Composite Tissue Allotransplantation

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ABSTRACT Composite tissue allotransplantation (CTA) recently took its first steps in the clinical arena in 1998 with the successful hand transplant performed in Lyons, France. That single operation represented a culmination of many years of laboratory research in multiple fields involving integumentary/musculoskeletal transplantation. Here we review the prerequisite developments in the field of immunology, microsurgery, and pharmacotherapy that helped bring CTA to clinical reality. This new field still has many unanswered questions which are addressed below. Additionally, new evolving research in CTA is also discussed.

KEYWORDS composite tissue allotransplantation, graft-vs-host disease, hand transplant, tolerance, vascularized bone marrow

Composite tissue allotransplantation (CTA) involves the transplantation of various tissues including integumentary/musculoskeletal, nerves, and vascular tissues, as opposed to a single separate organ in conventional solid organ transplantation (SOT). An example of CTA is limb transplantation, in which the transplanted graft includes skin, muscle, nerve, blood vessels, and bone. In SOT, such as a liver or kidney, allograft function is defined by the biochemical and physiologic properties of that particular organ. On the other hand, the function and the immunologic properties of the composite tissue transplant are more difficult to define, because each individual component tissue possesses its own unique characteristics that ultimately affect the successful outcome of the transplant.

The indications for solid organ transplantation are straightforward and little controversy exists as to whether or not whole-organ transplants should be performed. They are designed to restore the physiologic function of the particular organ, preserve life, and improve the quality of life. In contrast, most applications of CTA predominantly improve the quality of life for non-life-threatening conditions and aim to restore anatomic, cosmetic, and functional integrity. The benefits gathered by such procedures have to be balanced against the morbidity
of the surgical procedure itself and long-term immunosuppression therapy.

**HISTORICAL PERSPECTIVE**

The concept of limb transplantation dates back as far as the 4th century A.D. The legend of twin saints Cosmas and Damien described the restoration of an extremity by miraculous transplantation from a cadaver. This legend, well known in the transplant community, has represented the profound desire of humankind to achieve successful transplantation with full graft tolerance. Scientific research on transplantation began in the early 1870s with several reports of allogeneic skin graft attempts that met with variable successes [1]. Decades later in 1918, Masson first introduced the importance of donor–recipient compatibility [2]. However, this concept remained controversial until about 1930, when it became generally accepted that allogeneic skin transplants predictably rejected as opposed to syngeneic grafts. In 1944, motivated by the needs of burned pilots of the Royal Air Force, Peter Medawar performed a series of skin allograft experiments using a rabbit model with the collaboration of Thomas Gibson, a plastic surgeon [3, 4]. These works, and others, represented the groundbreakings in transplant immunology that set the stage for subsequent developments.

With advancements in vascular and microsurgical techniques as mentioned by Murray for various organ transplants [5], composite transplant models began appearing in the literature in the 1960s. One of the models first introduced by Schwind [6], the rat hindlimb allograft served as a popular model for many researchers in CTA. Others models subsequently developed included the canine hindlimb [7], hemimandibular graft in the rabbit and in the monkey [8, 9], laryngeal transplant in the rat [10], and hand allografts in the pig and primate [11, 12]. Clearly, technical hurdles have become manageable with the aid of microsurgical techniques and instrumentation.

The immunologic barriers presented a formidable challenge to CTA. In an attempt to understand and manipulate the dynamics of the immune system, researchers studied different methods of tolerance induction. In 1976, Poole et al. pioneered using immunologic enhancement prior to performing orthotopic limb transplants in rats [13]. Using recipient-derived antidonor antiserum, prolonged allograft survival was achieved by preoperative passive immunization. Whole blood administration was also attempted as a way of inducing transplant tolerance, though without benefit in rat hindlimb transplantation [14]. Donor irradiation also prolonged the survival of vascularized limb tissue allografts, thought the grafts ultimately rejected [15]. So far, no means of tolerance induction have been clinically practical.

An alternative route to prevent graft rejection is via immunosuppression with systemic medication. Limb allografts present an array of antigenic tissues that theoretically may require significantly higher doses of immunosuppression for graft survival [5, 16]. In 1979, Doi achieved long-term graft survival in two inbred strains of rats that had a strong antigenic mismatch at the major histocompatibility complex by using azathioprine and prednisolone [17]. However, all transplanted limbs ultimately rejected. The cyclosporine A (CsA) era began in the late 1970s when its immunosuppressive characteristics were discovered by Borel [18], and facilitated huge advances in SOT and CTA. CsA suppresses the immune response by inhibiting signal transduction pathways of calcineurin, a serine/threonine phosphatase. Inhibition of the target molecule in the cytosol completely blocks the translocation of nuclear factor of activated T cells (NF-AT), resulting in a failure to activate the genes regulated by the NF-AT transcription factor. These genes include those required for B-cell stimulation (IL-4) and CD40 ligand as well as those necessary for T-cell proliferation (IL-2) [19]. Black et al. successfully performed the first hindlimb allotransplants in rats across a strong antigenic mismatch using only CsA for immunosuppression in 1982 [20]. Their initial study prolonged allograft survival compared to the control group (18 days) to an average of 101 days, and a subsequent study demonstrated an even longer survival of up to 225 days [21]. With the discovery of new immunosuppressants and their wide clinical application in SOT, these drugs brought the reality of clinical CTA even closer.
IMMUNOSUPPRESSION IN COMPOSITE TISSUE ALLOTRANSPLANTATION

Although the technical feasibility of CTA was demonstrated over 20 years ago, clinical application was delayed due to the side effects of the immunosuppression regimens and the lack of a good animal model comparable with human physiology. Through the simultaneous developments of new and more effective drugs and the preclinical CTA animal trials, human hand transplantation was finally realized.

Glucocorticoids have served as a mainstay in immunosuppressive regimens, though they are unsuitable for use as solo agents due to increased side effects. Early successes of CsA in preventing CTA rejection generated optimism among researchers. Using the rat hindlimb CTA model, Furnas et al. used long-term CsA therapy postoperatively (8 mg/kg/day for the first 20 days followed by a maintenance dose of only 8 mg/kg twice a week) and reported one out of five recipients surviving without signs of rejection for more than 400 days [22]. Three of the remaining four rats survived 66–238 days postoperatively. In the same rat model, Hewitt et al. constructed a dose-response curve for CsA using low-dose CsA (4 mg/kg/day) for 20 days and observed purposeful withdrawal to painful stimulus of the transplanted limb up to 65 days posttransplant. At 8 mg/kg/day for 20 days, grafts survived an average of 74 days [23]. In a follow-up study, this group demonstrated that composite tissue allografts in the rat could achieve indefinite survival with low to moderate amounts of CsA (8 mg/kg/day subcutaneously for 20 days followed by 8 mg/kg/day orally). With that regimen the animals were able to maintain the allograft until the time of necropsy [24].

In primate studies, however, very high doses of CsA were necessary to control CTA rejection. Hovius et al. performed partial limb allotransplants in rhesus monkeys that received steroids and CsA at 25 mg/kg/day [25]. In spite of this therapy, 10 of 12 monkeys developed graft rejection. Other investigators produced similar results, and it was thought that clinical CTA may be prohibitive based on the toxic effects of immunosuppression in the primate models.

The development of mycophenolate mofetil (MMF), tacrolimus (FK506), sirolimus, and antibody immunosuppression continued to fuel the successful pursuit of human CTA. MMF is an antimitabolite that has largely replaced azathioprine. It is an inhibitor of de novo purine synthesis and preferentially inhibits both T- and B-cell proliferation. It has been used to reverse renal allograft rejection, and several experiments have indicated that it helps to prevent long-term rejection of all of the components of composite tissue allografts, including the skin [26]. Benhaim demonstrated that the combination of CsA and MMF allowed a lowered CsA dose and was superior to using each drug separately [26, 27].

Like CsA, FK506 also inhibits calcineurin via the FK binding protein [28]. Using FK506 as the primary therapy, Buttemayer et al. showed rejection-free survival up to 300 days postoperatively in rat hind limb allografts across a major antigenic mismatch [29]. In this experiment the animals received a 2-mg/kg/d dose for 14 days followed by 2 mg/kg twice a week. However, long-term animals did show mild signs of rejection. Complications of the immunosuppression were frequent, and bacterial pneumonia ultimately caused the death of all long-term animals.

Sirolimus is a TOR (target of rapamycin) inhibitor that also mediates IL-2 postreceptor signaling, though its immunosuppressive effects differs from CsA and FK506. It has been shown to be highly synergistic with the latter compounds, enabling reductions in toxicity in renal transplant patients [30]. Both polyclonal and monoclonal antibodies (e.g., Orthoclone OKT3) have been used as rescue drugs in acute rejection for SOT [31]. The toxicities of increase viral infections, cytokine release syndrome, and potentiation of lymphoproliferative disease limit their use on selected cases, but they are still important tools in the armamentarium. Though not currently used in CTA, these compounds’ demonstrated efficacies in preserving SOT grafts will certainly lend themselves to use in the future for CTA.

In investigating efficacies of these agents, it is important to select an appropriate animal model.
Among the multiple CTA animal models, the swine demonstrated an immune response to pharmacotherapy most similar to the human [32]. Based on the swine model, the combination drug regimen for the first U.S. hand transplant recipient consisted of prednisone, tacrolimus, and topical agents [33].

FUNCTIONAL RECOVERY

Composite tissue allotransplantation cannot be considered successful unless it results in significant functional recovery of the allograft. A limb with suboptimal motor and sensory function may lead to more harm than good, as seen in patients with diabetic neuropathies. Thus far in human hand transplants, motor and sensory recovery has not been complete but allows good function of the allografts. Measurements of progress included range of motion, grip and pinch strength, Tinel sign, and the Semmes-Weinstein and Carroll tests [34]. The latter test incorporates mobility and motor and sensory function in the functional performance to reflect the ability to perform activities of daily living. The Louisville hand transplant recipient scored 52/99 on the Carroll test at 12 months, while the Guangzhou hand recipients demonstrated 65/99 and 75/99 functional recovery at 7 months [34]. In other words, starting from a missing limb, the recipients are now able to feed, brush, write, and tie their shoes. To maximize functional recovery, the need for prolonged rehabilitation and physical therapy cannot be understated, requiring the dedication of both the medical team and the patient.

Intrinsic in functional recovery is the regeneration of axonal innervations. Mackinnon et al. performed nerve allografts in rats and monkeys using CsA treatment and demonstrated restoration of muscle function in both groups [35]. When immunosuppression was discontinued, donor Schwann cells were rejected but neural function persisted. This finding was thought to be due to the host’s own axonal regeneration, which in time replaced donor axons that served as initial conduits. Thus, only limited immunosuppression may be required for nerve allografting to bridge the interim of host axonal regeneration. This approach came to clinical fruition when Mackinnon performed a successful long nerve allograft in a 12-year-old boy with severe posterior tibial nerve injury [36]. In the context of CTA, this suggests that the nervous tissue may not be the component that requires long-term immunosuppression.

GRAFT-VERSUS-HOST DISEASE AND THE VASCULARIZED BONE MARROW TRANSPLANT

A unique component of CTA is the transfer of immunocompetent donor cells via the bone marrow and lymph nodes. This raises the theoretical problem of graft-versus-host disease (GVHD) where the donor immune cells reject the host. Yazdi et al. used a parental to F1-hybrid rat hindlimb transplant model across a semi-allogeneic barrier to study possible GVHD without immunosuppression [37]. All animals developed significant lymphoid chimerism over time. Thirty-seven and one-half percent of the rats developed a wasting syndrome consistent with GVHD and showed high levels of chimerism (60.2%). The remaining fraction of animals did not develop GVHD, became immunologically tolerant, and exhibited stable low levels of chimerism (18.3%).

The role of chimerism and GVHD in human CTA remains unknown. In the case of clinical hand transplantation, one graft was irradiated in order to reduce this potential problem. Thus far, no recipients have developed GVHD, nor demonstrated chimerism by flow cytometry [34]. The hypothesis is that a hand graft contains only small amounts of functionally active donor marrow, and therefore will not significantly affect a human recipient.

In order to study the immune contribution in a CTA to the host, Suzuki et al. developed an isolated vascularized bone-marrow transplant (VBMT) model [38]. Bone-marrow cells are engrafted along with their own stromal environment on a vascular pedicle, without muscle and tendon attachments as in the CTA. This model is unique from cellular bone-marrow transplantation (CBMT) in several ways. In CBMT, immunoablation is required to destroy malignant cells and to create a physical space
for the donor stem cells to seed and proliferate. In the case of VBMT, the cells are delivered within their stromal microenvironment, allowing for immediate engraftment. As part of a CTA model, it has been demonstrated that systemic immune reconstitution is drastically accelerated with a VBMT compared to CBMT of a comparable cell volume [39]. VBMTs have produced stable mixed T-cell chimerism in semi-allogeneic and allogeneic rats [40, 41] and may be responsible for the decreased incidence of GVHD and induction of tolerance in these animal models.

The isolated VBMT have several potential uses in the future. As previously mentioned, it may serve as an adjunct to conventional bone-marrow transplants by accelerating immune reconstitution in the recipient. Also, by potentially inducing tolerance via the process of a stable chimerism, VBMT may be used in conjunction with other solid organ transplants to ensure their survival. Further studies are needed to understand immune interactions with the host and mechanisms of tolerance induction for VBMT.

**COMPOSITE TISSUE ALLOTRANSPLANTATION ENTERS THE CLINICAL ARENA**

With the establishment of suitable surgical models and the development of novel immunosuppressive therapies, clinical experience with CTA took its first steps in the late 1990s. In November 1997, the International Symposium on Composite Tissue Transplantation was held in Louisville, KY, to discuss the main topic of possible human hand allotransplantation. The symposium concluded that with available medical regimens and surgical techniques, it was now appropriate to consider undertaking the procedure [42]. One year later, the first human hand transplant was performed in Lyons, France [43]. A team of surgeons transplanted the right hand and a distal forearm from a brain-dead donor to a 48-year-old man. The recipient initially received anti-thymocyte globulin (75 mg/day for 10 days), tacrolimus (to achieve blood levels of 10–15 ng/ml for the first month), mycophenolic acid (2 g/day), and prednisone (250 mg on day 1, then tapered to 20 mg/day). He was then maintained on tacrolimus (5–10 ng/ml), mycophenolic acid (2 g/day), and prednisone (15 mg/day at 6 months). The patient experienced mild rejection that coincided with decreased serum levels of tacrolimus, which clinically reversed with increased dosage. Six months after the transplant, the patient was reported to have “satisfactory” motor and sensory function [43].

Surgeons at the University of Louisville successfully duplicated this operation and performed the first U.S. hand transplant in January 1999. The patient was a 37-year-old man who lost his left hand in 1985. This patient also showed good functional recovery, and at 6-month follow-up demonstrated a positive Tinel sign at the level of the fingertips; by 11 months he reported pressure and temperature sensation in the fingertips. There was evidence of muscle innervation at 1 year. Overall the patient reported satisfactory function of the hand, although finger flexion and extension were incomplete. Similar to the initial recipient, this patient also experienced several episodes of moderate acute cellular rejection of the skin graft, which reversed with intravenous methylprednisolone combined with topical tacrolimus and clobetasol [44]. Since this time, the French team has performed a successful bilateral upper extremity transplant in January 2000. This patient is reportedly doing well. Other recent clinical endeavors involving CTA included a laryngeal allograft [45], three vascularized femoral diaphysis transplants [46], and four vascularized knee-joint allotransplants [47].

**TOLERANCE INDUCTION IN THE FUTURE**

Since CTA is an elective procedure, controversy revolves around the risks versus benefits, especially of the required immunosuppression. Although the development of pharmacological agents greatly contributed to the current success of CTA, the ultimate goal is to achieve tolerance across antigenic barriers without the need for immunosuppressive agents. Ideally, the recipient would gain donor specific tolerance to the graft, but otherwise remain totally
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immunocompetent. The mechanism of tolerance induction is not well understood at this point, and current research has focused on three main strategies to induce donor-specific tolerance in the recipient: genetic matching, costimulatory blockade, and development of mixed chimerism. Lee et al. have demonstrated long-term survival of musculoskeletal allografts from MHC-matched (major histocompatibility), minor antigen mismatched donors using only a 12-day course of CsA [48]. This model, however, did not contain a cutaneous component. In the clinical setting, MHC matching among unrelated individuals would be rare, making this approach unlikely.

Costimulatory blockade focuses on the T-cell requirement of multiple signals prior to antigen-stimulated proliferation. Several of these receptors and ligands have been defined, including CD28 and B7, and CD40 and CD154 molecules [49, 50]. By blocking the secondary signals using monoclonal antibodies, a long-lasting immune downregulation occurs with specificity to the bound antigen. In the laboratory, Elster et al. have shown prolonged allogeneic skin graft acceptance in primates utilizing anti-CD154 monoclonal antibodies [51]. This holds much promise in the context of CTA, as skin generally has been the most difficult component to manage with respect to rejection. Anti-CD154 has not yet been experimented in a CTA model, and its potential toxicity of inducing hypercoagulability [52] remains to be clarified.

Donor-specific tolerance remains the Holy Grail in transplantology. In the laboratory, stable chimeras have been created in rodents, large animals, and primates [53–55]. These protocols require a course of recipient preconditioning followed by immune reconstitution using T-cell-depleted bone-marrow grafts. Once a stable chimera has been created, limb and organ transplantations were possible without prolonged immunosuppression. However, these conditioning regimens are not clinically practical due to possible host toxicity of ablative conditioning, graft failure, GVHD, and the requirement of a waiting period for chimerism to develop prior to transplantation. Active research is ongoing in these areas to bring tolerance induction closer to clinical implementation.

OTHER APPLICATIONS OF CTA

The successful human hand transplants irrevocably marked the clinical reality of CTA. However, hand and limb transplants represent only a fraction of the clinical potential of CTA. In 1995, Anthony et al. first successfully studied heterotopic laryngeal transplants in dogs [56]. The carotid artery and external jugular veins served as the unilateral blood supply to the transplanted larynx, and the superior and recurrent laryngeal nerves supplied the innervation via microsurgical anastomoses. The animals received CsA, methylprednisolone, and mycophenolic acid. Laryngeal function and nerve conduction were evaluated by fiberoptic laryngoscopy and electromyographic studies. These animals were shown to remain rejection free for over 100 days postoperatively and none of the animals needed a tracheostomy [56]. In 1998, Birchall reported the first successful laryngeal transplant in a patient who suffered irreversible damage to his larynx in a motorcycle accident [45]. The revascularized allograft consisted of the larynx, thyroid, parathyroids, three tracheal rings, and 70% of the pharynx, and allowed the patient to speak for the first time in 19 years.

Many potential applications of CTA lie in the field of reconstructive and plastic surgery. Current methods of microvascular reconstruction for large tissue defects fall short in recreating normal sensation, function, and appearance. For example, mandibular reconstruction, performed using a variety of osteocutaneous flaps, such as radial forearm, fibula, and scapula, provides immediate coverage of the anatomic defect [57–59]. However, they may require prosthetic implants, fail to replace the muscles necessary for mastication, and often are insensitive. Though an aesthetic improvement, these reconstructions are not ideal, lacking the specialized motor and sensory functions of the face. These cases may be better served by CTA. Successful surgical models have already been developed, including CTA of the mandible in rabbits and monkeys [8, 9], and a hemimaxillary nose module and ear-calvarium units in the rabbit [60, 61]. In orthopedics, whole joint allografts are technically feasible and have been performed clinically in humans [62], though the problem of
lifelong immunosuppression and its associated risks remain.

THE FUTURE OF CTA AND THE RECONSTRUCTIVE TRANSPLANT SURGEON

As with any developing field, new technology requires new expertise and organization to move the advancements forward. Multidisciplinary collaboration is essential to successful application of CTA, and the components include the proper facility, an integrated preoperative and postoperative patient care team, and physicians trained in performing and managing CTAs.

At this early period of development, it is essential to direct potential CTA patients to specialized centers, which benefits the patient with the most experienced care and facilitates data collection for scientific analyses. In order to accommodate reconstructive challenges of the head and neck, face, upper and lower extremities, the future reconstructive transplant surgery is likely to evolve into its own specialty, possibly with training from dedicated fellowships.

Because the CTA center may not be near the patient’s home, follow up of the graft by a physician is an issue. CTA surveillance requires familiarity with the neurologic, dermatologic, vascular, and systemic manifestations of rejection and immunosuppression. Currently, the operating surgeon oversees the management of a CTA with consultants from multiple disciplines. However, this situation is evolving from a research environment and does not address the ultimate needs in the discipline when the patient base expands and CTA becomes common clinical practice.

CONCLUSION

Advances in research and successful clinical applications of CTA made great strides to ensure its place in the future of transplant surgery. Unlike SOT, which in most cases is lifesaving, CTA provides the possibility to improve the quality of life. These potential benefits need to be balanced with potentially toxic immunosuppression. Another concern is the ability to regain satisfactory function; otherwise, it may cause additional morbidities to the recipient as a useless limb. Thus far, both French and American teams have demonstrated successful functional recovery while maintaining well-tolerated immunosuppressive protocols. CTA will be a valuable addition to the armamentarium of reconstructive surgeons to potentially and dramatically improve the lives of people who are otherwise disfigured and/or crippled. Additional work is ongoing to improve immunosuppression, induce tolerance, and develop long-term patient care management. In the near future, CTA may ultimately become as commonly practiced as solid organ transplantation.

REFERENCES


