

A major barrier to the success of transplantation is graft rejection, wherein the transplanted tissue is destroyed by the transplant recipient's immune system. Graft rejection is very common in allografts, in which tissue is transplanted from one individual to another genetically non-identical individual of the same species because the transplant recipient's white blood cells (immune cells) recognize the donor tissue as "foreign" and attack it. In order to prevent graft rejection, immunosuppressive drugs are administered to suppress the allograft recipient's immune system. However, immunosuppressive drugs are expensive and they are associated with increased susceptibility to infection, increased risk of malignancy, nephrotoxicity (kidney toxicity), and cardiovascular complications.¹ Furthermore, immunosuppressive drugs do not completely eliminate the problem of graft rejection—for instance, chronic rejection still remains a leading cause of the late loss of renal allografts with a 3-5% annual rate of renal graft loss despite the use of immunosuppressive medication.² Hence, immunosuppressive drugs are not the ideal solution to the problem of graft rejection, and another method to achieve and sustain graft survival is desired. Current research aims to discover methods to induce transplant tolerance, in which the transplant patient can sustain the allograft without a need for ongoing immunosuppression. In this paper, two approaches to inducing transplant tolerance, mixed chimerism and regulatory T cell therapy, will be discussed in terms of their potential to solve the major public health problem of safely and effectively sustaining organ transplants.

Transplant tolerance relies on the induction of immune tolerance, a mechanism which prevents autoreactive (self-reactive) immune cells from destroying normal tissue in the individual. In central immune tolerance, autoreactive immune cells, specifically T cells and B cells, are deleted as they mature in primary lymphoid organs (i.e. the thymus and bone marrow).^{3,4} However, some autoreactive immune cells are able to escape this selection process. Peripheral immune tol-

erance, which occurs in tissue such as lymph nodes where T cells and B cells migrate after maturing, exists as a backup measure to deal with cells that have evaded central tolerance. One mechanism of peripheral tolerance involves the action of regulatory T cells (Tregs). Tregs help suppress the proliferation of auto-reactive cells through mechanisms that are not well-characterized.⁵ Both central and peripheral immune tolerance act to prevent autoimmunity, and it is hoped that inducing and manipulating these tolerance mechanisms may lead to the discovery of immunotherapy that can induce transplant tolerance.

Mixed chimerism refers to the co-existence of both donor and recipient cells in the host. Transplantation of bone marrow derived cells is often utilized to try to achieve mixed chimerism as a strategy to establish central immune tolerance. The bone marrow transplant can result in the recipient possessing both donor and recipient hematopoietic stem cells in the circulation.⁶ The donor hematopoietic stem cells provide a new source of immune cell, which allows the recipient's immune system to relearn what "self" is, so that the recipient now considers the donor tissue to be self and not foreign.⁶ Thus, alloreactive immune cells (i.e. the immune cells that react against the donor tissue) will now be eliminated in the primary lymphoid organs. The success of this concept was first demonstrated in the 1950s by Billingham, Brent, and Medawar⁷. The researchers injected donor cells into the fetuses of a pregnant mouse. Once the mice were born, each one of them was given a skin graft from the donor. Rejection of the skin graft occurred in three of the five mice that were born. The other two mice were able to sustain their skin grafts, even after 77 and 101 days, respectively⁷. This study demonstrated the success of inducing transplant tolerance through the injection of donor cells. They called this phenomenon, "actively acquired tolerance."⁷ This method of inducing transplant tolerance has shown further promising results in murine/rodent models.^{1,8} In one study, mixed chimerism was induced in mice by lethal irra-

diation of recipient mice and reconstitution of these mice with a mixture of host and donor bone marrow⁹. The tolerance induced by this technique permitted the long-term acceptance of genetically mismatched skin grafts in mice; these mice were able to fully tolerate grafts from the donor from which they received the bone marrow transplant and exhibited no signs of graft-versus-host disease, where the white blood cells from the donor bone marrow attack the recipient's body cells.⁹ Additionally, mixed chimerism has shown promising results in human kidney transplant patients. A study conducted by Scandling et al. at Stanford University followed twelve patients who received a kidney transplant along with enriched donor hematopoietic progenitor cells and T cells¹⁰. A conditioning regimen that included total lymphoid irradiation was used to prevent rejection of the donor immune cells. All patients were given cyclosporine as an immunosuppressive drug and all of them continued taking cyclosporine for at least six months following the renal transplant. In eight of the twelve patients, cyclosporine was discontinued and there was no evidence of graft rejection or graft-versus-host disease. Thus, in this clinical study, the majority of the patients were able to tolerate their kidney transplant following the induction of mixed chimerism.¹⁰

However, despite preliminary successful results in the clinic, mixed chimerism does have its shortcomings. Many bone marrow transplant techniques require the use of myeloablative conditioning, in which the recipient bone marrow is ablated via irradiation in order to prevent rejection of the donor hematopoietic cells. The Stanford study described above, for instance, utilized total lymphoid irradiation as part of the protocol for the bone marrow transplants¹⁰. There are many risks associated with myeloablative conditioning, since the host bone marrow is effectively destroyed. As a result, less toxic methods are desired.^{1,11} One such less toxic method is nonmyeloablative conditioning, which involves the administration of sufficient immunosuppression to permit the bone marrow transplant but the doses are low enough so that adverse effects are avoided.¹ Nonmyeloablative conditioning in mixed chimerism has been successful in preliminary studies with humans. In one study by Kawai et al., five transplant patients with end-stage renal disease were given a nonmy-

eloablative conditioning regimen, and they received an intravenous infusion of donor bone marrow following the kidney transplant. Four of the five patients developed tolerance to the kidney graft and were able to discontinue taking immunosuppressive drugs.¹² The success of nonmyeloablative conditioning has also been demonstrated in other studies. In a study by Leventhal et al., eight kidney transplant patients underwent a nonmyeloablative conditioning regimen prior to receiving a graft of bone marrow cells.¹³ After the kidney transplant, the patients received immunosuppression. Five patients exhibited durable chimerism and were able to tolerate their renal graft; they were taken off all immunosuppressive drugs one year after transplant. Another two patients exhibited transient chimerism and their immunosuppressive drug dosage was reduced. None of those patients produced anti-donor antibodies or exhibited engraftment syndrome or graft-versus-host disease.¹³ Together, the two clinical studies demonstrate that nonmyeloablative conditioning regimens in bone marrow transplantations can provide a viable and practical means of using mixed chimerism safely in the clinic. Future research should concentrate on refining the protocol for inducing mixed chimerism in the clinic so that a standard protocol, such as one that involves a safe and effective mechanism of inducing stable, long-term chimerism, can be constructed.

Regulatory T cell therapy is another method of inducing transplant tolerance. This method involves the induction of peripheral immune tolerance. Giving donor Tregs to a transplant patient may sustain graft survival as the donor Tregs can prevent the recipient cells from attacking the foreign graft tissue.⁵ The use of Treg therapy has shown positive results in mouse models. One recent study showed that therapies using donor Tregs prolonged the survival of a human pancreatic islet allograft in a humanized mouse model.¹⁴ When human donor Tregs were transferred into mice that contained a functioning human islet graft, only two of thirteen mice rejected the islet graft whereas nearly all the mice that didn't receive Tregs rejected the graft¹⁴. These results suggest that Treg therapy has the potential to protect a human allograft from rejection. In another study, sublethally irradiated mice were injected with Tregs from the donor. However, when the researchers tried perform-