism protocol that induced tolerance of kidneys failed to induce tolerance of isolated hearts (8). The majority of heart recipients developed anti-donor cellular and humoral immunity, resulting in severe rejection with CAV by day 175 (8). Recipients cotransplanted with heart and kidney allografts, however, became tolerant of both organs (Tonsho, M. manuscript submitted). Every heart/kidney recipient that successfully completed its conditioning regimen became tolerant. To our knowledge, these heart/kidney recipients represent the first nonhuman primates to become tolerant of cardiac allografts.

Potential mechanisms underlying organ-specific differences in tolerance induction

Why different organs exhibit different thresholds for tolerance induction is unknown. However, factors intrinsic to the organ as well as extrinsic factors such as exposure to the external environment in the case of lung and intestine, likely play a role. We hypothesize that the tolerogencity of a particular organ is a function of on how internal and external factors combine to promote the activation of host regulatory mechanisms and/or the inactivation of alloaggressive mechanisms, thereby shifting the balance of the overall immune response away from rejection and towards tolerance. Similar mechanisms have been put forth to explain the phenomenon of "immune priviledge" (65).

In terms of external factors, exposure to environmental antigens could contribute to the tolerance-resistance of lung and intestinal allografts. Stimulation of toll-like receptors (TLRs) by microorganisms could activate the innate and adaptive immune systems, thereby promoting rejection. This was suggested by studies showing that TLR signaling interferes with the immunomodulatory milieu established by T regulatory cells (66). Clinically, respiratory infections predispose lung grafts to immune-mediated failure (67) and infection with Pseudomonas aeruginosa results in neutrophilic graft infiltration that breaks established costimulation blockade-mediated lung allograft acceptance in mice (68). Low molecular weight hyaluronan, a damage-associated molecular pattern that is associated with chronic lung rejection in humans, breaks costimulation blockade-mediated acceptance of mouse pulmonary allografts (69). This abrogation of acceptance is dependent on neutrophilic graft infiltration and signaling via TLR2/TLR4 as well as MyD88 in the recipients.

Other important external factors include donor brain death (BD) and ischemia/reperfusion injury (IRI) which result in the elaboration of proinflammatory cytokines and chemokines leading to augmented graft antigenicity and increased susceptibility to host alloimmune responses. Indeed, there are many examples of how the deleterious effects of BD (70) and IRI (71) accelerate acute rejection in experimental models and negatively affect long-term graft survival in humans (72). However, while the effects of BD and IRI on organ quality have been studied extensively, investigations into the effect of brain death and ischemia on tolerance induction are extremely limited. In a rat kidney transplant model, brain death was shown to interfere with the beneficial effects of anti-CD4 mAb therapy on the long term graft function (73). Severe IRI abrogated costimulatory blockade-mediated induction of long-term acceptance of lung allografts in a mouse model (74). This was linked to neutrophilic graft infiltration that resulted in activation of donor antigen presenting cells and induction of Th1 responses. This observation is of potential clinical relevance as IRI-mediated primary graft dysfunction is a recognized risk factor for the development of

chronic lung rejection in humans (75). To our knowledge, the only large animal study to address this question is our earlier report in MHC inbred miniature swine showing that following traumatic brain death, proinflammatory cytokines were released systemically in the donor's serum and up-regulated in the donor's tissue. Furthermore, transplanting lung allografts procured from these brain dead donors resulted in the failure of a tolerance induction protocol that was otherwise successful when allografts were retrieved form non-brain dead donors (76). However, in contrast to lung recipients, brain death and ischemia did not prevent tolerance induction in analogously treated heart/kidney recipients (Michel S. manuscript submitted). This finding suggests that the effects of donor brain death and ischemia on tolerance induction also vary with the organ transplanted.

The striking difference in tolerogenicity between kidney and heart allografts combined with our finding that regulatory mechanisms play an important role in spontaneous tolerance and KICAT suggest that cells or cell products *intrinsic* to kidney but not heart allografts promote the activation and/or expansion of host Tregs which in turn, mediate tolerance of heart (and kidney) grafts. While the identity of those subpopulations is not clear, there are at least two cell populations present in kidneys capable of down-regulating alloimmune responses: 1) plasmacytoid dendritic cells (pDCs), capable of promoting the generation of Tregs and inducing tolerance to hearts in mice (77-79) and 2) renal tubular epithelial cells (RTECs), capable of inducing T cell unresponsiveness to self- and allo-antigens in mice and humans (80-85).

We have recently demonstrated that $CD11c^+$ cells isolated from naïve, untransplanted kidneys (from DBA/2 and BALB/c mice) contain a subpopulation of $PDCA-1^+B220^+$ pDCs not found in hearts from the same animals (Alessandrini et al., unpublished data). The presence of $PDCA-1^+B220^+$ pDCs in DBA/2 kidneys but not hearts may explain why DBA/2 kidneys are spontaneously accepted when transplanted into B6 WT recipients, whereas DBA/2 hearts are rejected (17,18).

Alternatively, it has been previously shown that Foxp3+ T cells are enriched in the tubules of mouse (86) and human (87) renal allografts. Frasca and colleagues have shown that IFNγ-treated human RTECs induce allospecific tolerance via a class II pathway (80). IFNγ induces PD-L1 and IDO on RTECs (88,89), and PD-L1 can induce the generation of allogeneic $Foxp3^+$ Tregs (90). Finally, RTECs are known to produce and activate TGFβ (91), which is an inducer of Foxp3+ Treg (92) and tolerogenic pDC (93) generation. High levels of TGFβ have been documented in accepted DBA/2 kidneys (17,18).

Recently, erythropoietin (EPO) was shown to inhibit T cell immunity *in vitro*, providing another possibly mechanism to explain the protective effects of kidney allografts (94).

Like kidney allografts, liver allografts are spontaneously accepted across certain mouse and rat strain combinations (10,21-24). The tolerogenic properties of livers suggest that, as in kidneys, cells or cell products intrinsic to the organ are important. Three cell populations present in liver have been shown capable of suppressing an alloimmune response; 1) pDCs, located within periportal areas and around central veins (95), 2) liver sinusoidal endothelial

cells (LSECs) that generate anti-inflammatory cytokines (96) and diminish the survival of CD8+ T cells (97-99) and 3) hepatic stellate cells (HSCs) that can induce Tregs (100,101).

Hepatic dendritic cells are less immunogenic than splenic dendritic cells and only 5% of splenic DCs are made up of pDCs while 19% of the liver DC population is made up of pDCs (102). Furthermore, circulating pDCs were increased relative to myeloid DCs (mDCs) in operationally tolerant pediatric liver allograft recipients as compared to patients maintained on chronic immunosuppression (103).

LSECs secreting IL-10 (96) are able to induce CD8+ T cell tolerance rather than immunity via cross-presentation of antigen to CD8+ T cells (97,98). LSEC-primed, naïve CD8+ T cells are initially induced to proliferate, to release cytokines, such as IL-2 and IFNγ, but eventually begin to secrete low levels of these cytokines and exhibit low cytotoxicity (97). The induction of CD8+ T cell tolerance has been correlated with and has been shown to be dependent on the induction of PD-L1 by LSECs (104).

Finally, HSCs store vitamin A and can produce TGFβ in response to inflammation and injury (104,105). Vitamin A-derived retinoic acid and TGFβ have been shown to participate in the conversion of CD4+ T cells to Tregs (106,107). Furthermore, activated HSCs express PD-L1 (108). Importantly, HSCs can confer unresponsiveness and long term survival to *islet* allografts by inducing Tregs (100) and myeloid-derived suppressor cells (109,110).

It has been the prevailing view that memory T cell infiltration into allografts is a barrier to tolerance induction and attempts have been made to eliminate this cell population (111). In fact, clinical immunosuppression protocols depleting T cells indiscriminately in the peri-operative period that have been successful in kidney transplantation have been adapted to lung transplant patients (112). However, we have recently shown that graft infiltration by central memory CD8+ T cells is critical to induce costimulatory blockade-mediated tolerance after lung transplantation in the mouse (113). Induction of tolerance was dependent on production of IFN-γ by central memory CD8+ T cells within the graft, which results in production of nitric oxide. One factor that needs to be considered when developing lung-specific tolerance protocols is that propagation of immune responses that lead to rejection or tolerance of lungs can occur in the graft itself (114,115). This is in contrast to other organs such as hearts where such responses depend on cellular trafficking to secondary lymphoid tissues (116,117).

Lymphoid neogenesis, the development of organized tertiary lymphoid tissue, has been observed at chronic sites of inflammation, including transplanted grafts. These structures play a role in generating local cellular and humoral immune responses. For example, the Lakkis group has shown that tertiary lymphoid organs are associated with graft rejection in murine heart transplants (20). In contrast, we have observed the induction of organized lymphoid structures in mouse lung allografts that are accepted long term after treatment with perioperative costimulatory blockade (114). These structures are rich in CD11c+ cells that express PD-L1 as well as Foxp3+ cells. In contrast to the "TOLs" observed in spontaneously accepted kidney allografts, high endothelial venules are induced in the tertiary lymphoid structures in accepted lung allografts.

Conclusion

Tolerance protocols already in use for clinical kidney transplantation will require modifications before becoming applicable to more stringent organs such as heart and lung. In fact, some approaches that have been developed for kidney transplants may be deleterious for thoracic organs (113). Modifications may include administration of immunomodulatory cellular therapy, blunting heterologous immunity, or donor-organ preconditioning. Studies focusing on the mechanisms of tolerance induction, and the differences between tolerance-prone versus tolerance-resistant organs, may provide further insight into ways to optimize immunosuppression-free survival in heart and lung transplant recipients.

Acknowledgements

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Key points

- Attaining tolerance of heart and lung allografts is more difficult than of liver and kidney allografts, reflecting organ-specificity in tolerance induction.

- The mechanisms underlying organ-specificity in tolerance are unknown but may be based on cells or cell products resident in tolerance-prone but not tolerance-resistant organs.

- Understanding the mechanisms underlying organ-specific alloimmune responses could contribute to the success of strategies aimed at extending tolerance to recipients of all tolerance-resistant organs.

- Separate clinical trials using more robust tolerance protocols will be required to achieve tolerance in heart and lung recipients.

Organ-specific differences in tolerance induction across a full MHC mismatch in miniature swine
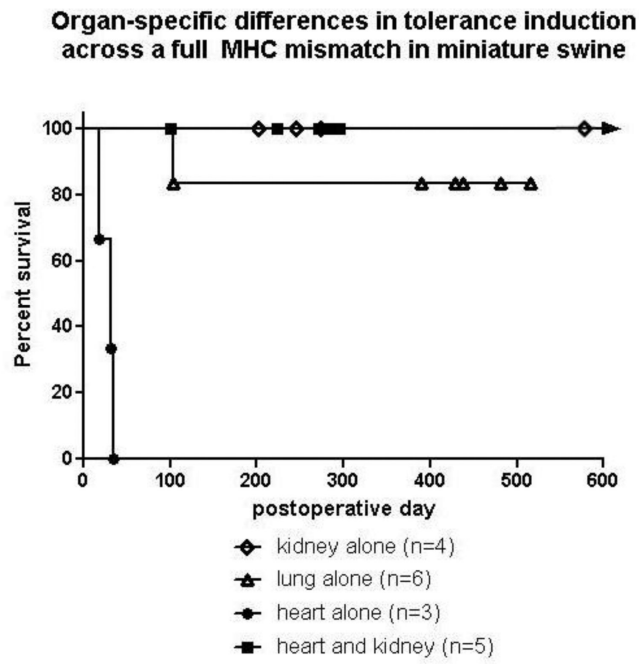
Graft survival data compiled as follows: kidney alone (38), lung alone (39), heart alone (40), heart and kidney (40).

Figure 1.
Graft survival data compiled as follows: kidney alone (38), lung alone (39), heart alone (40), heart and kidney (40).