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FORUM

COMPOSITE TISSUE ALLOTRANSPLANTATION

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INTRODUCTION

On May 17–18, 2000, the Second International Symposium on Composite Tissue Allotransplantation took place in Louisville, KY. We summarize the work presented at this historical meeting and in doing so provide the reader with an overview of the latest developments in the field of clinical composite tissue allotransplantation (CTA).

The concept of transplanting tissues for restoration of acquired or congenital deformities is not new. In fact, the first account of transplanting tissue from one individual to another appeared in 348 AD, when the transplantation of a lower extremity by two saints was reported (*1*). It was not until many centuries later, in 1964, that success in solid organ transplantation led a team of surgeons in Ecuador to perform the first human hand transplant. In this case, the immunosuppression used [azathioprine (AZA) and hydrocortisone] was insufficient and the hand rejected and was subsequently amputated 2 weeks posttransplant (*2*, *3*). In the late 1970s and early 80s, three separate groups tested the efficacy of the then revolutionary new drug cyclosporine in preventing rejection of extremity transplants in primates (*4–6*). Although rejection was suppressed for periods of up to 296 days, in these experiments the skin portion of the transplanted extremities was rejected within the first months after transplantation. The discouraging results in Ecuador, together with these primate studies, caused reconstructive surgeons to abandon further attempts to transplant hands for another decade.

In the early 1990s cyclosporine-AZA steroid-based regimens were used in a series of clinical CTAs to reconstruct nerves, tendons, muscle, bone, joint, and laryngeal defects. However, transplantation of composite tissue allografts with skin was still considered impossible when using this regimen. Finally, in 1997 tacrolimus/mycophenolate mofetil (MMF)/prednisone therapy was reported by our group to have successfully prevented composite tissue allograft rejection, while causing minimal systemic toxicity in a preclinical swine forelimb model. Based on these findings, between 1998 and 1999, teams in Lyon (France), Louisville (KY), and Guangzhou (China) performed the first four successful human hand transplants using tacrolimus/MMF/prednisone combination therapy (*7*). The early outcomes of these four hand transplants, as well as the first larynx, vascularized bone, joint, nerves, and tendons were presented by the respective transplant teams at the Louisville meeting and are summarized below. For a thorough review, the reader is directed to the December 2000 special issue of *Microsurgery* (*8*).

Human Hand Transplants

At the meeting in Louisville, four hand transplant procedures performed by three teams were presented: a right hand, transplanted in Lyon, France, on September 23, 1998; a left hand, transplanted in Louisville, KY on January 23, 1999, and two right hands, transplanted to two individuals in Guangzhou, China, on September 21, 1999. At the time this report was submitted for publication, to our knowledge seven additional hand transplant procedures had been performed in Lyon, France in January 2000 (bilateral); in Innsbruck, Austria in March 2000 (bilateral); in Kuala Lumpur, Malaysia, in May 2000 (unilateral); in Monza, Italy in October 2000 (unilateral); in Guangzhou, China, in October 2000 (bilateral), in Harping, China, in January 2001 (bilateral), and in Louisville, KY, in February 2001 (unilateral). In addition, the first recipient (Lyon, France) had requested that his transplanted hand be removed (due to immunotherapy noncompliance and rejection) on February 2, 2001, 2 years and 4 months posttransplant. The first four cases, presented at the meeting in Louisville, represented a complete series of hand transplants that had passed 6 months follow-up at the time of the meeting (*7*).

Clinical history. The ages of the hand recipients were; 48 years in Lyon, 37 in Louisville, and 39 and 27 in Guangzhou. All recipients (and donors) were men. The causes of hand loss were an accident with a saw in the Lyon recipient, an explosion in the Louisville recipient, and an explosion and a steel wire accident in the two Guangzhou recipients. The time elapsed between hand loss and transplant varied between the four recipients. In the Lyon recipient, 14 years had elapsed between hand loss and transplant. In the Louisville recipient 13 years had elapsed. For both Guangzhou recipients only 2 years separated the loss of their hands from the

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transplant. Of note in the medical history was that the Louisville recipient had a 13-year history of diabetes mellitus. However, no diabetic organ involvement had been detected before his hand transplant. The first Guangzhou recipient had elevated liver aspartate transferase (AST), but did not show any clinical correlation. The transplants in Lyon and Louisville were both performed between recipients and donors with six human leukocyte antigen (HLA) mismatches, whereas the HLA mismatches between the two Guangzhou recipients and their donors were three (*7*, *9*, *10*).

Immunosuppressive regimens. For induction, the Lyon and the two Guangzhou recipients were treated with antithymocyte globulin (ATG), whereas the recipient in Louisville received basiliximab. For maintenance, all teams used the same tacrolimus-based combination immunosuppressive therapy including tacrolimus (5–10 ng/ml blood levels in all cases), MMF (2000 mg/day in the Lyon, 2000–3000 mg/day in the Louisville, 750 mg/day in both Guangzhou cases), and prednisone (20–15 mg/day in the Lyon, 10 mg/day in the Louisville, 25 mg/day in both Guangzhou cases). Both Guangzhou recipients received higher doses of prednisone posttransplant than did the Lyon and Louisville recipients. In addition to the above, the Lyon recipient received basiliximab on days 26 and 100 posttransplant (*7*).

Immunological Outcomes. At the time of the meeting, the Lyon and the Louisville recipients were 20 and 15 months posttransplant, respectively, and each had experienced three rejection episodes. In the Lyon recipient these occurred at 2, 14, and 19 months. In the Louisville recipient rejection episodes occurred at 1, 5, and 7 months posttransplant. All rejection episodes were steroid-sensitive and reversible over a 1- to 2-week period. At the time of the Louisville meeting both Guangzhou recipients were 8 months posttransplant and neither were reported to have experienced detectable signs of rejection (*7*).

Immunosuppression-related complications. The Lyon recipient suffered from 1) insulin-dependent diabetes mellitus, which began at day 1, and was reversed by day 300, 2) reversible increase in serum creatinine when his tacrolimus dose was increased in response to periodic episodes of rejection, and 3) a herpes simplex infection at 8 weeks posttransplant, which was successfully reversed with acyclovir treatment. The Louisville recipient suffered from 1) a CMV infection at 15 weeks posttransplant, for which temporary i.v. and long-term oral gancyclovir was necessary, and 2) recurrent Tinea skin infections, for which topical treatment was sufficient. The first Guangzhou recipient suffered from 1) a single Tinea infection at 7 weeks that was successfully treated with topical fungistatics, 2) symptoms indicative of Cushing syndrome prior to the meeting in Louisville. The second Guangzhou recipient reportedly did not experience any immunosuppression-associated complications (*7*).

Functional outcomes. In an attempt to assess and compare functional recovery, the Louisville and the Guangzhou teams performed the Carroll test on their recipients. This test assesses activities of daily living (ADL) and consists of different tasks ranging from placing cubes on a shelf to manipulating different size metal balls between the thumb and other fingers. The results of this test were 52/99 for the Louisville recipient and 75/99 and 65/99 for recipients 1 and 2 from Guangzhou, respectively. These results are described as fair to good by the scheme developed by Russell (*11*), and are comparable to the results obtained after forearm replantation (*7*, *12*).

Human Larynx Transplant

On January 4, 1998 Strome et al. performed the only successful clinical larynx transplant reported to date (*13*).

Clinical history. The larynx recipient was a man, and at the time of transplantation he was 41 years old. His larynx was destroyed in a motorcycle accident involving a steel wire strung across a road. The time elapsed between the trauma and the transplant was approximately 20 years. At the time he received his transplanted larynx the recipient reported a history of hypertension but was otherwise healthy. The larynx recipient was a total (6/6) HLA match with the donor (*13*, *14*).

Immunosuppressive regimens. For induction, the patient received cyclosporine (10 mg/kg), AZA (5 mg/kg), solumedrol (1000 mg initially, then 50 mg/6 hr), and OKT3. At 1 month posttransplant AZA was substituted with MMF (2000 mg/day), and the cyclosporine regimen rapidly tapered. At 13 months posttransplant cyclosporine was substituted with tacrolimus. At the time of the meeting, the larynx recipient was receiving tacrolimus (10–15 ng/ml blood levels), MMF (2000 mg/day), and prednisone (8 mg/day) (*13*, *14*).

Immunological outcomes. A single rejection episode was reported at 13 months posttransplant that coincided with the tapering of the cyclosporine dose. It was steroid sensitive and resolved in approximately 5 days. At that time, the patient was switched from a cyclosporine to a tacrolimus-based regimen (*13*, *14*).

Immunosuppression-related complications. Preexisting hypertension accompanied by signs of nephrotoxicity was difficult to control in the initial phase posttransplant. However, the switch from cyclosporine to tacrolimus made hypertension controllable and resolved nephrotoxicity (*13*, *14*).

Functional outcomes. The functional results of the larynx transplant have been remarkable. Sensation was reported to have returned at 3–4 months, and motor function by 6 months posttransplant. In addition, the donor thyroid glands that were transplanted with the larynx were reported to be functional, and normal swallowing is possible. Although his laryngostomy had not been closed, 2 years after his transplant, the recipient had good pitch control (*13*, *14*). The true meaning of his functional recovery became apparent when the larynx recipient presented at the meeting, leaving the audience astound with the quality of his "transplanted" voice, which was difficult to discern from a normal one (Barker J.H., personal communication).

Human Femur and Knee Transplants

From 1994 to the time of the meeting in Louisville, Hofmann et al. had performed three vascularized femoral and five vascularized knee allotransplants (*15*).

Clinical history. All femur recipients were men, ages 21, 50, and 54 years. Indications were extensive bone loss after posttraumatic osteomyelitis (n=2) and a chondreosarcoma grade 1 without recurrence in 3 years (n=1). The lengths of the bone defects measured 12, 14, and 33 cm, respectively, and for all patients the alternative to transplantation was amputation (*15*).

From 1996 to the time of the Louisville meeting, the same

group performed five vascularized knee joint allotransplants in four males, ages 17, 23, 30, and 47 years, and one 34-year-old female. All indications were trauma resulting in bone defects of 10–15 cm in the distal femur and 5–10 cm in the proximal tibia, with destruction of the extensor apparatus. As in the case of the femur transplants, transplantation was the only alternative to amputation or joint fusion. All patients (both femur and knee recipients) received allotransplants from ABO matched and HLA mismatched (0/6) donors (*15*).

Immunosuppressive regimens. In all cases, recipients received 3 days of cyclosporine A (1.5 mg/kg i.v.), AZA (1.5 mg/kg i.v.), ATG (4 mg/kg i.v.), and methylprednisolone 250 mg. During the rest of the week methylprednisolone was reduced stepwise. In the 6 subsequent months, drug therapy consisted of cyclosporine A (6.0 mg/kg p.o.) and AZA (3.0 mg/kg p.o.). Thereafter, all patients received cyclosporine A monotherapy. In the femur recipients, immunosuppression was stopped 2 years posttransplant (*15*).

Immunological outcomes. The greatest hurdle associated with these procedures was reportedly the lack of ability to monitor bone rejection. Of several methods used to detect rejection, only single photon emission computed tomography (SPECT) was marginally successful. Using this method, decreases in perfusion and tracer uptake were interpreted as indications of bone rejection. Only one episode in one of the knee recipients was well documented. It is therefore possible that many rejection episodes went undetected (*15*).

Immunosuppression-related complications. The eight femur and knee recipients suffered many immunosuppression-related complications, mainly infectious in origin. Of the three femur recipients, one suffered from a transmitted CMV infection at 7 weeks posttransplant and a deep vein thrombosis in the operated leg. A second recipient required removal of the transplant at 18 weeks due to recurrent infections. No immunosuppression-related complications were reported in four of the five knee recipients. In the fifth patient, failure occurred and was reported to be due to infection that began within the first week posttransplant (*15*).

Functional outcomes. Between 6 and 15 weeks after transplantation, all patients achieved full weight bearing. All three femur transplants, despite several complications, regained a full weight-bearing limb. Of the five knee joint recipients, two have done well without major complications at 45 and 29 months. Two recipients required total knee arthroplasty at 15 and 24 months posttransplant. One knee transplant failed within the first week posttransplant due to infection. In this case, total knee arthroplasty was not possible and the patient was scheduled for a segment transport procedure with the aim of arthrodesis (*15*).

Human Nerve Transplants

Although clinical nerve allografting has been attempted since the late 19th century, clinical success was not achieved until the era of modern immunosuppressive therapy. The largest series of successful nonvascularized nerve allografts consists of seven patients and was performed by Mackinnon et al. (*16*, *17*).

Clinical history. Of seven nerve allografts, four were used in the upper extremities and three in lower extremities in patients ranging from 3 to 24 years of age. Nerve allografts were either used exclusively (2/7) or in combination with autografts (5/7). All patients had traumatic injuries of their extremities with massive peripheral nerve deficits that could not be reconstructed by conventional means (*16–19*).

Immunosuppressive regimens. In the first three cases triple therapy with cyclosporine (200–300 ng/ml blood levels), AZA (1.0–1.5 mg/kg/day), and prednisone (initially 0.25–0.5 mg/kg/day with max 30 mg/day and tapered off over 4 to 8 weeks) was used; in the four subsequent cases a combination of tacrolimus (5–15 ng/ml blood levels), AZA (1.0–1.5 mg/kg/day), and prednisone (initially 0.25–0.5 mg/kg/day with max 30 mg/day and tapered off over 4 to 8 weeks) was used. Six months after signs of nerve recovery, as evidenced by Tinel sign passing the graft and functional recovery, in all cases immunosuppression was withdrawn sequentially starting with prednisone and followed by cyclosporine or tacrolimus (*16–19*).

Immunological outcomes. One patient lost his allograft due to rejection, manifested by erythema, induration, swelling, and tenderness directly along the s.c. course of the allograft. This was misdiagnosed as infection and delayed antirejection treatment resulting in loss of the graft (*16–19*).

Immunosuppression-related complications. No significant immunosuppression-related complications were reported in any of the patients (*16–19*).

Functional outcomes. The longest follow-up is 10 years, and functional recovery in 6/7 allografts was reported to be successful as determined by the recovery of sensory and/or motor function. Although all had sensory recovery, three had motor recovery. None of the patients showed any evidence of deterioration of nerve function after the withdrawal of immunosuppression (*16–19*).

Tendon Allotransplantation

In 1988 and 1989, Guimberteau et al. performed the only two cases of vascularized tendon allotransplantation and presented them at the Louisville meeting. In one of these cases, a living donor was used although in the other the tendon was harvested from a cadaver. In both cases, immunosuppressive therapy consisted of cyclosporine monotherapy at 6 mg/kg/day for 6 months, and no immunosuppression-associated complications were reported. Although it was not possible to assess rejection, 11 and 12 years posttransplant both allografts were reported to function well (*20*, *21*). This coincided with findings 10 years posttransplant, when the allograft in one of the patients was revised and found to be healthy and well vascularized.

DISCUSSION

Immunosuppression in CTA

Despite the fact that composite tissue allotransplantation was conceptualized centuries before solid organ transplantation, the latter has become standard care although CTA continues to be experimental. Probably the two main reasons for this difference have been the relatively little immunosuppression that was achieved with primitive drugs (not allowing transplantation of skin) and the risk-versus-benefit debate in CTA. Although the risks of immunosuppression are considered to justify the benefits of "life saving" solid organ transplant procedures, by many the same risks are considered too high to justify "non-life-saving" CTA procedures (*22*). This is further complicated by the fact that within CTA

certain operations are considered more "justifiable" than others. Some argue, for example, that bilateral or blind upper extremity amputees are a better indication for transplantation than amputees who have lost only one hand (*23*). In a recent publication Edgell et al. (*24*) evaluated the risk-versus-benefit equation in CTA from a psychological as well as a sociological standpoint and concluded that in CTA this equation is not static, but rather individually determined by each individual's frame of reference. In short, the perception of the benefit of a transplanted hand is different from amputee to amputee, or from amputee to doctor. Similarly, the risks posed by immunosuppression are perceived differently by kidney transplant patients (who live with these risks), a hand amputee (who is told of the risks), a transplant surgeon (who treats the complications of these risks), a hand surgeon (who is indirectly aware of the risks), etc. In other words, one's perception of risk is based on his/her experience. Dialogue and compromise are therefore pivotal elements in the CTA risk-versus-benefit debate. The recent interest in clinical CTA, demonstrated by several new cases, has been fueled primarily by the reevaluation of the risk-versus-benefit equation, translated in the recognition of reconstructive surgeons that modern immunosuppressive drugs are capable of preventing acute CTA rejection with relatively low systemic toxicity. As in in solid organ transplantation, chronic rejection is likely to become a problem in CTA. However, due to the short follow-up and limited numbers of CTAs performed to date, to what extent this will be the case is difficult to assess. If CTA could be performed without immunosuppression, it would drastically change the risk-versus-benefit equation in favor of its benefits and likely pave the way to widespread clinical application. Consequently, as in solid organ transplantation, the vast majority of animal experiments in CTA focus on replacing the need for immunosuppression with methods of inducing transplantation tolerance.

Tolerance in CTA

Three important strategies for tolerance induction, and their relevance to CTA, were presented and discussed at the Louisville meeting: the use of anti-CD3 and deoxyspergualin (*25*), costimulatory blockade (*26*), and the use of mixed allogeneic chimerism (*27–29*). The focus of these strategies has traditionally been to achieve transplantation tolerance for solid organs. Whether they will be applied in clinical CTA and organ transplantation will eventually depend on their safety.

Forty years after the first solid organ transplants were performed, CTA has been introduced, or perhaps reintroduced into the clinical setting. The meeting in Louisville brought together protagonists and critics of CTA, allowing for the exchange of their views and experiences on this emerging clinical technology. Considering the excellent early results of CTA, it was the opinion of many at the meeting that the benefits of CTA were better than predicted, and warranted proceeding with select cases. A safe clinical tolerance protocol will likely be necessary to justify proceeding with widespread applications. Although the protagonists of CTA have often been criticized, the achievements of the first clinical CTA cases were applauded by those present from both the reconstructive and transplant communities, and perhaps best worded in Thomas Starzl's closing remarks: "I salute the renegades."

REFERENCES

1. Kahan BD. Cosmas and Damian revisited. Transplant Proc 1983; 15: 2211.
2. Gilbert R. Transplant is successful with a cadaver forearm. Med Trib Med News 1964; 5.
3. Anonymous. Hand transplanted from cadaver is reamputated. Med Trib Med News 1964; 5.
4. Daniel RK, Egerszegi EP, Samulack DD, Skanes SE, Dykes RW, Rennie WR. Tissue transplants in primates for upper extremity reconstruction: a preliminary report. J Hand Surg 1986; 11: 1.
5. Stark GB, Swartz WM, Narayanan K, Moller AR. Hand transplantation in baboons. Transplant Proc 1987; 19: 3968.
6. Hovius SE, Stevens HP, van Nierop PW, Rating W, van Strik R, van der Meulen JC. Allogeneic transplantation of the radial side of the hand in the rhesus monkey: I. Technical aspects. Plast Reconstr Surg 1992; 89: 700.
7. Francois CG, Breidenbach WC, Maldonado C, et al. Hand transplantation: Comparisons and observations of the first four clinical cases. Microsurgery 2000; 20: 360.
8. Microsurgery 2000; 20.
9. Jones JW, Gruber SA, Barker JH, Breidenbach WC. Successful hand transplantation: one-year follow-up. N Engl J Med 2000; 343: 468.
10. Dubernard JM, Owen E, Herzberg G et al. Human hand allograft: report on first 6 months. Lancet 1999; 1: 1315.
11. Russell RC, O'Brien BM, Morrison WA, Pamamull G, MacLeod A. The late functional results of upper limb revascularization and replantation. J Hand Surg 1984; 9: 623.
12. Graham B, Adkins P, Tsai TM, Firrell J, Breidenbach WC. Major replantation versus revision amputation and prosthetic fitting in the upper extremity: a late functional outcomes study. J Hand Surg 1998; 23A: 783.
13. Strome M, Stein J, Esclamado R, et al. Laryngeal transplantation and 40-month follow-up. N Engl J Med 2001; 344: 1676.
14. Strome M. Human laryngeal transplantation: considerations and implications. Microsurgery 2000; 20: 372.
15. Hofmann GO, Kirschner MH. Clinical experience in allogeneic vascularized bone and joint allografting. Microsurgery 2000; 20: 375.
16. Bain JR. Peripheral nerve and neuromuscular allotransplantation: current status. Microsurgery 2000; 20: 384.
17. Mackinnon SE, Doolabh VB, Novak CB, Trulock EP. Clinical outcome following nerve allograft transplantation. Plast Reconstr Surg 2001; 107: 1419.
18. Mackinnon SE, Hudson AR. Clinical application of peripheral nerve transplantation. Plast Reconstr Surg 1992; 90: 695.
19. Mackinnon SE. Nerve allotransplantation following severe tibial nerve injury. Case report. J Neurosurg 1996; 84: 671.
20. Guimberteau JC, Baudet J, Panconi B, Boileau R, Potaux L. Human allotransplant of a digital flexion system vascularized on the ulnar pedicle: a preliminary report and 1-year follow-up of two cases. Plast Reconstr Surg 1992; 89: 1135.
21. Guimberteau JC, Baudet J, Panconi B, Boileau R, Potaux L. Human allotransplant of a digital flexion system vascularized on the ulnar pedicle: a preliminary report and 1-year follow-up of two cases. Plast Reconstr Surg 1992; 89: 1135.
22. Lee WP, Mathes DW. Hand transplantation: pertinent data and future outlook. J Hand Surg 1999; 24: 906.
23. Herndon JH. Composite-tissue transplantation–a new frontier. N Engl J Med 2000; 343: 503.
24. Edgell SE, McCabe SJ, Breidenbach WC, Neace WP, La Joie AS, Abell TD. Different reference frames can lead to different hand transplantation decisions by patients and physicians. J Hand Surg 2001; 26: 196.
25. Thomas F, Ray P, Thomas JM. Immunological tolerance as an adjunct to allogeneic tissue grafting. Microsurgery 2000; 20: 435.
26. Elster EA, Blair PJ, Kirk AD. Potential of costimulation-based therapies for composite tissue allotransplantation. Microsurgery 2000; 20: 430.
27. Prabhune KA, Gorantla VS, Maldonado C, Perez-Abadia G, Barker JH, Ildstad ST. Mixed allogeneic chimerism and tolerance to composite tissue allografts. Microsurgery 2000; 20: 441.
28. Fuchimoto Y, Huang CA, Yamada K, et al. Mixed chimerism and tolerance without whole body irradiation in a large animal model. J Clin Invest 2000; 105: 1779.
29. Lee WP, Rubin JP, Bourget JL, et al. Tolerance to limb tissue allografts between swine matched for major histocompatibility complex antigens. Plast Reconstr Surg 2001; 107: 1482.

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