American Journal of Transplantation 2007; 7: 1753–1762
Blackwell Munksgaard

doi: 10.1111/j.1600-6143.2007.01837.x

First Forearm Transplantation: Outcome at 3 Years

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We here report on the surgical procedure, postoperative course and functional results at 3 years following the first bilateral forearm transplantation. A 41-year-old male underwent bilateral forearm transplantation on February 17, 2003. After ATG induction therapy, tacrolimus, prednisone and MMF were given for maintenance immunosuppression. At 16 months, MMF was switched to everolimus. Hand function, histology, immunohistochemistry, radiomorphology, motor and nerve conduction and somatosensory-evoked potentials were investigated at frequent intervals. A total of six rejection episodes required treatment with either steroids, basiliximab, ATG, alemtuzumab or tacrolimus dose augmentation. At 3 years, the patient is free of clinical signs of rejection despite a persisting minimal perivascular lymphocytic dermal infiltrate. No signs of myointimal proliferation in graft vessels were seen. Motor function continuously improved, resulting in satisfactory hand function. Intrinsic hand muscle function was first observed at 16 months and continues to improve. Although discrimination of hot and cold recovered, overall sensitivity remains poor. The patient is satisfied with the outcome. Bilateral forearm transplantation represents a novel therapeutic option after loss of forearms.

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Key words: Composite tissue, forearm, hand transplantation, outcome, transplantation

Received 02 January 2007, revised 02 March 2007 and accepted for publication 19 March 2007

Introduction

In the past 8 years, hand transplantation has become a clinical reality and raised hope for many patients suffering from amputation as well as various kinds of malformation. Although many hurdles are yet to be overcome, composite tissue allotransplantation (CTA) represents a major achievement in reconstructive medicine and transplantation.

The level of immunosuppression (IS) required in CTA is comparable to that for cardiac or pancreas transplantation. However, the unique situation that graft monitoring can be performed by visual inspection enables IS to be reduced to the particular level required, thereby preventing over-immunosuppression. Nevertheless, one or multiple acute rejections have been reported in most cases (1–7). Long-term IS remains indispensable and, hence, some patients are discouraged from seeking, and surgeons from routinely performing, hand transplantation.

Good, in some cases even excellent, functional results have been achieved with hand transplantation (1–5). Motor and sensory function has exceeded initial expectations and overall patient satisfaction has encouraged those centers performing hand transplantation to continue their programs. International experience and, even more, our own observations in the first patient receiving a bilateral hand transplant in Innsbruck prompted us to continue and take the next step by transplanting major parts of the upper extremity.

In contrast to hand transplantation, an amputation at the proximal forearm presents a more complex situation, namely one that usually leaves only short forearm muscles unsuitable for reconstruction with donor tendons and too short to provide enough range of finger movement. Such an injury was given in the patient described here. The surgical procedure therefore comprised transplantation of both forearms including the entire forearm muscles. This is the first report on a transplantation of this type.

Patient and Methods

A comprehensive description of the patient was given earlier (7). In brief, the 41-year-old male had lost both hands and two thirds of his forearms in

an electric current accident in March 2000. Myoelectrical prostheses were fitted after the amputation and used until transplantation.

For assessment of residual muscles, tendons, vessels and nerves both forearms were investigated clinically, by ultrasound, CT scan and MRI. Flexor as well as extensor muscles were considered too short for musculotendinous reconstruction and for providing sufficient hand function (Figure 4). Therefore, a novel surgical approach including transposition and fixation of forearm muscles to the recipient's humerus was designed and tested in a cadaver to clarify surgical details. As reversibility of the surgical intervention was considered a prerequisite, precautions were made to facilitate the use of myoelectrical prostheses in case of graft loss. Specifically, remnant recipient forearm muscles were kept intact together with their innervation in order to allow implementation of myoelectrical prostheses in case of graft loss (see Surgery for details).

The patient was informed in detail about the novelty of the surgical approach, the potential consequences of long-term IS for metabolic and organ function and the risk of infection and tumor development. The difficulty in predicting long-term outcome was explained. An independent expert as well as members of the hand transplant team appraised the patient as being psychologically stable and able to understand the complexity of the offered procedure. The patient was then given time to weigh the anticipated improvement in quality of life against the potential risk of transplant. Throughout the entire evaluation process as well as after repeated discussion of the aforementioned risks, the patient expressed his wish to undergo transplantation without any doubt.

Surgery

The surgical procedure was performed on February 17, 2003. Donor and recipient were matched for blood group, gender, age, bone size and cosmetic appearance.

Simultaneously, the recipient's stumps and the donor forearms were prepared by identifying and dissecting neurovascular structures and muscles. Subsequently, bone fixation was followed by revascularization (ulnar and radial artery, two deep and three superficial veins including the cephalic and basilica vein) and reconstruction of muscles, nerves and skin. Bone length was determined by inserting the medial donor epicondyle in its corresponding place on the recipient's humerus. For muscle reconstruction, a 'piggyback method' providing corresponding reinnervation of donor musculotendinous flexor and extensor units was employed. In order to ensure reversibility of the procedure, the recipient's muscle remnants medial (M. flexor carpi ulnaris) and lateral (M. extensor carpi radialis longus et brevis, M. brachioradialis) remained unattached and their nerve supply intact. Conservation of recipient forearm muscle stumps was considered to be of utmost importance, because they would be required for stimulation of myoelectrical prostheses in case of graft loss (lifeboat procedure). Ulnar and median nerve sutures were made distal to the respective (donor) motor branches. The dorsal extensor muscles were harvested together with fascia and fixed to the recipient by transosseous sutures. The posterior interosseus nerve was coapted at the supinator level. At the end of surgery skin flaps were adapted and sutured without any tension.

As the allografts contained a large quantity of skeletal muscle, which is known to be more sensitive to ischemia/reperfusion injury than are other compartments of the forearm, we aimed to keep ischemia time particularly short. This was achieved by precisely adjusting the time schedules and by having four surgical teams simultaneously operate on both donor and recipient forearms. Perfusion of the grafts with UW preservation solution (500 mL for each side) was started only after all required structures had been identified and prepared at the recipient's stumps. Immediately after perfusion and harvest of the forearms, bone union between graft and recipient was performed, followed by revascularization. This sequence resulted in ischemia times of 155 and 153 min for the right and left forearm, respectively.

Immunosuppression and infection prophylaxis

Donor serological testing was negative for hepatitis B and C, HIV, EBV, lues and toxoplasmosis, but positive for CMV. There was a four HLA-antigen donor/recipient mismatch, but the lymphocytotoxic crossmatch was negative. The initial immunosuppressive regime was published previously (7). For induction, anti-thymocyte globulin was given for 3 days together with methylprednisolone. For maintenance, tacrolimus, prednisolone and MMF were administered. Steroid dose was gradually tapered to 10 mg after 1 year. MMF was withdrawn after alemtuzumab had been given for an ATG-resistant rejection (see results). At 16 months, everolimus was added to the treatment protocol and tacrolimus slowly tapered to trough levels of 6–8 ng/mL. This protocol was repeatedly adjusted in response to the clinical situation (see results).

The patient received broad-spectrum antibiotics during the early postoperative period. Trimethoprim/sulfamethoxazol was given for 18 months. For CMV prophylaxis, ganciclovir (10 mg/kg) was administered i.v. for 1 week, followed by valganciclovir (ValGCV, 900 mg/day orally) until leukopenia necessitated withdrawal at day 137.

Graft monitoring

Monitoring of rejection included daily inspection of the skin and protocol skin biopsies performed at 1, 2, 3, 4, 8, 12, 24, 36 and 52 weeks, twice annually thereafter and whenever clinically indicated. Biopsies were graded according to a previously described scoring system for rejection of the skin in CTA (6,7).

To evaluate bone healing, X-rays of hands and forearms were performed. Graft vessels, nerves, muscles and tendons were investigated by ultrasonography at three-month intervals. Angiography and CT angiography were performed once a year and analyzed with particular regard to luminal narrowing/occlusion as potential signs of chronic rejection.

Histology and immunohistochemistry

Biopsy samples were embedded in paraffin and stained with H&E. In addition, antibodies against CD4, CD8, CD20, CD56 and CD68 were used for immunohistochemical investigation of any cellular infiltrate. C4 d staining (C4 d-Ab, #BI-RC4D, Biomedica, Austria) was performed to detect humoral rejection.

Rehabilitation program and evaluation of hand function

The rehabilitation program was based on early protective joint motion-EPM combined with Perfetti cognitive exercise training, electrotherapy and occupational therapy. Rehabilitation was initiated on the second postoperative day and continues until today. Various types of positioning and functional night and daytime splints were employed to protect the hand, avoid retraction and facilitate rehabilitation. In addition, the patient was trained in basic and free time-related activities of daily life.

The following tests were applied for evaluation and documentation: Tinel's test, Semmes-Weinstein monofilaments test, hot and cold temperature test, Weber static two-point discrimination test, Dellon moving two-point discrimination test, pinch gauge test, volumetric analysis, manual muscle strength testing using Kendall muscle power grading, range of motion measurement, grip strength using Jamar dynamometer, visual analog test, tactile and kinesthetic tests after Perfetti. In addition, functional results and subjective assessment were evaluated by means of the ARA test, the DASH score, the Ipsen classification and the International Registry Score System

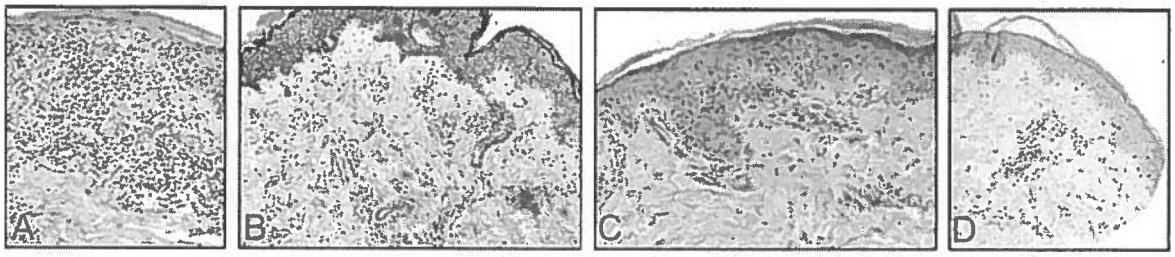

Figure 1: Histology of skin biopsy samples showing acute rejection grade III observed on day 345 (A), and two episodes of late acute rejection (grade I) seen on days 473 (B) and 972 (C). Despite absence of any skin lesions, a mild but persistent perivascular lymphocytic infiltrate was found in all skin biopsies from month 21 on (D).

proposed by the International Registry on Hand and Composite Tissue Transplantation (IRHCTT).

Electrophysiological studies

Electrophysiological studies were performed according to standard procedures using an Oxford Medelec Synergy (Witney, Oxfordshire, UK). Compound motor (CMAP) as well as sensory action potentials (CSAP) were measured at 3-month intervals starting at week 8.

Median nerve somatosensory evoked potentials (m-SSEP) were studied at 6 months and 3 years. Potentials were recorded over Erb's point, C5 and the somatosensory cortex according to standard procedures using a Nicolet Viking IV (Nicolet Biomedical, Madison, WI).

Results

Surgery

Wound healing was uneventful except for a small skin necrosis at the proximal end of each transplant requiring skin autografts at 1 week posttransplantation. Bone union was completed at 1 year. At 11 months, a small heterotopic paraarticular ossification (HPO) was observed at the level of the supinator muscle in the right forearm flexors with proximity to the donor proportion of the radius. This spike most likely arose from the periosteum. The HPO was dissected, but tendolysis was not needed. In response, functional recovery progressed more rapidly on this side. At the patient's request, a surgical intervention for correction of scars on both forearms was performed at 30 months after transplantation.

Immunosuppression and rejection

Within the first 6 months the patient experienced three rejection episodes, which were treated with methylprednisolone, basiliximab or alemtuzumab (see Ref.7). Thereafter, the patient remained free of clinical and histological signs of rejection for 8 months. At day 345, when lymphocyte count had risen to >10% of white blood cells, skin lesions characteristic for rejection were observed on both forearms and dorsal sides of both hands. Skin biopsy revealed rejection grade III (Figure 1A). As the rejection was severe and correlated with lymphocyte regeneration, alemtuzumab (20 mg), which has previously shown an effect superior to that of steroids or ATG, was administered. In response, the lymphocyte count again dropped to 0.5% of white blood cells, and skin lesions as well as histological signs of rejection disappeared over the following 2 weeks. Later, everolimus was added to the immunosuppressive regime after lymphocytes had again increased to over 10%. Two additional rejections were encountered on days 473 (Figure 1B) and 972 (Figure 1C). In contrast to previous episodes of acute rejection, maculopapular lesions on both forearms and the dorsal side of both hands progressed only slowly and erythema was less severe. Histology of skin biopsies showed grade I rejection in both cases. Both episodes where successfully treated with a transient tacrolimus dose increase in combination with topical application of tacrolimus ointment. At 3 years after transplantation, IS consists of tacrolimus (trough level 8 ng/mL), everolimus (trough level 6 ng/mL) and prednisone (5 mg/day). The patient is free of clinical signs of rejection, although a mild perivascular lymphocytic infiltrate in the skin persists (Figure 1D).

Tissue samples other than of the skin became available for histological evaluation when surgery was performed for scars (Figure 2). In these specimens, only single lymphocytes were found perivascularly in muscle and deeper connective tissue at 30 months after transplantation (Figure 3).

Immunohistochemistry and sensitization

Immunohistochemical analyses were performed whenever cellular infiltrate was observed and identified the majority of infiltrating cells as CD4-positive T lymphocytes (Figure 3, upper panel). CD8-positive T cells were found in a smaller number and accompanied by only few CD20-positive B-lymphocytes and a small proportion of CD56-positive NK cells. The number of macrophages increased with rejection progression and comprised a major subset of infiltrating leukocytes upon severe rejection.

In every biopsy performed throughout the observation period, immunohistochemical staining for C4d revealed a mild and unspecific signal at the endothelium of vessels in various tissues, comparable to staining in healthy, non-transplanted skin (Figure 3, lower panel). In addition,

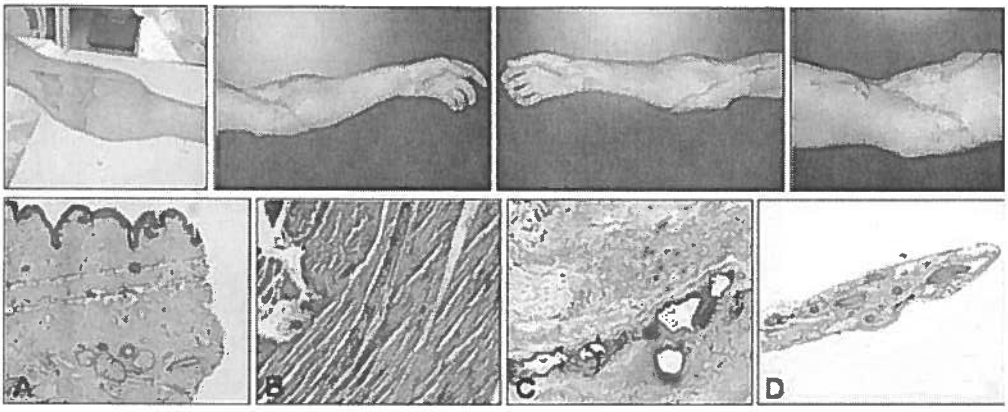

Figure 2: Samples from compartments other than the skin became available on surgical intervention for scars at 2.5 years after transplantation (upper panel). Despite the absence of any skin lesions a very mild perivascular lymphocytic infiltrate was observed in dermis (A). As for muscle (B), deeper connective tissue (C) and nerve (D) single perivascular lymphocytes were observed in otherwise healthy tissue.

testing for panel-reactive antibodies (ELISA) was negative at 3 years; no postoperative crossmatch was performed at that time point. Hence, no signs of humoral rejection were encountered.

Infections and side-effects

Shortly after ValGCV had to be withdrawn for neutropenia at day 137, the patient developed CMV infection. Treatment with cidofovir was commenced together with anti-CMV hyperimmunoglobulin and provided a sustained response (Ref.8; patient #4).

Five months later, multiple wart-like lesions on the dorsal side of the fingers and the distal hand were observed. Biopsy revealed HPV-associated lesions. Cidofovir ointment (1%) was applied on both hands, and regression of warts in size and number thereby achieved. Only few warts persist until today (9).

Two years after transplantation, the patient developed a small ulcerous lesion on his right thigh. Radiomorphological (MRI), histological and microbiological investigation revealed an infection with *Alternaria alternata* restricted to

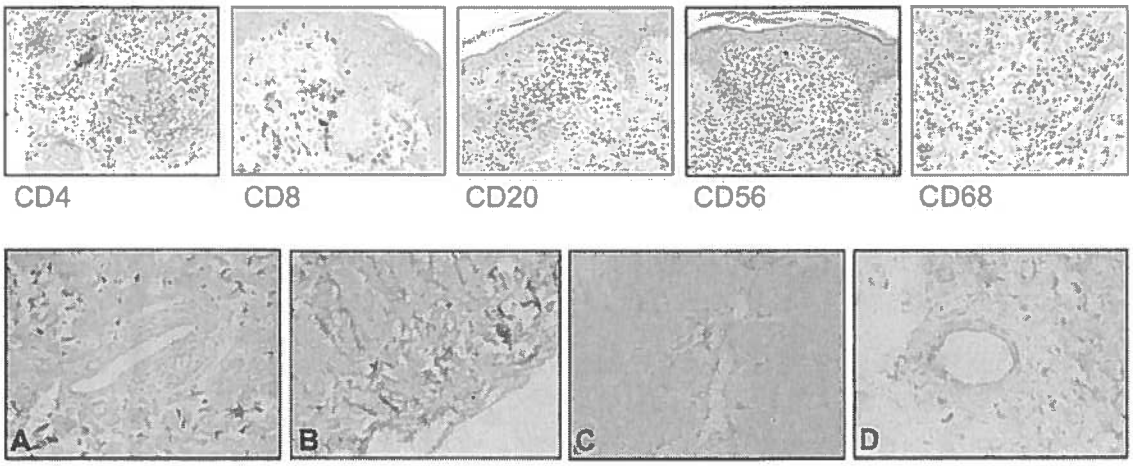

Figure 3: Upper panel: Immunohistochemical investigation of the cellular infiltrate (here shown for rejection grade III, Figure A, upper panel) revealed the majority of infiltrating cells to be CD4-positive T-lymphocytes together with a smaller number of CD8-positive T-suppressor cells. B-lymphocytes (CD20), NK cells (CD56) and macrophages (CD68) were found in a smaller number. Lower panel: Staining for C4 d revealed a mild and unspecific signal at the endothelium of vessels in dermis (A, B) and muscle (C). The signal was comparable to that for healthy, nontransplanted skin (D). No other signs indicating humoral rejection were seen.

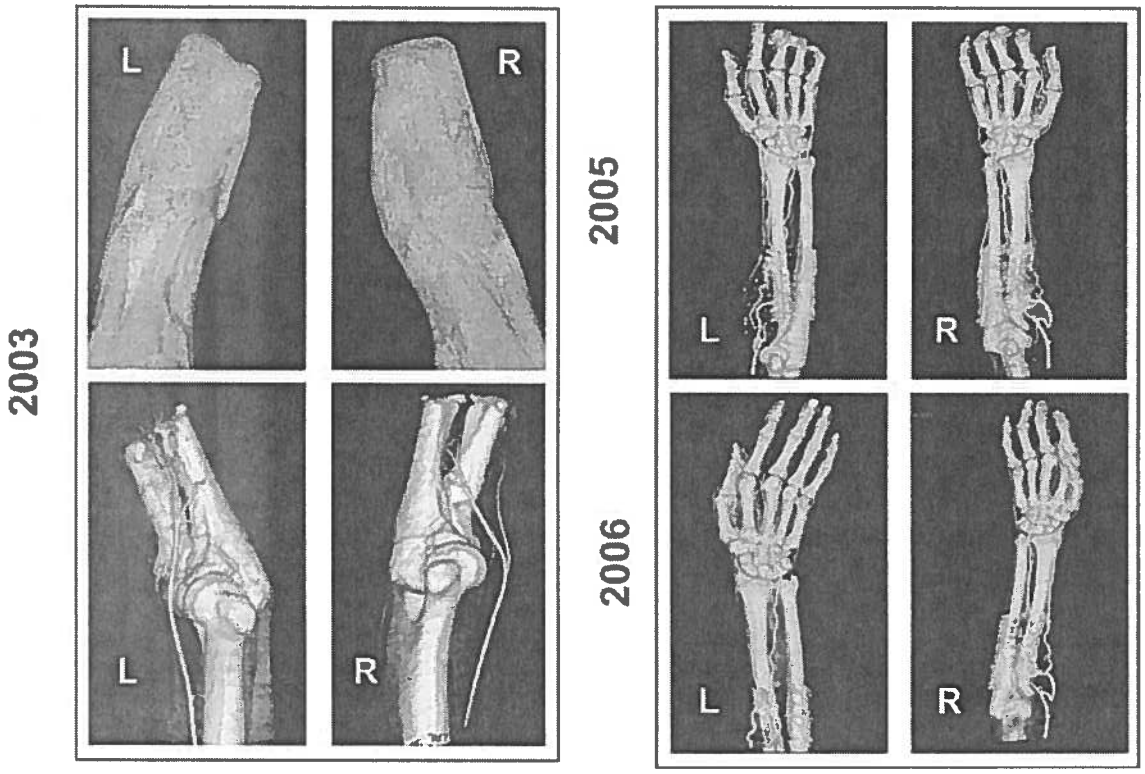

Figure 4: (2003) For CT angiography, 140 mL (5 mL/sec) of a non-ionic contrast medium was administered i.v. Smart Prep software (General Electric Medical Systems, Milwaukee, WI) was used to calculate scan delay. Volume rendering reconstructions after data were transferred to a three-dimensional rendering workstation (Advantage Windows 4.0; General Electric Medical Systems, Milwaukee, WI). Assessment of bone length, forearm muscles and vessels prior to transplantation revealed good vessel condition with sufficient caliber, but forearm muscles too short for reconstruction (2003). Investigations at 2 (2005) and 3 (2006) years after transplantation showed the radial, ulnar, and interosseous artery unchanged without any signs of luminal narrowing as indirect evidence of myointimal proliferation.

the skin. The lesion was excised and a splinter was found as the source of infection. Antifungal therapy consisted of liposomal amphotericin B (5 mg/kg) for 7 days followed by itraconazole (400 mg/day) for 3 months. The patient has been recurrence-free since then.

Hypertension causing recurrent episodes of headache was treated with rilmenidin (1 mg), losartan (12.5 mg), losartan-hydrochlorothiazide (50 mg/12.5 mg), doxazosin (1 mg) and urapidil (25 mg). Leukopenia persisted despite ValGCV withdrawal, with cell counts ranging from 2 to 4 G/L. Nephrotoxicity with a transient increase in creatinine levels (3.1 mg/dL) was observed under Foscarnet therapy, but returned to normal values thereafter. At 3 years, creatinine and urea are 1.3 and 60.2 mg/dL (1.0 and 58.7 mg/dL prior to transplantation) respectively. The calculated creatinine clearance (Cockroft-Gold) is 76.1 mL/min and lower than the 102.1 mL/min prior to transplantation. Glucose metabolism was disordered at an HbA1 C level of 6%–7.2% during the first 20 months. Glucose tolerance testing (dextrose, 75 mg) at 3 years was normal (blood glucose levels in detail: start 100 mg%, after 60 min 155 mg%, after 90 min 102 mg%, after 120 min 99 mg%). Urine glucose was normal and ketone negative. A bone scan showed results in the normal range before and 3 years after transplantation.

Radiomorphological studies

At 2 and 3 years angiography and CT angiography revealed normal blood flow through radial and ulnar arteries in the right and left hands and forearms. No luminal narrowing as an indirect sign of myointimal proliferation was observed; all arteries were preserved and unchanged in caliber in both arms (Figures 4C–F).

Graft function

During the first 3 years, active range of motion (ARM) of wrist, metacarpal (MCP) and interphalangeal (IP) joints continued to improve on both sides (Figure 5). Degree and speed of progression showed some differences related to side and joint. Elbow flexion was 120° on the left and 135° on the right side with unrestricted extension. Active

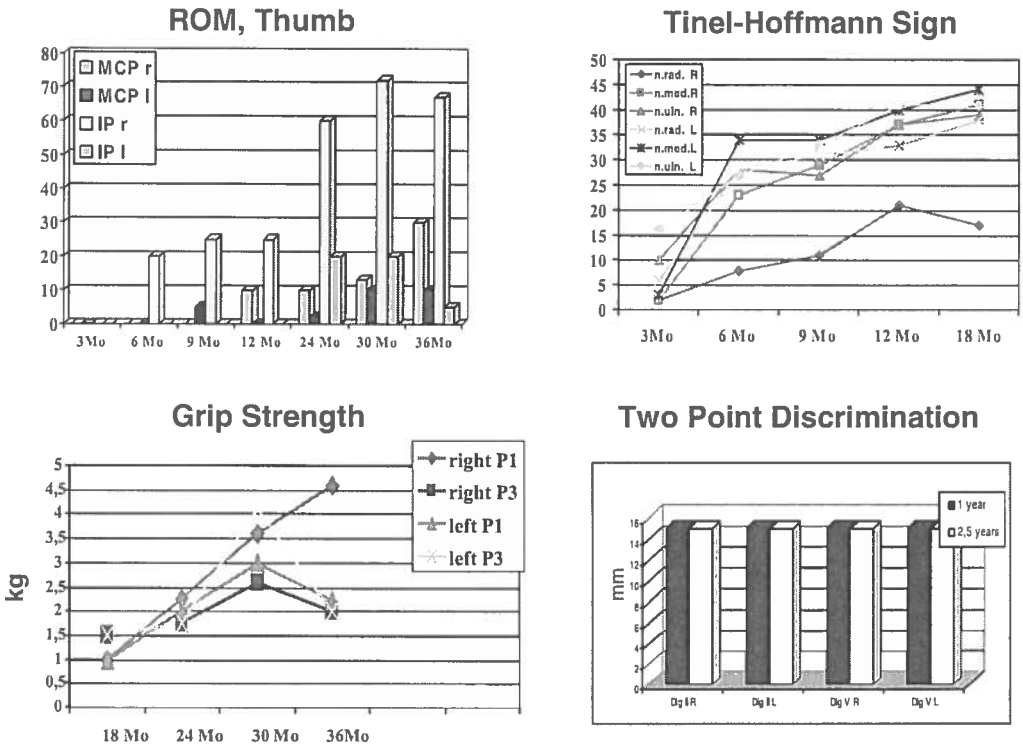

Figure 5: At 36 months after forearm transplantation ARM of wrist, MCP and IP joints on both sides is still constantly increasing with some differences related to side and joint. ARM of the right wrist is 85° and of the left wrist 80°. Total ARM for the index finger is 170° on the right side and 225° on the left side. The Hoffmann-Tinel sign reached the fingertips at 18 months. Grip strength ranges from 2.0 to 4.3 kg. Discriminative sensation remains poor, as reflected by a two-point discrimination of >15 mm.

forearm rotation measures 80/0/0 on the right and 75/0/20 on the left side. ARM of wrist and digits is depicted in Table 1. Total ARM of the wrist is 95° on both sides. Total ARM of the index finger, which describes the sum of active flexion measurements of the MP and IP joints, minus the sum of their active extension deficits is 165 on the right side and 230° on the left side. According to the Medical Research Council's Scale (0 no contraction, 1 no joint motion, but contraction palpable, 2 active movement, with gravity eliminated, 3 active movement against gravity, 4 active movement against gravity and resistance, 5 full muscle strength), muscle power of the left M. interosseus dors. I is M2. Muscle power of the M. interosseus palm. I is M1 on the right side and M2 on the left side. Key pinch is 1.66 kg on the right side and 1 kg on the left side on average (According to Mathiowetz, normal values for a healthy 42-year-old adult male are 11.6 kg (SD 1.2) and 11.4 kg (SD 1.8) for the right and left side, respectively.) Thumb opposition measures 6 (out of 10) on the right side and 4 on the left side according to the Kapandji score. This index assesses opposition of the thumb and flexion and extension of the long fingers by actively touching the four long fingers with the tip of the thumb. Hence, it does not require angular measures of joint mobility because the scoring system uses anatomic landmarks of the hand as references and allows determination of overall hand mobility. Active palm-pulp distance measures about 15 mm on average for all long fingers. The patient is able to perform pulp pinch on the right side, but unable to perform tip pinch. It is important to note that ARM as well as muscle strength varied with day and time (e.g. daytime) of assessment.

Discrimination for cold was first detected at 6 months, and discrimination for hot 2 months later. When evaluated using the two-point discrimination test, which examines the patient's ability to discriminate the sensory input of the quickly and slowly adapting fiber/receptor system, the overall sensory function was poor with no detectable two-point discrimination (Figure 5). To evaluate perception of light touch-deep pressure the Semmes-Weinstein Calibrated Test with monofilaments of increasing forces was used and revealed diminished protective sensation (4.31) with differences related to side and innervation. Occurrence of acute rejection was accompanied by transient stagnation or impairment of hand function.

Table 1: ARM of wrist and digits at 3 years after bilateral forearm transplantation

	Wrist			
	Extension	Flexion	Ulnar deviation	Radial deviation
Rigth	65	30	10	15
Left	75	20	10	10
	Digits			
Right	MP	PIP	DIP	IP
Thumb	0/15/45			0/15/80
Dig 2	0/0/60	0/40/105	0/0/40	
Dig 3	5/0/80	0/55/95	0/20/75	
Dig 4	10/0/90	0/35/95	0/15/65	
Dig 5	15/0/90	0/15/80	0/0/80	
Left	MP	PIP	DIP	IP
Thumb	0/35/45			0/15/20
Dig 2	0/0/70	0/20/100	0/5/85	
Dig 3	5/0/90	0/50/100	0/10/60	
Dig 4	20/0/90	0/45/90	0/10/80	
Dig 5	20/0/100	0/15/90	0/0/70	

MP = metacarpophalangeal joint; DIP = distal interphalangeal joint; PIP = proximal interphalangeal joint; IP = interphalangeal joint.

The functional test implemented proved useful in assessing functional parameters of the transplanted hands and forearms with relevance to their use in activities of daily life. According to the patient's subjective perception, hand function is slightly superior to that achieved with myoelectrical prostheses. The outcome according to the 'International Registry Score System' was 51.5/55.5 (ri./le.) at 1 year and 71/69.5 at 3 years. The DASH score is 64 at 3 years. The Disability of Arm, Shoulder and Hand (DASH) Version 2.0 questionnaire captures the subjective experience of patients regarding their own health. This 78-item instrument was developed to measure components of health status relevant to upper extremity conditions (4). The patient now describes his ability e.g. to wash his hair and body, shave, brush his teeth, eat, hold a glass or dress himself to be improved as compared to before surgery. He notes that the wider range of motion together with fine motor skills and an improved sense of body integrity are the primary benefits. However, some other functions requiring strength in only one particular direction, such as carrying a briefcase or shopping bag, opening a bottle with a tight top and turning a door key were easier with the prosthesis. These limitations are reflected in the DASH score, whereas other, more subjective perceptions remain disregarded using the DASH score system.

Electrophysiological studies

Eighteen months after transplantation CMAP from the hypothenar was recorded for the first time, whereas responses from the thenar were recordable only at 2 years after transplantation. At 3 years, all responses were considerably increased (Figure 6A). Sensory responses were recordable from the ulnar nerve at 2 years after transplantation. Sympathetic skin responses were absent at 1 year, but normal at 3 years (Figure 6B).

Reinnervation of the wrist and long finger flexors and extensors started 6 months after transplantation and progressed over time. Reinnervation of intrinsic hand muscles was first seen at 12 months and continued to improve thereafter. Three years after transplantation all muscles showed high-amplitude (up to 10 mV), long-duration motor unit potentials. Motor unit loss was seen in all muscles, but was more pronounced in intrinsic hand muscles.

Evoked potentials

At 1 year, cortical motor somatosensory evoked potentials (m-SSEP) were absent. Nevertheless, at 3 years good responses were recorded over the somatosensory cortex on both sides, while no potentials were recorded over Erb's point or the cervical spinal cord (Figures 6 C,D). Latencies of the cortical responses were 25.4 ms after right and 26.9 ms after left median nerve stimulation (normal <22.2 ms), and amplitudes were 1.0 and 0.4 μV (normal >1.5 μV). Findings not only confirm peripheral sensory reinnervation, but also indicate reorganization of the somatosensory cortex.

Discussion

It is common knowledge that the more proximal the level of arm amputation, the higher the degree of invalidity is

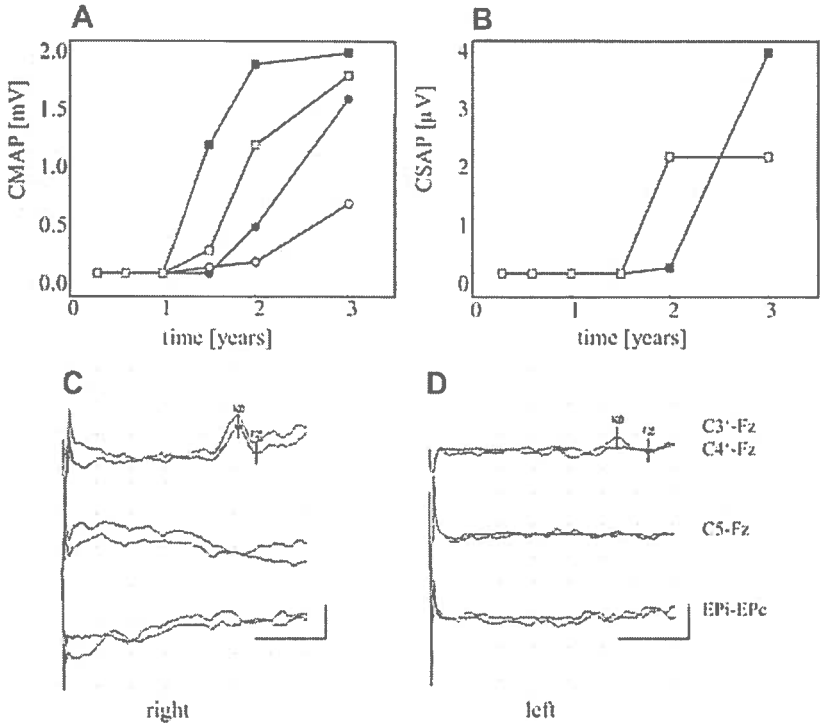

Figure 6: Compound motor action potentials (CMAP) were recorded using disposable surface electrodes placed on the abductor pollicis brevis and abductor digiti minimi muscles after stimulating the median and ulnar nerves at the wrist and elbow (A). Compound sensory action potentials (CSAP) were recorded from the index and fifth fingers using band electrodes. Electromyography was performed with concentric needle electrodes (B). ○ left median nerve; ● right median nerve; □ left ulnar nerve; ■ right ulnar nerve; Note the progressive increase in compound motor evoked potentials, whereas sensory evoked potentials were recordable only after ulnar nerve stimulation. Motor nerve conduction velocities in the transplanted forearm ranged from 20 to 28 ms^{-1} (normal > 49 ms^{-1}) at 3 years. Normal values for median and ulnar CMAP are 5 and 4mV, and for median and ulnar CSAP 10 and 8 μV, respectively. Left and right median nerve somatosensory evoked potentials 3 years after transplantation. Responses were absent over Erb's point (EP) and over the cervical spinal cord (C5, fifth cervical vertebra), but recordable over the somatosensory cortex of both hemispheres (C3′, C4′). Horizontal scaling bar, 10 ms; vertical, 1 μV. Latencies of cortical responses were 25.4 ms after right and 26.9 ms after left median nerve stimulation (normal <22.2 ms), amplitudes were 1.0 and 0.4 μV (normal >1.5 μV).

(10–12). After amputation of both hands and almost the entire forearms, the patient described here was equipped with myoelectrical prostheses. These allowed good elbow flexion up to 90°, but, despite all efforts to optimize their fit, the prostheses disconnected from the stumps when flexing any further. In addition, flexion as well as traction resulted in electrode displacement and subsequent loss of function. Arm-to-face contact requiring elbow flexion of more than 120° was restricted as grip patterns lost control. Additionally, loss of forearm rotation made it impossible for the patient to reach his back or the back of his head. Furthermore, the patient's sense of body integrity was disrupted, and loss of sensitivity made direct eye control a prerequisite for each grip.

Composite tissue transplantation is a novel and exciting therapeutic option after hand loss. Nevertheless, immunosuppression puts the recipient at risk for a variety of opportunistic infections and increases his tumor risk (13–15). Side-effects such as nephrotoxicity, hypertension or diabetes require additional treatment and entail the risk of long-term consequences such as renal failure and coronary heart disease (16–19). Therefore, the debate surrounding justification and indication for hand transplantation is ongoing, and the recent stagnation in the total number of hands transplanted, instead of an increase, indicates that major problems in this field remain unsolved. As very good results have been achieved with bilateral hand transplantation, the local selection committee decided to continue the CTA program 'with caution'. Based on the observation that motor function after hand transplantation was excellent and intrinsic muscles were found to be reinnervated in most patients, performance of bilateral forearm transplantation in the patient described here was considered a

promising therapy (1–6). In the approach to the transplantation, a surgical concept was designed that would allow the use of myoelectrical prostheses in case of graft loss as a lifeboat procedure.

By transplanting entire innervated musculotendinous units, function was restored. Ischemia time was kept very short in order to minimize muscular damage. As hand function was dependent on reinnervation of forearm muscles, the time frame observed for recovery of motor function differed from that for hand transplantation. In this context, early but not accelerated systematic rehabilitation is considered essential to obtain good functional results. Furthermore, the rehabilitation program needs to be adapted to the existing status of nerve regeneration. As for reconstruction, separate coaptation of the motor branches from the three main forearm nerves and independent reconstruction of sensitive branches proved beneficial, as the distance to the muscle was shortened, which resulted in shorter time for nerve regeneration. In this context, it is remarkable that reactivation of intrinsic muscles was observed despite the long distance between nerve junction and fingers. This contrasts with findings after ulnar nerve laceration at the forearm, where reconstruction usually fails to re-establish intrinsic muscle function. In the context of a transplant, however, donor nerves were kept long enough to allow nerve reconstruction without any tension, whereas reconstruction of an injured nerve often requires resection of small proportions of a nerve, resulting in short total length and tension on the suture. Also, the well-established beneficial effect of tacrolimus on nerve regeneration as well as the intense rehabilitation program might have facilitated nerve growth and rapid reinnervation of intrinsic muscles.

Activity of forearm muscles was observed early after transplantation and subsequently improved slowly but continuously. Reinnervation and reactivation of transplanted muscles providing good motor function has been proven and further emphasizes the potential of neuromuscular components to become integrated in the motor cortex. An increase in CMAP and CSAP was observed at 3 years. This is in line with observations made after hand transplantation, where motor and sensory function was found to improve during the first 7 years. In addition, these findings raise hope that sensitivity might also still improve. The patient states that 'now that side-effects (in particular repeated headaches) have been overcome, he is satisfied with the outcome'. Hand function is 'superior to that with prostheses and contributes to overall satisfaction'. He is happy that 'body integrity has been re-established'. The patient also points out, however, that 'the postoperative course was more challenging and exhausting than expected'. When evaluated according to the 'International Registry Score System', the outcome is less favorable than the outcome of 91.5 and 95 at 3 years after bilateral hand transplantation in our first patient, but comparable to results achieved in hand transplant recipients at other centers (4). As descriptions of ARM or grip strength are helpful in reaching a scientific judgment on such a procedure but fail to show its actual relevance, two short videos illustrating hand function are provided as complimentary files (supporting documents 1 & 2). Better than any description, these films provide a realistic picture of functional capacity as well as certain limitations. Standardized outcome assessment is essential for evaluating, monitoring and communicating performance after hand transplantation. The evaluation and documentation system not only influences the quality of individual treatment, but also impacts on comparability and quality control. We therefore believe that hand or forearm transplant recipients should be evaluated according to a scoring system that refers to the individual's ability to use his/her hands in activities of daily life.

As the CTA field progresses and patients with various types of amputation are requesting a transplant, the observations described here provide additional evidence that CTA may also be an option after amputations at a higher level. But what is the price of such an achievement? A challenging and exhausting postoperative course was complicated by multiple rejections, infections and severe side-effects requiring additional treatments. Infections remain challenging problems, with high-level immunosuppression known to be the most important risk factor. Although infections due to CMV, *Alternaria altenata* and HPV were successfully managed without withdrawal of immunosuppression, the clinical course described here underscores the risk for these otherwise healthy individuals. A long hospital stay, frequent outpatient visits, as well as exemplary patient compliance were needed until a stable condition was achieved without clinical signs of rejection or side-effects.

The transplanted forearms contain a large quantity of muscle and other connective tissues together with 2030 cm^2 of transplanted skin. This presumably very large antigen load is assumed to trigger a strong immune response and to have caused repetitive rejections. Such a statement, however, remains hypothetical as the number of hand transplants performed to date is too small to permit reliable conclusions to be drawn and the question whether graft size is related to immune response has not been addressed in an experimental CTA setting to date. Hence, the immunosuppressive regimen was not adjusted according to graft size, but had to be repeatedly adapted in response to the clinical situation. In comparison to our experience with more distal transplants, more severe and early acute rejections were observed here.

As for muscles and connective tissue, a mild perivascular lymphocytic infiltrate remains of unknown clinical relevance but contributes to the currently very limited knowledge on rejection in components other than the skin (20,21). For the time being, deep tissue biopsies do not seem to provide additional information, but bear the risk of damaging nerves, tendons or muscles or provoking

severe bleedings. Although the skin seems to be a 'sentinel component' of a CTA, tools for monitoring these compartments, other than biopsies, are desirable.

As for the humoral immune response, the histomorphological features and the relevance of antibody-driven rejection in CTA remain to be defined. In our experience, C4 d investigation has not been determined to be useful in this context, as the staining at the endothelium seems to be unspecific.

The course described here shows that the limitations and the problems to be overcome are of an immunological and not a surgical nature. In fact, the balance between drug-related toxicity and rejection is delicate and remains poorly defined. The ability to induce donor-specific tolerance would certainly revolutionize CTA. However, despite promising novel protocols, it is unlikely that long-term immunosuppression can be avoided in the near future. Instead, protocols avoiding steroid use or minimizing calcineurin inhibitors, which have been successfully applied in solid organ transplantation, might also be useful in CTA. In addition, the unique advantage in hand transplantation, namely the applicability of topical immunosuppression/immunomodulation, should be further addressed.

In conclusion, the case described here shows that a satisfactory functional outcome can indeed be achieved in forearm transplantation, but also confirms that the very challenging postoperative course may require an all-out effort by patient and surgeons to overcome repetitive rejections as well as side-effects.

Contributions

Surgery was performed by Markus Gabl, Milomir Ninkovic, Heribert Hussl and Hildegunde Piza-Katzer. Marina Ninkovic designed the rehabilitation program and conducted functional tests. Michael Rieger performed the radiomorphological studies; Wolfgang Loescher conducted all neurological studies. Bettina Zelger performed histological analysis including immunohistochemistry; she was assisted by Theresa Hautz. Stefan Schneeberger and Raimund Margreiter designed the immunosuppressive regime, postoperative follow-up and monitoring and coordinated the Innsbruck Hand Transplant Team. Gerald Brandacher, Claudia Boesmueller and Hugo Bonatti contributed to monitoring and postoperative follow-up. Hugo Bonatti coordinated the treatment of opportunistic infections.

Acknowledgments

We would like to thank the following staff members of Innsbruck Medical University Hospital, who performed the surgery and were involved in posttransplant monitoring and therapy: Surgical Team: Prof. Dr. Hildegunde Piza-Katzer (Head of the Department of Plastic and Reconstructive Surgery); Prof. Dr. Michael Blauth (Head of the Department of Trauma Surgery); Prof. Dr. M. Lutz, Prof. Dr. R. Zimmermann and Dr. P. Angermann (Department of Trauma Surgery); A. Lobgesang, D. Estermann (Chief Physiotherapists and their team); C. Neyer (Head Nurse and his team, Transplant Surgery).

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