Published in final edited form as:
Transplant Proc. 2009 March ; 41(2): 463–465. doi:10.1016/j.transproceed.2009.01.027.

Composite Tissue Allotransplantation: Past, Present and Future—The History and Expanding Applications of CTA as a New Frontier in Transplantation

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Abstract

Composite tissue allotransplantation (CTA) transplantation is currently being performed with increasing frequency in the clinic. The feasibility of the procedure has been confirmed in over 40 successful hand transplants, 3 facial reconstructions, and vascularized knee, esophageal, abdominal wall, and tracheal allografts. The toxicity of chronic, nonspecific immunosuppression remains a major limitation to the widespread availability of CTA and is associated with opportunistic infections, nephrotoxicity, end-organ damage, and an increased rate of malignancy. Methods to reduce or eliminate the requirement for immunosuppression would represent a significant step forward in the field. Mixed chimerism induces tolerance to solid organ and tissue allografts, including CTA. This overview focuses on the history and expanding applications of CTA as a new frontier in transplantation, and considers the important hurdles that must be overcome through research to allow widespread clinical application.

Composite tissue allotransplantation (CTA) transplantation involves the transplantation of various complex tissues, including integumentary/musculoskeletal, nerves, bone, tendon, and vascular tissues. Each year an estimated 7 million people in the United States could benefit from composite tissue reconstruction because of surgical excision of tumors, accidents and congenital malformations.[1] This review focuses on immunologic features relevant to CTA. To date, over 40 hand transplants have been performed and other anatomic parts such as face, larynx, and abdominal wall have been transplanted.[1] Seven of these are >8 years posttransplant and few graft failures have been reported.

RISKS ASSOCIATED WITH THE IMMUNOSUPPRESSIVE DRUGS IN CTA

Despite these promising outcomes, debate continues over the risks of immunosuppression. Immunosuppressive drugs broadly suppress the immune system, and their use is associated with an increased risk of neoplasms, opportunistic infections, and end-organ toxicity. The main challenge facing CTA is minimization of immunosuppression with the goal of donor-

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COI Statement: S. Ildstad has significant equity interest in Regenerex, LLC, a start-up biotech company based on the facilitating cell technology.

specific tolerance. Tolerance is defined as a state of donor-specific hyporesponsiveness in the recipient in the absence of immunosuppression.

ESTABLISHING TOLERANCE BY CHIMERISM

One of the best-studied approaches for establishing tolerance is hematopoietic stem cell (HSC) chimerism. In chimerism, tissues from two genetically distinct organisms co-exist in one organism. Two types of chimerism have been described: macrochimerism and microchimerism.[2] Macrochimerism usually occurs when bone marrow (BM) is transplanted in a conditioned recipient. The donor pluripotent HSC engrafts in the recipient and produces all its lineages, including the donor immune system. A new hybrid immune system is established in the recipient and reciprocal bidirectional donor:host tolerance results. As low as 1% donor chimerism is sufficient to induce robust tolerance to donor-specific organs, cells, and tissues.[3]

Microchimerism arises as a result of migration of passenger leukocytes from a transplanted allograft into an unconditioned recipient. Passenger leukocytes from the transplanted allograft interact with the recipient leukocytes and are hypothesized to lead to clonal exhaustion, resulting in donor-specific tolerance.[4] In microchimerism, donor pluripotent hematopoietic stem cells do not engraft, but alternatively hematopoietic-derived cells from the donor organ are produced and migrate systemically. Consequently, not all stem-cell-derived lineages are produced and very low levels of donor cells are found in the recipient's blood. Microchimerism has been demonstrated in liver and kidney transplant recipients. Starzl and Zinkernagel make a strong argument that microchimerism is essential for the maintenance of clonal exhaustion-deletion that is induced by the initial flood of passenger leukocytes during the first few weeks after transplantation. This may explain how some transplant recipients require significantly reduced levels or no immunosuppression over time.[5]

CHIMERISM CAN BE ACHIEVED THROUGH VASCULARIZED BONE MARROW TRANSPLANTATION (BMT)

Because hematopoietic tissue accompanies the bone in hand allografts, immunologists hypothesized that hand allografts may induce chimerism without conditioning. The donor bone marrow is transplanted within its own stromal microenvironment, and may provide a continuous supply of donor-derived hematolymphopoietic cells. Experimental studies in the rat model have confirmed that systemic immune reconstitution is significantly accelerated with a vascularized BMT compared with cellular BM transplantation of comparable cell numbers in F1 hybrid → parent transplants.[6] The limitation of this study is that success was achieved only if the two strains of rat were immunologically weak. Successful translation of these findings to more robust transplant models has not yet been achieved. In the clinical setting, Granger et al[7] showed transient peripheral microchimerism in hand transplantation.

MIXED CHIMERISM

In mixed chimerism, the donor and recipient hematopoietic systems co-exist.[3] Mixed chimerism is associated with donor-specific transplantation tolerance in vivo and in vitro[3] and has been shown to effectively induce donor-specific tolerance to a variety of allografts. In humans, BMT induced mixed chimerism has been shown to confer acceptance of donor-specific skin[8] and kidney allografts[9] without long-term immunosuppression. An additional advantage is that mixed chimerism prevents chronic rejection,[10] the major cause of late graft loss. Mixed chimerism has several advantages over fully allogeneic chimerism: (1) it is associated with a lower incidence and severity of graft-versus-host disease (GVHD); (2) it

retains immunocompetence for primary immune responses[11]; and (3) it can be induced through nonmyeloablative conditioning. Therefore, mixed chimerism represents a superior approach for the induction of durable transplantation tolerance.

HURDLES IN THE ESTABLISHMENT OF CHIMERISM

Despite the promising potential of mixed allogeneic chimerism, important considerations which must be addressed are (1) the risk of GVHD; (2) toxicity of the conditioning; and (3) the feasibility of simultaneous bone marrow and CTA transplantation. Cell-based therapies are under development to promote CTA and marrow acceptance.

FACILITATING CELLS (FC)

Ildstad et al[12] were the first to characterize graft FC as $CD8^+/TCR^-$. They utilized rare-event cell sorting to phenotypically and functionally define precisely which cell facilitates engraftment of purified allogeneic bone marrow stem cells in an MHC-specific fashion without causing GVHD. The FC population makes up only 0.4% of the total BM and comprises less than 1.6% of the total lymphoid gate. Recently, FC have been shown to exist in human marrow using a NOD/SCID immunodeficient mouse transplant model. Mechanistically, the FC induce tolerogenic regulatory T cells in vitro[13] and in vivo (manuscript submitted to Blood, 2009). As such, they may provide a novel approach to induce tolerance to CTA.

TOLEROGENIC REGULATORY T CELLS (T_{reg})

Another distinct subset of T cells that play an important role in peripheral regulation of GVHD are natural T_{reg}. The best studied are $CD4^+$ T cells arising during T cell development in the thymus. They constitutively express CD25, and constitute 5% to 10% of peripheral $CD4^+$ T cells in healthy mice and humans. The $CD4^+/CD25^{high}$ T_{reg} are best recognized by expression of the transcriptional regulator FoxP3, which serves as a master switch gene for T_{reg} development and function.[14] Hirahara et al[15] recently found that virtually all peripheral blood $CD4^+/CD25^{high}$ $Foxp3^+$ T_{reg} expressed high levels of the chemokine receptor CCR4, 80% expressed cutaneous lymphocyte Ag (CLA), and 73% expressed CCR6 homing receptors for the cutaneous immune system. Recent reports from the hand transplant experience have shown that $Foxp3^+$ T_{reg} infiltrate the skin of hand transplant recipients.[16] These findings may explain why rejection has not been a major limitation for this highly antigenic tissue burden.

A significant milestone in the potential clinical application of mixed chimerism to induce tolerance in CTA recipients occurred when mixed chimerism was successfully established in humans. Conditioning of recipients with 200 cGy TBI in combination with fludarabine and immunosuppression allows chimerism to be established in those too elderly or frail to tolerate ablative BMT for hematologic malignancies.[17] This nonmyeloablative conditioning approach is now widely used in treatment of a number of nonmalignant disorders as well. As a result, the morbidity and mortality associated with BMT has been substantially reduced.

FUTURE OUTLOOK

The tolerance induced by mixed chimerism has the potential to overcome the barriers which limit the application of CTA transplantation in clinical practice. The three major obstacles to the use of BM to induce tolerance to CTA are gradually being addressed through research efforts. It may be possible in the near future to engineer the composition of the donor BM in such a way that BM engrafts easily in the recipient without causing GVHD. FC and T_{reg} may achieve BM engraftment reliably without GVHD. Newer regimens of inducing mixed

chimerism, which further reduce or avoid recipient irradiation, need to be developed. A safe method of inducing tolerance through mixed chimerism will reverse the risk-vs-benefit ratio of immunosuppressive drugs and allow CTA transplantation to become a standard treatment for reconstructing large tissue defects. The ultimate goal of CTA transplantation for reconstruction is to be functional and life-enhancing. We believe that a safe protocol of inducing mixed chimerism based on bone marrow transplantation may minimize or eliminate the need for immunosuppressants and allow routine use of the CTA transplantation in clinical practice to achieve this aim.

Acknowledgments

Supported in part by NIH R01 DK069766 and NIH 5RO1 HL063442; JDRF 1-2005-1037 and JDRF 1-2006-1466; The Department of the Navy, Office of Naval Research; The Department of the Army, Office of Army Research (Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Office of Army Research. This publication was made possible by Award No. W81XWH-07-1-0185 from the Office of Army Research); the Commonwealth of Kentucky Research Challenge Trust Fund; the W. M. Keck Foundation; and The Jewish Hospital Foundation.

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