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The Science of Composite Tissue Allotransplantation

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Abstract

The science of composite tissue allotransplantation (CTA) is rooted in progressive thinking by surgeons, fueled by innovative solutions, and aided by understanding the immunology of tolerance and rejection. These three factors have allowed CTA to progress from science fiction to science fact. Research using pre-clinical animal models has allowed an understanding of the antigenicity of complex tissue transplants and mechanisms to promote graft acceptance. As a result, translation to the clinic has shown that CTA is a viable treatment option well on the way of becoming standard of care for those who have lost extremities and suffered large tissue defects. The field of CTA has been progressing exponentially over the past decade. Transplantation of hands, larynx, vascularized knee, trachea, face, and abdominal wall has been performed. A number of important observations have emerged from translation to the clinic. Although it was predicted that rejection would pose a major limitation, this has not proven true. In fact, steroid-sparing protocols for immunosuppression that have been successfully used in renal transplantation are sufficient to prevent rejection of limbs. Although skin is highly antigenic when transplanted alone in animal models, when part of a CTA, it has not proven to be. Chronic rejection has not been conclusively demonstrated in hand transplant recipients and is difficult to induce in rodent models of CTA. This review focuses on the science of CTA, provides a snapshot of where we are in the clinic, and discusses prospects for the future to make the procedures even more widely available.

Keywords

composite tissue; allotransplantation; hand; laryngeal transplant

Introduction

Clinical success in composite tissue allotransplantation (CTA) is the culmination of progress in two disparate surgical disciplines - plastic reconstructive surgery and transplantation.

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The science of replantation

The modern era of replantation in reconstructive surgery began in the 1960s with the reimplantation of the hand (1,2) and digits (3,4). Following the report of the first microvascular free tissue transfer of an omental flap (5), the free transfer of autologous tissue became the mainstay for the treatment of complex soft tissue defects.

The science of organ transplantation

The growth of solid organ transplantation parallels the emergence of newer immunosuppressive drugs (6). In the 1960s, azathioprine and prednisolone were used in renal transplantation. Polyclonal antithymocyte globulin (ATG) preparations became available in the 1970s (7). The introduction of cyclosporine A in the early 1980s improved three-year graft survival from 71.6% to 87.2% (7). Subsequently, the use of tacrolimus & sirolimus, have led to the current 94% one-year survival rate for kidney transplants and monoclonal antibodies have led to steroid-sparing immunosuppression (8).

Given these achievements, it was almost inevitable that by 1998 allogeneic tissue would be used for soft tissue reconstruction. This overview outlines the experimental and ethical issues encountered in the first successful hand transplants, the clinical outcomes to date, and the challenges ahead.

Animal Models

Preclinical models for CTA

Seminal studies in the 1960s elicited the hierarchy of antigenicity and cumulative effect of transplanting composite tissues (9). Murray found skin to possess the highest degree of antigenicity among all tissue tested (10). In contrast, Lee (11) found that no single tissue was dominant in primarily vascularized limb allografts.

Split tolerance

Split tolerance describes the process where different tissues from a donor generate contrasting immune responses, resulting in acceptance of one and rejection of another simultaneously (12). This was demonstrated in solid organ transplantation (13) in a miniature swine class I antigen mismatched renal allograft model sequentially exposed to skin grafts. The skin was rejected but the kidney was not. Musculoskeletal allografts in swine across minor histocompatibility barriers showed long-term survival with a short course of cyclosporine (14). Subsequent skin allografts from the same donor failed in half of the recipients despite continued acceptance of the musculoskeletal graft (15). Later studies demonstrated that the epidermis was rejected while the dermis survived (16). This is attributed to the highly immunogenic skin specific antigens in the epidermis (17) and the antigen presenting cells in the skin (18). Even bone marrow chimeras which accept transplanted heart may reject skin from the same donor (19).

Clinical experience with hand transplantation has corroborated the experimental data. The first human hand allograft was removed owing to rejection caused by noncompliance. The histological changes were most severe in the skin, with mild inflammation in muscles and tendons with sparing of bone and joints (20). However, contrasting findings with more intense rejection in muscle and nerve as compared to skin have been reported (21). A recent review noted that most of the tissue elements other than nerve were susceptible to a cellular immune response (22).

Rodent models of CTA

The most commonly used animal model in CTA is the rat. In 1984, Kim successfully used cyclosporine A in limb transplantation between BUF and LEW rats (23). Subsequent use of low-dose mycophenolate mofetil (MMF) (15 mg/kg/day) in combination with CsA (1.5 mg/kg/day) was also effective (24). Orthotopic mid-femur limb transplant from Brown-Norway donors into MHC-disparate F344 recipients was similarly accepted. Nonfunctional heterotopic hind limb allotransplantation models were developed to overcome technical difficulties in evaluating tolerance to the components of the transplanted tissue (25,26).

Co-stimulatory blockade between T cells and APC (Signal 2) during exposure to alloantigen (Signal 1) results in graft prolongation in xenogeneic pancreatic islet grafts, rat small bowel allografts and a murine heterotopic airway model (27-29). Similar graft prolongation has been achieved for hind limb allografts (30). Administration of CTLA4Ig or CD40Ig to Lewis (RT1^L) recipients of ACI (RT1A^a) limb transplants resulted in significant graft prolongation, and the combination of agents led to a synergistic prolongation of graft survival. Taken together, these data indicated that rejection of CTA may not be dissimilar to solid organ transplants.

Chimerism induces tolerance to CTA

A long-term goal in transplantation has been to induce donor-specific tolerance. Chimerism induces donor-specific tolerance to transplanted tissues and organs (31). Foster (32) prepared mixed chimeras by transplanting a mixture of T-cell depleted (TCD) syngeneic (WF) and allogeneic (ACI) bone marrow (BM) into WF recipients conditioned with 500-700 cGy of total body irradiation (TBI) and a single dose of anti-lymphocyte serum (ALS). Tacrolimus (FK506) was administered for 10 days post-operatively. Hind limb CTA was performed at 12 months. Chimerism levels of > 60% and < 20% were associated with tolerance and rejection respectively.

Further studies showed that CD28 blockade, in combination with tacrolimus (FK506), ALS, and 300 cGy TBI prior to transplantation of 100×10^6 TCD bone marrow established chimerism and induced tolerance to CTA without graft-versus-host disease (GVHD) (33). This success with non-myeloablative conditioning has great potential in human CTA.

The original rodent models involved a delay of at least one month between the BMT and CTA, rendering it impractical for the clinic. Performing mixed-allogeneic chimerism induction and rat hind limb allotransplantation *simultaneously* is needed. Prabhune (34) found that infusion of donor marrow cells into conditioned hosts immediately post-limb transplantation, combined with tacrolimus and MMF for 28 days also resulted in stable mixed chimerism and limb allograft tolerance.

GVHD has been observed in some models of CTA (35). Gorantla (36) found that ACI → WF chimeras with > 85% mixed-chimerism exhibited rejection-free survival of donor-specific hind limbs despite GVHD. However, transplantation with irradiated ACI hind limbs prevented GVHD. This is due to inactivation of GVHD causing donor cells present in the lymph nodes of the graft. Therefore, consideration should be given to lymphoid tissue included in various CTA with antibody pretreatment to prevent GVHD.

Other preclinical models for CTA

Facial transplantation has been evaluated in preclinical models for CTA. Demir introduced a rat hemifacial allograft model using Lewis-Brown Norway (RT1^l+n) and Lewis (RT1^l) rat strain combinations (37). Recipients were treated with CsA (16 mg/kg/day) during the first week, which was tapered to 2 mg/kg/day over the next 4 weeks. Five of six (83%) allografts

showed no rejection up to 240 days. Twenty-one days after transplantation, flow cytometric analysis showed low levels of peripheral microchimerism. Thus operational tolerance was induced by CsA monotherapy across this relatively weak MHC barrier. Successful translation of this model to a strong strain combination has not yet been reported.

Swine model of CTA

Ren (38) developed an osteomyocutaneous forearm flap model for the study of CTA in swine. Allograft recipients were able to ambulate immediately following the transplant. The authors concluded that this pre-clinical model was excellent for evaluating the effectiveness of immunotherapy.

The induction of tolerance to CTA with hematopoietic stem cell infusion was tested in an MHC-disparate miniature swine model (39). Seven recipients were T cell depleted (TCD) using antibody treatment *in vivo* and a short course of cyclosporine was initiated. Twenty-four hours later, a donor hematopoietic cell transplant consisting of cytokine-mobilized peripheral blood mononuclear cells or bone marrow cells, along with a heterotopic limb transplant were performed. All seven recipients accepted the musculoskeletal components of the CTA but rejected the skin. Six of seven animals (85%) displayed donor-specific unresponsiveness *in vitro*. The animals that received cytokine-mobilized peripheral blood mononuclear cells demonstrated macrochimerism and developed GVHD. The animals that received bone marrow cells showed neither stable chimerism nor GVHD. It was concluded that tolerance to the musculoskeletal elements of CTA is possible across an MHC barrier in miniature swine and that stable chimerism may not be necessary for functional tolerance.

Primate models for CTA

In 2005, Cendales reported a pre-clinical CTA model in nonhuman primates using a sensate osteomyocutaneous radial forearm flap (40). This was evaluated in nineteen monkeys that underwent auto- or allotransplantation, with or without sub-therapeutic immunosuppression, to allow characterization of rejection. Without immunotherapy, allografts were rapidly rejected with a perivenular T-cell infiltrate and alloantibody production causing graft thrombosis. Subtherapeutic immunosuppression caused alloantibody development and delayed graft rejection with a marked dermal lymphocytic infiltrate similar to human hand transplants. It is of note that antibody formation dominated if no immunosuppression was administered, and that cellular infiltrates only occurred in immunosuppressed recipients. One major limitation to the use of nonhuman primates is the difficulty in achieving therapeutic levels of immunosuppression without serious toxicity.

Ethical Considerations

CTA is not a life-saving procedure but aims to improve the *quality* of life. Simmons summarized the ethics of CTA as “the question is not just what can be done *for* a patient but what is being done *to* a patient” (41). One major controversy surrounding CTA is the toxicity of the immunosuppression (42-44) with an increased risk of cancer, organ failure, and opportunistic infections (45-47).

Another ethical concern is the risk/benefit ratio of the operation. Moore established six criteria for the introduction of innovative operations: 1) scientific background; 2) experience and skill of the surgical team; 3) ethical mores at the program; 4) ability for public evaluation; 5) openness of the team; and 6) discussions between the public and the professional teams involved (48,49). The first international symposium on CTA, held in Louisville, KY in 1997, discussed these points (50).

The psychological issues surrounding CTA cannot be underscored. The first hand transplant recipient became non-compliant and had his graft amputated (20). This raises the important issue of autonomy. Autonomy is a respect for individuals (51) with decision-making ability and capability of informed consent (52). In the emerging field of CTA with need for complex post-operative immunotherapy, there is a tendency to highlight the potential benefits and not the possible risks (41). Psychiatric screening for potential recipients is critical. The detailed interview, psychological testing, and assessment of issues inherent to CTA (realistic expectations: body image adaptation, anticipated comfort, among others), is necessary in evaluating the patient's decision-making capacity (53,54).

In 1997, when the team at the University of Louisville showed that the combination of prednisone, tacrolimus, and MMF could prevent rejection of an allograft limb transplant for 90 days in an adult swine model, it was concluded that CTA was feasible (55). These studies in rodents and swine led to the international symposium on CTA (50) with participation of reconstructive and microsurgeons, immunologists, scientists, and ethicists. Moore's ethical criteria for introduction of a new procedure suggested were carefully considered. The consensus was to proceed with hand transplantation using post-operative immunosuppression (55,56).

The Status of Hand Transplantation Worldwide

Table I summarizes the world hand transplant experience. The first hand transplant was first performed in Ecuador in 1964 using azathioprine and prednisone (57). The hand was amputated two weeks post-transplantation due to rejection (20). There was a hiatus until more efficient immunosuppression emerged. Dubernard performed the first successful hand transplant in September 1998 (58). The operation was successful but the graft was removed 29 months later due to rejection caused by non-compliance (20). The world's second hand transplant (currently the longest survivor) was performed in January, 1999 by Breidenbach in a 24-year-old man (59). The first double hand transplant was performed in France in 2000 (60). Although the initial hand transplants were limited to mid or distal forearm, success in these prompted proximal forearm transplants in Austria (61) and Poland (62). Fifteen hand transplants have been performed in China. Unfortunately, due to the lack of availability of immunosuppression, all have resulted in rejection (63). There is consensus that further transplants should not be performed without assurance of access to immunosuppression (6th International Symposium on CTA).

In Malaysia, an upper extremity transplant was performed at the level of the shoulder on a 28-day-old neonate born with congenital absence of one arm. The identical twin had a fatal brain anomaly and was the donor of the limb. The transplanted limb grew at the same rate as the native limb and is functional (64).

What Have We Learned To Date?

A number of important and unexpected findings have emerged as hand transplantation has become an established therapeutic option.

Immunosuppressive management

It was postulated by many in the transplant community that rejection in CTA would be difficult to control without high intensity immunosuppression (65). This has not proven to be the case. The majority of recipients have been maintained on immunosuppression, similar to that used in renal transplantation, consisting of a calcineurin inhibitor, MMF or rapamycin, and steroids (43,59,66-69). More recently, Campath-1H lymphodepletion induction and steroid-sparing maintenance with FK506 and MMF has been successfully utilized (70).

Rejection vs. graft infiltrating cells

The diagnosis of rejection in CTA is currently evolving. A histologic grading system for rejection in skin biopsies was proposed by Cendales (Table II) (22). Biopsies are scored from grade 0 to grade 4. In the early transplant recipients, even grade 1 rejection was treated with systemic anti-rejection therapy. More recently we and others have found that observational management or the use of topical agents is sufficient for grades 1 and 2 infiltrates. It is possible that the high number of acute rejection episodes reported in hand transplantation is due to the fact that the graft is visible and can be biopsied safely (70). There is evolving consensus that not all graft infiltrating cells are bad. Recent findings from the Lyon group and our own have investigated the nature of the cellular infiltrates. Notably, $CD4^+/CD25^+/Foxp3^+/CD127^-$ regulatory T cells (T_{reg}) comprise the majority of these graft-infiltrating cells in the skin up to 6 years post-transplantation and may be beneficial (71). Our understanding of rejection in CTA is far from complete. The questions that need to be answered include whether the involvement of different structures (adnexae, epidermis, vessels, etc.) signifies differences regarding outcome, or whether sampling induces diagnostic bias (22). There is thus a need for developing a new classification that combines both histological and clinical features.

As the understanding of rejection in CTA evolves, so does the strategy for management. Although most CTA recipients have had acute rejection episodes as defined by graft-infiltrating cells, no single group has enough experience to determine patterns of rejection on solely the histological level. Collectively, these findings contradict the proposed outcomes for CTA and challenge the current paradigms.

T_{reg} in CTA

T_{reg} cells in peripheral blood ($CD4^+/CD25^{high}/Foxp3^+$) have been shown to express both CCR4 and cutaneous lymphocyte Ag (CLA) (70). The presence of these functional skin homing receptors in the majority of circulating T_{reg} indicates that they home to normal skin. It is proposed that they suppress weak or moderately activated effector T cells, thereby maintaining peripheral tolerance to autoantigens and avoiding an inflammatory response to resident cutaneous microbial flora (72). The role of T_{reg} in promoting acceptance of hand allografts is only now being defined. Recent studies in hand transplant recipients have shown that high numbers of $CD4^+/CD25^+/Foxp3^+$ T cells infiltrate the donor skin from 4 months to as far out as 8 years (73). Although labeled as early rejection by the conventional nomenclature, this infiltrate may in fact be protective. In kidney transplantation, when Foxp3 expression has been noted in acute rejection, there is simultaneous elevation of cytokines such as perforin, TNF- α and IFN- γ . In contrast, when Foxp3 mRNA expression occurs in the absence of elevation of perforin, TNF- α or IFN- γ , but with increase of TGF- β or IL-10, it may indicate the process of regulatory immunomodulation (74,75). A comprehensive analysis of phenotype and function of graft-infiltrating cells needs to be performed to elucidate the exact role of T_{reg} in CTA. This may lead to the development of individualized approaches to manage CTA.

Chronic rejection

Chronic rejection limits the longevity of solid organ transplants (76). The universal feature is a progressive narrowing of hollow structures within the graft such as blood vessels, bile ducts or bronchioles. Subclinical immunological injury, drug toxicity, ischemia during the transplant procedure (77) or infections (such as CMV [78]) are thought to play a contributory role. Surprisingly, CTA grafts have shown little evidence of chronic rejection.

In renal transplantation, early acute rejection has been shown to be a critical antigen driven risk factor for later chronic events (79). Although acute rejection was noted in two-thirds of hand transplants worldwide (80), no evidence of chronic rejection has been noted in any of the

compliant recipients on long-term follow-up. This is significant, as 9 of the recipients have been followed up for over 5 years.

The first hand transplant recipient (Lyon, France) lost his graft following a long period of non-compliance with medication. The clinicopathological features of rejection were largely confined to the skin with milder involvement of muscle and tendon and sparing of bone and joints (20). Blood vessels were also severely affected, showing dense infiltration of their walls with inflammatory cells and partial disruption. Experimental data from a rat hind-limb allograft model showed changes consistent with chronic rejection such as neointimal thickening and luminal occlusion of graft arteries (81). However it took 11 ± 3 episodes of acute rejection after brief complete cessation of immunosuppression to induce these changes. In striking contrast, chronic rejection is readily induced in the rat to lung, cardiac, trachea, and aortic allografts without requiring cessation of immunosuppression (82,83). The data thus far suggest that CTA grafts are relatively resistant to chronic rejection. The reason for the privileged status of these grafts warrants further study.

Summary of Other CTA

Many other tissues have been successfully transplanted to restore tissue loss from trauma or tumor. Reports of success with nerve allografts (84), vascularized allotransplant of digital flexion system (85) and allogeneic vascularized knee transplantation (86) preceded the first successful hand transplant. Other CTA include tongue (87), penis (88) skeletal muscle for scalp reconstruction (89), face, and abdominal wall. These are discussed briefly below and summarized in Table III.

Abdominal wall transplantation

Simultaneous and sequential abdominal wall transplantation coincident with intestinal transplantation has been reported from the University of Miami (90). To date, 10 grafts have been performed in 9 patients (91). After induction with alemtuzumab, maintenance immunosuppression consisted of tacrolimus and a steroid taper. Five grafts were lost due to: sepsis (3 grafts), primary non-function (1 graft), and rejection (1 graft). Acute rejection occurred in 3 patients and was successfully treated with steroids.

Laryngeal transplant

A 40-year-old man received the first successful human laryngeal transplant in 1998 (92). An HLA matched laryngopharyngeal complex including thyroid, parathyroids, and five rings of trachea was transplanted along with anastomosis of both superior and one of the recurrent laryngeal nerves. At a follow-up of over 7 years, the patient had excellent function, normal swallowing, good phonation, and good quality of life (93). Tintinago has reported 13 laryngeal transplants with 90% graft survival at 2 years (94) using immunosuppression similar to renal transplantation.

Facial transplantation

Patients with severe disfigurement of face not amenable to reconstruction are likely to benefit from partial face transplantation. Three facial transplants have been performed to date. The first was a 38-year-old woman, disfigured by a severe dog bite, who received a central and lower facial transplant in 2005 (95). A sentinel skin graft was placed in the left inframammary area to monitor rejection. Sensitivity to temperature and light touch returned by 6 months, while motor recovery, allowing complete mouth closure, was achieved at 10 months. Despite 2 episodes of acute rejection and renal dysfunction requiring cessation of tacrolimus, the patient is satisfied with the aesthetic result and is maintained on sirolimus, MMF and prednisone (82).

Conclusions/Future Trends

The technical aspects of CTA are no longer the factor limiting widespread application of this treatment modality in the clinical setting. The feasibility of the procedure has been established, and functional outcomes have been excellent. The major challenge is at the immunologic level. While advancements in understanding the immune system have grown exponentially, much is still to be defined. The long-term goal is to induce donor-specific tolerance and avoid the toxicity of immunosuppression. Among some of the new and potentially beneficial approaches include using T_{reg}, tolerogenic dendritic cells (DC), and facilitating cells (FC) in tolerance induction.

The role of T regulatory cells

One cell population of interest that has emerged recently is the T regulatory cell (96). The best characterized T_{reg} are CD4⁺/CD25⁺. T_{reg} produce Foxp3, a transcription factor that is important in the function and development of T_{reg} cells for their role in maintaining tolerance (97). T_{reg} secrete interleukin 10 (IL-10) and TGF- β , which have been shown to suppress allograft rejection. Previous *in vitro* studies have shown that when T_{reg} are introduced into a mixed lymphocyte reaction, they suppress donor-directed T cell responses (71). T_{reg} suppress GVHD (98) and their use in the clinic is emerging in organ and bone marrow transplantation.

Dendritic cells

DC play an important role in both acquired and central immunity (99). However, their role in immunity is dependant on their maturational state. Under selected conditions, some DC are highly tolerogenic. While the mechanism is not clear, immature DC are capable of promoting transplant tolerance by generation of T_{reg} (100). Studies by Jonuleit showed that naïve CD4⁺ stimulated with immature DC resulted in generation of lymphocytes similar to T_{reg} that demonstrated low proliferation, secreted IL-10 and were able to inhibit specific immune responses (101). Studies by Turnquist showed that maturation of DC resulted in loss of support of T_{reg} function. Infusion of rapamycin-conditioned DC into recipient mice resulted in indefinite heart allograft survival (102). Rapamycin inhibits the maturation of DC and therefore promotes a tolerogenic milieu (103). One major concern regarding the clinical use of these cells is to prevent them from converting to be immunogenic *in vivo* (104). One unique subpopulation, CD8⁺/TCR⁻ FC maintain their tolerogenic properties *in vivo* (105,106) and significantly enhance the establishment of mixed chimerism. FC induce T_{reg} *in vivo* (manuscript in preparation) and *in vitro* (107) and therefore hold promise as an approach to tolerance induction.

In summary, CTA is rapidly emerging from an experimental model to standard of care. Progress made in the field of immunomodulation, namely conditioning protocols and immunosuppressive therapy, has significantly advanced the field of CTA. Treatment protocols and strategies utilized in CTA immunosuppression, and definition of rejection, have been successfully translated from the field of solid organ transplantation. But as is the uniqueness of the tissues in CTA, so too are the inherent adverse effects. In this sense; the field of CTA can not only learn from the solid organ transplant community, but perhaps it can teach as well. As we strive to define rejection, immunosuppressive strategies; and, just as importantly, quality of life and sense of self, the field of CTA can serve as a paradigm for transplantation. With cautious optimism and healthy critiques, the science of CTA promises to be a bright light.

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Abbreviations

(ATG), antithymocyte globulin; (BMT), bone marrow transplantation; (CTA), composite tissue allotransplantation; (DC), dendritic cells; (FC), facilitating cells; (GVHD), graft-versus-host disease; (MMF), mycophenolate mofetil; (T_{reg}), regulatory T cells; (TCD), T-cell depleted; (TBI), total body irradiation.

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TABLE I
27 TOTAL HAND TRANSPLANTS ON 20 PATIENTS

HAND TRANSPLANT	DATE	LOCATION	COMMENT	REFERENCE
1 st	September 23, 1998	Lyon, France	First hand transplant	(58)
2 nd	January 25, 1999	Louisville, KY	First performed in U.S.	(59)
3 rd and 4 th	September 21, 1999	Guangzhou, China	Performed simultaneously	(108)
5 th	January 12, 2000	Lyon, France	First double hand transplant	(60)
6 th and 7 th	January 2000	Guangxi, China		(80)
8 th	March 8, 2000	Innsbruck, Austria	Second double hand transplant	(109)
9 th	May 18, 2000	Malaysia	First arm/hand transplant	(64)
10 th	September, 2000	Guangzhou, China	Third double hand transplant	(80)
11 th	October, 2000	Monza, Italy		(80)
12 th	January, 2001	Harbin, China	Fourth double hand transplant	(80)
13 th	February 16-17, 2001	Louisville, KY	Second hand transplant in U.S.	(110)
14 th	October, 2001	Milan, Italy		(80)
15 th	June, 2002	Brussels, Belgium		(111)
16 th	June, 2002	Harbin, China		(80)
17 th	July, 2002	Nanjing, China		(80)
18 th	October, 2002	Harbin, China	Double hand	(80)
19 th	November, 2002	Milan, Italy		(80)
20 th	February, 2003	Innsbruck, Austria	Double hand	(112)
21 st	May, 2003	Lyon, France	Double hand	(80)
22 nd	February 2005	Nanjing, China	Digital transplantation	(80)
23 rd	February, 2006	Wroclaw, Poland		(113)
24 th	June, 2006	Innsbruck, Austria	Double hand	(80)
25 th	November, 2006	Louisville, KY	Third hand transplant in U.S.; 1st with induction therapy	www.handtransplant.com
26 th	December 1, 2006	Valencia, Spain	First female patient Double hand transplant	(114)
27 th	2006	Trzebnica, Poland	First forearm transplant Double extremity	(62)

TABLE II
HISTOLOGIC GRADE OF ACUTE REJECTION (22)

GRADE	SEVERITY	HISTOLOGIC CHANGES
0	Nonspecific changes	<ul style="list-style-type: none"> • Some epidermal atrophy • No inflammatory infiltrate is seen
1	Mild rejection	<ul style="list-style-type: none"> • Mild, superficial perivascular infiltrate • No infiltration of inflammatory cells into the epidermis • Few lymphocytes infiltrating the adnexal glands
2	Moderate rejection	<ul style="list-style-type: none"> • Inflammatory infiltrate is more intense • Still predominantly perivascular • Clear infiltration of lymphocytes into the epidermis and the hair shaft • Very little keratinocyte necrosis
3	Severe rejection	<ul style="list-style-type: none"> • Prominent perivascular inflammation • Band-like infiltrate just beneath the dermal-epidermal junction • Infiltration of the epidermis and adnexal glands is present

TABLE III**CASE SUMMARY OF CTA OTHER THAN HAND**

TISSUE	TOTAL #	REJECTION	RESULTS/ COMPLICATIONS	REFERENCE(s)
Abdominal wall	9	3	Death = 5	(91)
Face	2	1	Satisfactory aesthetic and functional results at 18 months from first partial face transplantation	(95,115,116)
Flexor tendon transfer	2		Acceptable range of motion at 1 year	(85)
Knee	6	5	Graft loss=5; Good function=1 (at 4 yrs)	(117)
Nerve	7	1	Sensory recovery=6; Motor recovery=3	(116)
Larynx	1	1	Satisfactory function at 7 years	(118,119)
Penis	1		Amputated at 14 days	(88)
Tongue	1		Functional at 8 months Patient succumbed to recurrence	(120)