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Transplantation Tolerance Through Mixed Chimerism: From Allo to Xeno

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Abstract

I would like to thank the organizers of this meeting for providing me with this opportunity to summarize the studies in which I have been involved for the past several decades directed toward the use of mixed hematopoietic chimerism to induce transplantation tolerance across allogeneic and xenogeneic barriers.

Tolerance across allogeneic barriers

The first demonstration of tolerance induced by mixed hematopoietic chimerism was an experiment of nature, reported in 1945 by Ray Owen (1). Owen was actually studying the basis for unusual blood types in Freemartin cattle, which are fraternal cattle twins born from a common placenta. The blood types of these animals could not be explained simply by Mendelian inheritance and Owen hypothesized that the reason for this anomaly was the mixing of blood from the fraternal twins in the placenta, with survival of both blood types thereafter into adult life. Sir Peter Medawar hypothesized that such survival of allogeneic blood cells in the absence of immunosuppression might indicate the existence of immunologic tolerance between the immune systems of these fraternal twins, presumably induced by the mixing of blood elements in utero. He and his colleagues then transplanted skin grafts between these cattle twins and found the survivals of these grafts to be markedly prolonged, supporting the existence of tolerance (2). They then carried out experiments in mice, injecting newborn animals with fully allogeneic hematopoietic cells within the first 24 hours after birth. Like the Freemartin cattle, these mice accepted skin grafts from the donor strain later in life, confirming the hypothesis that mixed chimerism early in life could lead to long-term transplantation tolerance (3). Since it is not clear at the time of birth who may need a transplant later in life, this information was not immediately relevant to the field of clinical transplantation, but it did lead scientists thereafter on a quest for ways to induce tolerance through mixed chimerism in adults.

In the field of transplantation, tolerance is probably best defined as the specific absence of a destructive immune response to a transplanted tissue in the absence of immunosuppression. The word "destructive" is included in this definition because it is now clear that tolerance may result not only from the absence of an immune response, but also from a non-destructive, down-regulatory immune response. Deletional tolerance, which leads to the absence of an immune response, occurs predominantly in the thymus, where potentially destructive T cells are eliminated by negative selection (4), while regulatory tolerance involves the turning off of potentially destructive immune responses by T cells that have escaped negative selection in the thymus by other, regulatory T cells or "Tregs" (5). As explained in more detail below, both forms of tolerance can be induced through mixed hematopoietic chimerism.

Our initial studies of tolerance induction in adult animals through mixed chimerism were carried out in mice (6). Recipient mice were lethally irradiated to eliminate all mature T cells and were then reconstituted with T cell-depleted bone marrow from both the recipient and a fully allogeneic donor strain. T cells needed to be depleted from the host bone marrow or else they were capable of eliminating the donor cell inoculum; and T cells needed to be depleted from the donor or else they were capable of inducing graft-versus-host disease. However, when both T cells were completely eliminated, new T cells of both host and donor strains developed in the irradiated thymus, leading to survival of both host and donor bone marrow-derived hematopoietic elements throughout the life of the recipient animal. Skin grafts from the donor strain placed months later were accepted for the life of these animals (6). While these studies demonstrated that induction of mixed chimerism following lethal irradiation would lead to transplantation tolerance, they also illustrated that this methodology would not be clinically applicable, because the side effects of lethal irradiation would be worse than the complications of immunosuppression. Such a protocol would therefore be unacceptable as a means of prolonging organ graft survival. In mice, the major side effect was premature graying and less than normal weight gain, but in large animals and humans, the side effects are much more pronounced and would only be acceptable if the lethal radiation were required for other reasons, such as the treatment of a malignancy.

Subsequent studies were therefore directed toward achieving mixed hematopoietic chimerism without lethal radiation. This goal was accomplished by infusing allogeneic bone marrow after treatment of recipients with a combination of monoclonal antibodies directed toward mature T cells, along with a low dose of irradiation and a boost of irradiation to the thymus. The thymic irradiation was added because we found that although anti-T cell antibodies were capable of eliminating mature T cells in the periphery, mature T cells in the thymus were coated by these antibodies but not eliminated (7). The animals, which became mixed chimeras and remained so for the rest of their lives, accepted subsequent donor skin grafts permanently (7). This "non-myeloablative" preparative regimen was much less toxic than lethal irradiation, and animals who received this regimen but no bone marrow recovered fully, because recipient stem cells were not eliminated. Premature graying was observed only where the thymic irradiation was administered (Fig. 1), leaving the animals otherwise normal. We therefore considered this non-myeloablative regimen to have potential clinical applicability as a way of inducing transplantation tolerance.

We next turned our attention to miniature swine and nonhuman primates as large animal models. Fig. 2 illustrates why we believed that applicability in large animal models was essential before attempting to take this treatment modality to the clinic. As illustrated in this figure, it is much easier to induce tolerance in mice than it is in primates. Indeed, among the methods by which tolerance has been induced in mice, the only one that has so far been successful in primates and in humans has been that of mixed chimerism. We used miniature swine as an intermediate model because of their immunologic and genetic reproducibility, their lower cost and the fact that, unlike mice, essentially all transplantation-related phenomena that have been demonstrated in swine have been able to be translated successfully to primates (8,9).

Fortunately, induction of transplantation tolerance through mixed chimerism has been successful in mice (6), miniature swine (10), monkeys (11) and most recently in the clinic (12). The mechanism by which tolerance has been induced in each of the species is not, however, the same. In mice, where essentially complete elimination of mature T cells is possible, mixed chimerism is permanent and predominantly deletional, since mixed chimeric donor cells reside in the thymus and are capable of negatively selecting anti-donor-reactive T cells throughout the animal's life. In large animals, including humans, such complete T cell depletion is difficult, if not impossible to achieve, especially across MHC barriers. Probably for that reason, the mixed chimerism that has been induced in our successful tolerance protocols has generally been only transient rather than permanent (11,12). Fortunately, at least for the kidney, as long as an organ transplant is performed while a recipient is chimeric, the tolerance persists even after chimerism has disappeared (11). The tolerance in this case becomes predominantly regulatory in nature, maintained by Tregs (13–15), and the presence of the kidney transplant appears to be essential in maintaining this tolerance (16). The kidney may also be responsible for the eventual deletion of donor-reactive T cells in the periphery, as suggested by high-throughput TCR sequencing techniques, which can track these cells (17).

Tolerance across xenogeneic barriers

As important as I believe that tolerance across allogeneic barriers will be, due mainly to its ability to improve the quality of life of transplant recipients, I think that tolerance across xenogeneic barriers will be even more important, because without tolerance, the amount of immunosuppression required will likely cause unacceptable toxicity. Our laboratory has therefore devoted considerable effort toward extending tolerance induction through mixed chimerism to xenografts.

The two approaches to xenotransplantation tolerance that we have explored in detail are: 1) vascularized thymus transplantation; and 2) mixed hematopoietic chimerism. As illustrated in Fig. 3, both of these approaches are based on the principle of inducing tolerance through establishing a mixture of host and donor cells (likely dendritic cells), capable of negative selection, in the thymus. In both cases, all mature T cells are first depleted by an appropriate preparative regimen. In the case of mixed chimerism (Fig. 3A), a bone marrow transplant from the donor leads to a mixture of host and donor dendritic cells entering the thymus and participating in negative selection of newly developing host T cells. In the case of thymus

transplantation (illustrated in Fig. 3B as transplantation of a vascularized "Thymokidney" (18), see below), the host thymus is removed and host bone marrow-derived dendritic cells enter the donor thymus, again allowing negative selection of newly developing T cells reactive to either host or donor.

While both of these approaches have been developed concurrently in our laboratory, the thymus transplantation approach advanced more rapidly, mainly because until recently, we were unable to establish mixed chimerism in the periphery, due to very rapid elimination of porcine donor bone marrow cells after their administration to baboons (19). Transplantation of vascularized thymic tissue, on the other hand, led to markedly prolonged survival of thymokidneys from GalT-KO miniature swine (20). These thymokidneys were prepared by implanting minced pieces of the donor pig's own thymus under its kidney capsule approximately two months before xenotransplantation (18). By the time of transplantation, the thymus tissue had re-organized into an intact, vascularized and functioning thymus under the kidney capsule, so that when the thymokidney transplant was performed, both the kidney and the thymus became vascularized immediately after successful arterial and venous anastomoses. Yamada and colleagues have recently reported survival of a bilaterally nephrectomized baboon, with normal renal function, for over six months after transplantation of a GalT-KO miniature swine thymokidney (21).

As mentioned above, the mixed chimerism approach has lagged behind the vascularized thymic transplant approach because of the difficulty in establishing mixed chimerism across a porcine to baboon barrier. Thus, even after the availability of GalT-KO miniature swine donors, for which natural anti-gal antibodies were no longer a problem, pig bone marrow administered to an appropriately prepared baboon recipient disappeared from the circulation very rapidly after intravenous injection (Fig 4). This result led us to search for other reasons besides natural antibody for the rapid loss of porcine cells following administration to primates. One likely candidate which we investigated is clearance of pig bone marrow cells through the CD47-SIRP α pathway (22,23). CD47 is a molecule expressed on bone marrow-derived cells that serves as a senescence marker within each species. It interacts with the SIRP α molecule on macrophages of the reticuloendothelial system to inhibit phagocytosis. Thus, when CD47 expression is lost through aging, cells are engulfed and removed from the circulation, a normal process to assure replacement of senescent cells with their newly derived counterparts. However, the CD47-SIRP α pathway is species-specific, so that even normal pig bone marrow-derived cells are phagocytized by primate macrophages.

We hypothesized that clearance of pig bone marrow-derived cells from the baboon circulation might be inhibited if primate CD47 were expressed on those cells. We therefore utilized nuclear transfer technology to produce a genetically engineered GalT-KO miniature swine transgenic for human CD47 (hCD47) and tested the ability of its bone marrow-derived cells to survive after administration to appropriately prepared baboons. For the first time, we were able to detect surviving porcine hematopoietic cells in the circulation of baboons for days rather than minutes (24). During the cloning procedure to produce this hCD47-expressing transgenic animal, a second cloned animal was produced that, despite having the same genetic makeup, did not express appreciable levels of hCD47 (Fig. 5). Intravenous administration of bone marrow-derived cells from this non-expressing animal into baboons

did not lead to detectable prolongation of survival of the porcine cells, thus confirming the role of hCD47 expression in preventing loss of porcine cells in baboons. Furthermore, the survival of skin grafts from the donor pig onto the baboons receiving hCD47-expressing cells were markedly prolonged (>50 days) compared to survival of skin grafts onto control baboons receiving either the non-expressing pig cells or no pig cells at all (<14 days) (24). Additional studies have demonstrated even greater prolongation of hCD47-expressing porcine bone marrow-derived cells in baboons following intra-bone injection of a portion of the inoculum ((25) and Yamada, et al, unpublished data).

Summary and Conclusions

1. Mixed chimerism is the only methodology that has been successful to date in inducing transplantation tolerance for allografts in man.
2. Mixed chimerism is more difficult to achieve for xenografts than for allografts.
3. When achieved, mixed chimerism has led to marked, specific prolongation of pig-to-baboon xenograft skin survival.
4. A combination of thymic and mixed chimerism approaches may be an effective means for achieving xenograft organ transplant tolerance and deserves further investigation.

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Fig. 1.
Mixed chimeric mouse prepared by non-myeloablative preparative regimen

Induction of Transplantation Tolerance across MHC Barriers

Method	Mice	Primates
Enhancement	+	-
DST	+	-
Peptides	+	-
Anti-MHC mAbs	+	-
Cyclosporin	+	-
ALS	+	-
Anti-CD4	+	-
Anti-CD25	+	-
TLI	+	-
Anti-CD3 toxin	+	-
Co-stimulatory blockade	+	-
Mixed Chimerism	+	+

Fig. 2.

Methods by which induction of tolerance has been attempted in mice and primates (+ = successful)

Two Approaches to Induction of Tolerance

Fig. 3.
Induction of tolerance through mixed chimerism (A) and vascularized thymic transplantation (B)

Tolerance through Mixed Chimerism

Why have attempts to induce pig->primate mixed chimerism failed?

Fig. 4.

Attempts to induce pig-to-baboon chimerism using GalT-KO bone marrow

hCD47 Expression in 18286 vs. 18289

Fig. 5.
FACS analysis of hCD47 expression in two cloned hCD47 transgenic GalT-KO swine vs. a wild-type GalT-KO swine