Transplantation Tolerance

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Summary: Tolerance following organ transplantation was first described in experimental models over 50 years ago. Reports of tolerance in clinical transplantation have appeared in the literature sporadically for decades. Despite this long-standing fascination with transplantation tolerance, the ability to reproducibly induce tolerance in humans undergoing organ transplantation has remained elusive. Recent advances in our knowledge of the mechanisms that contribute to the induction and maintenance of tolerance as well as those factors that oppose tolerance may allow the design of clinical trials aimed at introducing tolerance-inducing strategies into clinical transplantation.

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Although the work of work of Billingham et al[1] often is cited as providing the conceptual basis for tolerance, it should be noted that these experiments arose in large part from the earlier work of Owen,[2] who showed that the exchange of blood cells that occurred in utero in dizygotic cattle twins resulted in a state of persistent hematopoietic chimerism in each twin. Although Medawar et al first proposed using skin grafting as a means of distinguishing between monozygotic and dizygotic cattle twins, their repeated observation that dizygotic freemartin cattle accepted skin grafts from their dizygotic twin caused them to reformulate their hypothesis to postulate that the exchange of fetal blood in utero would promote tolerance to transplanted tissues in adult cattle. This hypothesis was directly tested in their seminal experiments in mice.

Briefly, a crude cell/tissue mixture from an allogeneic adult mouse was injected into 6 fetuses borne by a CBA female. Five healthy pups were born and 8 weeks later each underwent skin grafting with skin from the same allogeneic mouse strain used for the cell inoculation. Two of the 5 skin allografts were destroyed promptly (likely acutely rejected), 1 underwent a prolonged involution (likely chronic rejection), however, the final 2 allografts appeared perfectly healthy for 77 and 101 days. At this time, these 2 mice were challenged by implanting fragments of lymph nodes from mice immunized with donor antigen. This lead to the acute rejection of the 2 long-term surviving skin allografts. Attempts to reproduce this effect by inoculating neonatal mice with various tissues from mice of different strains were largely unsuccessful, with only 9 of 96 mice experiencing prolonged skin graft survival. Perhaps because of an incomplete appreciation of the details of these experiments, many in the field of transplantation took these results to indicate that a brief intervention before transplantation fundamentally could reset the immune system to promote the routine and nearly indefinite survival of organ allografts. However, as pointed out by Billingham et al,[1] the effect of this treatment was a continuum, with prolonged graft survivals ranging from a few days to indefinitely. Second, the regimen was largely ineffective in neonatal and adult mice and was consistently overcome by memory cells resulting from previous exposure to donor antigens.

The first functionally tolerant human transplant recipients were reported in 1975.[3] The term *functional tolerance* is used to distinguish the persistence of normal allograft function in

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the absence of immunosuppression in human beings from the more rigorous definition of *tolerance* used in experimental transplantation. In this series 6 patients who had been off immunosuppression for an average of 27 months were reported. Interestingly, only 2 rejections were noted. Owens et al went so far as to propose that in the absence of rejection, serious consideration should be given to not resuming immunosuppression. Similar to a subsequent report,[4] rejection, when it did occur, often was delayed for weeks or months. This group observed that the successful cessation of immunosuppression was more likely in the setting of human leukocyte antigen identical transplantation as identified by serotyping and mixed lymphocyte culture; the first use of an immunologic assay to predict tolerance.

Perhaps in response to the rarity of spontaneous tolerance and the limited effectiveness of available immunosuppressive agents and their toxicities, investigators began to explore strategies aimed at inducing tolerance in adults after transplantation. As early as 1955 Main and Prehn[5] created a state of full hematopoietic chimerism by injecting bone marrow into lethally irradiated allogeneic mice. To avoid some of the defects in protective immunity associated with the state of full hematopoietic chimerism, Ildstad and Sachs[6] devised an experimental strategy that resulted in a state of mixed hematopoietic chimerism in which both donor and recipient hematopoietic cells persisted long term. These mice displayed tolerance to donor-strain skin grafts. However, concerns about the toxicity associated with the conditioning regimens necessary to attain a state of stable hematopoietic chimerism has limited the enthusiasm for the routine clinical application of these approaches.

The next major advance toward achieving tolerance was based on the observation that signals resulting from engagement of the T-cell receptor alone were insufficient to promote complete activation of T lymphocytes. The additional or costimulatory signals necessary for full activation subsequently were shown to result from the engagement of receptors on T cells by their ligands, expressed largely by professional antigen-presenting cells. Two of the most widely studied costimulatory pathways and among the first described were the CD28/B7 (CD80 and CD86) and CD154/CD40 pathways. For a recent in-depth review of the ever-expanding known costimulatory pathways see Clarkson and Sayegh.[7] In 1992 it was reported that as a single intervention the short-term blockade of the CD28/B7 costimulatory pathway using cytolytic T lymphocyte-associated antigen immunoglobulin (CTLA4Ig) prolonged the survival of transplanted xenogeneic islets in mice and allogeneic hearts in rats.[8,9] Subsequently, it was reported that although blockade of either the CD28/B7 or CD154/CD40 pathways alone prolonged survival of heart allografts in mice, brief treatment with a combination of agents that blocked these 2 pathways prevented acute rejection and resulted in long-term allograft survival.[10] Although allografts from treated mice subsequently were shown to develop progressive damage suggestive of a chronic immunologic injury, combined costimulation blockade was viewed as the most immediate and feasible approach toward attaining tolerance to transplanted organs clinically.

TRANSLATION TO TRANSPLANTATION TOLERANCE IN HUMAN BEINGS: NONHUMAN PRIMATE STUDIES

Increasingly when possible the development of new therapeutic strategies in human beings is predicated on safety and efficacy data in preclinical models. Consequently, over the past decade many strategies aimed at inducing tolerance to transplanted organs and tissues have been investigated using nonhuman primate (NHP) models. Despite the promise and initial optimism surrounding numerous methods of inducing tolerance in rodent transplant models, subsequent experience has shown the sobering reality of how difficult it is to routinely induce tolerance in preclinical models and in clinical transplantation. Four major strategies for inducing transplantation tolerance have been investigated in-depth in NHP models including (1) blockade of costimulatory pathways, (2) bone marrow infusion/mixed chimerism, (3) pro-